FRESHWATER AQUATIC MACROPHYTES AS HEAVY METAL MONITORS - THE OTTAWA RIVER EXPERIENCE

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Abstract. The ability of freshwater aquatic vascular plants to accumulate heavy metals was examined in some detail during a five year study. Differences in uptake rate were found to depend on the species of plant, the seasonal growth rate changes and the metal ion being absorbed. Lead and mercury were concentrated to a greater extent than the lighter nickel and copper. Laboratory experiments were designed to establish uptake rate constants which were used to calculate water concentrations of mercury from the analyses of plant samples from the river. 'Background' levels of mercury in aquatic plants of 35–50 ng g^{-1} dry weight corresponded to a water concentration near 15 ng L^{-1} of total mercury of which 25–30% was methyl-mercury. Higher concentrations of mercury in the plants indicated a proportional increase in the mercury level in the water.

1. Introduction

The accumulation of heavy metal ions by aquatic macrophytes from the water in which they are growing has been documented by a number of authors. Dietz (1973) reported the concentrating capacity for several metallic ions absorbed by four species growing in the Ruhr River. He noted that the degree of enrichment depended both on the kind of metal and on the species of plant absorbing the metal. With two sampling stations in the Leine River, one above and one below the city of Gottingen, Abo-Rady (1980) observed increases in aquatic plant tissue concentrations of several heavy metals at the downstream station relative to the upstream station. He also noted that the accumulation seemed to depend both on the species and the metal. Ray and White (1979) found that the irregular distribution of the aquatic plants in the stream system draining a base metal mining area in New Brunswick, Canada limited the usefulness of these plants as monitoring agents but they did obtain similar results to the above with at least one species. Franzin and McFarlane (1980) did not find any correlation between the uptake by *Myriophyllum* and the concentration of 7 metallic ions in the sediments of 6 Canadian lakes. Eriksson and Mortimer (1975) had reported earlier that in laboratory experiments rooted aquatic plants absorbed mercury from the water into the submerged green parts and subsequently moved the metal to the roots. Dolar *et al.* (1971) studied the uptake of a variety of mercury compounds by *Myriophyllum* but their relatively high metal concentration (0.5 μg mL^{-1}) in the water induced toxicity in the plants. Also working at higher than environmental levels (0.025–0.5 μg mL^{-1}), Nakada *et al.* (1979) observed a decreasing enrichment factor with increasing metal concentration and a higher factor with lead than with cadmium, copper or zinc. Their plant, *Elodea nutallii*, also accumulated a higher concentration of metal in the growing tip than in the rest of the

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plant. Stokes *et al.* (1983) have recently reported the use of filamentous algae as accumulators of methylmercury from lake waters. Analyses of these plants for mercury content were correlated with methylmercury levels in fish from the same lakes.

The emphasis of these studies gradually shifted toward the use of aquatic plants as monitors for heavy metal water pollution. For example, Ray and White (1976) outlined a number of parameters such as widespread distribution of the plant species, required to achieve this objective. Say and Whitton (1983) also designed what they regard as a 'standard' procedure based on the use of the aquatic bryophyte, *Fontinalis antipyretica*, to monitor water systems in Great Britain and Belgium.

While studying the distribution and transport of pollutants in the Ottawa River system, we examined in some detail the accumulation of mercury compounds from the water into aquatic plants, (Ottawa River Project Group, 1979) coupling laboratory studies based on conditions observed in the river with analyses of samples taken from the river. Since the project extended over 4 yr (1972–1977), field data were collected over 3 or more growing seasons. During this period we also collected aquatic plants from the St. Lawrence River to compare levels of mercury in a different river system with those in the Ottawa River. The data presented in this paper will show that when the growth rates and heavy metal uptake characteristics of the aquatic plants have been established by laboratory experiments, samples of the same species taken from water systems can be used to estimate the concentration of and also the kind of mercury compounds in the water.

2. Materials and Methods

2.1. Laboratory studies

Uptake studies were carried out in a flowing water system similar to that described by Mortimer and Kudo (1975). Six 50 L glass aquaria were set up in parallel, with an outflow tube maintaining the water level in each aquarium at 45 L. Input water flow was adjusted to provide a complete exchange of water in 24 hr, (about 30 mL min^{-1}). The toxic metal input was metered into this stream through a pumping system delivering about 1 mL 10 min^{-1}. Thus to maintain an aquarium concentration of say 0.01 $\mu g\ mL^{-1}$ in the flowing water, the toxic metal solution concentration was set at 300 times this level or 3 $\mu g\ mL^{-1}$. New solutions were made up every 3 days and the reservoirs were held at +2 °C for the duration of the experiment.

Plants were set in these aquaria in about 2 cm of washed, white quartz sand uniformly spread over the bottom. Lighting was provided by 2–4 ft (122 cm) cool white fluorescent tubes plus 6–40 W light bulbs. Two sets of lights were required to illuminate 6 aquaria. All lights were about 40 cm above the water surface and were timed to a 16 hr day beginning at 6 AM. Water temperature was maintained at 21 °C minimum to about 25 °C maximum in mid summer. Plants were collected from the Ottawa River in the study site or in the case of *Elodea densa*, were purchased from a local aquarium supply house. Most laboratory experiments were carried out in the summer and completed

before the end of August, when the plants were entering senescence and uptake decreased drastically. *Elodea densa* could be used over a much longer season, almost year round, which made it the species of choice for several experiments.

When inorganic mercury or methylmercury were used, radioactive mercury (203) was added as the tracer. Solutions were made up with tracer amounts of the radioactive form and the required amount of unlabelled mercury salt. At the low levels used in some experiments the carrier in the radioactive salt set the lowest limit that could be used. The concentration of mercury in the plant tissue was then established by counting the radioactivity and multiplying by the known specific activity. All results were calculated on a dry weight basis. Aquatic plant samples could be oven-dried at 90 °C without loss of mercury in either the inorganic or methylmercury form. Samples (at least 10) of *Elodea* plants which had biologically incorporated radioactive inorganic or methyl mercury were counted before and after oven drying. There was no measureable loss for at least 48 hr drying. The usual drying period was 16 hr. Counting was accomplished in a Tracor Analytic 1197 Gamma Counter with a 3 inch sodium iodide crystal to a standard error of 2%.

Some comparative experiments were run with other toxic metals in the water. These included lead, cadmium, nickel and copper. The only difference from the procedures described concerned the analysis. Weighted samples of dried tissue (0.1–0.5 g) were digested in microkjeldahl flasks in a 50–50 mixture of perchloric and nitric acids. The digest was diluted to 10 mL in deionized water, filtered to remove the silica which was a significant constituent, particularly in *Elodea*, and finally diluted to the appropriate volume for analysis by flame atomic absorption (Jerrel Ash Model 850).

In all aquarium experiments, metals were added as the chloride or nitrate. However, at the dilutions employed the added anion probably had no significance. The pH of the water was 6.8 to 7.0, a value established by the growing submerged aquatic plants. The mean pH of the Ottawa River water during the period of this study was 7.2 (Ottawa River Project Final Report, 14.8).

Sediments were analysed for total mercury by permanganate/persulfate oxidation and water by extraction with dithizone in chloroform (Norstrom, 1977), followed by flameless atomic absorption spectrophotometry.

2.2. Field Studies

The study area was a 5 km stretch of the Ottawa River near the eastern edge of Ottawa. Several plant species were collected by digging all of the contents of a 0.25 m^2 quadrat set in an area of plant growth to a depth of 30 cm of sediment. Thus both roots and tops were available for analysis. These kilogram quantities of fresh plants were returned to the laboratory, sorted, washed in municipal water and oven dried. The oven-dried parts, usually tops (shoots) and roots, were then ground to 20 mesh particle size in a micro Wiley Mill. One gram portions of this well-mixed powder were weighed into the analysis cups for mercury determination by flameless atomic absorption. The analysis used the modification described by Norstom (1977) which reduced the detection limit to 10 ng or less. The mixing of the sample for analysis was important but difficult. Dried

plant powder segregates very easily into coarse and fine material because of static charges. The specific activity of the fine material was 3 to 5 times higher than that for the coarse parts. The latter were microscopically identifed as epidermal and fibre components.

Sampling sites were reached by a motorized boat and samples were collected by going over the side of the boat in hip waders. The depth of water ranged from a few centimeters to about one meter, which was the limit for the waders. Very few plants grew in deeper water, presumably because the water was too turbid. Collections were made at one and two week intervals from late May into September. Weather and water levels modified these dates by one or more weeks in some years. The bottom sediment was mostly sand but in areas sheltered from the current this sand was mixed with varying amounts of organic matter, presumably decayed plant parts from earlier years along with material deposited from the water among the plant stems. These plant samples were also used to determine productivity as a function of species, habitat and season. These data will be reported elsewhere.

For collections from the St. Lawrence River, a different technique was required, because the water was deeper and much clearer. Plants were growing at depths of at least 3 m. A weighted rake head on the end of a rope was thrown over the side of the boat into the plant bed, then drawn back into the boat usually with a good collection of plants. Sediments and roots were not collected with this set of samples.

3. Results

The data will be considered from several viewpoints. First the uptake rates as a function of plant species and age, then the uptake rates of different metallic ions and finally the use of this information to locate a point source of mercury input into a river.

3.1. Effect of plant species

The fact that different species have different uptake rates of the same metal under identical conditions is illustrated in Table I. Plants of *Elodea densa* and *E. canadensis* were set in the same aquarium with water containing about 0.7 $\mu g\ L^{-1}$ of inorganic mercury flowing through at 30 $mL\ min^{-1}$. Individual plants were removed at daily intervals, counted for radioactivity and weighed. The data clearly show that *E. canadensis* accumulates about 4 times as much mercury as *E. densa*. Under similar conditions, the uptake rates for 4 other species of submerged aquatics were measured. The data in Table II show that the concentration factor (μg Hg g^{-1} dry wt of plant absorbed per day μg^{-1} Hg mL^{-1} of water) varied from 0.9×10^3 to 3.3×10^3 depending on the species. The highest value achieved earlier (Mortimer and Kudo, 1975) with *Elodea densa* was about 5×10^3 for both inorganic and methylmercury. Although there are no data to illustrate the point, I recognize that actively growing plants of any species absorb metallic ions much faster than old or senescing plants. This is an important factor in aquarium experiments.

TABLE I

Absorption of inorganic mercury by two *Elodea* species from flowing water[a]

Day	*E. densa*	*E. canadensis*
2	0.012 μg g^{-1} [b]	0.032
3	0.046	0.108
4	0.058	0.149
5	0.085	0.184
6	0.101	0.230
7	0.120	0.265
10	0.143	0.406

[a] The average water concentration of radioactive mercury was about 0.7 μg L^{-1} at a temperature of 21 °C.
[b] Calculated from the specific activity of the radioactive mercury and the dry weight of the plants (about 230 mg dry weight).

TABLE II

Inorganic mercury uptake by submerged species of aquatic plants[a]

Species	Day			Conc. factor[b]
	4	10	14	
Utricularia	5.1	9.9	12.8	3.3×10^3
Ceratophyllum	1.5	3.9	5.1	1.3
Najas	1.4	2.9	3.5	0.9
Nitella			5.2	1.3

[a] Plants taken from the Ottawa River were floated or planted in aquaria with flowing water (30 mL min^{-1}) containing 203 Hg solution, to give a water concentration of 0.28 μg L^{-1}.
[b] Concentration factor: μg g^{-1} dry wt in plant/μg mL^{-1} in water day^{-1}.

3.2. Effect of tissue age

As indicated above there is an effect of physiological age of the plant tissue. This is illustrated in Table III. *Elodea densa* plants were dissected after 35 days of growth in aquaria containing inorganic or methylmercury. There was a particularly high concentration of methylmercury in the youngest tissue relative to the old tissue. A similar effect, but with less contrast, was found with inorganic mercury. The tissue weight data in this Table illustrate another observation, which is that growth in the presence of methyl mercury (518.4 mg) is much less than in the presence of inorganic mercury (836 mg). When field samples of aquatic plants taken from the Ottawa River were analysed for both inorganic and methylmercury, the proportion of methylmercury to total mercury was about 31% for the shoots or tops and about 10% for the roots (Table IV). That

TABLE III

Influence of tissue age on the absorption of mercury by *Elodea densa*[a]

Methylmercury			Inorganic mercury	
Wt/plant part mg dry wt	Hg/plant part cpm g^{-1}	Wt/plant part mg dry wt	Hg/plant part cpm g^{-1}	
56.0	106.9×10^3	Tip	78.8	7.6×10^3
47.0	52.3		91.9	5.6
49.5	32.0		66.2	7.7
31.9	38.0	Young	54.7	6.3
49.3	31.4		42.7	4.7
17.5	36.8		74.3	7.7
46.1	27.6	Old	80.3	4.7
60.4	26.2		88.5	4.7
89.0	20.1		91,9	3.6
62.7	16.3		74.1	4.6
			113.1	2.3
17.0	50.0	Root	6.3	3.3

[a] *Elodea* plants were set in separate aquari with flowing water (30 mL min^{-1}) containing an average 1 cpm mL^{-1} of ^{203}Hg for 35 days. Individual plants were cut into 6 cm sections, counted, dried and weighed. The values are the means of three plants for each treatment.

TABLE IV

Organic mercury content (% of total) of aquatic plant samples[a]

Species	Shoots	Roots	No. of samples
1975*Sagittaria latifolia*	38.6	8.7	4
Elodea canadensis	29.9		2
Sparganium angustifolium	30.2	14. 4	3
Sparganium eurycarpum	47.8	10.5	3
1976			
Sagittaria latifolia	27.4	12.0	9
Elodea canadensis	24.2	11.2	6
Sparganium angustifolium	32.7	7.6	8
Sparganium eurycarpum	22.9	7.5	4
Means	31.7	10.3	

[a] *S. latifolia* and *Sp. eurycarpum* are emergent species. Samples were from the total shoots for all species. Analysed by flameless atomic absorption. Organic mercury by difference between total and inorganic values. Each determination was replicated three times.

is, there is an internal fractionation of metallic ion species within the plant. Several plant species seem to be affected in a similar way. The seasonal changes in mercury concentration in the tissue are a combined effect of growth rates, transfer of material

TABLE V

Seasonal changes in mercury content of aquatic plants expressed as ng g^{-1} dry weight: shoots/(roots)[a]

	June		July				August[b]			
Sagittaria latifolia			93.9 (144.6)	77.0 (192.4)	74.6 (206.5)	58.5 (120.9)		63.9 (111.3)		53.7 (115.4)
Sparganium angustifolium	214.5 (251.5)	112.2	170.5 (97.4)	127.6	111.7 123.5)	70.5	211.3 (145.7)	121.7	121.5 (120.8)	80.5 (117.3)
Elodea canadensis				77.9	91.3 158.6)	78.5	225.1 (259.5)	192.4	103 (188.0)	64.8
Sparganium eurycarpum	109.1 (199.2)			164.4 (550.4)	62.2 (48.0)	92.5 (431.2)		50.0 260.1)		
Phalaris arundinacea	79.3	176.0 (275.6)		253.8 (213.4)		126.4 (211.2)		131.0 (447.9)		

[a] Each value is the mean of triplicate analyses of aliquots taken from 200 to 1500 g of dried plant tissue.

[b] Samples were collected at approximately weekly intervals during the 1975 growing season.

from root to shoot and back again and senescence in the fall. The accumulated data for 5 plant species collected from the same sites over a summer season are summarized in Table V. The total mercury concentration varied considerably within one month and also from month to month. Both tops and roots show the variability. We regard 50 ng g^{-1} dry wt as the background level so most of these samples indicate that there was contamination of the water by mercury at this time (1975). Whether the mercury found in the roots of the emergent species, *Sparganium eurycarpum*, was much higher than that in the tops because of absorption of mercury from the sediment was not resolved.

3.3. Effect of sediment

The relation between the mercury content of the sediments and the aquatic plants growing in them is shown as Figure 1. Plant mercury analyses include those shown in

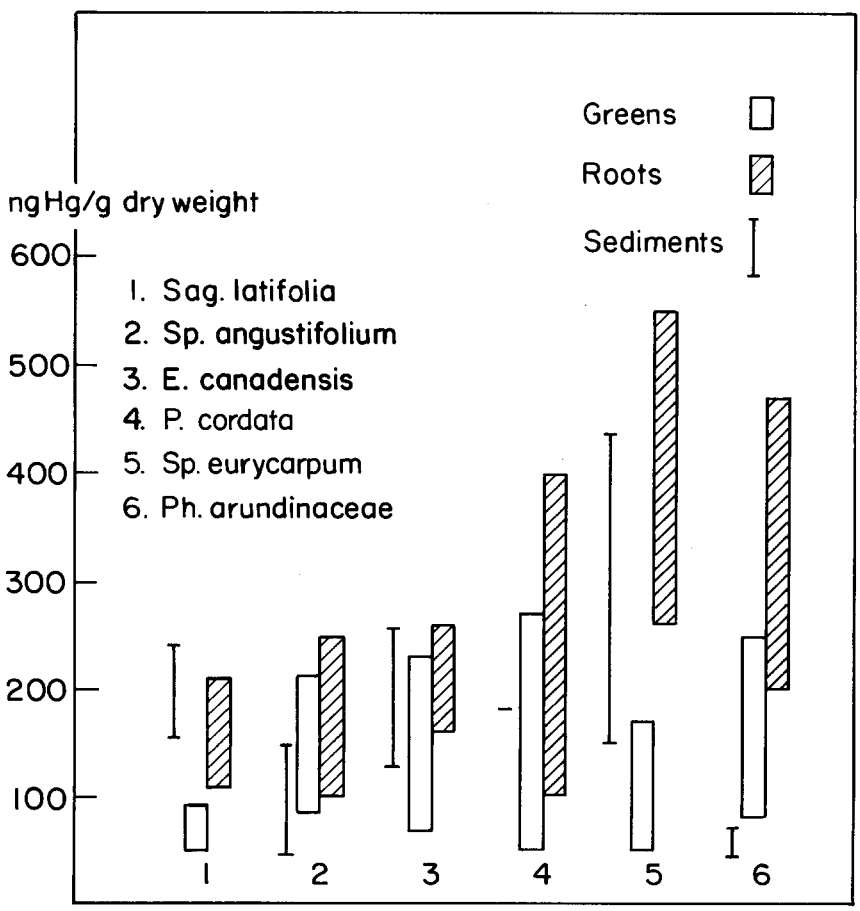

Fig. 1. The range of total mercury concentration over the 1975 growing season for 6 plant species and the sediment associated with each species. Each bar includes the means of triplicate analysis for 5 or 6 individual samples taken from a single site for each species, (#4 sediment, 1 only). The Ottawa River water was estimated to contain 15–20 ng L^{-1} total mercury at this time.

Table V with the addition of the corresponding mercury contents of sediments which were collected from the zone in which the plants were growing. The variability of the sediment mercury levels over the growing season suggest a relation to the plants. From earlier laboratory experiments we were able to conclude that mercury was absorbed from the water and transported to the roots within the plant (Eriksson and Mortimer, 1975). Thus the mercury found in the river sediments could have come from the decayed roots of earlier years plant productivity.

3.4. Effect of Metal Ion

The uptake of heavy metals from water by aquatic plants is also affected by the kind of ion. The comparative results in Table VI show that the concentration factor for lead is about 6 times that for nickel, while copper actually leaked out of the plants during this experiment. The copper content of the experimental water (1/10th Hoagland's medium in deionized water) was less than that in the aquaria where the plants were

TABLE VI

Uptake of heavy metals by *Elodea densa* from flowing water[a]

Metal[b]	Day				Conc. factor[c]
	0	3	8	12	
Copper	278	179	139	133	
Lead	14	29	55	182	1400
Cadmium	17	34	59	118	900
Nickel	37	41	59	63	200

[a] The flowing water was 1/10th strength Hoagland's medium in deionized water. Three 15 cm plants were used for each sample.

[b] The metal solution were metered into the flowing water stream to give an aquarium concentration of 0.01 $\mu g\ mL^{-1}$ of metal.

[c] Concentration factor: μg metal g^{-1} dry weight in plant/$\mu g\ mL^{-1}$ in water day^{-1}.

cultured in municipal water which flowed through copper pipes. Under similar conditions, inorganic mercury and methylmercury had concentration factors which were the same at 1500. It is interesting to note that the higher atomic weight ions, mercury and lead, were concentrated by *Elodea* much more effectively than the lower atomic weight ions, cadmium and nickel.

3.5. Effect of Water Source

The total mercury analyses of the samples collected from the St. Lawrence River are presented as Table VII and the collection sites are identified in Figure 2. The river has an average flow of 6.8×10^6 L sec^{-1}. with about 1/3 of this flowing through the North Channel around St. Regis Island. An earlier sediment survey of this region (Ontario Ministry of the Environment, 1977) had shown that the river sediments adjacent to the North shore in front of Cornwall townsite had elevated sediment mercury levels.

TABLE VII

Total mercury in aquatic plants[a] from the St. Lawrence River near Cornwall, Canada

Sample No.	North channel[b]			South channel		
	1976	1978	1978	1976	1978	1978
1		45	40[c]	70		
2		87				39
3			42		17	
4	247	316			40	
5		382			28	
6	207					24
7	68			96	24	
8		55			55	
9		115			34	54
10		317	30		54	
11		1600				
12		1235	1020			
13		53	104			
14	95	39				
15		29				
16		57	32			

[a] The plants collected were all a single species fo *Myriophyllum* which grew to heights of 1 to 3 m depending on water depth. About 50 cm of tip growth were taken for analysis. Samples were collected Oct. 10, 1976, July 15, and August 18, 1978.

[b] The sample numbers are the same as the site identifications marked on the map (Figure 2).

[c] ng mercury/g dry wt of plant sample. Mean of triplicate analyses.

Evidence for this may be seen in the 207 to 382 ng g^{-1} total mercury found in North channel samples 4, 5, and 6. The unusually high levels found in samples 11 and 12 (1600 ng g^{-1}) were unexpected. An additional surprise was that these samples were nearly 100% inorganic mercury instead of the usual 70%. We eventually found a 4 inch (10 cm) pipe coming out of the river bank just upstream from the sample 11 position. This pipe was traced back to a synthetic fibre factory which was using the alkali from the local chloralkali plant as part of its manufacturing process. Assuming that the factory was in an equilibrium state with respect to mercury content and that the incoming alkali had no more than the statutory 0.5 μg mL^{-1} of Hg^{++}, we were able to calculate, with the cooperation of the research management of the fibre factory, that about 40 g of mercuric ion were draining into the river from that pipe every day. It is interesting to note that this input was quickly diluted to background levels in the fast flowing water since downstream samples 13 to 16 were all at background levels of mercury.

Because all of the South Channel samples showed accumulations of 50 ng g^{-1} or less, corresponding to the background water concentration, we were able to conclude that there was not a significant input of mercury into the South Channel of the St. Lawrence River, and that in general, that river was less contaminated by mercury than the Ottawa River.

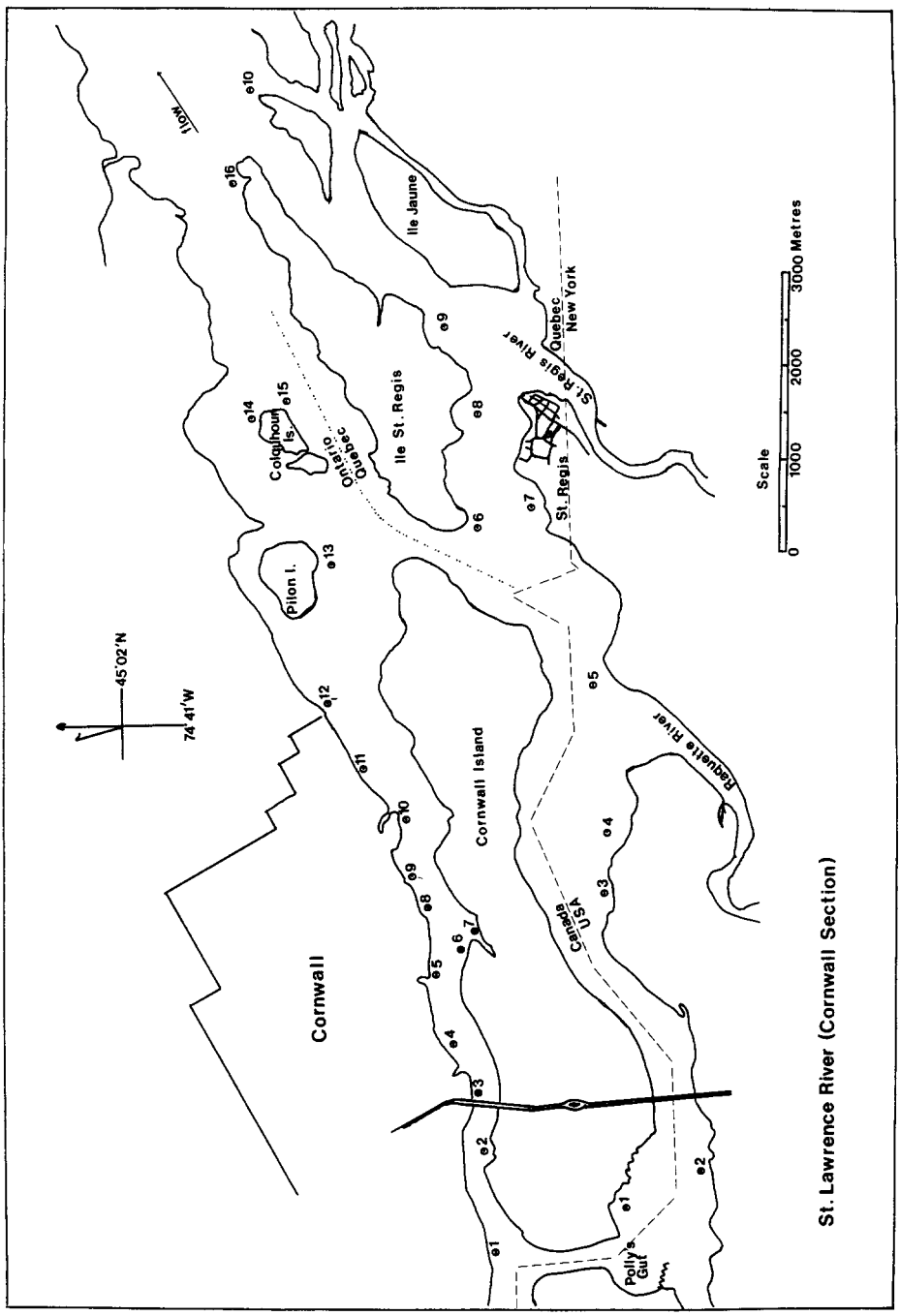

Fig. 2. Outline map of a 15 km section of the St. Lawrence River near Cornwall, Ontario. Numbers 1 to 16 identify the sites from which plant samples were collected for mercury analysis in 1976 and 1978. The analytical results are presented in Table VII.

4. Discussion

The changes in accumulation rates of heavy metals as a function of the season and physiological age of the plant, the localization of the accumulated metal in growing parts and the effect of metal concentration on the uptake rate all make relevant laboratory studies essential before aquatic plants can be used to monitor the heavy metal concentrations in water. Each of the heavy metals will have its own characteristics, and each plant species accumulates differently. From the data presented, it is clear that the higher molecular weight ions (lead and mercury) are accumulated much more effectively than are lower atomic weight elements such as nickel. This evidence is not consistent with current theories about movement of ions across membranes.

Sufficient laboratory data were obtained studying the characteristics of inorganic and methylmercury uptake to permit the use of plant sampling in river water as the means for estimating the amount and kind of mercury in the water. For example if an *Elodea* plant collected in mid-July contained 300 ng g^{-1} dry weight total mercury, then the water in which it was growing would contain about 20 ng L^{-1}. The calculations are based on a concentration factor of 1500. That is, one gram dry weight of plant tissue absorbs all of mercury from 1500 mL of water per day. The sample was the tip 10–15 cm, which would represent about 10 days growth at that time of year. Thus the uptake was about 30 ng/day, which divided by the uptake factor gives a water concentration of 20 ng L^{-1}. Since the 300 ng g^{-1} was about 30% methylmercury and since both species of mercury are absorbed at the same rate, then the water would contain about 6 ng L^{-1} of methylmercury and 14 ng L^{-1} of inorganic mercury. These values were later confirmed when Kudo *et al.* (1982) reported on methods developed for the direct analysis of water.

The sensitivity of the method was amply demonstrated by the samples collected from the St. Lawrence River. The current in this section was at least 1 m min^{-1} and the depth close to the shore was about 1.5 m. The *Myriophyllum* plants did accumulate the mercury draining into the river and the differential analysis did reflect the later discovery that the input source was indeed inorganic mercury. The rapid dispersal of such a point source in a large river was illustrated by the low mercury contents of downstream samples.

The relation between the heavy metal content of rooted aquatic plants and the sediment in which they are growing is by no means direct. The accumulated evidence from this study indicates that for the heavy metals there is probably no relation based on root absorption and upward transport. The experimental evidence suggests that movement in the reverse direction is more dominant. That is, metals contained in the water can be absorbed by the submerged stems and leaves, transported to the roots and added to the sediment concentration when the roots die.

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