Sampler systems for tracking emitters of phenanthrene in sewers

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ABSTRACT

Pollutants can accumulate in sewage sludge. In such cases, this sludge represents toxic waste and has to be disposed on specialized dumps or eliminated by incineration. The mechanisms of sorption and the sorption sites for such substances are varying according to their physico-chemical properties. Fact is that both polar and non-polar substances can be accumulated. The development of a monitoring approach for detecting pollutants along sewers represents a suitable solution for locating polluters upstream the waste water treatment plant (WWTP) and prevent the contamination of the sewage sludge. Phenanthrene, a polycyclic aromatic hydrocarbon, is one of the most common pollutants found in sewage sludge and was chosen in this work as a reference compound. It was reported that microbial biofilms can absorb and accumulate pollutants in aqueous environments, therefore sewer biofilms can be exploited as sampler system for detecting PAHs along the sewers. In addition to their role as a sink for pollutants, biofilms can desorb the absorbed compounds back into the aqueous phase and thus become a source of environmental pollution. The time between the absorption and the desorption processes is defined as the memory of the biofilm. It is necessary to estimate the memory of biofilms for phenanthrene in order to develop a systematic approach for monitoring PAH compounds in sewers. The investigation of the sorption properties of biofilms for phenanthrene was carried out in this study using polysaccharide gels as surrogate matrices. In fact pure polysaccharide gels and polysaccharide gels enriched with bacterial cells have been reported to display properties which are similar to biofilms regarding the diffusion of organic compounds. The sorption partitioning and the desorption kinetics of phenanthrene in polysaccharide gels with 98% (v/v) water content have been investigated here. The diffusion coefficients of phenanthrene in the gels ranged from 5 to 8 x 10^{-6} cm^2/sec, which is similar to the diffusivity of phenanthrene in pure water (7 x 10^{-6} cm^2/sec). In addition, the partition coefficients of phenanthrene between the gel and the water phase were measured. The organic carbon partition coefficient (K_{oc}), the gel-water partition coefficient (K_{gw}) and the distribution coefficients (Kd) were measured during both absorption and desorption partitioning experiments. The memory of a gel layer (1 cm x 3.5 cm x 0.1 cm) made of agar 1.5% (w/v) + gellan 0.5% (w/v) was calculated and experimentally determined under a continuous flow of deionized water. A polysaccharide gel memory of 2 hours for phenanthrene has been reported and discussed.

In addition, the research of a possible alternative sampler system was carried out for tracing phenanthrene in sewers by on-field measurements of fluorescence spectroscopy. Polydimethylsiloxane (PDMS) was chosen as sorbent phase for manufacturing a self-designed passive accumulation device (PAD). PDMS oil is hydrophobic and suitable for performing fluorescence spectroscopy measurements in order to detect fluorescent compounds absorbed into it, such as phenanthrene. The device was tested for the qualitative detection of phenanthrene in contaminated deionized water and surface water, using portable devices and optical fibers. The fluorescence spectra from the self-designed device were compared with those obtained from standard bench equipment. After these experiments, a new and optimized version of the device has been designed for future prospects. Using the Fick's laws of diffusion and the data available in literature, a simulation of the sorption kinetics of phenanthrene into the new PDMS device has been calculated, under conditions close to practice. A phenanthrene concentration of 9400 mg/L inside the PDMS oil was calculated after 30 hours of exposure to a continuous flow of contaminated water. The $K_{PDMS\text{-}water}$ of 10,000 (Sprunger et al., 2007) and the diffusivity of 1 x 10^{-6} cm^2/sec (DiFilippo and Egahouse 2010) of phenanthrene in PDMS were considered for the calculation of the simulation curve. When the contaminated flow ends and the phenanthrene concentration in the water drops down to 0, according to the simulation, no significant desorption occurs for a period of several weeks at 20-30 °C.

In conclusion this study provided first insights into the estimation of the biofilm memory for phenanthrene in aqueous systems. The sorption parameters and the desorption kinetics of phenanthrene under flow conditions from a polysaccharide gel layer were measured. The mass transfer of molecules inside biofilms is mainly governed by diffusion within the water phase and therefore the memory of biofilms for pollutants depends on the physical chemical properties of the compound itself. Nonpolar and hydrophobic compounds might display properties, that prevent them from being retained in hydrogel matrices such as polysaccharide gels and to some extent sewer biofilms for a long period of time. The development of an effective approach for monitoring PAHs in sewers by use of sampler systems is based on the memory of the sorbent phase. Long retention times are required for a suitable sampler system and PDMS was chosen as an alternative material to sewer biofilms for tracking PAHs in sewers. The preliminary experiments carried out revealed a high potential of this material for developing accumulation devices, which can be exploited for on field measurements by fluorescence spectroscopy.

1 INTRODUCTION

1.1 Pollution in sewage sludge and wastewater

Before the establishment of the modern wastewater treatment systems, most of the communities discharged their wastewater, or sewage, into streams and rivers with little if any treatment. As urban populations increased, the quality of streams and rivers began to deteriorate in many regions. In response to the water quality degradation and to concerns about healthcare issues, the need of suitable wastewater treatment systems arose.

"*By the end of the 18th century, all major northern European cities had built, or were building new systems to distribute water and evacuate liquid wastes (Reid, 1991). The realization that many diseases, such as cholera, were passed on by contaminated water was primarily behind the development of this "big pipe engineering approach" - for bringing drinking water into the city and for removing wastewater an d storm water from the city. Big pipe engineering became the standard water management technique*" (Vigneswaran et al., 2009)

The foundation of wastewater treatment plants and the establishment of waste water management strategies greatly improved stream and river water quality of the modern communities, but created another material to deal with: sewage sludge. The wastewater that enters a treatment plant is released from a variety of sources including homes, industries, medical facilities, rural activities, street runoff or businesses. Most of the water volume turns into clean effluent by the treatment processes, while the remainder portion is a dilute suspension of solids that has been captured by the primary, secondary and advanced treatment processes and includes grit, screenings and sludge. Of these constituents, sludge is by far the largest in volume, therefore handling methods and disposal practices of it are a matter of great concern. Since 1988, when the European legislation phased out the seawater disposal of the sludge (by the Urban Waste Water Treatment Directive), the main practices for handling the sewage sludge are: agricultural use (37%), incineration (11%), landfilling (40%), forest sulviculture and land reclamation (12%).

The latest trends in the field of sludge management are: combustion, wet oxidation, pyrolysis, gasification and co-combustion of sewage sludge with other materials. All of these new handling strategies have the aim to use the sludge as energy source. As previously mentioned the recycling of the waste sludge to land fertilization and land filling are the most common practices (Fytili and

Zabaniotou, 2008) and it is important to lower their contamination levels as much as possible in order to avoid any further environmental pollution. The pollutants present in the sludge can be divided in three groups:

1. Potential toxic elements (heavy metals)
2. Organic pollutants
3. Pathogens.

The fate of chemical contaminants entering a waste water treatment plant (WWTP) depends on both the nature of the chemical and the treatment processes (Zitomer and Speece, 1993). Because of the physical–chemical processes that are involved in activated wastewater sludge treatment, sludge tends to accumulate heavy metals existing in the wastewater. Heavy metals such as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg) and chromium (Cr) are principal elements restricting the use of sludge for agricultural purposes (US EPA 1993; The Sewage Sludge Directive 86/278/EEC; Hsiau and Lo; 1998).

The heavy metals are potential toxic elements (PTE) for human health and show long-term accumulation in soils and sediments (Wuana and Okieimen; 2011). The majority of PTEs entering the wastewater treatment plant transfer to the sewage sludge but between 20% and 40% can be released in the effluents from the treated wastewater. According to the EC policy of waste recycling, recovery and use, the application of sludge to agricultural land must be coupled with a constant monitoring of its contamination levels. This is a critical issue in view of the fact that the amount of sludge produced is going to increase and the governmental policies are going to be more stringent. Hence sludge quality must be protected and improved in order to secure the agricultural outlet as the most cost effective and sustainable option. The main source of PTE are the commercial and industrial activities. In Table 1 are shown the main inorganic pollutants occurring in sewage sludge along three European areas and the percentage of total incoming for each main source.

Table 1 Fytili and Zabaniotou; 2008 Contribution to the total PTE pollution of different sources, in reference areas in Europe (% values).

Pollutant	Country	Domestic wastewater	Commercial wastewater	Urban Runoff	Not identified
Cd	France	20	61	3	16
	Norway	40			
	UK	30	29	41	
Cu	France	62	3	6	29
	Norway	30			
	UK	75	21	4	
Cr	France	2	35	2	61
	Norway	20			
	UK	18	60	22	
Hg	France	4	58	1	37
Pb	France	26	2	29	43
	Norway	80			
	UK	43	24	33	
Ni	France	17	27	9	47
	Norway	10			
	UK	50	34	16	
Zn	France	28	5	10	57
	Norway	50			
	UK	49	35	16	

Organic chemicals may be volatilized, degraded (through biotic and/or abiotic processes), sorbed to sludge or discharged in the aqueous effluent. The main category of organic pollutants are: polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCB), di-2-ethylhexyl phthalate (DEHP) and Polychlorinated dibenzodioxins/dibenzofuran (PCDD/PCDF). These compounds are relatively hydrophobic and are strongly retained into particles and sludge. Most of them are not easily biodegraded during the treatments and can represent an environmental threat. Even though some of them are biodegraded, the by-products might be harmful and dangerous for the environment (European commission, *"Pollutants in waste water and sewage sludge"*, 2014, web). The main reason why these pollutants are a major concern is their harmful effect on human beings and other living organisms. Both the European community and the US environmental protection agency (EPA) established limit values of concentration for these pollutants within their own waste water framework policies and review these directives periodically in order to guarantee a suitable regulation according to the growing levels of industrialization and urbanization.

Since the time of the directive 86/278/EEC more pathogens associated with the food chain have been identified and new technologies have become available for sludge treatment. After separation from the wastewater, the sludge must be treated through one of a number of processes. Each of these has effects on the fate of both pathogens and the organic contaminants in the sludge

(Rogers, 1996). Heavy metals are not removed throw these steps and therefore their concentration must be evaluated before to address the sludge to land recycling purposes.

In order to produce sludge that is considered free of pathogens, it is exposed to thermophilic biological treatment and microbial digestion; or a combination of high pH and relatively high temperature (Table 2).

Table 2 Overview of the most used treatment processes of sewage sludge (European commission report; 2001).

Process	Parameters
Windrow composting: production of compost by piling biodegradable waste in long rows. This method is suited to producing large volumes of compost.	Batches of sludge (+/- bulking agent) to be kept at 55°C for 4 hours between each of 3 turnings, followed by maturation period to complete the composting process.
Aereted pile and invessel composting	The batch to be kept at a minimum of 40°C for at least 5 days and for 4 hours during this period at a minimum of 55°C. This to be followed by maturation period to complete the composting process.
Thermal drying	The sludge should be heated to at least 80°C for 10 minutes and moisture content reduced to < 10%.
Thermophilic digestion (aerobic or anaerobic)	Sludge should achieve a temperature of at least 55°C for a minimum period of 4 hours after the last feed and before the next withdrawal.Plant should be designed to operate at a temperature of at least 55°C with a mean retention period sufficient to stabilise the sludge.
Heat treatment followed by digestion	Minimum of 30 minutes at 70°C followed immediately by mesophilic anaerobic digestion at 35°C with a mean retention time of 12 days
Treatment with lime (CaO)	The sludge and lime should be thoroughly mixed to achieve a pH value of at least 12 and a minimum temperature of 55°C for 2 hours after mixing.

Concerning all the management strategies, which are used to handle the sewage sludge, pros and contras must be always considered carefully. Therefore, it is still a priority to monitor the waste water distribution systems and set suitable prevention strategies in order to lower the contamination at the source level.

1.1.2 Priority substances, polycyclic aromatic hydrocarbons (PAHs) and origin of contamination

1.1.2.1 Regulation

The priority substances are contaminants, which pose a significant risk to or via the aquatic environment. These substances are selected amongst those presenting higher index scores based on the occurrence in the environment and the toxicity on living organisms and also on human health (Lerche et al.,2002). Polycyclic aromatic hydrocarbons (PAHs) represent a category of highly common pollutants detected in water, soils, atmospheres and sewage sludge (Harrison et al., 2006, Perez et al., 2001). Some of them present an extremely low solubility in water and these are mostly accumulated in soil and living organisms. In addition, they display harmful effects on human beings, such as toxicity, mutagenicity and carcinogenicity.

The ecotoxicology of PAHs can be investigated by different tests involving the use of reference organisms like microorganisms, small invertebrates and plants (Table 3).

Table 3 Ecotoxicology tests of PAHs on contaminated soil samples (Eom et al.,2007).

	Tests (species)	Duration	Measured parameters	Test procedure
Microorganisms	Microtox® (*Vibrio fischeri*)	15, 30 min	Luminescence inhibition	ISO NF EN 11348-3 (1999)
	Mutatox® (*Vibrio fischeri* M169)	24 h	Genotoxicity	Microbics (1993)
	Ames (*Salmonella typhimurium* TA98, TA100)	72 h	Genotoxicity	Environment Canada (1993)
	Umu (*Salmonella typhimurium* TA1535/pSK1002)	4 h	Genotoxicity	ISO/DIS 13829 (2000)
Algae	Algae (*Pseudokirchneriella subcapitata*)	72 h	Growth inhibition	ISO 8692 (1996)
Aquatic invertebrates	Daphnid (*Daphnia magna*) Ceriodaphnid (*Ceriodaphnia dubia*)	24, 48 h 7 days	Immobilization reproduction	ISO 6341 (1996), AFNOR T90-376 (2000)
Terrestrial invertebrates	Collembola (*Folsomia candida*)	28 days	Survival, reproduction	ISO 11267 (1999)
	Earthworm (*Eisenia fetida*)	14 days 28, 56 days	Survival, reproduction	ISO 11268-1 (1993), ISO 11268-2 (1998)
Terrestrial plants	Lettuce (*Lactuca sativa*) Chinese cabbage (*Brassica chinensis*)	14–21 days	Germination, growth	ISO 11269-2 (1995)

Despite their high toxicity on most aquatic and terrestrial organisms, the priority in performing risk assessment investigations on contaminated soils and water streams is to evaluate the toxicity on mammals in reference to human beings. The toxicity of PAHs on mammals has been largely

studied by testing mice and rats in laboratory conditions and evaluating the LD50 (letal dose of 50% of the tested subjects) and other effects such as nephrotoxicity, oocyte and follicle destruction, testicular damage, carcinogenicity. In this regards Eisler 1987 provides a review that summarizes interesting results about the toxicity (Table 4) of both carcinogenic (Figure 1) and non-carcinogenic (Figure 2) PAHs.

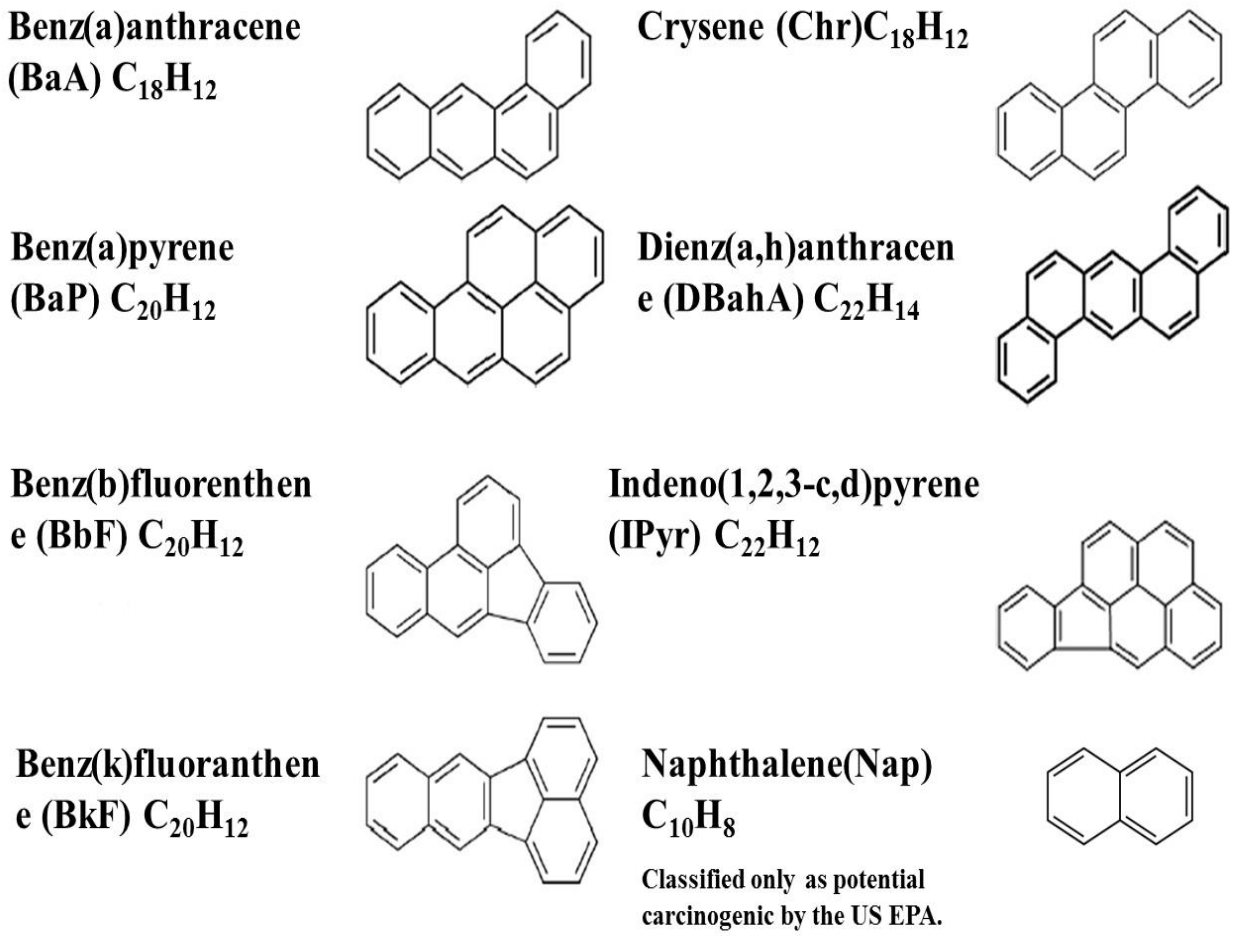


Figure 1 carcinogenic PAHs listed by the US EPA and by the European community. The name, the abbreviation and the structure are showed for each of the eight most regulated ones (Jennings et al.,2012).

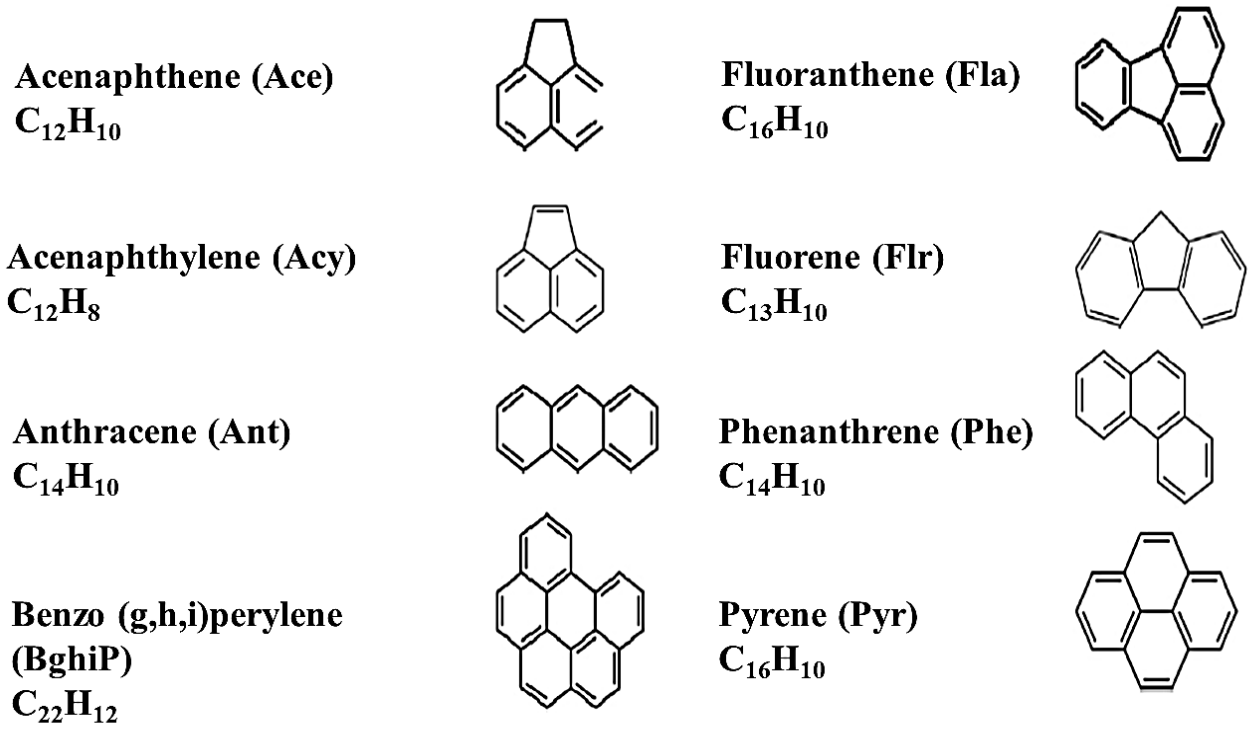


Figure 2 Non-carcinogenic PAHs listed by the US EPA and by the European community. The name, the abbreviation and the structure are showed for each of the eight most regulated ones (Jennings et al.,2012).

Table 4 Results of toxicity tests on rodents. In the right column are the values of PAH limit concentration for each toxicological test presented on the left column.

Tests

LD50 on rodents	**mg/kg of body weight**
Benzo-a-pyrene	50
Phenanthrene	700
Naphtalene	1780
Fluoranthene	2000
Carcinogenity oral administered	**mg/kg of body weight**
Benzo-a-pyrene	0.002
Chrysene	99
Anthracene	3300
Carcinogenity, topical administered	**mg**
Benzo-a-pyrene	0.001
Anthracene	0.08
Testicular damage oral administered	**mg**
Benzo-a-pyrene	100
Nephrotoxicity single intraperitoneal injection	**mg/kg of body weight**
Phenanthrene	150
Pyrene	150

In Europe the first policy notice about priority substances was included in the article 16 of the Water Framework Directive (2000/60/EC, WFD). This directive sets out "Strategies against pollution of water", outlining the need to establish, by way of Decision 2455/2001/EC, a first list of priority substances to become Annex X of the WFD. Later a more specific definition of priority substances was provided by the Directive on Environmental Quality Standards (Directive 2008/105/EC, EQS directive), which establishes environmental quality standards for the substances in surface waters (river, lake, transitional and coastal) and confirmed their designation as priority or priority hazardous substances. According to Annex V, point 1.4.3 of the WFD and Article 1 of the environmental quality standards directive, good chemical status is reached for a water body when it complies with the environmental quality standards for all the priority

substances and other pollutants listed in Annex I of the environmental quality standard directive (European commission, web page, Feb. 2014).

Each of the PAHs mentioned above, has been deeply studied and used as marker for environmental monitoring approaches. Although the establishment of reference environmental quality standards and the implementation of environmental guidelines by the European community and the US EPA, most of the regulation is still subject to country-specific laws.

For surface soil, the regulatory guidance values around the world can greatly vary from country to country. Jennings et al., 2012 provides a brief summary of these differences (Table 5). The Benzo(a)pyrene (BaP) is the reference compound for carcinogenic PAHs and the total concentration of the eight PAHs from Fig.2 is often used as reference for the limit concentration of non-carcinogenic PAHs.

Table 5 Limit values for carcinogenic (mg/Kg) PAHs in some of the countries mentioned by Jennings et al., 2012. The values which are not available are indicated with "-".

	Limit values (mg/ kg)						
Country	**BaA**	**BaP**	**BbF**	**BkF**	**Chr**	**DBahA**	**IPyr**
US EPA	0.15	0.015	0.15	1.5	15	0.015	0.15
Germany	-	2	-	-	-	-	-
Belgium	10.5	3.6	7	11.5	180	2.9	20
Italy	0.5	0.1	0.5	0.5	5	0.1	0.1
Brazil	20	1.5	-	-	-	0.6	25

Also in regard to the non-carcinogenic PAHs, the regulatory guidance values are provided within a broader range of higher concentrations (Table 6).

Table 6 Limit values for non-carcinogenic PAHs in some of the countries mentioned by Jennings et al., 2012. T is the total PAH concentration. The values which are not available are indicated with "-".

	Limit values (mg/ kg)							
Country	**Ace**	**Acy**	**Ant**	**BghiP**	**Fla**	**Flr**	**Phe**	**Pyr**
US EPA	3400	-	17000	-	2300	2300	-	1700
Germany	36-T	36-T	36-T	36-T	36-T	36-T	36-T	36-T
Belgium	14	1	70	3920	30	3950	65	395
Italy	-	-	5	0.2	5	-	5	5
Brazil	-	-	-	-	-		40	-

The regulations for PAHs levels in surface and drinking water are more uniform and shared at international level. In 1984 the World Health Organization (WHO) recommended values of total

PAH concentration around 0.7 µg/L as risk limit for human health. Hennion et al., 1994 reports values of 0.2-1 µg/L at European level, while later in time, within the WFD 2000/60/EC the value was lowered to 0.1 µg/L. The US EPA sets the limit of 0.2 µg/L. However, different specific caveats in the regulatory system of each country must be considered also for these values.

At European level the WFD 2000/60/EC the limit value for total PAH concentration in sewer sludge is equal to 6 mg/kg, after a deep investigation on the real levels of these compounds in various urban waste water treatment plants around Europe, ranging from 0.5 mg/kg to 27.8 mg/kg and updated by 2014.

1.1.2.2 Occurrence of PAH pollutants

PAH compounds are a major concern of pollution due to their toxicity and their ubiquitous occurrence in air, soil, surface water and waste water, from which they accumulate in the sewage sludge (Srogi; 2007, Baek et al.,1991).

On a global scale around 85% of the emitted PAHs is represented by low molecular weight PAHs, such as naphtalene, phenanthrene, acenaphtilene, acenaphthene, fluorine, fluoranthene, pyrene, anthracene, while the high molecular weight PAHs cover only the least of the overall emission (Table 7, Zhang and Tao; 2009).

Table 7 Percentage of each low molecular weight PAH in the whole global emission (Zhang and Tao 2009)

PAH	% global emission
Naphtalene	50
Phenanthrene	10
Acenaphtylene	10
Acenaphtene	5
Fluorine	3
Fluoranthene	3
Pyrene	4

The PAHs sources can be both natural and anthropogenic. PAHs are mostly formed during the incomplete combustion and pyrolysis of fossil fuels or wood, and from the release of petroleum products. Other sources are petroleum spills, oil seepage and diagenesis of organic matter in anoxic sediments. The main sources are divided in five categories: domestic, mobile, industrial,

agricultural and natural (Ravindra et al., 2008). Burning of coal, oil, gas, garbage and, to a lesser extent, other organic substances like food (e.g. char broiled meat) are the main processes belonging to the domestic source of PAH pollution. These processes are involved mostly in heating and cooking activities. The WHO estimated that 75% of the people in China, India and South east Asia burn solid fuels like wood, animal dung cakes and crop waste for daily heating and cooking. In a global scale these and the biofuel combustion are significant contributors to the overall emission of all the 16 PAHs listed by US EPA and European community as priority substances (Zhang and Tao, 2009, Table 8 and 9).

Table 8 The principal global emission processes of the 16 PAHs listed by the US EPA and the European community (Zhang and Tao; 2009).

Processes	Tons/year global emission
Open fires burning of wheat	10000
Indoor burning of corn	5000 – 10000
Indoor burning of rice	10000
Indoor burning of wheat	60000 – 80000
Forest fires	40000
Firewood burning	16000
Domestic combustion of coal	20000
Small scale coke production	10000 – 20000
Traffic gasoline	20000
Consumer products usage	20000-30000
Animal dung combustion	20000 – 30000
Grassland fires	20000 – 30000
Waste incineration	5000 - 10000

Table 9 Sources of PAHs. On the right is their percentage contribution on the global emission of the 16 PAHs listed by US EPA and European community as priority substances (Zhang and Tao 2009).

Sources of PAHs	% contribution
Biofuel burning	56.7
Wildfires	17
Consumer products	6.9
Traffic oil combustion	4.8
Domestic coal combustion	3.7
Industrial activities	10 (3.6% coke production)

The Environment Directorate-general of the European Community quantified the total emission of benzo(a)pyrene in Europe in 1990. The report for the economic evaluation of the air quality targets for PAHs published in 2001 shows that also in Europe, between 1990 and 2010, the main sourcesof benzo (a)pyrene were those involving domestic and small burning processes of coal and wood (about 200 Tons/year), while the industrial activities are just minor contributors (between 2 and 50 tons/year). Furthermore between 1990 and 2010 the European commission aimed a significant reduction of the industrial emission of B(a)P, but a relatively small decrease in the wood burning processes, mainly due to a not significant increase in the use of other bio fuels rather than wood, which still represent a significant source of B(a)P.

However the relative contribution of different PAH sources in the different countries depends on the energy structure, status of development, population density and vegetation cover of the country, so that big differences are observed for specific PAH sources between the European area, USA and the east of the world (Zhang and Tao, 2009).

Another important group of sources is represented by the mobile sources. These ones are considered all the transport systems, which involve the consumption of diesel or gasoline. Aircrafts, automobiles, ships, railway locomotives, off road vehicles and machinery belong to this category. Among these the ones which are fueled by diesel are the stronger PAH producers. The main industrial sources are aluminum production, coke production, waste incineration, cement manufacture, petrochemical industries, bitumen and asphalt industries and rubber tire manufacturing. In addition, agricultural activities are considered one of the main sources of PAHs in the environment, due to the open burning of biomass that is employed for residue disposal and

land preparation. All these procedures involve burning under sub optimum combustion conditions; therefore they are expected to significantly contribute to the overall PAH emission in the environment. The less important source of PAHs are natural processes such as forest and wood land fires (e.g. after lightning strikes) and volcano activities. Others are high temperature pyrolysis of organic matter, diagenesis of sedimentary organic material for fossil fuel formation and microbial biosynthesis.

As mentioned earlier PAHs are ubiquitous pollutants in the environment and cause a growing concern due to their significant harmful potential on human health. Toxicity, mutagenicity and carcinogenicity are the main effects on mammalians (Kim et al., 2013). When present in wastewater, high molecular weight PAHs molecules are slowly biodegraded in both activated sludge and compost piles and this limits their disposal for landfilling and for agricultural purposes. On the other hand low molecular weight PAHs ($\leq$ 3 aromatic rings) are not efficiently retained in the sewage sludge during the treatment steps (Charalabaki et al., 2005), therefore they can be found in the effluents of the WWTPs and this increases their diffusion in the environment (Charalabaki; 2005). Due to their high hydrophobicity, PAHs can be absorbed and can accumulate into particulates and soil fractions from contaminated water stream. The sorbent organic matter, which gathers these compounds can eventually sink and be a source of further pollution. This phenomenon has potentially harmful consequences for all the biota living downstream from the sinking. Furthermore, when retained in the soil or in aquatic organisms, these compounds can undergo a process of biomagnification, enter the food chain and increase the risk to human health (Ramesh et al., 2004; D'Adamo et al., 1997). Phenanthrene is one of the most common PAHs with low molecular weight detected in air, soil, wastewater streams and treated wastewater effluents (Wloka et al., 2013, Chang, 2006, Charalabaki et al., 2005). Processes leading to the formation of PAHs are common in urban and industrial areas. Therefore the occurrence of these pollutants is widespread in soil, surface water, air and wastewater. Due to the level of information and specific high occurrence in wastewater, phenanthrene has been chosen as reference compound for this study. Although it has not been proved to be carcinogenic for human beings, phenanthrene is often considered as analytical marker for evaluate the exposure to PAH sources , in relation to other more dangerous PAHs (Kuusimaki et al.,2004, Srogi 2007).

In Table 10 are listed examples of PAH occurrence in soil. It is possible to observe that the highest concentration of PAH in soil is found within industrial areas and more specifically where coal burning processes are performed. (Placha et al., 2009, Hussar et al., 2012). Furthermore, among all the PAHs, the reviewed studies confirm that phenanthrene is found to be the most common pollutant in both soil, water and sewage sludge (Table 10, 11, 12).

Table 10 Occurrence of PAHs in soil reported by literature references (Placha et al., 2009, Placha et al., 2010, Okedeyi et al., 2012, Hussar et al., 2012). dw= dry weight

Soil	∑PAHs (mg/kg dw)	Phenanthrene (µg/kg dw)	Reference
Soil near the Tiefa Coal mine, China	0.06 – 5.64	-	Liu et al.,2012
Plant for production of cement, Italy	0.1 - 96	5.3 - 33	Orecchio 2009
Coal tar refinery, Czech Republic	0.8 - 10	-	Placha et al.,2009
Forest soil near coal tar refinery, Czech Republic	0.7 - 79	-	Placha et al.,2009
Seine river basin, urban area, France	0.005 – 0.3	132	Motelay-Massei 2003
Seine river basin, industrial area, France	0.009 – 0.6	254	Motelay-Massei 2004
Urban area of Minsk, Belarus	0.6	-	Kukharchyk et al.,2013
Coal fired power plants, South Africa	9.7 – 6.1	-	Okedeyi et al.,2013
Industrial area of Chattanooga, Tennessee, USA	0.6 – 20.8	50 - 6600	Hussar et al.,2012

In water the occurrence of PAHs depends on the level of industrialization and urbanization of the monitored areas. The road run off, the industrial and household activities are mainly involved in the surface water pollution. A higher density of population and a high level of industrialization causes higher concentration of PAHs in the water bodies compared with areas where the industrialization is lower and better controlled (as for soil in Table 10).

Table 11 Occurrence of PAHs in surface water. Comparison between high population density areas and low density areas.

Surface water in urban and industrial areas	∑PAHs (µg/L)	Phenanthrene (ng/L)	Reference
Raba river, Urban and industrial area, Hungary	0.041 – 0.4	-	Nagy et al., 2013
mean from all the rivers, high density, China	0.003 – 38.1	-	Guo et al.,2012
Lake Maggiore, Italy	0.003	0.8 - 1.9	Olivella et al.,2006
Venice Lagoon, Italy	0.003	0.8 - 2.24	Manodori et al., 2006
Tväran river and Nemunas river, Lituania	0.0 8	7.7	Bergqvist et al.,2007

Also the concentration of PAHs in sewage sludge is strictly linked to the typology of the reference area. The range of concentrations is uniform between urban areas but differs largely from the small rural area described in Mansuy-Huault et al., 2009 (Table 12).

Table 12 Occurrence of PAHs in sewage sludge reported by literature references.(dw=dry weight)

Sewage sludge	∑PAHs (mg/kg dw)	Phenanthrene (µg/kg dw)	Reference
Rural district, Lorrain, France	0.8 - 60	0.08 - 6	Mansuy-Huault et al.,2009
Urban area, Poland, before composting	2 - 10	-	Oleszczuk 2008
Urban area Poland, after composting	1 - 7	800 - 5600	Oleszczuk 2009
Urban area Paris, France	14- 31	1500 - 3000	Blanchard et al.,2004
Urban area, Bejiing, China	2.4 - 26	48 - 466	Dai et al.,2007

1.1.2.3 Properties of PAHs and phenanthrene

The polycyclic aromatic hydrocarbons, also called polycyclic arenes, are organic compounds formed by the fusion of 2 or more benzene rings. There are several organic compounds classed as polycyclic aromatic hydrocarbons, but only about 16 are listed as reference compounds for the US EPA and the European Community (Table 13).

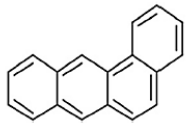
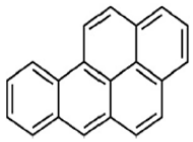
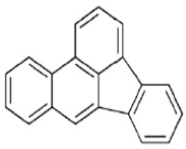
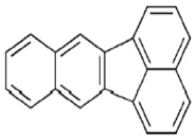
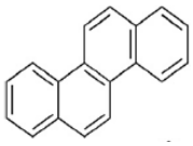
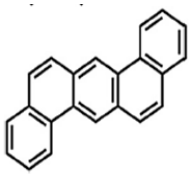
PAH have a common molecular electronic arrangement proper of conjugated systems. Conjugated molecules have π electrons that are not localized in individual double or triple bonds.

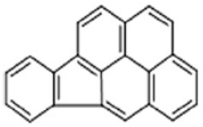
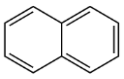
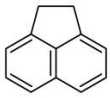
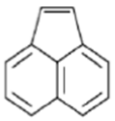
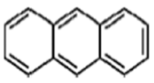
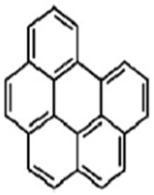
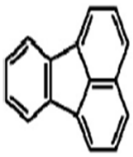
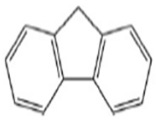
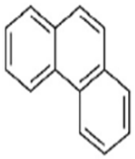
All physical-chemical properties of PAHs are related to the arrangement of π electrons. The main properties are (Lee et al., 1981):

- Low Solubility
- High vapour pressure
- High octanol-water partition coefficient
- High octanol-air partition coefficients

Briefly these properties are listed in Table 13, where the structure and the main properties of these compounds are presented (Sverdrup et al., 2002; Chiou, 1985.).

Table 13 The main chemical properties of all the PAHs listed by the US EPA and European community as priority substances (Sverdrup et al., 2002). Molecular weight (MW), water solubility (WS), octanol-water partition coefficients (L/Kg), vapour pressure (Pa) and structural formulas. Phenanthrene is highlighted as reference compound for this study.

Name	MW	WS (mg/L)	Log K_{ow}	Log K_{oa}	V_p / Pa	Structural formula
Benz(a)anthracene (BaA) $C_{18}H_{12}$	228.3	0.0094	5.8	10.28	2.80 x 10^{-5}	
Benz(a)pyrene (BaP) $C_{20}H_{12}$	252.3	0.0016	6.20	11.35	7.32 x 10^{-3}	
Benz(b)fluoranthene (BbF) $C_{20}H_{12}$	253.3	0.0015	6.4	11.34	6.67 x 10^{-5}	
Benz(k)fluoranthene (BkF) $C_{20}H_{12}$	252.3	0.0008	6.40	11.37	1.29 x 10^{-7}	
Crysene (Chr) $C_{18}H_{12}$	228.3	0.0020	5.80	10.30	8.31 x 10^{-7}	
Dibenz(a,h)anthracene (DBahA) $C_{22}H_{14}$	278.4	0.0025	6.50	12.59	1.27 x 10^{-7}	

Name	MW	WS (mg/L)	Log K_{ow}	Log K_{oa}	V_p / Pa	Structural formula
Indeno(1,2,3-c,d)pyrene (IPyr) $C_{22}H_{12}$	276.3	0.0002	6.70	12.43	1.67 x 10^{-8}	
Naphthalene(Nap) $C_{10}H_8$	128.2	31.0	3.32	5.19	1.13 x 10^{1}	
Acenaphthene (Ace) C12H10	154.2	3.9	3.94	6.44	2.87 x 10^{-1}	
Acenaphthylene (Acy) C12H8	152.2	16.1	4.07	6.46	8.91 x 10^{-1}	
Anthracene (Ant) C14H10	178.2	0.0434	4.50	7.70	8.7 x 10^{-4}	
Benzo (g,h,i)perylene (BghiP) C22H12	276.33	0.0003	6.6	12.55	1,33 x 10^{-8}	
Fluoranthene (Fla) C16H10	202.2	0.26	5.2	8.81	1.23 x 10^{-3}	
Fluorene (Flr) C13H10	166.2	1.69	4.2	6.85	8.0 x 10^{-2}	
Phenanthrene (Phe) C14H10	**178.2**	**1.3**	**4.6**	**7.64**	**1.61 x 10^{-2}**	
Pyrene (Pyr) C16H10	202.2	0.13	5.2	8.86	6.00 x 10^{-4}	

PAHs can be divided according to the molecular mass, in low molecular weight (LMW) having a number of benzene rings up to three and high molecular weight (HMW) having more than three benzene rings.

The LMW PAHs show higher solubility and volatility than the HMW PAHs. In addition the spatial arrangement of the aromatic rings within the molecule, provides highly different physical-chemical properties. PAHs can be formed by linearly fused rings or can present an angular disposition of the aromatic rings.

Greater thermodynamic stability arises from the delocalization of the π- electron density, so that liner fused PAHs are thermodynamically less stable than angular PAHs. Furthermore the higher are molecular weight and molecular arrangement, the stronger are the hydrophobicity and the electrochemical stability of the molecule and according to this principle, also the solubility, the vapour pressure, the chemical reactivity and the photochemical reactivity are significantly lowered.

As previously mentioned, higher molecular weight PAHs show stronger hydrophobicity and in the environment, this is in correlation with a lower biodegradability of the molecule. In fact the high molecular weight PAHs have a higher octanol-water partition coefficient (>4.5) and show a lower solubility in water , so that they are sequestered on particles of solid organic matter (SOM) and are less available for microbial communities to be degraded (Singh, "*Microbial degradation of xenobiotic*",Springer, 2012).

The vapour pressure decreases with increasing molecular weight and this causes the increase of the boiling temperature. Naphthalene (low molecular weight) has a vapour pressure equal to 11 Pa and a boiling point equal to 218 C, while BaP (high molecular weight) has a vapour pressure much lower around 7 $x10^{-2}$ Pa and a boiling point equal to 495 C.

PAHs are chemically classified as rather inert compounds, however when they do react, tend to retain their conjugated ring system and this arises the formation of derivate compounds by electrophilic substitution rather than addition reactions.

PAH compounds can absorb light both in the UV and in the visible regions of the spectrum. The absorption of light energy, leads to the excitation of the molecule. Firstly, the electrons of the molecule achieve upper energy states, then they return to the ground energy state gradually losing their energy. During this step, the PAH molecule can release the energy as photon (fluorescence

or phosphorescence). However, there are other possible pathways involved in this loss of energy from the excited state. Two of them are the energy transfer and the electron transfer to other molecules, which are present in the surrounding.

As previously mentioned, combustion processes (e.g. waste incineration) of organic matter is the main category of processes leading the distribution of PAHs in the overall environment. The industrial liquid effluents and the combustion gases are the main carriers which spread the PAH into the atmosphere, soil and aquatic systems. PAHs in the gas phase can undergo different processes depending on their volatility. Gas-particles partitioning is the main process that drives the distribution of PAHs in the atmosphere. Semi-volatile PAHs and all higher molecular weight PAHs are likely to be absorbed to particles of organic matter and adsorbed to soot, while volatile PAHs exist mainly in the gas phase (Keyte et al., 2013). Through the deposition of atmospheric particles, PAH can be accumulated in soil and plants. When PAHs occur in aqueous environments upon transport through industrial effluent, their solubility determines their distribution between the organic particles, the bottom sediments and the water column. The diffusion of PAH from the water streams into the soil matrix is another process increasing their accumulation in the environment. Higher molecular weight PAH are most likely found absorbed into the bottom sediments of water streams, while lower molecular weight PAHs are mostly absorbed on particles in the water phase (Readman et al., 1984). The fate of PAH compounds in the environment can be divided in four main categories of processes: abiotic oxidation, photo oxidation, sorption into organic matter and biodegradation. Temperature, turbidity, concentration of dissolved and particulate material and the nature and concentrations of microbial populations are the properties of the eco-system, which influence these transformation processes. PAHs can react with O_3, NO_3 radicals, NO_2, OH radicals, peroxides, sulphur oxides, chlorine and these reactions produce many different derivative compounds. All this processes rise much interest because the derivative compounds produced (e.g. oxygenated PAHs or polychlorinated-PAHs) display a significant toxic potential, in some case higher than the parent PAHs (Kochany and Maguire 1994). Linear PAHs undergo Diels-Alder reactions at the 9,10 positions and in presence of light might form endoperoxides when oxygen is available and photodimers in absence of oxygen. Angular PAHs are not subjected to Diels-Alder reactions and photodimerization, but can form endoperoxides (e.g 9,10-quinones) in presence of oxygen and under irradiation of light (Lee et al.,1981). These reactions increase the toxicity of PAHs, as proved by several studies on different organisms such as benthic invertebrates, aquatic vertebrates, plants and also mammals

(Arfsten et al., 1996). The presence of the skin in mammals allows the filtration of most of the light, limiting the toxicity to the out layer surface, causing photoallergies or non-immunologic induced skin reactions. Acute phototoxic effects have been observed after co-exposure of mice to PAHs and direct UV light. Mice painted with benzo(a)pyrene and exposed to sunlight for 30 minutes and 1 hour developed erythema and acute dermatitis (Arfsten et al., 1996). Furthermore, since the photo oxidized species are more water soluble than the parent PAHs, organisms could be exposed to higher concentrations of photomodified PAHs than the parent PAHs. This presents a greater toxic risk, because, as previously mentioned, oxidized PAHs are known to be more reactive and biologically damaging than the parent compounds (McConkey et al., 1997).

The conjugated nature of these molecules influences also their spectroscopic properties. Bigger conjugated systems absorb UV/Vis light at longer wavelength. This is easily observed for linear PAHs, but is valid for all the PAHs (figure 8, Reusch, 2013).

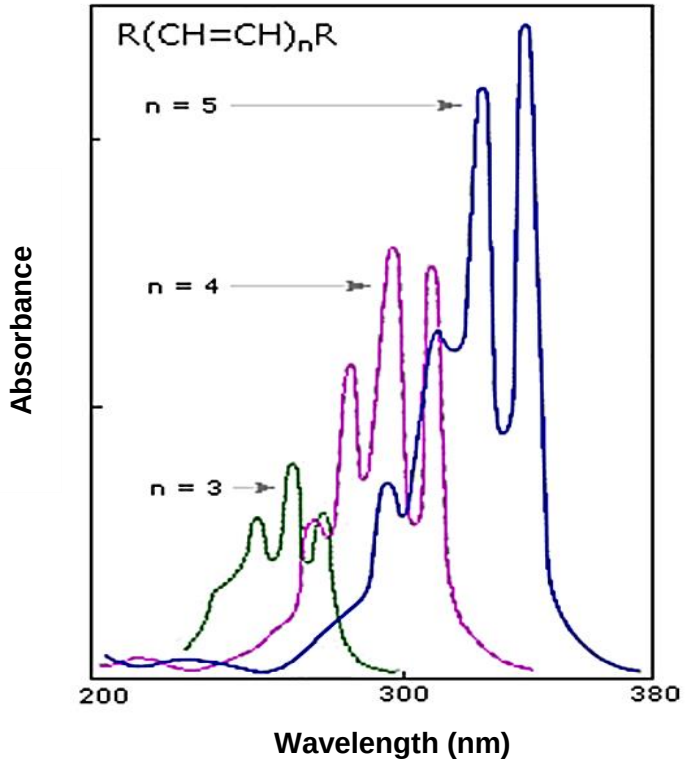


Figure 8 Absorbance spectra of the three linear polycyclic aromatic hydrocarbons in deionized water with a concentration of 0.5 mg/L (Reusch 2013). "n" indicates the number of aromatic rings composing the PAH compound characterized by each spectrum.

The absorption spectra of all PAHs are characterized by three main absorption bands: α, β, ρ. In a fluorescence emission spectrum each band is correlated to a different electron energy transition to

the exited state after a light excitation event. These three bands, which are common for all PAHs, shift to the longer absorption wavelength with increasing molecular weight of the PAH compounds. Therefore, it is possible to identify different PAHs by using the UV-absorption spectra. Furthermore the lower molecular weight PAHs need shorter excitation wavelength to be detected by UV light and higher molecular weight PAHs can be fluorescent at longer excitation wavelength. This phenomenon has been called annellation principle by E. Clar, who studied the correlation between the π electron density and the spectroscopic properties of aromatic compounds (Clar, 1964).

As previously mentioned PAH compounds represent a danger for human health. The carcinogenicity of PAHs has been largely investigated and has been found that the most potent carcinogens among PAHs are the ones resulted from reactions of addition or redox reaction on specific areas of the molecules. In fact, some PAH can have specific regions where they are more active. These regions are called “bay region” and “k-region”. Metabolites like arene oxides, hydroxyl, dihydroxy drivates, dihydrodiols and quinines are among the most carcinogenic pollutants (Jerina et al., 1976).

At 21°C temperature, phenanthrene has a water solubility between 1.2 and 1.5 mg/L (Eastcott et al., 1988) an octanol/water partition coefficient of around 4.3 - 4.6 (Cary et al.,1998) and a diffusion co-efficient in water of $7x10^{-6}$ cm²/s (Schüth, 1994). It is a semi-volatile organic compound with a vapour pressure of 6.8 $x10^{-4}$ mmHg. Phenanthrene shows UV-absorbance below 300 nm and fluorescence, between 345 nm and 382 nm with an excitation wavelength of 290 nm (PubChem-NCBI).

Although PAHs are commonly recognized as potential carcinogens, there are not experimental proves that phenanthrene is carcinogenic for mammalians, and more specifically for human beings (US EPA 2001). However Simmon et al., 1979 reported an oral median lethal dose (LD_{50}) of 750 mg/kg for mice. Single doses of 100 mg/kg/day of phenanthrene administered by gavage for 4 days suppressed carboxylestrase activity in the intestinal mucosa of rats, but did not produce other signs of gastrointestinal toxicity. Phenanthrene had no effect on hepatic or extrahepatic carboxylesterase activities (Nousiainen et al., 1984).

The importance of phenanthrene within environmental issues is represented by its high co-occurrence in water, soil and sewage sludge with more dangerous PAH compounds. Therefore

phenanthrene is listed as priority substance for its referential role in the environmental monitoring of polycyclic aromatic hydrocarbons.

Phenanthrene is also largely used for specific industrial products. It is involved in the synthesis of explosives, dyes, drugs and steroids (fact sheet by US Environmental Protection Agency, web, 2014). Phenanthrene based alkaloids are produced as antitumor substances (Wei et al., 2006), phenanthrene based tylophorine derivatives are used as antiviral agents, e.g. against the Tobacco mosaic virus (TMV) in plants (Wang et al., 2010). In the production of photovoltaics, specifically for bulk hetero conjunction (BHJ) solar cells, phenanthrene is a main component of the conjugated polymers. In the sintering industrial applications for the processing of thermoplastics, phenanthrene is a component of the sinterable polymers (e.g. US patent 2400099 A).

1.1.2.4 Octanol-water partition coefficient (Kow)

In the environment all chemicals are continuously transported and redistributed between solid, liquid and gaseous phases. The rate of transportation from one phase to another depend on the affinity of each chemical for each phase. In fact the actual issue about pollutants is their affinity for different environmental compartments. Risk assessment procedures are based on the transport of pollutants at water-solids, water-gas and gas-solid interphases. In this regard for each pollutant it is important to define specific parameters, which could describe their partitioning between these phases. These parameters are called partition coefficients. The general partition coefficients (K_p) are calculated by the ratio between the molar concentrations of the chemical in two immiscible phases (phase 1 and phase 2) at the equilibrium as follows:

$$K_p = \frac{C_1}{C_2} \qquad \text{(Eq.1)}$$

Solid phases refer to inorganic and organic matter. Part of the organic matter are soil, living organisms and other matrices such sediments, sludge and microbial biofilms. The aqueous phase and the gaseous phase are the most important phases for the transport of pollutants through the environment, while the solid phases play often the role of sinks and sources. A specific pollutant can be absorbed and accumulated into a solid phase and then, under different environmental conditions, can be desorbed back into the environment into the gas or liquid phase. In order to investigate the occurrence of a pollutant in the environment it is necessary to know its water-solvent phase partitioning. Since long time the partitioning of organic chemicals between pure water and n-octanol and the resulting octanol-water partition coefficient (K_{ow}) has been widely

used as first reference parameter for predicting the partitioning of organic chemicals between soil and water and between living organisms and water (Kenaga and Goring, 1978). The higher the K_{ow}, the more hydrophobic is the compound. Therefore this partition coefficient is a measure of the water solubility of chemicals. In fact Log K_{ow} (L/kg) values are generally inversely related to aqueous solubility and directly proportional to molecular weight (USGS, website, 2014). High values of K_{ow} indicate the tendency of a pollutant to be partitioned mostly into biota and soil rather than in water phases and therefore this parameter represent an important reference (Miller and Wasik 1985). Few methods are applied for measuring this parameter for different compounds, but it is possible to calculate it using an equation proposed by Chiou and co-workers (Chiou et al., 1982) and reviewed in Miller and Wasik 1985. This equation has been tested for 34 organic pollutants, resulting a predicting error less than one order of magnitude between the calculated and the measured values. Therefore it is possible to predict the octanol-water partition coefficient of a pollutant, knowing its water solubility and the activity coefficients of each compound in the two immiscible phases.

Given the K_{ow} as follows:

$$K_{ow} = \frac{(\gamma_{wo} \nu_{wo})}{(\gamma_{ow} \nu_{ow})} \quad \text{(Eq.2)}$$

The octanol-water partition coefficient is expressed as follows:

$$Log\ K_{ow} = Log\ Ws - \log Vo - \log \gamma o + \log \frac{\gamma w}{\gamma wo} \quad \text{(Eq.3)}$$

Where γ_w is the activity coefficient of the chemical in water, γ_{wo} the activity coefficient in water saturated with octanol, γ_{ow} is the activity coefficient of the chemical in n-octanol saturated with water, ν_{wo} is the molar volume of water saturated with octanol (m^3/mol) and ν_{ow} is the molar volume of octanol saturated with water (m^3/mol) V_o is the molar volume of the organic solvent and Ws is the water solubility of the chemical.

In 1989 De Bruijn et al., provided a good review about the methods used for the measurement of the octanol-water partition coefficient. The classical method for measuring the K_{ow} is the flask-shaking method. The chemical is mixed with an appropriate 1-octanol/water mixture and shaken for some given period during which equilibrium between both phases must be achieved. After both phases are allowed to separate, the concentration of the chemical in both phases is determined. Although this method has been widely used in the history, it has been proved to be

not accurate for the determination of the partitioning of the more hydrophobic compounds due to the formation of water/octanol emulsions which interfere with the partitioning procedure. Therefore other methods have been developed. Brooke et al., 1986 developed the so called "slow stirring" method. In this case the water and the octanol phase are equilibrated under conditions of slow stirring and the formation of emulsions can be prevented, resulting in a more precise measurement of the partitioning. The octanol-water partition coefficient is one of the most important parameters for predicting the environmental mobility of pollutants among the different compartments of the environment and it is taken in this study as a reference for modeling the sorption of phenanthrene into sewer biofilms.

1.1.2.5 Analytical methods to detect phenantherene

The procedures involved in the analysis of PAH compounds are different and depend on the nature of the sample analyzed. The analysis of organic pollutants from environmental samples is based on few necessary steps: extraction, separation, concentration and detection. Water, soil and sludge are three different matrices, which require different approaches. As reported by Manoli and Samara, 1999 the most widely used extraction techniques for water samples are the liquid-liquid extractions (LLE) and the solid phase extractions (SPE). According to the protocol EDIN38407 F18 the most suitable solvent for this extractions is n-hexane and alternatives are benzene, toluene, dichloromethane and cyclohexane. For marine water samples it is recommended to use a mixture of light petroleum and diethyl ether in order to perform the extraction of PAHs (Bruzzoniti et al., 2000).

Beside the good results and the low costs, the LLE implies the disposal of large volumes of toxic solvents, of environmental concern, and relatively long time procedures. On the other hand SPE does not requires large volumes of solvents and automatic procedures can be easily settled to decrease the analysis time. SPE implies the use of special cartridges packed with silica as solid phase on which the PAH molecules are adsorbed. The recovery of the targeted compounds is carried out by solvent elution. When following a prior steam distillation step, the SPE approach represents a valid choice. However the SPE procedure is suitable for „clean water" samples, because the presence of particle-bound PAHs affects its efficiency significantly.

A more effective approach for recovery of PAH from environmental samples is the solid phase microextraction (SPME). This technique involves the use of special fibers as solid phase, coated with polydimethylsiloxan (PDMS) and immersed into the sample while mechanical agitation,

stirring or ultrasonication is applied. The affinity between the fiber and the target substance will allow the efficient recovery even though on complex environmental samples. Since latest ´90s the column extraction of PAHs from water samples bythe use of immunosorbents has gained interest. The antibody is immobilized on a silica support and used as affinity ligand in order to bind the target analyte from the aqueous phase. Bouzige et al., 1998 proposed a more effective immunosorbent extraction method using an anti-fluorine assay followed by liquid chromatography with diode array detection (LC-DAD) on sediment and sludge samples. Although good results are summarized in literature, the efficiency of this technique is affected by the complexity of the sample, hence solvent extractions are preferable.

In this regard a broad range of methods are available since different types of samples require different solvents and conditions. For instance in case of marine water samples the best results are achieved by light petroleum diethyl ether mixture, followed by determination procedures based on high-performance liquid chromatography (HPLC). In case of soil, sludge or slurry samples the approaches are different.

The most widely used techniques are (Northcott and Jones, 2000; Song et al., 2002; Semple et al., 2003):

- Batch solvent shaking extraction.
- Soxhlet extraction.
- extraction after ultrasonic treatment.
- SPME followed by gas chromatography/mass spectrometry (GC/MS)
- Alternatives methods are (Camel, 2000).
- Microwave assisted solvent extraction.
- Supercritical fluid extraction.
- Accelerated solvent extraction.

After the hydrophobic organic compounds (HOC) have been recovered from the samples, the separation and the detection of the PAHs must be performed.

There are mainly two separation techniques: liquid chromatography (LC) or gas chromatography (GC).

The LC consists of a polar stationary phase and the substances are separated by the different affinity for the polar support. In the case of PAHs is common the use of a reverse LC method, in which the stationary phase is a nonpolar matrix and implies the use of an elution solvent to recover the molecules adsorbed on the stationary phase.

Nowadays the most common LC technique is the high pressure liquid chromatography (HPLC), that allows higher separation efficiency and higher resolution.

The GC for PAHs is usually employed with fused silica capillary columns coated by a liquid phase (e.g. methyl silicon).

These two kind of separation are coupled with different detection techniques:

- Flame ionization detection (FID).
- Fluorimetric detection (FLD).
- Ultraviolet detection (UVD)
- Mass spectrometry detection (MS).

The UVD can be performed by UV absorption or by photodiode array (PDA). The UV absorption is most widely used due to higher sensitivity (Robards et al., 1994).
UVD and FLD are most widely used coupled with LC, while MS might be used together with both LC and GC. According to the EDIN38407F18 and the US EPA method 610 the most suitable combinations for the wastewater analysis are:

- HPLC-FLD
- HPLC -UVD or-FLD
- GC-FID

According to current literature, the detection of phenanthrene by fluorescence spectroscopy on soil or sludge samples has not been applied, while for aqueous environmental samples has been performed only using pulsed laser technologies and by analyzing the fluorescence lifetime of the target molecule (Meidinger et al., 1993).

Furthermore fluorescence detection with pulsed laser followed by HPLC has been reported to be a valid alternative to the classical approaches (Manoli, 1999).

Although several clean up steps are needed to analyze the fluorescence of PAHs from environmental samples, Campiglia et al. in 1995 proposed the use of a laser excited synchronous luminescence device in order to trace Benzo(a)pyrene in various environmental samples. This technique has been proposed for its potential application for remote in situ sensing.

Other useful approaches can be (Patra, 2003):

- Excitation emission matrix fluorescence (EEMF)
- Synchronous fluorescence scan (SFS)
- Selective fluorescence quenching (SFQ)
- Time-resolved fluorescence spectroscopy (TRF)
- Phase-resolved fluorescence spectroscopy (PRFS)
- Fluorescence correlation spectroscopy (FCS)

Amongst all the methods mentioned, the SPME extraction or the liquid-liquid extraction coupled with the GC-MS analysis is the most suitable approach for the detection and quantification of environmental samples, although it can't be performed for in situ measurements. On the other hand the fluorescence spectroscopy methods, when applied on suitable surfaces, have the advantage to be performed on field using fiber optic devices for measurements. This methods would allow faster analyses than the other two procedures previously described and would increase the benefit of an environmental monitoring strategy. Furthermore since hydrophobic pollutants, such as phenanthrene, display low water solubility, the analysis of water samples is not a reliable approach for their detection in the environment. In this regard it is worth to exploit organic matrices, which can easily absorb this compounds from the water phase during their transportation. In this regard microbial biofilms might be suitable for detecting hydrophobic pollutants, such as PAHs in the environment. Microbial biofilms grow onto surfaces directly in contact with the contaminated aqueous systems and are proven to absorb hydrophobic pollutants. Although the use of microbial biofilms as bio-monitors for pollutants has been largely reviewed, a direct fluorescence spectroscopy application on this matrices for on field measurements has not been yet created. Because of all these reasons, the analysis of microbial biofilms seems to display a significant potential and it is the approach proposed in this study in order to detect PAHs in sewer systems.

1.2 Microbial biofilms

Biofilms are the main microbial form of life on the planet Earth (Flemming and Wingender, 2010). A biofilm is composed of microbial cells immersed in a matrix of self-produced polymeric substances such as proteins, polysaccharides, and nucleic acids, (Flemming and Wingender, 2010; Dunne, 2002; Lazarova and Manem, 1995; Frølund et al., 1995). Biofilms are ubiquitous and grow at all sort of interfaces (liquid/air, solid/air, solid/liquid) in lakes and streams. Thickness, water content, total density, polarity, chemical and microbial composition are among the most important parameters in the study of biofilm formation. Over recent decades, the scientific community substantially increased its interest in microbial biofilms for their role as active mediators of several environmental processes. Biofilms growing at the water/liquid interface can sequester particulate and dissolved matter present in the water, so that the acquisition of nutrients by the biofilm microorganisms is facilitated (Strathmann et al., 2007). In addition, through the sorption of solutes from the contact phases, biofilms play an important role on the fate and distribution of the pollutants in the environment (Flemming, 1995). The term sorption refers both to adsorption, absorption and desorption. Adsorption implies the retention of a solute on the surface of the particles of a material. Absorption in contrast involves the retention of a solute within the interstitial molecular pores of such particles. (Strathmann et al., 2007).
There are 3 main sorption sites in a general microbial biofilm matrix:

1. EPS (Extracellular polymeric substances) consisting of polysaccharides, proteins, nucleic acids and lipids providing hydrophilic and hydrophobic regions within the matrix.
2. Cell walls and lipid membranes again providing charged and not charged areas.
3. Cytoplasm as separate water phase

Each one of these sites displays specific physico-chemical properties and affinity for different kind of molecules; for instance, it has been already reported that heavy metals are strongly retained at the level of the cell walls, while bigger molecules like BTX are retained mainly inside the EPS layer. (Spaeth et al., 1998). Furthermore, the physico-chemical properties as the microbial composition of the biofilms are closely associated with the environmental conditions of which they are exposed to. For example, under dehydrated conditions and being deprived of

water, biofilms appear as crusts and show significantly different mechanical properties (Garcia-Pichel et al., 2003).

The knowledge attained so far about this kind of matrices brought significant improvements in many technical and scientific applications („Microbial Biofilms: Current Research and Applications "by Lear and Lewis, 2012).

1.3 Fate of PAHs in Sewers and role of sewer biofilms

Sewers are the main infrastructure for the transport and collection of wastewater in all European urban areas. Sewers can be represented by a simple model as composed of six different compartments:

- The wastewater suspension
- The particulate matter present in the aqueous phase.
- The bottom sediments
- The gas phase
- The fats, oils and grease portion (FOG), which accumulates on the surface of the aqueous suspension and at the sewer sidewalls.
- The microbial biofilms, which grow on the sewer surfaces both in contact or not with the water stream. In such an environment, hydrophobic pollutants (e.g. PAHs) encounter different fates.

Low molecular weight PAHs, composed of 1-3 aromatic rings, with a Log K_{ow} between 2 and 5 are mainly found in the water solution, constantly moving from the dissolved aqueous phase to the organic particles which are suspended in the water column and vice versa (Blanchard et al., 2004). The low molecular weight PAHs can also move from the aqueous phase to the gaseous phase depending on their molecular weight. Generally PAH with molecular weight lower than 234 g/mol (Gigliotti et al., 2002) and Henry's law constant greater than $1x\ 10^{-4}$ atm m^3/mol (Manoli and Samara 1999) are mainly found in the gaseous phase rather than in water. In fact the lower is the molecular weight of a PAH compound, the lower it is the water-air partition coefficient (Log K_{wa}) and therefore the more volatile is the compound. Another important process for low molecular weight PAHs is the bacterial biodegradation. In sewers, this process is significant only for compounds with less than 4 aromatic rings (Blanchard et al., 2004).

On the other hand the volatilization and bacterial biodegradation are not a significant removal processes for high molecular weight PAHs (more than 5 aromatic rings) present in wastewater. The higher Log K_{ow} and the lower solubility of these compounds allows their prevalent sorption into organic matrices such as sediments, fats, oils and lipids portions and of course microbial biofilms. Only a smaller amount of these compounds are found in the dissolved phase of the water column, accounting less than 20% of the total (Blanchard et al., 2004).

In general the sorption of hydrophobic compounds into organic matrices is described by the organic carbon partition coefficient (K_{oc}), which is calculated as follows:

$$K_{sw} = \frac{q_s}{C_w} \quad \text{(Eq.4)}$$

and then as follows:

$$K_{oc} = \frac{K_d}{f_{oc}} \quad \text{(Eq.5)}$$

Where K_{sw} is the equilibrium distribution factor of the compound between the water phase and the sorbent material, q_s is the uptake of the compound into the sorbent phase and C_w is the concentration of the pollutant in the water phase. f_{oc} is the fraction of organic carbon content of the sorbent material (Chiou et al.,1983, Wicke et al.,2007).

In order to predict the sorption of PAHs into organic matter, such as soil, sediments, biofilms or more general organic particles, two main models have been elaborated. Both of them relate the octanol-water partition (K_{ow}) coefficient with the organic carbon partition coefficient (K_{oc}).

For substituted PAH (e.g. polychlorinated PAHs) with a Log K_{ow} ranging from 2-5, Chiou et al., 1983 proposed the following linear relationship:

$$\text{Log } K_{oc} = 0.9 \text{ Log } K_{ow} - 0.543 \quad \text{(Eq.6)}$$

For PAHs with higher values of Log K_{ow}, Karickoff et al., 1979 proposed as follows:

$$\text{Log } K_{oc} = 1.0 \text{ Log} K_{ow} - 0.21 \quad \text{(Eq.7)}$$

These equations are important to model and predict the partitioning of PAHs in the open environment, but can be used in order to describe their partitioning also in sewers.

Also the deposits of fats, oils and lipids play a significant role in the distribution of PAHs in sewer systems. It is widely known that these substances display adhesive properties, which allows them to accumulate on the sewer walls and often pose a risk of sewer overflows due to

blockage events (He et al., 2013). In numerous works the significant increase that these substances gain on the sorption and the retention of PAHs into biota, food and soil has been pointed out (Bruner et al., 1994; Zhang and Tao, 2009; Moret and Conte, 2000). Therefore in this environment where they are an important portion of the organic matter, they must be taken under consideration.

An important linear relationship between the lipid-water partition coefficient (Log K_{lw}) and the octanol-water partition coefficients (K_{ow}) of PAHs has been proposed by Zhang and Tao, 2009:

$$\text{Log } K_{lw} = 1.23 \text{ Log } K_{ow} - 0.78$$

Also in this case of study the octanol-water partition coefficient linearly influence the sorption of different PAHs on lipid rich matrices.

1.3.1 Sewer biofilms

The abundance of organic and inorganic compounds present in waste water allows the formation of biofilm matrices in sewers (Jahn and Nielsen, 1998). The sewer biofilm system differs from the other biofilm systems in two important ways (Nielsen et al., 1992):

- Very high organic loading
- High shear stress at the biofilm surface

These two factors cause the formation of an extremely rough and thick biofilm matrix with high content of organic substances. Therefore the transportation of substrates and the EPS composition are significantly affected compared with different biofilm models. The total content of EPS produced by bacteria in sewer biofilms can differ highly depending on different environmental parameters. Sheng et al., 2010 summarized them as substrates, nutrient content, metal concentration, shear rate, aerobic and anaerobic conditions.

Although these factors are really difficult to be combined in order to create a suitable conceptual model, some investigation has been carried out and useful reference considerations have been suggested. Jahn et al., 1998 analyzed the biofilms from three different sewer lines from the Aalborg area in Denmark. The cell biomass was found to be the minor fraction of the total composition equal around to 2-12% of each sample, whereas proteins (50%), humic substances (1-10%), polysaccharides (30%) and uronic acids (5%) were found to be the most relevant components. Lipids and nucleic acids are not considered as main component of the EPS in this

study. Adav and Lee (Adav and Lee, 2008) studied the EPS composition of waste water sludge samples from a local municipal WWTP in Taipei, Taiwan and found that the content of lipids was equal to 8%-10% and the DNA was equal to 0.2% of the total volatile suspended solids. The polysaccharide/protein ratio (PN/PS) is also a reference parameter for investigations. Jahn and Nielsen 1998 calculated the PN/PS ratio equal to 0.6, confirmed by Zhang and Tao, 1999, which estimates it between 0.5 and 0.7 for biofilms grown in rotating angular reactors. Adav and Lee, found the PN/PS ratio equal to 0.9. So it seems likely that in sewer biofilms the proteins content is higher than the polysaccharide content. This specific characteristic needs to be further considered in regard to the sorption of organic pollutants by sewer biofilms. According to Jorand et al., 1998 and Celmer et al., 2008, the hydrophobic portion of a microbial biofilm is represented by proteins (Xu et al., 2011), therefore sewer biofilms are likely expected to have strong hydrophobic properties due the higher content in protein and humic substances than other biofilm systems.

Using molecular techniques for characterization of the microbial communities inside sewers, Vinke et al., 2001 found that bacterial and fungal communities are predominant, though bacteria are the main colonizers especially below the water level.

In this regard, the main phyla of bacteria in sewer biofilms are alpha, beta and gamma proteobacteria, acidobacteria and actinobacteria. (Satoh et al., 2009; Vincke et al., 2001).

1.3.2 Sorption properties of Biofilms

In aquatic environments, the fate of pollutants is strongly dependent on the sequestration process occurring at the solid-liquid interface. Biofilms cover most of the solid surfaces and therefore their sorption properties have an important role for the fate of organic and inorganic pollutants in sewers. In figure 6 is presented a general sewer model with all its sorptive compartments.

As previously mentioned (page 30, Figure 6), in sewers microbial biofilms represent one of the main sorbent phases for organic and inorganic substances. In biofilms the cells and the EPS layer represent important sorption sites (Flemming and Wingender, 2010).

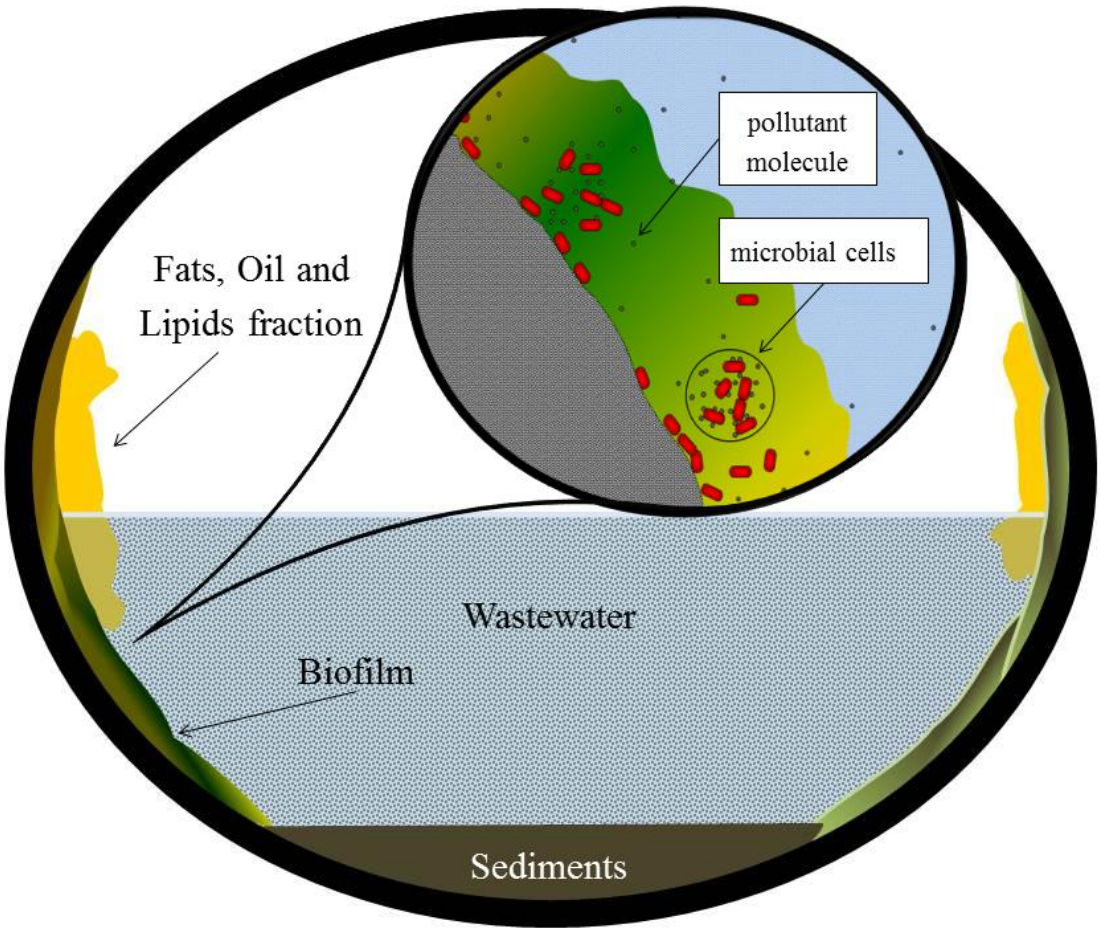


Figure 3 Sketch of a sewer and a sewer biofilm layer. The biofilm grows on solid surfaces (substrata) and is composed by EPS and microbial cells.

In this regard, two main observations must be evaluated:

1. Microbial biofilms are complex, dynamic and structured matrices. Each component of the matrix displays different sorption preferences, capacity and properties (Flemming 1995).
2. Microbial biofilms act both as sink and source of pollutants in aqueous environments.

The biofilm matrix contains several sorption sites (Table 14), such as extracellular polymeric substances (EPS), cell walls, cytoplasmatic membrane and cytoplasm. The main components of the dry mass of a biofilm are the EPS and the microbial cells. The EPS mainly comprise polysaccharides, protein, humic substances and nucleic acids. However the main component of a biofilm is water, which can reach over 98% of the total wet weight, while EPS represent the 1-2% w/w (Flemming 1995, Spaeth et al., 1998). The sorption and accumulation of heavy metals in biofilms has been largely reported and the cellular fraction, more specifically the cell wall have been identified as the main sorption site for this class of pollutants (Spaeth et al., 1998, Gutekunst 1989). The main processes governing the sorption of metal ions onto cell walls are ion exchange reactions, precipitation and complexation. In activated sludge and hence in sewer biofilms, the main process seems to be the ion exchange reaction (Sheng et al., 2010). Although the cell fraction seems to provide stronger affinity for metal sorption in surface water biofilms, Lie and Fang 2002 reported that in the wastewater framework, the hydrogen producing sludge and the sulfate-reducing bacteria (SRB) biomasses show EPS content of electrostatic binding sites 20-30 fold those reported for the bacterial cell fraction. In this case the EPS might play a major role in the sorption of heavy metals from the waste water solution.

Table 14 Sorption sites in biofilms (Flemming and Leis 2002)

Sorption sites in Biofilms
EPS (including capsules) mainly consisting of polysaccharides and proteins.
Charged groups, for example –cCOO-1, -SH-, -SO42-, -H2PO42-, -NH4+, -NRH2+ etc..
Apolar groups for example aromatics or aliphatics such as found in proteins; also hydrophobic regions in polysaccharides.
Cell walls:
Outer membrane of gram negative cells (lipids)
Murein or teichoic acid layer of gram negative and gram positive bacteria respectively
Cytoplasmatic membrane (lipids)
Cytoplasm

In nature microbial biofilms are occurring mostly as multispecies matrices, involving different species of microorganisms and often also different trophic groups like protozoa and metazoa (Wimpenny et al., 2000). Many strains form more than one strain-specific type of EPS, and the composition may change during their life cycle, so that the heterogeneity influences to the sorption properties of biofilms (Flemming, 1995). Dynes et al., 2006 studied the sorption of chlorexidine in river biofilms composed by algae and bacteria. Algae were found to show stronger sorption capacity for the chlorexidine due to the production of extracellular lipid droplets, which increased the solubility of the target compound. The differences in the bioaccumulation of chlorhexidine by diatoms and bacteria indicate that not all species in a natural biofilm will contribute to bioaccumulation to the same degree. Consequently, the degree of bioaccumulation in a biofilm will depend on the presence and abundance of certain species (Sutherland, 2001). The composition of polysaccharides of a biofilms depends on the microbial communities, which compose that biofilm. Different polysaccharides found in microbial biofilms can display different physical-chemical properties. Algal alginates differ from bacterial alginates for the level of acetylation. Bacterial polysaccharides are more acetylated than algal alginates, therefore within a biofilm matrix, the higher amount of bacterial alginates might lead the formation of nonpolar regions, where hydrophobic compounds would likely be retained (Sutherland 2001). On the other hand, a higher amount of algal alginates would increase the interaction with metal ions present in the liquid phase. The tertiary structure of the polysaccharides is another factor, which influences the sorption properties of a biofilm. Most of the polysaccharides found in biofilms bind high amounts of water, but some of them (e.g. bacterial cellulose, mutan or curdlan) produced by different bacterial strains (e.g. *Enterobacter agglomerans*) are rich in sequences of 1,3- or 1,4-b-linked hexose and form triple helices strongly bound together, able to exclude water and create portions completely insoluble (Sutherland 2001). These sub-regions might be a suitable accumulation sites for hydrophobic compounds. Furthermore, gram positive bacteria have been proved to display greater sorption capacity for metals due to their thicker layer of peptidoglycan (van Hullebusch et al., 2003). These bacterial cells exhibit additional acidic functional groups, such as phosphoryl from teichoic acids and carboxyl groups from teichuronic acids, which also provide stronger sorption capacity for heavy metals and metal ions (Beveridge et al., 1997, Sheng et al., 2010). Environmental conditions also influence the sorption of organic and inorganic substances from the liquid phase. Low pH values increase the sorption of metal ions (van Hullebusch et al., 2003), while high pH values increase

the sorption of hydrophobic compounds (Wang et al., 2002). The higher the ionic strength, the stronger will be the sorption of hydrophobic compounds, while low ionic strength increases the sorption of metal ions (van Hullebusch et al., 2003). Contrary to heavy metals, hydrophobic pollutants are retained in the EPS layer. Already in 1998, Spaeth and co-workers reported that BTX (benzene, toluene, and the xylene isomers), strongly hydrophobic pollutant, was absorbed and accumulated at the EPS level of a biofilm grown in a sequencing batch reactor. The same results are reported also for other compounds (Singh et al., 2006). The accumulation of dichlofop-methyl in biofilm produced by microorganisms from an activated sludge culture was reported to be caused by adsorption on EPS (Wolfaardt et al., 1995). The aromatic amino acids and the highly acetylated polysaccharides present in the EPS matrix interact with the hydrophobic compounds, causing their retention and accumulation. Within the EPS layer, van der Walls forces, electrostatic interactions, hydrogen bonds, hydrophobic interactions and London forces are responsible for the cohesion between the EPS components and the sorbed organic compounds (Flemming, 1995; Mayer et al., 1999; Kim et al., 2000). This interactions are useful to the microorganisms for the acquisition of nutrients, but can also protect the cells from the exposure to toxic substances. For instance it has been reported that *Enterobacter cloacae* strains, resistant to high concentration of lead, increase the production of EPS when are exposed to contaminated environments (Naik et al., 2012). The functional groups at the level of the polysaccharide chains play a major role in the sorption of lead. In this case the EPS matrix was characterized by high content of metal binding groups such as carboxyl, hydroxyl and amide groups along with glucuronic acid. Similar results are reported by White et al., 1998 for cadmium exposure of a SRB biofilm and by Priester et al., 2006 for chromium exposure of a *Pseudomonas putida* biofilm. In all cases it was remarked that the increase of EPS production, specifically proteins and polysaccharides, seems to be defensive mechanism to protect the biofilm cell from the toxic effect of certain organic and inorganic compounds.

As previously mentioned, biofilms are dynamic systems, so their composition can change in response to the sorption of various substances such as heavy metals, antimicrobial compounds and pollutants. Along with a change in composition also the sorption properties of the biofilms will change. Schmitt et al., 1995 studied the effect of toluene on the biofilm properties of *Pseudomonas putida* strain. At low concentrations (5 ppm) the presence of toluene correlated with the increase of EPS production and at higher concentration an increase of carboxylic group

was observed on the polysaccharide chains (Schmidt et al., 1995). Also the sorption of heavy metals can influence the properties of a biofilm.

Different experimental models for the sorption of organic compounds and metal ions in biofilms and activated sludge were proposed. The models are created mostly on the base of two experimental conditions: batch experiments and continuous packed bed systems. From batch experiments, the proposed models involve both Freundlich and Langmuir isotherms and the kinetics of sorption first order or pseudo second order kinetics. On the other hand the fundamental transport equations derived to model the fixed bed systems with theoretical rigor are differential in nature and usually require complex numerical methods to solve. Such a numerical solution is not usually difficult, but often does not fit experimental results especially well (Aksu, 2005). Once the pollutants are sorbed by the biofilms, they can be released back in the liquid phase through a number of mechanisms and this pose a risk on the environment downstream the breakdown site.

The detachment of whole portions of the EPS matrix is one of the mechanisms responsible for the remobilization of pollutants in the aqueous environments. There are four types of detachment reported in literature: abrasion, erosion, sloughing and predator grazing (Morgenroth and Wilderer, 2000). The erosion is caused by shear stress, abrasion by collision of biofilm support particles. The sloughing event can be induced by rapid changes in environmental conditions such as a sudden increase in shear force, sudden depletion in oxygen concentration or nutrients. However little is known about the causes of spontaneous sloughing events and the effect of subsequent biofilm development (Garny et al., 2009). Among all the detachment mechanisms, the sloughing involves larger portions of biofilm, compared with the other ones. Rice et al., 2005 observed that in the filamentous biofilm of a strain of *Serratia marcescens*, the sloughing off is controlled by quorum sensing in response to nutrient conditions.

The desorption is another important process which influences the remobilization of pollutants from the biofilms into the water phase. As previously mentioned, the sorption models for pollutants in biofilms and activated sludge depend on the experimental system used, whether a batch reactor or a continuous flow fixed bed reactor. The salinity and the pH of the water phase, and the occurrence of surfactants are the main environmental factors influencing the desorption of pollutants from microbial aggregates. The partitioning coefficients of organic pollutants between water and biofilm are important parameters in order to predict the fate of pollutants in

aqueous environments (Wicke et al., 2007; Headly et al., 1998). Several models have been proposed but further investigation are needed in order to better understand the desorption process of either metal ions and organic pollutants from microbial biofilms (Di Fabio et al., 2013; Laing et al., 2009; Aksu 2005; Tsezos and Bell, 1988; Headly et al., 1998; Fan et al., 1990).

1.4 Biodegradation of polycyclic aromatic hydrocarbons

Although PAH are major pollutants for water and air, the soil is the ultimate depository of these chemicals. Among all the pollutants, PAHs are not easily biodegraded in soil under normal conditions and their persistence increases with the molecular weight. Biodegradation is meant as the destruction of chemical compounds by the biological action of living organisms. Bigger PAH molecules are more hydrophobic and due to the sequestration into the soil particles they are less bioavailable to the microbial community for biodegradation (Haritash et al., 2009; Cerniglia 1992).

Microbial degradation is the main degradation process for PAH compounds and occurs both under anaerobic and aerobic conditions (Bumpus, 1989; Yuan et al., 2001; Seo et al., 2009). The anaerobic degradation of PAHs is characterized by slower kinetics and occurs naturally since the microbial communities in contaminated soils and sediments exist under dominant anaerobic conditions. The anaerobic biodegradation of PAHs in soil and sediments has attracted the interest of the scientific community for its potential to be managed in favor of bio removal and recovery purposes of contaminated grounds (Haritash et al., 2009). However the anaerobic degradation of PAHs has been proved to occur only on smaller aromatic compounds up to 3 aromatic rings, for bigger PAHs there is not enough data available.

The breakdown of the organic carbon takes place by the biotrasformation in less complex metabolites and through the mineralization into inorganic minerals, H_2O, CO_2 or CH_4. The PAH degradation can involve both bacteria, algae and fungi. Prokaryotic microorganism degrades PAHs by an initial dioxygenase attack to cis-dihydrodiols that are further oxidized to dihydroxy products. Eukaryotic microrganisms use monoxigenase to initially attack the PAH molecule to form arene oxides, followed by enzymatic addition of water to give trans-dihydrodiols (Cernaglia et al., 1989; Figure 5).

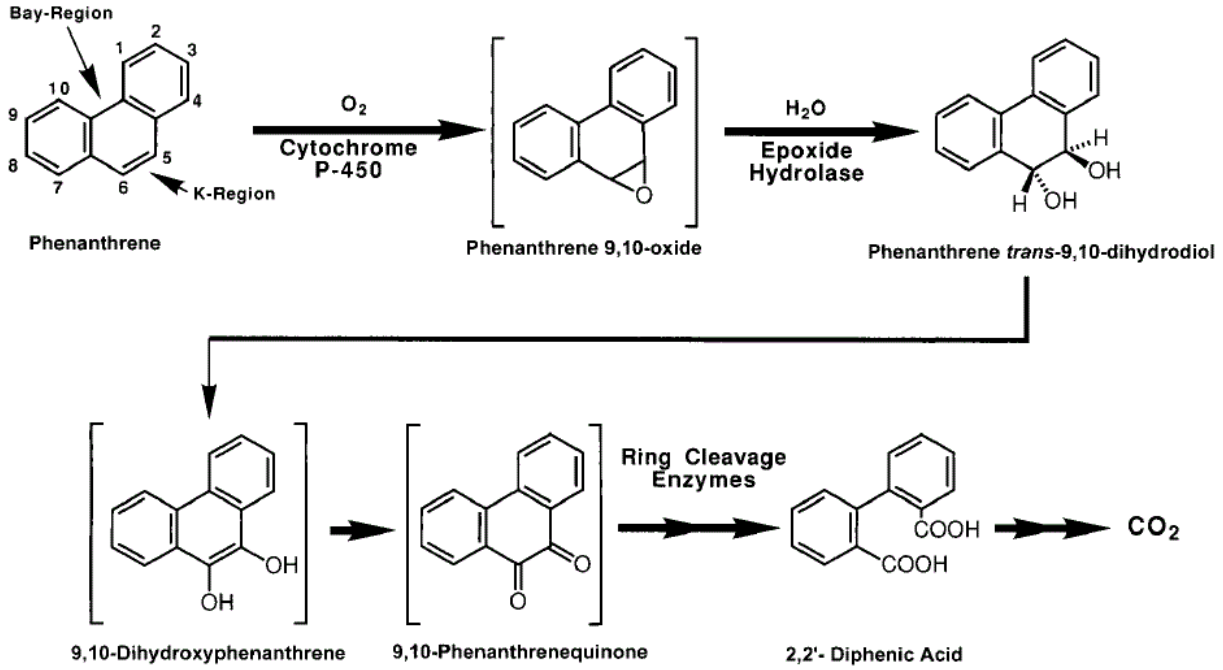


Figure 4 Proposed degradation pathway of phenanthrene by white rot fungus *P. ostreatus* (Bezalel et al., 1997)

The main factors affecting the biodegradation processes are pH, temperature, availability of oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties and chemical partitioning in growth medium (Singh and Ward, 2004). The bacterial species able to degrade PAH compounds are summarized in table 15 and the degradation pathways are presented in Figure 12. All the strains used in these studies were sampled from contaminated sites (Haritash et al., 2009).

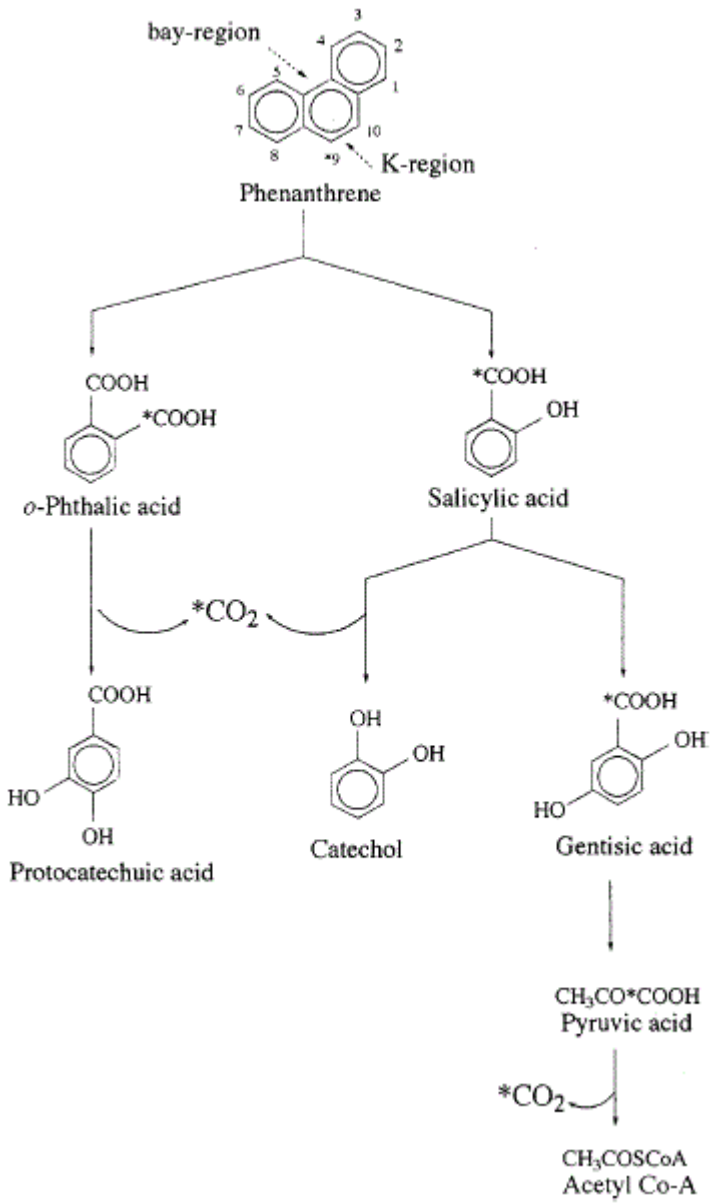


Figure 5 Proposed pathway for bacterial degradation of phenanthrene (Samanta et al.,1999)

Table 15 Summary of all PAHs, bacterial species and literature references related to the biodegradation of PAH pollutants (Haritash et al., 2009).

PAH	Microrganism	Reference	Dagradation rates
Benzo(a)pyrene	*Sphingomonas paucimobilis*	Ye et al.,1995	0.96 – 1.3 µg/mL day
	Agrobacterium spp. *Bacillus spp.* *Burkholderia spp.* *Pseudomonas spp.*	Aitken et al.,1998	Not available
Pyrene	*Rhodococcus sp.*	Walter et al.,1991	0.008 mg/mL day
	Mycobacterium sp.	Schneider et al.,1996	Not available
	Mixed culture Psuedomonas/Flavobacterium sp.	Trzesicka-Mlynarz et al.,1995	Not available
Pyrene	*Mycobacterium flavescens*	Dean-Ross et al.,2002	0.56 µg/mL min
	Mycobacterium sp.strain KR2	Rehmann et al.,1998	1.25 µg/mL hour
	Pseudomonas flurescens *Haemophilus spp.*	Yuan et al.,2002	Not available
Phenanthrene	*Paeubacillus spp.*	Daane et al.,2001	Not available
	Rhodotorula glutinis *Psudomonas aeruginosa*	Romero et al.,1998	Not available
Anthracene	*Rhodococcus spp.*	Dean-Ross et al.,2002	Not available

Among the fungal species, the ligninolytic fungi are proven to be able to degrade PAHs also under low oxygen conditions. Low molecular weight PAHs (LMW) are degraded by *Aspergillus sp.*, *Trichocladium canadense*, *Fusarium oxysporum*. High molecular weight (HMW) PAHs are degraded by *T.canadense*, *Aspergillus sp.*, *Verticillum sp*, *Achremonium sp*.

Also strains belonging to the white rot fungi (WRF) have been proved to be able to degrade benzo(a)pyrene in soil. Furthermore the presence of added surfactants to the mixture causes the increase of degradation because surfactants make PAHs easier to reach for the microorganism (Haritash et al., 2009). The most abundant fungi present in contaminated soils are yeasts. In presence of oxygen, this class of microrganisms can oxidize PAHs as alternative carbon sources. *Rhodotorula glutinis* showed high degradation rate similar to those observed for *Pseudomonas aeruginosa*. The biodegradation of PAHs by algal biomasses is mainly achieved by employing mixed algae-bacteria microcosms. In table 10 the main studies reported by Haritash et al., 2009 are summarized.

Several studies reported the biodegadation of low molecular weight PAHs using pure microbial cultures, but no significant results proved the degradation of high molecular weight PAHs by pure cultures of microorganisms. Since different PAHs (High molecular weight and low molecular weight) are simultaneously present in contaminated samples, it seems likely that the mineralization of PAHs inside heterogeneous media (e.g. soil, wastewater or sludge), is carried

out through cooperative metabolic activities of mixed microbial populations (Boonchan et al., 2000), rather than individual species of microorganisms.

The transport of PAHs inside such matrices is driven principally by the affinity that each molecule displays for the organic particles and this influences the bio-availability of the PAH molecule to the microbial populations. PAHs can be absorbed into particles, located in small pores inaccessible for bacteria, or otherwise occluded by the multitude of solid constituents. Since significant mixing forces are missing in such matrices, the diffusion of PAH molecules is reduced and their distribution is not uniform. Under these conditions, a physical barrier stands between PAH molecules and bacterial communities. The production of surfactants and EPS layers are two important strategies used by bacterial communities in order to increase the bio-availability of PAHs. Surfactants increase the diffusivity of PAHs in the water phase, enhancing their flux from the particle to the bulk liquid where the bacteria are. On the other hand, the production of EPS increases the accumulation of PAHs and provides a carbon storage for PAH-degrading communities (Johnsen and Harms, 2005).

In addition, the establishment of bacterial-eukaryotic consortia gives a further incentive to the biodegradation of PAHs in soil. In a first step ligninolitic and non ligninolitic fungi oxidize the PAHs by the action of unspecific exoenzymes and then bacteria can oxidize the more soluble by-products, through more specific PAH-dioxygenases. A first attack of the PAH molecules by fungi is more likely than a bacterial oxidation since bacterial enzymes are associated with the cell while fungal exoenzymes can diffuse better (Johnsen and Harms, 2005).

Algal populations are proved to facilitate the bio degradation of PAHs, furnishing O_2 for the metabolism of aerobic heterotrophic bacteria in wastewater maturation ponds (Haritash, 2009; Borde et al., 2003, Muñoz et al., 2003). In sewage sludge and soil mixed bacterial consortia are proved to drive the biodegradation of PAHs under both aerobic and anaerobic condition. Trzesicka-Mlynarz and Ward 1995 studied the biodegradation of BaP in soil by mixed culture of *Pseudomonas*and and *Flavobacterium* species. Ambrosoli et al., 2005 showed that in soil the biodegradation of Biphenyl ($C_{12}H_{10}$), fluorene ($C_{13}H_{10}$), phenanthrene ($C_{14}H_{10}$) and pyrene ($C_{16}H_{10}$) can occur under anaerobic conditions through fermentative and respiratory metabolism of mixed bacterial communities. Chang et al., 2003 reported that in municipal sewage sludge the anaerobic biodegradation of several PAHs (phenanthrene, acenaphthene, fluorene, pyrene and anthracene) is carried out by mixed bacterial communities composed mainly by methanogenic,

sulphate reducing bacteria (SRB) and nitrate reducing bacteria. Furthermore it is reported that co-metabolism is one of the main processes regulating the biodegradation of PAHs by mixed microbial communities. In fact the degradation of a PAH-mixture appears as a co-operative process involving a consortium of strains with complementary capacities. In addition, the presence of more different PAHs can lead to the inhibition or to the stimulation of microbial degradation of other PAHs. For instance Naphtalene was found to inhibit the phenanthrene degradation for a pure culture of *Sphingomonas sp*. (Shuttleworth and Cernaglia, 1996), while was found to stimulate its degradation for a pure culture of *Pseudomonas putida* strain KBM-1 (Haritash et al., 2009). Therefore, the co-metabolism process of PAHs is expected to complicate further the fate of these pollutants in heterogeneous media such as soil, sludge and wastewater.

1.5 Use the memory effect of biofilms to locate the pollution

A lot of effort is invested to improve the removal of pollutants by biological processes already existing in the wastewater treatment plants (WWTP), but this must be coupled with a suitable prevention strategy in order to limit the primary introduction of PAHs into the sewers. A problem is to determine the source of pollutants. In this regard, one of the most efficient solutions is the constant monitoring of pollution throughout the sewers and therefore a very cost effective monitoring approach would be required to allow the sewer biofilms to be used as monitoring devices for hydrophobic pollutants due to their natural affinity for these compounds. For a long time microbial biofilms have been studied as bio-monitors for organic and inorganic pollutants in lake, streams and rivers. The direct analysis of the biofilm matrix can provide the identity of the pollutants accumulated into it. Hydrophobic molecules display a very low solubility in water, therefore in aqueous systems they are not homogenously distributed.

This approach exploits the sorption capacity of microbial biofilm for organic and inorganic pollutants. The solvent extraction, the concentration and the detection of the pollutants are the main procedures practiced so far for the detection of pollutants in microbial biofilms (Kostel et al., 1999; Mahfoud et al., 2009, Houhou et al., 2009, Gasperi et al., 2010). An interesting example of this approach is given by Gasperi et al., 2010, a short review focused on the use of sewer biofilms as bio-monitors for tracing the sources of specific pollutants (PAHs and heavy metals) into the combined sewer system of a small catchment area in the centre of Paris, France. Through the analysis of sewer biofilms, it was possible to evaluate the individual contribution of wastewater, runoff and in-sewer processes, to the pollution of PAHs and heavy metals in the

Seine river downstream the catchment outfall. This study shows that biofilms might be used for tracing and addressing more precisely different sources of specific pollutants along a sewer.

Although many reported studies from literature are focused on the use of microbial biofilms as bio-monitors for environmental pollutants, most of them refers to relatively small areas, does not make use of a suitable on-field analytical methods and does not involve a systematic sampling strategy.

For these reasons, it would be worth to sample biofilms all along the wastewater distribution system within an urban and industrial area, from the WWTP catchment stream, upstream along all the sewers, in order to trace sources of organic and inorganic pollutants involving all the catchments connected to the main wastewater network (Figure 6). Furthermore, the establishment of a suitable on-field analysis method would improve the potential of this approach, decreasing the time needed for the detection of the pollutants. The analysis of different biofilm matrices sampled along the sewers can provide information about "when" and "where" the pollution started by localization of the contamination in reference to the flow direction. This is a suitable approach point for an effective prevention strategy by identification of the polluter.

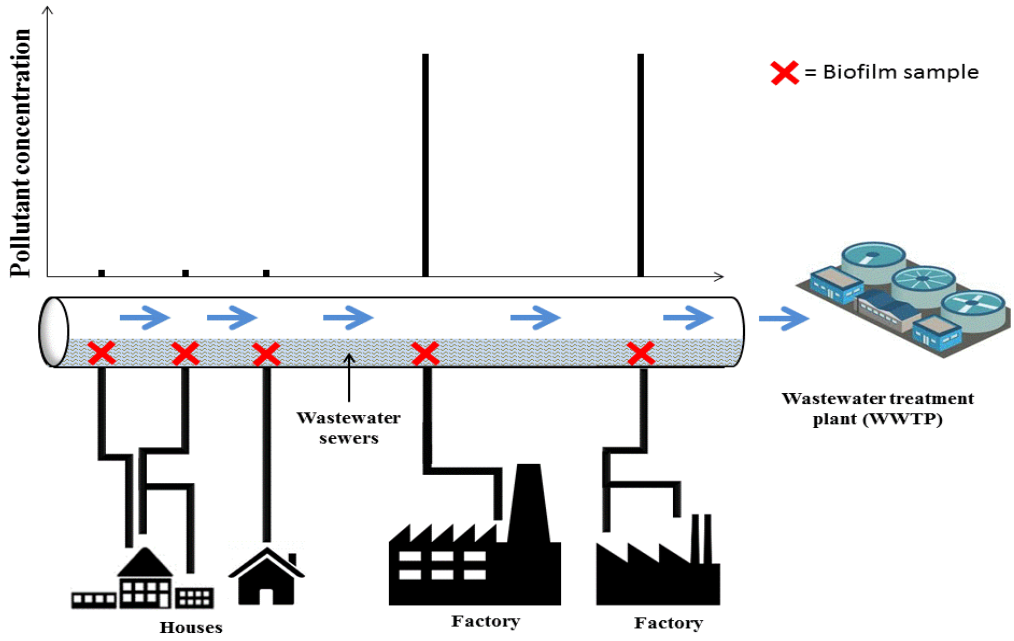


Figure 6 Sketch map of the approach proposed by this study. The measure of the pollutant concentration sequestered by the biofilms along the sewers can help to address the primary source of pollution.

In this regard a really interesting example is shown by Bendt et al., 2007. In this paper is reported the use of sewer biofilms as "sewer spies" for tracing the pollution of heavy metals into the waste water system upstream from the WWTP in the area of Wuppertal, Germany.

For this investigation, the metal load of each biofilm sample was calculated using the atomic absorption spectrometry (AAS) and the geographic information system (GIS, QGIS software) was used to establish the sampling points and facilitate the addressing of the polluters. The biofilms were grown on plastic supports immersed in the wastewater stream and the biomass was scratched off for the analyses.

This application has a high potential for detecting also the pollution of fluorinated suractants in sewers and has been further considered for editing new and more accurate environmental policies and regulations in Germany (Genuit and Block 2009).

In order to exploit microbial biofilms for tracing PAHs (phenanthrene) in sewers, it is necessary to estimate the memory of these matrices for such a compound. After the absorption of into the biofilm, how long is phenanthrene retained inside the matrix before to be released back into the aqueous phase by desorption processe? This information is necessary to understand whether biofilms can be used as accumulation devices for phenanthrene. A short memory would not facilitate the sampling procedure and all the monitoring approach would be compromised. Polysaccharide matrices are considered in this study as surrogates of biofilm, in order to calculate the memory for phenanthrene. Polysaccharide are easier to handle than real biofilm samples and present similar properties to the EPS matrix.

In order to provide the best possible monitoring procedure, it is necessary to develope an analytical approach which can allow on-field measurements. Although microbial biofilms display sorption capacity for both inorganic and organic pollutants, their matrix remain complex and difficult to analyse by on field measurements. All the methods for the analysis of microbial biofilms need to be performed in a laboratory and depending on the nature of the pollutant, some technical issue migh arise. Furthermore, the biofilm memory for PAH compounds might be too short and not exploitable for the establishment of such a systematic monitoring procedure.

When these problems can compromise the all idea, it is auspicable to consider other matrices, which could provide more suitable sorption properties and higher potential for this purpose.

Polydimethylsiloxane (PDMS) is an alternative suitable material for this kind of analysis on PAH pollution in aqueous systems. This oil material is widely used for coating SPME fibers for the extraction of hydrophobic pollutants in untreated environmental samples and displays suitable

properties for the employement of fluorescence spectroscopy as method for on field measurements. PDMS is a valid candidate for the creation of monitor devices which might be a good alternative to sewer biofilms.

In order to study the potential of a systematic approach for monitoring the pollution of sewers, this study has been focused on testing the memory of polysaccharide gels for phenanthrene and on the development of a novel analytical method. This part of the work has been attempted both on sewer biofilms and on alernative materials for detecting PAH pollutants in wastewater streams.

1.6 Aims of this study

The aims of this study were:

- Choose a reference PAH compound based on the environmental occurrence and its chemical properties.
- Choose the suitable analytical method for the experimental part of the work.
- Investigate the absorption and desorption kinetics of phenanthrene from biofilm model substances (hydrogels).
- Choose a suitable method in order to collect biofilm samples from wastewater streams.
- Design and construction of all the additional equipment needed for analyzing the phenanthrene into sewer biofilm suspensions using front-face fluorescence spectroscopy.
- Research of an alternative bio-monitor material rather than sewer biofilms for monitoring PAHs in wastewater streams and test it using the analytical method previously selected for the sewer biofilm analysis.
- Suggestion for monitoring devices

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Quartz suprasil (QS) glass cuvettes

Two kind of quartz suprasil (QS) glass cuvettes were used during the experiments: UV QS precision glass cuvettes macro (3.5mL volume, 1cm path length) from Carl Roth GmbH for the sorption experiments with polysaccharide gels (Figure 7).

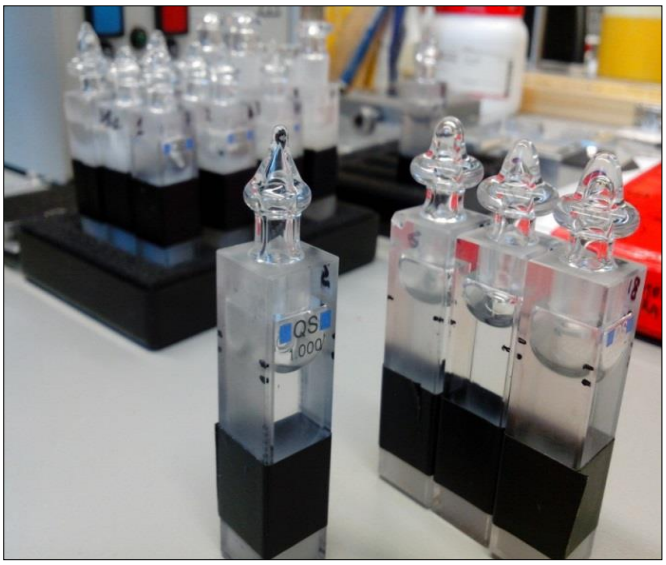


Figure 7. a) QS glass cuvettes used for the sorption experiments into polysaccharide gels.

2.1.2 Glass device for diffusion experiments

A self-designed glass device was manufactured at the University of Duisburg-Essen (Figure 8) in order to measure the diffusivity of phenanthrene through three different polysaccharide gels: agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v). The device is made of three parts. Two side tubes connected to a central chamber by standard taper joints 9/10 mm (Figure 8c). The volume capacity of the side vials is 6.5mL and of the central vial is 2.7mL. The inner diameter is 1 cm for all the vials.

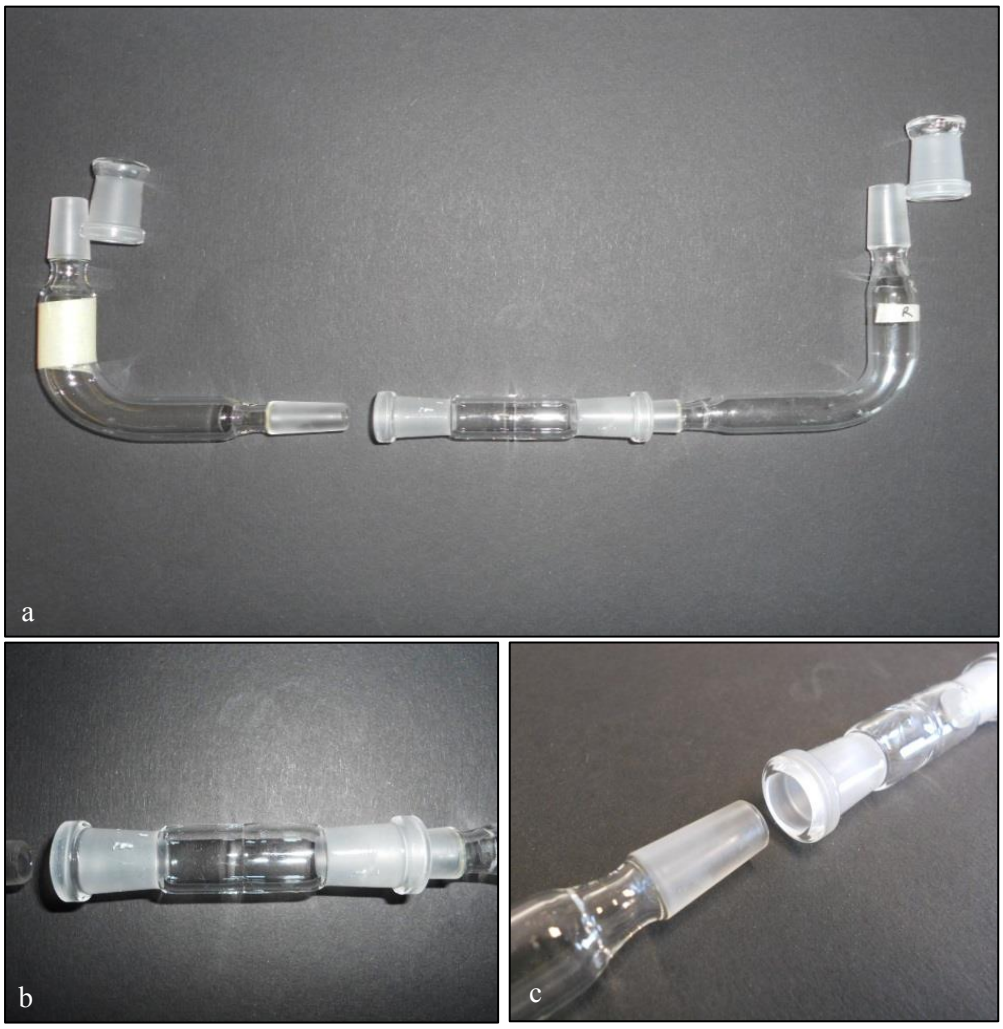


Figure 8 Diffusivity device. Glass device for diffusivity measurements. The whole device (a). The phenanthrene molecules diffuse from the right tube to the left tube, passing through the polysaccharide gel placed in the central chamber. All lids are standard taper joints 9/10 mm (b) (c) in order to avoid losses of phenanthrene by evaporation.

2.1.3 Polydimethylsiloxan (PDMS) monitor device

A self-designed device filled with PDMS oil was used for sorption experiments in deionized water and pond water solutions. The device is composed of three main stainless steel components: two external sealing frames and one internal chamber with a volume capacity of 40 mL. Two dialysis membranes foils (SERVA Visking dialysis membrane, 12-14 KDa porosity) were placed between the stainless steel sealing frames and the inner chamber as shown in Figure3b. In order to ensure the airtightness of the seal, two metal frames and two additional rubber frames, made of ethylene propylene diene-monomer rubber (EPDM from Schmidt & Bartl GmbH, 65 shore), were placed between the membrane foil and the internal chamber (Figure 9c). The dialysis membranes were washed for an hour in deionized water prior to use. The external stainless steel frames were then sealed with stainless steel screws. Once closed, the device was filled with PDMS oil. The total volume capacity of the device is 40mL and the size is 5cm x 4cm x 3 cm (length x height x thickness).

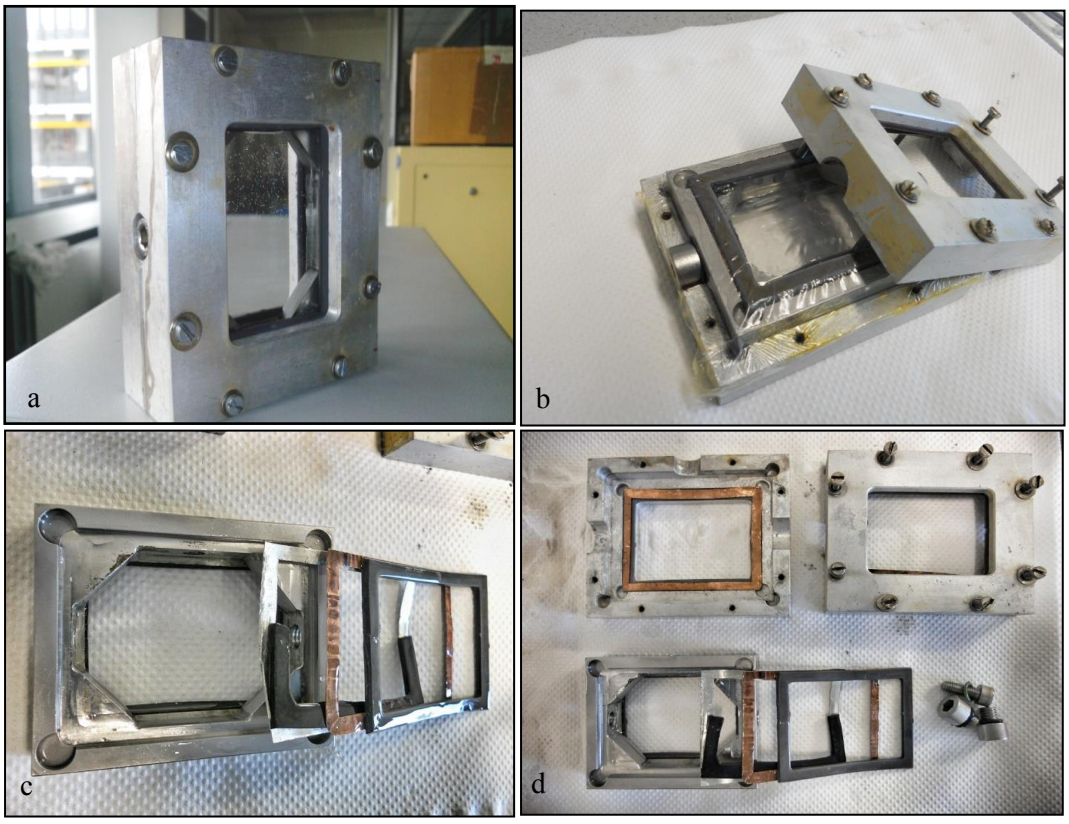


Figure 9 Prototype PDMS monitor device. a) Assembled device ready to be tested. b) external stainless steel frames and whole system with the dialysis membranes. c) The different sealing frames which placed between the inner chamber and the dialysis membrane; the first frame is made of tin (99%) the second is made of EPDM, the third is a copper frame and one more EPDM (black and grey color) d) a top view of all the components of the device before to be assembled.

2.1.4 Optical fiber platform

A self-designed fiber optic platform was manufactured at the University of Duisburg-Essen (Figure 10). This device is made of aluminum and is composed of two different planes. The optical fiber support can be moved at different height levels by a worm shaft mechanism. The sample plate can be moved on the x-axel also by a worm shaft mechanism. The angle between the two fiber optic collimators is fixed at 120° while the sample can be rotated in order to reach the better angle of incidence for the excitation light beam (Figure 10c). The base has size 12cm x 1.5cm x 5.5cm (length x height x thickness). The worm-shaft support on the bottom of the platform base, on the z-axe has size of 6cm x 2.5cm x 1cm (length x height x thickness).

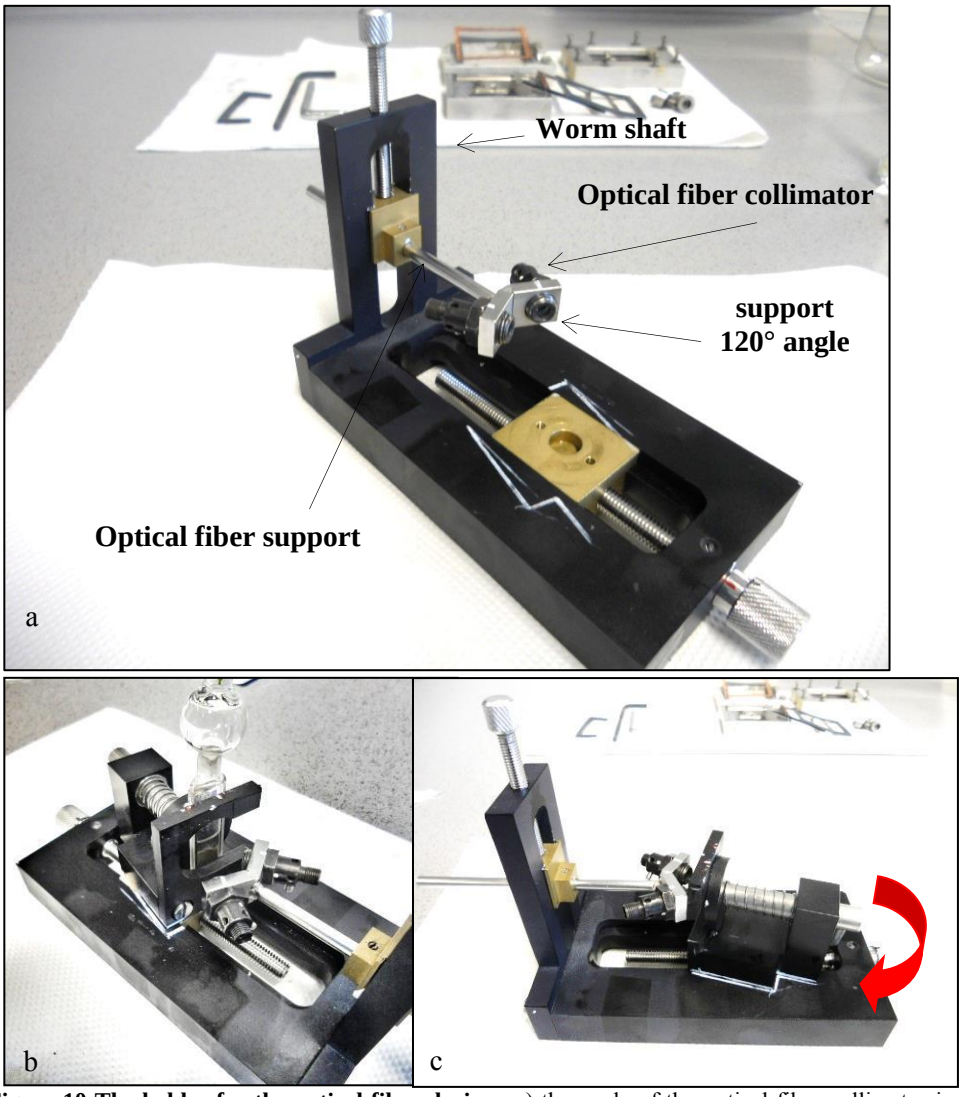


Figure 10 The holder for the optical fiber devices. a) the angle of the optical fiber collimator is set at 120°. The optical fiber support, the collimators and the worm shaft mechanism are indicated with the black arrows. b) the sample is placed in front of the optical fiber shaft. c) the sample holder can be twisted (red arrow) in order to achieve a better angle of incidence for the excitation of the sample. In this example is shown a cuvette holder.

2.2 Analytical Instruments

2.2.1 Bench fluorescence spectrometer

For the front face fluorescence calibration and measurement with 3.5mL QS glass cuvettes a Shimadzu RF-5301PC Shimadzu fluorescence spectrometer equipped with a xenon arc lamp was used The excitation wavelength was set at 290 nm.

2.2.2 USB fluorescence spectrograph

The fluorescence measurements on the sewer biofilms and on the PDMS device were performed with Ocean Optics USB QE65000 fiber optic USB spectrometer (Figure 11). Spectrasuit, a java based spectroscopy software, was exploited for the acquisition of the emission signal and the parameters were set as following (Table 16):

Table 16 Software parameters.

Sample	Integration time	Average scans per sec.	Boxcar width
Sewer biofilm	30sec	2 scans	4 nm
PDMS experiments	2 sec	2 scans	4 nm

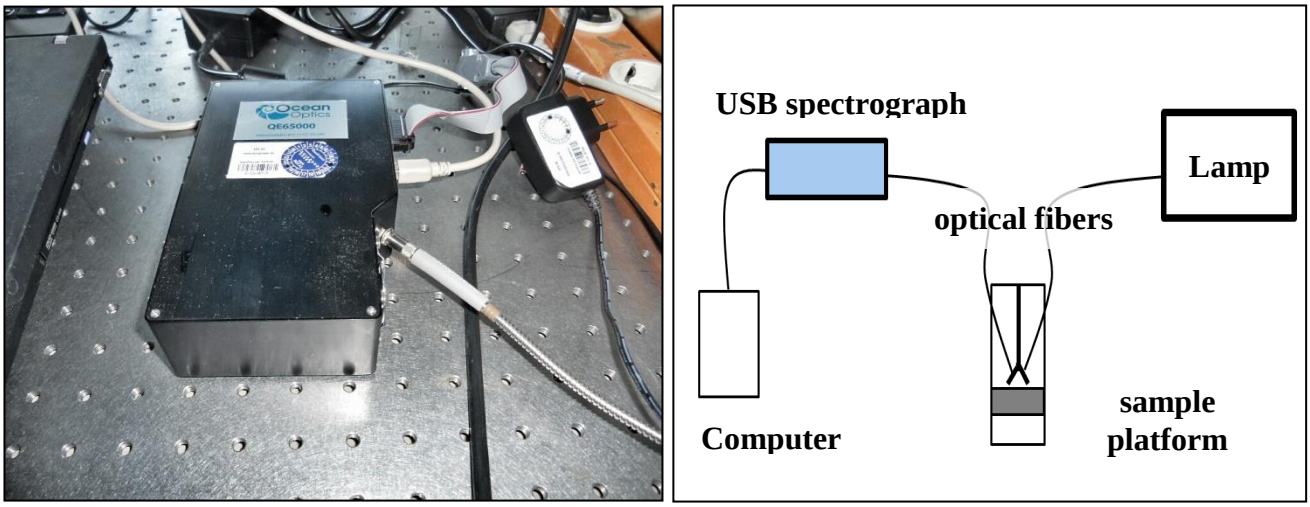


Figure 11 Ocean optics USB spectrometer and a sketch of the analytical system used for detecting phenanthrene in sewer biofilm suspensions and PDMS oil.

2.2.3 Total organic carbon (TOC) analyzer

The measurement of the total organic carbon fraction of the gel matrices was performed using a TOC-5000A bench analyzer from Shimadzu. The solutions analyzed were: agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v). A volume of 3mL for each sample was analyzed after the calibration of the instrument, performed using a solution of potassium hydrogen phthalate 1000 mg/L for the total carbon and a solution of both sodium bicarbonate (3.50 g) and sodium carbonate (4.41 g) at 1000 mg/L, for the inorganic carbon quantification. Before to prepare the solution, the sodium carbonate was dried in the oven for one hour at 285°C.

2.2.4 GC-MS device

The extraction of phenanthrene from the biofilm samples was performed according to the DIN38414-23 method. The GC-MS SIM mode analysis was performed according to Qian et al.,.2011 GC–MS (GCMS-QP5050, Shimadzu, Duisburg, Germany) under the following conditions: HP-5MS capillary column (0.25 mm , 30 m, 0.25 lm thick), helium 5.0 as carrier gas, flow rate at 1 mL/min, injector temperature at 250° C, interface temperature at 300° C, SIM mode, and electron ionisation (EI). The temperature program started at 160° C, held for 2 min and increased to 280 °C at a rate of 10° C per minute. After reaching 280° C, the temperature was held for 10 min.

2.3 Methods

2.3.1 Preparation of aqueous solutions of phenanthrene

2.3.1.1 Sorption experiments in polysaccharide gels

A methanol solution (HPLC grade from Fisher scientific UK) of phenanthrene (Supelco Analytical) of 10 mg/L was prepared inside a 10 mL matrass flask (Blau Brand duran glass), by diluting 100 times at 1000 mg/L methanol stock solution at. From this 10 mg/L methanol solution of phenanthrene, a volume of 1.5mL was diluted in 10mL of an autoclaved deionized water solution and stirred for 30 minutes using an autoclaved magnetic stirring bar (800 rpm). The whole preparation was performed under sterile conditions.

2.3.1.2 PDMS device experiment in deionized water solution

A methanol solution (HPLC grade from Fisher scientific UK) of phenanthrene at 100 mg/L was prepared inside a 10mL matrass flask (Blau brand duran glass) diluting a methanol stock solution of phenanthrene at 1000 mg/L. The methanol solution of phenanthrene at 100 mg/L of concentration was stirred for ten minutes and then mixed with 1 L of deionized water solution (previously autoclaved) in order to reach a phenanthrene solution concentration of 1.5 mg/L. The whole preparation was performed under sterile conditions.

2.3.1.3 PDMS device experiment in pond water solution

A volume of 2 L of water was sampled from a pond reservoir located in the city of Essen, Germany. The sample solution was divided in two volumes of 1 L and spiked with 1.5 mL of a methanol stock solution (HPLC grade from Fisher scientific UK) of phenanthrene at 1000 mg/L. Both solutions were stirred for 30 minutes with a magnetic stirrer (800 rpm). One solution was used for the absorption of phenanthrene in the PDMS device and the other solution was used as a blank in order to monitor the decrease of phenanthrene during the experiment without the use of the device.

2.3.2 Preparation of gel solutions

For the preparation of the gel agar 2% (w/v), 4g of agar powder (from Fluka analytical) was mixed in deionized water with a total volume solution of 200 mL and mixed for 20 minutes at 800 rpm. Then the solution was autoclaved.

For the preparation of the gel agar 1.5% (w/v) + gellan 0.5% (w/v), 3g of agar powder (Fluka analytical) and 1 g of gellan gum (Gelrite from Carl Roth GmbH) were mixed in a deionized water solution of total volume 200 mL and stirred for 20 minutes at 800 rpms. Then the solution was autoclaved.

For the preparation of the gel agar 1.5% (w/v) + gelatin 0.5% (w/v), 3g of agar (Fluka analytical) was mixed with a deionized water solution in a total volume of 200 mL and stirred at 800 rpm for 20 minutes. Then the solution was autoclaved at 120°C for 20 minutes. After that the agar solution was placed in a warm bath at 70°C, 1 g of gelatin powder (porcine skin type A powder gelatin 100G from Sigma-Aldrich) was added and the solution was stirred for 10 minutes at 900 rpm. All the procedure was performed under sterile conditions using a sterile bench for microbiology.

2.4 Sampling of the sewer biofilm

The biofilm samples were collected from the Bielefeld sewer system using a special plastic support (Figure12a) kept into the wastewater stream for 4 weeks. After this period, the sampler was taken out from the sewer and the biofilm was scratched (Figure13a) and collected in a sampling bottle. The samplers were placed along various sites of the sewer system in Bielefeld city, downstream to different wastewater sources (Figure 12b, Table 17).

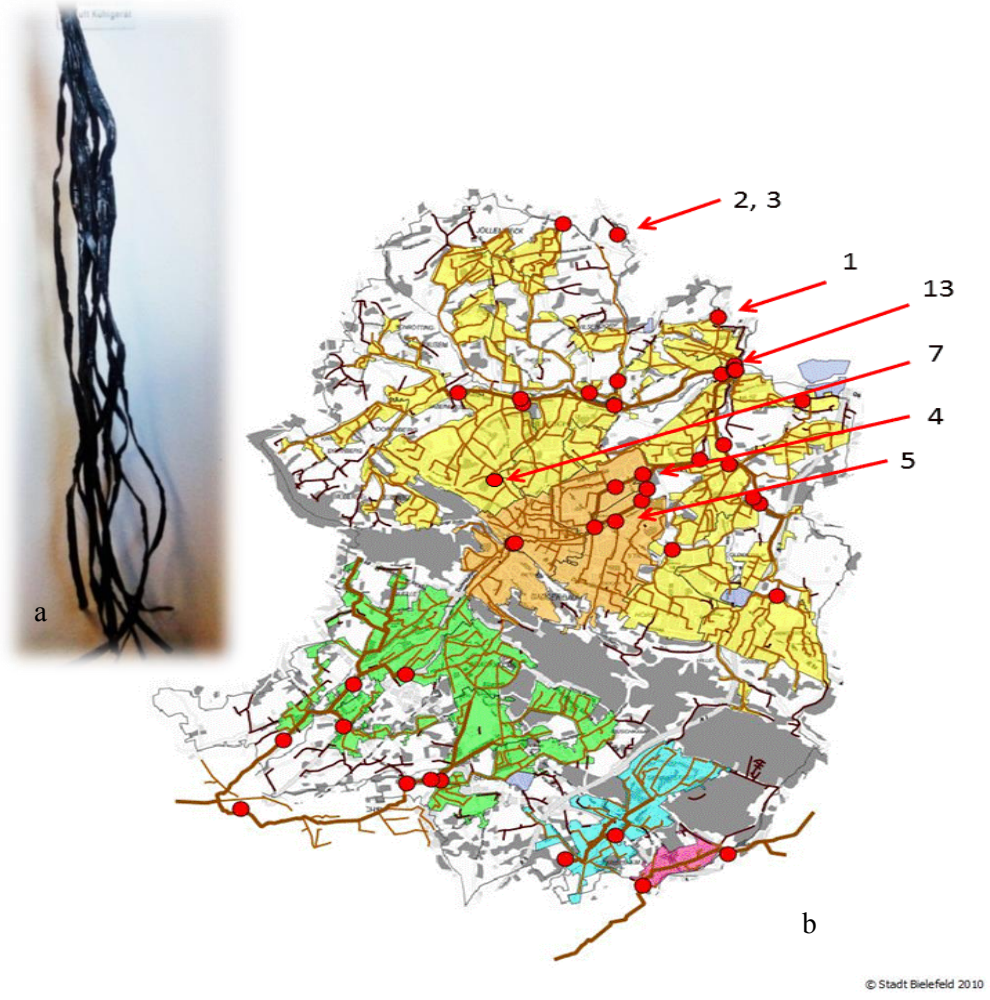


Figure 12 Overview of the sampling sites within the Bielefeld sewer system. The biofilm samples analyzed by GC-MS are indicated by the red arrows. On the left is shown the plastic sampler device used for collecting the biofilm from the sewers in collaboration with the Umweltamt of the Bielfeld city hall administration.

Table 17 Samples collected from the Bielefeld sewers by the Umweltamt city administration department. For each sample are indicated: the number, the name, the date of sampling and the specific upstream source of wastewater. Different sources might lead to a different composition of the biofilm matrix.

Number	Name	Date	Upstream wastewater source
1	419uth	7/8/2013	Metal (powder coating)
2	417EJ-DB	7/8/2013	Disposal site
3	417EJ-DS	7/8/2013	Disposal site
4	425hDrew	7/8/2013	Metal (powder coating + electroplating)
5	103WarG	7/8/2013	Metal (anodized) + screen printing
6	104nLWN	7/8/2013	Metal (Rinse + degreasing)
7	413GH	7/8/2013	Dentist
8	529JVA	7/8/2013	Laundry, Great kitchen
9	530nBAU	7/8/2013	Iron Foundry
10	424WS	13/8/2013	Mixed Municipal wastewater
11	425SP	13/8/2013	Mixed Municipal wastewater
12	419KA	13/8/2013	Mixed Municipal wastewater
13	400HN	13/8/2013	Mixed Municipal wastewater

Figure 13 Overview of the devices used for sampling sewer biofilms. a, b) the biofilm is scratched off from the sampler into a special tool. c) a closer look at the sewer biofilm attached on the PFT support used for the sampling procedure.

2.5 Front face fluorescence measurements

2.5.1 Sorption experiment in polysaccharide gels

For the fluorescence measurement of phenanthrene inside QS glass cuvettes (3.5mL), a standard front face cuvette holder for the RF-5301PC Shimadzu fluorescence spectrometer was used (Shimadzu GmbH). Using this holder, the angle of incidence of the excitation light beam was fixed at 60° as shown in Figure 14a. The gel and the water phase were selectively irradiated, sliding the QS cuvette up and down on the z-axe of the holder (Figure 14b). This procedure was tested and calibrated in order to guarantee reproducible results. The excitation slit of the instrument was made wider or narrower depending on the gel used. For the water phase analysis, the slit width was set to 1.5 nm for all the samples. For the agar 2% (w/v) was 3nm, for the agar 1.5% (w/v) + gellan 0.5% (w/v) was 3 nm, for agar 1.5% (w/v) + gelatin 0.5% (w/v) was 5nm. The width of the emission slit was fixed at 1.5 nm for all the experiments. Each polysaccharide matrix displays different intensity values for the fluorescence signal of phenanthrene.

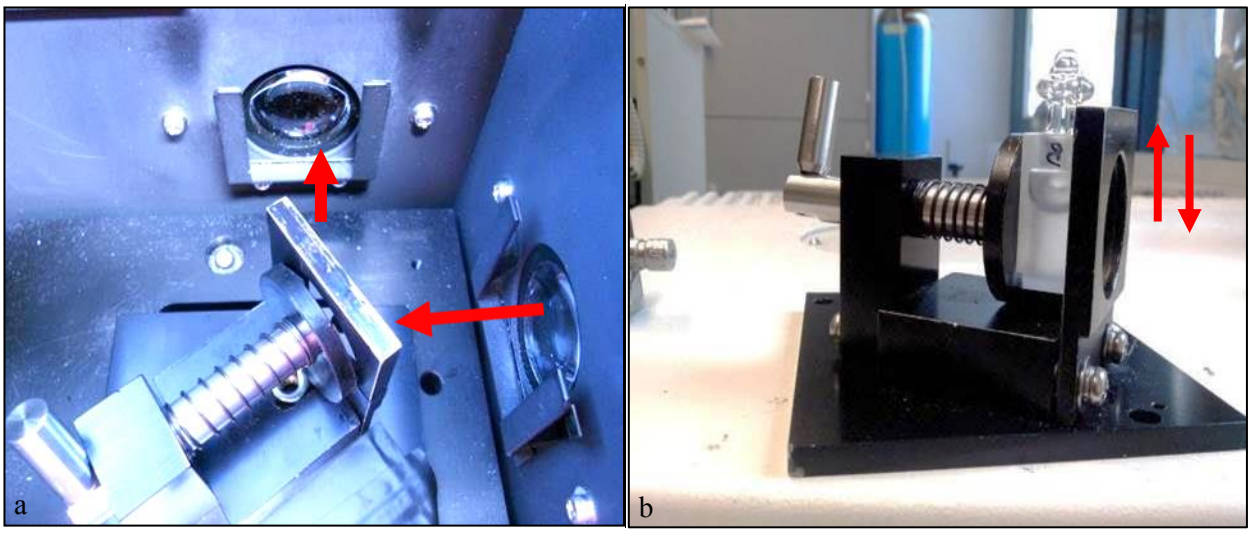
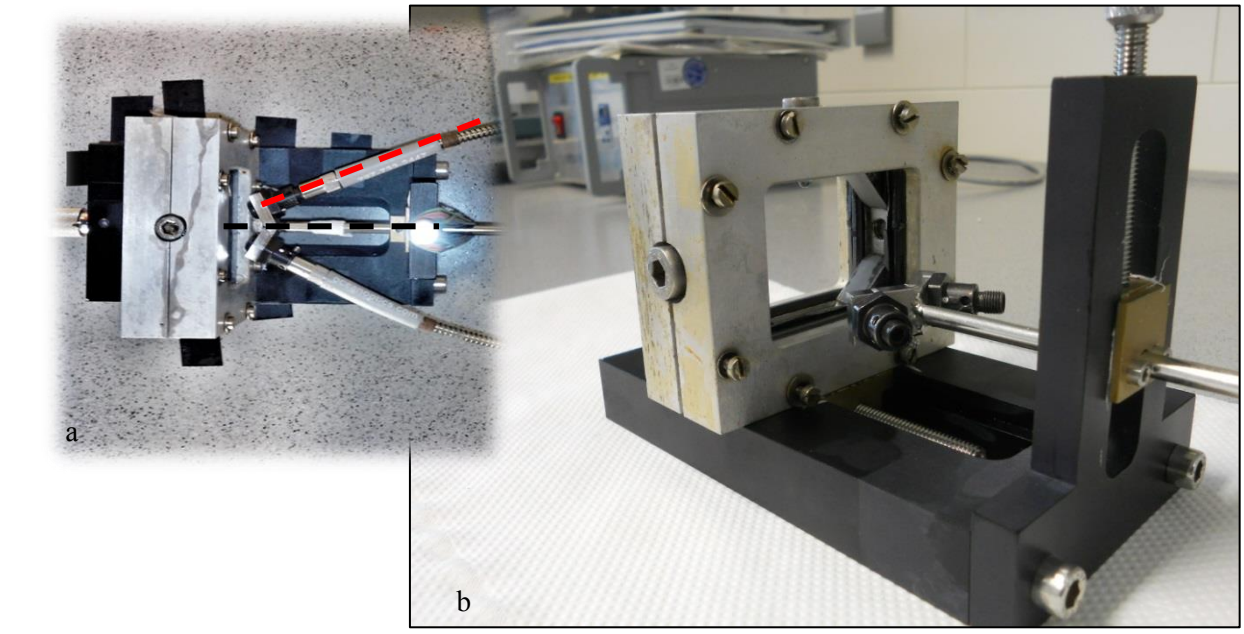


Figure 14 Overview of the inner chamber of the RF-5301PC Shimadzu fluorescence spectrometer. a) the excitation and the emission windows and the position of the sample inside the instrument. b) the QS cuvette was moved up and down the holder in order to selectively irradiate the two different phases.

2.5.2 PDMS device experiments

The PDMS prototype device was placed on the fiber optic platform, in order to achieve an angle of incidence equal to 30° for the excitation of the PDMS surface. This angle was chosen as the best arrangement in order to guarantee the reproducibility of the measurements (Figure 15).



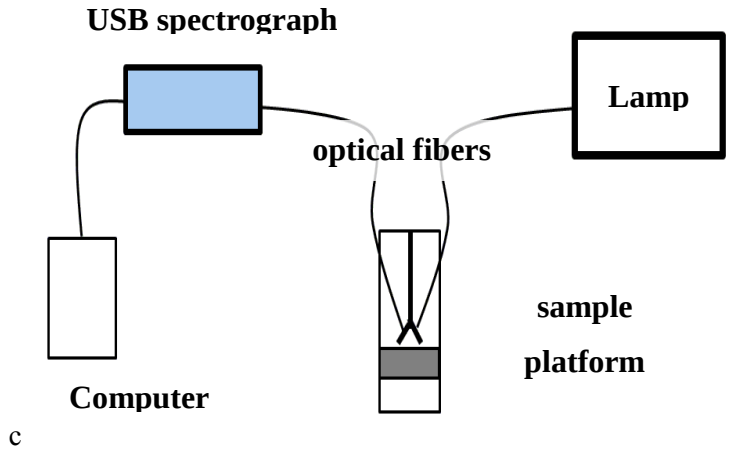


Figure 15 Top view of the analytical system arranged for the detection of phenanthrene inside the PDMS prototype device. a) The angle of incidence between the perpendicular to the target surface (black line) and the light beam (red line) was set at 30°. b) a closer view of the whole system. c) sketch of the experimental system.

2.6 Calibration procedures

2.6.1 The quantification of phenanthrene in deionized water solution.

A stock solution of 1000 mg/L of phenanthrene was prepared mixing 100 mg of phenanthrene crystals (Supelco Analytical, 99.1% purity) in 100mL of pure methanol (HPLC grade Fischer scientific UK). The solution was stirred for 15 minutes by magnetic bar at 800 rpm. Then a methanol solution of phenanthrene at 10 mg/L was prepared. This solution of phenanthrene was mixed with deionized water in order to prepare the standard solutions for the calibration procedure. The following phenanthrene standard solutions were prepared: 1.5 mg/L, 1 mg/L, 0.5 mg/L and 0.250 mg/L. The calibration was performed five times and each standard prepared in triplicate and analyzed in 3.5 mL QS glass cuvettes by front face mode using a RF-5301PC Shimadzu fluorescence spectrometer. The intensity value (a.u) of each standard solution used for the calibration curve, was calculated as the difference between fluorescence intensity (a.u) at 345 nm and 338nm.

2.6.2 The quantification of phenanthrene in polysaccharide gels.

For each gel used for the experiments, a calibration procedure for RF-5301PC Shimadzu fluorescence spectrometer was performed. The fluorescence spectra of phenanthrene in gel were recorded at the following concentrations: 0.25mg/L, 0.5 mg/L, 1 mg/L and 1.5 mg/L. Each gel solution was mixed with a methanol solution of phenanthrene at 10 mg/L in a total volume of 10 mL. Each mixture was stirred (1700 rpm) at 80° C for 2 minutes before to be placed in the QS cuvette for the further analysis. The gel was left 45 minutes in the QS cuvette to let it solidify and then analyzed using a RF-5301PC Shimadzu fluorescence spectrometer in front face mode. For the preparation of the gel standards which contain gelatin, the temperature was kept at 60-65 ° C to avoid the protein denaturation. Each standard was prepared in triplicate. The intensity value (a.u) of each standard solution used for the calibration curve, was calculated as the difference between fluorescence intensity (a.u) at 345 nm and 338 nm

2.6.3 Total organic carbon (TOC) analysis

For each gel solution, the total organic carbon concentration was measured with a TOC analyzer. The instrument was previously calibrated for both total carbon and inorganic carbon using a solution with 2.125 g potassium hydrogen phthalate ($C_8H_5KO_4$) at 1000 mg/L for the total carbon (Figure 17) and a solution of 3.50 g of sodium bicarbonate and 4.41g sodium carbonate ($NaHCO_3/Na_2CO_3$) at1000 mg/L for the inorganic carbon calibration (Figure 16). A volume of 100µL of each gel solution was diluted in a total volume of 20mL with deionized water and shacked for 10 minutes.1.5mL of each diluted solution was analyzed for the total carbon and the inorganic carbon content after the instrument calibration. According to the results, the organic carbon fraction was calculated for agar 1.5% (w/v) + gellan 0.5% (w/v), agar 2% (w/v), agar 1.5% (w/v) + gelatin 0.5% (w/v).

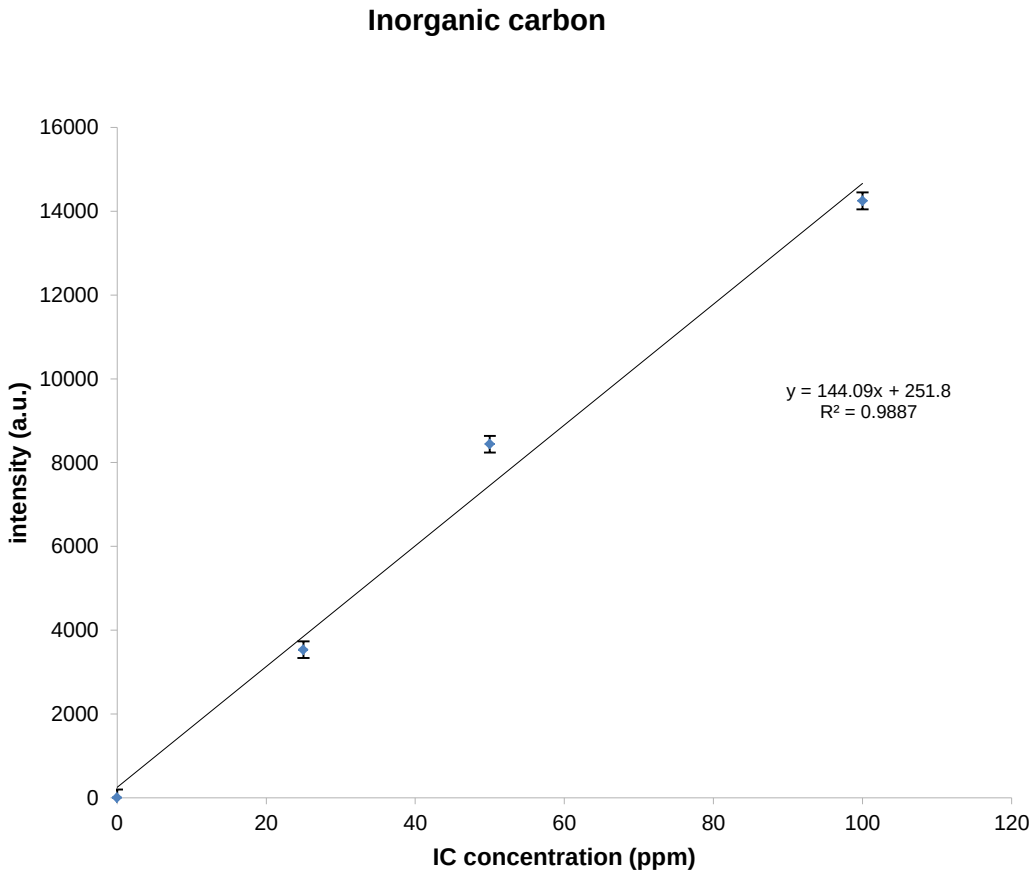


Figure 16 Inorganic carbon calibration curve. The standard concentrations were 0 mg/L, 25 mg/L, 50 mg/L, 100 mg/L.

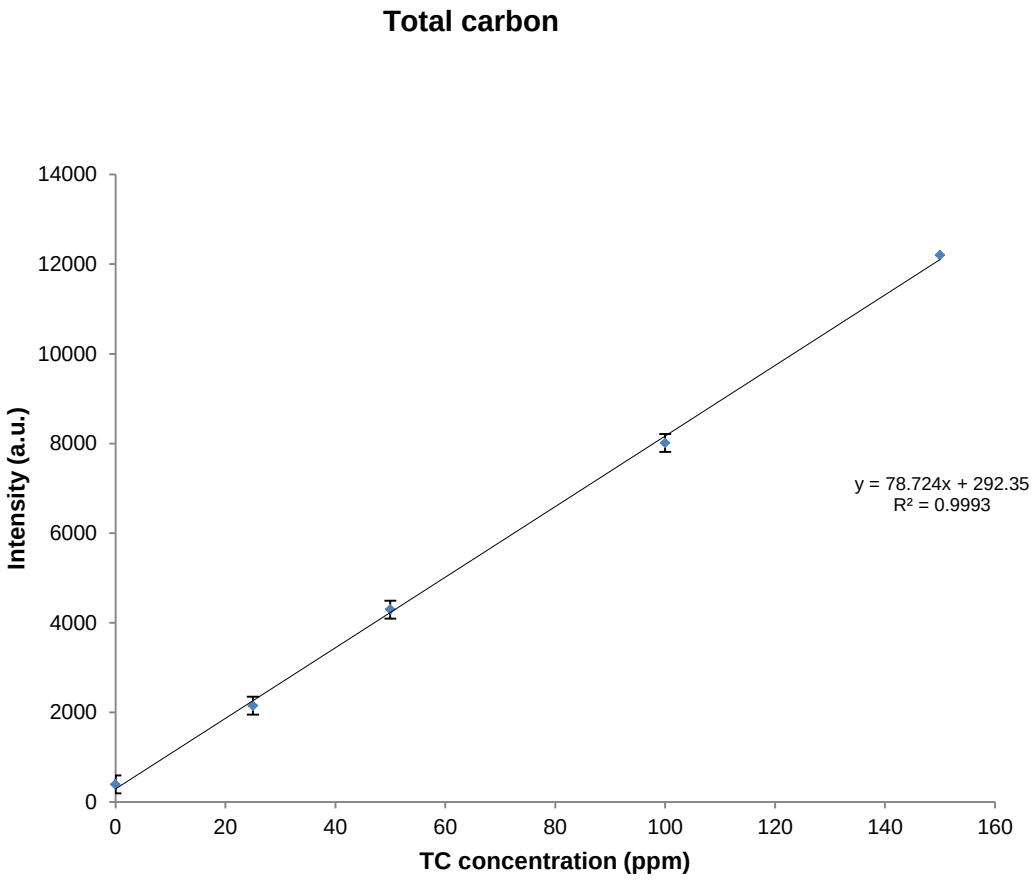


Figure 17 Total carbon calibration. The standard solutions were 0 mg/L, 25mg/L, 50 mg/L, 100 mg/L, 150 mg/L.

2.6.4 GC-MS device

A stock solution of phenanthrene at 1000ppm was prepared in methanol and diluted 10 times with acetone in a 10 mL flask. From this solution were prepared six standard solution of phenanthrene in acetone:. 1.5mg/L, 1.0 mg/L, 0.75 mg/L, 0.5 mg/L, 0.25 mg/L, and 0.125 mg/L. The standards were prepared in triplicate and analyzed using the previously described method. The area of the peaks was taken as reference for the calibration. The calibration curve is shown in Figure 18.

The limit of detection is equal to 1 µg/L and the limit of quantification is equal to 3 µg/L.

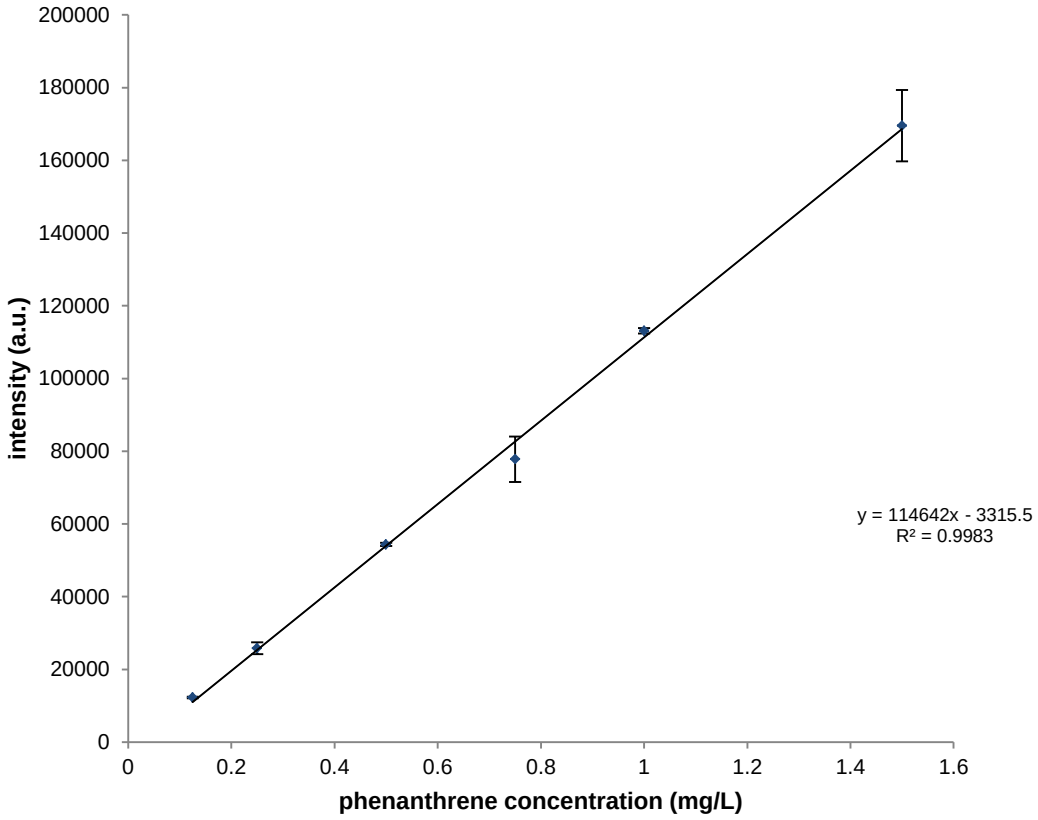


Figure 18 GC-MS calibration curve for the quantification of phenanthrene from sewer biofilm suspensions. The standard concentrations are 0.125 mg/L, 0.25 mg/L, 0.50 mg/L, 0.75 mg/L, 1.0 mg/L, 1.5 mg/L.

2.7 Experiments

2.7.1 Absorption partitioning of phenanthrene between polysaccharide gels and water 1:1 (v/v)

A volume of 1.5mL of gel was poured inside a QS glass cuvette (3.5mL) and left 45 minutes to solidify at room temperature. After 45 minutes a volume of 1.5mL of 1.5mg/L phenanthrene solution was added above the gel (Figure19). The QS glass cuvette was sealed air tightly with a glass cap. The experiment was carried out for all the matrices under static conditions. The cuvette was gently spin-shaken by hand before each measurement The fluorescence spectrum of both gel phase and water solution was collected at the beginning of the experiment and then each 24 hours till the equilibrium of the sorption process was reached. This kind of experiment was carried out in triplicate for agar 2% (w/v), agar 1.5% (w/v), agar 1.5% (w/v) + gellan gum 0.5% (w/v), agar 1.5% (w/v) + gelatin 0.5% (w/v).

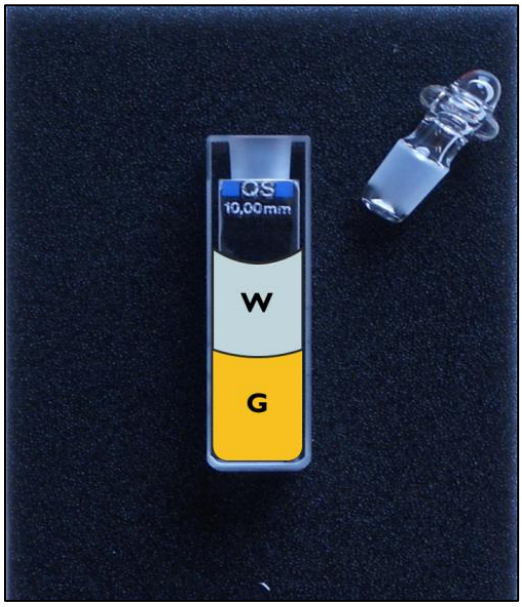


Figure 19 The experimental system used to evaluate the partitioning of phenanthrene between polysaccharide gels and water. The gel solution (G) was poured into the QS glass cuvette and the deionized water solution ofphenanthrene (1.5 mg/L) was added above (W). Also the glass stop is shown in the picture on the left side.

2.7.2 Desorption partitioning of phenanthrene between polysaccharide gels and water 1:1 (v/v)

After the equilibrium of the absorption process was reached, the water phase was removed under sterile conditions and replaced with 1.5mL of pure deionized water. The experiment was carried out under static conditions. The fluorescence spectrum of both gel phase and water solution was measured at the beginning of the experiment and then each 24 hours till the equilibrium of desorption process is reached. The cuvette was gently spin-shaken by hand before each measurement. The water phase was removed under sterile conditions and 1.5mL of a new deionized water solution was added above the gel phase. The fluorescence spectra of both gel phase and water solution were collected at the beginning of the experiment and then each 24 hours till the equilibrium is reached again. This kind of experiment was carried out in triplicate for agar 2% (w/v), agar 1.5% (w/v), agar 1.5% (w/v) + gellan gum 0.5% (w/v), agar 1.5% (w/v) + gelatin 0.5% (w/v), agar 1% (w/v) + gellan 0.5% (w/v) + gelatin 2% (w/v).

2.7.3 Absorption partitioning of phenanthrene between polysaccharide gels and water 1:100 (v/v)

A volume of 1.5mL of gel solution was poured inside a QS glass cuvette of 3.5mL volume capacity and let solidify for 45 minutes. Then a special glass vial was connected to the glass cuvette air tightly and filled with 150 mL of 1.5mg/L deionized water solution of phenanthrene above the gel phase. At the beginning and each 24 hours the concentration in both water and gel solutions was estimated by front face measurement. In order to measure the phenanthrene concentration in the water phase, a volume of 3.5 mL of solution was sampled and placed in a QS glass cuvette and analyzed with the fluorescence spectrometer. Before each sampling, the water phase was shaken by hand for 30 seconds. Then the water sample was replaced back in the glass vial. In order to measure the phenanthrene concentration in the gel phase, the cuvette was removed from the system and directly analyzed by the fluorescence spectrometer in front face mode. The experiment was performed only once for agar 2% (w/v), agar 1.5% (w/v) + gellan gum 0.5% (w/v), agar 1.5% (w/v) + gelatin 0.5% (w/v).

2.7.4 Desorption partitioning of phenanthrene between polysaccharide gels and water 1:100 (v/v)

After the absorption process reached the equilibrium, the water solution above the gel was removed and replaced with 150mL of deionized water. At the beginning of experiment and then each 24 hours the fluorescence of both solutions was measured as previously described for the absorption experiment. The experiment was performed only once for agar 2% (w/v), agar 1.5% (w/v) + gellan gum 0.5% (w/v), agar 1.5% (w/v) + gelatin 0.5% (w/v).

2.7.5 Diffusivity of phenanthrene in polysaccharide gels

A self-designed glass device was manufactured in order to perform diffusivity experiments and is presented in Figure 20. A volume of 2.7mL of gel solution was poured inside the central chamber of the device (gray sector) and let solidify for 45 minutes at room temperature under sterile conditions. Then a volume of 6.5mL of 1.5 mg/L deionized water solution of phenanthrene at was added in the right side vial (black solution) of the device and sealed thight with a glass stop. The left vial of the device was filled with 6.5mL of a pure deionized water (blue sector). At the beginning of experiment and then every 24 hours a sample of 3mL was taken from both water solutions using a 5ml air-tight glass syringe (Hamilton Co. Reno, NV) and replaced back in each vial after the analysis under sterile conditions. The concentration of phenanthrene in both samples, was measured by front- phase fluorescence spectroscopy using a QS glass cuvette and a fluorescence spectrometer (RF-5301PC Shimadzu). The measurements were carried out until the equilibrium of the process was reached. This experiment was carried out using agar 2% (w/v), agar 1.5% (w/v) + gellan gum 0.5% (w/v), agar 1.5% (w/v) + gelatin 0.5% (w/v).

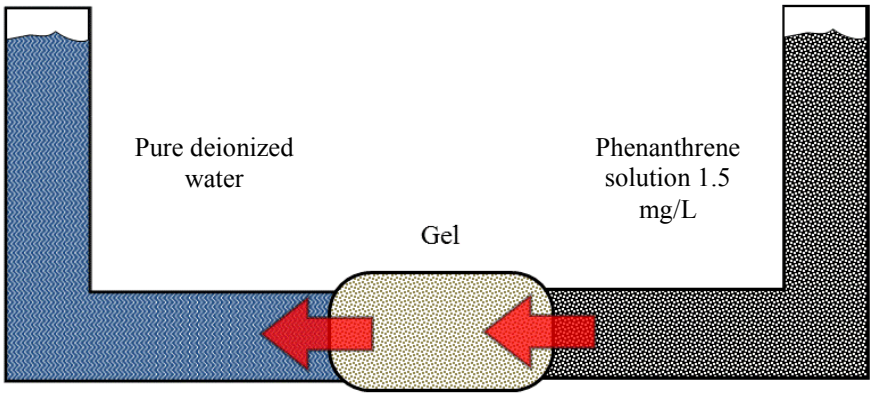


Figure 20 Sketch of the glass device used for the estimation of the diffusivity of phenanthrene in polysaccharide gels. The phenanthrene molecules diffuse through the gel from the right side to the left side (red arrows). All the glass junctions are provided with an airtight closure.

2.7.6 Kinetic experiment of phenanthrene between water and polysaccharide gels

A mixure of agar 1.5% (w/v) + gellan 0.5% (w/v) was chosen for this experiment (Figure 21). A volume of 1.5 mL of 10 mg/L methanol solution of phenanthrene was mixed with the gel solution in a final volume of 10 mL, at 90° C. The mixture was stirred for 2 minutes at 1200 rpm. A volume of 350 µL of the mixture was placed inside a QS glass cuvette (3.5mL), forming a thin layer of 0.1 cm thickness on the front inner surface of the QS cuvette. The cuvette was sealed with glass stops and the gel solution was let solidify for 30 minutes at 20 °C. Then the fluorescence of phenanthrene in the gel layer was analyzed by front face measurement as previously described. The resulting spectrum of phenanthrene in the gel was taken as reference for starting concentration. After the first measurement the QS cuvette was connected to a source of deionized water flow with a rate of 1.0 L/min and the fluorescence spectrum of phenanthrene inside the gel was collected every 10 minutes until the LOQ was reached. The fluorescence spectrum was collected using a RF-5301PC Shimadzu fluorescence spectrometer according to the methods previously described. After this first experiment, the flow rate was decreased to 20mL/min and three further experiment were carried out measuring the fluorescence of the gel layer every ten minutes till the LOQ was reached. The phenanthrene concentration inside the gel layer was plotted versus the time and the curves were compared with the theoretical model calculated using the diffusivity of phenanthrene within the agar 1.5% (w/v) + gellan 0.5% (w/v) layer. The calculations were performed using the first Fick's law of diffusion.

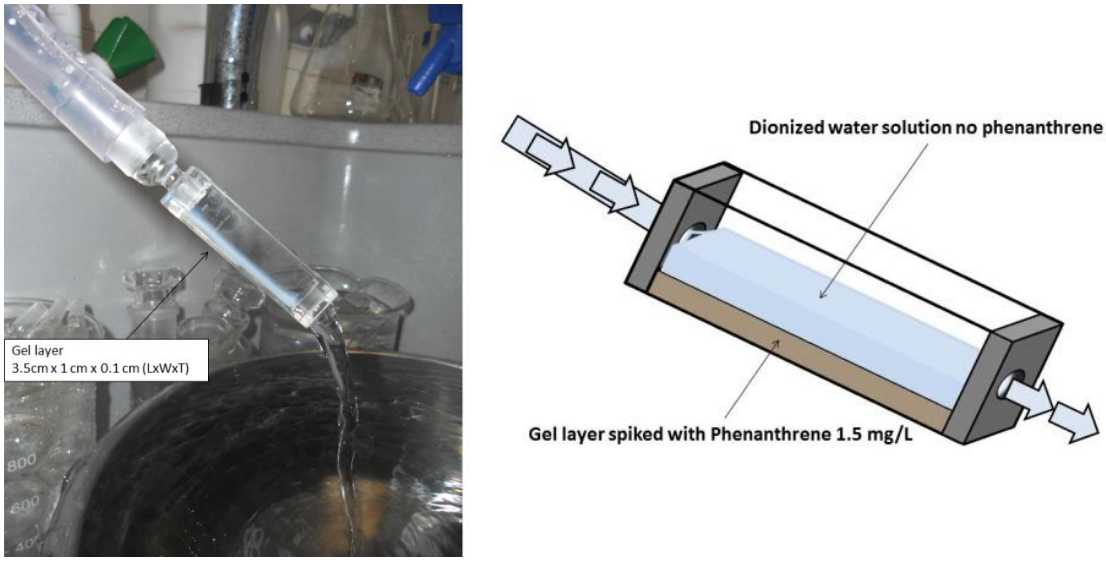


Figure 21 Experimental setting for investigating the memory of a polysaccharide gel for phenanthrene. The QS cuvette was connected to a continuous flow of deionized water to simulate real conditions.

2.7.8 Experiment with PDMS device in deionized water with phenanthrene

A volume of 40 mL of PDMS oil (WS Silikonöl V1000) was poured into the device that was plunged into deionized water solution (800mL) with phenanthrene (1.4 ±0.2 mg/L). The fluorescence emission spectra of phenanthrene absorbed by the device were collected each hour for the first nine hours and then each 24 hours till 72 hours. In order to perform the analysis, the device was removed from the water solution and left 10 minutes in the dark. In this time the semipermeable membrane, which encloses the PDMS oil, was getting dry and straight on its surface, so that allowed to be analyzed by front face fluorescence spectroscopy. After ten minutes the device was placed on the platform where optical fiber devices were used to perform the measurements.

2.7.8 Experiment with PDMS device in pond water with phenanthrene

A volume of 40mL of PDMS oil (WS Silikonöl V1000) was poured into the device that was plunged into a 1.4 mg/L pond water solution (800mL) of phenanthrene. The fluorescence emission spectra of phenanthrene absorbed by the device were collected after one hour and after 24 hours. In order to perform the analysis, the device was removed from the water solution and left 10 minutes in the dark. In this time the semipermeable membrane, which encloses the PDMS oil, was getting dry and straight on its surface, so that allowed to be analyzed by front face fluorescence spectroscopy. After ten minutes the device was placed on the optical fiber platform where optical fiber devices were used to perform the measurements.

2.8 Data elaboration procedures

2.8.1 Organic carbon partition coefficient (K_{oc})

The organic carbon partition coefficient was calculated following the method used by Wicke et al., 2007.

The fraction of sorbed phenanthrene in the gel matrices was calculated following the formula:

$$Q_{eq} = \frac{(C0 - Ceq.) * V}{ms} \quad \text{(Eq.8)}$$

where C_0 is the starting concentration of phenanthrene in the water phase, $C_{eq.}$ is the phenanthrene concentration in water at the equilibrium, V is the total volume of the water phase and ms is the dry weight of the gel matrix.

Then the partition coefficient was calculated by the ratio between the sorbed phenanthrene fraction in the matrix at the equilibrium ($Q_{eq.}$) and the concentration of phenanthrene in water at the equilibrium ($C_{eq.}$).

$$K_p = \frac{Q_{eq}}{C_{eq}} \quad \text{(Eq.9)}$$

The organic carbon partition coefficient was calculated by the ratio between the partition coefficient (K_p) and the organic carbon fraction of the gel matrix (f_{oc}), obtained by TOCanalysis .

$$K_{oc} = \frac{K_p}{f_{oc}} \quad \text{(Eq.10)}$$

2.8.2 Gel-water partition coefficient (K_{gw})

The partition coefficient of phenanthrene between the gel phase and the water phase for the absorption process was calculated as follows:

$$Q_{eq} = \frac{(Ceq - C0.) * V}{ms} \quad \text{(Eq.11)}$$

$$K_{gw} = \frac{Q_{eq}}{C_{eq}} \quad \text{(Eq.12)}$$

The partition coefficient of phenanthrene between the gel phase and the water phase (K_{gw}) for the desorption process was calculated as follows:

$$Q_{eq} = \frac{(C0 - Ceq.) * V}{ms} \quad \text{(Eq.13)}$$

$$K_{gw} = \frac{C_{eq}}{Q_{eq}} \quad \text{(Eq.14)}$$

where, *V* is the volume of the gel phase, C_0 is the starting concentration of phenanthrene in gel, *ms* is the dry mass of the gel phase, Q_{eq} is the total phenanthrene sorbed at the equilibrium and C_{eq} is the concentration of phenanthrene in water at the equilibrium.

2.8.3 Distribution coefficient (K_d)

The distribution coefficient of phenanthrene between the gel phase and the water phase during the partitioning experiments was calculated according to the following equation:

$$\frac{C_g}{C_w} = \boldsymbol{K_d} \quad \text{(Eq.15)}$$

where C_g and C_w are the equilibrium concentrations (mg/L) of phenanthrene in gel and in water respectively.

2.8.4 Diffusion coefficient (D) calculation

The concentration of phenanthrene inside both water phases was measured each 24 hours. As soon as the phenanthrene concentration reached a minimum value of 0.2 mg/L in the left glass vial, the diffusion coefficient was calculated using the mass transfer and the difference between the concentration at the equilibrium and at the time when phenanthrene reached 0.2mg/L in the left vial.

At first the mass transfer of phenanthrenhe into the left vial was calculated as follows:

$$\left[\frac{g}{s}\right] = \frac{(C_0 - C_{eq.})V}{s} \quad \text{(Eq.16)}$$

where C_{eq} and C_0 are the concentration of phenanthrene in the left vial at the equilibrium and at the beginning respectively. V is the volume of the solution in the left vial, s is the experiment duration in seconds and m^2 is the surface area (A) of the chamber where the gel is placed.

and then the diffusivity (D) was calculated following the equation:

$$\left[\frac{g}{m^2 s}\right] = D * A * \frac{Cr-Cl}{x} \quad \text{(Eq.17)}$$

where C_r and C_l are the phenanthrene concentration (in g/m^3) in the right and in the left vial respectively.

2.8.6 Polysaccharide gel memory simulation curve for phenanthrene

A theoretical phenanthrene concentration of 1.5 mg/L was estimated in a polysaccharide gel solution with volume 350µL, surface area (A) of 3.5 cm^2 and the thickness (x) of 0.1 cm. The phenanthrene concentration in the water phase (C_w) was set equal to 0 for each time step. The diffusivity of phenanthrene into the polysaccharide gel (D) was calculated by the previous experiments with agar 1.5% (w/v) + gellan 0.5% (w/v), equal to 8 x 10^{-6} cm^2/s

The decrease of phenanthrene concentration in the time was calculated by the following equation:

$$\left[\frac{g}{s}\right] = D * A * \frac{(Cg\frac{1}{K_d} - Cw)}{x} \quad \text{(Eq.18)}$$

2.8.7 Decay constant estimation for partitioning experiments

For all the sorption experiments carried out in this study the decrease of phenanthrene concentration both in water (absorption) and in gel (desorption) was investigated. The exponential decay curve from each experiment was fitted with the following exponential function using OriginLabpro9.1:

$$\boldsymbol{y = y_0 + Ae^{kt}} \quad \text{(Eq.19)}$$

Then the rate constant was calculated by the software using a Levemberg-Marquardt simulation for a r^2=0.99 for each curve.

3 RESULTS

This study is focused on sorption modeling of PAH compounds into microbial biofilms. Different polysaccharide gels have been used as surrogates of real biofilms and the partitioning of phenanthrene between the gel phase and the deionized water phase has been investigated. Fluorescence spectroscopy in front face mode is the analytical method chosen for studying the sorption of phenanthrene in polysaccharide gels. In order to study the partitioning process a quartz glass cuvette was filled with two immiscible phases, gel and deionized water. Each phase was selectively irradiated by an excitation light beam at 290 nm of wavelength in order to quantify the phenanthrene concentration during the partitioning event. In order to have a sufficient surface to be irradiated by the UV light beam, 1.5mL of each phase was placed in the cuvette. The polysaccharide gel was placed on the bottom of the cuvette and above it was poured the deionized water phase. The whole analytical procedure was optimized for the first time during the present study and represent a novel method for studying the partitioning of a UV-fluorescent substance between deionized water and an agar gel phase. This part of the work provides a unique analytical application for studying the sorption partitioning of phenanthrene between deionized water and polysaccharide gels.

3.1 Quantification of phenanthrene by fluorescence spectroscopy

3.1.1 Quantification of phenanthrene in deionized water solution by fluorescence spectroscopy in front face mode.

The calibration curve for the quantification of phenanthrene in deionized water is presented below (Figure 22) Due to the low solubility of phenanthrene in deionized water, the maximum concentration measured was 1.5 mg/L and the minimum was 0.25mg/L. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.05 mg/L and 0.1mg/L respectively.

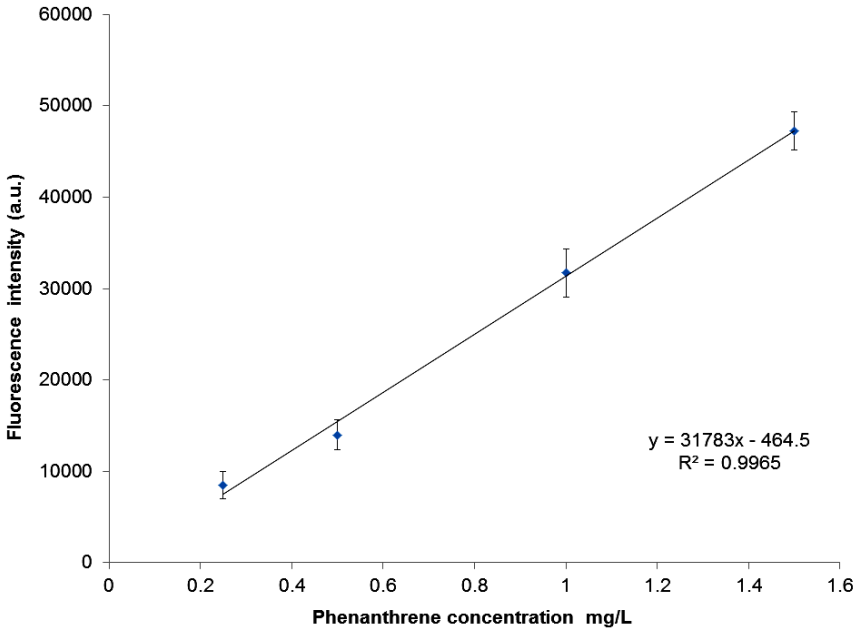


Figure 22 Calibration curve of phenanthrene in deionized water. The standard concentrations were 0.25mg/L, 0.5 mg/L, 1.0 mg/L and 1.5 mg/L .

It was possible to quantify phenanthrene in deionized water by using a self-designed front face measurements. All the following sorption experiments were focused on tracing phenanthrene during the absorption and the desorption across the gel and the water phase. This calibration curve is considered an important result and shows that following this procedure, the quantification of phenanthrene is reproducible and the values of concentrations in deionized water are reliable. Thank to this analytical method, it was possible to perform mass balance calculations during the sorption of phenanthrene into polysaccharide gels, keeping the experimental system isolated from external interferences. Without this approach, the sorption investigation would have required longer analyses through several steps of extraction and

concentration of the target compound and more material would have been necessary to set the experiments.

3.1.2 Quantification of phenanthrene in polysaccharide gels by fluorescence spectroscopy in front face mode.

The calibration curve for each gel is presented below (Figure 23). The limit of detection and the limit of quantification are presented in the Table 18. The standard concentrations are 0.25 mg/L, 0.5 mg/L, 1.0 mg/L and 1.5 mg/L.

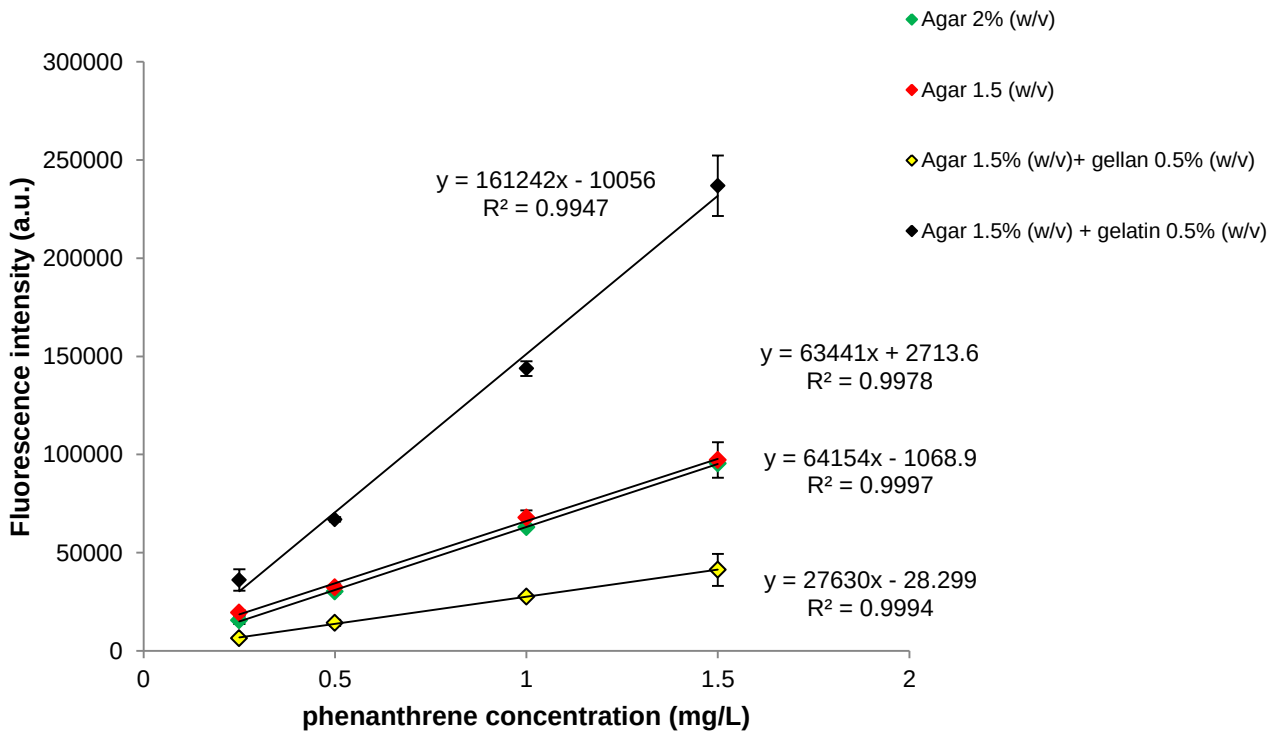


Figure 23 Calibration curves obtained with the RF-5301PC Shimadzu fluorescence spectrometer for the quantification of phenanthrene into polysaccharide gels. a) agar 2% w/v. b) agar 1.5% w/v + gellan 0.5% w/v. c), agar 1.5% w/v + gelatin 0.5% w/v. d) agar 1.5% w/v.

Table 18 Limits of detection and limits of quantification for each hydrogel matrix used in the calibration procedure.

Hydrogels	LOD (mg/L)	LOQ (mg/L)
Agar 1.5% (w/v)	0.09	0.3
Agar 2% (w/v)	0.03	0.12
Agar 1.5% (w/v) + gellan 0.5% (w/v)	0.05	0.17
Agar 1.5% (w/v) + gelatin 0.5% (w/v)	0.01	0.08

These results show that it was possible to quantify phenanthrene into each one of the polysaccharide gels used in the sorption experiments, which will be presented later in this section. The calibration of this procedure was a fundamental achievement, necessary for preforming correct mass balance elaborations during the partitioning of phenanthrene between water and gels. No significant differences were observed between the calibration curves of agar 2% (w/v) and agar 1.5% (w/v). These two mixtures show similar spectroscopic properties. The fluorescence intensity displayed by the agar 1.5% (w/v) + gelatin 0.5% (w/v) is higher than all the other mixtures, because the settings of the instrument were changed compared with the original settings used for the other gels. In this case the emission slit width was set narrower and the excitation one was set wider, in order to collect sharper spectra. In fact, the presence of gelatin inside the mixture caused a decrease of the resolution in the spectrum of phenanthrene due to the scattering effect on the emitted light. Gelatin display and excitation wavelength of 280 nm close to the wavelength used for the excitation of phenanthrene (290 nm) and its broad emission peaks are at 305 nm and 415 nm in pure solutions of deionized water (Liu et al., 2000). Therefore, the settings were arranged in order to decrease as much as possible the interference of the gelatin on the signal of phenanthrene. These settings were proven the best ones for achieving a reproducible quantification of phenanthrene in this kind of gel. The lowest fluorescence intensity was observed for the agar 1.5% (w/v) + gellan 0.5% (w/v). In this case the instrument settings were the same as in the analysis of the agar 2% (w/v). The mixture of agar and gellan has different optical properties than pure agar. It is opaque and less transparent, therefore the emitted fluorescence radiation is partially scattered inside the matrix and a lower intensity is observed in the spectra. However this arrangement of the instrument was enough to obtain reproducible results and therefore there was no need to change the instrument settings. In conclusion, this calibration curves are all the result of several steps of optimization in the gel preparation and in the analytical procedures and proves that this quantification of phenanthrene in the gels could be used for mass balance measurements during the following experimental part of the work.

3.2 Quantification of the organic carbon fraction of the polysaccharide gels

The calibration for the measurement of the inorganic carbon and the total carbon concentrations have been carried out for the following gels: agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v). The reference maximum standard deviation for each point was equal to 2% of each average value. For the inorganic carbon the limit of detection is 4 mg/L and the limit of quantification is 14 mg/L. For the total carbon the limit of detection was 7.6 mg/L and the limit of quantification is 25 mg/L. From the inorganic carbon and the total carbon content quantification, the organic carbon fraction of each gel was calculated. The organic carbon fraction (%) of each gel is presented in Table 19. The organic carbon fraction is a very important parameter that is exploited for measuring the partitioning of organic substances between different environmental compartments; it is used to calculate the organic carbon partition coefficient (K_{oc}) of several sorbents such as soil, sludge, sediments and biofilms in regard to the sorption of organic pollutants in aqueous and gaseous phases. Therefore, these results are considered a fundamental achievement of the work.

Table 19 The organic carbon fraction of the gel mixtures used during the experiments.

Gel mixture	Organic carbon fraction (%)
Agar 2% (w/v)	48 ± 4.6
Agra 1.5% (w/v) + gellan 0.5% (w/v)	52 ± 4.8
Agar 1.5% (w/v) + gelatin 0.5% (w/v)	40 ± 5.0

3.3 Sorption experiments with polysaccharide gels

This part of the work was aimed to model the memory of sewer biofilms for PAHs using polysaccharide hydrogels as biofilm surrogates. The sorption of hydrophobic pollutants into microbial biofilms influences greatly their fate and distribution in the environment. The absorption and the desorption of pollutant into and from the biofilm layer are processes which are not easy to investigate using real samples, therefore it is important to model them using surrogate matrices more easy to handle. At first it was necessary to estimate the partition coefficients and the diffusion coefficients of phenanthrene in the polysaccharide gels. Then the Fick's laws of diffusion were mathematically exploited using the parameters calculated and a predictive model of the gel memory was created. The partition coefficients and the diffusivity are two essential

parameters without which the diffusion modeling of a solute between two immiscible phases cannot be investigated properly. The last part of the work was focused on testing the actual desorption kinetics of phenanthrene from a polysaccharide gel layer and the comparison between the predictive model and the experimental data was finally carried out. The study of the partitioning coefficients, such as organic-carbon coefficient (K_{oc}), distribution coefficient (K_d) and gel-water coefficient (K_{gw}), of phenanthrene between the deionized water phase and the gel phase was a really important part of this modeling investigation. Both absorption and desorption partitioning coefficients were measured. The partitioning experiments were performed under static conditions. The static conditions were considered more suitable conditions for the practical arrangement of the experiments than mixing conditions. Furthermore no significant differences were observed applying mixing conditions to the system. In this regard, for agar 2% (w/v) under mixing conditions the equilibrium of the partitioning was reached after 4 days for both absorption and desorption. The values of Log K_{oc} were 2.03±0.3 and 2.02 ±0.3 for the absorption and desorption respectively. The values of K_d were 1.5 ±0.2 and 1.2 ±0.2 for desorption and absorption respectively. The values of partition coefficients at the equilibrium, obtained under these mixing conditions were equivalent to the values obtained under static conditions. Since the aim of these first experiments was to estimate the equilibrium partition coefficients and since no significant differences were observed to the values obtained under static conditions, all the other gels were tested under static conditions and the mixing was not further applied for this part of the work. Only a gentle shaking on the quartz glass cuvette was performed before each measurement. The gels used were mainly composed of agar. An addition of gellan and gelatin was performed separately to the gel in order investigate possible sorption effects. Agar gels have been previously used as surrogates for studying the diffusion of organic chemicals in microbial biofilms (Jouenne et al., 1994, Tresse et al., 1995, Westrin and Axelsson, 1991). Agar (Figure 24) consists of two fractions, agarose and agaropectin (Armisen and Galatas, 1987). Both residues are repeated alternately (Armisen and Galatas, 1987). Agarose and agaropectine togheter form a neutral hydrocolloid presenting polar moieties.

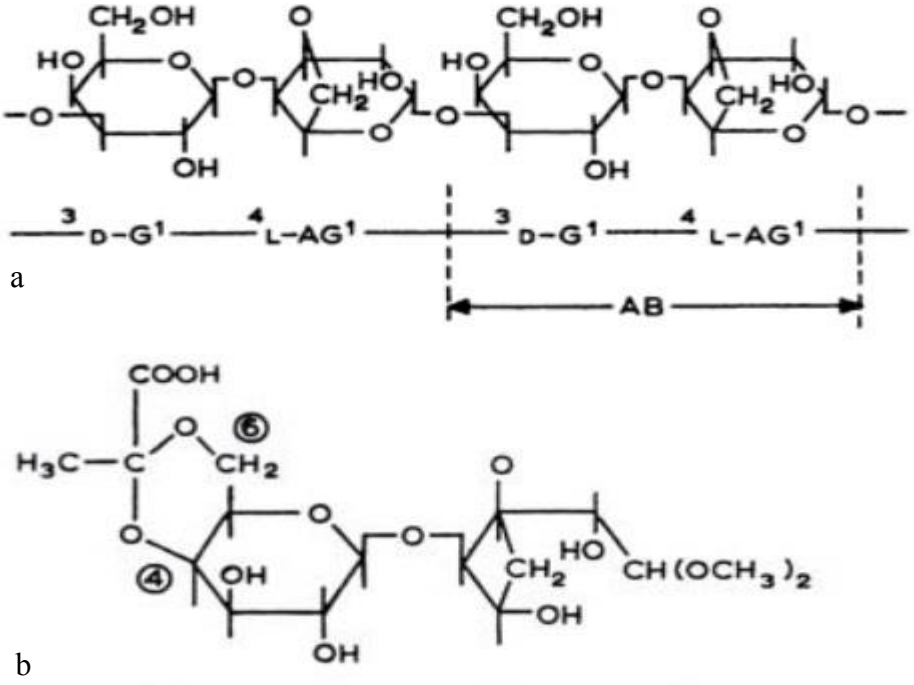


Figure 24 Agar, agarobiose and agaropectine.a) basic repeating unit of agar D-G = β-D-galactopyranose, L-AG = 3,6-anhydro-α-L-galactopyranose, AB = agarobiose. b) one of the compositional units of agaropectine. O-4,4-O-(1-carboxyethylidene)-(1→4) – 3,6-anhydro-L-galactose dimethylacetal(Dumitriu, 2012).

Gellan (Figure 25) is one of the most common polysaccharides found in microbial biofilms EPS (extra polymeric substances) and used in the food industry (Vu et al., 2009). In particular the gellan Gelrite (from Carl Roth GmbH) used in this study is a linear polysaccharide comprising glucuronic acid, glucose, rhamnose, and O-acetyl moieties. Gellan is a less polar matrix than agar and therefore it is expected to increase the sorption capacity of the agar gel for phenanthrene (Giavasis et al., 2000).

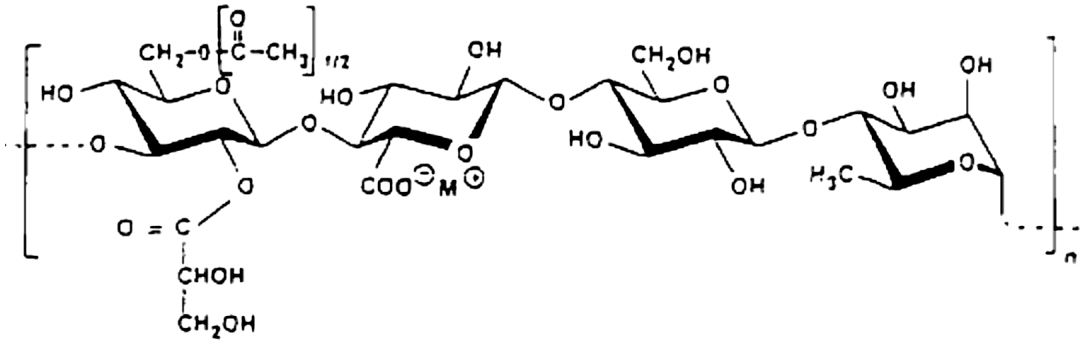


Figure 25 Chemical structure of Gellan (Giavasis et al., 2000).

Gelatin (Figure 26) is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen. Gelatin was used to investigate how the hydrophobic portion of the gel influences the sorption and the partitioning of PAHs from the water phase. It is a water soluble proteinaceous substance prepared by processes, which involve the destruction of the tertiary, secondary and to some extent the primary structure of native collagens (Fernandez-Diaz et al., 2001), specifically by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of animals (Mariod and Adam, 2013). The Type A gelatin, derived from porcine skin (as it is in this study), is prepared by acid processes. The amino acid composition particularly with respect to proline and hydroxyproline can vary from species to species but about 31% of the total content of amino acids is composed by nonpolar amino acids, such as alanine, glycine, valine, leucine, isoleucine, proline, phenylalanine, methionine (Easton, 1955).

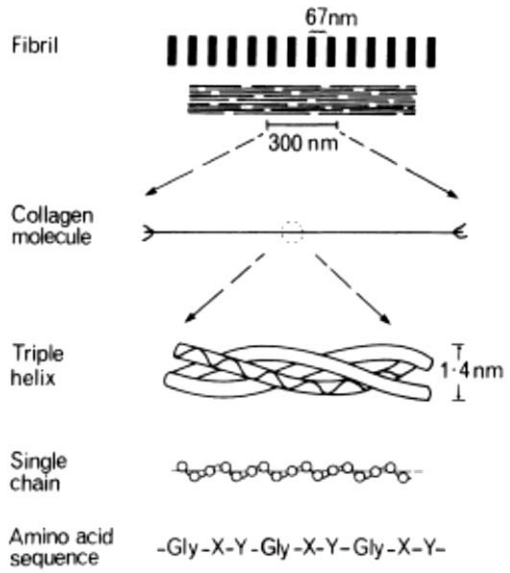


Figure 26 The structure of collagen molecules at different levels. From the top to the bottom : the typical striation of 67 nm of the collagen fibrils in native tissues, observed by electron microscopy ; the collagen rod triple helix ; the composition of a single chain, revealing the repetition of the sequence -(Gly-X-Y)-, X and Y being different aminoacids (Djabourov et al., 1988).

3.3.1 Absorption partitioning of phenanthrene between gel and water 1:1 (v/v)

The aim of this experiment was to evaluate the absorption partitioning of phenanthrene between the deionized water solution and different polysaccharide gel mixtures, which were taken as surrogates for microbial biofilms. The hypothesis is that the partitioning process between the two phases is mostly influenced by the water solubility of phenanthrene, which is expected to be similar to the solubility in the gel. Furthermore the gellan and the gelatin were added to the agar gels in order to evaluate their influence on the partitioning of phenanthrene between gel and

water. This partitioning test was carried out under static conditions and with a volume ratio equal to 1 between the gel and the water volumes. The volume ratio was chosen according to the analytical procedure optimized in this work. The two phases, gel and water, were placed inside a quartz glass cuvette of 3.5 mL capacity. In order to have enough surface exposed to the UV-light beam, the inner space of the cuvette was divided in two equal volumes occupied by each phase. This arrangement of the experiments was chosen, since the partitioning process does not depend on the volume ratio between two immiscible phases where a solute diffuses.

The agar 1.5% (w/v) and the agar 2% (w/v) showed similar results (Table 21). The equilibrium occurred after 7 days and the gel reached 0.1 mg/L of phenanthrene during the first 24 hours (Table 21). Agar 1.5% (w/v) + gelatin 0.5% (w/v) displayed a faster enrichment in phenanthrene compared with the other gels. The absorption equilibrium occurred after 7 days reaching a phenanthrene concentration of 0.2 mg/L in the gel during the first 24 hours.

Also the agar 1.5% (w/v) + gellan 0.5% (w/v) showed higher values than the pure agar 1.5% (w/v) and agar 2% (w/v), but lower uptake of phenanthrene in the first 24 hours than the agar 1.5% + gelatin 0.5% (Table 21). The concentration of the phenanthrene in water phase was measured and the decay rates were measured for each gel and are presented in Table 20. The faster absorption was observed using agar 1.5% (w/v) + gelatin 0.5% (w/v), with a value equal to 0.6 ±0.07 day^{-1}, while the slowest absorption process was observed with agar 1.5% (w/v) equal to 0.32 ±0.03 day^{-1}.

Table 20 Sorption parameters. 5 parameters have been chosen in order to compare the results obtained by using different gel solutions. The gel concentration of phenanthrene after 24 hours and at at the equilibrium, the time to the equilibrium, the distribution ratio and the rate of the absorption curve.

Gel matrix	PHE in gel after 24 h (mg/L)	PHE in gel at the equilibrium (mg/L)	Time to the equilibrium (days)	Distribution coefficient (K_d)	Decay constant (day^{-1})
agar 1.5% (w/v)	0.1±0.02	0.75±0.1	7	1.02±0.2	0.32±0.03
agar 2% (w/v)	0.1±0.04	0.62±0.2	7	1.02±0.5	0.42±0.07
agar 1.5% (w/v) + gellan 0.5% (w/v)	0.14±0.02	0.75±0.01	9	1.56±0.3	0.51±0.05
agar 1.5% (w/v) + gelatin 0.5% (w/v)	0.19±0.08	0.61±0.03	7	1.35±0.2	0.60±0.07

The distribution coefficient (K_d) of phenanthrene at the equilibrium was similar for agar 1.5% (w/v) and agar 2% (w/v) with values ranging from 0.9 to 1.0 (Table 21 and Figure 27). Agar

1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v) showed higher values equal to 1.5 and 1.3 respectively (Figure 27).

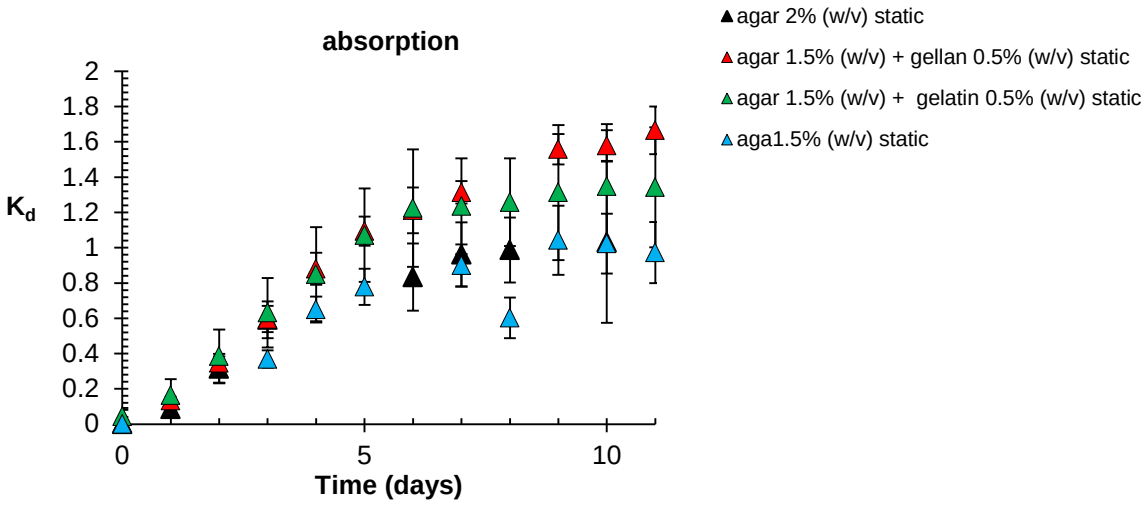


Figure 27 Distribution coefficient (K_d) values for each gel solution. Each value was calculated by the ratio between the concentration of phenanthrene into the water phase (mg/L) and the concentration of phenanthrene into the gel solution (mg/L).

For each experiment was performed a mass balance measurement of the phenanthrene concentration was performed in order to calculate the distribution coefficients and the partition coefficients. An example of this mass balance measurement is presented for the agar 1.5% (w/v) (Figure 28).

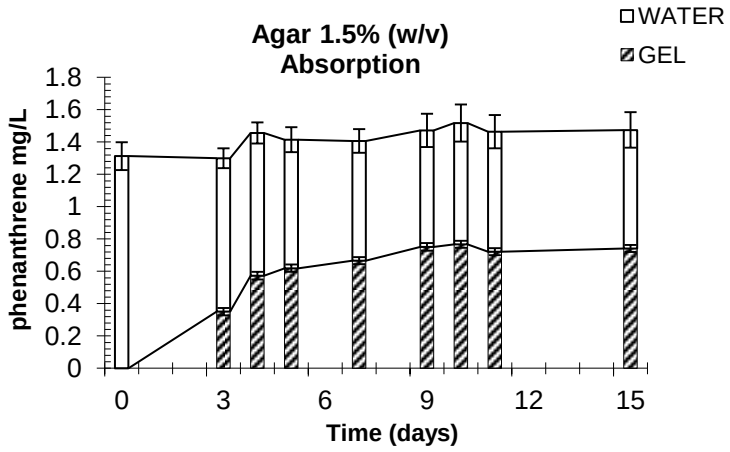


Figure 28 Representation of mass balance for agar 1.5% (w/v) under static conditions. The phenanthrene concentration decreased in water (white bars) and increased in the gel matrix (striped columns) during the absorption process and the equilibrium was reached after 6 days. The gel and the phenanthrene water solutions were dosed inside the quartz cuvette with a volume ratio of 1:1.

The organic carbon partition coefficients of phenanthrene was measured for agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v) according to Wicke et al., 2007. The agar 1.5% (w/v) displayed equal sorption properties to agar 2% and therefore was

not considered for this analysis. The results are shown in Table 22. Not significant difference was observed between the different gels with respect to the Log K_{oc}. All the values of Log Koc are equal to 2.3 (Table 21). The gel water partitioning coefficient (Log K_{gw}) is shown in Table 4. The highest Log K_{gw} value is obtained with Agar 1.5% (w/v) + Gelatin 0.5% (w/v), equal to 2.05 L/kg and the lowest with Agar 2% (w/v), equal to 1.86 L/kg. The differences between the gels used was more remarked by the K_{gw} and by the distribution coefficient K_d, than by the organic carbon partition coefficient (K_{oc}). In fact the calculation of the first two coefficients relates the loss of phenanthrene in the water phase to the dry weight and the volume of the sorbent phases, which are equal for all the different gels. Instead the organic carbon content differs from one gel to the other and the final partitioning coefficient is balanced within this differences. Through these experiments it was possible to estimate the absorption partitioning coefficients needed for editing the sorption model that will be presented later in this section (Results, page 96). All the experiments were carried out using a novel analytical procedure of fluorescence spectroscopy.

Table 21 Organic carbon partition coefficients. The value of each partition coefficient is reported as the Logarithm. The literatre references are shown in the left column.

Matrices	Log K_{oc} (L/Kg)	Log K_{gw} (L/Kg)
Agar 2% w/v (static, n=3)	2.27 ±0.7	1.86 ±0.4
Agar 1.5% w/v + Gellan 0.5% w/v (absorption,static, n=3)	2.3±0.2	1.91 ±0.6
Agar 1.5% w/v+ Gelatin 0.5% w/v(absorption,static, n=3)	2.3±0.1	2.05 ±0.4

3.3.2 Desorption partitioning of phenanthrene between gel and water 1:1 (v/v)

Once the equilibrium of the absorption partitioning was reached, the water phase above the gel was removed and replaced with the same volume of new deionized water solution. Then the quartz glass cuvette was sealed and the desorption partitioning was investigated. This experiment was performed in order to calculate the partitioning coefficients at the desorption equilibrium of phenanthrene from the gels to the water phase. The experiment was carried out under static conditions and with a volume ratio of 1:1. The desorption partitioning of phenanthrene between the water phase and the gel was investigated under the same conditions of the absorption experiments. In this way the results obtained from the two partitioning processes could be properly compared. Two desorption steps were performed for each gel matrix. Similar results were achieved for the investigation of agar 1.5% (w/v) and agar 2% (w/v). The concentration of phenanthrene in water was equal to 0.3 mg/L after 24 hours and 0.4 mg/L at the equilibrium, after

7 days, with a distribution coefficient (K_d) equal to 0.9-1.0. Higher values of distribution coefficient of 1.8 and 1.5 were observed using the agar 1.5% (w/v) + gellan 0.5% (w/v) and the agar 1.5% (w/v) + gelatin 0.5% (w/v) (Table 22). For the second step of desorption the distribution coefficient was 0.6 and 0.7 for the agar 2% and agar 1.5% respectively, but it was stable for the other two hydrogels, agar 1.5% + gellan 0.5% and agar 1.5% + gelatin 0.5% with values equal to 1.8 and 1.6 respectively. Also in the second step of desorption the highest retention of phenanthrene was observed for the agar 1.5% + gellan 0.5% and the lowest retention was observed with the agar 2% (Table 23). The concentration of the phenanthrene in the gel was determined and the resulting rate constants for each gel are presented in Table 22 and Table 23. The fastest desorption was observed using agar 1.5% (w/v) with a value equal to 0.66±0.07 day^{-1}, while the slowest desorption process was observed with agar 1.5% (w/v) + gelatin 0.5% (w/v) equal to 0.28 ±0.03 day^{-1}. For the second desorption step, no significant differences were observed in the decay constant values for each gel. The higher decay constant value was observed for agar 2% (w/v) equal to 1.0 ±0.2 day^{-1} and the lowest value was observed using agar 1.5% (w/v) + gelatin 0.5% (w/v) equal to 0.28 ±0.03 day^{-1}.

Table 22 First step of desorption. concentration of phenanthrene in water (mg/L), the equilibrium time, the decay constants of desorption ($days^{-1}$), the distribution coefficient ($K_d = C_w/C_g$) and the partition coefficient (K_{gw}) of phenanthrene between the water and the gel phase are presented here.

Hydrogel	PHE in water after 24 h (mg/L)	PHE in water at the equilibrium (mg/L)	Time to the equilibrium (days)	Distribution (K_d)	decay (day^{-1})
agar 1.5% (w/v)	0.3±0.05	0.4±0.1	7	1.0±0.3	0.66±0.13
agar 2% (w/v)	0.3±0.04	0.4±0.05	7	0.9±0.3	0.64±0.16
agar 1.5% (w/v) + gellan 0.5% (w/v)	0.2±0.007	0.3±0.008	4	1.8±0.3	0.63±0.14
agar 1.5% (w/v) + gelatin 0.5% (w/v)	0.16±0.02	0.25±0.003	4	1.5±0.2	0.28±0.01

Table 23 Second step of desorption. concentration of phenanthrene in water (mg/L), the equilibrium time, the decay constants of desorption (days-1), the distribution coefficient (Kd= C_w/C_g) and the partition coefficient (Kgw) of phenanthrene between the water and the gel phase are presented here.

Hydrogel	PHE in water at 24 h (mg/L)	PHE in water at equilibrium (mg/L)	Time to the equilibrium (days)	Distribution (K_d)	decay (day^{-1})
agar 1.5% (w/v)	0.21±0.03	0.25±0.03	6	0.7±0.4	0.72±0.04
agar 2% (w/v)	0.23±0.04	0.24±0.02	4	0.6±0.5	1.09±0.06
agar 1.5% (w/v) + gellan 0.5% (w/v)	0.12±0.006	0.17±0.002	7	1.8±0.1	0.6±0.03
agar 1.5% (w/v) + gelatin 0.5% (w/v)	0.1±0.001	0.1±0.01	6	1.6±0.2	0.28±0.06

As previously done for the absorption partitioning, also for the desorption experiments was performed the mass balance measurement for locating phenanthrene along both phases: gel and water. An example is provided from Figure 29.

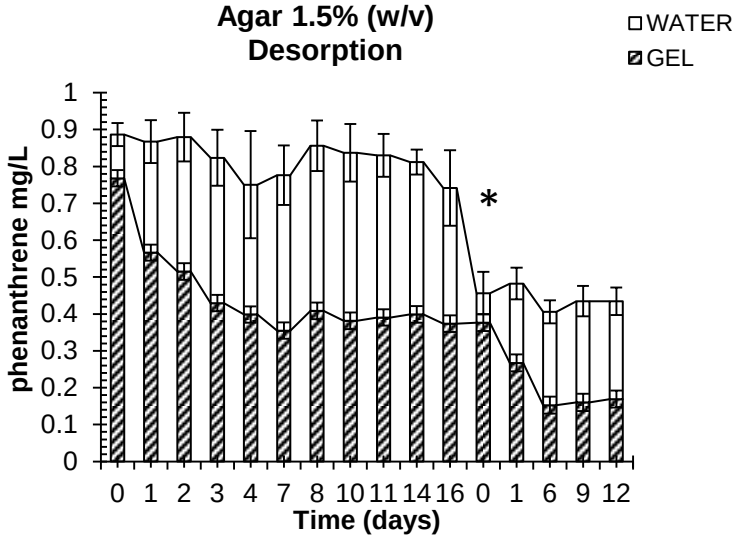


Figure 29 Representation of mass balance for agar 1.5% (w/v) under static conditions. The phenanthrene concentration decreased in the gel (striped bars) and increased in the water phase above (white bars) during the desorption process. The equilibrium of the first and the second desorption steps was reached after 7 days. The star icon (*) shows the beginning of the second desorption phase, when the water solution enriched in phenanthrene was replaced with a new deionized water solution. The gel and the water solution were dosed inside the quartz cuvette with a volume ratio 1:1.

The organic carbon partitioning coefficients (K_{oc}) were calculated for the desorption of phenanthrene from the gel and no significant differences were observed with the absorption organic carbon partitioning of phenanthrene between the gel and the water phase (Table 24). Considering both desorption steps, phenanthrene displays the lowest Log K_{oc} when exposed the agar 1.5% (w/v) + gelatin 0.5% (w/v), equal to 2.0 ±0.1 and 2.0 ±0.2 for the first and the second desorption respectively.

Table 24 organic carbon partition coefficients. The values of the main polysaccharide gels used for the study are presented as LogKoc for the first and the second step of desorption.

Matrices	Log K_{oc}(L/kg) Desorption I	Log K_{oc}(L/kg) Desorption II	Log K_{gw}(L/kg) Desorption I	Log K_{gw}(L/kg) Desorption II
Agar 2% w/v (n=3)	1.9 ±0.7	1.9 ± 0. 6	1.59±0.8	1.60 ±0.5
Agar 1.5% w/v + Gellan 0.5% w/v (n=3)	2±0.2	1.9± 0. 5	1.50±0.7	1.80±0.6
Agar 1.5% w/v + Gelatin 0.5% w/v(n=3)	2±0.1	2.0± 0.2	1.82±0.5	2.05±0.5

These values are important data for editing a predictive sorption model of phenanthrene into EPS layers, which contain proteins and gellan-like polysaccharides. The observed difference in the partitioning of phenanthrene into the different gels was taken in consideration for the final model calculations, which involve the Fick's laws of diffusion. The novel self-designed analytical procedure, exploited here, it is the same as described for the absorption experiments. As expected, gellan and gelatin increase the affinity of the polysaccharide gel for phenanthrene compared with pure agar gels.

3.3.3 Absorption partitioning of phenanthrene between gel and water 1:100 (v/v).

Three gel mixtures were selected in order to perform a single trial partitioning experiment using a volume of 1.5mL of gel and a volume of 150 mL of deionized water solution of phenanthrene with a concentration of 1.2-1.3 mg/L. The lower volume ratio (1:100 instead of 1:1) was arranged in order to investigate the equilibrium partitioning of phenanthrene between water and gel phase, under a higher gradient of concentration. The hypothesis is that the equilibrium concentration of phenanthrene inside the gel is determined by the partitioning properties observed during the previous experiments. In fact, the partitioning of a solute across two immiscible phases is independent from the volume ratio between the two phases. Therefore the following partitioning coefficients should have same values observed previously. This experiment lasted 33 days and was performed under static conditions. The water phase was shaken by hand 30 seconds before each measurement. Static conditions were considered more suitable for the arrangement of the experiment. The absence of mixing force influences the kinetics but not the equilibrium partition coefficients, as previously proven. For each gel the equilibrium occurred after 7 days and the concentration inside the gel solution varied from 1.6 mg/L inside agar 2% (w/v) and agar 1.5% (w/v) + gellan 0.5% (w/v) to 1.40 mg/L inside agar 1.5% (w/v) + gelatin 0.5% (w/v) (Fig.30).

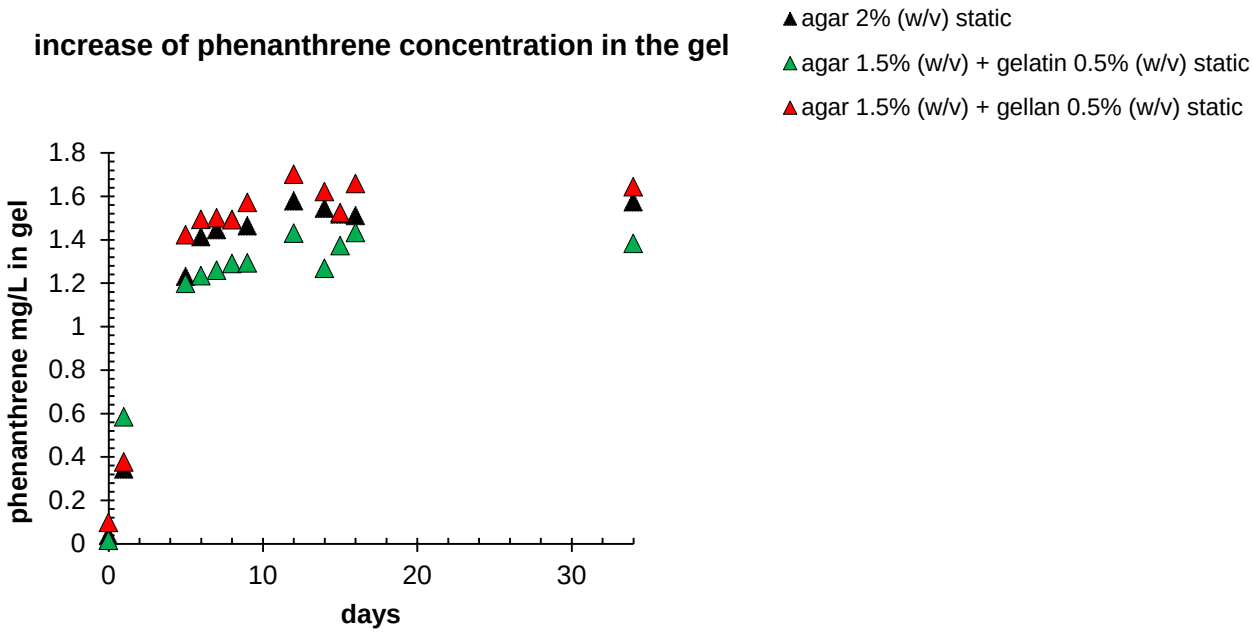


Figure 30 Absorption of phenanthrene in polysaccharide gels. The increase of phenanthrene concentration inside the gel solution (mg/L) is shown in the figure. Using a glass device manufactured at the University of Duisburg Essen, the gel and the phenanthrene solution were dosed inside the quartz cuvette with a volume ratio 1:100. All the experiments were performed under static conditions.

The concentration of phenanthrene inside the water phase was analyzed in parallel with the gel samples and a slight decrease of phenanthrene into the corresponding water phase was observed from 1.4 mg/L to 1.2 mg/L (Fig.31).

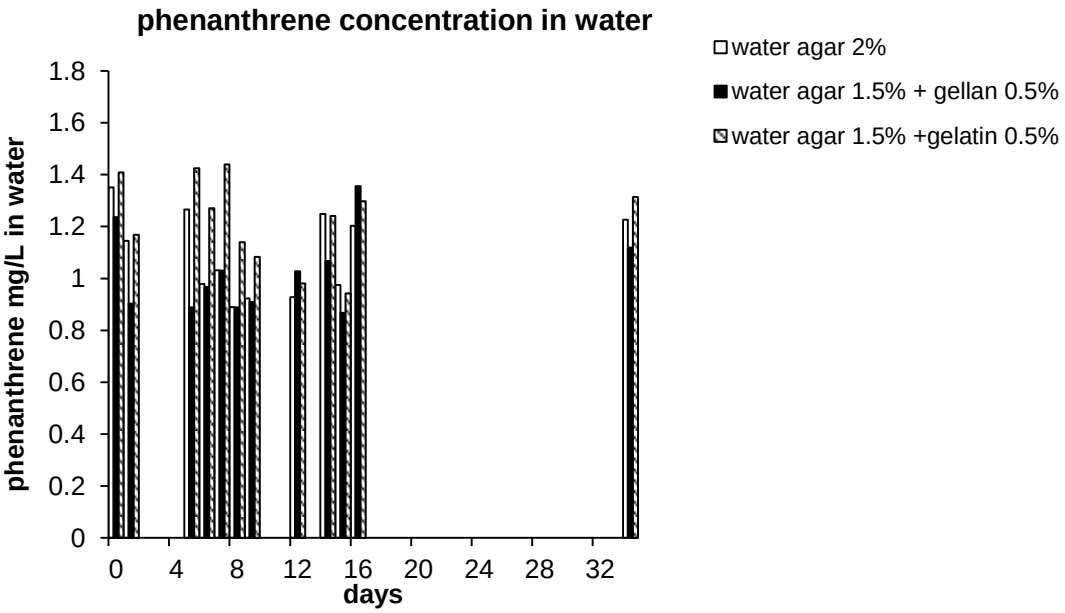


Figure 31 Adsorption of phenanthrene to the glass surface of the diffusivity device. The phenanthrene concentration inside the water solution (mg/L) is shown in the figure. Using a glass device manufactured at the University of Duisburg Essen, the gel and the phenanthrene solution were dosed inside the quartz cuvette with a volume ratio 1:100. All the experiments were performed under static conditions.

At the equilibrium the agar 1.5% (w/v) + gelatin 0.5% (w/v) displayed the lower values of distribution coefficient, ranging from 1.45 to 1.1, while the agar 2% (w/v) and the agar 1.5% (w/v) + gellan 0.5% (w/v) displayed higher values equal to 1.7-1.2 and 1.65-1.59 respectively (Fig.32).

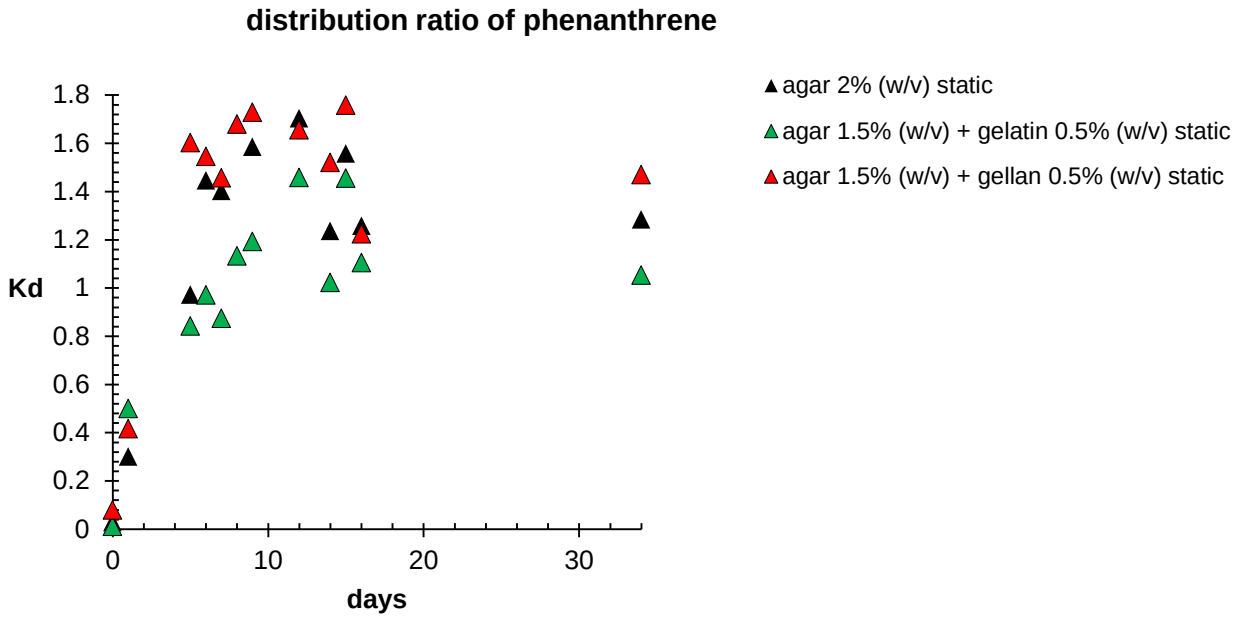


Figure 32 Distribution coefficients. During the absorption process, the distribution of phenanthrene between the two phases was evaluated through the distributon coefficient, the ratio between the concentration of phenanthrene in gel and in water. The values of distribution ratio for each model gel are shown in the figure. Using a glass device manufactured at the University of Duisburg Essen, the gel and the phenanthrene solution were dosed inside the quartz cuvette with a volume ratio 1:100. All the experiments were performed under static conditions.

The organic carbon partition coefficient (K_{oc}) was calculated for these experiment and the values observed for each gel mixture are similar to the values obtained previously with a volume ratio of 1:1 between the gel and the water phase (Table 25)

Table 25 Organic carbon partition coefficients of phenanthrene between water and gel during the experiment under 1:100 (v/v) volume ratio.

Matrices	Log K_{oc} (L/kg)	Log K_{gw} (L/kg)
Agar 2% w/v (static, n=3)	2.19	1.79
Agar 1.5% w/v + Gellan 0.5% w/v (absorption,static, n=3)	2.21	1.78
Agar 1.5% w/v+Gelatin 0.5% w/v(absorption,static, n=3)	2.15	1.76

The partitioning of phenanthrene does not change significantly with the decrease of the volume ratio between water and gel. Under such conditions, no significant difference was observed between the different gels. The solubility of phenanthrene in the gel matrix seems to be higher

than the water solubility, which is between 1.3 mg/L and 1.5 mg/L. The results obtained here replicate the results observed during the previous partitioning experiments and this is an important result that confirm the validity of the experimental procedure. In conclusion the absorption partition coefficients of phenanthrene between water and gel have been defined and confirmed.

3.3.4 Desorption partitioning of phenanthrene between gel and water 1:100 (v/v)

This experiment was performed in order to study the partitioning of phenanthrene between water and gel under a lower volume ratio between the gel and the water (1:100 instead of 1:1). The results obtained from the desorption experiments show that under these conditions all the matrices seem to display similar desorption properties for phenanthrene. The equilibrium is reached for all the gels after 9 days with a phenanthrene concentration of 0.18 mg/L using the agar 1.5% (w/v) + gellan 0.5% (w/v), and 0.10 mg/L for both agar 2% (w/v) and agar 1.5% (w/v)+gelatin 0.5% (w/v) (Fig.12). The decrease of phenanthrene in the gel was analyzed and a decay constant was calculated for each gel mixture. The values are 0.26, 0.30 and 0.31 day^{-1} for agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v) respectively. The phenanthrene equilibrium concentration in the mixture agar/gellan reached 0.2 mg/L, while in the other gels was around 0.1-0.15 mg/L. Most of the amount of phenanthrene is desorbed in the water phase. (Figure 33).

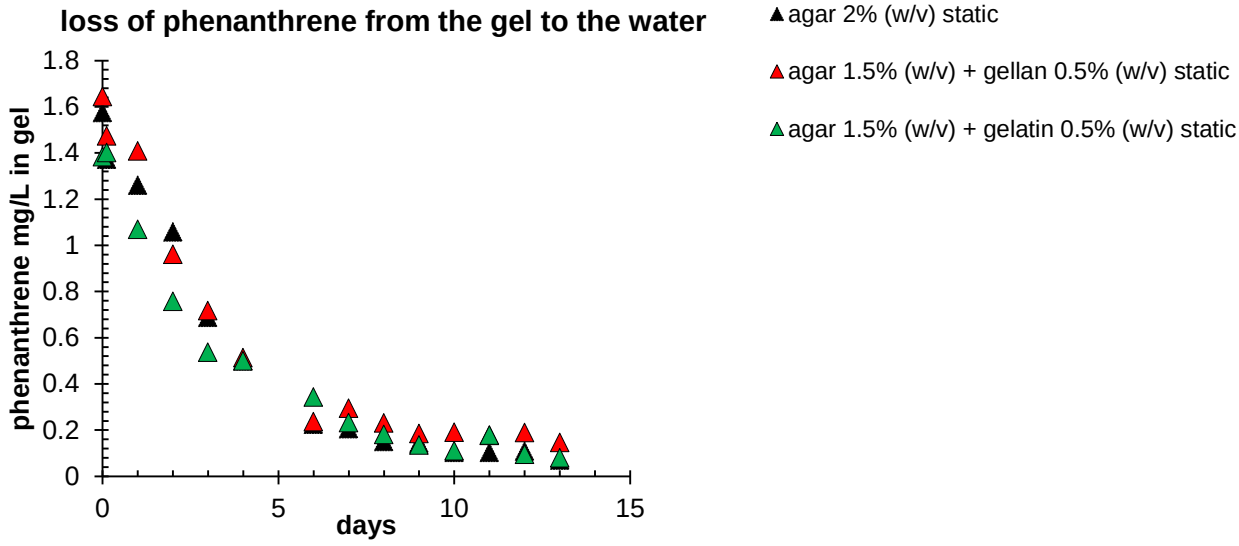


Figure 33 Desorption of phenanthrene from model gels. The decrease of phenanthrene concentration inside the gel solution (mg/L) is shown in the figure. Using a glass device manufactured at the University of Duisburg Essen, the gel and the phenanthrene solution were dosed inside the quartz cuvette with a volume ratio 1:100. All the experiments were performed under static conditions.

These results show that under conditions closed to practice, with higher gradient of concentration, the agar/gellan mixture displays higher retention of phenanthrene (about 0.2 mg/L) than the other gels (0.1-0.15 mg/L), in which the equilibrium concentration of phenanthrene was close to the limit of quantification.

3.3.5 Diffusivity of phenanthrene in polysaccharide gels

This experiment was carried out in order to calculate the diffusivity of phenanthrene through three gel mixtures: agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v). It is expected that the diffusivity of phenanthrene through a hydrogel matrix made of 98% of water is comparable to its diffusivity in water, which is equal to 6 $x10^{-6}$ cm^2/s. A volume of 2.3 ml of each gel solution was placed in a glass tube closed on both sides by glass vials containing two deionized water solutions, one spiked with phenanthrene on the right site and one with pure deionized water solution on the other side. Each one of volume 6.5mL. The experiment duration and the concentration of phenanthrene in both deionized water solutions are presented in Table 26.

Table 26 Diffusivity of phenanthrene in polysaccharide gels. The experimental parameters. Phenanthrene concentration in the right (R) and left (L) side of the device and the equilibrium time (days).

Gel	Days to equilibrium	R side at t_0 mg/L	R side 24h mg/L	R side at equilibrium mg/L	L side at equilibrium mg/L
Agar 2% (w/v)	34	1.5	1.2	0.5	0.30
Agar 1.5% (w/v) + gellan 0.5% (w/v)	28	1.7	1.07	0.5	0.26
Agar 1.5%(w/v) + gelatin 0.5% (w/v)	35	1.7	1.4	0.5	0.20

The highest diffusivity value was observed for agar 1.5% (w/v) + gellan 0.5% (w/v) equal to 8 x 10^{-6} cm^2/s, while the lowest diffusivity was observed for agar 1.5% (w/v) + gelatin 0.5% (w/v) equal to 4 x 10^{-6} cm^2/s. Agar 2% (w/v) displayed a diffusivity equal to 5 x 10^{-6} cm^2/s . These diffusivity values are similar to the diffusivity of phenanthrene in water, that is equal to 6 $x10^{-6}$ cm^2/s (Table 27).

Table 27 Diffusion coefficients of phenanthrene calculated for each one of gel matrices tested. The diffusivity of phenanthrene in water was taken from literature references (EPA 1989, Gustafson and Dickhut 1994, Say Kee Ong, Natural Processes and Systems for Hazardous Waste Treatment, ASCE Publications, 2008).

Sorbent matrix	Diffusivity
Agar 2% (w/v)	(5.3± 0.8) x10^{-6} cm²/s
Agar 1.5% (w/v) + gellan 0.5 (w/v)%	(8.0± 0.7) x10^{-6} cm²/s
Agar 1.5% (w/v) + gelatin 0.5% (w/v)	(4.0± 0.7) x10^{-6} cm²/s

A control experiment was performed using the same glass device and filling it with only a deionized water solution of phenanthrene at 1.4 mg/L of concentration. No significant loss of phenanthrene was observed during 24 days of time (Figure 34). Therefore, the quantification of phenanthrene during the sorption experiment was not affected by any significant adsorption of phenanthrene to the glass surface of the device and by the evaporation in the outer gas phase.

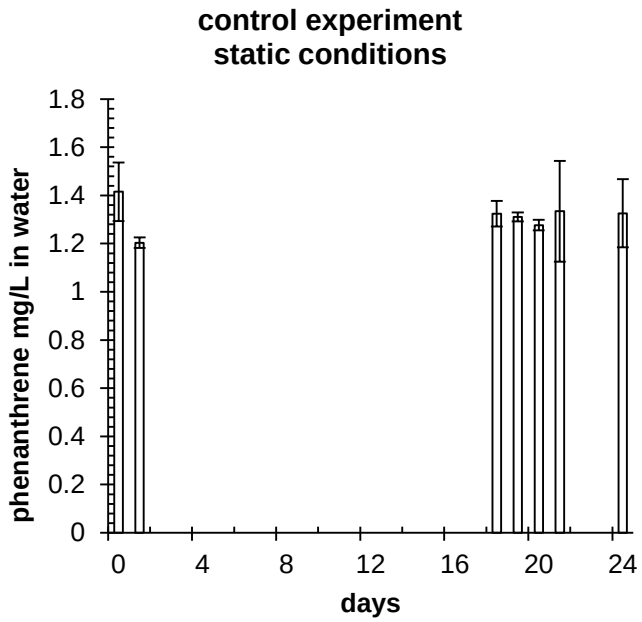


Figure 34 The phenanthrene concentration in deionized water (mg/L) during the control experiment is shown in the figure. A deionized water solution of phenanthrene was poured inside the glass device and closed air tightly. Not significant removal of phenanthrene was observed during 24 days.

From these results it was possible to observe that the diffusivity of phenanthrene in the polysaccharide gels exploited here as biofilm surrogates is similar but not equivalent to its water diffusivity. A polysaccharide gel is a hydrated phase mainly composed of so called bulk water. This kind of water is free to move and is not bound to the matrix. Microbial biofilms are composed both of free water, also called bulk water and bound water (Vogt et al., 2000). The

self-diffusivity of bulk water is similar to the diffusivity of pure water while the bulk water displays lower mobility (Vogt et al., 2000). In case of hydrogels, it is reasonable to assume that the transport of organic compounds inside hydrogel matrices is mainly driven by the diffusion of these compounds in water and therefore their diffusivity inside such matrices is similar to their diffusivity in pure water. From these results is confirmed that the diffusivity of phenanthrene in polysaccharide gels is close to its diffusivity in pure water. In particular for agar 1.5% (w/v) + gellan 0.5% (w/v) it is 3 x 10^{-6} cm^2/s higher than in water. In pure agar 2% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v) is lower than in water of 1 and 2 x 10^{-6} cm^2/sec respectively.

3.3.6 Sorption kinetics of phenanthrene between water and polysaccharide gels

After the calculation of the equilibrium partitioning coefficients (pages 82, 84-87, 90) and the diffusivity of phenanthrene (page 93) in polysaccharide gels, it was finally possible to perform the kinetic modeling of the gel memory for PAH compounds. A desorption experiment was carried out in order to evaluate the memory of a surrogate biofilm for phenanthrene under a continuous flow of deionized water. In parallel a predictive desorption curve was calculated using the partition and the diffusion coefficients, which were the object of the previous investigation. The comparison between the predictive model and the experimental results will validate the modeling procedure and all the mathematical assumptions formulated before. The agar/gellan mixture was used for this experiment. This sorbent matrix displayed more suitable optical properties, leading to a more reproducible detection of phenanthrene by front-face fluorescence spectroscopy. Furthermore, as previously said, is one of the matrices used for modelling the diffusion of organic compounds in biofilms and therefore its mimic potential turned itself in the best candidate for this experiment among the polysaccharide gels previously investigated in this study.

In real sewer conditions the flow rate of the wastewater can greatly vary in the time ranging from 1000 L/day (0.03 cm/min) to 30000 L/day (7.3 cm/min) day depending on the wastewater source and on the sewer pipe diameter (Boulos et al., 2006). Industrial effluents have higher flow rates than domestic discharges. In this case the relationship between the flow cell diameter and the flow rate was arranged proportionally to the real conditions of a sewer system which can vary the diameter from 300 mm to 1000mm. In this regard two different flow rate conditions were tested. A first test with a flow rate of 1L/min (flow velocity of 1300 cm/minute) was compared with a second experiment where the flow rate was 20 mL/min (flow velocity of 25.5 cm/minute). In

parallel to the experiment, also a theoretical model was calculated on the base of the diffusion coefficient and the distribution coefficient of phenanthrene in polysaccharide gels.

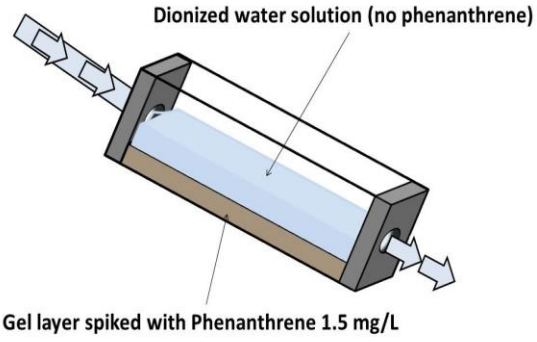
The hypothesis is that the sorption of phenanthrene onto the polysaccharide gels is influenced by the both water solubility of phenanthrene and by the distribution coefficient of phenanthrene between the gel and the water phase (K_d), and the sorption can be modeled using the Fick's law of diffusion, as follows:

$$F = D * A * \frac{(C_1 - C_2)}{x} \quad \text{(Eq.20)}$$

where F is the mass transfer (mg/s), D is the diffusion coefficient measured in this study (cm^2/sec), A is the surface area (cm^2), x is the thickness of the sorbent layer and C_1 and C_2 are the concentrations of the target molecules in the two different phases (mg/mL). The Equation 2 was used for simulating the desorption of phenanthrene from the gel layer during the exposure to a continuous flow of water, in which the phenanthrene concentration is constantly 0 mg/L:

$$F = D * A * \frac{\left(\frac{1}{K_d} C_g - C_w\right)}{x} \quad \text{(Eq.21)}$$

where K_d is the distribution coefficient calculated for the gel and presented in chapter 3.2.1, C_w is the water concentration of phenanthrene and C_g is the gel concentration of phenanthrene. For modeling the desorption, the concentration of phenanthrene in water was set as constantly at 0 mg/L. In this case the desorption distribution coefficient was used for modeling the desorption curve according to Equation 21. As it is possible to see in Figure 35b, the difference of flow velocities applied during the desorption experiment does not seem to influence significantly the kinetics of the process. The decay constant are similar each other, equal to 2.4 $h^{-1}$and 1.7 ± 0.8 h^{-1}



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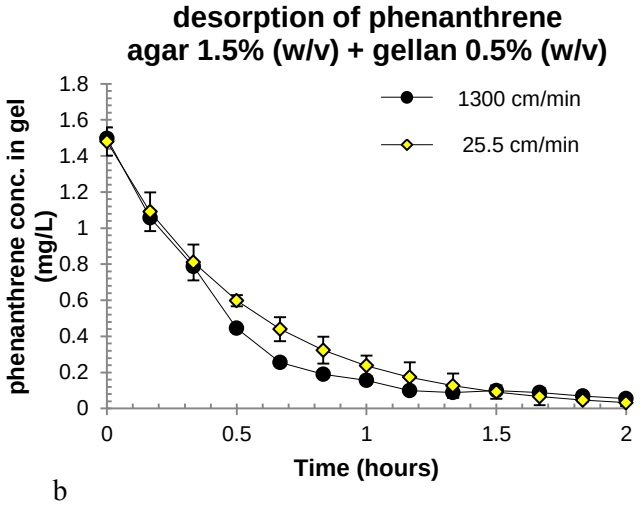

b

Figure 35 Desorption of phenanthrene under different flow rates. On the left (a), a sketch paint of the experimental system. A self-designed quartz glass cuvette was manufactured in order to measure the concentration of phenanthrene in gel during the desorption experiment. On the right (b),the observed gel concentration of phenanthrene under a flow velocity of 1300 cm/min (black dots) and under a flow velocity of 25.5 cm/min (yellow dots).

The desorption simulation curve obtained from Equation 21 matches well with the experimental results (Figure 36). The decay constants are equal to 1.65 h^{-1} for the simulation curve and 1.7 ± 0.8 h^{-1} for the desorption curve obtained from the experiment. The distribution coefficient and the diffusivity of phenanthrene in the gel, as previously mentioned, have a significant role in the process. In Figure 35 is also presented the desorption simulation curve obtained without considering the distribution coefficient in the calculations. This latter model curve is based only on the water diffusivity of phenanthrene and does not consider the desorption distribution coefficient that was estimated in this study. The decay constant of this simulation curve is equal to 3.9 h^{-1} that is significantly higher than the one obtained from the experiment. Other simulation curves for the desorption of phenanthrene from the gel layer have been investigated using the desorption patition coefficients obtained previously, but no significant correlation was found. Although the gel layer is composed of 98% of water, the water solubility of phenanthrene is not the only parameter influencing the desorption process.

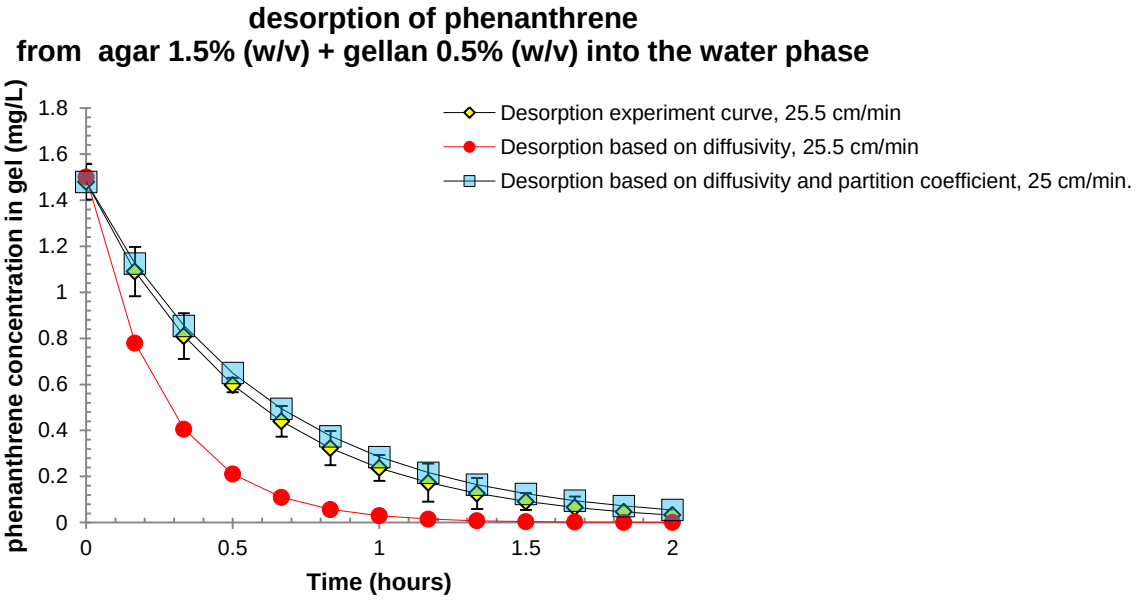

Figure 36 Sorption of phenanthrene from the surrogate biofilm in deionized water. The decrease of phenanthrene inside the gel matrix observed (yellow icons) and the simulated curve (transparent icons) calculated using both the distribution coefficient (K_d) and the diffusivity (D) values (green squares). In red is the simulation curve calculated using the only diffusivity (D) of phenanthrene into the gel mixture.

These data are the result of the experimental procedure that was used for modeling the gel memory for phenanthrene. All the equipment used for the experiments was self-designed and

manufactured during the present investigation. It was observed that the simulation curve based on the only diffusivity of phenanthrene in gels (red markers Figure 36), is not matching the experimental results. On the other hand the simulation curve that is based on both the diffusivity (D) in gel and the distribution coefficient (K_d) of phenanthrene between the water and the gel (blue markers Figure 36), coincides with the experimental data (yellow markers Figure 36). These results are important as they show that the partition coefficient of phenanthrene between the gel and the water phase it is a necessary parameter for predicting the desorption of phenanthrene from the gel layer under these experimental conditions. In addition these final results validate the new analytical procedure in this study and confirm all the mathematical modeling formulated. The memory of the polysaccharide gel for phenanthrene under conditions closed to the practice lasts about 2 hours. After this time, the concentration of phenanthrene in the gel is under the detection limit. When the polysaccharide gels are considered surrogates of microbial biofilms, the memory of these matrices for a nonpolar compound such as phenanthrene was found to be not significant for the establishment of a systematic monitoring of phenanthrene in sewers. The memory for phenanthrene is too short and alternative solutions are required.

In parallel with the establishment of a model for the sorption of phenanthrene in polysaccharide gels, it was attempted the invention of a novel analytical method for detecting phenanthrene into sewer biofilm suspensions by front-face fluorescence spectroscopy. This part of the work is presented below (Chapter 3.4) and it was aimed to provide a direct analytical method for in situ measurement of phenanthrene into sewers, as alternative choice to the sewer biofilms.

3.4 PDMS Device as pollutant sampler and analytical device

Since the steady state fluorescence spectroscopy is not suitable for detecting PAHs absorbed into sewer biofilms, it became necessary to find alternative and more suitable sorbent materials, which could be exploited by the same analytical method. It was necessary to find a UV transparent substance that could display significant accumulation of hydrophobic compounds such as PAHs and that could be analyzed by the analytical equipment designed and manufactured during this work. Polydimethylsiloxane (PDMS) displays these exact properties and therefore it was considered a suitable alternative to sewer biofilms and polysaccharide gels for the sorption and the detection of phenanthrene in sewers. The steady state fluorescence spectroscopy in front face mode is the analytical method of choice. In this part of the work, a prototype device was manufactured and exposed to aqueous solutions spiked with phenanthrene. During the

experiment, the device was removed from the solution and analyzed by front face fluorescence spectroscopy equipment. The aim of this preliminary experiment was to test the PDMS device in detecting phenanthrene both from deionized water and from a natural pond water solution. The hypothesis is that the steady state fluorescence spectroscopy is a suitable analytical method for the detection of phenanthrene in wastewater using the monitor device containing PDMS oil. At the end of these experiments the PDMS oil contained in the device and exposed to the contaminated aqueous solutions was analyzed through a standard front face fluorescence procedure using a bench spectrometer (RF-5301PC Shimadzu fluorescence spectrometer) and the resulting spectra were compared with a standard solution of phenanthrene at 1.5 mg/L (Fig.13). This comparison procedure was carried out in order to verify the presence of phenanthrene inside the PDMS oil. From the results (Figure 37) it seems that phenanthrene is present inside the PDMS oil after the exposure (24 hours) to the contaminated solutions. In the spectra of the PDMS oil the baseline is significantly higher than the baseline observed in the spectrum of the standard solution of phenanthrene. This might be due to interferences caused by specific components of the EPDM sealing frames, which are in contact with the oil and by other molecules present in the pond solution (e.g. humic susbtances).

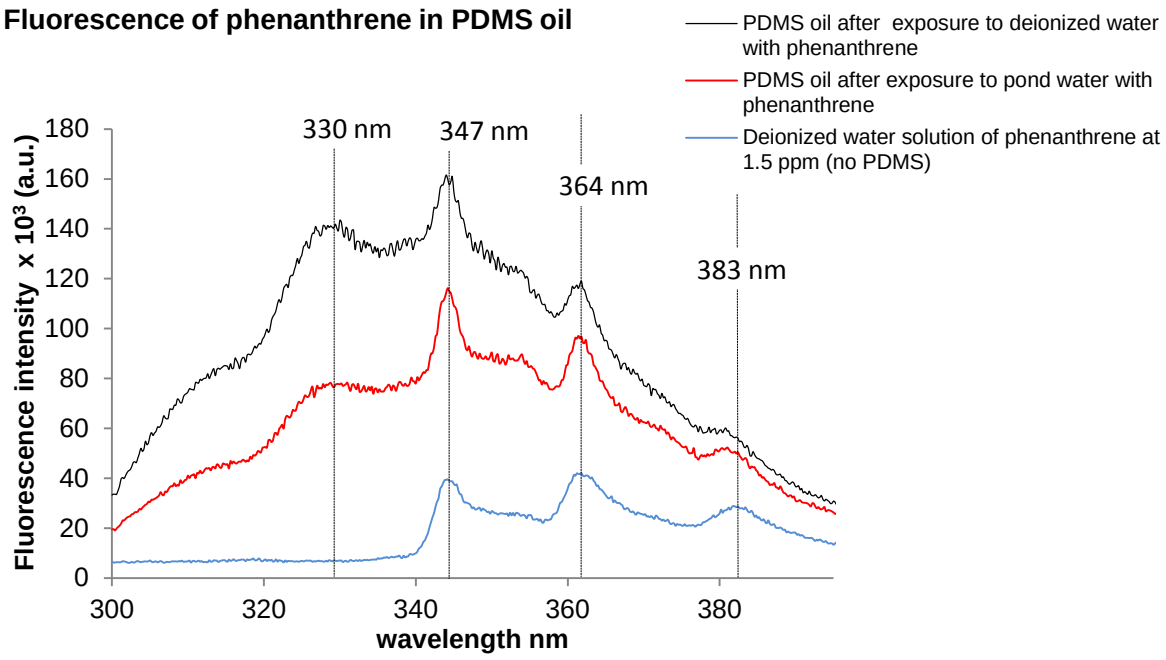

Figure 37 Phenanthrene fluorescence emission spectra obtained by standard procedure of fluorescence measurement in front face mode using a RF-5301PC Shimadzu fluorescence spectrometer. The spectrum of the phenanthrene standard (1.2ppm) is shown as a blue line, In the figure are shown also the PDMS oil spectra after the exposure to the pond water (red line) and to the deionized water (black line) spicked with phenanthrene. It is possible to identify the phenanthrene peaks in these latter spectra.

The analysis of the PDMS by standard procedure revealed the presence of phenanthrene, but the interference due to some component of the rubber sealing frames caused a decrease in the spectroscopic resolution (330 nm). As previously mentioned, the device was designed not only for the accumulation of phenanthrene into the PDMS oil, but also for performing front face measurements in order to identify phenanthrene using self-made analytical equipment. The comparison between the spectra obtained by using standard procedure (Figure 37) and the self-designed equipment (Figures 38, 39 and 40) was necessary in order to evaluate the efficiency of the device. In order to facilitate the comparison between the various fluorescence spectra, the peaks of intensity which were equal are marked by the green icon (ν) and the differences are highlighted by the red icon (x). For each experiment, in deionized water and in pond water, the device was analyzed before the exposure, after 1 hour and after 24 hours of exposure to the phenanthrene solution and to the blank solution of only deionized water. In Figure 17 are shown the PDMS spectra obtained during the experiment in pond water and in Figure 18 the spectra from the experiment in deionized water.

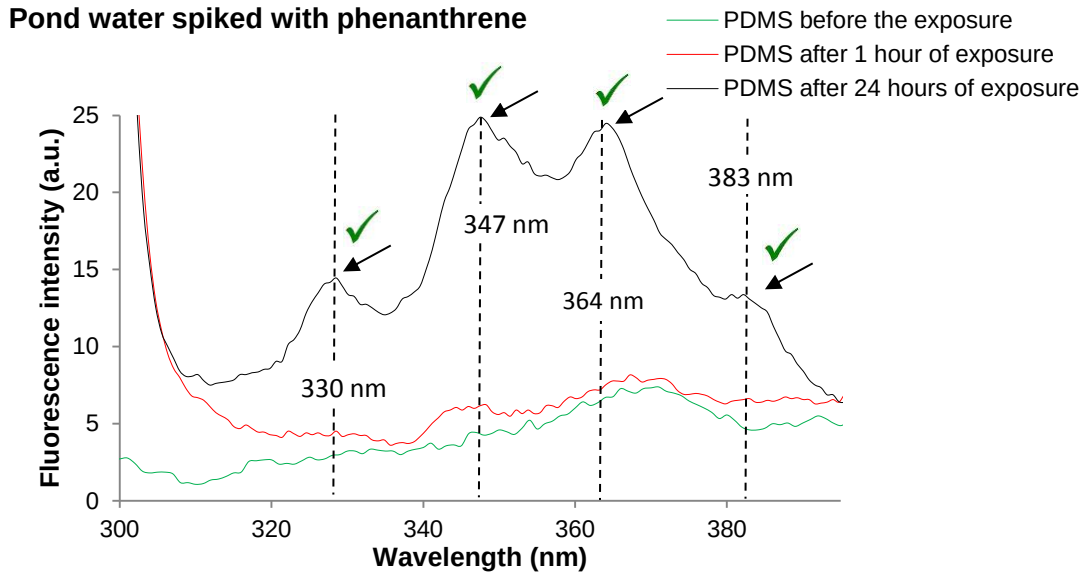

Figure 38 Fluorescence spectra of PDMS oil obtained exploiting the self-designed analytical equipment from the exposure of the device to the pond water spiked with phenanthrene. The green line is the spectrum before the exposure to phenanthrene; the red line is the spectrum after 1 hour of exposure and the black line is the spectrum after 24 hours of exposure. The dot lines indicate the four fluorescence peaks of the PDMS oil spectrum which were previously observed using the standard analytical procedure: 330 nm, 347 nm, 364 nm and 383 nm. The green icon (ν) indicate the corresponding peaks observed both in the the spectra obtained by standard and in those from the self-designed equipment.

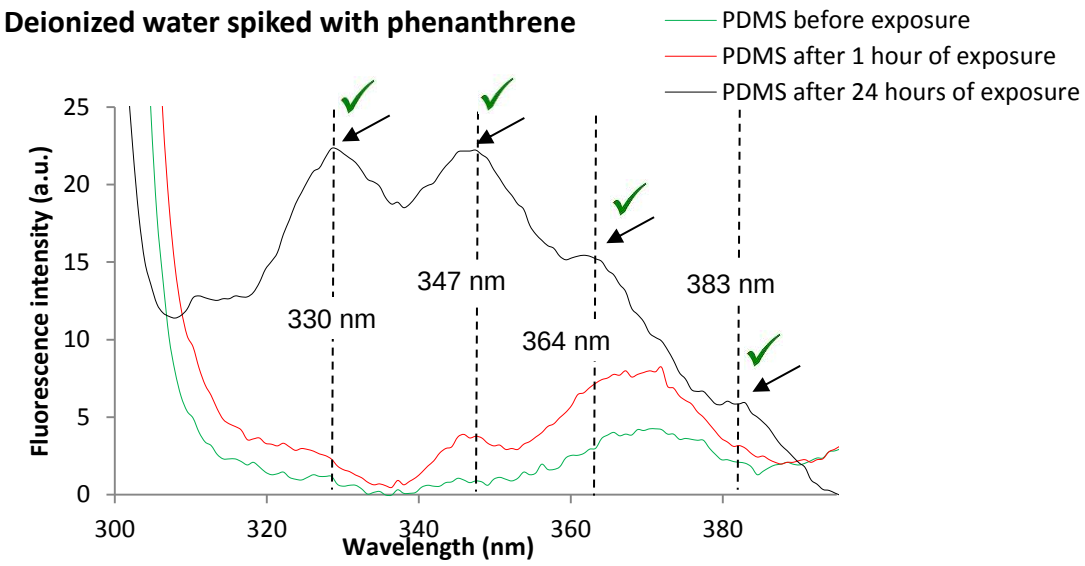

Figure 39 Fluorescence spectra of PDMS oil obtained exploiting the self-designed analytical equipment from the exposure of the device to the deionized water spiked with phenanthrene. The green line is the spectrum before the exposure to phenanthrene; the red line is the spectrum after 1 hour of exposure and the black line is the spectrum after 24 hours of exposure. The dot lines indicate the four fluorescence peaks of the PDMS oil spectrum which were previously observed using the standard analytical procedure: 330 nm, 347 nm, 364 nm and 383 nm. The green icon (✓) indicate the corresponding peaks observed in both the spectra obtained by standard and in those from the self-designed equipment.

The same peaks observed in Figure 37 are observed in Figure 38 and 39. In the case of Figure 39 the baseline is higher than in Figure 38 and this is also observed in Figure 37. The device used for all the experiment was the same. The experiments in deionized water were the first ones carried out, therefore it might be that in the PDMS oil, the amount of fluorescent EPDM component was more than the amount present in the PDMS oil after the exposure to the pond water. This is the reason why the spectrum in Figure 38 shows higher resolution of the phenanthrene signal than the spectrum in Figure 39. This is also confirmed in Figure 16. In order investigate the origin of the peak at 330 nm, a blank experiment was carried out in pure deionized water without phenanthrene and the spectra are shown in Figure 40. In this case only the peak at 330 nm is visible, while all the other peaks observed during the exposure to phenanthrene don't rise. This blank experiment was carried out using the same device and the peak at 330 nm displays similar intensity of the corresponding peak in Figure 38 but lower than in Figure 39. As previously said, most of the amount of the fluorescent compound contained into the EPDM sealing frames was extracted into the PDMS oil during the first experiments in deionized water, therefore during the experiments carried out in a second time (Figure 38 and 40) the peak at 330 nm displays a lower intensity.

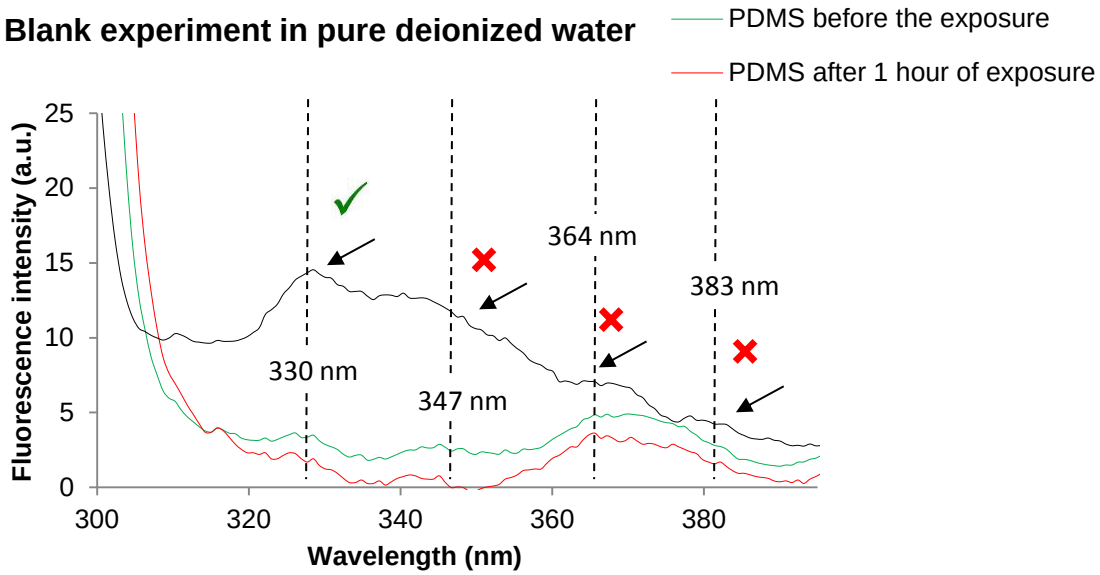

Figure 40 Fluorescence spectra of PDMS oil obtained exploiting the self-designed analytical equipment from the exposure of the device to the pure deionized water without. The green line is the spectrum before the exposure to phenanthrene; the red line is the spectrum after 1 hour of exposure and the black line is the spectrum after 24 hours of exposure. The dot lines indicate the four fluorescence peaks of the PDMS oil spectrum which were previously observed using the standard analytical procedure: 330 nm, 347 nm, 364 nm and 383 nm. The green icon (√) indicates the similarities observed between the spectra obtained by standard and self-designed equipment and the red icon (x) indicates the peaks, which were not observed in this case.

Furthermore, from Figures 38 and 39 it is possible to observe that after 1 hour of exposure, the first fluorescence peak of phenanthrene rose up at 347 nm. This peak was present only when phenanthrene was dissolved in the solution. In the blank experiment this peak does not appear in any one of the performed analyses (after 1 hour and after 24 hours). The similarities observed between the spectra obtained by the standard procedure and by the self-designed equipment, reveal a good level of resolution, although the interference observed in all the spectra affect the unequivocal identification of phenanthrene.

Once the device was emptied, the inner volume of the chamber was washed with deionized water and the fluorescence spectrum of the empty device was collected in order to check if phenanthrene was adsorbed on the external surface of dialysis membrane (Figure 41). The aim of this analysis was to confirm that the phenanthrene was absorbed all by the PDMS oil only. It was not possible to detect any of the fluorescence peak characteristic of phenanthrene in the spectrum of the dialysis membrane after the experiment. From the comparison of all the spectra obtained, it seems that the prototype device absorbed phenanthrene from the water phase only into the PDMS oil and not on the membrane. In fact, the spectrum of the membrane shows the only broad peak of cellulose between 360 nm and 380nm.

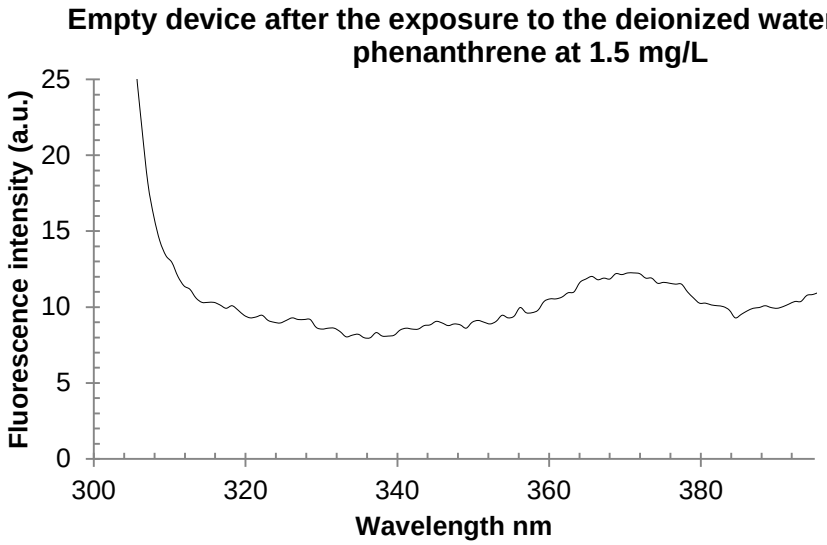

Figure 41 Fluorescence spectrum of the empty device. After the exposure to the deionized water containing phenanthrene at 1.5 mg/L of concentration. The broad peak of fluorescence intensity is the charachteristic peak of the membrane and similar spectra were collected from the device at the beginning of each experiment carried out in this part of the work.

In this regard, must be pointed out that all the spectra shown in the figures display a broad peak of fluorescence around 365 nm, which is the fluorescence spectrum of cellulose polymers when excited by a UV light at 290 nm (Plitt and Toner 1961). The dialysis membrane is made of cellulose, therefore this peak is present in all the measurements and is the only peak observed in Figure 41, when the membrane is analyzed without PDMS oil and phenanthrene.

The next part of the results is focused on the data obtained from the exposure of the device to the dionized water solution of phenanthrene at 1.5 mg/L (Figure 39). During the experiments the system was not hermetically isolated from the atmospheric gaseous phase and phenanthrene was subjected to both sorption into the device and evaporation in the gas phase. In real conditions this two processes would be influencing the detection of phenanthrene by the monitor device. The evaporation of phenanthrene in the gas phase does not affect the absorption into the prototype device.

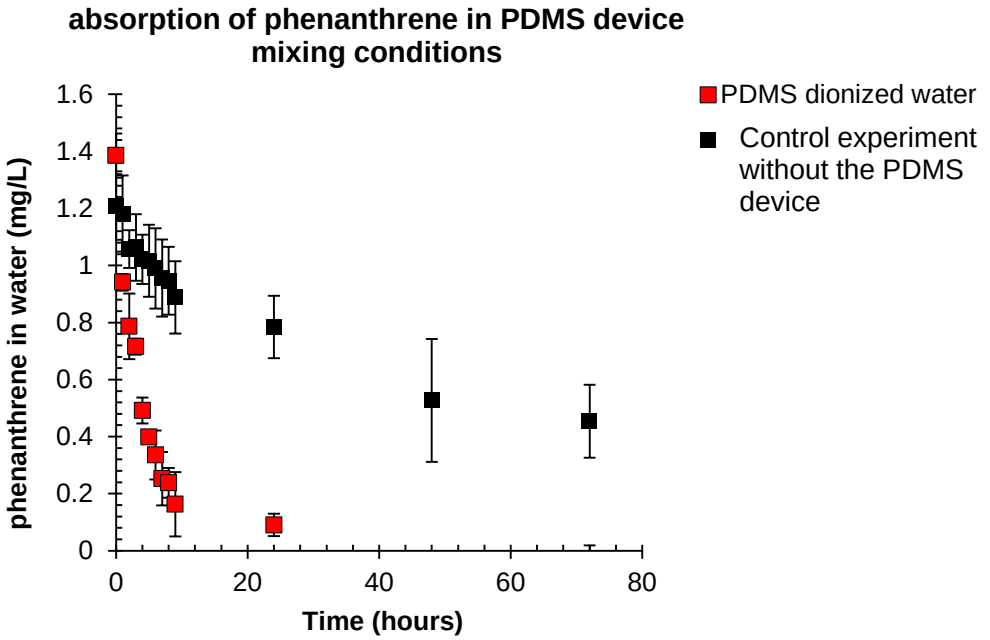

Figure 42 Phenanthrene concentration in water during the test with the device and during the control experiment. When the device is submerged into the dionized water solution of phenanthrene (red line), the decrease of phenanthrene is faster compared with the control experiment (black line). For the control experiment the deionizd water solution of phenanthrene was not in contact with the PDMS device. In both cases the solution was stirred at 900 rpm.

In Figure 42 it is possible to observe that the absorption process takes place mostly during the first 9 hours and overcomes the evaporation process. After 9 hours the device is saturated and the further loss of phenanthrene follows similar kinetics with or without the presence of the device. Presumably at this point the main process taking place is the evaporation of phenanthrene in the atmospheric gaseous phase. Using a linear fitting curve it is possible to observe that the decay costant of the phenanthrene concentration in the water phase after 8 hours has a comparable value between the two experiments (Figure 44). On the other hand during the first 8 hours the decay constants differ greatly between the two experiments, showing a much faster decrease of phenanthrene concentration when the device is submerged in the water solution (Fig.43).

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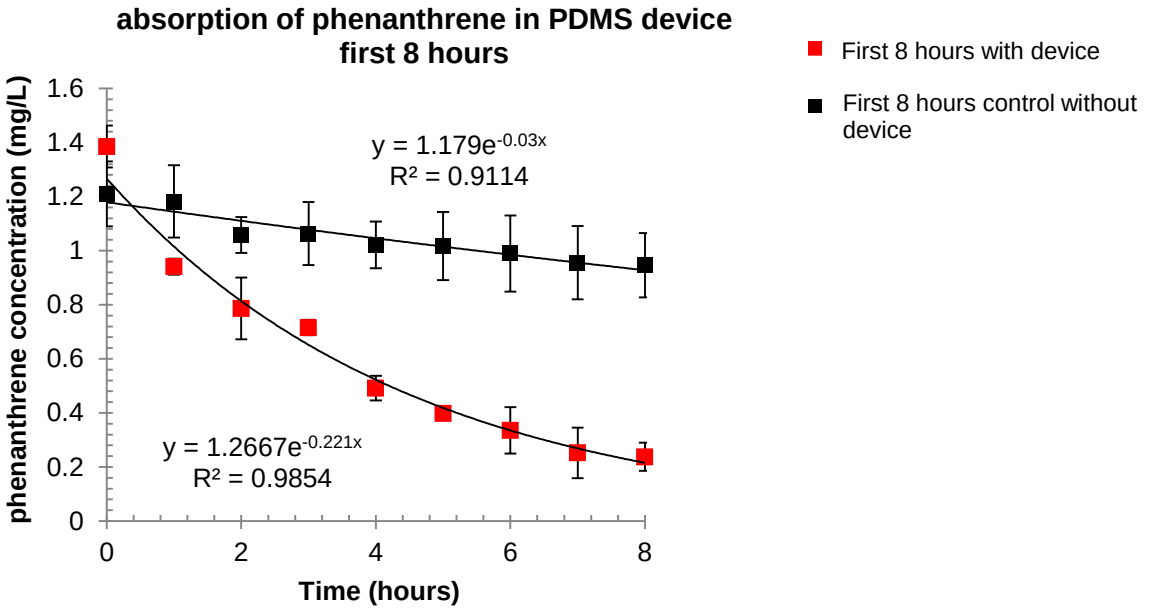

Figure 43 The phenanthrene concentration (mg/L) in the water during both the experiments: with (red line) and without (black line) PDMS device submerged in the solution. In the chart are shown the exponential equations for both decays, in order to compare the different rates**.** The decay rate is higher when the PDMS device is plunged into the phenanthrene solution.

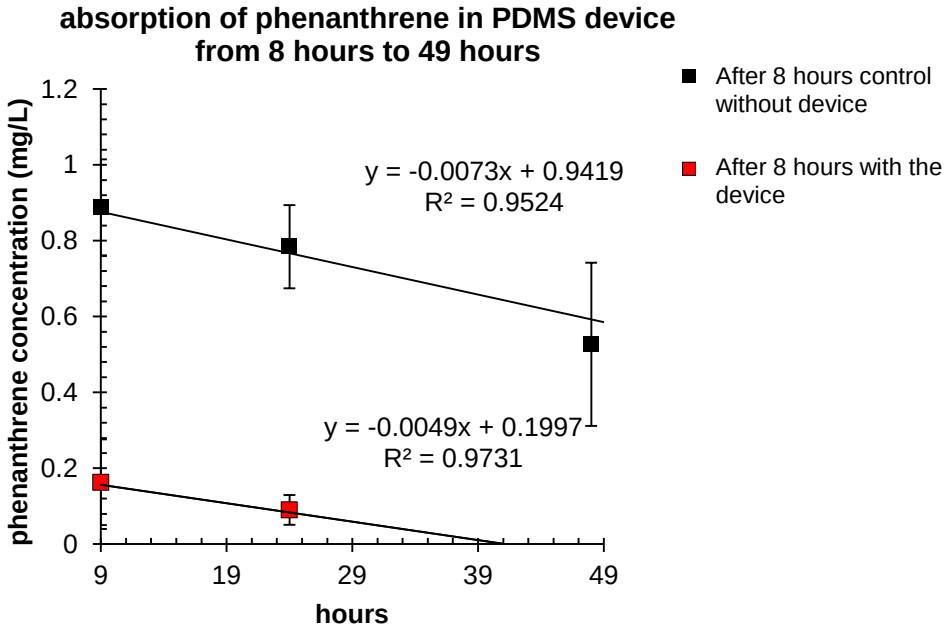

Figure 44 The phenanthrene concentration in the water during the second part of both experiments: with (red line) and without (black line) PDMS device present inside the phenanthrene solution. After the first 10 hours the decrease of phenanthrene in both solutions seems proceeding with similar rates, as shown by the equations

These results show that the use of PDMS oil combined with fluorescence spectroscopy for the detection of UV fluorescent pollutants in aqueous solutions is a suitable analytical approach. The spectra collected by using self-designed equipment display a high similarity with the spectra obtained by the standard analytical procedure. The characteristic structure of the emission signal of phenanthrene was observed within the same area of the emission spectra and the comparison to the standard solution allowed its identification. The aim of these measurements was not the quantification but the qualitative detection of phenanthrene from contaminated samples. Furthermore it was revealed that EPDM rubber frames are not a suitable material for manufacturing PDMS-based devices, since it contains compounds which cause interference with the emission spectra of the target molecule. Under the tested conditions the saturation of the device was reached within 9 hours of exposure to contaminated solutions. Under conditions more close to practice (e.g. lower volume ratio between the PDMS and the water phase), the device might display much faster kinetics. Although the front face spectroscopy proposed here displays a high potential for development of this kind of monitor devices, is a more complicate procedure than the classical full spectroscopy and it is not yet ready for on field applications. In conclusion, at the present time an ideal monitor device based on PDMS oil for the detection of PAHs in wastewater and surface water shall be designed for full-mode fluorescence spectroscopy equipment. In this regard, the next part of the work was focused on the optimized design of a more suitable prototype PDMS device. The aim was to provide a more efficient working mechanism than the original device.

Two possible versions are proposed for this device. One for smaller sewers pipes 200/500 mm diameter (Figure 46) and one by-pass version for combined sewers catchments or industrial discharge pipes, with a bigger diameter (Figure 47). The model shown in Figure 25 was chosen for the elaboration of a predictive sorption curve, which is useful to estimate its accumulation potential for phenanthrene from water. Using the partition coefficients of phenanthrene between PDMS materials and water, available in literature, the absorption curve of phenanthrene onto the PDMS device during the exposure in contaminated aqueous systems was simulated and is presented in Figure 45. The volume of PDMS oil exposed to the contaminated flow of water is 0.96 dm^3 and all the calculations were carried out on the basis of this specific size. This device is shown in Figure 25 and would be used for on-line measurements.

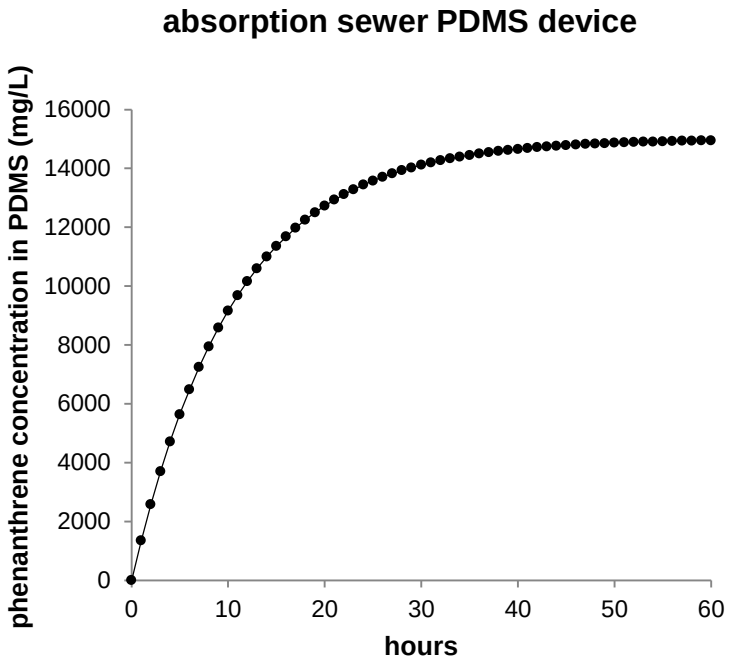

Figure 45 Aborption model for PDMS device. The absorption of phenanthrene into the PDMS device was estimated using the PDMS-water partition coefficient proposed by Sprunger et al., 2007 (Log $K_{PDMS\text{-}water} = 4$, k = 10000).

This simulation curve is based on the partition coefficient of phenanthrene between water and PDMS and its diffusivity in PDMS calculated by Sprunger and co-workers, using the solvation parameter model of Abraham (1 x 10^{-6} cm^2/sec, Sprunger et al., 2007). The partition coefficient of phenanthrene between water and PDMS ($K_{PDMS\text{-}water}$) is equal to 10000. This means that the equilibrium concentration of phenanthrene inside a PDMS phase, after the partitioning process took place, is 10000 times higher than the concentration in water. Considering a constant concentration of 1.5 mg/L in the water phase, it was estimated that after 30 hours, close to the equilibrium, the concentration inside the chosen device is equal to 14100 mg/L. According to the physical-chemical properties of PDMS, no significant desorption of phenanthrene from the PDMS device was accounted between 20° C and 30° C for a period of several weeks. The desorption occurs but so slowly that is negligible for practical purposes. Faster desorption kinetics can be achieved increasing the temperature over 100 °C. This is a process expressed by the van't Hoff equation, in which the partition coefficient of a compound between two immiscible phases depends on the temperature of the system. In the case of phenanthrene and PDMS the value of the partition coefficient does not change significantly up to 80-100 ° C

(DiFilippo and Egahouse 2010). In this case the desorption is not considered significant since the water temperature in combined sewers is usually about 20°C-30°C.

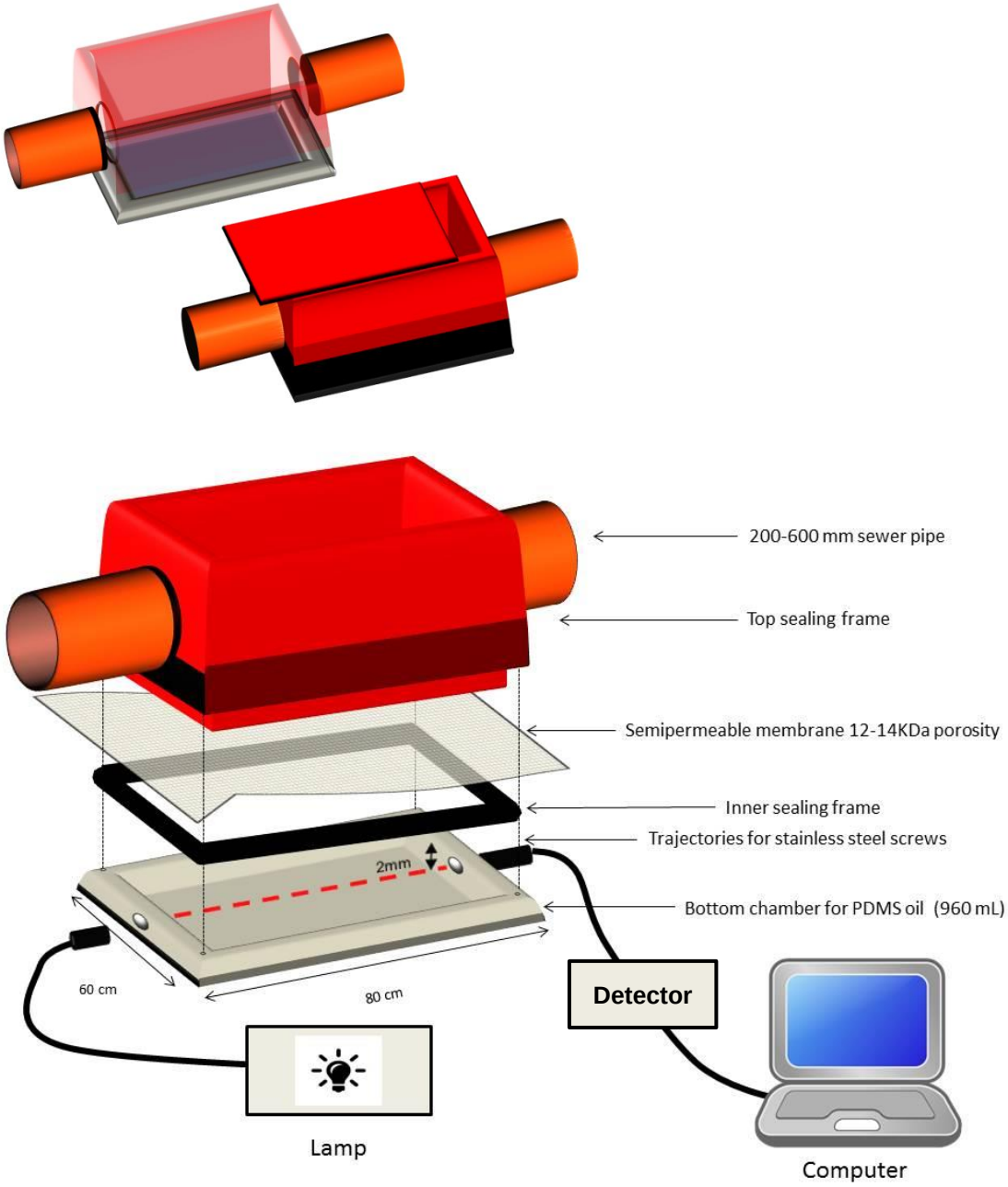

Figure 46 New prototype for monitoring hydrophobic pollutants in surface water and wastewater streams. The device is designed for small sewers with diameter 200mm, which is the minimum size according to the BS EN758:2008 regulation. It is composed of two external frames (stainless steel) which enclose hermetically (by use of screws) a PDMS oil chamber placed on the bottom of the device. The dot lines in the picture show the trajectory of the screws which link together the external frame and the bottom chamber. In order to ensure the complete closure of the inner chamber, a tin frame will be placed between the bottom chamber and the semipermeable membrane. Once the device is completely assembled, it will be constantly filled with wastewater and the gel on the bottom of the device will extract the pollutants from the water. Using fiber optic devices the oil will be analyzed from the bottom chamber and the emission spectra will be collected by use of specific softwares. It will be analyzed by fluorescence spectroscopy using portable equipment already available on the market.

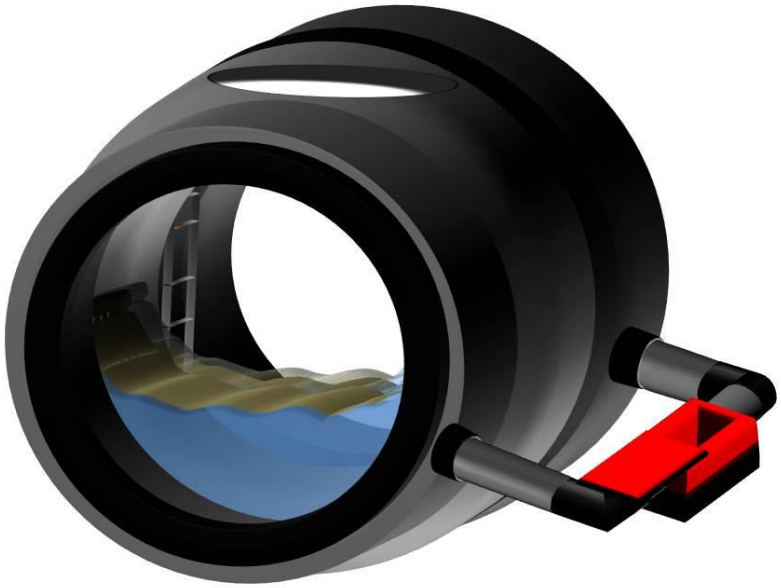

Figure 23 Combined sewer by-pass monitor device. The whole device is presented here completely assembled as a by-pass model prototype. The PDMS device presented before in Figure 25 is now linked to the wastewater stream by tubes. The use of pumps might be needed for keeping the low pressure inside the device and avoid damages.

According to this new design, a volume (3.5 mL) of the PDMS oil is analyzed from the bottom of the device by on-line connected UV fluorescence equipment in full mode. The UV light beam would pass across the all chamber and would be collected by fiber optic devices connected to a detector and a computer. This equipment is already available on the market and is used for similar on field measurements, therefore the development of such a technology would be easier to perform than the front-face application. The PDMS oil is a very interesting alternative to all the sorbent materials used for passive accumulation of pollutants in aqueous environments. It is a completely UV transparent material and therefore it allows on field measurements, which are faster than most of the laboratory procedures based on the extraction of the target compound from the sorbent phases.

4 DISCUSSION

4.1 Sorption experiments with polysaccharide gels

The aim of this part of the work was to create a model for estimating the memory of microbial biofilms for PAH compounds, using polysaccharide gels as surrogate matrices. In this study phenanthrene was chosen as a model compound. Phenanthrene is one of the most common PAHs found in soil, air, water and living organisms, such as plants, aquatic animals and human beings (Srogi, 2007). Phenanthrene is also found in high concentrations in PAH-contaminated environmental samples (de Barros Amorim et al., 2010). It has been widely studied and a large amount of literature is available about its physical-chemical properties (Ma et al., 2009; Keyte et al., 2013; NIST 2011; Wilcke, 2000; Floyd, 1976). For all these reasons, phenanthrene has been chosen as reference compound for the present study.

Steady state fluorescence spectroscopy in front face mode is the analytical method of choice for all the experiments carried out in this study. This method was proven to be suitable for studying the partitioning of phenanthrene between the gel and the water phase, providing a direct quantification of the target compound in the two different phases. No clean up, extraction and concentration procedures were needed for performing mass balance measurements during the sorption experiments. The final target here was to measure the memory of polysaccharide gels for phenanthrene under the effect of a flowing water phase. Polysaccharide gels have been exploited as surrogates of biofilms. In the environment, microbial biofilms act as sink and source of pollutants, through three main processes: absorption, desorption and the detachment. In particular the sorption processes were the subject of this investigation. Once absorbed, the pollutants can be desorbed back into the water phase. Memory is defined as the biofilm capacity of retaining the pollutant before to desorb it back in the water column. That said, the question arises: would it be possible to exploit the biofilm's memory for tracing pollutants in wastewater?

The first step for answering this question is to create a simple model system, which is composed of two phases only: the water phase and the sorbent matrix. In this case of study the water phase is deionized water solution spiked with phenanthrene. The sorbent phase is made of polysaccharide gels. Polysaccharides are main components of the EPS in microbial biofilms (Moore et al., 2014; Sutherland, 2001; Wingender et al., 1999), therefore a polysaccharide

sorbent phase has been chosen as the simplest form of surrogate biofilm for this study. Concerning sorption properties, biofilms are more complex matrices than polysaccharide gels. They are composed by the extracellular polymeric substances (such as proteins, polysaccharide, DNA and lipids), cell clusters and particles, which enter the biofilm from the water phase and are stabilized on it (e.g. clay particles; Reichert and Wanner, 1997; Eisenmann et al., 2001). Each of these components has an influence on the sorption processes of organic compounds into the biofilm, but only the polysaccharide/protein fraction has been investigated here. Since a model for estimating the memory of biofilms for PAHs has not been defined before, it was necessary to simplify it as much as possible. Agar gels have been previously used as surrogates for studying the diffusion of organic chemicals in microbial biofilms (Beyenal et Lewandowsky, 2000; Golmohamadi, 2012; Jouenne et al., 1994; Tresse et al., 1995; Westrin and Axelsson, 1991) and display structural properties, which are similar to those observed in biofilms. Agar consists of two fractions, agarose and agaropectin (Armisen and Galatas, 1987). Agarose is a long-chain molecule formed by β-D-galactopyranose residues connected through C-1 and C-3 with 3,6-anhydro-L-galactose residues connected through C-2 and C-4 (McHugh, 1987). Both residues are repeated alternately (Armisen and Galatas, 1987). Agaropectin has the same basic disaccharide-repeating units as agarose with some hydroxyl groups of 3,6-anhydro-α-l-galactose residues replacing by sulfoxy or methoxy and pyryvate residues (Fu and Kim, 2010). Agarose and agaropectine togheter form a neutral hydrocolloid presenting polar moieties.

As previously mentioned, the main parameter, which has been investigated here is the memory of polysaccharide gels for phenanthrene (page 96, Figure 36). For this purpose, the organic carbon partition coefficient (K_{oc}), the distribution coefficient during absorption and desorption processes (K_d) and the diffusion coefficients of phenanthrene (D) into the polysaccharide gels were measured (pages 82, 84-87, 90, 93). These values have been combined with the Fick's laws of diffusion and the gel's memory for PAH compounds has been simulated and tested (Equation 22 page 95).

The organic carbon partition coefficient (K_{oc}) was calculated exposing 1.5 mL of gel solution to 1.5 mL of deionized water with phenanthrene at 1.3-1.4 mg/L inside QS glass cuvettes. The partitioning of phenanthrene across the two phases was evaluated analyzing the two phases by front face fluorescence. The organic carbon partition coefficient (K_{oc}) has been previously used by other authors to express the sorption properties of different organic matrices (Seth et al., 1999;

Karickhoff, 1984). In Table 28 are presented values of Log K_{oc} measured in this study for the polysaccharide gels and in other studies for different sorbents.

Table 28 Log Koc values for phenanthrene, obtained in this study and in other articles.

Matrices	Log K_{oc}	Literature Reference
Agar 2% w/v (static, n=3)	2.27 ±0.7	
Agar 1.5% w/v + Gellan 0.5% w/v (absorption,static, n=3)	2.3±0.2	This study
Agar 1.5% w/v+Gelatin 0.5% w/v(absorption,static, n=3)	2.3±0.1	
Biofilm (*Sinorhizobium sp*.)	4.1	Wicke et al.,.2007
Sewage sludges	4.1-4.7	Oleszczuk, 2008

Compared with the reference values, polysaccharide gels seem to display a significantly lower affinity for phenanthrene. In case of biofilms, the K_{oc} of phenanthrene is the ratio between the absolute partition coefficient biofilm-water (K_{bw}) and the organic carbon fraction (f_{oc}) measured on the biofilm and equal to 0.48 (Wicke et al., 2007). In fact, the K_{oc} is the result of a double division of the phenanthrene concentration in the biofilm, first for the dry weight and then for the only organic fraction of it. As previously mentioned, in order to study the partitioning of chemicals in the environment, the K_{oc} is often used as reference parameter, although it displays some shortcomings in case of hydrogel matrices. When considering gel-like sorbents, such as polysaccharide gels, the use of partition coefficients, which take dry weight and organic carbon content as the main mathematical variables in the actual sorption processes of HOC, might lead to wrong predictive estimations. Wicke and co-workers (Wicke et al., 2007) studied the Log K_{oc} and the diffusivity of phenanthrene (D) in a pure biofilm of *Sinorhizobium sp.*. In this reference paper the Log K_{oc} is equal to 4.1, with a biofilm density assumed equal to 1 g/cm^3 and a biofilm water content of 89%. The biofilm was exposed to a 500 mL phenanthrene solution at 1.25 µg/L pumped inside a close system and the concentration of phenanthrene in the water solution was measured in the time. Then, from the K_{oc} value, the diffusivity is calculated according to Crank et al., 1956, who employs the Fick's law of diffusion (Equation 23) of a solute through a plane sheet of an isotropic sorbent in a steady condition as follows:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \text{(Eq.22)}$$

from which

$$\frac{q}{q_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2\lambda_n^2} \exp(-\frac{D\lambda_n^2 t}{l^2}) \quad \text{(Eq.23)}$$

where **q** and $\mathbf{q}_\infty$ are the **partial** and the **equilibrium uptake** of phenanthrene in the biofilm, **D** is the **diffusivity**, l is the **thickness** of the biofilm layer, **t** is the **time**, λ_n is the non-zero positive roots of

$\tan \lambda_n = -\alpha\lambda_n$,, and α is calculated as follows:

$$\alpha = \frac{V_l}{V_f K} \quad \text{(Eq.24)}$$

with V_f and V_l are the volumes of the biofilm and the water respectively and K is the partition coefficient. A value of Log K_{oc} equal to 4.1, expressed as K_{oc} is equal to 12589 and when this value is related to the Fick's laws of diffusion, it might lead to an underestimation of the diffusion coefficient and to an overestimation of the memory of a biofilm.

Microbial biofilms are matrices characterized by significantly high water content and the dissolution effect of water on the absorbed solute molecules have a crucial effect on the sorption kinetics. This effect must be considered for creating predictive models. Stewart (Stewart, 2003) explains well how the water phase of microbial biofilm has a major influence on the diffusion of organic molecules. Their model biofilm is mainly composed by a cluster of microbial cells (EPS and cells) interspersed with water channels through which the liquid phase flows. Also Wood et al., 2001 provides a similar model, which calculates the diffusivity by using a volume-averaged method. In both cases, the volume of the biofilm was used for the calculation of the solute concentrations in the different phases and the dry weight is not considered for estimating the final diffusion coefficients. Also Westrin and Axelsson, 1991 proposed a model for diffusion of solutes in polymer gels considering the volume concentration of the solutes in the gel for the calculation of the diffusivity (D). For all these reasons the main partition coefficient calculated in this study for simulating the memory of polysaccharide gels is the distribution coefficient K_d.

A special glass device was constructed for measuring the diffusivity of phenanthrene into the gels (Table 29). Two glass vials were connected (by standard taper joints 9/10 mm) to a central glass chamber where the gel solution was placed. One of the vials was filled with a deionized water solution of phenanthrene at 1.3-1.5 mg/L and the other side vial was filled with pure dionized water (page 50, materials and methods section). The concentration of phenanthrene in both the side vials was measured by fluorescence spectroscopy and the mass transfer of phenanthrene through the gel was calculated as follows:

$$F = D * A * \frac{Cr - Cl}{x} \qquad \text{(Eq.25)}$$

where **F** is the **mass transfer** (g/s), **D** is the **diffusivity** (cm^2/s), **A** is the **surface area** (cm^2), **x** is the **thickness** of the gel phase (cm) and $\mathbf{C_{r/l}}$ is the **concentration** of phenanthrene inside each of the side vials. The Diffusion coefficient was calculated by using the formula above starting from a mass transfer value (g/s) experimentally determined from the concentration of phenanthrene in the vial initially containing the pure deionized water solution. The diffusivity of phenanthrene into the polysaccharide gels presented in this study is similar to its water diffusivity ranging from 5 to 8 x 10^{-6} cm^2/sec. According to Wicke et al., 2007, the diffusivity of phenanthrene in biofilms of *Sinorhizobium sp*.is equal to 2.3 – 4.5 x 10^{-10} cm^2/s, while in water is about (7.0 ± 0.7) x10^{-6} cm^2/s. Between the two values there is a difference of 4 orders of magnitude. This big difference, as mentioned before, can be explained by the use of dry weight and organic carbon fraction for estimating the partitioning of phenanthrene between the water and the biofilm matrix. This is the reason why the distribution coefficient K_d has been used as partition coefficient. The K_d is calculated by the ratio between the volume concentration of phenanthrene in gel and water. Comparison with literature data (Tables 28, 29) might be useful to understand more about the diffusivity of phenanthrene in polysaccharide gels and in microbial biofilms. In order to confirm the results of the present study, it is necessary to compare the diffusivity of different solutes in polysaccharide gels and in water.

Lawrence et al., 1994 reported that the diffusivity of fluorescein (fluorescein isothiocyanate, FITC), measured by fluorescence recovery after photobleaching (FRAP) in agar gel 1.5% (w/v) was found equal to 4.7 x 10^{-6} cm^2/s, while its water diffusivity was reported in Galambos and Forsters 1998 between 5 and 6 x10^{-6} cm^2/sec. Also for the bovine serum albumine (BSA) a diffusivity of 0.6-0.7 x 10^{-6} cm^2/sec in agarose gels of 4%-7% volume fraction was reported in Johnson et al., 1996. In water the diffusivity of BSA is 0.4-0.7 x 10^{-6} cm^2/sec (Gibizova et al.,

2012). In both cases the diffusivity of the target compound in polysaccharide gels with high water content (>90%) is similar to the water diffusivity. These data confirm what has been observed here for phenanthrene. Miller and Allen (Miller and Allen 2004) determined a diffusion coefficient for α-pinene in agar gel 1.5% (w/v) equal to 3.4 x 10^{-6} cm^2/s, which is similar to its own water diffusivity and to the phenanthrene diffusivity in the agar gels used in the present study. The α-pinene is an organic compound of the terpene class produced by many kind of coniferous trees. It displays similar water diffusivity as phenanthrene, equal to 6.3 x 10^{-6} cm^2/s.

In order to understand the mass transfer of solutes in biofilms, it is necessary to compare the diffusivity of different compounds in water and in biofilms. The diffusivity of fluoresceine (FITC) in mixed biofilms is reported equal to 7.7 x 10^{-8} cm^2/s (Lawrence et al., 1994). In this case a decrease of two orders of magnitude (98%) is calculated between the diffusivity in water and in biofilms. Holden and co-workers (Holden et al., 1996) reported a diffusivity of 1.3 x 10^{-7} cm^2/sec for toluene in *Pseudomonas putida* biofilms. The diffusivity of toluene in water is 8.6 x 10^{-6} cm^2/sec. This is a similar decrease of 98% of the diffusivity in water, same as reported by Holden et al., 1996. This phenomenon has been explained as hindered diffusivity. Both the diffusivity and the hindrance effect for a macromolecule inside a porous matrix is size-dependent (Sharma and Yashonath 2007, Dechadilok and Deen 2006) and increases with the molecular weight and molecular radius of the molecule. In fact fluorescein isothiocyanate has a molecular radius of about 0.5 nm (Ambati et al., 2000) and a molecular mass of about 380 g/mol, while toluene displays a molecular radius of 0.3-0.4 nm (Bruggen et al., 1999) and a molecular weight of 92 g/mol. The hindrance observed on the diffusivity of toluene in biofilms is similar to the hindrance reported for FITC in biofilms. Phenanthrene displays a radius of 0.4 nm (Gotovac et al., 2007) and a molecular weight of 180 g/mol. Therefore its diffusivity in biofilms might be lower than its water diffusivity, but according to these assumptions, the value proposed by Wicke et al., 2007 (hindrance equal to four orders of magnitude) seems a not reliable estimation of the diffusivity of phenanthrene in biofilms.

From the reported results, it is possible to observe that polysaccharide gels display some limitations for fully modeling the sorption of macromolecules into microbial biofilms. The differences in the mass transport properties between biofilms and polysaccharide gels need to be discussed. Since it is a matter of molecular diffusion of phenanthrene in biofilms and

polysaccharide gels and since both matrices are composed for more than 90% of water, the first parameter that needs to be investigated is the water mobility inside these two sorbents.

Table 29 Diffusion coefficients of phenanthrene. Values of D (Diffusivity) for different compounds (left column) into different sorbents (second column from the left) are presented and the references are listed on the right column.

Compound	Sorbent matrix	Diffusivity (D) cm^2/s	Reference
phenanthrene	Water	$5–7x10^{-6}$	EPA 1989, Ong et al., 2008
phenanthrene	Biofilm (*Sinorhizobium sp*.)	$2.3 – 4.5 x10^{-10}$	Wicke et al.,.2007
phenanthrene	Agar 2% (w/v)	$5.3 \pm 0.8 x10^{-6}$	This study
phenanthrene	Agar 1.5% (w/v) + gellan 0.5 (w/v)%	$8.0 \pm 0.7 x10^{-6}$	This study
phenanthrene	Agar 1.5% (w/v) + gelatin 0.5% (w/v)	$4.0 \pm 0.7 x10^{-6}$	This study
α-pinene	Agar 1.5% (w/v)	$3.4 \pm 1.2 x 10^{-6}$	Miller 2004
α-pinene	water	$6.3 x 10^{-6}$	Miller 2004
Bovine serum albumin (BSA)	Agarose gels 4-7% (v/v)	$1.01 \pm 0.7 x 10^{-6}$	Johnson et al., 1996
Bovine serum albumin (BSA)	water	$0.5\text{-}0.7 x 10^{-6}$	Martinsen et al., 1992
Fluorescein isothiocyanate	Water	$5\text{-}6 x 10^{-6}$	Galambos and Forster 1998
Fluorescein isothiocyanate	Agar 1.5% (w/v)	$4.7 x 10^{-6}$	Wolfaardt et al., 1993
Fluorescein isothiocyanate	Mixed biofilms	$7.7 x 10^{-8}$	Lawrence et al., 1994
Toluene	Water	$8.6 x 10^{-6}$	
Toluene	Biofilm (*P.putida*)	$1.3 x 10^{-7}$	Holden et al., 1997

In both matrices, the mobility of water is mostly similar to its mobility in pure water. Beuling and co-workers (Beuling et al., 1998) used pulsed field gradient nuclear magnetic resonance (PFG-NMR) in order to measure the diffusivity of water inside microbial biofilms and agar gels. They observed that the diffusion coefficient of water in agar gels of 1.5% (w/v) - 2% (w/v) ranges between 90%-95% of their diffusivity in pure water ($20 x 10^{-6}$ cm^2/sec) linearly decreasing with increasing the gel concentration. Therefore in pure polysaccharide gels the mobility of water is not significantly affected. When 20%-30% (v/v) of polystyrene particles (0.9 μm diameter) and bacterial cells are added into two separate agar gels (1.5% w/v), the water diffusivity decreases

similarly to 85% of its value in pure water. The two kind of gels display same hindrance on the main fraction of water. Therefore, the cells seem to affect the mobility of water mostly by reduction of the diffusive volume and by steric interactions. However, from the PFG-NMR spectra obtained analysing the polysaccharide gels enriched with bacterial cells, it was possible to detect at least 2 different water fractions characterized from two different diffusivities. The main portion of the water displays similar diffusivity as observed in the pure polysaccharide gels and in the gels enriched with polystyrene particles. However the smallest water fraction displays a lower diffusivity, which the authors define as intracellular water. In gels enriched with bacterial cells, with a cell fraction of 5% v/v, the diffusivity of water is 90%-95% of its self-diffusivity in pure water. This hindrance decreases linearly with the cell volume fraction. In 2000 Vogt and colleagues reported new data about the water mobility in microbial biofilms (Vogt et al., 2000). In biofilms of *Ps.aeruginosa* after 24 hours of growth with a cell fraction of 1% v/v, the total water volume is divided in at least three fractions, each one characterized by a different self-diffusivity. The main portion of water displays a diffusivity equal to 85% of its value in pure water. This is the so called "free water fraction" in the biofilm. The second fraction (0.4% v/v) displays a diffusivity that is 10% of the pure water self-diffusivity. This is defined as intracellular water fraction. The third water fraction (<0.1% v/v) displays a self-diffusivity which is 1% of the value in pure water. This water is the water entrapped in the secondary structure of the EPS polymer matrix. In addition to this, within the same study, the diffusivity of glycerol was investigated in biofilms of *Ps. aeruginosa*. For glycerol two main portions were identified: the main fraction (90%) with a diffusivity of 80%-85% the diffusivity of glycerol in pure water, which is ascribed to the glycerol in the pores of the biofilm filled with water. The second portion (10%) displays a value of diffusivity equal to the 1% of that in pure water. This is assumed by the authors to be the glycerol diffusing in the EPS network and partially entrapped in the secondary structure of the polymer matrix. According to these results, the mass transfer of solutes in a biofilm occurs as diffusivity and the main fraction of a solute (glycerol) in such a matrix displays diffusivity values equal to 80%-90 % of its diffusivity in pure water and is addressed in the water filled pores of the matrix. A smaller fraction of the solute (10%) is entrapped in the thiner EPS networks and its diffusivity is significantly reduced. However such a decrease (99%) in the EPS was reported for only 1% of the water fraction. Glycerol might display much stronger ionic interactions with the polymers in of the EPS matrix than a nonpolar compound as phenanthrene. For phenanthrene the main decrease in the diffusivity through the EPS matrix is expected to be

due to steric interactions, tortuosity and weak electrostatic interactions, which are the same causes of diffusivity hindrance of water in polysaccharide gels. According to this, two main types of water have been defined in biofilms: free water and bound water (Beuling, 1998; Vogt et al., 2000). The mobility of the free water is similar to the mobility of pure water, while bound water displays a lower value of self-diffusivity and is assigned to intracellular water and water entrapped in the secondary structure of the polymer matrix. The free water is the most abundant type of water, while bound water represents the least fraction (1%). Beuling 1998 reported a self-diffusivity of water in biofilms equal to 11 x 10^{-6} cm^2/sec, compared with a pure water self-diffusivity of 20 x 10^{-6} cm^2/sec (between 20° C and 30°C). A fraction of 10 % of the water displayed a diffusivity of 19 x 10^{-6} cm^2/sec. The significantly decreased water self-diffusivity refers to about 1% of the all water volume and it is mostly located in the intracellular portion of the biofilm. Therefore, polysaccharide gels with immobilized bacterial cells display two different water phases: outside and inside the cells. The diffusivity of solutes in the cells can be significantly reduced (1 order of magnitude according to Vogt et al., 2000). Increasing the concentration of cells in a biofilms, the total diffusivity of a solute inside a biofilm decreases (Beuling et al., 1998). Cells and secondary structures of polymers are the main components responsible for the strongest hindrance of water diffusivity in biofilms. EPS matrix and polysaccharide gels display the same predominant water mobility and this reveals similar properties regarding the diffusion of macromolecules such as phenanthene (Vogt et al.; 2000). The mass transport of molecules in biofilms or hydrogels is driven by molecular diffusion in the water filled regions. Biofilms are heterogeneous matrices composed of different components with different physical-chemical properties (Table 30). The diffusion of a solute changes depending on which one of this phases interacts with it.

It seems reasonable that the mass transfer of macromolecules in the EPS matrix is well modeled by polysaccharide gels, althought the cells might be the most crucial component in the retention of phenanthrene between the biofilm and the water phase. This component can increase significantly the memory of a biofilm for hydrophobic compounds such as phenanthrene.

Table 30 Sorption sites in biofilms (Flemming and Leis 2002)

Sorption sites in Biofilms
5. EPS (including capsules) mainly consisting of polysaccharides and proteins.
a. Charged groups, for example –cCOO$^-$, -SH$^-$, -SO_4^{2-}, -$H_2PO_4^{2-}$, -NH_4^+, -NRH_2^+ etc..
b. Apolar groups for example aromatics or aliphatics such as found in proteins; also hydrophobic regions in polysaccharides.
6. Cell walls:
a. Outer membrane of gram negative cells (lipids)
b. Murein or teichoic acid layer of gram negative and gram positive bacteria respectively
7. Cytoplasmatic membrane (lipids)
8. Cytoplasm

The EPS matrix and the cell cluster are the two main fractions. Within the cell cluster fraction of a biofilm, there are different phases such as the cytoplasm, the membrane and the cell wall.

The interactions with the cell surface and with the EPS matrix can decrease the diffusion of hydrophobic macromolecules inside biofilms. In order to study the memory of biofilms for hydrophobic compounds it is necessary to investigate not only the diffusivity, but also the partition coefficients of these molecules between the water and the sorbent phase. The diffusivity gives the estimation of the mobility inside the biofilm, while the partition coefficient gives an estimation of the affinity between the biofilm and the sorbed molecule. The same solute might display equal diffusivity but different partition coefficients between two different sorbent phases and water. The diffusivity can be due to both intermolecular interactions and obstruction effect due to the polymeric chains composing the EPS matrix and the cells surface. Partition coefficients describe better the weak interactions (e.g. hydrogen bonds, hydrophobic effect, dispersion forces, electrostatic interactions) which can increase the retention time of a solute in a sorbent matrix (Flemming and Leis 2002, Gadd 2008). Therefore, the memory of a sorbent for a solute is related to both the diffusivity and the partition coefficient. However, in case of polysaccharide gels and hydrogels, it is wrong to use partition coefficients, which are based on the organic carbon fraction and on the dry weight of the sorbent phase. In order to model the memory of polysaccharide gels for phenanthrene in aqueous systems, another kind of partition coefficient has been calculated in this part of the work and is shown in Table 32. This coefficient is called here equilibrium distribution coefficient (K_d) and was calculated by the ratio of the phenanthrene concentration between the two volumes (gel and water). From the distribution

coefficients (K_d), it is possible to see that the sorption of phenanthrene into the agar 2% (w/v) and agar 1.5% (w/v) is fully reversible process, while a slight hysteresis was observed for the agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v). For these gels the equilibrium distribution coefficient of phenanthrene in absorption is equal to 1.5, while in desorption ranges from 1.6 to 1.8, which means that phenanthrene displays more affinity for these two mixtures of gels than for pure agar gels (Table 31).

Table 31 Distribution coefficients of phenanthrene in agar gels. The values of Kd for absorption and desorption experiments is reported in each column. Desorption I and II are the first and second steps of desorption respectively.

Gel matrix	Absorption Distribution coeff.(Kd)	Desorption I Distribution coeff.(Kd)	Desorption II Distribution coeff.(Kd)
agar 1.5% (w/v)	1.0±0.2	1.0±0.3	0.7±0.4
agar 2% (w/v)	1.0±0.5	0.9±0.3	0.6±0.5
agar 1.5% (w/v) + gellan 0.5% (w/v)	1.6±0.3	1.8±0.03	1.8±0.04
agar 1.5% (w/v) + gelatin 0.5% (w/v)	1.3±0.09	1.5±0.2	1.6±0.2

The hysteresis in the sorption of organic compounds into soil, sediments and sludge has been reported previously (Delle Site 2000, Conrad et al., 2005). The apparent higher retention of phenanthrene by gellan gum and gelatin than pure agar gels, must be discussed. Gelatin is a substantially pure protein food ingredient, obtained by the denaturation of collagen. The Type A gelatin, derived from porcine skin (as it is in this study), is prepared by acid processes. The amino acid composition particularly with respect to proline and hydroxyproline can vary from species to species but about 31% of the total content of amino acids consists of nonpolar amino acids, such as alanine, glycine, valine, leucine, isoleucine, proline, phenylalanine, methionine (Easton 1955). The content of hydrophobic amino acids, might explain the sorption hysteresis observed during the partitioning experiments. The hydrophobic interactions might be the cause of the retention of phenanthrene in gelatin-based gels. Also gellan gum (Gelrite from Carl Roth GmbH) displayed hysteresis in desorption partitioning tests. Gellan is a polysaccharides found in microbial biofilms and used in the food industry (Vu et al., 2009). The gellan gum present on the market is produced by the bactrerium S*phingomonas elodea* and can be found either in the native form or deacethylated (Milas and Rinaudo 1990). Gellan gum is composed by tetrasaccharide units, which consist of two residues of D-glucose and one of each residues of L-rhamnose and D-glucuronic acid (Jansson and Lindberg 1983, Giavasis et al., 2000).

In particular the gellan gum Gelrite (from Carl Roth GmbH) used in this study is a linear polysaccharide comprising glucuronic acid, glucose, rhamnose, and O-acetyl moieties. The tertiary structure involves the formation of three-fold left-handed double helices which involve hydrogen bonding between the glucuronic acid of one chain and the (1 → 4)-linked glucose and rhamnose molecules of its partner (Kasapis et al., 1999). Gellan/agar gels displayed higher K_d values than pure agar gels. The agar gels desorb phenanthrene with a distribution coefficient of about 1, which means that phenanthrene distributes equally between the two phases both in the absorption and desorption experiments. For gellan gum gels the value of the desorption distribution coefficient is 1.8. The phenanthrene holding capacity of gellan gum is higher than the agar gels. These results are in agreement with literature data. It has been reported that polycyclic aromatic hydrocarbons (phenanthrene, fluorene, pyrene and fluoranthene) display a higher solubility in gellan gum than in water. Johnsen and Karlson tested the partitioning of phenanthrene between gel and water phase and reported an equilibrium distribution coefficient of 1.5 (Johnsen and Karlson 2003). Wang et al., 2007 compared the absorption of carbazole from an aqueous solution between gellan gum and agar beads. Carbazole is a 3-ring heterocyclic aromatic compound, hydrophobic and almost insoluble in water. 2 mL gel beads of agar 2% (w/v) and gellan gum 1% (w/v) were placed in a 10mL solution of MSM (mineral salts medium) spicked with 3,340 μg of carbazole. The higher absorption of carbazole was observed using gellan gum beads, equal to 12.5 mg/g against the 4 mg/g obtained using agar gel beads. Phenanthrene is more soluble in gellan gum than in water and agar. When the gel is composed only of agar the phenanthrene diffuses equally between the gel and the water, while when one third of the gel composition is made of gellan gum, the solubility of phenanthrene in the gel increases and the compound is partially retained inside the gel.

Agar gels display a higher polarity than gellan gum due to the presence of functional groups such as sulfate esters, sulfoxy, methoxy and hydroxyl groups, while gellan gum displays only the less polar O-acetyl groups. Therefore when a portion of the agar gel is replaced by gellan gum, the total the affinity for a hydrophobic compound, such as phenanthrene, is increased. After this part of the work, the phenanthrene partitioning was investigated with a volume ratio between the phases equal to 1:100. This experiment was carried out under static conditions in order to investigate the absorption and desorption of phenanthrene into three gels: agar 2% (w/v), agar 1.5% (w/v) + gellan 0.5% (w/v) and agar 1.5% (w/v) + gelatin 0.5% (w/v). No significant differences were observed in the absorption partition coefficients obtained here. The absorption

partitioning is not dependent on the volume ratio between the two phases, therefore the values of K_d, K_{oc} and K_{gw} (pages 82, 84-87) were equal to the values obtained in the experiment with 1:1 volume ratio (Figure 32 page 89 and Table 26 page 90). The desorption experiment was carried out in order to evaluate any significant retention of phenanthrene by one of the gels used. The desorption process reached the equilibrium after 13 days. The phenanthrene concentration (mg/L) in the agar/gellan mixture was 0.2 mg/L and in the other gels was under the limits of quantification (between 0.08-0.1 mg/L). Therefore, it seems that under lower volume ratios between the water phase and the gel, as mimic of real conditions, a concentration of 0.2 mg/L is retained by the agar/gellan mixture and around 0.1 mg/L is retained by the other gels. However this experiment was carried out under static conditions, therefore cannot be interpretated as a valid measurement of the memory of polysaccharide gels for phenanthrene. This was not the aim of this experiment. The next step it was to determine how long would the surrogate-biofilm need to desorb completely the phenanthrene in the water column. The aim of the next part was to estimate the memory of such a sorbent for phenanthrene under conditions closer to practice.

In summary, different experiments were carried out for determining the equilibrium partition coefficients (K_d, K_{oc}, K_{gw}) and the diffusivity (D) of phenanthrene into gels and between the gels and the water phases. Then these coefficients were used for calculating the simulation desorption curves in the kinetic experiments carried out in the last part of this investigation. These last experiments will help to quantify the memory of polysaccharide gels for phenanthrene. In addition, a mathematical simulation of the memory was proposed and verified by the experimental results.

A layer of gel (0.1 mm) spiked with phenanthrene (1.5 mg/L) was allowed to solidify inside a QS glass cuvette (sketch paint page 96). The cuvette was connected to a continuous flow of deionized water and the concentration of phenanthrene inside the gel was measured every 10 minutes by fluorescence spectroscopy in front face mode (see materials and methods). From the calculated D and K_d values, a predictive model was formulated using the Fick's laws and the simulated desorption curve was compared with the experimental outcome. From the results is possible to see that the memory of a polysaccharide gel for phenanthrene, when exposed to a continuous flow of deionized water, lasts about 2 hours. After this time the phenanthrene in the gel reached a concentration lower than the limit of detection. This experiment helps to understand how the sorption of a hydrophobic pollutant such as phenanthrene by a polysaccharide hydrogel

layer would proceed under real conditions. In fact suitable flow conditions were chosen during this experiment in order to keep the phenanthrene concentration in the liquid phase equal to 0 mg/L. In a real condition, a plug flow of contaminated water would run only during a limited period of time. After this time the discharge of contaminated water would come to an end and the concentration of the pollutant in the water column would drop down to zero. At this point the desorption of the pollutant absorbed into the biofilm would start, regulated by the gradient of concentration, the diffusivity of phenanthrene in the gel and the partition coefficient between the two phases. When polysaccharide gels are employed to mimic microbial biofilms, then is possible to model and calculate their actual memory featuring the Fick's law of diffusion as follows:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \text{(Eq.26)}$$

and the distribution coefficients (equation 6):

$$F = D\frac{\left(\frac{1}{K_d}C_g - C_w\right)}{x} \qquad \text{(Eq.27)}$$

The simulated curve and the experimental data seem to be completely fitting each other (Figure 36, page 96). These results confirm the diffusivity and the partitioning profiles investigated during the first part of the work. These results provide a quantification of the desorption of phenanthrene from a polysaccharide hydrogel layer. The memory of the agar/gellan gel lasted about 2 hours. After this time the concentration of phenanthrene in the gel dropped under the limit of detection and it could not be further quantified. Phenanthrene might be retained in the gel for a longer time than 2 hours, but most of it is desorbed in this period. It must be pointed out that microbial and in particular sewer biofilms are complex matrices and might display higher sorption capacity than the surrogate matrices investigated in this study. Although the cell fraction occupies a smaller percentage of the whole sewer biofilm volume than the EPS matrix (about 15% according to Jahn and Nielsen, 1998), it might have a key role in the retention of compounds such as phenanthrene. Polysaccharide gels mimic the sorption properties of the EPS matrix for phenanthrene, but not of the whole biofilm. Microbial biofilms enriched on phenanthrene and exposed to a flowing aqueous system, might have a memory longer than 2 hours, as observed from the simulation. In order to confirm this assumption, other gel mixtures could be investigated in the future, adding further degrees of complexity to the surrogate matrices. The use of polysaccharide gels in modelling the sorption properties of microbial

biofilms is a useful scientific approach and this work provides a novel insight for achieving this aim.

4.3 Detection of PAHs in sewers

Wastewater, surface water and ground water are amongst the main carriers of pollutants in the open environment. Some pollutant, as strongly hydrophobic, is partitioned through the absorption into various organic phases such as soil, sediments, sludge, living organisms and microbial biofilms. Sewers are one of the main infrastructures for the distribution of wastewater. It has been reported that microbial biofilms grow abundantly on the surface of sewer pipes and in the sediments deposited on the bottom layer (Jahn and Nielsen 1998; Flemming and Wingender 2010). Microbial biofilms, as sorbent phases, are involved in the fate of pollutants in all aqueous systems (Spaeth et al., 1998, Headley et al., 1998) and also in sewers they are expected to fulfill this role (Rocher et al., 2003). In fact various authors reported that microbial biofilms as also sewer biofilms display high sorption capacity for hydrophobic organic compounds present in the water phase (Spaeth et al., 1998, Wicke et al., 2007, Antusch et al., 1995), but only few used biofilms for monitoring the pollution in sewers, following a systematic sampling approach (Genuit 2008; Genuit and Block 2009). One of the aims of this study was to use a systematic approach for sampling microbial biofilms from the sewers and analyze them for tracing the primary sources of polycyclic aromatic hydrocarbons. In order to achieve this target it was necessary to develop a suitable sampling procedure and a suitable analytical method. In collaboration with the environmental department of the Bielefeld city hall, the sampling of the biofilms was carried out using a plastic stripe support on which biofilm could grow, for a period of 4-6 weeks (Genuit 2008; Genuit and Block 2009). The sampling points have been chosen using a GIS map (geographic information system, QGIS software) of the sewers of Bielefeld city, in order to track down any possible pollution source. The analysis of the biofilm samples was carried out in order to detect phenanthrene, as marker compound for PAH pollution (Kuusimaki et al., 2004; Larsen and Baker 2003). The biofilms suspensions were analyzed using a liquid-liquid extraction method and the GC-MS analysis of the extracted solutions, prior calibration in methanol (Materials and methods, page 54). This part of the work was carried out according to the DIN 38414-23 protocol. This protocol was chosen according to the available instrumentation and to reported results for the analysis of wastewater and surface water (Lepom et al., 2009,

Krüger et al., 2012, Bercaru et al., 2006). As a result no significant trace of phenanthrene was observed in the extracts.

Few explanations might be considered: it is possible that no discharge of phenanthrene in the wastewater occurred during the monitoring period within the monitored area. Otherwise, although the discharges did occur, no significant retention of phenanthrene has been displayed from the biofilm layers. As previously reported, the partitioning of smaller PAH compounds in aqueous systems occurs mainly by sorption to particulate matter (PM) such as particles (Kim and Kwon 2010), which are present in combined wastewater streams or to the sediments accumulated on the bottom of the pipe. These latter ones could display higher organic carbon content, a higher distribution coefficient and higher density than the biofilms developed on the support and therefore a higher partition coefficient for these phases might have turned them in preferable sorbents for phenanthrene. Another reason is represented by the involved analytical procedures. Standard laboratory analyses of environmental samples are often long and complicated procedures, which involve several steps of clean up, extraction and concentration of the target compounds. Therefore, it might be the case for significant analyte losses during the procedures. However these findings are in agreement with the results shown in the previous section. A short time memory (e.g. few hours) would not allow to detect phenanthrene in the biofilm samples.

4.3.1 Monitor device for detection of phenanthrene in sewers

Another aim of the present study was the development of an analytical method, suitable for on field detection of phenanthrene present is sewers. A device was manufactured in order to achieve this aim. This device was designed for two main functions: passive accumulation of the target compound from the aqueous phase and detection of the pollutant directly from the device using optical fibers and UV fluorescence spectroscopy equipment. The device was submerged both in a deionized water solution of phenanthrene (1.3-1.5 mg/L) and in a natural pond water suspension spiked with phenanthrene (1.3-1.5 mg/L).

Passive accumulation devices have been widely used in monitoring pollutants in aqueous systems and various examples of devices have been reported in literature (Stuer-Lauridsen 2005). In this regard different materials have been used for such devices and different pollutants have been monitored. Most of them are reviewed in Steuer-Lauridsen 2005 and some of them are shown in Table 32.

Table 32 Passive accumulation devices reviewed in Stuer-Lauridsen 2005. Various sorbent/membrane combinations have been tested in each work.

Sorbent material/membrane	Target compounds	Measuement	Reference
Trimethylpentane/polyethylene	Chlordane and dieldrin	n.a.	Peterson et al., 1995
Polyurethane/fibreglass	Aromatic compounds	GC-MS	Madsen et al., 1996
Low-density polyethylene	PAHs and PCBs	GC-mass selective detector	Booij et al., 2000
Dowex Optipore L-493/ceramic membrane	BTEX and naphthalenes	GC-mass selective detector	Martin et al., 2003
Hexane/polyethylenemembrane	Organochlorines	GC-MS	Litten et al., 1993
XAD-7 TenaxTa/ /polycarbonatemembrane	Phenolic compounds	n.a.	Zhang and Hardy, 1989

In the last 15 years many PAD have been constructed for the detection of hydrophobic pollutants in aqueous systems. Most of these devices are designed for 1-4 weeks field deployment, where uptake is governed by first order kinetics providing a time weighted average of the exposure concentration (Stuer-Lauridsen 2005). Semipermeable membrane devices (SPMD) are the most used amongst the PADs. Here is a brief explanation of their functioning: a chemical compound in the water is carried to the sampler by convection; it diffuses across the boundary phase surrounding the sampler, and passes through the membrane pores by conduction. It is finally solubilized in the solvent or sorbed to a bonded receiving phase. The final phase is chosen to act as a sink for the chemical, thus ensuring an effective gradient across the sampler's interface to the ambient water. Obviously, the effectiveness of the sampler is related to the surface area, and to increase this several of the samplers allow the membrane to completely enclose the receiving phase forming a bag, tube or sandwich, thus forming a permeable housing (Stuer-Lauridsen 2005).

Different solvents and membranes can be used. Polyethylene, cellulose or ceramic membranes are the most common ones. Polyethylene is the preferred one for the accumulation of hydrophobic pollutants, since it displays more hydrophobic interactions and separates better the inner phase of the device from the water phase. Sorbent phases can greatly vary from solvents such as hexane, trimethylpentane, triolein to resins such as Dowex Optipore L-493 (Stuer-Lauridsen 2005).

Polydimethylsyloxane (PDMS) is a UV transparent material that is widely used for microfluidic chip analyses. Many PDMS devices that use fluorescence spectroscopy have been described (Thompson, 2005). PDMS can be found as additive in food, cosmetics, can be added as antifoam agent in lubricant solutions and in medicine for various purposes such as skin topical applications or wound dressings (ISO 10993, USP and European monographs). It is harmless for living organisms and the environment. From the wasterwater, it accumulates in the sewage sludge at the end of the wastewater treatment. PDMS is not easy biodegraded and when this sludge is used for landfilling, PDMS undergoes processes of fast soil hydrolysis (Graiver, 2003). These degradative processes produce low molecular weight silanols, which are volatile. In the athmosphere, these compounds are degraded by OH radicals as shown in Graiver et al., 2003. In this study the aim was to create a prototype analytical system that can be used for on field measurements by front face fluorescence spectroscopy. PDMS is highly hydrophobic and it is widely used as coating of solid phase micro extraction (SPME) fibers for detecting hydrophobic compounds in environmental samples (Laak et al., 2006; Yang et al., 2007). In this study PDMS oil was used for testing a prototype monitor device for PAH pollutants in sewers and surface water streams. The featuring of a passive accumulation device (PAD) containing PDMS oil with a front-face fluorescence spectroscopy method, is a novel idea for monitoring pollutants in water bodies.

Wang et al., 2001 reported the use of a SPMD based on triolein and enclosed with polyethylene tubing membrane for monitoring aromatic and chlorinated compounds within the wastewater treatment plant of Beijing city. The use of this polymeric membranes is inspired on the interactions which occur at the level of biological membranes of living organisms exposed to the contaminated water stream, such as fish, mosses or algae. All the SPMD analysis, reported by Wang and co-workers, involves an extraction procedure to take out the absorbed pollutant and analyze it by GC-MS and other methods. The advantage of using PDMS resides in its UV transparency. Using a suitable excitation light beam, it might be possible to detect a target PAH compound absorbed by the PDMS oil contained into the device.

The preliminary results obtained testing this device in deionized water and pond water (pages 98-100), show that the use of PDMS oil for front face fluorescence detection of phenanthrene after the exposure to contaminated environmental aqueous solutions, is a promising application. In the experiment carried out in the deionized water spiked with phenanthrene, under mixing conditions (900 rpm) and a volume ratio 1:25 between the device and the batch solution of phenanthrene, the

saturation of the device was reached after 9 hours and the signal of phenanthrene was well defined, considering this as a preliminary test. The optical fiber platform used for this experiment displayed good efficiency in collecting the emission signal from the device (page 99-100). The composition of the pond water suspension, during 24 hours of exposure, didn't influence the detection of phenanthrene into the device. Other hydrophobic molecules were expected to be partially absorbed into the PDMS, but these didn't cover the phenanthrene signal, collected directly from the device. After one hour of exposure it was already possible to observe the presence of phenanthrene inside the PDMS oil, by the arising of the first fluorescence peak of phenanthrene (345-350 nm). Unfortunately, the high fluorescence baseline didn't allow an optimal resolution of the signal. The explanation for such an effect might be attributed to the composition of the sealing frames made of ethylene propylene diene monomer (EPDM). The contact between the PDMS oil and the frames, caused the chemical extraction of some hydrophobic component of the frames and the arising of a fluorescence broad peak around 330 nm. This peak caused the rise of the whole baseline and affected the estimation of the peak areas of the phenanthrene signal. For further optimization of the device, metal frames, tin or cupper preferably, shall be used in order to avoid this kind of signal interference. The composition of the EPDM used is unknown and only some hypothesis can be formulated. EPDM is produced in a coordinative, anionic polymerization of ethylene, propylene and a non-conjugated diene such as hexadiene, dicyclopentadiene or ethylidene norbornene in a solution, suspension or gas phase technique (Röthemeyer and Sommer, 2006). Plasticizers are not usually present in EPDM rubber. Only for production of specific purposes they are added in the EPDM mixture (Wypych 2004):

- Pressure sensitive tapes used to join rubber membranes.
- Hose formulation.
- Cold shrinkable cable joint protection.
- Weather-stripping composition.

The most common plasticizers used for EPDM are (Wypych 2004):

- Polyisobutylene (the most frequently used non-migrating plasticizer of EPDM compositions).
- Paraffin oil.
- Dibutyl phthalate.
- Dioctyl phthalate.
- Vulcanized vegetable oil.

Due to their fluorescence properties, these compounds were not considered the cause of the observed interferences on the emission spectrum of phenanthrene. Other compounds of the EPDM rubber materials were investigated. Since various components of EPDM display absorbance in the UV range and are photo oxidized by UV light, in order to prevent the aging of EPDM material, anti-oxidants are often added to the matrix (Rivaton et al., 2005). These compounds absorb UV light in the range of 220-280 nm, and therefore these compounds might have caused the interference and the broad emission signal from 320 nm to 360 nm.

The use of steady state fluorescence spectroscopy for the detection of a specific compound in complex solutions present limitations when more than one compound can be excited at the same wavelength and emit fluorescence in the same spectral region. Interferences are one of the main limits of fluorescence spectroscopy when it comes to multicomponent mixtures such as environmental samples. Different approaches can be used in order to allow the direct measurement of specific compounds by on-field applications.

Using steady state fluorescence spectroscopy, the simplest approach is to investigate both the emission and the excitation spectra of a mixture. In this way, it is possible to distinguish different compounds, which display overlapping in the emission spectra at the same excitation wavelength. When this simple procedure is not sufficient, there are few other possible solutions in order to identify single compounds.

The excitation emission matrix fluorescence (EEMF) is a method based on the principle just mentioned. It allows plotting emission intensities at all combinations of excitation and emission wavelengths in a single three-dimensional graph (Patra 2003). The measurement is usually made by selecting one excitation wavelength and scanning the emission wavelength over the region of interest. Repeating this process for more than one excitation wavelengths, provides data that can

be presented as a three-dimensional surface, where x-axis is the emission wavelength, y-axis is the excitation wavelength and z-axis is the fluorescence intensity (Patra 2003).

The use of pulsed laser technology can be exploited for time-resolved measurements in order to identify a specific compound in complex mixtures. The fluorescence life-time is the time needed for a fluorophore in the excited state to reach the ground state. Each compound is characterized by a specific fluorescence life-time, therefore the comparison between the decay rates of the fluorescence signal can lead to the identification of a specific molecule in solutions composed by different fluorophores (Patra et al., 2003).

Another possible approach is the synchronous fluorescence scan (SFS). In this case both the excitation and the emission monochromators are scanned simultaneously. When the excitation and the emission profiles of a compound are known, then they can be compared with the data obtained by this method and the identification can be accomplished (Patra and Mishra 2001). All these analytical methods can increase the efficiency of the fluorescence spectroscopy as reference approach for the detection of different PAHs accumulated in the PDMS device proposed in this study.

As previously said this prototype was not designed only for the accumulation of phenanthrene from the water phase. It was manufactured also to be suitable for front face spectroscopic measurements. In particular this function is based on the surface optical properties of the dialysis membrane and this is a complex parameter that needs to be further optimized in order to display a higher degree of resolution. This is the reason why a new prototype device (shown in Figure 46 page 107) was designed for the analysis of the PDMS oil by fluorescence spectroscopy (optical fibers). This device represent a new idea for on field applications. Its high potential was presented here for the first time and might stimulate the interest of further investments. Once this new version of the PDMS device was designed, its absorption curve has been simulated under conditions close to practice, following the same simulation method carried out for the polysaccharide gels (Equation 22, page 95). As a result of this theoretical simulation, the exposure of 10 hours to a continuous flow of water saturated with phenanthrene (1.5 mg/L), would lead to an accumulation of about 8000-10000 mg/L of phenanthrene into the PDMS oil. At a temperature comprised between 30° C and 60° C, the desorption of phenanthrene from the device into pure water would take so long (several weeks) that it was considered negligible whithin a period of several weeks. The desorption and absorption curves of phenanthrene into

and from the device has been calculated considering pure water as second phase. It must be pointed out that, under real conditions, the presence in water of surfactants, particles and the deposition of sediments on the membrane of the device might significantly affect the memory of the device.

The use of PDMS oil for the fluorescence detection of phenanthrene in sewers has been proposed here. The potential and the weaknesses of this application have been discussed, but thanks to this latter part of the work, it is now highlighted the path to a novel strategy for monitoring PAHs in sewers. PDMS oil was never used for UV fluorescent detection of PAH pollutants in sewers and it seems to display the right properties for on field measurements.

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6 APPENDIX

6.1 List of abbreviations

AAS	Atomic absorption spectrometry
BHJ	Bulk hetero conjunction
BTX	Benzene, toluene and xylene
DEHP	Di-2-ethylhexylphthalate
DIN	Deutsches Institut für Normung
EEC	European economic community
EEMF	Excitation emission matrix fluorescence
EEMF	Excitation emission matrix fluorescence
EI	Electron ionization
EPA	Environmental protection agency
EPDM	Ethylene propylene diene-monomerrubber
EPS	Extracellular polymeric substances
EQS	Environmental quality standards
FCS	Fluorescence correlations pectroscopy
FID	Flame ionization detection
FITC	Fluorescein isothiocyanate
FLD	Fluorimetric detection
FOG	Fats, oils and grease portion
FRAP	Fluorescence recovery after photo bleaching
GC	Gas chromatography
GIS	Geographic information system

HMW	High molecular weight
HOC	Hydrophobic organic compounds
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
LC-DAD	Liquid chromatography with diode arraydetection
LD50	Letaldoseof50%
LLE	Liquid-liquid extractions
LMW	Low molecular weight
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry detection
NIST	National institute of standards and technology
PAHs	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyls
PCDD/PCDF	Polychlorinated dibenzodioxins/dibenzofuran
PDA	Photodiode array
PDMS	Polydimethylsiloxan
PM	Particulate matter
PN/PS	Polysaccharide/protein ratio
PRFS	Phase-resolved fluorescence spectroscopy
PTE	Potential toxic elements
QS	Quartz suprasil
SFQ	Selective fluorescence quenching
SFS	Synchronous fluorescence scan
SOM	Solid organic matter

SPE	Solid phase extractions
SPME	Solid phase micro extraction
SRB	Sulfate-reducing bacteria
TMV	Tobacco mosaic virus
TRF	Time-resolved fluorescence spectroscopy
USGS	United states geological survey
UV/Vis	Ultraviolet/visible
UVD	Ultraviolet detection
WFD	Water framework directive
WHO	World health organization
WRF	White rot fungi
WWTP	Wastewater treatment plant

6.2 Curriculum Vitae

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6.3 Statement

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Sampler systems for tracking emitters of phenanthrene in sewers"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, im Juli 2014

Giacomo Bertini

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