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Analysis of Lead in Freshwater Clams from the Embarrass River, Coles County, Illinois

Kwaku D. Nantwi
Eastern Illinois University

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ANALYSIS OF LEAD IN FRESHWATER CLAMS FROM THE EMBARRASS RIVER, COLES COUNTY, ILLINOIS

(TITLE)

BY

KWAKU D. NANTWI

B.S., EASTERN ILLINOIS UNIVERSITY

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

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IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

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YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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ANALYSIS OF LEAD IN FRESHWATER CLAMS FROM THE EMBARRASS RIVER, COLES COUNTY, ILLINOIS

Presented by

Kwaku D. Nantwi

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is acceptable.

ABSTRACT

Twenty-two freshwater clams of various genera were collected by hand from two sites on the Embarrass River located at 240 meters and 400 meters downstream from the dam at Lake Charleston and at depths of .4 and 2.5 meters. Ten grams of gill tissue from each clam was prepared by furnace ashing, dissolved in 30 ml. of acid mixture and analyzed for lead content using an atomic absorption spectrometer. The average lead concentration for the clams was 3.60 ppm, while that of the ambient water was 0.33 ppm indicating that these local bivalves show a lead biocentration of about ten times. The levels of lead found, and the proximity of the sites to motor routes, suggest that lead from automobile exhaust fumes finds its way into the River.

ACKNOWLEDGMENTS

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INTRODUCTION

The impact of heavy metal residues and other industrial wastes on the environment has been a major concern over the last few decades and a number of workers have studied this problem (Brooks, 1977; Brooks and Rumsby, 1966; Brown, 1968; Davies et al., 1976; Chisolm, 1971; Jones, 1938; Jovicie, 1973; Mayfield et al., 1973; Mullins, 1977; Nehring, 1976; Patrick and Loutit, 1975; Patterson et al., 1976; Rehwoldt, 1973; Shepar et al., 1978; Wurtz, 1962). The investigations of Watling and Watling (1976) demonstrate that increasing releases of wastes into the aquatic environment may have a toxic effect not only on the local flora and fauna, but also on humans as well.

Lead (Pb), a heavy metal, is very persistent in the environment. Its source of concern for humans lies in the many reported cases of lead poisoning, especially in children (Chisolm, 1971; Chisolm and Goyner, 1972). In the freshwater environment lead can be concentrated, over an extended period, to high levels in larger long-lived aquatic invertebrate animals (Anderson, 1974; Mathis and Cummings, 1973; Leland and McNurney, 1974).

Freshwater clams are sedentary, long-lived filter-feeding invertebrate animals that commonly occur in relatively large numbers in most inland aquatic habitats. This makes them feasible objects for investigating accumulation of heavy metal residue if a dependable, easily applied, method of analysis can be formulated. The Embarrass River in Illinois

supports a very diverse clam population and receives its share of industrial and agricultural residual waste from farmlands and bordering communities (Durham and Whitley, 1971). With this in mind the purpose of this study is as follows:

(1) To detect the presence, if any, of appreciable concentrations of lead in clams collected from the Embarrass River in Coles County, Illinois.

(2) To determine the applicability of the Atomic Absorption Spectroscopy method of Friend et al (1976) for analysis of lead residue in the tissue of freshwater clams and suggest adjustments that may improve the accuracy of the method.

(3) To determine the concentration of lead dissolved in the ambient water taken in the course of collecting clams.

LITERATURE REVIEW

Lead is a very ubiquitous metal that enters the environment through motor fuel, paints, varnishes and industry (Patterson, 1965, 1973; Davies et al., 1976; Langford, 1973; Ward et al., 1974; Patterson, Settle and Glover, 1976; Mullins, 1977). It is especially dangerous to children who are apt to eat peeling paint, a behavior termed pica.

According to the Committee on Biological Effects of Atmospheric Pollutants (1974), most research on lead poisoning has been restricted to cases involving occupational overexposure, lead-based paints being ingested by children, improperly lead-glazed earthenware vessels, discarded battery casings and illicitly distilled whiskey. In man the daily dietary intake of lead is 0.3mg. In excess, lead accumulates in the soft tissues and leads to a build up of aminolevulinic acid in urine (Chisolm, 1971). Lead has been implicated in ulcers and gastritis in those professions exposed to high concentrations (Jovicie, 1973). Scott et al., (1973) report that in high concentrations lead can inhibit Ca^{2+} uptake thus offsetting mitochondrial membrane transport mechanisms. Industrial waste is a source of concern and Watling and Watling (1976), report that increasing releases of wastes into aquatic environment may have a toxic effect not only on aquatic flora and fauna but also on humans. The most important mechanism of toxic action of metals is thought to be poisoning of enzyme systems (Pringle et al, 1968). Lead is known to be toxic to aquatic organisms with fish being especially

sensitive (Klein, 1962). It is not known to be an essential trace element in animal nutrition (Bowen, 1966). Most studies on the occurrence and accumulation of heavy metals in bivalves and other molluscs have been limited to saltwater forms (Ayling, 1974; Chow, Snyder and Snyder, 1975; Ireland, 1973; Kopfler and Mayer, 1973; Pentreath, 1973; Valiela and Banus, 1974; Windom and Smith, 1972; Brooks and Rumsby, 1965; Brooks, 1977). Reports on studies of heavy metal concentrations in freshwater bivalves are rather few. Mathis and Cummings (1973) report on heavy metal accumulation for three species of Unionidae collected in the Illinois River. Leland and McNurney (1974) have examined the transport and concentrations of lead in various components of a river ecosystem and report that the accumulation of lead by macro-invertebrates is a function of niche and habitat but it is not necessarily concentrated by a food chain mechanism. Anderson (1977) reports on the concentration of cadmium, copper, lead and zinc in six species of freshwater clams in Illinois and Wisconsin. One of the earliest studies on toxicity of metals was done by Jones in 1938. He calculated the various concentrations at which salts of lead and zinc showed adverse effects on the stickleback. He also showed that Ca^{2+} has antisynergistic effects on lead salts. Wurtz (1962) and Davies et al (1976) have since noted similar effects of Ca^{2+} on the toxicity of lead salts on mollusks.

Tatsumoto and Patterson (1963) reported the ability of invertebrate animals to concentrate high levels of lead. Shepar et al (1978) concluded that exposure of invertebrate animals to 32ug/L of lead for 28 days led to accumulations of 1000-9000 (fold). Goldberg (1965) reports bioaccumulations of 10^5 of many heavy metals by many lower members of food chains. However, the method of mechanism of such high ability to

concentrate heavy metals is not well understood although various theories have been proposed (Brooks and Rumsby, 1966). The toxicity of lead and cadmium to aquatic life has been investigated (Benoit et al, 1976; Davies et al, 1976; Holcombe et al, 1976; Shepar, 1976).

Research thus far indicates that many heavy metals entering aquatic environments are not magnified along straightforward trophic levels (Enk and Mathis, 1977). The relationship is more complex. Mathis and Cummings (1973) have demonstrated that bottom sediments act as a "sink" for heavy metals and organisms that dwell at the bottom have high concentrations. This is in agreement with the finding of Leland and McNurney (1974).

With the realization of the hazards of lead and other heavy metals on life, more recent and sophisticated analytical methods have been reported (Sprague, 1961; Mount and Brungs, 1967; Langford, 1969; Yeager et al, 1971; Mushak, 1977; Delves, 1978; Ediger, 1973; Friend et al, 1977).

MATERIALS AND METHODS

Site

The Embarrass River has its origin as a drainage ditch in the south campus of the University of Illinois in Champaign-Urbana. It flows south through the town of Villa Grove where it receives East Branch and Jordan Slough creeks. The enlarged flow continues southeast through Douglas, Coles, Cumberland and Jasper Counties. Two tributaries, Riley and Kickapoo creeks, flow into it carrying treated sewage effluents from Charleston and Mattoon, Illinois. The Embarrass empties into the Wabash River at Crawford County, Illinois.

Collection of Clams

Clams were collected at varying depths (10cm - 60cm) using a clam rake and by hand-dipping from two sites along the Embarrass River in Coles County, Illinois, during June and July, 1979. Collection sites were located at 400 meters and 240 meters downstream from the dam at Lake Charleston (used in this study as a reference point). Each clam was returned to the laboratory and immediately frozen at 0° C until ready for analysis.

Analysis Method

Determination of lead (Pb) was carried out using the procedure outlined by Friend et al (1976). The clam tissues were analyzed using a Beckman 485 Model Atomic Absorption Spectrometer. The underlying

principle of Atomic Absorption is that the sample components are converted into the elemental atomic vapor in a flame and this atomic vapor is imposed into a beam of radiation characteristic of the analyte element. For dilute solutions there is a direct correlation between the amount of radiation absorbed (Absorbance) in the flame and concentration of analyte in the solution. For high concentrations there is a broadening effect owing to the closeness of the analyte particles in the vapor state. This, in turn, is due to the interactions of particles with one another. Temperature has a direct effect in making available more particles in elemental form.

Preparation of Clam Tissue for Analysis

Each specimen was thawed, dissected and the gills removed. Ten grams of gill tissue were placed in a 250ml beaker to which was added 10 ml $Mg(NO_3)_2$ (0.1M) in 95 percent ethanol. The material was well mixed by agitation and the ethanol removed by evaporation on a hot plate. The sample was then placed in a muffle furnace that was controlled at 450° C/500° C and allowed to char for 20 hours. During this time, complete oxidation of organic matter was accomplished. The sample was then removed from the furnace and allowed to cool. A few drops of nitric acid were added to insure complete oxidation and the sample was warmed at 140° C - 150° C for an additional hour. The resulting ash was white in color.

The ash was dissolved in 30ml of an acid mixture (200ml of conc. HCl, 650ml H_2O and 150ml conc. HNO_3) with a little warming. The solution was filtered using a fritted glass filter (Porosity S). The filtrate was analyzed for lead by Atomic Absorption Spectrophotometry and the results reported in parts per million.

Preparation of Ambient H_2O for Analysis

A few drops of conc. HNO_3 were added to 30ml of ambient water to dissolve any particulate matter and bring the pH to an acid value. The solution was filtered and analyzed as described below.

Operation of Instrument

The aspiration rate of a test solution was controlled at about 5ml per (60 - 90) sec. After every aspiration of a sample or standard solution, distilled water was passed through the burner. This treatment was necessary to help clean away any precipitated debris that may have settled on the burner head and which would give rise to flame instability. Blank solutions of the acid mixture used in dissolving the clam tissue were also checked to detect the presence of any background lead, which would exaggerate the actual concentration of lead in the clam tissue.

The span of the instrument is a device that controls the scale of the instrument readout. A scale expansion for samples was used owing to the expected low levels of lead in the clam tissue. The scale was set so that the instrument read 0.200 absorbance units full scale. The scale expansion was obtained by setting the span control of the instrument to 2.00. With the expanded scale, the level of detection of lead was ± 0.03ppm.

A sample of the solution was transferred into a 5ml aspiration cup which was fitted in place, under the intake point to the flame. Aspiration was effected by a fine capillary tube between the burner and solution. As aspiration occurred, the absorbance of light in the flame was read on the output meter of the instrument. The zero absorbance of the

instrument was set prior to running any sample while operating distilled water into the flame. This zero point was checked periodically throughout the analysis. The instrument settings are presented in Table I below:

Table I

Instrument Settings

Hollow Cathode Lamp Current	20ma
Burner Height	1.2cm
C_2H_2 Pressure	4.0psig
Air Pressure	25.0psig
Analysis Wave Length	283.3nm
Slit Width	0.65mm

Preparation of Standards

A stock solution containing 100ppm Pb^{2+} was prepared by dissolving 0.1599g of $Pb(NO_3)$ in 0.1m HNO_3 and diluting with 0.1m HNO_3 to 100ml. Standards containing 1, 2, 4 and 10ppm lead were obtained by aliquot dilution of the stock lead solution.

RESULTS

Twenty-two clams of eight genera were collected from two sites in the Embarrass River. The species represented in the collection are presented in Table II.

Table II

Name and Number per Specimens of Freshwater Clams Collected from Embarrass River, Coles County, Illinois June-July 1979

Genus Species	Common Name	Number of Specimens
Quadrula quadrula	Maple Leaf	2
Tritogonia vericosa	Buckhorn	3
Strophitus rugosus	Squaws Foot	1
Lampsilis ventricosa	Pocket Book	6
Quadrula metanevra	Monkey Face	1
Lamsigona complanata	White Heel Splitter	2
Actinonaias carinata	Mucket	3
Anodonta grandis	Floater	4

The absorbance readings obtained for Pb^{2+} standards are presented in Table III and Figure I. The linearity observed in Figure I derives from a direct correlation between absorbance levels and concentration of solutions (if they are dilute). In general terms it is known as Beer's Law and is presented as $A = \epsilon bc$ where A is the absorbance, ϵ is the molar extinction coefficient, b is the thickness of the absorbing sample and c is the concentration of the absorbing species.

Figure I. Graph of Absorbance against Concentration for Standard Solutions of Pb (in ppm).

Fig. I

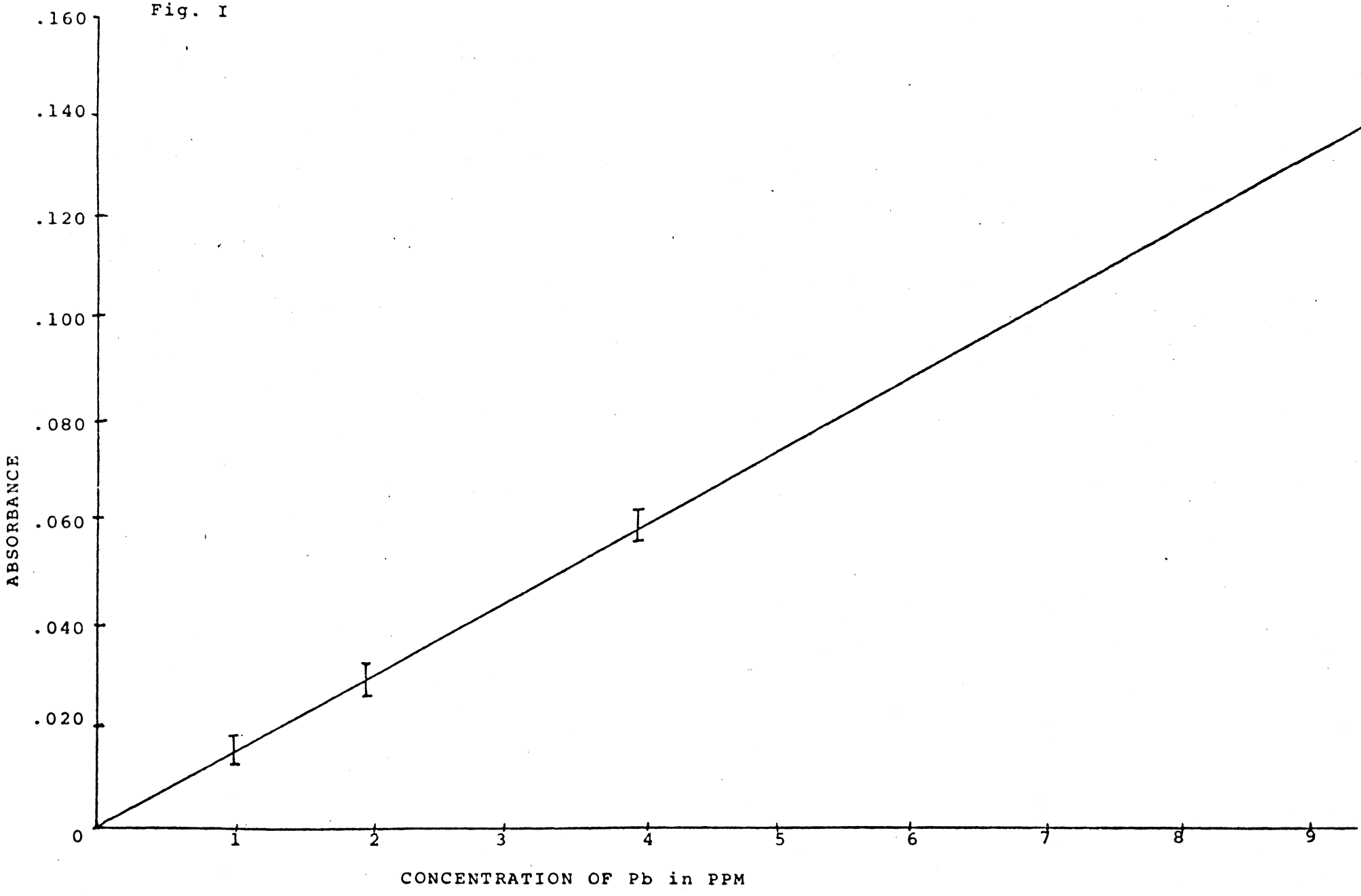

.160
.140
.120
.100
.080
.060
.040
.020
0
ABSORBANCE
1
2
3
4
5
6
7
8
9
CONCENTRATION OF Pb in PPM

Table III

Data Sheet (For Standards)

Concentration (ppm)		Absorbance
1.0		.018 ± .002
		.016 ± .002
		.018 ± .002
		.018 ± .002
	Average	.017 ± .002
2.0		.028 ± .002
		.028 ± .002
		.024 ± .002
		.028 ± .002
	Average	.027 ± .002
4.0		.045 ± .002
		.060 ± .002
		.062 ± .002
		.061 ± .002
	Average	.057 ± .002
10.0		.142 ± .002
		.142 ± .002
		.140 ± .002
		.144 ± .002
	Average	.142 ± .002

For each of the mean absorbance readings reported in Table IV for each solution of clam tissue, the concentration of lead in 30ml of solution can be read from Figure II. The concentration of lead in the clam tissue in ppm can then be computed thus:

$$\frac{\text{Concentration of } Pb^{2+} \text{ in ppm(vol. of sample Sol.)} \times 10^6}{10gm}$$

These results are reported in Table V.

Table IV

Comparison of Absorbance of Ambient H_2O and Clam Tissues for Individual Clams

Sampled Clams by Name and Number	Measured Absorbance	Mean Absorbance
Quadrula quadrula	.060	.060
	.060	
	.058	
	.060	
Lampsilis ventricosa	.028	.032
	.032	
	.032	
	.032	
Anodonta grandis	.032	.032
	.032	
	.032	
	.032	
Lampsilis ventricosa	.028	.028
	.028	
	.028	
	.028	
Actinonaias corinata	.028	.024
	.020	
	.024	
	.024	
Tritogonia vericosa	.020	.020
	.020	
	.024	
	.020	
	.016	
Quadrula quadrula	.020	.020
	.020	
	.020	
	.018	
Lampsilis ventricosa	.024	.020
	.020	
	.020	
	.020	
Lampsigona complanata	.020	.020
	.020	
	.020	
	.020	
Lampsilis ventricosa	.020	.020
	.020	
	.020	
Lampsilis ventricosa	.016	.016
	.016	
	.016	

Table IV (Con't.)

Sampled Clams by Name and Number	Measured Absorbance*	Mean Absorbance
Tritogonia vericosa	.016	.016
	.016	
	.016	
Anodonta grandis	.016	.016
	.016	
	.016	
Actinonaias corinata	.016	.016
	.016	
	.016	
	.016	
	.014	
Actinonaias corinata	.012	.012
	.012	
	.012	
Tritogonia vericosa	.012	.012
	.012	
	.012	
Anodonta grandis	.012	.012
	.012	
	.012	
Anodonta grandis	.010	.010
	.008	
	.010	
	.010	
Strophitus rugosus	.008	.008
	.008	
	.008	
Lampsilis ventricosa	.008	.008
	.008	
	.008	
Quadrula metanevra	.006	.006
	.006	
	.006	
Ambient H_2O	.004	.004
	.004	
	.004	

*The measured absorbances are known with the precision of ±.002 absorbance units.

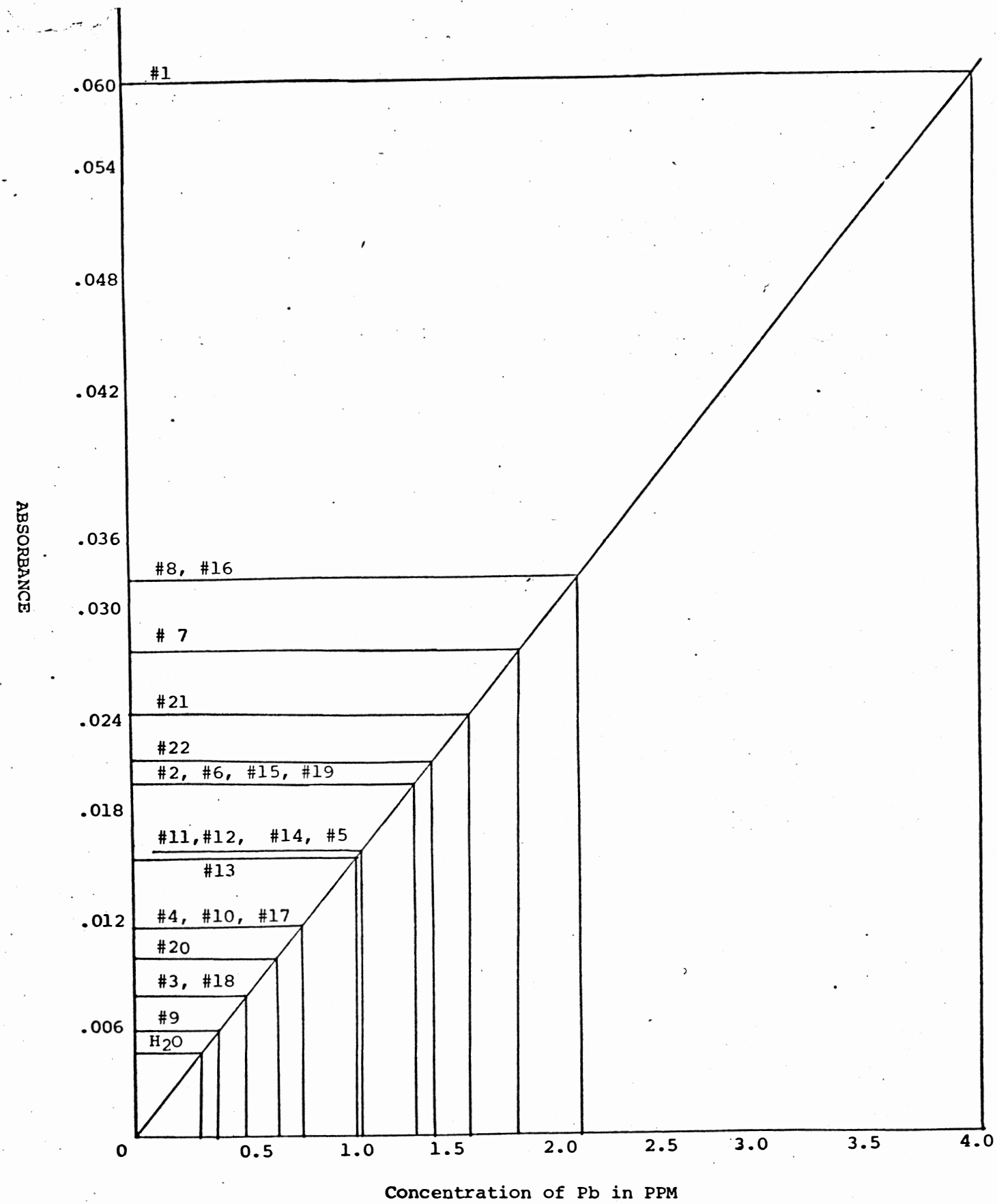
From Table IV it is observed that the mean absorbance of 30ml ambient H_2O was lowest of all the analyses. This is also reflected in the Pb^{2+} concentrations computed.

Table V

Average Concentrations of Pb^{2+} (ppm) in Clam Tissue According to Species

Genus Species	Conc. in ppm	Average Conc. in ppm	Sample # on Fig. II
Quadrula quadrula	12.00	8.02	1
	4.05		6
Quadrula metanevra	1.26	1.26	9
Tritogonia vericosa	2.28	3.21	10
	3.30		14
	4.05		2
Strophitus rugosus	1.53	1.53	3
Lampsilis ventricosa	1.53	4.34	18
	3.30		12
	4.05		15
	4.35		22
	5.34		7
	7.50		8
Lampsigona complanata	3.30	3.67	11
	4.05		19
Actinonaias corinata	2.20	3.07	4
	2.28		13
	4.80		21
Anodonta grandis	1.86	3.73	20
	2.28		17
	3.30		5
	7.50		16

Figure II. Absorbance of Level of Pb^{+2} in 10gm of Clam Tissue versus Concentration in ppm Pb^{2+} with Clams Numbered According to Table V.


#1
.060
.054
.048
.042
.036
#8, #16
.030
7
#21
.024
#22
#2, #6, #15, #19
.018
#11,#12, #14, #5
#13
.012
#4, #10, #17
#20
#3, #18
#9
.006
H2O
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
ABSORBANCE
Concentration of Pb in PPM

DISCUSSION

The finding here that ambient water contains low levels of lead (0.33ppm) is in general agreement with Mathis and Enk (1975) who found 0.5ppm lead in the Jubilee River--a permanent stream in rural Peoria County, Illinois--and in a similar study on the Illinois River Mathis and Cummings (1973) found low levels of lead in the ambient water (0.3ppm). A most comprehensive study on freshwater clams by Anderson (1975) in the Fox River between Big Bend, Wisconsin and Geneva, Illinois, however, shows rather unusually low levels of lead in the ambient water (.021ppm). These low levels of lead in ambient water support the finding by Mathis and Cummings (1973) that the bottom sediments act as a "sink" for most heavy metals. The bottom sediments consequently have the highest concentration of heavy metals, and this in turn reflects on bottom-dwelling macroinvertebrates (Leland, 1974). In general the low lead level in the streams could reflect both a solubility equilibrium between the metal ions and various salts in the sediment and adsorption of metal ions to the sediment clays. Mathis and Enk (1975) report high lead levels of 8.30ppm in the sediment of the Jubilee River. Anderson also reports lead sediment concentrations of 52 - 89.6ppm.

The results of this study also indicate fairly low levels of lead (1.26 - 12.00ppm) in the gills of local bivalves when compared to the Anderson study, which was carried out in the Fox River between Big Bend, Wisconsin and Geneva, Illinois. That study indicates lead levels of

20 - 50ppm in the whole clam and 10.6 - 26.5ppm in the gills. The section of the river studied by Anderson passes through both agricultural and urbanized areas and can be safely assumed to represent an urban-rural environment. Anderson also made a study comparing the shell, muscle, viscera and gills in ability to concentrate lead. This showed that the general relationship for metal concentrations between organs is shell $<$ muscle $<$ viscera $<$ gills for freshwater clams. The gills concentrate between 53 - 56 percent of the total body lead. This is due to the fact that the gills act both as a filtering mechanism for food material and as a respiratory organ. The use of the gills for analysis of lead in the present study was based on the selective accumulation of lead (and other heavy metals) in the gill tissue.

Lampsilis ventricosa, Lamsigona complanata and Strophitus rugosus were used in both studies. The lead levels found by Anderson for these species were 9.0, 13.4 and 13.2ppm. For the present study the lead levels were 4.34, 3.67 and 1.53ppm. This suggests that the higher levels in the Fox River clams is reflective of the exposure of the Fox River bivalves to greater amounts of industrial waste, effluents and exhaust gases from outboard boat motors. The Fox River site also probably has a high sorptive capacity for lead leading to a greater "sink" effect of the metal which in turn is reflected in the bottom-dwelling organisms.

No attempt has been made in the present study to categorize the species of clams with respect to their capability to bioconcentrate lead. This has been necessary owing to the small sample of each genus that was available at the time of the study. (See Table II) A greater sample number of (40 - 50) each genus would be required to adequately make such a distinction. The most variable levels of lead were found

in the genus Quadrula, three of which were analyzed. The lead concentrations in these samples were 1.26, 4.05 and 12.00ppm. These differences appear to be due more to the respective ages of the clams than the depth of the clam at a given location. Age appears to be a factor in this since these lead concentrations showed a direct relationship with increasing shell size. Older and bigger sized clams generally have experienced a longer exposure to an environment since clams are generally sedentary.

The average lead level in clam gills in the study is 3.60ppm whereas the level in ambient water is 0.33ppm. This indicates approximately a ten-fold bioconcentration in local bivalves. The source of this lead is unknown. It is not sewage-industrial waste from the Charleston - Mattoon area as these effluents enter the river below the sites at which the clams were collected. The most logical source of the lead is from automobile and agricultural engines owing to the exposure of the collection sites to motor vehicle routes and operating farm machinery.

CONCLUSION

This study does seem to point to the need for similar studies on the applicability of macroinvertebrates as biological monitors of environmental pollution. Ambient water generally has a lower metal concentration than the level in macroinvertebrates. This finding is in agreement with Mathis and Cummings (1973), Anderson (1974) and Mathis and Enk (1975).

The analytical method employed in this study is very feasible and does not require an extraction and concentration step for detection of lead in the parts per million range.

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