The Effects of Paraquat Used in Upland Rice and Maize Fields on Biomass of Attached Algae

Sutayot Yimpoolsap *, Natha Hungspreug, and Bundit Anurugsa
Department of Environmental Science, Thammasat University, Rangsit Campus, KhlongNueng, KhlongLuang, PathumThani 12120 Thailand

Abstract

Paraquat is a kind of herbicide widely used among Thai farmers especially those working in upland rice and maize fields in steep slope areas. In the rainy seasons, runoff water and sediment contaminated with paraquat drain into streams. This study, aimed to investigate the effects of paraquat, in concentrations of 0, 0.0276, 0.276, 2.76, 27.6, and 276 mg/l on the biomass of attached algae; the primary producers in an aquatic food chain. To conduct the experiment, the algal biomass was measured by the synthesis of chlorophyll a and c of the attached algae in six microcosms. Each microcosm was filled with water samples and attached algae from Mae Kham Stream in Mueng District, Phrae Province. The results of the study show that attached algae exposed to paraquat with concentrations ranging from 0.0276 to 0.276 mg/l synthesized less chlorophyll a and c than the control group that encountered 0 mg/l of paraquat. (The data represented a statistical significance of $P < 0.01$.) Regarding those exposed to paraquat concentrations of more than 2.76 mg/l, their biomass was adversely affected. In other words, the synthesis of attached algae was inhibited.

All in all, paraquat contamination of fresh water leads to destruction of aquatic organisms, especially phytoplankton and attached algae, which are considered vital producers in aquatic ecosystems.

Keywords : bipyridylium, periphytic algae, pigments.

1. Introduction

In Thailand, the widespread use of herbicides among farmers has led to the problem of high herbicide residue in the environment. Some herbicides are highly soluble in water; thus, they can easily contaminate numerous water resources by means of running off fields and draining into the water. Eventually, the accumulation of these toxic herbicides occurs in the sediment, which adversely affects aquatic ecosystems [3].

The majority of Thai farmers opt for paraquat, one of the bipyridilium herbicides which are fast-acting and non-selective. The Agricultural Regulatory Division (as cited in [6]) reiterated the excessive use of paraquat. In the year 2010, 21,028 tons of this sort of herbicide, which cost 1,662 million baht, were imported into Thailand. Theoretically, paraquat is very persistent in soil. Nonetheless, paraquat contamination in water results from interrill erosion and run-off water. This toxic herbicide is then adsorbed onto the river bed, causing destruction of aquatic organisms, especially phytoplankton and periphytic algae, which are considered vital producers in aquatic ecosystems.

*** Correspondence :** s.yimpoolsap@gmail.com

Attached algae stabilize substrata and function as a habitat for many other organisms. They also provide food for invertebrates, which in turn are fish food, in local and downstream ecosystems [4].

Axler& Reuter; Hagerthey & Kerfoot; Wurtsbaugh et al. (as cited in [9]) underlined the importance of attached algae. Since attached algae along with their surfaces are in charge of a substantial proportion of whole-lake primary productivity, they are considered a dynamic component of lake nutrient cycles.

Due to the fact that paraquat has become the herbicide of choice for countless Thai farmers before and in the middle of their farming process, runoff water and sediment contaminated with paraquat drain into streams in the rainy season. This study, hence, aimed to investigate the effects of paraquat on the amount of chlorophyll synthesized by periphytic algae in microcosms.

2. Materials and methods

2.1 Microcosm system

The microcosm system, which was applied in Debenest et al.'s experiment, [2], was a glass tank. Each microcosm was filled with 10.00 L of stream water in order to avoid any stress for attached algae. The microcosms were placed in a temperature-regulated room. Fluorescent tubes were put above each microcosm for algal growth and operated by timer switches. The experimental system was carried out with 12 hour-light cycles.

2.2 Freshwater attached algae collection

To collect the attached algae, stainless racks with clay tiles (100×100 mm) were immersed for one month (from May to June, 2013) in a site in Mae Kham Stream. This site was chosen due to its low concentrations of paraquat monitored during the field survey. At the end of the immersion, the racks were transferred to the laboratory in cool boxes containing stream water. Sixty liters of water used in the experiment were sampled from the site mentioned above.

2.3 Paraquat contamination

Attached algae were exposed to a single concentration of paraquat (commercial grade at 276,000 mg/l). Various concentrations were applied to the microcosms: 0.00, 0.02, 0.27, 2.76, 27.60 and 276.00 mg/l for one day followed by a four-day recovery period. These concentrations were adapted from Wong's experiment [10].

2.4 Attached algae samples in microcosms

The clay tiles were removed every day. The periphytic was scrubbed from the clay tiles with a toothbrush, and then from time to time, distilled water was poured over the tiles using a pipet. The solution obtained was filled up to 100 ml. From this solution, 30 ml aliquots were taken and filtered for chlorophyll a and c to be measured according to the trichromatic method [1]

2.5 Paraquat concentration measurement with a spectrophotometer

For each microcosm, 10 milliter of water was pipetted into a test tube. After that, two milliliters of 0.2% sodium dithionite were added, and the solution was mixed by shaking the tube twice. A portion was placed in a 1.0 cm. cell and absorbance at 396 nm was recorded [5].

2.6 Physical and chemical characterization

Throughout the experiment, a probe was employed daily to measure the pH, electrical conductivity, dissolved solids, and temperature.

2.7 Data analysis

The calculation of the mean and standard deviation was performed for physico-chemical parameters. Line graphs were drawn to depict the variations of attached algae biomasses. Referring to Debenest et al. [2], biomass inhibition (I) values were calculated according to:

$$I = \frac{Bc - Bt}{Bc} \times 100$$

Above "Bc" means the biomass concentration in the control microcosm while "Bt" refers to the biomass concentration in the treated counterpart on the same date. The results of the quantitative data were statistically analyzed by the analysis of variance (ANOVA) and Duncan's new multiple range test.

3. Results and Conclusions

3.1 Variations of the water quality in microcosms

The mean and standard deviation of the pH values of the water were 7.693±1.724; those of the electrical conductivity were 333.417±111.218 µs/cm; those of the dissolved solids were 165.917±56.365 mg/L, and those of the water temperature were 27.508±0.368 ˚C. The concentrations of paraquat measured in the microcosms are illustrated in Table 1.

3.2 Variations of attached algae biomass.

The line graphs (Figures1 and 2) and Table 2 depict the chlorophyll a and c synthesized by attached algae in six microcosms. Each one – microcosm A[1], B[2], C[3], D[4], E[5], and F[6]- was exposed to paraquat at a different concentration for one day (day 0 – day 1) followed by a recovery period of four days (day 1 – day 5).

3.2.1 Chlorophyll a

On the first day (day 0) when every microcosm was free of herbicide, the average of chlorophyll a synthesized by attached algae was 2.652 mg/m^2. The amount of chlorophyll a reached its peak in microcosm B at 2.971 mg/m^2 its lowest in microcosm D at 2.367 mg/m^2. In other words, the amounts of chlorophyll a in the microcosms were not significantly different. After a one-day exposure to paraquat, there was a sharp fall in the amount of chlorophyll a in every microcosm, except A. Microcosms B, C and D, which comprised 1.443, 0.960, and 0.676 mg/m^2 of chlorophyll a respectively, significantly surpassed microcosm F containing 0.286 mg/m^2 of the same pigment. However, the amount of chlorophyll a in microcosm F was not statistically different from that in microcosm E where the amount of chlorophyll a was at 0.340 mg/m^2. In contrast, the amount of chlorophyll a in microcosm A slightly increased on the following day and reached its zenith at 3.125 mg/m^2. In microcosms B, C, D, E, and F, the inhibition values were remarkable at 53.824, 69.216, 78.368, 89.120 and 90.848% respectively. These percentages demonstrate that the amount of chlorophyll

[1] Microcosm A was exposed to a paraquat concentration of 0 mg/L.
[2] Microcosm B was exposed to a paraquat concentration of 0.0276 mg/L.
[3] Microcosm C was exposed to a paraquat concentration of 0.276 mg/L.
[4] Microcosm D was exposed to a paraquat concentration of 2.76 mg/L.
[5] Microcosm E was exposed to a paraquat concentration of 27.6 mg/L.
[6] Microcosm F was exposed to a paraquat concentration of 276 mg/L.

a in every microcosm treated with paraquat plunged when compared with the control one from day 0 to day 1.

From the first to the fifth day of the experiment, chlorophyll a in microcosms D, E, and F tended to remain stable. The average amount of the pigment was 0.665, 0.344, and 0.262 mg/m^2, respectively. There was a statistically significant difference between chlorophyll a in microcosm D and that in the E and F counterparts. As shown in table 1, the fact that the amounts of chlorophyll a in microcosms D, E, and F leveled off was due to the great amounts of paraquat residue.

However, the amounts of chlorophyll a in microcosms B and C gradually rose from the first to the fifth day. On the second and the third days, the amount of the pigment in microcosm B, which was 1.979 and 2.116 mg/m^2 respectively, significantly surpassed that in microcosm C ,which was 1.094 and 1.507 mg/m^2, respectively.

Regarding microcosm A, there was a steady fluctuation throughout the five-day experiment. Eventually, the amounts of chlorophyll a in microcosms B and C on the fourth and the fifth days were more or less the same as those of the A counterpart.

3.2.2 Chlorophyll c

On the first day (day 0) when paraquat was not yet applied, the average amount of chlorophyll c from attached algae was 5.662 mg/m^2. Chlorophyll c was at a high in microcosm D at 5.814 mg/m^2, and it was the lowest in microcosm A at 5.395 mg/m^2. On day 0, the amounts of chlorophyll c in the microcosms were more or less the same. After a one-day exposure to paraquat, the amount of chlorophyll c dramatically dropped in every microcosm, except A. The amount of chlorophyll c in microcosm B (3.679 mg/m^2) was much greater than those in microcosms C, D, E, and F (1.859, 1.173, 0.348, and 0.143 mg/m^2, respectively). The amounts of chlorophyll c in microcosms C and D were remarkably higher than those in microcosms E and F. On day 1, on the other hand, there was a slight increase in the amount of chlorophyll c in microcosm A at 8.121 mg/m^2. In microcosms B, C, D, E, and F, the inhibition values were 54.697, 77.108, 85.356, 95.715 and 98.239% respectively. These percentages marked a dramatic inhibition of chlorophyll c synthesis in every microcosm exposed to paraquat when compared with the control counterpart from day 0 to day 1.

From the first to the fifth day of the experiment, there was very little change in the amount of chlorophyll c in microcosms E and F. The average amounts of chlorophyll c were 0.370 and 0.168 mg/m^2, respectively. In microcosm C, by contrast, the amount of chlorophyll c gradually climbed from 1.859 mg/m^2 on day 1 to 2.607 mg/m^2 on day 3, then remained stable. A similar pattern was observed in microcosm D where chlorophyll c had an upward trend from day 1 (1.495 mg/m^2) to day 3 (1.976 mg/m^2) and then leveled off.

As for microcosm B, the amount of chlorophyll c sky rocketed from day 1 (3.679 mg/m^2) to day 3 (6.775 mg/m^2), then remained more or less the same thereafter. From day 3 to day 5, the amount of the pigment in microcosm B was quite similar to that of the A counterpart. Regarding microcosm A, which was the control, there were mild fluctuations throughout the five days of the experiment.

Table 1. Different concentrations of paraquat (mg/L) in each microcosm from day 0 to day 5.

Concentration of Paraquat Expected (mg/L)	Concentration of paraquat (mg/L) Time (days)					
	0	1	2	3	4	5
0	0.002	0.002	0.001	0.001	0.000	0.000
0.0276	0.002	0.022	0.019	0.011	0.004	0.001
0.276	0.002	0.270	0.167	0.160	0.045	0.009
2.76	0.002	2.493	2.448	2.370	2.010	1.949
27.6	0.002	25.838	25.335	25.083	19.832	19.455
276	0.002	191.299	186.113	184.015	182.079	178.443

Paraquat was applied on day 0 right after the water evaluation.

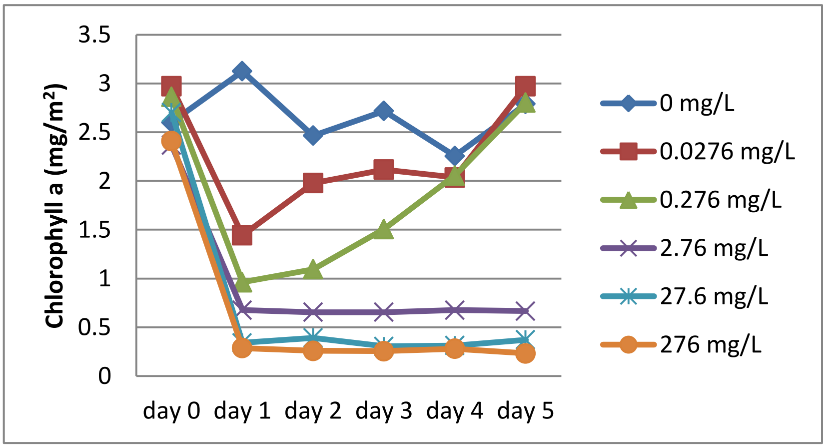

Fig .1. Chlorophyll a synthesized by attached algae after exposure to paraquat at different concentrations.

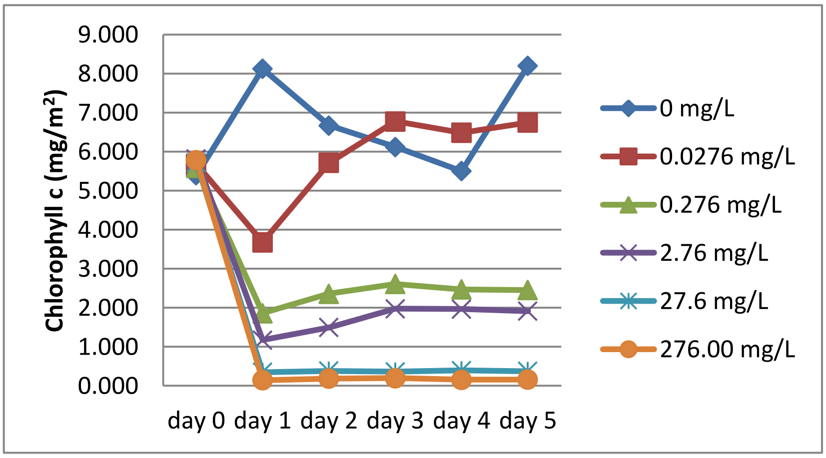

Fig .2. Line graph of chlorophyll c synthesized by attached algae after exposure to paraquat at different concentrations.

Table 2. Chlorophyll a and c (mg/m^2) content of attached algae in the presence of various concentrations of paraquat in the microcosms.

Concentration of paraquat Expected (mg/L)	Chlorophyll a and c (mg/m^2) Time (days)											
	0		1		2		3		4		5	
	Chl-a	Chl-c	Chl-a	Chl-c	Chl-a	Chl-c	Chl-a	Chl-c	Chl-a	Chl-c	Chl-a	Chl-c
0	2.601	5.395	3.125	8.121	2.464	6.667	2.718	6.121	2.255	5.499	2.789	8.198
0.0276	2.971	5.677	1.443	3.679	1.979	5.712	2.116	6.775	2.036	6.485	2.971	6.749
0.276	2.863	5.588	0.962	1.859	1.094	2.361	1.507	2.607	2.056	2.469	2.802	2.452
2.76	2.367	5.814	0.676	1.173	0.655	1.495	0.655	1.976	0.676	1.967	0.665	1.914
27.6	2.703	5.719	0.340	0.348	0.392	0.380	0.392	0.360	0.313	0.392	0.371	0.371
276	2.408	5.780	0.286	0.143	0.258	0.183	0.258	0.196	0.280	0.156	0.232	0.160

Chl-a refers to chlorophyll a.
Chl-c refers to chlorophyll c.

4. Discussion

According to the results, paraquat at different concentrations in every microcosm adversely affected attached algae by inhibiting the synthesis of chlorophyll a and c of attached algae. In agreement with this present study, Wong [10] clearly stated that paraquat in concentrations of 0.02 to 200 mg/l could lead to the inhibition of chlorophyll a synthesis of *Scenedesmus quadricauda* Berb 614 – a species of green algae. A study by Butler (as cited in [7]) reiterated the destructive effect of paraquat on some species of diatoms. This researcher reported that 0.1 mg/l of paraquat inhibited growth of *Navicula osteraria*, while 100 mg/l could stop the growth of *Phaeodactylum tricornutum*.

All in all, the application of paraquat in upland rice and maize fields should be critically reviewed. The presence of low concentrations (0.0276-0.276 mg/L) of paraquat could be detected by attached algae showing inhibiting effects to the synthesis of chlorophyll a and c. In consonance with this study, studies conducted by Ross et al.; Summers; Bauer; Dial & Bauer (as cited in [3]) revealed that sensitive aquatic plants may be adversely affected at levels between 0.019 and 0.372 mg/l of paraquat. However, this study shows that after the recovery period, paraquat would naturally degrade; thus, attached algae would resume synthesizing chlorophyll a and c. In line with this statement, the United States Environmental Protection Agency [8] has posited that paraquat be labeled as a non-selective herbicide that is hazardous to aquatic organisms, but can be applied at a concentration of 0.250 mg/l. Ross et al.; Summers; Bauer; Dial & Bauer (as cited in [3]) also recommended the use of 0.1 and 2.0 mg/l of paraquat for aquatic weed control.

Naturally, attached algae are attached to hard substrata namely rocks and pebbles. Consequently, they can be used as environmental indicators to evaluate paraquat contamination or residue in water, especially in Mae Kham watershed in Mueng Distract, Phrae Province, where paraquat is widely used among farmers working in upland rice and maize fields in steep slope areas. Further study on benthic algae diversity is recommended in order to develop bioindicators for evaluating paraquat contamination in lotic systems.

5. Acknowledgement

We sincerely thank the Thammasart University Research Fund who provided financial support for this research study.

6. References

[1] American Public Health Association, American Water Works Association and Water Environment Federation., Standard methods for the examination of water and wastewater, United Book Press, Inc, Baltimore, Maryland, 1998.

[2] Debenest, T., Pinelli, E., Coste, M., Silvestre, J., Mazzella, N., Madigou, C., and Delmas, F., Sensitivity of freshwater periphytic diatoms to agricultural herbicides, Aquat.Toxicol, Vol. 93, pp.11- 17, 2009.

[3] Eisler, R., Paraquat hazards to fish, wildlife, and invertebrates, in: A synoptic review, Contaminant Hazard Reviews, US Fish and Wild ServBiol Rep., Vol.85 (1.22), 28 pp., 1990.

[4] Finlay, J.C., Khandwala, S., and Power, M.E., Spatial scales of carbon flow in a river food web, Ecology,Vol. 83(7), pp. 1845-1859, 2002.

[5] Kuntom, A., Kifli, H., and Tan, Y.A., Method for the determination of paraquat residue in oil Matrix, Journal of Oil Palm Research, Vol. 2, pp. 57-62, 1999.

[6] Lerdsirivichai, C., Membrane technology based hybrid system to remove paraquat from agriculture run-off water, Master thesis, Asian Institute of Technology, 2011.

[7] Sáenz, M.E., Alberdi, J.L., Di Marzio, W.D., Accorinti, J., and Tortorelli, M.C., Paraquat toxicity to different green algae, Bull. Environ. Contam. Toxicol, Vol. 58, pp. 922-928, 1997.

[8] United States Environmental Protection Agency., Paraquat dichloride, registration eligibility decision(red), Office of Prevention, Pesticides and Toxic Substances, US Environmental Protection Agency, Washington. D.C., 1997.

[9] Vadeboncoeur, Y., Kalff, J., Christoffersen, K., and Jeppesen, E., Substratum as a driver of variation in periphyton chlorophyll and productivity in lakes, J.N. Am. Benthol. Soc, Vol.25(2), pp. 379-392, 2006

[10] Wong, P.K., Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis of *Scenedesmus quadricauda* Berb 614, Chemosphere, Vol. 41, pp. 177-182, 2000.