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Nutrient Removal Efficiency of *Rhizophora mangle* (L.) Seedlings Exposed to Experimental Dumping of Municipal Waters

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Received: 23 January 2018; Accepted: 12 March 2018; Published: 15 March 2018

Abstract: Mangrove forests are conspicuous components of tropical wetlands that sustain continuous exposure to wastewater discharges commonly of municipal origins. Mangroves can remove nutrients from these waters to fulfill their nutrients demand, although the effects of continuous exposure are unknown. An experimental greenhouse imitating tidal regimes was built to measure the efficiency of mangrove seedlings to incorporate nutrients, growth and above biomass production when exposed to three periodic wastewater discharges. The experiment totaled 112 d. Nutrient removal by the exposed group, such as phosphates, ammonia, nitrites, nitrates and dissolved inorganic nitrogen (97%, 98.35%, 71.05%, 56.57% and 64.36%, respectively) was evident up to the second dumping. By the third dumping, all nutrient concentrations increased in the interstitial water, although significant evidence of removal by the plants was not obtained ($p > 0.05$). Nutrient concentrations in the control group did not change significantly throughout the experiment ($p > 0.05$). Treated plants increased two-fold in stem girth when compared to the control ($p < 0.05$), although control plants averaged higher heights ($p < 0.05$). Biomass of treated group increased up to 45% against 37% of the control during the duration of the experiment ($p < 0.05$). We suggest that nutrient removal efficiency of mangroves is linked to the maintenance of oxic conditions in the pore-water because of oxygen transference from their aerial to their subterranean radicular system that facilitates the oxidation of reduced nitrogen compounds and plants uptake. Nevertheless, continuous inflows of wastewater would lead to eutrophication, establishment of anoxic conditions in water and soil, and lessening of nutrient absorption of mangroves.

Keywords: municipal wastewater; mangrove biofilter; nutrients removal; functional response

1. Introduction

Coastal environment researchers have highlighted as an important strategy the use of natural or artificial wetland habitats as filters for improving water quality discharges [1–4]. The first studies on biofilters were carried out in Germany during the 1950s, although the studies on wetlands as biofilters were initiated by American researchers during the 1960s [5]. It was not until 1990s that assessments of wetlands as biofilters, with high efficiency as pollutant removers and benefits for the economy, became more common [6].

Gersberg et al. [7] compared artificially the nutrient removal efficiency of the cattail (*Typha* sp.)—a common wetland plant—and the reed perennial grass (*Phragmites* sp.) using sand beds filled with domestic residual water. He reported a high removal rate of nitrogen compounds from freshwater in both cases, but *Typha* sp. was the most efficient converter, as it showed a higher biomass production of roots. Martínez-Cruz et al. [8] made artificial wetland habitats with *Scirpus americanus* (enrooted macrophytes) and *Lemna gibba*—an aquatic floating plant. In both cases, they obtained significant differences in nutrient removal rates compared with their controls. Habitats with *S. americanus* removed a total of 89%, 86% and 38% of nitrites, ammonia nitrogen and reactive phosphorous from water, respectively, whereas bed filters of *L. gibba* removed 90%, 80%, and 50%, respectively. Consequently, Huang et al. [9] stated that mangrove forests could be considered a tolerant group of plants for wastewater effluents due to their enormous nutrient demand. Apart from nutrient removal, Bayen [10] considers that mangroves play a key role in removing and/or degrading pollutants, such as heavy metals and pesticides from estuaries and coastal lagoons.

Robertson et al. [11] used mangrove species in artificial wetland habitats as biofilters for residual water discharges; they compared the nutrient removal rates (N and P) against a natural forest of *Rhizophora mangle*. From their results, they inferred that one-hectare of a semi-intensive or intensive artificial system has the equivalent nutrient removal efficiency of two to twenty hectares of natural forest. In natural environments, Wong et al. [12] used two intertidal sampling locations of *Kandelia candel* and *Aegiceras corniculatum* trees in order to test the removal rate of organic carbon and nitrogen; they concluded that these species displayed great biofilter potential as they removed up to 80% of the former compounds which in turn was reflected in biomass and basal area increments. Boonsong et al. [13] compared and analyzed phosphates removal rates ($PO_4{}^{-3}$ and total P) of a mangrove plantation of *Rhizophora* sp., *Avicennia marina*, *Bruguiera cylindrica* and *Ceriops tagal* with a natural mangrove forest receiving residual waters, and found higher values of removal rates than natural forest, therefore concluding that mangrove plantations are ideal biofilters for treating urban wastewaters. Due to this evidence, considerable attention has been directed towards the use of artificially constructed mangrove systems, as they provide an alternative low maintenance, cost-efficient and relative simple method for removal of nutrients and runoff pollutants from water [9,14–16].

The biofilter capacity of mangroves seedlings has also been tested and it seems that this capacity is affected by paramount environmental variables. Wu et al. [17] demonstrated that seedlings of *Aegiceras corniculatum* could remove high amounts of dissolved organic carbon, inorganic and organic ammonia (91%, 98% and 78% respectively), originating from residual water discharges of local areas when salinity was down to zero; however, when salinity was about 30 PSU, the removal efficiency was reduced to 71%, 83% and 56%, respectively. Wu et al. [18] also tested seedlings of *Kandelia candel* in controlled conditions and simulated tidal regimes as nutrient removers of Hong Kong municipal waters. They obtained removal rates of 70.4–76.4%, 75.1–79.1%, 76.2–91.8%, and 47.9–63.4%, of dissolved organic carbon, particulate organic carbon, dissolved inorganic ammonia and total nitrogen, respectively, probably achieving the closest approximations of natural environments removal rates [18]. Apparently, mangrove seedlings are also good removers of heavy metals as proved by Zhang et al. [19], who, using a simulated wetland of *Sonneratia apetala*, reported an individual removal rate of over 90% of total nitrogen, total phosphorus, copper, zinc, lead and cadmium; final biomass in that system displayed a linear positive correlation with the removal rate of the former elements. Moroyoqui-Rojo et al. [20] used an experimental silvofishery system planted independently with seedlings of *Rhizophora mangle* and *Laguncularia racemosa* imitating effluent conditions of aquaculture systems, and found that both plants were good nitrogen removers, while phosphorus removal was not significant.

It is largely known that hydroperiod, redox potential, salinity and temperature influence mangroves' physiological responses, and that they therefore may affect its removal capacity [21]. Consequently, we aimed to evaluate the nutrient removal rate and growth responses of *Rhizophora mangle* seedlings when submitted to successive wastewater discharges under controlled conditions and simulated tidal regimes. The variation of environmental cues was monitored during the duration of the experiment. Then, the specific response variables assessed were removal rate of nitrogen compounds NH_4^+, NO_2^-, NO_3^-, and phosphorous PO_4^{-3}, and growth in terms of height, diameter and above-ground biomass.

2. Materials and Methods

2.1. Facilities and Environment Emulation

2.1.1. System Built-Up and Stabilization

Six concrete tanks of 607.5 L capacity were constructed inside of a greenhouse of 2 m high occupying an area of 51.7 m^2. Hydraulic systems were individually fitted for each tank to habilitate the emulation of the local tidal regime: high tide by gravity and low tide by pumping (Figure 1). Shut-off valves and $\frac{3}{4}$″ horse power pumps connected to PVC tubes were installed to control speed and direction of circulating water.

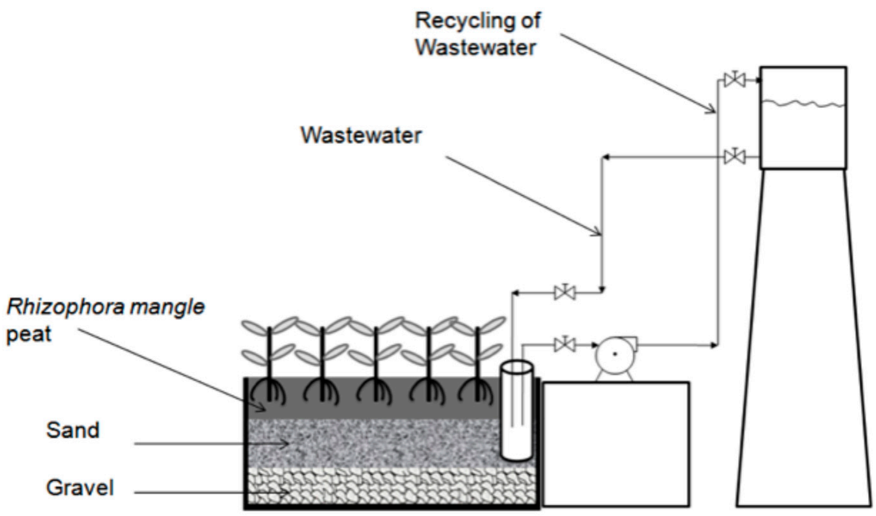

Figure 1. Diagram of the treatment system on secondary urban residual waste waters with subsurface recirculation.

Temperature was fixed at 26 ± 3 °C, with two air conditioners systems of 12,000 BTU each (English power units) and ventilators. Light incidence was ~50% reduced with a shadow mesh fixed at certain height that allowed air circulation avoiding the built-up of high temperatures inside the facilities. Filters in each tank were built from top to bottom with 0.11 m^3 of mangrove peat, 0.08 m^3 of sand and 0.08 m^3 of gravel. The mangrove peat was composed of 42% sand, 55% lime and 3% clay, which correspond to the soil texture category of silt loam, with sand grains and low content of clay. Other conditions, such as topography (ground leveling), frequency of flooding, as well as interstitial water salinity (26 ± 3 PSU) and redox potential (−195.9 ± 84 mV, oxygenic environment) were set to emulate the plant collection site, following historical records of Agraz-Hernández et al. [22]. Salinity adjustments were performed following the criteria of Orozco [23]. During a 40 d period, water parameters were monitored and afforestation was performed, ensuring that conditions were similar to the natural environment (Figure 2).

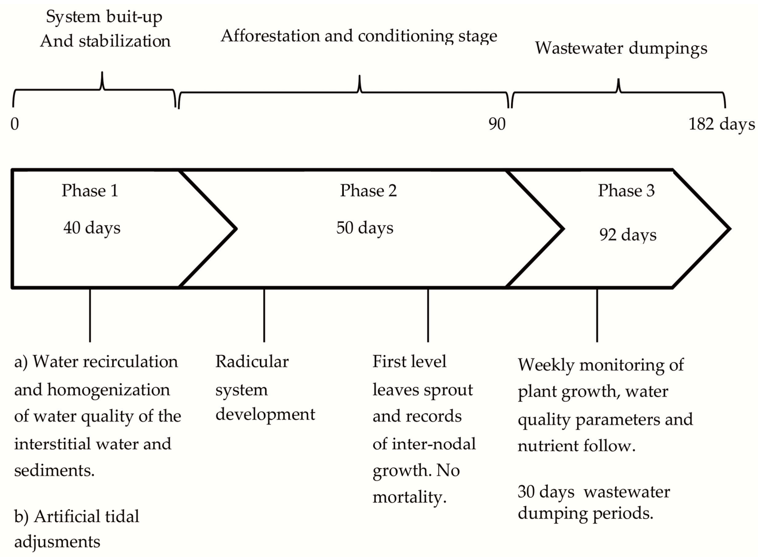

Figure 2. Experiment timeline events.

2.1.2. Afforestation and Conditioning Stage

Hypocotyls of *R. mangle* were collected from a monospecific forest located in Laguna de Terminos (18°42′378′′ N–91°37′621′′ W), a natural protected area in Campeche State, Gulf of Mexico. Hypocotyls were selected based in their good condition appearance. Any hypocotyls with signs of plague, drying or damage were culled. Hypocotyls were planted with a density of 100 per tank. During a 50 d period, the six tanks were observed for root system development and first sprout leave (Figure 2). During this stage, physico-chemical data were collected and tested by Multiple analysis of variance (MANOVA) considering data of individual tanks for comparison to each other. No significant differences were found ($p > 0.05$, Table 1). Internodal growth was recorded from all tanks and no plant mortality was detected at this stage. We assumed that homogeneous conditions were already set that would prompt nutrient absorption by the plant seedlings.

Table 1. Multiple analysis of variance (MANOVA) output of physicochemical parameters of pore water tanks during the afforestation and conditioning stage with *Rhizophora mangle* (Phase 2) ($p < 0.05$).

Physicoquimical Parameters	Criterion	Test Stadistic	*F* *	df Numerator	df Denominator	*p*-Values
pH, redox potential, temperature	Wilks	0.99495	0.024	3	14	0.995
	Lawley–Hotelling	0.00507	0.024	3	14	0.995
	Pillai's	0.00505	0.024	3	14	0.995
	* The values of s = 1, m = 1, n = 6					
PO_4^{-3}, NO_2^-, NO_3^-, NH_4^+	Wilks	0.79522	0.837	4	13	0.526
	Lawley–Hotelling	0.25751	0.837	4	13	0.526
	Pillai's	0.20478	0.837	4	13	0.526
	* The values of s = 1, m = 1.0, n = 5.5					

2.1.3. Experimental Discharges

The six-tank system was divided into two experimental groups; three tanks were submitted to ~30-day periods of wastewater discharges (experimental group) and the other three were kept only on marine water (control group). At the end of every period, waters were completely drained and replaced with new marine-water/wastewater. With the aid of a refractometer and a multiparametric data logger (HACH-HQ11d) salinity, dissolved oxygen and redox potential in pore-water were closely monitored.

2.2. Response Variables Follow-Up

2.2.1. Nutrients Removal Efficiency

Duplicated water samples during the experimental discharges stage were withdrawn from every tank on a weekly basis. A one-inch PVC tube was used to collect the water. These tubes were vertically inserted in each tank and water was collected with the aid of manual pumps. Water was kept in pre-washed (5% HCl^- and rinsed with DDH_2O) plastic containers. One drop of Phenol was added to one of the containers for ammonia testing. At the laboratory, water samples were put in cold storage (4 °C) until processing. All samples were processed within 2.5-month maximum. Processing consisted of letting the water samples warm-up to room temperature and filtering (47-mm GF/A micro-fiberglass filters) before being analyzed by ionic chromatography. Every chemical analysis was performed by duplicate. Dissolved inorganic nitrogen (DIN)—the sum of all concentrations of nitrite, nitrate and ammonia—was calculated as the first step for nutrient removal efficiency assessment, following the formula of Paniagua-Michel et al. [24]:

$$\%\mathrm{R} = \frac{(CE - CS)}{CE} \times 100, \quad (1)$$

where %R = nutrient removal efficiency; CE = inlet concentration of the effluent; and CS = outlet concentration of the effluent. Individual nutrient removal efficiency was also calculated by the former method.

2.2.2. Growth Responses

Growth responses from day 7 after every water discharge were weekly recorded. Twenty-five seedlings were randomly measured from each tank; internodal distance, stem diameter and total height were Vernier measured (0.02 mm digital precision) and recorded [25]. Also in a weekly fashion, two plants were extracted from every tank to measure above-ground biomass production. Stem and leaves of these plants were dehydrated in a convection oven for three days at 60 °C (Agraz-Hernández et al. [26]). A total of 324 samples were processed in this manner.

2.3. Statistical Analyses

Multiple variables variance analysis (MANOVA, $\alpha = 0.05$) were applied to the morphometric data (diameter increase and height growth rate of the seedlings), and to the physicochemical variables of the interstitial water (pH, redox potential, temperature and nutrients) collected between spills within and between treatments. Biomass data was tested at a significant level of $\alpha = 0.10$. All data were normalized [27]; square-root transformation was performed for proportional data departing from the normal distribution [28]. In addition, a linear regression analysis was carried out to determine the effect of the experimental treatment on the height and diameter growth rate of the seedlings. All statistical analyses were carried out using STATISTICA V.12 (©Copyright StatSoft, Inc., Palo Alto, CA, USA, 1984–2014) and SPSS 15.0 software for Windows (Copyright © 2006 of SPSS Inc., Chicago, IL, USA), and procedures criteria followed by Zar [28].

3. Results

3.1. Water Quality before and during the Wastewater Discharges

During the phase of afforestation and seedlings conditioning (Figure 1, 50 d), there was no significant variation of water quality parameters and nutrient concentration (Table 1; $p > 0.05$). Interstitial water maintained a slight alkaline (7.6 ± 0.1) and oxic condition (−60.9 ± 5.9 mV); temperatures ranged from (23.8 ± 0.3). Seedlings survival during this phase was 100%.

Water of both control and treated units during wastewater discharges displayed a neutral pH with a slight tendency towards alkalinity (6.9 ± 0.1 a 7.6 ± 0.1) and temperatures in the range of

23.5 ± 0.3 to 26.1 ± 1.1 °C. Redox potential of the interstitial water remained rather homogeneous and oxic (−60.9 ± 5.9 a 64.3 ± 7.5 mV) with no significant differences between treatments (Table 2, $p > 0.05$).

Table 2. Multiple analysis of variance (MANOVA), with a two-level factor, artificial wetland habitats with seawater (control) and urban residual wastes applied to physicochemical parameters of the interstitial water (Phase 3) ($p < 0.05$).

Physicoquimical Parameters	Criterion	Statistical Test	*F* *	df Numerator	df Denominator	*p*-Value
pH, redox potential, temperature	Wilks	0.85141	2.676	3	46	0.058
	Lawley–Hotelling	0.17453	2.676	3	46	0.058
	Pillai's	0.14859	2.676	3	46	0.058
	* The values of s = 1, m = 0.5, n = 22.0					
PO_4^{-3}, NO_2^-, NO_3^-, NH_4^+	Wilks	0.62140	7.464	4	49	0.0001
	Lawley–Hotelling	0.60927	7.464	4	49	0.0001
	Pillai's	0.37860	7.464	4	49	0.0001
	* The values of s = 1, m = 1.0, n = 23.5					

3.2. Nutrients of the Interstitial Water

Towards the end of the experiment (112 d) (right after and during the third wastewater dumping), increments in oxidized nitrogen concentrations were 2.6-fold in treated tanks (7710 μg L^{-1}) with respect to control tanks (2890 μg L^{-1}), while phosphorous (P-PO_4^{-3}) concentrations were 1.6-fold (250 ± 130 μgL^{-1}/150 ± 130 μg L^{-1}) coincidentally (Tables 3 and 4).

Ionized ammonia (NH_4^+) concentrations were similar when measured at the water outlet of treated/untreated tanks after the third wastewater spill (112 d); no significant differences were found ($p > 0.05$). However, significant differences were detected between treated/untreated tanks when ionized ammonia concentrations were measured between spills ($p < 0.01$). Lower concentrations of ammonia were registered after the second spill ($p > 0.05$; Tables 3 and 4).

Phosphate concentrations were significantly different between the first and second dumping; concentrations decreased in 87.1% in the outlet water ($p < 0.05$; Table 2), although phosphate recovered after the third dumping as concentrations were very similar to the first spill (Table 3). Nitrates increased 3.6-fold between the first and third spill although concentrations of nitrites kept homogeneous during wastewater spills (Table 3). No significant differences were found between nutrient concentrations in the interstitial water of the tanks of the control group ($p > 0.05$; Table 5).

Table 3. Nutrient concentration of interstitial water of tanks with *Rhizophora mangle* seedlings exposed to intentional periodic wastewater dumping.

Dumpings	Exposure (Days)	Concentration Right after the Spill	Concentration at System Outlet	Concentration Right after the Spill	Concentration at System Outlet	Concentration Right after the Spill	Concentration at System Outlet	Concentration Right after the Spill	Concentration at System Outlet
		NO_2^- (μgL^{-1})/± sd		NO_3^- (μgL^{-1})/± sd		PO_4^{3-} (μgL^{-1})/± sd		NH_4^+(μgL^{-1})/± sd	
First	30	2080 ± 70	1210 ± 50	5800 ± 100	1830 ± 120	1470 ± 190	310 ± 80	620 ± 0	20 ± 0
Second	31	4110 ± 40	1190 ± 30	7230 ± 920	3140 ± 160	1720 ± 30	40 ± 100	840 ± 50	14 ± 0
Third	31	3990 ± 80	1200 ± 50	10,640 ± 4150	6510 ± 300	1380 ± 150	250 ± 130	434 ± 20	21 ± 0

* sd = Standard deviation.

Table 4. Nutrient concentration of interstitial water of tanks with *Rhizophora mangle* seedlings with marine water (control).

Dumpings	Days after	NO_2^- (μgL^{-1})/± sd	NO_3^- (μgL^{-1})/± sd	PO_4^{3-} (μgL^{-1})/± sd	NH_4^+ (μgL^{-1})/± sd
First	30	1110 ± 50	1820 ± 120	170 ± 80	20 ± 0
Second	31	1190 ± 30	1850 ± 160	130 ± 100	10 ± 0
Third	31	1040 ± 50	1850 ± 300	150 ± 130	20 ± 0

* sd = Standard deviation.

Table 5. Multiple analysis of variance (MANOVA), with a two level factor, artificial wetland habitats with seawater (control) during a 112 d period (Phase 2, 3) ($p > 0.05$).

Nutrients	Criterion	Test Stadístic	*F* *	df Numerator	df Denominator	*p*-Value
PO_4^{-3}, NO_2^-, NO_3^-, NH_4^+	Wilks	0.7354	1.578	8	76	0.146
	Lawley–Hotelling	0.35078	1.622	8	76	0.133
	Pillai’s	0.2712	1.530	8	76	0.161

* The values of s = 2, m = 0.5, n = 18.0

3.3. Removal Rate of Dissolved Nutrients

DIN removal rate in the treated tanks during the first and second spill was similar (63.96% and 64.36%, respectively), but there was a decrease in the removal capacity during the third spill (48.71%) (Figure 3). Ammonia and phosphates were removed in higher rates than nitrite and nitrates (Figure 3). Ammonia and phosphate total removal rates were 96.12% and 78.98% at the end of the first dumping period; by the end of the second period, removal rates slightly increased for ammonia (98.35%) with a larger increase of phosphate clearance (97.68%) (Figure 3). Finally, during the third spill removal capacity decreased as ammonia was 91.15% and for phosphate was 81.83 (Figure 3). On the other hand, nutrient removal efficiency increased from the first (41.83%) to the second spill (71.05%) and slightly decreased (69.92%) towards the third spill. Nitrate removal efficiency followed a rather opposite trend, decreasing from the first to the second and third spill (68.46%; 56.57% and 38.88% respectively) (Figure 3).

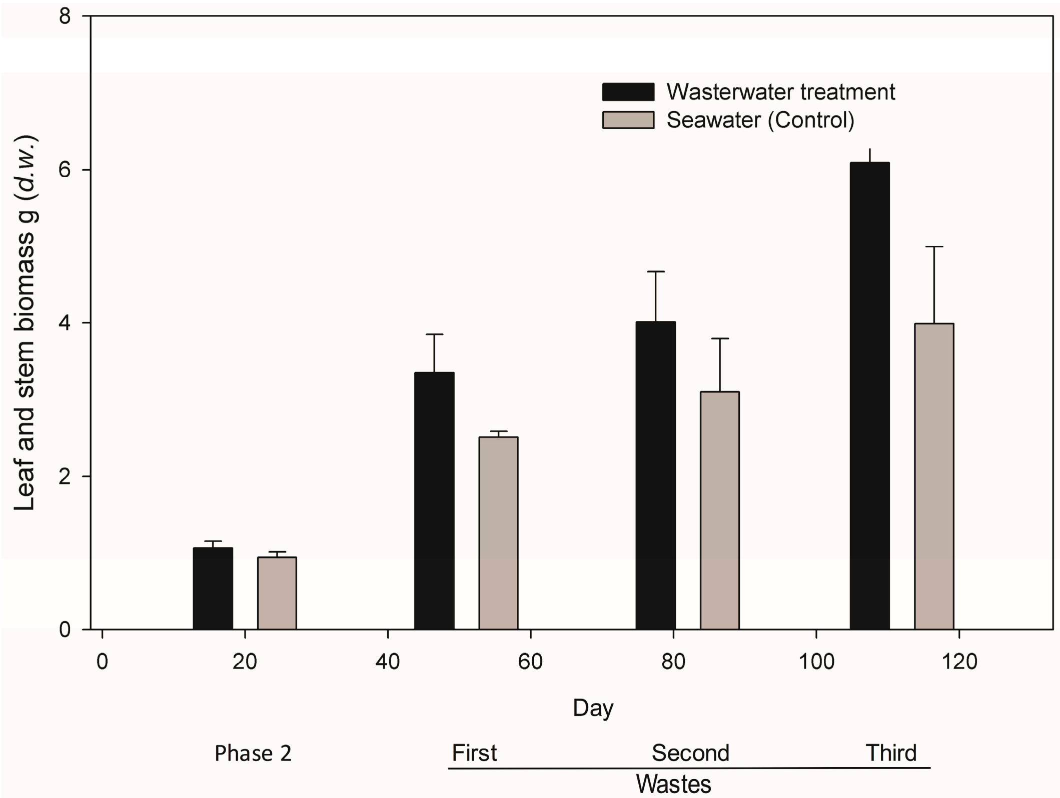

Figure 3. Efficiency of nutrient removal (nitrogen compounds and phosphates) in the interstitial water alters the first, second, third, waste of secondary urban residual water treated Phase II: Afforestation and conditioning stage.

3.4. Growth Responses

Single linear regression analysis defined the growth in height of treated and control plants with high concordance (R^2 = 0.99, p = 0.003 and R^2 = 0.96, p = 0.016, Figure 4a).

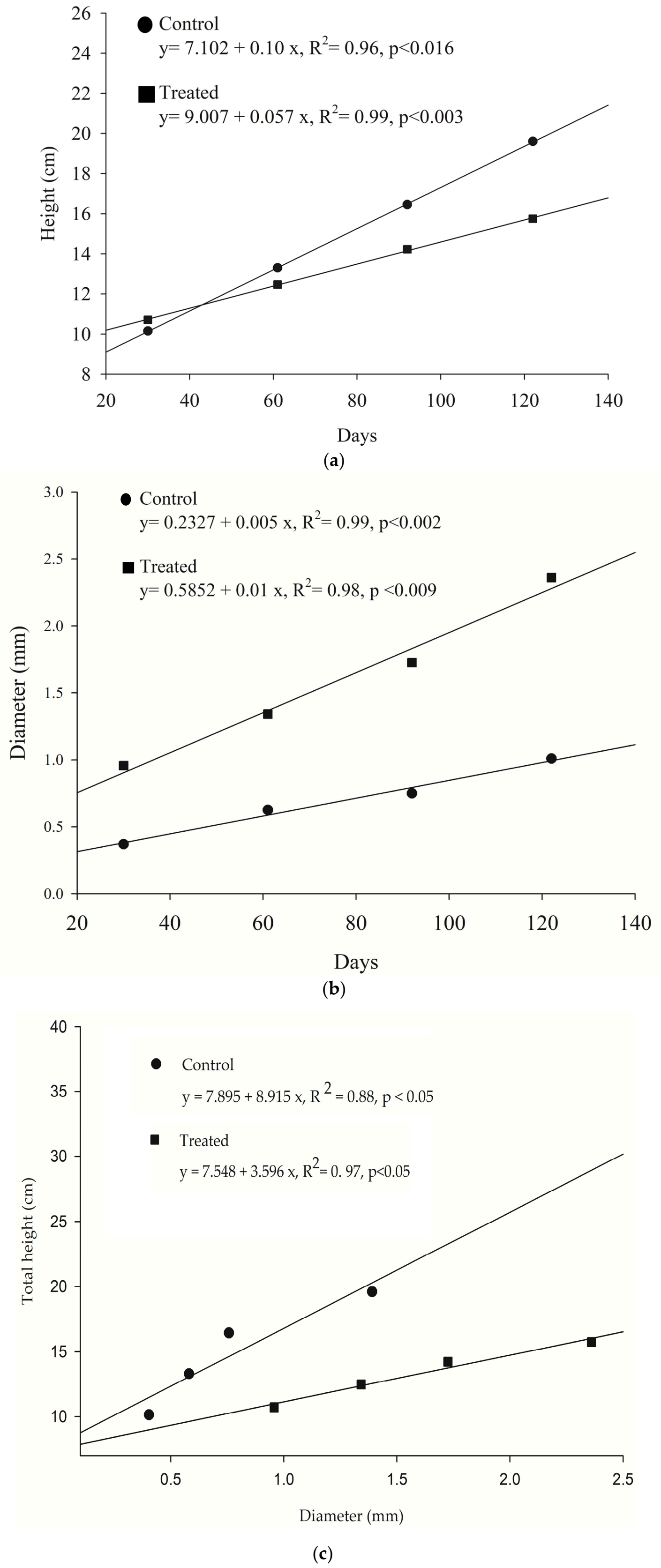

Figure 4. (**a**) Relationship between height and the time in treated plants and control; (**b**) Relationship between diameter and the time in treated plants and control; (**c**) Allometric relationship between height and diameter in treated plants and control.

Growth in height of treated plants was significantly inferior (15.8 ± 3.6 cm) when compared to the control group ((19.6 ± 5.5 cm) (Figure 4a). Consequently, height growth rate of untreated plants was 1.4-fold higher and significantly different than plants exposed to wastewater (0.10 y 0.067 cm day^{-1}, respectively ($p < 0.05$, Table 6). Girth growth linear regression analysis for control and treated plants were established at $R^2 = 0.99$, $p = 0.002$ and $R^2 = 0.98$, $p = 0.009$, respectively (Figure 4b).

Diameter growth of treated plants (2.36 ± 1.3 mm) at 112 d was two-fold and significantly different ($p < 0.05$, Table 6) against control group plants (1.39 ± 0.63 mm).

Lastly, single linear regression analysis defined that the type of growth either for the treated or control mangrove seedlings is allometric ($R^2 = 0.97$, $p < 0.05$ y $R^2 = 0.88$, $p < 0.05$, Figure 4c).

Significant differences were detected in biomass production between treated and untreated seedlings ($p < 0.10$). Plants exposed to wastewater spills displayed a biomass increment up to 45% between the first and third dumping (Figure 5), while the biomass increment for the same period of the control groups was about 37%.

Treated plants produced 25% leaves–stem biomass between the first and second spill and incremented to 35% from the second to the third dumping (Figure 5). Biomass increments for the control plants were only 19% and 22% respectively.

Table 6. Multiple variance analysis (MANOVA), with one-factor and two levels (control and treated) for the morphometric behavior of seedlings of *R. mangle* grown in treated artificial wetland habitats with urban residual wastes and control (only with sea water) ($p < 0.05$).

Morphometric Parameter	Criteria	Statistical Test	*F* *	df Numerator	df Denominator	*p*-Value
Diameter (mm), Growth height (cm)	Wilks	0.75918	11.023	4	139	0.0001
	Lawley–Hotelling	0.31720	11.023	4	139	0.0001
	Pillai's	0.24082	11.023	4	139	0.0001

* The value of s = 1, m = 1, n = 68.0

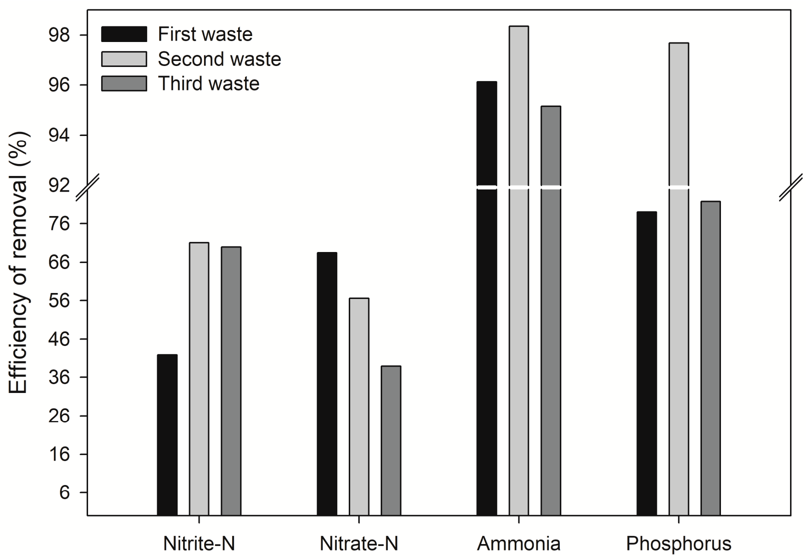

Figure 5. Above-ground biomass productions of seedlings of *Rhizophora mangle* in the wastewater treatment and seawater (control) artificial wetland habitats. See text for further details.

4. Discussion

The percentages of nutrients removal by *R. mangle* seedlings tested here under controlled conditions are very close to the removal capacity displayed by seedlings of other mangrove species, such as *Aegiceras corniculatum* at salinity of 30 PSU [17] (Table 7), and by seedlings of *Kandelia candel*

(2.1 PSU) [18], even though the plants of this study were maintained at a salinity of 26 PSU (Table 7). Moreover, seedlings of another mangrove species, *Sonneratia apetala* removes phosphate at the same magnitude as *R. mangle* [19]. Boonsong et al. [13] consider that mangroves of the *Rhizophora* genus besides other species are good biofilters with high removal rate of P-PO_4^{-3}, as part of their nutrient absorption metabolism needed for growth [29] observation that corresponds with the results obtained here.

Table 7. Comparison of nutrient removal capacity between seedlings plants of three species under controlled conditions.

Seedling	NID (%)	N-NH_4^+ (%)	P-PO_4^{-3} (%)	Reference
Rhizophora mangle	59.0 ± 9	96.5 ± 1.6	86.2 ± 10	present study
R. mangle (Firt dumpings)	63.96	96.12	78.98	present study
R. mangle (Second dumpings)	64.36	98.35	97.68	present study
R. mangle (Third dumpings)	48.71	95.15	81.83	present study
Aegiceras corniculatum	56.0	83	98.8	Wu et al. 2008a
Kandelia candel	63.34	91.8	97.0	Wu et al. 2008b

Rhizophora mangle seems to be a more efficient remover of phosphate and N-ammonia (Table 7) under oxic conditions when compared to lower removal values (below 50% and 65%) reported by Feller et al. [30] and Yi Ming et al. [31] achieved when seedlings thrive through under reduced conditions. In aerobic conditions (i.e., higher redox potential) nutrient removal rate is increased during the nitrification process, as consequence of the concomitant activity of the microbial community that oxidizes ammonia and free nitrogen [32,33], thus favoring nitrogen removal from the system.

Ideally, artificially constructed mangrove forests for waste treatment require tidal regimes (low and high tide) in order to generate aerobic and anaerobic conditions in the sediments which promotes nitrification and denitrification processes and facilitation of ammonia removal [34]. Ammonia is oxidized to NO_2 and then to NO_3 if oxic conditions prevail. However, there are other pathways for which nitrogen compounds are removed under oxic conditions, such as ammonification, ammonia volatilization, nitrification and plants absorption and adsorption processes [35]. In the absence of a tidal regime, reduced conditions would result in the sediments of artificial mangrove forests under wastewater dumping, with the consequent nitrogen built-up [36].

Results of the present paper also favor the notion that phosphates are more efficiently removed if oxic conditions are present, as phosphate removal was higher than reported from similar experiments in reduced conditions [30,31]. Phosphates are dissolved and retained in the uppermost surface (water–sediment interphase) in oxic conditions, allowing its solubilizing by bacteria associated to the root system, which is a necessary step before plant phosphates assimilation [19,37,38]. Furthermore, we consider that the nutrient removal capacity displayed by *R. mangle* seedlings is also associated to its ability for atmospheric oxygen transference from the aerial to the subterranean roots, facilitating the oxidation of reduced nitrogen compounds (i.e., ammonium-N), byproducts of bacteria nitrification. This mechanism supplies part of the nitrogen demands to the plants [17,39]. We noticed that removal rates of DIN and phosphates diminished from the first to the second and form the second to the third dumping (15.6% and 15.9%, respectively). This phenomenon in plants is known as luxury consumption, in other words, excess of nutrients in the immediate environment leads to sediment saturation tampering nutrient incorporation [29,40]. This negatively affects plants as has been extensively proved in crop yields and quality. Furthermore, the decreasing removal efficiency of nitrite and nitrate compounds could be attributed to natural reducing conditions occurring in the interstitial water as consequence of the combined effect of bacterial activity and organic matter oxidation. Reduction in the removal efficiency of nitrogen compounds of exposed mangroves over time were recently reported by [41]. Plant metabolism can also account for the nitrogen removal as they need this compound for their normal growth; in this experiment, biomass in the form of stem and leaves increased significantly between the first and second spill mainly. Similar results are reported for mangroves by Reef et al. (2010).

In the scenario where incoming wastewater continues, nutrient will built-up in sediments until saturation point. At this stage, surrounding waters will enter into eutrophication and the oxidation-reduction capacity in pore-water will turn the environment anoxic (redox potential < -300 mV). Other organic compounds proper to mangrove forests, such as methane, nitrous oxide, ammonia and sulphur are generated, which inhibit enzymatic activity and photosynthesis, situations that are not uncommon in mangroves [42]. Redox potential below -250 mV favors reduction of sulphates to hydrogen sulfide carbon dioxide and organic matter in sediments are reduced to methane (CH_4). All the former processes affect primary productivity. Tidal regimes associated to anoxic environment, such as places where residence times are high, would not allow oxidative processes in the water-sediment phase. About 20% the plants remove nutrient content and the 80% left is retained in the sediments [19]. Under constant nutrient inputs, saturation limit is achieved and plants lose their capacity of nutrient assimilation. We believe that biomass increment in leaves and stems observed in *R. mangle* seedlings of the treated group is the consequence of the absorption and assimilation of phosphates and ammonium-N made available between the first and third wastewater spillage. Plants incremented in 45% of their biomass; similar results have been reported for *Bruguiera gymnorrhyza* after incrementing its biomass before ammonia and nitrate rises [43]. *Sonneratia apetala* incremented its biomass after receiving inputs of municipal untreated waters with high concentrations of nitrogen and phosphorous [19]. It seems that during our experiment we unintentionally caused nutrient and saturation stress to our experimental subjects—*R. mangle* seedlings—since after the third spill, the nutrient removal rate and the growth rate (height) decreased significantly (29.4%). At this point, we must have achieved nutrient saturation in the interstitial water, propitiating the augmentation of reduced compounds [43].

Allometric positive growth between height and girth was verified to either seedlings growing in wastewater or marine water. However, the slope of the curve displayed a 46.9% decrease for the exposed plants ($R^2 = 0.97$, $p < 0.05$), when compared to the untreated group. It is known in plant science that growth increments do not maintain their initial proportions when exposed to constant nutrient uploads (the law of diminishing returns). Asymptotic growth is commonly achieved in this situation and growth stops despite having nutrient availability [44]. Primary production by the plants also stops; this effect is known as luxury consumption and it is believed that is caused by the imbalance of essential nutrients and toxic byproducts. Vaiphasa et al. [45] have also suggested that in these conditions, nutrients are accumulated in the aerial structures making the plants susceptible to water deficit [46].

5. Conclusions

Seedlings of *Rhizophora mangle* proved to possess a high nutrient removal capacity under oxic conditions. Oxic conditions occur during normal tidal regimes, in contrast to reported experiments where no tides were emulated. Nutrient excess, however, leads to sediment saturation, which decreases the removal capacity in the system. This scenario would occur if mangroves sustain wastewater dumping in a continuous manner, affecting foliar biomass yield and stem girth and height growth. As long as artificial mangrove forests are built in places exposed to normal tidal regimes, a good removal of nutrients form wastewater might be ensured. Further studies addressing the nutrient removal capacity of mangroves per species and number of individuals per afforestation area of impact are sorely needed.

Acknowledgments: This study was sponsored by The National Forest Commission of Mexico and The National Council for Research and Technology (CONAFOR; Comisión Nacional Forestal; CONACyT Consejo Nacional de Ciencia y Tecnología). We thank to Claudia Garcia Zaragoza, Roberto Arana Lezama, Manuel Rosado, Eleuterio Chan Canul, Xochil Rodríguez Muñoz, for their help during the monitoring.

Author Contributions: C.M.A.-H., R.E.d.R.-R., and C.A.C.-K. conceived of the study, and participated in its design and coordination and helped to draft the manuscript. R.E.d.R.-R., J.O.-S. and R.M.-S. advised throughout analyzed and interpreted data. C.M.A.-H., R.M.-S., J.O.-S., R.E.d.R.-R. and C.A.C.-K. collected, analyzed, and interpreted the data. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no competing of interest.

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