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Bioavailability and trophic transfer of humic-bound copper from bacteria to zooplankton

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ABSTRACT: The effect of humic acid (HA) on uptake and transfer of Cu by selected marine organisms from the microbial loop was determined. Bacteria grown with and without 15 µg Cu l^{-1} and with and without 10 mg Suwannee River Humic Acid (SRHA) l^{-1} were fed to *Uronema* sp. The *Uronema* were subsequently fed to *Acartia tonsa* to determine the effect of humic acid on the uptake and transfer of Cu from bacteria to copepods. The presence of 10 mg SRHA l^{-1} reduced Cu uptake in *A. tonsa* by an average of 54% and significantly reduced the negative effects of Cu on reproductive success of *A. tonsa*. The percentage of the total Cu residues in *A. tonsa* resulting from feeding was estimated by exposing *A. tonsa* to the same conditions with and without pre-exposed *Uronema* as food. The results indicate that approximately 50% of the Cu residue is due to feeding. Thus, SRHA seems to affect Cu uptake in *A. tonsa* through binding of free Cu in the water at the same rate as through the food chain. This study demonstrates the importance of complexation of metals by organic matter and trophic transfer processes for organisms critical to estuarine food webs.

KEY WORDS: Trophic transfer · Bioavailability · Humic acid · Zooplankton · Copper

INTRODUCTION

Macromolecules in dissolved organic matter (DOM) such as humic acid (HA) are known to adsorb onto the surfaces of microbes (Daniels 1980, Decho 1990). The fact that bacteria adsorb humic compounds (and therefore any compounds that may be complexed with the humics) onto their surfaces may be an important transport mechanism to higher trophic levels, such as bacterivores (e.g. ciliates) and zooplankton (e.g. copepods). Much research has been directed toward learning how HA effects the bioavailability of water-borne chemicals to higher aquatic organisms such as fish, molluscs and crustaceans (e.g. McCarthy et al. 1985, Alberts et al. 1989). Studies on the effect of HA on uptake have been inconsistent. Most of the existing research showed that binding to HA reduced the bioavailability of lipophilic compounds to organisms such as fish and amphipods in proportion to the fraction that was bound (e.g. McCarthy & Jimenez 1985, Landrum et al. 1987, Kukkonen et al. 1990), indicating that the bound fraction was not available for uptake by these organisms. Other research, however, has shown either no effect on uptake or that the presence of HA increased the uptake of chemicals, especially metals (e.g. Steinberg et al. 1992, Pempkowiak et al. 1994, Penttinen et al. 1995).

Comparatively little is known about how HA effects bioavailability to aquatic organisms at the bottom of the food chain. The fact that many of the previous examples of increased uptake of chemicals in the presence of HA have occurred in filter-feeding organisms that ingest microbes suggests that bacterial adsorption may be an important factor. Bacterivores feeding on bacteria that have macromolecules adsorbed to their surfaces may be ingesting significantly higher quantities of such compounds than they would otherwise. Bacterivores may then pass these materials on to higher zooplankton such as copepods. Wyman & O'Conner (1980) and McManus et al. (1983) demonstrated that feeding of PCB-contaminated phytoplankton to *Acartia tonsa* increased uptake of PCB.

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Few studies, if any, have evaluated the effect of HA on uptake and transfer of Cu by marine organisms of the microbial loop. Bacteria, ciliates and copepods are basic components of the microbial loop, a pathway that transfers carbon energy from dissolved organic matter to higher trophic levels (Azam et al. 1983, Sanders & Wickham 1993). Copepods are significant grazers of phytoplankton during certain periods of the year, especially during phytoplankton blooms (e.g. Durbin et al. 1990, Mallin & Pearl 1994, Dagg 1995). Ciliates are also a significant proportion of copepod diet during much of the year and may supply important nutritional components (e.g. Ohman & Runge 1994, Ederington et al. 1995, Jonasdottir et al. 1995). The importance of these organisms in marine food webs has been well documented, and copepods in particular provide a basic link to larval fish (Runge 1988, Sanders & Wickham 1993). If the adsorption of HA onto bacteria can carry significant amounts of adsorbed chemicals, then there could be a risk to higher organisms through the microbial loop. The use of ciliates and copepods in this study allowed the effects of HA on the uptake of Cu by bacteria to be linked with higher trophic levels, since ciliates and copepods are major links to higher trophic levels in the natural food chain (Stoecker & Capuzzo 1990).

Copper is commonly found as a pollutant in marine/estuarine waters due to its uses in antifouling paints, in treatment of marine lumber, and many other sources. The United States Environmental Protection Agency has established a water quality criterion of 2.9 µg Cu l^{-1} for marine waters (US EPA 1995). Sublethal effects in copepods have been demonstrated in the 1 to 10 µg l^{-1} range and reproductive effects are among the more sensitive of these sub-lethal indicators (Beers et al. 1977, Reeve et al. 1977, Sunda et al. 1990). The reported LC_{50} values for *Acartia tonsa* exposed to Cu were in the range of 17 to 40 µg l^{-1} (Sosnowski & Gentile 1978, Sosnowski et al. 1979, Sunda et al. 1990).

This study examined microbial adsorption and food chain transfer of humic bound Cu in marine waters. Research concentrated on understanding the effects of HA on bioaccumulation and trophic transfer of Cu through several food chain components, including bacteria, ciliates, and the copepod *Acartia tonsa*.

MATERIALS AND METHODS

Descriptions and sources of materials, such as Suwannee River Humic Acid (SRHA), artificial seawater, and organisms *Vibrio natriegens* and *Uronema* sp., along with culture conditions and methods of preparation can be found in Lores et al. (1999). A description of statistical methods is also described therein. The concentration of SRHA used in all experiments was 10 mg l^{-1}. It was chosen because it is well characterized (Averett et al. 1989), it is an aquatic humic extract, and is commercially available. SRHA is approximately 50% carbon, and the concentration used in these experiments represents a DOC concentration of 5 mg C l^{-1} This concentration was chosen to be high enough to cause measurable effects, but within the range of those found in estuaries (Moran et al. 1991). The choice to use artificial seawater was based on the desire to eliminate extraneous dissolved organics that may have been present in natural waters. The salinity of 16 was chosen for compatibility with the test organisms and to represent a median estuarine salinity. The concentration of Cu in these experiments was 15 µg l^{-1} and is below the LC_{50} concentration for *Acartia tonsa* of 17 µg Cu l^{-1}. This Cu concentration is higher than marine water quality criteria set by the Environmental Protection Agency (1985), but the higher concentration was needed to cause a measurable effect.

Copepods *Acartia tonsa* were collected from local waters using a 153 µm mesh 0.3 m diameter plankton net, then maintained in aquaria at ambient temperatures with sterilized filtered seawater from Santa Rosa Sound and fed a mixture of the unicellular algae *Thalassiosira* and *Isochrysis* prior to their use in the experiments. Only adult female or late stage copepedites were used in experiments. *A. tonsa* is found in estuarine waters throughout the United States. The use of *A. tonsa* in this study offered several advantages. The rapid rate of Cu uptake and egg production allowed both reproductive and bioavailability studies to be carried out in a matter of days. Their widespread and year-round availability was also advantageous.

Feeding/exposure experiments. To determine how humic binding affects bioavailability and food chain transfer, copepods were exposed to Cu and HA while being fed ciliates that were pre-exposed to the same conditions while feeding on bacteria. Three experiments were conducted, each consisting of 4 treatments and 3 replicates with and without Cu and HA. The 4 treatments consisted of a seawater control (CTL), 10 mg l^{-1} humic acid control (HACTL), 15 µg l^{-1} Cu exposure (CU) and a 15 µg l^{-1} Cu exposure with 10 mg l^{-1} humic acid (CUHA). Cultures (1 l) were incubated in the dark in 2 l Erlenmeyer flasks at 20 ± 1°C and were shaken at 60 rpm. The first 2 experiments consisted of 3 phases: a microbial growth phase, a ciliate growth phase and a copepod feeding phase. Bacteria were cultured in the 4 treatments listed above, then ciliates were added to consume the bacteria. When the ciliates reached stationary phase, they were collected and fed to copepods under the same conditions. The first experiment used mixed bacterial assemblages that was grown to stationary phase before allowing ciliates to feed. The bacterial assemblages were obtained from

cultures using 0.6 µm filtrates of Santa Rosa Sound water used as an inoculate. The effects of HA and/or Cu on survival and reproduction (nauplii production) in copepods were also investigated in the first experiment. The second experiment used washed *Vibrio natriegens*, pre-exposed to treatment conditions for 24 h, as a food source for the ciliates.

A third experiment was designed to determine how much Cu uptake was directly from water, allowing a determination of how much uptake was due to feeding. In the third experiment, Cu uptake was measured in copepods that were exposed to the same 4 treatments without ciliates as food to estimate the effect of SRHA on bioavailability of Cu through uptake pathways other than feeding.

Microbial growth phase. To start the first phase of the first feeding experiment, 100 ml of inoculate was added to 2 l Erlenmeyer flasks containing 900 ml of artificial seawater, 25 mg of yeast extract l^{-1}, HA and/or Cu as required (flasks and all ingredients except innoculate had been heat sterilized and cooled). Microbial cell density was measured 3 times d^{-1}. In the second experiment, an inoculate of *Vibrio natriegens* was used in place of the mixed inoculate; otherwise, conditions were the same.

Ciliate growth phase. After the microbial growth reached stationary phase, 100 ml of ciliates *Uronema* sp. at a density of approximately 10000 ciliates ml^{-1} were added to each microbial culture flask to begin the second phase. Samples for ciliate counts (0.5 to 2 ml) were collected 3 times d^{-1} and were preserved and counted as described in Lores et al. (1999). Ciliates were grown to stationary phase, collected by centrifugation (300 × *g*) and transferred to identical treatment flasks with 10 copepods in each, but without the yeast extract. A 50 to 100 ml subsample of the ciliate culture was taken for analysis of Cu in the ciliates.

In the second experiment, 1 ml of washed ciliates (1×10^5 ciliates ml^{-1}) was added to 500 ml flasks of washed *Vibrio natriegens* that had been pre-exposed to the treatment conditions for 24 h. The ciliates were allowed to feed on the bacteria for approximately 24 h before a 50 ml portion was taken for Cu analysis and a 100 ml portion was concentrated and transferred to 2 l Erlenmeyer flasks with 1 l of treatment solution and 10 copepods. The copepods were allowed to feed on the ciliates for 24 h before being collected for analysis of Cu residues. The remaining ciliate culture was sampled again for Cu analysis (50 ml) at 48 h.

Copepod exposures. Ten copepods were selected for each treatment flask and held in approximately 100 ml of artificial seawater for 2 to 4 h. Copepods were transferred to treatment flasks and allowed to feed on ciliates for 1 to 3 d (56 h in the first experiment and 24 h in the second). After exposure, copepods were collected by straining the contents of each flask through a 44 µm sieve. The contents of the sieve were washed into a petri dish with some of the strained water. Copepods were counted and survivors were then picked out with a glass pipette, washed with distilled water, and transferred to a plastic centrifuge tube for digestion and Cu analysis. The remaining solutions were preserved with rose bengal-formalin and nauplii were counted to determine reproductive success.

Copper analysis. For analysis of Cu residues in ciliates, a 50 ml sample was centrifuged (300 × *g* for 10 min) in a plastic centrifuge tube which had been rinsed in 5% HCl. The supernatant was decanted while holding a glass rod over the pellet; the pellet was dried and digested in 100 µl of concentrated nitric acid at 90°C. Live copepods collected at the end of each experiment were also dried and digested in 100 µl of nitric acid. Only adult copepods alive at the end of the experiment were used for these analyses. Digested samples were diluted as necessary with distilled water, and duplicate 10 µl aliquots were analyzed for Cu by Atomic Absorption Spectroscopy (AA) in a graphite furnace as described in Lores et al. (1999). The data was calculated on a per organism basis. Duplicate analyses were performed on all samples and standards and were repeated if the relative standard deviation was greater than 20%. Samples with Cu concentrations higher than the highest standard were diluted and re-analyzed. A matrix spike was run on every set of samples and all matrix spike recoveries were greater than 80%. Detection limit in the final solutions was 1 ng ml^{-1}.

RESULTS

Microbial growth curves (mixed inoculate) from the first experiment are shown in Fig. 1. Initial background absorbance in treatments containing HA was higher than in treatments without HA, and any decrease in absorbance due to loss of HA may have masked some growth; however, no significant differences in growth were apparent in any of the treatments.

At the end of the microbial growth phase, ciliates were added directly to the cultures, and the resulting *Uronema* sp. growth curves (Fig. 2) show growth on the mixed cultures for the first 36 h. The remaining portion of these curves shows the ciliate density after they were transferred to copepod flasks as prey. *Uronema* growth was not significantly affected by HA in this experiment. The drop in ciliate density at 40 h was due to losses as the ciliates were collected and transferred to copepod feeding flasks. The Cu residues in these ciliates at the time they were transferred to the copepod flasks ranged from 0.05 picograms $ciliate^{-1}$ in

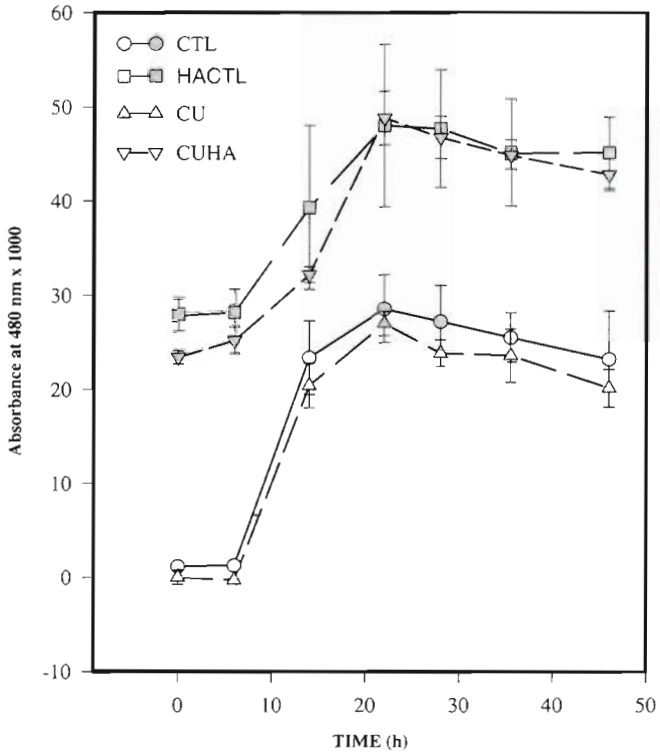

Fig. 1. Microbial growth curves estimated by optical density at 480 nm from experiment on effect of Suwannee River Humic Acid (SRHA) on transfer of Cu from bacteria to ciliates to copepods. CTL = control artificial seawater; HACTL = humic control (10 mg SRHA l^{-1}); CU = 15 µg Cu l^{-1}; CUHA = 15 µg Cu l^{-1} + 10 mg SRHA l^{-1} Values are means ±SD of triplicates

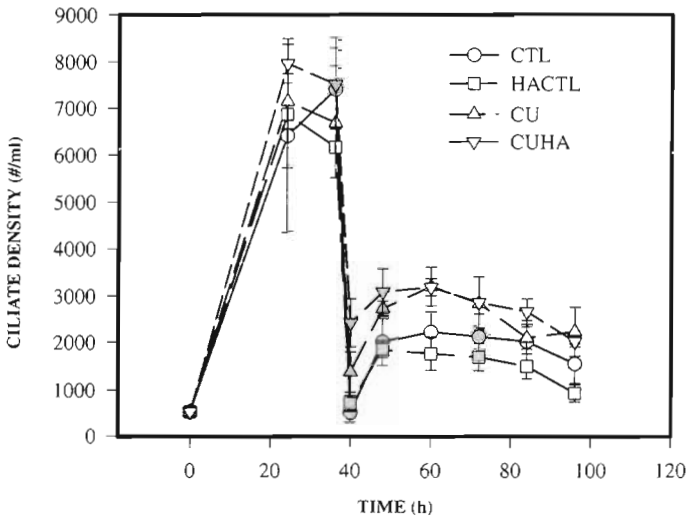

Fig. 2. *Uronema* sp. Ciliate growth curve from experiment on effect of Suwannee River Humic Acid (SRHA) on transfer of Cu from bacteria to ciliates to copepods. Treatments are the same as in Fig. 1. Values are means ±SD of triplicates

both controls to 0.14 and 0.29 picograms ciliate^{-1} in CUHA and CU treatments, respectively (Fig. 3). The difference between measured Cu residues ciliate^{-1} in the CU and CUHA treatments was statistically significant ($\alpha = 0.05$).

The Cu residues in copepods from this experiment ranged from 1.24 and 1.76 ng copepod^{-1} in HACTL and CTL, respectively, to 1.96 and 5.08 ng copepod^{-1} in CUHA and CU treatments, respectively (Fig. 3). There was a statistically significant difference ($\alpha = 0.05$) in Cu residues copepod^{-1} in the CU treatment and all other treatments, while the CUHA treatment was statistically different from HACTL , but not from CTL. In 1 of the samples from the CU treatment in the first experiment, there was only 1 copepod that survived; however, Cu residues were still detectable. In the other 2 CU samples, there were 5 and 6 copepods collected for analysis. In all other samples, at least 8 copepods were analyzed. In the first experiment (56 h), mean survival was 40% in the CU treatment and was greater than 87% in all other treatments (Fig. 4); however, there was no statistically significant differences ($\alpha = 0.05$). The mean number of nauplii collected from copepod flasks at the end of the 56 h experiment was significantly higher ($\alpha = 0.05$) in both controls and CUHA treatments compared with the total number of nauplii found in the CU treatment (Fig. 4).

In the second experiment, where copepods were exposed for only 24 h under the same conditions as in the first experiment, Cu residues in copepods were similar to results from the first experiment (Fig. 5). The Cu uptake was again lower in the presence of HA. Unlike the first experiment, copepod reproduction in this experiment was not significantly different, although the mean number of nauplii in the CU treatment was lower as in the first experiment. Statistical analyses of Cu residues in copepods from the first 2 experiments indicate that there are no statistical differences between experiments. Combining the results improved the level of significance (p-values), but did not change the overall significance of any of the pairwise comparisons.

In the second experiment, although ciliates were fed to copepods after 24 h exposure, ciliate exposure was continued for 48 h. The total Cu ml^{-1} of ciliates over the 2 d exposure, where ciliates were feeding on washed *Vibrio natriegens*, increased on Day 2 compared to Day 1 (Fig. 6). The difference in total Cu ml^{-1} of ciliates between the CU and CUHA treatments was statistically significant ($\alpha \leq 0.05$) on Days 1 and 2. The Cu ciliate^{-1} in the CUHA treatment over the same 2 d exposure shows a continuous increase over the period (Fig. 6). Copper ciliate^{-1} was significantly higher in the CU treatment than in the CUHA treatment in ciliates collected on Day 2.

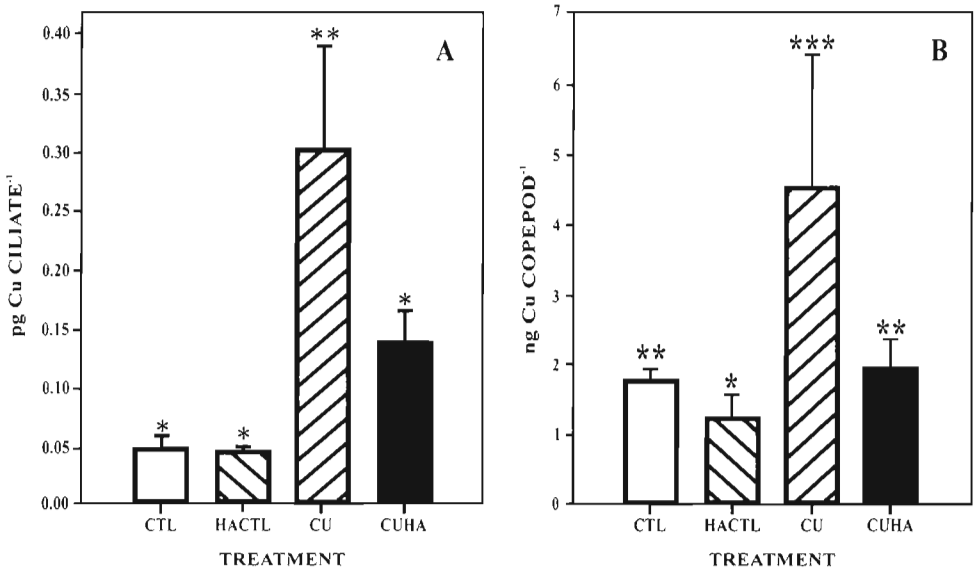

Fig. 3. Copper uptake by (A) ciliates after 24 h exposure while feeding on pre-exposed bacteria (B) copepods after 56 h exposure while feeding on ciliates. Treatments are the same as in Fig. 1. Values are means ±SD of triplicates. Difference in number of asterisks signifies statistical difference ($\alpha = 0.05$)

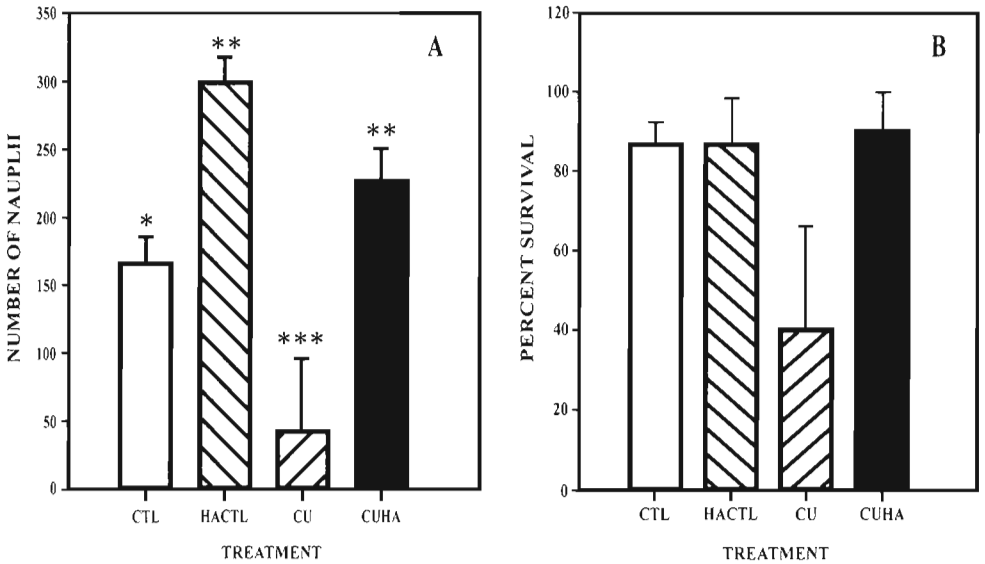

Fig. 4. *Acartia tonsa*. (A) Reproduction and (B) survival of adult female after 56 h exposure in experiment on effects of Suwannee River Humic Acid on transfer of Cu from bacteria to ciliates to copepods. Treatments are the same as in Fig. 1. Values are means ±SD of triplicates. Difference in number of asterisks signifies statistical difference ($\alpha = 0.05$)

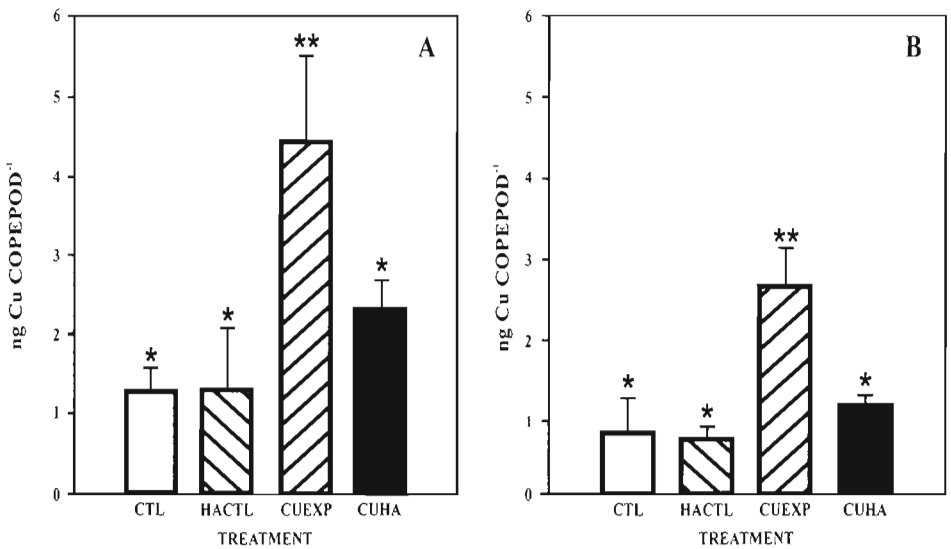

Fig. 5. Copper levels in (A) copepods feeding on pre-exposed ciliates and (B) unfed copepods after 24 h exposure. Treatments are the same as in Fig. 1. Values are means ±SD of triplicates. Difference in number of asterisks signifies statistical difference ($\alpha = 0.05$)

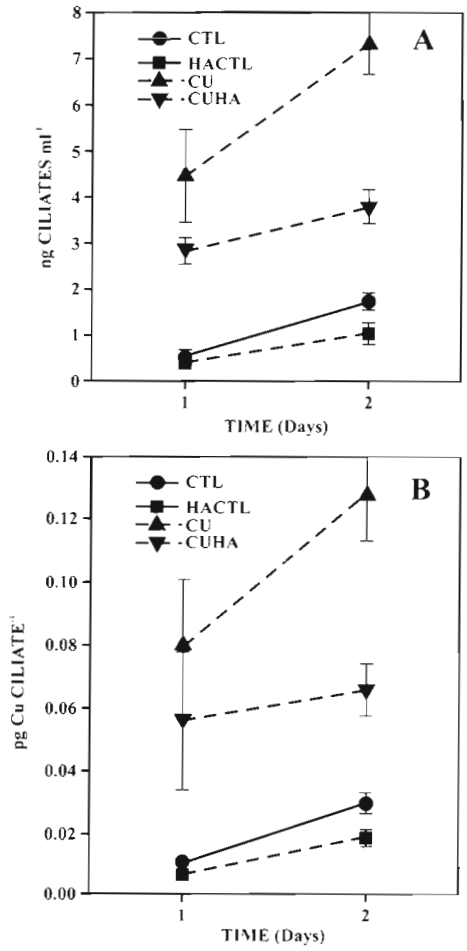

Fig. 6. *Uronema* sp. (A) Total Cu ml^{-1} of ciliate culture and (B) Cu uptake on a per ciliate basis over a 2 d exposure. Treatments are the same as in Fig. 1. Values are means ± SD of triplicates

The overall Cu residues in copepods from the third experiment, in which copepods were exposed for 24 h without food (ciliates), were reduced when compared to the first 2 experiments where copepods were fed (Fig. 5). As in the first 2 experiments, Cu uptake was significantly reduced in the presence of HA ($\alpha = 0.05$). In copepods exposed to Cu without HA, unfed copepods accumulated 2.6 ± 0.5 ng $copepod^{-1}$, while unfed copepods that were exposed to Cu plus HA accumulated only 1.1 ± 0.1 ng $copepod^{-1}$.

DISCUSSION

The microbial growth curves (Fig. 1) are based on optical density measurements. The difference between the initial and final absorbance (T_f) in the stationary phase can be used to estimate a net yield; however, it is possible that some of the HA may be utilized or adsorbed to cells, decreasing absorbance at the same time absorbance is increasing due to cell growth. Therefore, comparisons of the apparent yield between treatments with and without HA cannot be made, and though the differences in optical density are approximately the same, it is possible that the actual yield may be higher in the treatments with HA. Both treatments with HA do show a lag in growth during the log phase, but this too may be caused by binding of HA to cells. The Cu exposure levels in this experiment were not expected to have any effect on bacteria, and there did not seem to be any direct effect on microbial cultures caused by the Cu in this experiment, with the exception of a slightly more exaggerated lag in growth in the CUHA treatment. Copper residues in these mixed bacteria were not measured, but based on results with *Vibrio natriegens* (Lores et al. 1999), it is likely that the bacteria in the CUHA treatment contained less Cu to be transferred to their consumers, *Uronema* sp. in this case. The natural microbial mixtures in this experiment produced complex assemblages that were not reproducible. In addition, the actual cell density of a mixed culture cannot be estimated using optical density. Therefore, to reduce the complexity encountered in experiments with mixed cultures, further research with bacteria was restricted to *V. natriegens*.

The relative decrease in uptake of Cu by *Uronema* in CUHA treatments was approximately the same as in previous experiments (Lores et al. 1999) when ciliates were exposed to higher Cu levels. The decreased bioaccumulation of Cu by ciliates in the presence of HA suggests a decreased risk to ciliates and their consumers. The increased total ciliate Cu content (Fig. 6) in all treatments on Day 2 compared to Day 1 is probably due to movement of the Cu from the microbial pool (most of which is not collected by centrifugation at 300 × *g* for 10 min) to the ciliates. The increased uptake on Day 2 suggests that the response of the ciliate community as a whole may be slower than expected (days) relative to the doubling time of the population, which is only a few hours for *Uronema*.

In the first experiment, mean survival of copepods exposed to Cu was higher in the CUHA compared with the CU treatment (Fig. 4). The observed survival in the CU treatment is in agreement with the LC_{50} values reported by Sosnowski & Gentile (1978). Survival was not significantly different in any of the treatments from the two 24 h experiments. The number of nauplii collected at the end of the first experiment was higher in both treatments with HA; however, only the HACTL group was significantly higher than the CTL treatment. Reproduction was significantly lower in CU treatment and this group was statistically different from all other treatments. Similar effects from Cu on

reproduction were seen by Reeve et al. (1977) who reported marked reduction of egg production in *Acartia tonsa* exposed to Cu at 20 µg l^{-1} from the first day and suggested that exposure to levels as low as 5 µg l^{-1} can have reproductive effects over longer terms. Sunda et al. (1990) also investigated the bioavailability of complexed Cu to *A. tonsa* (using EDTA and NTA as complexing agents) and found that the complexation detoxified Cu and increased larval survival. In the second experiment, copepods were exposed to Cu for only 24 h and significant differences in survival and reproduction did not develop. There was a decrease in reproduction in the CU treatment as before, but the lack of a statistical difference in this case was probably related to the time of exposure.

In the first 2 experiments, the presence of SRHA reduced Cu uptake in copepods by 61 and 48%, respectively. In the third experiment, SRHA reduced Cu uptake in copepods by 57% in the absence of food. The humic binding measurements in Lores & Pennock (1998) suggest that approximately 60 to 80% of the Cu was bound to SRHA under the conditions similar to these experiments. However, pH was not measured in these experiments and small changes can significantly affect binding of Cu to HA. If the bound Cu was closer to 60%, these results suggest that little, if any, of the CuHA complex is available to copepods. However, if the bound Cu was near 80%, as was found when pH was maintained at 7.0, these results suggest that a small portion of the CuHA complex may be available.

The consistency of the Cu copepod^{-1} value suggests that measurement of individual copepod body burdens may be a useful indicator of environmental exposure. The fact that there seems to be little difference in body burden at 24 h compared with 56 h (Figs. 3B & 5A) suggests that they rapidly reach steady state with their environment. More measurements are needed to determine the relationship between copper concentration and uptake, and to accurately correlate effects with Cu body burden in copepods. If established, such relationships could be used to improve ecosystem risk assessment since copepods appear to accumulate Cu rapidly, but do not accumulate the HA-complexed Cu.

Combined, the results from these experiments show the effects of feeding and HA on Cu accumulation and Fig. 7 provides an overall summary of the conditions and uptake of Cu by the organisms (that is assuming a 50% binding to HA). In copepods exposed 24 h to Cu without HA, copepods that were not fed ciliates obtained approximately 40% less Cu than fed copepods. In copepods that were exposed to Cu plus HA, the unfed copepods accumulated approximately 50% less Cu than fed copepods. The higher Cu residues in fed copepods compared to unfed copepods suggest that the copepods are getting a significant amount of Cu through the feeding process, although it is possible that the uptake rate is just slower in unfed copepods. Reinfelder & Fisher (1991) suggested that copepods assimilate only the soluble fraction of their food. The fact that in the CUHA treatment unfed accumulated less Cu on a percentage basis suggests that those copepods are getting more through the feeding process and that, possibly, the ciliates from the CUHA treatment are converting Cu to a more available form.

It is possible to calculate the Cu/carbon ratio in these organisms based on literature carbon values for each organism. If we assume that 40% of the Cu was associated with bacteria in the CU treatment and 15% associated with the bacteria in the CUHA treatment (Lores et al. 1999), then based on the *Vibrio narreigens* cell carbon content of 0.2 pg in stationary phase (Ohman & Snyder 1991) and cell density of 3×10^7 ml^{-1}, the Cu/carbon ratios in bacteria are 1.0 and 0.2 ng µg^{-1} in CU and CUHA treatments, respectively. For *Uronema* sp., based on a cell carbon of 0.31 ng, the ratios are 0.93 and 0.45 ng µg^{-1}, respectively. And for *Acartia tonsa*, the ratios are 1.2 and 0.56 ng µg^{-1}, based on a carbon content of 4.1 µg C copepod^{-1} (Thompson et al. 1994). The values for the CU treatments are relatively constant, but the ratios seem to increase in the CUHA treatments, especially at the ciliate level, supporting the idea that the ciliates may be converting the CuHA complex into a more available form.

In all these experiments, Cu residues in ciliates and copepods in the CUHA treatment were significantly lower compared to the CU treatment. Thus, HA significantly reduced the bioavailability of Cu to these ciliates and copepods. However, some experiments suggest that ciliates may have active biological processes

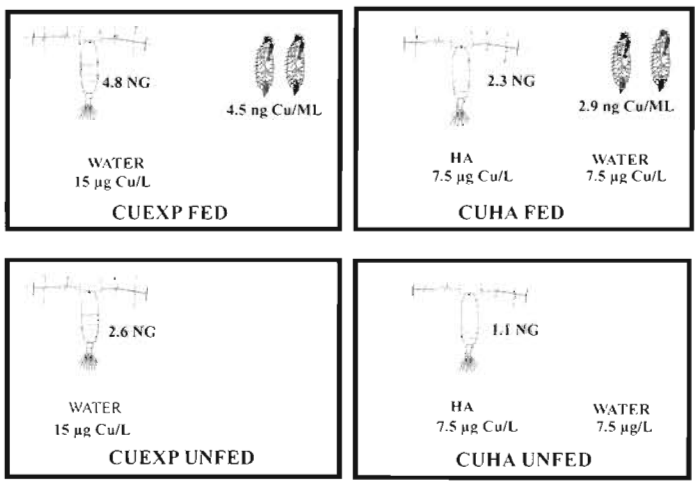

Fig. 7. *Acartia tonsa* and *Uronema* sp. Overall summary comparison of Cu uptake and conditions in experiments

that counteract the sequestration of Cu by SRHA. In Lores et al. (1999), uptake of Cu by a different ciliate, *Pleuronema* sp., was not reduced by HA. It is possible that organisms more adept at utilizing HA (such as flagellates or specific bacteria) may be at risk when toxic chemicals are adsorbed to HA. More experiments are needed to fully understand the interactions between various organisms of the microbial loop, their growth phases and binding of metals to HA.

From an overall perspective, each component of an ecosystem will compete for resources within the ecosystem, even xenobiotics. Many of the processes involved in this competition are passive physical and chemical processes such as physical transport and binding of chemicals to living and non-living components. Earlier experiments showed the binding affinity of SRHA for Cu can change dramatically depending on the pH and salinity of the water (Lores & Pennock 1998). Biological processes such as feeding, excretion, and population dynamics can be equally important. The microbial loop is an important component of estuarine and marine food webs. Most of the primary productivity of these waters goes through at least part of the microbial loop before it can be assimilated by larger organisms.

HA reduced Cu uptake by approximately half and improved survival and reproduction in copepods. The reproductive effects seen here and by others suggest that levels of Cu below the 15 $\mu g\ l^{-1}$ can have dramatic effects on these organisms at the population level. Copper is listed as one of the top 10 pollutants in the estuarine waters and sediments of the Gulf of Mexico (US EPA 1995) and has the potential to cause dramatic shifts in the population of these important organisms, especially in the absence of organic complexing agents such as HA. The major sources of HA in estuaries are the forested wetlands along rivers and salt marshes around estuaries. These wetlands, the sources of HA, can provide protection from the adverse impact of environmental pollutants in areas far removed from the wetlands.

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