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Patterns of Trace Metal Accumulation in Crayfish Populations

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The study of the occurrence and effects of heavy metals in aquatic systems has increased and an excellent review was presented by LELAND et al. (1974). Much of the information available on freshwater invertebrates, however, deals with lethal concentrations or the determination of tolerance limits. There are relatively few studies of heavy metals in freshwater invertebrates from field studies and these studies usually involve only a descriptive survey of the metal concentrations (MATHIS and CUMMINGS, 1973; NAMMINGA et al., 1974; ANDERSON, 1977). GALE et al. (1973) reported concentrations of Pb, Zn, Cu, Mn, and Cd in crayfish from Missouri's lead belt and indicated some of the effects of the variation of these metals in the environment on their concentration in organisms. VERMEER (1972), working with crayfish, and NEHRING (1976), working with insect nymphs, indicated these organisms could be used in the field to monitor heavy metal inputs. Our study investigated concentrations of Cd, Cu, Pb, and Zn in three populations of the crayfish, *Orconectes virilis* (Hagen). Two of the populations were collected at the same location but at sampling sites with different inputs of the trace metals. The third population was from a site on the same river but where metal input was low. These sites allowed an evaluation of the effects of different sublethal environmental concentrations on accumulation and concentration of the metals in crayfish. Various tissue concentrations from crayfish at the high input site were also examined to determine if particular body parts were sites of accumulation.

Methods

O. virilis were collected from two locations at Elgin, IL during the summer of 1974. The first sampling site was in the Fox River below a small flood control dam. The second site was a pond located on a mid-channel island adjacent to the river sampling location. Elgin is an industrial-urban complex and consequently discharge into the river is from industrial and domestic sewage, and storm water effluents. The island pond

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received water through ground seepage and directly from river water during high stages of the river. It was periodically dry during low river levels and extended dry periods. The third site was on the Fox River at Algonquin, IL. There was no significant industrial discharge or large sewage discharge immediately above this site. The urban population and area of storm drainage was also less than at Elgin. The major source of metal contamination would be from leaded gas used in motor boats.

All crayfish were collected with a 15 foot minnow seine and preserved in the field in 10 per cent buffered formalin. Crayfish samples were separated by sex and four size groups based on the length of the carapace which approximates age classes (MOMOT, 1967). The size categories were <1.0cm (0-0.5 yrs.), 1.0-2.5cm (0.5-1.5 yrs.), 2.5-4.0cm (1.5-2.5 yrs.), and >4.0cm (>2.5 yrs.). Some of the larger specimens from the Elgin river site were dissected to obtain gills, muscle, viscera, and exoskeleton for body part analysis.

Preparation for analysis of trace metals involved a modification of the dry ashing technique described by MIDDLETON et al. (1973). Dried samples were placed in a muffle furnace at 450°C for 12 hours. The resulting ash was dissolved in a 2:1 solution of nitric acid and distilled water. The mixture was then filtered through Whatman No. 44 ashless filter paper into 25ml volumetric flasks and the flasks brought to volume with distilled water. The digested samples in aqueous solution were analyzed for Cd, Cu, Pb, and Zn using a single beam Varian Techtron atomic absorption spectrophotometer, Model AA5, with direct aspiration of the sample into an air acetylene flame. The normal range of sensitivity was Cd, 0.5-5ppm, Cu and Pb, 0.5-10ppm. and Zn, 5-30ppm. All Values were corrected for sample preparation losses, proportional matric effects, and spectrophotometric background absorption as detailed in ANDERSON (1975). Statistical analysis of data used a K-level nested analysis of variance and Student Newmankuels multiple range test.

Results

Table 2 gives the concentrations of the studied trace metals in *O. virilis* from the sampling sites. The general relationship between the metal concentrations at the sites was $Cd < Pb < Cu \leq Zn$. This relationship does not reflect that found in the environment where $Cd < Cu \leq Pb < Zn$ (Table 1).

Differences between the metal concentrations at the sites were significant at the 0.01 level. Significantly higher concentrations of Cd and Pb were found in crayfish taken from the river at Elgin compared to those collected in the pool at Elgin or the river at Algonquin. There was no significant differences between the Elgin pool or Algonquin river sites ($p < 0.05$) and no significant differences in concentrations of Cu or Zn were found between any of the three populations of crayfish ($p < 0.05$).

No significant differences in metal concentrations were found between the sexes within sites, (Table 3). Sex Differences,

TABLE 1. Concentration of Cd, Cu, Pb, and Zn in the environmental components of the Fox River at Elgin and Algonquin, IL. Values are given in micrograms (ug) of metal per gram dry weight of smaple except for water which is micrograms per liter. Data from ANDERSON (1975).

Site	Metal	Water	Sediment	Detritus	Algae	Benthis Insects
Elgin						
	Cd	<0.00007	5.35	2.63	3.54	2.27
	Cu	0.001	25.29	124.75	12.58	14.91
	Pb	<0.022	161.66	209.19	13.17	28.60
	Zn	<0.002	101.90	272.10	30.25	225.63
Algonquin						
	Cd	<0.00007	0.54	1.47	2.89	1.35
	Cu	0.002	10.16	59.82	11.58	12.11
	Pb	<0.022	19.22	62.18	13.51	17.98
	Zn	<0.002	43.44	26.66	30.16	132.08

therefore, are not considered in relationship to uptake of these metals and no further distinction between sexes was made.

There are no consistent trends between the size classes for any of the studied metals. Size classes with the highest or lowest concentration of a particular metal varied between sites. Also no significant differences occurred between size groups for any of the studied metals (Table 4). There are overlapping sets of significant data revealed in the SNK multiple range test which prevents clear differences. As with the entire population and analysis by sex, however, the occurrence of higher concentrations of Cd and Pb from the Elgin river site is still very apparent when compared to Elgin pool and Algonquin.

As indicated in Table 5, specific relationships between tissues and metal concentrations were found. In general the lowest concentrations of any of the metals was found in muscle. The gills have significantly higher concentrations of Cu compared to exoskeleton and muscle. The exoskeleton has the highest Pb concentration followed by the gills and viscera, respectively. The highest concentrations of Zn were found in the gills and viscera. Consequently, with the exception of Pb, the gills had the highest concentration of the metals.

Discussion

The occurrence of higher concentrations of Cd and Pb in crayfish collected from the Elgin river site as compared to those at Algonquin and the Elgin pool was related to the input of these two metals into the habitat. Much higher concentrations of the metals were found in the substrate at Elgin and thus higher crayfish concentrations occur at this site. The other biotic components of the site also have higher Cd and Pb concentrations. Potential uptake by *O. virilis*, through water and food, was greater at Elgin and resulted in higher concentrations of the

TABLE 2. Concentration of Cd, Cu, Pb, Zn in *O. virilis* from each sampling site. Values given in µg of metal / g dry weight of organism (standard deviation).

Sampling sites	No. of Organisms	No. of Samples	Total Dry Wt. (g)	Cd	Cu	Pb	Zn
Elgin, river	50	29	42.99	2.22 (0.85)	64.27 (18.41)	27.39 (5.68)	78.30 (15.81)
Elgin, pool	318	32	67.40	1.04 (0.53)	61.24 (17.88)	8.41 (1.70)	87.07 (15.62)
Algonquin, river	144	29	46.11	1.09 (0.44)	71.32 (19.76)	11.33 (3.88)	101.30 (21.47)

TABLE 3. Concentration of the studied trace metals by sex. Values given in µg of metal / g dry weight of crayfish (standard deviation).

Sampling site	Sex	No of Organisms	No. of Samples	Total Dry Wt. (g)	Cd	Cu	Pb	Zn
Elgin, river	♂	11	11	21.62	2.07 (1.12)	68.30 (16.12)	23.97 (5.61)	83.61 (18.93)
Elgin, river	♀	11	11	16.04	2.38 (0.81)	70.30 (20.02)	28.64 (5.36)	70.09 (13.97)
Elgin, pool	♂	8	8	30.88	0.84 (0.20)	65.38 (27.18)	7.50 (1.10)	90.39 (20.58)
Elgin, pool	♀	9	9	29.53	0.89 (0.09)	61.99 (14.90)	7.83 (1.67)	79.61 (10.38)
Algonquin, river	♂	5	4	10.35	1.12 (0.50)	78.14 (33.54)	12.52 (6.87)	107.47 (15.57)
Algonquin, river	♀	13	8	23.35	1.27 (0.68)	77.63 (20.40)	13.12 (4.65)	92.57 (35.39)

TABLE 4. Concentration of trace metals found in general size groups of *O. virilis* at sampling sites. Size is based on the carapace length. Values in μg of metal / g dry weight of crayfish (standard deviation).

Sampling site	Size cm	No. of Organisms	No. of Samples	Total Dry Wt. (g)	Cd	Cu	Pb	Zn
Elgin, river	< 1.0	22	4	3.11	2.11 (0.41)	48.03 (12.08)	28.75 (3.16)	85.29 (7.23)
	1.0-2.5	10	7	3.91	2.38 (0.74)	56.68 (23.11)	28.73 (6.97)	80.97 (13.88)
	2.5-4.0	16	16	27.91	2.25 (1.02)	69.08 (15.11)	28.01 (4.02)	74.74 (17.52)
	> 4.0	2	2	7.49	1.66	83.29	14.86	83.54
Elgin, pool	< 1.0	263	10	6.50	1.30 (0.89)	50.71 (7.33)	9.31 (2.06)	93.46 (16.81)
	1.0-2.5	39	6	3.41	1.05 (0.11)	53.60 (7.27)	8 71 (1.00)	80.79 (5.86)
	2.5-4.0	9	9	27.78	0.83 (0.13)	70.61 (12.31)	7.76 (1.91)	82.22 (17.68)
	> 4.0	7	7	31.97	0.91 (0.18)	70.78 (29.05)	7.68 (0.50)	89.58 (15.54)
Algonquin, river	< 1.0	93	7	5.25	0.93 (0.32)	67.53 (14.61)	9.20 (0.89)	98.19 (14.14)
	1.0-2.5	41	12	8.52	1.00 (0.24)	67.30 (20.27)	11.14 (3.03)	105.50 (13.33)
	2.5-4.0	6	6	8.90	1.08 (0.73)	67.40 (16.63)	15.09 (4.70)	96.29 (25.12)
	> 4.0	4	4	20.31	1.66 (0.08)	95.74 (18.07)	9.98 (5.20	101.50 (45.38)

TABLE 5. Mean concentrations (standard deviation) of trace metals found in body parts of *O. virilis*. Tissues are from crayfish collected in the Fox River at Elgin, Illinois. Values in μg of metal / g dry weight of tissue.

Tissue	No. of Samples	Total Dry Wt. (g)	Cd	Cu	Pb	Zn
Exoskeleton	18	31.75	0.78 (0.13)	15.45 (5.82)	23.43 (8.89)	34.80 (8.89)
Muscle	18	9.92	<0.5	15.28 (5.63)	<4.0	52.50 (8.69)
Gills	7	3.07	1.47 (0.78)	120.67 (42.98)	12.93 (5.09)	81.98 (26.80)
Viscera	7	7.34	0.94 (0.20)	99.43 (32.04)	5.31 (2.46)	82.45 (19.98)

metals. This relationship of higher concentrations of metals occurring in organisms found in areas of greater input has been reported by PRICE et al. (1974) for terrestrial arthropods and GALE et al. (1973) for aquatic organisms.

A comparison of the Cd and Pb concentrations in the Elgin pool site to the Algonquin site showed similar concentrations in the crayfish. Although no data were collected on the water or sediment from the pool, it is likely that this area would have much lower concentrations of metals since its source of water was from ground water seepage and spring flooding. The river substrates, though, were being constantly exposed to contamination by the metals. Crayfish from the Elgin river site were exposed to high concentrations of metals as a result of input from storm water and treatment effluents. Crayfish in the pond normally receive the metals only from the dissolved or suspended fraction in the water or from areal fallout. Since the Fox River was alkaline, average pH 8.2, most of the metals are quickly precipitated and become incorporated in the detritus and sediment of the river. Consequently the crayfish in the pool were exposed to comparatively small concentrations of the metals as reflected by the low body burdens.

Unlike Cd and Pb, Cu and Zn are essential trace metals and similar concentrations occur between all three sampling sites. This indicates some regulatory ability of the organisms since input of these metals vary between the sites. The consistent concentrations of Cu were expected since the blood protein of *Orconectes*, hemocyanin, contains copper (WATERMAN, 1960). Similarly it has been found that the hepatopancreas of the crayfish absorbs Zn from food and that it regulates the body Zn content, maintaining it at a consistent level (BRYAN, 1966, 1967). The Zn concentrations for crayfish in the Fox River were very similar to those reported for the drainage basin in Missouri's lead belt (GALE et al., 1973). This was noted since potential environmental inputs in this area were often much higher than for the Fox River basin.

Copper concentrations in particular body parts were based on the relative amounts of hemocyanin present. Thus both the gills and viscera contained most of the hemocyanin in the organism. Relatively large viscera concentrations of Cu were also due to Cu accumulation in the midgut cells which serve as a storage reservoir (OGURA, 1959). The ability of the crayfish to regulate the Cu content was apparent from the fact that tissue concentrations were five to six times higher than water concentrations of Cu.

Like Cu, Zn concentrations were higher in the soft body parts than the exoskeleton. The occurrence of high Zn concentrations in the viscera were related to the hepatopancreas as discussed previously. Since tissue Zn concentrations were higher than environmental concentrations the hepatopancreas was probably the source of Zn regulation. Some Zn may also be adsorbed to the gill surfaces since a large volume of water actively moves across its relatively large surface area. Absorption of this Zn, however, may not readily occur. The low exoskeleton concentration reflects the fact that few metabolic processes occur in this hard body tissue and that it was not acting as a reservoir for metabolic Zn. Similar findings were reported by BRYAN (1964, 1966, 1968).

The highest Cd concentrations were found in the gills. Uptake of Cd on gill surfaces has been reported by investigators of Cd toxicity in fish and the fiddler crab (O'HARA, 1973; CEARLEY and COLEMAN, 1974). The large surface area for adsorption, the large volume of water passing over the gill surfaces, the adsorption on mucous sheaths secreted as a protective response to particulate matter in the water, and the relatively small biomass of the gills compared to their surface area, all result in comparatively high Cd concentrations in the gills. This adsorption does not result in accumulation unless water concentrations reach chronic toxicity levels since gill surfaces are shed during ecdysis. The small concentrations adsorbed to the exoskeleton is also lost during molting. Thus accumulation of toxic levels will not occur, particularly at the low water concentrations found in the Fox River, <0.00007 ug/ℓ.

The highest Pb concentrations were found in the exoskeleton and gills. As with Cd much of the Pb in the gills was a result of adsorption. Some absorption, however, does occur in the gills and gut. Part of the Pb in the exoskeleton was adsorbed but this would not account for the high values due to the low water concentration, <0.022 ug/ℓ, and molting activity of the crayfish. MACDONALD (1951) has shown that bone was the site of Pb deposition in vertebrates and a similar incorporation of Pb in the exoskeleton may account for the high concentrations. Again long term accumulation of potential toxic concentrations would not occur due to the periodic molt of the exoskeleton. The exoskeleton thus becomes a potential sink for the Pb and a method of eliminating soft tissue burdens of Pb. WISER and NELSON (1964) reported a similar function for crayfish integument with respect to the removal of cobalt-60 from body tissue.

The effectiveness of these crayfish as indicators of variation in trace metal input was dependent on the physiological role of the metal. This was particularly true with sublethal environmental concentrations. As shown, the animals can control body concentrations of physiologically important metals. Cd and Pb which do not have a physiological function occur in concentrations associated with environmental input. The crayfish, therefore, may be used to monitor inputs of these metals.

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References Cited

ANDERSON, R. V.: MS thesis, Northern Illinois University, DeKalb IL, 134p. (1975).

ANDERSON, R. V.: Bull. Environ. Contamination and Toxicol. (in press).

BRYAN, G. W.: J. Mar. Biol. Ass. U. K. 44, 549 (1964).

BRYAN, G. W.: Proceedings Int. Symp. on Radiological Concentration Processes. Pergamon Press, N.Y. pp1005-1016 (1966).

BRYAN, G. W.: J. Exp. Biol. 46, 281 (1967).

BRYAN, G. W.: J. Mar. Biol. Ass. U. K. 48, 303 (1968).

CEARLEY, J. E. and R. L. COLEMAN: Bull. Environ. Contamination and Toxicol. 11, 146 (1974).

GALE, N. L., B. G. WIXSON, M. G. HARDIE, and J. C. JENNETT: Water Res. Bull. 9, 673 (1973).

LELAND, H. V., E. D. COPENHAVER, and L. S. CORRILL: J. Water Poll. Control Fed. 46, 1452 (1974).

MACDONALD, N. S.: J. Biol. Chem. 189, 387 (1951).

MATHIS, B. J., and T. F. CUMMINGS: J. Water Poll. Control Fed. 45, 1573 (1973).

MIDDLETON, S. G., F. E. GILLIS, and J. G. GRAU: Ann. Entomol. Soc. of Amer. 66, 226 (1973).

MONOT, W. T.: Amer. Midl. Nat. 78, 55 (1967).

NAMMINGA, H. E., J. E. SCOTT, and S. L. BURKS: Proc. Okla. Acad. Sci. 54, 62 (1974).

NEHRING, R. B.: Bull. Environ. Contamination and Toxicol. 15, 147 (1976).

OGURA, K.: Annotat. Zool. Jap. 32, 133 (1959).

O'HARA, J.: J. Fish. Res. Bd. Can. 30, 846 (1973).

PRICE, P. W., B. J. RATHCKE, and D. A. GENTRY: Environ. Entomol. 3, 370 (1974).

VERMEER, K.: Can. Field-Naturalis 86, 123 (1972).

WATERMAN, T. H.: The Physiology of Crustacea. I. Academic Press, New York, 670p. (1960).

WISER, C. W. and D. J. NELSON: Amer. Midl. Nat. 72, 181 (1964).