FINAL REPORT

BIOACCUMULATION AND TOXIC EFFECTS OF LEAD AND SILVER IN HUDSON RIVER MICROZOOPLANKTON

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INTRODUCTION

We describe here the results of a study of the effects of two heavy metals, lead and silver, on planktonic ciliates. The study is motivated by the probable importance of this understudied component of the food web, and the presence of appreciable amounts of these two metals in the water column of the lower Hudson River.

Studies in a number of freshwater, estuarine and marine habitats indicate that the microzooplankton can be significant in the flow of matter and energy between planktonic algal and bacterial populations and the higher trophic levels (e.g., Capriulo et al 1991; Capriulo 1990, and references therein). Relatively little has been done so far, however, on the microzooplankton of the Hudson River estuary.

Heavy metals, including Pb and Ag, have been reported to be present at relatively high levels in the lower Hudson, and are therefore of potential importance as toxicants. It should be noted, however, that recent studies have called into question some of the published data on heavy metals in rivers, particularly in the dissolved state (Windom et al, 1991). It appears that the levels of dissolved metals are in general rather variable, and also much smaller than levels associated with suspended particulates in the water column. It has been suggested that the latter can provide a better indication of anthropogenic input (Windom et al 1991).

As part of the study, therefore, we have measured background levels of a suite of metals in various size-fractions of the particulate phase of Hudson River water samples. We also used added radionuclides (^{210}pb and ^{110m}Ag) to study the kinetics of accumulation from the dissolved to various size fractions of the particulate phase. Since both Ag and Pb are potentially toxic, and have been reported present at relatively high levels in the lower Hudson river, we have focussed on them. Ag, in particular, is relatively little studied, and yet is known to be a pollutant of major concern in some other estuarine systems, such as San Francisco Bay.

We also obtained basic hydrographic data on the water column during the period of the study, to provide an appropriate environmental background for the metals studies.

We looked at the effects of added Ag and Pb on ciliates in the laboratory, as seen in several parameters: survival, feeding rate, swimming rate and chemosensory behavior. It has frequently been suggested that behavior may be a more sensitive index of toxicity than growth or survival (e.g., Levandowsky and Hauser 1978), and we tested this possibility in the case of heavy metal stress in ciliates.

Since, in these assays, the metals were added in the dissolved (ionic) state, we studied the uptake by ciliates of added radionuclides, both from the dissolved state in the medium, and from labelled food algal cells. This allows us to relate the observed effects to the body burden of the metal.

Initially we used natural ciliate plankton populations, tinntinids and oligotrichs from the lower Hudson, as we had proposed. However, these proved difficult to culture, and also presented technical difficulties in measuring radionuclide uptake. Their delicacy and small size made it difficult to use techniques developed with invertebrates, and mechanically stronger algal cells (e.g. Fisher 1984, 1985, Fisher et al 1983, 1984). For these reasons much of our work, and most of our useful data involved the model estuarine ciliate Fabrea salina, a somwhat larger and more robust species. This has the added advantage of having been the object of several studies, so that a certain amount of information is available on its nutrition, behavior and life-cycle (e.g., Desimone and Repak 1991; Repak 1986, 1983; Demar-Gervais and Germont 1971; Ellis 1937; Kirby, Jr. 1934; Henneguy 1890).

There may be another advantage in using Fabrea in that, unlike the tinntinids, it lacks an inorganic shell, or lorica. Since we are looking at biological effects in the context of body burden of adsorbed metal, ambiguities could arise if metal is adsorbed onto the lorica's exterior, where it might not be in contact with the cell. Some metal might even be adsorbed onto empty loricas. Thus, it may be somewhat easier to interpret data from work with a naked cell, such as Fabrea.

Another difficulty that arose, which perhaps should have been anticipated, relates to the survival and growth of ciliates in appropriately diluted seawater during metals experiments. From the outset of work with marine cilioplankters, investigators have found that a small amount of metal complexing agent or chelator must be added to the medium to obtain growth. However, added complexing agent in experiments with heavy metals leads to ambiguity in interpretation of metal availability, since the uptake studies are done in the absence of such compounds. We found, as have others (e.g. Gold 1970), that we could not obtain growth without such complexing agents, and so we have concentrated on the effects of Ag and Pb on survival, rather than growth. Furthermore, even in the survival studies one should assume that the cells are probably under stress in the controls, and thus it is probably appropriate to view the addition of heavy metals as simply an added stress.

Thus, we have deviated somewhat in the details of the original protocol where this became necessary. Nevertheless we have been able to accomplish these essential goals:

1. We determined the effects of Pb and Ag on survival, feeding and behavior in a model estuarine ciliate.

2. This in turn can be related to our measurements of uptake and accumulation of these metals by the ciliate, from the medium and from food-organisms.

3. In order to relate this information to the environment of particular concern, we measured levels of Pb and Ag, as well as other metals, in particulate fractions of Hudson River water samples.

BEHAVIOR OF METALS IN THE ENVIRONMENT

I. BACKGROUND MEASUREMENTS OF ENVIRONMENTAL VARIABLES

As part of our effort to understand the effects of two heavy metals in the Hudson River, we measured several basic parameters in the water column at Pier 26 (the River Project), in lower Manhattan. This was done to obtain an understanding of the physicochemical matrix in which the processes of particular interest were occurring.

METHODS

On several occasions we used a Martek CTD sensor, belonging to the Marine Sciences Research Center, SUNY Stony Brook, NY, to measure the profile of temperature and salinity at the end of Pier 26 (The River Project), lower Manhattan. We also collected weekly samples by a messenger-operated PVC sampler, measuring temperature and salinity of surface and bottom water. The water at the end of the pier has a mean depth of 7 meters.

With the CTD, temperature was measured with a thermocouple and salinity with a magnetic coil conductivity meter. In the manually obtained samples, a mercury thermometer and a hydrometer set were used, respectively. Secchi disk depth was used as a crude measure of turbidity.

RESULTS

The water column at this point was usually stratified, with a fresher layer overlying a more saline wedge. Figure 1 gives the top and bottom (7m) salinities at weekly intervals during the period of the grant. Since these are usually rather different, it follows that the water column is usually strongly stratified at this site, though this tendency is somewhat reduced in the winter.

The Secchi disk values during the period of the grant were always

less than 2m, and usually less than 1m, indicating that the river is usually rather turbid (Fig. 2). Microscopic examinations indicate that the turbidity is due primarily to detritus rather than to phytoplankton.

Figure 3 shows top and bottom temperatures. Despite the stratification apparent in the water column, apparent from the salinity data (see above), the surface and bottom temperatures were usually within a degree or two of each other.

DISCUSSION

These observations emphasize the complexity of this habitat. Frequent stratification, and the prominence of the particulate phase suggest substantial diversity of microbial niches in this water column. They also reflect the variety of anthropogenic and natural conditions that may influence the microzooplankton here.

II. METALS CONCENTRATIONS IN THE PARTICULATE PHASE

Knowledge of environmental levels of heavy metals is an important component in assessing their significance in the ecology of the microzooplankton. As noted above, we focused on the particulate fractions as being more stable, and as considered to be more representative of anthropogenic contributions than the dissolved fraction. In addition to Pb and Ag, our main concern here, we also looked at Cu, Fe, Zn, Mn, Ni, Cd and Al. Of these, the first 5 are required nutritionally in in trace amounts, though Cu, Zn and Ni can be toxic at high levels. Cd is not a micronutrient but can be toxic. Cu and Zn occur naturally at potentially toxic levels in seawater, while high levels of Cd and Ni would generally be considered to arise anthropogenically. Al would come mainly from aluminosilicates (clay) in the suspended solids, and serves as an indicator of the relative importance of the inorganic (mineral) edaphic component of the detritus.

METHODS

Sample collection and filtration: Samples of surface water were collected from the end of Pier 26 in lower Manhattan (The River Project), in 2.5 liter polycarbonate containers that had been rinsed with 10% HCl, followed by deionized distilled water. The container opening was kept facing into the current during filling, to avoid the possibility of contamination.

Metals analyses: Particulates were digested using a HNO_3-$HClO_4$-HF acid digestion technique according to Trefry and Metz (1984). Metal analyses were performed using flame and flameless atomic absorption spectrometry (AAS) on a Perkin-Elmer Zeeman model 5000 equipped with an HGA 500 and an AS 40 autosampler. Lead, silver and copper analyses were performed using flameless AAS and, in the case of lead and silver, using ammonium phosphate and magnesium nitrate matrix modifiers, respectively. Zinc, iron, manganese and aluminum analyses were performed by flame

AAS. National Bureau of Standards 1643b Trace Metals in River Water was analyzed by furnace AAS along with the digest samples for lead, copper and silver to determine the accuracy of the AAS method. In addition, portions of the digest solutions were spiked with a known quantity of metal to measure the percent recovery of the metal from the digest solution.

RESULTS

Table 1 shows the concentrations of Pb, Ag and several other metals in particulate fractions of surface water collected on different dates. For comparison, average data from other rivers, the continental crust, and the Mississippi River are given.

DISCUSSION

From these data it is clear that in the winter samples Pb was present in Hudson River particulates at substantially higher concentrations than in the Mississippi and other rivers. Pb has many industrial uses, being a component of paints, printer's ink, some gasoline fuel, and many other common products that may enter the river in various ways. The great difference in Ag in the summer and winter samples is intriguing, and not understand.

Ag has been studied much less, and the few data cited by Martin and Maybeck (1979) have a wide spread, but within this poorly defined context, the levels in the Hudson were on the high side. Ag is less widely used than Pb. It is a component in some electronic systems, and in jewelry, but the major source would probably be from photography and the photographic publishing industry.

The analytic method used, atomic absorption, gives only the elemental composition, and tells nothing about the chemical state of the metals, or their biological availability or toxicity. Thus, while ionic Ag can be quite toxic, We do not know if the Ag in the Hudson is sufficiently available biologically to be toxic. Studies in the (freshwater) drainage downstream of the Eastman Kodak photographic industrial complex indicated that, while there was appreciable Ag in the water column, it was bound tightly to sulfide groups during the sewage treatment process, and was not toxic to fish in bioassay experiments. Similarly, the toxicity of Pb would depend very much on its chemical state in the particles, which we did not investigate.

Of the other metals, Cu is also significantly higher than the Mississippi and other rivers. Cd also appears to be high. These may represent the effects of anthropogenic sources. Otherwise, metals concentrations appear to be essentially comparable to those found elsewhere. The levels of Al, Mn, Ni and Fe appear to be comparable to crustal levels, and to those in other rivers.

The general decrease in levels of metals with increasing pore size no doubt reflects the decrease in surface area in the larger particulates.

From the Pb and Zn data, it is clear that the suspended particulates are both quantitatively and qualitatively different in the winter and the summer. This merits further study.

These data suggest that Pb and Ag were good choices of metals to investigate. Pb is clearly high in winter samples, and Ag is understudied. Both are potentially toxic, and therefore of environmental concern.

III. UPTAKE KINETICS OF DISSOLVED (IONIC) METALS BY PARTICULATES

To obtain an estimate of the quantitative significance of Pb and Ag uptake from the ionic state to particulates, including a colloidal fraction, an experiment was conducted using radiotracer additions, followed by ultrafiltration.

METHODS

Replicate aliquots from the same sample were labelled by incubation with gamma-emitting radionuclides, ^{110m}Ag and ^{210}Pb (both added in HCl solution), then filtered with acid-cleaned Nuclepore filters of several pore sizes: 10 um, 3 um, 1 um and 0.2 um. Filtrate from the 0.2 um filtrate was then subjected to ultrafiltration.

Ultrafiltration. The filtrate from 0.2 um Nuclepore filters was placed into an Amicon Model 8400 stirred ultrafiltration cell, under positive pressure of purified nitrogen, and passed through filters with cut-offs of 300 kDA (Amicon XM 300, acrylic polymer) and 50 kDA (XM 50, acrylic polymer). Experiments with these filters have shown that sorption to filter blanks is less than 2% for Ag.

Accumulations of radiolabel in the particulate fractions were measured with an automated gamma counter with a NaI(Tl) crystal (^{110m}Ag at 658 Kev and ^{210}Pb at 46 Kev).

RESULTS

Figures 4 - 6 show the partitioning of ^{110}Ag and ^{210}Pb among the various size fractions after 1 hr, 4hr and 24 hr. Since there is little change between 4 hr and 24 hr for most fractions, it appears likely that an equilibrium partitioning was achieved for most fractions within the the first 4 hours.

DISCUSSION

It is of interest that, after 24 hours, when the percentages in the various size fractions appear to have settled down, substantial proportions of the added metals remain in the < 50 kDa fraction, which we take operationally to represent a dissolved fraction (it may actually include small colloids however). This result might have changed over a longer time

period than 24 hours. However, since the conditions in the laboratory are different from those in the natural setting, it would be difficult to interpret such data. What we can say from this observation is that, in experiments such as those described below, in which ionic metals were added to cell suspensions, there was probably significant exposure to dissolved metal over the experimental period.

EFFECTS OF Pb AND Ag ON MICROZOOPLANKTERS

I. EXPERIMENTAL ORGANISMS AND MEDIA

As noted in the Introduction, we encountered several difficulties in working with wild population of ciliates, as follows:

First, methods developed for handling phytoplankton and invertebrate zoopolankton (e.g., Fisher 1985; Fisher et al 1984) in metal accumulation studies did not work well with typical wild ciliate plankters, such as tinntinnids and oligotrichs. These cells proved to be mechanically delicate, and difficult to concentrate by straining through fine nitex screens or filters.

In addition, it was difficult to culture these organisms in the laboratory. After many attempts we did obtain several tinntinnid and oligotrich cultures, but each of these only lasted a few months. This appears to be a typical experience in working with these organisms.

There is also an ambiguity associated with metal uptake measurements in the loricate forms (the tinntinnids), in that these could in some cases simply reflect adsorption to the inanimate lorica, rather than cellular accumulation.

For these reasons, after initial trials with wild populations we decided to employ a model estuarine ciliate, Fabrea salina in this study. This organism has several advantages: it is readily cultured in the lab for indefinite periods, it can be easily harvested by gently straining a cell suspension through a Nitex screen of appropriate mesh size, and it has no lorica. It is cosmopolitan, having been isolated from estuaries from California to Israel. We have used a strain originally isolated by Dr. D. Kahan of the Hebrew University.

For culture medium, we used diluted "clean" seawater collected from the Atlantic Ocean offshore from Shinnecock Bay, Long Island, with added EDTA and trace metals mix from Guillard's f_n series, added at the level of f_{20} (Guillard and Ryther 1962). For experimental medium, we used the same diluted seawater, without the trace metals-EDTA addition.

Both culture and experimental media were diluted with distilled/deionized water to 24 ppt, the salinity of the Hudson

River water used to study metals content and uptake by particulates.

II. UPTAKE AND BIOACCUMULATION OF RADIOISOTOPES

A. UPTAKE AND BIOACCUMULATION FROM THE MEDIUM

In order to properly evaluate the sensitivity of ciliates to added lead and silver, we needed to estimate the rate of uptake by the cell, and the resulting body burden.

METHODS

Ciliates were harvested by passing a healthy Fabrea culture through a nitex screen (35 um pore size) and placed in an experimental medium consisting of unenriched, filter-sterilized seawater. After allowing 3 hr for clearance of ingested food, cells were harvested again and put in fresh experimental medium with added radionuclide.

Acid-washed borosilicate glass vials were used, and controls without organisms were run to check for accumulation of adsorbed radiolabel by the container.

Bioaccumulation was measured as described in the previous section, using an automated gamma counter with a NaI(Tl) crystal.

RESULTS

Figure 7 shows adsorption of adsorption of Ag by the container. The high adsorption from a 20 uM solution probably reflects precipitation, which was observed microscopically in experiments containing levels greater than or equal to 10 uM. Pb adsorption to the container (not shown) was below 1% in the physiological range (see below).

Concentration of dissolved metals by cells is shown in Figs. 8 and 9 (for convenience,the same data have been plotted in arithmetic and logarithmic scales).

DISCUSSION

The diffference in concentration by cells of Ag and Pb from the dissolved state appears to be almost 2 orders of magnitude. This is of interest, in that it appears to parallel the difference in sensitivity of Fabrea to these two metals.

B. UPTAKE AND ACCUMULATION FROM FOOD ALGAE

While dissolved metals could have effects on cells, one might argue that another, at least equally likely route could be through the food chain, by ingestion of contaminated food organisms. To test this possibility we measured the uptake of ^{110m}Ag from radiolabeled food algal cells. The corresponding

experiment with ^{210}Pb was not done because of technical problems in labelling the food alga adequately for the experiment with the small amount of ^{210}Pb available to us.

METHODS

The food alga, Isochrysis galbana was grown in Guillard's F_2 medium, and exposed to ^{110m}Ag (added, as before, dissolved in HCl). The cells were washed by filtration and placed in unlabelled experimental medium. Aliquots of these suspensions were filtered over a period of 3 days to determine the rate at which label is lost from the cells to the medium (rate of depuration). Washed ciliates were placed in this suspension, harvested after feeding for various time intervals, and their accumulated radiolabel determined.

RESULTS

Depuration of the food alga, Isochrysis, is shown in Fig. 10. It appeared to be concentration dependent, and to involve two processes, one long-term and one short-term. From these data and those on uptake from the medium (see above), we calculate that uptake of Ag by ciliates from the dissolved state due to depuration of labeled food algae in unlabeled medium would represent less than 5% of total uptake from the labelled food cells.

Uptake of ^{110m}Ag from food cells is shown in Figures 8 and 9.

DISCUSSION

It is clear from these results that Ag can be accumulated by the ciliate from labelled food organisms. After 24 hours the concentration factor is comparable to that associated with uptake from the dissolved state.

III. EFFECTS ON SURVIVAL

As noted in the introduction, ciliates did not multiply in seawater without added complexing agent, presumably because of autochtonous levels of heavy metals, such as Cu and Zn. Thus, we could not examine the effects of added metals on growth rate without added complexing agent, and such added complexing would have affected the bioavailability of the added metals also, rendering the data ambiguous. For this reason, we were constrained to look simply at the effects of added metals on survival, rather than on growth rate.

METHODS

Experiments on the survival of Fabrea were conducted both at SUNY Purchase and at Haskins Labs, with slight differences in protocols. Fabrea were harvested by: (Haskins) filtration with a 35 um pore size Nitex screen) or (Purchase) picking by hand with

microcapillaries. They were then placed in filtered seawater, allowed to purge themselves for about 2 hours, then harvested again and put into clean filtered seawater, diluted to 24 ppt, to which an appropriate amount of metal had been added. These suspensions were incubated in acid-washed borosilicate vials for two days. These experiments were done with and without added food organisms. In one protocol, the added food algae (I. galbana or D. tertiolecta) were washed twice by centrifugation, followed by aspiration of supernatant and resuspension in fresh seawater (Purchase). In another protocol (Haskins) they were washed by gentle gravity filtration, using Nuclepore filters (1um pore size), to remove old medium before resuspension in fresh medium.

RESULTS

Some results of these experiments are shown in Fig. 11. In the absence of food organisms, Ag was lethal at levels ranging from 1uM - 0.1 uM in various experiments. In this range there was great variability in percent survival from one experiment to another.

Pb, under these conditions, had no clear effect on survival at ecologically meaningful concentrations. (At very high concentrations, $\geq$ 10 uM, where precipitation was often seen, there appeared to be some attrition, but at these concentrations conditions appeared to be unstable with regard to stability of the dissolved metal, and precipitates were often observed microscopically).

In the presence of washed food organisms, the results with Ag varied somewhat, depending on how the food organisms were treated. If these were harvested by successive centrifugation (SUNY Purchase), the ciliates tended to survive for 24 hours at 1uM, while if the food organisms were harvested by screening (Haskins Labs), the results were approximately the same as without food organisms.

In control suspensions, without added Ag or Pb, ciliates survived for two days, and sometimes longer, but did not multiply. If relatively small amounts of Na_2EDTA were added, at the level of Guillard's F_{20} medium, growth occurred in the presence of food organisms.

DISCUSSION

It's clear from these data that Ag is lethal to these ciliates at levels at least two orders of magnitude below Pb. Pb was, in fact, not lethal at any ecologically reasonable concentration. While this might be construed as a difference in sensitivity of the cell, the data on adsorption of the two metals suggest that differences in body burden, and thus availability is probably the principal factor here.

The manifest variability in experiments, particularly those where

food algae were introduced, may be due to variable amounts of secretion of organic complexing agents by either ciliates or their algal food. In particular, the difference between results at Purchase and Haskins may be due to this, and/or to differences in amount of complexing substances carried over in the different methods of harvesting.

Yet a third possibility is that food cells harvested by centrifuging may be healthier than those harvested by filtration, and may have different nutritional properties, leading to greater metal tolerance in the ciliates. These various possibilities merit further study.

In any case, we can make a rough calculation of what might be the lethal body burden. In the case of the lower threshold (10^{-6} M), assuming a concentration factor of 7×10^{3} (figure 8), we obtain an approximate intracellular concentration of 7×10^{-18} moles/um^3.

It's worth noting that the logarithm of this number is -17.15, and that comparable numbers found for Ag toxicity in 4 marine phytoplankter species were -16.3 (Thallassiosira pseudonana), -16.0 (Dunaliella tertiolecta) and -17.6 (Oscillatoria sp. and Emiliana huxleyi) (Fisher et al 1984).

IV. EFFECTS ON BEHAVIOR

In the preceding section we presented data on the severest possible response to toxic metals: death. It is however of interest to investigate the levels at which less extreme effects may occur. In particular, modifications in normal feeding behavior, motility and sensory responses, while less obvious in the laboratory, may have profound ecological effects. If the organism's ability to find and obtain food in competition with other species is impaired, it may not be able to survive in the natural setting.

A. FEEDING

We begin with the basic process of feeding. The approach is to quantify the end result (amount of particles ingested), without attempting to analyze the details of feeding behavior.

METHODS

Cultures of Fabrea were fed with suspensions of food algae (I. galbana or D. tertiolecta) and cornstarch granules in a ratio of 3:1, in a medium consisting of filtered seawater diluted to 24 ppt, to which appropriate amounts of ionic Pb or Ag were added.

Algal preparation: Young cultures grown in F2 medium were harvested by centrifuging for 20 minutes on a table-top centrifuge at low speed to achieve a cohesive pellet and a relatively clear supernatant. Supernatant was aspirated and cells

Cornstarch preparation: A small pinch of cornstarch was mixed in 24 ppt filtered seawater, then passed through a 20 um filter. The filtrate was passed through an 8 um filter, and the retained solids were resuspended in approximately 20 ml fresh seawater. An aliquot from this was stained with Lugol's iodine and counted, and an appropriate dilution was prepared.

Ciliate preparation: Cultures of Fabrea were gently washed through a 20 um cylindrical screen filter 3 times to remove food algae, then placed in a petri dish and picked by hand, using a hand-drawn microcapillary and a dissecting microscope. Twenty cells each were placed into 5 ml of feeding suspension in glass scintillation vials.

Two experiments were also done with wild ciliates (Tintinnopsis kofoidi and Strombidium sp.), picked by hand from a plankton tow of the Hudson River at the River Project. Procedures used were the same as with Fabrea.

Feeding: Ciliates were allowed to feed for 30-48 min (depending on the experiment). During this time vials were placed horizontally in a shaker at 20 C and agitated at a speed of 130 rpm, to insure that starch grains and algal cells would remain suspended and mixed.

Quantitation of feeding. Vials were removed at the designated time and preserved with a few drops of Lugol's iodine stain, then kept in a dark refrigerator until they could be counted. The contents of each vial were gently pipetted into a petri dish and examined with a dissecting microscope. Individual ciliates were picked out and examined at 40x under a compound microscope. The number of cornstarch grains in each cell was determined. Using the proportionality of starch grains to algal cells, and assuming that the latter were consumed in that proportion, the data were converted to number of algal cells ingested per minute.

RESULTS

Results were quite variable from one experiment to another (table 4). Feeding rates for Fabrea ranged over more than an order of magnitudein various experiments: from 5.4 to greater than 184 cells/hour/ciliate. The wild ciliates fell in the same range. Nevertheless, several qualitative conclusions are possible:

1. Data from such experiments have a high variance.

2. Even at abnormally high concentratons, Pb had no consistent effect on feeding.

3. Ag inhibited feeding above 1 uM.

DISCUSSION

Because of the high noise level in the data from feeding experiments, and the labor-intensive nature of the method that precluded doing large numbers of experiments, we cannot extract any firm quantitative conclusions on the effects of heavy metals on feeding rates here. Nevertheless, differences in the effects of Pb and Ag are quite clear, and follow the pattern seen in other indices: Pb had little discernible effect, even at environmentally absurd high levels. Ag had an inhibiting effect, but this is seen in these data only at levels much higher than would normally be encountered in the environment, and at levels that were lethal in the 24 hour experiments described earlier.

B. EFFECTS ON SWIMMING BEHAVIOR

In this section we present information on types of swimming behavior in Fabrea, and the effects of added Ag and Pb on these.

METHODS

Both ciliates from field samples (Tinntinnids and Oligotrichs - see above) and cultured Fabrea salina were studied. Cells were placed in filtered seawater (diluted to 24 ppt, with appropriate additions of ionic Ag and Pb) in Petri dishes, observed and recorded by videomicroscopy. Recordings were made at magnifications of 35x and 100x, and with a time resolution of approximately 30 frames/sec. Instantaneous swimming speeds were then obtained by playing back the videotape frame by frame and observing the change of position.

RESULTS

Normal Swimming Behavior

We observed several swimming modes with Fabrea. It rarely if ever approximated a random walk model, and software packages based on this model may therefore not be very useful for this species. Instantaneous swimming speeds could be obtained however, by the methods described above. The following 3 kinds of swimming behavior were commonly seen in healthy (control) suspensions of starved cells:

1. Steady spiralling along a straight axis for at least 10 body lengths. Swimming speeds (i.e., rate of progression along the axis) were usually in the range 0.1-0.8 mm/sec, as shown in Table 2.

2. Short excursions, usually less than 5 body lengths, punctuated by classic avoiding reactions: a sudden stop followed by a brief period of backward swimming, then forward swimming in a new direction.

3. Circular swimming in one place.

Although other kinds of swimming behavior were also observed, these three types were the most frequent, were relatively easily defined and distinguished, and are the focus of this study.

Effects of Metals on Swimming Behavior

1. Ag. At concentrations of 10^{-2} uM or higher, type 1 swimming behavior essentially disappeared. At levels near the lethal point (1 uM) virtually all movement consisted of type 3 behavior.

2. Pb. Effects were detected at 10 uM. Type 1 swimming was reduced in proportion to the other types, but not eliminated.

These effects are shown in table 2.

DISCUSSION

We did find effects on motility at levels below those at which the metals were lethal. To the extent that this reflects what occurs in the natural setting, we would expect that ciliates would not survive, or at least would not be able to remain suspended in the water column.

C. CHEMOSENSORY RESPONSES

In addition to simple survival, and general motility, we have focussed on sensory responses to chemical signals. We sought, and found responses to filtrates from suspensions of microalgae, including some food species. We reasoned that heavy metal exposure might affect such responses at concentrations below those that are lethal. If so, then, if such responses are a necessary component of finding food organisms, then metals could have significant impacts on ciliate populations at levels below the lethal concentrations determined above.

METHODS

Chemosensory responses were studied by observing ciliates swimming near cubes of 2.0% agar gel, made up with seawater to which putative chemical signals are added. Using videorecording, the number of cells within a given distance of a cube during a given time period was counted several times and comparisons between controls (cubes made up with seawater) and experimental cubes could then be made.

After initial trials, most experiments involved filtrate from suspensions of microalgal species in filtered seawater. Algal species used were: Dunaliella tertiolecta, Isochrysis galbana and Pavlova gyrans. Ciliates were starved overnight in f_{20} medium without food algae, then washed by micropipetting into filtered seawater, then into another aliquot of filtered seawater in the experimental dish. Subsequent behavior near agar cubes

was recorded by videomicroscopy.

RESULTS

Control populations: It was found that cells tended to remain in the vicinity of agar cubes made up with filtrate from Dunaliella and Isochrysis suspensions, and avoided those made up with Pavlova filtrate, in comparison to control gels made with filtered seawater. This is shown in Table 3. The mechanism for the positive response of to Dunaliella and Isochrysis filtrates seemed to be an increase in the frequency of type 2 and type 3 swimming in the vicinity of the cube, while type 1 swimming was more common in the vicinity of control cubes.

Effects of added metals: In cultures to which $AgNO_3$ was added, inhibition of the positive response to Dunaliella and Isochrysis was seen at concentrations above 10^{-2} uM. Mechanistically, this may be simply the result of an increase in type 2 and type 3 swimming in the entire culture, including in the vicinity of the control (filtered seawater) cubes. At 10^{-3} uM, where no effect was detected on swimming type, and type 1 swimming was common in the culture at large, chemosensory responses were essentially similar to those in controls lacking metals.

In cultures with added Pb, chemosensory responses were affected at 1 uM in some experiments, but not in others. Below this level chemosensory responses appeared to be unaffected.

DISCUSSION

The responses to filtrates by control cells appear reasonable given the fact that Fabrea uses both Dunaliella and Isochrysis as food (particularly the former), while Pavlova does not support growth (Repak, 1983).

From these observations, it appears that inhibition of the chemosensory response by metals could simply reflect a general effect on motility, and does not necessarily indicate the blocking or inactivation of receptors, or elements of a sensory transduction chain.

SUMMARY AND GENERAL DISCUSSION

Basic results from this study include the following:

(a). Suspended particulates in the Hudson River at times contain relatively high levels of lead and silver.

(b). Ionic Ag and Pb added to river water accumulate in the particulate fraction. This appears to involve two first order processes, with over 90 % of the metal in the particulate phase at equilibrium.

(c). Using the model estuarine species Fabrea salina, it was

found that ciliates accumulate both Ag and Pb from the dissolved (ionic) state, with concentration factors of approximately 6×10^3 and 2×10^2 (volume/volume), respectively.

(d). Ciliates accumulate Ag from food algae, with approximately the same concentration factor as from the dissolved state. Accumulation of Pb from food was not determined, for technical reasons (lack of availability of sufficient quantity of ^{210}Pb).

(e). When added in the dissolved, ionic state, Ag was lethal after 24 hours to ciliates at concentrations in the range 1.0 - 0.1 uM, with somewhat variable results from one experiment to another. Pb did not have effects at levels where precipitation did not occur. When washed food organisms were present, the lethal effects of Ag tended to occur at higher concentrations, depending on the method of washing employed.

(f). When added in the ionic state, Ag inhibited feeding at concentrations above 1 uM. No consistent effect was seen with Pb at levels where precipitation did not occur. Data from these experiments were quite variable.

(g). Three major types of swimming were observed with Fabrea. Type one (spiral swimming along a straight axis) was inhibited by Ag at levels above 10^{-2} uM, and by Pb above 1 uM.

(h). Chemosensory responses to filtrates of suspensions of algae were detected, using videorecordings of behavior near agar cubes incorporating the filtrates. Dunaliella tertiolecta and Isochrysis galbana elicited accumulation, whereas Pavlova gyrans-impregnated cubes were avoided.

(i). Ag and Pb added in ionic form inhibited these chemosensory responses, but this appeared to be through the effects on swimming noted already (see (h) above), i.e., the inhibition of swimming type one.

As noted above, the consistent, significant differences between the effects of added Pb and Ag on ciliates observed in this work may simply reflect the differences in accumulation of these two metals. It is in any case interesting that effects were seen on swimming behavior at concentrations significantly below those at which a lethal effect appeared.

Despite the fact that both Ag and Pb seem to be at relatively high concentrations in the particulate fraction of the Hudson River water column, we cannot necessarily conclude that the ciliate populations are impacted by this, since we do not know whether the chemical state of the metals in the river is comparable to that of the metals added to experimental populations. In the latter, the metals are added as ionic inorganic salts, and after a relatively short time they appear to reach a steady state in which a high proportion it adsorbed to the particulate fraction.