嘉南藥理科技大學專題研究計畫成果報告

不同脂肪酸種類之豐年蝦品種餵食幼魚之存活影響

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計畫編號：CNHN-91-02

執行期間：91 年 01 月 01 日至 91 年 12 月 31 日

計畫主持人：王瑞顯

共同主持人：

計畫參與人員：

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Survival Effects of fish larvae fed on different fatty acid profiles of *Artemia* species

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中文摘要

本實驗之數據顯示石斑(*Epinephelus* sp.)幼苗經由餵食Reference *Artemia* cysts (RAC)豐年蝦(Brine shrimp, *Artemia* sp.)活餌料28日後比較餵食*Artemia* of San Fransico Bay (SFB) and Great Salt Lake (GSL)皆有顯著較高($p<0.05$)之最終存活率specific growth rate (53.3%), 特別生長比值specific growth rate (14.3)與生物指數biomass index (62.7), 此可歸納於RAC豐年蝦比起其他兩豐年蝦種類有較高之16:1ω7 (13.30%), 18:1 (36.10%)及20:5ω3 (7.44%)脂肪酸種類，因此導致存活與生長之差異。

關鍵詞: 豐年蝦、石斑、存活率、幼魚苗。

ABSTRACT

The present study demonstrated that either the final survival (53.3%), specific growth rate (14.3) and biomass index (62.7) on grouper larvae (*Epinephelus* sp.) fed on Reference *Artemia* cysts (RAC) nauplii for 28 d were significantly higher ($p<0.05$) than those fed on the *Artemia* of San Fransico Bay (SFB) and Great Salt Lake (GSL). This may contribute to the relatively higher fatty acids found in 16:1ω7 (13.30%), 18:1 (36.10%) and 20:5ω3 (7.44%) from Reference *Artemia* cysts (RAC) compared to the other two *Artemia* species.

Keywords: Grouper larvae, *Epinephelus* sp., *Artemia salina*, survival.

INTRODUCTION

Live zooplankton, especially live brine shrimp (*Artemia*) nauplii, usually provide the best performing diet for rearing the larval stages of aquatic fish and crustaceans, since it is easily obtained or cultured, has an appropriate size and is nutritionally adequate (Simpson et al., 1983; Leger et al., 1986). However, various reports has also stated that using *Artemia* nauplii resulted in poor larval rearing, e.g. chlorinated hydrocarbon contamination (Olney, 1980) and lack of essential fatty acids (Schauer et al., 1980; Leger et al., 1985).

There is profound variation with respect to species in individual fatty acids and classes of fatty acids. Specially, it is utmost important for good fatty acid profiles existed in the diets for rearing and keeping good survival of larval stages of aquatic animals. However, several publications report significant variations in nutritional effectiveness *of Artemia* nauplii from different geographical origins. Thus, in the present study, three different batches of cysts, RAC *Artemia* (Reference *Artemia* cysts), San Francisco Bay *Artemia* and Great Salt Lake *Artemia* were fed to grouper larvae (*Epinephelus* sp.) for the survival differences resulting from different fatty acid profiles in different *Artemia* diets.

MATERIALS AND METHODS

Filtered seawater (0.45 μm) of 30‰ salinity was obtained from a local aquaculture farm. One gram of each dry *Artemia* cysts, RAC *Artemia* (Reference *Artemia* cysts), San Francisco Bay (SFB) and Great Salt Lake (GSL) was separately

hatched in a separatory glass funnel containing 2 L of filtered seawater (30‰ salinity) under continuous strong aeration at 25 ± 2°C for 24-h with light.

After 24-h hatching, *Artemia* nauplii were harvested under a 106 μm sieve and transferred to 10L glass tanks containing seawater. Fifty grouper at two-wk-old (obtained from a local aquaculture farm) were randomly collected and transferred to each of the 10L glass tanks under moderate aeration (20 ± 2°C). All the treatments were conducted replicated twice.

Every day before feeding, 1 L of water were siphoned to remove fecal detritus or dead *Artemia* nauplii and replaced with same volume of freshwater. After 28-d feeding with *Artemia* nauplii, grouper were collected, counted and weighed.

RESULTS AND DISCUSSION

Percent survivals of the fish larvae on the present 28-d feeding are shown in Table I. The daily percent survivals were derived by substrating daily mortality from the number of fish surviving on the previous day and then subtracted from the original number of fish. The grouper fed with RAC *Artemia* higher showed a significant higher survival, 53.3% than that of *Artemia* treatment with SFB (39.8%) or GSL (37.7%). The percent survivals of grouper larvae in the present trials were mostly higher than the study reported by Webster & Lovell (1990). During the 19-day posthatch feeding experiment in that study, survival for striped bass was greatest for fish fed with live *Artemia* (28.5%), followed by fish fed a dry diet (18.0%), freeze-dried *Artemia* (9.8%) and shock-frozen *Artemia* (9.3%). By comparsion with that report, the *Artemia* diet used in the present study can provide minimally satisfactory nutrition for the survival and growth of grouper larvae. For the cannibalism found in this study, the RAC group also showed a relatively lower percentage (6.7%) than the other groups suggesting lower mortality on the RAC group.

For the growth conditions in this 28-d feeding trial as shown in Table II, RAC *Artemia* treatments had the highest values of specific growth rate (SGR, 14.3) and biomass index (62.7) compared to all the other formulated diet treatments. Values for the SGR or biomass index on RAC diet treatments were also significantly different from the other two groups ($p < 0.05$). This is inconsistent with the predominant fatty acid profiles found from RAC *Artemia* nauplii as shown in Table III. The significant difference of the fatty acids is found to be on 20:5ω3, where RAC has a highest percentage of 7.44 compared to the other two species, SFB (3.44%) and GSL (0.66%).

Table I. Average final percentage survival and cannibalism of grouper larvae after feeding three different fatty acid profiles of *Artemia* for a 28-day experiment.

	RAC	SFB	GSL
Survival %	53.3^{a}*	39.8^{b}	37.7^{b}
Cannibalism%†	6.7^{c}	16.2^{d}	16.9^{d}

*Different letters denote pairs of groups significantly different at the 0.05 level for Duncan's multiple range test.
†% Cannibalism = (initial # of fish-total # of mortality fish recorded)/initial # of fish.

Table II. Average specific growth rate (SGR) and biomass index of grouper larvae after feeding three different fatty acid profiles of *Artemia* for a 28-day experiment.

	RAC	SFB	GSL
Specific growth rate†	14.3^{a}*	10.5^{b}	11.2^{b}
Biomass index‡	62.7^{c}	35.7^{d}	30.4^{d}

*Different letters denote pairs of groups significantly different at the 0.05 level for Duncan's multiple range test.
†SGR = Ln(final wet wt/initial wet wt.)x100/28 days.
‡Biomass index = ((Avg. final wet wt. x # survi.)-(Avg. initial wet wt. x initial # fish))/(initial# fish).

Table III. Fatty acid profiles as expressed as mean fatty acid methyl esters of 24-h freshly hatched RAC *Artemia* (Reference *Artemia* cysts), San Francisco Bay (SFB) and Great Salt Lake (GSL) in the present study.

Fatty acid methyl esters	RAC	SFB	GSL
14:0	1.61	0.68	0.53
16:0	12.59	11.42	9.22
16:lω9	0.28	0.32	0.48
16:lω7	13.30	5.37	3.64
18:0	4.48	2.84	3.82
18:1	36.10	30.80	29.17
18:2ω6	9.46	8.65	9.45
18:3ω3	1.70	21.78	26.83
20:1	0.37	0.37	-
22:1	0.59	0.77	-
20:5ω3	7.44	3.44	0.66
22:6ω3	0.04	-	-

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Table 1. Linear regression equations for the PCB congeners bioaccumulated in fish larvae when exposed to 1.0 ppb PCB contaminated water or fed with 1.0 ppb PCB contaminaed *Artemia* for 20 days, where y = PCB levels (ppb, wet wt. basis); x = days.

PCB congener	*Linear Regression Equations*	
	Exposed 1.0 ppb PCB contaminated water	Fed with 1.0 ppb PCB contaminaed *Artemia*
2,2'-PCB	$y = 18.08x - 38.4$ ($r^2 = 0.91$)	$y = 11.26x - 20.4$ ($r^2 = 0.95$)
2,2',4,4'-PCB	$y = 10.30x - 5.2$ ($r^2 = 0.99$)	$y = 6.38x - 12.6$ ($r^2 = 0.94$)
2,2',4,4',6,6'-PCB	$y = 8.14x - 19.8$ ($r^2 = 0.87$)	$y = 5.7x - 16$ ($r^2 = 0.86$)

第二階段(Phase II): 重金屬在水產幼苗經由食物鏈之轉移與蓄積

在水生食物鏈中當作食餌最基本與微小體積之滋養層(Trophic level) 浮游生物，例如:植物(Phytoplankton)與浮游動物(Zooplankton)，對於污染物有極其顯著之吸附性質。因此污染物在水生環境生態上被吸附然後經由食物鏈方式往上之傳遞轉移(Transfer)至較高層掠食生物佔有極其顯著與重要之因素。本研究第二階段(Phase II)計劃預計以第一階段(Phase I)幼豐年蝦食餌從水環境中吸附重金屬污染物之預先得知數據，接續將此污染之幼豐年蝦餵食水產幼魚苗-吳郭魚(*Chanos chanos*)，以探討重金屬污染物經由食物鏈轉移所造成此水產幼苗之蓄積程度。本研究計劃預計以實驗室模擬方式，依不同幼豐年蝦污染濃度與密度為組別，然後以幼吳郭魚苗之生長餵食不同過程時間，分別餵食不同污染濃度水缸組別之幼豐年蝦，然後於不同餵食期間收集餵食不同豐年蝦污染濃度之幼吳郭魚苗，再進行重金屬撿測分析，同時亦依幼魚苗成長過程，觀測可能經由污染食物餵食而可能遭受生長(体長與体重)與存活率之不同程度影響。

預期整個進行步驟預計摹擬Wang and Simpson (1996)方法如下；

1. 使用材料:

   重金屬，Cu、Pb、Zn，各1,000 ppm 標準品預計購自於 Sigma Co.，以30‰過濾海水鹽度加過濾淡水稀釋配製成各0.01、0.1、1.0、10 ppm 之5‰鹽度標準液，新鮮豐年蝦保久卵將購自本地零售商，幼魚苗-吳郭魚(*Chanos chanos*)將採用沾壁(PL 3)至青筋仔(PL 6-7)階段，豐年蝦卵於30‰過濾海水照光打氣24小時內孵化成幼蝦餌，幼魚苗、新鮮30‰過濾海水與過濾淡水將取自台南七股水產試驗分所。

2. 幼豐年蝦在重金屬之污染蓄積:

24小時內孵化成幼蝦餌以過濾網篩出，準確計算幼蝦餌依約每25、50、100、200隻/mL之密度，轉移投入已配製不同0.01、0.1、1.0、10 ppm 重金屬濃度之5‰鹽度250 mL標準液培養缸組別中，不同密度幼蝦餌分別在不同重金屬濃度2、4、8、16、24小時照光打氣暴露污染，不同暴露污染期間結束後以篩網收集幼蝦餌、並適當稱重以活餌投入培養缸中以以供餵食幼魚苗之用。

3. 重金屬污染之幼豐年蝦餵食幼魚苗之食物鏈轉移與蓄積培養:
幼魚苗每日投與稱重之污染幼豐年蝦活餌，依培養缸容量與幼魚苗密度打氣培養，每兩日吸取培養缸底部排泄物與死亡之活餌或幼魚苗，並換加1/2乾淨5‰鹽度水質。培養結束後依不同餵食重金屬濃度與成長過程組別，收集幼魚苗稱重、冷凍乾燥並儲存於-20°C以進一步化學分析，所有實驗組別與對照組別皆同時採三重覆以求平均值。

4. 重金屬之化學分析:
所有冷凍乾燥幼魚苗樣品將以設立於本食品衛生系之Questron Q-1000「微波消化爐」加濃HNO_3/HCl設定溫度與時間，直到分解樣品成透明澄清液，再以0.1N HCl稀釋至定量。再使用本校環境工程衛生系之火焰原子吸收光譜儀(GBC-908, flame AA spectrophotometer)進行重金屬之定量分析，並依重金屬標準液取得之線性迴歸曲線求出各檢體之準確含量與濃度。

5. 數據分析:
針對化學分析所得各組別之重金屬濃度與結合幼魚苗体長、体重與存活存活數據，以本食品衛生系之SigmPlot電腦套裝軟體做繪圖與統計分析其不同蓄積程度之影響與顯著性。

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第一階段(Phase I)：不同重金屬在幼豐年蝦(*Artemia* sp.)食餌之蓄積

豐年蝦(*Artemia* sp.)由於在水產養殖上為極廣泛的被使用在孵育魚貝幼苗的最佳生物活餌料，又由於此幼食餌本身極具耐污染性，在極低鹽度之條件下亦有高的存活率，實驗證明此幼活餌在低鹽度與廢污水中仍可吸附不同高低程度之重金屬與有機氯污染物，因此此幼食餌極為可能同時從水環境中吸附污染物而傳遞給被餵食之水產養殖魚貝幼苗，而有可能加深其污染濃度。本研究第一階段(Phase I)計劃預計以實驗室模擬方式配合水域中重金屬撿測值，依不同重金屬污染物種類、濃度、時間，與幼豐年蝦在重金屬污染液之不同密度與時間，組合不同暴露試驗條件組別，分析豐年蝦所造成重金屬之蓄積程度、計算生物濃縮係數(BCF)、與撿測毒性與存活影響。

預期整個進行步驟預計摹擬Wang and Simpson (1996)方法如下；

1. 使用材料:

重金屬，Cu、Pb、Zn，各1,000 ppm 標準品預計購自於 Sigma Co.，以30‰過濾海水鹽度加過濾淡水稀釋配製成各0.01、0.1、1.0、10 ppm 之5‰鹽度標準液，新鮮豐年蝦保久卵將購自本地零售商，豐年蝦卵於30‰過濾海水照光打氣24小時內孵化成幼蝦餌，新鮮30‰過濾海水與過濾淡水將取自台南七股水產試驗分所。

2. 幼豐年蝦在重金屬之污染蓄積:

24小時內孵化成幼蝦餌以過濾網篩出，準確計算幼蝦餌依約每25、50、100、200隻/mL之密度，轉移投入已配製不同0.01、0.1、1.0、10 ppm 重金屬濃度之5‰鹽度250 mL標準液培養缸組別中，不同密度幼蝦餌分別在不同重金屬濃度2、4、8、16、24小時照光打氣暴露污染，期間依不同濃度與時間組別取樣在顯微鏡下測量体長與計數存活率。不同暴露污染期間結束後以篩網收集幼蝦餌、稱重、冷凍乾燥並儲存於-20°C以進一步化學分析，所有實驗組別與對照組別皆同時採三重覆以求平均值。

3. 重金屬之化學分析:

所有冷凍乾燥幼蝦樣品將以設立於本食品衛生系之Questron Q-1000「微波消化爐」加濃HNO_3/HCl設定溫度與時間，直到分解樣品成透明澄清液，再以0.1N HCl稀釋至定量。再使用本校環境工程衛生系之火焰原子吸收光譜儀(GBC-908, flame AA spectrophotometer)進行重金屬之定量分析，並依重金屬標準液取得之線性迴歸曲線求出各檢體之準確含量與濃度。

4. 數據分析:

針對化學分析所得各組別之重金屬濃度與結合幼蝦体長與存活存活數據，以本食品衛生系之SigmPlot電腦套裝軟體做繪圖與統計分析其不同蓄積程度之影響與顯著性。

重金屬造成近海沿岸環境嚴重污染，使水產動植物之存活率與繁殖率有不同程度的抑制影響，並進而呈現毒性而造成生態破壞與水產食物之不堪食用問題，已有數多文獻與實驗報導(Macdonald et al., 1988; Emson and Crane, 1994; Wang et al., 1996)。而過去二、三十年來台灣隨著經濟快速成長，工商業發達，工廠也隨之林立，其中重金屬例如銅、鎘、鋅、鉛、、、等經由定點或非定點廢棄物之排放，透過渠道、河川、湖泊而排放注入海域，使近海水產養殖魚貝蝦受到輕重不等污染程度之影響，許多種水產品例如牡蠣、文蛤、蜆、、、等底棲魚貝類，亦已監測撿驗出含數種極高濃度之重金屬(Hung et al., 1983; Hung, 1988)，其中又以1989年於二仁溪畔水產養殖之「綠牡蠣」高含 4,401 ± 79 ppm 之銅濃度最受舉世矚目(Su et al., 1986; Han and Hung, 1990; Han et al., 1993)，雖然此飽受銅金屬污染之水產品在1990年後有濃度逐漸下降之趨勢，但其平均銅濃度 2,194 ± 212 ppm 對於長期攝取此水產品之本土消費者族群仍存在高潛在性之衛生與健康危害(Han et al., 1994)。根據文獻報導，加上其它重金屬例如砷、汞、、、在水產養殖養殖魚貝蝦之被分析撿驗出(邱等, 1990)，因此已證實重金屬為台灣本土水生環境中最為污染嚴重之課題(Han and Hung, 1989; Han and Hung, 1990)。

雖然水中之生物對於環境污染物蓄積通常經由三種管道；從水層進入鰓之直接分配(partitioning)，表皮吸收(absorption)與食餌(diets)，而在此不同蓄積過程之

管道，又常隨生物種類、密度、生活習性與污染物之濃度有密切關係(Scura and Theilacker 1977; Swartz and Lee, 1980; Borgmann et al., 1990)。雖然這些環境污染物在水生環境中濃度已被稀釋，而又由於污染物之疏水性質，一旦進入水域後即迅速吸附於微粒物質但仍會因吸附或吸收作用而使生物蓄積(Bioaccumulation)濃度增加，而再進一步被幼小動物掠食而傳遞並濃縮數千上萬濃度於較上層之食物鏈(Food Chain)之掠食生物(Predators)。因此在食物鏈中最基本與體積微小之滋養層(Trophic level)生物，例如:浮游植物(Phytoplankton)與浮游動物(Zooplankton)，在整個水生生態系統上對於污染物之吸附與往上傳遞給較高層之生物佔有極其顯著與重要角色。

而一些文獻報導顯示，對於極疏水性之污染物例如有機氯化合物係以食餌為主要方式，從最底部之滋養層向上至不同掠食動物之食物鏈，進而逐以傳遞增高其蓄積濃度(Thomann and Connolly, 1984; Rasmussen et al., 1990; Clark and Mackay, 1991; Wang and Simpson, 1996; Wang, 1998)。此基本供食者很容易地從水中蓄積高濃度的污染物，例如PCB、DDT及其它一些有機化合物而傳導到水生食物鏈中較高等的掠食動物(Predators)，使魚貝類的蓄積濃度增高;並造成這些掠食動物不同程度的存活率與繁殖率(McLean et al., 1987; van Sprang et al., 1991)。根據報導，浮游藻類在不同有機氯污染情形，可經由食物鏈關係而提升上一滋養層掠食者之濃度並顯著造成污染程度之濃縮(Lester and McIntosh, 1994)。對於常見之水產品，Wang et al.（1998)以本土極普遍之微矽藻*Nannochloropsis* 和 *Isochrysis* 餵食濾食性-文蛤三天後，可顯著地個別蓄積多氯聯苯濃度高達318.81 ppm 與22.55 ppm(依乾重計算)。Thomann and Connolly (1984)經由生態方式推算出90-99%之PCBs在水生動物體內係經由食物鏈方式而導致。Westin et al (1985)之分析數據表示以不同原有由母體而來之污染*Artemia*品種餵食幼條紋鑪(striped bass larvae)，可造成餵時後之此幼魚苗呈現低存活率與較高有機氯殘留。Wang and Simpson (1996)之研究亦報導在不同DDTs濃度預先污染之豐年蝦在餵食一個月大之溪鱒(brook trout)24天後，較高之污染濃度可顯著造成溪鱒蓄積較高之DDTs程度與較高之豐年蝦生物濃縮係數(Bioconcentration Factor, BCF)。這些文獻數據在在顯示有機污染物在水生環境生態上被吸附然後經由食物鏈方式往上之傳遞轉移(Transfer)佔有極其顯著與重要之因素。

而有關研究報導對於重金屬污染物在水生環境中經由滋養層之食物鏈方式蓄積與轉移較高等之掠食者，尤其在最底層生物之蓄積所知尚欠闕。依據Willis and Sunda (1984)對於兩種美洲海洋魚類之實驗，推估78~82%之^{65}Zn總蓄積量係由食物而來。Han and Hung (1990)亦報導牡蠣在污染水生實驗環境中曝露兩個禮拜後，銅金屬在此本土水產品之總蓄積量被預估為 214 μg/g day。然而這些研究之報導大多非反應實際水生環境之污染物濃度及確切生態上餌料至掠食動物所需求之時間階段。

依據其它一些文獻報導，豐年蝦(*Artemia* sp.)由於它容易取得、孵化簡單、大小適當與營養價值極高，因此至今在水產養殖上仍極廣泛的被使用在孵育魚貝幼苗的最佳生物活餌料(Simpson et al., 1983)。又由於豐年蝦本身極具耐污染性，

實驗證明此幼活餌在低鹽度與廢污水中仍可吸附不同高低程度之重金屬與有機氯污染物(MacRae and Pandey, 1991; Wang and Lee, 1997; Wang et al., 1997)。雖然水產魚貝幼苗可由水環境中直接分配或經由表皮吸收而造成其蓄積污染物之提升，然而幼苗對賴以存活與成長之餌料需求亦不可欠缺，因此對於在水產養殖極為廣泛使用之豐年蝦活餌亦可能同時從水環境中吸附污染物而傳遞給被餵食之水產魚貝幼苗，而有可能加深其污染濃度。

目前對於重金屬污染物在基本生物餌料蓄積情形，尤其豐年蝦對於重金屬污染物蓄積濃度的情形或毒性的承受所知有限。因此針對重金屬污染物在此基本水生餌料蓄積的不同程度，進而向上影響到本土整個或局部食物鏈的生態與蓄積情形，從整個水生食物鏈中最底層生物蓄積的基本探討實有其瞭解的必要。

為了要瞭解重金屬污染物在較次高等滋養層水生動物，例如高經濟價值之魚蝦幼苗之蓄積機構或轉移程度，實有必要對於最基層之微小浮游食餌所造成魚貝幼苗之蓄積程度開始進行一番研究與探討。因此本研究計劃預計模擬實際水生環境重金屬污染之情形，第一階段(Phase I)依不同重金屬污染物種類、濃度與時間在此基層之微小浮游食餌豐年蝦(*Artemia* sp.)所造成之蓄積程度、生物濃縮係數(BCF)、毒性與存活影響;與第二階段(Phase II)再以此重金屬蓄積不等濃度之浮游食餌餵食本土常見之水產養殖幼苗-吳郭魚(*Chanos chanos*)，依不同重金屬污染物種類、濃度、餵食時間與食餌密度，著眼於經食物鏈轉移重金屬所造成此水產幼苗之蓄積程度、生物蓄積係數(Bioaccumulation Factor, BAF)、毒性與存活率之不同程度影響。

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
執行期間：87 年 8 月 1 日至 88 年 7 月 31 日
主持人：王瑞顯　　執行機構及名稱：私立嘉南藥理學院 保健營養系

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 μg 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50μg mL^{-1}組別於2小時至22小時分別吸附37μg g^{-1}與48μg g^{-1}(濕重基準)之濃度;鎘金屬之污染亦以50 μg 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 μg mL^{-1} within different time periods. After 2-hr contamination, significant increases of the Pb concentrations in *Artemia* were found to be 37 μg mL^{-1} and 48 μg 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 μg 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

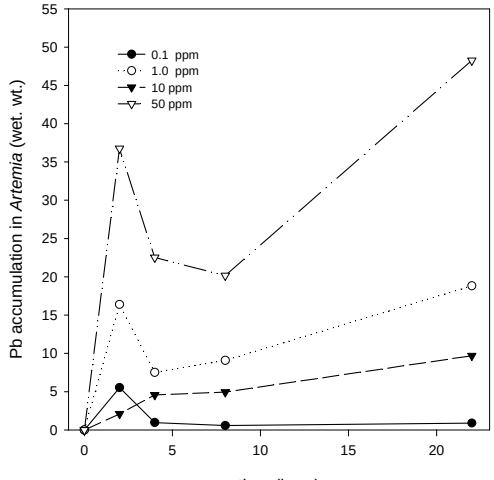
* Means ± standard deviation.

§ 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 μgmL^{-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.



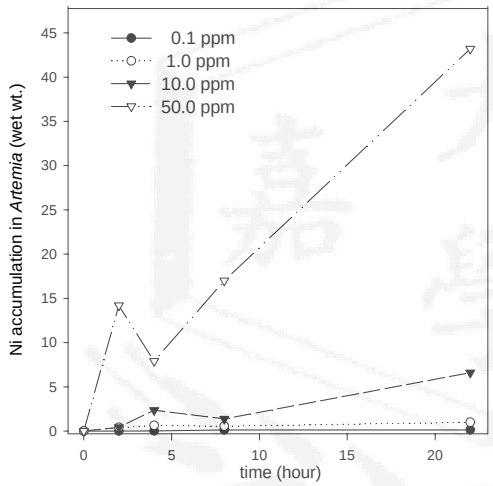


Fig. 2. Accumulation of Cd in *Artemia* after exposure to various Ni concentrations within 22 hr.

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