

## Enzyme and Microbial Activities in Paddy Soil Amended Continuously with Different Fertilizer Systems

Ravi Gadagi, Chang-Young Park<sup>1)</sup>, Geon-Jae Im<sup>1)</sup>, Dong-Chang Lee<sup>1)</sup>,  
Jong-Bae Chung<sup>2)</sup>, Olayvanh Singvilay and Tong-Min Sa\*

Department of Agricultural Chemistry, Chungbuk National University, Cheongju 361-763, Korea

<sup>1)</sup>National Youngnam Agricultural Experiment Station, RDA, Milyang 627-803, Korea

<sup>2)</sup>Department of Agricultural Chemistry, Daegu University, Kyongsan 712-714, Korea

(Received November 14, 2001. Accepted December 1, 2001)

**Abstract :** Soil enzyme and microbial activities are affected by fertilizer and compost applications and can be used as sensitive indicators of ecological stability. Microbial population and soil enzymes viz., dehydrogenase, urease, acid phosphatase and arylsulphatase were determined in the long-term fertilizer and compost applied paddy soil. Soil samples were collected from the four treatments (control, compost, NPK and compost+NPK). Long-term NPK+compost application significantly increased activities of urease, dehydrogenase and acid phosphatase than all other treatments. The compost application enhanced activities of urease, dehydrogenase and acid phosphatase than the NPK application. However, arylsulfatase activity was not significantly different between compost and fertilizer application. The highest microbial population was recorded in the NPK+compost treatment. The compost application also resulted in higher microbial population than the NPK application. The above results indicate that ecological stability could be maintained by application of compost alone or with NPK.

**Key words :** soil enzymes, microbial population, fertilizer, compost

### INTRODUCTION

Present day concerns over problems of environmental pollution and disturbance in soils due to the application of chemicals has stimulated researches in evaluation of the possible impacts of these stresses on ecosystem biota and long-term productivity. Soil biota is considered an important and labile fraction of soil organic matter involved in energy and nutrient cycling. The quality of any soil depends not only on the natural biological composition of the soil, but also on changes related to intensive cultivation and management<sup>1)</sup>.

The importance of microbial activity in cycling organic matter and regulating active nutrient pools in soils suggests that the effects of pollution on soil microorganisms are fundamentally related to the effects on crops natural vegetation and ecosystem productivity<sup>2,3)</sup>. Microbial activity measurements,

therefore, appear as good indicators of the degree of pollution of contaminated soils<sup>4,6)</sup>. Simultaneous measurement of enzyme activities in soil might be more valid for estimating the overall microbial activity and its response to diffuse pollution and environmental stress. It also gives information on the diversity of functions that can be assumed by the microorganisms in the soil, which is one of the main questions in the field of sustainable soil management<sup>7,8)</sup>.

Microbial activity is in fact a general term that includes all the metabolic reactions and interactions conducted by the microflora and microfauna in soil<sup>9)</sup>. It has been established that the more dynamic characteristics such as soil enzymes respond more quickly to changes in crop management practices or environmental conditions<sup>10,11)</sup>.

Dehydrogenase is considered as a good indicator of microbial activity in soil in relation to their mineralizing function<sup>12,13)</sup>. Urease and phosphatase activities in relation to the cycle of N (ammonification, nitrification, denitrification) or P (release of inorganic P) in soil have been used to evaluate the

Corresponding author :

Tel : +82-43-261-2561 Fax : +82-43-271-5921

Email : tomsa@cbucc.chungbuk.ac.kr

fertility of the soil or to describe the functioning of the ecosystem<sup>14,15</sup>.

The present study was undertaken to ascertain the effects of long term application of fertilizers on activities of dehydrogenase, urease, acid phosphatase and arylsulfatase, and population of bacteria, fungi, actinomycetes and  $N_2$  fixers.

## MATERIALS AND METHODS

Four treatments were selected from a long-term experiment at NYEAS, Milyang, Korea. The fertilizer application was started in 1967 on paddy soil. The fertilizer treatments included in the present study were T1 (control), T2 (10 t compost/ha), T3 (150-100-100 kg NPK/ha) and T4 (compost+NPK). The N, P and K were applied through urea, diammonium phosphate and muriate potash every year. Each treatment replicated thrice in a randomized block design.

Soil samples were collected from a depth of 5 to 10 cm from multiple locations in each plot and sieved at the field moist condition to pass a 2-mm screen, then mixed and sub-sampled for the determinations of enzyme activity and microbial population.

Activity of dehydrogenase (formazan release method) was determined by the method of Casida et al.<sup>16</sup>. Acid phosphatase (Orthophosphoric monoester phosphohydrolase EC 3.1.3.2; pH 6.5; 37°C) and urease (Urea amidohydrolase EC 3.5.1.5;  $NH_4$ -N release method; pH 9; 37°C) were determined by the methods of Tabatabai and Bremner<sup>17</sup> and Tabatabai and Bremner<sup>18</sup>, respectively. Arylsulfatase (arylsulfatase sulfohydrolase EC 3.1.6.1; pH 5.8; 37°C) was assayed by the method of Tabatabai<sup>19</sup>.

Microbial populations were enumerated using the serial dilution plate count technique with a selective medium for each organism. Total bacteria, total fungi and actinomycetes were enumerated on soil extract agar<sup>20</sup>, rose bengal agar<sup>21</sup> and Kuster's agar<sup>22</sup>, respectively. And free living  $N_2$  fixers were enumerated on Norris N-free agar<sup>23,24</sup>.

## RESULTS AND DISCUSSION

Soil dehydrogenase is an index of soil metabolism i.e. total oxidative activities of microflora in soil, and consequently the enzyme may be associated to be a good indicator of microbiological activity<sup>9</sup>. The application of compost plus NPK showed the maximum dehydrogenase activity (153.6 mg formazan /kg soil/hr), which closely followed by only compost and

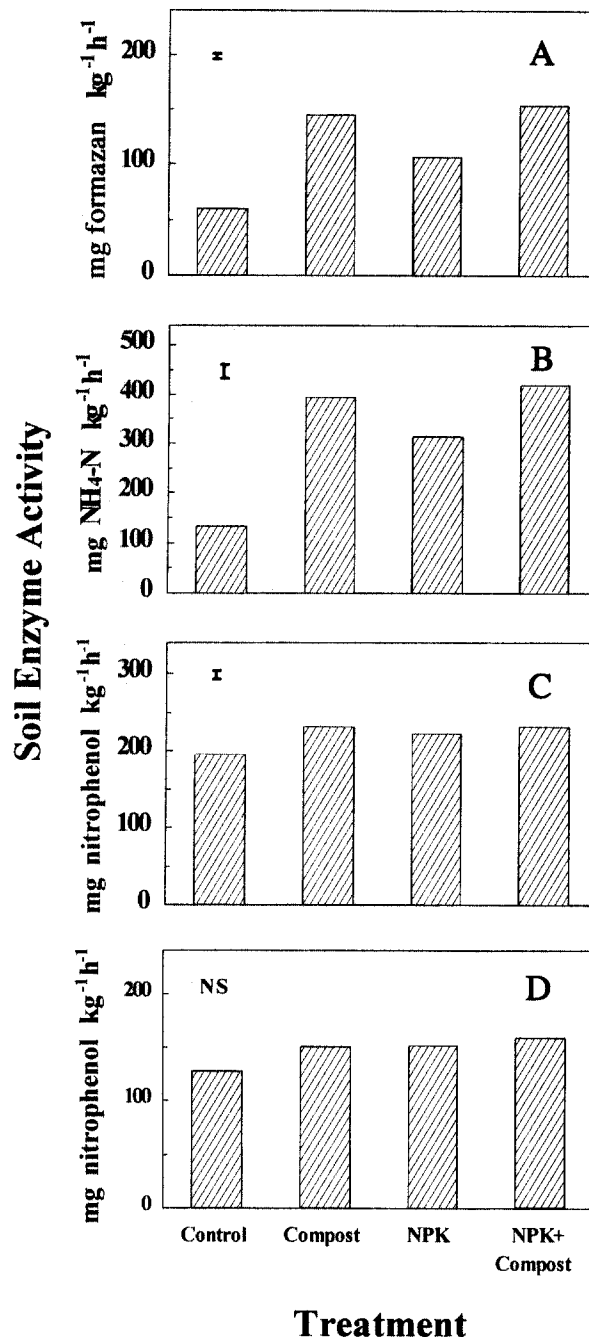


Fig 1. Effect of long-term applications of fertilizer and compost on soil dehydrogenase(A), urease(B), acid phosphatase(C) and arylsulfatase(D) activity. (Small bar represents  $LSD_{0.05}$ )

only NPK application (146.1 and 145.3 mg formazan/kg soil /hr, respectively) (Fig. 1-A). This is also supported by the higher total population of bacteria, fungi and actinomycetes in the compost-applied soils.

The long-term application of compost increased the dehydrogenase activity when compared to NPK application. This suggests that dehydrogenase activity is sensitive to long-term application of NPK and the enzyme activity can be slightly inhibited in those soils. Kanchikerimath and Singh<sup>25)</sup> reported similar results; heavy application of NPK for 26 years significantly inhibited dehydrogenase activity in soils. However, application of NPK with compost increased dehydrogenase activity, and this suggests the compost application would reduce the inhibitory effect of chemical fertilizers. Applications of compost improved the organic matter status of soils, and which was in turn reflected in the higher enzymatic activity. Similar conclusion was also made by Beyer et al.<sup>26)</sup>

Urea widely used as fertilizer of N source and the usefulness of urea depends on its breakdown into two molecules of ammonia and one of bicarbonate. This chemically difficult reaction is aided by the enzyme urease, which is present in many soil bacteria. The highest urease activity exhibited (418.2 mg NH<sub>4</sub>-N/kg soil/hr) in the NPK+compost treatment, followed by only compost application then only NPK application (Fig. 1-B). NPK+compost application increased the microbial population in the soil, which in turn might have enhanced urease activity. The lowest urease activity was found in the control treatment. Application of only NPK and only compost also increased the urease activity compared to control treatment. The availability of urea and urea related compounds present in the compost might have enhanced significantly the urease activity.

Major portion of the P in soil locked in the organic compounds, and this organic P is available to plants upon its degradation by phosphatase enzymes. Many plants and soil microorganisms produce the phosphatase enzymes, which in turn make organic P available to plants. The highest phosphatase activity was exhibited in the NPK+compost treatment (231.6 mg nitrophenol/kg soil/hr), which followed by only compost and only NPK applied treatment (Fig. 1-C). The soil amended with NPK and/or compost increased acid phosphatase activity compared to control treatment. Kanchikerimath and Singh<sup>25)</sup> reported that phosphatase activities were increased with application of NPK fertilizer and compost treatments as compared to control. Increase in organic matter content in the soil also increased the enzymatic activity. In this experiment, we also noticed the highest organic matter content in the NPK+compost soil. Similar acid phosphatase increase was also reported by Ajwa et al.<sup>27)</sup> in fertilized soils compar-

ed to unfertilized.

Arylsulfatase catalyzes the hydrolysis of organic sulfate ester with an aromatic radical<sup>28)</sup>. The soil amended with compost+NPK showed the highest arylsulfatase activity, which was followed by only compost application and then only NPK application (Fig. 1-D). The compost-applied soils exhibited higher arylsulfatase activity; this may be due to the S-containing organic compounds amended to soil. Ajwa et al.<sup>27)</sup> reported similar increase of arylsulfatase enzyme activity due to long-term fertilization. The lowest arylsulfatase activity was recorded in the control treatment.

The microbial population dynamics was also different with physiological growth stages of paddy crop. In the initial stages of plant growth microbial populations were not much increased (Table 1 to 4), however, in August microbial population was increased rapidly, which also coincidence with reproductive stage of the paddy crop. The populations of fungi, actinomycetes and N<sub>2</sub> fixers also followed the same trend.

The treatments receiving NPK+compost, only compost, and only NPK harbored significantly higher populations of bacteria, fungi, actinomycetes and N<sub>2</sub> fixers compared to the control treatment (Table 1 to 4). The highest microbial population was recorded in the treatment receiving NPK+compost, and followed by the only compost treatment. However, application of compost harbored significantly higher microbial population than NPK application. The control treatment harbored significantly less microbial population. Microbial populations were increased due to the application of compost, and this is mainly attributed to organic matter build-up in the soil<sup>29)</sup>.

**Table 1. Population dynamics of bacteria during cropping period of the paddy as influenced by the long term fertilizer and compost applications**

Treatments	may	June	July	Aug	Sept	Mean
( × 10 <sup>6</sup> /g soil )						
Control	24.21	35.66	36.29	48.13	34.12	35.68
Compost	72.62	53.63	60.06	82.20	56.36	64.97
NPK	67.46	41.78	62.30	73.24	47.25	58.39
NPK+compost	77.78	83.10	78.82	97.01	78.69	83.08
Mean	60.51	53.54	59.36	75.13	54.10	60.53
	Treatment (T)		Month (M)		T × M	
LSD <sub>0.05</sub>	6.08		6.80		13.60	

**Table 2. Population dynamics of fungi during cropping period of the paddy as influenced by the long term fertilizer and compost applications**

Treatments	May	June	July	Aug	Sept	Mean
( $\times 10^3$ /g soil )						
Control	26.49	20.06	41.50	53.84	22.12	32.71
Compost	73.40	46.07	96.96	101.25	47.68	73.07
NPK	67.46	34.12	95.08	98.76	35.29	66.16
NPK+compost	101.59	47.78	119.87	130.48	50.27	89.99
Mean	67.23	37.08	88.35	95.57	38.97	65.48
		Treatment (T)	Month (M)	T x M		
LSD <sub>0.05</sub>	0.68	0.76	1.53			

**Table 3. Population dynamics of actinomycetes during cropping period of the paddy as influenced by the long term fertilizer and compost applications**

Treatments	May	June	July	Aug	Sept	Mean
( $\times 10^2$ /g soil )						
Control	32.54	17.09	28.55	35.90	25.15	27.84
Compost	54.76	38.52	55.72	67.69	45.21	52.38
NPK	53.17	29.25	49.18	59.06	38.12	45.75
NPK+compost	60.20	40.86	73.00	87.76	48.25	62.01
Mean	50.16	31.43	51.61	62.60	39.18	46.99
		Treatment (T)	Month (M)	T x M		
LSD <sub>0.05</sub>	0.53	0.60	1.20			

**Table 4. Population dynamics of free-living N<sub>2</sub> fixers during cropping period of the paddy as influenced by the long term fertilizer and compost applications**

Treatments	May	June	July	Aug	Sept	Mean
( $\times 10^3$ /g soil )						
Control	40.48	52.75	63.17	77.31	51.25	55.99
Compost	68.25	114.05	117.22	130.44	98.25	105.64
NPK	57.14	91.92	116.81	122.18	84.24	94.45
NPK+compost	77.38	124.05	164.20	182.75	102.22	130.12
Mean	60.81	95.69	115.35	128.17	83.99	96.80
		Treatment (T)	Month (M)	T x M		
LSD <sub>0.05</sub>	0.64	0.71	1.43			

In conclusion, the enzyme and microbial activities were increased due to compost application compared to only NPK application as well as control over period of 37 years. The results indicate that ecological stability could be maintained by application of compost alone or with NPK. The favorable effect of soil enzymes and microbial activities on crop productivity can also be attributed to soil nutrient availability for plants, improvement of soil physical properties and efficiency of fertilizer nutrients by organic amendments. We can also reduce the excess application of NPK to soil which is coupled with various hazardous effects on human beings.

## ACKNOWLEDGEMENT

This work was supported by the Ministry of Agriculture and Forestry through the R&D Promotion Center for Agriculture and Forestry.

## REFERENCES

- Pierce, F. J. and Larsan, W. E. (1993) Developing criteria to evaluate sustainable land management. In Kimble, J. M. (Ed) Proceedings of the VIII international soil management workshop. Utilization of soil survey information for sustainable land use, Sacramento, CA. p.7-14.
- Zak, J. C., Willig, M. R., Moorhead, D. L. and Wildman, H. G. (1994) Functional diversity of microbial communities: a quantitative approach, *Soil Biol. Biochem.* 26, 1101-1108.
- Ladd, J. M., Foster, R. C., Nannipieri, P. and Oades, J. M. (1996) Soil structure and biological activity, In Stotzky, G. and Bollag, J. M. (Ed.) *Soil Biochemistry*: 9, Marcel Dekker Inc. New York, p.23-78.
- Tabatabai, M. A. (1977) Effects of trace elements on urease activity in soils, *Soil Biol. Biochem.* 9, 9-13.
- Insam, H., Hutchinson, T. C. and Reber, H. H. (1996) Effects of heavy metal stress on the metabolic quotient of the soil microflora, *Soil Biol. Biochem.* 28, 691-694.
- Kuperman, R. G. and Margaret, M. C. (1997) Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem, *Soil Biol. Biochem.* 29, 179-190.
- Burns, R. G. (1982) Enzyme activity in soil: location and a possible role in microbial ecology, *Soil Biol. Biochem.* 14, 423-427.
- Kennedy, A. C. and Smith, K. L. (1990) Soil microbial

- diversity and the sustainability of agricultural soils, In Collins H. P., Robertson, G. P. and Klug, M. J. (Ed.) *The Significance and Regulation of Soil Biodiversity*, Kluwer Academic Publisher, The Netherlands, p.75-86.
9. Nannipieri, P., Greco, S. and Ceccanti, B. (1990) Ecological significance of the biological activity in soil, In Stotzky, G. and Bollag, J. M. (Ed.) *Soil Biochemistry*, 6, 679-685.
  10. Dick, R. P. (1992) A review: Long-term effects of agricultural systems on soil biochemical and microbial parameters, *Agric. Ecosystem. Environ.* 40, 25-36.
  11. Doran, J. W., Sarrantonio, M. and Liebig, M. A. (1996) Soil health and sustainability, *Adv. Agron.* 56, 1-54.
  12. Haigh, S. D. and Rennie, A. F. K. (1994) Rapid methods to assess the effects of chemical on microbial activity in soil. *Environ. Toxicol. Water Qual.* 9, 347-354.
  13. Camina, F., Traser-Cepeda, C., Gil-Sotres, F. and Leiros, C. (1998) Measurement of dehydrogenase activity in acid soil rich in organic matter, *Soil Biol. Biochem.* 30, 1005-1011.
  14. Nannipieri, P., Muccini, L. and Ciardi, C. (1983) Microbial biomass and enzyme activities : production and resistance, *Soil Biol. Biochem.* 15, 679-685.
  15. Alef, K. and Nannipieri, P. (1995) *Methods in applied soil microbiology and biochemistry*, Academic press, London, p.576.
  16. Casida Jr., L. E., Klein, D. A. and Santoro, T. (1964) Soil dehydrogenase activity, *Soil Sci.* 93, 371-376.
  17. Tabatabai, M. A. and Bremner, J. M. (1972) Assay of urease activity in soils, *Soil Biol. Biochem.* 4, 479-487.
  18. Tabatabai, M. A. and Bremner, J. M. (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity, *Soil. Biol. Biochem.* 1, 301-307.
  19. Tabatabai, M. A. (1994) Soil enzymes, In Page A. L et al. (Ed.) *Methods of Soil Analysis*, SSSA, Madison, WI. p. 775-833.
  20. Allen, O. N. (1959) The isolation of *Azobacter* sp., In *Experiment in Soil Microbiology*, Burgess Publishing Company, Minneapolis, Minnesota, p.46-47.
  21. Martin, J. P. (1950) Use of acid, rose Bengal and streptomycin in plate method for estimating soil fungi, *Soil Sci.* 69, 215-232.
  22. Kuster, E. and Williams, S. T. (1964). Selection of media for isolation of *Streptomyces*, *Nature* 202, 926-929.
  23. Pikovskaya, R. I. (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species, *Microbiologiya.* 17, 362-370.
  24. Norris, J. R. (1959) The isolation and identification of *Azotobacter*, *Lab. Pract.* 8, 239-243.
  25. Kanchikerimath, M and Singh, D. (2001) Soil organic matter and biological properties after 26 years of maize-wheat-cowpea cropping as affected by manure and fertilization in a cambisol in semiarid region of India, *Agric. Ecosystem Environ.* 86, 155-162.
  26. Beyer, L., Wachendorf, C., Elsner, C. and Knabe, R. (1993) Sustainability of dehydrogenase activity assay as an index of soil biological activity. *Biol. Fert. Soil.* 16, 52-56.
  27. Ajwa, H. A., Dell, C. J. and Rice, C. W. (1999) Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization, *Soil Biol. Biochem.* 31, 769-777.
  28. Tabatabai, M. A. and Bremner, J. M. (1970) Factors affecting soil arylsulfatase activity, *Soil Sci. Soc. Am. Proc.* 34, 427-429.
  29. Benbi, D. K. Biswas, C. R., Bawa, S. S. and Kumar, K. (1998) Influences of farmyard manure, inorganic fertilizers, weed control practices on some physical properties in a long-term experiment, *Soil Use Manage.* 14, 52-54.