Nutrition and toxicity of inorganic substances from wastewater in constructed wetlands

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ABSTRACT

The use of constructed wetlands for purification of wastewater has received increasing attention around the world. A variety of wetland plant species (including ornamental ones) as either a monoculture or species mixes are used in constructed wetlands. Plants play an extremely important role in removing pollutants from wastewater. Although there is considerable information on plant productivity, biomass and nutrient dynamics in natural and fertilized wetlands, most studies on constructed wetlands for treatment of wastewaters have only addressed general aspects of plant growth and nutrient accumulation. Nutrition and toxicity of inorganic substances such as nitrogen, sulphur, salts and metals in wastewater on wetland plants has not been fully investigated and their interactive effects and environmental cycling in constructed wetlands remain poorly understood.

Nitrogen nutrition is the most important factor influencing plant performance in constructed wetlands, but higher NH_4-N may become toxic to wetland plants. Sulphur is

an essential nutrient for plant growth, but under waterlogged conditions sulphate is reduced to hydrogen sulphide that is highly toxic to wetland plants. Many metals in wastewater are essential micronutrients for wetland plants, but become toxic if their concentration exceeds a specific critical point. A proper amount of salts is essential for plant growth, but high concentrations of salts, particularly sodium chloride in wastewater have harmful effects on plant growth.

Wetland plant species have differential capacity to take up nutrients, different preference for nitrogen forms and have evolved various adaptive mechanisms protecting them against toxicity of inorganic substances. Given that plants are an integral part of constructed wetlands, the selection of suitable species, improvement of cultivations and determination of factors affecting growth are needed to produce healthy and effective wetland ecosystems. Understanding biogeochemical cycling in wetlands as well as nutrition and toxicity of inorganic substances from wastewater on plant development and function may help reduce performance variability and enhance pollutant removal in constructed wetlands.

INTRODUCTION

During the past decades, the world has experienced growing water stress in terms of both water scarcity and quality deterioration, which prompted the governments to enforce tough legislations on wastewater discharge and encourage efficient use of water resources, including widespread promotion of water reuse practices.

Treatment technology for wastewater encompasses a vast variety of options. Among them, constructed wetlands are considered a sustainable, low-investment and low-maintenance technology that can complement or replace conventional wastewater treatments. The use of constructed wetlands to treat wastewater from agricultural, mining, municipal and industrial sources etc has undergone dramatic development since the 1990s (Kadlec & Knight, 1996; Sundaravadivel & Vigneswaran, 2001; Scholz & Lee, 2005).

Wastewater quality varies widely among municipal, industrial, agricultural and stormwater categories. Different wastewater sources have unique mixtures of potential pollutants so that even a single wastewater source category such as municipal wastewater or urban runoff may vary depending on local, site-specific circumstance (Kadlec & Knight, 1996). Therefore, constructed wetlands receiving wastewaters are exposed to a wide range of pollutants with varying loads. Contaminants include major elements such as nitrogen (N) and phosphorus (P), mineral oils, pathogens, trace contaminants such as pesticides, heavy metals, radionuclides, and emerging pollutants such as brominated flame retardants, oestrogenic compounds, etc. The contaminants are transformed and distributed in multiple abiotic and biotic compartments, and may cause diverse ecological and ecotoxicological effects (Tack et al., 2007). However, the fate and behaviour of the wide variety of contaminants that enter constructed wetlands are not fully understood.

Wetland plants are often central to wastewater treatment in constructed wetlands (Scholz & Lee, 2005), in addition to the other design factors such as hydraulics, choice of substrate, etc. Macrophytes are assumed to be the main biological component of wetlands. They not only assimilate pollutants directly into their tissues from wastewater and substrates, but also act as catalysts for purification reactions by increasing the environmental diversity in the rhizosphere, and promoting a variety of chemical and biological reactions that enhance pollutant removal (Jenssen et al., 1993; Brix, 1997). However, in some cases the vegetation has either failed completely or proved difficult to establish in wetlands (Batty & Younger, 2004). The reasons for these problems are not thoroughly understood.

Wetland plants require optimum environmental conditions in each phase of their life cycles, including germination and initial plant growth, seasonal growth and senescence and decay. Wetland plants tolerate a wide range of water quality, but do have limits outside which they can not survive (Kadlec, 1999). The toxic inorganic substances such as ammonium, sulphides, salts and metals in wastewater may inhibit nutrient uptake and plant growth if present in concentrations exceeding those that wetland plants can tolerate.

The function of vegetation in constructed wetlands is a dynamic one, and requires a better understanding of plant nutrient requirements as well as plant tolerance to pollutants to optimise performance in terms of substance removal in constructed wetlands. The objective of this review is to increase the understanding of nutritional value as well as toxicity of inorganic substances in wastewater to wetland plant growth in constructed wetlands. Even though the plant system has a large bearing on microbial activity in constructed wetlands, there may be direct toxicities of inorganic substances to microbes, thus harming the wetland functions. Further limitations to growth of plants and microbes may arise from certain organic compounds such as polycyclic aromatic hydrocarbons (PAHs) in wastewater, but those potential toxicities to plants and microbes are beyond the scope of this review (but see Haberl et al., 2003; Chaudhry et al., 2007; Neculita et al., 2007; etc).

THE ROLE OF PLANTS IN CONSTRUCTED WETLANDS

Plants are vital for operation and maintenance of constructed wetlands. The most visible role of plants in a wetland is their impact on the aesthetics of the area and the quality of the wildlife habitat. However, it has been widely demonstrated that plants are also involved in almost every major function in the wetland treatment systems (Thullen et al., 2005). The main plant roles are:

(1) Providing the conditions for physical filtration of wastewater and a large surface area for microbial growth, as well as being a source of carbohydrates for microbes (Brix, 1997);

(2) Taking up nutrients and incorporating them into plant tissues. Although a portion of these nutrients is released when plants senesce and decompose, some nutrients remain in the un-decomposed litter that accumulates in wetlands, building organic sediments (Kadlec, 1995);

(3) Leaking oxygen into the sediments and creating a zone in which aerobic microbes persist and chemical oxidation can occur (Armstrong, 1978); and

(4) Having additional site-specific values by providing habitat for wildlife and making wastewater treatment systems aesthetically pleasing (Knight, 1997).

PLANT SPECIES USED IN CONSTRUCTED WETLANDS

A wide variety of aquatic plants can be used in constructed wetlands designed for wastewater treatment. Commonly, however, constructed wetlands are planned as marsh-type wetlands and are planted with emergent macrophytes (rooted plants that anchor to the substrate media) adapted to a water-dominated environment. Frequently used macrophytes species are cattails (*Typha sp.*), reeds (*Phragmites sp*.), bulrushes *(Scirpus sp*.) and sedges (*Carex sp.*) (Sundaravadivel & Vigneswaran, 2001).

The appropriate species for wastewater treatment wetlands depend on local conditions, the water depth, the design (surface or subsurface flow), and characteristics of the wastewater. Studies of wetland plant survival and effectiveness in constructed wetlands (Reddy & DeBusk, 1987; Hammer, 1994) have led to a list of general requirements that suitable plant species must satisfy (Tanner, 1996):

(1) Ecological acceptability, ie. no significant weed or disease risk or danger to the ecological or genetic integrity of surrounding natural ecosystems;

(2) Tolerance of local climatic conditions, pests and diseases;

(3) Tolerance of pollutants and hypertrophic and waterlogged conditions;

(4) Ready propagation, and rapid establishment, spread and growth (perennial habit); and

(5) High pollutant removal capacity, either through direct assimilation and storage, or indirectly by enhancement of microbial transformations, such as nitrification (via root-zone oxygen release) and denitrification (via production of carbon substrates).

Most frequently, it is optimal to use plant species that are found in nearby natural wetlands and have proven survival and purification capacity. However, in areas where some of the commonly used species are not locally found, local (indigenous) species should be tested for survival and effectiveness and used in preference to non-indigenous species (Maschinski et al., 1999). A variety of wetland plant species (emergent, submerged, floating and rooted floating-leaved species) used in constructed wetlands with high-nutrient loads were listed by Cronk & Fennessy (2001).

It is known that ornamental plants such as canna lily (*Canna flaccida*), calla lily (*Zantedeschia aethiopica*), elephant ear (*Colocasia esculenta*), ginger lily (*Hedychium coronarium*), and yellow iris (*Iris pseudacorus*) can be used in rock/plant filters to treat septic tank effluents (Wolverton, 1989). Belmont et al. (2004) reported that ornamental flowers (*Canna flaccida* and *Zantedeschia aethiopica*) with high economic value planted in the laboratory- and field-constructed wetlands performed as well as cattail (*Typha angustifolia*). Zurita et al. (2006) studied five ornamental species (*Anthurium andreanum, Canna hybrids, Hemerocallis dumortieri, Strelitzia reginae* and *Zantedeschia aethiopica*) in laboratory-scale subsurface-flow constructed wetlands and observed good quality of the effluent as well as good development of the plants.

Zhang et al. (2007a) have investigated ten emergent plant species, comprising six ornamental species: *Canna indica*, *Lythrum sp.*, *Alocasia macrorrhiza*, *Zantedeschia aethiopica*, *Iris louisiana*, *Zantedeschia sp.*, and four sedge species: *Carex tereticaulis*, *Baumea juncea*, *Baumea articulata* and *Schoenoplectus validus*. The plants were planted in the vertical-flow wetland microcosms and fed a simulated wastewater solution containing 17.5 mg N L^{-1} in the 1:1 proportion of NH_4-N and NO_3-N, and 10 mg P L^{-1} in the concentrations similar to the secondary-treated municipal wastewater. Different growth rates of ornamental species were observed, with *Canna indica* showing the most vigorous and healthy growth in the microcosms. Significant differences in both above-ground and below-ground biomass were found among plant species. Significant differences in the removal efficiencies of NH_4-N, NO_x-N and PO_4-P were detected among different species, with *Canna indica* achieving the relatively high nutrient removal efficiency. Although biomass of *Canna indica* was not the highest among the ornamental species, it has shown vigorous and healthy growth, and a relatively high potential of rooting-zone aeration and nutrient removal efficiency in the wetland microcosms (Zhang et al., 2007a).

Ornamental plants would provide economic benefits to the communities in addition to the efficiency of the wastewater treatment. Although ornamental plants have been tested in laboratory- and pilot-scale constructed wetlands, there is still not enough information about the growth and efficacy of ornamental species in constructed wetlands. Hence, the use of ornamental species in constructed wetlands should be further explored.

NUTRITION AND GROWTH OF WETLAND PLANTS

Like all plants, wetland plants require many macro- and micronutrients in proper proportions for healthy growth. Nitrogen (N) and Phosphorus (P) are key nutrients in the life cycle of wetland plants (EPA, 2000). However, the concentrations of inorganic substances, most importantly N and P, in the wastewater effluents (Kadlec & Knight, 1996; Tchobanoglous et al., 2003; Batty & Younger, 2004; Poach et al., 2004) and the loading rate to the constructed wetlands vary depending on the quality of wastewater, type of wastewater treatment facilities and the season. These changes to nutrient availability could influence plant growth responses and resource allocation in constructed wetlands (Tanner, 2001; Zhang et al., 2007b & 2008). Plants not only grow at a slow rate at low nutrient supply compared with high nutrient supply, but also increase their biomass allocation to roots (Poorter & Nagel, 2000) and reduce the nutrient concentrations in the biomass (Aerts & Chapin, 2000).

Wetland plants are able to tolerate high concentrations of nutrients and in some cases even to accumulate more nutrients than are needed for growth when supplemental nutrients are available (luxury uptake). Therefore, plant nutrient content is greater under high nutrient loads than under natural or background levels of nutrients. Greenway (1997) analysed eight common wetland plant species (emergent and floating-leaved) from both high-nutrient load and control wetlands. Plant N and P levels in the treatment wetlands averaged 7 g N kg^{-1} and 2 g P kg^{-1} dry weight more than in the control wetlands.

Different wetland plant species have a differential capacity to take up nutrients such as N and P from wastewater (Kadlec & Knight, 1996). Those considered efficient in assimilating nutrients have (i) rapid growth rates in resource-rich environments, and (ii) ability to concentrate luxury amounts of nutrients in their above- and below-ground biomass. The partitioning of nutrients between shoots and roots/rhizomes varies between species and seasons. Differences between species in biomass accumulation, and tissue N and P concentrations are likely to reflect species and developmental stage differences in efficiency of nutrient uptake and use (Tanner, 1996; Güsewell & Bollens, 2003).

The interactive effects of nutrients such as N and P can influence plant growth and removal efficiency in constructed wetland. Zhang (2008) has investigated the interactive effects of three levels of nitrogen (mg N L^{-1}) [5 low, 30 medium and 90 high in 1:1 ratio of NH_4-N and NO_3-N] and two levels of phosphorus (mg P L^{-1}) [3 low and 5 high] on growth and nutrient removal efficiency using *Canna indica* and *Schoenoplectus validus* in the vertical free surface-flow wetland microcosms. The plants in the high nutrient treatments outperformed those in the low nutrient treatment, growing taller and producing more stems, leaves and flowers; however, the growth was not significantly different between the medium N-low P and high N-low P treatments. The total plant biomass and concentrations of N and P in the plant tissues were significantly influenced by interaction of N and P treatments. The tissue concentrations of N increased with an increase in N application and decreased with an increase in P addition. For *Canna indica*, the tissue concentrations of P increased with an increase in P additions and decreased with an increase in N applications, whereas for *Schoenoplectus validus*, the tissue concentrations of P decreased with an increase in N applications in the low P treatment, but increased in the high P treatment.

For *Canna indica*, the growth performance was related to the physiological responses (Zhang et al., 2008). The photochemical efficiency measured as the chlorophyll fluorescence ratio (F_v/F_m) significantly increased with an increase in N additions. The photosynthetic rate increased from 13 to 16 μmol m^{-2} s^{-1} in the low-P treatments and from 14 to 20 μmol m^{-2} s^{-1} in the high-P treatments with an increase in N applications, but significant difference was only between low and medium N treatments, regardless of the P levels.

There was a significant interactive effect of N and P treatments on the removal efficiencies of NH_4-N, NO_x-N and PO_4-P (except for the removal of NO_x-N by *Schoenoplectus validus*) (Zhang, 2008). On average, more than 56% of nutrients taken up was allocated to the above-ground tissues, and, therefore, could be removed by harvesting. For *Canna indica*, plants were the major nutrient (N and P) removal pathway in the wetland microcosms (except P removal in the low N-high P treatments), whereas for *Schoenoplectus validus*, the plant uptake, substrate adsorption and other losses (such as denitrification) contributed similarly to N removal when N loading rates were relatively low, but other losses (such as denitrification) contributed more to N removal when N loading was relatively high. The P adsorption by substrate was the main contributor to P removal when P loading was relatively high, or when N and P loading rates were relatively low and plants were not intensively growing. However, plant uptake was the major factor responsible for P removal when N loading was relatively high and plants were vigorously growing. Hence, the appropriately high nutrient availability and optimum ratio of N and P are needed to stimulate the growth of wetland plants, resulting in preferential allocation of resources to the above-ground tissues, and enhancing the nutrient removal in constructed wetlands.

Although there is considerable information on plant productivity, biomass and nutrient dynamics in natural and fertilized wetlands (Mitsch and Gosselink, 2000; Cronk and Fennessy, 2001), most studies on constructed wetlands receiving wastewaters have only addressed general aspects of plant growth and nutrient content (Tanner, 2001). Plants are an integral part of constructed wetland and investigation of factors affecting growth are needed to produce the healthiest systems. By isolating growth factors in bench-scale studies, a more complete understanding of plant growth may help reduce performance variability and enable scientists to better predict treatment capability of various systems. Intensive studies of the growth and nutrients have predominantly been short-term and small scale (Hunter et al., 2000). Only a few studies have investigated plant growth in constructed wetlands on the field scale. For example, the growth characteristics and nutritional status of *Schoenoplectus validus* have been investigated by Tanner (2001), and the growth of *Phragmites australis* and *Phalaris arundinacea* has been compared by Vymazal and Krőpfelová (2005) in constructed wetlands for wastewater treatment. Hence, both short- and long-term studies on plant growth, development and management of various species in constructed wetlands receiving different sources of wastewater are needed in laboratory and field conditions.

UPTAKE OF NH_4-N AND NO_3-N BY WETLAND PLANTS

Of all the mineral nutrients, N is required in the largest quantities. Most plants get N from the water and substrate as either NH_4-N or NO_3-N, with some species showing a strong preference for one ionic form over the other (Kronzucker et al., 1997; Forde & Clarkson, 1999)**.** Plant species differ greatly in their capacities to utilise particular N forms, and these adaptations may contribute to the unique spatial and/or temporal distributions of these species (Bledsoe & Rygiewicz, 1986; Chapin et al., 1993; Kronzucker et al., 1997). The plant species preference for either NH_4-N or NO_3-N may have important ecological and practical implications (Forde & Clarkson, 1999). Wetland plants are suggested to favour NH_4-N rather than NO_3-N because the assimilation of NH_4-N has low energy cost.

NO_3-N participates in osmoregulation and can be stored in vacuoles without detrimental effects (Marschner, 1995). A widely grown variety of lowland rice was exceptionally efficient in absorbing and assimilating NO_3-N in contrast to NH_4-N compared with other plant species (Kronzucker et al., 1999, 2000). A modelling study by Kirk and Kronzucker (2005) implicated that wetland plants may be efficient in capturing NO_3-N formed in the rhizosphere. This raises the possibility that NO_3-N uptake by wetland plants is more important than generally thought.

The uptake of NH_4-N and NO_3-N by plants was influenced by pH. The uptake of NH_4-N generally decreased with decreasing external pH, but the uptake of NO_3-N is largely unaffected by pH or may even increase under slightly acidic conditions (Brix et al., 2002). In general, plant growth and nutrient uptake are also affected profoundly by pH (Rengel, 2002). For example, the growth of *Typha latifolia* almost completely stopped at pH 3.5 in the solution culture experiments. The growth inhibition at low pH was probably due to a reduced nutrient uptake and a consequential limitation of growth by nutrient stress (Brix et al., 2002).

Emergent wetland plant species may have variable nutrient uptake efficiency when grown in constructed wetlands for tertiary purification of wastewater. Zhang (2008) has compared the nutrient uptake kinetics of *Canna indica* and *Schoenoplectus validus*. The maximum uptake rate I_{max} was significantly lower for NH_4-N than NO_3-N in *Canna indica*, whereas the reverse was true in *Schoenoplectus validus*. The I_{max} for NH_4-N was significantly lower in *Canna indica* than *Schoenoplectus validus*, but no significant plant difference in I_{max} for NO_3-N uptake was observed. The significantly lower K_m for NO_3-N uptake was detected in *Schoenoplectus validus* compared to *Canna indica*.

Wetland plant species could have different preferences for inorganic nitrogen source. Zhang (2008) has observed that *Canna indica* preferred NO_3-N, but *Schoenoplectus validus* preferred NH_4-N, and was more capable than *Canna indica* to take up NO_3-N when the concentration of NO_3-N in the solution was relatively low. Fang et al. (2007) have found that out of four wetland plant species studied, two (*Bacopa monnieri* and

Azolla spp.) had preference for NO_3-N, whereas both N forms were required by *Ludwigia repens* for N uptake.

These findings have implications for the selection of wetland plants for the wastewater treatment in constructed wetlands. However, the preference for N forms is influenced by environmental factors, such as temperature, aeration, pH and composition of nutrients in solution, water and salt stress, and also by the plant growth stage and its ability to form a symbiosis with bacteria or fungi (Brix et al., 1994; Dyhr-Jensen & Brix, 1996; Garnett & Smethurst, 1999; Brix et al., 2002). Nevertheless, the mechanisms regulating the preference for different forms of N in aquatic species should be further characterised.

TOXICITY OF NH_4-N TO WETLAND PLANTS

Nitrogen as nutrient in wastewater may become a pollutant when present in excessive amounts. The ammonium concentration reported in most municipal or agricultural wastewater is between 12 and 50 mg L^{-1}, but undiluted second-cell anaerobic lagoon effluent from animal production operations has ammonium concentrations commonly exceeding 100 mg L^{-1} and reaching 400 to 500 mg L^{-1} (Hammer, 1992; Kadlec & Knight, 1996). As high as 2,074 mg L^{-1} NH_4-N concentration was recorded in the municipal landfill leachate (Kadlec, 1999). Wetland plant species may have reduced growth under high concentrations of NH_4-N (Hill et al., 1997; Clarke & Baldwin, 2002) and may develop ammonium toxicity syndrome, which is associated with accumulation of NH_4-N in tissues or a diminished cation (such as K^+, Mg^{2+} or Ca^{2+}) uptake (Mehrer & Mohr, 1989). Even species whose tolerance to NH_4-N is pronounced can suffer toxicity symptoms, given a high enough application of ammonium (Britto & Kronzucker, 2002).

Ammonia is a major concern in the operation of constructed wetlands receiving animal wastewater because of its toxicity to plants. Total ammonia in aqueous solution consists of two principal forms, the ammonium ion (NH_4^+) and un-ionised ammonia (NH_3), with relative concentrations being pH- and temperature-dependent. The un-ionised form is most toxic because it is uncharged and lipid soluble, thus traversing biological membranes more readily than the charged and hydrated NH_4^+ ions (Downing & Merkens, 1955). A number of studies, therefore, attributed the toxicity of total ammonia to the effect of NH_3 only (eg. Wang, 1991; Clement & Merlin, 1995). In other studies, both forms were reported to become toxic at high concentrations (eg. Litav & Lehrer, 1978; Monselise & Kost, 1993).

The response of duckweed to ammonium and ammonia has been reported extensively in the literature (Oron et al., 1985; Wang, 1991; Monselise & Kost, 1993; Clement & Merlin, 1995; Caicedo et al., 2000), but the conclusions are not always consistent because of different experimental conditions of temperature, pH, wastewater and medium composition and duckweed species. Wildschut (1984) and Oron et al. (1984) found 200 mg L^{-1} of total NH_4-N in domestic wastewater (pH 7) to be unfavourable to duckweed (*Lemna gibba*) growth. Wang (1991) studied the toxicity of the un-dissociated form (NH_3) on duckweed (*Lemna minor*), and a direct relationship was observed between un-dissociated ammonia concentration and the percentage of growth inhibition in renewal batch experiments with artificial substrate at initial pH = 8.5. An un-ionised ammonia concentration of 7.2 mg L^{-1} was calculated to cause 50% duckweed growth inhibition. In other plant species, Bitcover & Sieling (1951), using artificial growth medium, found toxicity effects on *Spirodela polyrrhiza* at concentrations above 46 mg N L^{-1} of total ammonia in the pH range 5–8, whereas Rejmankova (1979, as cited by Wildschut, 1984) reported tolerance up to 375 mg L^{-1} of total ammonia nitrogen.

The toxic effects of NH_4-N on emergent wetland plants are inconsistent in the literature, but indicate significant differences in tolerance to NH_4-N between wetland plant species. For instance, Surrency (1993) observed that *Typha latifolia* was stressed by ammonia concentrations of 160-170 mg L^{-1}, while *Schoenoplectus tabernaemontani* was not affected, whereas Humenik et al. (1999) found that *Juncus effuses* and *Schoenoplectus tabernaemontani* were unaffected by ammonium concentrations of 175 mg L^{-1} in a mesocosm study. Clarke & Baldwin (2002) found that the growth of *Juncus effuses*, *Sagittaria latifolia* and *Typha latifolia* was inhibited by ammonium concentration above 200 mg L^{-1}, whereas *Schoenoplectus tabernaemontani* was inhibited by ammonium concentration above 100 mg L^{-1}. Hill et al. (1997) tested ammonium effects on the biomass production of *Sagittaria latifolia*, *Phragmites australis*, *Scirpus acutus*, *Typha latifolia* and *Juncus roemerianus* in field-scale constructed wetlands at four ammonium concentrations of 20.5, 41.1, 61.6 and 82.4 mg L^{-1}. They found that only biomass of *Scirpus acutus* was significantly affected by ammonium concentration exceeding 61.6 mg L^{-1}, but there was no significant effect on other species.

Even though ammonia has been shown to be toxic to a variety of plant species (Britto & Kronzucher, 2002), few studies have investigated ammonia toxicity to wetland plants. Hence, the effects of ammonia on growth of various aquatic species should be further investigated.

EFFECTS OF SULPHUR ON WETLAND PLANT GROWTH

The acid mine drainage is often characterized by pH values as low as 2.5, concentrations of sulphate as high as 760 g L^{-1}, concentrations of total dissolved Fe and other soluble metal cations as high as 200 g L^{-1} (Wieder et al., 1990; Batty & Younger, 2004). The treatment of acid mine wastewater using constructed wetlands has increased recently.

Sulphur is an essential nutrient for plant growth. Sulphur occurs in a diverse range of forms necessary for plant function. Sulphur is a constituent of the amino acids (methionine, cysteine and cystine); its requirement in protein and co-enzymes containing these amino acids is the main biochemical role for sulphur in plants. In addition to its role in proteins and enzymes, sulphur affects the tertiary structure and hence function of polypeptides that may be important in protecting cells from heat or drought stress (Marschner, 1995).

The processes of sulphur cycling are determined by redox dynamics. Various sulphur compounds are present in native and constructed wetlands caused by natural and anthropogenic supply, particularly in industrial and mining wastewater. Sulphur in wetlands occurs in the soil solution, sorbed on variable charge surfaces as sulphate, as sulphate esters and bound in more recalcitrant forms of organic matter, and as sulphides of iron and other metals such as manganese and zinc. Sulphate ion is the ionic species absorbed by roots from the soil solution (Barber, 1984). Sulphate is the primary soluble sulphur species in aerobic soils, but is unstable at low redox potential that favours hydrogen sulphide (H_2S) formation. Provided active iron levels are sufficient, hydrogen sulphide reacts to form FeS as the main sulphide in the soil. However, sulphides of manganese and zinc also form at low redox potential (Bell, 2008).

Under waterlogged conditions in constructed wetlands, sulphate will be reduced to sulphide. Hydrogen sulphide is known to be highly toxic to wetland plants (Koch et al., 1990; Armstrong et al., 1996b; Armstrong & Armstrong, 2001; Van der Welle et al., 2007). It has been observed that the high sulphide levels resulted in the die-back of reed (*Phragmites australis*) in several natural wetlands (Armstrong & Armstrong, 2001; Fogli et al., 2002; Hotes et al., 2005).

Increased hydrogen sulphide concentrations can cause toxicity in aquatic plants, like root decay, reduced growth or even mortality at free sulphide concentrations between 10 μM and 1.4 mM (Van der Welle, 2006). For *Phragmites australis*, for example, below-ground tissues are sensitive to sulphide above 1 mM (Fuertig et al., 1996), and 1.4 mM sulphide causes stunted adventitious roots and fine laterals, bud death, callus blockages of gas pathways and blockages of xylem and phloem (Armstrong et al., 1996a). The concentration of 0.375 mM sulphide significantly lowered the ammonium uptake

(Chamber et al., 1998), and shoots were shorter when sulphide concentration was greater than 0.4 mM in soil (Chamber, 1997).

When sulphide is taken up by plants, it inhibits enzymes involved in photosynthesis and reduces the capacity of the roots to respire aerobically (Bagarinao, 1992). Sulphide may limit the generation of energy through anaerobic metabolism by inhibiting alcohol dehydrogenase activity (Koch et al., 1990). Koch et al. (1990) concluded that there was a significant negative effect of sulphide on the anoxic production of energy in wetland plant roots (*Spartina alterniflora* and *Panicum hemitomon*), and that an important negative effect of sulphide on plant growth is an inhibition of the energy-dependent process of N uptake.

Some wetland plants are more tolerant to sulphide than others. For instance, the addition of 1.0 mM sulphide significantly reduced culm, root and rhizome biomass in *Panicum hemitomon,* but only root biomass in *Spartina alterniflora* (Koch & Mendelssohn, 1989).

Wetland plants may utilise various mechanisms of coping with sulphide toxicity (Bagarinao, 1992). It has been suggested that the oxidized rhizosphere may enhance wetland plant tolerance to highly reduced soil environments (Armstrong & Boatman, 1967). For example, *Caltha palustris* is more sensitive to high sulphide concentrations than *Juncus effuses* because *J. effuses* can decrease sulphide concentrations in its rhizosphere by its much higher radial oxygen loss (Van der Welle et al., 2007). Plants may decrease toxic effects of sulphide by preventing high oxygen loss along most of the root, and allowing oxygen leakage only at the root tips of young roots, which are the most important parts for growth and nutrient uptake (Connell et al., 1999).

Sulphur reduction may be related to pollutant removal from wastewater in constructed wetlands. For instance, Wiessner et al. (2007) have found a clear correlation of the occurrence of reduced S-species with decreasing carbon and nitrogen removal performance and plant viability in the experimental constructed wetlands. Doubling the carbon load resulted in reducing sulphate, rising pH, increasing enrichment of S^{2-} and S^0 in pore water, and finally plant death and inhibition of nitrification by sulphide toxicity (Wiessner et al., 2007).

Understanding and treatments of sulphur-rich wastewater such as acid mine drainage to remove acidity and heavy metals are very important. However, sulphur cycling and its correlation to removal processes under dynamic conditions in the rhizosphere of wetland plants in constructed wetlands are still poorly understood.

EFFECTS OF HEAVY METALS AND METALLOIDS ON WETLAND PLANT GROWTH

Constructed wetlands have been used to detoxify heavy metals and metalloids such as cupper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), mercury (Hg), aluminium (Al), arsenic (As) and selenium (Se) in municipal wastewater and other types of polluted waters around the world (Terry et al., 2003).

Many metals such as Co, Cu, Fe, Mn, Mo, Ni and Zn are essential micronutrients for plants, because they are involved in numerous metabolic processes as constituents of enzymes and other proteins. However, they can become toxic if their concentration is higher than a specific critical point, as they can lead to a range of interactions at the cellular and molecular levels (Hall, 2002).

The effects of metals on wetland plant growth have been reported in numerous studies. Batty & Younger (2004) have found that the reduced growth of *Phragmites australis* in acidic spoil heap discharge contaminated wetlands was due to metal toxicity. As a mechanism, the presence of elevated concentrations of metals in the constructed wetland might inhibit uptake of nutrients such as Ca into plant tissues (Batty & Younger, 2004). The accumulation of Cr by aquatic plants in tannery wastewater influences the plant physiological process. It was found that a decrease in chlorophyll concentration occurred with an increase in metal Cr concentration (Rai et al., 1995; Sinha et al., 2002). The photosynthetic efficiency of plants growing under metal stress decreased with a concomitant change in composition of photosynthetic pigments (Sharma & Hall, 2002). The growth of *Elodea nuttallii* was decreased by Cu at 5.0 μM (Van der Werff & Pruyt, 1982). The high concentrations of heavy metals (Cu, Zn, Cd, Cr and Ni) present in wastewater were toxic, causing negative effects to both young mangrove (*Bruguiera gymnorrhiza*) and soil microbial activities (Yim & Tam, 1999). Photosynthetic intensity of *Canna indica* decreased with an increase in cadmium (Cd^{2+}) concentrations in the solutions (Cheng et al., 2002).

The toxic metal Al is a major constituent of many mine discharges; the more acidic the waters, the greater the mobility of the Al species. A relationship between root activity and Al behaviour has been demonstrated for *Typha latifolia*, the roots of which were proven to play a role in the cycling of Al in the mesocosms (Weider et al., 1990). The primary symptom of Al toxicity is a rapid inhibition of root growth, resulting in a reduced and damaged root system and limited water and mineral nutrient uptake, but some wetland plant species have developed Al tolerance (Barcelo & Poschenrieder, 2002).

Selenium is not known to be a required element for plant growth, but it may inhibit plant growth at high concentrations in the plant tissues (Wu et al., 1988). A field study on the Se removal and mass balance in a constructed wetland system was reported by Gao et al. (2003). The mass balance showed that on average about 59% of the total inflow of Se was retained within the wetland compartments, including 33% in the surface (0-20 cm) sediment, 18% in the organic detrital layer above the sediment, 2% in the fallen litter, <1% in the standing plants, <1% in the surface water and about 6% of unaccounted for. The Se outputs were outflow (35%), seepage (4%) and volatilization (2%).

The different forms (species) of the same metal can have different rates of uptake by, and different toxic effects on, wetland plants. For example, marsh sediments tended to rapidly reduce the very toxic Cr(VI) to the less toxic form, Cr(III) (Pardue & Patrick, 1995). Many bacteria can methylate arsenic, forming both volatile (methylarsines) and non-volatile compounds (methylarsonic acid and dimethylarsinic acid) (Bentley and Chasteen, 2002). Arsenic availability and toxicity were influenced by both concentration and forms (for a review see Quaghebeur & Rengel, 2005). For instance, monomethyl arsenic acid was the most phytotoxic species to *Spartina alterniflora* compared to arsenite, arsenate and dimethylarsinic acid (Carbonell et al., 1998).

Different wetland plant species have varied tolerance to toxicities of metals. For instance, three aquatic plants (*Myriophyllum aquaticum, Ludwigia palustris and Mentha aquatica*) were examined for their ability to remove heavy metals (Fe, Zn, Cu and Hg) from contaminated water. *Myriophyllum aquaticum* showed greater tolerance to toxicity than *Mentha aquatica* and *Ludwigia palustris.* The growth of *Ludwigia palustris* was significantly affected by heavy metal toxicity (Kamal et al., 2004). Iron toxicity to *Epilobium hirsutum*, but not to *Juncus subnodulosus* was observed both in the laboratory and field (Wheeler et al., 1985).

Wetland plant species have differential capacity to remove various metals. For instance, Sheoran (2006) investigated pollutant removal from acid mine drainage by three different aquatic plant species (*Typha angustifolia, Desmostachya bipinnata and Saccharum bengalense*) in bench-scale wetlands. It was found that the maximum metal removal occurred during the first 24 hours; *Typha angustifolia* was most effective for nickel, *Desmostachya bipinnata* for manganese and *Saccharum bengalense* for iron and lead. Hence, the selection of different plant species can play a major role in enhancing the metal treatment efficiency (Haberl et al., 2003).

It is striking that very few reports exist about effects of metals on wetland plants in the field conditions. Nevertheless, many wetland species can easily be established on metal-rich substrates and take up metals to concentrations several magnitudes higher than crop plants, suggesting that wetland plants may be innately tolerant to metals (Otte, 2001). When comparisons of the effects of copper on the growth, tolerance indices, mineral composition and metal uptake of reed (*Phragmites australis*) and maize (*Zea mays*) were conducted in hydroponics (Ait Ali et al., 2002), reed was significantly more tolerant to copper than maize, with a reduction in root length being a good indicator of copper toxicity.

Constructed wetlands comprise a complex ecosystem of plants, microbes and sediment that together act as a biogeochemical filter, efficiently removing contaminants (including metals) from wastewater. In general, wetland plants are not hyperaccumulators; they store metals in below-ground tissues (Batty & Younger, 2004). Therefore, it is very important to understand the metal biogeochemistry in the wetlands. Nevertheless, the current lack of knowledge of the interaction of plant tissues and metal biogeochemistry in wetland sediments inhibits the progress in advancing the efficiency of constructed wetland technology.

EFFECTS OF SALINITY ON WETLAND PLANT GROWTH

The aquatic farming, food-processing and domestic flush wastewater, especially in coastal areas, may comprise high salt concentrations designated as saline wastewater.

Sodium and other forms of salinity are the most difficult to remove from wastewaters. A proper amount of salts is essential for wetland plant growth, function and development. However, high salinity either limited plant growth or introduced open water areas that promoted the production of excessive amounts of algae and caused re-suspension of suspended solids in constructed wetlands (EPA, 2000). Extreme salt concentrations affect the function of biota such as plants and microorganisms (Klomjek & Nitisoravut, 2005).

The salinity in wastewater can influence plant growth in wetlands. Salinity (between 1 and 5 g L^{-1}) caused reductions in species richness and abundance of aquatic plants and zooplankton (Nielsen et al., 2003). However, little information exists on physiological effects of increased salinity on non-halophytic vascular aquatic plants. Hoching (1981) investigated the response of *Typha domingensis*, a large emergent macrophyte, under a range of salinity regimes in glasshouse trials. Plant growth after 8 weeks was reduced by salinities up to 2.9 g NaCl L^{-1}, indicating a sublethal effect at this concentration of salt. Between 2.9 and 5.9 g L^{-1}, growth was substantially reduced, with poor root growth, stunted shoots, and leaves with necrotic tips that subsequently died. Morris & Ganf (2001) observed that the growth of *Bolboschoenus medianus* was significantly reduced by the imposed salinities. Reductions in growth were reflected in lower number of leaves, lower leaf area per pot, fewer and shorter culms and lower rates of photosynthesis compared to non-saline control. It is important to note that one of the prominent plant responses to salinity was a change in biomass allocation from culms to tubers.

Different wetland plant species have differential tolerance to salinity. Warwick & Bailey (1997) investigated the effect of salinities of 2, 2 to 6 (increased over 64 days) and 6 g NaCl L^{-1} on the growth, leaf demography and ion concentrations of three wetland plants. *Potamogeton tricarinatus* was the most severely affected, showing significantly reduced dry weight and leaf size at 6 g L^{-1} together with a reduction in leaf appearance rate and an increase in leaf death. In comparison, *Triglochin procera* was not as severely affected, although leaf size was still reduced in plants grown at 6 g L^{-1}. *Amphibromus fluitans* was unaffected by salinity. The salt concentrations of 1.66 and 2.50 g L^{-1} were toxic to *Pistia stratiotes* and *Eichornia crassipes*, respectively (Haller et al., 1974). The order from least to most salt tolerant among four marsh macrophytes was *Panicum hemitomon* < *Sagittaria laucifolia* < *Eleocharis palustris* < *Scirpus americanus* in the laboratory; the ranking also reflected the field occurrence of these species along a gradient of increasing salinity in the northern Gulf of Mexico (Howard & Mendelssohn, 1999).

The selection of different plant species in tolerance to salinity can play a significant role in enhancing the treatment efficiency in constructed wetland. For example, Klomjek & Nitisoravut (2005) have investigated the plant growth and removal efficiency of eight emergent plants [*Typha angustifolia* (cattail), *Cyperus corymbosus* (sedge), *Brachiaria mutica* (water grass), *Digitaria bicornis* (Asia crabgrass), *Vetiveria zizaniodes* (vetiver grass), *Spartina patents* (salt meadow cordgrass), *Leptochloa fusca* (kallar grass) and *Echinodorus cordifolius* (Amazon)] under saline conditions in constructed wetlands. They observed that *Vetiveria zizaniodes* and *Brachiaria mutica* showed injury symptoms from the combination effect of high salt concentration and flood conditions, and were intolerant to sodium chloride (NaCl) at the conductivity of 14–16 mS cm^{-1}. However, other macrophytes were tolerant to salinity under the tested conditions and had high purification efficiency in the saline wastewater.

High salinity concentration is a major factor causing unexpectedly poor treatment performance. Normally, salinity has influence on the biotic functions that also take place in constructed wetlands for pollutant removal. However, the impacts of the salinity intensity and different salts on wetland performance are still unclear.

EHANCEMENT OF PLANT TOLERANCE AND TREATMENT PERFORMANCE BY MIXED CULTURE OF PLANT SPECIES

Improvements in plant selection and cultivation might make the constructed wetlands more efficient in pollutant removal from the wastewater. Mixed culture (intercropping) is commonly practiced in Asian and South American agriculture (eg. Li et al., 2001a & b). The practice of mixed culture has been applied in constructed wetlands. EPA (2000) pointed out that single plant systems (monoculture) are more susceptible to plant death due to predation or disease. Therefore, it is generally assumed that multiple species (mixture) and native plant systems are more resilient than monocultures in constructed wetlands (EPA, 2000). For organic carbon–limited free-surface wetlands, Bachand & Horne (2000) recommended a mixture of labile (submergent, floating) and more recalcitrant (emergent, grasses) plants as a reasonable approach to improving denitrification rates.

Few reports on the nutrient removal efficiency by various emergent species in mixed culture in constructed wetlands are inconsistent. Coleman et al. (2001) found that mixed culture of *Juncus effusus, Typha latifolia* and *Scirpus cyperinus* was effective in decreasing nutrient levels in the small-scale constructed wetlands receiving primary-treated wastewater. However, compared with monocultures, mixed culture of four wetland plant species (*Scirpus validus, Carex lacustris, Phalaris arundinacea* and *Typha latifolia*) in subsurface wetland microcosms did not increase the potential for N and P removal from mimicked domestic effluent (Fraser et al., 2004).

It is well known that positive, negative or indifferent relationship may occur between co-occurring plants of different species. The results of such competition might result in the preferential establishment and growth of certain species, and/or the suppression and extinction of others (Agami & Reddy, 1990). In recent years, several studies have been reported on the interspecies competition in wetlands. For example, Wetzel & van der Valk (1998) found *Phalaris arundinacea* to be an inherently better competitor than *Carex stricta* or *Typha latifolia*. Coleman et al. (2001) observed that *Typha latifolia* was the superior competitor compared with *Juncus effusus* and *Scirpus cyperinus* in the three-species mix in small-scale constructed wetlands. In plant mixtures consisting of *Carex flava, Centaurea angustifolia, Lycopus europaeus* and *Selinum carcifolia* grown in the sand culture with different total supplies of N and P in different N:P ratios, *Lycopus europaeus* performed best at low and intermediate N:P ratios, and *Carex flava* at a high N:P ratio (Güsewell & Bollens, 2003).

Few studies have investigated the impact of competition among species with different growth forms or significantly different morphologies. Nevertheless, Zhang et al. (2007b & c) have investigated the influence of mono- and mixed culture of *Canna indica* and *Schoenoplectus validus* on their growth in, and nutrient removal from, simulated wastewater in vertical free surface-flow wetland microcosms. Plants were grown for 50 days before imposing nutrient treatments that simulated secondary-treated municipal wastewater effluent with either low (17.5 mg N and 10 mg P per litre) or high (35 mg N and 20 mg P per litre) nutrient concentrations. After 65 days, the high nutrient treatment stimulated plant growth and resulted in allocation of more resources to the above-ground compared to below-ground tissues. The concentrations of N and P in plants (except P in above-ground parts) were significantly higher, whereas N and P use efficiencies were significantly lower in the high than the low nutrient treatment. The total biomass of *Canna indica* in the mixture increased significantly in the high nutrient treatment, but that of *Schoenoplectus validus* was significantly lower in the mixture than in the monoculture. Relative yield indicated that there was significant species competition between *Schoenoplectus validus* and *Canna indica* in mixtures, with the latter being the superior competitor. The growth of *Schoenoplectus validus* was significantly inhibited by the presence of *Canna indica*. Compared with monoculture, *Schoenoplectus validus* in the mixture had significantly higher percentages of root biomass and allocations of N and P to roots, whereas *Canna indica* was not significantly affected by the mixed culture (Zhang et al., 2007b).

The accumulation of N and P in above- and below-ground tissues largely reflected patterns of biomass allocation. Significant differences in nutrient removal efficiencies were observed between the planted and non-planted treatments, but no significant difference was detected between the nutrient treatments. Plant uptake was the major nutrient removal pathway in the wetland microcosms. Nutrient removal from simulated wastewater in mixed culture was not greater than in monocultures due to species competition. The results suggested that the growth and resource allocation of *Canna indica* and *Schoenoplectus validus* could be altered by differential nutrient availability and species competition in constructed wetlands (Zhang et al., 2007c).

Although numerous plant species have been tested in various constructed wetlands, few studies have given comparative data for evaluating the relative effectiveness of different plant species in improving effluent quality, and for testing whether species mixtures may be superior to monocultures in terms of pollutant removal in constructed wetlands (Coleman et al., 2001). If the effect of inter-species competition could be reduced, planting different species in mixed-culture may provide other benefits over monocultures, such as balanced pH and dissolved oxygen, improved aesthetic view, and an added economic value through harvesting ornamental species (Zhang et al., 2007c). It might also enhance tolerance to abiotic stress or improve treatment efficiency of toxins such as ammonia, sulphur, salts and metals. Hence, the intensive research in studying plant species mixtures between ornamental and other species at various combinations and in different planting densities is needed.

CONCLUSION

Plants are an important component of constructed wetlands for wastewater treatment. Macrophytes have several important functions that will improve purification efficiency and prolong the working life of constructed wetlands. Although macrophytes are widely used in constructed wetlands around the world, nutrient uptake and the toxic effects of inorganic substances (ammonium, salts, sulphide and metals etc) in wastewater on wetland plants and their biogeochemical cycling in constructed wetlands are not thoroughly understood. In particular, there is a lack of knowledge on interactive effects of these substances on wetland plants. The toxic inorganic substances in wastewater may inhibit nutrient uptake if present in concentrations exceeding those wetland plants can tolerate. Plant species have varied strategies and adaptations to tolerate toxicities of inorganic substances in wastewater. Further studies in laboratory and field conditions are needed on selecting plant species (particularly ornamental plants), improving plant cultivations (mono- or mixed culture) and identifying relevant factors influencing plant growth, development and management of various species in constructed wetlands receiving different sources of wastewater. A more complete understanding of nutrient uptake and plant growth, and their biogeochemical cycling in toxic environments may help reduce performance variability and enable scientists to better predict wastewater treatment capability of the wetland systems.

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