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Stable isotopes as a tool for nitrogen source identification and cycling in the Gulf of Trieste (Northern Adriatic)

Samo Tamše [a,b], Patricija Mozetič [c], Janja Francé [c], Nives Ogrinc [a,b,*]

[a] Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
[b] Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia
[c] Marine Biology Station, National Institute of Biology, Fornače 41, 6330 Piran, Slovenia

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ABSTRACT

Nitrogen concentrations and isotopic composition were used to identify nitrate sources and processes in the catchments of two Slovenian coastal rivers, Rižana and Dragonja, and to investigate whether they have any notable influence on the Gulf of Trieste. The sampling was performed from May 2010 to March 2011. The data indicated that $\delta^{15}N_{NO_3}$ values in rivers provided mainly information about sources of riverine nitrate derived from soil nitrification, sewage, and/or manure. The load weighted $\delta^{15}N_{NO_3}$ values of 4.3‰ and 9.4‰ determined at the mouth of Rižana and Dragonja, respectively, were largely controlled by land-use in the riverine catchment. Phytoplankton nitrate assimilation was found to be significant mainly at the mouth of Dragonja. The combined use of salinity, nutrient concentrations, and nitrate, particulate nitrogen and carbon isotopic compositions revealed that the seawater surface was influenced by mixing with different sources including seawater, rivers and sewage effluent. The site influenced by sewage effluent is relatively spatially isolated, which suggests that NO_3^- is not widely distributed by this point source. Besides mixing, phytoplankton uptake was the main process controlling the distribution and isotopic composition of NO_3^- in the marine system and was more extensive in spring 2010, while in autumn NH_4^+, not NO_3^-, was the dominant source of N for phytoplankton. In addition, our results are consistent with the occurrence of nitrification in the water column in autumn and winter. The nitrification activity was higher in autumn, while in winter it occurred in parallel to phytoplankton uptake. This study demonstrates how stable isotopes of nitrogen in nitrate along with concentration measurements can be relevant when trying to identify the sources and key processes such as assimilation and nitrification in rivers and coastal areas.

1. Introduction

Coastal seas are one of the most valuable habitats on Earth (Jickells, 1998; Harley et al., 2006) because of their elevated rates of primary production and active biogeochemical cycling of nitrogen (N) and carbon (C) (Walsh, 1991). However, there is a strong scientific consensus that these ecosystems are threatened by anthropogenic global climate change (IPCC, 2001; Meyers, 1997). In the North Adriatic Sea, climate change is expected to have significant consequences for the marine environment in terms of higher temperature, salinity and production (Alcamo et al., 2007). Seawater temperatures increased in the Gulf of Trieste during the period 1991–2003 (Malačič et al., 2006) while a decrease in nutrient concentrations and chlorophyll *a* (Chl *a*) was recently observed (Mozetič et al., 2010). Changes in the ecology and chemistry of the Gulf of Trieste are driven primarily by the input of riverine nutrients, which lead to phytoplankton blooms and, occasionally, the appearance of mucilage (Malone et al., 1999; Giani et al., 2005; Faganeli et al., 2009). Prior to the recent observed decreases in nutrient inputs (Mozetič et al., 2010), bottom waters in the gulf displayed periodic hypoxia and occasionally anoxia (Faganeli et al., 1991; Stachowitsch, 1991), resulting in massive mortalities of benthic fauna during these events (Stachowitsch, 1991). Cozzi and Giani (2011) estimated the input of total nitrogen (TN) and total phosphorus (TP) from rivers into the eastern part of the Gulf of Trieste for 2001 to be 645 t N yr^{-1} and 9 t P yr^{-1}, respectively, the former representing about 4% of total annual riverine N delivered to the Gulf of Trieste. These authors showed that nutrient transport from minor rivers, even though an order of magnitude lower than that from the River Po, was significant in relation to runoff.

In order to address the issue of nutrient (mainly nitrogen) loading in coastal ecosystems, we need tools for the identification

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[*] Corresponding author at: Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia. Tel.: +386 15885387; fax: +386 15885346.
E-mail address: nives.ogrinc@ijs.si (N. Ogrinc).

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of N nitrate (NO_3^-) sources. Estimates of NO_3^- sources, especially non-point (diffusive) ones are needed to guide management of aquatic environments. It is clear that NO_3^- inputs include both point and non-point pathways linking land to water, but source assignment remains a challenge. Non-point NO_3^- pollution poses a particular problem, since it is characterised by sources that are generally difficult to detect and by fluxes that are highly variable in time (Carpenter et al., 1998; Thornton et al., 1999). Furthermore, the fate of NO_3^- depends also on "internal" processes that may transform, immobilise or eliminate NO_3^- in the aquatic system, making source assignment even more challenging. Stable isotopes can be an effective and reliable tool to investigate the biogeochemical cycling of N as well as to identify NO_3^- sources in aquatic systems (Altabet, 1996; Harrington et al., 1998; Hebert and Wassenaar, 2001; Chang et al., 2002; Mayer et al., 2002; Voss et al., 2006; Xue et al., 2009). This is possible because the isotopic signature of nitrogen ($\delta^{15}N$) in NO_3^- from synthetic fertilizers, soil, and manure-derived sources differs sufficiently to enable an unambiguous distinction between them. For example, $\delta^{15}N$ values for NO_3^- from synthetic fertilizers range from -3 to 3‰ while those for NO_3^- derived from animal manure range from 10 to 25‰ (Kendall et al., 2008). NO_3^- is an important nutrient and electron acceptor and therefore very reactive in aquatic systems. Without understanding how NO_3^- cycling (e.g., denitrification, nitrification, assimilation) influences these source signatures, we are limited in our ability to distinguish precisely between different sources. Thus, an understanding of both source and processes is critical.

The main goal of this study was to determine the isotopic composition of nitrate N in two coastal rivers (Rižana and Dragonja) and at marine locations along the Gulf of Trieste where the greatest riverine impact was expected. The objective of the study was to identify nitrate sources and to determine whether there is any impact on the marine environment. We also determined the isotopic composition of C and N ($\delta^{13}C$ and $\delta^{15}N$) in particulate organic matter (POM) in order to identify the major mechanisms that influence the temporal variations of $\delta^{13}C$ and $\delta^{15}N$ in POM in the Gulf of Trieste.

2. Materials and methods

2.1. Study area

The study was carried out in the Gulf of Trieste, a shallow coastal area in the NE Adriatic Sea (Fig. 1). The Gulf of Trieste is a semi-enclosed basin with an area of approximately 500 km^2 and a maximum depth of only 25 m. Its oceanographic properties are under strong influence of freshwater inflows, mainly from the River Isonzo, and also from variable local meteorological events. However the flow of Isonzo River has reduced markedly in the last decades (Cozzi et al., 2012) with respect to previous data (average mean flow 123 m^3 s^{-1}; (Comici and Bussani, 2007)). It was found that the flow in the 2000s was around 51 m^3 s^{-1} (Cozzi and Giani, 2011) and is one of the causes of oligotrophication trends observed in the area. Surface salinity in its southern part usually varies between 30 and 38, while the water temperature is usually between 8 °C and 26 °C (Faganeli et al., 2009). A strong seasonal cycle results in the development of thermal stratification in the water column during summer, while in winter the water column is well mixed (Malačič, 1991).

Two major Slovenian coastal rivers that enter the Gulf of Trieste are the Rižana River (discharging into the Bay of Koper) and Dragonja River (discharging into the Bay of Piran). The Rižana is a 14 km long river with a drainage basin of approximately 212 km^2 and an average discharge of approximately 3.5 m^3 s^{-1}. The geological nature of its watershed is complex, featuring karstic hinterland with no surface streams in its eastern part, while the river's lower reaches consist of impermeable Eocene flysch bedrock. The groundwaters within the watershed emerge on this karst-flysch boundary as Rižana's karstic spring. The rest of the groundwater drains towards the Gulf of Trieste, recharging deep aquifer layers underneath the covering layers of flysh rocks and discharging at several submarine groundwater springs (Janža, 2010). A study by Faganeli et al. (2005) showed that the discharge of nutrients at these springs represents less than 4% of the total freshwater nutrient input. Therefore, it is widely accepted that surface freshwater discharges are the primary source of land-derived nutrient inputs to the Gulf of Trieste.

The Rižana River watershed consists of 67.2% forest, 28.8% agricultural land and 4.0% of urban and industrial areas (Table 2), with the majority of agricultural and industrial activity as well as population density being concentrated in the lower reaches of the river (CORINE Land Cover, 2006). Potential sources of nutrient pollution in the Rižana River include inadequately treated municipal and industrial wastewaters from the sewage treatment plant in Koper, located at the river mouth. The Rižana River is critical to Slovenian coastal communities as the only source of freshwater water in the region.

The Dragonja River is 27 km long with a drainage basin of approximately 90.5 km^2 and an average discharge of approximately 0.9 m^3 s^{-1}. Its watershed consists mostly of Eocene flysch deposits, except for its southernmost part, which consists of carbonate bedrock. The watershed consists of 59.8% forest, 39.3% agricultural land and 0.9% of urban areas (Table 2). The potential sources of pollution in Dragonja are agriculture, untreated sewage discharges from scattered settlements and industrial waste from a slaughterhouse operating in the area. As in the Rižana watershed, the majority of agricultural activity and population density is concentrated in the lower reaches of the river, where agricultural land represents $>50\%$ of the total area (CORINE Land Cover, 2006). Both rivers have Mediterranean flow regimes with highest average discharges in spring and autumn, and lowest during the summer.

2.2. Sampling procedure

Seawater samples were collected with Niskin bottles mounted on a Rosette sampler. Sampling took part in the SE part of the Gulf of Trieste and was conducted monthly from May 2010 to March 2011 at sampling locations F, K, and MA (Fig. 1). At each of these sites, samples were collected at the surface, and at 5, 10, 15 and 16 m depth, while at location ERI2, we sampled every second month during the study period and only at the surface and 5 m depth. Samples were filtered immediately upon arrival on shore (within 3 h of sampling) through 0.2 μm filters (Sartorius AG, Germany) into double deionized (DD) water rinsed HDPE bottles and frozen until analysis. Locations K, ERI2 and MA were selected due to an expected larger impact from Rižana and Dragonja, while location F was selected as a reference site because it receives almost no riverine inputs (Mozetič et al., 2008).

Temperature (T), salinity (S) and dissolved oxygen were measured in situ using the fine-scale CTD probe (Sea & Sun Technology GmbH). In May, August and November 2010 and March 2011 samples were taken for the determination of the isotopic composition of N in nitrate ($\delta^{15}N_{NO_3}$) and particulate nitrogen ($\delta^{15}N_{PN}$), and the isotopic composition of C in particulate organic carbon ($\delta^{13}C_{POC}$). 5 L of seawater from each depth were collected in HDPE bottles and filtered within 3 h of sampling through pre-combusted (480 °C for 4 h) Whatman GF/F glass-fibre filters. The filters were then dried at 40 °C and used for analyses of $\delta^{15}N_{PN}$ and $\delta^{13}C_{POC}$. The remaining water was