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Concentrations of Aluminum in Gut Tissue of Crayfish (*Procambarus clarkii*), Purged in Sodium Chloride

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Recent concern over the release of Al in the environment has prompted researchers and health officials to assess its effects on biological systems (Venturini and Berthon 1989; Dayde et al. 1990; Cochran et al. 1990). Aluminum, despite being the most abundant metal in earth's lithosphere, is normally complexed in soil and is therefore unavailable for biological assimilation. The recent advent of acid rain, however, has prompted Al release due to mobilization from surrounding sediments into the environment (Bohmer and Rahmann 1990). This is of particular concern in aquatic environments because organisms in aquatic food chains can access and concentrate sublethal levels of Al in their tissues relatively quickly (Clark and LaZerte 1985). The ingestion of affected organisms by humans may therefore pose a potential health risk.

One such organism, (Procambarus clarkii), is known to concentrate metals in a variety of tissues (Diaz-Mayans et al. 1986). In northern Louisiana, many people trap or fish for crayfish in lowland areas which lie adjacent to highways and secondary roadways. Water, soil, and crayfish from these areas are known to contain high levels of Al (Madigosky et al. 1991). Some tissues known to concentrate Al (muscle, hepatopancreas and intestine tissue and contents) are those which humans commonly consume. The ingestion of these tissues may therefore expose humans to elevated Al levels. Many people who eat crayfish often purge them in dilute concentrations (1-2%) of NaCl to rid them of contaminants and make them more palatable (*Personal Observation*). We are aware of no literature which corroborates the claim that purging removes contaminating metals.

The objectives of this study were to 1) document the amount of Al found in water, soil, and gut tissue of crayfish (P. clarkii) collected from a roadside wetland site, 2) determine the affect of NaCl purging on the release of Al in P. clarkii and 3) assess the differences in Al levels found between stomach tissue, stomach tissue contents, intestine tissue, and intestine contents in P. clarkii.

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MATERIALS AND METHODS

Crayfish, water, and soil samples were collected from a wetland site located 0.5 km north of the intersection of Louisiana Highways 1 and 509 in northwest Red River Parish (32° 7′ N, 9° 28′ 55″ W) in May 1990. The study area paralleled Route 1 for approximately 1.5 km and sample collections occurred in stagnant water 7 m west of the highway at 30-m intervals. Water depth averaged 1 m but fluctuated according to the amount of rainfall and highway runoff received. The width ranged from 6-10 m. Area soil is seasonally saturated and contains humic gley and argillic clay. Dense ground cover surrounding the impoundment consisted of several species of grasses, herbaceous annuals with few perennials, and deciduous broadleaves on the west side of the ditch.

Water samples were collected in the following manner. Eight separate samples were obtained by submerging 100-mL capped polyethylene test tubes and partially opening the cap 20 cm above the deepest point in the ditch. Caps were then immediately sealed. Soil was collected at identical sites in a similar manner. Soil was packed into each tube until nearly all water was displaced. Water and soil specimens were placed in an ice chest for transport to the laboratory at the Louisiana State University Medical Center, Shreveport, Louisiana. Specimens were stored 48 hr at 4° C prior to analysis. Hydrogen ion concentration was recorded in the field with a Cole/Parmer pH meter, model 5985-75, over a 1-mon duration and concurrent with sample collection.

Ninety eight adult intermolt male and female crayfish (P. clarkii) were collected with baited dip nets. Crayfish were immediately transferred to the laboratory, weighed (average wet weight = 11.93 g), and measured according to total carapace length (average length = 7.98 cm). Specimens were thoroughly rinsed in distilled water to remove debris from the exoskeleton. Crayfish were then divided equally into 2 groups (control and treatment). The control consisted of double distilled deionized water and the treatment group of 1.5% NaCl solution prepared using analytical grade NaCl in doubled distilled deionized water.

Forty eight crayfish in the control were divided equally into six sets and placed in 1-L polyethylene containers. Equal numbers of males and females were maintained in each set. Specimens were sacrificed at time 0, 45, 90, 180, 270, and 360 min. Similarly, specimens exposed to the treatment were maintained at the same schedule. At the completion of each time interval, crayfish were placed in polyethylene bags, labelled accordingly, frozen, and stored at -20° C until analyzed. After thawing, the stomach and intestine were dissected. Laboratory dissecting tools and containers used for tissue analyses were acid washed with 8 *M* double distilled nitric acid followed by .001 *M* ethylendiamine-tetracetate (EDTA) and rinsed with deionized water to minimize the possibility of contamination. Tissues were individually placed in 10-mL polyethylene containers, capped loosely and oven dried for 12 hr at 120° C to determine dry weight. Samples were digested with 1 mL concentrated doubled distilled nitric acid and incubated for 12 hr at 50° C.

Trace metal analyses were determined with a Perkin-Elmer 5000 atomic absorption spectrophotometer (AAS) coupled with a graphite furnace and AS-1 autosampler using specifications recommended by the manufacturer.

Aluminum (AAS) standards were prepared from certified stock solutions containing 1 mg/mL Al in 1% HNO_3 along with the matrix modifier Triton X-100 in 1% $MgNO_3$ (Sigma Chemical Co., St. Louis, Missouri). All reagents were made in double distilled chelex water. Blanks and ten standards were run within the linear working range. Statistical analyses, unless specified, were conducted with an Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Concentrations of Al in stomach and intestine including both tissue and contents of P. clarkii are presented in Figs. 1 & 2. The amount of Al found in stomach ranged from 1630-13802 μg/g in the control group and 4142-12677 μg/g in the treatment group. Al levels detected in the intestine of the control and treatment groups ranged between 3666-25266 μg/g and 6440-11388 μg/g respectively. The change in stomach Al content in both groups over 360 min revealed no conclusive trends. The apparent increase in Al found in crayfish stomach in the control group through 180 min may be a result of crayfish responding to osmotic changes. Our data, however, does not support this premise. It is doubtful that crayfish were appreciably effected by treatments employed in this study since crayfish are euryhaline and can tolerate a wide range of osmotic conditions. Additionally, the degree of exposure was ephemeral. Similarly, intestine Al content in the control group increased over time until 360 min when a significant decrease was observed ($p \leq 0.05$). Far fewer changes were seen in the treatment groups both for stomach and intestine. It is interesting to note that no statistical differences between the control and treatment groups were observed comparing crayfish at 0 and 360 min. The amount of Al detected in crayfish sacrificed at the various times indicates that crayfish do not release Al as a function of time in the presence of 1.5% NaCl, nor do they release Al in deionized water. The somewhat sporadic variation of Al detected in tissue within and between groups may partially reflect undigested material contained in the gut of each crayfish before dissection. Moreover, some variation may be accounted for by changing edaphic conditions observed throughout the study site. The relatively high level of Al detected in stomach and intestine are to a large extent a reflection of the ambient environment.

The amount of Al detected in the water in the experimental and control containers increased over the 360 min of exposure to NaCl or to water (Table 1).

Table 1. Mean concentration of Al in water samples.

Time (min)	Water (μg/L)	
	Control	Treatment
0	25	25
45	712	2533
90	925	3033
180	700	2815
270	3200	2635
360	3220	2633

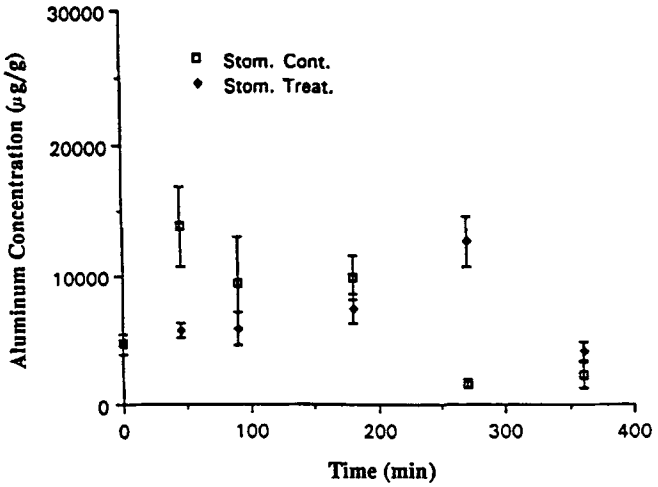

Figure 1. Mean concentration (± SE) of Al (μg/g dry wt.) in stomach of Procambarus clarkii (n=8 per sample).

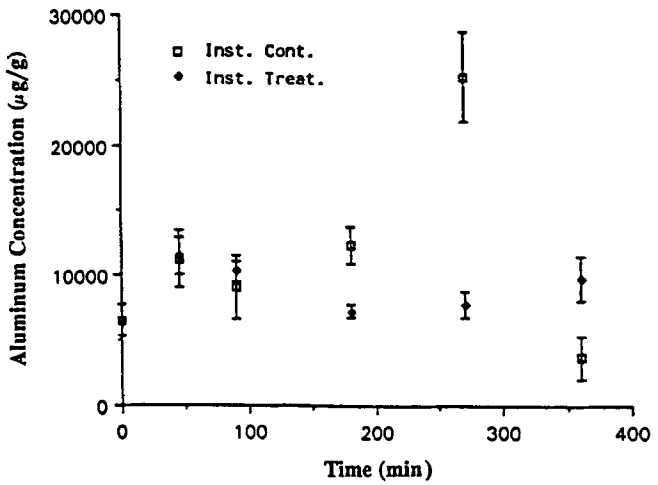

Figure 2. Mean concentration (± SE) of Al (μg/g dry wt.) in intestine of Procambarus clarkii (n=8 per sample).

Aluminum content in the treatment group was increased at 45 min, the first time point measured while the content rose in two steps in the control group. This may be an indication that crayfish are releasing Al from select tissue sites such as exoskeleton and branchial chamber, including the gills. Although differences in Al concentration were detected more rapidly in water analyzed from treatment groups, no statistical differences between the groups were present at 360 min. In addition, the increase of Al in water from both groups does not correlate with a reduction in stomach and intestine Al content. This suggests that Al is not being released from the gut. Increases in Al are most likely due to Al release from exterior tissues (gills) and

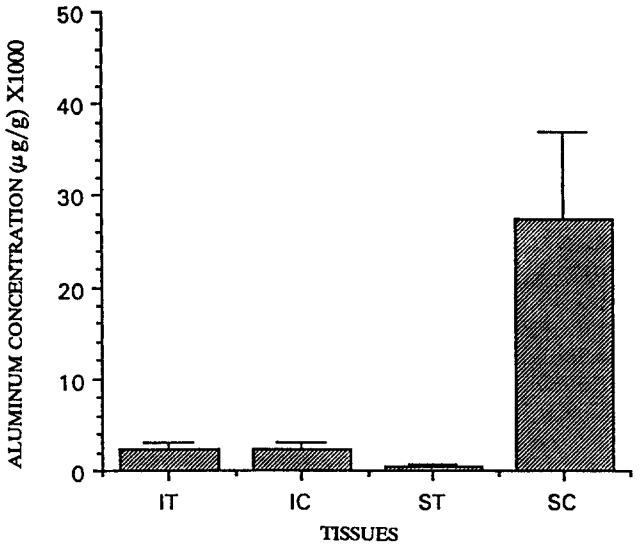

Figure 3. Mean concentration of Al (μg/g) in intestine tissue (IT), intestine contents (IC), stomach tissue (ST), and stomach contents (SC) in Procambarus clarkii (n=8 per sample)

possibly from the surface of the exoskeleton. Bohmer and Rahmann (1990) suggest that Al tends to precipitate on amphibian gill epithelium when found at elevated levels. The natural environment from which these crayfish were collected support this phenomenon. We conclude that purging crayfish in 1.5% NaCl before processing will not significantly reduce Al from gut tissue but may possibly promote release from exterior tissue sites. Furthermore, we did not observe this salt concentration to be an effective emetic during 360 min of exposure.

Comparisons between Al detected in intestine tissue, intestine contents, stomach tissue, and stomach contents are shown in fig. 3. The amount of Al detected in intestine tissue and contents is 2409 μg/g and 2342 μg/g, respectively. Highest levels of Al were found in stomach contents (27,388 μg/g). In comparison, stomach tissue contained 527 μg/g Al. A two-tailed T-test indicated significant differences between Al detected in stomach tissue and contents ($p \leq 0.05$). Marked differences were also found comparing stomach tissue with intestine tissue and contents ($p \leq 0.05$). The relatively high levels of Al found in crayfish gut are a direct result of assimilation from the environment and are therefore an indicator of ambient conditions.

The concentration of Al in intestine tissue and contents are similar. More Al is assimilated by the membrane tissue of the intestinal tract than by stomach tissue. The marked differences in Al concentration between stomach and intestine contents suggest that some Al is eliminated via the gut or is assimilated by other tissues. A substantial amount of Al is known to concentrate in the hepatopancreas (Madigosky et al. 1991). Other deposition sites include exoskeleton and to a lesser extent muscle tissue.

in the stomach and intestine. We speculate that only a small fraction of the total Al ingested by crayfish is absorbed by the gut, the remainder returned to the environment. This is substantiated by comparisons of Al found in tissue, surrounding soil, and water.

The concentration of Al in water collected from the field ranged from 250 μg/L to 1270 μg/L with an average of 412 μg/L. These levels are some of the highest ever recorded in North America and are similar to those observed in highly acidic lakes in northern New Jersey (Sprenger and McIntosh 1989), Germany (Bohmer and Rahmann 1990), and Ontario (LaZerte 1984). Aluminum levels detected in water from this site are atypical of levels found in unpolluted naturally buffered surface waters. Locked in soils, Al is usually concealed. In recent years, however, acid precipitation has enhanced Al release from soil (Robinson and Deano 1986; Weatherley et al. 1988). Lakes and other poorly buffered freshwater systems with acidity levels below pH 6 are especially at risk for Al toxicity (Baker et al. 1990). The pH of water recorded at our study site over a month duration averaged about 5.5 but levels as low as pH 4.0 were occasionally encountered. This is undoubtedly contributing to the release of Al into the environment. We believe that levels of Al detected in water, soil, and crayfish in this study are characteristic of conditions throughout northern Louisiana. Evidence from previous work (Madigosky et al. 1991) supports this premise.

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