

Review

Role of Plants in a Constructed Wetland: Current and New Perspectives

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Received: 20 February 2013; in revised form: 25 March 2013 / Accepted: 25 March 2013 /

Published: 8 April 2013

Abstract: The role of plants in the treatment of effluents by constructed wetland (CW) systems is under debate. Here, we review ways in which plants can affect CW processes and suggest two novel functions for plants in CWs. The first is salt phytoremediation by halophytes. We have strong evidence that halophytic plants can reduce wastewater salinity by accumulating salts in their tissues. Our studies have shown that *Bassia indica*, a halophytic annual, is capable of salt phytoremediation, accumulating sodium to up to 10% of its dry weight. The second novel use of plants in CWs is as phytoindicators of water quality. We demonstrate that accumulation of H₂O₂, a marker for plant stress, is reduced in the successive treatment stages, where water quality is improved. It is recommended that monitoring and management of CWs consider the potential of plants as phytoremediators and phytoindicators.

Keywords: *Bassia indica*; bioindicator; constructed wetland; phytoremediation; salinity; wastewater treatment

1. Introduction

A constructed wetland (CW) system is a man-made complex that mimics the structure of natural wetlands to serve as a wastewater filter [1]. Wastewater treatment by CWs has gained popularity in the last four decades as an alternative to conventional treatments such as activated-sludge systems. Four main components make up the CW—water, media, microbes and vegetation. Water is transferred through the filtering media, and contaminants are removed mainly by physical mechanisms, such as filtration or sedimentation, and biochemical interactions, such as microbial degradation. Among the most comprehensive reviews dealing with aspects of plants' roles in CWs we can count those by Brix [2,3], who pointed out plants' potential roles in CWs; Stottmeister *et al.* [4] who published an updated review and elaborated further on the physical effects of root structure; Brisson and Chazarenc [5], who focused on the effect of plant species composition in CWs; Langergraber [6] who concluded (based on a computer model) that nutrient uptake by plants has only a minor effect on water treatment in comparison to the applied loads, and very recently, Vymazal [7] who published a thorough literature review on plants' role in horizontal subsurface CWs. Plants are an important component of wetland systems [8]. However, the mechanisms by which macrophytes affect water treatment in CWs are still being debated [9]. Plant efficiency in promoting CW performance depends on several factors: CW type (e.g., vertical, horizontal, surface, or subsurface flow, with or without recirculation), quality and quantity of the wastewater loads [10], plant species and their combinations [5], climate, medium type, and plant management, such as harvesting regime. Moreover, plants' contribution to the CW's performance is a complex of various functions that are rarely studied. Unfortunately, most studies have not explored mechanisms, and therefore most of our information is restricted to observations describing the impact of the presence or absence of plants on water quality. For example, results reported in the literature indicate that mixed vegetation is more effective at pollutant removal than single-species vegetation [11,12], but information on the driving forces leading to this conclusion is scarce. Moreover, studies have made use of various experimental strategies with different plants, resulting in conflicting findings, with even more variation emerging when comparisons are made between different types of CW. On the other hand, studies exploring specific mechanisms often draw limited global conclusions. For example, Naylor *et al.* [13] found a correlation between plant biomass and N removal, but did not show which plant species contribute to this removal or the underlying mechanism. Nevertheless, most studies report a significant and positive effect of plants on CW performance. For example, Vymazal [7] summarized 22 articles that compared treatment efficiency of planted *versus* unplanted CWs: 20 of the reports (90%) showed a positive effect of at least some plants on some parameters of water quality.

2. Role of Plants in CWs

Plants can affect removal efficiency or contribute to the CW in the following ways (Table 1).

Table 1. Brief summary of plant roles in constructed wetlands (CWs), with references. New perspectives are in bold. Headings are in accordance with the functions listed in Section 2.

Role of plants in a constructed wetland	Reference
Physical effects of root structure	
Filtering effect	[7]
Velocity reduction, promotion of sedimentation, decreased resuspension	[7]
Prevention of medium clogging	[3]
Improved hydraulic conductivity	[2,14]
Macrophytes do not increase hydraulic conductivity and even contribute to clogging	[2,4]
No effect on removal of suspended solids	[7]
Roots as a base for microorganisms	
Provision of surface for microbial attachment	[2,7]
Root release of gas and exudates	
Oxygen leakage—addition of aerobic niches	[1,7,15,16]
Oxygen leakage—increased aerobic degradation	[17,18]
Oxygen leakage—supports precipitation of heavy metals	[7]
Oxygen leakage—increased nitrification	[2,11,19–21]
Excretion of carbon—increased denitrification	
Aerobic dynamics are very limited in horizontal flow CWs (HF)	[7]
Release of antibiotics, phytometallophores and phytochelatins	[7,22–24]
Root exudates promote metal chelation to prevent metal toxicity	[7]
Plant uptake	
Storage and uptake of nutrients	[7,25–28]
Plant nutrient uptake is negligible	[3,5–7,29–31]
Metal phytoremediation	[28,32,33]
Salt phytoremediation	[34]
Evapotranspiration	
Increased water loss	[35,36]
Microclimatic conditions	
Light attenuation reduces algal growth	[2]
Insulation from frost in the winter	[37–39]
Insulation from radiation in the spring	[3,40]
Reduced wind velocity	[7]
Stabilization of the sediment surface	[7]
Other functions of plants in the CW	
Elimination of pathogens	[41]
Insect and odor control	[42]
Enhanced mosquito reproduction	[43]
Wastewater gardens	[44,45]
Increased wildlife diversity	[2]
Aesthetic appearance of the system	[3,42]
Bioindicators	[46]

Table 1. Cont.

Role of plants in a constructed wetland	Reference
Plant production	
Ornamental plant production	[47–49]
Production of fiber for construction material	[7,50]
Bioenergy crops	[7,51]
Animal feed	(no reference, suggested in [52])

(a) Physical effects of root structure: Several authors have argued that the most important mechanism by which plants contribute to the CW treatment process is not in uptake but rather in the physical effects of root structure combined with aeration [5,53]. Root growth is well known to affect some soil hydraulic qualities [4,54]. Physical effects of roots include filtering, flow velocity reduction, improved sedimentation, decreased resuspension, and even the distribution of water and prevention of clogging [4,7].

(b) Rhizosphere as a base for microorganisms: Brix [2] and Vymazal [7] pointed out the significance of the rhizosphere in creating improved conditions for various microorganisms in CWs. Since microorganisms are considered key drivers in the treatment process, any factor that changes their composition, biodegradation efficiency or concentrations has a significant impact on the whole CW. However, this attribute has not been extensively studied.

(c) Root release of gas and exudates: Root release of liquid exudates and gas is likely a key component of plants' effects in CWs. Extensive work by Armstrong and others revealed various aspects of oxygen release by plant roots [16,55–58]. Root oxygenation occurs in the daylight and depends to some extent on photosynthetic activity [59,60]. Oxygenation by roots has been shown to have a significant impact on important mechanisms of wastewater treatment in CWs, including influence on redox potential [61], which is critical in determining nitrogen fate, oxidation of some phytotoxins [56], and enhancement of microbial activity [62]. Vymazal [7] argued that root-derived aerobic dynamics is very limited in horizontal CWs and its role is minor in periodically loaded vertical CWs with short hydraulic-retention times. However, others have reported oxygen leakage, which increases soil redox potential and aerobic niches, which in turn improves degradation, supports heavy-metal sedimentation, and increases nitrification (Table 1). The excretion of carbon from the roots has been reported to increase denitrification (Table 1). In addition, roots release antibiotics, as well as chelating components that further enhance metal precipitation.

(d) Plant uptake: By utilizing N, P, and other nutrients, plants can reduce the concentrations of elements that would otherwise be considered pollutants in CWs. Plants can also accumulate phytotoxic elements, such as heavy metals, in vacuolar or granular compartments. Thus, phytoremediation may be an important role for plants in CWs. Several authors have found that plant uptake in CWs is negligible [3,5–7,29–31]. Others have claimed that it is significant (Table 1). Another important aspect of plant uptake is the effect of plants on water balance in the CW.

(e) Evapotranspiration: Wetlands receive water through influx and rain, and lose water to outflow and evaporation. Plants have a critical role in determining the dynamics of water loss, mainly by dictating water loss through evaporation and plant transpiration, *i.e.*, evapotranspiration (ET). The ET of emergent macrophytes is a significant process in CWs [63] and may reach high levels—seven to

eight times higher than actual evaporation without plants. For example, ET from a CW in Morocco planted with *Arundo donax* was 40 mm day⁻¹ and nearly 60 mm day⁻¹ with *Phragmites australis*, as compared to 7 mm day⁻¹ in an unplanted horizontal-flow CW [64]. Borin *et al.* [36] found a similar amplification of water loss from a CW planted with *P. australis* in a rather humid area in Italy. Water loss from the CW through ET slows flow velocities which induces longer retention times, and increased pollutant and salt concentrations in the water [35]. The transfer of water to the atmosphere is sometimes an advantage, mainly in humid environments [65]. On the other hand, in arid regions, where treated wastewater is destined for reuse, water loss may be disadvantageous [64,66,67]. Nevertheless, data on ET rates in CWs in general, and in arid lands in particular, are still scarce [36,45].

(f) Microclimatic conditions: The physical structure of plants growing in the CW medium affects the microclimatic conditions in the system, which may then have a significant effect on various components of the system. The effect of plants on the microenvironmental conditions of the CW includes shade that prevents algal growth, and insulation from radiation in the spring and frost in the winter. Reduced wind velocity by the plants' upper parts may stabilize the sediment surface.

(g) Other functions of plants in the CW: These include aesthetic appearance, and the elimination of pathogens, insects, and offensive odors. However, some plant functions are negative. Knight *et al.* [43] reported enhanced mosquito reproduction in the presence of plants. Thomas *et al.* [68] mentioned that aerenchyma tissue plays a role in the emission of methane into the atmosphere through emergent wetland plants, supporting Reddy *et al.* [69] who revealed the role of aerenchyma in releasing, to the atmosphere, N₂ and N₂O produced by anaerobic denitrification of NO₃ in the wastewater. Brix [2] suggested that plants in CWs increase wildlife diversity, but we did not find any empirical study showing this effect or explaining this particular function.

(h) Plant production: CW plants can be used for the production of marketable goods. The literature includes reports on growing vegetation for fibers to be used in construction material (*Typha* spp. [50]), and bioenergy crops, as in the case of short-rotation willow coppice harvest in CWs [51]. Some plants are produced in CWs as submerged ornamental plants or conversely—some ornamental plants have been examined as CW plants, to enable wastewater treatment with the additional benefit of commercial value [48]. It is worth testing other potential products, such as water lilies and animal feed. With respect to the latter, no studies have specifically reviewed the advantages and obstacles of using CW plants as animal feed although such plants can also serve as good fodder. For example, *Bassia indica*, a potential CW plant, is reported to provide a fine addition to the main diet of lamb and sheep, by providing both palatability and nutrition [70,71].

(i) Novel roles for plants: Two novel roles have been the focus of recent studies by our team—the use of *B. indica*, a salt-tolerant plant (halophyte), for salt phytoremediation [34], and the use of plants as bioindicators [46]. These effects have been further studied and new results are presented in Section 3.

3. New Perspectives

In addition to the more traditional roles of plants in CWs, we present and discuss two unexplored functional mechanisms for plants in CWs. In the first, we demonstrate the role of a halophyte for salt phytoremediation and in the second, we test the role of plants as bioindicators of CWs.

3.1. Salt Phytoremediation in CW

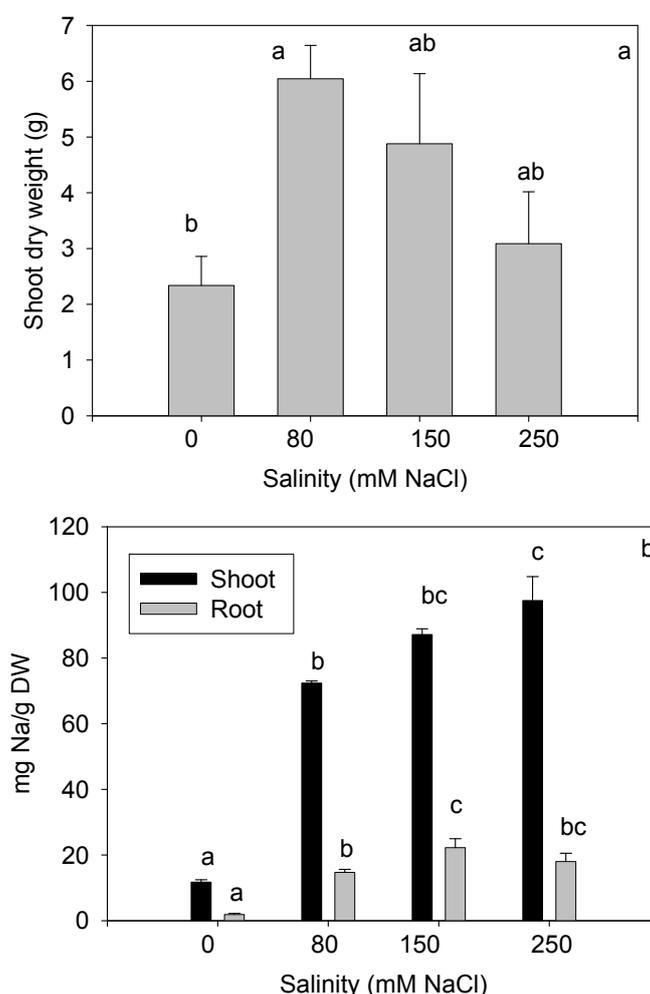
Most plants used in CWs are helophytes (plants with underwater buds [4]). Here we propose using halophytes (salt-tolerant plants) as well. Halophytes have long been suggested as nutrient filters for CWs that treat saline wastewater, such as aquaculture effluents [72]. However, the use of halophytes to reduce salinity is a novel strategy. To act as a salt accumulator, the plant has to tolerate a wide gradient of salinities, be able to grow in CWs, and most importantly, be able to accumulate enough ions within its tissues to significantly reduce the salinity of the wastewater. Our candidate halophyte to test the potential for salt phytoremediation was *Bassia indica* (Wight) A.J. Scott. *B. indica* is a halophytic annual that can gain a high amount of biomass (up to 9 kg of dry weight) in a short period of time (two to four months) and, therefore, has the potential to accumulate salts in amounts that will affect wastewater.

Plants were cultivated hydroponically in a greenhouse from January to March 2009. Young seedlings (18 days old) were grown in soil and then transferred to 4 L buckets. The buckets were aerated with an aquarium air pump to provide oxygen for the plant roots. Nutrients were supplied by liquid fertilizer—Long Ashton solution. Four solutions were used as treatments: 0, 80, 150 and 250 mM NaCl, two buckets for each solution, eight plants per bucket. Of these, a total of five plants per solution were sampled and measured. Plants were harvested after 72 days of growth in the solutions and immediately separated into roots and upper parts (shoots). Care was taken to ensure careful washing of the roots to avoid background measurement of the hydroponic solution. To measure Na concentrations in the tissues, five plant samples were dried (65 °C for 48 h), weighed (0.25 g) and digested overnight in 5 mL of an acidic mixture of perchloric acid and nitric acid (HClO₄:HNO₃ 15:85% v/v) in glass digestion tubes. Digestion was completed by gradually increasing the temperature from 60 to 195 °C according to Zhao *et al.* [73]. After cooling, 2.5 mL of 20% HCl was added, vortexed and warmed to 80 °C for 30 min. The final volume was brought to 10 mL with double-distilled water and rewarmed to 80 °C for another 30 min. Na concentrations were analyzed by fast sequential atomic absorption spectrometer (AA280 FS, Varian Inc., Palo Alto, CA, USA) following Welz and Sperling [74]. Data sets were tested for a Gaussian distribution by Shapiro-Wilk test. If a Gaussian distribution was detected, we used a parametric Student *t* test or Tukey procedure to test for significant differences. A non-parametric Mann-Whitney *U*-test was used whenever the data set did not fit the requirements of normal distribution. P values lower than 0.05 were considered as statistically significant. Calculations were conducted with SAS 10 (SAS Institute, Cary, NC, USA). Results showed that *B. indica* plants grow very well in salinities ranging from 0 to 250 mM NaCl (Figure 1a), with a preference for mild salinity levels (80 mM). Plants were found to accumulate significant amounts of Na, up to 10% of their dry weight (Figure 1b), and most of the accumulation occurred in the shoot.

Our results thus provided evidence of two basic traits for salt phytoremediation: survival of the plant in the treated media and high accumulation rate of the desired pollutant (NaCl). Based on these results, this approach was successfully tested in three on-site CWs in which the presence of *B. indica* reduced wastewater salinity as compared to “traditionally” planted controls [34]. In these experiments, *B. indica* developed successfully in the three different CW systems (hydroponic aerated containers in a greenhouse, vertical-flow CW, and recirculated vertical-flow CW). Salinities included mixed ion

solutions were reduced by 20% to 60% in CWs treated with *B. indica* as compared to systems without *B. indica* [34]. Regarding the fate of the salts accumulated in the phytoremediator plants, we suggested harvesting. We have just begun another study, aimed at examining the use of *B. indica* plants with accumulated salts for stock feed, a solution that is based on studies showing the potential of *B. indica* for feeding domesticated ruminants [70,71].

Figure 1. Performance of *Bassia indica* growing in hydroponic salt solutions. (a) Average dry weight of shoots ($n = 5$); (b) Accumulation of Na in roots and shoots. Vertical bars represent average \pm SE. Different letters denote significant differences between treatments ($p < 0.05$).



3.2. Plant Physiological Performance for CW Biomonitoring

Bioindication is based on the assumption that the performance of an organism reflects the conditions that prevail in its surroundings. Thus, plant performance can indicate the quality of the wastewater treatment. Accumulation of the highly reactive molecule hydrogen peroxide (H_2O_2) is potentially damaging and is related to the signaling of environmental stresses [75]. The connection between stress level and H_2O_2 concentrations spans over several orders of magnitude and the level of H_2O_2 change as response to stress can vary from pmol to mmol per gram fresh dry weight [76].

To test the role of plants as bioindicators in a CW, we studied plants in a CW located in a hyperarid region near Kibbutz Neot Smadar (30°02'45" N and 35°01'19" E) in Israel (Figure 2), where annual precipitation is less than 40 mm. Domestic and agricultural wastewater from the nearby kibbutzes were diverted into a sedimentation and oxidation pond and its effluent overflowed through a cascade of three horizontal-flow planted beds of 900 m² each, ordered A, B, and C from upstream to downstream [45]. Leaves were taken from plant species along the flow path in beds A and B, two leaves per species, and immediately frozen in liquid nitrogen, then stored at -80 °C for further analysis. The following plant species were sampled, analyzed, and pooled: *Phragmites nana*, *Spartina patens*, *Litrum salicaria*, *Schoenoplectus vallidus*, *Juncus acutus*, *Cyperus papyrus*, *Iris pseudoacorus*, and *Iris louisiana*. H₂O₂ accumulation in the plant tissues was assayed for each sample, using the ferrous ion oxidation method [77]. Leaves were homogenized in 5% (w/v) Trichloroacetic acid (TCA) (1 g tissue per 2 mL TCA) and then centrifuged for 10 min at 15,000 g. Aliquots of the supernatant (20 to 50 µL) were mixed with FOX 1 reagent containing 100 µM xylenol orange, 250 µM ammonium ferrous sulfate, 100 µM sorbitol and 25 mM H₂SO₄, incubated at room temperature for 30 min, and the absorbance was read at 560 nm. H₂O₂ content was calculated according to a standard curve (20 to 200 nmol H₂O₂/mLs). Data were analyzed with the same statistical tools and parameters described for the salt phytoremediation experiment (Section 3.1).

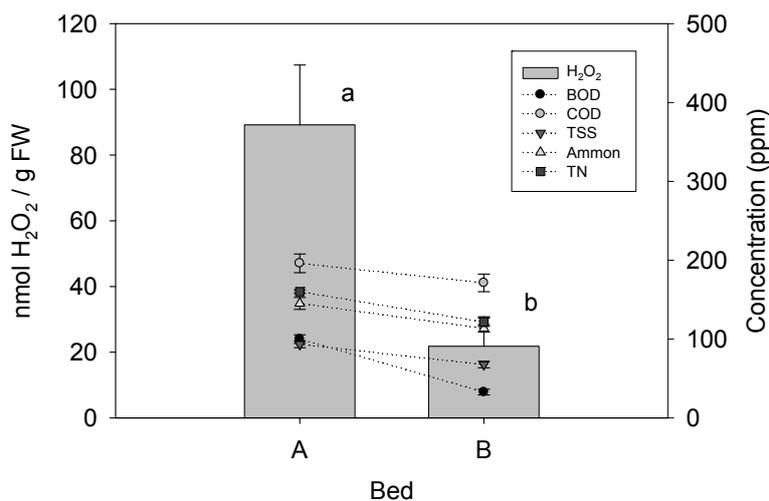
Figure 2. Submerged plant rows in the studied constructed wetland, Kibbutz Neot Smadar, Israel.



Analyzing H₂O₂ levels in plants in beds A and B along the wetland water flow demonstrated a 75% reduction in the downstream direction (Figure 3). The improvement in this physiological parameter was correlated to decreases in biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total ammonia N, total N, and *Escherichia coli* as measured over the years in the CW ([45,46], Figure 3). This observation, together with previous ones, showed that water quality in the studied wetland [46] is correlated with photochemical efficiency (Fv/Fm), net photosynthesis assimilation, and cell membrane stability. This supported our hypothesis that plant

performance can be used for CW monitoring, because it correlates with wastewater-quality improvement along the CW. Brisson and Chazarenc [5] argued that maximizing pollutant removal in CWs may be dependent on plant species selection. We recommend considering the use of plants as bioindicators of CW performance in the planning phase.

Figure 3. Effect of water quality on H₂O₂ concentration in plants. H₂O₂ concentrations are correlated to other chemical and physical parameters of water quality. Bed A is located upstream of Bed B. Different letters denote significant differences ($p < 0.05$).



4. Discussion

Studies related to plants' role in CWs provide a broad overview of their potential function. Herein we summarize the main contributions of plants to CWs as reported in the literature. We found that under some conditions, plants' benefits for CW performance are well established, whereas in others, their role is less significant. It is difficult to predict when and under what circumstances the plant contributions will be more salient, and this may be the reason for the controversy surrounding their actual roles. We hypothesize that this results from the fact that most studies relate to the overall effect of plants on CW systems, with much less focus on the specific plant species or mechanisms and their promotion of CW efficiency. We recognize that such studies are difficult to conduct under field conditions because of the complexity of the outdoor facility, with multiple variables affecting its performance (e.g., wastewater-influx quality, media composition, climate, fauna, and flora); however, such studies are crucial to better understanding and predicting plants' roles in CWs.

We go on to describe our research into two potentially novel roles for plants in CW systems—the use of halophytes for salt phytoremediation, and of plants as CW bioindicators. Both experiments involved measurements of specific mechanisms and were based on field experiments in an operating CW. The results showed that both salt phytoremediation and phytoindication are promising new functions of plants in CWs. These findings stress the importance of plants in CW practicum and studies. This is further emphasized by the fact that both perspectives were based on experimental setups that were not designed primarily to test them. *B. indica* was not chosen for this study as the best salt-accumulating halophyte, it was chosen due to our interest in its unique adaptations to salt stress [78], suggesting that other halophytes may also be used for salt phytoremediation and could

potentially provide even better results. Studies have demonstrated that halophytes can survive in extreme salinities and mitigate soil salinity [79–86], but studies on salt phytoremediation in water are still scarce. Overall, we concluded that salt phytoremediation is a plausible strategy in CWs. This is still a novel concept and further study is required to learn which plants are potential phytoremediators, what conditions will provide effective treatments, and what type of management will prevent resalinization through plant decay.

Similarly, plants in the CW in Kibbutz Neot Smadar were not chosen for phytoindication, but they appear to serve as potential bioindicators, suggesting that plants may offer even more options for phytoindication. It seems unlikely that H₂O₂ analysis or other plant measurements will replace the conventional measures of water quality (mainly BOD and COD). However, we suggest the use of phytoindicators as an additional index. The main advantage of biomonitoring is a continuous evaluation of water quality. Plants may serve as bioindicators of a combination of contaminants and physical conditions which would otherwise not be measurable in toxicity/chemical tests for a single contaminant or by hydrogeochemical assay [87,88].

5. Conclusions

Although most researchers agree that plants have a generally positive effect on wastewater treatment in CWs, the practical planning and maintenance of plantations is still premature as we lack the appropriate knowledge to direct these endeavors. This review clarifies the necessity of widening the scope of knowledge regarding the practical use of plants in CWs, which will lead to their better use in the treatment process. We recommend (1) concentrating on specific species and their mechanisms in CW dynamics and (2) in practice, being more mindful when planning the use of plants. Such a plan should include choosing the species, their composition, the order of planting and their spatial arrangement, as well as the educated practice of a harvest plan. The use of CW plants for commercial purposes should also be considered. These new perspectives expand the potential use of plants in CWs and add more factors to consider when planning vegetation and management practices for CWs.

Acknowledgments

We would like to thank Tanya Gendler for laboratory and field work, and Ahuva Vonshak for H₂O₂ and elemental analysis. We thank Kibbutz Neot Smadar for assisting with the constructed wetland sampling. The research was partly supported by the Rosenzweig-Coopersmith Foundation and The Jewish Colonization Association (ICA).

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