THE ASIAN-AUSTRALASIAN ASSOCIATION OF ANIMAL PRODUCTION SOCIETIES

Asian-Aust. J. Anim. Sci.
Vol. 22, No. 5 : 732 - 739
May 2009

www.ajas.info

Kinetics of Chemical Properties and Microbial Quantity in Soil Amended with Raw and Processed Pig Slurry

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ABSTRACT : Pig slurry is a good soil amendment not only because of its high organic matter content, but also because of its ability to provide various nutrients. The objective of this study was to estimate the influence of raw and processed pig slurry application on pot soil over chemical fertilizer and non-amended control soil. Change in the chemical parameters (pH, organic matter (OM), organic carbon (OC), macro and micronutrients) and microbial mass of the treated soils were monitored over 30 to 90 days. Pot soil was treated with the recommended dose of pig slurry and chemical fertilizer, and was sampled after 30, 60 and 90 days of incubation. The least significanct difference ($p<0.05$) was observed on Fe, Cu, Zn, available P and K between treatments. All treatments increased N, P and K content and microbial mass of soil over control soil. Interestingly, no significant effects were detected on OM, OC, total bacteria, actinomycetes and fungi mass in soil irrespective of treatments given. However fungal and bacterial counts, as well as available nutrients, were found to be higher in processed slurry (PS)-treated soil compared to other soils. In general a significant correlation existed between the fungal count and OM, OC, Zn, T Kjeldahl N (TKN), available P and K of soil. A strong negative correlation was observed between pH and Fe in soil. This study clearly demonstrated that the use of processed manure as a fertilizer could be a key for sustainable livestock agriculture. (**Key Words :** Pig Slurry, Chemical Fertilizer, Nutrients, Bacteria, Actinomycetes, Fungi)

INTRODUCTION

The conventional land application of raw and aerobically aged pig slurry has been increasing in Korea in response to the yearly increasing pig production rate (Dong, 2000). Pig slurry provides a valuable source of macro (N, P, K) and micronutrients (Cu, Zn, Fe, Mn) for crop growth, and represents a low-cost alternative to mineral fertilizers (Sharpley and Smith, 1995; Hall, 1999). Direct application of pig slurry to soil may induce changes in soil microbial and biochemical activities, which need thorough investigation (Bandick and Dick, 1999). Organic matter amendments to soils show particular interest, not only because of their effects on soil physical, chemical and biological fertility, but also because their buffering effect reducing soil pollution caused by an excessive or unbalanced use of mineral fertilizers (Rauhe, 1987, Hall, 1999).

The effect on microbial mass of soil by the application of organic amendments has thus become a very interesting subject of investigation, not only their importance in soil functioning and structure, but also because of changes in chemical properties, enzyme activities, biological quantity and be used as indicators of soil pollution (Tyler, 1982; Flieübach et al., 1994; Ong, 2000; Sasaki et al., 2009), and many sorts of organic residues might contain significant amounts of contaminants. If adequate quality is provided, pig slurry may act not only as a source of nutrients and organic matter, but also may increase the size of biodiversity and activity of the microbial population in soil, influencing structure, nutrient turnover. However, this practice has recently raised serious environmental concern due to the often excessive addition rates used for several consecutive years. Uncontrolled application of raw slurry to soil can generate, an excess of nitrates, salts, potentially harmful trace metals, undesirable xenobiotic organic compounds, pathogens and greenhouse gas emissions (Amberger, 1990; Dı´ez et al., 2001).

As analytical methods based on soil chemical data do not always predict overall soil functions, attempts have been made to use biological responses as indicators for evaluating possible impacts of biological wastes application into soil (Islam, 2003; Araujo, 2006; Kaku, 2004; Rahman, 2008). The application of fresh animal manures may produce diverse effects on soil properties including the

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Received July 28, 2008; Accepted December 27, 2008

Table 1. Details of fertilizer application as different forms

Treatment	Fertilization* (1st dose) (15th Feb 07)	Fertilization (2nd dose) (9th Mar 07)	Fertilization (3rd dose) (26th Mar 07)
C	NA[1]	NA	NA
CF	Urea- 109.7 mg Potase-285.4 mg	Urea- 80.8 mg Potase-39.58 mg	Urea- 80.8 mg Potase-39.58 mg
PS	26.13 ml (N-1.93 g/L, P-0.24 g/L Cu-13.8 mg/L, Zn-95 mg/L)	10.8 ml (N-3.44 g/L, P-0.28 g/L Cu-10.1 mg/L, Zn-70 mg/L)	NA
RS	13.71 ml (N-3.68 g/L, P-0.81 g/L Fe-68 mg/L, Mn-11 mg/L, Cu-15 mg/L, Zn-109 mg/L)	11.31 ml (N-3.28 g/L, P-0.21 g/L, Fe-56 mg/L, Mn-9 mg/L, Cu-11 mg/L, Zn-83 mg/L)	NA

[1] Not applicable. * per kg soil.

modification of the composition, size and activity of soil microflora. Microbiological activities are very important in regulating soil properties (Dick, 1992; Sasaki et al., 2009). The nutrient cycles in soil are driven by the activities of different microbial communities, which continuously influence physical structure, nutrient availability and organic matter turnover of soil (Gregorich et al., 1996; Hall, 1999; Rahman, 2008). Thus, the effects of any organic amendment on soil microbial activity under a given cultivation system and climatic condition need to be assessed to effectively evaluate this practice (Bandick and Dick, 1999; Hall, 1999).

The present study was carried out to observe the application of raw and processed (aerobically aged) pig slurry in soil planted with Spinach and consequence changes in soil chemical properties and soil microbial quantities. Results were compared to that of a different corresponding soil receiving chemical fertilization and no amended control soil. In addition an attempt was made to correlate chemical quantities and microbial quantity to investigate changes in soil properties due to the application of pig slurry.

MATERIALS AND METHODS

Experimental details

This experiment was conducted during February to early May 2007 under green house condition at Seoul National University, livestock farm, Suwon. The sandy clay loam surface soil (0-15 cm) was collected from the livestock farm and used (3 kg/pot) for this pot experiment. Spinach was used as a test crop (No sincere effort was made to evaluate the effect of manure application on cultivated spinach). The experiment was carried out with four treatments with three replications, viz., chemical fertilizer (CF), aerobically (30 days old) processed pig slurry (PS), raw pig slurry (RS), and control (C) without any nutrient supplement. All nutrients were applied (per kg soil) as per standard recommendation (Table 1) for spinach.

Soil sampling

Approximately 200 g of rhizopheric soil samples were collected at three different stages of crop growth at 30, 60, and 90 day after sowing on each pot. The samples were kept in sterile plastic bags and stored at 4°C.

Soil chemical analyses

The pH was measured using mixtures of soil:water (1:2.5), moisture content at 105°C for 24 h, TKN content was obtained by the Kjeldahl method (Bremmer, 1996), OM and OC contents by loss on ignition at 550°C for 5 h (Nelson and Sommers, 1996). The OC was estimated from the OM content using the conventional 'Van Bemmelem factor' of 1.724. This factor is based on the assumption that soil OM contains 58% C (Allison, 1965). Available P was determined colorimetrically by ammonium molybdate method (Olsen and Sommers, 1982). Available potassium and available micronutrients (Cu, Zn, Mn and Fe) were extracted with nitric acid digestion and determined by ICP-AES (Pauwels et al., 1992).

Soil microbiological analysis

A 20 g of soil sample was transferred to 180 ml of sterile distilled water containing sodium pyrophosphate (0.18%) and mixed well for 10min and left to settle the solids (5 min). 10 ml of this solution was added into 90 ml of sterile 25% Ringer solution (NaCl 2.25 g^{-1}, KCl 0.105 g^{-1}, $CaCl_2$ 0.045 g^{-1}, $NaHCO_3$ 0.05 g^{-1}, Citric acid 0.034 g^{-1}) and was subsequently serially diluted up to 10^{-9}. Estimation of total aerobic heterotrophic bacterial count was quantified by spread plating (0.1 ml) on Tryptic soy agar plates (in triplicate) and incubated at 37°C for 48 h (Zuberer, 1994). The fungal counts (Figure 1), 0.1 ml of each dilution were spread on Rose Bengal Agar (Difco) plates fortified with chloramphenicol (100 μg/ml). These plates were incubated at 25°C for 5 days. The actinomycetes counts (Figure 1), 0.1 ml of each dilution was spread on Starch-Casein-Nitrate agar (SCN) (Wellington and Toth, 1994) fortified with Rose Bengal (0.035 g/L) and cycloheximide (50 μg/ml). These plates were incubated at 27°C for 12 days.

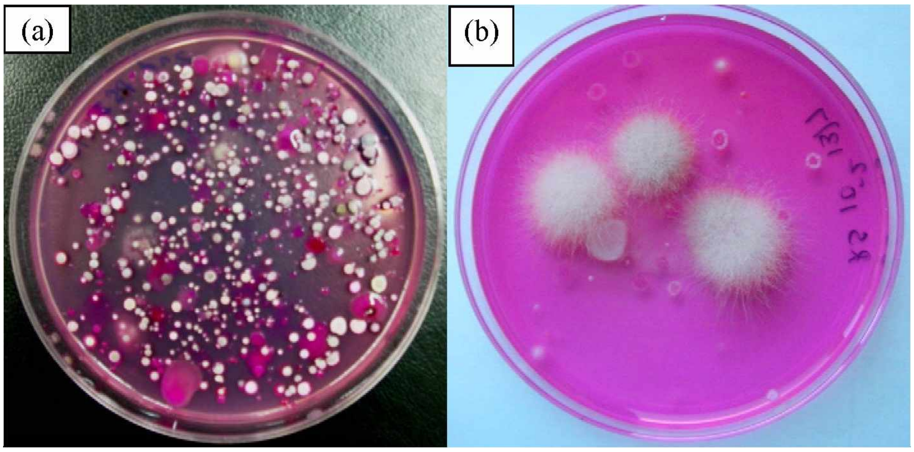

Figure 1. Different microbial colonies of soil samples (a) Actenomycetes on SCN agar plate, (b) Fungi on Rose Bengal agar plate.

Statistical analysis

An ANOVA procedure was used to analyze chemical properties and microbial quantities with different treatments. The relationships between the above parameters were analyzed using the Pearson product-moment correlation. Box plots were constructed to provide a visual summary of the distribution of microbial data collected from the treatments. The statistical analysis was done using SPSS 13.0, Inc., Chicago, IL, USA.

RESULTS AND DISCUSSION

Soil chemical analysis

The application of pig slurry caused significant differences (LSD) between treatments in pH, Fe, Mn, Cu, Zn, TN, available P and K at $p<0.05$ (Table 2, Figure 2). There was no significance observed between treatments for OM, OC and microbes. With respect to the control treatment, the pig slurry manure and CF application did not change the amount of OC and OM in soil. These results were in contrast with previous study (Ong, 2000; Grignani et al., 2007). In that study, the researchers found, application of farmyard manure at two different levels, markedly increased the soil C content and the effect of slurry was modest. This observation was surprising, since Korean pig slurry contains 70% organic matter. It might be consumed by microbes before first sampling (30 days), whereas macro and micronutrients are abundant in pig slurry and plants takes small quantity thus significant differences were observed between treatments. A reason that may have contributed to such an outcome may be that the sampling period was not effective for the sensitive chemical and microbial parameters. The observation of changes in microbes and sensitive chemical parameters (OM, OC) in soil should follow weekly sampling or less. pH was significantly differ between CF and RS only, because ammonia is a main cause for pH changes in soil after slurry application. Interestingly, Fe, Cu and Zn were significantly varied throughout all treatments. Nitrogen was significantly differ between C and CF only, where as phosphorous showed difference between C and PS, RS. In case of potassium, the significance was observed throughout all treatments.

The importance of sampling (days) factor could be seen in Figure 2 that shows TKN in control and CF treatments decreased steadily over the period from 30 to 90 days, but in PS and RS group, it sharply decreased from 30 to 60 days and maintained or slightly increased from 60 to 90 days.

Table 2. Effect of different nutrients application on micro and macro nutrients of soil used for spinach pot cultures

Chemical profiles	C				CF				PS				RS			
	30 D[1]	60 D	90 D	Average	30 D	60 D	90 D	Average	30 D	60 D	90 D	Average	30 D	60 D	90 D	Average
pH	6.33	6.58	6.65	6.52^{ab}	6.22	6.47	6.51	6.4^{a}	6.58	6.72	6.78	6.69^{ab}	6.48	6.88	7.22	6.86^{b}
OM (%)	2.27	2.08	1.78	2.04	2.21	2.22	2.3	2.24	2.59	2.20	2.21	2.33	2.41	2.31	2.30	2.34
OC (%)	1.32	1.21	1.03	1.19	1.28	1.29	1.33	1.30	1.50	1.27	1.28	1.35	1.40	1.34	1.33	1.36
Fe (mg/kg)	122	114	116	117^{a}	128	123	125	125^{b}	105	101	102	103^{c}	97	92	94	94^{d}
Mn (mg/kg)	2.26	2.96	3.07	2.76^{a}	3.89	6.74	5.26	5.30^{b}	3.82	4.35	5.40	4.52^{ab}	3.60	3.22	5.21	4.01^{ab}
Cu (mg/kg)	5.57	5.23	5.29	5.36^{a}	6.56	6.3	6.43	6.43^{b}	7.02	6.78	6.84	6.88^{c}	5.93	5.59	5.75	5.76^{d}
Zn (mg/kg)	5.05	4.75	4.8	4.87^{a}	6.72	6.45	6.6	6.59^{b}	7.06	6.82	6.89	6.92^{cd}	7.13	6.73	6.89	6.92^{cd}
TKN (g/kg)	6.4	5.6	4.1	5.3^{a}	14.5	11.4	8.3	11.4^{b}	12.5	7.6	8.1	9.4^{ab}	13.5	7.6	8.7	9.9^{ab}
Available P (mg/kg)	66.6	10.5	7.1	28.1^{a}	67.5	64.4	57.5	63.1^{abc}	85.8	73.1	71.7	76.9^{b}	98.0	68.7	60.2	75.6^{c}
Available K (mg/kg)	287	202	236	241^{a}	488	390	342	407^{b}	458	343	331	377^{b}	504	380	342	409^{b}

[1] Days, Numbers followed by the same letter within a row are not significantly different (LSD, $p<0.05$).

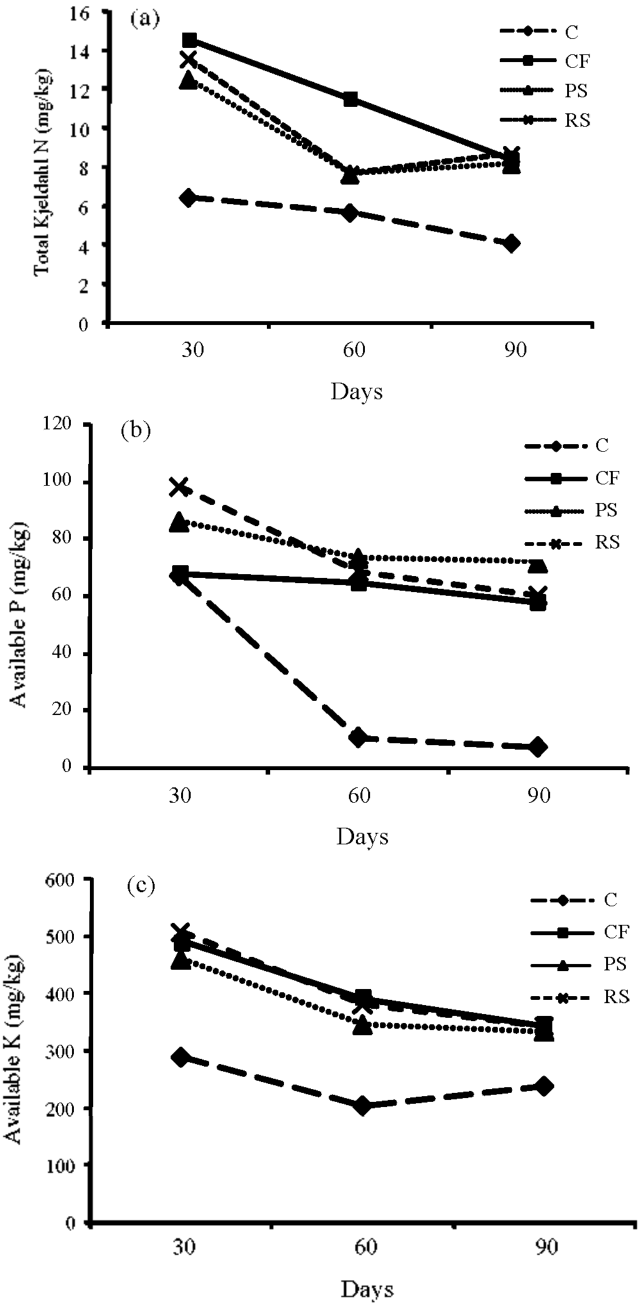

Figure 2. The kinetics of macronutrients on soil with treatments of raw, processed pig slurry, chemical fertilizer and control (a) Total Kjeldahl Nitrogen, (b) Available P, (c) Available K.

This difference in reducing pattern showed in Figure 2a suggests that pig slurry (organic form) exerted an action different from that of chemical fertilizer (inorganic form), that chemical fertilizer could be considered more "natural", at least from TKN point of view, since it expressed a similar reducing pattern as that of control. However, available P (Figure 2b) and available K (Figure 2c) were different trend, which showed drastic decrease in available P and modest decrease in available K from 30 to 60 days in control soil. Incase of other treatment soils observed modest decrease in both available nutrients. However the available P was maintained at 86 to 72 and 60 to 98 mg/kg from 30 to 90 days in PS and RS, respectively. With respect to the effect of sampling time on P and K availability and, significant different effects have been observed in soils respective to treatments (Table 2). Probably, phosphorous is, a one of the most difficult elements for fertilizing optimization in slurry applied soils, especially using organic residues, because of the heterogeneous nature of P into residues, the changes in P availability with pH, and the interaction between Ca-Mg and P compounds (Rodriguez et al., 2005).

In all treatment soils, CF amendments increased soil TKN content significantly at $p<0.022$ (Figure 2), over control soil, whereas PS and RS observed no significant increase at $p<0.095$, $p<0.066$, respectively. Whether manure is processed or not, it affects the TKN content after application (Table 2) as described by Rodriguez et al. (2005) and Kaku (2004). As expected, the application of manure was reflected in a significant increase in the available P content (Figure 2) in the PS ($p<0.017$) and RS ($p<0.019$) amended soils over control (Table 2) but no significant increase of CF was observed at $p<0.064$. The average value of available P was 28.1, 63.1, 76.9 and 75.6 mg/kg on C, CF, PS and RS treatment, respectively. Sharpley (2000) observed an increase in available P, ranging from 5 to 31 mg/kg for every 100 kg/ha of P added, in a review of P availability in soils treated with fertilizer or manure. The average values of available K in control soils was 242 mg/kg, while soils of CF, PS and RS observed at 407, 377, 409 mg/kg, respectively. In all treatment soils, increased the K content (Figure 2) over control soil at $p<0.020$, $p<0.044$ and $p< 0.019$ in CF, PS and RS soil, correspondingly. As for N and P, the application of different pig manure was reflected in a significant and proportional increase in the available K content in the soils (Rodriguez et al., 2005; Rahman, 2008).

The Pearson correlation was observed between all observed parameters of soil (Table 3), interestingly the pH was negatively correlated with Fe at $p<0.01$, R^2-0.76. Generally, the release of Fe, Mn, and Al is strongly dependent on pH, as these minerals compounds dissolve more easily in acid water (Berggren and Mulder, 1995). The significant correlation was observed between OC and OM at R^2 1.00. OC and OM was correlated considerably with, Zn, TN, available P, K and fungi nearly at R^2 1.00, 0.70, 0.64, 0.84, 0.69, 0.66, respectively. Cu showed strong positive correlations with Zn ($p<0.01$) at R^2 0.72, and available P ($p<0.05$) at R^2 0.63. All macronutrients were (N, P, K) significantly correlated with each other in soil. The source of variation was observed between treatment and days as main effects and its interaction with soil parameters (Table 4). It reveals significant variation in pH at $p<0.01$, $p<0.05$ in days and interaction, respectively. The significant

Table 3. Correlation coefficients (r pearson) and significance of chemical and microbial quantities (n = 12)

		pH	OM	OC	Fe	Mn	Cu	Zn	TKN	Avi.P	Avi.K	TBC	ACT.C	FC
pH	r	1	0	-0.01	-0.76**	0.23	-0.14	0.24	-0.35	-0.07	-0.24	0.46	0.25	-0.18
	Sig.		1	0.97	0.004	0.47	0.664	0.456	0.259	0.821	0.445	0.128	0.434	0.574
OM	r		1	1.00**	-0.31	0.15	0.52	0.70*	0.64*	0.84**	0.69*	-0.01	-0.07	0.66*
	Sig.			0	0.322	0.65	0.085	0.011	0.024	0.001	0.014	0.98	0.827	0.019
OC	r			1	-0.31	0.14	0.5	0.69*	0.64*	0.83**	0.68*	-0.02	-0.08	0.66*
	Sig.				0.331	0.671	0.096	0.013	0.024	0.001	0.014	0.943	0.805	0.02
Fe	r				1	0.06	0.02	-0.45	0.04	-0.34	-0.15	-0.14	-0.23	-0.06
	Sig.					0.863	0.95	0.146	0.904	0.281	0.649	0.667	0.478	0.866
Mn	r					1	0.52	0.53	0.31	0.24	0.23	0.55	0.5	0.12
	Sig.						0.081	0.074	0.32	0.455	0.48	0.063	0.1	0.702
Cu	r						1	0.72**	0.57	0.63*	0.57	0.37	0.21	0.48
	Sig.							0.008	0.052	0.028	0.054	0.235	0.511	0.115
Zn	r							1	0.71*	0.83**	0.80*	0.36	0.47	0.58*
	Sig.								0.01	0.001	0.002	0.251	0.122	0.05
TKN	r								1	0.72*	0.94**	0.03	0.27	0.75**
	Sig.									0.009	0	0.926	0.404	0.005
Avi.P	r									1	0.83**	-0.15	0.06	0.66*
	Sig.										0.001	0.646	0.849	0.02
Avi.K	r										1	0.01	0.26	0.73**
	Sig.											0.98	0.412	0.008
TBC	r											1	0.55	0.02
	Sig.												0.061	0.943
ACT.C	r												1	0.21
	Sig.													0.513
FC	r													1
	Sig.													

*, ** Significant at the probability level of 0.05 and 0.01, respectively.

variation was observed in treatment with Zn and fungal count, whereas days reveled significance with OM, TKN, available K and fungal count at $p<0.05$. However, interactions observed in pH and fungal count at $p<0.05$. The results indicates application of pig slurry (processed or raw) significantly affect the soil parameters (pH, OM, TKN, available K and fungal count) with respective to sampling days.

Soil microbiological analysis

One of the most commonly used parameters in soil biology is the microbial mass. As shown in Figure 3, there was no significant raise in microbial count although wide difference was observed in total bacterial count at 30days samples. The PS showed highest count at 7.9 $\log_{10}$ CFU/g, whereas CF at 7.6 $\log_{10}$ CFU/g, 6.3 $\log_{10}$ CFU/g in RS and 5.85 $\log_{10}$ CFU/g in control soil, respectively. However in 60 and 90 days samples did not show much difference in bacterial count. Fertilizer application has high availability of nutrients at initial days (with in 30 days) which supports bacterial growth (Rufete et al., 2006; Sasaki et al., 2009). In respective to sampling effects, the actinomycetes count was

Table 4. Analysis of general linear modal (Univariate) results for soil parameters of unamended (C), chemical Fertilizer (CF), processed slurry (PS) and raw slurry (RS) amended soils

Parameter	Source of variation					
	Treatment		Days		Treatment×days	
	F	p value	F	p value	F	p value
pH	3.84	0.113	83.32	0.001**	8.02	0.036*
OM	3.03	0.156	12.84	0.023*	4.47	0.091
OC	2.82	0.171	12.79	0.230	4.23	0.099
Fe	10.09	0.250	2.59	0.183	0.166	0.914
Cu	6.35	0.053	2.73	0.174	0.071	0.971
Zn	13.26	0.015*	2.58	0.184	0.06	0.976
TKN	2.38	0.211	12.33	0.025*	0.413	0.753
Available P	0.85	0.533	13.19	0.220	1.89	0.273
Available K	4.87	0.080	20.35	0.011*	0.83	0.541
Fungal count	7.01	0.045*	49.88	0.002*	7.52	0.040*

*, ** Significant at the probability level of 0.05 and 0.01, respectively.

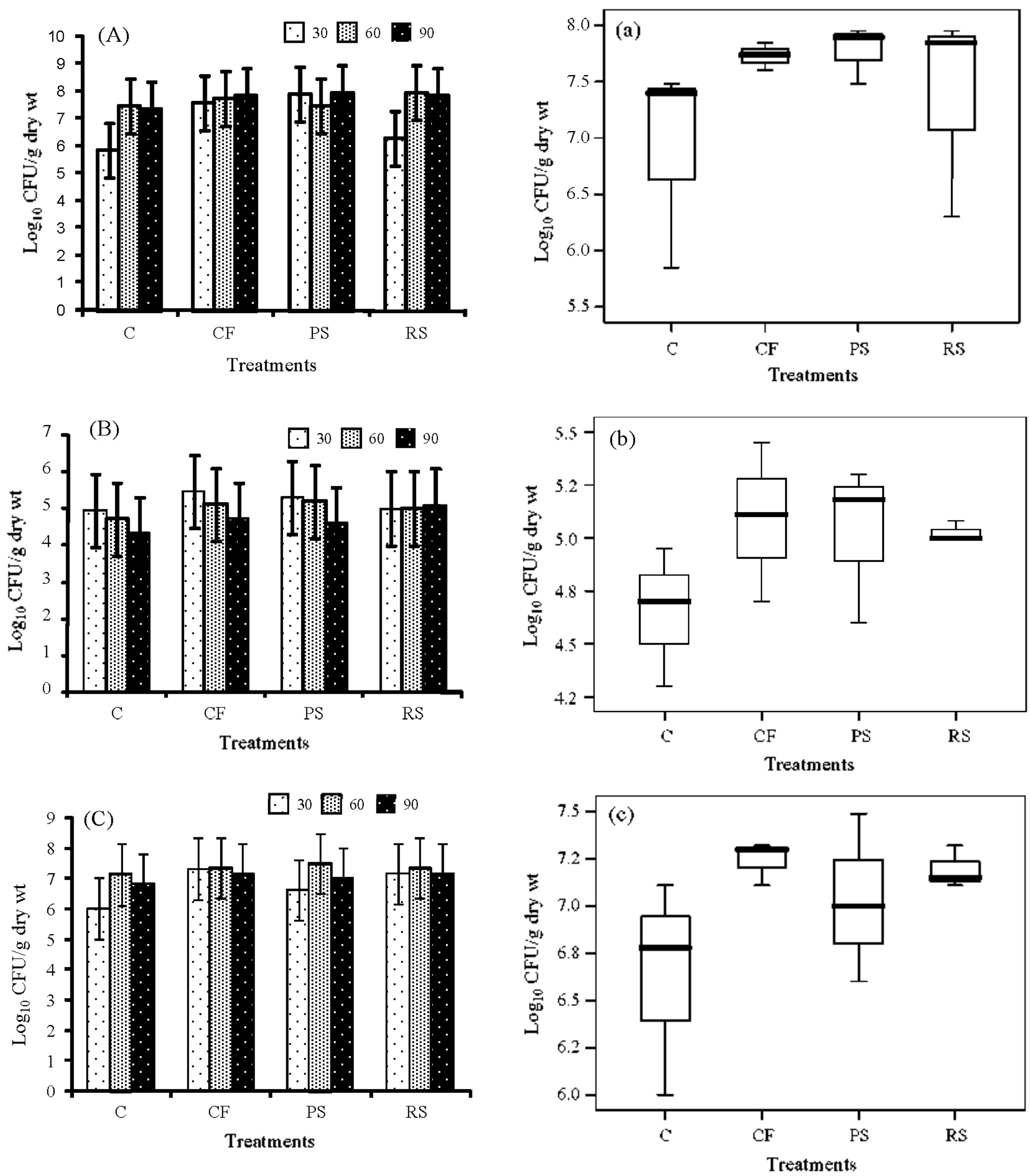

Figure 3. Kinetics of various microbial amounts in soil with application of different fertilizer as chemical, processed and raw slurry. (A, a) Total heterotrophic bacterial count, (B, b) Fungal count, (C, c) Actenomycetes count.

high in CF at 7.3 log_{10} CFU/g in 30 days, whereas in 60 days the high count was observed in PS at 7.49 log_{10} CFU/g. In case of 90 days very slight difference was noted in actenomycetes count in all treatment soils. It clearly indicates, the CF and PS support the actenomycetes growth initially in soil after application. The same effect was observed in fungal count, however, interestingly the 90 days observed elevated fungal count in RS at 5.08 log_{10} CFU/g, which suggested RS amended soil supports fungi later stages (after 60 days)(Van Gestel et al., 1993). The overall observation revealed a high average count of bacteria and fungi in treatment of PS at 7.78 and 5.02 log_{10} CFU/g. The actenomycetes was observed at 7.21 log_{10} CFU/g on CF treatment soil. However, in the soil amended with PS and CF observed larger microbial mass than the soil amended with RS and the control treatment. Even though raw slurry has great amount of microbes, which did not survive in the soil except the actenomycetes (7.15 log_{10} CFU/g). A reason for this may be due to the growth of soil microbiota in response to the presence of easily available nutrients (N, P, K, C) in PS and CF (Saviozzi et al., 2002). Changes in fungal, or bacterial mass, can have implications for ecosystem functions. For example, increases in fungi and nitrogen fixing bacteria could result in increased N being made available to host plants, thereby increasing annual net primary productivity (Cornelissen et al., 2001).

As the results indicated, all microbes were increased over control soil. The higher microbial biomass indicates high fertility of treated soil (due to different carbon contents). It is well known that soil microorganisms can mobilize as well as immobilize relatively high amounts of nutrients in their biomass (Diaz-Ravina, 1993). The decrease in fungal count from 30 to 90 days in CF, PS and control soil suggested the reduction of nutrients by plant uptake and microbes, whereas raw slurry maintained similar counts throughout sampling periods. The results are in agreement with the findings of soils amended with composted sewage sludge (Moreno et al., 1999; Selivanovskaya et al., 2001; Arauja and Monteiro, 2006; Sasaki et al., 2009). A strong positive correlation was observed (Table 3) between fungal count and available K, TN, available P, OM, Zn, and the source of variation divulged the significance against treatment, days and its interaction with fungal count (Table 4). However, no correlation was observed between bacteria and actenomycetes, since bacteria and actinomycètes populations are reported to have a protoplasmic C/N ratio of 5 whereas fungi have a ratio of 10 (Miller, 1991) thus, fungi have impact on soil nutrients.

CONCLUSIONS

The application of processed and raw pig slurry as fertilizer proved clear effect on pH, Fe, Cu, Zn, and available P and K in pot soil. All treatments increased the N, P and K content and microbes mass of soil over control. It is therefore recommended that a dose of calculated pig slurry should be applied only after taking into account the micro and macronutrients through appropriate soil analysis. No significant effects were observed on OM, OC, total bacteria, actenomycetes and fungi mass in soil respective to treatments. A reason that may have contributed to such an outcome may be that the sampling period was not effective for such sensitive chemical and microbial parameters. The observation of changes in microbes and sensitive chemical parameters (OM, OC) in soil should follow sampling at weekly or less. However the repeated addition of pig slurry might affect the biological characteristics of soil. In general, a significant correlation existed between the fungal count and OM, OC, Zn, TN, available P and K of soil. A strong negative correlation was observed between pH and Fe as Fe tends to dissolve more easily in acid water. To better understand the kinetic of soil with application of various choice (raw slurry, aerobically or anaerobically treated slurry) of pig slurry, more physiochemical and microbiological data needs to be obtained during the early stages with repeated application.

ACKNOWLEDGMENT

This research was supported by grants in part from ARPC, KIEST and RDA Korea. The authors would like to thank University animal farm, Seoul National University for allowing us to use green house for the experiment.

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