# 行政院國家科學委員會專題研究計畫初步成果報告

## 金屬污染物在基層水產生物之蓄積與轉移

**Uptake and transfer of metal pollutants in basic trophic level of aquatic biota**

**計畫編號：**NSC 88-2314-B-041-002

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### 中文摘要

**本初步研究經由一項短期間實驗室生態設計，暴露不同鉛與鎘由含**0.1, 1.0, 10, **至**50 $\mu g\ mL^{-1}$**之污染濃度於幼豐年蝦，並經由不同時間之吸附過程，以分析此豐年蝦所吸附此兩種鉛與鎘金屬之不同程度。豐年蝦在**2**小時之鉛污染後，發現有極顯著此金屬之吸附濃度，其中又以最高**50$\mu g\ mL^{-1}$**組別於**2**小時至**22**小時分別吸附****37**$\mu g\ g^{-1}$**與48**$\mu g\ g^{-1}$(**濕重基準**)**之濃度；鎘金屬之污染亦以**50 $\mu g\ mL^{-1}$**組別比起其它實驗組於**22**小時期間造成豐年蝦有顯著之上升。此實驗顯示不同金屬於數小時內即能造成此幼蝦體之明顯蓄積，且越高金屬濃度越可造成吸附之提升程度。**

**關鍵詞：** **豐年蝦、鉛、鎘、吸附、蓄積、轉移。**

### ABSTRACT

The uptake of two different heavy metals, Pb and Cd, by brine shrimp (*Artemia salina*) larvae was investigated in the present short-term laboratory ecosystem. The uptaken concentrations in brine shrimp were determined under various metal concentrations ranging from 0.1, 1.0, 10 to 50 $\mu g\ mL^{-1}$ within different time periods. After 2-hr contamination, significant increases of the Pb concentrations in *Artemia* were found to be 37 $\mu g\ mL^{-1}$ and 48 $\mu g\ mL^{-1}$ (wet wt.) at 2 hr and 22 hr, respectively. The resulting Cd concentrations in these larvae were also to be highest at 50 $\mu g\ mL^{-1}$ groups compared to other experimental groups within 22-hr period. Based on the preliminary results conducted in this study, apparent increasing concentrations can be accumulated in *Artemia* within several hr under various polluted metal concentrations and higher metal concentrations could cause higher degrees of accumulation in this aquatic biota.

**Keywords:** brine shrimp, *Artemia salina*, Pb, Cd, uptake, accumulation, transfer.

### INTRODUCTION

Heavy metals, such as Pb, Cu, Cd, Zn etc., have been widely released into environments through point or non-point locations and caused various levels of contamination in rivers, lakes and oceans. These heavy metals were treated as the most important pollutants of concern in Taiwan [1-3]. Once the pollutants adsorbed or absorbed by biota their concentrations may be increased in other higher trophic levels of aquatic biota due to the food chain transfer [4-7]. This may also result the toxicity in aquatic biota and endanger the food health and safety in human consumers [8].

In aquatic ecosystems, the basic food supplier --- plankton, plays its important role in adsorption with heavy metals and transfers these compounds to upper levels of pelagic predators through food chain [9]. Once the widely used food supplier, such as live

*Artemia* larvae, adsorbed with heavy metals, it will be most possible for this small size of zooplanktonic diets to uptake and transfer the pollutants to higher levels of predators.

Direct partitioning or adsorption of pollutants in the aqueous medium plays a major role in the uptake of pollutants by the lower level of small aquatic organisms, mainly planktons, because of the relatively higher biomass in the smaller sizes [10]. Zooplankton such as brine shrimp, *Artemia salina*, serves as the primary food source for the larval stages of many aquatic species. Various reports have shown that the *Artemia* are able to tolerate heavy metals, oil and oil dispersant, and this species has been used as an inexpensive system for the study of marine pollution due to its ready availability, low cost, and ease of culture [11-13].

However, information is scarce on the mechanism of metal accumulation in the lower levels of zooplanktonic biota or the role of these biota in transporting these pollutants to other larval stages of aquatic fish in the food chain system. The objective of this study was to assess the levels of metals accumulated by *Artemia* nauplii after exposure to various metal concentrations under a laboratory ecosystem. Through this study, the effects of the aquatic biota may be explored more clearly on the resulting transfer of metals to other higher aquatic trophic biota.

## MATERIALS AND METHODS

Standard stock solutions of Pb and Cu with 1000 μg $mL^{-1}$ were purchased from Sigma Co. Freshly filtered seawater (0.45 μm) of 25‰ salinity was obtained from local aquaculture farm. One gram of *Artemia* cysts (Great Salt Lake Brand) was hatched in a separatory glass funnel containing 2 L of filtered seawater (25‰ salinity) under continuous strong aeration at 25 ± 2°C for 24 hr.

Each of the either Pb or Cd standard stock standard was pipetted and transferred into Erlenmeyer flasks containing 200 mL of filtered seawater to reach 0.1, 1.0, 10, and 50 μg $mL^{-1}$ metal concentrations, respectively. Freshly 24-hr hatched *Artemia* nauplii were then evenly transferred to each flask and maintained under aqueous environments with moderate aeration at 25 ± 2°C for different time period. A control group without metal contamination was also prepared through the experiment. Each group for the time periods either from the contamination groups or control group were set at 2, 4, 8 and 22 hr. At each time period, live *Artemia* from each flask were siphoned and drained through a 250 μm sieve then rinsed with distilled water to remove any suspended solids or dead *Artemia* debris. The *Artemia* were then collected, weighed and stored under -20°C for further analysis. The collected samples were digested with 1 ml concentrated $HNO_3$ under a Questron Q-1000 microwave oven. The clear digestive solutions were dissolved in 5 ml of 1 N HCl for the metal determinations by using a GBC-908 flame atomic absorption spectrophotometer (AA). Each group was set up in duplicate to obtain average values.

Total suspended soilds (TSS) from the beginning of the aqueous solutions were determined according to APHA Standard Methods [14] by using glass fiber filters. Table 1 shows the characteristics of aqueous environments associated with the numbers of live *Artemia* nauplii used in this experiment. Amounts of the two metals and total suspended solids (TSS) were not found in the present filtered water with 25‰ of salinity. The live *Artemia* applied to each of the aqueous environment were to be at ca. 410 larvae $mL^{-1}$.

## RESULTS AND DISCUSSION

The preliminary results for the uptake of two different metal concentrations , Pb and Cd, in *Artemia* larvae within 22 hr are shown in Figure 1 and 2, respectively. The average uptakes of these two metals in *Artemia* were found to be significantly higher for both of the Pb and Cd 50 μg $mL^{-1}$

Table 1. Characteristics of aqueous solution for two different metals uptake in *Artemia* at the present experiment.

| | Aqueous solution |
|---|---|
| pH | 7.1 ± 0.2* |
| Salinity (‰) | 25 |
| TSS (mg $L^{-1}$) | n.d.§ |
| Pb (µg $mL^{-1}$) | n.d. |
| Ni (µg $mL^{-1}$) | n.d. |
| Live *Artemia* applied (larvae $mL^{-1}$) | 410 ± 20 |

* Means ± standard deviation.

§ Not detected.

treatment groups compared to other treatment groups (0.1, 1.0 and 10 µg $mL^{-1}$). This is in consistency with the report from Wang *et al.* [7] that degrees of PCB accumulation in basic aquatic biota, such as *Artemia* and microalgaes, is closely related to the pollutant concentrations from the aqueous solutions. However, the uptakes of Cd in *Artemia* were determined to be not significant for both of two treatment groups, 0.1 and 1.0 µg$mL^{-1}$ within 22-hr contamination experiment as shown in Figure 2. This may contribute to the different characteristic of the two different metals that the aquatic biota could adapt to different concentrations.

Based on the Figures 1 and 2, an increasing metal concentration was found for both metals in *Artemia* within 22-hr period with increasing metal concentrations in aqueous solutions. Meanwhile, the *Artemia* species were shown to increasingly uptake these two metals just within 2 hr. According to the results from Figure 1, *Artemia* could accumulate as high as 30, 17 and 6 µg $g^{-1}$ (wet wt.) from Pb contaminated aqueous solutions within 2 hr among 50, 10 and 1.0 µg $mL^{-1}$ concentrations respectively. However, the decreasing Pb concentrations in *Artemia* after 2 hr for each group may be explained by the resulting increase of dead *Artemia* numbers.

According to the report of Wang *et al.* [7], adsorption of high PCB residues from water in the outlayer of the nauplii could therefore cause high PCB residues in this small size and large biomass of zooplankton. Although, Franke *et al.* [15] reported that effective body burden concentrations in target tissues along with co-occurring adverse effects, such as mortality, are of much higher significance on bioaccumulation process for hydrophobic pesticides. However, the capability of *Artemia* to accumulate pollutants in the body may also depend on the numbers of the zooplankton present in aqueous environment [6]. Further studies, such as growth, survival conditions and the density of the aquatic species, are needed to explore in order to determine more precisely for the corresponding relation between biota burdens and contamination environments.

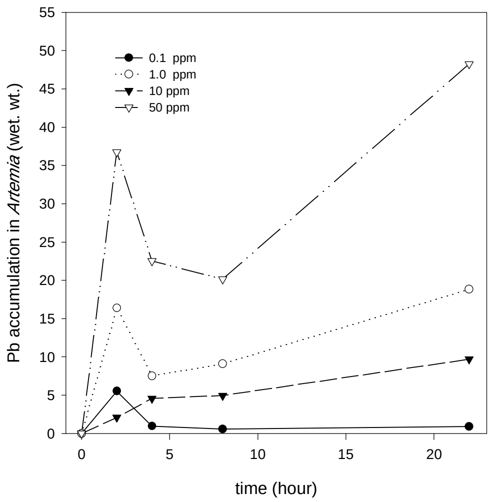


Fig. 1. Accumulation of Pb in *Artemia* after exposure to various Pb concentrations within 22 hr.

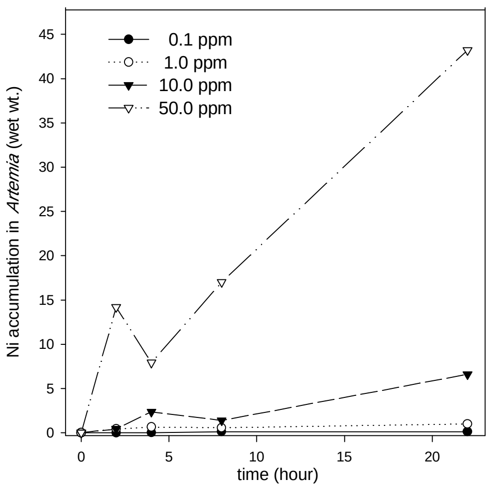


Fig. 2. Accumulation of Cd in *Artemia* after exposure to various Ni concentrations within 22 hr.

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