Determination of nitrate pollution sources in the Marano Lagoon (Italy) by using a combined approach of hydrochemical, isotopic, and remote sensing techniques

Bestimmung der Nitratquellen in der Lagune von Marano (Italien) durch kombinierte Anwendung von hydrochemischen, isotopischen und Fernerkundungsmethoden

P. Saccon[1,2], A. Leis[2], A. Marca[3], J. Kaiser[3], L. Campisi[3], M.E. Böttcher[4] & P. Escher[5]

[1] Waterdrop Consulting GmbH, Graz, Austria.
[2] JR-AquaConSol GmbH, Graz, Austria.
[3] School of Environmental Sciences, University of East Anglia, Norwich, United Kingdom.
[4] Leibniz Institute for Baltic Sea Research (IOW), Geochemistry & Isotope Geochemistry Group, Marine Geology Section, Warnemünde, Germany.
[5] Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

Korrespondierende Autoren: Pierpaolo Saccon, *office@waterdrop-consulting.eu;* Albrecht Leis, *albrecht.leis@jr-aquaconsol.at*

Zusammenfassung

Nitratbelastungen, die aus unterschiedlichen Quellen stammen können, stellen nach wie vor ein großes Problem für die europäischen Wasserressourcen dar. Die Erarbeitung und Umsetzung wirksamer Schutzmaßnahmen in Gebieten mit erhöhter Nitratbelastung erfordert einerseits die Identifizierung der wichtigsten Nitratquellen und anderseits die Abschätzung ihres Beitrages zu der Gesamtbelastung. Dies ist gleichzeitig von wesentlicher Bedeutung für die Ableitung zielgerichteter Sanierungsmaßnahmen für Entscheidungsträger in der Umweltpolitik. Durch die Einbeziehung von Isotopentechniken in das Ressourcenmanagement können potentielle Verschmutzungsquellen wesentlich besser identifiziert werden und dadurch geeignete Maßnahmen zur Reduktion der Nitratbelastung getroffen werden.

Im Jahr 2008 wurde in der Region Friaul-Julisch Venetien das gesamte Einzugsgebiet der Lagune von Marano als nitratgefährdetes Gebiet (NVZ) ausgewiesen und ein Aktionsprogramm zur Verringerung der Nitratauswaschung ins Leben gerufen. Bis zum Zeitpunkt der vorliegenden Studie wurden als Ursache für die Nitratbelastungen im genannten Gebiet ausschließlich Stickstoffeinträge aus landwirtschaftlichen Quellen angenommen und alle anderen potentiellen Nitratquellen als mögliche Verursacher von vornherein ausgeschlossen. Im Rahmen dieser Studie sollte untersucht werden, inwieweit dieser Ansatz haltbar ist oder ob neben den Stickstoffeinträgen aus der Landwirtschaft auch noch andere bedeutende Nitratquellen existieren. Da eine solche Differenzierung auf der alleinigen Grundlage herkömmlicher Methoden nur unzureichend möglich ist, wurde im Rahmen dieser Studie ein neuartiger, stark interdisziplinärer Ansatz zur Ermittlung der potentiellen Nitratquellen im Einzugsgebiet einer Lagune gewählt. Dieser bezieht neben der Auswertung traditioneller hydrochemischer Analysen (Haupt-Ionen und Nährstoffparameter) auch die Bestimmung der isotopischen Zusammensetzung von Nitrat ($\delta^{15}N$, $\delta^{18}O$ und $\delta^{17}O$), Bor ($\delta^{11}B$), Wasser ($\delta^{2}H$ und $\delta^{18}O$) und Sulfat ($\delta^{34}S$ und $\delta^{18}O$) in die Untersuchungen ein. Ergänzt wurde das Untersuchungsspektrum durch begleitende hydrologische Untersuchungen, Fernerkundung und GIS-Techniken.

Die Untersuchungen der stabilen Isotope im Nitrat erfolgten mit Hilfe der sogenannten „Denitrifizierer"-Methode. Diese Methode erlaubt die sensitive Isotopenmessung geringer Nitratmengen und ermöglicht die Differenzierung der Herkunft von Nitrat aus unterschiedlichen

terrestrischen N-Quellen (z. B. Güllestickstoff, Kunstdünger, Biomasse) und atmosphärischer Deposition. Gleichzeitig können mit Hilfe der Isotopensignaturen im Nitrat unterschiedliche Umsetzungs- und Transformationsprozesse unterschieden werden. Der Einsatz der Bor-Isotope gestattet hingegen die Identifikation der Auswirkungen kommunaler Abwässer auf das aquatische System. Für die Berechnung der Mischungsverhältnisse zwischen Süß- und Meerwasser wurden die stabilen Isotope des Wassermoleküls herangezogen. Die Umweltisotope des Wassers dienten außerdem zur ungefähren Abschätzung der Höhenlage der Einzugsgebiete einzelner Oberflächen- und Grundwässer. Über die Isotopensignaturen im gelösten Sulfat konnten einerseits Informationen über dessen Herkunft gewonnen werden und andererseits marines Sulfat von terrestrischen Anteilen abgegrenzt werden. Mit Hilfe von Fernerkundungsmethoden und GIS-Techniken wurden die räumlichen und zeitlichen Veränderungen der Algenverteilungen in der Lagune untersucht. Gleichzeitig dienten diese Methoden der näherungsweisen Bestimmung der Oberflächenwassertemperaturen und der Charakterisierung der vielfältigen morphologischen Veränderungen in der Lagune seit den 1970er Jahren.

Im Ergebnis der Isotopenuntersuchungen im Nitrat konnte gezeigt werden, dass die Nitratgehalte der Oberflächen- und Grundwässer im Bereich der Grundwasser–Aufstiegszone im Wesentlichen aus landwirtschaftlichen Quellen stammen. Gülledüngung stellt die maßgeblichste N-Quelle dar, während der Anteil von Kunstdünger eher gering ist. Im Gegensatz dazu lassen sich in einigen Flüssen, wie zum Beispiel bei Cormor und Corno, deutliche Beeinflussungen durch meist ungeklärte kommunale Abwässer nachweisen. In der Lagune selbst gestaltet sich die Quellendifferenzierung deutlich komplizierter. Dies ist dadurch bedingt, dass die ursprünglichen Isotopensignaturen durch komplexe Mischungsverhältnisse und nachträgliche Umsetzungsprozesse überlagert werden. Allerdings konnte anhand der signifikant erhöhten δ^{18}O-Werte bei zahlreichen Nitratproben aus der Lagune eine nachträgliche Nitratbildung nachgewiesen werden. Außerdem war es auf der Grundlage der δ^{17}O-Werte erstmals möglich, Nitrat aus atmosphärischen Quellen in der Lagune nicht nur nachzuweisen, sondern auch zu quantifizieren. Durch die zusätzliche Einbeziehung der Bor-Konzentrationen und -Isotope als weitere Tracer konnte ein Mischungsmodell mit verschiedenen Endgliedern erstellt werden, mit dem die wechselnden Anteile der einzelnen Verschmutzungsquellen an den unterschiedlichen Probenahmepunkten gut differenziert werden konnten. Die Daten zeigen, dass der überwiegende Teil der Proben Kontaminationen aus verschiedenen Quellen enthält. Die wesentlichsten Verschmutzungsquellen stellen die Landwirtschaft und ungeklärte kommunale Abwässer dar. Daneben ist bei einigen Proben im Bereich der Grundwasser-Aufstiegszone zusätzlich noch eine weitere Bor-Quelle erkennbar, die mit dem bestehenden Mischungsmodell nicht erklärt werden kann. Hier liegt der Verdacht nahe, dass diese Proben zusätzlich durch eine Kontamination aus einer anderen anthropogenen Borquelle - z. B. aus einer Deponie - beeinflusst werden.

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) fertilisers 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 deduced 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 characterise multiple nitrate pollution sources.

Nitrate (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 fertiliser 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 programme on the reduction of nitrate leaching was initiated. Thus, to identify and characterise 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 programme water samples from the lagoon, its tributary rivers, the groundwater upwelling line, groundwater, sewer pipes and the open sea were collected on a quarterly interval from 2009 to 2010 and subsequently analysed. The present study represents a novel approach on the identification and characterisation of potential sources of

nitrate pollution in a lagoon environment, which, besides the traditional hydrochemical analyses (main ions and nutrients), introduces the whole suite of stable isotopes of nitrate (δ^{15}N, δ^{18}O and δ^{17}O), the isotopic signature of boron (δ^{11}B), the stable isotopes of water (δ^{2}H and δ^{18}O), the stable isotopes of sulphate (δ^{34}S and δ^{18}O), 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 (Italy).

Investigation 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^2, 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 approximately 160 km^2, 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 is in the order of 1,750 and 1,500 m^3/s, respectively. 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 characterised by the urban and touristic areas of Lignano Sabbiadoro city, which is one of the main summer resorts in northern 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 7th 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^2. The fishing community of Marano Lagunare is situated in the northern lagoon sector and is the main urban centre within the lagoon perimeter with its 2007 inhabitants. 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 characterised 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^3/s), Turgnano (0.5 m^3/s), Cormor (9.3 m^3/s), Zellina (1.1 m^3/s), Corno (4.6 m^3/s) and Ausa with 10.0 m^3/s (RET, 2006). The groundwater upwelling 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 upwelling line in the left side of the Tagliamento River is about 80 m^3/s, which corresponds to a mean discharge of about 1.3 m^3/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 (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.
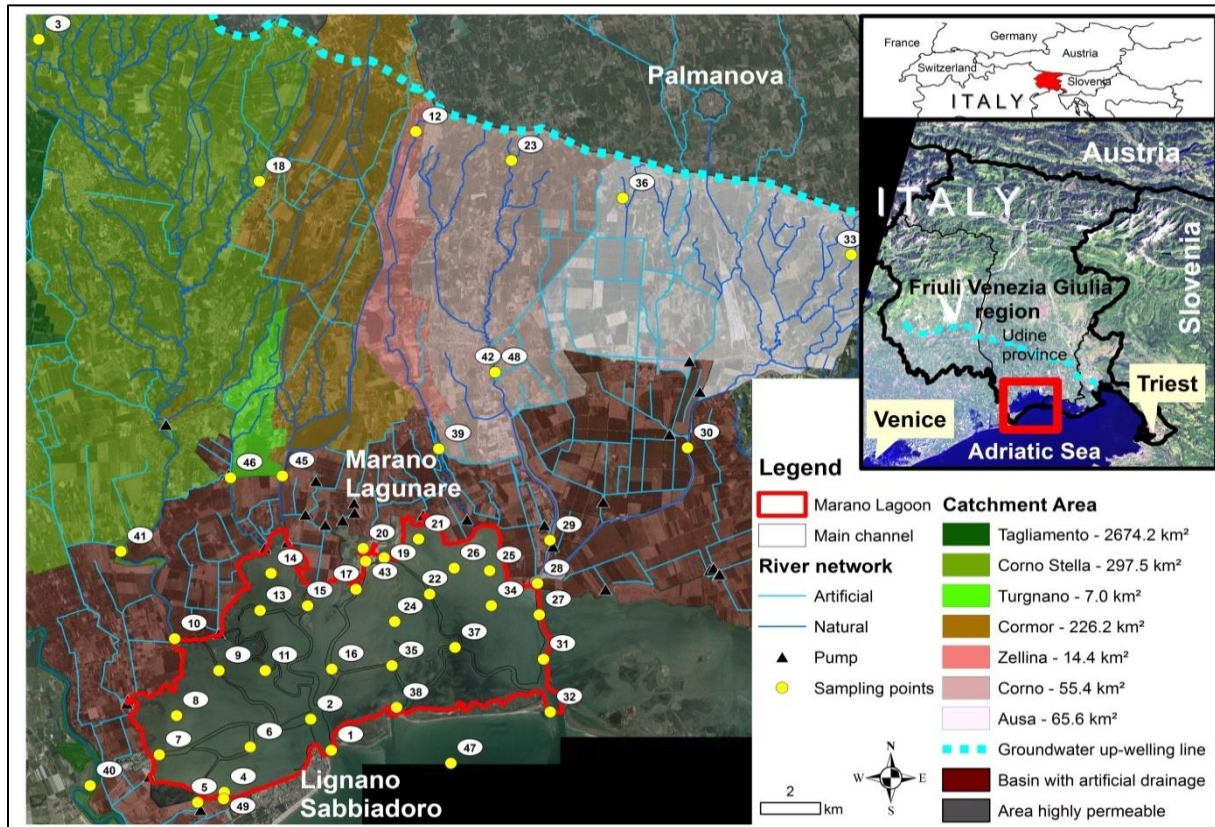

Figure 1: Study area: Marano Lagoon (red line).

Water sampling programme

In the water sampling programme 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 programme was carried out on a quarterly basis, as indicated in Tab. 1:

Table 1: Sampling programme schedule

Sampling campaign	Season	Date
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

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 upwelling 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.

Analytical methods

Hydrochemical analysis

For the chemical characterisation of the different water types included in the monitoring programme, 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 minimise 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 to take into account the local tide condition, wind speed and direction as well as the water current speed and its direction during each single sampling. 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 concentrations 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 analysed in Graz at the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research. Dissolved cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and anions (Cl^-, Br^-, 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.

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 & MCDONNELL, 1998). For example, among the hydrogen isotopes, deuterium (denoted as D or ^2H) 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 analysed 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 analysed by counting the number of disintegrations per unit time on gamma ray or beta particle counters, or analysed on mass spectrometers (KENDALL & CALDWELL, 1998).

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

– Nitrogen: AIR (atmospheric 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 analysed at the same time with this reference standard or with an internal laboratory standard that has been calibrated relative to the international standard.

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

δ (in ‰) = ($R_{sample}/R_{standard}$ – 1) x 10^3

where:

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

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

1) The oxygen and hydrogen isotopic composition of water ($\delta^{18}O$ and δ^2H) and the nitrogen isotopic composition ($\delta^{15}N$) of synthetic fertilisers, 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 ($\delta^{15}N$, $\delta^{18}O$ and $\delta^{17}O$) from the first sampling campaign (February 2009) were analysed in Grenoble (France) at UJF-Grenoble 1/CNRS-INSU, Laboratoire de Glaciologie et Géophysique de l'Environnement (LGGE), while for the other sampling campaigns the water samples were analysed 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 ($\delta^{11}B$) were carried out in Kiel (Germany) at the GEOMAR, Helmholtz-Zentrum für Ozeanforschung.

4) The analyses of the stable isotopes of sulphate ($\delta^{34}S$) 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). $\delta^{18}O$ values were measured at the Max Planck Institute for Marine Microbiology (MPI Bremen).

Stable isotopes of water: oxygen-18 and deuterium

The oxygen-18 (^{18}O) and deuterium (^2H) isotopes are integrated parts of the water molecule and are, therefore, well-suited for hydrological tracing and budget studies (SAETHER & DE CARITAT, 1997). The ^{18}O and ^2H contents are expressed as $\delta^{18}O$ and δ^2H and reported relative to the VSMOW (Vienna

Standard Mean Ocean Water) standard. Isotope hydrology provides complementary information on the type and origin 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, salinisation and discharge processes. Analysis of water for $\delta^{18}O$ and $\delta^{2}H$ can also provide extremely useful information about the sources of the nitrate and other solutes in water (Aravena & Robertson, 1998; McMahon & Böhlke, 2006). Frequently, different sources of nitrate in rivers and groundwater are associated with different water $\delta^{18}O$ and $\delta^{2}H$ values because of evaporation or because the waters are derived from very different geographic areas (Kendall et al., 2007). In this project deuterium ($\delta^{2}H$) was measured with a Finnigan DELTA^{plus} 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 ($\delta^{18}O$) of water was measured by the classic CO_2–H_2O equilibrium technique (Epstein & Mayeda, 1953) with a fully automated device adapted from Horita et al. (1989) coupled to a Finnigan DELTA^{plus} Mass Spectrometer.

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 $\delta^{15}N$ 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 & 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 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 oxygen-18 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 fertilisers), 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 20 ml 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_2O and the atmospheric gases (Fig. 2, right).

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

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_2O that may have been produced overnight. After the second purge, a volume of water sample of known nitrate concentration was injected into the bacteria suspension to produce 10 nmol of N_2O 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 N_2O was extracted, purified and measured using a Europa GEO 20-20 mass spectrometer on-line with a TGII prep system and Gilson autosampler. Water vapour was removed using three trapping systems: Nafion dryer, magnesium perchlorate trap and -80 °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 N_2O, as these gases are isobaric. Samples were analysed in triplicate, and together with them three international reference materials were analysed in quadruplicate: IAEA N3, USGS 34 and USGS 35. The raw data were 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 $\delta^{15}N$) and with respect to VSMOW (for $\delta^{18}O$). The precision of the analyses was generally better than 0.3 ‰ for both ^{15}N and ^{18}O measurements, based on the quadruplicate analyses of the three international standard materials measured.

For the oxygen-17 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 N_2O produced from the bacterial denitrification was 50 nmol, and (ii) prior to the mass spectrometer the N_2O gas was passed through a gold tube heated at 800 °C and converted to N_2 and O_2 gases, which were then passed through a molecular sieve GC column so that the N_2 gas was delayed with respect to the oxygen before going to the mass spectrometer. Using this protocol the ^{15}N, ^{17}O and ^{18}O isotopic compositions were measured using the nitrogen and oxygen gases, rather than the N_2O.

Stable isotopes of boron

Boron (B) has two naturally occurring stable isotopes, ^{10}B and ^{11}B. The large relative mass difference between the boron isotopes leads to a wide (ca. 90 ‰) natural range of $\delta^{11}B$ values (Barth, 1993). Because B is widely used in industrial, agricultural, cosmetics, and household products, $\delta^{11}B$ 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 ($NaBO_3$), 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, $\delta^{11}B$ 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); (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 $\delta^{11}B$ coupled with $\delta^{15}N$ 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), $\delta^{11}B$ was used to distinguish between two types of sewage that were indistinguishable using $\delta^{15}N$ alone: a high-B/low-NO_3/low-$\delta^{11}B$ type that is derived from washing powders, and a moderate-B/moderate-NO_3 type with $\delta^{11}B$ 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 $\delta^{11}B$ vs. 1/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).

Stable isotopes of sulphate: sulphur-34 and oxygen-18

Sulphur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) 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 a 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 °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 V_2O_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 via a Thermo Electron ConFlo IV split interface to a Thermo Scientific Flash 2000 elemental analyser. Silver sulphide isotope reference materials (IAEA-S-

1, IAEA-S-2 and IAEA-S-3) were used to link the mass spectrometric results (^{34}S/^{32}S) to the V-CDT scale (MANN et al., 2009). Replicate measurements agreed within better than ±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 VSMOW standard using NBS127, IAEA-SO-5 and IAEA-SO-6 as reference materials.

Remote sensing data analysis

Remote sensing is the science of acquiring information about the earth's surface without actually being in contact with it. This is done by sensing and recording reflected or emitted energy and processing, analysing, and applying that information (from: CCRS). Airborne and satellite remote sensing systems have known a remarkable evolution in the last years; this allowed the placing into orbit of increasingly sophisticated earth observation systems. The availability of a wider range of data with different spatial and spectral resolutions apparently allows the overcoming of a few past limitations concerning the chance of a detailed analysis of ecologically relevant but spatially limited environments (CARLEER et al., 2005; HAIPING et al., 2006; MASELLI et al., 2005; QIAN et al., 2006).

To assess the multi-temporal development as well as the spatial distribution of most superficial algal flora, the water temperature of the lagoon surface as well as the key environmental and morphological changes of the Marano lagoon, remotely sensed data collected since the beginning of the 1970s from the satellites Landsat-1 MSS, Landsat-5 TM, Landsat-7 ETM+ and recent orthophotos have been processed and interpreted. For a better characterisation of the environmental changes that occurred in the Marano lagoon and its catchment area in the last 35 years, as well as for a more accurate comparison among the different agricultural processes linked to the vegetative growth cycle of the crops, only imagery acquired between July and August has been used for this purpose. The results of the remotely sensed data analyses have been used as indirect indicators to better explain the main natural and anthropogenic processes that can trigger the eutrophication of the lagoon. The contributions of remote sensing analyses have then been integrated in the final interpretations of the hydrochemical and isotopic analyses.

Results

Hydrochemical characterisation of waters

The results of the hydrochemical analyses (SACCON et al., 2013) 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. From this statistical analysis it was possible to determine that: (i) 74.8 % of the lagoon surface are characterised by a mean annual nitrate concentration lower than 5 mg/l (dark blue area); (ii) 98.9 % of the lagoon surface have a concentration lower than 10 mg/l; and (iii) only 1.1 % of the area in question have 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.
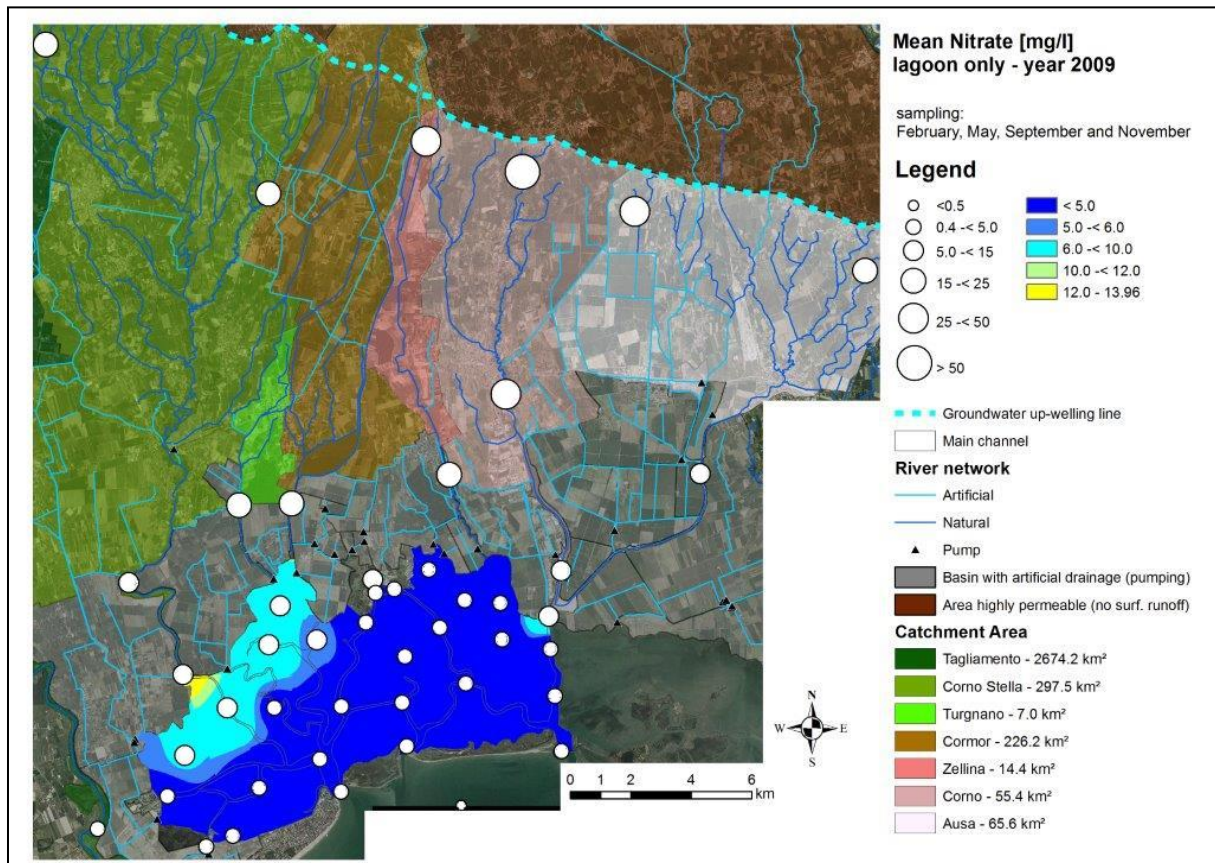

Figure 3: Spatial distribution of the mean annual nitrate concentration for 2009.

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 lower than 10 mg/l for 98.9 % of its surface, while at 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 upwelling line were always significantly higher and sometimes exceeded the limit imposed by the EU Directive for drinking water.

Relatively high ammonium and nitrite concentrations were detected in all the tributary rivers of the lagoon, while in the groundwater and along the groundwater upwelling 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 upwelling 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.

Nitrogen and oxygen isotopic composition of nitrate

The stable isotopes (δ^{15}N, δ^{18}O and δ^{17}O) in nitrate from the first sampling campaign (February 2009) were analysed at the Laboratoire de Glaciologie et Géophysique de l'Environnement in Grenoble (France), while the water samples from the other sampling campaigns were analysed at the Stable Isotope Laboratory of the School of Environmental Sciences, University of East Anglia, Norwich (United Kingdom). A comprehensive overview of all individual analytical measurements has been already published (SACCON et al., 2013).

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 fertilisers typically used in the Region Friuli-Venezia Giulia (FVG) have been measured. The analyses of the isotopic composition of nitrogen (δ^{15}N) in selected synthetic and organic fertilisers were conducted at the Laboratory Centre for Isotope Hydrology and Environmental Analytics of Joanneum Research in Graz (Austria). The δ^{15}N values in ‰ of these analyses are listed in Tab. 2.

Table 2: δ^{15}N values in ‰ of the main organic and synthetic fertilisers typically used in FVG.

δ^{15}N (‰)	Organic and synthetic fertilisers
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

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 fertilisers (NO_3^- and NH_4^+) are usually synthesised through industrial fixation of atmospheric N_2. Therefore, the isotopic composition of nitrogen in nitrate derived from synthetic fertilisers is close to atmospheric N_2 ($\delta \approx 0$). These values were consistent with the measured δ^{15}N of ammonium nitrate and urea reported in Tab. 2. None of the measured lagoon samples fell into the isotopic range typically observed for nitrate originating from synthetic fertilisers. This result indicates that synthetic fertilisers 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 hydrolysed 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 δ^{15}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 characterised by δ^{15}N values in the range of 3.7–8.5 ‰ (0.1 ‰) as shown in Fig. 4.
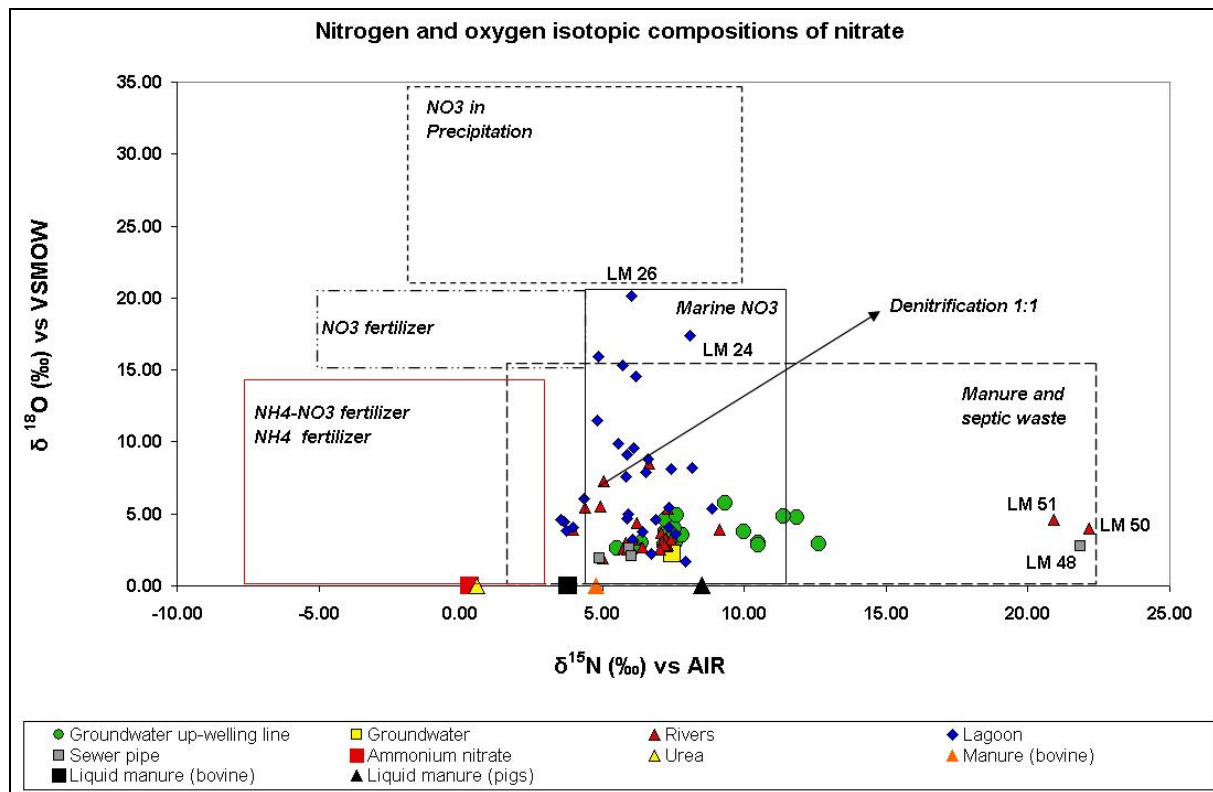
Figure 4: Nitrogen and oxygen isotopic composition of nitrate (modified after KENDALL et al., 2007).

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 fertilisers have been used as main reference value for the isotopic characterisation of the potential nitrate sources related to the agricultural activities. However, nitrate derived from urban sewage water is characterised by $\delta^{15}N$ 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 $\delta^{15}N$ 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 a 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 signatures as tracers 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 $\delta^{18}O$ values of nitrate from agriculture activities are in the range between 0 and 5 ‰, depending on the $\delta^{18}O$ 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 $\delta^{18}O$ will be more or less in the same range as described above. In the lagoon the characterisation 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 rainwater. 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 $\delta^{18}O$ 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 (remineralisation process) will have a higher $\delta^{18}O$ value.

This may mean that nitrate with $\delta^{18}O$ greater than 8 ‰ mainly originated from nitrification processes within the lagoon itself, especially since the small $\delta^{17}O$ values (mean 0.65 ‰, maximum 3 ‰) indicate an atmospheric nitrate contribution of no more than 3 % on average (but up to 10 % locally). $\delta^{17}O$ values of atmospheric nitrate fall in the range of 22 to 35 ‰, with the corresponding $\delta^{18}O$ values between 50 and 90 ‰. A contribution of 3 % atmospheric nitrate would therefore increase the $\delta^{18}O$ 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.

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 strong variations in $\delta^{11}B$ values from 15 to 30 ‰. Upwelling groundwater is influenced by agriculture and characterised by relatively low boron concentrations (0.11 to 0.23 mg/l). This is relatively close to the natural background content (< 0.1 mg/l) in Northern Italy (TARTARI & CAMUSSO, 1988). The $\delta^{11}B$ values of the upwelling groundwater samples analysed in this study show 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 $\delta^{11}B$ 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 characterised by a boron content of 4.2 mg/l and a $\delta^{11}B$ 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.

Most of the samples plot within the range of these mixing curves, which 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 characterised by low to moderate boron contents (0.1–0.45 mg/l) and high $\delta^{11}B$ values (36.2–39.6 ‰). This may be the result of the direct mixing of local recharged groundwater with seawater. Hence, 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 that determines the distribution of $\delta^{11}B$ in the catchment of the lagoon and in the lagoon itself. However, the higher boron concentrations in the seawater end-members result in non-linear (hyperbolic) boron concentration versus $\delta^{11}B$ mixing curves. That complicates the identification of the other pollution sources especially in the lagoon. Therefore, the $\delta^{11}B$ values have been normalised 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.
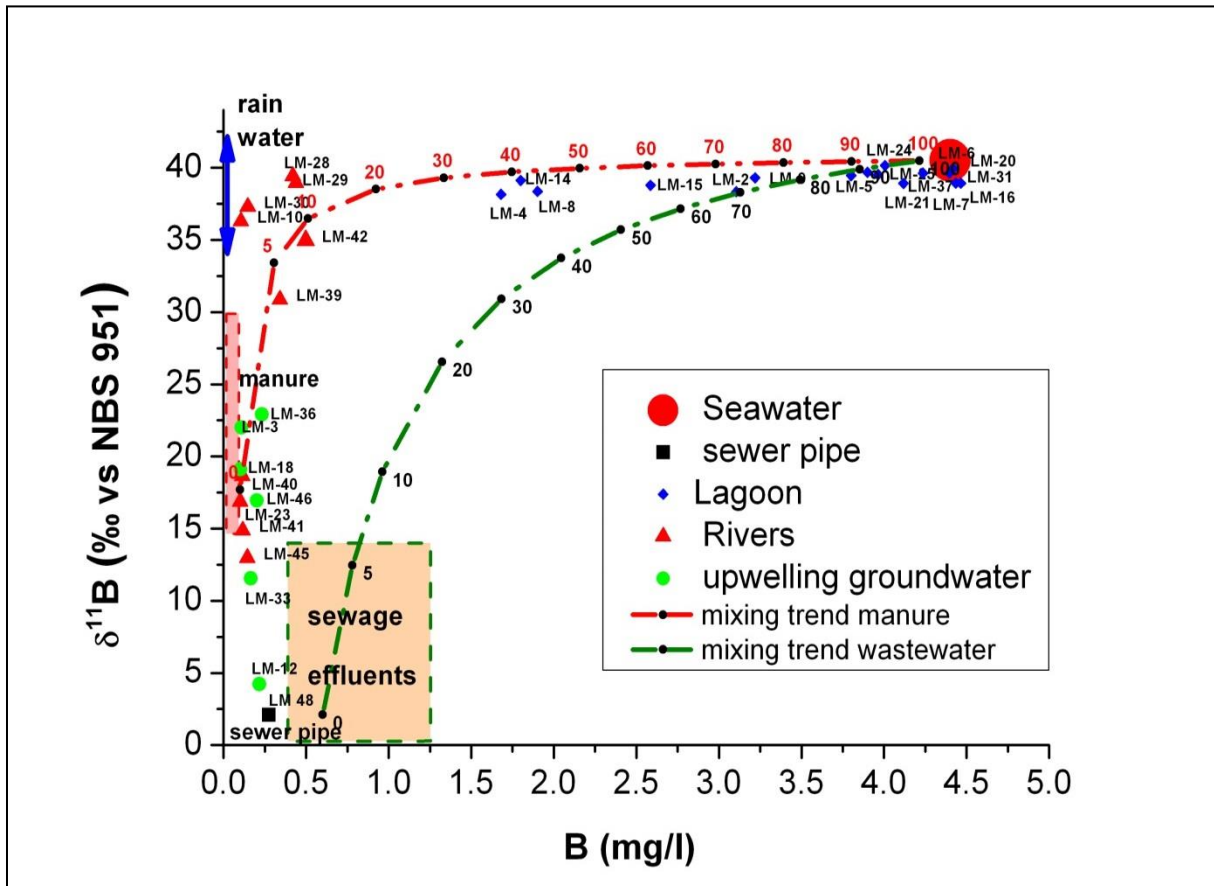

Figure 5: $\delta^{11}B$ vs. B content and calculated mixing curves during admixing among the end-members (i. e. seawater, urban wastewater, and manure).
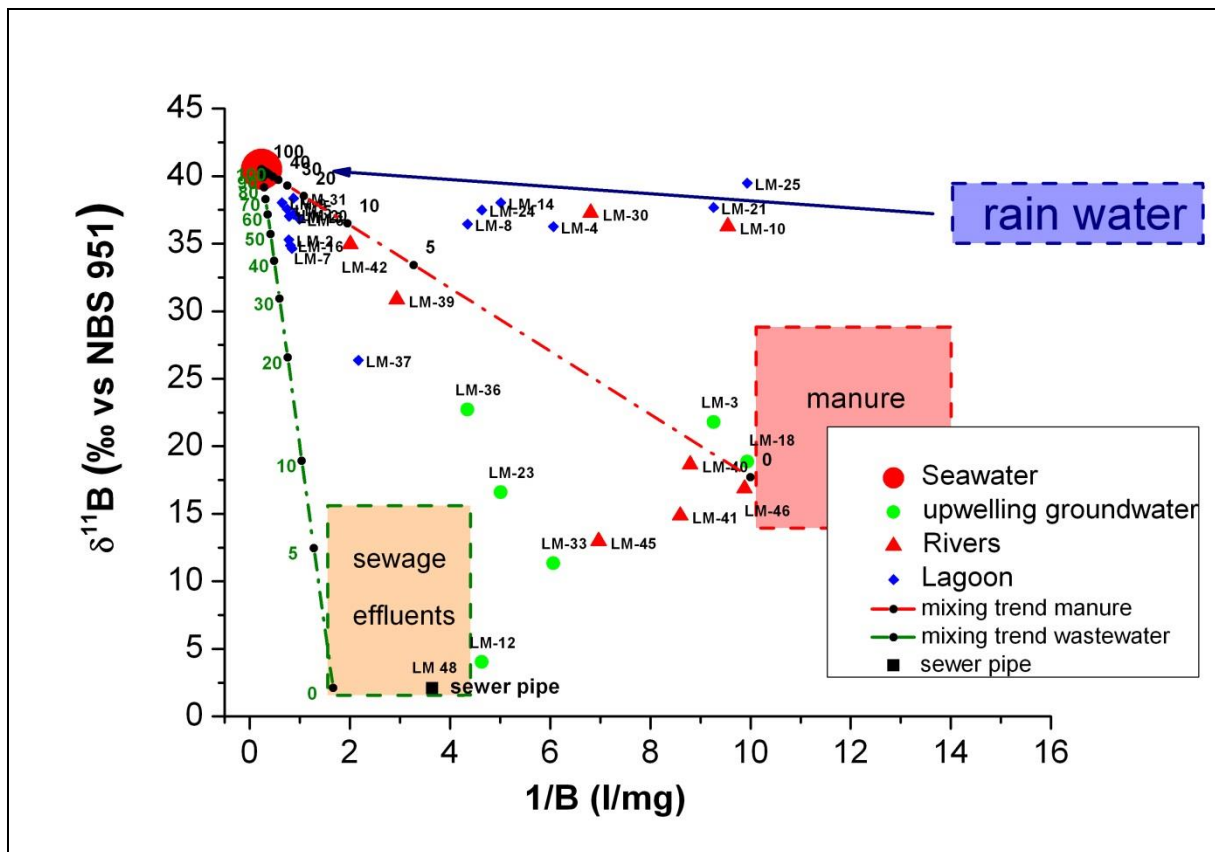
Figure 6: δ^{11}B vs. 1/B diagram showing normalised boron concentrations and δ^{11}B values.

Sulphur and oxygen isotopes

Dissolved sulphate in aquatic ecosystems may be derived from atmospheric (marine sea spray, anthropogenic influence), pedospheric (geogenic and fertilisers) 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 δ^{34}S and δ^{18}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 extent 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.
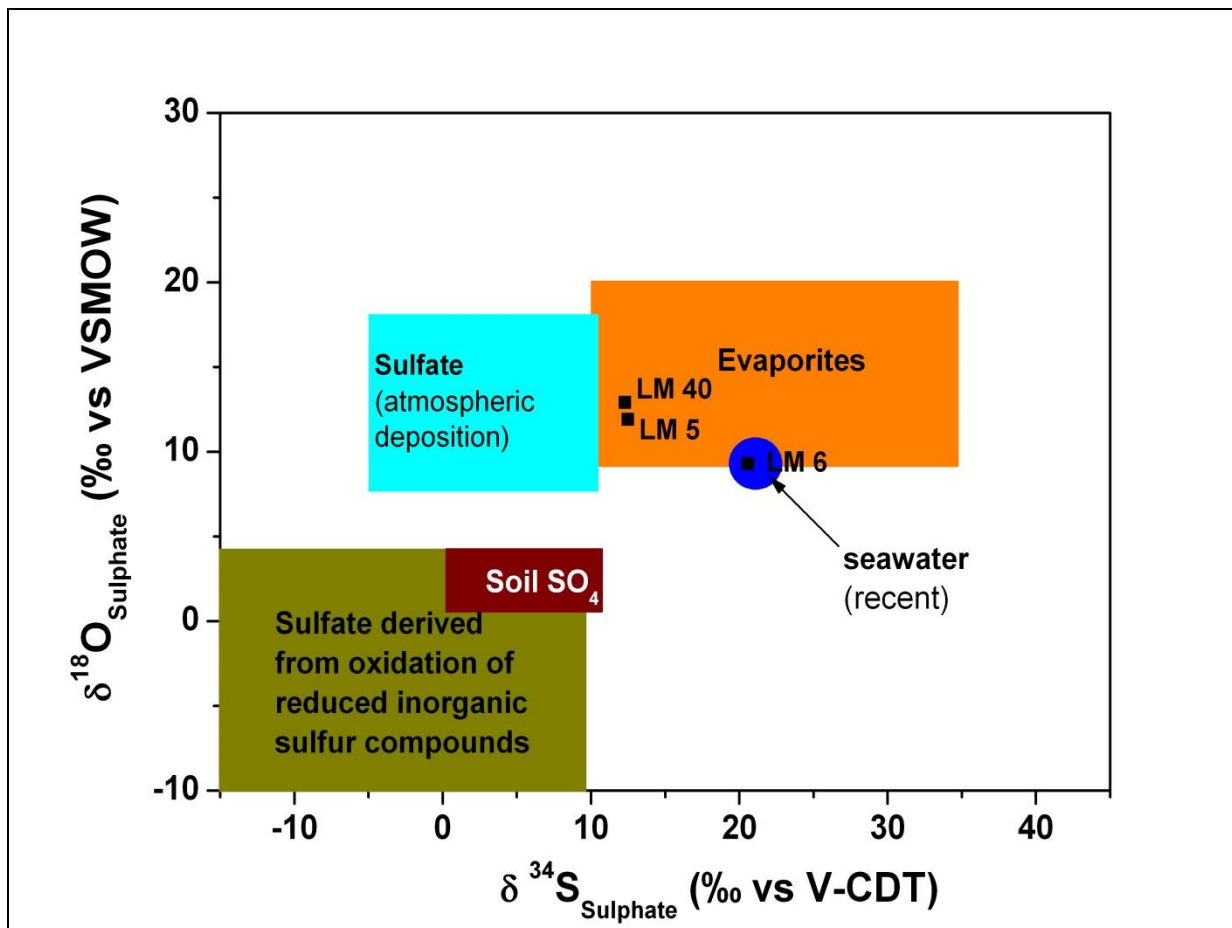
Figure 7: Commonly observed ranges of δ³⁴S and δ¹⁸O values for sulphate of different origin (after CLARK & FRITZ, 1997) and measured isotope values for dissolved sulphate in water samples at sites LM5, LM6 and LM40.

There is a sharp increase in sulphur isotope data with increasing chloride concentrations as expected for mixing between freshwater and seawater (e. g. FRY, 2002). The measured δ³⁴S and δ¹⁸O values for sulphate at the sampling point 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. While 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, which may have partly been modified by microbially induced transformations, cannot be completely ruled out and requires further investigations. Dissolved sulphate at the sampling point LM6 (lagoon) shows δ³⁴S and δ¹⁸O values of +20.6 ‰ (vs. V-CDT) and +9.3 ‰ (vs. VSMOW), 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.

Multi-temporal analysis using remotely sensed data

Using remotely sensed data, multi-temporal analyses on the spatial distribution of most superficial algal flora have been conducted, and the surface temperature of the lagoon and part of its

catchment area as well as the key environmental and morphological changes of the Marano lagoon have been analysed.

In order to cover a useful time interval for the multi-temporal analysis, satellite imagery acquired in the last 35 years (from 1972 to 2007) from Landsat-1 MSS, Landsat-2 MSS, Landsat-5 TM, Landsat-7 ETM+ (10 scenes in total) as well as recent orthophotos have been collected. Before proceeding to the satellite data processing, the images have been compared with the daily precipitation values of the month in which they were acquired. This was done to verify if the precipitation that occurred days prior to the satellite data acquisition could somehow influence the comparability of the data series used in the multi-temporal analysis. This comparison shows that the rainfall events in all four analysed cases were not relevant enough to compromise the comparability of the satellite data series. Therefore, the multi-temporal analyses should not be affected by any important changes on different ground surface characteristics related to the precipitation like, for instance, soil moisture, water interception by canopy, etc.

Surface temperature

Multi-temporal analysis of the spatial distribution of surface temperature (T_s) in the study area (lagoon and the surrounded mainland) was performed using the satellite data acquired by Landsat-5 TM in the following days: 11/07/1984 (09:20 GMT), 18/08/1992 (08:31 GMT), 17/08/2003 (09:29 GMT) and 27/07/2007 (09:45 GMT). It was not possible to analyse imagery from other Landsat satellites prior to 1982 (prior Landsat-4) because the thermal infrared channel necessary for this analysis was not yet included in the data set. The surface temperature expressed in °C was quantified using a part of the energy balance model (e. g. ALLEN & TASUMI, 2000; ALLEN et al., 2001; BASTIAANSSEN et al., 1998) dedicated to the software package ERDAS Imagine. The data sources required by the model for this computation were, in addition to the satellite images and their geometrical parameters, the digital terrain model (DTM) and various meteorological parameters (e. g. wind speed, dew point, ground roughness, etc.) collected from a meteorological station within the investigated area (SACCON, 2010). An example of this analysis is reported in Fig. 8.
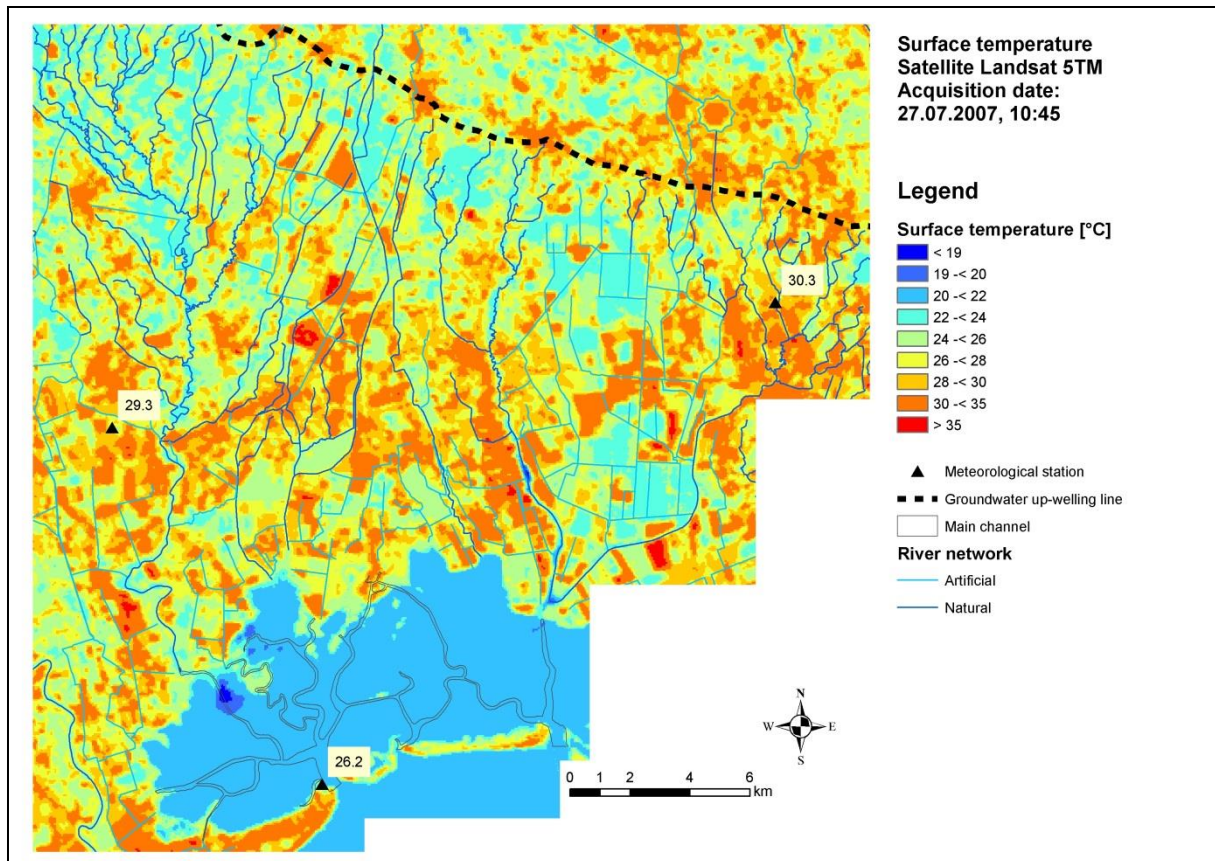
Figure 8: Surface temperature from 27/07/2007 (10:45 local time).

The results of each analysis were then compared and validated with the air temperature (T$_a$) values measured in several meteorological stations within the study area. The analyses of surface water temperatures in the lagoon clearly showed the temperature differences as well as the spatial distributions that freshwaters originating from the tributary rivers have within the lagoon perimeter in the different analysed periods. Moreover, the results of the image processing have been used as indirect verification tool to validate the degree of accuracy of the interpolated data using the "Inverse Distance Weighting" (IDW) interpolation method. To this purpose the surface water temperature and the spatial distribution of the nitrate concentration in the lagoon have been compared as shown in Fig. 9. The results of this comparison have demonstrated that both surface water temperature and nitrates coming from the Ausa-Corno river system were moving southwest instead of continuing their flow south within the Ausa-Corno Channel. This phenomenon has been observed not only related to the spatial distribution of nitrate concentration, but also in other analysed ions which confirms that the waters coming from the Ausa-Corno river system tend to flow towards the southwest. It should be noted that the remotely sensed data provide spatial information (pixel-by-pixel) of the earth's surface, thus continuous information and not punctual information as that collected in-situ and then used in the interpolation method. To conclude, we can consider this method of indirect comparison an essential tool for the verification and validation of the interpolated data produced using the selected interpolation method (SACCON, 2010).
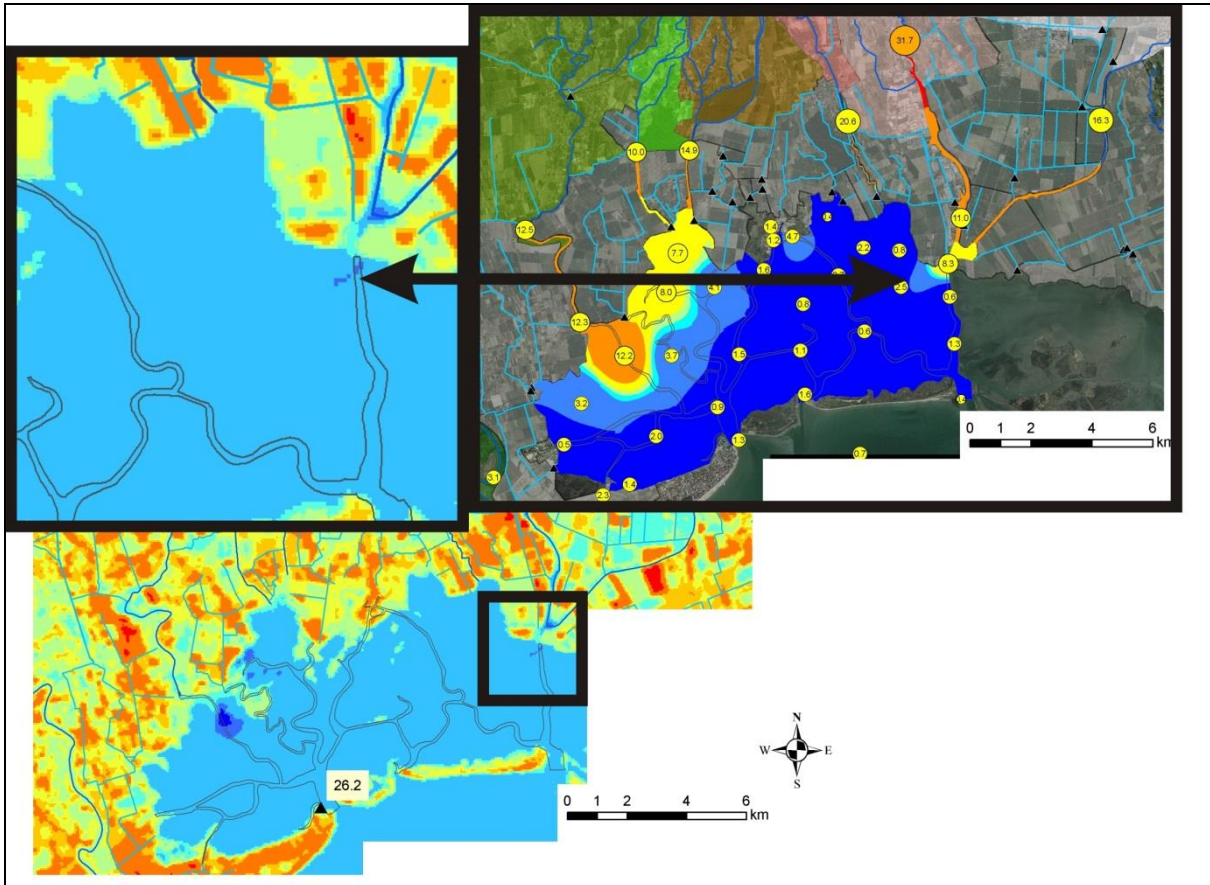
Figure 9: Comparison between the spatial distribution of the surface water temperature and nitrate concentration in the lagoon.

Algae distribution

Analysis of the most superficial algae distribution and, in particular, the mapping of the spatio-temporal evolution of seagrass meadows (*Posidonia Oceanica*) are very important indirect methods for the assessment of the presence of nutrient load causing the eutrophication of the lagoon. For this purpose, satellite imagery from 06/08/1973 (Landsat-1 MSS), 11/07/1984 (Landsat-5 TM), 17/08/2003 (Landsat-5 TM), and 27/07/2007 (Landsat-5 TM) as well as orthophotos acquired in summer 2007 have been processed and compared.

Taking into account the limitations of the classification method by identifying "objects" within water bodies, it is necessary to underline that the expected results are always strongly dependent on the local environmental conditions like, for instance, water transparency, wave motion, tide condition, water current direction, aqueous vapour in the air, as well as the cloud cover. The results obtained classifying the remotely sensed data have been verified directly in the lagoon during the water sampling campaigns. Only the results obtained processing the orthophotos from summer 2007 showed a more realistic spatial distribution of the algal cover that is at least comparable to the algal cover observed directly in the lagoon during the verification campaigns. An example of this analysis is reported in Fig. 10.
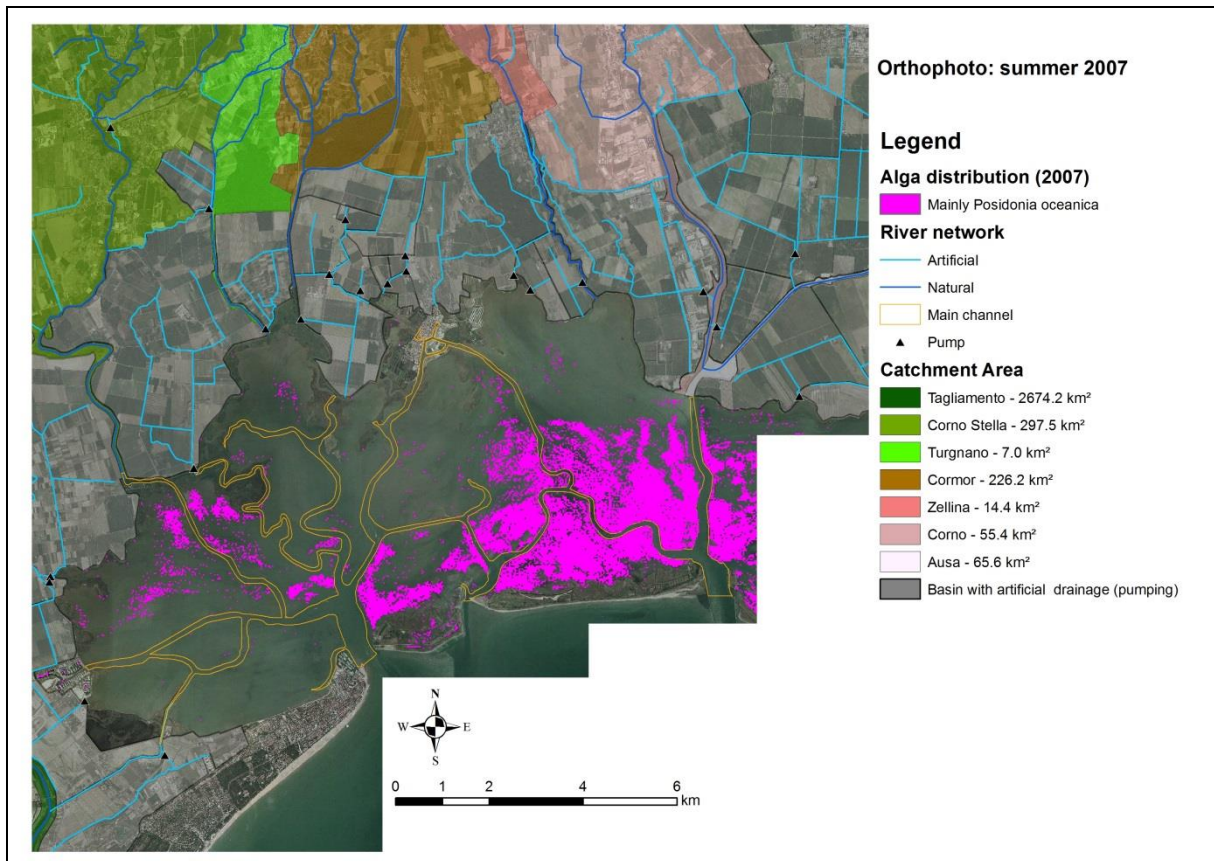
Figure 10: Classified image showing the algae distribution in the lagoon (areas marked in magenta colour).

Change detection

The multi-temporal analysis of the main morphological changes within the Marano lagoon and its surroundings that occurred from 1973 to 2007 have been analysed using the change detection algorithm included in the software package ERDAS Imagine. In this analysis, the algorithm identified the main multi-temporal differences in pixel values of two images, previously classified using the ISODATA algorithm, producing a new image with three classes which highlighted the areas that have been subjected to morphological changes from those that remained unchanged over time.

The raster image pairs used in the change detection analysis must have the following characteristics: (i) be perfectly overlapped with each other; (ii) have the same number of classes; and (iii) have the same cell size. Therefore, to allow the change detection analysis using satellite data from different Landsat series, the cell sizes of the Landsat-1 MSS's imagery have been resampled from 80 metres to 30 metres. In this way the cell sizes of both Landsat-1 MSS and Landsat-5 TM were identical and perfectly superimposable. It should also be noted that comparing information of the earth's surface acquired from sensors with different spectral resolutions like MSS (Landsat-1) and TM (Landsat-5) could reduce the final degree of accuracy of the multi-temporal analysis. The following pairs of satellite imagery have been geometrically corrected, classified and finally used in the change detection analysis:

– Landsat-1 MSS scene from: 06/08/1973 and Landsat-5 TM scene from: 18/08/1992;
– Landsat-5 TM scene from: 18/08/1992 and Landsat-5 TM scene from: 17/08/2003;
– Landsat-5 TM scene from: 17/08/2003 and Landsat-5 TM scene from: 27/07/2007;
– Landsat-1 MSS scene from: 06/08/1973 and Landsat-5 TM scene from: 27/07/2007.

In the last change detection analysis, the satellite imagery from 1973 and 2007 has been processed. In this way all main morphological changes that occurred in the lagoon and its surroundings in the last thirty-five years were identified and differentiated as shown in Fig. 11. The sites highlighted by the yellow and green circles indicate, respectively, the areas where the anthropogenic activities and the natural environmental processes have mainly transformed the morphology of the Marano lagoon. Therefore, it can be concluded that the human pressure through its different industrial, touristic and urban activities had a much higher environmental impact in comparison to those related to natural processes.
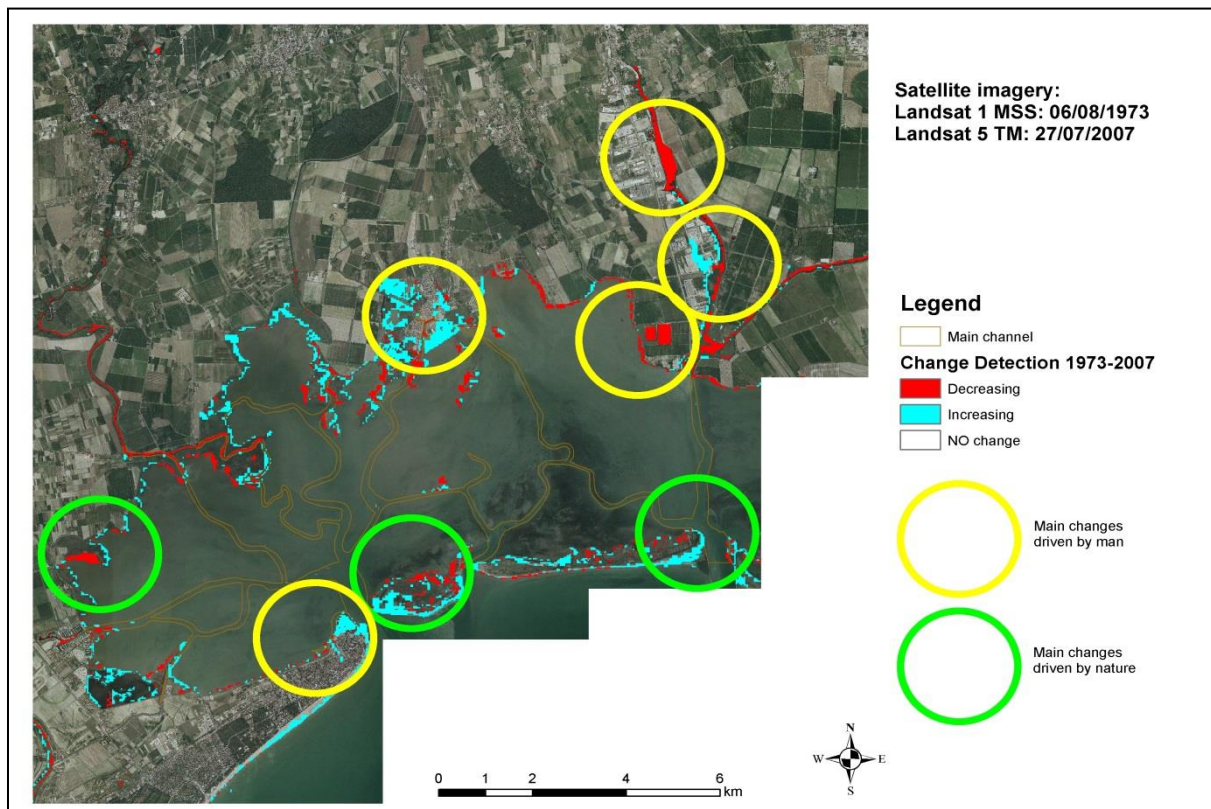

Figure 11: Multi-temporal analysis on the main morphological changes of the Marano lagoon and its surroundings from 1973 to 2007 (Landsat -1 MSS 06/08/1973; Landsat -5 TM 27/07/2007).

Summary and conclusion

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 for 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 programme 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, a priori excluding 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, besides the traditional hydrochemical analyses (main ions and nutrients), introduces the isotope signature of nitrate (δ^{15}N, δ^{18}O and δ^{17}O), boron (δ^{11}B), water (δ^2H and δ^{18}O), sulphate (δ^{34}S and δ^{18}O) as well as hydrological, remote sensing and GIS techniques. 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 the 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 fertilisers), 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 remote sensing and GIS techniques have been adopted to assess the spatial distribution of most superficial algal flora, surface water temperature as well as the key environmental and morphological changes of the lagoon since the beginning of the 1970s.

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 fertilisers. Nitrates detected in groundwater and along the groundwater upwelling 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, the Cormor and Corno rivers. In the lagoon, the characterisation of the origins and fate of nitrates was in general much more difficult to achieve because of complex mixing processes among different water types like seawater, river water and rainwater. However, it was possible to identify that nitrate can be formed in the lagoon by nitrification processes of ammonium coming from both anthropogenic sources and 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. Because of 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 δ^{11}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 show that in some parts of the lagoon the water is less affected by these two pollution sources. A few samples along the groundwater upwelling 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 characterise the mean altitude of the recharge area of the freshwater samples as well as to identify the origin of groundwater along the groundwater upwelling 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 upwelling line comes from local groundwater.

The isotopic signature of sulphate has been adopted to verify the possible connection between the Tagliamento and parts of the western lagoon sector through the Bevazzana artificial channel. The $\delta^{34}S$ and $\delta^{18}O$ 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 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 southwestern lagoon sector are influenced by the Tagliamento.

At last, the multi-temporal analysis of the remotely sensed data has shown that the main morphological changes of the lagoon and its surrounding 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.

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