Mangrove-based pollution mitigation in Thailand and Sri Lanka: engineering and microbiological aspects

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Abstract

The article deals with an *on-going* research on highly organically loaded mangrove-based wetland used for the incidental treatment of un-separated piggery wastes or anaerobic pond-treated municipal wastewater. In general terms, these wetlands are seen as buffers between anthropogenic pollution sources and nature reserves conserving coastal ecosystems (*Avicennia* mangrove and mudflats). In particular, a small holding pig farm (Samut Prakan province, Thailand) and a medium size city (Negombo, Sri Lanka) were the pollution sources to be dealt with before the attenuated nutrient loads entered the Bang Pu Nature reserve and the Kadol Kele Nature Reserve, respectively. It was shown that the wetlands play an important role in saving the integrity of the valued ecosystems from a major pressure imposed in terms of C, N and P. The survey of microbial diversity (microalgae and bacteria) demonstrated that residual post-treatment nutrients discharged onto the mudflats enhanced the primary productivity of the mudflats. Fluorescence *In Situ* Hybridization was used for the first time to elucidate microbial mechanisms behind the effective nutrient removal. It is argued that municipal and agricultural nutrients, if monitored, can enhance agri- and aquacultural production by conserved mangrove ecosystems without compromising their ecological integrity.

Keywords: mangroves, nature conservation, piggery wastes, wastewater treatment

1. Introduction

Threats faced by the Asia's diverse wetlands include drainage and reclamation for industrial and urban expansion, conversion to abandoned ponds by prawn farming, pollution, river diversion and logging. As the wetlands constitute a resource of great economic, cultural, scientific and recreational value, the loss of which would be irreparable, they should be therefore preserved and rehabilitated. As the sustainable development is an accepted approach in nature conservation, linking human activities such as nature education, agriculture/aquaculture with pollution abatement and with nature conservation/bio-diversification in the concept of the multi-purpose use of ecosystems can be seen as a major way for the future development of the urban and peri-urban areas (Nanda *et al.,* 2005). Mangrove-based wetlands commonly found along the coastlines in tropical and subtropical regions, appear to have a high resistance to impacts of domestic and agricultural pollutants as well as other environmental parameters and can possibly be developed as natural waste treatment systems (Wong *et al.*, 1997; Ye *et al.*, 2001; Boonsong *et al.*, 2003; Brenda, 2004.). Important function of pollution mitigation is thereby coupled to enhancement of mangrove productivity since nutrients, especially N and P, are known to be deficient for plant growth in many mangroves (Ye *et al.*, 2001). However, no or little information exists on the efficiency of treatment of N-rich livestock wastewater, as well as on the mechanisms of nutrient (C, N, P) removal in mangrove ecosystems in general, where microbial consortia undoubtedly play a major role. Of particular importance is to establish upper loading limits. The study presented is the on-going research evaluating the impact of the highest (to date) loads of piggery wastes and municipal anaerobically pretreated wastewater on mangrove ecosystems composed mainly of *Avicennia alba* and *A. marina* (i), microbial (ii) and other mechanisms (iii) of C, N, P removal and potential beneficial effects (iv) of such treatment on the conserved ecosystems.

2. Site description and methodology

The study areas are located in Thailand and Sri Lanka. The Thai site in the coastal Samut Prakarn province within the boundaries of Greater Bangkok, Bang Pu Nature Reserve, was a focal point. A popular destination for recreation/education of inhabitants of Bangkok, it has been recently declared the nature reserve and is under the aegis of WWF and the Royal Thai Army as a significant mangrove-dominated ecosystem. Situated in the recognize Priority Corridor within the Indochina Biodiversity Hotspot with a high urgency for conservation, the Reserve currently serves as a conservational and educational facility for thousands of school students, as well as Bangkok public at large. Though the Royal Thai Army and WWF patronize the Bang Pu Nature Reserve as an internationally important conservation spot and educational hub, it is still plagued by a number of serious problems, mainly due to its location in the urban area. The experimental (polluted) site I is situated in the northern corner of the Reserve (active zone S = 20,000 m^2). The small holding pig farm

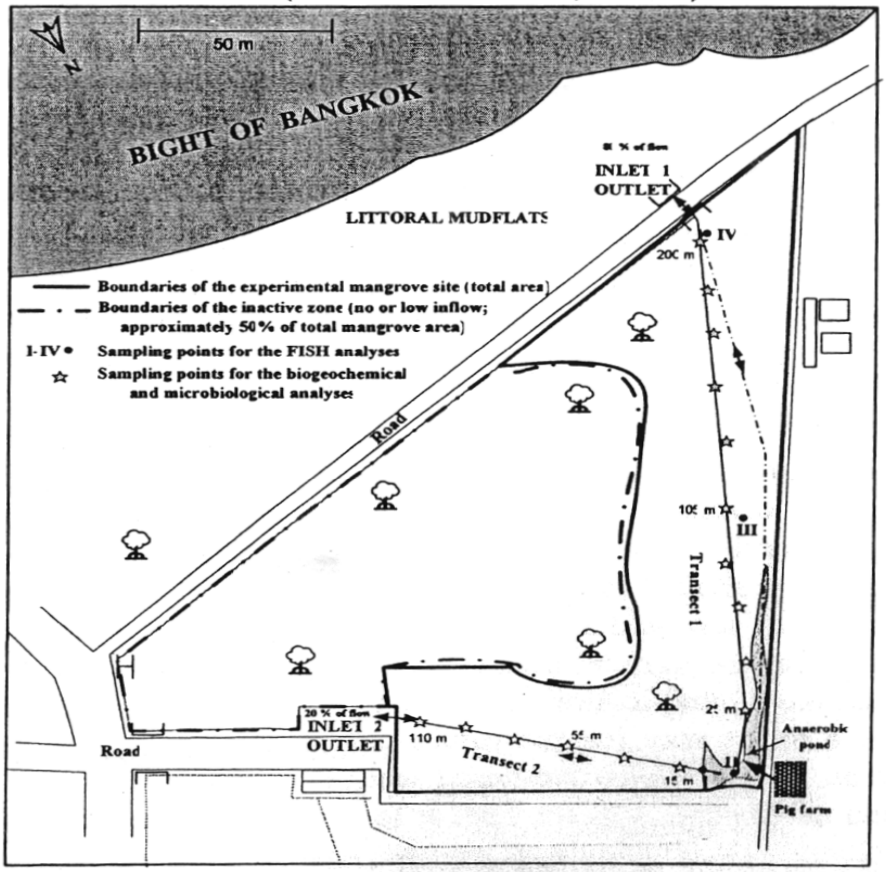

Fig.1. Layout of the experimental site treating piggery wastes of the small holding pig farm adjacent to and discharging into the Bang Pu Nature Reserve (Thailand). Site 1.

situated northwest of the site 1 contained from 50 to 95 pigs (Fig. 1). The un-separated wastes were discharged from the farm into the site at the C, N and P load of 67.2, 16.4, 6.9 kg/d, respectively. The characteristics of the wastes are presented in Table 1. Two transects with sampling points at regular intervals were selected. The natural site approx 1 km east within the boundaries of the Reserve was selected as a control to match the experimental site in terms of hydrology and size. The site 2 is a mangrove area of the Kadol Kele Nature Reserve (total area 20,000 m^2) receiving municipal wastewater at the active treatment zone 10,000m^2. The characteristics of the piggery wastewater and water in the sites were analyzed according to the Standard Methods (APHA, 1995). The Shannon-Weiner Diversity Index use for the calculation of micro alga diversity in the selected sites: $H' = -\sum p_i \log p_i$, where, p_i -proportion of total sample belonging to i^{th} category or species in community. Sampling for the FISH analysis of the microbial community structures was undertaken monthly. Due to a heterogeneous nature of the mangroves in which microorganisms are attached to a variety of surfaces, aerial mangrove root biofilms were used to represent diverse micro-habitats. Samples of biofilm (II-IV, Fig. 2) were proportionately averaged by homogenization to ensure that the representative microbial communities are reported. FISH technique data was generated according to the protocols described by Neef *et al.* (1996). Conventional Olympus microscope (Japan) with a reflected light fluorescence attachment was used. All FISH counts for 10 specific probes were expressed as percentages of EUB338 probe-related counts (all *Eubacteria*).

3. Results and Discussion

Table 2 shows the loading rates and removal efficiency of the sites 1 and 2 in the light of the previously studied mangroves, demonstrating that the loads applied and removals achieved by far exceed those reported elsewhere. Table 3 reports the balance of waste-related C, N and P in the experimental mangrove site 1, indicating an important role by plants and soil (microbial consortia). As evident from the data presented in Fig. 3, the main attenuation of C, N and P load occurs within first 50 meters from the discharge point (pig house) wile the anaerobic pond at the beginning of the transects plays a crucial role. On the other hand, there exists an intrinsic variability of mangroves' treatment efficiency depending on the mangrove species, soil type, hydraulic regime, *etc.,* (Brenda, 2004) showed that the mangrove root system facilitated the growth and development of bacteria that removed large amounts of nutrients by biological degradation. While the removal of carbonaceous and nitrogenous matter in mangrove wetlands appeared to be undertaken by microbial consortia, P removal is recognized to be mainly due to chemical absorption by soil mineral and organic matter.

The pioneering work on the FISH-generated microbial community structures of the anaerobic pond sludge and mangrove root surfaces (Fig. 2) showed a substantial presence of denitrifying and nitrifying bacteria involved in the effective mineralization of N-containing compounds of the discharged pig manure. It was previously found that the members of *Cytophaga-Flavobacterium* cluster were the most abundant group detected in the deep sea marine sediments (Glockner *et al*, 1999; Ishii *et al.,* 2004). However, the data contrasts with the prior observations that *Proteobacteria* (such as alpha, beta and gamma subgroups), *Planctomycetes* and *Actinomycetes* (HGC) were detected in low numbers. Abundance of microalgae and cyanobacteria common in mangroves (Kathiresan, 2000) and their primary productivity appears to correlate with loading rates (Table 4) with Shannon-Weiner diversity index being higher under pollution, which indicates adaptability of the ecosystem,

Table 1. Characteristics of un-separated piggery wastes discharged into the experimental mangrove site and water in the control natural mangrove site. Dry season 2006.

Parameter, mg l^{-1}	Piggery wastes discharged	Control (natural) site
pH	7.3 (6.5-7.3)	7.7 – 8.0
Conductivity, ms cm^{-1}	7.6 ± 1.2	19.5 ± 7.9
Salinity, g l^{-1}	6.5 ± 1.3	8.3 –20.3
TOC	30,058.0 ± 690.0	228.1 ± 24.5
TKN	2567 ± 461	20.2 ± 2.9
NH^{+}_{4}-N	804.3 ± 133.4	8.8 ± 6.4
P_{inorg}	1093.0 ± 192.3	16.1 ± 7.9

Table 2. Loading rates and removal efficiency of the currently studied mangrove-based wetlands treating piggery and municipal wastewater (n.a.- not available) in the light of the previously studied mangrove sites. Removals (%) are in brackets.

Site	Carbon (as TOC)	Nitrogen (as TN)	Phosphorus (as TP)
Royal Laem Phak Bia, Thailand **Settled municipal wastewater** **Loading rate, *g applied/m²/day*** **Removal efficiency, *g removed/m²/day*** **(Boonsong *et al*, 2003)**	0.67 0.34(51.1%)	0.32 0.15(50.4%)	0.1 0.05(28.3%)
Futian National Nature Reserve, Shenzhen, China **Settled municipal wastewater** **Loading rate, *g applied/m²/day*** **Removal efficiency, *g removed/m²/day*** **(Wong *et al*, 1997)**	0.14-0.44 0.07-0.22 (50.9%)	0.01-0.02 0.003-0.005 (27.1%)	0.008 0.006 (84.7%)
Pot greenhouse trials, Hong Kong **Piggery wastes** **Loading rate, *g applied/m²/day*** **Removal efficiency, *g removed/m²/day*** **(Ye *et al*, 2001)**	0.26 n.a.	0.14 0.13 (95.4%)	0.22 0.19 (92.9%)
Bang Pu Nature Reserve, Thailand **Un-separated pig wastes** **Loading rate, *g applied/m²/day*** **Removal efficiency, *g removed/m²/day***	Site 1 3.36 2.97(88.0%)	0.82 0.7(85.4%)	0.35 0.23(62.1[illegible]
Kadol Kele Nature Reserve, Sri Lanka **Anaerobic pond treated sewage** **Loading rate, *g applied/m²/day***	Site 2 1.8	0.45	0.15

Table 3. Balance of waste-related C, N and P in the experimental mangrove site (active zone S = 20,000 m^2). Dry season 2006.

LOAD PORTION	C (kg/d)	N (kg/d)	P (kg/d)
Total load onto the site	67.2	16.4	6.9
Flushed off into sea	6.9	2.2	2.9
Retained as	60.3	14.3	4.0
Plant-absorbed	*7.2*	*2.1*	*1.5*
Soil-removed	*53.1*	*12.2*	2.5
Load removal efficiency, %	88.0	85.4	62.1

Table 4. Abundance of microalgae and cyanobacteria in the experimental (highly loaded site) and the control (natural) site.

Group	Abundance and diversity			
	Polluted area (Experimental site)	%	Natural area (Control site)	%
Cyanobacteria (*Cyanophyceae)*	*Oscillatoria sp.*	14.9		
	Spirulina sp.	3.7		
	Microchatea sp.	1.8		
	Phytoconis sp.	7.4		
	Merismopedia sp.	1.1		
Bacillariophyceae (Diatoms)	*Cocconeis sp.*	5.6		
	Navicula sp.	5.5	*Navicula sp*	16.3
	Nitzschia sp.	6.0	*Nitzschia sp.*	12.5
	Plannularia sp.	4.2	*Plannularia sp.*	20.8
	Neidium sp.	4.5	*Neidium sp*	5.0
	Cymbella sp.	1.5	*Cymbella sp.*	15.1
	Gyrosigma sp.	19.3		
	Amphora sp.	2.0		
	Stichococcus sp.	1.1		
	Stauroneis sp.	1.9		
Chlorophyceae (Green algae)	*Chlorella sp.*	1.1	*Chlorella sp.*	15.6
	Eudorina sp.	0.7	*Eudorina sp.*	3.8
	Euglena sp.	1.9	*Euglena sp.*	2.5
	Staurastrum sp.	1.5	*Volvox sp.*	8.8
	Closterium sp.	0.9	*Pandorina sp.*	1.3
	Thalassiosira sp.	4.7		
	Skeletonema sp.	4.9		
Dynophyceae	*Ceratium sp.*	1.9		
Chlorophyll *a*, µg/l, Dry season (rainy season)	448.2 (435.9)		163.3 (90.8)	
Diversity Index (Shannon-Weiner) H'	1.8 ± 0.3		1.5 ± 0.2	

Table 5. Chlorophyll *a* concentration (µg/l) as an evidence of the enhanced primary productivity in the mudflats off the experimental site I (in comparison to the mudflats off the natural control site) due to the fertilizing effect of the residual nutrients originating from the site I.

Month	Mudflats off the site I	Mudflats off the natural site
August 2006	1572.5	230.0
October 2006	776.8	151.5

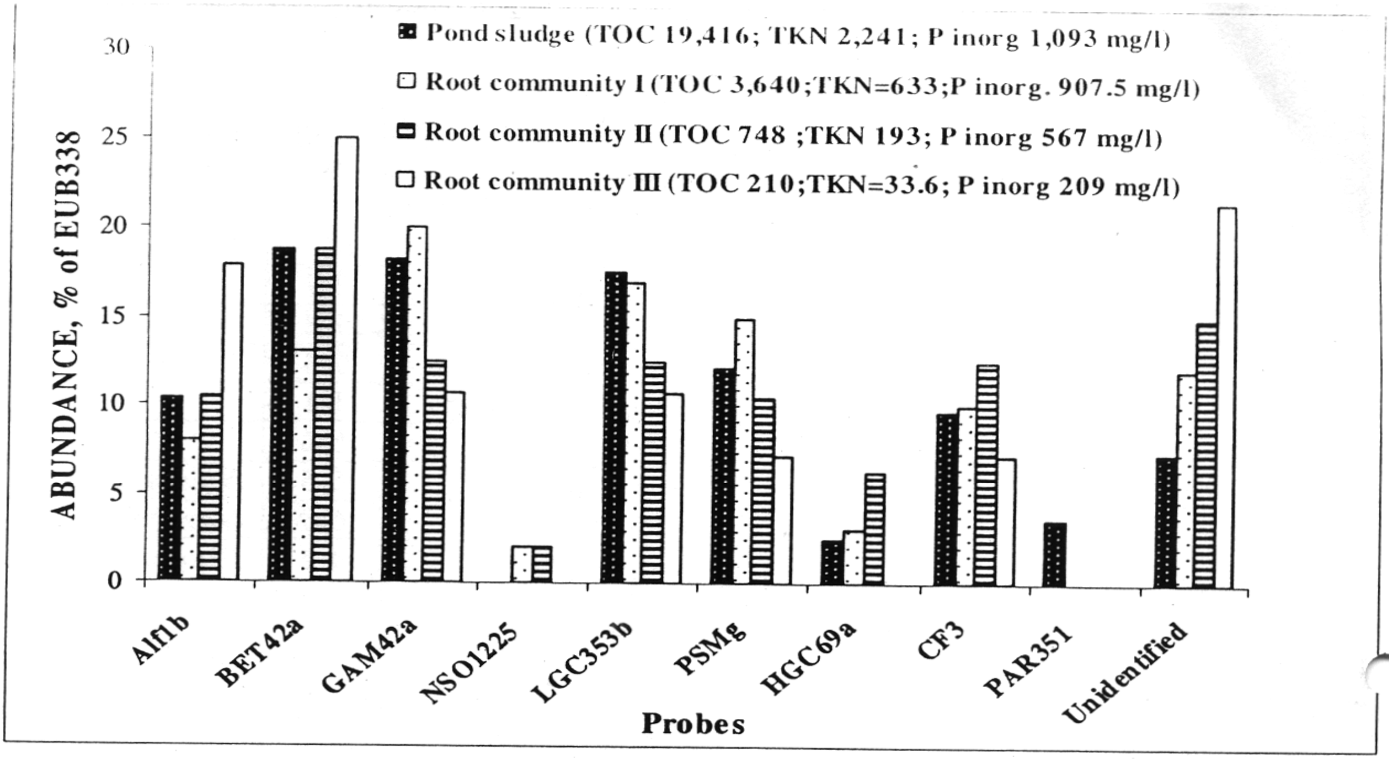

Fig 2. Microbial community structures of the anaerobic pond sludge and mangrove root surface at the sampling points I-IV (see Fig. 1). Ammonia-oxidizers (probe NSO1225); denitrifiers (probes PSMg and PAR351) were well represented.

at least at the microalgal level not losing its biodiversity. Elevated chlorophyll *a* concentrations (Table 5) were observed as a result of the enhanced primary productivity in the mudflats off the experimental site 1 in comparison to the mudflats off the natural control site demonstrating a fertilizing effect of the residual nutrients originating from the treatment site.

4. Conclusions

The data suggest that a viable, environmentally and socially sustainable approach is to apply the main pollution load on a relatively small area of the mangroves and the discharge the effluent, still containing safe amounts of residual C, N, P, into the main mangrove area with a beneficial impact in terms of enhancement of primary productivity and without compromising integrity of natural biodiversity. Since the newly-constructed mangrove systems may take too long to get established (Boonsong *et al.*, 2003), sequestering small size areas within the natural mangrove ecosystems may be justified for high intensity treatment in order to avoid impacting much larger areas. To the best of our knowledge there exists no available published information on the FISH-generated microbial structures of the mangroves, in particular, those treating wastewater. The pioneering data obtained pave the way to understanding the microbial mechanisms of treatment by mangroves used as vegetative buffers.

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