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Relationships of Eutrophication to the Distribution of Mercury and to the Potential for Methylmercury Production in the Peat Soils of the Everglades†

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Elevated mercury concentrations have been reported in fish and wildlife from the Everglades in recent years. The hypothesis that eutrophication caused by the impact of phosphorous- (P) rich agricultural runoff stimulated methylmercury accumulation was put forward because eutrophication had been shown to be a cause for methylmercury accumulation in other ecosystems. We tested this hypothesis by obtaining total mercury (Hg_T) concentrations and accumulation rates and estimating the potential for microbial methylation and methylmercury degradation in peat soils collected along a P gradient in water conservation area 2A (WCA-2A). A negative correlation observed between Hg_T and P concentrations in soils ($r^2 = 0.64$) was explained by increased peat accretion rates in a nutrient-enriched area (7.1–7.5 mm yr^{-1}) as compared to an unenriched area (1.92–2.50 mm yr^{-1}), estimated using lead-210 and cesium-137 dating. Total Hg accumulation rates (post-1964) were comparable for the enriched and unenriched sites (29–30 and 29–37 $\mu g\ m^{-2}\ yr^{-1}$, respectively). Thus, calculations of Hg_T accumulation rates are confounded by differences in peat accretion rates in the Everglades. Potential rates for both methylation (2.3–48.6 ng g^{-1} day^{-1}) and demethylation (6.5–113.2 ng g^{-1} day^{-1}) were higher in samples from WCA-2A than in samples collected in an area of the Everglades that had never been exposed to nutrients. However, trends suggesting the relationships of these activities to the P gradient in WCA-2A were not detected, and methylation to demethylation ratios did not correlate with soil P concentrations. The results suggest that (i) nutrient-enriched agricultural runoff originating upstream of WCA-2A did not contribute significantly to Hg_T built up in the northern Everglades and (ii) eutrophication did not affect the potential for net methylmercury formation in peat soils.

Introduction

The existence of a mercury (Hg) contamination problem in south Florida first surfaced in the late 1980s with reports of elevated mercury concentrations in fish ($\geq 0.5\ \mu g\ g^{-1}$) and other wildlife (*1*). Although the highest levels of mercury were measured in fish from the Everglades ecosystem, this problem is widespread throughout the state with concentrations ranging from 0.04 to 1.33 $\mu g\ g^{-1}$ (muscle tissue) in largemouth bass (*2*). This contamination prompted the State public health authorities to post advisories limiting or banning consumption of edible freshwater fish in Everglades and Big Cypress National Preserve and has possibly adversely affected this region's wildlife. Alterations in reproductive patterns (*3*) and poisoning (*4*) in wading birds and the death of at least one Florida panther (*Felis concolor coryi; 5*) have been attributed to mercury contamination.

The sources and processes responsible for this contamination are at present unknown. Increased atmospheric deposition of global and local origins and release of Hg stored in rapidly oxidized peat deposits in the intensely cultivated Everglades Agricultural Area (EAA) (*6*) are currently being investigated as possible sources for mercury in the Everglades ecosystem. Another plausible cause is the alteration of processes that control the amount of Hg that is available for accumulation by aquatic biota to favor formation of methylmercury (MeHg). The in situ synthesis and degradation of MeHg are a key issue in any Hg contamination problem because Hg in freshwater fish occurs almost exclusively in the methylated form (*7, 8*), but fish by themselves cannot methylate mercury (*9, 10*). Microbial transformations are largely responsible for the methylation of ionic mercury (Hg[II]) (*11*) and degradation of MeHg (*12*), but chemical methylation reactions are also known (*13*). Changes in the ecosystem that directly or indirectly increase methylation or decrease MeHg degradation may result in an elevated concentration of MeHg, its biomagnification in the aquatic food web, and a public health problem. Lake acidification due to acid deposition (*14, 15*) and increased oxidation of organic matter in newly flooded hydroelectric reservoirs (*16*) are examples where ecosystem alterations may result in increased MeHg production.

Historically the Everglades evolved under nutrient-limited conditions, specifically phosphorus (P; *17*). During the last century, large areas of the northern and eastern

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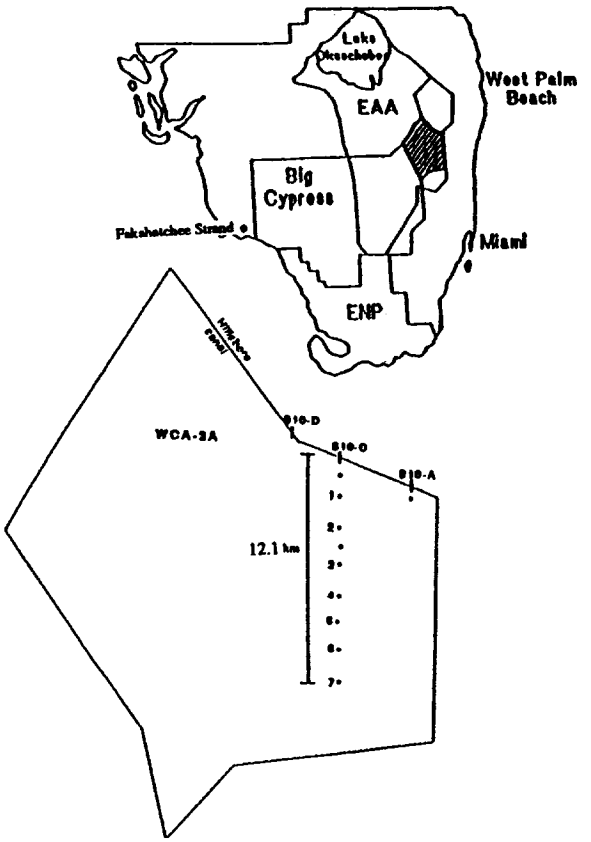

FIGURE 1. Map of water conservation area 2A (hatched area) of the northern Everglades showing the sampling locations (1•–7• denotes C1–C7). EAA, Everglades Agricultural Area; ENP, Everglades National Park.

Everglades have been drained for agricultural or urban land uses. The central Everglades (Figure 1) is fragmented into several units where the hydrology is regulated by pumps and canals (EAA, water conservation areas [WCA-1, WCA-2, and WCA-3], and Everglades National Park [ENP]). Nutrient-enriched agricultural drainage from EAA has been pumped southward since the 1960s resulting in eutrophication characterized by high concentrations of P in soil and surface water in some sections of the Everglades (*18*). Eutrophication is likely to affect net MeHg production by the following (i) increased microbial activities due to input of PO_4 (*19*), (ii) increased rates of nutrient accumulation (*20*) and labile organic matter production in soil (*20*), (iii) an increased soil anaerobiosis (*20*), and (iv) increased concentration of fulvic acid in the water column (*20*).

To study the potential effect of eutrophication on MeHg production in the Everglades ecosystem, we examined the total Hg (Hg_T), carbon (C), nitrogen (N), and P content and the potential for microbial methylation of Hg[II] and degradation of MeHg in peat soils collected along a P gradient in WCA-2A (Figure 1). Preliminary results showed lower Hg_T concentrations in eutrophied areas but a similar accumulation rate of Hg_T compared with oligotrophic soils. Potential rates of methylation and demethylation measured by the addition of radioactive substrates were not related to the eutrophication gradient and were similar to rates reported for other freshwater environments.

Materials and Methods

Study Area. Samples were collected in WCA-2A (Figure 1), a 574-km^2 marsh located in the Northern Everglades that receives 60 t of P and 1814 t of N annually, with EAA drainage through the S10 inflow structures A, C, D, and E (*21*). Nutrient-laden runoff has resulted in a north–south P enrichment gradient (*18, 22, 23*) characterized by changes in the species composition of aquatic macrophytes (*24*; Vaithiyanathan et al., manuscript in preparation), periphyton (*25*), and macroinvertebrates (*26*).

Sawgrass (*Cladium jamaicense*) marshes and Eleocharis sloughs (*Eleocharis* spp., *Nymphea odorata* Aiton, *Chara* spp., *Utricularia* spp., and associated periphyton) dominate the landscape in the oligotrophic areas of WCA-2A, whereas eutrophic areas primarily consist of cattail (*Typha domingensis*) stands (Vaithiyanathan et al., in preparation). Nutrient enrichment and prolonged hydroperiod have contributed to cattail proliferation in the eutrophic areas (*24*). Water levels within WCA-2A show seasonal fluctuations, greater than 1 m in magnitude, in response to rainfall and water management practices. Maximum water depth within the marsh typically ranges from 0.5 to 0.9 m during the late wet season (September/October), while some areas of the marsh are dry during March and April.

Soil Collection and Handling. Established transacts along the eutrophication gradient in WCA-2A were used in this study. A transect originating near the S10-C water control structure included seven sampling locations, and one additional sample was collected near the Hillsboro canal on the S10-A transect (Figure 1). Surface soil samples for potential methylation and demethylation determinations, Hg_T, P, C, and N analyses were collected in August 1993 and August 1994. A single sample was collected in August 1993 from each study location and was analyzed in triplicate for Hg_T content and potential for methylation and demethylation. In August 1994, triplicate samples collected at each location were subjected to a single determination of all parameters. Core samples for Hg_T and P analyses were collected in December 1993 at C1 (enriched site) and C6 (unenriched site).

Surface soil samples were collected using acid-rinsed (6 N HCl) 250-mL mason jars by scooping the top 2.5 cm of the soil. For microbiological analyses, the jars were totally filled with soil, and trapped air bubbles were carefully removed before capping. Glass jars used to collect soils for Hg_T analysis were baked at 200 °C overnight, capped with acid-rinsed (6 N HCl) lids, and stored in polyethylene bags before and after sampling. Samples for P, C, and N determinations were placed in polyethylene bags. Samples were stored on ice in a cooler during field collection and were refrigerated (5 °C) upon return to the laboratory. All analyses were performed within 2 months of collection. Repeated methylation and demethylation assays using the same sample showed no change in potential activities during this period of time. Core samples were collected using a 7.5 cm by 60 cm stainless steel box core with a removable wall (Richardson, unpublished design). Cores were divided into 5-cm sections and were stored refrigerated in polyethylene bags until analyses.

Soil Analyses. (i) Mercury. Samples collected in August 1993 were analyzed for Hg_T content by cold vapor atomic fluorescence spectrometry (CVAFD; Brooks Rand Ltd., Seattle, WA) as described by Saouter and Blattmann (*27*) after digestion in 1:2.5 (v:v) H_2SO_4 (98%) and HNO_3 (71%) at 90 °C for 6 h. Core samples and surface soils collected in August 94 were analyzed using a PS200 automated Hg analyzer (Leeman Labs Inc., Lowell, MA). Soils, 0.2–0.3 g (wet weight), were digested in a 3:1 mixture of concentrated

$HCl:HNO_3$ at 95 °C for 5 min, followed by oxidation in 1.4% $KMnO_4$ at 95 °C for 45 min. Excess $KMnO_4$ was reduced by the addition of $NH_2OH \cdot HCl$ (30% solution). Remaining solids were removed by centrifugation, and Hg_T was determined in the supernatant fraction. The wet weight of analyzed samples was multiplied by the percent of solids (determined by drying soils to constant weight at 150 °C) to obtain dry weight. The limit of detection by the Leeman PS200 analyzer was 30 ng of Hg_T g^{-1} (dry weight) soil. Analyses of standard material by CVAFS (TORT-1, National Research Council, Canada) and the PS200 analyzer (Buffalo River sediment NIST no. 2704) gave 365.5 ± 33.2 ng of Hg g^{-1} (certified value 330 ± 30 ng g^{-1}) (*27*) and 1.43 ± 0.03 ng g^{-1} (certified value 1.47 ± 0.07 ng g^{-1}), respectively.

(ii) Nutrients. Total P was determined by digesting 100 mg (dry weight) of ground soil in $HNO_3/HClO_4$ (*28*) and measuring phosphate in the digests using a TRAACS 800 autoanalyzer (Method 787-86T [*29*; Standard Reference Method 365-4], Branne-Luebbe Inc., Elmsford, NY). Total C and N were measured in 10–15 mg (dry weight) of soil using a Perkin-Elmer 2400 CHNS analyzer (Perkin-Elmer, Norwalk, CT). The standard general material pine needles (NBS no. 1575) were digested and analyzed for P using the same method. Buffalo River sediment (NBS no. 2704) and peach leaves (NBS no. 1547) were employed as standards for C and N, respectively. The measured values for these NBS standards were within 4% of the certified values.

(iii) Peat Accretion Rates. Estimation of peat accretion rate was carried out by ^{210}Pb and ^{137}Cs dating of the soil cores collected at C-1 and C-6 in June 1990. Total ^{210}Pb activity was measured by α-counting of its daughter, ^{210}Po. A ^{208}Po spike (used to monitor chemical yield) was added to 3 g of soil prior to conducting a sequential digestion with HNO_3 (72% of concentrated acid), HCl (37%), $HClO_4$ (70%), and HF (48%). After digestion, samples were taken to dryness and picked up in 1 mL of HCl. The acid solution was separated from the particles by centrifugation, and Po isotopes were electrodeposited on a silver planchet (1 cm^2). The constant total ^{210}Pb activity at depth in the core was assumed to represent the supported ^{210}Pb activity. Excess ^{210}Pb was calculated as the difference between total and supported ^{210}Pb.

Cesium-137 activity was measured in soil samples by counting γ-emissions at 661.62 keV. Samples were counted for 8 h using a high-purity germanium detector (2.08% efficiency; EG&G Ortec, Oak Ridge, TN). Counting efficiency was determined by counting a ^{137}Cs standard of the same matrix (Everglades peat).

Microbial Activities. Soil samples were mixed 1:1 (v:v) with artificial marsh water (*30*) and slurried in a Waring Blender (Hamilton Beach) using 3 × 30 s blending cycles at the lowest speed setup with 15-s breaks between cycles. Slurries were immediately dispensed to reaction chambers for determinations of potential methylation and demethylation rates. Obtained rates indicated potential rather than real rates because the addition of radioactive substrates exceeded in situ concentrations. The validity of the employed approach has been demonstrated (*14*, *15*). Samples collected during August 93 were assayed in three live replicates plus an acid-killed control (by the addition of 1 mL of 6 N HCl) and a procedural blank (addition of substrates after incubations and prior to analyses). Radioactivity in killed controls and procedural blanks did not exceed background readings. Only live samples were assayed for August 94 samples. All manipulations and incubations were performed under N_2 to minimize exposure of soil microbes to oxygen. A total of 5 mL of slurries was dried to constant weight at 100 °C for dry weight determinations.

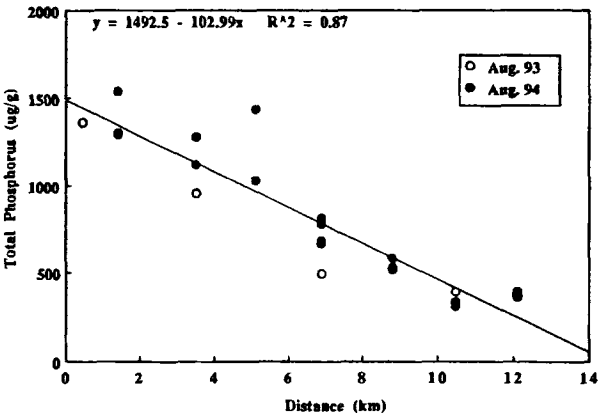

FIGURE 2. Total P distribution in the surface soils as a function of distance from the inflow structure S10-C.

(i) Methylation Assays. A total of 20 mL of slurry was placed in a 250-mL Pyrex centrifuge bottle and mixed with 1 μg (0.12–0.22 μCi) of $^{203}Hg(II)$ (as $HgCl_2$; Buffalo Materials Research Center, Buffalo, NY). The specific activities of substrates were 117 and 224 mCi g^{-1} Hg for August 93 and August 94 assays, respectively. Bottles were sealed with screw-cap lids and incubated at 29 °C for 24 h. Anaerobic conditions were maintained in the bottles throughout the incubation period. Reactions were terminated by the addition of 1 mL of 6 N HCl, and $Me^{203}Hg$ was extracted by the method of Furutani and Rudd (*31*) as modified by Korthals and Winfrey (*32*). Recoveries of MeHg by this procedure were at the 86–96% range. The variability of replicate extractions was <11%. $Me^{203}Hg$ was quantified using a Tri-Carb 2500 TR liquid scintillation counter (Packard Instruments Co. Inc., Meridien, CT).

(ii) Demethylation Assays. A total of 20 mL of slurry was mixed with 1 μg of Hg (as $^{14}CH_3HgI$; specific activity 57 mCi g^{-1} Hg; Amersham Laboratories, Buckinghamshire, England) in 125-mL glass bottles closed with silicone stoppers equipped with tygon inlet and outlet tubes. These tubes were clamped shut during the ensuing 24 h-incubation (29 °C). Reactions were terminated by the addition of 1 mL of 6 N HCl, and ^{14}C-labeled volatile products (resulting from $^{14}CH_3HgI$ degradation) were collected in Carbo-Sorb (Packard Instrument B. V., Groningen, The Netherlands) after oxidation of $^{14}CH_4$ to $^{14}CO_2$ by passage through a CuO tube furnace heated to 450 °C as described by Steffan et al. (*33*). The variability of replicate analyses was ≤20%.

Specific methylation and demethylation rates calculated as the percent of added substrates per day (*15*) were used to determine methylation to demethylation (M/D) ratios. Estimated absolute methylation and demethylation rates (ng g^{-1} sediment [dry weight] day^{-1}) were calculated using the known specific activities of the added substrates.

Data Analysis. Data were analyzed using the SAS System general linear model procedure (*34*).

Results

Phosphorus Gradient in WCA-2A. Total P concentration in the surface soils exhibited a linear decrease ($r^2 = 0.87$; $p < 0.01$) with distance from structure S10-C on the Hillsboro Canal (Figure 2). Soil P concentrations were

TABLE 1

Nutrient Ratios in Peat Soils along the North–South Gradient in WCA-2A[a]

distance from Hillsboro Canal (km)	C:N	C:P	N:P
0.45	13	347	26
1.40	15	323	22
3.50	17	399	24
5.10	14	363	26
6.90	14	634	46
8.80	18	783	45
10.50	15	1331	89
12.10	11	872	76

[a] Data obtained for August 1994 sampling. Ratios are between means of triplicate samples for each sampling site (largest standard deviations were 13%, 25%, and 23% for C, N, and P analyses, respectively).

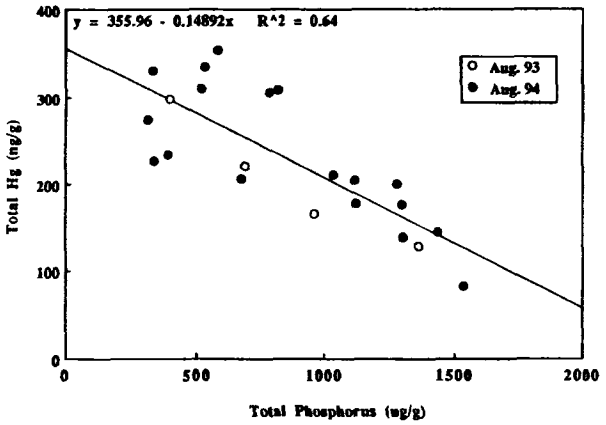

FIGURE 3. Relationships between total Hg and total P concentrations in surface soils collected along a eutrophication gradient in WCA-2A.

highest in the first 2 km south of the Hillsboro Canal (1376 ± 112 $\mu g\ g^{-1}$) and were nearly 3–4-fold above concentrations 10–12 km downstream (356 ± 31 $\mu g\ g^{-1}$). In comparison, Amador and Jones (*19*) have reported P levels of 231 and 1473 $\mu g\ g^{-1}$, respectively, for soils with low and high P enrichment in ENP. Thus, soils examined in this study represented a range of eutrophication levels characterized by varying degrees of P enrichment.

Soil C and N concentrations in the soils remained fairly uniform (C, 33–47%; N, 2.4–3.6%; C:N, 13–17) and were constant along the eutrophication gradient. Consequently, a trend showing a progressive increase in C:P and N:P ratios in the soils for the first 10.5 km of the north–south eutrophication gradient originating in S10-C was noted (Table 1).

Concentration of Hg_T in Soil and Its Relationship to Total P. Total Hg concentrations ranged from 83 (for C1, August 94) to 354 (C5, August 94) ng g^{-1}. These concentrations are well within the range reported for peat soils around the world (summarized in ref 35). Delfino et al. (*36*) reported a range of 17–411 ng g^{-1} Hg_T in Everglades surface soils. In WCA-2A, Hg_T content in the soils increased with distance from S10-C (Figure 3). Total Hg concentrations were low in the most P-enriched soils (83–177 ng g^{-1}) compared with the least unenriched soils (227–354 ng g^{-1}). Consequently, the surface soils exhibited an inverse relationship ($r^2 = 0.64$; $p < 0.01$) between Hg_T and total P. This relationships was confirmed by sampling medium-scale mesocosms constructed in the unimpacted areas of WCA-2A to test the effects of P dosing on ecosystem responses

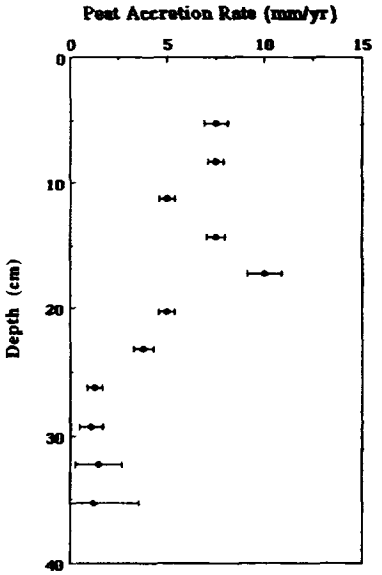

FIGURE 4. Peat accretion rate (calculated using ^{210}Pb dating) as a function of depth in the eutrophied site (C-1).

(Richardson et al., in preparation). Soil Hg_T levels in two experimental channels continuously dosed with 150 μg of P L^{-1} (comparable to P concentrations in the Hillsboro Canal) were lower (139 ± 26 and 169 ± 50 ng g^{-1}) than the concentrations in samples from control channels (no P addition; 238 ± 57 and 208 ± 31 ng g^{-1}; Richardson and Vaithiyanathan, unpublished data).

Two possible mechanisms can be invoked to explain the above trend: (i) dilution of deposited Hg in the eutrophic areas due to enhanced rates of peat accretion (*18, 37*) caused by algal and macrophyte productivity that is stimulated by the addition of PO_4 or (ii) mobilization and loss of soil-bound Hg in the northern reaches of the eutrophication gradient. We examined the relative importance of these mechanisms by estimating peat accretion rates and by calculating Hg accumulation rates in soil cores.

Peat Accretion Rates. Peat accretion rates estimated from ^{137}Cs and excess ^{210}Pb dating of the soil cores collected from the enriched (C1) and unenriched (C6) sites were in agreement with each other. At the enriched site, mean accretion rates for the post-1960s period corresponding to 25–0 cm in the soil profile (Figure 4) were 7.1–7.5 mm yr^{-1}, determined by ^{210}Pb and ^{137}Cs, respectively, and they were nearly 3-fold higher compared with the unenriched site (1.9–2.5 mm yr^{-1} by ^{210}Pb and ^{137}Cs analyses, respectively). Lead-210 dating of core sections revealed that at the enriched site peat accretion rates (Figure 4) had increased linearly ($r^2 = 0.672$) compared with a near uniform rate in the unenriched site (1.92 mm yr^{-1}, data not shown).

Total Mercury in Core Profiles. The soil core collected at the enriched site exhibited distinct zones with respect to Hg_T concentrations (Figure 5): (i) a zone (50–20 cm depth) with a linear increase (7–98 ng of Hg_T g^{-1}) and (ii) a zone (20 cm to surface) with a linear decrease (98–68 ng of Hg_T g^{-1}). Total Hg concentrations at the unenriched site generally increased through the soil profile (from 49 ng of Hg_T g^{-1} at 50 cm depth to 100 ng of Hg_T g^{-1} at 10 cm depth) with a region of declining levels at 20 cm. Total Hg values nearly doubled from 5 cm to the surface (179 ng of Hg_T g^{-1}) at the unenriched site. As was observed with surface soil samples (Figure 3), Hg_T concentration was lower in the top layers (0–20 cm) of the core collected at the

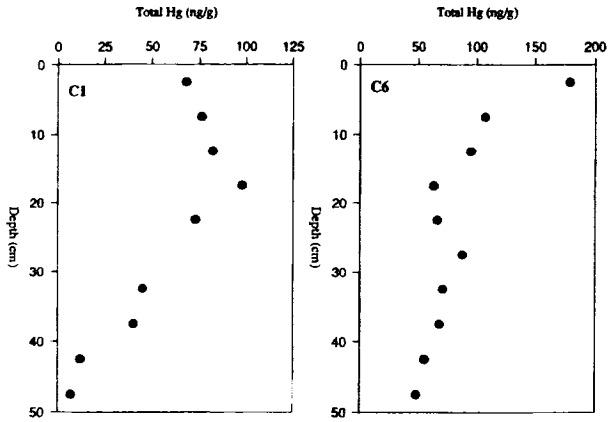

FIGURE 5. Depth distribution of Hg_T in soil cores collected at the enriched (C-1: left panel) and uneutrophied (C-6: right panel) sites. Triplicate samples were analyzed. The maximum variability was 16%.

TABLE 2

Total P and Hg Accumulation Rates in the Enriched and Unenriched Sites of WCA-2A

	time period	dating mehted	enriched site (C1)	unenriched site (C6)
total Hg ($\mu g\ m^{-2}\ yr^{-1}$)	post-1964	^{137}Cs	30	37
	post-1966	^{210}Pb	29	29
	1900–1960	^{210}Pb	16	18
total P ($mg\ m^{-2}\ yr^{-1}$)	post-1964	^{137}Cs	489	138
	post-1966	^{210}Pb	463	106
	1900–1960	^{210}Pb	59	62
total C ($g\ m^{-2}\ yr^{-1}$)	post-1964	^{137}Cs	158	128
	post-1966	^{210}Pb	150	98
	1900–1960	^{210}Pb	50	58

enriched site compared with those of the core collected at the unenriched site (Figure 5).

Total Hg accumulation rates at the enriched and unenriched sites were estimated from the rates of peat accretion (Table 2). Despite the differences in soil Hg_T concentrations between the enriched and unenriched sites (Figures 3 and 5), ^{210}Pb-determined Hg_T accumulation rates, which take into account differences in soil bulk density (i.e., weight per unit volume), were uniform in these two areas (Table 2). The ^{137}Cs data, however, showed an increased accumulation rate for Hg_T in the unenriched site. Phosphorus accumulation rates were approximately 4-fold higher at the enriched site, and C accumulation, an index of productivity, was also higher at the enriched site. Phosphorus, C, and Hg_T accumulation rates in C1 and C6 have increased in the post-1960 period (Table 2).

Potentials for Methylation and Demethylation in WCA-2A Soils. For all WCA-2A samples, potential rates for methylation varied between 2.3 and 48.6 ng of Hg(II) methylated $g^{-1}\ day^{-1}$ and for demethylation varied between 6.5 and 113.2 ng of MeHg degraded $g^{-1}\ day^{-1}$ (Table 3). Potential rates were determined in samples from two additional locations. A heavily eutrophied, an intermediately eutrophied, and a pristine site in ENP (R. Jones, personal communication) were sampled in the summer of 1991. In November 1993, samples were collected from a stream in Fakahatchee Strand State Reserve in the western reaches of the Everglades ecosystem (Figure 1). The latter served as a control, because no exposure to nutrient-rich agricultural drainage water was known for this location. Both potential methylation and demethylation were substantially higher in WCA-2A, although correlations of these activities to the P content of individual WCA-2A samples were not found (data not shown). A general increase in microbial activities in the organically rich environment of WCA-2A is the likely reason for these high methylation and demethylation rates. However, M/D ratios for WCA-2A and for Fakahatchee Strand samples had similar ranges, indicating that increased rates of the individual processes in the former had no effect on the potential for net MeHg accumulation. Lower M/D ratios in ENP samples suggested a lower potential for MeHg accumulation in this part of the unenriched Everglades. Potential activities for all locations were within the range reported for other freshwater sediments (*15*), with those of WCA-2A at the higher range, and with those for ENP and Fakahatchee Strand at the low to intermediate ranges. These rates were higher by 1–2 orders of magnitude than those reported by Matilainen et al. (*38*) for organically rich surficial lake sediments in Finland. Higher rates are expected for samples collected in a subtropical location and for assays that are performed at higher incubation temperatures (*39*).

TABLE 3

Ranges of Potential Methylation and Demethylation Rates in Samples Collected from Three Locations within the Everglades Ecosystem

location[a]	potential methylation rate ($ng\ g^{-1}\ day^{-1}$)	potential demethylation rate ($ng\ g^{-1}\ day^{-1}$)	M/D
WCA-2A (40)	2.30–48.60	6.50–113.20	0.14–2.03
ENP (6)	<0.004–4.50	0.95–7.82	<0.004–0.83
Fakahatchee Strand (3)	0.30–2.30	0.39–4.26	0.07–2.40

[a] Numbers in parentheses indicate the number of samples analyzed.

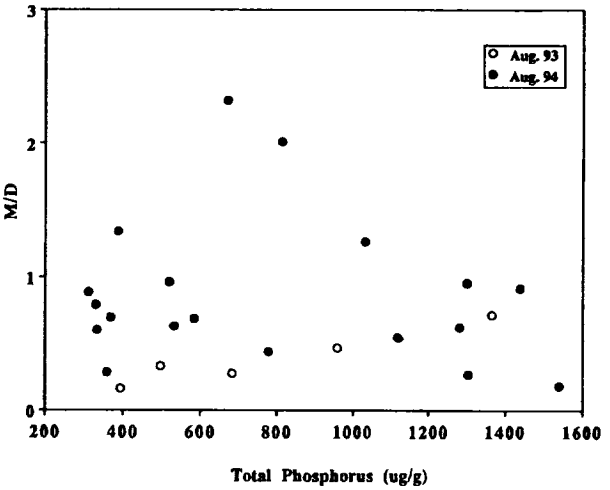

FIGURE 6. Relationships between M/D and total P concentration in surface soils collected along a eutrophication gradient in WCA-2A. Ratios between the means of triplicate methylation and demethylation are reported for August 1993 samples (maximum variability was 41%).

When all M/D ratios for samples collected at WCA-2A were plotted against total P content of the soils, no clear relationships emerged (Figure 6). A direct correlation ($r^2 = 0.91$; $p < 0.05$) of M/D to total P content was however noted for the five samples that were collected in August 93. We do not know why this correlation was not reproduced in August 94 and, therefore, conclude at this juncture that

eutrophication does not affect the potential for net MeHg production along the nutrient enrichment gradient in WCA-2A.

Discussion

An inverse correlation between Hg and P concentrations along a eutrophication gradient in WCA-2A was associated with enhanced peat accretion rates rather than with mobilization of Hg in nutrient-impacted soils. The employed experimental approach showed no relationships between eutrophication and the potential for net MeHg production although high rates of both methylation and demethylation were observed in WCA-2A as compared to other sites. Thus, the only detected effect of eutrophication on the geochemistry of Hg in the peat soils of the Everglades was a reduction in Hg concentration due to increased peat accretion rate. Whether this decrease affects the rates of MeHg production or not would depend on the fraction of available Hg in the Hg_T pool. Similar Hg_T accumulation rates in nutrient-impacted and unimpacted areas of WCA-2A suggest that Hg in runoff water from the EAA does not contribute significantly to the accumulation of Hg in Everglade biota.

Peat accretion rates in the surface layers of the enriched site (7.1–7.5 mm yr^{-1}) were nearly 3-fold higher than the near uniform rates observed in the unenriched site (1.9–2.5 mm yr^{-1}). Reduced concentrations of Hg in the surface layers of the soil core from the enriched site (Figure 5) and the negative correlation between total P and Hg in surface soil samples (Figure 3) are likely due to dilution by enhanced peat accumulation. In fact, when peat accretion rates were considered, similar Hg_T accumulation rates in the enriched and unenriched areas emerged (Table 2). The exponential decrease in Hg_T concentrations in surface soils along the eutrophication gradient of WCA-2A observed in this study (Figure 3), therefore, merely reflects the strong negative correlation between the rate of peat accretion and the distance from the Hillsboro Canal ($r^2 = 0.98$; $p = 0.001$; *20*), the point source for nutrient-enriched water. This observation suggests that soil Hg_T concentrations do not adequately describe the spatial and temporal patterns in Hg_T accumulation in the Everglades and may even be misleading, particularly in the P-impacted regions where stimulated macrophyte productivity has enhanced the rate of peat accretion (*17*, *18*).

Results of a pilot study in the canals of the Everglades ecosystem (*40*) have revealed clear north–south gradients (high to low) in total P, Hg_T, and MeHg in water and a gradient reversal from south to north for Hg_T in sediments. It needs to be verified (from sedimentation rates in the canals) whether in situ Hg dilution due to increased accretion rates may account for the reported south–north gradient of decreasing Hg_T concentration in canal sediments.

The temporal increases (post-1960) in Hg_T accumulation rates in both P-enriched and unenriched areas of WCA-2A (Table 2) is likely related to recent global and regional increases in atmospheric Hg deposition (*41*, *42*). The increase in P accumulation rates in WCA-2A (Table 2) is the consequence of agricultural runoff diversions from the EAA. Thus, the uniform rates of Hg accumulation in the enriched (C1) and unenriched (C6) sites exclude agricultural drainage input as an important contributor of Hg to WCA-2A. However, our results are based on single cores at the enriched and unenriched locations and require verification by examining additional sites along the nutrient enrichment gradient.

Relating M/D ratios to P concentrations suggested that eutrophication had no effect on net MeHg formation (Figure 6). However, eutrophication causes a variety of alterations (see Introduction) that might have opposing effects on MeHg formation. For example, labile organic matter (*20*) stimulates microbial activities but decreases the potential for methylation (*43*) possibly by reducing Hg availability for methylation (T. Barkay et al., manuscript in preparation). Likewise, anaerobic conditions stimulate methylation when methylating bacterial populations are established, yet the increased production of S^{2-} would inhibit methylation (*14*, *15*). Our results suggest that the effects of eutrophication on the variable factors and numerous interactions that influence the potential for MeHg production did not produce a clear trend to suggest a cause–effect relationship. However, Hg methylation and MeHg degradation might have been altered by exposure to agricultural drainage water and/or eutrophication as suggested by higher rates in WCA-2A as compared to other areas of the Everglades (Table 3). A more detailed examination of the isolated processes that directly or indirectly influence methylation and demethylation is needed to delineate how eutrophication affects MeHg accumulation in the Everglades.

The analogy with the case of MeHg accumulation in newly-impounded reservoirs (*44*, *15*) proposed to be driven by stimulated microbial activities due to increased availability of oxidizable organic matter (*16*, *46*) provided the rationale for hypothesizing that the input of PO_4 shown to stimulate microbial respiration in Everglades soils (*19*) might have contributed to MeHg accumulation. This is supported by increases in concentrations of microbial growth substrates found in the enriched site (C1) versus the unenriched site (*20*). However, the increased production of MeHg in impoundments is a transitory phenomenon (*16*, *47*) that is reversed when demethylation is stimulated and metal binding substances are produced (e.g., S^{2-}). The nutrient front in WCA-2A has been advancing from north to south, resulting in zones that are at different stages of the eutrophication process. This might imply lower M/D values in soils collected from the extreme ends of the eutrophication gradient, with higher values in soils with intermediate P content. This would mean that MeHg accumulation in the Everglades is a temporary phenomenon. A much larger data base is needed to statistically accept or reject this hypothesis.

Existing data on P concentrations in surface soils and on peat accretion rates in the entire Everglades (*18*; Richardson, unpublished data) indicate a general north–to-south P gradient and C accumulation rate in this ecosystem, but less extreme than the one observed in WCA-2A in the present study. Future investigations are necessary to examine whether the relationships of P enrichment to Hg dilution and/or mobilization in WCA-2A are true for the larger P gradient of the Florida Everglades.

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