WATER QUALITY INVESTIGATIONS OF THE RIVER LEA (NE LONDON)

by

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ABSTRACT

The Lea Navigation in the north-east of London, a canalised reach of the River Lea, is affected by episodes of very low levels of dissolved oxygen. The problem was detected by the Environment Agency in the stretch from the confluence with Pymmes Brook (which receives the final effluent of Deephams sewage treatment works) to the Olympic area (Marshgate Lane, Stratford). In this project, possible causes and sources of the poor water quality in the Lea Navigation have been investigated using a multi-parameter approach. A study of physico-chemical parameters, obtained from Environment Agency automated monitoring stations, gave a clear picture of the poor river water quality at three sites in this reach. River water ecotoxicity to the freshwater alga *Pseudokirchneriella subcapitata* was determined by algal growth inhibition tests, following the OECD guidelines. Moreover, a novel protocol was developed which involved the use of *E. coli* biosensors (CellSense) operating at a lower potential than the standard protocol and using pre-concentrated river water samples. This protocol is promising and it has the potential to be a useful tool to determine the toxicity of contaminants at environmental concentrations. Furthermore, the developed protocol is a rapid, easy to perform bioassay, with potential application in achieving the aims of the Water Framework Directive (WFD). In addition to the data from the Environment Agency automatic monitoring stations and the laboratory-based tests, two *in situ* monitoring approaches were performed: 1) a detailed spatial seasonal monitoring of physico-chemical parameters of river water at twenty-three sites, and 2) algal growth inhibition tests, with algae entrapped in alginate beads, at seven monitoring stations. Results showed chronic pollution, and identified polar compounds in the river water and high bacterial concentrations as possible causes of low dissolved oxygen levels. This study confirmed the negative impact of Deephams STW (throughout Pymmes Brook) on the water quality of the Lea Navigation. However, there was evidence of other sources of pollution, in particular Stonebridge Brook was identified as uncontrolled source of pollution and untreated wastewater. Other possible sources include Old Moselle Brook, diffuse pollution from surface runoff, boat discharges and other undetected misconnections. Finally, in the light of the WFD, this project provides a case study on the investigation of river water quality, providing evidence that the multi-parameter approach is reliable, and low cost approach for the monitoring of freshwater bodies.

DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of Bedfordshire.

It has not been submitted before for any degree or examination in any other University.

Name of candidate: Deborah Patroncini Signature:

Date:

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Abbreviations

3N-BBM+V	Bold-basal medium with 3-fold nitrogen and vitamins
ANOVA	Analysis of variance
AWQMS	Automated water quality monitoring station
BG11	Blue-green medium
CCAP	Culture collection of algae and protozoa
DO	Dissolved oxygen
EA	Environment Agency
FeCN	Potassium ferricyanide mediator
GQA	General Quality Assessment
MAD	Median absolute deviation
Met	Meteorological Office
NCIMB	National Collection of Industrial, food and Marine Bacteria
OD	Optical density
OECD	Organization for Economic Co-operation and Development
*p*BQ	*p*-Benzoquinone mediator
SD	Standard deviation
SEM	Standard error of the mean
SPE	Solid phase extraction
SS	Respiratory substrate cocktail
SSM	0.85% Saline + respiratory substrate medium
STW	Sewage treatment work
WFD	Water Framework Directive

1 Introduction

The River Lea is a major left-bank tributary of the Thames. It rises from springs in North Luton (Leagrave Common) and joins the River Thames near Bow, flowing through Bedfordshire, Hertfordshire and Greater London. Between the 1600's and the Mid 1800's, a length of the River Lea was canalised (from Hertford to the River Thames) to allow navigation, taking the name of Lea Navigation. Alongside this channel, other canals have been built for water abstraction and flood relief (Flood Relief Channel). In addition, several sewage treatment works (STW) discharge their final effluents into Lea water.

Urbanization has changed the physical aspect of the river and raised the quantity and the diversity of pollutants carried to receiving watercourses, especially in the Lower Lea catchment within the city of London. In particular, the dissolved oxygen level is almost persistently low in the stretch of the Lea Navigation downstream of Pymmes Brook, suggesting chronic pollution (Snook and Whitehead 2004).

Nevertheless, a good water quality level of the River Lea is needed for different reasons:

1. the Lea catchment includes local Nature Reserves and a Special Protection Area (SPA, Lea Valley Regional Park), which promote nature conservation but it is also a recreational area. It also contains aquatic habitat Sites of Special Scientific Interest (SSSIs) such as Rye Meads, Turnford and Cheshunt Pits, and Walthamstow Reservoirs, which are connected to the water resources of the area and will be affected by water level changes and water usage (Environment Agency 2006);
2. it has been for centuries an important source for the water supply in London, providing for around one-sixth of the population (Reid 1995 cited in Snook and Whitehead 2004);
3. in 2000 the European Union (EU) instituted a framework for water protection and management (European Water Framework Directive), which stated that all Community waters must accomplish "good ecological and chemical status" by 2015.

However, urban water pollution results from a large range of sources, which are generally difficult to detect, determine and manage (Defra 2012). The diffuse pollution of the water involves a wide variety of pollutants, often of unknown nature (emergent contaminants). Chemical analysis offers a quantitative measurement of selected chemicals in the water but it does not identify the presence of non-target pollutants (e.g. toxic metabolites). In addition, chemical assays do not give any indication about toxicity due to interactions between compounds. Complementary tools to chemical analysis are bioassays, which

provide an estimate of the potential toxicity of complex river water samples (Belkin 2003, Struijs *et al*. 2010).
Recently, Thames 21 (a waterway charity) has conducted an investigation of the water quality of the River Lea and its tributaries within the M25 perimeter. They concluded that the overall water quality was very poor and they reported the need to conduct long-term studies of chemical, biological, and physical water quality parameters (Thames 21 2011).

This study will use bioassays in the effort to better identify likely sources of water pollution in the reach of the channel under investigation, and at the same time, it will be one of the first attempts to give long-term results of water quality of the Lea Navigation.

1.1 The River Lea catchment

The River Lea catchment is mainly lowland. The northern area is dominated by agriculture (Upper Lea), while in the southern part urban development prevails (Lower Lea). The north-west of the catchment is characterized by chalk, and the geology in the south-east part of Lea catchment is mainly Tertiary deposits and London Basin (London clay) (Snook and Whitehead 2004).
As a consequence of the increasing urbanization, the Lea catchment water quality is affected by different factors: navigation, abstraction, misconnections, water runoff from roads and nearby areas (precipitation can cleanse animal faeces, foliage, waste, gravel and oil into rivers), and fish farm discharges effluents (the Gingercress Trout Farm and Westmill Trout Farm). Moreover, Luton Hoo Lakes, which collect runoff from Luton Airport and the surrounded urban areas, discharge into the Lea catchment. The same happens to the balancing reservoirs at Stansted Airport.
The River Lea is affected by thermal pollution since it receives cooling water released from the Central Electricity Generating Board (CEGB) at West Ham (Snook and Whitehead 2004). A large contribution to the pollution of the River Lea is from several sewage treatment works, which discharge their final effluent into Lea water: Luton East Hyde, Harpenden, Hatfield Mill Green and Rye Meads, Buntingford (via the River Rib) and Deephams STW (via Salmon Brook and Pymmes Brook).
Snook and Whitehead (2004) has reported changes in the River Lea flow regime, which could be summarized as following: 1) the flow has been changed in the Luton area; 2) upstream of Hertford the main flow is provided by sewage treatment effluent; 3) urbanisation is causing large alterations in flow during storm events, generating potential flood risks; 4) river water is widely abstracted for domestic supply and industrial processes in all the catchment; 5) lock gates along Lea Navigation slow the water flow and produce muddy substrate; 6) during summer period the river water flow level is low (due also to

drought conditions) and is mainly composed by sewage effluents, causing a reduction in the water quality.

Numerous studies have reported a decline of the quality both of the water and the aquatic habitats in the River Lea catchment, mainly connected to urban diffuse pollution.

The pollutants detected in the Lower Lea are mostly due to the increased number of urban areas and STWs discharges:

- heavy metals (Snook and Whitehead 2004);
- organic substances, such as herbicides, fungicides, insecticides, industrial substances (Snook and Whitehead 2004);
- nitrate and phosphorus. In 1998 the River Lea and the Lea Navigation were nominated “Eutrophic Sensitive Areas” (Flynn *et al*. 2002, Snook and Whitehead 2004, Environment Agency 2008a);
- pharmaceuticals and personal care products. However, some of the studies demonstrated that the STW effluent discharges were contributing to a dilution of compounds already present in the stream (Williams *et al*. 2003, Ashton *et al*. 2004, Ellis 2006).

Moreover, it has been noticed that sewage treatment work discharges raised the biological oxygen demand (BOD) in the Lea catchment which, combined with urban runoff and domestic misconnections, led to low DO levels in receiving waters (Snook and Whitehead 2004). The Environment Agency is continuously measuring key water physico-chemical parameters in the area affected by Deephams STW, using automated water quality monitoring stations (AWQMS). In particular, there is evidence of chronic pollution in the stretch of the Lea Navigation downstream of Pymmes Brook, since the dissolved oxygen level is almost persistently low. In order to solve this problem in the short term, a hydrogen peroxide oxidation unit has been positioned along the Pymmes Brook.

In the Lea Navigation and tributaries, the water quality is also compromised by coliform concentrations, which exceed the Bathing Water Directive standards, making these waters unsuitable for leisure purposes. However, tributaries exhibited higher coliforms levels than samples collected from sites along the Lea Navigation (Snook and Whitehead 2004).

The presence of pollutants, the regulated flow and the canalization of the river and some of its tributaries have resulted in depleted aquatic fauna (both macroinvertebrate and fish communities). Macrobiological surveys showed a very poor quality in the lower reaches of the Lea (GQA grade E), while Lea Navigation showed a good quality (grade A) (Snook and Whitehead 2004, Environment Agency 2008a). On the contrary, the heavily modified nature of the Lea Navigation has changed the distribution (the classical zonation does not occur in the channel), abundance and diversity of fish populations, with habitat niche overlap in the natural stretch upstream (Pilcher and Copp 1997, Watkins *et al*. 1997).

The water quality of the River Lea is also affected by a high level of suspended solids. In 2008 (Sodomková 2009), sediment analysis revealed that they were contaminated mainly by heavy metals and polycyclic aromatic hydrocarbons (PAH). This extensive sediment deposit was considered in part to be responsible for the low dissolved oxygen levels, because of the high sediment oxygen demand (SOD). For this reason between February and April 2009, dredging operations were undertaken in the reach from Pymmes Brook to Lea Bridge Weir (Sodomková 2009). However, studies carried out two months after the dredging were not able to determine if the dredging operations have improved the dissolved oxygen levels in the water (WRc 2009 cited in Sodomková 2009).

1.2 Urban diffuse pollution

Diffuse pollution results from a large range of sources, which are generally difficult to detect, determine and manage (Defra 2012). Urban water diffuse pollution could be determined by water from rain (stormwater), or human activities such as watering, car washing and irrigation (urban runoff); in both the cases runoff water can pick up many polluted substances and carry them into receiving water bodies (such as streams and lakes). Even floods lead to events of polluted runoff since large volumes of water drain off the surrounding landscape collecting pollutants along the way. The Water Framework Directive (EU, 2000 - article 10 and 11) focuses the attention at point and diffuse sources of pollution, recommending that measures to prevent or control the input of pollutants should be put in place within 12 years from the Directive being issued.
In the CIWEM report regarding diffuse pollution, d' Arcy *et al*. (2000 cited in Ellis and Mitchell 2006) showed that nonpoint urban runoff is responsible of the quality decline of 4-5 % of rivers in England and Wales, and 11 % of Scottish streams. Ellis and Chatfield (2002 cited in Ellis and Mitchell 2006) demonstrated that at least one third of the oil pollution events within the Thames Region were due to urban surface runoff, underlining the difficulty for regulatory bodies to manage the diffuse pollution. Sources of urban pollutants are various, such as residential/industrial/ highway runoff, garage/petrol/service stations, gardening, misconnections, sewer leaks, litter/waste disposal, pets/birds, car/vehicle emissions and the construction industry (Ellis and Mitchell 2006).

However, Defra and the Environment Agency have recognized five key sources (Defra 2012):

1. Road runoff;
2. Misconnections;
3. Contaminated sediment;
4. Industrial estate runoff;
5. Mines and combined sewer overflows.

In the UK, environmental regulatory agencies are suggesting the use of sustainable urban drainage systems (SuDS) for both surface water drainage and flood control. SuDS act like natural drainage and control the water above-ground, improving water quality and amenity (Environment Agency 2002). However, urban diffuse pollution is still a persistent problem. From the mapping of UK river basin districts, it emerged that almost one third of the UK rivers were heavily modified water bodies (such as Lea Navigation): 91 % of these channels received urban waters and showed degradation of their ecological, physical and water quality parameters (Ellis *et al*. 2012). Therefore, Ellis *et al*. (2012) demonstrated the need to implement the SuDS already in place especially in urban catchments, underlining the necessity to perform more studies to understand better the urban surface runoff quality and to improve the quality of receiving waters.

1.3 Chemical mixture in the aquatic system

As mentioned in the previous paragraph, diffuse pollution brings into the aquatic system a large range of compounds, with many pollutants coexisting in the same water body. There is strong evidence that chemical mixture effects are stronger than single compound effects (Kortenkamp *et al.* 2009). Recently, Gustavsson and Bachaus (2012) showed a high relation between the predicted risk of a chemical mixture and the average risk of the individual chemicals, underlining the necessity to go beyond the standard chemical-by-chemical compound assessment for the environmental risk assessment.

The approaches to evaluate chemical mixtures are applicable only to known chemical compositions. Nevertheless, the detection of each single compound of a mixture in the natural environment through chemical analysis has some limitations: 1) chemical monitoring cannot detect the high number of compounds potentially present into receiving waters, including metabolites resulting from substance transformation; and 2) it does not take into consideration the combined effects such as synergistic, additive or antagonistic. A review study, conducted by Leung (2012), stated that the combined ecotoxicity of antifouling biocides was in 80 % of the cases due to both additive and synergistic effects together.

Bioassay batteries offer a complementary rapid and inexpensive tool in monitoring programs, in order to measure also the toxic effects of unknown pollutants. Moreover, biological monitoring provides a trend in toxic pressure in receiving waters over a period of time (Struijs *et al*. 2010, 2012). Bioassays enable chemical stressors to be isolated from other stress factors present in the natural habitat. This is an advantage but, at the same time, it does not provide any information about interactions of chemical factors with physical and/or biological stressors in the environment. To complement the information obtained from laboratory tests, it is necessary to conduct biological monitoring *in situ*.

1.4 Bacterial contamination of surface waters

Wastewater treatment plants are the most common sources of bacterial pollution. However, bacterial contamination in receiving waters is also due to diffuse pollution from other sources such as surface runoff and combined sewer overflow. The most common indicator traditionally used to identify a recent bacterial contamination by sewage is the concentration of coliforms (such as *Escherichia coli*, Enterobacteriaceae family) since normally coliforms survive only hours or days outside their hosts. However, Health Canada website reports studies where it has been demonstrated that *E. coli* survival can be as long as 4-12 weeks at temperatures around 15-18 °C in waters with a moderate microflora (Health Canada 2009). *E. coli* is not pathogen but it was identified as good microbial indicator for different reasons: 1) it is the only coliform species present exclusively in human and warm-blooded animals, and usually is not present in the natural environment (in contrast with other species of coliforms); 2) it is excreted in large concentrations with faeces (10^9 per gram), so detectable even after dilution in receiving waters; 3) it has a life span similar to the pathogens of concern (Health Canada 2009). However, *E. coli* reliability has been questioned especially because they are not exclusive of human origin, but they could derive from pets, wildlife and livestock, which can contribute considerably to the thermotolerant coliforms and *E. coli* concentration. Ellis (2004) demonstrated that bacterial load in diffuse impermeable surface runoff was primarily due to domestic animals, rodents and birds within urban catchments in North London and Milton Keynes (UK), underlining the necessity to find alternative indicators of recent human contamination. For Hillebrand *et al*. (2012) an ideal anthropogenic marker should be source-specific and be detectable even after dilution in the environment. Carbamazepine, human-specific antibiotics and artificial sweeteners have been proposed as wastewater indicators, but they do not allow the distinction between untreated and treated wastewater. A good wastewater-specific marker seems to be caffeine (Buerge *et al*. 2006) which is mobile, is highly eliminated through wastewater treatment plants, but with a slow degradation rate in the environment of between 3 days and 3 months (Sauvé *et al*. 2012), and a high detection frequency (Hillebrand *et al*. 2012). Caffeine is exclusively human-specific, regularly consumed by people with coffee, tea, soft drinks, chocolate, and pharmaceuticals. Different studies shows that caffeine concentrations are 20-300 µg L^{-1} in raw sewage, 0.1-20 µg L^{-1} in treated wastewater effluents, 3-1500 ng L^{-1} in rivers, lakes and seawaters, and 10-80 ng L^{-1} in ground waters (Sauvé *et al*. 2012). Sauvé *et al*. (2012) found a strong correlation between faecal coliform counts and caffeine. Setting an arbitrary threshold of 400 ng L^{-1} caffeine, they identified water samples contaminated with faecal coliforms at concentration more than 200 cfu/100ml, which correspond to the limit used by the Environmental Ministry regulation in Canada.

Since the 1980s researchers have studied the potential of natural vegetation to decrease the bacterial concentration in polluted waters. Karim *et al*. (2008) reported different examples of the efficiency of wetlands in improving the water quality: 1) John (1984) documented a decrease of 99 percent in *E. coli*, coliforms and streptococci thanks to hyacinth in a Malaysian lagoon; 2) Karpiscak *et al*. (1996) reported a drop of 98 percent of total coliforms and 93 percent of faecal coliforms due to vegetation; 3) Rivera *et al*. (1997) recorded a decline of more than 99 percent for total and faecal coliforms present into wastewaters, which were forced to pass through the root zone of *Phragmites australis* and *Typha latifolia* set in a gravel base. Similar results were confirmed by the study of Karim *et al*. (2008) with the comparison of what happened to the bacteria population level in both a vegetated and an unvegetated scenario. There are two main explanations for the observed decrease of the coliforms levels in wetlands. The first is the competition for nutrients with natural microorganisms. In fact, all the photosynthate and organic compounds excreted by plants through their roots allow natural microorganisms to proliferate, depriving the coliforms of food. The second likely explanation is that coliforms are also predated by nematods (Mandi *et al*. 1993 cited in Karim *et al.* 2008). Other parameters which affect the rate of *E. coli* in surface waters, are temperature (low temperature, such as 4 °C, lead to a significant decrease), light (it has a negative effect), and heavy metals (negative effect on bacteria survival) (Wcisło and Chróst 2000).

1.5 Bioassay for toxicity assessment

1.5.1 Algal growth inhibition test

Recently, in the Directive 2008/105/EC the Council of the European Communities (2008) highlighted the importance of combining data on chemical concentration and toxicological evaluation for integrated assessment of the status (chemical and ecological) of water bodies. As a result, microbial bioassays are commonly used in ecotoxicological assessments of surface waters in line with the guidelines for the testing of chemicals (OECD 2006). They are also required in other European legislation, such as REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) for the evaluation of environmental hazard of chemicals.

Planktonic microalgae have been chosen as test organism for the following reasons (Lewis 1998, Källaqvist *et al*. 2008, Silva *et al*. 2009, Villem 2011):

1. in aquatic ecosystems algae occupy the first level of the food chain, as primary producers. This implies that disturbances in their productivity and structure may provoke changes in the structure of higher ecosystem levels;
2. they are easy to grow and maintain in the laboratory. Their short life cycle allow the measurement of toxic effect over many generations;

3. they are sensitive to modification of their environment, and they have been shown to be more responsive to different contaminants than invertebrates and fishes;
4. algal tests are characterised by high reliability, reproducibility and robustness, since algae are unicellular organisms.

Micro-algal bioassays have been used in many different situations to test pollution: 1) laboratory wastewater (Silva *et al*. 2009); 2) toxicity testing of heavy metals, pesticides, pharmaceuticals (Isidori *et al*. 2005, Tišler *et al*. 2009, Daus *et al*. 2010, Köck *et al*. 2010, Santos *et al*. 2010, Roberts *et al*. 2010); 3) chronic toxicity of river water, organic sediment extracts and sediment porewater to aquatic organisms (Källqvist *et al*. 2008).

In recent years, the use of algal bioassays as a complementary tool to chemical analysis in water quality assessment has increased, especially in the light of whole river chemical mixture monitoring, since chemical tests are not capable of detecting emergent contaminants, metabolites or synergetic/additive chemicals effects. Moreover, algal tests can offer long term monitoring, showing the trend in the water quality across different sampling sites over many years (Struijs *et al*. 2010).

Whilst algal growth inhibition tests take several days and require culturing facilities, rapid assessment of acute toxicity is possible with electrochemical whole cell biosensors (Rawson 1989 - UK and European Patent), in which the metabolic activities of algal and bacterial cells are monitored.

1.5.2 Whole CellSense biosensors

A biosensor is a form of bioassay that allows real time monitoring. Biosensors are defined by IUPAC as “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals” (IUPAC 2012). In other words, biosensors convert a biological event (e.g. metabolic activity) into a detectable signal, and the magnitude of the outcome signal is proportional to the metabolic status of the biocatalyst.

Biosensing offers different advantages, such as fast assays (in many cases it is not necessary to pretreat the sample), cost-effective analysis, portable equipment, and real-time measurements (Farré *et al*. 2009). Moreover, biosensors have been defined as green techniques by Farré *et al*. (2010), since they need small amount of samples and solvents. Biosensing techniques are potentially the right tool to complement chemical analysis, offering the way to implement the new European Union directives, such as the Water Framework Directive and the Marine Framework Directive (Farré *et al*. 2012).

In the environmental pollution monitoring, whole cell biosensors are used to measure the toxicity of both water and soil samples. One of the main advantages of using whole cells is that the biocatalyst does not require genetic manipulation, offering a simple method available to everybody. Examples are: 1) Chinese hamster ovary cells biosensor based on changes in UV absorption (Baumstark-Khan *et al*. 1999); 2) cultured fish cells biosensors based on metabolic changes (Polak *et al*. 1996); 3) fungal biosensor based on metabolic changes of yeast cells (Palmqvist and Berggren 1994); 4) screen-printed algal biosensor based on metabolic changes of *Chlorella vulgaris* cells (Shitanda *et al*. 2009), which is smaller, less expensive, and with shorter assay time of the conventional algal biosensors; 5) CellSense (Harvey-Coleman, Leeds), a mediated amperometric system which mostly use *Escherichia coli* cells as biocatalyst (Farré *et al*. 2001, Bathia *et al*. 2003). CellSense instrument has several advantages, listed as following: 1) it is easy to use; 2) it is not disrupted by turbidity (so it is a good tool to investigate wastewater effluent); 3) it takes only 20-30 minutes to run a test; 4) it is able to run up to 32 samples simultaneously; 5) and it gives the possibility to follow both inhibition and stimulation of the biocatalyst's metabolic activity in real time (measured at 4 s intervals). However, two literature works (dos Santos *et al*. 2002 and Farré and Barceló 2003) have reported some negative aspects of CellSense such as: 1) lack of reproducibility (different tests give different inhibition rates) and sensitivity for certain substances, 2) aggressive solvents and some substances could precipitate on the electrode, 3) electrochemical activity of the samples could alter the results. Nevertheless, as underlined by dos Santos *et al*. (2002), whole cell biosensors show the potential to provide a rapid technique for toxicity measurements.
In this project, CellSense whole cell biosensors were used to monitor the pollution in the Lea Navigation. Sensors employed here are formed of two electrodes: working and reference (Figure 2.3). The potential applied between the working and the reference electrode is constant, and it promotes a redox reaction, which results in a current flow in the external circuitry. This device uses chemical mediators to redirect electrons from the metabolic activities of the biocatalyst (such as respiration and/or photosynthesis) to the working electrode, converting the biochemical signal into an electrical signal.

The electron donation between the biocatalyst and the electrode is facilitated by the addition of chemical mediators. Prokaryotic biocatalysts can be monitored by non-penetrating mediators such as potassium ferricyanide, which has little impact on cell physiology; eukaryotic biocatalysts require lipophilic mediators, such as *p*-benzoquinone, that are capable of penetrating the plasma membrane. Long-term exposure to penetrating mediators can have a more pronounced impact on cellular events (Pandard and Rawson 1993). As illustrated in Figure 1.1, cellular redox events can result in the reduction of the oxidised mediator, which can in turn be re-oxidised at the surface of a suitably poised electrode, resulting in electron donation and current flow in the external circuit.

Figure 1.1 - Mediator redox activity in a prokaryotic cell (Rawson *et al*. 1989). Chemical mediators are reduced by cellular redox events, and then re-oxidised at the surface of poised electrode, resulting in electron donation and current flow in an external circuit.

Bacterial biosensor monitoring of metabolic status of cells allows the detection of acute toxicity within 30 minutes, and cyanobacterial or algal-based biosensors allow the detection of photosynthetic electron transfer disruption to be monitored in real-time as well as the respiratory chain activity.
So far, mostly *E. coli* based CellSense biosensors have been applied to investigate the toxicity of wastewaters and sewage sludge (Evans *et al*. 1998, Farré *et al*. 2001, Farré and Barceló 2003, Daniel *et al*. 2004). They can be very effective in giving a rapid assessment of surface water quality, and Rodiguez-Mozaz *et al.* (2004 and 2006) stated that the UK Environment Agency has proposed *E. coli* CellSense as a method for the Direct Toxicity Assessment (DTA) in 1999.

1.5.3 Alginate beads

Algal growth inhibition tests conducted in the laboratory are good tools to detect chemical pollution, but they have some limitations, such as the possibility that a contaminant disappears from the system due to adsorption events (OECD 2006). To avoid this inconvenience, algae can be used to monitor the water quality of a watercourse *in situ*.
The evaluation of *in situ* water quality at specific sites is possible using micro-algae immobilized in calcium alginate beads. The most widely used immobilization technique is gel entrapment, which can be performed using synthetic polymers, proteins, and natural polysaccharides. Alginate gel is a natural polysaccharide, which offers several advantages: low-cost, permeability, null toxicity, transparency of formed matrix, and immobilized cells do not experience extreme physical-chemical condition changes during

the immobilization process. CO_2 and nutrients can travel across the alginate barrier and simultaneously the physiological properties of the algal cells persists intact (Moreira-Santos *et al*. 2004). The most common cation used to form alginate beads is Ca^{2+} (Moreno-Garrido 2008). Depending on the environment in which alginate beads are used, their stability can change: gel degradation in freshwater occurs after a couple of weeks, whereas in a marine environment it happens after only a few days (Moreira-Santos *et al*. 2004). Moreover, the stability of beads can also depend on the algal species used (Moreno-Garrido *et al*. 2005). According to Moreira-Santos *et al*. (2004), *P. subcapitata* is a good test organism to be entrapped into alginate beads, since it grows in accordance with the control acceptability criteria stated by OECD guidelines. Entrapped *P. subcapitata* cells show less growth rate compared with free algae, because of the limited diffusion of light, CO_2 and nutrient through the alginate. Nevertheless, both free and immobilised algal cells respond in the same way to pollutants.

During the algal growth inhibition test, it is possible for the algal population to recover by the end of the bioassay, especially if the pollutant is present at low concentrations. An advantage in using entrapped algae *in situ* is that the algal cells are exposed continuously to pollution, and the population recovery can be avoided. However it has potential side effects: 1) the algal growth could be affected by differences in light and temperature, 2) the mesh where the algal beads are entrapped could be covered by sediment particle accumulation, 3) the alginate barrier could be disrupted by cation chelators (phosphate, surfactant, and citrate) (Moreira-Santos *et al*. 2004).

1.6 Definition of water quality parameters

1.6.1 Dissolved Oxygen (DO)

The dissolved oxygen (DO) is the amount of oxygen gas molecules in the water. Its concentration in streams is the results of production and depletion. The oxygen in fresh water is due to: 1) photosynthetic activity by algae or mosses, 2) the transfer of oxygen from the atmosphere to the water, 3) oxygenated water coming from tributaries. At the same time the oxygen is consumed by: 1) aquatic plants respiration, 2) the breakdown of organic matter by microbial organisms, producing a Biological Oxygen Demand (BOD), 3) chemical reaction (Chemical Oxygen Demand, COD), and 4) the oxygen demand by stream bed sediments (SOD)(Cox 2003).

The amount of oxygen in the water is usually measured as milligrams/litre, or as percentage saturation, that is the quantity of oxygen in a litre of water relative to the total of oxygen that the water can retain at that temperature (EPA 2011).

The concentration of dissolved oxygen in a stream is affected by different factors such as metabolic activity rates, diffusion, temperature (cold waters show higher DO levels than

warmer waters, Table 1.1) and proximity to the atmosphere (greater is the atmospheric pressure greater is DO level in the water).

Table 1.1 - Saturated dissolved oxygen concentrations vary with temperature (modified from EPA 2011).

Temp (°C)	DO (mg/L)	Temp (°C)	DO (mg/L)	Temp (°C)	DO (mg/L)	Temp (°C)	DO (mg/L)
0	14.60	11	11.01	22	8.72	33	7.16
1	14.19	12	10.76	23	8.56	34	7.16
2	13.81	13	10.52	24	8.40	35	6.93
3	13.44	14	10.29	25	8.24	36	6.82
4	13.09	15	10.07	26	8.09	37	6.71
5	12.75	16	9.85	27	7.95	38	6.61
6	12.43	17	9.65	28	7.81	39	6.51
7	12.12	18	9.45	29	7.67	40	6.41
8	11.83	19	9.26	30	7.54	41	6.41
9	11.55	20	9.07	31	7.41	42	6.22
10	11.27	21	8.90	32	7.28	43	6.13

Moreover DO levels increase with increasing water pressure (depth in the water) and decrease with increasing salinity (Dodds 2002). Dissolved oxygen level also varies with the time of the day, season, altitude and rate of flow (Behar 1997). At higher altitudes, streams have less dissolved oxygen. Usually in healthy stream the DO concentrations fluctuate daily: during the day time, the DO levels increase because the photosynthesis predominates over the consumption of oxygen, while during the night the DO concentration decreases because the photosynthetic activities have stopped and respiration, reaeration and other oxygen consuming activities are active (Hauer and Hill 1996, Behar 1997). Figure 1.2 shows an expected daily DO cycle, using data collected from the Environment Agency's automatic monitoring station located at Carpenters road.

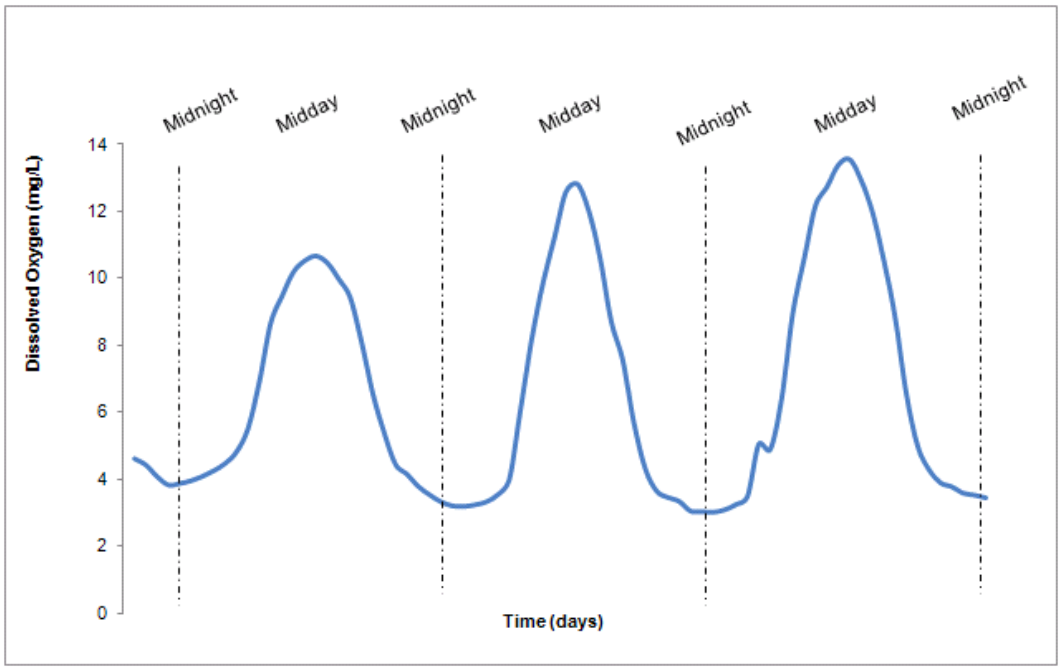


Figure 1.2 – Example of an expected daily curve of DO in river water with high ecological status. Data were collected from the Environment Agency's automatic monitoring station located at Carpenters road. During the day, the level of DO increases mostly because aquatic plants photosynthesize, with the maximum at midday. While during the night, the oxygen consuming activities are active and the DO levels decrease.

Dissolved oxygen levels in streams are often affected by human activities which affect it negatively by addition of organic waste (sewage), addition of nutrients (such as nitrates and phosphates), increasing the temperature (thermal pollution), adding chemicals, and changing the water flow (Behar 1997).

If the consumption of oxygen exceeds its production, the habitat can become anoxic (Dodds 2002). Behar (1997) declares that a very healthy stream should have a DO level ranged between 7 and 11 mg/l (Table 1.2).

Table 1.2 – Dissolved oxygen levels and their effect on the aquatic fauna (modified from Behar 1997)

DO (mg/l)	Effects on the aquatic biota
0 – 2	not enough oxygen to support life (anoxic)
2 – 4	only a few fish and aquatic insects can survive
4 – 7	good for many aquatic animals, low for cold water fish
7 – 11	very good for most stream fish

In UK, the Environment Agency has established a General Quality Assessment (GQA) scheme, composed of six quality grades for the dissolved oxygen level (DO). The quality classification is calculated on the 10^{th} percentile of the DO concentrations. DO levels should not fall below the standard presented in the classification more than 10% of the time. This classification was used by Thames 21 (Thames 21 2011) and the ranges are:

1. "bad" quality: ≤ 20%
2. "poor" quality: 20% - 49%
3. "fair" quality: 50% - 59%
4. "fairly good" quality: 60% - 69%
5. "good" quality: 70% - 79%
6. "very good" quality: ≥ 80%

1.6.2 Water temperature

Water temperature is an important parameter because it affects movement of molecules, fluid dynamics, saturation constants of dissolved gases in water (such as dissolved oxygen), and metabolic rates of organisms. Changes in water temperature are due to different factors: air temperature, stormwater runoff, groundwater inflows, turbidity, and exposure to sunlight (solar radiation is the main source of heat in surface water). Temperature variations in receiving water are also affected by human activity, such as STW effluent, domestic and industrial discharge. For those reasons heavily modified streams can present variable temperatures (Thames 21 2011).

The temperature varies naturally during the day and during the year, in both small and large rivers. These fluctuations in the temperature are important for the aquatic biota: reproduction and growth depend upon the temperature (Behar 1997, Hauer and Hill 1996). Aquatic fauna in UK streams normally necessitate temperatures below 20 °C with 8-10 °C as optimum (Thames 21 2011).

Running waters are rarely affected by thermal-stratification: only deep (> 15 m), slow moving rivers show significant differences in temperature between the bottom and the surface waters (Giller and Malmqvist 1998).

1.6.3 pH

pH is a measure of the concentration of hydrogen ions (H^+), so a measure of acidity. Ions H^+ and OH^- (hydroxide ions) formed by the dissociation of water molecules can interact with other compounds dissolved in the water and leave imbalance the equilibrium between the two ions. The water will be basic (pH > 7) if the concentration of OH^- is higher than H^+. Vice versa the water will be acidic (pH < 7) if the number of H^+ ions exceeds the number of OH^- anions. Most aquatic freshwater organisms live in a pH range between 6.5 and 8 (Behar 1997).

One of the factors, which affect the pH, is the capacity of rocks and soil in the catchment to buffer acidic precipitation caused by atmospheric pollution (the dissociation of carbonic acid in rainwater produce H^+). For example, calcareous rocks have a higher buffering capacity than granite (Giller and Malmqvist 1998).

pH level is also affected by the CO_2 concentration in the water: if CO_2 production through respiration is higher than its loss through dispersion to the atmosphere, the pH will decrease (Welch 1992). During photosynthesis, CO_2 is taken from the water, which results in an increase of pH levels.

The Freshwater Fish Directive (78/659/EEC) classifies pH ranges in streams as:

1. "too low": <6
2. ""good": ≥6 - ≤9
3. "too high": >9

1.6.4 Turbidity

Turbidity is an indicator of undissolved particles in the water and it is determinate by the amount of light that is deflected from that matter. The particles suspended in the water column could be inorganic such as silts, sands, clays, or organic such as plankton, or plant, microbes (Thames 21 2011). Normally, turbidity increases during and after a rainfall, because sediments become mixed with the water. High levels of turbidity are considered an indicator of poor water quality, since it is an indicator of matter in the water. Moreover, elevated turbidity can lead to "unhealthy" consequences for the watercourse such as damage to fish gills and eggs. High levels of turbidity block solar radiation preventing the photosynthesis, and lowering the dissolved oxygen levels. At the same time, the turbidity affects the water temperature since the particles suspended in the water absorb the heat of the solar radiation, contributing to decrease the oxygen levels (Behar 1997). However, even extremely clear waters are no guarantee of a healthy environment, since it could be an indicator of high levels of salinity or very acidic conditions (McCaffrey, 2012). In the past turbidity was measured by Formazine Attenuation Units (FTU), while nowadays the most common unit of measurement is NTU (Nephlometric Turbidity Units). Typical values for the turbidity measured in wastewater range from 70 to 2000 NTU; while in final outlet sewage treatment plant from 4 to 20 NTU (Daly 2007).

At present, the EU Water Framework Directive (2000/60/EC) does not specify a classification for turbidity as a parameter. However, as published by McCaffrey from Namoi Catchment Management Authority, Australia (2012), turbidity (NTU) could be classified in the following category:

1. "excellent": ≤10
2. "fair": 15-30
3. "poor": >30

1.6.5 Conductivity

Conductivity measures the quantity of dissolved electrolytes in the water. It is an indication that polluted water has flowed into the river under investigation, since dissolved ions concentration is increased. Normally conductivity is measured in microsiemens per centimetre (µS/cm). Ideally the conductivity in freshwater should range between 150 and 500 µS/cm to support aquatic biota, but most streams range between 50 to 1500 µS/cm (Behar 1997).

1.6.6 Nitrate and phosphate

The most common form of nitrogen in surface water is nitrate (NO^{3-}), which is derived from decomposing plants, animal waste, human sewage, and fertilizer. From the decomposition process, nitrogen is liberated as ammonium ion (NH^{4+}). Under aerobic conditions, nitrifying bacteria can oxidise the ammonium to nitrite (NO^{2-}) and then into nitrate. These reactions are oxygen consuming, due to the increased microbial respiration. At the same time, low dissolved oxygen levels could slow the oxidation process of ammonium, harming the aquatic life since ammonia (NH_3) is more toxic than nitrate. The equilibrium between the non-toxic ammonium ion and the toxic un-ionized ammonia depends on the pH and the temperature. The toxic un-ionized form increases at higher pH and higher temperatures. At similar pH, higher levels of ammonia are present in warmer water (Novak and Holtze 2005).

The unit of measurement for the nitrate is mg/L. Usually, the nitrate concentration in non-polluted waters is less than 1mg/L. Levels higher than 10 mg/L will affect the aquatic biota. For example for salmon, which is a sensitive fish, the nitrate level should be less than 0.06 mg/L (Behar 1997).

The Water Framework Directive (2000/60/EC) classification scale for the nitrate (mg/L) is:

1. “very low”: 5
2. “low”: 10
3. “moderate”: 20
4. “high”: 30
5. “very high”: 40
6. “excessively high”: >40

The readily bioavailable form of phosphorus is reactive phosphate or orthophosphate (PO_4^{3-}). It is not a risk for human health except if present at very high levels. As with nitrate, it is measured in mg/L. In general, concentrations greater than 0.1 mg/L negatively affect an aquatic system.

The Water Framework Directive (2000/60/EC) classification scale for the reactive phosphorus (orthophosphate, mg/L) is:

1. “very low”: 0.02
2. “low”: 0.06
3. “moderate”: 0.1
4. “high”: 0.2
5. “very high”: 1
6. “excessively high”: >1

Nitrogen and phosphorus are important components for the growth of plants and some microorganism, and therefore for the photosynthetic activity. Nevertheless, at the same time they are limiting elements. Their presence in water bodies could be due to single or diffuse sources, and the pollution caused by these two compounds is called eutrophication. High levels of nitrogen and phosphorus can cause toxic algal blooms (Thames 21, 2011).

1.7 Aims and main objectives

The literature describes the Lea Navigation as a heavily polluted channel which is affected by both point and diffuse pollution (treated sewage effluents, misconnections, run-off, etc), as it flows mostly through an urban environment (Snook and Whitehead 2004, Environment Agency 2008a). For this reason, the pollutants in the water are numerous and various, and it is difficult to investigate the complexity of that pollution just by chemical analysis (Belkin 2003, Struijs 2009). Moreover Thames 21 (2011) identified a lack of long-term data (biological and physical) of water quality of the "London" River Lea.

1.7.1 Aims

There were two main aims of this project:

1. to detect the likely causes and sources of the poor water quality in the Lea Navigation, downstream of the confluence with Pymmes Brook;
2. to use the investigation as a case study to evaluate a multi-parameter approach involving both conventional and novel monitoring techniques.

1.7.2 Key objectives

Key objectives of this study were:

1. Selection of monitoring sites for water sample collection.
   The area investigated in this project is the part of the Lea channel downstream of Pymmes Brook, which receives the final effluent of Deephams sewage treatment work (STW). To study the contribution of the STW discharge on the water quality of the Lea Navigation, water samples will be collected and analysed from Pymmes Brook (at the confluence with the channel) and two other stations downstream of the Brook (opposite Warwick reservoir and at Springfield Park, which is located downstream of the Marina). Tottenham Hale site, located in the Lea Navigation upstream of the confluence with Pymmes Brook will be investigated in order to use it as control station. Additional sampling sites may be added during the programme.

2. Collection and collation of physico-chemical data from the EA automated monitoring stations.
   In the present project, data over a period of two years are to be analysed with descriptive statistics on seasonal basis to investigate likely differences among stations, and perform a thorough correlation analysis. The main aim is to understand the general level of water quality and to identify the area to investigate. Physico-chemical data were collected from all the available stations:

1) near the Deephams STW; 2) at the tributaries, which discharge in the Lea Navigation; 3) upstream and downstream of Pymmes Brook confluence.

3. Investigation of water quality using conventional algal inhibition bioassays.
   To investigate the potential toxicity of the river water of the Lea Navigation and provide a trend in toxic pressure in the channel, standard algal assessments are to be performed. The utility of these bioassays is to study the ecotoxicity of the water samples, even if the nature of the pollutants is unknown. Algal growth inhibition tests will be carried out according to OECD (Organisation for Economic Co-operation and Development) guidelines, on a seasonal basis, employing river water samples collected at the selected monitoring sites. The test organism employed in those studies will be *Pseudokirchneriella subcapitata*, a green alga easy to grow in the laboratory and extremely sensible to water pollution.

4. Development a protocol for CellSense whole cell biosensor monitoring of water quality.
   The standard algal test is the one of the most common survey used to investigate water pollution, but it is time consuming. In order to develop a more rapid method, the exploitation of CellSense whole cells biosensors will be explored. River water samples collected from different sampling stations will be analysed with CellSense bacterial and algal biosensors.

5. Analysis of chemical and bacterial properties of water samples.
   Chemical assessments will be conducted at the Environment Agency laboratories in the effort to identify the likely causes of any chemical pollution, detected with the algal bioassays. At the same time coliform populations will be investigated to determine any link with the low dissolved oxygen levels present in the river water, coming out from Deephams STW effluent and misconnections.

6. Investigation of complementary assays, identification of including seasonal variations in physico-chemical properties, and the use of *in situ* algal monitoring.
   Physico-chemical surveys will be conducted seasonally at several points along the Lea Navigation, from upstream the confluence with Pymmes Brook to downstream Lea Bridge Weir, and along the River Lea, to optimize the water quality monitoring resolution in the Lea channel. The use of in situ bioassay monitoring using algae entrapped in alginate beads will be explored.

2 Materials and methods

In this current research a series of techniques were used for the investigation of the water quality of a stretch of the Lea Navigation in the North East London. An overview of the Lower Lea catchment was given by the analysis of raw data, such as physico-chemical parameters, rainfall, river flow, and Deephams sewage treatment work discharge provided by the Environment Agency, the Meteorological Office, and Thames Water. The river toxicity was tested by an algal growth inhibition test, whole cell CellSense biosensors, *in situ* measurements of physico-chemical parameters, and *in situ* biological assay with algae entrapped in alginate beads. All the laboratory experiments were carried out at the Institute of Biomedical and Environmental Science and Technology (iBEST) in Luton.

2.1 Sampling

2.1.1 Sampling sites

The study of the data available in the literature clearly showed that one of the major concerns regarding the water quality in the Lea Navigation was the Deephams sewage treatment works discharge, which flows in the Lea channel through Pymmes Brook. For this reason, the first algal growth inhibition tests were performed with water collected from four locations (Figure 2.1):

1. Lea Navigation, at Tottenham Hale upstream Tottenham Lock;
2. Pymmes Brook, at the confluence with Lea Navigation;
3. Lea Navigation, opposite Warwick Reservoir;
4. Lea Navigation at Springfield Park, downstream of the Marina.

The Tottenham Hale site was located upstream of the confluence between the Lea Navigation and Pymmes Brook. Generally, when monitoring the effects of inflow water in a receiving body, the water upstream of the incoming water is considered as control. Tottenham Hale monitoring station was chosen as the control site for four reasons: 1) it was located upstream of Pymmes Brook; 2) it presented the same physical characteristics of the sites downstream of Pymmes Brook; 3) it was easily accessible; 4) at this site, the water quality was better than the water quality downstream of Pymmes Brook, as described by historic data presented by the Environment Agency. The good quality of the river water at Tottenham Hale was further confirmed by ecotoxicity analysis and the collection of *in situ* physico-chemical parameters, which have been conducted in this study.

Results from the preliminary algal growth tests showed that the investigation of the Lea Navigation at the site opposite Warwick reservoir did not give any further information

about the water quality of the channel. For this reason, this station was not investigated further.

The collection *in situ* of physico-chemical data showed a detailed picture of the water quality in the area under investigation. Low dissolved oxygen levels were detected in the Lea channel downstream of the confluence with Pymmes Brook, in particular at the confluence with Stonebridge Brook and at Lea Bridge weir. Improved dissolved oxygen levels were detected in the river Lea downstream of the weir, whose water was tested to investigate any ecological improvement in the natural reach. As consequence of the *in situ* surveys, biological and chemical assessments were conducted at three more sites (Figure 2.1):

1. Lea Navigation, at the confluence with Stonebridge Brook;
2. Lea Navigation, at Lea Bridge weir;
3. River Lea, downstream the weir at Hackney Marshes.

The investigation of these six sampling sites allowed the investigation of in-stream variation of the water quality, as well as the study of discharges, showing how the Lea Navigation water quality was negatively affected by incoming flows such as Pymmes Brook and Stonebridge Brook.

Figure 2.1 – Map of the sampling sites for algal growth inhibition tests and CellSense biosensor tests. Six stations were located along the Lea Navigation from Tottenham Hale, upstream of Pymmes Brook, to the Lea Bridge weir. One station was located downstream of the weir, in the River Lea (The raster map is provided by OpenStreetMap - Creative Commons-Share Alike License [CC-BY-SA]).

2.1.2 Sampling procedure

A risk assessment was undertaken before collection of any river water samples. The containers used to collect the water were sterilized, screw-top, glass bottles. Samplings were performed from a footbridge where possible, or from the bank. The bottles were rinsed three times with the river water, and then filled to the top to avoid the diffusion of volatile compounds into any air space. Once the sample was collected the container was closed, dried with a paper towel, and labelled with date, time, and location. The water samples were brought to the laboratory and kept in the dark at 4°C until analysis; all the tests were conducted within 48 hours from the sample collection, according to Environment Agency guidelines (2008b).

2.2 Methodologies

2.2.1 Correlation analysis

To monitor the water quality along the Lea Navigation and some of its tributaries continuously, the Environment Agency (EA) had placed automated water quality monitoring stations (AWQMS) (Figure 2.2). Each station collects data approximately every 30 minutes of the following parameters: temperature, dissolved oxygen (mg/L and %), pH, total ammonia (mg/L), conductivity (μS/cm) and turbidity (NTU).
In this project, correlation analyses have been performed in order to identify any correlation among those physico-chemical parameters, over a period of 2 years (summer 2010-spring 2012). In particular, the focus was on the correlation of the dissolved oxygen, considered as the prime water pollution indicator.

In statistics, a correlation analysis is used every time it is required to establish the relation between two continuous variables. Correlation coefficients vary between -1.00 and +1.00. The direction of the correlation can be either positive or negative. If it is positive, the coefficient has a positive value and it means that with the increase of the variable X there is an increase in the variable Y. If it is negative, the coefficient has a negative value and with the increase in one variable the other decreases. The correlation magnitude is measured by the value of the coefficient. For perfect positive correlation, the coefficient value would be +1.00. On the other hand, for a perfect negative correlation the coefficient value would be -1.00. No correlation between the two variables under investigation is expressed by 0. Indicatively a correlation ± 0.50 suggests a significant relationship, even if managing correlations it is better to look at stronger relationships such as < - 0.70 or >0.70 (Reimann *et al*. 2008).

The most used correlations are: 1) Pearson (Galton, 1889, 1890), which could be used when data are normally distributed; 2) Spearman rank (Spearman, 1904) and Kendall-tau (Kendal, 1938), which are not sensitive to data distribution (Reimann *et al*. 2008).
In this project the Spearman rank correlation was performed, since most of the data were not normally distributed.

Rainfall data collected in the area of investigation were provided by the Meteorological Office (Figure 2.2) and were compared to the variations of the physico-chemical parameters.

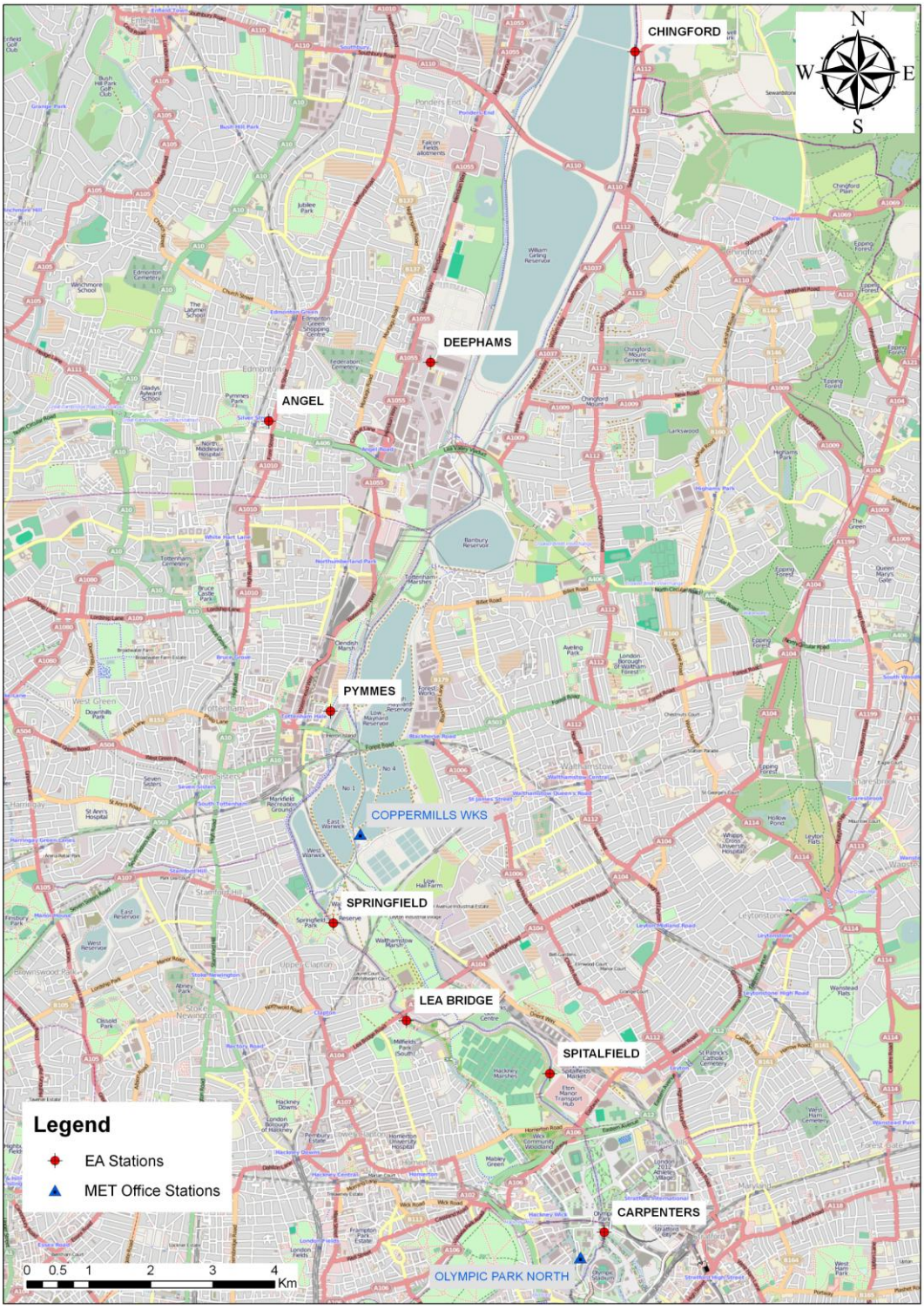


Figure 2.2 – Automated water quality monitoring stations (AWQMS) controlled by the Environment Agency and Met Office stations in the area under investigation (The raster map is provided by OpenStreetMap - Creative Commons-Share Alike License [CC-BY-SA]).

2.2.2 Algae and Cyanobacteria

2.2.2.1 *Pseudokirchneriella subcapitata*

Pseudokirchneriella subcapitata is a green alga (phylum Chlorophyta), eukaryotic unicellular, and motile. It is a planktonic species living in both oligotrophic and eutrophic freshwaters. Cells are solitary with helical shape, usually semi-circularly curved, and seldom clump together. Its chloroplasts are delimited by the double-membrane of the chloroplast envelope. *P. subcapitata* has both chlorophyll a and b (Lee 2008).

2.2.2.2 *Synechococcus leopoliensis*

Synechococcus leopoliensis is a cyanobacterium or blue-green alga (phylum Cyanophyta). The main photosynthetic pigment is chlorophyll a. It is a prokaryotic alga, so the photosynthetic thylakoids are not enclosed in organelles such as chloroplast. They are unicellular (order Chroococcales), free-living organisms able to fix atmospheric nitrogen. Photosynthesis and nitrogen fixation are alternated: during light period the photosynthetic activity is activated, while nitrogenase is inactivated; vice versa during dark period the nitrogen fixation starts and the photosynthesis stops. *S. leopoliensis* is a freshwater species (Lee 2008).

2.2.2.3 Culturing and maintenance

New algal cultures were set up every week to ensure healthy algal populations. The green micro-alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, CCAP 278/4) and the blue green algae *Synechococcus leopoliensis* (CCAP 1405/1) were obtained from the Culture Collection of Algae and Protozoa, Ambleside, Cumbria, UK. Three days before the start of the test, new cultures were established by adding 1 ml from a 1 week old algal culture (with optical density ≥ 1 at 550 nm) to 50 ml of 3N-BBM+V medium for *P. subcapitata* (Bold-Basal Medium with 3-fold Nitrogen and Vitamins; Appendix II) and to 50 ml of BG11 medium for *S. leopoliensis* (Blue-Green; Appendix I). The optical density was measured with an Eppendorf Biophotometer plus. Cultures were set up in sterile glass conical flasks, plugged with sterile, non-absorbent cotton wool to allow aeration. All the algal cultures were incubated in a shaking cabinet at 150 rpm (Model G25 – New Brunswick, Scientific CO. INC – Edison, New Jersey, U.S.A.), under continuous “cool-white” fluorescent illumination, and at a constant temperature of 23 ± 2 °C (OECD 2006). Both the algal species were used as a biocatalyst for biosensor tests, but only *P. subcapitata* was employed in growth inhibition tests.

2.2.3 Bacteria

2.2.3.1 Escherichia coli

Escherichia coli is a Gram-negative bacterium, rod-shaped, belonging to the Enterobacteriacae family. Many non-pathogenic strains of *E. coli* occur widely in nature (both soil and water) including the intestinal tracts of humans and other vertebrates, while some others are pathogenic and they can cause severe diseases under certain conditions. *E. coli* is one of the most studied bacteria and it is a key test organism in several research fields (including pollution monitoring) because of its fast growth rate, its large population size, and its easy and low-cost maintenance in the laboratory (Belkin 2003, Madigan and Martinko 2006).

2.2.3.2 Culturing and maintenance

Escherichia coli (NCIMB 8277) was selected as the biosensor biocatalyst because: 1) it is easy to handle in the laboratory (Ding 2009); 2) it has been described as a receptive bacterium to toxicants (Ding 2009); 3) it has been used in biosensors assays, including CellSense biosensors (Evans *et al*. 1998, Farré *et al*. 2001, Wex *et al*. 2006). A new bacterial culture was prepared daily. Using a sterile loop *E. coli* cells were transferred from an agar slope to a conical flask containing 20 ml of nutrient broth no.2 (Lab M). Then the flask was incubated in a shaking incubator (150 rpm) at 37 °C for 16 hours to ensure a good bacterial population for biosensor immobilization (Wex *et al.* 2006, Ding 2009). From the open slope, three subcultures were prepared (reference, closed and working cultures), left to grow at 37 °C for 48 hours, and then stored at 4 °C until required. Working slopes were used to set new bacterial population in growth medium, and so renewed weekly. New subcultures were prepared on monthly basis from closed cultures, to give fresh reference, closed and working slopes.

2.2.4 River water pre-concentration

2.2.4.1 Solid phase extraction

Solid phase extraction (SPE) was performed using silica based packing (ENVI-18,5 g, SUPELCO), which allowed a reversed phase separation, with a polar or moderately polar sample matrix (mobile phase) and a non-polar stationary phase. Organic analytes from polar mixture (as water) are retained because of non-polar – non-polar attraction forces (Van der Waals forces). The water samples were previously centrifuged for 10 minutes at 3500 rpm to remove particles and avoid the column blockage. According to the manufacturer's extraction protocol (Charlton Scientific, Independent Laboratory Suppliers, http://www.charltonsci.co.uk/) the column was first conditioned with 5 ml of methanol (Sigma 154903) and then washed with 5 ml of distilled water. Then 100 ml river water samples were passed though the column: the polar compounds were drawn out of the

cartridge, while the non-polar compounds were retained in the silica bed. All the 100 ml samples were collected to test the toxicity of the polar river sample fraction. Finally the non-polar analytes were eluted with 5 ml of acetone (Sigma 34480), a non-polar solvent. After acetone evaporation by heating and stirring, the solutes collected were re-suspended in 100 ml of distilled water, and this solution was used to test the non-polar river sample fraction toxicity by algal growth inhibition test and bacterial biosensors.

2.2.4.2 Rotary evaporator

A rotary evaporator was employed in the attempt to concentrate the contaminants dissolved in the river water samples. The key point of the rotary evaporator is to create an environment of low air pressure at a raised temperature, where the test solution will quickly boil off leaving only solutes. The pressure is controlled by a vacuum pump, while the temperature of the test solution is increased by immersion in a water bath. As the solvent begins to evaporate, the vapour condenses back to liquid, because of a condenser coil, and it has collected in another flask.

In this project, the first approach was to evaporate 200 ml of river water and the solutes were re-suspended in 5 ml of the same river water sample. However, the biosensor test did not show any significant changes in the outcome signals between samples and between the pre- and the post- addition of either 1 ml or 2 ml of the mixture to the test solution. Therefore, 100 ml of river water samples were evaporated and the solutes were dissolved into 1 ml methanol (Sigma 154903). Methanol samples were kept in screw-top centrifuge tube in the dark at 4 °C over night and then tested with bacterial CellSense biosensors. Pure methanol was used as a blank for the test and it was stored in screw-top centrifuge tubes at 4 °C in the dark.

2.2.5 Algal growth inhibition test

In this project, algal growth inhibition was used to measure the level of pollution in the stretch of Lea Navigation under investigation. River water samples were tested over a period of two years, giving long-term biological data, which has never been done for this part of the Lower Lea catchment before.

Tests were performed following the guidelines stated by the Organization for Economic Co-operation and Development (OECD 2006) and the Environment Agency protocol (Environment Agency 2008b). The test organism employed was the green alga *P. subcapitata*. River water samples were first pre-treated by centrifugation at 3500 rpm for 15 minutes in order to remove suspended solids; then all test solutions were supplemented with the same concentrations of OECD nutrient medium (Appendix III). Investigations were performed in 100 ml conical glass flasks containing 25 ml of the test sample, with at least 4 replicates for each testing sample. Flasks were capped with cotton air-permeable stoppers. The pH should increase between 0.5 and 1.5 units, as reported

by OECD guidelines (2006). Finally, an algal inoculum was added to give a starting optical density (OD) of 0.05 at 550 nm (corresponding to 12 x 10^4 cells/ml). Test vessels were placed in an incubator shaker (Model G25 – New Brunswick, Scientific CO. INC – Edison, New Jersey, U.S.A.) at 23 ± 2 °C, under continuous "cool white" fluorescent light, whilst being shaken at 150 rpm. The test duration was of 72 ± 4 hours and the OD was checked every 24 hours, with a spectrophotometer (Eppendorf Biophotometer plus) at 550 nm, using semi-micro cuvetts (PS,1 styrofoam, Fisher Brand). The cell density values were then converted into cell concentrations by the following equation:

$$\text{Cell concentration (cell/ml)} = (2{,}496{,}759 * OD_{550}) + 4{,}224 \qquad (2.1)$$

Details of the development of the equation are given in Appendix IV.

2.2.5.1 Data processing

The criteria for the validity of the test were checked, as reported in the OECD guidelines (2006):

- the mean algal cell density in the control should be increased by a factor of more than 16;
- the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35 %;
- the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.

The test endpoint is the inhibition of growth, i.e. the reduction in biomass compared to a control. In this project, the average specific growth rates were estimated as logarithmic increases in biomass during the 72 hour period. It was calculated for each single flask according to the following equation:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} \ (\text{day}^{-1}) \qquad (2.2)$$

where:

μ_{i-j} is the average specific growth rate from time *i* to *j*;

X_i is the biomass at time *i*;

X_j is the biomass at time *j.*

For each sampling site group and control group the growth rate average and the variance were calculated. According to the OECD guidelines, the average specific growth rate was calculated using a nominally inoculated biomass (i.e. 12 x 10^4 cells/ml) as the starting value, in order to have a greater accuracy.

From the average specific growth rates, the percent inhibition of growth rate was estimated for each sampling site, according the equation:

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \cdot 100 \qquad (2.3)$$

where:

$\%I_r$: percent inhibition in average specific growth rate;

μ_C mean value for average specific growth rate (μ) in the control group;

μ_T average specific growth rate for the treatment replicate.

2.2.6 Whole cell biosensors

Both algal (*P. subcapitata* and *S. leopoliensis*) and bacterial (*E. coli*) biosensors were used for rapid assessment of toxicity. The instrument used in this project was CellSense (Harvey-Coleman, Leeds), a mediated amperometric biosensor that allows real time and near continuous monitoring (measured at 4 s intervals) of metabolic status of the immobilised biocatalyst pre and post exposure to a pollutant.

2.2.6.1 Electrode component

The sensors used in this project incorporated two screen-printed electrodes: 1) a carbon working electrode, with a surface area of 5 mm in diameter, where the cells were immobilized; and 2) an Ag/AgCl electrode, both reference and counter (Figure 2.3).

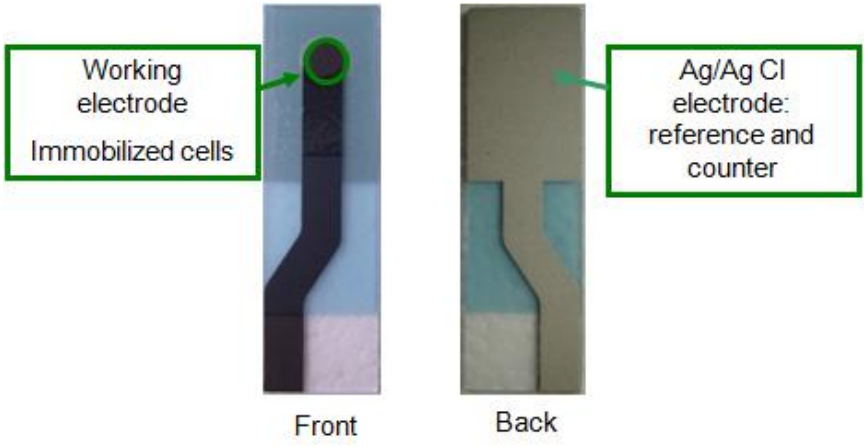


Figure 2.3 – Screen-printed sensor employed in this study. It was a two electrode system, with a carbon working electrode where cells were immobilised (front), and a Ag/AgCl reference electrode (back).

2.2.6.2 Immobilisation of *P. subcapitata* and *S. leopoliensis* biosensors

P. subcapitata and *S. leopoliensis* cultures at the exponential phase were diluted to 0.5 and 1 OD_{550} respectively, and 1 ml aliquots were harvested by centrifugation at 10,000 rpm for 6 minutes using an Eppendorf 5414 centrifuge. After centrifugation, the supernatant was discarded and the pellets were re-suspended in 100 µl of 0.85 % saline. Then 20 µl aliquots of cell suspension were immobilized onto a 0.4 µm pore size Anopore membrane (Whatman International, Maidstone, Kent), and placed facing the working electrode. The membrane was held in place by an adhesive area. Biosensors were held in growth medium for 30 minutes at room conditions before the start of the test.

2.2.6.3 Immobilisation of *E. coli* biosensors

E. coli cells were harvested from the culture prepared the day before and incubated at 37 °C and 150 rpm overnight. After measuring the optical density, which was between 1.5 and 1.7 at 490 nm, 1 ml of culture was pipetted into an Eppendorf tube and centrifuged for 2 minutes at 10,000 rpm. Then the supernatant was removed and the pellet was re suspended in 1 ml of 0.85 % saline. This procedure was repeated three times; the third time the pellet was re-suspended into a saline aliquot related to the optical density of the culture (e.g. 150 µl when the OD_{490} was 1.5). A 20 µl aliquot of that cell suspension was pipetted onto a 0.4 µm pore size Anopore membrane (Whatman International, Maidstone, Kent), which was pressed firmly onto the biosensors in order to position the *E. coli* cells in direct contact with the working electrode.

Monitoring *E. coli* biosensors involved the use of saline substrate medium (SSM), which was prepared dissolving a respiratory substrate cocktail (composed by equal concentrations of D-glucose, sodium succinate and sodium lactate) in 0.85 % saline. The final substrate concentration for each assay vial was of 5 mM.

Bacterial biosensors were held in SSM for 30 minutes at room conditions before the start of the test.

2.2.6.4 Redox mediators

Two different electrochemical mediators were used: potassium hexacyanoferrate(III), and *p*-benzoquinone.

Potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$) (Sigma 60299) is a non-penetrating mediator, and it was used to monitor *E. coli*. *E. coli* respiratory activity can be monitored by using a non-penetrating mediator, accessing redox events on the outer surface of the cell membrane. Potassium ferricyanide was dissolved in 0.85 % saline giving a stock concentration of 500 mM. During the tests 100 µl of the stock solution was added to each 10 ml assay vial to achieve a final concentration of 5 mM.

p-Benzoquinone (*p*BQ, $C_6H_4O_2$) (Sigma 12309) is a penetrating mediator, and it was employed to investigate *P. subcapitata*. To monitor the metabolism of eukaryotic organisms a penetrating mediator is necessary, as metabolic activities are located in complex organelles within the cells. The mediator was dissolved in 0.85 % saline giving a stock solution of 100 mM. During the experiments 100 µl of the stock mixture was added to each 10 ml assay vial to achieve a final dilution of 1 mM.

2.2.6.5 Operation of the CellSense instrument

Once the biosensors were acclimatised, they were placed into the connectors in the instrument lid. Closing the lid the biosensors electrodes were monitored in 10 ml stirred (700 rev min^{-1}) flat bottomed glass vials (Evans *et al*. 1998). First tests were conducted applying +550 mV potential to the working electrode, as suggested in literature (Evans *et al*. 1998, Farré *et al*. 2001). Later the potential was decreased to +200 mV to minimise the (electro)-chemical interference, which was masking the biological response.

Testing the photosynthesis response involved a red light-emitting diode (LED) illumination of the biosensors with a light intensity of 125 mcd and a peak wavelength of 635 nm.

Initially algal and bacterial biosensors were tested in growth media or SSM respectively to evaluate their metabolic activity under optimal conditions.

2.2.7 Biosensors for river water ecotoxicity assessment: standard protocol (protocol 1)

Bacterial and algal biosensors monitoring was performed following the standard operating protocol as reported in the literature (Evans *et al*. 1998). The first phase was to monitor biosensors in a solution providing optimal conditions for normal metabolic activity of the biocatalyst (SSM for *E. coli* and growth medium for *P. subcapitata* and *S. leopoliensis*). After a few minutes in these solutions, 100 µl of either *p*-benzoquinone (working with the alga and the cyanobacterium) or potassium ferricyanide (working with *E. coli*) were added to each assay vial in order to have a final concentration of 1 mM or 5 mM respectively. When the current signal stabilised, the lid was opened and the biosensors were exposed to mediator supplemented river water samples by replacing the SSM/growth medium vials with river water sample vials. Working with *E. coli,* the river water samples were supplemented with SSM. Generally, when the experiment involves the testing of known concentrations of toxicants or mixtures, these are added to the initial bathing solution through the ports located on the instrument's lid. However, working with environmental samples such as river water, involves exposing biosensors to the undiluted sample. At least four biosensors were used to test each sample. Biosensors bathed in either SSM or growth medium were used as control. The potential applied to the working electrode was +550 mV.

Nevertheless, testing environmental samples required changes to be made to the standard protocol (protocol 1), and in this project two different protocols were developed to test the river water toxicity from different sampling sites in the area under investigation. Details of Protocol 2 and Protocol 3 are given in Chapter 5.

2.2.8 Algal beads: immobilization and de-immobilization procedure

Standardised protocols, such as algal growth inhibition tests, are necessary to validate results, but they do not reflect all the variables presents in the natural environment (Twist *et al*. 1997). To monitor the water toxicity to *P. subcapitata* in the natural environment, *in situ* assays were conducted with *P. subcapitata* cells entrapped in sodium alginate (Moreira dos Santos *et al*. 2002). Alginate beads were prepared by modifying the protocols published by Rawson (1989) and Moreira dos Santos *et al*. (2002). A sodium alginate solution (2 % or 3 %) was prepared, stirring and heating until the complete dissolution of the alginate (alginic acid sodium salt, from brown algae, Sigma 71238). Aliquots of four days old algal cultures and with known optical density (~ 0.5 at 550 nm, 12 x 10^4 cells/ml) were centrifuged at 10,000 rpm for 6 minutes; 18 Eppendorf tubes of 1 ml volume were prepared each time. After centrifugation, most of the supernatant was removed except for a few microliters, used to re-suspend the pellets. This algal suspension was then mixed with 20 ml of sodium alginate at room temperature. Using a 20 ml syringe (fitted with a needle), the algae-alginate mixture was dropped into a calcium chloride solution at 4 °C (Sigma 223506), in order to allow the alginate to harden. The resulting beads were stood in the $CaCl_2$ (2 % or 4 %) stirred solution for about 30 minutes. Then they were washed four times with distilled water and kept at 4 °C in the dark in distilled water for 3 days, until their placement in the field.

To measure the bead optical density it was necessary to dissolve them into 3 % trisodium citrate (Sigma C3434). In order to have more consistency, at least 3 sets of 10 beads each (randomly selected) were dissolved in 1 ml 3 % trisodium citrate and checked for the optical density. A set of 10 alginate beads (without algae) was used as blank. The algal cells density was tested before the beads were placed in the field and at the end of the test (3 / 7 days).

The growth of *P. subcapitata* cells entrapped in 2 % sodium alginate and hardened into 2 % calcium chloride was first tested in the laboratory in both nutrient medium and river water samples. The river water was centrifuged and enriched with nutrients following the procedure used for the algal growth inhibition test. Forty beads were then placed into flasks with 50 ml of test solution and the optical density was measured every 24 hours. In order to monitor a situation similar to the natural environment and to expose the algal cells to pollutants present in the river water in a semi-continuous mean, test solutions were changed every 24 hours with fresh samples. The normality of the data set was tested with

Shapiro-Wilk test and a one-way ANOVA was performed to identify any significant difference between samples (Tukey was used as Post Hoc test).

2.2.9 *In situ* river water monitoring with algal beads

In order to monitor the water quality *in situ* using *P. subcapitata*, algae entrapped in alginate beads were positioned at the same six locations where river water samples were collected to be examined with bioassays (Figure 2.1):

1. Lea Navigation at Tottenham Hale;
2. Pymmes Brook at the confluence with Lea Navigation;
3. Lea Navigation at the confluence with Stonebridge Brook;
4. Lea Navigation at Springfield Park;
5. Lea Navigation at Lea Weir Bridge;
6. The River Lea at Hackney Marshes.

The beads (50 for each sampling site) were trapped in a nylon net, which was closed in a metal cage to avoid disturbance from aquatic birds and fishes and kept at 20 cm below the water surface to guarantee light provision (Corrêa *et al*. 2009). After 3 or 7 days of exposure, the beads were collected and brought in distilled water to the laboratory for the optical density measure.
Different concentrations of both alginate and calcium chloride were investigated in order to optimize the protocol (Table 2.1).

Table 2.1 – Different combinations of sodium alginate/calcium chloride/time tried in order to optimize the protocol for *in situ* monitoring with algal beads.

Sodium alginate (%)	Calcium chloride (%)	Time test
2	2	3 days / 7 days
2	4	3 days / 7 days
3	4	3 days / 7 days

2.2.10 *In situ* physico-chemical parameters monitoring

Alongside biological monitoring, physico-chemical parameters were monitored. In the area under study, the Environment Agency (EA) had three automated water quality monitoring stations (AWQMS). These stations are located in Pymmes Brook, and in the Lea Navigation at Springfield Park and at Lea Bridge Weir (Figure 2.2). Each station collects data approximately every 30 minutes of the following parameters: temperature, dissolved oxygen (mg/L and %), pH, total ammonia (mg/L), conductivity (μS/cm) and turbidity.

The preliminary analysis of the stations records indicates a clear pattern of pollution in the studied stretch of the river. It appears that Pymmes Brook is the most likely polluted input but other likely pollution sources could be causes of low DO levels at Springfield and Lea Bridge Weir area. In order to answer these questions and increase the spatial data resolution, physico-chemical parameters were determined at several locations along the river (Figure 6.1) using a multiparametric probe (YSI 6820), provided by the EA.
The parameters detected were:

1) dissolved oxygen (mg/l and %, optical sensor)
2) pH
3) conductivity (µS/cm)
4) total ammonia (mg/l)
5) temperature (°C).

Data were collected approximately every 50 m starting at Tottenham Hale site, sampling also in the proximity of likely working discharges. The final dataset included 23 sampling stations.
Measurements were carried out dipping the probe at a distance of 1.5 m from the bank (left or right depending upon the accessibility), or from the middle of footbridges where possible. Only one measuring point in the middle of the water column was taken.

The probe was calibrated by the EA and according to the manufactures instructions. The dissolved oxygen sensors were calibrated in the field prior to the data recording.
Surveys were conducted seasonally starting from summer 2011 for one year. Specifically the physico-chemical parameters were collected on:

- 22nd of August 2011 (summer)
- 31st of October 2011 (autumn)
- 09th of January 2012 (winter)
- 23rd of April 2012 (spring).

2.2.10.1 Data processing

The aim of this study was to answer questions raised by the preliminary analysis of the available data from the EA. There was a need for a more detailed physico-chemical dataset to explain the DO pattern seen in the Lea Navigation at Springfield Park and at Lea Bridge Weir.
The data collected in the field were used to produce a series of spatial maps of the stretch of the Lea under investigation. The data were interpolated with the inverse distance weighted algorithm. Using the following equation, a value can be predicted in an unsampled location using the nearby samples:

$$u = \sum_{i=0}^{N} \frac{w_i \cdot x_i}{\sum_{j=0}^{N} w_i} \quad (2.4)$$

Where u is a value in an unsampled location, x_i is a sample at the i^{th} location and w_i is the weight assigned to each sample point. The weights are assigned based only upon the distance of each sample to the point to be estimated, according to the following equation:

$$w_i = \frac{1}{d(u; x_i)^p} \quad (2.5)$$

Where $d(u; x_i)$ is the distance between the sample x_i and the unsampled location u and p is the power parameter. In this study, a power of 2 was used, because it is the most common power function employed in surface mapping and it gives higher weight to the surrounding points (compared with the weights of distant values), presenting a good level of closeness to the observed data (Schloeder *et al*. 2001, Perry and Hollis 2005).

With this interpolation method it was possible to estimate a regular grid of points with a horizontal resolution of 10 m from Tottenham Hale to the natural part of the river after Lea Weir. The results of this study are a series of maps, for each measured physico-chemical property and for each sampling survey, i.e. one map for each season.

2.2.11 River water chemical and bacterial analysis

Complementary to the biological monitoring, chemical and bacterial analyses were arranged in the attempt to identify a likely cause to the algal inhibition growth. For this reason, river water samples were collected in different sampling sites in the area of study and chemically analysed by Environment Agency's laboratories.

The focus was on two main chemical species:

- organic volatile compounds, since bioassays showed evidence of algal growth inhibition. The method applied was gas chromatography–mass spectrometry (GCMS);
- polar compounds, since there was evidence that polar pollutants were affecting the algal population. The method used was liquid chromatography–mass spectrometry (LCMS).

Those analyses were conducted on water samples collected on 28/06/2011 and on 07/11/2011.

In addition, coliforms concentration was analysed in river water samples collected on 30/01/2012. Total coliforms (cfu/100ml), faecal coliforms (cfu/100ml), and faecal

streptococci (cfu/100ml) were determined with NLS B T COLI ENV technique by membrane filtration (Environment Agency 2012).

3 River Lea lower catchment preliminary assessment

In this chapter an overview of the area under investigation is provided on: physico-chemical parameters recorded by automated monitoring station, historic river water quality (chemistry, biology, nutrients), and river flow rate provided by the Environment Agency; rainfall data registered by the Meteorological Office; and Deephams sewage treatment works (STW) flow discharge provided by Thames Water.

3.1 Physico-chemical parameters from Environment Agency automatic stations

The water quality of the lower Lea catchment is continuously monitored by the Environment Agency through automated monitoring stations located at several sites. The main intention of this survey was to examine and compare the differences in water quality parameters at points along the river and over the seasons. The variations of the water quality of this particular stretch of the Lea Navigation are due to both chronic contamination for contaminants present continuously in the water (even in small amount), and single events, for instance rainfall, which can cause acute effects on the water quality (sediments mixing, run-off, etc). The aim of this study was to investigate the chronic pollution in this particular stretch of the Lea Navigation.

Data from nine different automated monitoring stations (Figure 3.1) were collected and analysed over a two years period: from 21/06/2010 to 20/06/2012. The parameters considered were: temperature (°C), dissolved oxygen (%), pH, total ammonia (mg/L), conductivity (μS/cm) and turbidity (NTU). Values were grouped by season: summer 2010, autumn 2010, winter 2010-2011, spring 2011, summer 2011, autumn 2011, winter 2011-2012, and spring 2012.

The first part of the analysis consisted of removing the outliers and it was done in two steps: 1) data with a value of “mean ± 2 standard deviation” were deleted (Reimann *et al* 2008); then 2) values attributable to malfunctioning of the probe (e.g. negative data) were deleted (examples are presented in Appendix V). In some cases automated stations did not record data that had been classified here as NA (not available). Even after outliers had been deleted, the standard deviation of the mean was great on some occasions. For this reason, it was decided to use the median value results. Therefore, the median absolute deviation (MAD) was calculated as a measure of spread. MAD is a robust method equivalent to the standard deviation and it could be used even if the data set show deviations from the normal distribution. In addition minimum value, maximum value, and mean were estimated (Appendix VI). For the dissolved oxygen the 10^{th} percentile was also calculated (paragraph 3.1.1), in order to use the classification provided by the Environment Agency and as used by Thames 21 (Thames 21 2011).

Since there was evidence that the dissolved oxygen levels in the area under investigation were low and that it is known that the oxygen in the water is a useful indicator of pollution, correlation analyses (Spearman rank correlation) were performed with R software (open source software, available at http://www.R-project.org) to detect any relationship between physico-chemical water parameters and the dissolve oxygen.

For a better understanding of the results, the Environment Agency (EA) automated monitoring stations and the Meteorological Office (Met Office) operational sites are presented in Figure 3.1. All the stations are situated along concrete channels, except for Spitalfield and Carpenters which are located in the River Lea with non-concrete banks and bed.

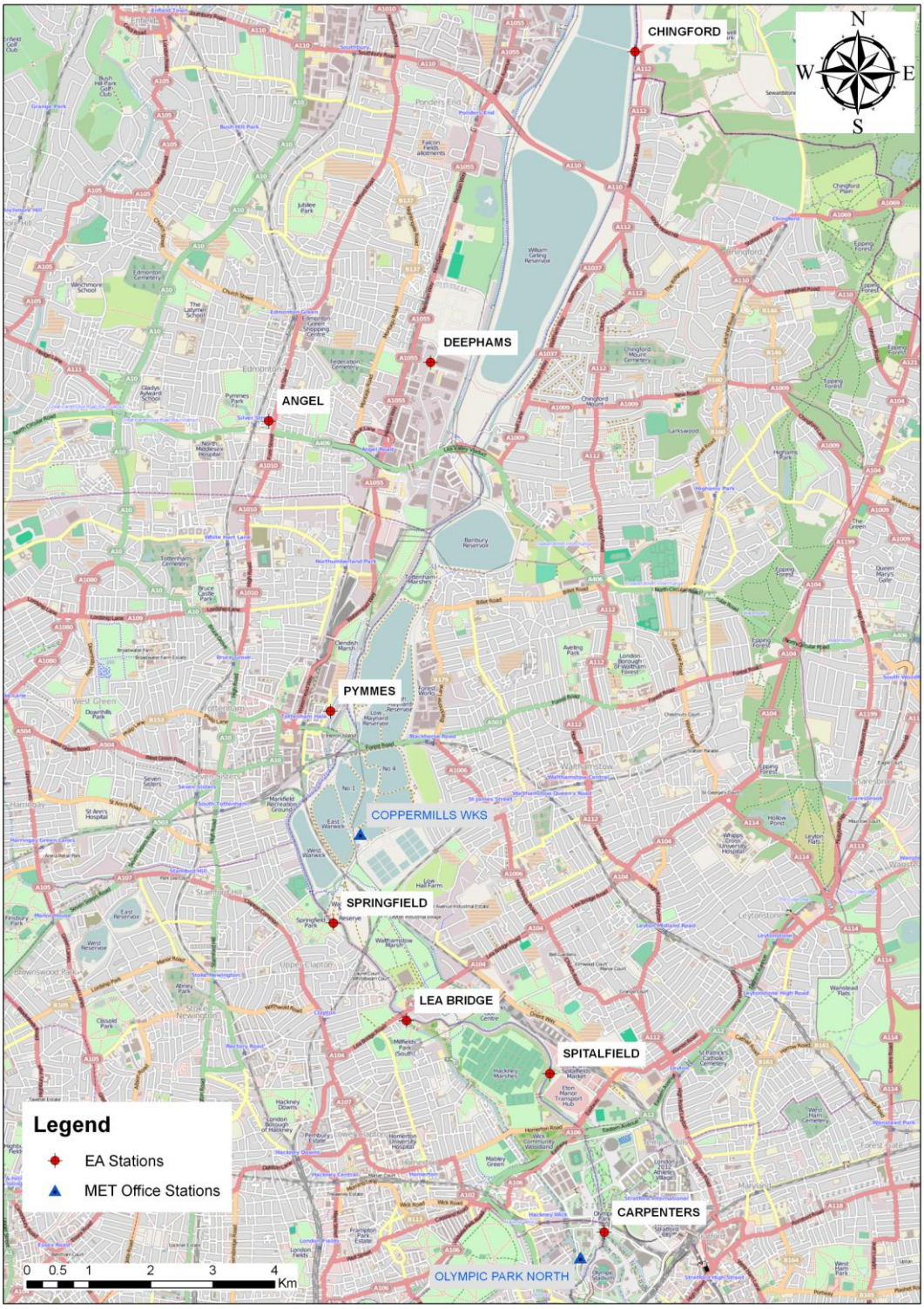


Figure 3.1 – Automated water quality monitoring stations (AWQMS) controlled by the Environment Agency and Met Office stations in the area under investigation (The raster map is provided by OpenStreetMap - Creative Commons-Share Alike License [CC-BY-SA]).

3.1.1 Dissolved oxygen (% and mg/l)

The dissolved oxygen (DO) data used in this project were classified by following the classification suggested in the Environment Agency General Quality Assessment scheme (GQA) and used by Thames 21 (Thames 21 2011). The GQA was a method used by the Environment Agency to estimate the water quality of river and canals. At the present, this method is under revision in order to be implemented in the light of the Water Framework Directive. The GQA classification for the dissolved oxygen is based on the 10th percentile, which indicates the value below which 10% of observations fall. Following the GQA scheme, DO concentrations should not fall below the standards presented in the classification more than 10 % of the time. Standard values are illustrated in Chapter 1 (paragraph 1.6.1). Table 3.1 gives the 10th percentile values estimated for each season at each monitoring stations.

Table 3.1 – 10th percentile values of dissolved oxygen (%), calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). DO values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. NA = data not available.

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	73	92	97	79	69	91	90	71
Angel	2	41	47	10	24	26	40	28
Deephams	38	44	46	43	43	9	31	NA
Pymmes E	34	16	16	2	12	3	2	2
Pymmes W	9	3	3	2	7	8	0	1
Springfield	13	29	57	3	15	16	45	30
Lea Bridge	14	24	42	15	12	16	33	21
Spitalfield	42	56	70	47	30	47	74	45
Carpenters	21	44	70	32	24	NA	60	45

Looking at the 10th percentile, the levels of dissolved oxygen saturation (%) ranged between “bad” (< 20 %) and “very good” (≥ 80 %). Chingford station, located along the Flood channel further upstream of the area of investigation, showed the highest DO concentrations with water quality between “good” (70 – 79 %) and “very good” (≥ 80 %). The lowest DO levels were recorded at Pymmes Brook, especially at the west site, indicating a “bad” condition (from 0 % to 16 %). An exception was recorded at Pymmes East during summer 2010 when the DO was 34 %. DO levels at Angel station, upstream Deephams effluent, exhibited values ranged between 2 % during summer (“bad”) and 47 % (“poor”) during winter. Deephams showed in general “poor” dissolved oxygen levels

(between 31 % and 46 %), except for autumn 2011 when the quality was “bad” (9 %). Lea Bridge and Springfield exhibited “bad-poor” quality (from 3 % to 45 %) with a peak at Springfield in winter 2010 (57 %, “fair”). Improved dissolved oxygen levels were recorded at Carpenters and Spitalfield where the quality was “poor” in summer and “good” during the winter. Most of the stations investigated showed higher dissolved oxygen levels during the two winter periods and lower ranges during the summers, in agreement with theoretically statements (Chapter 1, paragraph 1.6.1). However, this kind of tendency was not detected at Deephams and Pymmes Brook.

Figure 3.2 and Table 3.2 present the median values and the median absolute deviations (MAD) of dissolved oxygen (%) over the period of investigation. The dotted line in Figure 3.2 represents the seasonal trend calculated as mean of all the stations.

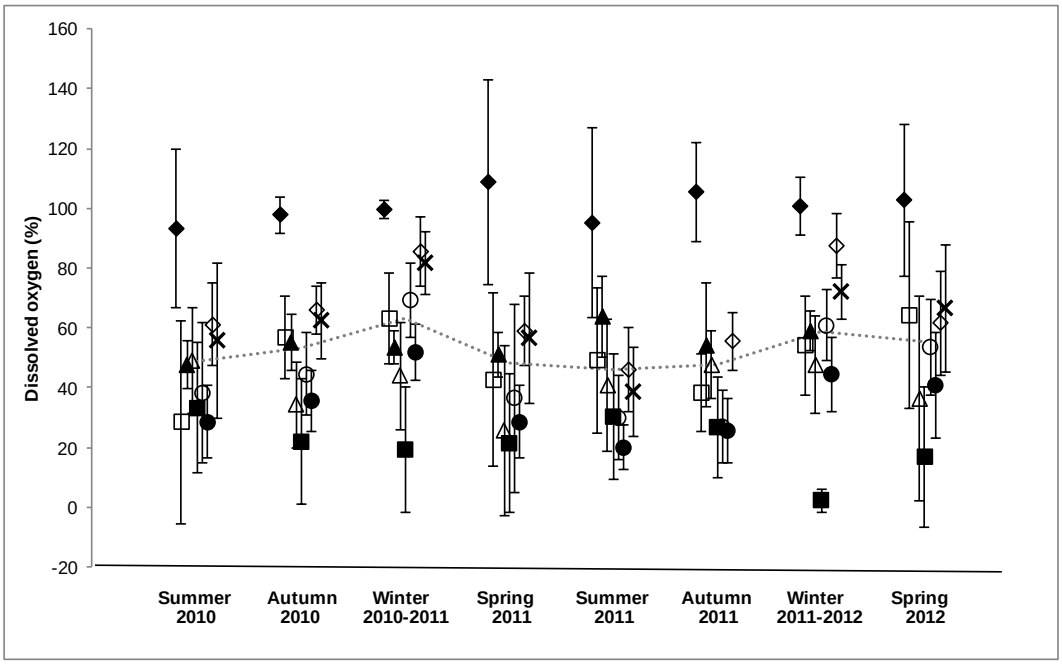


Figure 3.2 – Median and measure of spread (MAD) of the dissolved oxygen (%), calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). DO values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. Legend: ◆ Chingford; □ Angel; ▲ Deephams; △ Pymmes East; ■ Pymmes West; ○ Springfield; ● Lea Bridge; ◇Spitalfield; X Carpenters; ▪▪▪▪ seasonal trend.

Table 3.2 – Median and median absolute deviation (MAD) of dissolved oxygen (%) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). DO values were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up, and downstream.

Station name	Summer 2010		Autumn 2010		Winter 2010-2011		Spring 2011		Summer 2011		Autumn 2011		Winter 2011-2012		Spring 2012	
	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD
Chingford	94	26	98	6	100	3	109	34	95	32	106	16	101	10	103	25
Angel	29	34	57	14	63	15	43	29	50	24	39	13	55	16	65	31
Deephams	48	8	55	9	54	6	51	8	64	13	54	21	59	7	NA	NA
Pymmes E	49	17	35	14	44	18	26	28	41	22	48	11	48	16	37	34
Pymmes W	33	22	22	21	20	21	22	23	31	21	27	17	3	4	17	24
Springfield	39	24	45	14	70	12	37	31	30	14	27	12	61	12	54	16
Lea Bridge	29	12	36	10	52	9	29	12	20	7	26	11	45	12	41	18
Spitalfield	61	14	66	8	86	12	59	12	46	14	56	10	88	11	62	17
Carpenters	56	26	63	13	82	11	57	22	39	15	NA	NA	72	9	67	21

Values of dissolved oxygen (mg/l) enable the number of days when the dissolved oxygen levels were below the standards, suggested by Behar (1997, Chapter 1, paragraph 1.6.1), to be determined. The number of days when the DO was less than 4 mg/l, which indicates a stressful situation for the aquatic fauna, and the number of days when DO fell below 2 mg/l, indicating anoxic conditions, were determined (Table 3.3 and Table 3.4 respectively). Dissolved oxygen at Chingford station never decreased below the 4 mg/l.

Table 3.3 – Number of days when the DO < 4 mg/l, calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). DO values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. NA = data not available.

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	0	0	0	0	0	0	0	0
Angel	57	3	0	10	11	33	1	1
Deephams	36	3	0	2	8	38	11	NA
Pymmes E	2	54	31	61	52	13	24	48
Pymmes W	NA	74	65	81	61	81	75	70
Springfield	51	31	0	51	83	69	1	14
Lea Bridge	86	52	0	86	94	71	14	39
Spitalfield	6	0	0	0	41	0	0	1
Carpenters	21	4	0	12	46	NA	0	0

Table 3.4 – Number of days when the DO < 2 mg/l, calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). DO values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. NA = data not available.

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	0	0	0	0	0	0	0	0
Angel	12	0	0	9	0	0	0	0
Deephams	0	0	0	0	3	28	7	NA
Pymmes E	0	7	8	23	11	2	14	26
Pymmes W	NA	37	44	37	15	22	57	43
Springfield	16	1	0	32	20	22	0	1
Lea Bridge	21	4	0	22	54	21	0	2
Spitalfield	0	0	0	0	0	0	0	0
Carpenters	0	0	0	0	0	NA	0	0

Pymmes West site showed frequently low dissolved oxygen levels (DO < 4 mg/l), in particular during the two winter periods when days with anoxic conditions (DO < 2 mg/l) were 44 and 57 respectively over 90 day period (days within a season). In addition, Pymmes East exhibited days with stressful condition for the aquatic ecosystem over the two-year period. At Salmon (Angel station) and Deephams DO < 4 mg/l values were registered during summer 2010 and autumn 2011. Deephams presented anoxic condition in autumn 2011 (Figure 3.3).

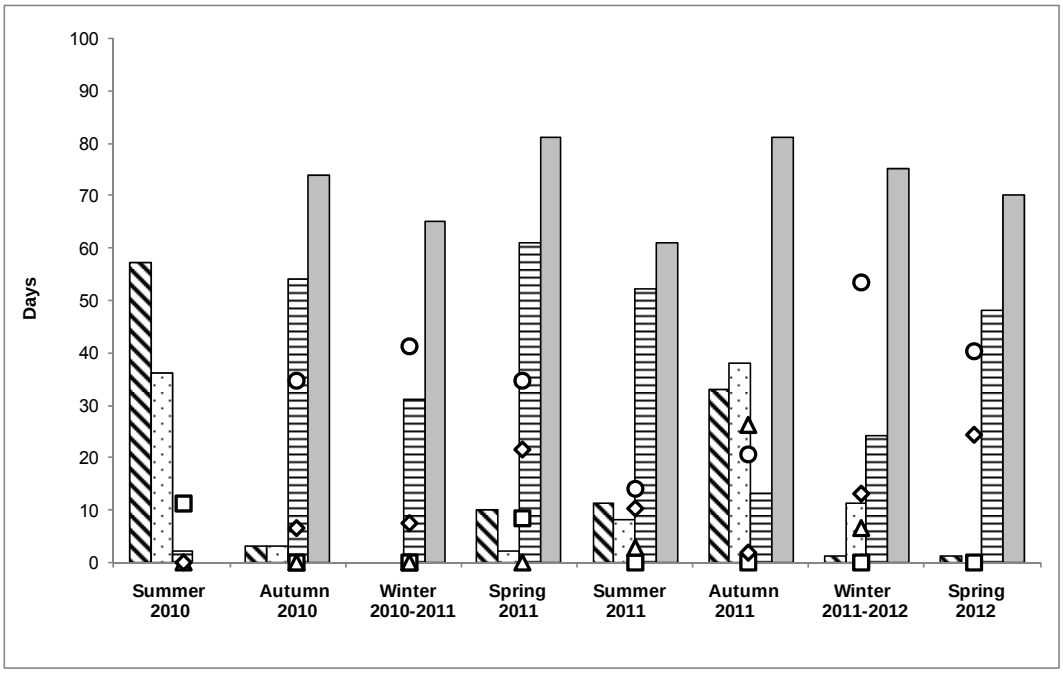


Figure 3.3 – Number of days when the DO (mg/l) levels were dangerously low over the two-year period (from 21/06/2010 to 20/06/2012) at Angel, Deephams, and Pymmes Brook sites. The bars represent the number of days when the DO < 4 mg/l. The symbols correspond to the number of days when the DO < 2 mg/l. DO values were recorded by EA automated monitoring stations. Legend: Angel; Deephams; Pymmes E; Pymmes W.

The two stations along the Lea Navigation (Springfield Park and Lea Bridge weir) showed the same trend, although the conditions at Springfield seemed to be better than at Lea Bridge. These stations presented stressful conditions (DO < 4 mg/l) throughout the two years of monitoring, being worse during spring 2011, summer 2011 and autumn 2011 when they presented several days with anoxic conditions. An exception to this trend was recorded during winter 2010-2011 and winter 2011-2012, when they exhibited "good" DO levels indicating possible dilution effects due to higher channel flow rate, and low water temperatures (Figure 3.4).

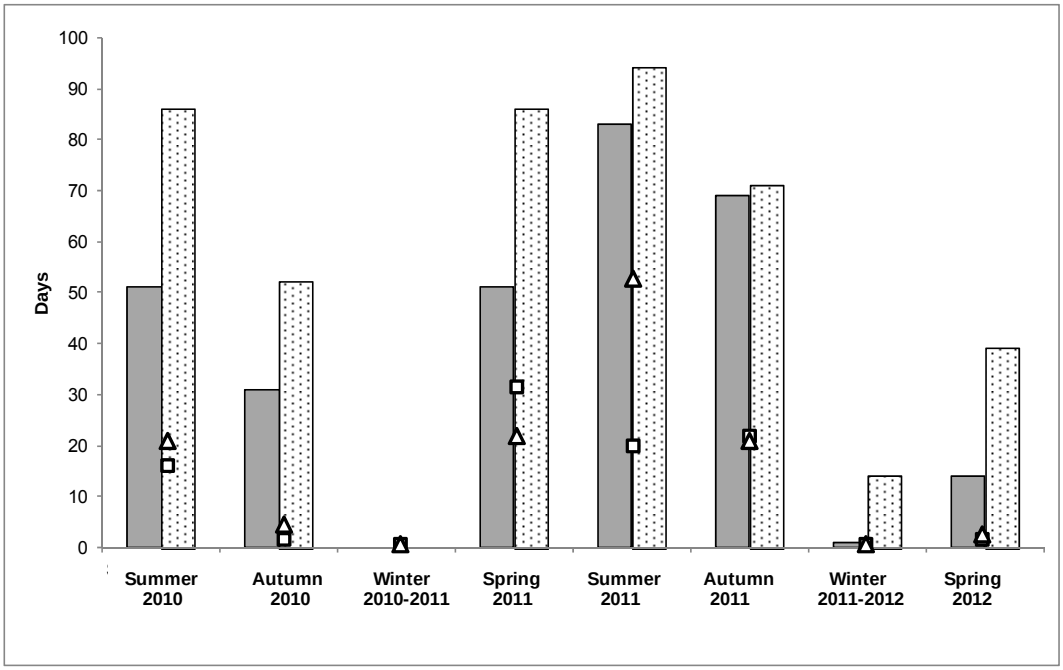


Figure 3.4 – Number of days when the DO (mg/l) levels were dangerously low over the two-year period (from 21/06/2010 to 20/06/2012) at Springfield Park and Lea Bridge weir sites. The bars represent the number of days when the DO < 4 mg/l. The symbols correspond to the number of days when the DO < 2 mg/l. DO values were recorded by EA automated monitoring stations. Legend: ■ □ Springfield; ⊡△ Lea Bridge.

Spitalfield and Carpenters stations, located downstream of the weir along the River Lea (with natural bed and banks), showed "good" dissolved oxygen levels. However, during the summer 2010 and the summer 2011 the number of days with DO < 4 mg/l increased, possible due to the high summer water temperatures. At Spitalfield and Carpenters, the dissolved oxygen levels never decreased below 2 mg/l (Figure 3.5).

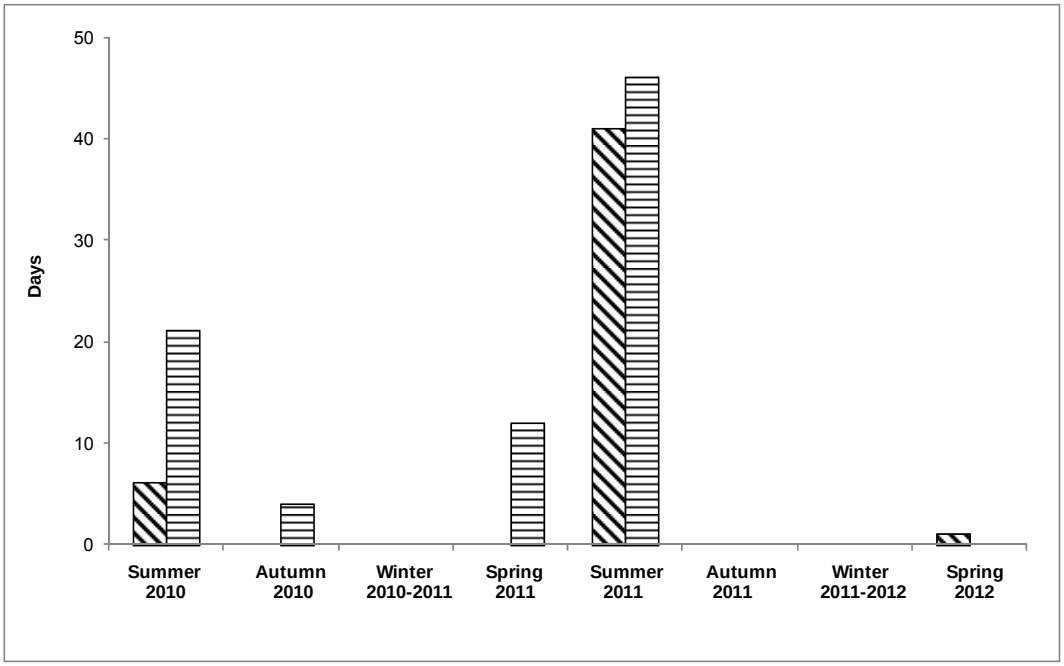


Figure 3.5 – Number of days when the DO < 4 mg/l over the two-year period (from 21/06/2010 to 20/06/2012) at Spitalfield and Carpenters sites. DO values were recorded by EA automated monitoring stations. Legend: Spitalfield; Carpenters.

The results obtained from the analyses of the dissolved oxygen (DO) levels gave the following picture of the area under investigation:

- Angel station, located upstream of Deephams sewage treatment work (STW) effluent discharge, and which flowed in an urban context often under the streets, showed "poor" DO levels. Moreover, Angel showed DO concentrations stressful for the aquatic environment (DO < 4 mg/l) during two seasons out of the eight.
- At Deephams station, the water quality ranged between "poor" and "fair", but DO levels were threatening for the aquatic environment only during two seasons of investigation (the same as for Angel station).
- Pymmes Brook, which received the water of Deephams STW effluent through Salmon Brook, showed very low oxygen levels. During the two years of study Pymmes Brook was a stressful environment for the aquatic life, since DO concentrations were below 4 mg/l. In addition, the DO levels at Pymmes Brook were below 2 mg/l for several days, indicating anoxic conditions especially in the concrete culvert (west side).
- The highest DO values were detected at Chingford station, which is placed along the Flood channel, further upstream of the confluence between Pymmes Brook and Lea Navigation. At Chingford, the DO levels never declined below 4 mg/l.
- The DO levels at Lea Bridge and Springfield, the two stations positioned along the Lea Navigation downstream Pymmes Brook, indicated "poor" water quality.

for several days the DO was very low (< 4 mg/l), indicating an almost continuous stressful environment for the aquatic fauna. Moreover, the DO decreased below 2 mg/l during numerous days in the two-year study.

- "Good" DO concentrations were recorded at Carpenters and Spitalfield, which were located downstream of the weir in the River Lea where the bed and banks were not concrete. At these two stations, days with stressful conditions were detected only during the two summer periods, but they never reached anoxic levels.

Dissolved oxygen levels showed a generally "poor" quality throughout the area under study. Exceptions were: 1) Chingford, in the Flood channel upstream of the confluence between Pymmes Brook and Lea Navigation; 2) Carpenters and Spitalfield, in the River Lea where the bed and banks were not concrete-made. The "bad" quality at Pymmes Brook indicated the likely influence of Deephams STW effluent and possible other uninvestigated pollution sources, since for example Salmon Brook (Angel station) also showed "poor" DO levels. A possible reason why the DO at Deephams ranged between "poor" and "fair" could be that during the digestive processes the wastewaters were continuously mixed helping the aeration, which could explain also the lack of DO seasonal trend at this station. The two monitoring sites located along Lea Navigation (Springfield Park and Lea Bridge) presented "poor" water quality, with several days of stressful conditions for the aquatic habitat, suggesting a likely effect of Pymmes waters. However, most likely the STW discharge was not the only cause of low dissolved oxygen levels in the Lea Navigation. In fact, low dissolved oxygen concentrations were also identified at Lea Bridge, which was located several kilometres downstream of Pymmes Brook and here the river water had the advantage of dilution. This could suggest the presence of other pollution sources, such as misconnections and runoff from the roads.

3.1.2 Temperature (°C)

Temperature trends were consistent with natural seasonal changes: higher in summer and spring, lower in winter and autumn. Nevertheless, as shown in Figure 3.6 and Table 3.5, the highest average temperatures over the entire period of study were at Deephams and Pymmes Brook East side, showing the influence of Deephams discharge and an alteration of the natural temperatures (thermal pollution). In fact, the lowest temperatures were detected at Angel and Chingford, which were not affected by Deephams effluent. Pymmes, Deephams, Angel and Chingford flowed in an urban context and they are concrete channels, so variations in temperature were not due to presence of vegetation. Going further downstream of the STW effluent, the water temperatures of Lea Navigation decreased slightly. This event is visible especially in the colder months (winter and autumn).

In summer 2010 and summer 2012, the temperatures ranged around 20 °C with the highest average temperatures being at Deephams and Pymmes East (21 °C), while the lowest were at Angel (18 °C and 17 °C respectively). In autumn 2010 and autumn 2012, the highest average values were recorded at Deephams (17 °C and 19 °C respectively) and the lowest at Chingford (9 °C and 11 °C respectively). During winter 2010-2011 and winter 2011-2012, once again the highest average measures were registered at Deephams (14 °C and 15 °C respectively) and the lowest at Chingford and Angel (7 °C). In spring 2011, Deephams and Pymmes East showed the highest average temperature (18 °C), while Angel had the lowest (14 °C). In spring 2012, the highest average temperature was at Deephams (17 °C), while the lowest was at Angel and Chingford (12 °C).
The dotted line in Figure 3.6 represents the seasonal trend calculated as mean of all the stations.

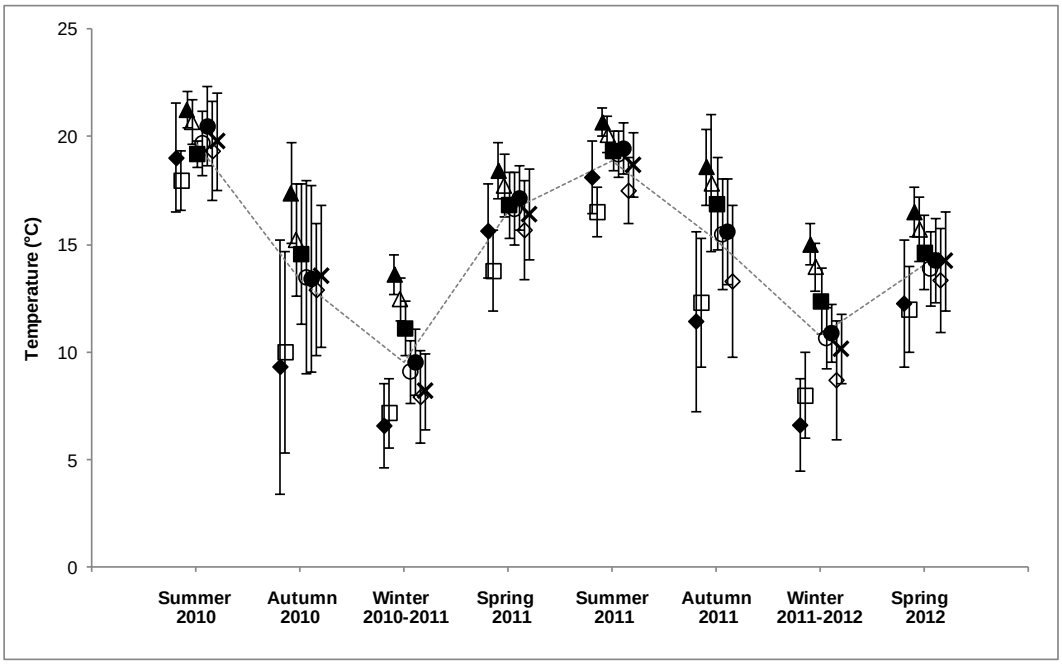


Figure 3.6 – Medians and measure of spread (MAD) of the temperature (°C), calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Temperature values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. Legend: ◆ Chingford; □ Angel; ▲ Deephams; △ Pymmes East; ■ Pymmes West; ○ Springfield; ● Lea Bridge; ◇Spitalfield; X Carpenters; ▮▮▮▮ seasonal trend.

Table 3.5 – Median and median absolute deviation (MAD) of temperatures (°C) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Temperature values were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up, and downstream.

Station name	Summer 2010		Autumn 2010		Winter 2010-2011		Spring 2011		Summer 2011		Autumn 2011		Winter 2011-2012		Spring 2012	
	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD
Chingford	19	3	9	6	7	2	16	2	18	2	11	4	7	2	12	3
Angel	18	1	10	5	7	2	14	2	17	1	12	3	8	2	12	2
Deephams	21	1	17	2	14	1	18	1	21	1	19	2	15	1	17	1
Pymmes E	21	1	15	3	12	1	18	1	20	1	18	3	14	1	16	1
Pymmes W	19	1	15	3	11	1	17	2	19	1	17	2	12	2	15	2
Springfield	20	2	14	4	9	1	17	2	19	1	16	3	11	1	14	2
Lea Bridge	21	2	13	4	10	2	17	1	19	1	16	2	11	1	14	2
Spitalfield	19	2	13	3	8	2	16	2	18	2	13	4	9	3	13	2
Carpenters	20	2	14	3	8	2	16	2	19	1	NA	NA	10	2	14	2

3.1.3 pH

The pH average values recorded at the nine monitoring stations were within the "good" quality level, ranging between 7.05 and 8.36 (Table 3.6) as stated in the Freshwater Fish Directive (78/659/EEC).

Differences between stations were not great. However, it was possible to identify small differences along the investigated channels (Figure 3.7). The highest average levels over the investigating period were at Chingford (average 8.16), while the lowest pH values were detected at Deephams (average 7.14). The two stations differed by one unit. One possible explanation was that bacteria produce acids during the nitrification process, decreasing the pH (EPA 2002). This theory was also supported by the pH at Angel (7.74), which was not affected by the STW effluent. Another explanation could be the lack of algal photosynthetic activity, since photosynthesis increases the pH levels by removal of CO_2 from the water, as mention in Chapter 1 (paragraph 1.6.3).

The dotted line in Figure 3.7 represents the seasonal trend calculated as mean of all the stations.

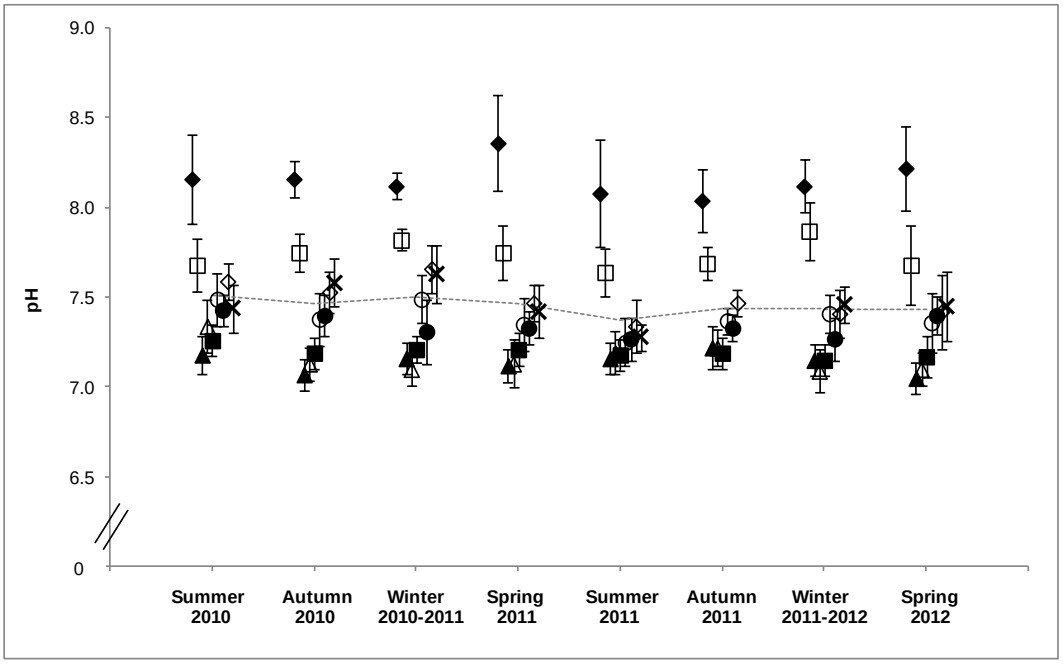


Figure 3.7 – Medians and measure of spread (MAD) of pH, calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). pH values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. Legend: ◆ Chingford; □ Angel; ▲ Deephams; △ Pymmes East; ■ Pymmes West; ○ Springfield; ● Lea Bridge; ◇Spitalfield; X Carpenters; ▪▪▪▪ seasonal trend.

Table 3.6 – Median and median absolute deviation (MAD) of pH calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). pH values were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up, and downstream.

Station name	Summer 2010		Autumn 2010		Winter 2010-2011		Spring 2011		Summer 2011		Autumn 2011		Winter 2011-2012		Spring 2012	
	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD
Chingford	8.16	0.25	8.16	0.10	8.12	0.07	8.36	0.27	8.08	0.30	8.04	0.18	8.12	0.15	8.22	0.24
Angel	7.68	0.15	7.75	0.10	7.82	0.06	7.75	0.15	7.64	0.13	7.69	0.09	7.87	0.16	7.68	0.22
Deephams	7.18	0.10	7.07	0.09	7.16	0.09	7.12	0.09	7.16	0.09	7.22	0.12	7.15	0.09	7.05	0.09
Pymmes E	7.34	0.15	7.13	0.09	7.10	0.09	7.13	0.13	7.19	0.12	7.22	0.10	7.09	0.12	7.10	0.09
Pymmes W	7.26	0.09	7.19	0.09	7.21	0.07	7.21	0.09	7.18	0.09	7.19	0.09	7.15	0.09	7.17	0.12
Springfield	7.49	0.15	7.38	0.15	7.49	0.13	7.35	0.15	7.25	0.13	7.37	0.07	7.41	0.10	7.36	0.16
Lea Bridge	7.43	0.09	7.40	0.12	7.31	0.18	7.33	0.09	7.27	0.12	7.33	0.07	7.27	0.12	7.40	0.10
Spitalfield	7.59	0.10	7.53	0.12	7.66	0.13	7.47	0.10	7.34	0.15	7.47	0.07	7.41	0.13	7.42	0.21
Carpenters	7.44	0.13	7.58	0.13	7.63	0.16	7.42	0.15	7.28	0.07	NA	NA	7.46	0.10	7.45	0.19

3.1.4 Conductivity (µS/cm)

Conductivity values registered over the studying period ranged between 800 and 1185 µS/cm. As Behar (1997) indicated, those values are far higher than the ideal situation in freshwaters (150-500 µS/cm), but still within a normal range (50-1500 µS/cm).

Figure 3.8 and Table 3.7 show the medians and the median absolute deviation (MAD) of the conductivity values. From the graph, it can be seen that the highest conductivity levels are at Deephams and Pymmes East, while the lowest at Chingford and Angel. The highest average concentrations were recorded at Deephams and Pymmes East (average 1154.38 and 1143.81 µS/cm respectively), due probably to the STW discharge. The lowest averages were noted at Chingford and Angel (average 865 and 903 µS/cm respectively) which are not affected by the STW effluent. Going further downstream of Deephams STW discharge, conductivity values decreased, with average around 1000 µS/cm.

The dotted line in Figure 3.8 represents the seasonal trend calculated as mean of all the stations.

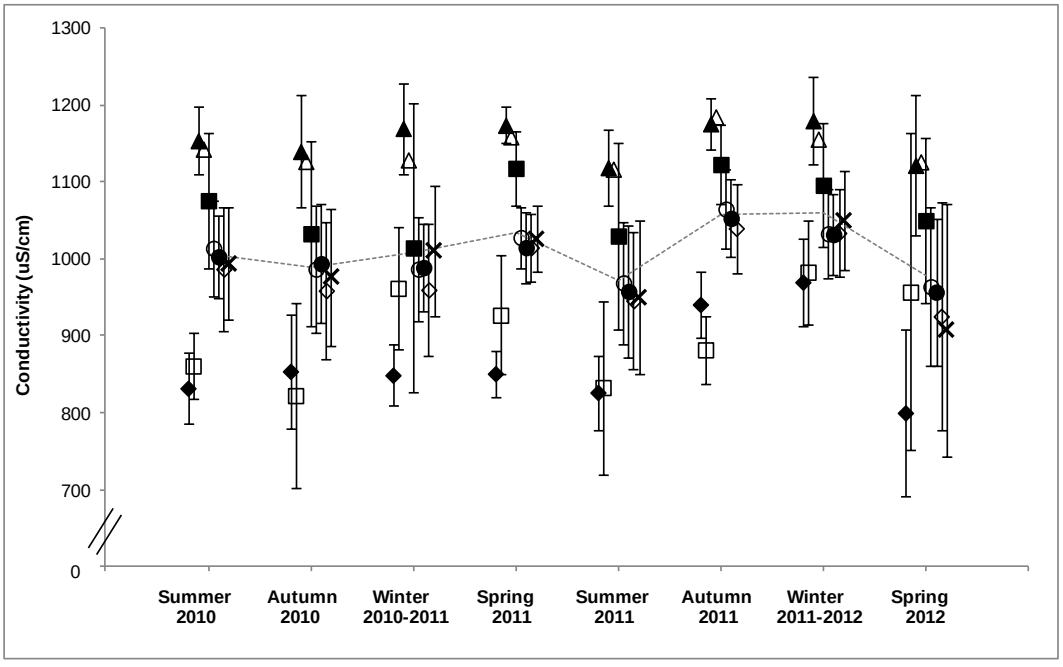


Figure 3.8 – Medians and measure of spread (MAD) of the conductivity (µS/cm), calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Conductivity values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. Legend: ◆ Chingford; □ Angel; ▲ Deephams; △ Pymmes East; ■ Pymmes West; ○ Springfield; ● Lea Bridge; ◇Spitalfield; X Carpenters; ▪▪▪▪ seasonal trend.

Table 3.7 – Median and median absolute deviation (MAD) of conductivity (µS/cm) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Conductivity values were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up, and downstream.

Station name	Summer 2010		Autumn 2010		Winter 2010-2011		Spring 2011		Summer 2011		Autumn 2011		Winter 2011-2012		Spring 2012	
	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD
Chingford	832	46	854	74	849	40	851	30	827	48	941	43	970	56	800	108
Angel	861	43	823	119	962	80	927	77	833	113	882	44	983	68	957	206
Deephams	1154	44	1140	73	1170	59	1174	24	1119	49	1176	34	1180	58	1122	90
Pymmes E	1143	42	1127	71	1129	68	1159	31	1117	68	1185	31	1156	61	1127	99
Pymmes W	1076	87	1033	120	1015	188	1118	49	1030	122	1123	52	1096	80	1050	107
Springfield	1014	62	987	83	987	68	1028	40	969	80	1065	52	1033	58	964	104
Lea Bridge	1003	53	994	77	989	58	1015	46	958	86	1053	50	1032	52	957	96
Spitalfield	987	80	959	89	960	86	1015	44	946	89	1040	58	1034	56	926	149
Carpenters	994	73	977	89	1011	85	1026	43	950	99	NA	NA	1050	65	908	165

3.1.5 Turbidity (NTU)

Pymmes Brook showed the highest level of turbidity compared to the other monitoring stations, with two evident peaks during winter 2010-2011 and winter 2011-2012 (Figure 3.9). The station located on the west side of Pymmes Brook, which is covered by concrete, exhibited the highest levels. Following the classification gave by McCaffrey (2012), the water quality at Pymmes was "poor" (> 30 NTU). All the others sites presented almost the same low average values of turbidity among different seasons, classifying their water quality as "excellent" (≤ 10), except for Springfield during the spring of 2012 (18 NTU). Also at Deephams STW effluent the turbidity was quite low, indicating "excellent" quality, in agreement with the range (between 4 and 20 NTU) presented by Daly (2007) for the conductivity in sewage treatment works discharges. Apart from Pymmes Brook, the turbidity in the other monitoring sites did not presented any seasonal variation.

Medians and the median absolute deviation (MAD) of the turbidity are given in Figure 3.9 and Table 3. The dotted line in Figure 3.9 represents the seasonal trend calculated as mean of all the stations.

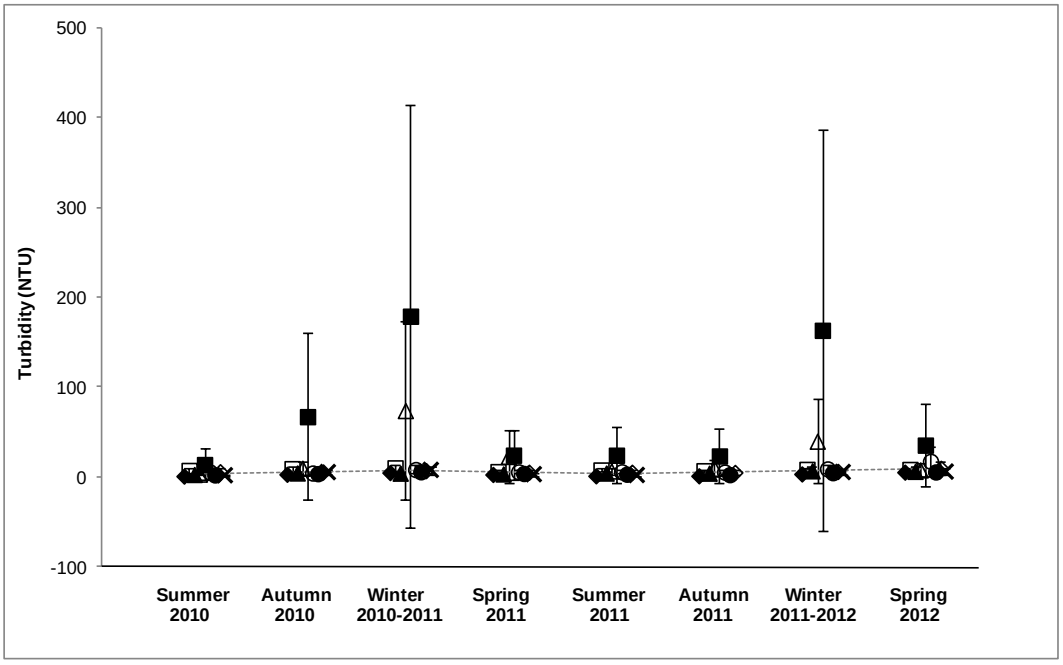


Figure 3.9 – Medians and measure of spread (MAD) of the turbidity (NTU), calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Turbidity values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. Legend: ◆ Chingford; □ Angel; ▲ Deephams; △ Pymmes East; ■ Pymmes West; ○ Springfield; ● Lea Bridge; ◇Spitalfield; X Carpenters; ▪▪▪▪ seasonal trend.

Table 3.8 – Median and median absolute deviation (MAD) of turbidity (NTU) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Turbidity values were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up, and downstream.

Station name	Summer 2010		Autumn 2010		Winter 2010-2011		Spring 2011		Summer 2011		Autumn 2011		Winter 2011-2012		Spring 2012	
	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD
Chingford	1	0	3	1	5	3	3	2	1	0	1	0	3	1	5	2
Angel	7	3	9	2	10	4	6	2	8	1	7	2	9	1	8	2
Deephams	3	1	5	1	4	2	3	2	4	2	4	1	7	4	6	6
Pymmes E	3	1	10	7	74	99	23	30	7	4	10	9	40	47	8	7
Pymmes W	14	17	67	93	179	235	24	28	24	31	23	30	164	224	36	46
Springfield	6	2	4	1	8	5	6	2	6	2	6	2	9	4	18	17
Lea Bridge	2	1	4	1	6	3	4	1	3	2	3	1	5	2	6	2
Spitalfield	6	3	6	3	7	3	5	2	5	2	5	3	6	3	10	8
Carpenters	2	1	5	3	8	4	3	1	2	2	NA	NA	5	3	6	4

3.1.6 Total ammonia (mg/l)

Levels of total ammonia did not have a clear seasonal trend, but there was evidence that the highest average concentrations of ammonia were recorded at Pymmes and the lowest at Chingford (Figure 3.10 and Table 3.9). Data showed a possible influence of Deephams discharge on the total ammonia levels downstream of its discharge into Pymmes Brook. In fact the station upstream of Deephams effluent confluence (Angel) showed lower total ammonia levels than the sites located downstream of Deephams discharge (such as Pymmes Brook). Across all nine stations, the range of the total ammonia is between 0.3 and 2.1 mg/l (Table 3.9).

The dotted line in Figure 3.10 represents the seasonal trend calculated as mean of all the stations.

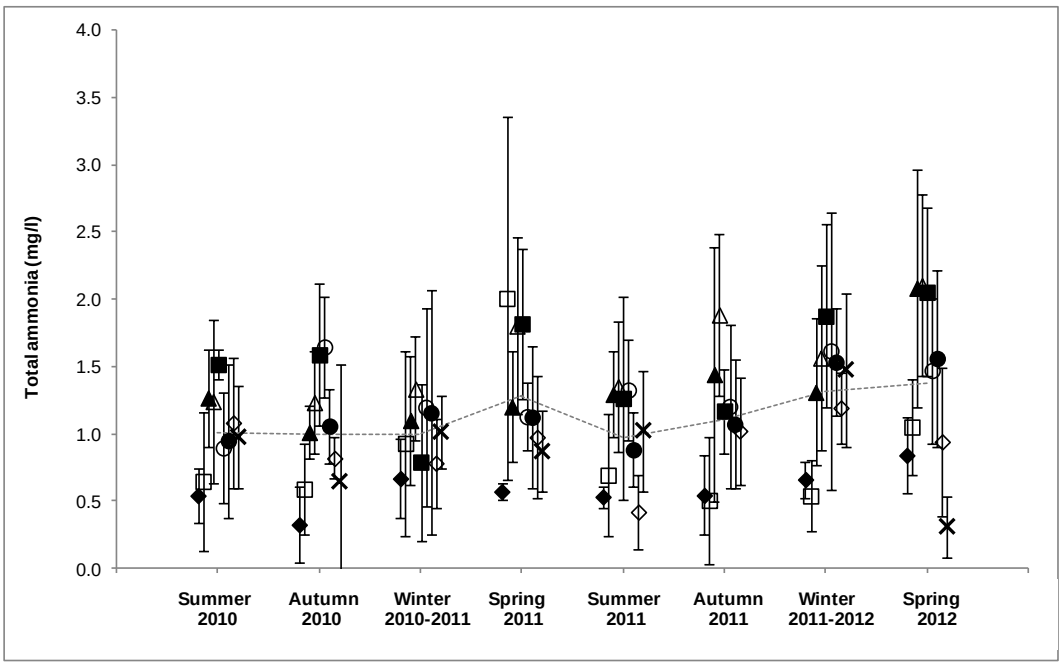


Figure 3.10 – Medians and measure of spread (MAD) of the total ammonia levels (mg/l), calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Total ammonia values were recorded by nine EA automated monitoring stations located in the area under investigation, and up, and downstream. Legend: ◆ Chingford; □ Angel; ▲ Deephams; △ Pymmes East; ■ Pymmes West; ○ Springfield; ● Lea Bridge; ◇Spitalfield; X Carpenters; ▪▪▪▪ seasonal trend.

Table 3.9 – Median and median absolute deviation (MAD) of total ammonia (mg/l) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Total ammonia values were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up, and downstream.

Station name	Summer 2010		Autumn 2010		Winter 2010-2011		Spring 2011		Summer 2011		Autumn 2011		Winter 2011-2012		Spring 2012	
	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD	Median	MAD
Chingford	0.5	0.2	0.3	0.3	0.7	0.3	0.6	0.1	0.5	0.1	0.5	0.3	0.7	0.1	0.8	0.3
Angel	0.6	0.5	0.6	0.3	0.9	0.7	2.0	1.3	0.7	0.5	0.5	0.5	0.5	0.3	1.1	0.4
Deephams	1.3	0.4	1.0	0.2	1.1	0.5	1.2	0.4	1.3	0.3	1.4	0.9	1.3	0.5	2.1	0.9
Pymmes E	1.2	0.6	1.2	0.4	1.3	0.4	1.8	0.7	1.4	0.5	1.9	0.6	1.6	0.7	2.1	0.7
Pymmes W	1.5	0.1	1.6	0.5	0.8	0.6	1.8	0.6	1.3	0.8	1.2	0.3	1.9	0.7	2.1	0.6
Springfield	0.9	0.4	1.6	0.4	1.2	0.7	1.1	0.2	1.3	0.4	1.2	0.6	1.6	1.0	1.5	0.5
Lea Bridge	1.0	0.6	1.1	0.3	1.2	0.9	1.1	0.5	0.9	0.3	1.1	0.5	1.5	0.4	1.6	0.7
Spitalfield	1.1	0.5	0.8	0.2	0.8	0.3	1.0	0.5	0.4	0.3	1.0	0.4	1.2	0.3	0.9	0.6
Carpenters	1.0	0.4	0.6	0.9	1.0	0.3	0.9	0.3	1.0	0.4	NA	NA	1.5	0.6	0.3	0.2

3.2 Correlation analysis

The Spearman correlation was used to identify any correlation between the dissolved oxygen (DO) and other parameters. Since there was evidence that the dissolved oxygen levels in the area under investigation were low and because the level of oxygen in the water is a good indicator of pollution, the main goal of these correlation analyses was to detect if variations of any other physico-chemical water parameters could explain variations in dissolved oxygen levels. The Spearman rank correlation coefficient (ρ_s) is not affected by the distribution of the data set (non-parametric test), since data are ranked.
The formula is:

$$\rho_s = 1 - \frac{6 \sum_i D_i^2}{N(N^2 - 1)} \qquad (3.1)$$

Where:
$D_i = r_i - s_i$, and it is the difference between the rank of the variable *r* and the rank of the variable *s* of the *i* observation,
N is the number of cases.

Spearman rank correlation coefficient varies between -1.00 and +1.00; for good positive correlation the ρ_s value would be +1.00, while for a good negative correlation the ρ_s value would be -1.00. No correlation between the two variables under investigation is express by 0. Indicatively a correlation ± 0.50 suggests a significant relationship, even if working with correlations it is better to look at stronger relationships such as < -0.70 and >+0.70 (Reimann *et al*. 2008). Correlations were estimated using data grouped in seasons.

Tables of results are presented in Appendix VII.

In summary:

1. Correlation between dissolved oxygen and pH:
   As explained in Chapter 1 (paragraph 1.6.3), the photosynthesis decreases the pH level by using CO_2 present in the stream water. Therefore, it will be expected to find positive relationships between the two variables. Most of the ρ_s values indicated a positive correlation between DO and pH. However, that trend was not detectable in all the stations at all the seasons investigated. For instance, there was a lack of strong relationship during autumn 2011 and winter 2011-2012 across all the monitored stations. Strong relationships between DO and pH were detected at Chingford, Angel, Spitalfield and Carpenters, but they were not seen in all seasons.

2. Correlation between DO and temperature:
   As mentioned in Chapter 1 (paragraph 1.6.1), dissolved oxygen solubility and temperature should be negative correlated: in cold waters, there is higher oxygen solubility than in warmer waters. Results showed only few significant negative correlation values during autumn 2010 and autumn 2011. Only one appeared to be strongly negative.
3. Correlation between DO and conductivity:
   The only significant relationships (negative) were detected during winter 2010-2011 at four stations out of nine.
4. Correlation between DO and turbidity:
   As explained in Chapter 1 (paragraph 1.6.4), these two parameters should be negative correlated, since high levels of turbidity indirectly cause low dissolved oxygen levels. Two are the main reasons: 1) turbidity reduces the incident light intensity resulting in lower photosynthetic activity, and 2) turbidity increases water temperature because the suspended particles absorb the heat of the sunlight (Behar 1997). It was not possible to identify any pattern, except for Angel, which presented positive correlations during three seasons out of eight.
5. Correlation between DO and total ammonia:
   During winter 2010-2011, four monitoring sites out of nine presented negative significant correlations, and only one was strong. No relationships were detected for the other seasons at any stations.

3.3 Seasonal trend vs daily trend

The main aim of the previous section was to detect any seasonal relationship between the dissolved oxygen and the other chemico-physical parameters. Spearman rank correlations did not show any consistent interaction between those variables, even when the correlation should theoretically occur. Quite strong and numerous relationships were identified only when the DO was related to the pH, even if they were not detected for all the seasons at all the stations. However, it is important to clarify that the absence of seasonal correlations does not mean absence of daily relationships between variables.

An example of the correlation between DO and temperature is presented. Seasonal trends and daily trends of temperature (°C) and dissolved oxygen (%) at Chingford station during autumn 2011 are shown in Figure 3.11 and Figure 3.12 respectively.

Figure 3.11 confirmed the lack of correlations on seasonal basis between the two variables, as previously detected by Spearman rank test (ρ_s = 0.20). In contrast, Figure 3.12 presents the daily relationship between temperature and dissolved oxygen, which is not distinguished by Spearman correlation. The graph shows peaks during the daytime and valleys during the nighttime for both the parameters. During the day the oxygen levels are highest due mostly to the photosynthetic activity, and the temperatures are highest due to the solar radiations.

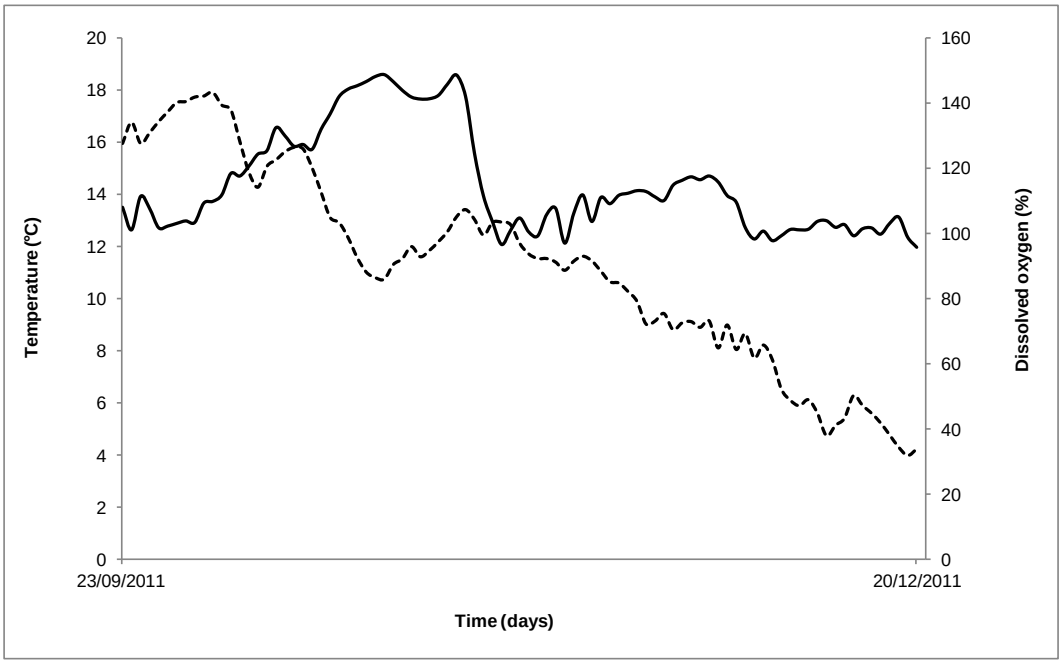


Figure 3.11 – Seasonal trends of temperature (°C) and dissolved oxygen (%) at Chingford during autumn 2011. No correlations were detected between the two variables (ρ_s = 0.20). Legend: ▬ dissolved oxygen; ▬ ▬ temperature.

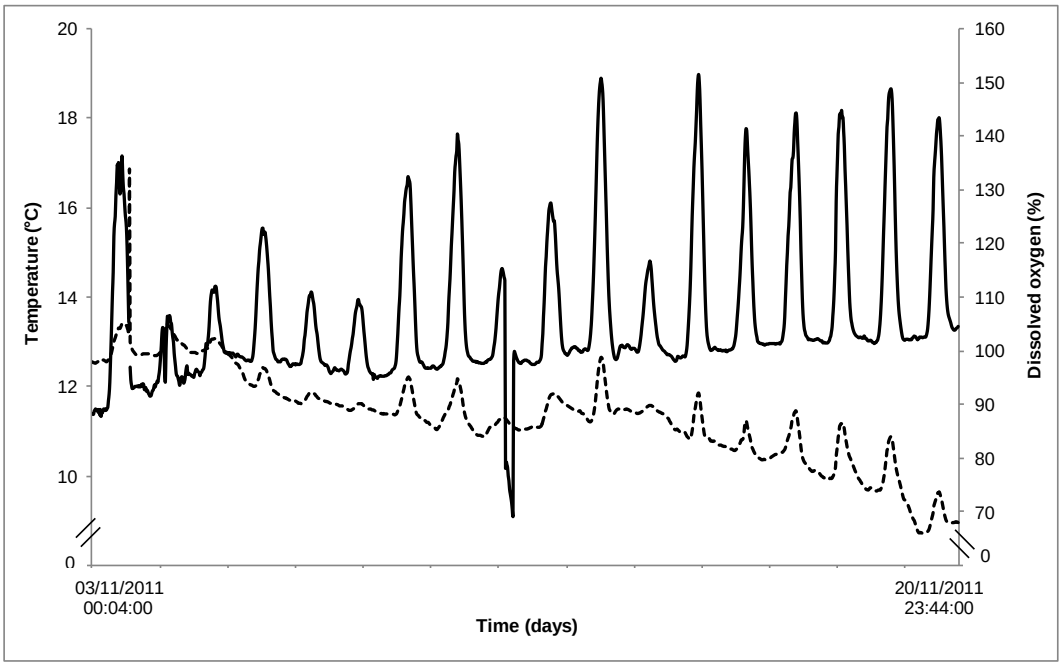


Figure 3.12 – Daily trends of temperature (°C) and dissolved oxygen (%) at Chingford station during autumn 2011. The peaks correspond to values registered during the day and the valleys are data recorded during the night. Legend: ▬ dissolved oxygen; ▬ ▬ temperature.

In conclusion, in this Chapter, Spearman rank tests were performed to identify any seasonal correlations between the dissolved oxygen and the other physico-chemical variables. Only few correlations were detected. However, this did not exclude the presence of daily correlations, as showed in Figure 3.12.

3.4 Flow data

Raw flow data of Lea Navigation recorded at Lea Bridge station were provided by the Environment Agency. In Table 3.10 mean, standard deviation, minimum and maximum values are shown for the stream flow data by season.

Table 3.10 – Mean, standard deviation (SD), minimum and maximum values of stream flow data (m^3/s) of Lea Navigation calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Flow rate values were recorded at the EA station of Lea Bridge.

Elaboration	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Mean	4.1	4.8	6.4	4.1	4.1	3.8	4.8	7.1
SD	1.7	1.8	3.5	1.1	1.2	1.2	1.0	4.3
Min	2.8	3.3	4.0	3.1	3.0	3.1	3.6	3.9
Max	12.0	17.1	24.9	9.1	10.3	11.0	10.5	31.1

Seasonal Lea Navigation flow means are plotted in Figure 3.13: during winter 2010-2011 (6.4 m^3/s) and spring 2012 (7.1 m^3/s) were recorded the highest flows at Lea Bridge. During the other seasons, the flow means were ~ 4 m^3/s.

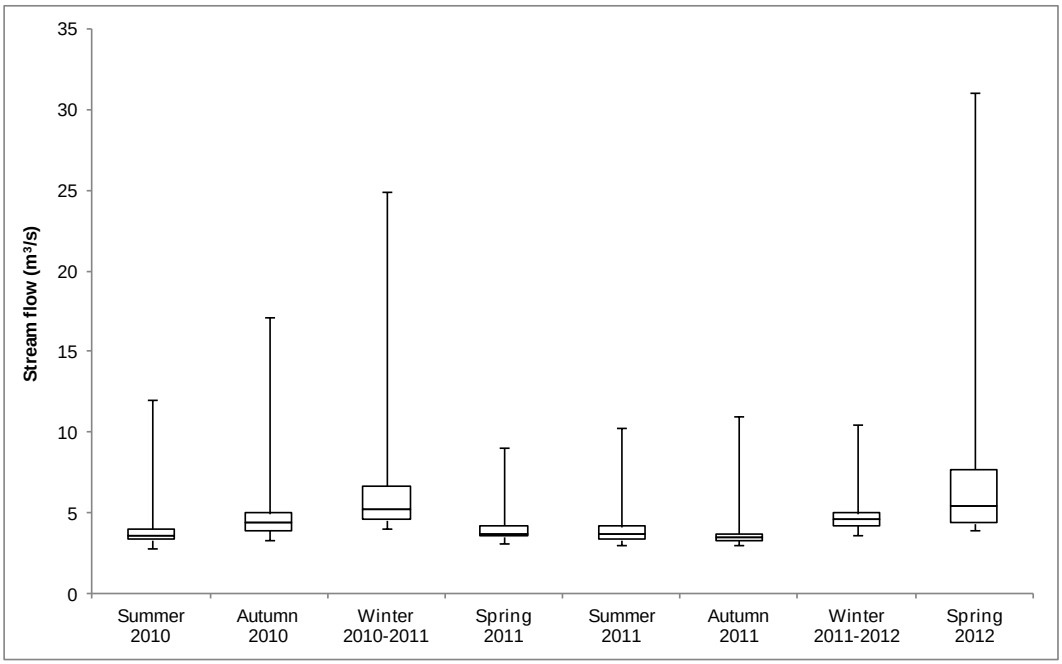


Figure 3.13 – Box plot representing Lea Navigation flow data (m^3/s) recorded over two years period (from 21/06/2010 to 20/06/2012). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Flow rate values were recorded at the EA station at Lea Bridge.

3.5 Rainfall data

Meteorological Office provided daily rainfall data (mm/day) from 21/06/2010 to 20/06/2012 (© Crown copyright Met Office 2011). Mostly the data used in this project were collected at Coppermills Water Works station (Walthamstow; 51.58 N, 00:.05 W), except for those collected in Spring 2012, that were recorded from the station at Olympic Park North (51.54 N, 00.02 W).

Table 3.11 shows total rainfall, mean, standard deviation, minimum and maximum values calculated for rainfall data by season.

Table 3.11 – Total rainfall, mean, standard deviation (SD), minimum and maximum values of rainfall data (mm/season) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Rainfall data were recorded by the Met Office at the station at Coppermills Water Works (Walthamstow; 51.58 N, 00:.05 W), except for those collected in Spring 2012, that were recorded at the station of Olympic Park North (51.54 N, 00.02 W).

Elaboration	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Total rainfall	140	133	129	100	187	81	107	337
Daily mean	1.5	1.5	1.4	1.1	2.0	0.9	1.2	3.7
SD	4.2	3.4	3.5	3.2	4.2	2.5	2.8	7.4
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	28.3	25.8	23.8	16.8	24.7	18.6	15.0	43.0

Seasonal rainfall is plotted in Figure 3.14: the highest rainfall was recorded in spring 2012 (337 mm/season), followed by summer 2011 (187 mm/season). The lower rainfall level was registered during autumn 2011 (81 mm/season).

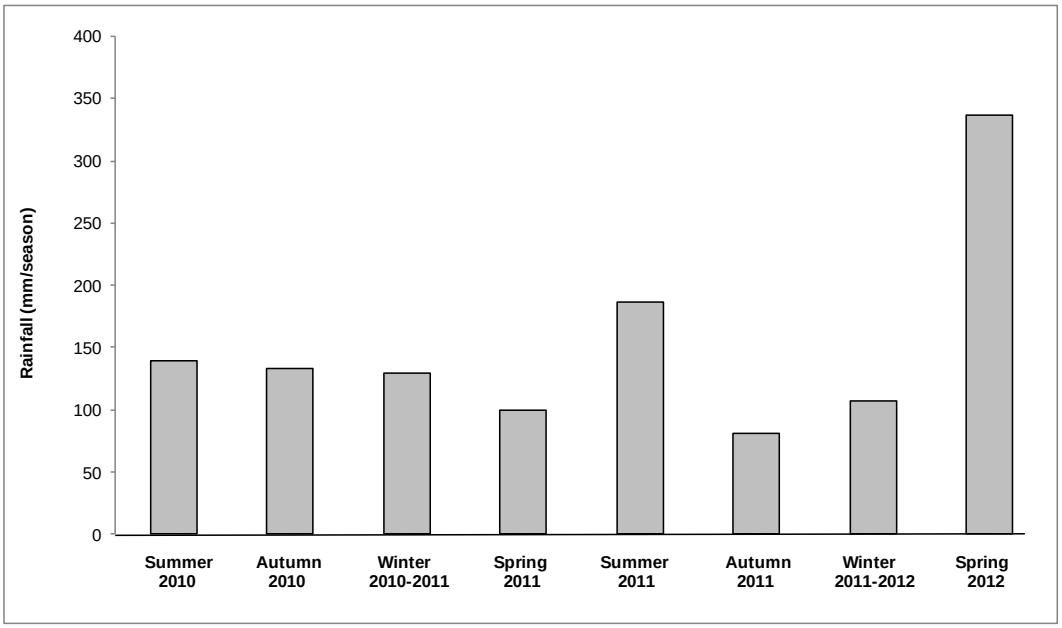


Figure 3.14 – Seasonal total rainfall values (mm/season) calculated over the two-year period (from 21/06/2010 to 20/06/2012). Rainfall data were registered at the station at Coppermills Water Works (Walthamstow; 51.58 N, 00:.05 W), except for those collected in Spring 2012, that were recorded at the station at Olympic Park North (51.54 N, 00.02 W).

3.6 Deephams STW discharge data

Daily data of the flow discharge from Deephams sewage treatment works (STW) were provided by Thames Water. In the 2011 data set, there were missing/incorrect data for the period 27/06 - 04/08 due to a meter failure.

In Table 3.12 mean, standard deviation, minimum and maximum values are given for discharge flow data of Deephams STW by season.

Table 3.12 – Mean, standard deviation (SD), minimum and maximum values of Deephams STW discharge flow data (m^3/s) calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). For the period 27/06/2011 - 04/08/2011 data were missing due to a meter failure.

Elaboration	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011
Mean	220923	237782	256858	216008	218094	215257
SD	42864	38999	52769	26509	32656	45921
Min	178655	199714	196931	190960	121521	178910
Max	439156	415103	444401	329232	311660	448996

The seasonal discharge flow data ranged between 215,000 m^3/s (autumn 2011) and 257,000 m^3/s (winter 2010-2011). The seasonal mean values of Deephams STW discharge are plotted in Figure 3.15.

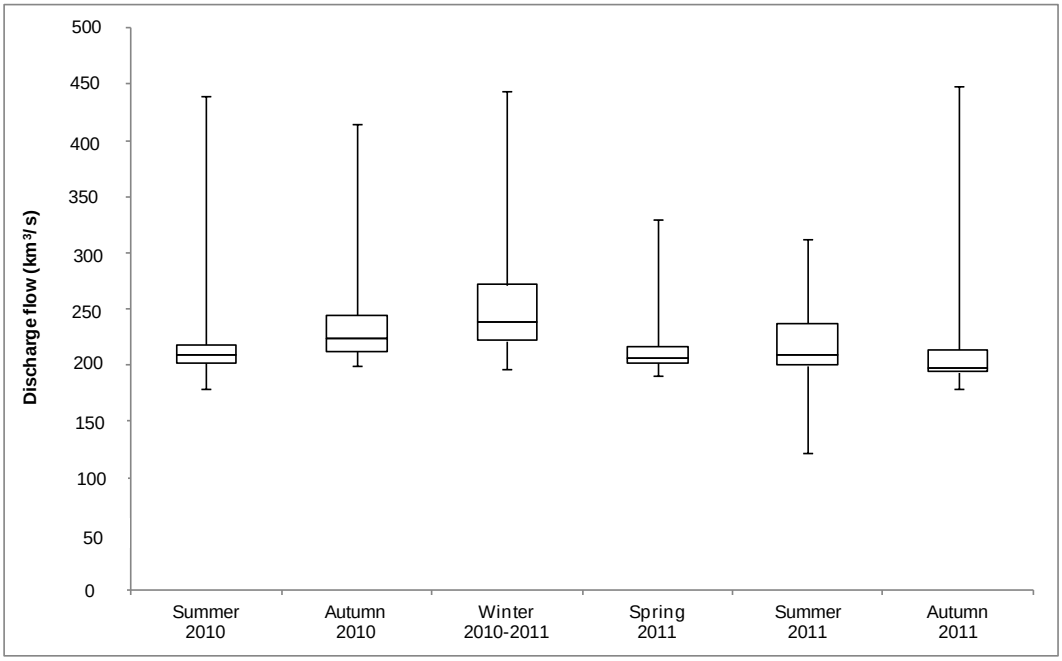


Figure 3.15 – Box plot representing Deephams STW discharge flow (km^3/s), calculated over the two-year period (from 21/06/2010 to 20/06/2012). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. For the period 27/06/2011 - 04/08/2011 data were missing due to a meter failure.

3.7 Comparisons between rainfall data, Lea Bridge flow data, STW discharge data, and dissolved oxygen

Lea Navigation water level is affected mainly by Deephams sewage treatment works (STW) discharge and rainfall, as remarked in Figure 3.16 with the data provided over the period of investigation.

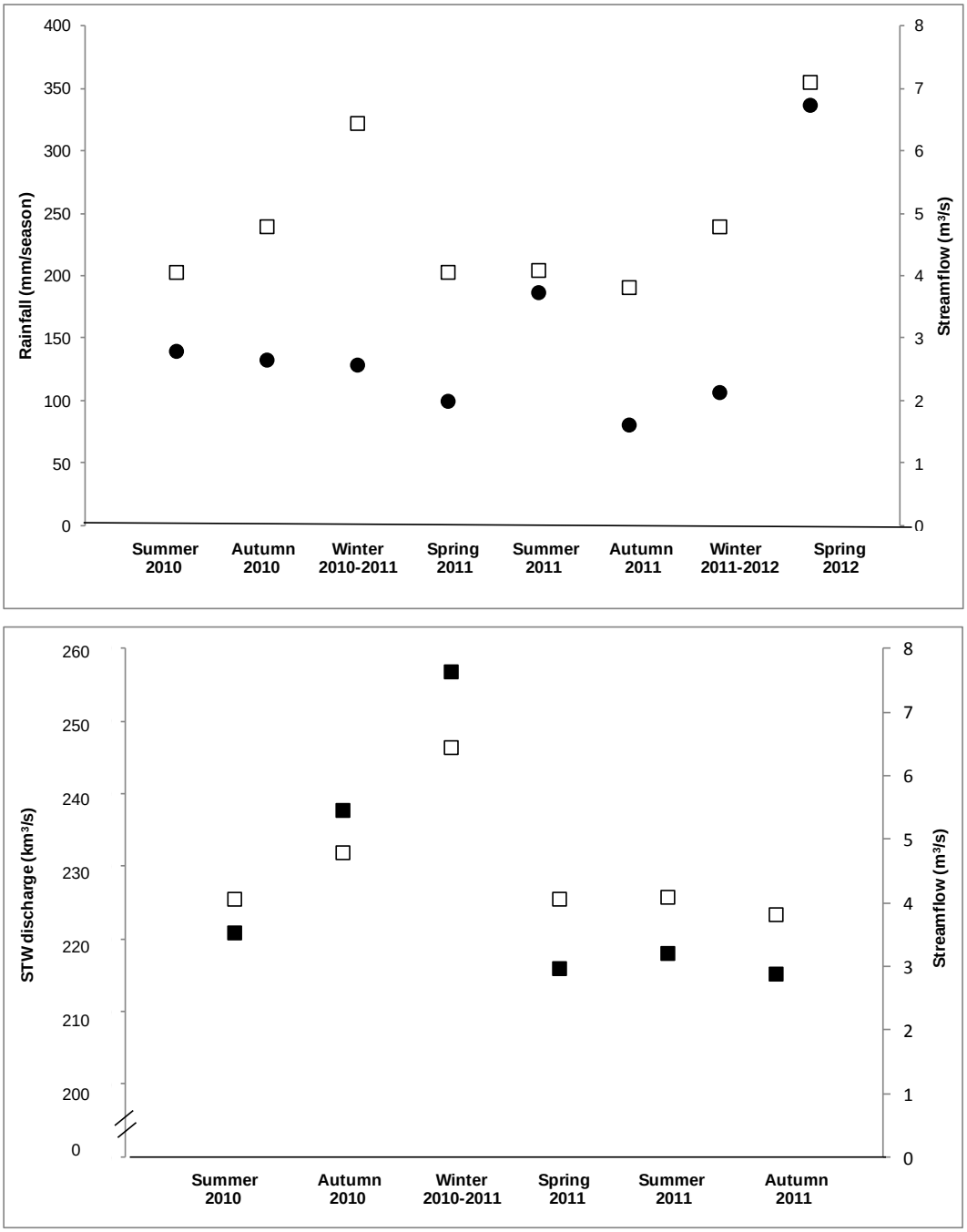


Figure 3.16 – Comparison between Deephams STW discharge means, Lea Navigation flow means (at Lea Bridge), and total rainfall data, calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Legend: ■ Deephams STW discharge; □ Lea Navigation flow at Lea Bridge; ● rainfall data.

Figure 3.17 shows a comparison between the Lea Navigation flow at Lea Bridge and the dissolved oxygen (DO) registered at the same station. The two variables presented the same trend: when the flow rate increased, the DO increased. However, this positive correlation was not seen during spring 2012, indicating a likely negative effect of the rain on the water quality due to possible channel sediments mixing and runoff from the surrounding areas. The rainfall effect was visible also during summer 2010 when the channel flow rate increased slightly, while the DO quality decreased a little. However, it was not possible to identify a decrease in the DO level associated with the peak in STW discharge flow during winter 2010, which may also be associated with the low water temperatures.

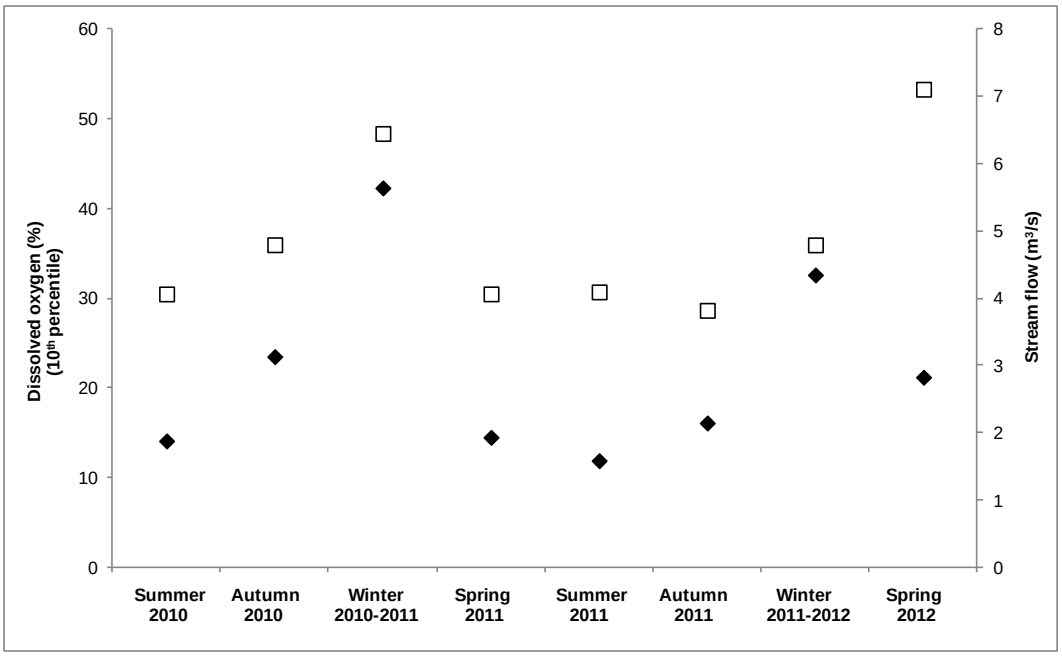


Figure 3.17 – Comparison between Lea Navigation flow mean (at Lea Bridge) and the dissolved oxygen (10th percentile) at Lea Bridge, calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Legend: □ Lea Navigation flow at Lea Bridge; ◆ dissolved oxygen (10th percentile) at Lea Bridge.

The above observations were also valid in the comparison between the river flow rate and the dissolved oxygen levels at Springfield Park station (Figure 3.18).

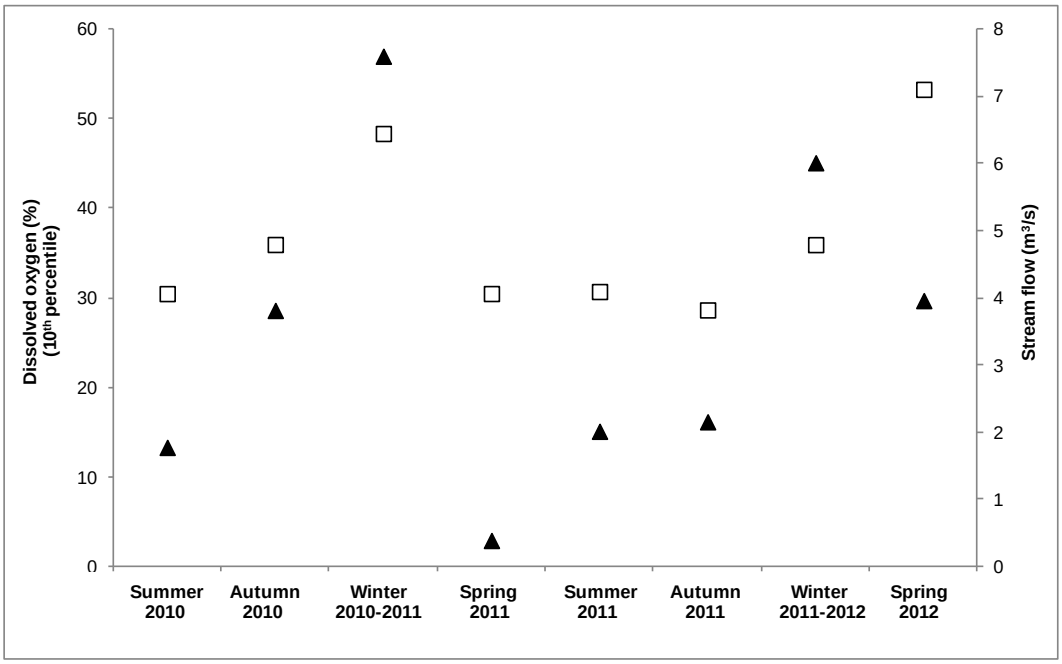


Figure 3.18 – Comparison between Lea Navigation flow mean (at Lea Bridge) and the dissolved oxygen (10th percentile) at Springfield Park, calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Legend: □ Lea Navigation flow at Lea Bridge; ▲ dissolved oxygen (10th percentile) at Springfield Park.

Figure 3.19 shows the STW discharge data and the dissolved oxygen registered at Pymmes Brook: no trend between the two data sets was identified.

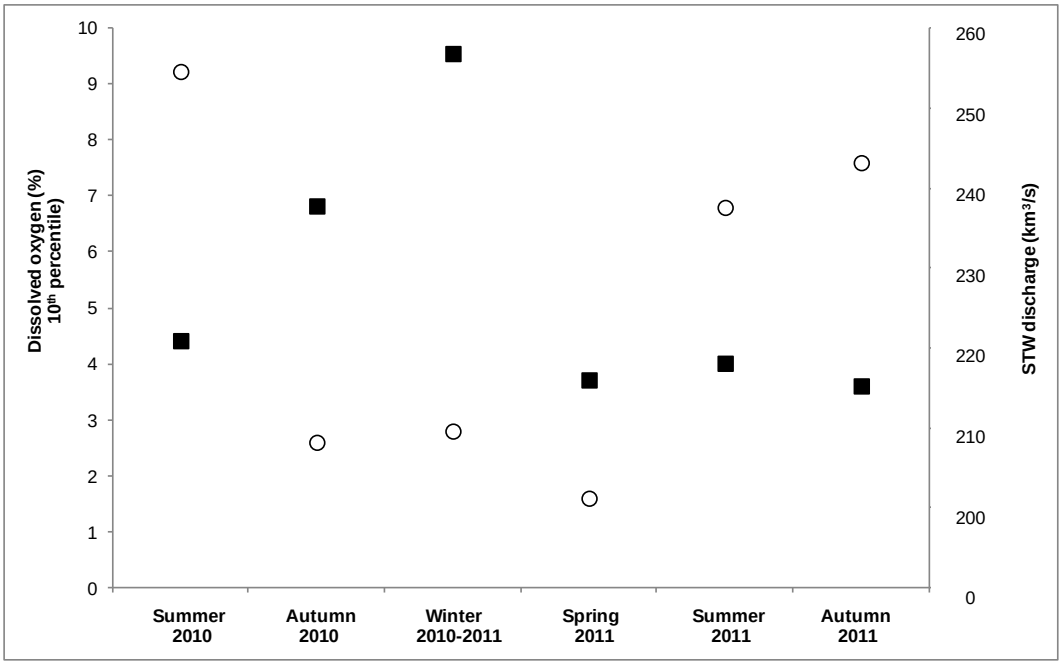


Figure 3.19 – Comparison between Deephams STW discharge mean and the dissolved oxygen (10th percentile) at Pymmes Brook, calculated on seasonal basis over the two-year period (from 21/06/2010 to 20/06/2012). Legend: ■ Deephams STW discharge; ○ dissolved oxygen (10th percentile) at Pymmes Brook.

3.8 Historic river quality

The Environment Agency conducts regularly river water analysis looking at the chemistry (ammonia, biochemical oxygen demand, and dissolved oxygen), biology (macroinvertebrate community), nitrate, and phosphate content. Chemistry, nitrate and phosphate are checked twelve times per year, and the biology is investigated every three years. Results are classified by the following grades, as indicated on the EA website (2012):

- Chemistry and biology - A to F (very good to bad)
- Nitrates and phosphates - 1 to 6 (very low levels to very high levels)

The descriptions of the above grades are given in Appendix XII.

High concentrations of nutrients may be naturally present in the stream and are not necessary indication of poor quality.

The chemical quality results from the combination of ammonia (mgN/l, calculated at the 90th percentile), biochemical oxygen demand (mg/l, calculated at the 90-percentile), and dissolved oxygen (%, elaborated at the 10th percentile). The biological quality is determinate by investigating the macroinvertebrate community.

Historic river quality data are public on EA web sites and are reported here in order to give a better overview of the area under investigation.

Table 3.13 lists the quality grades for the Lea Navigation upstream Tottenham Lock, and upstream of the confluence between Pymmes Brook and the Lea channel. From 2005 to 2009, the chemistry indicated an impacted system, with “moderate” nitrates concentration and “very high” phosphate levels. The biology quality decreased slightly in the later years, from “good” to “fairly good”.

Table 3.13 – Historic river quality grades of Lea Navigation upstream Tottenham Lock from 2005 to 2009, provided by Environment Agency. Chemistry (ammonia, biochemical oxygen demand, and dissolved oxygen) and biology (macroinvertebrate community): B = “good”; C = “fairly good”; D = “fair”. Nitrates: 4 = “moderate”; 3 = “moderately low”. Phosphates: 5 = “very high”.

Year	Chemistry	Biology	Nitrates	Phosphates
2005	D	B	4	5
2006	D	B	4	5
2007	D	C	4	5
2008	D	C	3	5
2009	D	C	4	5

The Lea Navigation quality declines downstream Tottenham Lock as illustrated in Table 3.14. The chemistry grades showed a poor ecosystem with “very high” nitrates and “excessively high” phosphate levels. The D grade for the biology quality indicated presence only of species tolerant to the pollution.

Table 3.14 – Historic river quality grades of Lea Navigation between Tottenham Hale locks (Lea Navigation) and Carpenters road (near Olympic area, in the River Lea) from 2005 to 2009, provided by Environment Agency. Chemistry (ammonia, biochemical oxygen demand, and dissolved oxygen) and biology (macroinvertebrate community): D = “fair”; E = “poor”. Nitrates: 6 = “very high”. Phosphates: 6 = “excessively high”.

Year	Chemistry	Biology	Nitrates	Phosphates
2005	E	D	6	6
2006	E	D	6	6
2007	E	D	6	6
2008	E	D	6	6
2009	E	D	6	6

Table 3.15 shows the water quality of Pymmes Brook between Salmon Brook and Lea Navigation. Pymmes Brook appeared to be an impacted channel (chemistry grade D) with “very high” nitrates and “excessively high” phosphate levels. The biology was not investigated.

Table 3.15 – Historic river quality grades of Pymmes Brook between Salmon Brook and Lea Navigation from 2005 to 2009, provided by Environment Agency. Chemistry (ammonia, biochemical oxygen demand, and dissolved oxygen): D = “fair”; E = “poor”. Nitrates: 6 = “very high”. Phosphates: 6 = “excessively high”. The biology (macroinvertebrate community) was not investigated.

Year	Chemistry	Biology	Nitrates	Phosphates
2005	E	--	6	6
2006	D	--	6	6
2007	D	--	6	6
2008	D	--	6	6
2009	D	--	6	6

Salmon Brook, which receives Deephams STW effluent and then flows into Pymmes Brook, showed “fair” chemistry quality and “bad” biology quality (grade F). Levels of nitrates were “very high” and concentrations of phosphate were “excessively high” (Table 3.16).

Table 3.16 – Historic river quality grades of Salmon Brook between Deephams STW and Pymmes Brook from 2005 to 2009, provided by Environment Agency. Chemistry (ammonia, biochemical oxygen demand, and dissolved oxygen) and biology (macroinvertebrate community): D = "fair"; E = "poor"; F = "bad". Nitrates: 6 = "very high". Phosphates: 6 = "excessively high".

Year	Chemistry	Biology	Nitrates	Phosphates
2005	E	D	6	6
2006	D	D	6	6
2007	D	F	6	6
2008	D	F	6	6
2009	D	F	6	6

Salmon Brook ecosystem upstream Deephams STW appeared to be impoverished (grade E for the chemistry) but with a "fairly good" biology quality. In addition, nitrates and phosphate levels were slightly better, since they were "moderate" and "high" respectively (Table 3.17).

Table 3.17 – Historic river quality grades of Salmon Brook upstream Deephams STW from 2005 to 2009, provided by Environment Agency. Chemistry (ammonia, biochemical oxygen demand, and dissolved oxygen) and biology (macroinvertebrate community): C = "fairly good"; D = "fair"; E = "poor". Nitrates: 4 = "moderate"; 3 = "moderately low". Phosphates: 5 = "very high".

Year	Chemistry	Biology	Nitrates	Phosphates
2005	E	D	4	5
2006	E	C	4	5
2007	E	C	4	5
2008	E	C	4	5
2009	E	C	3	5

3.9 Discussions and conclusions

A general overview of the area under investigation was achieved by collecting data of physico-chemical parameters (dissolved oxygen, temperature, pH, conductivity, turbidity and total ammonia), flow data, rainfall data, Deephams STW discharge flow data, and historic river quality data. Values were collected for a period of two years, from 21/06/2010 to 20/06/2012, grouped by season and analysed.

The dissolved oxygen trend over the period of investigation identified “poor” water quality in the stretch of the Lea Navigation between Pymmes Brook and Lea Bridge weir, with several days of oxygen levels below the threshold for stressful condition and, even worst, anoxic condition for the aquatic life. “Very bad” oxygen levels were detected at Pymmes Brook, which received the effluent of Deephams sewage treatment works through Salmon Brook, indicating a likely influence of Deephams STW effluent but also other not-investigated pollution sources, since for example also Salmon Brook (Angel station) showed “poor” DO levels. The influence of Deephams STW effluent in Pymmes Brook was detectable also by the higher temperature, conductivity, and total ammonia levels, and a lower pH compared to the other monitored stations. The DO levels improved in the River Lea, downstream of the weir, probably due to water mixing at the weir and the presence of plants on the banks and the bed of the river.

The dissolved oxygen (DO) measured in freshwater is a good indicator of pollution. One of the main aims of this chapter was to try to explain the low dissolved oxygen levels in Lea catchment waters through possible variations of other physico-chemical parameters. In this project, correlation analyses (Spearman rank correlation) were performed with the data provided over the two years of investigation in order to detect any seasonal relationship between the DO and the other water variables. Results did not show any strong seasonal correlation nor in those relationships where it would be expected, such as DO-temperature (negative correlation), DO-pH (positive correlation), and DO-turbidity (negative correlation). The highest number of significant correlations was detected between DO and pH, but that was not seen at all the stations over all the period of study. As demonstrated in the section 3.3, the fact that seasonal relationships were not identified does not exclude the possibility of daily correlations between variables.

Dissolved oxygen levels at Springfield and Lea Bridge were compared to rainfall data, Lea Navigation flow data, and Deephams STW discharge data. At both the monitoring stations DO concentrations showed to be positively correlated with the channel flow rate, particularly evident during the two winter periods: an increasing flow rate corresponded to increasing dissolved oxygen levels. From the data, it appeared that an increasing in the channel flow rate due to a greater STW discharge did not have negative effects on the

dissolved oxygen level, possibly helped by the low water temperatures. Conversely, from the data showed that the rainfall negatively affected the oxygen dissolved in the water, possibly due to channel sediments mixing and runoff from the surrounding areas, problem discussed in Chapter 1.

Finally in this chapter an overview of historic water quality data from 2005 to 2009 is given. From these data, it emerged that the Lea Navigation quality declines downstream of Pymmes Brook, both from a chemical and a biological point of view. Even the levels of nitrates and phosphates increased, indicating nutrient pollution. At the same time, the two tributaries Pymmes Brook and Salmon Brook are shown to be impacted ecosystems with “very high” nitrates and “excessively high” phosphate levels. The macroinvertebrate community was not investigated in Pymmes Brook, while in Salmon Brook only a small number of species very tolerant to the pollution were present. Salmon Brook ecosystem upstream of Deephams STW exhibited an impoverish chemistry (oxygen and ammonia levels), but with a “fairly good” biology quality (macroinvertebrates communities). Nitrates and phosphate levels were slightly better, since they were “moderate” and “high” respectively.

In conclusion, low DO levels were detected between Pymmes Brook and Lea Bridge weir (Lea Navigation), with several days of stressful conditions for the aquatic biota. There was evidence of the influence of the Deephams STW discharge, since levels of temperature, conductivity and total ammonia were higher at Pymmes than at the other stations, and the level of pH, which was lower, suggesting the presence of nitrifying bacteria or low photosynthetic activity. However, the levels of DO were not correlated to other physico-chemical parameters, even when a correlation would expect to be detected, such as DO-pH, DO-temperature, DO-conductivity. This indicated that the levels of oxygen in the channel, as well as the other physico-chemical parameters, were controlled by other variables. For instance, the low level of dissolved oxygen could be a consequence of low photosynthetic activity or high concentration of bacteria. Data showed that a higher river flow (combined with low temperature) influenced positively the DO in the channel, possibly due to a dilution effect. A peak in the STW discharge did not negatively affect the DO in the Lea Navigation, probably because the amount of discharge was not much greater than usual. On the other hand, rainfall was shown to affect negatively the water quality (Spring 2012) probably due to higher levels of surface runoff and sediment mixing. Unfortunately, STW discharge data were not available for spring 2012, so it was not possible to determine any influence of the STW effluent on the water quality of the Lea Navigation. The “bad” water quality downstream of Pymmes Brook was identified also by data collected by the Environment Agency (chemical and biological data) between 2005 and 2009.

4 Water quality monitoring with algal growth inhibition test and chemical analysis

Algal growth inhibition tests were performed as the standard method to investigate the water quality in the stretches of Lea Navigation, Pymmes Brook and River Lea (Figure 4.1).

The test organism was the green alga *Pseudokirchneriella subcapitata*, a planktonic unicellular organism easy to culture and sensitive to pollutants (Lewis 1998). Algal growth inhibition assay was chosen to test the river water because of its high reliability, reproducibility and robustness (Källqvist *et al.* 2008, Silva *et al.* 2009).

Tests were carried out following the guidelines set by the Environment Agency (Environment Agency 2008b) and by the Organization for Economic Co-operation and Development (OECD 2006), and the test procedure is explained in detail in Chapter 2 (paragraph 2.2.5). The test duration was of 72 ± 4 hours and the optical density (OD) was checked every 24 hours with a spectrophotometer at 550 nm, using semi-micro cuvetts. Optical density values were converted into cell concentrations by the experimental linear equation showed in “Materials and Methods” chapter (paragraph 2.2.5). After checking the validity of the test (Chapter 2, paragraph 2.2.5.1) as reported in the OECD guidelines, the average specific growth rates were estimated as logarithmic increases in biomass during the 72 hour period (OECD 2006). For each sampling site group and control group the average growth rate and the standard error of the mean (SEM) were calculated. To have a greater accuracy, the average specific growth rate was calculated using a nominally inoculated biomass (i.e. 12×10^4 cells/ml) as the starting value (OECD 2006). Finally, the percent inhibition of growth rate was estimated for each sampling site.

In order to detect the likely cause of the inhibition of the algal growth, chemical analyses of river water collected from different sampling sites were arranged with Environment Agency’s laboratories. The focus was on two main chemical species:

- organic volatile compounds, detected by gas chromatography–mass spectrometry (GCMS),
- polar compounds, identified by liquid chromatography–mass spectrometry (LCMS).

Analytical laboratories presented information only on the presence of specific compounds, but they did not give any quantitative data.

Figure 4.1 shows the sampling sites where water samples were collected to conduct algal growth inhibition tests and chemical analysis. The sampling was always conducted on the same day of the week (Monday) in order to have the similar level of discharge from

Deephams sewage treatment works (STW) on each occasion. The Environment Agency advised that during the weekends the discharge rate was higher than on weekdays.

Figure 4.1 – Map of the sampling sites for algal growth inhibition tests. Six stations were located along the Lea Navigation from Tottenham Hale, upstream of Pymmes Brook, to the Lea Bridge weir. One station was located downstream of the weir, in the River Lea (The raster map is provided by OpenStreetMap - Creative Commons-Share Alike License [CC-BY-SA]).

4.1 Preliminary investigations of the area under investigation

The study of Environment Agency's data and the literature highlighted major concerns that the water quality in the Lea Navigation was being affected by the Deephams sewage treatment works discharge. For this reason, preliminary investigations of the area were performed at four different locations around Pymmes Brook, which receives water from the effluent of the Deephams sewage treatment work:

1. Lea Navigation upstream of Tottenham Lock;
2. Pymmes Brook at the confluence with Lea Navigation;
3. Lea Navigation opposite Warwick Reservoir;
4. Lea Navigation at Springfield Park, downstream of the Marina.

The tests were conducted five times during autumn 2010, using fresh river water samples each time from those monitoring sites. Samples dates: 06/09/2010, 13/09/2010, 20/09/2010, 11/10/2010, 01/11/2010.

OECD nutrient medium was used as control. All the five tests showed high algal growth inhibition after 24 hours at Pymmes Brook, Lea Navigation opposite Warwick reservoir, and Lea Navigation at Springfield Park, compare to the algal growth in the nutrient medium. However, by the end of the test (after 72 hours) algal growth had recovered.

Figure 4.2 and Table 4.1 show an example of algal growth test results conducted with samples collected on 06/09/2010. Other graphs and data are given in Appendix VIII.

River water quality levels, detected throughout seven algal growth tests at the station opposite Warwick reservoir, did not give any additional information about likely pollution sources, since they were similar to those identified at both Pymmes Brook and Springfield Park (see Appendix VIII). For this reason, the site opposite Warwick reservoir was removed as a sampling station for future algal bioassays.

Table 4.1 – Example of results of algal growth inhibition test. The level of inhibition (%) was calculated with respect to the algal growth in the OECD medium (control). Four replicates were used for each test solution. The test was performed with water samples collected on 06/09/2010.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Tottenham Hale	4	2	4	1	3	2
Pymmes Brook	56	12	24	4	6	3
Lea Nav opposite Warwick reservoir	48	6	25	3	7	2
Lea Nav at Springfield Park	61	5	19	3	5	2

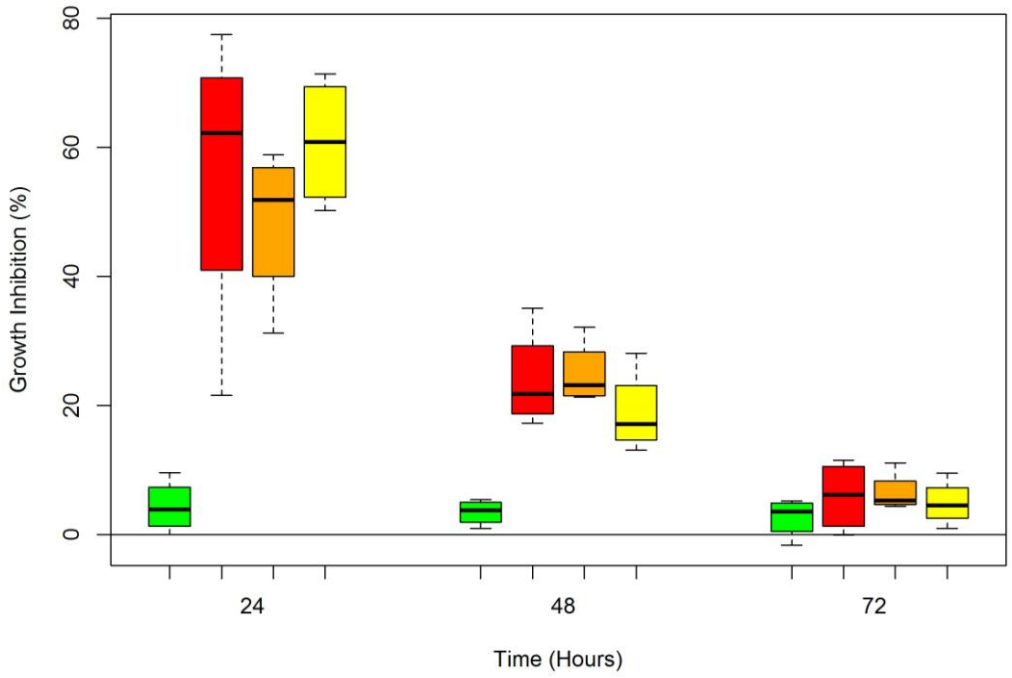


Figure 4.2 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 06/09/2010. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

4.2 Lea Navigation at Tottenham Hale station as control

The preliminary *P. subcapitata* growth inhibition tests showed that water sample collected from Tottenham Hale, which is located upstream of the confluence with Pymmes Brook, had a low level of inhibition (Table 4.2) and the algal growth was very similar to the growth in the nutrient medium. For these reasons, Lea navigation at Tottenham Hale was set as control site for the future tests. The *P. subcapitata* growth level in Tottenham Hale waters was found to be almost stable at around 1.3 units per day, after 24 hours of testing (Appendix VIII).

Table 4.2 – Examples of level of algal growth inhibition (%) in Tottenham Hale water samples after 24 hours. The inhibition was calculated with respect to the algal growth in the OECD medium (control). Four replicates were used for each test solution. The test was conducted with water collected on five different days.

Sampling date	Inhibition (%)	SEM
06/09/2010	4	2
13/09/2010	11	1
20/09/2010	-4	3
11/10/2010	19	4
01/11/2010	9	1

To give another visual interpretation regarding the comparability of the *P. subcapitata* growth trend in Tottenham Hale waters with the algal growth in OECD medium, data has been plotted as a box plot (Figure 4.3). In the graph, boxes represent growth values of Pymmes Brook, Lea Navigation opposite Warwick reservoir and Lea Navigation at Springfield Park all together. The red line is the algal growth in the medium and the green one is the algal growth in Tottenham Hale. After 24 hours, the algal growth in Tottenham Hale was more similar to the growth in the nutrient medium rather than to the other stations.

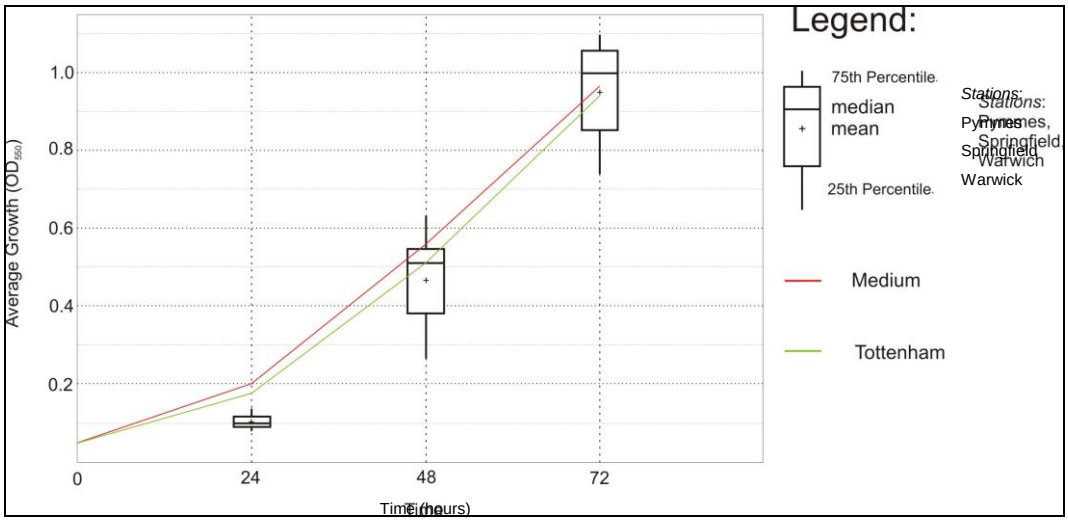


Figure 4.3 – Box plot of the results from five algal growth inhibition tests, conducted in autumn 2010. The boxes represent average combined growth values of Pymmes Brook, Lea Navigation opposite Warwick reservoir and Lea Navigation at Springfield Park all together. The red line is the algal growth in the medium and the green line is the algal growth in Tottenham Hale.

4.3 Algal growth variations at Tottenham Hale, Pymmes Brook, and Springfield Park stations over 2 years

Algal growth inhibition tests were conducted at Tottenham Hale, Pymmes Brook and Springfield Park over a period of almost 2 years. Samples were taken from September 2010 to July 2012.

Figure 4.4 shows the algal growth in water samples collected at Tottenham Hale after 24 hours of test. The *P. subcapitata* growth in Tottenham Hale was around 1.3 units per day.

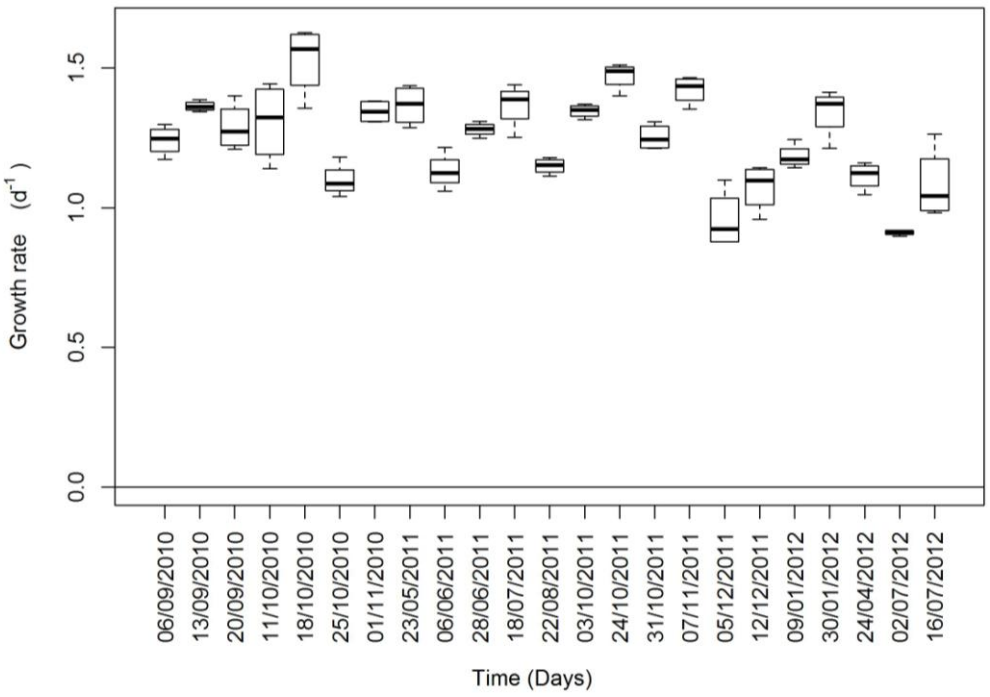


Figure 4.4 – Box plot representing *P. subcapitata* growth rate (d^{-1}) in water samples collected at Tottenham Hale, after 24 hours. Tests were conducted over two-year period, from September 2010 to July 2012. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points.

The algal growth at Tottenham Hale was considered as control and it was used to evaluate the level of inhibition (%) at Pymmes Brook station and Springfield Park site. Since the highest percentage of inhibition happened after the first day of test, only results obtained after 24 hour are given. Graphs with all the results are in Appendix VIII. Figure 4.5 shows the level of inhibition detected in Pymmes Brook and Springfield Park water samples during the period of investigation. Pymmes Brook water was not sampled on 18/07/2011.

Results did not show any seasonal trend in the two stations. Moreover, the level of inhibition at both the sampling sites was not higher than usual during summer, when

according to the literature the river water level should be low and composed mostly by sewage treatment work discharge.

The test conducted did not give any evidence of a clear pattern regarding which sampling station was the most polluted. In fact, most of the time, Pymmes Brook and Springfield Park did not present any significant differences in the level of inhibition. At the same monitoring station, different levels of inhibition were detected during sampling conducted in consequent weeks, indicating differences in the pollutant load coming into the two channels on a weekly basis. At both the stations, a decrease in the inhibition percentage was noticed during the last two samplings, likely due to a dilution effect due to heavy rainfall.

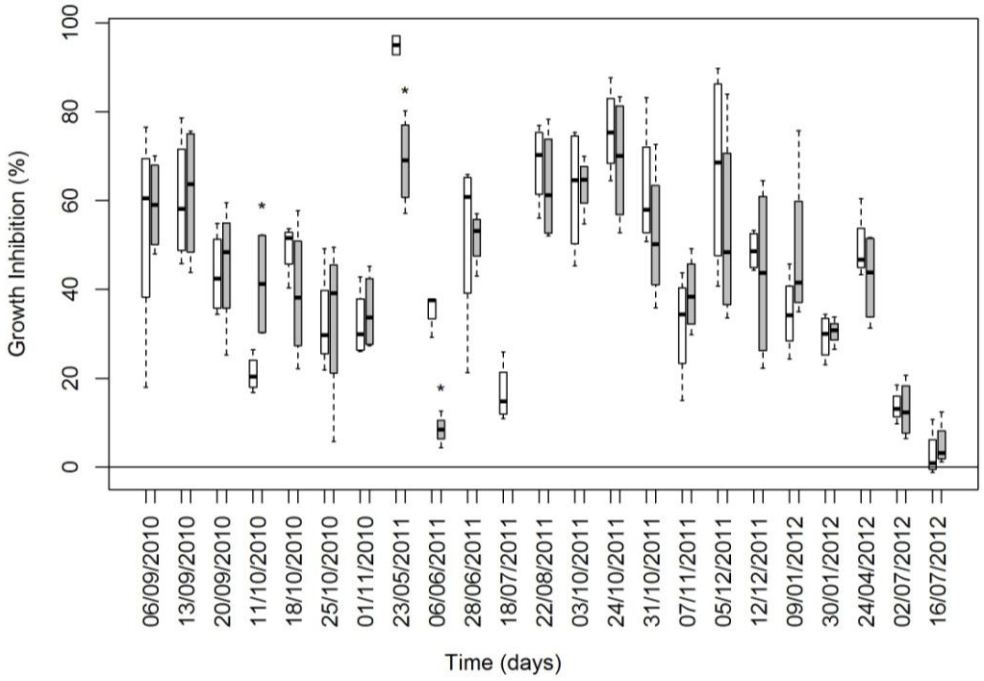


Figure 4.5 – Box plot representing *P. subcapitata* level of inhibition (%) in water samples collected at Pymmes Brook and Springfield Park, after 24 hours. The percentage of inhibition was calculated with respect to the growth in Tottenham Hale water samples (control). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. * The algal growth differs statistically ($p < 0.05$, t-test) between the two stations. Legend: ☐ Pymmes Brook; ▨ Lea Nav at Springfield Park.

4.4 Investigation of other sampling stations

After the first collection of physico-chemical parameters *in situ*, it was decided to monitor other sampling stations in addition to Tottenham Hale, Pymmes Brook and Springfield Park.

The additional stations were located at (Figure 4.1):

1. Lea Navigation at the confluence with Stonebridge Brook, which is situated between Pymmes Brook and the station at Springfield Park;
2. Lea Navigation at Lea Bridge weir, which is placed downstream of Springfield Park;
3. River Lea at Hackney Marshes, downstream of the weir, where banks and bed are not concrete.

River water samples at those sampling stations were collected from October 2011 to July 2012.

Figure 4.6 shows the level of inhibition in *P. subcapitata* population exposed to water samples collected from the Lea Navigation at the confluence with Stonebridge Brook. The graph presents only results after 24 hours of testing, when the highest level of inhibition was identified. On 05/12/2011, 12/12/2011 and 09/01/2012 the sampling at this site was not possible due to closure of the access to the monitoring station. Results showed that Stonebridge Brook was a source of pollution, in particular in two monitored events when the level of inhibition was higher than 100 % (30/01/2012 and 16/07/2012), indicating a lower algae concentration compared to the algal aliquot added the day before (time 0) at the river water sample. In the last two samplings (02/07/2012 and 16/07/2012), the inhibition at all the sampling stations was smaller than usual, while at Stonebridge Brook the percentage of inhibition was still high. Throughout all the samplings, the water coming out from Stonebridge Brook presented a whitish colour. Moreover, a smell of raw sewage was identified at this station during the last two sample collections, suggesting that Stonebridge Brook receives either water from combined sewer overflows (CSOs), misconnections, or both.

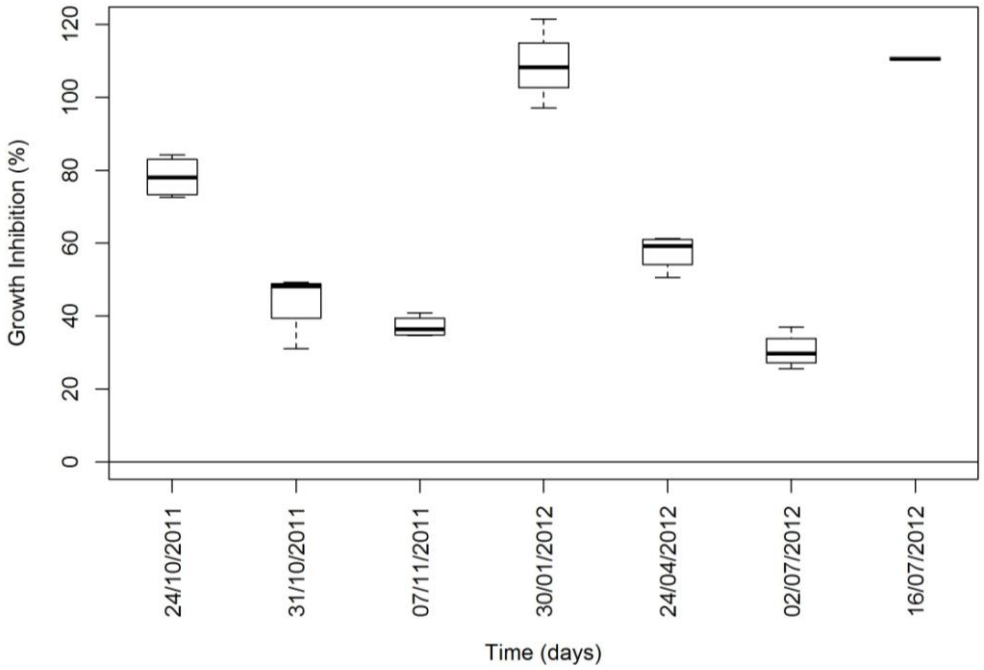


Figure 4.6 – Box plot representing *P. subcapitata* level of inhibition (%) in water samples collected from Lea Navigation at Stonebridge Brook, after 24 hours. The percentage of inhibition was calculated with respect to the growth in Tottenham Hale water samples (control). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points.

The results from the algal bioassay performed with water samples collected from the Lea Navigation at Lea Bridge weir are given in Figure 4.7. The highest inhibition level was slightly greater than 50 % compared to the algal growth in Tottenham Hale waters.

The percentages of inhibition for algal population grown in water samples collected from the River Lea at Hackney Marshes are given in Figure 4.8. The level of inhibition was greater than 50 % (52 %) only in one occasion, on 09/01/2012.

At both the stations, the level of inhibition in the last two samplings (02/07/2012 and 16/07/2012) was lower than usual, as in Pymmes Brook and Springfield Park samples.

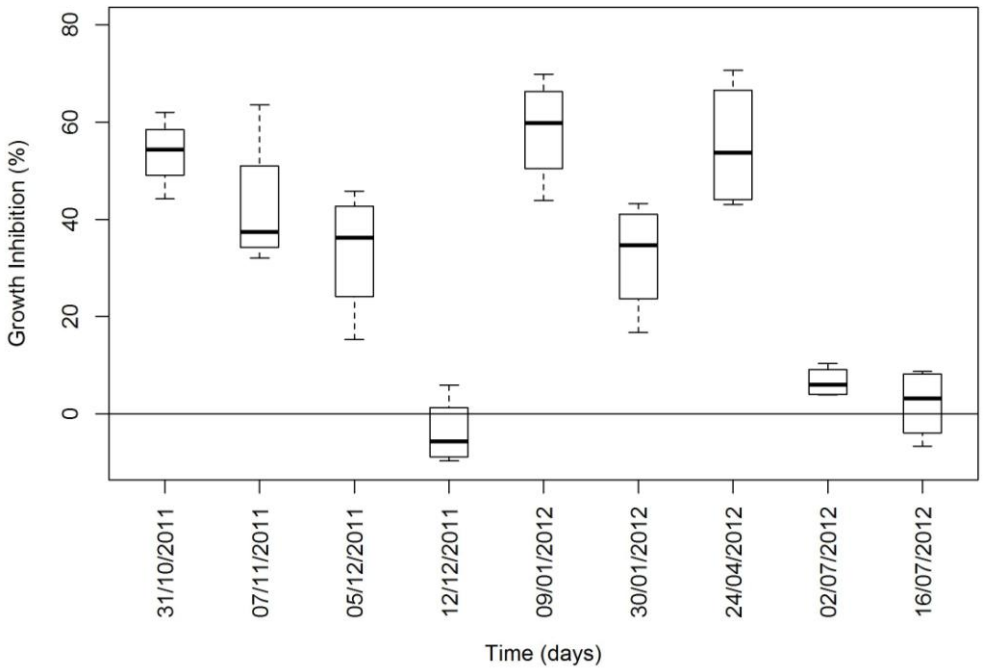


Figure 4.7 – Box plot representing *P. subcapitata* level of inhibition (%) in water samples collected from Lea Navigation at Lea Bridge weir, after 24 hours. The percentage of inhibition was calculated with respect to the growth in Tottenham Hale water samples (control). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points.

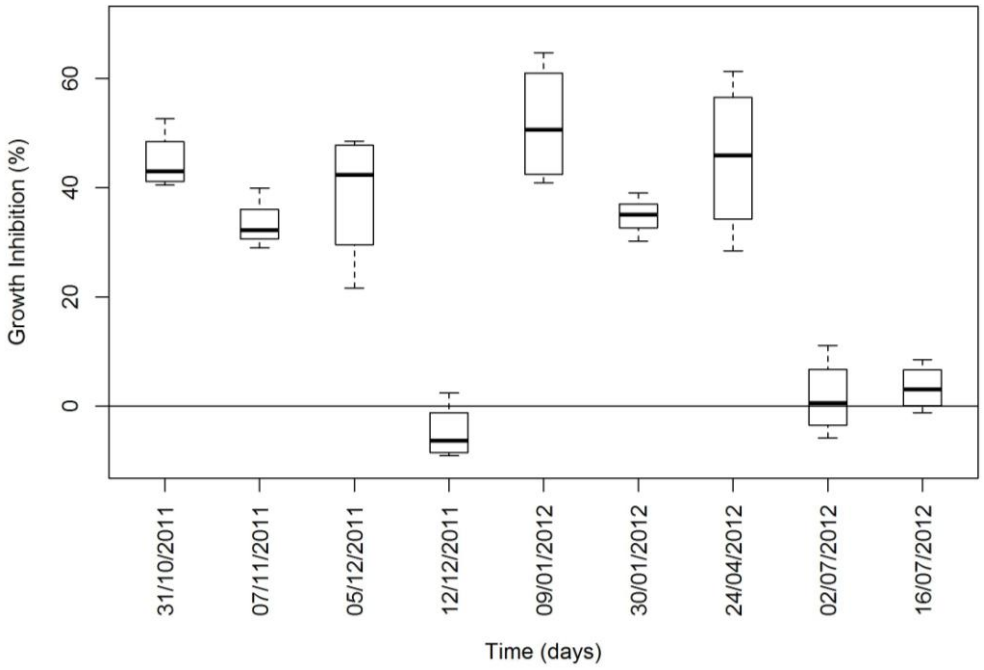


Figure 4.8 – Box plot representing *P. subcapitata* level of inhibition (%) in water samples collected from River Lea at Hackney Marshes, after 24 hours. The percentage of inhibition was calculated with respect to the growth in Tottenham Hale water samples (control). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points.

The algal tests conducted at six stations along the Lea Navigation showed an almost constant level of inhibition in this part of the channel over the two years of investigation. There was evidence that the pollutants were dissolved in the water column, since the algal assessments tested the river water. The chronic levels of pollution detected by the algal growth inhibition tests gave an indication of the impact contaminants present in the river water would have, on the primary producers in the channel (algae and aquatic plants) which would have contributed to the harmful reduction in dissolved oxygen levels.

4.5 Investigation of polar and non-polar river water fractions

Algal bioassays showed inhibition in algal growth at the monitoring stations downstream of Tottenham Locks, compared to the control site (Lea Navigation at Tottenham Hale). In order to further investigate the likely cause of algal inhibition, river water was pre-treated by Solid Phase Extraction (SPE). Using ENVI-18 columns, it was possible to separate polar compounds and non-polar compounds. Algal inhibition growth investigations were carried out with SPE pre-treated waters and untreated river samples in parallel, using river water samples collected from Lea Navigation at Tottenham Hale and from Lea Navigation at Springfield Park. The *P. subcapitata* growth in the OECD medium was used as control.
Since it was not possible to quantify the concentration of acetone (used to elute the non-polar water fraction) and methanol (used to elute the polar fraction) in the final eluents, their likely toxicity was tested with algal growth inhibition tests. Acetone showed to inhibit the algal growth over 72 hours; therefore, it was evaporated by stirring and heating. The methanol appeared to have little effect on the algal population, since the algal cells in Tottenham Hale polar water fraction showed just a small level of inhibition.
The test was repeated twice by analysing fresh water samples each time (collected on 11/04/2011 and 09/05/2011). *P. subcapitata* cells growth in OECD medium was used as control. Results from the two repetitions showed similar level of inhibition. There was evidence that the polar water fraction at Springfield Park was negatively affecting the algal population (Table 4.3 and Figure 4.9), since it showed the highest inhibition level compared to the other samples. Moreover, the inhibition percentage in Springfield Park polar fraction was much higher than the level of inhibition in Tottenham Hale polar fraction, indicating that polar pollutants were the likely major cause of negative effects on the algal population.
Inhibition was detected also in the non-polar water fraction of both Tottenham Hale and Springfield Park samples, but the level of inhibition was smaller than in Springfield Park polar fraction.

Table 4.3 – Example of algal growth inhibition test results with water pre-treated by solid phase extraction. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Four replicates were used for each test solution. The test was conducted with water collected on 09/05/2011 from the Lea Navigation at Tottenham Hale and at Springfield Park.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav Tottenham Hale	0	1	-2	0	-3	1
Lea Nav Tottenham Hale polar	4	1	1	0	0	0
Lea Nav Tott. Hale non-polar	17	1	9	1	3	0
Lea Nav Springfield Park	41	3	6	1	-2	0
Lea Nav Springfield Park polar	72	2	12	0	-5	3
Lea Nav Spr. Park non-polar	14	2	9	2	4	1

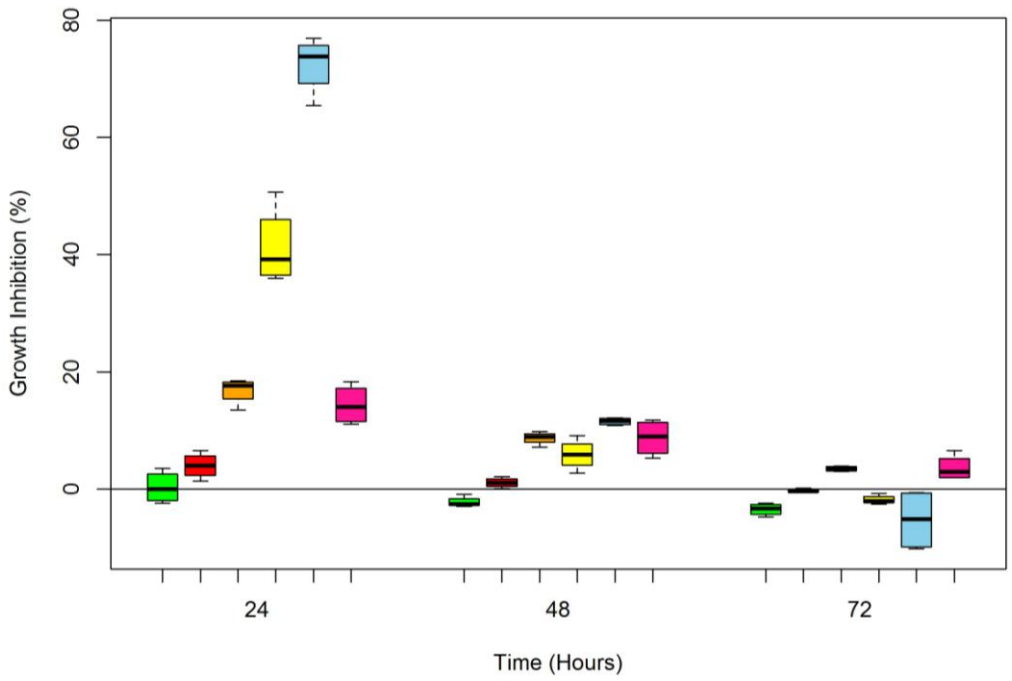


Figure 4.9 – Example of results of algal growth inhibition test conducted with river water pre-treated by solid phase extraction. The water samples were collected on 09/05/2011 from the Lea Navigation at Tottenham Hale and at Springfield Park. The percentage of inhibition was calculated with respect to the growth in the medium (control). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Legend: Lea Nav at Tottenham Hale; Lea Nav at Tottenham Hale polar fraction; Lea Nav at Tottenham Hale non-polar fraction; Lea Nav at Springfield Park; Lea Nav at Springfield Park polar fraction; Lea Nav at Springfield Park non-polar fraction.

4.6 Chemical analyses

Chemical analyses were performed three times: 28/06/2011, 07/11/2011 and 31/01/2012. Water samples were collected on 28/06/2011 from Lea Navigation at Tottenham Hale (upstream of Pymmes Brook), Pymmes Brook, and Lea Navigation at Springfield Park (downstream of Pymmes Brook). Table 4.4 shows a summary of the results. Detailed tables are presented in Appendix IX. None of the organic volatile compounds were detected at potentially toxic concentrations. Squalane (natural component of human sebo, used in cosmetics) was detected only at Springfield Park at a concentration of 15 µg/l. However, it has been showed that its acute animal toxicity is low (Christian 1982). In agreement with the European directive COM(2011)876, two priority hazardous substances were detected: anthracene at Springfield, and fluoranthene at all the three sites. However, the concentrations of both the chemicals were within the environmental quality standards listed in the European legislation.

The total concentration of all the detected organic compounds was largely higher at Pymmes Brook and Springfield Park than at Tottenham Hale. The inhibition of the algal growth could be explained by a synergistic effect of the chemicals. Regarding the polar species, a list of chemicals present at Pymmes and Springfield but absent at Tottenham is given in Table 4.5.

Table 4.4 – Summary of the chemical analysis results of water samples collected on 28/06/2011.

Analyte	Lea Nav at Tottenham Hale	Pymmes brook	Lea Nav at Springfield Park
Organic volatile compounds (Total concentration - µg/l)	2	13	93
Organic volatile compounds (Total count)	21	35	53
Polar compounds (Total count)	10	16	19

Table 4.5 – List of polar compounds present at Pymmes Brook and Springfield Park, but absent at Tottenham Hale. The water samples were collected on 28/06/2011. X = presence.

Chemical name (function)	Pymmes Brook	Lea Nav at Springfield Park
2,4-Dichlorophenoxyacetic acid (herbicide)		X
Mecoprop (herbicide)		X
Atenolol (medicine)	X	X
Diclofenac (medicine)	X	X
Mefenamic acid (medicine)	X	X
Sotalol (medicine)	X	X
Pirimiphos-methyl (surfactant)	X	X
Perfluorodecanoic acid (surfactant)	X	X
Perfluorohexane sulfonate (surfactant)	X	X
Perfluorononanoic acid (surfactant)	X	X
Perfluorooctane sulfonate (surfactant)	X	X
Perfluorooctanoic acid (surfactant)		X

A second set of chemical analyses was run on water samples collected on 07/11/2011 at six sites: Lea Navigation at Tottenham Hale, Pymmes Brook, Lea Navigation at Stonebridge Brook, Lea Navigation at Springfield Park, Lea Navigation at Lea Bridge weir, and River Lea at Hackney Marshes. During these analyses, general parameters were also measured, such as biological oxygen demand (BOD_5), ammoniacal nitrogen (NH_3-N), total oxidized nitrogen (N_{OX}), chloride, orthophosphate (reactive P), and turbidity. Table 4.6 presents a summary of the results. Detailed tables are presented in Appendix IX.

In the River Lea, at Hackney Marshes six priority hazardous substances (COM(2011)876) were identified: anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, and fluoranthene. The concentration of benzo[b]fluoranthene and benzo[ghi]perylene were higher than the maximum allowable concentration for inland surface waters (COM(2011)876). Fluoranthene was also detected at the other sampling stations but it was not at hazardous concentrations. The highest values of BOD_5, ammoniacal nitrogen (toxic form) and turbidity, and the highest number of organic volatiles were identified in the

Lea Navigation at the confluence with Stonebridge Brook, indicating this inflow channel as likely source of pollution. Fewer polar compounds were identified compared to the previous analysis (28/06/2011) and the highest number of polar substances was detected in the Lea Navigation at Stonebridge Brook. No polar pollutants were identified in the Lea Navigation at Springfield Park (Table 4.7).

Table 4.6 – Summary of the chemical analysis results of water samples collected on 07/11/2011. Legend: NH_3-N = ammoniacal nitrogen; N_{OX} = total oxidised nitrogen; BOD_5 = Biological Oxygen Demand.

Measure	Lea Nav at Tottenham Hale	Pymmes Brook	Lea Nav at Stonebridge Brook	Lea Nav at Springfield Park	Lea Nav at Lea Bridge weir	River Lea at Hackney Marshes
BOD_5 (mg/l)	<1	2.7	19.1	2.7	1.9	1.6
NH_3-N (mg/l)	0.046	0.823	2.8	0.97	0.466	0.398
N_{OX} (mg/l)	4.74	16.9	5.85	16.7	15.5	14.7
Chloride(mg/l)	88.8	109	90.7	110	104	108
Reactive P(mg/l)	0.122	3.06	1.88	3	2.72	2.66
Turbidity (NTU)	<1	6.4	9.7	2.51	9	3.8
Organic volatile compounds (Total concentration - µg/l)	4	22	194	18	14	16
Organic volatile compounds (Total count)	20	34	68	36	35	40
Polar compounds (Total count)	1	1	4	0	1	1

Table 4.7 – List of polar compounds present in water samples collected on 07/11/2011. X = presence.

Chemical name (function)	Lea Nav at Tottenham Hale	Pymmes Brook	Lea Nav at Stonebridge Brook	Lea Nav at Springfield Park	Lea Nav at Lea Bridge weir	River Lea at Hackney Marshes
Benzalkonium C10 (nitrogenous cationic surface-acting agent)			X			
Carbamazepine (medicine)	X	X	X		X	X
Hexadecyltrimethylammonium (cationic surfactant)			X			
Paracetamol (medicine)			X			

A third chemical analysis was carried out on river water samples collected on 30/01/2012 when BOD_5, NH_3-N, N_{OX}, chloride, reactive P, turbidity, and the load of coliform bacteria were determinated. Water samples were collected at the same six stations as the previous survey. Results are shown in Table 4.8. As detected from the previous analysis (07/11/2011), Lea Navigation at Stonebridge Brook showed to be the highest polluted station, also highly contaminated by faecal bacteria.

Table 4.8 – Chemical analysis results of water samples collected on 30/01/2012. Legend: NH_3-N = ammoniacal nitrogen; N_{OX} = total oxidised nitrogen; BOD_5 = Biological Oxygen Demand.

Measure	Lea Nav at Tottenham Hale	Pymmes Brook	Lea Nav at Stonebridge Brook	Lea Nav at Springfield Park	Lea Nav at Lea Bridge weir	River Lea at Hackney Marshes
BOD_5 (mg/l)	1.2	4.1	15.9	2.5	2	1.9
NH_3-N (mg/l)	<0.03	0.566	2.98	1.07	0.726	0.632
N_{OX} (mg/l)	5.92	13.2	7.49	13.6	14	13.9
Chloride (mg/l)	84.3	114	94.2	106	109	109
Reactive P (mg/l)	0.312	3.01	1.86	2.8	2.75	2.69
Turbidity (NTU)	1.7	2.7	8.2	2.6	2.4	3.3
Faecal Coliform (no./100ml)	450	35000	>100000	28000	12000	6500
Faecal Streptococci (no./100ml)	18	2100	27000	1636	937	856
Total Coliform (no./100ml)	1545	61000	>100000	77000	>100000	40000

In the UK there are no regulations on the coliform levels in streams, but since Lea Navigation is used for recreation purposes such as rowing, Table 4.9 gives the quality requirements for bathing water (directive 76/160/EEC).

Table 4.9 – Quality requirements for bathing water in UK (directive 76/160/EEC). G = guide, I = mandatory.

Parameters	G	I
Faecal coliforms / 100 ml	100	2000
Faecal streptococci / 100 ml	100	-
Total coliforms / 100 ml	500	10000

The Lea Navigation at Tottenham Hale met the quality requirements for bathing water, while the stations located downstream of Pymmes Brook presented higher levels than those recommended. Lea Navigation at the confluence with Stonebridge Brook shows the highest bacterial levels. Further downstream it was possible to identify decreasing concentrations of faecal bacteria indicating a likely low human and animal waste load.

As discussed in Chapter 1 (paragraph 1.4), the amount of faecal bacteria is not a strict indicator of human contamination, since the same kind of bacteria are present also in animal faeces. Some studies (Buerge *et al*. 2006, Hillebrand *et al.* 2012, Sauvé *et al.* 2012) demonstrated that caffeine is a good marker for human sewage contamination, since it is exclusively human specific. Chemical analysis conducted on sampling collected on 07/11/2011 showed that the concentration of caffeine was ~ 17 fold higher at Stonebridge Brook than at the other sampling stations (Appendix IX).The high caffeine concentrations and high faecal bacteria levels detected at Stonebridge Brook indicated a likely contamination by untreated wastewaters flowing from this Brook into Lea Navigation.

4.7 Discussions and conclusions

Algal growth inhibition tests were used to investigate the water quality in the Lea Navigation, upstream and downstream of the confluence with Pymmes Brook, which receives water from the discharge of Deephams sewage treatment work. As suggested by Struijs *et al*. (2009) ecotoxicological tests are useful tools to study the pollution in a stream even if the pollutants dissolved in it are unknown.

Early results showed inhibition after 24 hours, followed by algal recovery in the Pymmes Brook samples, and in the samples from Lea Navigation opposite Warwick reservoir and Springfield Park (both the stations were located downstream of Pymmes Brook). This indicated the presence of pollutants dissolved in the river water that were affecting algal populations. The most likely explanation regarding the recovery by 72 hours was that absorption of compounds resulted in the almost complete absence of bioavailable toxicant in the free water volume, as a result of working with low toxicant concentrations and small test solution volumes (OECD 2006). Phenomena of uptake by the algae, sorption, volatilization and degradation are described by Simpson *et al.* (2003) as causes of decrease of pollutants concentration during ecotoxicity test periods.

Preliminary investigations conducted in this study showed that the water collected from the Lea Navigation upstream of the confluence with Pymmes Brook (at Tottenham Hale), did not have a negative effect on the *P. subcapitata* population. The algal growth trend at Tottenham Hale was stable and it was very similar to the algal growth rate in the nutrient medium, which led to its use as control during the next test. Moreover, those results identified the channel stretch upstream of the confluence with Pymmes Brook as not being heavily polluted.

Tottenham Hale, Pymmes Brook and Springfield Park sites were investigated for almost 2 years. Algal growth tests results did not show any particular inhibition trend at Pymmes Brook and Springfield Park stations. The level of inhibition was not higher than usual during the two monitored summers, when the river water level should be low and composed mostly of sewage treatment work discharge. Results from algal bioassays conducted with water samples collected at the same sampling sites during consecutive weeks showed different levels of inhibition, indicating that the level of pollution was not constant.

The tests demonstrated that Pymmes Brook and Springfield Park had similar water quality, since they showed similar level of algal inhibition. However, algal bioassays conducted with water samples collected from the Lea Navigation at the confluence with Stonebridge Brook indicated this small inflow channel as another potential source of pollution. During the last two samplings (02/07/2012 and 16/07/2012) Stonebridge Brook samples presented high level of inhibition while the other monitoring stations showed decreased level of inhibition. Moreover, between Pymmes Brook and Stonebridge Brook a significant amount of water comes into the Lea Navigation through a stretch of the Old River Lea (Figure 1), which could dilute the water coming out from Pymmes Brook. A likely explanation of similarities in the pollution level at Pymmes and Springfield Park sites could be the combination of pollution sources (such as Stonebridge Brook, Marina, diffuse pollution from boats and runoff) and dilution effects (Old River Lea inflow) between the two sampling sites.

Downstream of Springfield Park, two other sites were monitored: one in the Lea Navigation at Lea Bridge weir and the second downstream of the weir on the River Lea at Hackney Marshes. Algal inhibition was detected at both stations, indicating the presence of other pollution sources, since they were located several kilometres downstream of its inflow. The inhibition level detected at the site located in the natural River Lea at Hackney Marshes suggested traces of pollution despite the presence of vegetation along the stream, which should act as a depuration filter.

In order to further investigate the nature of the inhibiting analyte, water samples (Tottenham Hale and Springfield Park) were pre-treated by solid phase extraction and separated into polar and non-polar fractions and algal growth tests were conducted. The highest level of inhibition was detected in Springfield Park polar fraction after 24 hours of testing. The level of inhibition identified in the polar fraction of the Springfield Park sample was noticeably higher than the percentage of inhibition detected in the polar fraction of the Tottenham Hale sample, indicating that polar compounds were likely to be the main responsible factor of the inhibition of the *P. subcapitata* growth in water samples collected at Pymmes Brook and downstream of its confluence with the Lea Navigation.

Results from chemical analyses confirmed that the Lea Navigation at Tottenham Hale (upstream of Pymmes Brook) had better water quality than the stations located

downstream of the confluence with Pymmes Brook. The two stations further downstream (Lea Navigation at Lea bridge weir and River Lea at Hackney Marshes) showed the same concentration of organic volatile compounds and the same amount of polar pollutants identified in the other sites, but the level of faecal bacteria was lower, indicating a likely low human sewage discharge (such as misconnections) or possible dilution. Whilst the Environment Agency has been concerned about Pymmes Brook and the likely pollution from Deephams STW, chemical analysis indicated Stonebridge Brook's uncontrolled pollution input (high BOD, coliforms level, and total organic volatile compounds concentration) to be an equally important contributor to the poor environmental quality in this reach of the channel.

In conclusion algal bioassays and chemical analyses confirmed the presence of pollution at Pymmes Brook and downstream of its confluence with the Lea Navigation, indicating a contribution of Pymmes Brook water to the level of pollution in the reach of the Lea Navigation under investigation. Differences in the inhibition levels at the same sampling point demonstrated evidence of periodical variations in the level of pollutants. However, no consistent seasonal trend was identified, during the summer when it would be expected to detect high inhibition levels due to higher pollutant concentrations in the river water since the river water presents a low level and it is mostly composed of sewage treatment work effluent. Further algal bioassays showed that polar pollutant(s) was the likely major cause of inhibition of *P. subcapitata* growth. However, the highest level of inhibition was detected after 24 hours of tests, followed by algal population recovery, indicating that the toxicant concentration was low. Both algal bioassays and chemical analyses indicated Stonebridge Brook as a potential source of pollution, which needs to be further monitored. In particular, the faecal bacteria levels and the caffeine concentrations suggested that Stonebridge Brook was a source of untreated wastewaters, possibly either from combined sewer overflows (CSOs) or misconnections or both. Inhibition was also detected with water samples collected from the two sites located further downstream of Springfield Park (Lea Bridge weir and Hackney Marshes). However, the levels of faecal bacteria contamination were lower than in the upstream sites, indicating either dilution effects or lower human and animal sewage discharge loads.

This project gave evidence of the importance of the ecotoxicological assessments in the investigation of the river water pollution. The results from the algal growth tests supplemented the results obtained by the analysis of physico-chemical parameters collected by the Environment Agency, underlining the importance of a multi-parameter approach for the investigation of the water quality of water bodies. The analysis of physico-chemical parameters collected by the Environment Agency (Chapter 3) showed a

general poor water quality of the Lea Navigation, detecting a negative influence of the Pymmes Brook on the receiving Lea channel waters. However, those data did not provide a good overview of the area under investigation. Only the improvement of the spatial monitoring data (with *in situ* collection of physico-chemical parameters) permitted the identification of another harmful source of pollution, which was Stonebridge Brook. The algal growth inhibition tests gave additional information to the physico-chemical parameters: 1) the pollution was chronic and it was due to pollutants present at low concentrations, and 2) the pollutants, which were affecting the primary producer in the river water, were probably polar. The subsequent chemical analysis and investigations of the coliform populations suggested that the chronic disturbance to the aquatic environment could be due to a combination of pollutants, which affected the algal population in the channel, and oxygen consumption of high levels of coliforms. However, the chemical analyses were not able to provide useful information on specific pollutants. The study of physico-chemical parameters in Chapter 3 focused on the effects of the chronic pollution, and the algal assessments identified a chronic contamination. However, over the two years of investigation, the algal inhibition growth tests showed variations in the pollution levels which could be due to single storm events, causing: 1) sediment mixing, re-dissolving pollutants in the water column, 2) urban run-off from the surrounded areas, and 3) overflow of a combined sewer.

This project demonstrated that it was possible to achieve a clear picture of the water quality in the Lea channel only by combining the information obtained from different methodologies, identifying Pymmes Brook and Stonebridge Brook as two major sources of pollution and detecting a contribution from the urban diffuse pollution to the water quality.

5 Water quality monitoring with CellSense whole cell biosensors

5.1 Biosensor environmental toxicity testing

CellSense whole cell biosensors were employed to investigate any evidence of metabolic disturbance in algal and bacterial cells (biocatalysts) when exposed to the river water. The biocatalysts employed were the alga *Pseudokirchneriella subcapitata*, the cyanobacterium *Synechococcus leopoliensis*, and the bacterium *Escherichia coli*. Monitoring the cells' metabolism with CellSense amperometric device uses chemical mediators, which facilitate electron donation between the biocatalyst and the sensor's working electrode. *p*-Benzoquinone was used to monitor eukaryotic organisms, such as *P. subcapitata*, since this mediator penetrates the cell membrane reaching the structures where the redox events occur. For prokaryotic organisms, such as *E. coli*, the non-penetrating mediator potassium ferricyanide was used.

The use of mediated amperometric biosensors offers several advantages, including low cost, ease of use and rapid detection of changes in metabolic activity (potentially within 30 minutes). Up to 32 samples can be monitored simultaneously, and both inhibition and stimulation of the biocatalyst's metabolic activity can be monitored in real time. The use of whole cells as biocatalyst (without the necessity of genetic manipulation), makes CellSense a simple device accessible to everybody. Moreover, the response is not affected by turbidity, making CellSense a good system to investigate river water and wastewater effluent.

In the environmental monitoring, CellSense has been used: 1) to study the toxicity of 3,5-dichlorophenol and other phenols in wastewater, with activated-sludge-based biosensors (Evans *et al*. 1998); 2) to test target analytes such as non-ionic surfactants, benzene and naphthalene sulfonates, which are present in wastewaters, using *E. coli* biosensors (Farré *et al*. 2001); 3) to analyse wastewater treatment works influent and effluent, using *E. coli* biosensors (Farré *et al.* 2001); 4) to investigate the toxicity of wastewaters and sewage sludge, with both *Pseudomonas putida* and *E. coli* biosensors (Farré and Barceló 2003); 5) to study trade effluents, with both activated-sludge-based and *E. coli* biosensors (Daniel et al. 2004). All these papers agreed that CellSense whole cell biosensors have the potential to provide a rapid technique for toxicity measurements in urban rivers, making this methodology an appropriate tool for this project.

Protocol 1 (standard operating protocol) was performed to test the water toxicity as described in "Materials and Methods" section (paragraph 2.2.7). However the biological response was masked by the (electro)-chemical activity of the river water samples hiding any biological response. To avoid this chemical interference two alternative approaches

were investigated, protocols 2 and 3. Protocol 2 was developed to prevent any interaction between river water and mediators, by exposing biosensors to river water without any chemical mediator and then, following this period of exposure, to monitor the metabolic activity by re-presenting the biosensors to the initial optimal solution supplemented with mediator: any changes in the outcome signal between the pre- and the post- exposure phase would reflect the impact of the exposure to river water. Protocol 3, developed alongside the protocol 2, used a lower potential at the working electrode, to try to monitor only the electron transfers due to redox reactions promoted by the mediator.

The water samples analyzed with the biosensors were collected from the same sampling sites as those used for the algal growth inhibition tests. The development of the three protocols used samples from three selected stations: Lea Navigation at Tottenham Hale Pymmes Brook and Lea Navigation at Springfield Park. The monitoring site at Tottenham Hale was considered as control, since during algal growth tests, it showed little or no inhibition compared to the other sampling stations. When protocol 3 had been developed and proven to be successful, samples from all the six monitoring sites (Lea Navigation at Tottenham Hale, Pymmes Brook, Lea Navigation at Stonebridge Brook, Lea Navigation at Springfield Park, Lea navigation at Lea Bridge weir, River Lea at Hackney Marshes) were screened with the CellSense system.

5.2 Detection limits for six chemicals using *E. coli* biosensors

The sensitivity of *E. coli* biosensors to chemicals has been tested by Rawson and his team in the past at the LIRANS laboratory at the University of Bedfordshire. The tests identified absolute threshold sensitivities of *E. coli* for the detection of the chemicals in saline medium.

Table 5.1 shows detection limits and EC_{50} values determined with *E. coli* biosensors for six chemicals. These values were never published but they were provided to users as guide concentrations for future testing.

Table 5.1 – Limits of detection (ppb) and indicative EC_{50} values (ppm) of *E. coli* biosensors for six chemicals. The values were detected in the laboratory of the University of Bedfordshire by Rawson and colleagues. N/d = not detected.

Chemical name	Limits of detection (ppb)	Indicative EC_{50} values (ppm)
2,5 chlorophenol	1200 µg/l	10 mg/l
Pentachlorophenol	9 µg/l	1 mg/l
Mercuric chloride	27 µg/l	1 mg/l
Zinc Chlorine	n/d	88 mg/l
Formaldehyde	n/d	156 mg/l
2,6 dinitro-orthocresol	500 µg/l	n/d
Tributyltin	11 µg/l	n/d

5.3 Preliminary tests

Initially biocatalysts were tested in growth media or in bacteriological saline to evaluate their metabolic activity under normal conditions and to optimise biocatalyst loading of the screen-printed sensors. Both algal and bacterial biosensors were tested applying a potential of +550 mV to the working electrode, and preliminary tests were conducted with differing amounts of cells loaded on the working electrode.

S. leopoliensis and *P. subcapitata* were tested in their growth media, i.e. BG11 and 3N-BBM+V respectively (CCAP, Culture Collection of Algae and Protozoa), supplemented with mediator, in order to provide the optimal conditions for the alga and the cyanobacterium. Photosynthetic activity was monitored during period of illumination by high intensity leds, wavelength 635 nm. Both the biocatalysts were monitored with *p*-benzoquinone (*p*BQ) during both light and dark periods.

As shown in Figure 5.1 and Figure 5.2, it was possible to monitor the photosynthetic activity during illumination, with the expected light and dark responses clearly seen. However, when *S. leopoliensis* was monitored in BG11 medium an increasing non-biological signal was detected that proved to be (electro)-chemical interference from the medium (paragraph 5.3.1).

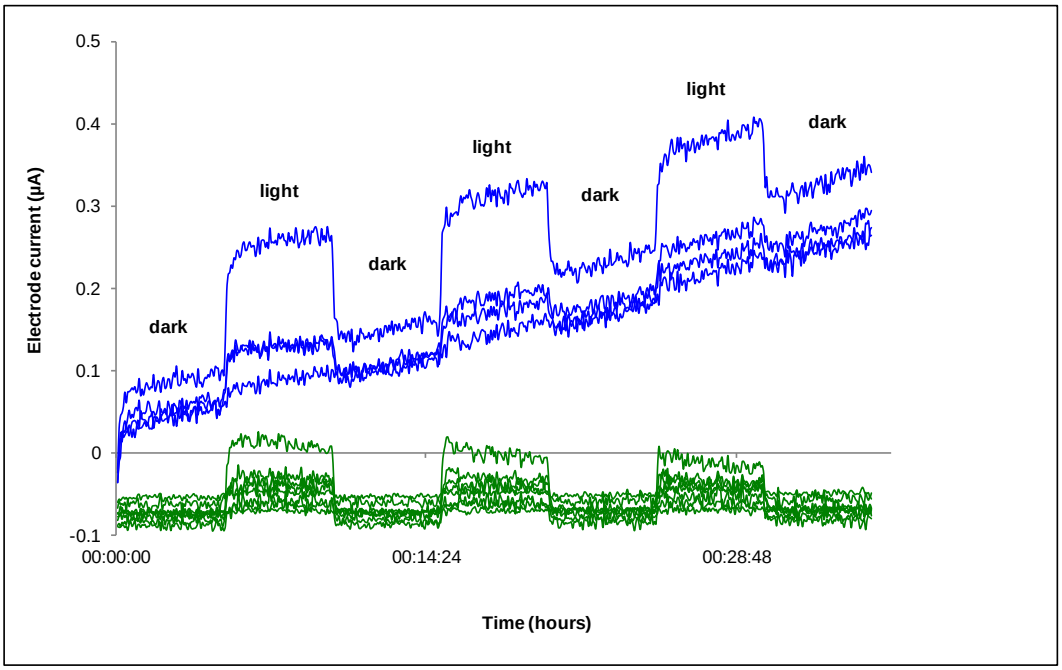


Figure 5.1 – Responses of *S. leopoliensis* and *P. subcapitata* biosensors in *p*BQ supplemented BG11 medium and 3N-BBM+V medium respectively to periods of light and dark. Legend: *P. subcapitata* in 3N-BBM+V medium; *S. leopoliensis* in BG11 medium.

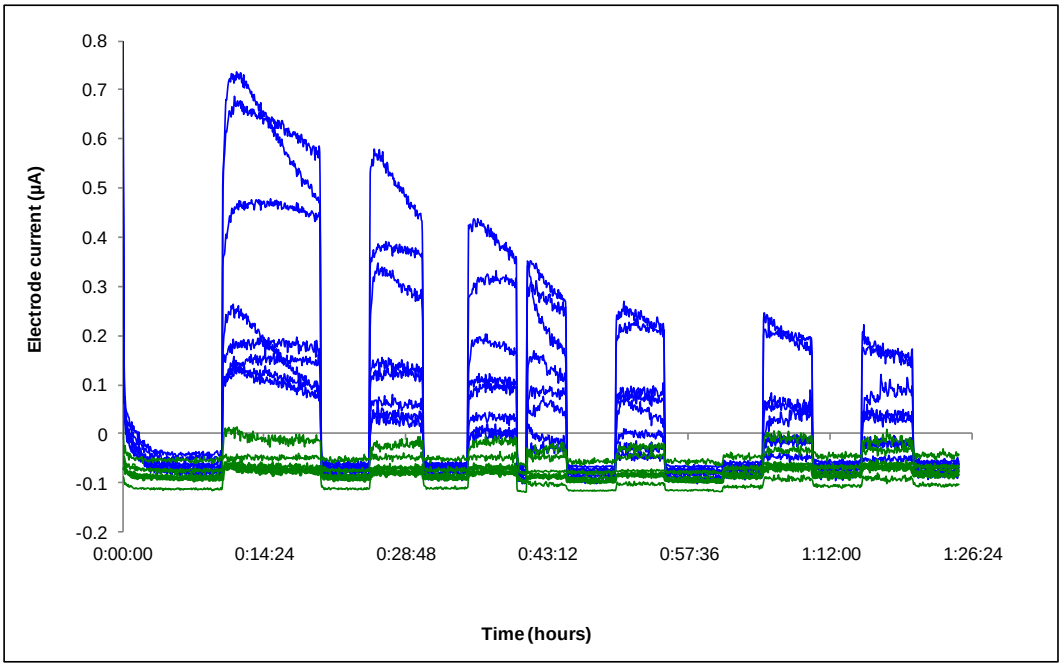


Figure 5.2 – Responses of *S. leopoliensis* and *P. subcapitata* biosensors in *p*BQ supplemented 3N-BBM+V medium to periods of light and dark. Legend: *P. subcapitata*; *S. leopoliensis*.

Metabolic activity of *E. coli* biosensors was monitored in 0.85% saline (Figure 5.3). The bacterial biosensors were immersed in 9.9 ml of 0.85% saline supplemented with potassium ferricyanide (FeCN). When the substrate cocktail (equal concentrations of D-glucose, sodium succinate, sodium lactate) was added through the ports on the instrument's lid, the biosensors responded rapidly to the presence of the respiratory substrate. The response was visible as a rapid increased signal, which became stable within 7 minutes.

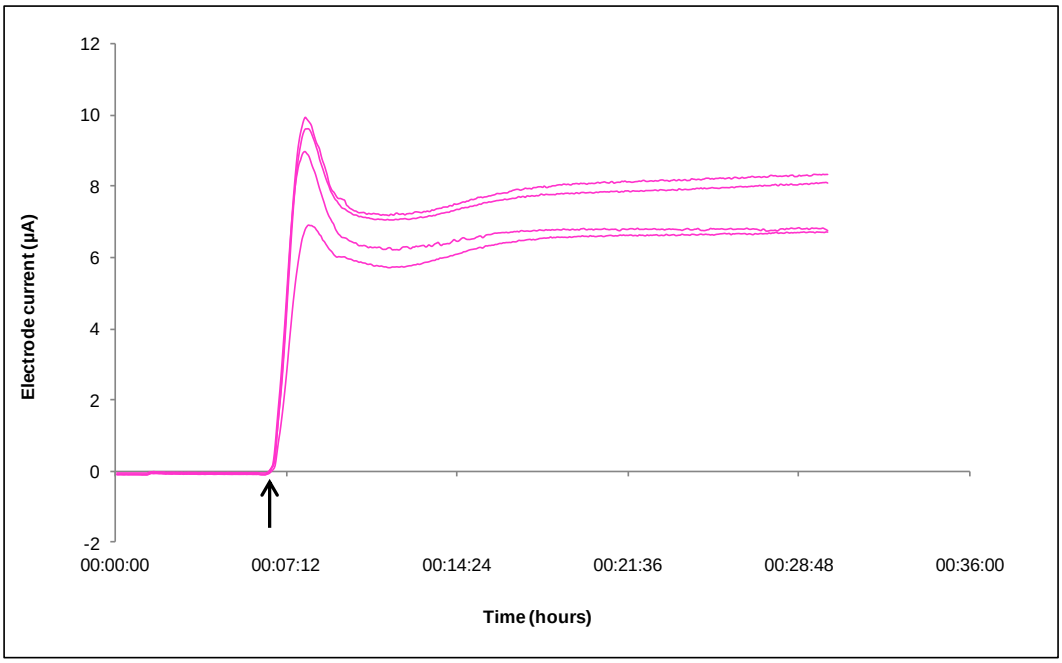


Figure 5.3 – Response of *E. coli* biosensors bathed in FeCN supplemented saline to the addition of a substrate cocktail (↑).

5.4 Protocol 1 (standard operating protocol)

Figure 5.4 shows a diagrammatic representation of Protocol 1, which is described in "Materials and Methods" section (paragraph 2.2.7).

The potential applied to the working electrode was +550 mV. Tests were performed with *P. subcapitata*, *S. leopoliensis* and *E. coli*.

The main aim was to detect any changes in the metabolic status of the biocatalyst when it was presented to river water samples.

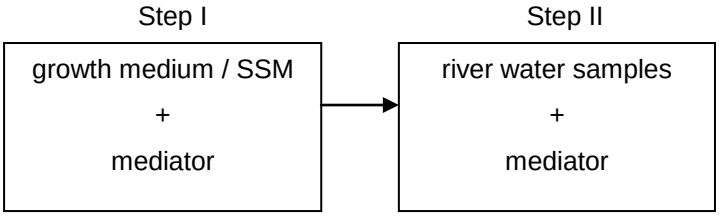


Figure 5.4 – Diagram of the protocol 1 (standard protocol) for river water monitoring with CellSense biosensors.

5.4.1 Using protocol 1 with river water samples

Figure 5.5 shows an example of the application of protocol 1 with *P. subcapitata* biosensors. The test started with each biosensor immersed in growth medium (3N-BBM+V), supplemented with *p*-benzoquinone (*p*BQ). The algal metabolic activities were monitored during both dark and light periods. After thirty minutes, vials were replaced and the algal biosensors were exposed to *p*BQ supplemented river water samples. After the switch, the metabolic activity was again monitored with light both on and off. The most significant outcome was the increasing background signal. Lea Navigation sample at Tottenham Hale increased rapidly, while the signal from the other two stations showed the same trend line but there was a lag time before the signal started to increase. The response of *P. subcapitata* biosensors expose to 3N-BBM+V medium did not show any increasing background response.

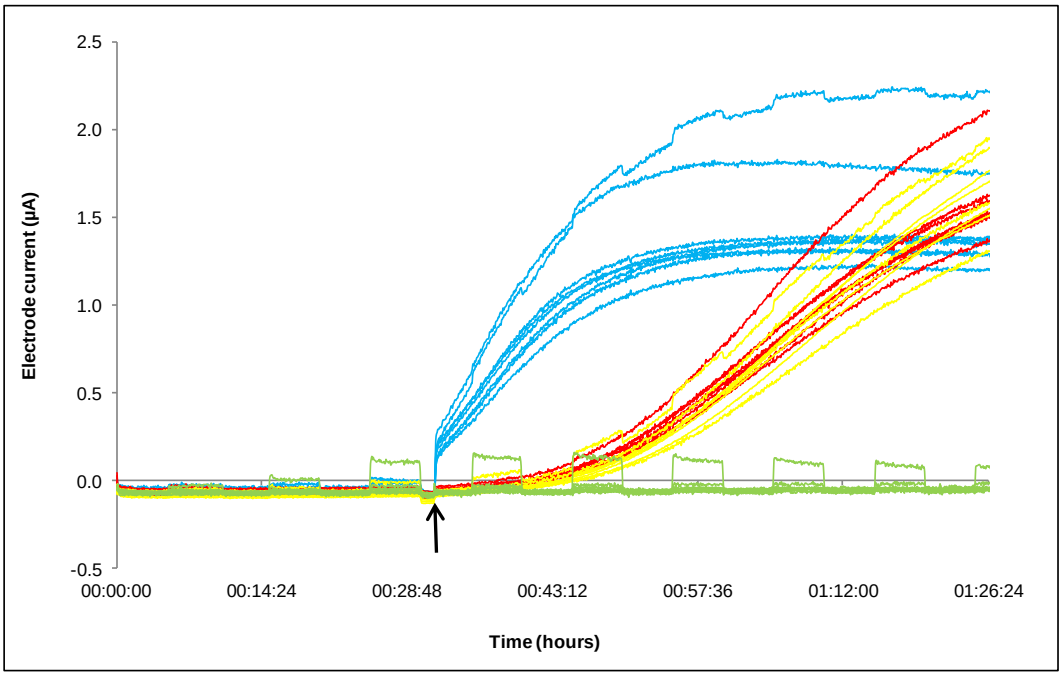


Figure 5.5 – Responses of *P. subcapitata* biosensors to exposure to river water samples (↑), using *p*BQ mediator. The test was conducted according to protocol 1. Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park; 3N-BBM+V (control).

The same test was performed with *S. leopoliensis* biosensors with BG11 control medium (Figure 5.6). First biosensors were bathed in *p*BQ supplemented BG11 medium and the biocatalyst metabolic status was recorded with the light off. After fifteen minutes, biosensors were exposed to river water samples supplemented with *p*BQ. Metabolic activity was monitored with light both on and off. Outcome signals showed similar increasing background signals as those resulted in the test with *P. subcapitata* biosensors. However, there was evidence of an increasing background signal with the BG11 control.

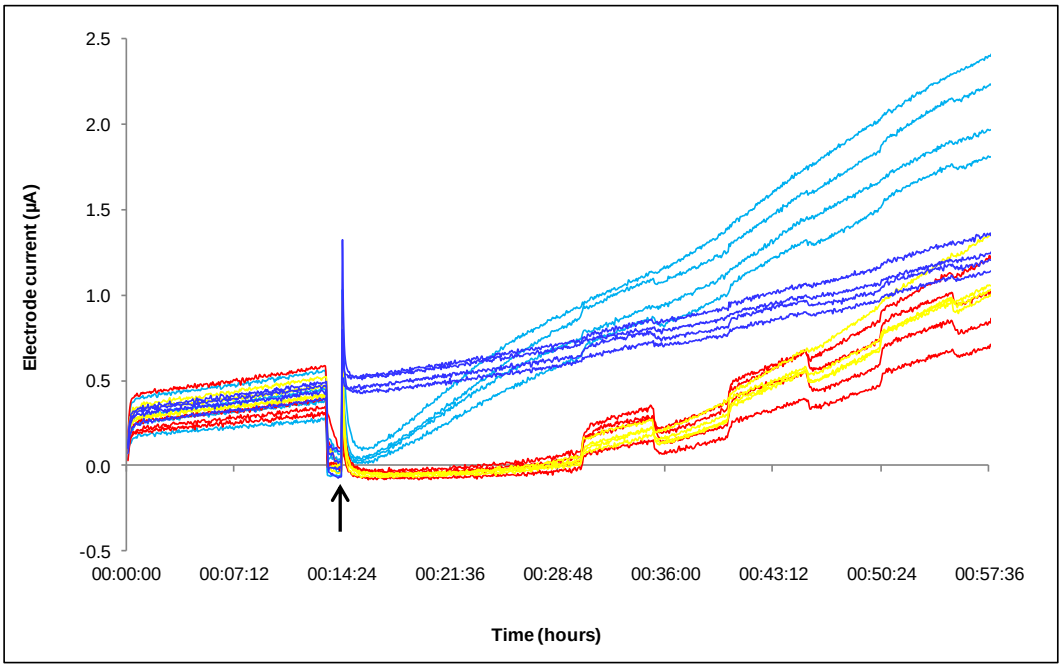


Figure 5.6 – Responses of *S. leopoliensis* biosensors to exposure to river water samples (↑), using *p*BQ. The test was conducted according to protocol 1. Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park; BG11 (control).

The non-biological response and the observation that the colour of the river water sample became brown/bronze after *p*BQ addition, indicated interactions between compounds present in the test solution (both river water and BG11 medium) and the mediator. Tests were carried out using blank electrodes (without biocatalysts) to determine if any redox reaction was present in the river water. Blank electrodes were first immersed in the test solutions: Lea navigation at Tottenham Hale, Lea Navigation at Springfield Park, Pymmes Brook, 3N-BBM+V medium and BG11 medium. After five minutes, the mediator was added to the vials by opening the lid. The electrochemistry of the samples was tested with both the mediators: *p*-benzoquinone (*p*BQ) and potassium ferricyanide (FeCN).

When *p*-benzoquinone was used as mediator, the samples tested showed an increasing signal except for the biosensors bathed in 3N-BBM+V medium (Figure 5.7). More importantly, the trends were the same as detected in the previous experiments with *P. subcapitata* and *S. leopoliensis* biosensors. This was a clear indication that the (electro)-chemical activity was affecting the results, by masking the biological response.

Using the potassium ferricyanide the (electro)-chemical signals were lower than those detected with *p*BQ (Figure 5.8). Moreover, this mediator looked to interact only with water from Pymmes Brook and Springfield Park, while Tottenham Hale showed only a small electric current increase.

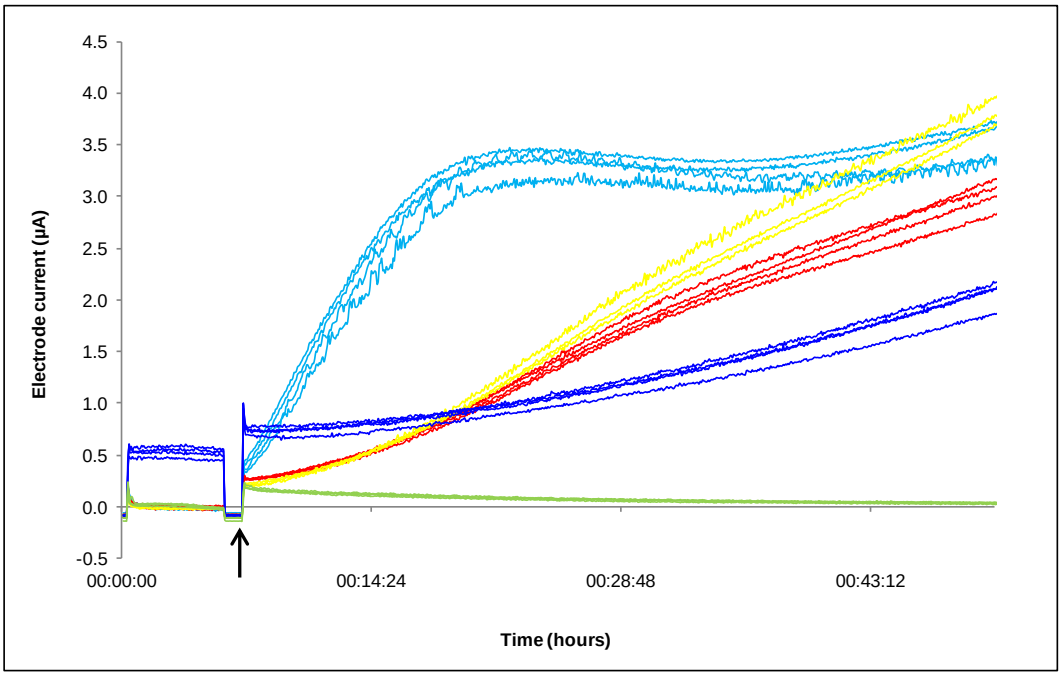


Figure 5.7 – (Electro)-chemical responses of river water samples and growth media to *p*BQ mediator addition (↑) tested with blank electrodes. Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park; BG11 medium; 3N-BBM+V.

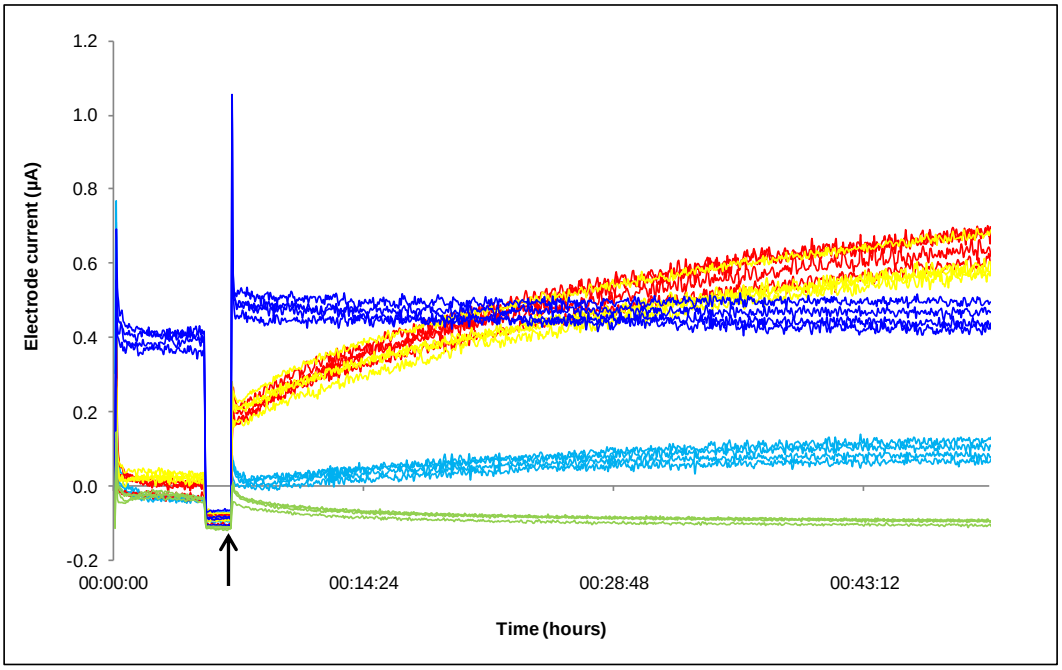


Figure 5.8 – (Electro)-chemical responses of river water samples and growth media to FeCN mediator addition (↑), tested with blank electrodes. Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park; BG11 medium; 3N-BBM+V.

These background current signals were found with both raw and centrifuged river water samples (3500 rpm for 15 minute). The water samples centrifugation was performed to remove any microorganisms present in the river water, in order to avoid monitoring their metabolic activity, which could give false results.
Since the increasing background signal was detected in BG11 and not in 3N-BBM+V, CellSense tests with blank electrodes were performed using fresh BG11 medium prepared without ammonium citrate, which was not present in the 3N-BBM+V medium. Test solution was supplemented with *p*BQ. However, an increasing current was still detected.

5.4.2 Using protocol 1 with *E. coli* biosensors and pre-treated river water samples

Parallel to the previous tests conducted with *P. subcapitata* and *S. leopoliensis* biosensors, *E. coli* (strain 8277) biosensors were also employed to test river water samples collected in the Lea Navigation at Springfield Park.
For these tests river sample was pre-treated by solid phase extraction (SPE). Since algal growth tests indicated a likely negative effect of the polar water fraction on the algal population, the main aim of this test was to investigate the two water fractions (polar and non-polar) using bacterial biosensors. A silica based packing (ENVI-18,5 g, SUPELCO) was used to perform the SPE, allowing a reverse phase separation with a polar or moderately polar sample matrix (mobile phase) and a non-polar stationary phase.
Therefore the samples investigated in the following tests were:

- SSM (as control for unaffected signal);
- Lea Navigation at Springfield Park (river water not treated by SPE);
- Lea Navigation at Springfield Park polar fraction;
- Lea Navigation at Springfield Park non-polar fraction.

The three water samples (Lea Navigation at Springfield Park, Lea Navigation at Springfield Park polar fraction, and Lea Navigation at Springfield Park non-polar fraction) were enriched with SSM to avoid any osmotic stress to the bacterial cells and to maintain nutrient levels. The two water fractions were analysed in two separate tests, but always in comparison with the untreated river sample and SSM.
Figure 5.9 shows the results from the run performed with the polar fraction. Following the procedure of the protocol 1, *E. coli* biosensors were first bathed in 0.85 % saline. After three minutes, the substrate cocktail (SS) was added through the ports located on the instrument's lid. After six minutes, the potassium ferricyanide (FeCN) was injected into the test vials. The addition of the mediator triggered redox reactions on the membrane of the bacterial cells, visible in an increasing electric current signal. After the stabilisation of the

signal (at 25 minute), the lid was opened and the vials were changed with vials containing the FeCN supplemented test samples under investigation. Four biosensors were used for each river sample, while four were bathed again in SSM as controls. Immediately after the closure of the lid, biosensors immersed in Springfield Park and in Springfield Park polar fraction showed a drop in the signal line. However, the signals increased within five minutes, suggesting that the results were affected by non-biological events.

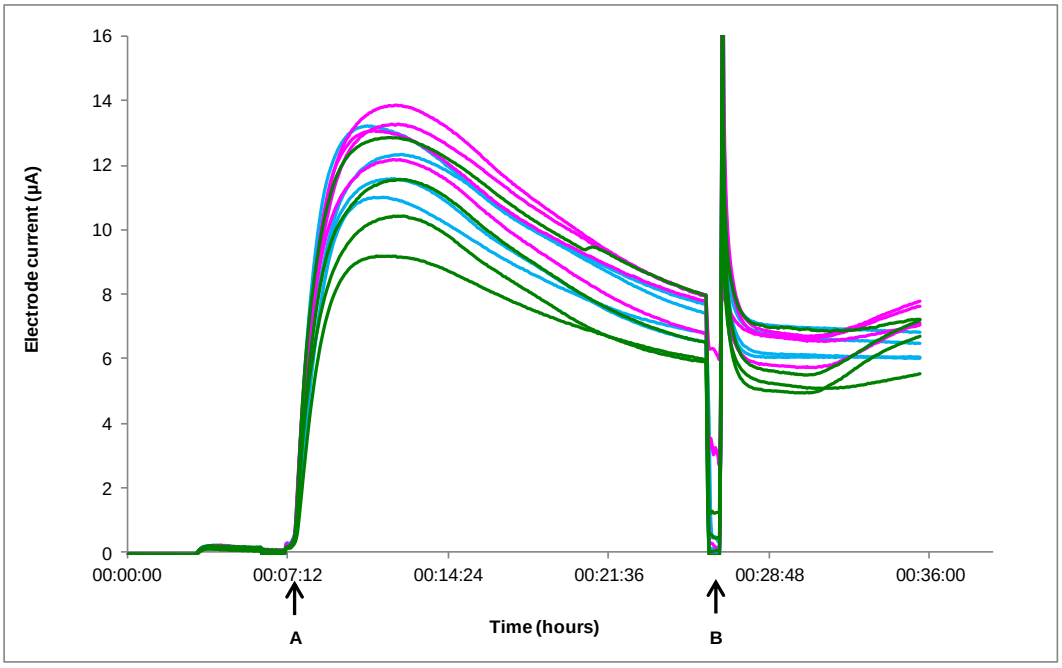


Figure 5.9 – Responses of *E. coli* 8277 biosensors to exposure to Springfield Park water POLAR fraction, and Springfield Park untreated water (B), using FeCN (A). River water samples were supplemented with SSM. The test was conducted according to protocol 1. Legend: SSM (control); Lea Nav at Springfield Park polar fraction; Lea Nav at Springfield Park.

The results from the analysis of the non-polar fraction are given in Figure 5.10. The test started with the biosensors immersed in FeCN supplemented SSM. After ten minutes, when the signal had stabilised, the lid was opened and the vials switched. The signal of the non-polar fraction sample did not show any change in the trend line, while Springfield Park sample presented a clear drop, followed signal increased, as resulted in the previous test.

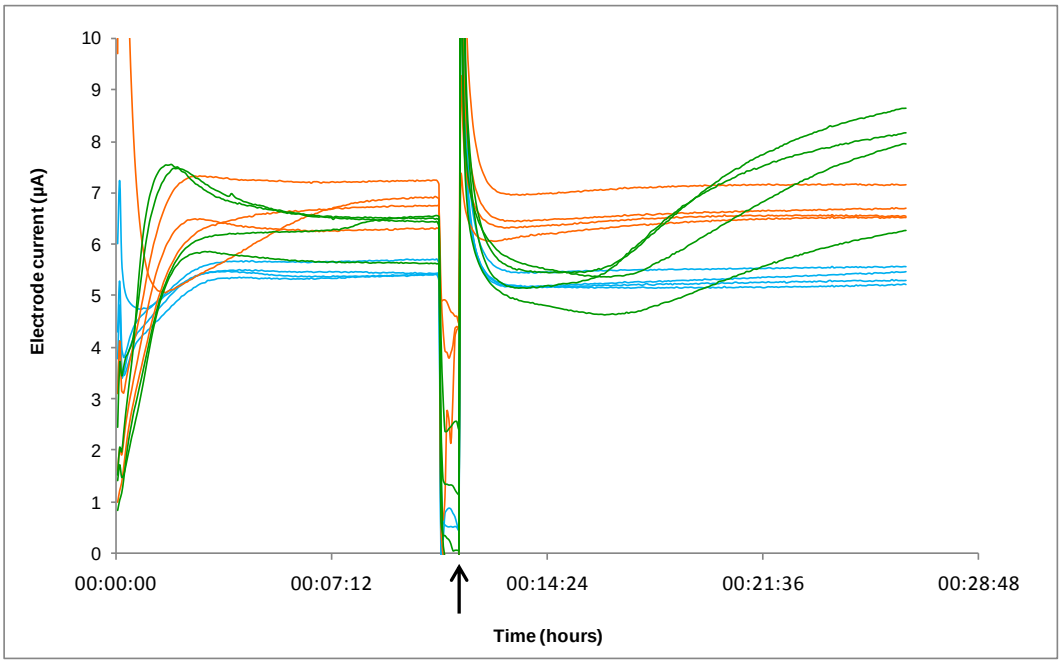


Figure 5.10 – Responses of *E. coli* 8277 biosensors to exposure to Springfield Park water non-polar fraction, and Springfield Park untreated water (↑), using FeCN. River water samples were supplemented with SSM. The test was conducted according to protocol 1. Legend: SSM (control); Lea Nav at Springfield Park non-polar fraction; Lea Nav at Springfield Park.

Results from the two tests gave indication of a possible negative effect of the polar fraction on the bacterial population, supporting the outcomes of the algal growth inhibition test (Chapter 4). However, no further tests were undertaken, because the window where the toxicity was seen was too small (five minutes) to be able to make any comparisons.

The increased signal was again investigated with blank electrodes (without biocatalyst) and with both the mediators: *p*-benzoquinone (Figure 5.11) and potassium ferricyanide (Figure 5.12). The test solutions analysed were: Lea Navigation at Springfield Park, Lea Navigation at Springfield Park polar fraction and Lea Navigation at Springfield Park non-polar fraction.

The non-polar fraction did not show any (electro)-chemistry neither with *p*BQ or FeCN, while the other two samples investigated presented increasing signal with both the mediators (Figure 5.11 and Figure 5.12).

Since it was demonstrated that the (electro)-chemical activity was altering the results, alternative approaches to monitoring the impact of river water using biosensors were developed: protocols 2 and 3.

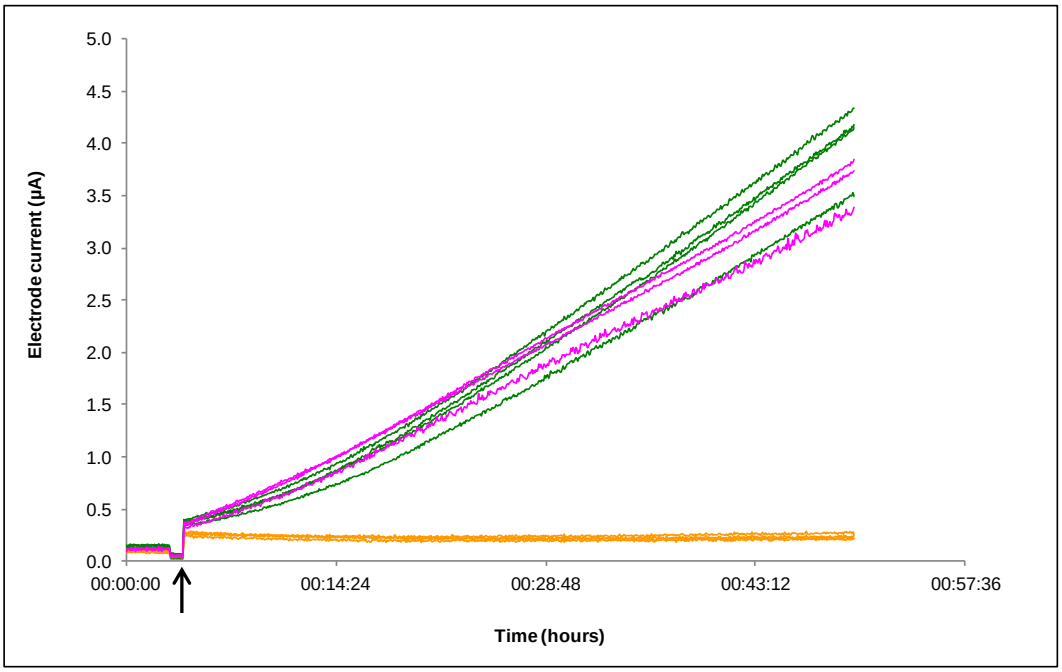


Figure 5.11 – (Electro)-chemical responses of Springfield Park waters and its two fractions (polar and non-polar) to *p*BQ mediator addition (↑), tested with blank electrodes. Legend: Lea Nav at Springfield Park polar fraction; Lea Nav at Springfield Park non-polar fraction; Lea Nav at Springfield Park.

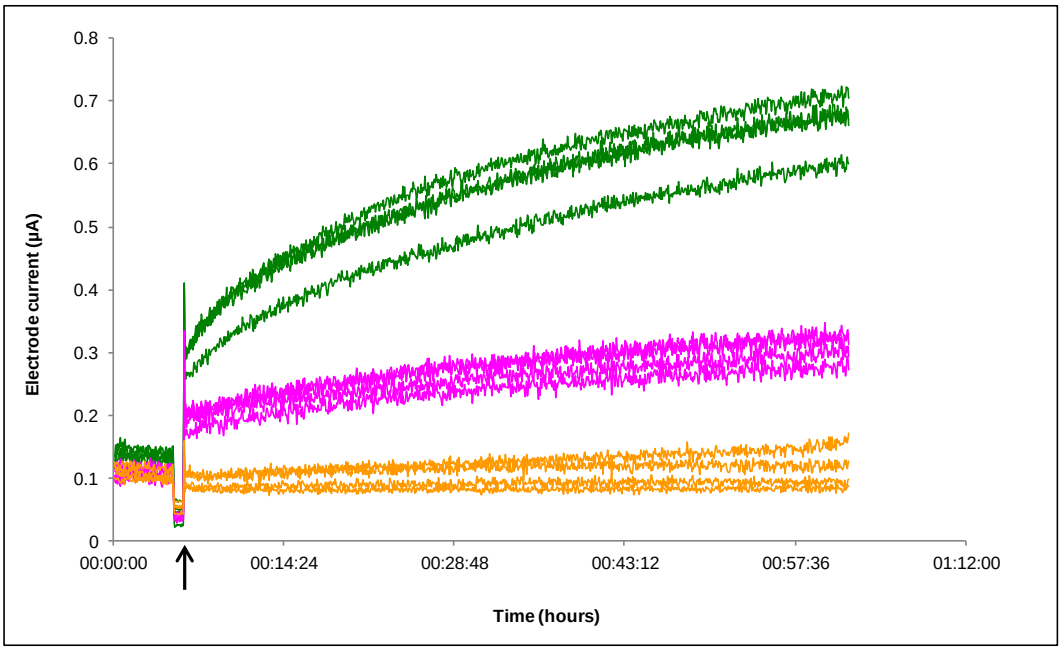


Figure 5.12 – (Electro)-chemical responses of Springfield Park waters and its two fractions (polar and non-polar) to FeCN mediator addition (↑), tested with blank electrodes. Legend: Lea Nav at Springfield Park polar fraction; Lea Nav at Springfield Park non-polar fraction; Lea Nav at Springfield Park.

5.5 Protocol 2

To avoid any (electro)-chemical interactions, in protocol 2 algal and bacterial biosensors were exposed to river water samples not supplemented with mediator. The protocol consisted of three steps: 1) pre-exposure period, where *P.subcapitata* and *E. coli* biosensors were monitored in either 0.85 % saline or growth medium; 2) exposure period, where biosensors were transferred to river water samples in the absence of mediator, for different time periods (30 minutes, 2 hours and 24 hours); 3) post-exposure period, where biosensors were returned to the same conditions used in step I (Figure 5.13).

The working electrodes were held at +550 mV potential. Each tested solution was monitored with at least four biosensors each time. *P. subcapitata* metabolic activities were investigated with light both on and off. During the pre-exposure stage of the tests with 2 hours and 24 hours of exposure, algal biosensors were bathed in 0.85 % saline to monitor the metabolic activities in neutral conditions without nutrients.

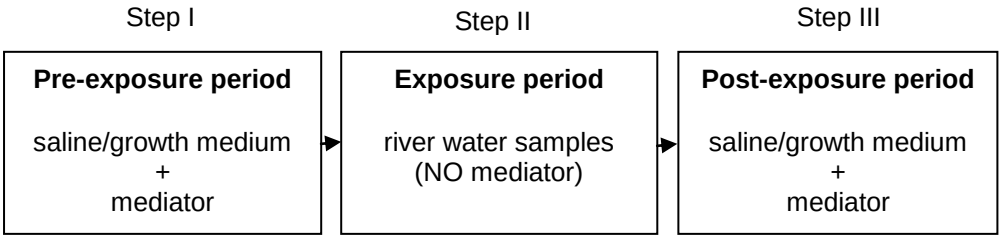


Figure 5.13 –Diagram of the protocol 2 for river water monitoring with CellSense biosensors.

5.5.1 Using protocol 2 with river water samples

Table 5.2 presents the results obtained by exposing *P. subcapitata* biosensors for 30 minutes to the river water samples collected from Lea Navigation at Tottenham Hale and Lea Navigation at Springfield Park. Four biosensors were monitored in the growth medium as control. The test started in the dark with biosensors immersed in 3N-BBM+V medium. After three minutes, *p*-benzoquinone (*p*BQ) was added through the ports located on the device's lid. When the signal was stable, the light was switched on and the biosensors were illuminated throughout the experiment. After fifteen minutes the vials were changed and the *P. subcapitata* biosensors were exposed to the two water samples and to 3N-BBM+V medium, without mediator. After 30 minutes of exposure to the test solutions, biosensors were re-placed in the vials used in the first step (*p*BQ supplemented 3N-BBM+V medium). In this way, data of the post-exposure phase were collected.

By comparing the last signal values recorded in the pre-exposure period with those registered at the first minute of the post-exposure period, it was not possible to detect any inhibition in the metabolic cell status (independent samples t-test). This leads to the conclusion that any pollution in the analysed samples was chronic and not detectable by a short term exposure of 30 minutes. Similar results were achieved exposing the algal biosensor to river water supplemented with growth medium.

Table 5.2 - Example of *P. subcapitata* biosensors response to 30 minutes exposure to Tottenham Hale and Springfield Park water samples, using *p*BQ mediator. The test was performed according to the protocol 2. The 3N-BBM+V medium was used as control. Pre-exposure current (µA) = last values recorded during the pre-exposure stage. Post-exposure current (µA) = values recorded at the first minute of the post-exposure stage.

Sample	Pre-exposure current (µA)	Post-exposure current (µA)
3N-BBM+V medium	-0.04	-0.03
Lea Nav at Tottenham Hale	-0.03	-0.02
Lea Nav at Springfield Park	-0.01	-0.01

In order to investigate any chronic pollution effects, further investigations were performed with longer exposure times to the river water.

Figure 5.14 shows an example of results of *P. subcapitata* biosensors exposed to 0.85% saline supplemented river water samples, for 2 hours. In order to monitor the water samples in as similar as possible to the environmental reality, algal biosensors were monitored without addition of nutrients throughout the three stages. During the pre-exposure period, the algal metabolic status was monitored, immersing the algal cells in 0.85 % saline supplemented with *p*BQ, with and without biosensor illumination. Then the device's lid was opened and the *P. subcapitata* biosensors were exposed to NaCl supplemented river water samples without mediator, in the dark. After 2 hours of exposure, the vials were changed again and the biosensors were re-immersed in *p*BQ supplemented saline. Results showed evidence of stimulation after the exposure to the river water samples, more evident when the light was turned on. The detected stimulation could be likely due to the presence of nutrients in the river water. Moreover, there was evidence of higher stimulation level in biosensors exposed to Springfield Park than to Tottenham Hale samples.

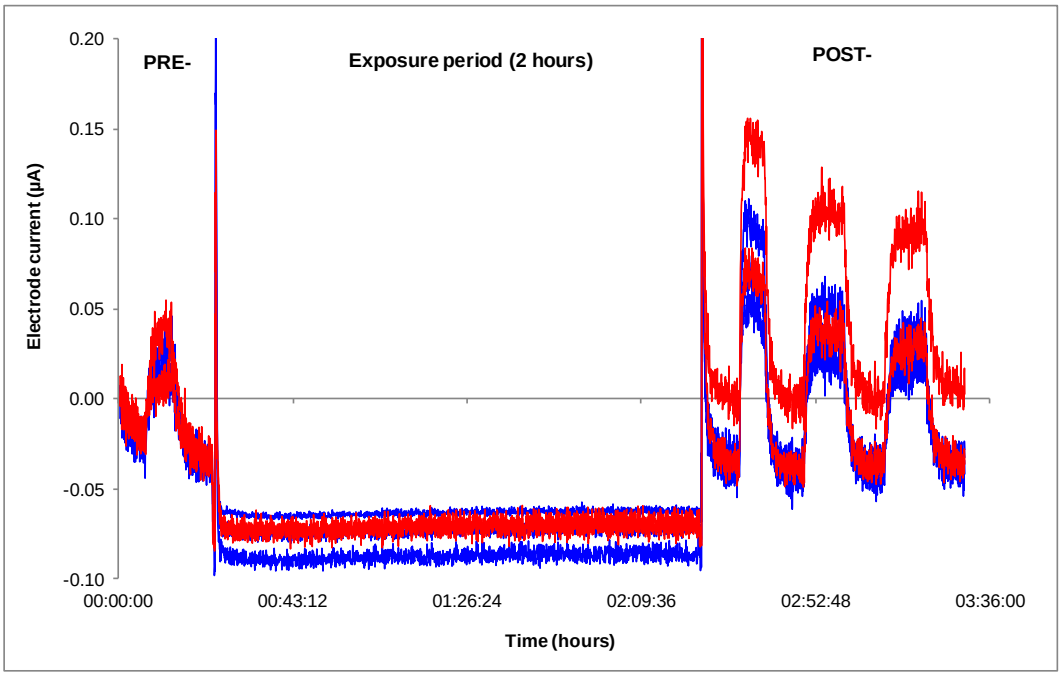


Figure 5.14 – Example of *P. subcapitata* biosensors responses to 2 hours exposure to Tottenham Hale and Springfield Park water samples, using *p*BQ. The test was performed according to the protocol 2. Legend: Lea Nav at Tottenham Hale; Lea Nav at Springfield Park.

The lack of detection of inhibition of the biocatalyst with an exposure time of 2 hours, confirmed that the toxicity in the river water was likely chronic. For this reason, the test was performed with a 24 hour exposure period, by testing samples from Lea Navigation at Tottenham Hale, Pymmes Brook and Lea Navigation at Springfield Park. *P. subcapitata* metabolic activities were tested in both light and dark periods, and the test was conducted as the previous one. During the 24 hours exposure period the biosensors were immersed in 10 ml of 0.85 % NaCl supplemented river water and kept in the incubator at 23 °C, under continuous illumination. However, the day after exposure the algal biosensors gave no response, suggesting that the algal cells were damaged, and the river water sample in the test vials was partially evaporated. Consequently, the test was repeated without supplementing the samples with 0.85 % saline, but adding growth nutrients (Figure 5.15). The growth medium employed in this test was OECD medium, the same used to carry out algal growth test. It was chosen to perform the experiment with this medium in order to compare the results with the algal growth inhibition outcomes.

As identified in the previous two experiments, after 30 minutes and 2 hours of exposure time, algal cells showed higher metabolic rates in the post-exposure stage than in the pre-exposure phase. In other words, the algal cells appeared to be stimulated by the exposure to the river water. Moreover, during the post-exposure period, algal cells presented to Pymmes Brook and Springfield Park samples presented a higher metabolic status than those algae exposed to the growth medium and Tottenham Hale sample. These results

disagreed with the trend detected performing algal growth inhibition tests where Pymmes Brook and Springfield Park water showed to negatively affect the algal population.
Inhibition was not detected after exposing the algal biosensors to river water samples for 24 hours. This could be due to the disappearance of toxicants from the experimental system by adsorption events, which happens with small water volumes and likely low pollutant concentrations. The detected stimulation was possibly associated to a higher concentration of nutrients in Pymmes Brook and the samples downstream of it, than in Tottenham Hale and in the growth medium.

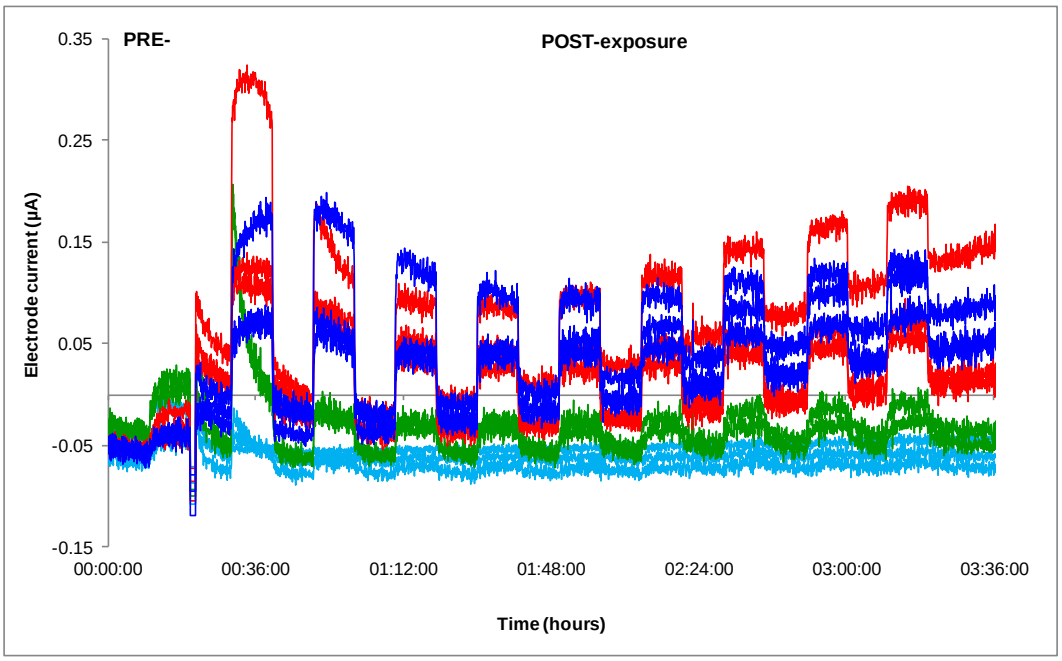


Figure 5.15 – Example of *P. subcapitata* biosensors response to 24 hours exposure to river water samples, using *p*BQ. The test was performed according to the protocol 2. Legend: OECD medium (control); Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park.

The same protocol was also used with *E. coli* biosensors. The test was conducted with 30 minute exposure time (Figure 5.16). The samples tested were Pymmes Brook and Lea Navigation at Tottenham Hale water samples. Bacterial biosensors were immersed in SSM. After three minutes potassium ferricyanide were added at each test vial through the ports. When the signals were stable (at fifteen minutes) the lid was opened, the test vials switched and the biosensors were bathed in river water supplemented with SSM for 30 minutes. Four bacterial biosensors were maintained in SSM, as control. After thirty minutes, the *E. coli* biosensors were placed back to the vials used in step I and the metabolic status were recorded. The outcome signals did not show evidence of inhibition or stimulation.

Since there was evidence that the protocol 2 was not successful, it was decided not to conduct further investigation with bacterial biosensors.

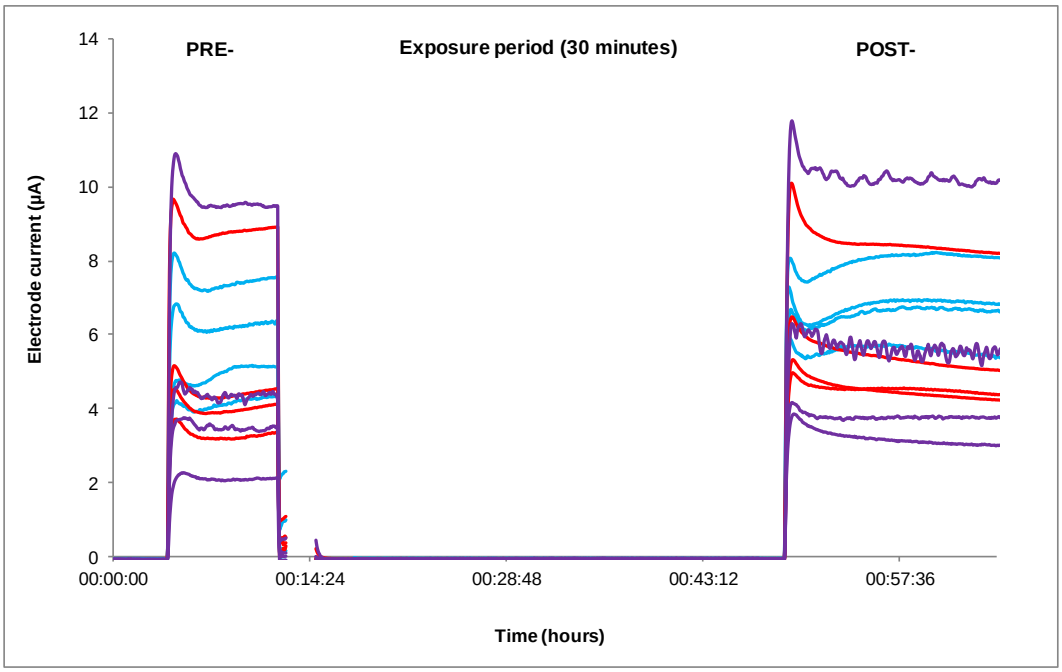


Figure 5.16 – Example of *E. coli* 8277 biosensors responses to 30 minutes exposure to river water samples, using FeCN. The test was performed according to the protocol 2. River water was supplemented with SSM. Legend: SSM (control); Pymmes Brook; Lea Nav at Springfield Park.

5.6 Protocol 3

Protocol 3 was developed in parallel with protocol 2, in an effort to find a method to reduce the (electro)-chemical disturbance. With this protocol, the problem was addressed by reducing the potential applied to the working electrode. Protocol 3 was performed only with *E. coli* biosensors because algal biosensors produced too small a response.

5.6.1 Change of the working potential

Previous tests (e.g. Figure 5.7 and Figure 5.8) showed that performing CellSense investigation at a working potential of +550 mV, the biological response was masked by an increased current signal, probably due to chemical interaction between the mediator (ferricyanide or *p*-benzoquinone) and chemicals dissolved in the river water samples. In order to decrease the (electro)-chemical disturbance, the lower oxidation potentials of the two mediators were investigated by cyclic voltammetry. The aim was to see if a lower applied potential could be identified that was adequate to monitor the cellular response while minimizing the (electro)-chemical disturbance.

The cyclic voltammetry (CV) was conducted with a screen-printed three-electrode sensor, with carbon working electrode. The two mediators tested were 1 mM ferricyanide dissolved in a phosphate buffered saline (PBS), and 1 mM *p*-benzoquinone dissolved in 0.85 % saline. The experiment conditions were as shown in Table 5.3.

Table 5.3 – Cyclic voltammetry conditions used to detect the lower oxidation potentials of the *p*BQ and FeCN mediators.

Experiment condition	*p*BQ	FeCN
Scan rate (mV s^{-1})	10	20
Scan number	3	3
Start potential(V)	+ 1.2	+ 0.7

Figure 5.17 and Figure 5.18 show the cyclic voltammograms for ferricyanide and *p*-benzoquinone respectively. From the two CV it was possible to identify that the oxidation potential for the ferrocyanide ($Fe(CN)_6^{4-}$) was ≥ +200 mV, while for the hydroquinone (HQ) it was ≥ -150 mV. Since it was decided to test the water with *E. coli* cells mediated with FeCN, the potential of +200 mV was selected to be applied to the working electrode.

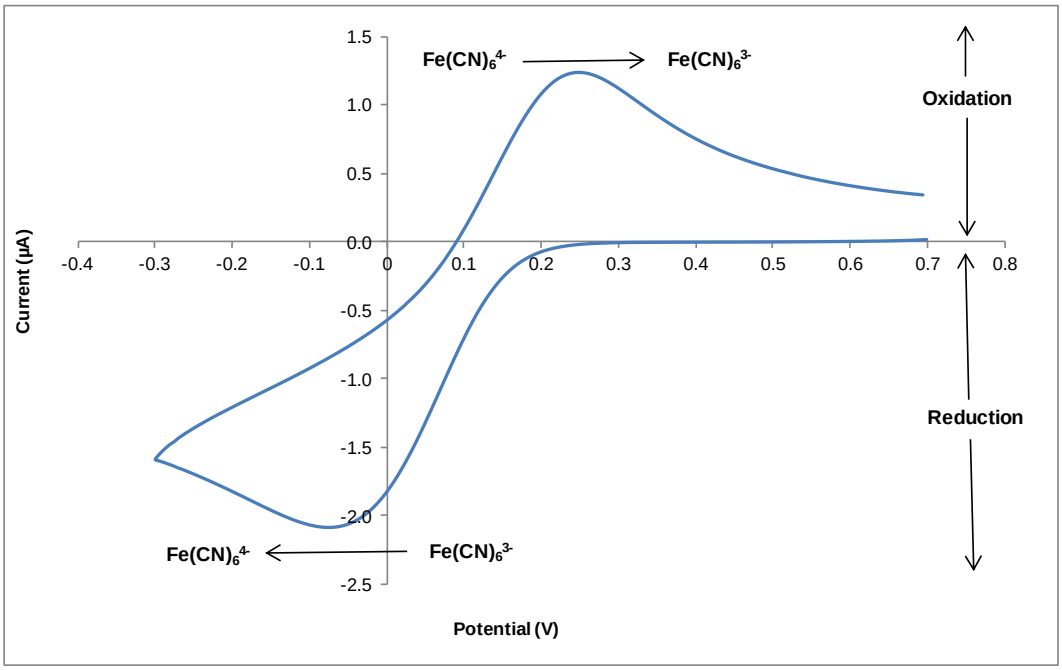


Figure 5.17 – Cyclic voltammogram for 1 mM ferricyanide in phosphate buffer saline (PBS). The start potential was + 0.7 V, and the scan rate of 20 mV s^{-1}.

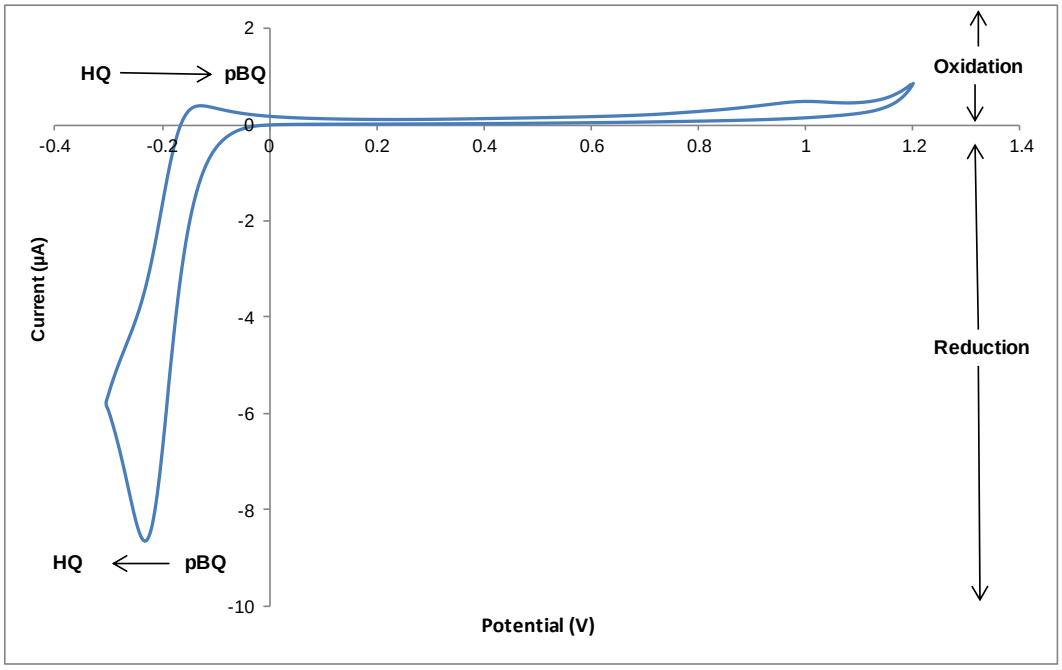


Figure 5.18 – Cyclic voltammogram for 1 mM *p*-benzoquinone in 0.85% saline. The start potential was +1.2 V and the scan rate was 10 mVs^{-1}. HQ = hydroquinone. *p*BQ = *p*-benzoquinone.

CellSense tests were performed with blank electrodes (without biocatalyst) in mediator supplemented Springfield Park water samples. Figure 5.19 presents a comparison with both ferricyanide and *p*-benzoquinone at potentials of +550 mV and +200 mV. The outcome signals at +200 mV mediated by FeCN did not show an increasing current

background, confirming that this working potential was able to avoid the non-biological (electro)-chemical response, when used with potassium ferricyanide.

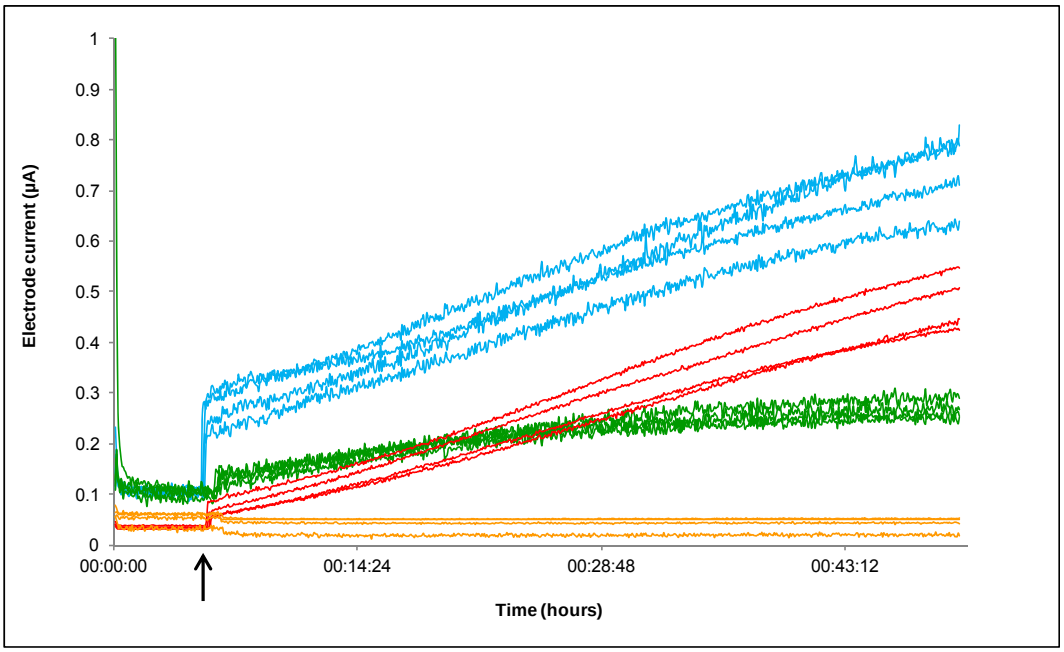


Figure 5.19 – (Electro)-chemical responses of Springfield Park waters to both FeCN and *p*BQ mediator addition (↑) at different working potentials. Legend: *p*BQ at +550 mV; *p*BQ at +200mV; FeCN at +550 mV; FeCN at +200 mV.

5.6.2 Testing river water with protocol 3

By using the reduced potential two main approaches were considered:

1. Untreated river water samples were tested. The procedure consisted of two steps. In step I the metabolic activity of the bacterial biocatalyst was checked in FeCN supplemented SSM. In step II the metabolic status was recorded by exposing the bacterial cells to river water supplemented with SSM and mediator (Figure 5.20).

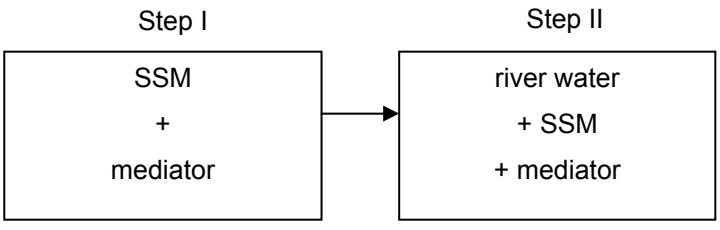


Figure 5.20 –Diagram of the protocol 3 for river water monitoring with CellSense biosensors, by testing untreated water samples.

2. Pre-concentrated river water samples were tested. River water samples were pre-concentrated by rotary evaporation in order to increase the likely low pollutant concentrations. The solutes were re-suspended in either:
    a. 5 ml of distilled water;
    b. 1 ml of methanol.

    The protocol consisted of 2 steps. During step I bacterial biosensors were monitored in SSM, supplemented with potassium ferricyanide. During step II concentrated river sample were added to the vials and the *E. coli* biosensors metabolic rate was monitored under this conditions (Figure 5.21).

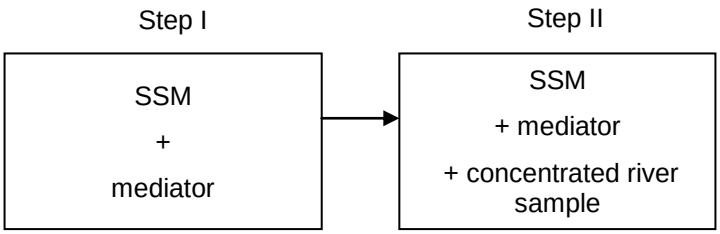


Figure 5.21 –Diagram of the protocol 3 for river water monitoring with CellSense biosensors, by testing pre-concentrated river water.

5.6.3 Using protocol 3 with river water samples

By applying a potential of +200 mV the first approach was similar to protocol 1, where first the biosensors were monitored in neutral conditions and then in river water samples. *E. coli* metabolic activities were first tested in SSM (Figure 5.22). After five minutes, potassium ferricyanide was injected through the ports of the device's lid. When the signal lines were stable, biosensors were presented to river water samples supplemented with SSM and FeCN. Some *E. coli* biosensors were held in SSM as control. However, the reproducibility of the test was problematic, because biosensors exposed to the river water did not give always the same response probably due to the presence of inorganic compounds in the samples, which worked as nutrient for the bacteria. In other words, there was evidence that the inhibition was masked by bacterial metabolic stimulation due to nutrients present in the water samples. The stimulation effect is visible in Figure 5.22, where the responses of *E. coli* biosensors showed increasing current signals after an initial drop when exposed to the water samples. In order to avoid stimulation of the bacterial metabolism and because initial results showed a likely chronic inhibition, river water samples were pre-concentrated.

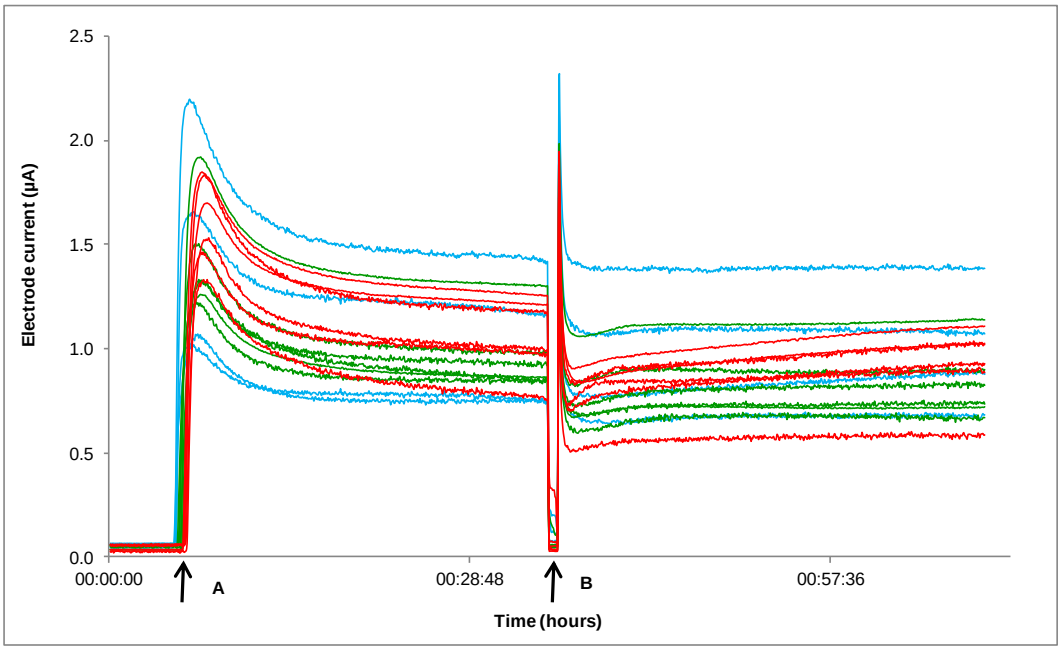


Figure 5.22 – Example of *E. coli* 8277 biosensors responses to exposure to Tottenham Hale and Pymmes Brook water samples (B), using FeCN (A). River water samples were supplemented with SSM. Legend: SSM (control); Lea Nav at Tottenham Hale; Pymmes Brook.

5.6.4 Using protocol 3 with pre-concentrated river water samples

A second approach was performed by testing pre-concentrated river water samples, always applying a potential of +200 mV to the working electrode.

First 200 ml of river water samples were evaporated by rotary evaporator and the solutes were re-suspended in 5 ml of SSM. However, when an aliquot of either 1 ml or 2 ml of the concentrated sample was added to the mixture of saline, substrate and potassium ferricyanide no detectable changes in *E. coli* biosensors response were seen.

For this reason, an alternative approach to re-suspend the concentrated solutes was tried. After evaporation of 100 ml of river water sample for each monitoring site, the solutes were re-suspended in 1 ml of methanol. The methanol mixtures were then kept in the dark at 4 °C overnight, and the CellSense test was carried out the following day. Pure methanol was stored in the same kind of vial at the same condition of the sample to use during the experiment as control. At the beginning of the experiment, biosensors were exposed FeCN supplemented SSM. After signal stabilisation, 100 µl of those methanol samples were added through the lid ports to the 9.9 ml SSM (100 fold dilution). The same amount of methanol was added to replicates of SSM and monitored in order to detect any methanol effect on the biocatalyst (methanol control). The test was carried out with water samples from Lea Navigation at Tottenham Hale, Pymmes Brook, Lea Navigation at Stonebridge Brook, Lea Navigation at Springfield Park, Lea Navigation at Lea Bridge weir,

and River Lea at Hackney Marshes (except for the first series of tests when for Lea Bridge weir and Hackney Marshes water samples were not tested).

The test was repeated three times with fresh water samples for each test.

The % inhibition was calculated by comparing the biosensors electric current before and after the addition of the sample mixture, following the equation:

$$\% \text{ Inhibition} = \frac{A - B_i}{A} * 100 \qquad (5.1)$$

where:

A is the last value recorded before the methanol mixture addition, and

B is the value registered after *i* minutes to the addition.

Inhibition was determined after 5, 10 and 30 minutes of the sample mixture injection. The normal distribution of the inhibition data was checked using Shapiro-Wallis test. Statistically significant differences were calculated with one-way ANOVA (Post Hoc: Tukey). Whenever data were not normally distributed, a Mann-Whitney test was performed to identify any differences of inhibition values between monitoring stations. In Figure 5.23 a subset of biosensor responses are presented for clarity, in order to show how the signal changed in the presence of contaminated water, such as Lea Navigation at Stonebridge Brook, during the test conducted with water collected on 02/07/2012. After a brief hormesis phase, where the bacterial population were responded to the low methanol concentration injected (visible as peak), the signal current started to decline.

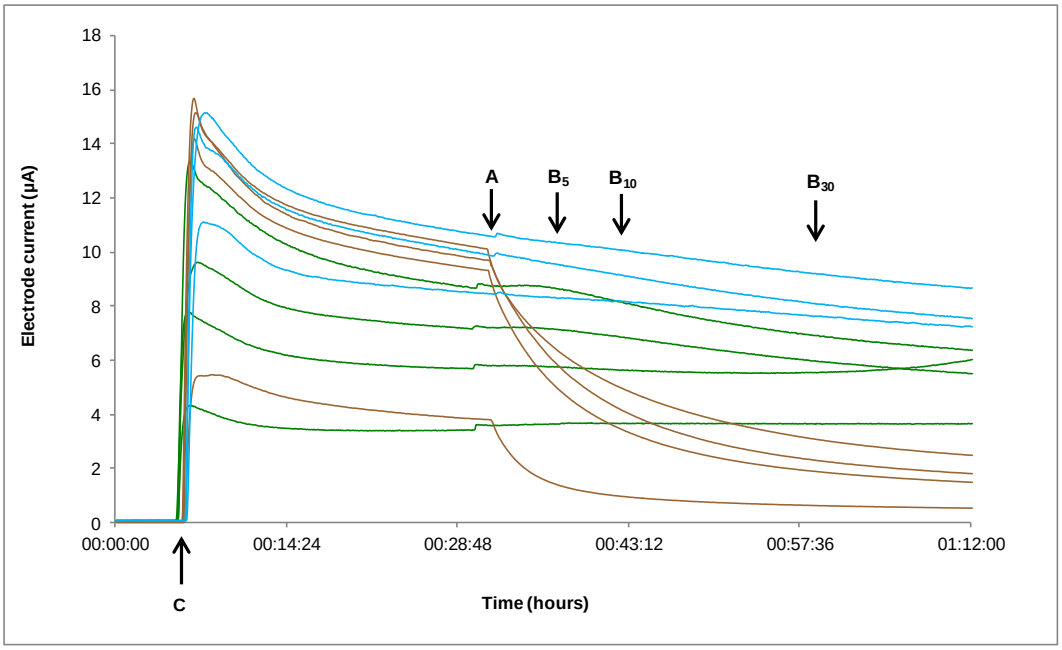


Figure 5.23 – Example of *E. coli* 8277 biosensors responses to exposure to SSM spiked with methanol sample mixtures (A), using FeCN (C). Legend: methanol control; Lea Nav at Tottenham Hale; Lea Nav at Stonebridge Brook; A = the last value recorded before the methanol mixture addition; $B_{5,10,30}$ = the value registered after 5, 10, 30 minutes of the methanol samples addition.

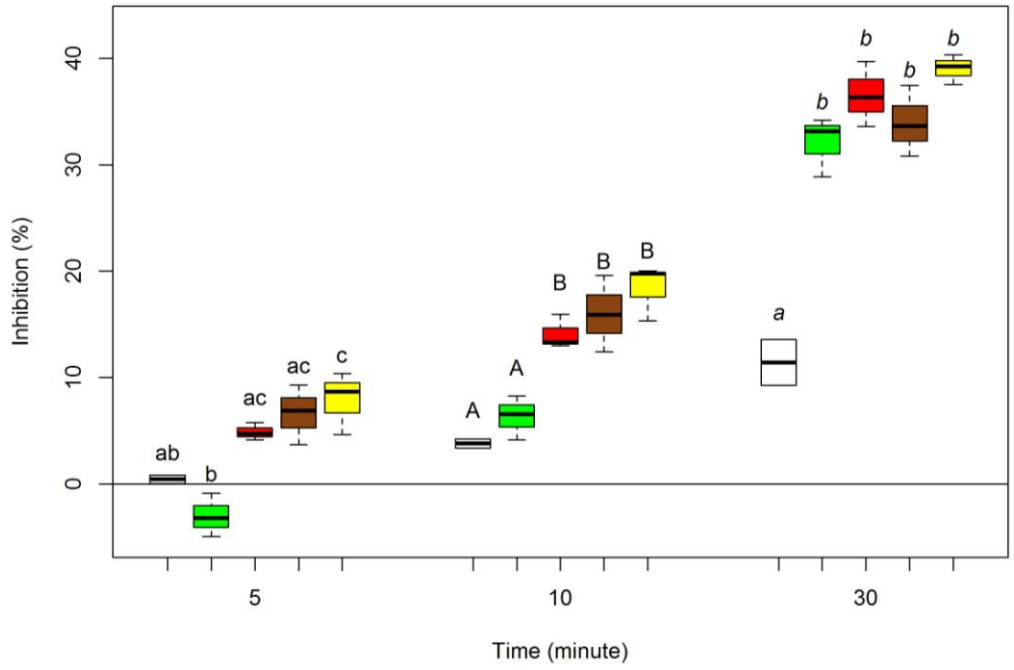
The comparison between Figures 5.22 and 5.23 clearly shows the lack of recovery of the bacterial population when using pre-concentrated water samples and dissolving the solutes in methanol, bypassing the metabolic stimulation of bacteria possible due to the presence of nutrients in river water samples.

Table 5.4 shows the *E. coli* biosensor inhibition (%) and the standard deviation (SD) values for the test conducted with water samples collected on 25/06/2012. The mean is calculated for the four biosensors employed for each test sample. Inhibition values (%) are presented in Figure 5.24, where graph A shows the total inhibition due to both methanol and river water toxicants, and graph B shows the inhibition values (%) without methanol effect in order to present only the percentage of inhibition due to toxicants present in the river water.

Table 5.4 – *E. coli* biosensor inhibition (%) and standard deviation (SD) calculated after 5, 10 and 30 minutes from methanol sample mixtures addition to SSM. Four biosensors were employed for each river water sample. The test was conducted with water collected on 25/06/2012.

Samples	Inhibition (%)					
	after 5 min		after 10 min		After 30 min	
	Mean	SD	Mean	SD	Mean	SD
Methanol control	0.4	0.6	3.8	0.6	11.41	3.1
Lea Nav at Tottenham Hale	-3.0	2.1	6.3	2.1	32.1	2.8
Pymmes Brook	4.9	0.8	14.1	1.6	36.6	3.1
Lea Nav at Stonebridge Brook	6.6	2.8	16.0	3.6	34.0	3.3
Lea Nav at Springfield Park	7.9	2.9	18.4	2.6	39.0	1.4

A)



B)

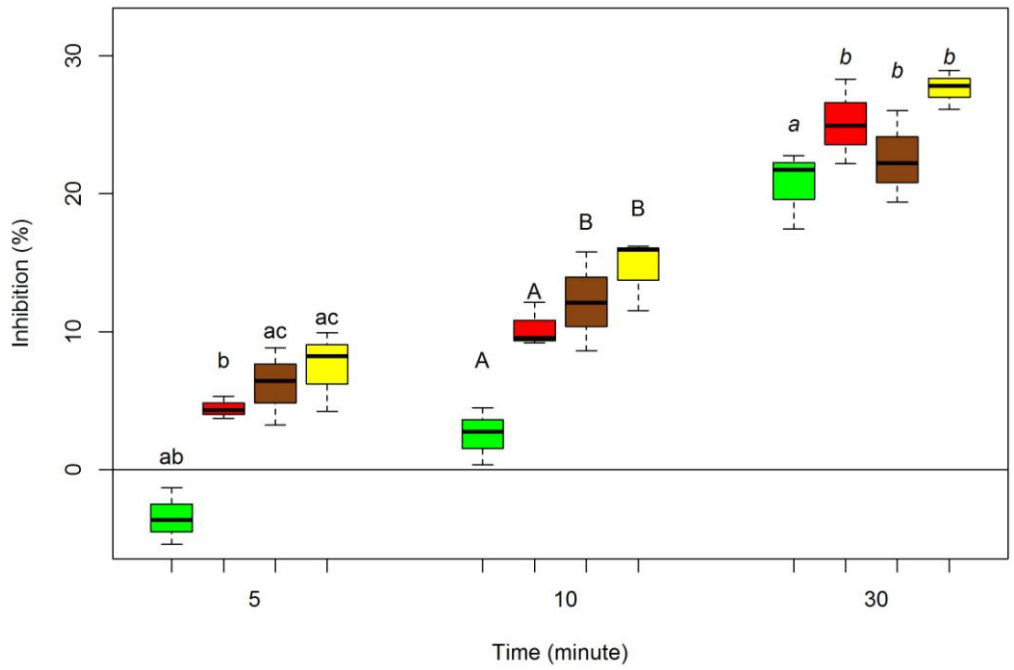


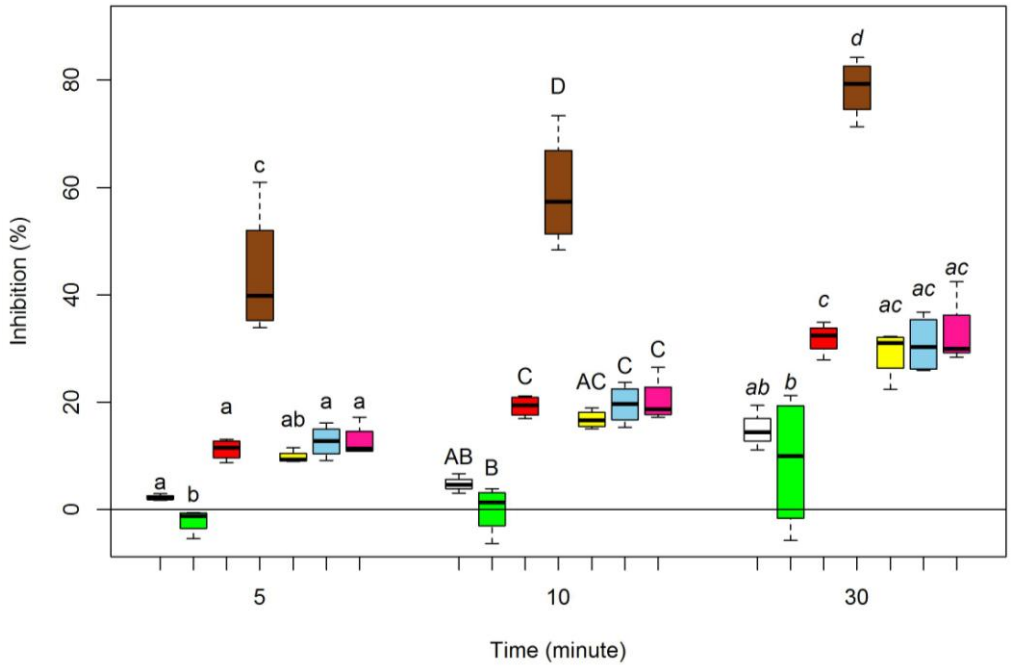
Figure 5.24 – Box plot representing *E. coli* biosensor inhibition (%) calculated after 5, 10 and 30 minutes from the addition of methanol samples to SSM. A) Inhibition due to both methanol and river water toxicants; B) Inhibition due to river water toxicants, without methanol effect. The test was conducted with water collected on 25/06/2012. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Different letters indicate significant differences ($p < 0.05$). Legend: ☐ methanol control; ■ Lea Nav at Tottenham Hale; ■ Pymmes Brook; ■ Lea Nav at Stonebridge Brook; ■ Lea Nav at Springfield Park.

Table 5.5 shows the *E. coli* biosensor inhibition (%) and the standard deviation (SD) values for the test conducted with water samples collected on 02/07/2012. The mean is calculated for the four biosensors employed for each test sample. Inhibition values (%) are presented in Figure 5.25, where graph A shows the total inhibition due to both methanol and river water toxicants, and graph B shows the inhibition values (%) without methanol effect in order to present only the percentage of inhibition due to toxicants present in the river water.

Table 5.5 – *E. coli* biosensor inhibition (%) and standard deviation (SD) calculated after 5, 10 and 30 minutes from methanol sample mixtures addition to SSM. Four biosensors were employed for each river water sample. The test was conducted with water collected on 02/07/2012.

Samples	Inhibition (%)					
	after 5 min		after 10 min		After 30 min	
	Mean	SD	Mean	SD	Mean	SD
Methanol control	2.3	0.6	4.8	1.8	15.0	4.2
Lea Nav at Tottenham Hale	-2.0	2.3	0.1	4.5	8.9	12.7
Pymmes Brook	11.2	1.9	19.3	2.0	31.9	2.9
Lea Nav at Stonebridge Brook	43.6	12.2	59.1	10.7	78.5	5.5
Lea Nav at Springfield Park	9.8	1.2	16.8	4.4	29.2	12.8
Lea Nav at Lea Bridge Weir	12.7	3.0	19.6	1.7	30.8	4.6
River Lea at Hackney Marshes	12.7	3.0	20.3	3.7	32.7	5.4

A)



B)

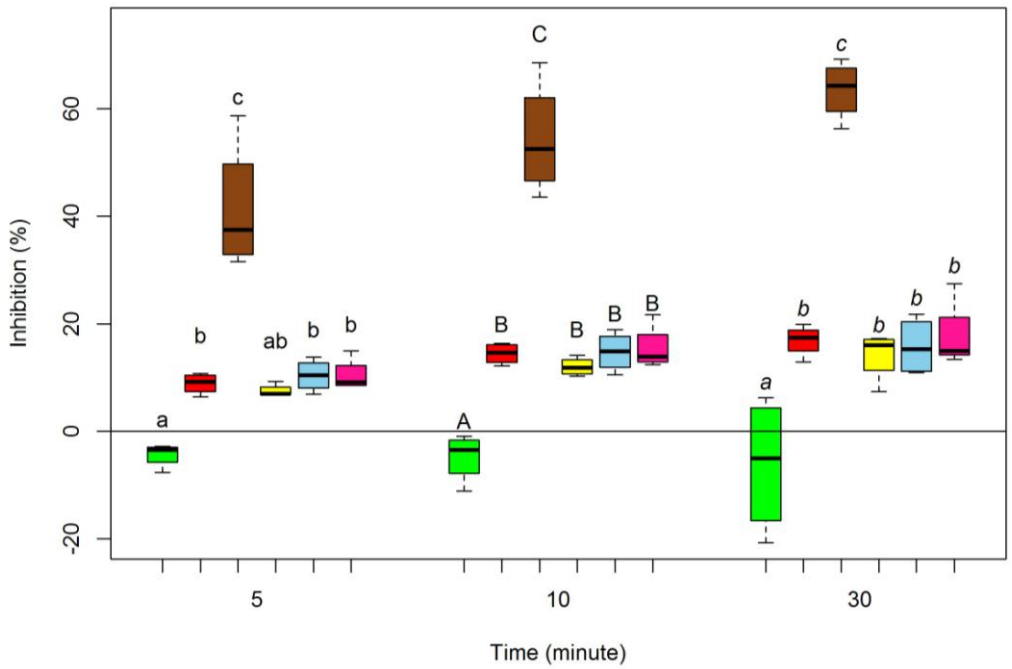


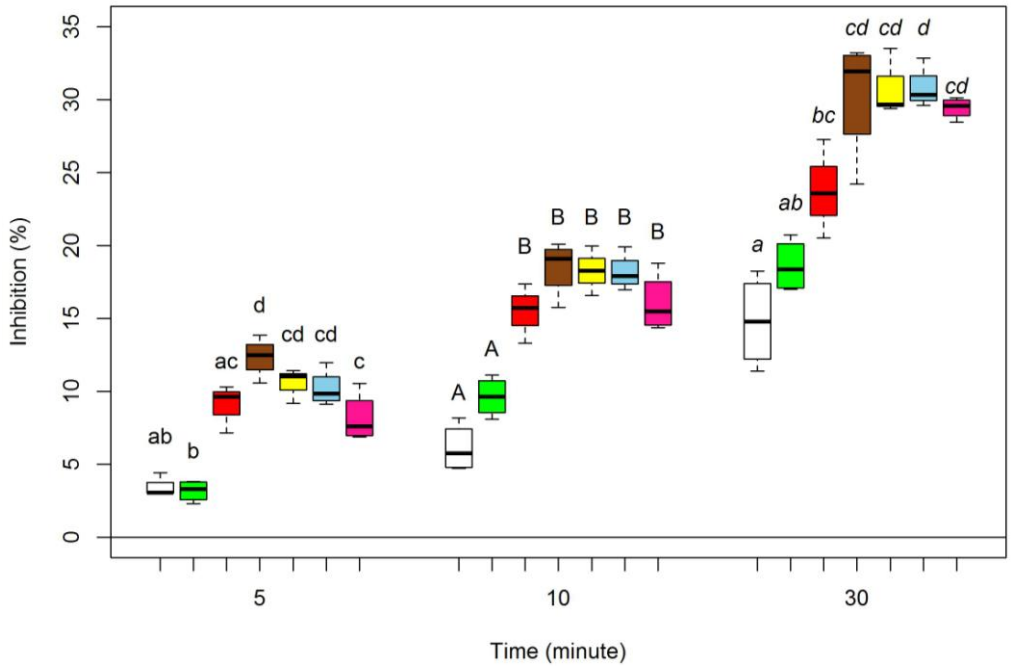
Figure 5.25 – Box plot representing *E. coli* biosensor inhibition (%) calculated after 5, 10 and 30 minutes from the addition of methanol samples to SSM. A) Inhibition due to both methanol and river water toxicants; B) Inhibition due to river water toxicants, without methanol effect. The test was conducted with water collected on 02/07/2012. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Different letters indicate significant differences ($p < 0.05$). Legend: ☐ methanol control; ■ Lea Nav at Tottenham Hale; ■ Pymmes Brook; ■ Lea Nav at Stonebridge Brook; ■ Lea Nav at Springfield Park; ■ Lea Nav at Lea Bridge weir; ■ River Lea at Hackney Marshes.

Table 5.6 shows the *E. coli* biosensor inhibition (%) and the standard deviation (SD) values for the test conducted with water samples collected on 16/07/2012. The mean is calculated for the four biosensors employed for each test sample. Inhibition values (%) are presented in Figure 5.26, where graph A shows the total inhibition due to both methanol and river water toxicants, and graph B shows the inhibition values (%) without methanol effect in order to present only the percentage of inhibition due to toxicants present in the river water.

Table 5.6 – *E. coli* biosensor inhibition (%) and standard deviation (SD) calculated after 5, 10 and 30 minutes from methanol sample mixtures addition to SSM. Four biosensors were employed for each river water sample. The test was conducted with water collected on 16/07/2012.

Samples	Inhibition (%)					
	after 5 min		after 10 min		After 30 min	
	Mean	SD	Mean	SD	Mean	SD
Methanol control	3.4	0.68	6.1	1.64	14.8	3.14
Lea Nav at Tottenham Hale	3.2	0.74	9.6	1.35	18.6	1.82
Pymmes Brook	6.5	5.35	11.8	7.55	17.9	12.16
Lea Nav at Stonebridge Brook	12.3	1.35	18.5	1.91	30.3	4.18
Lea Nav at Springfield Park	10.5	1.20	18.3	1.71	30.9	2.30
Lea Nav at Lea Bridge Weir	10.2	1.24	18.2	1.24	30.8	1.42
River Lea at Hackney Marshes	8.2	1.69	16.0	2.00	29.4	0.72

A)



B)

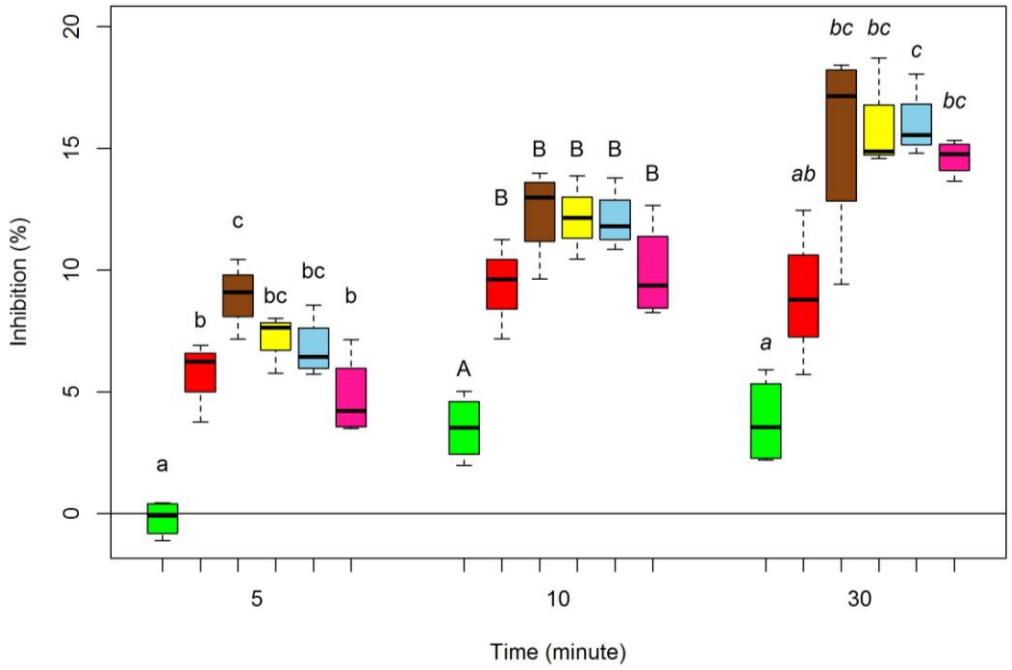


Figure 5.26 – Box plot representing *E. coli* biosensor inhibition (%) calculated after 5, 10 and 30 minutes from the addition of methanol samples to SSM. A) Inhibition due to both methanol and river water toxicants; B) Inhibition due to river water toxicants, without methanol effect. The test was conducted with water collected on 16/07/2012. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Different letters indicate significant differences ($p < 0.05$). Legend: ☐ methanol control; ■ Lea Nav at Tottenham Hale; ■ Pymmes Brook; ■ Lea Nav at Stonebridge Brook; ■ Lea Nav at Springfield Park; ■ Lea Nav at Lea Bridge weir; ■ River Lea at Hackney Marshes.

The three tests conducted showed inhibition at all the stations investigated. However, there was evidence that methanol was affecting the bacterial population, since inhibition was detected also for biosensors bathed in SSM and the level of inhibition increased over time. Nevertheless, river water samples showed higher levels of inhibition compared to the bacterial cells exposed to the methanol control, indicating that likely pollutants present in the water sample were affecting the *E. coli* cells. The results showed that bacterial biosensors exposed to Tottenham Hale water presented the lowest inhibition percentage between the monitoring stations, as previously seen in the algal bioassay.

The inhibition trend detected by exposing *E. coli* biosensors to methanol samples was similar to the inhibition trend found with *P. subcapitata* growth inhibition tests conducted with the water samples collected on the same day. These similarities were evident in particular in the experiments carried out with samples collected on 02/07/2012, where the highest inhibition percentage was identified in Stonebridge Brook waters. However, no significant similarities between the two bioassays were detected analysing the river water samples collected on 16/07/2012. No algal assessment was performed with water sampled on 25/06/2012.

Those discrepancies between the two experiments (biosensor assay and algal growth) could be likely due to the different test organism employed to test the toxicity. Moreover, the samples analysed with the bacterial biosensors were treated with methanol and this led to the investigation of concentrated polar pollutants soluble in methanol. Most importantly, inhibition was detected with the sample 100 fold diluted, suggesting that polar pollutants were investigated at the same concentration as in the river water and therefore the inhibition was not due to increased analyte concentrations.

In conclusion, protocol 3 conducted by exposing *E. coli* biosensors to water samples concentrated and treated with methanol, showed potential to investigate the toxicity of polar pollutants soluble in methanol. For this reason, it will be suggested to perform further test in order to improve the method.

5.7 Discussions and conclusions

Rapid acute toxicity tests were carried out using mediated amperometric whole cell biosensors (CellSense). Changes in the biocatalyst metabolic activity, due to the exposure to toxicants, results in a change in the electrode current response. Literature showed different examples of *E. coli* biosensors application to investigate the toxicity of wastewaters and sewage sludge (Evans *et al*. 1998, Farré *et al*. 2001, Farré and Barceló 2003, Daniel *et al*. 2004). They have been shown to be very effective in giving a rapid surface water quality assessment, and Rodiguez-Mozaz (2004 and 2006) stated that UK Environment Agency has proposed *E. coli* CellSense as a method for the Direct Toxicity Assessment (DTA) in 1999. In this project, it emerged that the use of whole cell mediated biosensors has two main limitations. The first is the necessity to customize the experiment based on the sample to be tested. For instance, the river Lea water showed to interact with the mediators and alternative protocols had to be developed to avoid this interaction. The second is the necessity to pre-treat the sample when testing chronic pollution. In fact, using the CellSense device, the biocatalyst is exposed to small volumes of test samples, which did not allow the detection of the toxicity of pollutants present at low concentrations. Dealing with chronic pollution, the use of pre-concentrate water samples is necessary for increasing the level of toxicity of the pollutants, especially when the toxicity is due to the persistent presence of low levels of contaminants. However, CellSense whole cells biosensors are low cost and rapid detection devices, which allow the toxicity testing by employing different test species, and are for these reasons powerful tools in environmental monitoring.

In this project, algal and bacterial biosensors were used to investigate the toxicity in river water samples, by performing three protocols.

1. Protocol 1. When both *P. subcapitata* and *S. leopoliensis* biosensors were exposed to river water samples, the result was an increased background current. This suggested a possible (electro)-chemical activity due to interactions between the mediator and some compounds dissolved in the river water, which was masking the biological response. This interpretation was supported also by the fact that interactions between *p*-benzoquinone and river water caused a change in the colour solution, which turned to brown/bronze. For these reasons, (electro)-chemical investigations were carried out by testing the mediated river water sample with blank electrodes (without biocatalyst). The resulting high current trends confirmed the presence of chemical reactions apparently due to interactions between the mediator and compounds present in the test solutions. When para-benzoquinone was used as mediator the (electro)-chemical responses were higher than with potassium ferricyanide. The relationship, if any,

between the toxic effect and the (electro)-chemical activity, needs to be determined as this may be a useful tool in future river water monitoring.

Bacterial biosensors were tested with river water pre-treated by solid phase extraction (SPE) in order to separate the polar fraction from the non-polar fraction. The results gave an indication of inhibition due to the polar fraction, supporting the results obtained with algal growth inhibition tests. However, the results were not reliable since there was evidence that the signal was affected by (electro)-chemical interactions.

Farré *et al*. (2001) employed successfully *E. coli* biosensors to investigate the toxicity of industrial effluents, but then in a paper published in 2003 the same authors recognised that in some cases the (electro)-chemical activity could alter the results. In this project, two alternative protocols were developed to bypass this inconvenience.

2. Protocol 2. Since there was evidence that the (electro)-chemical interferences were due to interaction between the mediator with compounds dissolved in the river water, biosensors were exposed to test samples without addition of mediator: *P. subcapitata* biosensors were exposed to water samples for different time period (30 minutes, 2 hours, and 24 hours). Results achieved did not show any significant level of inhibition. On the contrary, the outcome signals of biosensors exposed to polluted water samples showed evidences of stimulation compared to biosensors bathed in the growth medium (OECD) or Tottenham Hale water. According to Farré *et al*. (2001) stimulation of the metabolism could be due to: 1) low toxicant concentration; 2) the river water is a source of nutrients; 3) the river water works as a metabolic uncoupler.

3. Protocol 3. Another method to avoid the (electro)-chemical interferences was to change the potential at the working electrode from +550 mV to +200 mV. First, protocol 3 was carried out with untreated river water, but the reproducibility of the test was problematic, since there was evidence that the inhibition was masked by bacterial metabolic stimulation due to nutrients present in the water samples. To avoid stimulation of the bacterial metabolism and because initial results showed a likely chronic inhibition, river water samples were pre-concentrated. However, inhibition was detected by using river water samples 100 fold diluted, so pollutants were investigated at the same concentrations they had in the river water. The solutes re-suspended in methanol showed evidence of inhibition in the same stations, where there was evidence of inhibition by algal growth tests. Tests performed with solutes dissolved in water did not show any changes in the current

signal, which could be because some organic compounds have a significantly higher solubility in methanol than in water.

In conclusion, CellSense whole cell biosensors were employed in this project to investigate river water toxicity. Because the river samples were showing (electro)-chemical interaction with the mediator, alternative protocols were designed. Useful results were achieved decreasing the potential at the working electrode and using methanol to re-suspend solutes derived by water sample evaporation, which avoided the stimulation of the bacterial metabolism due to the presence of nutrients in the river water. The test performed with *E. coli* biosensors showed the potential to be a good tool to determine the toxicity of those pollutants present in the river water and soluble in methanol.

6 In situ river water quality monitoring

The river water quality was also monitored by *in situ* measurements in order to complement the results obtained with laboratory experiments.

Two different *in situ* monitoring assays were performed:

1. a spatial seasonal monitoring of physico-chemical parameters of river water at twenty-three sites between Tottenham Hale (Lea Navigation) and Hackney Marshes (River Lea);
2. *in situ* algal growth inhibition test with algae entrapped in alginate beads at six of these monitoring stations (Lea Navigation at Tottenham Hale, Pymmes Brook, Lea Navigation at Stonebridge Brook, Lea Navigation at Springfield Park, Lea Navigation at Lea Bridge weir, River Lea at Hackney Marshes).

6.1 Spatial monitoring of physico-chemical parameters

The Environment Agency had only three automated monitoring stations in the area under investigation, which were not enough to have an exhaustive picture of the water quality in that particular reach of the Lea channel. To improve the spatial data resolution, physico-chemical parameters were collected *in situ* by using a multiparametric probe.

The collection of physico-chemical parameters *in situ* had three main aims:

1. to increase the spatial data resolution of the data collected by the three automated monitoring stations of the Environment Agency, in an effort to identify likely sources of pollution;
2. to detect any seasonal trend in the variation of the physico-chemical data;
3. to produce spatial maps for each physico-chemical parameter.

Spatial maps are an “easy-to-read” presentation of the results. The interpolation of the data resulted in having a picture of the river water quality as close to reality as possible, even at the sampling sites not investigated. By estimating a value within two measured data, the interpolation method allowed to create a continuous dataset from punctual values, permitting to better distinguish the patterns of the different parameters in a spatial context. In this study, the interpolation method helped to focus the investigation at sites more polluted than others, which could be better identified looking at the map rather than reading listed data in a table.

Generally speaking, spatial maps could be useful tools to display data helping people to understand the water quality in the Lea Navigation. The arrangement of the results in a map helps people to relate the water quality of the channel with the nearest features (like streets, parks, and recreational areas), raising the public concern for the area and maybe raising awareness of the need to protect it.

Surveys were conducted on four occasions from summer 2011 to spring 2012. The physico-chemical parameters were monitored on:

- 22nd of August 2011 (summer)
- 31st of October 2011 (autumn)
- 09th of January 2012 (winter)
- 23rd of April 2012 (spring)

Data were collected at twenty-three sites from Tottenham Hale to the Lea Bridge weir, and further downstream in the Lea Navigation and in the River Lea at Hackney Marshes (Figure 6.1). In this Chapter, spatial maps for each physico-chemical parameter are presented by interpolation of the data, while collected values are listed in Appendix XI. More details about the data collection and the production of the maps are given in the "Materials and Methods" section.

Figure 6.1 – Map of the *in situ* physico-chemical monitoring sites. Physico-chemical parameters were collected at twenty-three sites between Tottenham Hale (Lea Navigation) and Hackney Marshes (River Lea). (The raster map is provided by OpenStreetMap - Creative Commons-Share Alike License [CC-BY-SA]).

6.1.1 Dissolved oxygen (%)

The highest dissolved oxygen levels were registered at Tottenham Hale and in the reach of the Old River Lea, which flows into the Lea Navigation a few metres downstream of Pymmes Brook confluence (Figure 6.2). Throughout the monitoring year at these two sampling sites, the dissolved oxygen saturation was > 80 %, indicating a “very good” quality level, except for October 2011 when the oxygen at the Old River Lea ranged between 70 - 79 %, showing a “good” quality of the water. The dissolved oxygen quality at Pymmes Brook was “poor” in October 2011, while it was “fairly good” during January 2012 and April 2012, and “fair” during August 2011. The Lea Navigation reach between Pymmes Brook inflow and the confluence with the Old River showed a “poor” quality in April 2012, while during the others seasons it was “fair” and “fairly good”. Downstream of the Old River Lea the oxygen levels were < 50 % indicating a “poor” quality, except for January 2012 when the quality in a section of the Lea Navigation was “fair”. It is important to underline that during two samplings (October 2011 and April 2012) “bad” water quality was found around Markfield Park where two Brooks comes into the Lea Navigation: Stonebridge Brook and Old Moselle Brook. Another site where a “bad” condition was seen was at the Marina where Coppermill flows in. The “bad” oxygen quality registered at Coppermill was likely due to the presence of stagnant water. A low dissolved oxygen level (< 20 %) was registered around Lea Bridge weir during April 2012. Downstream of the weir, in the natural reach of River Lea, the quality improved throughout the year, which could be due either to the influence of the vegetation in the water depuration from pollutants or to the greater water aeration because of both the water mixing at the weir and the waters were shallow and turbulent. During October 2011, at all the monitored stations the dissolved oxygen concentrations were the lowest compared to the other surveys, while the highest were recorded at January 2012. The spatial maps produced by interpolating dissolved oxygen values recorded during the four surveys are given in Figure 6.2. The colouring on the map and the classification were chosen in agreement with the Thames 21 paper (2011).

Figure 6.2 – Interpolated maps of dissolved oxygen levels (%) registered during four surveys.

6.1.2 Temperature (°C)

Water temperature measures (°C) were in agreement with the season: high in August 2011 (~ 20 °C), low in January 2012 (~ 8 °C), whilst it was 17 °C in October 2011 and 13 °C in April 2012. Measurements showed lowest temperatures at Tottenham Hale, the Old River Lea and Coppermill, suggesting a likely influence of Deephams sewage treatment work discharge on the Lea Navigation water temperatures. In fact, Pymmes Brook water and the area immediately downstream it showed the highest temperature compared to the other stations located along the Lea Navigation during all the four surveys (Figure 6.3).

Figure 6.3 – Interpolated maps of temperature (°C) registered during four surveys.

6.1.3 pH

At all the four sampling times pH values ranged between 6 and 9, indicating a “good” quality for the fish populations, in agreement with the Freshwater Fish Directive (78/659/EEC). However, it is possible to identify differences. For example, the pH level at Tottenham Hale and Old River Lea was > 8. Downstream of Pymmes Brook the pH ranged between 7 and 8, indicating a likely influence of the Deephams sewage treatment work discharge, in particular linked to the bacterial loading level, since aerobic process produces acids decreasing the pH (EPA 2002). However, downstream of Springfield Park the pH concentration slightly decreased and then increased downstream of the weir in the River Lea (Figure 6.4).

Data presented seasonal variations, with slightly higher pH values during April 2012 and August 2011 than October 2011 and January 2012, possibly due to the higher photosynthetic activity during spring-summer period. The lowest pH values were recorded in January 2012, which ranged between 7.14 and 8.02, while the highest pH values were registered in April 2012 (7.21 – 8.89). Values are reported in Appendix XI.

Figure 6.4 – Interpolated maps of pH levels registered during four surveys.

6.1.4 Conductivity (µS/cm)

Conductivity measurements (µS/cm) showed that Tottenham Hale, Old River Lea and Coppermill had the lowest values during all four surveys, whilst at Pymmes Brook the highest conductivity levels were registered, possibly due to the Deephams sewage treatment work discharge. Conductivity values were higher than the suitable level for the aquatic biota, which ideally should range between 150 and 500 µS/cm (Behar 1997). However measures were within the normal range for most of the streams (50 – 1500 µS/cm) as described by Behar (1997), except for the conductivity at Pymmes Brook in April 2012 (1900 µS/cm). During August 2011 and April 2012, conductivity values were lower than during October 2011 and January 2012 (Figure 6.5).

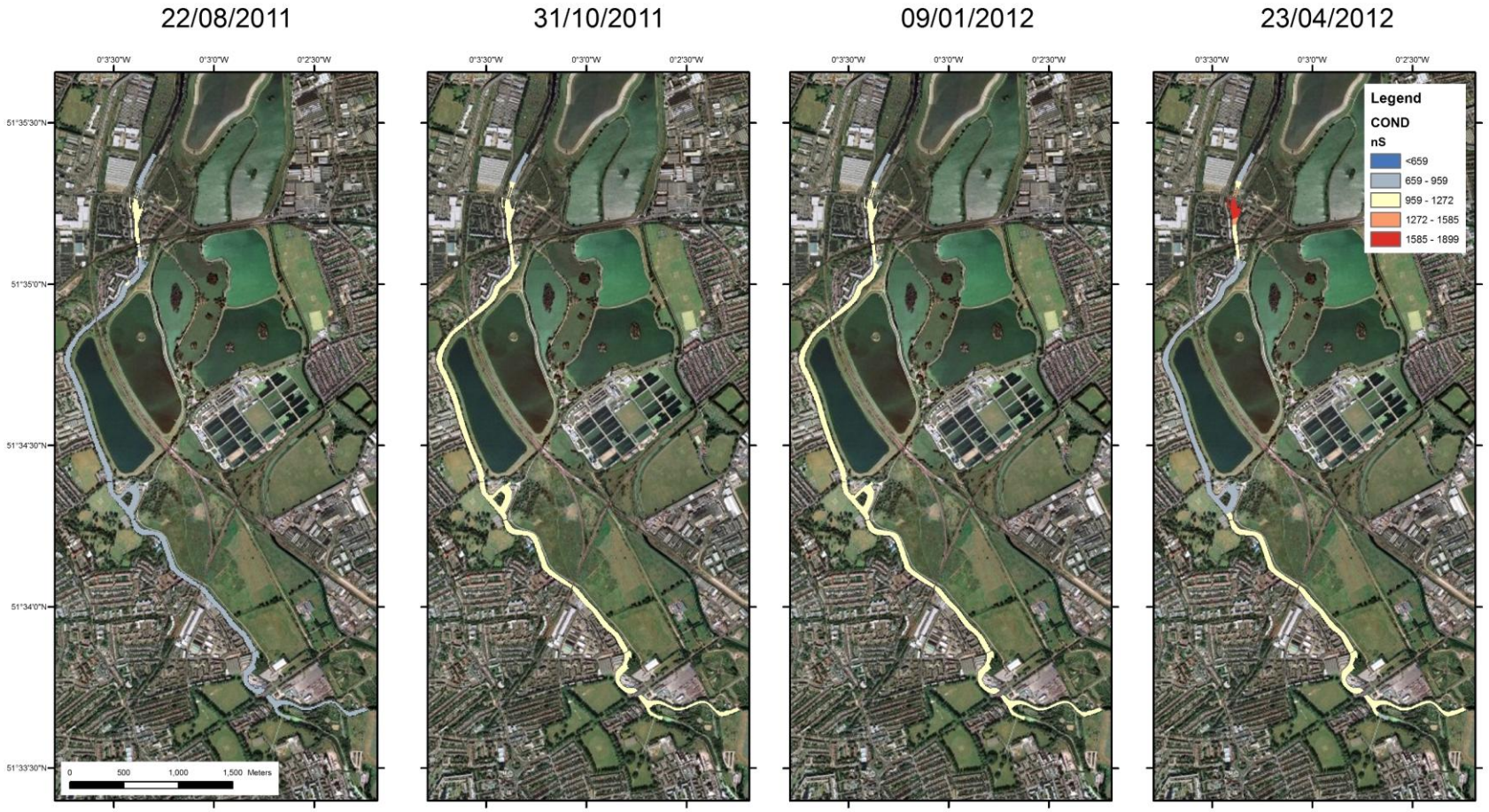


Figure 6.5 – Interpolated maps of conductivity (µS/cm) registered during four surveys.

6.1.5 Total ammonia (mg/l)

The total ammonia is the sum of the toxic un-ionized ammonia (NH_3) and the non toxic ammonium ion (NH_4^+). Values recorded ranged between 0.4 mg/l and 3 mg/l. During August 2011 and January 2012 Tottenham Hale, Old River Lea and Coppermill showed the lowest ammonia levels. During the other two surveys, no differences were detected. In October 2011 and January 2012, the total ammonia values were higher than during the August 2011 and April 2012 (Figure 6.6). These differences in total ammonia levels between surveys could be linked to the efficiency of Deephams STW, or runoff from the surrounded areas.

Figure 6.6 – Interpolated maps total ammonia (mg/l) registered during four surveys.

6.2 River water quality according to *in situ* physico-chemical parameters

In situ chemico-phisical surveys showed evidence that Lea Navigation water quality was affected by Pymmes Brook waters. However, there was indication of the presence of other likely pollution sources.

1. The dissolved oxygen saturation recorded downstream of Pymmes Brook was lower than upstream of Tottenham Hale locks and at Old River Lea. The low oxygen levels detected could be due to several factors such as: low photosynthetic activity, organic matter decomposition and nitrification by aerobic bacteria, high chemical oxygen demand (COD). Very low oxygen levels were identified in the Lea Navigation at the confluence with Stonebridge Brook and Old Moselle Brook, indicating these two brooks as likely sources of pollution. The detection of “bad” oxygen levels in the area of Lea Bridge weir during the survey of April 2012 suggested the presence of other sources of pollution, such as misconnections and/or surface runoff from the roads. The dissolved oxygen levels at Pymmes Brook were slightly higher than downstream of it, probably due to the shallower and more turbulent water flow. Higher dissolved oxygen concentrations were detected also in the River Lea downstream of the weir, which could be due both to physical phenomena, such as aeration of the water by the passage through the weir and by turbulent and shallow flow, and natural depuration from pollutants by the vegetation and microorganisms present along the banks and along the riverbed.
2. The temperature downstream of Tottenham Hale locks was higher than at upstream of the locks and at Old River Lea. The highest temperature was detected at Pymmes Brook and in Lea Navigation immediately downstream of its confluence. This could be explained by both the presence of sewage treatment work discharge waters and the lower water level in Pymmes Brook, which could facilitate the heat exchange with the air.
3. At Pymmes Brook and downstream of its confluence with the Lea Navigation the pH was lower than at Tottenham Hale and at Old River Lea. A possible explanation could be the presence of nitrifying organisms in the water, which produce acids during the oxidation process of ammonia into nitrite, decreasing the pH.
4. The highest conductivity levels, which indicate the presence of ions in the water and it could be linked to the total suspended solid, were detected at Pymmes Brook, suggesting the presence of water from the sewage treatment work discharge beside the contribution of surface runoff.

5. There was evidence of higher total ammonia downstream of Pymmes Brook than at Tottenham Hale, Old River Lea and Coppermill, even if it was not detected during all the four surveys. The equilibrium between the non toxic ammonium ion (NH_4^+) and the toxic un-ionized ammonia (NH_3) depends on the pH and the temperature. The toxic un-ionized form increases at higher pH and higher temperatures. At similar pH, higher levels of ammonia are present in warmer water. Moreover, surveys showed that the highest temperatures were at Pymmes Brook, which could results in the presence of toxic un-ionized ammonia.

6.3 *In situ* biological evaluation of water quality

To complement the data collected with algal growth inhibition tests conducted in the laboratory, *P. subcapitata* cells entrapped in alginate beads were employed to test the river toxicity *in situ*. Since experiments conducted in the laboratory do not reflect all the variables presents in the natural environment (Twist *et al*. 1998), the algal growth was monitored *in situ* by entrapping cells in sodium alginate beads and exposing them to continuous river flow. Algal beads were prepared by following modified protocols published by Rawson (1989) and Moreira dos Santos *et al*. (2002), as in the "Materials and Methods" section.

Algal beads were prepared by entrapping algal cells into 2 % sodium alginate and hardened into 2 % calcium chloride. The first stage was to test the growth of *P. subcapitata* cells entrapped in alginate beads in 25 ml nutrient OECD medium, and results showed that the growth was greater than 16 fold, in line with the criteria of validity listed in the OECD guidelines (2006). Algal beads were then tested in river water with experiments conducted in the laboratory to study the behaviour of algal cells entrapped in alginate beads when they were exposed to river samples. Figure 6.7 shows the results of a test conducted over 3 days with waters collected on 14/03/2011. Test solutions were enriched with nutrient medium and renewed with fresh sample from unopened bottles (enriched with OECD medium) every 24 hours. After 24 hours (day 1) no inhibition was detected, but after 48 hours (day 2) there was evidence of growth inhibition on the entrapped algae exposed to water collected from Pymmes Brook and from Lea Navigation at Springfield Park. Similar results were obtained by conducting the test for 5 days (Appendix X).

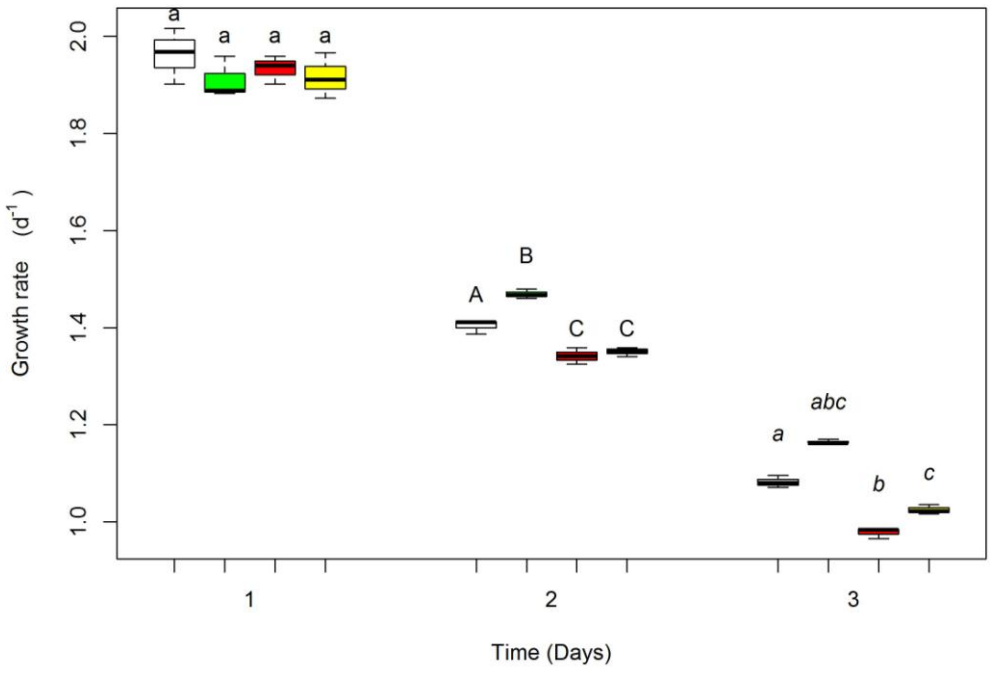


Figure 6.7 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples enriched with OECD medium. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Boxes with different letters differ significantly ($p < 0.05$). The test was conducted with water collected on 14/03/2011. Legend: ☐ OECD medium ■ Lea Nav at Tottenham Hale; ■ Pymmes Brook; ■ Lea Nav at Springfield Park.

In order to test conditions in the field, the *P. subcapitata* growth was tested with river water not supplemented with nutrients. The experiment was conducted with water collected on 21/11/2011 and the algal growth of both cells entrapped in alginate balls and free cells was investigated. Every 24 hours the test solutions were renewed with fresh river water samples from unopened bottles. River water samples with free algae were first centrifuged at 3500 rpm for 5 minutes and the pellet was re-suspended in 25 ml of fresh water. Results are given in Figure 6.8 and 6.9 respectively. As confirmation of previous tests, the growth in algal beads showed an inhibition effect with Pymmes Brook and Springfield Park waters from day 2, compared to the growth in Tottenham Hale sample. The standard algal growth test showed a different trend (described in Chapter 4) where the higher level of inhibition was detected after the first day of test, with complete or partial recovery from day 2. These results suggest a possible protective action of the alginate during the first stages of the test, acting as barrier to the pollutants present in the river water. At the end of the experiment, all the beads were undamaged. The growth in algal

population exposed to Tottenham Hale water (control) and entrapped in alginate beads was smaller than the growth of free algal cells (16 fold vs 46 fold), possibly due to reduce diffusion of nutrients and CO_2 across the alginate matrix.

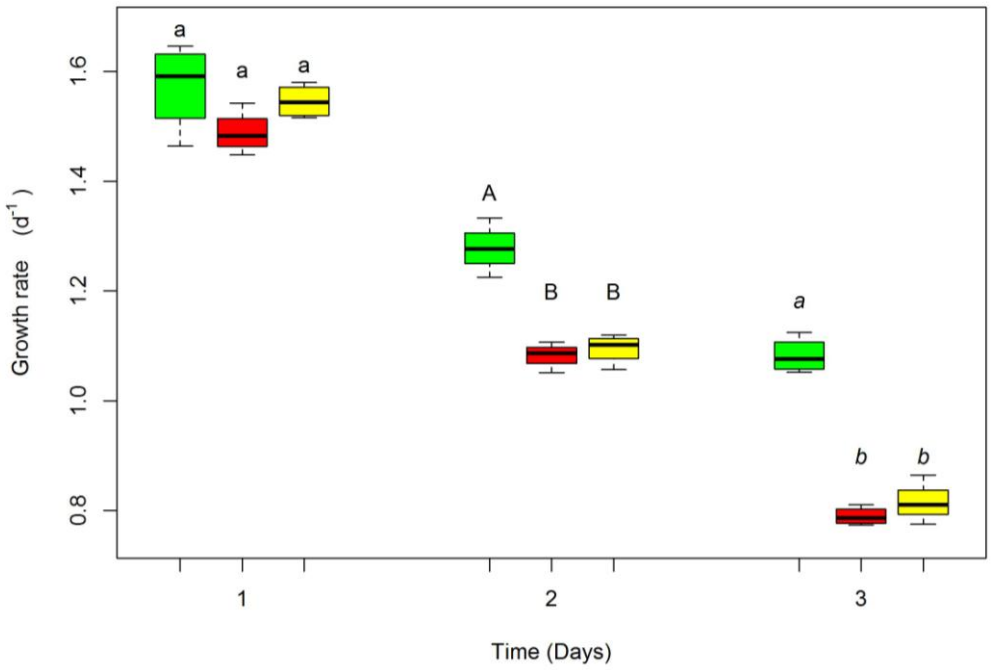


Figure 6.8 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples. The test was conducted with water collected on 21/11/2011. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Boxes with different letters differ significantly ($p < 0.05$).. Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park.

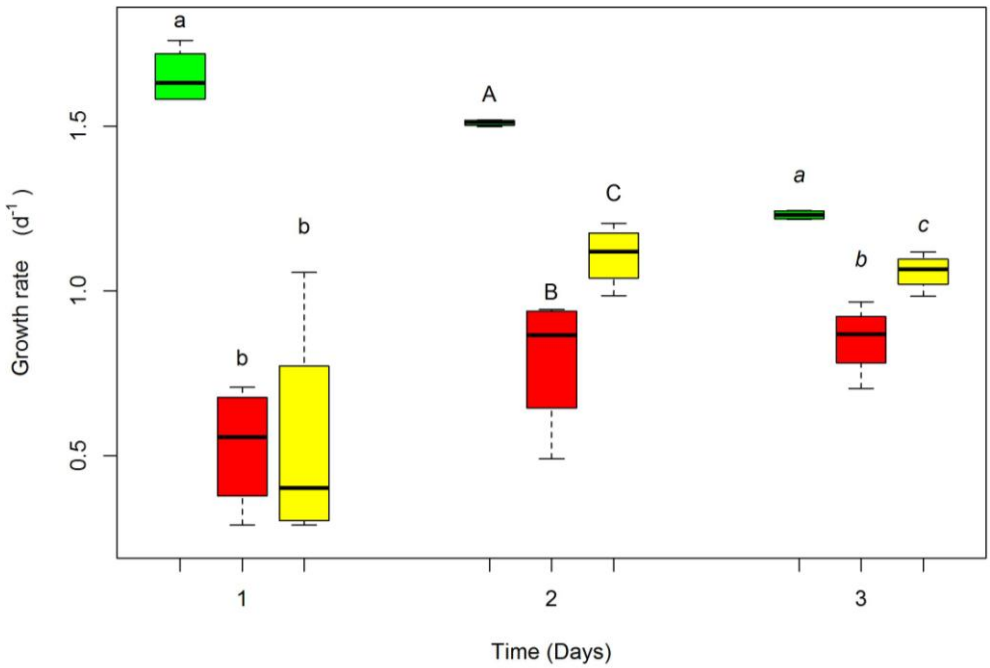


Figure 6.9 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* free cells and exposed to river water samples collected on 21/11/2011. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park.

After the laboratory tests, *P. subcapitata* beads were tested *in situ*. The procedure used for this monitoring is explained in the "Materials and Methods" section.

Beads entrapped in 2 % sodium alginate and hardened in 2 % calcium chloride were placed at six monitoring locations: Pymmes Brook, Lea Navigation at Tottenham Hale, Lea Navigation at the confluence with Stonebridge Brook, Lea Navigation at Springfield Park, Lea Navigation at Lea Bridge weir, and River Lea at Hackney Marshes. The test was conducted twice and the duration of the test was 7 days. Results from both the tests were incomplete since beads located at stations downstream of the confluence with Pymmes Brook were dissolved partially or completely (see Appendix X for the results). Moreover, the stations where the beads were undamaged did not show any statistically significant difference with Tottenham Hale, in disagreement with algal growth inhibition test results. The algal cells at Tottenham Hale grew 2 fold during the test, which are 8 fold less than the growth achieved with laboratory tests.

The resistance of the alginate ball was tested also by placing *in situ* blank alginate beads (without algal cells) for 3 days only, but even in this case the beads dissolved.

Consequently, harder beads were prepared, by using 3 % sodium alginate and 4 % calcium chloride. The algal growth was tested in the OECD medium with a test conducted over 3 days and the results showed a growth of 20 fold.

The beads were then placed at the six monitoring stations listed before for 3 days and at two stations (Tottenham Hale and Pymmes Brook) for 7 days. Beads did not dissolve, but results achieved after 3 days of test did not present any statistical difference between stations (Figure 6.10). After 7 days of exposure, algae exposed to Pymmes Brook waters exhibited a higher growth than algae placed at Tottenham Hale site (Figure 6.11). The *P. subcapitata* growth at Tottenham Hale station was of 1.5 fold after 3 days of test and 2.3 fold after 7 days.

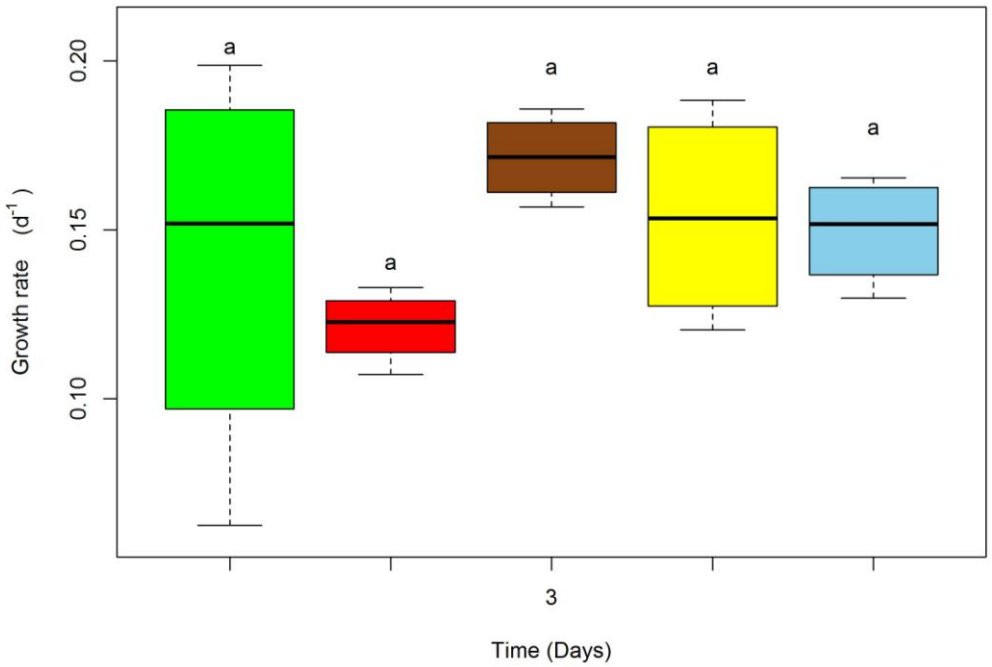


Figure 6.10 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride) exposed i*n situ* to river water for 3 days. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Boxes with different letters differ significantly (p < 0.05). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir.

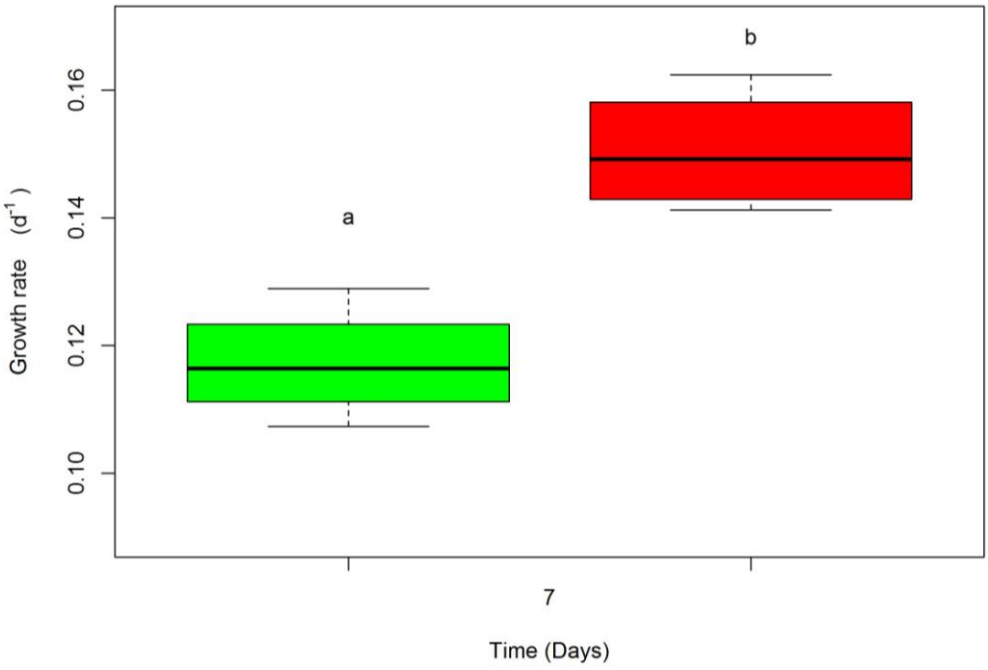
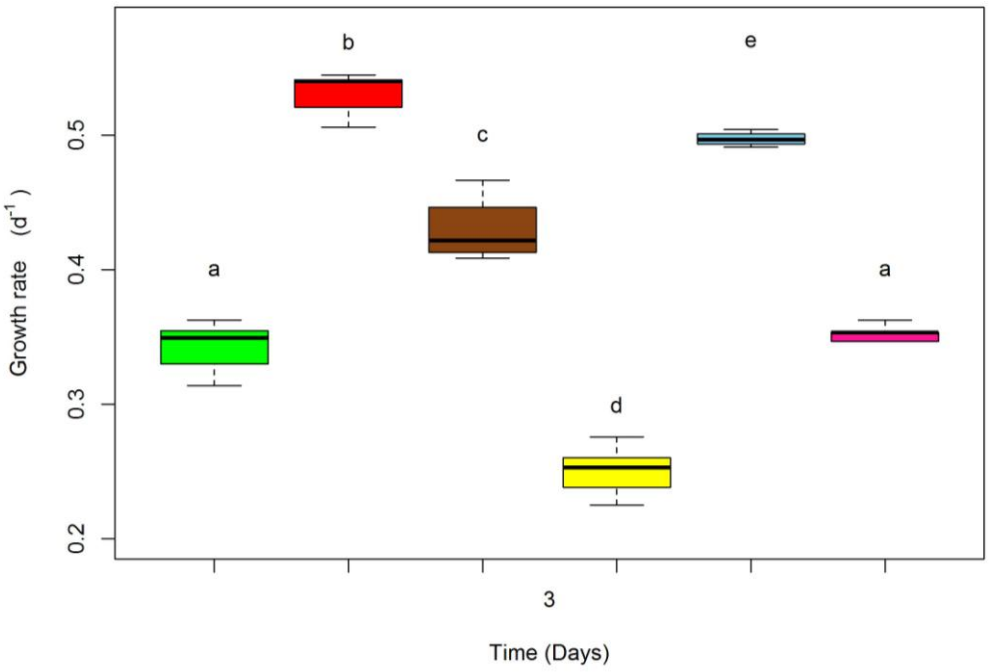


Figure 6.11 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride) exposed i*n situ* to river water for 7 days. Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Pymmes Brook.

Two possible explanations to the lower algal growth in beads exposed to Tottenham Hale compared to the other stations were: 1) high concentration of nutrient downstream of Pymmes Brook inflow; 2) the sodium alginate matrix was acting as barrier for the pollutants. For this reason beads with a thinner alginate barrier (2 %) but hardened in a high calcium chloride concentration (4 %) were prepared. Beads were placed at the six monitoring locations and the test was performed twice.

During the first test alginate beads did not dissolve but the algal growth at Tottenham Hale was still smaller than at the stations located downstream of Pymmes Brook, except for Springfield Park (Figure 6.12 - A). However, the inhibition at Springfield Park was not confirmed in the second test, which moreover resulted in dissolved beads at Pymmes Brook and Hackney Marshes after 3 days (Figure 6.12 - B). The growth at Tottenham Hale at the end of the test was of 2 and 3 fold in the first and the second test respectively.

A)



B)

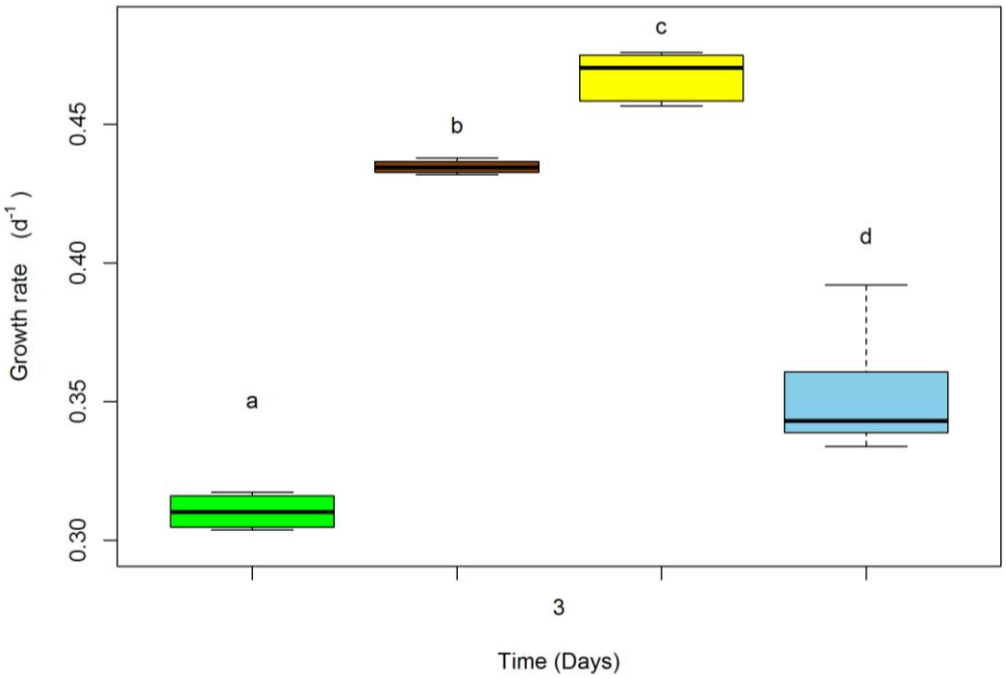


Figure 6.12 – Box plots representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride) exposed i*n situ* to river water for 3 days on two occasions (A) and (B). Box represents middle two quartiles; the horizontal line in the box represents the median. The ends of the whiskers represent the minimum and maximum data points. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

6.4 Discussions and conclusions

Two *in situ* monitoring approaches were performed to supplement the information gained from the Environment Agency automatic monitoring stations and the laboratory tests.

The first survey was to measure physico-chemical parameters using a multiparametric probe, in order to improve the spatial data resolution of the data collected by the three automated monitoring stations of the Environment Agency, in the effort to have a comprehensive picture of the river water quality and identify likely sources of pollution. Collected data were analyzed to investigate any seasonal trend of the physico-chemical parameters and to produce spatial maps, an “easy-to-read” way to present results. The detection of low dissolved oxygen levels downstream of Pymmes Brook showed that its water was affecting the Lea channel water. Also the other parameters suggested Pymmes Brook as a source of pollution: 1) the temperature of the water was higher at Pymmes Brook and the area immediately downstream; 2) the highest conductivity values were recorded at Pymmes Brook; 3) pH values were lower downstream of Pymmes Brook confluence than upstream of it (Tottenham Hale). The measurement of the total ammonia did not show any differences between stations. However, it is important to keep in mind that in warmer water there is more toxic ammonia than in cooler water, at any pH (Novak and Holtze 2005). This fact draws the attention to Pymmes and the surrounding area, where the temperature of the water was higher than in the other monitoring locations. In summary, the data of the *in situ* surveys detected a negative influence of Pymmes Brook’s water in the receiving stream, confirming the results obtained in Chapter 3.
Therefore, the performance of a spatially detailed survey allowed the detection of two potential source of contamination. In fact, the study of the dissolved oxygen levels gave evidence of other two likely punctual sources of pollution: Stonebridge Brook and Old Moselle Brook. Moreover, the low dissolved oxygen concentrations registered both above and after the Lea Bridge suggested the presence of other potential sources of contamination (runoff, misconnections, etc.).
Finally, the presentation of the results with maps allowed an immediate reading of the river water quality, whose variations during the space and the time were easily identifiable by changes in the colours.

The second type of *in situ* monitoring employed the exposure to river water of *P. subcapitata* cells entrapped in alginate beads, in order to avoid the recovery of algal population at the end of the test, as suggested by Moreira-Santos *et al*. (2004). The tests were conducted by following as far as possible the guidelines of the Organization for Economic Cooperation and Development (OECD) regarding the algal growth inhibition test. Laboratory assays conducted with algal beads showed evidence of inhibition by 48 hours in water samples collected from Pymmes Brook and Springfield Park compared to

the growth in water collected from Tottenham Hale, and in agreement with previous algal growth test (Chapter 4). When algal beads were placed *in situ*, at some sampling sites the alginate bead dissolved possibly due to the presence of chelants in the river water (such as phosphate, surfactants and citrate), which could interfere with the calcium used to hard the algal balls (Moreira-Santos *et al*. 2004).

Other combinations of sodium alginate and calcium chloride were tested in order to avoid the damage to the alginate matrix. However, results showed a lower *P. subcapitata* growth at the end of the 3 and 7 days tests at Tottenham Hale compared to the algal growth at Pymmes Brook and the sites located downstream of its confluence with the Lea Navigation, in disagreement with the algal growth test results. These trends could be explained by a likely higher concentration of nutrients (such as N and P) at Pymmes Brook and the stations downstream of its confluence, which could increase the algal growth (Corrêa *et al*. 2009). The lack of inhibition at the sites located downstream of Tottenham Hale locks could be due to the alginate barrier, which could prevent the pollutants reaching the algal cells, as suggested by Moreno-Garrido (2008). In this review the author reported evidence that: 1) metals could be entrapped in the alginate matrix (Awasthi and Rai 2005 cited in Moreno-Garrido 2008); 2) toxicants could have a low diffusivity in the beads (Jang 1994); 3) Glyphosate, Paraquat (Bozeman *et al*. 1989), and surfactant lineal alkylbenzene sulphonate (Moreno-Garrido *et al*. 2007) had more toxic effects in free algae than in entrapped algal cells.

The *in situ* biological assessments are very useful tools to monitor the chronic pollution in a stream. The algal growth tests are functional assays to standardise the results with guidelines and to compare the water quality data of different water bodies. However, as demonstrated in this study, the algal tests are not adequate for monitoring the river water toxicity due to pollutants present chronically and at low concentrations, since the toxicant levels decrease by the end of the test. By exposing the algal cells to the river water *in situ*, the algae would be exposed constantly to the pollutants and the results of the test would reflect the real situation, keeping in consideration all the natural variables.

However, results suggested that in this particular urban channel, it was difficult to monitor the toxicity with algal alginate beads *in situ*. The first challenge was regarding the positioning of the beads in the river. Several factors could affect the final result, such as: 1) illumination (boats parked along the channel banks and orientation of the river bank - North, South, West, East) 2) depth along the water column, 3) flow direction, 4) fine sediment particles accumulation and the mesh fouling. The second big challenge was how to present the test organisms to the river water. This project demonstrated that the use of sodium alginate (hardened with calcium ion) to entrap the algal cell was inappropriate to monitor the river water quality in this urban contest, because of chelants in the water were causing the disruption of the alginate matrix. However, it was possible to

detect the higher levels of nutrients downstream of the Pymmes Brook, due to the Deephams STW discharge.

In conclusion, both the *in situ* surveys confirmed a negative influence of Pymmes Brook waters on the Lea Navigation water quality. Physico-chemical parameters showed lower dissolved oxygen levels, higher temperature, lower pH levels, high conductivity and higher total ammonia at Pymmes Brook and downstream of its confluence compared to Tottenham Hale and Old River Lea stations. At the same time, by using algae entrapped in alginate beads, high levels of nutrients were identified at Pymmes Brook and downstream of its confluence compared to the nutrients levels at the Tottenham Hale site. However, the measurements of the dissolved oxygen levels showed other likely sources of pollution to monitor in the future: Stonebridge Brook and Old Moselle Brook. The spatially detailed collection of physico-chemical data *in situ* allowed the detection of polluted sites, whose ecotoxicity was monitored with bioassays. Since the use of *in situ* algal bioassay has the potential to provide more exhaustive information about the water toxicity than the algal test carried out in the laboratory, future work should develop an alternative method to entrapped algal cells to be exposed to the river water. This project demonstrated that appropriate river monitoring plans should combine: 1) the exploration of the area by collecting physico-chemical parameters, in order to detect critical sites; 2) biological bioassay to carry out both in the laboratory and *in situ*.

7 Discussions and conclusions

7.1 Introduction

A permanent low concentration of dissolved oxygen is found in the Lea Navigation downstream of Pymmes Brook (Snook and Whitehead 2004), which receives water from Deephams sewage treatment works final effluent, and as far downstream as the Olympic park area in Stratford (paragraph 2.1.1, and Figure 2.4). With urbanization, the original river Lea has experienced changes in both the physical characteristics, being canalised in sections for the navigation, and in the water quality, due to an increase in quantity and variety of pollutants entering the river. This chronic pollution is more evident in the Lower Lea catchment, since it mostly flows within the area of Greater London. The main factors affecting the Lea Navigation are: 1) diffuse urban pollution from surface runoff and untreated wastewaters (misconnections); 2) sewage treatment works final effluents; and 3) its waters are abstracted for domestic and industrial use.

At the same time the Lea catchment includes aquatic habitat Sites of Special Scientific Interest (Rye Meads, Turnford and Cheshunt Pits, Walthamstow Reservoirs), Special Protection Area (SPA, Lea Valley Regional Park), and local Nature Reserves, which need to be protected (Environment Agency 2006). Moreover, the Lea is an important water supply for London citizens (Reid 1995 cited in Snook and Whitehead 2004). These factors, combined with the necessity to accomplish the objectives stated by the European Water Framework Directive (2000), drive the necessity to improve the water quality of the Lea Navigation.

This project aimed to monitor the water quality of the Lea Navigation through the application of different techniques, in order to be able to determine the factors that are responsible for the poor water quality in the reach downstream of Pymmes Brook.

7.2 Overview of the findings

7.2.1 River Lea lower catchment preliminary assessment

The first stage of this project was to provide a general overview of the area studied by collecting and elaborating data collected over a period of two years (from 21/06/2010 to 20/06/2012), and grouped by seasons. Raw data were supplied by: 1) the Environment Agency, including physico-chemical parameters (dissolved oxygen, temperature, pH, conductivity, turbidity, and total ammonia), channel flow, and historic river quality data; 2) the Meteorological Office for the rainfall data; and 3) Thames Waters for Deephams STW discharge flow data.

A particular focus was on the dissolved oxygen (DO) concentrations, since low DO levels were the main problem in the Lea Navigation reach under investigation. Moreover, the level of dissolved oxygen was studied as indicator of pollution in the river water. Data showed that in the Lea Navigation between Pymmes Brook and Lea Bridge weir the DO concentration was low, presenting stressful conditions for the aquatic ecosystem over several days. In order to explain the low dissolved oxygen levels, possible variations of other physico-chemical parameters (such as temperature, conductivity, turbidity, total ammonia) were considered, and Spearman rank correlations performed. Results did not show consistent seasonal correlations between DO and the other parameters, nor when a correlation would have been expected to be detected, such as DO-temperature (negative correlation), DO-pH (positive correlation), and DO-turbidity (negative correlation). This indicated that the levels of oxygen in the channel, as well as the other physico-chemical parameters, were probably influenced by other variables, or a combination of variables for which control and detection is difficult. However, the DO showed the highest number of correlations with the pH, even if the relationships were not identified at all the stations throughout the period of study. The presence of both low dissolved oxygen and low pH levels could be due to two factors: 1) lack of photosynthetic activity, which decreases the DO concentration and increases the CO_2, increasing the pH level; 2) presence of nitrifying bacteria, which decrease the pH and consume DO during the nitrification process. Higher levels of DO were detected when the river flow was greater than usual and the temperature was low, suggesting a likely dilution effect. An increase of 20,000 m^3/s of the STW discharge did not show any negative effect on the DO concentration, probably because the increasing in the discharge flow was not significant compared to the normal discharge. However, the DO level decreased during Spring 2012, a period of heavy rainfall, but data regarding the STW discharge were not available for this season so it was not possible to determine any influence of it on water quality. The higher levels of temperature, total ammonia and conductivity, combined with the lower levels of pH at Pymmes Brook compared to the other stations, suggested a possible influence of the Deephams STW discharge. Finally, the “poor” quality of the Lea Navigation water downstream of Pymmes Brook was confirmed also by chemical and biological data

obtained from the Environment Agency for the period 2005-2009, which showed this area as an impacted ecosystem.

In conclusion, data showed problematic dissolved oxygen levels at Pymmes Brook, Lea Navigation at Springfield Park and Lea Navigation at Lea Bridge. However, there were no apparent correlations between DO and the other physico-chemical parameters. Since data in the Lea Navigation downstream of Pymmes Brook were collected from only two monitoring stations, more detailed surveys were needed and they were performed by collecting data *in situ* with a multiparametric probe.

7.2.2 Water quality monitoring with algal growth inhibition test and chemical analysis

Ecotoxicological bioassays, such as algal growth inhibition tests, have been demonstrated to be effective tools to investigate the pollution in freshwaters, even if the nature and the concentration of contaminants are not known (Struijs *et al*. 2010). In this project, *Pseudokirchneriella subcapitata* assessments were performed to investigate the water quality in the Lea Navigation stretch downstream of Pymmes Brook. The tests were conducted following the Organization for Economic Co-operation and Development (OECD 2006) guidelines and Environment Agency (2008b), with water samples collected over two-year period (September 2009 – July 2012). The control was water collected from Lea Navigation upstream of Pymmes Brook confluence (at Tottenham Hale), which is separated from the water below by locks, since algae cultured in this water showed little or no inhibition compared to the stations located downstream of Pymmes Brook confluence. Alongside these biological assays, chemical analyses were arranged with the EA's laboratories in order to further investigate the concentration and the nature of pollutants in the river samples, and to detect the likely cause of the *P. subcapitata* growth inhibition. Sample collection was always carried out on a Monday to have similar discharge levels from Deephams STW on each sampling, since Environment Agency information indicated that during the weekends the discharge rate was higher than on weekdays.

Results from both algal and chemical assessments showed evidence of a possible contribution of Pymmes Brook waters to the poor water quality in the Lea Navigation stretch under study. The levels of inhibition detected at the same monitoring sites showed differences even in samplings conducted during consecutive weeks, demonstrating periodical variations in the level of pollutants. However, seasonal trends of the algal inhibition were not identified, neither during the summers when it would be expected to detect higher inhibition levels due to higher pollutant concentrations in the river water since the river water presents a low flow rate and it is mostly composed of sewage treatment work effluent. Further assays, performed with polar and non-polar fractions of

the river water samples, showed that *P. subcapitata* growth was mainly affected by a polar contaminant(s). Both algal and chemical analyses gave evidence that Stonebridge Brook was a likely uncontrolled source of pollution, which should be further monitored. In particular, high levels of faecal bacteria and caffeine were detected at this site, suggesting the presence of untreated wastewater, possibly either from combined sewer overflows (CSOs) or misconnections or both. Algal bioassays also showed inhibition with water samples collected further downstream of Pymmes Brook, at Lea Bridge weir (Lea Navigation) and at Hackney Marshes (River Lea, downstream of the weir). However the concentrations of both faecal bacteria and caffeine were lower than at the monitoring stations located upstream of the weir, suggesting either lower human and animal sewage discharge load or dilution effects. Finally *P. subcapitata* growth assays showed highest levels of inhibition after 24 hours of exposure, followed by algal population recovery, indicating lower concentrations of bioavailable pollutants in the test system due to algae uptake, sorption, volatilization and degradation (Simpson *et al.* 2003). For this reason *in situ* monitoring by entrapped algae in alginate beads was performed.

In conclusion, data showed clear evidence of algal growth inhibition at Pymmes Brook and downstream of its confluence. Deephams STW (through Pymmes Brook) was shown to be a cause of the algal inhibition, but results also identified Stonebridge as another pollution source. There was evidence that the pollution was chronic and it was mainly due to the polar fraction of the river water.

7.2.3 Water quality monitoring with CellSense whole cell biosensors

Since bioassays, such as algal growth inhibition tests, take several days and require large laboratory facilities, the river water quality was also investigated using algal and bacterial CellSense biosensors (Rawson 1987 - UK and European Patent), which potentially provide a rapid assessment.

P. subcapitata, *S. leopoliensis* and *E. coli* cells were employed as biocatalysts to test the river water toxicity, investigating any change in their metabolic activity (both inhibition and stimulation) due to the exposure to pollutants.

Initial tests were conducted following the standard protocol (protocol 1) presented in literature (Evans *et al*. 1998, Farré *et al*. 2001, Farré and Barceló 2003, Daniel *et al*. 2004), where biosensors were initially bathed in mediator supplemented solutions providing optimal conditions for the normal metabolic activity of the biocatalyst and then exposed to mediator supplemented river water samples. However, since results indicated that the biological responses were masked by (electro)-chemical activity, due to interactions between mediators and compounds dissolved in the river water samples, alternative protocols (protocol 2 and 3) were developed. Nevertheless, there was evidence that the river water polar fraction was affecting the bacterial population, but the

"window" where the toxicity was evident was too small to be able to make any valid comparisons, because of (electro)-chemical interferences. Therefore, protocol 2 was designed, where biosensors were exposed to river water samples without chemical mediator supplement. Before and after the exposure to river water, biosensors were bathed in the optimal solution: any change in the outcome signal either between the pre- and the post- exposure stage or between the samples in the post-exposure phase, would reflect the impact of the exposure to river water. Results did not show any changes after an exposure time of 30 minutes, suggesting chronic pollution. Tests with longer exposure time (2 and 24 hours) showed stimulation for those algal biosensors exposed to river water samples collected at Pymmes Brook and downstream compared to those exposed to both Tottenham Hale water and OECD growth medium, which could indicate either low toxicant concentrations or high level of nutrients in the river water (Farré *et al*. 2001). Protocol 3 was developed alongside protocol 2 and it involved the use of a lower poised potential (+200 mV) to avoid (electro)-chemical interferences and to monitor only the electron transfers due to redox reactions promoted by the mediator. The procedure used in protocol 3 was the same as in protocol 1. However, the reproducibility of tests conducted with untreated water was problematic. Better results were obtained by pre-concentrating the river water by rotary evaporation and re-suspending the solutes in methanol, which was a further indication that the main cause of inhibition was due to the polar water fraction.
Whilst algal biosensors proved to be problematic since the signal was very low and noisy, the results achieved with *E. coli* biosensors were more promising and indicated that protocol 3 had the potential to be a useful tool to detect the toxicity of contaminants soluble in methanol. However further studies need to be performed.

In conclusion, data showed chronic pollution. Rapid biosensor assays were problematic in this project due to (electro)-chemical interactions in the river water samples. However, the problem was avoided by working at a lower potential and pre-concentrating the river water. Results showed inhibition at Pymmes Brook and downstream of its confluence, indicating Stonebridge Brook as the most polluted site between sampling stations. The water toxicity was evident at the 1:1 river water concentration, probably because the use of methanol to dissolve river water solutes avoided the toxicity of the river water being masked or neutralised by "beneficial" substances (such as inorganic compounds) for the biocatalyst, indicating also the major role of polar compounds in the water toxicity.

7.2.4 *In situ* river water quality monitoring

Laboratory bioassays are useful tools to detect chemical pollution, but at the same time, they have some restrictions, such as the possibility that pollutants are lost from the system due to algae uptake, sorption, volatilization and degradation (Simpson *et al.* 2003). In

order to avoid this problem, *P. subcapitata* cells entrapped in alginate beads have been employed *in situ* to monitor the freshwater quality. Physico-chemical parameters were also collected *in situ* with a multiparametric probe, in order to supplement data provided by the Environment Agency and monitor each potential pollution source in the Lea Navigation. Physico-chemical data were collected seasonally throughout one year (summer 2011-spring 2012).

Both of the assays showed that Pymmes Brook negatively affected the Lea Navigation water quality. In particular, the physico-chemical data showed lower dissolved oxygen levels, higher temperature, lower pH levels, high conductivity and higher total ammonia at Pymmes Brook and downstream of its confluence compared to Tottenham Hale and Old River Lea stations. However, there was evidence that Pymmes Brook was not the only source of pollution. Measurements of dissolved oxygen levels indicated Stonebridge Brook and Old Moselle Brook as likely sources of pollution that in future needed monitoring, as well as other unidentified sources in the area of Lea Bridge weir (probably diffuse pollution by surface runoff or misconnections), where also the pH levels decreased compared to the upstream area. In the River Lea (Hackney Marshes), downstream of the weir, a better level of dissolved oxygen was recorded, which could be due to either a greater water aeration due to both the water mixing at the weir and the waters being shallow and turbulent, or the influence of vegetation on contaminant depuration.

The *in situ* water quality monitoring with *P. subcapitata* cells entrapped in alginate beads was problematic, due to the dissolution of the alginate matrix possibly due to the presence of chelants (such as phosphate, surfactants and citrate) in the river water, which can replace the Ca^{2+} ions used to harden the sodium alginate barrier. However there was evidence of stimulation in the monitoring sites at Pymmes Brook and downstream of its confluence, indicating a possible change in the nutrient levels.

In conclusion, data showed evidence of the influence of Deephams STW (through Pymmes Brook) on the water quality of the Lea Navigation. However, results indicated also Stonebridge Brook and Old Moselle Brook as potential sources of uncontrolled pollution.

The use of algae entrapped in alginate beads is a promising method in monitoring the river water quality, but it is still challenging since the stability of the alginate matrix needs to be tested as well as the better positioning along the water column and along the river bank needs to be determined.

7.3 Final conclusions

This study investigated the water quality of a stretch of the Lea Navigation in the NE London with different techniques, in order to detect likely pollution sources and causes of the chronic low level of dissolved oxygen. Literature reviews indicated that one of the major pollution concerns in this part of the channel was the presence of the Deephams sewage treatment works, which discharges its final effluent in Pymmes Brook (through Salmon Brook) a tributary of the Lea Navigation. In this project, river water investigations were carried out for two years between 2010 and 2012, and consisted of the combination of standard assays (algal growth inhibition tests, chemical analysis, and collection and processing of physico-chemical parameters) with novel approaches (whole cell biosensor tests and *in situ* monitoring with *P. subcapitata* cells entrapped in alginate beads).

The present project suggested a reliable and low cost multi-parameter approach for the monitoring of freshwater bodies, both urban and natural, with respect to the Water Framework Directive (WFD). A successful monitoring program should start with the detailed investigation of the area by collecting the physico-chemical parameters, which could reveal the sites more polluted where to focus the analysis. For instance, in this study, the collection of the physico-chemical parameters *in situ* identified Stonebridge Brook as source of pollution, which was not detectable by the automated monitoring station of the Environment Agency. Moreover, the visualisation of the results with coloured maps allowed an "easy-to-read" presentation of the changes of the river water quality over the space and over the time.

Following that, the use of bioassays have shown to be a good method to investigate the toxicity of the river water when the cause of pollution is un-known, without resorting to expensive chemical analysis. In this project, algal assays were chosen to investigate the Lea water quality, mainly because algae are the primary producers, very sensitive to modification in their environments, and because there was evidence that the key problem in this part of the channel was the presence of chronic low dissolved oxygen levels. The results of the bioassays can give an indication of the possible sources of pollution. However, the standard algal bioassays took three days to complete, and they demonstrated to be inadequate for monitoring chronic pollution, since the concentration of contaminants decreased by the end of the test.

The present study developed a novel method to investigate the river water quality by employing CellSense whole cell biosensors. This novel technique was very important in the light of the WFD for two main reasons: 1) biosensors have been defined as "green methods", due to the small amount of samples and solvent that they use; and 2) the method elaborated here is a more rapid bioassay than the standard algal tests, since it gave results in about 30 minutes. The speed of the test is an important feature to fulfil the WFD, because of the necessity to monitor and keep monitored all the water bodies of Europe. Moreover, whole cell biosensors could be loaded with different organisms, and

they could potentially become a tool to use in the field. The novel *E. coli* biosensor protocol to monitor the quality of freshwater allowed the detection of toxicity due to polar compounds, avoiding the (electro)-chemical interferences, which were detected as problems in the study of Farré and Barceló (2003). Thanks to the pre-treatment of river water samples by concentrating the contaminants, this original protocol allowed the monitoring of chronic pollution due to pollutants present at low concentrations.

An overall monitoring of the river water quality should include a biological assay conducted *in situ*, in order to examine the toxicity in the real environment with all the variables involved. In this study, *P. subcapitata* cells entrapped in sodium alginate were employed to monitor the water quality of the Lea Navigation, giving an example of the use of this technique for investigations of the quality of a water body downstream of a sewage treatment work effluent.

The location of the algae directly in the channel allowed the possibility to expose the test organism to the pollutants constantly, avoiding the "loss" of contaminants, which happened with algal tests in the laboratory. Unfortunately, the use of algal alginate beads did not give the most useful of results when applied to the Lea channel because of the disruption of the alginate matrix, probably due to chelants present in the river water. However, the performance of *in situ* bioassays is recommended alongside the performance of tests in the laboratory, in order to provide results as close as possible to the real situation in the aquatic environment under investigation. Moreover, it will be useful in the future to develop a successful *in situ* technique to monitor urban channels like the Lea Navigation, by using the algae, organisms sensitive to pollutants to provide information on river water quality.

From the present study, it emerged that chemical analyses are not best suited for a primary role in the monitoring of the toxicity of the river water. Such analyses should be conducted after the investigation of the water by collecting physico-chemical parameters and by performing bioassays, which are low-cost methodologies. Chemical assays are expensive and they do not always give useful information, as demonstrated by the analyses carried out with Lea water. Specific investigations of the chemistry of the water should be carried out when there is evidence of pollution especially when a particular pollutant is suspected. In the specific case of the Lea channel, chemical analyses did not detect any pollutant at high concentrations, but a range of compounds were present at environmental levels, confirming the fact that often the urban pollution is chronic and possibly due to interaction between chemicals. Laboratory analyses were useful in tracking the levels of coliforms, which combined with the levels of caffeine allowed to identify Stonebridge Brook as source of untreated sewage.

In conclusion, in the current research the "poor" water quality of the Lea Navigation between Pymmes Brook and Lea Bridge weir was confirmed. The major sources of pollution were identified in Pymmes Brook, which receives the final effluent of Deephams

STW, and Stonebridge Brook, which is source of untreated sewage. The conclusions were that the low dissolved oxygen levels were likely due to both oxygen consuming by large bacteria populations, and inhibition of the photosynthetic activity because of polar pollutants in the river water.

7.4 Achievement of the aims

The first aim of this project was to detect the likely causes and sources of the poor water quality in the Lea Navigation. Results discussed above showed chronic pollution, identifying polar compounds dissolved in the river water and high bacterial concentrations as possible causes of low dissolved oxygen levels. This study confirmed the negative effect of Deephams STW (throughout Pymmes Brook) on the water quality of the Lea Navigation. However, there was evidence of other sources of pollution in particular Stonebridge Brook was identified as uncontrolled source of pollution and untreated wastewater. Other possible sources include Old Moselle Brook and diffuse pollution from runoff, boats discharges or other likely undetected misconnections.

The second aim was to provide a case study for the investigation of the river water quality using a multi-parameter approach, involving both standard and original techniques. This project showed the important role of both physico-chemical data to locate the polluted sites, and bioassays in the examination of pollutant toxicity, when the cause of the pollution is unknown. In particular, a novel protocol was developed, which involved the use of CellSense *E. coli* biosensors, a bioassay rapid and easy to perform, useful in the light of the WFD. The chemical analyses confirmed the presence of chronic pollution, but they did not provide useful information about the polar compounds in the samples, underling their secondary role in the river water monitoring. Finally, a comprehensive monitoring plan should include an *in situ* biological assay in order to provide results as close as possible to the real situation in the aquatic environment under study.

7.5 Originality of the approach

The originality of this research consisted mainly in:

- The use of a multi-parameter approach, which resulted in:
    - Identification of the likely cause of low dissolved oxygen in this part of the Lea Navigation: a combination of polar pollutants and bacteria.
    - Detection of Stonebridge Brook as uncontrolled source of pollution and untreated wastewater.
    - The assessment of a bioassay monitoring approach, involving algal growth inhibition testing, to monitor water quality over a two-year period. Such a study had not previous been used on this stretch of the Lea Navigation.

  - Conduct of *in situ* monitoring of the water quality with *P. subcapitata* cells entrapped in sodium alginate beads.
- The development of a novel protocol to test the toxicity of polar compounds dissolved in the river water and soluble into methanol with a rapid *E. coli* CellSense biosensor assessment.

7.6 Future work

This project has highlighted several areas for future research and implementation:

- Implementation of a protocol to monitor river water with *E. coli* CellSense biosensors. During this research, a protocol was developed to monitor the toxicity of polar compounds present in the river water by using *E. coli* CellSense biosensors. There was evidence that this protocol was detecting the toxicity, but still need to be improved with further investigations.
- Monitoring of Stonebridge Brook. Results clearly showed that Stonebridge Brook is a source of uncontrolled pollution and untreated wastewater. Therefore, it is strongly recommended to monitor it, investigating possible source(s) of pollution further upstream of its confluence with the Lea Navigation.
- Selected chemical analyses to investigate polar compounds in the Lea channel. Bioassays are useful tools to investigate the river water quality, but they need to be associated to chemical analyses to have a better understanding of the nature of the pollution present. In this project, only few chemical analyses were conducted. Since there was evidence that a polar compound was affecting the algal population, it would be useful to have detailed quantitative and qualitative data on the polar compounds present in order to determine the likely cause of the low photosynthetic activity and then of low dissolved oxygen levels in this part of the Lea Navigation.
- Implementation of *in situ* monitoring with algae entrapped in alginate beads to monitor the algal growth inhibition by exposing the organisms to the river water *in situ*. In this project, as in other published research, difficulties were faced with respect to matrix integrity disruption by chelant presence in the river water. Future research should be focused on the investigation of alternative methods to entrap the algal cells to expose to urban river water.
- It is suggested that possible interventions to decrease the bacterial levels in the Lea are considered, since bacteriological analyses showed high concentrations of coliforms in the Lea Navigation downstream of Pymmes Brook, which are a possible cause of dissolved oxygen depletion. Since there is evidence that the concentration of both total and faecal coliforms decrease in presence of aquatic plants (Decamp and Warren 2000, Karim *et al.* 2008), it is suggested that

advantage is taken (whenever possible) of establishing natural marshes between Springfield Park and Lea Bridge, and to divert part of the Lea channel water via a more natural course with vegetation, in order to further decrease the level of bacteria in the water and improve the dissolved oxygen levels. It would be useful to further investigate these solutions, looking at autochthon plants to depurate the water, especially to create a natural area below Springfield Park, where natural marshes already exist. High bacteria levels were identified in Pymmes Brook, suggesting a likely input of the coliforms from Deephams STW, which at the moment consists of primary sedimentation, 13 hours of activated sludge treatment, final settlement tanks and discharging in Salmon Brook (Ellis 2006). It is suggested that improvements to the STW are made in order to reduce the amount of coliforms in the final effluent.

8 References

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Appendix I: Preparation of BG11 growth medium for *Synechococcus leopoliensis* culturing

To culture *S. leopoliensis,* BG11 (Blue-Green Medium) growth medium was prepared following the guidelines given by Rippka *et al.* (1979). The final medium was prepared as showed in Table I.1 and it was autoclaved.

Table I.1 – Composition of BG11 growth medium for *S. leopoliensis* culturing.

Ingredient	Amount (g/l) in medium
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA	0.001
Na_2CO_3	0.02
Trace metal mix	1 ml/l
Deionised water	1000 ml

Table I.2 – Trace metal mix for the preparation of BG11 growth medium for *S. leopoliensis* culturing.

Ingredient	Amount (g/l)
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.222
$NaMoO_4 \cdot 2H_2O$	0.390
$CuSO_4 \cdot 5H_2O$	0.079
$Co(NO_3)_2 \cdot 6H_2O$	0.0494

Appendix II: Preparation of 3N-BBM+V growth medium for *Pseudokirchneriella subcapitata* culturing

To culture *P. subcapitata,* 3N-BBM+V (Bold Basal Medium with 3 fold Nitrogen and Vitamins; modified) growth medium was prepared following the guidelines given by Culture Collection of Algae and Protozoa (CCAP, http://www.ccap.ac.uk/media/documents/3N_BBM_V_000.pdf). The final medium was prepared as showed in table II.1 and it was autoclaved 15 psi for 15 minutes.

Table II.1 – Composition of 3N-BBM+V growth medium for *P. subcapitata* culturing.

Stock solution in g/1000 ml water	For 1 litre final medium
25.0 g $NaNO_3$	30 ml
2.5 g $CaCl_2 \cdot 2H_2O$	10 ml
7.5 g $MgSO_4 \cdot 7H_2O$	10 ml
7.5 g $K_2HPO_4 \cdot 3H_2O$	10 ml
17.5 g KH_2PO_4	10 ml
2.5 g NaCl	10 ml
trace element solution	6 ml
vitamin B1	1 ml
vitamin B12	1 ml
Deionised water	912 ml

Table II.2 – Trace metal mix for the preparation of 3N-BBM+V growth medium for *P. subcapitata* culturing.

Ingredient	Amount for 1 litre solution
Na_2 EDTA	0.75 g
$FeCl_3 \cdot 6H_2O$	97.0 mg
$MnCl_2 \cdot 4H_2O$	41.0 mg
$ZnCl_2$	5.0 mg
$CoCl_2 \cdot 6H_2O$	2.0 mg
$Na_2MoO_4 \cdot 2H_2O$	4.0 mg
$FeCl_3 \cdot 6H_2O$	97.0 mg

For the preparation of the vitamin B1, 0.12 g of Thiaminhydrochloride was dissolved in 100 ml of distilled water. The solution was sterilely filtered.

For the preparation of the vitamin B12, 0.1 g of Cyanocobalamin was dissolved in 100 ml of distilled water. From this solution, 1 ml was taken and it was added to 99 ml of distilled water. The solution was sterilely filtered.

Appendix III: Preparation of OECD growth medium for *Pseudokirchneriella subcapitata* culturing

OECD (Organisation for Economic Co-operation and Development) growth medium was prepared following the OECD guidelines (2006).

Table III.1 – Stock solution 1 (macro nutrients) for the preparation of OECD growth medium for *P. subcapitata* culturing.

Ingredient	Amount (g/l)
NH_4Cl	1.5
$MgCl_2 \cdot 6H_2O$	1.2
$CaCl_2 \cdot 2H_2O$	1.8
$MgSO_4 \cdot 7H_2O$	1.5
KH_2PO_4	0.16

Table III.2 – Stock solution 2 (iron) for the preparation of OECD growth medium for *P. subcapitata* culturing.

Ingredient	Amount (mg/l)
$FeCl_3 \cdot 6H_2O$	64
$Na_2EDTA \cdot 2H_2O$	100

Table III.3 – Stock solution 3 (trace elements) for the preparation of OECD growth medium for *P. subcapitata* culturing.

Ingredient	Amount (mg/l)
H_3BO_3	185
$MnCl_2 \cdot 4H_2O$	415
$ZnCl_2$	3
$CoCl_2 \cdot 6H_2O$	1.5
$CuCl_2 \cdot 2H_2O$	0.01
$Na2MoO_4 \cdot 2H_2O$	7

Table III.4 – Stock solution 4 (bicarbonate) for the preparation of OECD growth medium for *P. subcapitata* culturing.

Ingredient	Amount (g/l)
$NaHCO_3$	50

The stock solutions number 1 and 3 were sterilized by autoclaving (120 °C, 15 min). The stock solutions number 2 and 4 were sterilized by membrane filtration (mean pore diameter 0.2 μm). All the four stock solutions were stored in the dark at 4 °C.
The final medium was prepared as indicated in Table III.5.

Table III.5 – Preparation of the final OECD growth medium for *P. subcapitata* culturing.

Ingredient	Amount (ml)
Stock solution 1	10
Stock solution 2	1
Stock solution 3	1
Stock solution 4	1
Deionised water	987

Appendix IV: Experimental equation for the conversion of algal optical density to algal cells concentration

The count of algal cells in 1 ml of culture was performed with a Bürker chamber, following the standard operating procedure given by Biomatica© (win.biomatica.it/Public/SOA/CellCount_SOP.pdf).

Table IV.1 – Algal optical density (550 nm) and corresponding algal cell concentration (cells * 10^4/ml). The number of algal cells was counted by Bürker chamber at each optical density.

Algal optical density (550 nm)	Algal cell concentration (cells*10^4/ml)
1.007	324
0.544	134
0.439	112
0.229	58.7
0.118	32.4
0.062	13.4
0.050	11.2
0.019	5.7

The algal cells concentration, corresponding to 1.007 OD_{550} was considered an outlier, so it was not considered in the calculation of the equation. A possible explanation for the presence of the outliner could be operator error during the counting of the cells, since the number of cells corresponding to 1.007 OD550 was high.

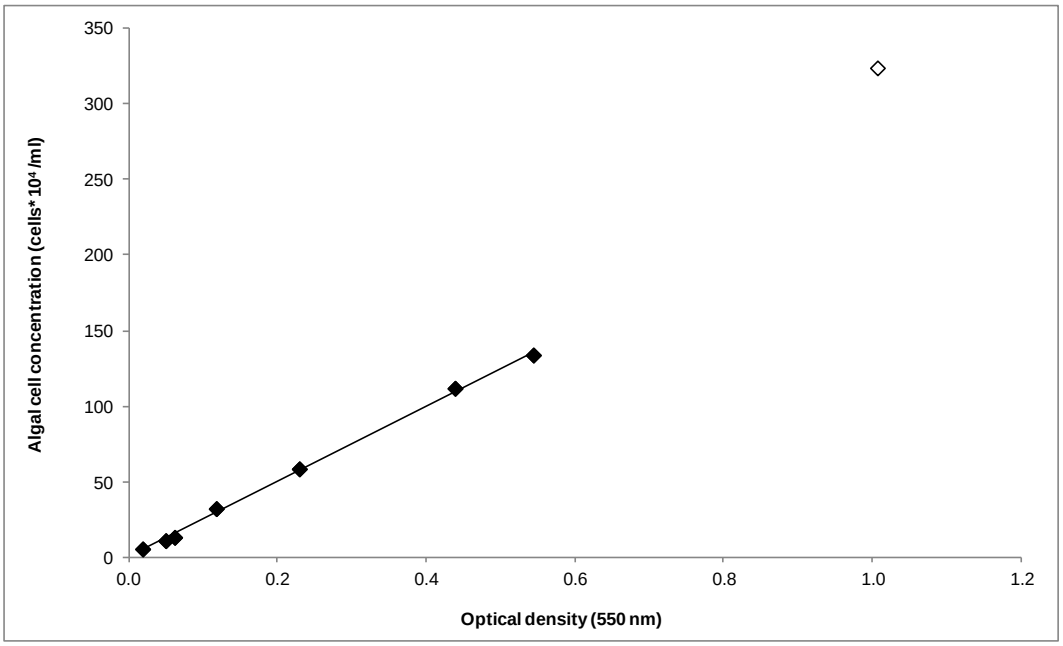

Figure IV.1 – Algal optical density (550 nm) plotted against algal cells concentration (cells*10^4/ml). The number of algal cells was counted by Bürker chamber at each optical density. Legend: ◇◆ measured data; ▬ regression line throughout measured data. NOTE. The datum corresponding to the unfilled symbol was not taken into account when calculating the trendline.

After plotting the data on Excel, the equation was obtained by linear least square regression:

$$\text{Cell concentration (cell/ml)} = [(2{,}496{,}759 \pm 44{,}908) * OD_{550}] + (4{,}224 \pm 12{,}718)$$

The r^2 of this equation was 0.9987.

Appendix V: Example of outliers in the analysis of physico-chemical parameters provided by the Environment Agency

The first part of the analysis of the data, recorded by automated monitoring stations of Environment Agency, consisted of removing the outliers and it was done in two steps: 1) data with a value of "mean ± 2 standard deviation" were deleted (Reimann *et al* 2008); then 2) values attributable to malfunctioning of the probe (e.g. zero or negative data) were deleted. Figures V.1 and V.2 show examples of outliers attributable to malfunctioning of the probe.

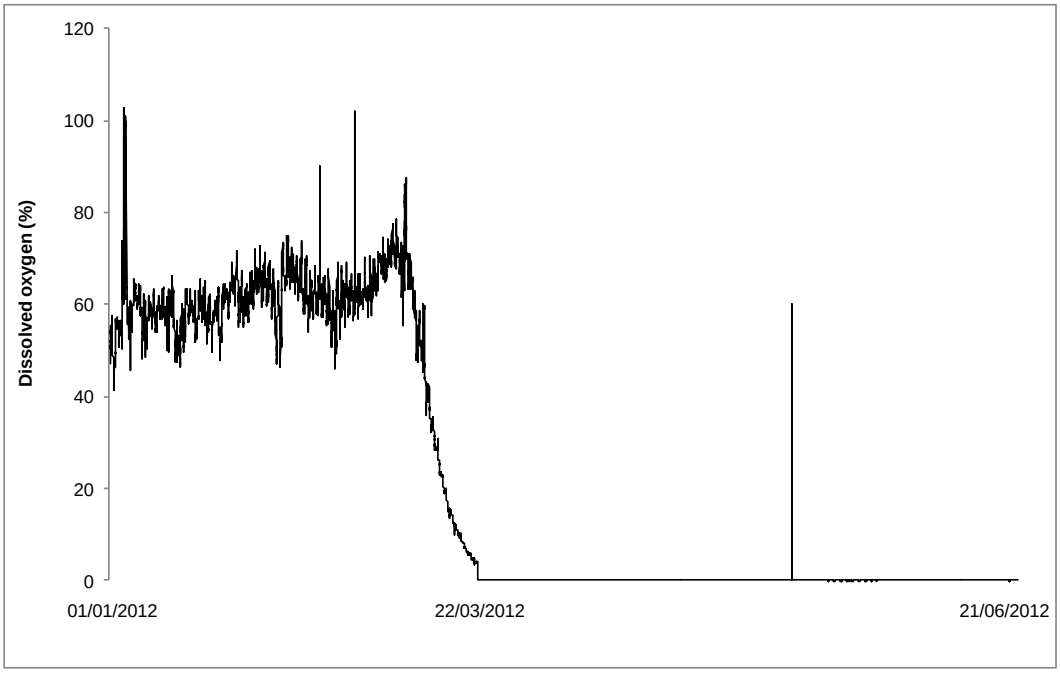

Figure V.1 – Example of outliers for the dissolved oxygen (%) at Deephams from 01/01/2012 to 21/06/2012. During the analysis of physico-chemical data collected from EA automated stations, data were considered outliers if they were ± 2 SD, or if clearly out of the trend for malfunctioning of the probe, as shown in the graph.

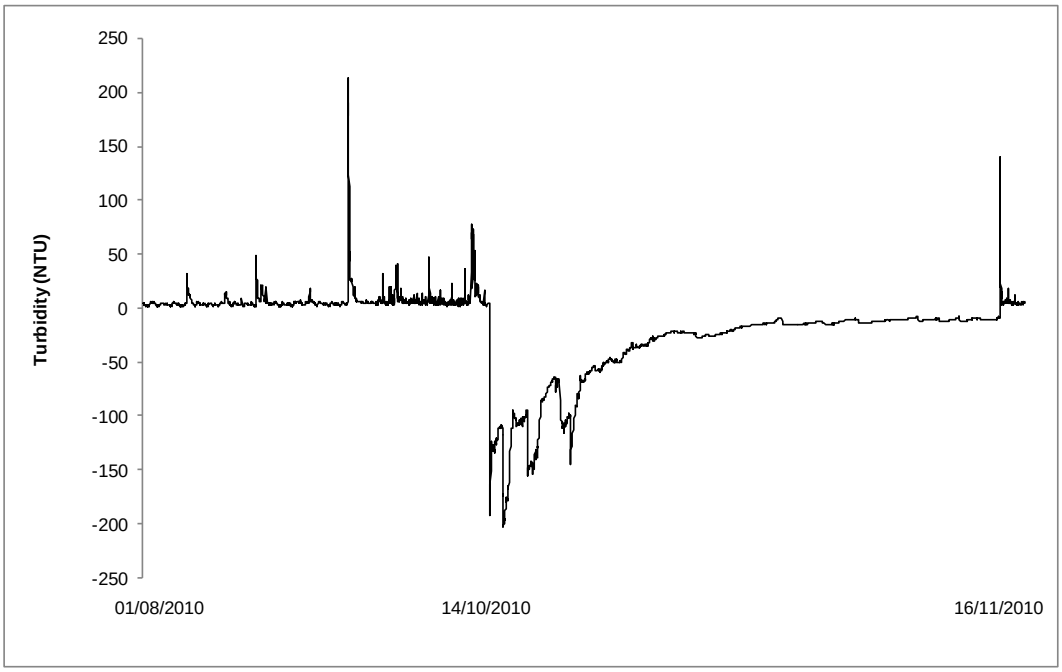

Figure V.2 – Example of outliers for the turbidity (NTU) at Pymmes East from 01/08/2010 to 16/11/2010. During the analysis of physico-chemical data collected from EA automated stations, data were considered outliers if they were ± 2 SD, or if clearly out of the trend for malfunctioning of the probe, as shown in the graph.

Appendix VI: Elaboration of physico-chemical parameters from Environment Agency automatic stations

Physico-chemical data collected from automatic monitoring stations of Environment Agency were processed to have a background of the area under investigation. The data here analysed were registered from 21/06/2010 to 21/06/2012. The following tables show minimum value, mean, maximum value and standard deviation (SD), for each parameter considered: temperature (ºC), dissolved oxygen (% and mg/l), pH, conductivity (µS/cm), total ammonia (mg/l), and turbidity (NTU). The calculations were performed grouping the data by seasons.

Table VI.1 – Minimum value, mean, maximum value and standard deviation (SD) of the temperature (ºC) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	14	19	23	2	1	9	17	4	0	6	10	2	8	15	23	2
Angel	13	18	22	1	2	10	17	4	3	7	10	1	8	14	19	2
Deephams	18	21	23	1	13	17	21	2	9	14	19	1	12	18	23	1
Pymmes W	17	21	23	1	11	15	20	2	9	12	15	1	14	18	22	1
Pymmes E	17	19	21	1	7	15	20	3	6	11	15	1	12	17	21	2
Springfield	17	20	23	1	6	13	19	3	4	9	12	1	10	16	20	2
Lea Bridge	16	20	23	1	6	13	19	3	7	10	13	1	12	17	21	2
Spitalfield	11	19	23	2	2	12	18	3	4	8	12	2	8	16	23	3
Carpenters	15	20	24	2	-3	13	19	3	0	8	12	2	10	16	22	2

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	15	18	23	2	4	11	23	4	1	7	12	2	8	13	23	3
Angel	14	17	21	1	6	12	17	3	2	7	11	2	6	12	19	3
Deephams	16	21	23	1	11	18	22	2	10	15	16	1	12	17	21	1
Pymmes W	16	20	23	1	9	17	22	3	10	14	16	1	10	16	22	2
Pymmes E	15	19	22	1	9	17	22	2	8	12	15	1	9	15	21	2
Springfield	17	19	22	1	9	15	20	3	1	10	13	2	10	14	20	2
Lea Bridge	17	20	23	1	8	15	20	3	6	11	13	2	10	15	21	2
Spitalfield	14	18	23	2	1	13	22	4	0	8	16	3	8	14	21	3
Carpenters	15	19	24	1	NA	NA	NA	NA	5	10	14	2	10	15	24	3

Table VI.2 – Minimum value, mean, maximum value and standard deviation (SD) of the dissolved oxygen (%) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	57	107	186	33	35	100	168	12	95	103	162	9	58	117	186	31
Angel	1	34	111	29	19	58	93	13	26	66	110	17	0	44	111	25
Deephams	2	47	84	8	4	55	80	9	21	54	93	7	8	52	94	8
Pymmes W	6	54	96	18	0	35	85	14	0	42	91	17	0	31	96	25
Pymmes E	0	35	75	19	0	24	75	17	0	25	75	19	0	25	75	20
Springfield	2	39	87	19	8	45	81	12	37	70	93	10	0	36	93	24
Lea Bridge	2	29	64	11	3	36	68	10	14	53	69	9	1	29	69	12
Spitalfield	17	60	102	13	36	66	92	8	60	85	102	10	31	61	102	12
Carpenters	6	55	110	24	19	63	107	14	42	82	110	10	1	58	110	22

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	47	107	186	36	69	113	186	22	79	104	184	15	51	106	186	27
Angel	14	50	111	20	10	42	90	15	12	58	108	16	6	62	111	25
Deephams	4	61	94	16	0	42	94	24	3	55	94	16	NA	NA	NA	NA
Pymmes W	0	41	96	21	0	47	96	14	1	43	96	20	0	37	96	26
Pymmes E	0	32	75	19	0	28	75	16	0	15	75	19	0	23	75	22
Springfield	0	32	91	14	8	30	90	14	8	61	92	12	5	53	93	17
Lea Bridge	1	21	66	7	8	29	69	12	4	46	69	11	1	41	69	15
Spitalfield	7	45	101	13	32	58	95	11	56	87	102	9	27	65	102	16
Carpenters	1	42	110	17	NA	NA	NA	NA	23	72	110	10	3	69	110	19

Table VI.3 – Minimum value, mean, maximum value and standard deviation (SD) of the dissolved oxygen (mg/l) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	5.00	11.05	23.68	4.08	3.63	11.65	17.83	1.75	11.21	12.69	18.23	0.97	5.54	12.14	23.50	3.36
Angel	0.05	3.38	11.93	2.91	1.84	6.57	11.41	1.64	2.97	7.81	12.00	1.87	0.00	4.37	11.84	2.74
Deephams	0.14	4.17	7.95	0.76	0.39	5.37	8.21	1.04	2.20	5.58	9.05	0.70	0.73	4.87	9.06	0.75
Pymmes W	0.56	4.99	8.97	1.74	0.03	3.50	8.38	1.43	0.00	4.42	8.97	1.85	0.00	2.42	8.97	2.44
Pymmes E	0.03	3.36	7.35	1.87	0.02	2.41	7.28	1.69	0.01	2.50	7.34	1.95	0.00	2.38	7.35	2.02
Springfield	0.15	3.57	7.45	1.74	0.80	4.76	8.77	1.58	4.05	7.93	10.30	1.18	0.00	3.21	10.30	2.75
Lea Bridge	0.22	2.63	5.92	1.07	0.28	3.84	7.55	1.30	1.56	5.84	7.61	0.97	0.11	2.87	6.63	1.19
Spitalfield	1.52	5.51	10.02	1.30	3.53	7.20	11.91	1.22	6.67	9.91	11.94	1.30	3.06	6.08	10.89	1.31
Carpenters	0.56	5.13	11.98	2.29	1.88	6.65	12.00	1.79	4.88	9.58	12.00	1.15	0.05	6.00	12.00	2.43

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	4.25	11.32	24.26	4.38	7.56	13.27	24.26	3.26	9.87	12.76	21.00	1.49	5.48	11.46	24.25	3.37
Angel	1.36	4.98	12.00	2.08	1.02	4.57	10.24	1.73	1.32	6.96	11.99	1.85	0.64	6.70	11.95	2.67
Deephams	0.32	5.45	9.06	1.42	0.00	3.89	8.97	2.37	0.33	5.60	8.99	1.59	NA	NA	NA	NA
Pymmes W	0.00	3.38	8.94	2.04	0.01	4.58	8.92	1.39	0.06	4.45	8.76	2.12	0.00	3.47	8.97	2.55
Pymmes E	0.00	2.66	7.35	2.06	0.00	2.68	7.33	1.59	0.00	1.43	7.34	1.98	0.00	2.32	7.35	2.20
Springfield	0.00	2.86	9.33	1.39	0.71	3.08	10.06	1.61	1.07	6.78	10.30	1.44	0.51	5.42	10.30	1.76
Lea Bridge	0.14	1.90	7.40	0.71	0.73	2.94	7.59	1.41	0.42	4.98	7.61	1.27	0.13	4.25	7.61	1.61
Spitalfield	0.68	4.30	9.54	1.20	3.32	6.26	11.79	1.62	6.45	9.83	11.94	1.23	2.77	6.86	11.94	1.92
Carpenters	0.11	3.90	11.54	1.56	NA	NA	NA	NA	2.58	8.17	11.96	1.25	0.34	7.16	12.00	2.07

Table VI.4 – Minimum value, mean, maximum value and standard deviation (SD) of the pH calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	7.80	8.25	8.77	0.25	7.83	8.16	8.61	0.11	7.83	8.15	8.71	0.14	7.84	8.36	8.77	0.23
Angel	7.19	7.75	8.81	0.25	7.41	7.74	8.01	0.10	7.54	7.82	8.01	0.07	7.33	7.77	8.33	0.17
Deephams	6.83	7.17	7.43	0.10	6.82	7.07	7.34	0.09	6.82	7.16	7.43	0.09	6.69	7.13	7.43	0.09
Pymmes W	7.00	7.35	8.48	0.16	6.91	7.13	7.49	0.09	6.83	7.10	7.46	0.09	6.86	7.13	7.56	0.13
Pymmes E	7.12	7.26	7.39	0.07	6.98	7.20	7.39	0.08	7.03	7.21	7.39	0.07	6.93	7.21	7.39	0.09
Springfield	7.17	7.49	7.74	0.13	7.07	7.39	7.74	0.14	6.30	7.49	7.74	0.11	6.82	7.32	7.74	0.16
Lea Bridge	7.20	7.43	7.60	0.09	7.11	7.40	7.60	0.11	6.93	7.29	7.60	0.16	7.04	7.32	7.53	0.08
Spitalfield	6.87	7.58	7.85	0.12	7.06	7.53	7.85	0.12	7.06	7.63	7.85	0.13	7.05	7.47	7.85	0.11
Carpenters	7.07	7.48	8.00	0.15	6.67	7.58	7.97	0.13	6.75	7.62	8.00	0.14	7.06	7.45	7.99	0.15

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	7.73	8.18	8.77	0.30	7.51	8.05	8.77	0.21	7.75	8.17	8.77	0.16	7.92	8.27	8.77	0.23
Angel	7.18	7.66	8.50	0.19	7.18	7.67	7.95	0.09	7.19	7.91	8.45	0.17	6.36	7.62	8.56	0.34
Deephams	6.91	7.15	7.41	0.08	6.90	7.21	7.43	0.11	6.90	7.15	7.40	0.09	6.82	7.06	7.38	0.09
Pymmes W	6.89	7.19	7.48	0.11	6.76	7.21	7.74	0.12	6.85	7.09	7.56	0.11	6.90	7.11	7.53	0.09
Pymmes E	6.93	7.19	7.39	0.09	6.99	7.19	7.39	0.08	6.93	7.15	7.39	0.08	6.88	7.17	7.39	0.11
Springfield	6.86	7.26	7.63	0.13	7.20	7.38	7.66	0.08	0.11	7.42	7.17	7.74	6.92	7.34	7.74	0.14
Lea Bridge	6.96	7.27	7.56	0.11	7.10	7.32	7.51	0.08	6.97	7.28	7.59	0.11	7.02	7.39	7.60	0.10
Spitalfield	6.68	7.32	7.74	0.15	7.15	7.47	7.70	0.08	7.13	7.47	7.85	0.15	6.96	7.43	7.85	0.18
Carpenters	6.95	7.28	7.99	0.10	NA	NA	NA	NA	7.16	7.45	7.99	0.10	6.08	7.48	8.00	0.18

Table VI.5 – Minimum value, mean, maximum value and standard deviation (SD) of the conductivity (µS/cm) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	426	819	915	61	643	855	1037	80	630	841	1130	84	752	855	949	32
Angel	135	798	1057	153	112	815	1416	200	247	936	1416	157	167	894	1107	143
Deephams	730	1136	1266	83	668	1128	1333	100	787	1162	1425	89	490	1153	1237	75
Pymmes W	192	1115	1346	92	211	1118	1346	96	104	1099	1346	108	405	1133	1342	91
Pymmes E	684	1072	1241	71	168	983	1379	168	197	930	1379	220	227	1078	1377	133
Springfield	274	981	1115	109	219	974	1325	116	508	973	1346	112	411	999	1136	95
Lea Bridge	303	974	1109	104	242	966	1205	112	515	966	1210	95	400	989	1106	93
Spitalfield	288	954	1110	122	234	926	1216	119	530	926	1096	102	416	983	1122	106
Carpenters	341	969	1154	109	247	952	1246	115	564	988	1247	108	522	996	1216	100

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	705	829	960	47	817	946	1095	45	813	975	1130	63	561	801	1012	98
Angel	127	760	1013	175	179	849	1358	114	256	990	1417	152	209	784	1380	353
Deephams	625	1098	1194	66	444	1161	1235	70	486	1175	1375	68	661	1092	1254	114
Pymmes W	307	1087	1326	103	448	1162	1300	83	564	1151	1346	80	384	1073	1346	150
Pymmes E	214	977	1246	161	251	1063	1218	167	315	1072	1379	124	326	985	1225	167
Springfield	334	942	1085	104	455	1045	1149	84	483	1027	1341	78	407	925	1094	127
Lea Bridge	357	930	1073	108	386	1034	1161	89	485	1021	1209	76	355	913	1077	124
Spitalfield	329	907	1057	126	466	1018	1208	91	512	1021	1215	90	417	884	1099	143
Carpenters	386	920	1089	120	NA	NA	NA	NA	527	1045	1247	91	445	875	1171	144

Table VI.6 – Minimum value, mean, maximum value and standard deviation (SD) of the total ammonia (mg/l) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	0.25	0.57	1.12	0.16	0.06	0.36	0.98	0.27	0.24	0.67	1.49	0.29	0.29	0.58	0.91	0.08
Angel	0.10	0.85	4.16	0.59	0.06	0.85	2.90	0.64	0.27	1.13	2.99	0.66	0.27	2.12	5.68	1.17
Deephams	0.55	1.46	17.45	0.90	0.57	1.21	5.48	0.59	0.46	1.79	41.14	2.59	0.38	1.42	12.78	0.98
Pymmes W	0.24	1.28	4.62	0.64	0.19	1.34	4.58	0.66	0.47	1.64	4.62	0.83	0.86	1.99	4.62	0.79
Pymmes E	1.10	1.57	35.52	0.92	0.50	1.67	5.65	0.64	0.12	1.13	7.83	1.09	0.33	2.01	7.96	0.96
Springfield	0.16	1.44	6.53	1.39	0.34	2.17	30.56	3.96	0.11	1.18	6.95	0.76	0.39	1.22	5.76	0.41
Lea Bridge	0.19	0.99	2.61	0.51	0.37	1.08	2.60	0.33	0.05	1.01	2.62	0.73	0.32	1.20	2.62	0.52
Spitalfield	0.13	1.21	7.36	0.70	0.29	1.17	6.77	1.18	0.34	0.81	4.02	0.34	0.45	1.03	3.32	0.41
Carpenters	0.04	0.98	6.24	0.40	0.02	0.74	19.72	0.94	0.51	1.09	3.79	0.34	0.24	0.88	2.53	0.30

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	0.25	0.63	1.71	0.27	0.06	0.46	11.41	0.35	0.50	0.83	2.19	0.34	0.43	0.85	2.43	0.25
Angel	0.11	0.73	2.78	0.38	0.02	0.53	2.11	0.41	0.18	0.53	5.35	0.35	0.22	1.29	13.99	1.34
Deephams	0.02	1.28	6.15	0.46	0.10	1.36	16.74	1.11	0.26	1.50	123.2	1.70	0.75	2.34	14.09	1.26
Pymmes W	0.41	1.46	4.57	0.58	0.64	1.91	4.61	0.69	0.81	1.88	4.62	0.85	0.85	2.28	4.62	0.76
Pymmes E	0.07	1.23	4.96	0.60	0.22	1.27	3.75	0.48	0.30	2.12	7.54	1.05	0.62	2.25	7.53	0.90
Springfield	0.35	1.32	4.05	0.37	0.00	1.00	3.59	0.73	0.34	1.54	3.63	0.79	0.24	1.46	3.65	0.49
Lea Bridge	0.23	0.92	2.62	0.36	0.38	1.22	2.62	0.46	0.70	1.57	2.62	0.37	0.41	1.54	2.62	0.52
Spitalfield	0.11	0.61	4.31	0.39	0.42	1.13	3.86	0.43	0.63	1.59	6.52	1.00	0.19	1.23	4.27	0.81
Carpenters	0.29	0.96	2.51	0.42	NA	NA	NA	NA	0.28	1.44	7.73	0.51	0.07	0.50	2.43	0.48

Table VI.7 Minimum value, mean, maximum value and standard deviation (SD) of the turbidity (NTU) calculated on seasonal basis over a period of two years (from 21/06/2010 to 20/06/2012). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites in the area under investigation, and up and down the stream. NA = data not available.

	Summer 2010				**Autumn 2010**				**Winter 2010-2011**				**Spring 2011**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	0	2	28	2	0	4	28	3	0	7	30	6	1	3	29	2
Angel	3	8	51	6	5	11	51	6	5	14	52	9	4	7	51	4
Deephams	1	4	68	4	1	6	62	5	0	7	77	9	0	6	74	8
Pymmes W	0	5	213	10	2	30	674	72	0	159	687	182	0	97	687	165
Pymmes E	1	34	323	40	2	216	931	254	0	300	931	284	0	77	915	125
Springfield	1	10	228	16	2	13	229	32	0	17	231	28	0	8	216	10
Lea Bridge	1	3	33	3	2	4	32	2	1	8	33	6	2	4	32	2
Spitalfield	1	10	138	13	2	8	133	9	2	13	143	17	1	9	146	13
Carpenters	1	3	65	4	0	7	110	6	1	14	164	18	0	3	95	3

	Summer 2011				**Autumn 2011**				**Winter 2011-2012**				**Spring 2012**			
	Min	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*	*Min*	*Mean*	*Max*	*SD*
Chingford	1	2	27	1	0	2	29	2	2	4	29	2	2	6	30	4
Angel	5	9	50	5	4	8	51	5	6	12	50	9	4	11	52	8
Deephams	1	6	75	6	0	5	75	5	0	10	76	8	1	13	77	15
Pymmes W	0	32	685	82	2	42	687	76	2	99	687	137	1	38	681	94
Pymmes E	2	83	929	135	0	104	929	168	0	246	932	258	1	124	927	189
Springfield	3	8	209	11	1	8	178	12	1	23	230	40	0	37	231	45
Lea Bridge	1	4	32	3	1	3	31	3	2	6	33	3	2	7	33	5
Spitalfield	1	7	137	8	1	8	146	9	1	8	127	8	2	22	147	27
Carpenters	0	3	121	7	NA	NA	NA	NA	0	8	168	10	0	14	169	23

Appendix VII: Correlation analysis (Spearman rank) results

Spearman correlation coefficients are given in Tables VII.1, VII.2, VII.3, VII.4, and VII.5.

Table VII.1 – Spearman correlation coefficient (ρ_s) calculated for DO (%) – pH. If ρ_s = ± 0.50 the correlation is significant; if ρ_s < -0.70 or >+0.70 the correlation is strong; if ρ_s = ± 1.00 the correlation is good. Significance of the test: * *p < 0.05 (2-tailed); *p < 0.01 (2-tailed). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites over a period of two years (from 21/06/2010 to 20/06/2012).

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	0.92*	0.64*	0.13*	0.90*	0.83*	0.11*	0.41*	0.69*
Angel	0.64*	0.36*	0.56*	0.40*	0.58*	0.67*	0.28*	0.67*
Deephams	0.34*	0.49*	0.54*	0.66*	-0.06*	-0.47*	0.27*	NA
Pymmes E	0.57*	0.47*	0.29*	0.63*	0.67*	0.31*	-0.09*	0.40*
Pymmes W	0.65*	0.46*	0.18*	0.41*	0.41*	0.35*	0.13*	0.21*
Springfield	0.76*	0.41*	0.53*	-0.15*	0.03**	0.36*	0.66*	0.59*
Lea Bridge	0.70*	0.26*	0.40*	0.68*	0.27*	0.08*	0.23*	0.20*
Spitalfield	0.56*	0.42*	0.50*	0.67*	0.06*	0.32*	0.71*	0.76*
Carpenters	0.74*	0.08*	0.78*	0.86*	0.48*	NA	0.53*	0.80*

Table VII.2 – Spearman correlation coefficient (r_s) calculated for DO (%) – temperature (°C). If ρ_s = ± 0.50 the correlation is significant; if ρ_s < - 0.70 or > + 0.70 the correlation is strong; if ρ_s = ± 1.00 the correlation is good. Significance of the test: * * p < 0.05 (2-tailed); * p < 0.01 (2-tailed). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites over a period of two years (from 21/06/2010 to 20/06/2012).

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	0.13*	-0.04*	0.48*	0.17*	0.14*	0.20*	0.61*	0.22*
Angel	-0.15*	-0.05**	-0.11*	0.18*	-0.01	-0.38*	-0.19*	-0.38*
Deephams	-0.36*	-0.53*	-0.19*	0.20*	-0.12*	-0.60*	-0.45*	NA
Pymmes E	0.02	-0.15*	-0.01	0.35*	0.20*	0.06*	-0.23*	0.14*
Pymmes W	0.54*	0.24*	-0.07*	0.44*	0.10*	0.05*	-0.17*	0.40*
Springfield	0.21*	-0.64*	-0.26*	-0.56*	-0.15*	-0.73*	-0.27*	-0.13*
Lea Bridge	-0.43*	-0.68*	-0.39*	-0.40*	-0.18*	-0.67*	-0.53*	0.14*
Spitalfield	-0.30*	-0.19*	-0.38*	-0.14*	-0.03**	-0.69*	-0.39*	-0.19*
Carpenters	-0.03*	-0.61*	-0.14*	-0.17*	0.18*	NA	-0.43*	0.13*

Table VII.3 – Spearman correlation coefficient (r_s) calculated for DO (%) – conductivity (µS/cm). If ρ_s = ± 0.50 the correlation is significant; if ρ_s < - 0.70 or > + 0.70 the correlation is strong; if ρ_s = ± 1.00 the correlation is good. Significance of the test: * * p < 0.05 (2-tailed); * p < 0.01 (2-tailed). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites over a period of two years (from 21/06/2010 to 20/06/2012).

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	-0.26*	-0.15*	0.09*	-0.31*	-0.13*	-0.18*	-0.39*	0.41*
Angel	-0.20*	-0.33*	-0.56*	-0.37*	-0.21*	-0.03	-0.07**	-0.03
Deephams	-0.17*	0.25*	0.09*	-0.43*	-0.12*	0.16*	0.11*	NA
Pymmes E	-0.23*	0.24*	-0.38*	-0.08*	0.09*	-0.06*	-0.22*	-0.24*
Pymmes W	-0.30*	-0.32*	-0.20*	-0.32*	-0.09*	0.06*	-0.01	-0.24*
Springfield	0.17*	0.29*	-0.50*	0.06*	-0.14*	-0.10*	-0.16*	-0.08*
Lea Bridge	-0.05*	0.29*	-0.30*	0.15*	0.02	-0.10*	0.16*	-0.13*
Spitalfield	-0.27*	0.16*	-0.67*	0.39*	0.01	0.10*	0.42*	-0.14*
Carpenters	0.13*	0.31*	-0.45*	0.46*	0.12*	NA	0.26*	-0.21*

Table VII.4 – Spearman correlation coefficient (r_s) calculated for DO (%) – turbidity (NTU). If ρ_s = ± 0.50 the correlation is significant; if ρ_s < - 0.70 or > + 0.70 the correlation is strong; if ρ_s = ± 1.00 the correlation is good. Significance of the test: * * p < 0.05 (2-tailed); * p < 0.01 (2-tailed). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites over a period of two years (from 21/06/2010 to 20/06/2012).

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	0.27*	-0.26*	-0.23*	0.25*	0.05*	-0.36*	0.03*	-0.39*
Angel	0.39*	0.45*	0.74*	0.16*	0.19*	0.37*	0.45*	0.37*
Deephams	-0.27*	-0.09*	-0.42*	-0.37*	0.00	0.15*	0.03*	NA
Pymmes E	-0.04**	-0.02	-0.31*	-0.17*	0.00	-0.04*	-0.15*	0.15*
Pymmes W	-0.03	-0.51*	-0.35*	0.06*	-0.12*	-0.24*	-0.31*	-0.37*
Springfield	0.44*	0.42*	0.38*	0.14*	0.43*	0.54	0.15*	0.03**
Lea Bridge	0.19*	0.27*	0.34*	-0.01	0.18*	0.17*	0.45*	0.36*
Spitalfield	0.16*	-0.21*	0.53*	-0.02	0.18*	-0.31*	0.07*	0.13*
Carpenters	0.41*	0.32*	0.27*	0.09*	0.12*	NA	0.20*	0.31*

Table VII.5 – Spearman correlation coefficient (r_s) calculated for DO (%) – total ammonia (mg/l). If ρ_s = ± 0.50 the correlation is significant; if ρ_s < - 0.70 or > + 0.70 the correlation is strong; if ρ_s = ± 1.00 the correlation is good. Significance of the test: * * p < 0.05 (2-tailed); * p < 0.01 (2-tailed). Data were recorded by automated monitoring stations of Environment Agency located at nine different sites over a period of two years (from 21/06/2010 to 20/06/2012).

Stations name	Summer 2010	Autumn 2010	Winter 2010-2011	Spring 2011	Summer 2011	Autumn 2011	Winter 2011-2012	Spring 2012
Chingford	0.05*	-0.32*	-0.62*	-0.02	0.04*	-0.24*	0.29*	0.28*
Angel	-0.36*	-0.34*	-0.75*	-0.51*	-0.28*	-0.21*	-0.40*	-0.21*
Deephams	-0.31*	-0.44*	-0.53*	-0.29*	-0.11*	0.72*	-0.46*	NA
Pymmes E	-0.36*	-0.11*	-0.42*	-0.40*	-0.36*	-0.12*	-0.20*	-0.38*
Pymmes W	-0.34*	-0.29*	-0.26*	-0.34*	-0.29*	-0.36*	-0.26*	-0.31*
Springfield	0.34*	0.30*	-0.16*	0.52*	-0.03	-0.51*	-0.31*	0.00
Lea Bridge	-0.53*	-0.02	-0.66*	-0.24*	-0.16*	0.22*	-0.56*	-0.07*
Spitalfield	0.09*	-0.04**	-0.26*	-0.15*	-0.05*	-0.49*	0.27*	-0.37*
Carpenters	-0.32*	0.26*	-0.46*	0.12*	-0.01	NA	-0.28*	-0.35*

Appendix VIII: Algal growth inhibition test results

The level of algal growth inhibition (%) was calculated following OECD guidelines (2006) and it was estimated over four replicates for each test solution.

The box plots were calculated with R software (open source software, available at http://www.R-project.org). The black horizontal line in the box represents the median. The box stretches out to the third quartile (above the median), and to the first quartile (below the median). The ends of the whiskers represent the minimum and maximum data points.

Results are given following.

Table VIII.1 and Figure VIII.1 show the results of the algal growth test conducted with water collected on 06/09/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium.

Table VIII.1 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 06/09/2010. The OECD medium was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Tottenham Hale	4	2	4	1	3	2
Pymmes Brook	56	12	24	4	6	3
Lea Nav opposite Warwick reservoir	48	6	25	3	7	2
Lea Nav at Springfield Park	61	5	19	3	5	2

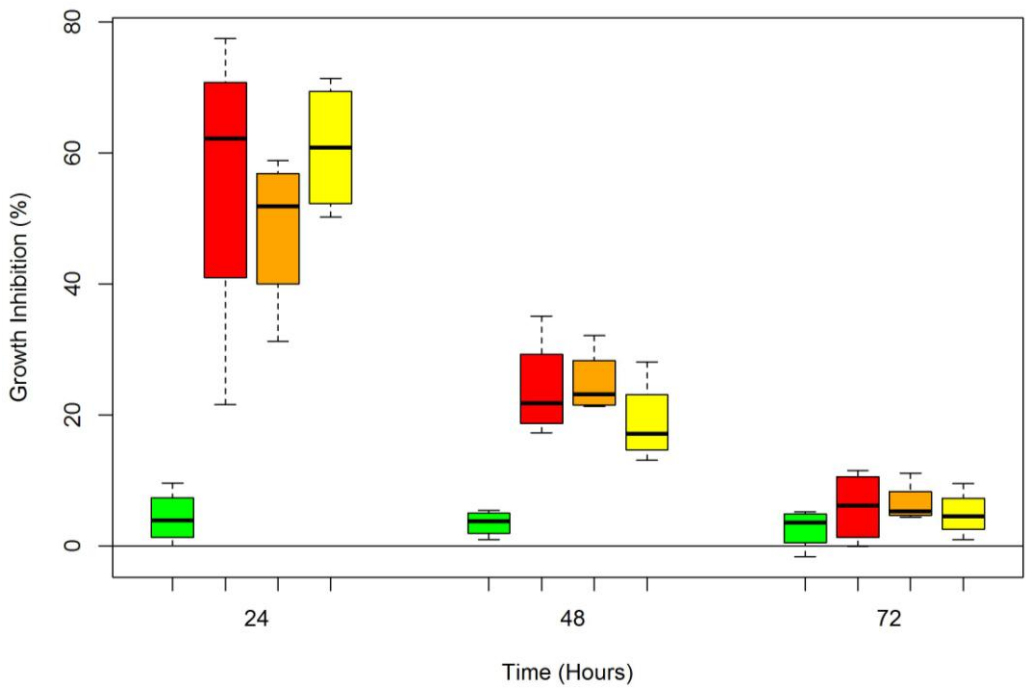

Figure VIII.1 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 06/09/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.2 and Figure VIII.2 show the results of the algal growth test conducted with water collected on 06/09/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.2 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 06/09/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	54	13	21	4	3	3
Lea Nav opposite Warwick reservoir	46	6	22	3	4	2
Lea Nav at Springfield Park	59	5	16	3	2	2

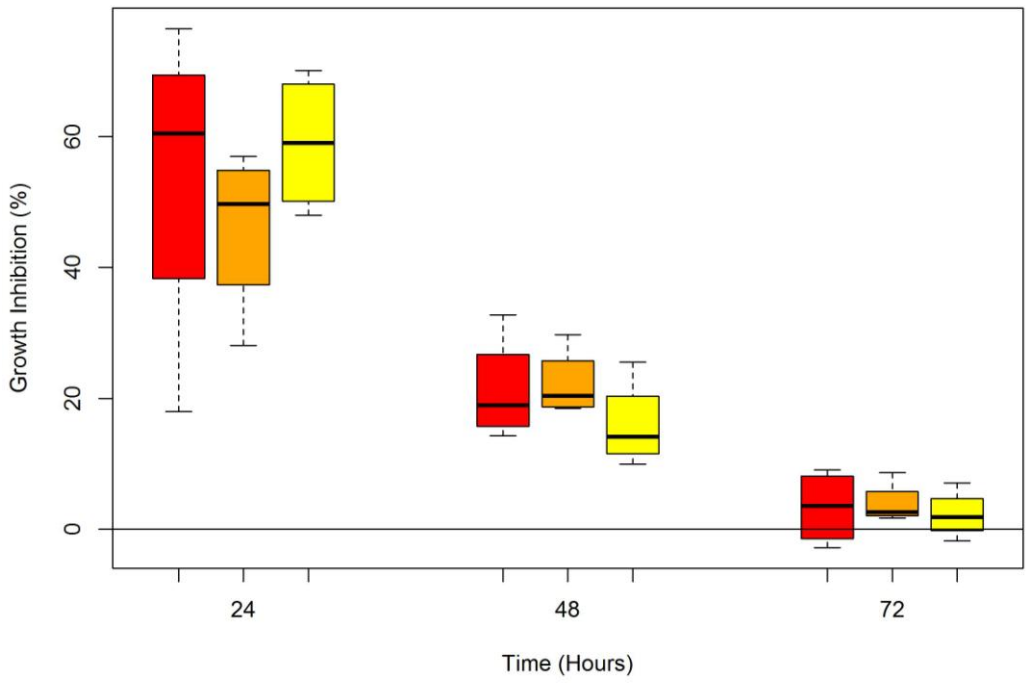

Figure VIII.2 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 06/09/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.3 and Figure VIII.3 show the results of the algal growth test conducted with water collected on 13/09/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium.

Table VIII.3 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 13/09/2010. The OECD medium was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Tottenham Hale	11	1	6	2	0	1
Pymmes Brook	64	6	23	4	4	1
Lea Nav opposite Warwick reservoir	50	0	7	1	-1	0
Lea Nav at Springfield Park	66	7	19	8	4	3

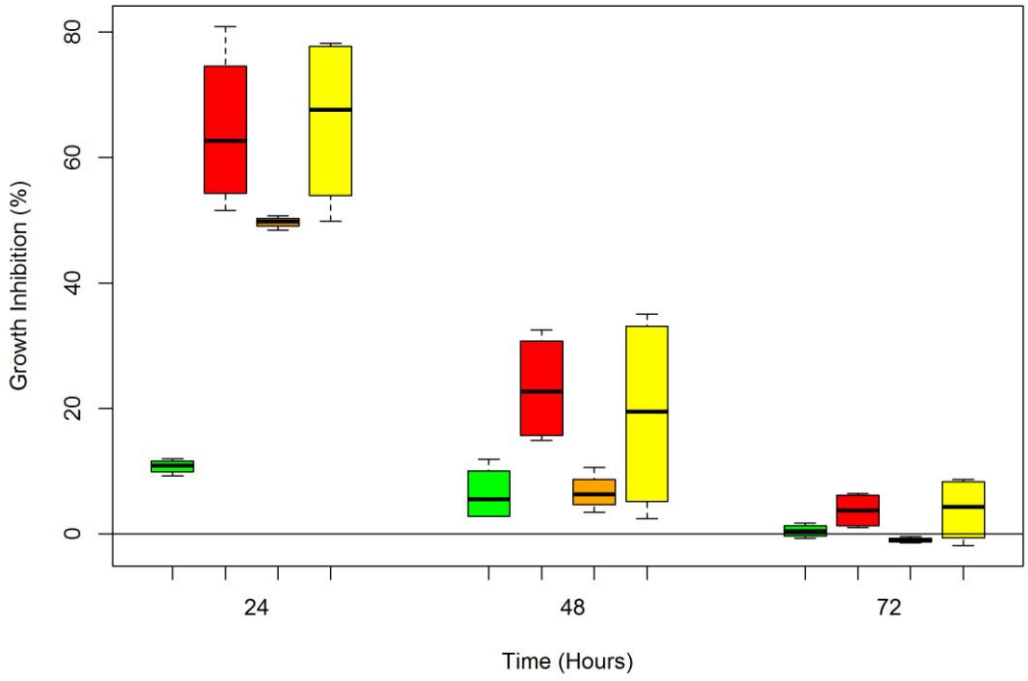

Figure VIII.3 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 13/09/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.4 and Figure VIII.4 show the results of the algal growth test conducted with water collected on 13/09/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.4 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 13/09/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	60	7	18	5	3	1
Lea Nav opposite Warwick reservoir	44	1	0	2	-1	0
Lea Nav at Springfield Park	62	8	14	9	3	3

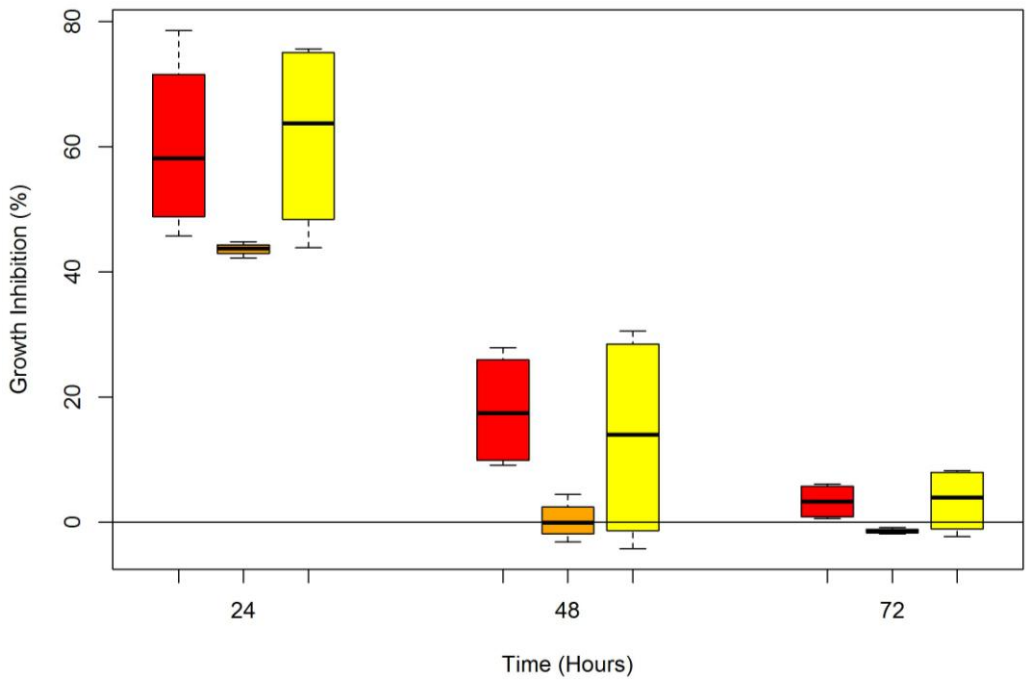

Figure VIII.4 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 13/09/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.5 and Figure VIII.5 show the results of the algal growth test conducted with water collected on 20/09/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium.

Table VIII.5 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 20/09/2010. The OECD medium was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Tottenham Hale	-4	3	-2	3	0	1
Pymmes Brook	41	5	-2	2	-3	1
Lea Nav opposite Warwick reservoir	44	7	-4	3	-4	1
Lea Nav at Springfield Park	43	8	-4	3	-4	1

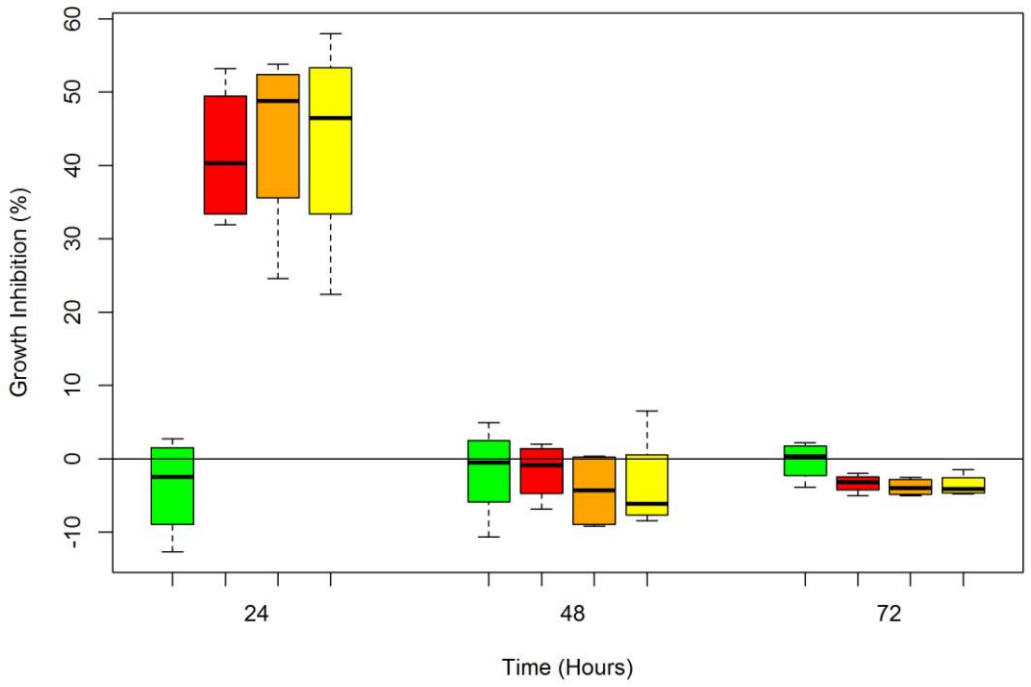

Figure VIII.5 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 20/09/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.6 and Figure VIII.6 show the results of the algal growth test conducted with water collected on 20/09/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.6 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 20/09/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	44	5	0	2	-3	1
Lea Nav opposite Warwick reservoir	46	6	-3	3	-4	1
Lea Nav at Springfield Park	45	7	-2	3	-3	1

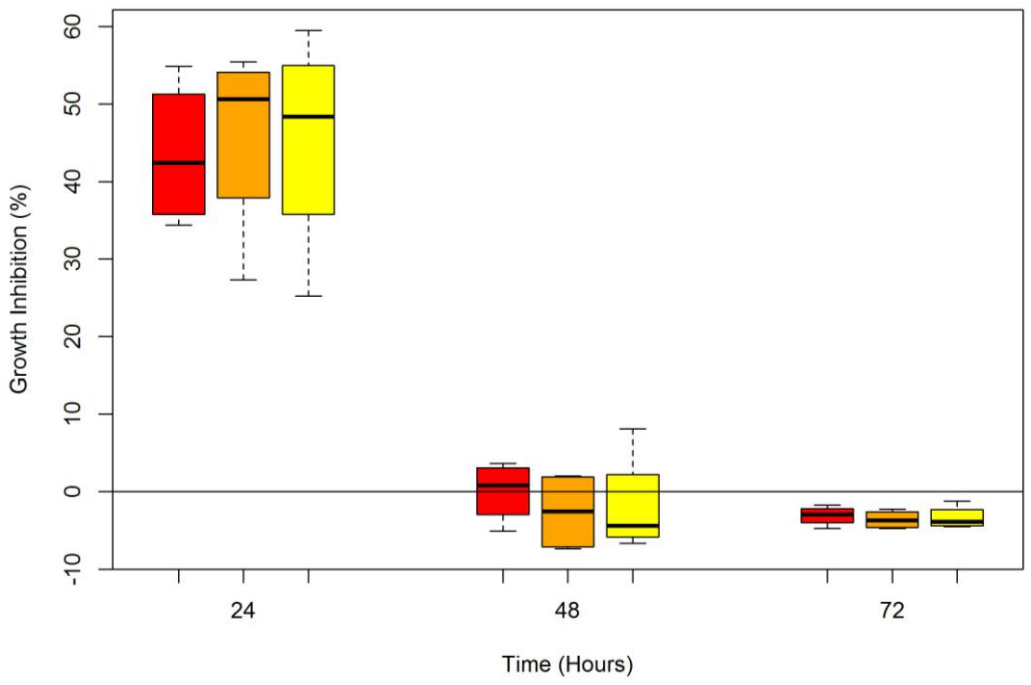

Figure VIII.6 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 20/09/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.7 and Figure VIII.7 show the results of the algal growth test conducted with water collected on 11/10/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium.

Table VIII.7 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 11/10/2010. The OECD medium was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Tottenham Hale	19	4	8	4	6	4
Pymmes Brook	36	2	0	1	-3	0
Lea Nav opposite Warwick reservoir	40	6	14	5	8	5
Lea Nav at Springfield Park	52	5	7	3	1	1

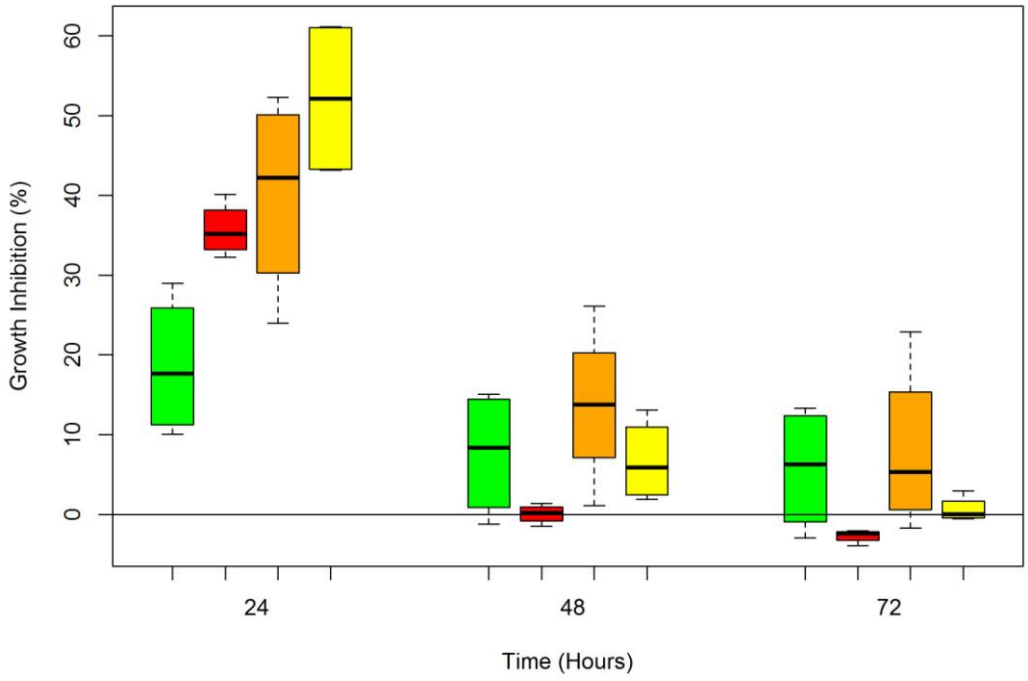

Figure VIII.7 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 11/10/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.8 and Figure VIII.8 show the results of the algal growth test conducted with water collected on 11/10/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.8 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 11/10/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	21	2	-8	1	-9	0
Lea Nav opposite Warwick reservoir	27	8	7	6	2	6
Lea Nav at Springfield Park	41	6	-1	3	-5	1

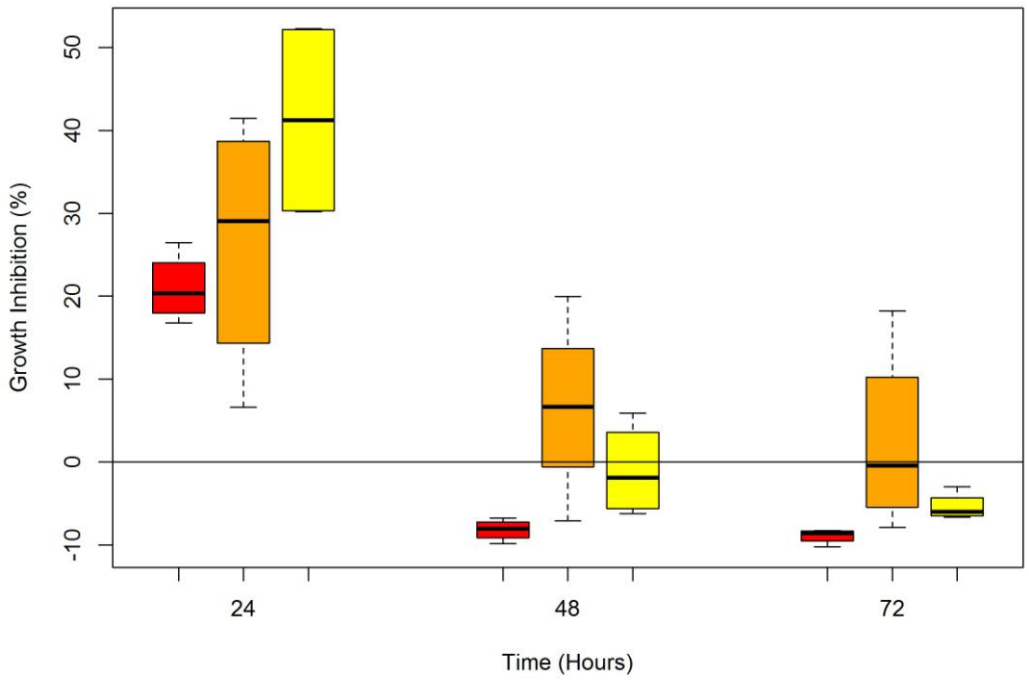

Figure VIII.8 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 11/10/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.9 and Figure VIII.9 show the results of the algal growth test conducted with water collected on 18/10/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.9 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 18/10/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	49	3	6	1	1	0
Lea Nav opposite Warwick reservoir	43	8	4	2	1	1
Lea Nav at Springfield Park	39	8	1	1	0	1

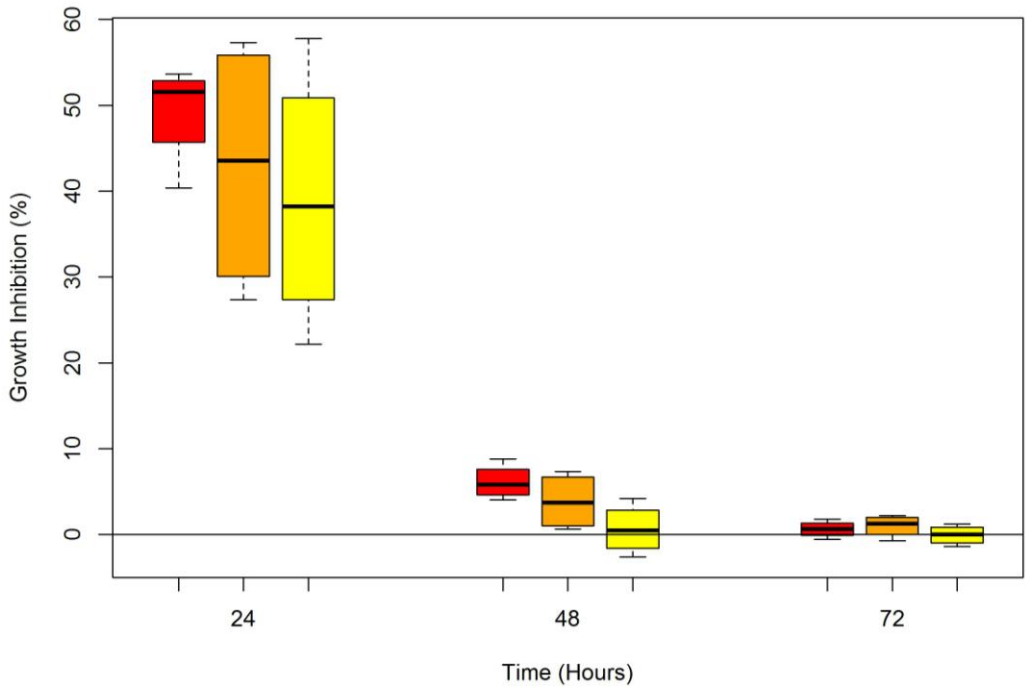

Figure VIII.9 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 18/10/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.10 and Figure VIII.10 show the results of the algal growth test conducted with water collected on 25/10/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.10 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 25/10/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	33	6	8	1	2	0
Lea Nav opposite Warwick reservoir	37	12	9	3	2	1
Lea Nav at Springfield Park	33	10	13	2	3	0

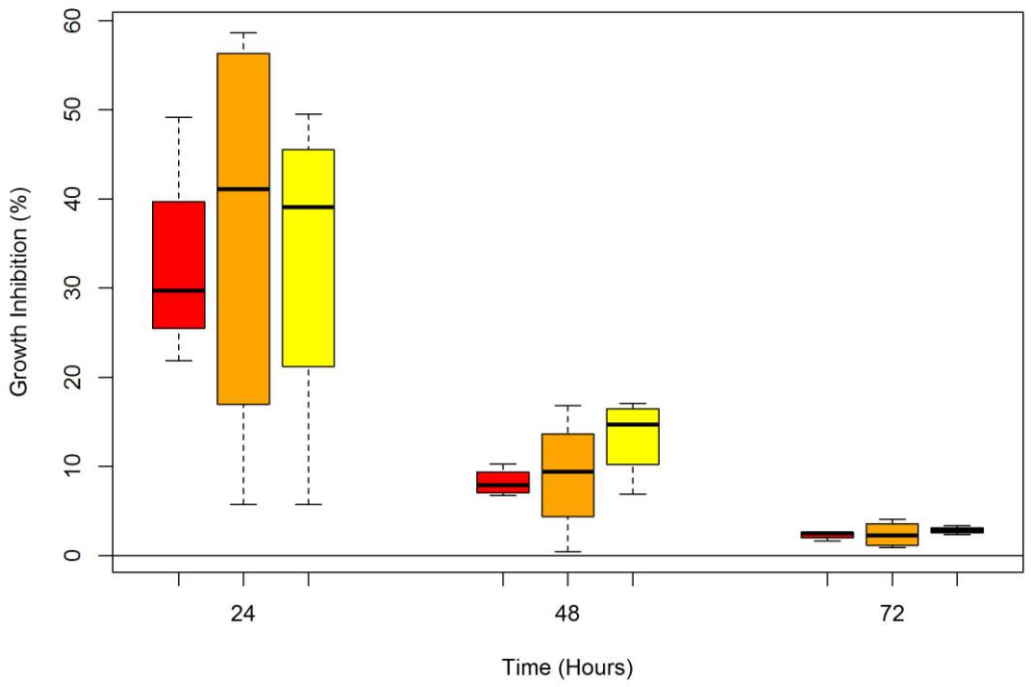

Figure VIII.10 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 25/10/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.11 and Figure VIII.11 show the results of the algal growth test conducted with water collected on 01/11/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium.

Table VIII.11 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 01/11/2010. The OECD medium was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Tottenham Hale	9	1	2	1	-3	0
Pymmes Brook	38	4	4	2	-2	1
Lea Nav opposite Warwick reservoir	29	6	0	1	-3	1
Lea Nav at Springfield Park	41	4	4	1	-1	0

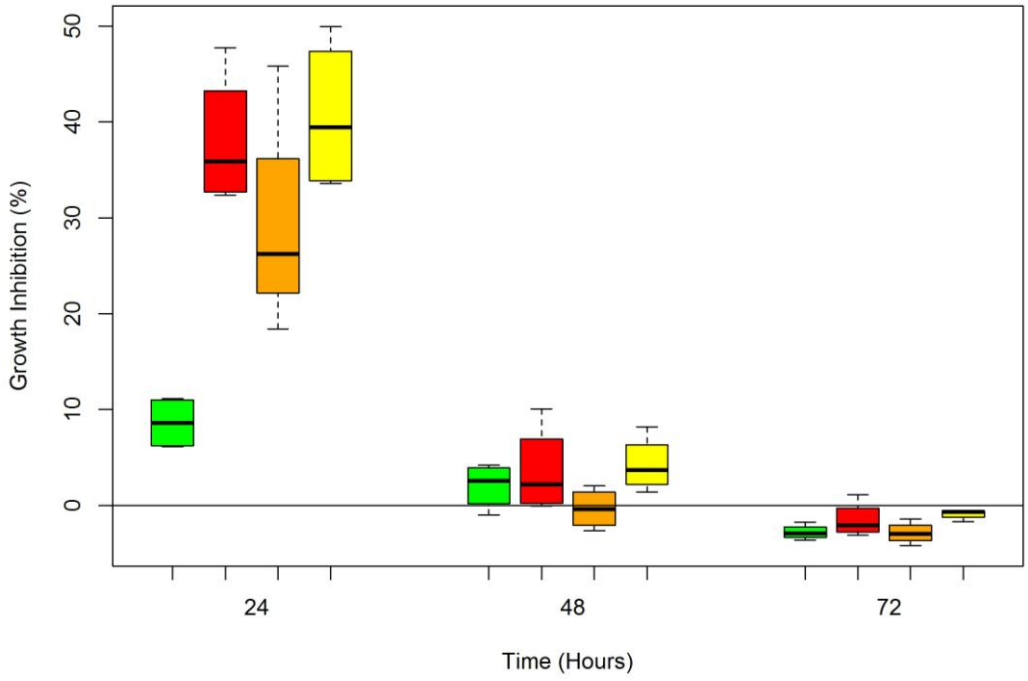

Figure VIII.11 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 01/11/2010. The level of inhibition was calculated with respect to the algal growth in the OECD medium (control). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.12 and Figure VIII.12 show the results of the algal growth test conducted with water collected on 01/11/2010. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.12 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 01/11/2010. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	32	4	2	2	1	1
Lea Nav opposite Warwick reservoir	22	6	-2	1	0	1
Lea Nav at Springfield Park	35	4	2	1	2	0

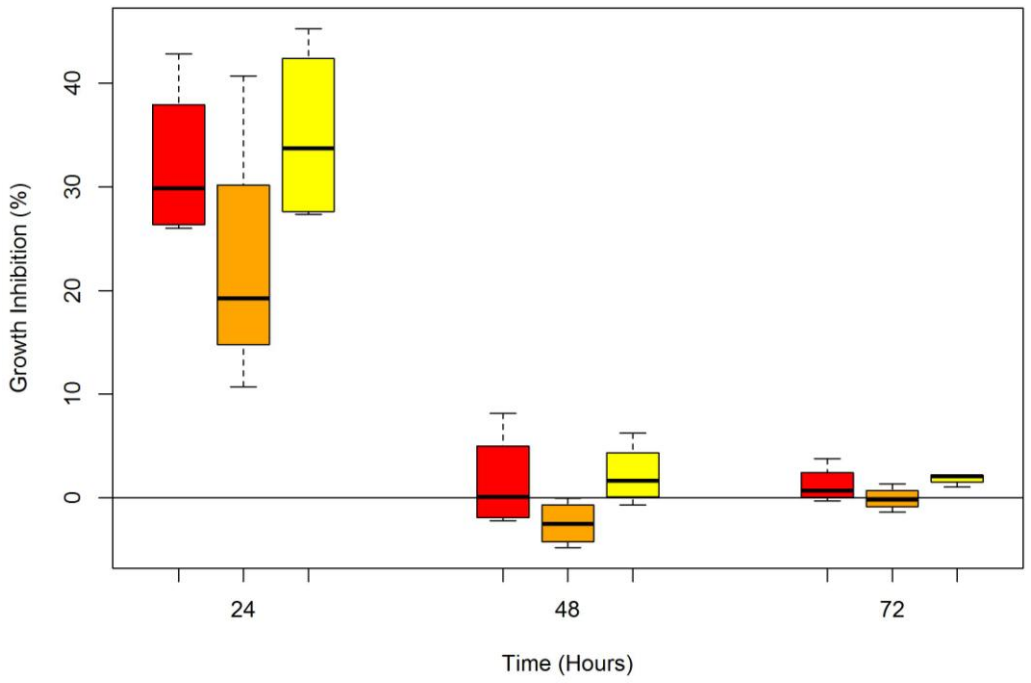

Figure VIII.12 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 01/11/2010. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Springfield Park.

Table VIII.13 and Figure VIII.13 show the results of the algal growth test conducted with water collected on 23/05/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.13 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 23/05/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	95	2	32	5	8	2
Lea Nav at Springfield Park	69	5	8	3	2	1

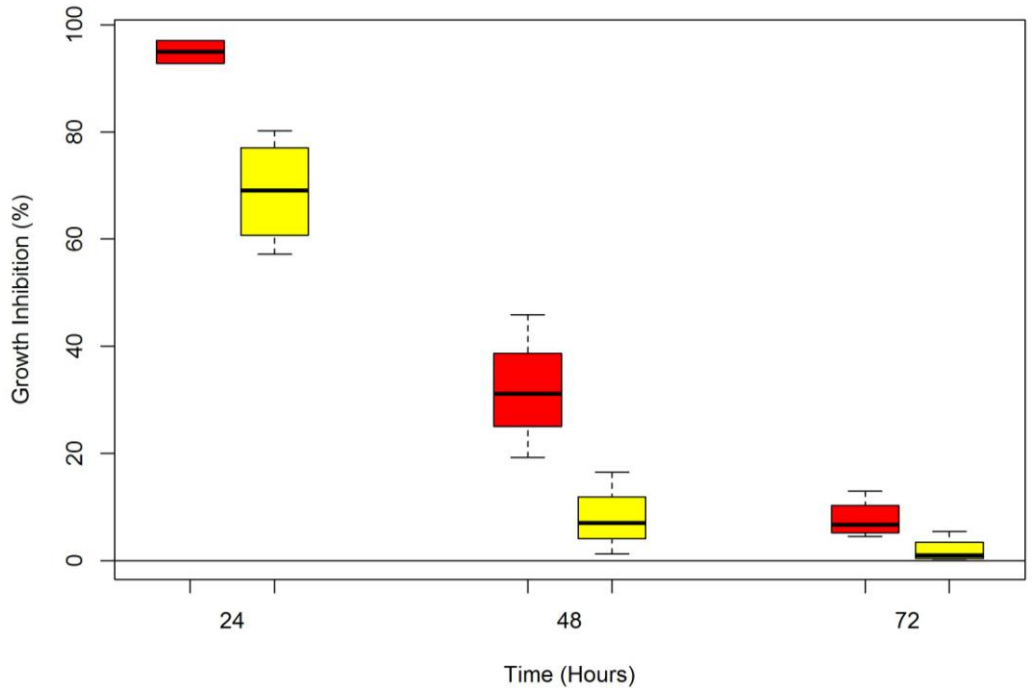

Figure VIII.13 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 23/05/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park.

Table VIII.14 and Figure VIII.14 show the results of the algal growth test conducted with water collected on 06/06/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.14 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 06/06/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	35	2	9	3	3	3
Lea Nav at Springfield Park	8	2	1	2	-1	1

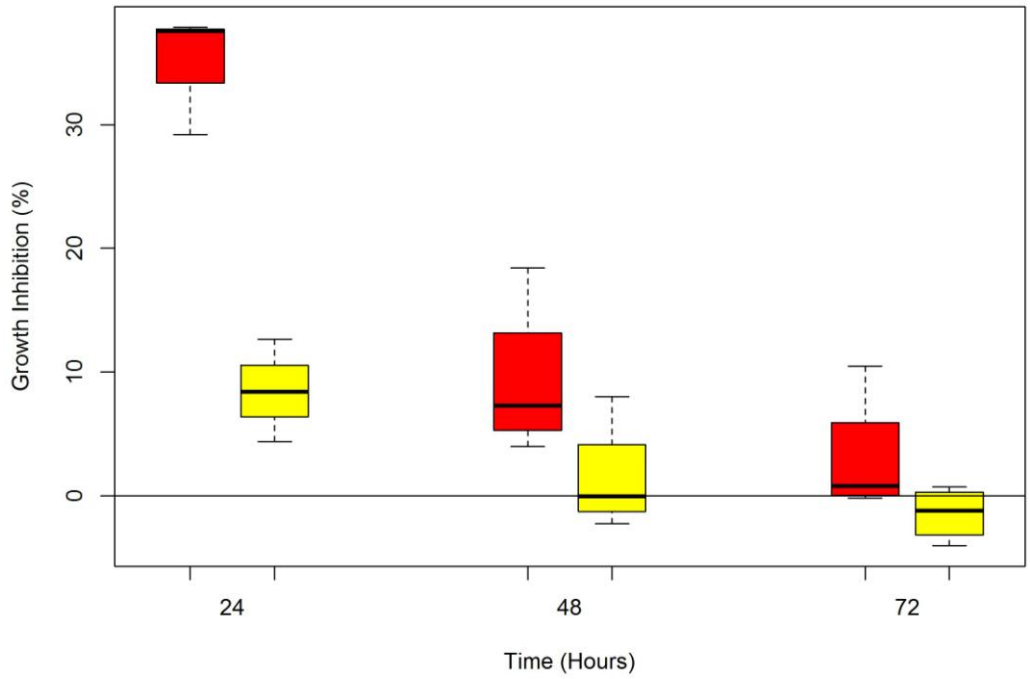

Figure VIII.14 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 06/06/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park.

Table VIII.15 and Figure VIII.15 show the results of the algal growth test conducted with water collected on 28/06/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.15 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 28/06/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	52	10	2	2	-2	1
Lea Nav at Springfield Park	52	3	10	1	0	0

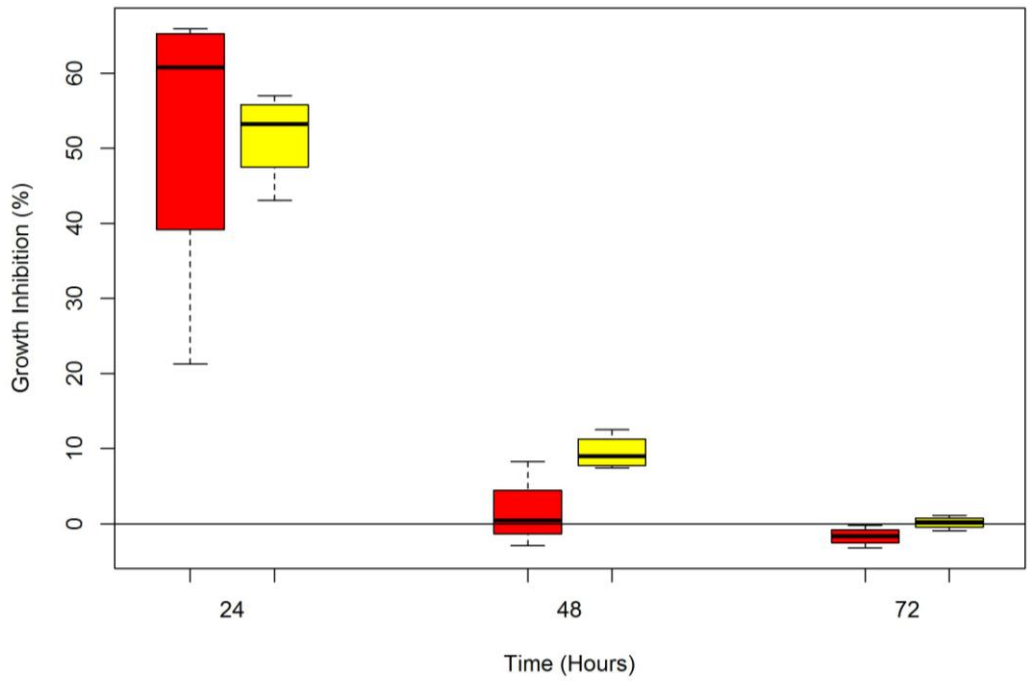

Figure VIII.15 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 28/06/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park.

Table VIII.16 and Figure VIII.16 show the results of the algal growth test conducted with water collected on 18/07/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.16 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on18/07/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Lea Nav at Springfield Park	17	3	10	2	2	2

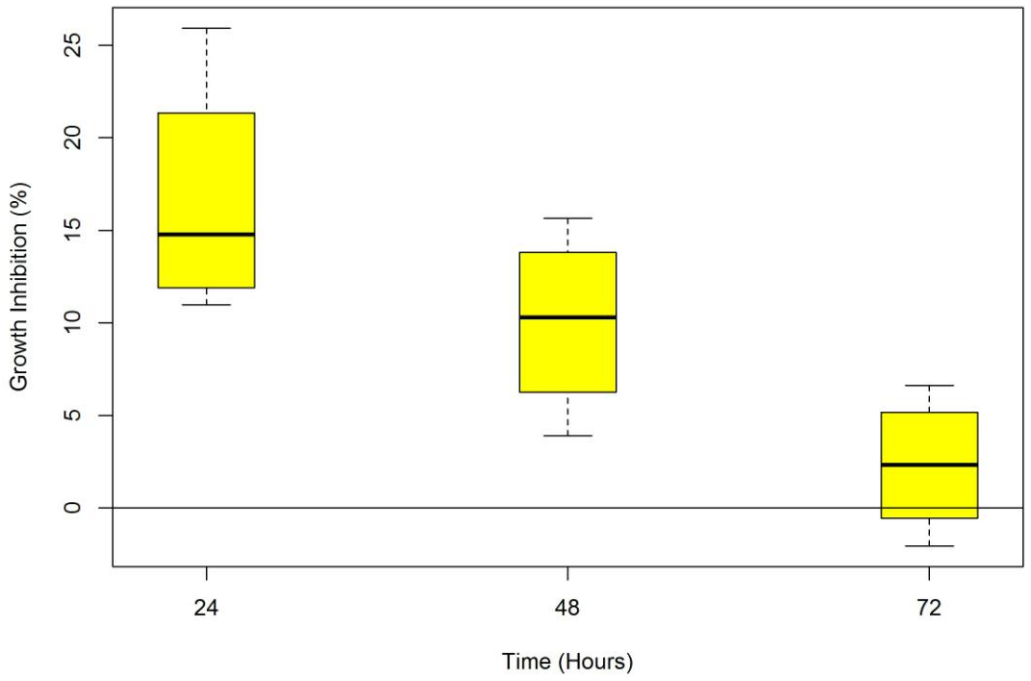

Figure VIII.16 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 18/07/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: ☐ Lea Nav at Springfield Park.

Table VIII.17 and Figure VIII.17 show the results of the algal growth test conducted with water collected on 22/08/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.17 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 22/08/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	68	5	-8	1	32	7
Lea Nav at Springfield Park	63	6	-13	2	6	2

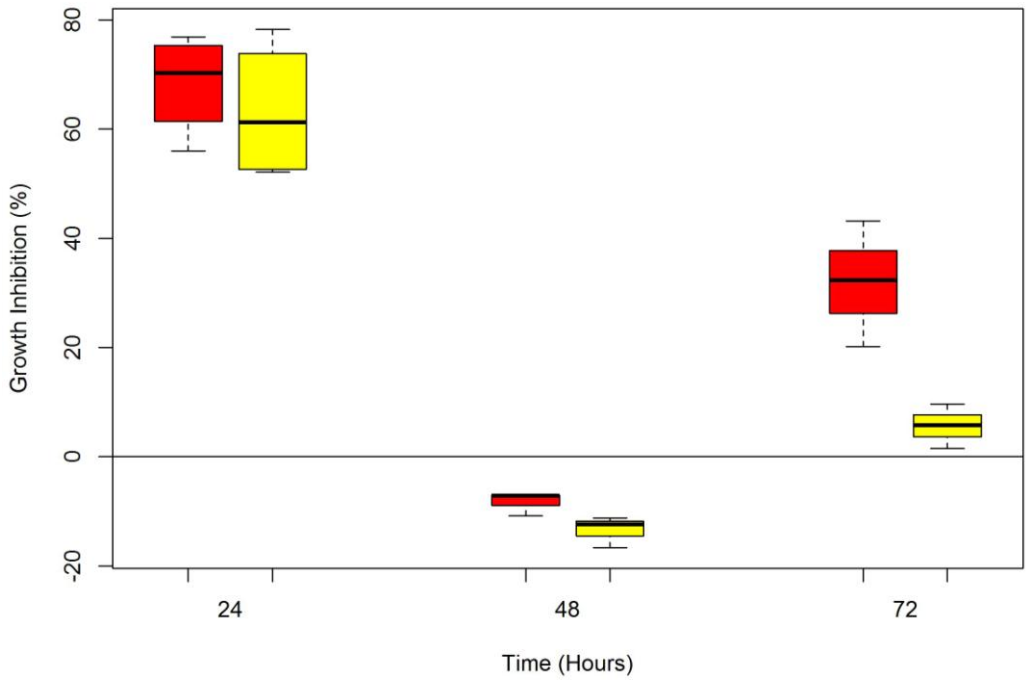

Figure VIII.17 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 22/08/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park.

Table VIII.18 and Figure VIII.18 show the results of the algal growth test conducted with water collected on 03/10/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.18 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 03/10/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	62	7	14	3	2	1
Lea Nav at Springfield Park	64	3	16	1	1	0

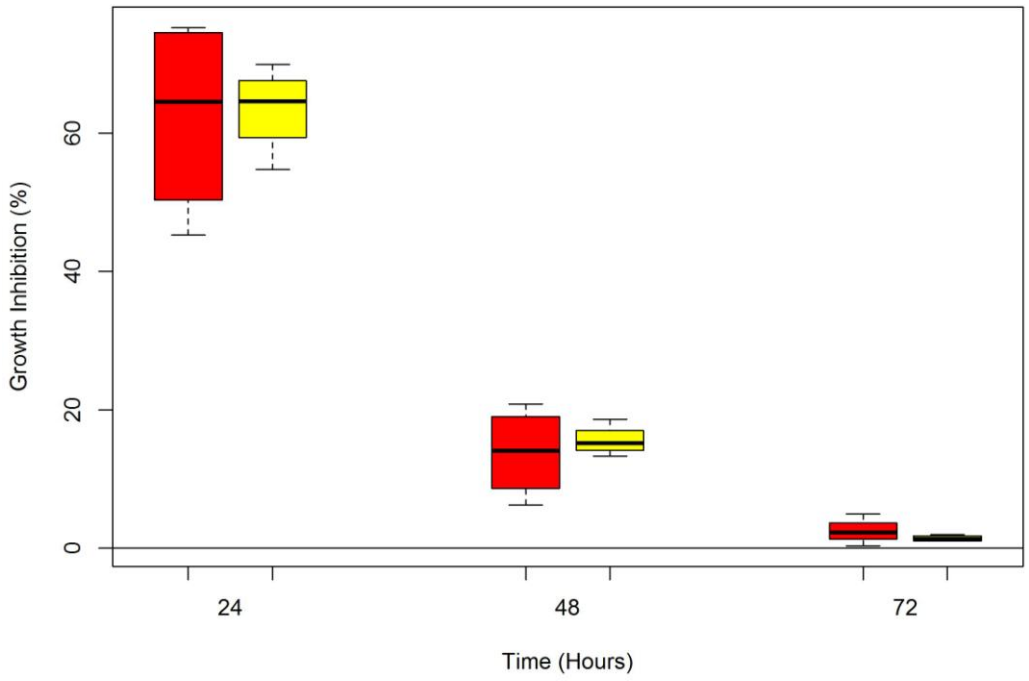

Figure VIII.18 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 03/10/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park.

Table VIII.19 and Figure VIII.19 show the results of the algal growth test conducted with water collected on 24/10/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.19 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 24/10/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	76	5	53	3	20	2
Lea Nav opposite Warwick reservoir	68	5	30	4	5	1
Lea Nav at Stonebridge Brook	78	3	41	3	8	2
Lea Nav at Springfield Park	69	7	29	5	5	1

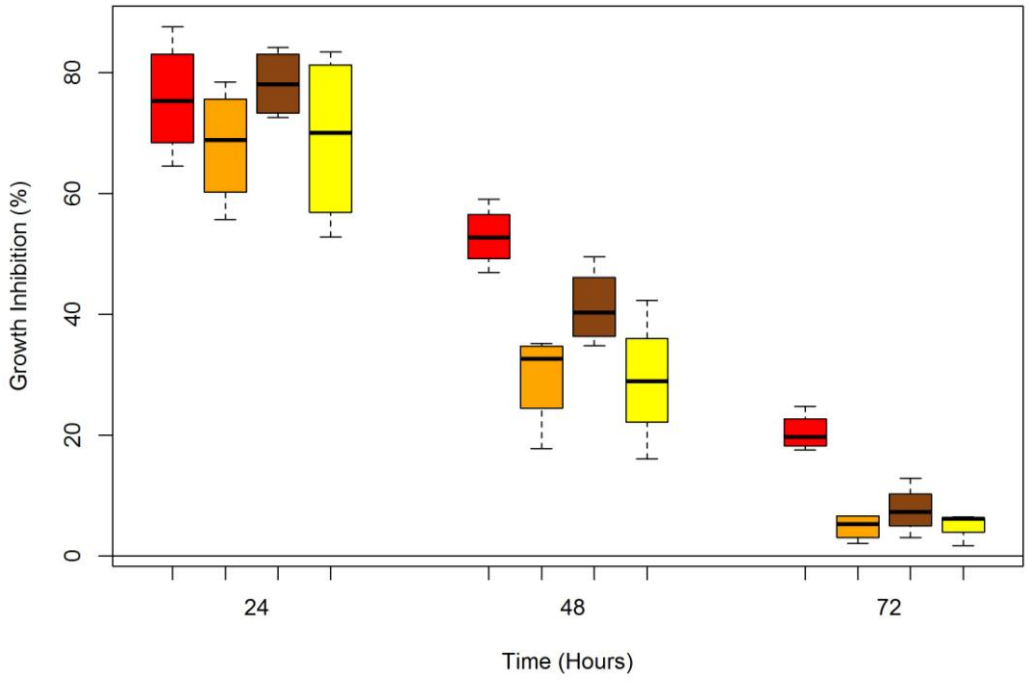

Figure VIII.19 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 24/10/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav opposite Warwick reservoir; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park.

Table VIII.20 and Figure VIII.20 show the results of the algal growth test conducted with water collected on 31/10/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.20 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 31/10/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	62	7	34	9	18	7
Lea Nav at Stonebridge Brook	44	4	23	2	9	1
Lea Nav at Springfield Park	52	8	19	3	7	2
Lea Nav at Lea Bridge weir	54	4	23	4	10	3
River Lea at Hackney Marshes	45	3	16	5	5	2

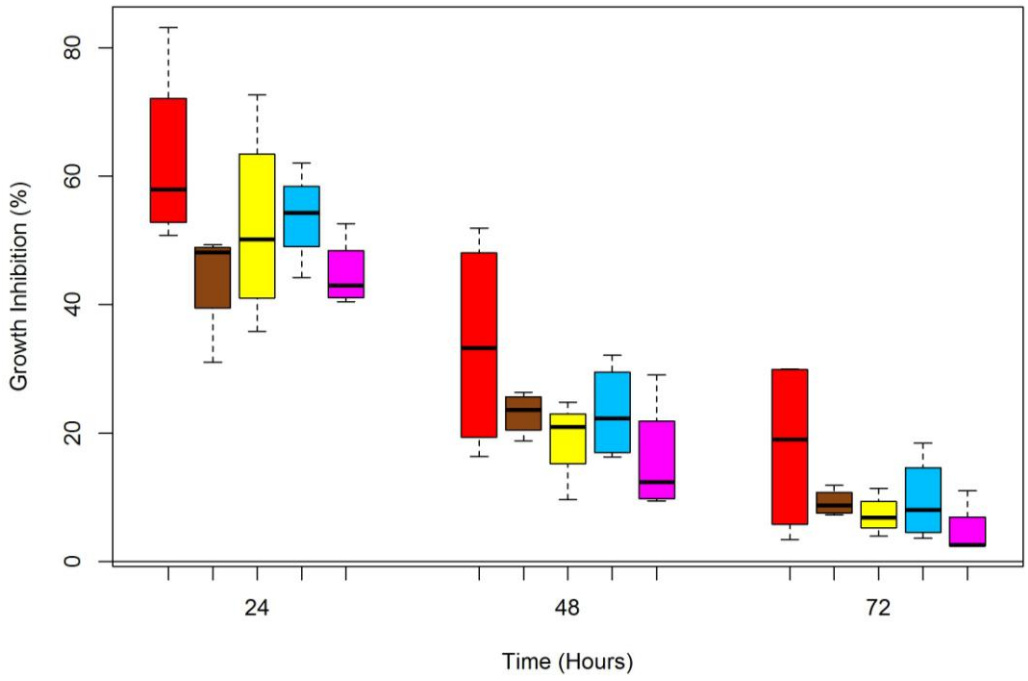

Figure VIII.20 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 31/10/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.21 and Figure VIII.21 show the results of the algal growth test conducted with water collected on 07/11/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.21– Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 07/11/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	32	6	15	1	3	0
Lea Nav at Stonebridge Brook	37	1	13	2	5	1
Lea Nav at Springfield Park	39	4	15	4	5	1
Lea Nav at Lea Bridge weir	43	7	16	4	5	1
River Lea at Hackney Marshes	33	2	8	3	3	1

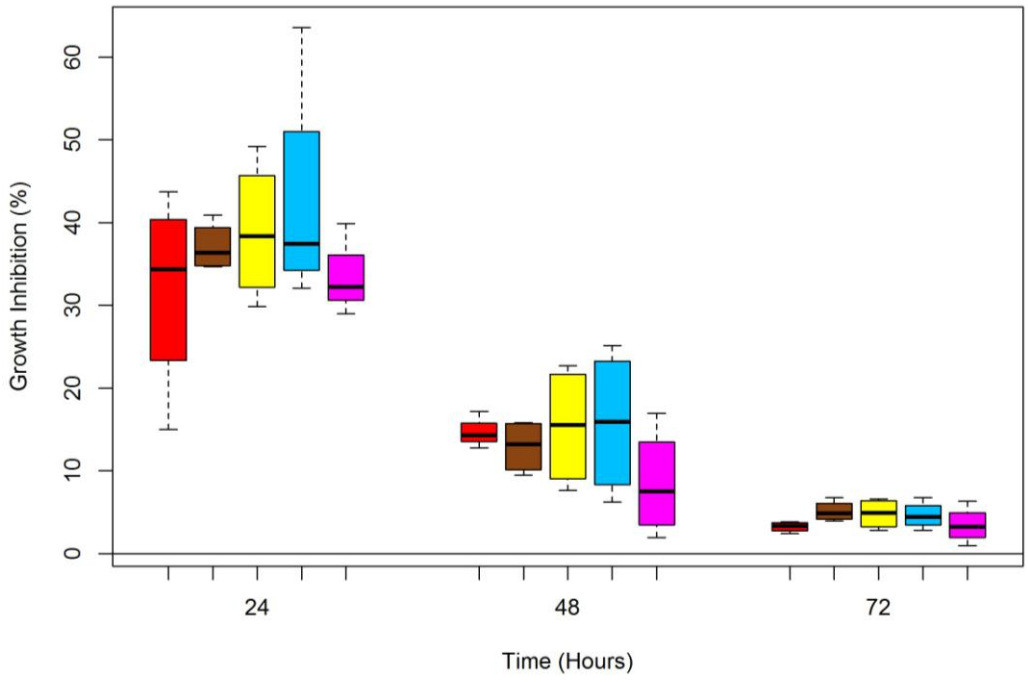

Figure VIII.21 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 07/11/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.22 and Figure VIII.22 show the results of the algal growth test conducted with water collected on 05/12/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.22 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 05/12/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	67	12	31	8	13	5
Lea Nav at Springfield Park	54	11	16	5	5	3
Lea Nav at Lea Bridge weir	33	7	6	2	2	1
River Lea at Hackney Marshes	39	6	7	3	2	1

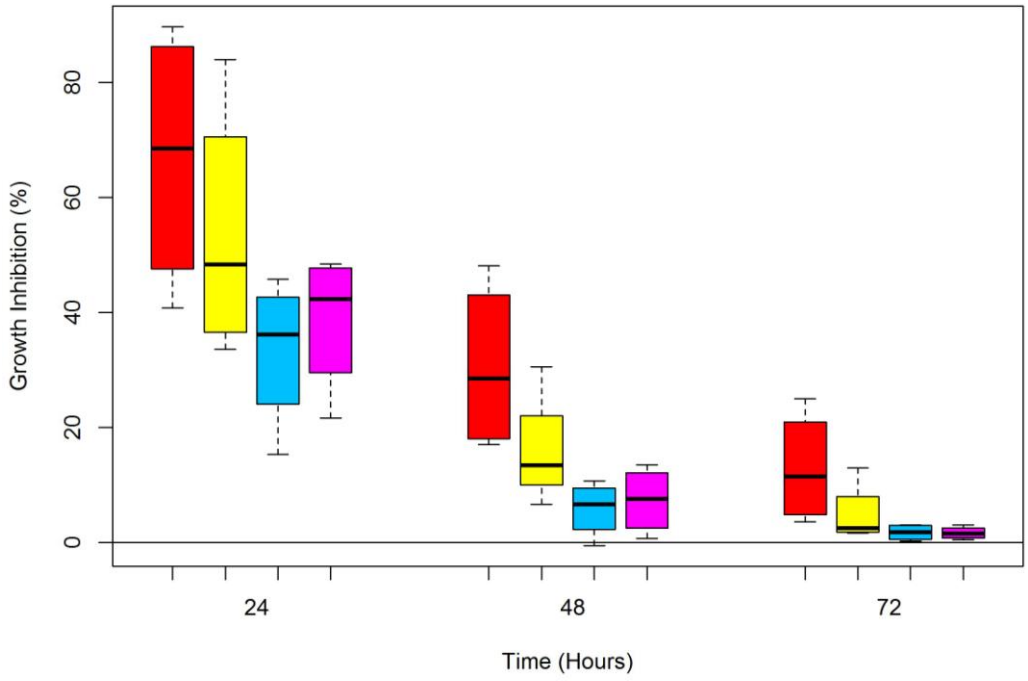

Figure VIII.22 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 05/12/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.23 and Figure VIII.23 show the results of the algal growth test conducted with water collected on 12/12/2011. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.23 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 12/12/2011. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	49	2	21	3	7	1
Lea Nav at Springfield Park	44	10	18	5	6	2
Lea Nav at Lea Bridge weir	-4	3	6	2	1	1
River Lea at Hackney Marshes	-5	3	8	2	0	1

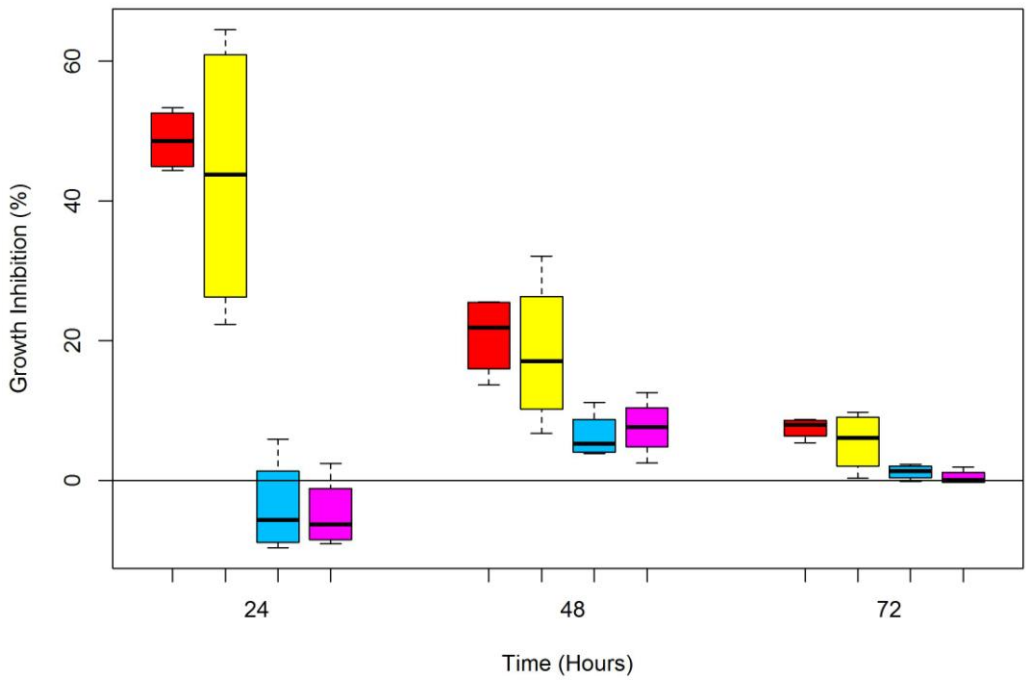

Figure VIII.23 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 12/12/2011. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.24 and Figure VIII.24 show the results of the algal growth test conducted with water collected on 09/01/2012. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.24 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 09/01/2012. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	35	4	10	3	3	1
Lea Nav at Springfield Park	48	9	14	5	6	2
Lea Nav at Lea Bridge weir	58	5	13	4	5	1
River Lea at Hackney Marshes	52	6	10	3	3	1

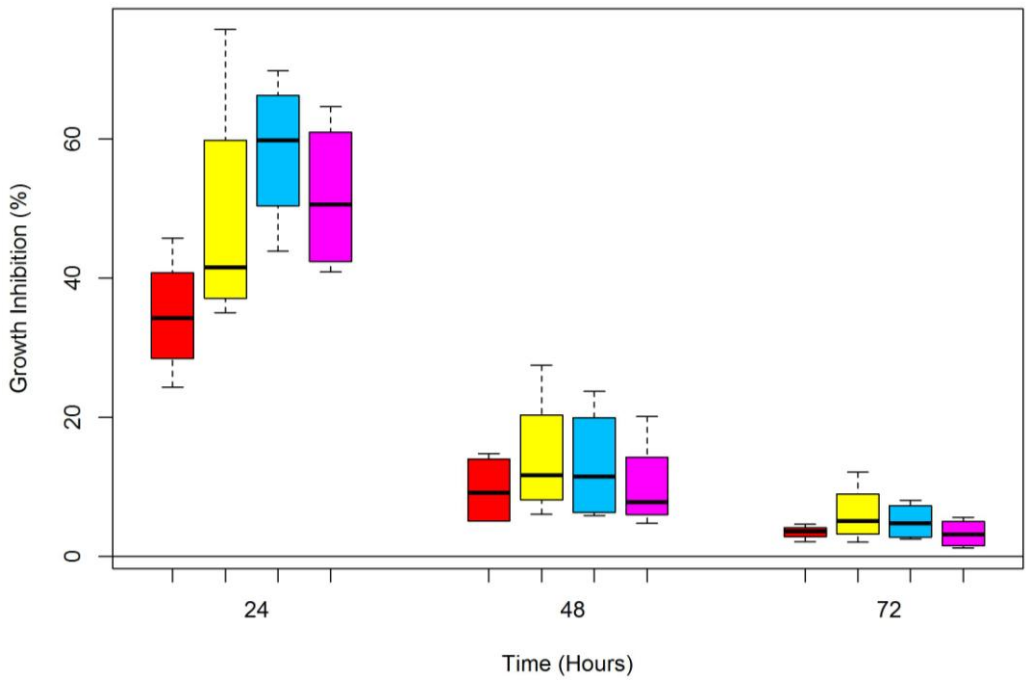

Figure VIII.24 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 09/01/2012. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.25 and Figure VIII.25 show the results of the algal growth test conducted with water collected on 30/01/2012. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.25– Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 30/01/2012. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	29	3	4	2	3	0
Lea Nav at Stonebridge Brook	109	7	34	3	13	2
Lea Nav at Springfield Park	30	2	11	3	5	1
Lea Nav at Lea Bridge weir	32	6	5	3	5	1
River Lea at Hackney Marshes	35	3	3	2	4	0

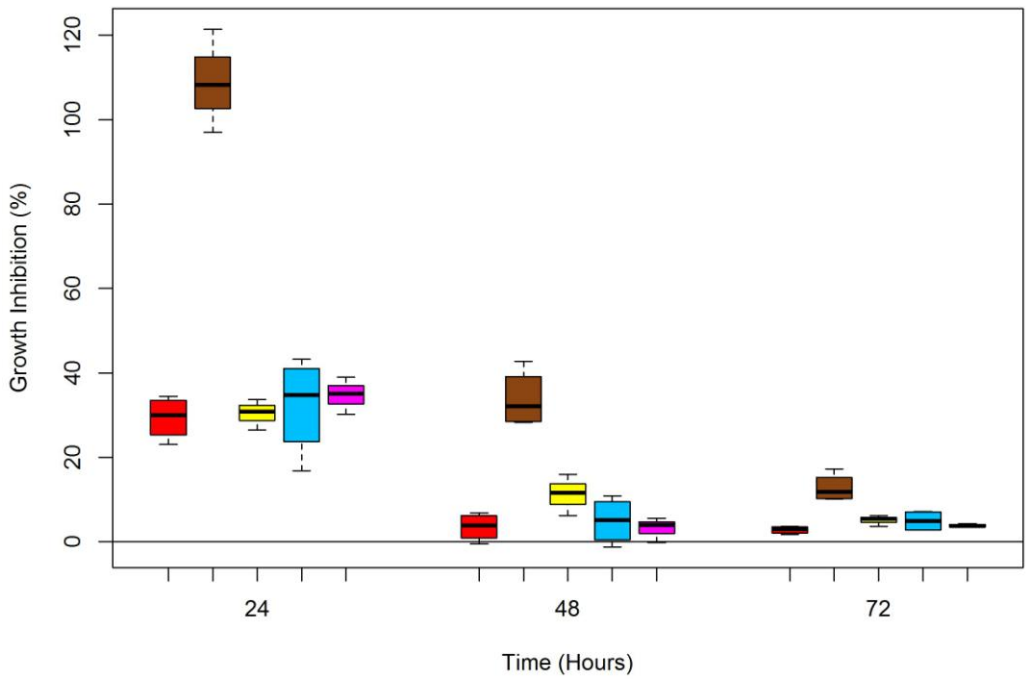

Figure VIII.25 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 30/01/2012. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.26 and Figure VIII.26 show the results of the algal growth test conducted with water collected on 24/04/2012. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.26 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 24/04/2012. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	49	4	23	1	11	1
Lea Nav at Stonebridge Brook	58	2	22	2	10	1
Lea Nav at Springfield Park	43	5	19	4	6	2
Lea Nav at Lea Bridge weir	55	7	23	3	7	2
River Lea at Hackney Marshes	45	7	21	3	7	2

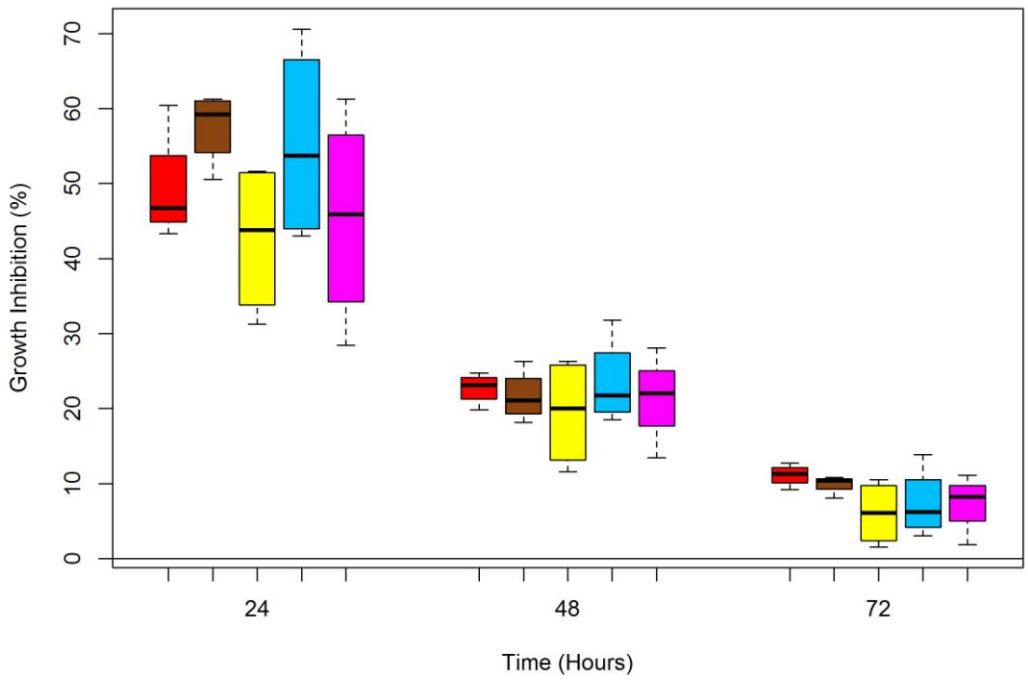

Figure VIII.26 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 30/01/2012. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.27 and Figure VIII.27 show the results of the algal growth test conducted with water collected on 02/07/2012. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.27 – Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 02/07/2012. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	14	2	8	3	5	1
Lea Nav at Stonebridge Brook	30	2	41	1	56	1
Lea Nav at Springfield Park	13	3	7	1	4	1
Lea Nav at Lea Bridge weir	7	2	5	2	5	2
River Lea at Hackney Marshes	2	4	4	1	4	1

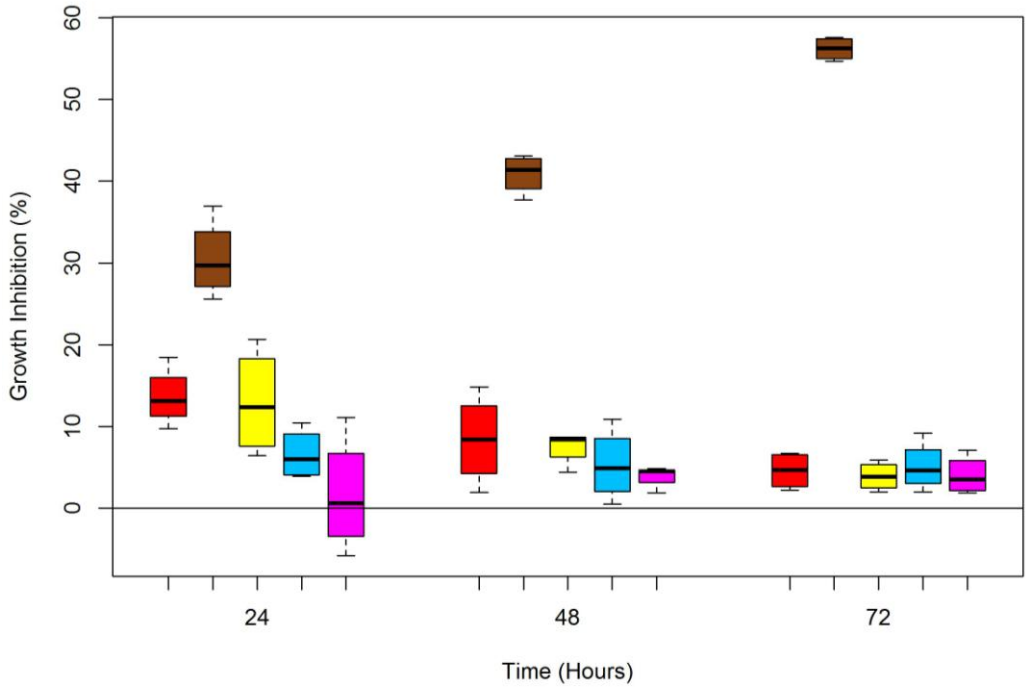

Figure VIII.27 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 02/07/2012. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.28 and Figure VIII.28 show the results of the algal growth test conducted with water collected on 16/07/2012. The level of inhibition was calculated with respect to the algal growth in the Tottenham Hale water sample.

Table VIII.28– Algal growth inhibition (%) and standard error of the mean (SEM) obtained with river water samples collected on 16/07/2012. A water sample from Tottenham Hale was used as the control.

Sampling station	24 hours		48 hours		72 hours	
	Inhibition (%)	SEM	Inhibition (%)	SEM	Inhibition (%)	SEM
Pymmes Brook	3	3	2	1	1	2
Lea Nav at Stonebridge Brook	111	0	83	9	89	2
Lea Nav at Springfield Park	5	3	2	2	2	1
Lea Nav at Lea Bridge weir	2	4	6	6	1	2
River Lea at Hackney Marshes	3	2	1	1	0	1

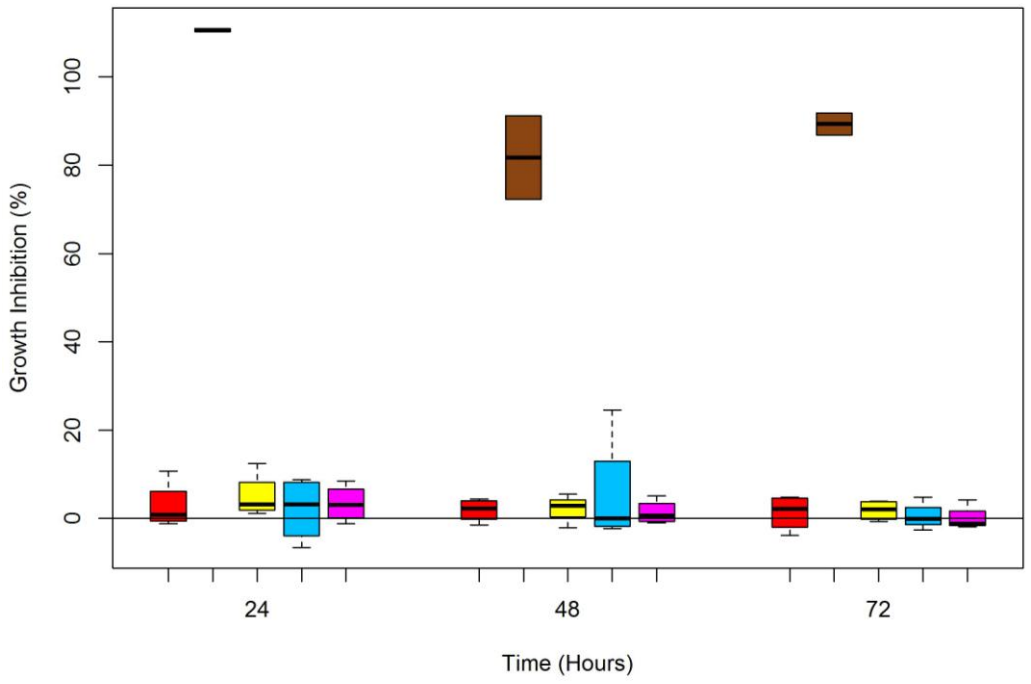

Figure VIII.28 – Box plot representing the results of *P. subcapitata* growth inhibition test conducted with water collected on 16/07/2012. The level of inhibition was calculated with respect to the algal growth in Tottenham Hale water sample (control). Legend: Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table VIII.29 and Figure VIII.29 show the *P. subcapitata* growth in Tottenham Hale water samples compared to the growth in the OECD medium.

Table VIII.29 – Comparison between growth rate (d^{-1}) of *P. subcapitata* cultured in OECD medium and Tottenham Hale water sample after 24 hours, in twelve occasions. Four replicates were used for each test solution.

Sampling date	OECD medium		Lea Nav at Tottenham Hale	
	Algal growth (d^{-1})	SEM	Algal growth (d^{-1})	SEM
06/09/2010	1.30	0.03	1.24	0.03
13/09/2010	1.53	0.02	1.36	0.01
20/09/2010	1.24	0.07	1.29	0.04
11/10/2010	1.61	0.05	1.31	0.07
01/11/2010	1.47	0.03	1.34	0.02
23/05/2011	1.41	0.04	1.37	0.04
06/06/2011	1.13	0.03	1.13	0.04
28/06/2011	1.32	0.03	1.28	0.01
18/07/2011	1.37	0.03	1.37	0.04
22/08/2011	1.24	0.03	1.15	0.01
03/10/2011	1.49	0.04	1.35	0.01

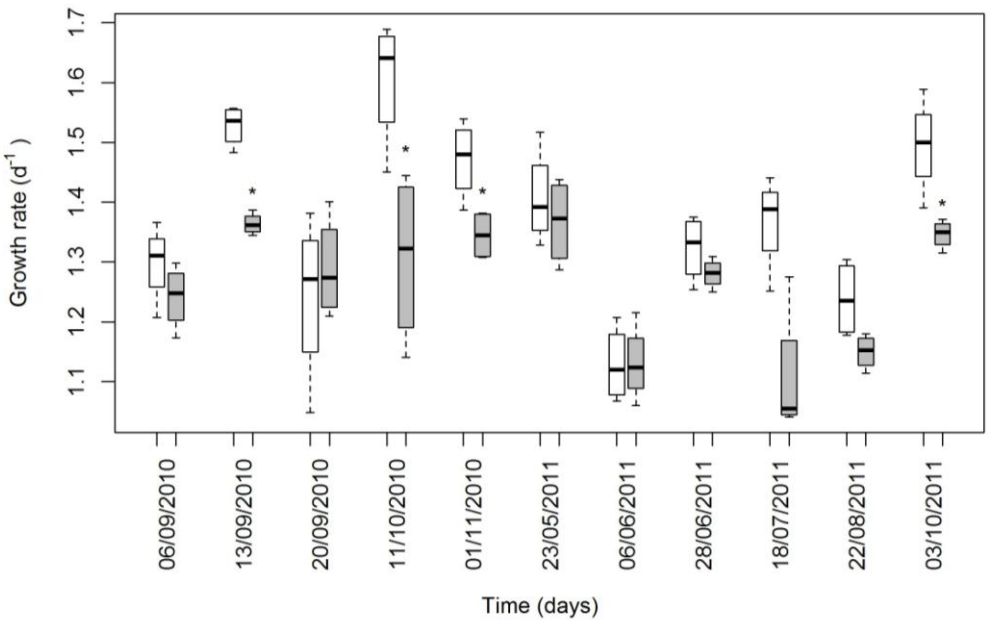

Figure VIII.29 – Box plot representing the comparison between the growth rate (d^{-1}) in OECD medium and Tottenham Hale water samples after 24 hours of testing. The test was conducted with water collected on different sampling days. * The algal growth differs statistically ($p < 0.05$, t-test) between the two test samples. Legend: ☐ OECD medium; ▨ Lea Nav at Tottenham Hale.

Appendix IX: Chemical analysis results

Chemical analyses were performed by Environment Agency's laboratories.

Table IX.1– Organic volatile compounds detected by gas chromatography–mass spectrometry (GCMS) in river water samples collected on the 28/06/2011 at Pymmes Brook and in the Lea Navigation at Tottenham Hale and Springfield Park. (1) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 0.1 µg/l. (2) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 0.12 µg/l.

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)
1(3H)-isobenzofuranone	87412			
1,3-dichlorobenzene	541731		0.09	0.06
1,4-dioxane	123911	0.14	0.4	0.35
1H-benzotriazole	95147		3.4	
2,2',3,3',4,4',5-heptachlorobiphenyl	35065306			0.01
2,2',3,4,4',5,5'-heptachlorobiphenyl	35065293			0.01
2,2',3,4,4',5'-hexachlorobiphenyl	35065282			0.26
2,2',3,4,5,5'-hexachlorobiphenyl	52712046			0.17
2,2',3,4,5'-pentachlorobiphenyl	38380028			0.08
2,2',3,5,5',6-hexachlorobiphenyl	52663635			0.02
2,2',3,5'-tetrachlorobiphenyl	41464395			0.06
2,2',4,4',5,5'-hexachlorobiphenyl	35065271			0.13
2,2',4,5,5'-pentachlorobiphenyl	37680732			0.02
2,2',5,5'-tetrachlorobiphenyl	35693993			0.16
2,3,3',4',6-pentachlorobiphenyl	38380039			0.37
2,3',4,4'-tetrachlorobiphenyl	32598100			0.16
2,3,5-trichlorophenol	933788		0.01	
2,4,7,9-tetramethyl-5-decyne-4,7-diol	126863	0.2	0.86	0.58
3,5-dimethylphenol	108689		0.02	
acenaphthene	83329			0.01

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)
alpha Isomethyl Ionone	127515			0.07
anthracene [(1)]	120127			0.01
bentazone	25057890	0.06		
benz[a]anthracene	56553	0.01		0.03
benzophenone	119619	0.03	0.12	0.16
benzophenone-3	131577		0.09	0.05
bis(2-ethylhexyl)phthalate (DEHP)	117817			3.7
bisphenol A	80057		0.05	0.08
bromodichloromethane	75274		0.1	0.1
bromoform	75252	0.07	0.1	0.13
butylated hydroxyanisole	25013165		0.2	0.14
caffeine	58082	0.16	0.56	0.48
carbamazepine	298464	0.17	0.6	
chlorobenzene	108907		0.01	
chlorodibromomethane	124481		0.25	0.2
chloroxylenol	88040		0.17	0.12
cholesterol	57885			66
chrysene	218019	0.01		0.04
crotamiton	483636	0.1	0.53	0.38
cyclohexanone	108941	0.1	0.06	0.09
dibromomethane	74953	0.02	0.04	0.03
dimethyl phthalate	131113			0.04
dimetridazole	551928		0.3	0.23
fipronil	120068373		0.03	

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)
fluoranthene [2]	206440	0.03	0.01	0.08
fluorene	86737			0.01
gabapentin	60142963	0.14	0.25	0.22
isophorone	78591	0.01		
lidocaine	137586		0.3	0.22
lilial	80546			0.08
metaldehyde	108623	0.06	0.07	0.07
N,N,N',N'-tetraacetylethylenediamine	10543574		0.8	0.66
N,N-Diethyl-m-toluamide	134623	0.14	0.38	0.23
phenanthrene	85018			0.03
pyrazine	290379	0.1		0.09
pyrene	129000	0.02	0.01	0.06
squalane	111013			15
sulfur (S8)	10544500			0.23
terpineol	98555		0.09	0.12
tetrachloroethylene	127184		1.8	0.15
tri-(2-chloroethyl) phosphate	115968	0.37	0.67	0.5
tributyl phosphate	126738		0.06	
triclosan	3380345		0.02	
triphenyl phosphate	115866	0.15		0.25
tris-(1,3-dichloroisopropyl) phosphate	13674878		0.15	

Table IX.2 – Polar analytes detected by liquid chromatography–mass spectrometry (LCMS) in river water samples collected on the 28/06/2011 at Pymmes Brook and in the Lea Navigation at Tottenham Hale and Springfield Park. Results are based on three criteria: retention time on column, accurate mass measurement, and sigma fit (statistical test of the isotopes present compared to the theoretical isotope pattern). Scores: 3 = all criteria passed (compound present); 2 = one of the criteria did not pass but still a likely hit; 1 = only one of the criteria passed but is a possible hit. (1) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 36 µg/l.

Chemical Name	Lea Navigation at Tottenham Hale	Pymmes Brook	Lea Navigation at Springfield Park
2,4-D			2
Atenolol		3	3
Azoxystrobin	3		
Carbamazepine	3	3	3
Carbendazim	3		
Celiprolol	2	2	2
Dichloroprop			
Diclofenac		3	3
Diuron	3	3	3
MCPA			
MCPP			3
Mefenamic acid		2	2
Paracetamol	3		
Perfluorodecanoic acid		3	3
Perfluorohexane sulfonate		3	3
Perfluorononanoic acid		3	3
Perfluorooctane sulfonate (1)		3	3
Perfluorooctanoic acid			3
Pirimiphos-methyl		2	2

Chemical Name	Lea Navigation at Tottenham Hale	Pymmes Brook	Lea Navigation at Springfield Park
Propranolol			
Sotalol		3	3
Sulfamethoxazole	3	3	3
Terbutryn	3	3	3
Thiabendazole	3	2	2
Trimethoprim	3	3	3

Table IX.3 – Organic volatile compounds detected by gas chromatography–mass spectrometry (GCMS) in river water samples collected on the 07/11/2011 at 6 sampling stations in the area under investigation. (1) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 0.1 µg/l. (2) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 0.27 µg/l. (3) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 0.017 µg/l. (4) Priority hazardous substance (COM(2011)876). Maximum allowable concentration (inland surface waters) = 0.0082 µg/l.

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Stonebridge Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)	Lea Navigation at Lea Bridge weir (µg/l)	River Lea at Hackney Marshes (µg/l)
1,3-dichlorobenzene	541731	0.01	0.1		0.1	0.1	0.08
1,4-Dioxane	123911	0.3	0.6	0.4	0.57	0.5	0.45
1,2,4-Trimethylbenzene	95636			0.1			
1,3-dichlorobenzene	541731			0.05			
1H-Benzotriazole	95147	1.37	2.3	1.9	1.6		1.3
1H-Benzotriazole-5-methyl	136856		1.04	1.8	0.7		0.3
2(3H)-Benzothiazolone	934349	0.09					
2H-Indol-2-one, 1,3-dihydro	59483			3			
2-Methoxynaphthalene	93049			0.1	0.01	0.01	
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	126863	0.23	0.63	0.6	0.38	0.23	0.18
4,7-Methano-1H-indenol, hexahydro	37275493			0.8			
9,12-Octadecadienoic acid (Z,Z)	60333			11			
acenaphthene	83329		0.01	0.01	0.01	0.01	0.01
acetophenone	98862			0.19			
alpha Isomethyl Ionone	127515		0.05	0.36	0.07	0.04	0.05

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Stonebridge Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)	Lea Navigation at Lea Bridge weir (µg/l)	River Lea at Hackney Marshes (µg/l)
Androstadiendione	1000335463			0.8			
anthracene [1]	120127						0.01
Benz[a]anthracene	56553					0.01	0.04
benzo[a]pyrene [2]	50328						0.03
benzo[b]fluoranthene [3]	205992						0.03
benzo[ghi]perylene [4]	191242						0.02
Benzophenone	119619	0.03	0.09	0.28	0.09		0.1
Benzophenone-3	131577			0.2			
Benzyl Salicylate	118581			0.67			
Bisphenol A	80057	0.05	0.1	0.47	0.09	0.09	0.1
Bromodichloromethane	75274		0.1	0.2	0.13	0.15	0.1
Bromoform	75252	0.04	0.13	0.18	0.14	0.08	0.08
Butylated hydroxyanisole	25013165		0.18	0.16	0.13	0.13	0.1
Butylated hydroxytoluene	128370		1.19				
Caffeine	58082	0.14	0.55	17	0.78	0.55	0.45
Carbamazepine	298464	0.26	0.64	0.45	0.54	0.54	0.5
cedrol	77532			0.3			
Chlorodibromomethane	124481		0.26	0.38	0.23	0.2	0.16
Chloroxylenol	88040		0.33	2.6	0.3	0.16	0.1
Cholesterol	57885		9	58	9	9	9
Chrysene	218019					0.01	0.04
cocaine	50362			0.86			
coumarin	91645			0.4			

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Stonebridge Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)	Lea Navigation at Lea Bridge weir (µg/l)	River Lea at Hackney Marshes (µg/l)
Crotamiton	483636	0.18	0.5	0.28	0.4	0.33	0.3
Cyclohexanone	108941			0.15	0.1	0.1	0.05
Cyclopentaneacetic, 3-oxo-2-pentyl-, methyl ester	24851987			1.5			
d-Limonene	5989275			0.2			
Dibromomethane	74953		0.06	0.05	0.05	0.04	0.03
Diethyl phthalate	84662			4			
dimethyl phthalate	131113			0.1			
di-n-butyl phthalate	84742		2	1.7			
eugenol	97530			0.9			
Ethylparaben	120478			0.2			
fluoranthene	206440	0.01	0.01	0.03	0.03	0.04	0.09
Fluorene	86737			0.01	0.01	0.01	0.01
Gabapentin	60142963	0.25	0.34	0.16	0.29	0.29	0.23
Geraniol	106241			0.12			
Hexyl Cinnamaldehyde	101860			0.1			
ibuprofen	15687271			0.06			
Isopropyl palmitate	142916			3.3			
Lilial	80546			0.18	0.09		0.05
Linalool	78706			1.7			
Methylparaben	99763			0.2			
n-Hexadecanoic acid	57103			11			
nicotine	54115			0.17			

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Stonebridge Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)	Lea Navigation at Lea Bridge weir (µg/l)	River Lea at Hackney Marshes (µg/l)
N,N,N',N'-Tetraacetylethylenediamine	10543574	0.12	0.92	25	1.1	0.77	0.6
N,N-Diethyl-m-toluamide	134623	0.34	0.24	0.24	0.17	0.13	0.13
Octyl-methoxycinnamate	5466773			0.19			
Oleic acid	112801			32			
p-Benzoquinone	106514			0.5			
p-Cresol (4-methylphenol)	106445			0.25			
phenanthrene	85018			0.03	0.02	0.02	0.03
phenol	108952			0.2			
Propylparaben	94133			0.38			
Pyrazine	290379	0.2				0.16	
pyrene	129000	0.01	0.01	0.03	0.02	0.03	0.08
Squalane	111013						
Sulfur (S8)	10544500		0.05	0.44	0.13	0.1	0.2
Terpineol	98555		0.15	3	0.23	0.1	0.06
Tetrachloroethylene	127184		0.2		0.25	0.2	0.2
toluene	108883			1.5			
Tri-(2-chloroethyl) phosphate	115968	0.13	0.29	0.27	0.19	0.19	0.19
Tributyl phosphate	126738	0.05					
Triclosan	3380345		0.02	0.09	0.02	0.02	0.02
Triclosan-methyl	4640011		0.01		0.01	0.01	
Triphenyl phosphate	115866	0.07	0.09	0.18	0.08	0.06	0.09

Chemical Name	Cas#	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Stonebridge Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)	Lea Navigation at Lea Bridge weir (µg/l)	River Lea at Hackney Marshes (µg/l)
Tris-(1,3-dichloroisopropyl) phosphate	13674878		0.09	0.28			

Table IX.4 – Polar analytes detected by liquid chromatography–mass spectrometry (LCMS) in river water samples collected on the 07/11/2011 at 6 sampling stations in the area under investigation. Results are based on three criteria: retention time on column, accurate mass measurement, and sigma fit (statistical test of the isotopes present compared to the theoretical isotope pattern). Scores: 3 = all criteria passed (compound present); 2 = one of the criteria did not pass but still a likely hit; 1 = only one of the criteria passed but is a possible hit.

Chemical Name	Lea Navigation at Tottenham Hale (µg/l)	Pymmes Brook (µg/l)	Lea Navigation at Stonebridge Brook (µg/l)	Lea Navigation at Springfield Park (µg/l)	Lea Navigation at Lea Bridge weir (µg/l)	River Lea at Hackney Marshes (µg/l)
Benzalkonium C10				2		
Carbamazepine		3	3	3		3
Hexadecyltrimethylammonium				2		
Paracetamol				3		

Table IX.5– General parameters analysed in river water samples collected on the 07/11/2011 at 6 sampling stations in the area under investigation. BOD_5 = Biological Oxygen Demand. NH_3-N = ammoniacal nitrogen. N_{OX} = total oxidized nitrogen. Reactive P = orthophosphate.

Parameters	Lea Navigation at Tottenham Hale	Pymmes Brook	Lea Navigation at Stonebridge Brook	Lea Navigation at Springfield Park	Lea Navigation at Lea Bridge weir	River Lea at Hackney Marshes
BOD_5 (mg/l)	<1	2.7	19.1	2.7	1.9	1.6
NH_3-N (mg/l)	0.046	0.823	2.8	0.97	0.466	0.398
N_{OX} (mg/l)	4.74	16.9	5.85	16.7	15.5	14.7
Chloride (mg/l)	88.8	109	90.7	110	104	108
Reactive P (mg/l)	0.122	3.06	1.88	3	2.72	2.66
Turbidity (NTU)	<1	6.4	9.7	2.51	9	3.8

Table IX.6 – General parameters analysed in river water samples collected on the 31/01/2012 at 6 sampling stations in the area under investigation. BOD_5 = Biological Oxygen Demand. NH_3-N = ammoniacal nitrogen. N_{OX} = total oxidized nitrogen. Reactive P = orthophosphate.

Parameters	Lea Navigation at Tottenham Hale	Pymmes Brook	Lea Navigation at Stonebridge Brook	Lea Navigation at Springfield Park	Lea Navigation at Lea Bridge weir	River Lea at Hackney Marshes
BOD_5 (mg/l)	1.2	4.1	15.9	2.5	2	1.9
NH_3-N (mg/l)	<0.03	0.566	2.98	1.07	0.726	0.632
N_{OX} (mg/l)	5.92	13.2	7.49	13.6	14	13.9
Chloride (mg/l)	84.3	114	94.2	106	109	109
Reactive P (mg/l)	0.312	3.01	1.86	2.8	2.75	2.69
Turbidity (NTU)	1.7	2.7	8.2	2.6	2.4	3.3
Faecal Coliform (NO/100ml)	450	35000	>100000	28000	12000	6500
Faecal Streptococci (NO/100ml)	18	2100	27000	1636	937	856
Total Coliform (NO/100ml)	1545	61000	>100000	77000	>100000	40000

Appendix X: River water monitoring with *P. subcapitata* cells entrapped in alginate beads

The algal growth rate (d^{-1}) was calculated following OECD guidelines (2006) and it was estimated over four replicates for each test solution.

The box plots were calculated with R software (open source software, available at http://www.R-project.org). The black horizontal line in the box represents the median. The box stretches out to the third quartile (above the median), and to the first quartile (below the median). The ends of the whiskers represent the minimum and maximum data points.

Results are given following.

Table X.1 and Figure X.1 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride), tested in the laboratory with water samples collected on 09/03/2011. River water samples were enriched with OECD medium and changed every 24 hours. Test duration: 5 days.

Table X.1 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples enriched with OECD medium. The test was conducted with water collected on 09/03/2011.

Sampling station	Day 1		Day 2		Day 3	
	Growth rate (d^{-1})	SEM	Growth rate (d^{-1})	SEM	Growth rate (d^{-1})	SEM
OECD medium	1.5	0.02	1.1	0.03	0.6	0.01
Lea Nav at Tottenham Hale	1.5	0.03	1.1	0.01	0.6	0.00
Pymmes Brook	1.5	0.03	1.0	0.00	0.5	0.00
Lea Nav opposite Warwick reservoir	1.5	0.01	1.1	0.01	0.5	0.00
Lea Nav at Springfield Park	1.5	0.01	1.1	0.01	0.5	0.00

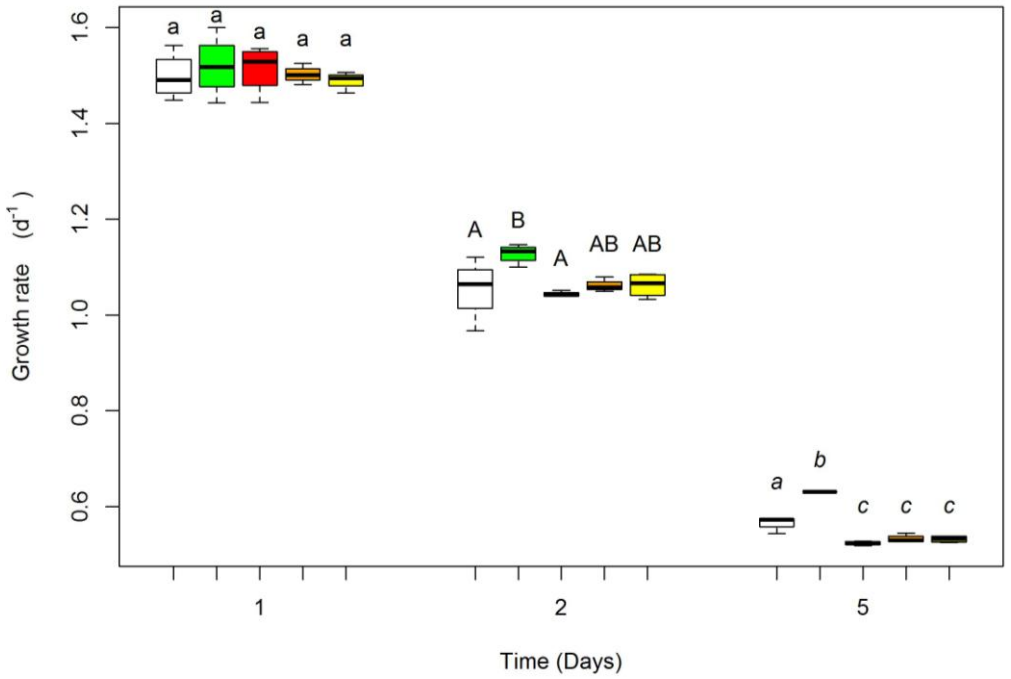

Figure X.1 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples enriched with OECD medium. Boxes with different letters differ significantly ($p < 0.05$). The test was conducted with water collected on 09/03/2011. Legend: ☐ OECD medium ☐ Lea Nav at Tottenham Hale; ☐ Pymmes Brook; ☐ Lea Nav opposite Warwick reservoir; ☐ Lea Nav at Springfield Park.

Table X.2 and Figure X.2 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride), tested in the laboratory with water samples collected on 14/03/2011. River water samples were enriched with OECD medium and changed every 24 hours. Test duration: 3 days.

Table X.2 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples enriched with OECD medium. The test was conducted with water collected on 14/03/2011.

Sampling station	Day 1		Day 2		Day 3	
	Growth rate (d^{-1})	SEM	Growth rate (d^{-1})	SEM	Growth rate (d^{-1})	SEM
OECD medium	2.0	0.03	1.4	0.01	1.1	0.01
Lea Nav at Tottenham Hale	1.9	0.02	1.5	0.01	1.2	0.00
Pymmes Brook	1.9	0.02	1.3	0.01	1.0	0.01
Lea Nav at Springfield Park	1.9	0.03	1.4	0.01	1.0	0.01

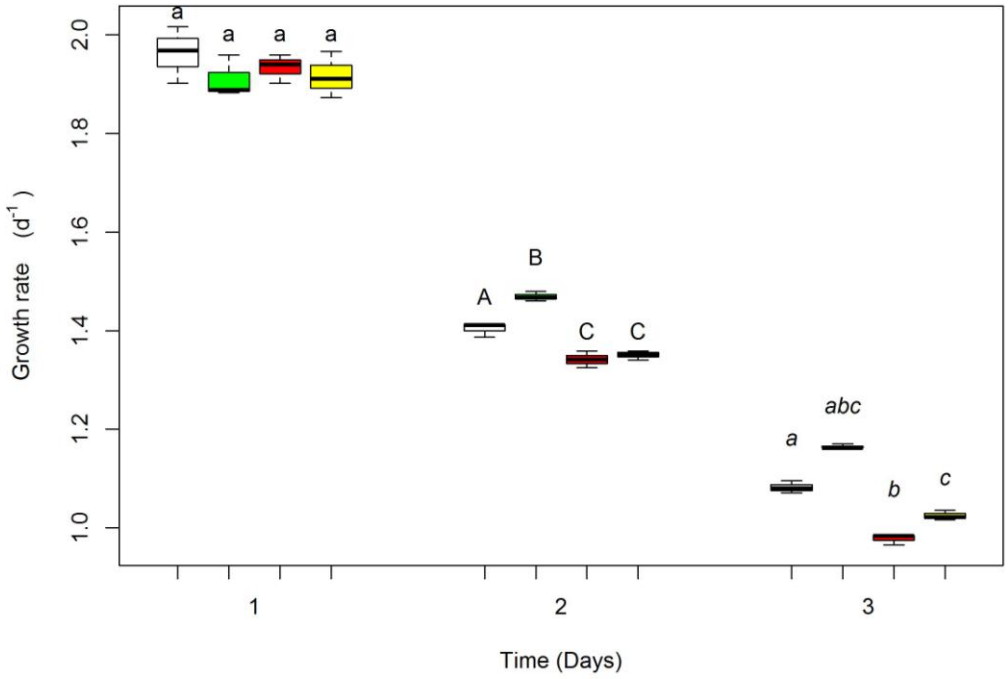

Figure X.2 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples enriched with OECD medium. Boxes with different letters differ significantly ($p < 0.05$). The test was conducted with water collected on 14/03/2011. Legend: ☐ OECD medium ■ Lea Nav at Tottenham Hale; ■ Pymmes Brook; ■ Lea Nav at Springfield Park.

Table X.3 and Figure X.3 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride), tested in the laboratory with water samples collected on 21/11/2011. Test duration: 3 days.

Table X.3 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples. The test was conducted with water collected on 21/11/2011.

Sampling station	Day 1		Day 2		Day 3	
	Growth rate (d^{-1})	SEM	Growth rate (d^{-1})	SEM	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	1.6	0.04	1.3	0.02	1.1	0.02
Pymmes Brook	1.5	0.02	1.1	0.01	0.8	0.01
Lea Nav at Springfield Park	1.5	0.02	1.1	0.01	0.8	0.02

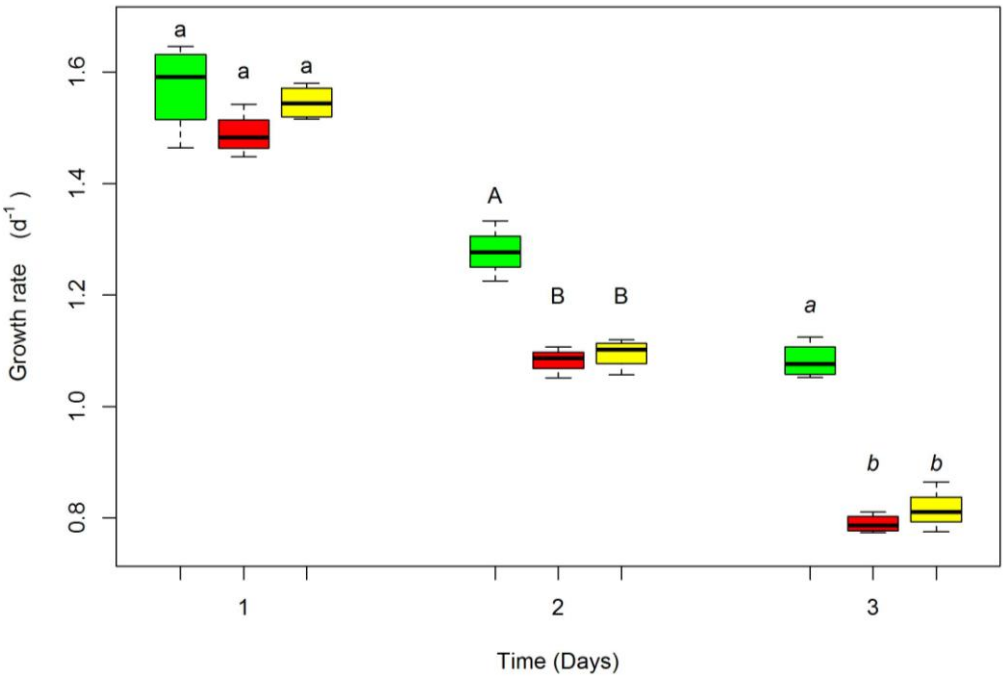

Figure X.3 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed to river water samples. Boxes with different letters differ significantly ($p < 0.05$). The test was conducted with water collected on 21/11/2011. Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Springfield Park.

Table X.4 and Figure X.4 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride), tested *in situ*. Beads were placed on 28/11/2011. Test duration: 7 days. Beads at Pymmes Brook and Lea Navigation at Stonebridge Brook were dissolved at the end of the test.

Table X.4 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed *in situ* to river water samples for 7 days. Beads were placed in the river on 28/11/2011.

Sampling station	Day 7	
	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	0.11	0.01
Lea Nav at Springfield Park	0.05	0.01
Lea Nav at Lea Bridge weir	0.11	0.00
River Lea at Hackney Marshes	0.10	0.00

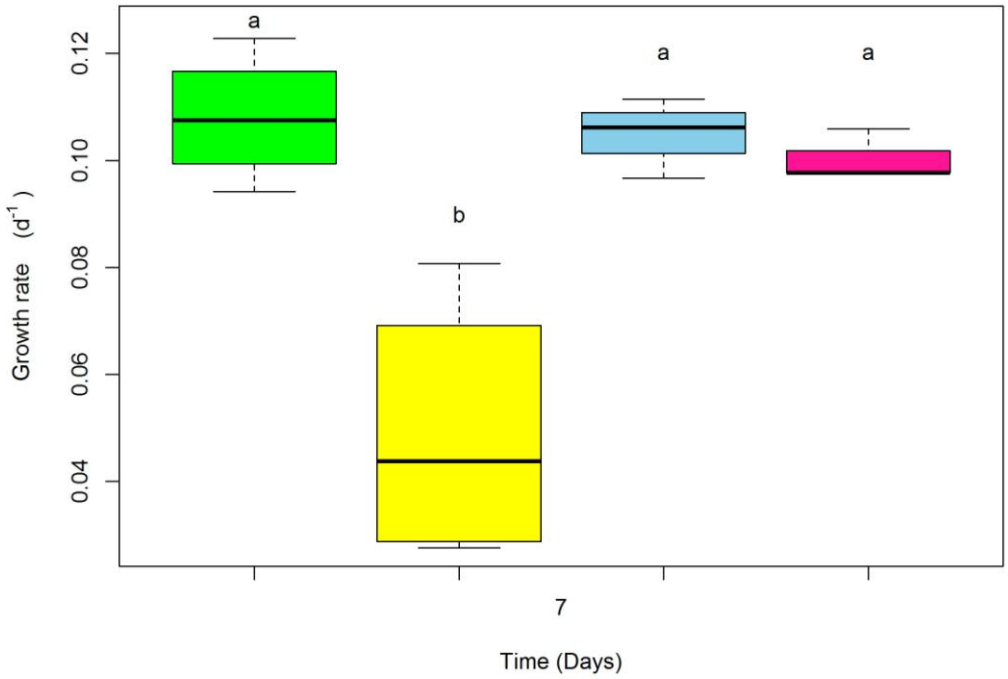

Figure X.4 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed *in situ* to river water samples for 7 days. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table X.5 and Figure X.5 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride), tested *in situ*. Beads were placed on 05/12/2011. Test duration: 7 days. Beads at Pymmes Brook, Lea Navigation at Stonebridge Brook, at Springfield Park, and at Lea Bridge weir were dissolved at the end of the test.

Table X.5 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed *in situ* to river water samples for 7 days. Beads were placed in the river on 05/12/2012.

Sampling station	Day 7	
	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	0.13	0.00
River Lea at Hackney Marshes	0.13	0.00

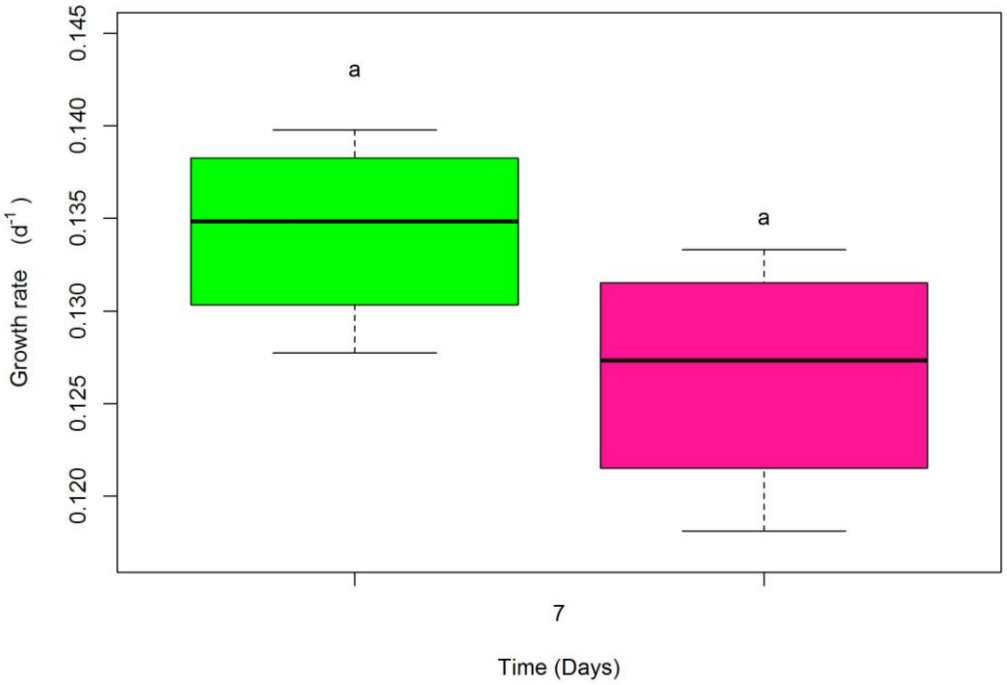

Figure X.5 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 2 % calcium chloride) and exposed *in situ* to river water samples for 7 days. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; River Lea at Hackney Marshes.

Table X.6 and Figure X.6 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride), tested *in situ*. Beads were placed on 20/02/2012. Test duration: 3 days. Beads at Hackney Marshes were stolen.

Table X.6 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 3 days. Beads were placed in the river on 20/02/2012.

Sampling station	Day 3	
	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	0.14	0.03
Pymmes Brook	0.12	0.01
Lea Nav at Stonebridge Brook	0.17	0.01
Lea Nav at Springfield Park	0.15	0.02
Lea Nav at Lea Bridge weir	0.15	0.01

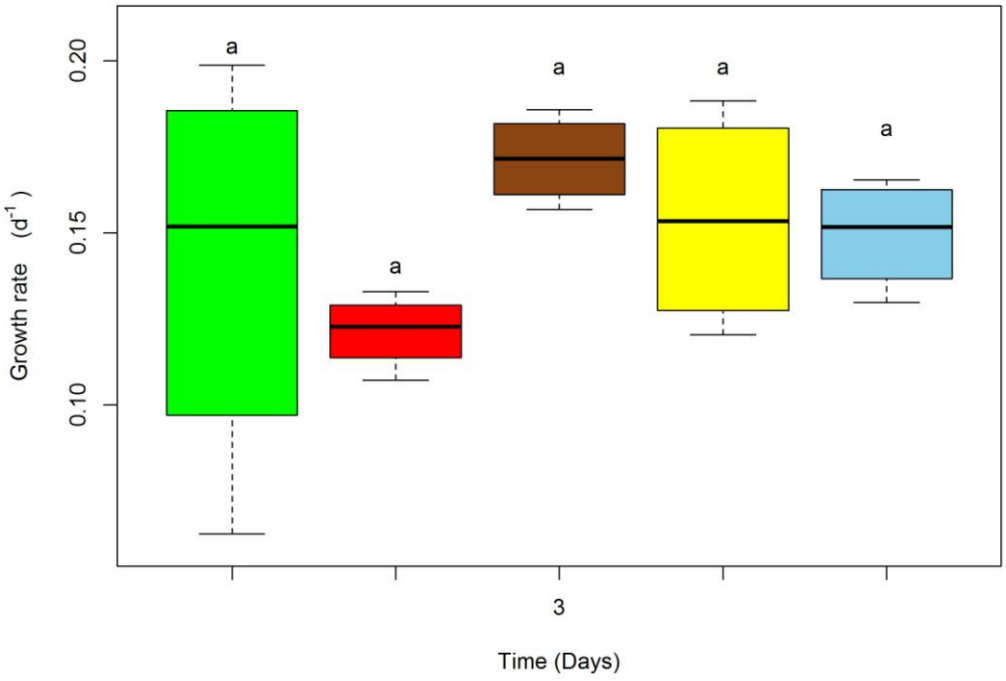

Figure X.6 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 3 days. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir.

Table X.7 and Figure X.7 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride), tested *in situ*. Beads were placed on 20/02/2012. Test duration: 7 days.

Table X.7 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 7 days. Beads were placed in the river on 20/02/2012.

Sampling station	Day 7	
	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	0.12	0.00
Pymmes Brook	0.15	0.00

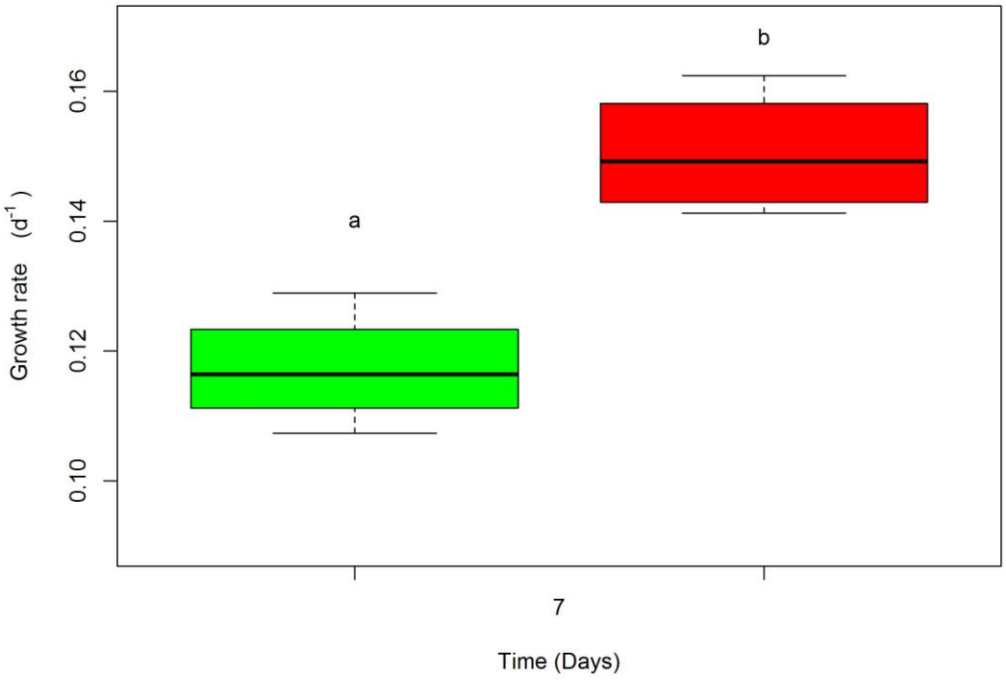

Figure X.7 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (3 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 7 days. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Pymmes Brook.

Table X.8 and Figure X.8 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride), tested *in situ*. Beads were placed on 12/03/2012. Test duration: 3 days.

Table X.8 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 3 days. Beads were placed in the river on 12/03/2012.

Sampling station	Day 3	
	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	0.34	0.01
Pymmes Brook	0.53	0.01
Lea Nav at Stonebridge Brook	0.42	0.01
Lea Nav at Springfield Park	0.25	0.01
Lea Nav at Lea Bridge weir	0.49	0.01
River Lea at Hackney Marshes	0.35	0.00

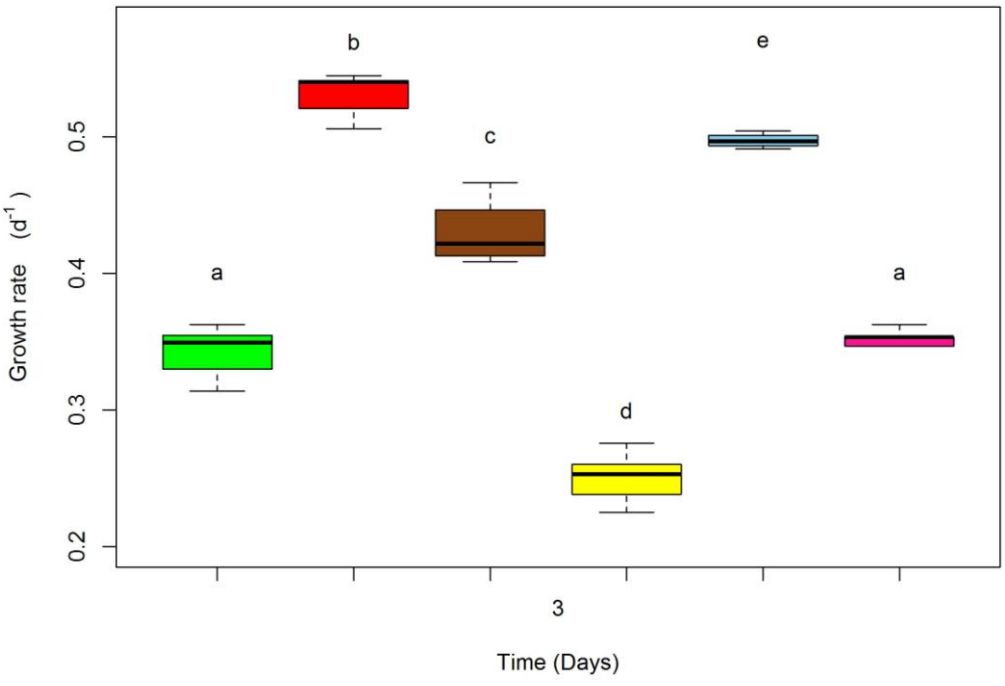

Figure X.8 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 3 days. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Pymmes Brook; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir; River Lea at Hackney Marshes.

Table X.9 and Figure X.9 show the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride), tested *in situ*. Beads were placed on 30/04/2012. Test duration: 3 days. Beads were dissolved at Pymmes Brook and Hackney Marshes.

Table X.9 – Growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 3 days. Beads were placed in the river on the 30/04/2012.

Sampling station	Day 3	
	Growth rate (d^{-1})	SEM
Lea Nav at Tottenham Hale	0.32	0.01
Lea Nav at Stonebridge Brook	0.43	0.00
Lea Nav at Springfield Park	0.47	0.00
Lea Nav at Lea Bridge weir	0.35	0.01

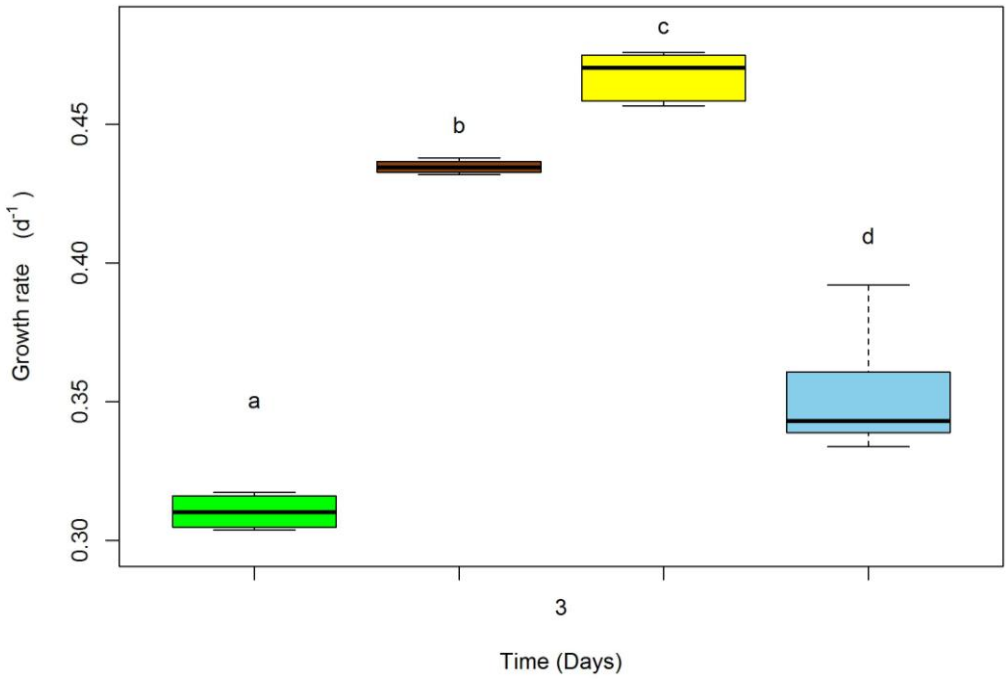

Figure X.9 – Box plot representing the growth rate (d^{-1}) of *P. subcapitata* cells entrapped in alginate beads (2 % sodium alginate and 4 % calcium chloride) and exposed *in situ* to river water samples for 3 days. Boxes with different letters differ significantly ($p < 0.05$). Legend: Lea Nav at Tottenham Hale; Lea Nav at Stonebridge Brook; Lea Nav at Springfield Park; Lea Nav at Lea Bridge weir.

Appendix XI: *In situ* physico-chemical monitoring

Physico-chemical parameters were collected at several locations along the river using a multiparametric probe (YSI 6820), provided by the Environment Agency, during four surveys. Results are given in Tables XI.1, XI.2, XI.3, and XI.4.

Table XI.1 – Physico-chemical parameters collected *in situ* on the 22/08/2011 at twenty-three sites in the area under investigation.

Sampling site	Time (hours)	Temperature water (°C)	Conductivity (µS/cm)	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	pH	Total ammonia (mg/L)
0 – Tottenham Hale	12:28	19	761	119	11.0	8.37	0.49
2 – Pymmes Brook	12:20	21	1027	66	5.9	7.38	1.13
4b	13:46	21	993	67	5.9	7.40	0.98
5	12:09	20	993	57	5.1	7.36	1.14
6 – Old River Lea	13:26	20	645	115	10.4	8.82	0.38
8	11:48	20	964	42	3.8	7.28	1.19
9	11:38	20	937	43	3.9	7.30	1.13
10	11:24	19	892	48	4.4	7.36	0.99
11 – Old Moselle Brook	11:13	19	879	46	4.2	7.36	0.98
11b	11:04	19	891	49	4.5	7.37	0.96
12 – Stonebridge Brook	10:54	19	884	48	4.4	7.38	0.96
13	10:41	19	882	48	4.4	7.37	0.93
15	10:23	19	911	45	4.1	7.30	0.99
17	10:16	19	912	45	4.1	7.30	1.01
19	09:46	19	913	44	4.0	7.28	1.03
20 - Coppermill	09:56	16	738	28	2.8	7.25	0.44
21 – Springfield Park	09:34	19	911	43	4.0	7.28	1.03
22	14:26	20	895	43	3.9	7.28	1.04
24	14:44	20	904	41	3.7	7.28	0.98
26	15:03	20	910	39	3.5	7.28	0.98
27 – Lea Bridge weir	15:14	20	911	37	3.3	7.28	0.96
28	15:25	20	883	28	2.5	7.30	1.01
29 – River Lea	15:42	20	907	57	5.2	7.36	0.95

Table XI.2 – Physico-chemical parameters collected *in situ* on the 31/10/2011 at twenty-three sites in the area under investigation.

Sampling site	Time (hours)	Temperature water (°C)	Conductivity (µS/cm)	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	pH	Total ammonia (mg/L)
0 – Tottenham Hale	09:43	13	900	87	9.2	8.13	1.11
2 – Pymmes Brook	10:20	18	1156	48	4.5	7.26	2.19
4b	12:20	18	1156	53	5.0	7.28	1.72
5	12:26	18	1162	53	5.0	7.27	1.61
6 – Old River Lea	09:56	14	795	84	8.8	7.93	0.97
8	12:14	17	1089	45	4.4	7.35	1.93
9	12:08	17	1090	44	4.3	7.35	1.91
10	12:00	17	1073	44	4.2	7.33	2.06
11 – Old Moselle Brook	11:44	16	1043	40	3.9	7.34	2.01
11b	11:39	16	1024	32	3.2	7.37	2.06
12 – Stonebridge Brook	11:30	16	1014	20	2.0	7.38	2.17
13	11:25	16	1057	37	3.6	7.30	2.22
15	11:16	17	1086	34	3.3	7.26	2.32
17	11:07	17	1092	33	3.2	7.24	2.32
19	10:49	17	1089	34	3.3	7.25	2.48
20 - Coppermill	10:56	15	1010	19	1.9	7.27	1.47
21 – Springfield Park	12:57	17	1079	34	3.3	7.27	2.05
22	13:21	17	1093	32	3.0	7.26	2.14
24	13:29	17	1097	32	3.1	7.26	2.05
26	13:40	17	1097	31	3.0	7.27	1.09
27 – Lea Bridge weir	13:50	17	1093	30	2.9	7.28	1.62
28	13:55	17	1089	30	2.9	7.31	1.45
29 – River Lea	14:12	17	1090	62	6.0	7.42	1.41

Table XI.3 – Physico-chemical parameters collected *in situ* on the 09/01/2012 at twenty-three sites in the area under investigation.

Sampling site	Time (hours)	Temperature water (°C)	Conductivity (µS/cm)	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	pH	Total ammonia (mg/L)
0 – Tottenham Hale	09:42	4	918	92	12.0	8.02	1.64
2 – Pymmes Brook	09:51	10	1125	57	6.3	7.26	2.77
4b	10:14	10	1097	48	5.4	7.20	2.86
5	10:07	10	1101	52	5.8	7.22	2.75
6 – Old River Lea	11:07	4	781	99	13.0	8.03	1.38
8	11:34	10	1091	47	5.3	7.21	2.45
9	11:40	9	1063	56	6.4	7.27	2.41
10	11:50	9	1070	53	6.0	7.24	2.50
11 – Old Moselle Brook	11:56	9	1026	55	6.4	7.29	2.41
11b	12:02	8	1008	54	6.4	7.28	2.55
12 – Stonebridge Brook	12:07	8	1015	57	6.6	7.28	2.58
13	12:15	9	1019	55	6.3	7.26	2.55
15	12:22	8	1013	56	6.6	7.28	2.51
17	13:05	8	1007	53	6.2	7.27	2.42
19	13:11	8	995	56	6.6	7.29	2.38
20 - Coppermill	13:19	6	965	30	3.6	7.29	2.01
21 – Springfield Park	13:29	8	1000	54	6.3	7.25	2.41
22	13:37	9	1027	43	5.0	7.18	2.65
24	13:48	9	1039	41	4.8	7.17	2.78
26	14:21	9	1047	40	4.5	7.16	2.69
27 – Lea Bridge weir	14:30	9	1054	34	3.9	7.14	2.79
28	14:36	9	1054	34	3.9	7.15	2.69
29 – River Lea	14:50	9	1052	69	8.0	7.28	2.64

Table XI.4 – Physico-chemical parameters collected *in situ* on the 23/04/2012 at twenty-three sites in the area under investigation. Measurements were performed after some days of rainfall and it was raining also during the sampling.

Sampling site	Time (hours)	Temperature water (°C)	Conductivity (µS/cm)	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	pH	Total ammonia (mg/L)
0 – Tottenham Hale	10:20	11	817	136	14.6	8.89	1.25
2 – Pymmes Brook	10:28	14	1900	50	5.0	7.31	1.83
4b	10:38	14	1003	42	4.3	7.25	1.67
5	10:33	14	1001	43	4.2	7.21	1.62
6 – Old River Lea	10:57	12	830	90	9.6	7.96	0.80
8	11:29	13	921	51	5.3	7.45	1.36
9	11:34	13	928	53	5.4	7.43	1.15
10	11:38	13	977	44	4.4	7.32	1.25
11 – Old Moselle Brook	11:44	11	659	25	2.7	7.39	0.66
11b	11:48	12	873	40	3.8	7.46	0.50
12 – Stonebridge Brook	11:52	12	931	36	3.7	7.45	0.41
13	12:02	13	950	48	5.0	7.41	0.79
15	13:04	13	955	46	4.7	7.40	1.31
17	13:11	13	953	39	4.0	7.38	1.12
19	13:18	13	955	37	3.8	7.36	1.12
20 - Coppermill	13:24	11	838	58	6.2	7.52	0.68
21 – Springfield Park	13:31	13	964	32	3.3	7.33	1.08
22	13:40	13	972	33	3.4	7.33	1.19
24	13:50	13	978	30	3.0	7.31	1.25
26	14:29	13	984	27	2.8	7.28	1.57
27 – Lea Bridge weir	14:40	13	987	25	2.5	7.27	1.31
28	14:44	13	956	22	2.3	7.27	1.08
29 – River Lea	15:05	13	986	51	5.3	7.38	1.15

Appendix XII: River water quality classifications

To classify the freshwater quality, the Environment Agency used a General Quality Assessment scheme (GQA), which is now under revision.

The scheme consisted of:

1. chemistry quality;
2. biological quality;
3. nutrient status.

Chemistry classification method

The water samples are examined for ammonia, biochemical oxygen demand (BOD) and dissolved oxygen. Average and percentile are calculated for each site, and the values are compared to the limits set for the six classes shown in Table XII.1. The quality category assigned to the water is the lowest status reached in any of the three tests. The classification is taken from the Environment Agency website (http://www.environment-agency.gov.uk/homeandleisure/37815.aspx).

Table XII.1 – Chemistry classification method (from Environment Agency website).

Classification	Likely uses and characteristics
A – very good	All abstractions Very good salmonid fisheries Cyprinid fisheries Natural ecosystems
B - good	All abstractions Very good salmonid fisheries Cyprinid fisheries Ecosystems at or close to natural
C - fairly good	Potable supply after advanced treatment Other abstractions Good cyprinid fisheries Natural ecosystems, or those corresponding to good cyprinid fisheries
D - fair	Potable supply after advanced treatment Other abstractions Fair cyprinid fisheries Impacted ecosystems
E - poor	Low grade abstraction for industry Fish absent or sporadically present, vulnerable to pollution Impoverished ecosystems
F - bad	Very polluted rivers which may cause nuisance Severely restricted ecosystems

Biology classification method

Rivers can be classified into one of the six grades shown in Table XII.2, by the comparison of the macroinvertebrates found in the sampling site with those expected if the river was not contaminated. The classification is taken from the Environment Agency website (http://www.environment-agency.gov.uk/homeandleisure/37817.aspx).

Table XII.2 – Biology classification method (from Environment Agency website).

Classification	Description
A – very good	Biology similar to that expected for an unpolluted river
B - good	Biology is a little short of an unpolluted river
C - fairly good	Biology worse than expected for unpolluted river
D - fair	A range of pollution tolerant species present
E - poor	Biology restricted to pollution tolerant species
F - bad	Biology limited to a small number of species very tolerant of pollution

Nutrients classification method

Water samples are tested for nitrates and orthophosphates. A grade is assigned for each nutrient as shown in Table XII.3 and XII.4.

The classification is taken from the Environment Agency website (http://www.environment-agency.gov.uk/homeandleisure/37813.aspx).

Table XII.3 – Phosphate classification method (from Environment Agency website).

Classification for phosphate	Grade limit average (mgP/l)	Description
1	0.02	Very low
2	0.06	Low
3	0.1	Moderate
4	0.2	High
5	1.0	Very high
6	>1.0	Excessively high

Table XII.4 – Nitrate classification method (from Environment Agency website).

Classification for phosphate	Grade limit average ($mgNO_3/l$)	Description
1	5	Very low
2	10	Low
3	20	Moderately low
4	30	Moderate
5	40	High
6	>40	Very high