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Aquatic phytoremediation: Novel insights in tropical and subtropical regions*

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Abstract: An overview of the state of the art in phytofiltration of nutrients and heavy metals (HMs) from wastewaters using tropical and subtropical plants in constructed wetlands (CWs) and lagoons is presented. Various mechanisms to remove these pollutants are discussed, in regard to three different types of systems: surface flow constructed wetlands (SFCWs), subsurface flow constructed wetlands (SSFCWs), and lagoons with floating plants. Only recent reports at laboratory, pilot and full scale, especially in tropical regions, are discussed. Most of the experiences around the world have shown that these systems are efficient and high removal percentages have been reported for both, nutrients and metals. However, there are still several unsolved or partially understood issues. Long-term studies at the mesocosms or large scale, in order to gain a full insight of the various mechanisms occurring in each system, are required. The understanding of the fate or compartmentalization of the pollutants in these complex artificial ecosystems, especially in the case of HMs, will permit us to establish the frequency of harvesting and the advantages of the use of specific species. The huge biodiversity that is commonly found in tropical and subtropical regions represents a challenge for finding new species with outstanding characteristics for tolerance to toxic and recalcitrant pollutants or to extreme environmental conditions, such as high temperature or salinity.

Keywords: aquatic plants; constructed wetlands; lagoons; metals; nutrients.

INTRODUCTION

Pollutants from inorganic [heavy metals (HMs), radionuclides, nitrogen (N), phosphorus (P), etc.] and organic (fuels, solvents, explosives, pesticides, herbicides, chemical and petrochemical compounds, etc.) origin may contaminate surface water and groundwater as a consequence of natural and human activities [1]. N and P inputs can trigger undesirable eutrophication, which is the most widespread water quality problem in the world. In some regions, eutrophication is a common problem due to the lack of infrastructure for wastewater treatment [2]. The negative impact of excessive nutrients on riverine and palustrine systems, estuaries and coastal waters is recognized as a serious global problem [3] while agriculture is, in many regions of the world, the largest single source of N emissions to the aquatic environments [4]. Upstream agroindustrial pollution sources, mainly the sugar and alcohol industries, have been found as major contributors of N and P into the Mexican coastal area of the Gulf of Mexico [5].

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On the other hand, the release of HMs into the environment presents a serious threat [6]. HMs can be absorbed by living organisms and enter the food chain [7], causing cytotoxic, mutagenic, and carcinogenic effects on human beings and wildlife [8]. Their elimination from contaminated waters has become a major topic of research in recent years [9].

Nutrients such as N and P can be removed from wastewater by a variety of physicochemical and biological processes, the latter being more effective and less expensive [10]. Chemical precipitation, coagulation–flocculation, flotation, ion exchange, and membrane filtration can be employed to remove HMs from contaminated wastewater [7]. However, they have inherent limitations in application mainly due to lack of economical feasibility for treating a large volume of water with a low metal concentration. Furthermore, the major disadvantage of conventional technologies is the production of sludge [11].

Due to the above-mentioned constraints of conventional technologies, the biological treatment of metals, especially phytoremediation, is becoming a more attractive alternative. Phytoremediation is defined as the use of plants and their associated microbes to remove, reduce, degrade, or immobilize environmental pollutants from soil and water, thus restoring contaminated sites to a relatively clean, nontoxic environment. Phytoremediation includes various strategies, and all of them are promising, cost-effective, and environmentally friendly technologies. A variety of polluted waters can be phytoremediated, including sewage and municipal wastewater, agricultural runoff/drainage water, industrial wastewater, coal pile runoff, landfill leachate, mine drainage, and groundwater plumes [12].

Phytofiltration, a specific strategy of phytoremediation, is the use of plants to remove contaminants from water and aqueous waste streams. Three different systems can be considered within this strategy: (a) rhizofiltration (the use of hydroponically cultivated plant roots) [6,13,14]; (b) constructed wetlands (CWs) and lagoons; and (c) bioadsorbent-based systems [1]. Preparation stages such as growth of terrestrial plants by means of hydroponic cultures are required in rhizofiltration, and growth, drying, and size reduction of plant biomass are required in the bioadsorbent-based systems before they can be used for pollutants removal. These extra requirements may increase the investment and operational costs. On the contrary, the lagoons and CWs are designed to process the influents in one single stage.

This work is aimed at presenting an overview of the state of the art in phytofiltration of nutrients and HMs from wastewater, using tropical and subtropical plants in CWs and lagoon systems. It is considered that this type of plant require special attention since they may be used in areas where wastewater treatment infrastructure is still missing. Work performed with other phytofiltration systems such as rhizofiltration and bioadsorbent-based systems is outside the scope of this review and can be consulted elsewhere.

REMOVAL OF NUTRIENTS

Constructed wetlands

CWs are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages for wastewater treatment within a more controlled environment [15]. They have many advantages for treating wastewater and runoff. They are a cost-effective and technically feasible technology. The expenses of operation and maintenance (energy and supplies) are low, requiring only periodic, rather than continuous, on-site labor. CWs are tolerant to fluctuations in flow and facilitate water reuse and recycling. Additionally, they provide habitat for many wetland organisms and benefits to wildlife habitat [16].

The basic classification of CWs is based on the type of water flow regime and type of plants that are suitable for each system (Fig. 1). Nitrogen removal in CWs is carried out mainly by nitrification/denitrification, volatilization, adsorption, and plant uptake processes. On the other hand, P removal occurs due to processes such as adsorption, absorption, complexation, and precipitation.

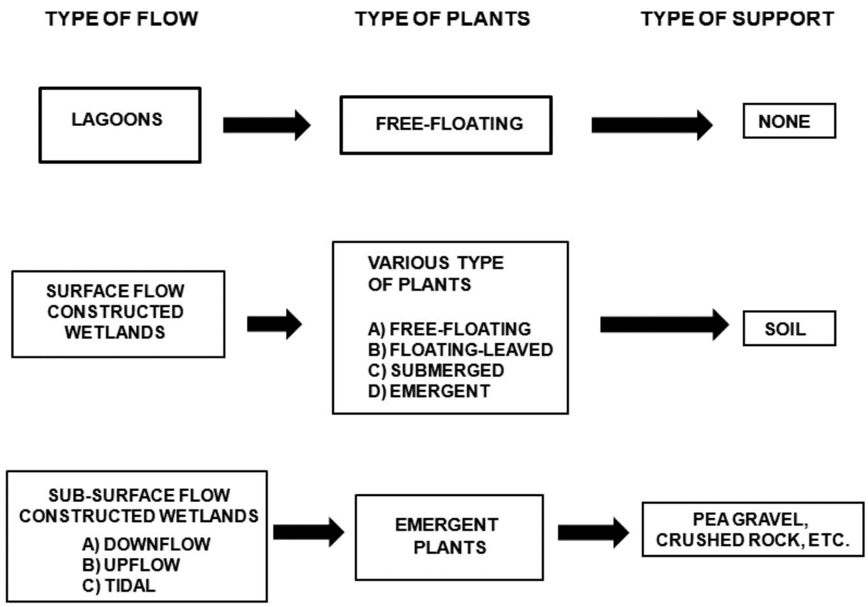

Fig. 1 Classification of phytofiltration systems for wastewater treatment using aquatic plants.

Although N and P removal through plant uptake is negligible, this mechanism may play a more significant role in tropical and partially subtropical regions where the growth seasonality and nutrient translocations between above- and below-ground parts are minimal, unlike limitations occurring in temperate and colder regions [16,17].

Surface flow constructed wetlands (SFCWs)

SFCWs are shallow sealed basins or sequence of basins, containing 20–30 cm of rooting soil, with a water control structure that maintains a shallow depth of water (less than 0.4 m). The water surface above the soil is aerobic, while the deeper waters and substrate are usually anaerobic [16]. Dense emergent vegetation covers usually more than 50 % of the surface. However, leave-floated, submerged, and floating macrophytes are also found [18]. Their capital and operating costs are low. However, their main disadvantage is that a larger land area is required than in other systems [16].

Recent reports on the evaluation of this type of system indicate that there are still many unsolved issues and that there are many environmental factors that affect the performance of the systems. During the evaluation of the performance of SFCWs at laboratory scale for the treatment of saline wastewater [19], although cattail and Asia crabgrass were tolerant to saline wastewaters, the nutrient and organic removal in the system (NH_4–N: 18.0–65.3 %, total P: 12.2–40.5 %) was not enough to have an acceptable effluent quality and then a post-treatment was required. The authors suggested that flourishing growth of algae and plankton in the surface flow system was responsible for the reduction in the CW performance. In regard to the comparison between mono and mixed cultures [20], no differences were found for the case of *Canna indica* and *Schoenoplectus validus* for the treatment of simulated secondary-treated municipal wastewater.

In tropical regions, evapotranspiration is a very important factor to take into account. Bojcevska and Tonderski [21] found that total P mass removal rates were 50–80 % higher when evapotranspiration was only estimated, instead of using the direct data from pan evaporation, during the performance of an SFCW for the treatment of sugar factory stabilization pond effluent. These results illustrate the importance of accurate estimations of evapotranspiration for pollutant mass removal rates in CWs in tropical climates. Additionally, they found that systems planted with *Cyperus papyrus* resulted in more efficiency than those with *Echinochloa pyramidalis* in terms of NH_4–N removal. Other recent experiences have also shown the feasibility of using SFCWs for the removal of pollutants from diluted seafood processing wastewater. *C. involucratus*, *Thalia deabata*, and *Typha augustifolia* proved to achieve an acceptable efficiency in terms of nutrient uptake rates, which were in the range of 1.43–2.30 g N m^{-2} day and 0.17–0.29 g P m^{-2} day^{-1}, respectively at a hydraulic retention time (HRT) of 3 days. The highest treatment performances were found at an HRT of 5 days, total N: 72–92 %, total P: 72–77 % [22].

The majority of full-scale experiences reported recently are related to the treatment of non-point wastewaters. In Italy, Borin et al. [23] evaluated SFCWs that received drainage water from 6 ha of land managed, where maize, sugarbeet, winter wheat, and soybean were cultivated. Over 5 years, the system showed an apparent removal efficiency of about 90 %. The main N removal mechanisms were plant uptake (1110 kg ha^{-1}) and soil accumulation (570 kg ha^{-1}), whereas the contribution of denitrification was estimated at around 7 %. In contrast with these results, in an SFCW established in a subtropical zone in China (2800 m^2) only 14 % of the N load was incorporated into the plant biomass (*Zizania caduciflora* and *Phragmites australis*), 39 % of it was discharged and 47 % of the N load was inferred to be removed by nitrification/denitrification exchange with groundwater/ammonia adsorption/bacteria or algae assimilation [24]. The authors mentioned that this eco-technology could be used effectively for water quality enhancement in China and other areas with a similar climate. In South Florida, SFCWs have also proved to be efficient for P reduction from the Everglades Agricultural Area runoff using emergent vascular plant-cattail (*T. latifolia*), submerged aquatic vegetation (*Najas guadalupensis*, *Chara* sp., *Ceratophyllum demersum*, and *Hydrilla verticillata*), or algal periphyton (mixed with *Eleocharis cellulosa* and *Utricularia* spp. in the south site only). Under a constant hydraulic loading rate (9.27 yr^{-1}), P removal efficiencies were 56–65 % at the north site. Soluble reactive P (SRP) and particulate P were the major forms at inflow and were removed effectively by all of the test cells. Direct plant uptake, wetland filtering, microbial degradation, and coprecipitation with calcium carbonate were mechanisms thought to be responsible for P removal in these systems [25].

Subsurface flow constructed wetlands (SSFCWs)

SSFCWs are gravel and/or soil/sand-filled trenches, channels, or basins with no standing water, which support emergent vegetation. In the horizontal-flow SSFCW (HFCW), the wastewater flows slowly through the bed in a relative horizontal path and comes into contact with a network of aerobic, anoxic, and anaerobic zones. On the other hand, vertical-flow CWs (VFCWs) are fed intermittently to flood the surface and wastewater, then gradually percolate down through the bed and is collected by a drainage network at the base [17]. The advantages of SSFCWs over SFCWs are: greater cold tolerance; minimization of pests, such as mosquito larvae, and odor problems; and, possibly, greater assimilation potential per unit of land area, which results in a smaller requirement of land for the same volume of wastewater. On the other hand, SSFCWs are more expensive to construct and may be more difficult to regulate than SFCWs. Furthermore, maintenance and repair costs are generally higher. Clogging and unintended surface flow problems have been also reported for this kind of system [16].

Nutrient removal in SSFCWs has been widely reported. In this section, only some recent reports dealing with removal of N and P at laboratory, mesocosms, pilot and full scale, especially in tropical regions, are discussed (Table 1). Microcosm experiences have shown the effectiveness of SSFCWs for non-point and point water pollution. In Thailand, Kantawanichkul et al. [26] demonstrated that a hybrid (an upflow VFCW followed by a downflow VFCW) as a post-treatment system for anaerobic effluents from pig wastewater was suitable for treating wastewater with a high ammonium concentration and that the denitrification in the up-flow bed and the nitrification in the down-flow bed were noticeable. However, further treatment was also needed for nitrate reduction in the final effluent. On the other hand, SSFCWs have been also used as a secondary treatment to treat municipal wastewater in Hong Kong, resulting in high nutrient removals [27].

Table 1 Nutrient removal efficiency in SFCWs for different wastewaters.

Wastewater	Plant(s)	Removal percentage (%)	Ref.
Municipal wastewater	*Typha latifolia*	NH_4–N: 92 and 95; PO_4–P: 79 and 72; DOC: 68 and 72	26
Anaerobic effluents from pig wastewater	*Typha angustifolia, Cyperus alternif*	NH_4–N: 62.7; TN: 56.8 COD: 68.4	27
Diluted sugarcane molasses stillage from ethanol production	*Pontederia sagittata*	BOD_5: 80.24–80.62; COD: 82.2–87.31; TKN: 73.42–76.07 NO_3–N: 56–58.74	28
Municipal wastewater	*Scirpus grossus*	NH_4–N:71; BOD_5: 69	31
Highly polluted river water	*T. latifolia*	NH_4–N: 25.1; TN: 10.0 SRP: 7.7; TP: 7.4	32
Municipal wastewater	*Zizaniopsis bonariensis*	TN: 68; P: 79–81 COD: 69–98; BOD_5: 73–98	33

TN: total nitrogen
TKN: total kjeldahl nitrogen
NH_4–N: ammonia
TP: total phosphorus
COD: chemical oxygen demand
NO_3–N: nitrates
SRP: soluble reactive phosphorus
BOD_5: biochemical oxygen demand
DOC: dissolved organic carbon

Concerning the treatment of effluents with a very high organic matter load, our research group has recently reported the use of SSFCWs at the mesocosm level, vegetated with *Pontederia sagittata* for the treatment of diluted sugarcane molasses stillage from ethanol production [28]. Stillage is a very difficult-to-treat effluent since it contains not only very high organic matter content (chemical oxygen demand, COD, in the range of 22–45 g l^{-1} and biochemical oxygen demand, BOD_5, in the range of 12–20 mg l^{-1}), but also, very high contents of sulfate (in the range of 400–3730 mg l^{-1}), potassium (in the range of 800–3817 mg l^{-1}) and toxic/recalcitrant compounds called melanoidins [29]. *Pontederia sagittata* is a tropical plant chosen primarily because it grows where most of the alcohol factories are located in the State of Veracruz, México. In a start-up stage, it was compared with *Pistia stratiotes*, growing in lagoons, also at mesocosms scale. Stillage was not subjected to any pretreatment apart from being diluted and adjusted to pH 6.0. A much better performance of *P. sagittata* in SSFCWs was obtained compared to that of *P. stratiotes* in lagoons in terms of organic matter and nutrient removal (unpublished results). Based on these results, only SSFCWs with *P. sagittata* were operated in a second stage [28]. It was found that a very high organic matter removal percentage (80.43 % of COD and 84.75 % of BOD_5) occurred, considering that the systems were fed at very high surface COD loading rates (47.26 and 94.83 g COD m^{-2} d^{-1}). These results reflect the presence of tolerance and/or degradation mechanisms of toxic and non-easily degradable compounds such as those present in the molasses stillage. Regarding nutrients, total Kjeldahl nitrogen (TKN) was removed at a high proportion during the 55 days of operation, especially at the higher HRT tested of 5 d (73.42–76.07 %) compared to NO_3–N (56–58.74 %). On the contrary, phosphate and potassium were not removed. Finally, the fact that the CWs removed very efficiently sulfates (in an average of 69 %) may indicate the presence of a very active sulfate-reducing bacteria population that might have contributed to the high removal rate of organic matter. It is known that when input sulfate concentrations are high and redox conditions are favorable, a considerable fraction of the organic carbon removal may be attributed to sulfate reduction [30].

At pilot scale, *Scirpus grossus* has been shown to have great potential in removing pollutants from domestic wastewater in SSFCWs in tropical regions. Such a system, evaluated over 14 months, achieved high removal percentages for NH_4–N and fecal coliforms, whereas total P and NO_3–N were removed at a lower extent [31]. In China, studies on the contribution of intermittent artificial aeration to nutrient removal from a eutrophied river water, have been also developed using SSFCWs planted with *Typha latifolia* L. Results showed that aeration at the bottom of the supporting media enhanced ammonia–N, total N, SRP, and total P removal. Furthermore, additional total N removal of 116 kg N ha^{-1} and 126 kg N ha^{-1} by harvesting above-ground plant biomass, was observed when intermittent artificial aeration was applied in the middle and at the bottom of the wetland substrate, respectively [32].

Philippi and coworkers [33] evaluated the treatment performance of four SSFCWs in rural areas of Brazil under a subtropical climate. All systems consisted of a septic tank followed by an HFCW (450, 84, 50, and 40 m^2, respectively) planted with *Zizaniopsis bonariensis*. The authors pointed out that treatment efficiency of all systems was good and stressed that SSFCWs are easy to operate and maintain, and therefore these systems have great potential in rural areas. However, for systems with low specific area (<1 m^2 person connected to the $system^{-1}$) there was a strong trend of substrate clogging.

Lagoons with floating plants

The lagoons with floating plants must be deep enough to prevent emergent plants from growing, but shallow enough to ensure adequate contact between the roots of the floating plants and the wastewater (depth range: 0.9–1.5 m) [34].

Nitrogen removal in systems with free-floating plants is carried out through plant uptake, ammonia volatilization, and nitrification–denitrification processes. The latter occurs by the presence of nitrifiers attached to the plant roots and when dissolved oxygen levels of the water are adequate to support activity of nitrifying bacteria in the water column. Phosphorus can be removed from these systems by plant uptake, microbial assimilation, precipitation with cations, or adsorption onto clays or organic matter. Harvesting is essential to avoid detritus of plants releasing P into water during decomposition [17].

Many species of free-floating plants have been used for nutrient removal. The systems based on duckweed present some advantages such as: rapid growth rates, high levels of nutrient removal, easy harvest, high protein content, and low fiber content. All these characteristics make these systems as cost-effective for recycling nutrients as fertilizer and animal fodder [35]. The lagoons with duckweed have been studied for treating different kinds of wastewaters, including raw and diluted sewage, secondary effluents, dairy waste, sewage ponds, and those from fish culture. Reviews of the results from laboratory experiments using small-scale duckweed-covered batch systems are available in the literature [36,37].

Salvinia minima, an aquatic fern, offers several advantages for the phytoremediation of wastewater [38]: (a) it has a wide geographical distribution within the tropical and subtropical regions of the world; (b) it outgrows duckweed (lemnaceas) in a mixed culture and may reach very high productivities; (c) it has shown an annual average productivity of 32 ton ha^{-1} yr^{-1} in a chemically defined medium under the climatic conditions of Florida; (d) it reached an average productivity of 28 ton ha^{-1} yr^{-1} in a full-strength effluent of a coffee processing plant containing a rather high ammonium–N concentration (62.4 mg l^{-1}) under summer subtropical conditions in México, showing a simultaneous removal percentage of 74 % for NH_4–N and 75 % for PO_4–P [39]. In the latter work, some conditions were recommended for optimal operation: (a) the pH of the wastewater has to be adjusted to 6.0 to avoid inhibition of growth at alkaline pHs; (b) the depth of the ponds should be adjusted to around 0.3 m for reaching the maximum absolute removal efficiency (considering the total volume of the reactor) and the maximum productivity. During the late autumn and winter period, the anaerobic effluents should be diluted 1:2 in order to enhance the ammonia–N removal. It has also been shown that *S. minima* is a better option than *Spirodela polyrrhiza* for treating high-strength wastewater [38], since it showed a 2.3-fold higher productivity in a high-strength synthetic wastewater. The recommended conditions for lagoon operation when treating high-strength wastewater with *S. minima* batchwise such as anaerobic effluents from pig waste, are: (a) the maximum initial ammonium–N concentration should be 70 mg l^{-1} at a pH of 5.0 or 6.0; (b) the initial density of the plant should be maintained in the range of 7–15 g dry weight (dw) m^{-2}.

Eichhornia crassipes has been one of the most studied free-floating macrophyte for nutrient removal. In a recent work, the efficiency of *E. crassipes*, *P. stratiotes*, and *Salvinia molesta* ponds was evaluated for the treatment of effluents from Nile tilapia culture ponds. Macrophytes were placed in 2000-l outdoor concrete tanks (4.0 m^2 surface area) with continuous water flow. The results showed that *E. crassipes* and *P. stratiotes* were more efficient especially in total P removal (82.0 and 83.3 %, respectively) than *S. molesta* (72.1 %). Total N removal ranged from 42.7 to 46.1 % for all systems. Although the treatments with *E. crassipes* and *P. stratiotes* presented similar efficiency for nutrient removal, authors pointed out some considerations. If there is interest in using the biomass of aquatic macrophytes as plant compost, biogas production, or animal feed, *E. crassipes* should be preferred for treatment of aquaculture effluents, since its weight gain was about 2.7 times higher than that of *P. stratiotes*. On the other hand, if these goals are not of interest, *P. stratiotes* is recommended because of the lower gain of mass, thus reducing problems from excess of biomass [40]. Even more recently, the same group evaluated such systems to treat effluents from shrimp culture, achieving total P removal of 71.6 % by *E. crassipes*; 69.9 % by *P. stratiotes*, and 72.5 % by *E. crassipes* plus *P. stratiotes* [41].

The potential of water hyacinth (*E. crassipes*), pennywort (*Hydrocotyle umbellata*), and water lettuce (*P. stratiotes*) to improve the water quality of anaerobically digested flushed dairy manure wastewater (ADFDMW) was also evaluated. It was necessary to carry out a 1:1 dilution of this high-strength organic matter wastewater (COD = 2010 mg l^{-1}) to allow the growth of the plants, which was robust only for water hyacinth. Nutrient removal was performed at the highest rate in systems with *E. crassipes*, compared to those with water lettuce and pennywort ponds. TKN was reduced by 91.7 %, ammonium by 99.6 %, total P by 98.5 %, and SRP by 96.5 % in a 31-day batch operation. A polyculture of the three plant species in 1:1 diluted ADFDMW exhibited the next best performance [42].

REMOVAL OF METALS

SFCWs and SSFCWs

The metal removal in wetlands is the result of a complex interaction of physical, chemical, and biological processes such as settling, sedimentation, sorption, co-precipitation, cation exchange, photodegradation, phytoaccumulation, biodegradation, microbial activity, and plant uptake. The extent to which these reactions occur depends on the composition of the supporting media, sediment, pH, type of wastewater, and plant species [43]. Recent reports of the use of SFCWs and SSFCWs for metal removal mainly at full scale are presented in Table 2. Different issues were investigated, such as the identification and characterization of rhizosferic microorganisms [44], the role of plants other than metal adsorption [45], the fate of metals among various compartments in CWs [46,47] and the effect of temperature, season, and COD load on sulfate reduction and metal removal [30]. Evaluation of the performance of CWs with different kinds of plants for different wastewaters at full scale, especially in tropical areas, was also considered [48–53].

Table 2 Metal removal in CWs for different type of wastewater.

Wastewater	Plant(s)	Observations	Ref.
SFCWs			
Tannery wastewater	*Typha* sp., *Scirpus americanus*	Heterotrophic sulfur-oxidizing bacteria (SOB): 104–106 cells g^{-1} sediment Sulfate-reducing bacteria (SRB): 104–106 and 102–105 cells g^{-1} sediment	44
Copper-contaminated water	*Schoenoplectus californicus*	A primary function of the plant was to produce OC for removal of copper by provision of organic ligands and to use OC as an energy source for sulfate production	45
Municipal wastewater	*Carex, Phormium*, *Juncus*, *Schoenoplectus, Bolboschenus, Lythrum hyssopifolia*	Cu: Precipitation with organics/ sulfides Zn and Pb showed an additional strong affinity for hydroxides	46
Agricultural and industrial run off	First section: a riparian-swamp ecosystem; second section: a riparian and wet ecosystem; third section: a marsh ecosystem.	Zn, Cr, Cu, and Ni were linked with sulfides. The formation of insoluble carbonates was another potential removal process detected.	47
Wastewater from metallurgic industry	*P. stratiotes*, *E. crassipes, Salvinia rotundifolia, Cyperus alternifolius*, *P. elephantipes*, *Thalia geniculata*, *Polygonum punctatum*, *Pontederia cordata*, *Pontederia rotundifolia*, *Typha domingensis, Aechmea distichantia*.	Small scale: Cr: 81 %, Ni: 66 %, Fe: 82 % removal. Full scale: Cr: 86 %, Ni: 67 %, Fe: 95 % removal.	48
Metallurgic plant wastewater	*E. crassipes*, *T. domingensis*, and *Pontederia cordata* L	Dominance *E. crassipes* Removal: Cr 88 %, Ni 93 %, Zn 98 %	49
Treatment plant wastewater	*Acorus, Typha*	Removal: Cu 47.16 %, Ni 25.42 %, Zn 65 %	50
SSFCWs			
Synthetic wastewater	Vetiver grasses	Main mechanism of As removal: entrapment into the porous of supporting media (50–57 % of total fraction).	51
Municipal wastewater	*P. australis*	Main metal compartment: sediments. 0.5 % Cu and 1.4 % Zn mass load in the influent was accumulated in the above-ground biomass.	52
Synthetic wastewater	*P. australis, T. latifolia*	Removal: *T. latifolia* 75 %; *P. australis* 95 %	53

(*continues on next page*)

Table 2 (*Continued*).

Wastewater	Plant(s)	Observations	Ref.
Mining wastewater	*T. latifolia* *Schoenoplectus acutus*	Temperature affected sulfate removal and there was Zn-sulfide precipitation	30

OC: organic carbon

Lagoons with floating plants

Plant uptake is the main pathway of metal removal in this kind of system [54,55], especially when media is free of strong ligands which may chelate the metals in solution [56]. Adsorption to surface plant, translocation, and intracellular accumulation have been described as the main removal mechanisms [56,57]. The majority of reports dealing with this type of system have been carried out in single-metal microcosms in batch-operated systems.

Salvinia minima Baker is a small free-floating aquatic fern native to México, and Central and South America. Our research group described this plant, for the first time, as hyperaccumulator of Cd (II) [58] and Pb (II) [56]. Zayed et al. [59] proposed a bioconcentration factor (BCF) higher than 1000 and a tissue metal concentration higher than 1 % (dw) as criteria to define an aquatic plant as hyperaccumulator. Thus, the results obtained for *S. minima* met widely such criteria (Cd BCF > 2718, 1.1 %; Pb BCF = 2065, 2.74 %) under environmental controlled conditions using small batch-operated lagoons. Studies evaluating the effects of environmental factors and nutrients on the various possible removal mechanisms (surface adsorption, intracellular accumulation and precipitation to sediments) and partitioning of lead among various compartments (plant biomass, water column, and sediments) were also carried out [56]. Surface adsorption was found to be the main Pb removal mechanism in the system when the medium was free of ligands such as ethylenediaminetetraacetic acid (EDTA) and phosphates. It was concluded that the mechanisms of lead removal by *S. minima*, and the compartmentalization of this metal in the microcosm of batch-operated lagoons, are primarily a function of the presence of certain nutrients and chelants, with secondary dependence on environmental conditions. In addition, the relevance of using a compartmentalization analysis complementary to the use of BCF and metal removal kinetics by plants was demonstrated [56]. Recently, and based on such a compartmentalization analysis [60], a bioadsorption (BAF) and an intracellular accumulation factor (IAF) were proposed in order to gain full insight into the hyperaccumulating metal capacity of aquatic plants, using *S. minima* exposed to lead as an experimental model. It was clear that in this model, adsorption of lead to the plant surface was the major removal mechanism, since the BAF was significantly higher than the IAF (780–1980 vs. 57–1007). This process followed a pseudo-second-order kinetics and was dependent on the initial metal concentration (from 0.8 to 28.40 mg Pb l^{-1}). Such high capacity to adsorb Pb was most likely due to its exceptional physicochemical characteristics such as a very high surface area (264 $m^2\ g^{-1}$) and a good content of carboxylic groups (0.95 mmol $H^+\ g^{-1}$ dw). Surprisingly, the ability of *S. minima* to accumulate the metal into the cells was not inhibited at concentrations as high as 28.40 ± 0.22 mg Pb l^{-1}.

On the other hand, in lab systems with *Hydrocotyle umbellata,* the accumulation of Pb(II) and Cr(VI) was found not to be linear with the exposure time and metal concentration. Both metals were accumulated mainly in the roots. The results also indicated a higher accumulation potential of Pb(II) than Cr(VI) in this plant [61].

Removal assessments in multi-metal systems have been reported using synthetic solutions and wastewaters. *Lemna minor* was shown to remove efficiently metals such as Pb(II) and Ni(II) (85–95 %, respectively) when they were in a mixture solution [62]. Furthermore, the potential competition between these two metals was also examined and no synergistic/antagonistic effect was found for the multiple metal experiments, in terms of metal removal [63]. El-Gendy [64] found that removal (24–80 %

of total HMs) of metals in mixtures [Cd(II),Cr(VI),Cu(II),Pb(II),Ni(II)] from municipal landfill leachate was mainly due to accumulation into biomass, which was a function of the initial metal concentration in leachate (from 0.18 to 5.50 mequiv l^{-1}). The average specific content of metals accumulated in the whole plant ranged from 23.86 ± 2.2 to 748.7 ± 93.6 mequiv kg^{-1} of plant. The nonlinear kinetics of HMs disappearance suggests that the plant roots employed different mechanisms for metal removal being the sorption one of the most important. *P. stratiotes*, *Spirodela intermedia*, and *L. minor* were also found highly effective in the simultaneous removal of several HMs [65]. Although the removals percentages achieved in systems with *P. stratiotes* were very high [ca >85 % Pb(II),Cr(III),Mn(II),Zn(II)], *S. intermedia* showed the highest rate coefficients and concentration factors resulting to be the most appropriated for metal removal. *L. minor* did not survive until the end of the experiment (15 days). A competition with the $PbCrO_4$ precipitation process was observed. As the rate of sorption gradually diminished after the first hours, and became negligible, plants should be harvested regularly, making the water purification a continuous process. In another group of experiments [66], the effectiveness of *P. stratiotes* and *S. polyrrhiza* was tested for the removal of five HMs. Results revealed high removal of Fe(II), Cu(II), and Zn(II) for both plants (76–96 %), whereas Cd(II) and Cr(VI) were removed at a lower proportion (70–82 %). Plants accumulated HMs in their tissues without signs of toxicity or growth reduction. Thus, they can be used for large-scale removal of HMs from wastewater.

The use of free-floating aquatic plants for metal removal at the mesocosmos level is little documented. In this regard, *E. crassipes* and *L. minor* have been tested for the removal of HMs from the coal mining effluent in ponds. The highest removal efficiency (>60 % for Fe, Cr, Cu, Cd, and Zn) was found in the combination of *E. crassipes* and *L. minor*, probably due to preferential higher absorption capacities of each plant [67]. Experimental sets containing only *E. crassipes* removed the highest concentration of HMs. Low metal translocation factors (TFs) were observed, which can be associated with protection of photosynthesis from toxic levels of trace elements [68]. No symptoms of metal toxicity were found; therefore, the authors suggest that this method can be applied to a large-scale treatment of wastewater in which the metal concentrations are low [67].*E. crassipes*, *P. stratiotes*, *L. minor*, *Azolla pinnata*, and *S. polyrhiza* were tested for their HM removal capacity from the secondary treated municipal wastewater (150 l-volume systems). The aquatic plants showed metal tolerance, and, surprisingly, the secondary treated municipal wastewater promoted their growth. *E. crassipes* was the most efficient accumulator, removing up to 70 % of Fe(II) and 59 % of Ni(II). TFs, in general, were less than 1, indicating that the metals were largely accumulated in the roots in comparison with the leaves; the highest TF was obtained for *L. minor* for Fe (0.94). Maximum removal at a 20-day HRT and a decreasing trend after that, indicated that aquatic plants should be harvested every 20 days for wastewater treatment. This technology is highly recommendable for tropical wastewaters where sewage is mixed with industrial effluents [69].

Finally, Jayaweera and co-workers [70] found that Fe was removed mainly from Fe-rich industrial wastewaters, by *E. crassipes* through an uptake process and chemical precipitation of Fe_2O_3 and $Fe(OH)_3$, followed by flocculation and sedimentation. A high Fe accumulation of 6707 Fe mg kg^{-1} dw was observed in the plant tissue. Active effluxing of Fe back to the wastewater at intermittent periods was a key mechanism to avoid Fe phytotoxicity in the plant cultivated in all nutrient conditions. It was concluded that water hyacinth grown under nutrient-poor conditions is ideal for removing Fe from wastewaters with a HRT of approximately 6 weeks.

CONCLUSIONS

CWs and lagoons of various types, using tropical and subtropical plants, have shown to be efficient for nutrient and HM removal from wastewaters. However, there are still several issues unsolved or partially understood. It is recommended to set up long-term studies at the mesocosms or large scale, in order to gain full insight into the various mechanisms occurring in each system. The understanding of the fate or compartmentalization of the pollutants in these complex artificial ecosystems, especially in the case of HMs, will allow drawing recommendations on the convenience and frequency of harvesting and on the advantages of the use of specific species. The huge biodiversity that is commonly found in tropical and subtropical regions represents a challenge for finding new species with outstanding characteristics for tolerance to toxic and recalcitrant pollutants or to extreme environmental conditions, such as high temperature or salinity.

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