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Metal Accumulation in Crayfish, *Procambarus clarkii,* Exposed to a Petroleum-Contaminated Bayou in Louisiana

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Red swamp crayfish (*Procambarus clarkii*) were placed for a period of 7 days in Bayou Trepagnier, a Louisiana waterway which received petroleum-laden effluents from a manufacturing complex several decades ago. However, coolant water from the plant continued to be released into the bayou until 1995. Analyses of sediments at the exposure site revealed heavy contamination by lead, chromium, and copper, while concentrations of arsenic and cadmium were very low. Significant bioaccumulation of lead was observed in the hepatopancreas and gills of 7-day-exposed crayfish, whereas chromium accumulated the most in the gills and blood. Concentrations of copper in the crayfish did not change during the course of the study, suggesting that this essential metal constituent of the respiratory pigment is successfully regulated even when crayfish are exposed to relatively high levels of copper in the sediments. There was no metal accumulation in the abdominal muscle of the crayfish. That damage to the hepatopancreas occurred during the exposure is suggested by histopathological studies which revealed swollen and vacuolated R cells and an increase in the pH of the digestive juices. Blood glucose levels, as well as ovarian and hepatopancreatic indices, were unchanged. This study demonstrates that accumulation of nonessential metals in crayfish tissues in a wetland environment contaminated by mixed pollution (metals and hydrocarbons) reflects the concentrations of metals in the sediment.

INTRODUCTION

Bayou Trepagnier, a 3.5-mile-long waterway in the LaBranche Wetlands near Norco, Louisiana, received a petroleum-laden waste stream from a manufacturing complex at its headwaters during the 1950s and 1960s. Although discharge of the petroleum wastes was discontinued more than 25 years ago, petroleum residues that impact on the quality of life of the native species are still present in the sediments and dredge spoil banks. Additionally, discharge of coolant water from the nearby manufacturing plant continued until February 1995 when this discharge was diverted from the bayou to the Mississippi River because of the high content of copper in the discharge. Analyses by this laboratory and others have identified heavy metals (lead, chromium, zinc, and copper) and a wide variety of straight-chain, branched, and polyaromatic hydrocarbons as contaminants of Bayou Trepagnier sediments (LaDEQ, 1989; EA, 1991; Flowers *et al.,* 1994).

Bayou Trepagnier provides an environmental laboratory in which to study the impact of mixed pollution (metals and hydrocarbons) on the biota indigenous to the wetlands of Louisiana. This paper reports on the effects of exposure of red swamp crayfish, *Procambarus clarkii,* to a highly contaminated site within Bayou Trepagnier. Bioaccumulation of heavy metals (lead, chromium, copper, arsenic, cadmium) was evaluated in various crayfish tissues, as well as measurement of blood glucose levels and assessment of hepatopancreatic function by measurement of the pH of the gastric juice and histopathologic study of the hepatopancreas.

MATERIALS AND METHODS

Characterization of Study Site

The study site is located about 2 miles downstream from the origin of the waterway. In a recent geological survey, the area is designated by Marker 105 (Flowers *et al.,* 1994). At this site in the bayou, the water depth averages 4 feet and the width is about 25 feet. Strong organic odors emanate from the sediments and petroleum residues are present in them. Currently, rainwater runoff from the wetlands on the eastern side of the bayou is its greatest source of water. Also, diurnal tides cycle water into and out of the wetlands from a neighboring 625-square-mile lake (Lake Pontchartrain) into which the bayou empties. Water levels are also influenced by the direction of the winds. The water in the bayou is mildly brackish.

Collection of Sediment and Water Samples

Each sediment sample was collected from the study site by driving a 3-in.-diameter acrylic tube into the bayou bed and plugging the upper end of the tube with a rubber stopper, so that the enclosed sediment and water column were withdrawn intact. When the water was siphoned away and the cores were extruded from the tubes, undisturbed specimens of the surface sediments were obtained. Water from the water column was filtered, placed in plastic jars, and brought to a pH of less than 2 with nitric acid for metal analyses.

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Crayfish

Mature red swamp crayfish (*P. clarkii*) were purchased from a local seafood vendor. They were maintained in aerated, plastic, laboratory aquaria for 4 days prior to use in the study. During this interval, they were fed commercial crayfish chow (People's Moss Gin Co., Inc., Palmetto, LA) and were maintained at a controlled temperature (25°C). Adult crayfish with a carapace length of 24 to 40 mm were selected for the field exposure study.

Field Exposure Study

Crayfish were placed in two plastic-coated steel mesh cages (approximately 90 crayfish/cage). The cages were totally submerged and tethered to the bed of the bayou. This permitted contact of the crayfish with the sediment and allowed for circulation of water.

Ten crayfish were removed from the cage for study after 1, 2, 4, and 7 days of exposure. In addition, 10 crayfish were selected in the laboratory as the zero time control at the start of the experiment. Gastric juice for determination of pH and blood for glucose determinations were collected upon harvest of the crayfish in the field to avoid any variations that might have been induced during transport of the crayfish to the laboratory. By use of a syringe equipped with a 27-gauge hypodermic needle, blood was drawn from the pericardial area. Digestive juice was drawn from the stomach through the mouth. The crayfish were then immediately taken back to the laboratory for further evaluation. The crayfish were weighed, and the hepatopancreas and ovaries were collected and weighed for determination of the hepatopancreatic and ovarian indices (weight of organ/weight of crayfish × 100). The hepatopancreas was preserved in Bouin's fixative for histopathological study.

Ten additional crayfish were selected at Time 0 and another 10 from those that had been exposed for 7 days in the bayou to determine tissue concentrations of metals of interest (lead, chromium, arsenic, cadmium, copper). After these crayfish were weighed and blood was collected in the manner described above, gills, hepatopancreas, and abdominal muscle were collected for metal analyses. These tissues were stored at −20°C until processed for metal determinations.

Determination of pH of Gastric Juices and Blood Glucose Concentrations

The pH of the gastric juices was determined using an Orion Research pH meter (Orion, Cambridge, MA) and blood glucose concentrations were determined using a Sigma glucose oxidase diagnostic kit (Procedure No. 510) (Sigma Chemical Co., St. Louis, MO). Twenty-five microliters of blood was used for each determination.

Histological Procedures

The hepatopancreas was washed and dehydrated in increasing concentrations of ethanol, cleared in xylene, and infiltrated and embedded in paraffin. Sections of 5 to 7 μm in thickness were cut using a paraffin rotary microtome and affixed to clean glass slides, stained with hematoxylin and eosin, and coverslipped. Images of the hepatopancreas were captured with a Kodak Megaplus II digital camera and NIH image software. Illustration formatting was performed using Photoshop 4.0.

Metal Analyses

Certified standards and independent certified controls were used in the preparation and validation of the standard curves. Metal standards were obtained from EM Science (Gibbstown, NJ) and controls were obtained from Aldrich Chemical Co. (Milwaukee, WI). EM Science trace metal grade nitric acid and HPLC grade methylene chloride (Curtin Matheson Scientific, Houston, TX) were used to prepare samples for analyses. Matrix modifier reagents were obtained from Perkin–Elmer (Norwalk, CT). Laboratory grade (Type II) water was prepared using a Life Scientific Inc. water purification system (St. Louis, MO).

Tissues (~0.5 g) and sediments (~1.5 g) were prepared for metal analyses by digestion in 10 ml of nitric acid solution using a CEM MDS-2000 microwave digestion system with advanced composite, Teflon vessels. Acid concentrations were 17% for the tissues and 34% for the sediments. The 50-min digestion was performed using 60% microwave power, with pressure settings progressively ramped through 20, 40, 85, 135, and 175 psi at 10-min intervals. Complete digestion of the tissue was accomplished. A digested reagent blank prepared with each 11 samples demonstrated less than 5 ppb metal background. Analyses were performed on a Perkin–Elmer Model 5100 ZL atomic absorption spectrometer equipped with Zeeman background correction and a graphite furnace with autosampler. Furnace parameters used were those recommended by the manufacturer. The matrix modifier solution for lead is ammonium dihydrogen phosphate (20 g/liter) and magnesium nitrate hexahydrate (1 g/liter); for chromium, a 1:1 mixture of calcium nitrate (5 g/liter) and magnesium nitrate (5 g/liter); and for arsenic, cadmium, and copper, magnesium nitrate (2 g/liter) and palladium nitrate (3 g/liter). Analyses of spiked digests demonstrated better than 95% recovery of added metal. Lead, chromium, and copper were selected for study because these metals have previously been identified as contaminants in Bayou Trepagnier (LaDEQ, 1989; EA, 1991). Cadmium and arsenic were selected even though these metals have not been identified as major contaminants of the bayou.

Statistical Evaluation

Data are presented as means plus or minus the standard error of the mean (SEM) and have been statistically evaluated using a two-tailed, unpaired Student t test ($P < 0.05$). Analyses of tissue digests with negligible metal content sometimes resulted in slightly negative values. In the statistical evaluation of these results, the negative sign was maintained to reflect the normal analytical variability around the zero point.

RESULTS

Metal Concentrations in Sediments and Water

The results of analyses of the two surface sediment samples collected from different spots at the exposure site confirmed the state of contamination of the bayou at this location (Table 1). The differences in the concentrations of contaminants in the two sediment samples demonstrate the patchy distribution of the contaminants. Previous sediment evaluations by the authors and others (LaDEQ, 1989; EA, 1991) support this observation. Lead, copper, and chromium are present in large amounts in the sediments, while arsenic and cadmium are present at very low levels. Metal concentrations in the water columns above the sediments were low: cadmium, 33 ppb; chromium, ≤5 ppg; lead, ≤5 ppb; copper, ≤5 ppb; arsenic, nondetectable.

Metal Bioaccumulation in Crayfish Tissues

The metal concentrations in the tissues of the crayfish on Days 0 and 7 of the experiment are presented in Table 2. Significant bioaccumulation of lead was observed in the gills and hepatopancreas but not in the abdominal muscle or blood of the crayfish after 7 days of exposure in the bayou. After exposure, the gills contained the highest concentration of lead, followed by the hepatopancreas.

Significant bioaccumulation of chromium was observed in the gills and blood after the 7-day exposure period, while the chromium concentrations in the hepatopancreas and abdominal muscle were not significantly different from those observed at Time 0.

No accumulation of arsenic, cadmium, or copper was observed in any of the crayfish tissues evaluated. In fact, the tissue concentrations of arsenic and cadmium in the hepatopancreas were significantly greater at Time 0 than after 7 days of exposure in the bayou, amounting to about a 40% loss of arsenic and a 60% loss of cadmium from the hepatopancreas during the exposure period. Since the sediment concentrations of these two metals were extremely low, the crayfish were actually downloading cadmium and arsenic from these tissues during the 7 days of the experiment. There were no significant changes in copper in any of the tissues evaluated.

TABLE 1
Concentrations of Metals in the Surface Sediments at Crayfish Exposure Site[a]

Contaminant	Sediment Sample 1 (μg/g, dry wt)	Sediment Sample 2 (μg/g, dry wt)
Arsenic	4	2
Cadmium	2	1
Chromium	43	63
Copper	128	41
Lead	6027	433

[a] Two separate samples were taken at the same time at different spots at Marker 105.

pH of Gastric Juices, Blood Glucose Levels, and Somatic and Ovarian Indices

During the 7-day experimental period of exposing the crayfish to the bayou, the pH of the gastric juice increased one pH unit, indicating a disturbance in hepatopancreatic function. Blood glucose concentration and ovarian and hepatopancreatic indices did not change significantly during the course of the exposure study (Table 3).

Histopathology

The metal-storing cells in the hepatopancreatic tubules of the crayfish are R (resorptive) cells which are present throughout the tubules and F (fibrillar) cells located primarily in the distal portion of the tubules (Icely and Nott, 1992; Rolden and Shivers, 1987). The hepatopancreata of crayfish exposed to the toxic conditions of the bayou were disorganized, cells were swollen, and R cells exhibited increased vacuolization (Fig. 1). The formation of intracellular vacuoles in cells of the hepatopancreas in response to metal insult has been identified as a mechanism which is utilized by these cells to sequester and detoxify metals (Coombs and George, 1978).

DISCUSSION

Analyses of sediment samples at the study site in the bayou revealed the presence of significant amounts of lead, chromium, and copper with very small amounts of these metals present in the water columns above the sediments. The sediment is rich in acid-volatile sulfide which binds and holds the metals in the sediment (Flowers *et al.*, 1994). Thus, metal bioaccumulation in the crayfish occurred mainly as a result of crayfish contact with the sediment rather than the water which contained very low levels of the metals. Whereas only metal accumulations in crayfish tissues were evaluated in this study, the crayfish were simultaneously exposed to many hydrocarbons.

After 7 days of exposure of the crayfish to the mixed pollution present in the bayou sediment, there were significant accumulations of lead and chromium in the gills, of lead in the hepatopancreas, and of chromium in the blood ($P < 0.05$). Accumulation of arsenic, copper, or cadmium was not significant in any of the crayfish tissues evaluated. Since arsenic and cadmium concentrations in the sediment are extremely low, accumulation would not be expected. While the sediment contained relatively high amounts of copper, the concentrations of this metal in the crayfish tissues remained unchanged during the course of the exposure period. Copper, unlike the other four metals studied, is an essential metal for crayfish. It is present in hemocyanin, the respiratory pigment. The physiological mechanisms to regulate the levels of this important metal are well developed in crayfish and apparently enable them to successfully cope with exposure to higher levels of copper in their environment without exhibiting tissue bioaccumulation. Dickson *et al.* (1979) found that essential metals did not exhibit comprehensive species differences in tissue concentrations.

TABLE 2
Concentrations of Metals in Crayfish Tissues

	Lead (ng/g)		Chromium (ng/g)		Arsenic (ng/g)		Cadmium (ng/g)		Copper (μg/g)	
Tissue type	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Gills	26 ± 1	205 ± 14*	4 ± 2	74 ± 14*	2 ± 2	37 ± 22	64 ± 33	4 ± 17	36 ± 4	29 ± 2
Hepatopancreas	4 ± 3	104 ± 24*	18 ± 6	42 ± 15	388 ± 30	146 ± 11	295 ± 63	99 ± 19	5.0 ± 0.4	5.0 ± 0.6
Blood	42 ± 9	59 ± 5	0	30 ± 9*	51 ± 34	4 ± 2	0 ± 7	0 ± 13	7.4 ± 2.9	6.7 ± 0.9
Muscle	9 ± 3	0	14 ± 5	16 ± 5	81 ± 22	90 ± 15	26 ± 15	21 ± 12	1.8 ± 0.5	1.2 ± 0.4

Note. Data expressed as means ± SEM, wet weight.
* Indicates significant increase from Day 0.

The abdominal muscle did not demonstrate accumulation of any of the metals evaluated.

Other field and laboratory studies involving the bioaccumulation of metals in tissues of different crayfish species demonstrated similar patterns of metal accumulation to what is reported herein for *P. clarkii* (Anderson *et al.*, 1978; Dickson *et al.*, 1978; Knowlton *et al.*, 1983; Stinson and Eaton, 1983; Pastor *et al.*, 1988; Madigoski *et al.*, 1991; Meyer *et al.*, 1991; Anderson *et al.*, in press; Bollinger *et al.*, in press). Upon exposure of crayfish to metals, the following order of tissue accumulation is observed: gills and exoskeleton > hepatopancreas/viscera > abdominal muscle. In general, tissue accumulation of nonessential metals (lead, cadmium, aluminum, arsenic) reflects environmental concentrations.

The concentrations of both arsenic and cadmium in the hepatopancreas were significantly higher on Day 0 than after 7 days of exposure in the bayou. Presumably, because these two metals were extremely low in the sediment, they were released from this organ during the exposure period.

Metal–metal interactions with regard to bioavailability and bioeffects have not been elucidated for crayfish and other animals. One way that nonessential metals may exert their toxic effects is to compete with essential metals (copper, zinc) and each other in transport and metabolic processes. Meyer *et al.* (1991), in a study involving the exposure of crayfish to low levels of lead and cadmium, found that the tissue concentration of either metal was lower when the crayfish were exposed to both metals simultaneously than when exposed to each metal alone.

In this study the crayfish exoskeleton was not considered a good biomarker of metal accumulation since the crayfish were covered with an oily residue due to the presence of grease and oil in the sediment. As a result, the crayfish required washing to remove the oily residue. In addition, metal uptake by the crayfish exoskeleton is variable depending upon the molt cycle and the metal under consideration. In particular, lead content in the exoskeleton appears to be mainly due to adsorption rather than incorporation (Knowlton *et al.*, 1983; Anderson *et al.*, in press).

One of the first tissues to demonstrate metal accumulation is the gills (Anderson and Brower, 1978; Pastor *et al.*, 1988; Meyer *et al.*, 1991; Anderson *et al.*, in press; Bollinger *et al.*, in press). The gills are in direct contact with the environment and are involved in exchange of gases and regulate ion fluxes. They have mechanisms which permit them to make necessary adjustments in response to changes in their aquatic environment. Heavy metals interfere with the respiration and osmoregulation functions of the gills in fish and crustaceans (Baker, 1969; Eisler and Gardner, 1973; Jones, 1975). Lead not only causes a decrease in oxygen uptake by the whole crayfish, but also produces a significant decrease in oxygen consumption by excised gills (Torreblanca *et al.*, 1987). Low concentrations of lead and/or cadmium cause a decrease in activity of oxidative enzymes in the gills (Meyer *et al.*, 1991). In addition, the gills form a mucous sheath as a protective response which in turn adsorbs the metals (Anderson, 1978; Anderson and Brower, 1978). Lead and chromium have also been demonstrated to damage the gills as demonstrated by histopathological study (Torreblanca *et al.*, 1987; Anderson *et al.*, in press; Bollinger *et al.*, in press).

The hepatopancreas is involved in a variety of physiological processes which include the secretion of digestive juices, absorption and storage of digested food, and the detoxification and storage of heavy metals (Icely and Nott, 1992). The hepatopancreas has the ability to concentrate metals from both the hemolymph and the digestive tract and to store them in intracellular vacuoles in the R and F cells (Rolden and Shivers, 1987). Cells of the hepatopancreas have been found to respond to toxic insult by forming vacuoles (Coombs and George,

TABLE 3
Gastric Juice Acidity, Blood Glucose Levels, Hepatopancreas, and Ovarian Indices of Crayfish Exposed to Bayou Trepagnier

Sampling day	pH of gastric juice	Blood glucose (mg %)	Hepatopancreatic index	Ovarian index
0	5.22 ± 0.44	6.0 ± 0.45	7.64 ± 0.42	0.063 ± 0.002
1	6.04 ± 0.08	5.5 ± 0.95	7.04 ± 0.45	0.061 ± 0.002
2	5.92 ± 0.05	6.0 ± 0.66	—	—
4	6.21 ± 0.06	6.0 ± 0.92	8.43 ± 0.37	0.062 ± 0.004
7	6.22 ± 0.08	7.0 ± 0.85	7.74 ± 0.55	0.066 ± 0.003

Note. Values expressed as means ± SEM, $n = 10$.

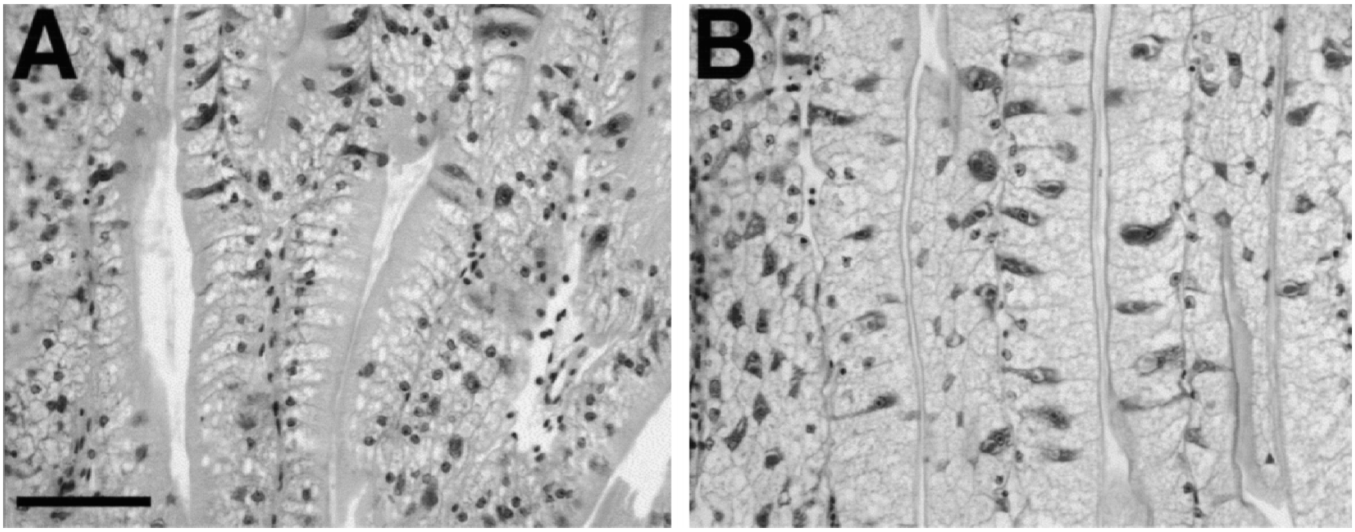

FIG. 1. Light micrographs of hematoxylin- and eosin-stained sections of crayfish hepatopancreas. Bar = 100 μm. (A) Hepatopancreas from a control crayfish collected at Time 0. (B) Hepatopancreas from a crayfish after 7 days of exposure in the bayou.

1978). In this study, an increase in the vacuolization of the cells in the hepatopancreas of crayfish exposed to the bayou was observed as well as an increase in the pH of the digestive juices. The pathological changes in the hepatopancreas in the current study suggest that the physiological roles of this organ were impaired, which is consistent with the observation of Reddy and Fingerman (1994) that cadmium exposure results in decreased output of amylase from the hepatopancreas into the digestive juice and an elevation of the pH of this juice. Cadmium exposure also induced hyperglycemia in the crayfish (Reddy *et al.,* 1994), which was not observed in the current study.

There are few reports concerning metal concentrations in the blood of crayfish. This parameter was measured in order to assess more fully true organ/tissue accumulation. Elevated concentrations of metals in the blood relative to organ concentrations suggest that the metal is binding to a blood component or cell. In the current study, the chromium concentration in the blood increased significantly during the exposure period, but at the end of the exposure period it was not significantly different from the chromium concentrations in the hepatopancreas and abdominal muscle of the crayfish that had been in the bayou for 7 days (Table 2). Studies in this laboratory (Bollinger *et al.,* in press) demonstrate that blood concentrations of chromium increase in proportion to the increasing exposure concentrations of chromium. Lead studies (Anderson *et al.,* in press) do not demonstrate such a phenomenon; blood levels of lead do not significantly differ between the concentration groups.

No accumulation of metals was observed in the abdominal muscle in this study. The abdominal muscle has consistently been found in the literature to be the tissue containing the lowest concentrations of metals. In earlier laboratory studies concerning the accumulation of lead or chromium in *P. clarkii* over exposure periods of 4 to 7 weeks, low levels of metal accumulation were also noted in the abdominal muscle (Anderson *et al.,* in press; Bollinger *et al.,* in press). This may be of importance to human health since abdominal muscle is the edible portion of the crayfish. Lead concentrations in the muscle, while low, exceeded the FDA safe limit for human consumption of 0.3 μg Pb/g tissue. With increased time of exposure in the bayou to the reported high concentrations of lead and chromium present in the sediment, accumulation of these metals in the abdominal muscle would be expected.

The rapidity with which changes in the concentrations of lead, chromium, arsenic, and cadmium occurred in the crayfish tissues indicates that there is a sensitive and dynamic equilibrium between the crayfish and their environment in dealing with heavy metals. The tissue concentrations of the metals reflect the concentrations of the metals in the sediments containing mixed pollution. Thus, the concentrations of metals in crayfish tissues make these tissues useful biomarkers for the presence of metals in contaminated wetlands.

CONCLUSION

The sediments in Bayou Trepagnier, a stream that received metal and hydrocarbon effluents from a manufacturing plant, were analyzed. Although the flow of effluents from the plant into the bayou has been discontinued since 1995, analyses of the sediments indicated there are still high concentrations of lead, chromium, and copper. Analyses of tissues of caged red swamp crayfish, *P. clarkii,* placed in the bayou for 7 days revealed that during this exposure period lead accumulated in the gills and hepatopancreas, whereas chromium accumulated in the gills and blood. This exposure also resulted in histopathologic changes in the hepatopancreas and an increase in the pH of digestive juice of the stomach. The concentrations of heavy metals in crayfish tissues, especially the gills and hepatopancreas, appear to serve as practical biomarkers of pollution.

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