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# Effects of pretreated domestic wastewater supplies on leaf pigment content, photosynthesis rate and growth of mangrove trees: A field study from Mayotte Island, SW Indian Ocean

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## A B S T R A C T

After 12 and 18 months of daily wastewater discharge into mangrove plots in Mayotte Island, SW Indian Ocean, leaf pigment content, photosynthesis rate and growth of *Rhizophora mucronata* and *Ceriops tagal* mangrove trees were evaluated and compared with similar individuals from control plots. Chlorophyll and carotenoid contents, measured using an HPLC analyser, were significantly higher in leaves of mangrove trees receiving wastewater discharges. Photosynthesis and transpiration rates, analysed using an LCI portable system, increased significantly for mangrove trees in impacted plots. Measurements of leaf areas, young branch length and propagule length showed significant increases in plots receiving wastewater. These results suggest a beneficial effect of domestic wastewater on *R. mucronata* and *C. tagal* mangrove tree functioning. Analyses and observations on mangrove ecosystems as a whole – taking into account water and sediment compartments, crab populations and nitrogen and phosphorus cycles – are nevertheless necessary for evaluation of bioremediation capacities of mangrove ecosystems.

**Keywords:**  
Wastewater  
Mangroves  
Photosynthesis rate  
Chlorophyll  
Growth  
Bioremediation

## 1. Introduction

### 1.1. Mangroves and bioremediation

The utilisation of mangrove swamps as natural systems for wastewater treatment has been proposed as an efficient and low-cost solution for tropical coastal areas. Characterised by a high primary production and biomass and established as often as not on nutrient-poor sediments, mangrove ecosystems are considered able to absorb nutrients in excess contained in sewage, without any major structural or functional disturbance (Saenger, 2002).

Nedwell (1975) showed that the discharge of pretreated wastewater into a mangrove swamp in Fiji could be a means of reducing eutrophication in coastal waters, and therefore suggested that mangroves might be used as the final stage in sewage treatment. Clough et al. (1983) published one of the first review articles dealing with the impact of sewage on mangrove ecosystems. These authors established that the capacity of mangroves to remove nutrients from sewage was largely determined by hydrodynamic

factors in the short term and that the efficiency of the processes was largely dependent on the sediment properties and biological characteristics of the ecosystem in the longer term. Corredor and Morell (1994) demonstrated that the excess nitrogen coming from a sewage treatment plant in Puerto Rico could be absorbed by the mangrove ecosystem through natural denitrification processes, without any damage.

In an exploration of the different aspects of the role of mangrove swamps as sinks for wastewater-borne pollutants through numerous experiments conducted in the Hong Kong and Shenzhen area (South China), Tam and Wong (1995, 1996) successively showed that mangrove soils are good traps to fix phosphorus and certain heavy metals from wastewater; that no significant change was observed in the plant community structure or in leaf nutrient content of a mangrove site receiving wastewater discharges for 1 year (Wong et al., 1995, 1997); and that litter production and decomposition were not perturbed (Tam et al., 1998). The addition of wastewater to mangrove soils also seems to stimulate the growth of microbial populations, probably through nutrients and carbon components present in wastewater (Tam, 1998). More recently, these authors showed that a mangrove plant community growing in constructed microcosms receiving wastewater was effective in removing organic matter, nitrogen and phosphorus (Wu et al., 2008; Tam et al., 2009). Analysing a natural mangrove area in Thailand, Wickramasinghe et al. (2009) arrived to similar

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conclusions, demonstrating the efficiency of mangrove ecosystem in waste treatment, with an enhancement of mangrove growth and abundance of invertebrate populations

### 1.2. Mangrove tree growth and nutrient enrichment

While the use of mangrove ecosystems for removing pollutants from sewage discharges is becoming rather well documented, the response of mangrove plants themselves in terms of growth should be analysed and controlled, and results in this domain are still contradictory. Henley (1978) reported that mangrove tree growth in the Darwin area, Australia, was not affected when they received sewage discharges, and Clough et al. (1983) concluded that nutrient enrichment of a mangrove ecosystem through wastewater supply did not appear harmful and in some cases might have a beneficial effect on growth and productivity. Kelly (1995), investigating the impact of sewage effluents on mangroves dominated by *Avicennia marina* in Australia, found that the N and P leaf concentrations were higher at impacted sites, but no clear growth-enhancing effects were noted at these same sites. From similar experiments concerning the two mangrove species *Kandelia candel* and *Aegiceras corniculatum*, Wong et al. (1995) did not find any significant differences in plant growth after 1 year of sewage discharges, but noted that effects – positive or adverse – on vegetation functioning could become apparent only over a longer term. More recently, Lovelock et al. (2009) established that nutrient enrichment (N and P) could increase the mortality of mangroves in sites characterised by low annual rainfall and high sediment salinity. These authors added that mortality rates were significant in landward scrub forests and no tree deaths occurred in fringe forests. Lovelock et al. (2004) and Martin et al. (2010) specified that N and P enrichment significantly increased mangrove tree growth, but in certain salinity conditions might alter the structure of mangrove forests.

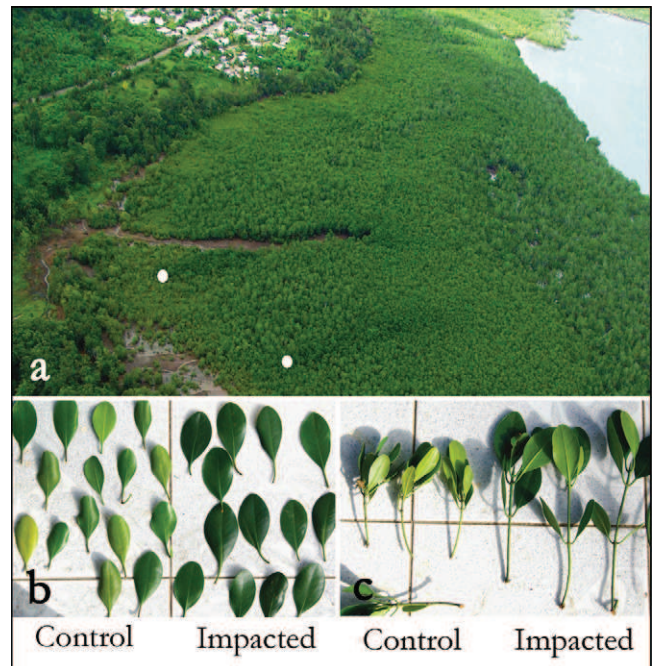
### 1.3. Mangrove tree functioning and environmental stresses

Relationships between nutrient enrichment and metabolic processes in mangroves are still little documented. Peculiarly, data on photosynthesis rate in mangrove trees as a functional marker of their health state are rare; such data are generally linked to hydrological and salinity parameters and take into account propagule populations in greenhouse conditions (Ball and Farquhar, 1984; Youssef and Saenger, 1998; Kao and Tsai, 1999; Kao et al., 2001; Krauss and Allen, 2003). Some studies considered the links between mangrove structure (scrub vs. fringe mangrove), mangrove tree height and photosynthesis characteristics (Lin and Sternberg, 1992; Lovelock et al., 2004), and Naidoo and Chirkoot (2004) established in a specific context that photosynthetic performance of *A. marina* was reduced when coal dust was deposited on the leaf surface of the mangrove trees.

In other studies, pigment content of mangrove leaves has been analysed in relation to the light environment of the mangrove forest canopy (Lovelock and Clough, 1992; Moorthy and Kathiresan, 1997). Rajesh et al. (1998) established correlations between growth rate, photosynthetic and pigment characteristics, and salinity levels for *Ceriops tagal* populations. MacFarlane and Burchett (2001) showed that photosynthetic pigment concentration decreased in *A. marina* populations impacted by heavy metals, and MacFarlane (2002) suggested that photosynthetic pigments could be considered as biological indicators of stress for mangrove trees.

### 1.4. Mayotte Island context and bioremediation project

The Mayotte Archipelago, West Indian Ocean, is currently experiencing environmental degradation linked to a very important

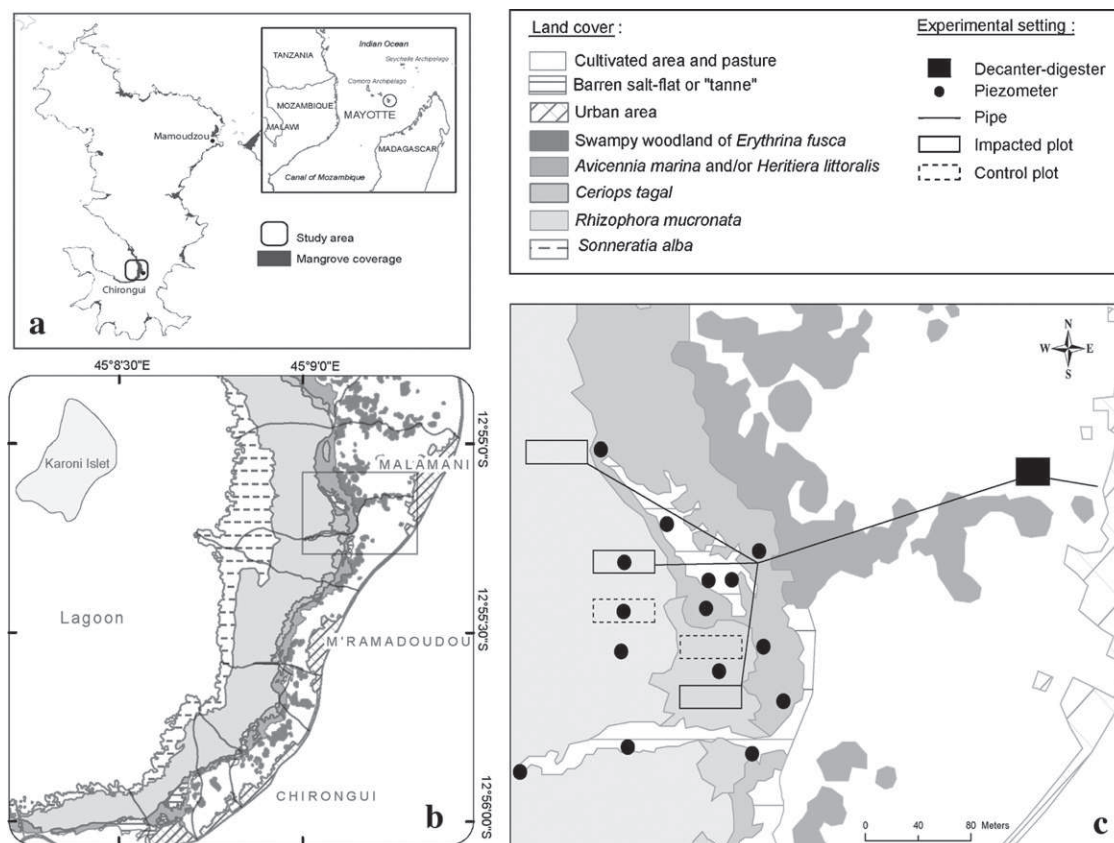


**Fig. 1.** (a) The study site, aerial photography from ultralight. The two impacted plots are characterised by a strong green colour (white circles mark the upper limit of plots). (b) Colour changes in *Ceriops tagal* leaves between control and impacted plots. (c) Growth differences in *Ceriops tagal* branches between control and impacted plots. All pictures: March 2009, i.e. after 12 months of daily wastewater discharge.

increase of population and rapid economic development. Sewage treatment is largely deficient in Mayotte and constitutes a major problem for the local authorities. Only one sewage plant, built in 2001 and recently renovated (2010), treats wastewater in Mamoudzou, the main town of Mayotte; however, the majority of effluent flows – directly or after having crossed mangrove swamps at the ends of bays – into the vast coral reef lagoon surrounding the island.

In this context, experiments have been launched at Malamani, SW Mayotte, to evaluate the bioremediation capacities of a mangrove swamp receiving, in controlled conditions, pretreated domestic wastewater. Water bodies, sediment, vegetation and fauna (crab populations) of mangrove ecosystems have been taken into account and analysed (Herteman, 2010; Herteman et al., submitted for publication) and experiments are still in progress at the Malamani study site.

We now report investigations concerning mangrove vegetation functioning after 12 and 18 months of daily wastewater discharge. An aerial survey of the study site clearly showed a change in the colour of the mangrove canopy, turning from light green to strong green, corresponding to mangrove plots receiving wastewater (Fig. 1a). This change appeared 6 months after the first sewage discharges in the mangroves and persisted 12 months later; the change in leaf colour clearly corresponds to the discharge. Observations in the field confirmed the colour change of the mangrove leaves and also showed obvious differences in branch length between control and impacted plots (Fig. 1b and c). To analyse such changes in vegetation and evaluate the impact of wastewater, photosynthetic pigment concentrations, photosynthesis rate and growth of mangrove trees were followed in impacted and non-impacted mangrove plots, in two different facies, respectively, dominated by *Ceriops tagal* (Perr.) C.B. Robinson and *Rhizophora mucronata* Lam.



**Fig. 2.** The study area and the experimental site. (a) Mayotte Island, SW Indian Ocean. (b) The study site, SW Mayotte Island, between Malamani village and the lagoon. (c) Experimental setting: decanter collecting domestic wastewater from Malamani; pipe network; impacted and control plots in *Ceriops tagal* and *Rhizophora mucronata* mangrove facies; piezometer network for water analyses.

## 2. Materials and methods

### 2.1. Study area

Mayotte Island is a dependent French overseas territory in the Comoro Archipelago, located in the Mozambique Channel, SW Indian Ocean (Fig. 2). The little volcanic island (376 km<sup>2</sup>) is surrounded by an almost continuous barrier reef system enclosing one of the largest lagoons in the world (1500 km<sup>2</sup>). Mangrove swamps are developed at the ends of bays on around 650 ha. The tide range is high for an oceanic island, reaching up to 4 m in spring tides. Mayotte's climate is maritime tropical, with a warm wet season from November to April (mean seasonal rainfall and temperature: 1200 mm and 27.2 °C, respectively) and a cooler dry season from May to October (210 mm and 25.1 °C).

The study area is located in Chirongui Bay, southwest of Mayotte (12°55'S, 45°09'E). A primary treatment unit sized for 400-equiv. inhabitants daily receives domestic wastewater from Malamani village. Wastewater is decanted and stored, and then carried through a pipe network to the mangrove area. Time delivery and discharge volumes are automatically controlled by a SOFREL processing system. Wastewater is then delivered every second low tide onto two mangrove plots respectively dominated by *C. tagal* and *R. mucronata* at the rate of 10 m<sup>3</sup> per 24 h on each 45 m × 15 m plot. A third 45 m × 15 m plot connected to the pipe network automatically receives wastewater in excess, particularly in the rainy season when discharge volumes exceed 20 m<sup>3</sup> per day (Fig. 2c).

Photosynthetic pigment concentration, photosynthesis rate and growth of mangrove trees were analysed 12 and 18 months after commencement of wastewater discharges in the two impacted

plots, and in two equivalent control plots. The average composition of the wastewater is given in Table 1, and the vegetation structure of the four plots is presented in Table 2.

### 2.2. Photosynthetic pigment analyses

Mature and healthy leaves of 12 random patches in each of the four plots were collected in January (wet season) and April (beginning of dry season) 2009 and rapidly stored in a cold place (cooler box during transport, then -80 °C freezer in laboratory). Three disks 18 mm in diameter were cut from each leaf patch sample, crushed with 50 mg Fontainebleau sand, rinsed with 20 ml methanol, and then placed under ultrasound for 3 min. Mixtures were stored for 15 min at -20 °C, and then spin-dried (5 min in -1 °C centrifuge at 3500 rpm). Samples of the supernatant were taken (1 ml), filtered through 0.2 µm syringe filters, and then analysed using HPLC.

### 2.3. Photosynthesis and transpiration rates

The net photosynthetic rate was measured on intact, mature *C. tagal* and *R. mucronata* leaves with a portable photosynthesis system (ADC Bioscientific Ltd portable), equipped with a 6.25 cm<sup>2</sup> leaf chamber. We measured 150 and 120 leaves, respectively, in each *C. tagal* and *R. mucronata* 45 m × 15 m plot. Three successive measurements were made for each sampled leaf at intervals of 25 s. All measurements were made between 10:00 and 13:00 h, on sunny days and under the following conditions: photosynthetically active radiance: 1000–2000 µmol m<sup>-2</sup> s<sup>-1</sup>, relative humidity: 65 ± 5%, temperature: 30 ± 2 °C.

**Table 1**  
Nutrient composition (mg l<sup>-1</sup>) of domestic wastewater after pre-treatment in decanter. Analyses realised on July 02, 2009, SIEAM Laboratory (Mayotte); April 01 and October 10, 2009, ARVAM Laboratory (La Réunion Island).

	NO <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub> + NO <sub>2</sub>	NH <sub>4</sub>	PO <sub>4</sub>
July 02, 2009	1.40	0.17	1.57	–	8.40
April 01, 2009	1.09	0.01	1.10	1.18	5.61
October 10, 2009	0.01	0.02	0.03	1.95	12.55

## 2.4. Growth rate measurements

**Leaf measurements:** In each control and impacted plot (*C. tagal* and *R. mucronata* stands), 90 mature and healthy leaves were randomly collected, and their lengths and widths measured. Fresh and dry weights were measured, and leaf areas were calculated using ImageJ software and leaf digitisation. Leaf area-weight relationships were determined. Measurements were made in April and October 2009.

**Branch measurements:** In each *C. tagal* plot, 60 branches were measured on 15 trees, i.e. four branches per tree, distributed in the upper, middle and lower parts of the control and impacted plots. In the *R. mucronata* plots, 39 branches were measured on 13 trees, i.e. three branches per tree, distributed throughout the control and impacted plots. Measurements were made in April and October 2009.

**Propagule measurements:** In each control and impacted plot (*C. tagal* and *R. mucronata*), 90 propagules were randomly collected from 9 trees, i.e. 10 propagules per tree, distributed in the upper, middle and lower parts of plots. Propagule length was measured in October 2009.

## 2.5. Statistical analyses

The Shapiro test was conducted on each dataset (pigment concentration, photosynthesis and transpiration rates, growth rate measurements) and showed that data were normally distributed. Mean values and standard deviation were calculated.

One-way ANOVA (for  $p \leq 0.05$  and  $p \leq 0.01$ ) was employed to test the significance of differences between control and impacted plots, between dates and between species, for each dataset except propagule lengths, which were analysed using Student's *t*-test ( $p \leq 0.05$ ).

All analyses were performed using the PAST software, version 1.94b (Hammer et al., 2001).

## 3. Results

### 3.1. Vegetation structure

Mangroves on the study site are developed over a length of about 600 m with a classical zonation according to inundation and salinity gradients, i.e. from landward to seaward: a degraded *Heritiera littoralis* Dryand. stand at the upper limit of tidal influence, followed by a barren salt flat or "tanne" surrounded by old *A. marina*

(Forssk.) Vierh. trees, a dense and a low *C. tagal* stand progressively mixed with *R. mucronata* individuals, a high and important *R. mucronata* stand including scattered patches of *Bruguiera gymnorhiza* (L.) Lam., and finally on the lagoon side a well-developed *Sonneratia alba* J. Smith zone.

Experiments were conducted in two mangrove facies, chosen for their representativeness and their important development in most mangrove stands in Mayotte, namely *C. tagal* and *R. mucronata* facies. Structures are described in Table 2. The *C. tagal* facies were largely dominated by the eponymous species, which represented 90% of the specific composition, with 9% for *R. mucronata* and a few individuals of *A. marina* in the upper part of the stand and rare *B. gymnorhiza* in the lower part. Total density is very high with 69,500 ind ha<sup>-1</sup> and 62,750 ind ha<sup>-1</sup> for *C. tagal*. *C. tagal* individuals are small trees with 2.2 ± 1.1 cm trunk diameter and 1.7 ± 0.9 m in height. The second facies is dominated by *R. mucronata* (79%) with *C. tagal* individuals in the upper part (16%) and patches of *B. gymnorhiza* (5%). Total density is lower, with 7900 ind ha<sup>-1</sup> and 6250 ind ha<sup>-1</sup> for *R. mucronata*. The mean trunk diameter for dominant individuals of *R. mucronata* is 16.1 ± 5.2 cm with a height of 7.1 ± 2.1 m.

It is important to note that the vegetation structure was analysed successively in November 2006, before the first sewage discharges, and in November 2008, 6 months after discharges began. No significant difference was observed within the period. We also noted that the vegetation structure had not changed after 12 and 18 months of discharges when functional analyses were made, in terms of density or mortality rates. Regeneration seems to be enhanced in impacted plots and density of canopy as well. Analyses are currently underway to quantify these processes.

### 3.2. Photosynthetic pigment concentration

Table 3 and Fig. 3 show the results of analyses of chlorophyll a and b, carotene, and xanthophyll pigments extracted from *C. tagal* and *R. mucronata* leaves sampled in control and impacted plots (January 2009, April 2009).

Pigment content appears to be significantly higher in plots having received wastewater than in control plots, for all pigment types, for the two dates analysed and for both *C. tagal* and *R. mucronata* stands, except for chlorophyll b, for which results are not significant for *R. mucronata* in January 2009.

Pigment concentration increased around twofold between *C. tagal* control and impacted stands and for the two dates, i.e. from 1.47 to 2.88 mg g<sup>-1</sup> dw (January 2009) and 1.23 to

**Table 2**  
Structural analyses of mangrove plots, before wastewater discharge (November 2006).

Facies	Species	Specific dominance (%)	Density (n ha <sup>-1</sup> )	Dbh (<10 cm)	Dbh (>10 cm)	Height (m) (Ø < 10 cm)	Height (m) (Ø > 10 cm)	Basal area (m <sup>2</sup> ha <sup>-1</sup> )	Dead ind. (n ha <sup>-1</sup> )
<i>C. tagal</i>	<i>A. marina</i>	0.7	500	5.8	–	2.8	–	2.8	250
	<i>B. gymn.</i>	0.3	250	3.2	–	0.6	–	0.4	0
	<i>C. tagal</i>	90.0	62,750	2.2	–	1.7	–	31.1	3500
	<i>R. mucr.</i>	9.0	6000	4.8	11.1	2.7	3.5	18.2	250
<i>R. mucr.</i>	<i>B. gymn.</i>	5.0	350	3.9	21.7	2.2	5.8	0.29	50
	<i>C. tagal</i>	16.0	1300	4.1	–	2.6	–	2.04	50
	<i>R. mucr.</i>	79.0	6250	6.4	16.1	3.7	7.1	71.5	200

**Table 3**Leaf pigment content ( $\text{mg g}^{-1}$  dw) of *C. tagal* and *R. mucronata*, in control and impacted plots January and April 2009.

	<i>Ceriops tagal</i>				<i>Rhizophora mucronata</i>			
	January 2009		April 2009		January 2009		April 2009	
	Control	Impacted	Control	Impacted	Control	Impacted	Control	Impacted
Chlor a	1.47 ± 0.59	2.88 ± 0.76**	1.23 ± 0.41	3.28 ± 0.68**	2.08 ± 0.68	2.87 ± 0.68**	3.01 ± 0.67	4.01 ± 0.75
Chlor b	0.43 ± 0.19	0.89 ± 0.26**	0.34 ± 0.12	1.03 ± 0.22*	0.60 ± 0.1	0.88 ± 0.23**	0.9 ± 0.21	1.31 ± 0.29
Chl a:b	3.46 ± 0.2	3.25 ± 0.15	3.64 ± 0.15	3.18 ± 0.14	3.46 ± 0.15	3.28 ± 0.15	3.33 ± 0.21	3.09 ± 0.18
β-carotene	0.43 ± 0.16	0.79 ± 0.18**	0.36 ± 0.12	0.87 ± 0.2**	0.58 ± 0.08	0.76 ± 0.2**	0.82 ± 0.20	1.04 ± 0.18
Xanth.	0.07 ± 0.02	0.13 ± 0.03**	0.06 ± 0.02	0.15 ± 0.03**	0.1 ± 0.01	0.14 ± 0.03**	0.14 ± 0.03	0.19 ± 0.03

Significant differences between control and impacted plots with \* $p \leq 0.05$  and \*\* $p \leq 0.01$ .  $n = 12$  for each modality.

3.28  $\text{mg g}^{-1}$  dw (April 2009) for chlorophyll a. In comparison, the increase in *R. mucronata* is significant but moderate, i.e. from 2.08 to 2.87  $\text{mg g}^{-1}$  dw (January 2009) and from 3.01 to 4.01  $\text{mg g}^{-1}$  dw (April 2009) for chlorophyll a.

In *C. tagal* plots, we note that differences between the two control plots and between the two impacted plots are not significant between January and April, for all pigments. For instance, changes were from 1.47 to 1.23 (control plots) and 2.88 to 3.28  $\text{mg g}^{-1}$  dw (impacted plots) for chlorophyll a, 0.43 to 0.36 (control) and 0.79 to 0.87 (impacted) for β-carotene, and 0.07 to 0.06 (control) and 0.13 to 0.15 (impacted) for xanthophylls. Similar comparisons for *R. mucronata*, however, show significant increases between dates, with 2.08–3.01 (control) and 2.87–4.01 (impacted) for chlorophyll a, 0.58–0.82 (control) and 0.76–1.04 (impacted) for β-carotene, and 0.1–0.14 (control) and 0.14–0.19 (impacted) for xanthophylls.

### 3.3. Photosynthesis and transpiration rates

Table 4 and Fig. 4 summarise results for both parameters, for measurements made in April 2009 (end of wet season) and October 2009 (end of dry season).

Photosynthesis rate appears significantly higher in plots receiving wastewater than in control plots, in April (6.14 vs. 9.86  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and October (5.82 vs. 9.53  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for *C. tagal* plots and in October 2009 only (8.68 vs. 10.66  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for *R. mucronata* plots.

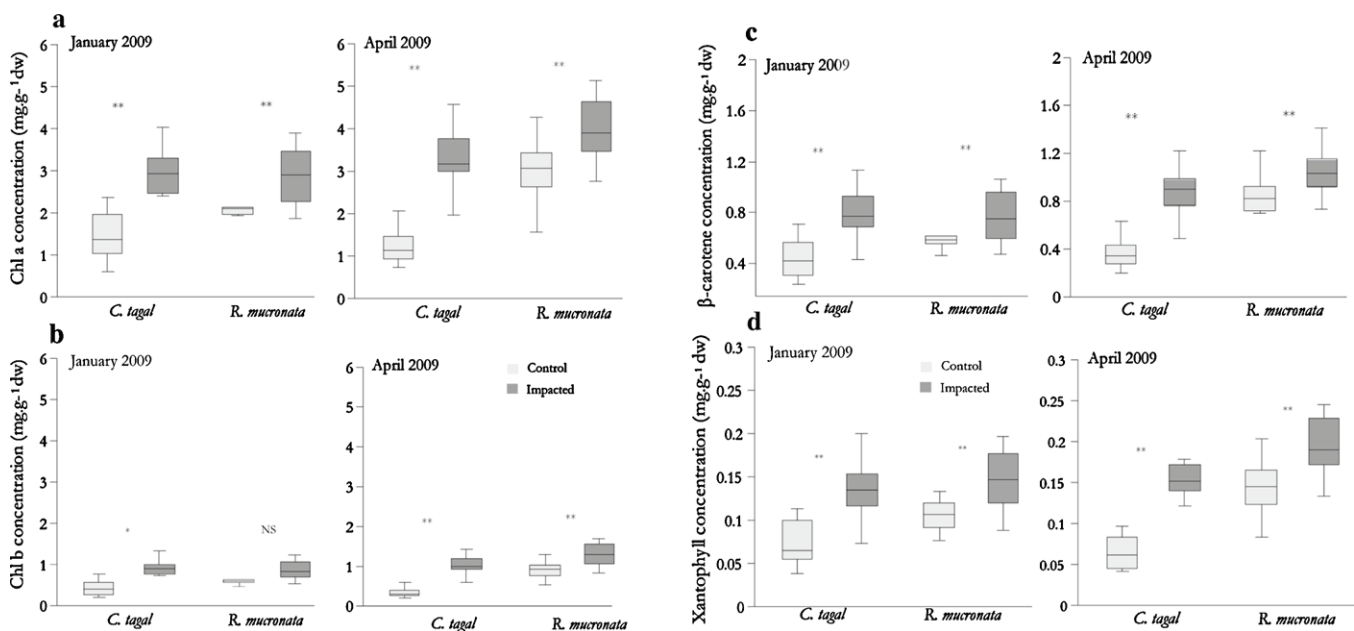
Comparisons between species show that the photosynthesis rate is significantly higher in *R. mucronata* than in *C. tagal*, in both control (12.52 vs. 6.14  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in April, 8.68 vs. 5.82  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in October, respectively) and impacted plots (12.62 vs. 9.86 and 10.66 vs. 9.53) and for each of the dates considered. The photosynthetic rate also appears slightly but significantly higher for both species at the end of the wet season (April) than at the end of the dry season (October).

If we compare transpiration rates between control and impacted plots, measurements indicate significant differences for both species in April with higher values in plots receiving wastewater (3.95 vs. 2.47  $\text{mmol m}^{-2} \text{s}^{-1}$  for *C. tagal*, and 4.1 vs. 3.53  $\text{mmol m}^{-2} \text{s}^{-1}$  for *R. mucronata*, respectively), while the differences are not significantly different in October (3.64 vs. 3.65 for *C. tagal* and 3.27 vs. 3.64 for *R. mucronata*).

### 3.4. Growth rate measurements

Results for leaf (length, width, weight, surface area), branch and propagule (length) measurements after 12 and 18 months (April 2009 and October 2009) are presented in Table 5 and Fig. 5.

Except for *R. mucronata* measurements in April, leaf length and width and consequently leaf area are significantly higher for samples collected in impacted plots for both species and dates, i.e. for leaf areas, respectively 15.9 (control plots) and 37.9  $\text{cm}^2$  (impacted



**Fig. 3.** Pigment concentration in *Ceriops tagal* and *Rhizophora mucronata* leaves, collected in control and impacted plots, Malamani study site, January and April 2009. (a) Chlorophyll a. (b) Chlorophyll b. (c) β-Carotene. (d) Xanthophyll. Unit:  $\text{mg g}^{-1}$  dw.

**Table 4**  
Photosynthesis rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) in leaves of *C. tagal* ( $n: 150$ ) and *R. mucronata* ( $n: 120$ ), in control and impacted plots, April and October 2009 (mean  $\pm$  Sd).

	<i>Ceriops tagal</i>				<i>Rhizophora mucronata</i>			
	April 2009		October 2009		April 2009		October 2009	
	Control	Impacted	Control	Impacted	Control	Impacted	Control	Impacted
Photosynthesis rate	6.14 $\pm$ 1.9	9.86 $\pm$ 1.52	5.82 $\pm$ 1.3	9.53 $\pm$ 1.35	12.5 $\pm$ 2.7	12.62 $\pm$ 2.39	8.68 $\pm$ 4.02	10.66 $\pm$ 2.98
Transpiration rate	2.47 $\pm$ 0.94	3.95 $\pm$ 0.82	3.65 $\pm$ 0.89	3.64 $\pm$ 0.89	3.53 $\pm$ 1.11	4.1 $\pm$ 1.18	3.64 $\pm$ 1.11	3.27 $\pm$ 0.75

plots) for *C. tagal* and 63.2 (control) and 89.5 (impacted) in October for *R. mucronata*.

The leaves of both species are slightly heavier (dry weight) for both species, and leaf area-to-weight ratios are significantly higher in impacted conditions than in controlled ones.

Concerning branch length, all results are significant with important increases, i.e. 4.68–13.38 cm for *C. tagal* and 16.04–20.06 cm for *R. mucronata* in October. No significant seasonal change appeared in branch length from April to October for either species or plot conditions.

Finally, the impact of wastewater supply in mangrove plots significantly increased propagule length in both species, i.e. 16.4–32.1 cm for *C. tagal* and 32.1–39.1 cm for *R. mucronata*.

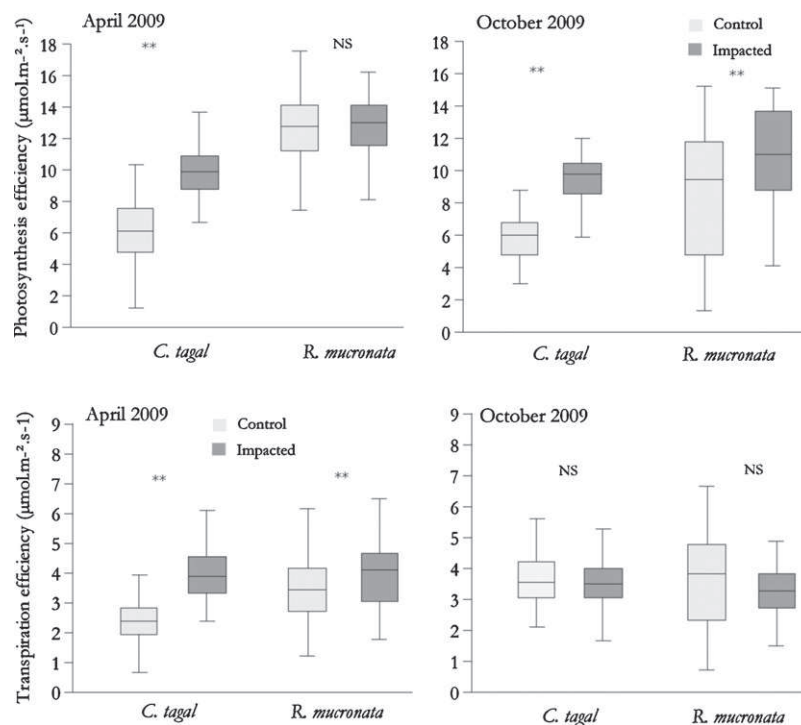
## 4. Discussion

### 4.1. Leaf pigment concentration in mangrove trees and wastewater effects

In natural conditions in Mayotte Island, we established that pigment concentration was significantly higher in *R. mucronata* than in *C. tagal* leaves, particularly for chlorophyll a, the trigger element of photochemical processes. Chlorophyll a:b ratios, considered to be a significant index of photosynthetic functioning, exhibit very stable and similar values for both species and dates, corresponding to val-

ues given by Das et al. (2002) for *R. apiculata* and *B. gymnorhiza*, and by Basak et al. (1996) for *R. mucronata* and *C. decandra*. As noted by these authors, carotenoid content is very low in the *Rhizophoraceae* family, as we observed in Mayotte Island in natural conditions.

The supply of wastewater to mangrove plots enhances pigment concentration in mangrove leaves, with clear increases for chlorophylls,  $\beta$ -carotene and xanthophylls in both species. While no data were found in the literature directly concerning the relationships between pigment concentration in mangrove leaves and wastewater supplies, many authors have considered pigment concentration in relation to environmental factors. Medina and Francisco (1997) established that chlorophyll content appeared to be higher in mangrove leaves of riverine mangrove stands and lower in leaves of mangroves from dry sites, and added that N and P leaf concentrations and leaf areas varied in the same way between dry and wet sites. Authors interpreted such results as interactions between salinity and water stresses, in relation to nutrient supplies and photosynthetic productivity. MacFarlane and Burchett (2001) and MacFarlane (2002) showed links between leaf chlorophylls (a + b) and carotenoid content of *A. marina* and heavy metal concentration in mangrove sediment. Ye et al. (2003) examining the effects of waterlogging on growth and physiological characteristics of *B. gymnorhiza* and *Kandelia candel* (*Rhizophoraceae*), showed that chlorophyll and carotenoid concentrations increased when waterlogging duration and intensity increased.



**Fig. 4.** Photosynthesis rate and transpiration rate measured on *Ceriops tagal* and *Rhizophora mucronata* leaves, in control and impacted plots, Malamani study site, April and October 2009. Unit:  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

**Table 5**Shoot length (cm), internode number and leaf number per shoot for *C. tagal* and *R. mucronata*, in control and impacted plots, April and October 2009 (mean  $\pm$  Sd, n = 60).

	<i>Ceriops tagal</i>				<i>Rhizophora mucronata</i>			
	April		October		April		October	
	Control	Impacted	Control	Impacted	Control	Impacted	Control	Impacted
Shoot length	4.68 $\pm$ 2.5	13.38 $\pm$ 4.26	5.01 $\pm$ 3.44	12.82 $\pm$ 1.5	16.04 $\pm$ 2.11	20.06 $\pm$ 2.63	9.16 $\pm$ 6.19	8.88 $\pm$ 6.02
Internodes number	1.3 $\pm$ 0.5	3.3 $\pm$ 0.9	2.1 $\pm$ 0.7	3.3 $\pm$ 0.9	3.7 $\pm$ 1.4	2.8 $\pm$ 0.5	17.3 $\pm$ 1.2	21.2 $\pm$ 2.0
Leaf number per shoot	8.6 $\pm$ 5.5	9.6 $\pm$ 7.3	-	5.2 $\pm$ 1.3	5.9 $\pm$ 1.5	5.3 $\pm$ 1.0	4.7 $\pm$ 1.7	4.2 $\pm$ 1.1

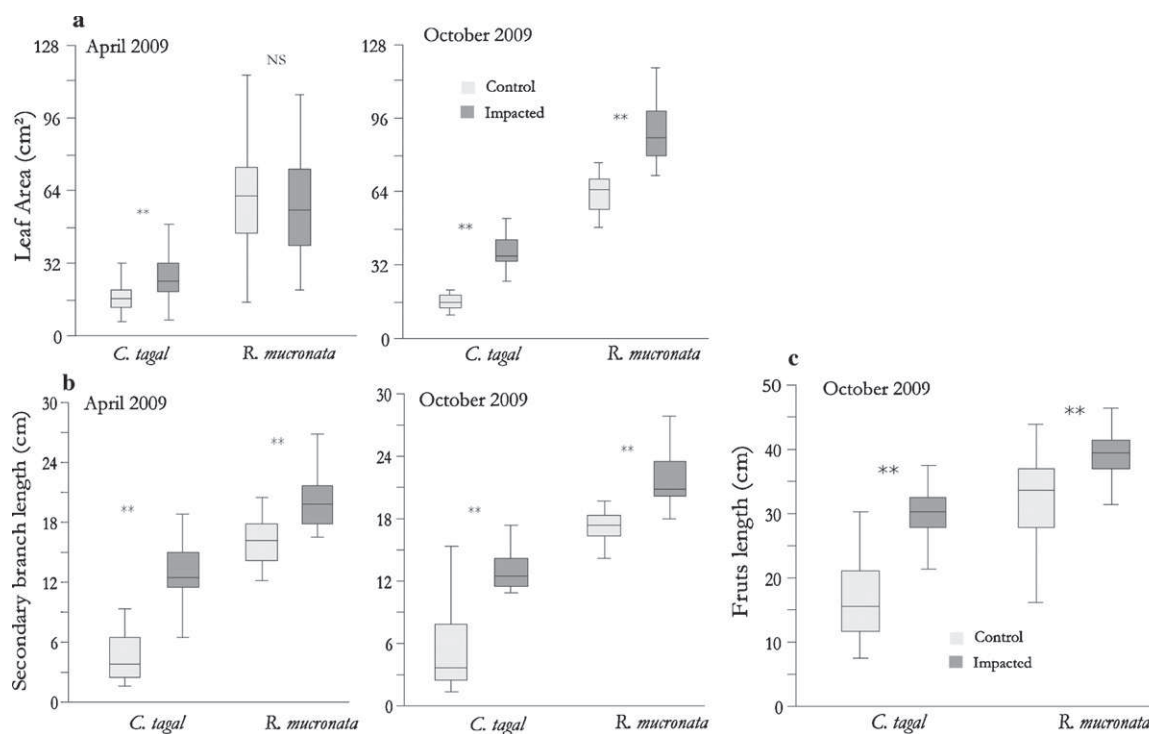
Wastewater supply to impacted mangrove plots in Malamani contributes both to lower salinity level – fresh water is added to the ecosystem – and to increased N and P levels. The average composition of sewage (Table 1) indicates the amount and the nature of nitrogen and phosphorus compounds delivered daily to mangrove plots. Moreover, we established that wastewater delivered to mangroves at low tide rapidly seeps into sediment and is progressively absorbed by vegetation, and that N and P compounds are at least partially used by mangrove trees (Herteman, 2010; Herteman et al., submitted for publication). The change in the colour of the vegetation of impacted plots (Fig. 1) also reflects these processes and corresponds to the increase in leaf pigment concentration. As proposed by the authors cited above, pigment concentration may thus be considered a marker of stress conditions for mangrove trees, or a marker of change in mangrove functioning, revealing pollution with heavy metals (MacFarlane and Burchett, 2001; MacFarlane, 2002) or an excess of nutrient, as we demonstrated in our Malamani experiments. From a more general point of view, studies of pigment content in higher plants as biomarkers are rare, and essentially concerned micro-algae, where pigment content is directly linked to biomass (Wilhelm et al., 1995). Brain and Cedergreen (2009), in a recent review on biomarkers in aquatic plants, indicated advantages for considering pigment content as a biomarker: it is an easy-to-measure and robust parameter and, furthermore, visual observation, as in our Malamani experiments, may preclude

the necessity of measuring pigment content. These authors added that chlorophylls and carotenoids were the primary light-capturing pigments in higher plants, absorbing light energy for photosynthesis. Nutrient status, with light intensity or temperature, is one of the factors affecting the content of photosynthetic pigments. At high nutrient availability, and particularly with excess N, pigment content increases and enhances carbon fixation.

#### 4.2. Photosynthetic processes

Pigment concentration is directly linked to photosynthetic activity, and photosynthetic rates and pigment content, i.e. chlorophyll a:b ratio, have been found to be correlated (Anderson et al., 1988; Das et al., 2002).

Measurements of photosynthesis rate in control plots in Malamani clearly indicate differences between species, with the highest values obtained in *R. mucronata*, where the highest pigment concentrations were also found. Theuri et al. (1999) obtained similar results with higher values for *R. mucronata* than for *C. tagal* in mangrove stands in Kenya. Nevertheless, these authors globally found lower values in Kenyan mangroves than in Mayotte (around 1.5 and 1.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for *R. mucronata* and *C. tagal*, respectively) and important seasonal variation, with twofold values in the wet season (around 4.0 and 3.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively) while seasonal changes in Malamani were not significant. Transpiration rate



**Fig. 5.** Changes in leaf area (a), branch length (b) and propagule length (c) of *Ceriops tagal* and *Rhizophora mucronata* in control and impacted plots, Malamani study site, April and October 2009. Units:  $\text{cm}^2$  and cm.



levels are also greater in Mayotte (2.45–3.65 mm m<sup>-2</sup> s<sup>-1</sup>) than in Kenyan mangrove (0.78–0.94 mm m<sup>-2</sup> s<sup>-1</sup>).

Clough et al. (1997) and Clough (1998) found high values of photosynthetic rates for *R. apiculata* (average rate for the whole canopy: 9.0 μmol m<sup>-2</sup> s<sup>-1</sup>) and different Rhizophoraceae (6.13–12.9 μmol m<sup>-2</sup> s<sup>-1</sup>), with higher values for *Rhizophora* spp. and lower values for *C. australis*. These authors added that rates of photosynthesis may be substantially lower in mangrove stands characterised by higher aridity and salinity conditions with values around 4–5 μmol m<sup>-2</sup> s<sup>-1</sup>.

In the mangrove stands of Malamani, wastewater supplies clearly contribute to increased photosynthetic rates in impacted mangrove plots as they lead to an increase in pigment concentrations. As we noticed above, wastewater contributes to lower salinity rates, enriches the mangrove ecosystem in N and P nutrients, and consequently enhances photosynthesis rate. Sobrado (2000) and Li et al. (2008) indicated similar relationships between salinity conditions and photosynthesis processes for different mangrove trees including Rhizophoraceae, and Kao et al. (2001) showed that an increase in N availability increased photosynthetic rates for the Rhizophoraceae *K. candel.* Li et al. (2008) added that high levels of Na concentration in mangrove trees inhibited electron transport in photosynthetic processes and consequently led to a decrease in photosynthetic efficiency.

#### 4.3. Mangrove growth rates and wastewater supplies

Increased mangrove growth rates (leaf dimensions and surface areas, branch length) observed in impacted plots at the study site are a direct effect of enhancement in photosynthesis rate and of the increase in leaf pigment concentration. The supply of freshwater and nutrients (N and P), particularly through wastewater discharges, is known to induce an increase in mangrove tree growth (Clough et al., 1983) by acting as a fertiliser supply in the ecosystem (Boto and Wellington, 1983). Onuf et al. (1977) also observed that a *Rhizophora* mangrove stand naturally enriched with guano from a bird colony exhibited significant enhancement of growth. Clough et al. (1983), analysing all these results, concluded that “nutrient enrichment from fertilization or from sewage effluent is not likely to be deleterious to mangroves, and may be beneficial where the nutrient status of the mangroves is low”. Lin and Sternberg (1992), and more recently Lovelock and Feller (2003) and Lovelock et al. (2004), while analysing functional differences between scrub and fringe mangroves, established that CO<sub>2</sub> assimilation rate and photosynthetic efficiency seem to be lower in scrub facies, also characterised by high salinity levels. Conversely, fertilisation by N and P supplies may induce significant shoot growth in dwarf mangrove stands.

Recent papers, however, have emphasised potential negative consequences of excessive nutrient enrichment in mangroves. They established, for instance, that excessive N supply might induce changes in root-to-shoot ratio (development of shoots at the expense of roots) and increase the vulnerability of mangrove stands in high-saline environments (Martin et al., 2010), or even lead to the death of mangrove trees in high salinity and low rainfall conditions (Lovelock et al., 2009). In this last study, nutrient enrichment seems to have been through a single, massive supply annually or biannually, i.e. 300 g of urea or phosphate into holes cored on either side of the tree stems. Notice that these amounts correspond to the total amount of N provided to our impacted plots in a whole year, but delivered daily every second low tide to our experimental mangrove stands. The kinetics of absorption and assimilation of nutrients is then certainly different in the two cases, and thus the consequences on mangrove

tree metabolism will be different as well. While no negative effect on mangrove vegetation appeared after 18 months of wastewater supplies in the mangroves of Malamani, we will still require continuing control experiments to assess the long-term efficiency of bioremediation through mangrove ecosystem. In another domain, Penha-Lopes et al. (2010) indicated, from mesocosm experiments, that sewage contamination caused disturbances to gastropod populations (*Terebralia palustris*) associated with mangrove trees. In the Malamani study site, preliminary results did not show any change in crab populations impacted by wastewater (Herteman, 2010), but further experiments are planned to evaluate potential effects over a longer term.

## 5. Conclusions

The present study showed that domestic wastewater discharges induced important changes in mangrove vegetation. In particular, the wastewater:

- increased leaf pigment content in *C. tagal* and *R. mucronata* stands impacted with 12–18 months of daily supplies;
- enhanced significantly photosynthetic activity and transpiration rate; and
- induced significant increase in leaf area and branch length of impacted mangrove stands.

At the same time, no evident modification appeared in general structure or functioning of mangrove vegetation.

If our results seem to demonstrate that pretreated domestic wastewater may have beneficial effects on mangrove functioning, a survey of the literature nevertheless shows that N and P excess, brought through domestic wastewater or experimental supplies, could in certain conditions and over a long term induce dysfunctioning in mangrove vegetation.

Further experimentation and analyses are necessary before we can clearly define the possible role of mangrove ecosystems in bioremediation of domestic wastewater.

Such experimentation is currently in progress in the Malamani study site, taking into account the different compartments of the mangrove ecosystem and their interactions, i.e. salty and freshwater bodies, sediment, crab populations and the structure and functioning of the mangrove vegetation. While the preliminary results in this paper show that wastewater is effectively absorbed by mangrove trees and induces enhancement of mangrove tree functioning, global N and P balances must be established for better quantification. Another avenue of research, also in progress, is to improve wastewater treatment in the primary treatment unit before its discharge into mangrove stands.

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## References

- Anderson, J.M., Chow, W.S., Goodchild, D., 1988. Thylakoid membrane organization in sun/shade acclimation. *Aust. J. Plant Physiol.* 1, 11–26.

- Ball, M.C., Farquhar, G.D., 1984. Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long term salinity and humidity condition. *Plant Physiol.* 74, 1–6.
- Basak, U.C., Das, A.B., Das, P., 1996. Chlorophylls, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangrove. *Bull. Mar. Sci.* 58, 654–659.
- Boto, K.G., Wellington, J.T., 1983. Nitrogen and phosphorus nutritional status of a northern Australian mangrove forest. *Mar Ecol. Prog. Ser.* 11, 63–69.
- Brain, R.A., Cedergreen, N., 2009. Biomarkers in aquatic plants: selection and utility. *Rev. Environ. Contam. Toxicol.* 198, 49–109.
- Clough, B., 1998. Mangrove forest productivity and biomass accumulation in Hinchinbrook Channel, Australia. *Mangrove Salt Marshes* 2, 191–198.
- Clough, B.F., Boto, K.G., Attiwill, P.M., 1983. Mangroves and sewage—a re-evaluation. In: Teas, G.H. (Ed.), *Proceedings of the 2nd International Symposium on Biology and Management of Mangroves*, Papua New Guinea. W. Junk Publisher, The Hague, pp. 151–161.
- Clough, B.F., Ong, J.E., Gong, W.K., 1997. Estimating leaf area index and photosynthetic production in canopies of the mangrove *Rhizophora apiculata*. *Mar. Ecol. Prog. Ser.* 159, 285–292.
- Corredor, J.E., Morell, J.M., 1994. Nitrate depuration of secondary sewage effluents in mangrove sediments. *Estuaries* 17, 295–300.
- Das, A.B., Parida, A., Basak, U.C., Das, P., 2002. Studies on pigments, proteins and photosynthetic rates in some mangroves and mangrove associates from Bhitarkanika, Orissa. *Mar. Biol.* 141, 415–422.
- Hammer, O., Harper, D.A.T., Ryan, P.D., 2001. PAST: palaeontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 1–9.
- Henley, D.A., 1978. An investigation of proposed effluent discharge into a tropical mangrove estuary. In: *Proceeding of International Conference on Water Pollution Control in Developing Countries*, September 1978, Thailand, pp. 43–64.
- Herteman, M., 2010. Evaluation des capacités bioremédiatrices d'une mangrove impactée par des eaux usées domestiques. Application au site-pilote de Malamani, Mayotte. Thèse Université de Toulouse.
- Herteman, M., Lambs, L., Fromard, F., Sanchez-Perez, J., Muller, E. Water circulation and storage capacity in a mangrove swamp of Mayotte Island, submitted for publication.
- Kao, W.Y., Tsai, H.C., 1999. The photosynthesis and chlorophyll a fluorescence in seedlings of *Kandelia candel* (L.) Druce grown under different nitrogen and NaCl controls. *Photosynthetica* 37 (3), 405–412.
- Kao, W.Y., Tsai, H.C., Tsai, T.T., 2001. Effect of NaCl and nitrogen availability on growth and photosynthesis of seedlings of a mangrove species, *Kandelia candel* (L.) Druce. *J. Plant Physiol.* 158, 841–846.
- Kelly, T., 1995. Effects of long-term discharges of treated sewage on the nutrient status of adjacent mangrove communities. Unpublished Thesis, Southern Cross University, Lismore, Australia.
- Krauss, K.W., Allen, J.A., 2003. Influences of salinity and shade on seedling photosynthesis and growth of two mangrove species, *Rhizophora mangle* and *Bruguiera sexangula*, introduced to Hawaii. *Aquat. Bot.* 77, 311–324.
- Li, N.Y., Chen, S.L., Zhou, X.Y., Li, C.Y., Shao, J., Wang, R.G., 2008. Effect of NaCl on photosynthesis, salt accumulation and ion compartmentation in two mangrove species, *Kandelia candel* and *Bruguiera gymnorhiza*. *Aquat. Bot.* 88, 303–310.
- Lin, G., Sternberg, L. da S.L., 1992. Comparative study of water uptake and photosynthetic gas exchange between scrub and fringe red mangrove, *Rhizophora mangle* L. *Oecologia* 90, 399–403.
- Lovelock, C.E., Clough, B.F., 1992. Influence of solar-radiation and leaf angle on leaf xanthophyll concentrations in mangroves. *Oecologia* 91, 518–525.
- Lovelock, C.E., Feller, I.C., 2003. Photosynthetic performance and resource utilization of two mangrove species coexisting in a hypersaline scrub forest. *Oecologia* 134, 455–465.
- Lovelock, C.E., Feller, I.C., McKee, K.L., Engelbrecht, B.M., Ball, M.C., 2004. The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama. *Funct. Ecol.* 18, 25–33.
- Lovelock, C.E., Ball, M.C., Martin, K.C., Feller, I.C., 2009. Nutrient enrichment increases mortality of mangroves. *PLoS ONE* 4 (5), e5600, doi:10.1371/journal.pone.0005600.
- MacFarlane, G.R., 2002. Leaf biochemical parameters in *Avicennia marina* (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems. *Mar. Pollut. Bull.* 44, 244–256.
- MacFarlane, G.R., Burchett, M.D., 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Mar. Pollut. Bull.* 42, 233–240.
- Martin, K.C., Dan Bruhn, Lovelock, C.E., Feller, I.C., Evans, J.R., Ball, M.C., 2010. Nitrogen fertilization enhances water-use efficiency in a saline environment. *Plant Cell. Environ.* 33 (3), 344–357.
- Medina, E., Francisco, M., 1997. Osmolality and delta C-13 of leaf tissues of mangrove species from environments of contrasting rainfall and salinity. *Estuar. Coast. Shelf Sci.* 45, 337–344.
- Moorthy, P., Kathiresan, K., 1997. Influence of ultraviolet-B radiation on photosynthetic and biochemical characteristics of a mangrove *Rhizophora apiculata*. *Photosynthetica* 34, 465–471.
- Naidoo, G., Chirkoot, D., 2004. The effects of coal dust on photosynthetic performance of the mangrove, *Avicennia marina* in Richards Bay, South Africa. *Environ. Pollut.* 127, 359–366.
- Nedwell, D.B., 1975. Inorganic nitrogen-metabolism in a eutrophicated tropical mangrove estuary. *Water Res.* 9, 221–231.
- Onuf, C.P., John, M., Teal, J.M., Valiela, I., 1977. Interactions of nutrients, plant growth and Herbivory in a mangrove ecosystem. *Ecology* 58 (3), 514–526.
- Penha-Lopes, G., Bartolini, F., Limbu, S., Cannicci, S., Mgaya, Y., Kristensen, E., Paula, J., 2010. Ecosystem engineering potential of the gastropod *Terebralia palustris* (Linnaeus 1767) in mangrove wastewater wetlands: a controlled mesocosm experiment. *Environ. Pollut.* 158 (1), 258–266.
- Rajesh, A., Arumugam, R., Venkatesalu, V., 1998. Growth and photosynthetic characteristics of *Ceriops roxburghiana* under NaCl stress. *Photosynthetica* 35, 285–287.
- Saenger, P., 2002. *Mangrove Ecology Silviculture and Conservation*. Kluwer Academic Publishers, Dordrecht.
- Sobrado, M.A., 2000. Relation of water transport to leaf gas exchange properties in three mangrove species. *Trees Struct. Funct.* 14, 258–262.
- Tam, N.F.Y., 1998. Effects of wastewater discharge on microbial populations and enzyme activities in mangrove soils. *Environ. Pollut.* 102 (2–3), 233–242.
- Tam, N.F.Y., Wong, Y.S., 1995. Mangrove soils as sinks for wastewater-borne pollutants. *Hydrobiologia* 295, 231–241.
- Tam, N.F.Y., Wong, Y.S., 1996. Retention and distribution of heavy metals in mangrove soils receiving wastewater. *Environ. Pollut.* 94, 283–291.
- Tam, N.F.Y., Wong, Y.S., Lan, C.Y., Wang, L.N., 1998. Litter production and decomposition in a subtropical mangrove swamp receiving wastewater. *J. Exp. Mar. Biol. Ecol.* 226, 1–18.
- Tam, N.F.Y., Wong, A.H.Y., Wong, M.H., Wong, Y.S., 2009. Mass balance of nitrogen in constructed mangrove wetlands receiving ammonium-rich wastewater: effects of tidal regime and carbon supply. *Ecol. Eng.* 35, 453–462.
- Theuri, M.M., Kinyamario, J.I., Van Speybroeck, D., 1999. Photosynthesis and related physiological processes in two mangrove species, *Rhizophora mucronata* and *Ceriops tagal*, at Gazi Bay, Kenya. *Afr. J. Ecol.* 37, 180–193.
- Wickramasinghe, S., Borin, M., Kotagama, S.W., Cochard, R., Anceno, A.J., Shipin, O.V., 2009. Multi-functional pollution mitigation in a rehabilitated mangrove conservation area. *Ecol. Eng.* 35, 898–907.
- Wilhelm, C., Volkmar, P., Lohmann, C., Becker, A., Meyer, M., 1995. The HPLC-aided pigment analysis of phytoplankton cells as a powerful tool in water-quality control. *J. Water Supply Res. Technol. Aqua* 44, 132–141.
- Wong, Y.S., Tam, N.F.Y., Lan, C.Y., 1997. Mangrove wetlands as wastewater treatment facility: a field trial. *Hydrobiologia* 352, 49–59.
- Wong, Y.S., Lan, C.Y., Chen, G.Z., Li, S.H., Chen, X.R., Liu, Z.P., 1995. Effect of wastewater discharge on nutrient contamination of mangrove soils and plants. *Hydrobiologia* 295, 243–254.
- Wu, Y., Chung, A., Tam, N.F.Y., Pi, N., Wong, M.H., 2008. Constructed mangrove wetland as secondary treatment system for municipal wastewater. *Ecol. Eng.* 34, 137–146.
- Ye, Y., Tam, N.F.Y., Wong, Y.S., Lu, C.Y., 2003. Growth and physiological responses of two mangrove species (*Bruguiera gymnorhiza* and *Kandelia candel*) to waterlogging. *Environ. Exp. Bot.* 49 (3), 209–221.
- Youssef, T., Saenger, P., 1998. Photosynthetic gas exchange and accumulation of phytotoxins in mangrove seedlings in response to soil physico-chemical characteristics associated with waterlogging. *Tree Physiol.* 18, 317–324.