EFFECTS OF WASTEWATER DISCHARGE ON ROOT ANATOMY, RADIAL OXYGEN LOSS PATTERNS AND FORMATION OF IRON PLAQUE ON THE ROOT SURFACE OF MANGROVE PLANTS AND THEIR SIGNIFICANCE IN WASTEWATER TREATMENT

PI NA

DOCTOR OF PHILOSOPHY
CITY UNIVERSITY OF HONG KONG
JANUARY 2011
CITY UNIVERSITY OF HONG KONG
香港城市大學

Effects of wastewater discharge on root anatomy, radial oxygen loss patterns and formation of iron plaque on the root surface of mangrove plants and their significance in wastewater treatment
污水排放對紅樹植物根部解剖學特徵、放氧方式、鐵鞘形成的影響以及它們在污水處理過程中所起的重要作用

Submitted to
Department of Biology and Chemistry
生物及化學系
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
哲學博士學位

by

Pi Na
皮娜

January 2011
二零一一年一月
Abstract

Mangrove wetlands have been proposed to be low-cost and effective wastewater treatment systems for the removal of pollutants from municipal wastewater. However, mangrove wetlands are often challenged by oxygen stress caused by: i) periodic or permanent flooding; ii) aerobic degradation of nutrients and organic matter and iii) oxidation of heavy metals. Continuous discharge of wastewater rich in nutrients, organic matter and heavy metals, exaggerate the oxygen-stress problem and cause damage to the plants. Mangrove plants have adapted to the anaerobic conditions by developing special features: i) root anatomy, including aerenchyma air spaces and outer layers; ii) radial oxygen loss (ROL) from aerenchyma air spaces and iii) formation of iron (Fe) plaque on their root surfaces. Aerenchyma air spaces provide the internal pathway for oxygen transfer. ROL allows roots to create an aerobic
protective rhizosphere for plants when growing in anaerobic environments. The outer layers prevent excessive ROL and protect plant roots against toxic pollutants. The Fe plaque that forms on the root surface has a high capacity to immobilize toxic substances, thus prevents the excessive uptake by plants and contributes to the wastewater treatment.

So far, the studies on root anatomy, ROL and Fe plaque formation have mainly focused on important crops, grasses and common wetland plants. Little is known with regards to mangrove plants, a very important group of inter-tidal wetlands in tropical and sub-tropical regions. The effects of wastewater discharge on root anatomy, ROL and Fe plaque formation in mangrove plants have never been reported. The present research aimed: i) to examine the root anatomical features and ROL patterns of seedlings of eight true mangrove species in Hong Kong, namely *Avicennia marina* (Forsk.) Vierh., *Acanthus ilicifolius* L., *Aegiceras corniculatum* (Linn.) Blanco, *Bruguiera gymnorrhiza* (L.) Poir, *Excoecaria agallocha* L., *Heriteria littoralis* Dryand. ex W. Ait., *Kandelia obovata* Sheue, Liu & Yong and *Lumnitzera racemosa* Willd.; ii) to investigate the effects of wastewater discharge on root anatomy and ROL patterns in three true mangrove species, *B. gymnorrhiza*, *E. agallocha* and *A. ilicifolius*, with and without tidal flushing and iii) to determine the variation of Fe plaque formation on root surface and heavy metals immobilization in Fe plaque when receiving wastewater containing both nutrients and heavy metals.

The spatial patterns of ROL of the eight true mangrove species was comparable, with more oxygen lost from the tip than that from the basal and mature zones, but
this extent was species-specific. The roots of *A. marina* and *A. ilicifolius* had the largest areas of aerenchyma air spaces but the weakest outer layer. On the other hand, *H. littoralis* had the least longitudinal oxygen transfer because of its smaller area of aerenchyma air spaces in roots. The tolerance of mangrove species to waterlogged soil followed the order of *A. marina* (most foreshore species) > *A. ilicifolius* > *K. obovata* > *A. corniculatum* > *B. gymnorrhiza* > *E. agallocha* > *L. racemosa* > *H. littoralis* (most landward species), which is related to their anatomical features and ROL.

The effects of wastewater discharge on root anatomy and ROL varied among three mangrove species. The rates of ROL from roots of *B. gymnorrhiza* were the highest in the root tip and declined in the basal and mature zones, indicating the ‘tight barrier’ to ROL, and there was no significant difference among the three treatments, FW (freshwater, control), NW (with concentrations of dissolved organic carbon (DOC), ammonium-N (NH₄⁺-N), nitrate-N (NO₃⁻-N), total Kjeldahl N (TKN) and inorganic phosphate (PO₄³⁻-P), which is the same as that in the primary settled municipal sewage in Hong Kong), and 10NW (10 times the pollutant concentrations of NW). The ROL patterns of *E. agallocha* in FW and NW treatments exhibited a ‘tight barrier’, but changed to a ‘partial barrier’ when receiving 10NW, with similar rates along a lateral root. The effects of wastewater discharge on ROL of *A. ilicifolius* were the most obvious, with the ‘tight barrier’ in FW and the ‘partial barrier’ in NW, but shifted to a ‘weak barrier’ in 10NW, with much higher rates in the mature zone than in the tip. Wastewater discharge, without tidal flushing, induced more ROL from
roots for all three species, the stronger the wastewater, the more ROL. However, this induction was not exhibited under the tidal flushing condition due to the formation of Fe plaque on root surface, which was not found in roots without tidal flushing. The correlation between ROL and Fe plaque was positive in FW but changed to negative in 10NW.

When receiving wastewater containing both nutrients and heavy metals, the concentrations of Fe plaque formed on root surface increased with wastewater discharge for all three species; the stronger the wastewater, the more the Fe plaque formed. Among the three species, the concentration of Fe plaque formed on the root surface was the least in *B. gymnorrhiza* in 5SW (synthetic wastewater with concentrations of DOC, NH$_4^+$-N, NO$_3^-$-N, TKN, PO$_4^{3-}$-P and heavy metals, including Fe$^{3+}$, Ni$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Cr$^{6+}$, Cd$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$, five times of that in the primary settled municipal sewage in Hong Kong) and 10SW (double the pollutant concentrations in 5SW). More Fe plaque was formed on roots of *A. ilicifolius* when receiving 5SW, but the plants which received 10SW were all dead at the end of 75-day experiment. The concentrations of heavy metals immobilized in Fe plaque were positively correlated with the concentration of Fe plaque formed, although the correlation coefficient varied from species to species.

These results suggested that *B. gymnorrhiza* was the most tolerant species to pollutants as the root anatomy and ROL were least affected by wastewater discharge, followed by *E. agallocha* and *A. ilicifolius* was the most susceptible species, and thus, was not suitable for treating wastewater which contains high concentrations of
pollutants. More ROL induced more Fe plaque to form on the root surface. However, excessive ROL from the root led to plant death. On the other hand, Fe plaque formation prevented excessive ROL and acted as a reservoir to immobilize toxic pollutants, thus protecting roots against these pollutants and contributing to the wastewater purification.
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.1 General introduction</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.2 Aim and objectives</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.3 Research plan</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1.4 Layout of the thesis</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Literature Review</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1 Mangroves in Hong Kong SAR</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1.1 What are mangroves?</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1.2 Distribution of mangroves in Hong Kong SAR</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.1.3 Mangrove plants in Hong Kong SAR</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.2 Potential use of mangrove plants for wastewater treatment</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.2.1 Wastewater pollution problems</td>
<td>14</td>
</tr>
</tbody>
</table>
2.2.2 Wastewater treatment methods: conventional methods vs constructed wetland

2.2.3 Mechanisms of constructed wetland for wastewater treatment

2.2.4 Mangrove wetlands for wastewater treatment

2.3 Oxygen stress of mangrove plants to wastewater treatment

2.4 Root anatomical features

2.4.1 Aerenchyma air spaces

2.4.1.1 What are aerenchyma air spaces?

2.4.1.2 Formation of aerenchyma air spaces

2.4.1.3 Function of aerenchyma air spaces

2.4.2 Outer layers

2.5 Radial oxygen loss (ROL)

2.5.1 What is radial oxygen loss (ROL)?

2.5.2 Effects of ROL

2.5.2.1 Positive effects of ROL

2.5.2.2 Negative effects of ROL

2.5.3 Oxygen diffusion in roots

2.5.4 Methods used to evaluate ROL from roots

2.6 The ‘barrier’ to radial oxygen loss (ROL)

2.6.1 Anatomical and physiological basis of the ‘barrier’ to ROL in roots
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6.2 Constitutive and inducible ‘barriers’ to ROL</td>
<td>44</td>
</tr>
<tr>
<td>2.6.3 Adaptive significance of a ‘barrier’ to ROL for roots in anaerobic substrates</td>
<td>45</td>
</tr>
<tr>
<td>2.6.4 Types of ‘barrier’ to ROL</td>
<td>47</td>
</tr>
<tr>
<td>2.7 Root structure and their functional ‘barrier’ to ROL</td>
<td>48</td>
</tr>
<tr>
<td>2.8 Formation of (iron) Fe plaque on root surface</td>
<td>51</td>
</tr>
<tr>
<td>2.8.1 What is iron (Fe) plaque?</td>
<td>51</td>
</tr>
<tr>
<td>2.8.2 Effects of Fe plaque</td>
<td>52</td>
</tr>
<tr>
<td>2.8.3 Factors affect the formation of Fe plaque</td>
<td>55</td>
</tr>
<tr>
<td>2.9 Research on root anatomy, radial oxygen loss and Fe plaque formation</td>
<td>58</td>
</tr>
</tbody>
</table>

**Chapter 3. Root anatomy and spatial pattern of radial oxygen loss (ROL) of eight true mangrove species in Hong Kong**

3.1 Introduction                                                       | 60   |
3.2 Materials and methods                                              | 63   |
3.2.1 Preparation of plant materials                                  | 63   |
3.2.2 Measurements of plants                                          | 64   |
3.2.2.1 Measurement of dry biomass                                    | 64   |
3.2.2.2 Investigation of Radial Oxygen Loss (ROL) patterns             | 65   |
3.2.2.3 Observation of root anatomy

3.2.3 Statistical analyses

3.3 Results

3.3.1 Plant dry biomass

3.3.2 Spatial pattern of radial oxygen loss (ROL)

3.3.3 Root anatomical features

3.3.3.1 Cortex

3.3.3.2 Outer layer

3.4 Discussion

3.5 Conclusion

Chapter 4. Effect of wastewater discharge on root anatomy and radial oxygen loss (ROL) patterns of three mangrove species in Hong Kong SAR

4.1 Introduction

4.2 Materials and methods

4.2.1 Preparation of plant materials

4.2.2 Design of the experiment

4.2.3 Sample collection and measurements

4.2.3.1 Measurement of concentrations of nutrients and organic matter in the effluent

4.2.3.2 Determination of redox potential ($Eh$) of the soil
4.2.3.3 Investigation of rate and pattern of radial oxygen loss

4.2.4 Statistical analyses

4.3 Results

4.3.1 Plant dry biomass

4.3.2 Nutrients and organic matter in the effluent

4.3.3 Redox potential ($Eh$) of the soil

4.3.4 Rates and patterns of radial oxygen loss (ROL)

4.3.5 Root anatomical features

4.3.5.1 Cortex

4.3.5.2 Outer layer

4.3.6 Relationships between root anatomy, ROL, nutrients and organic matter removal and plant growth

4.4 Discussion

4.5 Conclusion

Chapter 5. Effects of wastewater discharge on formation of Fe plaque on root surfaces and radial oxygen loss (ROL) of mangrove roots

5.1 Introduction

5.2 Materials and methods

5.2.1 Preparation of plant materials
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2.2</td>
<td>Design of the experiment</td>
<td>138</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Sample collection and measurements</td>
<td>141</td>
</tr>
<tr>
<td>5.2.3.1</td>
<td>Determination of Fe plaque formation on root surface and phosphorus (P) immobilization in Fe plaque</td>
<td>142</td>
</tr>
<tr>
<td>5.2.3.2</td>
<td>Investigation of phosphorus (P) uptake by plants</td>
<td>143</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Statistical analyses</td>
<td>143</td>
</tr>
<tr>
<td>5.3</td>
<td>Results</td>
<td>144</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Redox potential ($Eh$) of the soil</td>
<td>144</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Plant dry biomass</td>
<td>144</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Rates and patterns of radial oxygen loss (ROL)</td>
<td>149</td>
</tr>
<tr>
<td>5.3.4</td>
<td>Root anatomical features</td>
<td>152</td>
</tr>
<tr>
<td>5.3.4.1</td>
<td>Cortex</td>
<td>152</td>
</tr>
<tr>
<td>5.3.4.2</td>
<td>Outer layers</td>
<td>157</td>
</tr>
<tr>
<td>5.3.5</td>
<td>Fe plaque formation on root surface and phosphorus (P) immobilization in Fe plaque</td>
<td>158</td>
</tr>
<tr>
<td>5.3.6</td>
<td>Correlations between rate of ROL, root anatomy and Fe plaque</td>
<td>164</td>
</tr>
<tr>
<td>5.3.7</td>
<td>Relationship between Fe plaque formation and P immobilization</td>
<td>166</td>
</tr>
<tr>
<td>5.4</td>
<td>Discussion</td>
<td>168</td>
</tr>
<tr>
<td>5.5</td>
<td>Conclusion</td>
<td>170</td>
</tr>
</tbody>
</table>
Chapter 6. Formation of Fe plaque on wastewater-treated mangrove roots and its role in wastewater treatment

6.1 Introduction

6.2 Materials and methods

6.2.1 Preparation of plant materials

6.2.2 Design of the experiment

6.2.3 Sample collection and measurements

6.2.3.1 Measurement of concentrations of heavy metals in the effluent

6.2.3.2 Investigation of Fe plaque formation on root surface, and heavy metals and P immobilized in Fe plaque

6.2.4 Statistical analyses

6.3 Results

6.3.1 Heavy metals, nutrients and organic matter in the effluent and removal efficiencies

6.3.2 Redox potential ($E_h$) of the soil

6.3.3 Plant dry biomass

6.3.4 Variations of Fe plaque formed on root surface

6.3.5 Variations of heavy metals and P immobilized in Fe plaque
6.3.6 Correlations between Fe plaque formation and immobilization of wastewater-borne pollutants 202
6.3.7 Correlations between Fe plaque formation and nutrients and organic matter removal 203

6.4 Discussion 217
6.5 Conclusion 221

Chapter 7. General discussion and conclusions 222

7.1 Criteria for selecting plant species for wastewater treatment based on root anatomy and radial oxygen loss (ROL) 222
7.2 Significance of Fe plaque formation on wastewater treatment and on tolerance of wetland plants to pollutants 227
7.3 Contribution to original knowledge 230
7.4 Limitations of the present study and future research 231
7.5 Conclusions 233

References 236

Conferences and publications 259
List of Tables

Table 2.1 Eight true mangrove plants in Hong Kong SAR (*: *L. racemosa* had inconspicuous root knees and pneumatophores) 13

Table 2.2 Summary of methods used to determine ROL from roots (Colmer, 2003) 37

Table 3.1 Background properties of Sai Keng mangrove soil used in the present study (Mean and standard error of 24 replicates; data were presented in air dried soils, except salinity) 65

Table 3.2 Plant dry biomass (dried weight) of one-year old seedlings of eight true mangroves harvested from pots grown in the greenhouse 71

Table 3.3 Intensity of the blue color at various sections along the same root of eight true mangrove species indicating variations in spatial patterns of ROL (+++: very deep blue, +++: deep blue, ++: blue, +: light blue, -: colorless) 74

Table 3.4 Types and areas of aerenchyma in three sections along a lateral root of eight true mangrove species and results of one-way ANOVA with Tukey test at a probability level of 0.05 (% cross-sectional area = area of aerenchyma air spaces / area of root x 100%; mean and standard error of three replicates are shown; S: schizogenous aerenchyma, L: lysigenous aerenchyma, NS: no significant difference) 81

Table 3.5 F value of two-way ANOVA results showing the differences of the percentages of cross-sectional areas of aerenchyma air spaces and outer layers (E+H) among plant species and root sections (The respective degrees of freedom for Plant Species and Root Sections was 7 and 2; *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001) 82

Table 3.6 Characteristics of outer layers, epidermis (E) and hypodermis (H), in three sections along a lateral root of eight true mangrove species (C: Closely packed, L: Loosely packed, +++: very thick, ++: thick, +: thin, -: Not present) 85

Table 3.7 Areas of outer layers, epidermis (E) and hypodermis (H), in three sections along a lateral root of eight true mangrove
species and results of one-way ANOVA with Tukey test at a probability level of 0.05 (% cross-sectional area of (E+H) = area of (E+H) / area of root x 100%; mean and standard error of three replicates are shown; NS: no significant difference)

Table 3.8 Ratio of proportional cross-sectional area between aerenchyma air spaces and outer layers (epidermis (E) and hypodermis (H)) in three sections along a lateral root of eight true mangrove species and results of one-way ANOVA with Tukey test at a probability level of 0.05 (ratio = % area of aerenchyma air spaces / % area of (E+H), mean and standard error of three replicates are shown)

Table 4.1 General Properties of artificial municipal wastewater (NW) (* except pH)

Table 4.2 Standard method for water sample preservation according to Eaton et al. (1995) (*: Storage at 4°C in the dark, P: plastic container, G: glass container)

Table 4.3 Amounts of nutrients and organic matter in the effluent at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; Bg: B. gymnorrhiza, Ea: E. agallocha, Ai: A. ilicifolius; the value, an average of four replicates, followed by different letters at the superscript position within each row of the same treatment indicated that they were significantly different at p ≤ 0.05 according to one-way ANOVA test)

Table 4.4 Redox potential (Eh value) of the rhizosphere soil at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; the value, an average of four replicates, followed by different letters at the superscript position of each species indicated that they were significantly different at p ≤ 0.05 according to one-way ANOVA test)

Table 4.5 F value of three-way ANOVA results showing the differences in the rates of radial oxygen loss (ROL) from roots, the differences in the percentages of cross-sectional areas of aerenchyma air spaces and outer layers (E+H), among three species, different wastewater treatments and sections along the same root at the end of the experiment (The respective degrees of freedom for Species, Sections and Treatments was
2, 4 and 2; *: \( p \leq 0.05 \), **: \( p \leq 0.01 \), ***: \( p \leq 0.001 \)

Table 4.6 Characteristics of epidermis (E) and hypodermis (H) in three sections of the same root at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; +++: very thick, ++: thick, +: thin, -: not present; C: closely packed, L: loosely packed)

Table 4.7 Ratios between areas of aerenchyma air spaces and outer layers (E+H) in three sections of the same root at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; the value, an average of four replicates, followed by different letters at the superscript position within each row of the same section indicated that they were significantly different at \( p \leq 0.05 \) according to one-way ANOVA test)

Table 4.8 Simple correlation coefficient (r) matrix among plant factors and amounts of nutrients and organic matter removed (ROL: radial oxygen loss, Ar: aerenchyma air spaces, E+H: epidermis and hypodermis, PDB: plant total dried biomass; \( n = 12 \), *: \( p \leq 0.05 \), **: \( p \leq 0.01 \), ***: \( p \leq 0.001 \))

Table 4.9 Multiple regression model and coefficient of multiple determination \( (R^2) \) for *Bruguiera gymnorrhiza* and *Acanthus ilicifolius* (Y: amounts of nutrients and organic matter removed; \( X_1 \): ROL (radial oxygen loss), \( X_2 \): Ar (area of aerenchyma air spaces), \( X_3 \): (E+H) (area of epidermis and hypodermis), B: partial regression coefficients, C: constant; \( n=12 \), *: \( p \leq 0.05 \), **: \( p \leq 0.01 \), ***: \( p \leq 0.001 \))

Table 5.1 Redox potential \( (Eh \) value) of the rhizosphere soil at the end of 105 days of wastewater treatment (FW: Freshwater, 10NW: Strong Wastewater; the value, an average of triplicates, followed by different letters at the superscript position of each species indicated that significantly difference was found between wastewater treatments at \( p \leq 0.05 \) according to independent sample t-test)

Table 5.2 F value of three-way ANOVA results showing the differences in the rates of radial oxygen loss (ROL) from roots, the differences in the percentages of cross-sectional areas of aerenchyma air spaces and outer layers (E+H), among three species, different wastewater treatments and sections along
the same root at the end of the experiment (The respective degrees of freedom for Species, Sections and Treatments was 1, 2 and 1; *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001, NS: not significant)

Table 5.3 Characteristics of aerenchyma, epidermis (E) and hypodermis (H) in three sections of the same root at the end of 105 days (FW: Freshwater, 10NW: Strong Wastewater; SA: Schizogenous Aerenchyma, LA: Lysigenous Aerenchyma; +++: very thick, ++: thick, +: thin, -: not present; C: Closely packed, L: Loosely packed)

Table 5.4 Mean ratio of area between aerenchyma air spaces and outer layers (epidermis (E) and hypodermis (H)) in three sections of the same root, root tip, basal zone (4 cm from the tip) and mature zone (8 cm from the tip) of the two mangrove species at the end of 105 days (FW: Freshwater, 10NW: Strong Wastewater; values followed by different superscripts within each row in each root section indicated that they were significantly different at a probability level of 0.05 according to independent sample t-test)

Table 5.5 F value of three-way ANOVA results showing the differences in the concentrations of Fe plaque formed on root surface and P immobilized in Fe plaque among three species, different wastewater treatments and sections along the same root at the end of the experiment (The respective degrees of freedom for Species, Sections and Treatments was 1, 2 and 1; *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001)

Table 5.6 Simple correlation coefficient (r) values between radial oxygen loss and other root factors, including root anatomy and concentration of Fe plaque on root surface (ROL: radial oxygen loss, Ar: cross-sectional area of aerenchyma air spaces, E+H: cross-sectional area of epidermis and hypodermis, Fe: iron plaque; *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001, a: the simple correlation coefficients between ROL and Ar, E+H for both species were calculated based on the results in the preliminary study described in Chapter 3, Section 3.3.3; n = 9 for all r values)

Table 5.7 Relationships between formation of Fe plaque on root surface and immobilization of P in Fe plaque in two mangrove species (FW: Freshwater, 10NW: Strong Wastewater; n = 9; *: p ≤
0.05, **: p ≤ 0.01, ***: p ≤ 0.001, the values of B followed by different letters at the superscript position of the same species indicated that they were significantly different at p ≤ 0.05 according to one-way ANOVA test)

Table 6.1 General properties of artificial synthetic wastewater (SW) 175

Table 6.2 Mean concentrations of Mn$^{2+}$ in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated significant differences at p ≤ 0.05 among three plant species according to one-way ANOVA test; removal percentage (%) = (concentration in influent – concentration in effluent) / concentration in influent × 100% )

Table 6.3 Mean concentrations of Ni$^{2+}$ in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated significant differences at p ≤ 0.05 among three plant species according to one-way ANOVA test; -: were not detected, the detection limit for Ni was 0.38 μg L$^{-1}$respectively; removal percentage (%) = (concentration in influent – concentration in effluent) / concentration in influent × 100%)

Table 6.4 F value of ANCOVA results showing the effects of treatment time, plant species and wastewater treatment on the concentrations of pollutants in the effluent (The respective degrees of freedom for Time, Plant species and Wastewater treatment was 1, 2 and 2; *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001, NS: not significant)

Table 6.5 Mean concentrations of NH$_4^{+}$-N in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater,
5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated significant differences at $p \leq 0.05$ among three plant species according to one-way ANOVA test; removal percentage ($\%) = \frac{\text{concentration in influent} - \text{concentration in effluent}}{\text{concentration in influent}} \times 100\%$

### Table 6.6
Mean concentrations of NO$_3^-$-N in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated significant differences at $p \leq 0.05$ among three plant species according to one-way ANOVA test; removal percentage ($\%) = \frac{\text{concentration in influent} - \text{concentration in effluent}}{\text{concentration in influent}} \times 100\%$)

### Table 6.7
Mean concentrations of TKN in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated significant differences at $p \leq 0.05$ among three plant species according to one-way ANOVA test; removal percentage ($\%) = \frac{\text{concentration in influent} - \text{concentration in effluent}}{\text{concentration in influent}} \times 100\%$)

### Table 6.8
Mean concentrations of PO$_4^{3-}$-P in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated
significant differences at $p \leq 0.05$ among three plant species according to one-way ANOVA test; removal percentage ($\%$) = (concentration in influent – concentration in effluent) / concentration in influent $\times 100\%$)

Table 6.9 Mean concentrations of DOC in effluents and removal percentages of three replicates at different sampling times during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: *Bruguiera gymnorrhiza*, Ea: *Excoecaria agallocha*, Ai: *Acanthus ilicifolius*; in each treatment and in each period, the mean value of triplicates followed by different letters at the superscript position indicated significant differences at $p \leq 0.05$ among three plant species according to one-way ANOVA test; removal percentage ($\%$) = (concentration in influent – concentration in effluent) / concentration in influent $\times 100\%$)

Table 6.10 F value of ANCOVA results showing the effects of treatment time, plant species and wastewater treatment on the redox potential ($E_h$ value) of the rhizosphere soil, biomass of each plant part and Fe plaque formation on root surface (The respective degrees of freedom for Time, Plant species and Wastewater treatment was 1, 2 and 2; *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, NS: not significant)

Table 6.11 F value of ANCOVA results showing the effects of treatment time, plant species and wastewater treatment on the immobilization of pollutants in Fe plaque (The respective degrees of freedom for Time, Plant species and Wastewater treatment was 1, 2 and 2; *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, NS: not significant)

Table 6.12 Total amounts of Fe plaque formed, pollutants added and removed from medium synthetic wastewater (5SW), and pollutants immobilized in Fe plaque in three mangrove species at the end of 75 days of wastewater treatment (Bg: *Bruguiera gymnorrhiza*, Ea: *Excoecaria agallocha*, Ai: *Acanthus ilicifolius*; *: percentages of total amount of wastewater-borne pollutants immobilized in Fe plaque to total amount removed ($\%$) = total amount immobilized in Fe plaque / total amount removed $\times 100\%$)

Table 6.13 Total amounts of Fe plaque formed, pollutants added and
removed from strong synthetic wastewater (10SW), and pollutants immobilized in Fe plaque in three mangrove species at the end of 75 days of wastewater treatment (Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; *: percentages of total amount of wastewater-borne pollutants immobilized in Fe plaque to total amount removed (%) = total amount immobilized in Fe plaque / total amount removed × 100%; the data for A. ilicifolius was the end of 60 days of wastewater treatment as all plants died at Day 75)

Table 6.14  Relationships between formation of Fe plaque on root surface and pollutant immobilized in Fe plaque in three mangrove species (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; n = 15, except for A. ilicifolius in 10SW treatment, which n = 12 as all plants died at Day 75; the B values of each plant species for each pollutant followed by different letters at the superscript position indicated that they were significantly different among three wastewater treatments at p ≤ 0.05 according to one-way ANOVA test; *, ** and *** indicate the R² values were significant at p ≤ 0.05, 0.01 and 0.001, respectively)

Table 6.15  Relationships between removal of nutrients/organic matter and formation of Fe plaque on root surface in three mangrove species (Bg: Bruguiera gymnorrhiza, Ea: Excoecaria agallocha, Ai: Acanthus ilicifolius; n = 15, except for A. ilicifolius in 10MW treatment, which n = 12 as all the plants died at day 75; *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001, the value of B followed by different letters at the superscript position within each column of the same pollutant indicated that they were significantly different at p ≤ 0.05 according to one-way ANOVA test; -: not calculated as r value was insignificant)
**List of Figures**

Fig. 1.1  Flow diagram of the research plan 8

Fig. 2.1  Mangrove wetlands 10

Fig. 2.2  Distribution of mangroves in Hong Kong SAR (Tam and Wong, 2000) 12

Fig. 2.3  Eight true mangrove plants in Hong Kong SAR 14

Fig. 2.4  Two types of aerenchyma (Visser et al., 2000b) 26

Fig. 2.5  Processes involved in the formation of aerenchyma air spaces (Evans, 2003) 27

Fig. 2.6  Structure and major functions of root 31

Fig. 2.7  Types of ‘barrier’ to ROL in roots 48

Fig. 2.8  Formation of Fe plaque on root surface 52

Fig. 2.9  Immobilization of heavy metals in Fe plaque 53

Fig. 3.1  Root morphology and depth (cm) of one-year old seedlings of eight true mangroves grown in greenhouse with average temperature, humidity and light intensity of 27.5°C, 68% and 27.54 $\mu$mol m$^{-2}$ s$^{-1}$, respectively. All the eight mangrove species have taproot. Root type: 1, taproot; 2, lateral root 73

Fig. 3.2  Intensity of the blue color at various sections along the same root of mangrove species indicating variations in spatial patterns of ROL 75

Fig. 3.3  Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *Acanthus ilicifolius* and *Avicennia marina* (Cross sections with thickness of 10 $\mu$m were made and photographed; scale bars equal to 200 $\mu$m; E+H: epidermis and hypodermis, Ar: aerenchyma air spaces, Ct: cortex) 78

Fig. 3.4  Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *Kandelia obovata*, *Aegiceras corniculatum* and *Heritiera littoralis* (Cross sections with thickness of 79
Fig. 3.5 Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *Excoecaria agallocha*, *Lumnitzera racemosa* and *Bruguiera gymnorrhiza* (Cross sections with thickness of 10 µm were made and photographed; scale bars equal to 200 µm; E+H: epidermis and hypodermis, Ar: aerenchyma air spaces, Ct: cortex; SW: suberized walls)

Fig. 4.1 Set-up of the experiment

Fig. 4.2 Set-up of the experiment in greenhouse

Fig. 4.3 Set-up of the equipment for the measurement of ROL from mangrove roots

Fig. 4.4 Diagram of the experimental assembly for the measurement of radial oxygen loss from roots (Gaynard and Armstrong, 1987)

Fig. 4.5 Seedling of *A. ilicifolius* at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater)

Fig. 4.6 Dry biomass of individual plant part of each mangrove species at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; the value, an average of four replicates, having different letters within each plant part of each species indicated that they were significantly different at p ≤ 0.05 according to one-way ANOVA test)

Fig. 4.7 Seedling of *E. agallocha* at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater)

Fig. 4.8 Seedling of *B. gymnorrhiza* at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater)

Fig. 4.9 ROL patterns along a lateral root of each mangrove species at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; mean
and standard deviation of four replicates are shown)

Fig. 4.10 Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *B. gymnorrhiza* at the end of 105 days of wastewater treatment (Cross sections with thickness of 10 µm were made and photographed; scale bars equal to 200 µm; E+H: epidermis and hypodermis, Ar(S): schizogenous aerenchyma air spaces, Ar(L): lysigenous aerenchyma air spaces, Ct: cortex, SW: suberized walls)

Fig. 4.11 Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *E. agallocha* at the end of 105 days of wastewater treatment (Cross sections with thickness of 10 µm were made and photographed; scale bars equal to 200 µm; E+H: epidermis and hypodermis, Ar(S): schizogenous aerenchyma air spaces, Ar(L): lysigenous aerenchyma air spaces, Ct: cortex, SW: suberized walls)

Fig. 4.12 Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *Acanthus ilicifolius* at the end of 105 days of wastewater treatment (Cross sections with thickness of 10 µm were made and photographed; scale bars equal to 200 µm; E+H: epidermis and hypodermis, Ar(S): schizogenous aerenchyma air spaces, Ar(L): lysigenous aerenchyma air spaces, Ct: cortex)

Fig. 4.13 Percentages of cross-sectional area of aerenchyma air spaces to total area of root in each of the three sections of the same root at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; mean and standard deviation of four replicates having different letters within each section of each species indicated that they were significantly different at $p \leq 0.05$ according to one-way ANOVA test)

Fig. 4.14 Percentages of cross-sectional area of outer layers, epidermis (E) and hypodermis (H), to total area of root in each of the three sections of the same root at the end of 105 days of wastewater treatment (FW: Freshwater, NW: Normal Wastewater, 10NW: Strong Wastewater; mean and standard deviation of four replicates having different letters within each section of each species indicated that they were significantly different at $p \leq 0.05$ according to one-way ANOVA test)
Fig. 5.1 Set-up of the experiment 140

Fig. 5.2 Set-up of the experiment in greenhouse 141

Fig. 5.3 Seedling of *E. agallocha* at the end of 105 days of wastewater treatment (FW: Freshwater, 10NW: Strong Wastewater) 146

Fig. 5.4 Dried biomass of leaf, stem and root of each species at the end of 105 days of wastewater treatment (Mean of triplicates are shown; the values followed by different letters within each plant part of each species indicated that they were significantly different at a probability level of 0.05 according to independent sample t-test; FW: Freshwater, 10NW: Strong Wastewater) 147

Fig. 5.5 Seedling of *B. gymnorrhiza* at the end of 105 days of wastewater treatment (FW: Freshwater, 10NW: Strong Wastewater) 148

Fig. 5.6 ROL patterns along a lateral root of two mangrove species at the beginning (day 0) and at the end of the experiment (day 105) (FW: Freshwater, 10NW: Strong Wastewater; mean and standard deviation of triplicates are shown) 151

Fig. 5.7 Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *B. gymnorrhiza* at the end of the experiment (day 105) (Cross sections with thickness of 10 µm were made and photographed; scale bars equal to 200 µm) 153

Fig. 5.8 Cross-sections of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of *E. agallocha* at the end of the experiment (day 105) (Cross sections with thickness of 10 µm were made and photographed; scale bars equal to 200 µm) 154

Fig. 5.9 Percentages of cross-sectional area of aerenchyma air spaces to total area of root in each of the three sections of the same root at the end of the experiment (day 105) (FW: Freshwater, 10NW: Strong Wastewater; mean and standard deviation of triplicates were shown; different letters in each root section indicated that they were significantly different at $p \leq 0.05$ according to independent sample t-test) 156
Fig. 5.10 Percentages of cross-sectional area of outer layers (epidermis and hypodermis, E+H) to total area of root in each of the three sections of the same root at the end of the experiment (day 105) (FW: Freshwater, 10NW: Strong Wastewater; mean and standard deviation of three replicates were shown; different letters in each root section indicated that they were significantly different at $p \leq 0.05$ according to independent sample t-test)

Fig. 5.11 Concentration of Fe plaque formed on the surface of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of each mangrove species at the beginning (day 0) and at the end of the experiment (day 105) (FW: Freshwater, 10NW: Strong Wastewater; mean and standard deviation of three replicates were shown; different letters in each root section indicated that they were significantly different at $p \leq 0.05$ according to independent sample t-test)

Fig. 5.12 Concentration of P immobilized in Fe plaque of root tip, basal zone (4 cm from the root tip) and mature zone (8 cm from the root tip) of each mangrove species at the beginning (day 0) and at the end of the experiment (day 105) (FW: Freshwater, 10NW: Strong Wastewater; mean and standard deviation of three replicates were shown; different letters in each root section indicated that they were significantly different at $p \leq 0.05$ according to independent sample t-test)

Fig. 6.1 Set-up of the experiment

Fig. 6.2 Set-up of the experiment in greenhouse

Fig. 6.3 Redox potential ($Eh$ value) of the rhizosphere soil during the 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater)

Fig. 6.4 Effect of wastewater discharge on dry biomass of plant leaf for each mangrove species during 75 days of the experiment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown; all the plants of $A. ilicifolius$ at Day 75 were dead)

Fig. 6.5 Effect of wastewater discharge on dry biomass of plant stem
for each mangrove species during 75 days of the experiment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown; all the plants of *A. ilicifolius* at Day 75 were dead)

Fig. 6.6 Effect of wastewater discharge on dry biomass of plant root for each mangrove species during 75 days of the experiment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown; all the plants of *A. ilicifolius* at Day 75 were dead)

Fig. 6.7 Variations of the Fe plaque formed on root surface for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.8 Variations of Zn immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.9 Variations of Mn immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.10 Variations of Cu immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.11 Variations of Ni immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.12 Variations of Pb immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)
species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.13 Variations of Cd immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.14 Variations of Cr immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)

Fig. 6.15 Variations of P immobilized in Fe plaque for each mangrove species during 75 days of wastewater treatment (FW: Freshwater, 5SW: Medium Synthetic Wastewater, 10SW: Strong Synthetic Wastewater; mean and standard deviation of triplicates are shown)