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TRACE ELEMENTS IN ESTUARINE ENVIRONMENTS: MIXING, PARTITIONING AND FATE IN THE MAIN ITALIAN AND SLOVENIAN RIVER MOUTHS (GULF OF TRIESTE, NORTHERN ADRIATIC SEA)

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Abstract

Estuaries are complex aquatic systems at the land-sea interface where the interaction between different water masses leads to strong physico-chemical gradients. Before reaching the coastal zone, the river-transported material must pass through the estuarine environment. In this context, estuaries act as a filter for the freshwater inputs of both dissolved and particulate components. The original river-transported signals can then emerge from the mixing zone in a form which has been notably modified with respect to that which entered the estuarine system. Trace element distribution, mobility and fate heavily depends on the physical, geochemical and biological processes occurring in the estuarine mixing zone, which also affect the equilibria between solid and dissolved phases. The study of trace element phase partitioning is essential to gain a thorough knowledge of their natural cycles, although significant alterations may be due to the high anthropogenic pressure that often affects estuarine environments.

This research includes two main topics. The first concerns trace element (As, Cd, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb and Zn) phase partitioning in the main estuarine environments of the Gulf of Trieste (northern Adriatic Sea). In detail, estuarine water samples were collected at the Isonzo/Soča, Timavo/Reka, Rižana and Dragonja River mouths under different seasonal conditions and size fractionation allowed for the isolation of three fractions: suspended particulate matter, colloidal material and the truly dissolved fraction. Further information regarding the investigated estuarine systems was provided by dissolved and colloidal organic carbon, total nitrogen and nutrients including nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), ammonium ($N\text{-}NH_4^+$), soluble reactive silicate (SRSi) and soluble reactive phosphorous (SRP).

Suspended particulate matter plays a crucial role as the main vehicle of trace elements from terrestrial sources to marine coastal areas. Generally, Cr, Cu, Fe, Hg, Ni and Pb are mainly partitioned in the suspended particles and partially bound to the colloidal material. Conversely, As and Cs are preferably associated with the truly dissolved fraction, whereas major differences are observed in the partitioning behaviour of Co, Mn and Zn. Colloidal organic carbon is found to be scarcely present at the river mouths, since the hydrodynamic conditions may weaken the aggregation of very fine particles, thus suggesting that colloids play a minor role.

Regarding nutrients, $N\text{-}NO_3^-$ prevails among the forms of dissolved inorganic nitrogen as a result of runoff from soils where agricultural activities take place. In terms of water quality, the DIN:SRP molar ratio indicates that P-limitation prevails at the Isonzo/Soča, Timavo/Reka and Dragonja River mouths. Conversely, the Rižana estuarine system switches from P-limitation, prevailing in autumn and winter, to N-limitation in summer.

The second topic of this research is focused on the karstic estuarine system of the Timavo/Reka River where several industrial activities are located. In order to provide a geochemical characterisation, trace elements (Al, As, Ba, Cd, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb, V and Zn) were measured in different environmental matrices (water, suspended particles and sediments) as well as dissolved organic carbon, total nitrogen and nutrients. Principal component analysis was employed to depict disparities among the different sectors of the estuarine system. In particular in the innermost sector, sediments were found to be enriched in trace elements and potential adverse ecotoxicological effects were identified for Ni and Hg. Moreover, potential anoxic conditions at the sediment-water interface suggest that removal of trace elements via sulphide precipitation could occur although recycling of sinking Fe and Mn-oxy-hydroxides appears to affect dissolved trace element concentrations in the anoxic water layer.

Riassunto

Gli estuari rappresentano complessi ambienti di transizione tra le terre emerse e le aree marino-costiere. Nella zona di mescolamento tra acque fluviali e marine, le sostanze contaminanti potenzialmente veicolate dai corsi d'acqua sono soggette a svariati processi fisici, geochimici e biologici. Infatti, le condizioni chimico-fisiche di contorno, specialmente in termini di salinità, sono estremamente variabili nello spazio e nel tempo e concorrono alle complesse dinamiche sedimentarie degli ambienti di foce fluviale. Inoltre, i processi che avvengono nella zona di mescolamento svolgono un ruolo fondamentale nella regolazione degli equilibri tra la fase solida e quella disciolta. Per questi motivi, gli estuari sono spesso considerati trappole sedimentarie e fungono da filtro naturale per le sostanze contaminanti, inclusi elementi in tracce potenzialmente tossici.

L'attività di ricerca comprende due argomenti principali. Il primo riguarda lo studio del partizionamento degli elementi in tracce (As, Cd, Co, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb e Zn) nel particellato in sospensione, nel materiale colloidale e nella frazione veramente disciolta. Questo aspetto è stato valutato nei principali ambienti estuarini del Golfo di Trieste: fiumi Isonzo/Soča, Timavo/Reka, Rižana e Dragonja. Ulteriori informazioni riguardo alla qualità delle acque sono state fornite dalla determinazione del carbonio organico disciolto e colloidale, azoto totale e nutrienti quali nitrati ($N\text{-}NO_3^-$), nitriti ($N\text{-}NO_2^-$), ammonio ($N\text{-}NH_4^+$), silice reattiva (SRSi) e fosforo reattivo (SRP).

I risultati evidenziano che il particellato in sospensione svolge un ruolo fondamentale nella distribuzione degli elementi in tracce, i quali spesso tendono ad essere partizionati nella fase solida. Generalmente, Cr, Cu, Fe, Fe, Hg, Ni e Pb sono preferenzialmente associati al particellato in sospensione e, al tempo stesso, mostrano una discreta affinità con il materiale colloidale. Al contrario, As e Cs sono preferibilmente presenti in forma disciolta, mentre maggiori differenze, in termini di partizionamento tra fase solida e disciolta, sono emerse nel caso di Co, Mn e Zn.

Per quanto riguarda i nutrienti, la prevalenza dei $N\text{-}NO_3^-$, tra le forme dell'azoto inorganico, è riconducibile a processi di dilavamento da suoli interessati da attività agricole. Per quanto concerne la generale qualità dell'acqua, il rapporto molare DIN:SRP indica che nelle foci fluviali dei fiumi Isonzo/Soča, Timavo/Reka e Dragonja prevalgono condizioni di P limitazione. Al contrario, condizioni di N limitazione sono state riscontrate in estate nel sistema estuarino del fiume Rižana, sebbene condizioni di P limitazione prevalgano in autunno e in inverno.

Il secondo argomento indagato in questa ricerca riguarda il sistema estuarino del fiume Timavo/Reka, sede di diverse attività industriali. Allo scopo di fornire una caratterizzazione geochimico-ambientale, svariati elementi in tracce (Al, As, As, Ba, Cd, Co, Cr, Cs, Cu, Fe, Hg,

Mn, Ni, Pb, V e Zn) sono stati determinati in diverse matrici ambientali (acqua, particellato in sospensione e sedimenti) così come carbonio organico disciolto, azoto totale e nutrienti. L'analisi delle componenti principali è stata utilizzata allo scopo di valutare eventuali differenze tra i diversi settori dell'area di studio, mettendo in luce le maggiori criticità dal punto di vista ambientale. Nel settore più interno, i sedimenti sono risultati arricchiti in diversi elementi in tracce e potenziali effetti ecotossicologici per gli organismi bentonici sono emersi nel caso di Ni e Hg. Inoltre, condizioni di solfato-riduzione all'interfaccia acqua-sedimento potrebbero favorire la precipitazione di solfuri con conseguente accumulo di elementi in tracce nella frazione residuale del sedimento. Tuttavia, si suppone che ossidi e idrossidi di Fe e Mn svolgano un ruolo fondamentale nella regolazione delle concentrazioni disciolte di elementi in tracce lungo la colonna d'acqua.

Chapter 1 – Introduction

1.1 The estuarine system

Among natural aquatic systems, estuaries are crucial environments connecting land to oceans and seas. One of the first definitions was provided by Fairbridge (1980) who stated that an estuary is "an inlet of the sea reaching into a river valley as far as the upper limit of the tidal rise". This leads to the common distinction between the lower sector of the estuary which is dominated by seawater; the middle estuary, home to the interaction between freshwater and seawater, and the upper estuary where river freshwater prevails (Chester, 1999). The complexity of the estuarine system is mainly due to mixing processes between river freshwater and seawater, both of which are very different in composition (de Souza Machado et al., 2016). This leads to strong gradients in the physico-chemical boundary conditions, especially in terms of salinity, which are subject to both spatial and seasonal variations in the estuarine mixing zone (Elliott and McLusky, 2002; Fiket et al., 2018).

In microtidal and mesotidal estuarine environments, the occurrence of a salt wedge intrusion is, in fact, a common condition whereas permanent mixing along the water column prevails in macrotidal estuarine systems. In the case of microtidal and mesotidal estuaries, the extension of the salt wedge intrusion towards the different sectors of the estuary is modulated by several factors such as river discharge, tides, rainfall events and winds (Geyer and Farmer, 1989; Perales-Valdivia et al., 2018). All these factors hugely affect the fate of suspended particles (Meade, 1972) which tend to concentrate in the maximum turbidity zone (Menon et al., 1998; de Souza Machado et al., 2016), settling down towards the bottom sediments as a result of the decrease in the river flow velocity that generally occurs at the river mouth (Wright, 1977; Menon et al., 1998). Therefore, estuaries are generally thought to act as a sort of natural filter for the sediment carried mainly in suspension by the riverine inputs and, as a consequence, for all the potentially toxic compounds adsorbed on the surface of the finest particles (Benoit et al., 1994; Turner, 1996; Fu et al., 2013).

Moreover, due to their geographic position between land and sea, estuaries are often suitable environments for intense urbanisation, industrial settlements and economic activities (e.g. industries, harbours, marinas, aquaculture activities). As a result, estuaries are often affected by high levels of anthropogenic pressure, which renders them especially fragile among natural aquatic systems (Garcia-Ordiales et al., 2018; Petranich et al., 2018b). There has been increasing interest in these ecosystems over the last decades and considerable efforts have been made to assess the occurrence and distribution of a variety of potentially toxic compounds supplied to the estuarine environment from different anthropogenic sources (e.g. Casado-Martínez et al., 2006; Neto et al., 2006; Botwe et al., 2017). Among these contaminants, trace elements are of major concern since the physico-chemical and biological processes occurring in the estuarine mixing zone could heavily affect their speciation, mobility and fate (Deycard et al., 2014; Domingos et al., 2015).

1.2 Sources of trace elements and pathways to estuarine systems

Rivers represent the main effective vehicle in transporting organic and inorganic chemical species to the estuarine environment. According to the hydrological cycle, rainfall events, groundwater inputs, decomposition of organic material, surface rainwater runoff and infiltration are the main pathways of water to the river system (Figure 1).

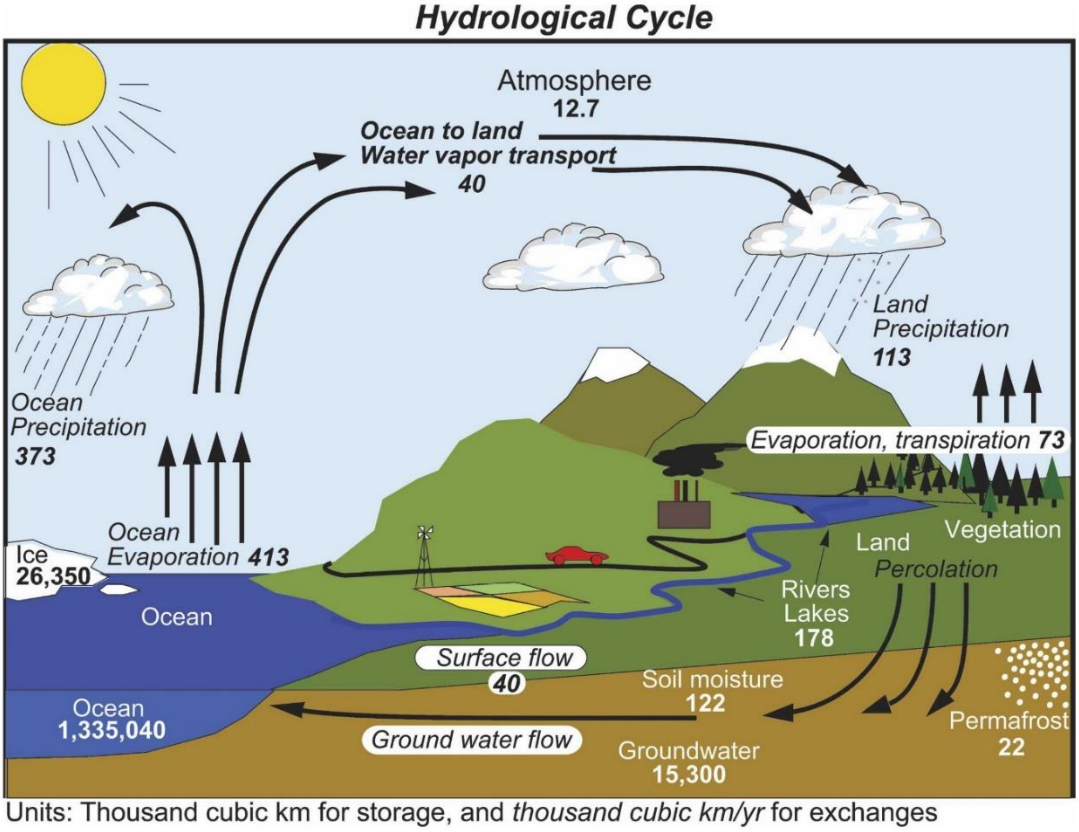

Figure 1. The hydrological cycle. Estimates of the main water reservoirs, given in plain font in 10^3 *km*^{3}*, and the flow of moisture through the system, given in slant font (*10^3 *km*^{3} *yr*$^{-1}$*), equivalent to Eg (*10^{18} *g) yr*$^{-1}$*. From Trenberth et al. (2007).*

Trace elements supplied to estuarine environments by river freshwater inputs may derive from several natural and anthropogenic sources (Bradl, 2005). First of all, the amounts of trace elements in natural water bodies heavily depend on the geology of their drainage basins. Indeed, different lithological formations outcropping in the continental area may actively influence the chemical composition of the river freshwater, since natural weathering of rocks and soils can easily mobilise major and trace elements, both in dissolved and particulate forms. In this context, leaching of trace elements and their release dynamics from a riverine soil into water can be notably affected by several factors such as redox potential (Eh), pH, composition of dissolved organic carbon (DOC) and Fe-Mn oxides (Rinklebe et al., 2016). This is especially relevant in floodplain ecosystems where periodical inundation can favour frequent changes in the redox potential (Eh) (e.g. Frohne et al., 2015; Shaheen et al., 2017) which can be a crucial factor influencing trace element mobility and fate from soil to solution (Frohne et al., 2015).

In order to provide a global overview, the average composition of continental rocks, soils as well as dissolved and particulate material carried by river freshwater proposed by Martin and Meybeck (1979) is reported in Table 1.

Table 1. Average composition of crustal rocks, soils and dissolved and particulate river material (Martin and Meybeck, 1979).

	Continents		Rivers			Continents		Rivers	
	Rock μg g^{-1}	Soils μg g^{-1}	Dissolved μg L^{-1}	Particulate μg g^{-1}		Rock μg g^{-1}	Soils μg g^{-1}	Dissolved μg L^{-1}	Particulate μg g^{-1}
Ag	0.07	0.05	0.3	0.07	Mg	16400	5000	3100	11800
Al	69300	71000	50	94000	Mn	720	1000	8.2	1050
As	7.9	6	1.7	5	Mo	1.7	1.2	0.5	3
Au	0.01	0.001	0.002	0.05	Na	14200	5000	5300	7100
B	65	10	18	70	Nd	37	35	0.04	35
Ba	445	500	60	600	Ni	49	50	0.5	90
Br	4	10	20	5	P	610	800	115	1150
Ca	45000	15000	13300	21500	Pb	16	35	0.1	100
Cd	0.2	0.35	0.02	1	Pr	9.6	-	0.007	8
Ce	86	50	0.08	95	Rb	112	150	1.5	100
Co	13	8	0.2	20	Sb	0.9	1	1	2.5
Cr	71	70	1	100	Sc	10.3	7	0.004	18
Cs	3.6	4	0.035	6	Si	275000	330000	5000	285000
Cu	32	30	1.5	100	Sm	7.1	4.5	0.008	7
Er	3.7	2	0.004	3	Sr	278	250	60	150
Eu	1.2	1	0.001	1.5	Ta	0.8	2	<0.002	1.25
Fe	35900	40000	40	48000	Tb	1.05	0.7	0.001	1
Ga	16	20	0.09	25	Th	9.3	9	0.1	14
Gd	6.5	4	0.008	5	Ti	3800	5000	10	5600
Hf	5	-	0.01	6	Tm	0.5	0.6	0.001	0.4
Ho	1.6	0.6	0.001	1	U	3	2	0.24	3
K	24400	14000	1500	20000	V	97	90	1	170
La	41	40	0.05	45	Y	33	40	-	30
Li	42	25	12	25	Yb	3.5	-	0.004	3.5
Lu	0.45	0.4	0.001	0.5	Zn	127	90	30	250

Another notable source of trace elements is dry atmospheric deposition from volcanic dust and industrial emissions. Indeed, trace elements also occur in the atmosphere where they can be present under particulate, aerosol and gaseous chemical forms.

Natural cycles of trace elements among the main environmental compartments (atmosphere, hydrosphere and biosphere) are regulated by physical, geochemical and biological processes but strongly affected by human interactions. Indeed, anthropogenic inputs can considerably influence trace element natural cycles resulting in local or regional contamination of soils, rivers, groundwater and sediments (e.g. Horvat et al., 1999; Guéguen and Dominik, 2003; Amini et al., 2008; Carr et al., 2008) leading to a general deterioration of water quality with subsequent adverse effects on the biological community (Long et al., 1995; Giani et al., 2012) and humans in the worst case scenario (e.g. Ayotte et al., 2003; Rahman et al., 2009; Azizullah et al., 2011; Khan et al., 2013; Bhowmik et al., 2015). Among the principal anthropogenic sources of trace elements, smelting of mining ores, industrial emissions and exploitation of fossil fuels directly impact the atmosphere (Nriagu, 1988), although dry deposition of particles may also have effects on the surrounding soils and water systems. Discharge of animal wastes, irrigation drainage from agricultural soil, food waste, domestic and industrial waste water, urban runoff and discharge of sewage sludge represent other diffuse anthropogenic sources of contaminants in general (Luoma and Rainbow, 2008).

In view of the above, trace elements are increasingly present in estuarine systems (Mason et al., 2006; Monterroso et al., 2007; de Souza Machado et al., 2016; Garcia-Ordiales et al., 2018; Liu et al., 2019) and, according to Förstner and Wittmann (1979), estuaries are one of the most heavily polluted aquatic environments at the global level. This contributes to make these aquatic systems of great scientific interest as they require specific management strategies.

1.3 Phase partitioning of trace elements in the estuarine system

In estuarine environments, the concentration of particulate and dissolved trace elements depends on several factors, among which the most essential are the geological characteristics of the river drainage basin, the potential anthropogenic inputs and the equilibria between solid and aqueous phases in the estuarine mixing zone. As previously mentioned, trace elements are involved in several physico-chemical and biological processes in the estuarine mixing zone, where the interaction between different water masses may affect their phase partitioning and speciation, which in turn contribute to their toxicity, reactivity and bioavailability (Benoit et al., 1994). The study of the phase partitioning of trace elements is fundamental in order to better understand their geochemical behaviour, as well as their potential impact on the quality of the estuarine ecosystem (Benoit et al., 1994; Vignati et al., 2009). Generally, trace elements can be preferentially partitioned in the solid phase or be present under dissolved chemical forms. Since the suspended particles are known to play a crucial role in regulating trace element distribution (Turner et al., 1991; Turner, 1996), several studies have dealt with their transport and dispersion during river flood events (e.g. Gagnon and Saulnier, 2003; Covelli et al., 2004, 2007; Yao et al., 2016) as well as their interaction with the dissolved phase (Town and Filella, 2002; Zhang et al., 2008; Fu et al., 2013; Savenko et al., 2014; Fiket et al., 2018).

However, there are three main fractions to which organic and inorganic material can be associated: the suspended particulate matter (SPM), the truly dissolved fraction and the colloidal material (more detailed information about these fractions is provided in Chapter 3). Indeed, colloids can settle extremely slowly and tend to aggregate in the maximum turbidity zone where they may be involved in several estuarine processes (e.g. coagulation and flocculation) responsible for the removal of trace elements from solution (Benoit et al., 1994; Guentzel et al., 1996). Moreover, colloids may also have a role in regulating trace element transport, behaviour and bioavailability in natural aquatic systems (Vignati et al., 2005).

1.4 Main processes governing trace element behaviour and fate in estuaries

As previously mentioned, one of the main characteristics of estuaries is the strong gradients in the physico-chemical boundary conditions (e.g. salinity, density, flow velocity, composition of the suspended matter) (Elliott and McLusky, 2002; Du Laing et al., 2009). This is of fundamental importance regarding trace element behaviour and fate since physico-chemical and biological processes can greatly modify the equilibria between solid and dissolved phases (Figure 2).

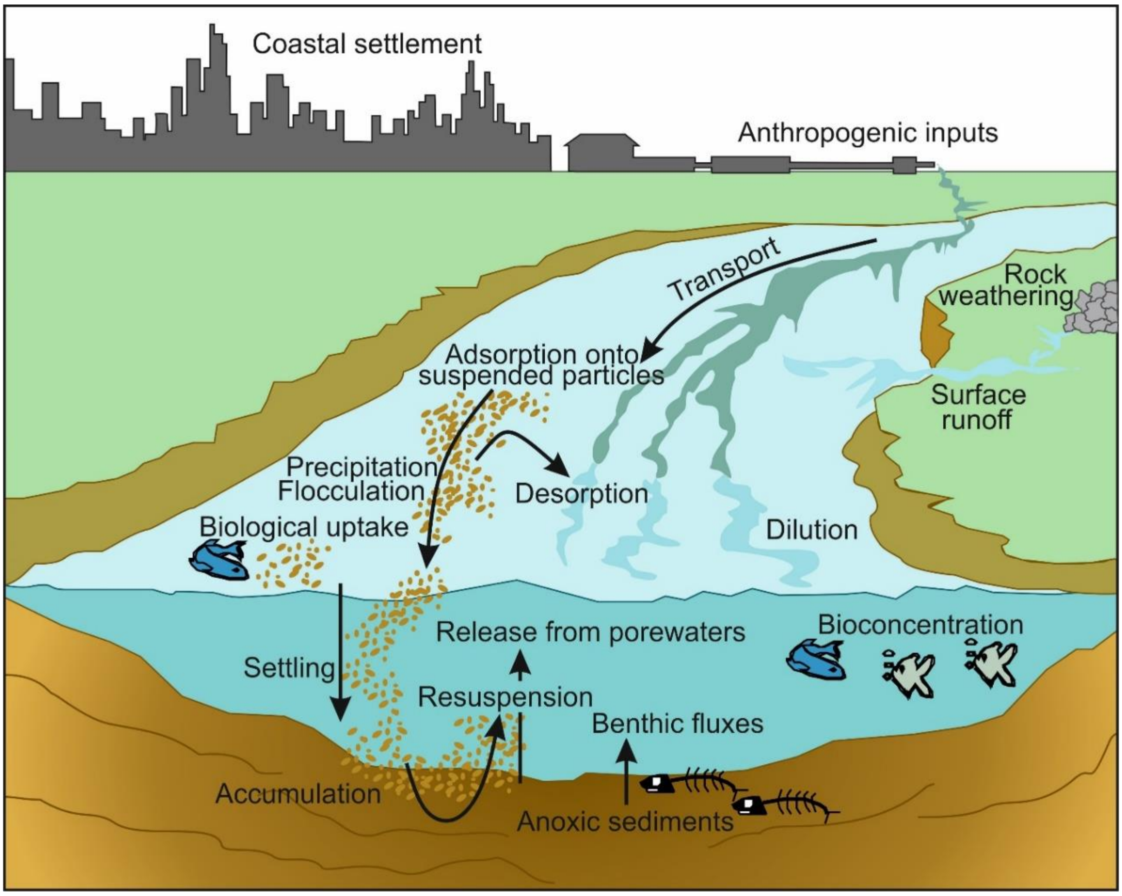

Figure 2. Schematic representation of the main physico-chemical and biological processes in estuarine environments.

In the estuarine mixing zone, a chemical specie can behave conservatively or non-conservatively (Chester, 1999) although different behaviours may be observed for the same chemical compound depending on the specific characteristics of the estuary. Generally, conservative behaviour is observed when the dissolved or particulate concentration of a chemical constituent changes linearly with increasing salinity, demonstrating that its distribution along the water column is mainly modulated by physical mixing. On the contrary, behaviour is defined as non-conservative when removal and/or addition processes set in. Indeed, the suspended particles can be considered both a source and a sorptive surface for release and adsorption of dissolved trace elements, respectively.

Desorption and dissolution from the suspended matter, as well as complexation and chelation reactions mediated by organic and inorganic ligands, may contribute to add trace elements in the dissolved phase. Conversely, the removal of trace elements from the dissolved phase may be due to flocculation, precipitation, adsorption and biological uptake, which are the main mechanisms responsible for trapping trace elements in the suspended particles and, following their settling down, into the bottom sediments. All these processes are mainly mediated by organic matter, clay minerals and Fe and Mn oxy-hydroxides, which play a key role in regulating the removal of trace elements from the water column and their accumulation in the bottom sediments (Jones-Lee and Lee, 2005; Du Laing et al., 2009; Yakushev et al., 2009; Smrzka et al., 2019). Since estuaries are notably dynamic and complex water systems, an active interaction is generally present among the described processes which often occur simultaneously and heavily depend on the speciation of dissolved trace elements and on the chemical composition and concentration of the suspended particles (Turner, 1996).

The sediment compartment represents an internal reservoir for contaminants (Monterroso et al., 2007; Liu et al., 2019; Zonta et al., 2019), including trace elements transported to estuarine environments by river freshwater inputs. However, sediments are globally considered a secondary source of contamination due to remobilisation processes that occur at the sediment-water interface (SWI) (Warnken et al., 2001; Du Laing et al., 2009; Petranich et al., 2018b). Early diagenetic processes such as benthic organism respiration, reduction of nitrates, Fe and Mn oxy-hydroxides and sulphides followed by organic matter decomposition (Froelich et al., 1979) contribute to the formation of metastable phases acting as sources of dissolved trace elements in the overlying water. Indeed, trace elements stored in the sediment compartment can be easily transferred to porewaters via seawater diffusion at the SWI (Smrzka et al., 2019). In this context, resuspension events are of major concern and may be due both to natural processes such as the action of tidal currents and waves, storms and bioturbation (Sanford et al., 1991; Arfi et al., 1993) and to anthropogenic activities including dredging, shipping and trawling (Schoellhamer, 1996; Lewis et al., 2001; Roberts, 2012; Wasserman et al., 2016; Silveira et al., 2017). As a result, the release of dissolved trace elements from porewaters to the water column may occur (Simpson et al., 1998; Caplat et al., 2005) and changes in the physico-chemical boundary conditions can heavily influence the mobility and bioavailability of trace elements (Cotou et al., 2005; Piou et al., 2009).

1.5 Research aim

The geochemical behaviour of trace elements in different environmental compartments represents a broad field of research, and considerable interest on the part of the scientific community continues to be directed towards natural aquatic systems. Trace element mobility and bioavailability in aquatic environments depend on several factors, among which the most relevant are their chemical form and the physico-chemical boundary conditions. Knowledge of the major geochemical processes that regulate trace element behaviour, mobility and fate is fundamental in order to provide scientific support for the environmental management and risk assessment often needed in transitional water systems. Indeed, the behaviour of trace elements in coastal environments are of key importance with reference to the goals of the European Union's Marine Strategy Framework Directive (adopted on 17 June 2008 and revised in 2017) which aims to achieve a good environmental status of the marine water systems by 2020. In this context, estuaries are of major concern for two principal reasons. The first is linked to one of the main estuarine characteristics: their natural function acting as a filter for suspended particles and, as a result, for trace elements and other contaminant species adsorbed on their reactive surface. Secondly, estuaries are often affected by high anthropogenic pressure resulting in the modification of trace element natural cycles, contamination issues and potential adverse effects on the water quality and biota.

The occurrence, distribution and the partitioning behaviour of trace elements in the Gulf of Trieste (northern Adriatic Sea) have not been thoroughly addressed. The main intent of this doctoral research activity is to provide further information about the estuarine water quality looking for potential critical conditions and common environmental mechanisms controlling trace element mobility and fate.

Specifically, trace element occurrence and partitioning among different fractions was investigated in the main Italian (Isonzo/Soča and Timavo/Reka Rivers) and Slovenian (Rižana and Dragonja Rivers) estuarine environments of the Gulf of Trieste, as well as the major processes controlling trace element mixing behaviour and distribution along the water column. Secondly, another aim of this research is to provide a geochemical characterisation of the Timavo/Reka River mouth, evaluating trace element occurrence and mobility among different environmental matrices.

Indeed, this doctoral research activity is in the scope of recent European Union's frameworks and directives and represents a step towards further understanding the behaviour and fate of trace elements supplied to the estuarine environments in association with freshwater inputs affected by several anthropogenic sources.

Chapter 2 – Study Area

This section provides a brief description of the main estuarine environments of the Gulf of Trieste (northern Adriatic Sea) which were investigated for this research, and which are reported on in more detail in the later sections (Chapter 5).

The northern Adriatic Sea is a semi-enclosed marine area subjected to a large runoff and affected by several urban and industrial areas. The rivers flowing into the northern Adriatic Sea generally show two periods of high flow conditions during the year, largely due to precipitation events and snowmelt. Periods of intense discharge usually occur in autumn and spring, whereas low discharge prevails during winter and summer (Cozzi et al., 2012). The freshwater supply mainly comes from the Po River, which is responsible for the input of approximately one-fourth of the material into the northern Adriatic Sea (Frignani et al., 2005).

Despite SPM often being considered the main vehicle for the transfer of trace elements (Turner et al., 1991; Beck et al., 2013) from land to marine and coastal areas, and estuaries being recognised as acting as filters for contaminants in general (Elliott and McLusky, 2002; de Souza Machado et al., 2016), little information is currently available regarding the behaviour of trace elements in the estuarine environments of the northern Adriatic Sea.

Scientific articles written by Juračić et al. (1986, 1987) were two of the earliest contributions regarding the estuarine system of the Adige River, the second most important supplier of freshwater and SPM to the Adriatic Sea (Boldrin et al., 1989). According to the authors, similarities in the physico-chemical, mineralogical and geochemical properties were observed between riverine suspended material and the surface sediments at the river mouth. This means that the majority of the particulate trace elements was accumulated in the prodelta area (Juračić et al., 1987). In addition, the same authors also reported notably high concentrations of Cr (220 $\mu g\ g^{-1}$) likely related to industrial activities from tanners located upstream in the river drainage basin (Juračić et al., 1986). The occurrence and distribution of particulate trace elements, their interaction with the dissolved phase and the role of colloids were investigated at the Po river estuarine system (Tankéré and Statham, 1996; Zago et al., 2002; Vignati and Dominik, 2003; Vignati et al., 2005).

The Gulf of Trieste is a semi-enclosed marine basin located in the north-eastern sector of the Adriatic Sea (Italy), it covers an area of approximately 500 km^2 and the water depth is generally shallow, reaching a maximum depth of 25 m in its central sector. Typical marine salinity values are recorded in the Gulf, ranging between 33.0 and 38.5 and between 36.0 and 38.5 in the surface and bottom water layers, respectively (Olivotti et al., 1986). The anticlockwise circulation of the Adriatic Sea primarily dominates the circulation of the water masses in the Gulf of Trieste and tides, winds and seasonal variations in the freshwater inflow further affect hydrodynamic conditions (Olivotti et al., 1986; Malačič and Petelin, 2009).

The rivers entering the Gulf of Trieste generally display two periods of high flow during the year, primarily due to snowmelt and rainfall events (Cozzi et al., 2012). The main freshwater input is represented by the Isonzo/Soča River with an average annual flow rate at the river mouth estimated to be approximately 82 m^3 s^{-1} in the period from 1998 to 2008 (Cozzi et al., 2012). The river discharge is affected by notable seasonal variations and two main flood periods associated with a high level of SPM occur during the year as a result of snowmelt and rainfall (Covelli et al., 2004). Due to long term *cinnabar* (HgS) extraction from the Idrija mining district (Horvat et al., 1999; Hines et al., 2000; Faganeli et al., 2003) located in the Slovenian sector of the river drainage basin, the Isonzo River is well-known as the main source of Hg in the Gulf of Trieste (Covelli et al., 2001, 2007).

The Timavo/Reka River is the other principal source of freshwater in the Gulf of Trieste and one of its main characteristics consists in its particular river path since it flows underground for a long sector of its course. The mouth of the TV River is located in the Bay of Panzano, which is impacted by several anthropogenic and industrial activities.

The Rižana and Dragonja Rivers are the main freshwater systems flowing in the Slovenian sector of the Gulf of Trieste, although their river discharge is notably low if compared to that of the IS and TV Rivers. The RZ River mouth is located in the Bay of Koper, site of one of the most active ports in the northern Adriatic Sea, whereas the DG River flows into the Bay of Piran.

More detailed information regarding the environmental setting of the four investigated river mouths is provided in Chapter 4 (sections 4.1, 4.2 and 4.4).

Chapter 3 – Research plan and methodological issues

The research activity was focused on the main Italian and Slovenian estuarine environments of the Gulf of Trieste and has been divided into two main topics. The first topic regards trace element behaviour and partitioning among particulate, colloidal and truly dissolved fractions. In addition, the occurrence and behaviour of nutrients, organic carbon and total nitrogen were evaluated in the colloidal material and in the truly dissolved fraction. This issue has been investigated at the Isonzo/Soča, Timavo/Reka, Rižana and Dragonja River mouths under different seasonal conditions (Chapter 4 – sections 4.1, 4.2 and 4.3). It was decided to assign a specific section (Chapter 4 – section 4.1) regarding the phase partitioning and mixing behaviour of trace elements at the mouth of the Isonzo/Soča River. Indeed, although the behaviour of Hg has been intensively studied, little information is currently available regarding other trace elements supplied by the river freshwater into the Gulf of Trieste.

The second topic is the distribution, mobility and fate of trace elements in the karstic estuarine system of the Timavo/Reka River (Chapter 4 – section 4.4). As previously mentioned, the Timavo/Reka River is another notable freshwater source entering the Gulf of Trieste, it has a karstic river-path and its mouth is affected by several anthropogenic activities. For these reasons we performed an additional sampling campaign during summer in order to provide a geochemical characterisation of this distinctive estuarine system.

In the following Figures (Figures 1, 2 and 3), schematic plans of the research activity, of sampling and sample handling, of the cleaning procedures to maintain ultraclean conditions and of the analytical measurements are schematically described. Further details regarding the water sample size fractionation, mass balances and analytical quality control procedures are briefly described in the following paragraphs and specific details are reported in Chapter 4.

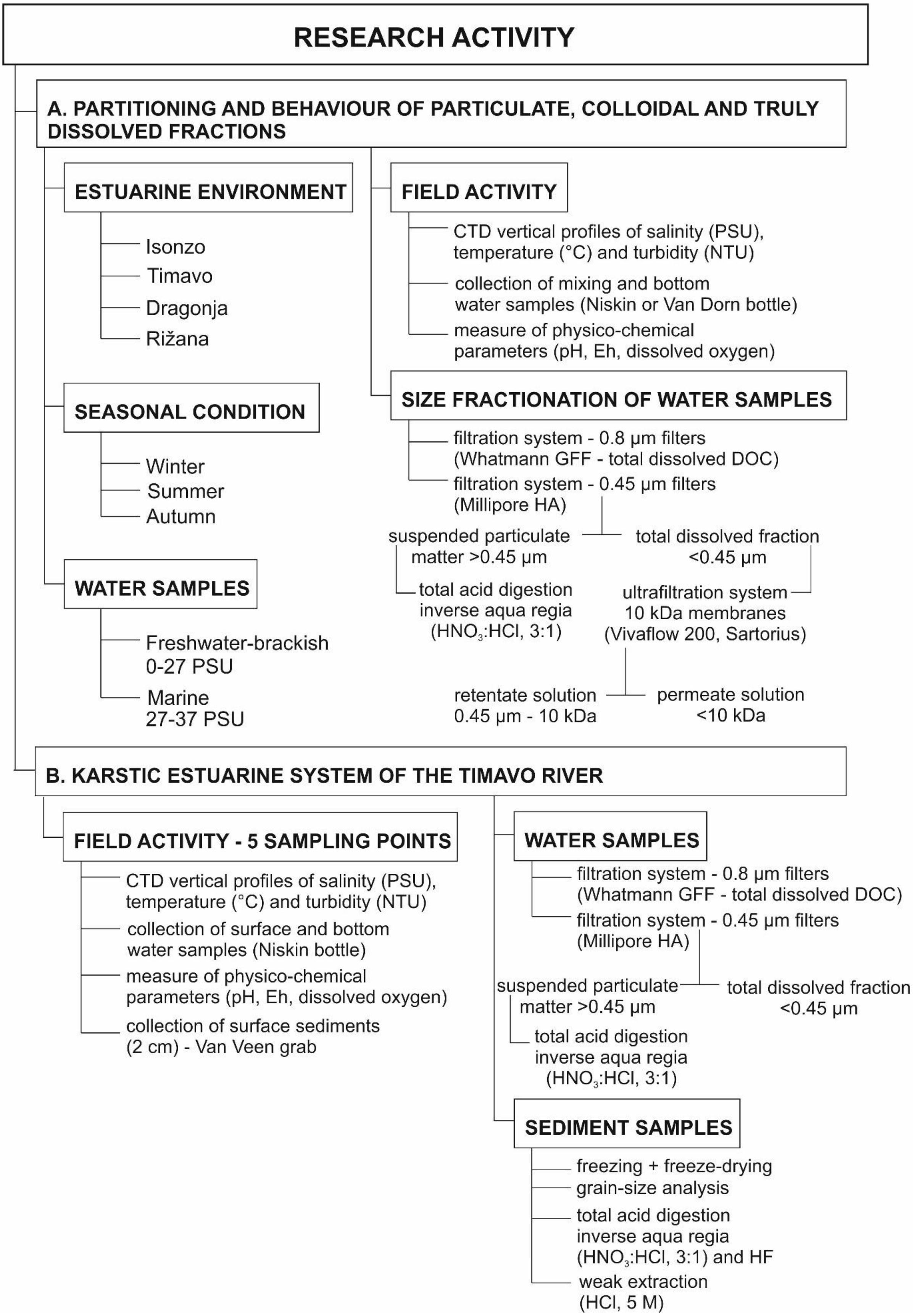

Figure 1. Schematic description of sampling and laboratory activities A) performed seasonally on estuarine water samples collected at the Isonzo, Timavo, Rižana and Dragonja River mouths and B) on estuarine water and sediment samples from the Timavo estuarine system.

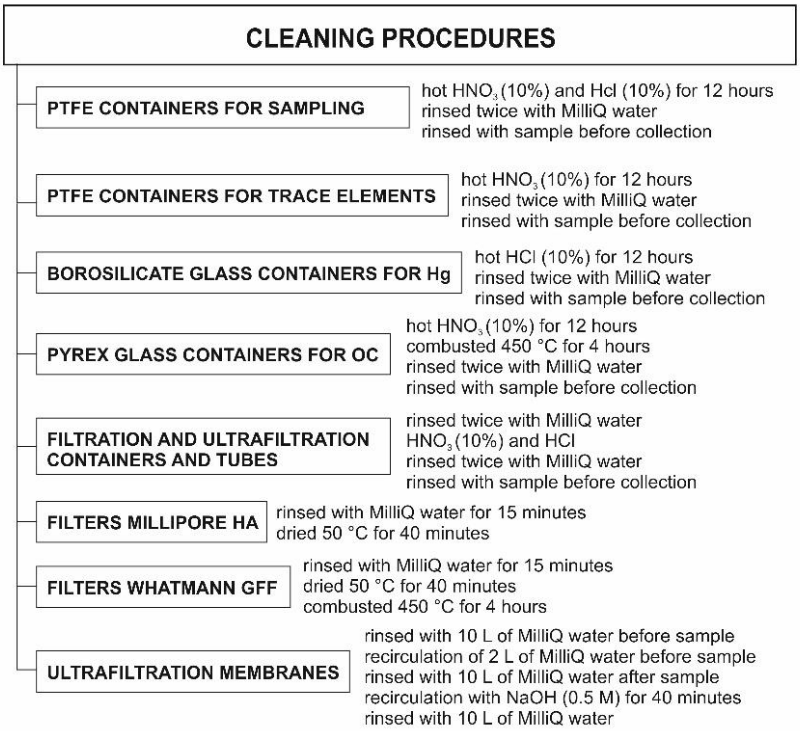

Figure 2. Schematic description of cleaning and pre-conditioning procedures.

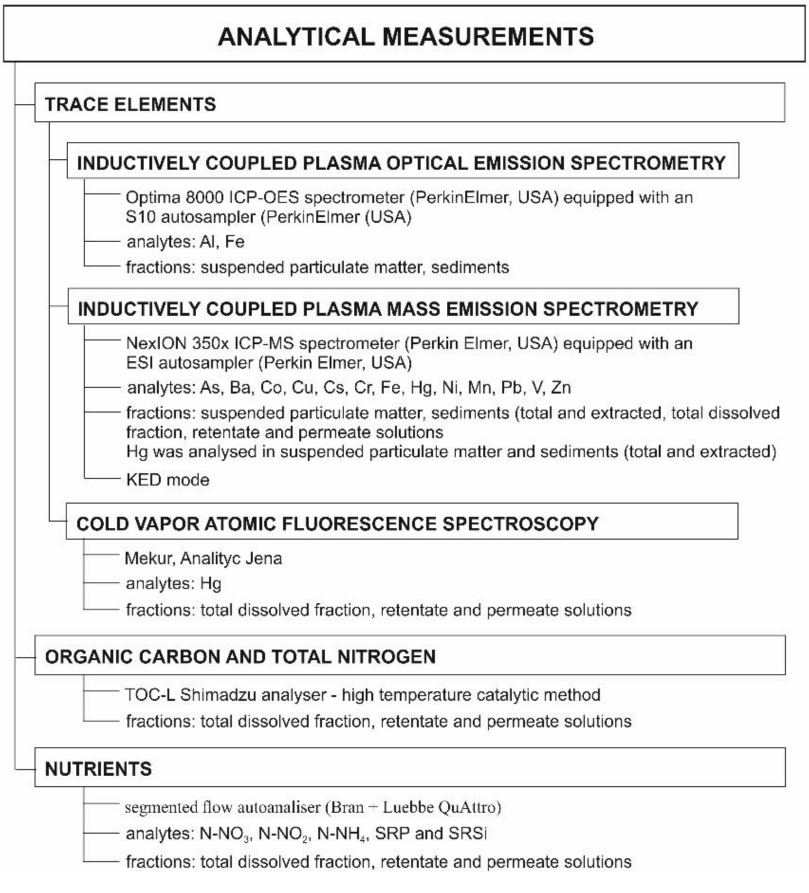

Figure 3. Schematic description of analytical determinations of trace elements, organic carbon, total nitrogen and nutrients performed on sediments, suspended particulate matter, total dissolved fraction, retentate and permeate solutions.

3.1 Size fractionation of estuarine water samples: pre-filtration and ultrafiltration systems

This section provides a complete description on the fractionation procedure used to isolate the three main dimensional fractions evaluated in this research: SPM, high molecular weight colloids and the truly dissolved fraction. With regards to phase partitioning, organic and inorganic matter in aquatic systems can be divided into three classes: particulate, colloidal and dissolved fractions (Guo and Santschi, 1997) (Figure 4).

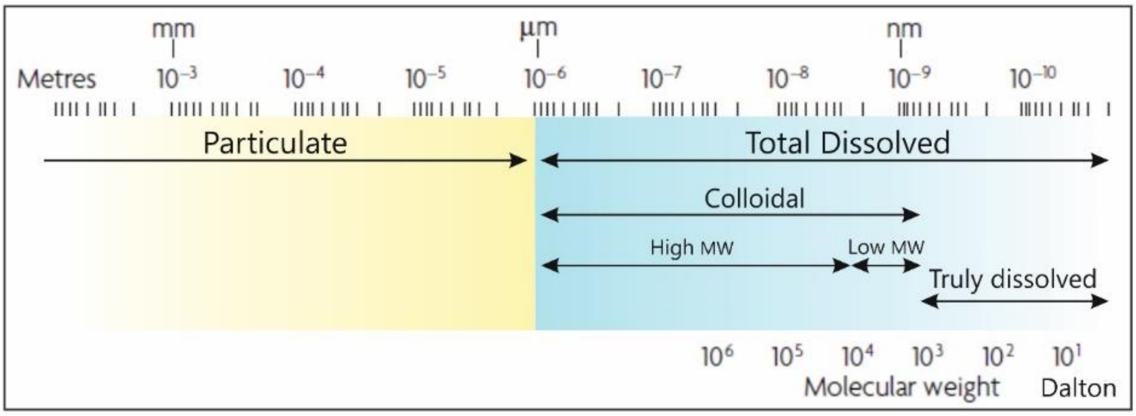

Figure 4. Size spectrum of chemical species in aquatic environments.

The lower limit of the suspended particles is conventionally defined of being between 0.4 and 0.5 µm, although the upper dimensional limit has not been well-established (Wilding et al., 2004) and the value of 100 µm has been proposed by Visher (1969) as the limit between the particles that move by saltation under a laminar flow regime and those transported in suspension. A value of 0.45 µm is commonly employed as the limit between the suspended particles and the traditionally defined total dissolved fraction. However, values of 0.2, to exclude bacteria, and 1.2 µm were also used (Table 1).

According to Guo and Santschi (2007), colloids are conventionally defined as macromolecules and nanoparticles having sizes ranging between 1.00 nm (approximately to 1.00 kDa) and 1.00 µm.

A similar description has also been provided by the International Union of Pure and Applied Chemistry (IUPAC) which reported that the term *colloids* includes microparticles, macromolecules and molecular assemblies defined by molecular weight sizes between 1.00 nm and 1.00 µm.

The lower limit of colloids is not well defined and there is a lack of consistency regarding the boundary between the colloidal material and the so-called truly dissolved fraction (Guo and Santschi, 1997; Wells et al., 2000; Town and Filella, 2002; Wilding et al., 2004).

Although there is still debate regarding optimal sampling operations, ultrafiltration has become one of the most widely employed techniques used to isolate physical fractions of chemical species in water samples. Ultrafiltration devices can be employed for size fractionation of samples collected from a variety of natural water systems such as marine, estuarine, coastal and riverine environments, including sediment porewaters and aquifers. Filtration and ultrafiltration procedures can be utilised using different filter pore sizes and ultrafiltration membranes with different molecular weight cut-offs (MWCO) (Table 1).

Table 1. Pre-filtration and ultrafiltration cut-offs employed in this research and in other studies as reported in the literature.

Pre-filtration cut-off	Ultrafiltration cut-off	Sample category	Reference
0.45 µm	10 kDa	estuarine, marine	This study
0.2 µm	10 kDa	estuarine, marine	Sañudo-Wilhelmy et al., 1996
0.4 µm	10 kDa	estuarine, marine	Krapiel et al., 1997
0.2 µm	10 kDa	river freshwater	Jaïry et al., 1999
0.45 µm	1, 10 kDa	estuarine, marine	Wen et al., 1999
0.45 µm	10 kDa	river freshwater	Sigg et al., 2000
0.2 µm	1, 8 kDa	estuarine, marine	Wells et al., 2000
0.4 µm	10 kDa	river freshwater	Babiarz et al., 2001
1.2 µm	1 kDa	river freshwater	Guéguen and Dominik, 2003
0.22 µm	1, 5, 10, 100 kDa	estuarine, marine	Ingri et al., 2004
0.45 µm	1 kDa	river freshwater	Jarvie et al., 2012
0.7, 0.22 µm	5, 10, 30 kDa	marine	Koron et al., 2013
0.22, 0.45 µm	5 kDa	marine	Klun et al., 2015
0.2 µm	3 kDa	estuarine, marine	Wang and Wang, 2016
0.22, 0.45 µm	5 kDa	estuarine, marine	Klun et al., 2019

Generally, filtration of water samples through 0.20 – 1.00 µm pore size filters typically accomplishes the isolation of aquatic colloids by means of ultrafiltration membranes. Regarding ultrafiltration, values of 1 and 10 kDa are actually the most commonly used, since the value of 10 kDa represents the limit between high molecular weight colloids and low molecular weight colloids (Figure 4).

In this research, estuarine water samples (approximately 15 L) were filtered using 0.45 µm filters (preconditioned and pre-weighted Millipore, HA) using a vacuum filtration system. The total dissolved fraction (<0.45 µm) was collected into preconditioned containers whereas the SPM was retained on the filters. Blanks were prepared for each sample by filtrating 500 mL of MilliQ water. All filters were first dried at room temperature, stored on silica gel for 5-6 days, weighed for SPM determination and acid digested through a total dissolution using inverse *aqua regia* (HNO_3 and

HCl, 3:1) in a closed microwave system (Multiwave PRO, Anton Paar) according to the modified EPA Method 3052 (2007).

Sample aliquots for analytical determination of dissolved organic carbon (DOC) and total nitrogen (TN) in the total dissolved fraction were isolated by means of glass-fiber filters (Whatmann, GFF, pore size 0.8 µm).

A typical ultrafiltration system usually consists of a pre-filtration system, pump(s), ultrafiltration membrane(s), reservoir(s), tubes and fittings (Figure 5).

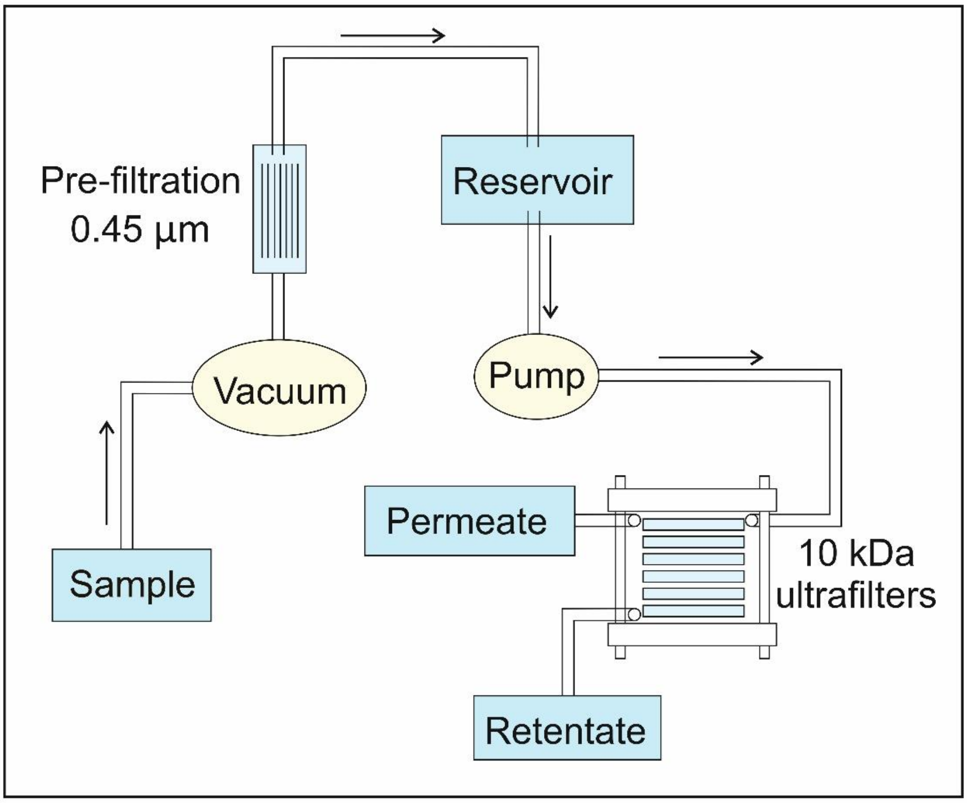

Figure 5. Pre-filtration and ultrafiltration system.

During ultrafiltration, hydrostatic pressure drives colloids having a molecular weight lower than the MWCO through the membrane (Buesseler et al., 1996). In this way, the colloidal material unable to pass through the membrane concentrates in the defined retentate solution, whereas the permeate solution, also known as ultrafiltrate solution or truly dissolved, is collected in a different container.

In this research, the colloidal retentate solution was isolated through two ultrafiltration membranes (Vivaflow 200, Sartorius) having a MWCO of 10 kDa. Ultrafiltration was carried out under recirculation mode using a concentration factor (cf) of approximately 60. The cf is defined as the ratio between the initial volume and the retentate volume for each ultrafiltration run and is necessary to define the cf (Guo and Santschi, 2007). The definition of the optimal cf for successful ultrafiltration system is a difficult problem to solve. High cf (>40) has been suggested for sampling marine colloids on the basis of several laboratory experiments (Guo and Santschi, 1996).

Blanks were prepared for each sample by ultrafiltrating 2 L of MilliQ water under recirculation mode. Regarding trace elements, blank samples of different fractions (SPM, total dissolved, retentate and permeate solutions) were found to be mainly below the LOD. The only exception was Zn, hich was occasionally found to be higher than 0.50 µg L^{-1}, as previously reported by Sigg et al. (2000).

3.2 Concentration of colloids and mass balances

According to other authors (e.g. Buesseler et al., 1996; Sigg et al., 2000; Ingri et al., 2004; Wilding et al., 2004), the colloidal concentration of a chemical compound (X_{coll}) is calculated according to the following equation (1):

$$(1) \quad X_{coll} = \frac{C_{rx} - C_{px}}{cf}$$

where C_{rx} and C_{px} are the concentrations of the chemical compound x in the retentate and permeate solutions and cf is the concentration factor. This approach was employed to calculate colloidal trace elements, nutrients and total nitrogen.

Mass balances (recovery %) were calculated as follows (equation 2) in order to evaluate the performance of the fractionation system for trace elements, nutrients and total nitrogen:

$$(2) \quad R_{\%} = \frac{C_{rx} + C_{px}}{C_{TDx}} \cdot 100$$

where C_{rx}, C_{px} and C_{TDx} are the concentrations of the chemical compound x in the retentate, permeate and total dissolved fractions. Mass balances were calculated only when the chemical specie was detectable in all the sample fractions (total dissolved, retentate and permeate solutions). Acceptable recoveries were obtained for trace elements varying averagely between 84 and 113%, although some contamination/losses did occur especially for Fe and Hg (Table 2). Unfortunately, acceptable mass balance was not achieved for Hg. We suspect this could be due to the effects of bubbling during filtration and ultrafiltration, which become significant at very low concentrations (ng L^{-1}) and considering that Hg may be present in its dissolved gaseous form in coastal waters

(Floreani et al., 2019). Although some contamination/losses did occur, reasonable recoveries were also obtained for TN and nutrients varying averagely between 94 and 127% (Table 3).

Table 2. Average recovery (%) of As, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb and Zn and related number of samplings (n) suitable for mass balance calculation.

	As	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	Zn
average	99	113	90	101	91	111	296	94	103	84	91
SD	14	27	27	21	45	102	302	19	48	20	37
n	24	22	11	20	16	17	5	20	14	12	12

Table 3. Average recovery (%) of TN, N-NO$_3^-$, N-NO$_2^-$, N-NH$_4^+$, SRSi and SRP and related number of samplings (n) suitable for mass balance calculation.

	TN	N-NO$_3^-$	N-NO$_2^-$	N-NH$_4^+$	SRSi	SRP
average	123	127	121	108	102	94
SD	54	84	61	39	21	25
n	24	24	22	23	24	21

Regarding organic carbon (OC), acceptable blank values (approximately 20 µmol L^{-1}) were obtained for the total dissolved fraction, whereas contamination issues unfortunately occurred during ultrafiltration. Since the ultrafiltration was performed under recirculation mode (pre-concentration), OC content in the retentate sample solution was found to be significantly high. Conversely, more unreliable contents were found in the permeate solution. This forced us to estimate the OC concentration in the permeate solution (C_{pOC}) as follows (equation 3)

$$(3) \quad C_{pOC} = \frac{(C_{rOC} \cdot V_r) + (C_{TD\,OC} \cdot V_{TD})}{V_p}$$

where C_{rOC} and $C_{TD\,OC}$ are the concentrations of OC in the retentate and total dissolved fractions and V_r, V_p and V_{TD} are the volume of the retentate, permeate and total dissolved fractions.

3.3 Analytical quality control

The quality of the analytical measurements was evaluated by means of Certified Reference Materials (CRMs), laboratory-fortified samples and laboratory-fortified blanks.

Regarding nutrients, certified standard solutions (Inorganic Ventures Standard Solutions and MOOS-2, NRC) were used for quality control. Furthermore, proficiency testing (PT) exercises proposed by the European network of PT providers (QUASIMEME programmes AQ1 and AQ2) were periodically performed to ensure the accuracy of the analytical performance.

In the case of DOC and TN, quality control was ensured by analysing CRMs (Certified Reference Material, University of Miami, Florida) and the precision of the method was <3%.

Regarding the determination of trace elements in the solid phase (SPM and sediments), Certified Reference Material (PACS-3 Marine Sediment Certified Reference Material, NRCC, Canada) was mineralised in the same batch as the sediment and particulate samples and acceptable recoveries were obtained varying between 104 and 116% overall (Table 4).

Table 4. Average recovery (%) of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, V and Zn in the Certified Reference Material (PACS-3 Marine Sediment Certified Reference Material, NRCC, Canada) for the analysis of the solid phase.

	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	V	Zn
Average	116	109	106	106	109	104	104	110	108	111
SD	1	10	12	10	16	5	10	10	0	3

In addition, laboratory-fortified samples were prepared by spiking an amount of either 1 or 5 µg L^{-1} of trace elements into actual estuarine water and particulate samples, which were then analysed for quality control. For this purpose, a different multistandard solution from that used for instrument calibration was used. Allowable recoveries from 6 spiked samples were obtained for both estuarine water (ranging between 77 and 104%) and particulate samples (ranging between 90 and 116%) (Table 5).

Table 5. Average recovery (%) of As, Ba, Cd, Cr, Cu, Hg, Mn, Ni, Pb, V and Zn in the laboratory-fortified samples employed for the quality control during the analysis of estuarine water samples and SPM.

		As	Ba	Cd	Co	Cr	Cs	Cu	Fe	Mn	Ni	Pb	V	Zn
Water	average	96	91	75	91	99	81	76	96	98	89	77	104	91
	SD	6	3	6	4	9	3	5	8	6	5	5	6	8
SPM	average	105	98	97	103	107	91	98	95	98	116	90	102	95
	SD	1	12	15	1	2	3	17	10	2	9	3	6	5

Generally, the performance of the analysis over time was evaluated by means of laboratory-fortified blanks, which consisted of a standard solution of 1 µg L^{-1} prepared by the dilution of a multistandard solution different from that used for instrument calibration.

Regarding nutrients, the limits of detection (LOD) were 0.02 µM for N-NO$_3^-$, N-NO$_2^-$ and N-NH$_4^+$ and 0.01 µM for SRP and SRSi. The LOD for Hg in water sample aliquots was 0.6 ng L^{-1}.

In the case of ICP-MS, water sample aliquots, SPM and sediment samples were diluted 1:10, 1:20 and 1:50 respectively and the instrumental LOD for each element is reported in Table 6.

Table 6. Limits of detection (LOD) for As, Ba, Cd, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb, V and Zn of the analysis performed by means of ICP-MS.

LOD	As 75	Ba 138	Cd 111	Co 59	Cr 52	Cs 133	Cu 65	Fe 57	Hg 202	Mn 55	Ni 60	Pb 208	V 51	Zn 66
	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}
	0.006	0.004	0.005	0.001	0.008	0.001	0.01	0.09	0.003	0.01	0.01	0.003	0.002	0.04

Chapter 4 – Results and Discussion

4.1 Partitioning and mixing behaviour of trace elements at the Isonzo/Soča River mouth (Gulf of Trieste, northern Adriatic Sea)

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Abstract

Estuaries are unique water systems and represent a vital link between land and sea. River transported contaminants, among which trace elements are of major concern, are subjected to a variety of physical, chemical and biogeochemical processes in the estuarine mixing zone.

The Isonzo/Soča River is the main source of freshwater into the Gulf of Trieste (northern Adriatic Sea) and is known as the primary source of Hg due to long term cinnabar (HgS) extraction from the Idrija mining district (Slovenia). Mercury distribution, cycling and speciation have been intensively studied at the Isonzo/Soča River mouth. Still, little information is currently available regarding other trace elements (As, Co, Cu, Cs, Cr, Fe, Ni, Mn, Pb and Zn). Indeed, this research aims to evaluate trace element occurrence, mixing behaviour and partitioning among suspended particulate matter (>0.45 µm), colloidal material (0.45 µm – 10 kDa) and the truly dissolved fraction (<10 kDa) at the mouth of the Isonzo/Soča River.

Generally, trace elements are mainly associated with suspended particulate matter, which represents their main effective vehicle to coastal environments. In addition, dilution effects between riverine and marine particles are responsible for the decrease in particulate trace element concentrations along the water column. Mercury was notably present in winter in the freshwater, as expected under conditions of high river discharge. As opposed to other trace elements, particulate Mn was found to be high in the marine water, particularly in summer when high water temperatures could promote oxidation and precipitation processes. The Isonzo/Soča River mouth is characterised by a strong salinity gradient and geochemical processes appear to be responsible for the non-conservative behaviour of Fe, Mn and Cu, which also displayed the highest concentration in the colloidal material. Conversely, conservative behaviour was found for As and Cs, which are often present in ionic dissolved forms in natural water systems.

Keywords: trace elements, estuary, suspended matter, colloids, North Adriatic

Introduction

Estuaries and river mouths are crucial environments for the transfer of chemicals from land to open sea (Fu et al., 2013; de Souza Machado et al., 2016; Yao et al., 2016; Fiket et al., 2018). In this context, mixing between river freshwater and marine saltwater represents a key process for the regulation of the fate and distribution of contaminants, among which trace elements are of major concern (Yiğiterhan and Murray, 2008; Deycard et al., 2014; Domingos et al., 2015). Indeed, estuaries generally show strong physico-chemical gradients (salinity, density, flow velocity) which may affect trace element speciation and their partitioning between solid and dissolved phases (Savenko et al., 2014). In addition, organic matter production and oxidation (de Souza Machado et al., 2016), the oxygenation state and potential release of trace elements from the sediment-water interface (SWI) (Petranich et al., 2018b and references therein) are also influential factors.

Organic and inorganic matter in natural aquatic systems can be associated with three main fractions: suspended particulate matter (SPM), colloids and the truly dissolved fraction. By convention, particulate matter in suspension is defined as the material retained on a filter with a nominal porosity of between 0.4 and 0.5 µm. The upper dimensional limit of the particles is not well-defined, although Visher (1969) proposed a value of 100 µm, which is the limit between the particles that move by saltation under a laminar flow regime and those transported in suspension. Colloids are generally defined as submicron particles enclosed within the traditionally defined dissolved fraction (<0.45 µm) (Guo and Santschi, 1997).

The role of SPM as the main carrier for trace elements in estuarine environments has been investigated in several studies (e.g Turner and Millward, 2000; Cenci and Martin, 2004; Fu et al., 2013). As a consequence of SPM settling, bottom sediments represent an important sink for contaminants associated with fine particles. However, bottom sediments may act as a secondary source when processes such as bioturbation and resuspension are responsible for the release of trace elements back to the water column (Sigg et al., 2000).

Trace elements bound to the colloidal material may have a different fate. Indeed, very small particles can settle extremely slowly or be trapped in the commonly defined maximum turbidity zone or may eventually form larger aggregates (Guo and Santschi, 2007). Colloidal material can also influence the transport, bioavailability and fate of trace elements and other contaminants in aquatic systems (Benoit et al., 1994; Guentzel et al., 1996). In addition, colloids represent essential intermediaries in several removal processes such as coagulation and flocculation (Savenko et al., 2014) that often take place in estuarine environments where strong physico-chemical gradients occur, especially in terms of salinity.

The Isonzo River represents the primary freshwater input entering the Gulf of Trieste (northern Adriatic Sea) and it is known to be the main source of Hg (Covelli et al., 2001, 2007) due to long-term cinnabar (HgS) extraction from the Idrija mine, located in the Slovenian sector of the river drainage basin. During mining operations, mineralised tailings generated by HgS roasting processes were dumped along the banks of the Idrijca River, swept away by floodwaters towards the Isonzo River and finally transported into the Gulf of Trieste. According to Baptista-Salazar et al. (2017), the amount of Hg in the soil ranged between 0.08 and 46.3 μg g^{-1} and the maximum concentrations were found in the vicinity of the mining district, most likely due to the presence of ore tailings. Furthermore, the same authors also stated that the amount of Hg associated with the SPM in the Idrijca River freshwater is significantly affected by hydrological conditions. Indeed, particulate Hg ranged between 4.13 to 37.2 μg g^{-1} during rain events whereas significant variations of dissolved Hg were not detected during low and high flow conditions (Baptista-Salazar et al., 2017).

In addition, along the Isonzo River drainage basin, the decommissioned Pb-Zn mining district of Raibl (Cave del Predil village, northern Italy) represents a source of Zn, Pb and other trace elements since the major drainage of the mine discharges directly into the Isonzo River in Slovenia (Petrini et al., 2016). Waste water discharge from a cement factory (near Kanal, Slovenia) as well as rainwater runoff from cropping areas of the Isonzo River alluvial plain (Comici and Bussani, 2007; Cozzi et al., 2012) may further contribute to increase the amount of dissolved and particulate trace elements in the river freshwater.

The behaviour of Hg in the Gulf of Trieste has been intensively studied with regard to Hg contamination in coastal sediments (Covelli et al., 2001), transport and dispersion of particulate Hg (Covelli et al., 2007) and Hg cycling at the SWI (Emili et al., 2011). With the only exception being Hg, the occurrence and distribution of other trace elements and their partitioning between solid and dissolved phases have not yet been thoroughly addressed in the eastern sector of the northern Adriatic Sea, as well as the Gulf of Trieste.

The aim of the present work is to assess trace element partitioning among SPM, colloidal and truly dissolved fractions in estuarine water at the mouth of the Isonzo River, where a strong salinity gradient occurs. Secondly, another goal of this research is to investigate the major processes and factors controlling trace element mixing behaviour and distribution along the water column.

Materials and methods

Environmental setting

The Gulf of Trieste is a semi-enclosed marine basin located in the north-eastern Adriatic Sea (Italy). The water depth is generally shallow, reaching a maximum depth of 25 m in the central area of the Gulf where the Isonzo/Soča River represents the main freshwater input (Figure 1).

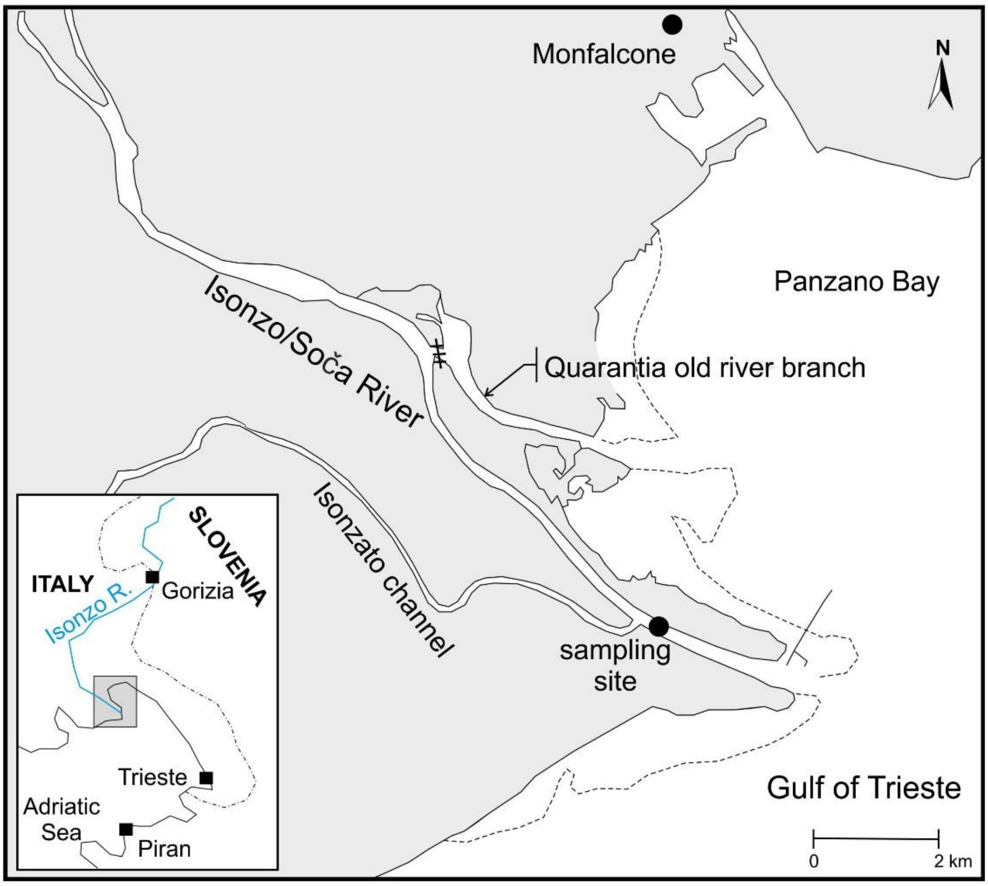

Figure 1. Map of the study area and location of the sampling station at the Isonzo/Soča River mouth.

The average annual flow rate at the river mouth is assessed to be about 82 m^3 s^{-1} in the period from 1998 to 2008 (Cozzi et al., 2012). The Isonzo River mouth can be defined as a microtidal, low-energy and fine-grained deltaic system (Covelli et al., 2004). It is affected by significant seasonal variations in the river discharge with two main flood periods during the year related to snowmelt and rainfall. Furthermore, brief peaks of intense discharge associated with a high level of SPM are followed by extended periods of low-medium riverine flow. As previously mentioned, the Isonzo River is the main source of Hg entering the Gulf of Trieste and Covelli et al. (2001) reported that high Hg concentrations are restricted mainly to sandy-silty sediments near the Isonzo River mouth, where the element is present in detrital form (HgS, cinnabar). However, suspended fine sediments strongly affect the distribution of Hg associated with the SPM as a result of resuspension events involving the silty bottom sediments (Covelli et al., 2006b). In addition, the same authors stated that most of the Hg transported by the river freshwater was associated with SPM, especially after periods of river plume when particulate Hg influx into the Gulf ranges between 37.0 and 112 ng L^{-1} (Covelli et al., 2007).

Sampling activity and water sample size fractionation

Estuarine water samples were collected at the Isonzo River mouth under different seasonal conditions (winter, summer and autumn). Before sampling, vertical profiles of salinity (PSU, Practical Salinity Unit), temperature (°C) and turbidity (NTU, Nephelometric Turbidity Unit) were recorded by means of a CTD multiprobe (Hydrolab H2O with a 0.10 dbar pressure step) in order to identify the water masses. Freshwater-brackish (0-27 PSU) and marine (27-37 PSU) water samples (approximately 15 L) were alternatively collected using a Niskin or a Van Dorn bottle depending on the thickness of the water layer to sample. In addition, the main physico-chemical parameters (pH, redox potential (Eh) and dissolved oxygen) were promptly measured on ship using portable probes (pH-meter PH25 and Conductivity-meter CM 35+ by Crison Instruments).

Fractionation of water samples allowed for the isolation of three size fractions: 1) suspended particulate matter (SPM) fraction is defined as particles having a diameter larger than 0.45 µm; 2) colloidal retentate solution which consists of submicron particles and macromolecules having a diameter < 0.45 µm and a molecular weight > 10 kDa; 3) dissolved permeate solution. The last fraction is also known as ultrafiltrate solution or truly dissolved (Guo and Santschi, 1997; Wells et al., 2000; Town and Filella, 2002), which consists of molecules and ions able to pass through an ultrafiltration membrane having a theoretical MWCO (Molecular Weight Cut-Off) of 10 kDa.

Size fractionation was performed by vacuum filtration (pre-conditioned and pre-weighed membrane filters, Millipore HA, ø 47 mm, 0.45 µm pore size) and cross-flow ultrafiltration (ultrafiltration membranes, Sartorius Vivaflow 200, MWCO 10 kDa) in order to isolate the SPM and the colloidal retentate solution, respectively. Specifically, ultrafiltration was performed under recirculation mode and using a concentration factor of approximately 60. The ultrafiltration system was rinsed with NaOH (0.5 M) and 10 L of MilliQ water after each sample.

According to other studies (Sigg et al., 2000; Ingri et al., 2004), recovery (R%) and colloid concentrations were estimated as follows (equations 1, 2):

$$(1)\ R\% = \{((permeate) + (retentate))/(total\ dissolved)\} * 100$$

$$(2)\ Colloid = \{(retentate) - (permeate)/cf\}$$

where *cf* is the concentration factor of the single ultrafiltration run (equation 3).

$$(3)\ cf = Volume_{tot}/Volume_{\ retentate}$$

The recovery (%) (Table S1) was not calculated when the concentration was below the limit of detection (LOD, Table S2) for one or more fractions. Reasonable mass balances were obtained although losses/contamination did occur. Unfortunately, it was not possible to provide mass balance for Hg since its concentration in the total dissolved fraction was often found to be below the LOD. We suspect this could be due to the effects of bubbling during filtration and ultrafiltration which become significant at very low concentrations (ng L^{-1}) and considering that Hg may be present as dissolved gaseous form in coastal waters (Floreani et al., 2019).

Sample aliquots for trace element determinations in total dissolved, colloidal retentate and truly dissolved fractions were collected into pre-conditioned (hot suprapure HNO_3, ≥ 69%, 10%) PTFE containers and acidified with nitric acid (suprapure HNO_3, ≥ 69%, 1%) to preserve sample composition. For Hg detection, samples were collected into pre-conditioned (hot suprapure HCl, ≥ 37%, 10%) borosilicate glass containers and immediately oxidised with bromine chloride (BrCl, 500 µL/100 mL until the samples turned yellow) following EPA Method 1631 (2002).

Moreover, blank samples were collected at each step to validate both filtration and ultrafiltration systems. Blank values were mainly below LOD. The only exception was represented by Zn, which in some cases was observed to be higher than 0.50 µg L^{-1}, as already reported by Sigg et al. (2000).

Regarding SPM samples, filters were acid-digested in PTFE vessels through a total dissolution using inverse aqua regia (HNO_3 and HCl, 3:1) in a closed microwave system (Multiwave PRO, Anton Paar) according to EPA Method 3052 (2007) for trace element detection.

Analytical measurements

Major elements (Al, Fe, Ca and Mg) in the SPM were detected by means of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using an Optima 8000 ICP-OES Spectrometer (PerkinElmer, USA) equipped with an S10 Autosampler (PerkinElmer, USA). Instrument calibration was performed using standard solutions (ranging between 1 and 100 mg L^{-1}) prepared by dilution of a multistandard solution (100 mg L^{-1}) for ICP analysis (Instrument Calibration Standard 2, PerkinElmer, USA).

Trace element (As, Co, Cu, Cs, Cr, Fe, Ni, Mn, Pb and Zn) concentrations in the sample solutions (total dissolved, colloidal retentate and truly dissolved) as well as in the SPM were detected by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a NexION 350x Spectrometer (PerkinElmer, USA) equipped with an ESI SC Autosampler. Water samples were diluted 1:10 with acidified (1% HNO_3) Milli-Q to avoid the problems caused by high salt concentrations. The instrument was calibrated using standard solutions (ranging between 0.5 and 10 µg L^{-1}) obtained by dilution of a multistandard solution (10 mg L^{-1}) for ICP analysis (Periodic Table MIX 1, Sigma Aldrich). Analysis was performed in KED mode (Kinetic Energy Discrimination) to control and reduce cell-formed polyatomic ion interference. Quality control was performed by the analysis of laboratory-fortified samples prepared by spiking 1 µg L^{-1} of trace elements into actual estuarine water and particulate samples. For this purpose, we diluted a multistandard solution (10 mg L^{-1}) different from the solution used for instrument calibration. Allowable recoveries on 6 spiked samples of both estuarine water and particulate samples were obtained (ranging between 77 and 104% and between 90 and 116% for water and particulate samples, respectively) (Table S2).

The performance of the analysis over time was evaluated by means of laboratory-fortified blanks which were analysed every 10 samples. Those laboratory-fortified blanks consisted of standard solutions of 1 µg L^{-1} (depending on trace element concentrations in the current batch) prepared by the dilution of a multistandard solution (10 mg L^{-1}) different from the solution used for instrument calibration.

Mercury determination in the water samples was conducted by means of Cold Vapor Atomic Fluorescence Spectrometry coupled with a gold trap pre-concentration system (CV-AFS Mercur, Analytic Jena). Specifically, Hg determination was performed through a pre-reduction using NH_2OH-HCl (250 µL/100 mL sample) until the yellow colour disappeared, followed by a reduction with $SnCl_2$ (2% in HCl 2%) according to EPA Method 1631e (2002). Instrument calibration was performed using standard solutions (ranging between 1 and 50 ng L^{-1}) prepared by dilution of a Hg standard solution (mercury standard solution, Merck Millipore, 1000 mg L^{-1}) and acidified with BrCr (0.5 %) in order to avoid matrix effects. Certified reference solution (ORMS-5, Canada) was analysed in the same batch as the water samples for quality control and allowable recovery was obtained (105%).

A summary of major and trace element concentrations in the different fractions (SPM, colloidal material and truly dissolved) of the water samples collected at the Isonzo River mouth are reported in Tables S3 and S4.

Results and discussion

Physico-chemical characterisation of the water column

River discharge was found to be notably higher in winter (275 m^3 s^{-1}) and autumn (230 m^3 s^{-1}) rather than in summer (20 m^3 s^{-1}). Two distinct layers in terms of salinity were detected in the water column at the river mouth, as a consequence of the interaction between the freshwater input and the salt-wedge intrusion (Figure 2)

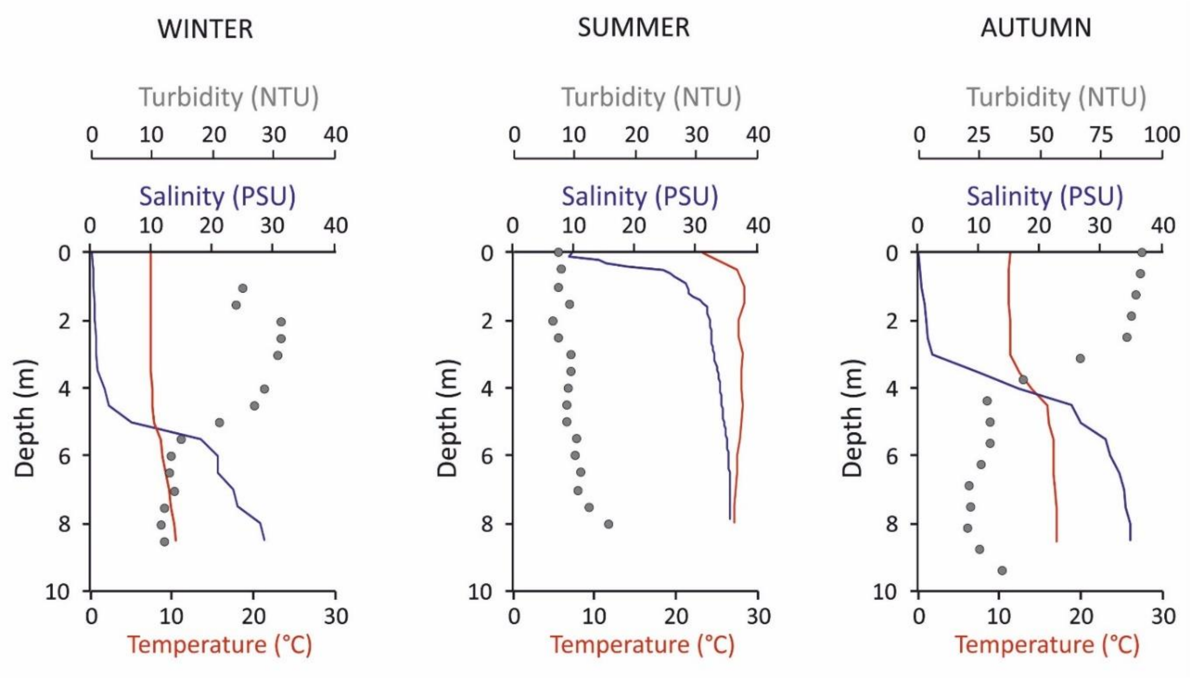

Figure 2. Vertical profiles of salinity (PSU, Practical Salinity Unit), temperature (°C) and turbidity (NTU, Nephelometric Turbidity Unit) recorded at the Isonzo River mouth under different seasonal conditions.

Indeed, salinity vertical profiles displayed a sharp halocline, as previously reported by Covelli et al. (2004). The surface water layer showed salinity values typical of riverine waters (< 2 PSU) in winter and autumn, whereas marine salinity values were recorded at the bottom (35.8 PSU and 35.0 PSU in summer and autumn, respectively). However, brackish water (28.2 PSU) was recorded at the bottom in winter along with freshwater prevalence up to a depth of 4 meters due to the heavy rainfall which occurred for several days before sampling.

The water column stratification was also confirmed by the turbidity trend, especially in autumn (ranging between 20.3 and 93.4 NTU) and, to a lesser extent, in winter (ranging between 11.4 and 32.5 NTU). Here, turbidity reaches higher values in the freshwater-brackish water decreasing with depth as a consequence of the river flood events that occurred in these two seasons. Conversely, the turbidity trend appears to be more homogeneous with increasing depth in summer (ranging between 6.37 and 15.5 NTU), when a slight increase in the turbidity values was observed most likely due to resuspension induced by tidal currents. Regarding the temperature, slight variations were observed along the water column and among the different seasons. The lowest temperatures were obviously recorded in winter (7.37 and 10.2 °C in the surface and bottom water layers, respectively), whereas warm water prevails in summer both at the surface (25.1 °C) and at the bottom (27.2 °C). Conversely, the vertical temperature profile acquired in autumn shows intermediate values ranging between 11.4 °C and 17.1 °C in the surface and bottom water layers, respectively (Figure 2).

Values of dissolved oxygen, pH and redox potential (Eh) measured at the Isonzo River mouth under different seasonal conditions are shown in Figure 3.

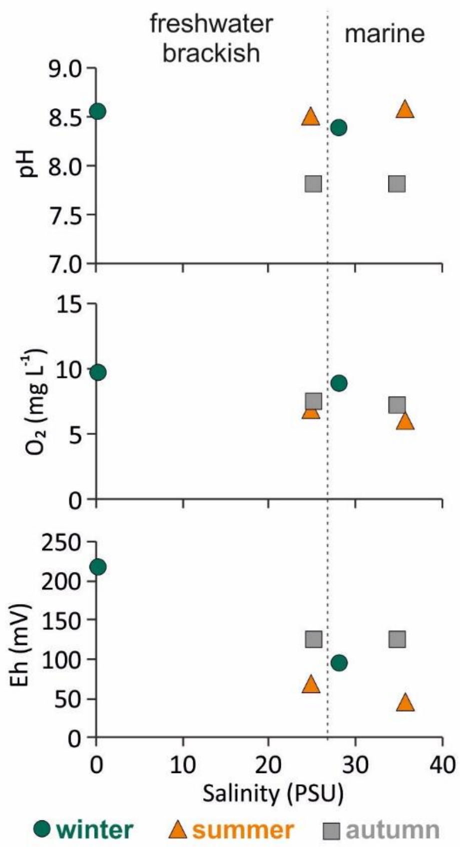

Figure 3. pH, dissolved oxygen and redox potential in the estuarine water samples collected at the Isonzo River mouth under different seasonal conditions.

Significant disparities in terms of pH were not found, although water samples collected in autumn show slightly lower pH. Dissolved oxygen and redox potential are significantly correlated (r = 0.793) and decrease with increasing salinity. In particular, lower values of both parameters were recorded in summer, when the strong stratification of the water column does not allow for mixing between the water masses.

Occurrence of trace elements in the suspended particulate matter

Generally, the freshwater-brackish water samples showed higher amounts of particulate trace elements compared to the marine water at the bottom (Figure 4, Table 1).

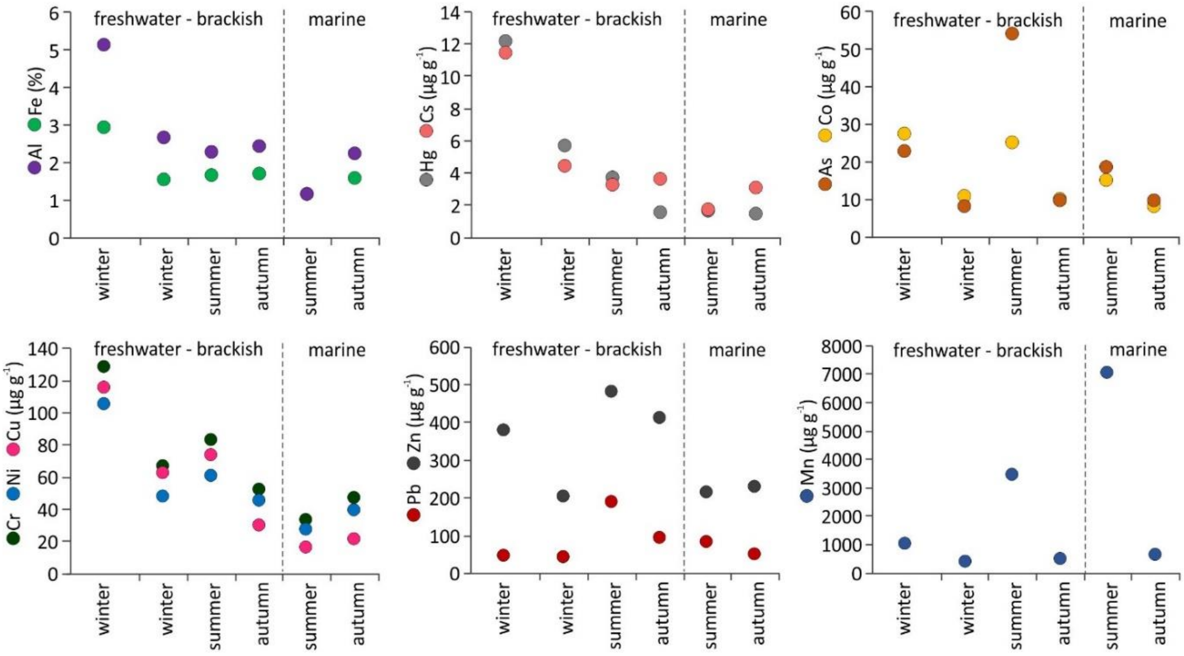

Figure 4. Concentrations of particulate Al, Fe (%), As, Co, Cr, Cs, Cu, Hg, Mn, Ni, Pb and Zn (µg g^{-1}) in the estuarine water samples collected at the Isonzo River mouth under different seasonal conditions.

As it has been suggested that mixing processes play a crucial role in regulating trace element occurrence and distribution in estuarine environments (Turner, 1999; Hatje et al., 2001), the commonly observed decline in particulate trace element concentrations along the water column could account for dilution effects between riverine and marine particles. Indeed, in our sampling, surface freshwater showed high amounts of trace elements compared to the marine bottom water due to high river water discharge (Figure 4).

The highest concentrations of Al (5.16 %), Co (27.4 µg g^{-1}), Cr (128 µg g^{-1}), Cs (11.4 µg g^{-1}), Cu (116 µg g^{-1}), Fe (2.94 %), Hg (12.1 µg g^{-1}) and Ni (105 µg g^{-1}) in the SPM were found in winter, most likely due to the river flood period which occurred for several days before sampling. Conversely, As and Mn reached the highest concentrations in summer both in the brackish (54.2 and 3445 µg g^{-1} of As and Mn, respectively) and marine (18.5 and 7055 µg g^{-1} of As and Mn, respectively) water samples.

The commonly observed decline in particulate trace element concentrations along the water column could account for dilution effects between riverine and marine particles (Turner, 1999; Hatje et al., 2001).

*Table 1. Concentrations of Al, Fe (%), As, Co, Cr, Cs, Cu, Hg, Mn, Ni, Pb and Zn ($\mu g\ g^{-1}$) in the suspended particulate matter collected at the Isonzo River mouth and in analogous estuarine environments as reported in the literature (nd: not determined; *: average).*

Site	Season	Salinity	Al	As	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Reference
Isonzo River[1]	winter	freshwater	5.16	22.8	27.4	128	11.4	116	2.94	12.1	1017	105	45.0	377	This work
		brackish	2.67	8.08	10.7	67.2	4.44	62.9	1.58	5.71	414	47.9	41.3	201	
	summer	brackish	2.29	54.2	25.2	83.5	3.25	73.5	1.68	3.71	3445	61.1	189	478	
		marine	1.18	18.5	15.0	33.3	1.71	16.8	1.16	1.59	7055	28.0	80.9	214	
	autumn	brackish	2.46	9.50	10.1	52.4	3.59	30.2	1.72	1.57	505	45.6	91.8	410	
		marine	2.24	9.73	8.11	47.0	3.11	21.5	1.58	1.45	628	39.4	49.3	228	
Isonzo River[1]	winter	freshwater	nd	nd	nd	nd	nd	nd	nd	10.20	nd	nd	nd	nd	Covelli et al., 2006b
		brackish	nd	nd	nd	nd	nd	nd	nd	10.20	nd	nd	nd	nd	
		marine	nd	nd	nd	nd	nd	nd	nd	9.94	nd	nd	nd	nd	
	summer	freshwater	nd	nd	nd	nd	nd	nd	nd	11.0	nd	nd	nd	nd	
		brackish	nd	nd	nd	nd	nd	nd	nd	20.7	nd	nd	nd	nd	
		marine	nd	nd	nd	nd	nd	nd	nd	24.9	nd	nd	nd	nd	
Isonzo River[1]	winter	freshwater	17.8	nd	nd	60.3	nd	69.9	nd	7.48	nd	205	315	nd	Covelli et al., 2002
		brackish	9.50	nd	nd	36.7	nd	25.0	nd	4.13	nd	5.19	273	nd	
		marine	3.59	nd	nd	38.9	nd	15.7	nd	1.34	nd	10.7	25.6	nd	
Adige River[1]	summer	bulk	nd	nd	nd	150	nd	35	nd	nd	nd	57	90	225	Boldrin et al., 1989
Adige River[1]	autumn	bulk	5.24			207		108	2.79		507	38	62	329	Juračić et al., 1987
Adige River[1]		freshwater	nd	nd	nd	126-159	nd	47-50	nd	nd	nd	32-42	49-78	213-270	Juračić and Pravdić, 1991
Krka River[2]			nd	nd	nd	150	nd	180	nd	nd	nd	150	92	210	
Po River[1]	winter	freshwater	nd	nd	1.77 - 7.66	nd	nd	nd	0.26 - 0.83	nd	824 – 1373	18.8 - 43.4	nd	nd	Camusso et al., 1997
	summer		nd	nd	5.30 - 10.0	nd	nd	nd	0.45 - 0.58	nd	989 – 1209	35.8 - 93.9	nd	nd	
Adriatic Sea	winter	freshwater	nd	nd	1.18 - 5.89	nd	nd	nd	0.14 - 0.45	nd	220 - 604	12.3 - 17.0	nd	nd	
	summer		nd	nd	2.36 - 10.0	nd	nd	nd	0.13 - 0.43	nd	714 – 1428	29.4 - 35.2	nd	nd	
Raša River[3]	summer	freshwater	nd	nd	nd	129	nd	161	nd	nd	518	nd	38	55	Sondi et al., 1994
		marine	nd	nd	nd	216	nd	274	nd	nd	633	nd	281	165	
	autumn	freshwater	nd	nd	nd	237	nd	71	nd	nd	568	nd	129	133	
		marine	nd	nd	nd	129	nd	66	nd	nd	935	nd	93	121	
Mejerda River[4]	winter		4.46	nd	18.2	106	nd	<LOD	1.70	nd	10.1	82	67.8	143	Helali et al., 2016
	wummer		3.01	nd	8.70	61.2	nd	37.3	1.77	nd	78.0	65.5	61.3	264	
Guanabara Bay[5]	winter	freshwater	1.00	nd	nd	180	nd	nd	0.80	1.90*	3000	nd	79.6*	1040*	de Melo et al., 2015
		marine	2.20	nd	nd	270	nd	nd	1.50		1000	nd			

[1] Italy; [2] Slovenia; [3] Croatia; [4] Tunisia; [5] Brazil

Manganese was found to be relatively high in the marine water, especially in summer (7055 µg g^{-1}), as also stated by Sondi et al. (1994) in another estuarine system (Raša River estuary, Croatia). Remobilisation of particulate Fe and Mn in reducing sediments and/or resuspension of bottom sediments (Hatje et al., 2001; Petranich et al., 2018b) are known to be two of the most important processes influencing trace element mobility in the aquatic compartment. Moreover, Fe and Mn oxy-hydroxides are important host phases for contaminants due to their high adsorptive capacities (Gagnon and Saulnier, 2003). The notably high concentration of particulate Mn observed in summer (7055 µg g^{-1}) could be related to oxidation processes and the subsequent precipitation of Mn oxy-hydroxides promoted by high water temperatures (Morris et al., 1982; Hatje et al., 2001). As opposed to what was found for particulate Fe, concentrations of particulate Mn displayed significant disparities among seasons. Indeed, the Fe/Mn ratio would indicate the relative abundance of each sorbent phase throughout the year. This ratio is considerably high in winter both in the freshwater (28.9) and in the brackish water (38.1) samples, thus revealing the concomitant decrease in particulate Mn and increase in particulate Fe. This ratio remained of the same order of magnitude in autumn, whereas it reached values of 4.88 and 1.65 in the brackish and marine water samples respectively in summer, testifying to the increase in particulate Mn.

If compared with the results obtained from other studies focused on the Isonzo River and other estuarine environments (Table 1), particulate Hg concentrations (ranging overall between 1.45 and 12.1 µg g^{-1}) confirmed the results obtained by Covelli et al. (2006b) although the authors reported higher particulate Hg concentrations in the marine water in summer. The river is still an active source of Hg in particulate form, which was found to be high in the freshwater and brackish water samples (12.1, 5.71 and 3.71 µg g^{-1}, in winter, summer and autumn, respectively) decreasing in the marine water (1.59, 1.57 and 1.45 µg g^{-1}, in winter, summer and autumn, respectively). In this study, Hg content is comparable to that measured at Guanabara Bay (de Melo et al., 2015), a shallow water basin along the Brazilian coast subjected to high anthropogenic impact. Regarding the other elements, we measured comparable particulate concentrations with respect to preliminary measurements performed by Covelli et al. (2002). The only exception is represented by the freshwater sample collected in winter found to be enriched in several trace elements such as Cr, Cu, Hg and Pb, likely due to the higher river flow conditions during sampling (Table 1).

However, the suspended particles collected at the Isonzo River mouth were not particularly enriched in Cr (the only exception being the freshwater sample collected in winter that showed a value of 128 µg g^{-1}) when compared to other Adriatic estuarine environments (Juračić and Pravdić, 1991; Sondi et al., 1994) (Table 1). On the other hand, SPM samples generally showed higher concentrations of Fe, Al and Mn with respect to those reported for the estuarine system of the Po River, whereas comparable content was found for Co, Cu and Zn (Zago et al., 2002).

Trace element phase partitioning between suspended particles and the dissolved fraction

The distribution coefficient (K_D) has often been used in order to evaluate the tendency of a chemical constituent to be preferentially partitioned in the solid or in the aqueous phase, as a result of sorption and precipitation (EPA, 1999; Gagnon and Saulnier, 2003; Guéguen and Dominik, 2003; Wang and Wang, 2016). Although K_D values does not provide any specific information on the kind of metal associations, K_D could be considered an important index to better understand circulation of contaminants among different environmental compartments (Gagnon and Saulnier, 2003; La Colla et al., 2015). According to Turner (1996), K_D (L kg^{-1}) was calculated as the ratio of particulate concentration to total dissolved concentration and expressed on a logarithmic scale (Table 2) using the following equation (4)

$$(4)\quad Log\ K_D = [TE_{SPM}]/[TE_{DISS}]$$

where TE_{SPM} and TE_{DISS} are the trace element concentrations in the SPM and in the total dissolved fraction, respectively.

Table 2. Log K_D (L kg^{-1}) values for As, Co, Cr, Cs, Cu, Fe, Hg, Ni, Mn, Pb and Zn in estuarine water samples collected at different depths under different seasonal conditions at the Isonzo River mouth.

Water sample	Season	As	Co	Cr	Cs	Cu	Fe	Hg	Ni	Mn	Pb	Zn
Freshwater	Winter	4.88	6.00	5.82	6.70	5.36	7.32	6.55	6.18	5.80	6.48	5.32
Brackish	Winter	3.85	5.17	6.17	4.60	5.22	7.29	6.98	5.43	4.75	6.44	5.89
	Summer	4.66	5.66	6.22	4.52	4.72	7.03	5.76	6.83	5.80	5.82	4.72
	Autumn	3.72	5.69	4.94	4.42	4.71	6.90	5.60	4.83	4.87	5.64	3.84
Marine	Summer	3.88	5.21	5.82	3.95	4.04	6.98	6.73	6.49	5.88	5.91	4.52
	Autumn	3.59	4.69	5.14	4.17	4.48	6.60	5.54	4.68	4.55	4.94	3.80

In addition, the relative percentages of As, Co, Cr, Cs, Cu, Fe, Hg, Ni, Mn, Pb and Zn in the SPM and in the total dissolved fraction from the water samples collected at the Isonzo River mouth under different seasonal conditions are plotted in Figure 5 in order to better highlight disparities between seasons and water masses.

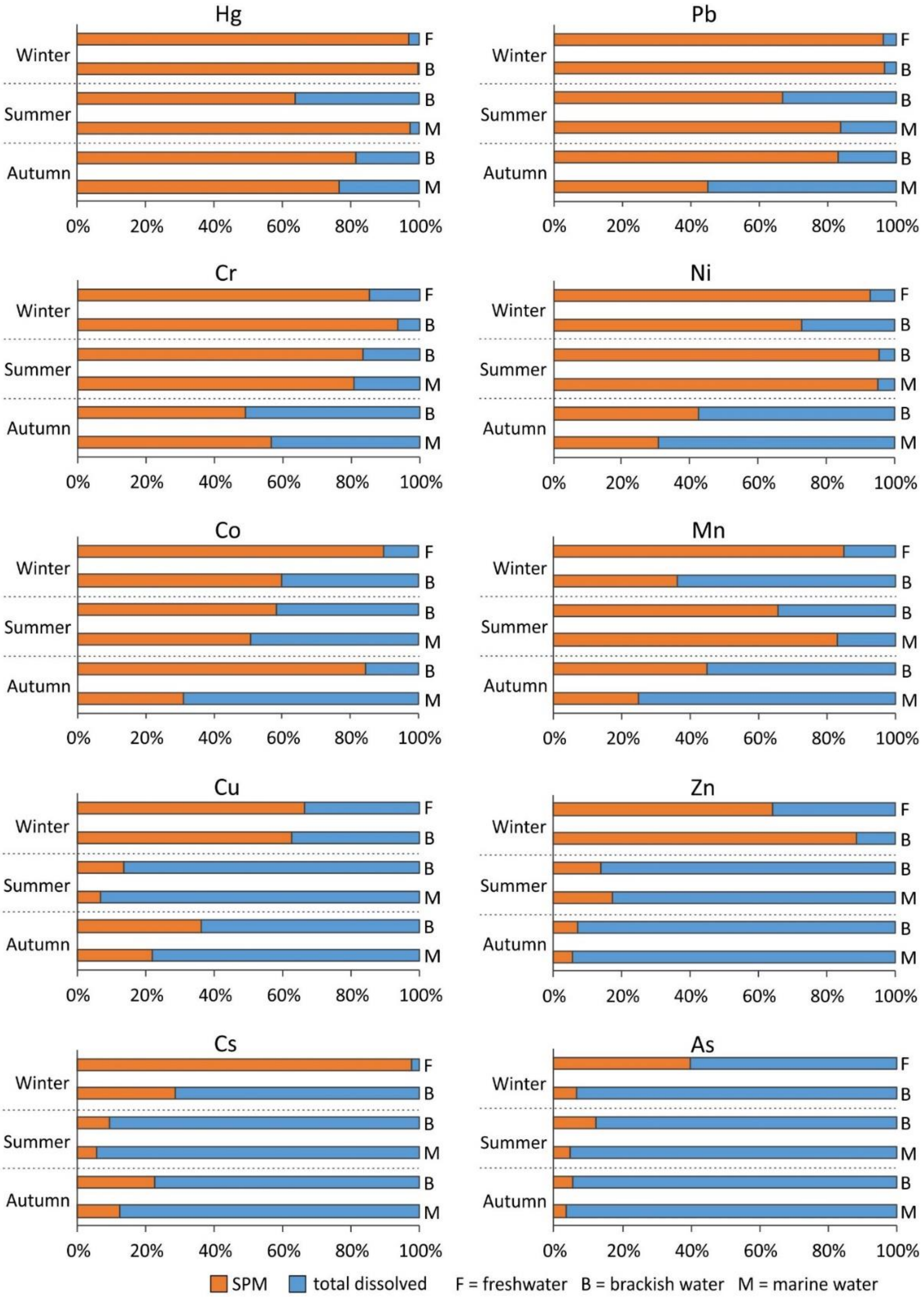

Figure 5. Percentages of trace elements (Hg, Pb, Cr, Ni, Co, Mn, Cu, Zn, Cs, As) associated with suspended particulate matter and with the total dissolved fraction in estuarine water samples collected at the Isonzo River mouth under different seasonal conditions.

The highest Log K_D values were observed for Fe, Hg and Pb, followed by Cr and Mn, reflecting their high reactivity of these elements to the SPM. Generally, trace elements appear to be mainly associated with the suspended particles, especially in the water samples collected during winter river discharge. This is particularly evident for Hg and Pb, which are almost completely partitioned in the solid phase both in the freshwater (96.8 % and 96.3 %, for Hg and Pb respectively) and in the brackish water (99.5 % and 96.5 %, for Hg and Pb respectively). This is also confirmed by Log K_D values which were found to be quite elevated both for Hg (6.55 L kg^{-1}) and Pb (6.48 L kg^{-1}) in the freshwater collected in winter. Regarding Hg, this behaviour confirms the results found by Covelli et al. (2007) who stated that the contribution of particulate Hg into the Gulf of Trieste still remains considerable, although extreme flood events are not frequent during the year. In addition, the evidence that Hg conveyed by the Isonzo River is mainly associated with the solid phase is also confirmed by the element particulate percentages recovered in autumn and summer. Indeed, Hg remains considerably present in the suspended particles, showing on average a Log K_D equal to 6.19 L kg^{-1} to the detriment of the total dissolved fraction, which reaches the maximum percentage of only 36.4 % in the brackish water sample collected in summer.

If compared with the results obtained from other studies focused on the Isonzo River and other estuarine environments, dissolved Hg concentrations were found to be lower than those reported by Covelli et al. (2006b), likely due to disparities in the river flow conditions during sampling (Table 3). However, the estuarine water samples showed dissolved Hg concentrations which were notably higher than those reported by Kwokal and Lovrić (2006) (Krka River estuary, Croatia) for an estuarine system not affected by Hg anthropic sources near the river mouth or along the river drainage basin.

Similar behaviour is also evident for Pb, Cr and Ni, which are strongly associated with the suspended particles in winter and summer, showing quite elevated values of Log K_D (5.87, 5.68 and 5.74 L kg^{-1} on average for Pb, Cr and Ni). Consequently, those elements displayed quite low concentrations in the aqueous phase. To a lesser extent, this behaviour is also evident for Co, which shows a Log K_D of 6.00 L kg^{-1} in the freshwater collected in winter. However, dissolved Co percentages increased in summer (41.8 % and 49.2 % in the brackish and marine water samples, respectively) and autumn, especially in the marine water (55.1 % and Log K_D of 4.69 L kg-1). Similar concentrations of dissolved Co were also found in different estuarine environments (Zago et al., 2002; Fiket et al., 2018) (Table 3).

Although particulate percentages are quite high in the freshwater collected in winter, Cu, Zn, and especially Cs and As, appear to be mainly present in dissolved form. Among those elements, the lowest values of Log K_D (4.10 L kg^{-1} on the average) and particulate percentages (12.1 % on the average) are reached by As, confirming its affinity to the aqueous phase.

*Table 3. Dissolved concentrations of As, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn (µg L^{-1}) and Hg (ng L^{-1}) at the Isonzo River mouth and in analogous estuarine environments as reported in the literature (nd: not determined; *: average).*

Site	Season	Salinity	As	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Reference
Isonzo River[1]	winter	freshwater	0.19	0.03	<LOD	<LOD	0.28	<LOD	6.25	1.07	<LOD	<LOD	<LOD	This work
		brackish	1.29	0.10	<LOD	0.13	0.25	<LOD	4.09	7.97	0.24	<LOD	<LOD	
	summer	brackish	1.13	0.07	0.06	0.09	1.77	6.64	8.60	2.97	<LOD	0.30	9.99	
		marine	2.10	0.12	<LOD	0.19	1.74	<LOD	<LOD	6.09	<LOD	0.08	6.93	
	autumn	brackish	1.69	<LOD	0.29	0.13	0.15	1.78	1.85	6.75	0.81	<LOD	57.9	
		marine	2.31	0.13	0.31	0.17	0.41	3.51	4.16	16.9	0.70	<LOD	31.2	
Isonzo River[1]	winter	freshwater	nd	nd	nd	nd	nd	nd	15.4	nd	nd	nd	nd	Covelli et al., 2006b
		brackish	nd	nd	nd	nd	nd	nd	10.4	nd	nd	nd	nd	
		marine	nd	nd	nd	nd	nd	nd	5.59	nd	nd	nd	nd	
	summer	freshwater	nd	nd	nd	nd	nd	nd	17.0	nd	nd	nd	nd	
		brackish	nd	nd	nd	nd	nd	nd	28.5	nd	nd	nd	nd	
		marine	nd	nd	nd	nd	nd	nd	33.4	nd	nd	nd	nd	
Isonzo River[1] *		bulk	0.07	0.03	0.21	0.01	0.51	41.9	0.001	0.93	0.35	nd	5.03	Klun et al., 2019
Gulf of Trieste[2] *		marine	1.50	0.06	0.62	0.27	2.61	12.5	0.001	1.54	0.88	nd	4.64	
Venice Lagoon[1]	summer	freshwater	nd	nd	nd	nd	0.27 - 0.56	0.18 - 0.63	nd	0.44 - 1.53	0.65 - 1.13	0.004 - 0.03	nd	Martin et al., 1995
		brackish	nd	nd	nd	nd	0.29	0.15	nd	0.45	0.39	0.013	nd	
		marine	nd	nd	nd	nd	0.24 - 0.29	0.08 - 0.14	nd	0.27 - 0.36	0.38 - 0.48	0.007 - 0.010	nd	
Po River[1]	summer		nd	nd	nd	nd	1.59	nd	nd	nd	1.76	nd	0.39	Tankéré and Statham, 1996
Po River[1]	summer	freshwater	nd	0.04	nd	nd	0.44	<LOD	nd	0.91	nd	nd	0.86	Zago et al., 2002
		marine	nd	0.03	nd	nd	0.30	<LOD	nd	1.03	nd	nd	1.71	
	winter	freshwater	nd	0.05	nd	nd	0.66	0.20	nd	2.69	nd	nd	0.42	
		marine	nd	0.03	nd	nd	0.34	0.08	nd	0.48	nd	nd	0.39	
Po River[1]	winter	freshwater	nd	nd	nd	nd	3.65	2.34	nd	2.75	3.06	0.32	6.15	Tankéré et al., 2000a
	summer	freshwater	nd	nd	nd	nd	2.63	2.10	nd	1.41	2.8	0.24	3.06	
Po River[1]	winter	freshwater	nd	0.01 - 0.05	nd	nd	nd	1.01 - 4.86	nd	2.27 - 8.36	0.59 - 0.94	nd	nd	Camusso et al., 1997
	summer		nd	0.01 - 0.02	nd	nd	nd	0.67 - 1.12	nd	0.54 - 1.46	1.41 - 2.88	nd	nd	
Adriatic Sea	winter	freshwater	nd	0.02 - 0.06	nd	nd	nd	0.28 - 1.84	nd	2.10 - 5.66	0.29 - 1.17	nd	nd	
	summer		nd	0.01 - 0.04	nd	nd	nd	0.34 - 0.95	nd	1.40 - 3.67	0.29 - 0.76	nd	nd	
Krka River[3]		brackish	nd	nd	nd	nd	nd	nd	0.50 - 0.60	nd	nd	nd	nd	Kwokal and Lovrić, 2006
		marine	nd	nd	nd	nd	nd	nd	0.90 - 1.10	nd	nd	nd	nd	
Zrmanja River[3]		freshwater	0.25	0.01	0.22	0.01	<0.05	6.14	nd	0.13	0.04	54.3	nd	Fiket et al., 2018
		marine	1.74	0.26	1.10	0.20	1.04	10.1	nd	3.22	6.36	127	nd	

[1] Italy; [2] Slovenia; [3] Croatia

Redox conditions are found to be one of the most important factors in regulating the behaviour of As and other trace elements in natural water systems (Smedley and Kinniburgh, 2002). In addition, Andreae et al. (1983) stated that processes such as diffusion from sediment porewaters, coprecipitation with Fe oxides or anthropogenic input could be responsible for the occurrence of As in estuarine systems. Moreover, Smedley and Kinniburgh (2002) suggested that the simultaneous effects of dissolution and desorption processes can lead to an increase of dissolved As. Since the adsorption and desorption characteristics of As in estuaries are strongly salinity dependent (Benoit et al., 1994; Balzer et al., 2013), Hong et al. (2018) reported that a high concentration of salt in water can weaken adsorption of As on the surface of the suspended particles due to the formation of weak As dissolved complexes.

As previously mentioned, Mn is the element which shows the highest disparities between different water masses and seasons both in the SPM and in the total dissolved fraction (Figure 6).

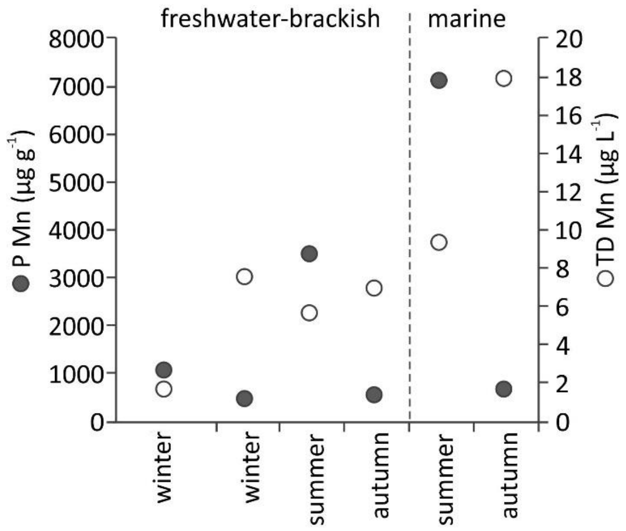

Figure 6. Concentration of Mn in the suspended particulate matter (PMn, µg g^{-1}) and in the total dissolved fraction (TDMn, µg L^{-1}) in the estuarine water samples collected at the Isonzo River mouth under different seasonal conditions.

Manganese is a redox sensitive element, and the fact that particulate Mn increases when total dissolved Mn decreases and vice versa is likely due to the relative prevalence of oxidation and reduction or dissolution processes. This is in agreement with other Adriatic estuarine environments where dissolved Mn was found to be relatively high, ranging from 0.55 and 3.22 µg L^{-1}, with a general increase towards the SWI (Tankéré et al., 2000b; Fiket et al., 2018).

This element is mainly partitioned in the solid phase in the freshwater collected in winter (84.6 % of PMn and 15.4 % of TDMn with a Log K_D value of 5.80 L kg^{-1}), whereas it is mainly present in dissolved form in the brackish water sample (36.0 % of PMn and 64.0 % of TDMn with a Log K_D

value of 4.75 L kg^{-1}). According to de Melo et al. (2015), hydrodynamic conditions with a strong stratification of the water column and low water temperature could be responsible for the relatively low concentrations of particulate Mn in the marine water. Furthermore, the oxidation of dissolved Mn is influenced by the low water temperature, which slows this process down (Turner et al., 1991; Hatje et al., 2001). This was confirmed in the present study when the low temperature (10.3 °C) measured in the brackish water in winter may not have promoted the oxidation of dissolved Mn resulting in a lower concentration of the element in the SPM (4.15 µg L^{-1}). At the same time, Mn shows a quite high concentration in the aqueous phase (7.38 µg L^{-1}) which could be due to dissolution processes from oxy-hydroxides. Indeed, although dissolved oxygen shows a value of 8.87 mg L^{-1}, the brackish water sample is characterised by quite a low value of redox potential (Eh = 93 mV) if compared to the river freshwater (216 mV).

Water samples taken in summer showed the opposite situation, although Log K_D remained quite high (5.80 and 5.88 L kg^{-1} in the brackish and marine water samples, respectively), thus testifying to the prevalence of particulate Mn (45.2 µg L^{-1} at the bottom) likely due to oxidation and the subsequent precipitation favoured by high water temperatures (27.2 °C), or remobilisation from surface sediments by resuspension processes (Hatje et al., 2001). The relatively low value of the redox potential (only 46.0 mV) could be responsible for dissolution or desorption processes which led to the increase in the dissolved Mn concentration (9.24 µg L^{-1}), although there was no evidence of reductive conditions and the amount of dissolved oxygen was equal to 6.10 mg L^{-1}. A similar situation was also found in the estuarine environment of the Po River, where quite elevated concentrations of dissolved Mn were detected at the bottom despite oxidative conditions (90-150%) (Tankéré et al., 2000b). Moreover, the aggregation and accumulation of mucilage and marine snow (which consists of mucus and large flakes of polymeric exudates and dead phytoplankton cells) at the pycnocline is favoured by a stratified water column and low turbulence (Fajon et al., 1999; Granéli et al., 1999). These organic aggregates are rapidly colonised and degraded by bacteria (Fogg, 1995; Serratore et al., 1995) through biological processes that lead to the consumption of the dissolved oxygen causing suboxic conditions (Tankéré et al., 2000b).

The highest percentages of dissolved Mn were found during autumn, both in the brackish (55.1 %) and in the marine (75.1 %) water samples, likely due to a slight stratification and an increase in mixing favouring exchanges between the water masses. The occurrence of dissolved Mn in autumn is also confirmed by lower Log K_D, which reaches values of 4.87 and 4.55 L kg^{-1} in the brackish and marine water, respectively.

Iron was almost completely partitioned in the suspended particles, reporting particulate percentages higher than 90%. Indeed, the high Fe reactivity to SPM was also confirmed by the Log K_D values (7.02 L kg^{-1} on average) if compared to those reported for the other elements.

The role of salinity in trace elements phase partitioning

Estuarine environments are characterised by strong physico-chemical gradients in salinity, density and flow velocity, and varied composition of the suspended particles (Elliott and McLusky, 2002). The interaction of these environmental gradients, especially in terms of salinity, and geochemical processes that often occur in the estuarine mixing zone are often responsible for the non-conservative behaviour of several trace elements (Benoit et al., 1994; de Souza Machado et al., 2016).

In order to evaluate the behaviour of trace elements at the Isonzo River mouth, mixing diagrams for Mn, Fe, Cu, Ni, As, Co and Cs are reported in Figures 7 and 8, although few estuarine water samples were collected and results provide only a snapshot of trace element behaviour during mixing. In addition, different fractions (the SPM, truly dissolved and colloidal material) were shown to better evaluate removal and/or addition processes among different phases.

Generally, a conservative or quasi-conservative behaviour was found for As, Co and Cs at the Isonzo River mouth (Figure 7).

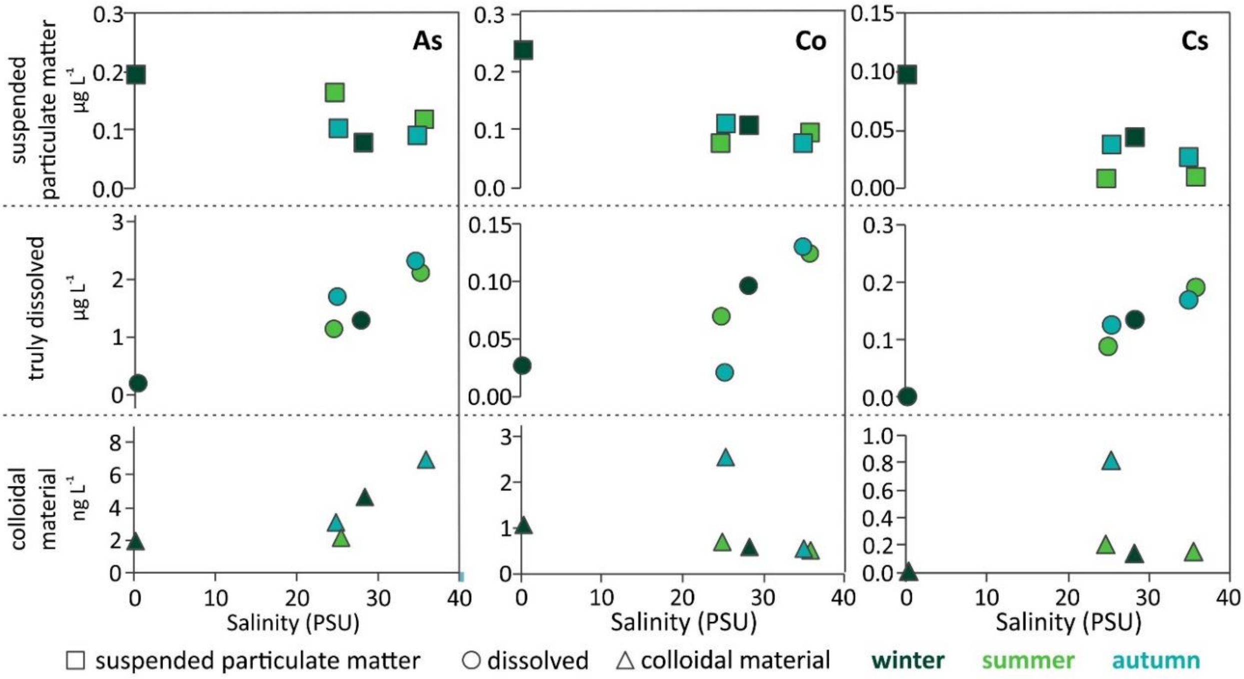

Figure 7. Arsenic, Co and Cs in the suspended particulate matter (µg L^{-1}), truly dissolved (µg L^{-1}) and colloidal material (ng L^{-1}) plotted as a function of salinity at the Isonzo River mouth under different seasonal conditions.

Arsenic concentration in the suspended particles were quite low and the element appears to behave conservatively showing a linear decrease with increasing salinity (0.20 and 0.12 µg L^{-1} for the two salinity end-members) as also seen in other estuarine environments (Zrmanja River Croatia) (Fiket et al., 2018). In this study, As appears to be preferentially partitioned in the truly dissolved phase, showing concentrations below 4 µg L^{-1}, typical for estuarine waters under natural conditions according to Smedley and Kinniburgh (2002). In addition, the prevalence of dissolved As could be confirmed by the slight removal of this element depicted from the mixing diagram for colloidal material, although very low colloidal concentrations were found.

The mixing behaviour of Co and Cs is similar to that reported for As, with the only exception being the brackish water taken in autumn. Although the colloidal material generally shows very low concentrations, which are an order of magnitude lower compared to the other fractions, an increase in the colloidal concentration of these elements is evident in this season. This is particularly marked for Co, where the increase in the colloidal material corresponds to a decrease in the truly dissolved fraction. On the contrary, Mn, Fe and Cu appear to behave non-conservatively during mixing and simultaneously show a higher content of colloidal material if compared to that reported for trace elements that behave conservatively (Figure 8).

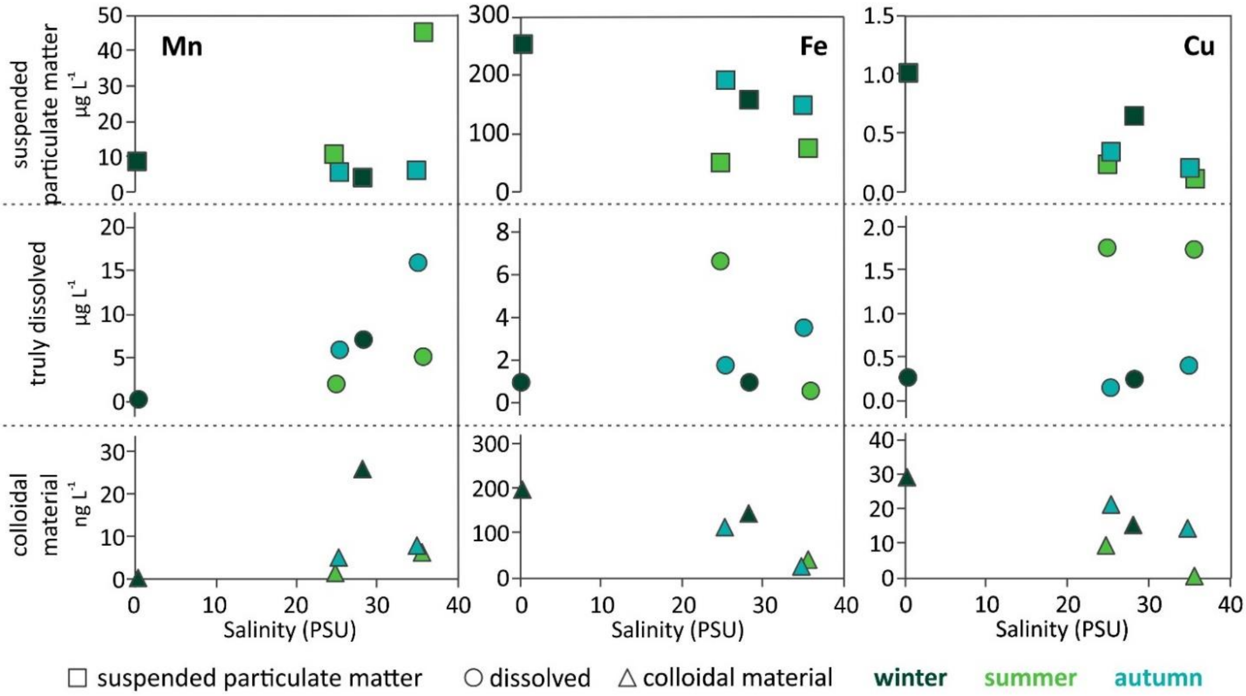

Figure 8. Manganese, Fe and Cu in the suspended particulate matter (µg L^{-1}), truly dissolved (µg L^{-1}) and colloidal material (ng L^{-1}) plotted as a function of salinity at the Isonzo River mouth under different seasonal conditions.

In addition, despite no data currently being available for the amount of trace elements associated with the colloidal material in the estuarine environments of the northern Adriatic Sea, a comparison between the Isonzo River mouth and the Venice Lagoon is provided in Table 4, as well as other estuarine and riverine systems worldwide.

*Table 4. Colloidal concentrations of As, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn (ng L^{-1}) at the Isonzo River mouth compared to other estuarine and riverine environments as reported in the literature (nd: not determined; *: average).*

Site	Season	Salinity	As	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Reference
Isonzo River[1]	winter	freshwater	1.94	1.06	27.5	0.01	29.1	196	0.16	0.50	19.2	nd	nd	This work
		brackish	4.72	0.58	14.1	0.14	15.0	142	0.08	26.3	34.8	nd	nd	
	summer	brackish	3.11	0.69	7.28	0.20	9.41	nd	0.22	1.83	21.1	1.64	nd	
		marine	7.03	0.49	5.01	0.15	0.16	39.3	0.03	6.66	14.9	1.71	6.11	
	autumn	brackish	2.21	2.54	32.7	0.82	20.8	114	0.16	5.40	51.6	5.01	nd	
		marine	nd	0.56	7.60	nd	14.3	25.6	0.08	7.97	10.9	0.52	nd	
Isonzo River[1] *		bulk	4.50	1.47	26.5	3.99	165	1016	0.80	33.0	45.8	nd	172	Klun et al., 2019
Gulf of Trieste[2] *		marine	52.4	2.95	41.6	3.99	254	949	0.80	33.0	29.3	nd	111	
Gulf of Trieste[2]	summer	marine	89.9	nd	31.2	nd	445	nd	1,003	22	47.0		196	Koron et al., 2013
Venice Lagoon[1]	summer	freshwater	nd	nd	nd	nd	274 - 530	2843 - 62642	nd	422 - 1451	101 - 281	19.5 - 203	nd	Martin et al., 1995
		brackish	nd	nd	nd	nd	75	391	nd	257	4.7	0.41	nd	
		marine	nd	nd	nd	nd	120 - 416	291 - 1142	nd	247 - 860	12.9 - 221	2.28 - 14.5	nd	
Penzé Estuary[3]		brackish	nd	nd	nd	nd	136 - 182	nd	nd	Nd	nd	6.21 - 55.9	nd	Waeles et al., 2008
Marne River[3]	summer	freshwater	nd	nd	nd	nd	230 - 1300	nd	nd	700 - 24600	nd	nd	nd	Jarïy et al., 1999
Vistula River[4]	autumn	freshwater	nd	7.00 - 24.0	55.0 - 338	nd	nd	18 - 347	0.1 - 8.5	230 - 2820	nd	18.0 - 68.0	210 - 3450	Guéguen and Dominik, 2003
Ekhagen Bay[5]	winter	freshwater	nd	7.66	nd	nd	477	nd	nd	49.4	299	nd	nd	Ingri et al., 2004
	summer	freshwater	nd	10	nd	nd	235	nd	nd	<LOD	211	nd	nd	
San Francisco Bay[6]	winter	freshwater	nd	nd	nd	nd	50.8	815	nd	1,099	13.5	nd	19.6	Sañudo-Wilhelmy et al., 1996
		brackish	nd	nd	nd	nd	19.1	278	nd	nd	nd	nd	6.54	
		marine	nd	nd	nd	nd	3.18	145	nd	54.9	nd	nd	3.27	

[1] Italy; [2] Slovenia; [3] France; [4] Poland; [5] Sweden; [6] United States

As expected, lagoon water samples (Martin et al., 1995) displayed concentrations of colloidal Cu, Fe, Mn, Ni and Pb, which are notably higher, especially in the surface freshwater and at the seawater-freshwater interface, than those reported for the mouth of the Isonzo River. This could be related to lower turbulence conditions, which could lead to a local source for colloidal Fe, Mn, Cd and Pb due to biological activity and physicochemical boundary conditions.

As previously mentioned, particulate Mn is highly present in the marine water in summer (45.2 µg L^{-1}) due to oxidation and precipitation, which are processes enhanced by high water temperatures. This markedly high concentration in the marine end-member seems to highlight a non-conservative behaviour of the other samples, thus suggesting that the release of dissolved Mn may have occurred. Indeed, remobilisation of dissolved Mn from the SPM and/or resuspension of surface sediments could be responsible for the increase in the concentration of dissolved Mn (Tankéré et al., 2000b).

In addition, reduction processes at the SWI or bacterial degradation of particulate organic matter could have led to an increase in the dissolved Mn in the marine water (Fiket et al., 2018).

An increase in dissolved Mn is clearly evident only in the marine water collected in autumn. On the contrary, the removal of particulate Mn in the brackish water collected in winter appears to correspond to an increase in the colloidal concentration, thus suggesting that the element could be partially present in colloidal form. Moreover, colloidal Mn concentrations are significantly lower than those reported for other estuarine environments (Sañudo-Wilhelmy et al., 1996; Guéguen and Dominik, 2003) likely because the element tends to be more linked to the suspended particles at the Isonzo River mouth. Our results are comparable to those found in the Bay of Piran (Slovenia) during summer (Koron et al., 2013). Iron associated with the suspended particles reaches the maximum value of 254 µg L^{-1} in the freshwater and it significantly decreases with depth upon increasing salinity. Moreover, Fe concentrations in the truly dissolved fraction (<LOD) and in the colloidal material (196 ng L^{-1}) suggest that colloidal Fe precipitates could have formed in the freshwater as a result of coagulation and/or flocculation processes (Förstner and Wittmann, 1979; Kraepiel et al., 1997). However, the brackish water shows a slight enrichment in particulate Fe with the only exception of the sample collected in summer, showing a lower concentration of particulate Fe. In addition, the relatively low Fe concentrations in the truly dissolved fraction and the quite high content in the colloidal material would confirm the fact that the element is preferentially partitioned in the solid phase.

The only exception is the brackish water collected in summer, which displays a truly dissolved concentration of 6.64 µg L^{-1} likely due to the dissolution of Fe oxy-hydroxides. In addition, processes such as reduction mediated by biological activity and diffusion from the SWI could be responsible for the increase in dissolved Fe in oxic seawater (Hopwood et al., 2015).

The behaviour of Cu is similar to that described for Fe, although its concentration in the SPM appears to decrease linearly with increasing salinity. Copper plays an important role in the chemistry of Fe and it was demonstrated that the Fe oxidation process is favoured by the presence of Cu (González et al., 2016). In addition, the non-conservative behaviour of Cu was also found in other transitional systems, especially regarding the truly dissolved phase (Martin et al., 1995). However, a depletion in truly dissolved Cu was clearly evident in autumn and winter in the brackish and marine water. In addition, the enrichment in terms of colloidal material confirms that this element is preferentially partitioned in the solid phase with the only exception of the brackish water sample collected in summer, which displayed a truly dissolved Cu concentration of 1.77 µg L^{-1}.

Conclusions

In the Isonzo/Soča estuarine system, trace elements appear to be mainly associated with the suspended particulate matter, thus confirming its crucial role in the transfer of contaminants from land to coastal and marine environments. Dilution processes between riverine and marine particles occurring at the river mouth are responsible for the decrease in the particulate trace element concentrations along the water column. However, significant levels of particulate Mn were detected in the marine water in summer, when the warm water conditions could promote oxidation and consequent precipitation of Mn oxy-hydroxides.

Regarding Hg, the results discussed herein are in agreement with previous studies attesting that the Isonzo/Soča River still represents an active source of particulate Hg. Furthermore, the element appears to be mainly partitioned in this solid phase, especially after river flood events.

The strong physico-chemical gradients, especially in terms of salinity, lead to a non-conservative behaviour for Fe, Mn and Cu, which are also partially partitioned in the colloidal material. Conversely, As and Cs behaves conservatively and were found to be primarily present under ionic dissolved forms showing a linear increase at increasing salinity.

Acknowledgements

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Supplementary material

Table S1. Average recovery (%) for As, Co, Cr, Cs, Cu, Hg, Fe, Mn, Ni, Pb and Zn and related number of samplings (n). Recovery was not calculated when the concentration was below LOD for one or more fractions (nd: not determined).

	As	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	Zn
n	6	5	2	5	6	2	nd	6	3	2	4
average	93±16	116±26	74±29	98±16	78±38	84±5	nd	83±22	119±36	94±19	102±10

Table S2. Limits of detection (LOD) for As, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb and Zn of the analysis performed by means of ICP-MS as well as average recovery (R%) and residual standard deviation (RSD%) in the laboratory-fortified samples employed for the quality control during the analysis of estuarine water samples and SPM.

	As μg L^{-1}	Co μg L^{-1}	Cr μg L^{-1}	Cs μg L^{-1}	Cu μg L^{-1}	Fe μg L^{-1}	Hg μg L^{-1}	Mn μg L^{-1}	Ni μg L^{-1}	Pb μg L^{-1}	Zn μg L^{-1}
LOD	0.006	0.001	0.008	0.001	0.01	0.09	0.003	0.01	0.01	0.003	0.04
water R%	96 ± 6	91 ± 4	99 ± 9	81 ± 3	76 ± 5	96 ± 8	nd	98 ± 6	89 ± 5	77 ± 5	91 ± 8
RSD%	< 3%	< 2%	< 3%	< 2%	< 3%	< 5%	nd	< 3%	< 3%	< 2%	< 3%
SPM R%	105 ± 1	103 ± 1	107 ± 2	91 ± 3	98 ± 17	95 ± 10	91 ± 8	98 ± 2	116 ± 9	90 ± 3	95 ± 5
RSD%	< 3%	< 2%	< 3%	< 3%	< 3%	< 5%	< 2%	< 3%	< 3%	< 2%	< 3%

Table S3. Concentration of Al, Ca, Fe and Mg in the three analysed fractions of estuarine water collected at the Isonzo River mouth under different seasonal conditions (nd: not determined).

			Winter		Summer		Autumn	
Element	Fraction		Freshwater	Brackish	Brackish	Marine	Brackish	Marine
Al	SPM	%	5.16	2.67	2.29	1.18	2.46	2.24
		μg L^{-1}	445	267	69.9	75.3	272	209
	C	ng L^{-1}	nd	nd	nd	nd	nd	nd
	D	μg L^{-1}	nd	nd	nd	nd	nd	nd
Ca	SPM	%	18.2	6.86	4.77	3.12	17.1	11.1
		μg L^{-1}	1571	687	145	200	1893	1038
	C	ng L^{-1}	nd	nd	nd	nd	nd	nd
	D	μg L^{-1}	nd	nd	nd	nd	nd	nd
Fe	SPM	%	2.94	1.58	1.68	1.16	1.72	1.58
		μg L^{-1}	254	158	51.2	74.5	190	148
	C	ng L^{-1}	196	142	nd	39.3	114	25.6
	D	μg L^{-1}	<LOD	<LOD	6.64	<LOD	1.78	3.51
Mg	SPM	%	3.52	3.24	3.73	3.44	3.81	3.79
		μg L^{-1}	304	324	114	220	421	354
	C	ng L^{-1}	nd	nd	nd	nd	nd	nd
	D	μg L^{-1}	nd	nd	nd	nd	nd	nd

SPM = suspended particulate matter C = colloidal material D = truly dissolved

Table S4. Concentration of As, Co, Cr, Cs, Cu, Hg, Mn, Ni, Pb and Zn in the three analysed fractions of estuarine water collected at the Isonzo River mouth (nd: not determined).

			Winter		Summer		Autumn	
Element	Fraction		Freshwater	Brackish	Brackish	Marine	Brackish	Marine
As	SPM	μg g^{-1}	22.8	8.08	54.2	18.5	9.50	9.73
		μg L^{-1}	0.20	0.08	0.17	0.12	0.11	0.09
	C	ng L^{-1}	1.94	4.72	3.11	7.03	2.21	nd
	D	μg L^{-1}	0.19	1.29	1.13	2.10	1.69	2.31
Co	SPM	μg g^{-1}	27.4	10.7	25.2	15.0	10.1	8.11
		μg L^{-1}	0.24	0.11	0.08	0.10	0.11	0.08
	C	ng L^{-1}	1.06	0.58	0.69	0.49	2.54	0.56
	D	μg L^{-1}	0.03	0.10	0.07	0.12	<LOD	0.13
Cr	SPM	μg g^{-1}	128	67.2	83.5	33.3	52.4	47.0
		μg L^{-1}	1.11	0.67	0.25	0.21	0.58	0.44
	C	ng L^{-1}	27.5	14.1	7.28	5.01	32.7	7.60
	D	μg L^{-1}	<LOD	<LOD	0.06	<LOD	0.29	0.31
Cs	SPM	μg g^{-1}	11.4	4.44	3.25	1.71	3.59	3.11
		μg L^{-1}	0.10	0.04	0.01	0.01	0.04	0.03
	C	ng L^{-1}	0.01	0.14	0.20	0.15	0.82	nd
	D	μg L^{-1}	<LOD	0.13	0.09	0.19	0.13	0.17
Cu	SPM	μg g^{-1}	116	62.9	73.5	16.8	30.2	21.5
		μg L^{-1}	1.00	0.63	0.22	0.11	0.33	0.20
	C	ng L^{-1}	29.1	15.0	9.41	0.16	20.8	14.3
	D	μg L^{-1}	0.28	0.25	1.77	1.74	0.15	0.41
Hg	SPM	μg g^{-1}	12.1	5.71	3.71	1.59	1.57	1.45
		μg L^{-1}	0.10	0.06	0.01	0.01	0.02	0.01
	CM	ng L^{-1}	0.16	0.08	0.22	0.03	0.16	0.08
	D	ng L^{-1}	6.25	4.09	8.60	<LOD	1.85	4.16
Mn	SPM	μg g^{-1}	1017	414	3445	7055	505	628
		μg L^{-1}	8.78	4.15	10.5	45.2	5.59	5.86
	C	ng L^{-1}	0.50	26.3	1.83	6.66	5.40	7.97
	D	μg L^{-1}	1.07	7.97	2.97	6.09	6.75	16.9
Ni	SPM	μg g^{-1}	105	47.9	61.1	28.0	45.6	39.4
		μg L^{-1}	0.91	0.48	0.19	0.18	0.50	0.37
	C	ng L^{-1}	19.2	34.8	21.1	14.9	51.6	10.9
	D	μg L^{-1}	<LOD	0.24	<LOD	<LOD	0.81	0.70
Pb	SPM	μg g^{-1}	45.0	41.3	189	80.9	91.8	49.3
		μg L^{-1}	0.39	0.41	0.58	0.52	1.02	0.46
	C	ng L^{-1}	nd	nd	1.64	1.71	5.01	0.52
	D	μg L^{-1}	<LOD	<LOD	0.30	0.08	<LOD	<LOD
Zn	SPM	μg g^{-1}	377	201	478	214	410	228
		μg L^{-1}	3.25	2.02	1.46	1.37	4.53	2.13
	C	ng L^{-1}	nd	nd	nd	6.11	nd	nd
	D	μg L^{-1}	<LOD	<LOD	9.99	6.93	57.9	31.22

SPM = suspended particulate matter C = colloidal material D = truly dissolved

4.2 Trace elements in estuarine systems: a chemometric approach to depict partitioning and behaviour of particulate, colloidal and truly dissolved fractions

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Abstract

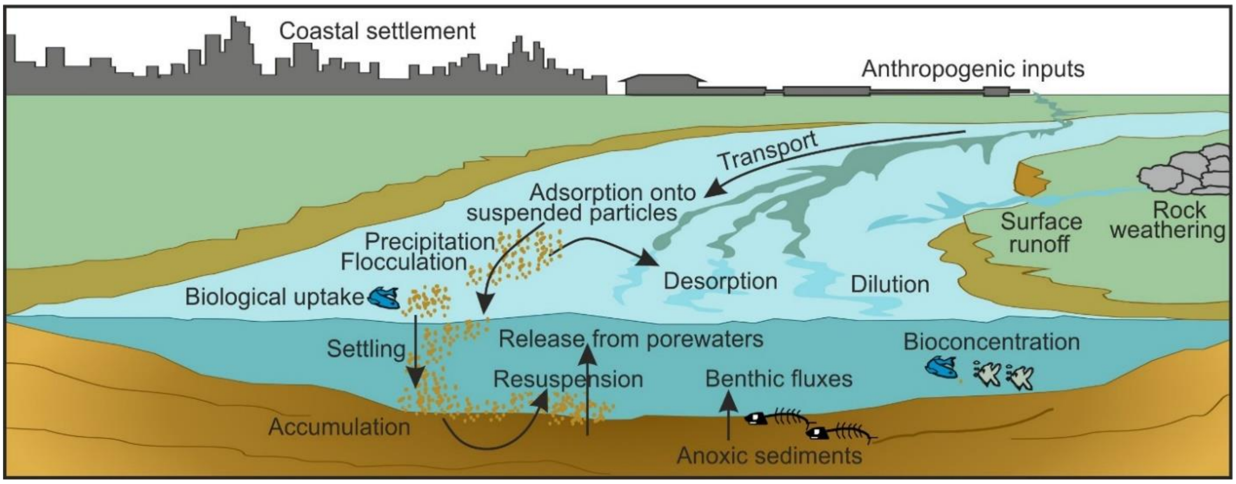
Estuaries are transitional water systems where the hydrodynamic processes governing water circulation actively influence suspended particle transport and deposition. In the estuarine mixing zone, the strong physico-chemical gradients resulting from the interaction between river freshwater and seawater may affect the distribution, mobility and fate of several potentially toxic compounds, among which trace elements are of major concern.

In this study, trace element occurrence and phase partitioning among suspended particulate matter (>0.45 µm), colloidal material (0.45 µm – 10 kDa) and the truly dissolved fraction (<10 kDa) were investigated in the main Italian and Slovenian estuarine environments of the Gulf of Trieste (northern Adriatic Sea, Italy).

Multi-way principal component analysis was employed in order to depict disparities among sampling sites, water layers and seasonal conditions and to evaluate trace element phase partitioning. Results indicated that the suspended particulate matter acts as the main effective vehicle for Cu, Cr, Fe, Ni and Pb, and enhanced adsorption processes resulted in elevated partitioning coefficients (K_D), especially for Fe and Pb. Although disparities occurred between sampling sites and seasons, trace elements showing affinity for the solid phase appeared to be partially bound to the colloidal fraction. Conversely, As and Cs prevailed in the truly dissolved fraction, especially in seawater and showed scarce affinity for both the suspended particles and colloids.

Keywords: trace elements, estuary, suspended matter, colloids, multi-way data analysis

Graphical abstract



Introduction

Among natural aquatic systems, estuaries are often affected by significant anthropogenic pressure which heavily contributes to render them particularly fragile (Caplat et al., 2005; Casado-Martínez et al., 2006; Vale et al., 2008; Guo et al., 2010; Deycard et al., 2014). Estuaries can also be considered a sedimentary trap since the decrease in flow velocity occurring at the estuarine mixing zone leads to fine particle aggregation and settling (Wright, 1977; Menon et al., 1998). These natural processes can easily involve a variety of potentially toxic compounds adsorbed on the surface of the suspended particles such as trace elements, organometallic compounds and persistent organic pollutants (e.g. Neto et al., 2006; Botwe et al., 2017), which tend to accumulate in the sediments. Moreover, resuspension events, changes in the boundary conditions at the sediment-water interface (SWI) and mixing processes between different water masses may affect the partitioning of trace elements between solid and dissolved phases and their fate in the estuarine system (Fu et al., 2013; Domingos et al., 2015; de Souza Machado et al., 2016; Wang and Wang, 2016; Fiket et al., 2018).

Three fundamental fractions are generally involved in the phase partitioning of organic and inorganic matter in natural aquatic systems: suspended particulate matter (SPM), colloids and the truly dissolved fraction. The SPM is known as the main carrier for trace elements entering the estuarine environment (e.g. Turner and Millward, 2000; Cenci and Martin, 2004; Fu et al., 2013) though the upper dimensional limit of the particles is not well established and the lower limit has been conventionally defined as being between 0.4 and 0.5 µm. According to Guo and Santschi (1997), the traditionally defined total dissolved fraction (TD<0.45 µm) incorporates the truly dissolved fraction (D) and colloidal material (C). Colloids consist of submicron particles having a molecular weight lower than 10 kDa (high molecular weight colloids) (e.g. Sañudo-Wilhelmy et al., 1996; Kraepiel et al., 1997; Sigg et al., 2000) or 1 kDa (low molecular weight colloids) (e.g. Wen et al., 1996; Wells et al., 2000; Guéguen and Dominik, 2003). In addition, the strong physico-chemical gradients occurring in estuaries may favour the aggregation of colloids as a result of coagulation and flocculation processes (Guo and Santschi, 2007; Savenko et al., 2014) which can influence the biogeochemical cycles of trace elements (Benoit et al., 1994; Guentzel et al., 1996). These macroaggregates exhibit a reactive surface area for adsorption processes (Filella, 2007) with the subsequent removal of trace elements from the dissolved phase.

The shallow marine area of the northern Adriatic Sea is subject to intense freshwater discharge during autumn and spring (Cozzi et al., 2012) mainly from the Po River, which is the source of the majority of the SPM (Frignani et al., 2005), followed by the Adige and Brenta Rivers (Cozzi and Giani, 2011).

Regarding the easternmost sector of the northern Adriatic Sea, the main freshwater input is represented by the Isonzo/Soča River followed by the Timavo/Reka River (Cozzi et al., 2012) whereas lower contributions come from the Rižana and Dragonja Rivers, located along the Slovenian coast (Frignani et al., 2005).

The mixing behaviour of trace elements and their partitioning between solid and dissolved phases in the estuarine environments of the northern Adriatic Sea has not yet been thoroughly addressed. The role of SPM as the primary carrier of trace elements was investigated at the Po, Adige and Isonzo Rivers (Juračić et al., 1987, 1986; Juračić and Pravdić, 1991; Pavoni et al., submitted(a)), whereas few studies have examined the potential release of trace elements in the aqueous phase (Tankéré and Statham, 1996; Tankéré et al., 2000a; Zago et al., 2002; Pavoni et al., submitted (a)). This research aims to assess trace element occurrence and phase partitioning among SPM, colloids and the truly dissolved fraction in the main Italian and Slovenian estuarine environments of the Gulf of Trieste, evaluating their mixing behaviour and distribution along the water column. In this context, to fully exploit the extraction of the information embodied within data, a multi-way principal component analysis (MW-PCA) was employed in order to discern the main differences and relations between estuarine environments.

Materials and methods

Environmental setting

The semi-enclosed coastal environment of the Gulf of Trieste is located on the north-eastern sector of the Adriatic Sea (Italy), it covers an area of approximately 500 km^2, reaching its maximum depth (25 m) in the central sector. The circulation of water masses in the Gulf is primarily dominated by the anticlockwise circulation of the Adriatic Sea, and further influenced by tides, winds and seasonal variations in the freshwater inflow (Malačič and Petelin, 2009).

Along the Italian coast, the Isonzo/Soča River represents the main source of freshwater entering the Gulf of Trieste, followed by the Timavo/Reka River, whereas minor freshwater inputs are conveyed by the Rižana and Dragonja Rivers along the Slovenian sector, discharging into the Bay of Koper and the Bay of Piran, respectively (Figure 1).

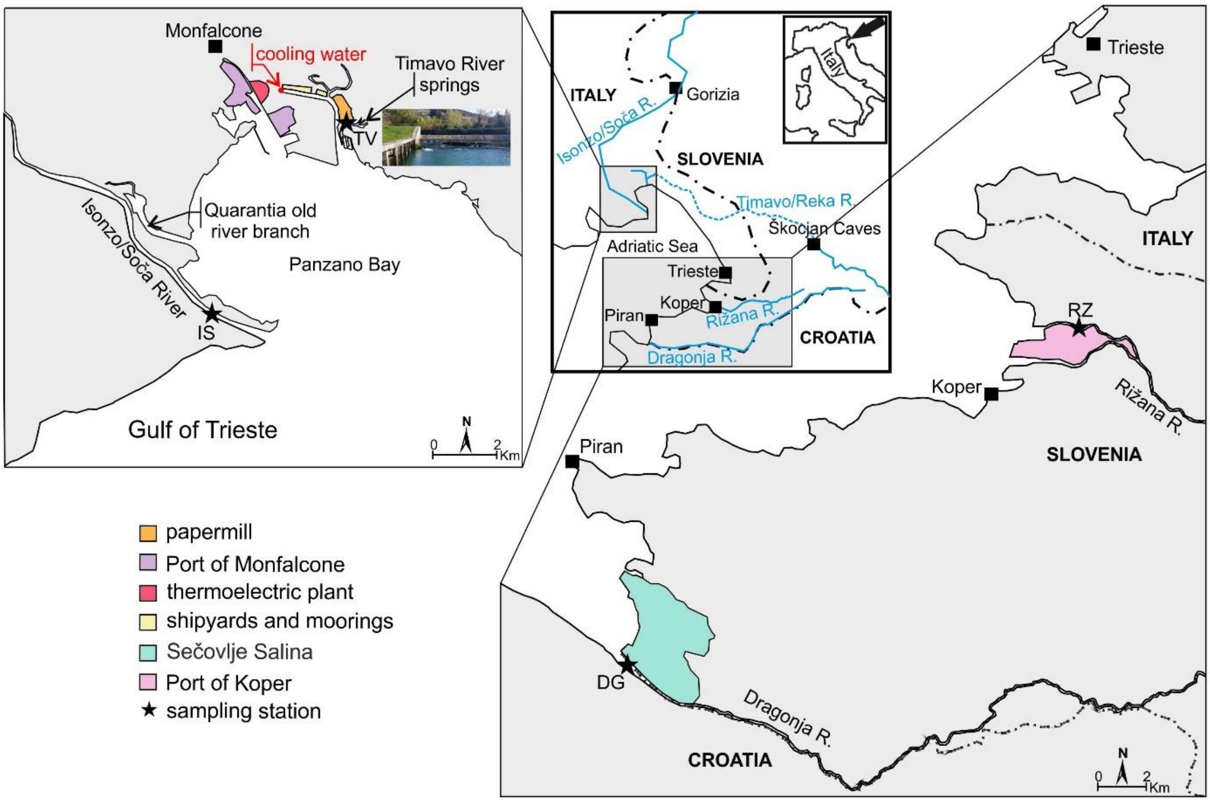


Figure 1. Map of the study area and location of the sampling stations at the main estuarine environments of the Gulf of Trieste: the Isonzo (IS) and Timavo (TV) River mouths along the Italian coast and the Dragonja (DG) and Rižana (RS) River mouths in the Slovenian sector.

The Isonzo River (IS) generally shows significant seasonal variations in the river discharge with an average flow of 82 $m^3 s^{-1}$ (Cozzi et al., 2012) and two main flood events usually occur during late spring and autumn, mainly influenced by rainfall and snowmelt. The IS River represents an active source of mercury (Hg) which appears to be primarily bound to the suspended particles (Covelli et al., 2004, 2006b, 2007). Moreover, the impact of long-term *cinnabar* (HgS) extraction activity from the Idrija mining district (Slovenia) (Hines et al., 2000) is well known with regards to the occurrence of dissolved Hg in groundwater (Cerovac et al., 2018), Hg contamination of surface sediments (Covelli et al., 2001) and Hg cycling at the SWI (Emili et al., 2011).

The Timavo River (TV) originates in Croatia (Snežnik, Slovenia-Croatia border area), it flows on a flysch formation outcropping in the Slovenian sector before sinking into the Karst Plateau at the Škocjan Caves (Slovenia) (Gabrovšek et al., 2018). Its underground river path is approximately 38 km before it re-emerges from several springs with an average discharge of 30.2 $m^3 s^{-1}$ (Gabrovšek and Peric, 2006) in the innermost sector of the Panzano Bay, home to several industrial and harbour activities (Pavoni et al., submitted (b)) (Figure 1).

The Rižana River (RZ) is one of the shortest rivers in Slovenia (14 km); it originates under the Kraško-Slavniška plateau draining directly into the Bay of Koper, which is subject to a variety of industrial settlements (Mandac and Žagar, 2018) (Figure 1). The anthropogenic pressure present in this area is mainly due to the intense activity at the Port of Koper, which is one of the biggest harbours in the northern Adriatic Sea and where potentially toxic compounds, including trace elements, were found to be notably present in bottom sediments (Šmuc et al., 2018).

The Dragonja River (DG) begins in Croatia, it flows on flysch terrain for approximately 30 km and its course follows the border between Slovenia and Croatia until discharging into the Bay of Piran (Figure 1). Its river path is markedly branched and meandered; the river discharge is generally low and often dries up during summer (Brečko Grubar and Kovačič, 2010).

According to Cozzi et al. (2012), the average discharge of the RZ and DG Rivers ranges between 1 and 4 m^3s^{-1}.

Field activities and water sample size fractionation

Water samples were collected at the TV, RZ and DG estuarine systems under different seasonal conditions (winter, summer and autumn). A CTD multiprobe (Hydrolab H_2O with a 0.10 dbar pressure step) was used to depict the water column stratification in terms of salinity (PSU, Practical Salinity Unit), temperature (°C) and turbidity (NTU, Nephelometric Turbidity Unit).

According to the water stratification present in each sampling area, either a Niskin or Van Dorn bottle were alternatively employed to collect large volume (approximately 15 L) of freshwater-brackish (0-27 PSU) and marine water (27-37 PSU). Furthermore, additional physico-chemical parameters such as pH and redox potential (Eh) were measured *in situ* by means of portable probes (pH-meter PH25 and Conductivity-meter CM 35+ by Crison Instruments).

Vacuum filtration was performed using pre-conditioned and pre-weighed membrane filters (Millipore HA, ø 47 mm, 0.45 µm pore size) in order to isolate the SPM (>0.45 µm), which was then acid digested through a total dissolution (inverse *aqua regia*, suprapure HNO_3 (≥69%) and HCl (≥37%), 3:1) in a closed microwave system (Multiwave PRO, Anton Paar) following the modified EPA Method 3052 (2007) for the analytical determination of trace elements.

Further sample fractionation involved the total dissolved fraction, which was ultrafiltered (ultrafiltration membranes, Sartorius Vivaflow 200, Molecular Weight Cut-Off 10 kDa) allowing the isolation of the colloidal retentate solution (10 kDa – 0.45 µm) from the permeate solution (<10 kDa), also known as ultrafiltrate solution or truly dissolved (Guo and Santschi, 1997; Wells et al., 2000; Town and Filella, 2002). Every ultrafiltration run was carried out under recirculation mode, applying a concentration factor of approximately 60 and NaOH (water solution, 0.5 M) followed by large amounts of MilliQ water which were used to rinse the ultrafiltration system.

Mass balances and concentrations of the colloidal material were estimated according to the following equations (1, 2) (Sigg et al., 2000; Ingri et al., 2004) and acceptable recoveries were obtained varying between 75 and 113 %.

(1) $R\% = \{((permeate) + (retentate))/(total\ dissolved)\} * 100$
(2) $Colloid = \{(retentate) - (permeate)/cf\}$

where cf is the concentration factor of the single ultrafiltration run (equation 3).

(3) $cf = Volume_{tot}/Volume_{retentate}$

Sample aliquots for trace element determination were promptly acidified with suprapure HNO_3 (1 mL/100 mL sample) and stored in pre-conditioned PTFE containers, whereas borosilicate glass containers were preferred for Hg storage and detection. The water samples used for Hg detection were immediately oxidised with bromine chloride (BrCl Hg-free from Brooks Rand Instruments, 500 μL/100 mL until the sample turned yellow) according to the EPA Method 1631e (2002).
Blank samples were collected at each step and were found to be mainly below the limit of detection (LOD) with the only exception being Zn, which was occasionally found to be higher than 0.50 μg L^{-1}, as previously reported by Sigg et al. (2000).

Analytical measurements

Concentrations of As, Co, Cu, Cs, Cr, Fe, Ni, Mn, Pb and Zn in the different sample fractions (SPM, colloidal retentate solution and truly dissolved fraction) were quantified by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a NexION 350x Spectrometer (PerkinElmer, USA) equipped with an ESI SC Autosampler. Standard solutions (0.50 – 100 μg L^{-1}) obtained via the dilution of a multistandard solution for ICP analysis (10 mg L^{-1}, Periodic Table MIX 1, Sigma Aldrich) were analysed in order to calibrate the instrument and the analysis was performed in KED mode (Kinetic Energy Discrimination) to avoid and minimise cell-formed polyatomic ion interference.
Particulate Al and Fe were detected by means of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using an Optima 8000 ICP-OES Spectrometer (PerkinElmer, USA) equipped with an S10 Autosampler (PerkinElmer, USA). Instrument calibration was performed using standard solutions (ranging between 1 and 100 mg L^{-1}) prepared by the dilution of a

multistandard solution (100 mg L^{-1}) for ICP analysis (Instrument Calibration Standard 2, PerkinElmer, USA).

Mercury analysis was preceded by a pre-reduction using NH_2OH-HCl (250 µL/100 mL sample until the yellow colour disappeared), followed by a reduction via $SnCl_2$ (2% in HCl 2%) (EPA Method 1631e, 2002) and performed by means of Cold Vapor Atomic Fluorescence Spectrometry coupled with a gold trap pre-concentration system (CV-AFS Mercur, Analytic Jena).

The instrument was calibrated using standard solutions (1-50 ng L^{-1}) prepared by the dilution of a Hg standard solution (mercury standard solution, Merck Millipore, 1000 mg L^{-1}) and stabilised with BrCl (500µL/100 mL).

Laboratory-fortified samples prepared by spiking 1 µg L^{-1} of trace elements into actual estuarine water and particulate samples were analysed for quality control. For this purpose, a different multistandard solution from that used for instrument calibration was used. Allowable recoveries from 6 spiked samples were obtained for both estuarine water (ranging between 77 and 104%) and particulate samples (ranging between 90 and 116%). In addition, laboratory-fortified blanks were analysed every 10 samples in order to evaluate the performance of the analysis over time. Those laboratory-fortified blanks consisted of a standard solution of 1 µg L^{-1} prepared by the dilution of a multistandard solution different from that used for instrument calibration.

Exploratory multivariate data analysis

Experimental data were analysed using a 5-way principal component analysis (5W-PCA) by means of the Tucker-3 algorithm (Smilde et al., 2004), as an unsupervised exploratory tool for the visual identification of relationships within the five investigated modes (matrices, water layers, seasons, rivers and variables) (Oliveri et al., 2020). A Log-transform was applied to all the variables to normalise their frequency distribution. Subsequently, column autoscaling (j-scaling) (Smilde et al., 2004) was applied to data matrices in order to minimise systematic differences between the experimental variables (Oliveri et al., 2019).

Results and discussion

Water column characteristics and physico-chemical boundary conditions

River discharge was found to be notably higher at the TV River mouth (40.0, 18.3 and 17.3 $m^3 s^{-1}$ during winter, summer and autumn sampling campaigns, respectively) rather than at the estuarine systems of the RZ (0.43, 0.23 and 6.55 $m^3 s^{-1}$ during the winter, summer and autumn sampling campaigns, respectively) and DG (2.65, 0.33 and 0.64 $m^3 s^{-1}$ during the winter, summer and autumn sampling campaigns, respectively). The water column stratification was found to vary among different sampling sites and seasons, although the salt-wedge intrusion can be clearly identified (Figure 2).

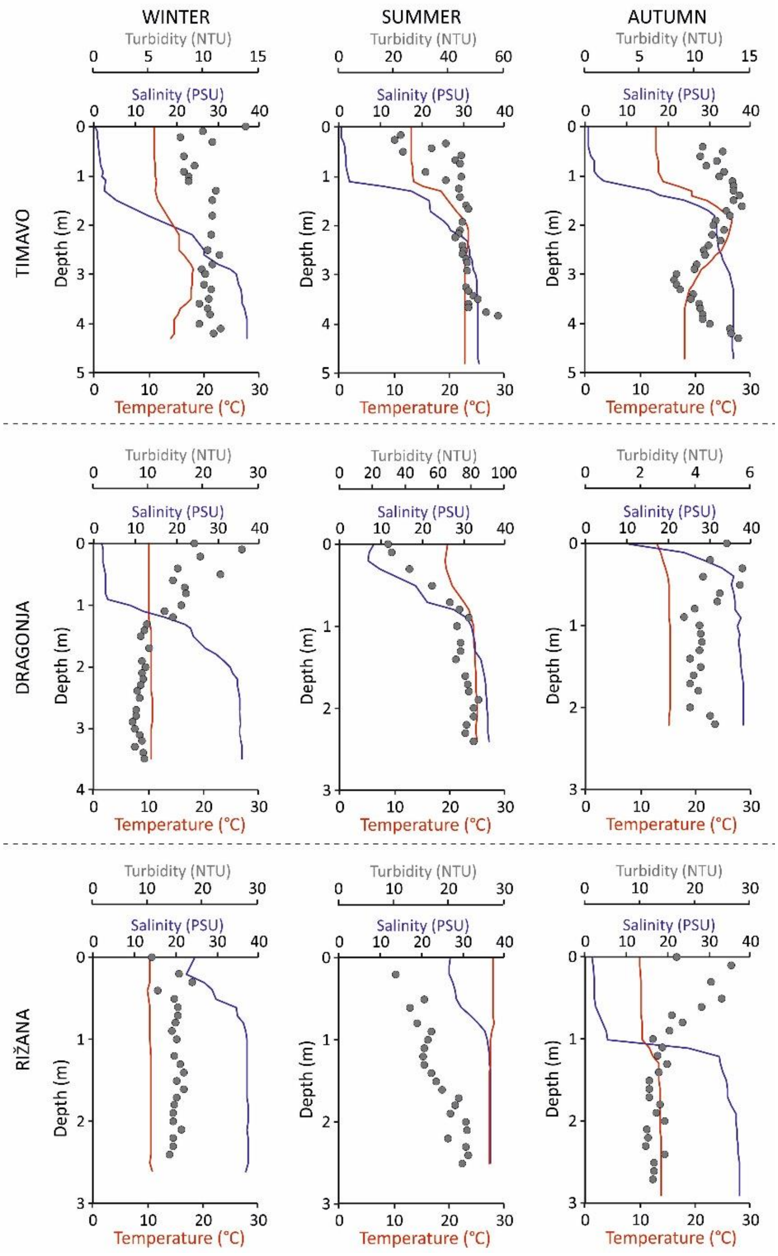


Figure 2. Vertical profiles of salinity (PSU, Practical Salinity Unit), temperature (°C) and turbidity (NTU, Nephelometric Turbidity Unit) recorded at the Timavo (TV), Dragonja (DG) and Rižana (RZ) River mouths under different seasonal conditions.

Indeed, salinity vertical profiles displayed a sharp halocline at the TV River mouth, attesting to the evident and permanent salt-wedge intrusion under different seasonal conditions. The surface water layer was dominated by the river freshwater (<10 PSU), whereas salinity values were typical of seawater at the bottom (37.1, 34.0 and 32.4 PSU during winter, summer and autumn, respectively). Results were similar for the mouth of the DG River, where a strong stratification occurred during the year, although the surface freshwater layer demonstrated varied thicknesses among different seasons (Figure 2). The freshwater contribution was clearly evident in winter, followed by summer, when an increasing mixing occurred, and autumn, when the river freshwater was confined to the first 20 cm of the water column. The strongest salt-wedge intrusion was found at the RZ River mouth where the brackish water was found in the surface layer of the water column during both winter (23.8 PSU) and summer (27.2 PSU). River freshwater (1.80 PSU) prevailed in the surface water layer up to a depth of 1 m only in autumn.

Turbidity did not display significant differences with depth in winter and autumn, whereas a slight increase in turbidity values was evident in summer at both TV (22.6 and 55.7 NTU in the surface and bottom water layers, respectively) and RZ River mouths (14.0 and 30.0 NTU in the surface and bottom water layers, respectively). The highest turbidity values were measured at the DG river mouth, especially in summer when turbidity gradually increased with depth (30.5 and 79.6 NTU in the surface and bottom water layers, respectively). Indeed, since the DG estuarine system is very shallow, it is often exposed to resuspension events followed by re-deposition of fine resuspended particles to the estuarine sediments.

The highest differences in temperature were found at the TV River estuarine system, where warm water was recorded in the mixing and bottom layers due to the discharge of cooling water from a thermal plant, as previously reported in Pavoni et al. (submitted (b)). The increase in temperature was notably evident among different seasons, reaching a maximum value of 27.0 °C in the mixing layer (approximately 2 m depth) in autumn. On the contrary, slight variations were observed at the DG and RZ estuarine systems, where the temperature was rather homogeneous with increasing depth in winter (10.5 and 10.4 °C at the DG and RZ River mouths, respectively) and autumn (15.1 and 12.6 °C at the DG and RZ River mouths, respectively), whereas higher values were measured in summer, as expected. This is especially evident at the RZ River mouth where the shallow water depth and scarce water circulation led to a temperature equal to approximately 28 °C.

Significant disparities in terms of pH were not found among the sampling sites and among different seasons, although the bottom water layer generally showed slightly higher pH values (Table S1).

The redox potential testified to oxidative conditions in the brackish water and slightly lower values were generally found at the bottom. The only exception was the RZ estuarine system which showed reductive conditions in the whole water column in winter (Eh = -36 and -139 mV in the brackish and marine water samples, respectively) and autumn (Eh = -28 and -72 mV in the brackish and marine water samples, respectively), whereas slightly higher Eh values were found in summer (Eh = 21 and 31 mV in the brackish and marine water samples, respectively).

Trace elements in the suspended particulate matter

At the three investigated estuarine systems, the brackish water layer generally displayed high concentrations of particulate trace elements, whereas lower values were found in the marine bottom water (Table S2) likely due to dilution effects and mixing processes between freshwater and seawater (Turner, 1999; Hatje et al., 2001). This trend of general dilution was evident for most of the investigated trace elements at the TV, RZ and DG River mouths in winter and autumn, whereas minor differences between brackish and marine water were found in summer.

The highest concentrations of particulate trace elements were found at the RZ River mouth, especially in the brackish water in autumn, followed by the TV estuarine system where the maximum values of Cu (399 µg g^{-1} in the brackish water in autumn) and Pb (6237 µg g^{-1} in the brackish water in summer) were detected. Conversely, the estuarine system of the DG River showed lower concentrations, especially in the marine water in the summer season (Table S2).

Particulate Mn was found to be notably high in summer at the three investigated estuarine systems (1182, 1839 and 1074 µg g^{-1} at the TV, RZ and DG River mouths, respectively) compared to the concentrations measured in the brackish water in winter (586, 473 and 744 µg g^{-1} at the TV, RZ and DG River mouths, respectively) and autumn (551, 1061 and 180 µg g^{-1} at the TV, RZ and DG River mouths, respectively). As reported in other studies (Morris et al., 1982; Sondi et al., 1994; Hatje et al., 2001; Pavoni et al., submitted (a)), considerably high amounts of particulate Mn in summer could be due to oxidation and precipitation processes promoted by high water temperatures.Furthermore, the concentrations of particulate Mn were generally found to be of the same order of magnitude as those reported for other estuarine systems of the northern Adriatic Sea, such as the Isonzo River (Pavoni et al., submitted (a)), the Po River (Camusso et al., 1997) and the Raša River (Sondi et al., 1994).

Moreover, the redox behaviour of Mn and Fe are strongly linked in natural aquatic systems where Fe and Mn oxy-hydroxides are important host phases for adsorption processes due to their high adsorptive capacities (Tankéré et al., 2000b; Gagnon and Saulnier, 2003). For these reasons, the Fe/Mn ratio represents a useful index to understand their relative prevalence under different seasonal conditions. In this study, this ratio was noticeably high in winter and autumn for the three investigated estuarine systems, varying collectively between 38.7 and 160 in the brackish water and between 42.2 and 117 at the bottom. Conversely, lower values were found both in the brackish water (15.6, 11.1 and 16.5 at the TV, RZ and DG River mouths, respectively) and at the bottom (18.9, 23.3, 11.8 at the TV, RZ and DG River mouths, respectively) in summer, thus revealing the concomitant decrease in particulate Fe and increase in particulate Mn.

Particulate Hg was mainly below LOD, except for the TV River mouth, where Hg was high in the brackish water (1.33 - 9.79 µg g^{-1}) and low at the bottom (0.90 – 1.17 µg g^{-1}). The occurrence of Hg at the TV River mouth has previously been reported (Pavoni et al., submitted (b)) and could be explained by the role of the Isonzo River, which is known to be the main vehicle for particulate Hg entering the Gulf of Trieste (Covelli et al., 2007). Indeed, stream losses from the IS River may act as a groundwater source for the unconfined aquifer of the IS River alluvial plain (Cerovac et al., 2018) and mixing processes between the two types of fluvial waters could occur, especially under conditions of low discharge from the IS River (Calligaris et al., 2018).

In addition, Cr has previously been reported at high levels in the bottom sediments of the Gulf of Trieste (Faganeli et al., 1991; Covelli and Fontolan, 1997), suggesting that river inputs may act as the source of this element. Indeed, high concentrations of particulate Cr were found at the TV (45.4-137 µg g^{-1}), RZ (66.4-288 µg g^{-1}) and DG (30.5-153 µg g^{-1}) River mouths. The same was true for particulate Ni, which ranged between 27.9 and 132 µg g^{-1}, between 45.2 and 161 µg g^{-1} and between 22.5 and 121 µg g^{-1} at the TV, RZ and DG estuarine systems. Indeed, Cr and Ni are lithogenic and their origin is due to runoff and erosion of the flysch formation outcropping in the continental area (Lenaz and Princivalle, 1996; Zupančič and Skobe, 2014). Moreover, particulate Cr was generally higher in the brackish water (114-137 µg g^{-1} at the TV River mouth, 97.3-288 µg g^{-1} at the RZ River mouth and 54.8-153 µg g^{-1} at the DG River mouth) and comparable to results reported by Pavoni et al. (submitted (a)) for the IS estuarine system where the concentration of Cr ranged between 33.3 and 128 µg g^{-1}. The same is also true for particulate Ni, the concentrations of which in the suspended particles were found to be higher in the brackish water (73.8-132 µg g^{-1}, 98.6-161 µg g^{-1} and 44.1-121 µg g^{-1} at the TV, RZ and DG River mouths, respectively) and comparable to other estuarine environments of the northern Adriatic Sea (Juračić and Pravdić, 1991; Pavoni et al., submitted (a)).

The common lithogenic origin of Cr and Ni was also confirmed by the strong correlation ($r=0.883$, $N=18$, $p<0.001$) between these two elements in the brackish and marine water at the three investigated estuarine systems (Figure 3). Moreover, equally significant correlations were also found between Ni, Cr and Co ($r=0.905$, $N=18$, $p<0.001$ and 0.861, $N=18$, $p<0.001$ for Cr and Ni, respectively) since Co could also derive from alteration processes of flysch terrains (Frančišković-Bilinski et al., 2014) (Figure 3).

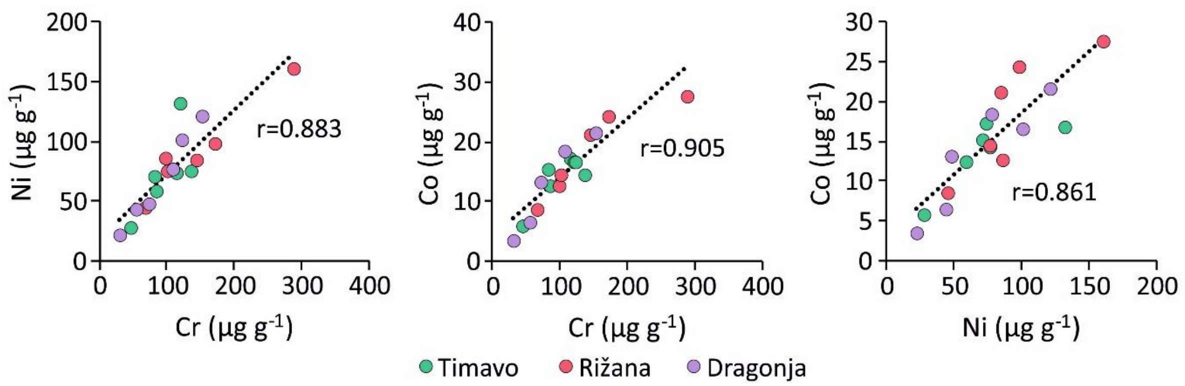


Figure 3. Correlations between Cr, Ni and Co (µg g^{-1}) in the SPM isolated from estuarine water samples collected at the Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Trace element partitioning between solid and dissolved phases

The partitioning between solid and dissolved phases is essential for the evaluation of trace element fluxes carried by riverine freshwater to estuarine environments (e.g. Morris et al., 1982; La Colla et al., 2015; Wang and Wang, 2016). A 5W-PCA was employed for the evaluation of trace element partitioning between the solid phase, represented by the SPM and colloidal material, and the truly dissolved fraction (Tables S3 and S4), depicting relations and differences between sampling sites, water masses and seasons. Although more detailed information about trace element phase partitioning and mixing behaviour for the IS River has previously been reported elsewhere (Pavoni et al., submitted (a)), it was decided to include those results in the multi-way model since the IS River represents the main freshwater input entering the Gulf of Trieste.

The 5W-PCA was executed after data normalisation and column autoscaling (j-scaling), and 72.4% of the total variance was explained by Factor 1 and Factor 2 (Figure 4).

In addition, distribution coefficients (K_D) (Turner, 1996; EPA, 1999; Gagnon and Saulnier, 2003; Guéguen and Dominik, 2003; Wang and Wang, 2016) and the relative percentages of As, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb and Zn in the SPM and in the truly dissolved fraction are reported in Table S5.

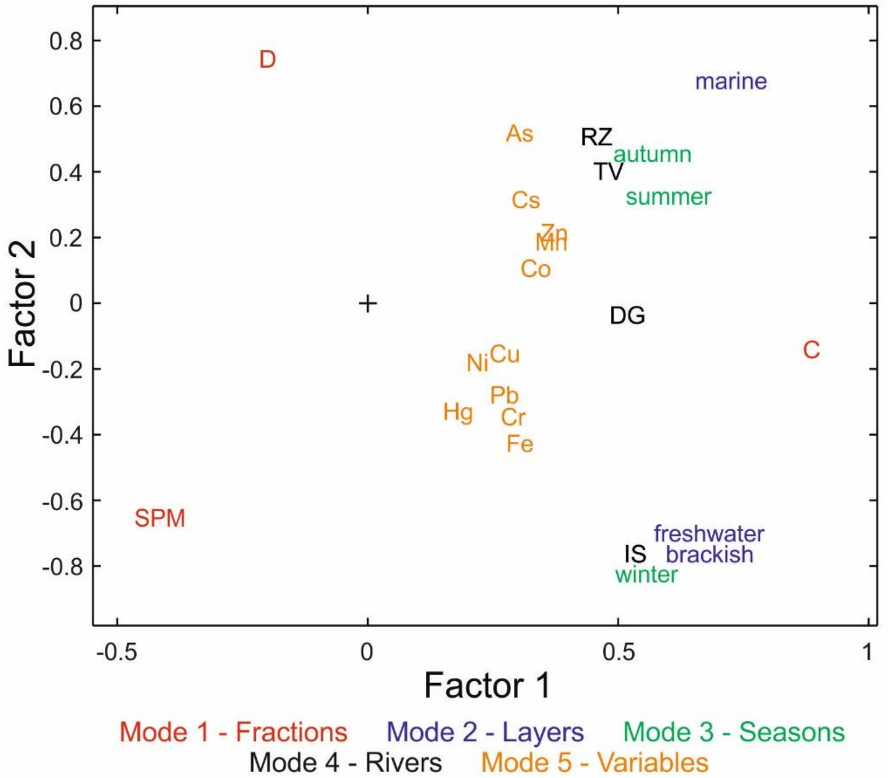


Figure 4. Plot resuming the results of 5W-PCA performed on the trace element phase partitioning among suspended particulate matter (SPM), colloidal material (C) and the truly dissolved fraction (D) in the estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

The 5W-PCA clearly denotes the difference between the three sample fractions (SPM, colloids and truly dissolved) and between the brackish and marine water. Moreover, the distribution of trace elements in the plot (Figure 4) appears to be governed by their partitioning behaviour. In fact, trace elements found to be mainly associated with the solid phase (Fe, Hg, Cr, Pb, Ni and Cu) were clearly distinguished from those prevailing in dissolved forms (As and Cs). The SPM is known to play a crucial role as the main vehicle for several trace elements from continental areas to coastal environments (Turner and Millward, 2000; Vignati and Dominik, 2003; Beck et al., 2013; Helali et al., 2016). This is notably evident for Fe, Pb and Cr which are almost completely partitioned in the solid phase, as seen by their elevated percentages in the SPM (>90% for Fe, between 71.3 and 99.4 % for Pb and from 54.1 to 98.5% for Cr). This was also confirmed by the K_D values which were found to be high for Fe and Pb, followed by Cr, Cu and Ni, reflecting their high affinity to the suspended particles. According to Benoit et al. (1994), Pb is highly particle reactive and rapid adsorption onto the suspended particles, as well, removal processes such as co-precipitation with Fe oxy-hydroxides may easily occur in the estuarine mixing zone (Fu et al., 2013; Fiket et al., 2018). Conversely, lower values were found for As and Cs, which are generally present under dissolved ionic forms in uncontaminated aquatic systems (Smedley and Kinniburgh, 2002).

The 5W-PCA output clearly demonstrated that the SPM was the dominant matrix for Fe, Cr and Pb, especially in the brackish water in winter. Indeed, the highest concentrations of particulate Fe were found in the river freshwater and in the brackish water at the IS (254 µg L^{-1}) (Pavoni et al., submitted (a)) and DG River (281 µg L^{-1}) mouths, whereas dissolved Fe was found to be below LOD in winter as evidenced by the fact that the truly dissolved fraction is plotted on the opposite side in the 5W-PCA output. This is similar to Cr and Pb, which were found to be mainly below LOD in the truly dissolved fraction. Furthermore, slightly higher concentrations of dissolved Fe were found in the Zrmanja River (Croatia) (4.71-10.0 µg L^{-1}, Fiket et al., 2018), in the Bay of Piran (12.5 µg L^{-1}) and in the IS River freshwater (42.0 µg L^{-1}) (Klun et al., 2019) whereas comparable concentrations were reported by Zago et al. (2002) for the Po River (<LOD-0.20 µg L^{-1}). Regarding dissolved Cr, the results were found to be of the same order of magnitude as those measured at the Zrmanja River (0.58-1.18 µg L^{-1}, Fiket et al., 2018) and at the IS River (0.62 µg L^{-1}, Klun et al., 2019).

With the only exception being at the mouth of the IS River, dissolved Hg was mainly below LOD and primarily associated with the solid phase confirming the role of the IS River as the main source of particulate Hg in the Gulf of Trieste (Covelli et al., 2007). Indeed, the relationship between particulate Hg and the IS River is clearly evident in the 5W-PCA output, from their closeness in the plot of Figure 4, since the maximum concentrations of particulate Hg were reached at the IS River mouth in winter, both in the river freshwater (105 ng L^{-1}) and at the bottom (57.3 ng L^{-1}) (Pavoni et al., submitted (a)).

To a lesser extent, the solid phase was found to be the dominant fraction in the case of Cu and Ni, which appeared to be strongly associated with the suspended particles in winter, especially in the brackish water of the DG River (98.9 and 98.8% for Cu and Ni, respectively). Conversely, the dissolved Cr and Ni were below the LOD in winter and generally prevailed at the bottom (71.0, 51.1 and 72.2% of Cu at the TV, RZ and DG River mouths) in summer (Figure 4).

Arsenic and Cs appeared to be almost completely partitioned in the truly dissolved phase, showing Log K_D values ranging between 3.16 and 4.33 and between 3.94 and 4.96 for As and Cs, respectively. Both elements were found to be higher in the marine water, especially in summer and autumn, as indicated by the 5W-PCA output (Figure 4). In addition, As and Cs are plotted on the opposite side with respect to the SPM (Figure 4) suggesting that a weak relationship occurred between these elements and the solid phase. Indeed, As and Cs showed low concentrations in the solid phase compared to the truly dissolved fraction, especially at the TV and RZ River mouths.

The partitioning behaviour described for As and Cs and their occurrence in the dissolved phase is in agreement with other estuarine environments of the northern Adriatic Sea such as the Zrmanja River (Fiket et al., 2018) and the IS River (Klun et al., 2019; Pavoni et al., submitted (a)).

Moreover, dissolution and desorption processes can favour the increase of dissolved As (Smedley and Kinniburgh, 2002), especially in estuarine and marine environments where high concentration of salts in the water can weaken adsorption on the surface of the suspended particles (Hong et al., 2018).

Major differences in the partitioning between solid and dissolved phases were found for Co, Zn and Mn, as confirmed by Log K_D values ranging between 4.75 and 5.57 for Co, between 4.12 and 6.79 for Zn and between 4.34 and 5.28 for Mn (Table S5). According to the 5W-PCA output, a strong relationship is evident between Mn and Zn in summer (Figure 4). Indeed, dissolved Mn and Zn were generally found to be higher both in the brackish water (12.8, 30.4 and 19.8 µg L^{-1} for the TV, RZ and DG Rivers, respectively) and at the bottom (14.2, 11.8 and 20.4 µg L^{-1} for the TV, RZ and DG Rivers, respectively) in summer.

Trace elements in the colloidal material

The concentrations of trace elements in the colloidal material were notably lower than those measured in the SPM and in the truly dissolved fraction (Tables S3 and S4) and in agreement with the results obtained at the IS River mouth (Pavoni et al., submitted (a)). Moreover, trace element contents in the colloidal material was found to be generally low if compared to other studies focused on river and estuarine systems (e.g. Sañudo-Wilhelmy et al., 1996; Jaïry et al., 1999; Guéguen and Dominik, 2003; Ingri et al., 2004).

The highest percentages bound to colloids were reached by those elements (Cr, Cu, Fe, Ni and Pb) that showed relatively low concentrations in the truly dissolved fraction (Table S6). Coagulation and/or flocculation processes (Kraepiel et al., 1997; Fu et al., 2013) could be responsible for increasing colloidal Fe in the brackish water. This is particularly evident at the DG River mouth where 887 and 421 ng L^{-1} of colloidal Fe were detected in summer and autumn, respectively (Figure 5).

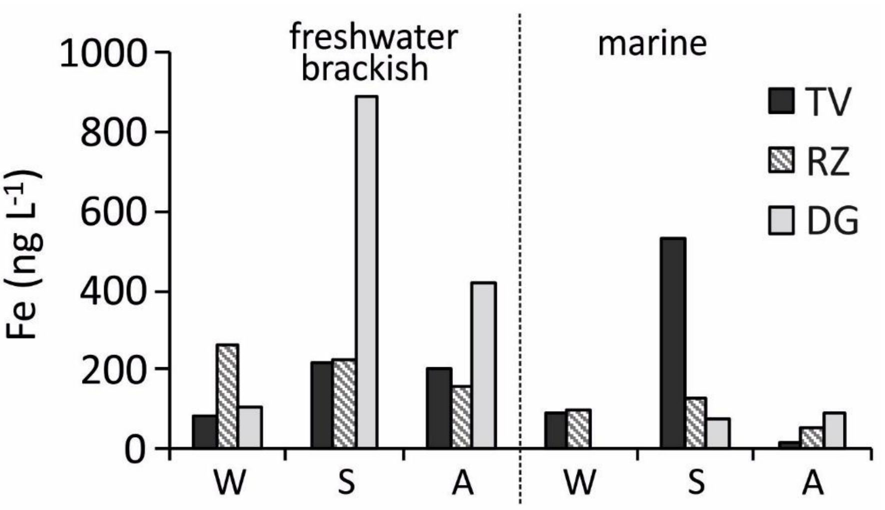


Figure 5. Concentration of colloidal Fe (ng L^{-1}*) in the estuarine water samples collected at the Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (W=winter, S=summer, A=autumn).*

The same is verified for the TV River mouth, where colloidal Fe prevailed both in the brackish water (219 ng L^{-1}) and at the bottom (527 ng L^{-1}) in summer. Furthermore, colloidal Fe was found to be of the same order of magnitude as the results for the Bay of Piran (424 ng L^{-1}, Klun et al., 2019) and for the IS River mouth (196 ng L^{-1}, Pavoni et al., submitted (a)), and lower if compared to water samples from the Lagoon of Venice, where colloidal Fe, Mn, Ni and Pb were found to be notably present, especially in the surface freshwater (Martin et al., 1995). Indeed, the higher amounts of organic matter that generally occur in lagoon aquatic systems could favour colloid aggregation with subsequent high amounts of trace elements in the colloidal material.

Colloidal Cu, Ni and Cr prevailed at the TV River mouth followed by the RZ River mouth, whereas lower contents were found at the estuarine system of the DG River (Figure 6).

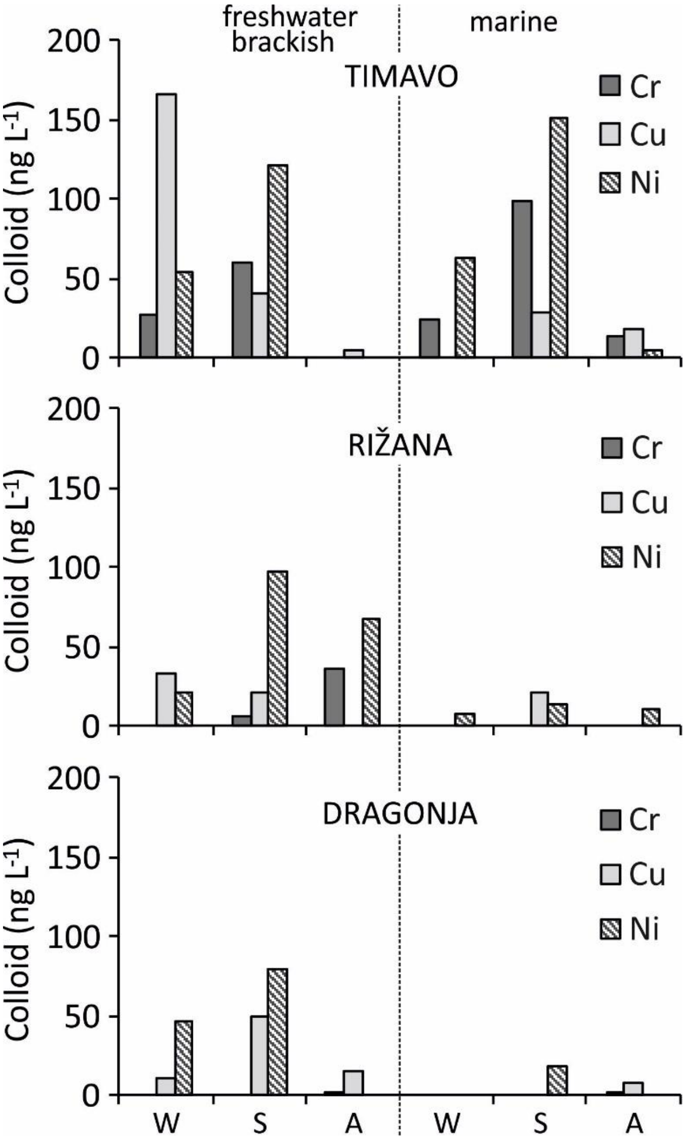


Figure 6. Concentration of colloidal Cr, Cu and Ni (ng L^{-1}) in the estuarine water samples collected at the Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (W=winter, S=summer, A=autumn).

Although colloidal Cu reached low concentrations compared to those of colloidal Fe, it was demonstrated that Cu plays an important role in the chemistry of Fe especially regarding oxidation and precipitation processes (González et al., 2016). In this context, colloidal Cu appeared to follow the behaviour of colloidal Fe at the DG estuarine system where both elements increased in the brackish water in summer (50.2 and 887 ng L^{-1} of Cu and Fe, respectively) becoming undetectable or minimally present in the bottom marine water. The same is also true for the RZ River mouth whereas, although colloidal Cu clearly prevailed in the brackish water, the maximum concentration (166 ng L^{-1}) was found at the mouth of the TV River in winter.

Despite colloidal Cr occasionally not being detectable in the marine water of the DG and RZ Rivers, extremely similar behaviour was found for Cr and Ni, which clearly prevailed both in the brackish water and at the bottom in summer, especially at the TV River mouth (60.2 and 99.0 ng

L^{-1} of Cr and 122 and 151 ng L^{-1} of Ni). As previously mentioned, the common lithogenic origin of Cr and Ni (Lenaz and Princivalle, 1996; Zupančič and Skobe, 2014) could explain the strong correlation in the suspended particles, whereas the concentrations in the truly dissolved fraction were generally low and often below LOD. Moreover, their preferential partitioning in the solid phase was also notably evident in the colloidal material, especially at the TV River mouth ($r=0.944$, $N=5$, $0.01<p<0.05$) (Figure 7). In addition, strong correlations were also found between colloidal Ni, Cr and Fe ($r=0.905$, $N=5$, $0.01<P<0.05$ and 0.986, $N=5$, $p<0.001$ for Ni and Cr, respectively) and Mn ($r=0.902$, $N=5$, $0.01<P<0.05$ and 0.794, $N=5$, $p<0.05$ for Ni and Cr, respectively) (Figure 7).

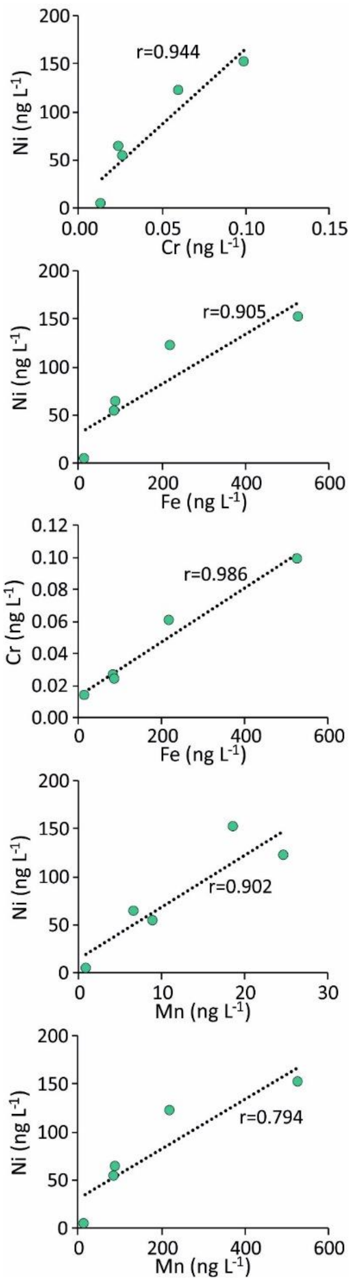


Figure 7. Correlations between Cr, Ni, Fe and Mn (µg L^{-1}) in the colloidal material in the estuarine water samples collected at the Timavo (TV) River mouth under different seasonal conditions.

Conclusions

Trace element phase partitioning among different fractions was evaluated by means of a multi-way principal component analysis, which was confirmed as an effective chemometric tool for depicting disparities among different freshwater inputs and estuarine systems. The suspended particulate matter played a crucial role in regulating trace element distribution and was the main carrier from terrestrial sources towards marine coastal environments of the Gulf of Trieste. Indeed, among the investigated trace elements, Cu, Cr, Fe, Hg, Ni and Pb appeared to be mainly associated with the suspended particles and showed a high affinity for the colloidal material.

A common lithogenic origin was ascertained for particulate Cr and Ni, which derive from alteration processes and runoff from flysch terrains outcropping in the continental area. Conversely, the truly dissolved fraction was identified as the dominant phase for As and Cs, especially in the marine water, whereas major differences in the partitioning behaviour were observed for Co, Mn and Zn.

Generally, colloidal Cr, Cu and Ni prevailed at the Timavo River mouth, where elevated average percentages of Cr (40.3%), Cu (21.0%) and Ni (25.7%) were found. However, compared to the suspended particulate matter and the truly dissolved fraction, colloids were scarcely present at the investigated river mouths, where the hydrodynamic conditions presumably inhibit aggregation processes involving very fine particles. Results suggested that the colloidal material played a minor role in regulating trace element partitioning at the river mouths of the Gulf of Trieste.

Future research should address to investigate the interaction of dissolved and colloidal organic matter with trace elements in estuarine environments and in the nearby coastal lagoons, which are highly productive and organic-rich water systems.

Acknowledgements

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Supplementary material

Table S1. Redox potential (Eh, mV) and pH in the estuarine water samples collected at the estuarine systems of the Timavo (TV), the Rižana (RZ) and Dragonja (DG) Rivers under different seasonal conditions.

	River	TV		RZ		DG	
	Water sample	F-B	M	F-B	M	F-B	M
Eh	winter	80.0	55.0	-36	-139	82.0	50.0
	summer	81.0	55.0	21	31	76.0	64.0
	autumn	178	167	-28	-72	130	117
pH	winter	7.99	8.36	8.09	8.09	8.24	8.51
	summer	8.31	8.52	8.26	8.52	8.16	8.53
	autumn	7.53	7.90	7.70	7.73	7.78	8.29

F-B=freshwater-brackish; M=marine

Table S2. Concentration of Al, Fe (%), As, Co, Cr, Cs, Cu, Hg, Mn, Ni, Pb and Zn (µg g^{-1}) in the suspended particulate matter collected at the estuarine systems of the Timavo (TV), the Rižana (RS) and Dragonja (DG) Rivers under different seasonal conditions (<LOD: below the detection limit).

	River	TV		RS		DG	
	Water sample	F-B	M	F-B	M	F-B	M
Al	winter	4.20	3.92	2.80	1.75	4.06	2.31
	summer	2.58	2.92	1.76	2.57	2.39	1.97
	autumn	3.54	2.09	5.78	3.23	4.26	1.68
As	winter	15.8	13.5	21.0	15.1	12.2	9.51
	summer	15.1	14.9	11.9	12.4	18.0	16.8
	autumn	18.9	11.7	20.3	15.6	3.03	2.44
Co	winter	17.3	12.6	24.4	8.63	18.4	13.2
	summer	16.8	15.3	12.7	14.6	21.7	16.6
	autumn	14.4	5.94	27.6	21.3	6.57	3.46
Cr	winter	114	84.6	172	66.4	108	72.1
	summer	119	81.6	97.3	101	153	123
	autumn	137	45.4	288	145	54.8	30.5
Cs	winter	5.21	4.48	3.59	2.21	6.01	3.71
	summer	3.51	3.82	2.51	3.55	8.01	6.68
	autumn	5.99	2.19	6.82	3.89	4.17	1.74
Cu	winter	77.4	56.1	262	127	72.4	54.5
	summer	128	76.6	126	109	66.7	42.8
	autumn	399	161	137	86.8	63.7	68.2
Fe	winter	2.50	2.21	2.54	1.48	2.93	1.59
	summer	1.84	2.21	2.04	1.96	1.77	1.38
	autumn	3.26	2.41	4.10	2.36	2.87	1.15
Hg	winter	1.33	0.90	<LOD	<LOD	<LOD	<LOD
	summer	1.35	1.11	<LOD	<LOD	<LOD	<LOD
	autumn	9.79	1.17	<LOD	<LOD	<LOD	<LOD
Mn	winter	586	437	473	205	744	354
	summer	1182	1167	1839	843	1074	1167
	autumn	551	205	1061	559	180	138
Ni	winter	73.8	58.9	98.6	45.2	78.0	47.7
	summer	132	70.6	86.5	75.9	121	101
	autumn	76.0	27.9	161	84.4	44.1	22.5
Pb	winter	1035	80.7	449	29.6	151	111
	summer	6237	122	57.3	154	98.3	57.3
	autumn	3918	86.3	1802	61.5	27.9	27.1
Zn	winter	241	143	1179	2360	264	132
	summer	386	296	1066	561	238	228
	autumn	875	238	465	459	150	93.5

F-B=freshwater-brackish; M=marine

Table S3. Concentrations of As, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb, Zn ($\mu g\ L^{-1}$) and Hg ($ng\ L^{-1}$) in the suspended particulate matter (SPM) and in the truly dissolved fraction (D) collected at the estuarine systems of the Timavo (TV), the Rižana (RS) and Dragonja (DG) Rivers under different seasonal conditions (<LOD: below the detection limit).

River		TV				RS				DG			
Water sample		F-B		M		F-B		M		F-B		M	
		SPM	D	SPM	D	SPM	D	SPM	D	SPM	D	SPM	D
	winter	0.10	1.36	0.14	1.81	0.06	1.01	0.11	1.67	0.12	0.93	0.09	1.86
As	summer	0.08	1.50	0.14	1.98	0.09	1.55	0.12	2.46	0.16	1.15	0.40	1.99
	autumn	0.07	0.89	0.10	1.26	0.12	1.24	0.11	1.69	0.02	1.22	0.03	1.69
	winter	0.11	0.11	0.13	0.06	0.07	0.10	0.06	0.10	0.18	0.10	0.13	0.10
Co	summer	0.09	0.07	0.14	0.08	0.09	0.23	0.14	0.16	0.19	0.10	0.39	0.14
	autumn	0.05	0.04	0.05	0.06	0.17	0.11	0.15	0.10	0.05	0.10	0.04	0.05
	winter	0.71	<LOD	0.88	<LOD	0.49	<LOD	0.50	<LOD	1.03	<LOD	0.69	<LOD
Cr	summer	0.63	<LOD	0.77	<LOD	0.70	0.11	0.98	<LOD	1.35	<LOD	2.90	<LOD
	autumn	0.50	0.43	0.39	0.28	1.76	<LOD	1.01	<LOD	0.41	0.25	0.38	0.11
	winter	0.03	0.14	0.05	0.17	0.01	0.10	0.02	0.17	0.06	0.08	0.04	0.19
Cs	summer	0.02	0.14	0.04	0.18	0.02	0.15	0.03	0.20	0.07	0.09	0.16	0.18
	autumn	0.02	0.08	0.02	0.14	0.04	0.12	0.03	0.17	0.03	0.11	0.02	0.20
	winter	0.81	<LOD	0.61	<LOD	0.75	0.93	0.95	<LOD	1.09	<LOD	0.66	<LOD
Cu	summer	0.67	0.62	0.72	1.77	0.90	<LOD	1.07	1.11	0.59	1.75	1.01	2.62
	autumn	1.47	0.69	1.39	0.61	0.84	<LOD	0.60	<LOD	0.48	0.58	0.85	0.16
	winter	158	<LOD	234	<LOD	72.2	2.80	110	3.12	281	<LOD	151	8.68
Fe	summer	96.9	1.70	208	<LOD	147	6.14	191	0.73	156	<LOD	326	1.54
	autumn	120	3.91	207	4.79	251	2.14	164	3.30	215	12.1	145	0.93
	winter	8.28	<LOD	9.32	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Hg	summer	7.07	<LOD	10.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	autumn	36.0	<LOD	10.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	winter	3.64	4.42	4.54	5.51	1.35	9.80	1.53	8.11	7.12	4.98	3.36	4.58
Mn	summer	6.21	12.8	11.0	14.2	13.2	30.4	8.21	11.8	9.49	19.8	27.5	20.4
	autumn	2.03	2.87	1.77	2.95	6.49	6.27	3.88	9.18	1.35	8.31	1.72	1.36
	winter	0.46	0.45	0.61	0.58	0.28	0.55	0.34	0.58	0.75	<LOD	0.45	2.61
Ni	summer	0.69	0.86	0.67	<LOD	0.62	3.20	0.74	0.29	1.07	0.01	2.38	0.29
	autumn	0.28	<LOD	0.24	0.50	0.98	0.46	0.59	0.71	0.33	<LOD	0.28	<LOD
	winter	6.43	1.85	0.84	<LOD	1.28	<LOD	0.22	<LOD	1.45	<LOD	1.05	0.11
Pb	summer	32.8	9.06	1.15	0.46	0.41	<LOD	1.50	0.28	0.87	0.08	1.35	0.25
	autumn	14.4	2.95	0.74	<LOD	11.0	1.67	0.43	<LOD	0.21	<LOD	0.34	<LOD
	winter	1.50	13.5	1.48	0.47	3.35	11.2	17.7	4.65	2.52	8.46	1.25	<LOD
Zn	summer	2.03	7.43	2.79	19.1	7.67	15.7	5.47	21.3	2.10	6.88	5.39	17.3
	autumn	3.22	<LOD	2.05	14.6	2.84	7.11	3.19	11.9	1.12	<LOD	1.17	<LOD

F-B=freshwater-brackish; M=marine

Table S4. Concentrations of colloidal As, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb and Zn (ng L^{-1}) in estuarine water samples collected at the estuarine systems of the Timavo (TV), the Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (nd: not detected).

	River	TV		RS		DG	
	Water sample	F-B	M	F-B	M	F-B	M
	winter	2.52	1.11	3.78	5.73	2.49	nd
As	summer	nd	2.40	2.34	nd	2.21	3.07
	autumn	nd	6.44	1.27	1.82	nd	6.46
	winter	0.30	1.24	0.75	0.60	3.31	0.04
Co	summer	0.99	1.20	1.31	0.78	1.12	0.58
	autumn	nd	0.66	nd	0.45	nd	0.10
	winter	26.6	24.1	nd	nd	nd	nd
Cr	summer	60.2	99.0	6.95	nd	nd	nd
	autumn	nd	13.6	36.6	nd	0.96	0.59
	winter	nd	0.25	1.67	2.89	nd	nd
Cs	summer	0.07	nd	2.48	3.42	0.03	nd
	autumn	nd	0.79	2.04	2.93	nd	nd
	winter	166	nd	33.6	nd	10.5	nd
Cu	summer	40.9	29.2	21.0	21.9	50.2	nd
	autumn	4.72	18.0	nd	nd	15.0	7.48
	winter	85.2	89.3	262	101	103	nd
Fe	summer	219	527	224	124	887	77.2
	autumn	206	15.7	159	50.2	421	88.2
	winter	nd	0.20	nd	nd	nd	nd
Hg	summer	0.43	0.05	nd	nd	nd	nd
	autumn	0.10	nd	nd	nd	nd	nd
	winter	9.04	6.63	5.17	nd	4.87	nd
Mn	summer	24.8	18.7	17.1	11.6	21.6	53.5
	autumn	nd	0.88	3.59	3.52	nd	nd
	winter	54.6	63.8	21.8	8.42	46.2	nd
Ni	summer	122	151	97.3	14.5	80.1	18.9
	autumn	nd	4.85	67.7	10.2	nd	nd
	winter	6.61	nd	3.10	nd	11.0	nd
Pb	summer	108	8.13	6.17	5.04	3.20	0.03
	autumn	19.4	nd	51.2	nd	1.40	nd
	winter	nd	84.2	nd	16.3	12.7	nd
Zn	summer	35.2	111	61.7	48.9	18.7	nd
	autumn	nd	nd	nd	nd	nd	nd

F-B=freshwater-brackish; M=marine

Table S5. Log K_D values for As, Co, Cr, Cs, Cu, Fe, Ni, Mn, Pb and Zn and their percentages in the SPM and truly dissolved fraction in estuarine water samples collected at the estuarine systems of Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

TIMAVO RIVER

Season	WINTER						SUMMER						AUTUMN					
Water sample	freshwater - brackish			marine			freshwater - brackish			marine			freshwater - brackish			marine		
	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D
As	4.07	5.25	94.8	3.87	4.84	95.2	4.00	6.63	93.4	3.88	5.03	95.0	4.33	10.0	90.0	3.97	9.91	90.1
Co	5.18	48.5	51.5	5.32	68.4	31.6	5.35	54.1	45.9	5.27	63.7	36.3	5.57	57.6	42.4	5.01	46.9	53.1
Cr	6.40	94.0	6.02	6.27	95.1	4.91	6.42	93.2	6.77	6.25	94.4	5.57	5.51	54.1	45.9	5.21	58.0	42.0
Cs	4.57	18.8	81.2	4.42	21.4	78.6	4.40	11.6	88.4	4.32	16.4	83.6	4.90	22.5	77.5	4.19	11.7	88.3
Cu	6.83	98.6	1.41	6.69	98.1	1.86	5.32	52.3	47.7	4.64	29.0	71.0	5.76	68.0	32.0	5.42	69.5	30.5
Fe	7.72	99.8	0.20	7.67	99.7	0.30	7.03	99.8	0.23	7.67	98.3	1.73	6.92	97.7	2.26	6.70	96.8	3.16
Mn	5.12	45.1	54.9	4.90	45.2	54.8	4.96	32.6	67.4	4.92	43.7	56.3	5.28	41.4	58.6	4.84	37.5	62.5
Ni	5.21	50.3	49.7	5.00	51.2	48.8	5.19	44.8	55.2	6.89	98.7	1.35	6.92	96.8	3.15	4.75	32.5	67.5
Pb	5.75	77.6	22.4	6.95	98.9	1.07	5.84	78.3	21.7	5.42	71.3	28.7	6.12	83.0	17.0	6.98	98.8	1.21
Zn	4.25	10.0	90.0	5.48	76.0	24.0	4.72	21.5	78.5	4.19	12.7	87.3	6.79	95.8	4.19	4.21	12.3	87.7

RIZANA RIVER

Season	WINTER						SUMMER						AUTUMN					
Water sample	freshwater - brackish			marine			freshwater - brackish			marine			freshwater - brackish			marine		
	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D
As	4.32	5.59	94.4	3.95	6.31	93.7	3.88	5.24	94.8	3.70	4.68	95.3	4.21	9.10	90.9	3.96	6.02	94.0
Co	5.38	40.6	59.4	4.94	39.7	60.3	4.75	28.6	71.4	4.96	47.0	53.0	5.40	60.6	39.4	5.33	59.8	40.2
Cr	6.58	91.5	8.48	6.16	91.6	8.38	5.94	86.2	13.8	6.35	95.6	4.43	6.80	97.5	2.51	6.50	95.7	4.32
Cs	4.58	9.68	90.3	4.11	8.75	91.2	4.22	10.8	89.2	4.24	14.6	85.4	4.74	25.2	74.8	4.35	13.6	86.4
Cu	5.45	44.6	55.4	7.04	98.8	1.21	7.04	98.7	1.26	4.99	48.9	51.1	7.07	98.6	1.36	6.88	98.1	1.88
Fe	6.96	96.3	3.73	6.67	97.3	2.75	6.52	96.0	4.02	7.43	99.6	0.38	7.28	99.2	0.85	6.85	98.0	1.97
Mn	4.68	12.1	87.9	4.40	15.9	84.1	4.78	30.3	69.7	4.85	41.0	59.0	5.23	50.9	49.1	4.78	29.7	70.3
Ni	5.25	33.7	66.3	4.89	36.7	63.3	4.43	16.3	83.7	5.42	72.0	28.0	5.55	68.3	31.7	5.08	45.3	54.7
Pb	7.69	99.3	0.71	6.51	96.1	3.95	6.80	97.8	2.15	5.75	84.5	15.5	6.03	86.8	13.2	6.83	97.9	2.08
Zn	5.02	23.1	76.9	5.71	79.2	20.8	4.83	32.8	67.2	4.42	20.5	79.5	4.82	28.6	71.4	4.59	21.1	78.9

DRAGONJA RIVER																		
Season	WINTER						SUMMER						AUTUMN					
Water sample	freshwater - brackish			marine			freshwater - brackish			marine			freshwater - brackish			marine		
	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D	KD	%SPM	%D
As	4.12	11.2	88.8	3.71	4.64	95.4	4.19	12.1	87.9	3.93	16.6	83.4	3.40	1.87	98.1	3.16	1.79	98.2
Co	5.28	64.3	35.7	5.14	56.5	43.5	5.35	66.2	33.8	5.06	72.9	27.1	4.82	33.2	66.8	4.85	46.7	53.3
Cr	6.37	95.8	4.23	6.20	93.8	6.21	6.53	96.7	3.26	6.43	98.5	1.54	5.34	61.8	38.2	5.43	77.2	22.8
Cs	4.87	41.6	58.4	4.28	15.3	84.7	4.96	44.8	55.2	4.57	46.7	53.3	4.56	21.5	78.5	3.94	9.74	90.3
Cu	6.80	98.9	1.05	6.67	98.3	1.73	4.58	25.2	74.8	4.21	27.8	72.2	5.04	45.1	54.9	5.63	84.3	15.7
Fe	7.79	99.8	0.17	6.26	94.6	5.42	7.57	99.7	0.30	6.95	99.5	0.47	6.38	94.7	5.32	7.09	99.4	0.64
Mn	5.17	58.8	41.2	4.89	42.3	57.7	4.74	32.4	67.6	4.76	57.4	42.6	4.34	14.0	86.0	5.01	55.9	44.1
Ni	6.93	98.8	1.20	4.26	14.8	85.2	7.12	99.2	0.84	5.55	89.3	10.7	6.69	97.3	2.68	6.39	96.9	3.12
Pb	7.22	99.4	0.62	5.99	90.4	9.65	6.10	91.7	8.32	5.37	84.6	15.4	6.49	95.8	4.16	6.48	97.4	2.60
Zn	4.49	23.0	77.0	5.97	89.9	10.1	4.54	23.4	76.6	4.12	23.8	76.2	6.03	88.9	11.1	5.82	89.3	10.7

Table S6. Percentages of colloidally bound As, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb and Zn in estuarine water samples collected at the estuarine systems of the Timavo (TV), the Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (nd: not detected).

	River	TV		RZ		DG	
	Sample	F-B	M	F-B	M	F-B	M
As	winter	0.19	0.06	0.37	0.24	0.27	nd
	summer	nd	0.12	0.15	nd	0.19	0.15
	autumn	nd	0.51	0.10	0.11	nd	0.38
Co	winter	0.26	2.02	0.73	0.60	3.29	0.05
	summer	1.30	1.43	0.57	0.49	1.14	0.40
	autumn	nd	1.12	nd	0.45	nd	0.19
Cr	winter	36.9	34.6	nd	nd	nd	nd
	summer	57.0	68.6	5.82	nd	nd	nd
	autumn	nd	4.59	44.6	44.6	0.38	0.52
Cs	winter	nd	0.15	1.73	1.65	nd	nd
	summer	0.05	nd	1.63	1.66	0.04	nd
	autumn	nd	0.55	1.62	1.67	nd	nd
Cu	winter	93.5	nd	3.50	nd	47.7	nd
	summer	6.23	1.62	64.4	1.93	2.79	nd
	autumn	0.68	2.87	nd	nd	2.53	4.48
Fe	winter	15.3	15.9	8.56	3.12	17.9	nd
	summer	11.4	52.8	3.52	14.6	65.3	4.78
	autumn	5.00	0.33	6.90	1.50	3.36	8.65
Hg	winter	nd	56.8	nd	nd	nd	nd
	summer	74.3	24.8	nd	nd	nd	nd
	autumn	39.6	nd	nd	nd	nd	nd
Mn	winter	0.20	0.12	0.05	nd	0.10	nd
	summer	0.19	0.13	0.06	0.10	0.11	0.26
	autumn	nd	0.03	0.06	0.04	nd	nd
Ni	winter	10.7	9.85	3.79	1.42	83.6	nd
	summer	12.4	94.3	2.95	4.80	89.8	6.20
	autumn	nd	0.96	12.9	1.42	nd	nd
Pb	winter	0.36	nd	25.4	nd	54.8	nd
	summer	1.17	1.73	40.4	1.79	3.91	0.01
	autumn	0.65	nd	2.96	nd	13.4	nd
Zn	winter	nd	15.3	nd	0.35	0.15	nd
	summer	0.47	0.58	0.39	0.23	0.27	nd
	autumn	nd	nd	nd	nd	nd	nd

F-B=freshwater-brackish; M=marine

4.3 Dissolved and colloidal organic carbon and nutrients in the estuarine environments of the Gulf of Trieste (northern Adriatic Sea)

Occurrence and distribution of dissolved organic carbon and nitrogen

In marine coastal environments, dissolved organic carbon (DOC) is recognised as one of the main reservoirs of partially reactive carbon (Hopkinson et al., 1997; Zhao and Gao, 2019) and plays a crucial role in its geochemical cycle (Guo et al., 1995; Packard et al., 2000; Barrón and Duarte, 2015). Several physico-chemical and biological processes are involved in the regulation of the occurrence of DOC in estuaries, where the boundary conditions are extremely variable and can greatly affect DOC vertical distribution among different seasonal conditions (De Vittor et al., 2008; Meng et al., 2017). Moreover, the biological production and consumption of organic carbon, as well as the interaction between DOC and particulate organic carbon (POC) in the estuarine mixing zone can notably influence the final fluxes of carbon from land to coastal environments (Guo et al., 1995; Wang et al., 2012; Zhao and Gao, 2019).

Total DOC and nitrogen (TN) were measured in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (winter, summer and autumn) (Table 1).

Table 1. Concentrations of total DOC and TN (μmol L^{-1}) and DOC:TN molar ratio in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

	River	IS		TV		RZ		DG	
	Layer	F-B	M	F-B	M	F-B	M	F-B	M
DOC	winter	100	133	73.7	76.5	145	97.2	85.1	81.2
	summer	87.6	108	70.3	96.9	261	180	110	112
	autumn	105	108	72.8	91.2	83.7	80.4	149	109
TN	winter	67.4	78.7	45.8	24.4	44.4	30.9	31.2	13.2
	summer	96.1	32.9	38.0	21.3	93.1	37.3	107	31.5
	autumn	36.7	32.8	65.2	34.8	34.8	25.3	41.1	11.1
DOC:TN	winter	1.49	1.69	1.61	3.14	3.26	3.15	2.72	6.15
	summer	0.91	3.29	1.85	4.56	2.80	4.82	1.03	3.55
	autumn	2.86	3.30	1.12	2.62	2.40	3.18	3.62	9.82

F-B: freshwater-brackish; M=marine

The concentrations of DOC in the total dissolved fraction varied overall between 70.3 and 261 μmol L^{-1}, whereas TN ranged between 11.1 and 107 μmol L^{-1} and slight disparities in the DOC concentrations occurred among the investigated estuarine systems (Table 1, Figure 1).

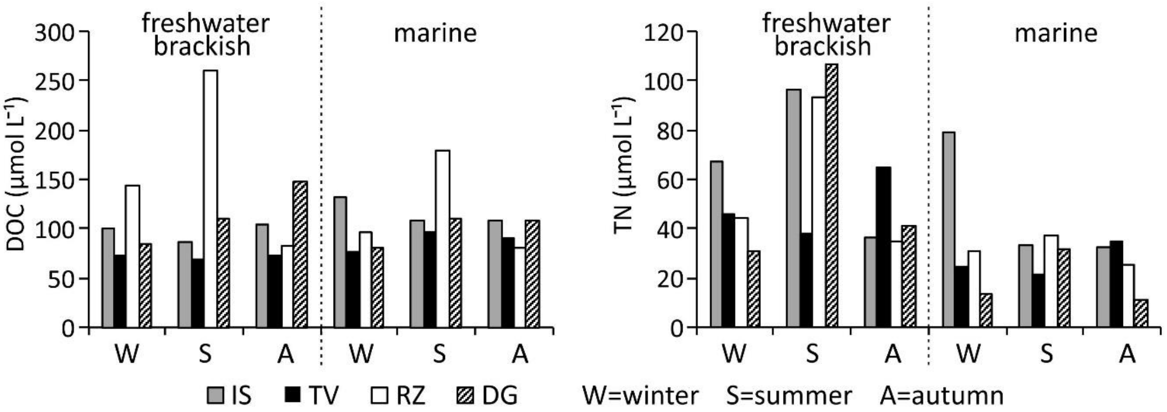

Figure 1. Concentrations of DOC and TN (µmol L^{-1}) in the total dissolved fraction of the estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Concentrations of total DOC at the IS River mouth (87.6-108 µmol L^{-1}) were generally found to be of the same order of magnitude as those measured by Klun et al. (2019) at the river mouth (66-101 µmol L^{-1}) and higher if compared to the average annual values reported for the Gulf of Trieste (ranging between 98 and 83 µmol L^{-1} from 1999 and 2003) (De Vittor et al., 2008). This was also clearly evident for the RZ and DG estuarine environments where DOC ranged between 80.4 and 261 µmol L^{-1} (RZ) and between 81.2 and 112 µmol L^{-1} (DG). Conversely, slightly lower values were measured at the TV estuarine system, where total DOC ranged from 70.3 and 96.9 µmol L^{-1}. These data are consistent with geochemical processes that often occur in the estuarine mixing zone, such as degradation of the POC along with its dissolution and/or desorption, which could be responsible for the increase in DOC in the vicinity of the river mouths (Guo et al., 2012; Barrón and Duarte, 2015; Zhao and Gao, 2019).

In the case of TN, the brackish water showed higher concentrations compared to the marine bottom water at all sites, especially in summer (Table 1, Figure 1).

Marine coastal areas are usually subjected to large freshwater inputs as a result of rainwater runoff, precipitation events and snowmelt and DOC may have a terrestrial or marine origin (Bodineau et al., 1999). The DOC:TN molar ratio is commonly employed to evaluate the origin of the organic matter (OM) which could derive from both marine production or riverine inputs. In this study, the DOC:TN molar ratio was found to be higher in the marine bottom water rather than in the freshwater and brackish water and lower than 10, attesting to the marine origins of the OM (Table 1).

Occurrence and distribution of nutrients in the truly dissolved fraction

Since shallow marine coastal areas are often the site of intense human urbanisation (de Souza Machado et al., 2016), anthropogenic activities (e.g. agriculture, aquaculture, industrialisation and so on) represents one of the main sources of nutrients in riverine freshwater systems, lagoons and estuaries (e.g Conley, 1999; Roselli et al., 2009; Acquavita et al., 2015). In these aquatic environments, conditions of excessive enrichment in nutrients (eutrophication phenomena) can easily lead to a general decline in water quality with adverse effects on the aquatic organisms (Nixon, 1995; Nixon et al., 2001; McGlathery et al., 2007; Acquavita et al., 2015). Moreover, the intense proliferation of phytoplankton biomass, and the depletion of dissolved oxygen in the bottom layer of the water column could occur (Conley, 1999). Under these conditions, recycling processes at the SWI can act as a secondary source of nutrients for the overlying water column, which may cause a further decrease in the environmental quality of the entire ecosystem (Petranich et al., 2018a).

Truly dissolved soluble reactive silicate (SRSi), nitrate (N-NO_3^-), nitrite (N-NO_2^-), ammonium (N-NH_4^+), dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) were measured in estuarine waters collected at the IS, TV, RZ and DG River mouths under different seasonal conditions (Table 2).

Table 2. Concentrations of SRSi, N-NO_3^-*, N-*NO_2^-*, N-*NH_4^+*, DIN and SRP (µmol* L^{-1}*) in estuarine water collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.*

River		IS		TV		RZ		DG	
Water sample		F-B	M	F-B	M	F-B	M	F-B	M
SRSi	winter	76.9	37.9	57.7	29.0	15.4	6.08	45.9	21.5
	summer	70.4	29.4	23.4	21.1	78.2	26.3	146	48.2
	autumn	35.4	18.4	41.7	37.2	29.3	22.7	90.9	14.0
N-NO_3^-	winter	61.7	22.0	37.1	17.1	32.8	7.43	25.3	27.9
	summer	60.1	4.86	1.76	2.37	33.1	25.9	64.2	15.9
	autumn	19.8	9.38	56.7	23.4	9.76	11.4	37.8	3.15
N-NO_2^-	winter	0.05	1.15	0.08	0.12	0.30	0.19	<LOD	0.03
	summer	0.42	0.51	<LOD	0.09	6.89	1.64	1.47	0.47
	autumn	0.13	0.12	0.10	0.23	0.24	0.84	0.21	0.48
N-NH_4^+	winter	0.07	0.42	0.24	0.28	2.46	8.34	0.06	0.16
	summer	0.25	1.79	0.32	0.31	2.96	0.55	1.46	0.43
	autumn	0.45	0.74	0.38	0.47	0.22	0.50	0.08	<LOD
DIN	winter	61.9	23.6	37.5	17.5	35.5	16.0	25.3	28.1
	summer	60.7	7.17	2.09	2.77	43.0	28.1	67.2	16.8
	autumn	20.3	10.2	57.2	24.1	10.2	12.8	38.1	3.64
SRP	winter	0.21	0.15	0.21	0.09	1.16	1.64	0.07	<LOD
	summer	<LOD	<LOD	0.04	<LOD	9.85	2.68	0.61	0.03
	autumn	0.10	0.11	0.36	0.16	0.24	0.52	0.25	0.03

F-B=freshwater-brackish; M=marine

The N-NO_3^- was generally found to be the predominant form of DIN among riverine, brackish and marine water samples, reaching the maximum percentage of 99.8% in the river freshwater collected at the IS River mouth in winter and remained elevated both in the brackish (ranging overall between 77.1 and 99.7%) and marine water samples (ranging overall between 46.6 and 99.3%) (Figure 2). Conversely, N-NO_2^- and N-NH_4^+ were less represented in the river freshwater at the IS River mouth (0.09 and 0.12 % of N-NO_2^- and N-NH_4^+, respectively) as well as in both brackish (ranging overall between 0.04 and 16.0% for N-NO_2^- and between 0.21 and 15.2% for N-NH_4^+) and marine (ranging overall between 0.12 and 13.1% for N-NO_2^- and between 0.28 and 52.3% for N-NH_4^+) water samples (Figure 2).

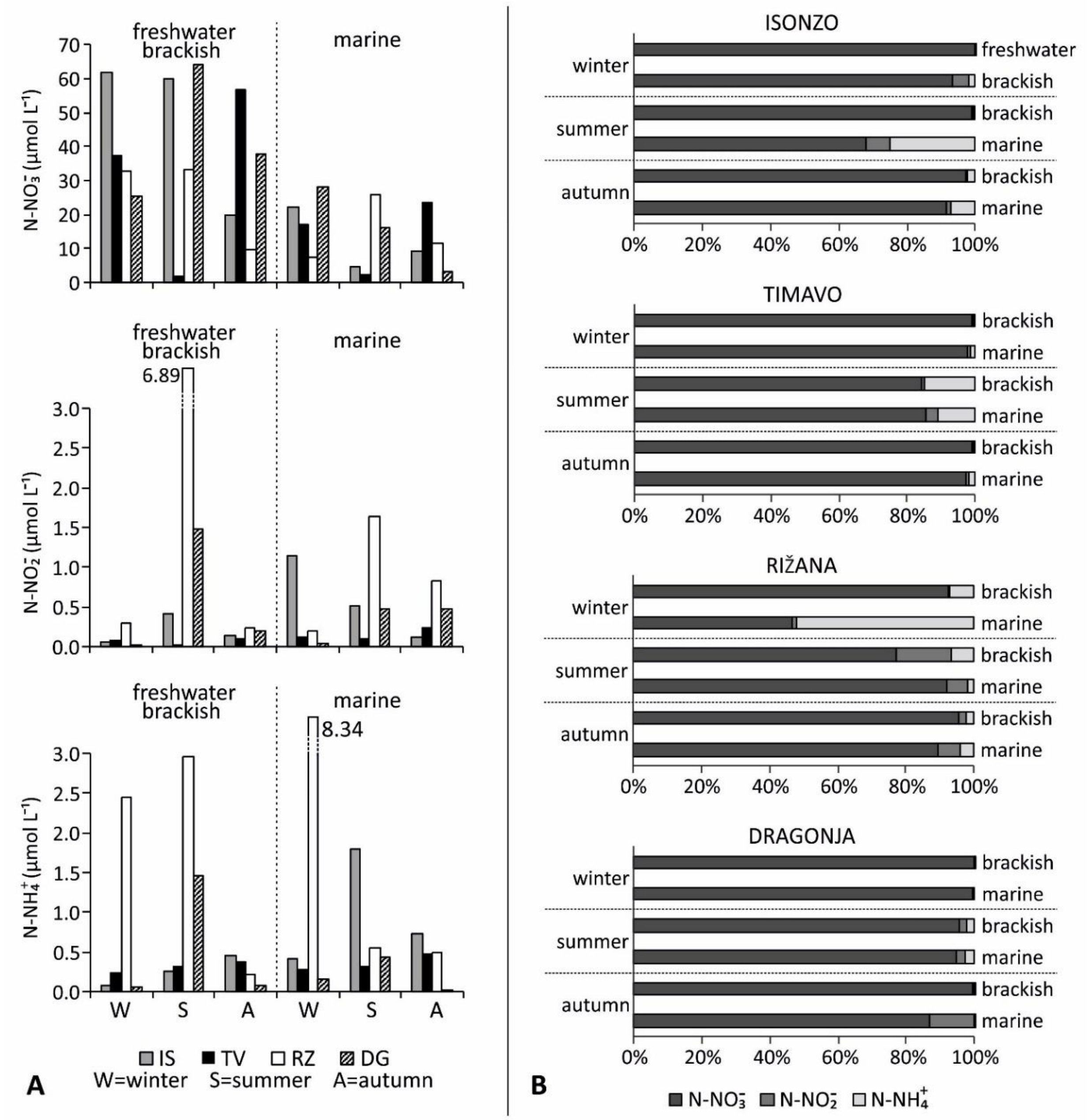

Figure 2. Truly dissolved concentrations of N-NO$_3^-$, N-NO$_2^-$ and N-NH$_4^+$ (µmol L^{-1}) (A) and their relative percentages (B) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

At the IS River mouth, N-NO$_3^-$ clearly prevailed in the surface freshwater (61.7 µmol L^{-1}) and in the brackish water collected in autumn (19.8 µmol L^{-1}) whereas low concentrations were constantly found in the marine water, especially in summer (4.86 µmol L^{-1}). This could be due to the high river discharge (275 and 230 m^3 s^{-1} in winter and autumn, respectively) that occurred for several days before sampling. Despite the low river discharge (20 m^3 s^{-1}), notably elevated concentrations of dissolved N-NO$_3^-$ were also measured in summer, especially in the mixing layer (60.1 µmol L^{-1}).

This suggests that dilution does not appear to have an effect on the occurrence of N-NO_3^-, the inputs of which still remains elevated under periods of low discharge. In this context, the occurrence of dissolved N-NO_3^- could be the result of rainwater runoff from soils where agricultural activities take place (Roselli et al., 2009) since fertilisers often consists of soluble chemical forms of N that are easily leached out during precipitation events and/or irrigation (Addiscott, 1996). In addition, concentrations of dissolved N-NO_3^- were found to be higher than those measured in the Gulf of Trieste (Faganeli et al., 2009) most likely due to the enhanced influence of the riverine inputs.

Nitrite (N-NO_2^-) and N-NH_4^+ concentrations were one or two orders of magnitude lower than N-NO_3^- and appeared to be slightly higher in the marine water (Table 2, Figure 2). The highest value of N-NH_4^+ was observed in summer (1.79 µmol L^{-1}), whereas low concentrations were found in both winter and autumn (0.42 and 0.74 µmol L^{-1}). The OM transported by the river freshwater during flood events occurring in winter accumulates in the surface sediments, where its decomposition could contribute to N-NH_4^+ production, which is enhanced in summer (Brogueira and Cabeçadas, 2006).

Similar to the IS River mouth, high amounts of N-NO_3^- were measured in the brackish water at the TV, DG and RZ estuarine systems although notably lower values occurred in summer at the TV River mouth (1.76 and 2.37 µmol L^{-1} in the mixing layer and at the bottom, respectively) and at the mouth of the RZ River (7.43 and 9.76 µmol L^{-1} in the brackish and marine water, respectively). Moreover, estuarine water from the RZ River mouth showed the highest values of both N-NO_2^- (6.89 and 1.64 µmol L^{-1} in the brackish and marine water in summer) and N-NH_4^+ (2.46 and 8.34 µmol L^{-1} in the brackish and marine water in winter). The reductive conditions measured along the water column in winter (-36 and -139 mV in the brackish and marine water, respectively) and autumn (-28 and -72 mV in the brackish and marine water, respectively) and the relatively low values of the redox potential observed in summer (48 and 27 mV in the brackish and marine water, respectively) suggested that potential ipoxic or anoxic conditions could occur at the SWI. This may be responsible for increased fluxes of nutrients at the SWI, since dissolved N-NO_2^- and N-NH_4^+ are released during denitrification and OM degradation processes (Kemp et al., 1997; Emili et al., 2011; Acquavita et al., 2015).

Soluble reactive silicate (SRSi) ranged overall between 6.08 and 146 µmol L^{-1} and generally reached high concentrations in the river freshwater and brackish water samples (Table 2). Distinct trends were not evident during the year, although slight differences were observed among the investigated river mouths. In detail, SRSi appeared to be high in summer at the mouths of the RZ and DG Rivers, whereas SRSi generally prevailed at the IS and TV estuarine systems in winter (Figure 3).

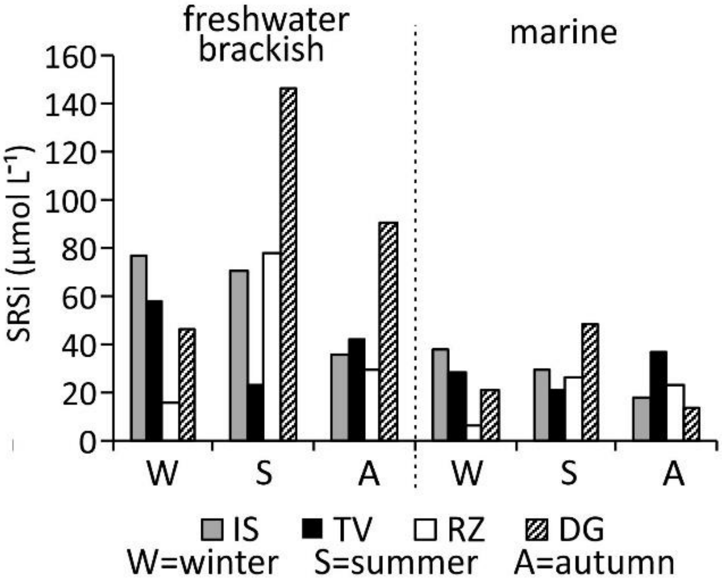

Figure 3. Concentrations of SRSi (µmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

According to Zhang et al. (2013), dissolution processes involving diatom siliceous structures in sediments could be responsible for the release of dissolved SRSi at the SWI. However, since SRSi was found to be prevalent in the surface and the brackish water, river freshwater inputs were supposed to play a major role as the main vehicle of SRSi, following the behaviour previously described for N-NO_3^- and DIN. This was also confirmed by the significant correlations found between SRSi and both N-NO_3^- ($r=0.752$, $N=24$, $p<0.001$) and DIN ($r=0.758$, $N=24$, $p<0.001$) (Figure 4), thus suggesting that both SRSi and N-NO_3^- may have terrestrial origins (Roselli et al., 2009).

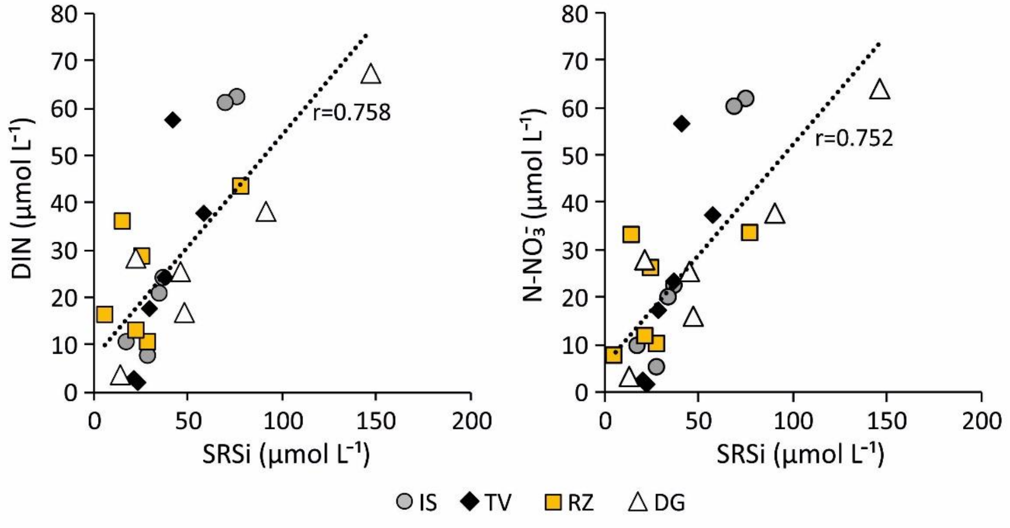

Figure 4. Correlation between SRSi (µmol L^{-1}) and both DIN (µmol L^{-1}) and N-NO_3^- (µmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) estuarine systems under different seasonal conditions.

The SRSi:DIN molar ratio represents a useful index to evaluate the relative prevalence of different algae classes in natural aquatic systems (Conley et al., 1993). Although this issue is not the main aim of this research, the SRSi:DIN molar ratio (Table 3) was calculated and found to be mainly higher than 1, especially during summer when the excess of SRSi could promote the growth of diatoms.

Table 3. SRSi:DIN molar ratio in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

River		IS		TV		RZ		DG	
Layer		F-B	M	F-B	M	F-B	M	F-B	M
SRSi:DIN	winter	1.24	1.61	1.54	1.66	0.43	0.38	1.81	0.77
	summer	1.16	4.11	11.2	7.61	1.82	0.94	2.18	2.86
	autumn	1.74	1.79	0.73	1.54	2.87	1.78	2.38	3.86

F-B: freshwater-brackish; M=marine

Soluble reactive phosphorous (SRP) was generally low, varying overall between <LOD and 9.85 µmol L^{-1} (Table 2) and there were no notable differences observed considering the different water masses and seasons at the IS (<LOD-0.21 µmol L^{-1}), TV (<LOD-0.21 µmol L^{-1}) and DG (<LOD-0.61 µmol L^{-1}) River mouths. Conversely, concentrations higher than one order of magnitude were found at the estuarine system of the RZ River, especially in summer when 9.85 and 2.68 µmol L^{-1} were measured in the brackish and marine water, respectively (Figure 5).

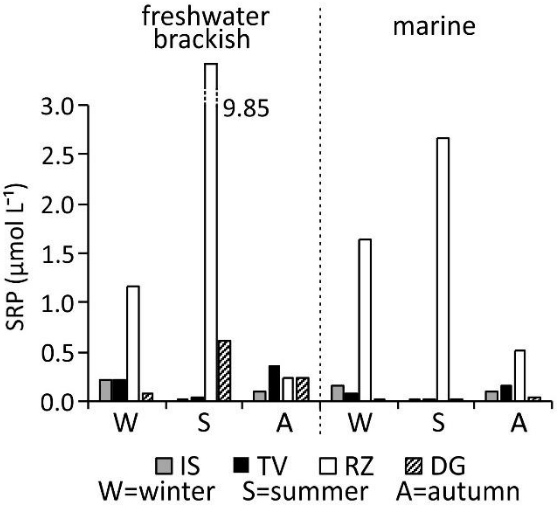

Figure 5. Concentrations of SRP (μmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

In the RZ estuarine mixing zone, the warm water temperature measured in summer (28.1 and 27.4 °C in the mixing and marine water layers, respectively) could promote the release of SRP as a result of dissolution and/or desorption processes of Fe oxy-hydroxides (Sundby et al., 1992; Conley, 1999; Ogrinc and Faganeli, 2006). Indeed, SRP can precipitate in association with Fe oxy-hydroxides in winter and spring, when condition of weak sulphate reduction occurred (Jensen et al., 1995). Subsequently, remobilisation of SRP from sediments to the overlying water column could be enhanced in summer, as a result of the intense OM degradation.

In this context, the N-NH_4^+:SRP molar ratio may provide evidence as to the relative prevalence of release or adsorption of SRP from/to the solid phase (Anschutz et al., 2007; Petranich et al., 2018a). Generally, the estuarine water collected at the RZ River mouth displayed values of N-NH_4^+:SRP molar ratio (Table 4) lower if compared to the sites, especially in summer (0.30 and 0.21 in the brackish and marine water, respectively) thus suggesting that the release of dissolved SRP from reduced Fe oxy-hydroxides may develop (Sundby et al., 1992).

Table 4. N-NH_4^+:SRP molar ratio in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

River		IS		TV		RZ		DG	
Layer		F-B	M	F-B	M	F-B	M	F-B	M
N-NH_4^+:SRP	winter	0.35	2.74	1.13	3.21	2.11	5.09	0.80	31.5
	summer	50.4	359	8.05	62.2	0.30	0.21	2.39	14.6
	autumn	4.61	6.84	1.05	2.88	0.91	0.95	0.32	0.29

F-B: freshwater-brackish; M=marine

Estimation of water quality: the DIN:SRP molar ratio

The role of nutrients in limiting the primary production in natural aquatic systems was investigated by Conley (1999). The author stated that N is generally considered the limiting factor for freshwater systems whereas conditions of P-limitation generally prevail in marine environments. Nonetheless, according to the same author, estuaries can show both N and P limitation in different seasons. In this context, the DIN:SRP molar ratio can provide evidence as to the relative influence of N and P as factors limiting the primary production in aquatic environments (e.g. Ogrinc and Faganeli, 2006; Roselli et al., 2009; Acquavita et al., 2015; Petranich et al., 2018a). In this research, the DIN:SRP molar ratio (Table 5) was found to be mainly higher that 16 (Redfield et al., 1963) thus suggesting the prevalence of P-limitation at the IS, TV and DG estuarine systems.

Table 5. DIN:SRP molar ratio in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

River		IS		TV		RZ		DG	
Layer		F-B	M	F-B	M	F-B	M	F-B	M
	winter	293	155	177	197	30.5	9.74	344	5621
DIN:SRP	summer	12148	1434	53.1	555	4.36	10.5	110	569
	autumn	207	94.7	157	149	41.8	24.3	155	106

F-B: freshwater-brackish; M=marine

The only exception is represented by the RZ River mouth where the DIN:SRP molar ratio was found to be lower than 16 in summer (4.36 and 10.5 in the brackish and marine water, respectively) and in winter (marine water, 9.74). Conversely, higher values of the DIN:SRP molar ratio were observed in autumn, both in brackish and marine water, and in brackish water during winter. This suggested that the estuarine system of the RZ River may switch from conditions of P-limitation, prevailing in autumn and to a lesser extent in winter, to N-limitation which is generally strongest in summer (Conley, 1999).

According to the European Environmental Agency (EEA 2001, 2003), the DIN:SRP molar ratio represents a useful index to assess the trophic status of aquatic environments. In addition, water quality can be estimated following the four classes (*good, fair, poor* and *bad*) proposed by the European Environmental Agency (EEA 2001, 2003) with respect to dissolved concentrations of both DIN and SRP. Regarding DIN (Figure 6A), most of the estuarine samples showed *bad* water quality since DIN, which was often hugely represented by N-NO_3^-, reached higher concentrations rather than those of SRP.

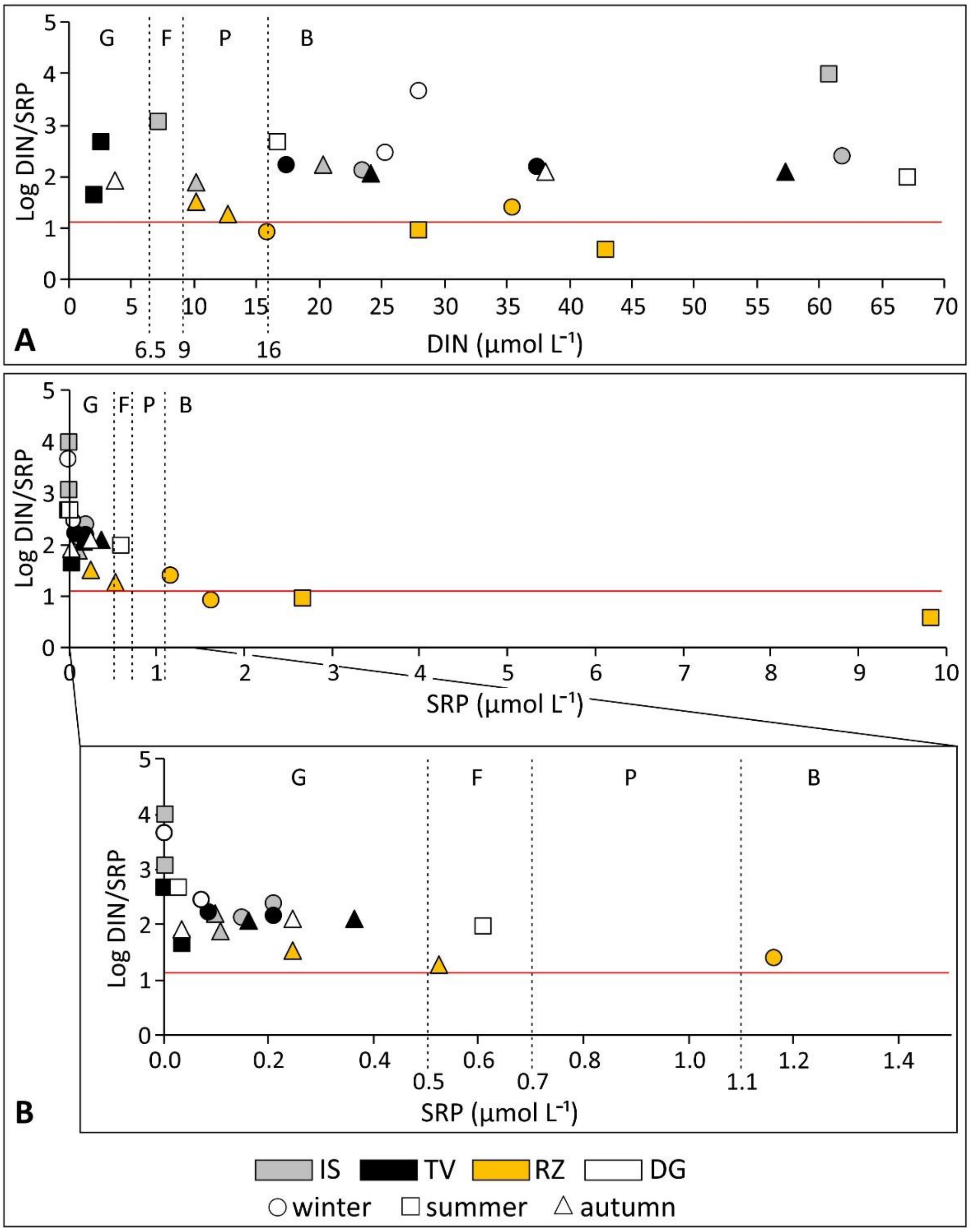

Figure 6. The DIN:SRP ratio (expressed on a logarithmic scale) plotted against dissolved concentrations of DIN (A) and SRP (B) measured at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths. In both plots, water quality classification (G=good, F=fair, P=poor, B=bad) proposed by EEA (2001, 2003) was applied and the red line represents DIN:SRP =16 (Redfield et al., 1963).

With the only exception of the marine water collected in summer, which was of *fair* quality, the results highlighted a *bad* water quality at the IS River mouth. This was especially evident in the river freshwater collected in winter and in the brackish water sampled in summer. Indeed, these samples showed the highest concentrations of DIN (61.9 and 60.7 µmol L^{-1} in winter and summer, respectively). A slightly similar condition was observed at the mouth of the DG River, where *bad* water quality occurred especially in the brackish water in summer (DIN=67.2 µmol L^{-1}) whereas *good* water quality was reached by the TV estuarine system in summer since the concentrations of DIN were notably low (2.09 and 2.77 µmol L^{-1} in the brackish and marine water, respectively) compared to those measured in winter (37.5 and 17.5 µmol L^{-1} in the brackish and marine water, respectively) and autumn (57.2 and 24.1 µmol L^{-1} in the brackish and marine water, respectively) where *bad* water quality occurred. As previously mentioned, the mouth of the RZ River showed the lowest values of the DIN:SRP molar ratio, especially in summer when the system was N-limited (2.91 and 3.29 in the brackish and marine water, respectively).

In case of SRP (Figure 6B), *good* water quality was achieved at the IS, TV and DG estuarine systems with the only exception being the brackish water collected at the DG River mouth in summer, when the highest concentration of SRP was found (0.61 µmol L^{-1}) leading to *fair* water quality. Conversely, results highlighted *bad* water quality for most of the estuarine water samples collected at the RZ River mouth, especially in summer when 9.85 and 2.68 µmol L^{-1} of SRP were found in the brackish and marine water, respectively.

Occurrence and distribution of colloidal organic carbon, nitrogen and nutrients

The strong physico-chemical gradients that often occur in estuarine regions are responsible for the water column stratification, but can also affect sedimentation, precipitation and flocculation of particles and colloids (Förstner and Wittmann, 1979). Indeed, aggregation of colloids is enhanced in the commonly defined maximum turbidity zone (Lead et al., 1997; de Souza Machado et al., 2016) where the occurrence of colloids strongly depends on the concentration of the total suspended particulate matter (Benoit et al., 1994).

Colloidal organic carbon (COC) represents a significant portion of the bulk DOC in seawater and may have a crucial role in marine biogeochemical cycles (Guo and Santschi, 1996, 1997; Jaïry et al., 1999) influencing the distribution, chemical speciation and fate of a variety of potentially toxic compounds (Wells et al., 1998; Guo et al., 2000; Ran et al., 2000). Colloidal organic carbon (COC) and colloidal nitrogen (CN) were detected in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (Table 6).

Table 6. Concentrations of COC, CN (μmol L^{-1}), COC:CN and COC:DOC (%) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

River		IS		TV		RZ		DG	
Water Sample		F-B	M	F-B	M	F-B	M	F-B	M
	winter	3.14	7.77	3.12	7.02	11.1	9.28	5.67	6.88
COC	summer	16.3	12.9	10.1	12.0	17.1	17.1	8.61	12.8
	autumn	7.05	9.64	3.87	9.32	7.69	7.07	9.39	8.41
	winter	0.23	0.55	0.29	2.48	1.25	2.80	0.21	2.47
CN	summer	2.85	1.65	1.69	0.74	1.79	3.49	0.75	1.41
	autumn	0.74	1.41	0.39	0.61	0.74	0.84	0.47	0.51
	winter	13.6	14.2	10.8	2.83	8.91	3.32	26.7	2.79
COC/CN	summer	5.71	7.83	5.96	16.1	9.55	4.89	11.5	9.12
	autumn	9.57	6.83	9.92	15.2	10.4	8.42	20.1	16.6
	winter	3.19	5.92	4.30	9.31	7.81	9.70	6.77	8.61
%COC:DOC	summer	18.9	11.9	14.5	12.5	6.65	9.64	7.96	11.7
	autumn	6.82	9.05	5.40	10.4	9.34	8.92	6.41	7.82

F-B: freshwater-brackish; M=marine

The contents of both COC and CN were found to be lower if compared to the truly dissolved fraction, thus suggesting that scarce aggregation of colloidal material likely occurred at the investigated estuarine systems. This could explain why significant interaction between COC and colloidal trace elements, which in turn were found to be scarsely present (Chapter 4.1 and 4.2), were not found at the investigated river mouths although COC might have a role in regulating trace element mobility and fate in estuarine environments. Similarly, low COC values were reported by Sigg et al. (2000) in the freshwater of the Thur River (Switzerland) where the truly dissolved fraction prevailed.

In detail, COC represented only a small fraction of the bulk DOC at the IS (9.29 ± 5.54% on average), TV (9.40 ± 3.97% on average), RZ (8.68 ± 1.21 % on average) and DG (8.20 ± 1.88 % on average) and no clear difference between water layers and seasonal sampling was found (Table 6). Colloidal organic carbon (COC) varied overall between 3.12 and 17.1 μmol L^{-1} whereas CN showed values ranging overall from 0.21 to 3.49 μmol L^{-1} (Table 6; Figure 7).

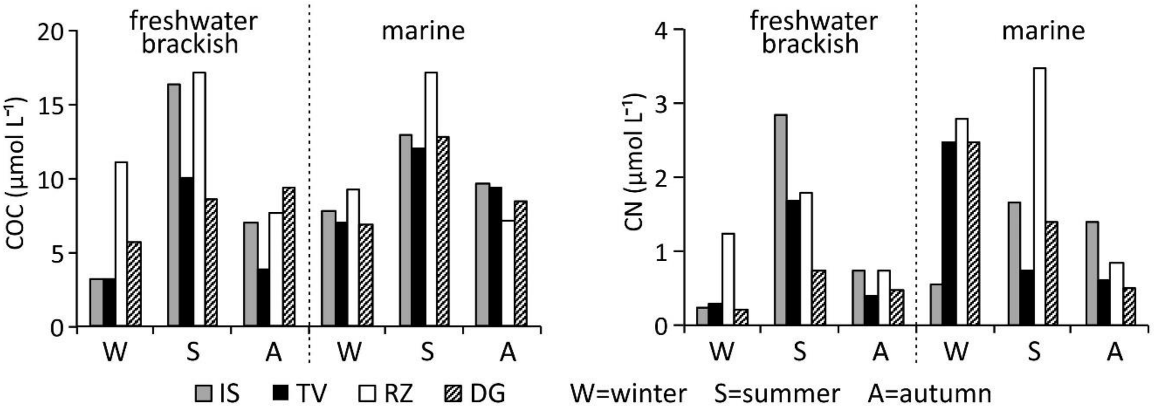

Figure 7. Concentrations of COC and CN (µmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Despite the scarce abundance of colloidal material, a significant correlation (r=0.852, N=21, p<0.001) (Figure 8) was found between COC and CN. The only exception was represented by the marine water samples collected at the TV (2.48 µmol L^{-1}), RZ (2.80 µmol L^{-1}) and DG (2.47 µmol L^{-1}) estuarine systems in winter, which appeared to be slightly enriched in CN.

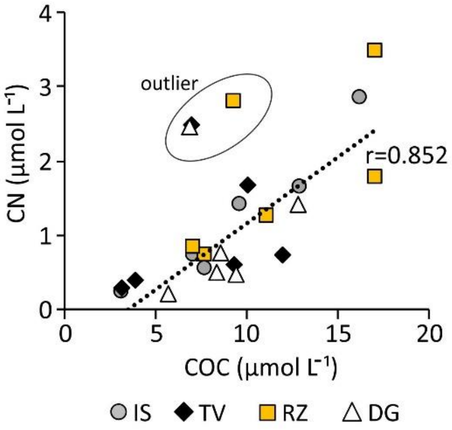

Figure 8. Correlation between COC (µmol L^{-1}) and CN (µmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) estuarine systems under different seasonal conditions.

The highest concentrations of COC and CN were generally found in the marine water and an increase in summer was found (Klun et al., 2019) with the highest values reached at the estuarine system of the IS (16.3 and 12.9 µmol L^{-1} in the brackish and marine water, respectively), TV (10.1 and 12.0 µmol L^{-1} in the brackish and marine water, respectively), RZ (17.1 µmol L^{-1} in both brackish and marine water, respectively) and DG (8.61 and 12.8 µmol L^{-1} in the brackish and marine water, respectively) Rivers (Figure 7). A similar seasonal increase in colloid levels was reported by Klun et al. (2019) for the Gulf of Trieste, where COC concentrations increased by nearly doubled from winter to summer. As previously mentioned, the degradation of POC leads to high DOC concentrations in summer when the aggregation of colloids may also be favoured (Wells et al., 2000; Ingri et al., 2004). This trend was especially evident at the IS and DG estuarine systems, where a significant correlation was found between COC and temperature (r=0.879, N=12, p<0.001) (Figure 9), and to a lesser extent, at the mouth of the TV and RZ Rivers although at these sites, slight disparities in the COC concentrations were observed at the RZ River mouth in both winter and autumn.

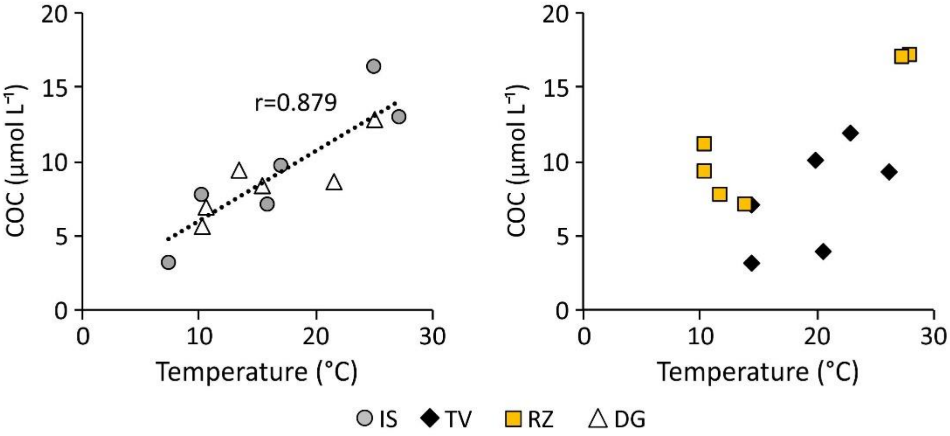

Figure 9. Correlation between COC (μmol L^{-1}) and temperature (°C) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Colloidal N showed very similar behaviour to that of COC and a significant correlation with temperature (r=0.798, N=11, 0.001<p<0.01) was found at the IS and DG River mouths with the only exception of the marine water collected at the DG estuarine system in autumn which appeared to be enriched in N (2.47 μmol L^{-1}) with respect to the other estuarine samples (Figure 10). Conversely, the temperature did not appear to affect the distribution of CN at the TV and RZ River mouths, as previously mentioned in the case of COC.

Since there is lack of knowledge on the occurrence and distribution of nutrients in the colloidal material, concentrations of colloidal SRSi, N-NO$_3^-$, N-NO$_2^-$, N-NH$_4^+$ and SRP were also investigated (Table 7). As also observed for COC and CN, colloidal nutrients were lower than those found in the truly dissolved fraction.

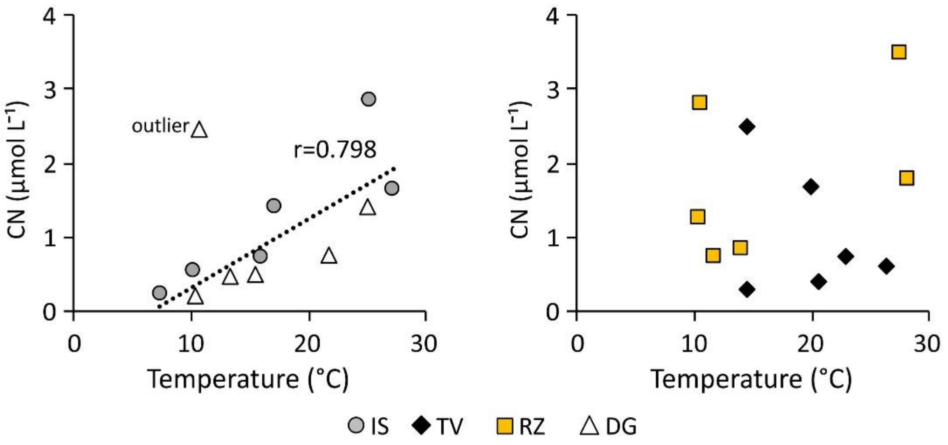

Figure 10. Correlation between CN (µmol L^{-1}) and temperature (°C) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Table 7. Concentrations of colloidal SRSi, N-NO$_3^-$, N-NO$_2^-$, N-NH$_4^+$ and SRP (nmol L^{-1}) in estuarine water collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions (nd: not determined).

River		IS		TV		RZ		DG	
Layer		F-B	M	F-B	M	F-B	M	F-B	M
SRSi	winter	117	83.3	115	88.7	32.8	29.6	1243	150
	summer	119	90.0	202	88.4	129	74.5	136	115
	autumn	nd	2.35	167	49.5	438	nd	471	76.2
N-NO$_3^-$	winter	nd	64.6	11.3	1651	24.8	895	442	982
	summer	1995	432	53.2	8.03	40.4	991	22.4	1411
	autumn	10.5	1404	32.8	nd	303	18.9	nd	2.69
N-NO$_2^-$	winter	1.75	1.34	0.82	3.05	0.68	2.38	2.73	1.15
	summer	0.66	0.66	2.55	1.90	0.82	3.95	1.16	0.91
	autumn	nd	0.25	0.66	1.17	5.79	nd	nd	0.50
N-NH$_4^+$	winter	2.05	nd	0.79	4.64	13.4	nd	2.43	1.22
	summer	14.1	0.79	5.36	1.99	1.63	nd	1.97	10.0
	autumn	nd	8.00	nd	nd	1.54	nd	nd	nd
SRP	winter	nd	nd	nd	0.91	5.01	4.55	1.24	0.91
	summer	0.74	0.41	0.99	1.16	21.3	11.9	2.48	0.99
	autumn	0.25	nd	1.00	nd	3.14	nd	nd	0.59

F-B: freshwater-brackish; M=marine

Nitrate (N-NO$_3^-$) clearly prevailed among colloidal N forms and ranged between 10.5 and 1995 nmol L^{-1} at the IS River mouth, between 11.3 and 1651 nmol L^{-1} at the TV River mouth, between 24.8 and 991 nmol L^{-1} at the RZ River mouth and between 22.4 and 1411 nmol L^{-1} at the DG River mouth (Table 7, Figure 11).

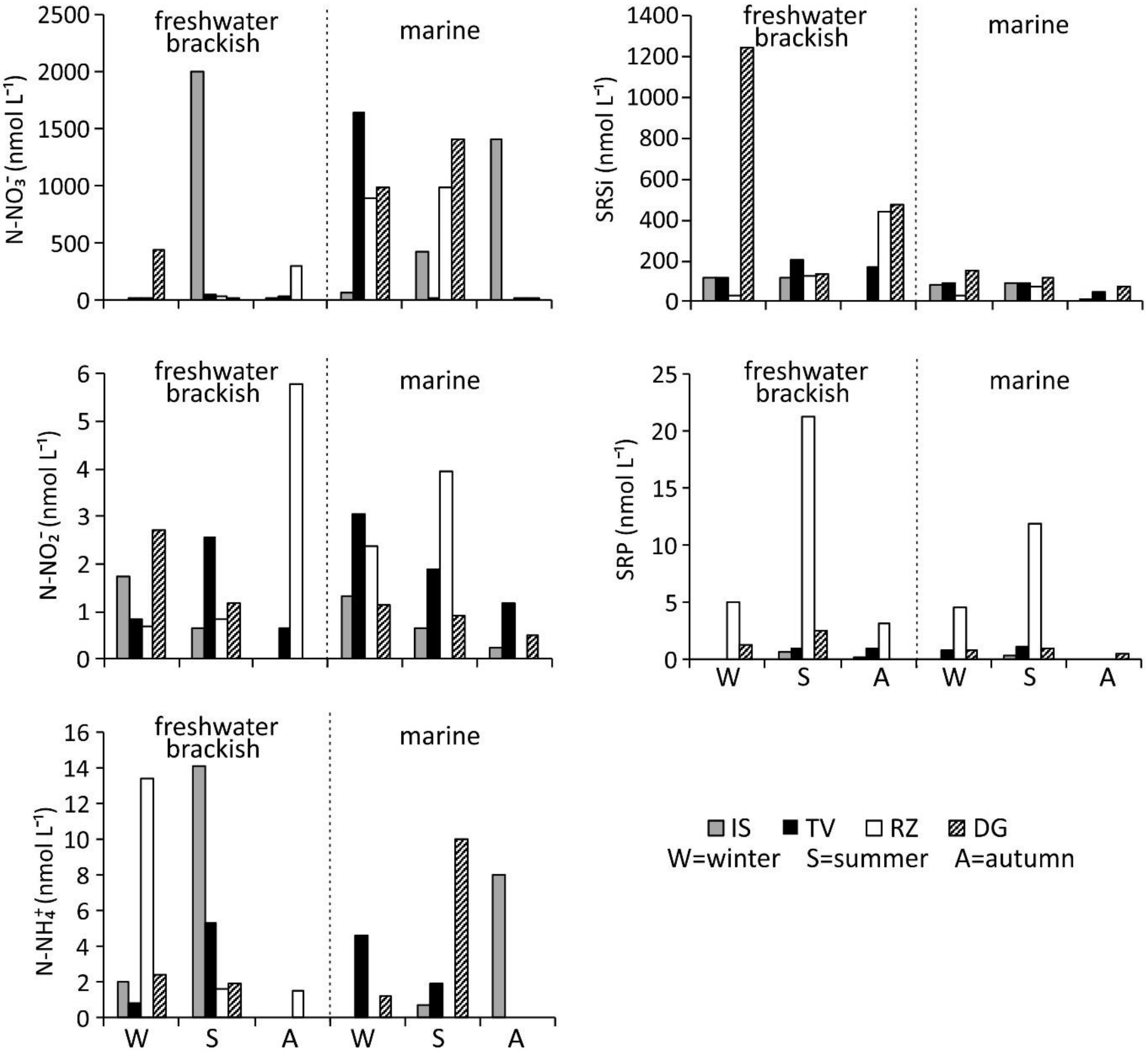

Figure 11. Concentrations of colloidal N-NO$_3^-$, N-NO$_2^-$, N-NH$_4^+$, SRSi and SRP (nmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

The only exception being the brackish water collected at the IS River mouth (1995 nmol L^{-1}), colloidal N-NO$_3^-$ was found to be higher in the bottom marine water (Figure 11). As previously mentioned, due to rainwater runoff from soils subjected to agricultural activities elevated concentrations of dissolved N-NO$_3^-$ were found, especially along the IS River drainage basin. Taking into consideration the colloidal fraction, this was less evident but it could be observed that N-NO$_3^-$ showed the maximum concentration in summer at the IS (1995 nmol L^{-1}), RZ (991 nmol L^{-1}) and DG (1411 nmol L^{-1}) systems since it has been demonstrated that the aggregation of colloids can be enhanced by warm temperatures. The only exception was the TV River mouth, where colloidal N-NO$_3^-$ was also found to be notably present in winter (1651 nmol L^{-1}).

Despite the limited number of samples considered and the low concentrations found, the colloidal material appeared to have a role in transporting N-NO$_3^-$ which showed a discrete correlation with

COC ($r=0.757$, $N=5$, $p<0.05$) at the IS River mouth (Figure 12). Conversely, this was not evident at the TV, RZ and DG estuarine systems.

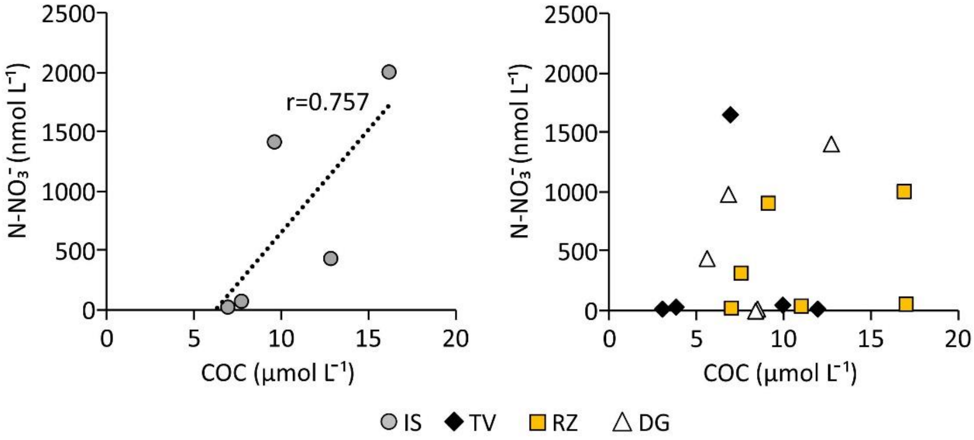

Figure 12. Correlation between colloidal N-NO$_3^-$ (nmol L^{-1}) and COC (µmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Nitrite (N-NO$_2^-$) and N-NH$_4^+$ ranging overall from 0.25 to 5.79 nmol L^{-1} and between 0.79 and 14.1 nmol L^{-1} of N-NO$_2^-$ and N-NH$_4^+$, respectively (Table 7, Figure 11) and, thus accounted for a lower percentage of total N pool. A significant correlation was found between colloidal N-NH$_4^+$ (nmol L^{-1}) and COC (µmol L^{-1}) at the IS and TV River mouths ($r=0.969$, $N=6$, $p<0.001$) with the exception of the summer sampling. This suggested that COC could represent a quite effective adsorptive phase for colloidal N-NH$_4^+$ in winter and autumn. Conversely, this was not evident at the RZ and DG estuarine systems where colloidal N-NH$_4^+$ was generally found to have very low concentrations (Figure 13).

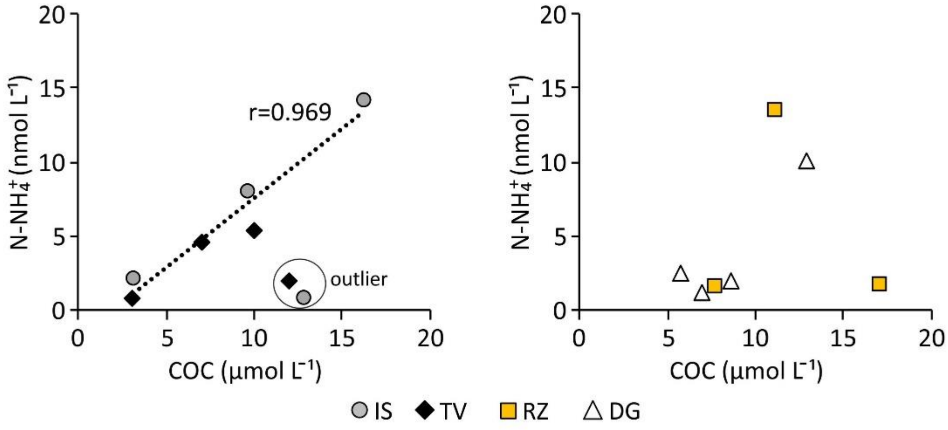

Figure 13. Correlation between colloidal N-NH$_4^+$ (nmol L^{-1}) and COC (µmol L^{-1}) in estuarine water samples collected at the Isonzo (IS), Timavo (TV), Rižana (RZ) and Dragonja (DG) River mouths under different seasonal conditions.

Taking into consideration the colloidal SRP, when detectable, and low concentrations were found at the IS (0.25-0.74 nmol L^{-1}), TV (0.91-1.16 nmol L^{-1}) and DG (0.59-2.48 nmol L^{-1}) estuarine systems, whereas the RZ River mouth showed slightly elevated values ranging between 3.14 and 21.3 nmol L^{-1} (Table 7, Figure 11). Conversely to what was observed at the IS, TV and DG River mouths, where notable differences were not observed among different seasons, there were some exceptions: at the RZ River mouth colloidal SRP was found to be higher in summer both in the brackish (ranging between 3.14 and 21.3 nmol L^{-1}) and marine (nd-11.9 nmol L^{-1}) water.

4.4 Distribution, mobility and fate of trace elements in a karstic estuarine system under anthropogenic pressure (northern Adriatic Sea)

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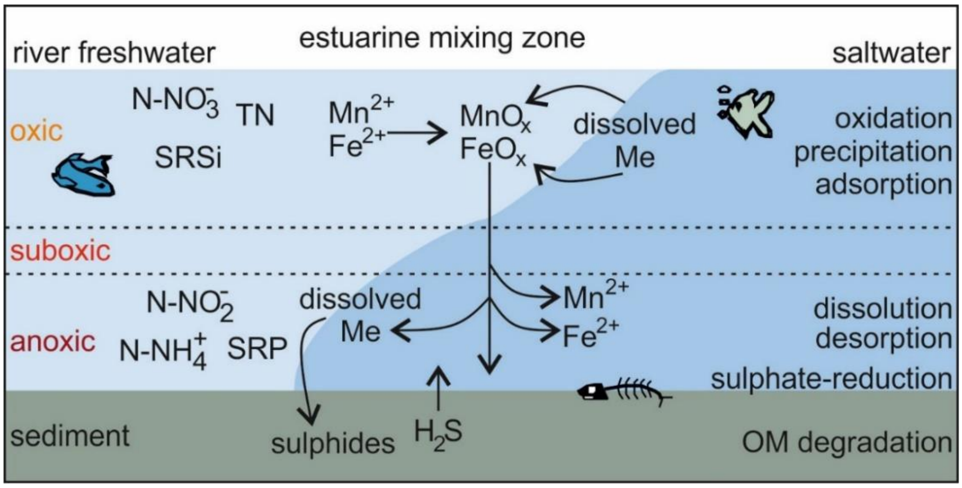
Abstract

The accumulation of contaminants and their potential mobility represent two of the main environmental issues facing coastal environments. Sediments often act as “reservoirs” of contaminants, including potentially toxic trace elements, but they can also be considered a secondary source due to remobilisation processes at the sediment-water interface, which may affect the quality of the coastal water and aquatic biota. This work aims to provide a preliminary geochemical characterisation of the Timavo/Reka River mouth, focusing on the occurrence of trace elements in different environmental matrices.

The reductive conditions measured at the bottom in the innermost sector of the estuarine system testify to potential anoxia at the sediment-water interface. Principal component analysis was employed in order to reveal disparities among the different sectors of the estuarine system. The surface sediments were found to be enriched in trace elements, whose concentrations often exceeded the Italian regulatory threshold limits, especially in the innermost sector and the lithogenic contribution of the Timavo/Reka River can be identified as the primary source of Cr and Ni. Sulphate-reductive conditions occurred at the bottom in the innermost sector, driving trace element accumulation in the residual fraction of sediments. However, Fe and Mn redox-behaviour appears to have a crucial role in the recycling of dissolved trace elements in the water column. Conversely, oxidative conditions prevail in the surface freshwater. Here, oxidation and precipitation processes are responsible for trace element partitioning between the solid and dissolved phases.

Keywords: trace elements, sediment, partitioning, water quality, principal component analysis, estuary

Graphical abstract



Introduction

Estuaries are recognised worldwide as crucial water systems where strong freshwater-seawater mixing occurs (Savenko et al., 2014; de Souza Machado et al., 2016; Fiket et al., 2018). The hydrodynamic processes governing the estuarine water circulation actively influence suspended particle transport and deposition (Meade, 1972). In the estuarine mixing zone, the decrease in flow velocity and the occurrence of sharp density and salinity gradients lead to fine particle aggregation and settling (Wright, 1977; Menon et al., 1998). For this reason, estuaries can be considered a sedimentary trap. This is of relevant concern as suspended particulate matter (SPM) is known to play a crucial role in the transfer of potentially toxic trace elements and other contaminants in estuarine and coastal environments (Turner et al., 1991). In addition, trace elements are subjected to a variety of physico-chemical and biogeochemical processes which may strongly affect their distribution, speciation and fate.

Marine coastal areas are often affected by high anthropogenic pressure due to extended urban areas and industrial, harbour and tourism activities (Casado-Martínez et al., 2006; Petranich et al., 2018b). The derived impact leads to the accumulation of contaminants including potentially toxic trace elements, organometallic compounds and persistent organic pollutants (e.g. Neto et al., 2006; Botwe et al., 2017). In this context, sediments are largely thought to act as a sink for several toxic compounds and are also of great concern, becoming a secondary source of contamination (Monterroso et al., 2007; Santos-Echeandia et al., 2009; Acquavita et al., 2010). Indeed, resuspension events due to tidal currents or caused by dredging operations (Wasserman et al., 2016; Silveira et al., 2017) and changes in the physico-chemical boundary conditions at the sediment-water interface (SWI) (Piou et al., 2009) could lead to remobilisation and the subsequent release of potentially toxic trace elements in solution (Warnken et al., 2001; Caplat et al., 2005). The mobility of these contaminants is influenced by several factors including the chemical form in sediments, the redox state at the SWI, the microbial activity and the occurrence of organic matter (OM). Biogeochemical processes at the SWI could also be responsible for the transformation of contaminants into more toxic and dissolved bioavailable forms with a subsequent transfer to the aquatic trophic chain (Point et al., 2007; Siot et al., 2008). These processes are usually enhanced in aquatic coastal environments characterised by shallow water, such as lagoons and semi-closed estuarine environments. Here, the increasing aerobic microbial activity often results in oxygen depletion favouring anoxic/hypoxic conditions at the SWI, which can lead to recycling processes of organic carbon and nutrients (e.g. De Vittor et al., 2012; Ospina-Alvarez et al., 2014; Petranich et al., 2018a).

The Timavo/Reka river mouth is a semi-closed estuarine system located in the northeastern sector of the Adriatic Sea and little information is currently available on river-mouth processes involving potentially toxic trace elements (Reisenhofer et al., 1996, 1998). For this reason, the principal aim of this research is to provide a geochemical characterisation of this estuarine system. Trace element occurrence and distribution in different environmental matrices were investigated by means of principal component analysis (PCA) in order to depict differences among different sampling sites and to evaluate the potential mobility of trace elements between water column and sediment compartments.

Materials and methods

Environmental setting

The Timavo/Reka River has its origins in Croatia (Snežnik, Slovenia-Croatia border area) and flows on flysch terrains in Slovenia before sinking into the Karst Plateau at the Škocjan Caves (Reisenhofer et al., 1998; Gabrovšek et al., 2018) (Figure 1).

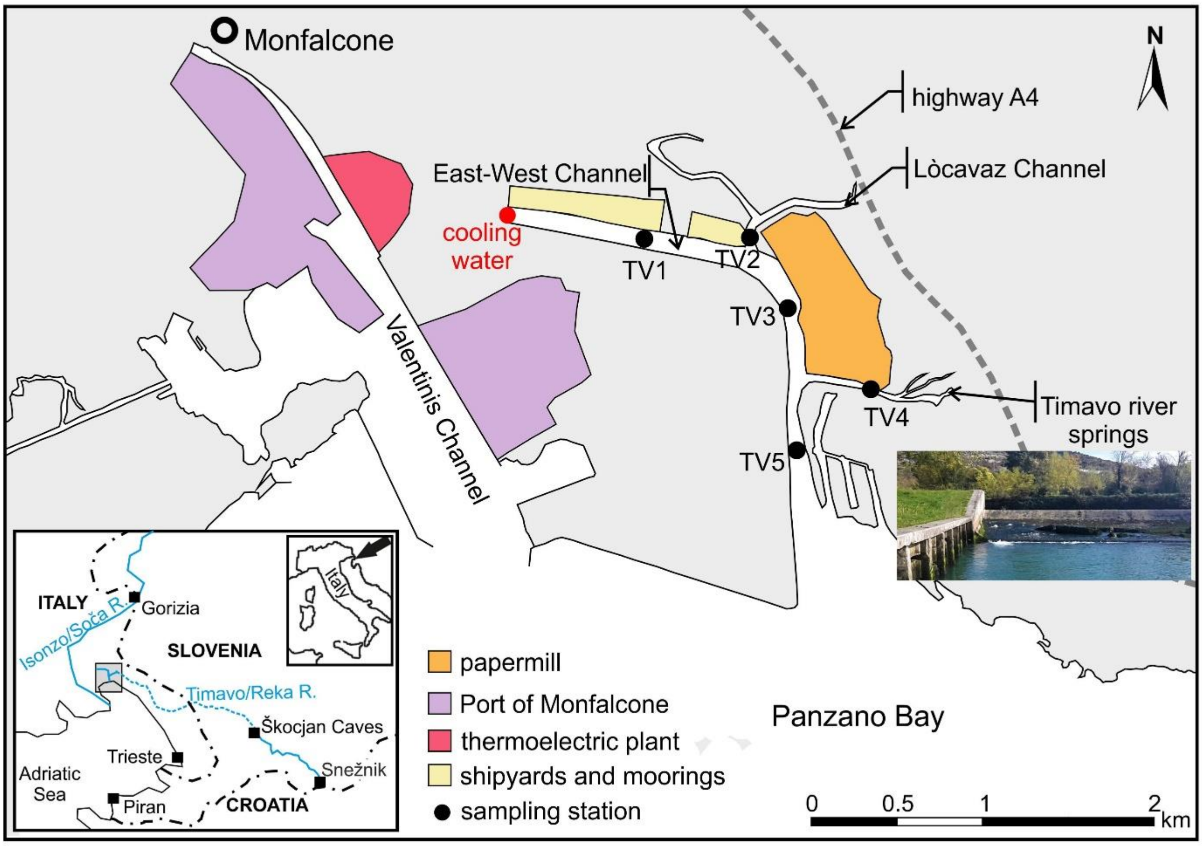


Figure 1. Index map of the study area and location of the sampling sites at the mouth of the Timavo/Reka River.

After an underground path of approximately 38 km, the river re-emerges from several springs 2 km from the sea and flows into the Panzano Bay in the northwest sector of the Gulf of Trieste (Figure 1). Those springs represent a secondary source for water supply for the nearby build-up areas and the average discharge was measured to be 30.2 m^3 s^{-1} (Gabrovšek and Peric, 2006).

In addition, the aquifer also discharges through other smaller springs in the vicinity, many of which are below sea level. Regarding the mouth of the Timavo River, two additional water channels (the Lòcavaz and the East-West channels) contribute to the water supply of the river itself.

The Gulf of Trieste is a semi-closed shallow-water basin with a maximum water depth of 25 m in its central sector. The water in the Gulf typically reports marine salinity values ranging between 33.0 and 38.5 ‰ (Olivotti et al., 1986). The anticlockwise circulation of the Adriatic Sea dominates the water circulation in the Gulf and tides, seasonal variation in the freshwater inflow and winds also play a crucial role in regulating the vertical water circulation (Malačič and Petelin, 2009).

The main freshwater input entering the Gulf of Trieste is represented by the Isonzo/Soča River (average flow rate of 1.61 ± 0.59 103 m^3 yr^{-1} in the period from 2004 to 2007 (Cozzi and Giani, 2011), followed by the Timavo River. According to the same authors, the Timavo River average annual freshwater contribution was assessed to be approximately 0.82 ± 0.15 10^3 m^3 yr^{-1}, ranging between 0.59 10^3 m^3 yr^{-1} and 1.02 10^3 m^3 yr^{-1}.

The Bay of Panzano, as well as the estuarine system of the Timavo River, is subject to strong anthropogenic pressure. Firstly, the occurrence of Hg in the Gulf of Trieste is well documented (Horvat et al., 1999; Covelli et al., 2001; Faganeli et al., 2003) since the Isonzo River is known as the main source of particulate Hg (Covelli et al., 2007) due to long term *cinnabar* (HgS) extraction activity at the Idrija mining district (Slovenia) (Hines et al., 2000). Moreover, human activities increased during the 20th century with rising mariculture, port and industrial activities including the thermal plant near the town of Monfalcone and sewage discharge.

Significant levels of trace elements and persistent organic pollutants (PAHs and PCBs) were found in the area of the Port of Trieste (Adami et al., 2000a; Pozo et al., 2009; Acquavita et al., 2010), which was recognised as the most contaminated area of the Gulf, followed by the Bay of Koper and the Bay of Panzano (Notar et al., 2001).

Sampling strategy

Water and sediment samples were collected at five sites in the estuarine system of the Timavo River during summer 2018 (Figure 1). Specifically, by moving from the coastal area close to the Panzano Bay (TV5), samples were collected just before the Timavo River freshwater input (TV3) and along the main branch of the Timavo River itself (TV4). Finally, water and sediments were sampled at the confluence with the Lòcavaz Channel (TV2) and in the innermost sector of the study area (TV1), where shipyards and moorings for touring boats are located.

Continuous salinity (PSU, Practical Salinity Unit), temperature (°C) and turbidity (NTU, Nephelometric Turbidity Unit) vertical profiles were recorded using a CTD multiprobe (Hydrolab H_2O with a 0.10 dbar pressure step). Subsequently, surface and bottom water samples were collected using a Niskin bottle and pH and redox-potential (Eh) were measured *in situ* with portable probes (pH-meter PH25 by Crison Instruments). Unfortunately, the determination of dissolved oxygen was not possible immediately after sampling. However, this parameter was measured *in situ* (oxygen meter OXI 45P by Crison Instruments) during an additional sampling campaign (November 2018) in the innermost sector (TV1 and TV2), where reductive conditions were observed in the bottom layer of the water column during summer.

Furthermore, surface sediments were collected by means of a stainless steel Van Veen grab (1.5 L). The first 2 cm of the sediment surface were promptly scraped off using a stainless steel spoon and homogenised on ship, collected in polyethylene containers, stored in an appropriate cool portable fridge and transported to the laboratory.

Analytical determinations: dissolved fraction

Water samples were filtered (Millipore Millex HA, 0.45 µm pore size) in the field to isolate the dissolved trace elements and nutrients. In addition, glass microfiber filters (Whatman GF/F, ø 25 mm, 0.8 µm pore size) were employed to collect sample aliquots for the determination of dissolved organic carbon (DOC) and total dissolved nitrogen (TN).

Sample aliquots for trace element determinations were immediately acidified (1%) with suprapure HNO_3 ($\geq 69\%$) to preserve sample composition. With the lone exception of Hg, trace elements (As, Ba, Co, Cu, Cs, Cr, Fe, Ni, Mn, Pb, V and Zn) were detected by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a NexION 350x Spectrometer (PerkinElmer, USA) equipped with an ESI SC Autosampler. The calibration curves were achieved by using standard solutions (ranging between 0.50 and 10 µg L^{-1}) obtained by the dilution of a multistandard solution

(10 mg L^{-1}) for ICP analyses (Periodic Table MIX 1, Sigma-Aldrich). Kinetic Energy Discrimination (KED) mode was preferred in order to minimise polyatomic ion interference.

Borosilicate glass containers were used to store water aliquots for dissolved Hg and water samples were immediately oxidised with bromine chloride (BrCl, 0.50 %, 500 µL/100 mL until the sample colour turned yellow) following the EPA Method 1631e (2002).

Dissolved Hg was determined by means of Cold Vapor Atomic Fluorescence Spectrometry coupled with a gold trap pre-concentration system (CV-AFS Mercur, Analytic Jena). In detail, the analysis was performed through a pre-reduction using NH_2OH-HCl (250 µL/100 mL sample) until the yellow colour disappeared, followed by a reduction with $SnCl_2$ (2% in HCl 4%) according to EPA Method 1631e (2002). The instrument was calibrated using standard solutions (ranging between 1 and 50 ng L^{-1}) prepared by the dilution of a Hg standard solution (mercury standard solution, Merck Millipore, 1000 mg L^{-1}) and acidified with BrCl (0.5 %) in order to avoid matrix effects. In addition, certified reference solution (ORMS-5, Canada) was analysed in the same batch as the water samples for quality control.

Furthermore, matrix effects were evaluated by means of laboratory-fortified samples prepared with 1 µg L^{-1} (diluted in 1% v/v HNO_3) for trace elements and 10 ng L^{-1} (diluted in 0.5% v/v BrCl) for Hg. Acceptable recoveries on 6 spiked samples were obtained (77-104%). Moreover, laboratory-fortified blanks (1 µg L^{-1} and 10 ng L^{-1} for trace elements and Hg, respectively) were analysed every 10 samples for quality control.

The analytical determination of dissolved nutrients, including nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), ammonia ($N\text{-}NH_4^+$), soluble reactive phosphorous (SRP) and soluble reactive silicate (SRSi), was performed using a segmented flow autoanalyser (Bran + Luebbe QuAttro) following the method reported by Grasshoff et al. (1999) and modified for the specific instrument. The limits of detection (LOD) were 0.02 µM for $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ and 0.01 µM for SRP and SRSi. Certified standard solutions (Inorganic Ventures Standard Solutions and MOOS-2, NRC) were used for quality control. Furthermore, proficiency testing (PT) exercises proposed by the European network of PT providers (QUASIMEME programmes AQ1 and AQ2) were periodically performed to ensure the accuracy of the analytical performance.

Dissolved organic carbon (DOC) and TN were analysed by means of a high temperature catalytic method using TOC-L Shimadzu analyser (Sugimura and Suzuki, 1998). The instrument was calibrated using potassium phthalate and quality control was ensured by analysing certified reference material (Consensus reference material, University of Miami, Florida). The precision of the method was <3%.

Analytical determinations: solid matrix

After freezing and freeze-drying (CoolSafe 55-4 SCANVAC), two aliquots of the sediment samples were collected for chemical and grain size analyses, respectively. The latest (15-20 g) were treated with H_2O_2 (3%) for 24 h to eliminate a majority of the organic matter. Subsequently, the samples were wet-sieved through a 2 mm sieve and the resulting <2 mm fraction was analysed by means of a laser granulometer (Malvern Mastersizer 2000).

For trace element determination, sediments were sieved to <2 mm, finely ground and acid-digested in PTFE vessels through a total dissolution in a closed microwave system (Multiwave PRO, Anton Paar) using inverse aqua regia (suprapure $HNO_3 \geq 69\%$ and $HCl \geq 37\%$, 3:1) and HF (EPA Method 3052, 2007). Two heating steps were performed for the mineralisation and H_3BO_3 was added in order to buffer the excess HF in the second step. After mineralisation, the solutions were diluted up to a volume of 25 mL by adding Milli-Q water and filtered (Millipore Millex HA, 0.45 µm pore size) before analysis. Moreover, Certified Reference Material (PACS-3 Marine Sediment Certified Reference Material, NRCC, Canada) was digested in the same batch as the sediment samples to ensure the accuracy of the procedure. Acceptable recoveries were obtained varying between 104 and 116%.

In addition, the adsorption of trace elements on the surface of the finest fraction of sediment as well as their amount associated with Fe and Mn oxy-hydroxides, carbonates and phosphates were evaluated by performing a weak extraction (HCl, 0.5 M) (Adami et al., 1999). For this purpose, 2 g of sediment and 20 mL of the extraction solution were rotated at room temperature for 18 ± 2 h. Samples were then centrifuged (3000 rpm for 10 minutes) and the obtained solutions were filtered (Millipore Millex HA, 0.45 µm pore size) and diluted up to a final volume of 25 mL by adding Milli-Q water.

The SPM concentrations were measured via vacuum filtration through pre-conditioned and pre-weighed membrane filters (Millipore HA, ø 47 mm, 0.45 µm pore size). Subsequently, filters were acid-digested using inverse aqua regia (suprapure $HNO_3 \geq 69\%$ and $HCl \geq 37\%$, 3:1) according the modified EPA Method 3052 (2007) and the obtained solutions were treated in the same manner described for the sediment samples.

The concentrations of major (Al and Fe) and trace (As, Co, Cu, Cs, Cr, Fe, Hg, Ni, Mn, Pb and Zn) elements in sediments (total and extracted samples) and in the SPM were detected by means of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using an Optima 8000 Spectrometer (PerkinElmer, USA) equipped with a S10 Autosampler (PerkinElmer, USA) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS, NexION 350x equipped with an ESI SC autosampler, PerkinElmer), respectively. The instrument calibration, settings and quality control were conducted according to the same criteria described for the analysis of the dissolved fraction.

Exploratory multivariate data analysis

Experimental data were analysed by principal component analysis (PCA), as an unsupervised exploratory tool for the visual identifications of relationships within samples (PC scores and score plots), within variables (PC loadings and loading plots), and between samples and variables (biplots) (Oliveri et al., 2020). Prior to multivariate analysis, data matrices were pre-processed via the application of column autoscaling, aimed at minimising systematic differences (e.g., scale effects) between the experimental variables (Oliveri et al., 2019). Multivariate data processing was performed using the CAT (Chemometric Agile Tool) package, based on the R platform (The R Foundation for Statistical Computing, Vienna, Austria) and freely distributed by Gruppo Italiano di Chemiometria (Italy) (Leardi et al., 2019).

Results and discussion

Water column characterisation

The vertical variability of the main physico-chemical parameters (turbidity, salinity and temperature) is depicted in Figure 2.

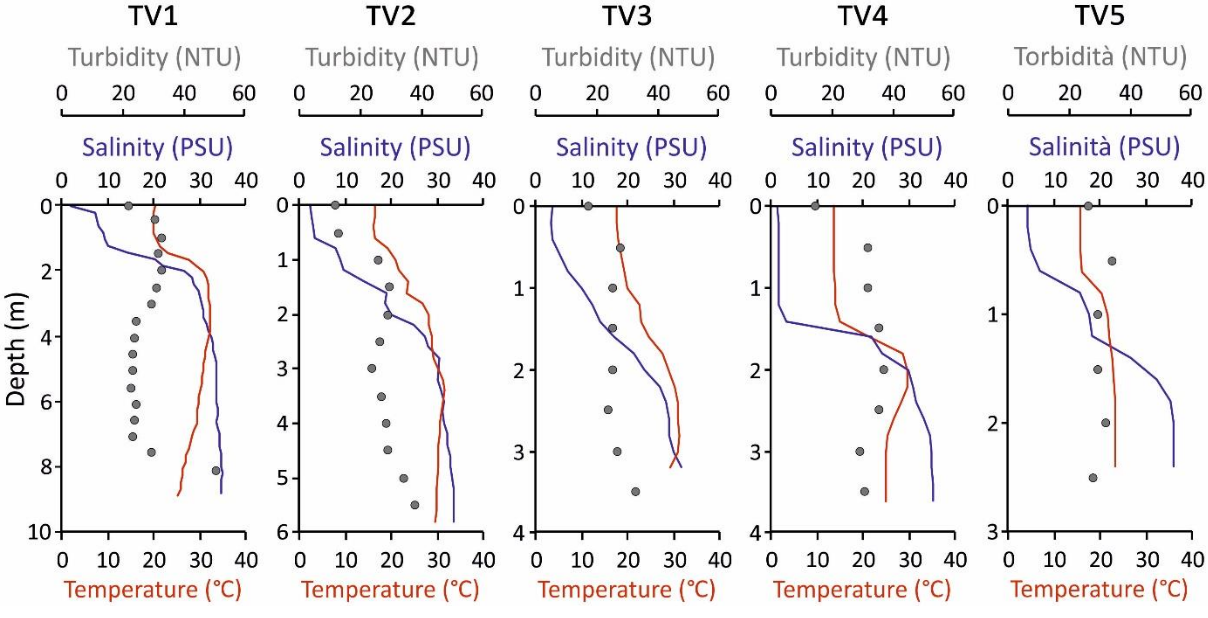


Figure 2. Vertical profiles of turbidity (NTU), salinity (PSU) and temperature (°C) recorded in the estuarine system of the Timavo River (sampling stations TV1, TV2, TV3, TV4 and TV5).

The estuarine system of the Timavo River showed an evident salt-wedge intrusion and a sharp halocline was recorded at different sampling sites. Although site TV4, which is representative of the main branch of the Timavo River, displayed a strong prevalence of surface freshwater showing a thickness of approximately 1.5 m, the salinity generally increased with depth reaching values typical of marine waters at the bottom (34.3 PSU). On the other hand, an increased mixing between riverine and marine waters was also clearly evident downstream at the bottom of the sampling site TV3 (28.8 PSU). A similar condition of mixing was also recorded in the external sector (TV5), where the salinity gradient appears to be more affected by the coastal marine waters (35.0 PSU at the bottom).

Regarding turbidity, substantial variations along the water column and among different sampling sites were not found. Indeed, turbidity values were quite constant with increasing depth (25-30 NTU).

Due to the discharge of cooling water from a thermal plant, warm water was found in the mixing and bottom water layers, reaching maximum values equal to 32.1 °C (TV1) and 31.4 °C (TV2), between a depth of 3 and 4 m in the innermost sampling sites and the increase in temperature was also quite evident at the sampling site TV3 (30.3 °C between 2 and 3 m depth). However, the Timavo River freshwater input (TV4) and the external area (TV5) showed relatively lower values (27.1 °C and 23.4 °C between a depth of 2 and 3 m at TV4 and TV5, respectively), most likely due to dilution effects related to the increasing mixing conditions between river freshwater and seawater.

Despite significant differences in terms of pH not being recorded, the bottom layer generally showed higher values of pH, as a consequence of the presence of the salt-wedge intrusion. The highest differences between the surface and the bottom water layers were measured at sites TV3, TV4 and TV5, where the freshwater contribution prevailed (Figure 3).

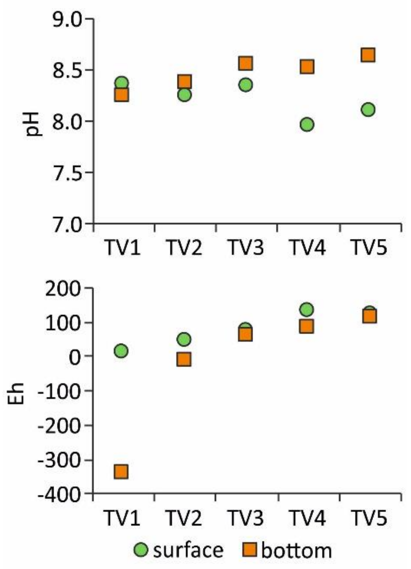


Figure 3. pH and redox-potential (Eh) in the surface and bottom water layers measured at the estuarine system of the Timavo River (sampling sites TV1, TV2, TV3, TV4 and TV5).

The redox potential showed a gradual increase by moving from the innermost sampling sites (TV1 and TV2) to the external sector (TV5), both in the surface layer and at the bottom. Although significant differences between the water layers were not observed at sites TV3, TV4 and TV5, where oxidising conditions prevailed, reductive conditions were found at the bottom of the innermost sampling sites. Here, the bottom water displayed Eh of -341 mV and -15 mV in TV1 and TV2 respectively, thus testifying to potential anoxic conditions at the SWI. On the contrary, the surface freshwater displayed more oxidising conditions (12 and 45 mV in TV1 and TV2, respectively) (Figure 3).

As previously mentioned, the dissolved oxygen was unfortunately not detected during sampling (July 2018). However, quite low oxygenation (2.87 and 4.69 mg L^{-1} in TV1 and TV2, respectively) and redox potential (32.7 and 31.4 in TV1 and TV2, respectively) were measured at the bottom during an additional survey (November 2018). These results suggest that potential anoxia at the bottom could have occurred during summer as already reported in similar aquatic environments (Turner et al., 1987; Riedel et al., 1999; Petranich et al., 2018b).

Surface sediments: grain size composition and occurrence of trace elements

Grain size was found to be different among sampling sites despite the innermost sector of the study area (TV1 and TV2) showing very similar grain size spectra and composition (Figure 4).

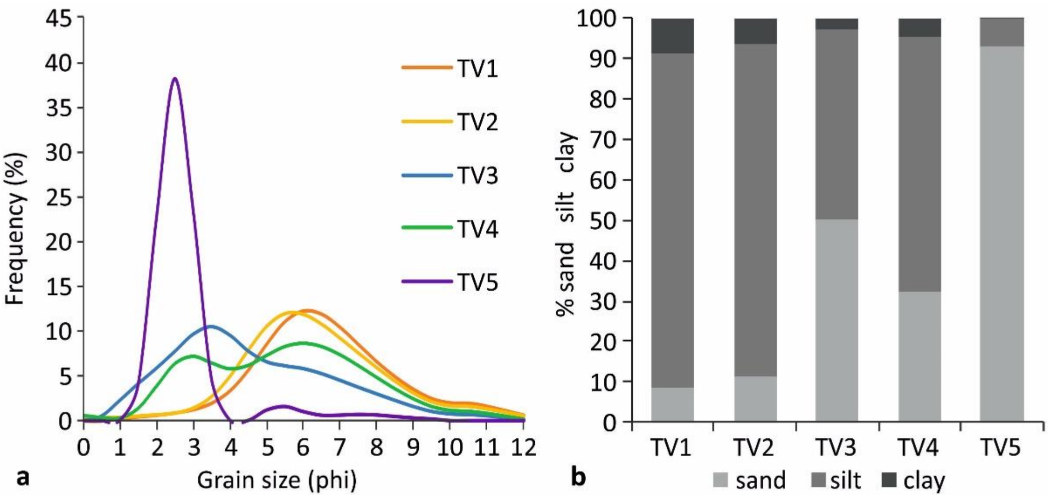


Figure 4. a) grain size spectra and b) grain size composition (sand, silt and clay) expressed in percentages of the surface sediment samples collected at five different sites near the mouth of the Timavo River.

According to Shepard's (1954) classification, TV1 and TV2 consisted mostly of silt (82.6 and 82.0 %, respectively) and showed the highest percentage of clay (8.68 and 6.60 % in TV1 and TV2, respectively) whereas the sandy fraction was less represented. The latest fraction clearly increased by moving from the innermost area (TV1 and TV2) to the external sector (TV5) where the percentage of sand was equal to 92.8 %. The silty fraction was dominant in the surface sediments from the Timavo River freshwater inflow (62.6 % in TV4), followed by the sandy fraction (32.5 %) and clay (4.90 %).

Sediments often act as a reservoir of contaminants, including potentially toxic trace elements (Piou et al., 2009; Acquavita et al., 2010). The surface sediments of the Timavo River were found to be enriched in several trace elements (Table S1), reaching concentrations that often exceeded the Italian regulatory threshold limits (Italian legislative decree 260/2010), especially for Hg, Ni and Cr (Figure 5).

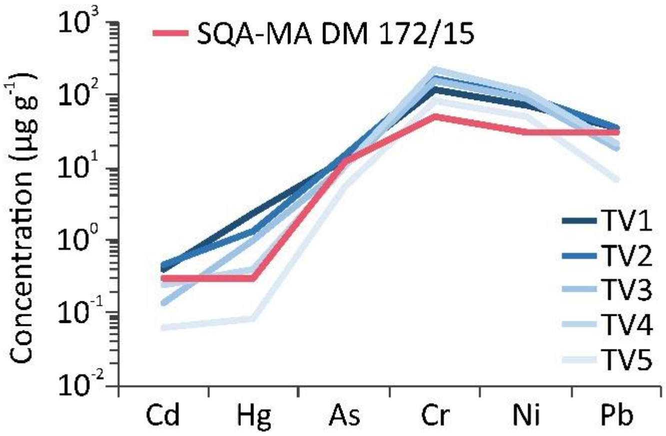


Figure 5. Comparison between Cd, Hg, As, Cr, Ni and Pb contents (μg g^{-1}) in the surface sediments collected near the mouth of the Timavo River and the Italian regulatory threshold limits (Italian legislative decree 172/15 according to EU Directive 2000/60/EC).

Nickel and Cr reached the maximum values at site TV4 (109 and 216 μg g^{-1} of Ni and Cr, respectively) exceeding the threshold limit values (30 and 50 μg g^{-1} for Ni and Cr, respectively) in the whole study area. Moreover, Hg levels were found to significantly exceed the threshold limit value (0.30 μg g^{-1}) with the only exception of the external sector of the area (0.08 μg g^{-1} in TV5). Trace element contents were found to be comparable with those reported for the nearby Panzano Bay and with the exception of Hg, slightly higher than those measured in front of the Isonzo River mouth (Acquavita et al., 2010). Indeed, according to Acquavita et al. (2010), the surface sediments in front of the Isonzo River mouth displayed Ni concentrations ranging between 62 and 63 μg g^{-1}, which are significantly lower compared to those measured in the estuarine system of the Timavo River (48.8 – 109 μg g^{-1}). This is also true for Cr, which showed concentrations between 81.6 and 216 μg g^{-1}, whereas contents for the same metal at the mouth of the Isonzo River were found from 73 to 84 μg g^{-1} (Acquavita et al., 2010). The enrichment in Cr in the easternmost sector of the Gulf of Trieste has previously been reported by Faganeli et al. (1991) and Covelli and Fontolan (1997) as the result of alteration processes of flysch formation outcropping in the continental area (Lenaz et al., 1996).

Since the Timavo River estuarine system is affected by several industrial and human activities, a comparison can be made with the results obtained by Petranich et al. (2018b) in two tourist port areas of the Gulf of Trieste. Trace element contents were of the same order of magnitude found at the marina of Lucija (Slovenian sector of the Gulf of Trieste) and significantly lower than the marina of San Rocco (Italian sector of the Gulf of Trieste), which appeared to be the most contaminated showing significantly higher concentrations of As (151-1253 μg g^{-1}), Cu (329-1185 μg g^{-1}), Hg (73.8-888 μg g^{-1}), Pb (680-3458 μg g^{-1}) and Zn (759-1959 μg g^{-1}).

However, the potential anthropogenic contribution and the degree of contamination in the surface sediments cannot be fully assessed through the total concentration of one or more contaminants. Indeed, disparities in grain size and mineralogical composition are also important factors defining the state of the quality of the bottom sediments. In this work, trace elements were normalised with respect to Al and the Enrichment Factor (EF) was calculated in order to evaluate the enrichment or depletion of Cu, Cr, Fe, Hg, Mn, Ni, Pb and Zn. The EFs were calculated using linear regression parameters between trace elements and Al as proposed by Covelli and Fontolan (1997) in order to calculate the regional baselines and for Hg EFs were calculated according to the following equation (1)

$$(1)\ EF = (Me/Al)_{sample}/(Me/Al)_{background}$$

where the background level corresponds to the average Hg concentration found in the uncontaminated levels of the deepest sections of three sediment cores from the Gulf of Trieste (Covelli et al., 2006a). The results are reported following the five classes proposed by Sutherland (2000) according to the EF value in Table 1.

Table 1. Enrichment factors (EFs) for Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn in the surface sediments collected at the mouth of the Timavo River. The classification proposed by Sutherland (2000) was applied.

Site	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
TV1	1.38	6.07	1.62	24.3	0.78	0.87	0.36	2.98
TV2	1.61	4.25	1.63	10.3	0.73	0.92	0.33	2.36
TV3	2.03	2.65	1.36	12.3	0.54	1.15	0.21	1.61
TV4	2.53	2.49	1.59	4.04	0.78	1.33	0.23	1.62
TV5	1.38	0.63	1.10	1.95	0.61	0.92	0.09	1.38

EF (Sutherland 2000)
< 2 deficiency to low enrichment
2-5 moderate enrichment
5-20 significant enrichment
20-40 very high enrichment
> 40 extremely high enrichment

According to this classification, the highest enrichment was reached by Hg (EF=24.3) in the innermost area (TV1) and decreased to significant (TV2 and TV3) and moderate (TV4 and TV5) by moving towards the external sector of the study area. The occurrence of Hg in the estuarine system of the Timavo River can be related to two different sources: the first is linked to the role of the Isonzo River, which is known to be the primary source of particulate Hg (PHg) entering the Gulf of Trieste (Covelli et al., 2007). In fact, it has been demonstrated that stream losses from the

Isonzo River, especially under conditions of low discharge, may act as one of the main sources of groundwater in the unconfined aquifer of the Isonzo River alluvial plain (Cerovac et al., 2018) and influence the Classical Karst groundwater (Doctor et al., 2000; Calligaris et al., 2018). Moreover, according to Doctor et al. (2000), although karstic groundwater showed quite low dissolved Hg (DHg) concentrations, significant variability occurred under different hydrological conditions. Consequently, despite the hydrochemical seasonal variability of the discharge at the Timavo River springs is mainly related to the hydrochemical variability of the upper course of the river (Cucchi et al., 1997), the occurrence of Hg suggests that mixing processes involving Isonzo River freshwater could occur. On the other hand, it cannot be excluded that the high concentrations of Hg in sediments may be related to the role of the tidal fluxes. Indeed, resuspension events occurring in the Panzano Bay (THg=5.54 $\mu g\ g^{-1}$) (Acquavita et al., 2010), lead to the transport of Hg associated with the finest fraction into the innermost sector of the Timavo River estuarine system (TV1 and TV2). Here, low hydrodynamic conditions may favour the settling of the suspended particles and accumulate this contaminant at the bottom.

Potential mobility of toxic trace elements from the surface sediments to the water column

Sediments act as a sink and also a secondary source of contaminants due to remobilisation processes that occur at the SWI (Caplat et al., 2005; Zonta et al., 2019), thus also affecting water quality as well as the aquatic biota. The potential adverse biological effects to benthic organisms were evaluated according to the guidelines published by NOAA (National Oceanic and Atmospheric Administration, USA) by means of the Effect Range-Low (ERL) and the Effect Range-Median (ERM) which are two indices derived from toxicity tests (Table 2) (Long et al., 1995). According to those indices: i) concentrations below the ERL value indicate minimal ecotoxicological effects or conditions in which effects would rarely occur ii) concentrations between ERL and ERM identify possible ecotoxicological effects or conditions in which effects would be occasionally observed iii) concentrations equivalent or above the ERM testify to probable effects or conditions in which effects frequently occur (Long et al., 1995).

Table 2. Comparison of the Effect Range-Low (ERL) and Effect Range-Median (ERM) indices (Long et al., 1995) with concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb and Zn ($\mu g\ g^{-1}$) in the surface sediments collected from the estuarine system of the Timavo River (sampling sites TV1, TV2, TV3, TV4 and TV5).

	Long et al., 1995		This study				
Element	ERL	ERM	TV1	TV2	TV3	TV4	TV5
	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
As	8.20	70.0	13.3	14.7	10.3	10.5	5.40
Cd	1.20	9.60	0.39	0.44	0.13	0.24	0.06
Cr	81.0	370	119	163	160	216	81.6
Cu	34.0	270	135	110	53.9	54.7	9.75
Hg	0.15	0.70	2.40	1.34	1.03	0.39	0.08
Ni	20.9	51.6	71.9	92.2	86.0	109	48.8
Pb	46.7	218.0	33.9	34.7	18.3	21.0	6.72
Zn	150	410	222	214	109	121	66.0

< ERL

ERL ≤ x < ERM

> ERM

Generally, the innermost sector (TV1 and TV2) displays the worst conditions in terms of potential ecotoxicological risk. In detail, Ni and Hg contents were found to be higher than the ERM set threshold, thus suggesting probable adverse ecotoxicological effects. In the case of Hg, this result can be of major concern as anoxic surface sediments may act as a significant source of the more toxic and potentially bioaccumulable form of Hg (methylmercury) (Bloom et al., 1999; Mason et al., 2006; Emili et al., 2014; Cossa et al., 2017): reductive conditions at the SWI, especially in TV1 and TV2, could favour methylation processes promoted by sulfate-reducing bacteria (Ullrich et al., 2001; Du et al., 2019).

Possible ecotoxicological effects were also observed for Cr, the concentration of which was always found to be between the ERL and the ERM values, whereas minimal risk rarely occurred for Cd and Pb.

The total concentration is not enough to clearly assess the potential environmental risk linked to the occurrence of potentially toxic trace elements stored in sediments (Gleyzes et al., 2002; Zonta et al., 2019). Indeed, the chemical form is one of the main factors affecting the potential release of trace elements and other contaminants from sediments to porewaters and to the water column (Riedel et al., 1999; Eggleton and Thomas, 2004). Moreover, the boundary conditions, especially the redox state, also play a crucial role in regulating trace element mobility (Riedel et al., 1999; Shaheen et al., 2016). For this reason, single or sequential extractions are generally applied in order to investigate the potential mobility of contaminants from sediments to the overlying water (Adami et al., 1999, 2000b, Gleyzes et al., 2002; Sahuquillo et al., 2003).

Principal component analysis (PCA) was performed on chemical (total and extracted concentrations of trace elements) and grain-size data in order to reveal differences among the investigated sampling sites. PCA was executed after column autoscaling and 88.9 % of the total variance was explained by PC1 and PC2 (Figure 6).

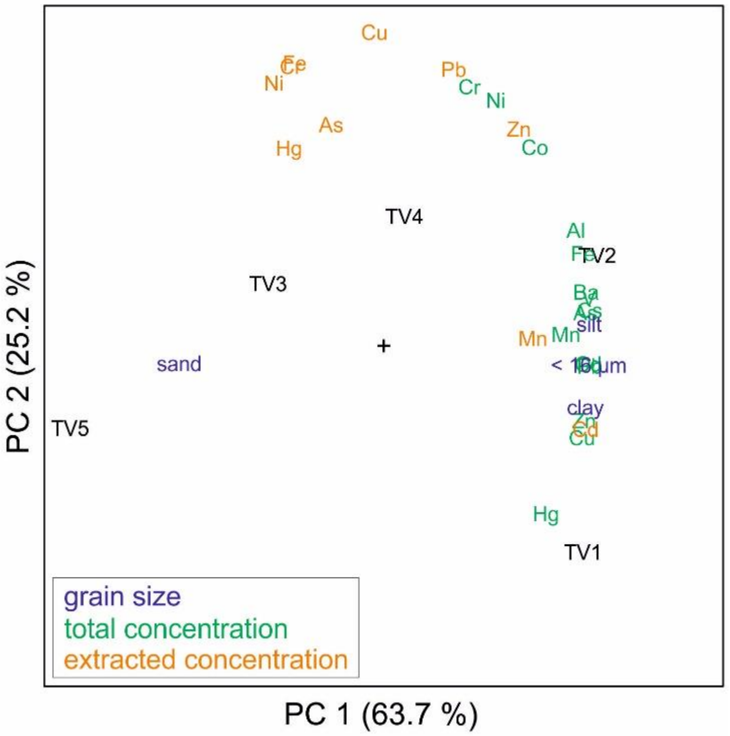


Figure 6. Biplot resuming the results of PCA performed on grain size composition, total and extracted trace element concentrations detected in the surface sediments collected at the mouth of the Timavo River (sampling sites TV1, TV2, TV3, TV4 and TV5).

The PCA output (Figure 6) clearly denotes that several trace elements are strongly associated with the silty-clay fraction, which prevails in the innermost sector (TV1 and TV2). Conversely, the sediment consists mostly of sand in the external area (TV5), where total trace element concentrations are significantly lower. This trend is markedly evident for Hg, which reaches a value of 2.40 µg g^{-1} in the innermost sampling site (TV1) and gradually decreases to a concentration of 0.08 µg g^{-1} in the external sector (TV5).

According to several authors (e.g. Covelli et al., 2001; Yao et al., 2016), the clay and fine silt fractions (<16 µm) exhibited the highest adsorption capacities for several trace elements. This is in agreement with the results of this research due to the strong positive correlations between total trace element contents and the <16 µm fraction with the exception of Cr and Ni (Figure 6).

The high concentrations of Cr and Ni observed in the surface sediments at site TV4 (216 and 109 µg g^{-1} of Cr and Ni, respectively) may originate from alteration processes of the Cr-spinel enclosed in the bauxite veins found in the Late Maastrichtian-Paleocene bauxite layer (Lenaz et al., 2002).

In fact, Cr-spinel is a common heavy mineral in the Quaternary reddish-brown silt-clayey soils depleted in OM (Lenaz et al., 1996) which was found to be similar to flysch in terms of $^{87}Sr/^{86}Sr$ isotopic ratio and mineralogical composition (Lenaz et al., 1996). Runoff from these soils and erosion from flysch formation would then be responsible for the enrichment of Cr and Ni the fine suspended load carried by the Timavo River.

At site TV1, scarce mobility was observed for most of the trace elements investigated (Cr<Pb<Fe<Hg<Cu<As<Ni<Zn<Cd<Mn) most likely due to the prevalence of refractory phases such as sulphides or strong bindings with OM (Sahuquillo et al., 2003; Emili et al., 2016). Conversely, although reductive conditions occurred (Eh = -15 mV), significant levels of labile trace elements were observed at site TV2 (Pb>Mn>Cu>Zn>Cd>Fe>Ni>As>Cr>Hg). In this case, reductive conditions at the SWI might have been responsible for the reduction of Fe and Mn oxy-hydroxides with the subsequent release in solution of other potentially associated trace elements (Mason et al., 2006). Since the reduction of Mn and Fe oxy-hydroxides usually occurs in the first centimetres of the bottom sediments, dissolved Fe, Mn and trace elements in the porewater profiles often reach their maximum concentration in the surface layer of sediments. These processes may promote effusive fluxes of the trace elements at the SWI where sediments are eventually depleted in their mobile phases (Warnken et al., 2001; Caplat et al., 2005).

A similar situation was identified in two tourist port areas in the Gulf of Trieste (Petranich et al., 2018b). The authors observed that the high percentages of the labile trace elements were reached in the sediments of the Lucija marina although total trace element contents were far lower compared to the more contaminated site of the San Rocco marina.

On the whole, the labile fraction of several trace elements (As, Cu, Fe, Hg, Mn, Ni, and Pb) increases by moving from the innermost sector (TV1) to the external sector (TV3 and TV5), thus testifying to the presence of more available chemical forms moving downstream in the estuarine zone (Mn>Pb>Cu>Cd>Fe>Ni>Zn>As>Hg>Cr in TV5) (Figure 6). A significant increase is evident for Hg, the labile percentage of which varies between 0.05% (TV1) and 13.6% (TV5), and other trace elements such as As (19.2%), Cr (10.7%) and Ni (45.6%) which achieved the maximum labile percentages at site TV5.

Dissolved phase: processes controlling dissolved trace elements and nutrients

Although trace element concentrations in the dissolved fraction did not exceed the Italian regulatory threshold limits, substantial differences occurred between different water layers (surface and bottom) and among sampling sites (Table S2).

The N-NO$_3^-$ appears to be the predominant form of dissolved inorganic nitrogen (DIN) in the surface freshwater (99.5-100%) whereas N-NH$_4^+$ (0.01-0.24%) and N-NO$_2^-$ (0.01-0.22) were less represented (Table S3). The N-NO$_3^-$ also prevailed in the bottom layer of the water column varying between 99.2% (TV5) and 49.6% (TV1), whereas N-NH$_4^+$ and N-NO$_2^-$ reached elevated concentrations at site TV1 representing 47.2% and 3.21% of the DIN, respectively.

The relative importance of N and P in regulating the primary production is generally described by the DIN:SRP molar ratio (Acquavita et al., 2010; Petranich et al., 2018a), which was found to be higher than 16 (Redfield et al., 1963) suggesting that the system was P-limited. The only exception is represented by the bottom water at site TV1, which showed a DIN:SRP molar ratio equal to only 2.30. Significant disparities in the concentrations of DOC and TN were not observed along the water column and among different sampling sites (Table S3). The DOC:TN molar ratio was found to be higher at the bottom and lower than 10, attesting to the marine origins of the OM.

In order to evaluate the distribution of dissolved trace elements and nutrients in the estuarine system of the Timavo River, PCA was performed (Figure 7). After column autoscaling, 83.3% of the total variance was explained by PC1 and PC2.

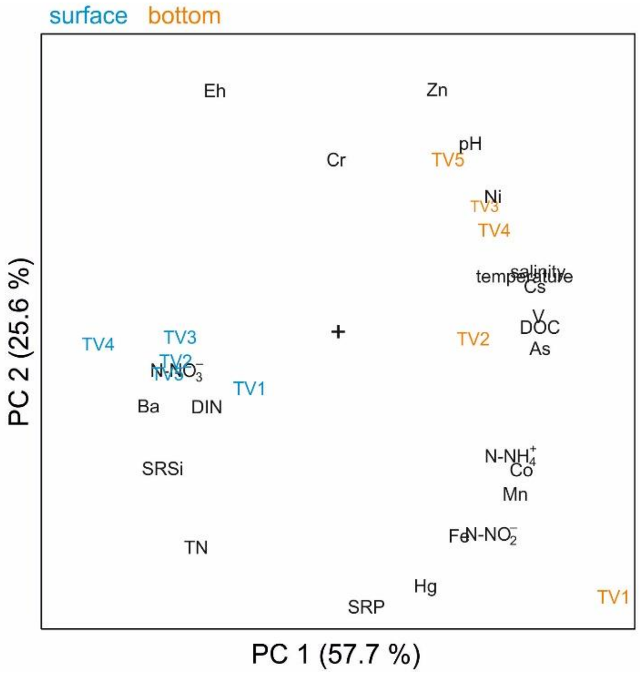


Figure 7. Biplot resuming the results of PCA performed on physico-chemical parameters, dissolved concentrations of trace elements, nutrients, DOC and TN detected in the surface and bottom water samples collected at the mouth of the Timavo River (sampling sites TV1, TV2, TV3, TV4 and TV5).

The PCA output clearly denotes the difference between the surface freshwater and the salt water at the bottom. The surface freshwater was dominated by the presence of N-NO_3^- and also showed the highest concentrations of Ba (15.2 – 23.3 µg L^{-1}), TN (83.8 – 96.8 µmol L^{-1}) and SRSi (73.9 – 99.8 µmol L^{-1}).

The strongly reductive conditions (Eh = -341 mV) measured in the bottom water at site TV1, as evidenced by anoxic conditions at the SWI, could be responsible for the precipitation of sulphides with the subsequent removal of dissolved trace elements from the water column (Huerta-Diaz and Morse, 1992; Tribovillard et al., 2006; Scholz et al., 2013). This is in agreement with the results of the weak extraction that did not indicate significant trace element mobility at site TV1.

According to Tribovillard et al. (2006), anoxic conditions in the water column may be favoured by scarce water circulation and renewal and are typical of confined aquatic systems where dissolved oxygen is consumed by intense OM degradation. Under these conditions, OM degradation is driven by secondary oxidant sources such as nitrate, Fe-Mn oxy-hydroxides and sulphate (Froelich et al., 1979). In addition, the extremely negative values of Eh indicated conditions of sulphate-reduction where hydrogen sulphide (H_2S) occurs within the water column due to reduction processes mediated by sulphate-reducing bacteria (Froelich et al., 1979; Tribovillard et al., 2006).

Although the sampling done in this study only provides a snap-shot of these processes, it is reasonable to suppose that a certain amount of H_2S may occur above the SWI at site TV1. This could allow for the precipitation of sulphides and the subsequent storage of trace elements into the residual fraction of the sediments.

The bottom layer displayed elevated concentrations of dissolved Mn (366 µg L^{-1}) and Fe (25.6 µg L^{-1}), followed by As (2.97 µg L^{-1}) and Hg (5.10 ng L^{-1}). The redox cycling of Fe and Mn are strictly connected (Tribovillard et al., 2006; Dellwig et al., 2010) although two different behaviours occur: a) Fe is efficiently involved in sulphide precipitation whereas Mn does not show the same affinity to organic or sulphide phases (Huerta-Diaz and Morse, 1992; Algeo and Maynard, 2004) and b) Fe and Mn display different oxidation kinetics since the Fe oxidation process is faster (Konovalov et al., 2004; Tribovillard et al., 2006).

According to Konovalov et al. (2004), the redox cycling of Fe and Mn in stratified (oxic-anoxic) water column are driven by two fundamental processes: the oxidation of Mn(II) and Fe(II) in the upper oxic layer and the reduction-dissolution processes of sinking Fe and Mn oxy-hydroxides in the lower anoxic water layer. Moreover, under conditions of high stratification of the water column elevated concentrations of dissolved Mn occur immediately below the oxygen-H_2S interface (Tribovillard et al., 2006; Yakushev et al., 2009) due to re-dissolution of MnO_x particles in sulfidic water (Dellwig et al., 2010).

Depletion in dissolved oxygen at the SWI could also promote the reduction of As and subsequent desorption from Fe and Mn oxy-hydroxides (Smedley and Kinniburgh, 2002) increasing dissolved As in the innermost sector (2.97 and 2.31 µg L^{-1} in TV1 and TV2, respectively). In addition, McLaren and Kim (1995) stated that the increase in dissolved As could be the result of temperature-controlled microbial reduction of As(V) to As(III). Although no information about As speciation was provided, this could be in agreement with our results. In fact, dissolved As was one order of magnitude higher in the bottom saltwater, where the temperature was significantly higher compared to that in the surface freshwater.

Since the reductive forms of N prevails under anoxic conditions, a significant increase of N-NO_2^- and N-NH_4^+ was evident in the bottom water layer at site TV1. Denitrification processes and degradation of OM (Acquavita et al., 2010; Emili et al., 2011) could result in a significant decrease in N-NO_3^-, the concentration of which was quite low at site TV1 (8.19 µmol L^{-1}), leading to an increase in N-NH_4^+ (7.78 µmol L^{-1}).

Moreover, the bottom water also displayed a significant concentration of SRP, which is one order of magnitude higher (7.18 µmol L^{-1}) compared to the other sampling sites and to the surface freshwater. Under these boundary conditions, the release of Fe and Mn have already been reported (Mason et al., 2006; Emili et al., 2011) and could originate from reduction-dissolution of particulate Fe and Mn oxy-hydroxides, Fe phosphate and Fe-Mn-P particle aggregates (Dellwig et al., 2010) sinking to the SWI.

Trace element phase partitioning between the suspended particles and the dissolved phase

The trace element concentrations in the suspended particles were found to be quite constant in the surface freshwater and among different sampling sites (Table S4). Differences were found between the surface and the bottom water layers, especially regarding particulate Al, As, Cu, Fe and Mn, Zn, which appeared to be higher at the bottom. Particulate Mn was notably present at the bottom ranging between 4.48 µg L^{-1} (TV3) and 34.1 µg L^{-1} (TV1), followed by particulate Fe (104-286 µg L^{-1} in TV3 and TV2, respectively).

Since trace element behaviour is influenced by several physical and biogeochemical processes in estuaries, their sole concentration in the SPM is generally not enough to clearly describe their mobility and phase partitioning. The Log K_D represents a useful index for evaluating trace element fractionation between the dissolved fraction and the suspended particles (Turner, 1996; EPA 1999; Gagnon and Saulnier, 2003). As reported in Table 3, the Log K_D values were found to be generally high in the surface freshwater, especially at sites TV3, TV4 and TV5.

Table 3. Log K_D values for As, Ba, Co, Cr, Fe, Mn, Ni and V in water samples (surface and bottom) collected in the estuarine system of the Timavo River (TV1, TV2, TV3, TV4 and TV5).

Element	Layer	TV1	TV2	TV3	TV4	TV5
As	surface	4.58	4.77	4.86	5.00	4.90
	bottom	3.89	4.07	3.82	4.17	4.24
Ba	surface	4.10	3.94	4.21	4.17	4.26
	bottom	3.70	4.00	4.02	4.23	4.05
Co	surface	5.87	5.97	6.28	6.58	6.27
	bottom	4.61	5.06	5.24	5.38	5.41
Cr	surface	5.91	5.56	5.69	5.88	6.13
	bottom	5.85	5.47	5.02	5.21	5.74
Fe	surface	6.95	6.84	7.16	7.51	7.41
	bottom	5.74	6.71	6.92	6.64	6.93
Mn	surface	5.03	5.46	5.52	5.91	5.57
	bottom	3.85	5.16	4.97	5.08	5.29
Ni	surface	5.58	5.76	5.67	5.99	5.74
	bottom	4.75	5.06	4.92	5.02	5.07
V	surface	5.16	5.29	5.54	5.48	5.44
	bottom	4.41	4.62	4.51	4.79	4.76

Indeed, the surface freshwater layer is dominated by oxidative conditions which could promote oxidation and precipitation processes of the dissolved phase thus increasing the partitioning in the SPM. On the contrary, the reductive conditions and potential anoxia at the bottom layer led to the preferential partitioning of several trace elements in the dissolved phase (Mn<As<V<Co<Ni<Fe), which show the lowest Log K_D value especially at site TV1.

As a final approach, PCA was performed on the whole dataset (trace elements, nutrients, DOC and TN) and matrices (sediment, SPM and dissolved fraction) in order to provide a complete overview of the occurrence of trace elements and to investigate the major processes controlling their mobility and partitioning between solid and aqueous phases. PCA was performed after column autoscaling and 69.6% of the total variance was explained by PC1 and PC2 (Figure 8).

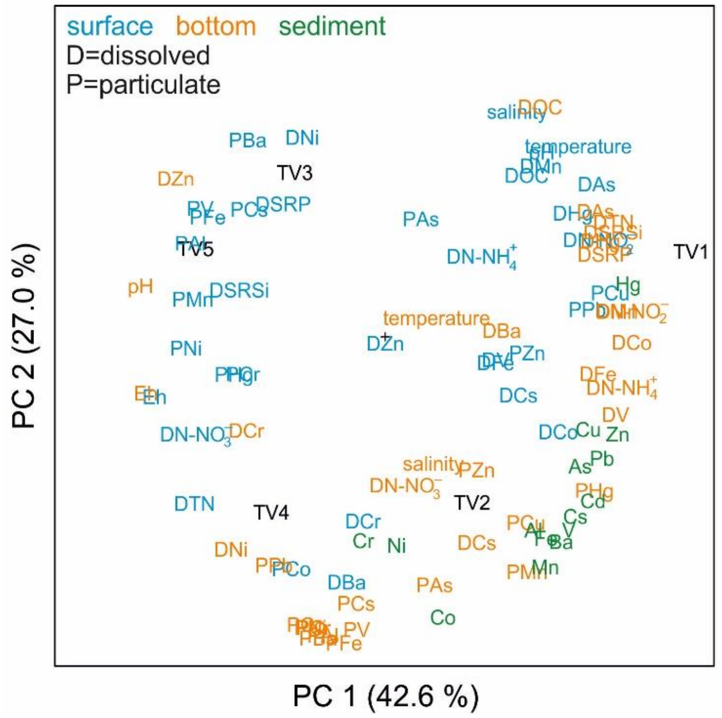


Figure 8. Biplot resuming the results of PCA performed on physico-chemical parameters and concentrations of trace elements, nutrients, DOC and TN detected in different environmental matrices (surface sediments, SPM and dissolved fraction) collected at the mouth of the Timavo River (TV1, TV2, TV3, TV4 and TV5).

As previously mentioned, dissolved trace elements prevailed at the bottom in the innermost sector (TV1 and TV2) where the reductive conditions also promoted an increase in N-NO_2^-, N-NH_4^+ and SRP. Conversely, oxidative conditions prevailed in the surface freshwater and in the whole water column at sites TV3 and TV5 where oxidation and/or precipitation processes led to trace element partitioning in the suspended particles.

Moreover, particulate trace elements were also notably present in the bottom layer most likely due to resuspension events. The Timavo River is confirmed as the primary source of Cr and Ni, which appear to be preferentially partitioned in the suspended particles at site TV4, along the main branch of the river, reaching concentrations equal to 1.11 and 0.92 µg L^{-1}, respectively. PCA also denotes a quite strong correlation between particulate Cr and Ni and their concentrations in the surface sediments, thus suggesting the potential resuspension effects involving the finest fraction of sediments.

An analogous situation was found at site TV2, where particulate Mn, Cu and As show significant correlations to the total trace element content in the surface sediments.

Conclusions

The estuarine system of the Timavo/Reka River was found to be heterogeneous in terms of occurrence and distribution of potentially toxic trace elements in spite of the limited extension of the investigated area. Oxidation and precipitation processes prevailed in the surface freshwater driving to trace elements association with the suspended particles. The innermost sector was recognised as the most critical area. There, elevated concentrations of potentially toxic trace elements were detected in the surface sediments and probable adverse ecotoxicological effects were found for Ni and Hg.

In addition, potential anoxic conditions at the SWI appear to be responsible for the removal of dissolved trace elements from the water column with a subsequent accumulation in sediments. However, recycling of sinking Fe and Mn oxy-hydroxides was thought to play a crucial role in the anoxic water layer. These results suggest that a better understanding of the chemical status of this aquatic environment is needed, especially in the innermost sector. Specifically, future research should investigate trace element speciation in sediments, as well as biogeochemical cycling of nutrients and potentially toxic trace elements at the SWI and their impact on the water column.

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Supplementary material

Table S1. Concentration of major (%) and trace elements (µg g^{-1}) in the surface sediments collected at the mouth of the Timavo River.

Element		TV1	TV2	TV3	TV4	TV5
Al	%	4.45	5.85	3.77	4.38	1.94
Ca	%	10.1	6.28	3.71	2.72	1.09
Fe	%	2.84	3.49	2.13	2.75	1.17
Mg	%	2.95	2.28	2.16	1.43	0.66
As	µg g^{-1}	13.3	14.7	10.3	10.5	5.40
Ba	µg g^{-1}	172	200	120	158	89.8
Cd	µg g^{-1}	0.39	0.44	0.13	0.24	0.06
Co	µg g^{-1}	13.1	16.6	12.1	16.9	10.1
Cr	µg g^{-1}	119	163	160	216	81.6
Cs	µg g^{-1}	5.87	7.12	3.11	4.53	1.20
Cu	µg g^{-1}	135	110	53.9	54.7	9.75
Hg	µg g^{-1}	2.40	1.34	1.03	0.39	0.08
Mn	µg g^{-1}	377	376	252	376	259
Ni	µg g^{-1}	71.9	92.2	86.0	109	48.8
Pb	µg g^{-1}	33.9	34.7	18.3	21.0	6.72
V	µg g^{-1}	105	123	60.7	89.8	30.4
Zn	µg g^{-1}	222	214	109	121	66.0

Table S2. Concentrations of As, Ba, Cd, Co, Cr, Cs, Cu, Hg, Fe, Mn, Ni, Pb, V and Zn in the dissolved fraction of surface and bottom water samples collected at the Timavo River mouth (sampling sites TV1, TV2, TV3, TV4 and TV5).

Element		Layer	TV1	TV2	TV3	TV4	TV5
As	µg L^{-1}	surface	0.78	0.58	0.62	0.38	0.49
	µg L^{-1}	bottom	2.97	2.31	1.95	1.88	1.79
Ba	µg L^{-1}	surface	15.2	23.3	18.4	19.3	17.5
	µg L^{-1}	bottom	8.30	8.94	8.17	7.64	7.16
Cd	µg L^{-1}	surface	<LOD	<LOD	<LOD	<LOD	<LOD
	µg L^{-1}	bottom	<LOD	<LOD	<LOD	<LOD	<LOD
Co	µg L^{-1}	surface	0.04	0.04	0.03	0.02	0.03
	µg L^{-1}	bottom	0.19	0.12	0.09	0.09	0.07
Cr	µg L^{-1}	surface	0.22	0.57	0.33	0.37	0.26
	µg L^{-1}	bottom	0.06	0.33	0.45	0.78	0.19
Cs	µg L^{-1}	surface	0.05	0.04	0.03	0.02	0.04
	µg L^{-1}	bottom	0.19	0.22	0.18	0.19	0.19
Cu	µg L^{-1}	surface	2.31	<LOD	<LOD	<LOD	<LOD
	µg L^{-1}	bottom	<LOD	<LOD	<LOD	<LOD	<LOD
Hg	ng L^{-1}	surface	2.27	<LOD	<LOD	<LOD	0.78
	ng L^{-1}	bottom	5.10	<LOD	<LOD	<LOD	<LOD
Fe	µg L^{-1}	surface	3.96	6.58	2.33	1.96	2.77
	µg L^{-1}	bottom	25.6	5.06	3.34	7.37	2.94
Mn	µg L^{-1}	surface	9.00	3.25	3.66	2.20	5.89
	µg L^{-1}	bottom	366	21.8	8.14	19.1	8.91
Ni	µg L^{-1}	surface	0.36	0.29	0.22	0.25	0.56
	µg L^{-1}	bottom	0.54	0.72	0.62	1.00	0.73
Pb	µg L^{-1}	surface	<LOD	<LOD	<LOD	<LOD	<LOD
	µg L^{-1}	bottom	<LOD	<LOD	<LOD	<LOD	<LOD
V	µg L^{-1}	surface	1.18	1.03	0.91	0.95	1.14
	µg L^{-1}	bottom	3.34	3.32	2.69	2.82	2.64
Zn	µg L^{-1}	surface	<LOD	3.30	<LOD	<LOD	<LOD
	µg L^{-1}	bottom	<LOD	4.47	6.76	11.6	23.5

Table S3. Concentrations of nutrients ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, SRP and SRSi), DOC and TN in the dissolved fraction of surface and bottom water samples collected at the Timavo River mouth (sampling sites TV1, TV2, TV3, TV4 and TV5).

		Layer	TV1	TV2	TV3	TV4	TV5
$N\text{-}NO_3^-$	µmol L^{-1}	surface	60.0	82.5	68.8	85.4	100
	µmol L^{-1}	bottom	8.19	114	14.7	9.66	13.1
$N\text{-}NO_2^-$	µmol L^{-1}	surface	0.14	<LOD	<LOD	<LOD	<LOD
	µmol L^{-1}	bottom	0.53	0.27	0.15	0.10	<LOD
$N\text{-}NH_4^+$	µmol L^{-1}	surface	0.14	0.19	0.11	<LOD	0.15
	µmol L^{-1}	bottom	7.78	1.76	0.54	0.70	0.10
SRP	µmol L^{-1}	surface	0.25	0.28	0.27	0.19	0.45
	µmol L^{-1}	bottom	7.18	0.38	0.10	0.27	<LOD
SRSi	µmol L^{-1}	surface	75.6	75.4	73.9	80.6	99.8
	µmol L^{-1}	bottom	42.9	23.1	26.2	18.0	14.7
DOC	µmol L^{-1}	surface	101	85.6	76.6	53.8	90.9
	µmol L^{-1}	bottom	172	144	166	132	142
TN	µmol L^{-1}	surface	83.8	93.1	87.8	96.2	96.8
	µmol L^{-1}	bottom	86.6	33.7	36.0	21.8	24.6

Table S4. Concentrations of Al, As, Ba, Cd, Co, Cr, Cs, Cu, Hg, Fe, Mn, Ni, Pb, V and Zn in the SPM of surface and bottom water samples collected at the Timavo River mouth (TV1, TV2, TV3, TV4 and TV5).

Element		TV1		TV2		TV3		TV4		TV5	
		surface	bottom	surface	bottom	surface	bottom	surface	bottom	surface	bottom
Al	%	5.17	1.31	6.74	3.59	9.10	3.42	9.50	4.30	10.5	2.98
	µg L^{-1}	76.6	117	96.6	393	122	145	97.7	376	106	211
Fe	%	3.56	1.40	4.55	2.61	6.48	2.45	6.39	3.25	7.16	2.49
	µg L^{-1}	52.8	126	65.2	286	87.0	104	65.8	284	71.9	176
As	µg g^{-1}	29.7	23.1	34.0	27.1	42.3	16.8	37.5	27.6	38.6	31.4
	µg L^{-1}	0.04	0.21	0.05	0.30	0.06	0.07	0.04	0.24	0.04	0.22
Ba	µg g^{-1}	192	41.6	205	88.7	279	93.0	288	130.4	321	80.2
	µg L^{-1}	0.28	0.37	0.29	0.97	0.38	0.39	0.30	1.14	0.32	0.57
Cd	µg g^{-1}	<LOD	5.54	<LOD	6.03	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	µg L^{-1}	<LOD	0.05	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Co	µg g^{-1}	29.2	7.88	36.1	14.2	27.2	13.1	85.8	20.4	54.6	17.2
	µg L^{-1}	0.04	0.07	0.05	0.16	0.04	0.06	0.09	0.18	0.05	0.12
Cr	µg g^{-1}	176	41.4	208	98.6	194	69.8	280	127	351	105
	µg L^{-1}	0.26	0.37	0.30	1.08	0.26	0.30	0.29	1.11	0.35	0.75
Cs	µg g^{-1}	7.45	<LOD	10.6	4.93	15.7	4.86	12.9	5.34	15.4	<LOD
	µg L^{-1}	0.01	<LOD	0.02	0.05	0.02	0.02	0.01	0.05	0.02	<LOD
Cu	µg g^{-1}	479	167	251	143	113	120	178	140	286	163
	µg L^{-1}	0.71	1.49	0.36	1.56	0.15	0.51	0.18	1.22	0.29	1.16
Hg	µg g^{-1}	1.96	2.57	2.88	1.78	2.68	2.08	3.28	1.99	4.28	1.38
	µg L^{-1}	0.003	0.02	0.004	0.02	0.004	0.01	0.003	0.02	0.004	0.01
Mn	µg g^{-1}	966	2576	942	3115	1164	1056	1810	2281	2174	1727
	µg L^{-1}	1.43	23.1	1.35	34.1	1.56	4.48	1.86	19.9	2.18	12.3
Ni	µg g^{-1}	135	30.7	163	83.2	168	55.5	247	106	308	86.8
	µg L^{-1}	0.20	0.28	0.23	0.91	0.23	0.24	0.25	0.92	0.31	0.62
Pb	µg g^{-1}	160	49.4	154	66.0	117	66.5	132	148	183	124
	µg L^{-1}	0.24	0.44	0.22	0.72	0.16	0.28	0.14	1.29	0.18	0.88
V	µg g^{-1}	171	85.6	202	138	267	92.1	285	172	316	152
	µg L^{-1}	0.25	0.77	0.29	1.51	0.36	0.39	0.29	1.50	0.32	1.08
Zn	µg g^{-1}	683	535	769	262	583	327	688	553	947	462
	µg L^{-1}	1.01	4.80	1.10	2.87	0.78	1.39	0.71	4.83	0.95	3.28

Chapter 5 – Conclusions and future perspectives

In this research, trace element phase partitioning and fate was investigated in the main Italian and Slovenian estuarine environments of the Gulf of Trieste (northern Adriatic Sea). Two main topics were investigated during the research activity.

The first topic regards the evaluation of trace element (As, Cd, Co, Cr, Cs, Cu, Fe, Hg, Mn, Ni, Pb and Zn) occurrence, behaviour and phase partitioning among suspended particulate matter, colloidal material and the truly dissolved fraction at the river mouths of the Isonzo/Soča, Timavo/Reka, Rižana and Dragonja. In addition, further information regarding the different estuarine systems was provided by the analysis of organic carbon, total nitrogen and nutrients including nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), ammonium ($N\text{-}NH_4^+$), soluble reactive silicate (SRSi) and soluble reactive phosphorous (SRP).

The evaluation of the partitioning behaviour of trace elements, as well as disparities among different riverine inputs and estuarine systems is supported by a MW-PCA, which is an efficient chemometric tool for these purposes. Results highlight that the major role in the transport and distribution of trace elements is played by the suspended particulate matter, and that dilution processes between different water masses occurring in the estuarine mixing zone are responsible for the decrease in the particulate trace element concentrations along the water column.

Alteration processes and rainwater runoff from flysch formation outcropping in the continental area have been recognised as the main sources of particulate Cr and Ni, which are notably present at the Timavo/Reka (maximum values of 137 and 132 $\mu g\ g^{-1}$ of Cr and Ni, respectively) Rižana (maximum values of 288 and 161 $\mu g\ g^{-1}$ of Cr and Ni, respectively) and Dragonja (maximum values of 153 and 121 $\mu g\ g^{-1}$ of Cr and Ni, respectively) estuarine systems.

Regarding Hg, results are in agreement with previous studies and the Isonzo/Soča River still represents an active source of this element, which appears to be mainly partitioned in the solid phase. This is particularly evident after river flood events as confirmed by the elevated concentration of particulate Hg measured in winter (12.1 $\mu g\ g^{-1}$). Surprisingly, particulate Hg was also found at the Timavo/River estuarine system (maximum value of 9.79 $\mu g\ g^{-1}$), suggesting that stream losses from the Isonzo/Soča River and mixing between the two types of riverine water in the karstic groundwater system may be a reasonable explanation for the presence of Hg in this estuary.

Regarding the phase partitioning, Cr, Cu, Fe, Hg, Ni and Pb commonly tend to be associated with the suspended particles and the elevated K_D values testify to enhanced adsorption processes. To a certain extent, those elements also show high affinity to the colloidal material, although the low percentages of COC suggest that colloids are scarcely present and play a minor role in regulating trace element distribution at the investigated river mouths. Conversely, As and Cs clearly prevail in the truly dissolved fraction, especially in the marine water as a result of dissolution and/or desorption processes, whereas Co, Zn and Mn show major differences in their partitioning behaviour. High levels of particulate Mn are detected in the marine water, especially during summer (7055, 1182, 1839 and 1074 $\mu g\ g^{-1}$ at the IS, TV, RZ and DG River mouths, respectively) when oxidation and precipitation processes may be promoted by warm water temperatures.

Among dissolved inorganic nitrogen (DIN) forms, $N\text{-}NO_3^-$ clearly prevails due to rainwater runoff from soils affected by agricultural activities along the river drainage basins and the maximum percentage (99.8%) was measured in the freshwater at the Isonzo/Soča River mouth in winter. Conversely, $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ are generally less represented, show concentrations notably lower than those of $N\text{-}NO_3^-$ and the maximum concentrations were found at the Rižana estuarine system (6.89 and 8.34 $\mu mol\ L^{-1}$ of $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$, respectively).

Soluble reactive phosphorous (SRP) was generally low and the maximum concentrations were measured at the Rižana estuarine system in summer (9.85 $\mu mol\ L^{-1}$) suggesting that dissolution and/or desorption of Fe oxy-hydroxides may occur. The DIN:SRP molar ratio was employed to evaluate the relative influence of N and P as factors limiting the primary production. Results indicate that P-limitation prevails at the Isonzo/Soča, Timavo/Reka and Dragonja River mouths, whereas the Rižana estuarine system appears to switch from P-limitation in winter and autumn to N-limitation in summer.

The second topic investigated in this research regards the karstic estuarine system of the Timavo/Reka River which is subject to high anthropogenic pressure due to industrial and harbour activities. A geochemical characterisation is provided concerning the distribution, mobility and fate of trace elements in different environmental matrices (water, SPM and sediments). For this purpose, a PCA was employed to depict disparities among the sampling sites allowing for the identification of the most critical condition in the innermost sector. There, sediments were found to be enriched in trace elements which reached concentrations that, especially for Hg (2.40 $\mu g\ g^{-1}$), Ni (71.9 $\mu g\ g^{-1}$) and Cr (119 $\mu g\ g^{-1}$), exceeded the Italian regulatory threshold limits with probable adverse ecotoxicological effects to benthic organisms.

Moreover, potential anoxic conditions at the sediment-water interface (SWI) seem to promote precipitation of sulphides with subsequent removal of dissolved trace elements although recycling of sinking Fe and Mn oxy-hydroxides are suspected of playing a crucial role in the anoxic water at the bottom.

The results discussed in this doctoral study will help further understand the behaviour of trace elements transported to the estuarine environments of the Gulf of Trieste by river freshwater inputs affected by several anthropogenic sources. Future research should address the role of colloids in regulating trace element mobility in lagoon water systems where the organic matter production is higher, and in the sediment porewater, since colloids may hugely affect the total dissolved concentrations of trace elements released to the overlying water during resuspension events.

Regarding the distinctive estuarine system of the Timavo/Reka River, results suggest that further investigation is needed, especially in the innermost sector. Scientific interest should be focused on trace element speciation, both in sediments and along the water column, in order to reach a thorough understanding of the biogeochemical cycles of nutrients and trace elements at the SWI and their impact on water quality.

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Scientific outreach and didactic activity

Publication of IF Journal

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Oral presentations

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Poster presentations

Covelli S., Garcia-Ordiales E., Braidotti G., Petranich E., Pavoni E., Sanz-Prada L., Roqueñi N., Loredo J., 2019. Simulation of the effects of dredging on the mobility of mercury and arsenic in resuspended contaminated estuarine sediments (Asturias, Spain). XV International Estuarine Biogeochemistry Symposium, Book of Abstracts p. 53, Vigo (Spain), 4-5 June 2019. Presenting author: Prof. Stefano Covelli

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Pavoni E., Petranich E., Crosera M., Adami G., Baracchini E., Rusalen M., Lenaz D., Emili A., Higueras P., Covelli S., 2015. Bioaccumulation of trace metals in plants growing nearby a decommissioned Zn-Pb mine (Salafossa, Northeastern Italian Alps). XXV Congresso della Divisione di Chimica Analitica della Società Chimica Italiana, Book of Abstracts p. 320, Trieste (Italy), 13-17 September 2015. Presenting author: Dr. Elena Baracchini

Pavoni E., Petranich E., Crosera M., Adami, G., Baracchini E., Lenaz D., Emili A., Higueras P., Covelli S., 2015. Bioaccumulation of Thallium and other heavy metals in Biscutella laevigata nearby a decommissioned Zn-Pb mine (Salafossa, Northeastern Italian Alps). Il Pianeta Dinamico: sviluppi e prospettive a 100 anni da Wegener – Congresso congiunto SIMP-AIV-SoGel-SGI, Book of Abstracts p. 432, Firenze (Italy), 2-4 September 2015. Presenting author: Dr. Elisa Petranich

Pavoni E., Covelli S., Emili A., Lenaz D., Petranich E., Crosera M., Adami G., Cattelan R., Higueras P., 2015. Geochemical characterization of drainage waters after closure of sulphides extraction activity (Salafossa, Northeastern Italian Alps). Il Pianeta Dinamico: sviluppi e prospettive a 100 anni da Wegener – Congresso congiunto SIMP-AIV-SoGeI-SGI, Book of Abstracts p. 431, Firenze (Italy), 2-4 September 2015. Presenting author: Dr. Elisa Petranich

Pavoni E., Colle Fontana M., Cattelan R., Esbrì J.M., Petranich E., Emili A., Higueras P., Covelli S., 2014. Environmental issues related to abandoned zinc-lead mining operations in the Northeastern Italians Alps. Energy and Environment Knowledge Week Congress – E2KW 2014, p. 163-165, Toledo (Spain), 30-31 October 2014. Presenting author: Prof. Pablo Higueras

Didactic activity

Tutoring activity for the BSc course in Chimica Analitica II for a total of 50 hours of work. University of Trieste, Department of Chemical and Pharmaceutical Sciences

Co-supervisor of BSc thesis

Cristiano Mastroianni 2018-2019. Mobilità di metalli in tracce potenzialmente tossici da residui di lavorazione di minerali sulfurei. BSc degree in Geochimica Ambientale, BSc Course in Geologia. Supervisor: Prof. Stefano Covelli. Co-supervisor: Prof. Davide Lenaz, Dr. Elena Pavoni

Susanna Mesghez 2018-2019. Caratterizzazione mineralogica e geochimica dei sedimenti del Dosso di Santa Croce. BSc degree in Monitoraggio Ambientale, Marino e Atmosferico, BSc Course in Scienze e Tecnologie per l'Ambiente e la Natura. Supervisor: Dr. Stefano Cirilli. Co-supervisor: Prof. Davide Lenaz, Dr. Elena Pavoni

Giulia Zaia 2016-2017. Determinazione analitica di metalli potenzialmente tossici nel particellato in sospensione di ambienti estuarini. BSc degree in Chimica Analitica, BSc Course in Chimica. Relatore: Prof. Gianpiero Adami. Correlatori: Dr. Matteo Crosera, Dr. Elena Pavoni

Simone Visintin 2016-2017. Evidenze micropaleontologiche e geochimiche del passaggio Cretacico-Paleogene nella zona del Monte San Michele (GO). BSc degree in Paleontologia, BSc Course in Geologia. Relatore: Dott. Romana Melis. Correlatori: Dr. Sara Biolchi, Dr. Elena Pavoni

Co-supervisor of MSc thesis

Giuseppe Guerra 2018-2019. Elementi potenzialmente tossici in acque di sorgente nel settore occidentale delle Apli Carniche: origine e mobilità. MSc degree in Geochimica Ambientale, MSc Course in Geoscienze. Relatore: Prof. Stefano Covelli. Correlatori: Dr. Matteo Crosera, Dr. Elena Pavoni

Silvia Pisana Reinotti 2017-2018. Metalli in tracce e loro partizionamento in colonna d'acqua in un ambiente estuarino (Fiume Timavo, Golfo ti Trieste). MSc degree in Geochimica Ambientale, MSc Course in Scienze e Tecnologie per l'Ambiente e il Territorio. Relatore: Prof. Stefano Covelli, Correlatori: Dr. Matteo Crosera, Dr. Elena Pavoni

Greta Braidotti 2017-2018. Simulazione degli effetti della risospensione indotta dal dragaggio sulla mobilità dei metalli in tracce in sedimenti estuarini contaminati (Asturie, Spagna). MSc degree in Geochimica Ambientale, MSc Course in Scienze e Tecnologie per l'Ambiente e il Territorio. Relatore: Prof. Stefano Covelli, Correlatori: Dr. Efrén Garcia Ordiales, Dr. Elena Pavoni

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