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POTENTIAL OF NITRIFICATION RATE IN SEDIMENTS OF GREY MANGROVE (*AVICENNIA MARINA*) LOCATED AT DIFFERENT STATIONS ALONG KARACHI COAST, PAKISTAN

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Abstract

Mangrove acts as a vital source/sink pool for biogeochemical processes (nitrogen cycle) in the ecosystem. Owing to the importance of nitrification process in mangrove sediments the present study was undertaken to assess seasonal variation in potential nitrification (PN) rate in the mangroves along the Karachi coast. Sediments samples were retrieved in triplicate from the rhizosphere at four sites during pre-monsoon, monsoon, and post-monsoon season and divided into the top, middle, and bottom (3 cm each) of the core. Overall, the potential nitrification rate was high in the post-monsoon season as compared to pre-monsoon season and monsoon season. In terms of sections of sediment cores, PN rate was high in the middle and bottom sections as compared to the top section. Among sites, at Sandspit and Korangi Creek, the highest PN rate was observed in the middle section of the sediment at 45mM and 15mM chlorate concentrations at 6 hours of incubation. However, at Manora Channel and Port Qasim, it was found high in the bottom section at 60mM and 15mM at 6 hrs. of incubation, respectively. A distinct seasonal variation in Physico-chemical variables including temperature, salinity, pH, and dissolved oxygen was observed at all four stations. In the case of nutrients ion, the highest value of NO_2 was observed in pre-monsoon, and NO_3 and PO_4 ions were in post-monsoon season at all stations. While the maximum value of NH_4 ion was recorded in pre-monsoon followed by post-monsoon and monsoon seasons. In conclusion, the present study revealed a strong potential nitrification phenomenon observed at all stations with a slight variation that may be due to the relevance of anthropogenic activities along the backwaters of the Karachi coast.

Key words: Anthropogenic, Mangrove ecosystem, Nutrients, Potential nitrification rate, Backwaters.

Introduction

Mangroves are evergreen forests and coastal wetlands mostly found between 25°N and 30°S latitude in the tropical and subtropical regions of the world (Kathiresan & Bingham, 2001). They occur in 118 countries of the world and covered approximately 137,760 km^2 areas of the world (Giri *et al*., 2010). About 60% of the world's population is living in coastal areas where mangrove forests play an important role in their livelihood and protection from natural disasters such as tsunamis, tropical cyclones, storms, erosion, and maintaining water quality (Nguyen, 2014). Mangrove forests are the most important source of timber, fuel-wood, and fodder for many grazing animals and they provide shelter and serve as breeding and nursery ground for a wide variety of organisms including fish and shellfish (Gayathre *et al*., 2021). The mangrove ecosystem acts as a vital source/sink pool and also plays a significant role in the biogeochemical processes, such as biogeochemical reactor, transforming and releasing nutrients in coastal and marine ecosystems (Zhao *et al*., 2021). The primary yield of the mangrove ecosystem is quite high as compared to other coastal ecosystems due to the occurrence of a distinctive microbial community (i.e. bacteria, cyanobacteria, fungi, diatoms, and other protozoans). Mangroves are nitrogen and phosphorus limited (Vazquez *et al*., 2000), but the proficient microbial transformation of detrital organics provides essential nutrients including numerous nitrogen, and thus maintains balance in the ecosystem (Yousaf *et al*., 2021).

Nitrogen is known to be the most constraining factor in the mangrove ecosystem (Reis *et al*., 2017). Dinitrogen fixation and denitrification are the most important processes which balance the nitrogen level in the marine sediments (An *et al*., 2021). The nitrogen transformation (nitrogen fixation and denitrification) in the mangrove ecosystem is mediated by microorganisms (Semblante *et al*., 2017). Where nitrogen fixation is done by diazotrophic bacteria and cyanobacteria whereas, denitrification is facilitated by heterotropic facultative anaerobes (Zhou *et al*., 2021). Generally, nitrification occurs under aerobic conditions (Reef *et al*., 2010) as well as under anaerobic conditions in the presence of manganese and iron (Reef *et al*., 2010). High organic carbon may decrease the nitrification process by transferring nitrogen from the nitrifiers to heterotrophs (Strauss & Lemberti, 2000). During denitrification microbes (ammonia and nitrite-oxidizing bacteria) reduce nitrate and nitrite to form gaseous nitrous oxide and nitrogen which are eliminated from the system (Inamori *et al*., 2008). The Nitrification process changes immobile ammonium nitrogen to mobile nitrate (Reef *et al*., 2010). Sediment comprises about 140-106 ammonium oxidizing bacteria per gram of soils, and $\leq 10^{-7}$ archaeal ammonia oxidizers per gram of sediment (Okano *et al*., 2004; Leininger *et al*., 2006). In the marine ecosystem, anaerobic ammonium oxidation (anammox) has significant importance in nitrogen transformation (Zheng *et al*., 2020) through oxidation of NH_4^+ to N_2 (Meyer *et al*., 2005), whereas in nitrogen cycle, nitrite (NO_2^-) is produced either by heterotrophic NO_3 reduction or nitrification by anammox (Francis *et al*., 2005). About 55% of the nitrogen is lost by denitrification and about 67% of nitrogen is removed by nitrification processes (Chiu *et al*., 2004). Decomposition and re-mineralization of the dead organic matter is another important source to increase nitrogen concentration in mangrove sediment (Carugati *et al*., 2018). This nitrogen released during the recycling process is eventually used by the phytoplankton to fulfill their nitrogen requirements. Potential nitrification is largely controlled by nutrients and oxygen levels in the soil (Yao *et al*., 2011) as well as temperature (Lu *et al*., 2020), Salinity (Chi *et al*., 2021), pH (Han *et al*., 2021), dissolved oxygen (Liu *et al*., 2019; Wu *et al*., 2021), the concentration of NH_4 (Triska *et al*., 1994), organic carbon and iron (Krishnan & Bharathi, 2009), moisture content, the texture of ediment (Wang *et al*., 2014) and C:N ratio (Gao *et al*., 2019).

According to Anon., (2007) report, the total extent of mangrove forest is about 157,000 hacter along the coast of Pakistan. Karachi is the largest city/ hub to other cities and is located on the coastal belt of Pakistan. It has two seaports namely; Karachi Port and Bin Qasim collectively handle more than 90 percent of all external trade in Pakistan. Sandspit (SP) backwaters are located approximately at 24º 49′ N and 66º 56′ E between Manora and Hawksbay. They receive seawater through Manora Channel (MC) which contains a considerable amount of domestic and industrial waste entering the mangrove forest through the Layari River (Ahmed *et al*., 2017). MC is adjacent to SP and extremely polluted because untreated effluents of industrial and domestic waste come through Layari which contains large quantities of untreated and semi-treated domestic wastes. The Layari River outfall waters mainly contain significant inorganic pollution in terms of nutrients, heavy metals, PCB's and PAH's etc. (Saher *et al*., 2019). A number of Cargo ships, fish trawlers, and other bots dumped their trash into the seawater as well as cleaning oil tankers and fish processing units at the harbor responsible for the discharge of solid waste and effluents. This backwater area of MC also receives untreated domestic wastes from five villages (Bhaba Island, Bhit Island, Shams Pir, and Kakka Pir) located at Manora Island. MC area is now considered to be the most heavily polluted marine site in Pakistan (Khan *et al*., 2015). On the other hand, Korangi creek (KC) is situated on the southeast coast of Karachi and depicts an environment which is also subjected to a type of anthropogenic stress. It receives industrial effluents (heavy metals) from Korangi and Landhi industrial area. In addition to these effluents, the untreated wastewater from domestic, agricultural pollutants is released into the creeks. Ultimately these all toxic pollutants reach the backwaters of mangrove areas which may pose serious and potential health hazards to fisheries (Farooq & Siddiqui, 2020). Various industrial areas, including chemical, textile, pharmaceutical, automobile, oil refineries, tanneries, and KE Bin Qasim power plant situated on the fringes of the main city located at Port Qasim (PQ) which is also a second port of Karachi (Amjad *et al*., 2007).

Apart from the significance of mangroves coastal belt of Karachi, is getting highly polluted and needs to address the issues, especially with reference to biogeochemical changes that occur in the mangrove ecosystem. Recently an effort has been made to understand the major contribution of the nitrification process in the biogeochemical cycle at Sandspit backwaters (Ahmed *et al*., 2017). Therefore, due to the importance of the nitrification process in mangrove sediments, the present study was undertaken to investigate the seasonal variation in the rate of potential nitrification in mangrove sediment occurring in other backwaters including Sandspit along the Karachi coast. Furthermore, the results of the present study will provide a comparative baseline for future research.

Materials and Methods

Study sites: Mangrove sediment samples were collected from four different locations i.e. Sandspit backwaters (SP; 24º.83'04" N, 66º.92'82"E), Manora Channel (MC; 24º.51'303" N, 66º.55'528"E), Port Qasim (PQ; 24º.51'305"N, 67º. 18'458"E and Korangi Creek (KC; 24º.46'99"N, 67º.23'83"E)) during 2018-2019 (Fig. 1).

Fig. 1. Maps showing four stations Sandspit (St1), Manora Channel (St 2), Port Qasim (St 3) and Korangi Creek (St 4) along Karachi coast.

Sampling: Sediments samples were retrieved in triplicate from the rhizosphere at each sites during three seasons, i.e. pre-monsoon (PRM), monsoon (MON) and post-monsoon (POM). Sediment samples retrieved using sterile PVC pipe corer (50 mm diameter) were separately wrapped in aluminum foil placed on ice in, an icebox, and immediately transported to the laboratory.

Analysis of potential nitrification: The rate of potential nitrification was determined by employing modified method as described earlier (Belser & Mays 1980; Hoffmann *et al*., 2007). Each sediment core sample was divided and three parts (3 cm each) were taken from the top (T), middle (M), and bottom (B) of the core. From each section, sediment (3 gm) slurry was made by adding 15ml of phosphate buffer and amended with 1mM $(NH_4)_2SO_4$ solution separately.

Because chlorate inhibits the nitrification process therefore, different concentrations of sodium chlorate (15mM, 30mM, 45mM, and 60mM) were added (15ml) in each flask respectively whereas, the control set of flasks did not contain Sodium chlorate. All the flasks were incubated on an orbital shaker at 150 rpm for 6 hours. The supernatant of slurry (2ml) was pipetted out after 2hrs. of regular intervals over the incubation period, and centrifuged at 3000 rpm for 5 minutes and filtered through 0.45µm (25 diameters, Whattman). Finally, the supernatant of each sample was analyzed spectrophotometrically for N determination according to Strickland & Parsons, (1972). The levels of potential nitrification during different seasons were observed by the standard curve of nitrite with time and concentrations.

Physico-chemical parameters: Parameters, such as temperature (air, water, and sediment), salinity, pH were recorded with the help of a standard mercury thermometer), refractometer (ATAGO 0161633, Japan), and pH meter (ELEMETRON, CP-401), respectively, and dissolved oxygen was measured by modified Winkler method as described by Strickland & Parson, (1972).

Statistical analysis

Analyses of variance (ANOVAs) were used to investigate differences in physicochemical parameters of water with sites, seasons, and PN rate as factors by using SPSS version 14.0. Pearson coefficient correlation was applied to observe seasonal variation in the rate of potential nitrification in different concentrations at the top, mid, and bottom sections with a concentration of nutrients in water using PAST version 2.13 (Hammer *et al*., 2001).

Results

In the present study, potential nitrification (PN) rate of four different sites of Karachi coastal area were recorded. Overall, potential nitrification rate was high in POM season as compared to PRM season and MON season. In terms of sections of sediment cores, PN rate was high in the middle and bottom sections as compared to the top section. At SP, the highest potential nitrification (PN) rate was observed in the middle section of the sediment (0.39 µg NO_2N g $w^{-1}h^{-1}$) on 45mM chlorate concentration at 6 hrs. of incubation which was followed by bottom and top sections (0.341 µg NO_2N g $w^{-1}h^{-1}$,0.308µg NO_2N g $w^{-1}h^{-1}$) respectively at 60 mM concentration during POM season (Fig. 2). Whereas, the lowest nitrification rate (bottom 0.197 µg NO_2N g $w^{-1}h^{-1}$) was found in the PRM season at 60 mM concentration (Fig. 2c). The rate of PN at MC, was found high (0.447 µg NO_2N g $w^{-1}h^{-1}$) in the bottom section followed by mid (0.425 µg NO_2N g $w^{-1}h^{-1}$) and top (0.393 µg NO_2N g $w^{-1}h^{-1}$) during the POM season in 60 mM and 30 mM concentration at 6 hrs of incubation time (Fig. 3). A low PN rate (0.113 µg NO_2N g $w^{-1}h^{-1}$) was recorded in the M season at 60 mM concentration in the middle section (Fig. 3). The highest values of PN rate (0.373 µg NO_2N g $w^{-1}h^{-1}$) were observed at PQ, again during POM season in the bottom section and lowest in the middle section (0.167 µg NO_2N g $w^{-1}h^{-1}$) of sediment sample at 45 mM concentration at 6 hrs of incubation (Fig. 4). In the case of KC, maximum value of PN (0.257 µg NO_2N g $w^{-1}h^{-1}$) was observed in the middle section at 15 mM chlorate concentration during POM season whereas, it was low in the bottom section (0.193 µg NO_2N g $w^{-1}h^{-1}$) at 15mM concentration at 6 hrs of incubation (Fig. 5).

ANOVA table indicating significant effect of variable factors such as seasons, stations, time duration on potential nitrification rate (Table 1). In case of rate of PN all stations, revealed a significant correlation between NO_3^- and PO_4^{3-} concentration and rate of potential nitrification at various chlorate concentration (Table 2). A significant correlation was also observed between ammonium ion and potential nitrification rate at 15, 30 and 45 mM concentration of chlorate in KC area (Table 2). A highly significant correlation was also observed between PN rates at different concentrations of chlorate with respect to the depth of the sediment samples (Table 2). Overall results showed that the highest PN was recorded in the bottom section at MC and PQ stations whereas it was high in the middle section at SP and KC stations. Seasonally, data showed that there was a high potential nitrification rate recorded in a post-monsoon season which was followed by pre-monsoon and monsoon seasons respectively.

A distinct seasonal variation in physico-chemical conditions of water was observed at all four study sites (Table. 3). Temperature values ranged between 19-26°C, 19-35°C , 28-36°C, and 18-28°C at SP, MC, PQ, and KC, respectively, with the highest value observed in the MON season at all stations except MC (Table 3). The maximum value of salinity was recorded in PRM season at all stations (SP; 40 PSU, PQ; 44 PSU, KC; 38 PSU) except for MC where the highest value (36 PSU) was observed in POM season. The pH values varied from 6.75 to 7.86, with the lowest value (6.74) recorded at SP in the POM and the highest value (7.86) at PQ during the MON season (Table 3). Dissolved oxygen had considerable variation (0.004-1.152 mg/L) where the highest value was observed in monsoon season at all stations (SP; 1.126 mg/L, MC; 0.929 mg/L, PQ; 0.829 mg/L, KC; 1.152 mg/L).

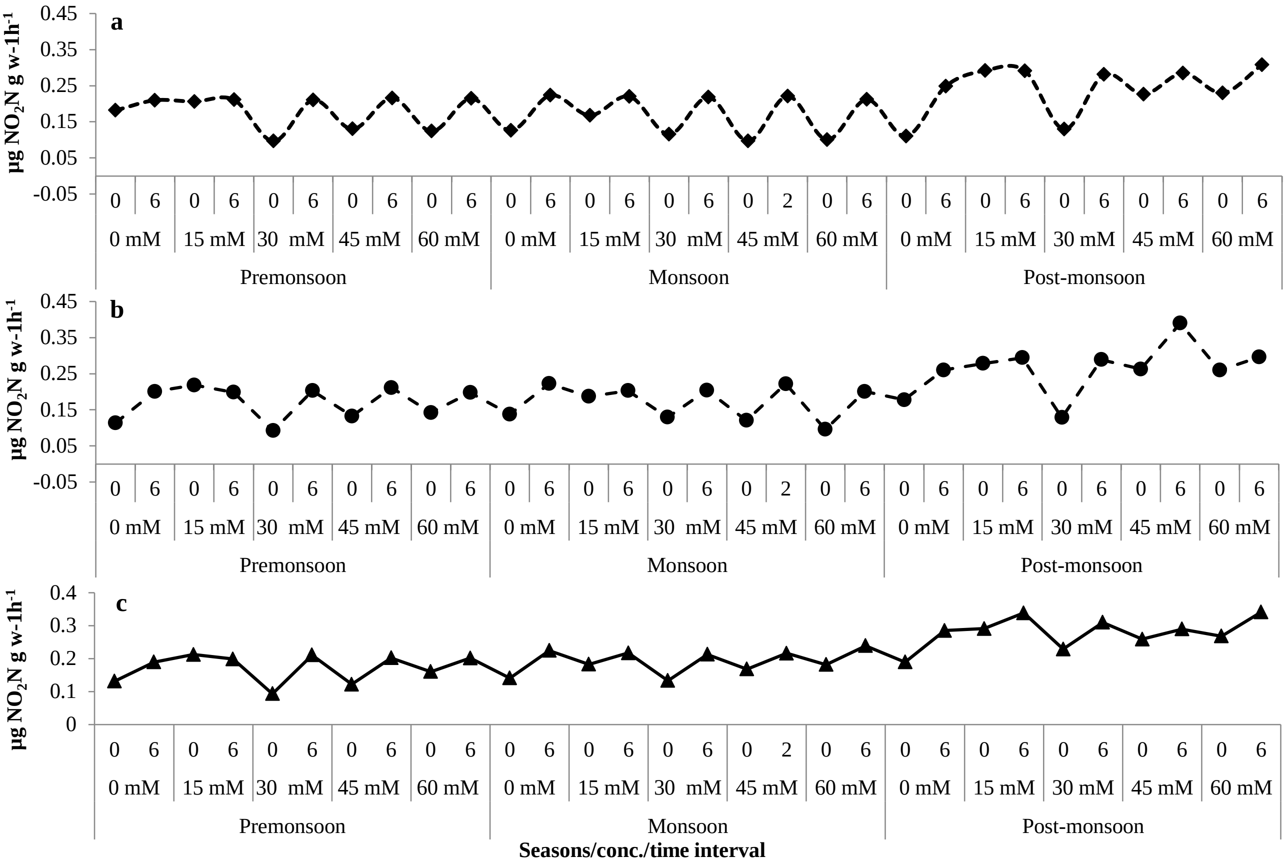

Fig. 2. Seasonal variation in the rate of potential nitrification activity in sediment samples a) top, b) mid and c) bottom of Sandspit backwater during 0 and 6 hours of incubation period at 0,15,30,45,60mM chlorate concentration.

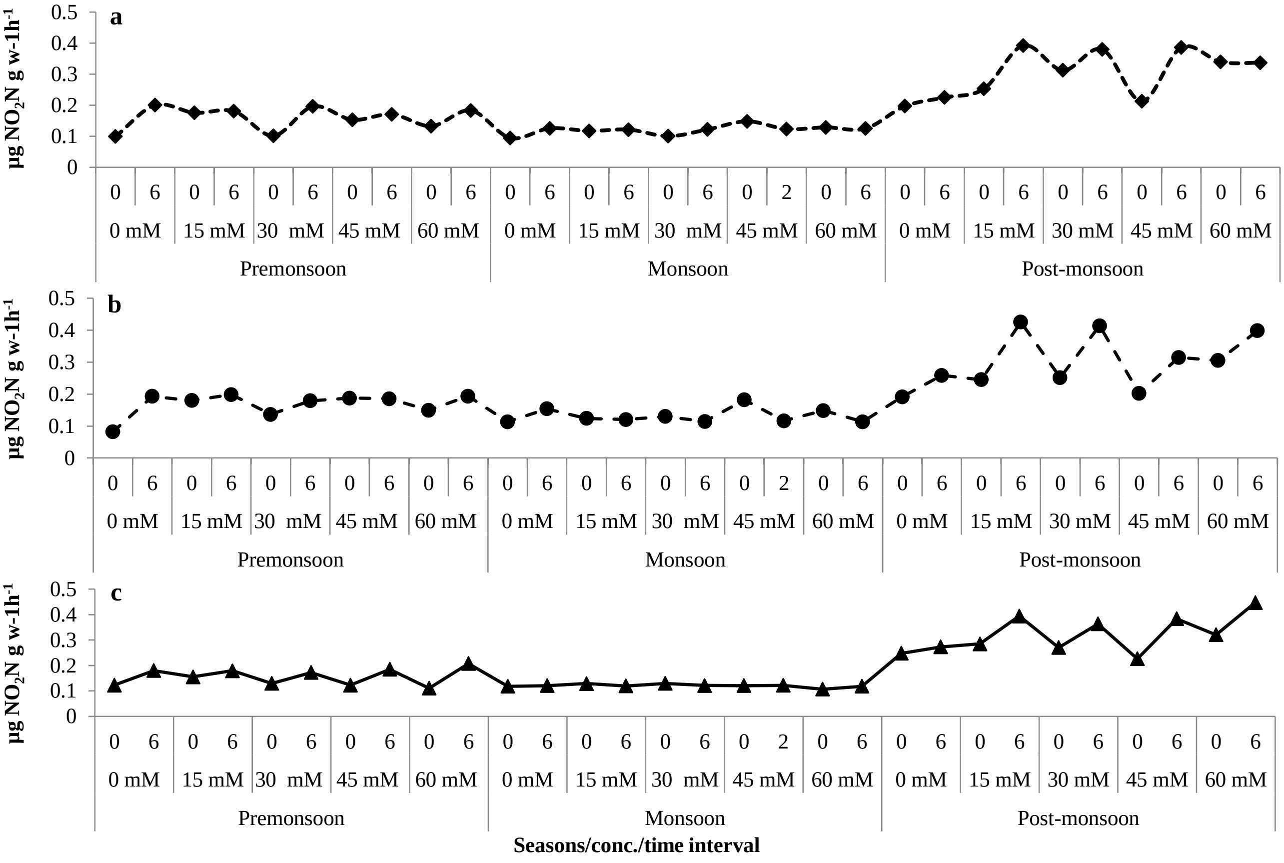

Fig. 3. Seasonal variation in the rate of potential nitrification activity in sediment samples a) top, b) mid and c) bottom of Manora channel during 0 and 6 hours of incubation period at 0,15,30,45,60mM chlorate concentration.

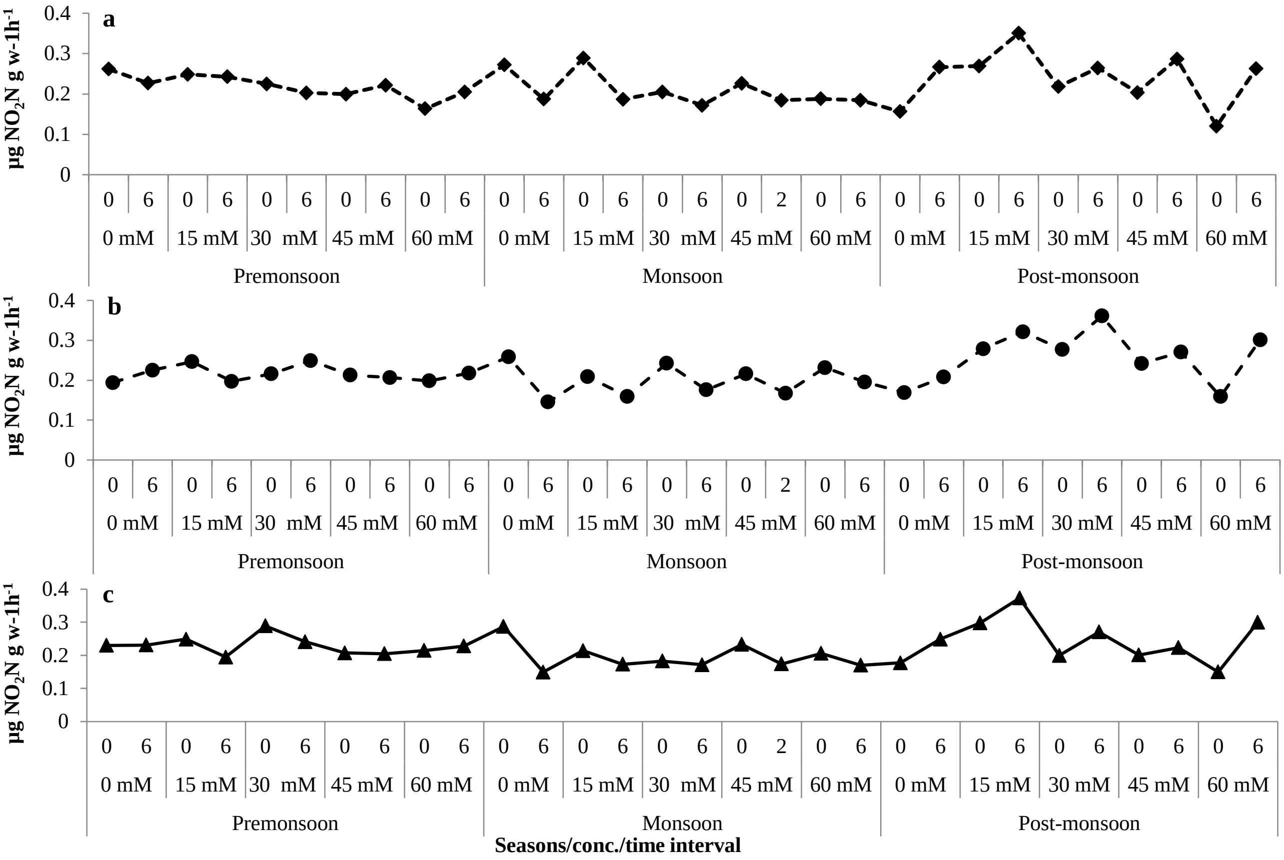

Fig. 4. Seasonal variation in the rate of potential nitrification activity in sediment samples a) top, b) mid and c) bottom of Port Qasim during 0 and 6 hours of incubation period at 0,15,30,45,60mM chlorate concentration.

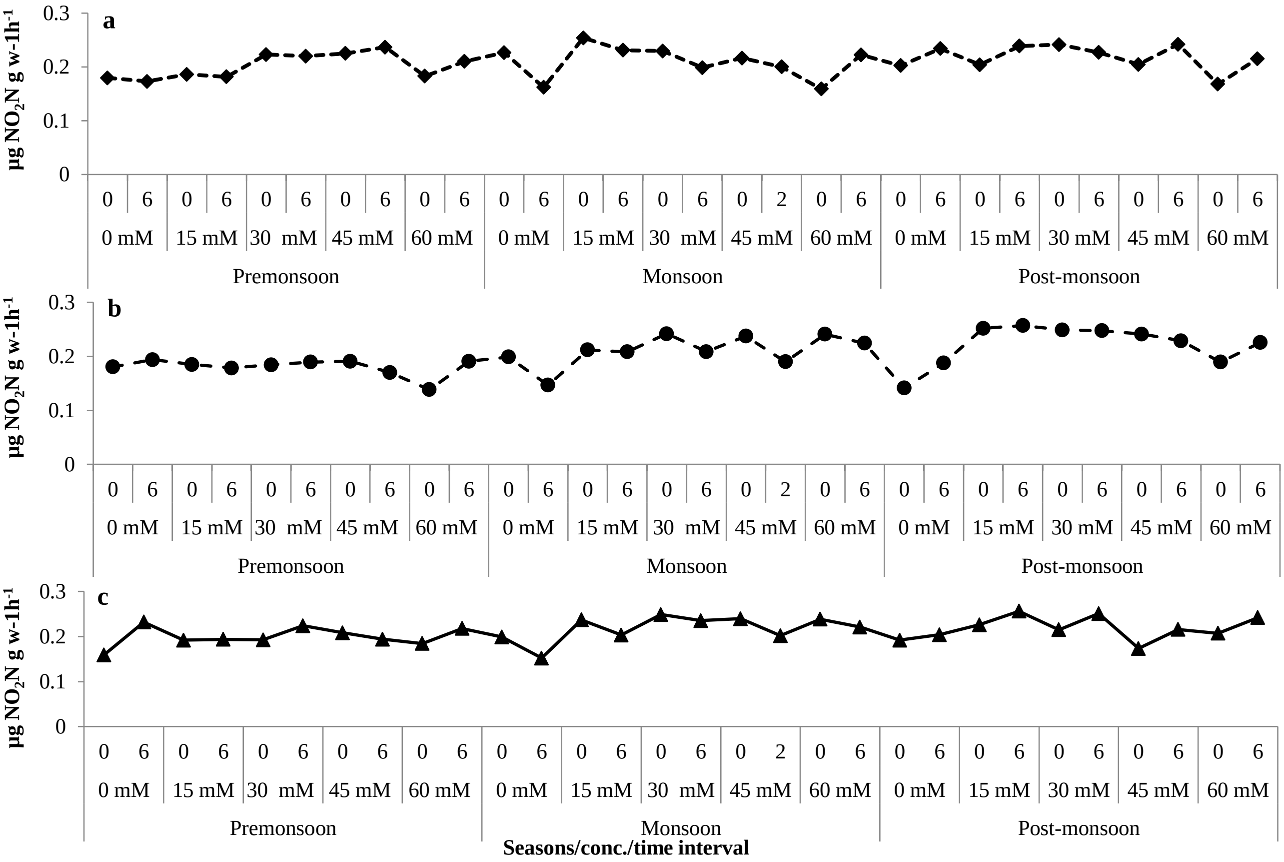

Fig. 5. Seasonal variation in the rate of potential nitrification activity in sediment samples a) top, b) mid and c) bottom of Korangi Creek during 0 and 6 hours of incubation period at 0,15,30,45,60mM chlorate concentration.

Table 1. Four-way ANOVA indicating individual and combined effect of stations, seasons, time and concentration on potential nitrification (PN) rate.

Factors	F-value
Station	**4.59
Season	***197
Concentration	***7.59
Time	***21.99
Station * Season	***33.28
Station * Concentration	*2.12*
Station * Time	**3.13
Season * Concentration	***4.61
Season * Time	**3.59
Concentration * Time	**2.90
Station * Season * Concentration	*1.79
Station * Season * Time	***8.45
Station * Concentration * Time	ns 1.13
Season * Concentration * Time	ns 1.49
Station * Season * Concentration * Time	*1.43

Where, *** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$; non-significant (ns) = $p > 0.05$

Nutrients ion concentration of NH_4^+, NO_2^-, $N0_3^-$, PO_4^{3-} and SiO_3^- were observed at all stations and seasons. The highest value of NO_2^- (SP; 0.46 µg/L, MC; 0.159 µg/L, PQ; 1.694 µg/L, KC; 1.980 µg/L) were observed in PRM and $N0_3^-$ (SP;1.395 µg/L, MC;0.958 µg/L, PQ; 3.385 µg/L, KC; 2.226 µg/L) and PO_4^{3-} ion (SP;0.6 µg/L, MC;0.35 µg/L, PQ; 1.221 µg/L, KC; 0.796 µg/L) were in POM season at all stations. The maximum value of NH_4^+ ion was recorded in PRM (SP; 15.175 µg/L and MC; 21.302 µg/L) and in POM (PQ; 1.451, MC; 21.3 µg/L) and (KC; 3.375 µg/L) seasons (Table 3). However, concentrations of SiO^-_4 were high in the MON season at (SPB; 1.45 µg/L, PQ; 1.62 µg/L, and KC; 1.56 µg/L), except MC; 3.665 µg/L in PRM (Table 3).

Two-way ANOVA indicated the individual effect of stations and seasons as well as their effect combined effect on physic-chemical parameters (temperature, salinity, pH and DO) recorded for air, water, and sediment (Table 4). However, Table 5 showed the individual and combined effect of station and season on the variability of nutrient ion concentration. Pearson correlation coefficient showed a significant positive correlation between temperature and salinity at stations 2 and 3, pH and DO at stations 1, 2, and 3 (Table 6). Whereas in the case of nutrient ions significant correlation was recorded between nitrate and phosphate ions at all four stations. pH and dissolved oxygen were also positively correlated with silicate at stations 1, 3, and 4 (Table 6).

Discussion

Nitrification play a crucial role in global nitrogen cycling. This nitrification is process has two phases in which ammonium converts into nitrite and then nitrate. In the present study, PN activity was observed high in the post-monsoon season at all study sites. According to previous studies, the high temperature often supports PN activity (Lu *et al.*, 2020), but it is dissimilar to the present study in which the PN rate was high in the winter season as compared to the summer (monsoon) season. The study reveals that a lower PN rate was found in the monsoon season due to high precipitation which may possibly decrease the coupled nitrification and denitrification activity as described earlier (Fu *et al.*, 2022).

Table 3. Seasonal variation in physicochemical parameters in the backwaters of Sandspit (SP), Manora Channel (MC), Port Qasim (PQ) a nd Korangi Creek (KC) along Karachi coast.

Stations	Seasons	AT	Water parameters									
			WT	ST	Sal.	pH	DO	NO_2^-	NO_3^-	NH_4^+	PO_4^{3-}	SiO_3^-
SP	PRM	25 ± 1.00	19 ± 1.15	20 ± 0.58	40 ± 0.58	6.88 ± 0.06	0.015 ± 0.00	0.849 ± 0.01	0.653 ± 0.09	15.175 ± 0.73	0.258 ± 0.04	0.795 ± 0.03
	MON	27 ± 0.58	26 ± 0.58	26 ± 0.58	40 ± 0.58	7.24 ± 0.06	1.126 ± 0.00	0.032 ± 0.02	0.561 ± 0.10	2.335 ± 0.40	0.193 ± 0.02	1.45 ± 0.10
	POM	26 ± 0.58	26.0 ±10	26 ± 0.58	38 ± 0.58	6.74 ± 0.05	0.154 ± 0.00	0.46 ± 0.02	1.395 ± 0.07	10.109 ± 0.70	0.6 ± 0.02	0.82 ± 0.01
MC	PRM	30 ± 0.58	20 ±0.57	19 ± 0.58	34 ± 0.58	7.17 ± 0.04	0.004 ± 0.00	0.888 ± 0.00	0.764 ± 0.02	21.302 ± 0.03	0.28 ± 0.03	3.665 ± 0.11
	MON	34 ± 0.58	27 ±0.58	26 ± 1.15	35 ± 0.58	7.57 ± 0.10	0.929 ± 0.00	0.111 ± 0.00	0.209 ± 0.03	20.514 ± 0.89	0.103 ± 0.01	1.09 ± 0.05
	POM	35 ± 0.58	28 ±0.58	28 ± 0.58	36 ± 0.58	7.28 ± 0.03	0.205 ± 0.00	0.118 ± 0.02	0.958 ± 0.11	8.684 ± 0.04	0.35 ± 0.05	0.925 ± 0.03
PQ	PRM	31 ± 0.58	30 ±0.57	29 ± 0.58	44 ± 0.58	7.54 ± 0.02	0.246 ± 0.00	1.694 ± 0.01	2.354 ± 0.46	1.451 ± 0.03	0.553 ± 0.16	0.85 ± 0.02
	MON	36 ± 0.58	29 ±0.58	29 ± 0.58	42 ± 0.58	7.86 ± 0.10	0.829 ± 0.00	0.71 ± 0.03	0.271 ± 0.16	3.325 ± 0.24	0.206 ± 0.07	1.62 ± 0.05
	POM	34 ± 0.58	29 ±1.53	28 ± 0.58	34 ± 0.58	7.38 ± 0.01	0.163 ± 0.00	0.371 ± 0.02	3.385 ± 0.25	4.168 ± 0.87	1.221 ± 0.08	0.61 ± 0.05
KC	PRM	18 ± 0.58	19 ±0.57	18 ± 0.58	38 ± 0.58	7.74 ± 0.06	0.265 ± 0.00	1.980 ± 0.02	1.999 ± 0.03	3.375 ± 1.14	0.646 ±0.11	1.01 ± 0.05
	MON	28 ± 0.58	28 ±0.58	28 ± 0.58	37 ± 0.58	7.79 ± 0.04	1.152 ± 0.00	0.108 ± 0.00	0.309 ± 0.05	1.241 ± 0.33	0.135 ± 0.014	1.56 ± 0.01
	POM	27 ± 0.58	27 ±0.58	27 ± 0.58	38 ± 0.58	7.23 ± 0.03	0.35 ± 0.00	0.222 ± 0.01	2.226 ± 0.12	4.60 ± 0.12	0.796 ± 0.09	0.695 ± 0.03

PRM = Pre-monsoon, MON = Monsoon, POM = Post-monsoon, AT = Air temperature, WT = Water temperature, ST = Sediment temperature, DO = Dissolve oxygen, NO_2^-= Nitrite, NO_3^-= Nitrate,NH_4^+= Ammonia,PO_4^{3-}= Phosphate,SiO_3^-= Silicate

Table 2. Pearson correlation coefficient showing the relationship between nutrients ion concentration of water and potential nitrification rate in sediment sample four different stations 1) Sandspit, 2) Manora channel, c) Port Qasim and d) Korangi Creek with incubation time (0-6 hrs.) and chlorate concentration.

	NO_2^-		NO_3^-		NH_4^+		PO_4^{3-}		SiO_3^-	
	Stations									
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
NH_4^+	**0.99**	0.54	-	-	-	-	-	-	-	-
PO_4^{3-}	-		**0.99**	**0.99**		-	-	-	-	-
SiO_3^-	-	1				0.59	-	-	-	-
PNTI	-	-	**0.96**	0.76	-	-	**0.98**	0.78	-	-
PNM1	-	-	**0.99**	0.74	-	-	**0.99**	0.76	-	-
PNB1	-	-	**0.96**	0.73	-	-	**0.95**	0.75	-	-
PNT2	-	-	**0.90**	0.99	-	-	0.88	**0.99**	-	-
PNM2	-	-	**0.89**	0.91	-	-	0.87	**0.92**	-	-
PNB2	-	-	0.88	0.92	-	-	0.86	**0.93**	-	-
PNT3	-	-	**0.97**	0.83	-	-	**0.96**	0.85	-	-
PNM3	-	-	**0.98**	0.85	-	-	**0.98**	0.87	-	-
PNB3	-	-	**0.97**	0.83	-	-	**0.96**	0.85	-	-
PNT4	-	-	**0.97**	0.87	-	-	**0.96**	0.88	-	-
PNM4	-	-	**0.99**	0.83	-	-	**0.98**	0.84	-	-
PNB4	-	-	**0.99**	0.82	-	-	**0.98**	0.84	-	-
PNT5	-	-	**0.98**	0.81	-	-	**0.97**	0.82	-	-
PNM5	-	-	**0.98**	0.9	-	-	**0.97**	**0.91**	-	-
PNB5	-	-	**0.96**	0.84	-	-	**0.95**	0.86	-	-
PNT6	-	-	**0.99**	0.69	-	-	**0.99**	0.72	-	-
PNM6	-	-	**0.99**	0.7	-	-	**0.98**	0.72	-	-
PNB6	-	-	**0.93**	0.7	-	-	**0.91**	0.71	-	-
	Stations									
	3	**4**	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**
NH_4^+	-	-	-	**0.96**	-	-	-	-	-	-
PO_4^{3-}	-	-	**0.93**	**0.99**	-	**0.98**	-	-	-	-
SiO_3^-	-	-	-	-	-	-	-	-	-	-
PNTI	-	-	-	-	-	-	-	-	**0.90**	0.80
PNM1	-	-	-	-	0.76	-	-	-	0.63	-
PNB1	-	-	-	-	-	-	-	-	-	0.75
PNT2	-	-	**0.98**	0.70	-	0.85	**0.98**	0.77	-	-
PNM2	-	-	0.86	**0.97**	-	0.88	0.61	**0.94**	-	-
PNB2	-	-	**0.98**	0.89	-	0.75	0.86	0.84	-	-
PNT3	-	-	**0.93**	-	-	-	**1.00**	-	-	-
PNM3	-	-	0.88	-	0.56	-	**0.99**	-	-	-
PNB3	-	-	0.81	-	0.66	0.68	**0.97**	0.57	-	-
PNT4	-	-	**0.93**	**0.99**	-	**0.99**	**0.99**	**0.99**	-	-
PNM4	-	-	**0.95**	-	-	0.53	**0.99**		-	-
PNB4	-	-	**0.99**	-	-	-	**0.91**		-	-
PNT5	-	-	**0.94**	1	-	**0.96**	**0.99**	**0.99**	-	-
PNM5	-	-	**0.95**	-	-	0.52	**0.99**	-	-	-
PNB5	-	-	**0.99**	-	-	-	**0.94**	-	-	-
PNT6	-	-	0.89	-	0.54	-	**0.99**	-	-	0.69
PNM6	-	-	0.86	-	0.59	-	**0.98**	-	-	-
PNB6	-	-	**0.97**	-	-	0.7	**0.99**	0.58	-	-

*Significant, PN = Potential nitrification rate, T = Top, M= Middle, B = Bottom, 1=0 mM chlorate concentration, 2=15 mM chlorate concentration, 3=30 mM chlorate concentration, 4=45 mM chlorate concentration, 5=60 mM chlorate concentration), NO_2^- = Nitrite, NO_3^- = Nitrate, NH_4^+= Ammonia, PO_4^{3-} = Phosphate and SiO_3^-= Silicate

Table 4. Two-way ANOVA indicating individual and combined effect stations and seasons on physic-chemical parameters (air, water, sediment temperature; salinity, pH and DO).

Factors	Parameters	F-value
Station	Air temperature	ns 1.97
	Water temperature	***10.01
	Sediment temperature	ns 0.244
	Salinity	***146
	pH	***214
	DO	***882
Season	Air temperature	***834
	Water temperature	***430
	Sediment temperature	***728
	Salinity	***60
	pH	***516
	DO	***156
Station * Season	Air temperature	***19.90
	Water temperature	*2.88
	Sediment temperature	***13.44
	Salinity	***67.60
	pH	***12.55
	DO	***3405

Where, *** = $p<0.001$; ** = $p\leq0.01$; * = $p\leq0.05$; Non-significant (ns) = $p>0.05$

Table 5. Two-way ANOVA showing the individual and combined effect of stations and seasons on nutrient ion (NO_2^-; NO_3^-; NH_4^+; PO_4^{3-} and SiO_3^-) concentrations.

Factor	F-value
Stations	***29.66
Seasons	***27.10
Stations * Seasons	** 28.09

The process of PN can take place in both oxic and anoxic environments (Reef *et al.*, 2010; Zheng *et al.*, 2020). This may have a reflection on our observations where high PN rates were observed in upper (2 to 4 cm) sediment layer as compared to the deeper sediment (below 4 cm). Our results correspond well with previous reports that PN decreases gradually with increasing sediment depth (Lin *et al.*, 2021; Zhao *et al.*, 2021). Nitrification activity in the deeper layers of sediment indicates the presence of the nitrifying organism. It has been shown that nitrifying bacteria can withstand anoxic conditions (Semblante *et al.*, 2017). There are several other factors that might be responsible to support this phenomenon such as bioturbation, which disrupts sediment stratigraphy, alters sediment-water exchange, porosity, and permeability, and thus oxygenate the sediments (Berner, 2020; An *et al.*, 2021). Variability in PN values at the different sites may be due to the variations in the microbial community and is directly proportional to their abundance (Zhou *et al.*, 2021).

It is well documented that exposure to biogeochemical processes particularly nitrogen transformations is being influenced by anthropogenic activities (Lugendo & Kimirei, 2021). Assessment of the potential nitrification rates provides in-depth information about the processes related to the biogeochemical cycle in the sediment substrate of mangroves. Anthropogenic alterations in the mangrove habitat can enhance inputs of nitrogenous products, however, mangroves show their ability to react as natural sewage treatment plants. This phenomenon has also been reported by Clough *et al.*, (1983) and Ramos *et al.*, (2021). Although, the vulnerability of mangrove ecosystems to contaminants other than nutrients, such as organic matter, heavy metals, and pesticides may suppress the habitat but mangrove ecosystems have a tendency to speed up the nitrification process and absorb the discharge of sewage effluents and minimize their effects (Cochard, 2017). Overall results of the present study specify that the potential nitrification rate is high at anthropogenically affected sites for example Manora Channel and Sandspit as described earlier (Qari *et al.*, 2015; Chaudhary *et al.*, 2021). It may be suggested that long-term experiments to be conducted for the assessment of pollutant's effect on the nitrification process.

In general, environmental factors are known to affect the PN rate in sediment. We observed some conflicting results with respect to PN and its correlation with other observed parameters. For example, although increasing salinity reduces the PN rates (Chi *et al.*, 2021), despite the variability in salinity during different seasons we did not observe a significant effect which is in agreement with a previous study (Magalhães *et al.*, 2005). Similarly, variable observations suggest that elevated pH level (>9) may decrease or inhibit the nitrification activity (Yao *et al.*, 2011), It was also reported that there are no effects on nitrification rate between pH 7.85 to 6.45 whereas, highly acidic or highly alkaline pH completely inhibits the nitrification rate (Takahashi *et al.*, 2020) and that nitrifying bacteria show better growth in the acidic pH <5.5 while denitrifying bacteria favors alkaline pH (Blum *et al.*, 2018). Under the alkaline condition, ammonium ions have a negative relationship with PN (Masoud *et al.*, 2019). The negative relationship between PN and DO observed in the present study coincides with a previous report (Liu *et al.*, 2019) which showed higher DO levels increase PN rate.

Ammonium ion (NH_4) is known to be the main substrate of nitrification activity (Seitzinger *et al.*, 2005). High concentration of ammonia (NH_3) may be lethal to nitrifying microorganism and may hinder their growth and disturb their enzyme efficiency (Urakawa *et al.*, 2019). According to Zhang & Li, (2019), a low concentration of pore water NH4 increases the potential nitrification rate which is in conformation to our results. Our results also show that nitrite and nitrate concentration had a direct relation to the potential nitrification rate. Reduction in nitrate concentration and immobilization of NH_4 may cease the nitrification activity (Sahrawat, 1989). PO_4 ion concentration is another factor that affects the PN, as the nitrification process increases the soluble P in the sediment. We also observe, in agreement with some previous studies (Zhang *et al.*, 2019), a positive correlation between the rate of potential nitrification activity and soluble P in anoxic sediments.

Table 6. Pearson correlation coefficient showing relationship between physico-chemical parameters at four different stations 1) Sandspit, 2) Manora channel, 3) Port Qasim and 4) Korangi Creek.

	WT		ST		Sal.		pH		DO		NO_2^-		NO_3^-		NH_4^+	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
ST	-	**0.99**		**0.96**	-	-	-	-	-	-	-	-	-	-	-	-
Sal.	-	**0.92**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	-	0.62	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-
DO	0.59	-	0.61	-	-	-	**0.92**	**0.99**	-	-	-	-	-	-	-	-
NO_2^-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO_3^-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NH_4^+	-	-	-	-	-	-	-	-	-	-	**0.99**	-	-	-	-	-
PO_4^{3-}	-	-	-	-	-	-	-	-	-	-	-	-	**0.99**	**0.99**	-	-
SiO_3^-	-	-	-	-	-	-	**0.95**	-	**0.99**	-	-	**0.99**	-	-	-	0.59
	3	**4**	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**
ST	-	**0.99**		-	-	-	-	-	-	-	-	-	-	-	-	-
Sal.	0.65	-	**0.98**	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	-	-	0.76	-	0.62	-	-	-	-	-	-	-	-	-	-	-
DO	-	0.65	0.69	0.64	-	-	0.97	-	-	-	-	-	-	-	-	-
NO_2^-	**0.97**	-	-	-	0.82	0.54	-	-	-	-	-	-	-	-	-	-
NO_3^-	-	-	-	-	-	**0.99**	-	-	-	-	-	-	-	-	-	-
NH_4^+	-	-	-	-	-	**0.9**	-	-	-	-	-	-	-	**0.97**	-	-
PO_4^{3-}	-	-	-	-	-	**0.98**	-	-	-	-	-	-	**0.93**	**0.99**	-	**0.99**
SiO_3^-	-	-	0.69	-	-	-	**0.99**	**0.83**	**0.99**	**0.89**	-	**0.99**	-	-	-	0.59

*Significant <0.05, NO_2^- = Nitrite, NO_3^- = Nitrate, NH_4^+= Ammonia, PO_4^{3-} = Phosphate and SiO_3^- = Silicate

Conclusion

In conclusion, the present study revealed a strong potential nitrification phenomenon observed at all four stations with a slight variation that may be due to the relevance of anthropogenic activities along the backwaters of the Karachi coast. Furthermore, it is recommended to compare the PN rate from unpolluted sites along the other mangroves backwaters of Pakistan.

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