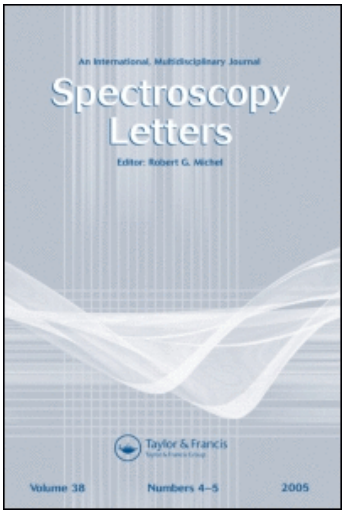

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Determination of Copper, Iron, and Zinc in Crayfish (*Procambrus clarkii*) by Inductively Coupled Plasma–Optical Emission Spectrometry

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ABSTRACT Inductively coupled plasma–optical emission spectrometry was used to determine copper, iron, and zinc in both whole Crayfish and tail meat of crayfish from selected areas in southwestern Louisiana. Also included was a comparison between male and female crayfish. Results showed no significant difference between male and female crayfish and individuals from different sites. Iron was approximately four times greater in concentration for whole crayfish compared with tail meat.

KEYWORDS copper, crawfish, crayfish, ICP-OES, iron, zinc

INTRODUCTION

Crayfish (*Procambrus clarkii)*, also known as crawfish or crawdads, are a Louisiana cultural icon and are most frequently served boiled or the tail meat (digestive tract) used in a variety of edible dishes (etouffee, bisque, etc.). In 2004, there were approximately 118,000 acres in southwestern Louisiana devoted to crayfish with a catch of around 70 million pounds (economic value of $48 million). Louisiana produces around 90% of the nation's crayfish with approximately 70% being consumed locally. A select No. 1 crayfish will weigh approximately 1 oz with the tail meat approximately 15–20% by weight of the crayfish.

Crayfish are grown in ponds and feed mainly on aquatic vegetation. Therefore, they are in contact with sediments and soils. During the 1940s, refining of fossil fuels and production of chemicals was introduced into southwestern Louisiana. The absence or poor enforcement of environmental regulations caused widespread pollution and led to the introduction of much of this pollution into these areas. Although improvements in environmental control in the 1970s occurred, there is still some ongoing concern over pollution, particularly as it affects the food chain. Further complications occurred when Hurricane Rita hit southwestern Louisiana on September 24, 2005.

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The object of this study was to determine copper, iron, and zinc in crayfish in ponds in southwestern Louisiana. Copper and zinc are considered essential for crayfish metabolism but no recent data is available about concentration levels in southwestern Louisiana crayfish. Iron was a pollutant is this area in past years. A previous study[1] concentrated on lead, but unpublished data showed concentrations of copper, iron, and zinc in crayfish from southwestern Louisiana. Crayfish have been investigated as an indicator of bioavailability of heavy metals in environmental monitoring[2,3] but work has been primarily limited to cadmium, lead, and arsenic. A recent review from this laboratory provides information on metals in fish and seafood, in general, from southwestern Louisiana.[4]

MATERIALS AND METHODS

Collection of Crayfish

Three different crayfish farms were selected from the southwestern Louisiana area for the crayfish collection based on their environmental surroundings: crayfish from a crayfish farm near Interstate I-10 (a highly traveled highway) and from crayfish farms in more pristine areas (further away from emissions of motor vehicles). Exact coordinates of sampling were obtained using a Global Positioning System (GPS). Crayfish samples were collected in early June 2006.

Group 1 (samples 1–6; Table 1) crayfish were collected in Gillis, Louisiana, at 30°24.4′N, 93°11.7′W, approximately 3000 meters away from the I-10 highway, exposed to minimal emissions. Group 2 (samples 7–13; Table 1) crayfish were collected in Ragley, Louisiana, at 30°29.4′ N, 93°12.7′W, approximately 1000 meters away from the I-10 highway. Group 3 (samples 14–21; Table 1) crayfish were collected from a farm in Jennings, Louisiana, at 30° 29.4′N, 90°12.7′W, which was approximately 100 meters from the I-10 highway.

To prevent or minimize contamination, latex gloves were used to collect the live crayfish in all steps of the procedure from collection to preparation of the samples. Each crayfish was placed in a separate Ziploc bag, labeled, and placed in an iced container until sample preparation.

TABLE 1 Results for the Determination of Copper, Iron, and Zinc in Crayfish

Sample no.	Mass (g)	Part used	Sex	Bomb Mass (g)	Cu (μg/mL)	Fe (μg/mL)	Zn (μg/mL)	Cu (μg/mL)	Fe (μg/mL)	Zn (μg/mL)
1	46.02	t	f	0.3178	0.38	0.48	0.60	60	76	94
2	39.51	t	m	0.2844	0.30	0.74	0.82	52	129	144
3	36.30	t	m	0.3017	0.42	0.61	0.74	70	102	122
4	45.05	w	m	0.2983	0.43	2.48	0.63	72	416	104
5	42.46	w	f	0.3037	0.39	2.24	0.47	65	369	77
6	36.29	w	m	0.3161	0.38	2.25	0.49	60	352	77
7	36.24	w	f	0.2950	0.33	4.03	0.58	56	683	99
8	45.20	t	f	0.3048	0.33	0.66	0.61	54	108	100
9	46.82	w	m	0.3146	0.53	2.54	0.53	56	403	85
10	56.05	t	m	0.2945	0.60	0.58	0.60	52	99	101
11	41.75	w	m	0.3071	0.60	4.21	0.60	57	686	98
12	42.83	w	f	0.3089	0.68	2.44	0.68	61	395	110
13	50.00	w	f	0.3178	0.65	3.51	0.65	73	553	102
14	38.58	w	f	0.3135	0.65	2.90	0.65	83	462	103
15	46.30	t	f	0.3079	0.64	0.81	0.64	74	132	103
16	64.25	w	m	0.3223	0.62	4.18	0.62	66	649	96
17	67.07	t	f	0.3034	0.66	0.71	0.66	65	117	109
18	66.17	t	f	0.3079	0.72	0.55	0.72	63	90	116
19	37.03	w	m	0.3244	2.04	3.58	2.04	67	551	314
20	50.12	w	f	0.3021	0.62	7.61	0.62	74	1260	103
21	58.61	t	m	0.3056	0.65	0.78	0.65	58	128	106

t = tail meat, w = whole, f = female, and m = male.

Preparation of Crayfish

Once collected, each live crayfish was rinsed with distilled water to remove debris. Once cleaned, the crayfish were boiled in distilled water as follows: to boil the crayfish, 400 mL distilled water was placed in a 600-mL beaker, supported on a ring stand, and positioned above a Bunsen burner. Once the water was heated to a boil, a single live crayfish was placed in the boiling water for 5 min then taken out. After each boiling, the beaker was cleaned, rinsed, and refilled with fresh distilled water to ensure that there was no or minimal contamination from each crayfish. Several samples of the water used to boil the crayfish were analyzed for copper, iron, and zinc, and concentrations at or below the detection limit (less than 0.10 parts per million) were found.

After boiling, the crayfish were air-dried. Two variations of samples were prepared to test for the metals and the possible location of metals in the crayfish; the whole crayfish and the tail meat only. Each sample was placed on a separate watch glass and weighed. This setup was labeled and placed in an oven for 24 h. The temperature of the oven was 135°C. After 24 h, the samples were weighed, then placed in the oven once again for a further 24 h. Once again the samples were weighed to check for significant change in weight. If there is a significant difference in the weights, there may still be water in the sample, so it is heated for 24 more hours. Likewise, if there is not a significant change, further heating is not necessary.

Once completely dried, the samples were ground down to small particles to make the digestion efficient. Each sample was placed in a mortar and ground with a pestle. The mortar and pestle was cleaned between each sample grinding. Once the sample was ground, it was placed in a labeled Ziploc bag for storage. The bag was vacuum-packed and placed in a freezer until used.

Digestion Procedure

Approximately 0.3000 g of ground crayfish was accurately weighed and placed in a microwave digestion vessel (Teflon Digestion Vessel #578B; Savillex Corporation, Minnetonka, MN). A 4.0-mL aliquot of trace metal grade concentrated nitric acid (Alfa Aesar, Ward Hill, MA; HNO_3 70% ACS, CAS 7697-37-2) was added, followed by the addition of 2.0 mL hydrogen peroxide. The digestion vessel was then capped and tightened.

The digestion vessel was placed in a commercial microwave (GE Turntable Microwave Oven, P/No. DE68-003070A) at 20% power level for 3 min. The vessel was allowed to cool for 5 min completely sealed. The vessel was heated at 30% power level for 5 min, and allowed to cool for 5 min. Once again it was completely sealed during cooling. Once cooled, the bomb was heated a final time for 5 min at 50% power level. It was allowed to cool for 5 min completely sealed. After 5 min, the pressure release on the bomb was slightly opened for 2 min under a fume hood and gradually opened more and more. (Note, different microwaves have different calibrations.)

Once the vessel was completely depressurized, the contents of the vessel were poured into a 50-mL volumetric flask using a filtered funnel. The vessel, along with its cap, was rinsed with distilled water with the rinse going into the flask. The flask was filled with distilled water to the 50.0-mL line, sealed, and labeled for metal determination.

Quality Control

Accuracy was assessed by comparison with National Institutes of Science & Technology Standard Reference Material (NIST-SRM) 1577b bovine liver (NIST, Gaithersberg, MD) and found to be acceptable. Approximately 0.2500–0.3000 g of the liver was accurately weighed and digested exactly as was the dried crayfish. The results for triplicate determination of copper was 150 ± 10 μg/g, iron was 200 ± 12 μg/g, and zinc was 116 ± 8 μg/g. This compared favorably with the NIST certified values of 158 ± 7 μg/g for copper, 194 ± 20 μg/g for iron, and 123 ± 8 μg/g for zinc. Recoveries were determined periodically throughout the study and found to be around 95% in all cases.

Instrumentation

All quantitative metal determinations were performed using inductively coupled plasma–optical emission spectrometry (ICP-OES). Instrument settings (PS series; Leeman Labs, Lowell, MA) and a discussion on the procedure are detailed elsewhere.[5] Results of the metals from the digested and diluted solutions

were determined as parts per million (ppm) and converted into μg/g (ppm× volume [mL]/sample mass [g]).

RESULTS AND DISCUSSION

Results of the study are shown in Table 1. Based on these results, it was concluded that there were no major differences in Cu, Fe, and Zn concentrations from the three different sampling areas. Therefore, the following discussion pools all 21 samples. Using the combined sample sites results, a mean copper concentration of 64 (range 52–83) μg/g, mean iron concentration of 370 (range 76–1260) μg/g, and mean zinc concentration of 113 (range 77–314) μg/g were obtained. However, it is obvious that the mean iron concentrations from the tail of 109 (n = 9; range 76–132) μg/g were significantly different from the mean iron concentrations of the whole crayfish of 565 (n = 12; range 352–1260) μg/g. Comparison between the female and male tail meat for mean copper concentration of 63 (range 54–74) versus 58 (range 52–70) μg/g, mean iron concentration of 105 (range 76–132) versus 115 (range 99–129) μg/g, and mean zinc concentration of 105 (range 90–116) versus 115 (range 101–144) μg/g were considered not to be significant. Comparison between the female and male whole crayfish for mean copper concentration of 69 (range 56–83) versus 58 (range 60–72) μg/g, mean iron concentration of 620 (range 395–1260) versus 510 (range 352–686) μg/g, and mean zinc concentration of 99 (range 77–110) versus 92 (range 85–104) μg/g were considered not to be significant. The result of mean zinc concentration for whole male for sample 19 was excluded in the above calculation as it was considered abnormally high.

CONCLUSIONS

Based on the this study and the results presented, it can be concluded that for copper, iron, and zinc concentrations in crayfish, there is no significant difference between a pristine and less pristine site and no significant difference between male and female individuals. Whereas there is no significant difference between tail meat and whole crayfish for copper and zinc, there is a significant difference in iron, about four times as much, for whole crayfish compared with the tail meat. This could be due to the fact that the shell of the whole crayfish must contain a high level of iron compared with the tail meat.

This work has provided valuable information and background to a recently initiated study involving several crayfish ponds of various soils and a more extensive list of metals. The study was performed during prime crayfish season (in southwestern Louisiana) from February through late May 2007. Results of this work will presented in due course[6].

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