Ecological Applications, 11(1), 2001, pp. 215–225

BIOMONITORING SITE QUALITY IN STRESSED AQUATIC ECOSYSTEMS USING *VALLISNERIA AMERICANA*

Kelly Potter and Lesley Lovett-Doust[1]

Department of Biological Sciences, University of Windsor, 401 Sunset Ave., Windsor, Ontario, Canada N9B 3P4

Abstract. Leaf-to-root surface area ratios in *Vallisneria americana* have been shown to provide a simple and inexpensive relative measure of sublethal effects of organochlorine contamination. The present study was conducted to determine whether this index of surface area could be used as an effective biomonitor of overall site quality in stressed aquatic ecosystems. The leaf-to-root surface area ratio was determined for samples of *V. americana* collected from 225 microsites within 12 Areas of Concern (environmentally contaminated areas designated by the International Joint Commission) throughout the Laurentian Great Lakes of Ontario. Statistical analyses indicate that 77% of the variation in the surface area index could be attributed to differences among microsites, with only 23% of variation occurring among plants within a microsite. A multiple regression equation was developed for predicting the leaf-to-root surface area ratio from several measures of microsite quality. Significant parameters affecting the surface area ratio included plant density, light intensity, and an index of sediment contamination. In contrast, measures of water contamination did not show any correlation with leaf-to-root surface area ratio. These observations support the hypothesis that *V. americana* accumulates contaminants primarily from the sediments and that the leaf-to-root surface area ratio can be used to construct contours of point source impact zones in Areas of Concern. The regression model developed here provides a simple, inexpensive means for monitoring overall site quality throughout the Great Lakes.

Key words: American wild celery; Areas of Concern; biomonitoring; Great Lakes; heavy metals; organochlorines; plant density; point source impact zones; radiation, photosynthetically active; sediment contamination; Vallisneria americana.

Introduction

As the burden of persistent toxic compounds discharged into waterways continues to increase, there is a growing need for simple, inexpensive methods to assess site quality in aquatic ecosystems and to identify degraded microsites requiring remediation (Dolan and Hartig 1996). Analytical methods for detecting organic and metallic pollutants in sediment, water, or tissue are so costly that it is impractical to use them for routine repeated assessments; and in any event such measurements don't necessarily reflect bioavailability of contaminants. An alternative environmental management tool involves the use of living organisms as biomonitors. Biomonitoring can be defined as, ''the use of organisms in situ to identify and quantify toxicants in an environment'' (Chaphekar 1991). This procedure takes advantage of the ability of living organisms to accumulate contaminants in their tissues through bioconcentration (uptake from the ambient environment) and biomagnification (uptake through the food chain). In contrast to chemical analyses of abiotic samples that merely measure the concentration of contaminants present in an area, the ability of biota to accumulate contaminants enables them to indicate the total pollution loadings present in an environment (Lovett-Doust et al. 1994*b*). The present study investigates the use of the aquatic macrophyte *Vallisneria americana* as a direct biomonitor of site quality.

The potential use of plants as biomonitors is not a new idea. Over fifty years ago, studies suggested that the metal content of terrestrial plants could be used to predict the location of ore deposits for the purpose of prospecting (Cannon 1960). However, biomonitoring studies involving aquatic plants have been largely confined to the last few decades. These studies have investigated the biomonitoring potentials of various species of aquatic plants, as well as diverse biomonitoring methods and endpoints. Sediment toxicity has been tested in laboratory bioassays with the floating duckweed, *Lemna* sp., in terms of the number of fronds, chlorophyll production, root length, and carbon-14 uptake (Taraldsen and Norberg-King 1990, Huebert and Shay 1993). Through assays of various single contaminants and effluent mixtures, it was found that duckweed showed comparable (or even greater) sensitivities than other commonly used test species, such as *Ceriodaphnia dubia* and *Pimephales promelas* (Taraldsen and Norbert-King 1990). However, Huebert and Shay (1993) have warned that such bioassays must adhere to strict growing condition criteria if they are to be useful, and only comparisons of relative growth rates, not final yields, may be made between studies. Root

Manuscript received 10 June 1999; revised 12 July 1999; accepted 3 September 1999; final revision 4 February 2000.

[1] Address correspondence to this author.

and shoot length, peroxidase or dehydrogenase activity, as well as chlorophyll production, have all been suggested as suitable measures of sediment toxicity in the rooted submersed macrophyte *Hydrilla verticillata* (Klaine et al. 1990). Walsh et al. (1982) found that changes in the photosynthesis/respiration ratio of the sea grass *Thalassia testudinum* occurred in response to the presence of atrazine and pentachlorophenol. Root elongation in *Panicum miliaceum* has been used in toxicity testing of phenolic compounds with sensitive, regular, and predictable toxic responses (Wang 1986). The submersed, rooted, aquatic macrophyte *Vallisneria americana* has also been proposed as a potential biomonitor of water quality (Biernacki et al. 1995*a*, *b*) and sediment quality (St-Cyr and Campbell 1994), as well as being used as a biomonitor in pesticide toxicity testing (Swanson et al. 1991, Solomon et al. 1996). St-Cyr and Campbell (1994) found that metal concentrations in the leaves of naturally occurring *V. americana* reflected the spatial variations in bioavailable sediment concentrations of cadmium, chromium, copper, nickel, lead, and zinc. They suggested that *V. americana* showed promise as a bioindicator species for trace metals. Metal concentrations in the sediment have also been found to be reflected in the metal content of aquatic plants such as *Potamogeton* sp., *Najas flexilis*, *Myriophyllum exalbescens*, *Elodea canadensis*, and *Ceratophyllum demersum* (Pip 1990). Giblin et al. (1980) found a relationship between marsh sediment concentrations and *Spartina* sp. tissue concentrations of cadmium, chromium, copper, and zinc; but found no correlation for lead, iron, and manganese. Although advances have certainly been made through these studies, there is still a need to incorporate more aquatic macrophyte biomonitors into toxicity testing and risk assessment programs (Smith 1991, Swanson et al. 1991, Hughes 1992).

Vallisneria americana has shown particular promise as a biomonitor of aquatic contaminants. In controlled greenhouse experiments, trichloroethylene (TCE), a degreasing solvent, was shown to effect changes in the leaf-to-root surface area ratio of *V. americana* plants when introduced into the water column, with higher concentrations of TCE resulting in higher ratios (Biernacki et al. 1995*b*). The study also used four sediment treatments, each containing a different proportion of silica sand. The leaf-to-root surface area ratio of the plants was observed to decrease in sediments with higher sand content. However, in the control treatment, where no TCE was added, no significant differences were observed among plants grown in different sediments. This suggests that sediment type only affected plant growth form in the presence of the contaminant. TCE is extremely hydrophobic and tends to accumulate in sediments where it adsorbs to organic particles. Hence it was hypothesized that *V. americana* plants accumulated the TCE primarily through their roots, where it likely remained, since organochlorine contaminants typically have very limited mobility within plant tissues (Guilizzoni 1991). The resultant increase in leaf-to-root surface area ratio of the plants was due to decreased root length and diameter causing a proportionately greater allocation of biomass to the leaves, which were surrounded by the relatively less contaminated water column (Biernacki et al. 1996). The study also examined the change in leaf-to-root surface area ratios over the 40-d duration of the experiment. Ratios were observed to increase over the first 10 d of exposure, then remained relatively constant until the end of the study. In further field studies, Biernacki et al. (1996) examined the leaf-to-root surface area ratio of *V. americana* collected from 243 natural populations in the Huron–Erie corridor of the Great Lakes. The ratio was found to be significantly correlated ($P < 0.001$) with the ranking of the collection sites in terms of concentrations of various organochlorine contaminants reported independently in the published literature, for both sediments and biota (Biernacki et al. 1996). These studies, however, did not investigate the relationship between the leaf-to-root surface area ratio of *V. americana* and other types of contaminants (such as heavy metals), or other physical and chemical descriptors of site quality, such as levels of macronutrients, organic matter, light availability, and etc.

The general purpose of the present study was to determine whether the leaf-to-root surface area ratio of *V. americana* was an indicator of only organic contamination, or whether it would respond, for example, to differences in metallic contaminant levels, or any of the array of standard limnological parameters, and could therefore be useful as a general metric of overall site quality. We also wished to develop a simple model for predicting site quality using the leaf-to-root surface area ratio in *V. americana*, a model which could be applied in the field with minimal cost and effort. Finally, we sought to test the efficacy of this metric over the broad geographic range of the Laurentian Great Lakes.

Methods

Test organism

Vallisneria americana (var. *americana* Michx.; family Hydrocharitaceae) is a perennial, submersed freshwater macrophyte indigenous to eastern North America (Catling et al. 1994). It is characterized by a rosette of long, ribbon-like leaves and fibrous, unbranched roots. Sexual reproduction in this dioecious species (i.e., the species has separate male and female individuals) follows upon water pollination (Cox 1993). Extensive clonal growth occurs via the production of underground stolons and overwintering turions (Lovett-Doust and LaPorte 1991).

Study sites and sampling protocol

During the period 25 July–22 August 1995, sampling was conducted at 225 nearshore microsites throughout

PLATE 1. A sampling site in Spanish River, Lake Huron. Photograph by Kelly Potter.

the Laurentian Great Lakes (see Plate 1). For the purposes of this study, a microsite is defined as a localized sampling site ~5 m^2 in area. These microsites were located within 12 rivers, harbors, or bays which had previously been designated by the International Joint Commission (1987) as ''Areas of Concern'' (AOC; Fig. 1). Areas were sampled sequentially from the south to the north in order to sample plants at similar stages of development. At almost all of the microsites plants were observed to be flowering, thus confirming that the plants were being collected at similar points in their developmental cycle. Individual microsites within an AOC were selected by locating beds of *V. americana* at evenly spaced intervals around the perimeter of the AOC where depths ranged between ~0.5 and 1.0 m. Of course, not all sites originally considered for study could be used, due to the absence of plants at some locations. At each sampling microsite, the sediment particle size composition, the density of *V. americana* plants, and the depth of the water column were deter-

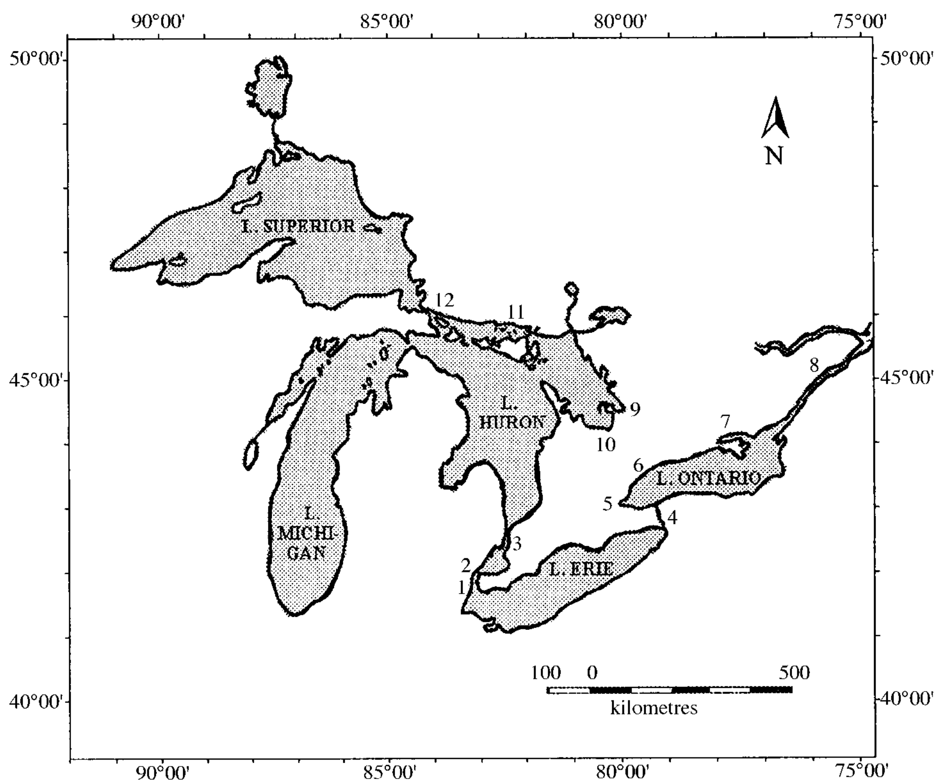

FIG. 1. Location of Great Lakes ''Areas of Concern'' sampled in 1995: (1) Detroit River, (2) Rouge River, (3) St. Clair River, (4) Niagara River, (5) Hamilton Harbour, (6) Toronto Harbour, (7) Bay of Quinte, (8) St. Lawrence River, (9) Severn Sound, (10) Collingwood Harbour, (11) Spanish River, and (12) St. Marys River.

mined. For sediment composition, the results for each microsite were converted to a coded value between 1 (particle composition most coarse) and 6 (most fine). Measures of plant density were determined by first counting the number of plants in three different 0.1-m^2 quadrats within the microsite. A mean of these counts was then calculated and multiplied by 10 to give an estimate of the number of plants per square meter. Photosynthetically active radiation at a microsite was measured at both the water column/sediment interface and the water column/air interface using a LI-COR waterproof spherical quantum sensor (model LI-193SA) attached to a LI-COR quantum/radiometer/photometer (model LI-189; LI-COR, Inc., Lincoln, Nebraska, USA). From these measurements, the proportion of incident light intensity reaching the sediment surface was calculated. Ramets of *V. americana* were extracted with a shovel and all excess sediment gently washed from the roots. Five intact, undamaged plants were used to represent each microsite. Plants were preserved in 1-L glass jars containing a 4% formaldehyde solution and stored at room temperature until analysis. Previous studies have shown that this method of preservation does not alter the surface area of the plants (Biernacki et al. 1996).

Calculation of plant measures and microsite contamination indices

The total length and mean width of all leaves, and the length and mean diameter of all roots were measured for each ramet using electronic digital calipers (Mitutoyo Digimatic Caliper; Mitutoyo America, Aurora, Illinois, USA). The leaf surface area was calculated by doubling the product of the length and the mean width (to account for both sides of the leaf) and totaling all leaves. Root surface area was calculated as for a cylinder (the roots were cylindrical rather than conical), as length $\times$ mean diameter $\times$ π. The leaf-to-root surface area ratio calculated for each microsite was the mean leaf area divided by the mean root area of each microsite's five replicate plants.

In addition to the site data collected in the field, an extensive literature review was also conducted in order to construct a comprehensive database containing independently collected information on limnological and contaminant parameters for each microsite. This information was compiled from a large pool of previously published, peer-reviewed papers and government reports (see Potter 1998). Maps of contaminant sampling sites from the literature were compared with maps of the plant sampling sites. Sites were considered to coincide if they were within 100 m of each other. Contaminant data incorporated into the database for each microsite potentially included both water column and sediment concentrations for aluminum, arsenic, barium, calcium, cadmium, chromium, copper, iron, mercury, manganese, nickel, lead, zinc, aldrin/dieldrin, chlordane, hexachlorocyclohexane (α- and β-BHC), lindane (γ-BHC), dichlorodiphenyl dichloroethylene (DDE), dichlorodiphenyl trichloroethane (DDT), endosulfan, endrin, heptachlor, hexachlorobenzene, total chlorobenzenes, methoxychlor, mirex, octachlorostyrene, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and phenol. Also included were sediment concentrations of silver, cobalt, molybdenum, oil, and tetrachlorodibenzodioxins (TCDD). Using published governmental water and sediment quality guidelines as a basis (U.S. Environmental Protection Agency 1977, 1990, 1993, Sullivan et al. 1985, Canadian Council Of Resource And Environment Ministers 1987, Environment Ontario 1988, 1989, Fitchko 1989, Newell 1989, Persaud et al. 1991, Environment Canada 1992, New York State Department Of Environmental Conservation 1993, Ontario Ministry Of Environment And Energy 1994), individual contaminant data were then assigned to one of five classes, corresponding to levels in terms of their concentration in water or sediment (value of 1 = least contaminated, 5 = most contaminated). For example, concentrations of mercury in sediment $>$2 mg/kg were assigned a score of 5, concentrations between 1.0 and 2.0 mg/kg were assigned a score of 4, concentrations between 0.3 and 1.0 mg/kg were assigned a score of 3, concentrations $<$0.3 mg/kg were assigned a score of 2, and if no mercury was detected a score of 1 was assigned. By comparison, aluminum, which is considered safe at far higher concentrations, was assigned a score of 5 if sediment concentrations were $>$30 000 mg/kg. An overall microsite contamination index was calculated, separately for each of the sediment and water phases, by dividing the sum of the contaminant codes for a site, by the maximum possible score (i.e., the number of individual contaminant indices determined times five [the maximum individual score]). Using the overall contamination indices, it was possible to compare relatively heterogeneous sites, for which we might have data on different numbers and kinds of contaminants. Furthermore, we suggest this categorical approach should diminish the effect of instrumentation and investigator variation between different studies.

Statistical analyses

Statistical analyses were carried out using SYSTAT 6.0 (SPSS 1996). Parameters were either natural log- or square root-transformed as needed to normalize their distributions, and to remove any heteroscedastic variation (Sokal and Rohlf 1995). A one-way analysis of variance was conducted to determine the relative contributions of differences both among and within microsites to variation in the leaf-to-root surface area ratio. In order to develop an equation to predict the leaf-to-root surface area ratio from measures of site quality, a multiple regression analysis was conducted. The plants' leaf-to-root surface area ratios were regressed against various measures of site quality. Parameters considered for use in the regression included measures

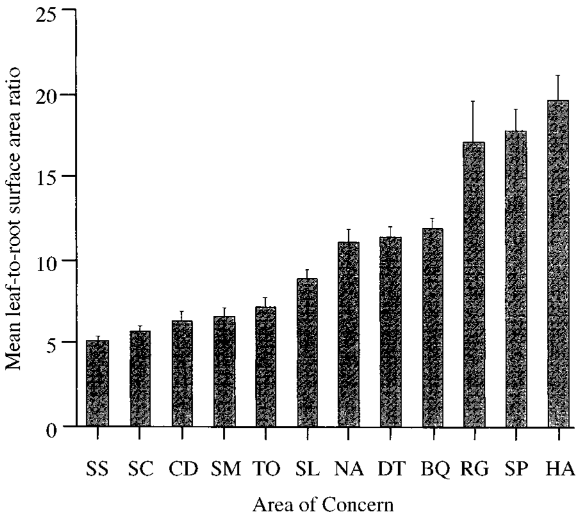

FIG. 2. Mean (+1 SE) leaf-to-root surface area ratios for plants collected from each of 12 Areas of Concern: SS = Severn Sound, SC = St. Clair River, CD = Collingwood Harbour, SM = St. Marys River, TO = Toronto Harbour, SL = St. Lawrence River, NA = Niagara River, DT = Detroit River, BQ = Bay of Quinte, RG = Rouge River, SP = Spanish Harbour, HA = Hamilton Harbour.

of the physical properties of water and sediment and observed concentrations of organic contaminants and heavy metals. Percent loss on ignition of sediments, water column conductivity, concentration of suspended solids, water current, total phosphorus, total Kjeldahl nitrogen, and chlorophyl *a* concentrations were not included in the regression due to the fact that these data were not available for all microsites. This also eliminated many problems due to the nonindependence of some of these variables (e.g., conductivity and suspended solids). We ended up with a subset of nine site parameters for use in the regression: water depth, plant density, PAR (the proportion of incident photosynthetically active radiation reaching the plants), sediment composition (coded in terms of particle size), sediment contamination, water contamination, water temperature, latitude, and rivermile (the downstream distance, in kilometers, of each site from the furthest upstream site sampled, with Sault Ste. Marie on the St. Marys River as rivermile zero, and Cornwall on the St. Lawrence River as rivermile 1350).

TABLE 1. Results of multiple linear regression, with leaf-to-root surface area ratio of *V. americana* as the dependent variable.

Parameter	Coefficient	SE	P value (two-tailed)
Sediment contamination	2.61	0.75	0.001***
Plant density	0.03	0.01	0.001***
PAR†	−0.08	0.02	0.002**
Rivermile‡	3.7×10^{-4}	1.2×10^{-4}	0.003**
Water depth	1.05	0.60	0.081
Constant	−0.86	0.83	0.304
Parameters rejected:			
Sediment composition	−0.01		0.919
Water contamination	−0.01		0.941

† Photosynthetically active radiation.
‡ Distance (km) downstream in the Great Lakes–St. Lawrence system, with Sault Ste. Marie as rivermile zero and Cornwall as rivermile 1350.
** $P < 0.01$; *** $P < 0.001$.

To further illuminate the relationship between contaminant concentrations and the leaf-to-root surface area ratio, a Spearman rank correlation was conducted with both the sediment and water contaminant indices. A Spearman rank correlation was also used to compare the leaf-to-root surface area ratios observed for plants in this study with those of a previous study which included plants from some of the same microsites harvested two years earlier.

RESULTS

Variation in leaf-to-root surface area ratio

Through one-way analysis of variance, microsite was determined to have a highly significant effect on the leaf-to-root surface area ratio ($P < 0.001$; $n = 1095$; $R^2 = 0.816$). Approximately 77% of the variance in leaf-to-root surface area ratio was accounted for by differences among microsites, with 23% of the variance occurring among individual plants within a microsite. The leaf-to-root surface area ratio ranged from 0.9 to 97.3, with a mean ratio over all sampled plants of 10.3 ($n = 1095$; 1 SE = 0.28). The mean surface area ratios for plants sampled within each of the areas of concern ranged from 5.1 for Severn Sound, to 19.6 for Hamilton Harbour (Fig. 2).

Multiple regression

A stepwise multiple regression was conducted (backward selection, P value of 0.10 to enter or remove, Sokal and Rohlf 1995). A statistically significant regression line was obtained with an R^2 of 0.294 ($n = 133$, $P < 0.001$). In other words, more than one quarter of the variation in the leaf-to-root surface area ratio could be explained by these parameters (Table 1). Water contamination, latitude, temperature, and sediment composition were then rejected from the regression due to their high P values ($P > 0.10$). The remaining parameters, which were significant in the regression, included the sediment contamination index ($P = 0.001$), plant density ($P = 0.001$), photosynthetically active radiation ($P = 0.002$), and rivermile ($P = 0.003$). The regression equation was:

$$\begin{aligned}\log(\text{leaf-to-root surface area ratio}) &= 2.608(\sqrt{\text{sediment contamination}}) \\ &\quad + 1.049(\sqrt{\text{water depth}}) + 0.031(\sqrt{\text{plant density}}) \\ &\quad - 0.075(\sqrt{\text{PAR}}) + 3.7 \times 10^{-4}(\text{rivermile}) \\ &\quad - 0.858. \qquad (1)\end{aligned}$$

TABLE 2. Comparison of regression coefficients for the two model equations.

Parameter	Equation 1 coefficient	Equation 2 coefficient	Difference between equation values
Sediment contamination	2.608	1.767	0.841
Plant density	0.031	0.019	0.012
PAR†	−0.075	−0.071	0.004
Rivermile‡	3.7×10^{-4}	-2.2×10^{-4}	5.9×10^{-4}
Water depth	1.049	−1.171	2.220

† Photosynthetically active radiation.
‡ Distance (km) downstream in the Great Lakes–St. Lawrence system, with Sault Ste. Marie as rivermile zero and Cornwall as rivermile 1350.

A Spearman rank correlation was also conducted between the leaf-to-root surface area ratio and the sediment and water contaminant indices. This again showed a significant correlation (0.257; $P < 0.01$; $n = 138$) between sediment contaminant indices and the leaf-to-root surface area ratios, but there was no significant correlation with water-borne contaminants.

Regression using additional data

Seventy-three cases were originally excluded from the analysis; these cases were missing data on water contamination, but had data for all remaining variables. Since water contamination was not found to be a necessary component, and therefore was excluded from the final regression equation, we were able to conduct a second multiple regression using only the ''extra'' 73 cases, and incorporating the same parameters as in the first regression equation. Through a standard multiple regression, the following equation was obtained:

$$\begin{aligned}\log\ &(\text{leaf-to-root surface area ratio})\\ &= 1.767(\sqrt{\text{sediment contamination}})\\ &\quad - 1.171(\sqrt{\text{water depth}}) + 0.019(\sqrt{\text{plant density}})\\ &\quad - 0.071(\sqrt{\text{PAR}}) - 2.2 \times 10^{-4}(\text{rivermile})\\ &\quad + 2.137. \qquad (2)\end{aligned}$$

Comparison of the coefficients obtained for each of the regression analyses are shown in Table 2. To test whether there was a significant difference in the slopes of the two lines, a paired comparison t test of the regression coefficients was conducted. For a t value of 1.417, there was no significant difference between the two sets of coefficients (critical t value for four degrees of freedom at a probability of 0.05 is 2.776). Hence, since the two lines had similar slopes, we pooled the data from the two regressions to produce a single regression equation:

$$\begin{aligned}\log\ &(\text{leaf-to-root surface area ratio})\\ &= 1.352(\sqrt{\text{sediment contamination}})\\ &\quad + 0.246(\sqrt{\text{water depth}}) + 0.028(\sqrt{\text{plant density}})\\ &\quad - 0.087(\sqrt{\text{PAR}}) + 1.054. \qquad (3)\end{aligned}$$

Results of the pooled regression are shown in Table 3. With all cases combined, similar results were obtained as for the initial model. Parameters that displayed statistical significance in the regression were, again, sediment contamination ($P < 0.01$), photosynthetically active radiation ($P < 0.001$), and plant density ($P < 0.001$).

Comparison of leaf-to-root surface area ratios with previous studies

Many of the sites sampled from the Detroit River, Rouge River, and St. Clair River in this study corresponded with sampling sites used by Biernacki et al. (1996). To test whether the surface area ratios of *V. americana*, when determined by different researchers and in different years, would still show the same relative ranking of site quality, a Spearman rank correlation was conducted. In total, 56 microsites were found to be shared between this study and that of Biernacki et al. (1996). The Spearman rank correlation statistic, 0.45, was much larger than the critical value of 0.26 ($n = 56$; $P < 0.05$) and was significant at $P < 0.001$. Both studies showed the highest ratios (and potentially poorest site quality) in three sites: the Trenton Channel, at the mouth of the Rouge River, and at the mouth of the Ecorse River in the Huron–Erie corridor.

Regressions using separate contaminant indices for metal and organic compounds

A significant regression effect due to an overall sediment contamination index was observed (Table 3). We then explored whether this truly was a combined effect due to both metals and organics. We subdivided the contamination index into two separate components: sediment metals and sediment organics. Table 4 shows the result of the multiple linear regression, but with the two separate contamination components. Again the overall regression was highly significant ($P < 0.001$; $n = 138$; $R^2 = 0.283$), and the parameters of plant density and light intensity were significant ($P = 0.007$ and $P = 0.003$, respectively). The index for sediment metal contamination was positive and highly significant

TABLE 3. Results of regression using pooled data.

Parameter	Coefficient	SE	P value (two-tailed)
Sediment contamination	1.352	0.482	0.006**
Plant density	0.028	0.007	0.000***
PAR†	−0.087	0.017	0.000***
Rivermile‡	1.00×10^{-4}	9.17×10^{-5}	0.277
Water depth	0.246	0.486	0.613
Constant	1.054	0.565	0.063

† Photosynthetically active radiation.
‡ Distance (km) downstream in the Great Lakes–St. Lawrence system, with Sault Ste. Marie as rivermile zero and Cornwall as rivermile 1350.
** $P < 0.01$; *** $P < 0.001$.

TABLE 4. Results of multiple linear regression using separate sediment contamination indices for metals and organics.

Parameter	Coefficient	SE	*P* value (two-tailed)
Sediment metals	1.019	0.217	0.000***
Sediment organics	−0.135	0.146	0.356
Plant density	0.022	0.008	0.007**
PAR†	−0.059	0.019	0.003**
Rivermile‡	1.08×10^{-5}	1.19×10^{-4}	0.928
Water depth	0.588	0.555	0.291
Constant	2.279	0.566	0.000***

† Photosynthetically active radiation.

‡ Distance (km) downstream in the Great Lakes–St. Lawrence system, with Sault Ste. Marie as rivermile zero and Cornwall as rivermile 1350.

** $P < 0.01$; *** $P < 0.001$.

in the regression ($P < 0.001$), but the index for sediment organic contamination was not significant.

DISCUSSION

This study consisted of a wide-scale survey in which many variables could not be controlled, and in which there was, no doubt, variation in unmeasured environmental factors from site to site. This may explain why the R^2 value of the regression, although statistically significant, was somewhat low, accounting for only one quarter of the variation. Nevertheless, in spite of all the background noise in terms of other factors which may affect plant growth, we did still detect highly significant effects of the four environmental parameters: sediment contamination concentration, *V. americana* plant density, and photosynthetically active radiation, as well as rivermile (the distance of an area downstream of Sault Ste. Marie).

The regression coefficient for PAR is negative. This likely reflects an etiolation response by the plants, i.e., at lower radiation levels the leaves must grow longer to obtain enough light for photosynthesis, resulting in higher leaf-to-root surface area ratios. Other researchers have also found that shoot length in *V. americana* increases at lower light intensities (Twilley and Barko 1990, Barko et al. 1991). An etiolation response may also explain why the regression coefficient for plant density is positive. As Titus and Stephens (1983) found, the presence of neighboring plants influenced the growth pattern of *V. americana* (through competition for light due to shading), resulting in taller individuals.

The high significance of sediment contamination in the regression is of particular interest. This result suggests that there is just as strong a response of plant growth form to contaminants in the sediment as there is to light intensity and plant density. Since there was no relationship between concentration of water contamination and growth form of the plants, it is likely that the main source of contaminant uptake for the plants is from the sediment, via the roots. Numerous researchers have reported that rooted, submersed macrophytes primarily derive their nutrients from the sediment (e.g., Carignan and Kalff 1980, Barko and Smart 1981, Pip 1990). Except in extreme hypereutrophic waters, uptake from sediments has been found to account for almost all phosphorus uptake in *V. americana* (Carignan and Kalff 1980). Aquatic plants have also been found to accumulate trace metals primarily through the roots. Arsenic, lead, zinc, chromium, mercury, nickel, cadmium, and copper have all been found to accumulate to a greater extent in the roots than in the shoots of submersed plants (Ray and White 1976, Pip and Stepaniuk 1992, Reimer and Duthie 1993, Gupta and Chandra 1994, Dushenko et al. 1995). Organic contaminants have likewise been reported to accumulate primarily in the roots of *V. americana,* suggesting that for these substances, also, most uptake occurs from the sediments (Lovett-Doust et al. 1994*a,* Biernacki et al. 1996). St-Cyr and Campbell (1994) found that the spatial pattern of heavy metal contamination of *V. americana* corresponded with the spatial distribution of heavy metal contamination in surficial sediments. However, it must also be noted that many factors can influence the bioavailability and uptake of contaminants to aquatic plants. Giblin et al. (1980) found that sediment concentrations of some metals were reflected by the vegetation, but there was also a lack of correlation for other metals between plant and sediment concentrations. They suggested that factors such as pH, salinity, and redox potential might affect movement of metals from sediments to plant tissue.

In addition to the above, sediment contamination may appear to affect the leaf-to-root surface area ratio more than water contamination because measurements of contamination in the sediment are more reliable and indicative of site quality, while concentrations of pollutants in the water column are transient and can fluctuate greatly over short periods of time. Periodic measures of contaminant concentrations in the water column will only describe current conditions, and may not give an accurate representation of site quality if contaminants are being diluted or released in pulses (Lovett-Doust et al. 1993).

Using the third (overall) equation developed in this study (which included all microsites), and measures of PAR, plant density, and the leaf-to-root surface area ratio of *V. americana,* it should also be possible to predict the severity of sediment contamination. The use of this model could provide a valuable, inexpensive resource management tool for evaluating site quality. The site-specificity of the leaf-to-root surface area ratio would also allow for evaluation of sediment toxicity among individual microsites within areas of concern, perhaps providing a means for mapping point source impact zones and pinpointing point sources (see International Joint Commission 1987). For example, when plotted on a map of the area, the leaf-to-root surface area ratios for microsites in the Bay of Quinte showed several potential localized ''hotspots'' (Fig. 3). The lo-

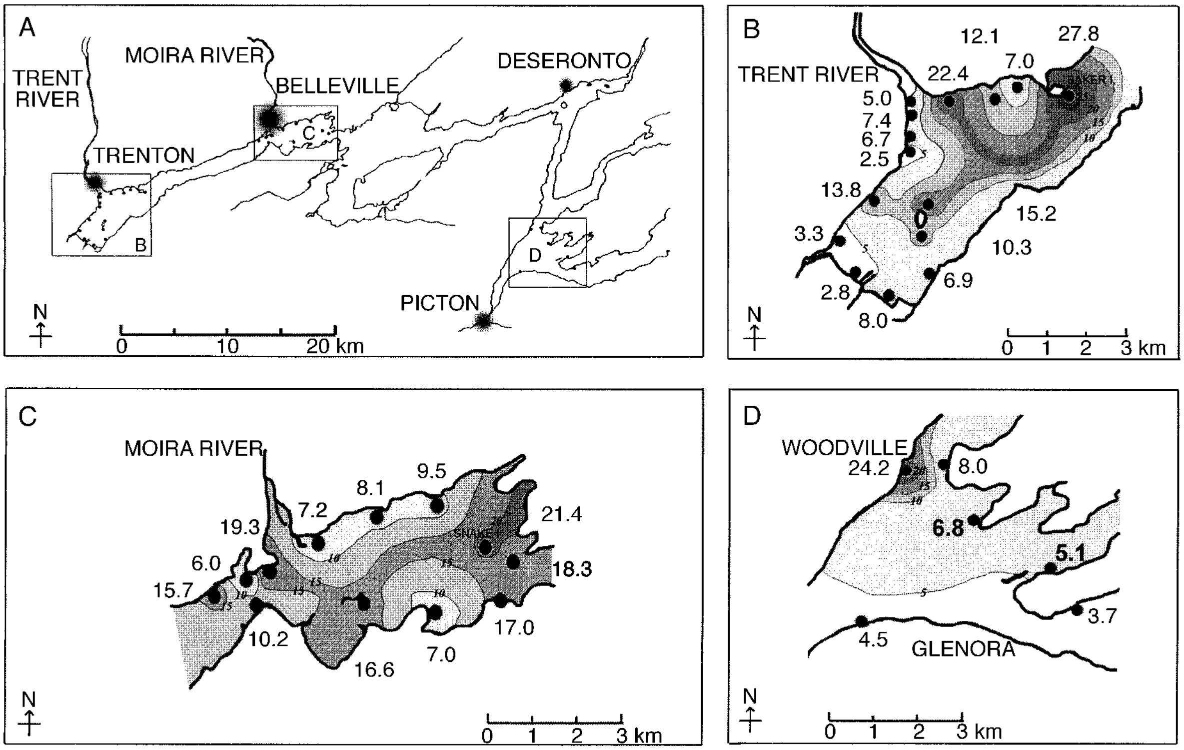

FIG. 3. Maps of potential point source impact zones from the leaf-to-root surface area ratios of *Vallisneria americana.* (A) Map of the Bay of Quinte Area of Concern. Microsite sampling was conducted within the three boxed areas. (B, C, D) Enlarged views of the sampling areas near Trenton, Belleville, and Picton, respectively. Microsite locations are indicated by dots, and mean leaf-to-root surface area ratios for plants collected from each of the sampled microsites are shown. Based on these measured ratios, interpolated isoclines of plant morphology have been mapped to indicate the contours of potential point source impact zones.

cations showing *V. americana* with the highest surface area ratios, and presumably the poorest site quality, appear on the downstream side of the mouth of the Trent River, at Baker Island, at Snake Island, and beside Woodville. Several of these sites are located downstream or adjacent to known pollution point sources. The site at the mouth of the Trent River is directly downstream from both a packaging plant and a sewage treatment plant. The Baker Island site is located adjacent to the Canadian Forces Base Trenton, whose sewage treatment plant has been cited as a point source for nutrient loading into the bay (Hartig and Law 1994). The Snake Island site is directly adjacent to a manufacturer of phenolic resins.

The implementation of our environmental monitoring procedure based on leaf-to-root surface area ratio is relatively simple and could therefore be easily applied as a routine biomonitoring program for the purpose of ranking the severity of impact at AOCs, tracking remediation, and identifying point source impact zones within an AOC. Within the present protocol we are seeking to improve the efficiency of making surface area measurements. Preliminary results suggest that computer image analysis can speed up the measurement process 400% over the manual methods used here, thereby reducing the processing time from 2 h/plant, by manual methods, to ~30 min/plant (J. VanDerWal, *unpublished data*). We have also shown that the relative ranking of sites by different investigators, as well as from year to year, are relatively consistent and can be validly compared. There was a highly significant agreement between the ranking of our leaf-to-root surface area ratio measurements for plants collected in 1995, and those of Biernacki et al. (1996) collected from 56 of the same sites in 1993. The fact that both of these studies were able to pinpoint the same locations as having poor site quality further reinforces the reliability of this method. Biernacki et al. (1996, 1997) have also shown that the leaf-to-root surface area ratio could be used in comparing samples collected in different growing seasons. We would suggest, however, that for the purpose of comparing ratios between years, studies should always be conducted at the same stage of plant development. The leaf-to-root surface area ratio of *V. americana* does change with time, increasing up to maturity as the plants grow (Biernacki et al. 1996). Therefore, plants collected at different stages of maturity cannot be reliably compared. Also, rates of contaminant uptake may change over the course of a growing season, with maximal uptake likely occurring during periods of peak biomass production. Seasonal differences in the metal content of *Spartina* sp. growing on contaminated marsh sediment were observed by Giblin et al. (1980). These differential rates of contaminant uptake would also likely affect the leaf-to-root surface area ratios.

Our regression analyses with separate sediment metal and organic contaminant indices indicated a highly significant effect due to metals, but no significant effect by the organics. These results appear to contradict the findings of Biernacki et al. (1996) who did find a significant correlation between sediment organochlorine contamination and the leaf-to-root surface area ratio of *V. americana*. However, Biernacki et al. did not examine metal contamination in their study.

Of the Canadian AOCs examined in this survey, Hamilton Harbour and Spanish Harbour contained plants with the highest mean leaf-to-root surface area ratios (Fig. 1), suggesting that they also may be the locations with the poorest overall site quality. High ratios were also observed for the Bay of Quinte, the Detroit River, and the Niagara River. Many of these areas of concern are geographically extensive and have numerous different site impairments that may require a variety of targeted remedial approaches. Consequently, it may be of even greater importance for governments or organizations funding remediation projects to pinpoint the smaller areas within an AOC that are particularly problematic. Of the microsites examined in this survey, the locations which produced plants with the highest leaf-to-root surface area ratios include the area directly adjacent to Fort Erie on the Niagara River, the northeast corner of Hamilton Harbour, the northeast corner of Spanish Harbour where the Spanish River enters, the Trenton Channel of the Detroit River, and the mouth of the Rouge River where it empties into the Detroit River at Zug Island. All of these locations are either adjacent to, or directly downstream from, major industrial discharges. The Fort Erie site is directly adjacent to a manufacturer of lead acid storage batteries, and in the same vicinity as a chemical plant, and a manufacturer of airplane and satellite components (Ontario Ministry Of Environment And Energy 1993). The Hamilton Harbour site is located directly across from two large steel manufacturers. The Spanish Harbour site is downstream from a pulp and paper mill (Spanish Harbour RAP Team 1993). The Trenton Channel is lined with more than a half dozen industries including a steel factory, an oil refinery, a car manufacturer, a chemical plant, and a power plant (Michigan Department Of Natural Resources and Ontario Ministry Of Environment And Energy 1991). The mouth of the Rouge River in Michigan is the site of a large steel plant and a coal-burning power plant (Southeast Michigan Council Of Governments 1988). Although the model generated in this study does not directly prove that discharges from these point sources are the cause of the larger surface area ratios observed in *V. americana* plants collected from these sites, it seems to us plausible that these point sources may be responsible for impaired site quality at these locations.

Future work should include collection of a new, independent data set to test the validity of the pooled regression model developed here. Further sampling could be conducted at the four Canadian Areas of Concern on Lake Superior which were not sampled during this study. The focus so far has been on sites identified by the federal governments of Canada and the USA as ''Areas of Concern''. It would be useful to collect data from ''clean'' reference sites as well, in order to set a baseline and targets for remediation. There may also be additional sites that merit assessment as candidates for designation as AOCs, and our survey approach could identify or eliminate such sites from further consideration. It would also be advantageous to collect direct field measurements of additional physical parameters that may influence the surface area ratio. By adding other parameters to the model, it may be possible to obtain a greater R^2 value, and to obtain a model that will account for a greater fraction of the variation observed in the plants.

Acknowledgments

We thank Maciej Biernacki for help with field sampling and advice on data analysis. Jon Lovett-Doust and David Dolan provided valuable input at various stages of this work. Additional statistical advice was offered by Jan Ciborowski and Robyn Nease. This work was supported by an NSERC grant to L. Lovett-Doust, and a grant from Ontario Ministry of Environment and Energy to J. Lovett-Doust and L. Lovett-Doust.

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