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The combination technique of bioaugmentation and phytoremediation on the degradation of paraquat in contaminated soil

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Abstract

Paraquat is a common herbicide that is wildly used in agricultural areas thanks to its rapid action and applications to a wide range of plants. However, overuse and long–term use of paraquat can lead to the problem of paraquat contamination in agricultural areas and environment. A study on the effect of bacterial consortium, isolated from rhizosphere soil collected from the field on which paraquat was used. The experimental design was divided into 3 treatments: natural attenuation (NA), bioaugmentation (BA), and control group (Control). Soil samples with an initial concentration of paraquat of 80 mg/kg were analyzed for paraquat residue by spectrophotometer after each treatment. After 60 days, the degradation efficiency in BA and NA treatments were 76% and 44%, respectively. Having half-life of paraquat less than 20 days for BA suggested that an important role of isolated bacterial consortium on paraquat degradation. Isolated bacterial consortium together with phytoremediation was introduced to contaminated soil in order to increase the degradation efficiency for 45 days. Among the 5 plants tested, African sesbania showed the highest paraquat degradation efficiency (97.14%), followed by jack bean and pinto peanut (96.43% and 95.71%, respectively) (p<0.05). Higher degradation efficiency after combination of microbial consortium and phytoremediation can be attributed to a certain extent of medication of the soil by plants that could enhance microbial activities, such as rooting, which allowed for more oxygen, water, and nutrients to penetrate to the lower level of the soil, resulting in the appropriate conditions for bacteria to degrade the paraquat.

Keywords: Pesticide, Bioremediation, Biodegradation, Rhizosphere remediation

1. Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is a non-selective contact herbicide in the group of quaternary nitrogen compounds. It is a post-emergence herbicide that has the ability to disrupt the photosynthesis of the leaves when sprayed onto the green plant tissue [1]. Paraquat is widely used in agricultural areas because it gives a rapid result, is convenient to use, and has a low cost [2]. Based on the import statistics of pesticides in Thailand, paraquat is in the top ten of imported substances. In 2019, paraquat held the fifth place in the ranking with a total amount of 9,943,932.80 kg [3]. The increase in use of paraquat causes dispersion and accumulation into the environment, which has both direct and indirect effects on living beings and human life. Pesticide residues may cause harm to living organisms due to their highly toxic effect on the nervous system, respiratory system, and reproductive system. Due to its high water-solubility (700 mg/L at 20 °C) and high organic matter affinity (K_{oc} = 15-106), paraquat can be easily spread and readily adsorbed to the organic matters in the environment. The fraction of adsorbed paraquat could be leached toward run-off areas and consequently cause contamination to the aquatic environment. Since there have been reports that paraquat is extremely biologically active and toxic to plants and

animals and ecotoxic to the aquatic environment, the remediation of paraquat from a contaminated environment is needed.

Bioremediation is a pollutants elimination technique using activities of living organisms such as microorganisms or plants. This technique has been used for the treatment of environmental contaminants as it is more cost-effective, easier to maintain and more effective with low contaminant concentrations, in comparison to other chemical and physical methods [4]. Bioaugmentation is one bioremediation technique where microorganisms are used to improve the biodegradative capacities of contaminated sites in order to accelerate the removal of undesired compounds [5]. Research has reported that bioaugmentation is the most advantageous method for cleaning up soil contaminated with pesticides. Önneby [6] found that bioaugmentation with *Sphingobium* sp. T51 has significantly enhanced the degradation of herbicide (4-chloro-2-methylphenoxyacetic acid) compared with uninoculated soil. Plangklang [7] found that bioaugmentation of carbofuran in contaminated soil by isolated degraders could enhance the degradation of carbofuran. The addition of *Burkholderia cepacia* PCL3 could reduce the half-life of carbofuran in paddy soil from 58 to 12 days. Obuotor et al. [8] reported that paraquat dichloride was degraded by 70% when adding fermented corn steep, the source of paraquat degrading microorganism, in soil contaminated with paraquat dichloride (25 mg/kg) while the control sample degraded by 30.1%. In the actual environment, biodegradation of pesticides, which is complex substances, by the microbial consortium has a higher possibility of success in biodegradation than that of a single strain because microbial consortia were combined interactions of diverse species and performing of complicated enzymatic catalysis. For this reason, microbial consortia are flexible and adaptive capabilities to encounter complex environmental stresses. Similarly, the report by Perruchon et al. [9], found bacterial consortium capacity biodegradation for neonicotinoid insecticides in an unstable environment.

Phytoremediation, which uses plants to remove, immobilize, or decompose pollutants in the environment, is another bioremediation strategy. It is an *in situ*, cost-effective, easy to adopt and eco-friendly approach for environmental remediation that has entered an inactive developmental phase [10]. For example, Yoshitomi and Shann [11] reported that an increase in pyrene mineralization was obtained from the addition of corn root exudates. Although bioremediation is effective in degrading contaminant compounds in laboratory-scale studies, its effectiveness often decreases when applied under environmental conditions. Bioaugmentation has limitations such as rapid decrease of bacterial viability and abundance after inoculation, as well as limited dispersal of the inoculated bacteria in the soil matrix. Phytoremediation also has its disadvantages such as a very slow and seasonally effective treatment method. Therefore, the combination of bioaugmentation and phytoremediation techniques may decrease the limitations of each technique. The rhizosphere was considered as a treatment zone for contaminant degradation plants [12]. The enhanced rate of biodegradation in the rhizosphere could be attributed to co-metabolism and/or the larger microbial populations stimulated by root exudates, root turnover, and improved soil moisture, oxygen, and nutrient conditions. Roots also sorb pesticides onto their surfaces, and dead roots add organic matter to soil, which can enhance the sorption of pesticides onto soil humic matter where microbial transformation may occur. From the past research, it has been found that there is little research on bioremediation by combination bioaugmentation and phytoremediation technique in Thailand. Although previous reports showed that the combination of both techniques has good effectiveness on paraquat degradation, there are limitations in the variety of plants used in remediation. These reports also focused more on plant growth and drought tolerance-promoting bacterium than remediation treatment. Therefore, this research aims to focus on increasing the efficiency of bioremediation on paraquat by combining bioaugmentation and phytoremediation techniques that using more diversity of plants. After the research has been completed, an effective method for treating paraquat in soil would be found and can be a guideline for the treatment of contaminated pollutants in the environment, especially the treatment of soil contamination with pesticides by use of biological methods. Moreover, the research team aims to create a quick and effective treatment process for the degradation of environmental pollutants.

2. Materials and methods

2.1 Soil sampling

Soil samples to be used in soil microcosm and soil pots experiments were collected from the field having a history of paraquat application in Muang district, Kalasin province. Soil samples were collected at a depth of 0-15 cm from the surface level and sifted through a sieve of 2 mm to remove wood, stone, and grass. It was dried at room temperature until the moisture content was less than 10%. It was kept at 4 °C until it was used to maintain the activity of microorganisms. The characteristics and compositions of the soil i.e., soil texture, organic matter content, and soil pH were analyzed. In the control treatment, the soil sample was sterilized by autoclave at 121 °C 15 lb/in^2 for 15 min. The autoclave procedure was repeated three times with a 24 h incubation period at room temperature in between [13].

2.2 Isolation of paraquat degrading bacterial consortium microbial community analysis

Bacterial consortiums were isolated from the rhizosphere soil which was collected from the field that had a history of paraquat application. The enrichment technique was applied to increase the number of bacterial consortiums. Ten grams of rhizosphere soil were infused in mineral salt medium supplemented with paraquat as a nitrogen source. The sample was cultured in 250 mL Erlenmeyer flask at 30 °C and 150 rpm. Sub-culturing was conducted every 7 days in order to obtain a bacterial consortium capable of paraquat degradation. The paraquat degradation efficiency was examined at the end of incubation for each sub-culture (7 days). The sub-culture was repeated until the paraquat degradation efficiency was higher than 50%. The obtained bacterial consortiums were then used in further experiments. The microbial community in the bacterial consortium was examined by Polymerase Chain Reaction Denaturing Gradient Gel Electrophoresis (PCR-DGGE) technique. The 16s ribosomal DNA was amplified as previously described [14]. Moreover, the 16s recombinant DNA (rDNA) sequences were determined using standard nucleotide sequencing. The DNA sequencing data were analyzed using The Basic Local Alignment Search Tool (BLAST) program in the National Center for Biotechnology Information (NCBI) database.

2.3 Bioremediation of paraquat in soil microcosm

The bioremediation of paraquat in soil with natural attenuation (NA) and bioaugmentation (BA) were investigated in soil microcosms. Fifty grams of soil were placed in 240 mL glass bottles and mixed with paraquat with an initial concentration of 80 mg/kg soil. Sterile distilled water was used to adjust the moisture content of the soil to about 20%. In the process of BA, the isolated bacterial consortium was added to the soil with a concentration of 100 g/kg soil. The components in the glass bottle were mixed and the bottles were covered by the prior and incubated at room temperature without light. The moisture content of the soil was examined every 3 days by comparing the bottle weight at day 0 and adjusted to 20% when moisture content was lower than 10% by the addition of sterile distilled water. Soil samples were sacrificed at days 0, 3, 7, 14, 21, 28, 35, and 60 to extract the remaining paraquat in the soil using solid-liquid extraction method and to analyze the concentration of paraquat by UV Spectrophotometer. The autoclaved soil mixed with 80 mg/kg of paraquat was conducted as a control treatment.

2.4 Combination of bioremediation technique on paraquat degradation in soil pots

The bioremediation of paraquat in controlled soil (soil without plant and microbial consortium, bioaugmentation (soil with microbial consortium), phytoremediation (PR), and combined bioaugmentation and phytoremediation (BAP) were investigated in plastic pots. Phytoremediation of paraquat in soil was further investigated using five plants i.e., sunflowers (*Helianthus annuus*), marigold (*Tagetes erecta* L), pinto peanut (*Arachis pintoi* Krapov. & W.C. Greg.), jack bean (*Canavalia ensifomis* (L.) DC.) and African sesbania (*Sesbania rostrata* Brem and Oberm.). These plants are characterized by rapid growth, easy to grow, and easy to maintain with a short life cycle. In addition, a variety of plants and being indigenous plants are also criteria to select plants for this study. This research, therefore, selected plants that are flowering plants and legumes which are not commonly used as food, to reduce the problem of contamination of pollutants entering the food chain. In addition, the combined bioaugmentation and phytoremediation treatments (BAP) were also investigated for the paraquat removal from soil. The seeds of 5 plant types were soaked in distilled water for 3 h and then cultivate in soil in a seeding tray. These samples were nursed at room temperature for 10 days under natural light and were given water daily to maintain constant soil moisture concentration. The BAP experiment was assigned to 12 treatments and 3 replicates by Completely Randomized Design (CRD). In the phytoremediation treatment (PR), two kg of soil mixed with 80 mg/kg of paraquat was added into an 8-inch diameter pot before planting with each of the plant saplings. For the BAP treatments, the bacterial consortium was added to the soil with a concentration of 100 g/kg soil before planting. The pots were given water daily to control soil moisture concentration at about 60% [15]. Then the samples were sacrificed at days 0, 5, 10, 15, 25, 35, and 45 to extract the remaining paraquat in the soil using the solid-liquid extraction method and analyze the concentration of paraquat by UV spectrophotometer. Natural attenuation of soil mixed with 80 mg/kg of paraquat was conducted as a control.

2.5 Extraction and analysis of paraquat

The extraction of paraquat from soil samples was conducted with the modified method from Wong et al. [16]. Fifty grams of the dry weight of soil and 100 mL methanol were put into 300 mL cover glass bottle for extraction at 150 rpm on the horizontal shaker for 4 h. This extraction procedure was repeated 3 times. The extracted solution was collected, pooled together, and filtered through Whatman No. 42 filter paper. The extracted solution was subjected to methanol evaporation in a hot air oven at 80 °C for 24 h. The dried extract was dissolved with 1.0

mL deionized water and mixed with 3 mL of DI water and 1 mL of 1% $Na_2S_2O_4$ in 0.1 M NaOH solution. The supernatant was then analyzed for the concentration of paraquat using UV Spectrophotometer UV-1800 SHIMADZU (Japan) at the absorbance of 600 nm [17]. The recovery percentage in analysis of paraquat concentration by UV Spectrophotometer was 93%. Paraquat concentration was calculated to compare with the standard curve of paraquat concentration. The efficiency of paraquat extraction method was not less than 90%.

2.6 Statistical analysis

The data were statistically analyzed by using the variance analysis (ANOVA) with Duncan's New Multiple Range Test (DMRT). The Statistical Product and Service Solutions (SPSS) 10.0 program was used for statistical analysis. $p<0.05$ was measured statistically significantly.

3. Results and discussion

3.1 Soil properties

The physical properties of soil samples were determined and tabulated in Table 1. The results indicated that soil texture is loamy sand, and the color of the soil is brown. The pH is 8.1 and a low organic matter content concentration of 0.82% was observed in the soil. Initial paraquat residues in soil samples were under the detectable limit (< 0.1 mg/L).

Table 1 Properties of soil used in experiments.

Soil physicochemical properties	Values
Organic matter content (%)	0.82
Soil texture	Loamy sand
Sand (%)	80.91
Silt (%)	12.91
Clay (%)	6.19
pH	8.10

3.2 Isolation and identification of paraquat degrading bacterial consortium

The community structure of a bacterial consortium capable of degrading paraquat was analyzed by PCR-DGGE. The isolated consortium exhibited a greater than 92% identity to the sequences deposited in the databases which were closely matched to 6 genera i.e., *Sphingomicrobium marinum* (97%), *Ferrovibrio xuzhouensis* (93%), *Azospirillum lipoferum* (93%), *Altererythrobacter xinjiangensis* (94%) *Xanthobacter autotrophicus* (92%) and *Azospirillum amazonense* (99%). The report of the researchers found microorganisms i.e., *Sphingomicrobium* sp. [18], *F. xuzhouensis* [19], *A. lipoferum* [20], *X. autotrophicus* [21] capable of degrading the recalcitrant natural, anthropogenic compounds and different herbicides and pesticides.

3.3 Effect of bioaugmentation on paraquat degradation in soil

This research studied the addition of bacterial consortium on the efficiency of degradation of paraquat in soil. Figure 1 shows the breakthrough curve of C/C_0 versus time. Table 2 illustrates the paraquat removal efficiency with various bioremediation techniques. Paraquat removal in the control sample (with autoclaved soil) was only 9%, while it was 44% in the NA treatment. Results indicated that the indigenous microorganisms have the capability to degrade paraquat which can be the result of microbial adaptation to paraquat application in the field. BA treatment resulted in a rapid decrease in the concentration of paraquat in soil. BA was the most efficient treatment giving 76% of paraquat removal on day 60 of the experiment (Table 2). The half-life of the paraquat in treatments with BA was shorter than 20 days. This study indicated that the addition of bacterial consortium resulted in better efficiency of paraquat degradation. In addition, the efficiency of paraquat degradation increases with the addition of nutrients in combination with addition of bacteria. These results were in line with the study of Dams et al. [22] who found that the bioaugmentation of *Sphingomonas chlorophenolica* ATCC 39723 into Pentachlorophenol (PCP) contaminated soil accelerated the complete decomposition of contaminants. Elväng et al [23] reported that complete degradation of 4-chlorophenol (4CP) contaminated soils occurred when *Arthrobacter chlorophenolicus* A6 was added. In addition, the maximum number of chlorobenzene removal from soil of 80% could be achieved with the addition of *Burkholderia* sp. strain PS14 [24].

Table 2 Effect of biological treatment process on the efficiency of paraquat degradation in contaminated soil.

Treatment	k (day^{-1})	$t_{1/2}$ (days)	r^2	Paraquat removal at day 60 (%)[a]
Natural attenuation (NA)	0.011	63.0	0.89	44.12±5.09[b]
Bioaugmentation (BA)	0.042	16.5	0.86	76.47±1.27[a]
Control	0.001	495	0.80	8.82±2.21[d]

[a] Comparison between treatments in columns is significantly different (Duncan, $p \leq 0.05$) if marked with different lowercase letters.

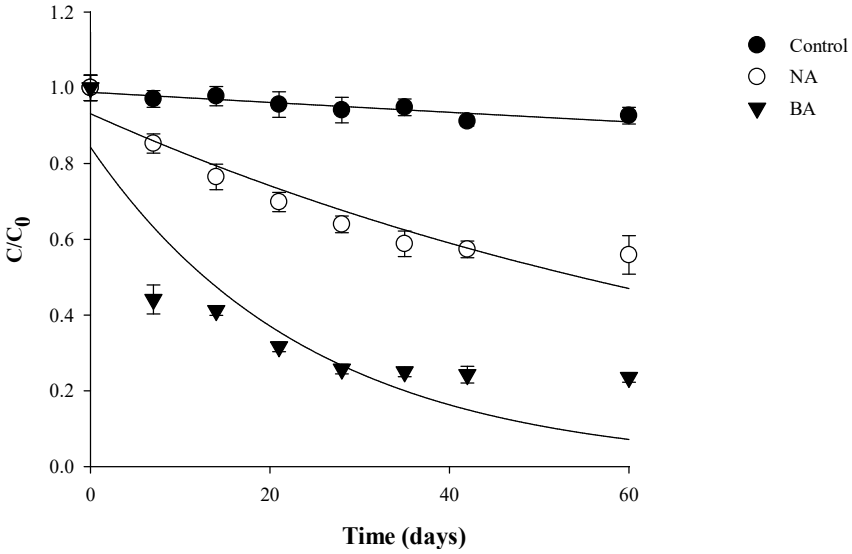

Figure 1 The breakthrough curve for varying bioremediation methods at NA: natural attenuation, BA: bioaugmentation, control: autoclaved soil without any treatment, and initial paraquat concentration of 80 mg/kg.

3.4 Effect of bioaugmentation and phytoremediation on degradation of paraquat in contaminated soil

The efficiencies of paraquat removal from soil with PR and BAP treatments were investigated in this study. The percentage of paraquat removal was only 46% in the control treatment (soil without planting and without isolated consortium) while 51.43% of paraquat removal was observed in the treatment with the addition of an isolated consortium (bioaugmentation). Among the plants used for bioremediation treatments, African sesbania, jack bean, and pinto peanut had a high efficiency of paraquat remediation with removal percentages (in PR treatments) greater than 64%. BAP treatments gave the higher paraquat degradation efficiency with removal percentages greater than 75% as compared to the PR treatments (removal percentages in the range of 64-89%). The BAP treatment with African sesbania showed the highest paraquat removal rate of 97.14%, followed by BAP treatment with jack bean (96.43%) and BAP treatment with pinto peanut (95.71%). However, the result found that BAP treatment with African sesbania, jack bean, and pinto peanut on the percentage of paraquat removal was not significantly different. On the contrary, the percentage of paraquat removal between PR and BAP treatment of pinto peanut was higher than other treatments. This indicated that pinto peanut is a plant that causes better rhizosphere augmentation when compared with those of four plants. In other words, this plant provides rhizodeposits to drive force in the stimulation of activity on plant roots, which perform better performance than other plants.

The higher percentages obtained from BAP treatment with African sesbania, jack bean, and pinto peanut as compared to BA treatments indicated the improvement of paraquat degradation in soil by these three plants. The results were in accordance with the study of Chouychai et al. [25] which found that growing corn resulted in 80-90% degradation of phenanthrene in soils and 60-70% of non-cultivated soils within 60 days. Somtrakoon et al. [26] found that the anthracene content in the soil surrounding the roots decreased by 90% and 45% when growing sweet corn and long beans. It was also found that planting mustard plants could decrease PAH content from 2,203.4 mg/kg to 1,370.4 mg/kg [27] while the planting of oats resulted in a 68% reduction in the amount of pyrene in soil within 32 days [28]. A study of weed to be used in fluoranthene phytoremediation by Chouychai and Somtrakoon [29] found that *Cyperus rotundus* could decrease fluoranthene content in soil about 41.8% more than non-planted soil (37.4%) after 30 days. The mechanism by which plants treat most soil contaminants is to stimulate degradation by microorganisms in rhizosphere soil.

When considering the comparison between phytoremediation treatment (PR) and the combined bioaugmentation and phytoremediation treatments (BAP), BAP treatment was found to have a higher efficiency than the PR treatment. In which, the differences between the percentages of paraquat removal from contaminated soil of BAP treatment and PR treatment were statistically significant. Moreover, the addition of *Sphingomonas* which is a bacterium isolated from rhizosphere soil is contaminated with hexachlorocyclohexane together with crops plantation. This can reduce the amount of linden that is contaminated in soil by 30% within 25 days. While soil with crops plantation and no added *Sphingomonas* can reduce the amount of linden that is contaminated in soil by less than 3%. Soil that does not have crops plantation can reduce the amount of linden that is contaminated in the soil by only 2%. It can be indicated that the plant helps *Sphingomonas* survive in contaminated soil and improves the efficiency of the bacterial degradation of linden [30]. In addition, the study of Alvarez et al. [31] confirmed that root exudates from plants help support the growth and degradation of the linden by *Streptomyces* sp. strains A5 and M7 bacteria. The bacteria can use root exudates as their carbon and energy sources and can decompose more linden**.** This is expected to be caused by the process of co-metabolism.

In the controlled treatment with no crop plantation and bacterial consortium addition, it was found that paraquat was eliminated from soil by 46% only while the controlled treatment with the addition of bacterial consortium was able to get rid of paraquat compounds slightly higher at 51.43%. Considering previous experiments that studied the effect of the addition of bacterial consortium on the efficiency of degradation in soil in microcosm, it was found that experimental set without the addition of bacteria and nutrients (NA) could eliminate 44% of paraquat. This is due to the soil used in the experiment came from the area with paraquat history. Therefore, it can be suggested that the indigenous microorganism is able to adapt and decompose paraquat. The addition of bacterial consortium, therefore, increases the number of microorganisms that are capable of paraquat degradation. This results in higher efficiency of paraquat degradation in controlled treatment with the addition of bacterial consortium than that of the controlled experiment. However, since the experiment at the greenhouse level involves an environment that is different from the experiment in microcosm, it may cause bacterial consortium to grow poorer than at the laboratory level. As a result, the efficiency of paraquat removal in the greenhouse is lower than that in the laboratory. In the experimental set with crops plantation and the addition of bacteria consortium, the efficiency of paraquat degradation is higher because plants can stimulate microbial activity. The plant roots help improve the physical structure of the soil such as the penetration and deepening of plant roots. It contributes to an increase of oxygen in the soil which facilitates the spread of nutrients and water into the lower soil. This is considered to be a change of conditions to be suitable for the work of microorganisms that decompose toxic substances [32-33]. The substances secreted from the plant roots also stimulate the decomposition of pollutants. Substances that are secreted from plant roots can be surfactants, enzymes, nutrients, or supplements that can stimulate microorganisms to use the nutrients to decompose toxins [32]. Examples of chemicals secreted from plant roots are amino acids, sugars, and phenols. In which, the substances secreted from plant roots can stimulate the surrounding bacteria to produce enzymes that do not have specificity to decompose contaminants with similar structures [32-33].

Table 3 The effect of Combined bioaugmentation and phytoremediation by various plants on degradation of paraquat in contaminated soil at day 45 of the experiment.

Treatment	k (day^{-1})	$t_{1/2}$ (days)	r^2	paraquat removal at day 45 (%)[a]
Control (soil without plant and microbial consortium)	0.0153	45.29	0.99	46.43±2.47[a]
Soil with microbial consortium (bioaugmentation)	0.0172	40.29	0.98	51.43±1.24[b]
Phytoremediation (PR)				
Sunflower	0.0284	24.40	0.98	72.14±3.71[d]
Marigold	0.0219	31.64	0.99	64.29±1.24[c]
Pinto peanut	0.0366	18.93	0.97	82.86±2.14[e]
Jack bean	0.0401	17.28	0.96	88.57±1.24[f]
African sesbania	0.0405	17.11	0.96	89.29±2.14[f]
Combined bioaugmentation and phytoremediation (BAP)				
Sunflower with microbial consortium	0.0376	18.43	0.94	83.57±1.24[e]
Marigold with microbial consortium	0.0296	23.41	0.99	75.00±3.27[d]
Pinto peanut with microbial consortium	0.0476	14.56	0.95	95.71±2.14[g]
Jack bean with microbial consortium	0.0480	14.44	0.94	96.43±2.47[g]
African sesbania with microbial consortium	0.0465	14.90	0.95	97.14±1.24[g]

[a] Comparison between treatments in columns is significantly different (Duncan, $p \leq 0.05$) if marked with different lowercase letters.

The comparison of the results obtained in this study and those found in the literature was shown in Table 4. Using bioaugmentation technique on remediation paraquat in contaminated soil, the paraquat removal achieved in this study (76.47%) was close to the results obtained when fermented corn steep, source of bacteria and fungi which present for the degradation of paraquat dichloride contaminated soil that was used (70%) [8]. On the other hand, Murray et al. [34] reported that the removal of paraquat (initial concentration of 50 mg/kg) after 21 days of experience was not a significantly enhanced result on treatments of nonsterile soil (48%), microbial mat (39.4%) and microbial mat with isolated soil bacteria (43.7%). The limitation in effective bioaugmentation was the ability

of the inoculated microbes and the bioavailability of the tightly soil-bound pollutants. The degradation of paraquat by microorganisms occurred due to the process of utilizing paraquat as nitrogen or carbon source and co-metabolism process for transforming paraquat into a less toxic compound. Phytoremediation, one of the bioremediation techniques, can decrease the impact of herbicides on the environment. In this research, more than 64% of phytoremediation of paraquat in contaminated soil occurred in planting with sunflower, marigold, pinto peanut, jack bean, and African sesbania. The best paraquat phytoremediation results were achieved with African sesbania treatment (89.29%). Similarly, Inthama et al. [35] who studied plant growth and drought tolerance-promoting bacterium for bioremediation of paraquat pesticide residues in agriculture soils, reported that cowpea (*Viga unguiculata*) planting in non-sterilized soil with paraquat had a high degradation (99%). Although the use of phytoremediation technique has increased paraquat degradation, the combined bioaugmentation and phytoremediation had the better result. The percentage of paraquat in phytoremediation by African sesbania treatment (89.29%) was lower than treatment with planting African sesbania with microbial consortium (97.14%). This is in accordance with the report of Inthama et al. [35] which found that paraquat residue in non-sterilized soil with cowpea and *Bacillus aryabhattai* strain MoB09, was lower than that in non-sterilized soil with cowpea and without *B. aryabhattai* strain MoB09. It was because plants could activate microbial activities by soil modification means such as rooting and deep rooting to increase oxygen in the soil, leading to more nutrients and water penetration into the lower level of soil. This could make soil appropriate for bacteria to degrade paraquat. The synergistic plant-microbe interaction in the rhizosphere can be utilized for the development of new bioremediation strategy, defeating the disadvantage of individual bioaugmentation or phytoremediation procedures.

Table 4 Comparisons biodegradation of paraquat using bioremediation technique.

Bioremediation technique		Paraquat removal (%)	Reference
Bioaugmentation	fermented corn steep	70	[8]
	microbial mat (consortium of cyanobacteria and bacteria) with isolated soil bacteria	44	[34]
	isolated bacterial consortium	76	This study
Phytoremediation	cowpea	99	[35]
	African sesbania	89	This study
Combined bioaugmentation and phytoremediation	African sesbania with microbial consortium	97	This study

4. Conclusion

The isolated strains include *Sphingomicrobium marinum*, *Ferrovibrio xuzhouensis*, *Azospirillum lipoferum*, *Altererythrobacter xinjiangensis*, *Xanthobacter autotrophicus*, and *Azospirillum amazonense* were capable to degrade paraquat in contaminated soil. The half-life for paraquat in soil from treatment BA in microcosm (16.5 days) was shorter than the half-life for paraquat from soil with microbial consortium treatment in soil pots (40.29 days). This was because the bioaugmentation with degrading microbes performed strong powerful for the herbicide cleanup in various studies. On the other hand, the performance in real field cases is occasionally unstable. The half-life for paraquat in soil without plant and microbial consortium, bioaugmentation, and phytoremediation treatment in soil pots were average 45.29, 40.29, and 21.87 days, respectively. The results also showed that the half-life for paraquat in treatment combined bioaugmentation and phytoremediation (BAP) in soil pots (17.14 days) was lower than phytoremediation treatment.

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6. References

[1] Oliveira DRJ, Remião F, Carmo H, Duarte JA, Navarro AS, Bastos ML, Carvalho F. Paraquat exposure as an etiological factor of Parkinson's disease. Neurotoxicology. 2006;27(6):1110-1122.
[2] Maksymiv I. Pesticides: benefits and hazards. J Vasyl Stefanyk Precarpath Nat Univ. 2015;2(1):70-76.
[3] The Office of Agriculture Regulation. Summary report of the top 10 hazardous substances imported in the year 2019 [Internet]. 2019 [cited 2021 Feb 14]. Available from https://www.doa.go.th/ard/wp-content/uploads/2020/02/HASTAT62_03.pdf.
[4] Vangnai A. Bioremediation. 1st ed. Bangkok: Chulalongkorn University Press; 2010.

[5] Goswami M, Chakraborty P, Mukherjee K, Mitra G, Bhattacharyya P, Dey S, Tribedi P. Bioaugmentation and biostimulation: a potential strategy for environmental remediation. J Microbiol Exp. 2018;6(5):223-231.

[6] Önneby K. Bioaugmentation for reduction of diffuse pesticide contamination [thesis]. Uppsala: Swedish University of Agricultural Sciences; 2013.

[7] Plangklang P. Degradation of carbofuran by rhizosphere soil microorganisms [thesis]. Bangkok: Chulalongkorn University; 2004.

[8] Obuotor TM, Yahaya PB, Akinloye OA, Sakariyau AO. Biodegradation of paraquat dichloride-contaminated soil with fermented corn steep. Uniosun J Sci. 2016;1(1):67-73.

[9] Perruchon C, Pantoleon A, Veroutis D, Blanco SG, Laurent FM, Liadaki K, Karpouzas DG. Characterization of the biodegradation, bioremediation and detoxification capacity of a bacterial consortium able to degrade the fungicide thiabendazole. Biodegradation. 2017;28:383-394.

[10] Cheema SA, Khan MI, Shen C, Tang X, Farooq M, Chen L, et al. Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. J Hazard Mater. 2010;177:384-389.

[11] Yoshitomi KJ, Shann J. Corn (*Zea mays* L.) root exudates and their impact on 14C-pyrene mineralization. Soil Biol Biochem. 2001;33(12):1769-1776.

[12] Garcıa CS, Pande P, Seguin A, Arnaud M, Yergeau E. Rhizoremediation of petroleum hydrocarbons: a model system for plant microbiome manipulation. Microbial Biotechnology. 2018;11:819-832.

[13] Zamani J, Hajabbasi MA, Alaie E. The effect of steam sterilization of a petroleum contaminated soil on PAH concentration and maize (*Zea mays* L.) growth. Int J Curr Microbiol Appl Sci. 2015;4(8):93-104.

[14] Suksong W, Jehlee A, Singkhala A, Kongjan P, Prasertsan P, Imai T, et al. Thermophilic solid-state anaerobic digestion of solid waste residues from palm oil mill industry for biogas production. Ind Crops Prod. 2017;95:502-511.

[15] Gadissa M, Woldemichael A, Yimer F. Optimal soil moisture depletion levels for the production of Vernonia (*Vernonia galamensis* L.) and it's effect on growth, yield and yield components. J Irrig Drain Eng. 2017;6(3):1-7.

[16] Wong YC, Norsyamimi N, Nurdiyana WA. Determination of paraquat (herbicide) residue level in sandy clay loam soil using high performance liquid chromatography. J Basic Appl Sci. 2013;9:566-577.

[17] Lima TL, Nicoletti MA, Munhoz C, Abreu GR, Magalhães JZ, Ricci EL, et al. Determination of paraquat in several commercially available types of rice. Food Nut Sci. 2018;9(12):1368-1375.

[18] Stolz A. Molecular characteristics of xenobiotic-degrading Sphingomonads. Appl Environ Microbiol. 2009;81(5):793-811.

[19] Song M, Zhang L, Sun B, Zhang H, Ding H, Li Q, et al. *Ferrovibrio xuzhouensis* sp. nov., a cyhalothrin-degrading bacterium isolated from cyhalothrin contaminated wastewater. Antonie van Leeuwenhoek. 2015;108(2):377-382.

[20] Romeh AA, Hendawi MY. Bioremediation of certain organophosphorus pesticides by two biofertilizers, *Paenibacillus* (*Bacillus*) polymyxa (*Prazmowski*) and *Azospirillum lipoferum* (*Beijerinck*). J Agri Sci Technol. 2014;16:265-276.

[21] Sharma A, Bhatt P, Khati P, Gangola S. Microbial degradation of pesticides for environmental cleanup. In: Bharagava RN, Saxena G, editors. Bioremediation of industrial pollutants. 1st ed. New Delhi: Write & Print Publications; 2016. p. 178-205.

[22] Dams RI, Paton G, Killham K. The effect of rhizosphere on growth of Sphingomonas chlorophenolica ATCC 39723 during pentachlorophenol (PCP) biodegradation in batch culture and soil. Biotemas. 2006;19(4):13-22.

[23] Elväng AM, Westerberg K, Jernberg C, Jansson JK. Use of green fluorescent protein and luciferase biomarkers to monitor survival and activity of *Arthrobacter chlorophenolicus* A6 cells during degradation of 4-chlorophenol in soil. Environ Microbiol. 2001;3(1):32-42.

[24] Rapp P, Timmis K. Degradation of chlorobenzenes at nanomolar concentrations by *Burkholderia* sp. strain PS14 in liquid cultures and in soil. Appl Environ Microbiol. 1999; 65(6):2547-2552.

[25] Chouychai W, Tongkukiatkul A, Upatham S, Lee H, Pokethitiyook P, Kruatrachue M. Plant-enhanced phenanthrene and pyrene biodegradation in acidic soil. J Environ Biol. 2009;30(1):139-144.

[26] Somtrakoon K, Wongsomsee W, Wannarat J, Chouychai W. Phytoremediation of anthracene-contaminated soil by yard long bean and sweet corn. J Sci Tech UBU. 2012;14(4):1-12.

[27] Liste H, Prutz I. Plant performance, dioxygenase-expressing rhizosphere bacteria and biodegradation of weathered hydrocarbons in contaminated soil. Chemosphere. 2006;62(9):1411-1420.

[28] Liste H, Alexander M. Accumulation of phenanthrene and pyrene in rhizosphere soil. Chemosphere. 2000; 40:11-14.

[29] Chouychai W, Somtrakoon K. Weed selection for phytoremediation of fluoranthene. J Agric Res Ext. 2019;36(2):1-10.

[30] Boltner D, Godoy P, Rojas MJ, Duque E, Morillas MS, Sanchez L, et al. Rhizoremediation of lindane by root-colonizing *Sphingomonas*. Microb Biotechnol. 2008;1(1):87-93.
[31] Alvarez A, Yañez LM, Benimeli CS, Amoroso MJ. Maize plants (*Zea mays*) root exudates enhance lindane removal by native Streptomyces strains. Int Biodeterior Biodegradation. 2012;66:14-18.
[32] Cheema SA, Khan MI, Shen C, Tang X, Farooq M, Chen L, et al. Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. J Hazard Mater. 2010;177:384-389.
[33] Lee S, Lee W, Lee C, Kim J. Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes. J Hazard Mater. 2008;153(1-2):892-898.
[34] Murray R, Phillip P, Bender J. Degradation of pesticides applied to banana farm soil: comparison of indigenous bacteria and a microbial mat. Environ Toxicol Chem. 1997;16(1):84-90.
[35] Inthama P, Pumas P, Pekkoh J, Pathom-aree W, Pumas C. Plant growth and drought tolerance-promoting bacterium for bioremediation of paraquat pesticide residuce in agriculture soils. Front Microbiol. 2021; 12:1-14.