CHAPTER 5

TOXICITY OF DEGRADED CHLORPYRIFOS AND WASTEWATER AFTER WASHED CHILLI BY ULTRASONICATION AND OZONATION

5.1 Introduction

The toxicity of the by-products from pesticide degradation using ultrasonication and ozonation should be evaluated. Bioassay can be used in a laboratory in order to determine toxicity by the estimation of the median lethal concentration fifty (LC_{50}) which have been reported for a series of toxins, pesticides and other contaminants (Kanwar, 2007). Ultrasonication in combined with ozonation process was employed to pretreated heterocyclic pesticide wastewater for increasing biodegradability and reducing biological toxicity (Xiong *et al*., 2011). The purposes of the research reported below were to investigate the effect of ultrasonication, and its combination with ozone, and to study the toxicity of degraded pesticides.

5.2 Materials and Methods

5.2.1 Toxicity of degraded chlorpyrifos solution after ultrasonication and ozonation by bioassay method

Toxicity estimation becomes an important tool to test the treatment efficiency. Toxicity of degraded chlopyrifos after ultrasonication and ozonation was conducted against brine shrimp (*Artemia salina* L.) as shown in Figure 5.1. Brine shrimp eggs were hatched in artificial sea water (3 % marine salt of water) with oxygen bubbles by air pump, and the adults stage of brine shrimps were available for this experiment 3 weeks after sowing by brine shrimp lethality test method followed Kanwar (2007). The 10 adults of brine shrimps were put into a vial containing 5 ml of the degraded chlorpyrifos concentration at 0, 0.1, 1.0, 10.0 and 100 mg/l after using ultrasonication (108, 400, 700 kHz and 1 MHz), ozonation and the combination treatment, with marine salt 1.5 mg / 5 ml of the solution. The experiment was done in 5 replicates.

Temperature of solutions was in the ranged from 25 - 30 °C.

Mortality of adult brine shrimps has been checked every 6 h eventually after 18 h. LC_{50} of the chlorpyrifos solution was calculated. Brine shrimp mortality in each concentration was calculated using following formula:

$$Mm_{ct} = \frac{N_{Mm} \times 100}{N_0}$$

where:

M_{mct} is the mortality of individuals in time t (%)

N_{Mm} is the average number of died individuals

N_0 is the initial number of living individuals put into every concentration at the test start

Brine shrimp mortality percentage was plotted against log concentration of chlorpyrifos and from this graph the LC_{50} values were evaluated by linear regression analysis.

Figure 5.1 Materials and method of brine shrimp hatching: marine salt (A), eggs of brine shrimp (B), hatching set-up (C) and adult brine shrimp (D).

5.2.2 Toxicity of wastewater from chilli washing with ultrasonication and ozonation by bioassay method

The brine shrimp at 3 weeks after hatching was the same above method. The 10 adult of brine shrimps were put in a vial containing 3 % marine salt and 5 ml of the wastewater from chilli washings after using 1 MHz ultrasonication, ozonation and their combination treatment. Samples were tested in 5 replicates. Percentage of mortality of the brine shrimp was checked every 6 h up to 18 h. The LC_{50} value was calculated as in experiment 5.2.1.

Statistical analysis

All experiments were evaluated with a regression procedure using the SPSS version 17, while the differences among various treatments by Duncan's New Multiple Range test. The significant difference at $p < 0.05$ was assigned by statistical method.

5.3 Results and discussion

5.3.1 Toxicity of degraded chlorpyrifos solution after ultrasonication and ozonation by bioassay method

The size and weight adult of brine shrimp after hatching for 3 weeks in this experiment were measured by a vernier scale and balance. It was found that the average size of brine shrimp was 0.40 cm width, 1.00 cm length, and weight of brine shrimp was 9.60 mg.

The logarithm plots of chlorpyrifos concentration and the mortality percentage of brine shrimp were given in Appendix; (Figures 6 - 9), and the calculated LC_{50} values at 18 h of brine shrimps with different chlorpyrifos treatments were shown in Table 5.1. Toxicity was slightly reduced after 1 MHz ultrasonic treatment as revealed by the LC_{50} values which increased from 12.29 to 14.25 mg/l. The LC_{50} of ozonation and the combination treatments increased up to 52.75 and 383.12 mg/l, respectively (Figure 5.2).

Using ultrasonic for chlorpyrifos degradation effectively reduced the yields of toxic intermediate chlorpyrifos. According to Yao *et al*. (2011), more than 90 % toxicity of dimethoate (organophosphate insecticide) was reduced within 45 min ultrasonic irradiation. Figure 5.3 and 5.4 were shown the mortality percentage of brine shrimps after treated chlorpyrifos solution with ultrasonication, ozonation and the combination treatments at 0 and 18 h respectively, various standard chlorpyrifos concentrations at 0, 0.1, 1.0, 10 and 100 mg/l. At the initial time, the brine shrimps of all treatments were still alive, whereas almost all of brine shrimps died in 100 mg/l of standard chlorpyrifos after 6 h. Every 6 h eventually till 18 h, the percentage of brine shrimp mortality were increased with increasing contact time. It may be due to degradation of chlorpyrifos concentration by ultrasonication as less toxicity than the initial chlorpyrifos was observed. Likely, Naddeo *et al*. (2010) showed decreases in toxicity to *D. magna* during ultrasound irradiation of 40 mg/l diclofenac in water. *A. salina* was non-toxic after 48 h exposure time of ultrasonication, thus indicating the decomposition of diclofenac by-products. Zhang *et al*. (2011) also reported the toxicity evaluation which indicated that the toxicity of diazinon decreased after ultrasonic irradiation.

Table 5.1 LC_{50} values at 18 h to the brine shrimp (*Artemia salina* L.) toxicity of chlorpyrifos after ultrasonication or/and ozonation treatments for 60 min.

Treatment	**Regression equation**	R^2	**log concentration** (where; y = 50)	**LC_{50} (mg/l)** ($10^{\text{log concentration}}$)
Control	y = 24.6x + 23.2	0.9566	1.09	12.29d
1 MHz	y = 26x + 20	0.9399	1.15	14.25c
O_3	y = 18x + 19	0.9975	1.72	52.75b
1 MHz/O_3	y = 14.4x + 12.8	0.8816	2.58	383.12a

The same letter in the column do not differ significantly at p = 0.05 using the least significant difference test.

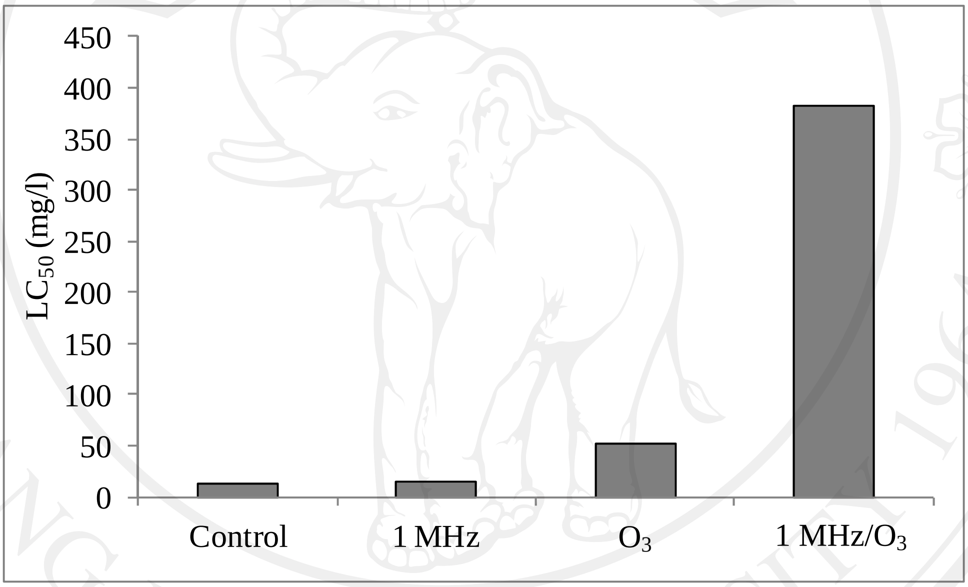

Figure 5.2 LC_{50} values to the brine shrimp (*Artemia salina* L.) toxicity of chlorpyrifos after ultrasonication or/and ozonation treatments for 60 min.

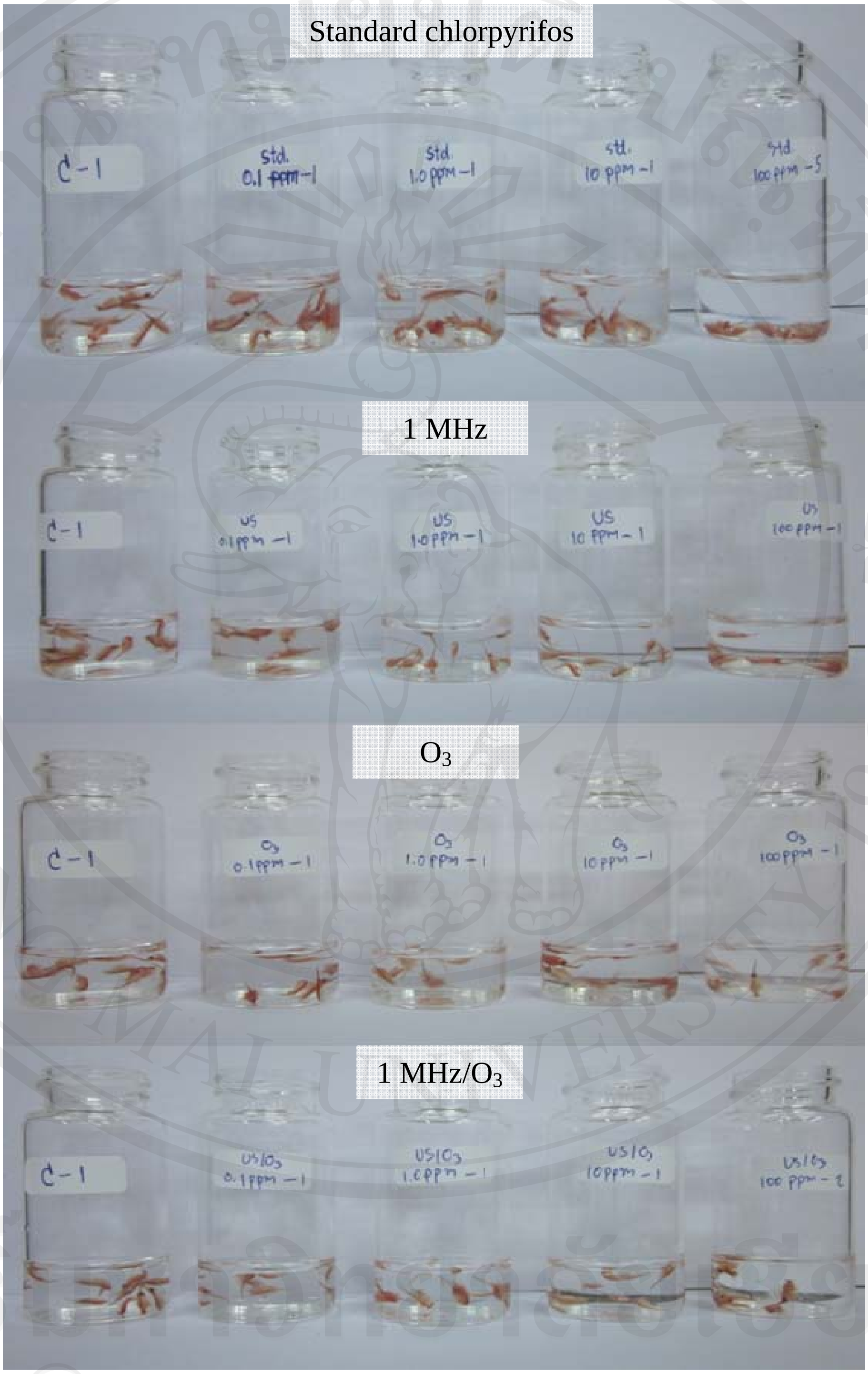

Figure 5.3 The brine shrimps (*Artemia salina* L.) at initial time (0 h) in standard chlorpyrifos solutions (0, 0.1, 1.0, 10 and 100 mg/l) and treated chlorpyrifos solutions with 1 MHz, O_3 and 1 MHz/O_3 treatments.

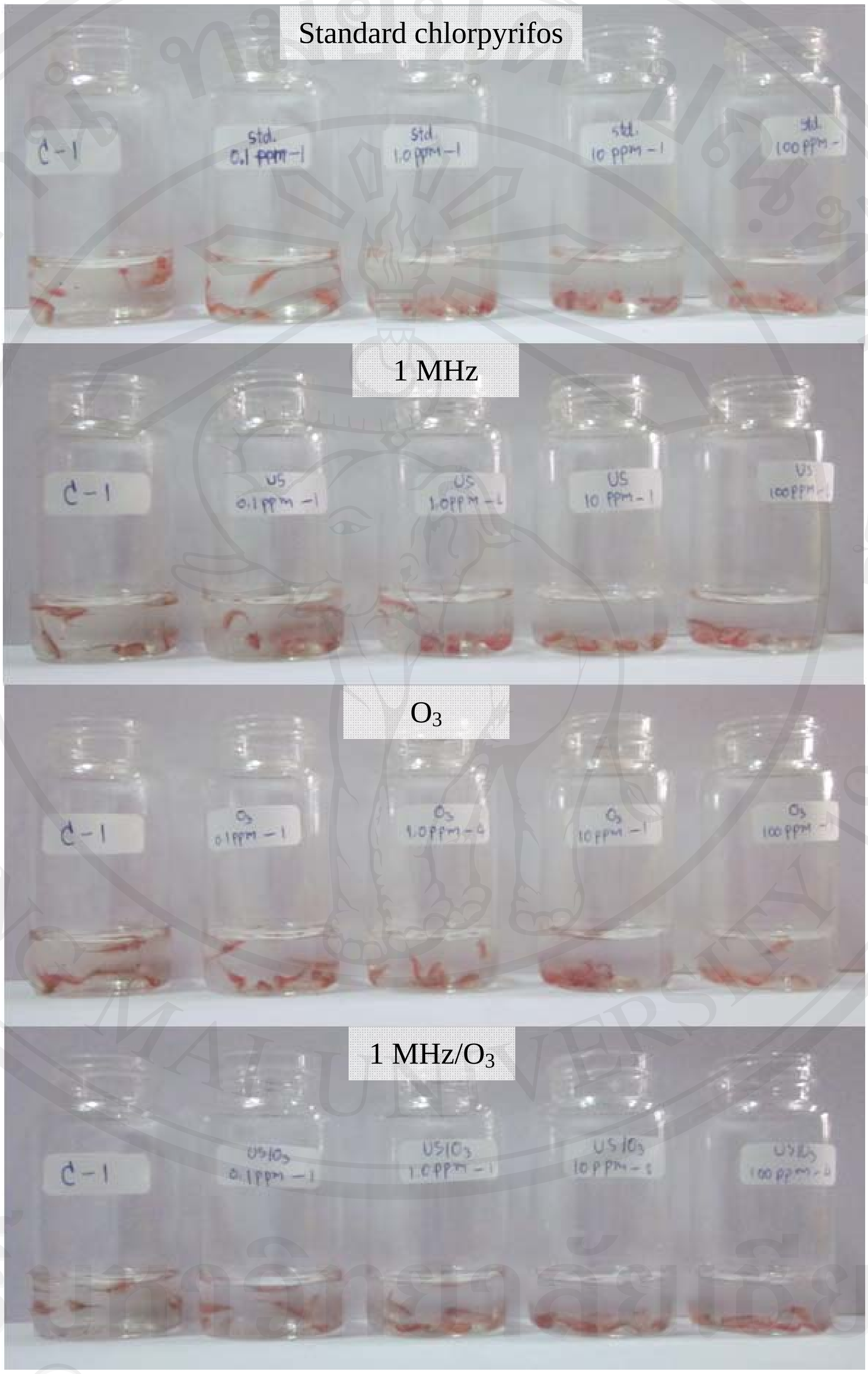

Figure 5.4 The brine shrimps (*Artemia salina* L.) at 18 h in standard chlorpyrifos solutions (0, 0.1, 1.0, 10 and 100 mg/l) and treated chlorpyrifos solutions with 1 MHz, O_3 and 1 MHz/O_3 treatments.

In this experiment, chlorpyrifos residues in wastewater was decreased with increasing contact time, correspond to the increase of chlorpyrifos degradation, and was the wastewater was less toxic than initial time. The toxicity of wastewater after treatments was confirmed by bioassay method in the next experiment.

5.3.2 Toxicity of wastewater from chilli washing after ultrasonication and ozonation by bioassay method

The mortality percentages of brine shrimp in wastewater samples from chilli washing (ultrasonication, ozonation and their combination) were also determined the mortality percentage of brine shrimp at initial time throughout observed period. The mortality percentages of the brine shrimp were slightly increasing, when time increased. At 18 h, the mortality percentage of brine shrimp in wastewater from the combination treatment was significantly reduced, compared to the other treatments (Figures 5.9 - 5.10 and Appendix; Table 18). It could be suggested that using ultrasonication combined with ozonation, which was the most efficiency to reduce toxic. Tsuda *et al*. (1997) reported that fish and other aquatic organism were low concentration of diazinon and other organophosphorus pesticides in the environment. According to Schneider *et al*. (2003), the reduction in *Karenia brevis* toxin concentration by ozone in sea water displayed a positive correlation with the reduction of toxicity as determined by a fish (*Cyprinodon variegatus*) bioassay. In addition, the toxicity to *Daphnia magna* of sonicated petrochemical wastewater samples decreased significantly, by the sonication alone (Sponza and Ozteking, 2011). Naddeo *et al*. (2010) reported that diclofenac and its by-products after ultrasound irradiation treatment did not inflict toxicity to *A. salina*. Similarly, the toxicity of ciprofloxacin (antibacterial drug) clearly decreased when the solutions were treated towards the green algae *Pseudokirchneriella subcapitata* longer than 20 min, which suggested the toxic degradation products were destroyed by further sonolysis (De Bel *et al*., 2009).

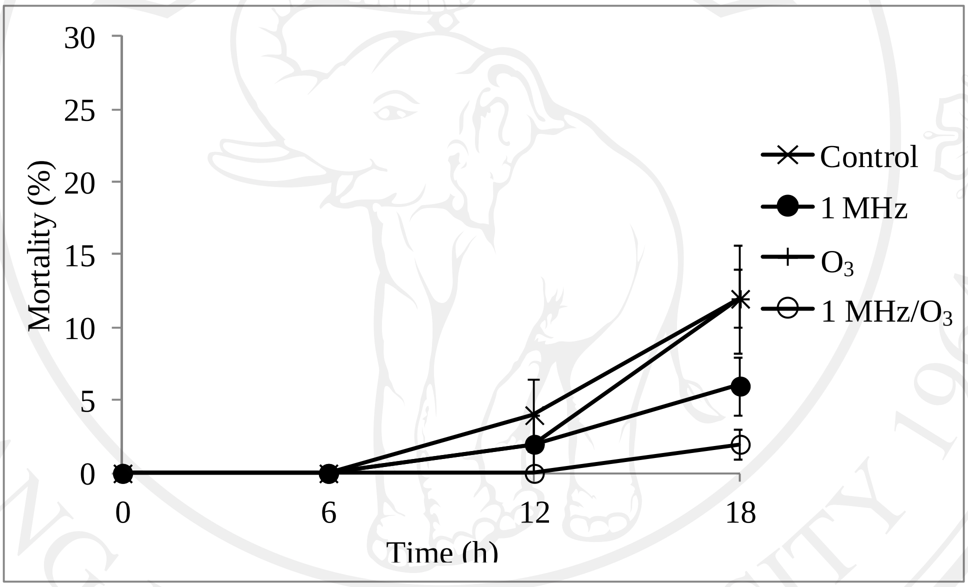

Figure 5.5 Bioassay toxicity test of wastewater from different chilli washing treatments against brine shrimp (*Artemia salina* L.).

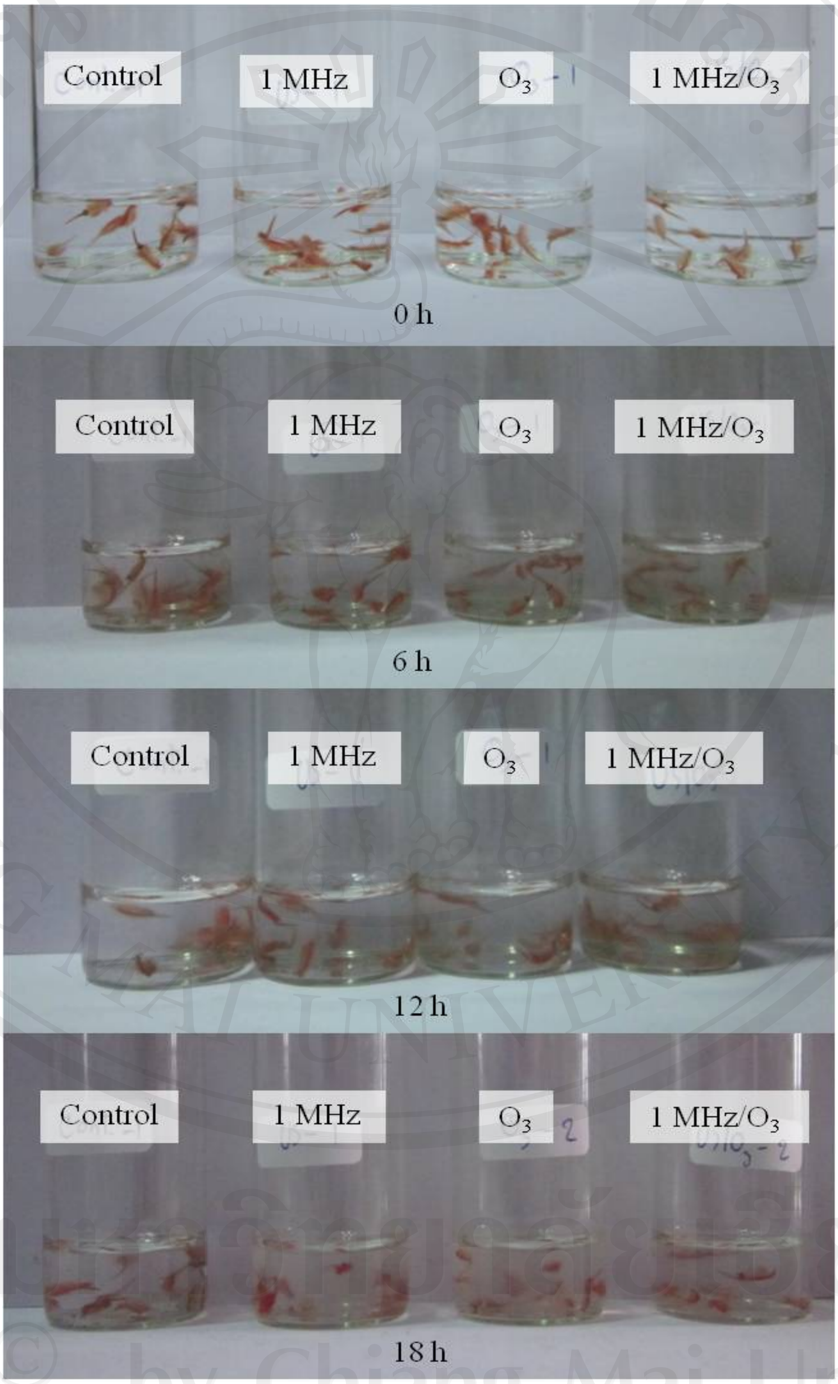

Figure 5.6 Bioassay toxicity test set-up for wastewater from different chilli washing treatments against brine shrimp time 0 - 18 h.

5.4 Conclusion

Ultrasonication and ozonation enhance reduction of the standard chlorpyrifos toxicity in solution. The washing water of chilli fruit treated with 1 MHz of ultrasonication combined with ozonation for 60 min was significantly reduced brine shrimp mortality, compared to ozonation and 1 MHz of ultrasonication alone.