Vascular Hydrophytes for Bioassay of Phosphate Enrichment in Fresh Waters: A Pilot Study

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Abstract

Vascular hydrophytes were shown to have the potential to be useful for straightforward, low-technology, bioassay of fresh water quality, specifically in relation to phosphate enrichment by effluent from sewage treatment works. Field-collected shoots of *Elodea canadensis* and *Callitriche* sp. made greater extension growth when incubated in canal water from downstream of discharges, indicating enrichment. This was supported by phosphate analysis and by conventional *Selenastrum* bioassay. Growth of shoots incubated in phosphate-augmented water from upstream of discharges equalled that in downstream water, confirming that bioassay, using vascular plants, is effective in detecting enrichment by phosphate.

Introduction

Eutrophication of fresh waters is an international problem. Its causes include inputs of phosphate from sewage-treatment works (STWs), inorganic nitrogen in runoff from fertilized agricultural land, and the release of diverse inorganic nutrients from inundated terrestrial vegetation and soils, following reservoir construction. Its detrimental effects include excessive growth of macrophyes, for example, the overgrowth of tropical African reservoirs by floating masses of *Salvinia molesta* Mitchell and *Eichhornia crassipes* (Mart.) Solms, and the replacement of submerged vascular plants by phytoplankton including nuisance blooms of cyanobacteria in lakes. Ultimately, eutrophication can lead to deoxygenation, fish kills, foul smells, threat to domestic water supplies, and to economic loss (Moss, 1988; Wetzel, 2001; Mason, 2002).

Monitoring programmes for fresh waters, that seek to recognize changes in water quality that are potentially related to eutrophication, routinely analyse for inorganic nutrients. For example, in the UK the Environment Agency, which has statutory responsibility for monitoring water quality, routinely samples watercourses and undertakes analysis for a wide range of chemical determinands that include inorganic phosphate, nitrate, nitrite and ammonia (Goulder, 2008). Prediction of the response of freshwater organisms to changes in water quality shown by chemical analysis is not, however, straightforward. For this reason monitoring might preferably include bioassay alongside chemical analysis.

Micro-algae, notably the unicellular *Selenastrum capricornutum* Printz (Chlorophyta, Chlorococcales), are widely used for bioassay of fresh water quality. *Selenastrum* bioassay has been used, for example, to show enrichment of rivers by discharges from fish farms (Carr & Goulder, 1990) and STWs (Kang & Goulder, 1996), to investigate the toxic effects of herbicide pollution in river water (Hatakeyama *et al.,*

1994), and to identify the nutrients that limit productivity of lake phytoplankton (Forsberg *et al.,* 1975; Groeger, 2007). In contrast, vascular hydrophytes are less often used as bioassay tools (Lewis, 1995) although they have, for example, found favour for testing the potential toxicity of herbicides (Wendt-Rasch *et al.,* 2003; Coors *et al.,* 2006; Knauer *et al.,* 2006) and the growth-promoting effects of enhanced inorganic-nitrogen availability (Ozimek *et al.,* 1990, 1993). Potentially, vascular hydrophytes have a practical advantage over bioassay using *Selenastrum* and other micro-algae in that the infrastructure for microbiological laboratory work is not required.

In the present communication, a pilot study which suggested that vascular hydrophytes may to be used for bioassay of water quality, specifically in the context of phosphate enrichment by STWs' effluent, is described. The sites used for the study were two watercourses in north-east England: the Pocklington Canal and the Driffield Canal. These are artificial channels, excavated for commercial navigation in the 18th and 19th centuries, which are now part derelict and part occasionally used for recreational boating. They are both of substantial wildlife conservation value and have luxuriant aquatic vegetation that includes submerged, floating-leaved and emergent vascular plants (Goulder, 2003). These canals are fed by unpolluted water from headstreams but they also receive effluent from Pocklington or Driffield sewage treatment works. These works, which largely treat domestic sewage, have modern activated-sludge plants but are not equipped with phosphate-stripping, tertiary-treatment facilities.

Effluent from the Pocklington works is discharged into a stream that feeds the canal at UK National Grid Reference SE 761 444; effluent from the Driffield works is discharged directly into the canal at TA 032 568. The extension growth (i.e. increase in length) of vascular-hydrophyte shoots was measured in canal water from upstream and downstream of the discharges and parallel phosphate analysis and *Selenastrum* bioassay were undertaken. Bioassays were also done on water samples experimentally enriched with phosphate to explore whether enhanced growth was linked to higher phosphate concentrations found downstream of the discharges.

Materials and methods

Surface water was collected in glass bottles at distance intervals upstream and downstream of the discharges. Submerged vascular hydrophytes (*Elodea canadensis* Michx. from the Pocklington Canal and a non-determined *Callitriche* L. species from the Driffield Canal) were collected from upstream of the discharges using a grapnel and transported in polythene bags. Shoot apices were cut to 50 mm length with a razor blade against a ruler and rinsed in pure water. Batches of 20 replicate shoot apices were transferred to 1 litre of freshly-collected canal water in 2-litre beakers and were incubated at 20 °C under continuous illumination provided by white fluorescent lamps (approx. PAR 76 mol/m^2/s).

At about thrice weekly intervals the shoots were gently removed from their beaker, blotted dry, measured against a ruler, and returned to the beaker; any shoots that fragmented during incubation were discarded. At weekly intervals the water was

renewed, using canal water, that had been stored in darkness, to avoid algal growth, at about 5 °C. The incubations ran for 3–4 weeks.

Concentration of reactive phosphate was determined in unfiltered water samples using the spectrophotometric, molybdate method (Mackereth *et al.,* 1978). *Selenastrum* bioassay, based on Miller *et al.* (1978) followed the procedure used by Linton & Goulder (1998). Canal water samples were filtered (Whatman No. 1 filters), boiled and cooled to kill native micro-algae, and 50 ml aliquots were transferred to 100-ml sterile, loose-stoppered, conical flasks. The flasks were inoculated with 1 ml of a stationary-phase culture of *Selenastrum capricornutum* (CCAP 278/4, Culture Centre of Algae and Protozoa, Ambleside, UK) that had been three times centrifuged and re-suspended in pure water, to prevent carry-over of nutrients in the inoculum. The flasks were incubated at 20 °C under continuous illumination, as described above, and were swirled daily. *Selenastrum* cells were counted in twice-weekly samples, using a Fuchs-Rosenthal haemacytometer, until an asymptote was reached after 2–4 weeks.

Enrichment of water from upstream of the discharges, so that PO_4-P concentration equalled that determined in downstream water, was achieved by addition of an appropriate volume of a 100 mg PO_4-P l^{-1} solution of disodium hydrogen phosphate.

Results

The extension growth of the vascular hydrophytes was greater in canal water from downstream of the STWs' discharges (Fig. 1). For example, the mean increase in length

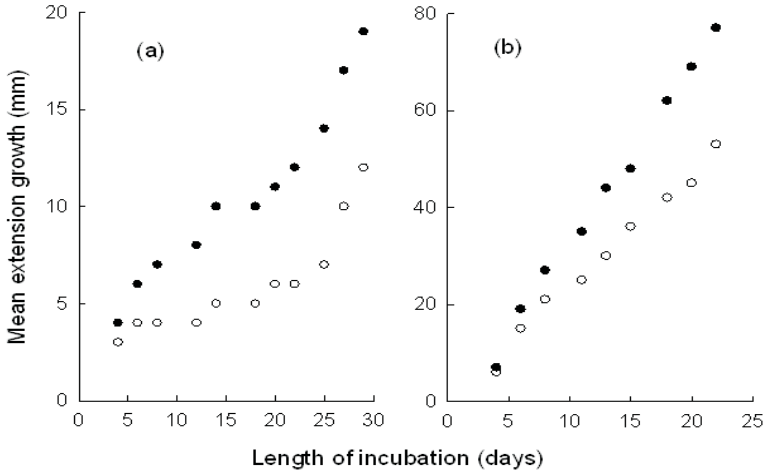

Fig. 1. Mean extension growth of (a) *Elodea canadensis* shoots in Pocklington Canal water and (b) *Callitriche* sp. shoots in Driffield Canal water from upstream (o) and downstream (●) of STW discharges. Growth in downstream water was significantly greater than in upstream water; for *E. canadensis* after 22 days incubation $P < 0.05$, for *Callitriche* sp. $P < 0.01$ (Mann-Whitney U-test). Water from the Pocklington Canal was collected on 18 May 2006 at 1000 m upstream of the discharge and 100 m downstream; water from the Driffield Canal was collected on 30 May 2006 at 600 m upstream of the discharge and at 50 m downstream (see Table 1 for PO_4-P concentrations).

by *Elodea canadensis* shoots after 22 days growth in Pocklington Canal water from downstream of the discharge was 12 mm compared to 6 mm in upstream water. That of *Callitriche* sp. shoots in Driffield Canal water from downstream of the discharge was 77 mm compared to 53 mm in upstream water. Extension growth had not ceased after 22 days incubation (Fig. 1); instead, the shoots in all treatments were continuing to increase in length.

The greater growth of vascular hydrophytes in downstream water was accompanied by higher phosphate concentrations (Table 1). PO_4-P in Pocklington Canal water from downstream of the discharge was up to 59 g l^{-1} which contrasted with a maximum of 38 g l^{-1} in upstream water, while in the Driffield Canal the concentration downstream of the discharge was up to 144 g l^{-1} compared to a maximum of 88 g l^{-1} in upstream water. Furthermore, *Selenastrum* bioassay showed that there was greater algal growth potential in water from downstream of the discharges (Table 1). *S. capricornutum* concentration after 20 days growth in Pocklington Canal water from downstream of the discharge reached 3.3×10^6 cells/ml compared to 2.5×10^6 cells/ml in upstream water, while for the Driffield Canal concentration in downstream water reached 3.6×10^6 cells/ml compared to 2.2×10^6 cells/ml in upstream water.

When plant shoots were incubated in

TABLE 1

Phosphate concentration and Selenastrum *growth in canal water from upstream and downstream of STW discharges*

	Upstream		*Downstream*		
Pocklington Canal					
	1000 m	50 m	100 m	500 m	
PO_4-P concentration (g l^{-1})					
28 March	23	38	53	59	
18 May	9	10	18	23	
21 June	8	–	56	–	
4 July	21	–	56	–	
Selenastrum bioassay (10^6 cells/ml)					
18 May	2.4	2.5	3.0	3.3	
Driffield Canal					
	600 m	200 m	50 m	5000 m	7500 m
PO_4-P concentration (g l^{-1})					
30 May	88	85	126	134	144
4 July	24	–	110	–	–
Selenastrum bioassay (10^6 cells/ml)					
30 May	2.2	2.2	2.8	3.6	3.6

PO_4-P concentrations are means of two determinations, range < 35% of the mean; *Selenastrum* cell concentrations are means from counts on two replicate flasks after 20 days incubation, range < 10% of the mean; samples were collected March-July 2006.

upstream water, downstream water, and upstream water augmented with PO_4-P to the concentration determined in downstream water it was found that extension growth in augmented upstream water was not significantly different from that in downstream water (Table 2). Similarly, with *Selenastrum* bioassay, a similar cell concentration was achieved in both augmented upstream water and downstream water (Table 2).

TABLE 2

Extension growth of hydrophyte shoots and Selenastrum *growth in canal water from upstream and downstream of STW discharges and in upstream water augmented with phosphate*

Upstream water	*Upstream water + PO_4*	*Downstream water*
Pocklington Canal		
Mean extension growth by *Elodea canadensis* shoots (mm)		
11.7 (4.9)	14.1 (5.4)	15.0 (5.4)
Selenastrum bioassay (10^6 cells/ml)		
2.5 (0.2)	3.3 (0.3)	3.3 (0.4)
Driffield Canal		
Mean extension growth by *Callitriche* sp. shoots (mm)		
46.9 (22.0)	77.9 (33.0)	77.9 (21.7)
Selenastrum bioassay (10^6 cells/ml)		
2.4 (0.2)	3.5 (0.3)	3.6 (0.3)

Shoot extension growth was from measurements made after 26 days incubation; *Selenastrum* cell concentrations are means from counts on 10 replicate flasks after 21 days incubation. Standard deviations are in brackets. There were no significant differences between shoot growth and *Selenastrum* cell concentrations in upstream water + PO_4 and downstream water ($P > 0.05$, Mann-Whitney *U*-test). Water from the Pocklington Canal was collected at 1000 m upstream and 100 m downstream of the discharge, that from the Driffield Canal was from 600 m upstream and 50 m downstream. Water for *Selenastrum* bioassay was collected from the Pocklington Canal on 21 June and from the Driffield Canal on 4 July 2006; shoots and water for hydrophyte bioassays were collected from both canals on 4 July 2006. The PO_4-P concentration in the augmented upstream water was raised to equal that in the downstream water (see Table 1).

Discussion

The pilot study suggests that vascular hydrophytes have the potential to be used as a straightforward, low-technology, bioassay tool. The greater extension growth of macrophyte shoots in water from downstream of STWs (Fig. 1) was supported by both chemical analysis, which showed increased phosphate concentration (Table 1), and by conventional *Selenastrum* bioassay in which higher cell concentrations were achieved (Table 1).

The shoots used in the study were collected from relatively un-enriched canal sites upstream of the discharges. This was because fresh water macrophytes, when in a nutrient-rich environment, can assimilate nutrients and store them in their tissues for use in subsequent extension growth. This was shown, for example, by Goulder &

Boatman (1971), who found that extension growth of shoots of *Ceratophyllum demersum* L. collected from a pond decreased through spring-summer in parallel with decrease in tissue nitrogen content, and by Carr & Goulder (1990), who found that extension growth of *Ranunculus penicillatus* (Dumort.) Bab. ssp. *pseudofluitans* (Syme) S.D. Webster, collected from a river enriched by fish-farm effluent, increased with increase in tissue phosphorus content. It follows that shoots to be used for bioassay of water quality should all be collected from the same site which should preferably be un-enriched. The observation that in the present study the rate of extension growth did not fall off with time (Fig. 1) suggests that extension growth was supported by nutrients from the regularly-replenished surrounding water rather than from stored nutrients in the plant tissues.

The fragmentation of some shoots during incubation, on average 3.7 out of 20 shoots per beaker after 19–20 days incubation, was accompanied by obvious colonisation by epiphyton. Boiling and cooling of test water before use would be a straightforward approach to countering this problem; the substitution of axenic shoots for field-collected material would be much more complex.

The observation that growth of the bioassay organisms (vascular hydrophytes and *Selenastrum*) in upstream water augmented with phosphate equalled that in water from downstream of the discharges (Table 2) indicated that greater growth in downstream water (Fig. 1, Table 2) probably was due to the extra phosphate. The result confirmed that bioassay using vascular plants may be useful for detection of increased phosphate and the potential for eutrophication in fresh waters.

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