Methods for estimating cell death and toxicity using acridine orange staining for environmental toxicology: Responses to exposure and ingestion of particulate pollutants

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ABSTRACT

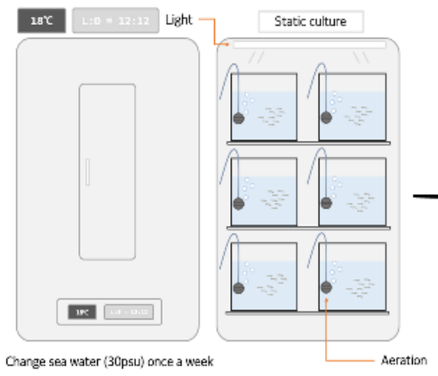
There are methods for determining apoptosis, such as enzyme-based methods and imaging-based methods. Among these, acridine orange staining has been used for a long time. However, the experimental techniques that must be applied vary depending on the variable nature of AO, and different results are shown depending on the concentration, exposure time, and cell condition. This study complements the existing AO staining method and provides AO usage methods suitable for each experimental condition. When AO is treated at 10-6 M, green fluorescence in the area where RNA conditions have changed becomes stronger. The intensity of the green spot is presented as evidence of apoptosis. When AO is treated at a concentration of 10-5 M, damaged cells emit RED. As a large amount of lysosomes and destroyed cells are generated, AO accumulation becomes stronger. Therefore, apoptotic and destroyed cells can be seen through RED. Since toxicity experiments require changes that occur in a short period of time, it is expected that easy and fast AO staining will allow us to quickly determine the degree of toxic effects on organisms.

OBJECTIVES

- In order to image the degree of cell death of zooplankton, appropriate exposure concentration and time of AO are derived.
- Utilizing the variability of AO, it is proven that there is an AO concentration setting according to the experiment.
- By treating it with actual toxic substances, it is proven that the method using AO staining is an easy and convenient method for imaging cell death.

MATERIALS AND METHODS

- **Control fluorescence test_ ship wastewater**



Neomysis awatschensis

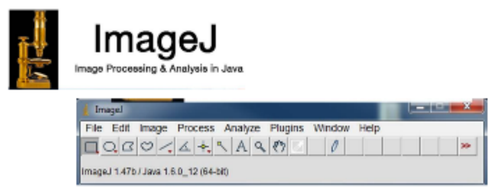
10^{-6} M
10^{-5} M
10^{-4} M

1 min
5min
10min
15min

AO staining

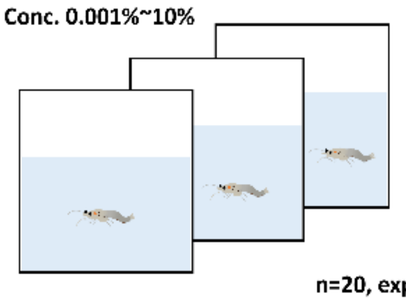
Comparing green and red exposure value
Setting the appropriate staining level and time according to concentration

- **Fluorescence intensity analysis using Image J**



- **Acute toxicity test_ ship wastewater**

Conc. 0.001%~10%



n=20, exposure time 3 h

After calculating the toxicity value, the experiment was conducted at the highest concentration

10^{-6} M
10^{-5} M
10^{-4} M

10 min

AO staining

Decision of exposure value and exposure time
Creating relevant and meaningful images

- **Apoptosis and cell death test caspase-3 activity test**

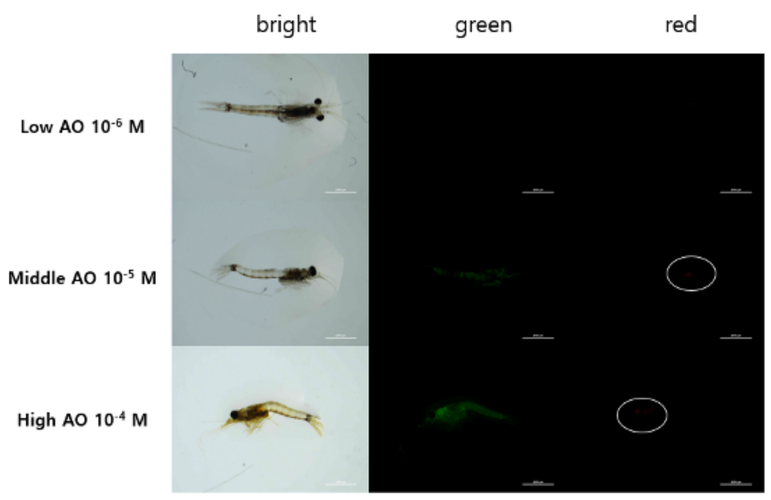
Control
0.001%
0.01%
0.1%
1%
2%
10%

Prepare by diluting wastewater
Identify the most influential cell death concentration through caspase-3 test

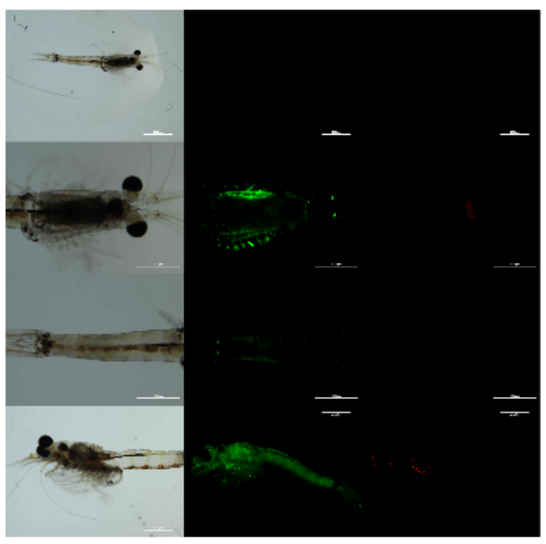
RESULTS

Control fluorescence test

Control

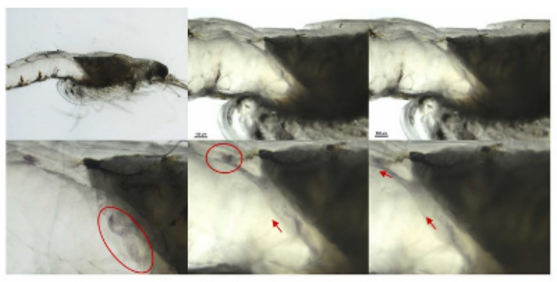


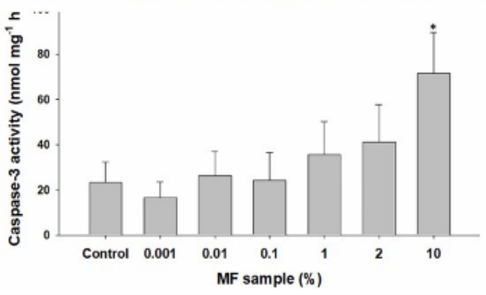
Low AO 10^{-6} M 15min

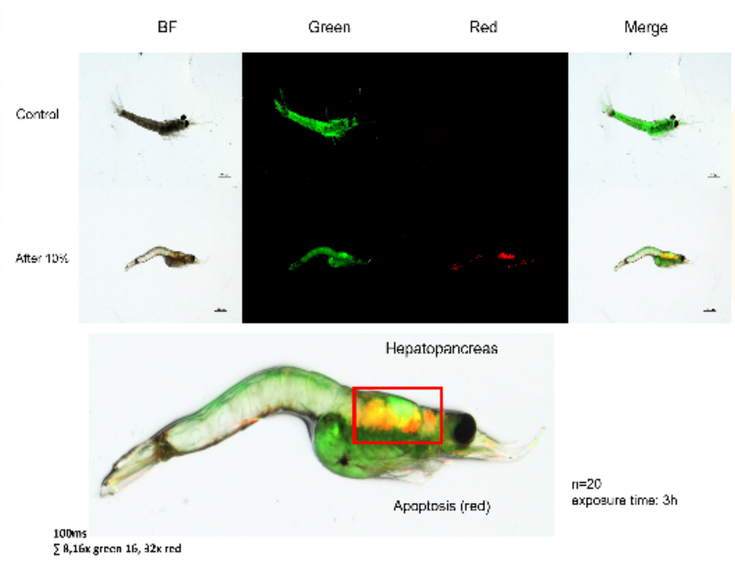


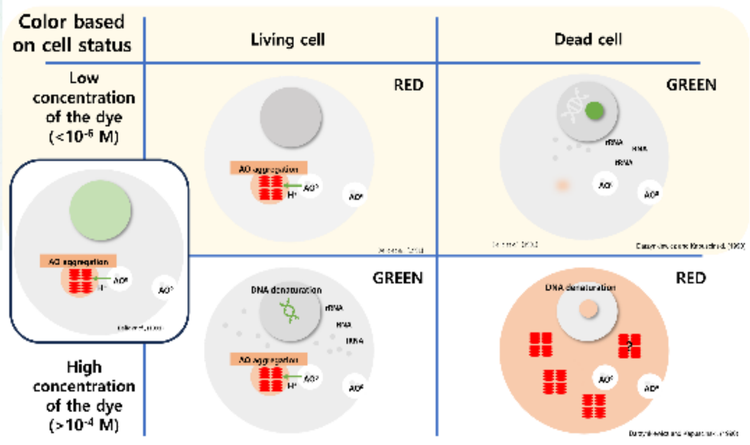
High AO 10^{-4} M 15min

Confirmation of apoptosis









Concentration	10^{-5} M	
Exposure time	5-10 min	
Exposure value	100 ms	
∑ value	green	8 16 x
	red	16-32 x

CONCLUSION

Even within the control, when the concentration of AO increases, green and red fluorescence appears, but the intensity is very weak. Therefore, individuals exposed to toxic substances were able to extract significant red fluorescence using a concentration of 10^{-5}. It was found that individuals that consumed high concentrations of hull cleaning discharge showed strong RED fluorescence when stained with AO. In addition, when looking at the effect of caspase-3 activity, it was most active, so it can be inferred that a response to apoptosis occurred. Therefore, AO staining is a convenient material that can visualize the degree of influence by environmental toxic substances, and it can be easily used in the *in vivo* environment and zooplankton. If a concentration of 10^{-5} is used, red fluorescence can be visualized to easily identify apoptosis.