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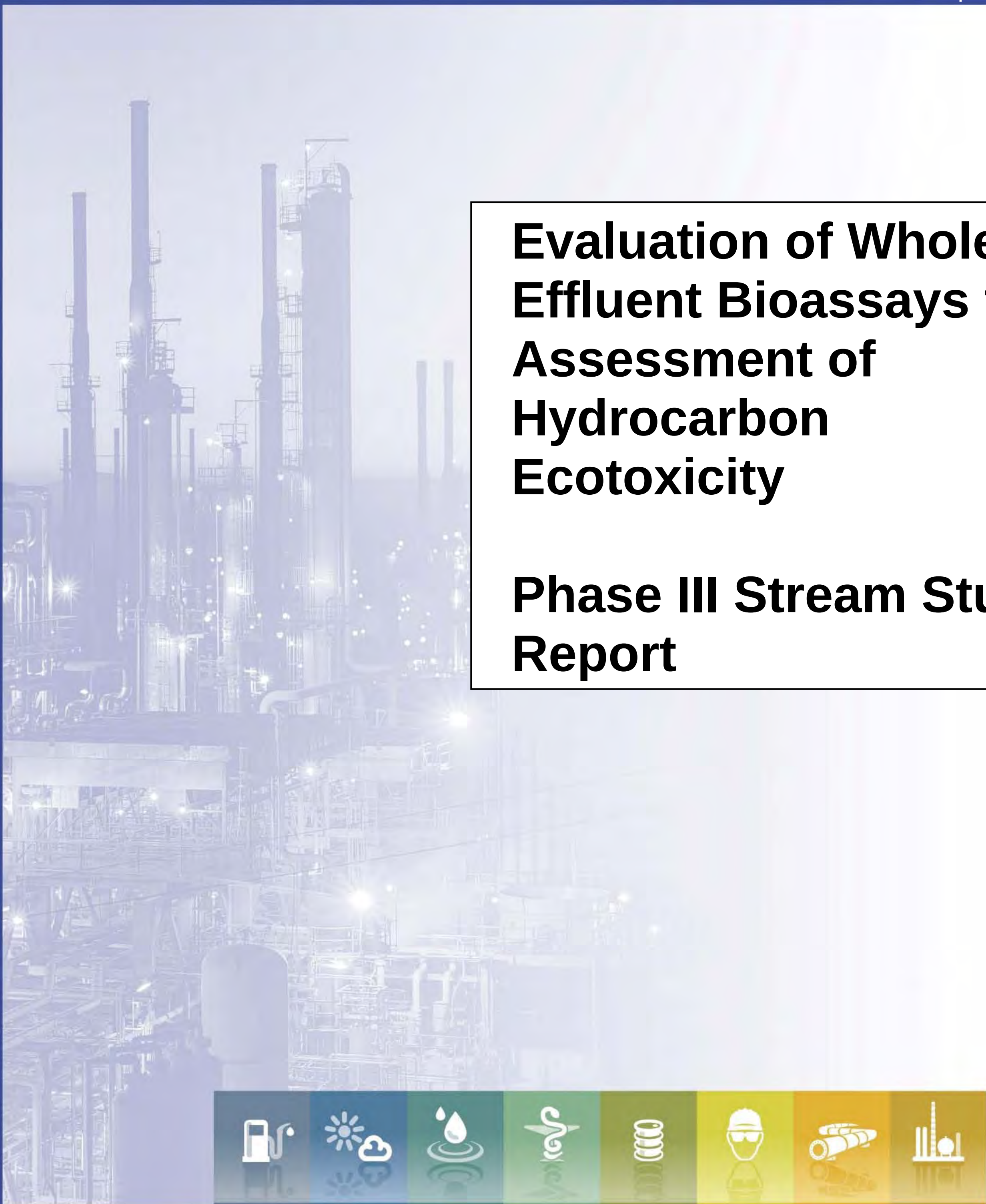

Evaluation of Whole Effluent Bioassays for Assessment of Hydrocarbon Ecotoxicity

Phase III Stream Study Report

Environmental science for the European refining industry

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Evaluation of Whole Effluent Bioassays for Assessment of Hydrocarbon Ecotoxicity

Phase III Stream Study Report

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ABSTRACT

Toxicity predictions based on laboratory- based Whole Effluent Toxicity (WET) tests have been assessed by dosing outdoor stream mesocosms with unmodified and fortified refinery effluents. The tests performed allowed the process effluents to be classified on the basis of their toxicity to a range of aquatic organisms and endpoints. Duplicate stream channels were exposed to three different profiles of hydrocarbon contamination: an unfortified effluent (A) containing low levels of mono aromatics and naphthenic mono aromatics, 10mM of Potential Bioaccumulative Substances (PBS) and low levels of biodegradable substances; effluent (B) fortified with kerosene and containing very high levels of hydrocarbon contamination (mainly normal and iso paraffins, mono and di-naphthenes and mono aromatics) with very high PBS levels (≈700 mM); and effluent (C) fortified with diesel and containing high levels of hydrocarbon contamination (mainly normal and iso paraffins and mono naphthenes) with high PBS levels (≈300 mM).

Measured PBS concentrations in the six exposed streams were 0.91 ± 0.4 mM (A), 3 ± 1.15 mM (B) and 2.1 ± 1.6 mM (C). Little or no variation in hydrocarbon concentrations was observed for the 3 treatments during the 21 day study. Unfortified effluent (A) had no impact on either benthic invertebrate or primary production in stream mesocosms. Effluents (B) and (C) fortified with kerosene or diesel had no short term effects but significant long term effects on both benthic invertebrate and primary production in stream mesocosms. However, within 30 days of ceasing treatment, a rapid partial or total recovery was observed in the streams treated with the fortified effluents.

In WET tests the unfortified effluent (A) exhibited no acute or chronic toxicity in any of the three tests (*Vibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella subcapitata*). Effluent B fortified with kerosene to a PBS level of ≈ 700 mM exhibited chronic toxicity to both crustacean and microalgae but no acute toxicity (except for *Vibrio fischeri*) and effluent C fortified with diesel to a PBS level of ≈ 300 mM exhibited both acute and chronic toxicity to crustacean and microalgae.

When considering a specific biological compartment (ie. Bacteria, Invertebrates, algae or primary production), WET tests were found to either over-predict the effects in the streams, or to predict effects similar to those observed in the streams. In this study, the prediction of *in- situ* impact using WET tests never gave a false negative (i.e. under-prediction of toxicity effects).

These results suggest that environmental impact assessment based solely on data obtained from laboratory WET assays is likely to be conservative. i.e. the biological impact would be less in a more realistic exposure system.

KEYWORDS

Whole Effluent Toxicity, Bioassays, Mesocosms, Hydrocarbons

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SUMMARY

Ecological status is classically measured using parameters associated with that specific ecosystem. Those applicable to rivers focus mainly on the abundance and taxonomy of communities of diatoms and benthic macro-invertebrates. The parameters have been formalised into indices that are derived using standard methods with the following pre-requisites for deriving reliable and relevant values: access to the specific site and relevant habitat for sampling, knowledge that the site is only being potentially impacted by the industrial release under consideration and access to a close reference site not impacted by the industrial releases

It can be difficult to meet these requirements for both technical and practical reasons. This is particularly true in water-courses receiving inputs from multiple sources. In such circumstances, Whole Effluent Toxicity (WET) or Whole Effluent Assessment (WEA) methods can provide a basis for deriving data that can provide a valid assessment of the likely contribution of the effects of a particular pollutant input to a water-course.

This report describes the third phase of a programme of research to compare results from laboratory-based WET assays and *in-situ* assay methods. Differences in outcome from using the two methods are assessed by examining actual effects observed on communities living in semi-natural outdoor artificial stream mesocosms. Work began in 2009 on the phase I project, which was to develop and test the experimental protocols. In phase 2 of the study, stream experiments were carried out to assess the effect of a single refinery effluent on stream mesocosm communities. The key finding from phase 2 was that the effluent was not sufficiently toxic to produce a dose- response relationship over the applied dilution range (from 200 to 1500, v/v), with only slight effects observed at the lowest dilution. Consequently, it was not possible to clearly conclude whether the results of the WET assays overestimated or underestimated the impact to aquatic ecosystem.

For this phase III study, effluents from three refineries were sampled, before biological treatment and after the DAF (Dissolved Air Flotation) or API (American Petroleum Institute) separator units. The three effluents were evaluated using WET bioassays and in the stream mesocosms. One effluent was used as supplied (i.e. sampled after the DAF but before biological treatment) and the other two were used after fortification with distillate hydrocarbon fractions (diesel or kerosene) to ensure that hydrocarbon concentrations were relatively constant and sufficiently high to induce observable effects on the biota.

As observed in phase II, unfortified effluent sampled in the refinery before biological treatment had no impact on either benthic invertebrate or primary production in stream mesocosms, probably due to the effluent dilution factor in the streams (140, v/v). Effluent fortified with kerosene or diesel had no short term effects but significant long term effects on both benthic invertebrate and primary production in stream mesocosms. However, within 30 days of ceasing treatment, a rapid partial or total recovery was observed in the streams treated with the fortified effluents. The results therefore clearly demonstrate that stream mesocosms can be used to study the potential effects of refinery effluents on aquatic ecosystems, especially those resulting from long-term (chronic) exposures.

The toxicity of the unfortified and fortified effluent was also assessed using WET tests. The results show that unfortified effluent (A) exhibited no acute or chronic toxicity in any of the three tests (*Vibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella*

subcapitata). Effluent B fortified with kerosene to a PBS level of ≈ 700 mM exhibited chronic toxicity to both crustacean and microalgae but no acute toxicity (except in *Vibrio fischeri*) and effluent C fortified with diesel to a PBS level of ≈ 300 mM exhibited both acute and chronic toxicity to crustacean and microalgae.

When considering a specific biological compartment (ie. Bacteria, Invertebrates, algae or primary production), WET tests were found to either over-predict the effects in the streams, or to predict effects similar to those observed in the streams. In this study, the prediction of *in- situ* impact using WET tests never gave a false negative (i.e. under-prediction of toxicity effects).

These results suggest that biological impact assessments based only on data obtained from laboratory Whole Effluent Toxicity (WET) tests are likely to be conservative. i.e. the biological impact would be less in a more realistic exposure system. Three laboratory WET tests were assessed in this project (*Vibrio fischeri* for bacteria, *Pseudokirchneriella subcapitata* for micro-algae and *Daphnia magna* for invertebrates). Further studies are planned to assess whether other WET test methods are similarly conservative.

1. INTRODUCTION

1.1. CONTEXT

1.1.1. Regulatory

The European commission has made protection of the quality of natural water bodies and sources of water for abstraction a high priority over the last few decades. Consequently prevention and where necessary mitigation of water pollution has been a key area of regulatory focus.

Early European legislation began with the adoption of legislative instruments addressing pollution from urban and industrial waste-water and included the Directive for Integrated Pollution Prevention and Control (IPPC). The IPPC was adopted in Europe (1996) in order to prevent, reduce, and as far as possible eliminate, pollution by giving priority to intervention at source and ensuring prudent management of natural resources. On 21 December 2007 the European Commission adopted the Directive on Industrial Emissions, which recast seven earlier Directives including the IPPC Directive. Regulation of emissions from industrial installations is expected to play a significant role in the reduction of pollutant inputs to the environment.

The Water Framework Directive (WFD) was adopted by the European Parliament on 23 October 2000. The purpose of the WFD was to establish a European-wide regulatory framework for cleaning up polluted water bodies and ensuring that clean waters are kept clean. The WFD includes general requirements for protecting the biology and ecology of all surface waters ("good ecological status") and complying with minimum chemical standards ("good chemical status"). Procedures for determining and monitoring ecological water quality status have been proposed or are in development. Chemical standards are based on suites of known hazardous substances and analytically determined levels that can be compared with established safe concentrations.

Over the past few years, member state governments within the EU have progressively sought the adoption of Best Available Techniques (BAT) to control pollutant emissions to air, soil and water from a range of industrial sectors, including the oil industry. Refinery wastewaters can be compositionally complex and can be challenging for assessing their hazard and risk to the aquatic environment. There is also increasing recognition by regulators that there are limitations to the “substance-specific approach” for assessing and controlling the environmental fate and effects of effluents because of the potential for substances to act additively or interactively. Concawe also recognises this issue and has proposed that the effects of pollutant emissions can be assessed, where possible, using direct measurements made in the receiving environment or a model of that environment. Such a strategy is consistent with the WFD which requires the potential impact of a refinery effluent on receiving environment (river, estuary…) to be assessed and/or monitored in order to identify both chemical and ecological status.

1.1.2. Technical

Ecological status is classically measured using parameters associated with that specific ecosystem. Those applicable to rivers focus mainly on the abundance and taxonomy of communities of diatoms and benthic macro-invertebrates. The parameters have been formalised into indices that are derived using standard methods with the following pre-requisites for deriving reliable and relevant values:

- access to the specific site and relevant habitat for sampling
- knowledge that the site is only being potentially impacted by the industrial release under consideration.
- access to a close reference site not impacted by the industrial releases

It can be difficult to meet these requirements for both technical and practical reasons. This is particularly true in water-courses receiving inputs from multiple sources. In such circumstances, Whole Effluent Toxicity (WET) or Whole Effluent Assessment (WEA) methods can provide a basis for deriving data that can provide a valid assessment of the likely contribution of the effects of a particular pollutant input to a water-course.

Whole Effluent Toxicity (WET) is a term used to describe the aggregate toxic effect of an aqueous sample (e.g., whole effluent wastewater discharge) as measured by an organism's response upon exposure to the sample (e.g., lethality, impaired growth or reproduction). WET tests replicate the total effect and actual environmental exposure of aquatic organisms to toxic pollutants present in an effluent without requiring the identification of the specific pollutants. WET testing is a vital component of the water quality standards implementation through the US NPDES (National Pollutant Discharge Elimination System) permitting process and supports meeting the goals of the Clean Water Act [1]. WET tests are designed to predict the impact and toxicity of effluent discharges from point sources into U.S. receiving waters.

There are both scientific and regulatory concerns that using WET tests to regulate industrial effluents may result in either false positive and/or false negative values [2] because the exposure conditions may not fully replicate those of the receiving environment. In order to realistically predict the effect of an effluent on the receiving environment the test should be as representative as possible of the conditions in the receiving environment. Consequently, many regulators are seeking even more holistic techniques, such as Whole Effluent Assessment (WEA) to supplement existing approaches. Even in countries where whole effluent toxicity (WET) is already assessed there is growing desire to address other issues including persistence and bioaccumulation of effluent components.

WEA is the instrument for the “whole sample approach” developed for effluents [3]. WEA increases the understanding of the combined effects of all known and unknown substances within effluents, especially in complex mixtures. WEA utilises the broader approach of toxicity (T parameter) along with additional parameters, persistence (P parameter) and the potential to bioaccumulate (B parameter) [4].

In many schemes WEA approaches are seen as new (developing) tools for assessing effluent quality that should be applied in combination with (and not instead of) the substance-oriented approach. Within Europe, WEA-type schemes are generally seen as supporting the hazardous substance strategies of OSPAR and as a tool within IPPC and potentially in support of the WFD. As with any initiative there are advantages and disadvantages of WEA approaches. One of the principal advantages of WEA is that the effluent can be assessed as a whole and this can improve the information relating to environmental effect of poorly characterised and complex effluents (i.e. those containing unknown mixtures of chemicals). Disadvantages could potentially occur if the tests are inappropriate and/or incorrectly applied and interpreted, leading to demands for unjustified measures to reduce environmental effect.

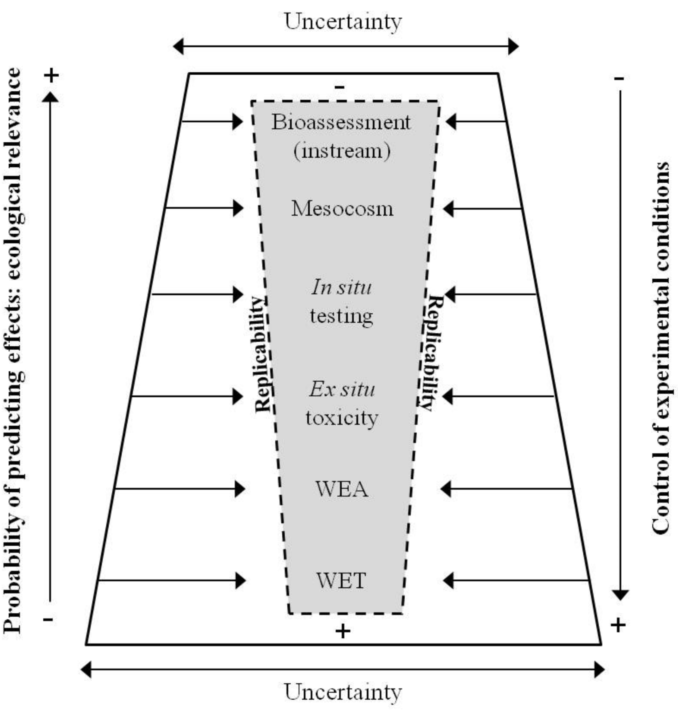
1.2. AIMS OF THIS STUDY

Standardized tests to evaluate effluent toxicity have been used for many years in the United States under the NPDES and in Europe within the framework of Integrated Pollution Prevention and Control (IPPC). However, there are concerns regarding the validity of extrapolating from test data obtained for single indicator species under controlled laboratory condition to responses that could be expected in complex aquatic ecosystems [5, 6]. These concerns may well be justified since the single-species tests were initially conceived in order to provide data on relative hazard rather than to provide quantitative descriptors of likely ecosystem responses.

Several earlier studies have focused on validating the use of single species toxicity test results for predicting effects in aquatic ecosystems. They have looked at the relationships between effects observed on WET test endpoints in the laboratory and those observed on in-stream biological condition. The latter has been assessed in natural streams and in stream mesocosms [2, 7] by studying effects on benthic macro-invertebrates, periphyton and fish. In so doing these studies have identified some limits on drawing definitive conclusions from such comparisons:

- Difficulty in distinguishing between contaminant-caused effects on aquatic populations and those resulting from other causes (dissolved oxygen, temperature, habitat changes…)
- Lack of sample replication
- Bio-assessment methods only providing qualitative data
- Variation in exposure conditions arising from fluctuating and varying effluent discharges.

Figure 1: Predicting receiving stream impacts from effluent discharge (modified from [8])



Mesocosm studies present several advantages compared to WET methods when it comes to investigating the potential differences in outcomes for the assessment of environmental effects. WET methods are inherently conservative because of need to factor in uncertainty associated with extrapolating from the lab to the field. The level of uncertainty is much less when extrapolating from a mesocosm to the field and is even less when data are obtained from in-stream measurements (**Figure 1**). It could even be argued that data obtained from mesocosm studies is even more useful for extrapolating to more general in-stream effects because exposure conditions can be controlled to the extent that extraneous variability arising from incidental factors can be limited (realistic controlled exposure conditions, interspecies interaction…). However it is important to remember that multispecies assessments, which include those conducted in streams and in mesocosms, do have limitations arising from [9, 10, 11, 12]:

- Non-standardized protocols
- More variability between replicates compared with single species toxicity tests
- Less repeatability between experiments

The effects of refinery effluents or hydrocarbon distillates on communities present in stream mesocosms have so far only been reported by Bayona et al. (2014) [13, 14]. These studies were designed to define and to test the sensitivity of structural and functional descriptors of aquatic communities for the environmental risk assessment of organic chemicals (petroleum middle distillate and fungicide) in pond and stream mesocosms.

The aim of the present study was to investigate the potential differences in outcomes for assessments of effects based on WET methodology and on *in situ* impact measurement in outdoor artificial stream mesocosms. This project was designed and undertaken in three phases:

Phase I: Experimental design (selection/storage of the effluent, aging)
Phase II: Feasibility assessment of testing effluents in outdoor stream mesocosms
Phase III: Understanding and comparing the biological responses in effluents and mesocosms.

Phase II of this project jointly organised by TOTAL and Concawe addressed the feasibility of testing refinery effluents in outdoor mesocosms and allowed a standard protocol for conducting such experiments to be developed.

In phase III the potential differences in outcomes for assessment of environmental effects based on WET methodology and one based on *in-situ* impact measurement were investigates using experiments performed in outdoor stream mesocosms located at Lacq, France.

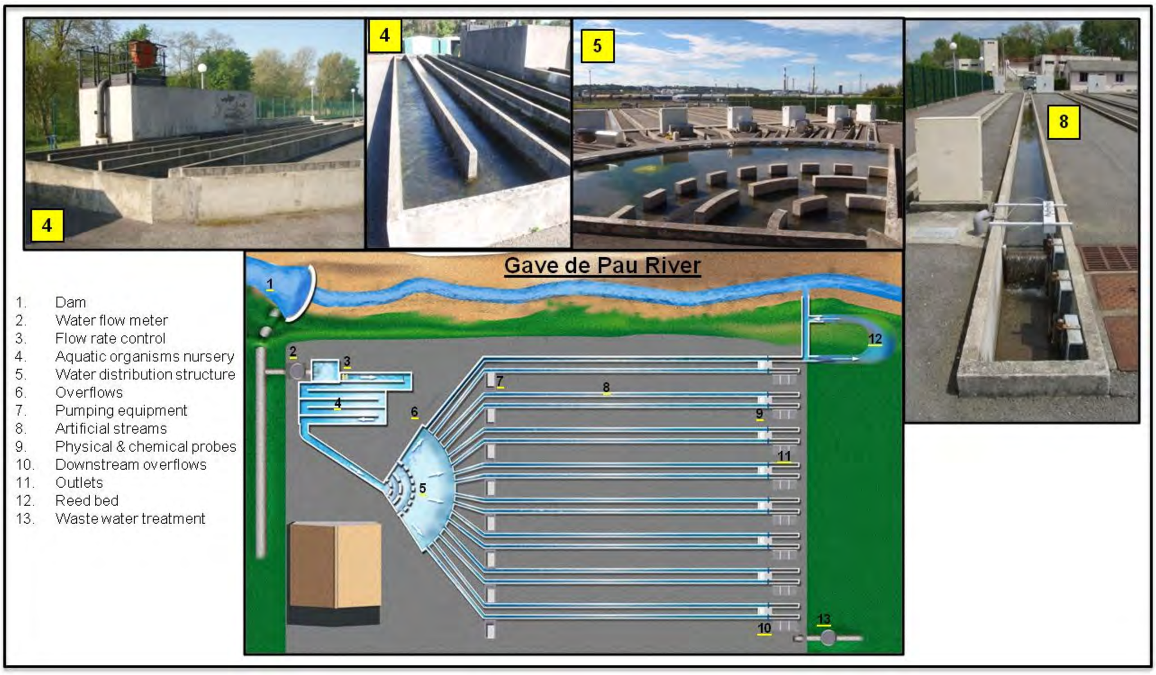
This report mainly covers phase III but also refers to some of the works performed in phase I and II. More details of phase I and II could be found in [15] and [16].

2. MATERIALS AND METHODS

2.1. DESCRIPTION OF THE MESOCOSMS

TOTAL's experimental mesocosm system is located in the south west of France, close to the Pyrenees. The water that supplies the mesocosms ("Pilot Rivers") comes from the Gave de Pau River. The system is fed from the Artix' Dam located 5 km upstream, allowing gravity flow to feed the mesocosms or "Pilot Rivers". The water passes through a flow regulation system, then circulates through a "supply nursery" in which aquatic plants (macrophytes), benthic organisms (invertebrates) and micro-algae are present as a result of natural colonization (**Figure 2**). These organisms subsequently colonize the sixteen mesocosms. Each of the mesocosms is 40 m long and 50 cm wide and a water depth ranging from 10-to 30 cm. A two month colonization period precedes treatment of the streams and commencement of an experiment. The whole system has been in operation for more than ten years and over this period the supply nursery has never dried out.

Figure 2: Scheme of the "Pilote Rivers"



The flow in each stream is identical and depends on the setting of the overflow and the total water flow entering the system. This flow can be regulated to between 0 and 200 m^3 / hour (the latter figure corresponding to a 12.5 m^3/ hour flow in each stream). During phase II of this project, the mesocosms were operated with the maximum water flow entering the system. Taking into account the maximum volume of refinery effluent that could be stored on site (20 m^3) and taking into account the duration of the treatment period (21 days), the minimum dilution factor during phase II was approximately 300.

The experimental treatments used in phase II resulted in few detectable effects on the stream communities. Concawe STF32 members therefore raised the following, perhaps conflicting, questions:

- Since it is not possible to store larger volumes of the effluent on the Pilot Rivers site, would it be possible to decrease the water flow circulating the system in order to decrease the dilution factor of the effluent in the streams?
- The diversity of the benthic invertebrate community in the streams is directly linked to the water velocity which itself depends on the water flow entering the streams [17, 18]. Would it be possible to modify the structure of the streams in order to increase the diversity of the benthic invertebrate community such that the international EPT index (Ephemeroptera, Plecoptera, Trichoptera index), which is based on sensitive species from three different orders, could still be calculated even with a lower water flow entering the system?

In order to answer these two questions, a series of experiments were designed to increase both (1) the water velocity in the artificial streams and (2) the abundance and diversity of benthic invertebrates. The experiments were carried out between March and July 2011 (TOTAL internal report) and are summarised below.

Three sets of experimental conditions were examined in parallel in eight of the streams:

- Four artificial streams were established under “normal” conditions (i.e. 10 cm of calibrated rocks at the bottom of each stream, all along the streams)

  Four others streams were set up with new conditions (“modified” streams). These new conditions consisted of dividing the streams into two parts, creating two different types of habitat (**Figure 3**):

  - upstream, with higher water velocity
  - downstream, with lower water velocity

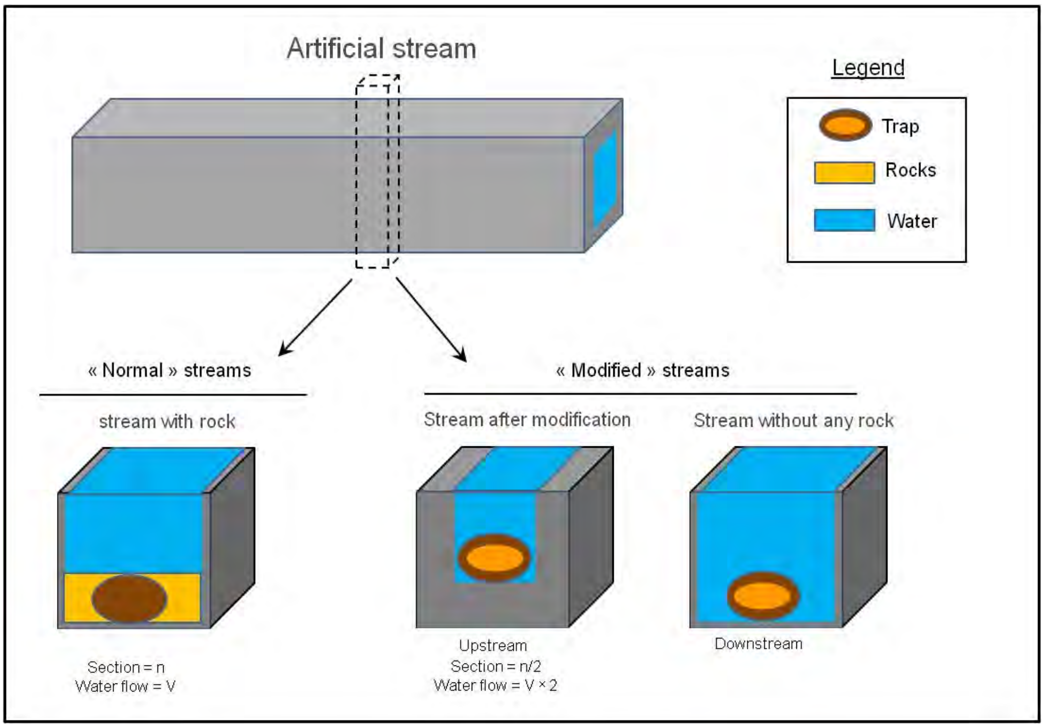
  The four “normal” streams were prepared by introducing benthic invertebrate traps and adding 10 cm of rocks at the bottom of each stream above the traps (streams 1, 6, 10 and 12). In parallel, four other “modified” streams (streams 3, 8, 11 and 16) were prepared taking into account the modifications described below:

- The first section of the four “modified” streams, the upstream “rapid” area, was partially filled with breeze-blocks in order to reduce the cross-section of the streams and by so doing increase the water velocity compared to that in the “normal” streams
- The second section of the four “modified” streams, the downstream “slow” area, was left completely empty (with no rocks) in order to obtain a low velocity area compared to that in the “normal” streams.

Benthic invertebrate traps were added to the “modified” streams in both the high (upstream) and low (downstream) flow velocity areas. For streams T0, T14 and T35, only traps with “rocks + leaves” were used. For streams T21, two types of benthic invertebrate traps were tested (both upstream and downstream):

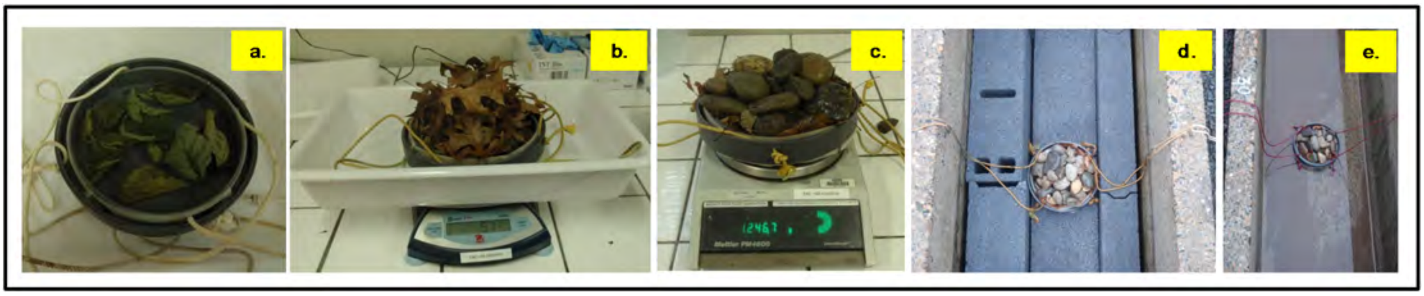
- Half of the traps were filled with “rocks + leaves”
- Half of the traps were filled only with rocks.

Figure 3: Scheme of the tests performed in order to improve the benthic invertebrate biodiversity in the pilot Rivers, in comparison with "usual" stream conditions



To ensure consistency across the streams the quantities of leaves (15 g) and rocks (2 kg) were weighed in the lab before they were added to the streams (**Figure 4**).

Figure 4: Preparation of the benthic invertebrate traps (a. selection of the leaves, b. weighing of the leaves in the lab, c. weighing of the rocks in the lab, d. installation of the traps in the high velocity area (upstream), e. installation of the traps in the low velocity area (downstream)).



No leaves were put into the traps in the four "normal" streams before the start of sampling (i.e., at T0, T14, T21 and T35).

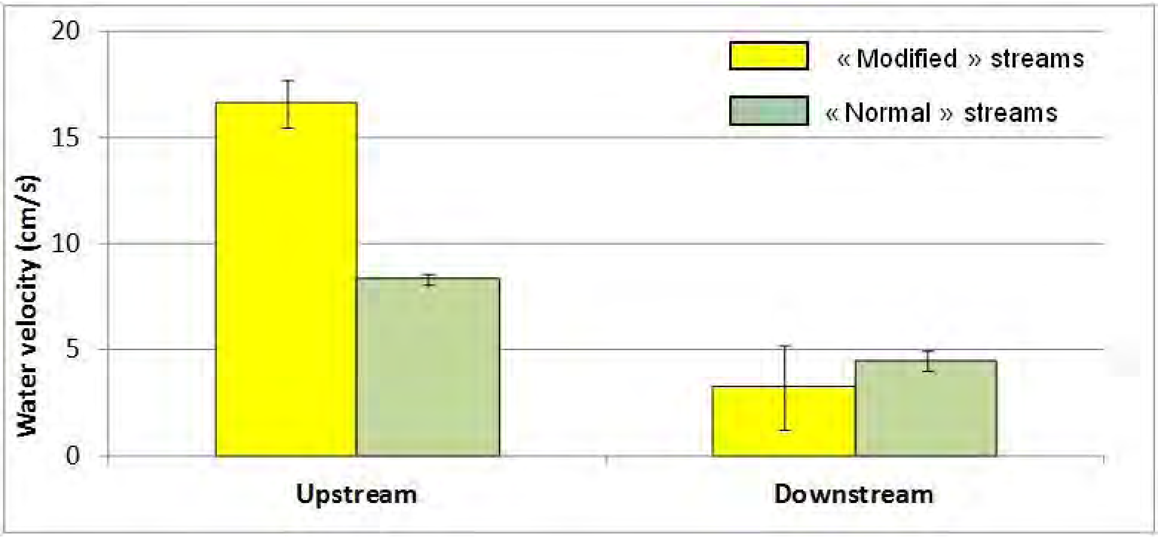
The colonization of the streams by benthic invertebrates lasted two months following the introduction of the traps into the "normal" and "modified" streams. Sampling of the benthic invertebrate traps commenced after this period as described in **Table 1**.

Table 1: Detail of benthic invertebrate's sampling during the experiment

	« normal » streams	« modified » streams			
		Upstream (rapid area)		Downstream (slow area)	
	Without leaves	With leaves	Without leaves	With leaves	Without leave
T0	1 sample/stream	1 sample/stream		1 sample/stream	
T14	1 sample/stream	1 sample/stream		1 sample/stream	
T21	1 sample/stream	1 sample/stream	1 sample/stream	1 sample/stream	1 sample/stream
T35	1 sample/stream	1 sample/stream		1 sample/stream	

The water velocities measured in the streams are shown in **Figure 5**. The results indicate that the upstream modification increased the flow velocity by a factor 2 (16.6 cm/s ± 1.1) relative to the "normal" streams (8.4 cm/s ± 2). These results also indicate that two water velocity habitats were created in the "modified" streams; a high velocity area 16.6 cm/s ±1.1 upstream and a low velocity area 3.2 cm/s ± 0.28 downstream.

Figure 5: Water velocity in "Normal" streams and in "Modified" streams (both upstream and downstream) (mean for the four stream ± standard deviation)

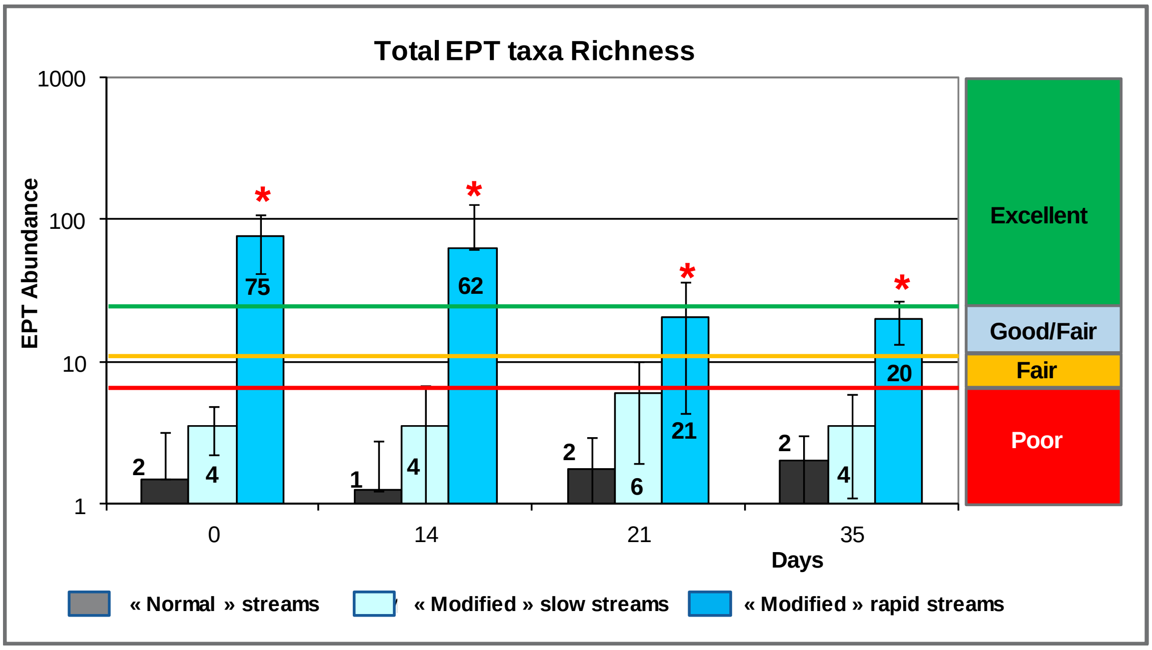


Total EPT abundance values in both the "normal" and "modified" streams are presented in **Figure 6**. Only the results obtained in the traps with leaves in are presented for the "modified" streams.

Total EPT abundance values in the high flow velocity area of the "modified" streams from T0 to T35 ranged between 20 ± 7 to 75 ± 34 organisms/trap. These values correspond to a good/fair to excellent water quality score according to Lenat's classification **[**19]. The scores obtained in these traps are significantly higher than the ones obtained in the "normal" streams and in the low flow velocity area of the "modified" streams.

The alterations to the normal procedures that were tested in the "modified" streams, especially the increase of the water velocity, allowed colonization by Ephemeroptera and Trichoptera species in the traps. These modifications significantly increased the water quality score of the stream mesocosms, according to the EPT taxa richness criteria [19], with a good to excellent water quality score in the "modified" streams. Therefore the best solution to obtaining higher EPT species scores in the mesocosms was to create a high velocity area in the streams. However, these modifications did not allow significant colonization by Plecoptera species in the traps; the general increase of EPT index scores in the streams was mainly due to colonization by Ephemeroptera and Trichoptera species in the high velocity area.

Figure 6: Total EPT richness in the "usual" streams and in the "modified" streams (From T0 to T35 days) (* represent significant difference in comparison with conventional streams, p<0.05)



With the above results in mind it was therefore decided to modify the stream mesocosms for phase III accordingly so that:

- colonization by EPT species was enhanced and
- the cumulative water flow and the dilution factor of the effluent were decreased; this would result in higher exposure concentrations of the effluents.

In so doing it was anticipated that there would be a greater chance of detecting and examining effects on the stream communities in general and on EPT species in particular.

2.2. SUBSTANCE TESTED IN THE MESOCOSMS

2.2.1. Technical considerations

Concawe has been working consistently to establish relationships between the bioaccumulation potential and simple narcotic toxicity of hydrocarbons [20]. This relationship is important because it takes into account bioavailability and simulates bio-concentration. The work has been based on the assumption that the narcotic toxic effects of Potentially Bio-accumulating Substances (PBS) are mainly responsible for the observed toxicity of refinery effluents and that the measurement of PBS is a suitable surrogate for toxicity tests at the screening stage [21, 22, 23]. Indirect assessment of this toxicity might therefore be addressed by measuring the extractable hydrocarbons (PBS) obtained by solid phase micro- extraction (SPME) and correlating this with known measured toxicity data. PBS levels could then be used to select the effluents to be tested in the phase III mesocosm experiments [24]. On the basis of the results of a literature review (**Table 2**) a PBS threshold of 8 mM [24] could be expected to be correlated with observed chronic toxicity in the stream mesocosms.

Table 2: Critical benchmarks (acute and chronic) of Cfiber (Total molar concentration in the fiber) for trout, algae and zooplankton. Chronic narcotic effect was estimated using the ratio of acute to chronic (ACR) effects of 1/5 ([4] from [24]).

Endpoint	Critical Cfiber (mM)	Reference
Acute narcotic effect trout	77	Parkerton et al., 2001
Acute narcotic effect algae	57	Parkerton et al., 2001
Acute narcotic effect *Daphnia magna*	42	Parkerton et al., 2001
Chronic narcotic effect *Daphnia magna*	8	Estimated based on ACR of 1/5*

The experimental set-up for phase III, and in particular the type of sample to be tested, was discussed with Concawe STF32 members before starting the project. In view of the low PBS levels measured in the pure <u>effluent</u> samples that were examined in phase II, it was decided to test samples of waste-water treatment plant <u>influent</u> instead since these contained higher levels. The study protocol was also modified to include more replicates of fewer influent samples so that higher PBS levels could be tested. From here after in the report the test material will be refer to as “effluent” and “fortified effluent”.

Three parameters were taken into account when selecting the effluent samples:

- The minimum PBS level to be achieved in the streams was to be 8 mM
- The maximum volume of each effluent that could be stored on site was (20 m^3)
- The water flow entering the system was 200 m^3/h

On the basis of the results obtained in phase II, the phase III effluents could still only be minimally diluted if effects on aquatic organisms were to be observed in the streams. Applying the modified operational procedure described in section 2.1, the water flow entering the system would be half that used in phase II (100 m^3/h). Taking this into account and the maximum flow of effluent for a 21 day exposure (20000/(21*24), the minimum PBS level that would need to be present in the effluent samples would be defined as:

Minimum PBS level in the stream × dilution factor = 8 mM × (6500/ (20000/(21×24)) = 1310.48 mM

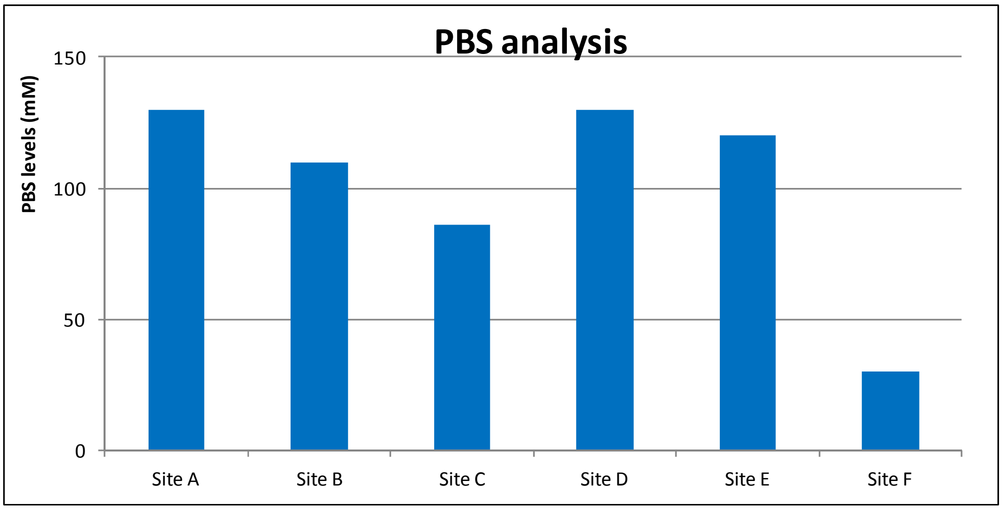
This figure is much higher than the PBS level of 100 mM in phase II. For phase III, it was therefore decided to decrease the number of replicates from 3 to 2 compared with what was initially planned. This was done in order to increase the flow of the effluents injected into the streams and therefore achieve higher PBS levels in the exposures. With this new modification, the minimum PBS level of the effluent injected into the streams would be approximately 870 mM, which is again far from what we expected for phase III. Taking into account the minimum dilution factor in the artificial streams and PBS levels measured in samples from various industrial sites, a threshold of 8 mM could not be maintained for a sufficient period of time with the available effluent sample volumes.

An additional modification of fortifying some of the effluent samples with an appropriate petroleum distillate, so as to increase the PBS concentration in the streams, was therefore adopted. Middle distillates (Gas oil, Diesel and Jet Fuel) were selected, on the basis that they have a carbon range of C9 to C25 and contain all the hydrocarbons detected in European refinery discharges [25](mainly normal and iso-paraffins (C9-C24), mono-naphthenes (C9-C20) and mono-aromatics (C6-C14 and olefins).

2.2.2. Selection of the refineries for sampling

Initially, all Concawe member refinery locations in France and its bordering countries were free to contribute samples to phase III. One of the objectives of this study was to test effluents with a high PBS concentration from several different refineries. After an initial assessment of the sites, ten were selected as possible sample contributors but only six of them eventually supplied samples for a first stage evaluation. These six sites belong to three refining companies: four from one company (sites B, D, E, and F) and two sites from two different refining companies. For this evaluation, a one litre sample was required to assess the PBS content of the effluent (Figure 7). Four of the six effluents evaluated had similar PBS content (sites A, B, D and E with PBS content ranging from 110 to 130 mM). Three sites belonging to the three O&G companies were selected from the six as having samples that had high enough PBS levels to use in Phase III. Sites A and C were selected because these two sites belong to two O&G companies that supplied only one sample for the evaluation. Site B was selected because this site belongs to the third O&G Company and is located closed to the Pilote River site. Site B had also relatively high PBS content (110 mM). Site D which belongs to the same O&G Company than site B was initially selected but was not able to supplied large volume for the experiment due to an unexpected operational shut down.

Figure 7: PBS levels in six refinery sites



2.2.3. Sampling, Transport and Storage of the Effluents

The effluents from the three refineries were sampled before biological treatment and after DAF (Dissolved Air Flotation) or API (American Petroleum Institute) separator treatments. The sampling critical requirements were:

- The location should allow sampling of a 60 m^3 volume
- H2S should not be present in the sample

The effluent samples were transported to the stream mesocosm facility in Lacq by Group SAMAT who are specialists in transport logistics for liquid chemical products. The samples were transported at ambient temperature using 25-m^3 stainless steel tank trucks (Photo 1). The samples arrived within 24 hours of collection.

Photo 1: Tank trucks used for transportation

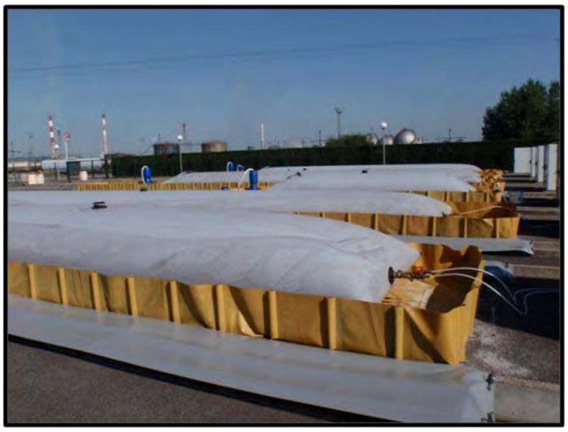
The effluents were stored in nine 20 m^3 purpose made and pillow shaped flexible tanks that can easily be carried and set up and offer entire protection of the stored liquid from contact with air and light. The tanks are normally used for the storage of, for example, chemical waste, sludge and fertilizer. This storage method was chosen for the large volumes of effluent that were needed in order to be consistent with the phase I findings which showed that settlement of suspended particles, volatilization into the headspace of the storage tanks and exposure to light resulted in a decrease in the toxicity of effluents over the storage period.

The tanks were supplied by ABEKO, specialists in fluid storage solutions, and were made of plastomer-coated fabric manufactured by high frequency welding. The fabric has high resistance to mechanical damage. Coating and design engineering features confer chemical resistance properties and water tightness. The plastomer fabric (ALCRYN, which allow lower adsorption) was selected depending on the characteristics of the liquid to be stored. The external surface is given a treatment to resist damage by UV radiation. The tanks are fitted with equipment for filling, emptying and degassing.

The advantages of the flexible tanks in comparison with other storage solution like milk tanks for example are summarised below:

- High volumes stored
- High resistance to climatic conditions
- No risk of condensation and liquid contamination
- Protection of the liquid (no evaporation, oxidation or crystallization)
- Confinement of odours.

Photo 2: Flexible tanks used for the storage of the effluents



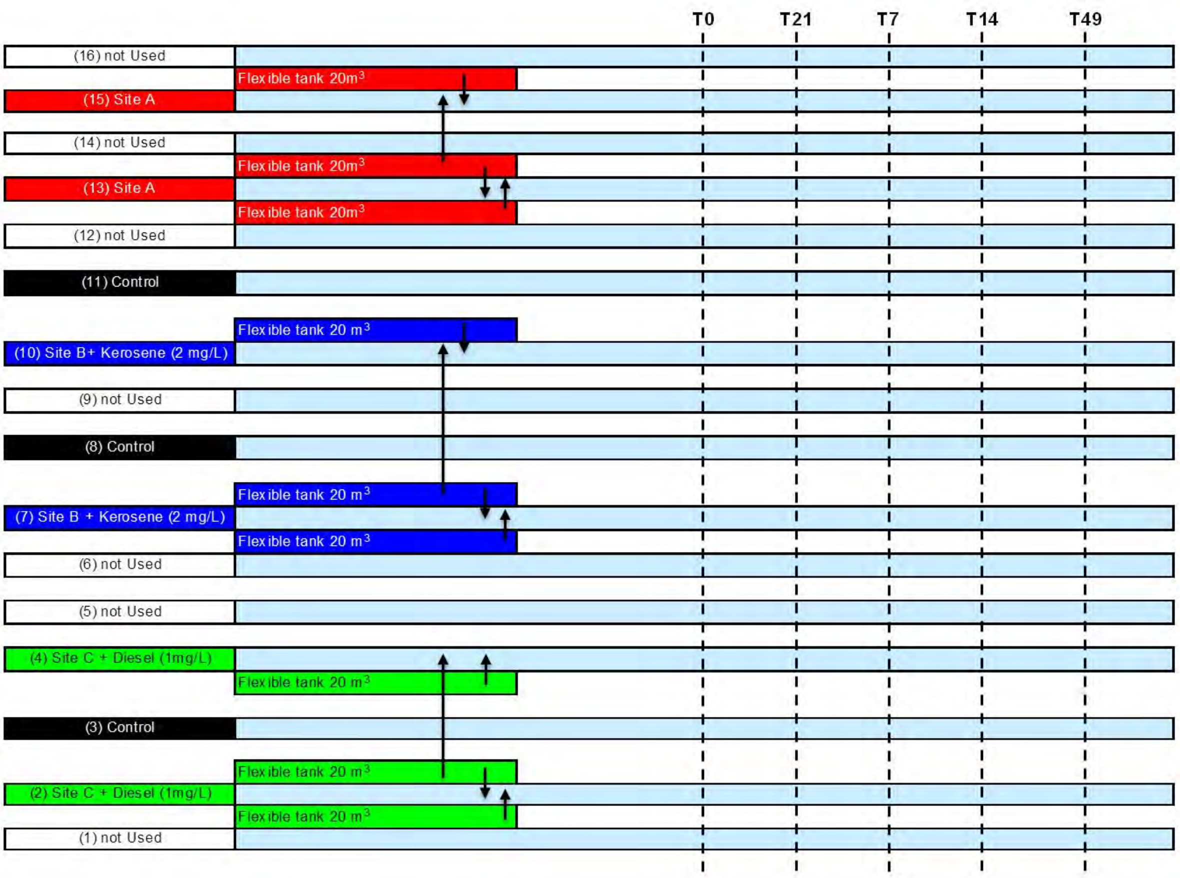
Smaller versions of the tanks were sent to the sampling sites prior to commencing the definitive part of the study to check their suitability for storing the samples. After one month no leakage was observed.

For the phase III, the toxicity of the effluent samples was assessed throughout the study from the sampling in the refinery and throughout storage in the flexible tanks. A significant initial decline in toxicity was observed over the storage period in the flexible tanks. To allow this decline to stabilize, the samples from the three sites were stored in the tanks for one week before they were injected into the streams.

2.3. EXPERIMENTAL SETUP

The effluents from the different refineries were tested in the mesocosms. Each effluent treatment was duplicated. Three “control” streams, with no effluent addition, were established in parallel. A total of nine of the 16 available stream mesocosms were therefore used for this experiment. The nine streams were distributed across the whole facility as shown in **Figure 8**.

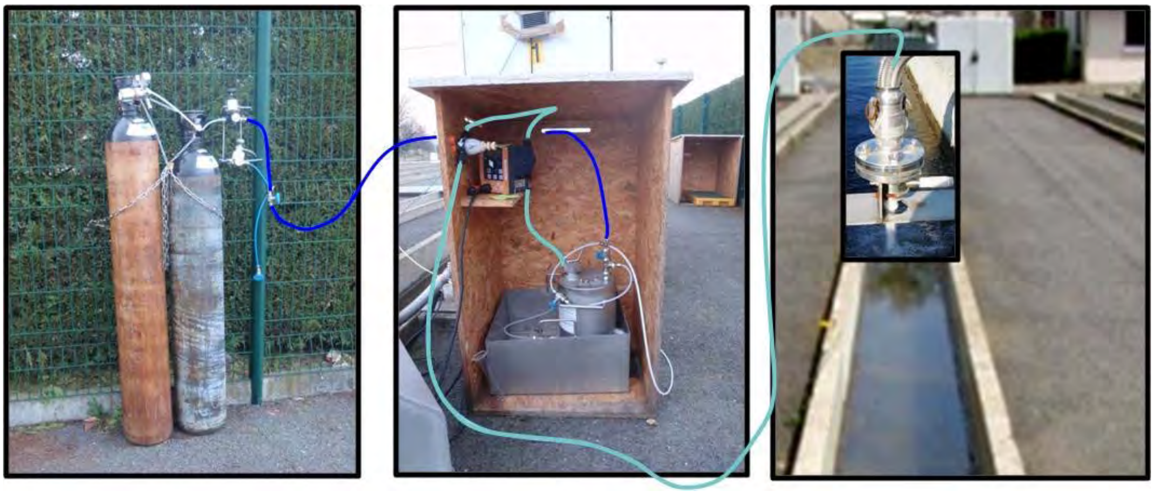
Figure 8: Distribution of the experimental conditions among the sixteen artificial streams (dotted lines indicate the sampling location at each sampling date)



The streams were continuously treated upstream for 21 days using pumps. The effluent with the highest PBS level (130 mM) was not fortified whereas the other two were fortified with petroleum distillates; effluent B with Kerosene (2 mg/l) and effluent C with Diesel (1 mg/l). Kerosene and diesel concentrations were selected based on previous experiments performed in the stream mesocosms with these petroleum distillates. The concentrations used were realistic relative to average Oil in Water concentrations in the respective refinery effluents.

Special equipment was used for the treatments with the effluents fortified with petroleum distillates (**Figure 9**) to improve dissolution of hydrocarbons in the water. First, a high pressure-pump was used that pumped the water upstream in the channel and then inject it downstream using a mixing valve. A second pump was used to pump the effluent from the flexible tank and inject it into the high-pressure flow of stream water before the mixing valve. A third pump was used to pump the petroleum distillates from their storage containers and inject them into the mixture of effluent and stream water before the mixing valve. The headspace in the containers was continuously filled with nitrogen in order to avoid volatilization of the distillates in the tanks during the experiments (**Figure 9**).

Figure 9: Equipment used to treat the streams



2.4. MEASUREMENTS

Physical-chemical and biological parameters were measured over the course of the study in both the streams and the flexible tanks. The parameters were evaluated throughout the 21-day treatment period. They were also measured again 30 days after ceasing treatment in order to investigate the recovery of the stream biota from any treatment related effects.

2.4.1. Sampling of the Water

Three types of water samples were sampled for physical-chemical and biological analyses:

- Effluents stored in the flexible tanks
- Stream water
- Effluents fortified with the petroleum distillates

For pure effluent analyses, the treated water was sampled using the pump that injected it into the streams. Sampling was a little more complex for the effluents fortified with petroleum distillates because three pumps were used for this treatment. The effluent was first sampled using the pump that injected it into the streams and then, the petroleum distillate was sampled using the pump that injected it into the mixture of effluent and stream water. Before analysis these samples were mixed in the lab using high shear mixing (Ultra Turrax ® IKA T25) in order to mimic the effect of the mixing valves. For stream water analyses, the water was sampled directly in the artificial streams.

Each water sample was collected in specific containers depending on the type of analysis to be performed (glass bottles for TPH, PBS and bioassay analyses and 10 L stainless steel containers for GCGC).

2.4.2. Physical-Chemical Parameters

2.4.2.1. General parameters

The physical-chemical quality of European rivers is periodically monitored by governmental water agencies. In France the quality evaluation system links the physical-chemical water quality to the ecological status of rivers as shown in Table 3. This evaluation system, which can help to understand the results of the biological monitoring, was used in phase III. The physical-chemical (O_2, pH, temperature and conductivity) parameters were monitored continuously using physical-chemical sensors located at the end of each stream. The turbidity was also continuously measured in one stream (Stream 1). All these measurements were recorded continuously, 24 hours a day, using an acquisition loop on a computer.

Table 3: Physical-chemical parameter thresholds corresponding to the different ecological classes of the French surface freshwater (Methods and criteria of evaluation of ecological status, chemical status, and ecological potential of surface waters, arêté ministeriel du 25 janvier 2010)

Parameters	Limits				
	very good	good	medium	mediocre	bad
oxygen assessment					
Dissolved oxygen (mg.O_2/L)	8	6	4	3	
Oxygen saturation (%)	90	70	50	30	
BOD5 (mg.O_2/L)	3	6	10	25	
Dissolved organic carbon (mg.O_2/L)	5	7	10	15	
Temperature					
Salmonicol water	20	21.5	25	28	
Cyprinicol water	24	25.5	27	28	
Nutrients					
PO_4^{3-} (mg PO_4^{3-}/L)	0.1	0.5	1	2	
Total phosphorous (mg P/L)	0.05	0.2	0.5	1	
NH_4^+ (mg NH_4^+/L)	0.1	0.5	2	5	
NO_2^- (mg NO_2^-/L)	0.1	0.3	0.5	1	
NO_3^- (mg NO_3^-/L)	10	50	+	+	
Acidification					
Minimum pH	6.5	6	5.5	4.5	
Maximum pH	8.2	9	9.5	10	
Salinity					
Conductivity	+	+	+	+	
Chlorides	+	+	+	+	
Sulphates	+	+	+	+	

Temperature, pH, O_2 and conductivity of the process effluents were also measured using portable multi-parameter probes. Measurements were made when the effluents arrived at the Lacq facility, after one week of storage in each flexible tank (T0) and once a week in each flexible tank during the study (T4, T14, T21 and T42).

A set of additional parameters listed below were also measured weekly during the experiment in the three "control" streams to characterize biodegradable organic matter (oxidation), pollutants containing nitrogen and pollutants containing phosphorous and chlorides:

- BOD5 according to the EN 1899-1 method
- COD according to the ISO 15705 method
- Nitrites according to the EN 26777 method
- Ammonia according to the NF T 90-015-2 method
- Total *Kjeldahl Nitrogen according to the* EN 25663 method
- Nitrates *according to the* EN ISO 10304 method
- Orthophosphate *according to the* NF T 90-023: 1982 method
- Total phosphorous *according to the* NF T 90-023: 1982 method
- Chlorides EN ISO 10304 method

BOD_5 and COD were also measured once a week in the effluents stored in the flexible tanks.

2.4.2.2. Substance characterisation

The effluents and the petroleum distillates injected into the streams were complex mixtures, particularly in terms of hydrocarbon composition. Characterisation of the water in the streams and flexible tanks was necessary in order to assess whether the organisms were exposed to constant concentrations of the effluents. For this reason, hydrocarbons were measured both in the streams and tanks using different methods.

- **Hydrocarbon Block characterisation**

Each petroleum distillate fraction can contain thousands of individual chemical substances, which can be grouped together into blocks or groups of substances (hereafter referred to as “hydrocarbon blocks”) that share similar physical and chemical properties. The substances within these blocks can be collectively assessed for hazard and risk using the Hydrocarbon Block Method [23].

Comprehensive high-resolution two-dimensional gas chromatography (GC×GC) using a method developed by Shell [26] has been employed for the detailed characterisation of complex middle-distillate fuel fractions. Concawe has also applied this analytical method to refinery effluents [25]. In GCxGC the petroleum sample is subjected to two independent (orthogonal) GC separations, providing far better discrimination of the numerous components than is possible by conventional GC. The first separation is based on volatility differences (i.e. carbon number) between the components whilst the second separation exploits differences in polarity (i.e. functionality). All components are quantified using the universal flame ionization detector (FID), so calibration standards of the individual components are not required for accurate quantitative analysis.

For the speciation of HC blocks the effluent was extracted in 5 L closed glass bottles without headspace. Each bottle contained 250 ml of dichloromethane. The extraction process lasted for 48 hours. The sample was then fractionated into an aliphatic and an aromatic fraction and analyzed by GCxGC-FID. An Agilent 7890A gas chromatograph (GC) was used with capillary flow technology as modulator, and flame ionization detection (FID) with a scan speed of 200 Hz. A full description of the methods used can be found in Concawe report No 9652 [27].

An external laboratory (Institute for Environmental Studies, IVM of the VU University of Amsterdam) was contracted in phase III to perform GCxGC analyses of Hydrocarbon blocks in the streams and in the effluent stored in the flexible tanks. Hydrocarbon Block measurements were performed on Days 2, 7 and 21 of treatment in three streams exposed respectively to effluent A (stream 15), effluent B fortified with Kerosene (stream 10) and effluent C fortified with Diesel (stream 4). Hydrocarbon Block measurements were also performed at the beginning (Day 0) and at the end (Day 21) of treatment in the effluents stored in the flexible tanks, both without fortification (Days 0, 7 and 21) and with fortification with the petroleum distillates.

- **TPH analysis**

Analysis of Total Petroleum Hydrocarbons (TPH) in water samples represents the total content of C10 through to C40 hydrocarbon structures. This range encompasses the major components of a number of widely used petroleum products such as kerosene, jet and diesel fuels. The analysis method involves manual liquid-liquid extraction (LLE) of the water samples. The extract is dried using specially prepared anhydrous sodium sulphate, concentrated through an evaporation step, and purified with florisil (magnesium silicate). The purified extract is then concentrated through another evaporation step before analysis using a gas chromatograph coupled to a flame ionization detector (GC-FID).

The IVM laboratory was used to perform the TPH analysis of both the stream waters and in the effluents stored in the flexible tanks. TPH measurements were performed in all the streams before (Day 0), and during the treatment of the effluent (Days 2, 7 and 21). TPH analysis was also performed on the effluents stored in the flexible tanks. The analysis was carried out on both the fortified and unfortified samples. The analyses were carried out at the start (Day 0) and end (Day 21) of treatment.

- **PBS analysis**

Potentially bioaccumulative substances (PBS) were analysed for in the effluent stored in the flexible tanks by a contract laboratory (Institute for Environmental Studies (IVM) of the VU University of Amsterdam). A partitioning method based on bio-mimetic solid phase micro-extraction (SPME) was used. The method was based on a protocol used in the OSPAR inter-laboratory study [12]. Briefly, SPME fibres (100 µm PDMS (poly(dimethylsiloxane)) were exposed to 250 ml of effluent, with agitation, for 24 hours in a closed glass bottle. Measurements were performed in triplicate for each sample. After 24 hours of exposure the fibres were removed from the effluent solution and dried with a tissue and directly injected into a gas chromatograph (GC) equipped with flame ionization detection (FID). A DB-1 (210 m x 0.25 mm x 0.1 µm) GC column was used. For quantification 2,3-dimethylnaphthalene was used as an external standard. The total peak area of the chromatogram was integrated (between C9 and C38) and the molar concentration was calculated [28].

The IVM laboratory was used to perform PBS analysis in phase III. Analyses were performed on stream waters at the start of treatment (Day 0) and on Day 7. Samples were analysed from three streams exposed respectively to effluent A (stream 15), effluent B fortified with Kerosene (stream 10) and effluent C fortified with Diesel (stream 4). Additional PBS analyses were performed on samples from all the streams on days 14 and 21 of treatment. PBS measurements were also performed on the effluent samples stored in the flexible tanks. Samples were analysed both with (Day 0 and Day 21) and without (Days 0, 7 and 21) fortification with the petroleum distillates.

2.4.3. Biological Parameters

2.4.3.1. *In situ* measurements into the streams

➢ **Bacteria**

Densities of heterotrophic aerobic bacterial were estimated during the treatment in the sediment pore water of each stream by means of the most probable number (MPN) method described in guideline NF T 90-413. This method consists of taking samples from the liquid, incubating each sample in a suitable culture medium, and observing whether any growth of the bacterial colonies has taken place. The estimation of density is based on an application of the theory of probability to certain assumptions (a random distribution of bacteria throughout the liquid). This endpoint was measured in the streams throughout the treatment period on days 0, 1, 2, 7, 14 and 21. Heterotrophic aerobic bacterial density was also measured in each stream 2, 7 and 30-days after ceasing treatment.

➢ **Primary production and diatom abundance and biodiversity**

Diatoms are the main primary producers in rivers and they are good indicators of short- and long-term changes in water quality. Many methods of characterizing diatom populations have been developed in Europe and in other parts of the world.

- Primary production was estimated on the basis of samples of periphyton deposited naturally on glass plates (5×10 cm^2 for each face) that were left submerged in the stream throughout the treatment period. Several glass plates were placed in each stream. These glass plates were sampled and taken back to the laboratory in order to measure the quantity of chlorophyll using a spectrophotometer according to guideline NF T 90- 117. This endpoint was measured in the streams on days 0, 7, 14 and 21 of treatment. Primary production was also measured in each stream 30 days after ceasing treatment.
- One additional glass plate/stream was also left submerged throughout the whole of the experiment. These glass plates were used to estimate the primary production by measuring the chlorophyll using a bbe fluoroprobe (Biological Biophysical Engineering, Moldaenk) with an additional Benthos adapter. This probe allowed the kinetics of the chlorophyll content of one plate/stream to be followed throughout the study. In addition to determining the chlorophyll content, the probe also detects the presence of algae and allocates them to various colour spectrum classes (blue-green algae/ cyanobacteria, green algae, diatoms / dinoflagellates / chrysophytae, cryptophytae). This endpoint was measured in each stream on days 0, 7, 14 and 21 of treatment. Chlorophyll content was also measured in each stream 7 days and 30 days after ceasing treatment.
- Diatom communities were also studied using additional submerged glass plates (5×10 cm^2 for each face). Several glass plates were placed in each stream and sampled during the treatment. Diatoms were collected from the surface of the plates and a taxonomic list drawn up which indicated impacts on the diatom community. Both diatom biodiversity and abundances were estimated from the samples. These endpoints were measured in each stream during treatment on days 0, 7, 14 and 21. The same endpoints were also measured in each stream 30 days after ceasing treatment. ASCONIT consultants (an independent consulting organisation, France) were contracted for the identification and counting of the diatoms.

Both diatom biodiversity and abundance results were used to calculate ecological indices. Some of these are based on the saprobic system (which is based on 4 zones of gradual self-purification: the polysaprobic zone, the α-mesosaprobic zone, the β-mesosaprobic zone, and the oligosaprobic zone), some on auto-ecological knowledge (affinity for abiotic and biotic factors) and others on community structure. More recent methods, such as the IBD (Biological Diatom Index) [29; 30] and the French IPS or SPI index (Specific Pollution Sensitivity Index) [31], have been developed on the basis of the relationship between chemical parameters and diatom abundance.

The standardised French IBD index has been widely used in water quality monitoring programs since 1999 [32]. Sampling is carried out, where possible, in the area of the river with the highest flow. Five to ten stones are selected depending upon their size and diatoms are collected from their surface. Exactly 400 individuals are counted and identified. Taxonomic lists obtained at each site are compared to a base list appended to the IBD method. The list is made up of 209 diatom taxa. The index is calculated, taking into account both the abundance of the taxa and their tolerance for both organic pollution and nutrients levels (nitrogen and phosphorus). On the basis of the IBD scores water bodies are characterized as five quality classes, from very low to very good water quality [29].

The IPS index is determined similarly to the IBD index. Exactly 400 individuals are counted and identified. IPS index is correlated with parameters related to organic pollution, ionic strength, and eutrophication and gives an indication of water quality. IPS is determined taking into account the abundance and the susceptibility of all diatom species of the sample. A decrease of the both IBD and PSI scores characterizes an increase of organic pollution *in situ*.

Trophic Diatom Index (TDI) is a measure of the effect of nutrients (predominately phosphorus) on stream communities [33]. The TDI is based on the weighted average equation of Zelinka and Marvan (1961):

$$Index = \sum_{j=1}^{n}(a_j s_j v_j) \,/\, \sum_{j=1}^{n}(a_j v_j)$$

Where aj = abundance or proportion of valves of species j in sample, sj = pollution sensitivity (1-5) of species j and vj = indicator value (1-3). Values of sensitivity (s) are as follows:

1 = favoured by very low nutrient concentrations

2 = favoured by low nutrient concentrations

3 = favoured by intermediate nutrient concentrations

4 = favoured by high nutrient concentrations

5 = favoured by very high nutrient concentrations.

The TDI has been developed to aid investigation focusing on particular point discharges and is developed for basic screening of large sewage in UK. The index has a scale of 0 – 100, with higher values indicating progressively higher levels of nutrients. TDI is also used by some as an index of organic pollution [34]. An increase of the TDI score characterizes an increase of organic pollution *in situ*.

IPS, IBD and TDI indices were calculated using Omnidia v4 software.

- **Benthic invertebrate abundance and biodiversity**

Invertebrate communities that colonized the stream mesocosms were sampled using specific traps (0.1 m^2). These traps were made from 160 µm mesh net that allowed sampling of benthic organisms larger than 160 µm. Five traps, each one corresponding to one sampling date, were placed in each of the 12 streams at the start of the colonization phase. The traps were filled with standard substrate (coarse pebbles) and provided a habitat for benthic invertebrates. The traps were sampled during the treatment period without disturbing the surrounding substrate. Once sampled, the substrate was cleaned with water. This water and the fine sediment were then filtered using 160 µm sieves to remove any invertebrates that were present. The invertebrates were fixed in formalin prior to identification and counting. Benthic invertebrate biodiversity and abundance were assessed. These endpoints were measured in each stream on days 0, 7, 14 and 21 of treatment. The same endpoints were also measured in each stream 30 days after ceasing treatment. AQUABIO (an independent consulting organisation) were contracted for the identification and counting of the benthic invertebrates.

Both benthic invertebrate biodiversity and abundance results were used to calculate ecological indices (French IBGN & EPT indices).

The French version of the IBGN index (Indice Biologique Global Normalisé) is commonly used for monitoring biotic integrity in French rivers. The index takes into account the presence of both sensitive and resistant taxa. Eight habitats per site are normally sampled using this method in order to encompass the diversity of the site. Taxonomic lists obtained at each site are compared to a base list appended to the IBGN method. The list is made up of 152 benthic invertebrate taxa of which 38 are classified into 9 indicator groups according to their increasing tolerance to organic pollution and associated hypoxia. The IBGN scores are obtained from a reference table appended to the IBGN method. The table contains the 9 indicator groups and 14 classes of species richness and the IBGN score is calculated taking into account the species richness of each indicator group [35].

EPT species present in the sampled benthic invertebrates were specifically targeted and an EPT index, corresponding to the total number of EPT taxa detected, was calculated for each sample.

2.4.3.2. WET performed on the effluents stored in the flexible tanks

- **Microtox® (EN ISO 11348-3)**

The Microtox® bioassay (with bacterium *Vibrio fischeri*) was carried out to assess the toxicity of the effluents according to guideline EN ISO 11348-3. Toxic inhibition of normal metabolism of the bacterium results in a reduction in bioluminescence which can be assessed by measuring light output using the Microtox® equipment. The assay was run across a range of concentrations of the stored effluent samples and EC50 values (volume %) were estimated from the light output data for 5, 15 and 30 minute exposure periods using statistical programs developed for the Microtox® test system. The EC50 value is defined as the concentration, which (after a certain exposure period) reduces the bioluminescence by 50% in comparison with the control. The EC50 value then provides a measure of the toxicity of the samples that can be compared with similar data for other samples. The procedure is rapid, simple and cheap. These results might provide a useful measure of relative toxicity across a range of samples. Microtox® assays were carried out with the effluents that were stored in the flexible tanks, both without fortification (Days 0, 7, 14 and 21) and with fortification with the petroleum distillate fractions at the beginning (Day 0) and at the end (Day 21) of treatment. CARSO Group, which is a lead provider in France in analytical services for quality of the environment and life, was contracted to perform the tests.

➢ **Daphnia magna acute toxicity test (EN ISO 6341)**

Acute toxicity tests were performed with *Daphnia magna* according to ISO guideline 6341 to assess the toxicity of the effluents. Immobility of the organisms was assessed after 24 hours exposure to the effluent. Test organisms were considered to be immobile, if they did not respond within 15 seconds to gentle agitation of the test vial. EC50 values were estimated, using the statistical program (Regtox: http://www.normalesup.org/~vindimian/fr_index.html). The EC50 value is defined as the concentration which (after a certain exposure period) causes 50% immobilization of the organisms. The EC50 value provides a measure of the toxicity of the samples that can be compared with similar data for other samples. *Daphnia magna* acute bioassays were conducted on the effluents stored in the flexible tanks, both with and without fortification with the petroleum distillate fractions (Days 0 and 21). CARSO Group were contracted to perform the tests.

➢ **Micro-algae test (EN ISO 8692)**

Chronic toxicity tests were performed with *Pseudokirchneriella subcapitata* according to ISO guideline 8692 to assess the toxicity of the effluents. Cell densities were measured in the tests after specified time intervals and growth rates (R) were calculated for each test concentration and replicate. EC50 values were estimated using the statistical program (Regtox: http://www.normalesup.org/~vindimian/fr_index.html). The EC50 value is defined as the concentration which (after a certain exposure period) causes 50% inhibition of algal growth rate in comparison with the control. The values are then used to indicate the toxicity of the effluent samples to algae in a multi-generation test system. *Pseudokirchneriella subcapitata* chronic toxicity bioassays were conducted on the effluents stored in the flexible tanks, both with and without fortification with the petroleum distillate fractions (Days 0 and 21). CARSO Group were contracted to perform the tests.

2.5. HIGHER LEVEL DATA ANALYSIS

2.5.1. Community effects

The species abundance data (benthic invertebrates and diatoms) obtained in the Pilot Rivers was analyzed using the Principal Response Curve (PRC) ordination method. The analyses were performed using the CANOCO statistical software (Windows 4.5 package). PRC is a multivariate data analysis method that allows the visualization of community data with an emphasis on dimension reduction (ordination), regression analysis, and a combination of the two.

The PRC method is based on redundancy analysis (RDA), adjusted for overall changes in community response over time, as observed in control test systems. This adjustment allows the method to focus on the time-dependent treatment effect.

Redundancy analysis is a type of Principal Components Analysis (PCA) that is applied to the fitted species data. In RDA the sample scores are constrained to be linear combinations of the explanatory variables. PCA is therefore performed first and then RDA is derived from it. RDA allows variables (ordination axes) to be identified that represent the best predictor of the abundance values for all the species. In contrast to PCA, RDA extracts information from the explained variance only; the RDA axes represent a percentage of that variance. RDA therefore allows the effect of a treatment on a community of organisms to be explained more simply over time. The principal component is plotted against time, yielding a PRC of the community for each treatment.

The PRC method distils the complexity of time-dependent, community-level effects of pollutants into a graphic from that can be appreciated more readily than the results of other currently available multivariate techniques [36]. The PRC method also enables a quantitative interpretation of effects at the species level. The RDA is used to identify the variance in the data sets which is due to the treatment, the time or a combination of the time × the treatment. In CANOCO, the RDA is used in conjunction with a Monte Carlo permutation test. The Monte Carlo test is a test of statistical significance that is performed by repeatedly and randomly permuting the related samples (the species data in one block, for instance in one treatment). Each permutation leads to a new dataset from which we can calculate the test statistic. The reference distribution therefore is the distribution of the test statistic in the permuted data sets. The new data sets can be obtained by random Monte Carlo permutations if the samples in the species data are independent and exchangeable. The power of the test to detect a significant effect increases with the number of permutations.

In this study the Monte Carlo test was used to calculate the statistical significance of the impact of the explanatory variables on the species compositions of the samples. This allowed the significance of the treatment regimes to be tested on each sampling date. ANOVA and multivariate Dunnet *post-hoc* tests were then performed on the sample scores (using Statistica v10 software) to identify which treatments differed significantly from the control and thereby define a NOEC for the species community.

2.5.2. Effects on Taxa

In view of the low number of replicates (two for treated streams and three for the control streams), the taxa abundance data for the streams treated with the effluent were expressed as a percentage of the same taxa abundance in the control streams. Coefficients of variation were calculated for each treatment. These results were plotted in charts that allowed differences between control and treated streams to be visualised.

2.5.3. Effects in Toxicity tests and Bioassays

To clarify the following discussion it is important to distinguish between a toxicity test and a bioassay and the ways in which the results are expressed and interpreted.

2.5.3.1. Toxicity tests

A toxicity test determines the concentration of a defined substance that cause an effect (e.g. mortality, immobilisation or reduced reproduction) on a defined proportion of an exposed population (e.g. 50%) within a specified time period. The result can be expressed as ECX, LCX, NOEC or LOEC values depending upon the type of test and its duration. The result of a toxicity test is often expressed as a concentration of the substance, for example as mg/l in water or mg/kg in soil or sediment.

2.5.3.2. Bioassays

A bioassay determines the toxicity of a sample relative to that of a reference or control sample; is the test sample more or less toxic than the reference or control sample. The result of a bioassay is normally expressed as a percentage of the control or reference sample response.

2.5.3.3. Expressing results as Toxic Units

Toxic units (TU) are an alternative way of expressing the toxicity of a substance or sample. Two types of TU values can be determined depending upon whether or not the substances present in a test sample have been chemically characterised.

a) *Toxic units for a specific chemical substance which is present in a test medium at a known concentration*

For a specific chemical substance with established toxicity test data (acute and/or chronic) a measured exposure concentration of that substance in a sample can be expressed as TU by dividing the measured concentration by the acute and/or chronic toxic threshold concentration (expressed as, for example, the known EC50, LC50 or NOEC value). It is important that both values are expressed in terms of the same units, for example mg/. If the ratio of the two values is say 2, then 2 toxic units of the substance are present in the sample.

TU values for defined chemical substances in test media can range from <1 to >1 depending upon the concentration of the substance in a medium. If the concentration is less than the toxic threshold concentration then the TU value will be <1. If it is above the threshold concentration it will be >1.

For substances that share a common mode of action it is possible to sum the TU values for all the substances that are present in a sample and determine an overall sum of TU. This is a helpful way of visualising the overall toxicity of a sample containing multiple chemical contaminants in terms of a standardised parameter.

b) *Toxic units for a water sample of undefined chemical composition*

If the test substance is a water sample of undefined chemistry it is still possible to define the results of a bioassay conducted on it in terms of acute and/or chronic TU. In this case the calculations are performed using bioassay results, which are expressed as percentage concentrations of a test sample corresponding to the relevant toxic threshold effect levels, and one or both of the following expressions

$$\mathbf{TU_{acute} = 100/EC50}$$
$$\mathbf{TU_{chronic} = 100/EC10 \text{ or } NOEC}$$

Hence, if the EC50 value for the test sample is 50% then 2 TU are present (i.e. 100/50 = 2).

TU values for a sample of undefined composition, such as an effluent, can range from 1, which signifies that the sample is no more or less toxic than the reference or control sample, to >1 where the sample is more toxic than the reference or control sample.

Bioassay results are presented in this report and so TU values have all been calculated using one or other of the formulae given above.

Interpretation of TU values derived from bioassay data for effluent and waste-water samples

In the Canadian Regulation [37], industrial effluents with TU>1 are considered to be toxic (acutely or chronically). However, depending on the country and the specific circumstances, industrial permits could allow the discharge of effluents with TU>1 provided that the dilution factor is high enough. Toxic Unit thresholds defined by SATL in Ireland [38] and presented in **Table 4** have been used to interpret the results of the phase III study.

Table 4: Toxicity classification according to SATL

Toxic Units	Description
< 3	Non Toxic
3 - 10	Slightly Toxic
10 - 50	Toxic
50 - 100	Very Toxic
> 100	Extremely Toxic

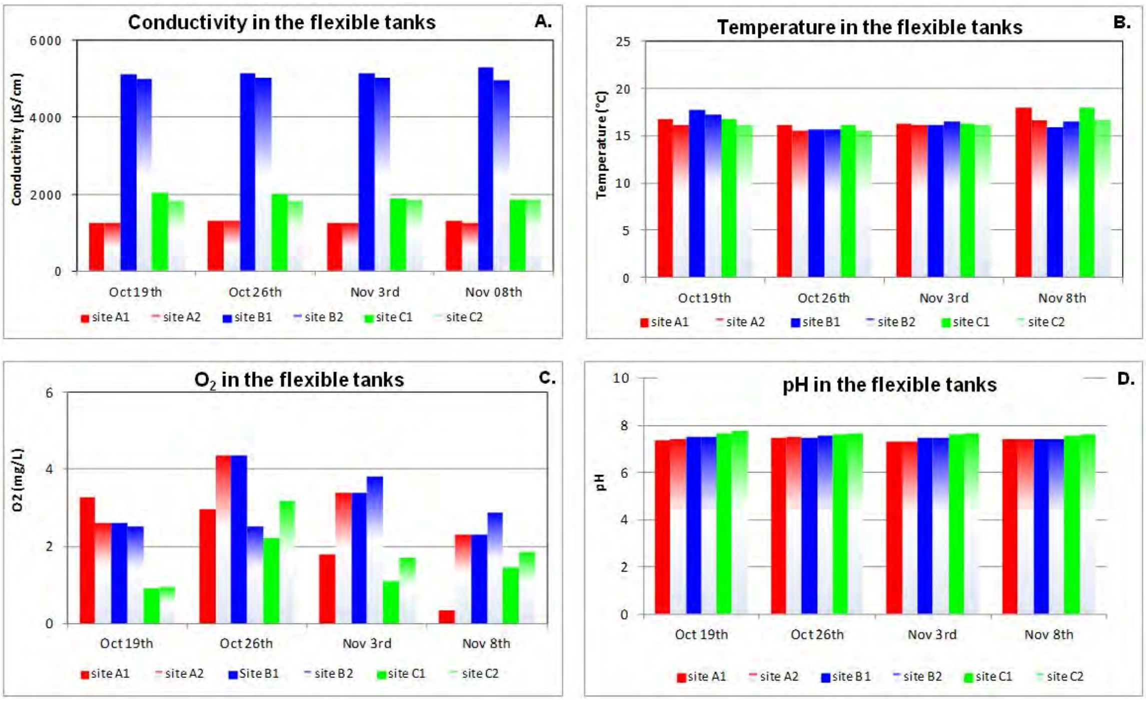
3. RESULTS

3.1. PHYSICAL AND CHEMICAL PARAMETERS

3.1.1. Flexible Tanks

Conductivity, temperature, oxygen and pH were measured once a week in the effluent stored in the flexible tanks and the results are presented in the **Figure 10**.

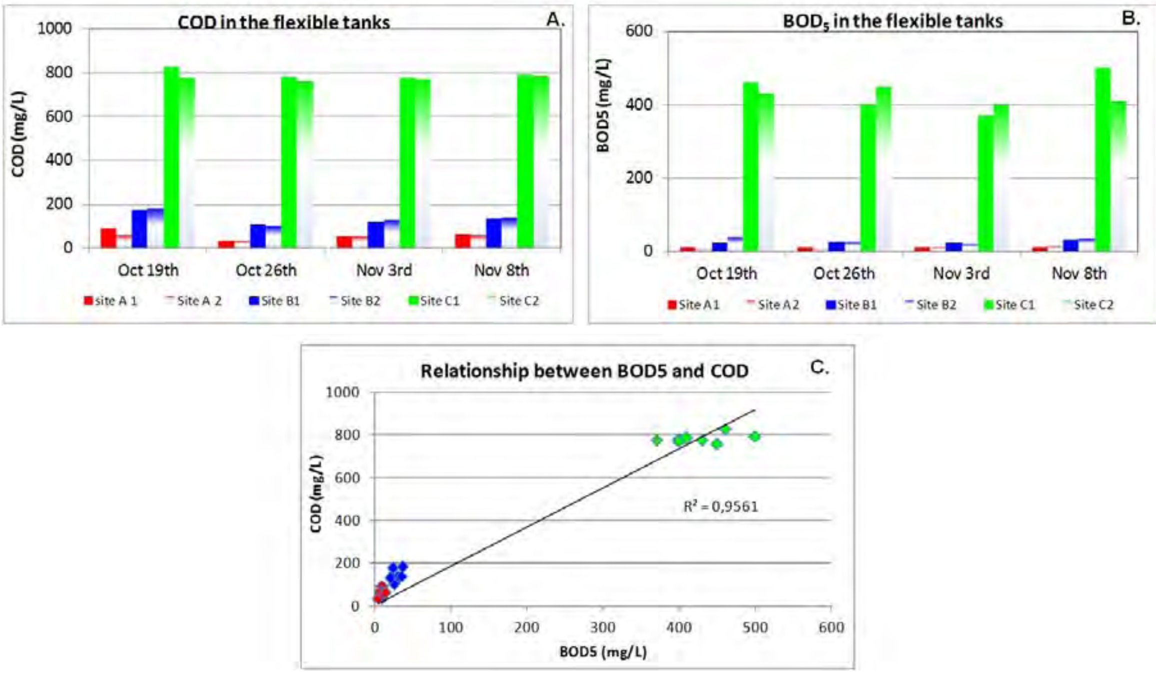
Figure 10: Conductivity (A), Temperature (B), Oxygen (C) and pH (D) measured once a week in the effluents stored in the flexible tanks.



No or little change in these parameters was observed in the effluents over the course of the 21-day experiments. The results also show that effluent B was characterized by a higher (2.5 times higher) conductivity than the other two.

BOD_5 and COD were also measured once a week in the effluents stored in the flexible tanks and the results are presented in **Figure 11**. No changes in these parameters were observed over the 21-day experiments. The results also show that effluent C was characterized by higher COD and BOD_5 levels than the other two. Effluent C had a high level of biodegradable substances in comparison with the other two effluents.

Figure 11: COD (A), BOD5 (B) and the relationship between BOD_5 and COD (C) measured once a week in the effluents stored in the flexible tanks.



TPH and PBS levels were measured on days 0 and 21 in the effluent stored in the flexible tanks (**Figure 12**). These two parameters were also measured in the fortified effluent injected into the streams (**Figure 12**). The results show that the samples from the three sites exhibited different levels of TPH and PBS. Site A was the least contaminated whereas site B was the most contaminated. In terms of TPH and PBS content, little or no change was observed in the effluents stored in the flexible tanks over the 21-day period of the experiment. The fortified process effluents injected into the streams presented no difference in terms of TPH and PBS levels between day 0 at the beginning of the experiment and day 21 at the end of the experiment.

The streams exposed to the highest TPH and PBS levels were the ones treated with site B's effluent fortified with Kerosene; the levels were twice as high as those in the streams exposed to site C's effluent fortified with Diesel.

Figure 13 shows that there was a good correlation between measured TPH and PBS levels in the same effluent stored in the same flexible tanks (both before and after being fortified with the petroleum distillates).

Figure 12: PBS (A and B) and TPH (C and D) levels in the effluents before and after fortification with the petroleum distillates

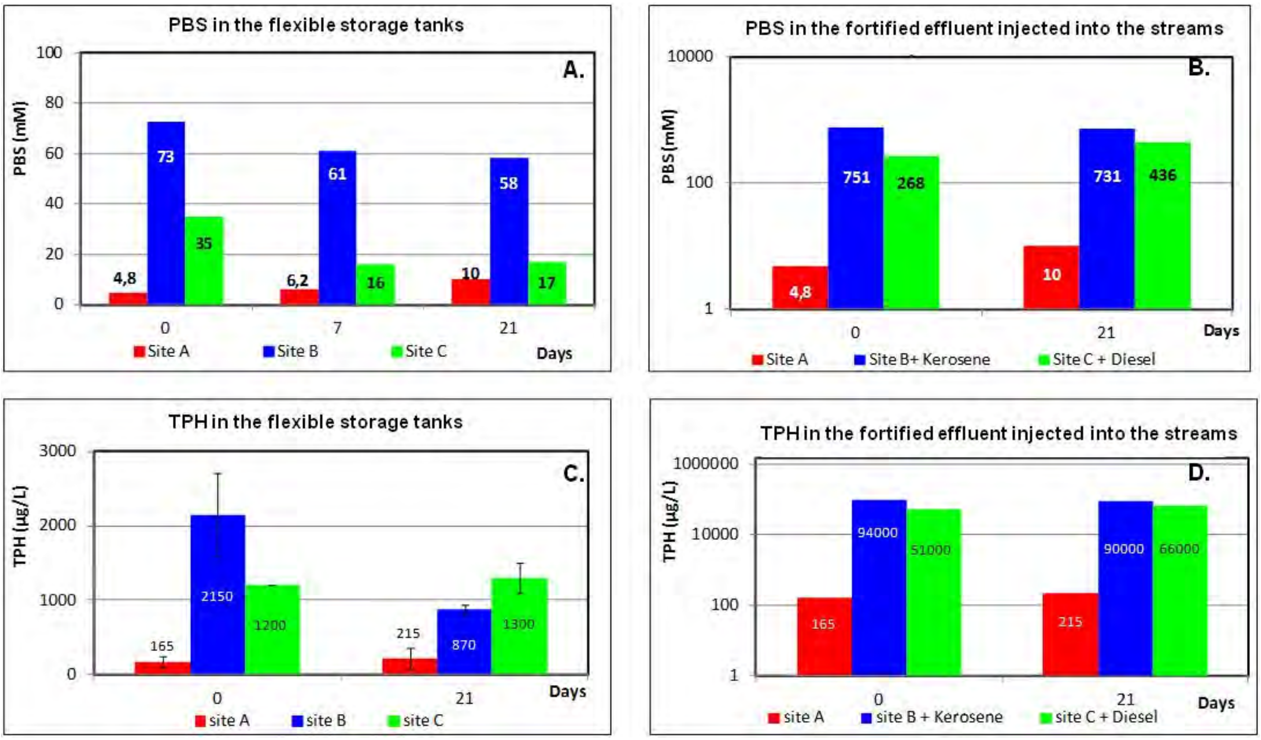
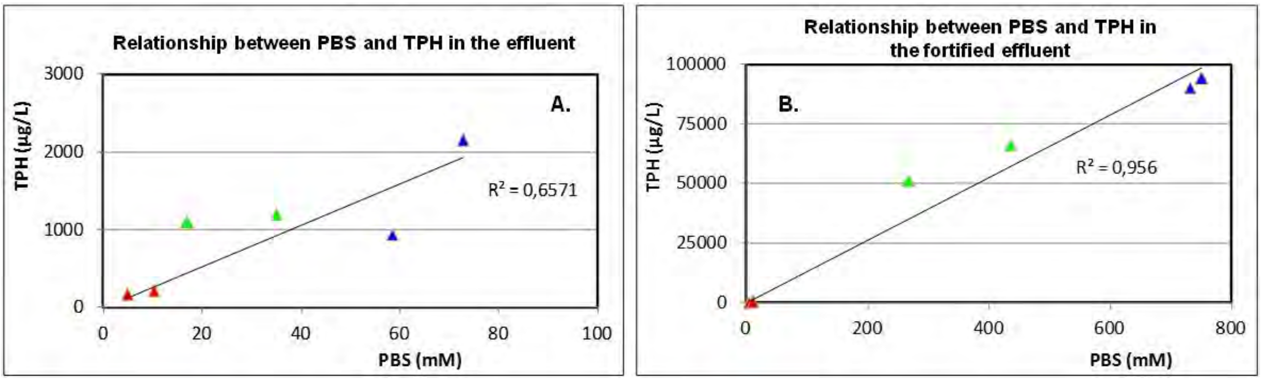
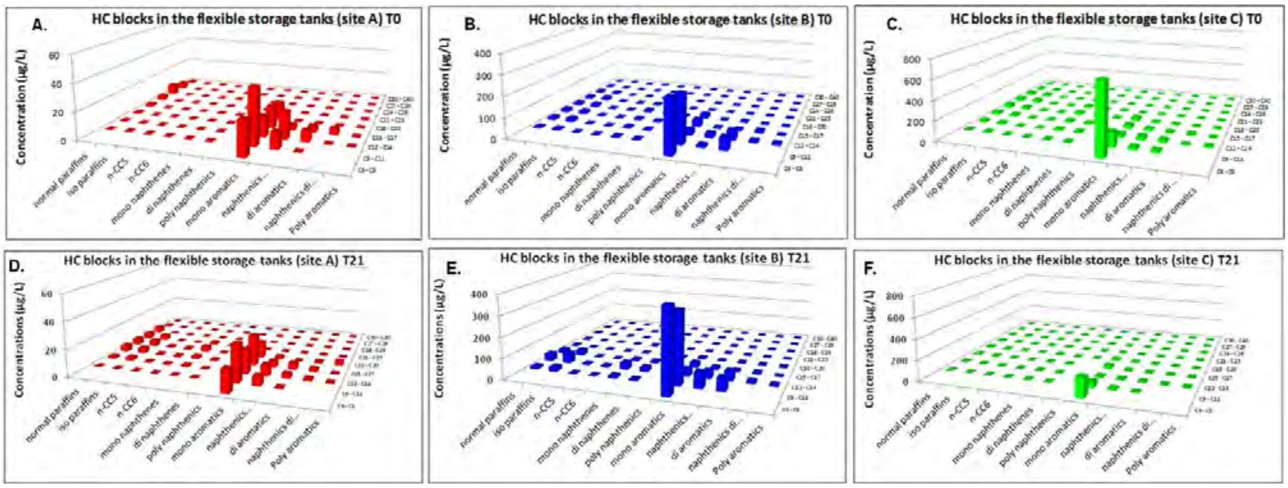


Figure 13: Relationship between measured PBS and TPH content in the effluents before (A.) and after fortification (B.) with the petroleum distillates



Hydrocarbon profiles were also measured using GCGC on day 0 and day 21 in the effluents (with and without fortification with petroleum distillate fractions) that were stored in the flexible tanks. The results are shown in **Figure 14**.

Figure 14: Hydrocarbon blocks obtained using GCGC for the three effluents stored in the flexible tanks (A. and D. for site A, B. and E. for site B and C. and F. for site C).

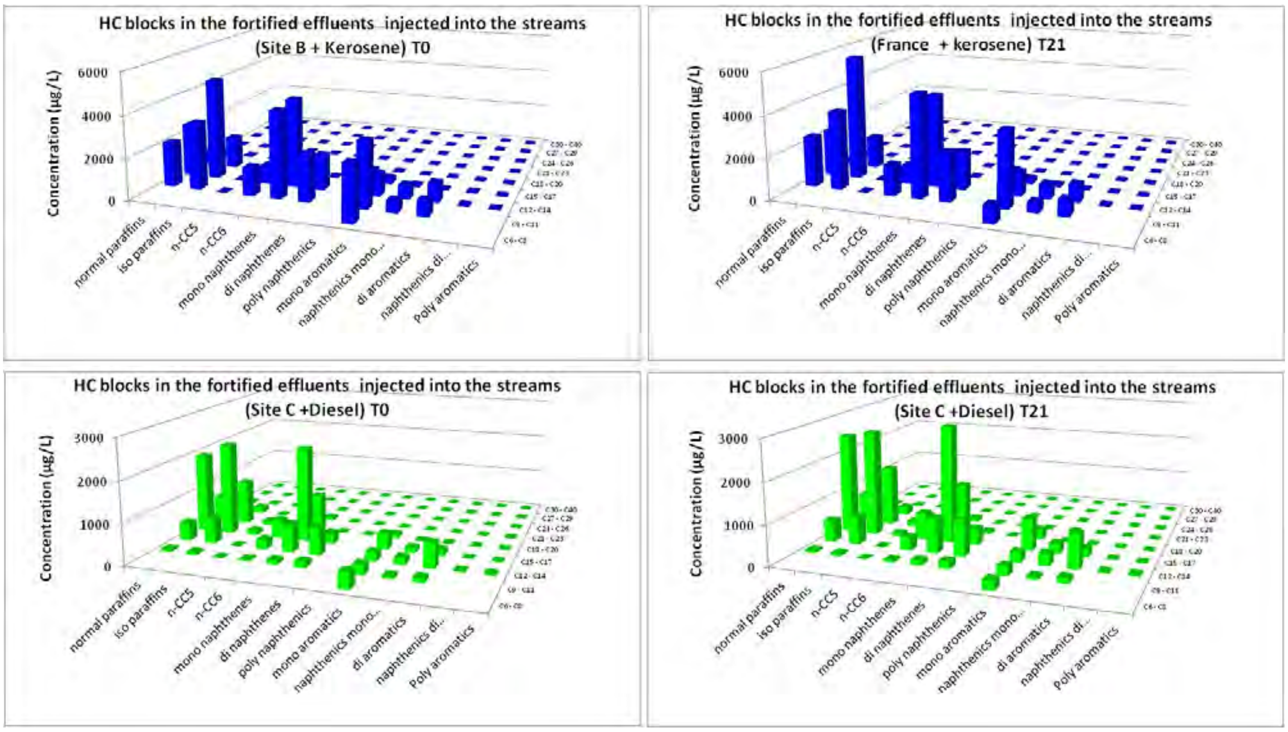


No or little variation was apparent in the hydrocarbon profiles of the effluent samples stored in the flexible tanks between the beginning of the study on day 0 and the end of the experiments on day 21. This was true both in terms of the overall concentrations of the hydrocarbon blocks (expressed in terms of µg/l) and the composition of the blocks. Effluent A contained mainly mono aromatics (C6 to C20) and naphthenic mono aromatics (C9-C14). Effluent B contained mainly mono aromatics (C6 to C11) and di-aromatics (C11-C14). Effluent C contained mainly mono aromatics (C6 to C8).

For the fortified effluents injected into the streams, the results of the analyses performed on day 0 and day 21 are presented in **Figure 15**.

For the fortified effluents injected into the streams (site B fortified with kerosene and site C fortified with diesel), no variation was also observed between day 0 and day 21, both in terms of hydrocarbon block levels and composition of the blocks (**Figure 15**). Effluent B fortified with kerosene contained mainly normal and iso paraffins (C9 to C14), mono and di-naphthenes (C9 to C14) and mono aromatics (C6 to C11). Effluent C fortified with diesel contained mainly normal and iso paraffins (C15 to C20) and mono naphthenes (C14 to C20).

Figure 15: Hydrocarbon blocks obtained using GCGC for fortified effluents injected into the streams (A. and B for site B, C and D. for site C).

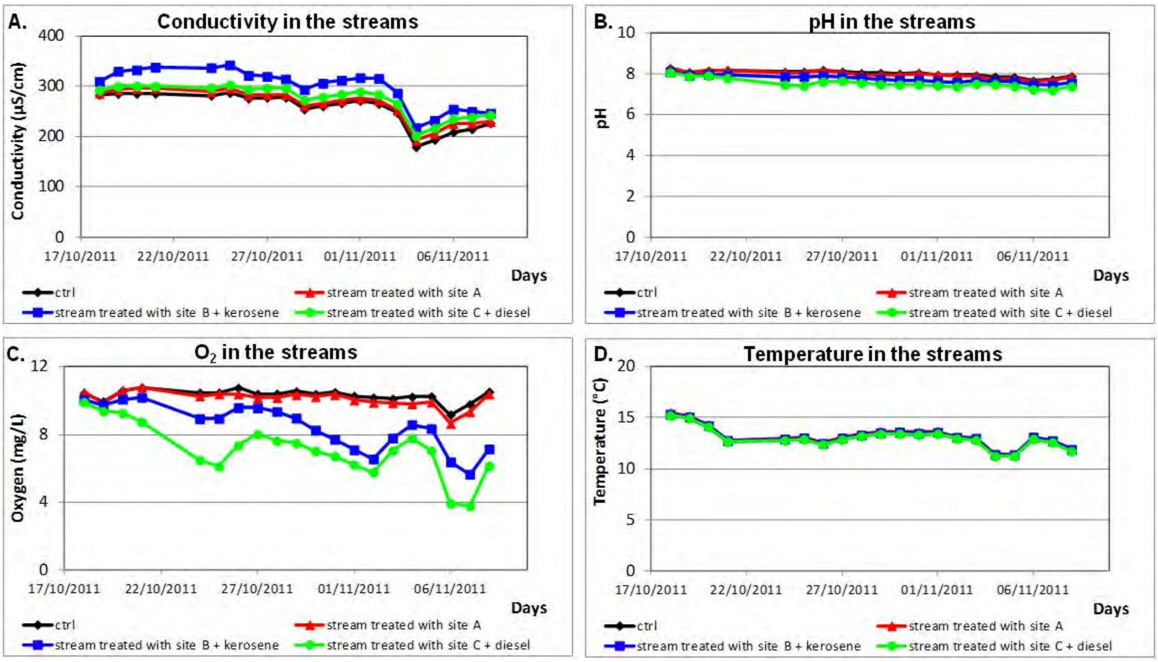


In conclusion, no significant changes were observed in the three stock solutions injected into the streams during 21 days of exposure. The treated streams were exposed to three different profiles of hydrocarbon contamination. Two streams were exposed to low levels of hydrocarbon contamination (mainly mono aromatics and naphthenic mono aromatics) with low PBS levels (10 mM) and low levels of biodegradable substances. Two streams were exposed to very high levels of hydrocarbon contamination (mainly normal and iso paraffins, mono and di-naphthenes and mono aromatics) with very high PBS levels (≈700 mM). Two streams were exposed to high levels of hydrocarbon contamination (mainly normal and iso paraffins and mono naphthenes) with high PBS levels (≈300 mM).

3.1.2. Streams

Conductivity, temperature, dissolved oxygen and pH were measured in both the untreated and treated streams. The results are presented in **Figure 16**.

Figure 16: Conductivity (A), Temperature (B), Dissolved oxygen (C) and pH (D) in the effluents stored in the flexible tanks.

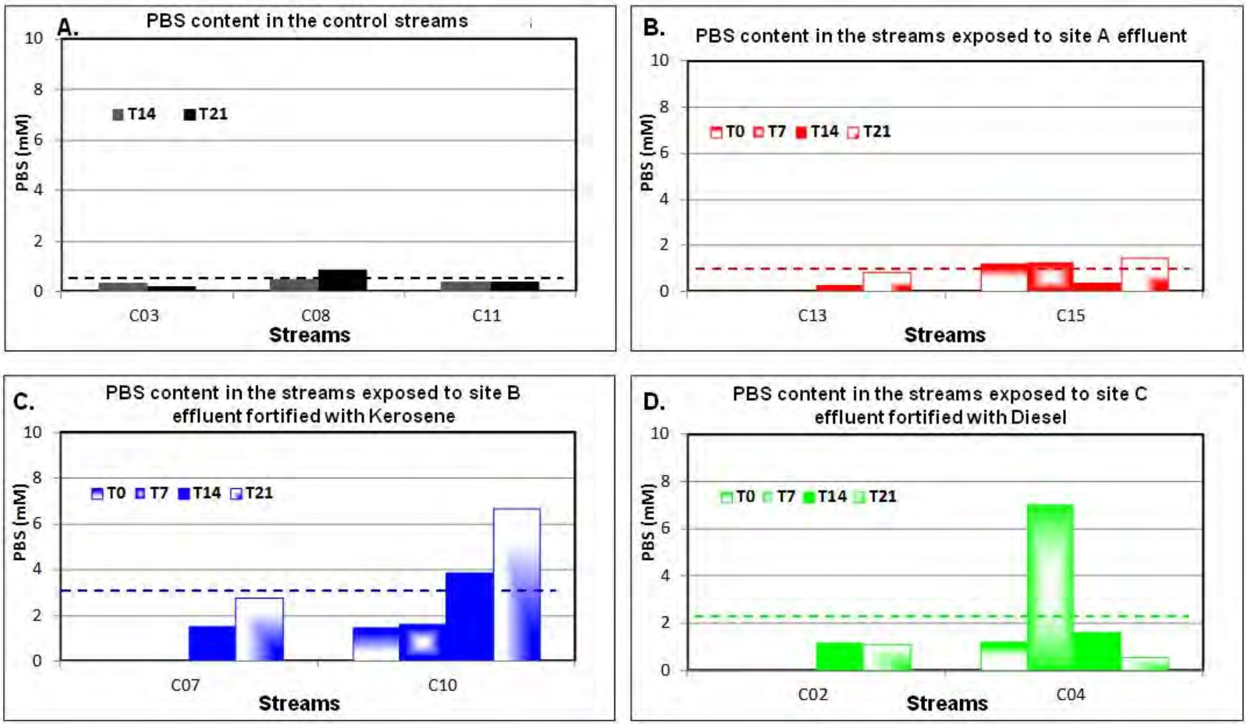


The measured water temperature in all the streams decreased from 15 to 12°C over the course of the exposure. However, no differences in temperature were observed between the treated and the control streams. No significant changes in the pH of the streams were noted over the course of the exposure period and no differences in pH were noted between the treated and control streams. The conductivity of the stream water remained constant except for the last three days of the treatment period when a decrease in conductivity was noted. The decrease is explained by a reduction in the flow that was due to the partial plugging of the water feed pipe. However, no or little difference in conductivity was observed between the treated and control streams.

Similar dissolved oxygen concentrations were observed in the control streams and in the streams treated with effluent A. However, over the 21-day exposure period, no variation in dissolved oxygen concentration was observed in the control streams and the streams treated with effluent A. The streams treated with effluent B fortified with kerosene and those treated with effluent C fortified with diesel had dissolved oxygen concentrations lower than those measured in the control streams. The decreases were mainly due to a Biofilm which was observed at the surface of the probes. After removing the biofilm, the concentration of dissolved oxygen increased for a short period of time and then declined again as the biofilm was re-established.

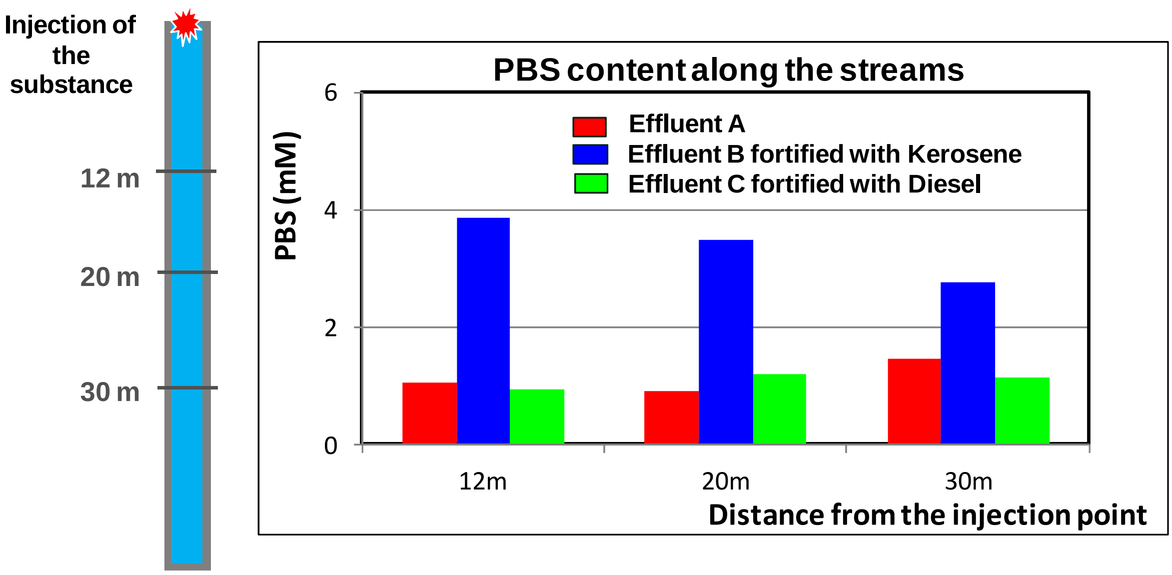
PBS levels were measured once a week in the streams during the experiments (**Figure 17**). The results show that PBS levels were low in the control streams (Average: 0.46 ± 0.15 mM) and in the streams exposed to effluent A (Average: 0.91 ± 0.4 mM). Levels in the streams exposed to effluent C fortified with diesel were higher; average PBS level of 2.1 ± 1.6 mM. The highest PBS levels were observed in the streams exposed to effluent B fortified with kerosene; the minimum levels were close to 2 mM (Average: 3 ± 1.15 mM).

Figure 17: PBS concentrations in the control streams and in the streams exposed to effluents (dotted lines represent the average TPH concentration calculated for each treatment)



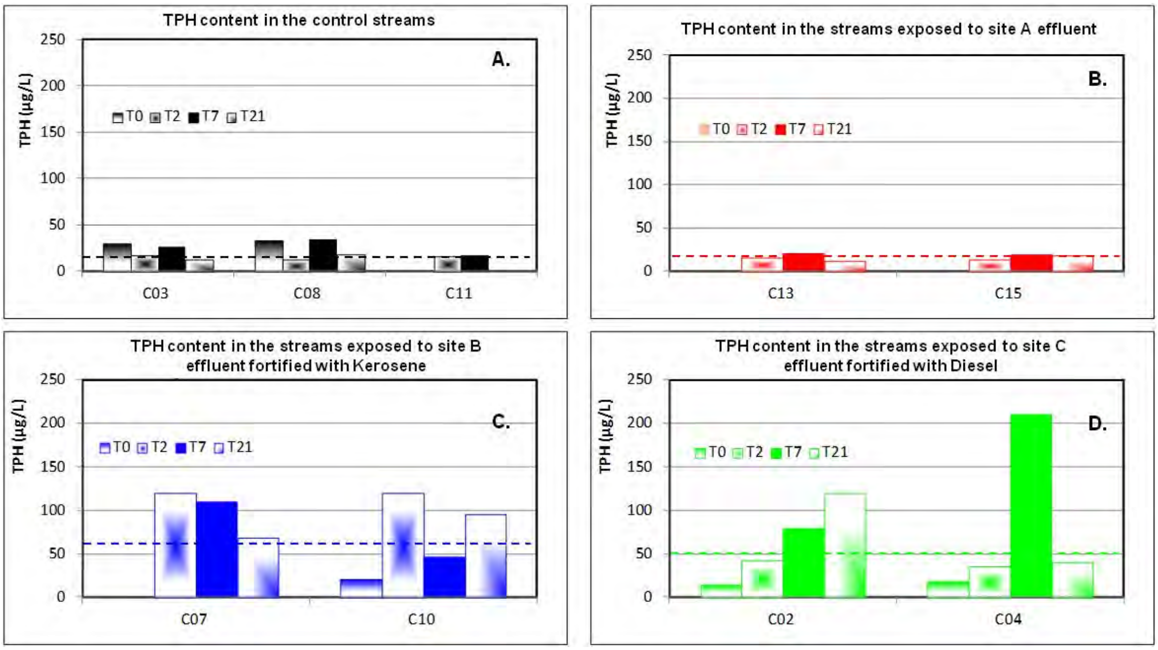
Water samples were taken for analysis at 10, 20 and 30 m downstream from the injection point to confirm that PBS levels were the same for each treatment along the streams. The results are shown in **Figure 18**. The streams were exposed to relatively constant PBS levels along their length.

Figure 18: PBS contents along the downstreams



TPH levels were also measured once a week in the streams during the experiments and the results are given in **Figure 19**. Low and similar TPH levels were measured in the control streams (Average: 22 ± 8 µg/l) and in the streams exposed to effluent A (Average: 17 ± 4 µg/l).

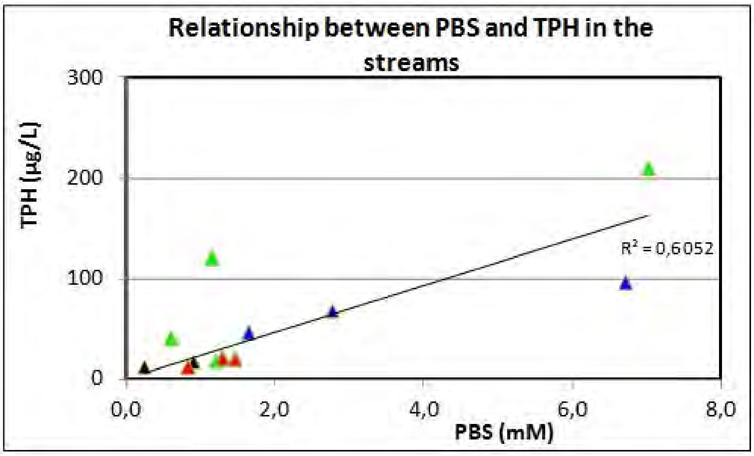
Figure 19: TPH concentrations in the control streams and in the streams exposed to effluents (dotted lines represent the average TPH concentrations calculated for each treatment).



The streams exposed to effluent from site C that was fortified with diesel contained on average 70 ± 66 µg/l of TPH. In keeping with PBS levels, the highest TPH concentration of 83 ± 39 µg/l was also observed in the streams exposed to site B effluent fortified with kerosene.

Figure 20 shows the relationship between PBS and TPH levels measured in water samples obtained from the streams.

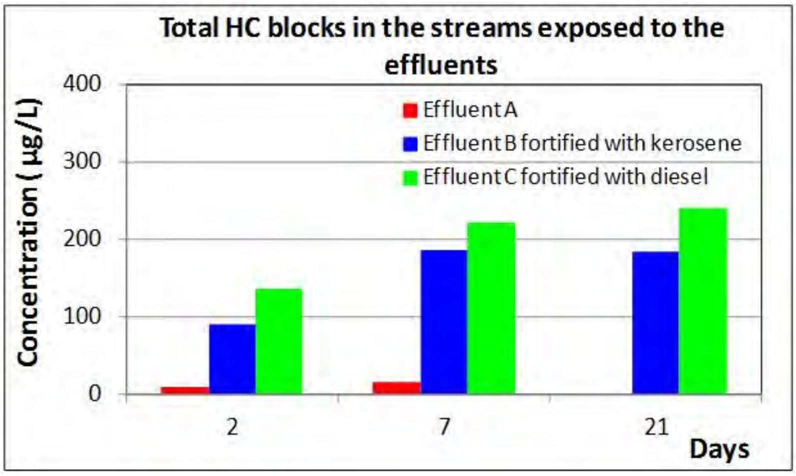
Figure 20: Relationship between measured PBS and TPH levels in the streams.



There was, with one exception, a consistent relationship between PBS and TPH levels measured in water samples obtained from the streams. The exception was one sample from the stream containing effluent C which had a high TPH level relative to PBS level.

Levels of hydrocarbon contamination were also measured in the streams using GCGC. This method allowed total hydrocarbon block constituent concentrations to be measured in the treated streams. The results are given in **Figure 21**.

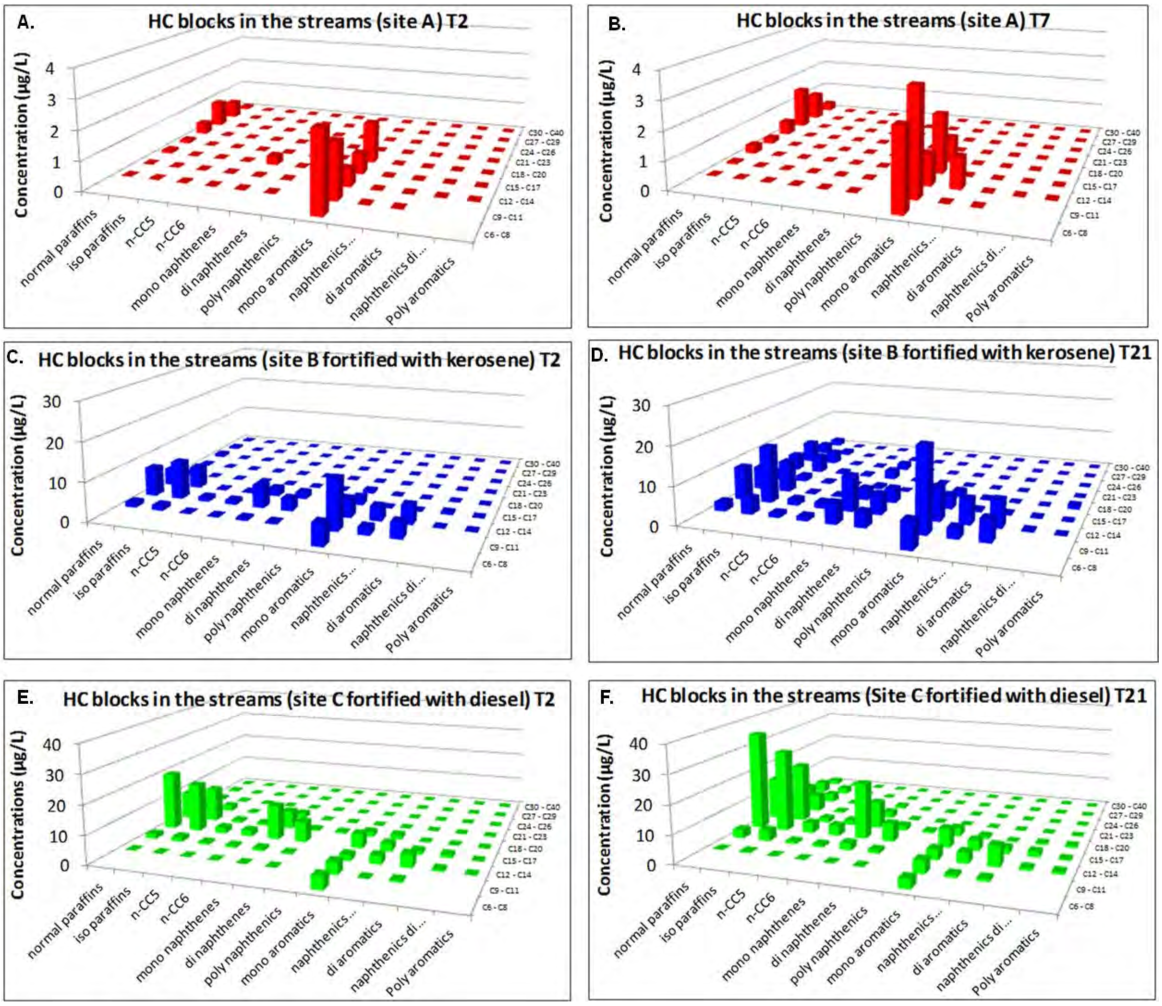
Figure 21: Measured total HC Block concentrations in the streams exposed to the effluents.



The results show that the streams exposed to the highest total HC block concentrations were the ones that were treated with the effluent fortified with diesel (Average: 200 ± 42 µg/l). The streams treated with the effluent fortified with kerosene were exposed on average to 153 ± 42 µg/l of total hydrocarbon blocks. Low levels of total hydrocarbon blocks were measured in the streams treated with effluent A (Average: 13 ± 3 µg/l). These low values were at least ten times lower than the ones measured in the streams treated with the fortified effluents. The total hydrocarbon concentrations in the treated streams were consistent throughout the exposure period.

Profiles of the hydrocarbon blocks that were present in the stream water samples at the start and end of the experiments are presented in **Figure 22**. The data show that there was little variation over the course of the study in hydrocarbon blocks that were present and their concentrations.

Figure 22: Measured HC block concentrations in the streams exposed to effluents



The streams treated with effluent A were mainly exposed to mono aromatics (C6 to C20) but also to low concentrations of normal paraffins (C24-C29). Most of the blocks detected in effluent A were also detected in the streams treated with the effluent. The streams treated with effluent B fortified with kerosene were mainly exposed to normal and iso paraffins (C12 to 17), to mono and di-naphthenes (C9 to C14) and to mono and di-aromatics (C06-C14). Most of these blocks were also detected in the streams treated with the fortified effluent. The streams treated with effluent C fortified with diesel were mainly exposed to normal and iso paraffins (C15 to 20), to mono and di-naphthenes (C15 to C17) and to mono and di-aromatics (respectively C6 to C17 and C12-C14). Again the results show that most of the blocks detected in the effluent C fortified with diesel were also detected in the streams treated with the effluent.

The results are summarised below. It is concluded that the treated streams were exposed to three different hydrocarbon contamination profiles. Two streams exhibited low levels of hydrocarbon contamination and four others presented high levels. Little or no variation in hydrocarbon contamination was observed for the treatments during the 21 day study.

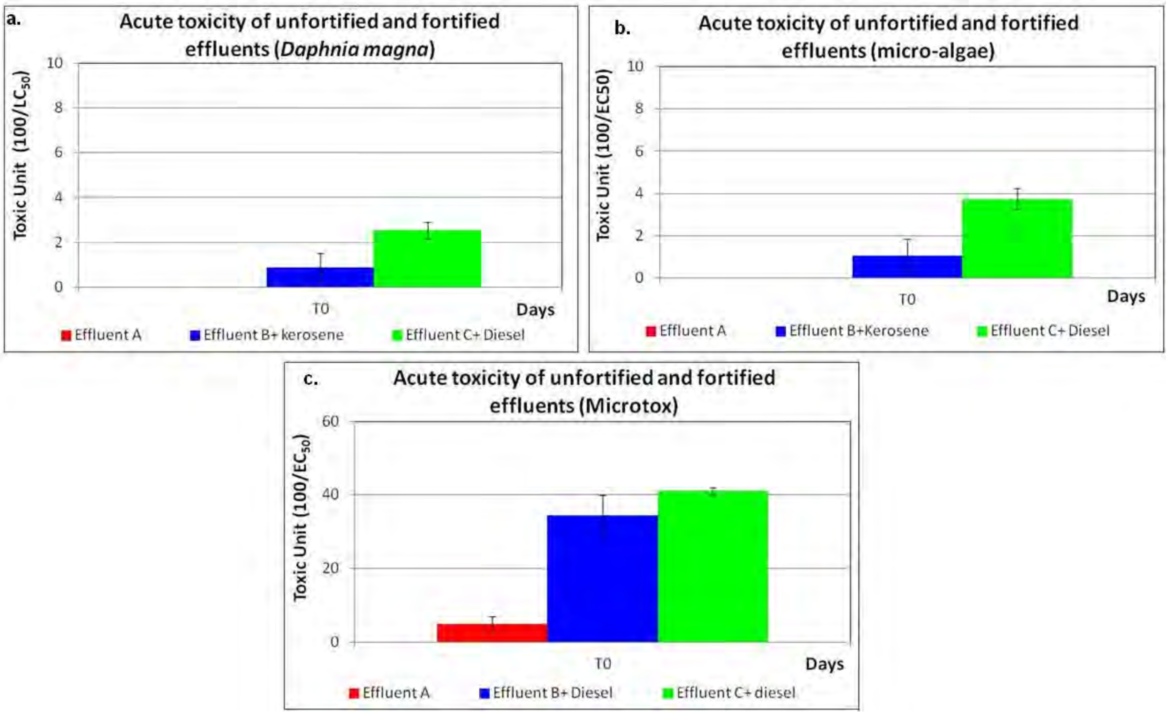
	Effluent A	Effluent B fortified with Kerosene	Effluent C fortified with Diesel
TPH levels	Low (17 ± 4µg/L)	Medium (83 ± 39 µg/L)	Medium (70 ± 66 µg/L)
PBS Levels	Low (0.9 ± 0.4 mM)	Medium (3 ± 1.15 mM)	Medium (2.1 ± 1.6 mM)
Total HC Blocks	Low (13 ± 3µg/L)	High (153 ± 42 µg/L)	High (200 ± 42 µg/L)
Main HC Blocks	Monoaromatics (C6 to C20)	Normal and iso paraffins (C12-C17) mono-naphthenes (C9-C14) di-naphthenes (C9-C14) Monoaromatics (C6-C14) Di-aromatics(C6-C14)	Normal and iso paraffins (C15-C20) mono-naphthenes (C15-C17) di-naphthenes (C15-C17) Monoaromatics (C6-C17) Di-aromatics(C12-C14)

3.2. BIOLOGICAL ENDPOINTS MEASURED DURING THE EXPERIMENTS

3.2.1. Bioassays performed on the Water Samples stored in the Flexible Tanks and Comparison with PBS Levels

Bioassays were performed with three species (*Daphnia magna*, *Pseudokirchneriella subcapitata* and *Vibrio fischeri*). The results are presented in **Figure 23**.

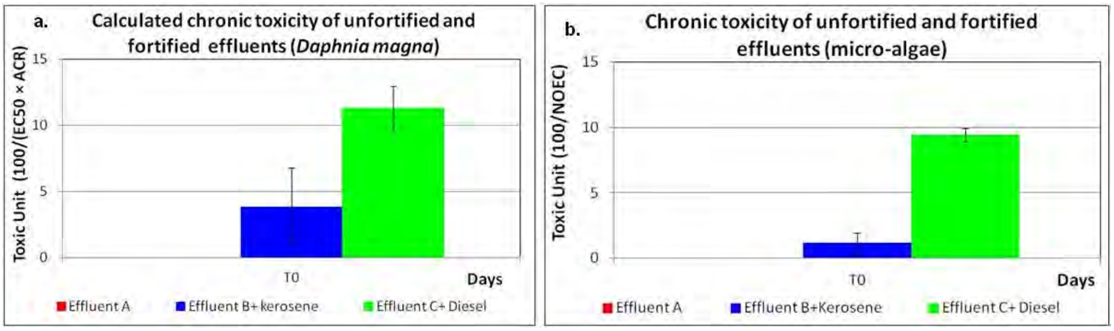
Figure 23: Acute aquatic toxicity of the effluents and fortified effluents to (a) *Daphnia magna*, (b) micro-algae (*Pseudokirchneriella subcapitata*) and (c) *Vibrio fischeri*.



The results show that effluent A was slightly toxic (5 TU) to *Vibrio fischeri* but was not toxic (<4 TU) to *Daphnia magna* and *Pseudokirchneriella subcapitata*. Effluent C fortified with Diesel was most toxic to *Vibrio fischeri* (41 TU) but exhibited little toxicity in the other two bioassays (<4 TU). Effluent B fortified with kerosene was also toxic to *Vibrio fischeri* (35 TU) but not toxic in the other two assays (<1 TU).

One chronic bioassay was performed with microalgae and the results are shown in **Figure 24 (a)**. To provide a basis for comparing the results of the acute bioassay conducted with Daphnia magna (**Figure 23 (a)**) with effects on invertebrates observed in the streams, the acute bioassay data has been converted to chronic toxicity threshold values using the default Acute to Chronic Ratio (ACR) of 4.47 specified in PETROTOX version 3.05 (PETROTOX Users guide). The converted values are shown in **Figure 24 (b).**

Figure 24: Chronic toxicity of the effluents and of the fortified effluents to (a) Daphnia magna (calculated from the acute data using an ACR of 4.47) and (b) Micro-algae (Pseudokirchneriella subcapitata)



The results show that effluent C fortified with diesel exhibited chronic toxicity to both *Daphnia magna* (11 TU) and *Pseudokirchneriella subcapitata* (9.4 TU). Effluent B fortified with kerosene exhibited slight toxicity to *Daphnia magna* (4 TU) but effectively no toxicity to *Pseudokirchneriella subcapitata* (1.2 TU).

Figure 25: Relationship between effluent toxicity and PBS levels

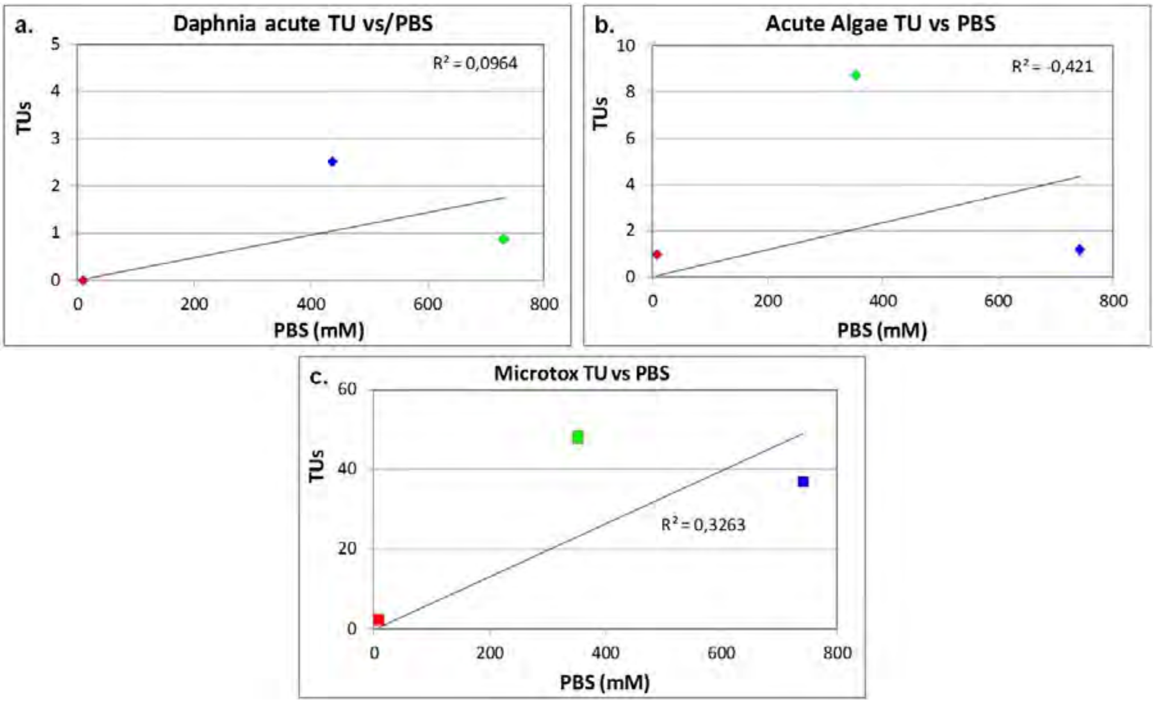


Figure 25 shows the TU values calculated from the results of the bioassays plotted against the corresponding PBS values for the unfortified and fortified effluents. No statistical trends could be determined in the data because only three data points were available for each effluent.

In conclusion, the results of the bioassays that are summarised below allowed the process effluent samples to be ordered according to their toxicity. The most toxic sample was process effluent C fortified with diesel. Process effluent A showed no toxicity.

	Effluent A	Effluent B fortified with Kerosene	Effluent C fortified with Diesel
Microtox	Low	Toxic	Toxic
Acute *Daphnia magna* test	No	Low	Slight
Chronic *Daphnia magna* test	No	Slight	Toxic
Acute *Pseudokirchneriella*	No	Low	Slight
Chronic *Pseudokirchneriella*	No	Low	Toxic

3.2.2. Ecological Endpoints measured in the Streams

In this paragraph, all the effects measured in the streams will be considered as acute or chronic. Acute effects are short term effects measured after one week of exposure. Chronic effects are long term effects measured after more than one week of exposure (14 and 21 days of exposure).

3.2.2.1. Taxa

The results obtained in the streams were first analysed at the taxa level. The results are presented for selected taxa in **Figure 26 to Figure 30**. Some of the taxa that colonized the streams were clearly negatively impacted by the effluents that were fortified with the petroleum distillate fractions (**Figure 26 to 29**); insect larvae, crustacea, oligochaeta and Cnidaria (Hydrozoa). Only data for the most impacted taxa are presented in this report.

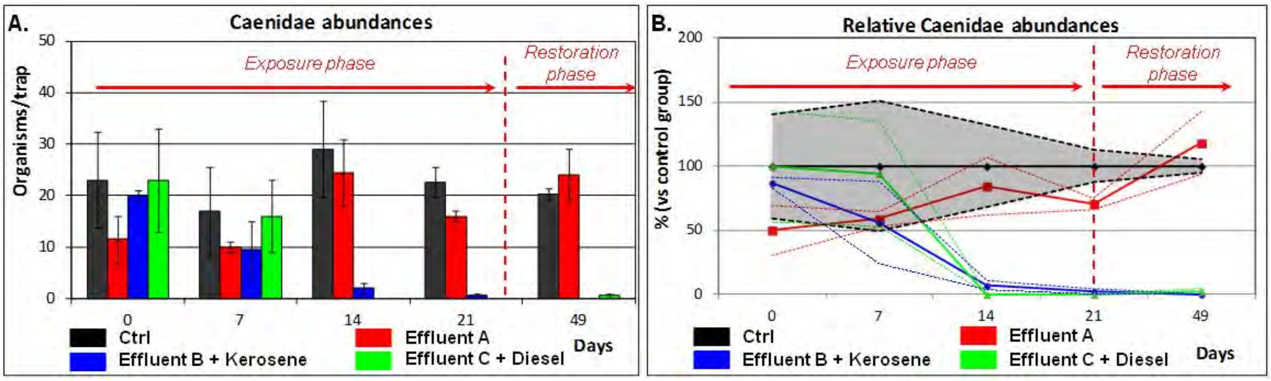
Among the susceptible insect larvae that colonized the streams, Ephemeroptera and Trichoptera were severely impacted.

The population of Caenidae (Ephemeroptera) decreased from an initial value of 20 ± 10 individuals/trap) to a value 50% lower after seven days in the streams treated with effluent B fortified with kerosene. In comparison little effect on the population of Caenidae was apparent in the streams treated with effluent C fortified with Diesel. After 14 and 21 days, almost no Caenidae were sampled in the streams treated with effluents B fortified with Kerosene and C fortified with Diesel.

More severe effects were observed on Heptageniidae populations. After 7 days, the population had decreased from an initial value of 12 ± 9 individuals/trap to 50% of that value in the streams treated with effluent C fortified with Diesel and to 5% of that value in the streams treated with effluent B fortified with Kerosene. Heptageniidae populations were not detected in the streams exposed to fortified effluents after 14 and 21 days. No significant acute or chronic effect was observed in the streams treated with the unfortified effluent A.

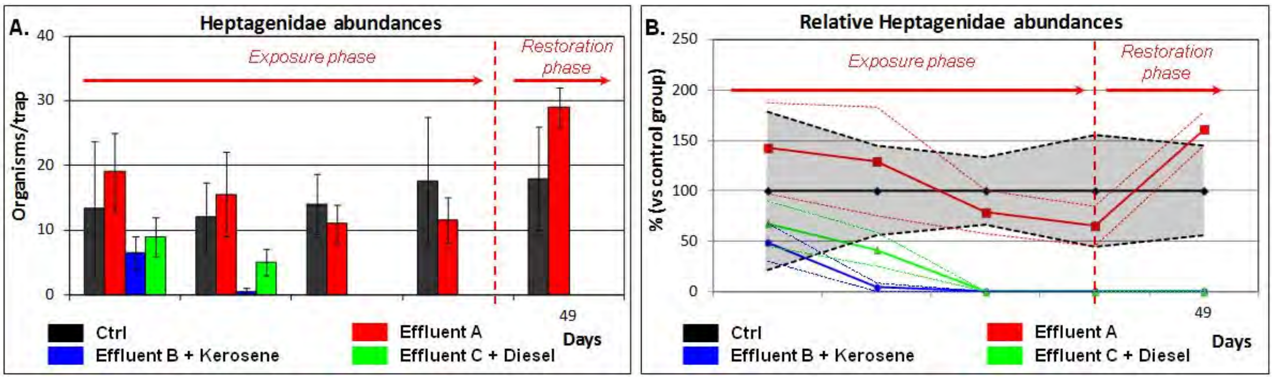
No variation was observed in the populations of Caenidae (22 ± 9 individuals/trap) and Heptageniidae and (15 ± 9 individuals/trap) in the control streams over the treatment period.

Figure 26: (A) Caenidae (Ephemeroptera) abundance mean values (± standard deviation) and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



No recovery in the populations of Caenidae and Heptageniidae was apparent 30 days after ceasing treatment with the fortified effluents.

Figure 27: (A) Heptageniidae (Ephemeroptera) abundance mean values (± standard deviation) and (B.) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



Gammaridae taxa (crustacea) were also susceptible to hydrocarbons (**Figure 28**) and a significant acute (short term) effect was observed after 7 days exposure in all the streams treated with the fortified effluents. The initial gammaridae population (103 ± 31 individuals/trap) decreased by 50% in the streams treated with effluent C fortified with Diesel and by 90% in the streams treated with effluent B fortified with Kerosene.

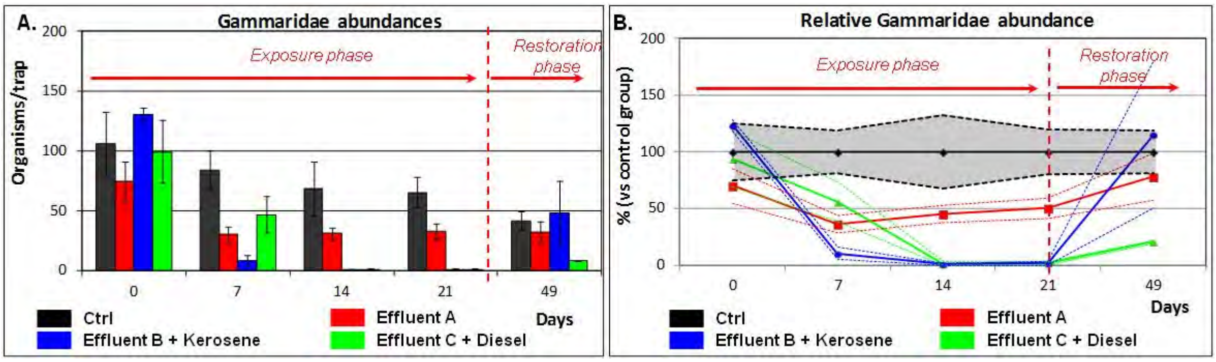
A significant chronic (long-term) effect on gammaridae was also determined after 14 and 21 days of exposure in all the streams treated with the fortified effluents.

A total rapid recovery in gammaridae populations was observed within 30 days of ceasing treatment in the streams previously treated with effluent B fortified with Kerosene. A low level of recovery was observed in the streams previously treated with effluent C fortified with Diesel.

No significant acute or chronic effect was observed in the streams treated with the unfortified affluent A.

Almost no change in gammaridae populations in the control streams (73 ± 30 individuals/trap) was observed over the study period.

Figure 28: Gammaridae abundance (A) mean values (± standard deviation) and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).

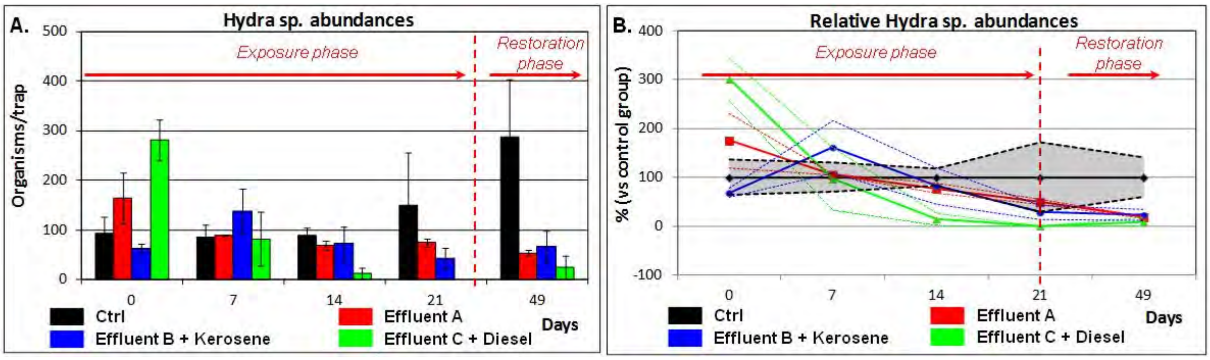


No acute effects were determined on Hydra sp. in all the streams treated with fortified effluents after 7 days of exposure. However, a significant chronic effect was observed in the streams treated with effluent C fortified with Diesel after 14 and 21 days of exposure.

A chronic effect on Hydra sp. was only observed in the streams treated with effluent B fortified with Kerosene after 21 days. Almost no effect was observed in the streams treated with the unfortified effluent. Within 30 days of ceasing treatment, no recovery was observed in Hydra sp. abundance in the streams treated with the fortified process effluents.

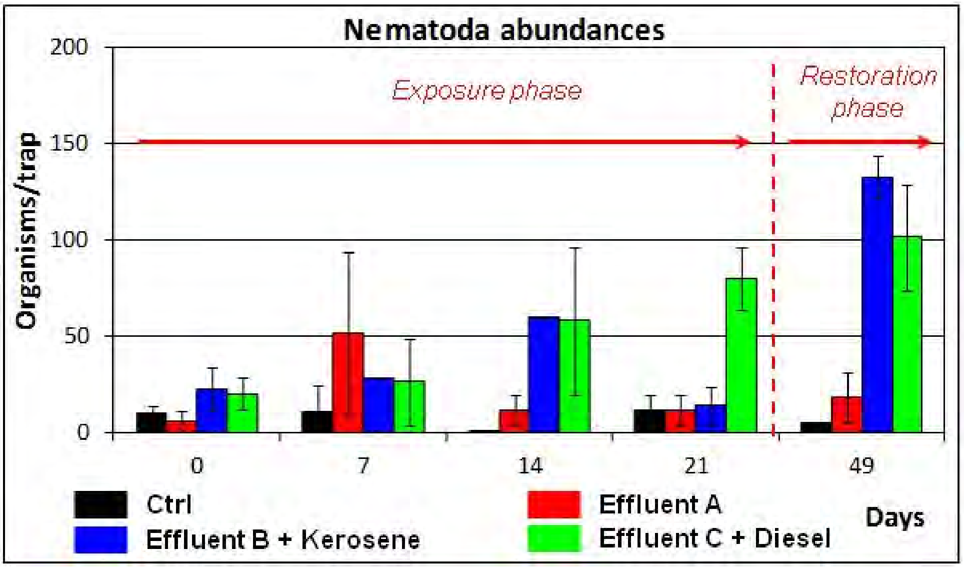
Little variation was observed in Hydra sp. abundance in the control streams over the course of the study.

Figure 29: Hydra sp. abundance (A) mean values (± standard deviation) and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



Other taxa were positively affected by the effluents fortified with the petroleum distillates. For example, **Figure 30** shows that no effects were observed on Nematode abundance in all the treated streams after 7 days exposure. After 14 and 21 days exposure the populations had significantly increased compared with the controls in all the streams treated with the fortified effluents. No recovery to pre-treatment levels of nematode abundance was observed 30-days post-treatment in the streams treated with the fortified effluents.

Figure 30: Nematode abundance mean values (± standard deviation)



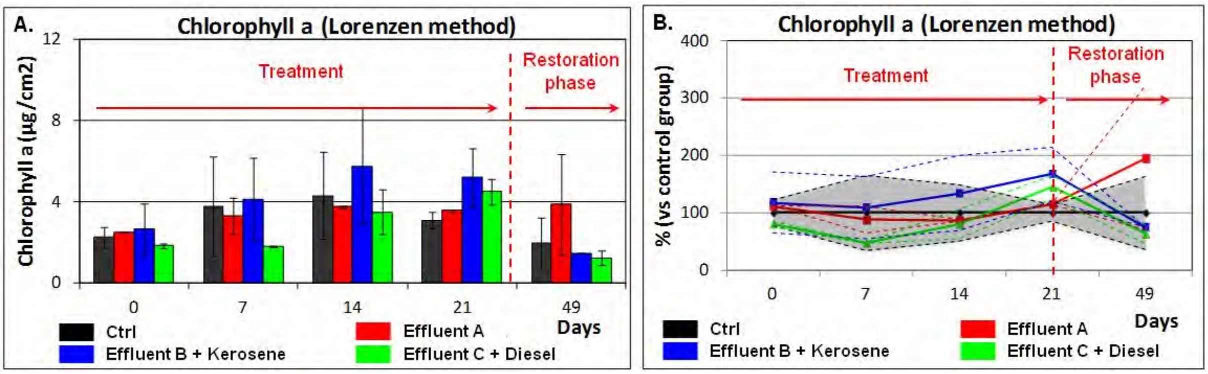
3.2.2.2. Measurements at the community level

Microalgae and diatoms

The results are presented in **Figure 31** and **Figure 32** respectively. Similar results were obtained at the beginning of the experiments using the two different methods; 2.3 ± 0.6 µg/cm^2 and 1.9 ± 1.0 µg/cm^2 respectively.

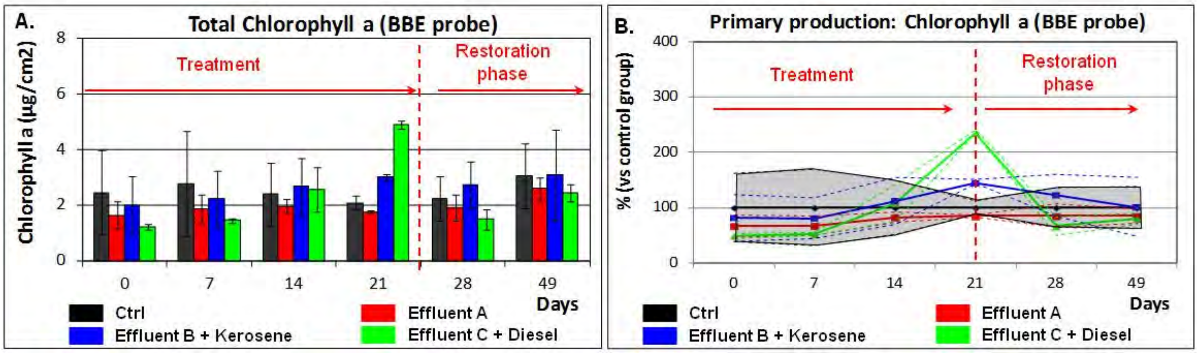
The measurements obtained using the Lorenzen method showed low variation between the control streams and treated streams. No acute (short-term) or chronic (long-term) impact was measured in the streams treated with the unfortified effluents. No acute impact was observed in all the streams treated with fortified effluents after 7 days of exposure. A significant chronic impact was determined after 21 days in all the streams treated with the fortified effluents, with no difference between the streams treated with effluent B fortified with Kerosene and the streams treated with effluent C fortified with the Diesel. In these streams, a statistically significant increase in chlorophyll a was observed after 21 days of exposure. However, the increase was small and could only be identified because of the low variability in the control streams. Within 30 days of ceasing treatment, a rapid recovery in the level of primary production was observed in the streams treated with the fortified effluents.

Figure 31: Total chlorophyll a measured using the Lorenzen Method



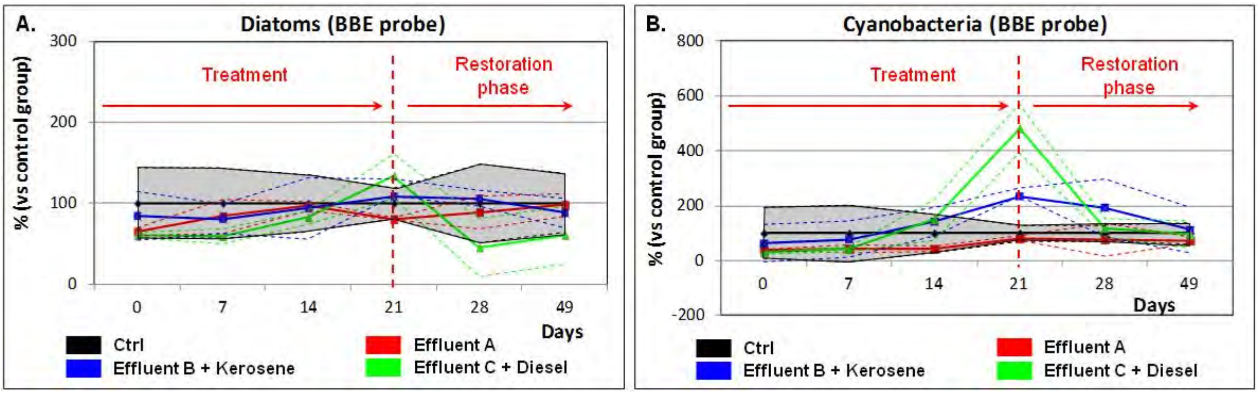
The BBE probe measurements showed similar results to those obtained using the Lorenzen method. Very little variation was observed in the control streams. No acute or chronic impact was measured in the streams treated with the unfortified effluents. No acute impact was observed after 7 days of exposure in all the streams treated with the fortified effluents. A significant chronic impact was determined after 21 days of exposure in all the streams treated with the fortified effluents. The highest impact was observed in the streams treated with the effluent fortified with Diesel. At the end of the treatment, the chlorophyll a levels were respectively 2.5- and 1.4-times higher than the control levels in the streams treated with the effluents fortified with Diesel and Kerosene. A rapid recovery in chlorophyll a levels to those in the controls was observed within 7 days of ceasing treatment in the streams treated with the fortified effluents.

Figure 32: Total chlorophyll a measured using the BBE probe



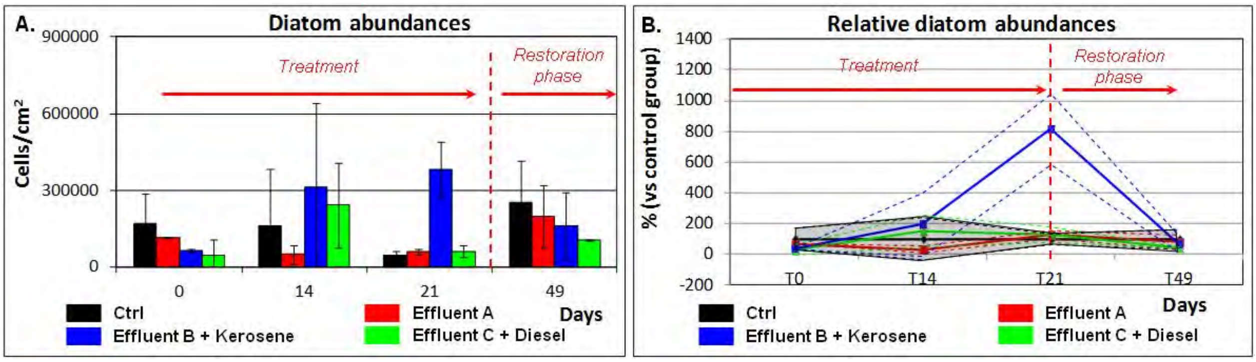
The BBE probe also allocates algae to various spectral classes. Three classes were determined using the probe; green algae, blue green algae or cyanobacteria and diatoms. Only diatom and cyanobacteria results are presented in **Figure 33** since no variation in the abundance of green algae was detected. The results show no significant acute or chronic impact on diatoms in all the treated streams. No significant acute impact on cyanobacteria was observed in all the treated streams. However, a significant chronic impact on cyanobacteria was observed in all the streams treated with the fortified effluents after 21 days of exposure.

Figure 33: Relative abundance of diatoms and cyanobacteria measured using BBE probe



After 21 days of treatment cyanobacteria levels in the streams treated with effluents fortified with Diesel and Kerosene were respectively 4.8 and 2.3 times those of the controls. Rapid recovery to control levels was observed within 7 days of ceasing treatment in the streams treated with the fortified effluents.

Figure 34: Diatom abundance measured using a microscope (A) mean values and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



Diatoms communities were also specifically sampled and studied using submerged glass plates and the results are presented in **Figure 34, 35 and 36**. The results show that no significant impact was observed on diatom abundance in the streams treated with the unfortified effluent and in the streams treated with effluent C fortified with Diesel. A highly significant chronic impact was determined in the streams treated with effluent fortified B with Kerosene after 21 days of exposure. This result is different from the one obtained using the BBE probe which showed no significant impact on diatom density in any of the treatments. It is not certain why this difference should be apparent however it may have something to do with differences in the methods used to sample the diatoms. When using the BBE probe the development of diatom abundance was determined on the same glass plates on days 0, 7, 14, 21, 28 and 49, whereas diatom densities estimated using the microscope were obtained from different glass plates collected on each sampling occasion.

Figure 35: Diatom biodiversity in the streams (A) mean values and (B) relative values versus the control streams (the shaded zone represents the coefficient of variation in the control streams).

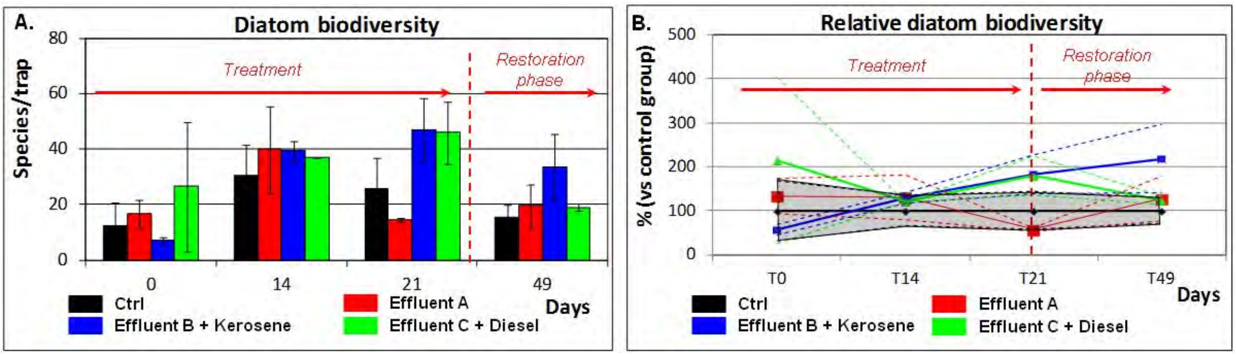


Figure 35 shows that, after 14 days of exposure, no significant impact was observed on diatom biodiversity in all the treated streams. A significant increase in diatom biodiversity was measured after 21 days of exposure in the streams treated with the effluent B fortified with Kerosene and effluent C fortified with Diesel.

A rapid recovery to pre-treatment levels of biodiversity was observed within 30 days of ceasing treatment in the streams treated with effluent C fortified with Diesel. A lower level of recovery was observed in the streams treated with effluent B fortified with Kerosene.

Figure 36: PRC of the diatom community (Dunnett Test scores: * p<0.05, ** p<0.01 and *** p<0.001)

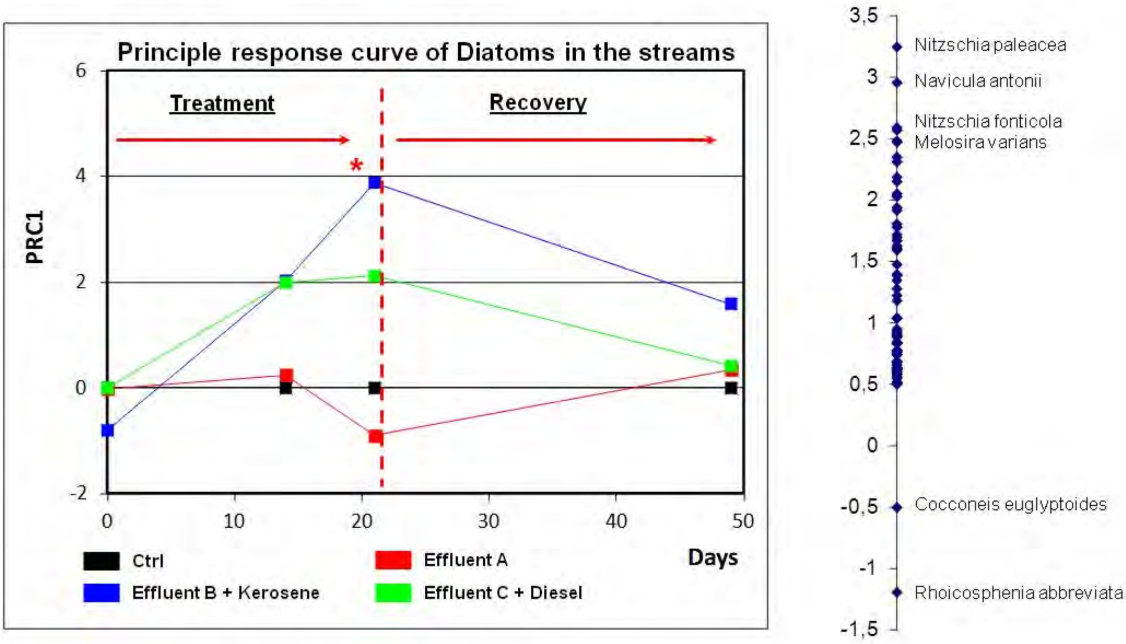


Figure 36 shows the impact of the treatments on diatom communities expressed as PRC values. No effect was observed at the community level in the streams exposed to the unfortified effluent. An apparent chronic effect of the fortified effluents was

observed after 14 days of exposure. However, this effect was not statistically significant. A significant chronic effect (Dunnet test: p=0.017) was determined after 21 days only in the streams treated with the effluent fortified with Kerosene. Within 30 days of ceasing treatment, a rapid recovery was observed in the streams treated with the fortified effluents.

The PRC values also show that some diatom species (*Cocconeis euglyptoides* and *Rhoicosphenia abbreviata*), were negatively impacted and some others (mainly *Nitzschia paleacea*, *Navicula antonii*, *Nitzschia fonticola*, *Melosira varians*), were positively impacted. These species contributed most to modifying the community structure in the streams exposed to the fortified effluents. Cocconeis species are susceptible to alterations in water quality and are generally characteristic of good quality. However, Nitzschia species, and especially *Nitzschia pleacea*, are heterotrophic diatoms which are tolerant of organic contamination; this could explain their increased abundance in the streams treated with all the fortified effluents.

Benthic invertebrates

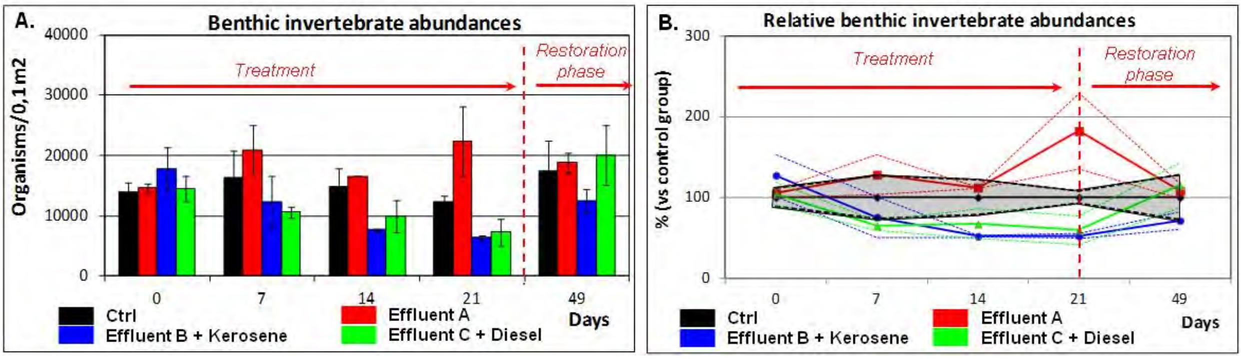
Benthic invertebrate abundance and diversity data are presented in **Figure 37** and **Figure 38**.

The results show a significant increase in invertebrate abundance in the streams treated with the unfortified effluent but only after 21 days of exposure.

No significant acute effect on invertebrate abundance was observed in the streams treated with the fortified effluents after 7 days of exposure.

A significant chronic effect was determined after 14 and 21 days in the streams treated with effluent B fortified with Kerosene. A chronic effect was also determined after 21 days in the streams treated with effluent C fortified with Diesel.

Figure 37: Benthic invertebrate abundances in the streams (A.) mean values and (B.) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



A rapid and complete recovery in invertebrate abundance and diversity was observed within 30 days of ceasing treatment in the streams previously treated with the effluent fortified with Diesel. A lower level of recovery was observed in the streams treated with the effluent fortified with Kerosene.

Irrespective of sampling time, no significant acute or chronic effect on benthic invertebrate diversity was observed in the streams treated with the unfortified effluent.

No acute effect on diversity was observed in the streams treated with fortified effluents after 7 days of exposure.

A significant chronic effect on diversity was observed in the streams treated with fortified effluents after 14 and 21 days of exposure. Most significantly, a reduction of 50% was apparent compared with the control streams after 21 days.

Within 30 days of ceasing treatment, a partial recovery was observed in all the streams treated with the effluent fortified with Diesel and in the one fortified with Kerosene.

Figure 38: Benthic invertebrate biodiversity in the streams (A) mean values and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).

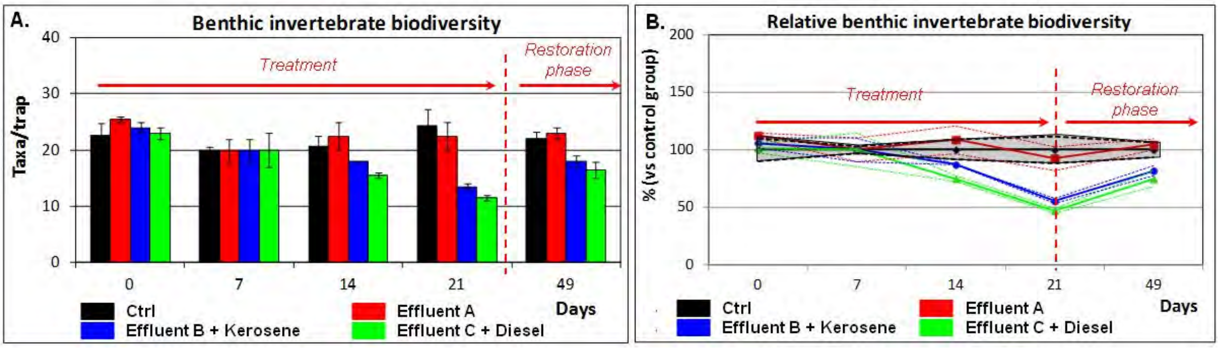


Figure 39 shows the effects of the treatments on the benthic invertebrate communities.

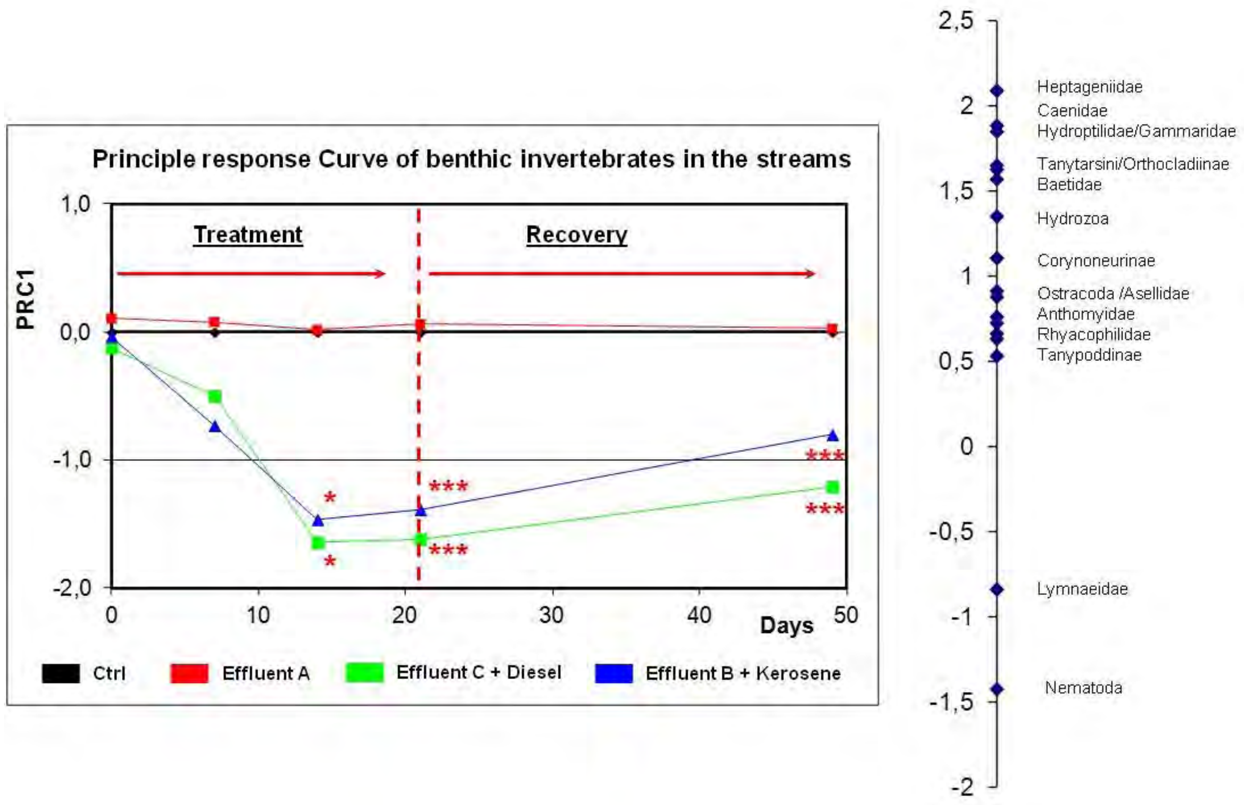
No effect was observed at the community level in the streams exposed to the unfortified effluent.

An apparent acute effect of all the fortified effluents was observed after 7 days of exposure. However this effect was not statistically significant.

A significant chronic effect on community structure was determined after 14 and 21 days in all the streams treated with the fortified effluents. Greater effects were observed in the streams treated with effluent C fortified with Diesel than in the ones treated with effluent B fortified with Kerosene.

Within 30 days of ceasing treatment, a rapid partial recovery was observed in the streams treated with the fortified effluents. The PRC also shows that EPT species and crustaceans (gammaridae), which were negatively impacted, and molluscs and nematodes, which were positively impacted, contributed most to the modification of the community structure in the streams exposed to the fortified effluents.

Figure 39: PRC of benthic invertebrate communities (Dunnett Test scores: * p<0.05, ** p<0.01 and *** p<0.001)

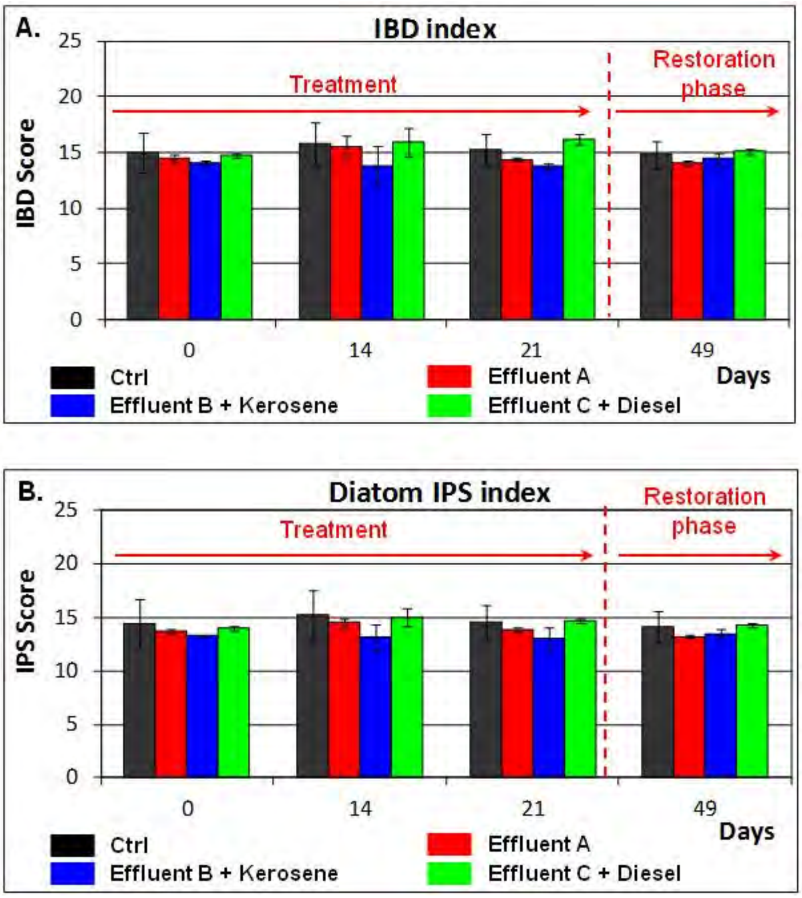


3.2.2.3. Environmental indices

Several ecological indices were calculated based on the results obtained in the artificial streams. Three of these were related to diatoms (**Figure 40** and **Figure 41**). Two others were related to benthic invertebrates (**Figure 42** and **Figure 43**) and one was used to characterize heterotrophic aerobic bacterial densities (**Figure 44**).

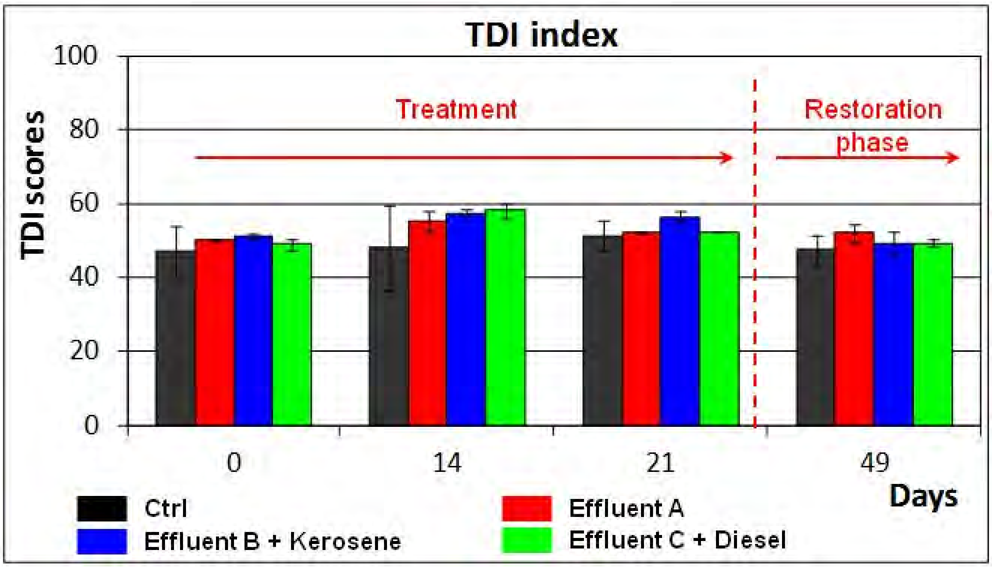
In this study, diatoms were sampled using glass plates that were submerged in the streams; the diatoms colonised these plates. The IBD and IPS index scores that have been calculated from the resulting data are presented in **Figure 40**.

Figure 40: Diatom IBD (A) and IPS (B) indices calculated for each treatment (± standard deviation)



No significant variation in IBD and IPS scores was observed in the control streams during the 49 days of the experiment; the average IBD and IPS scores were respectively 15.2 ± 1.4 and 14.6 ± 1.8. No significant differences were observed between the control and the treated streams. These data show that the diatom communities present in the streams were not very susceptible to the hydrocarbons present in the effluents.

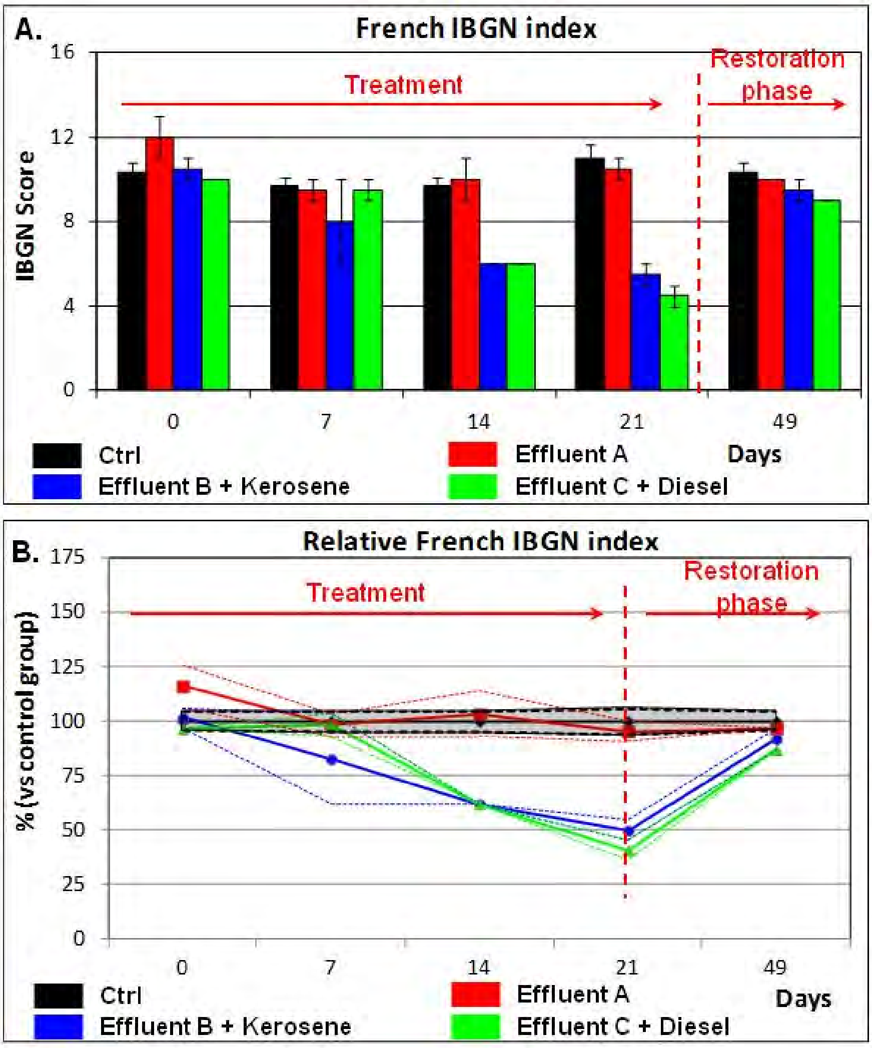
Figure 41: Diatom TDI index calculated for each treatment (± standard deviation)



The TDI scores calculated for each treatment are presented in **Figure 41**. No variation of the TDI scores was observed in the control stream, during the 49 days of the experiments; average TDI score of 48 ± 6. No significant difference was observed between the control and the treated streams. These results again show that the diatom communities present in the streams were not very susceptible to the effects of the hydrocarbons present in the effluents.

In this study only one habitat was sampled/stream/sampling time which explains the relatively low IBGN score. The IBGN scores calculated for each treatment are presented in **Figure 42**.

Figure 42: French IBGN index calculated for each treatment (A) mean values (± standard deviation) and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



No variation in the IBGN index score was observed in the control stream during the 49 days of the experiments; the average IBGN score was 10.2 ± 0.8.

No difference was observed in the streams treated with effluent A (Mean IBGN score: 10.4 ± 1.2).

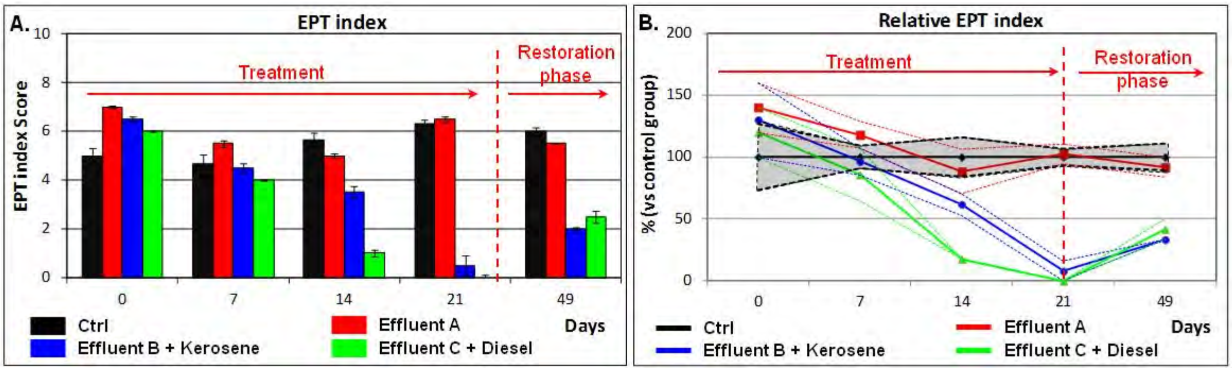
There was an apparent acute effect on benthic invertebrates after 7 days in the streams exposed to effluent B fortified with kerosene. However, this effect was not statistically significant.

A significant chronic effect was determined after 14 and 21 days in all the streams treated with the fortified effluents.

Within 30 days of ceasing treatments, no significant difference could be determined in the French IBGN index scores for the benthic invertebrate communities in the treated and control streams.

The EPT index scores calculated for each treatment are shown in **Figure 43**. In contrast with the IBGN index, the EPT index focuses on the community richness of sensitive benthic invertebrate species (*Ephemeroptera, Plecoptera, Trichoptera*). Good water quality is characterised by a high EPT score and a low score corresponds to poor water quality.

Figure 43: EPT index calculated for each treatment (A) mean values (± standard deviation) and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



Low variation of the EPT score was observed in the control streams over the 49 days of the experiment; the average EPT score was 5.5 ± 1.1.

There was no significant difference in the EPT score for the streams treated with effluent A (Mean EPT score: 5.9 ± 1.1) and the score for the control streams.

There was an apparent acute effect on EPT species after 7 days in the streams exposed to the fortified effluents. However, this effect was not statistically significant.

A significant chronic effect was determined after 14 and 21 days in all the streams treated with the fortified effluents. The highest effect on EPT species was measured in the streams exposed to effluent C fortified with diesel. At the end of exposure (day 21), no EPT species were detected in any of these streams which explains the low EPT scores. Within thirty days of ceasing treatments, EPT species began to recolonize the mesocosms but a significant difference was still measured between the control streams and the streams treated with the fortified effluents.

Densities of heterotrophic aerobic bacteria determined for each treatment are shown in **Figure 44**.

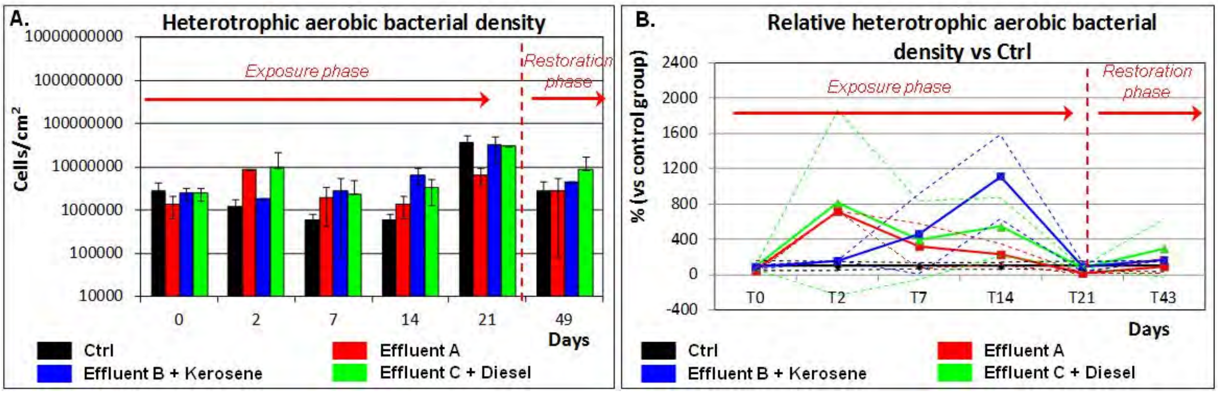
Low variation was observed in the densities in the control streams during the 49 days of the experiments.

Higher heterotrophic aerobic bacterial densities compared with those in the control streams were observed in the streams treated with effluent A after two days exposure. Bacterial densities then decreased and reached similar densities to those in the control streams after 21 days.

After 2, 7 and 14 days higher bacterial densities were measured in the streams treated with fortified effluents than in the control streams. However, no difference was observed in all the treated streams in comparison with control streams after 21 days.

The data show that densities of aerobic bacterial were impacted in all the treated streams but no clear trend was apparent.

Figure 44: Heterotrophic aerobic bacterial densities for each treatment (A) mean values (± standard deviation) and (B) relative values versus control streams (the shaded zone represents the coefficient of variation in the control streams).



It is concluded that the tests performed in the artificial streams allowed the process effluents to be classified on the basis of their toxicity to a range of aquatic organisms and endpoints. No significant effects of the unfortified effluent were observed on bacteria, benthic invertebrate and diatom communities. Effluent C fortified with diesel was most toxic to the invertebrate community. The most significant effects on the diatom community were determined in the streams treated with effluent B fortified with Kerosene. The communities in the affected streams had recovered within 30-days of ceasing the treatments. The results are summarized in the following table.

	Effluent A	Effluent B fortified with Kerosene	Effluent C fortified with Diesel
Benthic invertebrate species abundances	No impact	EPT species (↘↘); gammaridae (↘↘↘) Lymnaeidae (↗); Nematoda (↗)	EPT species (↘↘); gammaridae (↘↘) Lymnaeidae (↗); Nematoda (↗↗)
Diatom species abundances	No impact	Cocconeis euglyptoides (↘↘); Rhoicosphenia abbreviata (↘↘) Nitzschia paleacea (↗↗); Navicula antonii (↗↗)	Cocconeis euglyptoides (↘↘); Rhoicosphenia abbreviata (↘↘) Nitzschia paleacea (↗↗); Navicula antonii (↗↗)
Benthic invertebrate community	No impact	(↘)	(↘↘)
Diatom community	No impact	(↗↗)	(↗)
Primary production	No impact	(↗)	(↗)
ecological indices based on benthic invertebrates	No impact	IBGN (↘); EPT (↘↘)	IBGN (↘↘); EPT (↘↘↘)
ecological indices based on Diatoms	No impact	No impact	No impact
Heterotrophic aerobic bacteria	(↗)	(↗↗)	(↗)
Cyano-bacteria	No impact	(↗)	(↗↗)
Recovery of benthic invertebrate community	-	(↗↗)	(↗)
Recovery of diatom community	-	(↗)	(↗↗)

4. DISCUSSION

4.1. STREAM RESULTS

Few experimental microcosm and mesocosm studies have investigated the effects of effluents from the oil industry on benthic invertebrate or diatom communities [39, 40, 41]. However, some studies have reported on the *in situ* impacts of hydrocarbon wastes from the oil industry on aquatic communities [42, 43, 44, 45, 46, 47, 48, 49]. Wake (2005) reviewed the ecological impacts of oil refineries on the aquatic environment both in term of toxicity tests and of field surveys [49]. The majority of the studies she reviewed have looked at the impact on the estuarine or marine macrobenthos community.

Most of the studies have reported that a decrease of the number of benthic invertebrate individuals and of the biodiversity occurred in a limited area close to the effluent outfall into the aquatic ecosystems [50, 51, 52, 53]. Often the impacted area is limited to a specific distance from the discharge point. In this area, two distinct groups of organisms could be defined based on the level of impact. The species that are found close to the refinery outfalls are mainly opportunistic species and are typical species found in organically enriched areas. Sensitive species that are not found close to the outfall are not able to survive or have drifted to another location [53, 54].

A quantity of papers also examined oil spill impacts in freshwater [55, 56] and in marine water [57, 58, 59]. They have reported the presence of species (both benthic invertebrates and algae) that are sensitive to or tolerant of hydrocarbon contaminations. Some other studies reported the impact of petroleum distillates on communities using experimental systems [13, 14, 60, 61, 62, 63]. However, most of these publications gave no indication of the composition of the petroleum distillates (with or without additives) used in the experiments and this makes it difficult to draw meaningful comparisons with the results of this study. Most of the studies also reported a relatively rapid recovery of benthic invertebrate communities after hydrocarbon exposure.

Similar results were observed in the Concawe WEA stream project. Benthic invertebrate community changed after being treated with refinery effluents fortified with petroleum distillate. A decrease of sensitive species (Ephemeroptera, Plecoptera and, Trichoptera species) and an increase of opportunistic species (Nematodes) were observed. Some workers have shown that Nematodes are not very susceptible to hydrocarbon exposure compared to other invertebrate taxa including daphnids [64]. Furthermore their populations can even increase if the abundance of their prey increases. Free-living nematodes mainly feed on bacteria, algae and fungi and increases of both bacteria and diatoms were observed in the streams treated with the fortified effluents. These observations could explain why populations of nematodes were not adversely affected in this study. However, other workers [65] have observed that some nematodes are adversely affected by Diesel exposure while others are “Diesel resistant”. The picture is therefore not totally clear.

In this study, other species (Lymnaeidae, Physidae) were not susceptible to refinery effluent fortified with petroleum distillates exposure. Lymnae sp. are aquatic pulmonate snails that are capable of breathing air through lung-like sacs. As a consequence they are not very susceptible to the decreases in dissolved oxygen concentrations that were apparent in the streams treated with the fortified effluents. Moreover, these molluscs feed on micro-algae, the density of which increased over the treatment periods. Brendelberger [66] showed that radix species which feed rather unselectively on whatever food is available (diatom, green algae and cyanobacteria).

Wake (2005) [49] also reviewed the ecological impacts of oil refineries on the flora. In some cases, algal growth has been seen to increase near the effluent outfall. Algae are notably abundant around the outfalls in the Littlewick Bay close to the refinery outfall [67]. However, a decrease of productivity of periphyton communities was observed downstream below the pollution outfalls in skeleton Creek, Oklahoma, a stream receiving domestic and oil refinery effluents [45]. Similar impact has been reported in [68]. Growth pattern in five micro-algae test species was examined in a laboratory study [69]. This study showed that growth pattern was negatively impacted in the 5 species after exposure to high concentrations of a petrochemical effluent. However, the lowest concentrations also stimulated the growth of these 5 species. Walsh et al. [70] also showed that industrial effluents can cause stimulation or toxicity and structural damages to algae. Low molecular weight hydrocarbons were found to stimulate phytoplankton growth depending upon the species [71]. Bayona et al. (2014) [63] showed an increase of diatom diversity after treatment to hydrocarbon emulsion (petroleum distillate) in stream mesocosms with an increase of taxa tolerant to organic pollution.

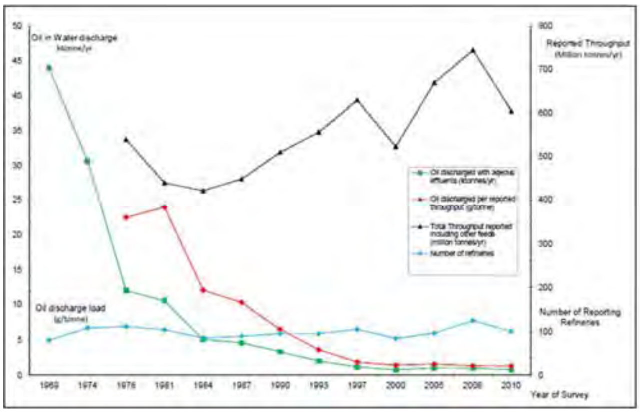
Similar results were observed in this project. An apparent chronic effect of the fortified effluents was observed on diatom community with an increase of taxa tolerant to organic pollution. An increase of diatom abundance and biodiversity were also observed in streams treated with the fortified effluents. One factor that could explain the increase in diatom biodiversity in the streams treated with the fortified effluents was the increased frequency of abnormal diatoms (abnormal valve or form) that was found in these streams after 21 days of exposure. Another factor could be an increase in the number of opportunistic species that took advantage of the environment that was created by the hydrocarbon exposures. This was confirmed by the increase of cyan bacteria in streams exposed to the fortified effluents. Several studies have reported high abundance of Cyan bacteria in area contaminated by refinery effluent or oil spill [72]. Most of these studies reveal that higher amount of phosphates and nitrates, with sufficient amount of oxidizable organic matter and limited dissolved oxygen contents played a vital role in the distributional pattern of cyanobacterial populations [73, 74]. Cyanobacteria have also been recorded directly in industrial effluents like oil refinery [74].

Contrary to what was observed in the streams treated with fortified effluents, no significant impact was measured in the streams treated with pure refinery effluent on both benthic invertebrate and diatom communities, probably due to its dilution factor in the streams (140, v/v).

The three main treatment processes for effluents before discharge are gravity separation (API separators, tank separation), advanced treatment (flocculation, sedimentation, filtration) and biological treatment (biofilters, activated sludge, Dissolved Air Flotation: DAF) [75]. However, the effluent tested in this study has been sampled after the DAF but before biological treatment in the refinery which implies that this effluent is likely to be more toxic that a similar effluent discharged into the environment. As not all refineries have the same treatment processes the effluents that are produced will have different chemical compositions and concentrations depending on the type of treatment they receive. Petroleum refinery wastewaters are made up of many different chemicals, which include oil, phenols, sulphides, ammonia, suspended solids, cyanides, nitrogen compounds and heavy metals. Concawe has summarized survey data on oil refinery effluent water quantity, quality and treatment processes in Europe (EU-27 countries and those in Croatia, Norway and Switzerland)

[75]. The main conclusions drawn from this study were that the amount of oil, ammonia, total nitrogen (TN) and phenols discharged in effluents from reporting installations has seriously decreased and continued to decrease (**Figure 45**).

Figure 45: Discharges of oil (1969-2010) ammonia, total nitrogen & phenols (1993-2010).



Year	Ammonia[1]	Total Nitrogen	Phenols
	tonne/year (Number of Refineries reporting)		
1993	5,202 (82)	n.a.	179 (77)
1997	3,210 (82)	n.a.	161 (73)
2000	1,715 (46)	1,884 (46)	61 (55)
2005	1,959 (64)	4,778 (80)	180 (84)
2010	454[2] (26) 22[3] (3)	2,307[2] (66) 56[3] (8)	31[2] (76) 5.2[3] (8)
	g/tonne throughput		
1993	10.4	n.a.	0.41
1997	8.0	n.a.	0.32
2000	5.7	7.4	0.16
2005	5.5	10.0	0.35
2010	0.82[2] 8.15[3]	4.2[2] 21[3]	0.058[2] 1.9[3]

(1) Ammonia may also be included in the figures for total nitrogen since many refineries measure this rather than ammonia. Although related, the two measurements are required for different reasons, ammonia can cause acute aquatic toxicity depending upon the pH and temperature, whereas TN is a measure of the potential for eutrophication of the aquatic environment.

(2) Figures for direct discharges from installations, (3) Figures for discharges after transfer to, and treatment by, offsite WWTP.

There are few cases where no ecological effect was also detected in an area close to an effluent discharge [51, 76]. However, changes in distribution and abundance of species are often very localised and in some cases may result from behavioural responses rather than direct toxic effects.

4.2. COMPARISON BETWEEN WET BIOASSAYS AND BIOLOGICAL ENDPOINT MEASURED IN THE STREAMS

Few studies have investigated the effects of effluents, and at least none for refinery effluents, using both *in situ* ecological monitoring and bioassays. Some studies have investigated the effects of effluents using different approaches at the same time (*in situ* bioassays, macro invertebrate and diatom communities monitoring, multispecies toxicity evaluation) [77, 78, 79, 80, 81], but most of the time only one of those approaches was considered. The overall objective of the phase III project was to compare the results of bioassays conducted on refinery effluent samples with the results of applying various methods of measuring the effects of the same effluents in stream mesocosms. The results of making these comparisons are presented in **Figure 46 to 49**.

4.2.1. Comparison of laboratory-derived acute and chronic bioassay data for Daphnia magna with effects observed on benthic invertebrates in the stream mesocosms

No significant acute toxicity of the unfortified and fortified effluents to benthic invertebrates was determined in the stream mesocosms. The results of the laboratory acute bioassays conducted with *Daphnia magna* showed that the unfortified effluent and the effluent fortified with Kerosene were not acutely toxic (TU = 0 and TU = 0.9

respectively) whereas the effluent fortified with Diesel was acutely toxic (TU = 2.5). These data suggest that the acute Whole Effluent Toxicity (WET acute) bioassays conducted in the laboratory yielded conservative results for the latter effluent compared to the observed outcome in the streams.

Figure 46: Comparison between bioassays and ecological indicators: acute toxicity on benthic invertebrate in the streams versus *Daphnia magna* acute toxicity bioassays

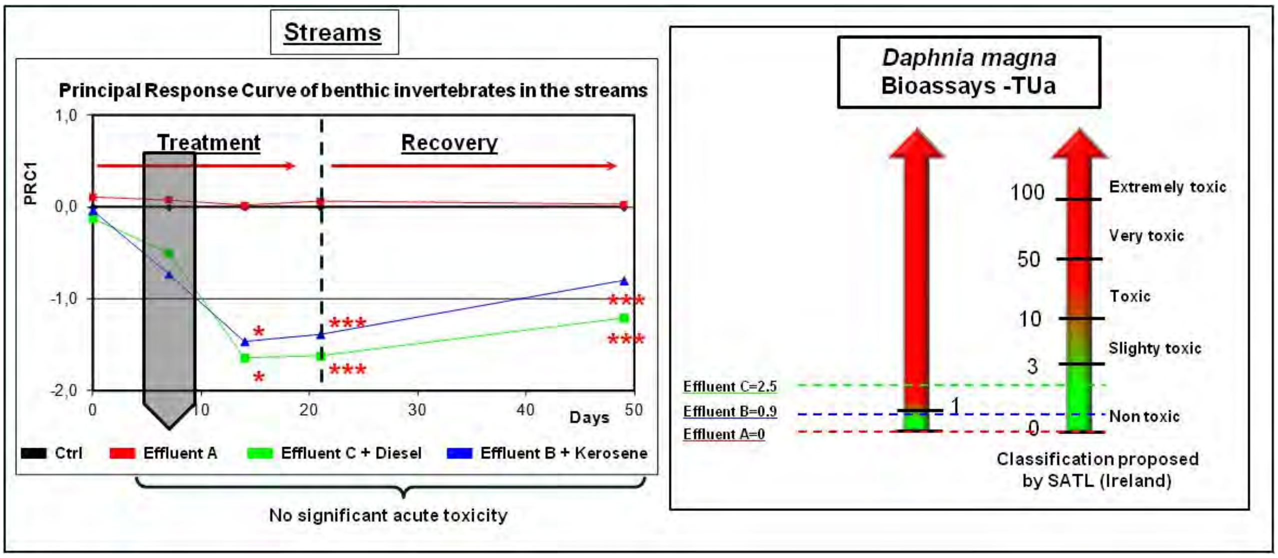
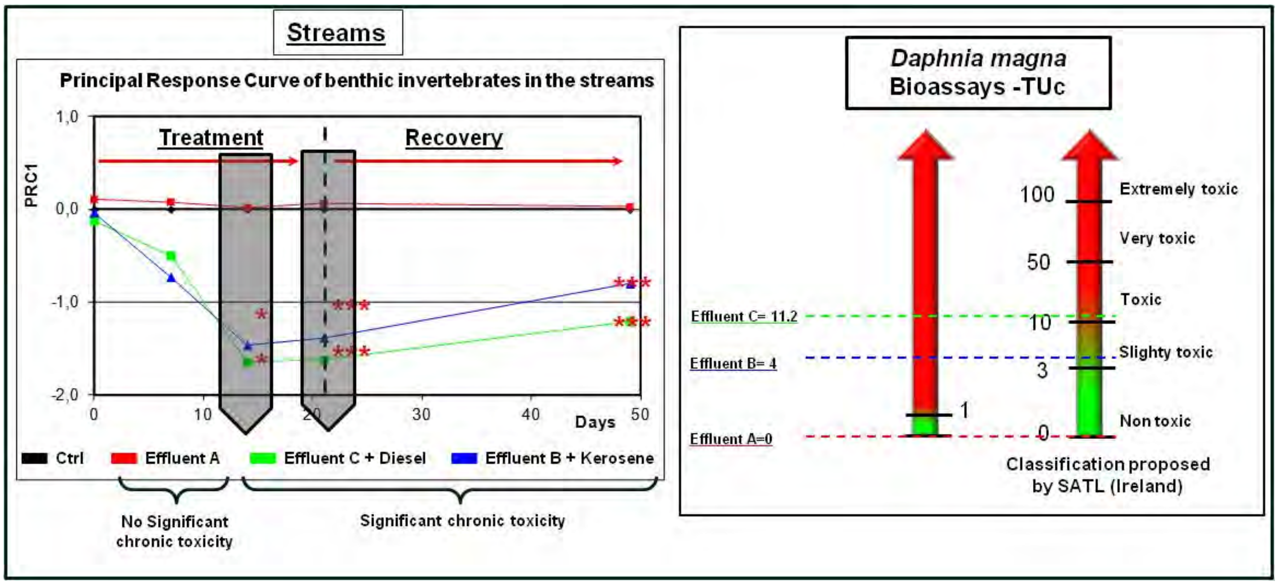


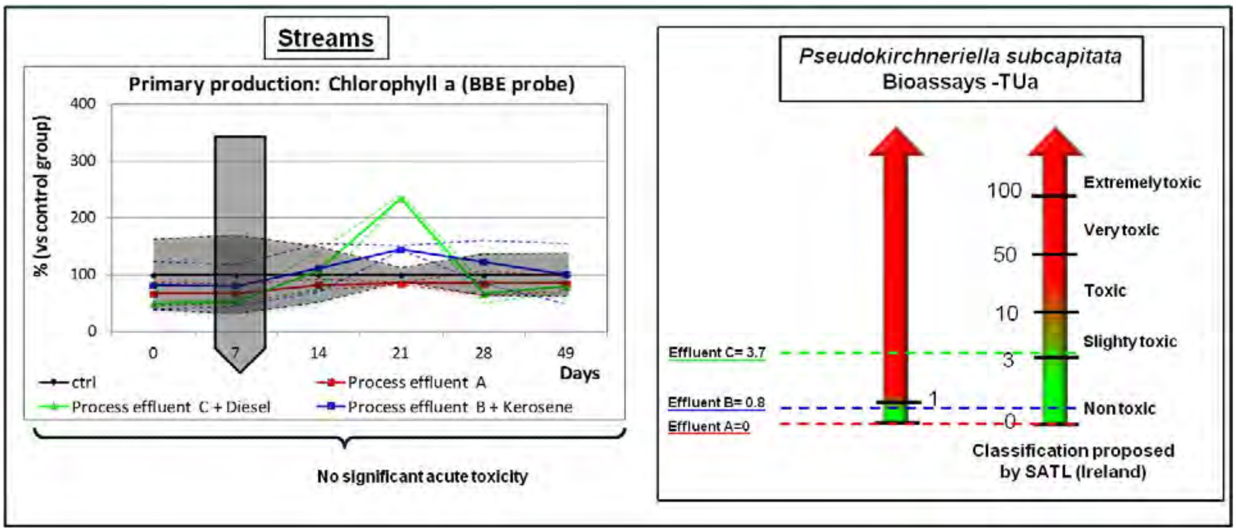
Figure 47: Comparison between bioassays and ecological indicators: chronic toxicity to benthic invertebrate in the streams with *Daphnia magna* chronic toxicity bioassays



No chronic toxicity to benthic invertebrates was determined in the stream study carried out with the unfortified effluent. In contrast, the fortified effluents exhibited significant chronic toxicity to benthic invertebrates in the stream study. The extrapolated chronic data show that the unfortified effluent was unlikely to be chronically toxic whereas it is likely that the fortified effluents would be (respectively TU = 4 for the effluent fortified with Kerosene and TU = 11.2 for the effluent fortified with Diesel). These data suggest that the chronic Whole Effluent Toxicity (WET chronic) bioassays conducted in the laboratory are likely to yield conservative results compared with the outcome observed in the streams. It would have been beneficial to confirm these results by carrying out chronic toxicity bioassays on the same samples with *Daphnia magna*.

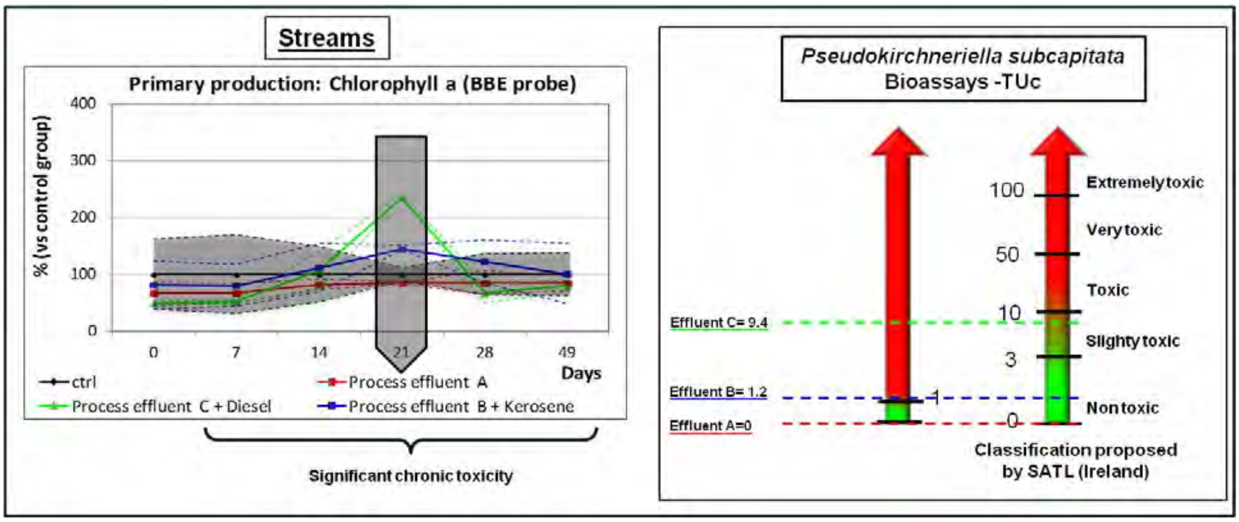
4.2.2. Comparison of laboratory-derived acute and chronic bioassay data for Pseudokirchneriella subcapitata with effects observed on primary production in the stream mesocosms

Figure 48: Comparison of acute effects on primary production observed in the streams with the results of *Pseudokirchneriella subcapitata* acute bioassays



No significant acute effects of the unfortified and fortified effluents on primary production were determined in the streams. The laboratory bioassays conducted with *Pseudokirchneriella subcapitata* showed that the unfortified effluent and the effluent fortified with Kerosene were not acutely toxic (TU = 0 and TU = 0.8 respectively) whereas the effluent fortified with Diesel was acutely toxic (TU = 3.7). These results indicate that data obtained from acute Whole Effluent Toxicity (WETacute) tests conducted in the laboratory with micro-algae are likely to be conservative compared to the observed outcome in stream mesocosms.

Figure 49: Comparison between the results of applying bioassays and ecological indicators of toxicity: comparing chronic effects on primary production observed in the streams with the results of *Pseudokirchneriella subcapitata* chronic bioassays



No chronic effects of the unfortified effluent and the effluent fortified with Kerosene were determined on primary production in the streams. However the effluent fortified with Diesel did cause effects.

The laboratory bioassays conducted with *Pseudokirchneriella subcapitata* showed that unfortified effluent was not chronically toxic whereas fortified effluents were chronically toxic (respectively TU = 1.2 for the effluent fortified with Kerosene and TU = 9.4 for the effluent fortified with Diesel). These results indicate that data obtained from Whole Effluent chronic laboratory bioassays with micro-algae are likely to be conservative in relation to outcome observed in stream mesocosms.

Results obtained by applying laboratory bioassays to refinery effluents are qualitatively compared in the following table with those obtained by applying ecological measures of effect in stream mesocosm experiments. The results of the comparison suggest that risk assessment based solely on data obtained from laboratory Whole Effluent Toxicity (WET) assays conducted in the laboratory are likely to be conservative relative to outcomes observed in more realistic exposure systems.

	Effluent A		Effluent B fortified with Kerosene		Effluent C fortified with Diesel	
	Stream mesocosms	Bioassays	Stream mesocosms	Bioassays	Stream mesocosms	Bioassays
Invertebrate acute effect	0	0	0	0	0	+
Invertebrate chronic effect	0	0	+	+	+	+
Primary production acute effect	0	0	0	0	0	+
Primary production chronic effect	0	0	+	+	+	+
Bacteria	0	+	+	+	+	+

5. CONCLUSIONS

This study was carried out to compare differences in the outcomes of biological impact assessments of refinery effluents based on results obtained using laboratory-based Whole Effluent Toxicity (WET) assay methods and those obtained using *in-situ* assay methods. The differences in outcome from using the two methods have been assessed by examining actual effects observed on communities living in semi-natural outdoor artificial stream mesocosms.

The first series of experiments performed in the stream mesocosms (Concawe stream study phase II) did not provide evidence of a clear dose response because the effluent was insufficiently toxic. Only slight effects were determined at the lowest dilution. Consequently it was not possible to clearly conclude whether the results of the WET assays overestimated or underestimated the impact to aquatic ecosystem. The lower than expected toxicity of the phase II effluent, which was sampled after the DAF but before biological treatment, was probably due to its dilution factor in the streams (from 200 to 1500, v/v). Ageing of refinery effluent could also have a contributory factor, since abiotic degradation (i.e. photo-oxidation by limiting exposure to the light and volatilisation by limiting or avoiding headspace in the containers) could be reduced but not completely avoided: In phase I and phase II it was found that volatilization of VOCs during sampling, transport and filling of the flexible tanks followed by settlement of suspended particles resulted in a decrease in the toxicity of the effluent over the storage period (one week). Phase II showed the feasibility of conducting large scale experiments with refinery effluents using stream mesocosms with one limitation: the ageing of the effluent over the storage period. Strategies were therefore identified to address this issue, including on-site chronic toxicity assessment and the use of artificial refinery effluents consisting of natural water or aged refinery effluent fortified with petroleum distillates [82].

In phase III of the study, which is the subject of this report, effluents from three refineries were sampled before biological treatment and after the DAF (Dissolved Air Flotation) or API (American Petroleum Institute) separator units. The 3 effluents were evaluated using WET bioassays and in the stream mesocosms. One effluent was used as supplied (i.e. sampled after the DAF but before biological treatment) and the other two were used after fortification with distillate hydrocarbon fractions (diesel or kerosene) to ensure that hydrocarbon concentrations were relatively constant and sufficiently high to induce observable effects on the biota.

As observed in phase II, unfortified effluent sampled in the refinery before biological treatment had no impact on either benthic invertebrate or primary production in stream mesocosms, probably due to the effluent dilution factor in the streams (140, v/v). Effluent fortified with kerosene or diesel had no short term effects but significant long term effects on both benthic invertebrate and primary production in stream mesocosms. However, within 30 days of ceasing treatment, a rapid partial or total recovery was observed in the streams treated with the fortified effluents. The results therefore clearly demonstrate that stream mesocosms can be used to study the potential effects of refinery effluents on aquatic ecosystems, especially those resulting from long-term (chronic) exposures.

The toxicity of the unfortified and fortified effluent was also assessed using whole effluent toxicity (WET) tests. The results show that unfortified effluent (A) exhibited no acute or chronic toxicity in any of the three test organisms (*Vibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella subcapitata*). Effluent B fortified with kerosene to a PBS level of ≈ 700 mM exhibited chronic toxicity to both crustacean and microalgae but no acute toxicity (except *Vibrio fischeri*) and effluent C fortified with diesel to a PBS level of ≈ 300 mM exhibited both acute and chronic toxicity to crustacean and microalgae.

When considering a specific biological compartment (ie. Bacteria, Invertebrates, algae or primary production), WET tests were found to either over-predict the effects in the streams, or to predict effects similar to those observed in the streams. In this study, the prediction of *in- situ* impact using WET tests never gave a false negative (i.e. under-prediction of toxicity effects).

These results suggest that biological impact assessments based only on data obtained from laboratory Whole Effluent Toxicity (WET) tests are likely to be conservative. i.e. the biological impact would be less in a more realistic exposure system. Three laboratory WET tests were assessed in this project *Vibrio fischeri* for bacteria, *Pseudokirchneriella subcapitata* for micro-algae and *Daphnia magna* for invertebrates). Additional studies are planned to assess whether other WET test methods are similarly conservative, for example fish embryo development and *Daphnia* sp. reproduction for chronic toxicity.

The WET assay results give a conservative estimate of the toxicity of the effluent samples to stream ecosystems. However the results clearly do not cover the range of compositional variability that could arise in refinery effluents from different sources. Confidence in the findings could be increased by testing a larger set of refinery effluents, with fortification if required to achieve the target hydrocarbon profile. The outcome of such a work programme could be used to draw more widely applicable conclusions regarding the applicability of using WET assays in biological impact assessing the effects of refinery effluents.

6. PERSPECTIVES

Several concerns still exist regarding the outcomes for the assessment of environmental effects of refinery effluents based solely on WET tests. A first series of experiments, jointly organised by TOTAL and Concawe, has shown that outdoor stream mesocosms could be used to investigate the potential differences in outcomes for assessments of effects based on Whole Effluent Toxicity (WET) methods, and one based on *in situ* effects measurement.

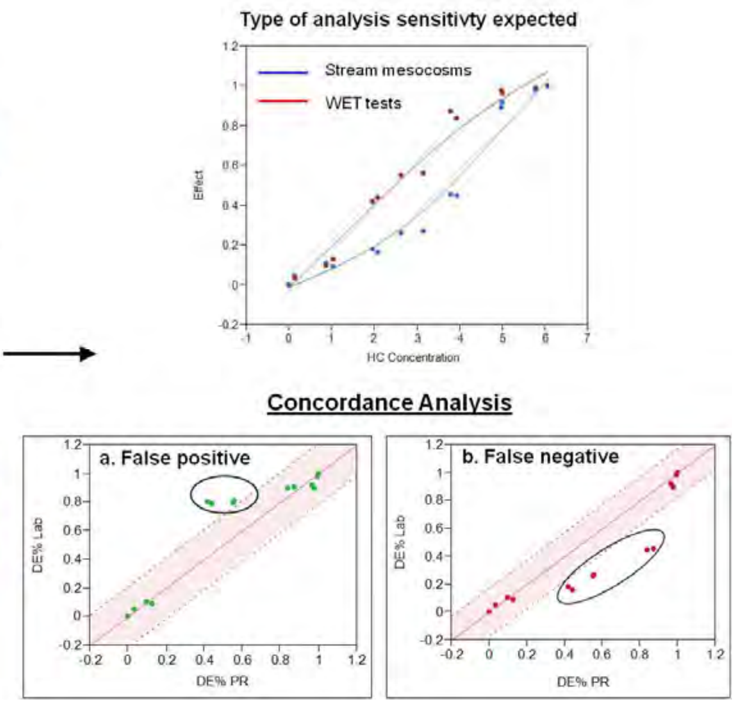
The first series of experiments performed in the stream mesocosms did not provide evidence of a clear dose response (phase II). Consequently it was not possible to clearly conclude whether the results of the WET assays overestimated or underestimated the impact to aquatic ecosystem. However, whilst it may not be possible to accurately assess the magnitude of toxicity present in the streams using the laboratory assays, it is possible to evaluate whether they can reliably distinguish between signal and noise. Two types of error can occur in the interpretation of the results:

- A false positive (Type-I error) is a statistically significant negative effect, or an observed value of an endpoint that is not “real” e.g. a spurious, artifactual or unrelated observation (Figure 50).
- A false negative (Type-II error) can represent the failure of the test organisms to respond to a real toxic challenge or the failure of the method of measurement to detect a real effect because of the lack of sensitivity of the method (**Figure 50**).

Both are errors in accuracy. Why are false positives and false negatives of importance for such studies? They are important because it is useful to know how often the experimental system can be expected to give “wrong” answers. Unreliable answers may lead to unnecessary regulatory action or undetected environmental harm, an issue that has received some attention in the literature [1, 3, 4, 83].

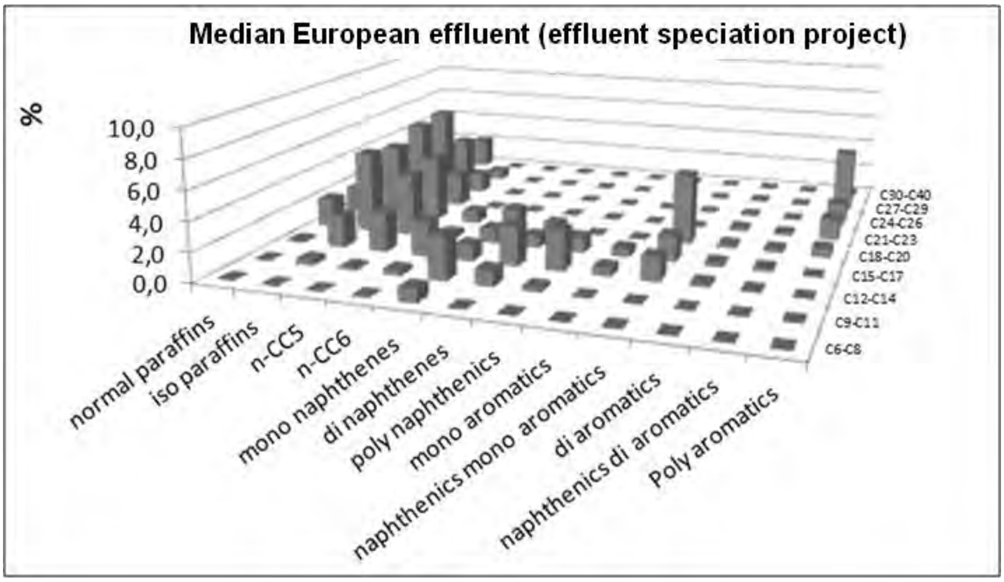
Figure 50: Proposal to study false positive and false negative on WET tests

Stream code	effluent	HC	Loading (PBS)
S1	yes	HC1	1
S2	yes	HC1	2
S3	yes	HC1	3
S4	yes	HC1	4
S5	yes	HC1	5
S6	yes	HC1	6
S7	yes	HC1	1
S8	yes	HC1	2
S9	yes	HC1	3
S10	yes	HC1	4
S11	yes	HC1	5
S12	yes	HC1	6
S13	yes		0
S14	no	River Water	0
S15	no	River Water	0
S16	no	River Water	0



In an earlier project sponsored by Concawe, 111 Effluent discharge samples from 105 Concawe refineries in Europe were obtained and analyzed in the period June 2008 to March 2009 for metals, standard effluent parameters (including COD, BOD), oil in water, BTEX and volatile organic compounds [25]. The parameters measured included standard effluent parameters as well as their speciated hydrocarbon composition, which could then be assigned to hydrocarbon blocks (HCBs) included in the Hydrocarbon Block Method (HBM).The hydrocarbon blocks were determined in those refinery effluents by high resolution GCxGC analytical method [27]. This study allowed determining the median concentrations of the hydrocarbon blocks in refinery effluents discharging to the freshwater environment in Europe (**Figure 51**).

Figure 51: Median composition (%) of hydrocarbons in effluents discharging to the freshwater environment [25]



An artificial refinery effluent mixture similar to the median refinery effluent discharging to the freshwater environment determined in the Concawe effluent speciation project could be prepared with petroleum distillates.

The toxicity of this artificial mixture could then be assessed using the experimental systems described in this report and the type of analysis illustrated in Figure 50. The outcomes of those two methodologies could then be compared to delineate false-positive and false negative results when using WET tests and to quantify their magnitude.

The Concawe effluent speciation project [25] also showed differences in hydrocarbon block patterns between refineries. The results presented in this report showed that the WET assay results gave a conservative estimate of the toxicity of the effluent samples to stream ecosystems. However those results clearly do not cover the whole range of variability that could arise from refinery effluents obtained across the whole range of potential sources as determined in the Concawe speciation project [25].

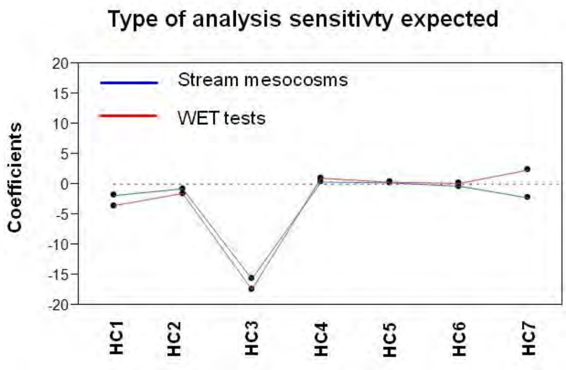
A larger set of refinery effluents or reconstituted refinery effluents with variable composition could be tested (6 to 13) using the experimental systems described in this report and the type of analysis illustrated in **Figure 52** in order to draw wider conclusions regarding the conservatism of WET tests. The outcomes of such a work could be used to determine the frequency of false negatives and positives. The strength of the conclusions drawn will be directly linked to the number of effluent patterns tested.

Figure 52: Proposal to study the impact of the refinery effluent variability on WET tests

Stream code	12 HC
S1	HC1
S2	HC2
S3	HC3
S4	HC4
S5	HC5
S6	HC6
S7	HC7
S8	HC8
S9	HC9
S10	HC10
S11	HC11
S12	HC12
S13	Effluent
S14	River Water
S15	River Water
S16	River Water

Or

Stream code	6 HC Duplicated
S1	HC1
S2	HC2
S3	HC3
S4	HC4
S5	HC5
S6	HC1
S7	HC2
S8	HC3
S9	HC4
S10	HC5
S11	HC6
S12	HC1
S13	Effluent
S14	River Water
S15	River Water
S16	River Water



More interpretation of the data obtained in phase III and Phase IV of this project could be conducted with respect to PETROTOX, a model developed under REACH [84, 85]. PETROTOX is a spreadsheet-based programme that is designed to calculate the toxicity of petroleum substances and products to aquatic organisms, using the HBM. The methodology used is based on the analytical method of 2-dimensional Gas Chromatography or GCxGC. Petroleum substances are complex mixtures of hydrocarbons that exert a narcotic mode of toxic action, which is assumed to be additive. This model calculates the solubility of a petroleum product and then uses the Target Lipid Model [86] and toxic unit theory of additivity to calculate the toxicity or environmental risk limits of these mixtures. Concawe has used PETROTOX model [87] to predict the ecotoxicity of the refinery effluent spot samples [88] taken and analysed during a previously reported effluent speciation project [25] on the basis of their hydrocarbon composition.

Another study has been carried out by Concawe to determine whether the toxicity of refinery effluents, that was determined in laboratory tests and mesocosm experiments, could be predicted using PETROTOX. Those predictions were in reasonable agreement with the effect measured in laboratory tests and in stream mesocosms fed with those refinery effluents. However, the magnitude of the effects predicted by PETROTOX needs further investigations.

7. GLOSSARY

API	American Petroleum Institute
IBD	Biological Diatom Index
BOD_5	Biological Organic Demand
COD	Chemical Organic Demand
Concawe	Conservation of Clean Air and Water in Europe
DAF	Dissolved Air Flotation
ECX	Effect concentration
EPT	Ephemeroptera, Plecoptera, Trichoptera
FID	Flame Ionization Detector
GCGC	Two-dimensional gas chromatography
HBM	Hydrocarbon Block Method
IBGN	Indice Biologique Global Normalisé
IPPC	Integrated Pollution Prevention and Control
LOEC	Lowest Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NOEC	No Observed Effect Concentration
PBS	Potentially Bio-accumulating Substances
PCA	Principal Component Analysis
PRC	Principal Response Curve
SPME	Solid Phase Micro- Extraction
SPI index	PolluoSensitivity Index
TPH	Total Petroleum Hydrocarbons
TU	Toxic Unit
TDI	Trophic Diatom Index
WFD	Water Framework Directive
WEA	Whole Effluent Assessment
WET	Whole Effluent Toxicity

8. ACKNOWLEDGEMENTS

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APPENDIX 1 TPH ANALYSIS IN THE EFFLUENT (WITH AND WITHOUT FORTIFICATION)

Effluent without fortification (Flexible tanks)			
	IVM LIMS nr.	Sample code	TPH (ug/l)
Effluent A	11/1190	TPH T0 CL+CH	90
	11/1283	TPH 21, CH+CL	<50
Effluent A	11/1189	TPH T0 CG+CH	240
	11/1282	TPH 21, CG+CH	350
Effluent B	11/1191	TPH T0 CF+CE	1600
	11/1281	TPH 21, CE+CF	940
Effluent B	11/1194	TPH T0 CD+CE	2700
	11/1280	TPH 21, CD+CE, CITERNA	800
Effluent C	11/1192	TPH T0 CC+CB	1200
	11/1279	TPH 21, CB+CC	1100
Effluent C	11/1193	TPH T0 CA+CB	220
	11/1278	TPH 21 CA+CB,	1500
Effluent with fortification (Flexible tanks+petroleum distillates)			
Effluent B + Kerosene		TPH 0, CE+CF	94000
		TPH 21, CE+CF	90000
Effluent C + Diesel		TPH T0 CC+CB	51000
		TPH T21 CC+CB	66000

APPENDIX 2 TPH ANALYSIS IN THE STREAM MESOCOSMS

stream mesocosm samples			
	IVM LIMS nr.	Sample code	TPH (ug/l)
Ctrl streams (03, 08, 11)	11/1186	TPH 0 C03	30
	11/1163	TPH 2 C03	17
	11/1172	TPH 7C03	26
	11/1270	TPH 21 C03	13
	11/1181	TPH 0 C08	33
	11/1166	TPH 2 C08	13
	11/1175	TPH 7 C08	34
	11/1273	TPH 21 C08	18
	11/1183	TPH 0 C11	<10
	11/1168	TPH 2 C11	16
	11/1177	TPH 7 C11	17
	11/1275	TPH 21 C11	<10
stream 02 treated with effluent C + diesel	11/1180	TPH 0 C02	15
	11/1162	TPH 2 C02	43
	11/1171	TPH 7 C02	79
	11/1269	TPH 21 C02	120
stream 04 treated with effluent C + diesel	11/1182	TPH 0 C04	18
	11/1164	TPH 2 C04	35
	11/1173	TPH 7 C04	210
	11/1271	TPH 21 C04	40
stream 07 treated with effluent B + Kerosene	11/1188	TPH 0 C07	<10
	11/1165	TPH 2 C07	120
	11/1174	TPH 7 C07	110
	11/1272	TPH 21 C07	68
stream 10 treated with effluent B + Kerosene	11/1187	TPH 0 C10	21
	11/1167	TPH 2 C10	120
	11/1176	TPH 7 C10	46
	11/1274	TPH 21 C10	96
stream 13 treated with effluent A	11/1184	TPH 0 C13	<10
	11/1169	TPH 2 C13	16
	11/1178	TPH 7 C13	21
	11/1276	TPH 21 C13	12
stream 15 treated with effluent A	11/1185	TPH 0 C15	<10
	11/1170	TPH 2 C15	13
	11/1179	TPH 7 C15	20
	11/1277	TPH 21 C15	19

APPENDIX 3 SPME ANALYSIS (PBS LEVELS) IN THE EFFLUENTS AND STREAM MESOCOSMS

Effluent without fortification (Flexible tanks)			
	IVM LIMS nr.	Sample code	PBS (mM)
Effluent B	11/1147	SPME PBS T0 CF+CE	73
	11/1139	SPME PBS T7 CF+CE	61
	11/1259	SPME PBS T21, CF+CE	58
Effluent A	11/1148	SPME PBS T0 CL+CH	4,8
	11/1140	SPME PBS T7 CH+CL	6,2
	11/1260	SPME PBS T21, CL+CH	10
Effluent C	11/1149	SPME PBS T0 CC+CB	35
	11/1138	SPME PBS T7 CB+CC	16
	11/1258	SPME PBS T21, CB+CC	17
Effluent with fortification (Flexible tanks+petroleum distillates)			
Effluent B + Kerosene		SPME 0, CE+CF	751
		SPME 21, CE+CF	731
Effluent C + Diesel		SPME T0 CC+CB	268
		SPME T21 CC+CB	436
stream mesocosm samples			
	IVM LIMS nr.	Sample code	PBS (mM)
Ctrl streams (03, 08, 11)	11/1262	SPME PBS T14 C03	0,3
	11/1246	SPME PBS T21 C03	0,2
	11/1264	SPME PBS T14 C08	0,5
	11/1251	SPME PBS T21 C08	0,9
	11/1266	SPME PBS T14 C11	0,4
	11/1253	SPME PBS T21 C11	0,4
stream 07 treated with effluent B + Kerosene	11/1263	SPME PBS T14 C07 30M	1,6
	11/1248	SPME PBS T21 C07 12M	3,9
	11/1249	SPME PBS T21 C07 20M	3,5
	11/1250	SPME PBS T21 C07 30M	2,8
stream 10 treated with effluent B + Kerosene	11/1144	SPME PBS T0 C10	1,5
	11/1142	SPME PBS T7 C10	1,6
	11/1265	SPME PBS T14 C10	3,9
	11/1252	SPME PBS T21 C10	6,7
stream 04 treated with effluent C + diesel	11/1145	SPME PBS T0 C04	1,2
	11/1141	SPME PBS T7 C04	7,0
	11/1298	SPME PBS T14 C04	1,6
	11/1247	SPME PBS T21 C04	0,6
stream 02 treated with effluent C + diesel	11/1261	SPME PBS T14 C02 30M	1,2
	11/1243	SPME PBS T21 C02 12M	0,9
	11/1244	SPME PBS T21 C02 20M	1,2
	11/1245	SPME PBS T21 C02 30M	1,2
stream 13 treated with effluent A	11/1267	SPME PBS T14 C13	0,3
	11/1254	SPME PBS T21 C13	0,8
stream 15 treated with effluent A	11/1146	SPME PBS T0 C15	1,2
	11/1143	SPME PBS T7 C15	1,3
	11/1268	SPME PBS T14 C15 30M	0,4
	11/1255	SPME PBS T21 C15 12M	1,0
	11/1256	SPME PBS T21 C15 20M	0,9
	11/1257	SPME PBS T21 C15 30M	1,5

APPENDIX 4 GCGC ANALYSIS (SPECIATION) IN STREAM MESOCOSMS

2D GC T2 C04	IVM LIMS nr 11/1154	stream 04 treated with effluent C + diesel (Day2)										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	di aromatics	naphthenics di aromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							4.5					
C9 - C11	<0.03	<0.2	<0.03	<0.03	<0.2	<0.2	4.2	<1	0.6			
C12 - C14	1.6	1.6	0.7	0.7	0.9	0.3	2.1	2.9	4.6	0.6	<1	
C15 - C17	20	17	2.1	2.1	12	7.2	5	2.7	1.3	1.1	<1	
C18 - C20	9.5	12	0.9	0.8	6.3	0.5	1.7	1.6	<1	<0.5	<1	0.5
C21 - C23	1.1	2	0.08	0.1	1.8	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	0.9	0.3	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	0.6	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	0.1	<0.2	<0.03	<0.03	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5
2D GC T7 C04	IVM LIMS nr11/1150	stream 04 treated with effluent C + diesel (Day7)										
C3 - C5												
C6 - C8							3.3					
C9 - C11	<0.03	<0.2	<0.03	<0.03	<0.2	<0.2	3.6	1.2	0.8			
C12 - C14	4.1	3	1.36	1.4	1.7	0.7	2.8	3.1	6.4	0.7	<1	
C15 - C17	36	30	4.03	4	23	6.7	7.5	3.5	2	1.7	<1	
C18 - C20	17	19	1.5	1.4	10	3.5	2.3	1.9	<1	<0.5	<1	<0.5
C21 - C23	1.9	3.4	0.2	0.1	1.2	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	2.3	1	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	1.8	0.6	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	0.9	<0.5	<0.1	<0.1	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5
2D GC T21, C04	IVM LIMS nr 11/1284	stream 04 treated with effluent C + diesel (Day21)										
C3 - C5												
C6 - C8							3.4					
C9 - C11	0.06	0.4	<0.03	<0.03	<0.2	<0.2	4.6	1,0	1,0			
C12 - C14	3.1	3.8	0.9	0.9	2.5	1.2	3.8	3.8	7.3	0.9	0.8	
C15 - C17	35	29	3.7	3.7	20	6.5	6.7	3.6	2.2	1.9	0.4	
C18 - C20	15	21	1.6	1.7	9.6	1.8	2.9	1,0	<1	0.5	0.7	0.6
C21 - C23	3.9	6.2	0.5	0.4	2.2	<0.2	<1	<1	<1	<1	<5	<0.5
C24 - C26	5.4	3.1	0.1	0.1	0.9	<0.2	<1	<1	<1	<1	<5	<0.5
C27 - C29	3.9	2.2	<0.03	<0.03	<0.2	<0.2	<1	<1	<1	<1	<5	<0.5
C30 - C40	1.6	0.4	<0.1	<0.1	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5

2D GC T2 C10	IVM LIMS nr 11/1153	stream 10 treated with effluent B + Kerosene (Day 2)										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	di aromatics	naphthenics di aromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							5.6					
C9 - C11	1.3	1.3	0.2	0.2	0.6	0.4	13	1.9	4.1			
C12 - C14	7.5	9.8	1.1	1.1	5.9	3.2	4.8	3.7	4.9	<0.5	<1	
C15 - C17	4.4	5.6	0.2	0.2	2,0	1.7	2,0	<1	<1	0.5	<1	
C18 - C20	0.2	<0.2	<0.03	<0.03	<0.2	<0.2	0.7	<1	<1	<0.5	<1	<0.5
C21 - C23	0.3	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	0.9	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	0.6	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	0.2	<0.2	<0.1	<0.1	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5
2D GC T7 C10	IVM LIMS nr 11/1151	stream 10 treated with effluent B + Kerosene (Day 7) (mislabelling with with Stream 15 treated with effluent A) (Day21)*										
C3 - C5												
C6 - C8							<2					
C9 - C11	0.4	1.2	<0.03	<0.03	<0.2	<0.2	5.6	<1	<0.1			
C12 - C14	0.6	0.7	<0.03	0.04	<0.2	0.2	9.5	1.6	<1	<0.5	<1	
C15 - C17	3.1	0.7	0.2	0.2	2.5	<0.2	13	<1	1.7	<0.5	<1	
C18 - C20	13	9.8	2,0	1.3	4.5	0.3	13	<1	1.2	0.5	10	1.5
C21 - C23	12	15	2,0	1.3	8,0	<0.5	<1	<1	<1	<1	<5	0.7
C24 - C26	14	8.2	0.2	0.2	2,5	<0,5	<1	<1	<1	<1	<5	<0,5
C27 - C29	9.9	5.1	<0,03	<0.03	<0.2	<0,5	<1	<1	<1	<1	<5	<0.5
C30 - C40	5.7	2.4	<0.1	<0.03	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5
2D GC T21, C10	IVM LIMS nr 11/1285	stream 10 treated with effluent B + Kerosene (Day 21)										
C3 - C5												
C6 - C8							7.1					
C9 - C11	2.4	4.2	1.1	1.1	5,4	4,0	22	2,7	5.8			
C12 - C14	8.8	15	1.6	1.6	9.4	5.3	8.7	6.6	7.2	0.3	<1	
C15 - C17	6.1	8.3	0.3	0.3	3.0	3.8	3.6	0.5	0.4	0.2	<1	
C18 - C20	2.2	1.5	0.4	0.3	0.7	<0.2	1.7	0.3	<1	<0.5	1,0	<0.5
C21 - C23	4.3	4.6	0.6	0.5	2.4	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	5.6	3.3	0.2	0.2	1.7	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	2.6	1.1	<0,03	<0.03	0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	1.7	<0.5	<0.1	<0.1	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5

2D GC T 2 C 15	IVM LIMS nr 11/1155	stream 15 treated with site A (Day2)										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	di aromatics	naphthenics di aromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							2.7					
C9 - C11	<0.03	<0.2	<0.03	<0.03	<0.2	<0.2	1.9	<1	<1			
C12 - C14	<0.03	<0.2	<0.03	<0.03	<0.2	<0.2	0.6	<1	<1	<0.5	<1	
C15 - C17	0.1	<0.2	<0.03	<0.03	0.3	<0.2	0.7	<1	<1	<0.5	<1	
C18 - C20	0.1	<0.2	<0.03	<0.03	<0.2	<0.2	1.4	<1	<1	<0.5	<1	<0.5
C21 - C23	0.4	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	0.9	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	0.6	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	0.1	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
2D GC T 7 C 15	IVM LIMS nr 11/1152	stream 15 treated with site A (Day7)										
C3 - C5												
C6 - C8							2.8					
C9 - C11	<0.03	<0.2	<0.03	<0.03	<0.2	<0.2	3.7	<1	<0.1			
C12 - C14	0.06	<0.2	<0.03	<0.03	<0.2	<0.2	1.1	1.1	<1	<0.5	<1	
C15 - C17	0.3	<0.2	<0.03	<0.03	<0.2	<0.2	2.1	<1	<1	<0.5	<1	
C18 - C20	0.2	<0.2	<0.03	<0.03	<0.2	<0.2	0.9	<1	<1	<0.5	<1	<0.5
C21 - C23	0.5	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	1.5	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	1.0	<0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	0.3	<0.5	<0.1	<0.1	<0.5	<0.5	<1	<1	<1	<1	<5	<0.5
2D GC T21, C15	IVM LIMS nr 11/1286	stream 15 treated with site A (Day21) (mislabelling with with Stream 10 treated with effluent B + kerosene day 7)*										
C3 - C5												
C6 - C8							12					
C9 - C11	2.7	4.5	1.1	1.1	5.0	3.7	25	3.4	7.5			
C12 - C14	11	18	2.1	2.1	13	9.0	12	5.9	8.1	0.5	0.2	
C15 - C17	7.2	13	0.5	0.5	4.9	7.8	3.9	0.9	0.6	0.4	0.4	
C18 - C20	0.4	0.3	0.03	0.03	0.1	<0.2	1.1	0.5	<1	<0.5	0.5	<0.5
C21 - C23	0.5	0.7	0.1	0.1	0.11	<0.5	<1	<1	<1	<1	<5	<0.5
C24 - C26	1.0	0.8	<0.03	<0.03	0.1	<0.5	<1	<1	<1	<1	<5	<0.5
C27 - C29	0.6	0.2	<0.03	<0.03	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5
C30 - C40	0.3	<0.5	<0.1	<0.1	<0.2	<0.5	<1	<1	<1	<1	<5	<0.5

APPENDIX 5 GCGC ANALYSIS (HC BLOCKS FROM PETRORISK AND RISK ASSESSMENT) IN STREAM MESOCOSMS

Stream mesocosm samples (priority HC blocks of PetroRisk blocking system)										
		stream 04 treated with effluent C + diesel			stream 10 treated with effluent B + Kerosene			stream 15 treated with effluent A		
Sample code		2D GC T2 C04	2D GC T7 C04	2D GC T21, C04	2D GC T2 C10	2D GC T7 C10 (mislabelling with with Stream 15 treated with effluent A day 21)*	2D GC T21, C10	2D GC T2 C15	2D GC T7 C15	2D GC T21, C15 (mislabelling with with Stream 10 treated with effluent B + kerosene day 7)*
IVM LIMS nr.		11/1154	11/1150	11/1284	11/1153	11/1151	11/1285	11/1155	11/1152	11/1286
HC block		µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
A C9-C11 n-/i-paraf	A	<0.2	<0.2	0,5	2,6	1,7	6,6	<0.2	<0.2	7,2
B C12-C14 n-/i-paraf	B	3	7,1	6,9	17	1,3	24	<0.2	<0.2	29
C C9-C11 mNaphth	C	<0.2	<0.2	0,1	0,9	<0.2	7,5	<0.2	<0.2	7,1
D C12-C14, mNaphth	D	2,3	4,5	4,4	8,1	0,2	13	<0.2	<0.2	17
E C9-C11 diNaphth	E	<0.2	<0.2	<0.2	0,3	<0.2	4,0	<0.2	<0.2	3,7
F C12-C14 diNaphth	F	0,3	0,70	1,2	3,2	0,20	5,3	<0.2	<0.2	9,0
G C15-C17 diNaphth	G	7,2	6,7	6,5	1,7	<0.2	3,8	<0.2	<0.2	7,8
H C15-C17 mArom	H	5,0	7,5	6,7	2,0	13	3,6	0,7	2,1	3,8
I C15-C17 Naphth mArom	I	2,7	3,5	3,6	0,5	0,3	0,4	<1	<1	0,9
J C12-C14, diArom	J	4,5	6,4	7,3	4,9	0,3	7,2	<1	<1	8,1
K C15-C17 diArom	K	1,1	2,0	2,2	<1	1,5	0,4	<1	<1	0,6
Sum priority HC blocks of PetroRisk blocking system		26	38	39	41	19	76	1	2	94
All GCxGC peaks		180	270	320	130	350	250	<50	56	250
All HC blocks		140	220	240	93	180	180	15	21	190
% sum priority blocks of all GCxGC peaks		15	14	12	32	5	30	-	4	38
% sum priority blocks of all HC peaks		19	17	16	44	10	42	5	10	50

Stream mesocosm samples (priority HC blocks from the risk assessment)										
Sample code		2D GC T2 C04	2D GC T7 C04	2D GC T21, C04	2D GC T2 C10	2D GC T7 C10	2D GC T21, C10	2D GC T2 C15	2D GC T7 C15	2D GC T21, C15
		stream 04 treated with effluent C + diesel			stream 10 treated with effluent B + Kerosene			stream 15 treated with effluent A		
IVM LIMS nr.		11/1154	11/1150	11/1284	11/1153	11/1151	11/1285	11/1155	11/1152	11/1286
HC block		µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
A C9-C10 n-/i-paraffins	A	<0.1	<0.1	0,1	0,5	0,6	3,0	<0.1	<0.1	2,8
B C11 n-/-i-paraffins	B	<0.05	<0.05	0,3	2,1	1,1	3,6	<0.05	<0.05	4,4
C C12-C14 n-/-i-paraffins	C	3,1	7,1	6,9	17	1,3	24	<0.2	<0.2	29
D C9-C11 mono-naphthenes	D	<0.2	<0.2	0,1	0,9	<0.2	7,5	<0.2	<0.2	7,1
E C12-C13 mono-naphthenes	E	2,3	4,4	4,0	6,0	0,1	8,5	<0.1	<0.1	11,7
F C11 di-naphthenes	F	<0.05	<0.05	<0.05	0,3	<0.05	2,2	<0.05	<0.05	1,9
G C12-C13 di-naphthenes	G	0,3	<0.05	1,1	2,6	0,2	3,5	<0.05	<0.05	6,5
H C14-C16 di-naphthenes	H	4,5	5,5	6,7	3,7	0,1	6,1	<0.1	<0.1	13
I C15-C16 mono-aromatics	I	3,2	5,2	4,6	1,5	11	2,8	0	1,4	3,0
J C15-C16 naphthenic mono-aromatics	J	2,1	2,6	2,7	0,5	0	0,4	<0.5	<0.5	0,7
K C14-C15 di-aromatics	K	2,1	3,1	3,3	0,5	<0.3	0,6	<0.5	<0.5	0,9
Sum original HC blocks		18	28	30	36	15	62	0	1,4	81
All GCxGC peaks		180	270	320	130	350	250	<50	56	250
All HC blocks		140	220	240	93	180	180	15	21	190
% sum original blocks of all GCxGC peaks		10	10	9	27	4	25	1	3	32
% sum original blocks of all HC blocks		13	13	12	38	8	35	2	7	42

APPENDIX 6 GCGC ANALYSIS (ALL HC BLOCKS) IN STREAM MESOCOSMS

Stream mesocosm samples (all HC blocks)										
		stream 04 treated with effluent C + diesel			stream 10 treated with effluent B + Kerosene			stream 15 treated with effluent A		
Sample code		2D GC T2 C04	2D GC T7 C04	2D GC T21, C04	2D GC T2 C10	2D GC T7 C10	2D GC T21, C10	2D GC T2 C15	2D GC T7 C15	2D GC T21, C15
IVM LIMS nr		11/1154	11/1150	11/1284	11/1153	11/1151	11/1285	11/1155	11/1152	11/1286
Chemical functionality	HC block	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
All GCxGC peaks		180	270	320	130	350	250	<50	56	250
ALL HC blocks		140	220	240	93	180	180	15	21	190
C9 normal and iso paraffins	A	<0.05	<0.05	0,1	0,06	0,5	0,9	<0.05	<0.05	0,7
C10 normal and iso paraffins	A	<0.05	<0.05	0,04	0,4	0,1	2,1	<0.05	<0.05	2,1
C11 normal and iso paraffins	A	<0.05	<0.05	0,3	2,1	1,1	3,6	<0.05	<0.05	4,4
Total A		<0.2	<0.2	0,5	2,6	1,7	6,6	<0.2	<0.2	7,2
C12 normal and iso paraffins	B	0,06	0,1	0,5	3,8	0,7	5,3	<0.05	0,05	6,3
C13 normal and iso paraffins	B	0,6	1,1	1,7	5,6	0,2	8,3	<0.05	<0.05	10
C14 normal and iso paraffins	B	2,5	5,8	4,7	7,9	0,4	10	<0,05	<0.05	13
Total B		3,1	7,1	6,9	17	1,3	24	<0.2	<0.2	29
C9 mono naphthenes	C	<0.05	<0.05	<0.05	<0.05	<0.05	1,4	<0.05	<0.05	1,2
C10 mono naphthenes	C	<0.05	<0.05	0,04	0,1	<0.05	3,0	<0.05	<0.05	2,3
C11 mono naphthenes	C	<0.05	<0.05	0,06	0,8	<0.05	3,1	<0,05	<0.05	3,7
Total C		<0.2	<0.2	0,09	0,9	<0.2	7,5	<0.2	<0.2	7,1
C12 mono naphthenes	D	<0.05	0,07	0,3	2,2	0,1	4,0	<0.05	<0.05	5,1
C13 mono naphthenes	D	0,4	0,7	0,8	3,2	<0.05	4,2	<0.05	<0.05	5,7
C14 mono naphthenes	D	1,9	3,7	3,2	2,8	0,1	4,3	<0,05	<0.05	6,0
Total D		2,3	4,5	4,4	8,1	0,2	13	<0.2	<0.2	17
C9 di naphthenes	E	<0.05	<0.05	<0.05	<0.05	<0.05	0,8	<0.05	<0.05	0,6
C10 di naphthenes	E	<0.05	<0.05	<0.05	<0.05	<0.05	1,1	<0.05	<0.05	1,2
C11 di naphthenes	E	<0.05	<0.05	<0.05	0,3	<0.05	2,2	<0,05	<0.05	1,9
Total E		<0.2	<0.2	<0.2	0,3	<0.2	4,0	<0.2	<0.2	3,7
C12 di naphthenes	F	<0.05	<0.05	0,1	0,7	<0.05	1,8	<0.05	<0.05	2,4
C13 di naphthenes	F	<0.05	<0.05	0,1	0,5	0,1	0,9	<0.05	<0.05	1,2
C14 di naphthenes	F	0,3	0,7	1,0	2,1	0,08	2,6	<0.05	<0.05	5,3
Total F		0,3	0,7	1,2	3,2	0,2	5,3	<0.2	<0.2	9,0
C15 di naphthenes	G	1,8	1,0	2,5	1,4	<0.05	3,3	<0.05	<0.05	4,5
C16 di naphthenes	G	2,4	3,8	3,2	0,2	<0.05	0,2	<0.05	<0.05	2,8
C17 di naphthenes	G	2,9	1,9	0,8	0,2	<0.05	0,3	<0.05	<0.05	0,5
Total G		7,2	6,7	6,5	1,7	<0.2	3,8	<0.2	<0.2	7,8
C15 mono aromatics	H	1,3	2,2	2,2	0,8	7,4	1,7	0,2	0,7	2,0
C16 mono aromatics	H	1,9	3,0	2,4	0,7	3,6	1,1	0,1	0,7	1,0
C17 mono aromatics	H	1,8	2,4	2,2	0,5	2,4	0,8	0,4	0,8	0,9
Total H		5,0	7,5	6,7	2,0	13	3,6	0,7	2,1	3,8
C15 naphthenics mono aromatics	I	1,3	1,4	1,7	0,5	<0.3	0,4	<0.3	<0.3	0,7
C16 naphthenics mono aromatics	I	0,8	1,2	1,0	<0.3	0,3	<0.3	<0.3	<0.3	<0.3
C17 naphthenics mono aromatics	I	0,6	0,9	0,9	<0.3	<0.3	<0.3	<0.3	<0.3	0,2
Total I		2,7	3,5	3,6	0,5	0,3	0,4	<1	<1	0,9
C12 di aromatics	J	1,4	1,9	2,2	3,0	<0.3	4,6	<0.3	<0.3	5,0
C13 di aromatics	J	1,9	2,7	3,0	1,4	0,3	2,0	<0.3	<0.3	2,3
C14 di aromatics	J	1,3	1,9	2,1	0,5	<0.3	0,5	<0.3	<0.3	0,7
Total J		4,5	6,4	7,3	4,9	0,3	7,2	<1	<1	8,1
C15 di aromatics	K	0,8	1,2	1,2	<0.3	<0.3	0,1	<0.3	<0.3	0,2
C16 di aromatics	K	0,3	0,5	0,7	<0.3	0,9	0,2	<0.3	<0.3	0,3
C17 di aromatics	K	<0.3	0,3	0,2	<0.3	0,5	0,07	<0.3	<0.3	0,1
Total K		1,1	2,0	2,2	<1	1,5	0,4	<1	<1	0,6

APPENDIX 7 GCGC ANALYSIS (SPECIATION) IN THE EFFLUENTS (WITHOUT FORTIFICATION)

day0	IVM LIMS nr 11/1159	Effluent A										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	diaromatics	naphthenics diaromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							25					
C9 - C11	<0.2	<1	<0.2	<0.2	<2	<2	41	12	<5			
C12 - C14	0.3	<1	<0.2	<0.2	<2	<2	15	20	7.7	<5	<5	
C15 - C17	0.9	<1	<0.2	<0.2	<2	<2	16	3.8	4.7	4.4	<5	
C18 - C20	1.8	<1	<0.2	<0.2	<2	<2	13	<5	<5	<5	<5	<5
C21 - C23	3.4	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C24 - C26	7.6	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	4.5	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	1.1	<5	<0.5	<0.5	<5	<5	<10	<10	<10	<10	<50	<5
day7	IVM LIMS nr 11/1158	Effluent A										
C3 - C5												
C6 - C8							17					
C9 - C11	<0.2	<1	<0.2	<0.2	<2	<2	28	6.5	<5			
C12 - C14	<0.2	<1	<0.2	<0.2	<2	<2	11	7.4	3.4	<5	<5	
C15 - C17	1.5	<1	<0.2	<0.2	<2	<2	14	<5	<5	<5	<5	
C18 - C20	1.1	<1	<0.2	<0.2	<2	<2	11	<5	<5	<5	<5	<5
C21 - C23	1.1	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C24 - C26	2.2	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	1.2	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	<1	<5	<0.5	<0.5	<5	<5	<10	<10	<10	<10	<50	<5
day21	IVM LIMS nr 11/1289	Effluent A										
C3 - C5												
C6 - C8							17					
C9 - C11	<0.2	<1	<0.2	<0.2	<2	<2	28	6.9	0.4			
C12 - C14	1.7	2.6	0.5	0.5	1,0	<2	18	8.1	3.4	1.5	0.4	
C15 - C17	4.6	3.9	0.8	0.8	1.9	0.7	23	4.4	4.5	2.1	<1	
C18 - C20	4.8	4.7	1,0	1,0	3.8	<2	12	1.9	<5	1,0	2.8	<5
C21 - C23	4.1	3.7	<0.2	<0.2	1.8	<5	<10	<10	<10	<10	<50	<5
C24 - C26	4.4	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	1.4	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	<1	<5	<0.5	<0.5	<2	<5	<10	<10	<10	<10	<50	<5

day0	IVM LIMS nr 11/1160	Effluent B										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	di aromatics	naphthenics di aromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							260					
C9 - C11	6.2	8,0	1,0	1,0	4.5	3.1	240	12	60			
C12 - C14	13	18	1.5	1.5	8.4	4.3	27	20	47	9.7	9.3	
C15 - C17	18	17	1.7	1.5	7,0	4.6	34	3.8	6.8	12	<5	
C18 - C20	14	16	2.1	2,0	12	<2	20	<5	3.9	<5	<5	<5
C21 - C23	12	15	1.9	1.7	5.9	<5	<10	<10	<10	<10	<50	<5
C24 - C26	10	10	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	4.6	1.6	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	1,0	<5	<0.2	<0.2	<5	<5	<10	<10	<10	<10	<50	<5
day7	IVM LIMS nr 11/1157	Effluent B										
C3 - C5												
C6 - C8							330					
C9 - C11	0.8	2.3	0.1	0.1	<1	<2	330	84	89			
C12 - C14	6,0	7.2	0.8	0.8	2.8	<2	22	35	61	11.6	7.88	
C15 - C17	8.3	7.1	0.5	0.5	2.6	<2	22	7.5	5.2	9.19	<10	
C18 - C20	5.2	4.4	1.1	1.1	4.6	<2	14	<5	<10	<5	<10	<5
C21 - C23	3.2	4.0	0.3	0.3	<2	<5	<10	<10	<10	<10	<50	<5
C24 - C26	2.9	2.0	<0.3	<0.3	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	1.2	<2	<0.3	<0.3	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	1.9	<2	<1	<1	<5	<5	<10	<10	<10	<10	<50	<5
day21	IVM LIMS nr 11/1288	Effluent B										
C3 - C5												
C6 - C8							400					
C9 - C11	13	22	2,0	2,0	13	6.7	350	77	64			
C12 - C14	37	50	5.6	5.6	33	20	35	37	49	13	6.1	
C15 - C17	20	26	1,0	1,0	9.4	8.9	23	10	5.4	7.4	<5	
C18 - C20	4.2	0.4	<0.2	<0.2	1.3	<2	7.4	1,0	2.8	2.5	2.8	<5
C21 - C23	3.4	<1	<0.2	<0.2	3,0	<5	<10	<10	<10	<10	<50	<5
C24 - C26	3,0	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	1.2	<1	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	<1	<5	<0.5	<0.5	<2	<5	<10	<10	<10	<10	<50	<5

day0	IVM LIMS nr 11/1161	Effluent C										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	diaromatics	naphthenics diaromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							710					
C9 - C11	7.7	6.2	0.5	0.5	1.2	2.7	150	30	23			
C12 - C14	18	14	2,0	2,0	15	12	36	38	28	4.6	14	
C15 - C17	31	21	3.3	3,0	19	15	41	23	11	15	3.1	
C18 - C20	38	25	4.8	4.4	26	<2	32	4.5	1.5	4.2	4.3	7.7
C21 - C23	34	30	4.2	3.6	18	<5	<10	<10	<10	<10	<50	5.7
C24 - C26	30	29	1.7	1.6	11	<5	<10	<10	<10	<10	<50	<5
C27 - C29	16	13	<0.2	<0.2	3,0	<5	<10	<10	<10	<10	<50	<5
C30 - C40	4.1	<5	<0.2	<0.2	<5	<5	<10	<10	<10	<10	<50	<5
day7	IVM LIMS nr 11/1156	Effluent C										
C3 - C5												
C6 - C8							180					
C9 - C11	1.5	<2	<0.3	<0.3	<2	<2	82	16	5.4			
C12 - C14	4.7	2.5	0.4	0.4	<2	<2	26	20	12	<5	8.6	
C15 - C17	8.6	3.8	0.7	0.6	<2	<2	38	<5	7	6.5	<10	
C18 - C20	9.7	5.6	1.0	1,0	3.9	<2	34	<5	<10	<5	<10	<5
C21 - C23	9.2	7.6	0.9	1,0	3.9	<5	<10	<10	<10	<10	<50	<5
C24 - C26	8.6	7.8	0.3	0,3	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	4.5	2.8	<0.3	<0.3	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	1,0	<2	<1	<1	<5	<5	<10	<10	<10	<10	<50	<5
day21	IVM LIMS nr 11/1287	Effluent C										
C3 - C5												
C6 - C8							190					
C9 - C11	1.3	<1	<0.2	<0.2	<2	<2	53	14	3.9			
C12 - C14	5.4	3.5	0.5	0.5	1,0	<2	15	10	6.5	2.0	2.3	
C15 - C17	10	4.3	0.9	0.8	1.7	1.7	29	2.4	5.1	3.8	2.2	
C18 - C20	9.6	5.2	1,0	1,0	5.7	<2	16	2.4	<5	<5	<5	<5
C21 - C23	9,0	6.6	1,0	0.9	3,0	<5	<10	<10	<10	<10	<50	<5
C24 - C26	7.3	6.1	0.2	0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	3.2	1.9	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	0.4	<5	<0.5	<0.5	<2	<5	<10	<10	<10	<10	<50	<5

APPENDIX 8 GCGC ANALYSIS (SPECIATION) IN THE EFFLUENTS (WITH FORTIFICATION)

day0	IVM LIMS nr 11/1147	Effluent B + Kerosene										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	diaromatics	naphthenics diaromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							2716.2					
C9 - C11	2155	3248.7	3	1366.2	4170.8	2274	3180.9	586.3	738.9			
C12 - C14	2547	4881	4.5	883.1	4269.9	1678	1077.7	665.4	830.6	18.8	9.3	
C15 - C17	618.8	1520.6	5.1	82.9	526.8	10.6	275.3	82.3	18.5	36	<5	
C18 - C20	31,8	40.7	2,3	3,8	26,7	<2	34,2	<5	<5	<5	<5	<5
C21 - C23	20,6	24.4	2,3	2,7	7,7	<5	<10	<10	<10	<10	<50	<5
C24 - C26	15,2	16.9	0.15	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	7.6	2,2	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	2.7	<5	<0.2	<0.2	<5	<5	<10	<10	<10	<10	<50	<5
day21	IVM LIMS nr 11/1259	Effluent B + Kerosene										
C3 - C5												
C6 - C8							817.4					
C9 - C11	2432,7	3777,8	2,0	1487,5	4961,7	2357,4	3638,5	486,7	776,2			
C12 - C14	2271,2	6000,9	5,6	890,6	4475,1	1842,5	1168,7	723,5	881,7	9,4	2,8	
C15 - C17	161,7	1651,7	1,0	91,5	940.4	6,9	254	68,7	14,1	<5	<50	
C18 - C20	1.1	9,1	<0.2	0.05	3,2	<5	13	<10	<5	<10	<50	<5
C21 - C23	1,0	1.2	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C24 - C26	0.7	0.3	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	0.5	0.09	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	<0,1	<0.1	<0.5	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
day0	IVM LIMS nr 11/1149	Effluent C + Diesel										
	normal paraffins	iso paraffins	n-CC5	n-CC6	mono naphthenes	di naphthenes	mono aromatics	naphthenics mono aromatics	diaromatics	naphthenics diaromatics	Poly aromatics	poly naphthenics
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
C3 - C5												
C6 - C8							381.4					
C9 - C11	54.4	55.7	0	40.9	102.8	150.6	239.3	59.2	101.5			
C12 - C14	452.4	612.3	0.0	228.3	687	678.6	225	174.6	608.9	15.4	26.7	
C15 - C17	1981.7	2284.5	120.5	458.5	2340.8	270.2	401.3	117.5	145	<5	1.9	
C18 - C20	636.3	1094.6	91.2	78.2	914.8	<2	39.9	<5	<5	<5	<5	1.8
C21 - C23	89.8	162.6	7.6	7.2	69	<5	<10	<10	<10	<10	<50	0.4
C24 - C26	9.6	10.4	<0.2	0.5	0.5	<5	<10	<10	<10	<10	<50	<5
C27 - C29	4.1	0,6	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	1.3	<5	<0.2	<0.2	<5	<5	<10	<10	<10	<10	<50	<5
day21	IVM LIMS nr 11/1258	Effluent C + Diesel										
C3 - C5												
C6 - C8							231.7					
C9 - C11	60.3	57.3	<0.2	50.6	120.4	181.5	221.1	63.1	127.5			
C12 - C14	536.6	706.4	0.5	322.2	962.2	891.9	251.7	301.1	846.3	17.5	39.9	
C15 - C17	2508.4	2641.2	76.5	665.8	2926.7	410	790.6	249.5	192.4	3.8	3.5	
C18 - C20	746.8	1501.2	115.7	109.9	1229.1	52.9	261.6	<10	<5	<5	<50	<5
C21 - C23	106	207.1	9.3	14.2	88.6	<5	<10	<10	<10	<10	<50	<5
C24 - C26	7.9	13.4	0.3	0.2	<2	<5	<10	<10	<10	<10	<50	<5
C27 - C29	4.2	2	<0.2	<0.2	<2	<5	<10	<10	<10	<10	<50	<5
C30 - C40	0.4	<0.1	<0.5	<0.5	<2	<5	<10	<10	<10	<10	<50	<5

APPENDIX 9 GCGC ANALYSIS (HC BLOCKS FROM PETRORISK AND RISK ASSESSMENT) IN THE EFFLUENTS

Effluent samples (priority HC blocks of PetroRisk blocking system)													
	Effluent C			Effluent B			Effluent A			Effluent B + Kerosene		Effluent C + Diesel	
Sample code	**2D GC T0 CC+CB**	**2D GC T7 CB+CC**	**2D GC T21, CB+CC**	**2D GC T0 CF+CE**	**2D GC T7 CF+CE**	**2D GC T21, CF+CE**	**2D GC T0 CL+CH**	**2D GC T7 CH+CL**	**2D GC T21, CI+CH**	**CF+CE (Day0)**	**CE+CF (Day21)**	**CC+CB (Day0)**	**CB+CC (Day21)**
IVM LIMS nr.	**11/1161**	**11/1156**	**11/1287**	**11/1160**	**11/1157**	**11/1288**	**11/1159**	**11/1158**	**11/1289**	**11/1160**	**11/1259**	**11/1149**	**11/1287**
HC block	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**
A C9-C11 n-/i-paraf	14	0,9	1,3	14	3,1	34	<1	<1	<1	5404	6211	110	118
B C12-C14 n-/i-paraf	31	7,2	9	31	13	87	<1	<1	4,3	7428	8272	1065	1243
C C9-C11 mNaphth	2,2	<1	0,3	6,6	1,2	17	<1	<1	<1	5536	6449	144	171
D C12-C14, mNaphth	18	1,7	2,1	11	3,4	44	<1	<1	2	5152	5366	915	1185
E C9-C11 diNaphth	2,7	<1	<1	3,1	0,6	6,7	<1	<1	<1	2274	2357	151	182
F C12-C14 diNaphth	12	<1	<1	4,3	<1	20	<1	<1	<1	1678	1843	679	892
G C15-C17 diNaphth	15	<1	1,7	4,6	1,2	8,9	<1	<1	0,7	14	7	270	409
H C15-C17 mArom	41	38	29	34	22	23	16	14	23	275	254	401	791
I C15-C17 Naphth mArom	23	3,4	1,5	5,7	6,7	10	3,8	<5	4,4	60	69	118	249
J C12-C14, diArom	28	12	6,5	47	61	49	7,7	<5	3,3	931	882	609	846
K C15-C17 diArom	11	6,1	4,6	6,8	<5	4,8	4,2	<5	4,5	20	14	145	192
Sum priority HC blocks of PetroRisk blocking system	198	69	56	169	112	304	32	14	42	28773	31723	4606	6277
All GCxGC peaks	2200	1100	880	1600	1600	2100	520	<500	410	46069	47745	22730	26001
All HC blocks	1700	540	450	1100	1100	1400	190	110	180	40707	42123	16286	20803
% sum priority blocks of all GCxGC peaks	9	6	6	11	7	14	6	3	10	62	66	20	24
% sum priority blocks of all HC peaks	12	13	12	15	10	22	17	13	23	71	75	28	30
Effluent samples (priority HC blocks from the risk assessment)													
Sample code IVM LIMS nr.	**2D GC T0 CC+CB 11/1161**	**2D GC T7 CB+CC 11/1156**	**2D GC T21, CB+CC 11/1287**	**2D GC T0 CF+CE 11/1160**	**2D GC T7 CF+CE 11/1157**	**2D GC T21, CF+CE 11/1288**	**2D GC T0 CL+CH 11/1159**	**2D GC T7 CH+CL 11/1158**	**2D GC T21, CI+CH 11/1289**	**CF+CE (Day0) 11/1160**	**CE+CF (Day21) 11/1259**	**CC+CB (Day0) 11/1149**	**CB+CC (Day21) 11/1287**
HC block	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**
A C9-C10 n-/i-paraffins	7,0	<0.5	0,3	6,6	1,7	21	<0.5	<0.5	<0.5	3202	3760	45	45
B C11 n-/-i-paraffins	6,9	0,9	1,0	7,7	1,3	13	<0.3	<0.3	<0.3	2201	2450	66	73
C C12-C14 n-/-i-paraffins	31	7,2	8,9	31	13	87	<0.5	<0.5	4,3	7428	8272	1065	1243
D C9-C11 mono-naphthenes	2,2	<0.5	0,3	6,6	1,2	17	<0.5	<0.5	<1	5536	6449	144	171
E C12-C13 mono-naphthenes	13	1,6	1,7	7,9	3,4	30	<0.5	<0.5	1,9	3345	3425	770	999
F C11 di-naphthenes	2,1	<0.3	<0.3	1,8	0,6	4,7	<0.3	<0.3	<0.3	828	812	75	93
G C12-C13 di-naphthenes	10	<0.5	<0.5	3,0	<0.5	14	<0.5	<0.5	<0.5	1568	768	494	656
H C14-C16 di-naphthenes	18	<0.5	1,7	7,1	1,2	19	<0.5	<0.5	0,7	110	122	443	602
I C15-C16 mono-aromatics	30	21	20	20	12	18	9,1	7,9	17	201	162	242	372
J C15-C16 naphthenic mono-	17	3,4	1,5	5,7	6,7	8,1	3,8	<2	2,3	60	69	118	249
K C14-C15 di-aromatics	13	7,3	4,6	10	6,6	9,1	4,4	<2	2,4	64	70	232	339
Sum original HC blocks	150	41	40	107	48	240	17	8	29	24545	26360	3693	4841
All GCxGC peaks	2200	1100	880	1600	1600	2100	520	<500	410	46069	47745	22730	26001
All HC blocks	1700	540	450	1100	1100	1400	190	110	180	40707	42123	16286	20803
% sum original blocks of all GCxGC peaks	7	4	5	7	3	11	3	2	7	53	55	16	19
% sum original blocks of all HC blocks	9	8	9	10	4	17	9	7	16	60	63	23	23

APPENDIX 10 GCGC ANALYSIS (ALL HC BLOCKS) IN THE EFFLUENTS

Effluent samples (all HC blocks)													
	Effluent C			Effluent B			Effluent A			Effluent B + Kerosene		Effluent C + Diesel	
Sample code	**CC+CB (Day0)**	**CB+CC (Day 7)**	**CB+CC (Day 21)**	**CF+CE (Day 0)**	**CF+CE (Day 7)**	**CF+CE (Day 21)**	**CL+CH (Day 0)**	**CH+CL (Day 7)**	**CI+CH (Day21)**	**CF+CE (Day0)**	**CE+CF (Day21)**	**CC+CB (Day0)**	**CB+CC (Day21)**
IVM LIMS nr	**11/1161**	**11/1156**	**11/1287**	**11/1160**	**11/1157**	**11/1288**	**11/1159**	**11/1158**	**11/1289**	**11/1160**	**11/1259**	**11/1149**	**11/1287**
Chemical functionality	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**	**µg/l**
All GCxGC peaks	2200	1100	880	1600	1600	2100	520	<500	410	1600	47745	22730	880
ALL HC blocks	1700	540	450	1100	1100	1400	190	110	180	1100	42123	16286	450
C9 normal and iso paraffins	2,3	<0.3	<0.3	1,7	1,0	14	<0.3	<0.3	<0.3	1060	1264	14	13
C10 normal and iso paraffins	4,7	<0.3	0,3	4,9	0,7	7,0	<0.3	<0.3	<0.3	2142	2496	30	32
C11 normal and iso paraffins	6,9	0,9	1,0	7,7	1,3	13	<0.3	<0.3	<0.3	2201	2450	66	73
Total A	14	0,9	1,3	14	3,1	34	<1	<1	<1	5404	6211	110	118
C12 normal and iso paraffins	8,3	1,9	2,4	7,9	2,8	19	<0.3	<0.3	0,6	2252	2397	136	158
C13 normal and iso paraffins	11	2,5	2,9	11	4,7	30	<0.3	<0.3	1,6	2739	2883	317	385
C14 normal and iso paraffins	12	2,9	3,6	12	5,6	39	<0.3	<0.3	2,1	2437	2992	611	701
Total B	31	7,2	8,9	31	13	87	<1	<1	4,3	7428	8272	1065	1243
C9 mono naphthenes	0,2	<0.3	0,3	2,1	0,6	1,1	<0.3	<0.3	<0.3	1341	1769	16	19
C10 mono naphthenes	0,6	<0.3	<0.3	2,3	0,6	6,5	<0.3	<0.3	<0.3	2332	2649	50	58
C11 mono naphthenes	1,4	<0.3	<0.3	2,2	<0.5	9,5	<0.3	<0.3	<0.3	1864	2031	78	94
Total C	2,2	<1	0,3	6,6	1,2	17	<1	<1	<1	5536	6449	144	171
C12 mono naphthenes	5,2	<0.3	0,4	3,5	<0.3	14	<0.3	<0.3	<0.3	1806	1941	145	186
C13 mono naphthenes	7,3	0,7	0,6	3,9	1,7	16	<0.3	<0.3	0,8	1927	1988	305	365
C14 mono naphthenes	6,0	1,0	1,1	4,0	1,7	14	<0.3	<0.3	1,1	1418	1437	466	634
Total D	18	1,7	2,1	11	3,4	44	<1	<1	2,0	5152	5366	915	1185
C9 di naphthenes	0,2	<0.3	<0.3	0,8	<0.3	0,9	<0.3	<0.3	<0.3	408	440	12,8	15
C10 di naphthenes	0,3	<0.3	<0.3	0,5	<0.3	1,1	<0.3	<0.3	<0.3	1038	1105	62,4	74
C11 di naphthenes	2,1	<0.3	<0.3	1,8	0,6	4,7	<0.3	<0.3	<0.3	828	812	75	93
Total E	2,7	<1	<1	3,1	0,6	6,7	<1	<1	<1	2274	2357	151	182
C12 di naphthenes	2,5	<0.3	<0.3	1,3	<0.3	7,0	<0.3	<0.3	<0.3	952	1075	184	236
C13 di naphthenes	3,9	<0.3	<0.3	0,5	<0.3	3,5	<0.3	<0.3	<0.3	618	653	272	320
C14 di naphthenes	5,9	<0.3	<0.3	2,5	<0.3	10	<0.3	<0.3	<0.3	108	115	222	335
Total F	12	<1	<1	4,3	<1	20	<1	<1	<1	1678	1843	679	892
C15 di naphthenes	7,4	<0.3	1,2	2,7	1,2	8,0	<0.3	<0.3	0,7	6	4	137	195
C16 di naphthenes	4,3	<0.3	0,5	1,9	<0.3	0,8	<0.3	<0.3	<0.3	5	3	84	73
C17 di naphthenes	3,1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	49	142
Total G	15	<1	1,7	4,6	1,2	8,9	<1	<1	0,7	11	7	270	409
C15 mono aromatics	15	10	9,3	7,0	4,5	8,7	4,8	5,3	8,3	97	115	129	137
C16 mono aromatics	15	11	11	13	7,4	9,1	4,3	2,6	9,0	104	47	112	235
C17 mono aromatics	11	17	8,1	14	10	4,7	7,2	5,6	6,0	74	92	159	419
Total H	41	38	29	34	22	23	16	14	23	275	254	401	791
C15 naphthenics mono aromatics	8,8	<2	<2	5,7	6,7	8,1	2,0	<2	1,5	50	55,3	59	124
C16 naphthenics mono aromatics	8,3	3,4	1,5	<2	<2	<2	1,8	<2	0,8	11	13,4	58	125
C17 naphthenics mono aromatics	5,5	<2	<2	<2	<2	1,4	<2	<2	2,1	<2	<2	0	<2
Total I	23	3,4	1,5	5,7	6,7	10	3,8	<5	4,4	60	69	118	249
C12 di aromatics	11	3,9	2,2	27	38	29	2,4	<2	1,0	620	557	215	288
C13 di aromatics	9,4	3,6	2,0	14	16	14	2,5	<2	1,1	253	266	259	351
C14 di aromatics	7,5	4,2	2,2	6,8	6,6	6,1	2,8	<2	1,2	56	59	135	207
Total J	28	12	6,5	47	61	49	7,7	<5	3,3	931	882	609	846
C15 di aromatics	5,1	3,1	2,4	3,0	<2	3,0	1,6	<2	1,2	8	11	97	132
C16 di aromatics	4,5	3,0	2,2	2,8	<2	1,8	2,6	<2	2,5	8	2,7	48	60
C17 di aromatics	1,1	<2	<2	<2	<2	<2	<2	<2	0,8	<2	<2	<2	<2
Total K	11	6,1	4,6	6,8	<5	4,8	4,2	<5	4,5	20	14	145	192

APPENDIX 11 BENTHIC INVERTEBRATE TAXONOMIC LIST

			Ephemeroptera (insecta)										
Sampling day	stream number	Produit	Baetidae	Caenidae	Ephemerellidae	Ephemeridae	Heptageniidae	Leptophlebiidae	Oligoneuriidae	Polymitarcidae	Potamanthidae	Prosopistomatidae	Siphlonuridae
0	2	effluent C + Diesel	8	13	0	0	6	0	0	0	0	0	0
0	3	Control	35	35	0	0	29	0	0	0	0	0	0
0	4	effluent C + Diesel	9	33	0	0	12	0	0	0	0	0	0
0	7	Effluent B + Kerosene	4	19	0	1	4	0	0	0	0	0	0
0	8	Control	0	25	0	0	3	0	0	0	0	0	0
0	10	Effluent B + Kerosene	6	21	0	0	9	0	0	0	0	0	0
0	11	Control	13	9	0	0	8	0	0	0	0	0	0
0	13	Effluent A	38	7	0	0	13	1	0	0	0	0	0
0	15	Effluent A	29	16	0	1	25	4	0	0	0	0	0
7	2	effluent C + Diesel	4	23	0	1	7	0	0	0	0	0	0
7	3	Control	11	30	0	0	19	0	0	0	0	0	0
7	4	effluent C + Diesel	3	9	0	0	3	0	0	0	0	0	0
7	7	Effluent B + Kerosene	0	4	0	1	0	0	0	0	0	0	0
7	8	Control	2	8	0	0	4	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	15	0	0	1	0	0	0	0	0	0
7	11	Control	22	13	0	0	13	0	0	0	0	0	0
7	13	Effluent A	27	11	0	0	9	0	0	0	0	0	0
7	15	Effluent A	15	9	0	1	22	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	19	43	0	1	21	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	1	3	0	1	0	0	0	0	0	0	0
14	8	Control	9	28	0	0	8	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	1	0	0	0	0	0	0	0	0	0
14	11	Control	6	16	0	0	13	0	0	0	0	0	0
14	13	Effluent A	22	31	0	0	8	0	0	0	0	0	0
14	15	Effluent A	6	18	0	1	14	0	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	25	27	0	0	24	0	0	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	1	0	0	0	0	0	0	0	0	0
21	8	Control	4	19	0	0	3	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	10	22	0	0	26	0	0	0	0	0	0
21	13	Effluent A	15	15	0	2	8	0	0	0	0	0	0
21	15	Effluent A	20	17	0	0	15	0	0	0	0	0	0
49	2	effluent C + Diesel	22	1	0	0	0	0	0	0	0	0	0
49	3	Control	115	22	0	0	29	0	0	0	0	0	0
49	4	effluent C + Diesel	14	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	34	0	0	0	0	0	0	0	0	0	0
49	8	Control	10	20	0	0	6	0	0	0	0	0	0
49	10	Effluent B + Kerosene	72	0	0	0	0	0	0	0	0	0	0
49	11	Control	7	19	0	0	19	0	0	0	0	0	0
49	13	Effluent A	54	29	0	0	32	0	0	0	0	0	0
49	15	Effluent A	27	19	0	0	26	0	0	0	0	0	0

			Plécoptera (insecta)							Trichoptera (insecta)			
Sampling day	stream number	Produit	Capniidae	chloroperlidae	Leuctridae	Nemouridae	Perlidae	Perlodidae	Taeniopterygidae	Baaerdae	Brachycentridae	Ecnomidae	Glossosomatidae
0	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	3	Control	0	0	0	0	0	0	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	8	Control	0	0	0	0	0	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	11	Control	0	0	0	0	0	0	0	0	0	0	0
7	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	8	Control	0	0	0	0	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	11	Control	0	0	0	0	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	0	0	0	0	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	0	0	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	11	Control	0	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

			Trichoptera (insecta)										
Sampling day	stream number	Produit	Goeridae	Helicopsychidae	Hydropsychidae	Hydroptilidae	Lépidostomatidae	Leptoceridae	Limnephilidae	Molannidae	Odontoceridae	Philopotamidae	Phyganeidae
0	2	effluent C + Diesel	0	0	0	18	0	0	0	0	0	0	0
0	3	Control	0	0	16	68	0	0	0	0	0	0	0
0	4	effluent C + Diesel	0	0	1	9	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	16	0	0	0	0	0	0	0
0	8	Control	0	0	0	47	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	5	16	0	0	0	0	0	0	0
0	11	Control	0	0	0	4	0	0	0	0	0	0	0
0	13	Effluent A	0	0	1	10	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	19	0	0	0	0	0	0	0
7	2	effluent C + Diesel	0	0	0	1	0	0	0	0	0	0	0
7	3	Control	0	0	18	15	0	0	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	7	Effluent B + Kerosene	0	0	1	0	0	0	0	0	0	0	0
7	8	Control	0	0	1	16	0	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	3	5	0	0	0	0	0	0	0
7	11	Control	0	0	0	6	0	0	0	0	0	0	0
7	13	Effluent A	0	0	0	12	0	0	0	0	0	0	0
7	15	Effluent A	0	0	1	68	0	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	1	0	0	0	0	0	0	0
14	3	Control	0	0	14	41	0	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	8	Control	0	0	0	19	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	1	1	0	0	0	0	0	0	0
14	11	Control	0	0	0	23	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	39	0	0	0	0	0	0	0
14	15	Effluent A	0	0	0	48	0	0	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	0	0	2	71	0	0	0	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	0	0	0	18	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	0	0	1	13	0	0	0	0	0	0	0
21	13	Effluent A	0	0	1	5	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	66	0	0	0	0	0	0	0
49	2	effluent C + Diesel	0	0	0	4	0	0	0	0	0	0	0
49	3	Control	0	0	8	106	0	0	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	4	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	20	0	0	0	0	0	0	0
49	8	Control	0	0	0	2	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	60	0	0	0	0	0	0	0
49	11	Control	0	0	3	32	0	0	0	0	0	0	0
49	13	Effluent A	0	0	1	4	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	16	0	0	0	0	0	0	0

			Trichoptera (insecta)					Coleoptera (insecta)					
Sampling day	stream number	Produit	Polycentropodidae	Psychomyidae	Rhyacophilidae	Sericostomatidae	Thremmatidae	Curculionidae	Donaciidae	Dryopidae	Dytiscidae	Eubriidae	Elmidae (L)
0	2	effluent C + Diesel	0	1	0	0	0	0	0	0	0	0	0
0	3	Control	1	0	0	0	0	0	0	0	0	0	0
0	4	effluent C + Diesel	0	1	8	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	6	1	1	0	0	0	0	0	0	0	0
0	11	Control	1	0	1	0	0	0	0	0	0	0	0
0	13	Effluent A	2	1	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	3	Control	0	0	0	0	0	0	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	7	Effluent B + Kerosene	1	0	0	0	0	0	0	0	0	0	0
7	8	Control	0	0	0	0	0	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	1	0	0	0	0	0	0	0	0
7	11	Control	0	0	0	0	0	0	0	0	0	0	0
7	13	Effluent A	0	1	0	0	0	0	0	0	0	0	0
7	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	2	0	0	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	1	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	1	0	0	0	0	0	0	0	0	0	0
14	8	Control	1	0	0	0	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	11	Control	0	0	4	0	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	15	Effluent A	0	0	1	0	0	0	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	1	0	4	0	0	0	0	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	4	0	1	0	0	0	0	0	0	0	1
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	0	0	2	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	1	0	0	0	0	0	0	0	0
21	15	Effluent A	0	1	2	0	0	0	0	0	0	0	0
49	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	1	4	0	0	0	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	1	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	11	Control	0	0	2	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	2	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	3	0	0	0	0	0	0	0	0

			Coleoptera (insecta)										
Sampling day	stream number	Produit	Elmidae (A)	Gyrinidae	Haliplidae	Helodidae (L)	Helodidae (A)	Helophoridae	Hydraenidae	Hydrochidae	Hydrophilidae	Hydroscaphidae	Hygrobiidae
0	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	3	Control	0	1	0	0	0	0	0	0	0	0	0
0	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	1	1	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	3	Control	0	0	0	0	0	0	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	8	Control	0	0	0	0	0	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	11	Control	0	0	0	0	0	0	0	0	0	0	0
7	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	8	Control	0	0	0	0	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	11	Control	0	0	0	0	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	0	0	0	0	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	0	0	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	1	0	0	0	0	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	11	Control	0	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

			coleoptera (insecta)		Diptera (insecta)								
Sampling day	stream number	Produit	Limnebiidae	Spercheidae	Anthomyidae	Athericidae	Blphariceridae	Ceratopogonidae	Chaoboridae	Chironomidae	sF Corynoneurinae	sF Prodiamesinae	sF Tanypoddinae
0	2	effluent C + Diesel	0	0	2	0	0	0	0	575	4	0	2
0	3	Control	0	0	12	0	0	0	0	641	18	0	5
0	4	effluent C + Diesel	0	0	0	0	0	0	0	347	12	0	4
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	527	15	0	10
0	8	Control	0	0	1	0	0	0	0	757	8	0	0
0	10	Effluent B + Kerosene	0	0	1	0	0	0	0	1210	12	0	8
0	11	Control	0	0	1	0	0	0	0	1057	98	0	0
0	13	Effluent A	0	0	1	0	0	0	0	1467	45	0	0
0	15	Effluent A	0	0	1	0	0	0	0	1081	0	0	5
7	2	effluent C + Diesel	0	0	0	0	0	0	0	241	4	0	1
7	3	Control	0	0	1	0	0	0	0	378	6	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	364	0	0	0
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	234	0	0	0
7	8	Control	0	0	7	0	0	0	0	768	37	0	50
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	304	7	0	3
7	11	Control	0	0	0	0	0	0	0	866	0	0	3
7	13	Effluent A	0	0	0	0	0	0	0	910	8	0	8
7	15	Effluent A	0	0	0	0	0	0	0	2139	0	0	1
14	2	effluent C + Diesel	0	0	1	0	0	0	0	53	0	0	2
14	3	Control	0	0	2	0	0	0	0	383	7	0	1
14	4	effluent C + Diesel	0	0	2	0	0	0	0	167	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	1	0	55	0	0	5
14	8	Control	0	0	0	0	0	0	0	835	60	0	0
14	10	Effluent B + Kerosene	0	0	1	0	0	0	0	13	0	0	1
14	11	Control	0	0	3	0	0	0	0	901	112	0	0
14	13	Effluent A	0	0	0	0	0	0	0	1044	80	0	12
14	15	Effluent A	0	0	2	0	0	0	0	1288	16	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	108	2,2	0	0
21	3	Control	0	0	10	0	0	0	0	735	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	174	0	0	1
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	27	0	0	5
21	8	Control	0	0	3	0	0	0	0	597	0	0	8
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	17	0	0	0
21	11	Control	0	0	7	0	0	0	0	1090	0	0	4
21	13	Effluent A	0	0	0	0	0	0	0	1198	0	0	4
21	15	Effluent A	0	0	1	0	0	0	0	1416	0	0	4
49	2	effluent C + Diesel	0	0	1	0	0	0	0	129	0	0	5
49	3	Control	0	0	7	0	0	0	0	840	0	0	2
49	4	effluent C + Diesel	0	0	0	0	0	0	0	110	0	0	1
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	411	0	0	0
49	8	Control	0	0	8	0	0	0	0	924	0	0	102
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	393	0	0	0
49	11	Control	0	0	9	0	0	0	0	1412	10	0	6
49	13	Effluent A	0	0	2	0	0	0	0	979	6	0	21
49	15	Effluent A	0	0	3	0	0	0	0	1928	8	0	13

			Diptera (insecta)										
Sampling day	stream number	Produit	sF Diamesinae	sF Orthocladiinae	sF Chironominae	dont tr Chironomini	dont tr. Tanytarsini	Culicidae	Dixidae	Dolichopodidae	Empididae	Ephydridae	Limonidae
0	2	effluent C + Diesel	0	465	104	2	101	0	0	0	0	0	0
0	3	Control	0	439	179	3	176	0	0	0	0	0	0
0	4	effluent C + Diesel	0	221	109	4	105	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	302	200	0	200	0	0	0	0	0	0
0	8	Control	0	575	174	0	174	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	826	364	0	364	0	0	0	0	0	0
0	11	Control	0	829	131	0	131	0	0	0	0	0	0
0	13	Effluent A	0	1099	324	0	324	0	0	0	0	0	0
0	15	Effluent A	0	970	106	16	89	0	0	0	0	0	0
7	2	effluent C + Diesel	0	180	56	8	48	0	0	0	0	0	0
7	3	Control	0	284	88	0	88	0	0	0	0	0	0
7	4	effluent C + Diesel	0	255	109	1	108	0	0	0	0	1	0
7	7	Effluent B + Kerosene	0	77	157	1	156	0	0	0	0	0	0
7	8	Control	0	487	194	0	194	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	246	48	0	48	0	0	0	0	0	0
7	11	Control	0	765	98	3	96	0	0	0	0	0	0
7	13	Effluent A	0	691	203	0	203	0	0	0	0	0	0
7	15	Effluent A	0	1836	302	0	302	0	0	0	0	0	0
14	2	effluent C + Diesel	0	48	3	0	3	0	0	0	0	0	0
14	3	Control	0	364	11	0	11	0	0	0	0	0	0
14	4	effluent C + Diesel	0	163	4	0	4	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	13	37	1	36	0	0	0	0	0	0
14	8	Control	0	597	178	5	173	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	3	9	0	9	0	0	0	0	0	0
14	11	Control	0	666	123	0	123	0	0	0	0	0	1
14	13	Effluent A	0	745	208	4	204	0	0	0	0	0	0
14	15	Effluent A	0	1120	152	0	152	0	0	0	0	0	0
21	2	effluent C + Diesel	0	105	1	1	0	0	0	0	0	0	0
21	3	Control	0	529	206	5	201	0	0	0	0	0	0
21	4	effluent C + Diesel	0	163	10	1	9	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	12	10	0	10	0	0	0	0	0	0
21	8	Control	0	420	169	0	169	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	12	5	0	5	0	0	0	0	0	0
21	11	Control	0	1043	43	0	43	0	0	0	0	0	0
21	13	Effluent A	0	1067	127	0	127	0	0	0	0	0	0
21	15	Effluent A	0	1200	212	1	211	0	0	0	0	0	0
49	2	effluent C + Diesel	0	120	4	0	4	0	0	0	0	0	0
49	3	Control	0	676	162	0	162	0	0	0	0	0	0
49	4	effluent C + Diesel	0	101	8	0	8	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	304	107	0	107	0	0	0	0	0	0
49	8	Control	0	494	328	1	327	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	286	107	1	106	0	0	0	0	0	0
49	11	Control	0	1200	196	3	192	0	0	0	0	0	0
49	13	Effluent A	0	790	162	1	161	0	0	0	0	0	0
49	15	Effluent A	0	1840	69	2	67	0	0	0	0	0	0

			Diptera (insecta)										
Sampling day	stream number	Produit	Psychodidae	Ptychopteridae	Rhagionidae	Scatophagidae	Sciomyzidae	Simuliidae	Stratiomyidae	Syrphidae	Tabanidae	Thaumaleidae	Tipulidae
0	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	1	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	1
7	3	Control	0	0	0	0	0	0	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	8	Control	0	0	0	0	0	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	11	Control	0	0	0	0	0	1	0	0	0	0	1
7	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	1
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	8	Control	0	0	0	0	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	11	Control	0	0	0	0	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	1	0	0	0	0	0
14	15	Effluent A	0	0	0	0	0	1	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	1
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	0	0	0	0	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	0	0	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	2	effluent C + Diesel	0	0	0	0	0	5	0	0	0	0	0
49	3	Control	0	0	0	0	0	1	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	0	0	1	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	1	0	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	1	0	0	0	0	0
49	11	Control	0	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

			Odonata (insecta)										Megaloptera (insecta)
Sampling day	stream number	Produit	Aeschnidae	Calopterygidae	Coenagrionidae	Cordulegasteridae	Corduliidae	Gomphydae	Lestidae	Libellulidae	Platycnemididae	Zygoptère	Sialidae
0	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	3	Control	0	0	0	0	0	0	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	8	Control	0	0	0	0	0	0	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
7	11	Control	0	0	0	0	0	0	0	0	0	0	0
7	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
7	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	8	Control	0	0	0	0	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	11	Control	0	0	0	0	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	0	0	0	0	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	11	Control	0	0	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	11	Control	0	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

			Insecta				Crustacea						
Sampling day	stream number	Produit	Osmylidae	Sysyridae	Hyménoptères	Pyralidae	F. Limnadiidae	Gammaridae	Asellidae	Astacidae	Atyidae	Grapsidae	Cambaridae
0	2	effluent C + Diesel	0	0	0	0	0	126	0	0	0	0	0
0	3	Control	0	0	0	0	0	146	2	0	0	0	0
0	4	effluent C + Diesel	0	0	0	0	0	73	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	136	1	0	0	0	0
0	8	Control	0	0	0	0	0	95	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	126	1	0	0	0	0
0	11	Control	0	0	0	0	0	78	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	91	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	58	1	0	0	0	0
7	2	effluent C + Diesel	0	0	0	0	0	32	0	0	0	0	0
7	3	Control	0	0	0	0	0	95	0	0	0	0	0
7	4	effluent C + Diesel	0	0	0	0	0	62	0	0	0	0	0
7	7	Effluent B + Kerosene	0	0	0	0	0	13	1	0	0	0	0
7	8	Control	0	0	0	0	0	98	0	0	0	0	0
7	10	Effluent B + Kerosene	0	0	0	0	0	4	0	0	0	0	0
7	11	Control	0	0	0	0	0	60	0	0	0	0	0
7	13	Effluent A	0	0	0	0	0	37	0	0	0	0	0
7	15	Effluent A	0	0	0	0	0	24	24	0	0	0	0
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	83	4	0	0	0	0
14	4	effluent C + Diesel	0	0	0	0	0	2	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
14	8	Control	0	0	0	0	0	88	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	0	1	1	0	0	0	0
14	11	Control	0	0	0	0	0	35	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	36	0	0	0	0	0
14	15	Effluent A	0	0	0	0	0	26	29	0	0	0	0
21	2	effluent C + Diesel	0	0	0	0	0	2	0	0	0	0	0
21	3	Control	0	0	0	0	0	53	5	0	0	0	0
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
21	8	Control	0	0	0	0	0	59	10	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	2	0	0	0	0	0
21	11	Control	0	0	0	0	0	85	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	39	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	27	49	0	0	0	0
49	2	effluent C + Diesel	0	0	0	0	0	9	0	0	0	0	0
49	3	Control	0	0	0	0	0	30	0	0	0	0	0
49	4	effluent C + Diesel	0	0	0	0	0	8	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	21	0	0	0	0	0
49	8	Control	0	0	0	0	0	45	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	75	0	0	0	0	0
49	11	Control	0	0	0	0	0	50	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	41	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	24	4	0	0	0	0

			Mollusca										
Sampling day	stream number	Produit	Corbiculidae	Dreissenidae	Sphaeriidae	Unionidae	Bithinellidae	Ancylidae	Bithynidae	Hydrobiidae	Lymnaeidae	Neritidae	Physidae
0	2	effluent C + Diesel	0	0	0	0	0	0	0	1	420	0	26
0	3	Control	0	0	0	0	0	0	0	0	315	0	87
0	4	effluent C + Diesel	0	0	0	0	0	0	0	0	66	0	9
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	133	0	83
0	8	Control	4	0	0	0	0	0	0	0	77	0	12
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	8	0	66
0	11	Control	0	0	0	0	0	0	0	0	25	0	14
0	13	Effluent A	0	0	0	0	0	0	0	0	1	0	144
0	15	Effluent A	0	0	0	0	0	0	0	0	32	0	60
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	268	0	11
7	3	Control	0	0	0	0	0	0	0	0	222	0	42
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	127	0	3
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	75	0	42
7	8	Control	0	0	0	0	0	0	0	0	85	0	23
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	14	0	112
7	11	Control	0	0	0	0	0	0	0	0	5	0	1
7	13	Effluent A	0	0	0	0	0	0	0	0	0	0	60
7	15	Effluent A	0	0	0	0	0	0	0	0	13	0	55
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	182	0	4
14	3	Control	0	0	0	0	0	0	0	0	120	0	72
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	74	0	16
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	120	0	43
14	8	Control	0	0	0	0	0	0	0	0	66	0	6
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	9	0	66
14	11	Control	0	0	0	0	0	0	0	0	10	0	4
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	72
14	15	Effluent A	0	0	0	0	0	1	0	0	33	0	65
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	186	0	21
21	3	Control	0	0	0	0	0	0	0	0	118	0	52
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	66	0	22
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	153	0	109
21	8	Control	4	0	0	0	0	1	0	1	95	0	38
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	1	9	0	104
21	11	Control	0	0	0	0	0	0	0	0	44	0	62
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	192
21	15	Effluent A	1	0	0	0	0	0	0	0	37	0	83
49	2	effluent C + Diesel	0	0	0	0	0	0	0	0	117	0	1
49	3	Control	0	0	0	0	0	0	0	0	105	0	32
49	4	effluent C + Diesel	0	0	0	0	0	0	0	0	16	0	72
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	153	0	73
49	8	Control	0	0	0	0	0	0	0	0	51	0	21
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	21	0	30
49	11	Control	0	0	0	0	0	0	0	0	22	0	18
49	13	Effluent A	0	0	0	0	0	1	0	0	0	0	59
49	15	Effluent A	0	0	0	0	0	0	0	0	54	0	105

			Mollusca			Annelida				Tricladia			
Sampling day	stream number	Produit	Planorbidae	Valvatidae	Viviparidae	Erpobdellidae	Glossiphoniidae	Hirudidae	Piscicolidae	Dendrocoelidae	Dugesiidae	Planariidae	Oligochaetae
0	2	effluent C + Diesel	0	0	0	0	0	0	0	0	2	0	322
0	3	Control	0	0	0	0	0	0	0	0	0	0	460
0	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	568
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	161
0	8	Control	0	0	0	0	0	0	0	0	1	0	352
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	603
0	11	Control	0	0	0	0	0	0	0	0	0	0	692
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0	387
0	15	Effluent A	0	0	0	0	0	0	0	0	1	1	688
7	2	effluent C + Diesel	0	0	0	0	0	0	0	0	1	0	429
7	3	Control	0	0	0	0	0	0	0	0	0	0	373
7	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	723
7	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	402
7	8	Control	0	0	0	0	0	0	0	0	0	0	522
7	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	1	0	377
7	11	Control	0	0	0	0	0	0	0	0	0	0	376
7	13	Effluent A	0	0	0	0	0	0	0	0	0	0	607
7	15	Effluent A	0	0	0	0	0	0	0	0	0	0	711
14	2	effluent C + Diesel	0	0	0	0	0	0	0	0	1	0	163
14	3	Control	0	0	0	0	0	0	0	0	0	0	499
14	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	162
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	241
14	8	Control	0	0	0	0	0	0	0	0	4	0	167
14	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	1	0	257
14	11	Control	0	0	0	0	0	0	0	0	0	0	416
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	1022
14	15	Effluent A	0	0	0	0	0	0	0	0	2	0	489
21	2	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	325
21	3	Control	0	0	0	0	0	0	0	0	1	0	701
21	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	177
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	208
21	8	Control	0	0	0	0	0	0	0	0	2	0	467
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	48
21	11	Control	0	0	0	0	0	0	0	0	0	0	699
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	920
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	2021
49	2	effluent C + Diesel	0	0	0	0	1	0	0	0	0	0	1790
49	3	Control	0	0	0	0	0	0	0	0	2	0	540
49	4	effluent C + Diesel	0	0	0	0	0	0	0	0	0	0	4171
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	1	505
49	8	Control	0	0	0	0	0	0	0	0	0	0	1696
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	2	1	354
49	11	Control	0	0	0	0	0	0	0	0	1	0	1224
49	13	Effluent A	0	0	0	0	0	0	0	0	4	0	2054
49	15	Effluent A	0	0	0	0	0	0	0	0	2	0	612

Sampling day	stream number	Produit	Nemathelminthes	Hydrachnidiae	Hydrozoa	Porifera	Ectoprocta	Nemertea
0	2	effluent C + Diesel	12	20	240	0	0	0
0	3	Control	5	82	128	0	0	5
0	4	effluent C + Diesel	29	0	322	0	0	0
0	7	Effluent B + Kerosene	12	4	72	0	0	0
0	8	Control	13	0	43	0	0	0
0	10	Effluent B + Kerosene	34	4	56	0	0	0
0	11	Control	13	0	109	0	0	1
0	13	Effluent A	1	1	112	0	0	4
0	15	Effluent A	11	29	216	0	0	0
7	2	effluent C + Diesel	49	4	27	0	0	0
7	3	Control	0	24	56	0	0	0
7	4	effluent C + Diesel	4	0	137	0	0	0
7	7	Effluent B + Kerosene	29	0	92	0	0	0
7	8	Control	32	0	123	0	0	0
7	10	Effluent B + Kerosene	28	0	183	0	0	0
7	11	Control	0	0	76	0	0	0
7	13	Effluent A	94	1	91	0	0	0
7	15	Effluent A	9	1	89	0	0	2
14	2	effluent C + Diesel	20	0	24	0	0	0
14	3	Control	0	49	77	0	0	0
14	4	effluent C + Diesel	96	0	1	0	0	0
14	7	Effluent B + Kerosene	60	0	106	0	0	0
14	8	Control	0	0	77	0	0	0
14	10	Effluent B + Kerosene	60	0	40	0	0	0
14	11	Control	1	0	112	0	0	0
14	13	Effluent A	4	48	78	0	0	0
14	15	Effluent A	20	0	60	0	0	0
21	2	effluent C + Diesel	64	0	0	0	0	0
21	3	Control	24	8	67	0	0	0
21	4	effluent C + Diesel	96	0	0	0	0	0
21	7	Effluent B + Kerosene	24	0	64	0	0	0
21	8	Control	8	0	73	0	0	0
21	10	Effluent B + Kerosene	4	5	21	0	0	0
21	11	Control	4	4	310	0	0	0
21	13	Effluent A	4	1	82	0	0	0
21	15	Effluent A	20	9	67	0	0	0
49	2	effluent C + Diesel	74	4	0	0	0	0
49	3	Control	5	4	113	0	0	4
49	4	effluent C + Diesel	129	0	49	0	0	0
49	7	Effluent B + Kerosene	144	4	99	0	0	1
49	8	Control	4	0	306	0	0	0
49	10	Effluent B + Kerosene	121	33	34	0	0	0
49	11	Control	6	0	444	0	0	0
49	13	Effluent A	31	0	60	0	0	0
49	15	Effluent A	6	4	48	0	0	0

APPENDIX 12 ZOOPLANKTON TAXONOMIC LIST

			zooplankton						
Sampling day	stream number	Produit	Copepoda	Chydoridae	Daphniidae	Macrothricidae	Tardigrada	Colembolla	Ostracoda
0	2	effluent C+	930	1458	12	0	0	0	29
0	3	Control	610	727	14	0	0	0	21
0	4	effluent C+	651	1003	0	12	0	0	16
0	7	Effluent B+	1168	1161	0	24	0	0	81
0	8	Control	566	1031	15	4	0	0	32
0	10	Effluent B+	1131	2087	35	0	0	0	20
0	11	Control	706	1374	8	4	0	0	38
0	13	Effluent A	884	733	5	0	0	0	8
0	15	Effluent A	484	736	4	4	0	0	38
7	2	effluent C+	820	971	18	0	0	0	29
7	3	Control	705	514	4	4	0	0	7
7	4	effluent C+	604	417	2	0	0	0	4
7	7	Effluent B+	647	532	1	0	0	0	9
7	8	Control	1046	1489	17	4	0	0	12
7	10	Effluent B+	1831	1307	9	4	4	0	9
7	11	Control	1445	2836	21	0	0	0	8
7	13	Effluent A	1120	1282	0	0	0	0	40
7	15	Effluent A	568	2550	10	0	0	0	62
14	2	effluent C+	654	737	6	4	0	0	1
14	3	Control	481	819	20	0	0	0	9
14	4	effluent C+	813	1841	0	0	20	0	10
14	7	Effluent B+	809	481	0	32	0	0	32
14	8	Control	722	1499	0	0	0	0	40
14	10	Effluent B+	800	641	0	8	0	0	0
14	11	Control	964	2370	4	12	0	0	80
14	13	Effluent A	981	809	1	0	0	0	12
14	15	Effluent A	644	1449	8	12	0	0	21
21	2	effluent C+	1055	594	0	20	0	0	40
21	3	Control	435	598	1	4	0	0	20
21	4	effluent C+	432	321,6	0	0	0	0	5
21	7	Effluent B+	674	368	0	48	0	0	48
21	8	Control	722	763	0	8	0	0	29
21	10	Effluent B+	645	672	0	4	0	0	4
21	11	Control	403	680	0	0	0	0	21
21	13	Effluent A	645	1045	0	8	0	0	62
21	15	Effluent A	572	2691	10	8	0	0	49
49	2	effluent C+	857	806	0	0	0	0	30
49	3	Control	164	432	0	0	0	0	0
49	4	effluent C+	379	1364	0	0	56	0	0
49	7	Effluent B+	1059	1121	0	12	0	0	0
49	8	Control	405	1201	0	240	0	0	122
49	10	Effluent B+	975	480	0	0	8	0	5
49	11	Control	1008	1234	0	0	0	0	209
49	13	Effluent A	1214	640	0	0	0	0	20
49	15	Effluent A	405	1040	0	8	0	0	15

APPENDIX 13 DIATOM TAXONOMIC LIST

Sampling days	stream number	substance	Achnanthes biasolettiana	Achnanthes rupestoides	Achnanthidium atomoides	Achnanthidium eutrophilum	Achnanthidium latecephalum	Achnanthidium minutissima	Achnanthidium minutissimum	Achnanthidium pyrenaicum	Achnanthidium rivulare	Achnanthidium sp.
0	2	Effluent C+ Diesel	0	0	85	0	0	0	1288	271	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	9442	0	220	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	678	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	822	0	0	0
0	11	Control	0	0	0	0	0	0	158794	4391	0	5854
0	13	Effluent A	0	0	287	287	0	0	10621	1148	0	0
0	15	Effluent A	0	0	0	0	0	0	6105	0	0	0
14	2	Effluent C+ Diesel	0	0	6110	0	0	0	9470	18329	0	0
14	3	Control	0	0	49	0	0	0	1759	586	0	0
14	4	Effluent C+ Diesel	0	839	7554	839	0	0	78059	21823	0	0
14	7	Effluent B + Kerosene	0	0	18670	4001	0	0	21337	13336	0	0
14	8	Control	0	0	1382	106	0	0	7549	1595	0	0
14	10	Effluent B + Kerosene	0	0	579	0	0	0	9065	3857	0	0
14	11	Control	0	0	0	0	0	0	317071	4848	0	0
14	13	Effluent A	0	179	3040	0	0	0	8405	10551	0	1431
14	15	Effluent A	0	0	115	0	0	0	3212	344	0	0
21	2	Effluent C+ Diesel	0	0	3268	182	182	0	8353	9805	0	182
21	3	Control	0	0	0	0	0	0	428	143	0	0
21	4	Effluent C+ Diesel	0	0	641	0	0	0	12283	2243	107	0
21	7	Effluent B + Kerosene	0	0	5192	0	0	0	42281	10385	0	3709
21	8	Control	0	0	344	0	0	0	2132	1032	0	0
21	10	Effluent B + Kerosene	0	0	7662	0	0	0	41595	20797	0	0
21	11	Control	0	0	532	0	0	0	24761	1864	0	0
21	13	Effluent A	0	0	0	0	0	0	623	249	0	0
21	15	Effluent A	0	0	0	0	0	0	3697	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	18454	527	0	0
49	3	Control	0	0	0	0	0	0	1725	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	24461	263	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	1361	510	0	0
49	8	Control	0	0	240	0	0	0	7904	240	0	0
49	10	Effluent B + Kerosene	2504	0	626	0	0	0	90141	4382	626	626
49	11	Control	0	0	0	0	0	0	168419	1947	0	0
49	13	Effluent A	0	0	269	269	0	0	4036	0	0	0
49	15	Effluent A	0	0	0	0	0	0	3198	0	0	0

Sampling days	stream number	substance	Achnanthidium subatomus	Achnanthidium subhudsonis	Adlafia Moser	Adlafia sp.	Amphora copulata	Amphora montana	Amphora ovalis	Amphora pediculus	Amphora veneta	Caloneis bacillum	Caloneis molaris	Caloneis schumanniana
0	2	Effluent C+ Diesel	0	0	0	0	0	0	0	34	0	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	0	0	0	0	0	2749	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	293	0	0	0	0
14	4	Effluent C+ Diesel	0	0	0	0	0	0	0	2518	0	0	0	0
14	7	Effluent B + Kerosene	1334	0	0	0	0	0	0	4001	0	0	0	0
14	8	Control	0	0	0	0	106	0	0	1170	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	0	193	0	0	579	193	193	0	0
14	11	Control	0	0	0	0	0	0	0	3879	0	970	0	0
14	13	Effluent A	0	0	0	0	0	0	179	1252	0	179	0	0
14	15	Effluent A	0	0	0	0	57	0	0	0	0	0	57	0
21	2	Effluent C+ Diesel	545	363	0	0	0	0	0	1634	0	0	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	143	0	0
21	4	Effluent C+ Diesel	427	0	0	0	0	0	0	427	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	742	0	0	0	3709	0	0	0	0
21	8	Control	69	0	0	0	0	0	0	344	0	0	0	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	0	7662	0	0	0	0
21	11	Control	0	133	133	0	0	133	0	399	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	249	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	1582	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	263	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	510	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	240	0	0	0	0
49	10	Effluent B + Kerosene	626	0	0	0	626	0	0	1878	0	0	0	626
49	11	Control	974	0	0	0	0	0	0	16550	0	0	0	0
49	13	Effluent A	807	0	0	0	0	0	0	1076	0	269	0	0
49	15	Effluent A	0	640	0	0	0	0	0	640	0	0	0	0

Sampling days	stream number	substance	Cocconeis	Cocconeis euglyptoides	Cocconeis pediculus	Cocconeis placentula Ehrenberg var.euglypta	Cocconeis placentula Ehrenberg var.lineata	Cocconeis pseudolineata	Craticula molestiformis	Cyclostephanos invisitatus	Cyclotella cyclopuncta	Cyclotella meneghiniana	Cyclotella ocellata
0	2	Effluent C+ Diesel	0	0	102	2543	68	17	0	0	0	0	17
0	3	Control	0	0	188	74586	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	878	75535	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	715	53184	2859	143	0	0	0	0	0
0	8	Control	0	0	1696	130605	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	4605	59539	0	0	0	0	0	0	0
0	11	Control	0	0	5854	80495	0	732	0	0	0	0	0
0	13	Effluent A	0	0	1148	90426	0	0	0	0	0	0	0
0	15	Effluent A	0	0	1388	98795	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	3055	6110	305	1527	0	0	0	0	0
14	3	Control	0	0	440	13531	0	0	0	0	0	49	0
14	4	Effluent C+ Diesel	0	0	839	7554	0	839	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	1334	16003	4001	4001	0	0	0	4001	0
14	8	Control	319	106	319	7123	0	532	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	386	5786	193	1157	0	0	0	0	0
14	11	Control	0	0	1939	17453	0	970	0	0	0	0	0
14	13	Effluent A	0	0	715	2682	2325	715	0	0	0	179	0
14	15	Effluent A	0	0	401	10209	0	172	0	0	0	0	0
21	2	Effluent C+ Diesel	0	0	908	3632	1089	1089	0	0	0	0	0
21	3	Control	0	0	1142	51816	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	107	3632	214	214	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	2225	31896	742	3709	0	0	0	0	0
21	8	Control	0	0	481	16092	0	206	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	1095	17514	3284	1095	1095	0	0	0	0
21	11	Control	0	266	1065	7455	0	0	133	0	133	0	0
21	13	Effluent A	0	125	3366	44757	0	0	0	0	0	0	0
21	15	Effluent A	0	0	1008	54109	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	527	527	72496	0	0	0	0	0	0	0
49	3	Control	0	1150	1150	220858	0	2876	0	0	0	0	0
49	4	Effluent C+ Diesel	0	1315	526	65756	526	789	0	0	0	0	0
49	7	Effluent B + Kerosene	0	3573	1021	51220	340	2042	0	0	0	0	0
49	8	Control	0	240	958	79758	479	1437	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	1878	40063	0	0	0	0	0	0	0
49	11	Control	0	0	3894	190810	0	15576	0	0	0	0	0
49	13	Effluent A	0	0	3767	79369	0	5112	0	0	0	0	0
49	15	Effluent A	0	0	1279	262879	640	5756	0	0	0	0	0

Sampling days	stream number	substance	Cymbella excisa	Cymbella excisa	Cymbella subleptoceros	Cymbella tumida	Cymbopleura kuelbsii	Denticula tenuis	Diadesmis confervacea	Diadesmis contenta	Diatoma ehrenbergii	Diatoma vulgaris	Discostella pseudostelligera
0	2	Effluent C+ Diesel	17	0	0	17	0	0	0	0	0	85	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	678	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	732	0	0	0	0	0	0	732	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	574	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	1222	9470	0
14	3	Control	0	0	0	0	0	0	0	0	0	195	0
14	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	2518	12590	0
14	7	Effluent B + Kerosene	0	0	1334	0	0	0	0	0	0	9335	0
14	8	Control	0	0	0	0	0	106	0	0	106	425	0
14	10	Effluent B + Kerosene	193	0	0	0	0	0	0	0	0	386	0
14	11	Control	0	0	970	0	0	0	0	0	0	1939	0
14	13	Effluent A	0	0	179	0	0	0	0	0	0	2325	179
14	15	Effluent A	0	0	57	57	0	0	0	0	0	57	0
21	2	Effluent C+ Diesel	182	0	182	0	182	0	0	0	182	1634	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	107	0	0	0	0	107	214	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	742
21	8	Control	0	0	0	0	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	0	1095	0	0	0	0	0	0	5473	0
21	11	Control	0	133	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	168	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	1252	0
49	11	Control	0	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	269	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

Sampling days	stream number	substance	Discostella stelligera	Discostella woltereckii	Encyonema minutum	Encyonema prostratum	Encyonema silesiacum	Encyonema species	Encyonema ventricosum	Encyonema vulgare	Eolimna comperei	Eolimna minima	Eolimna subminuscula
0	2	Effluent C+ Diesel	0	0	0	17	17	0	0	0	0	322	34
0	3	Control	0	0	0	0	0	0	0	0	0	188	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	220	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	339	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	732	0	0	0	0	11708	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	574	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	0	0	2138	0	0	0	0	7332	305
14	3	Control	0	0	0	0	49	0	0	0	0	147	98
14	4	Effluent C+ Diesel	0	839	839	0	2518	0	0	0	0	9233	839
14	7	Effluent B + Kerosene	0	0	0	0	2667	0	0	0	0	6668	0
14	8	Control	0	0	106	0	425	0	0	0	0	3615	0
14	10	Effluent B + Kerosene	0	0	0	0	386	0	0	0	0	4822	193
14	11	Control	0	0	970	0	0	0	0	0	0	38785	0
14	13	Effluent A	0	0	536	0	1252	0	0	0	0	8047	0
14	15	Effluent A	0	0	0	0	0	0	0	0	0	688	0
21	2	Effluent C+ Diesel	0	0	908	0	1453	0	0	0	0	3632	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	214	0	214	0	0	0	0	2884	0
21	7	Effluent B + Kerosene	0	0	0	0	1484	0	0	0	0	11868	742
21	8	Control	0	0	0	0	138	0	0	0	0	344	0
21	10	Effluent B + Kerosene	0	0	2189	0	3284	0	3284	0	0	21892	1095
21	11	Control	0	0	0	0	266	0	0	0	0	4926	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	168	0	0	0	0	0	0	504	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	1318	0
49	3	Control	0	0	0	0	0	0	0	0	0	575	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	526	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	510	0
49	8	Control	0	0	0	0	0	0	0	0	0	1916	0
49	10	Effluent B + Kerosene	0	0	3130	0	0	0	0	0	0	10642	0
49	11	Control	0	0	0	0	0	0	0	0	0	7788	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	4574	269
49	15	Effluent A	0	0	0	0	0	0	0	0	0	1279	0

Sampling days	stream number	substance	Epithemia adnata	Fallacia lenzi	Fallacia subhamulata	Fistulifera saprophila	Fragilaria arcus	Fragilaria capucina	Fragilaria capucina	Frustulia rhomboides	Geissleria acceptata	Geissleria decussis	Gomphonema angustatum
0	2	Effluent C+ Diesel	0	0	0	0	0	0	17	17	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	287	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	611	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0	49
14	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	1334	0	0
14	8	Control	0	0	213	0	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	193	0	0	0	0	0	0	0	0
14	11	Control	0	0	970	0	0	0	0	0	0	0	0
14	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	2	Effluent C+ Diesel	0	0	0	182	0	0	0	0	363	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	214	0	0	107	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	742	0	0	742	0	0	0	0
21	8	Control	69	0	0	0	0	69	138	0	0	0	0
21	10	Effluent B + Kerosene	1095	0	1095	1095	0	0	1095	0	0	0	0
21	11	Control	0	0	0	0	0	0	0	0	133	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	125	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	170	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	1252	0	0	0	0	0	0	0	0
49	11	Control	0	0	0	0	974	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

Sampling days	stream number	substance	Gomphonema angustivalva	Gomphonema auritum	Gomphonema exilissimum	Gomphonema gracile	Gomphonema hebridense	Gomphonema lagenula	Gomphonema minutum	Gomphonema olivaceum	Gomphonema parvulum	Gomphonema parvulum
0	2	Effluent C+ Diesel	0	0	0	0	0	0	102	0	34	0
0	3	Control	0	0	0	0	0	0	0	0	188	0
0	4	Effluent C+ Diesel	0	0	0	0	220	0	659	220	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	143	0
0	8	Control	0	0	0	0	0	0	339	0	1018	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	329	0	0	0
0	11	Control	0	0	0	1464	0	0	5122	0	3659	732
0	13	Effluent A	0	0	0	0	0	0	5167	287	574	0
0	15	Effluent A	0	0	0	0	0	0	3053	278	833	0
14	2	Effluent C+ Diesel	0	0	0	0	0	0	2749	916	1222	0
14	3	Control	0	0	0	0	0	0	293	0	1026	0
14	4	Effluent C+ Diesel	0	0	0	0	0	0	7554	5036	20984	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	10669	1334	14669	2667
14	8	Control	0	0	0	0	0	0	1170	425	1382	0
14	10	Effluent B + Kerosene	0	0	0	0	0	0	1157	1157	1543	193
14	11	Control	0	0	0	0	0	0	1939	1939	3879	0
14	13	Effluent A	179	0	0	0	0	0	4292	358	179	0
14	15	Effluent A	0	0	0	0	0	0	2810	115	1434	0
21	2	Effluent C+ Diesel	0	0	0	0	0	0	1634	0	726	0
21	3	Control	0	0	0	0	0	0	856	285	2141	0
21	4	Effluent C+ Diesel	0	0	0	0	0	0	961	0	2457	107
21	7	Effluent B + Kerosene	0	0	0	0	0	0	11127	742	14835	5192
21	8	Control	0	69	0	0	0	0	1100	0	894	0
21	10	Effluent B + Kerosene	0	0	0	0	0	0	3284	3284	14230	0
21	11	Control	0	0	0	0	0	0	2263	0	2396	0
21	13	Effluent A	0	0	0	0	0	0	249	0	0	0
21	15	Effluent A	0	0	0	0	0	0	3361	168	1008	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	3954	0	2900	0
49	3	Control	0	0	0	0	0	0	575	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	3419	526	3945	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	1191	340	1191	0
49	8	Control	0	0	0	0	0	0	240	240	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	4382	0	5008	626
49	11	Control	0	0	0	0	0	0	5841	0	974	0
49	13	Effluent A	0	0	0	0	0	0	1614	1345	538	0
49	15	Effluent A	0	0	0	0	0	0	1919	0	640	0

Sampling days	stream number	substance	Gomphonema pseudoaugur	Gomphonema pumilum	Gomphonema pumilum	Gomphonema pumilum	Gomphonema rhombicum	Gomphonema subclavatum	Gomphonema tergestinum	Gomphonema truncatum	Gomphonema utae
0	2	Effluent C+ Diesel	0	0	0	0	0	17	0	34	0
0	3	Control	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	339	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	164	0	0	0
0	11	Control	0	0	0	0	0	2195	0	1464	0
0	13	Effluent A	0	0	0	0	0	287	0	287	0
0	15	Effluent A	0	0	0	0	0	833	0	555	0
14	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	98	0	0	0
14	4	Effluent C+ Diesel	0	0	839	0	0	1679	0	4197	0
14	7	Effluent B + Kerosene	0	0	0	0	0	5334	0	2667	0
14	8	Control	0	0	0	0	0	0	0	213	0
14	10	Effluent B + Kerosene	0	0	0	0	0	193	0	579	0
14	11	Control	0	0	0	0	0	1939	0	970	0
14	13	Effluent A	0	0	358	0	0	0	0	894	0
14	15	Effluent A	0	0	0	0	0	287	115	631	0
21	2	Effluent C+ Diesel	0	0	908	0	0	0	182	182	0
21	3	Control	0	143	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	10385	0	1484	0
21	8	Control	0	0	138	0	0	0	0	138	0
21	10	Effluent B + Kerosene	0	0	1095	0	0	0	1095	0	0
21	11	Control	0	0	0	0	0	0	0	666	0
21	13	Effluent A	0	0	0	125	0	0	0	125	0
21	15	Effluent A	0	0	0	0	0	1008	0	1008	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	263	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	170	0	340	0
49	8	Control	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0
49	11	Control	0	1947	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0

Sampling days	stream number	substance	Gyrosigma attenuatum	Gyrosigma nodiferum	Hippodonta capitata	Karayevia oblongella	Luticola goeppertiana	Mayamaea atomus	Mayamaea atomus	Melosira varians	Navicula antonii	Navicula associata
0	2	Effluent C+ Diesel	0	0	0	0	0	0	0	102	102	0
0	3	Control	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	1464	3659	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	278	0
14	2	Effluent C+ Diesel	0	0	0	0	305	0	0	5193	1222	0
14	3	Control	0	0	0	0	0	0	0	98	98	0
14	4	Effluent C+ Diesel	0	0	0	0	0	0	839	1679	6715	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	4001	9335	0
14	8	Control	0	0	0	0	0	0	0	319	744	0
14	10	Effluent B + Kerosene	0	0	0	0	0	193	0	579	9065	1736
14	11	Control	0	0	0	0	0	0	0	0	2909	0
14	13	Effluent A	0	0	179	0	0	0	715	1252	1609	179
14	15	Effluent A	0	0	0	0	0	0	0	0	172	0
21	2	Effluent C+ Diesel	0	0	0	0	0	0	182	908	2724	0
21	3	Control	0	0	0	0	0	0	0	0	143	0
21	4	Effluent C+ Diesel	0	0	0	0	0	0	107	320	2884	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	742	742	12610	0
21	8	Control	0	0	69	0	0	0	0	206	344	0
21	10	Effluent B + Kerosene	0	1095	0	0	0	0	2189	3284	28460	2189
21	11	Control	0	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	1845	0
49	3	Control	0	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	170	510	681	0
49	8	Control	0	0	0	0	0	0	240	240	719	0
49	10	Effluent B + Kerosene	0	0	0	0	0	0	0	1878	7512	0
49	11	Control	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	269	0
49	15	Effluent A	0	0	0	0	0	0	0	0	640	0

Sampling days	stream number	substance	Navicula capitatoradiata	Navicula catalanogermanica	Navicula caterva	Navicula concentrica	Navicula cryptocephala	Navicula cryptotenella	Navicula exilis	Navicula germainii	Navicula gregaria	Navicula lanceolata
0	2	Effluent C+ Diesel	17	0	0	0	34	85	0	17	34	0
0	3	Control	0	0	0	0	0	377	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	329	0	0	0	0	0	0	0	0	0
0	11	Control	732	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	278	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	1833	0	0	305	0	2749	0	0	916	0
14	3	Control	0	0	0	0	49	195	0	0	0	0
14	4	Effluent C+ Diesel	3357	0	0	0	0	4197	0	0	0	1679
14	7	Effluent B + Kerosene	5334	0	0	0	0	2667	0	0	0	0
14	8	Control	106	0	0	0	0	213	0	0	0	0
14	10	Effluent B + Kerosene	1350	0	0	0	0	1929	0	0	386	0
14	11	Control	0	0	0	0	0	0	0	0	0	0
14	13	Effluent A	536	0	179	0	0	1073	0	179	179	0
14	15	Effluent A	57	0	0	0	0	229	0	57	0	0
21	2	Effluent C+ Diesel	2542	0	0	0	182	1089	0	363	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	427	0	0	0	0	214	0	0	0	0
21	7	Effluent B + Kerosene	2225	742	0	0	742	2967	0	0	0	0
21	8	Control	481	0	0	0	0	69	0	0	0	0
21	10	Effluent B + Kerosene	3284	0	1095	0	1095	5473	0	1095	1095	0
21	11	Control	133	0	0	0	0	932	0	0	266	0
21	13	Effluent A	0	0	0	0	0	125	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	264	0
49	3	Control	0	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	0	0	0	240	0	0	0	0
49	10	Effluent B + Kerosene	626	0	0	0	0	0	0	0	0	0
49	11	Control	974	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	538	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0

Sampling days	stream number	substance	Navicula moskalii	Navicula novaesiberica	Navicula reichardtiana	Navicula rhynchocephala	Navicula rostellata	Navicula sp.	Navicula tridentula	Navicula tripunctata	Navicula trivialis	Navicula upsaliensis	Navicula veneta
0	2	Effluent C+ Diesel	0	17	0	0	0	0	0	17	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	611	0	305	0	0	1222	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0	0
14	4	Effluent C+ Diesel	0	0	0	0	0	0	0	839	0	0	0
14	7	Effluent B + Kerosene	0	0	0	1334	0	0	0	1334	0	0	0
14	8	Control	0	0	106	0	0	0	0	319	0	0	106
14	10	Effluent B + Kerosene	0	0	579	0	0	0	0	386	0	193	0
14	11	Control	0	0	970	0	0	0	0	0	0	0	0
14	13	Effluent A	0	179	179	0	0	0	0	1073	0	0	0
14	15	Effluent A	0	0	115	0	0	0	0	0	0	0	0
21	2	Effluent C+ Diesel	182	0	182	0	182	0	0	545	182	0	0
21	3	Control	0	0	0	0	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	0	0	0	0	0	214	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	0	0	742	0	0	0
21	8	Control	0	0	69	0	0	0	0	138	0	0	0
21	10	Effluent B + Kerosene	0	1095	4378	0	0	0	0	3284	0	0	0
21	11	Control	0	0	0	0	0	0	133	0	133	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	263	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	626	0	0	0	0	1878	0	0	0
49	11	Control	0	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	640	0	0	0	0	0	0	0

Sampling days	stream number	substance	Navicula viridula	Navicula viridulacalcis ssp. neomundana	Nitzschia amphibia	Nitzschia dissipata	Nitzschia fonticola	Nitzschia inconspicua	Nitzschia linearis	Nitzschia palea	Nitzschia paleacea	Nitzschia sociabilis	Nitzschia solgensis
0	2	Effluent C+ Diesel	0	0	0	17	542	0	0	0	339	34	0
0	3	Control	0	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	659	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	5122	0	0	0	1464	0	0
0	13	Effluent A	0	0	0	287	2009	0	0	0	287	0	0
0	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	0	916	27494	0	0	0	3360	0	0
14	3	Control	0	0	0	0	782	0	0	49	0	0	0
14	4	Effluent C+ Diesel	0	0	0	4197	89810	0	0	839	47843	0	0
14	7	Effluent B + Kerosene	0	0	0	1334	248044	0	0	5334	97351	0	0
14	8	Control	106	0	0	319	7761	0	0	319	2020	0	106
14	10	Effluent B + Kerosene	0	0	579	386	22951	0	0	0	2314	0	0
14	11	Control	0	0	970	0	7757	0	0	0	1939	0	0
14	13	Effluent A	0	0	0	0	11802	0	179	0	179	0	0
14	15	Effluent A	0	0	57	0	1090	0	0	0	0	0	0
21	2	Effluent C+ Diesel	0	0	182	726	16706	0	0	182	2361	0	0
21	3	Control	0	0	0	0	143	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	0	534	7797	0	320	854	748	0	0
21	7	Effluent B + Kerosene	0	0	0	2967	100881	0	0	0	4451	0	0
21	8	Control	0	0	69	344	1994	0	69	0	138	0	0
21	10	Effluent B + Kerosene	0	0	1095	2189	167475	0	0	3284	27365	0	0
21	11	Control	0	0	266	133	3594	0	0	0	399	0	0
21	13	Effluent A	0	0	0	0	249	0	0	0	0	0	0
21	15	Effluent A	0	0	0	168	336	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	264	1582	0	527	264	791	264	0
49	3	Control	0	0	0	0	1150	0	0	575	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	263	0	0	526	526	789	0
49	7	Effluent B + Kerosene	0	0	0	0	340	0	0	0	340	0	0
49	8	Control	0	0	0	0	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	626	1878	45071	626	0	626	5008	0	0
49	11	Control	0	0	0	0	974	0	0	0	0	0	0
49	13	Effluent A	0	0	0	807	1345	0	0	269	538	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0	0

Sampling days	stream number	substance	Nitzschia subcurvata	Nitzschia vermicularis	Parlibellus protractoides	Planothidium frequentissimum	Planothidium lanceolatum	Planothidium rostratum	Pseudostaurosira parasitica	Puncticulata radiosa	Reimeria sinuata	Rhoicosphenia abbreviata
0	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	33,90086169
0	3	Control	0	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	1463,539135
0	13	Effluent A	0	0	0	0	0	0	0	0	0	287,0670774
0	15	Effluent A	0	0	0	0	0	0	0	0	0	555,0268958
14	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	3360,38295	305,4893591
14	3	Control	0	0	0	0	0	0	0	0	0	48,84819605
14	4	Effluent C+ Diesel	0	839	0	0	0	0	0	0	1678,687	0
14	7	Effluent B + Kerosene	0	0	1333,572158	0	0	0	0	0	5334,28863	0
14	8	Control	0	0	0	0	0	0	0	0	637,919885	212,6399616
14	10	Effluent B + Kerosene	0	0	0	0	192,8652168	0	192,8652168	0	771,460867	0
14	11	Control	0	0	0	0	0	0	0	0	969,636927	969,6369267
14	13	Effluent A	0	0	0	0	0	0	0	0	894,117846	357,6471386
14	15	Effluent A	0	0	0	0	0	0	0	0	172,059895	0
21	2	Effluent C+ Diesel	0	0	0	363	0	0	0	0	907,908013	0
21	3	Control	0	0	0	0	0	0	0	0	0	428,2328634
21	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	747,678703	320,4337297
21	7	Effluent B + Kerosene	0	0	0	0	742	0	0	0	2225,30671	0
21	8	Control	0	0	0	0	0	0	0	0	206,3021	343,8368328
21	10	Effluent B + Kerosene	0	0	0	1095	0	0	0	0	3283,81534	0
21	11	Control	133	0	0	0	0	0	0	0	266,24811	399,3721654
21	13	Effluent A	0	0	0	0	0	0	0	0	124,6713	249,3425995
21	15	Effluent A	0	0	0	0	0	0	0	0	0	1008,234634
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	575,151735	0
49	4	Effluent C+ Diesel	0	0	0	0	263,0259777	0	0	0	263,025978	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	170,166345	340,3326898
49	8	Control	0	0	0	239,5149262	0	0	0	0	0	239,5149262
49	10	Effluent B + Kerosene	0	0	0	0	625,9809709	0	0	625,980971	1877,94291	625,9809709
49	11	Control	0	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	269,0460606	0	0	0	0	538,092121	269,0460606
49	15	Effluent A	0	0	0	0	0	0	0	0	0	0

Sampling days	stream number	substance	Sellaphora bacillum	Sellaphora pupula	Staurosira construens	Staurosira construens	Staurosira venter	Staurosirella pinnata	Surirella angusta	SURIRELLA	Ulnaria ulna
0	2	Effluent C+ Diesel	0	17	0	0	102	34	0	0	0
0	3	Control	0	0	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0
0	8	Control	0	0	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	0	0	0	164	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	0	0	287	0	0	0	0
0	15	Effluent A	0	278	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	305	0	305	0	305	305	0	0	0
14	3	Control	0	0	0	0	49	0	0	0	0
14	4	Effluent C+ Diesel	0	839	0	0	0	0	0	0	0
14	7	Effluent B + Kerosene	0	0	0	0	0	0	0	0	0
14	8	Control	0	106	0	0	0	0	0	0	0
14	10	Effluent B + Kerosene	0	0	0	193	0	0	0	0	0
14	11	Control	0	0	0	0	0	0	0	0	0
14	13	Effluent A	0	179	0	0	179	0	0	0	0
14	15	Effluent A	0	115	0	0	0	57	0	0	0
21	2	Effluent C+ Diesel	0	363	0	0	182	0	0	0	0
21	3	Control	0	0	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	107	0	0	0	320	0	0	0	0
21	7	Effluent B + Kerosene	0	0	0	0	0	742	0	0	0
21	8	Control	0	69	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	1095	0	0	0	2189	0	0	0	1095
21	11	Control	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
49	3	Control	0	0	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	0	0	0	0	170	0	0
49	8	Control	0	240	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	1252	0	0	0	0	626	0	0
49	11	Control	0	0	0	0	0	0	0	0	0
49	13	Effluent A	0	0	0	0	0	0	0	0	0
49	15	Effluent A	0	0	0	0	0	0	0	0	0

			Abnormal forms									
Sampling days	stream number	substance	Achnanthidium eutrophilum	Achnanthidium pyrenaicum	Cocconeis placentula var.euglypta	Diatoma vulgaris	Eolimna minima	Fragilaria capucina var.vauch eriae	Gomphon ema parvulum	Navicula antonii	Navicula capitatora diata	Nitzschia fonticola
0	2	Effluent C+ Diesel	0	17	51	0	17	0	0	0	0	34
0	3	Control	0	0	188	0	0	0	0	0	0	0
0	4	Effluent C+ Diesel	0	0	659	0	0	0	0	0	0	0
0	7	Effluent B + Kerosene	0	0	286	0	0	0	0	0	0	0
0	8	Control	0	0	2035	0	0	0	0	0	0	0
0	10	Effluent B + Kerosene	0	0	822	0	0	0	0	0	0	0
0	11	Control	0	0	0	0	0	0	0	0	0	0
0	13	Effluent A	0	0	861	0	0	0	0	0	0	0
0	15	Effluent A	0	0	833	0	0	0	0	0	0	0
14	2	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
14	3	Control	0	0	0	0	0	0	0	0	0	0
14	4	Effluent C+ Diesel	0	0	0	0	0	1679	0	0	0	0
14	7	Effluent B + Kerosene	0	1334	2667	0	0	0	0	0	0	8001
14	8	Control	0	106	106	0	0	0	0	0	0	532
14	10	Effluent B + Kerosene	0	0	771	0	0	0	0	0	0	0
14	11	Control	0	0	970	0	970	0	0	0	0	0
14	13	Effluent A	0	715	179	0	358	0	0	0	0	179
14	15	Effluent A	0	0	115	0	0	0	0	0	0	0
21	2	Effluent C+ Diesel	0	363	0	182	182	0	0	0	0	182
21	3	Control	0	0	428	0	0	0	0	0	0	0
21	4	Effluent C+ Diesel	0	0	107	0	0	0	0	0	0	107
21	7	Effluent B + Kerosene	0	742	0	0	0	0	0	0	0	6676
21	8	Control	0	0	0	0	0	0	0	0	0	0
21	10	Effluent B + Kerosene	0	2189	0	1095	1095	0	0	1095	1095	15324
21	11	Control	0	0	0	0	0	0	0	0	0	0
21	13	Effluent A	0	0	0	0	0	0	0	0	125	0
21	15	Effluent A	0	0	0	0	0	0	0	0	0	0
49	2	Effluent C+ Diesel	0	0	264	0	0	0	0	0	0	0
49	3	Control	0	0	4026	0	0	0	0	0	0	0
49	4	Effluent C+ Diesel	0	0	0	0	0	0	0	0	0	0
49	7	Effluent B + Kerosene	0	0	851	0	0	0	0	0	0	0
49	8	Control	0	0	719	0	0	0	0	0	0	0
49	10	Effluent B + Kerosene	0	0	1252	0	2504	0	626	0	0	6260
49	11	Control	0	0	6815	0	0	0	0	0	0	0
49	13	Effluent A	0	0	2960	0	0	0	0	0	0	0
49	15	Effluent A	0	0	1919	0	0	0	0	0	0	0

			Abnormal forms	
Sampling days	**stream number**	**substance**	**Nitzschia paleacea**	**Reimeria sinuata**
0	2	Effluent C+ Diesel	0	0
0	3	Control	0	0
0	4	Effluent C+ Diesel	0	0
0	7	Effluent B + Kerosene	0	0
0	8	Control	0	0
0	10	Effluent B + Kerosene	0	0
0	11	Control	0	0
0	13	Effluent A	0	0
0	15	Effluent A	0	0
14	2	Effluent C+ Diesel	305	0
14	3	Control	0	0
14	4	Effluent C+ Diesel	0	0
14	7	Effluent B + Kerosene	0	0
14	8	Control	319	0
14	10	Effluent B + Kerosene	0	0
14	11	Control	0	0
14	13	Effluent A	179	0
14	15	Effluent A	0	0
21	2	Effluent C+ Diesel	0	0
21	3	Control	0	0
21	4	Effluent C+ Diesel	0	0
21	7	Effluent B + Kerosene	1484	0
21	8	Control	0	0
21	10	Effluent B + Kerosene	0	0
21	11	Control	0	0
21	13	Effluent A	0	0
21	15	Effluent A	0	0
49	2	Effluent C+ Diesel	0	0
49	3	Control	0	0
49	4	Effluent C+ Diesel	0	0
49	7	Effluent B + Kerosene	0	0
49	8	Control	0	0
49	10	Effluent B + Kerosene	1252	626
49	11	Control	0	0
49	13	Effluent A	0	0
49	15	Effluent A	0	0

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