Effect of Manure and Cocoa Shell Biomass Addition on Soil Chemical Properties Under Laboratory Incubation Conditions

Christos Lykas*, Nikolaos Gougoulias, Ioannis Vagelas
University of Thessaly, Department of Agriculture Crop Production and Rural Environment, Fytokou St., N. Ionia, GR-38446 Magnesia, Greece

Abstract
In this study was investigated the effect of manure and cocoa shell biomass addition on soil chemical properties and microflora, when the above mentioned organic materials areused in different proportions in soil mixtures. For this reason different amounts (0.2, 0.4, and 0.6g) of air dried cocoa beans residue, were mixed with 50g of soil and 5.0g of manure. The control mixtures that contained only soil and manure as well as the mixtures which contained different amount of cocoa shells, were placed in incubator at 28^{o}C for a period of 21 weeks. The organic matter and the concentration of nutrient elements in the soil, manure, cocoa shells biomass and in their mixtures, were measured before and after the incubation period. The results showed that the addition of more than 1% of cocoa shell biomass to soil-manure mixtures, may influence the mineralization of N, C and K, and reduce the time needed for mixtures maturation. The higher organic P content measured in mixtures contained lower than 1% cocoa shell biomasswhere the microbial activity was still high. However the concentration of the available P was not affected from the addition of cocoa shell biomass to the soil-manure mixtures.

Keywords : *Organic waste, cocoa residue, soil microflora, biodegradation, soil mixtures, organic matter, mineralization*

I. INTRODUCTION

The intensive crop production systems leads to the uncontrolled disposal of agricultural waste and the excess use of nutrients in order to achieve high yields. However, the recycling of nutrients as organic soil amendments can lead to the sustainable use of agricultural waste and may become a practice with economic and environmental benefits [1], [2], [3], [4]. The application of organic materials as soil amendments, can improve soil quality characteristics and increase nutrients availability through mineralization of N and C, as well as the immobilization of the added materials[5], [6], [7], [8], [9].Due to their high content of organic matter, their addition to the soil, canalso activate micro-organisms and affect its chemical and physical properties [10], [11], [12]. The available C and N into the soils, where the organic materialwas added, depends on the decomposition rates and synchrony of nutrient mineralization [13]. However both the amount and the rate of nutrients released from plant residues werestrongly dependent on their biochemical composition [14].

Nowadays, a variety of wastes in the form of straw, pulp, stover, husk etc, are generated from food and agricultural products processing industries such as olive oil mills, sugar industries, wineries, coffee and chocolate industries [15], [16]. World production of cocoa beans by the main producing countries (Brazil, Nigeria, Ecuador, Ghana, Côte d'Ivoire, Cameroon, and Indonesia) during 2013-2014 harvest period, was 4.3 million tons and has increased over the last decade [17]. Since the commercial interest is focused on cocoa nibs, the dried cocoa shells which is the main by-product from the chocolate industry, is usually burnt for fuel or used as a mulch to increase soil fertility [18]. Cocoa shells are rich in polysaccharides mainly cellulose and little lignin, as well as in phenolic compounds which exert antioxidant and antimicrobial effect [19], [20], [21], [22], [23]. In addition many studies have shown that cocoa shells can remove several heavy metals from very acidic solutions [24], [25], [26] and can absorb and hold a significant amount of water that can be released gradually to the ground, preventingplants dehydration. They can be used to increase soil content in Ca, K, and several microminerals such as Cu, Fe, Mn and Zn, since shells mineral analysis shows that they contain significant amount of these elements [27], [28], [29].

For this reason, cocoa shells can be used in mixtures with other organic materials to produce substrates for plants cultivation. Alternatively, cocoa shells can be used to produce material for soil amendment, since its addition to the soil as bio-fertilizers, increases soil fertility and crop productivity [22], [30].

However, the addition of plant material to the soil, seems to have a different effect on organic carbon mineralization depending on plant species from which it originates. In laboratory experiments the presence of *Ocimum basilicum* L. dry matter in soil mixtures increased the soil organic carbon mineralization, while the presence of *Origanum vulgare* L. dry matter decreased it [31], [32]. Cocoa shells have rather low content in N and P compared totheir content in C [33], depending on the region where cocoa was produced. Based on the ratio of carbon to nitrogen, the organic waste materials can be characterized as nitrogen source materials and carbon source materials. Although a mature compost should have C/N ratio <25 [34], in many cases C/N ratio in cocoa shells composts exceeds the above mentioned value [33], [35]. This may cause nitrogen limitation of plants, especially when this material is added in soils with low content in N.

In this work was investigated the effect of manure enriched with dried cocoa beans shells, on soil microflora and chemical properties, when used in mixtures with different proportions as soil amendments.

II. MATERIALS AND METHODS

A. Incubation experiment:

Amounts of 0.2, 0.4 and 0.6 g of fine cocoa shells powder, originated from cocoa shells that dried in a circulated air oven (80°C), were used for the preparation of three mixtures ($CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ respectively) with 5.0 g of sheep manure and 50 g of air-dried sieved sandy loam soil. Themanure was digested for three months before the mixture preparation, while the soil was collected from a permanent grassland plot (0–10 cm depth). Manure-soil mixturesthat did not contain cocoa shell powder were served as control(CS_0).

Four samples from the above mentioned mixture, 40 g each one, were placed in an incubator at 28^{o}C for a period of 21 weeks. During the first three weeks of the incubation period, the moisture was maintained at two-thirds of mixtureswater holding capacity (WHC), while for the next three weeks they were left to dry. This process was repeated until the end of the incubation period following the methodology of relevant works [36]. At the end of the incubation period the chemical analysis of the samples was performed.

B: Methods of analysis:

Organic matter was oxidized with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$. Soil pH and electrical conductivity, (EC) was measured in (1:5) soil/water extract.Both ammonium and nitrate nitrogen were extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) were extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy.Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol L^{-1} H_2SO_4 and all forms were measured by spectroscopy. Exchangeable forms of K were extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK). Available forms of Mn, Zn, and Cu were extracted with solution containing DTPA (diethylene triamine pentaacetic acid) 0.005 mol L^{-1}, $CaCl_2$ 0.01 mol L^{-1} and triethanolamine 0.1 mol L^{-1} and measured by atomic absorption. For the determination of total concentration of Mn, Cu and Zn, the amount of 1 g of hydratedsoil, manure, cocoa shell powder as well as of the above mentioned mixtures respectively, were digested at 350 ^{0}C with solution which contained 10 ml HNO_3 and 5 ml $HClO_4$. The samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), using air-acetylene flame.

C. Estimations:

Soil Exchangeable Sodium Percent (ESP), and Sodium Adsorption Ratio (SAR) were determined according to the Eq. (1) [37], [38]and Eq. (2) respectively [39].

$$ESP = (Na^+ / CEC)*100$$

Where:
ESP = Exchangeable sodium percentage (%)
Na^+ = Measured exchangeable Na (cmol/kg)
CEC = Cation exchange capacity (cmol/kg)

and

$$0.015*SAR = ESP/(100-ESP)$$

E. Soil microflora:

To investigate the effect of added material on the soil microflora (bacterial communities), a small amount of soil was spread onto potato dextrose agar plates and incubated for 2 days at 25°C in darkness. After the incubation period, the number of the bacterial colonies was counted.

F. Statistical analysis:

The experiment was performed following a completely randomized design with four replications. Tukey's procedures were used to detect and separate the mean treatment differences at P= 0.05. Statistical analyses were performed by the statistical program MINITAB.

III. RESULTS AND DISCUSSION

The initial organic matter content in sheep manure and cocoa shells were high, 40.5% and 49.9% respectively. In these materials the total amount of Nand K was also high, ranged in similar levels as shown in Table 1, while the P content was lower in cocoa shells compared to that of manure. The measured values concerned the content of cocoa shells in organic matter, N, P and K that are presented in Table 1 were similar to those referred by several authors [27], [33], [35].The soil used in this experiment had a very low content (0.95%) in organic matter, since the organic fraction of soils typically accounts proportion of 5 to 10% of soil mass [40].

Table 1. Chemical properties of soil, sheep manure and of cocoa shells

Property	Soil	Sheep manure	Cocoa shells
Texture	Sandy loam		
Organic matter (%)	0.95 ± 0.05	40.5 ± 2.25	49.9 ± 2.91
NH_4^+-N (mg kg^{-1})	21.6 ± 4.55		
NO_3^--N (mg kg^{-1})	79.1 ± 7.46		
N –total (g kg^{-1})	1.49 ± 0.07	11.76 ± 0.90	11.60 ± 0.73
P -Olsen (mg kg^{-1})	9.4 ± 2.43		
K-exchangeable (mg kg^{-1})	206.8 ± 12.11		
Na-exchangeable (mg kg^{-1})	92.1 ± 4.57		
CEC (cmol kg^{-1})	18.4 ± 1.02		
pH (1:5)	7.83 ± 0.39	8.39 ± 0.32	5.73 ± 0.30
EC (1:5) dS m^{-1}	0.28 ± 0.02	2.96 ± 0.16	2.02 ± 0.13
$CaCO_3$ (%)	8.2 ± 0.34		
Cu -DTPA (mg kg^{-1})	0.89 ± 0.04		
Zn -DTPA (mg kg^{-1})	0.73 ± 0.04		
Mn -DTPA (mg kg^{-1})	4.62 ± 0.47		
Na-total (g kg^{-1})	0.302 ± 0.04	8.10 ± 0.47	0.189 ± 0.01
K-total (g kg^{-1})	4.009 ± 0.16	18.86 ± 0.99	21.156 ± 0.96
P -total (g kg^{-1})	0.396 ± 0.02	6.22 ± 0.39	1.71 ± 0.11
Cu -total (mg kg^{-1})	30.05 ± 2.12	33.1 ± 2.76	59.0 ± 4.28
Zn -total (mg kg^{-1})	37.29 ± 2.08	187.6 ± 12.46	53.7 ± 3.98
Mn -total (mg kg^{-1})	458.2 ± 29.66	226.6 ± 18.81	<0.1

Note:± represent SD (n)=4.

After 21 weeks of incubation, mixtures with higher cocoa shell powder percentage ($CS_{0.6}$), had increased EC (1.79 dS m^{-1}) in comparison to the CS_0, $CS_{0.2}$ and $CS_{0.4}$ mixtures that had similar EC values as shown in Table 2 (1.66, 1.64 and 1.66 dS m^{-1} respectively).In contrast the pH of the mixtures decreased progressively from 7.6 (C_0) to 7.4 ($CS_{0.6}$), as their content in cocoa shell powder increased (Table 2).The higher EC and the lower pH values of $CS_{0.6}$ mixtures in comparison to CS_0, is probably due to the increased concentration of several nutrient elements and to the oxidation of the NH_4^+-N to NO_3^{-}-N respectively.

In specific, a strong correlation (R^2=0.98, P<0.05) between NH_4^+-N and NO_3^{-}-N concentration was observed in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$, mixtures (Fig.1).

At the end of the incubation period NH_4^+-N concentration in CS_0, $CS_{0.2}$, $CS_{0.4}$ mixtures was similar to that in the soil before the addition of the manure and the cocoa shells powder. In contrast in $CS_{0.6}$ mixtures the NH_4^+-N concentration was 35.2% and 42.8% lower compared to that of the soil and CS_0 respectively.At the same period the NO_3^{-}-N concentration increases in a range of 101.2% (in $CS_{0.2}$ and $CS_{0.4}$ mixtures) to 131.6% (in $CS_{0.6}$ in mixtures), compared to that in the soil and from 10.8% (in $CS_{0.2}$ and $CS_{0.4}$ mixtures) to 22.5% (in $CS_{0.6}$ mixtures) compared to CS_0.

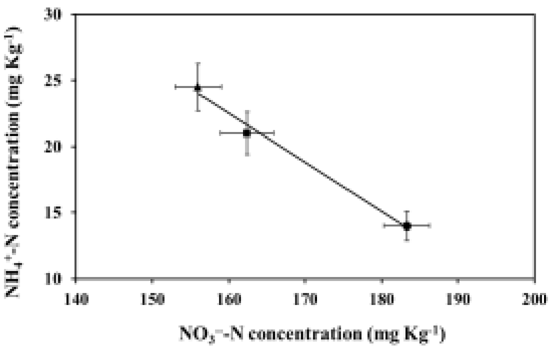


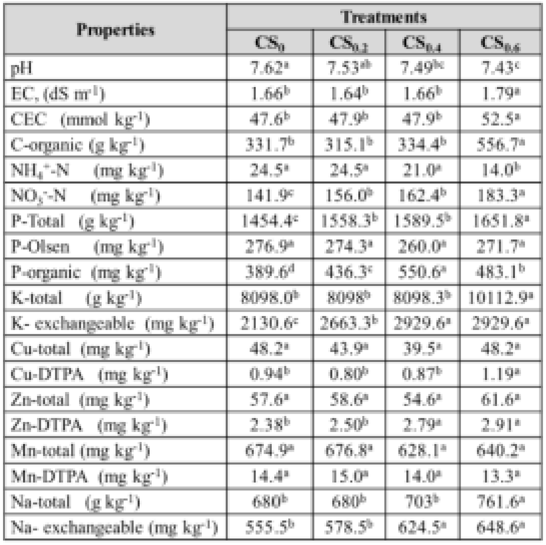
Fig. 1:Correlation (R^2=0.98, P<0.05)of average NH_4^+-N to NO_3^{-}-N concentration in $CS_{0.2}$ (▲), $CS_{0.4}$ (■) and $CS_{0.6}$ (●) mixtures, at the end of the incubation period. Vertical and horizontal bars indicates SD.

According to the results of several relative works, a temporary increase of NH_4^+-N concentration is usually observed after the addition of organic amendments to soil, for a relatively short period of time (several hours to few days or weeks) during the early stage of the incubation period. The initial increase of NH_4^+-N is later followed by their gradual reduction, (maturation period), usually up to the levels that the soil had before the addition of the organic amendment [7], [41]. In the present work, at the end of the incubation period, both the rate of NH_4^+-N decrease and the NO_3^{-}-N increase was higher in CS_{60} mixtures, where the greater amount of cocoa shell powder was added. This indicates a higher rateof N mineralization in CS_{60} mixtures as compared to CS_0, $CS_{0.2}$ and $CS_{0.4}$ mixtures (Table 2).The results presented in Tables 1 and 2 also indicate that the addition of manure in a proportion of 10% in thesoil, did not alter significantly soil's ammonium nitrogen after 21 weeks of incubation. The addition of 0.2 or 0.4 g of cocoa shell powder to the manure-soil mixtures did not altertheir ammonium nitrogen content, but increased the nitrate nitrogen concentration in $CS_{0.2}$ and $CS_{0.4}$ mixtures almost the same (9.9 and 14.4% respectively), at the end of the incubation period (Table 2).Therefore,it can be considered that a similar N mineralization rate, was performed in CS0.2 and CS0.4 mixtures.

The NH_4^+-N: $NO3^{-}$-N ratio is used to evaluate the state of compost, since NH_4^+:NO_3^{-} ratio equal or lower to 0.16 is used as an indicator for compost maturity[42], [43], [44].The $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures studied in this work, which contained cocoa shells powder,hadNH_4^+-N: $NO3^{-}$-N ratio equal or lower to 0.16 (0.16, 0.13 and 0.08 respectively), while mixtures that contained only manure had a greater to 0.16 NH_4^+:NO_3^{-} ratio (0.17), after 21 weeks incubation period.The results also indicated that the higher the content of the mixtures in cocoa shells powder the lower the NH_4^+-N:NO_3^{-}-N ratio at the end of incubation period.

The increased nitrification rate in mixture where cocoa shell powder was added, reduced the time needed for $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$mixtures maturation, compared to CS_0. This resulted probably from the higher microorganism activity developed in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures compared to CS_0 (Fig 2).The addition of cocoa shells in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures may increase the microbial content of these mixtures at the initial stage of incubation period. This is because organic materials have a significant microbial biomass.However, the major cause for microbial population's increment in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures, must be attributed to the increased concentrations of NH_4^+-N and C, which are the main nitrogen and energy sources for microorganisms in the substrates. This allowed microorganism populations of the above mentioned mixtures to multiply faster [45], [46]. In several studies [7], [41], [47]a significant correlation of the N and C mineralized to the microbial biomass and the microbial activity was referred. As it was expected, the results of this experiment revealed a strong correlation between the number of bacterial colonies developed in plates with potato dextrose agar, where small quantities of $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures were spread, with NO_3^{--}-N (R^2= 0.90, P<0.05) and NH_4^+-N (R^2= 0.96, P<0.05) concentration, as shown in Fig.3a and b respectively.

Table 2. Chemical properties and available forms of elements of the mixtures at the end of the incubation period



Properties	Treatments			
	CS_0	$CS_{0.2}$	$CS_{0.4}$	$CS_{0.6}$
pH	7.62^{a}	7.53^{ab}	7.49^{bc}	7.43^{c}
EC, (dS m^{-1})	1.66^{b}	1.64^{b}	1.66^{b}	1.79^{a}
CEC (mmol kg^{-1})	47.6^{b}	47.9^{b}	47.9^{b}	52.5^{a}
C-organic (g kg^{-1})	331.7^{b}	315.1^{b}	334.4^{b}	556.7^{a}
NH_4^+-N (mg kg^{-1})	24.5^{a}	24.5^{a}	21.0^{a}	14.0^{b}
NO_3^--N (mg kg^{-1})	141.9^{c}	156.0^{b}	162.4^{b}	183.3^{a}
P-Total (g kg^{-1})	1454.4^{c}	1558.3^{b}	1589.5^{b}	1651.8^{a}
P-Olsen (mg kg^{-1})	276.9^{a}	274.3^{a}	260.0^{a}	271.7^{a}
P-organic (mg kg^{-1})	389.6^{d}	436.3^{c}	550.6^{a}	483.1^{b}
K-total (g kg^{-1})	8098.0^{b}	8098^{b}	8098.3^{b}	10112.9^{a}
K- exchangeable (mg kg^{-1})	2130.6^{c}	2663.3^{b}	2929.6^{a}	2929.6^{a}
Cu-total (mg kg^{-1})	48.2^{a}	43.9^{a}	39.5^{a}	48.2^{a}
Cu-DTPA (mg kg^{-1})	0.94^{b}	0.80^{b}	0.87^{b}	1.19^{a}
Zn-total (mg kg^{-1})	57.6^{a}	58.6^{a}	54.6^{a}	61.6^{a}
Zn-DTPA (mg kg^{-1})	2.38^{b}	2.50^{b}	2.79^{a}	2.91^{a}
Mn-total (mg kg^{-1})	674.9^{a}	676.8^{a}	628.1^{a}	640.2^{a}
Mn-DTPA (mg kg^{-1})	14.4^{a}	15.0^{a}	14.0^{a}	13.3^{a}
Na-total (g kg^{-1})	680^{b}	680^{b}	703^{b}	761.6^{a}
Na- exchangeable (mg kg^{-1})	555.5^{b}	578.5^{b}	624.5^{a}	648.6^{a}

Note: The values in the lines of table with the same letter do not differ significantly according to the Tukey's test (P=0.05)

A similar correlation (R^2= 0.84, P<0.05) was revealed between the number of bacterial colonies of $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures with the organic C at the end of incubation period(Fig.3c), when $CS_{0.6}$ mixturescontained 40.4 % more organic C compared to C_0mixtures (Fig. 4).

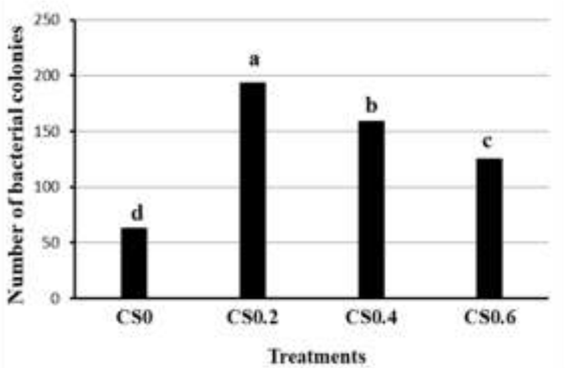


Fig. 2: The number of bacterial colonies developed in plates with potato dextrose agar where small quantities of $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures were spread. Bars with the same letters do not differ significantly according to Tukey's test (P = 0.05).

In specific, in $CS_{0.6}$ mixtures the higher organic C and NO_3^{--}-N concentration, were combined with the lower number of microorganisms, whereas in $CS_{0.2}$ mixtures the lower organic C and NO_3^{--}-N concentration were combined with the higher number of microorganisms at the end of the incubation period. The microbial biomass in amended soils is higher during the early stages of the composting, when there are sufficient nitrogen and carbon sources in the substrate, than at the latest stages [48], [49].The above mentioned, in combination with the NH_4^+-N:NO_3^{--}-N ratios, estimated for each one of $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures, reinforces the aspect that after 21 weeks period of incubation, the N and C mineralization in $CS_{0.2}$ and $CS_{0.4}$ mixtures may be still in progress, while in $CS_{0.6}$ mixtures probably it was completed.

The addition of manure and cocoa shell powder increased soil's total K content from 50.5 % (in the case of CS_0, $CS_{0.2}$ and $CS_{0.4}$ mixtures) to 60.3% ($CS_{0.6}$ mixture). This is because potassium in manure and compost is highly available. According to relative works, K availability in soil mixtures after the application of different manure types or after the addition of composted manure,may range from 24 to 152% [50], [51], [52].The addition of 0.2, 0.4 and 0.6 g of cocoa shells powder in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures respectively, which contained a standard proportion of manure and soil,it would be expected to increase proportionally their content in total K, since cocoa shells powder have a high concentration in K (Table 1).Total K content was significantly higher only in $CS_{0.6}$ mixtures (10112.9 g Kg^{-1}), while it was the same in CS_0, $CS_{0.2}$ and $CS_{0.4}$ mixtures (8098 g Kg^{-1}) at the end of incubation period (Table 2). The addition of 0.2 and 0.4 g of cocoa shell powder in 55 g of soil-manure mixture (otherwise 0.36 to 0.72% content in cocoa shell powder), wasn't adequate to increase significantly their total K content, despite that potassium is the most abundant mineral element in cocoa shells [53], [54]. In contrast the total K content of the soil-manure mixtures increased significantly when more than 1% of cocoa shell powder was added in the mixture.

The exchangeable form of potassium was increased in mixtures contained cocoa shell powder in comparison to the CS_0.This increase ranged from20% to 30% by the addition of 0.2 and 0.4 or 0.6 g of cocoa shells powder respectively. Differences in exchangeable K content between CS_0, $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$, shown in Table 2, are probably caused by the different microbial activity in these mixtures.

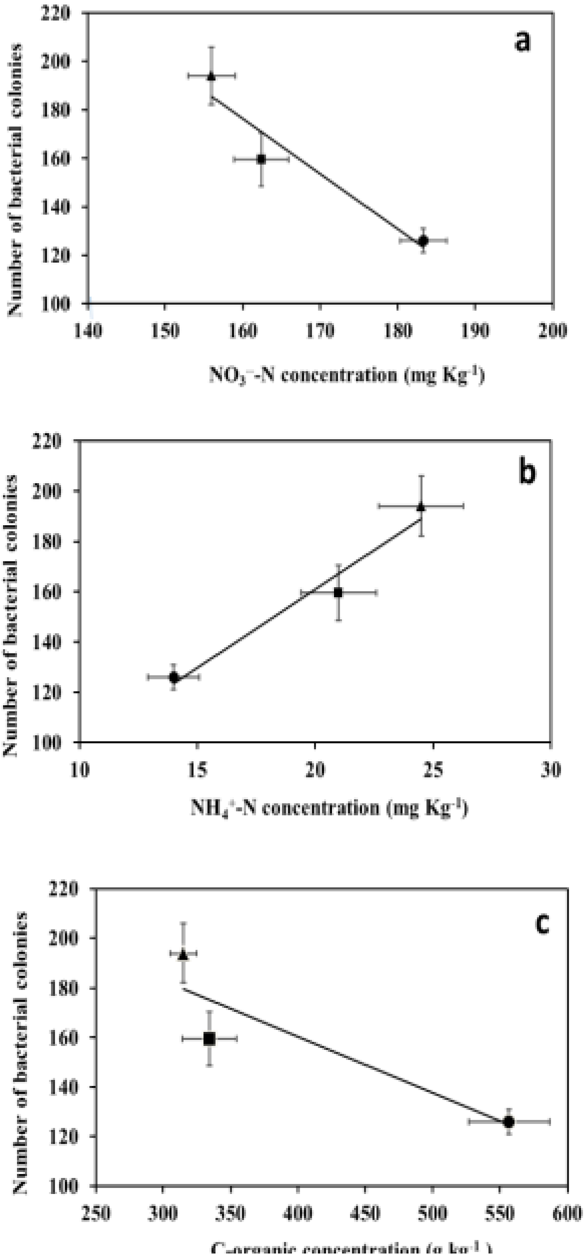


Fig. 3:Correlation of average bacterial colonies developed in plates with potato dextrose agar where small quantities of $CS_{0.2}$ (▲), $CS_{0.4}$ (■) and $CS_{0.6}$ (●) mixtures were spread, with: a) NO_3^{-}-N (R^2=0.9, P<0.05), b) NH_4^{+}-N (R^2=0.96, P<0.05) and c) C-organic concentration (R^2=0.8, P<0.05). Vertical and horizontal bars indicates SD.

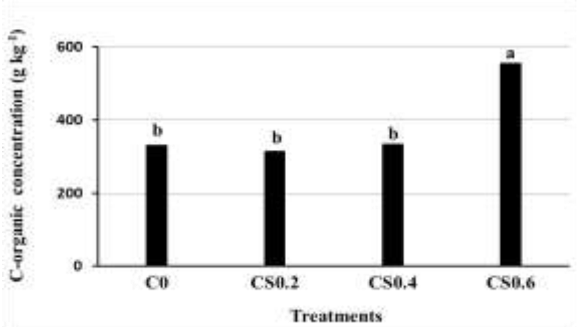


Fig. 4:Effect of cocoa shells powder addition on organic carbon mineralization of CS_0, $CS_{0.2}$ or $CS_{0.4}$ and $CS_{0.6}$ mixtures. Columns with the same letter do not differ significantly according to the Tukey's test (P=0.05).

Potassium solubilizing microbes such as Pseudomonas, Bacillus, and Aspergillus, that are present in soil, excrete organic acids (oxalic acid, citric acid and formic acid) which solubilize the unavailable potassium, converting the insoluble or mineral structural potassium compounds into soluble forms [55], [56], [57], [58]. Accordingly, the microbial activity in the above mentioned mixtures, should be followed by their acidification, caused from the accumulation of organic acids during the incubation period. This is in accordance with the mixtures pH gradation, shown in Table 2, as well as with the assumption that the nutrient elements mineralization in $CS_{0.2}$ and $CS_{0.4}$ mixtures may be still in progress, while in $CS_{0.6}$ mixtures probably it was completed after 21 weeks of incubation period.

Chemical analysis also shown that the variation of total andexchangeable sodium in CS_0, $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$mixturesfollowed a pattern similar to that of potassium. As shown in Table 2, the maximum increase of the total and exchangeable sodium was occurred in $CS_{0.6}$ mixtures (10.71% and 14.35% respectively, in comparison to the CS_0mixtures). However the increase of exchangeable sodium did not cause any sodicity risk of mixtures. As shown in Table 2, mixturesESP and SAR values estimated from Eq (1) and (2) respectively as well as their measured pH values,were lower to critical values thatare specified by the relevant literature(15 %, 13 and 8.5 respectively)[59], [60].

The soil used in this work can be classified as low in P, since both the total P content (0.396 g kg^{-1}) and the Olsen-P value (9.4 ppm) were lower than the thresholds of 0.6g kg^{-1} and 10 ppm respectively [61], [62] as shown in Table 1. In contrast manure contained a significant amount of phosphorus(6.22 g Kg^{-1}), whereas P was in lower concentration in cocoa shell powder, similar to those that has been reported in several works [51], [63], [64], [65], [66]. Although manure and composts contain more inorganic (63 to 92%) than organic P (5 to 25%) [67], the inorganic and organic forms of P, as well as the nature and partition of P forms, depends on manure type, animal diet and the type of organic amendment.

Consequently the addition of manure and cocoa shell powder in mixtures increased significantly ($P = 0.05$) the total-P in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures by 6.6, 8.5 and 12% respectively compared to CS0 (Table 2). The highest total phosphorus content was measured in $CS_{0.6}$ mixtures, where more organic matter was added (manure plus cocoa shell powder). These are in agreement with the results presented in several works [68], [69], [70], [71], where increases in inorganic P are reported,after the application of manure to the soil.Differences in organic P content were observed among CS_0, $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures (Table 2), while the higher organic P content was measured in $CS_{0.4}$ mixtures and the lower in CS_0 mixtures.Factors that may contribute to the organic phosphorus content of the $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures compared to CS_0, are both the inorganic P pool of the mixtures and the microbial activity. The number of microorganism colonies grown in the substrate is therefore critical for phosphate solubilization, since the latest is affected by organic acids produced from these microorganisms [72], [73]. However the addition of organic material in soil mixtures can stimulate the synthesis of soil microbial biomass, increasing their demand for P. This amount of P is pumped from the phosphorus pool contained into the soil mixture and immobilized on the microbial cells [74]. For this reason the correlation of the organic P produced from the microbial activity during the incubation period and the total P which is contained in the substrate is not linear [75]. A similar nonlinear relation was observed in this work between the number of microorganism's colonies and the organic P contained in CS_0, $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$(Fig. 5).The above mentioned can explain why the highest concentration of organic P was observed at the intermediate rate of manure and cocoa shells additions ($CS_{0.4}$), where a smaller number of microorganisms colonies was developed compared to $CS_{0.2}$. However the increment of total P in $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures did not affect their content in available P, since the Olsen-P values were similar in all mixtures.

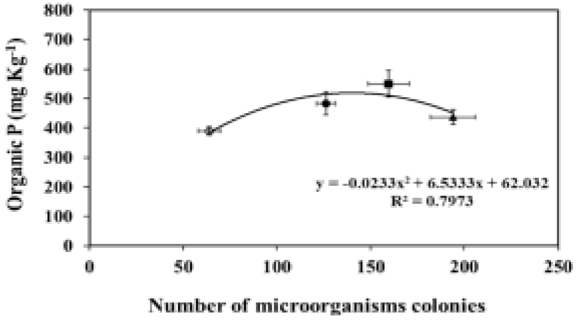


Fig. 5: Correlation (R^2=0.8, P<0.05) between the organic P concentration and the number of microorganisms colonies developed in plates with potato dextrose agar where small quantities of CS_0 (○), $CS_{0.2}$ (▲), $CS_{0.4}$ (■) and $CS_{0.6}$ (●) mixtures were spread.Vertical and horizontal bars indicates SD.

The concentration of the total Cu, Zn and Mn, in CS_0, $CS_{0.2}$, $CS_{0.4}$ and $CS_{0.6}$ mixtures was similar, while the concentration of the available form of the above mentioned microelements, was affected in a different way by the addition of cocoa cell powder to the soil manure mixtures (Table 2). In specific the concentration of the available form of Cu was increased by 26.6% in $CS_{0.6}$ mixtures, while the concentration of the available form of Zn was increased in both $CS_{0.4}$ and $CS_{0.6}$ mixtures by 17.2% and 22.3% respectively, compared to those of CS_0 mixtures. In contrast the concentration of the available form of manganese was not affected from the addition of the manure and the cocoa cell powder into the soil-manure mixtures.

IV. CONCLUSIONS

The addition of cocoa shell biomass to soil-manure mixtures up to 1% did not affect their NH_4^+-N concentration while it increased the NO_3^{-}-N concentration. Addition of greater than 1% cocoa shell biomass to these mixtures, reduced the NH_4^+-N and increased the NO_3^{-}-N concentration as well as the organic C and the exchangeable Kcontent, after a 21-week incubation period. This indicates that the addition of cocoa shell biomass to soil-manure mixtures in excess of 1% may strongly influence the mineralization of N, C and K.

The results also revealed that the higher the content of the mixtures in cocoa shells powder, the lower the time needed for soil-manure mixtures maturation. After an incubation period of 21 weeks, mixtures that contained lower than 1% cocoa shell powder had a high microbial load, probably because in these mixtures the nutrients mineralization were still in progress. In contrast, in mixtures that contained higher than 1% cocoa shell powder the microbial load was low, possibly because the nutrients mineralization was already completed. The cocoa shell powder in the soil manure mixtures therefore seems to act as accelerator for N, K and C mineralization.

The highest total P content was measured in mixtures where more organic matter was added, while the higher organic P content was measured in mixtures thatcontained lower than 1% cocoa shell powder,where the microbial activity was still high. However the concentration of the available P was not affected by the addition of cocoa shell powder to the soil-manure mixtures.

The variation of total andexchangeable sodium in soil-manure-cocoa shell powder mixturesfollowed a pattern similar to that of potassium. However the increase of exchangeable sodium did not cause any sodicity risk of mixtures.The concentration of the total Cu, Zn and Mn, was not affected by from the addition of the cocoa cell powder to the soil-manure mixtures, while the concentration of their available form was affected in a different way.

REFERENCES

[1] Poulsen, P.H.B., Magid, J., Luxhøi, J. and de Neergaard, A. (2013). Effects of fertilization with urban and agricultural organic wastes in a field trial–waste imprint on soil microbial activity. Soil Biology and Biochemistry, 57, 794-802.

[2] Fernández-Hernández, A., Roig, A., Serramiá, N., Civantos, C.G.O. and Sánchez-Monedero, M.A. (2014). Application of compost of two-phase olive mill waste on olive grove: effects on soil, olive fruit and olive oil quality. Waste Management, 34, 1139-1147.

[3] Hernández, T., Chocano, C., Moreno, J.L. and García, C. (2014). Towards a more sustainable fertilization: combined use of compost and inorganic fertilization for tomato cultivation. Agriculture, Ecosystems and Environment, 196, 178-184.

[4] Odlare, M., Pell, M., Arthurson, J.V., Abubaker, J.and Nehrenheim, E. (2014). Combined mineral N and organic waste fertilization–effects on crop growth and soil properties. The Journal of Agricultural Science, 152, 134-145.

[5] Campos, A. C., Etchevers, J. B., Oleschko, K. L. and Hidalgo, C. M. (2013). Soil microbial biomass and nitrogen mineralization rates along an altitudinal gradient on the Cofre De Perote Volcano (Mexico): the importance of landscape position and land use. Land Degradation and Development, 25(6), 581-593.

[6] Novara, A., Gristina, L., Rühl, J., Pasta, S., D'Angelo, G., La Mantia, T. and Pereira, P. (2013). Grassland fire effect on soil organic carbon reservoirs in a semiarid environment. Solid Earth, 4, 381-385.

[7] Baldi, E. and Toselli, M. (2014). Mineralization dynamics of different commercial organic fertilizers from agro-industry organic waste recycling:an incubation experiment. Plant Soil Environ.Vol. 60(3), 93-99.

[8] Hueso-González, P., Martínez-Murillo, J. F. and Ruiz-Sinoga, J. D. (2014). The impact of organic amendments on forest soil properties under Mediterranean climatic conditions. Land Degrad. Dev., 25, 604-612.

[9] Oliveira, S. P., Lacerda, N. B., Blum, S. C., Escobar, M. E. O. and Oliveira, T. S. (2014). Organic carbon and nitrogen stocks in soils of northeastern brazil converted to irrigated agriculture, Land Degrad. Dev., 26, 9-21.

[10] Koyama, S., Urayama, H., Karunaratne, K.M.P.D. and Yamashita, T. (2009). Effects of Coir Application on Soil Properties and Cucumber Production as a Reuse Model of Organic Medium Used in Soilless Culture. Tropical Agriculture and Development, 53(1), 7-13.

[11] Gougoulias, N., Vagelas, I., Papachatzis, A., Kalfountzos, D., Giurgiulescu, L. and Chouliara, A. (2014). Mixture of solid with water soluble, olive oil mill waste application, as soil amendment in greenhouse cultivation of vegetables (case study). Carpathian Journal of Food Science & Technology, 6(2), 63-68.

[12] Gougoulias, N., Vagelas, I., Giurgiulescu, L., Touliou, E., Kostoulis, V. and Chouliara, A. (2017). The coir substrate for soilless cultures, reused as soil amendment (study in vitro and in vivo). Carpathian Journal of Food Science and Technology, 9(4), 61-70.

[13] Murungu, F. S., Chiduza, C., Muchaonyerwa, P. and Mnkeni, P. N. S. (2011). Decomposition, nitrogen, and phosphorus mineralization from residues of summer-grown cover crops and suitability for a smallholder farming system in South Africa. Commun. Soil Sci. Plant Anal., 42, 2461-2472.

[14] Abbasi, M.K., Tahir, M.M., Sabir, N. and Khurshid, M. (2015). Impact of the addition of different plant residues on nitrogen mineralization immobilization turnover and carbon content of a soil incubated under laboratory conditions. Solid Earth, 6, 197-2015.

[15] Hossain, Md. Z., von Fragstein, P., Heß, N. and Heß, J. (2016). Plant Origin Wastes as Soil Conditioner and Organic Fertilizer: A Review. J. Agric. & Environ. Sci., 16 (7), 1362-1371.

[16] Chatterjee, R., Gajjela, S. and Thirumdasu, K. R. (2017). Recycling of Organic Wastes for Sustainable Soil Health and Crop Growth. Int. J. Waste Resour., 7:3.doi: 10.4172/2252-5211.1000296.

[17] International Cocoa Organization (2017). Quarterly Bulletin of Cocoa Statistics. Volume XLIII No. 1, Cocoa Year 2016/17.

[18] Jokić, T., Gagi, T., Knez, Ž., Šubarić D. and Škerget, M. (2018). Separation of Active Compounds from Food by-Product (Cocoa Shell) Using Subcritical Water Extraction. Molecules, 23(6), 1408.doi: 10.3390/molecules23061408.

[19] Redgwell, R., Trovato, V., Merinat, S., Curti, D., Hediger, S. and Manez, A. (2003). Dietary fibre in cocoa shell: characterization of component polysaccharides. Food Chemistry, 81(1), 103-112.

[20] Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L. and Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (Theobroma cacao L.). Food Chemistry, 104(3), 948-954.

[21] Agbor, R. B., Ekpo, I. A., Osuagwu, A. N., Udofia, U. U., Okpako, E. C. and Antai, S. P. (2017). Biostimulation of microbial degradation of crude oil polluted soil using cocoa pod husk and plantain peels. Journal of Microbiology and Biotechnology Research, 2(3), 464-469.

[22] Kuppusamy, S., Venkateswarlu, K. and Megharaj, M. (2017). Evaluation of nineteen food wastes for essential and toxic elements. International. Journal of Recycling of Organic Waste in Agriculture, 6(4), 367-373.

[23] Okiyama, D. C., Navarro, S. L. and Rodrigues, C. E. (2017). Cocoa shell and its compounds: Applications in the food industry. Trends in Food Science and Technology, 63,103-112.

[24] Fiset, J. F., Tyagi, R. D. and Blais, J. F. (2002). Cocoa shells as adsorbent for metal recovery from acid effluent. Water quality research journal of Canada, 37(2), 379-388.

[25] Meunier, N., Laroulandie, J., Blais, J.F and Tyagi, R.D. (2003). Cocoa shells for heavy metal removal from acidic solutions. Bioresource technology, 90, 255-263.

[26] Meunier, N., Blais, J. F. and Tyagi, R. D. (2004). Removal of heavy metals from acid soil leachate using cocoa shells in a batch counter-current sorption process. Hydrometallurgy, 73(3), 225-235.

[27] Aregheore, M.E. (2002). Chemical Evaluation and Digestibility of Cocoa (Theobroma cacao) Byproducts Fed to Goats. Tropical Animal Health and Production, 34, 339-348.

[28] Adejobi, K. B., Famaye, A. O., Akanbi, O.S.O., Adeosun, S. A., Nduka, A. B. and Adeniyi, D. O. (2013). Potentials of cocoa pod husk ash as fertilizer and liming materials on nutrient uptake and growth performance of cocoa. Research Journal of Agriculture and Environmental Management, 2(9), 243-251.

[29] Campos-Vega, R., Nieto-Figueroa, H. K. and Oomah, B. D. (2018). Cocoa (Theobroma cacao L.) pod husk: Renewable source of bioactive compounds. Trends in Food Science & Technology, 81, 172-184.

[30] Sadasivuni, S., Bhat, R. and Pallem, C. (2015). Recycling potential of organic wastes of arecanut and cocoa in India: a short review. Environmental Technology Reviews, 4(1), 91-102.

[31] Chouliaras, N., Gravanis, F., Vasilakoglou, I., Gougoulias, N., Vagelas, I., Kapotis, T. and Wogiatzi, E. (2007). The effect of basil (Ocimum basilicum L.) on soil organic matter biodegradation and other soil chemical properties. Journal of the Science of Food and Agriculture, 87, 2416-2419.

[32] Gougoulias, N., Vagelas, I., Vasilakoglou, I., Gravanis, F., Louka, A., Wogiatzi, E. and Chouliaras, N. (2010). Comparison of neem or oregano with thiram on organic matter decomposition of a sand loam soil amended with compost, and on soil biological activity. Journal of the Science of Food and Agriculture, 90, 286-290.

[33] Chai, E.W., H'ng, P.S., Peng, S.H., Wan-Azha, W.M., Chin, K.L., Chow M.J. and Wong, W.Z. (2013). Compost feedstock characteristics and ratio modelling for organic

waste materials co-composting in Malaysia. Environmental Technology 34 (20), 2859-2866.

[34] Sangamithirai, K.M., Jayapriya, J., Hema, J. and Manoj, R. (2015). Evaluation of in-vessel co-composting of yard waste and development of kinetic models for co-composting. Int. J. Recycl. Org. Waste Agricult. 4, 153-165.

[35] Wijaya, M. M. (2014). Characterization chemical compound based pyrolysis process from cacao wastes. Proceeding of International Conference On Research, Implementation And Education Of Mathematics And Sciences, Yogyakarta State University, 18-20 May, 97-102.

[36] Wu, J. and Brookes, P.C. (2005). The proportional Mineralisation of Microbial Biomass and Organic Matter caused by air-drying and rewetting of a grassland soil. Soil Biology Biochemistry, 37, 507-515.

[37] Zare, M., Ordookhani, K., Emadi, A. and Azarpanah, A. (2014). Relationship Between Soil Exchangeable Sodium Percentage and Soil Sodium Adsorption Ratio in Marvdasht Plain, Iran. Int. J. Adv. Biol. Biom. Res, 2 (12), 2934-2939.

[38] Habel, A. and Al, O. (2013). Pedotransfer Functions for Estimating ESP & SAR for Arid Soils in the Eastern Region of Libya. Alexandria Science Exchange Journal, 34 (1), 106-112.

[39] Kamphorst, A. and Bolt, G.H. Saline and sodic soils in Bolt G.H. and Bruggenwert M.G.M., Eds. Soil chemistry. A. Basic elements. 2nd ed. Elsevier Scientific, Amsterdam 1978.

[40] Haynes, J. R. (2005). Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Advances in Agronomy, 85, 211-268.

[41] Flavel, T.C. and Murphy, D. V. (2006). Carbon and Nitrogen Mineralization Rates after Application of Organic Amendments to Soil. J. Environ. Qual. 35, 183-193.

[42] Riffaldi, R. J., Levi, M., Pera, A. and Bertoldi, M. (1986). Evaluation of compost maturity by means of chemical and microbial analysis. Wast Manag Res 4, 387-396.

[43] Bernal, M.P., Paredes, C., Sánchez-Monedero, M.A. and Cegarra, J. (1998). Maturity and stability parameters of composts prepared with a wide range of organic wastes. Bioresource Technology, 63(1), 91-99.

[44] Chukwujindu, I., Egun, A.C., Emuh, F.N., Isirimah, N.O. (2006). Compost Maturity Evaluation and its Significance to Agriculture. Pakistan Journal of Biological Sciences 9 (15), 2933-2944.

[45] Sastre, I., Vicente, M.A. and Lobo. M.C. (1996). Influence of the application of sewage sludges on soil microbial activity. Bioresour. Technol. 57, 19-23.

[46] Daebeler, A., Bodelier, P.L., Yan, Z., Hefting, M.M., Jia, Z. and Laanbroek, H.J. (2014). Interactions between Thaumarchaea, Nitrospira and methanotrophs modulate autotrophic nitrification in volcanic grassland soil. The ISME Journal. 8, 2397-2410.

[47] Haney, R. L., Franzluebbers, A. J., Porter, E. B., Hons, F. M. and Zuberer D. A. (2004). Soil Carbon and Nitrogen Mineralization. Soil Science Society of America Journal. 68(2), 489-492.

[48] Mondini, C., Sanchez-Monedero, A., Letita, L., Bragato, G. and De Nobli, M. (1997). Carbon and ninhydrin-reactive nitrogen of the microbial biomass in rewetted compost samples. Commun. Soil Sci. Plant Anal. 23, 113-122.

[49] Sanchez-Monedero, M.A., Mondini, C., de Nobili, M.,Leita, L. and Roig. A. (2004). Land application of biosolids. Soil response to different stabilization degree of the treated organic matter. Waste Manage. 24, 325-332.

[50] Motavalli, P.P., Kelling, K.A. and Converse. J.C. (1989). First year nutrient availability from injected dairy manure. Journal of Environmental Quality 18, 180-185.

[51] Wen, G., Winter, J.P., Voroney, R.P. and Bates, T.E. (1997). Potassium availability with application of sewage sludge, and sludge and manure compost in field experiments. Nutrient Cycling in Agroecosystems 47, 233-241.

[52] Eghball, B., Wienhold, B. J., Gilley, J. E. and Eigenberg, R. A.(2002). Mineralization of Manure Nutrients. Journal of Soil and Water Conservation 57(6), 470-473.

[53] Afoakwa, E. O., Quao, J., Budu, A. S. and Saalia, F. K. (2013). Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. J Food Sci Technol. 50(6), 1097-1105.

[54] van Vliet, J., Slingerland, M. and Giller, K. Mineral nutrition of cocoa: a review. Plant Production Systems Group, Wageningen University, 2015.

[55] Sheng, X.F. (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. Soil Biol Biochem 37, 1918-1922.

[56] Han, H.S. and Lee, K.D. (2005). Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. Res J Agric Boil Sci 1(2), 176-180.

[57] Zeng, X., Liu, X., Tang, J.,Hu, S., Jiang, P., Ii, W. and Xu, L. (2012). Characterization and Potassium-Solubilizing Ability of Bacillus Circulans Z 1–3. Advanced Science Letters. 10. 173-176.

[58] Teotia, P., Kumar, V., Kumar, M., Shrivastava, N. and Varma, A. Rhizosphere Microbes: Potassium Solubilization and Crop Productivity-Present and Future Aspects in Meena, V.S., Maurya, B.R., Verma, J.P. and Meena, R.S. (ed.) Potassium SolubilizingMicroorganisms for Sustainable Agriculture Springer India 2016, pp 315-325.

[59] Sparks, L.D. The Chemistry of Saline and Sodic Soils, Environmental Soil Chemistry (Second Edition), Academic Press, 2003, pp: 285-300.

[60] Choudhary, O. and Kharche, V. Soil Salinity and Sodicity. In book: Soil Science: An Introduction, 2018, pp: 353-385.

[61] Tripathi, B.R., Tandon, H.L.S. and Tyner, E.H.(1970). Native inorganic phosphorus forms and their relation to some chemical indicies of phosphate availability for soils of Agra district. Soil Science 109(2), 93-101.

[62] Marx, E.S., Hart, J. and Stevens, R.G. Soil Test Interpretation Guide, Oregon State University, 1999.

[63] Cooperband, L.R. and Good, L.W. (2002). Biogenic phosphate minerals in manure: implications for phosphorus loss to surface waters. Environ Sci Tech. 36, 5075-5082.

[64] Onwuka, M.I., Osodeke, V.E. and Okolo, N.A. (2007). Amelioration of soil acidity using cocoa husk ash for maize production in Umudike area of south east Nigeria. Trop subtrop. agroecosyst. 7, 41-45.

[65] Miller, J. J., Beasley, B. W., Drury, C. F. and Zebarth, B. J. (2010). Available nitrogen and phosphorus in soil amended with fresh or composted cattle manure containing straw or woodchip bedding. Can. J. Soil Sci. 90, 341-354.

[66] Rao, R. (2017). Enriched cocoa pod composts and their fertilizing effects on hybrid cocoa seedlings. International Journal of Recycling of Organic Waste in Agriculture. 6(2), 99-106.

[67] Sharpley, A.N. and Moyer, B. (2000). Forms of Phosphorus in Manures and Composts and Their Dissolution during Rainfall. Journal of Environmental Quality, 29, 1462-1469.

[68] Campbell, C. A., Biederbeck, V. O., Selles, F., Schnitzer, M. and Stewart, J. W. B. (1986). Effect of manure and p fertilizer on properties of a black chernozem in southern Saskatchewan. Canadian Journal of Soil Science, 66(4), 601-614.

[69] Mafongoya, P.L., Barak, P. and Reed, J.D. (2000). Carbon, nitrogen and phosphorous mineralization of tree leaves and manure. Biol. Fertil. Soils 30, 298-305.

[70] Pramanik, P., Ghosh, G.K., Ghosal, P.K and Banik, P. (2007). Changes in organic – C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. Bioresource Technology 98, 2485-2494.

[71] Gichangi, E.M. and Mnkeni, P.N.S. (2009). Effects of goat manure and lime addition on phosphate sorption by two soils from the Transkei Region, South Africa. Commun. Soil Sci. Plan. Anal. 40(21-22), 3335-3347.

[72] Fankem, H., Nwaga, D., Deubel, A., Dieng, L., Merbach, W. and Etoa, F.X. (2006). Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree

(Elaeis guineensis) rhizosphere in Cameroon. Afr. J. Biotechnol., 5, 2450-2460.

[73] Hu, X., Chen, J. and Guo, J. (2006). Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J.Microbiol. Biotechnol., 22, 983-990.

[74] Ayaga, G., Todd, A. and Brookes, P.C.(2006). Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. Soil Biol. Biochem., 38, 81-90.

[75] Gichangi, E.M., Mnkeni, P.N.S. and Brookes, C.P. (2009). Effects of goat manure and inorganic phosphate addition on soil inorganic and microbial biomass phosphorus fractions under laboratory incubation conditions. Soil Science and Plant Nutrition (2009) 55, 764-777.