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UPTAKE OF KEPONE BY OYSTERS EXPOSED TO CONTAMINATED SEDIMENTS MIXED WITH LIGNITE

by

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1978

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Preliminary studies conducted by personnel of the Allied Chemical Corporation at Morristown, N. J. suggested that coal would be used to adsorb Kepone from an aquatic substrate. The possibility of using coal to bind Kepone contaminating the natural environment indicated further preliminary exploration of the matter.

The Virginia Institute of Marine Science undertook a laboratory study with oysters under contract with the Allied Chemical Corp. to explore that possibility. Lignite was mixed on a 1:10 dry-weight ratio with sediments contaminated with Kepone.

In one study, oysters were exposed to a lignite-sediment mixture flowing over them in suspension using sediments from Jordan Point in the James River. In a second type of experiment the oysters were laid, partially buried, in a bed of lignite-sediment mixture (also in a 1:10 dry-weight ratio) using sediments collected inside Bailey's Creek in Hopewell. Appropriate control experiments were conducted simultaneously.

The period covered by these experiments was 1-22 February, 1978.

Materials and Methods

Apparatus used in experiments with sediments in suspension

A diagram of the apparatus used to conduct experiments with flowing suspended sediments is shown on Figure 1. York River water was piped into a constantly-overflowing box (A) from which it was pumped through heat exchangers (C) into a rectangular cascading trough (D). The latter served to allow bubbles created by the escape of dissolved gases to dissipate before reaching the animal trays. Heated water then flowed into a rectangular trough (E) which was suspended from the ceiling directly above the wet table that held the experimental trays. Water depth in the trough was maintained at 20 cm by a drain standpipe of that height.

Water to supply the experimental trays was siphoned out of trough E with plastic tubing. Flows were regulated by the bore size of the plastic tubing used for siphons. Siphons were cleaned daily and flow measurements made before and after the siphons were cleaned.

Water from the siphons entered a cylindrical mixing chamber made of acrylic plastic (I), 14 cm in diameter and 13 cm in height.

Kepone-contaminated sediment suspensions entered the mixing chamber simultaneously with the York River water. Sediment suspensions were kept well mixed in flasks (H) by magnetic stirrers (J). They were metered into the mixing chamber at a constant rate by peristaltic pumps (G).

River water and sediment suspensions were mixed in the mixing chamber by magnetic stirrers. Observation showed that the mixing was complete before the mixture flowed out of the mixing chamber. There was no sedimentation in the chamber. The diluted sediment suspensions flowed into the experimental trays (K) through a standpipe located at the end opposite to the one through which water and sediments entered the chamber. The system set up was the same for trays holding control animals except for elimination of components G, H and J.

The trays used were made of acrylic plastic and were 49 cm long, 26 cm wide and 8 cm high. The overflow end was 6 cm high and that also was the water depth in the tray. Oysters laid directly on the bottom of the tray.

A system of sediment traps was used to insure that no contaminated sediments from the overflowing trays escaped into the floor drain which emptied into the York River. The first component was the wet table on which the experimental trays were set (L in Figure 1). A standpipe about 2.5 cm high inserted in the drain hole of the wet

table converted the table into a sediment trap. A plastic circular tank (50 cm high and 30 cm in diameter) received water from the wet table through a pipe reaching close to the bottom. The tank overflowed near its top into a series of three rectangular boxes (114 cm long and 25 cm wide), each with a 15 cm high standpipe overflow. The third box overflowed into the floor drain. The sediments and other excess solids obtained in the experiments were collected in carboys for disposal.

Apparatus used in experiments with oysters in a sediment bed

The experiment in which oysters were held partially buried in a bed of sediments contaminated with Kepone was conducted using a recirculating water system (Figure 2). Two long troughs made of acrylic plastic (141 cm long, 15 cm wide and 6 cm deep) were filled with sediments to a depth of 4 cm. York River water was pumped to the head of the troughs through plastic tubing by a submersible pump from a sump located under the end of the troughs. The water flowed over the sediment bed and overflowed into the sump. A variable transformer was used to regulate the pumping rate of the submersible pump and thus regulate the water flow velocity.

A fresh supply of York River water at ambient temperature (between 2 and 5 C) was added continuously at the head of the troughs at a rate of 30 ml/min. The added

water served to maintain an adequate supply of food and oxygen in the system and to keep water temperature from exceeding 30 C. Once a day, the flow of York River water was increased to between 50 and 60 ml/hr for a period of one hour to further ensure that the quality of the water in the system did not deteriorate.

The water overflowing the sumps was collected in 13-gal plastic carboys. Small volumes of a liquid laundry bleach solution were added to the water accumulating in the carboys. This caused percipitation of suspended sediments and allowed siphoning of the clear supernatant from the carboys.

Before oysters were placed on the sediment bed in the troughs, water velocity was increased until sediments were stirred into suspension. After about two minutes, water velocity was reduced to a level that would not disturb the sediment bed. The sediments in suspension were allowed to settle out over the bed. Then oysters were placed on the sediment bed with their anterior end (hinge end) pressed into the sediments at an angle of about 30° so that about one-third of the oysters' height was below the sediment surface level. Water flowing over the animals was two to three cm deep. Once oysters had been placed in the troughs, water velocity was increased until there was a slight scouring of the upper 2-3 mm sediment bed with

transport of a very light load of suspended sediments. Water velocity was 4 cm/sec.

Source of experimental animals

Oysters used were obtained from areas free of Kepone in the Piankatank River. They were acclimated to the experimental temperature and salinities under flowing-water conditions at least one week prior to use. Analysis before start of each experiment showed them to be free of contamination with Kepone.

Preparation of sediment suspensions

Figure 3 presents a flow chart outline of the steps taken in preparation of Kepone-contaminated sediment suspensions. Sediments contaminated with Kepone were collected with a sediment grab sampler at Jordan Point, in the James River at Hopewell, and represented the top 6 cm of the bottom. They were transported to the laboratory in large plastic bags. These sediments were mixed in a large tub. Small plastic bags were filled to a volume of approximately 500 ml. The bags were numbered in succession in the order they were filled and stored in a freezer until needed. Only sediments collected on the same date were used.

When needed, a bag of sediments was thawed, mixed with well water and shaken mechanically in flasks for 12 hours or more. The sediments were then wet-sieved through

a 63μ screen and the resulting suspension diluted up to 6,000 ml with well water. This volume was labelled as a stock suspension with its identification number. It was maintained in suspension by continuous agitation with a magnetic stirrer and bar.

To insure homogeneity in dosage, stock suspensions were paired together using bags at opposite ends of the numerical progression; for example, the first bag filled was paired with the last one filled. Thus, the paired samples showed an increasing progression from the lowest number up and a decreasing progression from the highest number down.

The sediments in the paired stock suspensions were combined by siphoning them simultaneously into a series of smaller flasks in equal measured volumes. Siphoning was alternated from flask to flask in small volumes in a cycle that was repeated until each flask had been filled to the desired volume. The samples in two of the flasks were used to determine the concentration of Kepone in the suspension and the dry weight per unit of volume of the sediments in the suspension, respectively.

Sediments used in preparation of sediment beds

Sediments used in preparation of sediment beds in long troughs were collected inside Bailey's Creek in Hopewell. They were mixed together and transferred unsieved

into the troughs. Depth of the sediments was approximately 4 cm.

Addition of lignite to sediments

a) Sediments in suspension. Fine-size lignite (<100 mesh) was added to the sieved suspended sediments in flasks in a ratio of 1:10 by dry-weight. After the dry-weight per unit volume of the sediments in the suspension was determined, the proper amount of lignite was weighed and poured into the flask holding the suspension. The mixture was stirred continuously over a magnetic stirrer for three days before it was used in the experiment.

In each pair of flasks with sediment suspension, one had lignite added to it while the other did not. One tray of oysters received the suspension mixed with lignite and another tray received the suspension without lignite. A third tray receiving York River water with no added sediments suspension served as experimental control.

b) Sediment bed in long troughs. Two troughs holding oysters in a sediment bed were used. Lignite of an 8-20 mesh size was thoroughly mixed with the sediments in one of the troughs before any water

was added. The dry-weight ratio of lignite to sediment was 1:10 and was based on the dry-weight per unit volume of several samples of sediments taken from the trough.

No lignite was added to the second trough.

Sampling of animals in trays

Samples of the animals were analyzed for Kepone at the start of each experiment and at approximately weekly intervals thereafter for the three-weeks duration of the experiment. Each sample consisted of from three to five animals.

Kepone analysis

Analysis of all samples for concentration of Kepone were done by personnel of the Department of Ecology and Pollution in their laboratories. The method used was soxhlet extraction, fluorosil cleanup and electron-capture gas cromatography.

Determination of Kepone concentration in sediments

a) Experiment with sediments in suspension. The concentration of Kepone in the diluted sediment suspension flowing over the experimental animals was determined by computation of the product of four factors:

$$K_c = (s_c)\ (k_c)\ (d)$$

where

K_c = computed Kepone concentration in diluted suspension, in ppb

s_c = sediment dry weight per unit volume in stock suspension, in Kg/l

k_c = Kepone concentration determined analytically for stock suspension, in ppm

d = factor by which the suspension being pumped into mixing chambers was diluted; determined by the flow rate at which it was being pumped and the flow rate of York River water flowing simultaneously into the mixing chamber.

The factor d was controlled by selection of a peristaltic pump setting that would deliver the desired flow rate of the sediment suspension into the mixing chamber. In this experiment the flow from the peristaltic pump was 3.75 ml/min. The flow of river water into the mixing chamber was 420 ml/min.

b) Sediment bed in long troughs. Samples of sediments, in the long troughs, collected from six different places of each trough, were combined for Kepone analysis prior to addition of the lignite.

Environmental conditions

Mean water temperature and salinity during the three-weeks duration of the experiment using sediments in suspension were: 1st week, 17.0 C and 14.2°/oo; 2nd week, 17.6 C and 14.9°/oo; and 3rd week, 20.4 C and 14.5°/oo.

Mean water temperature and salinity during the eighteen-days duration of the experiment with oysters on a sediment bed were 22.8 C and 14.4‰, respectively.

Results and Discussion

The study where oysters were laid on a sediment bed lasted 17.9 days; the one in which they received sediments in suspension lasted 21.4 days. During that time oysters in the sediment bed were sampled twice (after 8.6 and 17.9 days) and oysters exposed to sediments in suspension were sampled three times (after 6.9, 13.9 and 21.4 days). Duplicate samples analyzed from each source and time interval consisted of between three to six oysters.

Results of these experiments are presented in Tables 1 and 2; Figures 4 and 5.

With one exception, in both types of experiments the concentration of Kepone in the meats of oysters exposed to contaminated sediments mixed with lignite was lower than the concentration in oysters exposed to similar sediments but without the admixture of lignite. The exception was found in the samples analyzed after 21.4 days from oysters exposed to sediments in suspensions (Figure 4). In that instance, the mean concentration of Kepone was nearly the same in oysters receiving sediments with lignite and in those exposed to sediments not mixed with lignite.

It is difficult to assess the statistical validity of these differences because of the small number of samples analyzed. However, there is a strong possibility that the differences are statistically significant.

Mixing lignite with the contaminated sediments appeared to reduce the accumulation of Kepone in oyster meats. However, the levels found were still within the range of values expected for the corresponding concentrations in the sediment suspensions, as established in previous experiments (Figure 6). Figure 6 shows the relationship between the mean hourly concentration of Kepone in sediment suspensions (analyzed at approximately weekly intervals) and the concentration in oyster meats exposed to those sediments for the same weekly periods. The data in Figure 6 come from three series of experiments similar to, but conducted previous to the experiments with lignite. The data are few at the upper end of the regression line, but they still provide a strong suggestion of the existing relationship.

The fact that the concentrations in oysters exposed to sediments in suspension are below the action level set by the EPA is a direct result of the concentration in the sediments to which they were exposed. Had that concentration been higher, the concentration in oysters would likely have also been higher as is indicated by the regression line in Figure 6 and as has been the case in other experi-

ments conducted since the time that Figure 6 was prepared (Figure 7).

The high concentrations found in oysters partially buried in a bed of sediments was due to the extremely high concentration of Kepone in the sediments. Those sediments were collected inside Bailey's Creek in Hopewell and analysis of two unsieved samples from the two experimental troughs showed them to contain 24.98 and 22.00 ppm, respectively. Analysis of a separate sample collected from the same location showed that the concentration in the unsieved sediments was 14.97 ppm. When sieved, the same sediments showed a concentrationof 8.85 ppm in the material >63μ and 93.0 ppm in the material <63μ.

This preliminary study suggests that mixing lignite with sediments contaminated with Kepone appears to reduce, but will not prevent contamination with Kepone of the bottom-dwelling fauna in the James River, especially those organisms that use particulate matter as their food source, when exposed to those sediments.

Table 1. Concentration of Kepone in the meats of oysters held in laboratory trays receiving contaminated sediments in suspension, with and without addition of fine-sized lignite. 1-22 February 1978.

Exposure Period (days)	Kepone conc. in sediments (ppb)[1] Range[2]	Hourly Mean	No. Oysters in Sample	Mean Size (cm)	Kepone Conc. in Oyster Meats (ppm)	Mean Concentration Factor
A. Sediments mixed with lignite						
6.9	0.09-0.15	0.12	4	7.9	0.16	1291
			4	8.2	0.15	
13.9	0.07-0.23	0.12	4	8.1	0.16	1291
			4	8.3	0.15	
21.4	0.01-0.29	0.15	3	7.9	0.16	937
			3	7.2	0.16	
B. Sediments not mixed with lignite						
6.9	0.06-0.20	0.11	4	8.0	0.21	1773
			4	8.4	0.18	
13.9	0.08-0.19	0.11	4	8.2	0.25	2182
			4	8.6	0.23	
21.4	0.08-0.28	0.16	4	9.0	0.14	1032
			3	7.5	0.17	

[1] Kepone concentrations given are those for each of the three weekly periods and not for the total exposure period.

[2] Range does not include values of 0, representing short periods of time when the trays were receiving only York River water without added suspended sediments. However, those values of 0 were used in computation of the mean hourly concentration for the weekly period.

[3] Mean of two oyster meat samples X 1000/Hourly mean concentration in sediments.

Table 2. Concentration of Kepone in oysters held partially buried in beds of contaminated sediments with and without addition of lignite. 5-23 February 1978.

Exposure Period (days)	No. Oysters in Sample	Mean Size (cm)	Kepone Conc. in Meats (ppm)
Trough A (Lignite added)			
8.6	5	7.4	1.00
	5	6.7	Lost
17.9	6	7.1	1.01
	6	6.7	0.82
Trough B (No Lignite)			
8.6	5	7.2	1.10
	5	7.1	1.26
17.9	6	7.3	0.80
	6	7.3	1.26

Key to identification of components in Figure 1.

A. Constantly-overflowing box providing York River water supply to system.

B. Submersible pump.

C. Heat exchanger system.

D. Cascading trough used to allow escape of gases coming out of suspension as result of river water being heated up.

E. Constantly-overflowing overhead trough from which water from experimental trays was siphoned.

F. Plastic tubing siphon.

G. Peristaltic pump used to meter out sediment suspension.

H. Flask holding sediment suspension.

I. Mixing chamber receiving simultaneously York River water and sediment suspension.

J. Magnetic stirrer.

K. Experimental tray holding oysters.

L. Wet table holding experimental trays.

M. Drain pipe maintained a water level of about 2.5 cm on wet table. This served as first component of a series of sediment trays.

N. Water from wet table overflowed into a series of three other sediment traps.

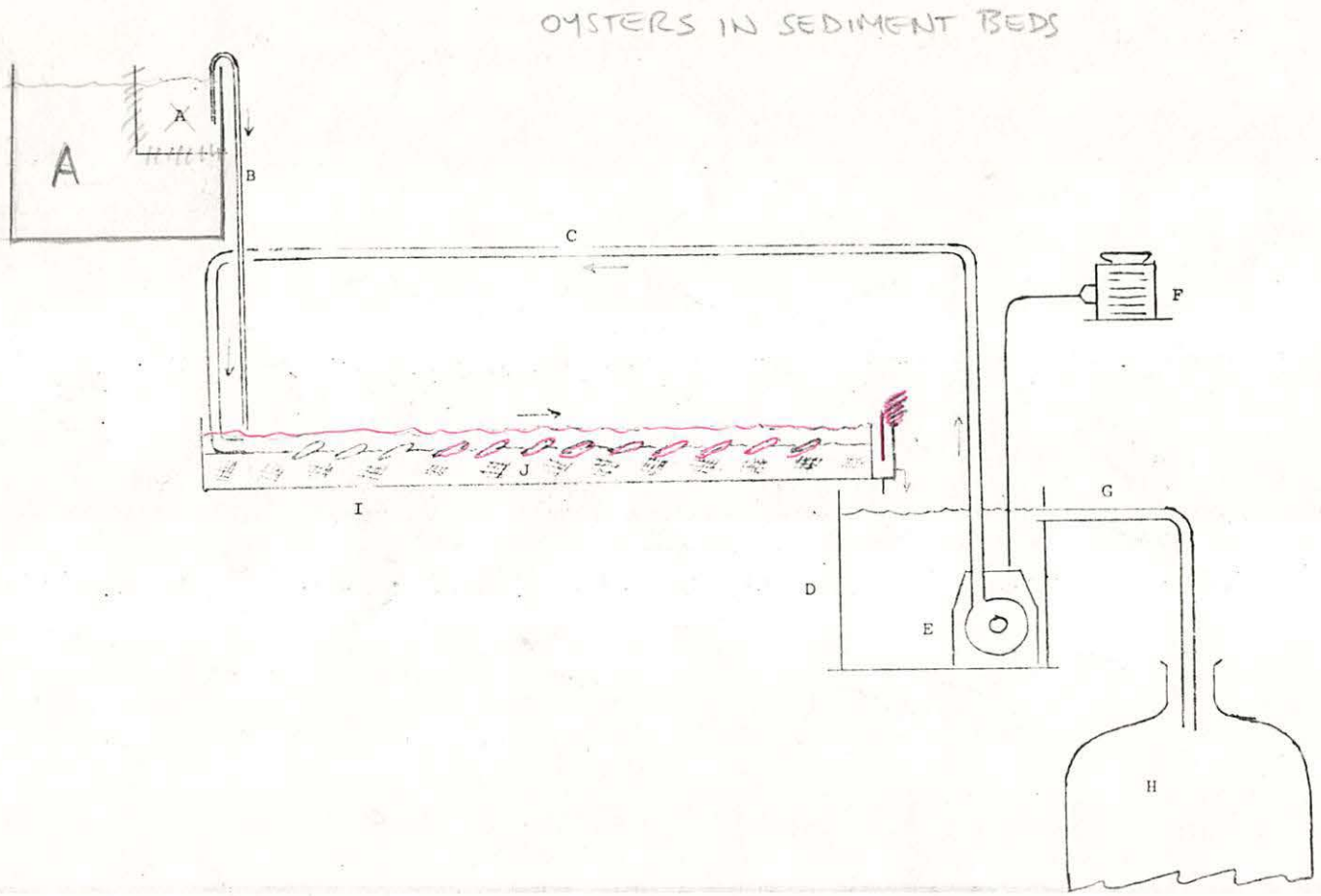

Figure 2. Diagram showing apparatus used in experiments where oysters were partially buried in a bed of contaminated sediments in a long trough. Duplicate apparatus were used for sediments mixed with lignite and sediments without addition of lignite. Identification of components appears on next page.

Key to identification of components in Figure 2.

A. Constantly overflowing trough with ambient York River water.

B. Plastic tubing siphon.

C. Plastic tubing from pump to head of tray.

D. Sump.

E. Submersible pump.

F. Variable voltage transformer.

G. Sump overflow tube.

H. 13-gal carboy receiving overflow water.

I. Long trough with oysters in sediment bed.

J. Sediment bed.

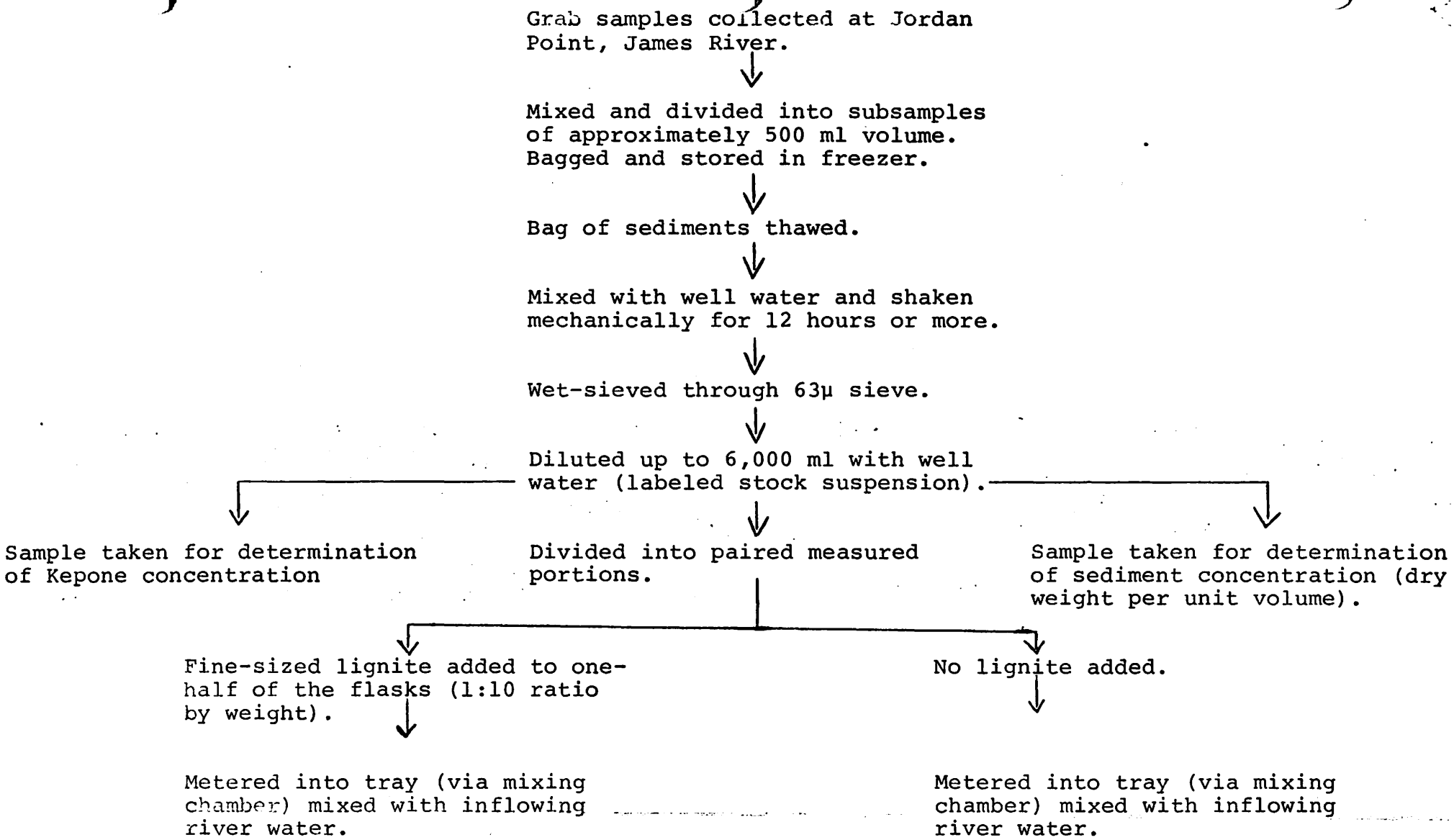

Figure 3. Flow chart showing steps in preparation of sediment suspensions introduced into trays holding experimental oysters.

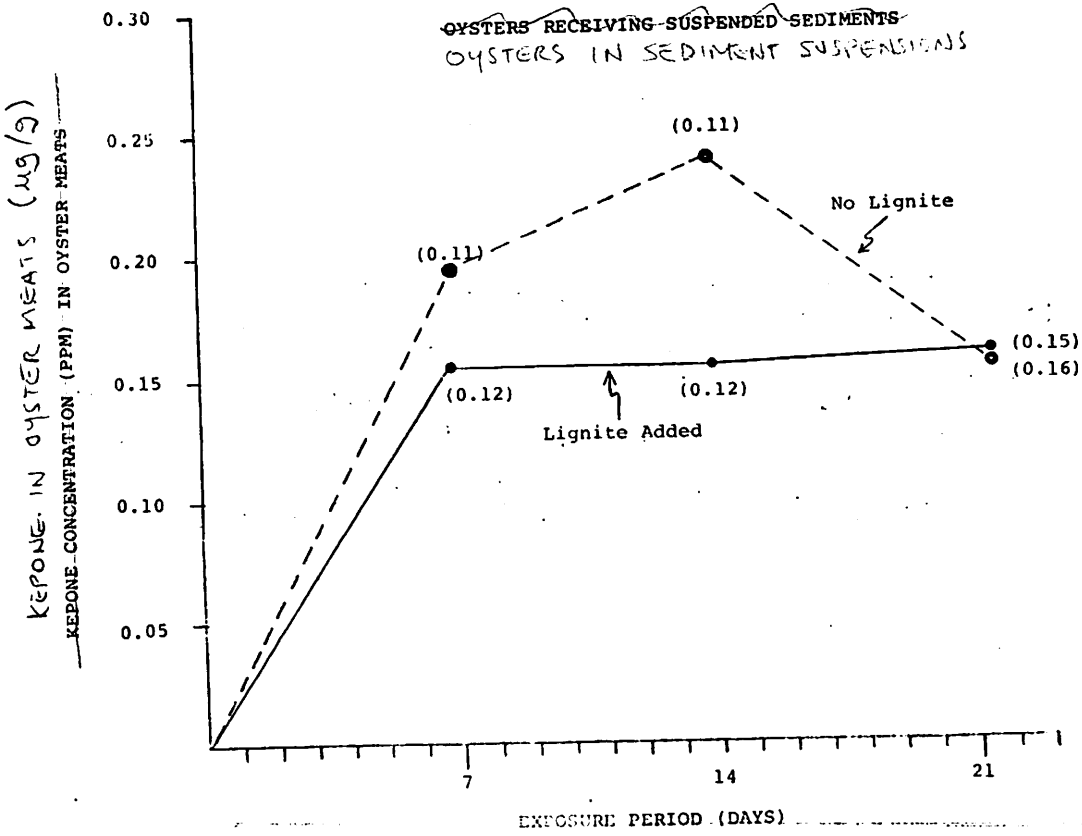

Figure 4. Mean concentration of Kepone in meats of oysters exposed to contaminated oysters in suspension. Experiments conducted between 1-22 February 1978. Figures in parentheses are mean hourly concentration of Kepone for weekly period ending at that point.

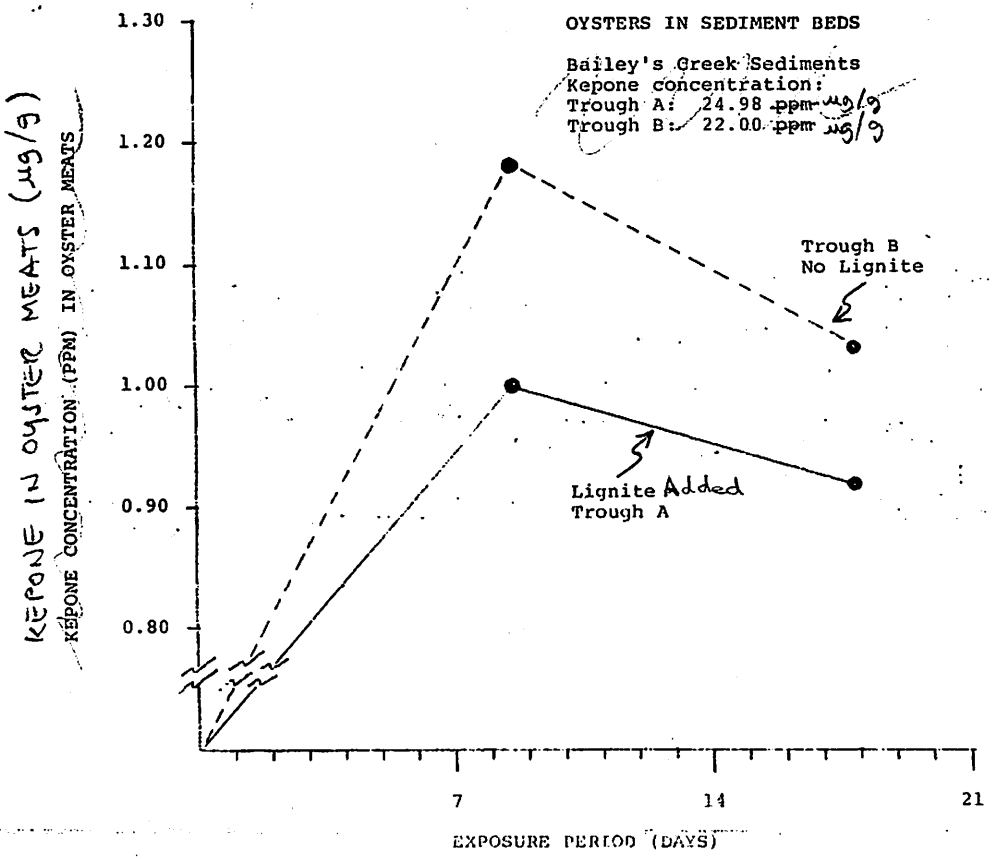

Figure 5. Mean concentration of Kepone in oysters held partially buried in contaminated sediment beds in laboratory troughs. Experiment conducted between 5-23 February 1978.

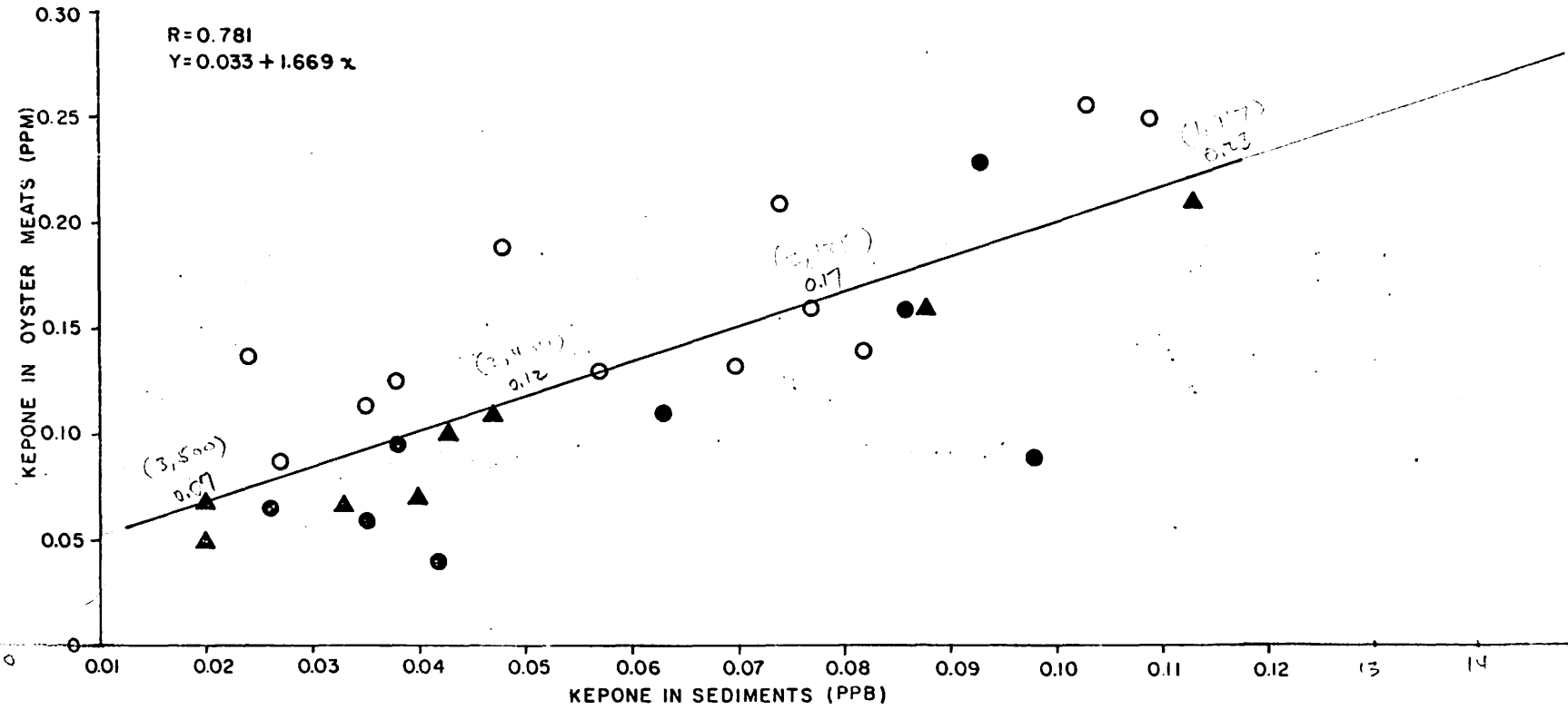

Figure 6. Regression of concentration of Kepone in oyster meats on mean hourly concentration of Kepone in suspended sediments for weekly periods in three series of experiments. Open circles: first series, closed circles: second series, triangles: third series.

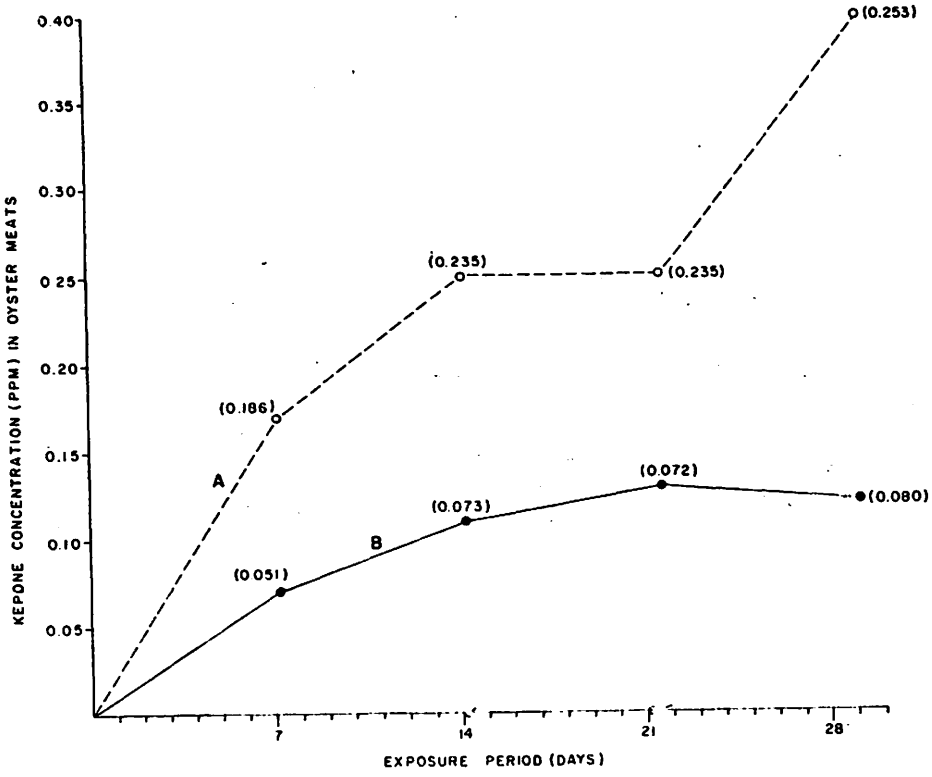

Figure 7. Mean concentration of Kepone in meats of oysters exposed to contaminated sediments in suspension. Experiments conducted between October-November, 1977. Figures in parentheses are mean hourly concentration of Kepone for weekly period ending at that point.