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*Original Research*

# Differential Response of Green Algal Species *Pseudokirchneriella subcapitata*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Chlorella vulgaris* and *Chlorella pyrenoidosa* to Six Pesticides

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### Abstract

This work examines the effects of six pesticides on five green algae: *Pseudokirchneriella subcapitata*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Chlorella vulgaris* and *Chlorella pyrenoidosa*. The results indicate that the decreasing order of the average toxicity to five algae was: fluazinam > propineb > maneb > mancozeb > zineb > bromoxynil octanoate. However, according to sensitivity magnitude, the decreasing order of the ecological risk was: Maneb > bromoxynil octanoate > propineb/fluazinam > zineb/mancozeb. There was a strong variance between toxicity and ecological risk.

**Keywords:** pesticides, green algal sensitivity, acute toxicity tests

## Introduction

Large amounts of pesticides enter aquatic ecosystems as a result of agriculture. Adverse effects of pesticides on non-target plants are of particular concern because of the annual, widespread, and increasingly worldwide use of these chemicals [1]. Algae are essential components of aquatic ecosystems. They produce oxygen and organic substances on which most other life forms depend by providing food for other organisms, including fish and invertebrates. Toxic chemical effects on algae can directly affect the structure and function of an ecosystem, resulting in oxygen depletion, and decreased primary productivity [2]. Pesticides can affect the structure and function of aquatic communities through altering species composition of an algal community [3]. This work was done to examine the effects of six pesticides on five green algae: *Pseudokirchneriella Subcapitata*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*. In order to compare the sensitivity among various green algae, the acute toxicity tests have been devised. In this study, six pesticides were tested to examine their effect on five green algae and compare their differential sensitivity.

## Experimental Procedures

All pesticides were purchased from Limin Chemical Co., Ltd. in China and their REG. NO. and formulation are shown in Table 1. The pesticides were dissolved by acetone or distilled water. The concentration of acetone in the medium was less than 0.05%. The United States Environmental Protection Agency recommends maximum allow-

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Table 1. Selected pesticides, their REG. NO., formulation and test concentrations.

| Test concentrations /Algae | *C. vulgaris* | *C. pyrenoidosa* | *S. obliquus* | *S. quadricauda* | *P. Subcapitata* | Reg. No. | **Formulation |
|---|---|---|---|---|---|---|---|
| Maneb | 0.002-0.5 | 0.001-0.1 | 0.2-20 | 0.002-0.5 | 0.05-5 | 12427-38-2 | 87% TC |
| Zineb | 0.02-5 | 0.1-10 | 0.1-20 | 0.02-5 | 0.02-2 | 12122-67-7 | 88%TC |
| Propineb | 0.002-1 | 0.02-2 | 0.05-20 | 0.005-1 | 0.02-1 | 12071-83-9 | 87%TC |
| Mancozeb | 0.02-10 | 0.01-5 | 0.1-20 | 0.02-20 | 0.02-10 | 8018-01-7 | 92%TC |
| Fluazinam | 0.01-0.5 | 0.005-0.1 | 0.01-1 | 0.005-0.5 | 0.005-1 | 79622-59-6 | 99%TC |
| Bromoxynil octanoate | 0.2-20 | 50-1000 | 0.05-50 | 0.1-20 | 0.2-20 | 1689-99-2 | 96%TC |

*Test concentrations range such as 0.002-0.5 denotes 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 mg/L, the other is similar; **TC denotes technical grade product.

able limits of 0.05% solvent for acute tests and 0.01% for chronic tests. This concentration of acetone was not significant with regard to toxicity [4]. Five green algal species were obtained from the Institute of Wuhan Hydrobiology, the Chinese Academy of Science. The medium for the algal growth inhibition test was prepared in accordance with The Chinese National Environmental Protection Agency Guidelines 201, using HB-4 medium. The culture medium was sterilized at 121, 1.05 kg $cm^{-2}$ for 30 min [5].

Green algal cells were propagated in a 250 mL Erlenmeyer flask containing 100 mL liquid HB-4 medium and kept on a rotator shaker (100 rpm) at 25°C, and illuminated with cool-white fluorescent lights at a continuous light intensity of 450μmol $m^{-2}$ $s^{-1}$. 20 mL medium containing algal cells (initial cell concentration OD680nm=0.008, equal to $5\times10^5$ cells/mL) were distributed to sterile 50 mL Erlenmeyer flasks [5]. A wide range of concentrations was examined in a pretest in order to determine the appropriate test concentrations for each pesticide. Actual test concentrations are shown in Table 1. The medium was then treated with various pesticide concentrations, and incubated for 96 h on an orbital shaker (100 rpm) at the same temperature and light intensity. Cell counts were correlated with absorbance over time for 96 h on a Shimadzu UV-2401PC spectrophotometer. The most suitable wavelength to use for monitoring culture growth was 680 nm. Each pesticide concentration was replicated three times. Appropriate control systems containing no pesticide but equal acetone solvent was included in each experiment. In each experiment, percent inhibition values, relative to growth in control systems, were calculated using optical density.

The 96h $EC_{50}$ values were calculated using linear regression analysis of transformed pesticide concentration as natural logarithm data versus percent inhibition ratio. The NOEC (no observed effect concentration) is the test concentration immediately below the lowest significant concentration. A significant concentration is interpreted to mean a concentration exhibiting a statistically significant reduction in biomass (at $P < 0.05$) when compared with the control. Weighted analysis of variance was used, followed by a one-sided Dunnett's test using a 5% significance level to obtain the LOEC (lowest observed effect concentration) [6]. CV (chronic value) was geometric mean of NOEC and LOEC. Statistical analysis was conducted using SPSS version 11.0.

## Results

The acute toxicities of the pesticides are in Table 2. The 96 h $EC_{50}$, LOEC and NOEC of maneb to five green algae varied around 0.1-5.3 mg/L, 0.002-2 mg/L and 0.001-1 mg/L, respectively. That of zineb varied around 0.3-3.9 mg/L, 0.1-1 mg/L and 0.05-0.5 mg/L respectively. That of propineb varied around 0.1-4.1 mg/L, 0.02-0.2 mg/L and 0.01-0.1 mg/L respectively. Mancozeb varied around 0.5-2.7 mg/L, 0.05-1 mg/L and 0.02-0.5 mg/L respectively. Fluazinam varied around 0.01-0.13 mg/L, 0.001-0.05 mg/L and 0.0005-0.02 mg/L respectively. Bromoxynil octanoate varied around 5-325 mg/L, 0.5-100 mg/L and 0.2-50 mg/L respectively. The decreasing order of the toxicity (owing to $EC_{50}$) to five green algae of six pesticides was: fluazinam > propineb > maneb > mancozeb > zineb > bromoxynil octanoate.

Wide variations occured in response to the pesticides among individual species (Table 3). Among various species, the sensitivity of *P. subcapitata*, *S. quadricauda* and *C. pyrenoidosa* to pesticides were the highest, *C. vulgaris* was the middle and *S. obliquus* was the lowest. For maneb, according to $EC_{50}$, the decreasing order was: *C. pyrenoidosa* > *S.quadricauda* / *C. vulgaris* > *P. subcapitata* > *S. obliquus*, the sensitivity between *C. vulgaris* / *S. quadricauda* and *S. obliquus*, when exposed to maneb varied over one order of magnitude; between *C. pyrenoidosa* and *S. obliquus* varied over two orders of magnitude. According to magnitude of CV, the decreasing order also was: *C. pyrenoidosa* > *S. quadricauda* / *C. vulgaris* > *P. subcapitata* > *S. obliquus*, the sensitivity of various species of algae – between *C. pyrenoidosa* / *P. subcapitata* and *S. quadricauda* / *C. vulgaris*, when exposed to maneb varied over one order of magnitude; between *C.*

Table 2. The effect of various pesticides to five green algae.

| Pesticides | Regression equation* | CC* | SL* | $EC_{50}$ (mg/L) | LOEC (mg/L) | NOEC (mg/L) |
|---|---|---|---|---|---|---|
| Maneb | (1)Y=3.5209+0.1883X | 0.99 | 0.00 | 0.11 | 0.02 | 0.01 |
| | (2)Y=5.2572+0.2679X | 0.99 | 0.01 | 0.02 | 0.002 | 0.001 |
| | (3)Y=3.8569+0.2761X | 0.98 | 0.02 | 5.24 | 2 | 1 |
| | (4)Y=3.5419+0.1912X | 0.99 | 0.00 | 0.12 | 0.02 | 0.01 |
| | (5)Y=4.0646+0.2636X | 0.99 | 0.00 | 1.34 | 0.5 | 0.2 |
| Zineb | (1)Y=2.6118+0.1549X | 0.96 | 0.00 | 1.20 | 0.1 | 0.05 |
| | (2)Y=5.3817+0.3689X | 0.98 | 0.00 | 1.79 | 1 | 0.5 |
| | (3)Y=3.4462+0.2361X | 0.97 | 0.01 | 3.81 | 1 | 0.5 |
| | (4)Y=2.6184+0.1564X | 0.96 | 0.00 | 1.31 | 0.1 | 0.05 |
| | (5)Y=4.1034+0.2440X | 0.99 | 0.00 | 0.38 | 0.1 | 0.05 |
| Propineb | (1)Y=2.9094+0.1542X | 0.98 | 0.00 | 0.16 | 0.02 | 0.01 |
| | (2)Y=5.7230+0.3495X | 0.96 | 0.00 | 0.32 | 0.2 | 0.1 |
| | (3)Y=2.3654+0.1503X | 0.96 | 0.00 | 4.07 | 0.2 | 0.1 |
| | (4)Y=2.7146+0.1432X | 0.98 | 0.00 | 0.19 | 0.02 | 0.01 |
| | (5)Y=5.1773+0.2992X | 0.98 | 0.00 | 0.16 | 0.1 | 0.05 |
| Mancozeb | (1)Y=2.9696+0.1863X | 0.98 | 0.00 | 1.75 | 0.2 | 0.1 |
| | (2)Y=7.1515+0.4610X | 0.96 | 0.04 | 0.54 | 0.05 | 0.02 |
| | (3)Y=3.7745+0.2552X | 0.99 | 0.01 | 2.68 | 1 | 0.5 |
| | (4)Y=2.9966+0.1893X | 0.98 | 0.00 | 1.87 | 0.2 | 0.1 |
| | (5)Y=3.2332+0.1924X | 0.99 | 0.00 | 0.68 | 0.2 | 0.1 |
| Fluazinam | (1)Y=4.778+0.2625X | 0.99 | 0.00 | 0.08 | 0.02 | 0.01 |
| | (2)Y=5.3063+0.2706X | 0.99 | 0.01 | 0.02 | 0.001 | 0.0005 |
| | (3)Y=3.0717+0.1664X | 0.95 | 0.00 | 0.19 | 0.05 | 0.02 |
| | (4)Y=4.9870+0.2765X | 0.99 | 0.00 | 0.09 | 0.02 | 0.01 |
| | (5)Y=4.3705+0.2433X | 0.98 | 0.00 | 0.12 | 0.01 | 0.005 |
| Bromoxynil octanoate | (1)Y=2.6197+0.1756X | 0.96 | 0.00 | 5.72 | 1 | 0.5 |
| | (2)Y=2.0062+0.1875X | 0.91 | 0.03 | 324.55 | 100 | 50 |
| | (3)Y=3.1492+0.2246X | 0.97 | 0.00 | 7.54 | 2 | 1 |
| | (4)Y=2.8105+0.1917X | 0.98 | 0.00 | 5.82 | 0.5 | 0.2 |
| | (5)Y=3.4670+0.2407X | 0.97 | 0.01 | 4.43 | 1 | 0.5 |

Y and X stand for percent inhibition and natural logarithm of concentration, respectively. CC and SL denote coefficient correlation and significance level, respectively. (1) *C. vulgaris* (2) *C. pyrenoidosa* (3) *S. obliquus* (4) *S. quadricauda* (5) *P. Subcapitata*

Table 3. Compare the pesticide toxicity and algal species toxicity ($EC_{50}$ value).

| Species | Pesticide toxicity rank | *Average pesticide toxicity (Mean±SD) | Pesticide | Species toxicity rank | *Average species toxicity (Mean±SD) |
|---|---|---|---|---|---|
| C. vulgaris | 0.08-1.75 | 1.50±2.18 | Maneb | 0.02-5.24 | 1.37±2.23 |
| C. pyrenoidosa | 0.02-1.79 | 55±132 | Zineb | 0.38-3.81 | 1.70±1.28 |
| S. obliquus | 0.19-5.24 | 3.92±2.46 | Propineb | 0.16-4.07 | 0.98±1.73 |
| S. quadricauda | 0.08-1.75 | 1.57±2.21 | Mancozeb | 0.54-2.68 | 1.50±0.89 |
| P. Subcapitata | 0.12-1.34 | 1.19±1.65 | Fluazinam | 0.02-0.19 | 0.10±0.06 |
| | | | Bromoxynil octanoate | 0.02-5.24 | 70±143 |

95% confidence interval of the difference

*pyrenoidosa* and *P. subcapitata,* between *S. obliquus and S. quadricauda / C. vulgaris* varied two orders of magnitude; between *C. pyrenoidosa* and *S. obliquus* varied three orders of magnitude. i.e. the sensitivity of five green algae is much higher.

As to zineb, the decreasing order of the sensitivity was: *P. subcapitata* > *S. quadricauda* / *C. vulgaris* > *C. pyrenoidosa* > *S. obliquus*, the sensitivity exposed to zineb varied small. Refer to CV, the decreasing order also was: *C. vulgaris / S. quadricauda* / *P. subcapitata* > *C. pyrenoidosa* / *S. obliquus*, the sensitivity of various species of algae—between *C. vulgaris / S. quadricauda* / *P. subcapitata* and *C. pyrenoidosa* / *S. obliquus,* when exposed to zineb varied one order of magnitude.

For propineb, the order of sensitivity was: *C. vulgaris* / *S. quadricauda* / *P. subcapitata* > *C. pyrenoidosa* > *S. obliquus*, the sensitivity between *C. vulgaris / S. quadricauda* / *P. subcapitata* and *S. obliquus*, when exposed to propineb varied one order of magnitude. According to CV, the order of the sensitivity also was: *C. vulgaris* / *S. quadricauda* > *P. subcapitata* > *C. pyrenoidosa* / *S. obliquus*, the sensitivity between *C. vulgaris* / *S. quadricauda* and *C. pyrenoidosa* / *S. obliquus* when exposed to propineb varied one order of magnitude.

With respect to mancozeb, the order of sensitivity was: *C. pyrenoidosa* > *P. subcapitata* > *S. quadricauda* / *C. vulgaris* > *S. obliquus,* the sensitivity of various species of algae exposed to mancozeb varied. Consulted CV, the order also was: *C. pyrenoidosa* > *C. vulgaris / S. quadricauda* / *P. subcapitata* > *S. obliquus*, the sensitivity between *C. pyrenoidosa* and *S. obliquus* when exposed to mancozeb varied one order of magnitude.

As to fluazinam, refer to $EC_{50}$, the order of the sensitivity was: *C. pyrenoidosa* > *S. quadricauda* / *C. vulgaris* > *P. subcapitata* > *S. obliquus,* the sensitivity between *C. pyrenoidosa and S. obliquus,* when exposed to fluazinam varied over one order of magnitude. Refer to CV, the order also was: *C. pyrenoidosa* > *P. subcapitata* > *S. quadricauda / C. vulgaris* > *S. obliquus,* the sensitivity between *C. pyrenoidosa* and *S. obliquus* / *S. quadricauda* / *P. subcapitata / C. vulgaris* when exposed to fluazinam varied over one order of magnitude.

As for bromoxynil octanoate, consulted $EC_{50}$, the order of sensitivity was: *P. subcapitata* > *S. quadricauda / C. vulgaris* > *S. obliquus* > *C. pyrenoidosa,* the sensitivity between *C. pyrenoidosa* and *S. obliquus* / *S. quadricauda* / *P. subcapitata / C. vulgaris,* varied over one order of magnitude. Consulted CV, the order also was: *S. quadricauda* / *P. subcapitata / C. vulgaris* > *S. obliquus* > *C. pyrenoidosa,* the sensitivity between *S. obliquus* and *C. pyrenoidosa* varied over one order of magnitude; between *C. pyrenoidosa* and *S. quadricauda* / *P. subcapitata C. vulgaris* varied two orders of magnitude.

## Discussion of Results

Algal water blooms is attributed to the overabundance of algal growth and the gradual shift of algal community structure. The latter usually indicates a gradual shift from dominance by an algal species to dominance by another [7]. Whether there exists the pollutants factor that among algal species has greater differential sensitivity. If it is true, the contamination may result in a shift of algal group structure and would experience higher ecological risk [9]. The results indicate that the decreasing order of the average toxicity to five algae was: fluazinam > propineb > maneb > mancozeb > zineb > bromoxynil octanoate. However, according to sensitivity magnitude, the decreasing order of the ecological risk was: Maneb > bromoxynil octanoate > propineb/fluazinam > zineb/mancozeb. There was a strong variance between toxicity and ecological risk.

Single-species toxicity test has historically been the source of biological data for risk evaluation. However, it has been discussed as to whether information from these tests alone is suitable to predict effects at the ecological level [10]. Furthermore, multiple-species toxicity tests such as microcosm and mesocosm tests enable the observation of the indirect effects of chemicals caused by interactions among species. Outdoor aquatic ditch mesocosms were treated with a range of pesticides to simulate various spray drift rates resulting from a typical crop protection program used in the cultivation of potatoes by Arts et al. [11] and their aims were to provide information on the fate and ecological effects of drift of the pesticides into surface water and to evaluate the effectiveness of drift-reduction measures in mitigating risks. The effects of a pesticide mixture (asulam, fluazinam, lambda-cyhalothrin, and metamitron) on aquatic ecosystems were investigated in 20 outdoor aquatic microcosms by Wendt-Rasch et al. [12], they show that the structure of the ecosystem influences the final effect of pesticide exposure. However, conducting mesocosm tests to assess the impact of chemicals on ecosystems involves skilled labor and is time consuming, expensive, and not easy to interpret. Therefore, we think that we should select a lot of special genus organisms, through single-species toxicity test respectively and using mathematical mode describing ecosystem instead of multiple-species toxicity tests such as microcosm and mesocosm tests in risk evaluation.

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