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PHOSPHATASE AND UREASE INSTABILITY CAUSED BY PESTICIDES PRESENT IN SOIL IMPROVED BY GROUNDED RICE STRAW

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ABSTRACT

Phosphatase and urease were involved to the mineralization of phosphorus and nitrogen compound in soil. In this observation, these enzymes examined through 12 weeks incubation of the soil containing pesticide and compared to free pesticide one. The treatment executed in greenhouse for incubation. Phosphatase had been lower activity compared to urease of all action. These activities fluctuated in the beginning and decline to 12 weeks incubation. Phosphatase and urease increased in 2 weeks incubation, 2.45 and 49.25 unit/g soils, respectively. Urease was more responsive to soil containing pesticide evaluated to phosphatase activity. Carbon dioxide release as caused of soil microbial respiration most advantageous at 4 weeks incubation in soil free (B soil; 2.96 mg/g/hour) and long-lasting healing with pesticide (A soil; 2.55 mg/g/hour), compared to the soil containing fresh pesticides which was peaked later at 6 week incubation (1.82 mg/g/hour). Organic substance (grounded rice straw) amended into soil was strongly to implicate respiration rate, phosphatase, and urease activities concerning to soil containing pesticide. The result should make representative work in effort to evaluate phosphatase and urease related to mineralization process within and without pesticide degradation in soil, as well as caused by rice straw organic compound augmentation to the soil containing pesticide.

Keywords: phosphatase, urease, respiration, organic substance, pesticide.

INTRODUCTION

Measurements of microbial biomass and its various enzyme systems have been widely used to diagnose the soil state and to describe the effect of different influences of pollutants, agricultural management, and land use. Possible indicator value of the microbial parameters for environmental stress in general had investigated through microbial possessions including biomass and soil enzyme processes (Bandick and Dick, 1999; Tscherko and Kandeler, 1999). Analyses of the activity of soil enzymes provide information on the biochemical processes occurring in the soil. Enzyme activity in soil is regulated by pH and microbial biomass (Dick *et al*., 1988), which is correlate to soil organic matter, and soil moisture content (Harrison, 1983), as well as to soil compaction (Karaca *et al*., 2000). Soil enzyme activity is variable in time and limited by available substrate supply (Degens, 1998; Tateno, 1988), and may provide useful linkage between microbial community composition and carbon processing (Waldrop *et al*., 2000). Information of soil enzyme activities used to determine soil microbiological characteristics are very important for soil quality and healthy. Enzymatic activities as caused by soil microbial activities were sensitive indicators to detect changes occurring in soils (Gonzalez *et al*., 2007).

Phosphatases find widely in bacteria to mammals, and indicate their importance in fundamental biochemical processes (Posen, 1967). The term phosphatase in soil is used to describe a group of enzymes that are responsible for the hydrolytic cleavage of a variety of ester-phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate. Acid and alkaline phosphatases particularly hydrolyse the ester bonds binding P to C (C-O-P ester bonds) in organic matter. During the process, inorganic P is released from organically bound P such as leaf litter, dead root systems, and other organic debris without concomitant release of C (Harrison, 1983). Phosphatase is concentrated in the surface layer and rhizosphere where most of the fresh and less humified organic matter is prevailing (Rojo *et al*., 1990; Tarafdar *et al*., 2001). Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms, and thus in the cycling of it within the soil (Schneider *et al.*, 2001).

Information on the nature of urease activity in soil was beneficial to develop and employ strategies for nitrogen management. Urease hydrolysis activity is elevated in aerobic condition, and its hydrolysis varied to the plant growth stage within green manure amendment to the crop (Pattnaik *et al*., 1999). Urease activity is not well when the bioavailability in the soil is troubled (Nadgórska-Socha et *al*., 2006), and Saliha *et al.* (2006) confirm that urease activity increased along with microbial population in soil amended with liquid organic substrate. Askin and Kizilkaya (2005) used enzymatic activity to the certain landscape for producing current map of soil quality through soil urease surveillance.

Diversity of metabolic pathways is available to certain microbes for its metabolism. Soil microbes such as fungi, bacteria, actinomycetes, *etc.* would involve in enhanced degradation, and may be capable of rapidly degrading anthropogenic chemical like pesticides. Some certain indigenous microbial enzymes may respond to defeat the organic substance as carbon source involve to its metabolite pathway (Degens, 1998). Available of organic compound for the organisms present in soil is requiring in order decomposing pesticides residue, and those processes are called cooxidation (Hill and Wright, 1978). Oxidation processes of the enzymes often makes the pesticide more amenable to degradation at the extracellular level and then proceed further at the intracellular level that causing increase its water solubility,

and lastly increasing bioavailability. That residue in soil so might to turn and may develop into organic substance and valuable to the plant.

The objective of this work was partly to study soil microbial enzymatic activities in the soil, in the present of excessive quantities of pesticide in soil. Approach method was setting in a greenhouse environment to observe phosphatase, urease, bacteria and fungal population, and soil induce respiration that is the keys for input parameters. Organic compound augmented into soil containing pesticide expectantly helps its degradation. The result was meaningful to reference as representative work of rehabilitation in agriculture soil.

MATERIALS AND METHODS

Soil collected in bulk sample from Horticulture Research Station Department of Agriculture in Cipanas, West Java, Indonesia; at 1200 meters elevation, on March 2006. Group of A soil deprive from agriculture plot that intensively take care with pesticide basis for crop production. Sampling of B group soil gathered from the garden at the same area as free pesticide soil. For each sample bulked and packed in a polyethylene bag. The fresh samples were stored at 4^0C until laboratory measurements commenced. Soil physic and chemical character describe as noted in Table-1. Chopped rice straw were grounded and then altering to soil as organic compound for treatment. Grounded rice straw (GRS) consists of 34.25% and 1.27% as carbon (C) and nitrogen (N) component, respectively. Insecticides were identified as deltrametrin (commercially named DECIS), and fungicide probineb known as ANTRACOL, were used for soil treatment in the experiment.

Table-1. Soil chemical and its physic character.

Soil condition	Soil sampling site	
	Long-lasting healing with pesticide (A soil)	**Free pesticide soil** (B soil)
pH	5.70	5.50
Moisture cont. (%)	6.67	6.42
C (%)	2.54	2.95
N (%)	0.19	0.25
Ca ($cmol_{(+)}$/kg)	13.56	11.82
Mg ($cmol_{(+)}$/kg)	1.29	1.65
K ($cmol_{(+)}$/kg)	0.30	0.68
Na ($cmol_{(+)}$/kg)	0.28	0.14
P_2O_5 (mg/100g)	380	91
K_2O (mg/100g)	24	45
Sand (%)	44	43
Silt (%)	38	35
Clay (%)	18	22

Soil trial was set as follows:

a. Twenty gram of GRS mixed into 1000 g soil (A and B soil) prepared inside polyethylene-bag (SPB), and the other group treatment was void of GRS.
b. The same treatment provided to other B soil group but mixed up with Decis and Antracol at the same quantity (11.3 mg), and the other group of soil keeps without pesticide as control.
c. Twenty gram of GRS also amended to B soil group as (b) setting treatment with pesticide only.

All of SPB (each treatment) then placed in the greenhouse, and let the tops opening along incubation. Initial soil moisture performs with spraying by water daily to remain for original weight. Soil samples were taken (30 g per pot or SPB) from all treatment after 0, 2, 4, 6, 8, and 12 weeks incubation. Samples were stored at refrigerator waiting to use for respiration measurement, enzymes analysis, and microbial plate count. Intensive observation on 0, 3, 7, 14, and 28 day's incubation perform throughout B soil treated with pesticide, and compared to that soil amended with organic matters (GRS). Every part of SPB groups' remains in triplicate to each treatment. Analysis of correlation and least significant differences (LSD) among mean values were calculated at $P<0.05$ confidence level (Parker, 1979). Acid phosphatase measurement pursues to Margesin (1996) method. One gram of soil sample collected from SPB in the greenhouse, then placed into 100-ml-Erlenmeyer flask for control (C) and treatment (T) groups as well respectively. One ml of *p*-nitrophenil-phosphate (10.8 mM) substrate put into T's group flask, and then added with 4 ml tris(hydroximethyl) aminomethane buffer (pH 6.5). Those flasks then shacked, and incubated in 37^OC waterbath for 60 minutes.

After incubation, 1 ml 0.5M $CaCl_2$ and 4 ml 0.5M NaOH added to the flasks, and then dissolved it with 90 ml aquadest. Color density of the solution observed through spectrophotometer with 400 nm absorbance. The same procedure was terminated to C's group flask, but quantity of 1 ml p-nitrophenil-phosphate as substrate additional to C's flask was executed after dispensing of 1 ml 0.5M $CaCl_2$. Phosphatase activity calculated in unit/g soil.

Urease measurement adapted from Kandeler (1996) method. Five grams of each sample collected from SPB in the greenhouse then put into 100-ml-Erlenmeyer flask. These flasks divided into control (C) and treatment (T) groups. Urea (0.48 g urea/100ml H_2O) substrate added to T's group flask (2.5 ml), and the same quantity of aquadest put into C's group flask, too. The entire T and C group of flask incubated in 37^OC for 2 hours.

Same quantity of substrate and aquadest then also added through the reverse flask group after incubation. It means that 2.5 ml of urea substrate give to C's group and 2.5 ml of aquadest confer to T's group flask. All T and C group of flask solved with 50 ml of 1M KCl, shacked for 30 minutes, and filtered. One ml of each filtrate put in every test-tube, add with 0.2 ml Nessler solution, and finally liquefied with 9 ml of aquadest. All solution then

settled in 10 minutes, and observed with spectrophotometer in 420 nm absorbance. Urease activity quantified to unit/g soil.

Soil Induce Respiration (SIR) measured to follow Beck *et al.* (1996) method. Soil sample (20 g) induced with glucose solution (0.08%) to become 50% of field soil capacity, wrapped and kept hanging down the string inside 250-ml-Schottbotle that was already filled with 20 ml 0.05 N KOH. Carbon dioxide release as cause of microbial respiration trapped with KOH; and CO_2 captured then titrated with 0.1 N HCl solutions.

Phenolphthalein (1 gram phenolphthalein dissolved to 100 ml Ethyl alcohol) indicator used as the first titrate solution to designate that KOH left in solution is reacted with HCl. The secondary indicator is Methyl-orange (one gram Methyl-Orange per 100 ml H_2O) solution to indicate CO_2 release caused of soil respiration and captured to become bicarbonate acid as the last reaction, as caused by HCl titration. Every single ml of titrated HCl in the second titration was equal to 2.20 mg CO_2, and equivalent to 20.6 mg C-biomass that liberated from 100 g of soil. Carbon dioxide release in mg/g soil calculated for one-hour incubation.

Most probable number (MPN) method pursued to Carvalhal *et al*. (1991). Microbial counts were determined using nutrient agar (3g beef extract, 5g peptone, 15g agar, and 1000 ml aquadest) and malt extract agar (30g malt extract, 5g peptone, 15g agar, and 1000 ml aquadest) for soil bacterial and fungal growth, correspondingly. The colony forming unit (CFU) of fungal and bacteria determined in one gram of soil sample gathered from SPB in the greenhouse, by using a ten fold serial sterilized aquadest dilution technique, and followed by inoculation of drop of each dilution onto the surface of the appropriate agar plate in petridish after a 48-hour-incubation at 28ºC. Microbial growth observed under magnified plate counter.

RESULTS AND DISCUSSIONS

Phosphatase and urease activities, as well as soil microbial respiration were fluctuation at the beginning then tendentiously reduced to 12 weeks incubation (Figure-1). Phosphatase activity in soil free pesticide was superior to soil containing pesticide in the period of 6 to 12 weeks incubation. Refer to urease activity proved that A soil release more these enzyme after 4 weeks incubation, compared to B soil. Microbial respiration becomes an indicator of biological activity in soil. In this observation, soil-containing pesticides appear to have affected to soil respiration. However, stimulatory effect of pesticide was remark here to agitate microbial respiration, compared to soil free pesticide ones at 4 weeks incubation. Phosphatase increased significantly in agriculture soil improved with municipal waste compost, and dissimilarly urease was in low activities (Crecchio *et al*., 2004). Phosphatase gets lower on its activity while urease was stable during ten month in the soil with sewage sludge application for olive grove (Gasco *et al*., 2004). Soil organic content positively correlated with the activities of phosphatase studied, and had significantly higher phosphatase activity in soil that received supplementary tillage (Duran *et al*., 2006). Organic substance obtains from dairy sewage sludge combine with lignite ash and use to managed plant cultivation caused to stimulate urease activity in soil (Jezierska and Fryc, 2006). Soil aeration was emphasizing balance between soil, inside atmosphere, water, and microbial activities. Aerobic microbial activity increases when the soil reaches highest humidity, and followed with maximum microbial transpiration, nitrification, and mineralization. Urease and phosphatase as hydrolyze enzymes keenly rise to maximum level (Karaca *et al*., 2000). GRS organic substance in soil in the experiment setting was able to increase urease activity.

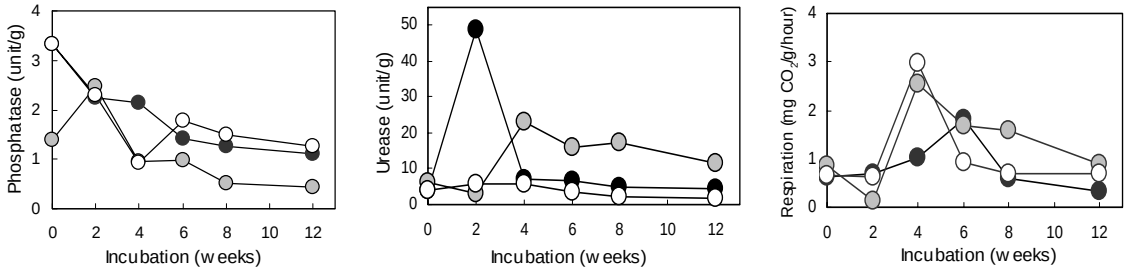

Figure-1. Parameters performs to evaluate A soil (●), free pesticide B soil (○), and soil refinement with pesticide of B soil(◌) along with incubation period

Pesticide added to B soil and its implication after addition of GRS specifically examined. Those moments become important to analysis soil reaction to GRS augmentation in soil receiving fresh pesticides. Incubation after four-week, soil biological activities was relatively decline to all parameters. Otherwise, intensive analysis of 0, 3, 7, 14, and 28 days acceleration activities along incubation have reviewed with considering correlation connecting to treatments (Table-2). Increasing of urease and phosphatase activities proved strong correlation as caused by GRS amplification to B soil containing pesticides. Jastrzêbska and Kucharski (2007) find out that dehydrogenases and urease activities inhibited with increasing dose of fungicide in non-cropped soil as treatment, but it did not influence to the soil cropped with barley in the greenhouse experiment.

Table-2. Intensive observation (0, 3, 7, 14, and 28 day's incubation) hought B soil treated with pesticide compared to that soil amended with organic matters (GRS).

Correlation	Value for p 0.05
Soil induce respiration	NS*
Phosphatase	0.9797
Urease	0.8906

*NS = not significant ($P_{0.05} = 0.75$)

Long-term organic amendments increased the capacity of the small-sized fractions to protect soil microorganisms; urease activity was mainly located in that fraction (Kandeler *et al*., 1999), and the activities of total urease significantly correlated with microbial biomass-C (Klose and Tabatabai, 1999). GRS augmentation was rising firmly to respiration rate in 2 weeks incubation in the observation that makes its correlation coefficient fall insignificant. Acceleration in respiration was considerable since the beginning of treatment to B soil containing pesticide. In the other hand, GRS treatment takes contrary effect to phosphatase and urease measurement. Improving soil by GRS in this observation showed that urease considerably increases but small reaction to phosphatase activity and its soil respiration in soil containing pesticide (Figure-2). When pesticide repeatedly used in soil, some opportunist microorganisms may well develop the capability to use this toxic compound. This adaptation can provide strains with advantage competitive over other microbes in terms of sources its energy (Singh *et al.*, 2000). That ecological adaptation verified to pesticide-degrading bacterium caused as intensively practices insecticide in soil (Singh *et al.*, 2003).

Biological and chemical transformations influence the fate of pesticides degradation. Abiotic transformation of pesticide may happen through hydrolysis, oxidation-reduction, dehydrohalogenation, and photolysis (Wolfe *et al*., 1990). Beck and Jones (1995) recognized that microbes degrading caused limit pesticide in soil was being initiation with the processes of sorption, desorption, and solubilization. Microorganisms can metabolize organic substance if they are bioavailability (e.g. water soluble and not adsorbed) and if they have a chemical structure compatible with the organisms' enzymes that catalyze of the biodegradation process. Chemical transformations occur less frequently, but arise to make the first move the degradation process, producing intermediate compounds

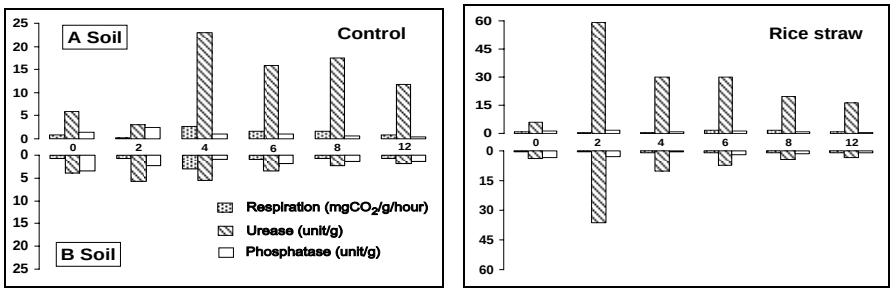

Figure-2. Pattern of respiration and soil enzymes activities in contaminated soil (A soil) compared to soil refinement with pesticide (B soil), after treated with grounded rice straw.

Capacity of Trametes versicolor as native soil microflora was able to degrade pesticide after amended with organic compound of stubble corn and sorghum as bioaugmentation for treatment (Graciela Ruiz-Aguilar and Refugio Rodriguez-Vazquez, 2006). Extensive degradation on pesticide was successful by Actinomycetes isolates which grown on all pesticide compounds. Most of the fungal isolates were not inhibiting any of the pesticides. Overall, endosülfan inhibited growth of the greatest number of microorganisms except for Actinomycetes sp. (Digrak and Ozçelik, 1996). Those results have implications in stimulating development of a bioremediation strategy in agriculture land within the status of crisis xenobiotic contamination trough microbial enzymatic assessment.

Decomposition of agriculture residue, in particular lignocelluloses, interacts to control microbial N mineralization-immobilization processes (Heal *et al.*, 1997). Biochemical quality of substrate as residue and its particle size affect to longevity of decay rate (Bending and Turner, 1999). Additional of simple organic substrate (GRS) to soil change in the phenomenon along 12 weeks soil incubation, and implicate to the monitored parameters (Figure-2). GRS treatment give strong impact to urease activity, as well as to phosphatase and carbon dioxide release, as it showed with narrow scale for control (25 scale) parameters compared to treated one (60 scale).

Urease activity in a soil is advanced then B soil for both of control and GRS treatment. Source of A soil is cultivated area that may contain nitrate and ammonium fertilizer. Studies of the effects ammonium and nitrate as source of N on urease production in soils amended with organic C showed that although microbial activity, as measured by CO_2 production, urease production in the experiment repressed by these forms of N (McCarty *et al.,* 1992). Same result showed that hydrolysis urease in soil also increase as caused by green manure augmentation (Pattnaik *et al*., 1999).

Performance of fungal and bacterial population in relation to soil respiration and phosphatase activities evaluated. The influence of GRS conditions upon the microbial properties estimated by correlation analyses through out pesticide adjustment to B soil (Figure-3).

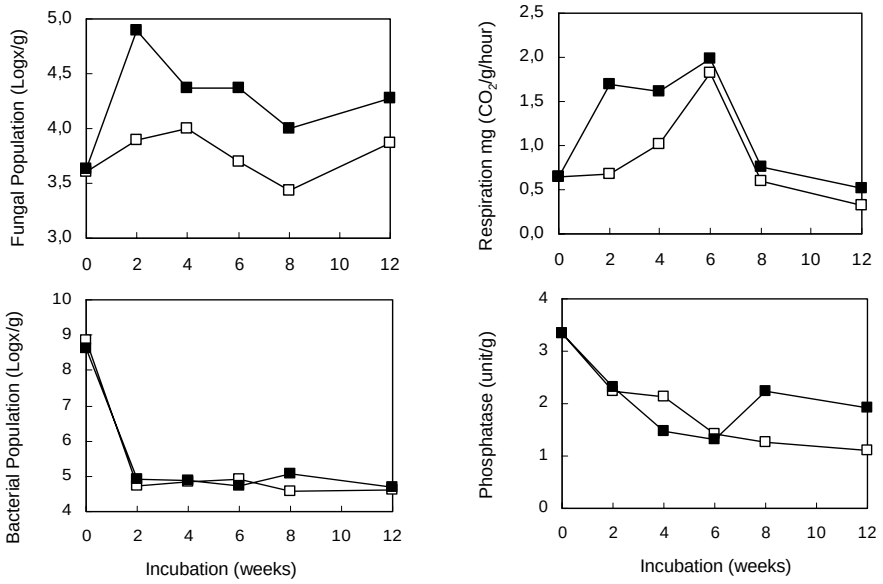

Figure-3. Representation on fungal and bacteria population (CFU/g soil) in B soil (with pesticide refinement) amended with GRS (■) and compared to soil without GRS (□)in 12 weeks incubation.

Significant correlation in fluctuation throughout incubation period showed in fungal populations and respiration when B soil amended with GRS. Input of GRS to soil stimulate fungal population in the second week's incubation and respiration strongly increases later in 6 weeks, but weakly performance on bacterial population and its phosphatase activity. Decrease in bacterial population followed with declining of phosphatase activity. Wyszkowska and Kucharski (2000) describe that barley straw application in soil containing petroleum able to stimulate triticale growth and its yield as well, and increasing urease but it was not significant to acid and alkaline phosphatase activities.

Population of fungi and bacteria in soil with long-lasting healing by pesticide (A soil) fluctuated along its incubation time. Range of fungal inhabitants (0.16 to $3{,}16x10^4$ CFU/g soil) was lower then bacterial population (0.1 to $1x10^5$ CFU/g soil). Fungal CFU in this observation was higher in soil with GRS revises compared to untreated ones. The GRS treatment was only able to accelerate bacterial population six to twelve week's incubation (Figure-4). Urease activity become elevated as caused of GRS treatment to a soil.

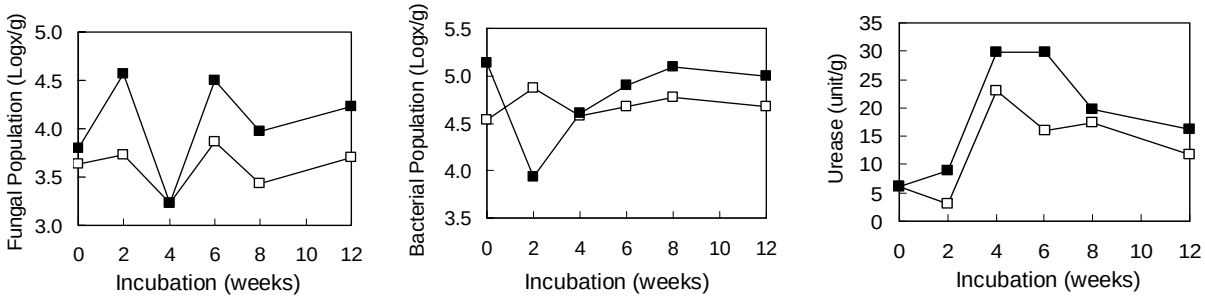

Figure-4. Representation on fungal and bacteria population (CFU/g soil) in A soil amended with GRS (■) compared to soil without GRS (□) in 12 weeks incubation, match up to urease activities.

Recognizing of soil hydrolyze enzyme such as urease may important role in soil fertility as well as to its microbial population. Nitrogen mineralization to plant absorption and as do to carbon degradation by microbes should well understand. Soil enzymes regulate ecosystem functioning and in particular play a key role in nutrient cycling. Ureolytic activity in soil is carried out by extracellular urease, which is stabilized by immobilization on organic and mineral soil colloids (Makoi and Ndakidemi, 2008). GRS as organic compound put into a soil prepared urease activity and microbial population in soil raise up above control.

CONCLUSIONS

- Phosphatase and urease activities are instability in the beginning of 2 to 4 weeks incubation along with decomposing of mixed pesticide of deltrametrin and probineb in soil, and decline to last part of 12 weeks incubation;
- Urease is strongly active after two weeks incubation in soil with long healed with pesticide (A soil) as agriculture soil, particularly in soil conditioned with organic compound of grounded rice straw; and
- Respiration rate of microbial biomass in B soil within pesticide refinement is representing with fungal population at some point in incubation period.

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