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Bioaccumulation of Mercury in Fish Exposed to Experimentally Contaminated Water and Sediment

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Mercury, like the majority of contaminants when released into the aquatic environment, is deposited at the bottom of the ecosystem and adsorbed by the sediment. Although the sediment can be considered the final deposition of contaminants in the aquatic environment, physical, chemical, biological and also anthropogenic processes can occur that lead to the desorption of pollutants, thereby resulting in a secondary source of contamination of water (REYNOLDSON and DAY, 1993).

The biomethylation of inorganic mercury compounds in the sediment and their subsequent accumulation in the form of methyl mercury by aquatic biota, allows deleterious effects to reach animals of higher trophic levels (WALDOCK, 1994). In fish, Hg content is particularly important because it represents the first road to the contamination of their consumers, especially humans.

According to REYNOLDSON and DAY (1993), the interactive effect of complex mixtures of chemical pollutants and the toxic effects of available fractions in sediments, can be investigated by bioassays developed under controlled conditions in the laboratory or by field tests.

The aim of the present study was to determine the role of sediment in the deposit and release of mercury, using bioassays for toxicity in fish based on indicators of mortality and Hg bioaccumulation.

MATERIAL AND METHODS

The fish utilized in the experiments were tilapia fingerlings, *Oreochromis niloticus* (0.53g ± 0.29g), and zebrafish, *Danio rerio* (0.30g ± 0.03g), obtained from commercial fish farms. The two experiments were carried out simultaneously, with five treatments and five simultaneous replicates: C = control (only dilution water, without sediment), SC = sediment control (sediment + dilution water), SSHg = sediment experimentally contaminated (spiked) with 1.0 mg /L Hg, SHg0.2 = sediment maintained at in continuous contact with dilution water, contaminated with mercury at a concentration of 0.2 mg/L, Hg0.2 = only dilution water, contaminated with mercury at a concentration of 0.2mg/L.

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In treatment SSHg, the sediment was contaminated with a solution of 1.0 mg /L Hg for a period of 24 h. Afterward, excess solution was removed and the sediment was utilized in the tests. The mercury concentration of 0.2 mg/L utilized in two of the treatments is the $LC_{50\text{-}96h}$, determined in previous studies conducted with *O. niloticus* (ISHIKAWA, 2003). Mercury ($HgCl_2$, *Sinth*™) was the product used for experimental contamination.

The study was conducted in the Aquatic Toxicology Laboratory at the Instituto de Pesca, São Paulo, Brazil. The methodology for conducting the bioassays was standardized in accordance with recommendations outlined by APHA et al. (1998), by BURTON (1992) and BURTON and MACPHERSON (1995) for toxicity tests with sediment. The assays were performed twice (Experiment 1 and Experiment 2). Dechlorined tap water was used as source of the dilution water.

Samples of sediment were collected from the bottom of a natural lake by Ekman dredge, the samples was previously analyzed and considered of good quality (lake with its own wellspring, known to be free of influence from farming/livestock, industrial or urban activities). The samples of the whole sediment were stored without any manipulation at a low temperature (4^0C), where the toxicity bioassays were carried out within six weeks after the collection of the material.

Before assays were performed, the fish were acclimated for 10 days to the same conditions as in the laboratory in which the experiments were conducted. Toxicity assays were seven days long and were carried out in 600- mL beakers provided with 100 g of sediment (wet weight) and 400 mL of dilution water, thus in a proportion of 1:4 (sediment:water). The beakers were artificially aerated and stocked with six fish in each unit. During the experiments the fish were given daily flocculated commercial ration ad libitum. The system utilized was semi-static, with a change of 1/3 of the water every 24 h. Only dilution water was used in all replacements, excepting the two treatments SHg0.2 and Hg0.2, which stock solution of 0.2 mg/L was previously prepared. Each test consisted of seven days exposure to sediment.

After checking the normality of data, the differences among more than two treatments were determined by analysis of variance (ANOVA) and the Student's t-test for comparison only between two treatments. The results were considered significant when $p<0.05$ (ZAR, 1999). Temperature, dissolved oxygen, pH and electrical conductivity of the water were recorded every 24 h. Hardness, alkalinity and total ammonia (mg NH_4/L) were recorded at the beginning and end of the experiments. Mortality was recorded every 24 h and dead individuals were removed from the beakers.

Hg concentration was determined in the sediments (dry weight) and in the fish that survived the 7-d exposure (wet weight). Samples were processed by acid digestion in a block digestor (open system). The chemical analysis was performed with blank of the reagents. Total Hg was assayed with a Merlin atomic fluorescence spectrophotometer (minimum detection level of 1 ng). The sediment in treatment SSHg was analyzed before and after the experiment to determine the

occurrence of desorption of Hg.

RESULTS AND DISCUSSION

Table 1 gives the means of the chemical and physical variables of water observed in the bioassays, which were similar among the different treatments of each experiment, suggesting that these variables did not interfere with results obtained.

Table 1. Means of physical and chemical variables of water used in bioassays carried out with sediment experimentally contaminated with Hg. Test organisms: *Danio rerio* and *Oreochromis niloticus*.

Trial	Temperature (°C)	Dissolved Oxygen (%saturation)	pH	Electric Conductivity (µS/cm)	Hardness mg $CaCO_3$ /L	Amonium mg NH_3 / L
			Experiment 1			
C	23.92 /23.70	90.93 /90.33	7.47 /7.66	130.26 /123.82	56.45 /57.89	0.067 /0.070
SC	23.38 /23.51	87.87 /88.30	7.47 /7.51	126.75 /108.14	56.45 /56.45	0.067 /0.070
SSHg	23.47 /23.69	89.47 /86.97	7.44 /7.66	113.36 /110.64	58.79 /57.37	0.072 /0.076
SHg0.2	23.51 /23.56	90.17 /88.87	7.64 /7.62	113.21 /114.36	51.89 /57.37	0.077 /0.075
Hg0.2	23.44 /23.78	90.30 /89.30	7.60 /7.53	116.49 /119.27	50.26 /55.58	0.072 /0.072
			Experiment 2			
C	21.70 /21.63	90.20 /85.37	7.60 /7.81	131.50 /218.82	22.22 /19.60	0.022 /0.021
SC	21.26 /21.41	90.43 /86.17	7.46/7.83	114.98 /190.80	23.20 /19.60	0.022 /0.021
SSHg	21.57 /21.98	88.57 /87.00	7.57 /7.85	103.17 /156.52	19.80 /23.21	0.020 /0.022
SHg0.2	21.51 /21.90	89.57 /87.73	7.61 /7.86	103.46 /143.77	21.90 /19.60	0.012 /0.016
Hg0.2	21.27 /21.31	92.80 /82.27	7.65 /7.83	115.97 /160.04	19.60 /19.60	0.020 /0.018

C = Control (only water for dilution); SC = sediment + water for dilution, SSHg = sediment contaminated with 1.0 mg/L Hg; SHg0.2 = sediment kept in continuous contact with water contaminated with mercury, at a concentration of 0.2 mg/L; Hg0.2 = only dilution water contaminated with mercury at a concentration of 0.2 mg/L.

The mortality rates observed over the course of the experiments were relatively low and did not differ significant among treatments ($p>0.05$). The mortality values for the control groups (C and SC) were below 10%, in accordance with the established limit for toxicity tests (APHA et al., 1998). Treatment SSHg (sediment experimentally contaminated with mercury) also showed mean mortality values of 10%. Higher mortality indices were recorded for treatment Hg0.2, that is, 23.33 and 16.66% for *O. niloticus* and *D. rerio*, respectively. These levels were always higher when compared with treatment at the same concentration of mercury but in the presence of sediment (13.33 and 3.33%).

Based on the mortality data obtained, treatments that contained mercury did not cause death in exposed organisms, since there was no statistical difference when compared to the mortality rate of control groups. Meanwhile, it was possible to observe a greater mortality in fish exposed to treatment with a concentration of 0.2 mg/L Hg in the absence of sediment when compared to treatment with the same concentration in the presence of sediment. Hg could have been incorporated into the sediment, diminishing

its availability in the water column, which would be reflected in lower mortalities in treatment SHg0.2 (13.33 and 3.33%), when compared to Hg0.2 (23.33 and 16.66%) for *O. niloticus* and *D. rerio*, respectively. However, these differences did not prove to be statistically significant. Hg could also have been adsorbed to the beakers, but it would altered the results, since this material was similar to both treatments.

JAHANBAKHT et al. (2002) observed an increase in the lifespan of fish exposed to Hg concentration of 0.095 mg /L in the presence versus the absence of sediment, where fish stayed alive for 35 h when there was sediment and only 5 h without sediment present.

Table 2 shows the quantities of mercury determined in the sediments. As expected, Hg was not detected in the samples of control sediment. This sediment, when experimentally contaminated (spiked with 1.0 mg Hg/L), adsorbed 0.06 and 0.23 μg Hg/g, experiments 1 and 2, respectively. Those differences may be attributed to the intrinsic characterists of the two samples of sediment. However, both samples do not revealed any difference among aspects analyzed in this study. Analysis of the sediment in treatment SHg0.2 showed that part of the Hg that was in the water (0.2 mg/L) became incorporated into the sediment after seven days exposure (0.01 μg/g in experiment 1 and 0.1 μg/g in experiment 2).

Table 2. Mean concentration of Hg (μg/g dry weight) in sediment experimentally contaminated with Hg, in bioassays performed with *O. niloticus* and *D. rerio*

	Experiment 1	Experiment 2
SC	ND	ND
SSHg'	0.06	0.23
SSHg''	0.02	0.22
SHg0.2	0.01	0.10

C = Control (only water for dilution); SC = sediment + water for dilution, SSHg' = sediment contaminated with 1.0 mg/L Hg before the experiment; SSHg'' = sediment contaminated with 1.0 mg/L Hg after the experiment; SHg0.2 = sediment kept in continuous contact with water contaminated with mercury, at a concentration of 0.2 mg/L; Hg0.2 = only dilution water contaminated with mercury at a concentration of 0.2 mg/L. ND = not detected

In all the experiments carried out in the present study, the values for Hg adsorbed in the sediment (Table 2) did not surpass the limit of 1.0 μg/g, considered by the USEPA (1976) as safe for this compartment of the aquatic ecosystem. According to MACDONALD et al., (2000), the concentration of 1.0 μgHg /g in the sediment can cause adverse effects to aquatic organisms, while 0.20 μgHg/g can be considered as lowest effect level (LEL). The concentration of 0.23μg/g observed in the present study was slight higher than LEL stated by those authors.

The Hg present in the sediment in treatment SSHg appears to have been poorly available to the fish, at least in the experimental time utilized here (seven days), since there was no difference in Hg concentration before and after the experiment. This result is corroborated by the bioaccumulation data for fish exposed to this treatment, where only *O. niloticus* accumulated Hg (0.02μg/g) in the two experiments. This difference between the two species tested may be due to the

habit of *O. niloticus* of stirring up the sediment, establishing a more direct contact with this compartment and increasing the possibility of absorption of pollutants.

Assays for bioaccumulation of mercury demonstrated that there was a statistically significant difference ($p<0.05$) between treatments Hg0.2 and SHg0.2 for the two species of fish (Figure 1). In all experiments, fish exposed to a concentration of 0.2mg Hg /L without sediment accumulated more Hg when compared with fish submitted to treatment containing sediment. Hg /L without sediment accumulated more Hg when compared with fish submitted to treatment containing sediment.

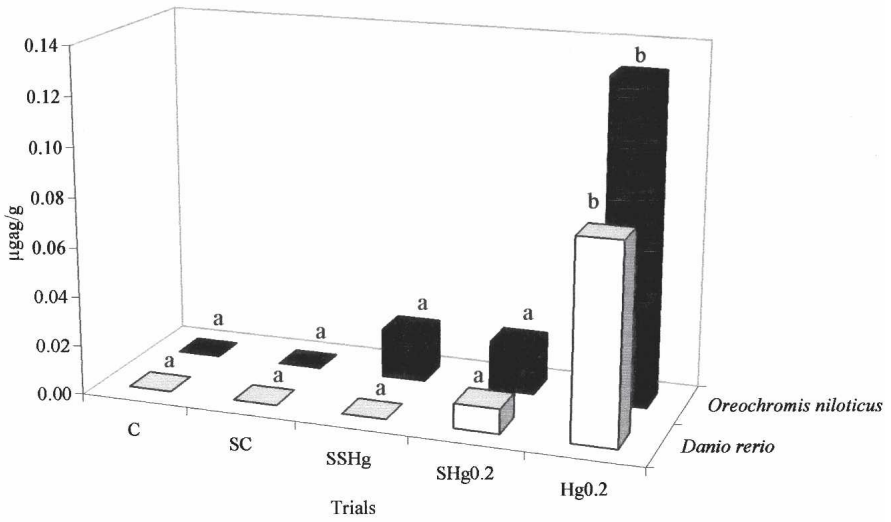

Figure 1. Bioaccumulation of Hg in the survived fish after 7-d exposure (wet weight) during assay carried out with sediment experimentally contaminated. Mean of experiment 1 and experiment 2

C = Control (only water for dilution); SC = sediment + water for dilution, SSHg = sediment contaminated with 1.0 mg/L Hg; SHg0.2 = sediment kept in continuous contact with water contaminated with mercury, at a concentration of 0.2 mg/L; Hg0.2 = only dilution water contaminated with mercury at a concentration of 0.2 mg/L. Bars with the same superscript letters are not statitiscally different ($p<0.05$)

The short time of exposure (7 days) can be one of the factors contributing to the finding that mercury was not available to the water column in large amounts. GILLESPE (1972) contaminated sediment with 50 mg Hg/kg and exposed fish to this sediment for 97 days. Analysis of the exposed fish after this period showed bioaccumulation levels on the order of 1.0µg Hg/g tissue. The time utilized by this author is much longer than that used in the present work. According to BURTON (1992), storage of the sediment at 4°C does not block microbial activity. Therefore, bacteria could theoretically methylate the mercury present in thesediment. CALLISTER and WINFREY (1986) using radioactive mercury demonstrated that when added to the sediment, 98% of metal was rapidly

incorporated and 3% methylated in 10 days. It is seen therefore that although the standardization of bioassays of sediment calls for a period of seven days of exposure (BURTON, 1992), this is perhaps not ideal or sufficient to permit the desorption of pollutants in the sediment and their consequent distribution in the water column.

With respect to adsorption, as demonstrated in Table 2, control sediment did not show any contamination by Hg. Thus, it is clear that mercury detected in the sediment in treatment SHg0.2 is due to the adsorption of this metal, after seven days exposure to Hg concentration of 0.2 mg/L in the water.

JAHANBAKHT et al. (2002) showed that the sediment in water that had a Hg concentration of 0.050 mg /L adsorbed 0.6 μg/g in 30 days, and at the end of the experiment the sediment adsorbed more than 96% of all the Hg dissolved in the water.

The bioaccumulation of Hg determined in the present study was very below the limit of 0.5 μg Hg/g established by WHO (1991) as being acceptable for the consumption of fish. It should be pointed out, however, that in the present study, the fish were conditioned to low concentrations of this metal and mainly to a short exposure period. BOUDOU and RIBEYRE (1983) reported that fingerlings of *Salmo gairdneri* accumulated 0.57μg Hg/g in 30 days of exposure to 0.001 mgHg/L. On the other hand, rainbow trout (*Salmo trutta)* of 30 g accumulated 5.0 μg Hg /g when exposed to 0.150 mg Hg /L for 5 days (SKAK e BAATRUP, 1993). After 60 days of exposure *Pimephales promelas* accumulated mercury concentrations ranging from 0.80μgHg/g at the lowest water concentration (0.31μgHg/L) to 4.18μgHg/g at the highest concentration (4.51μgHg/L) (SNASRKI e OLSON, 1982).

The adsorption of mercury by the sediment accounted directly for the bioaccumulation in fish exposed to a concentration of 0.2 mg/L in the presence and absence of sediment. In the beakers containing sediment, there was partial adsorption of mercury from the water column, reducing therefore the bioaccumulation of this metal in fish. These results demonstrate that sediment, in the first instance, has a very important and efficient role in the adsorption of mercury. In the natural environment, this characteristic of sediment often accounts for the concentration of a pollutant being higher in the sediment than in the water column (BURTON and SCOTT, 1992). If the physical and chemical conditions of this compartment does not favor the methylation of Hg, the availability of this metal to biota remains low, where it is stored in the sediment until physical, chemical or biological factors cause the mercury to be bioavailable.

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