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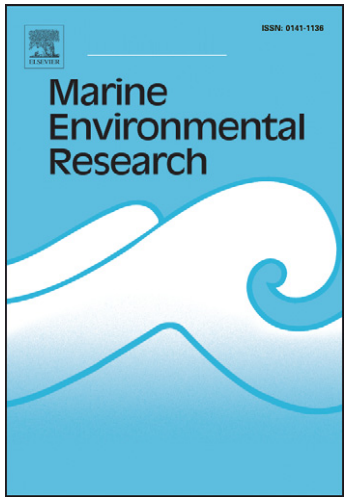

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Mercury speciation in sediments at a municipal sewage sludge marine disposal site

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Abstract

Mercury speciation was performed in excess activated sewage sludge (ASS) and in marine sediments collected at the AAS disposal site off the Mediterranean coast of Israel in order to characterize the spatial and vertical distribution of different mercury species and assess their environmental impact. Total Hg (HgT) concentrations ranged between 0.19-1003 ng/g at the polluted stations and 5.7-72.8 ng/g at the background station, while the average concentration in ASS was 1181±273 ng/g. Only at the polluted stations did HgT concentrations decrease exponentially with sediment depth, reaching background values at 16-20 cm, the vertical distribution resulting from mixing of natural sediment with ASS solids and bioturbation by large populations of polycheates.

Average Methyl Hg (MeHg) concentration in ASS was 39.7±7.1 ng/g, ca. 3% of the HgT concentration, while the background concentrations ranged between 0.1-0.61 ng/g. MeHg concentrations in surficial polluted sediments were 0.7-5.9 ng/g (ca. 0.5% of the HgT) and decreased vertically, similar to HgT. A positive correlation between MeHg and Hg only at the polluted stations, higher MeHg concentrations at the surface of the sediment and not below the redoxline, and no seasonality in the concentrations suggest that the MeHg originated from the ASS and not from *in situ* methylation.. By

doing selective extractions, we found that ca. 80% of the total Hg in ASS and polluted sediments was strongly bound to amorphous organo-sulfur and to inorganic sulfide species that are not bioavailable. The fractions with potential bioaccessible Hg had maximal concentrations in the range in which biotic effects should be expected. Therefore although no bioaccumulation was found in the biota in the area, the concentration in the polluted sediments are not negligible and should be carefully monitored.

Keywords: Mercury, Marine sediments, Sewage sludge, Bioavailability, Speciation, Eastern Mediterranean.

Introduction

Mercury is one of most studied metals in environmental and human health research. It has a very complex biogeochemical cycle, is bioaccumulative, can biomagnify along the food web and is toxic, impacting environment and man (Beckvar *et al.*, 1996; Benoit *et al.*, 1999a; Benoit *et al.*,1999b; Covelli *et al.*,1999; Hammerschmidt & Fitzgerald, 2004; Horvat, 1997; Manaham, 2003; Morel *et al.*,1998; Sunderland *et al.*, 2004; US EPA, 2000; Weber, 1993). Methylmercury (MeHg) is known to be the most toxic species of Hg and poses high risk to human health, mainly through the consumption of polluted fish (Baldi, 1997; Morel *et al*., 1998). Mercury methylation occurs mostly in anoxic sediments as a result of sulfate reducing bacteria (SRB) biosynthesis (Mason *et al.*, 1993; Morel *et al*., 1998). Abiotic methylation may also occur in the environment, especially mediated by humic organic matter (Weber, 1993). MeHg enters the food web by fast diffusion and strong bonding to sulfhydryl

groups present as part of biological molecules (Manaham, 2003). More than 90% of total Hg in muscle tissue of top marine predators is MeHg (Baldi, 1997).

Transformations among different mercury species can have a major effect on the metal's mobility and bioavailability, affecting it's potential for methylation and hence bioaccumulation (Benoit *et al*., 1999a; Benoit *et al*., 1999b; Bloom *et al.,* 2003; Hsu & Sedlak, 2003; Morel *et al*., 1998). Bioavailable species like $HgCl_2$ or polysulfide complexes (HgS_x) can efficiently penetrate efficiently through cellular membranes (Morel *et al*., 1998), while species like organo-mercury complexes or $HgS_{(S)}$ are not bioavailable (Benoit *et al*., 1999a; Hsu & Sedlak, 2003).

In Israel, mercury is introduced into the marine environment by disposal of activated sewage sludge (ASS). The ASS is produced at the Dan region wastewater project that treats the municipal sewage of ca. 2 million inhabitants (Kress *et al.*, 2004). Half of the mercury in the sewage can be traced to municipal households, dental clinics, drinking water supply system and industrial sources; especially caustic soda production. The source of the other half is unknown (Veber *et al.*, 2001). Since 1987, approximately 16,000 m³ of excess ASS are discharged daily to the marine environment at a disposal site located 5 km off the Israeli Mediterranean coast at ca. 38 m water depth (Fig.1). Monitoring studies at the disposal site found total Hg (HgT) concentrations up to 1.4 µg/g (dry wt.) in the sediments (Kress *et al.*, 2004), much higher than the normal Hg concentration found along the Mediterranean coast of Israel (0.05 µg/g).

Until now, only HgT concentrations in surface sediments were measured. No data exist on the vertical distribution of Hg concentrations in the sediments nor on the Hg species present at the disposal site, so it was impossible to estimate its environmental impact. Thus, the objective of this research was to identify the mercury species present in the ASS and in the sediments at the disposal site, characterize their vertical distribution, and assess, based on the results, their environmental impact.

Materials and Methods

Study area

Four sampling stations, from the outfall and northwards were chosen: Station 0 at the outfall, station 3 (200 m from the outfall), station 21 (1,500 m from the outfall) and station 29 (5,500 m from the outfall) (Fig. 1). It is known that there is a preferential northwards dispersion of the ASS in agreement with the currents in the area (Kress *et al.*, 2004), therefore the stations represent a gradient of decreasing ASS influence. Stations 0, 3, 21 will be referred to as polluted stations and station 29, not affected by the ASS, as the background station.

Sampling

The stations were sampled 6 times between 1999 and 2003 (August 1999, June 2000, August 2001, May 2002, October 2002 and September 2003) on board the Research Vessel "Shikmona". During each survey, seawater, suspended particulate matter (SPM) and sediment cores for Hg analysis and speciation were sampled. Seawater for SPM was sampled by a membrane pump ("FLOWJET") into clean plastic containers. Seawater samples from the box-corer were sampled as well into polypropylene sterile

tubes ("SARSTEDT"). Sediments were sampled by a 0.062 m^2 box-corer with an effective penetration of 40 cm (Ocean Instruments model 700 AL). Sub cores were taken from the box corer with hollow Perspex cylinders. The sub cores were sliced on board to 0.5-3 cm wide slices which were placed in clean plastic containers, frozen until chemical analysis in the laboratory.

Samples for HgT determination in seawater were preserved immediately upon sampling by oxidation with 0.1N BrCl (US EPA Method 1631, 2002). Seawater samples for HgT determination in SPM were filtered on board through a 0.45 µm pre-weighted membrane filters (Herut & Kress, 1997).

Activated sewage sludge (ASS) was sampled prior to its disposal at sea at the wastewater treatment plant into acid cleaned plastic containers. The ASS was freeze-dried for 48 hours after centrifugation (3500 RPM for 20 min) and the solid deposit was separated. The dry ASS was homogenized and kept in clean dry plastic containers until analysis of Hg species. In addition, during 2002-2003, routine analysis of HgT in ASS was performed on a monthly basis (n=151). In this case, the wet ASS was acidified to pH 2 with HCl and kept refrigerated for up to a week until analysis of HgT. Water content was determined by drying in sub-samples.

Laboratory analyses

Water and Organic carbon content in the sediments and ASS: Water content was calculated after drying at 105^0C overnight (SM-2540 B). Organic carbon was determined by potentiometric titration after digestion with potassium dichromate (Avnimelech ,1989; Gaudette *et al.*,1974).

Hg Speciation: in the laboratory, the frozen sediment samples were lyophilized for 48 hours and then dry sieved through a 1000 µm sieve to extrude extraneous components such as seeds, broken shells, etc. Hg speciation was performed on dry sediment and dry ASS at least in duplicates. Speciation included measurements of HgT, MeHg and ethyl Hg, and Hg fractionation as measured by sequential selective extractions and by pyrolysis.

HgT in seawater and wet ASS was measured by oxidation and cold vapor atomic fluorescence spectrometry (US EPA Method 1631). Dry sediments and ASS were analyzed after digestion with aqua regia for 1 hour at 160^0C (Bloom, Preus *et al.*, 2003; PSA Application 013). SPM filters were dried and digested with concentrated HNO_3 at 140^0C in stainless steel Teflon-lined pressure decomposition vessels (Herut & Kress, 1997). Hg analyses were performed by cold vapor atomic fluorescence spectrometry (CVAFS) with a Merlin Millenium system (PS Analytical, UK), after $SnCl_2$ reduction and purging with high purity argon. Quality control and quality assurance of the results was performed with standard reference materials from the US National Institute of Standards and Technology (NIST 2781), the National Research Council of Canada (NRCC-MESS-2), and the International Atomic Energy Agency (IAEA-405). The standard reference materials were digested and analyzed in the same manner as the samples, with each analytical run. The results were within 5% of the certified values.

MeHg was extracted from the sediment or the ASS as a halide salt with dichloromethane, followed by a cleanup procedure involving the formation of a water soluble adduct, its extraction into an aqueous phase which was separated and oxidized

by BrCl for Hg measurement (Cai, *et al.*, 1997; Longbottom *et al.*, 1973; Sakamoto *et al.*, 1992). At the early stages of this work both MeHg and Ethyl Hg were measured by HPLC after solvent extraction (Cai *et al.*, 1997; Hintelmann & Wilken, 1993; PS Analytical, application 10.025, 2001; Wu, 1991). However, because no Ethyl Hg was detected in any of the samples, all organic Hg was assumed to be MeHg. Therefore, the separation step by HPLC was skipped and the aqueous extract was oxidized with BrCl and Hg measured as in the seawater samples.

Selective Extractions: Hg in different biogeochemical fractions was measured by a five-step sequential extraction (Bloom *et al.*, 2003). This method differentiates among different Hg species based on their biogeochemical behaviors and includes the following fractions: water-soluble species (F1) leached with deionized water; 'human stomach acid' soluble species (F2) leached with acetic acid; organo-chelated species (F3) leached with 1N KOH ; strong-complexed species (F4) leached with 12N HNO_3 (elemental and/or Hg bound up in amorphous organo-sulfur, Hg-Ag amalgams, or crystalline Fe/Mn oxide phases); and mercuric-sulfide (F5) leached with aqua-regia. After each step Hg was measured as HgT in the leaching media by CVAFS as described above. For quality control and determination of method recovery, each sample was analyzed for HgT as well. In all cases there were no significant differences ($p<0.05$) between measured HgT and the sum of Hg concentrations in all fractions. Selective extractions were performed in duplicate samples from sediment cores sampled in May 2002, October 2002, and September 2003. Speciation was performed also in the following certified reference materials (CRM): domestic sludge (NIST 2781) and estuarine sediment (IAEA-405).

Pyrolysis: Pyrolitic Hg analyses were performed in 16 representative samples by Dr. Harald Biester (Institute of Environmental Geochemisty, University of Heidelberg, Germany) by the method described in Biester & Scholz, 1997 and; Biester *et al.*, 2000. The purpose of the analyses was to identify qualitatively the presence or absence of some Hg species in the samples.

Results and Discussion

Water and Organic Carbon content in the sediments

Water content in the sediments is a simple but effective parameter to identify ASS presence, since ASS is essentially a liquid (99% water content) that coagulates and sinks to the bottom following contact with seawater (Hunt, 1990). The average water content in the sediments at the background station was 33.3% ± 8% from the surface and down to 25 cm depth. At the polluted stations maximal water content (70-80%) was observed at the surface, and decreased gradually to background values at 5-10 cm depth (Fig.2). The content of water at the upper layer of the polluted stations was higher in fall. A similar pattern was observed for organic carbon concentrations: homogeneous vertical profile at the background station (0.4-0.95%), in contrast to a decreasing gradient at the polluted stations with maximal values at the surface (6%) down to background concentration at a depth of 10-20 cm (Fig.2). Also considering that the concentration of organic carbon in the ASS was 31.4% ± 6.6% (n=4), these results indicate mixing between sediment and ASS down to 10-20 cm at the polluted stations. In the spring the fraction of the ASS in the mixed layer was smaller than in the fall. This is in agreement with the fact that ASS accumulates from spring to fall at the site while winter storms resuspend and disperse the ASS (Kress *et al.*, 2004).

Total Hg (HgT)

HgT in SPM from the polluted stations was in the range of 206-1621 ng/g. This range corresponds to 0.2-6.1 ng/L in seawater with an average concentration of 4.5 mg/L SPM. The concentrations of HgT in the seawater from just above the sediment surface were up to 100 ng/L. The concentrations of HgT in the seawater at the ASS plume were 22.0 ± 17.6 ng/L (Shoam-Frider, 2005) These concentrations are very low compared to those measured in the ASS and sediments.

Average HgT concentration in the ASS was 1181±273 ng/g, dry weight (*n*=151). In the sediments, concentrations ranged between 0.19-1003 ng/g at the polluted stations and 5.7-72.8 ng/g at the background station, with averages of 263±239 ng/g (*n*=132) and 24.8±12.1 ng/g (*n*=51), respectively. Table 1 presents the average (6 surveys) vertical distribution of HgT in the sediments down to 30 cm depth. At the background station, HgT concentration was essentially constant at all depths, at background levels (22.5 ng/g, Table 1). At the polluted stations, the depth distribution of HgT in the sediments showed an exponential decrease with depth (Fig.3), reaching background levels at 16-20 cm depth, and was similar to the depth distribution of organic carbon. The concentrations decreased probably due to natural mixing processes of sediment with ASS solids deposited at the site. Moreover, large populations of polycheates (3,000-10,000 specimens/ 0.0124 m^3) are usually observed in the spring at the polluted stations (Kress *et al.*, 2004), therefore it is reasonable to assume that bioturbation also plays a major role in the mixing. It is known that oligochaetes and other epifaunal species are responsible for the active sediment mixing down to about 10 cm (Thibodeaux & Bierman, 2003).

It can be seen (Fig.3) that the vertical decrease of HgT concentrations with sediment depth was similar at stations 0 and 3 and different at station 21 which had a more moderate decrease. For instance, at 10 cm depth, the HgT concentrations were ca.100 ppb at stations 0 and 3 while ca.300 ppb at station 21. This shows that a higher accumulation of HgT occurs at station 21, located 1500 m northwards from the outfall, than at stations 0 and 3, located closer to the outfall (Fig.1). The maximal accumulation of Hg was found at station 21 and not at the outfall, in agreement with dispersion models (Hunt, 1990) and with the average northward current along the Israeli shore with velocities between 15 to 90cm s^{-1} (Rosentraub, 1990). The accumulation decreased from station 21 northwards and at station 29 (the background, located 5.5 km northwards from the outfall) the Hg concentrations reached natural values.

No seasonal differences in HgT concentrations were observed ($p<0.05$) at the polluted stations, even though it is known that during winters the sewage sludge is dispersed from the area and it re-accumulates from spring to winter (Kress *et al.*, 2004). Lower HgT concentrations were measured in the winter, compared to spring and fall. It is not clear which mechanism causes some of the Hg to remain in the area. One possible explanation could be the retention of colloidal phase Hg bound to organo-sulfur in pore waters in the area while Hg associated with solid particles is dispersed (Guentzel *et al.*, 1996; Sunderland *et al.*, 2006).

Methyl Hg (MeHg)

Average MeHg concentration in the ASS was 39.68±7.06 ng/g (dry wt., *n*=4) and it constituted about 3% of the HgT present in the ASS. MeHg in sewage sludge might

originate from biotic and/or abiotic methylation processes. Biotic methylation is a bacterial process that involves the growth factor B_{12}, which is the main substrate for MeHg synthesis (Baldi, 1997). It is known that the B_{12} is produced by a wide range of aerobic and anaerobic bacteria; nearly all are present in sewage sludge (Metcalf & Eddy, 2003). Abiotic methylation of Hg involves organic humic matter, which is present in the aquatic environments and in sewage (Weber, 1993).

At the polluted sediments the MeHg constituted an average 0.46% of HgT. MeHg concentrations in the surface of the sediments ranged between 0.7-5.90 ng/g with no seasonal differences, and changed with sediment depth a pattern similar to HgT (Fig. 4). A positive correlation was found between MeHg and HgT (Fig. 5). On the other hand, at the background station MeHg concentrations (0.09-0.61 ng/g) were higher in fall than in spring, and constituted 2.5% and 0.23% of HgT, respectively. No significant correlation between MeHg and HgT was found at the background stations.

Several findings in this study suggest that MeHg found in the sediments of the polluted stations originated from the ASS and not from *in situ* methylation: a positive correlation between MeHg and HgT, higher MeHg concentrations at the surface of the sediment and not below the redoxline, and the lack seasonality of the concentrations. Past studies show that MeHg concentrations are dependent mostly on environmental factors (i.e. temperature, redox potential, organic carbon, sulfide) Kelly *et al.*, 1995), however, positive correlations between HgT and MeHg concentrations in sediments were found in other marine areas where current and historical pollution by Hg was documented (Hammerschmidt & Fitzgerald, 2004, Sunderland *et al.*, 2004; Sunderland *et al.*, 2006). In this study, a positive correlation between MeHg and HgT

was found only at the polluted stations and not at the background station. Furthermore, the relation between HgT and MeHg was more significant at station 0 (R^2=0.76, p<0.0001, Fig. 5) positioned at the outfall itself, where HgT concentration and accumulation of sludge were not maximal, but the sewage sludge there is "fresh". It is known that methylation is mediated by SRB and that MeHg will occur below the redoxline where the sediments become reduced (Davis *et al.*, 1997; Gagnon *et al.*, 1996). In this study, maximal MeHg concentrations were found at the surface of the sediments of the polluted stations. Moreover, there were no seasonal differences in MeHg concentrations at the polluted stations in contrast to other studies (Bloom *et al.*, 1999; Covelli *et al*., 1999; Leermakers *et al.*, 2001). At the background station (St.29), MeHg concentrations were significantly higher in fall than in spring while HgT was essentially constant. The higher concentrations of MeHg in the fall can be the result of higher natural methylation when water temperatures are higher (Hammerschmidt & Fitzgerald, 2004; Leermakers *et al*., 2001).

Hg in biogeochemical fractions

Hg concentrations in the different biogeochemical fractions of polluted sediments, background sediment and ASS are presented in Tables 2-3 and Figure 6. Pyrolysis results showed that Hg was released mostly in the temperature range of 230-280^{0}C, typical to Hg associated with humic acids (organic fraction). In a few cases, Hg was released at 300-340^{0}C, typical of Hg release from cinnabar. None of the polluted sediment samples contained Hg^0 which is typically released at a temperature of 100^{0}C (Biester & Scholz, 1997; Biester *et al*., 2000).

The selective extractions results showed that in all samples 99% of the Hg was located at fractions F3-F5 (Fig. 6, Table 2); the organo-chelated species (F3), the strong-complexed species (F4) and the mercuric-sulfide (F5). However, there were differences among the relative contributions of the F3 and F4 fractions in the ASS, the background station and the polluted stations (Table 3). The relative content of F3 was the highest at the background station, followed by the ASS and the polluted stations. The F4 relative content was the highest at the polluted stations, followed by the ASS and the lowest at the background station. Dominance of the F4 fraction was also found in marine sediments from the bay of Trieste with HgT concentration of 132 ppm present mostly as mercury sulfides (Kim *et al.*, 2003). There were no significant differences in the F5 content between the background and the polluted stations. The relative distribution of Hg in ASS was very similar to that found in sludge CRM while relative distribution in the background station was very similar to estuarine sediment CRM and to that found in natural sediments elsewhere (Bloom *et al.*, 2003). The relative distribution of the F3-F5 fractions in polluted stations had a high similarity to that at the ASS and differed significantly from the background, indicating the ASS as the source of the Hg in the area.

The F3 fraction in environmental samples is known to contain mainly Hg associated with humic organic matter and with living and dead biota (Bloom *et al.*, 2003). The F4 fraction, which contained more than 70% of the HgT at the polluted stations and the ASS, may include Hg^0, Hg bound to amorphous organo-sulfur, Hg-Ag amalgams, or crystalline Fe/Mn oxide phases (Bloom *et al.*, 2003; Revis *et al.*, 1989). Pyrolysis of representative samples in our study found that most of the Hg was bonded to humic acids and no presence of Hg^0 was detected. These findings suggest that the Hg in the

F4 fraction is bonded to humic matter through organic S functional groups that are stronger and more stable than other functional groups like carboxylic or oxide groups (Allard & Arsenie, 1991; Mierle & Ingram, 1991; Weber, 1993). Recent advances in spectroscopic techniques and indirect evidence suggest strong interactions between Hg and dissolved organic matter, most likely through covalent bonding at thiol-type functional groups in organic matter (Ravichandran, 2004). Evaluation of the bond-strength between Hg and humic and fulvic acids found a range of values which can be explained only if complexation involves reduced RSH groups (Amirbahman *et al.*, 2002; Hintelmann *et al.*,, 1997; Karlsson & Skyllberg, 2003). In addition, several studies (Di Giulio & Ryan, 1987; Dyrssen & Wedborg, 1991; Hsu & Sedlak, 2003; Laurier *et al.*, 2003; Ravichandran *et al.*, 1998; Ravichandram, 2004) have showed that Hg, present in organic-rich matrixes like sludge or polluted sediments, forms strong complexes with S-containinig organic ligands, and these complexes are stable, inert and unavailable to chemical and biological transformations, like methylation. Therefore, it is reasonable to assume that Hg bonded to non-RSH functional groups in humic acids would be released more easily and be present in the F3 fraction, while the stronger S-bonded Hg in humic acids would be present in the F4 fraction. It is hypothesized that the Hg present in the F4 fraction, in the ASS and polluted sediments (more than 75%), is not readily bioavailable.

Fluctuations with no clear tendencies were observed in the vertical distribution of the relative Hg content in the F3-F5 fractions in the sediments from the polluted stations (Fig. 7). Only the relative Hg content in the F1 fraction (the water-soluble, mobile, bioavailable Hg species) increased significantly with increased depth of the sediment (Fig. 7; $n=39$, $p<0.0001$) but the concentrations remained very low. A weak but

significant negative correlation was found as well between the F1 and F5 fractions (Fig.8) and when a multi variable regression was applied ($n=38$, $p<0.05$, not presented), a significant negative relation was found between the F1 fraction and the other three fractions together (F3+F4+F5). That leads us to assume that a small portion of the Hg released from the other fractions, and especially the F5 fraction remains as water-soluble species in the sediment. When the relationships between the fractions were tested by linear regression, a negative correlation was also observed between F5-F4 and F4-F3 (Fig. 8). No correlation was observed between F5 and F3. These relationships showed that Hg could be exchanged between F3-F4, F4-F5, and F1-F3, F4, F5. This dynamics is in agreement with the differences in the relative content of the fractions found among the different samples: the content of F3 decreased in the polluted sediments compared to the AAS while F4 increased. It seems that at sea the organic fraction (F3) decomposes and Hg is re-adsorbed mostly by the F4 fraction.

Bioavailability and environmental impact

Speciation is the key to understanding mercury behavior and to assess it's accessibility, bioaccumulation and toxicity. Speciation determinations were performed in ASS and sediments. Although no speciation test was performed in seawater, the HgT concentrations in the seawater (maximum 0.1 µg/L) were lower than the Israeli guideline of 0.16 µg/L for Mediterranean seawater and lower than the US EPA criteria (0.9 and 1.8 µg/L for continuous and maximum concentrations, respectively) (US EPA, 2002). Therefore, Hg in seawater is not expected to be detrimental to the environment.

Mercury speciation in ASS and sediments at the marine outfall showed that most of the Hg (ca. 80%) was strongly bound to amorphous organo-sulfur (F4) and to sulfide (F5). This Hg is neither available nor accessible directly to the biota, nor is it available to methylation processes (Bloom *et al*., 2003). The F1-F2 fractions that contained the bioaccessible Hg in the polluted sediments and the F3 fraction that can decompose and release accessible Hg to the environment contained 0.1-2.4% and 20-24% of the HgT, with maximal concentrations of 10 ng/g and 227 ng/g, respectively. Long *et al*. (1995) suggested marine sediment guidelines, based on the potential to induce toxic effects in marine organisms: ERL (Effects Range Low) and ERM (Effects Range Median) that for HgT are 150 and 710 ng/g, respectively. These guidelines were adopted by many countries and serve as a basis in risk assessment. In this study, the maximal concentration of Hg in the bioaccessible fractions, F1-F3, of 240 ng/g was between the ERL-ERM, therefore biotic effects should be expected and the concentration in the polluted sediments are not negligible.

MeHg is the species that is taken up by marine organisms, bioaccumulates and biomagnifies. This study suggests that the Hg in F3 was not available to methylation in contrast to other studies (Bloom *et al*., 2003), and that the ASS was the source of MeHg found in the polluted sediments. An anoxic incubation of polluted sediments from the study area with ASS in seawater showed no significant changes in MeHg concentrations from 6 weeks to 5 months (Shoam-Frider, 2005).

The low concentrations of MeHg in the polluted sediments (0.7-5.90 ng/g) and the assumption of a lack of *in-situ* methylation, are in agreement with the fact that until

today no accumulation of mercury was found in marine biota collected at the ASS marine outfall area (Kress, Galil & Herut, 2003). The concentrations (<5-100 ng/g wet weight) were similar to those found in the same species at a control areas. Assuming that all of the Hg is in the form of MeHg, it is still much lower than the strict criterion of 300 ng/g MeHg fish tissue (wet wt.) (US EPA, 2001), indicating no Hg threat to human health due to the consumption of fish from the area. Nevertheless, the bioavailable MeHg reaching the marine environment directly with the ASS and the concentrations found at the bioaccessible fractions might pose an environmental risk and should be carefully monitored.

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Table 1: Concentrations of HgT (ng/g, dry wt.) in sediment cores from the polluted (0, 3, 21) and background (29) stations.

	HgT ng/g dry weight											
Depth	**Station 0**			**Station 3**			**Station 21**			**Station 29**		
cm	*n*	Average	STD	*n*	Average	STD	*n*	Average	STD	*n*	Average	STD
0-2	9	451	279	9	516	212	13	552	218	12	31.7	17.2
2-4	6	295	195	8	447	240	8	448	141	8	28.3	6.8
4-6	5	227	125	5	257	181	6	366	221	5	21.9	5.3
6-8	5	225	163	4	120	119	6	248	96	4	19.9	8.3
8-10	4	107	68.1	4	84.4	102	6	269	182	4	16.5	9.1
10-12	5	88.3	50.9	3	44.3	35.9	5	176	109	4	21.1	10.4
12-14	3	53.0	21.8	3	12.1	4.06	4	140	122	2	27.0	9.3
14-16	3	66.6	106	3	8.32	5.83	5	141	89.9	3	22.2	13.8
16-18	4	22.9	22.2	1	16.7		5	97.5	101	3	17.1	10.7
18-20	2	3.20	4.25	2	11.6	2.12	3	31.2	23.7	2	19.0	3.7
20-30	6	15.2	14.0				8	33.9	44.8	7	23.3	12.9

Table 2: Average and standard deviation of Hg concentrations (ng/g, dry wt.) and relative content (%) calculated from HgT at the F1-F5 fractions in the sediment cores from the polluted stations, activated sewage sludge (ASS) and background sediments (*n*=number of samples).

		F-1		F-2		F-3		F-4		F-5	
n	Depth (cm)	**Hg ng/g**	**%**	**Hg ng/g**	**%**	**Hg ng/g**	**%**	**Hg ng/g**	**%**	**Hg ng/g**	**%**
15	0-2	0.32±0.6	0.1±0.1	0.13±0.3	0.03±0.07	52.5±30.4	15.4±6.6	344±330	75.5±9.8	37.3±35.7	9.0±8.8
13	2-4	0.07±0.1	0.02±0.05	0.11±0.2	0.02±0.05	55.7±45	15.6±9.8	253±89	75.2±8.8	30.5±23.2	9.1±7.2
12	4-6	0.62±0.7	0.3±0.3	0.26±0.5	0.07±0.13	61.3±55	17.4±10	260±240	71.9±9.6	29.3±20.1	10.4±9.1
14	6-8	0.68±0.7	0.5±0.9	0.06±0.1	0.05±0.11	48.3±75.5	16.1±9.5	203±208	72.4±12.5	33.4±45.8	10.9±12.9
14	8-10	1.23±2.3	0.7±1.1	0.05±0.1	0.06±0.10	31±30.5	13.9±5.6	180±206	73.9±10.1	53.3±104	11.5±13.4
8	10-12	0.32±0.53	0.3±0.6	0.04±0.07	0.05±0.10	7.33±6.48	14.0±12.1	66.3±46.3	79.8±14.1	3.72±5.7	5.8±5.6
6	12-14	1.09±1.23	2.9±3.2	0.05±0.08	0.57±0.66	7.62±6.1	11±3.3	75.2±75.2	83±3.6	3.96±5.6	2.8±2.6
6	14-16	0.67±0.64	1.3±1.3	0.1±0.09	0.31±0.37	7.59±6.83	11.1±4.1	50.6±30.2	85.8±3.1	0.93±1.5	1.5±2.4
6	16-18	0.92±0.66	1.4±0.2	0.1±0.08	0.53±0.69	7.88±6.79	12.3±6.2	57.1±38.9	83.4±4.8	2.24±3.45	2.5±3.9
8	18-22	0.64±0.37	3.4±1.3	0.07±0.08	0.36±0.40	2.94±1.03	17.4±5.1	13.4±6.7	70.9±8.4	1.22±1.05	7.9±7.1
5	ASS	0.46±0.7	0.03±0.05	11.5±14.7	1.0±1.3	271±55	21.7±2.8	867±48	70.3±3.9	87.8±44.7	6.9±3.1
10	Background	0.13±021	0.63±0.92	0.06±0.06	0.44±0.62	9.3±5	50.5±16.8	8.28±5.59	43.7±16.2	1.43±2.94	4.8±7.1

Table 3: Comparison among the relative distributions of Hg (%) in fractions F3-F5, at the polluted stations (P.S), the ASS and the background station (B.S).

Matrix	**F3**	**F4**	**F5**
P.S	14.9 ± 7.9	75.9 ± 10.3	8.2 ± 9.3
ASS	21.7 ± 2.8	70.3 ± 3.9	6.9 ± 3.2
B.S	50.5 ± 16.8	43.7 ± 16.2	4.8 ± 7.1
	B.S>ASS>P.S	P.S>ASS>B.S	B.S=P.S=ASS

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Fig. 7: Vertical distribution of relative Hg content (%) in each fraction, at the polluted stations.

Fig. 8: Linear regression between relative Hg content (%) in the different fractions (n=50) at the polluted stations. Note different y axis for F1 vs. F5.

Figure 1: Area of study and location of the sampling stations at the marine sewage sludge outfall.

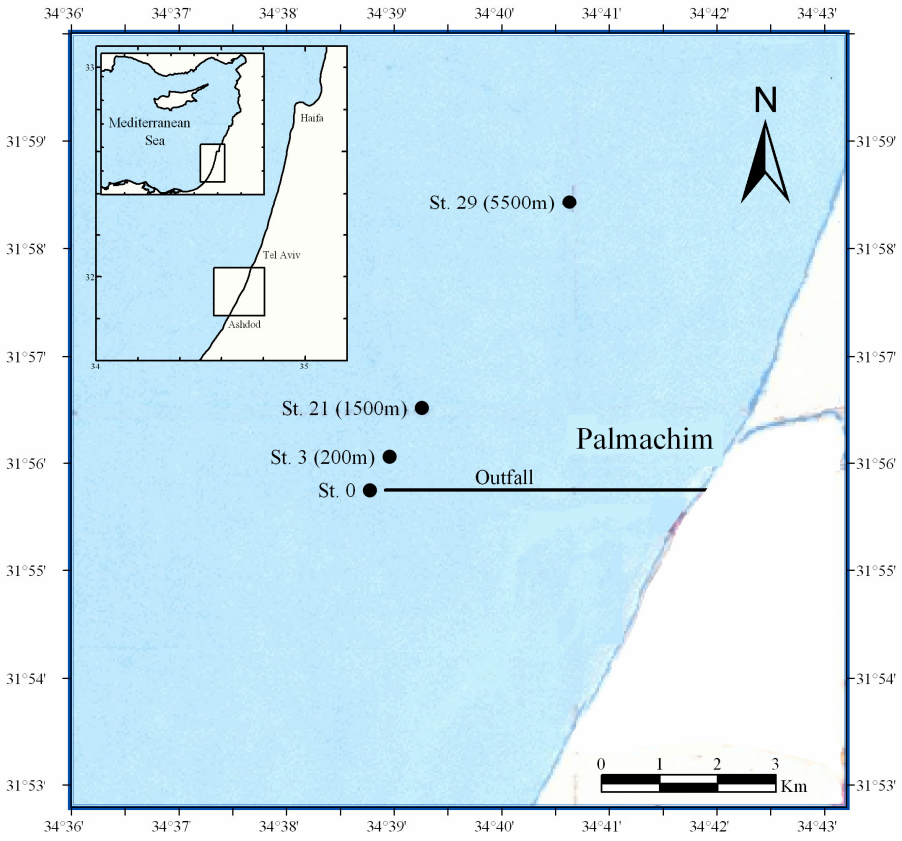


Fig.2: Water and organic carbon content in the sediments from the polluted stations (0, 3, 21) and the background station (29), at representative surveys of spring (May) and fall (August-October) conditions.

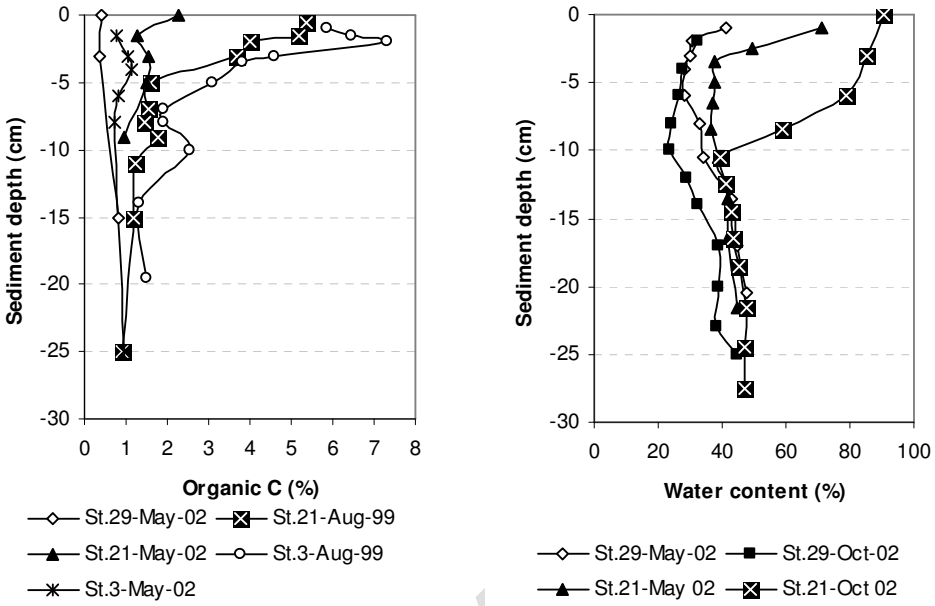


Fig.3 HgT concentrations in sediment cores at the polluted stations (0, 3, 21) and background station (29), in all surveys. Note different X scale at station 29.

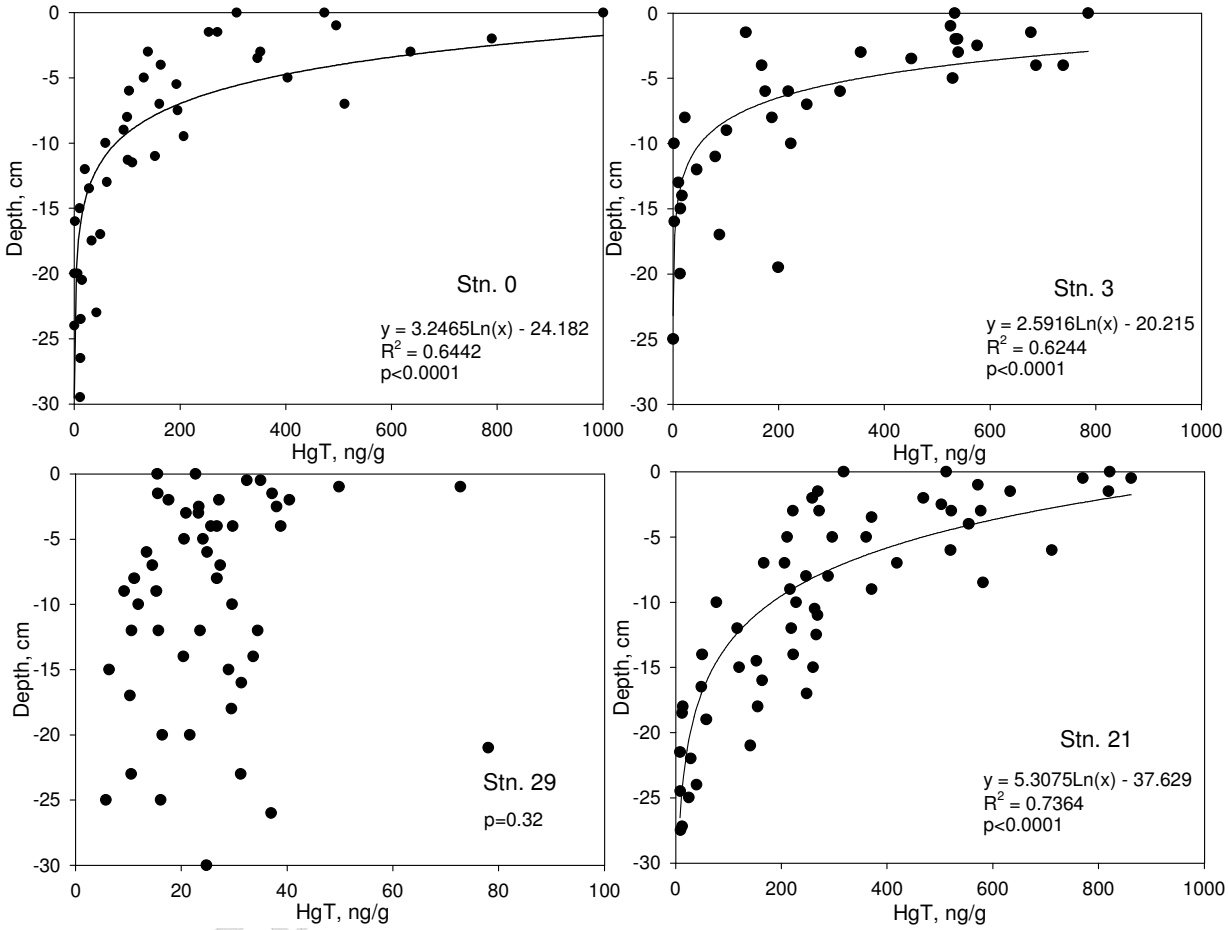


Fig. 4: Vertical distribution of average MeHg concentrations in sediments from the polluted stations (0, 21) and the background station (29) (May-02, October-02 and September-03).

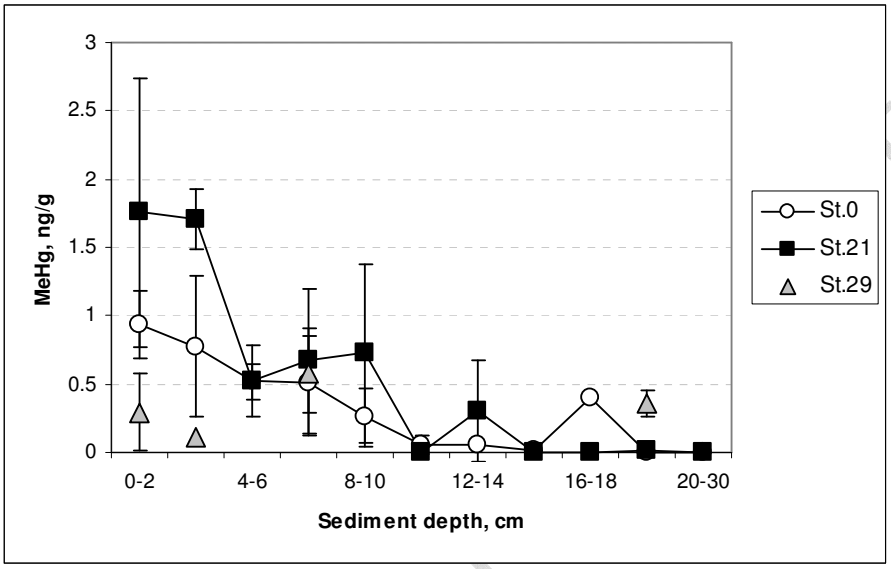
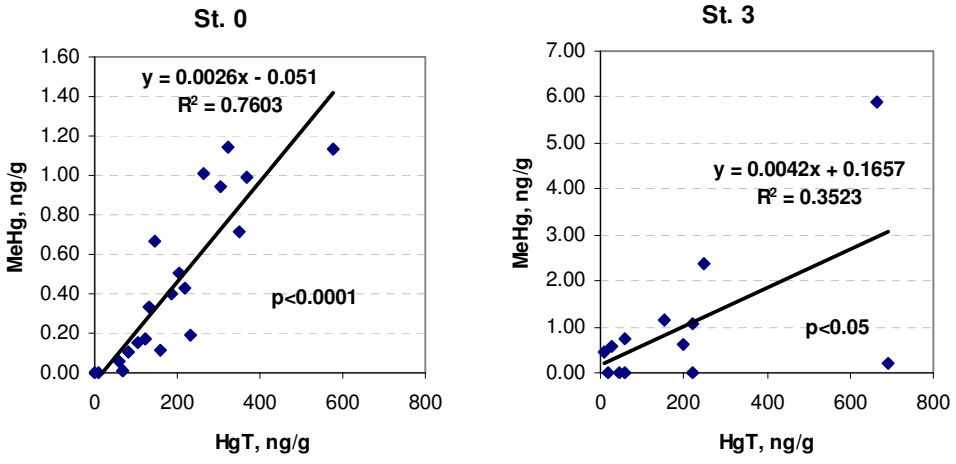


Fig: 5: Linear regression between MeHg and HgT (ng/g dry wt.) in sediment cores from the polluted stations 0 (n=21), 3 (n=13), 21 (n=21) and the background station 29 (n=6), from all surveys. Note different Y axis scales.



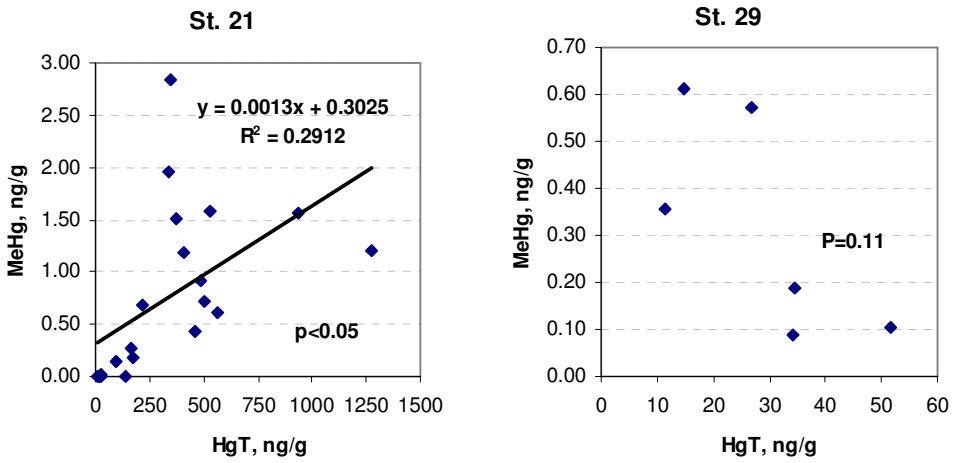


Fig.6: Hg distribution among F1-F5 fractions at the background (BG) station, the polluted stations, activated sewage sludge (ASS) and 2 certified reference material: NIST-2781=Domestic Sludge, and IAEA-405=Estuarine sediment (n=number of samples; at the polluted stations the average of all results is presented).

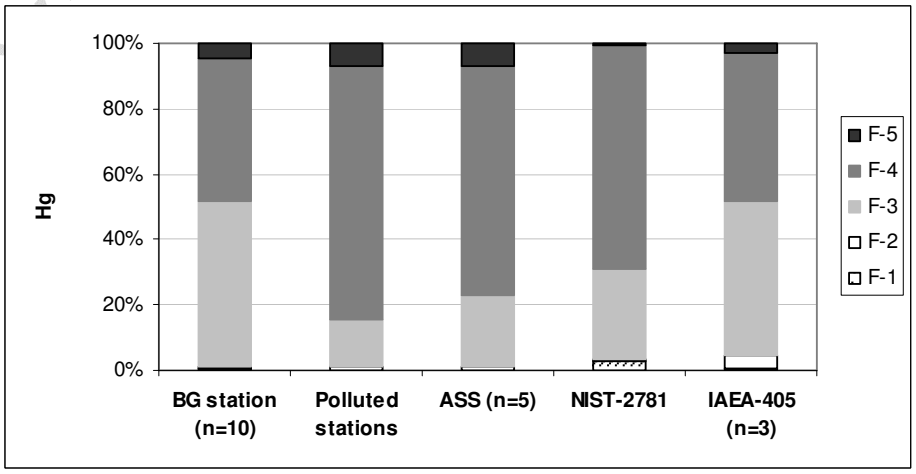


Fig. 7: Vertical distribution of relative Hg content (%) in each fraction, at the polluted stations.

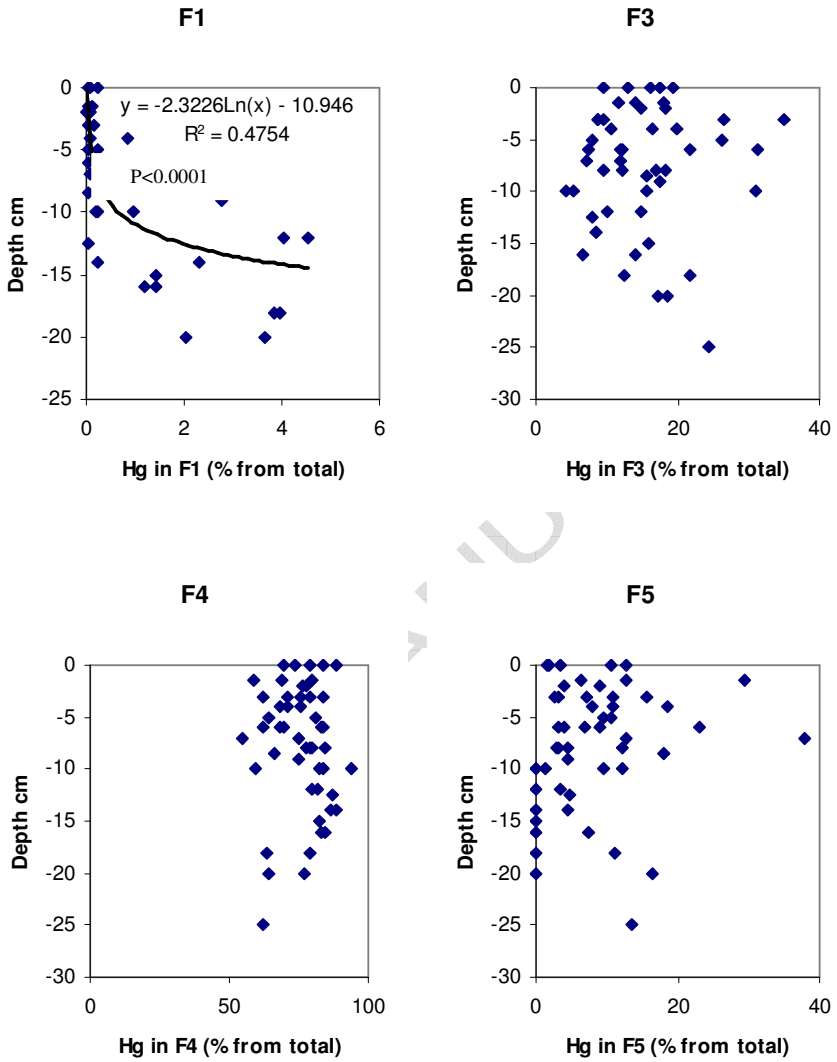


Fig. 8: Linear regression between relative Hg content (%) in the different fractions (n=50) at the polluted stations. Note different Y axis for F1 vs. F5.