嘉南藥理科技大學專題研究計畫成果報告

不同 pH 值之重金屬溶液對豐年蝦之耐受與存活探討

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執行單位：保健營養系

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Study of resistance and survival for *Artemia* in various heavy metal solutions with different pH values

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中文摘要

本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 μg mL^{-1}之污染濃度於幼豐年蝦，並經由不同金屬溶液之pH值與暴露時間之過程，以檢視兩種鉛與鎘金屬對豐年蝦所產生不同之耐受與存活程度。初步發現豐年蝦隨著暴露越高濃度之鉛與鎘溶液數小時之後，發現有極顯著之越低存活率；其中發現鉛與鎘金屬溶液之pH值為7.0時豐年蝦之存活率最佳，此亦意謂於此pH值有最好之耐受程度。

關鍵詞: 豐年蝦、鉛、鎘、存活、耐受。

ABSTRACT

This study was conducted according to a short-term laboratory ecosystem for exoposure brine shrimp (*Artemia salina*) in two different metal solutions, Pb and Cd, by using various concentrations ranging from 1 ng mL^{-1} to 200 ng mL^{-1} and pH values to determine the resistance and survival of this biota. Preliminary results showed that lower survivals were found in *Artemia* under higher concentrations of these two metal solutions within several hours. Meanwhile, a highest survival rate was found when exposure *Artemia* to the metal solutions at a pH value around 7.0. This may also mean that *Artemia* have a higher resistance at a metal concentration for around pH 7.0 compared to other pH values.

Keywords: Artemia, Pb, Cd, resistence, survival.

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. 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. This may also result the toxicity in aquatic biota and endanger the food health and safety in human consumers.

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. 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. 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.

However, information is scarce on the concentrations of metal solutions exposure to the lower levels of zooplanktonic biota. Once the aquatic areas were dumped or contaminated for some kinds of various metal solutions with different pH values. This aquatic biota might encounter certain degrees of growth or survival not only from the xenobiotic pollutants but also from the accompanied conditions, such as fluctuating pH values. The objective of this study was to assess various pH values of metals 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 upon different pH values of metals contamination. Focus is taken on study for the resistance and survival on this aquatic biota, *Artemia*.

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 20‰ 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 (20‰ 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. Each metal concentration was further adjusted its pH value by using 1.0 N HCl to the values of 5.0, 6.0, 7.0 and 1.0 NaOH to the values of 8.0, 9.0, 10.0. Freshly 24-hr hatched *Artemia* nauplii were then evenly transferred to each flask and maintained under these various pH values of 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 group were counted under a microscope by pipetting 50 μL from the culture for measuring the numbers of survival. Each group was set up in triplicate to obtain average values.

RESULTS AND DISCUSSION

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 20‰ of salinity. The live *Artemia* applied to each of the aqueous environment were to be at ca. 330 larvae 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 (‰)	20
TSS (mg L^{-1})	n.d.§
Pb (μg mL^{-1})	n.d.
Cd (μg mL^{-1})	n.d.
Live *Artemia* applied (larvae mL^{-1})	330 ± 15

* Means ± standard deviation.

§ Not detected.

Any changes in growth produced by exogenous chemicals will have multiple causes. Heath (1987) suggested a pollutant may cause an increase in maintenance cost which then requires a greater food intake if the energy balance is to be maintained. Decreased growth of aquatic animals when exposed to xenobiotics is well documented in various reports. According to Mauck et al., at 48 d after hatching, brook trout growth was significantly decreased by Aroclor 1254 concentration ≥ 1.5 μg/l. Hansen et al. (1976) reported channel catfish fingerlings fed a diet containing 20 ppm Aroclor 1242 for 20 weeks responded with reduced weight gain. Westin et al. (1985) reported that inherited and dietary chlorinated hydrocarbons could affect survival of striped bass larvae after yolk absorption. Also, according to Olney et al. (1980) fish survival is probably a definitive indicator of pollutant-induced stress.

In the present study, survival numbers of *Artemia* nauplii after exposure to various metal concentrations at different pH values for 4 hr were shown in Table 2. Since the pH value for filtered seawater (20‰ salinity) was determined to be at 7.1 and freshly adjusted to different pH values of metal solutions, therefore no other affecting factors can cause any survival effects. Apparently, survival numbers of *Artemia* were found to be lower at the higher metal concentrations ranging from 0.1, 1.0, 10, to 50 μg mL^{-1}, either on Pb or Cd with different pH values of the solutions. Highest survival was found on the treatment with both of Pb and Cd at pH value of 7.0 to be 330 larvae mL^{-1}. Based on the Table 2, a decreasing survival number of the aquatic biota was apparent for the pH values either lower or higher than 7.0 on both of the metal solutions. A consistently decreasing survival number of *Artemia* was also found to be in consistence to the decreasing or increasing pH value at 7.0. Meanwhile, both of the metal solutions with highest concentrations at 50 μg mL^{-1} for Pb and Cd were shown to have the lowest survival number to be 43 and 88 larvae mL^{-1}, respectively.

In conclusion, an apparent pH changes either higher or lower than 7.0 can cause a decreasing survival number of *Artemia* for both of Pb and Cd solutions in the present study. This also means that *Artemia* nauplii can only resistant to the pH values at around 7.0. Although, Franke *et al.* 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 xenobiotics. However, the capability of *Artemia* to accumulate pollutants in the body may also depend on the numbers of the zooplankton present in aqueous environment. Further studies, such as 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.

Table 2. Survival numbers of *Artemia* after exposure to various metal concentrations at different pH values for 4 hr. (The initial number of *Artemia* at the beginning of the study was 330 larvae mL^{-1}).

	Survival numbers of *Artemia* (larvae mL^{-1})							
	Pb concentrations (μg mL^{-1})				Cd concentrations (μg mL^{-1})			
pH value	0.1	1.0	10	50	0.1	1.0	10	50
5.0	146	125	85	43	202	198	103	88
6.0	312	167	103	83	269	245	147	110
7.0	330	263	219	106	330	308	207	159
8.0	256	233	160	89	241	214	185	107
9.0	201	147	141	71	209	171	163	93
10	155	166	95	68	178	136	117	71