



A TRIAL FOR DEVELOPMENT OF NON CLASSIC FERTILIZATION PRACTICES

[23]

Heba, A.M. Abou-Zeid¹; A.E. El-Leboudi¹; M.A. Mostafa¹; E.M. Abd- Elmoniem¹
and U.A. El-Behairy²

- 1- Soils Department, Faculty of Agriculture, Ain Shams University, Shobra El-Khema, Cairo, Egypt
2- Horticulture Department, Faculty of Agriculture, Ain Shams University, Shobra El-Khema, Cairo, Egypt

Keywords: Organic manures; Manure extracts; Biofertilizers; Rock phosphate; Soil chemical characteristics; Element availability

most of the studied parameters, application of rock phosphate to the mentioned treatments being also favorable.

ABSTRACT

The current study was carried out to evaluate certain non-conventional techniques including the use of organic manures, either residues left from soaking cattle and chicken manures applied to the soil or spray of their extracts on the plant biomass; biofertilizers as well as natural resources of rock phosphate were also used, inorganic fertilizers being included for comparison. Such evaluation was thought to be performed through evaluating responses of soil characteristics represented by both chemical characteristics and element availability; responses of developed plants to applied treatments were thought to be not included in the present study. This study was conducted under conditions of greenhouse located in Soils Department, Faculty of Agriculture, Ain Shams University; it represents the application of results obtained from the two pot experiments previously conducted. Cucumber seedlings of 15 days old were planted after being inoculated with liquid culture of the used biofertilizers and let to be grown three months up to harvest. The studied soil samples were taken out before flowering stage of grown cucumber plants to evaluate the concerned responses of the indicated soil samples. Results showed that organic manure application practices were favorable compared to either original untreated soil or inorganic control treatments; application of the used biofertilizers added, either separately or in combinations, to manuring was favorable for

1. INTRODUCTION

A number of studies have indicated the beneficial effects of organic manures on improving soil properties and plant growth. Several organic amendments were frequently used to achieve such goal of clean agriculture. Organic amendments are currently used in agricultural lands to improve their physical, chemical and biological properties; soil organic matter (SOM) includes several components and it is not clear which one is modified when organic inputs are used. Organic manures can be valuable inexpensive soil conditioners and source of plant nutrients; positive effect of organic wastes on soil has been reported in several studies (Odlare *et al* 2008).

Many organic wastes or by-products after composting process as well as their use through non-conventional practices have been found to be suitable for use as growing organic substrates in the soilless agriculture. However, there is a complex nature for production of non-conventional forms of certain organic substrates, such non-conventional forms presenting a challenge for the adjustment of available element contents in the supplied nutrient culture to match plant requirements. In the eco-organic type soilless culture, the important factor affecting the indicated adjustment of nutrient composition of the supplied nutrient solution should be the extent of ionic release and balance with capacity of adsorption by organic substrate. Several investigations were presented for suggesting the

use of extracts, obtained by soaking different used organic manures, either as a nutrient solution (Abd-Elmoniem and El-Shinawy, 2002) applied directly to the soils beside the grown plants, (Abou-Hussein *et al* 2002) or as a foliar spray on the grown plants (Al-Mughrabi, 2007).

Man has added organic and inorganic amendments to soil for centuries to improve soil fertility and increase crop yield (Kábana, 1986). Zmora-Nahum *et al* (2007) reported that a proper world for future generations needs farming systems that give high crop yields with limited pollution risks and minimal nutrient depletion of natural, not renewable resources (soil, water and atmosphere). In the last 60 years, a wrong land use and incorrect agricultural practices have often lowered the soil content of organic matter; today a rise of this soil component is required almost everywhere.

Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritional elements from relatively difficult available to available form through biological processes (Vessey, 2003). Multi-microbial inoculation has been proposed as a way for protecting plants against environmental stress and increasing the sustainability for plant production; biofertilizers play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormones production and ammonia excretion as well as to control various plant diseases (Vestberg *et al* 2004).

Mycorrhizal colonization of roots usually results in an increase in the surface area for nutrient acquisition; the extrametrical fungal hyphae can extend several centimeters into the soil and absorb large amounts of nutrients for the host root (Khan *et al* 2000). In an attempt to reduce environmental risks with fertilizer uses, N and P biofertilizers have been considered as possible substitutes for traditional mineral nitrogenous and phosphatic fertilizers (El-Sirafy *et al* 2006).

Several natural resources may be used in agriculture practice to improve both element availability and plant growth either directly through their contents of certain essential elements, or indirectly through their effects on the soil properties either directly through nutrient supply or indirectly through interactions with the concerned practices of fertilization; rock phosphate (R) was used frequently for those purposes (Duponnois *et al* 2005).

The present study was conducted to evaluate the possibility of adopting certain non-conventional fertilization practices; such evaluation was thought

to be performed through evaluating soil responses to applied amendments including organic manures and their application practices, bio-fertilizers as well as natural resource of rock phosphate.

2. MATERIALS AND METHODS

A field trial was conducted to evaluate soil responses to the used amendments including the use of best treatments encountered with the previous two successive pot experiments, as a base diet, (unpublished data). The studied treatments included the use of both extracts and residues left from soaking either cattle or chicken manure either alone or accompanied with the used biofertilizers, applied either separately or in combinations. Such indicated biofertilizers, including N-fixing bacteria containing *Azotobacter chroococum* and *Azospirillum lipoterum* (Z) known under commercial name of "nitrobein", phosphate dissolving bacteria involving *Bacillus megatherium* (P) known under commercial name of "phosphorein" and arbuscular mycorrhizal fungi (M) with or without addition of rock phosphate. Organic extracts were prepared by using extraction ratio of 1 manure: 10 water; cucumber seedlings, 15 days old, were inoculated with liquid cultures of either the nitrobein, phosphorein or both taken from Agriculture Research Center (ARC) but mycorrhizal fungi were, however, collected from soil close to rhizosphere of maize plants grown on the Experimental Station of Soils Department, Faculty of Agriculture, Ain Shams University. The studied treatments were represented by four groups, with three replicates for each treatment, A, B, C and D, three control treatments being also included.

Group A

This group included three treatments, including the inoculation with the indicated biofertilizers in separate forms.

Z = inoculated with nitrobein.

P = inoculated with phosphorein.

M = inoculated with mycorrhizal fungi.

Group B

This group included four treatments including the combination of the used biofertilizers.

Z+P = inoculated with nitrobein accompanied with phosphorein.

Z+M = inoculated with nitrobein along with mycorrhizal fungi.

P+M = inoculated with phosphorein accompanied with mycorrhizal fungi.

Z+P+M = inoculated with nitrobenin accompanied with phosphorein and mycorrhizal fungi.

Group C

This group included three treatments, similar and correspondent to group A, but enriched with rock phosphate.

Group D

This group included four treatments, similar and correspondent to group B, but again, enriched with rock phosphate.

Control treatments

These treatments contained three treatments including control 1, only receiving the suggested dose of inorganic NPK fertilizers, control 2, receiving the suggested dose of inorganic NPK fertilizers accompanied with the manure extracts sprayed on grown plants, and control 3 receiving the residues left from the soaked concerned organic manures accompanied with the indicated manure extracts on the plant biomass.

The current study was carried out to investigate the effects of different studied treatments on responses of soil which were evaluated through evaluating the chemical characteristics represented by electrical conductivity EC, soil pH and organic matter OM content; element availability was also included; nitrogen (N) and phosphorus (P) being selected as representing the macronutrients, Fe being selected to represent the micronutrients and Pb to stand for the heavy metal pollutants.

2. 1. Soil analyses

2.1.1. Chemical characteristics

Electrical conductivity EC (dSm^{-1} at 25 C) was determined in 1: 5 (soil: water) extract, using EC meter, as described by **Jackson (1973)**.

Soil pH was assayed in 1:2.5 (soil: water) suspension using glass electrode pH meter according to **Jackson (1973)**.

Organic matter (OM) content was determined according to Walkely and Black method using α -phenanthroline as indicator (**Black et al 1965**).

2.1.2. Element availability

Available nitrogen was extracted using 2 M KCl solution and determined by steam distillation procedure using Mg devarda alloy according to **Black et al (1965)**.

Available phosphorus was determined according to **Olsen et al (1954)** using 0.5 M NaHCO_3 solution for extraction following ascorbic acid method.

Finally, available iron Fe and lead Pb were assayed, as being represented by Fe and Pb DTPA-extractable, using atomic absorption according to **Chapman and Pratt (1961)**.

Surface soil samples, representing the selected area of investigation were, initially taken out, correspondent samples from the concerned organic manures being also included. Analyses have been undertaken for selected soil and manure samples following the standard procedures reported by **Black et al (1965)**. Some characteristics, including physical, chemical and biological, of the indicated soil samples are shown in (**Table 1**); correspondent analyses for the concerned organic manures are shown in (**Table 2**).

The same indicated analyses were performed for the soil samples subjected to different treatments, such samples being taken out (from surface soil) just before flowering stage of developed cucumber plants.

The suggested dose of organic manures was 20 ton fed^{-1} from each used one; the suggested dose of inorganic fertilizers, on the other hand, was 300 kg fed^{-1} ammonium sulfate (15.5 %N), 150 kg fed^{-1} super phosphate (15 % P_2O_5) and 100 kg fed^{-1} potassium sulphate (48 % K_2O).

2.2. Statistical analysis

Obtained data were subjected to analyses of variance (ANOVA) and the differences among the means were determined using the Tukey test by M- Stat computer program.

3. RESULTS AND DISCUSSION

A field trial was, as previously mentioned, conducted to evaluate responses of soil characteristics to application of both cattle and chicken manures, which were selected as the best studied manures of the two studied pot experiments (unpublished data) regarding both chemical characteristics and element availability with the final goal of evaluating organic manuring, bio-fertilization and rock phosphate application.

Table 1. Some physical, chemical and biological properties of the used soil

Physical properties			Chemical properties			Biological properties	
Particle size distribution			CaCO ₃	%	3.41	Available elements (mgkg ⁻¹)	
Sand	%	11.3	OM	%	1.76	N	19.2
Silt	%	17.2	*EC dSm ⁻¹		0.33	P	18.3
Clay	%	71.5	Soluble cations (mrq/l)			Fe	16.4
Soil texture	(Clay)		Ca ⁺⁺		0.96	Pb	4.23
SP	%	68.0	Mg ⁺⁺		0.68		
			Na ⁺		0.84		
			N ⁺		0.81		
			Soluble anions (meq/l)				
			CO ₃ ⁻		Nil		
			HCO ₃ ⁻		0.86		
			Cl ⁻		1.83		
			SO ₄ ⁻		0.69		
			**pH		8.20		

* = Soil extract (1 soil: 5 water).

** = Soil suspension (1 soil: 2.5 water).

Table 2. Some characteristics of the used organic manures

Characteristics Organic manure	*EC dS.m ⁻¹	**pH	OM %	OC %	C/N ratio	N %	P %	Fe mgkg ⁻¹	Pb mgkg ⁻¹
Cattle manure	6.57	7.12	40.4	23.4	13.9	1.68	0.49	680	10.2
Chicken manure	7.10	6.46	33.7	19.6	4.73	4.14	0.38	540	20.1

* = Manure extract (1manure:10 water).

** = Manure suspension (1manure: 2.5 water).

3.1. Soil chemical characteristics

As previously mentioned, soil chemical features, including electrical conductivity (EC), soil reaction (pH) and organic matter content (OM) were evaluated as regarding to responses to the investigated treatments including those of control; values are shown in **Table (3)**.

3.1.1. Electrical conductivity

Obtained results showed that EC values of all the studied treatments were higher, compared to both original untreated soil (**Table 1**) and "cont 3" that only received the base diet which was, how-

ever, slightly higher compared to both "cont 1" and "cont 2", receiving inorganic fertilizers applied separately or accompanied with spray of manure extracts on the plant biomass, respectively, "cont 1" being the lowest. Obtained results are not unexpected due to the relatively high content of soluble salts in the used organic manures (**Table 2**) which should be expected to raise soil salinity. This agrees with results of **Badran et al (2000)** who pointed out that use of organic manures, especially at high rates, as well as inorganic fertilizers may raise soil salinity indirectly due to mineralization process of the applied manures or directly through soluble ions released from inorganic fertilizers.

Table 3. Effect of the used organic manures on the studied soil characteristics.

A- Cattle manure

Treatments		Chemical characteristics			Available element (mgkg ⁻¹)			
		*EC dSm ⁻¹	**pH	OM%	N	P	Fe	Pb
Inorg.	cont 1	0.45 abc	7.84 f	1.92 a	50.4 a	19.5 a	18.5	4.86 gh
Inorg.+Ext.	cont 2	0.46 abcd	7.81 cde	1.94 ab	57.5 b	20.6 a	20.9 b	4.88 h
Res.+Ext.	cont 3	0.51 abcdef	7.78 abcde	2.49 j	60.7 c	22.4 b	22.6 c	4.79 defgh
A	Z	0.53 cdef	7.73 ab	2.21 fgh	73.1 g	24.1 v	28.1 de	4.69 bcde
	P	0.47 abcdef	7.75 abcd	2.25 gh	65.2 d	27.6 d	27.1 d	4.66 bc
	M	0.43 a	7.71 a	2.35 hi	67.6 e	29.4 e	29.2 efg	4.53 a
B	Z+p	0.56 f	7.76 abcd	2.11 de	75.3 h	29.8 e	30.2 gh	4.74 cdefg
	Z+m	0.54 def	7.74 abc	2.16 ef	76.8 h	30.6 ef	31.6 ij	4.68 bcd
	P+M	0.52 bcdef	7.71 a	2.29 hi	69.3 ef	31.4 fg	31.3 hij	4.66 bc
	Z+P+M	0.50 abcdef	7.79 bcde	2.16 ef	82.6 k	35.1 j	33.1 k	4.78 cdefg
C	Z+R	0.55 ef	7.76 abcd	2.15 de	76.3 h	25.4 c	28.8 ef	4.73 cdef
	P+R	0.46 abcd	7.76 abcd	2.17 efg	69.4 f	29.2 e	28.2 de	4.69 bcde
	M+R	0.44 ab	7.73 ab	2.27 hi	71.5 g	31.4 fg	30.4 ghi	4.57 ab
D	Z+P+R	0.58 fg	7.78 abcde	2.07 cd	78.6 i	32.6 gh	30.1 fgh	4.81 efg
	Z+M+R	0.56 f	7.75 abcd	2.09 cd	81.2 j	33.5 hi	30.7 hij	4.72 cdef
	P+M+R	0.55 ef	7.74 abc	2.14 ef	81.8 g	34.8 ij	31.9 jk	4.69 bcde
	Z+P+M+R	0.52 bcdef	7.83 ef	2.11 de	85.9 kl	36.4 jk	36.4 jk	4.83 fgh

Table 3. Cont.

B- Chicken manure

Treatments		Chemical characteristics			Available element (mgkg ⁻¹)			
		*EC dSm ⁻¹	**pH	OM%	N	P	Fe	Pb
Inorg.	cont 1	0.45 abc	7.84 f	1.92 a	50.4 a	19.5 a	18.5 a	4.86 fg
Inorg.+Ext.	cont 2	0.47 abcd	7.79 de	1.96 a	60.2 b	20.8 b	21.2 b	4.89 fgh
Res.+Ext.	cont 3	0.49 bcdef	7.75 abcd	2.57 e	65.3 c	22.8 c	23.1 c	4.81 defg
A	Z	0.51 cdefg	7.71 abc	2.22 a	76.5 f	24.6 d	29.3 def	4.75 cdef
	P	0.46 abc	7.73 abcd	2.19 cd	67.1 c	28.9 f	28.6 d	4.69 bc
	M	0.41 a	7.68 a	2.16 cd	69.7 d	30.2 g	30.4 efg	4.56 ab
B	Z+p	0.54 efg	7.76 bcd	2.21 d	78.1 fg	30.7 gh	30.8 fgh	4.78 cdef
	Z+m	0.53 defg	7.73 abcd	2.24 d	79.6 gh	31.4 hi	31.9 gh	4.71 bcd
	P+M	0.51 cdefg	7.71 abc	2.22 d	71.4 de	32.1 ij	31.7 gh	4.69 bc
	Z+P+M	0.48 bcde	7.68 ab	2.12 bc	87.4 j	36.8 i	33.6 i	4.72 defg
C	Z+R	0.53 defg	7.74 abcd	2.18 cd	78.2 fg	28.8 e	29.1 de	4.77 cdef
	P+R	0.48 bcde	7.78 cde	2.11 bc	70.3 d	30.1 g	28.5 d	4.73 bcde
	M+R	0.43 ab	7.73 abcd	2.07 b	71.6 de	31.8 hij	30.9 fgh	4.62 bc
D	Z+P+R	0.57 h	7.79 de	2.12 bc	81.2 hi	31.9 hij	30.6 fgh	4.83 cdef
	Z+M+R	0.55 gh	7.75 abcd	2.17 cd	83.1 i	32.7 jk	30.8 fgh	4.76 cdef
	P+M+R	0.53 defg	7.74 abcd	2.18 cd	73.6 e	33.8 k	32.3 hi	4.73 bcde
	Z+P+M+R	0.49 bcdef	7.71 abc	2.06 b	89.3 k	37.2 j	34.8 j	4.85 fg

Values followed by the same letters are not significantly different.

* = Soil extract (1soil:5water) ** = Soil suspension (1soil:2.5water) Inorg. = inorganic fertilizers

Ext. = manure extract Res. = manure residues Z = Nitrobein P = Phosphorein

M = Mycorrhizal fungi R = Rock phosphate

Results also revealed that application of the previously mentioned base diet to the used bio-fertilizers of Z, P or M each applied separately, compared to "cont 3" treatment, had a depressive effect on the EC values; "M" treatment involving mycorrhizal fungi had the lowest values, with "Z" treatment being the highest. "Z+P" treatment, representing one of biofertilizers combinations of "B" treatments, was superior to "A" treatments; addition of "M" was, however, depressive.

Relatively lower EC values may be attributed to absorption of different elements by grown plants through activation role of microorganisms, particularly those nutritionally required, and thus element depletion should occur especially with mycorrhizal fungi. Encountered higher values, particularly with "Z" treatment, on the other hand, may be a resultant of improvement of mineralization of applied organic manures and thus release more soluble ions.

Addition of rock phosphate to "A" treatments (i.e. "C" treatments) was, to some extent, favorable possibly due to its important role of interaction with applied microorganisms in solubilization of P as well as Ca from the applied rock phosphate. Correspondent effect of rock phosphate added to combinations of the concerned biofertilizers (i.e. "D" treatments) was more obvious, compared to both "B" and "C" treatments. This is true in spite of the inferiority of "Z+P+M" treatment due to the relatively more nutritional requirements of the combined microorganisms; such requirements should be taken out from the soluble form in soil, additive effect for the three microorganisms should be of particular role.

According to the previously mentioned presentation, it may be worth to conclude that, application of either inorganic fertilizers, organic manures separately or accompanied with natural resource of rock phosphate along with biofertilizers each separately or combined, was favorable for EC values which were relatively higher; combinations of the three used biofertilizers were, however, depressive for such values.

3.1.2. Soil pH

Obtained results showed, as expected, relative decreases in soil pH values for the studied treatments, compared to both original untreated soil (**Table 1**) and "cont 3" treatment which had the lowest pH values, compared to either "cont 1" or "cont 2" probably due to addition of residues of the soaked organic manures, to soil, which should

produce some organic acids through their decomposition process.

"A" treatments, compared to "cont 3", had relatively lower pH values; "M" treatment was the lowest, followed by "Z" treatment, with "Z" treatment having the highest values; lower pH values may be due to the role of "M" in producing some organic acids. "B" treatments, particularly that involving "Z+P+M" microorganisms, compared to "A" treatments had, opposite to those of cattle manure, relatively lower pH values in case of chicken manure; this may be due to the previously indicated role of the applied biofertilizers in improvement of mineralization process of organic materials present initially in the soil or produced from added organic manures, all giving organic acids leading to the indicated decreases in pH values. Relatively higher values obtained with cattle manure may be a resultant of relatively higher pH values of the raw material of this manure compared to chicken manure.

Addition of rock phosphate to fertilization system of "A" treatments (i.e. "C treatments), gave relatively higher pH values. Such results are not unexpected; rock phosphate should be considered to be a some sort of buffer which can react with any acidity source. This may be due to interaction between the indicated rock phosphate and combinations of applied microorganisms particularly what concerning the above mentioned buffering action of the indicated rock phosphate. Correspondent additions of rock phosphate to the applied combinations of the used biofertilizers ("D" treatments), generally, gave higher values, compared to both "B" treatments (having no rock phosphate) and "C" treatments (involving the separate additions of the concerned biofertilizers), "Z+P+M+R" being inferior with chicken manure opposite to that of cattle manure.

It may be worth to mention that, treatments receiving the used biofertilizers, opposite to that of natural resource of rock phosphate, were relatively lower in pH than those of "cont 3", receiving none of such materials. This may confirm the previously mentioned role of interactions between biofertilizers, natural resource of rock phosphate and applied manures.

3.1.3. Soil organic matter content

As expected, OM content of all studied treatments increased, compared to the original untreated soil (**Table 1**). Such studied treatments were, however, lower than "cont 3", in spite of its superi-

ority, compared to other control treatments ("cont 1" and "cont 2") probably due to application of organic manures.

"A" treatments, compared to "cont 3", had relatively lower values of organic matter content. This may go along with results of **Gagnon (2004)** who pointed out that OM content in soil decreased due to improvement of mineralization process by microorganisms which should give efficient loss of carbon as CO₂ through microbial oxidation particularly at relatively long time period close to that of present study (before flowering stage of plant growth). Combinations of the used biofertilizers ("B" treatments), particularly "Z+P+M" treatment, led to decreases in OM content with cattle manure, opposite to that of chicken manure, compared to "A" treatments possibly due to relatively more efficient improvement of mineralization process, particularly the easily decomposable chicken manure, as a resultant of increasing number of living microorganisms.

Addition of rock phosphate to "A" treatments (i.e. "C" treatments) gave relatively lower values of OM content probably due to the presence of rock phosphate which is considered representing the natural resource for P required by microorganisms and thus enhanced their ability to decompose the applied materials more efficiently and that finally could be reflected on decreasing such organic matter content of soil. Correspondent effect of rock phosphate, added to combinations of the concerned biofertilizers ("D" treatments) was more depressive compared to both previously mentioned "B" and "C" treatments.

Finally, it may be worth to mention that, biofertilizers as well as natural resource of rock phosphate was depressive for the OM content of the studied soil samples; the latter combined with "Z+P+M" combinations of biofertilizers gave the lowest values.

3. 2. Element availability

Organic materials have a definite role in improving soil fertility. Organic manures, produced from residues of either plants, animals or both, mostly contain several nutrients, essential for plant growth, including both macro and micronutrients. Such organic manures also produced organic acids, which usually made most nutrients available to developed plants. However, some of the macro nutrients, such as nitrogen, phosphorus and potassium may be not present in adequate amounts required by plants, unless the organic manures are

supplied in large quantities (**Rynk et al 1992**). In spite of that, such manures may include some heavy metals as pollutants hazardous for developed plants (**Abd- El-Kawy, 2003**).

As previously mentioned in the section of Materials and Methods, element availability was evaluated through evaluating available nitrogen (N) and phosphorus (P) as macronutrients, iron (Fe) as a micronutrient and lead (Pb) as a heavy metal pollutant.

Regarding to both available nitrogen and phosphorus, as representatives for macronutrients, obtained results of **Table (3)** showed that all studied treatments, compared to both original untreated soil (**Table 1**) and "cont 3" treatment, had higher available values; in spite of that, "cont 3" gave higher values compared to those of the other control treatments ("cont 1" and "cont 2"). Superiority of all studied treatments, compared to both original untreated soil and "cont 3" is expected due to favorable effects of applied fertilization system representing manures, applied natural resource of rock phosphate and biofertilizers, the application practices may be included. Highest values obtained with the latter "cont 3", compared to other control treatments ("cont 1" and "cont 2") may be attributed to the role of applied manures.

"A" treatments, compared to "cont 3", had higher available values of both N and P. The highest values of available N were obtained with "Z" treatment involving nitrobein microbes; in spite of that, correspondent highest values of available P was obtained with "M" treatment involving mycorrhizal fungi. The highest N values of "Z" treatment may be possibly due to the presence of nitrobein microbes and their role in biological fixation to supply the developed plants by their required N. These results agree with those obtained by **Siddiqui (2004)** who reported that *Azotobacter* not only provides nitrogen, but also produces a variety of growth promoting substances among which are indol acetic acid, gibberellins and B vitamins; these substances stimulate, at least to some degree, the production of root exudates. In addition, other important characteristic of *Azotobacter* associated with plant improvement is excretion of ammonia in the rhizosphere in the presence of root exudates (**Narula and Gupta, 1986**).

Favorable effect of mycorrhizal fungi on available P values, on the other hand, may be attributed to their essentiality for enhancing root surface area which could improve P availability in soils as a resultant of more chances given for adsorption of P on either the surface of plant roots or on hyphae of

mycorrhizal fungi and thus saving from interactions with different environmental conditions and let it more suitable to be released into soil to finally result in better ion balance. Important role of Mycorrhizal fungi (**Malajczuk and Cromack, 1982**), in producing several organic acids such as citric and oxalic acids which should lead to decrease in soil pH that would increase element availability, should not be ignored. The role of root exudates for solubilizing calcium phosphates should be taken in consideration. The superiority of both "M" and "P" treatments was recorded by **Wu et al (2005)** who found that AMF colonization as well as P- solubilizing bacteria can play an important role in improving P bioavailability. However, the effect of P- solubilizing bacteria was less significant when compared with AMF. In spite of absorption of both N and P from the soil by grown plants, the indicated high available values of both indicated elements in soil, reflected the ion balance occurring as a resultant of element release from the applied organic manures through their decomposition process especially in the presence of nitrobenzoin microbes and mycorrhizal fungi; of course, all used biofertilizers, particularly mycorrhizal fungi are expected to give more chances for suitable conditions process through enhancing root growth.

"B" treatments, particularly "Z+P+M", had higher available values of both N and P, compared to "A" treatments. Higher values obtained with both N and P due to bio- fertilizers combinations of "B" treatments, particularly "Z+P+M" treatment, are possibly attributed to higher numbers of living microorganisms and their efficient role in increasing the availability of both indicated elements through improvement of decomposition process of organic matter initially present in soil or produced from the applied organic manures. These results may go along with those of **Narula and Gupta (1986)** who found that dual or more combinations of biofertilizers were more efficient in increasing element availability than single inoculation.

Addition of rock phosphate to "A" treatments, (i.e. "C" treatments), again, gave higher values; the highest values of available N were, however, encountered with "Z+R" treatment, correspondent highest values of available P being obtained with "M+R" treatment. Correspondent effect for rock phosphate added to combinations of biofertilizers (i.e. "D" treatments) improved the role of the concerned combinations ("B" treatments), particularly "Z+P+M+R" whose available values of both N and P were the highest. Enhancement effect of rock phosphate application, particularly, if combined

with bio-fertilizer applied separately, is probably due to efficient interactions between the concerned rock phosphate and used biofertilizers especially nitrobenzoin for N and mycorrhizal fungi for P. Importance of rock phosphate as a source for P, required by both concerned microorganisms and developed plants, reflected on ion balance and thus giving a balanced diet for the applied biofertilizers should not be ignored. Interactions between roles imposed by both high numbers of microorganisms in combinations form and presence of rock phosphate ("D" treatments) are of importance for activity of both plants and such microorganisms to release more available elements from the concerned applied manures, rock phosphate and sparingly soluble salts initially present in soil.

Finally, it may be worth to mention that, biofertilization, particularly with "Z+P+M" treatment seemed to be a suitable technique for nutrient availability, addition of rock phosphate being beneficial. In addition, there is an importance for selecting the suitable applied microorganisms in order to help in providing the developed plants by their requirements of essential elements, nitrobenzoin and mycorrhizal fungi being the most suitable for both N and P, respectively. It may be also worth to mention that, responses of N to the applied practices were more obvious than those of P possibly due to the relatively high initial available P in soil.

With regard to responses of available iron, as representative for micronutrients, to the studied treatments, obtained data revealed an almost similar pattern to those of N and P. In spite of that; such responses were different to N, "M" along with "M+R" treatments being the superior instead of "Z" and "Z+R" treatments. Obtained results may be, again, attributed to the important role of mycorrhizal fungi in improving root growth along with extension of its biomass in soil which should be reflected on more aeration required by both useful microorganisms and grown plants; depressing soil pH as a resultant of organic acids produced by mycorrhizal fungi may be other possibility. Besides, interactions between roles imposed by both higher numbers of microorganisms and presence of rock phosphate, release of Fe from FePO_4 into soil solution seemed to be also obvious. Relatively initial high Fe content of the used organic manures along with their application practices are of importance.

Frequent presence of both Fe and P together in several compounds, particularly those of iron phosphate, phosphate adsorbed on both CaCO_3 and sesquioxides R_2O_3 along with some clay minerals should not be ignored. Besides, it is well

known that responses of both Fe and phosphates to changes in soil pH are generally similar (Singh and Dahiya, 1980).

Finally, with regard to responses of available lead, as representative for heavy metal pollutants, to the designed treatments, obtained results showed that values of available Pb form in all studied treatments, similar to all studied nutritional elements N, P and Fe, were higher compared to those of original untreated soil (Table 1), but, different from the indicated nutritional elements, had lower values than "cont 3" treatment which was, however, the inferior among all control treatments with "cont 2" being the highest. Superiority of the studied treatments, compared to the original untreated soil, may be a resultant of applied fertilization design involving the use of manuring interacting with natural resource of rock phosphate and presence of biofertilizers. Their inferiority, compared to "cont 3", on the other hand, may be attributed to the favorable effect of both rock phosphate and biofertilizers on development of grown plants which should be more efficient in absorption of elements including Pb whose renewal of available form from the difficulty available one, different to studied nutritional elements, seemed to be relatively slow as to finally result in its depletion from soil. Inferiority of "cont 3", compared to other control treatments ("cont 1" and "cont 2") is possibly due to effects of applied inorganic fertilizers which should be contaminated with different pollutants such as Pb; other possibility may be the presence of organic materials in "cont 3" which may help in plant development, particularly root which may grow better with more chances for releasing exudates able to enhance the mobility and absorption of its indicated Pb. Lower values of "cont 1" compared to "cont 2", on the other hand, may be a resultant for absence of manure extracts; in spite of that, "cont 3" was the inferior even if compared to "cont 1" due to organic source of the former and inorganic source of the latter. These results were in accordance with those obtained by Zawadzka *et al* (1990) who found that inorganic fertilizers contain high concentration of heavy metals which may represent a major source of contamination for both soil and developed vegetables, as to suggest the safety of organic manures compared to inorganic fertilizers. Usman *et al* (2004) mentioned that organic matter makes strong bonds with heavy metals including Pb and thus decreased their availability. In fact, all mentioned practices seemed to be interacting as to give the indicated variations among different treatments.

"A" treatments, particularly "M", compared to "cont 3", had lower values of available Pb possibly due to the previously mentioned role of microorganisms, directly or indirectly, on the organic matter behavior of the soil as to create strong bonds with present Pb regardless the known favorable effect on root growth. In fact, Liao *et al* (2003) pointed out that corpuscles of mycorrhizal fungi may accumulate certain heavy metals as to be relatively non available for plants; such effect was dependent on the concerned element and mycorrhizal type. Combinations of the used bio-fertilizers "B" treatments, particularly "Z+P+M", showed no great differences in values of available Pb, which were relatively higher compared to the separate addition of such biofertilizers ("A" treatments) possibly due, again, to the role of high numbers of living microorganisms in increasing availability of the studied elements; in fact, bio-fertilizers seemed to help each other, particularly if containing mycorrhizal fungi, when being in combinations, in the indicated favorable effect on Pb availability.

Application of rock phosphate to "A" treatments (i.e. "C" treatments), particularly "Z+R", improved the role of applied biofertilizers for increasing Pb availability possibly due, again, to the role of Azotobacter microbes in improvement the mineralization process of organic matter initially present in the soil or produced from the applied organic manures; besides, rock phosphate, which is considered a source for P required by the applied microorganisms, should be expected to help solubilization of elements such as Pb which may be presented initially in the applied rock phosphate as contaminants. "M" treatment was the lowest probably due to the previously mentioned role of mycorrhizal fungi in improvement root growth as well as their biomass and thus reflected on more efficient absorption or adsorption for the concerned element. Correspondent activating role of rock phosphate to biofertilizer combinations ("D" treatments), particularly "Z+P+M+R", gave relatively higher Pb values, compared to both "B" and "C" treatments possibly due, again, to the interactions between applied rock phosphate and high numbers of used biofertilizers.

It may be worth to mention that Pb behavior, regarding responses to the studied treatments, was, as expected, generally different from that of Fe due to the variations in their chemical features as to give different chemical behavior for both elements. Of course, interactions among different elements, particularly the studied two elements Pb and Fe, should be taken in consideration.

It may be also worth to mention that, separate application of biofertilizers especially mycorrhizal fungi, particularly when accompanied with organic manure residues as well as spray of their extracts on the plant biomass, seemed to have a definite role in limiting the availability of Pb directly or indirectly through effects of absorption, adsorption, translocation and retention processes of Pb within both plant roots and soil. Besides, such biofertilizers appeared to be generally favorable for increasing availability of the studied elements of N, P and Fe. Accordingly, recommendations should be introduced, emphasizing the importance of biofertilization as a suitable technique particularly with the known fact of its economical returns.

Finally, a comparison between the studied soil chemical features and those of element availability showed that the responses of the latter to the studied treatments were more affected than those of the formers, possibly due to the relatively short period of the present study as to be not enough for great changes.

4. REFERENCES

- Abd-Elmoniem, E.M. and M.Z. El-Shinawy. (2002). Feeding of lettuce plants by chicken manure under hydroponic system. *Egypt. J. Hort.* 29: 513-524.
- Abd El-Kawy, T.F.A. (2003). **The Effect of some Organic and Inorganic Fertilizers on Plant Growth and Fruit Quality of Cucumber Plant under Plastic Houses**. pp. 43-44. M.Sc. Thesis, Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo.
- Abou-Hussein, S.D.; I. El-Oksh; T. El-Shorbagy and U.A. El-Behairy. (2002). Effect of chicken manure, compost and bio-fertilizers on vegetative growth, tuber characteristics and yield of potato crop. *Egypt. J. Hort.* 29: 135-149.
- Al-Mughrabi, K.I. (2007). Suppression of *Phytophthora infestans* in potatoes by foliar application of food nutrients and compost tea. *Aust. J. Basic and Applied Sci.* 1: 785-792.
- Badran, Nadia; M.E.A. Khalil and M.A.A. El-Emam. (2000). Availability of N, P and K in sandy and clayey soils as affected by the addition of organic materials. *Egypt. J. Soil Sci.* 40: 265-283.
- Black, C.A.; D.D. Evans; L.E. Ensminger; J.L. White; F.E. Clark and R.C. Dinauer. (1965). **Methods of Soil Analysis. II: Chemical and Microbiological Properties**. pp. 163-165. Amer. Soc. Agron. Inc. Bull., Madison, Wisconsin, USA.
- Chapman, H.D. and P.F. Pratt. (1961). **Methods of Analysis for Soils, Plant and Water**. pp. 643-658. Calif. Univ., USA.
- Duponnois, R.; A. Colombet; V. Hein and J. Thioulouse. (2005). The mycorrhizal fungus *Glomus intradices* and rock phosphate amendment influence on plant growth and microbial activity in the rhizosphere of *Acaia holosericea*. *Soil Biol. Biochem.* 37: 1460-1468.
- El-Sirafy, Z.M.; H.J. Woodard and E.M. El-Norjar. (2006). Contribution of bio-fertilizers and fertilizer nitrogen to nutrient uptake and yield of Egyptian winter wheat. *J. Plant Nutr.* 29: 587-599.
- Gagnon, B. (2004). Contribution of farmyard manure and industrial composts on soil pH and enrichment in available nutrients and metals. *Can. J. Soil Sci.* 84: 439-445.
- Jackson, M.L. (1973). **Soil Chemical Analysis**, pp. 178-182. Prentice-Hall of India Private Limited, New Delhi.
- Kábana, R.R. (1986). Organic and inorganic nitrogen amendments to soil as nematode suppressants. *J. Nematol.* 18: 129-135.
- Khan, A.G.; C. Kuek; T.M. Chaudhry; C.S. Khoo and W.J. Hayes. (2000). Plant mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* 41: 197-207.
- Liao, J.P.; X.G. Lin; Z.H. Cao; Y.Q. Shi and M.H. Wong. (2003). Interaction between arbuscular mycorrhizal and heavy metals under sand culture experiment. *Chemosphere* 50: 847-853.
- Malajczuk, N. and J.K. Cromack. (1982). Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. *New Phytologist*, 92: 527-531.
- Narula, N. and K.G. Gupta. (1986). Ammonia excretion by *Azotobacter chroococcum* in liquid culture and soil in the presence of manganese and clay minerals. *Plant and Soil* 93: 205-209.
- Odlare, M.; M. Pell and K. Svensson. (2008). Changes in soil chemical and microbiological properties during 4 years of application of various organic residues. *J. Waste Management* 28: 1246-1253.
- Olsen, S.R.; C.V. Cole; F.S. Watanable and L.A. Dean. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. **USDA Circulars No. 939, 19pp**. U.S. Government Printing Office, Washington, D.C.
- Rynk, R.; M. Van de Kamp; G.B. Willson; M.E. Singley; T.L. Richard; J.J. Kolega; F.R. Gouin; L.L. Jr; K. David; D.W. Murphy; H.A.J. Hoitink and W.F. Brinton. (1992). **On Farm Composting Handbook**. Technical editor: Marty Sailus, North-

- east Regional Agric. Engineering Service. 152 Riley Robb Hall Cooperative Extension. Ithaca, NY., USA. **C.F. Lela, Kh. M.M. 2005. Studies on Mixing of Bio and Organic Fertilizers on Nutrient Availability and Crop Production. p. 40.** M.Sc. Thesis, Soils Department, Faculty of Agriculture, Al-Azhar University, Cairo.
- Siddiqui, Z.A. (2004).** Effects of plant growth promoting bacteria and composed organic fertilizers on the reproduction of *Meloidogyne incognita* and tomato growth. **Bioresour. Technol. 95: 223- 227.**
- Singh, R. and S.S. Dahiya. (1980).** Effects of farmyard manure and iron on dry matter yield and nutrients uptake by oats (*Avena sativa*). **Plant and Soil 56: 403-412.**
- Usman, A.R.A.; Y. Kuzyakov and K. Stahr. (2004).** Dynamics of organic C mineralization and the mobile fraction of heavy metals in a calcareous soil incubated with organic wastes. **Water, Air and Soil Pollution. 158: 401-418.**
- Vessey, J.K. (2003).** Plant growth promoting rhizobacteria as biofertilizers. **Plant and Soil 255: 571- 586.**
- Vestberg, M.; S. Kukkonen; K. Saari; P. Parikka; J. Huttunen; L. Tainio; N. Devos; F. Weekers; C. Kevers; P.Thonart; M.C. Lemoine; C. Cordier; C. Alabouvette and S. Gianinazzi. (2004).** Microbial inoculation for improving the growth and health of micropropagated strawberry. **Applied Soil Ecology 27: 243- 258.**
- Wu, S.C.; Z.H. Cao; Z.G. Li; K.C. Cheung and M.H. Wong. (2005).** Effects of biofertilizers containing N-fixers, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. **Geoderma 125: 155- 166.**
- Zawadzka, T.; H. Mazur; K.M. Wojciechowska Mazure and K. Strska. (1990).** Contents of metals in vegetables from different regions of Poland in the years 1986- 1988. 1- Contents of lead, cadmium and mercury. **Roczniki Panstwowego Zakadu Higieny 41: 111-131.**
- C.F. Abd-El-Kawy (2003).**
- Zmora-Nahum, S.; Y. Hadar and Y. Chen. (2007).** Physico-chemical properties of commercial composts varying in their source materials and country of origin. **Soil Biol. Biochem. 39: 1263- 1276.**