Adams et al 1981

# The use of the three-ridge clam (Amblema perplicata) to monitor trace metal contamination

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Keywords: Cadmium, zinc, pollution, molluscs, monitoring

## Abstract

The three-ridge clam *Amblema perplicata* was used to monitor two streams for the presence of zinc (Zn) and cadmium (Cd) derived from an industrial source. Clams were collected from a relatively uncontaminated area in one river and transported to four study sites in the two contaminated streams. The clams were placed into polyethylene cages and left in these streams for one week. Control clams were treated in a similar manner and left in the uncontaminated river.
The highest mean concentration of Zn (956 μg/g dry wt) was found in the gill tissue of clams from the most contaminated site. The highest mean Cd concentration (18.6 μg/g dry wt) was found in digestive glands of clams, also from the most contaminated site. Mean concentrations at contaminated sites were significantly higher than background and control levels; the findings suggest that these particular organs of clams may be useful in monitoring levels of Zn and Cd in other freshwater systems.

## Introduction

The contamination of streams and lakes with heavy metals and other potentially toxic substances has created the need for periodic monitoring of contaminant levels in these systems. Many investigators have proposed that molluscs be used as biological monitors (Bedford *et al.* 1968; Butler 1973; Boyden 1974; Darracott & Watling 1976; Foster & Bates 1978; Phillips 1977, 1978; Walker *et al.* 1975). Molluscs have been used mostly to assess the degree of contamination in marine and estuarine ecosystems. However, Bedford *et al.* (1968) successfully used confined freshwater mussels (*Lampsilis* spp.) to monitor levels of organochlorine insecticides in the Red Cedar River in Michigan, USA. Recently, Foster & Bates (1978) used caged *Quadrula quadrula* to monitor copper contamination in an Ohio stream.

It was the purpose of this study to evaluate the use of the three-ridge clam (*Amblema perplicata*) as a monitor of zinc (Zn) and cadmium (Cd) contamination in two freshwater streams.

## Materials and methods

Williamson Ditch and Trimble Creek, located in northcentral Indiana, were selected for study (Fig. 1). An electroplating plant descharged wastes that contained Zn and Cd into Williamson Ditch approximately 3 km from Palestine Lake. Although most of the metals are deposited in the sediments of the lake (McIntosh *et al.* 1978; Wentsel *et al.* 1977), Trimble Creek, which is the only effluent stream of the lake, is also contaminated with metals (Adams *et al.* 1980). Trimble Creek flows about 6 km northward from the lake through wooded areas of low geographic relief, where it eventually empties into the Tippecanoe River.

Three-ridge clams were collected during the months of August and September, 1977 from a

Hydrobiologia 83, 67–72 (1981). 0018-8158/81/0831-0067/$01.20.

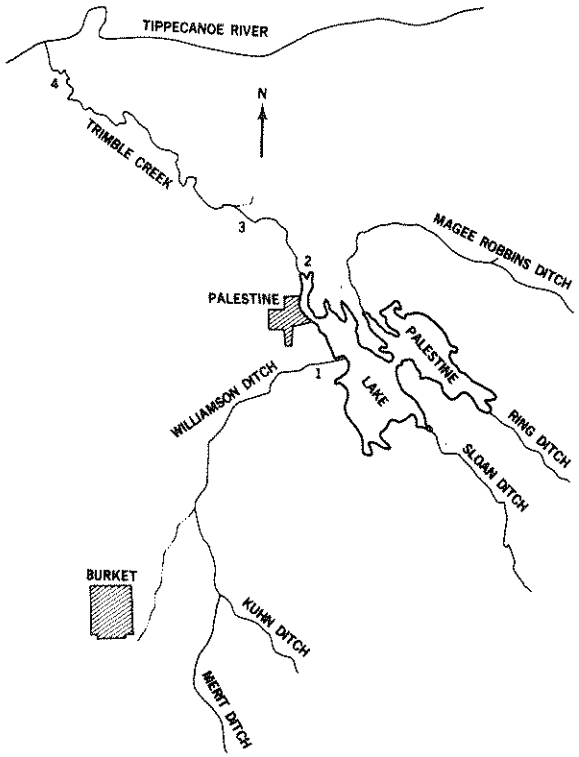


*Fig. 1.* Diagrammatic map of Williamson Ditch, Palestine Lake, Trimble Creek and a portion of the Tippecanoe River showing water flow patterns and sampling sites.

relatively uncontaminated site in the Wabash River and transported to four selected sites in the study area (Fig. 1). At each site, 6 clams were placed in large meshed polyethylene containers with the open end resting in the bottom substrate to insure natural exposure of clams to metals in both the water and sediment. Control clams were handled in a similar manner and left where they were collected in the Wabash River. Background concentrations of Zn and Cd were immediately determined in clams collected from the uncontaminated site.

After an exposure time of 7 days, all clams were collected from the study sites and returned to the laboratory for Zn and Cd analysis. The soft body tissue was removed from the shell, rinsed in deionized water, drained, and dissected into separate tissue components. Wet weights were recorded and, after drying at 105 C until a constant weight was attained, dry weights were determined. Tissues were then wet ashed with a 2:1 mixture of $HNO_3$-$HClO_4$, extracted with ammonium-1-pyrrolidinedithiocarbamate (APDC) and methyl isobutyl ketone (MIBK), and the extract was analyzed for Cd by atomic absorption spectrophotometry (AAS) (Atchison *et al.* 1977). Zn concentrations were determined in the aqueous solution by AAS before extraction. Concentrations were calculated by comparisons to standards made from stock solutions of Cd and Zn.

Logarithmic transformations of metal concentrations were used to satisfy the assumption of normality within each site and to improve the homogeneity of variance between sites. One-way analyses of variance and Newman-Keuls sequential range tests at the $P < 0.05$ level were used to evaluate the data.

Concentration factors (X-factor) were calculated based on the methods of Coughtrey and Martin (1976). The formula used was the following

$$\text{X-factor} = \frac{\%\text{ of metal content in particular tissue}}{\%\text{ of total weight of that particular tissue}}$$

The X-factor provides a convenient comparison between the ability of each tissue type to concentrate metals within a single sample of clams.

On the day that the clams were removed from the study sites and control area, water and sediment samples were collected for Zn and Cd analysis. Surface water samples were collected in acid-washed polyethylene bottles and immediately placed on dry ice for transport to the laboratory. The samples were then thawed and filtered through 0.45 $\mu$ membrane filters. The filterable fraction was acidified with 5 ml of concentrated distilled nitric acid.

Zinc concentrations in the filterable fractions of water were determined by direct aspiration into the spectrophotometer. The concentration of Cd was determined as described by Sullivan *et al.* (1977). Potassium acid phthalate buffer (0.5 M) was added to 200 ml of filtered water and the pH adjusted to 4.2. APDC and MIBK were used to extract the Cd. The organic layer containing the Cd was aspirated directly into the spectrophotometer.

The non-filterable fractions of the water samples were treated in a manner identical to the process used to analyze Zn and Cd in clams.

Duplicate grab samples of bottom sediment were collected at each site, placed in separate plastic containers and stored on dry ice for transport to the laboratory. Approximately 25 g of each sample was

*Table 1.* Average cadmium and zinc concentrations in the filterable and non-filterable fractions of water (n = 1) and the sediment (n = 2; standard error of mean in parentheses) from various sites in Williamson Ditch and Trimble Creek (see Fig. 1 for site locations). All samples were taken on the date of clam removal from these sites after 7 days of exposure.

| Site | Metal | Water | | |
|---|---|---|---|---|
| | | filterable (μg/l) | non-filterable (μg/l) | Sediment (μg/g dry wt) |
| 1 | Cd | 4.44 | 3.16 | 35.4 (0.35) |
| | Zn | 196 | 20.9 | 272 (5.02) |
| 2 | Cd | 1.29 | 6.58 | 16.5 (0.75) |
| | Zn | 59.9 | 50.8 | 217 (5.95) |
| 3 | Cd | 1.17 | 7.86 | 16.8 (0.93) |
| | Zn | 48.1 | 40.3 | 253 (0.18) |
| 4 | Cd | 1.29 | 1.86 | 9.53(2.34) |
| | Zn | 59.3 | N.D.* | 185 (40.8) |

*N.D. = Not detectable

dried at 105 C to a constant weight. Subsamples of 0.5 g of dried sediment were then placed into Kjeldahl flasks and digested for 6 hours in 20 ml concentrated $HNO_3$. The digested samples were filtered through Whatman No. 40 filter paper and diluted to volume for analysis by AAS.

## Results and discussion

Sediment samples from Williamson Ditch (Site 1) were significantly ($P < 0.05$) more contamined than samples from the remaining sites (Table 1). The lowest concentrations of Zn and Cd in the sediment were found at Site 4, the site furthest from Palestine Lake. Mayes (1972) found average sediment values of 32.2 μg Zn/g and 0.60 μg Cd/g in the Wabash River near our control site.

Concentrations of Zn and Cd in the filterable fraction of water were highest at Site 1 (Table 1). The same trend held true for Zn in the non-filterable fraction but non-filterable and total Cd were highest at Sites 2 and 3. More extensive water and sediment analyses for these study sites have been published (Adams *et al.* 1980) and they indicate that Site 1 is consistently more contaminated than Sites 2, 3 and 4 but that there is a great deal of variation in both levels found over time and in the percentage of Zn and Cd found in filterable or non-filterable fractions at any one time. Data reported in this paper represent conditions found during the test period only.

As demonstrated by the data presented in Table 1 and by Adams *et al.* (1980), Williamson Ditch and, to a lesser extent, Trimble Creek were contaminated by Cd and Zn above background levels (refer to Enk & Mathis 1977; Mathis & Cummings 1973;

*Table 2.* Accumulation (μg/g dry wt) of zinc in tissues of caged clams (*Amblema perplicata*) after 7 days exposure in Williamson Ditch, Trimble Creek or a control area.

| Tissue | Site | | | | | Background[b] |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Control[a] | |
| Foot | 170[c] (12.2) | 177 (15.8) | 149 (7.68) | 146 (7.60) | 93.4 (7.81) | 134 (7.54) |
| Mantle | 538 (71.3) | 257 (18.5) | 298 (30.3) | 309 (48.3) | 161 (9.35) | 171 (10.7) |
| Gill | 956 (72.1) | 622 (53.8) | 531 (37.6) | 513 (51.6) | 407 (44.7) | 462 (35.0) |
| Digestive gland | 208 (5.20) | 201 (26.5) | 247 (14.8) | 173 (30.3) | 145 (6.18) | 118 (7.89) |
| Viscera | 232 (11.5) | 201 (29.0) | 214 (10.9) | 191 (18.9) | 89.5 (11.0) | 114 (6.78) |
| Whole body | 388 (40.7) | 251 (27.8) | 259 (8.14) | 241 (23.8) | 127 (9.77) | 162 (8.21) |

[a] Control: uncontaminated site in Wabash River.
[b] Background: based on a subsample (N = 6) of the Wabash River clams before exposure to contaminated areas.
[c] Mean (Standard error of mean) based on N = 6.

*Table 3.* Accumulation (μg/g dry wt) of cadmium in tissues of caged clams (*Amblema perplicata*) after 7 days exposure in Williamson Ditch, Trimble Creek or a control area.

| Tissue | Site | | | | | Background[b] |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Control[a] | |
| Foot | 1.54[c] | 0.48 | 0.77 | 0.99 | 0.62 | 0.41 |
| | (0.13) | (0.05) | (0.06) | (0.11) | (0.11) | (0.09) |
| Mantle | 9.08 | 1.98 | 1.31 | 2.51 | 2.00 | 1.36 |
| | (0.59) | (0.23) | (0.10) | (0.11) | (0.23) | (0.25) |
| Gill | 16.5 | 1.47 | 1.27 | 2.23 | 1.80 | 1.44 |
| | (2.03) | (0.24) | (0.11) | (0.21) | (0.14) | (0.22) |
| Digestive gland | 18.6 | 2.94 | 4.28 | 4.43 | 1.78 | 2.85 |
| | (2.75) | (0.22) | (0.48) | (0.93) | (0.19) | (0.19) |
| Viscera | 3.69 | 1.23 | 1.34 | 1.76 | 0.94 | 0.69 |
| | (2.47) | (0.17) | (0.23) | (0.07) | (0.06) | (0.21) |
| Whole body | 7.78 | 1.44 | 1.43 | 1.98 | 1.31 | 1.08 |
| | (0.61) | (0.08) | (0.13) | (0.08) | (0.09) | (0.16) |

[a] Control: uncontaminated site in Wabash River.
[b] Background: based on a subsample (N = 6) of the Wabash River clams before exposure to contamined areas.
[c] Mean (Standard error of mean) based on N = 6.

*Table 4.* Distribution (x-factor[a]) of accumulated zinc after 7 days of exposure in Williamson Ditch, Trimble Creek or a control area.

| Tissue | Site | | | | | Background[b] |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Control | |
| Foot | 0.45[c] | 0.75 | 0.57 | 0.63 | 0.76 | 0.82 |
| | (0.03) | (0.08) | (0.04) | (0.07) | (0.09) | (0.10) |
| Mantle | 1.53 | 1.06 | 1.14 | 1.24 | 1.36 | 1.06 |
| | (0.15) | (0.04) | (0.10) | (0.08) | (0.14) | (0.05) |
| Gill | 2.59 | 2.12 | 2.03 | 2.13 | 3.27 | 2.84 |
| | (0.18) | (0.28) | (0.10) | (0.15) | (0.29) | (0.24) |
| Digestive gland | 0.57 | 1.22 | 0.94 | 0.71 | 1.34 | 0.65 |
| | (0.02) | (0.40) | (0.05) | (0.11) | (0.19) | (0.08) |
| Viscera | 0.62 | 0.81 | 0.82 | 0.79 | 0.76 | 0.73 |
| | (0.01) | (0.04) | (0.04) | (0.02) | (0.09) | (0.05) |

[a] $x = \frac{\%\text{ of total metal content in a particular tissue}}{\%\text{ of total weight of that particular tissue}}$
[b] Background: based on a subsample (N = 6) of the Wabash River clams before exposure to contaminated areas.
[c] Mean (Standard error of mean) based on N = 6.

and Namminga & Wilhm 1977, for comparison).

During the current study, heavy rains created elevated stream flows which diluted Cd and Zn in the water and scoured, to some degree, the bottom sediments, thus metal levels in water and sediment reported here for some sites are lower than those previously reported for these same stream sites during periods of lower flow (Adams *et al.* 1980). However, *A. perplicata* still concentrated Zn and Cd to levels higher than those found in the water

and in general reflected the concentrations present in their immediate surroundings. Mean whole body concentrations were significantly higher ($P < 0.05$) in clams from Williamson Ditch, which was in general the most contamined site, than in specimens from all other less contaminated sites (Tables 2 and 3). No significant differences were found among clams from sites 2, 3 and 4.

Mean metal concentrations in individual tissues within clams from the contaminated sites were highest in the gill for Zn (Table 2) and in the digestive gland for Cd (Table 3). Based on X-factors, Zn was preferentially concentrated to a significant degree ($P < 0.05$) in the gill tissue (Table 4). For Cd, the X-factor of digestive gland was significantly higher ($P < 0.05$) in clams from all sites except Site 1, where both gill and digestive gland tissue had high X-factor values (Table 5).

The importance of the gill in concentrating metals was expected, since the gill acts not only as a filtering mechanism for food and particulate matter, but also as a respiratory organ which absorbs and adsorbs metals from both sediment and water (Anderson 1977; Bryan 1976).

The digestive gland's affinity for Cd is also related to an adsorption/absorption process. Small particles are taken into the gland by either phagocytosis or pinocytosis. Once absorbed, the Cd may be mediated by a carrier system and bound to calcium or magnesium granules, with subsequent storage in the digestive gland (Bryan 1976; Walker *et al.* 1975).

In view of the elevated concentrations of Zn and Cd found in gills, digestive glands, and whole body tissues of exposed clams and the tendency of these tissues to reflect rather will the levels of metals at Site 1, we conclude that *A. perplicata* does have potential as a monitoring organism in the assessment of trace metal contamination in freshwater systems. However, as pointed out by Foster & Bates (1978) and Phillips (1977), more research must be done to provide the background and basis for the use of freshwater molluscs as routinely-used tools in water quality assessment.

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*Table 5.* Distribution (x-factor[a]) of accumulated cadmium after 7 days of exposure in Williamson Ditch, Trimble Creek or a control area.

| Tissue | Site | | | | | Background[b] |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Control | |
| Foot | 0.22[c] | 0.29 | 0.55 | 0.51 | 0.49 | 0.38 |
| | (0.02) | (0.05) | (0.07) | (0.07) | (0.05) | (0.06) |
| Mantle | 1.28 | 1.32 | 0.94 | 1.28 | 1.73 | 1.25 |
| | (0.12) | (0.15) | (0.13) | (0.07) | (0.13) | (0.23) |
| Gill | 2.39 | 0.89 | 0.85 | 1.10 | 1.51 | 1.45 |
| | (0.22) | (0.21) | (0.10) | (0.07) | (0.08) | (0.33) |
| Digestive | 2.54 | 2.90 | 3.01 | 2.19 | 2.56 | 2.88 |
| gland | (0.25) | (0.87) | (0.39) | (0.37) | (0.57) | (0.40) |
| Viscera | 0.55 | 0.81 | 0.87 | 0.89 | 0.86 | 0.75 |
| | (0.05) | (0.08) | (0.10) | (0.03) | (0.09) | (0.24) |

[a] $x = \frac{\%\text{ of total metal content in a particular tissue}}{\%\text{ of total weight of that particular tissue}}$

[b] Background: based on a subsample ($N = 6$) of the Wabash River clams before exposure to contaminated areas.

[c] Mean (Standard error of mean) based on $N = 6$.

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Received September 11, 1980.