論文の内容の要旨
Abstract of Dissertation

論文題目
Title of Dissertation Effect of Heavy Metal Distribution in the Food Organisms to Freshwater Ostracod (*Heterocypris incongruens*) in Whole Sediment Toxicity Test

（全底質毒性試験における淡水カイミジンコ（*Heterocypris incongruens*）に対する餌生物中の重金属分布の影響）

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（本文）(Abstract)

Aquatic organisms maybe exposed to toxicants through aqueous (dissolved) phase and/or through direct contact (dietary) with metal contaminated particles. It has been found that the amount of contaminant accumulated by aquatic organisms was influenced by the biological factors such as food quality and quantity, partitioning of contaminants on the food particles, digestive physiology of the organisms, contaminant characterization within the animal tissue and its associations with different geochemical fractions in food particles. Recent studies have shown that exposure to metal-contaminated diets can be harmful to various freshwater invertebrates thus, might observed reduction in survival, growth and reproduction. Therefore, there is growing evidence that dietborne metal toxicity might be important in aquatic ecosystems.

The current understanding of dietborne metal toxicity is, however insufficiently developed and, as a consequence, the dietary exposure route is generally not considered explicitly in most existing regulations or risk assessment. Regulatory assessments of metal toxicity in freshwaters are mostly based on dissolved metal concentrations, assuming that toxicity is caused by waterborne metal only. Previously, the dissolved phase was thought to be the main source of metal exposure for planktonic organisms, but recently a number of studies have demonstrated that dietary exposure is also an important route for metal accumulation.

A better understanding of the fate of sediment-associated metals and metal contaminated food are needed because if dissolved metal concentrations alone are used to predict toxicity, then environmental risk could be underestimated if there is bioavailable metal associated with these. It is presumed that organisms do not take up metal from food or food is not considered as metal source.

The main objective of this study was to investigate the effect of heavy metal distribution in the food organisms to freshwater ostracod *Heterocypris incongruens* in the whole sediment toxicity test. Specifically, to determine bioaccumulation and toxicity of heavy metals to ostracod from metal-contaminated microgreen algae *Scenedesmus acutus*, *Chlorella vulgaris,* and *Ankistrodesmus falcatus*, to evaluate the metal uptake and its subcellular distribution in different microgreen algae and to determine the potentially available fraction of heavy metals for possible trophic transfer to benthic ostracod, and to analyze the effect of different food type and light condition on heavy metal toxicity to benthic ostracod. This study will give information to understand the mechanisms of ostracod toxicity test which was recently listed in the ISO publication (ISO 14371:2012 (E)). Ostracod toxicity test has been cited in literatures but the actual exposure routes in the test were not simple thus giving a complex cause of toxicity.

Preliminary modifications of different test conditions were done from the standard ostracod toxicity test such as the use of quartz sand as the control sediment, photoperiod exposure of the test (i.e. 24-h dark and light/dark conditions) and different food sources (i.e. TetraMin®, *Chlorella vulgaris* and *Ankistrodesmus falcatus*). These fundamental investigations were necessary for further understanding the effect of different exposure routes in the sediment toxicity test. The toxicity test using quartz sand exposed both under 24-h dark and light/dark conditions showed that mortality of ostracod were relatively comparable to the reference sediment. However, the measured mean growth of ostracod was smaller than in the reference sediment (266±53 and 365±48 µm under 24-h dark and light/dark conditions, respectively). Thus, quartz sand can be used as the control sediment since measured mean growth and mortality were within the acceptable criteria based on the standard procedure. The mortality of ostracod under light/dark condition for reference sediment and quartz sand were 2% and 0%, respectively. This implied that the toxicity test can also be exposed to different photoperiod. Light/dark condition was tested since this condition simulates the actual environmental condition wherein aquatic organisms were exposed as well in light condition. In order to determine whether *Chlorella vulgaris* and *Ankistrodesmus falcatus* can be possible food for ostracod, different algal concentrations were prepared and tested both under 24-h dark and light/dark conditions. Algal suspension preparation for the new algal species followed the standard protocol (ISO 14371:2012 (E)). The following algal cell concentrations will be used based on the optimum measured mean growth and mortality of ostracod after the 6-day toxicity tests – *C. vulgaris*: 6.0×10^8 cells/well and *A. falcatus*: 3.0×10^6 cells/well. The results of the modifications of test conditions were used for the next stage of experiments.

Scenedesmus acutus and *H. incongruens* were used to evaluate the effect of different food and different light condition on ostracod sensitivity under different concentrations of copper and zinc. Effect of using different food on the growth of ostracod shows that as the dosage of TetraMin® available for ostracod increased, the mean growth also increased from 289±74 to 384±71 µm under 24-h dark condition. No mortalities were observed at TetraMin® dosages of 0.5 mg/well and 1.0 mg/well but mortality of 7% was observed at 2.0 mg/well. Though better growth was observed at 2.0 mg/well TetraMin®, consequently dosage of 1.0 mg/well TetraMin® was used. Therefore, TetraMin® can be used as possible food supplement in ostracod sediment toxicity test as results met the criteria both in terms of the mortality and growth inhibition of the ostracod. Furthermore, the results of the different light conditions tests revealed that light/dark condition had no significant influenced on the ostracod sensitivity to zinc when fed with TetraMin® (T2 and T3) and *S. acutus* but had a significant effect when fed with *C. vulgaris* and *A. falcatus*. On the other hand, light condition had a significant influenced in copper toxicity to ostracod in all different experiments. Lastly, comparison of the determined LC50 of copper and zinc using different food type and food amount showed that ostracod were more sensitive to zinc than copper.

Heavy metal contamination can enter the aquatic food chain either through direct consumption of water or biota or through aquatic exposure. Metal in water can be uptaken by algae and distributed in several fractions in the algal cell. Thus, it is important to know the available fractions of metal that can have potential risk to higher trophic aquatic organisms. Therefore, different algae were exposed to different concentrations of metals to determine the distribution of metals in their cells and to determine the hypothetical fractions of metals available for trophic transfer to ostracod. *Scenedesmus acutus, C. vulgaris* and *A. falcatus* were exposed to different concentrations of copper and zinc. After the 10-day exposure and the subsequent metal fractionation of algal cells, the copper in the algal cells were distributed in almost similar proportion for each algal species. *Scenedesmus acutus, C. vulgaris* and *A. falcatus* accumulated most of the copper in their algal cells (1.1 to 6.9×10^2 µgCu/L). Zinc accumulation in the algal cells varies among the three species - *S. acutus* (7.8 to 2.9×10^2 µgZn/L), *C. vulgaris* (7.0 to 2.4×10^2 µgZn/L) and *A. falcatus* (5.2 to 1.4×10^2 µgZn/L). Additionally, most of zinc in the cells of *S. acutus* were internally bound (intracellular soluble: 5.9 to 2.0×10^2 µgZn/g and intracellular insoluble: 35 to 4.9×10^2 µgZn/g). *Chlorella vulgaris* accumulated zinc inside their cells mainly as intracellular insoluble form (46 to 1.0×10^3 µgZn/g). On the other hand, in *A. falcatus* most of zinc was in cell-surface

exchangeable (3.5 to 3.3 x 10^2 µgZn/g) and intracellular insoluble forms (99 to 6.5 x 10^2µgZn/g). Copper in the cells of *S. acutus, C. vulgaris* and *A. falcatus* were distributed in intracellular soluble (0.4 to 1.3 x 10^3 µgCu/g, 4.6 to 1.4 x 10^3 µgCu/g, and 2.3 to 7.8 x 10^2 µgCu/g, respectively) and intracellular insoluble fractions (12 to 9.7 x 10^3 µgCu/g, 14 to 1.1 x 10^4 µgCu/g, and 26 to 1.0 x 10^4 µgCu/g, respectively). Hence, distribution of zinc incorporated in exposed algae varied among the three species while accumulated copper were mostly in intracellular soluble and intracellular insoluble fractions for the three algae. From these results, we may conclude that bioavailability of metals is dependent on the algal species.

Hypothetical trophically available metal (TAM) was determined as the sum of the cell-surface exchangeable and the intracellular soluble fractions in the algal cells. Estimated trophically available zinc varied with zinc concentration, Z1（420 µgZn/L） having the highest estimated TAM corresponding to 36% (*S. acutus*), 33% (*C. vulgaris)* and 55% (*A. falcatus*). *Chlorella vulgaris* had the highest percentage of trophically available copper in their cells around 12 to 25%, compared to *S. acutus* and *A. falcatus* which accumulated only about 4 to 15% and 5 to 8 %, respectively.

Different metal contaminated algal food were prepared and used in the toxicity test to determine the possible dietary metal effect to ostracod. Based on the dose response relationship, the total zinc content increased almost 19 times (43 to 8.0 x 10^2 µgZn/g) but the mortality of ostracod did not significantly increased (33 to 52 %). On the other hand, as the total zinc accumulated in the algal cells of *C. vulgaris* increased, the toxicity to ostracod increased (23 to 83%) more clearly than the case of *S. acutus*. In C1 and C2 tests, significant mortality was observed for ostracod fed with Cu-exposed *S. acutus* and *C. vulgaris*. On the other hand, similar results were observed with Zn-exposed and Cu-exposed *A. falcatus*, high mortality was observed at control (clean algal cells) (68% and 57%, respectively) condition and maybe due to food insufficiency. Additional experiments were suggested to determine the possible source of high toxicity.

Based on dose response relationship when ostracod was exposed to different zinc and copper concentration through aqueous exposure (Chapter 5), the measured zinc and copper in the overlying water was not high enough to cause lethal toxicity to ostracod, thus the observed mortality should be considered as the result of the metal-exposed food (dietary metal).

There was almost no difference among the different fractions of metals to determine the possible toxic effect of dietary metal to ostracod especially when exposed to copper based on the dose response curves thus, total metal or trophically available metal may be used to discuss the toxicity of contaminated algae to ostracod.

To conclude, the current study provides significant implications for understanding the dietary toxicity of copper and zinc in the benthic ostracod. The study highlights the importance of dietborne metal and suggests that dietary exposure should be incorporated when assessing the ecological effects of contaminants in the sediments where contaminated food is one of the potential exposure pathways. This recently standardized toxicity test was believed to be utilized by more researcher for evaluation of sediment toxicity thus, the developed solid-phase dose response curves can be give fundamental information for the interpretation of future ostracod toxicity test results. This study also indicates that a clearer understanding of the effects of both aqueous and dietary exposure routes as well as of interactions between them is necessary for further refinement of the chronic metal toxicity test for *H. incongruens*.