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DETERMINATION OF MANGANESE IN NAIAD MOLLUSC SHELLS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Naiads from two locations 11 river miles apart on the lower Muskingum River in Ohio were studied to determine whether location, calendar year and species could be correlated with the manganese concentration in the shells of the freshwater bivalve molluscs. Neutron activation analysis was used to determine the manganese concentration. The effect of each of these variables on the manganese content of the naiad shells was observed and discussed.

INTRODUCTION

Since molluscs are relatively immobile and tend to incorporate some constituents of their aqueous environment into their shells and soft parts, they are potential pollution indicators of any stream, river, lake, or ocean in which they live.

The streams of the Muskingum River basin receive drainage from many inactive and active coal mines in the area (US Department of the Interior, 1969). Analysis of water samples from selected sites on these streams reveals the presence of manganese (US Department of the Interior, 1969).

Mathis & Cummings (1973), in their analysis of naiad soft parts from an industrially polluted river, found significant concentrations of metals. Nielson & Nathan (1975) demonstrated the presence of heavy metals in New Zealand molluscs and Phillips (1977) has reviewed the use of biological indicator organisms to monitor trace metal pollution and found molluscs to be a good subject for study.

It therefore seemed likely that the naiad molluscs of the Muskingum River might serve as effective monitors of the manganese in the water.

The present work is a study of the manganese content of the shells of two species of naiads from the Muskingum River at Beverly, Ohio and five species of naiads at

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Lowell, Ohio, 11 miles downstream in the same river. The objective is to determine whether calendar year, location, or species influences the ability of these animals to deposit manganese in their shells.

EXPERIMENTAL

Instrumentation

The Ohio State University Research Reactor: a pool type reactor using flat-plate, fully enriched fuel elements, having several irradiation facilities and licensed to operate at a maximum power of 10 kW.

4000—Channel Analyser: Canberra Industries, Inc., Model 8180.

Ge (Li) Detector: Princeton Gamma Tech., Model LGC 14SD with an efficiency of 14% and a peak to compton ratio of 44:1.

Minicomputer: Digital Equipment Corp., Model PDP11/05 on line with the analyser.

Sample preparation

Each shell was scoured with a firm-bristled toothbrush and common kitchen scouring powder. This was done to prevent contamination of the samples and to distinguish the annular rings which differentiate the yearly layers of the shell.

The second annual layer was chosen for analysis because it is usually the largest of any layer present. The peripheral nacre was selected since it represents the largest and most easily handled part of the second annular layer. Only the right valve was used for the analysis. The left valve was kept unbaked to have a reference for annular rings, which the periostracum provides, for the dissection of the annular layers in the right valve.

The right valve was put in a muffle furnace at 538 °C for 10 min. The heating destroys the annular ring markings in the periostracum (Fig. 1). During the 10 min heating period the periostracum burned off. The remains of the prismatic layer were scraped off.

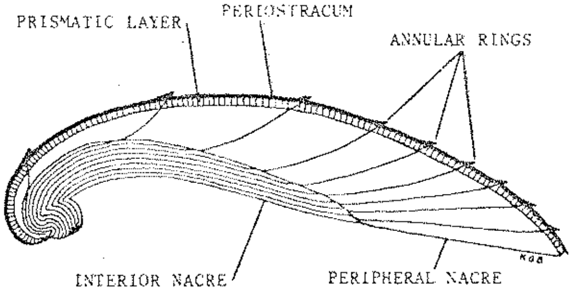

Fig. 1. Cross-section of naiad shell.

The left valve was used as a guide to divide the peripheral nacre in the right valve into its annular layers. The second annular layer of the peripheral nacre was removed from the rest of the right valve which generally pulls apart into layers quite easily at this point. A sharp knife can be wedged between the layers to help separate them.

The second annular layer of peripheral nacre was then weighed. The weight generally varies between 0·5 and 2·0 g. This weight gives an indication of how much sample is available for analysis and how much the naiad grew during that particular growing season. The second annular layer of peripheral nacre was then pulverised with a porcelain mortar and pestle which was easily cleaned between samples to prevent cross contamination.

Samples of the order of 0·5 g each were used for the analysis. An accurately weighed amount of the powder was placed in a plastic vial suitable for neutron activation analysis (Ernest F. Fullam, Inc., No. 1161). The vials were labelled with a black grease pencil and filled to the top with heated paraffin. Neither the grease pencil markings nor the paraffin caused interferences in the analysis, as determined in a blank run.

The paraffin seal was used to keep the sample in the vial and to fill most of the void space in the container. Air in the vial was kept to a minimum because the ^{40}Ar in the air produces a significant amount of radioactive ^{41}Ar when irradiated. The gamma rays from ^{41}Ar interfere with the determination of gamma rays from the samples if the amount of air in the vial is greater than 0·5 ml.

Standards were prepared by accurately weighing (to the nearest μg) approximately 1000 μg of MnO_2 (99·9 % pure) into the same type plastic vials. The standards were sealed and labelled using the same procedure described above for the samples.

Sample irradiation

The samples were placed in a small plastic basket in such a way as to be bracketed by standards. The basket contained three levels of vials. The centre level was placed in the centre of the reactor core where the highest neutron flux exists. The variation of the neutron flux along the length of the basket was found to be less than 10 % based upon the resultant activities of the standards after irradiation. The samples were irradiated at full power (10 kW) for 20 min in a thermal neutron flux of $2{\cdot}0 \times 10^{11}$ neutrons/(cm^2 sec). After irradiation, the samples were removed from the reactor and placed in a lead cave to allow short lived isotopes to decay. The radioactivity of a batch of eight samples and four standards immediately after irradiation was of the order of 200 mrems/h.

Gamma ray analysis of samples

After about five hours, the samples were analysed using a gamma ray spectrometer consisting of a Ge(Li) detector and a multichannel analyser (MCA)

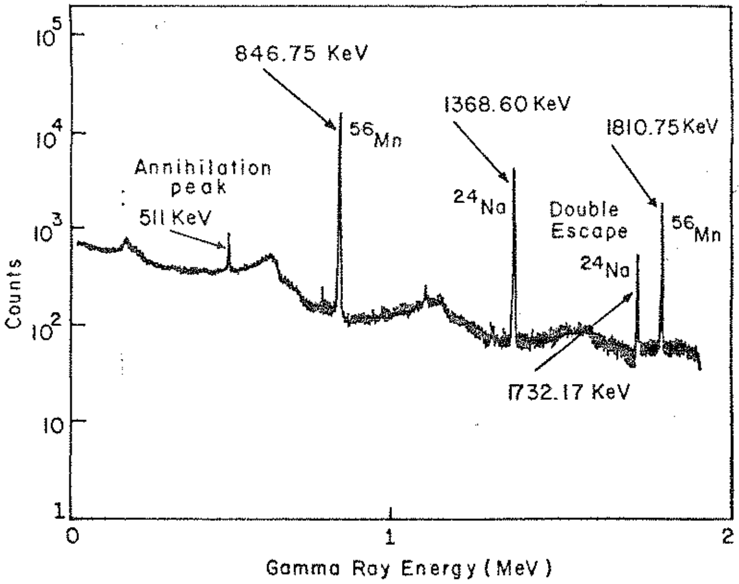

Fig. 2. Gamma ray spectrum of shell material.

(Fig. 2). The analysis was performed with the aid of a minicomputer connected directly to the MCA. The minicomputer has a program (ANALYZ) available on disc, provided by Canberra, which analyses spectra in the MCA based on a library chosen by the user. The information needed in the library for the computer to analyse each spectra for ^{56}Mn is the energy (846·8 keV), the half-life (2·54 h), and the yield of the isotope (99 %).

RESULTS AND DISCUSSION

The five species sampled were *Quadrula quadrula* (Rafinesque, 1820), *Amblema plicata plicata* (Say, 1817), *Obovaria subrotunda* (Rafinesque, 1820), *Obliquaria reflexa* (Rafinesque, 1820), and *Quadrula pustulosa* (Lea, 1831). Two collections from the Ohio State University Museum of Zoology were used in this study. The first was collected on 27 September 1969 by D. H. Stansbery and J. Ditmars. The shells were gathered from the Muskingum River at Lowell, Adams Twp., Washington Co., Ohio. The second set was collected on 17 October 1969 by D. P. Tanner. These shells were gathered from the Muskingum River at Beverly near the mouth of Wolf Creek, Waterford Twp., Washington Co., Ohio.

The sex of these species cannot be determined from shell characters, so this was an uncontrolled variable. A previous study (Saville, 1975), however, shows no significant correlation between sex and Mn concentration in naiad shells.

A significant correlation between age and Mn concentration has been noted (Sterrett, 1975) and thus only the second annular layer of each shell was used in this analysis. Since one purpose of this study was to determine whether a correlation between calendar year and Mn concentration exists, individuals of different ages at the time of collection were analysed. This means that their second annular layers correspond to different calendar years. The ages of the individuals analysed were three, four, five or six years. Their second annular layers correspond to the summers of 1968, 1967, 1966 and 1965, respectively. A three-year-old individual refers to a naiad that was in its third year of growth in the summer of 1969.

This method of keeping age constant accounts for some of the random variation in the observed results. Although the second annular layer of each individual is analysed, the age level of each individual when it deposits the second annular layer may vary up to a maximum of about 6 months. For example, if individual A starts life in the autumn of the year and individual B starts life in the spring of the year, the first annular layer of A will be smaller than for B, and the ability of A to concentrate Mn in its second annular layer may be different from the ability of B to concentrate Mn in its second annular layer.

A summary of the experimental values for Mn concentrations in the naiad shell samples is given in Table 1. The availability of the shells dictated the choice of samples.

Using the two way analysis of variance with replications, the following conclusions were reached.

In a consideration of the species *O. reflexa* and *Q. pustulosa* at Beverly, Ohio in the years 1965, 1966, and 1967, it was found that there was a difference in the manganese concentration among years at the 5% level. As shown in Fig. 3, the highest average manganese concentration in both species was in the year 1966. In addition, it was found that the concentration was not dependent on species at the 5% level. Although the average manganese concentration in each of these years at Beverly Ohio is greater for *O. reflexa* than for *Q. pustulosa*, the observed differences in the means are not significant as a result of the variations in the experimental data.

In a consideration of the species *O. reflexa* and *Q. pustulosa* at Lowell, Ohio in the years 1965 and 1966, it was found that there is a difference in the manganese concentration between species at the 1% level but not between years. As shown in Fig. 4, the average manganese concentration of *O. reflexa* is over twice as large as that of *Q. pustulosa* in both 1965 and 1966.

In a consideration of the species *Q. pustulosa*, *O. subrotunda*, *A.p. plicata*, and *Q. quadrula* at Lowell, Ohio in the years 1967 and 1978, it was found that there is no difference between years at the 5% level.

In a consideration of *Q. pustulosa* at Beverly and Lowell, Ohio in the years 1965, 1966, 1967, and 1968, it was found that there is a difference between locations at the

TABLE 1

MANGANESE CONCENTRATIONS OF NAIAD SHELLS FROM THE MUSKINGUM RIVER AT BEVERLY AND LOWELL

Location	*Species*	*Age of naiad at time of collection (growing seasons)*	*Year in which 2nd layer was formed*	*Individual number*	*Mn concentration ppm*	*Av. Mn concentration ppm*
Beverly	*O. reflexa*	4	1967	1	651	636
				2	621	
Beverly	*O. reflexa*	5	1966	1	1218	1008
				2	669	
				3	1138	
Beverly	*O. reflexa*	6	1965	1	462	529
				2	605	
				3	520	
Beverly	*Q. pustulosa*	3	1968	1	379	408
				2	436	
Beverly	*Q. pustulosa*	4	1967	1	556	591
				2	511	
				3	706	
Beverly	*Q. pustulosa*	5	1966	1	426	694
				2	958	
				3	697	
Beverly	*Q. pustulosa*	6	1965	1	784	509
				2	435	
				3	308	
Lowell	*Q. quadrula*	3	1968	1	497	412
				2	328	
Lowell	*Q. quadrula*	4	1967	1	226	304
				2	382	

Lowell	*A. p. plicata*	3	1968	1	437	479
				2	521	
Lowell	*A. p. plicata*	4	1967	1	390	366
				2	342	
Lowell	*O. subrotunda*	3	1968	1	439	354
				2	269	
Lowell	*O. subrotunda*	4	1967	1	436	396
				2	356	
Lowell	*O. reflexa*	5	1966	1	904	750
				2	772	
				3	574	
Lowell	*O. reflexa*	6	1965	1	730	862
				2	803	
				3	1052	
Lowell	*Q. pustulosa*	3	1968	1	338	403
				2	489	
				3	381	
Lowell	*Q. pustulosa*	4	1967	1	331	328
				2	324	
Lowell	*Q. pustulosa*	5	1966	1	335	320
				2	306	
Lowell	*Q. pustulosa*	6	1965	1	276	276

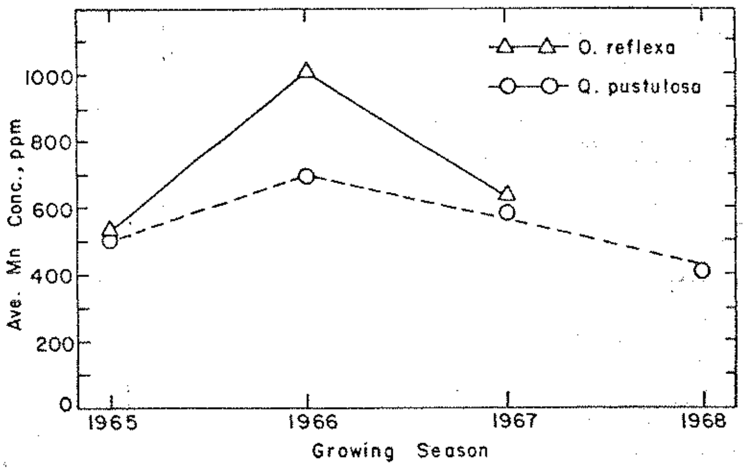

Fig. 3. Variation of manganese content with growing season at Beverly, Ohio.

2% level, indicating different growing conditions (e.g. different manganese concentrations in the river water) at these two locations during this time period (Fig. 5).

The significance level indicated in the above discussion is the probability of observing a difference at least as large as the one observed if no true difference exists.

Crystalline calcium carbonate constitutes the bulk of the bivalve shell and calcite and aragonite dominate the bivalve shell mineralogy (Taylor *et al.*, 1969). The

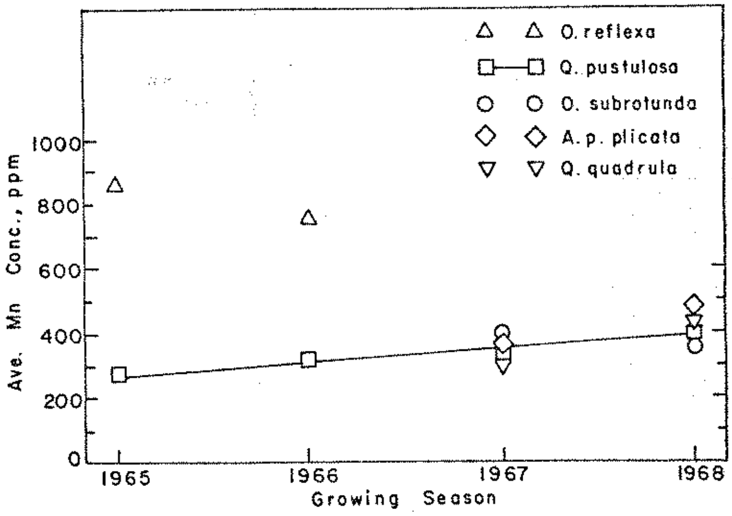

Fig. 4. Variation of manganese content with growing season at Lowell, Ohio.

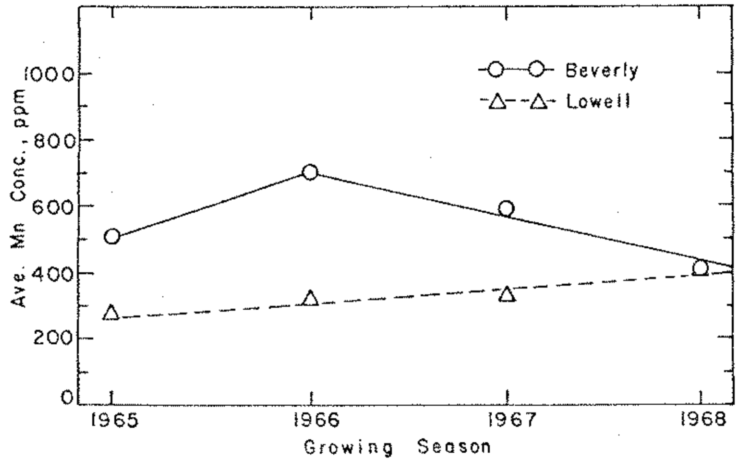

Fig. 5. Variation of manganese content of *Quadrula pustulosa* with location and growing season.

calcium carbonate of bivalve shells invariably contains small amounts of trace elements isomorphously replacing calcium (Taylor *et al.*, 1969). It is a reasonable possibility that the manganese found in the naiad shells is present as a result of the replacement of calcium by manganese (II) in the calcium carbonate matrix.

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