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Assessing the Native Plant Species for Phytoremediation of Freshwater Bodies in Southern Ontario, Canada

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Abstract

Many Canadian freshwater ecosystems are polluted by agricultural runoff, impairing their function with increased nutrient levels. Here, we simulated the water filtration function of wetlands, which uses aquatic plant species to create a phytoremediation system that can address the contamination of freshwater ecosystems with excess nutrients. We collected the water samples from three of Ontario's freshwater bodies: the Holland Marsh, a highly agricultural area; the Nottawasaga River, a river in a rural area and part of a greater Nottawasaga watershed and Lake Ontario, near industrial sites in the Niagara region. To filter nitrogen (N), phosphorus (P) and potassium (K) from the collected samples, we determined the effectiveness of five local wetland and agricultural plant species: duckweed (*Lemnoideae*), watercress (*Nasturtium officinale*), coontail (*Ceratophyllum demersum*), thyme (*Thymus praecox*) and parsley (*Petroselinum crispum*). During a five-month experiment, plants were grown in collected water samples to determine their ability to uptake N, P and K. Along with monitoring their effectiveness in lowering nutrient levels, we tracked the health and growth of each plant species. The results showed that duckweed was the most tolerant to high nutrient concentrations and the most effective at an overall nutrient reduction. From the Holland Marsh sample with the highest nutrient concentrations among all collected samples, the duckweed reduced N, P, and K by 11%, 53%, and 21%, respectively, compared to the control sample (*i.e.*, with no plant). This filtration system allows for ecosystem restoration and prevention of further damage and contamination from agricultural runoff and nutrient pollution.

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Introduction

Worldwide, nutrient pollution combined with global climate change is increasing the vulnerability of freshwater ecosystems [1, 2]. Agricultural lands are a major contributor of nutrient loading to aquatic ecosystems that leads to harmful algal blooms and eutrophication [3, 4]. Canada's highest-quality farmland is within the province of Ontario, where agriculture is an important industry. Less than two percent of all farms across Ontario use regenerative agricultural practices that focus on maintaining soil health and preventing runoff pollution and erosion [5]. As a result, nutrient loss from the oversaturation of fertilizers has continuously been damaging freshwater ecosystems [6]. While the need to reduce nutrient pollution and its associated harmful impacts on freshwater is increasingly recognized, economically feasible, eco-friendly and sustainable remediation and restoration measures for freshwater bodies are yet to be established and implemented at regional scales [7, 8].

Wetlands are widely recognized for their function of filtering pollutants out of water bodies naturally [9]. The ability of wetlands to improve water quality has become a primary argument for their protection and restoration throughout the world [10, 11]. Through their system of aquatic plants and microorganisms, wetlands absorb sediments and excess nutrients to supply clean water with lower levels of nutrients and microorganisms [12]. Wetlands sequester nutrients [13] and purify water through physical (sedimentation), chemical (adsorption, precipitation, chelation), and biological (plant uptake) processes [14, 16-18]. The latter process of wetlands is replicated to remove, detoxify, or immobilize nutrients from freshwater bodies, which is called phytoremediation. Phytoremediation can effectively improve water quality to create functioning ecosystems, which provide many ecosystem services and benefits to society such as water supply, wildlife habitat, fish production, places for recreational activities and nutrient cycling [14]. Various aquatic plant species have been recognized and used for their efficiency to uptake inorganic and organic contaminants from the water via hydroponic or field applications [15]. Phytoremediation is an effective and popular technique to reduce nutrients in contaminated water bodies; thus, it restores freshwater bodies and other aquatic environments. At a large spatial scale, improving and restoring contaminated water bodies requires huge investments [16]. In such cases, the implementation of phytoremediation is pragmatic as it involves a floating system in the form of a buoyant mat or raft that helps plants to grow above the water with their roots in the water [17]. However, the first step toward the application of the phytoremediation technique is to identify the plants that have a high efficacy to accumulate dissolved nutrients and other contaminants from contaminated waters [14].

Because nitrogen (N), phosphorus (P) and potassium (K) are essential nutrients in fertilizers, we usually observe these three nutrients in freshwater samples. While these elements are required in certain amounts for the healthy functioning of ecosystems, their oversaturation in freshwater ecosystems creates an imbalance, killing important species and increasing harmful algal blooms [18]. For this high degree of environmental change turning freshwater ecosystems to eutrophic conditions, the most effective method of ecosystem restoration must involve the reduction of high-concentration nutrients [18]. On the national scale, the effectiveness of certain Canadian wetland plants in the uptake and removal of nutrients is well-documented, such as the common rush, common great bulrush and common reed [19]. However, there are very few instances where the studies [25] have determined the efficacy of plant species native to natural wetlands and agriculture in southern Ontario (Canada). Therefore, our understanding of plant species that can be used in phytoremediation to solve water quality problems in southern Ontario, is limited. Thus, in this study, we performed experiments on different aquatic plant species native to natural wetlands and agriculture in southern Ontario and attempt to determine their effectiveness for nutrient uptake from the freshwater ecosystems. Both wetland species and agricultural plant species were selected for this study. Wetland species were selected as they are excellent candidates for this filtration due to their environmental adaptations. Agricultural plants were selected as they are herbs that can be grown hydroponically. We collect water samples from three freshwater bodies in southern Ontario that receive runoff largely from nonpoint sources of pollution [21].

Materials and Methods

Water samples

We designed hydroponic systems to reduce targeted nutrients in freshwater ecosystems, including marshes, rivers and lakes. One reason for selecting these ecosystem types is that they are affected differently by contamination and have different

Table 1 The criteria used to assign health scores to the plants used in the study.

Score	1	2	3	4	5
General statement	Overall health is bad.	Overall health is poor.	Overall health is fair.	Overall health is good.	Overall health is excellent.
Watercress specifications	Most leaves are discolored, and yellowish dead. Few leaves are present, are sparse and have low density. Most plant growth is above water.	Little leaf presence underwater, some leaves yellow or discolored, lower amounts of leaves, and sparse arrangement.	Some leaf presence underwater, generally leaves are the same shade of green, some yellowing or pale, the lower density of and leaf quantity.	Leaves growing underwater and are mostly the same shade of green, leaves either high density or evenly distributed.	A significant amount of the growth is underwater, leaves are green, and there is a high density of evenly distributed leaves.
Duckweed specifications	The quantity of duckweed decreases, and there is a significant amount of white or discolored plants.	The quantity of duckweed decreases slightly, and some of the plants are discolored or white.	There is a small increase in the quantity of duckweed, and there are only some discolored plants.	There is an increase in the quantity of duckweed, and the leaves are all mostly the same green color.	There is a vast density increase in the plant's quantity, and the leaves are all a uniform color.
Coontail specifications	The plant is floating near the surface and is brown.	The plant is brown.	The plant is green-brown.	The plant is green in color, with slight brown on it.	The plant is green in color.
Parsley and thyme specifications	The plant is shriveling up and/or dying.	The plant is brown, and the quantity of leaves has significantly decreased.	The plant has the same quantity of leaves as at the beginning of the study, but much remains the same.	The plant is bright green, leaf growth has increased slightly and leaf distribution is even.	The plant has several more stems, and an abundance of leaves, and is not exhibiting signs of reaching such as elongated stems and sparse growth.

Table 2 The criteria used to assign growth scores to the plants used in the study.

Score	1	2	3	4	5
General statement	Overall growth is bad.	Overall growth is poor.	Overall growth is fair.	Overall growth is good.	Overall growth is excellent.
Watercress specifications	No increase in leaf density, or a decrease in leaf density.	Little increase in leaf density, little growth underwater.	Some increase in leaf density, and some leaf growth and development underwater.	Significant increase in leaf density growing underwater.	High increase in leaf density, a large proportion of leaves growing underwater.
Duckweed specifications	There is a substantial decrease in plant quantity.	There is no increase in the plant quantity or a slight decrease.	There is little to no increase in the quantity of the plant.	There is an increase in the quantity of the plant.	There is a large increase in the quantity of the plant.
Coontail specifications	The plant has died.	The plant has lost leaves or those have decreased in size.	The plant has remained the same size.	The plant has increased in size and/or sent off small shoots.	The plant has increased substantially in size and/or sent off many shoots.
Parsley and thyme specifications	The plant has died.	The plant has lost substantial leaves, stems are pale green and are reaching.	The plant has remained the same size, but leaf distribution is sparse.	The plant has increased in size and height and/or sent off small shoots.	The plant has increased substantially in size, has sent off many shoots, and has dense leaf distribution.

processes for the filtration of contaminants based on their specific features. Sample sites included the Holland Marsh (abbreviated HM) (44°06'40.2"N 79°32'57.8"W), a marsh located in a highly agricultural area, the Nottawasaga River (abbreviated NR) (44°09'11.0"N 79°51'25.2"W), a river which is part of a greater watershed surrounded by a rural area, and Lake Ontario (abbreviated LO) (43°10'52.2"N

79°21'01.1"W) in the Niagara region near to industrial sites. Each of these water samples had high nutrient concentrations, which were not within the acceptable range for safe drinking water in Canada. We collect and test water samples for initial N, P and K values from these ecosystems located in southern Ontario, Canada, during the study period of five months. We also collected and tested tap water collected from Alliston, Ontario. The control sample for each of the four water samples is the one in which we do not grow plants. The control sample serves as a baseline and is used to compare the changes in the

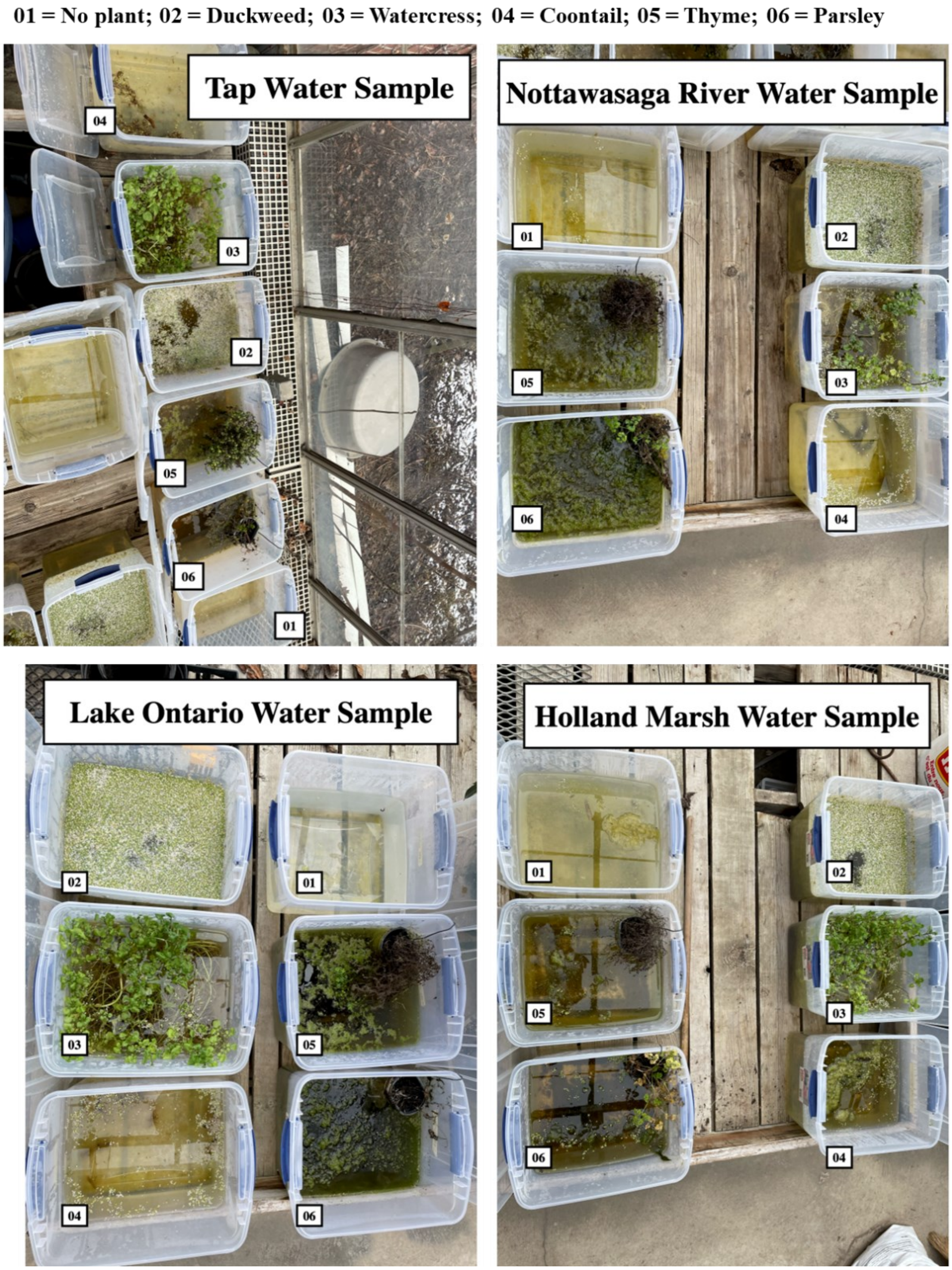

Fig. 1 Plant species grown in six water samples from four water sources. The numbers indicate the name of the plant in the sample.

nutrient levels in the samples treated with different plant species. To run a test with each plant in each water sample, the collected samples from each water body were divided into six portions in containers (one control sample and five samples with plants) of 26 liters each.

Selected plant species

Different aquatic plant species possess varying filtration capabilities for water samples with oversaturated nutrient levels. Therefore, we tested five different plant species, belonging to freshwater wetlands and agricultural plant species in Ontario. Wetland plant species are excellent candidates for filtering nutrients from freshwater ecosystems due to their environmental adaptations, whereas agricultural plants are herbs that can be grown hydroponically. The selected wetland plant species were duckweed (*Lemnoideae*), watercress (*Nasturtium officinale*), and coontail (*Ceratophyllum demersum*); the agricultural plant species used in the study were thyme (*Thymus praecox*) and parsley (*Petroselinum crispum*). These plant species are rife in southern Ontario's wetlands and agricultural landscape. In the aquatic species, coontail is classified as submerged whereas duckweed and watercress as floating plant species. For phytoremediation, the submerged species are suitable for deep water bodies and floating species for shallow water bodies. Previous studies have shown that different plant species can effectively remove excess nutrients from the water, and our selection of plant species was based on their demonstrated ability to remove nitrogen and phosphorus from water [22]. Equal quantities of selected plant species were distributed into each of the 26 liters samples (maintaining 26 liters division of each sample as a control sample, without the addition of plant matter) creating a total of 24 samples (Fig. 1). The study was conducted in a greenhouse-controlled conditions, which allowed the study to be run in the winter months with the same environmental conditions. All the samples were maintained at the same temperature, CO_2 levels, lighting, airflow and humidity for the duration of the study (five months). To prevent substantial evaporation of the samples, lids were kept on the samples except when monitoring and collecting data.

The effects of each plant type on each of the water samples were observed for five months. Every third day the concentrations of N, P and K were measured using a digital nutrient intelligent test kit (NPK Soil Sensor, Fish Hawk, USA). The kit probes were inserted into water samples at an approximate depth of three centimeters and gently swirled to agitate the water. Then the highest concentration values observed were recorded. In addition to the changes in concentration of N, P, and K values, the changes in both plant health and growth over time were observed and scaled using a criterion developed for each plant for this study (Tables 1 and 2). The scaling of plants' health and growth allowed us to determine whether each species was well adapted for survival in water bodies with high nutrient concentrations. All 24 samples were photographed every week, allowing for the qualitative observation of changing plant growth and health. Because phytoremediation is finally implemented in the field [23], we conducted this experiment in a greenhouse.

Results and Discussion

The results obtained over a period of five months for changes in the concentrations of N, P and K over a period of five months are given in Table 3. The results show that the nutrient levels of most samples increased throughout the study due to the decreasing quantity of the solutions resulting from water evaporation. The health and growth scores for the plants are given in Table 4. The results show that the health of most plants in both the Holland Marsh and the Nottawasaga River was good in comparison with the others, while the growth of most plant species was good in Lake Ontario, the Holland Marsh, and the Nottawasaga River water samples. Because farmlands are the largest contributor to pollution and degradation of freshwater bodies [24], intensive cultivation is deteriorating freshwater ecosystems in southern Ontario, Canada. In this study, we investigate the capacity of five plant species to reduce nutrient (N, P, and K) levels in water samples taken from three freshwater bodies and a tap water sample. Despite the nutrients being consumed by the plants, the concentration of the nutrients in the most of the solutions show a net increase due to water evaporation from the samples. The quantity of evaporated water was proportionally greater than the quantity of nutrients consumed by the plants, increasing the nutrient concentrations. Therefore, we calculated the percent change in nutrients using the initial (base) and final concentrations for each water sample. Then we compared the percent change of each sample with the plants to the percent change in the control sample (without plants) for the corresponding water body/source to assess the effectivity of the plants for reducing nutrient levels. In the tap water, the coontail and thyme were effective in reducing the K levels compared to the

Table 3 Initial and final nutrient concentrations for the control sample and samples with plants. The concentrations in all samples increase due to water evaporation.

Water Sample	Plant	Nitrogen		Phosphorous		Potassium	
		Initial level (ppm)	Final level (ppm)	Initial level (ppm)	Final level (ppm)	Initial level (ppm)	Final level (ppm)
Tap water	Control	5	10	10	12	23	39
	Parsley	5	18	10	23	23	55
	Thyme	5	13	10	14	23	36
	Watercress	5	25	10	35	23	69
	Duckweed	5	13	10	27	23	41
	Coontail	5	12	10	17	23	30
Holland Marsh	Control	35	28	36	78	120	166
	Parsley	35	47	36	113	120	196
	Thyme	35	43	36	96	120	181
	Watercress	35	57	36	79	120	201
	Duckweed	35	24	36	59	120	141
	Coontail	35	34	36	90	120	178
Nottawasaga River	Control	62	105	118	153	326	362
	Parsley	62	102	118	162	326	343
	Thyme	62	103	118	158	326	359
	Watercress	62	95	118	158	326	337
	Duckweed	62	85	118	137	326	249
	Coontail	62	109	118	168	326	301
Lake Ontario	Control	7	14	12	24	35	71
	Parsley	7	15	12	31	35	53
	Thyme	7	12	12	23	35	68
	Watercress	7	26	12	57	35	101
	Duckweed	7	29	12	21	35	38
	Coontail	7	23	12	20	35	58

control sample (with no plants). However, the other plants resulted in an increase in the N, P, and K compared with the control sample.

In the Holland Marsh, the duckweed and coontail plants were effective in reducing the N levels with respect to the control sample (Fig. 1). The other plant species resulted in increases in the N concentration with respect to the control sample (Fig. 1). The duckweed was also effective in reducing P and K concentrations with respect to the control sample, while all other plants were not (Fig. 1). In the Nottawasaga River, all plant species except the coontail were able to reduce the concentrations of N with respect to the control sample. The duckweed was the only plant able to reduce the P levels compared to the control sample, all others produced an increase. All the plant species were effective in reducing the K levels with respect to the control sample (Fig. 1). For the Lake Ontario water sample, the thyme was effective in reducing the N levels with respect to the control sample, whereas none of the other species reduced the N levels. The duckweed, coontail, and thyme were all effective in reducing the P levels with respect to the control sample. For reducing the K concentrations with reference to the control sample, the duckweed, coontail, thyme and parsley were effective. The aquatic plant species, commonly found in wetlands, tested were far more effective at reducing the percent increase of N, P, and K concentrations than agricultural plant species. Therefore, wetland plant species have better suitability for filtration and nutrient uptake, which may be due to their physiological adaptations [25]. Submerged aquatic species, the coontail, uptake nutrients from the water column through the leaves, whereas floating species (duckweed) and semi-submerged (watercress) species primarily utilize their root systems for nutrient uptake. These different nutrient uptake processes influence each species' ability to absorb nutrients, which is why some species are more effective at reducing nutrient levels than others [25]. The concentrations of N, P, and K in the water samples from the Nottawasaga River were 62, 118, and 326 mg/L and from Holland Marsh were 35, 36, and 120 mg/L, respectively. These high nutrient concentrations can be intolerable for certain plant species and other species may thrive in the high nutrient environments. For example, thyme had low health and growth scores for the Nottawasaga River and Holland Marsh water samples; however, duckweed is well suited for this level of nutrients and therefore has high health and growth scores for

Table 4 Plant health and growth scores. High scores (3-5) indicate relatively better-observed health and better-observed growth patterns.

Plant species	Water sample	Health score	Growth score
Watercress	Tap Water	4	3
	Holland Marsh	5	5
	Nottawasaga River	3	3
	Lake Ontario	4	5
Duckweed	Tap Water	1	2
	Holland Marsh	4	4
	Nottawasaga River	4	5
	Lake Ontario	4	5
Coontail	Tap Water	3	3
	Holland Marsh	4	3
	Nottawasaga River	4	5
	Lake Ontario	2	3
Thyme	Tap Water	5	5
	Holland Marsh	2	2
	Nottawasaga River	2	2
	Lake Ontario	3	3
Parsley	Tap Water	1	1
	Holland Marsh	4	4
	Nottawasaga River	4	4
	Lake Ontario	1	1

Nottawasaga River and Holland Marsh water samples. Similarly, low health and growth scores for the duckweed in the tap water samples were the result of low initial nutrient levels: 5 ppm of N, 10 ppm of P, and 23 ppm of K (Table 3). Agricultural plant species (*i.e.*, thyme and parsley) had low health and growth scores in water samples with high nutrient concentrations. One reason for low scores may be the highly acidic environment formed by high nutrient levels that becomes harmful to the plant's health and growth [26]. In addition, plant's anatomical structure (in particular, the mechanical resistance) becomes weaker with an increase in nutrient abundance, and therefore, it increases the plant's risk of mechanical failure [27].

The relative health and growth of each plant in different water samples varied substantially, and this was mainly caused by the concentrations of nutrients within each water sample. The plant species with lower growth and health scores were placed in the water samples with high nutrient levels. Therefore, the plants with low scores were not well adapted and had lower chances of survival in high nutrient concentration environments as compared to those with higher scores. Therefore, we can conclude that plants with low health and growth scores are unable to tolerate the oversaturation of nutrients in water samples [28]. On the other hand, the plants in water samples with low concentrations of nutrients obtained high health and growth scores. Alternatively, the relatively high health and growth scores of other plants indicate that they need nutrient-rich environments for their growth [29]. Overall, the results of this study show the capacity of five native aquatic plants to remediate the common agricultural pollutant from the freshwater ecosystems. Under different conditions, each plant possesses nutrient uptake abilities, with unique characteristics to tolerate polluted freshwater ecosystems. Future studies should use a combination of the most effective plants to determine their phytoremediation potential.

Conclusions

Freshwater ecosystem degradation due to agricultural nutrient pollution is a major environmental concern in southern Ontario - a region with the most productive agricultural lands in Canada. Aquatic phytoremediation is a nature-based solution to help remediate polluted freshwater bodies. For the application of phytoremediation, we investigated the nutrient assimilation capacity of five aquatic plant species: three wetland species (duckweed, watercress and coontail) and two agricultural species (thyme and parsley). The results of our experiment on water samples from four water sources show that duckweed tolerates high-nutrient environments and takes up the nutrients in the largest quantities among all the species considered in the study. Because the aquatic plant species performed better than the agricultural plant species, they can be used in phytoremediation as an effective filter for reducing high N, P and K levels in polluted water bodies across Southern

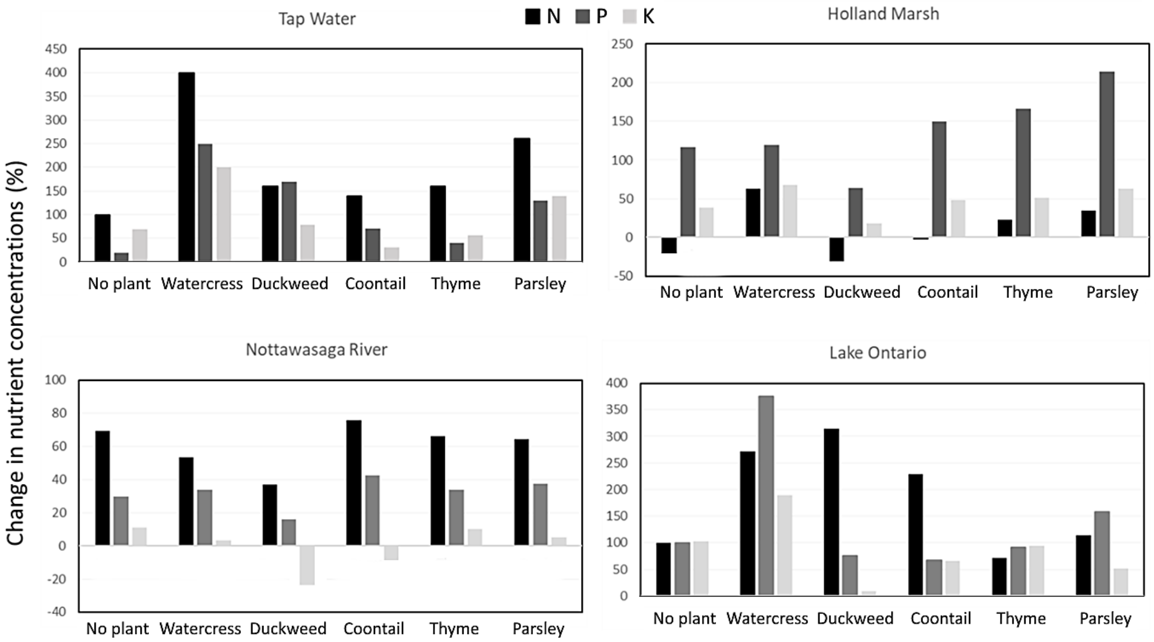

Fig. 2 Percent change in the final concentrations (with respect to the initial (base) concentration in each water sample) for nitrogen (N), phosphorus (P), and potassium (K) in four water samples. 'No plant' indicates the control sample values.

Ontario. Future work should investigate the phytoremediation potential of using a combination of these plants for pollution remediation from nutrient-enriched waters.

Conflict of interest

The authors declare no conflict of interest.

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