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LABORATORY ASSAY OF SEDIMENT PHYTOTOXICITY USING THE MACROPHYTE *VALLISNERIA AMERICANA*

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Abstract—In contrast to their ecological importance, submersed rooted macrophytes have been overlooked in environmental science. Presently, the array of standard phytotoxic bioassays includes only one free-floating vascular macrophyte (*Lemna*) and several algal species. A short-term and inexpensive assay was studied for feasibility in evaluating sediment quality. Cloned ramets of the macrophyte *Vallisneria americana* were used to test phytotoxicity of sediments collected at different locations in the Detroit River. Ramets were planted in sediment samples and placed in greenhouse aquaria. After a week of exposure, ramets of *V. americana* were destructively sampled and preserved. The leaf and root surfaces areas were determined, and plant biomass was recorded for each ramet. An index of the leaf-to-root surface area ratio was a reliable predictor of sediment phytotoxicity; the ratio of leaf-to-root mass was also useful but proved less consistent. Ramets grown in sediments that were relatively less contaminated with organic compounds had lower values of the leaf-to-root surface area ratio, while plants grown in more contaminated sediments had greater values. Results of analyses of variance indicated that the index of leaf-to-root surface area ratio responded to sediment quality but was not significantly affected by either variation in plant genotype or interaction between sediment and plant genotype. There was a significant correlation ($p < 0.001$) between rank-ordered results of the present greenhouse study and results of leaf-to-root surface area ratios for plants previously surveyed in the field.

Keywords—American wildcelery Submersed plant Genet Contamination Leaf/root surface areas

INTRODUCTION

The role of rooted macrophytes in the aquatic ecosystem

Submersed rooted macrophytes play an important role in aquatic ecosystems. They form part of the base of the trophic food web. Rooted plants also provide shade, substrate, shelter, nursing areas, and food for a host of organisms [1–4]. Macrophytes also yield high-quality detritus that is very important in benthic and pelagic food webs and may serve to buffer seasonal variations in available plankton [5]. Macrophytes increase sedimentation and decrease soil erosion; they increase water clarity and, by linking elements of the sediment with the water column, are important in macro- and microelement uptake, storage, and cycling in aquatic environments [6–8]. The importance of functional roles that submersed macrophytes typically provide to surrounding components of the ecosystem is even more significant and evident in highly disturbed and contaminated areas, such as the designated areas of concern in the Great Lakes of North America [9]. In areas of concern, beds of submersed macrophytes have been reported to support the greatest diversity and highest density of young-of-the-year of commercially important fish species [10].

Potential of submersed macrophytes as biomonitors in aquatic ecosystems

Recently, submersed rooted macrophytes have been shown to be involved in the uptake, bioaccumulation, and movement of toxic metals and organochlorine contaminants in aquatic ecosystems [11–17]. The ability of plants to bioaccumulate and, in some cases, biotransform toxic compounds into less toxic forms is largely unexplored. However, some recent 'phytoremediation' studies have documented great potential for aquatic plants to bioaccumulate nutrients, metals, and organics from contaminated sediments and/or water [18–21]. Living at the boundary between the sediment layer and the water column, rooted plants have great potential for biomonitoring toxic metals [13,14] and organochlorine contaminants [15,16,22–24]. They may be superior to algae or free-floating plants when contaminated sediments are the major source of impairment. Some studies have shown that species of rooted macrophytes may be more sensitive to lower concentrations of herbicides and organochlorine contaminants than free-floating plants [16,25].

Despite the many functional roles that plants play in aquatic ecosystems and their demonstrated ecological importance, submersed rooted macrophytes tend to be overlooked in remediation studies, rarely used in ecosystem assays, and not required in standard screening procedures for the introduction and registration of new chemicals, including herbicides that target vascular plants [25–28]. Many reasons exist for this neglect. Some obstacles to their use in laboratory assays are their large size, relatively slow growth, relatively long growth cycle, technical difficulties in studying roots, the lack of standard analytical methods for tissue contaminant analyses, the lack of standarized plant material, and the lack of established test methods and assay endpoints. In several recently published studies, *Vallisneria americana* was used, in both greenhouse experiments [22,23] and field studies [15–17,24], and a number of the above problems were resolved. These studies showed that *V. americana* is noticeably responsive to changes in environmental quality and potentially very useful in both short- and long-term studies of environmental quality. Plant growth can be standardized and a number of growth assays that mea-

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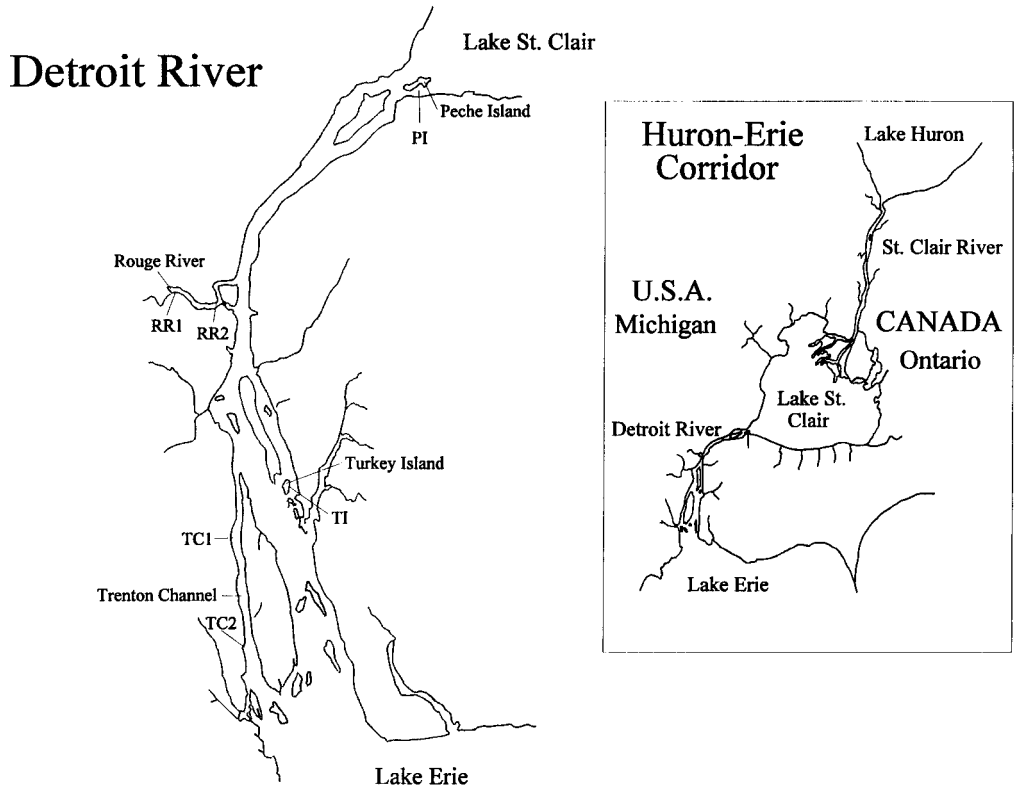

Fig. 1. Map of the Detroit River indicating sites of sediment sampling. Sites are labeled in the Rouge River as RR1 and RR2, in the Trenton Channel as TC1 and TC2, at Turkey Island as TI, and at Peche Island as PI. Insert shows location of Detroit River in the Huron–Erie corridor.

sure levels of contaminants in both the external environment and plant tissues can be utilized.

Several studies have shown a significant correlation between rates of root and/or shoot elongation in plants exposed to contaminants and contaminant concentrations [29,30]. Our earlier studies showed that contaminants affect not only elongation of roots and leaves but also changes in the number of leaves and roots per ramet, leaf width, root diameter, and patterns of biomass allocation to leaves and roots [22,23]. Some of these changes in plant morphology may be reflected in changes in the leaf and root surface areas of a plant. Shifts in leaf and root dimensions may occur very rapidly; significant differences can be observed within less than 1 week [23,29]. (For comparison, significant changes in other measures of plant performance, such as rate of clonal growth, frequency of flowering, and leaf production per ramet, have been observed after 5 weeks of exposure [22].) Furthermore, even at very low concentrations of organic contaminants, a significant change in relative root and shoot growth can occur before measureable increases in enzyme activity [29].

The objectives of this study were to develop a simple method for controlled sediment evaluation, to estimate the potential of selected clones of *V. americana* as a short-term bioassay, and to evaluate the reliability and utility of the method by comparison with field results.

MATERIALS AND METHODS

An experiment was carried out in the greenhouse of the University of Windsor (Windsor, ON, Canada) in June 1995. Ramets (shoots) of *V. americana* were planted in sediments collected at different locations. During the experimental period water temperature ranged from 22 to 24°C (measured at noon at bottom of aquaria). Plants were exposed to the natural photoperiod, and light quanta ranged from 2,000 μmol s^{-1} m^{-2} on sunny days to 400 μmol s^{-1} m^{-2} on cloudy days (measured at noon at floor level at the bottom of aquaria). Dechlorinated tap water (pH 7.1) was used, and no additional nutrients were given. To minimize evaporation, aquaria were covered with Plexiglas®.

Sediment

Sediments were collected from six sites in the Detroit River (see Fig. 1). Two samples were taken from the Trenton Channel (sites TC1 and TC2, see Fig. 1), two were taken from the Rouge River (RR1 and RR2), one was taken off Turkey Island (TI), and one was taken off Peche Island (PI). Samples of sediment were collected at a water depth of 1 m (approx. the top 12–15 cm of the sediment layer), placed in sealed polyethylene bags, and stored for transport in large, dark plastic containers. Within 2 to 3 h following collection, sediments were stored in a cold room at 6 °C. Within 1 week sediments were placed in 500-ml glass jars (9.5 cm tall) and set up in the aquaria filled earlier with water. Each aquarium (capacity, 175 L; 92 cm [length] × 31 cm [width] × 62 cm [height]) contained six jars filled with sediment originating from the same site. Three replicate aquaria were used for each sediment treatment (each with six jars). A total of 18 aquaria were used (with a total of 108 ramets planted individually in jars).

Experimental plants

Plants of *V. americana* var. *americana* (native to North America) and of *V. americana* var. *biwaensis* (native to South America and Asia) (Hydrocharitaceae) (classified after Lowden [31]) were used in the study. Ramets of selected genets

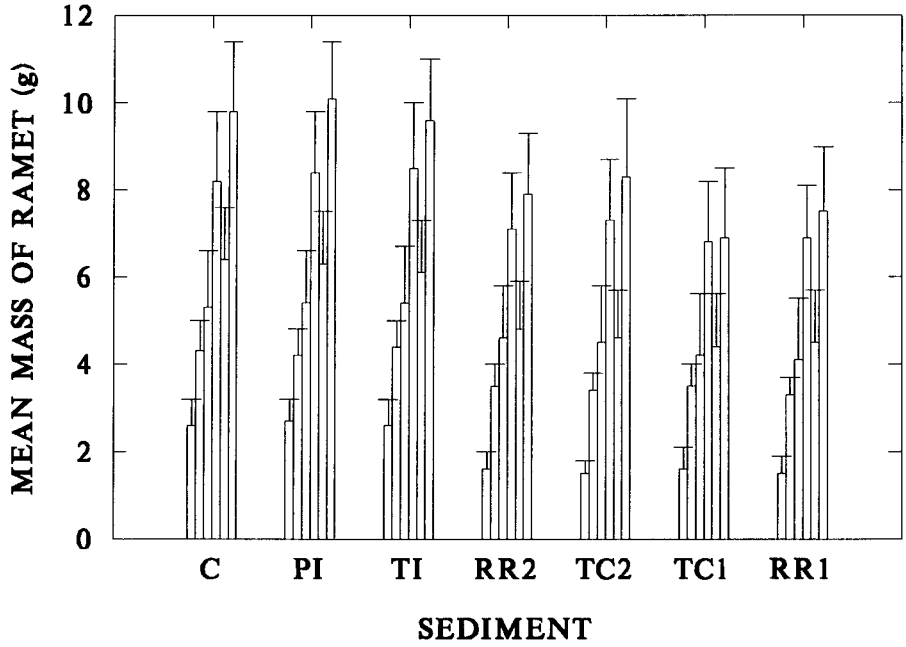

Fig. 2. Mean biomass (±SE) of ramets planted in different sediments: C, control before initiation of experiment; PI, Peche Island sediment; TI, Turkey Island sediment; TC1 and TC2, Trenton Channel sediments; and RR1 and RR2, Rouge River sediment. Each of the six bars per sediment represents one of the six genetic lines (from 1 on the left to 6 on the right; see ''Materials and Methods'' for descriptions) used in the study.

had been cultivated prior to the study (see Biernacki [17] for details). Individual genets are capable of producing many identical ramets each growing season [4]. Only well-developed, undamaged ramets were used in the study. The following genets were selected: (1) a male plant of *V. americana* var. *americana*, originating from the Chenal Ecarte area of the St. Clair River and found previously to be tolerant of high concentrations of trichloroethylene [22] and subsequently cultivated in the greenhouse; (2) the same genetic individual as in item 1, but subsequently cultivated in the field; (3) a female plant of *V. americana* var. *americana*, originating from the mouth of the Rouge River and cultivated in the greenhouse; (4) the same genetic individual as in item 3, but cultivated in the field; (5) a female plant of *V. americana* var. *americana*, originating from Turkey Island in the Detroit River and cultivated in the field; and (6) a plant of *V. americana* var. *biwaensis*, of unknown sex (plant did not flower) and cultivated in the greenhouse. In order to standardize initial plant conditions, all ramets of each genet (regardless of their cultivation history) were planted in the greenhouse in containers filled with a mix of 80% sand by volume, 18% silt, and 2% clay (pH 7.2) and grown for 2 weeks. Ramets selected for the study initially had four or five leaves. One ramet was planted per jar. Each aquarium had plants of the six genetic lines.

Data collected

Prior to the experiment, samples of three ramets from each of the six genetic lines were preserved in 4% formaldehyde to enable determination of initial levels of growth. After 1 week of exposure to the experimental sediments, ramets from each of the six genetic lines were removed from the aquarium and preserved. When ramets were removed, sediment around the roots was carefully washed in order to get intact plants and undamaged roots. The preserved plants were subsequently analyzed individually, and the number of leaves, the width and length of each leaf, the number of roots, the diameter and length of each root, and the biomass of leaves and roots were determined. Leaf and root dimensions of harvested ramets were measured using a digital micrometer. A total of 126 ramets was measured: 18 ramets (representing the six genetic lines) that had been collected prior to the experiment (controls, indicated on Figs. 2 and 3 as ''C'') and 108 ramets exposed to sediments. Surface areas of leaves and roots were calculated, and the mean of three ramets per genetic line was used for all subsequent analyses. Measures of plant performance were used to estimate adverse effects of each sediment on plant growth.

Comparison of laboratory-derived data with field observations

To extend conclusions from this short-term laboratory test, results were compared with data for leaf and root surface areas from an independent survey of *Vallisneria* ramets in the field, at the same sites of sediment collection, carried out in 1993 [24]. Plants surveyed in 1993 in the field at sites of subsequent sediment collection were measured, and leaf-to-root surface area ratios were calculated in the same way as for plants in the present laboratory test. To compare field and laboratory studies we used Spearman rank correlation analysis; for both studies sediments were ranked increasingly from those that caused the greatest leaf-to-root surface area ratio to those that induced the lowest ratio.

Comparison with Microtox® assay

Giesy et al. [32] reported results of a Microtox® assay for 136 sediment samples from the Detroit River, including the Trenton Channel and Rouge River. Site RR1 in our study was the same as site 203 in the study of Giesy et al. [32] our site RR2, site 198, our site TC1, site 110; and our site TC2, site 42. Site 83 in Giesy et al. [32] study was very close to our site TI. However, it is important to note that sediments for the two studies were collected in different years; hence, we compared results using a nonparametric Spearman rank correlation analysis on the relative ranks of sediment toxicity reported in both studies. Sediments from Giesy et al. [32] were ranked in

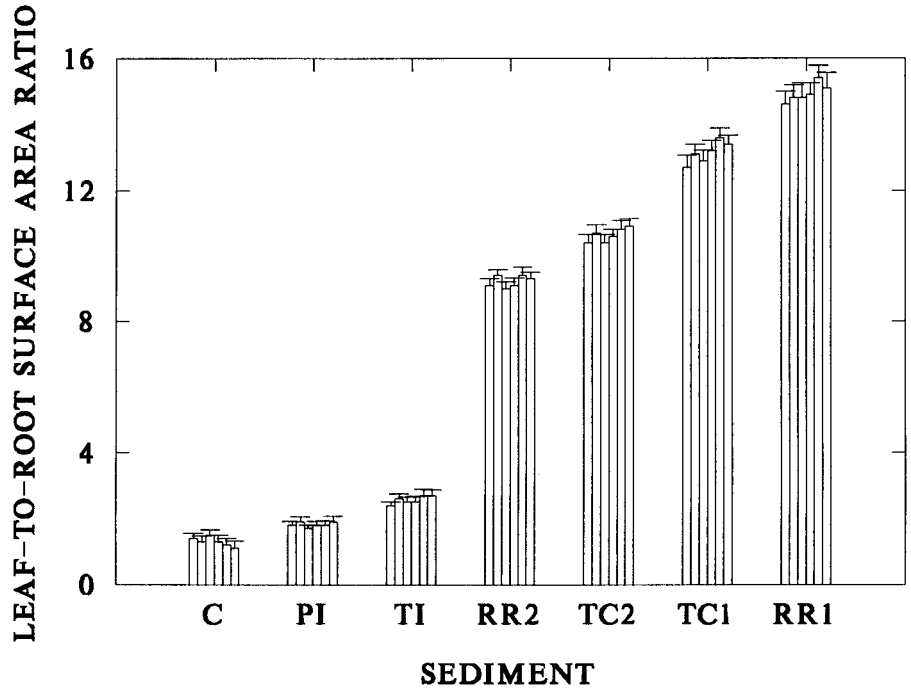

Fig. 3. Mean leaf-to-root surface area ratios (±SE) of ramets planted in different sediments: C, control before initiation of experiment; PI, Peche Island sediment; TI, Turkey Island sediment; TC1 and TC2, Trenton Channel sediments; and RR1 and RR2, Rouge River sediment. Each of the six bars per sediment represents one of the six genetic lines (1 to 6) used in the study.

increasing order from the most toxic to least toxic, and sediments in our study were ranked also in order from the one that induced the greatest leaf-to-root surface area ratio in *Vallisneria* to that with the lowest.

Statistical analyses

Data were analyzed using SYSTAT® for Windows®, version 5.03 [33], (analysis of variance), and, where appropriate, differences between mean values were tested for significance using Tukey's honestly significant difference pairwise comparison tests.

RESULTS

Analysis of variance revealed significant effects of the sediment, genetic line, and, though less significant, their interaction for nearly all variables (see Table 1). The number of leaves per ramet did not change significantly in ramets. Plant measures most significantly affected were the indices of leaf-to-root surface area ratio, the leaf-to-root biomass ratio, the surface area of leaf per gram of leaf tissue, and the surface area of root per gram of root tissue. However, the only measure that responded to sediment effects but was not affected by other factors (genetic line and interaction) was the ratio of

Table 1. Summary of analysis of variance of effects of sediment and genetic line on morphological parameters in *Vallisneria americana*[a]

Variable	Sediment	Genetic line	Sediment × genetic line
Number of leaves	NS	0.041*	NS
Number of roots	0.011*	0.023*	NS
Leaf-to-root number ratio	0.037*	0.019*	NS
Leaf mass	0.042*	0.021*	0.025*
Root mass	0.011*	0.038*	0.016*
Ramet biomass	0.036*	0.024*	0.029*
Leaf-to-root mass ratio	0.0041**	0.031*	NS
Root diameter	0.014*	NS	0.039*
Surface area of a leaf	0.033*	0.027*	0.026*
Surface area of a root	0.012*	0.042*	0.037*
Surface area of leaves per ramet	0.034*	0.035*	0.038*
Surface area of roots per ramet	0.012*	0.027*	0.035*
Leaf-to-root surface area ratio	>0.0001***	NS	NS
Surface area of leaves per gram of leaf tissue	0.0053**	0.026*	0.019*
Surface area of roots per gram of root tissue	0.0029**	0.037*	0.017*

[a] Analyses were based on measurements of 108 ramets.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. NS, not significant ($p > 0.05$).

Table 2. Regression analyses of changes in leaf-to-root surface area ratio and other morphological parameters in *Vallisneria americana* (only significant relations are shown)[a]

Variable	r^2	p
Number of roots	0.09	*
Leaf-to-root number ratio	0.11	*
Root mass	0.09	*
Leaf-to-root mass ratio	0.31	***
Root diameter	0.10	*
Surface area of a leaf	0.14	*
Surface area of a root	0.09	*
Surface area of leaves per ramet	0.19	**
Surface area of roots per ramet	0.12	*
Surface area of leaf per gram of leaf tissue	0.18	***
Surface area of roots per gram of root tissue	0.21	**

[a] Analyses were based on measurements collected from 108 ramets. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

leaf-to-root surface area. Further analyses showed no significant differences between female and male genets ($p = 0.51$) and no significant differences in leaf-to-root surface area ratio between plants that had been cultured in the field and those cultured in the greenhouse ($p = 0.45$). However, plants cultured in the field (genetic lines 2 and 4) had significantly ($p < 0.0001$) greater biomass than plants cultured in the greenhouse (genetic lines 1 and 3) (see also Fig. 2).

Regression analyses of various measures of plant performance on leaf-to-root surface area ratio revealed significant functional relationships (Table 2). The leaf-to-root biomass ratio and the surface area of leaf per gram of leaf tissue were significantly affecting changes in leaf-to-root surface area ratio. Across all the ramets we observed, changes in leaf-to-root biomass ratio accounted for 31% of the variance in leaf-to-root surface area ratio. Further analysis including only smaller ramets (below 4 g) indicated that changes in leaf-to-root biomass ratio explained nearly 65% of the variance in leaf-to-root surface area ratio.

Ramet mass changed measurably over the relatively brief study period. Final biomass of ramets tended to be lower following growth in sediment originating from the Rouge River and the Trenton Channel than in sediment from Turkey Island or Peche Island (Fig. 2). However, only for smaller ramets (those below 4 g, $n = 44$) were these differences significant ($p = 0.0063$). Overall there were no significant differences in final biomass of ramets, and changes in biomass of ramets were not used in ranking sediment quality.

Sediment type significantly affected changes in leaf-to-root surface area ratio (Table 1 and Fig. 3). The ratio ranged from 2 to 15 following 1 week of exposure to sediment treatments. The lowest value of leaf-to-root surface area ratio was with the Peche Island sediment sample; the highest was for Rouge River sediment collected at site RR1. Values of the ratio were three to five times greater for Trenton Channel and Rouge River sediment samples than for Peche Island and Turkey Island sediment samples.

Comparison of laboratory-derived data with field observations

A significant difference ($p < 0.0001$) between the absolute value of leaf-to-root surface area ratio of *Vallisneria* ramets collected in a field survey in 1993 and the absolute value of the ratio found in ramets in the present laboratory study was observed. In a large field survey of leaf-to-root surface area ratios in natural populations of *Vallisneria*, values ranged from four (at the Peche Island location) to 89 (at site RR1) [24]. In the present laboratory test, values ranged from 2 to 15 for the six different sediment samples studied. However, no significant difference was observed between the relative rankings of sediment quality in the field survey and the present laboratory test (Spearman rank correlation analysis, $p_s < 0.0001$; with Bartlett χ^2 statistics, $p < 0.001$).

Comparison of Vallisneria *phytotoxicity test with Microtox assay*

Spearman rank correlation analysis carried out on the rankings of relative toxicity of sediments in a Microtox assay, reported by Giesy et al. [32] for sediments sampled at the same locations as in the present study, and on the rankings of relative phytotoxicity of these sediments to *Vallisneria* in the present study revealed a marginally significant correlation between the two sets of data ($P_s = 0.032$, with Bartlett χ^2 statistics, $p < 0.05$). Ranking of sediments according to toxicity to *Photobacterium phosphoreum* in the Microtox assay and ranking of sediments according to phytotoxicity to *Vallisneria* in our test thus were similar for sediments sampled at the same locations in the two studies.

DISCUSSION

In an earlier laboratory study we showed an organic contaminant (trichloroethylene) added into the water column of aquaria was subsequently adsorbed to sediments and caused a rapid increase in the leaf-to-root surface area ratio in *V. americana* [23]. Plants responded differently to different contaminant concentrations, and each contaminant concentration was associated with characteristic leaf-to-root surface area ratios in exposed plants. Sediments adsorbed contaminant to greater concentrations than the water column, so ramets were exposed to higher concentrations through root tissues than through leaves. Subsequently in contaminated treatments, turnover of roots and leaves increased because of decreases in their life spans [22,23]. Ramets exposed to contaminants allocated more biomass to new leaf tissues than to new root tissues, and their leaf surface area increased and root surface area decreased, depending on contaminant concentrations found in the water column and sediment pore water [23]. Also, we confirmed in the field survey that increased concentrations of organic contaminants at different sites were significantly correlated with increased leaf-to-root surface area ratios in ramets collected throughout the Huron–Erie corridor of the Great Lakes of North America [24]. In a long-term field study, a significant correlation of the leaf-to-root surface area ratio with other measurements of plant growth, development, reproduction, and survivorship were observed [17].

Recently, environmental managers and, in particular, wetland conservation authorities, have been studying approaches to the assessment of freshwater environmental quality using submersed macrophytes [25,27,28]. Measurement of the leaf-to-root surface area ratio in *Vallisneria* ramets seems to have much potential as a biomonitoring tool for laboratory and field studies of pollution monitoring and remediation. Many benefits would occur if a more standardized protocol for the submersed macrophyte assay were utilized together with other useful laboratory assays.

In our study we compared results of the short-term test with results of a 1993 survey of *Vallisneria* sampled from many natural populations in the Detroit River [24] and with results

of sediment Microtox toxicity tests carried out in the same areas [32]. It is possible that since the 1988 study was carried out, absolute contaminant concentrations could have changed, but long-term studies have suggested that relative contamination of sites changes little over time [34]. A nonparametric test was used to evaluate correlations between our results and those of Giesy et al. [32] and gave a significant result. All six sites for sediment sampling in this study were also sampled in an earlier survey [24]. Again, a significant correlation was observed between the two patterns, suggesting that the present laboratory test may be used to predict the relative levels of contamination of sediments in the field using *Vallisneria*. Testing polluted sediments or soil samples could proceed at sites where *Vallisneria* does not occur naturally (e.g., dredged sediments, retrieved sediments from greater depth, terrestrial soils, and underground sediments) or at times of the year when *Vallisneria* does not actively grow (late fall, winter, and early spring). The present assay has potential in the testing of new chemicals in laboratory-prepared sediments. We observed lower values of leaf-to-root surface area ratio in the greenhouse than in the field. This is likely a result of the fact that plants in the field had spent their entire lives living in their sediment, not just 1 week as in the bioassay test group, and were thus more acclimated to the conditions. Other differences existed between greenhouse and field conditions. In the greenhouse temperature was higher. Temperature could promote increased mobility and bioavailability of contaminants in the sediment. Furlong et al. [35] reported a range of organic contaminants and their concentrations found in sediment samples taken at the same locations as in our study, including many polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polychlorinated naphthalenes, and polychlorinated triphenyls. Local contaminant concentrations could have changed over time since the study by Furlong et al. [35], but due to continuous high loadings of contaminants (over 6 million m^3 of effluent daily) from Detroit municipal sewage treatment plants, power plants, steel mills, petroleum refineries, chemical manufacturing plants, and the automobile and plastic industries into the Detroit River, contaminant concentrations in sediments and biota remain very high [36,37]. Overall, the greatest contaminant concentrations in the Detroit River (including tributaries) were found in the Rouge River and Trenton Channel sediments [37].

Submersed plants have been shown to improve aspects of the aquatic environment by purification of water and sediment, cycling of essential nutrients, and increased sedimentation of suspended particles within macrophyte beds [4,6,7,12]. Furthermore, plants provide shelter and nursery areas for numerous biota; all these services are even more important and evident in highly polluted areas [1,9,10]. It has been concluded that remediation of degraded freshwater bodies is not possible without including recovery of submersed aquatic macrophytes as a major part of the process [38,39] and that sustainable fisheries are not possible without recovery of beds of aquatic macrophytes [39,40].

Currently, routine tests using aquatic plants are not required for the registration of new pesticides, even newly introduced herbicides that specifically target higher vascular plants need not be tested with submersed plants [25,28]. Comparative studies of toxicity are seldom done. Some aquatic plants have been reported to be orders of magnitude more sensitive to herbicides (e.g., atrazine) than, for example, zooplankters or fish [41]. In comparison with seven other species of aquatic macrophyte (*Potamogeton perfoliatus, P. pectinatus, Lemna gibba, L. minor, Myriophyllum spicatum, Elodea canadensis,* and *Ceratophyllum demersum*), *V. americana* was reported to be the most sensitive to atrazine [25,41]. Possibly because of that inherent sensitivity and because of direct contact with the substrate, submersed rooted macrophytes may be particularly suitable for testing contaminated sediments [15,16,22,24,27,28]. A modified form of the present test using artificial substrates could be developed for testing extracts derived from environmental samples.

Toxicologists have indicated that results of tests in algae (*Selenastrum capricornutum* was the most frequently used) may be representative for higher plants; however, many tests using algae did not predict the responses of aquatic macrophytes [13,25,27,28]. This may explain the only marginally significant correlation between rankings of sediment quality using the Microtox bacterial assay and rankings of *Vallisneria* leaf-to-root surface area ratios in the present study. Also, the number of comparisons was low, which greatly affects the power of the test.

The genetic line of *V. americana,* as well as interaction between genetic line and sediment, had a significant effect on most measures of plant performance (Table 1). Of all measures, only the index of leaf-to-root surface area was not affected by variation in the genetic line of the plant or its interaction with sediment quality. The use of genetically identical ramets in biomonitoring studies may increase the precision and accuracy of toxicity testing [42,43]. Standardization of plant genotypes would improve comparability of results from different places as well as facilitate the use of particular traits (e.g., size of ramets, sex, and tolerance of particular chemicals). Genets of *V. americana* var. *biwaensis* continue to produce ramets throughout the entire year. By comparison, *V. americana* var. *americana* typically produces turions (overwintering buds) in the fall and does not initiate new growth until May or June of the following year [17]. However, fully developed ramets of both varieties of *V. americana* may be stored for a long period in the greenhouse at the light compensation point and thus be available over the full year [17].

We observed significant prior-cultivation effects for the two genetic lines in which we considered this. Ramets cultured in the field had greater biomass compared with the same genetic individual precultured in the greenhouse. Smaller plants may be more useful for management purposes as they are easier to maintain. Furthermore, smaller ramets (below 4 g) are particularly useful for measurement of leaf-to-root mass ratio as a substitute for leaf-to-root surface area ratio, since in these plants changes in leaf-to-root mass ratio accounted for nearly two-thirds of variance in leaf-to-root surface area ratio. Mass of leaves and roots may be easier to determine than leaf and root surface areas. In our study, the surface areas of leaves and roots were measured manually; however, measurement of surface areas may be simplified by using photometric techniques [44] or image analysis techniques [45,46].

In summary, the submersed aquatic macrophyte *V. americana* has great potential as a biomonitor and should be considered for toxicity screening of new chemicals or in environmental samples. We have shown that a laboratory assay using *V. americana* may be simple, quick, and cost-efficient and can deliver field-relevant results.

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