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Bioaccumulation of lead nitrate in Red Swamp Crayfish (*Procambarus clarkii*)

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Abstract

Crayfish were exposed to intermediate concentrations of lead nitrate (150 $\mu g l^{-1}$ and 1100 $\mu g l^{-1}$) for periods up to 7 weeks. Lead clearance was monitored at 3 weeks following the 7 week exposure to the lower concentration. Lead bioaccumulation was demonstrated to be a time- and dose-dependent phenomenon in gills, hepatopancreas and abdominal muscle, but not the exoskeleton. The tissue concentrations of lead in soft tissues, in decreasing order were gills > hepatopancreas > muscle > hemolymph. Lead clearance was significant in all tissues evaluated except the hepatopancreas, the organ of metal storage and detoxification. © 1997 Elsevier Science B.V. © 1997 Elsevier Science B.V.

Keywords: Red Swamp Crayfish (*Procambarus clarkii*); Lead; Bioaccumulation

1. Introduction

Contamination of our environment with heavy metals from industrial and household sources has become a significant issue with respect to our ecosystems as well as human health. A primary consideration is the bioaccumulation of metals such as lead, cadmium, chromium etc. into tissues of both plants and animals which result in toxic effects [1,2]. These metals have long biological half-lifes in animal tissues, which in the case of lead

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is 10–30 years in bone tissue [3,4]. Lead can produce a wide range of pathologies associated with hemoglobin synthesis, renal function, nervous system function and reproduction. Lead continuously accumulates in the bone of humans over the course of exposure which subsequently provides a pool of lead for its slow release over an extended period of years [5,6]. Lead exposure and accumulation in children are especially serious in that lead is incorporated into the matrix of rapidly growing bone [7,8]. Consequently, upon long term, low level release, there may be sustained biological effects such as neurological damage.

Exposure to lead can occur through food, water and/or air. The introduction of lead into the food chain can thus provide a potential pathway for complete exposure to this metal. Aquatic species such as fish and crayfish are part of the food chain which have previously been studied for bioaccumulation of lead from the environment. Crayfish are unique in that they are bottom feeders, scavengers, ubiquitous to wetlands and thus are good biomodels for assessment of contaminants such as lead.

Previous studies in both the field [9–13] and laboratory [14–17] have addressed lead accumulation into tissues of a variety of strains of crayfish following exposure to either very low (20 $\mu g l^{-1}$) or very high (10 000–150 000 $\mu g l^{-1}$) lead concentrations. However, studies addressing bioaccumulation following exposure to lead at intermediate concentrations found in many contaminated environments (100–2000 $\mu g l^{-1}$) have not been conducted. The current study was conducted to evaluate the bioaccumulation of lead in various tissues of the red swamp crayfish (*Procambarus clarkii*) upon chronic exposure to lead in the water at concentrations of 150 $\mu g l^{-1}$ and 1100 $\mu g l^{-1}$.

2. Materials and methods

2.1. Chemicals

Certified atomic absorption spectrometry lead standards (EM Science, Gibbstown, NJ, and Aldrich Chemical, Milwaukee, WI) were used in specimen analysis, in the spiking of the exposure water for the crayfish, as well as for an independent control for validation of the standard curve. Specimens for metal analyses were digested in instrumental grade nitric acid (EM Science Tracepur Plus). Reagents used in the preparation of the matrix modifier for lead analysis (magnesium nitrate hexahydrate [99.995%] and ammonium dihydrogen phosphate [99.999%]) were also obtained from Aldrich Chemical.

Reagents for the hemolymph anticoagulant solution were obtained from the following sources: sodium chloride (S-671) and citric acid (A940) were from Fisher Scientific (Pittsburgh, PA); glucose (G5250), trisodium citrate dihydrate and ethylenediaminetetraacetic acid (E5134) were from Sigma Chemical (St. Louis, MO).

Buffered formalin (1:10) was obtained from Curtin Matheson Scientific (Houston, TX).

Laboratory grade (Type II) water was obtained using a Life Scientific (St. Louis, MO) water purification system (Part 2618S2).

2.2. Test Crayfish

Red swamp crayfish (*Procambarus clarkii*) were purchased from a local vendor and separated according to size and gender. Mature, intermolt, female crayfish with carapaces measuring 20–48 mm in length were used in this study. Twenty to 24 crayfish were maintained in individual plastic aquaria containing 4 l of constantly aerated tap

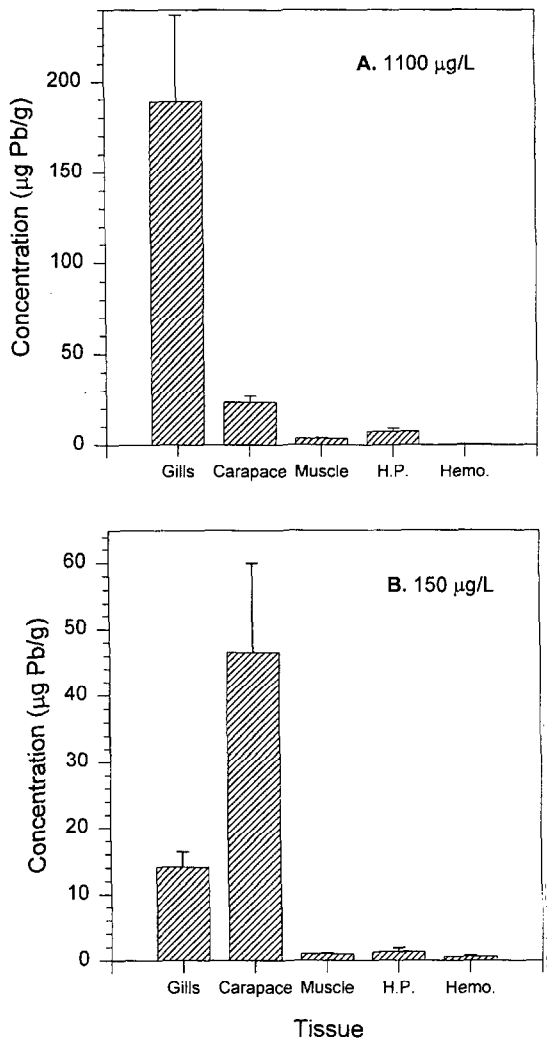

Fig. 1. Tissue-concentrations of lead after 4 weeks of exposure. (A) Tissue-concentrations of lead in crayfish exposed to 1100 $\mu g Pb l^{-1}$, $N = 5$. (B) Tissue-concentrations of lead in crayfish exposed to 150 $\mu g Pb l^{-1}$, $N = 10$. All values are expressed as mean $\pm$ S.E.M. All tissue-concentrations of lead are significantly different from one another except for the hepatopancreas and muscle which exhibit similar lead-concentrations. H.P. = hepatopancreas, Hemo = hemolymph.

Table 1
Three week clearance study after 7 weeks of exposure to 150 $\mu g Pb l^{-1}$

	Lead concentrations ($\mu g g^{-1}$ or $\mu g ml^{-1}$)		
	Time 0	7 weeks	3 weeks clearance
Exoskeleton	0.27 ± 0.05	29.79 ± 10.63	3.77 ± 0.69 [a]
Gills	0.31 + 0.06	13.19 ± 2.78	6.66 ± 0.65 [a]
Hepatopancreas	0.01 ± 0.01	1.69 ± 0.25	1.32 ± 0.45
Abdominal muscle	0.03 ± 0.01	0.43 ± 0.04	0.09 ± 0.03 [a]
Hemolymph	0.01 ± 0.01	0.27 ± 0.10	0.01 ± 0.00 [a]

Values are expressed as mean ± S.E.M.
[a] Concentrations are significantly different ($P \leq 0.05$) from their corresponding 7 week exposure concentrations.

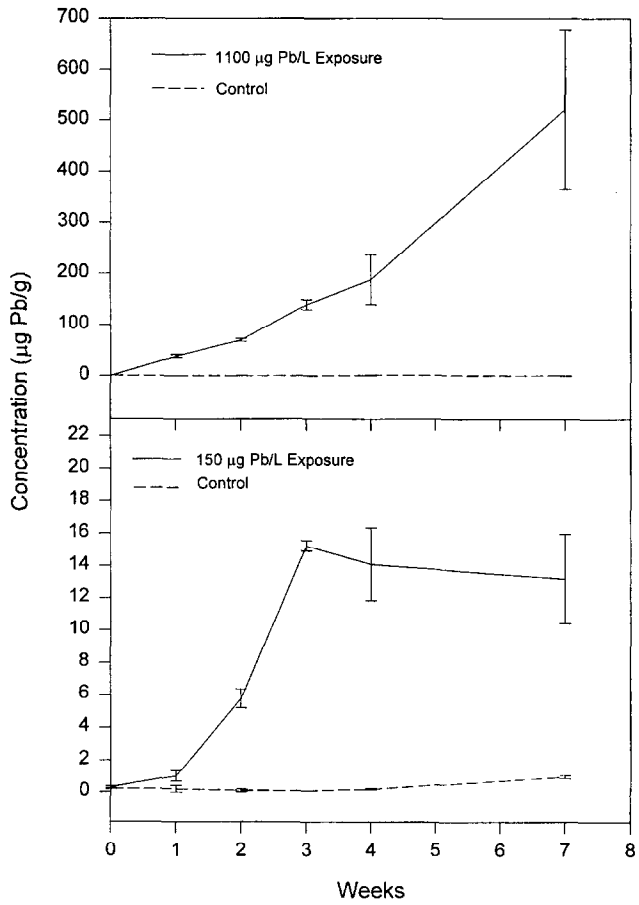

Fig. 2. Lead concentration in gills of crayfish exposed to 1100 $\mu g Pb l^{-1}$ ($N = 5$) and 150 $\mu g Pb l^{-1}$ ($N = 10$). All values are expressed as the mean ± S.E.M.

water which contained < 5 $\mu g Pb l^{-1}$. All crayfish were housed under controlled conditions of temperature (24°C) and light (12 h light/12 h dark). Crayfish were fed commercial crayfish food (People's Moss Gin, Milwaukee, WI) three times a week. All crayfish were housed under the above conditions for 2 weeks before being included in the lead exposure study.

2.3. Lead exposure study

Crayfish were exposed to two different concentrations of lead (150 $\mu g l^{-1}$ and 1100 $\mu g l^{-1}$) in the form of lead nitrate for 1–7 weeks. All lead solutions were prepared in tap water and control crayfish were maintained in tap water. Preliminary studies revealed that lead concentrations in water occupied by crayfish under the conditions

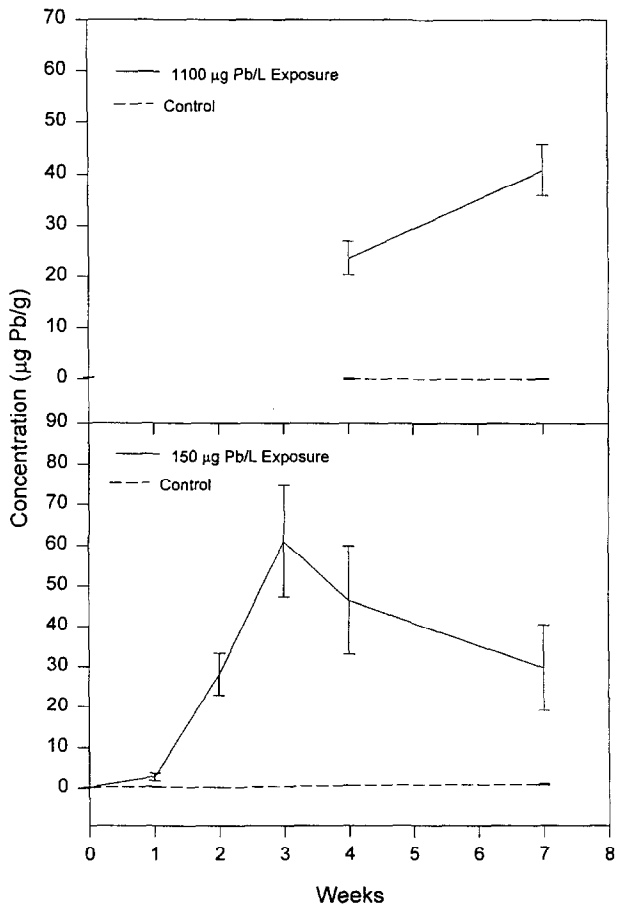

Fig. 3. Lead concentrations in exoskeleton (carapace) of crayfish exposed to 1100 $\mu g Pb l^{-1}$ ($N = 5$) and 150 $\mu g Pb l^{-1}$ ($N = 10$). All values are expressed as the mean ± S.E.M.

described above were substantially reduced (~ 57%) within a 24 h period. As a result, solutions in experimental and control aquaria were changed daily over the duration of the study.

At the end of each exposure period, the following protocol was followed. Crayfish were weighed and hemolymph was collected using a 3 cc syringe coated with hemolymph anticoagulant (0.14 M sodium chloride, 0.1 M glucose, 30 mM trisodium citrate, 26 mM citric acid and 10 mM EDTA adjusted to a pH of 4.6). Volume of hemolymph was recorded (approximately 1.0 ml) and was then added to a plastic tube containing 0.33 ml of the anticoagulant solution. Crayfish were then sacrificed and the following tissues collected and weighed for metal analyses and/or histopathological evaluation: hepatopancreas, gills, abdominal muscle and exoskeleton (carapace).

Crayfish exposed to 150 $\mu g\,Pb\,l^{-1}$ for 7 weeks were placed in tap water for a period

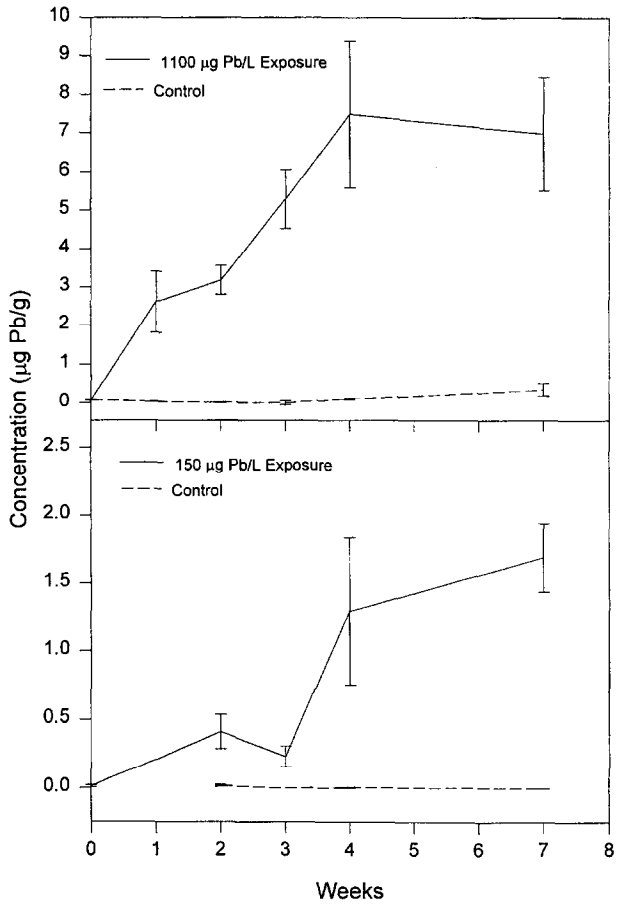

Fig. 4. Lead concentrations in hepatopancreas of crayfish exposed to 1100 $\mu g\,Pb\,l^{-1}$ ($N = 5$) and 150 $\mu g\,Pb\,l^{-1}$ ($N = 10$). All values are expressed as the mean ± S.E.M.

of 3 weeks for a clearance study. Water was changed on a daily basis. At the end of the three weeks, the crayfish were evaluated as described above.

2.4. Lead analyses

Weighed tissues (~ 0.5 g) and hemolymph samples were digested in 10 ml of 17% trace metal nitric acid using a CEM MDS-2000 Microwave Digestion System.

Lead analyses were performed on a Perkin Elmer Model 5200 ZL atomic absorption spectrometer equipped with Zeeman background correction and a graphite furnace with autosampler. High purity argon was used. The composition of the matrix modifier solution was ammonium dihydrogen phosphate (20 $g l^{-1}$) and magnesium nitrate hexahydrate (1 $g l^{-1}$).

The analyses of tissue specimens spiked with lead demonstrated 97% recovery. Lead

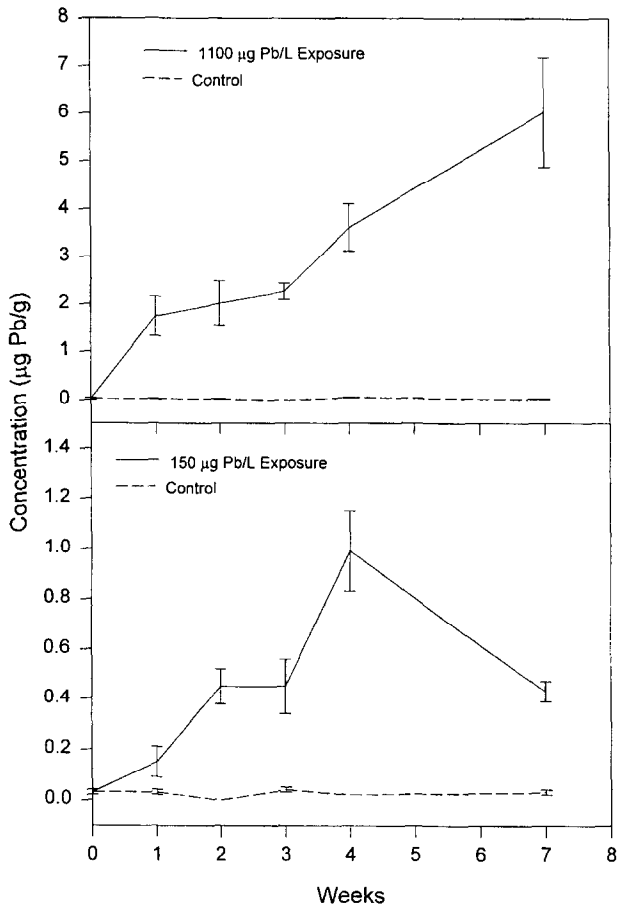

Fig. 5. Lead concentrations in abdominal muscle of crayfish exposed to 1100 $\mu g Pb l^{-1}$ ($N = 5$) and 150 $\mu g Pb l^{-1}$ ($N = 10$). All values are expressed as mean ± S.E.M.

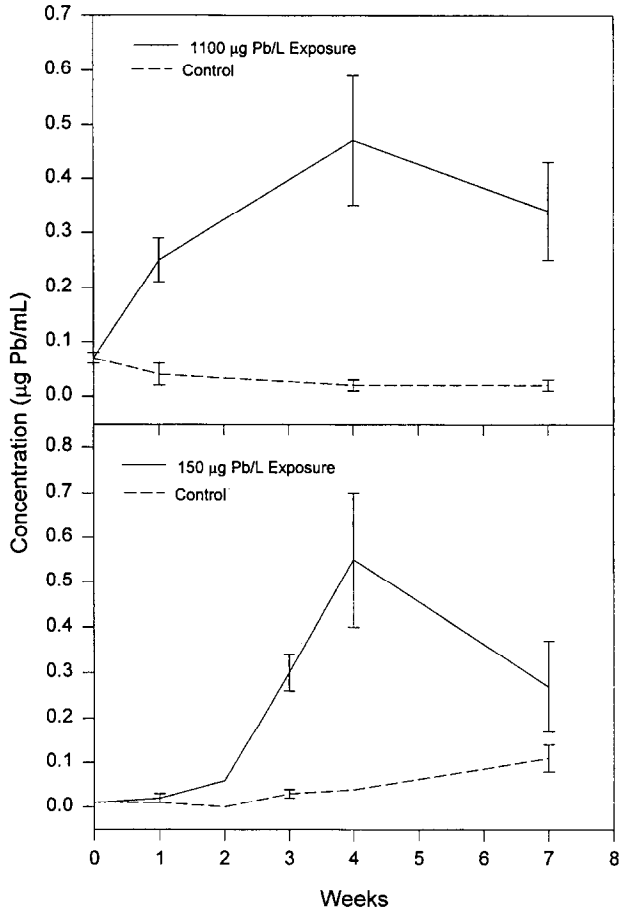

Fig. 6. Lead concentrations in hemolymph of crayfish exposed to 1100 $\mu g\,Pb\,l^{-1}$ ($N = 5$) and 150 $\mu g\,Pb\,l^{-1}$ ($N = 10$). All values are expressed as mean ± S.E.M.

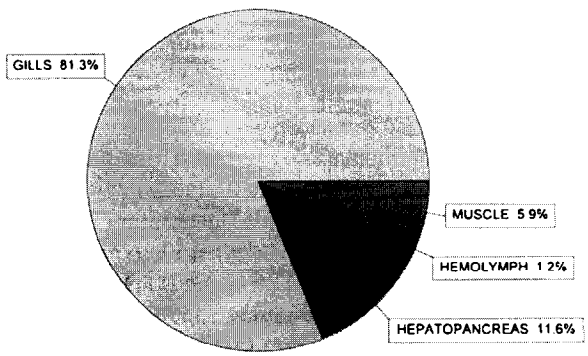

Fig. 7. Distribution of lead in soft tissues of crayfish expressed as a percentage of total soft tissue lead content.

content of the tissues was calculated in terms of μg of lead per g of tissue or μg of lead per ml of hemolymph.

2.5. Histopathology studies

All tissues were fixed in 10% buffered formalin. Tissues were dehydrated, cleared and infiltrated in paraffin using an MVP I Tissue Processor and embedded in paraffin. Tissue sections of 5–7 μm were prepared using a Microm HM 335 E Microtome and affixed to clean glass slides. Mounted sections were stained with hematoxylin and eosin and viewed with a Nikon light microscope.

2.6. Statistical Analyses

Data were analyzed statistically using a two-tailed non-paired *t*-test for single group analyses. Analysis of variance (ANOVA) test was utilized for multi-group data followed by Scheffe's test for post hoc comparisons.

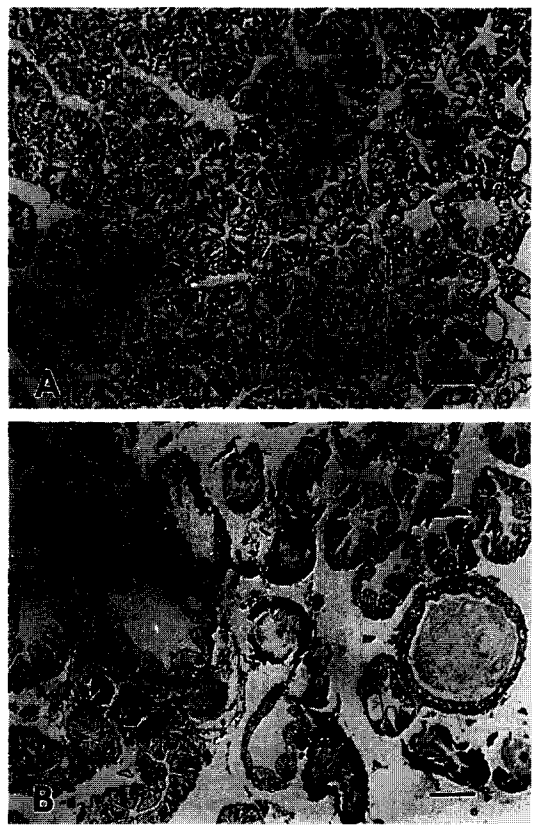

Fig. 8. Hematoxylin and eosin stained section of crayfish hepatopancreas. (A) Section of control hepatopancreas. Only normal appearing tubules are present. (B) Section of hepatopancreas from a crayfish exposed to 1100 μg $Pb l^{-1}$ for 7 weeks. Tubules are in various stages of degeneration. Bar = 30 μm.

3. Results

3.1. Lead accumulation in crayfish tissues

The results of this study demonstrate a dose- and time-dependant bioaccumulation of lead in most of the tissues evaluated. In crayfish exposed to the higher lead concentration (1100 $\mu g Pb l^{-1}$), the order of magnitude of tissue concentrations of lead is gills > exoskeleton > hepatopancreas > abdominal muscle > hemolymph for both 4 and 7 week exposures (Fig. 1A). In the lower lead exposure group (150 $\mu g l^{-1}$) the order of lead tissue concentration is exoskeleton > gills > hepatopancreas > abdominal muscle > hemolymph after 4 and 7 weeks of exposure (Fig. 1B and Table 1). All tissue concentrations within a particular exposure-group after both 4 and 7 week exposures are significantly different from one another except for the hepatopancreas and abdominal muscle which demonstrate similar levels of lead bioaccumulation.

Lead bioaccumulation in gills is time- and dose-dependant (Fig. 2). At the lower

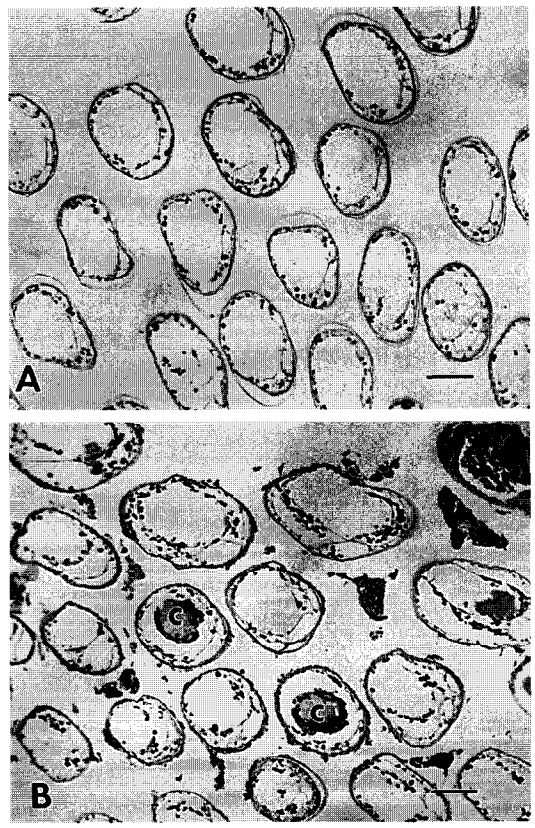

Fig. 9. Hematoxylin and eosin stained sections of crayfish gills. (A) Section of control gill filaments. Only normal appearing filaments are present. (B) Section of gills from a crayfish exposed to 1100 $\mu g Pb l^{-1}$ for 7 weeks. Gill filaments are disorganized and demonstrate the formation of capsules. Bar = 30 μm.

exposure concentration of lead (150 $\mu g l^{-1}$), the gills reach a plateau with respect to accumulation of lead after 3 weeks of exposure, while accumulation continued in the higher lead concentration group throughout the entire 7 week period.

Crayfish exposed to the lower lead concentration (150 $\mu g l^{-1}$) demonstrate a time-dependant accumulation of lead in the carapace through the first 3 weeks of exposure (Fig. 3). After 4 and 7 weeks of lead exposure there was a slight but not significant decrease in lead content from the concentrations observed after 2 and 3 weeks of exposure. The levels of lead measured in the carapace of crayfish after 4 and 7 weeks of lead exposure to the higher lead concentration (1100 $\mu g l^{-1}$) are not significantly different from those concentrations measured in the carapace of crayfish exposed to the lower lead concentration (Fig. 3). Thus, no dose-response accumulation in the exoskeleton (carapace) is observed in this study.

Lead bioaccumulation in the hepatopancreas appears to be time- and dose-dependant (Fig. 4). At the higher lead concentration (1100 $\mu g l^{-1}$), lead accumulation in this organ appears to reach a maximum between 3 and 4 weeks of exposure. At the lower concentration (150 $\mu g l^{-1}$) bioaccumulation of lead continues over the entire 7 week exposure period, although the tissue concentrations between 4 and 7 weeks are not significantly different.

Lead bioaccumulation in the abdominal muscle of crayfish is dose-dependent (Fig. 5). A time-response for accumulation is observed throughout the 7 weeks of exposure to lead in the group exposed to the higher lead concentration (1100 $\mu g l^{-1}$) while crayfish exposed to the lower lead concentration (150 $\mu g l^{-1}$) demonstrate a time-response accumulation only through the first 4 weeks of the study. After 7 weeks of exposure to 150 $\mu g Pb l^{-1}$, this group demonstrates a significant decline in abdominal muscle levels of lead from the 4 week values.

Concentrations of lead in hemolymph reflect circulating levels (Fig. 6). After 4 and 7 weeks of lead exposure, concentrations in hemolymph are not significantly different between the higher (1100 $\mu g l^{-1}$) and the lower (150 $\mu g l^{-1}$) lead exposure groups.

The percentage of the lead present in the soft tissues of the crayfish is presented in Fig. 7. While the exoskeleton would be expected to contain the greatest percentage of total body lead based on the lead concentration and the bulk of this tissue, it was not possible in this study to obtain an accurate value for the total weight of the exoskeleton. The percentage of the total lead in soft tissues is greatest in the gills, followed by hepatopancreas and muscle.

The results of the 3 week study of lead clearance from the tissues of crayfish exposed to 150 $\mu g Pb l^{-1}$ for 7 weeks are seen in Table 1. All tissues, except the hepatopancreas, reveal a significant decrease in tissue concentrations of lead. The most dramatic clearance of lead is observed in the exoskeleton (87% clearance). The other tissues demonstrate the following clearances of lead: gills (50%), abdominal muscle (79%) and hepatopancreas (22%). Hemolymph concentrations of lead returned to pre-exposure levels indicating little or no lead present in the circulation of the crayfish.

3.2. Measurement of lead water concentrations over a 24 h period

Lead concentrations were measured in the water of the higher lead exposure group (1100 $\mu g l^{-1}$) before and after a 24 h exposure period. This was done twice a week

throughout the 7 weeks of the study. The lead concentration decreased approximately 57% in a 24 h interval. For this reason tank solutions were changed daily to maintain exposure concentrations.

3.3. Histopathologies of hepatopancreas and gills

The histopathological studies demonstrate that there was lead induced damage in both the hepatopancreas (Fig. 8) and the gills (Fig. 9) at both lead concentrations tested and occurred by 4 weeks even at the lower concentration of 150 $\mu g l^{-1}$. Degenerating and/or disorganized tubules of the hepatopancreas are observed among normal appearing tubules. Gills collected from lead exposed crayfish exhibited a disorganization of the epithelial cells lining the afferent and efferent branchial vessels of the gill filaments. Capsules are also present in gills of the lead exposed crayfish. No degenerative changes were observed in the hepatopancreas or gills of control crayfish.

4. Discussion

This study demonstrates a dose- and time-dependent bioaccumulation of lead in various crayfish tissues with the only exception being the exoskeleton (carapace). Lead concentrations in gills and muscle after 4 weeks of lead exposure appear to reach saturation at the lower lead concentration but lead continues to accumulate in the gills and muscle at the higher concentration throughout the 7 weeks of exposure. This suggests that the uptake of lead by these two tissues is tissue specific and biphasic. However, saturation of lead concentrations in the hepatopancreas plateaus after 4 weeks exposure at both lead concentrations.

Previous laboratory studies have addressed the accumulation of lead in crayfish tissues [15,17] but very different lead concentrations were used and different protocols were followed.

Pastor et al. [15] evaluated the accumulation of lead in adult, intermolt crayfish (*Procambarus clarkii*) exposed to lead-concentrations of 10000, 50000 and 100000 $\mu g l^{-1}$ in distilled water for 96 h. These concentrations are 10–100 times greater than those used in the current study. Tissues evaluated for lead included gills, midgut (hepatopancreas), antennal glands and muscle. Results were expressed as ppm ($\mu g/g$ dry weight). All tissues were seen to accumulate lead in relatively high concentrations, but no dose-response was observed due to the high lead concentrations tested and no time-response was included in the study.

In a study of crayfish (*Astacus astacus L.*) exposed to low levels of lead (20 $\mu g l^{-1}$) and/or cadmium (2 $\mu g l^{-1}$) over a 10 week period, the accumulation of lead in the tissues was rapid within the first 2 weeks, plateauing and remaining constant after that time [17]. This suggests that the crayfish are able to quickly reach equilibrium with the low metal concentration in their environment. The only exception was the hepatopancreas in which lead continued to accumulate over the duration of the experiment. A function of this organ is to concentrate and store minerals [18]. The lead concentration tested by these investigators is approximately 1/7 of our lower concentration of 150

$\mu g\,l^{-1}$. They also reported lead concentrations to be highest in carapace and gills ($\sim$ 3.0 $\mu g\,g^{-1}$) followed by hepatopancreas ($\sim$ 2.0 $\mu g\,g^{-1}$) and muscle ($\sim$ 0.1 $\mu g\,g^{-1}$).

While the concentrations of lead tested in the above two studies [15,17] employed lead concentrations much higher and lower than the levels evaluated in this study, the order of tissue concentrations is similar: gills > hepatopancreas > muscle. The percent of total lead in soft tissues follows this same order. Neither of the previous two studies reported initial tissue concentrations of lead before lead exposure commenced nor did they measure hemolymph concentrations of lead which represent circulating levels of lead as opposed to real accumulation in the crayfish tissues. Tissue concentrations in our current study are reported as $\mu g/g$ wet weight of tissue while values reported by other investigators [15,17] are reported as $\mu g/g$ dry weight of tissue.

Results of field studies [9–13] concerning lead concentrations in different crayfish tissues is in agreement with the laboratory studies in that the exoskeleton and gills tend to exhibit the highest lead tissue levels while muscle exhibits the lowest lead tissue concentrations. Hepatopancreatic lead concentrations tend to be somewhat higher than the concentrations found in muscle. One group of investigators described significant sex differences in lead accumulation in crayfish exoskeleton and muscle [9] while other groups did not observe any such gender differences in lead accumulation [8,11,13]. The gender difference in lead accumulation was explained as being a phenomenon of differences in the molt cycle of the sexes [9]. In the current study, only female crayfish were evaluated and molting did not play a role since crayfish which molted during the course of the experiment were eliminated from the study.

In a laboratory experiment involving exposure of crayfish and macrophytes to contaminated sediment, it was observed that the exoskeleton accumulated much higher levels of lead than the muscle and that the accumulation in the exoskeleton was mainly through adsorption [14]. It was further reported that 80% of the lead in crayfish exposed to lead-treated sediment was present in the exoskeletons of intermolt crayfish and that the lead was lost from this tissue through molting. By use of chelating agents, it was demonstrated that most of the lead present in the exoskeleton was due to adsorption. Since the results of the current study did not demonstrate a dose- or time-dependent accumulation of lead in the carapace of lead-exposed crayfish and since there were no differences in exoskeleton lead concentrations between the two concentration groups, it suggests that lead is concentrated in the exoskeleton mainly by adsorption to a finite number of sites which are saturated after 2 weeks of exposure.

The gills contained the highest concentrations of lead among the soft tissues studied and histopathological studies revealed damage to the gills. The gills are involved in the exchange of gases and control ion fluxes. As a result they are responsible for making necessary adjustments in their function to meet the changes in their aqueous environment [18]. The permeability of the gills thus renders them susceptible to toxic ions such as lead. It has been reported that sublethal concentrations of lead (100–400 $mg\,Pb\,l^{-1}$) significantly decrease oxygen uptake by crayfish gills [19].

While the lead levels in muscle are considered as being low relative to the other tissues, accumulation is still significant as lead content exceeds the FDA safe limits for human consumption (0.3 $\mu g\,Pb/g$ tissue).

The clearance study demonstrates that a significant loss of lead from all tissues

occurs except from the hepatopancreas. It is of interest to note that the functions of the hepatopancreas are multiple, including roles in the digestive process, mineral storage and metal detoxification [16,20]. Histological studies revealed damage to the hepatopancreas even at the lowest lead concentration tested. Furthermore, it has been reported by Roldan and Shivers [16] that lead is stored in metal-containing vacuoles of cells of the hepatopancreas.

This study on lead nitrate clearly demonstrates that crayfish are excellent species for evaluating the lead contamination of our wetland environments. Lead accumulation in crayfish is dose- and time-dependant which may be reflective of the levels of lead present in contaminated wetlands. This accumulation may be of concern for human health and the ecosystem in that crayfish can survive in a lead-contaminated environment and through the food chain could impact on the health of both humans and animals.

Acknowledgements

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