THE IMPACT OF PARAQUAT REPEATED APPLICATION IN PEAT SOIL ON MICROBIAL POPULATION

Erni Martani*, Bambang Hendro Sunarmin**, Laili Fitri Yeni ***, and Medhina Magdalena***

ABSTRACT


Paraquat herbicide has been applied periodically in peat land agricultural production. This research was conducted to study the fate of paraquat in peat soil after its repeated application, and its possible influence on the population of microorganisms that play an important role in soil fertility.

Paraquat (20 ppm) was sprayed periodically to three types of peat soil, i.e. fibric, hemic and sapric. They were treated with time to increase soil pH around 5.5. During incubation time of 6 months, paraquat residue in soil was analyzed periodically, and microbial population was measured. Ammonifier and nitrifier were measured using The Most Probable Number (MPN) method, and Phosphate stabilizer was counted using plate count method on a specific medium.

During repeated application, accumulation of paraquat residue in the soil increased. The accumulation level depended on soil types, namely the level was higher in undecomposed peat soil (fibric). Microbial measurement showed that microbial tolerance to paraquat was species-specific. In this case, ammonifier was not significantly affected by the increase of paraquat concentration, and nitrifier population decreased from 10^8 to 10^7 MPN/g; but phosphate stabilizer decreased from 10^8 to 10^7 CFU/g. Further experiments showed that timing treatment decreased paraquat residue. These data show that time addition supported the growth of paraquat degrader and their degradation activity. The timing also increased the population of nitrifier and phosphorus stabilizer. These data are important for minimizing the impacts of paraquat application on non-target organisms.

Key words: paraquat, repeated application, peat soil, microbial population.

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INTISARI

Herbisida dengan bahan aktif paraquat digunakan secara terjadwal di lahan gambut, namun belum pernah dilakukan kemungkinan akumulasi dan pengaruhnya terhadap organisme bukan sawaran. Penelitian ini dilakukan untuk mengatasi masalah (fase) paraquat dalam gambut setelah diaplikasikan beberapa kali dan pengaruhnya terhadap populasi mikrobia tanah yang berperan dalam kesuburan.

Tiga jenis tanah gambut, yaitu: fibrik, hemik dan saprik, digunakan dan diperlakukan dengan paraquat (koncentrasi 20 ppm) sebanyak tiga kali. Kapur ditambahkan untuk meningkatkan pH tanah sampai sekitar 5.5. Secara periodik dilakukan pengamatan residu paraquat serta populasi mikrobia yang berperan dalam kesuburan tanah. Bakteri amonifikasi dan nitrifikasi diteruk dengan metoda MPN (The Most Probable Number), sedangkan mikrobia pelarut fosfat secara Plate count.


Pengapuran juga meningkatkan populasi mikrobia yang terperang dalam kesuburan tanah. Hasil penelitian ini mempunyai arti penting dalam usaha memperbaiki kualitas lingkungan, terutama yang terkait dengan pelaksanaan pertanian yang berwawasan lingkungan.

Kata kunci: Paraquat, Aplikasi terjadwal, Tanah gambut, Populasi mikrobia.

INTRODUCTION
Weeds are the most severe and widespread biological constraint to agricultural production (Naylor, 1996). For controlling weed's growth, herbicides have been applied for years. However, misuse of these agrochemicals may influence non-target organisms (Anderson, 1978; Naylor, 1996; Roger & Simpson, 1996). Paraquat (N, N'-dimethyl bipyridilium dichloride) is an active agent of herbicides widely and repeatedly applied in peat land agricultures.

Studying the fate and accumulation of paraquat in soil after its repeated application is important, because paraquat was not degraded for six years in soil (Alexander, 1999). In peat soil, paraquat was not degraded for more than two months (Margino et al., 2000). Paraquat persistence in soil was due to its chemical stability in low pH environments (Anonymous, 1984) and other limiting factors in soil that not supported the growth of paraquat degrader (Margino et al., 2000; Martani & Soto, 1991a). In the preliminary studies concerning peat soil, Martani et al., (2002a) reported that bacterial inoculation failed to accelerate paraquat degradation, unless the peat soil extract medium was increased its pH value to 5 or 6 by addition of lime. As widely known that pH value and nutrient status in peat soil was low. Additionally, peat soil also contained heavy metals and toxic substances, such as phenolic compounds (Sahibam et al., 1997) which can influence plant growth (Tadano et al., 1992). Slow paraquat degradation in soil may cause paraquat accumulation. Therefore, repeated application of paraquat may increase concentration of its residue and influence non-target organisms.

Studies concerning effects of paraquat and its persistence in peat soil have been conducted. Paraquat changed the population dynamics of Rhizobium in culture medium (Martani, 2002b), and other microorganisms in soil (Anderson, 1978; Margino et al., 2000; Setyaningsih et al., 2001). In greenhouse experiments, application of paraquat in peat soil inhibited the growth of crops and decreased the yield (Martani et al., 2000; Martani et al., 2001a; Martani et al., 2001b). Higher paraquat concentration gave greater toxicity (Martani, 2002b).

This research was conducted to study the possibility of paraquat accumulation in peat soil and its influence on non-target microorganisms. Paraquat was applied periodically in three types of peat soils, namely fibric, hemic and sapric soils.
MATERIALS AND METHODS

Soil sample and characterization.

Three types of peat soil were used in this study, i.e. fibric, hemic and saphric peat soils. The soil was obtained from Pang kok, Kapuas District, Central Kalimantan. Some physico-chemical analyses were done to know the characteristics of each type of the soil sample.

Paraquat

In this study, paraquat was applied by using Gramoxone® (Zeneca Ltd; active agent 200 mg of paraquat ion/l).

Paraquat and Liming Treatments

The soil was put into plastic pot and treated with paraquat and/or lime. Paraquat solution was added to final concentration of 20 mg paraquat ion/g soil. Application of paraquat was repeated every two months. Lime (Ca(OH)₂) was added at 35.71 g/kg, 34.48 g/kg, and 24.69 g/kg of fibric, hemic and saphric soil, respectively to reach the pH value around 5.5. Paraquat and lime were added into the soil and mixed homogenously. During incubation for six months, the soil was kept at its field capacity.

Paraquat analyses

Paraquat residue was analyzed periodically during incubation time. Paraquat residue was measured periodically using UV-spectrophotometric method with a standard curve of paraquat and λ = 603.2 nm (Pack, 1967).

Microbial population measurement

Microbiological examinations were conducted periodically to count the number of important microorganisms in nitrogen biotransformation and phosphate solubilisation. Ammonifier and nitrifier microorganisms were counted based on The Most Probable Method (MPN) using specific media (Rao, 1994). After incubation time of a week and three weeks, the media of ammonifier and nitrifier were added with Nessler and diphenylamine sulphuric acid reagents, respectively. The existence of ammonifier and nitrifier was indicated by the formation of brownish-yellow and blue colour of each medium (Rao, 1994).

Phosphate solubilizing bacteria was counted using plate count method in Picovaskaya medium (Rao, 1994). Colony of phosphate solubilizer was characterized by formation of clear zone around the colony.

RESULTS AND DISCUSSION

Soil Analyses

Three types of peat soil were used in this study, i.e. saphric, hemic and fibric peat soil. Fibric is undecomposed peat soil, and saphric is the most advanced decomposed peat soil (mature peat), while the organic decomposition level in hemic is between fibric and saphric. Due to decomposition level, peat soil has some differences in physical and chemical characters (Siradz, 1991). Therefore, before soil treatment, all types of the soil were analyzed for the important soil characteristics.

Table 1 shows that there are some differences in physical and chemical characters among the peat soils. Field capacity was higher in decomposed soil (saphric), but level of porosity was lower than that of other peat soils. This means that there was decomposition of organic substances of plant residues in saphric. The low pH in saphric (3.68) might be caused by

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fibric</th>
<th>Hemic</th>
<th>Saphric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field capacity (%)</td>
<td>270.95</td>
<td>383.6</td>
<td>440.80</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>47.42</td>
<td>45.39</td>
<td>27.03</td>
</tr>
<tr>
<td>pH₂O₉₀</td>
<td>4.02</td>
<td>3.69</td>
<td>3.48</td>
</tr>
<tr>
<td>Total Organic concentration (%)</td>
<td>89.00</td>
<td>87.66</td>
<td>81.82</td>
</tr>
<tr>
<td>Carbon organic concentration (%)</td>
<td>68.53</td>
<td>67.49</td>
<td>63.00</td>
</tr>
<tr>
<td>Total Nitrogen (%)</td>
<td>1.99</td>
<td>2.00</td>
<td>3.98</td>
</tr>
<tr>
<td>Available Nitrogen (%)</td>
<td>0.0361</td>
<td>0.012</td>
<td>0.0072</td>
</tr>
<tr>
<td>C-N ratio</td>
<td>34.44</td>
<td>33.74</td>
<td>15.83</td>
</tr>
<tr>
<td>Ash concentration (%)</td>
<td>11.13</td>
<td>12.79</td>
<td>18.26</td>
</tr>
<tr>
<td>Humic (%)</td>
<td>56.29</td>
<td>50.39</td>
<td>48.88</td>
</tr>
<tr>
<td>Fulvic (%)</td>
<td>17.09</td>
<td>21.17</td>
<td>32.00</td>
</tr>
</tbody>
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synthesis of organic acids from organic decomposition. Sabithum et al. (1997) reported that benzoic acids and phenolic substances in decomposed peat soil were higher than in undecomposed one.

Decreasing of total organic and C-organic concentration, and the increasing of ash concentration (Table 1), show that organic substances in saphric were decomposed more advance than other soils. The advanced decomposition was shown also by the higher levels of humic and fulvic substances in saphric than fibric or hemic.

Difference in physico-chemical characteristics of soil may influence the fate of pesticides. Paraquat and other positively charged pesticides would be adsorbed on clay minerals and organic substances of soil (Riley & Wilkinson, 1970; Dyson, 1995). Adsorption of paraquat on clay minerals was stronger than those on organic substances. The adsorbed paraquat is non-bio-available and could not be degraded by soil microorganisms, or sometimes was degraded very slowly (Carr et al., 1985; Katayama & Kuwatsuka, 1992). This means that paraquat may persist in soil for a long period.

**Paraquat Residue**

The residue of paraquat was analyzed periodically. The results are shown in Table 2.

**Table 2. Effect of liming on paraquat residue after its repeated application**

<table>
<thead>
<tr>
<th>Paraquat Application</th>
<th>Paraquat residue in Fibric (ppm)</th>
<th>Paraquat residue in Humic (ppm)</th>
<th>Paraquat residue in Saphric (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlimed</td>
<td>Limed</td>
<td>Unlimed</td>
</tr>
<tr>
<td>First</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24.1</td>
<td>24.1</td>
<td>27.7</td>
</tr>
<tr>
<td>4</td>
<td>20.6</td>
<td>17.9</td>
<td>23.1</td>
</tr>
<tr>
<td>8</td>
<td>21.7</td>
<td>19.9</td>
<td>20.1</td>
</tr>
<tr>
<td>8</td>
<td>43.7</td>
<td>41.9</td>
<td>58.7</td>
</tr>
<tr>
<td>Second</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>43.0</td>
<td>40.8</td>
<td>60.5</td>
</tr>
<tr>
<td>16</td>
<td>43.6</td>
<td>41.9</td>
<td>61.4</td>
</tr>
<tr>
<td>Third</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>44.0</td>
<td>41.9</td>
<td>61.4</td>
</tr>
<tr>
<td>10</td>
<td>39.4</td>
<td>39.4</td>
<td>63.8</td>
</tr>
<tr>
<td>20</td>
<td>39.9</td>
<td>38.4</td>
<td>61.5</td>
</tr>
</tbody>
</table>

Almost no paraquat degradation, or slow degradation was detected in all types of peat within eight weeks (Table 2). Therefore, the addition of paraquat at the second (week eight) and third time (week sixteen) increased paraquat residue in the following incubation time. Margino et al. (2000) reported that paraquat in peat soil was not degraded within two months. Another report showed that six years were needed for paraquat degradation in soil (Alexander, 1999). Dyson (1995) reported that adsorbed paraquat residue in soil will be accumulate after more than ten years of periodical application. However, the accumulation depends on the type of soil.

There was a tendency that paraquat accumulation was higher in decomposed peat (saphric) than in undecomposed peat soil (fibric). These results were supported by Martani et al. (2002). Paraquat degradation by bacterial isolates was faster in fibric soil extract medium than in saphric extract medium.

Paraquat was adsorbed on clay particles or organic substances. In peat soil, paraquat was adsorbed especially by functional groups of organic substances. As shown in Table 1, organic concentration was around 70%, and Sabithum et al. (1997) reported that phenolic and benzoic acids in decomposed peat was higher than those in undecomposed soil. Katayama and Kuwatsuka (1992) insisted that adsorbed paraquat was inactive and could not be degraded by soil microorganisms.

Inspite of the soil type, xenobiotic degradation in environments was influenced by ecological factors, such as pH value and available nutrients (Alexander, 1999; Martani and Seto, 1991b; Martani et al., 2002b). Therefore, biodegradation of xenobiotics could be accelerated by optimization of environmental conditions. Martani (2002) reported that paraquat degradation in peat soil extract medium by bacterial isolates was detected when the pH of medium was enhanced from 3.5 to around 5 or 6. As shown in Table 1, pH value of the peat soil was around 3.7 to 4.0. These low pH values were responsible to the slow degradation of paraquat.

The effect of liming on paraquat accumulation was shown in Table 2. Accumulation of paraquat residue was higher in control.
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(unlimed) than in limed treated soil. This means that paraquat was not degraded in control soil, but there was an acceleration of paraquat degradation in limed peat soil. Although not significant, these results showed that addition of lime increased paraquat degradation. The low pH value of peat soil is a limiting factor in paraquat degradation (Martani et al., 2002b). From the soil chemical few point, the lime (CaCO₃) will be adsorbed by the negatively charged soil particles, which will cause the release of paraquat (desorption). These desorbed paraquat were in free condition to be degraded by paraquat degrader. Therefore, paraquat degradation in limed peat soil was faster then those in unlimed soil (Table 2).

Another possible mechanism is that addition of CaCO₃ caused better environmental condition for paraquat degrader by the increase of pH value and nutritional addition. In turn it will support the growth and their activity to degrade paraquat.

The positive effect of liming treatment in paraquat degradation is important for minimizing the impact of paraquat on non-target organisms. In greenhouse experiments, paraquat inhibited plant growth and reduced yield; and liming treatment reduced the impact of paraquat on the crops (Martani et al., 2000; 2001; 2002a).

**Microbial Measurements**

Although some researches reported that pesticides, including paraquat influenced the growth of microbial community (Anderson, 1978; Margino et al., 2000). Biedeback et al. (1992) showed that there is no significant effect of paraquat to soil and plants. These discrepancies were due to the differences in soil type. The same pesticides may give different effects to microorganisms and other non-target organisms in different type of soil. Physical and chemical characteristics of soil is suggested to have responsibility for these different phenomena.

Figure 1 to Figure 3 show the change of some microbial population in peat soil.

The figures show that the effect of paraquat on microorganisms was species-specific. Figure 1 shows that ammonifier were tolerant to paraquat, although paraquat was applied repeatedly and its concentration was accumulated to more than 50 ppm (Table 2). These phenomena were observed both in fibric and sapric peat soils.

Ammonification is catalyzed by heterotrophic bacteria, fungi and actinomycetes (Alexander, 1999); which has different tolerance to paraquat (Margino et al., 2000). Due to the heterogenous microbial species of ammonifier that has different tolerance to paraquat, ammonifier did not show significant effect to this herbicide (Figure 1).

Stolp (1981) insisted that ammonification is done well in environments with pH value around 7, and optimal at 8 - 9. Liming enhanced the soil pH from 3.7 or 4.0 (Table 1) to around 5.5 to 6.0. Although it was not reach the optimal pH for ammonification, there was slightly increase of ammonifier population in fibric and sapric peat soils treated with lime (Figure 1). It was not only due to the increase of pH, but also the Calcium added as lime (CaCO₃) could be used also as additional microbial nutrition. The heterogenous microbial ammonifier (Alexander, 1999) also has different optimal pH value. Many bacteria and actinomycetes grew well at neutral or alkaline condition, but fungal growth optimal at low pH condition. Therefore, the number of ammonifier detected in unlimed and limed peat soils may be accounted.
by different microbial species. Namely, bacteria and actinomycetes will dominate in relatively high pH, and fungi will dominate in low pH value. These phenomena may caused liming treatment did not significantly increased the ammonifier population.

Different data were observed in case of nitrifying bacteria (Figure 2). In control soil (FC and SC), during six months incubation period nitrifying population decreased slightly from $10^3$ to $10^5$ MPN/g soil (Figure 2). These decrease might be due to the limiting available nutrient in the soil after it was incubated for six months. However, the decrease of nitrifier in parquat treated soil (FQ and SQ), was greater than control (FC and SC), namely from $10^3$ to $10^1$ MPN/g soil. These phenomena might be due to both limiting nutrient and increasing concentration of parquat residue to more than 50 ppm (Table 2). Biederbeck et al. (1992) was shown that routine parquat application in dark brown soil within years did not influence the population of ammonifier and nitrifier in a dark brown soil. This different data should be caused by different type of soil.

![Figure 2](image)

**Figure 2.** Influence of liming and repeated application of parquat on the population of nitrifier bacteria in fibrich (A) and saphric (B) peat soil

Notes: FC = Fibrich control; FQ = Fibrich + parquat; FLQ = Fibrich + lime + parquat

The addition of lime to increase soil pH to around 5.5 enhanced the population level of nitrifying bacteria until $10^6$ or $10^7$ MPN/g in both peat soils (Figure 2). This stand to reason as nitrifying bacteria, such as Nitrosobacter, Nitrosomonas, and Nitrobacter, grow well and active optimally in pH value around neutral or slightly alkaline condition (Stolp, 1988). Additionally, CaCO$_3$ that was added to increase pH value, could be used also as their nutrition. Figure 2 also shows that population of nitrifying bacteria changed during incubation time, from $10^4$ or $10^5$ decreased to $10^6$ MPN/g. This decrease was due to the limited nutrients in soil, and the higher parquat residue at the end of incubation (Table 2).

Figure 3 shows that population of phosphate-solubilizing microorganisms in the control pot (FC and SC) decreased from $10^4$ to $10^3$ CFU/g soil. These phenomena were similar to both ammonifier (Figure 1) and nitrifier (Figure 2), which caused by nutrient limitation. The addition of parquat (FQ and SQ) caused faster decrease in population level of $PO_4$-sulabili-

![Figure 3](image)

**Figure 3.** Influence of liming and repeated application of parquat on the population of phosphate solubilizing bacteria in fibrich (A) and saphric (B) peat soil

Notes: FC = Fibrich control; FQ = Fibrich + parquat; FLQ = Fibrich + lime + parquat
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from 10^5 to 10^6 and to 10^5 CFU/g soil, in sapric and fibrec peat soil respectively. Liming treatment (FLQ and SLQ) supported the growth of phosphate solubilizer and caused the increase of their population density than other treatments (Figure 3). However, within six months the population decreased from 10^6 to 10^5 CFU/g soil. These results were coincided with the report of Suyatiningsih et al. (2001). These results reflected that phosphate-solubilizing microorganisms were more sensitive to paraquat than those of ammonifier and nitrifier.

Bacterial species from the genera of Alcaligenes, Bacillus, Escherichia, Flavobacterium, Pseudomonas and Thiobacillus; and the fungi of Aspergillus, Fusarium, and Penicillium, were able to solubilize phosphate (Rao, 1994). Those bacteria grow optimally at pH value ranged from neutral to alkaline; and the fungi will grow well in pH value around 4.5–5.5. The pH value of limed–treated peat soil was around 5.5. This value would be better for the growth of both phosphate-solubilizing bacteria and fungi (Figure 3).

Positive effect of liming on microbial population means the addition of lime enhanced microbial population especially of nitrifier and phosphate solubilizer although the soil was added with paraquat (Figure 2 and Figure 3). At the end of incubation time, in which the pH value was around 4 (data not shown), the population decreased to a level around the same as the level before incubation. These phenomena showed that the increase of population during incubation was due to the increase of pH and nutritional conditions.

The effect of paraquat on microorganisms is species-specific. For example, paraquat degrader should be tolerant to paraquat, at least until certain concentration. The tolerance of microorganisms to this herbicide depends on their ability to neutralize paraquat toxicity. Carr et al. (1985) and Katayama and Kuwatsuka (1992) reported that the enzymes of superoxide-dismutase and catalase neutralize paraquat toxicity. The ability to synthesize these enzymes was depended on genetic characteristics of each species.

CONCLUSIONS
1. Paraquat repeated application caused paraquat accumula-

tion in peat soil, and the accumulation level was depended on the type of peat soil. Namely, accumulation was higher in decomposed than those in undecomposed peat soil.
2. Liming slightly decreased paraquat accumulation, but this decrease was not able to reduce the effect of paraquat on microbial population significantly.
3. The influence of paraquat on microbial populations is species-specific. Phosphate solubilizer was the most affected by paraquat than nitrifier and ammonifier. In general higher paraquat concentration caused higher toxicity to microorganisms.

REFERENCES