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Chapter 8

Nutrient Fluxes and Their Dynamics in the Inner Izmir Bay Sediments (Eastern Aegean Sea)

Ebru Yesim Ozkan, Baha Buyukisik and Ugur Sunlu

Additional information is available at the end of the chapter

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1. Introduction

Nitrogen, which is the most limiting nutrient for marine productivity of the ecosystem, is an essential element contributing to the biological process of all organisms (Capone and Knapp 2007; Bertics et al. 2012). Nitrification, the sequential oxidation of amonia to nitrite and then nitrate by nitrifiers, is also a critical step in the biological removal of nitrogen from the wastewater treatment process. In coastal ecosystems, nitrification is often coupled to denitrification (Jenkins and Kemp 1984; Sebilo et al. 2006), ultimately resulting in nitrogen being returned to the atmosphere (Bernhard and Bollmann 2010). Most transformation reactions recognized so far in the benthic N cycle are catalyzed by a suite bacteria and include the release of ammonium during the degradation of organic matter, the aerobic oxidation of ammonium to nitrite and nitrate(nitrification) and the bacterial denitrification of nitrite and nitrate to N_2 under anaerobic conditions. Denitrification and nitrification are two of the main bacterial pathways responsible for inorganic nitrogen removal and speciation in estuaries (Rao et al. 2007). Denitrification, dissimilatory reduction of NO_3^- and NO_2^- to N_2 and N_2O, is recognized as the key process to maintain nitrogen limitation for primary production in marine environments. As a consequence, denitrification is important in controlling the eutrophication level in coastal environments that are increasingly affected by nutrient inputs (Cloern 2001; Poulin et al. 2007). The exposure of nutrient inputs removed by denitrification in sediments of continental margins leads to high bacterial mineralization. In addition, microbially mediated nitrogen transformations have a potential impact on coastal eutrophication and estuarine oxygen status (Balls et al. 1996; Sanders et al. 1997; Barnes and Owens 1998).

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Sediments play a key role in the global N cycle, especially in regard to the sinks and sources of fixed N (Capone and Knapp 2007; Carpenter and Capone 2008), is incomplete and often debated (Bertics et al. 2012). In sediments impacted by bioturbation, nitrification and denitrification are closely coupled yet spatially separated by the oxic/anoxic interface, and the interactions of aerobic and anaerobic processes lead to a relatively complex overall regulation of N_2 formation (Nishio et al. 1983; Jenkins and Kemp 1984; Seitzinger 1988; Christensen et al. 1989, Rysgaard et al. 1994; Thamdrup and Dalsgaard 2000).

The dependence of benthic denitrification derived from in situ nitrification and nutrient fluxes from overlying water into sediments may be controlled by several factors such as temperature, salinity, pH, dissolved oxygen and mineralization. Additionally, burrowing sediment infauna can also greatly influence the oxic conditions of surface sediments, especially by transporting oxygen down into the sediment, otherwise anoxic zones can develop in the sediment (Christensen et al. 2003). Several studies have stressed the important role of nitrification, denitrification and nutrient flux in coastal marine sediments (Jenkins and Kemp 1984; Rysgaard et al. 1994; Christensen et al. 2003;, Capone and Knapp 2007), but this study is the first report on İzmir Bay. This paper was,

1. To measure sediment-water fluxes of nitrogen and phosphorus at sites sampled
2. To quantify nutrient fluxes across the sediment-water interface in order to assess the relative importance of benthic nutrient recycling
3. To compare fluxes and denitrification measurements among the sampling sites.

2. Materials and methods

2.1. Study area

İzmir Bay is one of the great natural bays of the Mediterranean. It is divided into three sections (outer, middle and inner) according to topographic points of view (Figure 1). It is an important semi-enclosed basin and has been increasingly polluted with massive loads of contaminants discharged from various anthropogenic sources.

This research was conducted in the inner İzmir Bay which is located 38° 20N and 38° 40N latitude and 26° 30E and 27° 10E longitude. Inner İzmir Bay is a shallow estuarine with a surface area of 66,68 km^2 located in the eastern Aegean Sea. Fine-grained sediments with high values of water content are the characteristics of the inner bay. It is suggested that the eastern Aegean Sea acts as an effective trap for nutrients and autochthonous particulate organic matter. The quality of the water and sediment in the inner İzmir Bay is seriously affected by pollutants which enter through drains that bring domestic as well as industrial effluents and discharge into the river and also from the sewage system that pumps treated effluent into the bay water. The main industries in the city include food processing, beverage manufac-

turing and bottling, tanneries, oil soap and paint production, chemical industries, paper and pulp factories, textile industries, metal and timber processing (Küçüksezgin 2001). The inner part of the Izmir Bay, has an average depth of 15 m, a small volume relative to the other parts of the Bay. The main fresh water sources of the bay are Melez, Bayraklı, Bostanlı and Poligon rivers. The rivers' flow typically peaks in late winter and early spring and is at a minimum in the summer to fall months.

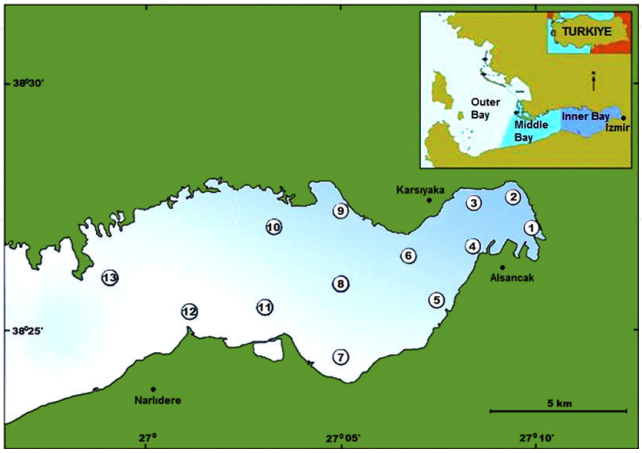

Figure 1. Map of inner İzmir Bay and the sampling stations.

2.2. Analytical procedure

The sediment samples used in this study were taken by a 4.0 cm ID gravity corer with removable acrylic liners. In addition, water samples were collected vertically and temperature, salinity, pH and dissolved oxygen content were measured. Based on the knowledge gained from a full seasonal study in the inner bay, sampling was chosen at 13 stations in three sampling seasons (summer, autumn and summer) during 2007-2008. Every trial has been replicated. After the lower lids of the cores were closed, they were filled with bottom water. Each of the water levels over the sediment in the acrylic cores was measured in millimeter (mm). Surface water was passed from the two incubation tanks by a submerged pump to stabilize water temperature. Each of the sediment cores was placed in two tanks, where nutrient fluxes were measured in the dark condition (Figure 2). As a starting process (t=0), 50 ml HDPE (high density polyethylene) bottles were filled with water and then put into an ice bag at -78°C. The reduced water level in the cores was compensated by bottom water. All the sediment cores taken from the 13 stations were coated with dark acetate papers. Starting and finishing times were noted and incubation times of the cores were around 24 hours. Control incubations were made with cores including only bottom water. The core tubes were closed by their lids with two Luer lock injectors on, one of which was filled with bottom water and the other pressed empty.

Figure 2. The incubation of core liners in the constant temperature tank on the vessel.

When the injector filled with bottom water was pushed to homogenize the overlying water in the core tube, the water was elevated in the other injector to stabilize pressure. This process was repeated prior to both starting and finishing trials. After 24 hours the lids were removed and the 50 ml sample was siphoned out of the core and frozen on dry ice until further analysis (Ozkan and Buyukisik 2012).

2.3. Nutrients and flux measurements

The pore waters were obtained after the incubation experiments. The first 10 cm part of the sediment from the core samples was put into a sediment pore water squeezer system and compressed through a two-layer Whattman GF/C glass filter paper to obtain clear water. The water samples were diluted 10-100 fold prior to nutrient analysis. The nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, PO_4^{-3}) were measured according to Strickland and Parsons (1972).

Seawater temperature was recorded by an electronic thermometer, the pH of the samples was measured on-site using a pH-meter, and also dissolved oxygen was determined by using the Winkler method immediately after sampling.

The nutrient fluxes were measured from both the controls and experimental series calculated as μmol/m^2 day. Flux measurements were made by formula,

$$J_{gross} = V_t \left(C_t - C_0 \right) \ / \ \Delta t \ A \tag{1}$$

where J_{gross} : gross nutrient flux (μmol/m^2 day), V_t : water volume on the core surface at time (t), C_t: solute concentration at time (t), C_0: initial solute concentration in the experiment, Δt: time of sampling (day with the begining of the experiment being 0), A: core surface area (cm^2).

The rate of flux measured was regarded as the gross flux. In addition, it measured only nutrient flux of overlying wateras a control (C_{cont}). The net flux was calculated from the difference between solute concentration at time (t) and solute concentration in control at time (t). So net flux was calculated by

$$J_{net} = \left(C_t - C_{cont}\right).\ 24.\ h.\ 10000 /\ \Delta t\ A \tag{2}$$

where J_{net} : net nutrient flux (μmol/m^2 day), V_t : water volume on the core surface at time (t), C_t: solute concentration at time (t), C_{cont}: solute concentration at time (t) in control cores, Δt: time of sampling (day with the begining of the experiment being 0), A: core surface area (cm^2), h: height of overlying water (m).

2.4. Statistical analysis

The data set has been subjected to factor analysis for elucidating the relationships between nutrient fluxes and physicochemical parameters such as temperature, pH, dissolved oxygen and salinity.

3. Results

The results from incubation tests were classificated as three groups. The first one of them is measurement of flux from processes at overlying water (control). The second one is measurement of gross flux. The third one is calculated as net flux from sediment. NO_3^- contribution of overlying water via nitrification was seen at most of the stations (Figure 5). In the bottom water, negative flux values for NO_3^- can be assumed as dark uptake for phytoplankton if dissolved oxygen is sufficient. The values of gross fluxes from direct measurements of incubations show that there is a NO_3^- flux directed from sediment to water at most of the stations in winter. The highest values of gross fluxes were observed in stations 2 and 9 (Figure 6). In summer and autumn, gross flux values are lower than they are in winter. Net NO_3^- fluxes from sediment to overlying water were given in Figure 5. Negative values were seen at many stations. The loss of nitrate by denitrification in sediment was at a maximum at stations 6 and 12 in winter and at station 3 in summer. In Figure 6, the NH_4^+ flux values of overlying water were generally negative, indicating a nitrification process. Positive values at station 6 in summer, and at station 7 and 12 in winter were observed indicating mineralization of organic matter. Many of the core samples were showed positive gross NH_4^+ fluxes, explaining the mineralization of organic matter (Figure 5). The negative values of net NH_4^+

fluxes were relatively lower at stations 6 and 7 and higher at station 12, indicating nitrification in sediment (Figure 6). In Figure 3 and 4, controls and net fluxes were plotted for ammonium and nitrate. The first 10 stations showed that mineralization and nitrification processes had been taken place in sediment and overlying water respectively. A denitrification process was taking place in sediment. Negative nitrate fluxes in overlying water can be explained by diffusive processes to sediment for denitrifying. The general trend in changing fluxes from summer to winter was a decreasing of nitrification in overlying water and mineralization in sediment. Convective water mixing in winter caused increased diffusive transport of the dissolved species and increased denitrification. Also nitrification is taking place in sediment and overlying water.

In winter, net 289 kg NH_4^+ per day nitrified in sediment of the complete Inner Bay area when 643 kg per day is nitrified in overlying water. 1135 kg NH_4^+-N per day is produced in sediment of the Inner Bay area. 45% of total NH_4^+-N flux is nitrified in overlying water. In summer, 4147 kg NH_4^+-N loading from sediment was found. 50% of ammonium loading is nitrified in overlying water.

In summer, 479 kg NO_3^--N per day is produced by nitrification in water. 574kg per day is removed by denitrification when 856 kg/day is released to water. Net nitrate loading is 282 kg/day. 67% of nitrate loading is removed by the denitrification process. In winter, 2583 kg nitrate per day is produced in overlying water. 2145 kg nitrate per day is produced by nitrification in sediment while 1398 kg /day is removed by denitrification. 65% of nitrate loading is removed by denitrification.

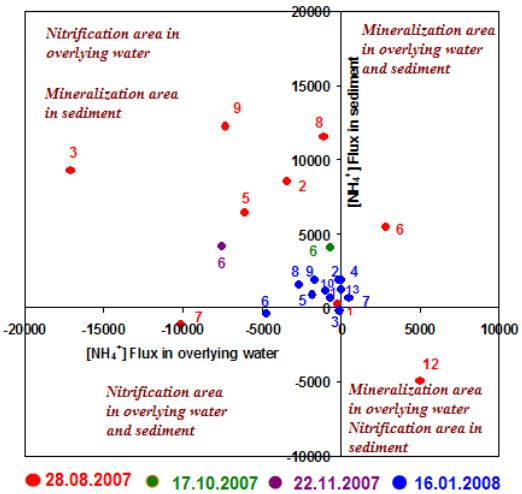

Figure 3. The plot of NH_4^+ fluxes of overlying water against net NH_4^+ fluxes in sediment.

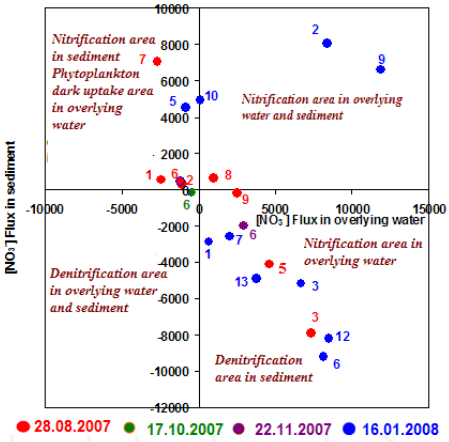

Figure 4. The plot of NO_3^- fluxes of overlying water against net NO_3^- fluxes in sediment (µmolN/m²day).

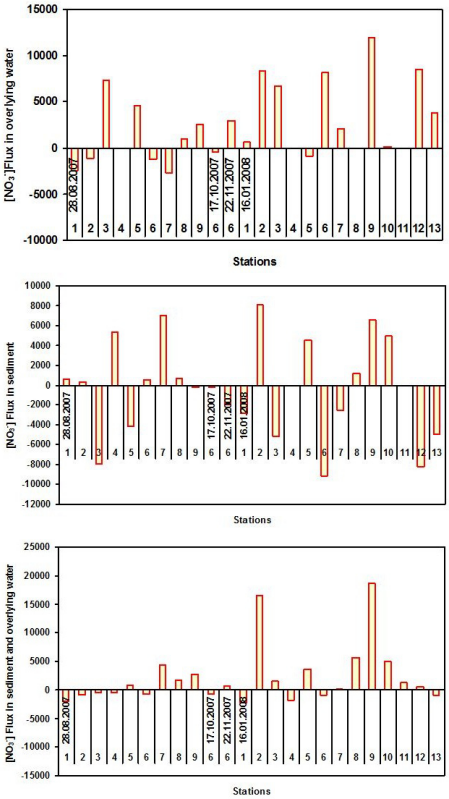

Figure 5. Control, gross and net NO_3^- fluxes of experimental core samples (µmol/m² day).

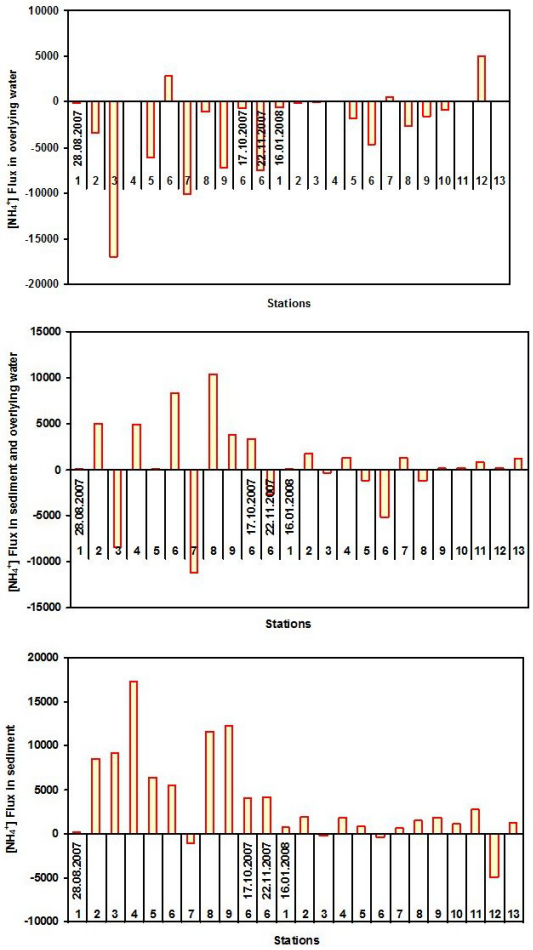

Figure 6. Control, gross and net NH_4^+ fluxes of experimental core samples (μmol/m^2 day).

3.1. Factor Analysis

Factor analysis is a powerful statistical technique, which delineates simple patterns distributed among complex data by evaluating the structure of the variance-covariance matrix and extracting a small number of independent hypothetical variables (R mode) or sample (Q mode) referred to as factors (Klovan and Imbrie 1971, Duman et al. 2006). Principal component analysis (PCA) were used as type of factory. PCA seeks a linear combination of variables such that the maximum variance is extracted from the variables. It then removes this variance and seeks a second linear combination which explains the maximum proportion at the remaining variance, and soon. This method results in uncorrelated factors.

Data variables were chosen as: NH_4FluxGross (sediment+water), NH_4FluxWater, NH_4Flux-Sediment, NO_3FluxGross (sediment+water), NO_3FluxWater, NO_3FluxSediment, RP, NO_3, TIN (total inorganic nitrpgen), pH, DO (dissolved oxygen),T (temperature), S (salinity). The factor analysis results for the raw data show that five factors extracted account for 86,99% of the total variance. Remaining variance was assumed to be random. In Table 1, eigen values, percentage of variances and cumulative variances were given. The eigen value for a given factor measures the variance in all the variables which is accounted for by that factor. Kaiser criterion was chosen for dropping all components with eigen values under 1.0 as usual in most statistical software. The factor analysis results for the raw data show that five factors account for a total of 86.98% of the total variance. Remaining 13.02% of the variance cannot be explained by factor analysis and is assumed to be random. Factor loading matrix after varimax rotation was also given in Table 2. Factor loadings are the correlation coefficients between the variables and factors.

Factor Number	**Eigenvalue**	*Percent of Variance*	*Cumulative Percentage*
1	4,2628	32,791	32791
2	2,48938	19,149	51940
3	1,92521	14,809	66,749
4	1,61264	12,405	79,154
5	1,01821	7,832	86,986
6	0,838686	6,451	93,438
7	0,402971	3,100	96,538
8	0,275157	2,117	98,654
9	0,0934268	0,719	99,373

Table 1. The eigen values and variances and extracted factors.

The 3D plot and 2D plot of factor loadings were seen in Figure 7. Factor 1 explains 32,79% of total variance and includes the mineralization and nitrification processes in the overlying water and the related variables as pH, DO and temperature. Factor 1 can be described as " overlying water factor ". Factor 2 explains 19,15% of total variance and includes bottom water nitrate concentrations, total inorganic nitrogen (TIN) and the bottom water salinity. In Figure 8a, inverse relationships were found between sediment pore water NH_4^+ concentrations and bottom water salinity. NH_4^+ release from clay minerals in high salinities can be important. This explaines the decreased NH_4^+ pore water concentrations in high salinity bottom waters because of the obtaining of pore water samples after the core incubation ex-

periments. Factor 2 can be described as "anthropogenic factor ". Factor 3 accounts for 14,81% of total variance and includes gross and net sediment fluxes and overlying water processes for NO_3^-. The nitrification process in overlying water has a negative effect. Factor 3 can be described as "NO_3^- flux factor ". Factor 4 accounts for 12,41% of total variance and includes gross fluxes in nitrogen species, mineralization in overlying water and sediment, nitrification in overlying water and denitrification in sediment. Factor 4 can be described as " biological processes on nitrogen species ". Factor 5 explaines 7,8% of total variance and includes reactive phosphorus (RP) in bottom water. Ammonium and RP releases from decomposition of organic matter in 16:1 ratio and ammonium is consumed and oxidized to nitrate in the same N:P ratio. In denitrification, this ratio was reported as 104:1 and inorganic nitrogen distributions are effected. Nitrate deficite values (=16xRP-NO_3^-) do not reflect the truth. The second term in the right hand side of the equation for inner Izmir Bay waters was much lower than the first term. This situation has been explained by Ozkan and Buyukisik (2012) by means of RP release from sediment via Fe mobilization. Factor 5 can be described as "iron and RP mobilization factor".

	Factor	*Factor*	*Factor*	*Factor*	*Factor*
	1	**2**	**3**	**4**	**5**
NH4FluxGross	-0,215254	-0,194004	0,00849782	**0,845738**	0,104213
NH4FluxW	**0,484817**	-0,0461229	0,0452335	**0,840061**	0,0817993
NH4FluxSed	**-0,778075**	-0,168951	-0,0475222	0,00121901	0,0257112
NO3FluxGross	0,262306	-0,120293	**0,737525**	**-0,417474**	0,0243472
NO3FluxW	**0,453693**	-0,162346	**-0,719379**	**-0,34443**	0,0872775
NO3FluxSed	-0,190756	0,0252568	**0,973636**	0,091898	-0,0353923
RP	-0,0563268	-0,0754223	-0,0494444	0,135157	**0,965347**
NO3	0,109249	**0,901002**	0,00312072	0,183428	-0,132118
TIN	0,0905194	**0,892973**	0,0267146	**-0,329392**	-0,174481
pH	**0,917485**	0,148011	-0,189637	0,0547122	-0,10158
DO	**0,879905**	0,0185277	-0,092712	-0,127195	0,183948
T	**-0,913059**	-0,210555	0,068695	-0,0684956	0,159605
S	-0,259059	**-0,815551**	-0,0017208	0,173195	-0,184399

Table 2. Factor Loading Matrix After Varimax Rotation.

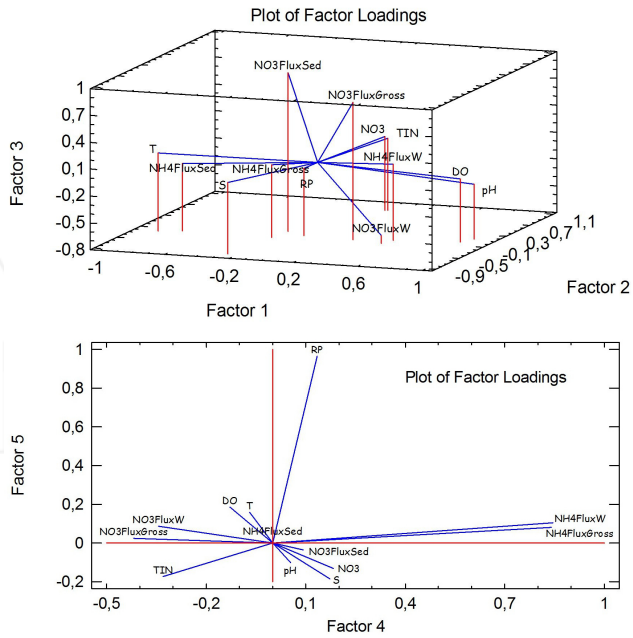

Figure 7. and 2D representation of factor loadings.

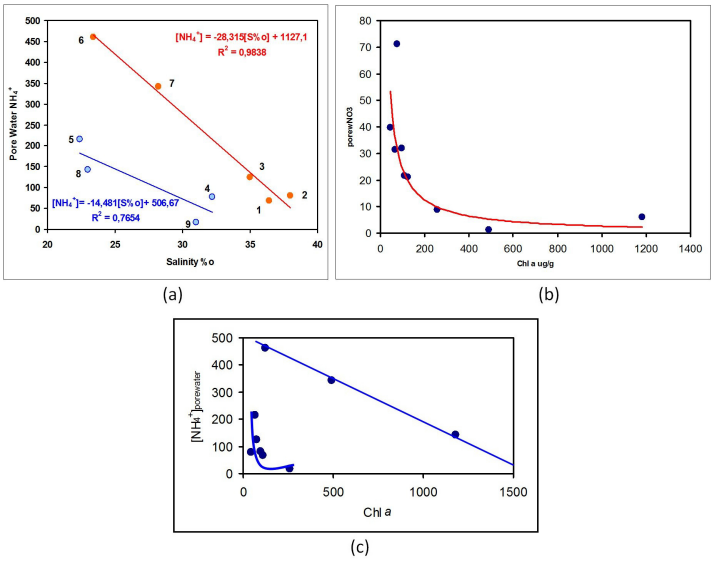

Figure 8. a)The relationships of salinity against the pore water NH_4^+ concentration in sediment samples after the incubation experiments. The plot of surface sediment Chl a (µg/g) values against pore water NO_3^- concentrations (b) and pore water ammonium concentrations (c).

Relative importance of ANAMMOX for dinitrogen producing inversely related with remineralized solute (NH_4^+)production, benthic oxygen demand and surface sediment chlorophyll a values (Engström et al. 2005). In Figure 8 b and 8c, Chl-*a* values in sediment surfaces at stations 1 to 9 changed in the range of 43.9 - 1180.9 μg/g. Anammox process is not expected as an important process in stations 1 to 9. Pore water nitrate and ammonium concentrations decreased hyperbolically/linearly with increasing Chl a values in sediment (Figure 8b, 8c).

4. Conclusion

Although the waste water treatment plant reduces ammonia inputs, creeks have high ammonium concentrations (Ozkan et al. 2008) and enrich increasingly the surface waters via rainfall. In the bottom waters, another ammonium sources results from the sinking of organic matter produced by primary production of phytoplankton in the surface waters and mineralization of it in sediment by bacteria. NH_4^+ production is released to bottom water in relation to bottom water salinity and contributed to bottom water reserves. Nitrification in the bottom water is the dominant process except at station 12. Nitrate is produced by this process and diffused in to the sediment. A denitrification process is taking place in suboxic sediments and produces dinitrogen gas as a loss process of nitrogen. Some of the mineralized ammonium in sediment is oxidized to N_2 gas by the anammox process if the sediment contains MnO_2 and it does not reach to ≥1 μgChl-a/g sediment and ≥2 μMNH_4^+/h (Engström et al. 2005) in the bay. Only in station 12 at the boundary of inner Izmir Bay, ammonia and nitrate loss can be attributed to anammox and the denitrification process. Creeks provide MnO_2 to stations 1,2,3,4 and 9 upto 1,5 μMMnO2/g sediment. Sediments of other stations (except station 12) do not have Mn because hypereutrophication caused anoxia in the bottom waters before the wastewater treatment plant and mobilization of reduced Fe and Mn may have been transported out of the inner Izmir Bay (Ozkan and Buyukisik 2012). Natural treatment of nutrients in the benthic area can contribute to reduced nitrogen levels in the inner Izmir Bay after the enrichment of sediments with Mn and Fe, but it will take some time.

Factor analysis discriminates five factors with a low number of variables. Mineralization and nitrification in the overlying water is affected by pH, DO and temperature (factor 1:overlying water factor). Bottom water nitrate and TIN is negatively effected by salinity (factor 2: anthropogenic factor). Factor 3 was described as "NO_3^- flux factor". The nitrification process in overlying water affects the factor 3. Factor 4 explains the biological process on nitrogen species (TIN). Factor 5 clarifies the RP does not statistically effect on the other variables and supports that RP fluxes are related with Fe and RP mobilization from sediment. RP coming from mineralization of organic matter comprises 0.3-6.9 % of bottom water. RP concentrations cannot be used for the evaluation of denitrification, nitrification and N_2 fixation processes (Tyrell and Lucas, 2002).

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Author details

Ebru Yesim Ozkan*, Baha Buyukisik and Ugur Sunlu

*Address all correspondence to: ebru.yesim.koksal@ege.edu.tr

Ege University, Faculty of Fisheries, Dept. of Hydrobiology, Turkey

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