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PARAQUAT HERBICIDE IN PEAT SOIL: I. ITS EFFECTS ON THE DYNAMICS OF MICROBIAL POPULATION

HERBISIDA PARAQUAT DALAM LAHAN GAMBUT: I. PENGARUHNYA TERHADAP DINAMIKA POPULASI MIKROBIA

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INTISARI

Paraquat merupakan bahan aktif herbisida yang dipakai secara meluas dan terjadwal di lahan gambut. Senyawa ini bersifat stabil di lingkungan masam, sehingga dimungkinkan menjadi persisten dalam tanah gambut. Penelitian dilakukan untuk menelaah pengaruh paraquat terhadap dinamika populasi mikrobia di dalam tanah gambut. Paraquat ditambahkan ke dalam tanah pada konsentrasi 20 ppm. Inkubasi dilakukan selama 2 bulan dalam suhu kamar. Untuk mengetahui peran pengapuran, sebagian tanah gambut dikapur dan diinkubasikan. Penghitungan populasi bakteri, aktinomisetes dan jamur serta residu paraquat dilakukan secara periodik. Hasil pengamatan menunjukkan bahwa di dalam tanah gambut yang tidak dikapur, paraquat tidak menyebabkan perubahan secara nyata pada dinamika populasi mikrobia tertentu. Perubahan populasi bakteri terlihat nyata pada tanah gambut yang dikapur. Perlakuan pengapuran juga mengubah dinamika populasi aktinomisetes. Bila paraquat ditambahkan ke dalam tanah yang dikapur, tingkat populasi bakteri dan aktinomisetes menurun. Dalam penelitian ini juga terlihat bahwa tidak terjadi perubahan yang nyata pada dinamika populasi jamur sebagai akibat perlakuan paraquat dan/atau pengapuran. Pengapuran sampai pH sekitar 5,5 tidak menurunkan persistensi paraquat di dalam tanah gambut.

Kata kunci: paraquat, tanah gambut, populasi mikrobia

ABSTRACT

Paraquat has been used widely and periodically in peat soil. It is stable in acid environments, therefore its application in peat soil which represents an acid environment, might prolong its persistence. Liming treatment has known to reduce peat soil acidity. This research was conducted to study the effect of paraquat and liming treatments on the dynamics of microbial population in peat soil. Unlimed and limed peat soil were treated with paraquat to a final concentration of 20 ppm, and incubated for 2 months. Microbiological analysis, consisting of counting of bacterial, actinomycetes, and fungal population were done weekly. The changes of pH value and paraquat residue were also measured. The results showed that in unlimed peat soil, paraquat treatment did not influence microbial population. However, when paraquat was added into limed peat soil, the number of microbial population decreased; especially the population of bacteria. Liming treatment increased bacterial population and changed the population dynamics of actinomycetes. No significant difference of fungal population in peat soil treated with paraquat and lime. Additionally, there was no significant difference in paraquat resistance between limed and unlimed peat soil.

Key words: paraquat, peat soil, microbial population

INTRODUCTION

Minimum-tillage agricultural system in peat soil and some other rice cultured area require the use of herbicides to kill weeds. Inspite of that, the high cost and lack of agricultural labor, supported the needs of herbicides. Paraquat (1,1-dimethyl-4,4-bipyridinium) is an active agent of herbicides (Riley & Wilkinson, 1976), such as Gramoxone and Dextrone X, which were widely used in peat land and other rice cultured area for killing broad and narrow leaf weeds.

Peat soil in Kalimantan was formed in high concentration of water, low in temperature, pH value, and oxygen concentration. Inspite of that, it has low concentration of minerals but high in Al and Fe. These conditions cause soil nutritions to be unavailable for plants. Additionally, the population and activities of microorganisms in peat soil are not in optimal condition. As known widely, soil microorganisms play many important roles in agriculture and soil ecosystem. Some of them are plant disease causing organisms. Another group is important in plant protection, namely by the mechanism of antagonism, they destroy soil borne plant diseases. Many soil microorganisms are also required by plants due to their contribution in biotransformation of nutrients (Alexander, 1967). Heterotrophic organisms also important in decomposition of organic matter, in which resulted in the release of plant nutrition.

Paraquat is stable in acid environments (Anonymous, 1984), and it is bounded tightly by organic substances (Zweig, 1967). Positively charged paraquat could be adsorbed in peat soil which have high concentration of organic matter and Cation Exchange Capacity (CEC). This adsorption is responsible to paraquat inactivation and persistence in soil (Glonn *et al.*, 1982; Pasi, 1978). In these conditions, paraquat is not degraded by soil microorganisms (Katayama & Kuwatsuka, 1991). Due to its temporary effect on weed plants, paraquat is applied periodically. Periodic application of some pesticides could influence the growth of soil microorganisms (Rao, 1982). Laboratory research done by Katayama & Kuwatsuka (1991), showed that high concentration of paraquat decreased the microbial population, especially bacteria and actinomycetes. As mentioned before, microorganisms have significant contributions in soil ecosystem. Therefore, the changes of the dynamics of microbial population would influence the whole conditions in soil ecosystem.

Paraquat is not degraded by plants. However, in the availability of sun light, O_2 and water, it is transformed to free ionic radicals of H_2O_2, O_2^-, OH^-, and O_2 (Ashton & Crafts, 1981; Glonn *et al.*, 1982). These transformation products was potentially toxic, especially to aerobic microorganisms (Glonn *et al.*, 1982). However, the mechanism is complicated and still unknown (Carr *et al.*, 1986).

Liming would increase pH value and effectivity of fertilization (Radjagukguk, 1983). Additionally, liming will support the growth of soil microorganisms, including paraquat degrader, which could mineralyze paraquat in soil. Usually, mineralization of paraquat resulted in non-toxic inorganic substances (Carr *et al.*, 1985; Hata *et al.*, 1986). Therefore, hopefully it will reduce toxic effect of paraquat on soil micro-organisms other than paraquat degrader. In peat soil, liming treatment is usually done to increase pH value to around 5.5.

Although paraquat is toxic to many microorganisms, Katayama & Kuwatsuka (1991) reported that several micro-organisms tolerant to paraquat until 1000 mg/l, for examples *Escherichia coli* K-12 W3110, *Pseudomonas* sp. strain TT01 and

L. strarkeyi. The fungal of *Aspergillus niger* and *Penicillium frequentans* also still grew in soil treated with 2000 ppm of paraquat. These results represented that certain microorganisms have special mechanism to detoxify paraquat. The enzyme of superoxide dismutase (SOD) was reported neutralized the toxicity of paraquat (Carr *et al.*, 1986).

Degradation of paraquat was influenced by available oxygen and a broad range of pH value and temperature, namely 4.8–7.2 and 26–34°C, respectively (Carr *et al.*, 1985). Direct oxidative cleavage of the ring structures will form methylamine and dialdehyde succinate (Hill & Wright, 1978; WHO, 1984). These substances would be mineralized to form CO_2, NH_3 dan H_2O (Dyson, 1995). *Clostridium pasteurianum, Corynebacterium fascians* (WHO, 1984), and *Achromobacter* sp. (Hill & Wright, 1978) degraded paraquat. *Nocardia* sp., and *Streptomyces* sp. were known as paraquat degrading actinomycetes (Hill & Wright, 1978; Carr *et al.*, 1985). Inspite of that, a soil isolated yeast, *Lipomyces starkeyi* (WHO, 1984; Hata *et al.*, 1986; Katayama & Kuwatsuka, 1991) was widely studied in paraquat degradation. It does not known yet, whether these paraquat degrader were found in peat soil, due to the non-supportive conditions of this soil.

In this study, we observed the dynamics of microbial population in peat soil treated with paraquat. Due to the possible effect of low pH on paraquat persistence, the peat soil was treated with lime to reach pH value around 5.5.

MATERIALS AND METHODS

Soil sample. The saphric peat soil which was obtained from Pangkoh, Kapuas Distric, Province of Central Kalimantan. Its specific characteristics were: Water holding capacity 410,6%; Total Nitrogen 3,98%; Total Carbon 63,00%; C/N ratio 15,83; Available N (as mg NH_4^+, NO_3^-) 0,07; pH value 3.0; CEC 333 me/100 g soil. A part of the soil samples were sterilized at 110–115° C for 15 min. Sterilization was conducted three times every 24 hours allowing microbial spores to germinate.

Herbicide used. Gramoxone ® herbicide (PT ZENECA Agri Product of Indonesia under-license ZENECA Limited, U.K) with paraquat concentration of 276 g/l (± 200 g/l of paraquat ion) was used in this research. A stock solution of paraquat in sterilized distilled water was added to the soil at desired concentration.

Liming treatment. To increase pH value, a solution of $Ca(OH)_2$ was added to the peat soil at 40 ton/ha. The final pH of limed peat soil was 5.47.

Microbiological experiments. Paraquat herbicide (final concentration 20 ppm of active agent) was added to the limed and unlimed soil samples. The soil was incubated for 2 months at room temperature, and was kept at its water holding capacity during incubation time. Microbiological analysis were done periodically. The bacterial population was measured using nutrient agar with pH value around 6. Dextrose-nitrate agar was used to measure Actinomycetes population, and Bengal Rose Agar added with streptomycin sulphate at final concentration 100 ppm, was used for counting fungal population.

Analysis of paraquat residue. The residue of paraquat during incubation time was measured based on the method of Lane *et al.* (1997).

RESULTS AND DISCUSSION

Paraquat is stable in acid environments (Zweig, 1967). In the other hand, peat soil has low pH value namely around 3.0 (Figure 1). Therefore, it was suggested that paraquat is stable and persist for a longer period in peat soil. Multiple applications of paraquat will increase paraquat residue in soil environments. Fryer *et al.* (1977) insisted that paraquat persistence decreased the growth of soil microflora. In natural environments, some of soil microorganisms play an important role in pest management and the other supported soil fertility. If paraquat influence these beneficial soil microorganisms, incorrect application of paraquat may decrease soil fertility and the agricultural yields (Rao, 1982).

Liming directly increased pH value, and indirectly availability of plant nutritions and supported decomposition of organic substances in peat soil (Kuswandi, 1993). In preliminary study, several dosages of lime (10–40 ton/ha) were tried. The results showed that 40 ton/ha (42.006 g/5 kg soil), gave pH value 5.28. Within 4 days, this pH value decreased to 4.8, then stable at 4.7 until 2 weeks incubation (data not shown). Decreasing of the pH value was due to the production of organic substances, humic and fulvic acids from the decomposition of organic matter (Rajagukguk, 1983). Therefore, in this research dosage 40 ton/ha was used to increase pH value of the peat soil. Paraquat was added into the limed soil which was incubated for 4 days. The experiment was started at this time.

Figure 1 shows the effect of paraquat on the pH value of limed and unlimed peat soils. In unlimed peat soil, addition of paraquat slightly decreased pH value. For instance, fluctuation of pH without paraquat addition was in the range of 3,0–3,2; while soil added with paraquat was 2,8–3,0 (Fig. 1). Similar pattern was found in limed peat soil. These might be due to the reaction between cations of the lime and the negatively charges of paraquat.

Figure 2 shows the effects of paraquat on the growth of bacterial population in limed and unlimed peat soil. Addition of paraquat at 20 ppm did not significantly influence the dynamics of bacterial population. There was a slight increase of bacterial number at the first week incubation, but relatively stable after that time. Liming treatment significantly increased bacterial population. However, when paraquat was added to this limed soil, the enhancement of bacterial population was lower. These data reflected that principally paraquat is toxic to bacteria.

The toxic effect of paraquat was also observed in other microorganisms. Paraquat addition caused longevity of lag phase at the growth of a soil yeast *Lipomyces starkeyi* in N-source medium (Hata *et al.*, 1986). Paraquat also decreased the growth of soil *Azotobacter* which play an important role in non-symbiotic N_2 fixation in soil (Calderbank & Slade, 1976).

Change in the dynamics of bacterial population and increase of its number, especially were due to the increase of pH value in limed soil. It was widely known that bacterial growth was influenced by pH value. In general, bacterial growth decreases in acid environments, higher pH values will increase the growth of bacteria, and its optimal condition was around neutral (Kuswandi, 1993).

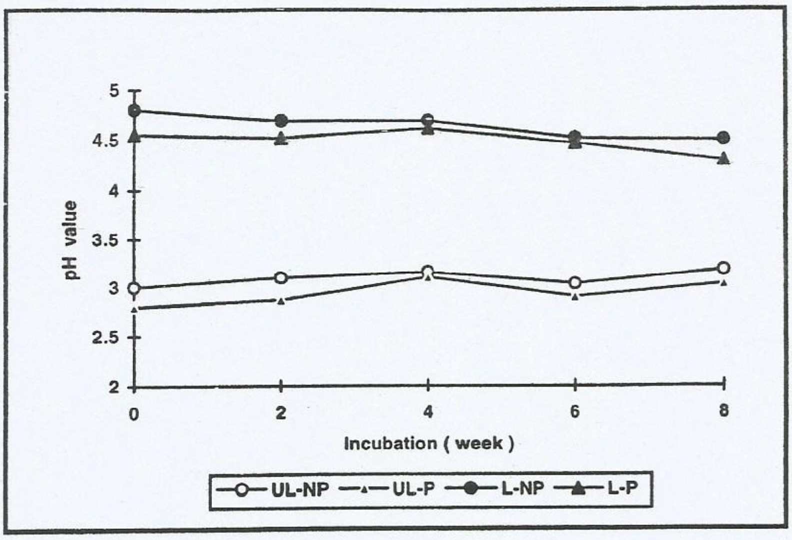

Figure 1. The value of pH in treated and untreated peat soil
Note: UL-NP = Unlimed - non paraquat;
UL-P = Unlimed - added paraquat;
L-NP = Limed - non paraquat;
L-P = Limed - added paraquat.

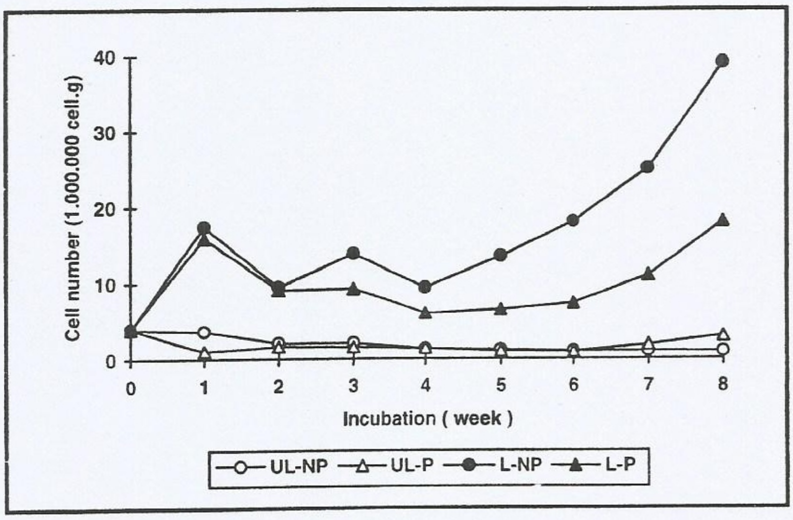

Figure 2. Dynamics of bacterial population in peat soil.
Note: UL-NP = Unlimed - non paraquat;
UL-P = Unlimed - added paraquat;
L-NP = Limed - non paraquat;
L-P = Limed - added paraquat.

Although in this research, the range of pH value of limed peat soil was still lower than neutral conditions (around 4.8); it could support the growth of bacteria and caused the increase of bacterial population. Inspite of the direct effect of pH on enzymatic activity of cell; indirectly, addition of lime also increased the availability of trace elements required by plants and microbial growth (Kuswandi, 1993). Liming also neutralized the toxicity of Al and Mn in peat soil (Rajagukguk, 1983; Anonymous, 1991). Higher pH in peat soil will increase the availability of Ca and Mg which important for many organisms. Therefore, the change of dynamics and number of bacterial population in limed peat soil, with and without paraquat addition, were detected in this study.

The influence of paraquat and/or lime treatments on actinomycetes population was different with those of bacterial population. Both treatments significantly changed the dynamics of actinomycetes population, but there was no significant difference in the maximal and minimal cell number of actinomycetes (Fig. 3). The dynamics of actinomycetes population was significantly different in limed peat soil. They grew faster in limed soil with or without paraquat. During the first 4 weeks incubation period they reached its number to 1.0–1.4 x 10^6 cell/g of soil; which reflected that liming treatment gave better conditions for the growth of actinomycetes. Addition of paraquat to limed peat soil reduced the number of cell compared with limed peat soil without paraquat addition. These data was coincided with those of Fig. 2, in which paraquat had negative effect to bacterial population. Other researches also showed these similar data (Calderbank & Slade, 1976; Hata *et al.*, 1986).

The dynamics of population growth of actinomycetes in untreated peat soil might be caused by the changes of physical and chemical characters of the soil during incubation period. Sunarminto *et al.* (2000) reported that there were changes of physical and chemical characters of peat soil during 8 weeks incubation period in a glass house. The changes of physical – chemical conditions of soil after it was taken from a natural soil bulk and incubated in the glass house might also had responsibility to these population dynamics.

In unlimed peat soil, paraquat addition did not significantly influence the dynamics of actinomycetes population. Only at the second weeks, paraquat increased population to 0.7x10^{10} cell/g, relatively higher than those in limed soil without paraquat (0.4x10^{10} cell/g). Significant increase of population in unlimed peat soil with and without paraquat were detected after the third week. However, at the 8 week incubation, its population decreased to the same level as its initial period. These data showed that liming treatment has higher effect on the dynamics of actinomycetes population. Rao (1982) insisted that the growth of actinomycetes was influenced by pH value. Actinomycetes growth rate was reduced in environments with pH value under 5.0.

In this study, addition of lime only increased pH value to 4.8; and liming treatment did not increase the total number of cell population, but changed the dynamics of population. It was suggested that change of population dynamics, especially due to the higher pH value of soil microenvironments. Inspite of the increasing of pH value, liming treatment also means addition of certain mineral required by microorganisms.

Many species of microorganisms have high tolerance to paraquat up to 1000–2000 ppm (Katayama & Kuwatsuka, 1991). In this research, addition of paraquat did not significantly influence actinomycetes population. The data showed that actinomycetes was tolerant to 20 ppm of paraquat. These phenomena gave an important informations in the relation with the role of actinomycetes in plant protection in natural soil environments. It is known that many species of actinomycetes could synthesize substances having anta-gonistics effect to other microorganisms (Alexander, 1967).

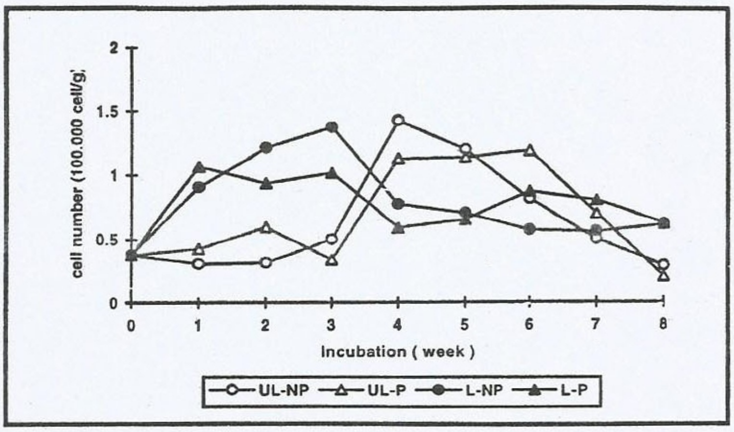

Figure 3. Dynamics of actinomycetes population in peat soil.
Note: UL-NP = Unlimed - non paraquat;
UL-P = Unlimed - added paraquat;
L-NP = Limed - non paraquat;
L-P = Limed - added paraquat.

Figure 4 showed the effects of paraquat on fungal population. The difference of the dynamics of fungal population between treatment and control was detected only around the second and third weeks. In that period, addition of paraquat into unlimed peat soil increased fungal population compared with in the control soil. Additionally, liming treatment did not significantly influenced the fungal population. After the third weeks fungal population in all treated and untreated peat soil were relatively constant at the same level.

Many soil fungi have high tolerance to herbicides, including paraquat. For instances, paraquat as high as 2,000 ppm did not inhibit the growth of *Aspergillus niger* and *Penicillium frequentans* (Katayama & Kuwatsuka, 1991). Fungal mycorrhizae in a synthetic medium has been known to be resistant to paraquat at concentration 2,000 ppm (Hill & Wright, 1978). Carr *et al.,* (1986) reported that many species of microorganisms were able to synthesize superoxide dismutase enzyme (SOD); an enzyme which neutralize toxic effect of anionic O_2^- formed from paraquat oxidation (Ashton & Crafts, 1981), There is possibility that this enzyme was synthesized by several microorganisms growing in the peat soil.

Many studies reported that O_2^- negatively influenced microorganisms especially aerobic microorganisms (Carr *et al.,* 1986). However, in this research paraquat addition did not significantly influenced the dynamics of microbial population in peat soil, especially bacteria and Actinomycetes. These might be due to the relatively low toxicity of 20 ppm of paraquat to microorganisms. Katayama & Kuwatsuka (1991) showed that many kinds of microorganisms have tolerance to paraquat as high as 1,000 mg/kg of soil. These data proved that the tolerance of microorganisms to xenobiotic substances depend on species of the microorganisms.

Inspite of that, another possible mechanism for the resistance of fungi to paraquat was due to tightly adsorbed of paraquat to soil particles, which cause inactivity of paraquat to microorganisms (Riley & Wilkinson, 1976).

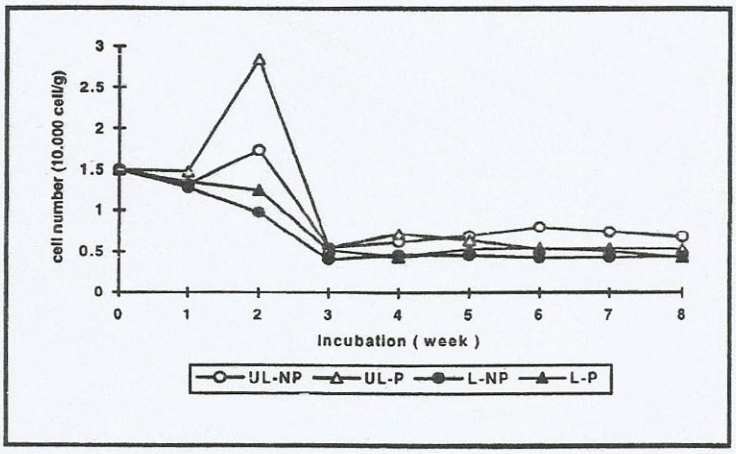

Figure 4. Dynamics of fungal population in peat soil.
Note: UL-NP = Unlimed - non paraquat;
UL-P = Unlimed - added paraquat;
L-NP = Limed - non paraquat;
L-P = Limed - added paraquat.

In this study it was shown that the change in the dynamics of microbial population in peat soil treated with paraquat and lime depended on the microbial species. Generally, within 8 weeks incubation period, paraquat had negative effects on certain microorganisms, especially in limed peat soil. During this period, paraquat residue was still detected at relatively the same concentration with its initial time (Fig. 5). No difference of paraquat degradation in sterile and nonsterile peat soil, which means that microorganisms in peat soil unable to degrade paraquat. Nonsupportive conditions in peat soil might have responsibility to the failure of soil microorganisms to degrade xenobiotics, including pesticides. Many researches reported that biodegradation of pesticides were highly influenced by environmental conditions, such as abiotic factors, available nutritions and interaction among natural microbial community in each environments (Martani & Seto, 1991; Martani, 1995).

Paraquat could be adsorbed by the soil particles and organic matters within a few time after the paraquat solution was added into the soil (Brian *et al.*, 1958; Riley & Wilkinson, 1976; Anonymous, 1984). Due to the electric charge of pesticides and the surface of soil molecules, adsorption will cause immobility of pesticides (Hill & Wright, 1978). Organic matters has an important role in adsorption of many pesticides (Hill & Wright, 1978). Peat soil contain high concentration of reactive humic and fulvic acids, such as COOH, phenolic, enolic, heterocyclic and aliphatic OH. Adsorbed paraquat by soil particles caused it to be unavailable for soil microorganisms (Carr *et al.*, 1985; Katayama & Kuwatsuka, 1991). Inspite of that, paraquat was quickly adsorbed by soil colloids, which caused its stability in soil. Therefore, peat soil microorganisms could not degrade paraquat and paraquat is still persisted in peat soil.

Paraquat was stable in low pH value environments, and unstable in alcaline solutions (Staiff *et al.*, 1981). However, in this study, addition of lime did not significantly reduce paraquat persistence in peat soil (Fig. 5). This phenomena might be due to the still relatively low pH value of limed peat soil. Final pH was around 4.8 (Fig. 1), which means that still in the acid pH range. The increase of pH value of peat soil to around neutral will influence chemical reaction and nutrition availability in the peat soil (Donahue *et al.*, 1975).

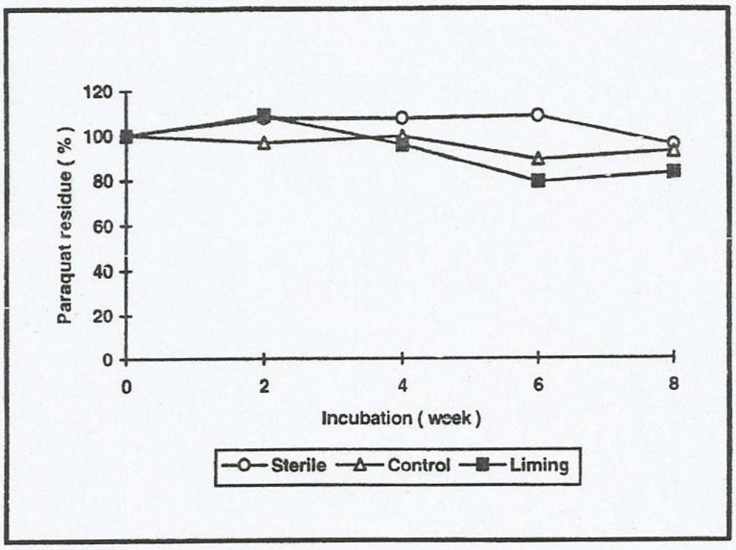

Figure 5. Degradation of paraquat in peat soil with several treatments

CONCLUSION

Based on the data obtained from this research it could be concluded that paraquat application in peat soil influenced the dynamics and the number of certain soil microorganisms, especially in limed peat soil. Liming treatment also changed the dynamics of microbial population. However, the role of these microorganisms to the growth and yield of crops were still unclear. Recently, we are still doing research concerning the influence of this herbicide on the peat soil fertilization characteristics and on the growth of several crops.

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