

UNIVERSITY OF NOVA GORICA  
GRADUATE SCHOOL

**POLYCYCLIC AROMATIC HYDROCARBONS IN THE  
SEAWATER AND SEDIMENTS OF THE GULF OF  
TRIESTE**

MASTER'S THESIS

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*»Persist.  
Because with an idea,  
determination and the right tools,  
you can do great things.  
Let your instincts, your intellect  
and let your heart guide you.«*

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## ABSTRACT

Concentrations, spatial distribution and sources of polycyclic aromatic hydrocarbons (PAHs) were determined in surface seawater and sediments of the Gulf of Trieste, northern Adriatic. Fluorescence spectroscopy and gas chromatography techniques were used for the determination of PAHs in seawater, while gas chromatography (GC) was used for sediments. Total PAH concentrations ( $\Sigma 24$  PAHs) analyzed with GC varied from 78 ng L<sup>-1</sup> to 302 ng L<sup>-1</sup> in seawater, and from 297 ng g<sup>-1</sup> to 115,854 ng g<sup>-1</sup> (dry weight) in sediments. The surface seawater and sediments of the Gulf of Trieste are moderately to highly contaminated by PAHs compared with other marine areas through out the world. The PAHs were present in higher levels in semi-enclosed areas such as marinas and harbours, due to more intensive pollution and higher accumulation of pollutants as a consequence of the limited circulation of seawater. The seasonal variation of total PAHs in seawater measured with fluorescence spectroscopy indicated higher average concentrations in summer compared to winter. The total PAH concentrations varied from 0.07  $\mu\text{g L}^{-1}$  to 34.32  $\mu\text{g L}^{-1}$  in winter and from 0.01  $\mu\text{g L}^{-1}$  to 48.51  $\mu\text{g L}^{-1}$  in the summer period. The seawater samples were dominated by low molecular weight PAHs (2-3 ring PAHs), while sediments were dominated by high molecular weight PAHs, in particular 4- and 5-ring PAHs. Composition pattern analysis suggested that PAHs in seawater were mainly of petrogenic origin, while PAHs in sediment derived mostly from pyrogenic sources. In conclusion, a good correlation ( $r^2 = 0.94$ ;  $n=7$ ) in relative PAH concentrations (range between 1  $\mu\text{g L}^{-1}$  and 6  $\mu\text{g L}^{-1}$ ) was obtained when comparing results obtained by fluorescence spectroscopy and gas chromatography. On the other hand an important overestimation ( $\sim 20$  times) of absolute PAH concentrations was obtained by fluorescence measurement. It has been mentioned that this linear relationship was not observed at higher PAH concentrations.

**Key words:** polycyclic aromatic hydrocarbons (PAH), seawater, pollution, sediment, PAH sources, Gulf of Trieste

## POVZETEK

Magistrsko delo z naslovom »Policiklični aromatski ogljikovodiki v morski vodi in sedimentu Tržaškega zaliva« obravnava onesnaženost površinske morske vode in sedimenta s policikličnimi aromatskimi ogljikovodiki (PAH) v Tržaškem zalivu. V raziskavi so bile določene koncentracije, prostorska razporeditev, sezonsko nihanje in viri onesnaženja s PAH. Fluorescenčna spektroskopija in plinska kromatografija sta bili uporabljeni za določitev PAH v morski vodi, plinska kromatografija pa za določitev PAH v sedimentu. Koncentracije PAH izmerjene s plinsko kromatografijo so bile v območju med  $78 \text{ ng L}^{-1}$  in  $302 \text{ ng L}^{-1}$  v površinski morski vodi ter med  $297 \text{ ng g}^{-1}$  in  $115,854 \text{ ng g}^{-1}$  v sedimentu. Morska voda in sediment Tržaškega zaliva sta v glavnem zmerno onesnažena s PAH (nekatera zaprta območja so zelo onesnažena) v primerjavi z ostalimi obalnimi morskimi območji po svetu. Visoke koncentracije PAH so bile zaznane v delno zaprtih območjih (marine, mandračji) kot posledica intenzivnejšega onesnaževanja in večjega kopičenja onesnažil. Sezonska sprememba v koncentracijah PAH v površinski morski vodi analizirana s fluorescenčno spektroskopijo kaže na višje povprečne vrednosti v poletnem času v primerjavi z zimo. Koncentracije PAH v vodi so bile v območju med  $0.07 \text{ } \mu\text{g L}^{-1}$  in  $34.32 \text{ } \mu\text{g L}^{-1}$  pozimi ter med  $0.01 \text{ } \mu\text{g L}^{-1}$  in  $48.51 \text{ } \mu\text{g L}^{-1}$  poleti. V površinski morski vodi prevladujejo nizko molekularni policiklični aromatski ogljikovodiki (2-3 aromatski obroči) medtem ko v sedimentu prevladujejo visoko molekularni policiklični aromatski ogljikovodiki (predvsem 4-5 aromatski obroči). Analiza sestave vzorcev nakazuje, da v morski vodi prevladujejo PAH petrogenega izvora medtem, ko v sedimentu prevladujejo PAH pirogenega izvora. Pri relativnih koncentracijah (v območju med  $1 \text{ } \mu\text{g L}^{-1}$  in  $6 \text{ } \mu\text{g L}^{-1}$ ) je bila ugotovljena dobra korelacija ( $r^2= 0.94$ ;  $n=7$ ) med fluorescenčno spektroskopijo in plinsko kromatografijo. Podobna korelacija pa ni bila ugotovljena pri višjih koncentracijah. Potrebno je poudariti, da je bilo ugotovljeno, da so absolutne koncentracije, dobljene s fluorescenčno spektroskopijo, v povprečju 20 krat višje v primerjavi s tistimi dobljenimi s plinsko kromatografijo.

**Ključne besede:** policiklični aromatski ogljikovodiki (PAH), onesnaženje, morska voda, morski sediment, viri PAH, Tržaški zaliv.

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## 1 INTRODUCTION

Hydrocarbons are a wide spread class of environmental chemical pollutants (McElroy et al., 1989; Harvey, 1996; Liu et al., 2008; Valavandis et al., 2008) which enter the marine environment through natural pathways and anthropogenic processes. Natural sources include forest fires, natural petroleum seeps and post-depositional transformation of biogenic precursors (Young and Cerniglia, 1995). The most important anthropogenic sources are petrogenic and pyrogenic, the latter usually being largely prevalent in aquatic environments (Zakaria et al., 2002). Anthropogenic sources include urban runoff, sewage disposal, industrial effluents, oil production and transport activities (Kim et al., 1999).

Polycyclic aromatic hydrocarbons (PAHs), a class of ubiquitous contaminants with two or more fused aromatic rings, are mainly introduced into the environment via anthropogenic processes (Fulton et al., 1993; McGroddy and Farrington, 1995; Qiu et al., 2009) such as the combustion of fossil fuels and the direct release of oil and oil products, with a smaller contribution arising from forest fires and agricultural burn-off (Law and Biscaya, 1994). Anthropogenic inputs of PAHs have caused significant accumulation of PAHs in the marine environment (Simpson et al., 1996; Heath et al., 2006). Several studies (Pereira et al., 1999; Arzayus et al., 2002; Frignani et al., 2003; Heath et al., 2006) reveal that the increased anthropogenic inputs of pyrogenic PAHs in sediments correspond to industrial and urban development and fossil fuel usage since the industrial revolution.

PAHs are known to enter the marine environment through the spillage of petroleum, atmospheric fallout and urban runoff (Neff, 1979; Sharma et al., 1997; Gevaio et al., 1998; Vieites et al., 2004). Upon entering the aquatic system PAHs distribute between different phases including truly dissolved, colloids, suspended particulate matter, surface sediments and biota (Readman et al., 1987; Zhou et al., 1998). The way in which PAHs are distributed between these different phases is controlled by their intrinsic physicochemical properties including solubility, vapour pressure and lipophilicity (Zhou et al., 1998).

Because of their low water solubility (Nesterova et al., 1982) and hydrophobicity (Karcher, 1988) they tend to adsorb to particulate material (Gearing et al., 1980; Chiou et al., 1998) and deposit in the sediments (Guzzella and de Paolis, 1994; Quintero and Diaz, 1994). In this form they are more persistent to biodegradation (Readman et al., 1982; Soclo et al., 2000) in comparison to dissolved PAHs. The degradation intensity differs depending of biological, chemical and physicochemical parameters (Jovančićević et al., 2007).

Earlier studies (Hardy, 1982, 1987; Liu and Dickhut, 1997) indicate that hydrophobic organic compounds such as PAHs are often enriched in the sea surface and sediments. In the water column most PAHs tend to adsorb to particles and to be deposited in the underlying sediments (Law and Biscaya, 1994). Degradation of PAHs in sediments is generally slow, particularly with the higher molecular weight PAHs (Readman et al., 1982) and when sediments are anaerobic (Meador et al., 1995). In deep water depositional areas the sediments can be regarded as a sink for PAHs, but in shallower and more dynamic environments significant remobilization can occur (Law and Biscaya, 1994). Accumulation of PAHs in sediments can be remobilized in the seawater and become bio available to indigenous aquatic organisms (Wetzel and Van Vleet, 2004). Concentrations of PAHs at the sea surface and in sediments are of toxicological importance to both benthic and pelagic marine organisms. Thus accumulation of pollutants represents a potential hazard to the organisms (Hardy et al., 1987; Bihari et al., 2006).

As pollutants, they are of great concern since some of them have been identified as carcinogenic and mutagenic (Sander and Wise, 1988; Menzie et al., 1992; Armstrong et al., 1994; Simpson et al., 1996; Kennish, 1992; Durant et al., 1996). Moreover, PAHs can induce oxidative stress and oxidative DNA damage through metabolic activation and the generation of reactive oxygen species (Ohnishi and Kawanishi, 2002). The principal pathways of the metabolic activation of PAHs are generated from diol epoxides, formation of radical cations (Stegeman and Lech, 1991; Cavalieri and Rogan, 1995; Baird, 1995) and formation of reactive redox *o*-quinones (Penning et al., 1996).

Polycyclic aromatic hydrocarbon toxicity is very structurally dependent, with isomers varying from being nontoxic to being extremely toxic. Toxicity of those substances depends upon the number and configuration of the benzene rings and the presence and position of their substituents (Harvey, 1996). Many high molecular mass PAHs are known to be carcinogenic and mutagenic. A PAH compound, benzo[a]pyrene, is notable for being the first chemical carcinogen to be discovered. PAHs known for their carcinogenic, mutagenic or teratogenic properties are: benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, coronene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene and ovalene (Simpson et al., 1996). The PAH naphthalene and its methyl-substituted derivatives 1- and 2-methyl-naphthalenes have been described as some of the most acutely toxic and water soluble components of crude oils (Heitkamp and Cerniglia, 1987).

Maximum acceptable concentration of PAHs in drinking water is  $0.1 \mu\text{g L}^{-1}$  for the sum of five PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene) and  $0.01 \mu\text{g L}^{-1}$  for benzo[a]pyrene (Ur.l. RS, 2004). However, maximum acceptable concentration values of PAHs in seawater and seafood products are not reported.

The composition of PAHs usually reflects the sources that produced the PAH. Some molecular indexes play a major role in establishing the origin of PAHs in marine waters and sediments. In particular, the ratios between low and high molecular weight PAHs (Neff, 1979; Gschwend and Hites, 1981; Sicre et al., 1987; Wise et al., 1988; Soclo et al., 2000; Magi et al., 2002) or the ratio of some isomers, e.g. phenanthrene/anthracene (Gschwend and Hites, 1981; Sicre et al., 1987; Budzinski et al., 1997; Baumard et al., 1998a; Baumard et al., 1998b; Soclo et al., 2000; Magi et al., 2002), fluoroanthrene/pyrene (Gschwend and Hites, 1981; Sicre et al., 1987; Colombo et al., 1989; Baumard et al., 1998b; Magi et al., 2002), fluoranthrene/(fluoranthrene plus pyrene) (Budzinski et al., 1997; Gogou et al., 1998; Magi et al., 2002), pyrene/benzo[a]pyrene (Manoli and Samara, 1999; Macias-Zamora et al., 2002) chrysene/benzo[a]anthracene (Soclo et al., 2000; Bertolotto et al., 2003) and methyl-phenantrenes/phenantrene (Youngblood and Blumer, 1975; Zakaria et al., 2002) are distinctive of the parent process leading to such

hydrocarbons. PAHs isomeric composition is temperature dependent because it is governed by thermodynamic properties in low temperature processes (e.g. formation of petroleum) or by kinetic factors in high temperature processes (e.g. pyrolysis of organic matter). On the other hand, petrogenic contamination is characterized by the predominance of the lower molecular weight fraction, while the higher molecular weight PAHs prevail in pyrolytic PAHs.

A wide variety of instrumental and non-instrumental techniques are currently used in the analyses of hydrocarbons. Gas chromatography (GC), high performance liquid chromatography (HPLC), synchronous fluorescence spectroscopy (SFS) and fluorescence spectroscopy are the most widely used.

There are several studies on PAH determination in different environmental matrices, which use gas chromatography (Hoffman et al., 1984; Simpson et al., 1996; Notar et al., 2001; El Nemr and Abd-Allah, 2003; González et al., 2006; De Luca et al., 2005; Manodori et al., 2006; Heath et al., 2006; Qiu et al., 2009), high performance liquid chromatography (Theobald, 1989; Law et al., 1997; Jonsson et al., 2004; Liu et al., 2008), synchronous fluorescence spectroscopy (Jonsson et al., 2004; Sharma et al., 2007) and fluorescence spectroscopy (Ehrhardt and Knap, 1989; Ehrhardt and Petrick, 1989; Dujmov and Sučević, 1989; Picer, 1998; Jonsson et al., 2004; Pikkarainen and Lemponen, 2005; González et al., 2006; Lim et al., 2007).

Chromatographic techniques, specially GC-FID and GC-MS are very frequently used in analytical procedures for the separation, determination and identification of a wide spectrum of analytes present in samples with complex and sometimes variable matrices. In addition, Krahn et al. (1993) reviewed the application of rapid HPLC methods to screen for aromatic components in sediments, bile and tissue samples. These methods have been used to assess damage to natural resources after the Exxon Valdez oil spill.

From the analytical point of view, hydrocarbons have very good fluorescence properties under UV excitation, which allow their determination to be carried out at very low concentrations. Two simpler and less time consuming methods, fluorescence spectroscopy and synchronous fluorescence spectroscopy (SFS) were

introduced for the investigation of the state of the marine environment. Because of the method's simplicity, selectivity, sensitivity and rapidity (Gordon et al., 1978; Law, 1981; Falcon et al., 1996; Li et al., 2000; Giessing et al., 2003) has been often employed for the estimation of concentrations of PAHs in seawater (Mackie et al. 1978; Law, 1978; Picer, 1998; Sharma et al., 2007).

Fluorescence spectroscopy is a simple and non-expensive technique for the determination of total PAH background levels in seawater which provides excellent sensitivity for PAH quantification. The method is based on the difference in solubility, in non-polar organic solvents such as hexane, of high molecular weight fluorescent organic seawater constituents and the presence of hexane soluble, fluorescent aromatic hydrocarbons in petroleum (Ehrhardt and Petrick, 1989). This method is useful for the determination of the presence of trace concentrations of dissolved and/or finely dispersed petroleum residues in seawater. Its weakness could be that it can only provide information concerning the classes of compounds (Mason, 1987) whereas its application for the determination of the concentrations of individual compounds is rather limited (Erhardt and Knap, 1989). On the other hand, the fluorescence may be also due to degradation products generated from oil in the natural environment (Erhardt and Knap, 1989). These compounds are important constituents of the unresolved complex mixture (UCM), determined usually as a background signal in GC chromatograms. The sum of resolved and UCM was considered as a better measure of the level of petroleum hydrocarbons than the resolved components (Farrington et al., 1982).

In proceeding the term »total PAHs« was used to express concentrations of PAH in seawater. It should be mentioned that fluorescence spectroscopy at defined wavelengths detect all the fluorescing matter. By an adequate selection of emission and excitation wavelengths in a narrow range a satisfactory selectivity can be achieved. This is the reason why it is usually to use the expression »total PAHs« instead of total fluorescing matter.

The use of fluorescence spectroscopy for the estimation of natural and waste-water pollution by hydrocarbons has been published in numerous papers from an analytic point of view and applied for monitoring purposes (Ehrhardt and Knap, 1989a;

Ehrhardt and Petrick, 1989b; Dujmov and Sušević, 1989; Picer, 1998; Jonsson et al., 2004; González et al., 2006; Lim et al., 2007). Chrysene and crude oil of different origin were most frequently used as standards (Ehrhardt and Petrick, 1989b; Dujmov and Sušević, 1989). The estimations of concentrations of hydrocarbons in marine sediments were also published (Al Lihabi and Al Omran, 1996; Badawy et al., 1993; Ehrhardt and Burns, 1993; Petersen et al., 2002; Gonzales-Macias et al., 2007).

In contrast with fluorescence spectroscopy, synchronous fluorescence spectroscopy is known to be a powerful tool for simultaneous analysis of multi-component samples without pre-treatment of samples (Dissanayake, 2004; Sharma, 2007). The synchronous fluorescence introduced by Lloyd (1971) has been used to characterize complex mixtures, providing fingerprints of complex samples, such as crude oils of different origins (Lloyd, 1971; Clark et al., 1987) and extracts and pyrolysis tars of coals (Katoh et al., 1980; Li et al., 1994). The synchronous fluorescence technique is now being used more and more frequently in the investigations of aromatic components of mixtures of various origins (Dissanayake and Galloway, 2004; Jonsson et al., 2004; Sharma et al., 2007; Hua et al., 2007).

The distribution of PAHs in seawater and sediments of the Adriatic Sea has been the subject of several studies (Marcomini et al., 1986; Dujmov and Sušević, 1989; Guzzella and de Paolis, 1994; Bajt, 2000; Notar et al., 2001; Manodori et al., 2006; Heath et al., 2006; Cibic et al., 2008) but relatively few of these (Bajt, 2000, Notar et al., 2001, Manodori et al., 2006; Heath et al., 2006; Cibic et al., 2008) have focused on the PAHs in seawater and sediments in the Gulf of Trieste, northern Adriatic.

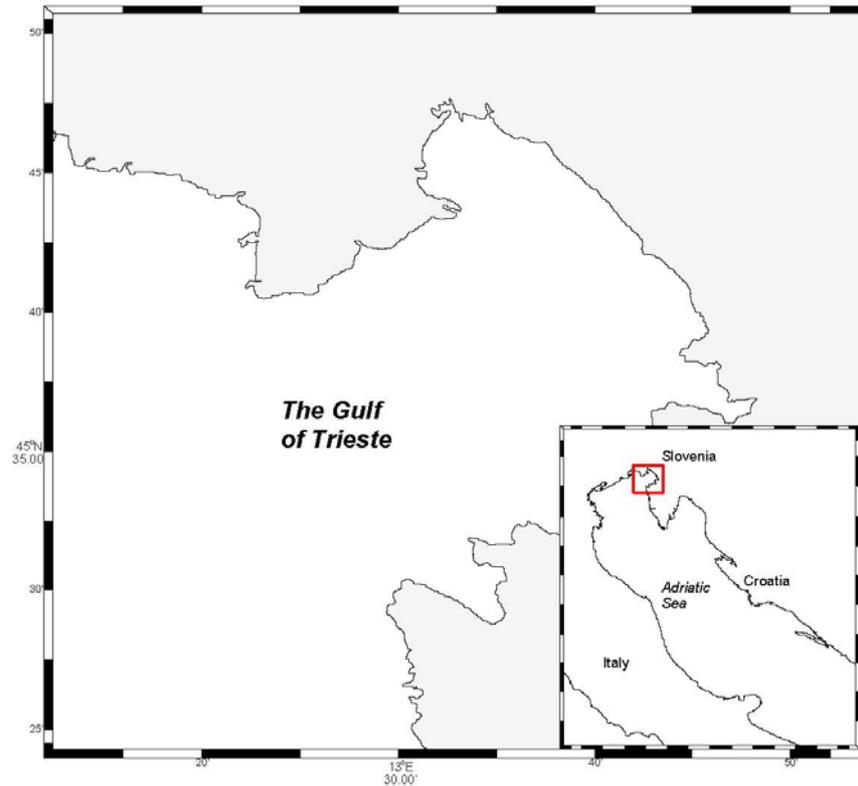
The aim of the present study was to investigate and establish the pollution with PAHs in surface seawater and sediments of the Gulf of Trieste, northern Adriatic. Two main techniques were applied for this purpose, UV fluorescence spectroscopy (UVF) and gas chromatography (GC). UV fluorescence spectroscopy was applied as a screening method for the determination of total PAHs in surface seawater across a range of estuaries, marinas, coastal and offshore areas in order to identify »hot spots«, while GC method for the individual PAHs compounds identification at the most polluted sites. Because surface seawater samples often reflect the momental situation of PAH pollution, which usually depends on hydrological and

meteorological conditions, sediment samples at sites with higher concentrations of PAHs in surface seawater were also analysed. It is well known, that sediments better reflect the long term accumulation of PAHs. The research was focused on distribution, seasonal variation, composition and sources of PAHs in surface seawater and sediments of the Gulf of Trieste. Different diagnostic ratios were applied to elucidate the sources of the investigated compounds.

Results of the present study provide informations about the contamination of surface seawater and sediments with PAHs, which are considered as one of the most dangerous group of environmental pollutants, in an industrialized and very sensitive area in the northern Adriatic. The results of this study will contribute to the better understanding of the state and sources of pollution with PAHs in the Gulf of Trieste.

## 2 STUDY AREA

The Gulf of Trieste is the northernmost part of the Adriatic Sea with a surface area of about 600 km<sup>2</sup> and a maximum depth of 26 m (Fig. 1).

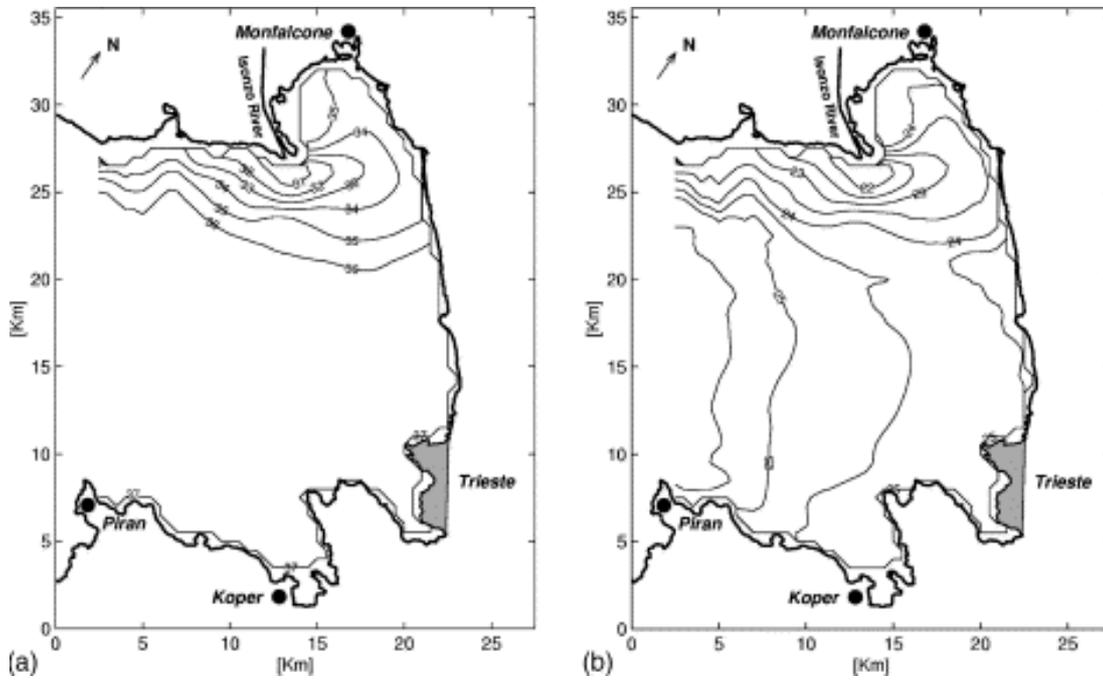


**Figure 1:** *Gulf of Trieste, North Adriatic Sea.*

The Gulf is bounded by Slovenia to the southeast and Italy to the north and west. It is partially isolated from the rest of the northern Adriatic Sea by a shoal extending northeast and southwest from the Istrian peninsula to the Grado lagoon.

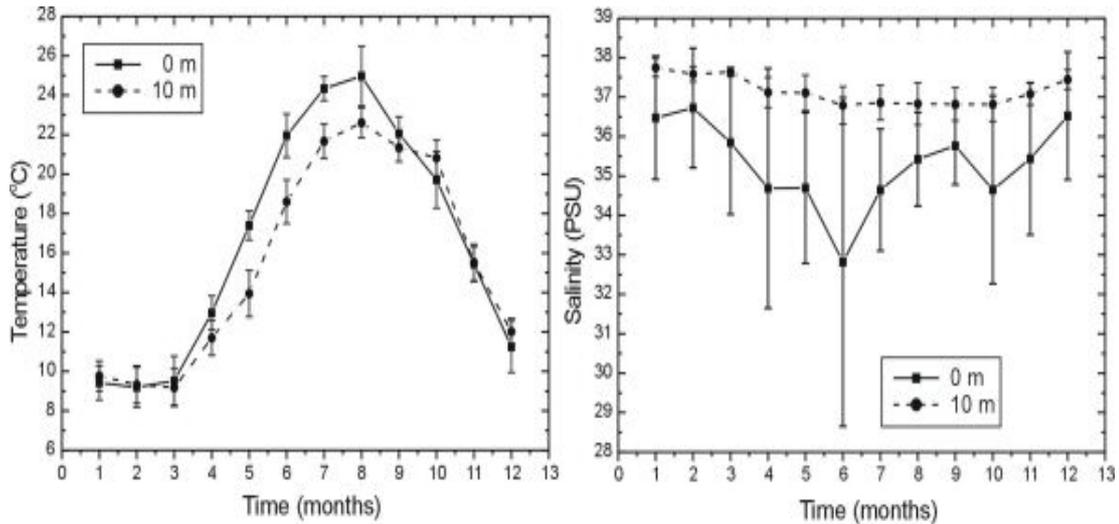
The Gulf is significantly affected by the generally anticlockwise circulation pattern of the Adriatic Sea that brings oligotrophic waters from the south (Turk and Potočnik, 2001). The generally anticlockwise circulation pattern in the Gulf of Trieste is modulated by local winds, tidal currents, density currents and inertial effects (Stravisi, 1983; Malačič and Petelin, 2001).

Freshwater inputs show high temporal variability that affect salinity, whose values range from 32-38.5 PSU in the surface layer and 36-39 PSU in the bottom water (Malej and Malačič, 1995).



**Figure 2:** Simulated surface patterns of (a) salinity (PSU) and (b) temperature (°C) in summer, during a period of scarce Isonzo River outflow ( $50 \text{ m}^3 \text{ s}^{-1}$ ) (Cozzi et al., 2004).

During late spring and summer the water column is thermally stratified and the freshwater inputs mainly affect the superficial layers, while in autumn and winter the water column tends to become homogeneous (Cardin and Celio, 1997). The density stratification in the water column starts in spring and intensifies until the late summer and is often associated with hypoxia/anoxia in bottom waters (Malej and Malačič, 1995). Water temperature shows an annual pattern with winter minima even lower than  $8^\circ\text{C}$  in February and summer maxima of  $25^\circ\text{C}$  (Cardin and Celio, 1997).



**Figure 3:** Annual variations of monthly temperatures (left) and salinities (right), near the surface (0 m, or 0.0 m - 0.5 m; full lines), and at a depth of 10 m (9.5 m - 10.5 m; dashed lines) in the Gulf of Trieste (Malačić et al., 2004).

The seasonal variation of water circulation is moreover controlled by fluctuations of freshwater inflow. Freshwater enters the system from several rivers, e.g., the Dragonia and Rižana rivers in the southeast, and the Isonzo and Timavo rivers in the northwest. The main freshwater inflow is from the Isonzo River (Olivotti et al., 1986). The flow regime of the Isonzo River is generally characterized by high flows in early spring and autumn and low flow in early winter and summer. Episodic short-lived pulses of high flow may occur in late spring and summer as result of local storms (Malej et al., 1995).

Due to its semi-enclosed position and shallowness, the Gulf of Trieste is an exceptionally sensitive ecosystem which is strongly influenced by human activities. The gulf's peculiar geomorphologic and hydrologic conditions make it prone to the accumulation of pollutants (Solis-Weiss et al., 2004; Cibic et al., 2008) which mainly enter the marine environment through anthropogenic activities.

The Gulf of Trieste is one of the most industrialized and urbanized area in the northern Adriatic. The principal industries around the Gulf of Trieste include the iron and steel industry, petroleum production, metal manufacturing, production of chemicals and the food industry. Economic development caused regression in agriculture activities that now mainly include wine, fruit and vegetable cultivation.

In the Gulf of Trieste three central treatment plants are active (Koper, Piran and Servola). Besides the above mentioned treatment plants there are also some minor outlets discharging sewage from smaller towns on the coast. It has to be mentioned that new central treatment plants in the municipality of Koper and Piran are under construction. The new treatment plant at Koper will connect the sewage systems of the municipality of Koper and Izola, while the treatment plant at Piran will replace the existing one. The existing sewage system in the municipality of Koper with secondary treatment has a central sewage treatment plant with a capacity of 84,000 PE, with a total yearly effluent of about  $3.8 \times 10^6 \text{ m}^3 \text{ year}^{-1}$  (ARSO, 2009). About 66% of the wastewater is from household and about 34% from industry/enterprises/public sector. The sewage effluent is discharged into the estuary of the Rižana river. The sewage system in the municipality of Piran with only primary treatment has a central sewage treatment plant with a capacity of 33,000 PE and total yearly effluent of about  $3.6 \times 10^6 \text{ m}^3 \text{ year}^{-1}$  (ARSO, 2009). After mechanical treatment (screening, sand and grease removal, sedimentation) the sewage water is discharged into the sea, through two submarine pipes, 3450 m and 3600 m from the shore, with diffusers at the end. The sewage of the community of Izola is collected in a treatment basin and discharged without treatment into the sea about 300 m from the shore, with a flow rate of about  $1.3 \times 10^6 \text{ m}^3 \text{ year}^{-1}$  (Turk and Potočnik, 2001). Estimated yearly freshwater input into the Gulf of Trieste from the Slovenian coast is  $206 \times 10^6 \text{ m}^3$ . The total quantity of urban and industrial wastewater is  $11 \times 10^6 \text{ m}^3 \text{ year}^{-1}$ , taking into account that the existing measured flow contains rain water as well as intrusion seawater (Turk and Potočnik, 2001).

The Servola sewage disposal plant is the most important sewage plant of the Italian coastal area in the Gulf of Trieste, serving up to 270,000 inhabitants (Cibic et al., 2008). Until the early 1980s, the Servola sewage plant, in place since 1938, and calculated for a maximum of 100,000 inhabitants, served the whole area. Its primary treatment has been based on chemical precipitation since 1992. The treated water is discharged via two adjacent submarine ducts (6.5 and 7.5 km) ending at 23 m depth, with several diffusers (Novelli, 1996). The sewage disposal plant of Servola discharges on average  $43.8 \times 10^6 \text{ m}^3 \text{ year}^{-1}$  of wastewater, but its flow rate depends on precipitation in the area (Cozzi et al., 2004).

One of the main problems concerning wastewater discharge is the rapid growth of the population as well as industrial and tourism development. In the last decades this area has been exposed to strong development pressure shown in the rapid growth of population, town planning and development of the business sector (traffic, trade, tourism, agriculture). The increase in population directly and indirectly influences sewage discharge and the pollution of the gulf. The Slovenian coastal region which extends to an area of 384 km<sup>2</sup> (about 1.7% of the total national territory) has a population of slightly over 84,000 people which is about 4% of the total population of Slovenia (STAT, 2009). The population density of the area (232 inhabitants/km<sup>2</sup>) is more than twice the national average (98 inhabitants/km<sup>2</sup>). Most of the coastal population (>80%) lives within a 1.5 km wide strip (Turk and Potočnik, 2001). Average summer seasonal increase of the population in the municipalities of Koper, Izola and Piran is 5.4%, 16.6% and 81.4%, respectively (Turk and Potočnik, 2001). The Italian coastal area of the Gulf of Trieste, extending between the cities of Trieste and Grado and including the province of Trieste, Gorizia and Pordenone, has a population of approx. 675,000 people with a population density of 1,122 inhabitants/km<sup>2</sup> in the province of Trieste, 302 inhabitants/km<sup>2</sup> in the province of Gorizia and 131 inhabitants/km<sup>2</sup> in the province of Grado (ISTAT, 2006). The municipality of Trieste has a population of 206,564 inhabitants with a population density of 2,445 inhabitants/km<sup>2</sup> (ISTAT, 2006).

The Gulf of Trieste hosts three ports: Port of Trieste, Port of Koper and Port of Monfalcone. Because of good inland transport connection the ports of Trieste and Koper have become the most important ports for Central Europe. In the year 2008 Port of Trieste handled 48.3 million tons of cargo (35.9 million tons of crude oil), Port of Koper over 16 million tons (2.8 millions tons of oil products) and Port of Monfalcone about 4.5 million tons of cargo. The total handling of those three ports in year 2008 was about 68.8 million tons of cargo, among them over 38.7 million tons of oil and oil products.

In addition to the ports there are also many marinas. The Marina of Portorož with 1,257 berths, the Marina of Sistiana and Porto Franco Nuovo with 600 berths each, are the biggest marinas in the northern Adriatic. Other marinas in the area are: the Marina of Izola (620 berths), the Marina of San Rocco (525 berths), the Marina of

Grignano (350 berths), the Marina of Monfalcone (300 berths), the Marina San Giusto (226 berths) and the Marina of Koper (75 berths).

The most severe problems concerning the marine environment in this region are intense maritime traffic, seasonal tourism (including nautical), industrial and municipal wastewaters and agricultural activities in the hinterland (Turk and Potočnik, 2001; Cozzi et al., 2004). Maritime traffic through the ports of the northern Adriatic increased dramatically in the last decade. For example, between 1998 and 2008 total cargo handling in the Port of Koper increased 80 % (from 8.6 to 16 millions tons). In the same period the quantities of oil products increased from 1.6 million tons to 2.8 million tons. Also nautical tourism, specially in summer influence the marine pollution in this area. For example, between 2003 and 2008 the number of moorings (wet) in the Slovenian sea increased from 5400 (STAT, 2004) to 5774 (STAT, 2009).

Seawater of the Gulf of Trieste is also affected by urban runoff as well as atmospheric deposition. Urban runoff has been recognized as an important pathway of PAH introduction to the water environment and aquatic ecosystems (Hoffman et al., 1984). PAHs are of particular concern because of their relatively high concentration level, diverse sources, and toxicity to aquatic organisms (Beasley and Kneale, 2002). The results of a previous study (Bajt, 2000; Bajt and Penko, 2009) in the Gulf of Trieste revealed a significant impact of runoff pollution from coastal roads on the concentrations of hydrocarbons in seawater and sediments.

### **3 MATERIALS AND METHODS**

#### **3.1 Chemicals**

»For residue analyses« quality hexane, methanol and dichloromethane (Fluka) were used for the extraction of hydrocarbons. All other chemicals were of analytical grade, purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Na<sub>2</sub>SO<sub>4</sub> was precleaned by Soxhlet extraction with methanol and hexane and additionally precombusted at 400°C for 6 hours. SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> were precleaned by Soxhlet extraction with methanol and hexane for 8 hours and then dried at 105 °C. Before use, both oxides were activated at 200 °C for 4 hours and then partially deactivated by adding 5% of precleaned water. Cu was activated with HCl and rinsed several times with distilled water, acetone and hexane. It was kept in hexane before use.

#### **3.2 Sampling**

The surface seawater samples (0.2m) were collected at twenty-seven coastal and four offshore sites in the Gulf of Trieste, northern Adriatic (Fig. 4). Samplings were performed between 2008 and 2009. Seawater samples were taken with a pre-cleaned glass bottle. The samples were kept in a dark and cold place until delivered to the laboratory for analyses.

The sediment samples were collected using a gravity corer sampler at six coastal and four offshore sites along the Slovenian coast of the Gulf of Trieste (Fig. 10). Samplings were performed in 2008. The upper 2 cm layer of each sediment sample was taken for analyses. The sediment samples were stored in deep freezer at -30°C.

#### **3.3 Samples extraction**

The extraction of seawater samples was performed according to UNEP/IOC procedure (UNEP/IOC, 1984). Two litres of seawater were extracted three times with 30 ml of *n*-hexane in a precleaned separatory funnel. The extracts were collected in a precleaned flask and concentrated on a rotary evaporator to about 10 ml. The reduced extracts were dried with the addition of Na<sub>2</sub>SO<sub>4</sub> and analysed with the fluorescence

spectroscopy. For GC analyses internal standards were added to the seawater samples prior to extraction.

The extraction of sediment samples was performed according to UNEP/IOC procedure (UNEP/IOC, 1982). Samples were frozen and after that freeze dried. After freeze-drying, about 10g of sediment were transferred in a Soxhlet apparatus and extracted with 200ml of hexane and methylenechloride (1:1) for 8 hours. The extracts were dried with  $\text{Na}_2\text{SO}_4$ , concentrated on a rotary evaporator and additionally under a nitrogen stream. Sulphur was removed overnight by the addition of activated Cu. After additional concentration of extracts under the nitrogen stream the partitioning in aliphatic and aromatic fractions was performed with column chromatography (Silica gel, Alumina). Two fractions were eluted, the first one (aliphatic ) with 20 ml of hexane and the second one (aromatic), first with 30 ml of hexane/methylenechloride (90:10) and then with 20 ml of hexane/methylene (50:50). The aromatic fraction was concentrated and used for the analyses.

### **3.4 Analyses**

Fluorescence spectroscopy and gas chromatography analyses were used to analyze PAHs in surface seawater and sediment samples. Fluorescence was measured using a Perkin-Elmer LS 45 Fluorescence Spectrometer. The excitation and emission wavelengths were 310 nm and 360 nm, respectively (with 10 nm excitation and emission slit). Background intensity was measured and subtracted. Fluorescence measurements were made in a standard 1x1cm quartz cell. Crysene was chosen as the external standard.

Gas chromatographic analyses were performed using an HP 5890 gas chromatograph equipped with on column injector and flame ionization detector (FID). The HP Ultra 2 column (25 m x 0.32 mm, 0.17  $\mu\text{m}$  film thickness) was used for analyses. The GC temperature program was as follows: column initial temperature of 60 °C, increased at 3°C min<sup>-1</sup> to 290 °C and maintained at 290 °C for 15 minutes. The initial injector (on column) temperature was 63 °C, increased at 3°C min<sup>-1</sup> up to 293 °C and maintained until the end of the analysis. The injection volume was 1  $\mu\text{L}$ . The detector temperature was 300 °C. The carrier gas (Helium) flow rate was 1 ml min<sup>-1</sup>.

### 3.5 Quality assurance

During the work, quality control procedures were applied. Procedural blanks were performed at the same time of the analyses. For recovery determination internal standards hexamethylbenzene and cadalene were added. Recoveries ranged from 60%-85% for seawater and from 75%-93% for sediment samples. Concentrations were calculated using an external standard solution of determined PAH in the case of GC. A five point calibration curve was applied. The response was linear in the applied concentration range (from 1 to 30 ng g<sup>-1</sup>). Repeatability was calculated by analysing three samples from the same sampling site. The relative standard deviation measured with GC for individual PAH compounds was between 5% and 20%. The LOD was determined by spiking blank samples and presented in Table 1.

*Table 1: LOD for different PAHs (ng g<sup>-1</sup>)*

PAH	LOD
Naphthalene	0.4
1-methylnaphthalene	0.6
1-ethylnaphthalene	0.6
Acenaphthylene	0.4
Acenaphthene	0.4
Fluorene	0.4
Phenanthrene	0.5
Anthracene	0.5
2-methylphenanthrene	0.5
1- methylphenanthrene	0.5
3,6-dimethylphenanthrene	0.5
Fluoranthene	0.5
Pyrene	0.5
1- methylpyrene	0.6
Chrysene	0.6
Perylene	0.8
Benzo(a)anthracene	0.6
Benzo(b)fluoranthene	0.7

Benzo(k)fluoranthene	0.6
Benzo(e)pyrene	0.6
Benzo(a)pyrene	0.7
Indeno(1,2,3-c,d)pyrene	0.7
Dibenzo(a,h)anthracene	0.7
Benzo(g,h,i)perylene	0.7

For fluorescence measurement chrysene was used as an external standard. Two calibration curves were applied, first one in the range from 0.01 to 0.1  $\mu\text{g L}^{-1}$ , and the second one from 0.1 to 1.0  $\mu\text{g L}^{-1}$ . The response in these ranges was linear. Repeatability of fluorescence measurements was performed by analysing three samples from the same sampling sites. The relative standard deviation was 20%. The LOD, determined on blank samples was 0.01  $\mu\text{g L}^{-1}$ .

For sediment samples a CRM IAEA-408 was also analysed to test the accuracy of our work. The results are presented in Table 2.

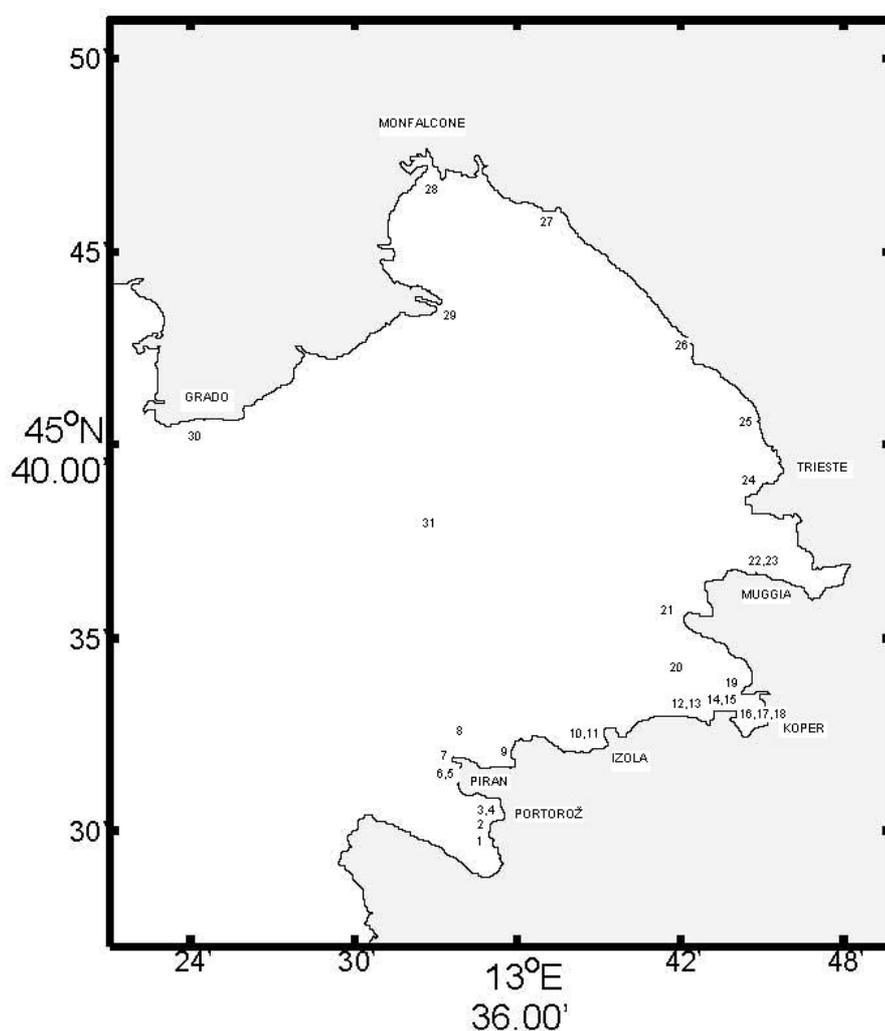
**Table 2:** Results of CRM IAEA-408 analysis ( $\text{ng g}^{-1}$  dry weight)

<b>Polyaromatic hydrocarbon</b>	<b>Median value</b>	<b>95 % confidence interval</b>	<b>Obtained value</b>
Phenanthrene	35	21-43	22
Anthracene	9.8	8-13	12
Chrysene	35	25-56	54
Fluoranthene	84	53-110	99
Pyrene	77	57-93	88
Benzo(b)fluoranthene	46	32-69	69
Benzo(k) fluoranthene	46	26-61	60
Benzo(a)anthracene	53	35-60	38
Perylene	320	140-420	180
Benzo(e)pyrene	55	51-81	69
Benzo(a)pyrene	48	30-63	68
Naphthalene	27	16-47	14
Benzo(g,h,i)perylene	38	20-52	48

## 4 RESULTS AND DISCUSSION

### 4.1 Seasonal variation and distribution patterns of total PAHs in surface seawater

Twenty-seven coastal and four offshore surface seawater samples were collected between winter and summer 2008-2009 in the Gulf of Trieste, northern Adriatic and analysed by fluorescence spectroscopy (Fig. 4).



*Figure 4: Seawater sampling stations within the investigated area.*

The total PAH concentrations of seawater samples, determined by fluorescence spectroscopy (UVF) for winter and summer 2008-2009, are presented in Table 3.

**Table 3:** Total PAH concentrations ( $\mu\text{g L}^{-1}$ , chrysene equivalents) in surface seawater at different sampling stations in the Gulf of Trieste for winter and summer 2008-2009 measured by UVF

No.	Station	Location	Concentration	
			Winter	Summer
1	LC1	Cape Lucija	0.17	0.22
2	LC2	Camp Lucija	0.32	0.87
3	MP1	Marina of Portorož –inside	0.73	48.51
4	MP2	Marina of Portorož – entrance	0.17	0.23
5	PI1	Municipal harbour of Piran	8.57	26.28
6	PZ	Bay of Piran	0.10	0.13
7	PI2	Cape Piran	0.16	0.26
8	PP	NorthEast part off Piran	0.07	0.10
9	ST	Bay of Strunjan	0.09	0.18
10	IZ1	Marina of Izola	0.67	0.40
11	IZ2	Municipal harbour of Izola	14.84	32.50
12	ZU	Small harbour of Molet	1.72	0.69
13	SE	Municipal harbour of Semedela	2.38	1.62
14	KP1	Municipal harbour of Koper	0.79	43.23
15	KP2	Marina of Koper	0.14	0.47
16	LK1	Port of Koper (pool I)	0.22	2.05
17	LK2	Port of Koper (pool II)	0.78	1.32
18	LK3	Port of Koper (pool III)	0.68	2.23
19	SK	Municipal harbour of St. Katarina	4.05	0.31
20	KZ	Bay of Koper	0.10	0.14
21	DB	Cape Debeli Rtič	0.65	0.20
22	MI1	Marina of San Rocco	0.92	1.38
23	M2	Small harbour of Muggia	0.89	3.52
24	TS1	Marina of San Giusto	34.32	27.06
25	TS2	Small harbour Barcola	2.00	0.70
26	MR	Marina of Grignano	2.22	20.44
27	SI	Marina of Sistiana	1.26	1.87
28	MO	Marina of Monfalcone	0.26	0.14
29	SO	River Isonzo - estuary	0.34	0.79
30	GR	Grado – canal of the Schiusa Island	0.49	1.06
31	CZ	Center of the Gulf of Trieste	0.09	0.01

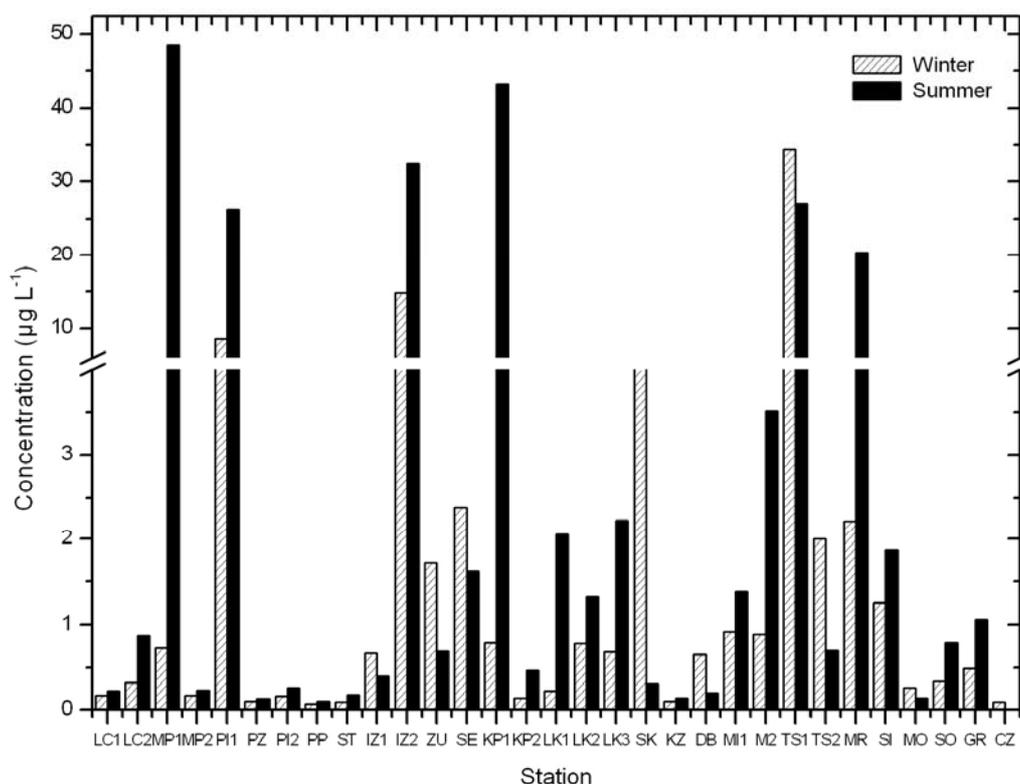
The total PAH concentrations in surface seawater of the Gulf of Trieste differed markedly in the winter and summer seasons. The concentration of total PAHs in winter ranged from  $0.07 \mu\text{g L}^{-1}$  to  $34.32 \mu\text{g L}^{-1}$ . The highest PAH concentration was found in the Marina San Giusto (Station TS1). Higher concentrations ( $8\text{-}15 \mu\text{g L}^{-1}$ ) were also found in the municipal harbours of Izola (Station IZ2) and Piran (Station PI1). Elevated concentrations ( $1\text{-}5 \mu\text{g L}^{-1}$ ) were found in the municipal harbour of St. Katarina (Station SK), the Marina of Grignano (Station MR), the small harbour of Barcola (Station TS2), the Marina of Sistiana (Station SI), the small harbour of Molet (Station ZU) and in the municipal harbour of Semedela (Station SE). At all other stations the recorded values were under  $1 \mu\text{g L}^{-1}$ . Low concentrations ( $<0.1 \mu\text{g L}^{-1}$ )

were recorded in the Bay of Piran (Station PZ), the Bay of Strunjan (Station ST), the Bay of Koper (Station KZ) and in the center of the Gulf of Trieste (Station CZ). The lowest concentration ( $0.07 \mu\text{g L}^{-1}$ ) was obtained in the northeast part off Piran (Station PP).

The concentration of total PAHs in surface seawater in summer ranged from  $0.01 \mu\text{g L}^{-1}$  to  $48.51 \mu\text{g L}^{-1}$ . The highest concentrations were observed in the Marina of Portorož (Station MP1) and the municipal harbour of Koper (Station KP1) with a value of  $48.51 \mu\text{g L}^{-1}$  and  $43.23 \mu\text{g L}^{-1}$ , respectively. Very high concentrations ( $20\text{--}33 \mu\text{g L}^{-1}$ ) were also recorded in the municipal harbours of Izola (Station IZ2) and Piran (Station PI1), in the Marina of San Giusto (Station TS1) and in the Marina of Grignano (Station MR). Elevated concentrations ( $1\text{--}5 \mu\text{g L}^{-1}$ ) were detected in the small harbour of Muggia (Station M2), Port of Koper (stations LK1, LK2, LK3), the Marina of Sistiana (Station SI), the Marina of San Rocco (Station MI1), the municipal harbour of Smedela (Station SE) and in the Grado canal (Station GR). A low concentration ( $<0.1 \mu\text{g L}^{-1}$ ) was recorded in the northeast part off Piran (Station PP). The lowest concentration ( $0.01 \mu\text{g L}^{-1}$ ) was recorded in the center of the Gulf of Trieste (Station CZ).

Several previous studies (Halsall et al., 1997; Witt, 2002; Park et al., 2002) were carried out in order to determine the seasonal variability of PAH concentrations in seawater and sediments. The results of previous studies suggest that seasonal variations in PAH concentrations are generally connected to temperature. Lower concentrations of PAH were observed during summer, while higher concentrations during winter. Witt (2002) observed that decreasing concentrations in summer could be a result of accelerated degradation due to the higher water temperature that also affects the rate by which PAHs are degraded by microorganisms or due to higher photooxidation. Additionally, previous studies (Witt, 2002; Sharma et al., 2007) have also suggested that seasonal variation in PAH concentrations could be also attributed to hydrological and meteorological conditions.

In contrast to previous studies (Witt, 2002; Park et al., 2002) the results of our study show on average higher total PAH concentrations in summer compared to winter (Fig. 5).



**Figure 5:** Comparison of the total PAH concentrations in surface seawater ( $\mu\text{g L}^{-1}$ , chrysene equivalents) in the Gulf of Trieste between winter and summer 2008-2009 at different sampling stations measured by UVF.

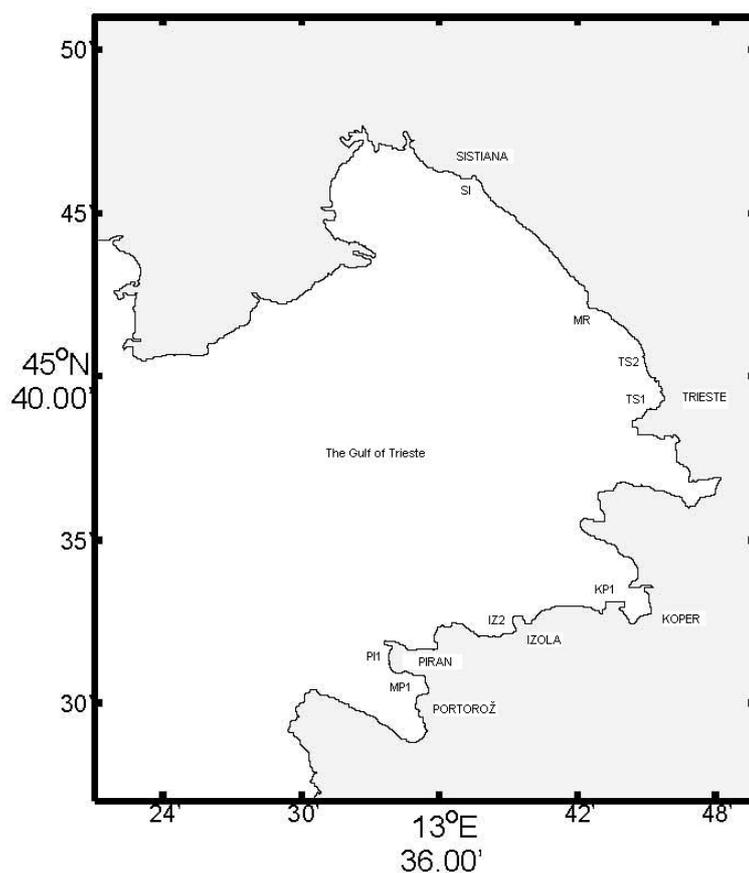
Higher concentrations of PAHs (measured with fluorescence spectroscopy) detected in summer could be attributed, among PAHs, to fluorescence of different degradation products present in surface seawater, while in general, the levels of PAHs determined in previous studies took into account only resolved PAH compounds.

In accordance with our expectations, higher concentrations of PAHs were obtained in semi-enclosed sites such as marinas and harbours. The Marina of Portorož (Station MP1), the Marina of San Giusto (Station TS1), the Marina of Grignano (Station MR) and the municipal harbours of Koper (Station KP1), Izola (Station IZ2) and Piran (Station PI1) were the most polluted stations in the investigated area. The reason lies in more intensive pollution and limited circulation of seawater, which leads to a higher accumulation of pollutants, arising from boats engine losses, atmospheric deposition and urban runoff. On the other hand, the off shore sites are less subjected to pollution pressure and at the same time, the mixing of water masses leads to the dilution of dissolved/dispersed compounds.

## 4.2 Individual PAHs in surface seawater

### 4.2.1 Spatial distribution of PAHs

Eight surface seawater samples were collected in the Gulf of Trieste between May and July 2009 and analyzed by gas chromatography in order to determine the pollution sources (Fig. 6).



*Figure 6: Seawater sampling stations.*

24 PAH compounds (including 16 US EPA PAHs) were grouped in four groups according to their number of aromatic rings and analysed. The concentrations of PAHs representing 2-3-ring, 4-ring, 5-ring and 6-ring PAHs are shown in Table 4.

**Table 4:** Concentrations of PAHs in surface seawater from the Gulf of Trieste measured by GC (ng L<sup>-1</sup>)

Compounds	Station								
	MP1	PI1	KP1	IZ2	SI	MR	TS1	TS2	
2-3-ring PAHs	Naphthalene	*	*	*	*	*	10	*	
	1-methylnaphthalene	*	*	*	*	*	*	*	
	1-ethylnaphthalene	*	*	*	*	*	*	*	
	Acenaphthene	*	*	*	*	*	*	*	
	Acenaphthylene	*	*	*	*	*	*	*	
	Phenanthrene	44	36	20	32	21	18	29	32
	Anthracene	*	5	*	*	*	*	*	*
	Fluorene	*	8	23	*	*	*	8	11
	1-methylphenanthrene	4	34	*	25	16	15	54	5
	2-methylphenanthrene	3	12	*	18	11	12	28	7
3,6-dimethylphenanthrene	*	*	*	*	*	*	10	*	
4-ring PAHs	Fluoranthene	5	8	15	4	2	*	29	1
	Pyrene	6	15	18	20	4	43	30	6
	1-methylpyrene	*	8	*	6	*	*	5	*
	Chrysene	25	26	39	9	18	20	13	2
	Benzo[a]anthracene	*	7	*	*	*	*	*	*
5-ring PAHs	Benzo[b]fluoranthene	3	11	3	2	2	10	2	7
	Benzo[k]fluoranthene	9	15	2	2	8	6	2	4
	Benzo[e]pyrene	3	1	1	*	13	3	1	3
	Benzo[a]pyrene	5	3	2	6	10	4	2	2
	Dibenzo[a,h]anthracene	*	1	4	*	*	*	7	*
	Perylene	125	13	52	24	16	13	56	7
6-ring PAHs	Indeno[1,2,3-c,d] pyrene	1	2	2	4	*	*	12	*
	Benzo[g,h,i]perylene	*	2	*	*	*	4	4	*
Total PAH	233	207	181	152	121	148	302	78	

\* below detection limits

Levels of PAHs in the surface seawater from the eight sites of the Gulf of Trieste showed that twenty PAHs can be identified at detectable levels from the twenty-four PAHs ( $\Sigma 24$  PAH) investigated and only seven PAHs were detected at all stations. The total amount of PAHs examined ranged from  $78 \text{ ng L}^{-1}$  to  $302 \text{ ng L}^{-1}$ , with a mean value of  $178 \text{ ng L}^{-1}$  (Table 3). The Marina of San Giusto (Stations TS1), the Marina of Portorož (Station MP1) and the municipal harbour of Piran (Station PI1) showed the highest PAH concentrations ( $>200 \text{ ng L}^{-1}$ ). Elevated PAH concentrations ( $100\text{-}200 \text{ ng L}^{-1}$ ) were observed in surface seawater collected in the municipal harbour of Koper (Station KP1), the municipal harbour of Izola (Station IZ2), the Marina of Grignano (Station MR) and the Marina of Sistiana (Station SI). The lowest concentration ( $78 \text{ ng L}^{-1}$ ) was recorded in the small harbour of Muggia (Station TS2).

Compared with the results from the literature the investigated area of the Gulf of Trieste in general could be considered as moderately contaminated by PAHs in seawater (Table 4).

**Table 5:** Summary of literature data about total PAH concentrations in seawater from various marine sites in the world

Location	N <sup>a</sup>	N <sup>b</sup> PAH	Concentration range ( $\text{ng L}^{-1}$ )	Method	Reference
Gulf of Trieste, northern Adriatic	7	18	78-302	GC-FID	This work
Deep Bay, China	-	15	31.7-118.8	GC-MS	Qiu et al., 2009
Saronikos Gulf, Greece	-	17	103-459	HPLC	Valavanidis et al., 2008
Western Xiamen Sea, China	9	16	106-945	GC-FID	Zhou et al., 2000
Daya Bay, China	63	16	4228-29,325	GC-FID	Zhou and Maskaoui, 2003
Jiulong River Estuary and Western Xiamen Sea, China	19	16	6960-26,920	GC-FID	Maskaoui et al., 2002
Baltic Sea	-	14	0.3-0.59	GC-MS	Maldonado et al., 1999
Seawater around England and Wales	63	15	<1-24,824	HPLC	Law et al., 1997

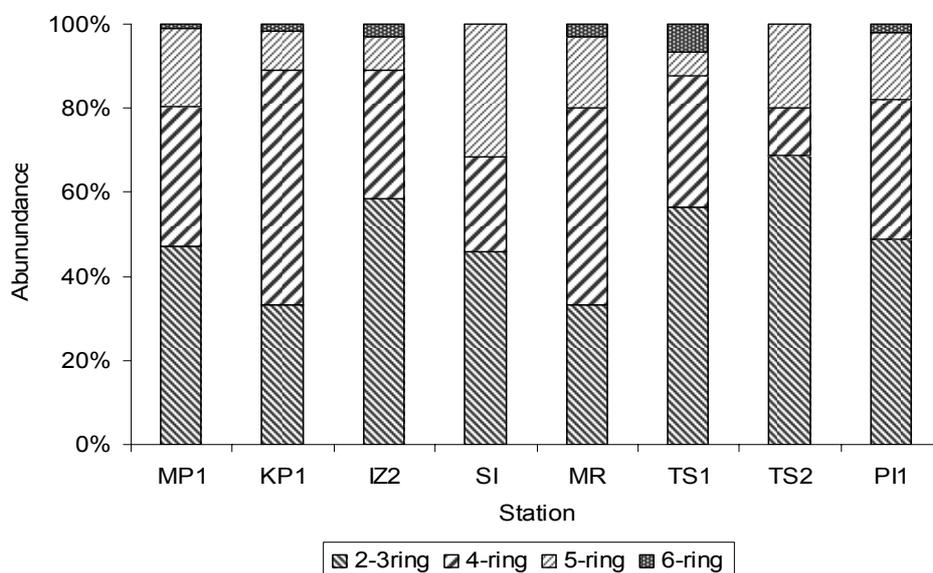
<sup>a</sup> N= the number of samples

<sup>b</sup> N PAH = the number of PAH compounds analysed in each study

In comparison with the results from some other studies, the concentrations of total PAHs ( $\Sigma 24$  PAHs) detected in the seawater of the Gulf of Trieste were significantly higher than that of the Baltic Sea ( $<0.59 \text{ ng L}^{-1}$ , Maldonado et al., 1999) and higher than that of the Deep Bay ( $<118 \text{ ng L}^{-1}$ , Qiu et al., 2009), but were comparable to those reported in Saronikos Gulf, Greece ( $<459 \text{ ng L}^{-1}$ , Valavanidis et al., 2008) and lower than those found in the Western Xiamin Sea ( $<945 \text{ ng L}^{-1}$ , Zhou et al., 2000), Daya Bay ( $<29,325 \text{ ng L}^{-1}$ , Zhou and Maskaoui, 2003) and in seawater around England and Wales ( $<24824 \text{ ng L}^{-1}$ , Law et al., 1997). It should be noted that a different number of target analytes were employed in different studies.

#### 4.2.2 PAH composition and sources

The distribution of PAHs in surface seawater of the Gulf of Trieste according to the number of aromatic rings is shown in Fig. 7. It is clear that 2-3- ring PAHs are the most abundant PAHs in seawater.



**Figure 7:** Distribution of PAHs in surface seawater of the Gulf of Trieste according to the number of aromatic rings.

Predominance of low molecular weight PAH (2-3 ring PAHs) over high molecular weight PAHs (4-6 ring PAHs) in seawater is in accordance with some other studies (Qiu et al., 2009; Zhou and Maskaoui, 2003). 2-3- ring PAHs showed dominance at

stations MP1, IZ2, SI, TS1, TS2, PI1. In addition, 4-ring PAHs showed dominance at stations KP1 and MR.

Among the individual PAH compounds identified (excluding perylene-natural origin) 2-3 ring compounds phenanthrene, 1-methylphenanthrene and 2-methylphenanthrene were dominant at stations MP1, PI1, IZ2, SI, TS1 and TS2, while the 4-ring compounds pyrene and chrysene dominated at stations KP1 and MR.

PAH compounds anthracene and benzo[*a*]anthracene were observed only at station PI1, while 1-methylnaphthalene, 1-ethylnaphthalene, acenaphthene and acenaphthylene were below the detection limit at all sampling stations. Some carcinogenic compounds, e.g. benzo[*a*]anthracene, benzo[*a*]pyrene and benzo[*e*]pyrene were in low concentrations or below detection limits.

The composition of PAHs in a particular sample usually reflects the sources that produce the PAH. There are three major types of PAH which differ by their genesis: petrogenic, pyrogenic and biogenic. PAHs with 2-3 rings are generally of petrogenic origin, related to petroleum including crude oil and its refined products (Liu et al., 2008; Valavanidis et al., 2008). High concentrations of phenanthrene and methylphenanthrenes found at stations IZ2 TS1 and TS2 indicate the presence of petrogenic sources. The presence of naphthalene at station TS1 suggests fresh petroleum pollution (Robertson, 1998). PAHs with 4-6 ring hydrocarbons are generally of pyrogenic origin and generated by the combustion of fossil fuels and of recent organic material (Liu et al., 2008). High concentrations of fluoranthene, pyrene and chrysene found at stations KP1, MP1, PI1, MR and SI suggest a pyrogenic origin.

Besides anthropogenic PAHs at some stations, natural PAH (perylene) was also found at high concentrations. Perylene is a five-ring PAH that has been found widely in both marine and freshwater sediments (Muri et al., 2003). The existence of perylene has been reported, and it is believed that perylene was formed from biogenic precursors, e.g. perylenequinone pigments (Zakaria et al., 2002) during early diagenesis, while only small amounts of perylene are produced during combustion (Silliman et al., 1998; Luo et al., 2006). Muri and Wakeham (2009) also

suggest that redox conditions may also play a role in perylene formation. Most PAH molecules have fairly well known origins and therefore are useful indicators of their sources, delivery routes, and alteration conditions. The origin of perylene, in contrast, is obscure, even though it is found widely in sediments and often is one of the major PAHs. Relative concentrations of perylene higher than 10% of total penta-aromatic isomers suggest a probable diagenetic input, otherwise a probable pyrogenic origin of the compounds is indicated (Baumard et al., 1998b; Baumard et al., 1998c). In the present study, perylene occurred at elevated levels (7-125 ng L<sup>-1</sup>) and was with phenanthrene the most abundant compound of PAHs in our study. The percentage of perylene higher than 10% of total penta-aromatic isomers recorded at stations MP1 (53%), KP1 (29%), TS1 (18%), IZ2 (16%) and SI (13%), indicate a diagenetic input of perylene in surface seawater.

Several methods can be used to determine the most probable sources of PAHs (Table 6). In many studies (De Luca et al., 2005; Manodori et al., 2006; Heath et al., 2006; Liu et al., 2008; Qiu et al., 2009) the sources of PAHs in various environmental samples have been determined by various molecular diagnostic ratios.

One of the most used approaches is to compare the sum of several pyrogenic PAHs (4-6-ring PAHs) with a group of petrogenic PAHs (2-3-ring PAHs), since the 2-ring and 3-ring PAHs are more abundant in petroleum, while highly condensed PAHs (4-6-ring PAHs) are significant combustion products (Gogou et al., 2000; Maldonado et al., 1999). Pyrogenic sources, such as the combustion-derived particles present in urban atmospheric dust, were depleted in low molecular weight 2–3 ring PAHs (LPAHs) and enriched in high molecular weight 4–6 ring PAHs (HPAHs), which led to an LPAHs/HPAHs ratio <1. Petrogenic sources, such as fuel oil or light refined petroleum products, were dominated by low molecular weight PAHs and have LPAHs/HPAHs ratio > 1. Indeed the higher the LPAHs/HPAHs ratio, the higher the prevalence of petrogenic sources (Neff, 1979; Wise et al., 1988; Soclo et al., 2000; Rocher et al., 2004; Wang et al., 2006).

The sources of PAHs may be assessed also by using the ratios of individual PAH concentrations (Gschwend and Hites, 1981; Readman et al., 1987; Budzinski et al., 1997; Yunker et al., 2002; Manodori et al., 2006; Katsoyiannis et al., 2007, Brändly

et al., 2007). Different authors use different methods for such purposes (Gogou et al., 2000; Brändly et al., 2007). Widely used ratios are fluoranthene to pyrene (Flu/Py), methylphenanthrenes to phenanthrene (MPhe/Phe), methylepyrene to pyrene (MPy/Py) and phenanthrene to anthracene (Phe/Ant).

The Flu/Py ratio  $> 1$  suggests pyrogenic sources, while Flu/Py  $< 1$  indicate petrogenic sources (Blumer, 1976; El Nemr and Abd-Allah, 2003; Kim et al., 2008). An MPhe/Phe ratio  $< 1$ , indicates combustion sources, while an MPhe/Phe ratio  $> 2$  suggests fossil-fuel sources (Prah and Carpenter, 1983). An MPy/Py ratio  $< 2$  suggests pyrogenic sources, while MPy/Py  $> 2$  indicates petrogenic sources. The ratio between phenanthrene and anthracene was also extensively used to infer the nature of PAH pollution (Gschwend and Hites, 1981; Budzinski et al., 1997; Soclo et al., 2000; Magi et al., 2002). A Phe/Ant ratio  $< 10$  suggests pyrogenic origin while contamination from petrogenic sources is characterized by Phe/Ant  $> 10$ .

Besides the above mentioned ratios some other PAH isomer ratios, such as anthracene to anthracene plus phenanthrene (Ant/(Ant+Phe)), benzo[*a*]anthracene to benzo[*a*]anthracene plus chrysene (BaA/(BaA+Chr)), fluoranthene to fluoranthene plus pyrene (FlA/(FlA+Py)), indeno[1,2,3-*cd*]pyrene to indeno[1,2,3-*cd*]pyrene plus benzo[*g,h,i*]perylene (IPy/(IPy+BPE)) and benzo[*a*]pyrene to benzo[*g,h,i*]perylene (PaP/BPE), have been developed for a more detailed interpretation of PAH composition and the inference of possible sources (Yunker et al., 2002; Katsoyiannis et al., 2007; Brändly et al., 2007).

**Table 6:** Characteristic PAH molecular diagnostic ratios

	<b>Petrogenic</b>	<b>Pyrogenic</b>
LPAHs/HPAHs	$>1$	$<1$
Flu/Py	$<1$	$>1$
Phe/Ant	$<10$	$>10$
MPy/Py	$>2$	$<2$
MPhe/Phe	$>2$	$<1$
Ant/(Ant + Phe)	$<0.1$	$>0.1$

	<b>Petrogenic</b>	<b>Pyrogenic</b>
BaA/(BaA + Chr)	<0.2	>0.35
Flu/(Flu + Py)	<0.4	>0.4
IPy/(IPy + BPE)	<0.2	>0.2
	<b>Petrogenic</b>	<b>Pyrogenic</b>
	Fuel combustion	Grass/coal/wood combustion
Flu/(Flu + Pyr)	0.4–0.5	>0.5
IPy/(IPy + BPE)	0.2–0.5	>0.5
	<b>Non-traffic</b>	<b>Traffic</b>
BaP/BPE	<0.6	>0.6

Ant: Anthracene; Phe: Phenanthrene; BaA: Benzo[a]anthracene; Chr: Chrysene; Flu: Fluoranthene; Py: Pyrene; IPy: Indeno[1,2,3-cd]pyrene; BPE: Benzo[g,h,i]perylene; BaP: Benzo[a]pyrene; BPE: Benzo[g,h,i]perylene; LPAHs: Low molecular weight PAHs; HPAHs: High molecular weight PAHs.

For detailed determination of PAHs origin some authors (Budzinski et al., 1997; Baumard, 1998b; El Nemr and Abd-Allah, 2003; De Luca et al., 2005; Qiu et al., 2009) used a cross plot of various PAH ratios. They joined the information provided by two PAH ratios by plotting Phe/Ant against Flu/Py and suggested that PAH contamination of pyrogenic origin is characterized by Phe/Ant <10 and Flu/Py >1 while contamination from petrogenic sources is characterized by Phe/Ant >10 and Flu/Py <1, as subsequently confirmed by other authors (El Nemr and Abd-Allah, 2003; Baumard, 1998). Furthermore, Baumard (1998b), suggested that only the evaluation of both ratios Phe/Ant and Flu/Py could prevent a misleading estimate of PAH sources.

In the present study for the identification of PAHs origin in surface seawater the ratios of LPAHs/HPAHs, Flu/Py and MPhe/Phe were examined (Table 7), which have been suggested by other researchers (Budzinski et al., 1997; Yunker et al., 2002; Manodori et al., 2006; Katsoyiannis et al., 2007; Brändly et al., 2007).

**Table 7:** PAH ratios of surface seawater samples in the Gulf of Trieste

Station	Ratio		
	LPAHs/HPAHs	Flu/Py	MPhe/Phe
PI1	0.96	0.53	1.28
MP1	0.89	0.83	0.16
KP1	0.50	0.84	n.q.
IZ2	1.42	0.20	1.34
SI	0.84	0.50	1.28
MR	0.50	n.q.	1.50
TS1	1.30	0.97	3.17
TS2	2.20	0.17	0.37

n.q. – non quantified

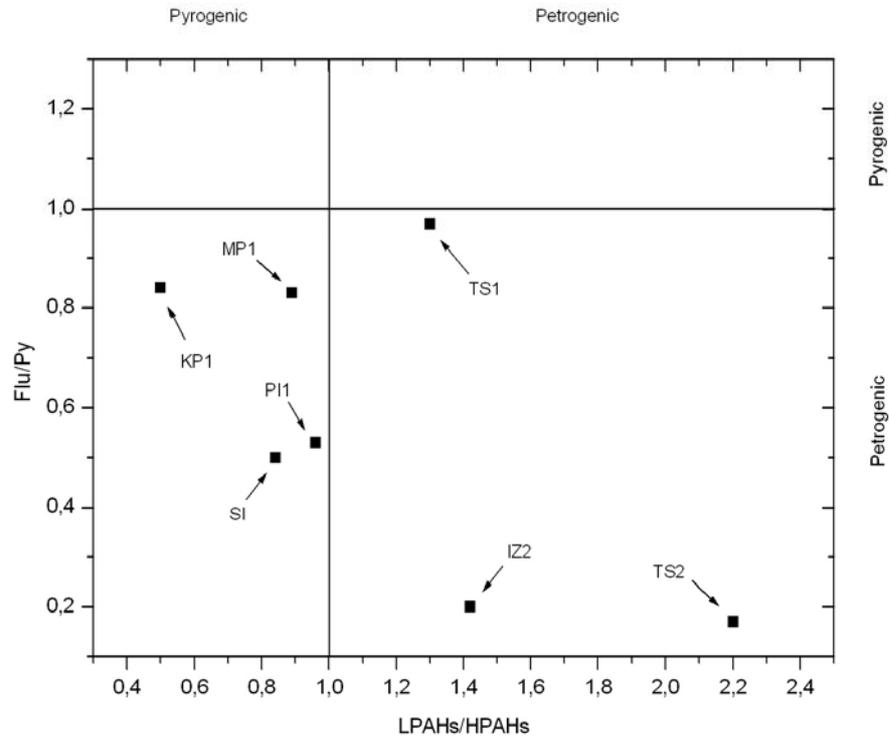
LPAHs: Low molecular weight PAHs; HPAHs: High molecular weight PAHs; MPhe: Methylphenanthrene; Phe: Phenanthrene; Flu: Fluoranthene; Py: Pyrene

The ratios of low molecular weight PAHs (2- and 3-rings PAHs) and high molecular weight PAHs (4-, 5-, 6-ring PAHs except perylene) calculated for the eight surface seawater samples in our study were between 0.5 and 2.2, suggesting both pyrogenic and petrogenic origin (Table 7). LPAHs/HPAHs ratio  $<1$  calculated for samples PI1, MP1, KP1, SI and MR, suggests pyrogenic origin, while ratio LPAHs/HPAHs  $>1$  calculated for samples IZ2, TS1 and TS2 suggests petrogenic origin.

According to Gschwend and Hites (1981), Sicre et al. (1987) and Magi et al. (2002), the Flu/Py ratio is also useful in the attribution of PAH pollution sources. A Flu/Py ratio  $<1$  suggesting petrogenic origin were found at all sampling stations (Table 7).

For better determination of PAH sources the ratios of LPAHs/HPAHs against Flu/Py were plotted (Fig. 8). Seawater samples TS1, TS2 and IZ2 with LPAHs/HPAHs ratio  $>1$  and Flu/Py ratio  $<1$  suggested petrogenic sources, while sample KP1, MP1, PI1 and SI with LPAHs/HPAHs ratio  $<1$  and Flu/Py ratio  $<1$  suggested mixed sources (petrogenic and pyrogenic). High concentrations of phenanthrene, 1-methylphenanthrene and 2-methylphenanthrene, which were observed at stations TS2 (56% of total PAHs) IZ2 (49% of total PAHs) and TS1 (37% of total PAHs) confirmed the petrogenic source, most probably leaking of petroleum products from boats or cargo vessels. The MPhe/Phe ratio  $<1$  found at station MP1 suggest the

prevalence of pyrogenic origin, while the Phe/Phen ratio between 1 and 2 found at stations PI1 and SI indicate mixed sources of pollution with PAHs.



**Figure 8:** PAH cross plots for the ratios of LPAHs/HPAHs versus Flu/Py.

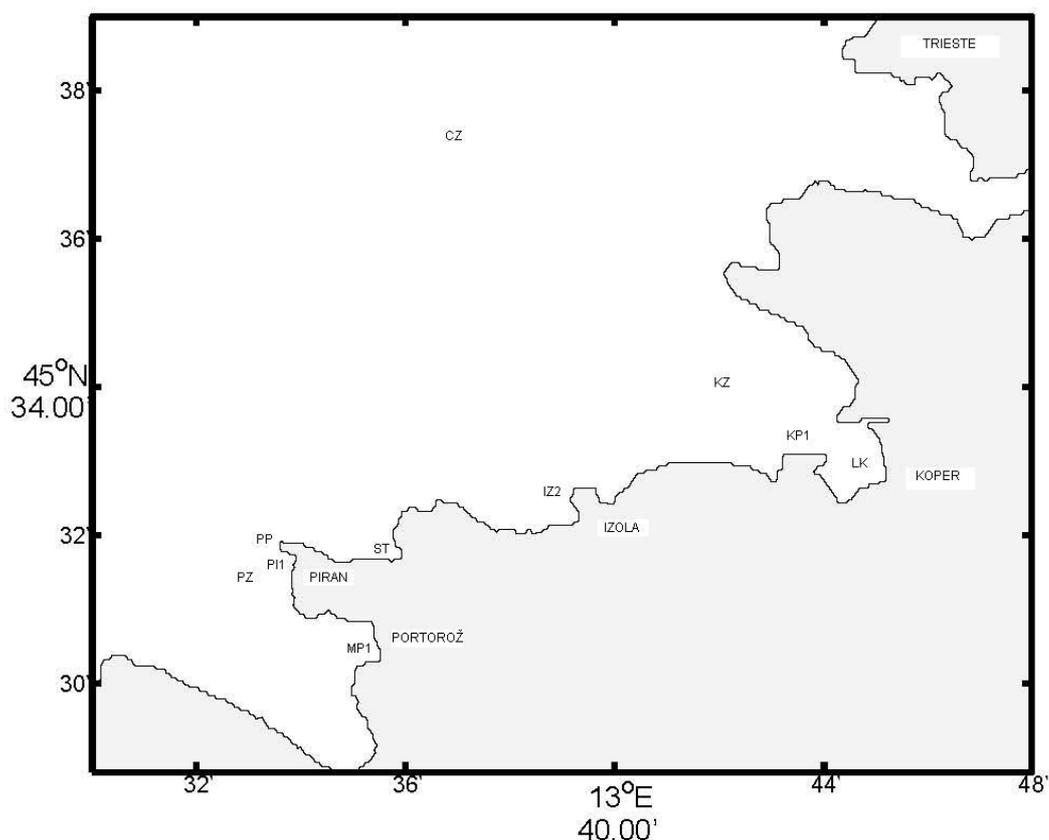
The absence of anthracene and methylpyrene at almost all stations make impossible the determination of phenanthrene to anthracene ratio (Phe/Ant) and methylpyrene to pyrene ratio (MPy/Py). However, in general no more than two criteria are used to determine possible sources (Notar et al., 2001; Pereira et al., 1999; Benlanchen et al., 1997) because using more criteria can lead to a different and ambiguous interpretation of the results.

The present results, considering all the studied source definition criteria indicate the prevalence of a petrogenic source in the seawater of the investigated area. Some contribution of pyrogenic sources is also evident at some sampling sites (KP1, MP1, PI1, SI). The results regarding seawater sources found in our study are very similar to those found by Qiu et al. (2009), King et al. (2004) and Zhou and Maskaoui (2003).

### 4.3 PAHs in sediments

#### 4.3.1 Spatial distributin of PAHs

Ten surface sediment samples were collected in 2008 and analysed by gas chromatography (GC) in order to determine individual PAH compounds (Fig. 9).



**Figure 9:** Sediment sampling sites within the investigated area.

Twenty-four PAH compounds were grouped in four groups according to their number of aromatic rings and analysed. The concentrations of PAHs representing 2-3-ring, 4-ring, 5-ring and 6-ring PAHs are shown in Table 8.

**Table 8:** Concentrations of PAHs in sediments from the Gulf of Trieste measured by GC (ng g<sup>-1</sup>, dry weight)

		Sampling station									
		PI1	ST	IZ2	KP1	LK	MP1	CZ	KZ	PZ	PP
2-3-ring PAHs	Naphthalene	*	56	*	22	10	28	*	16	*	*
	1-methylnaphthalene	*	*	*	*	9	11	13	31	*	*
	1-ethylnaphthalene	*	*	*	*	*	*	*	*	*	*
	Acenaphthene	881	33	46	7	*	47	*	30	*	11
	Acenaphthylene	*	*	*	*	*	24	*	36	*	12
	Phenanthrene	11107	464	1494	289	17	28	17	13	18	10
	Anthracene	1121	70	644	57	6	1	11	5	21	6
	Fluorene	*	31	85	7	*	*	*	16	*	*
	1-methylphenanthrene	311	64	470	59	11	5	30	39	11	12
	2-methylphenanthrene	3242	46	437	100	29	7	21	19	*	*
	3,6-dimethylphenanthrene	*	*	86	*	*	22	*	*	*	
4-ring PAHs	Fluoranthene	18713	568	3523	482	27	88	44	30	26	5
	Pyrene	12201	505	3043	487	33	93	49	42	21	2
	1-methylpyrene	*	14	458	20	*	*	*	*	*	*
	Chrysene	4020	365	2169	446	80	82	49	37	4	3
	Benzo[a]anthracene	2919	294	2077	316	22	36	28	20	14	8
5-ring PAHs	Benzo[b]fluoranthene	12686	419	1945	464	40	93	33	41	30	31
	Benzo[k]fluoranthene	19717	215	1615	239	125	198	101	88	65	65
	Benzo[e]pyrene	7658	268	1706	260	35	50	53	48	32	48
	Benzo[a]pyrene	7765	298	2310	269	25	50	42	43	27	55
6-ring PAHs	Dibenzo[a,h]anthracene	1610	24	134	28	43	56	22	63	19	3
	Indeno[1,2,3-c,d]pyrene	5942	349	63	259	57	60	61	92	30	14
	Benzo[g,h,i]perylene	5961	226	513	156	30	72	24	94	27	12
Total PAH	115854	4309	22878	3967	599	1051	598	803	345	297	

\* below detection limit

The total amount of sediment PAHs examined ranged from 297 ng g<sup>-1</sup> to 115,854 ng g<sup>-1</sup> (Table 8). The highest concentration was observed in the municipal harbour of Piran (Station PI1) and in the municipal harbour of Izola (Stations IZ2). Very high PAH concentrations (1,000 ng g<sup>-1</sup>–5,000 ng g<sup>-1</sup>) were observed in surface sediment collected in the Bay of Strunjan (Station ST), the municipal harbour of Koper (Station KP1), and the Marina of Portorož (Station MP1). The total PAH concentration in surface sediment found at station PI1 was several orders of magnitude higher than those found at other stations. Concentrations in the municipal harbours of the three coastal cities (stations KP1, IZ2 and PI1) are even significantly higher than those found in the Port of Koper (Station LK) and the Marina of Portorož (Station MP1), which are usually thought to be the most polluted areas in the Slovenian sea. However, in the case of the Port of Koper dredging activities can affect the content of different pollutants in marine sediments and direct comparison could be sometimes difficult. The high concentration of total PAHs in the Bay of Strunjan (Station ST) was surprisingly much higher than expected. This area is a protected area and it was usually used as a reference site. One of the possible sources of pollution with PAH could be runoff water from the crossroad on the main coastal road to Croatia. Comparison of the total PAH concentrations in the Bay of Koper (Station KZ) and the Bay of Piran (Station PZ) reveals that the second is less polluted. The Bay of Koper is probably affected by pollution from the maritime traffic through the Port of Koper, as from the industrial and sewage outfalls from the city of Koper. The offshore area around the city of Piran emerges as a rather unpolluted part of the Slovenian sea. This is in agreement also with low concentrations of PAH in surface seawater in this area. The relatively high concentrations of PAHs found at station CZ are most probably due to the influence of intensive maritime traffic through the ports and marinas of the north Adriatic, as well as industrial and sewage discharges from the coastal cities in this region.

A comparison of PAH concentrations in surface sediments collected from the different marine areas is given in Table 9. The investigated area of the Gulf of Trieste is moderately to highly contaminated by PAHs in surface sediments compared with other marine areas through out the world. Similar high PAH concentrations were observed by Simpson et al. (1996) in the Kitimat Harbour and by Hong et al. (1995) in Xiamen Harbour.

**Table 9:** A comparison of PAH concentrations in sediments collected from different marine areas

Location	N <sup>a</sup>	N <sup>b</sup> PAH	Concentration range (ng g <sup>-1</sup> , d.w.)	Method	References
Gulf of Trieste, Northern Adriatic	10	24	297-115,854	GC-FID	This work
Yanbtze Estuary, China			107-633	HPLC	Liu et al., 2008
Santander Bay, Spanish			20-34,460	HPLC and GC-MS	Viguri et al., 2002
Todos Santa Bay, Mexico			7.6-813	GC-MS	Macias-Zemora et al., 2002
Gulf of Trieste, Northern Adriatic	17	22	34.67–682.29	GC-MS	Notar et al., 2001
Gulf of Trieste, Northern Adriatic	3	22	600-800	GC-MS	Heath et al., 2006
Western Xiamen Bay, China	8	16	247-480	GC-FID	Zhou et al., 2000
Western Baltic Sea	22	14	3-30,100	GC-MS	Baumard et al., 1999
Masan Bay, Korea	28	16	41.5-1,100	GC-MS	Khim et al., 1999
Kitimat Harbour, Canada	25	15	310-528,000	GC-FID and GC-MS	Simpson et al., 1996
San Francisco Bay, USA	16	17	2,653-27,680	GC-MS	Pereira et al., 1996
Casco Bay, USA	65	23	16-20,748	GC-MS	Kennicut et al., 1999
Victoria Harbour, Hong Kong, China	12	8	1,200-14,000	GC-FID	Hong et al., 1995
Xiamen Harbour, China	9	8	2,900-61,000	GC-FID	Hong et al., 1995

<sup>a</sup>N – the number of samples

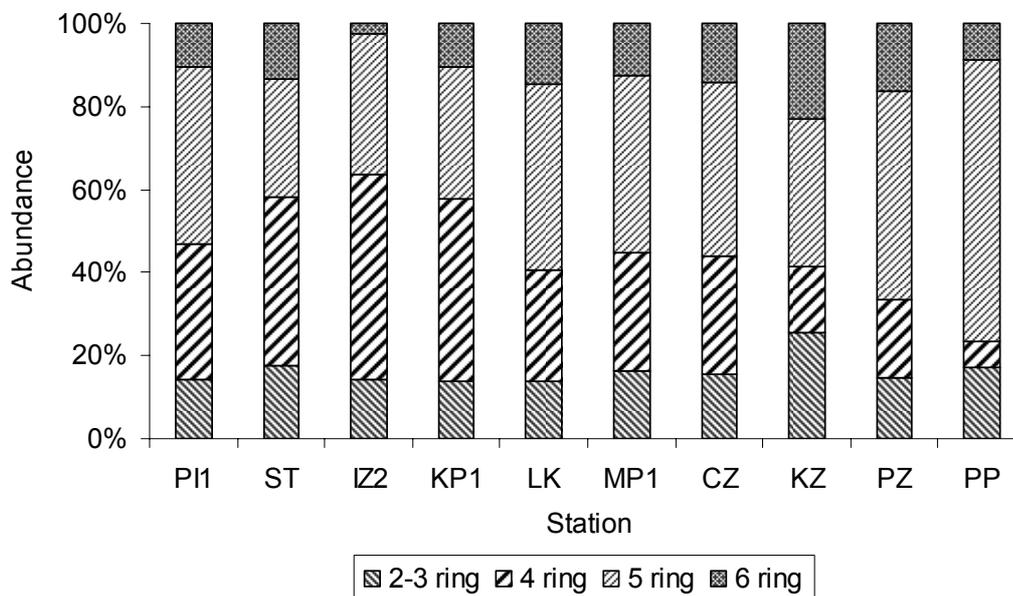
<sup>b</sup>N PAH- the number of the PAH compounds analyzed in each study

In comparison with the results of previous studies carried out in the Gulf of Trieste, the concentrations of total PAHs were similar with those found by Notar et al. (2001)

and Heath et al. (2006) in the vicinity of our sampling sites (central part of the Gulf of Trieste, Bay of Koper, Bay of Piran and northeast part off Piran).

#### 4.3.2 PAH composition and sources

The composition pattern of PAH by ring number is shown in Fig. 10. As shown in Fig. 10 the surface sediment is dominated by high molecular weight PAHs (4-ring and 5-ring PAHs).



**Figure 10:** Distribution of PAHs in surface sediments of the Gulf of Trieste according to the number of aromatic rings.

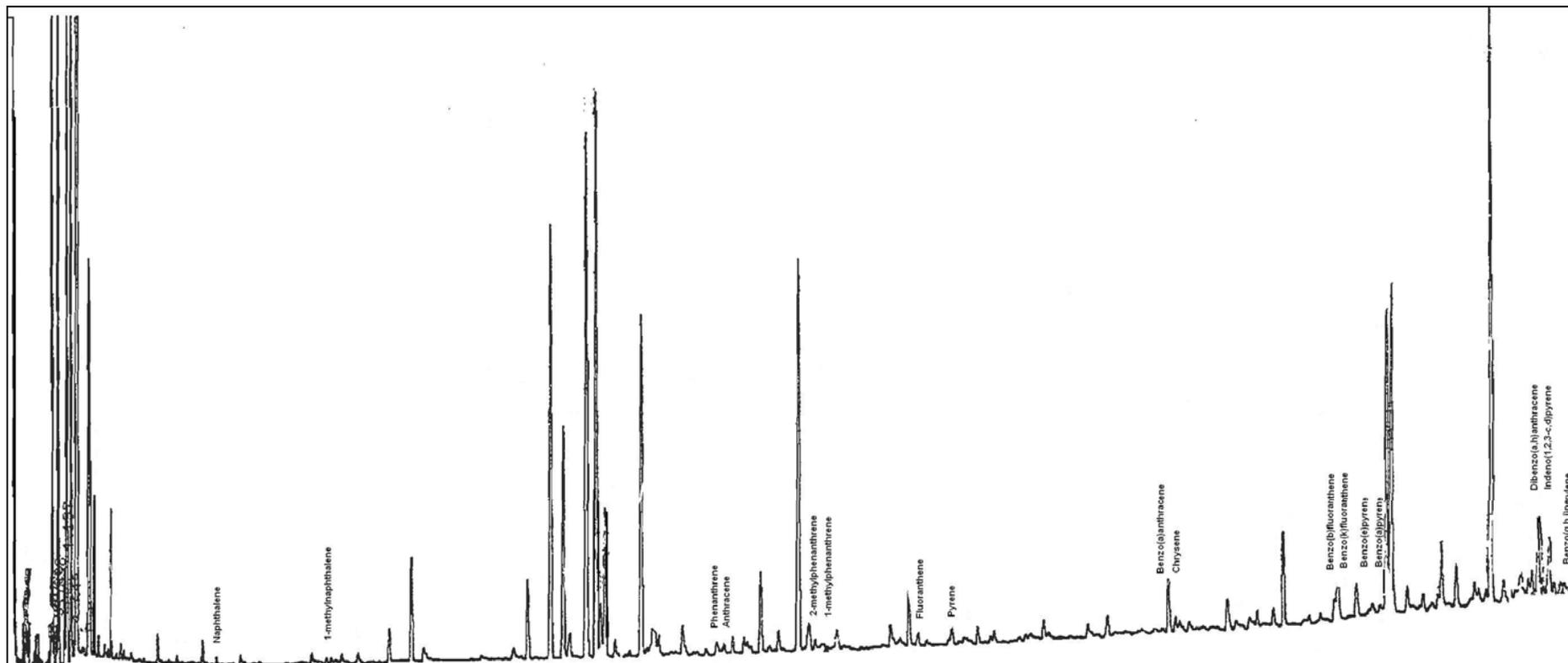
Usually, high molecular weight PAHs predominate in sediment samples, while low molecular weight PAHs predominate in seawater (Qiu et al., 2009; Zhou and Maskaoui, 2003). Such a difference in contaminant abundance by different PAH assemblages is expected due to at least two factors. First, the water column receives direct PAH inputs from various sources, including both petrogenic and pyrolytic. In the surface seawater of the Gulf of Trieste, the petrogenic sources may be more widespread and dominant than pyrolytic ones, leading to the abundance of 3-ring PAHs in seawater. Second, only those PAHs that are stable enough will reach the sediment bed. Such PAHs are quite likely to be of relatively high molecular mass and hence more resistant to degradation, evaporation and dissolution processes. In addition, processes such as biodegradation will attack PAHs leading to the

accumulation of those PAHs more resistant to degradation. Again, it is mainly the high molecular mass PAHs that are more stable. As a result, the water and sediment samples were dominated by different PAHs.

The higher concentration of higher molecular weight PAHs has been commonly observed in sediments from river and marine environments (Magi et al., 2002; Zhou and Maskaoui, 2003; Qiu et al., 2009). Pereira et al. (1996) also showed that 4-ring PAHs dominated PAH distribution in sediments from San Francisco Bay.

Among the individual PAH compounds identified the 4-5 ring compounds fluoranthene, pyrene and benzo[*k*]fluoranthene dominated at stations PI1, ST, IZ2, KP1, LK, MP1, PZ and PP, while 6-ring compounds indeno[1,2,3-*c,d*]pyrene prevailed at station KZ.

The most abundant 2-3 ring compounds were phenanthrene, anthracene and methylphenanthrenes. Naphthalene and 1-methylnaphthalene were also present at stations LK, ST, KP1, MP1, CZ and KZ.



*Figure 11: The chromatogram of the aromatic hydrocarbon fraction at station Luka Koper (LK)*

For the identification of the PAHs origin in surface sediments the ratios of low molecular weight PAHs to high molecular weight PAHs (LPAHs/HPAHs), methylephenanthrenes to phenanthrene (MPhe/Phe), phenanthrene to anthracene (Phe/Ant), fluoranthene to pyrene (Flu/Py), fluoranthene to fluoranthene plus pyrene (Flu/(Flu+Py)) and anthracene to anthracene plus phenanthrene (Ant/(Ant+Phe)) were examined. PAH ratios of surficial sediment samples are presented in Table 10.

**Table 10:** Calculated ratios for source identification in sediment samples

Station	Ratio					
	LPAHs/HPAHs	MPhe/Phe	Phe/Ant	Flu/Py	Flu/(Flu+Py)	Ant/(Ant+Phe)
CZ	0.18	3.00	1.54	0.89	0.47	0.39
LK	0.16	2.36	1.17	0.82	0.45	0.26
MP1	0.17	1.21	28.00	0.94	0.49	0.03
KZ	0.34	4.46	2.60	0.71	0.41	0.28
PZ	0.17	0.61	0.86	1.24	0.55	0.50
PP	0.21	1.20	1.67	2.50	0.71	0.38
PI1	0.20	0.29	9.91	1.53	0.60	0.09
ST	0.22	0.23	6.63	1.12	0.53	0.13
IZ2	0.17	0.66	2.32	1.16	0.54	0.30
KP1	0.16	0.55	5.07	0.99	0.50	0.16

LPAHs: Low molecular weight PAHs; HPAHs: High molecular weight PAHs; Phe: Phenanthrene; MPhe: Methylphenanthrene; Flu: Fluoranthene; Py: Pyrene; Ant: Anthracene

The LPAHs/HPAHs ratio  $<1$  and Flu/(Flu+Py) ratio  $>0.4$  calculated for the ten surface sediments suggest pyrogenic sources at all stations. In contrast the Flu/Py ratio  $<1$ , which indicates petrogenic origin, was recorded at stations CZ, LK, MP1, KZ and KP1, while Flu/Py ratio  $>1$  suggesting pyrogenic origin was recorded at stations PZ, PP, PM1, ST and IZ2. The MPhe/Phe ratio  $<1$  found at stations PZ, PM1, ST, IZ2 and KP1 suggest pyrogenic origin, while MPhe/Phe ratio  $>2$  found at stations CZ, LK and KZ suggest petrogenic origin. The MPhe/Phe ratio between 1 and 2 found at stations MP1 and PP suggest mixed sources. In addition, Phe/Ant ratio  $<10$  and Ant/(Ant+Phe) ratio  $>1$  indicating pyrogenic sources was found at all stations except at station MP1 while station PI1 in both cases was near the boundary between pyrogenic and petrogenic sources.

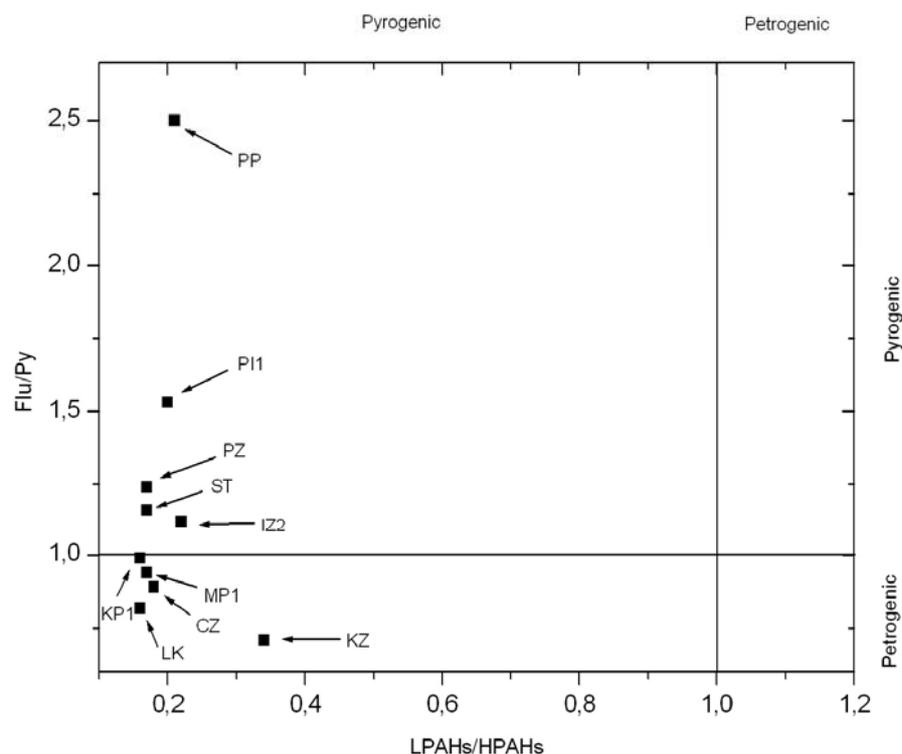
For better determination and demonstration LPAHs/HPAHs ratio against Flu/Py ratio, Phe/Ant ratio against Flu/Py ratio, Ant/(Ant+Phe) against Flu/Py and Ant/(Ant+Phe) against Flu/(Flu+Py) were plotted. Different PAH cross plot ratios of surficial sediment samples are presented in Table 11.

**Table 11: Determination of PAH pollution sources of sediment samples**

Station	Ratio			
	LPAHs/HPAHs vs. Flu/Py	Phe/Ant vs. Flu/Py	Ant/(Ant+Phe) vs. MPhe/Phe	Ant/(Ant+Phe) vs. Flu/(Flu+Py)
	Sources			
CZ	Mix	Mix	Mix	Pyro
LK	Mix	Mix	Mix	Pyro
MP1	Mix	Petro	Petro	Mix
KZ	Mix	Mix	Mix	Pyro
PZ	Pyro	Pyro	Pyro	Pyro
PP	Pyro	Pyro	Pyro	Pyro
PI1	Pyro	Mix	Mix	Mix
ST	Pyro	Pyro	Pyro	Pyro
IZ2	Pyro	Pyro	Pyro	Pyro
KP1	Mix	Mix	Pyro	Pyro

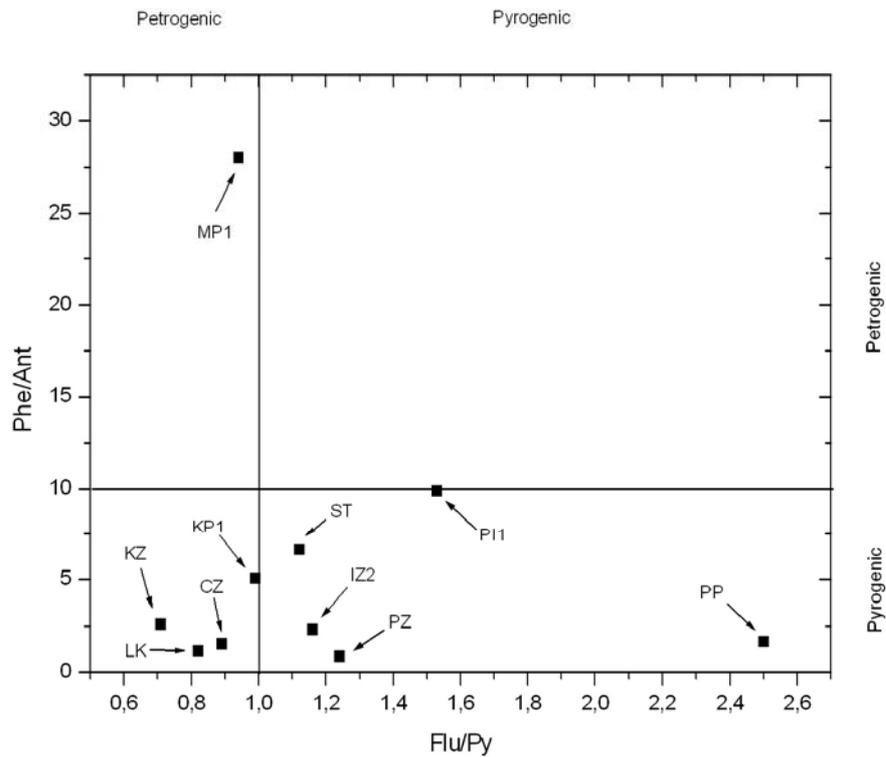
Pyro: Pyrogenic; Mix: Mixed; Petro: Petrogenic

The PAH cross plots of the ratios of LPAHs/HPAHs against Flu/Py indicate different PAH origins (Fig. 12). Sediments PP, PI1, PZ ST and IZ2 with LPAHs/HPAHs ratio <1 and Flu/Py ratio >1 suggest pyrogenic origin, while sediments KZ, LK, KP1, MP1 and CZ with LPAHs/HPAHs ratio <1 and Flu/Py ratio <1 suggest mixed origin (pyrogenic and petrogenic).



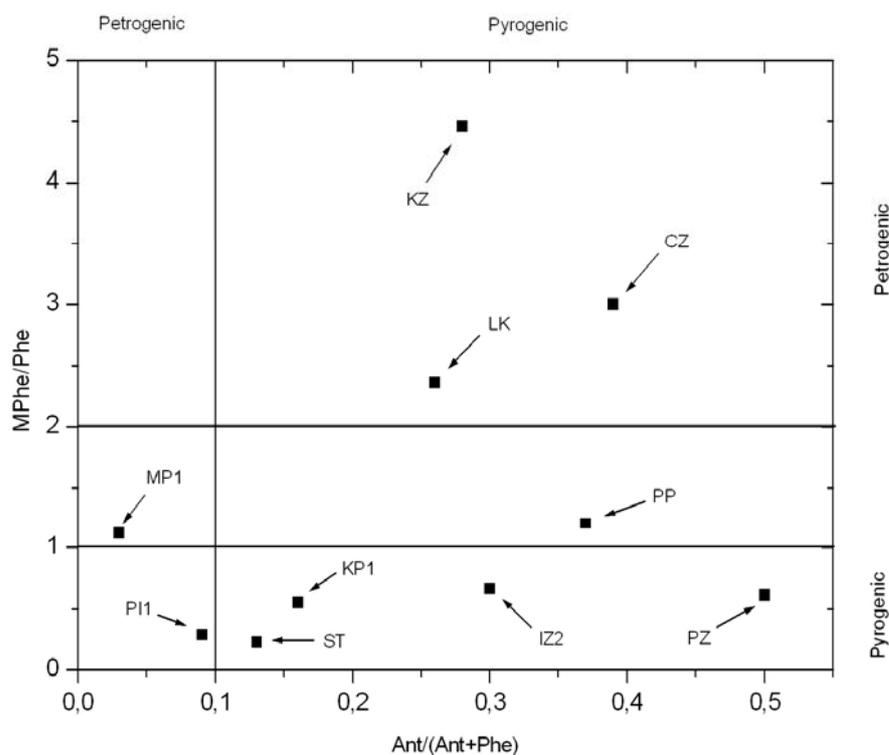
**Figure 12:** Cross plot of the LPAHs/HPAHs versus Flu/Py ratio.

The origin of PAHs in surficial sediment was also examined by plotting Phe/Ant ratio against Flu/Py ratio (Fig. 13). The Phe/Ant ratio < 10 and Flu/Py ratio > 1 found in sediments PP, ST, IZ2 and PZ suggest contamination from combustion processes, confirmed by a cross plot of LPAHs/HPAHs against Flu/Py, while Phe/Ant ratio > 10 and Flu/Py ratio < 1 found in sediments LK, KZ CZ and KP1 confirmed mixed origin. The Phe/Ant ratio against Flu/Py ratio found in sediment PI1 suggests mixed origin, while sediment MP1 indicates the predominance of petrogenic origin.



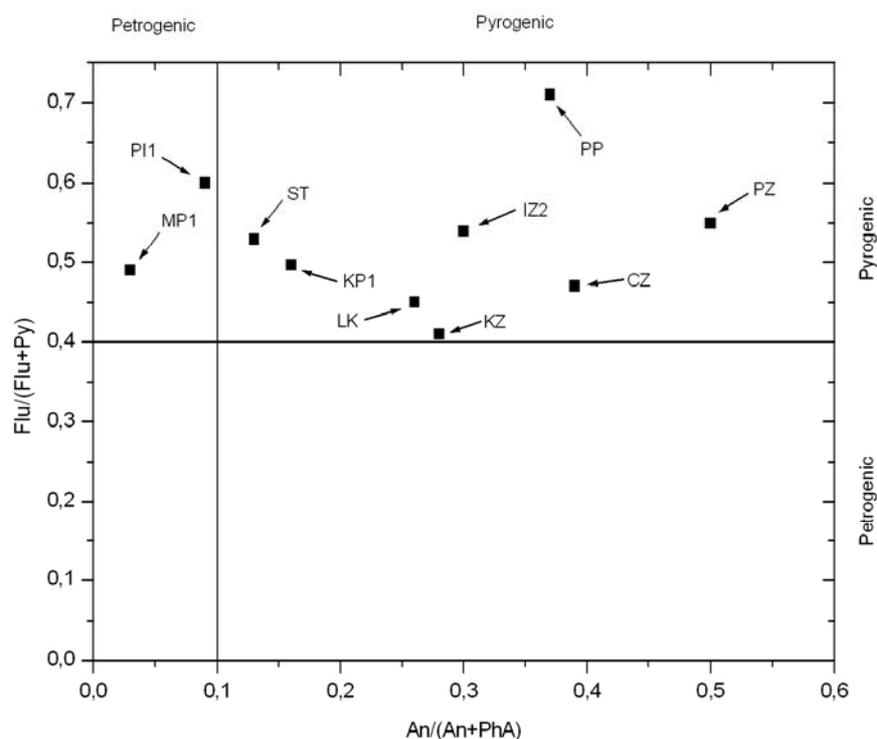
**Figure 13:** Cross plot of the Phe/Ant versus Flu/Py ratio.

In addition, ratios of Ant/(Ant+Phe) against MPhe/Phe were plotted (Fig. 14). In accordance with previous results, the Ant/(Ant+Phe) against MPhe/Phe cross plot confirmed mixed origin (sediments CZ, LK, KZ), as pyrogenic (sediments PZ, PP, ST and IZ2) and petrogenic (station MP1). Sediment PI1 suggested mixed origin, while sediment KP1 indicated the prevalence of pyrogenic origin.



**Figure 14:** Cross plot of the  $Ant/(Ant+Phe)$  versus  $MPhe/Phe$  ratio.

In order to determine PAH sources in sediments, where different ratios and plots show mixed origin (e.g. stations CZ, LK, KZ and KM) the ratios  $Ant/(Ant+Phe)$  against  $Flu/(Flu+Py)$  were plotted (Fig. 15). The cross plot of the  $Ant/(Ant+Phe)$  versus  $Flu/(Flu+Py)$  ratio reveals that sediment samples CZ, LK, KZ and KP1 indicate mainly pyrogenic origin and confirmed pyrogenic origin in sediment PZ, PP, ST and IZ2 which were previously reported in this study.



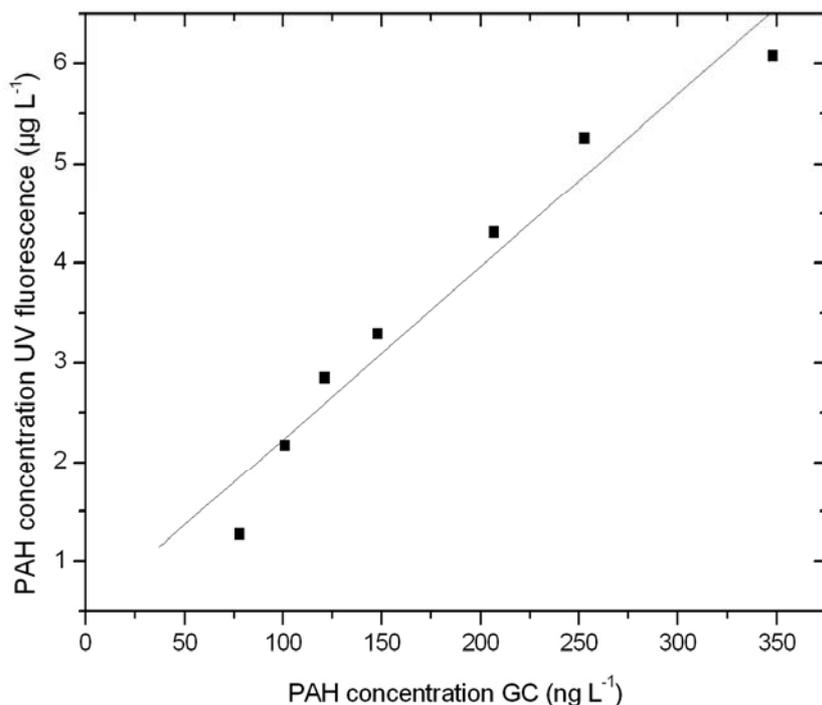
**Figure 15:** Cross plot of the  $Ant/(ant+Phe)$  versus  $Flu/(Flu/Py)$  ratio).

In conclusion, based on the studied source definition criteria the main source of sediment contamination with PAH in the investigated area is of pyrogenic origin. Some contribution of a petrogenic source is also evident at some sampling stations. The results of source definition in our study are comparable with those found by Cibic et al. (2008), Heath et al. (2006) and Notar et al. (2001) in the Gulf of Trieste.

#### **4.4 Fluorescence spectroscopy and gas chromatography relationship**

Determination of seawater contamination is time consuming and costly. The delineation of contamination requires systematic sampling within a predetermined sampling grid at multiple sites, depths and with appropriate replication, generating a large number of samples. The development of cost-effective, semi quantitative screening methods for the determination of organic contamination has received an attention of different authors. Fluorescence spectroscopy has been used as screening method for PAHs detection by several researchers (Wakeham, 1977; Owen et al., 1995). Some monitoring programs such as MARPOLMON (IOC-UNESCO, 1982) have also opted to employ the fluorescence spectroscopy as a screening method to detect the presence of petroleum hydrocarbons in seawater. Fluorescence spectroscopy is very sensitive, quick and relatively inexpensive for the estimation of total PAH concentrations, but has generally been shown to overestimate total PAH concentrations, since it is sensitive also to various PAH metabolites and possible high humic acid levels (Knopp et al., 2000). The fluorescent matter could also be a degradation product or products generated by micro-organisms or abiotically generated from oil or other chemicals, and the fluorescence believed to indicate the presence of oil residues maybe connected to some other compounds. It has been recommended (Farrington et al., 1982) that the fluorescence spectroscopy screening method is best employed in combination with chromatography techniques because of their inherent sensitivity and ability to detect resolved hydrocarbons and other compounds.

In this work fluorescence spectroscopy and gas chromatography techniques were applied at same time for the analyses of PAHs in seawater. The correlation between concentrations of total PAHs measured with fluorescence spectroscopy and GC is shown in Fig. 16.



**Figure 16:** Cross plot of PAH concentrations obtained by fluorescence spectroscopy and GC.

The results show good correlation of relative PAH concentrations ( $r^2=0.94$ ;  $n=7$ ) obtained with fluorescence spectroscopy and GC. It has to be mentioned that this linear relationship was not observed at higher PAH concentrations. On the other hand an important overestimation ( $\sim 20$  times) of absolute PAH concentrations was obtained by fluorescence measurements. Higher concentrations measured with fluorescence spectroscopy are probably due to the fluorescence of different residues (not only PAHs) present in the surface seawater. These degradation products are usually considered as unresolved complex mixture (UCM) in the GC chromatogram. In addition, we calculated the UCM to examine the relationship between the concentrations obtained by fluorescence measurements and the sum of resolved PAHs and UCM obtained by GC. Unfortunately, the results were very scattered and no relationship could be determined. Further studies should be conducted to elucidate this relationship.

## 5 CONCLUSIONS

This work has provided important data about PAH concentrations, distribution and sources in seawater and sediments of the Gulf of Trieste, northern Adriatic. Total PAH concentration ( $\Sigma 24$  PAHs) varied from 78 ng L<sup>-1</sup> to 302 ng L<sup>-1</sup> in surface seawater, and from 297 ng g<sup>-1</sup> to 115854 ng g<sup>-1</sup> (dry weight) in surficial sediments. The levels of PAHs in seawater were comparable to those found in many other marine systems, although PAHs in sediment, at some stations were relatively high in comparison to other urbanized coastal areas worldwide. The PAH distribution profile in the investigated area indicated potential source dependence, as the levels were generally higher in the vicinity of marinas and harbours. The most contaminated sites were the Marina of San Giusto, the Marina of Portorož and the municipal harbours of Koper, Izola and Piran.

In addition, seasonal variation of total PAH concentrations in surface seawater of the Gulf of Trieste measured with fluorescence spectroscopy indicates higher concentrations in summer compared to winter. The total PAH concentrations (in chrysene equivalents) in winter ranged from 0.07  $\mu\text{g L}^{-1}$  to 34.32  $\mu\text{g L}^{-1}$ , while in summer they ranged from 0.01  $\mu\text{g L}^{-1}$  to 48.51  $\mu\text{g L}^{-1}$ . Higher concentrations of PAHs (measured with fluorescence spectroscopy) recorded in summer could be attributed not only to PAH, but also to fluorescence of different degradation products, while in general, the levels of PAHs took into account only resolved PAH compounds. In accordance with our expectations higher concentrations were recorded in semi-enclosed areas such harbours and marinas as a result of more intense pollution and higher accumulation of pollutants.

The PAH group profile shows the predominance of 2-3 ring PAHs in surface seawater and 4-5 ring PAHs in surface sediments. Among the individual PAH compounds identified, the most abundant PAH compounds in surface seawater (excluding perylene) were 1-methylphenanthrene, 2-methylphenanthrene, pyrene and chrysene, while in sediments were fluoranthene, pyrene, benzo[k]fluoranthene and benzo[g,h,i]perylene. The presence of relative high content of perylene in seawater indicated terrestrial inputs of hydrocarbons.

Two main sources of PAHs, petrogenic and pyrogenic, were determined. The main sources of seawater contamination in the Gulf of Trieste appear to be petrogenic, presumably due to oil spills from shipping, while pyrogenic prevailed in sediments.

The comparison of the results obtained by fluorescence spectroscopy and gas chromatography revealed a good correlation of relative PAH concentrations ( $r^2=0.94$ ;  $n=7$ ) in the range between  $1 \mu\text{g L}^{-1}$  and  $6 \mu\text{g L}^{-1}$ . It has to be mentioned that this linear relationship was not observed at higher PAH concentrations. On the other hand an important overestimation ( $\sim 20$  times) of absolute PAH concentrations was obtained by fluorescence measurements. Fluorescence spectroscopy appeared as suitable screening methods for the assessment of the contamination of seawater with PAHs. However, further studies should be conducted to evaluate its applicability.

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