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The Role of Filter-Feeding Organisms in Concentration of Suspended Solids Containing Kepone Into Bottom Deposits.

by

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INTRODUCTION

Filter-feeding marine animals such as molluscs, tunicates and barnacles filter particles as small as 1 micron from suspension during their feeding process and void them as fecal pellets. These settle at much faster rates than their component particles. Feces or pseudofeces which settle are termed biodeposits. Eighty-two to 93 percent by volume of the particles in the biodeposits of several species of molluscs are smaller than 4 microns; the range in size of those particles is about 1 to 10 microns (Haven and Morales-Alamo, 1973).

Commonly occurring marine filter feeders deposit large quantities of biodeposits. Verwey (1952) calculated that, in the Waddensea, the bivalve <u>Cardium</u> deposited 100,000 metric tons annually. Oysters may produce up to 2400 kg per

hectare weekly. Many other marine filter feeders produce similar quantities (Haven and Morales-Alamo, 1966b).

An analysis of the biodeposits of oysters (those of other filter feeders have similar characteristics) indicates that clay minerals make up from 77 to 91% of each sample. The major constituents were illite, chlorite, mixed layer of clays, with quartz, feldspar, and montmorillonite the minor minerals (Haven and Morales-Alamo, 1973). These minerals are also the major components of sediments found in suspension in Chesapeake Bay tributaries (Nelson, 1960; Nichols, 1972).

Removal of fine particles in the 1 to 10 micron range by oysters and other filter feeders may be an important factor in sedimentary processes affecting the physical and chemical characteristics of bottom substrates (Haven and Morales-Alamo, 1966a and 1966b). Organic substances such as Kepone adsorbed onto clay mineral particles, or incorporated into bacterial or algal cells or organic detritus will be removed from suspension in the water and deposited on the bottom by filter feeders. Once the biodeposits are on the sediment surface, they may be mixed into subsurface sediments by benthic invertebrates to a depth from 10 to 15 cm (Haven and Morales-Alamo, 1973).

The inference which may be drawn from the preceding paragraphs is that when Kepone is associated with algal,

bacterial or inorganic clay particles, and is removed from suspension by filter feeders, it may accumulate on the bottom near the filter feeders where they would be subject to the bioturbation activity of the benthic infauna. Therefore, sediments in that area (to a depth of about 10 cm) may contain higher levels of that compound than similar nearby areas devoid of filter feeders. The persistence of Kepone in that environment will depend on how rapidly it disappears from the upper 10 cm of bottom. This latter aspect will be related to hydrographic conditions which affect the stability of the sediments and the types of sediment-mixing invertebrates inhabiting the area.

In view of the adverse impact Kepone is having in the James and in Chesapeake Bay, it is important to know if Kepone is being concentrated and the role of filter feeders in this process. Moreover, it is of major importance to know how rapidly Kepone will be dissipated from these deposits.

MATERIALS AND METHODS

The experiments reported here consisted of two phases. First, biodeposits (feces and pseudofeces) produced by oysters exposed to sediments contaminated with Kepone were collected in laboratory trays using the apparatus shown in Figure 1. The units labelled A through D were used only

when ambient river water temperature was below 10°C most of the time. York River water was piped into a constantlyoverflowing 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. When river water temperatures were above 10°C, York River water was piped directly into a rectangular trough (E) which was suspended from the ceiling above the wet table that held the experimental trays. Water to supply the experimental trays was siphoned out of trough E with plastic tubing into a cylindrical mixing chamber (I), 20.3 cm in diameter, where it was mixed with a heavy suspension of Kepone-contaminated sediments collected from the James River at Hopewell. sediments were maintained in suspension in a 6000-ml flask (H) by a magnetic stirrer (J). They were metered into the mixing chamber at a constant rate by peristaltic pumps (G). Water flow rates were controlled by glass flowmeters (F).

The river water and the sediment suspension were mixed in the mixing chamber by a magnetic stirrer. Observation showed that the mixing was complete before the mixture flowed out of the mixing chamber. Sedimentation in the chamber was negligible. The diluted sediment suspension flowed into the experimental tray (K) through a standpipe located at the end opposite to the one through which water and sediments entered the chamber.

The acrylic plastic tray was 81 cm long, 55 cm wide and 9 cm deep. A 9-cm high baffle with a 2.5 cm plastic mesh screen insert in its lower half was placed across the width of the tray 3.5-cm in front of the water entry point. The screen inset served to distribute the incoming water evenly through the width of the tray. An overflow baffle, 8-cm high, located at the opposite end of the tray controlled water depth in the tray. The tray was divided into 25 compartments by 2.5-cm high partitions which separated biodeposits produced by one oyster from those produced by oysters in the adjacent compartments.

Feces and pseudofeces produced by oysters were collected separately every day and each was combined in a container from the period of collection (usually one week). River water salt was washed off biodeposits by decanting after material had settled out, filling container with tap water and repeating the procedure after re-settling. Biodeposits were analyzed for Kepone concentration at the laboratories of the VIMS Department of Ecology-Pollution. They were stored wet in a refrigerator until the time they were used in the second phase of the study.

A system of sediment traps was used to insure that no contaminated sediments from our experiments escaped into the floor drain which emptied into the York River.

The first component was the wet table on which the experi-

mental trays were set. A standpipe about 2.5 cm high inserted in the drain hole of the wet table converted the table into a sediment tray. 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.

The second phase of the study consisted of exposure of benthic organisms in aquarium sediment beds to biodeposits ontaminated with Kepone. These biodeposits were placed on surface of the sediment beds. Sediments from the interal zone of the York River at Gloucester Point were transed to acrylic plastic cylindrical aquaria in the laboratory.

The aquaria were 30.5 cm in diameter and 26 cm

Depth of the sediment bed in each aquarium was 18 cm.

ved into the aquaria through small-bore tubing at

400 ml/min. A surface baffle was used to prevent

stream from impinging directly on the surface

and to direct it evenly around the aquarium area.

e was submerged just below the water surface.

rflowed out of the aquaria evenly over their

Animal populations in the sediment beds were established in three ways. Some of the aquaria were left undisturbed to represent the natural fauna in the sediments in terms of composition and density. All other aquaria were treated to kill the animals in the sediment bed. This was accomplished by adding fresh water to the aquaria, allowing the water to stand for 24 hrs, decanting it at the end of that period and repeating the procedure for each of five consecutive days. Following this treatment, York River water was allowed to flow through the aquaria for 24 hours. Then, selected animals collected from the same area that the sediments came from were added to some of the aquaria. Other aquaria received no new animals and were separated as controls. The fresh-water killing procedure was repeated for control aquaria for a 24-hr period at the end of every week.

After an acclimation period of three to four weeks, when there was ample evidence of the normal activity of the animals (and lack of it in the control aquaria). Evidence of that activity consisted of the presence of mounds, depressions and tube openings as well as burrows visible through the tank sides. The Kepone-contaminated biodeposits were then introduced into the aquaria to start the experiment. Table 1 summarizes the information on the quantities of biodeposits added to the different aquaria and the quantity of Kepone associated with them.

Prior to addition of the biodeposits, water flow was stopped and the water level lowered to about 3 cm from the aquarium top. The surface of the sediment bed was gently stirred up to level it and eliminate any mounds or depressions created by the animals. The sediments stirred into suspension were then allowed to settle out for 12 to 24 hours before the biodeposits were added.

Biodeposits added to each squarium were measured volumetrically, weighed wet and then mixed with river water. The slurry was added slowly and gently mixed with the water in the aquarium in such a manner that it was distributed evenly through the aquarium but without stirring up the sediment bed surface. Water flow into the aquaria was restored after 24 hours when the water column in the aquarium was clear, indicating that the biodeposits had settled out over the sediment bed.

The aquaria were held in a water table, undisturbed, until the time when core samples of the sediment beds were collected. Water flow, temperature and salinity were monitored during that period.

Core samples were collected at pre-determined times using 5-cm-diameter plastic core tubes. The coring tubes were centered around the entrance of an animal's burrow, over the center of conical mounds of sand built up by animals or around depressions or craters caused by the animals' activity.

All core tubes were thrust in place and plugged with a rubber stopper before any of them were removed. Removal was accomplished by digging into the sediment bed, placing a flat piece of plastic under the bottom of the tube and lifting it out. A rubber stopper was substituted for the piece of plastic at the bottom of the core tube.

Contents of each core were extruded upward in steps up to selected depth intervals with a rubber stopperpiston inserted through the bottom of the tube. intervals selected for analysis were the immediate surface layer (approximately 2-3 mm), the next two 0.5 cm sections in succession, 4-5 cm, 9-10 cm and 14-15 cm. Sediment sections between these were cut off and discarded. Each section selected for sampling was extruded above the upper opening of the core tube, the sides cut away so that only a cube remained in the center of the core and the cube sliced off the core and transferred to a pre-labelled clean glass jar. Cut-off of the section sides assured that the fraction analyzed for Kepone had not been contaminated accidentally by sediments from other depths due to the sliding of the core tube through the sediment mass.

Samples thus collected were analyzed for Kepone content in the laboratories of the VIMS Department of Ecology-Pollution. The method used was soxhlet extraction, fluorosil cleanup and electron-capture gas cromatography.

A shallow cylindrical aquarium similar to those used for holding the sediment beds was used in the first and third series of experiments as a sedimentation control. It was 30.5 in diameter, as were the others, but it was only 8 cm deep. The aquarium received water through the same baffle arrangement and at the same rate as the other aquaria. Thus, this aquarium represented the part in the other aquaria that was filled only with water. The bottom of the sedimentation control aquarium corresponded to the surface of the sediment bed in the other aquaria. This sedimentation control was used to measure the accumulation of sediments settling out of suspension from the water onto the surface of the sediment beds.

Samples of the sediment composing the sediment beds in the experimental aquaria were collected for particle size analysis from cores taken out of several of the aquaria. A longitudinal segment representing one-fourth of the volume of each core from the surface to a depth of 10 cm was sliced out. The whole segment was analyzed as one sample following the soil sieve and pipette methods outlined by Krumbein and Pettijohn (1938).

BENTHIC INVERTEBRATES USED TO STUDY BIOTURBATION

Phylum NEMATODA

Nematodes

Free-living marine nematodes are generally limited to burrowing in the substratum. They occur in large numbers where the bottom is rich in organic matter and are considered one of the most abundant groups of infauna. They feed on algae, diatoms and gungi and detritus depending on the species and its buccal apparatus. Those used in this study were not identified because the taxonomy of the 'group is very difficult.

Phylum PHORONIDA

Phoronis architecta

This thin and very elongated worm-like animal lives in a chitinous tube covered with sand grains buried vertically in the substrate. It is a member of the phylum Phoronida which is characterized by possession of a food-catching organ that surrounds the mouth (lophophore). Somewhat like a basket with ciliated tentacles, this organ operates as a filter-feeding mechanism by creating water currents. The intestine is U-shaped and ends in an anus at the anterior end of the body at the same level as the lophophore.

The tubes are 1.5 mm in diameter and up to 20 cm in length. Although the body of the animal is free inside the tube, only the anterior end ever emerges out of the opening. The animals used in this study were 6-8 cm long.

Phylum ANNELIDA

Arabellids

Burrowing errant polychaete worms of the family
Arabellidae. They are threadlike, very thin, and very long.
The ones we used were difficult to measure because of their length which was estimated as about 30 cm.

Glycera americana (bloodworm)

Glycerid polychaetes are raptorial feeders that live within a gallery system of tubes below the sediment surface. The system contains numerous loops which open to the surface. Detecting a prey on the surface <u>Glycera</u> moves to the burrow opening, extends out of the tube partially or completely, and seizes the prey with the jaws in its proboscis. Although it does not ingest sediments, the construction of a tube system and its movements in and out through the tubes can contribute to the mixing of the surface sediments into subsurface layers.

Clymenella torquata (bamboo worm)

This annelid inhabits a vertical sand-grain tube 2-3 cm in diameter which in mature animals may be between

15 and 20 cm long. The animal may reach a length of 16 cm. Animals used in this study were about 6-8 cm long. It is an important sediment mixer because it feeds below the redox potential discontinuity and defecates at the sediment surface. It has been called a "conveyor-belt" species by Rhoads (1974). They live upside down in the tube and ingest the substratum at the bottom of the tube. Following a distinct rhythum, the feeding halts and the worm backs up to the top of the tube to deficate the material passed through the qut.

Bioturbation by <u>C. torquata</u> has been well studied and its importance in the process has been shown by Mangum (1964), Rhoads and Stanley (1966), and Rhoads (1967).

Nereis succinea

The crawling polychaete N. succinea lives on the surface of sediment beds and also in U-shaped burrows it will build for itself or in the abandoned burrow of other animals. It can be found in tunnels as deep as 20 cm below the surface. It is a very active animal and does not occupy any of those burrows permanently. It feeds primarily on detritus material in the substratum.

Loimia medusa

This annelid of the family Terebellidae inhabits a membranous U-shaped burrow 15-20 cm long and feeds on

surface deposits by means of long slender tentacles which may extent up to 15-30 cm away from the burrow entrance. Surface deposit material adheres to mucous secretions on the surface of the tentacles and is then conveyed to the mouth along ciliated tracts or grooves. At intervals, ingested sediments are ejected back into the water column and settle unto the surface around the entrance to the burrow where they aggregate to form a mound.

Besides its potential for causing surface sediments to be mixed into subsurface layers <u>L</u>. <u>medusa</u> can concentrate contaminated material scattered over the surface into the mound formed around its burrow entrance (Haven and Morales-Alamo, 1967).

L. medusa may attain a length of 3 cm and those used in this study were about that size.

Phylum MOLLUSCA

Mercenaria mercenaria (hard clam)

The hard clam is an infaunal burrower which moves about to some degree by digging with their foot. No permanent burrow or connection with the surface is maintained, although in relatively stable substrates a hole for extension and retraction of the siphons is often kept unobstructed. Feeding is accomplished by filtering water from above the surface to the sediment bed. Mixing of sediments is accomplished by

the burrowing activity and extension and retraction of the siphons. The species commonly attains a length of 10 cm. In our experiments we used small clams between 1 and 3 cm in length.

Macoma balthica

M. balthica is a lamellibranch characteristic of the intertidal and shallow-water fauna in muddy sand. It is apparently restricted to the mesohaline part of our estuaries and is a true estuarine animal. It burrows only a few cm below the surface and remains at that depth depending on extension of its siphons for feeding at the surface. It is usually a deposit feeder that scours the surface sediments with its siphon to obtain food. At times it may feed as a suspension feeder by holding its siphons upright above the surface sediment layer.

The animals in this study were 2.5-3 cm long.

Tagelus plebeius (stout razor clam)

T. plebeius is a burrowing clam which has been seen to feed on surface sediments on occasion but is usually considered to feed primarily as a suspension feeder. It moves up and down within its burrow and does not rely completely on its extensible siphons when feeding. It can attain a length of 9 cm but those used in this study ranged between 2-4 cm.

Mya arenaria (soft clam)

The soft clam is a bivalve that may reach a length of up to 10 cm as an adult. The animals used in this study were 2-4 cm in length. It may bury as deep as 30 cm into the substrate with the siphons extending upward to the sediment-water interface. As far as is known, it is entirely a filter feeder relying on the simple extension of its siphon into the water column for feeding. Major lateral movements by the animal are apparently absent once the animal establishes its burrow. Its burrow can be 20-30 cm deep.

Phylum ECHINODERMATA

Leptosynapta tenuis (sea cucumber)

This echinoderm (Class Holothuroidea) has an elongated worm-like shape and ranges in size up to a length of 15 cm. Our experimental samples were between 8 and 10 cm. It has no permanent tube but burrows through the subsurface sediments ingesting and voiding sediments as it moves along. Myers (1977) and Powell (1977) have studied in detail feeding and sediment mixing by this species. They illustrated and described the construction of the U-shaped burrow, a process which involves construction of a funnel-shaped depression. For that reason it has been called a funnel feeder.

Phylum HEMICHORDATA

Saccoglossus kowalewskii (acorn worm)

S. kowalewskii is a sluggish worm-like animal with limited locomotor powers that burrows into mud and sand, usually in shallow water. Its musus-lined burrows are U-shaped with two openings to the surface. It is a mucus-ciliary feeder that will ingest sediment deposits. It piles up its fecal material in a coiled mound at the posterior opening of its burrow.

The animals used in this study were around 8 cm in length.

RESULTS

The data collected are grouped into three series of experiments according to the dates that cores were collected from the aquaria sediment beds. The first two series were started on the same date but cores were collected after 12 weeks (83 days) in the other. The third series of experiments was started four months later and extended for about 5 weeks (33 days).

The number of aquaria involved and number of cores collected in each series were, respectively, as follows: first series, 3 and 16; second series, 5 and 23; and third series, 5 and 23. The results of analyses for

Kepone at different depths in each core for the three series appears in Tables 2-4. Except where specified by a footnote, no animals were found within the cores collected.

First Series (36 days) - The three aquaria included in this series of experiments included one containing only the fauna dug up with the sediments, and one control aquarium in which the fauna was killed by exposure to fresh water. The third aquarium contained 12 animals of four different species as shown in Table 5: five Phoronis achitecta, three Loimia medusa, and two each of Leptosynapta tenuis and Glycera americana.

The distribution of Kepone with depth in those three aquaria was not greatly different from one to the other (Table 2). There was no strong indication that the presence of animals in two of the aquaria affected the distribution of Kepone differently than was found in the control aquarium without animals. In most cores Kepone was found only in the surface and top half-centimeter sediment layers. In two instances (cores no. 3 and 7 in aquarium no. 8), there was Kepone in the 0.5-1 cm layer although the concentrations in both cases were extremely low. In one core (no. 1, aquarium no. 8), Kepone was found at a depth of 9-10 cm. Strangely enough, in the latter core Kepone was found only at that depth (9-10 cm). There was no measurable amount of Kepone at the surface or 0.5 cm depth.

Kepone was found only in the surface slurry in five of the 13 cores collected from the two aquaria containing animals.

Three of the cores in the aquaria with animals showed evidence that the cores traced in depth burrows made by some of the animals. In core no. 5 of aquarium 1 we found a live phoronid (Phoronis architecta) extending from a depth of 0.5 cm to a depth of 5 cm. In cores no. 2 and 3 of aquarium 8 the tubes of Loimia medusa individuals were traced through the core from the surface to a depth of 11 cm in one and to a depth between 5 and 10 cm in the other. In the latter two cores, however, no trace was found of the animals that built the tubes. The tubes, however, curved away from the core section in both cases.

The concentrations of Kepone found at the surface and 0-0.5 cm depth were considerably lower than the concentration in the biodeposits added to the sediment bed. The concentration in sediments at those depths was between 0 and 2.9% of that in the biodeposits in the aquaria with animals. In the control aquarium the range of the difference was 0-5.2%.

Second series (83 days) - Five aquaria were included in this series of experiments. One was a control without animals. The other four involved different combinations of benthic species added to the aquaria after the original population had been killed with fresh water. Table 5 lists the species present in each of the aquaria. This series actually represents

an extension of the first series. Both groups of experiments were started at the same time, but the second series was allowed to continue for a longer period of time.

Results showed that in none of the cores collected was Kepone found at depths greater than 1 cm (Table 3).

Kepone was found at the 0.5-1 cm depth in only 6 of the 21 cores collected from aquaria with animals. In four instances no Kepone was found below the immediate surface of cores from the aquaria with animals even though there was evidence of animal activity at deeper levels, including even the presence of parts of a worm.

As in the first series, the concentration of Kepone in the core sediments of the surface and at a depth of 0-0.5 cm was considerably lower than the concentration in the biodeposits spread over the sediment bed at the start of the experiment. The range of the percent difference between the two was 0-14.2% in aquaria with animals and between 0-7.5% in the control aquarium.

Third series (33 days) - The results of the third series of experiments paralleled those of the first two. Cores from three aquaria with animals and two control aquaria without animals showed that in almost every core Kepone was found only in the surface and 0-0.5 cm samples (Table 4). There were only two instances in which Kepone was found at a greater depth and then it was only at a depth of 0.5-1 cm. As in the other two series, the concentration of Kepone found

in the individual core samples was much lower than the concentration in the biodeposits added to the surface of the sediment beds.

Two coring tubes were inserted into the sediment bed in aquarium no. 4 at the start of this series (3) of experiments. Aquarium 4 was a control without animals. The coring tubes extended about two inches above the overflow level of the water in the aquarium. Therefore, the surface sediments inside the coring tubes would not be affected by the accumulation of sediments settling out of suspension into the surface of the sediment bed and none could be transported out of the tube. The coring tubes remained in place in the sediment bed throughout the duration of the experiment.

At the end of the experiment those two cores were extracted and treated in the same manner as the other cores collected from the same aquarium and other aquaria. The distribution of Kepone with depth in the core sections analyzed from those two cores did not differ from other control samples.

A sedimentation control aquarium was included in this series of experiments to determine the extent to which sediments settling out of suspension from the water might dilute the concentration of Kepone on the surface of the other experimental aquaria. The control aquarium used was the same used as a sedimentation control in the first series.

A volume of pseudofeces comparable to that introduced into aquarium 4 was introduced into the sedimentation control aquarium. Thus, at the start of the experiment this aquarium contained only a layer of pseudofeces over the bottom.

Thickness of the layer was approximately one mm.

Visual observation indicated that the quantity of sediments on the bottom of the aquarium at the end of the experimental period was greater than at the beginning. The layer of deposits on the bottom was then approximately two mm thick. All the deposits were collected into a jar and oven-dried. A sample analyzed for Kepone showed that the concentration was 0.37 ppm, representing only 27% of the original concentration in the pseudofeces (Table 5).

Water Temperature

Low ambient water temperatures during the period between December 1978 and March 1979 required the use of a heat exchanger system to raise the river water temperature to a level which would permit the animals in the aquaria to remain active. The first two series of experiments were conducted during that period. Temperature was usually measured five times every day between 0800 and 2300 hours.

Between December 13, 1978 and January 18, 1979 the daily temperature of the water in the aquaria averaged 15.7°C with a range of 12.0-19.5°C in individual measurements (Table 6). Between January 18 and March 6, 1979, the mean temperature was 16.0°C with a range of 9.6-20.7°C.

The experiments included in the period May 31 and July 3, 1979 were conducted at ambient river water temperatures with a mean of 23.4°C and a range of $20.9-26.0^{\circ}\text{C}$.

Water Salinity

Water samples for salinity analysis were collected daily. During the period December 13, 1978 to January 18, 1979 salinity averaged 20.3% with a range in daily values of 19.1-21.5% (Table 6).

Between January 18 and March 6, 1979 the mean salinity was 18.1%oo with a range of 12.6-20.8%oo. During the latter period salinity stayed around 19-20%oo between January 18 and January 30, around 17-18%oo between January 19 and February 28 and around 13-15%oo between March 1 and March 6. Between May 31 and July 3, 1979 salinity averaged 15.7%oo with a range of 14.0-19.8%oo.

Size distribution of particles in the sediment beds

Seven cores were collected for particle size analysis from six of the aquaria used in the first and second series of experiments at the same time that cores for Kepone analysis were collected. Duplicate samples were collected from one aquarium and single samples from five other aquaria.

Only slight differences were found between cores from the different aquaria or the different series of experiments (Table 7). In all samples 90-95% by weight of the

particles were larger than 63μ . Sixty-eight to 86% by weight of the particles were between 125 and 500μ . The size class with the highest percentage of the total weight shifted between the $125-250\mu$ and $250-500\mu$ classes from sample to sample. The percentage of the particles for the remaining three size classes, <63, 63-125 and >500 was similar in most of the samples, with a range of 1-16%.

Visual examination of the cores collected for Kepone analysis when they were being sectioned showed that surface was covered by a very soupy layer of muddy sediment which was probably composed of particles in the silt-clay sizes. The depth of this layer, which was not easy to measure, was estimated at 2-3 mm. Below that depth the sediments were definitely sandy.

DISCUSSION

Benthic animals that live in bottom sediments and move within the subsurface layers are known to be capable of mixing and recycling large quantities of surface materials into the subsurface layers (Rhoads, 1963, 1967, 1974; Mangum, 1964; Gordon, 1966; Jacobsen, 1967; Rhoads and Young, 1970, 1971, Cadee, 1976; Kraeuter, 1976; Powell, 1977; Myers, 1977a, b). This mixing process is termed bioturbation. It is also known that filter-feeding invertebrates deposit significant

quantities of feces and pseudofeces (combined under the term biodeposits) on the surface of bottom sediments (Verwey, 1952; Lund, 1957; Kuenzler, 1961; Haven and Morales-Alamo, 1966b, 1972; Rhoads, 1967; Young, 1971; and Kraeuter, 1976).

Oysters and other bivalve molluscs which reside on or in the bottom of the James River have been shown to accumulate substantial amounts of Kepone in their tissues when exposed to sediment suspensions contaminated with that chemical (Haven and Morales-Alamo, 1979). Biodeposits produced by these animals were also contaminated with Kepone. The biodeposits produced increased significantly the total weight of the material that accumulated in experimental trays holding the animals when compared to trays without animals into which only suspended sediments settling out by gravity were being deposited.

It is reasonable then to assume that the combination of uptake of Kepone by oysters and other filter feeders with biodeposition and bioturbation would result in the displacement of Kepone from the surface of the sediment bed to subsurface layers. The studies reported here were conducted based on that premise.

Results obtained from our experiments did not show mixing of Kepone into subsurface layers deeper than 0.5 cm, with a few exceptions. In all but one of the exceptions the maximum depth at which Kepone was found was 1.0 cm. Previous

studies using radioactive tracers and fluorescent particles with some of the same animals in York River sandy sediments such as those used in the present experiments indicated that the zone of active sediment mixing was 2-4 cm although mixing could be detected deeper than 10 cm (Kraeuter, Haven and Morales-Alamo, MS in preparation). In aquaria with a mud substrate the zone of active mixing was 5-7 cm. The water content profile data of Young and Rhoads (1971) and Rhoads (1974) showed most of the activity in the top 2-3 cm. It appears, therefore, that the absence of Kepone from depths greater than 0.5 cm is anomalous.

Observation of the activity of the animals in the sediment beds and of the different types of sedimentary structures created by the animals (burrows, tunnels, depressions, mounds, fecal pellets, etc.) indicated that they were active and apparently in good health. Furthermore, Tables 1-3 present evidence of burrowing activity by some of the animals and even the presence of animals - at depths as great as 10 cm.

The only explanation we can give for the absence of Kepone at depths greater than 0.5 cm is that the quantity of Kepone added to the surface of the aquarium sediment beds was insufficiently large to permit detection of any fractions mixed with the sandy sediments of the sediment beds.

The quantities of biodeposits added in the first and second series of experiments were intended to represent the production of biodeposits by one oyster for periods of one to three weeks scattered evenly over an area of 729 sq. cm (the surface area of the aquarium sediment bed). This was considered an environmentally realistic accumulation of biodeposits based on the average deposition by one oyster established by us as 1.6 g/week in Haven and Morales-Alamo, (1966b). As a result of the concentration of Kepone in the different samples of biodeposits used there was a fairly wide range in the weight of Kepone contained in the biodeposits, from four to 34 g.

In the third series of experiments, the quantities of some of the biodeposits added were increased to the equivalent of up to nearly 13 weeks in three cases but held to an equivalent of three weeks in the other two. The 13-weeks equivalent was desired to compensate for the low concentration of Kepone in those biodeposits.

It appears that those quantities of Kepone added were not sufficient to permit their detection in sediment samples from the subsurface layers, due primarily to dilution by the much heavier sandy sediments of the substrate. Oyster biodeposits are composed of very fine particles (95% less than 3µ, Haven and Morales-Alamo, 1966), while the sandy sediments in the aquaria were 85-90% larger than 63µ. Therefore,

any small fraction of biodeposits (with its small weight of Kepone) would be reduced to insignificance by the much larger weight of the sandy substrate when mixed into it.

The sedimentation control (Aquarium no. 6, third series) provides evicence of this dilution effect. The pseudofeces added to that shallow aquarium weighed 18.1 g dry and had a Kepone concentration of 1.36 μ g/g (Table 1). At the end of the thirty-three days duration of the experiment, the material in the tray weighed 71.7 g and had a Kepone concentration of 0.37 μ g/g. There was a 75 percent increase in the weight of the material in the pan and a 73 percent reduction in the concentration of Kepone. Obviously, the accumulation of sediments settling out of suspension in the shallow aquarium added substantally to the weight of the material in the aquarium and consequently diluted the Kepone introduced with the biodeposits.

The mixing of Kepone into the 0-0.5 cm deep sediment layer cannot be ascribed to the action of the animals exclusively, because the control samples showed similar results. Most of the mixing at that depth can be attributed to mechanical mixing, possibly during the collection of the samples. The reduction in Kepone concentration from the level in the biodeposits and that in the core samples is probably due to the dilution effect mentioned in the preceding paragraph.

Despite our negative results, it is reasonable to assume that Kepone is mixed into subsurface layers by the benthic fauna that lives and moves within the subsurface layers of sediments in the contaminated bottom of the James River. Tracer labels of Kepone or much higher concentrations of the chemical will probably be required to establish the extent to which that may be occurring. In experiments in which higher concentrations of Kepone are used consideration should be given to possible lethal or sub-lethal effects of the Kepone on the infauna.

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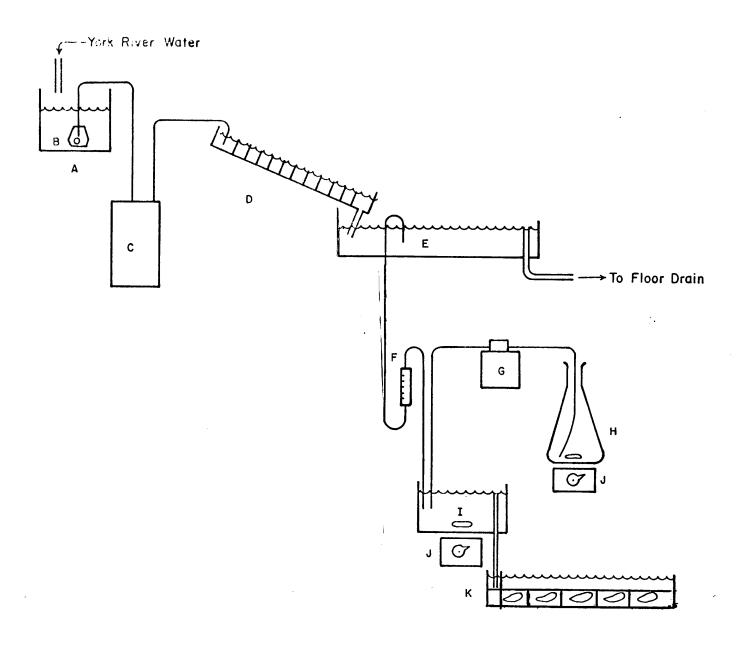


Figure 1. Diagram of the apparatus used to expose oysters to Kepone-contaminated sediments for the purpose of collection of their contaminated biodeposits. Key to identification of individual components appears on next page.

Table 1. Volume and weight of biodeposits added to laboratory aquaria in bioturbation studies and the amount of Kepone associated with the biodeposits.

Aquarium No.	Type of Biodeposit Added	Volume Added (ml)	Wet Weight (g)	Equivalent Dry Weight ¹ (g)	Equivalent Production Period ² (Weeks)	Kepone Conc. in Biodeposits (µg/g)	Weight of Kepone (µg)
First Series	<u>s</u>						
1 7 8	Pseudofeces Pseudofeces Feces	12 17 25	13.2 20.6 26.7	2.8 5.3 5.0	1.7 3.3 3.1	2.39 3.88 6.89	6.7 20.6 34.4
Second Serie	<u>es</u>						
2 3 4 5 6	Feces Feces Feces Feces	14 16 18 16 24	14.7 16.6 21.0 17.4 27.6	2.0 2.0 2.9 2.6 3.4	1.2 1.2 1.8 1.6 2.1	2.17 2.17 2.93 2.93 2.17	4.3 4.3 8.5 7.6 7.4
Third Serie	<u>s</u>						
1 2 3 4 5 Sedimentati	Feces Pseudofeces Pseudofeces Pseudofeces Feces on Pseudofeces	27 85 80 67 25	28.6 91.8 82.4 79.7 28.0	4.6 20.4 19.2 19.1 4.2	2.9 12.7 12.0 11.9 2.6	11.9 1.36 1.36 1.36 11.9	54.7 27.7 26.1 26.0 50.0

¹ Determined by drying and re-weighing previously weighed wet sub-samples.

 $^{^2}$ Number of weeks required for one oyster to produce a similar weight of biodeposits (feces and pseudofeces), based on average dry weight of 1.6 g/week/oyster as given by Haven and Morales-Alamo (1966b).

Table 2. Distribution of Kepone with depth in cores collected from sediment beds holding benthic organisms in laboratory aquaria. Kepone-contaminated biodeposits added to sediment surface on 13 December 1978. Cores collected on 18 January 1979.

			Type of biodeposit	Sampling depths (cm)						
Aquarium No.	Core No.	Features of core surface	added and Kepone conc. in μg/g	Surf.	0-0.5	0.5-1	4-5	9-10	<u>14-15</u>	
1	1	Hole surrounded by pellets Sea squirt buried one cm.	Pseudo., 2.39	0.036	0.044	ND	ND	ND	ND	
	2	Small hole, probably phoronid tube		0.02	0.036	ND	ND	ND	ND	
	3	Small hole, probably phoronid tube		0.069	0.017	ND	ND	ND	ND	
	4	Small hole, probably phoronid tube		0.049	ND	ND	ND	ND	ND	
	5	Phoronid		0.016	0.022	ND ¹	\mathtt{ND}^{1}	ND	ND	
	6 .	Small hole, probable phoronid tube		0.17	ND .	ND	ND	ND	ND	
7	1	Control (no animals)	Pseudof., 3.88	0.204	0.019	ND	ND	ND	ND	
	2	Control (no animals)		0.023	ND	ND	ND	ND	ND	
	3	Control (no animals)		0.052	ND	ND	ND	ND	ND	
8	1	Surface crater (0.5 cm depression)	Feces, 6.89	ND	ND	ND	ND	0.026	ND	
	2	<u>Loimia</u> tube		0.020	ND ²	ND ²	ND ²	ND ³	ND	
	3	Loimia tube		0.21	0.009 ²	0.007 ²	ND ²	ND ⁴	ND	

Table 2 (Contd.)

			Type of biodeposit		Sampling depths (cm)					
Aquarium No.	Core No.	a c	Surf.	0-0.5	0.5-1	4-5	9-10	<u>14-15</u>		
	4	Fine fecal strings around 1-mm opening	Feces, 6.89	0.071	ND	ND	ND	ND	ND	
	5	1-mm opening and mound		ND	0.005	ND	ND	ND	ND	
	6	1-mm opening	•	0.005	ND	ND	ND	ND	ND	
	7	Fine fecal strings around 1-mm opening		ND	0.005	0.003	ND	ND	ND	

 $^{^{1}\}mathrm{One}$ live phoronid found at these depths.

²Tube present at this depth.

³Animal tube bent away from core at 11-cm depth.

 $^{^4\}mathrm{Animal}$ tube no longer present at 10-cm depth.

Table 3. Distribution of Kepone with depth in cores collected from sediment beds holding benthic organisms in laboratory aquaria. Kepone-contaminated biodeposits added to sediment surface on 13 December 1979. Cores collected on 6 March 1979.

			Type of biodeposit added and Kepone conc. in µg/g			Sampling depths (cm)						
Aquarium No.	Core No.	Features of core surface			Surf.	0-0.5	0.5-1	4-5	9-10	<u>14-15</u>		
2	1	1-mm hole and 2-mm tube	Feces,	2.17	0.094	ND ¹	ND ¹	ND ¹	ND	ND		
	2	Two 2-mm tubes			0.046	ND^2	ND ·	ND	ND	ND		
	3	Two 2-mm tubes, black sand around them			0.031	0.002	ND^2	ND	ND	ND		
	4	One 2-mm tube			0.064	0.003 ²	ND	ND	ND	ND		
	5	One 2-mm tube			0.050	ND^3	ND ¹	ND ¹	ND	ND		
3	1	Phoronid tube	Féces,	2.17	0.034	0.020	ND :	ND	ND			
	2	Phoronid tube			0.094	0.021	ND	ND	ND			
	3	Two 3-4 cm deep craters next to 6-8 cm mound			0.015	0.039	0.013	ND	ND			
	4	4-5 cm high mound next to 3-5 cm depression			ND	0.006	ND	ND^2	ND			
	5	Three phoronid tubes			0.042	0.031	ND	ND	ND			
4	1	<u>Loimia</u> tube	Fecės,	2.93	· ND	0.043	NDl	ND^{1}	ND1	ND ¹		
	2	Depression near core #1			0.28	ND	ND	\mathtt{ND}^{1}	ND	ND		
	3	3-cm diam. mound, 3-4 cm high			ND	0.033	ND	ND	ND	ND		
	4	Similar to #3			0.032 ¹	0.019	0.0131	nd ⁴	ND^2	ND		

Table 3 (Contd.)

	_		Type of biodeposit added and Kepone			Sampling depths (cm)						
Aquarium <u>No.</u>	Core No.	Features of core surface		kepone n μg/g	Surf.	0-0.5	<u>0.5-1</u>	4-5	9-10	14-15		
	5	2.5-3.0 cm depression			0.052	0.017	ND	ND	ND	ND		
5	1	Control (no animals)	Feces,	2.93	0.22	0.019	ND	ND	ND	ND		
	2	Control (no animals)			0.062	ND	ND	ND	ND	ND .		
6	1	Two small mounds (1-cm diam, 2-3 mm high) of black sediments	Feces,	2.17 %	0.013	0.019	ND	ND	ND	ND		
	2	Five pinholes in slightly raised area			0.175	0.309	0.011	ND	ND	ND		
	3	3-mm hole			0.011	0.017	0.009	ND	ND ⁵	ND		
	4	l-mm hole surrounded by fine fecal strings			0.063	0.014	ND	ND	ND	ND		
	5	1.5-mm hole, hard clam			0.012	0.018	0.0156	ND^2	ND	ND		
	6	1 mm hole surrounded by 6 mm-high mound			0.005	0.108	0.146	ND	ND	ND		

 $^{^{1}}$ Tube present at this depth.

 $^{^2\}mathrm{No}$ tube hole evident at this depth or below.

³Part of worm found at this depth.

⁴Two worm tubes found at this depth.

⁵Nereis worm between 10-11 cm depth.

 $^{^{6}}$ 1.6-cm Mercenaria between 0.5-1.0 cm depth.

Table 4. Distribution of Kepone with depth in cores collected from sediment beds holding benthic organisms in laboratory aquaria. Kepone-contaminated biodeposits added to sediment surface on 31 May 1979. Cores collected on July 3, 1979.

			Type of biodeposit		Sampling depths (cm)					
Aquarium No.	Core <u>No.</u>	Features of core surface	added and Kepone conc. in µg/g	Surf.	0-0.5	0.5-1	4-5	9-10	14-15	
1	1	Mya clam at 10 cm depth	Feces, 11.9	0.04	0.04	ND	ND	ND		
	2	Two 1.5 mm holes		0.03	0.09	ND	ND	ND		
	3	Mya clam at 8 cm depth		0.01	0.09	ND	ND	ND		
	4	Mya clam at 16 cm depth, mound 2 cm diam., 3-4 mm high		ND	ND	ND	Lost	ND		
	5	Part of clam siphon at 5 cm depth, mound 2 cm-diam., 3-4 mm high		0.05	0.17	ND	ND	ND		
	6	Black mound, 1.5 cm diam., 2 mm high		0.03	0.03	ND	ND	ND		
	7	Mya clam at 5-7 cm; mound 2 cm diam., 3-4 mm high		ND	0.10	ND	ND	ND		
	8	Black mound 1.2 cm diam., 2 mm high		0.14	0.29	ND	ND	ND		
2	1	Two 2-mm holes	Pseudof., 1.36	0.17	0.04	ND	ND	ND		
	2	Several holes on surface (1-2 mm)		0.20	0.01	ND	ND	ND		
	3	2 mm hole and 6 x 12 mm elliptical depression about 3-4 mm deep		0.04	0.05	ND	ND	ND		

Table 4 (Contd.)

			Type of biodeposit		Sampling depths (cm)						
Aquarium No.			added and Kepone conc. in µg/g	Surf.	0-0.5	0.5-1	4-5	9-10	<u>14~15</u>		
	4	2 mm hole in depression about 14 mm diam., 4 mm deep; Mya siphon in hole		0.07	0.03	0.014	ND	ND	·		
•	5	3 mm hole near 15-16 mm depression, 4 mm deep Leptosynapta at 10-13 cm depth		0.04	0.11	ND	ND	ND	 		
3	1	Loimia tube down to 11 cm deep	Pseudof., 1.36	0.05	0.03	ND	ND	ND	 ·		
	2	<u>Loimia</u> tube down to 10 cm deep		0.04	0.14	0.009	ND	ND	—		
	3	<u>Loimia</u> tube down to 11 cm deep		0.045	0.02	ND	ND	ND			
	4	<u>Loimia</u> tube down to 9 cm deep		0.03	0.018	ND	ND	ND			
4	1	Control (no animals) Core in place at start of expt.	Pseudof., 1.36	0.06	ND	ND	ND	ND	·. 		
	2	Control (no animals) Core in place at start of expt.		0.11	0.01	ND	ND	ND			
	3	Control (no animals)	•	0.19	0.07	ND	ND	ND			
	. 4	Control (no animals)		0.22	0.11	ND	ND	ND	<u></u>		

Table 4 (Contd.)

			Type of biodeposit			Sampling d	epths (cm)		
Aquarium No.	Core No.	Features of core surface	added and Kepone conc. in µg/g	Surf.	0-0.5	0.5-1	4-5	9-10	14-15
5	1	Control (no animals)	Feces, 11.9	0.08	0.10	ND	ND	NĎ	
	2	Control (no animals)		0.06	0.36	ND	ND	ND	
6		Sedimentation Control	Pseudof., 1.36	0.37*					

^{*}Kepone concentration in mixture of pseudofeces and sediments settling out of suspension.

Table 5. Compositon of the animal populations in individual aquaria used in studies of mixing of Kepone-contaminated biodeposits into subsurface layers by benthic organisms. January-July 1979.

Date Aquaria Sampled	Aquarium No.	Faunal Composition Species	No. in Aquarium
18 Jan	1	Natural Fauna	
	7	Control (No Animals)	
	8	Phoronis architecta Loimia medusa Glycera americana Leptosynapta tenuis	5 3 2 2
6 Mar	2	Phoronis architecta Loimia medusa Clymenella torquata	6 5 4
	3	Phoronis architecta Saccoglossus kowalewskii Glycera americana Leptosynapta tenuis	10 6 3 3
	4	Nemerteans Leptosynapta tenuis Saccoglossus kowalewskii Loimia medusa	4 4 3 2
	5	Control (No Animals)	
	6	Phoronis architecta Glycera americana Nereis succinea Arabellids Mercenaria mercenaria	6 2 2 2 2
3 Jul	1	Macoma balthica Tagelus plebeius Mya arenaria (small)	7 3 3
	2	Phoronis architecta Leptosynapta tenuis Clymenella torquata Arabellid	6 2 2 1

Table 5 (Contd.)

Date		Faunal Composition	
Aquaria Sampled	Aquarium <u>No.</u>	Species	No. in <u>Aquarium</u>
3 Jul	3	Loimia medusa	7
	4	Control (No Animals)	
	5	Control (No Animals)	

Table 6. Temperature and salinity of York River water flowing into experimental laboratory aquaria holding animals in sediment beds. December 1978-July 1979.

Dates Included		ature (C)	Salinity (^O /oo) Mean Range			
Included	<u>Mean</u>	Range	Mean			
First Series						
13 Dec 1978- 18 Jan 1979	15.7*	12.0-15.9	20.3	19.1-21.5		
Second Series						
18 Jan- 6 Mar 1979	16.0*	9.6-20.7	18.1	12.6-20.8		
Third Series		÷				
31 May- 3 Jul 1979	23.4	20.9-26.0	15.7	14.0-19.8		

^{*}Temperature raised with heat exchangers.

Table 7. Percent size distribution of sediments collected from sediment beds in cylindrical aquaria used to study relationship between bioturbation and mixing of Kepone-contaminated biodeposits into subsurface layers. 8 March 1979.

			cent comp e fractio		f differer in micror	
Aquarium No.	Date Collected	>500	500-250	250-125	125-63	<63
1	18 Jan	2.57	25.25	56.88	8.54	6.75
1	18 Jan	3.58	32.22	51.68	6.66	5.85
7	18 Jan	4.50	46.96	38.91	5.46	4.16
8	18 Jan	10.29	36.93	42.41	5.59	4.77
3	8 Mar	1.31	28.53	58.95	6.55	4.66
5	8 Mar	16.54	41.22	27.42	5.09	9.73
6	8 Mar	1.16	28.18	57.19	5.87	7.60