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# SEASONAL VARIATIONS IN POTENTIAL NITRIFICATION RATES IN MANGROVE SEDIMENT AT SANDSPIT BACKWATERS, KARACHI, PAKISTAN

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**Abstract**

Sandspit coastal area is located between Hawksbay and Manora channel, south of Karachi. The area opposite to sandy coast is covered by mangrove forest at Sandspit backwaters. The potential nitrification rates in sediment from rhizosphere and non-rhizosphere at Sandspit backwater mangrove forest was examined using sodium chlorate inhibition method. Samples collected during pre-monsoon (Jan), monsoon (June) and post-monsoon (Nov) seasons were analyzed using two different concentration of inhibitor. The higher inhibitor concentration (30mM) was found to be more effective than lower (15mM) concentration used. The potential nitrification rates were slightly higher during monsoon as compared to other seasons. The potential nitrification values ranged from 0.06 (post-monsoon) to 1.20 μg $NO_2^-$ N $gw^{-1}h^{-1}$ (monsoon). The potential nitrification rates had significant correlation with the incubation time ($p<0.005$, n=3). The nitrifiers in mangrove sediments appear to tolerate a range of physical and chemical parameters, such as, temperature (17-28 ºC), Salinity (36-42), pH (6.8-8.9), and DO (3.7-6.5 mg/L) and maintain steady potential nitrification rates, as observed during experimentation in two sediments types during all seasons.

**Key Words:** Mangrove, Potential nitrification, Sodium Chlorate, Sandspit, Pakistan

## Introduction

Mangrove belt of Sandspit is highly productive (Siddiqui *et al*., 2008; Faroouqi *et al*., 2012; Shafique *et al*., 2013) which is located in the harbor area of Karachi that stretches from Hawksbay to Mannora channel. It is an estuarine area with a tidal range of -0.4 up to 3.2m. This area is an integral part of the fan shaped Indus delta and dominated by monospecific *Avicenna marina* which provides food, fodder, medicine and coastal protection to nearby local community area. Sediments are transported via Indus river to estuarine parts which consequently develops and sustains this region (Saifullah *et al*., 1997; Siddiqui *et al*., 2008). The primary productivity of mangrove area is relatively high as compared to other coastal systems due to the presence of a unique ecosystem having microbial community that efficiently transforms and provides essential nutrient supplies including various forms of nitrogen (Kristensen *et al*., 1998; Tam *et al*., 2009). Microbial oxidation of ammonia to nitrite and then to nitrate is the nitrification process. Soil nitrification is a vital biogeochemical process as the microbes belonging to the family Nitrobacteraceae are responsible for recycling of nitrogen. Nitrogen is considered to be a limiting factor in ecosystems (Rabalais 2002; Elser *et al.,* 2007). The oxidized nitrogen is used as a source of energy for growth. This process is under the influence of various environmental parameters such as temperature salinity and pH (Olsson & Falkengren-Grerup, 2000; Isnansetyo *et al*., 2011). The nitrite which is produced during nitrification is required for nitrogen leaching in soil and directly involved in the production of gaseous nitrogen greenhouse compounds and removal of N from soil (Cleemput & Samater, 1995; Yan *et al*., 2015). A recent study conducted in Germany revealed that nitrite acts as the direct precursor of nitric oxide (NO) formation under both anaerobic and aerobic state respectively (Russow *et al*., 2009). In Southeast Asia and China it was observed that the potential nitrification rates in mangrove area were influenced by salinity (Miranda *et al*., 2008), organic carbon (Krishnan & Bharathi, 2009), soil moisture content and nutrients (Singh & Kashyap, 2007) and tidal cycle (Hou *et al*., 2007). In Pakistan the significance of potential nitrification process have been examined mostly on agricultural soils ( Amin & Flower, 2004; Gill *et al*., 2006; Ahmedani *et al*., 2007; Ali *et al*., 2008; Ali *et al*., 2012; ) but there is no study related to mangrove areas.

The objective was to observe the seasonal effect on the rate of potential nitrification (PN) of two sediment types, mangrove (Rhizoidal area , R) and fringe area (non-rhizoidal, NR) by using the sediment slurry method of Belser & Mays, (1980). The sodium chlorate was used as a nitrite oxidation inhibitor. It enabled to measure $NO_2$-N production in samples and after treatment, they were finally analyzed spectrophotometrically.

## Material and Methods

Sampling was conducted from study site (Fig. 1) during late November 2014 till late June 2015. Sediment samples rhizoidal (R) and non-rhizoidal (NR) regions were collected during pre-monsoon (January), monsoon (June) and post -monsoon (November) seasons respectively. The samples were cored with 50.8 mm diameter sterile plastic corers wrapped in aluminum foil and immediately kept under crushed ice in an icebox and transported to laboratory. The potential nitrification rate was determined by modified method of Belser & Mays, (1980) (Kristensen *et al*., 1998; Hoffmann *et al*., 2007).

All samples were examined in triplicate. Briefly, each of the core samples were divided into 3 cm interval top (T), middle (M) and bottom (B) sediment sections. Sediment slurry (3g) was made in 15ml of phosphate buffer (pH 7.5). These samples were then amended with substrate of ammonium sulphate 1mM (Merck). Because chlorate acts as an inhibitor of nitrification process (Belser & Mays, 1980), the samples tested against two different concentrations of sodium chlorate, i.e., 15mM and 30Mm (BDH Chemicals) respectively. The control set consists of flasks with no treatment of chlorate. The flasks were sealed with stopper and incubated at room temperature on shaker at 150 rpm for 6h. Supernatant of slurry samples were pipetted out after 2 hrs regular intervals over the incubation period and filtered through 0.45 µm (25 mm diameter, Whatman) and centrifuged at 3000 rpm for 5 min. The samples were then immediately frozen until further analysis. Finally slurry samples for N determination were analyzed spectrophotometrically by modified method of Strickland & Parsons (1972). The potential nitrification levels were observed by standard curve of nitrite with time and concentrations during different seasons. Field parameters such as, temperature of air, water and sediment were recorded using standard mercury thermometer. Salinity was observed by Refractometer (ATAGO, 0161633; Japan) and pH was noted via pH meter (CP-401; ELEMETRON). The dissolved oxygen was determined using modified method of Strickland & Parsons (1972). The statistical analyses such as Pearson correlation, box plots were computed using the Minitab Version, 17.1.0.


Fig. 1: Map showing study site at Sandspit backwaters Mangrove forests, Karachi.

## Results

Sandspit Mangrove sediment is made up of different combination of clay, sand and silt. The rhizoidal (R) area is that part of the forest floor where topsoil is covered by thick microbial mat and pneumatophores protrude out from the ground. That area is always moist, muddy and often water logged. In contrast, the non- rhizoidal (NR) area which is present on the outskirts of mangrove vegetation having no microbial mat or pneumatophores, is relatively less moist and comparatively loose with visibly low clay and high sand contents. The potential nitrification rates along with physico-chemical parameters were observed for these two types of sediment for all seasons. Maximum values recorded were temperature 28 ºC (monsoon), salinity 42 (pre-monsoon), DO 6.5 mg/L (monsoon) and pH 8.9 (post-monsoon) respectively (Figs. 2 A, B).

The overall the potential nitrification rates in the top layer (T) of both sediment (R 0.43, NR 0.38µg $NO_2^-$ Ng $w^{-1}h^{-1}$) samples were relatively high as compared to the middle (M) (R 0.27, NR 0.27 µg $NO_2^-$ Ng $w^{-1}h^{-1}$) and bottom (B) (R 0.30, NR 0.25 µg $NO_2^-$ N $gw^{-1}h^{-1}$) sections. The rhizoidal (R) samples have higher potential nitrification rates as compared to non-rhizoidal (NR) sediment samples (Fig 3.). During 6h incubation maximum PN rates of R sediment sections were found to be 1.20(top, 0-3cm), 0.50(mid, 3-6cm), 0.60(bottom, 6-9 cm) µg $NO_2^-$ N $gw^{-1}h^{-1}$., and the highest rates of NR sediment sections were 0.60(top, 0-3cm), 0.50(mid, 3-6 cm) and 0.50(bottom, 6-9cm) µg $NO_2^-$ Ng $w^{-1}h^{-1}$ respectively.

More fluctuations were observed in PN values of Rhizoidal-T section as compared to rest of the sections. Similarly, the NRT section has more activity as compared to NRM and NRB sections. The nitrite accumulation vs incubation period showed significant correlation. Different concentrations of sodium chlorate 15mM and 30mM at equal time interval were also examined. It was found that the inhibition of $NO_2$-N was affected more by 30mM than 15mM concentration. There was a significant correlation between dissolved oxygen and temperature. The pH of water and sediment was also found to be highly significant (Table 1).

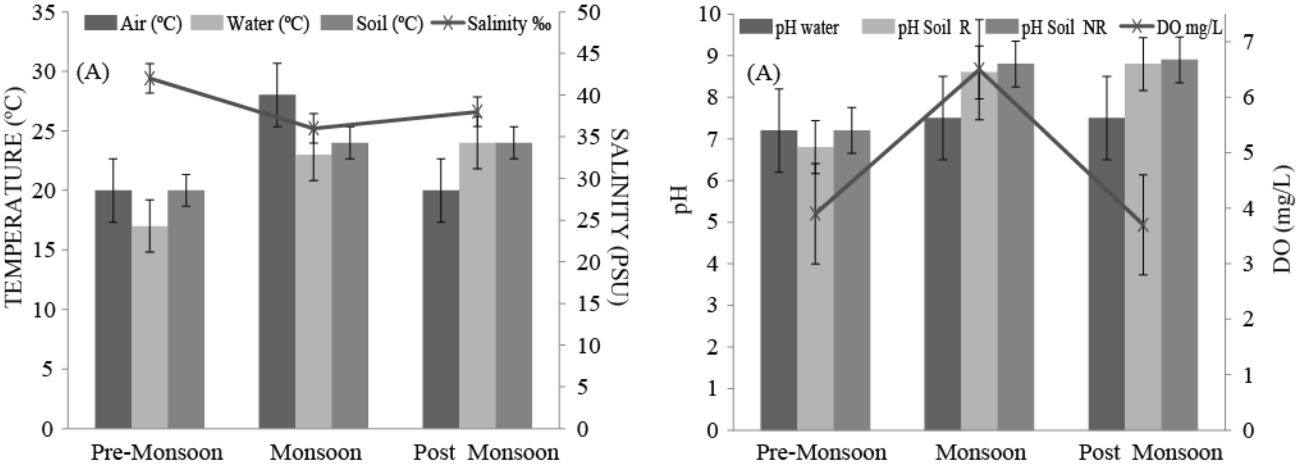


Fig. 2. Seasonal observations of field parameters of study site, A= rhizoidal area(R), B= non-rhizoidal area (NR). (N=3, ±S.D. error bars)

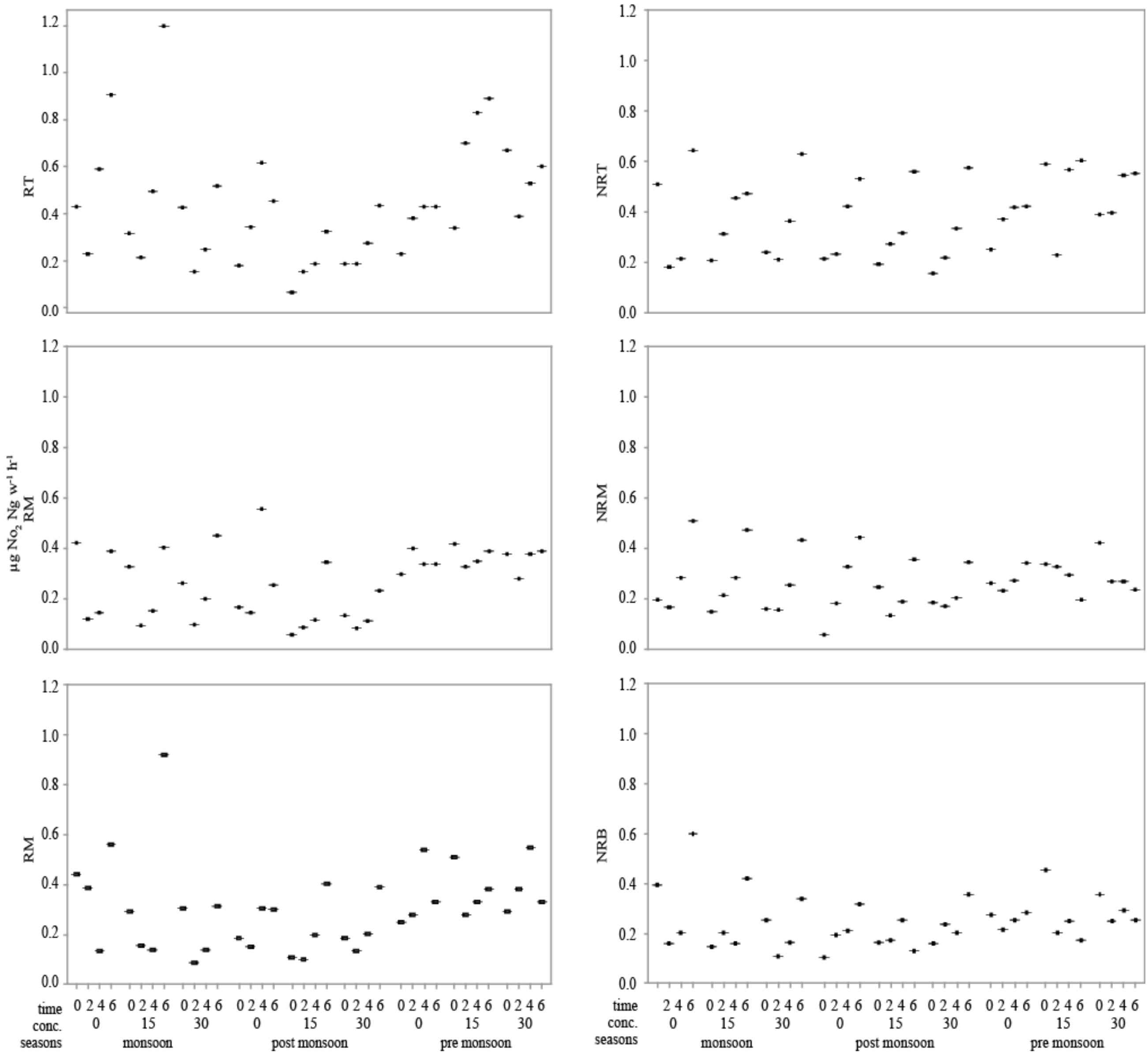


Fig 3. Box plot showing the difference in terms of Potential Nitrification activity (PN) activity between Rhizoidal (R) and Non-Rhizoidal (NR) top 0-3cm (RT, NRT) mid 3-6cm (RM, NRM) and bottom 6-9cm (RB, NRB) sections during 0-6h incubation time interval and 0,15,30mM concentration of Sodium Chlorate in Monsoon, Pre-Monsoon and Post-monsoon seasons (N=3)

**Table 1. Pearson correlation coefficient matrix showing relationship between top mid bottom Rhizoidal and Non-Rhizoidal sections with time, chlorate concentrations and physicochemical parameters.**

| | RT | RM | RB | NRT | NRM | NRB | Time hr. | Conc. mM | DO mg/L | Air °C | Water °C | Sedimen °C | pH water | pH R Sed. | pH NR Sed. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RM | 0.63* | | | | | | | | | | | | | | |
| RB | 0.66* | 0.65* | | | | | | | | | | | | | |
| NRT | 0.58* | 0.69* | 0.57* | | | | | | | | | | | | |
| NRM | 0.62* | 0.54* | 0.53* | 0.62* | | | | | | | | | | | |
| NRB | 0.53* | 0.54* | 0.65* | 0.60* | 0.695* | | | | | | | | | | |
| Time hr | 0.54* | 0.31 | 0.34 | 0.65* | 0.543* | 0.20 | | | | | | | | | |
| Conc. mM | -0.03 | -0.05 | -0.08 | 0.06 | -0.031 | -0.10 | -0.06 | | | | | | | | |
| DO mg/L | 0.12 | -0.11 | 0.07 | -0.05 | 0.044 | 0.13 | 0.03 | 0.10 | | | | | | | |
| Air °C | 0.10 | -0.14 | 0.05 | -0.07 | 0.032 | 0.12 | 0.03 | 0.09 | 0.99* | | | | | | |
| Water °C | -0.31 | -0.47 | -0.3 | -0.32 | -0.162 | -0.21 | -0.04 | -0.11 | 0.41 | 0.46 | | | | | |
| Sediment °C | -0.27 | -0.45 | -0.27 | -0.30 | -0.146 | -0.18 | -0.03 | -0.09 | 0.52* | 0.57* | 0.99* | | | | |
| pH water | -0.27 | -0.45 | -0.27 | -0.30 | -0.146 | -0.18 | -0.03 | -0.09 | 0.52* | 0.57* | 0.99* | 1.00* | | | |
| pH R Sed. | -0.30 | -0.47 | -0.29 | -0.31 | -0.157 | -0.20 | -0.03 | -0.10 | 0.44 | 0.50 | 0.99* | 0.99* | 0.99* | | |
| pH NR Sed. | -0.29 | -0.46 | -0.28 | -0.31 | -0.153 | -0.20 | -0.03 | -0.09 | 0.48 | 0.53* | 0.99* | 1.00* | 1.00* | 0.99* | |
| Sal. water | 0.17 | 0.39 | 0.18 | 0.25 | 0.096 | 0.09 | 0.01 | 0.03 | -0.76 | -0.79 | -0.91 | -0.95 | -0.95 | -0.92 | -0.93 |

*= Significant at $p<0.005$, Rhizoidal top (RT) mid (RM) bottom (RB), Non-Rhizoidal top (NRT), mid (NRM), bottom (NRB), Time (h), Chlorate Concentration (mM).

Seasons significantly affected the potential nitrification rates. During monsoon season the values varied between 1.20 µg $NO_2^-$ N $gw^{-1}h^{-1}$ (RT,15 mM, 6h) to 0.08 µg $NO_2^-$ N $gw^{-1}h^{-1}$ (RB, 30mM, 2h). The rates decreased during pre-monsoon season 0.89 µg $NO_2^-$ N $gw^{-1}h^{-1}$ (RT, 15 mM, 6h) to 0.17µg $NO_2^-$ N $gw^{-1}h^{-1}$ (NRB, 15 mM, 6h) and were lowest during post monsoon season 0.58 µg $NO_2^-$ N $gw^{-1}h^{-1}$ (NRT, 30mM, 6h) to 0.06 µg $NO_2^-$ N $gw^{-1}h^{-1}$ (RM,15 mM, 2h) respectively.

**Discussion**

Present study is the first attempt to record potential nitrification (PN) rates in sediment from mangrove backwaters, Pakistan. During experimentation, it was observed that PN rates were higher in rhizoidal sediment (which was covered with microbial mat) as compared to non-rhizoidal (devoid of microbial mat) area of mangroves. These results were in agreement with earlier study conducted by Luo *et al*., 2014 that also reported higher nitrification rates in mangrove areas as compared to mud flats. Nitrification is a 2-step process in which nitrite is an intermediate product and due to its fast reaction rate sodium chlorate $NaClO_3$ was used as a specific nitrite oxidation inhibitor. This type of inhibitor is useful in understanding the process of nitrification and its associated bacteria as it permits the accumulation of nitrite by inhibiting the oxidation. The rate of inhibition is proportional to the concentration of nitrite (Belser & Mays, 1980). Potential nitrification is an indirect method to observe the nitrifying bacteria population (Hansen *et al.,* 1981; Schmidt & Belser, 1982). The increased rate of reaction in RT section indicates high rate of bacterial population (Abbasi *et al*., 2001). Certain environmental factors were involved in the regulation of nitrification process such as organic matter, dissolved oxygen, temperature, salinity, pH (Isnansetyo *et al.,* 2011).

The PN rates were found to be fairly constant throughout all seasons indicating that the nitrifying bacteria involved in these processes are hardy and tolerant to variety of extreme physical and chemical factors. The salinity tends to fluctuate during seasons due to waste water runoff, rainfall input and evaporation rates. The physicochemical observations concur with the study of Miranda *et al.,* 2008 conducted in Kochi backwaters. The sediment pH values were usually above 8.5 suggesting that alkaline conditions favoured nitrification process which was found in earlier studies (Brierley & Wood, 2001; Isnansetyo *et al.,* 2011). The nitrification process manifested in sediments of up to 6-9 cm throughout all seasons which proved that the nitrifiers were present at such depth. The PN rates do not decrease with the passage of incubation time at chlorate concentration of 15 mM but at 30 mM, the rates gradually started to deplete. The inhibitor started to take visible effect after $5^{th}$ hour of incubation. The PN rate is increased in R sediment because the Sandspit mangrove area have muddy soil and high clay content (Farooqui, 2012 ) and clay due to its small size and potential to exchange cations results in the ability to exhort a positive effect on microbial processes associated with soil and water (Macura & Stotzky, 1980; Hoffmann *et al.,* 2007). NR sediment have relatively low water potential which can somewhat limit the bacterial movement (Berg & Rosswall, 1987) and result in less PN values. The amount of substrate which is flowing continuously and may be changing slowly with seasons can also be the factor to control decrease in rates through pre-monsoon, increase in monsoon and then decrease again post-monsoon. Another reason for year-long PN activity is the presence of these bacteria in form of very loose to tough slime layer creating a restrictive barrier between water stress and air drying. The formation of micro colonies within soil particles helps nitrifier survival up to 9 cm deep in soil (Marshall, 1976; Klemedtsson *et al.,* 1987). There was no significant relationship between nitrification rates and physicochemical parameters, however, there was a strong correlation between physicochemical factors such as water-soil pH and dissolved oxygen-temperature. It was also observed that there was a significant correlation in between PN rate and incubation time. The PN rates of two sediment types (R and NR) were also positively correlated with sections (T, M, B) respectively ($p<0.005$).

In future, multidisciplinary approach is required to understand the role of nitrifiers in nitrification and their relationship between various physico-chemical aspects as limiting factors such as carbon content, total nitrogen, chlorophyll and nutrient ion content. This may gain better understanding of nitrification process taking place in Sandspit backwaters mangrove forests.

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**References**

Abbasi, M.K., Z. Shah and W.A. Adams. 2001. Mineralization and nitrification potentials of grassland soils at shallow depth during laboratory incubation. *J. Plant Nutr. Soil Sci.,* 164: 497-502.

Ahmedani, M.A., S. Yasmin, M.A. Arain, K.H. Shah, H. Abro, and M.A. Nahyoon. 2007. Effect of bactericidal plants treatments on urea hydrolysis and nitrification in soils of different cropping histories. Part-2. Nitrification. *Pak. J. Bot.,* 39(7): 2673-2680.

Ali, R., H. Kanwal, Z. Iqbal, M. Yaqub, J.A. Khan and T. Mahmood. 2012. Evaluation of some nitrification inhibitors at different temperatures under laboratory conditions. *Soil Environ.,* 31(2): 134-145.

Ali, R., J. Iqbal, G.R. Tahir and T. Mahmood. 2008. Effect of 3, 5-dimethylpyrazole and nitrapyrin on nitrification under high soil temperature. *Pak. J. Bot.,* 40: 1053-1062.

Amin, M. and T. Flowers. 2004. Effect of two applications of substrate on nitrification and pH of soils. *J. Res. Sci.,* 15(3): 263-269.

Belser, L. W. and E.L. Mays. 1980. Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Appl. Environ. Microbial.,* 39(3): 505-510.

Berg, P. and T. Rosswall. 1987. Seasonal variations in abundance and activity of nitrifiers in four arable cropping systems. *Microb. Ecol.,* 13(1): 75-87.

Brierley, E.D.R. and M. Wood. 2001. Heterotrophic nitrification in an acid forest soil: isolation and characterisation of a nitrifying bacterium. *Soil Biol. Biochem.,* 33(10): 1403-1409.

Cleemput, O.V. and A.H. Samater. 1995. Nitrite in soils: accumulation and role in the formation of gaseous N compounds. *Fert. Res.,* 45(1): 81-89.

Elser, J.J., M.E. Bracken, E.E. Cleland, D.S. Gruner, W.S. Harpole, H. Hillebrand, J.T. Ngai, E.W. Seabloom, J.B. Shurin, and J.E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Let.,* 10(12): 1135-1142.

Farooqui, Z. 2012. *Studies on Mangrove ecosystem with respect to Biodiversity, Productivity and Nutrient dynamics*. Institute of Marine Sciences, University of Karachi, Karachi, Pakistan.

Farooqui, Z., S. Shafique, K.L. Khan, A. Ali, P. Iqbal and P.J.A. Siddique. 2012. Assessment of litter production in semi-arid mangroves forests near active Indus river mouth (Hajambro creek) and Karachi backwaters, Pakistan. *Pak. J. Bot.,* 44(5): 1763-1768.

Gill, S., M. Abid, and F. Azam. 2006. Root-induced changes in potential nitrification and nitrate reductase activity of the rhizospheric soil of wheat (*Triticum aestivum* L.) and chickpea (Cicer arietinum L.). *Pak. J. Bot.* 38.4: 991.

Hansen, J.I., K. Henriksen and T.H. Blackburn. 1981. Seasonal distribution of nitrifying bacteria and rates of nitrification in coastal marine sediments. *Microb. Ecol.,* 7(4): 297-304.

Hoffmann, H., M., Schloter and B.M. Wilke. 2007. Microscale-scale measurement of potential nitrification rates of soil aggregates. *Biol. Fert. Soils*, 44(2): 411-413.

Hou, L.J., M. Liu, S.Y. Xu, D.N. Ou, J. Yu, S.B. Cheng, X. Lin and Y. Yang. 2007. The effects of semi-lunar spring and neap tidal change on nitrification, denitrification and N 2 O vertical distribution in the intertidal sediments of the Yangtze estuary, China. *Estuarine, Coastal & Shelf Sci.*, *73*(3): 607-616.

Isnansetyo, A., N.D. Thien and M. Seguchi. 2011. Potential of mud sediment of the Ariake Sea tidal flat and the individual effect of temperature, pH, salinity and ammonium concentration on its nitrification rate. *J. Environ. Earth Sci.* 3: 587-599.

Klemedtsson, L., P. Berg, M. Clarholm, J. Schnürer and T. Rosswall. 1987. Microbial nitrogen transformations in the root environment of barley. *Soil Biol. Biochem.,* 19(5): 551-558.

Krishnan, K.P. and P.L. Bharathi. 2009. Organic carbon and iron modulate nitrification rates in mangrove swamps of Goa, south west coast of India. *Estuarine, Coastal & Shelf Sci.*, 84(3): 419-426.

Kristensen, E., M.H. Jensen, G.T. Banta, K. Hansen, M. Holmer, and G.M. King. 1998. Transformation and transport of inorganic nitrogen in sediments of a Southeast Asian mangrove forest. *Aquat. Microb. Ecol.,* 15: 165-175.

Luo, Z., Z. Qiu, Q. Wei, G.D. Laing, Y. Zhao and C. Yan. 2014. Dynamics of ammonia-oxidizing archaea and bacteria in relation to nitrification along simulated dissolved oxygen gradient in sediment–water interface of the Jiulong river estuarine wetland, China. *Environ. Earth Sci.*, 72(7): 2225-2237.

Macura J. and G. Stotzky. 1980. Effect of montmorillonite and kaolinite on nitrification in soil. *Folia Microbiol.*, 25: 90-105.

Marshall, K.C. 1976. *Interfaces in microbial ecology*. HUP, Cambridge, Mass., USA.

Miranda, J., K.K. Balachandran, R. Ramesh and M. Wafar. 2008. Nitrification in Kochi backwaters. *Estuar. Coastal & Shelf Sci.,* 78: 291-300.

Olsson, M.O. and U. Falkengren-Grerup. 2000. Potential nitrification as an indicator of preferential uptake of ammonium or nitrate by plants in an oak woodland understorey. *Ann. Bot.,* 85(3): 299-305.

Rabalais, N.N. 2002. Nitrogen in aquatic ecosystems. *AMBIO,* 31(2): 102-112.

Russow, R., C.F. Stange and H.U. Neue. 2009. Role of nitrite and nitric oxide in the processes of nitrification and denitrification in soil: results from 15 N tracer experiments. *Soil Biol. Biochem.* 41(4): 785-795.

Saifullah, S.M. 1997. *Management of the Indus Delta mangroves.* In: *Coastal zone Management Imperative for Maritime Developing Nations*. (Eds.): B.U. Haq, S.M. Haq, G. Kullenberg and J.H. Stel. 333-346. Kluwer-Academic-Publ., Dordrecht-The-Netherlands.

Schmidt, E.L. and L.W. Belser. 1982. *Nitrifying bacteria: Methods of soil analysis. Part 2. Chemical and microbiological properties.* (2nd Ed.): 1027-1042. ASA, SSSA.

Shafique, S., P.J.A. Siddiqui, R.A. Aziz and N. Shoaib. 2013. Variations in carbon and nitrogen contents during decomposition of three macroalgae inhabiting Sands pit backwater, Karachi. *Pak. J. Bot.*, 45(3): 1115-1118.

Siddiqui P.J.A., S. Farooq, S. Shafique, Z. Burhan and Z. Farooqi. 2008. Conservation and management of biodiversity in Pakistan through the establishment of marine protected areas. *Ocean & Coastal Mang.*, 51: 377-38.

Singh, J.S. and A.K. Kashyap. 2007. Variations in soil N-mineralization and nitrification in seasonally dry tropical forest and savanna ecosystems in Vindhyan region, India. *Trop. Ecol.*, 48(1): 27-36.

Strickland, J.D.H and T.K. Parsons. 1972. *A practical hand book of sea water analysis*. (2nd Ed.) Bull. Fish, Res. Bd. Canada.

Tam, N.F.Y., A.H.Y. Wong, M.H. Wong and Y.S. Wong. 2009. Mass balance of nitrogen in constructed mangrove wetlands receiving ammonium-rich wastewater: effects of tidal regime and carbon supply. *Ecol. Engineering*, 35(4): 453-462.

Yan, D., Q. Wang, L. Mao, T. Ma, Y. Li, C. Ouyang, M. Guo, and A. Cao. 2015. Interaction between nitrification, denitrification and nitrous oxide production in fumigated soils. *Atmospheric Env.* 103: 82-86.

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