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## **Individuazione e caratterizzazione delle potenziali fonti dei nitrati nella Laguna di Marano e messa a punto di un piano di monitoraggio ambientale. Progetto FONIMAR. Parte II.**

**Riassunto:** Come già anticipato nella prima parte pubblicata nel numero 34 del Bollettino della Società Naturalisti "Silvia Zenari" (Dicembre, 2010) questo progetto che ha avuto come obiettivo principale quello d'individuare oltrechè differenziare le potenziali fonti dei nitrati in soluzione presenti nella laguna di Marano e parte del suo bacino drenante è stato coordinato dallo Joanneum Research di Graz per conto dell'ERSA del Friuli-Venezia Giulia. In questa seconda parte vengono esposti i risultati finali del progetto **FONIMAR** (Individuazione e caratterizzazione delle potenziali **FO**nti dei **NI**trati nella Laguna di **MAR**ano) ottenuti nei due anni di monitoraggio e campionamento delle acque. Questo studio è da considerarsi un esempio di ricerca integrata dove sono state combinate assieme diverse discipline scientifiche quali: l'idrochimica, la geochimica isotopica, l'idrologia ed il telerilevamento. Va sottolineato che per poter individuare l'origine dei nitrati in un ambiente acquatico estremamente complesso come quello lagunare è stato necessario utilizzare gli ultimi ritrovati della tecnologia presenti attualmente in Europa. Il metodo multi-isotopico adottato in questo studio per indagare l'origine dei nitrati si basano sull'abbondanza degli isotopi stabili di azoto e ossigeno della molecola di nitrato ( $^{15}\text{N}$ ,  $^{18}\text{O}$  e  $^{17}\text{O}$ ), idrogeno e ossigeno della molecola d'acqua ( $^2\text{H}$ ,  $^{18}\text{O}$ ),

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boro disciolto ( $6^{11}\text{B}$ ), zolfo e ossigeno della molecola di solfato ( $6^{34}\text{S}$ ,  $6^{18}\text{O}$ ) mentre per la datazione delle acque di falda è stato utilizzato il metodo isotopico del Trizio-Elio ( $3\text{H}$ - $3\text{He}$ ). L'analisi e l'interpretazione di questa serie d'isotopi rendono possibile l'individuazione e la differenziazione delle diverse fonti dei nitrati oltreché l'origine, provenienza e datazione delle acque così come segue: (i) nitrati presenti nell'acqua meteorica; (ii) nitrati di sintesi industriale (concimi sintetici); (iii) nitrati sintetizzati nel suolo dai processi microbici; (iv) nitrati provenienti dall'ossidazione della materia organica legata alle attività agricole (letame) o urbane (fosse settiche, fognature, etc.); (v) provenienza e miscibilità delle acque; (vi) datazione dell'acqua di falda. L'analisi delle acque campionate ha riguardato oltre all'analisi isotopica degli elementi e molecole sopracitate anche l'analisi della concentrazione dei seguenti ioni: nitrato, nitrito, ammonio, cloruro, bromuro, calcio, magnesio, potassio, sodio, solfato, orto-fosfato, fosfato totale, silicio totale, e boro totale oltreché i parametri chimico-fisici quali pH, conducibilità elettrica, ossigeno disciolto, salinità e temperatura.

Per quest'ultima serie di parametri è stato eseguito, per ogni punto campionato, anche un profilo verticale ad intervalli di 0.5m che ha permesso d'individuare e caratterizzare la stratificazione della colonna d'acqua. La parte analitica del programma di monitoraggio è stata integrata con le tecniche del telerilevamento. L'analisi multi-temporale dei dati telerilevati dai satelliti Landsat-1 MSS, Landsat-5 TM, Landsat-7 ETM+ e ortofoto recenti ha permesso di ricostruire l'evoluzione spazio-temporale di: (i) flora algale; (ii) temperatura dell'acqua e (iii) dei principali cambiamenti morfologici della laguna a partire dagli anni '70 così come già ampiamente riportato nel numero 34 del Bollettino della Società Naturalisti "Silvia Zenari" (Saccon, 2010).

Tra i principali risultati ottenuti da questo primo studio scientifico applicando il metodo multi-isotopico è emerso, ad esempio, che nelle acque campionate nel periodo 2009-2010 non è stata rilevata la tipica composizione isotopica dei nitrati derivanti da concimi sintetici mentre i nitrati individuati lungo la linea delle risorgive vanno principalmente riferiti all'uso di concimi organici. Nella zona di studio esistono comunque altri nitrati che provengono, invece, principalmente da reflui urbani così come quelli individuati in diversi corsi d'acqua superficiali quali il Cormor ed il Corno.

Nelle acque lagunari campionate è stato in genere più difficile poter caratterizzare l'origine dei nitrati essendo la laguna di Marano un ambiente acquatico molto complesso caratterizzato dalla mescolanza di acque di diversa natura quali appunto le acque marine, fluviali e meteoriche. Nonostante ciò è stato possibile identificare che i nitrati in laguna si possono formare anche dai processi di nitrificazione che coinvolgono sia l'ammonio di origine antropica che da quello prodotto in laguna attraverso i processi di rimineralizzazione oltreché quelli di origine atmosferica. Alla luce di questi dati si può concludere che in laguna il carico da nitrati così come individuato nel periodo di monitoraggio 2009-2010 non è da attribuire solamente alle pratiche agricole ma anche da altre origini come appunto le acque reflue urbane, processi di nitrificazione e da depositi atmosferici.

Visto che il boro è un elemento che viene associato ai nitrato di origine antropica gli isotopi del boro sono stati utilizzati in questo studio quale tracciante aggiuntivo per l'identificazione dell'impatto antropico in ecosistemi acquatici. Pertanto per quest'analisi sono state selezionate tre fonti di riferimenti (end-members) principali quali appunto le acque

reflue urbane, i concimi organici oltreché l'acqua marina stessa. Dalla firma isotopica del boro oltreché dalla sua concentrazione chimica è emerso che le acque campionate sono caratterizzate dalla mescolanza di boro di origine antropogenica derivante da acque reflue urbane oltreché da boro originato da concimi organici e da acqua marina.

Dai risultati ottenuti si è visto che la maggior parte delle acque campionate sono influenzate dalla presenza di entrambe fonti inquinanti e cioè quelle da reflui urbani e da concimi organici evidenziando però che alcune zone della laguna sono meno contaminate da questi due inquinanti. Utilizzando il modello degli "end-members" non è stato possibile spiegare l'origine del boro di una serie di punti di campionamento lungo la linea delle risorgive ipotizzando l'esistenza di altre potenziali fonti inquinanti. In aggiunta alle analisi isotopiche di nitrato e boro è stata anche determinata la composizione isotopica dell'acqua. Questa ha permesso di stabilire la provenienza delle acque in termini di quota media del bacino imbrifero, per quel che riguarda le acque superficiali, mentre per le acque di falda e di risorgiva si è potuto determinare la zona di ricarica delle stesse confermando ad esempio che le acque campionate lungo la linea delle risorgive derivano direttamente dalla falda freatica locale.

Per poter stabilire l'influenza delle acque del Fiume Tagliamento che s'immettono nel settore occidentale della laguna di Marano attraverso il canale artificiale di Bevazzana si è fatto uso, oltre che della forma isotopica dell'acqua, anche quella del solfato. Infatti, i valori di  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  del solfato misurato al punto LM40 (Fiume Tagliamento) e LM5 (Canale di Bevazzana) indicano che l'origine del solfato in questi due punti sarebbe di tipo litosferico (dissoluzione di evaporiti) mentre nel punto di campionamento LM6 (in laguna) l'origine del solfato è da riportare a quella dell'acqua marina del Mediterraneo. Questo risultato va a confermare che le acque del Fiume Tagliamento raggiungono la laguna attraverso il canale artificiale di Bevazzana. Infine, per poter determinare il tempo di residenza medio delle acque di falda (età dell'acque di falda) è stato utilizzato il metodo isotopico di datazione del Trizio-Elio. I risultati di quest'analisi condotta in tre pozzi dell'Alta Pianura Friulana hanno evidenziato che il tempo di residenza medio delle acque di falda nel punto 55-PO27 in prossimità di Chiasellis (UD) è di  $1,3 \pm 0,5$  anni mentre nel punto 56-PO30 in prossimità di Gris (UD) è di  $1,4 \pm 0,5$  anni. Nel punto di campionamento 57-PO101 in prossimità di Mereto di Capitolo (UD) questo valore è di  $4 \pm 1$  anni.

Sulla base di questi risultati scientifici si è potuto individuare oltreché caratterizzare meglio l'origine dei nitrati presenti nella laguna di Marano e parte del bacino drenante della laguna stessa ricordando che, prima di questo studio, i nitrati sono stati imputati ad un'origine legata esclusivamente alle pratiche agricole escludendo a priori altre possibili fonti inquinanti. Nonostante questo sia stato uno dei primi studi condotti in Europa per l'individuazione e la caratterizzazione delle fonti dei nitrati in ambiente lagunare utilizzando la suddetta metodologia di ricerca integrata questo non vuole essere un punto d'arrivo ma bensì un punto di partenza per poter approfondire alcuni nuovi aspetti emersi da questa prima indagine scientifica.

**Abstract:** Detection and characterisation of potential nitrate pollution sources in the Marano Lagoon and set-up of an environmental monitoring plan – FONIMAR project – Part II. *The aims of this study were mainly: (i) the identification and differentiation of the main anthropogenic nitrogen sources in the Marano Lagoon (Italy) and its catchment area; and (ii)*

*the assessment of the intra-lagoonal water circulation, the morphological development of the lagoon and its anthropogenic pressure by applying a combined approach of hydrochemical, isotopic, hydrological and remote sensing techniques. To achieve the aforementioned targets analyses of stable isotope signatures of nitrate, boron, water and sulphate have been used. Moreover the residence times of groundwater were determined by the tritium-helium dating method. To characterize the chemical composition of the different water types the concentrations of the major ions and nutrients as well as the physicochemical parameters have been measured. Remote sensing techniques have been applied to assess the spatial distribution of most superficial algal flora, water temperature as well as the key environmental and morphological changes of the lagoon since the beginning of the 1970s as already reported in the previous issue (N. 34, December 2010) of the "Bollettino della Società Naturalisti Silvia Zenari" (Saccon, 2010).*

## **1 Introduction**

Nitrate pollution is still a major concern in many European aqueous resources, which may originate from multiple sources: excess application of mineral nitrogen (N) fertilizers and animal manure in agriculture, discharges from urban or industrial N bearing waste water, septic leachate in unsewered areas, atmospheric deposition or decomposition of soil organic N. In addition to the existence of multiple potential nitrate sources, nitrate pollution may originate from present as well as from historical land use, which complicates the problem. In the framework of the Nitrates Directive (91/976/EEC) and the Urban Wastewater Directive (91/271/EEC), several policy measures with major socio-economic impact have been implemented to reduce nitrate inputs from agriculture or urban and industrial waste water. The Water Framework Directive (2000/60/EC) and Nitrates Directive require that the chemical water quality (concentration of nitrate and other ions) is monitored by the national environmental agencies to evaluate the effectiveness of the measures taken (EUR 23658 EN, 2008). The objectives of the directive are to ensure that the nitrate concentration in freshwater and groundwater supplies does not exceed the limit of 50 mg/l, and to control the incidence of eutrophication. European legislation on nitrate has been around for a long time; nonetheless nitrate pollution remains an issue.

The development and implementation of effective remediation measures in nitrate polluted areas requires the identification of the main nitrate pollution sources and the estimation of their contribution is essential for environmental policy makers to implement effective, source-oriented as well as sustainable remediation measures. This information cannot be deducted from the application of a single investigation method like water quality monitoring alone (concentration measurements) without the introduction of an integrated and interdisciplinary water resources management approach. A multi-isotope method in combination with hydrochemical, hydrological and remote sensing investigations is a powerful, scientifically based tool to identify and characterize multiple nitrate pollution sources.

Nitrate ( $\text{NO}_3^-$ ) is found naturally at moderate concentrations in many aquatic environments, but is often enriched to high levels by anthropogenic activities involving nitrogenous compounds such as mineral fertilizer and by-products of organic compounds from agriculture, septic systems and poultry, hog or cattle manure (Berger *et al.*, 1976; Heaton *et al.*, 1983; Mariotti *et al.*, 1988; Ostrom *et al.*, 1998; Williams *et al.*, 1998). In spite of the increasing efforts at national and European levels to reduce nitrate inputs from intensive agriculture (EC Directive 91/976/EEC), nitrate is still one of the major contaminants of aquatic systems (Widory *et al.*, 2004). Traditional approach for environmental management and control of water quality regarding nitrate is merely based on the sole monitoring of pollutant concentrations. However, chemical data alone does not enable one to establish unambiguously the type, location and contribution of different nitrate pollution sources. In particular, differentiating urban and agricultural origin of nitrate is extremely difficult (even by increasing the number of monitoring stations or samples). The study area is located in the Marano lagoon in the Friuli-Venezia Giulia region (North-east Italy) where nitrates from intensive agricultural and anthropogenic activities are responsible for its pollution. In 2008, the entire catchment area of the Marano lagoon was designated as a nitrate vulnerable zone (NVZ) where an action program on the reduction of nitrate leaching was initiated. Thus, to identify and characterize multiple-sources of nitrate pollution of agricultural and urban origin, a water monitoring network in the Marano lagoon and its catchment area was set-up. In the monitoring program water samples from the lagoon, its tributary rivers, the groundwater up-welling line, groundwater, sewer pipe and open sea on a quarterly interval from 2009 to 2010 were collected and analyzed.

The present study represents a novel approach on the identification and characterization of potential sources of nitrate pollution in a lagoon environment, which, beside the traditional hydrochemical analyses (main ions and nutrients), introduces the whole suite of stable isotopes of nitrate ( $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  and  $\delta^{17}\text{O}$ ), the isotopic signature of boron ( $\delta^{11}\text{B}$ ), the stable isotopes of water ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ), the stable isotopes of sulphate ( $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$ ), the tritium-helium ( $^3\text{H}$ - $^3\text{He}$ ) isotopic method as well as hydrological, remote sensing and GIS techniques.

The project has been funded by the ERSA (Regional Agency for Rural Development) of the Friuli-Venezia Giulia region.

## 2 Study area

The Marano lagoon is located in the Northern Adriatic Sea (Northeast Italy) and it is entirely included in the Province of Udine of the Friuli-Venezia Giulia region as represented in Fig. 1. The lagoon has a surface of about 77 km<sup>2</sup>, with a length of nearly 14 km and an average width of 5.5 km. To the east of the Marano lagoon, the Grado lagoon is situated, which is the second part of the

Grado-Marano lagoon system. The area of the entire lagoon system is of approximately 160 km<sup>2</sup>, with a length of almost 32 km and an average width of 5 km. The two lagoons are divided by the administrative border between the Udine and Gorizia provinces where the Ausa-Corno Channel is also located. In the south the Marano lagoon is connected to the Adriatic Sea through three lagoon inlets (Porto Lignano, Sant'Andrea and Porto Buso). A total mean water exchange rate between the lagoon and the Adriatic Sea through the Porto Lignano and Porto Buso inlets are respectively in the order of 1750 and 1500 m<sup>3</sup>/s. The tide in the lagoon has a mean height of approximately 0.6 m with a maximum of about 1.0 m. The tide propagates along the major channels with a velocity of approximately 40-60 cm/s and then enters in the shallow areas where the velocity quickly decreases to below 10 cm/s (Ferrarin *et al.*, 2010). The mean bathymetry in the shallow lagoon areas is roughly 0.8 m, while in the navigable channels it is about 3.2 m. The overall mean bathymetry of the lagoon is in the order of 1.95 m. In the southwest, the lagoon is characterized by the urban and touristic areas of Lignano Sabbiadoro city, which is one of the main summer resorts in northern

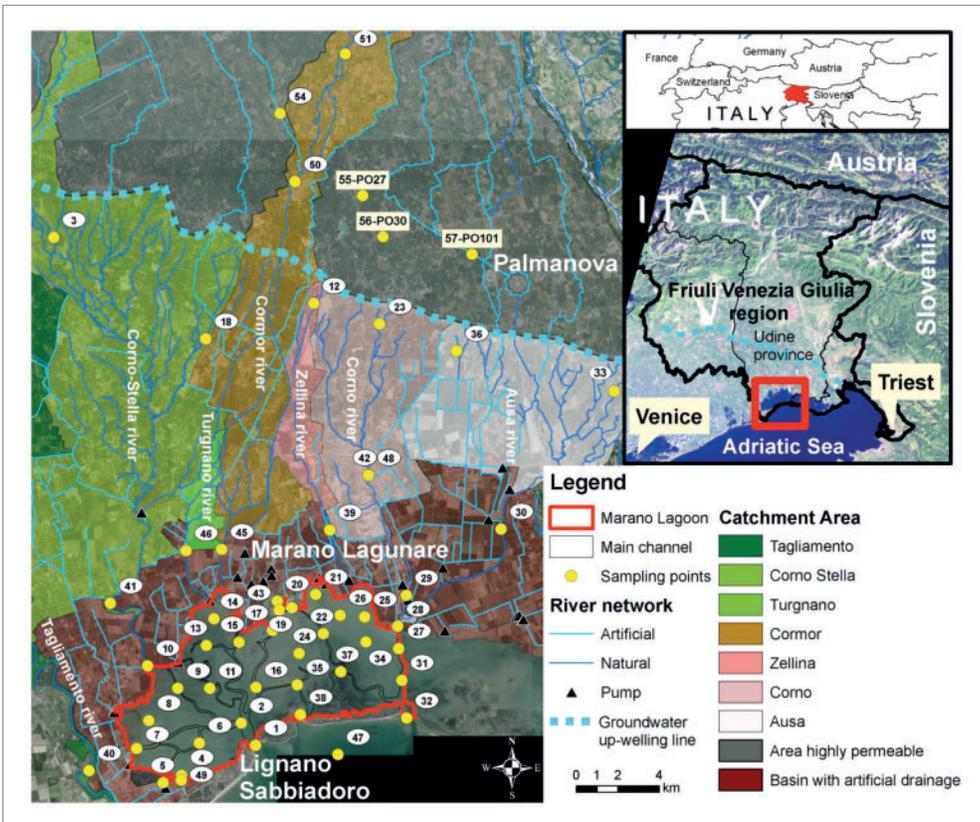


Fig. 1 - Study area: Marano Lagoon (red line).  
Area di studio: Laguna di Marano (linea rossa).

Italy, and by the Aprilia Marittima resort. In this lagoon sector, the artificial channel of Bevazzana is located, which is responsible for the connection between the Tagliamento River and the lagoon.

The Tagliamento River is a 7<sup>th</sup> order river that flows unimpeded by high dams for 172 km into the Northern Adriatic Sea. The Tagliamento River is the most important river of the Friuli-Venezia Giulia region with a catchment area of approximately 2674 km<sup>2</sup>. The fishing community of Marano Lagunare is situated in the northern lagoon sector and with its 2007 inhabitants is the main urban centre within the lagoon perimeter. About 5 km east of Marano Lagunare city, the “Aussa-Corno” industrial site (ZIAC) is located. This important industrial area spans over 1000 ha and includes 87 factories comprising the following main categories: chemical, mechanical engineering, naval, iron and steel production, textile, food, wood, etc. The northern sector of the Marano lagoon is also characterized by the discharge of freshwater from its tributary rivers. The lagoon tributary rivers and their mean discharges are as follows: Corno-Stella (35.4 m<sup>3</sup>/s), Turgnano (0.5 m<sup>3</sup>/s), Cormor (9.3 m<sup>3</sup>/s), Zellina (1.1 m<sup>3</sup>/s), Corno (4.6 m<sup>3</sup>/s) and Ausa with 10.0 m<sup>3</sup>/s (Ret, 2006). The springs of Stella River are located along the groundwater up-welling line. After the confluence with the Corno torrent, the Stella River becomes one of the main tributary rivers to the Marano lagoon. The catchment area of the Corno-Stella River is about 300 km<sup>2</sup>, while those of the other rivers originating from the groundwater up-welling line like the Rivers Turgnano, Zellina, Corno and Ausa are respectively 7 km<sup>2</sup>, 14.4 km<sup>2</sup>, 55.4 km<sup>2</sup> and 65.6 km<sup>2</sup>. The Cormor River's springs are located at an altitude of 250 m in the Mt San Lorenzo near Santo Stefano di Buia (UD) and after flowing for 51 km through the glacial moraines (Würmian), the Upper Friulian Plain and the groundwater up-welling line reach the Marano lagoon. The catchment area of the Cormor River is about 226 km<sup>2</sup>. The groundwater up-welling line divides the Friuli-Venezia Giulia Plain into two units, the Upper Friulian Plain and the Lower Friulian Plain. The mean discharge of the groundwater up-welling line in the left side of the Tagliamento River is about 80 m<sup>3</sup>/s, which corresponds to a mean discharge of about 1.3 m<sup>3</sup>/s/km. The Friuli-Venezia Giulia Plain groundwater systems are located in sediments that are underlain by an Eocene to Oligocene succession deposited above a 7 km thick Mesozoic carbonate platform (Cati et al., 1987; Carulli, 2006). In the Marano lagoon, there are two nature reserves, the “Riserva delle foci del fiume Stella” (Reserve of the Stella River mouths) that takes the entire delta of the Corno-Stella River and the “Riserva della Valle Canal Novo” (Canal Novo Valley reserve) that covers a fishing area of around 35 hectares. They are protected by the Ramsar convention on wetlands of international interest. Also, according to the EU Birds Directive (2009/147/EC) on the conservation of wild birds and the EU Habitats Directive (92/43/EEC) on the conservation of natural habitats and of wild fauna and flora, the whole lagoon system is included in the Natura 2000 network.

### 3 Water sampling program

In the water sampling program the main climatic, environmental, and seasonal changes, as well as all relevant agricultural, livestock, anthropogenic and touristic activities that potentially influence the distribution, concentration and origin of nitrates have been taken into account. The 2009-2010 water sampling program was carried out on a quarterly basis, as indicated in Tab. 1:

<b>Sampling campaign</b>	<b>Season</b>	<b>Date</b>
1	Winter	02-06/02/2009
2	Spring	25-29/05/2009
3	Summer	31/08-03/09/2009
4	Autumn	02-06/11/2009
5	Winter	15-19/02/2010
6	Spring	25-29/05/2010; 15/06/2010

Tabella 1 - Sampling program schedule.  
Piano del programma di campionamento.

To achieve the project's goals and due to the necessity of doing extensive investigations on some particular and interesting areas the initial 47 sampling points were progressively increased to 55 points. The total sampling points were distributed as follows: 16 sampling points along the lagoon's navigation channels; 14 sampling points along the main tributary rivers of the lagoon; 6 sampling points along the groundwater up-welling line (linea delle risorgive); 11 sampling points in shallow water areas of the lagoon; 3 sampling points in the lagoon inlets to the Adriatic Sea; 1 sampling point in the open sea; 4 sampling points in groundwater.

## 4 Analytical methods

### 4.1 Hydrochemical analysis

For the chemical characterization of the different water types included in the monitoring program their chemical compositions have been determined using the following analytical methods:

1. In-situ chemical analysis and measurement of the physicochemical parameters;
2. Post-sampling analyses carried out in Graz at the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research.

For this particular study, the water sampling depth has been fixed at approximately mid-water depth. By operating in this way, it was possible to minimize the unwanted contributions of: (i) rainfall in the upper layers of the water column; and (ii) various physicochemical as well as microbiological processes linked to the lagoon bottom.

Moreover, in determining the optimal sampling depth it was necessary, during each single sampling, to take into account the local tide condition, wind speed and direction, as well as the water current speed and its direction. Water samples were gathered using a Ruttner water sampler equipped with a drop messenger that could be closed at any desired depth.

The in-situ chemical analysis and measurement of the physicochemical parameters took place directly in the field including the following activities: (i) measurement of pH, water temperature, electrical conductivity, dissolved oxygen and salinity using a portable multi-parameter meter; (ii) measurement of nitrate, nitrite, and ammonium concentration using a portable spectrophotometer “WTW FC photoFlex/Turb 430 LED”; (iii) measurement of orthophosphate concentration using a second portable spectrophotometer “Merck SQ 118”; and (iv) storage of all collected water samples for the later analyses in the laboratories. In all cases water samples collected during the field campaigns were analyzed in Graz at the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research. Dissolved cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ) and anions ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ ) were analysed by ion chromatography (Dionex ICS-3000). Alkalinity was measured by titration and total phosphorus (P-total) was determined photometrically in the laboratory after digestion of deep frozen water samples.

## 4.2 Isotopic analysis and analytical techniques

Isotopes are atoms of the same element that have different numbers of neutrons. Differences in the number of neutrons among the various isotopes of an element mean that the various isotopes have different masses (Kendall and McDonnell, 1998). For example, among the hydrogen isotopes, deuterium (denoted as D or  $^2\text{H}$ ) has one neutron and one proton. The superscript number to the left of the element designation is called the mass number ( $A$ ) and is the sum of the number of protons and neutrons in the isotope. Stable isotopes are analyzed either on gas- or solid-source mass spectrometers, depending on both the masses of the isotopes and the existence of appropriate gaseous compounds stable at room temperature. Radioisotopes can be analyzed by counting the number of disintegrations per unit time on gamma ray or beta particle counters, or analyzed on mass spectrometers (Kendall and Caldwell, 1998).

The ratio between two isotopes is computed with the heavier isotope in the numerator (e.g.  $R = n(^{15}\text{N})/n(^{14}\text{N})$ , where  $n$  represents the amount of isotope). To avoid handling  $R$  values with many decimals, a relative  $\delta$  notation using the ratio between  $R_{\text{sample}}$  and  $R_{\text{standard}}$  is used (see below). The main internationally accepted stable isotope standards for hydrogen, oxygen, nitrogen, boron and sulphur are as follows:

- Nitrogen: AIR (atmospheric  $\text{N}_2$ )
- Hydrogen and oxygen: VSMOW (Vienna Standard Mean Ocean Water)
- Boron: NBS-SRM 951 (boric acid)

- Sulphur: V-CDT (Vienna-Canyon Diablo Troilite)

Samples are either analyzed at the same time as this reference standard or with an internal laboratory standard that has been calibrated relative to the international standard.

$\delta$  values are computed as follows and expressed in parts per thousand (denoted as ‰, pronounced "per mil"):

$$\delta \text{ (in ‰)} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 10^3 \text{ where:}$$

- $R_{\text{sample}}$  is the ratio of the heavy isotope to the light isotope in the sample (e.g.  $^{15}\text{N}/^{14}\text{N}_{\text{sample}}$ );
- $R_{\text{standard}}$  is the ratio of the heavy isotope to light isotope in the international reference material;
- (pronounced delta) is the relative isotope ratio difference between sample and standard.  $\delta$  will be positive if the measured sample is enriched in heavy isotopes relative to the standard ( $R_{\text{sample}} > R_{\text{standard}}$ ) and will be negative in the reverse case ( $R_{\text{sample}} < R_{\text{standard}}$ ).

The isotopic analyses of the water samples were carried out via the following phases:

1. The oxygen and hydrogen isotopic composition of water ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) and the nitrogen isotopic composition ( $\delta^{15}\text{N}$ ) of synthetic fertilizers, sewage and manure (collected in some farms of the Region Friuli-Venezia Giulia) were measured in Graz at the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research.
2. The stable isotopes of nitrate ( $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  and  $\delta^{17}\text{O}$ ) from the first sampling campaign (February 2009) were analyzed in Grenoble (France) at UJF-Grenoble 1/CNRS-INSU, Laboratoire de Glaciologie et Géophysique de l'Environnement (LGGE) UMR 5183, St.-Martin-d'Hères, while for the other sampling campaigns the water samples were analyzed in the United Kingdom at the Stable Isotope Laboratory of the School of Environmental Sciences, University of East Anglia, Norwich.
3. The analyses of the stable isotopes of boron ( $\delta^{11}\text{B}$ ) were carried out in Kiel (Germany) at the Leibniz-Institute of Marine Sciences (Leibniz-Institut für Meereswissenschaften an der Christian-Albrechts Universität zu Kiel - IFM-GEOMAR).
4. The analyses of the stable isotopes of sulphate ( $\delta^{34}\text{S}$ ) were conducted in Germany at the Leibniz-Institute for Baltic Sea Research, Geochemistry & Isotope Geochemistry Group, Marine Geology Section, Warnemünde (Leibniz-Institut für Ostseeforschung Warnemünde – IOW).  $\delta^{18}\text{O}$  values were measured at the Max Planck Institute for Marine Microbiology (MPI Bremen).
5. Tritium and helium isotopes were analyzed in Germany, at the University of Bremen, Institute of Environmental Physics - Section of Oceanography (Universität Bremen, Institut für Umweltphysik – IUP).

#### 4.2.1 Stable isotopes of water: oxygen-18 and deuterium

The oxygen-18 ( $^{18}\text{O}$ ) and deuterium ( $^2\text{H}$ ) isotopes are integrated parts of the water molecule and are, therefore well-suited for hydrological tracing and budget studies (SAETHER AND DE CARITAT, 1997). The  $^{18}\text{O}$  and  $^2\text{H}$  contents are expressed as  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , and reported relative to the VSMOW (Vienna Standard Mean Ocean Water) standard. Isotope hydrology provides complementary information on the type, origin and age of water. If the isotopic content does not change within the aquifer, it will reflect the origin of the water, particularly the location, period and processes of recharge. If the isotopic content changes along groundwater paths, this will reflect the history of the water, particularly the mixing, salinization and discharge processes. Analysis of water for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  can also provide extremely useful information about the sources of the nitrate and other solutes in water (ARAVENA & ROBERTSON, 1998; MCMAHON & BÖHLKE, 2006). Very often, different sources of nitrate in rivers and groundwater are associated with different water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values because of evaporation or because the waters are derived from very different geographic areas (KENDALL *et al.*, 2007). In this project deuterium ( $\delta^2\text{H}$ ) was measured with a Finnigan DELTA<sup>plus</sup> XP continuous flow stable isotope ratio mass spectrometer by chromium reduction using a ceramic reactor slightly modified from MORRISON *et al.* (2001). The oxygen isotopic composition ( $\delta^{18}\text{O}$ ) of water was measured by the classic  $\text{CO}_2\text{-H}_2\text{O}$  equilibrium technique (EPSTEIN AND MAYEDA, 1953) with a fully automated device adapted from HORITA *et al.* (1989) coupled to a Finnigan DELTA<sup>plus</sup> Mass Spectrometer.

#### 4.2.2 Stable isotopes of nitrate: nitrogen-15, oxygen-18 and oxygen-17

The isotopic composition of the dissolved nitrogen (N) species has been used extensively to better constrain the sources and fate of N in groundwater (PANNO *et al.*, 2001). The possibility of quantifying both the origin and the secondary processes affecting N concentrations by means of a single tracer appears more limited however. Nitrogen cannot be considered conservative because it is biologically modified through nitrification and denitrification reactions, both during infiltration of the water and in the groundwater body, causing isotopic fractionation that modifies the  $\delta^{15}\text{N}$  signatures of the dissolved N species (VOGEL *et al.*, 1981; MARIOTTI *et al.*, 1988; Böttcher *et al.*, 1990; Smith *et al.*, 1991; FEATS *et al.*, 1998; ARAVENA AND ROBERTSON, 1998; PAUWELS *et al.*, 2000, TORRENTÓ *et al.*, 2010; ZHANG *et al.*, 2010). In some cases, the interference between the isotopic fractionation (linked to denitrification) and the mixing processes (from the combination of multiple nitrate sources) might hamper the identification of nitrate sources, since the associated fractionation processes can alter the isotope composition of dissolved nitrate. Discriminating between multiple nitrate sources by their N isotopic composition alone becomes impossible wherever heterogenic or autogenic denitrification occurs, thus

there is a need for establishing co-migrating discriminators of nitrate sources (WIDORY *et al.*, 2004). Some of these limitations can be tackled by including the isotopic signature of tracers that migrate along with nitrate, e.g. boron, strontium, sulphate S and/or O (e.g. WIDORY *et al.*, 2004; VITORIA *et al.*, 2008; ZHANG *et al.*, 2010). In the frame of this study the nitrate nitrogen and 18-oxygen isotopic compositions were analysed using the denitrifying bacteria procedures (denitrifier method) described in SIGMAN *et al.*, 2001; CASCIOTTI *et al.*, 2002; KAISER *et al.*, 2007 and MORIN *et al.*, 2009 and have been used to differentiate among nitrate coming from agriculture (synthetic and natural fertilizers), airborne nitrate and nitrate from nitrification processes in soils. For this analysis the *Pseudomonas aureofaciens* bacteria strain has been used, which was first grown on agar plates, from where single colonies were used to inoculate 500 ml media bottles containing tryptic soy broth with nutrients. Bottles were kept on a shaker table in the dark for minimum 5 days to maximum 10 days. After the growth period, bacteria were isolated from the nutrient media by centrifuging (Fig. 2, left), then re-suspended in a volume of nutrient free media (NFM) and a few drops of antifoaming agent. Aliquots of 2 ml of *Pseudomonas aureofaciens* in NFM were pipetted into 20ml volume glass vials, then crimp-sealed using butyl stoppers and aluminium caps. These vials were purged for 45 minutes using helium gas to remove traces of N<sub>2</sub>O and the atmospheric gases (Fig. 2, right).



Fig. 2 - Left: Isolated *Pseudomonas aureofaciens* bacterial; Right: Helium injection for degasification of the vials.

Sinistra: isolamento di batteri *Pseudomonas aureofaciens*; Destra: iniezione di Elio per la degassificazione delle fiale.

The vials were kept overnight in the dark on a shaker table at room temperature. As bacteria were in NFM, any potential oxidised nitrogen contamination was consumed by the bacteria overnight. The following day the vials were purged the second time with helium for 45 minutes to remove traces of residual N<sub>2</sub>O that may have been produced overnight. After the second purge, a volume

of water sample of known nitrate concentration was injected over the bacteria suspension, to produce 10 nmol of  $\text{N}_2\text{O}$  gas. After about two hours, 0.1 ml of 6 mol/l NaOH solution was added to each vial to kill the bacteria. The evolved  $\text{N}_2\text{O}$  was extracted, purified and measured using a Europa GEO 20-20 mass spectrometer on-line with a TGII prep system and Gilson auto-sampler. Water vapour was removed using three trapping systems: Nafion dryer, magnesium perchlorate trap and  $-80^\circ\text{C}$  cryogenic trap. The remaining carbon dioxide was mostly removed using a carbosorb trap. Additionally, prior to the mass spectrometer, gases were passed through a GC column in order to separate carbon dioxide traces from the analyte  $\text{N}_2\text{O}$ , as these gases are isobaric. Samples were analyzed in triplicate, and together with them three international reference materials were analyzed in quadruplicate: IAEA N3, USGS 34 and USGS 35. The raw data was corrected for drift, blank, oxygen isotopic exchange with the parent water oxygen, isobaric interferences, scale conversion and stretch (SIGMAN *et al.*, 2001; CASCIOTTI *et al.*, 2002; BÖHLKE *et al.*, 2003 and KAISER *et al.*, 2007). Data was reported with respect to AIR (for  $\delta^{15}\text{N}$ ) and with respect to VSMOW (for  $\delta^{18}\text{O}$ ). The precision of the analyses was generally better than 0.3 ‰ for both  $^{15}\text{N}$  and  $^{18}\text{O}$  measurements, based on the quadruplicate analyses of the three international standard materials measured.

For the 17-oxygen analyses of nitrate the procedure used was identical to the one described above, but (i) the water sample volume injected over bacteria was five times higher, so the amount of  $\text{N}_2\text{O}$  produced from the bacterial denitrification was 50 nmol, and (ii) prior to the mass spectrometer the  $\text{N}_2\text{O}$  gas was passed through a gold tube heated at  $800^\circ\text{C}$  and converted to  $\text{N}_2$  and  $\text{O}_2$  gas, which were then passed through a molecular sieve GC column so that the  $\text{N}_2$  gas was delayed with respect to the oxygen before going to the mass spectrometer. Using this protocol the  $^{15}\text{N}$ ,  $^{17}\text{O}$  and  $^{18}\text{O}$  isotopic compositions were measured using the nitrogen and oxygen gases, rather than the  $\text{N}_2\text{O}$ .

#### 4.2.3 Stable isotopes of boron

Boron (B) has two naturally occurring stable isotopes,  $^{10}\text{B}$  and  $^{11}\text{B}$ . The large relative mass difference between the boron isotopes leads to a wide (ca. 90‰) natural range of  $\delta^{11}\text{B}$  values (BARTH, 1993). Because B is widely used in industrial, agricultural, cosmetics, and household products,  $\delta^{11}\text{B}$  is a useful tool for determining sources of pollutants including nitrate (EISENHUT *et al.*, 1996; BARTH, 2000). The main industrial source of B to waters is sodium perborate ( $\text{NaBO}_3$ ), which is used in laundry detergents (primarily as a bleaching agent) and in household cleaners; consequently, B is commonly found in household sewage. Purification of waters in sewage treatment plants generally removes little or no B (BARTH, 2000); hence,  $\delta^{11}\text{B}$  is a conservative tracer of a wastewater source. While B isotopes are not affected by denitrification, they are fractionated through processes such as adsorption on clays.

Boron isotopes have been shown to be useful for identifying anthropogenic B sources in surface water and shallow groundwater systems: (i) municipal wastewater and sewage (BASSETT, 1990; VENGOSH *et al.*, 1994, 1999; BASSETT *et al.*, 1995; EISENHUT *et al.*, 1996; BARTH, 1998; VENGOSH, 1998; SEILER, 2005); (ii) irrigation return flows (BASSETT *et al.*, 1995); (iii) fertilizer-affected irrigation waters from various agricultural settings (KOMOR, 1997); (iv) domestic solid waste deposit leachates from landfills (EISENHUT & HEUMANN, 1997; BARTH, 2000); (v) mixed agricultural sources dominated by animal waste (WIDORY *et al.*, 2004); (vi) fly ash deposit leachates from a coal-fired power plant (DAVIDSON & BASSETT, 1993).

Use of  $\delta^{11}\text{B}$  coupled with  $\delta^{15}\text{N}$  has proved to be an effective means for tracing agricultural nitrate sources (e.g. hog manure, cattle feedlot runoff and synthetic fertilizers) in surface and groundwaters (BASSETT *et al.*, 1995; KOMOR, 1997; WIDORY *et al.*, 2004). In a recent study (WIDORY *et al.*, 2004),  $\delta^{11}\text{B}$  was used to distinguish between two types of sewage that were indistinguishable using  $\delta^{15}\text{N}$  alone: a high-B/low- $\text{NO}_3$ /low- $\delta^{11}\text{B}$  type that is derived from washing powders, and a moderate-B/moderate- $\text{NO}_3$  type with  $\delta^{11}\text{B}$  values close to animal (probably human) manure. Some separation of different animal sources of B (e.g. sewage, cattle, hogs and poultry) is seen on plots of  $\delta^{11}\text{B}$  vs.  $1/\text{B}$  (WIDORY *et al.*, 2004). The measurements of boron isotopes were performed on a Thermo Fisher AXIOM MC-ICP-MS (Multicollector-Inductively Coupled Plasma Mass Spectrometer) equipped with an ESI New Wave Research LFC (large format cell) (FIETZKE *et al.*, 2010).

#### 4.2.4 Stable isotopes of boron

Sulphur and oxygen isotopes in sulphate can be used to trace natural and anthropogenic sources of sulphur. The dual isotope approach often provides a better separation of potential sulphur sources (BÖTTCHER, 1999). Moreover, this method provides information on the processes involved in sulphur cycling of the atmosphere, and terrestrial and marine ecosystems (VAN STEMPVOORT & KROUSE, 1994; BÖTTCHER *et al.*, 1998; 2001). In this study isotope signatures of dissolved sulphate were used to distinguish between sulphate from geogenic, marine and anthropogenic origin in the catchment of the Marano lagoon. Dissolved sulphate was precipitated from acidified filtered samples by the addition of barium chloride solution. The precipitate was filtered, carefully washed and dried in a drying oven. For oxygen isotope measurements, the precipitate was additionally heated in an oven at  $500^\circ\text{C}$  to remove potential contaminations derived from DOC. Aliquots for sulphur isotope ratio measurements were combusted by flash combustion in Sn cups together with p.a. grade  $\text{V}_2\text{O}_5$  in a stream of He. Stable sulphur isotope measurements were carried out with a Thermo Finnigan MAT 253 gas mass spectrometer that was connected to via a Thermo Electron ConFlo IV split interface to a Thermo Scientific Flash 2000 elemen-

tal analyzer. Silver sulphide isotope reference materials (IAEA-S-1, IAEA-S-2 and IAEA-S-3) were used to link the mass spectrometric results ( $^{34}\text{S}/^{32}\text{S}$ ) to the V-CDT scale (MANN *et al.*, 2009). Replicate measurements agreed within better than  $\pm 0.3\%$ . Stable oxygen isotope measurements of sulphate were carried out with a Thermo Finnigan Delta+ gas mass spectrometer after conversion in a Thermo Quest TCEA system. Samples were combusted in Ag cups and isotope results are given towards the V-SMOW standard using NBS127, IAEA-SO-5 and IAEA-SO-6 as reference materials.

#### 4.2.5 Tritium-helium method for groundwater dating

The combined use of tritium ( $^3\text{H}$ ) and its radioactive decay product helium ( $^3\text{He}$ ) provides an excellent tool for groundwater dating. The tritium-helium ( $^3\text{H}$ - $^3\text{He}$ ) method is based on analysis of radioactive hydrogen ( $^3\text{H}$ ) in the water molecule and its decay product helium-3 ( $^3\text{He}$ ) and it is one of the most frequently used dating techniques for shallow groundwater (SCHLOSSER *et al.*, 1988; SOLOMON *et al.*, 1995). The advantage of the  $^3\text{H}$ - $^3\text{He}$  dating method over the use of tritium alone is that the  $^3\text{H}$ - $^3\text{He}$  dating is independent of the  $^3\text{H}$  input function and the mean groundwater residence time (groundwater age) can be determined from an individual point within the aquifer. The major application of the non-atmospheric noble gas components in groundwater is dating; i.e. the determination of the mean residence time of the water in the subsurface. Dating applications fall into two major categories: (i) young groundwater with mean residence times between a few months to about 50 years can be studied with the  $^3\text{H}$ - $^3\text{He}$  technique as well as with the anthropogenic radioisotope  $^{85}\text{Kr}$ . The natural radioisotope  $^{222}\text{Rn}$  can be used to extend the age range to the study of very young groundwater (up to 20 days); and (ii) old groundwater with mean residence times of thousands to millions of years is studied by using the accumulation of stable radiogenic  $4\text{He}$  and  $40\text{Ar}$ , or by the long-lived radioisotope  $^{81}\text{Kr}$ . Another radioisotope,  $^{39}\text{Ar}$ , covers the time range between about 100 and 1000 years that otherwise is very difficult to access (KIPFER *et al.*, 2002). Measurements of tritium ( $^3\text{H}$ ) were carried out using the  $^3\text{He}$ -ingrowth method at the noble gas laboratory of the Institute of Environmental Physics / Section of Oceanography, University of Bremen, Germany (SÜLTENFUSS *et al.*, 2009). Samples of 500g of water were degassed and stored for the accumulation of the  $^3\text{H}$  decay product ( $^3\text{He}$ ) in dedicated He-free glass bulbs. After a storage period of several months,  $^3\text{He}$  was analyzed with the mass spectrometric system. This method achieves a detection limit of 0.01TU, which clearly allows the detection of the residue of pre-bomb  $^3\text{H}$  tests. The uncertainty is typically less than 3% for samples of  $>1\text{TU}$  and 0.01TU for very low concentrations. For ideal samples, analytical conditions allow a time resolution of about 3 months for apparent ages less than 10 years. For the He and Ne isotope analysis, the gases were extracted from water. He and Ne were separated from other gases with a cryo-system, kept at 25 K and 14 K.  $^4\text{He}$ ,  $^{20}\text{Ne}$  and

$^{22}\text{Ne}$  were measured with a quadrupole mass spectrometer (Balzers QMG112A). Helium isotopes were analyzed with a high-resolution sector field mass spectrometer (MAP 215–50) (SÜLTENFUSS *et al.*, 2004). The system was calibrated with atmospheric air and controlled for stable conditions for the He and Ne concentrations and the  $^3\text{He}/^4\text{He}$  ratio. The precision of the He and Ne concentrations is better than 1% and for the  $^3\text{He}/^4\text{He}$  ratio is better than 0.5%. For most of the samples duplicate measurements have been conducted.

## 5 Results and Discussion

### 5.1 Hydrochemical characterisation of waters

The results of the hydrochemical analyses have shown that the spatial distribution of nitrate concentration in the different lagoon sectors is very heterogeneous. To explain this phenomenon it has to be mentioned that the water circulation in the lagoon is very complex and influenced by many factors like, for example, the interaction and mixing processes between seawater, freshwater and rain water, tide, wave motion, water current direction, wind speed, discharge of the tributary rivers, lagoon bottom morphology, bathymetry as well as by the geometry and orientation of the intra-lagoon channels. Due to the reasons mentioned, in the eastern lagoon sector, the concentrations of nitrates coming from the Rivers Ausa and Corno were strongly reduced by both dilution with seawater that flows into the lagoon through the Ausa-Corno Channel, which is directly connected to the Adriatic Sea and by the absorption of nutrients by the algal flora. Due to reduced dilution and mixing processes with seawater from the lagoon inlet of Porto Lignano, the spatial distribution of nitrate coming from the Corno-Stella, Turgnano and Cormor rivers was much more extended in the western lagoon sector, even though the concentrations of this ion were in general lower in comparison to those detected in the eastern lagoon sector. The mean annual nitrate concentration for 2009 is reported in Fig. 3. In order to quantify more precisely the mean annual nitrate concentration in each lagoon sector, five different concentration classes have been selected and then related to the lagoon surface as indicated in the diagram and Tab. 2.

From this statistical analysis it was possible to determine that: (i) 74.8% of the lagoon surface is characterized by a mean annual nitrate concentration lower than 5 mg/l (dark blue area); (ii) 98.9% of the lagoon surface has a concentration lower than 10 mg/l; and (iii) only 1.1% of the area in question has a mean annual nitrate concentration greater than 10 mg/l, whereby this area is located at the estuary area of the Ausa and Corno rivers. Generally, in 2009 the entire lagoon surface had a mean annual nitrate concentration of 3.95 mg/l.

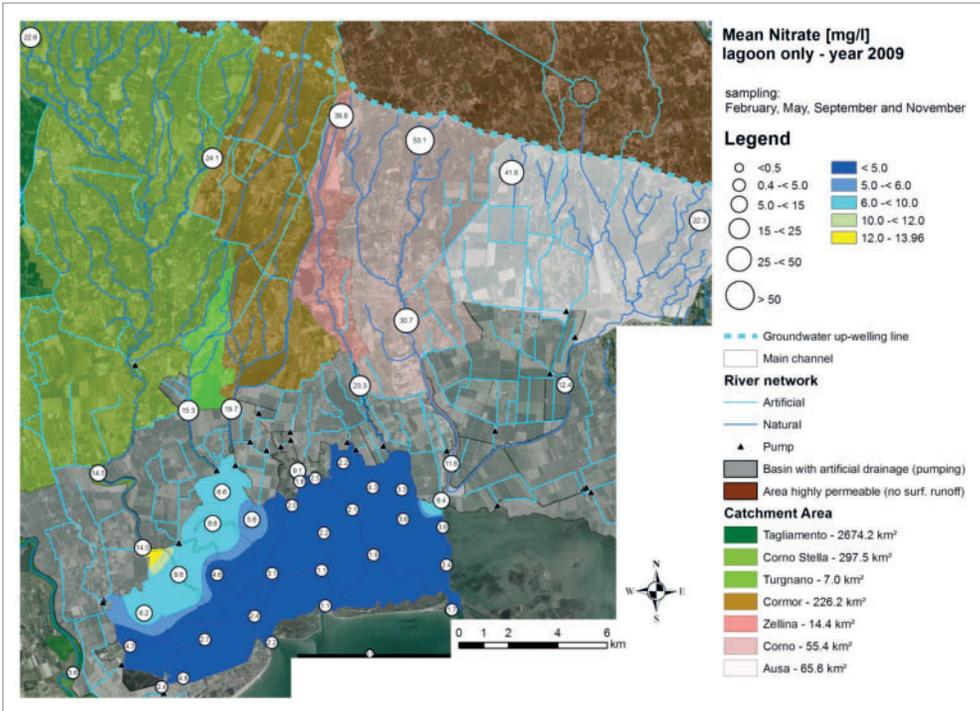


Fig. 3 - Spatial distribution of the mean annual nitrate concentration for 2009.  
Distribuzione spaziale della concentrazione media annuale di nitrati per l'anno 2009.

During the whole monitoring period 2009-2010, the highest nitrate concentrations in the lagoon and in its tributary rivers were observed in November 2009. In this period of the year all crops have already been harvested, therefore the combination of bare soils and high precipitation rates increases the soil nitrate leaching. However, the mean annual nitrate concentration within the lagoon perimeter was for 98.9% of its surface lower than 10 mg/l, while in the monitoring point located in the open sea the nitrate concentration was less than 1 mg/l (mean annual value = 0.5 mg/l). In contrast, the nitrate concentrations in groundwater and along the groundwater up-welling line were always significantly higher and it sometimes exceeded the limit imposed by EU Directive for drinking water.

Relatively high ammonium and nitrite concentrations were detected in all the tributary rivers of the lagoon, while in groundwater and along the groundwater up-welling line their concentrations were below the detection limit. The presence of these two ions in the river system indicates that local pollution sources of anthropogenic origin (e.g. untreated urban and/or industrial wastewaters) have directly contaminated the surface waters. It is important to stress that nitrification processes involving these two nitrogen compounds will consequently increase the nitrate concentration in the lagoon. Additionally, phosphate was detected in all

tributary rivers of the lagoon, while it has never been detected in groundwater and along the groundwater up-welling line. This clearly indicates that the only source of water contamination by phosphates comes from anthropogenic pollution, mainly from urban wastewaters. In the western lagoon sector, relatively high phosphate concentrations were detected. Due to the fact that this nutrient is the most important trigger of eutrophication in aquatic systems it has to be treated much more carefully than nitrate itself.

Conc. NO3 [mg/l]	Classes	Cells	%	Groups [%]	Area [m <sup>2</sup> ]	Area [km <sup>2</sup> ]	Area [ha]
< 5.0	1	575310	74.8	74.8	57531000	57.53	5753.10
5.0 -< 6.0	2	52372	6.8		5237200	5.24	523.72
6.0 -< 10.0	3	133087	17.3	98.9	13308700	13.31	1330.87
10.0 -< 12.0	4	4630	0.6		463000	0.46	46.30
12.0 - 13.96	5	3916	0.5	1.1	391600	0.39	39.16
<b>Total</b>		769315	100.00	100.00	76931500	76.93	7693.15

Conc. Nitrate [mg/l]		
Min	Max	Mean
1.13	13.96	3.95

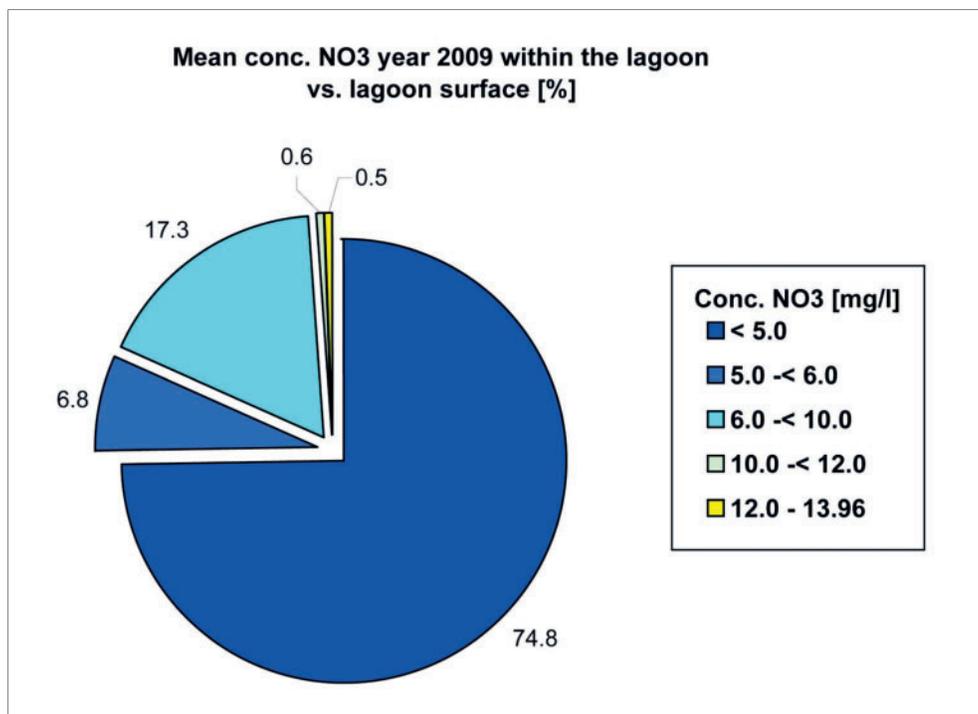


Tabella 2 - Statistical analysis of the mean annual nitrate concentration for 2009.  
 Analisi statistica della concentrazione media annuale di nitrati per l'anno 2009.

## 5.2 Nitrogen and oxygen isotopic composition of nitrate

The stable isotopes ( $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in nitrate from the first sampling campaign (February 2009) were analyzed at the Laboratoire de Glaciologie et Géophysique de l'Environnement in Grenoble (France), while the water samples from the other sampling campaigns were analyzed at the Stable Isotope Laboratory of the School of Environmental Sciences, University of East Anglia, Norwich (United Kingdom).

In order to better determine the potential origin of nitrate identified within the lagoon and its catchment area, the isotopic signatures of the main synthetic and organic fertilizers, typically used in the Region Friuli-Venezia Giulia (FVG), have been measured. The analyses on the isotopic composition of nitrogen ( $\delta^{15}\text{N}$ ) in selected synthetic and organic fertilizers have been done at the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research in Graz (Austria). The  $\delta^{15}\text{N}$  values in ‰ of these analyses are listed in Tab. 3.

$\delta^{15}\text{N}$ ‰	Organic and synthetic fertilizers
4.79	Manure (bovine) from Marianis farm
3.77	Liquid manure (bovine) from Marianis farm
8.52	Liquid manure (pigs)
0.59	Urea
0.30	Ammonium nitrate

Tabella 3 -  $\delta^{15}\text{N}$  values in ‰ of the main organic and synthetic fertilizers typically used in FVG. Valori di  $\delta^{15}\text{N}$  in ‰ dei principali fertilizzanti organici e sintetici utilizzati in Friuli Venezia Giulia.

The isotopic compositions of different anthropogenic and natural nitrate sources as well as the values of measured samples are reported in Fig. 4. Both nitrogen compounds in mineral fertilizers ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) are usually synthesized through industrial fixation of atmospheric  $\text{N}_2$ . Therefore, the isotopic composition of nitrogen in nitrate derived from synthetic fertilizers is close to atmospheric  $\text{N}_2$  ( $\delta \approx 0$ ). These values were consistent with the measured  $\delta^{15}\text{N}$  of ammonium nitrate and urea reported in Tab. 3. None of the measured lagoon samples fell into the isotopic range typically observed for nitrate originating from synthetic fertilizers. This result indicates that synthetic fertilizers have not entered the lagoon directly or, if present, must have undergone additional biological processing. Nitrogen in manure (both liquid and solid) is mainly in the form of urea, which is hydrolyzed to ammonium and converted to nitrate in the soil zone. The hydrolysis of urea produces a temporary rise in pH, which favours the formation of ammonia, easily lost to the atmosphere. These processes lead to an enrichment of  $\delta^{15}\text{N}$  values of the remaining ammonium in liquid manure. The isotopic

compositions of manure samples (both liquid and solid) collected in the study area were characterized by  $\delta^{15}\text{N}$  values in the range of 3.7 – 8.5‰ (+/- 0.1‰) as shown in Fig. 4.

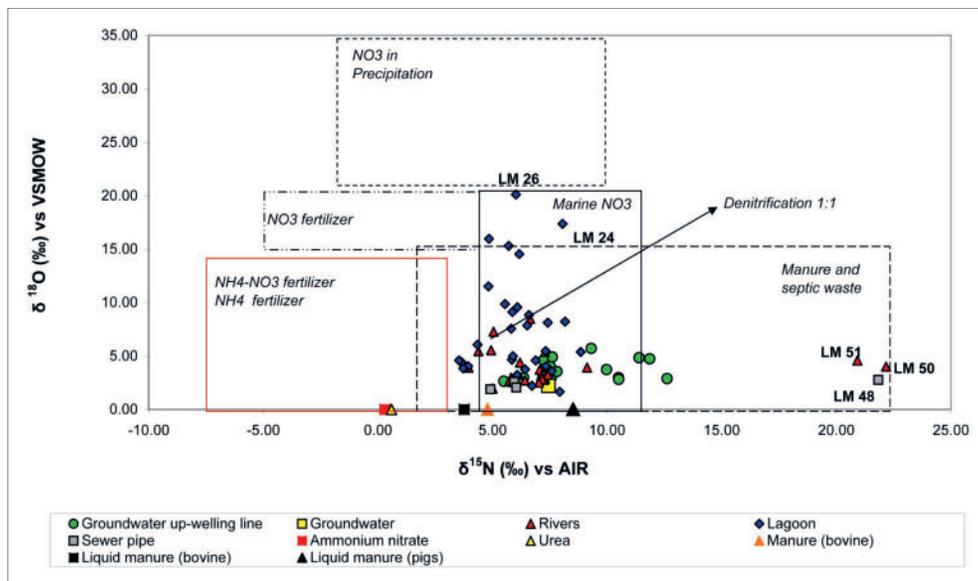


Fig. 4 - Nitrogen and oxygen isotopic composition of nitrate (modified after KENDALL *et al.*, 2007). Composizione isotopica dell'azoto ed ossigeno del nitrato (modificata da KENDALL *et al.*, 2007).

During the nitrification processes the nitrogen in manure (both liquid and solid) will be converted to nitrate where the isotopic values of this newly formed nitrate will be in a similar range or slightly more enriched as described above. Therefore, the isotopic compositions of the different local organic fertilizers have been used as main reference value for the isotopic characterization of the potential nitrate sources related to the agricultural activities. However, nitrate derived from urban sewage water is characterized by  $\delta^{15}\text{N}$  values in the same range, and therefore it is not possible to distinguish it from agricultural nitrogen sources.

The isotopic composition of nitrate measured in the rivers was in the typical range of animal manure and urban sewage water but in the case that urban sewage water flows directly into the river system the  $\delta^{15}\text{N}$  values can be much higher. This phenomenon has been detected in the Cormor River south of Udine city (sampling points: LM50 and LM51) and in the Corno river (sampling point LM48 at sewer pipe). Moreover, these isotopic values fit very well with the high concentrations of nitrate, nitrite, ammonium and phosphates measured at the same sampling points. Since nitrite, ammonium and phosphates are typical indicators of local and direct anthropogenic pollution it could be concluded that, in

this case, the main nitrate sources come from urban wastewaters. Normally, one of the main difficulties in using N isotopic signature as a tracer of nitrate sources lies in the interference between two processes that can lead to an isotopic shift. These processes are dilution of the polluted surface water and natural denitrification. However, in our case the impact of the denitrification process is negligible due to the oxic conditions found in both the lagoon and river waters. The typical  $\delta^{18}\text{O}$  values of nitrate from agriculture activities are in the range between 0 and 5‰ depending on the  $\delta^{18}\text{O}$  value in soil water. If nitrate in the river system is formed from the nitrification of nitrogen compounds (i.e. ammonium) from urban sewage water the isotopic signature  $\delta^{18}\text{O}$  will be more or less in the same range as described above. In the lagoon the characterization of origin and fate of nitrate is in general much more difficult to achieve because of complex mixing processes among different water types like seawater, river water and rain water. Since water circulation in the lagoon is strongly influenced by seawater (as confirmed by the analyses on the spatial distribution of bromide as well as by the isotopic composition of water) and since the  $\delta^{18}\text{O}$  of seawater is higher than that of freshwater, the nitrate directly produced in the lagoon from nitrification of ammonium coming from both anthropogenic sources as well as from mineralisation of local dead biomass (remineralization process) will have a higher  $\delta^{18}\text{O}$  value.

This may mean that nitrate with  $\delta^{18}\text{O}$  greater than 8‰ mainly originated from nitrification processes within the lagoon itself, especially since the small  $\Delta^{17}\text{O}$  values (mean 0.65‰, maximum 3‰) indicate an atmospheric nitrate contribution of no more than 3% on average (but up to 10% locally).  $\Delta^{17}\text{O}$  values of atmospheric nitrate fall in the range of 22 to 35‰, with the corresponding  $\delta^{18}\text{O}$  values between 50 and 90‰. A contribution of 3% atmospheric nitrate would therefore increase the  $\delta^{18}\text{O}$  of lagoon nitrate by only about 2‰. Thus, the isotopic signatures of nitrates detected in the lagoon clearly showed that the nitrate load was not only derived from the agriculture activities but also from other sources like urban wastewaters, nitrification processes in the lagoon itself and from atmospheric deposition.

### 5.3 Boron isotopes

To assess the impact of different boron sources in a catchment area an “end-member mixing model” has to be used. The most important pollution end-members selected for this study were: manure, urban wastewater and seawater. Manure has low boron concentrations (<0.1 mg/l) and big variations in  $\delta^{11}\text{B}$  values from 15 to 30‰. Upwelling groundwater is influenced by agriculture and characterized by relatively low boron concentrations (0.11 to 0.23 mg/l). This is relatively close to natural background content (<0.1 mg/l) in Northern Italy (Tartari & Camusso, 1988). The  $\delta^{11}\text{B}$  values of the upwelling groundwater samples analyzed in this study showed large variations from 4 to 22.7‰ (median value 17.7‰). In contrast, urban wastewater has higher boron concentrations

(0.46 to 1.1 mg/l) and lower  $\delta^{11}\text{B}$  values from 0 to 12.9‰ (Vengosh et al., 1994). No significant differences in boron contents and isotopic compositions have been found between raw and treated sewage. Therefore, boron isotope variations can be applied for tracing contamination of groundwater by both raw and treated sewage effluents. The sampled seawater in the Adriatic Sea at sampling point 47 was characterized by a boron content of 4.2 mg/l and a  $\delta^{11}\text{B}$  value of 40.5‰. Fig. 5 shows the different end-members and the theoretical mixing curves during admixing of seawater with manure and urban wastewater.

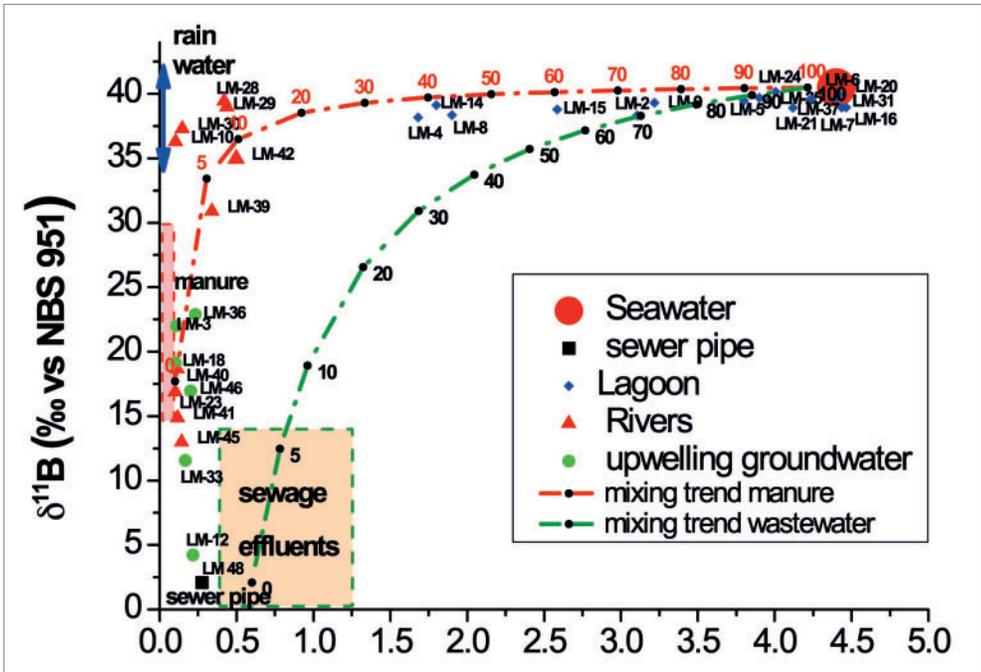


Fig. 5 -  $\delta^{11}\text{B}$  vs. B content and calculated mixing curves during admixing among the end-members (i.e. seawater, urban wastewater and manure).

Valori di  $\delta^{11}\text{B}$  vs. B e curva di mescolamento calcolata durante il rimescolamento degli end-members (cioè: acqua marina, reflui urbani e concimi organici).

Most of the samples plot within the range of these mixing curves can be explained by ternary mixing among the three end-members. However, some of the river water samples were above the mixing lines. This group of data is characterized by low to moderate boron contents (0.1 – 0.45 mg/l) and high  $\delta^{11}\text{B}$  values (36.2 – 39.6‰). This may be the result of the direct mixing of local recharged groundwater with seawater. From this, it can be concluded that the mixing of river water boron with anthropogenic boron from manure and wastewater as well as with boron from seawater is the major factor, which determines the distribution of  $\delta^{11}\text{B}$  in the catchment of the lagoon and in the lagoon itself.

However, the higher boron concentrations in the seawater end-member result in non-linear (hyperbolic) boron concentration versus  $\delta^{11}\text{B}$  mixing curves. That complicates the identification of the other pollution sources especially in the lagoon. Therefore, the  $\delta^{11}\text{B}$  values have been normalized using bromide concentration in water as a second tracer for admixing of seawater as shown in Fig. 6. The data plotted below indicate that most of the samples were affected by both agriculture and urban wastewater pollution sources. Moreover, the data shows that in some areas of the lagoon the waters were less influenced by these pollution sources. This identifies rain water as an additional mixing end-member.

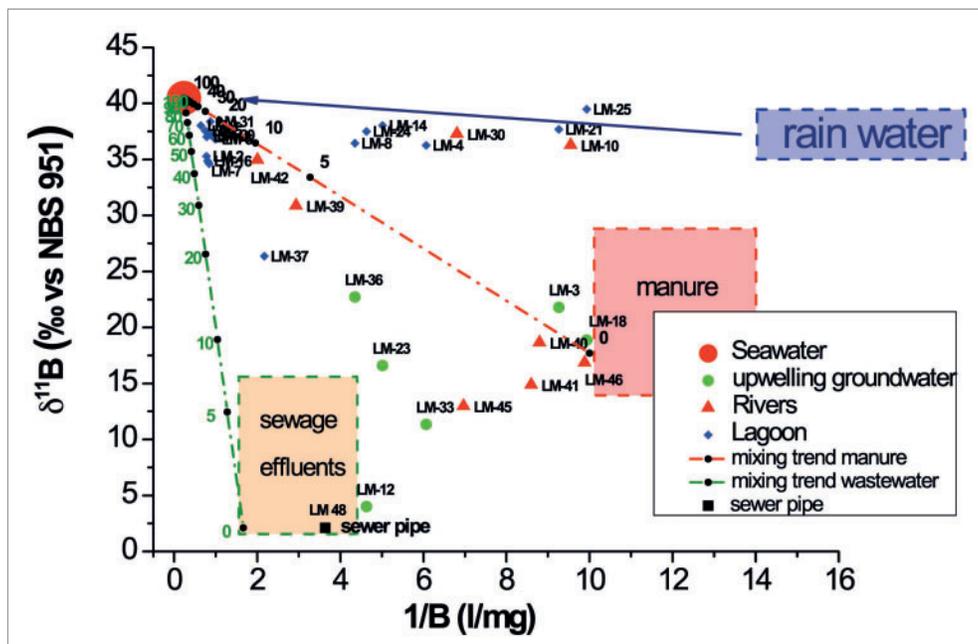


Fig. 6 -  $\delta^{11}\text{B}$  vs.  $1/\text{B}$  diagram showing normalised boron concentrations and  $\delta^{11}\text{B}$  values. Diagramma del  $\delta^{11}\text{B}$  vs.  $1/\text{B}$  inerente alle concentrazioni di boro normalizzate ed i valori di  $\delta^{11}\text{B}$ .

#### 5.4 Sulphur and oxygen isotopes

Dissolved sulphate in aquatic ecosystems may be derived from atmospheric (marine sea spray, anthropogenic sources), pedospheric (geogenic and fertilizers) and lithospheric (evaporates and oxidation of sulphide minerals) sources. Moreover, sulphur compounds in liquid industrial waste products are a potential additional source of sulphate. Fig. 7 shows the commonly observed fields of  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values for sulphates depending on their geogenic or anthropogenic origin. The different sulphates are introduced to the aquatic ecosystem by groundwater discharge to rivers and direct mixing with seawater and to a smaller

extend by precipitation (sea spray and atmospheric sulphate). Oxygen isotope values of dissolved sulphate are far from the isotope exchange equilibrium with water. Therefore, sulphur and oxygen isotopes can be used to trace natural and anthropogenic sources of sulphur in agricultural watersheds as well as in coastal systems. Except for one sampling campaign at site LM40 (Tagliamento river), the sulphur isotope data indicate that seawater sulphur is the dominant sulphate source.

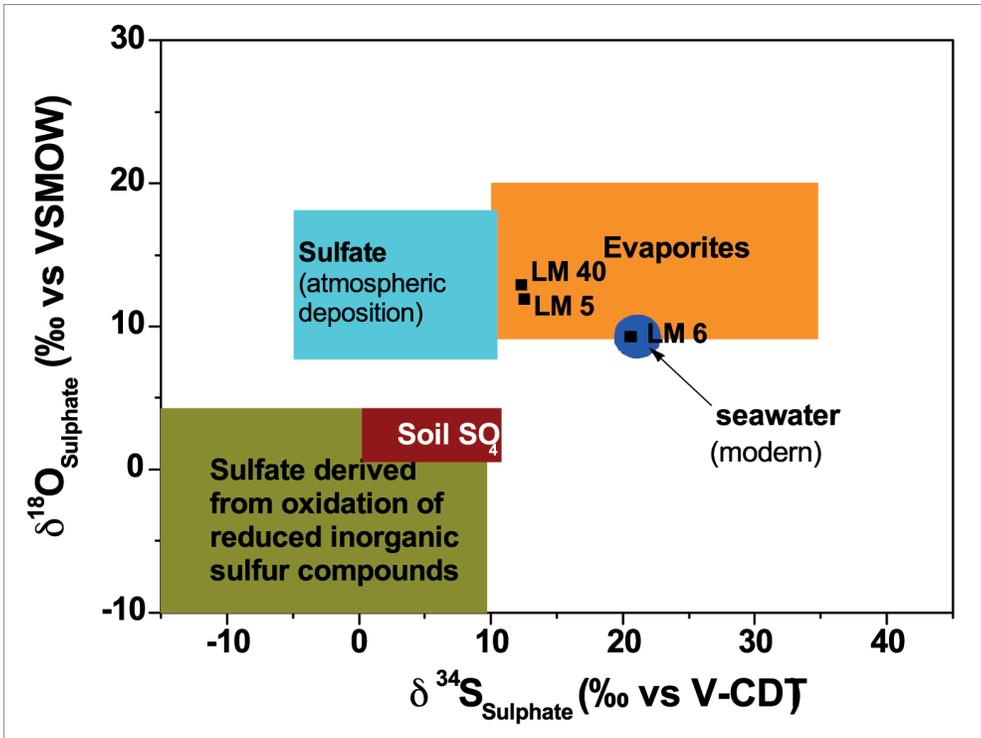


Fig. 7 - Commonly observed ranges of  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values for sulphate of different origin (after CLARK & FRITZ, 1997) and measured isotope values for dissolved sulphate in water samples at sites LM5, LM6 and LM40.

Campi comunemente rilevati del  $\delta^{34}\text{S}$  e  $\delta^{18}\text{O}$  per solfati di origine diversa (da CLARK & FRITZ, 1997) e valori isotopici misurati del solfato in soluzione nei punti di campionamento LM5, LM6 e LM40.

There is a sharp increase in sulphur isotope data with increasing chloride concentrations (Fig. 8) as expected for mixing between freshwater and seawater (e.g., Fry, 2002). The measured  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values for sulphate at the sampling points LM40, on the other hand, were +12.3‰ and +12.9‰, respectively. This most likely indicates a lithogenic origin in the low-concentrated solutions, e.g. from the dissolution of Permian evaporites (BÖTTCHER, 1999). This is

in agreement with the ion balance of the dissolved components found at these sites. Whereas pyrite oxidation induced by oxidants like, nitrate or oxygen, can be neglected as a significant sulphate source (ZHANG *et al.*, 2010), mixing between different geogenic and anthropogenic sulphur sources, that may have partly been modified by microbially induced transformations, cannot completely ruled out and requires further investigations. Dissolved sulphate at the sampling point LM6 (lagoon) shows  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values of +20.6‰ (vs V-CDT) and +9.3‰ (vs V-SMOW) respectively, that are typical for Mediterranean seawater (BÖTTCHER *et al.*, 1998) without indication for significant net sulphate reduction or an intense reduction-oxidation cycle (BÖTTCHER *et al.*, 2001). The stable isotope analyses of sulphate confirm that the water of the south-western lagoon sector is under influence of the Tagliamento river.

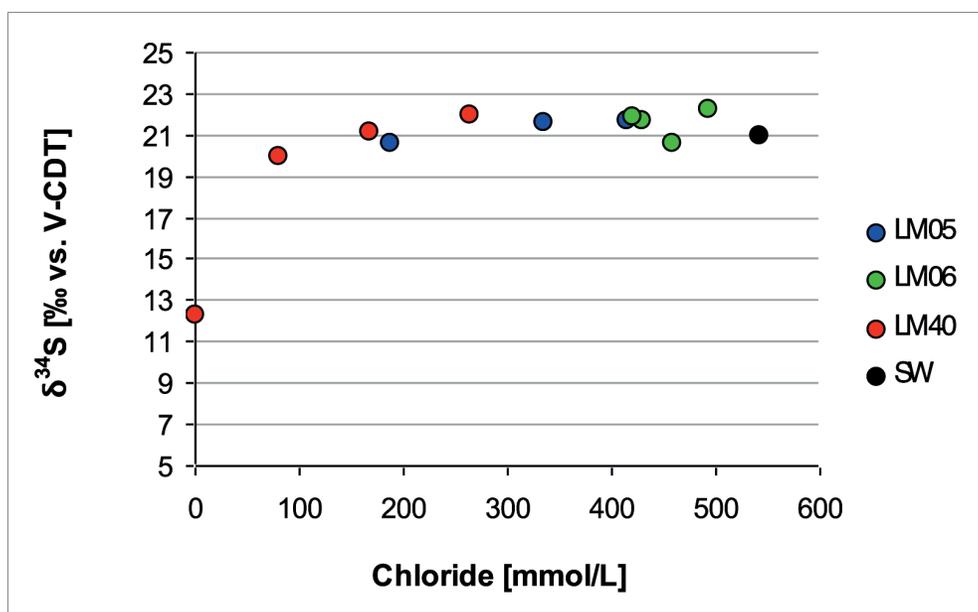


Fig. 8 - Covariation of  $\delta^{34}\text{S}$  data of dissolved sulphate with dissolved chloride concentrations, as a measure for the impact of seawater. Lighter Isotope data are found at low chloride concentrations.

Covarianza tra i valori di  $\delta^{34}\text{S}$  del solfato in soluzione e la concentrazione del cloruro in soluzione quale misura dell'impatto dell'acqua marina. Valori isotopici più leggeri vengono trovati a basse concentrazioni di cloruro.

## 5.5 Groundwater dating

Measurements of tritium ( $^3\text{H}$ ) of the groundwater sampled in June 2010 were carried out using the helium-ingrowth ( $^3\text{He}$ ) method at the noble gas laboratory of the Institute of Environmental Physics / Section of Oceanography, University of Bremen (Germany). The dating method utilized for these analyses is described e.g. by Kipfer *et al.* (2002). The groundwater in the Upper Friulian Plain was

sampled at different irrigation wells where two samples of water for  $^3\text{H}$  measurements were collected at each sampling point.

The characteristics of the selected wells as total well depth, groundwater table depth, groundwater sampling depth and depth of the installed pumps are described in Tab. 4, while the results of the analysis for tritium and helium isotopes for groundwater dating are reported in Tab. 5.

Sample ID	Sampling date	Total well depth [m]	Groundwater table depth [m]	Sampling depth [m]	Depth of pump 1 [m]	Depth of pump 2 [m]
55-PO27	15.06.10	26	16.12	18	21.35	22
56-PO30	15.06.10	33.6	10.61	13	15.5	17.5
57-PO101	15.06.10	35	14.35	17	21	35

Tabella 4 - Characteristics of the selected groundwater sampling wells.  
Caratteristiche dei pozzi selezionati per il campionamento della falda.

Sample ID	$^3\text{H}$ [TU]	Excess Ne [%]	$^4\text{He}$ radiogenic [ccSTP/kg] Error: 0.2 E-6	$^3\text{He}$ tritogenic [TU]	Age [years] normalized to June 15. 2010
55-PO27	4.95±0.1	48	0.0 E-6	0.4	1.3±0.5
56-PO30	5.1±0.1	44	0.0 E-6	0.4	1.4±0.5
57-PO101	4.85±0.1	34	0.0 E-6	1.2	4.0±1.0

Tabella 5 - Results of the analysis for tritium and helium isotopes for groundwater dating.  
Risultati delle analisi isotopiche del Trizio ed Elio per la datazione delle acque di falda.

The data reported in the table above were calculated with assumed infiltration temperature of 10°C, altitude 0m a.s.l. and scaled to the sampling date of June 15<sup>th</sup> 2010. Analytical errors of all data were negligible. For most of the samples double measurements have been conducted. Tritium concentrations and ages refer to 15<sup>th</sup> June 2010. Ages were calculated with the assumption of piston flow conditions in the aquifer. None of the samples show  $^4\text{He}$  from radiogenic sources, which is used as an indicator for waters with ages much above 50 years. The concentrations of tritium and tritogenic helium ( $^3\text{He}_{\text{tri}}$ ) in the samples match the concentrations of tritium in precipitation quite well. The portion of excess Ne - compared to solubility equilibrium concentration - is rather high, namely in the order of 34 – 48% of the equilibrium value. This indicates that an equilibration of the dissolved gases in the water with the atmosphere after recharge could be excluded. Hence, the low concentration of  $^3\text{He}_{\text{tri}}$  and the derived young ages are a reliable feature of the investigated aquifer. As shown in Fig. 9, the groundwater ages calculated at the three sampling points is reported.

The calculated groundwater ages, normalized to June 15<sup>th</sup> 2010, are respectively  $1.3 \pm 0.5$  years at the point 55-PO27 in Chiasellis and  $1.4 \pm 0.5$  years at the point 56-PO30 in Gris, practically the groundwater age at these two points is the same. Instead, the calculated groundwater age at the point 57-PO101 in Mereto di Capitolo is  $4 \pm 1$  years. The Upper Friulian Plain includes calcareous and dolomitic gravels with a well-developed phreatic aquifer, with an aquifer transmissivity of approximately  $10^{-2}$  to  $10^{-4}$  m<sup>2</sup>/s in a southerly direction (Mosetti, 1983; Carniel, 1999). To explain the groundwater age differences between the first two sampling points (55-PO27 and 56-PO30) and the point 57-PO101 it must be noted that shortly before sampling groundwater at point 57-PO101 the two pumps installed in the irrigation well were running. Therefore, mixed waters between upper groundwater and deeper groundwater layers (older groundwater) have been sampled. In the other two irrigation wells (55-PO27 and 56-PO30) the pumps were, for many days, out of service, therefore the sampled waters from these two wells were not mixed with deeper water as in the first case.

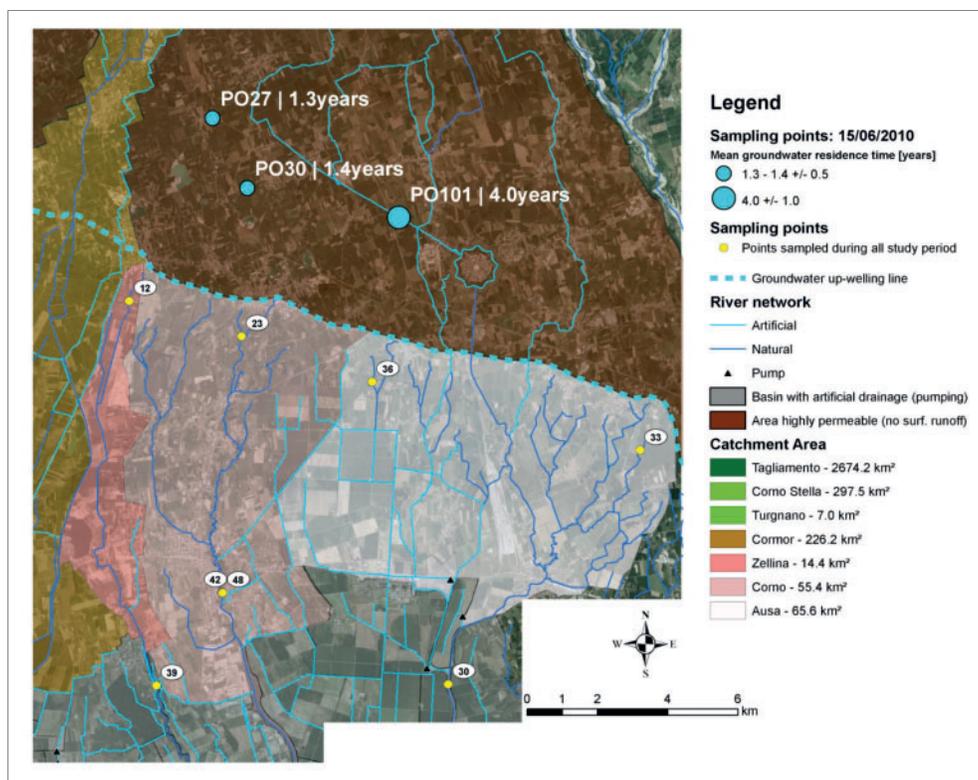


Fig. 9 - Location of the groundwater sampling points and calculated mean groundwater residence time [years].

Localizzazione dei punti di campionamento della falda e valori calcolati del tempo medio di residenza dell'acqua di falda [anni].

## 6 Conclusions

Nitrate pollution is still a major concern in many European aqueous resources, which may originate from multiple sources. The development and implementation of effective remediation measures in nitrate polluted areas requires identification of the main sources and estimation of their contribution is essential for environmental policy makers to implement effective, source-oriented remediation measures. For this reason the need of an integrated water resources management policy becomes fundamental in order to improve the management and the quality of water. Isotope data can help to identify nitrate pollution sources, to investigate the fate of nitrate in the environment and to come up with appropriate measures to reduce nitrate pollution. In 2008, in the Friuli-Venezia Giulia Region the entire catchment area of the Marano lagoon was designated as a nitrate vulnerable zone (NVZ) where an action program on the reduction of nitrate leaching was launched. Before this study all the remediation measures adopted within the NVZ were addressed to reduce pollution caused only by nitrates from agricultural activities excluding a priori other potential nitrate pollution sources.

This study represents a novel interdisciplinary research approach to the determination of potential nitrate sources in a lagoon environment, which, beside the traditional hydrochemical analyses (main ions and nutrients), introduces the isotope signature of nitrate ( $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ), boron ( $\delta^{11}\text{B}$ ), water ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ), and sulphate ( $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$ ), the tritium-helium ( $^3\text{H}$ - $^3\text{He}$ ) isotopic method as well as hydrological, remote sensing and GIS techniques. The methodology adopted in this project represents also an example of integrated water resources management, where information and knowledge from different productive sectors have been combined and implemented in the final interpretation of the project results. Moreover, this research study provides a new important scientific contribution in terms of identification of potential nitrate pollution sources that the water management authorities of the Friuli-Venezia Giulia Region have to implement in their future water policy in order to improve water quality of the Marano lagoon and its catchment area.

The stable isotopes in nitrate measured by the denitrifier method have been used to differentiate among nitrate coming from agriculture (synthetic and natural fertilizers), airborne nitrate and nitrate from nitrification processes in soils. Boron isotopes have been used to identify the impact of domestic wastewaters to the aquatic system. The stable isotopes in water have been used to calculate mixing ratios between sea and fresh water and to get information about the mean altitude of the recharge area of surface waters. The stable isotopes in sulphate have been adopted to determine both its origin and the marine and terrestrial contributions, while the tritium-helium isotopic method has been used for groundwater dating. Remote sensing and GIS techniques have been adopted to assess the spatial distribution of most superficial algal flora, surface water tempe-

perature as well as the key environmental and morphological changes of the lagoon since the beginning of the 1970s as already reported in the last “*Bollettino della Società naturalisti Silvia Zenari*”, N.34, December 2010 (SACCON, 2010).

The isotopic compositions of different anthropogenic and natural nitrate sources as well as the isotopic signatures of nitrate in numerous water samples collected in the catchment area of the lagoon and in the lagoon itself were measured. None of the samples fell into the isotopic range typically observed for nitrate originating from synthetic fertilizers. Nitrates detected in groundwater and along the groundwater up-welling line are mainly related to the use of manure (both liquid and solid), while other nitrate sources come from urban wastewaters as detected in some rivers like, for instance, Cormor and Corno rivers. In the lagoon the characterization of the origins and fate of nitrate was in general much more difficult to achieve because of complex mixing processes among different water types like seawater, river water and rain water. However, it was possible to identify that nitrate can be formed in the lagoon by nitrification processes of ammonium coming from both anthropogenic sources as well as from remineralisation. Therefore, it can be concluded that the nitrate load in the lagoon, as detected during the monitoring period 2009-2010, was not only derived from agriculture activities but also from other sources like urban wastewaters, nitrification processes in the lagoon as well as from atmospheric deposition. Due to the fact that boron represents a co-migrant of nitrate in anthropogenic pollution sources, boron isotopes have been used as an additional tracer to identify different human impacts in aquatic ecosystems. Manure, urban wastewater and seawater were the three end-members used for this analysis. From the concentration values and from the isotopic signatures of boron found in the sampled waters, it can be concluded that the distribution of  $\delta^{11}\text{B}$  in the lagoon and its catchment area was the result of mixing anthropogenic boron from urban wastewaters, manure (liquid and solid) and from boron in seawater. The measured data indicate that most of the samples were influenced by both pollution sources, i.e., agriculture and urban wastewaters. Moreover, the data shows that in some parts of the lagoon the water is less affected by these two pollution sources. Few samples along the groundwater up-welling line could not be explained by the use of the end-member mixing model. Therefore, these samples may be affected by the contamination of a different anthropogenic boron source such as landfill. The isotopic compositions of water have been used to characterize the mean altitude of the recharge area of the freshwater samples as well as to identify the origin of groundwater along the groundwater up-welling line. Additionally, the water isotopes were used to identify mixing processes between freshwater and seawater within the lagoon. From these results it was possible to confirm that the sampled water along the groundwater up-welling line comes from local groundwater.

The isotopic signature of sulphate has been adopted to verify the possible connection between Tagliamento River and part of the western lagoon sector

through the Bevazzana artificial channel. The  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  values of sulphate measured at the sampling points LM40 (Tagliamento River) and LM5 (Bevazzana channel) indicate that at both sites the sulphate may originate from the similar lithospheric sources (e.g., the dissolution of evaporites), whereas at the sampling point LM6 (lagoon) the sulphate originates from modern Mediterranean seawater. The sulphur and oxygen isotope analyses confirm that the waters of the south-western lagoon sector are influenced by the Tagliamento River.

Moreover, the tritium-helium method was used for calculating the mean groundwater residence time (groundwater age). The results of this analysis conducted in three groundwater wells in the Upper Friulian Plain have shown that groundwater age is respectively  $1.3 \pm 0.5$  years in the point 55-PO27 in Chiasellis and  $1.4 \pm 0.5$  years in the point 56-PO30 in Gris. Instead, the calculated groundwater age in the point 57-PO101 in Mereto di Capitolo is  $4 \pm 1$  years. At last, the multi temporal analysis of the remotely sensed data has shown that the main morphological changes of the lagoon and its surroundings mainland that occurred during the period of 1973-2007 were much more influenced by the numerous human activities (i.e. industrial, touristic and urban) in comparison to those related to the natural transformation processes (SACCON, 2010).

**Acknowledgments:** Many institutions and individuals have contributed to make this work possible.

First and foremost, we would like to thank the Regional Agency for Rural Development (ERSA) of the Friuli-Venezia Giulia region for funding the project and for the scientific support in all the project phases. We express our very sincere gratitude to the Regional Forestry Corps (Corpo forestale Regionale) and to the Regional agency for rural, agro-food and forest resources (Direzione centrale risorse rurali, agroalimentari e forestali) for providing logistical support in the fields and the very qualified personnel.

We are also thankful to the technical and scientific staff of all laboratories involved in the isotopic analyses and in particular to the colleagues Barbara Zirngast of the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research, Ines Scherff (Leibniz-Institut für Ostseeforschung Warnemünde – IOW) and Thomas Max (Max Planck Institute for Marine Microbiology – MPI, Bremen). The research leading to the results carried out by the School of Environmental Sciences of the University of East Anglia have benefited from the research findings in the IntraMIF project in the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement number n°237890. Moreover, the CNRS, the INSU and the LEFE-CHAT program are acknowledged for their scientific support of the present study mainly through the activities of Joël Savarino, and Joseph Erbland.

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