行政院國家科學委員會專題研究計畫　成果報告

台灣烏腳病疫區地下水養殖虱目魚之砷累積及風險評估研究

計畫類別：個別型計畫
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行政院國家科學委員會補助專題研究計畫 **■ 成 果 報 告**
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台灣烏腳病疫區地下水養殖虱目魚之砷累積及風險評估研究

Bioaccumulation and Risk Assessment of Arsenic in Milkfish (*Chanos chanos*) from Farms using Groundwater in Blackfoot Disease Area, Southwest Taiwan

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成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：南華大學　通識教學中心

中　華　民　國　九十二　年　十　月　三十　日

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Bioaccumulation and Risk Assessment of Arsenic in Milkfish (*Chanos chanos*) from Farms using Groundwater in Blackfoot Disease Area, Southwest Taiwan

中文摘要

本計畫針對烏腳病疫區地下水養殖虱目魚進行砷生物累積、急性毒及風險評估研究。根據野外採樣之養殖魚類及池水含砷量，求得虱目魚對砷的生物濃縮因子 (BCF) 為 112.66± 17.74。另外，藉由實驗室模擬得知，砷對虱目魚的半致死濃度與時間的關係為 LC_{50} (t) = $134.69t^{-0.7175}$，其中 LC_{50} 為半致死濃度 (μg ml^{-1})；t 為曝露時間 (h)。半數致死時魚體砷含量與水中砷濃度成比，其關係式為 $C_{L,50} = 63.972+0.9202C_w$，其中 $C_{L,50}$ 為半數致死時魚體砷含量 (μg g^{-1})。台灣西南沿海烏腳病疫區居民食用養殖虱目魚而暴露於砷污染之終身致癌風險 (TR) 為：布袋 $2.18\times10^{-04}\pm2.98\times10^{-04}$；義竹 $2.41\times10^{-04}\pm2.56\times10^{-04}$；學甲 $6.07\times10^{-05}\pm 4.48\times10^{-05}$；北門 $1.21\times10^{-04}\pm1.3\times10^{-04}$，其值均大於安全標準 1×10^{-06}，顯示食用以含砷地下水養殖之虱目魚，會對人體造成危害。

關鍵詞：砷，養殖，生物累積，烏腳病，地下水，虱目魚，風險評估

英文摘要：

Bioaccumulation, acute toxicity and risk assessment of Arsenic (As) in cultured milkfish (*Chanos chanos*) from the ponds in blackfoot disease (BFD) area were studied. Bioconcentration factor (BCF) of milkfish was calculate from the ratio of the As concentration in the fish to that in the water of the pond. The tolerance of As toxicity was examined by exposing the fish to varied concentrations ranging from 0 to 1000 μg ml^{-1} As, under laboratory conditions. The resulting value of BCF for As accumulation in the milkfish was 112.66± 17.74. The median lethal concentration (LC_{50} value) for milkfish showed a significant allometric, negatively correlation with the exposure time as LC_{50} (t) = $134.69t^{-0.7175}$, where LC_{50} (t) is the median lethal concentration (μg ml^{-1}) and t is the exposure time (h). Exposure concentrations followed by exposure times to deaths are associated with internal lethal concentrations in fish.

Lethal internal residue of As in fish body that causes 50% mortality ($C_{L,50}$ value) is a liner function of As concentration in water as $C_{L,50} = 63.972+0.9202C_w$, where $C_{L,50}$ is the lethal internal residue of As in fish body that causes 50% mortality ($\mu g\ g^{-1}$) and C_w is As concentration in water ($\mu g\ ml^{-1}$). It showed that the $C_{L,50}$ value increased with C_w; whereas the time to death of the fish was decided by the As concentrations in the target tissues of fish. The TR values of comsuming the milkfish from Pu-Dai, Yi-Chu, Sheh-Cha and Pei-Men were $2.18\times10^{-04}\pm2.98\times10^{-04}$, $2.41\times10^{-04}\pm2.56\times10^{-04}$, $6.07\times10^{-05}\pm 4.48\times10^{-05}$, and $1.21\times10^{-04}\pm1.3\times10^{-04}$, respectively; which were higher than 1×10^{-6}, indicating that the consumption of As-polluted milkfish from BDF area might pose a risk to human health.

Key words: Arsenic, aquaculture, bioaccumulation, blackfoot disease, groundwater, milkfish, risk assessment

INTRODUCTION

Milkfish (*Chanos chanos*) is one of the most commercially important aquacultural species in Taiwan. Most milkfish ponds are located in the southwest coasts of Taiwan, where the inhabitants used to suffer from the blackfoot disease (BFD) (Chen et al. 1985; Lin et al. 2001). BFD, a peripheral vascular disorder, was reported to correlate with the consumption of groundwater that contains high concentration of arsenic (Chen et al. 1980, 1985, 1986). Arsenic has been well documented as one of the major risk factors for black-foot disease as well as cancers of the lung, liver, and bladder among residents in BFD area (Chen et al. 1986). A significant exposure-response relationship between As concentration and the mortality from various cancers has been reported (Wu et al. 1989). Arsenic was known to increase the risk of cancer (Chiou 1995). Chen et al. (1985) demonstrated that both the standardized mortality ratios (SMRs) and cumulative mortality rate for cancers of bladder, kidney, skin, lung, liver and colon in the residents from BFD area were significantly greater than that in the general population in Taiwan.

Nowadays, people living in these areas do not drink water from wells; however, the groundwater is still used for aquaculture (Lin et al. 2001). Milkfish culture needs a high amount (38,000-49,000 ton ha^{-1}) of freshwater; and therefore, the fish in the ponds using groundwater may be contaminated with As. Arsenic was reported to be toxic to fish (Donohue and Abernathy 1999). In addition, As can be accumulated in fish tissues, and humans who consume these tissues may be threatened by As (Lin et al. 2001). Since milkfish is common seafood in Taiwan, ingestion of contaminated fish could result in As accumulation in humans and lead to adverse health effects. Thus, it is important to determine the As content in fish from these culture ponds. The tolerance of milkfish to As toxicity as well as the As accumulation from the ambient water in the fish need to be determined.

The process of accumulation of water-borne chemicals by fish and other aquatic animals through nondietary routes is defined as bioconcentration (Franke et al. 1994). The bioconcentration factor (BCF), relating the concentration of a chemical in water to its concentration in the aquatic animal at steady-state equilibrium, is generally used to estimate the propensity of an organism to accumulate chemicals (Franke et al. 1994). Fish are targets for BCF assessments because of their importance as a human food source and the availability of standardized testing protocols. Measured or predicted BCFs are a requisite component for both environmental and human risk assessment. In this work, the bioaccumulation of As in milkfish was studied to assess the potential hazards of As in the aquacultural environment. The acute toxicity of As and the BCF value of the fish were determined.

MATERIALS AND METHODS

Samples of juvenile milkfish (range 4.0-6.0 cm in length and 0.41-1.41 g in weight) and ambient water were obtained from 9 culture ponds in BFD area. Three fish and three 500 ml water samples per pond were collected. The milkfish were placed on ice immediately, and kept at 4°C during transfer to the laboratory. The water samples were fixed by adding 5 ml 1N HNO_3.

A total of 80 non-polluted milkfish (body lengths range from 4 – 6 cm) were collected from the Tainan Fisheries Research Institute for laboratory exposure experiments. The fish were transferred into 6 tanks of approximately 80 L volume, containing 60 L of filtered water. The temperature, salinity, pH and dissolved oxygen were maintained at 24.0±0.5°C, 0, 7.0±0.2 and 8.0±0.1 μg ml^{-1}, respectively; which were similar to the conditions of the culture ponds in BFD area (24.0±3.2°C, 0, 7.2±1.1 and 8.0±0.9 μg ml^{-1}, respectively). The milkfish were held for 2 weeks before they were exposed to As.

Acute toxicity assays were conducted to determine the median lethal time (LT_{50} value) and lethal time (LT_{100} value), as well as the median lethal concentration (LC_{50} value) for milkfish. The tolerance of As toxicity was examined by exposing fish to As concentrations ranging from 0 to 1000 μg ml^{-1}. The concentrations were prepared from arsenite (Na_3AsO_3). Eight healthy milkfish were exposed to As for each concentration. The mortality was recorded every 1 h for the first 12 h and every 3 h thereafter up to 4 d. Death was defined as cessation of opercular movement; and dead fish were removed from the tanks immediately and kept at -20°C before they were analyzed. During the experiments, water samples were taken daily from each tank, acidified by adding 5 ml 1N HNO_3, and stored for analysis of As concentration. After sampling, the tank water was renewed immediately to maintain the As concentration.

Samples were sent to the Super Micro Mass Research and Technology Center, Cheng Shiu Institute of Technology for analysis of total As. The frozen muscle of milkfish were dehydrated in a dryer (40°C) for 96 h and grounded into powder. Aliquots of dry muscle powder weighing 0.5 g were placed into a 250 ml beaker. Nitric acid (65%, 10 ml) was added and then covered with a glass for an overnight digestion.

After the initial digestion, the beaker was heated with a water bath at 70-80°C for 2-4 h to reduce the total volume to 1-2 ml. This volume of solution was transferred to a volumetric flask (50 ml). The rinsed solution (5 ml of 0.01N of HNO_3) for the watch glass was also added

to the flask. The flask was then filled with 0.01N of HNO_3 to make a 50 ml of final solution. After filtration, this 50 ml solution was transferred to test tubes for As analysis. Arsenic analysis was carried out by using an Agilent 7500a ICP-MS. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference materials (DORM-2, Dogfish Liver-2-organic matrix, NRC-CNRC, Canada). Recovery rates ranged from 95% to 97%.

The bioconcentration factor derived using dry weight (BCFD), relating the concentration of As in water to its concentration in the fish was used to estimate the propensity of accumulating ability of milkfish: $\mathrm{BCFD} = C_b / C_w$, where C_b (μg g^{-1} dry wt) is the As concentration in biota, i.e., milkfish; C_w (μg ml^{-1}) is the As concentration in water.

Table 1. The median lethal time (LT_{50} value), lethal time (LT_{100} value) and the median lethal concentration (LC_{50} value) for milkfish acutely exposed to waterborne As

Nominal As concentration (μg ml^{-1})	Mean (SE) assayed As concentration (μg ml^{-1})	LT_{50} value (h)	LT_{100} value (h)	LC_{50} value (μg ml^{-1})
0	ND[a]	> 96	> 96	15.20
5	4.8 (1.1)	> 96	> 96	40.80
10	10.2 (2.3)	61.33	> 96	89.40
15	14.6 (3.7)	20.50	> 96	86.02
20	19.5 (2.4)	23.25	34.58	117.72
25	25.5 (3.0)	4.50	20.12	97.38
50	51.9 (2.2)	2.33	14.04	94.42
00	104 (10.1)	1.00	1.26	173.63
250	237 (17.2)	0.52	0.90	284.86
500	487 (16.5)	0.20	0.25	550.57
1000	1014 (22.2)	0.10	0.12	971.14

[a] ND: not detectable

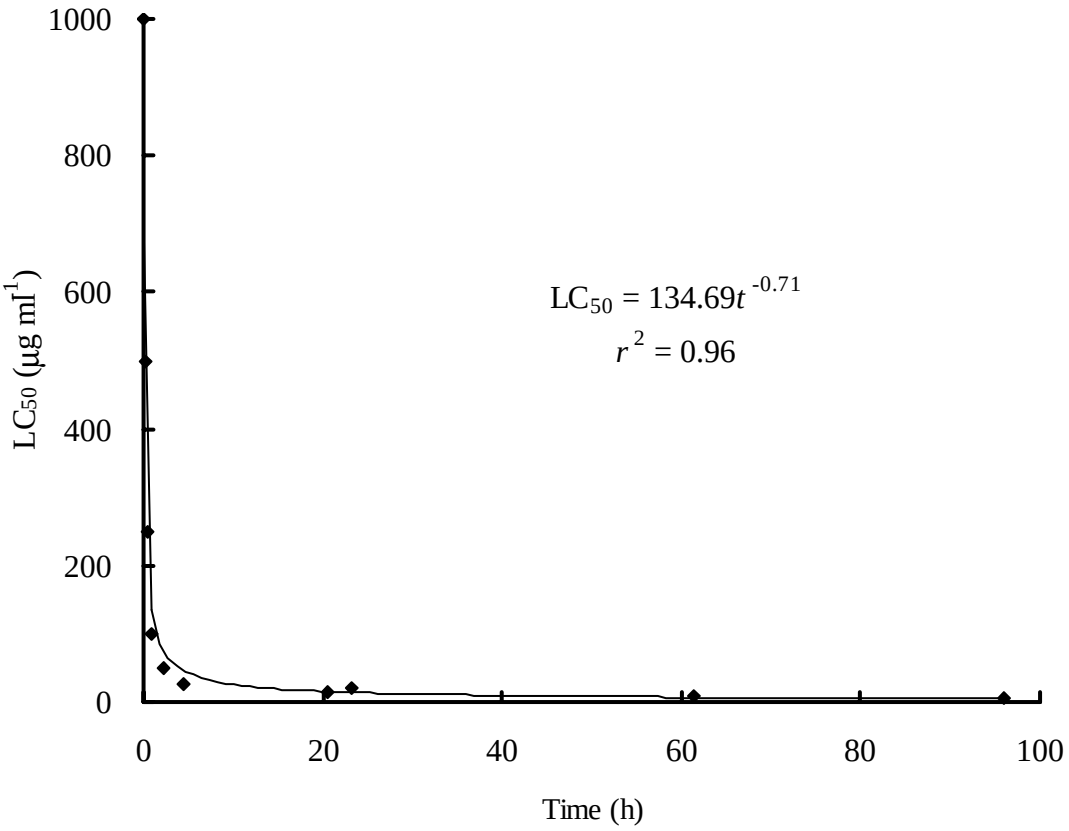

Figure 1. Plot of the relation between the median lethal concentration (LC_{50} value) for milkfish and the exposure time.

RESULTS AND DISCUSSION

Field samples showed that the level of As in the pond water was 0.027 ± 0.001 μg ml^{-1} (Mean ± SD) and that in milkfish was 15.20 ± 5.10 μg g^{-1} dry wt. The value of BCFD of milkfish was 556.16 ± 187.98 dry wt.

The actual arsenic concentrations assayed, the median lethal time (LT_{50} value) and lethal time (LT_{100} value) are listed in Table 1. The median lethal exposure concentration (LC_{50} value) for milkfish showed a significant negative relation to exposure time: LC_{50} (t) = $134.69t^{-0.7175}$ (Fig. 1), where LC_{50} (t) is the median lethal concentration (μg ml^{-1}) and t is the exposure time (h). Milkfish can tolerate As concentration in water higher than 10 μg ml^{-1} for more than 96 h.

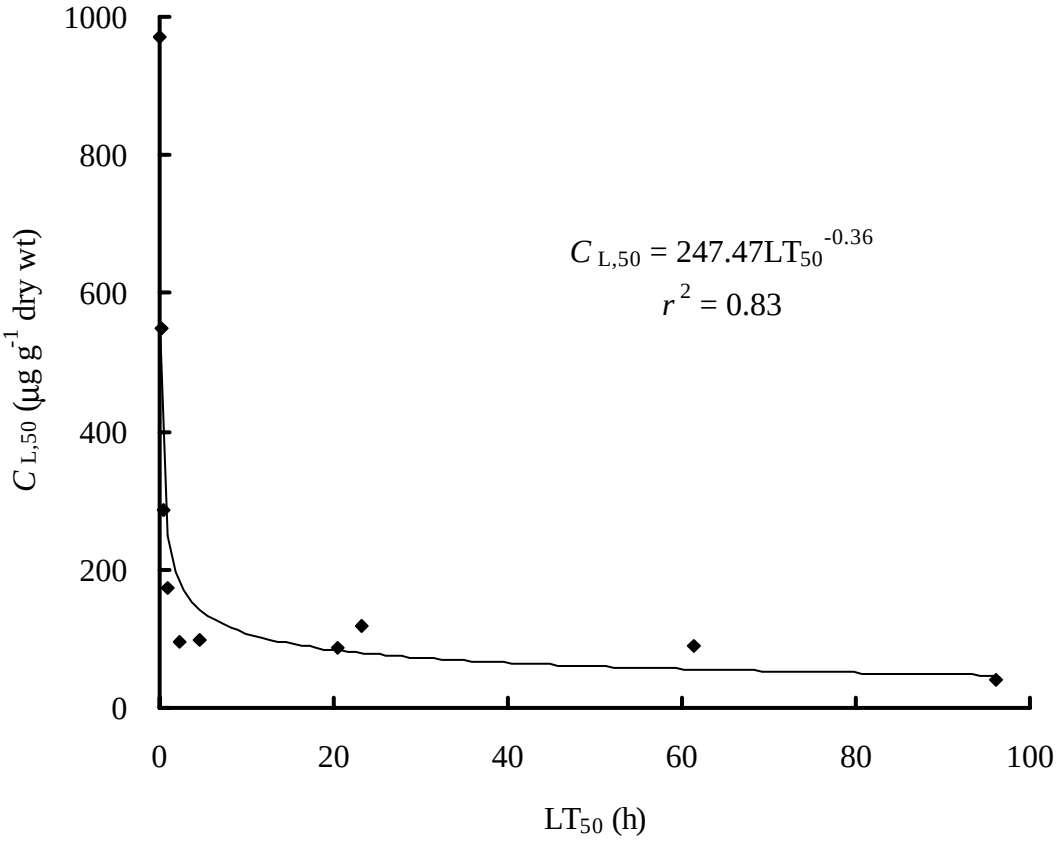

Figure 2. Plot of the lethal internal residue of As in milkfish body that causes 50% mortality ($C_{L,50}$ value dry wt) and the median lethal time (LT_{50} value).

Lethal internal residue of As in fish body that causes 50% mortality ($C_{L,50}$ value) is negatively related to median lethal time (LT_{50} value) as $C_{L,50} = 247.47LT_{50}^{-0.36}$ (Fig. 2), $C_{L,50}$ is the lethal internal residue of As in fish body that causes 50% mortality (μg g^{-1} dry wt) and LT_{50} is the median lethal time. The lethal internal residue of As in fish body that causes 50% mortality ($C_{L,50}$ value) is a linear function of As concentration in water as $C_{L,50} = 63.97+0.92C_w$ (Fig. 3), where C_w is As concentration in water (μg ml^{-1}).

Due to dense human population and industrial expansion, the aquatic environments of Taiwan are suffering an ever-increasing impact from human activities (Lin and Liao 1999). In spite of natural As in the

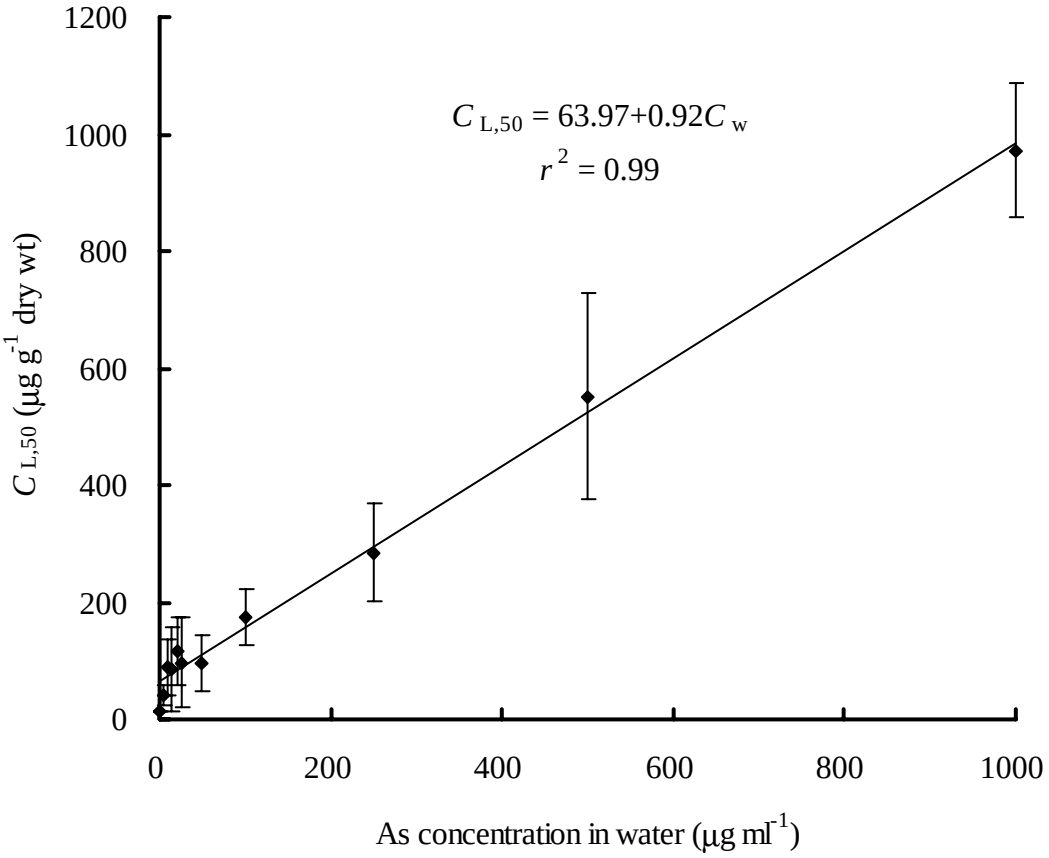

Figure 3. Plot of the lethal internal residue of As in milkfish body that causes 50% mortality ($C_{L,50}$ value dry wt) and the As concentration in water (μg ml^{-1}).

underground water, pollutants of As were discharged into the underground water from polluted rivers,

sewage outfalls or local industries because of those human activities. The resulting data indicated that the pond waters in BFD area are contaminated by As. The value of BCFD showed that the milkfish taken from the ponds accumulated a high level of water-borne As. The high tolerance of As for milkfish showed that the fish can accumulate high concentrations of As before they are harmed.

Thus far no adverse effect on health of the people in BFD area due to exposure to As contaminated seafood is evidenced. A wider study

involving As analyses of milkfish from non-BFD areas, as well as other aquacultural products should be investigated to assess the extent of As contamination in seafood. The daily intake of As compounds from fish and other aquacultural products need to be studied to determine the relative risk of As toxicity in the population.

The TR values of comsuming the milkfish from Pu-Dai, Yi-Chu, Sheh-Cha and Pei-Men were $2.18\times10^{-04}\pm2.98\times10^{-04}$, $2.41\times10^{-04}\pm2.56\times10^{-04}$, $6.07\times10^{-05}\pm4.48\times10^{-05}$, and $1.21\times10^{-04}\pm1.3\times10^{-04}$, respectively; which were higher than 1×10^{-6}, indicating that the consumption of As-polluted milkfish from BDF area might pose a risk to human health.

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