*Coastal Systems Research Group*
*Ecosystem Services Competence Area*

*Final Report for comment*

# Assessment of the inshore subtidal environment in the vicinity of the PetroSA outfall in Vleesbaai: November 2012 survey

**August 2013**

**Prepared for**

The Petroleum Oil and Gas Corporation of South Africa (SOC) Ltd

Herein referred to as "PetroSA"

**Prepared by**

Weerts SP*, Newman BK*, Blair AG and Atkinson LJ**

* Coastal Systems Research Group
Ecosystems Services Competence Area
CSIR
PO Box 17001
Congella 4013
Durban

** South African Environmental Observation Network
Egagasini Node
Private Bag X2
Roggebaai
Cape Town 8012

**Contact Person:**

Steven Weerts
Telephone: (031) 242 2356
Telefax: (031) 261 2509
Email: sweerts@csir.co.za

For bibliographic purposes this report should be cited as:
Weerts SP, Newman BK, Blair AG and Atkinson LJ (2013) Assessment of the inshore subtidal environment in the vicinity of the PetroSA outfall in Vleesbaai: November 2012 survey. CSIR Report CSIR/NRE/CO/ER/2013/0061/C.

# Executive Summary

Wastewater from the PetroSA refinery near Mossel Bay is discharged through a deepwater outfall into the marine environment of Vleesbaai, situated just west of Mossel Bay. The managed discharge of wastewater (effluent) to the marine environment is recognised as an acceptable disposal option from multiple perspectives, including human and environmental health, social acceptability, and economic prudence. The PetroSA effluent nevertheless contains a range of contaminants that have the potential to impair the ecological functioning of the Vleesbaai receiving environment, or pose a risk to the health of human users of this environment, including ammonia, metals and oils. To ensure the integrity of an effluent receiving environment is not unacceptably compromised, the South African government issues effluent discharge licenses that stipulate the conditions under which a discharge is authorised. A common condition is that the effluent discharger must implement and report on an environmental monitoring programme designed to identify the impact, or lack thereof, of the discharge on the ecology of the receiving environment and human health. This report discusses the findings of a survey performed in November 2012 to assess the impact of effluent discharge through the PetroSA outfall on the Vleesbaai receiving environment.

Three components of the Vleesbaai receiving environment were assessed for potential impact resulting from the effluent discharge. These were water quality, sediment quality, and invertebrate communities living within sediment (benthic macrofauna and meiofauna). *In situ* monitoring of water quality and the collection of water, sediment and benthic macrofaunal and meiofaunal community samples took place in November 2012. The monitoring and sample collection was performed at sites situated to the east and west of the outfall diffuser section. A wide suite of parameters were analysed in water samples, and in one effluent sample collected prior to discharge. The grain size composition, total organic content, and concentrations of metals, BTEX and Total Petroleum Hydrocarbons were analysed in sediment samples. The abundance and biomass of macrofauna (>0.5 mm) were recorded, whilst the Nematode/Copepod ratio of meiofauna (>45 µm) was determined for comparison with previous studies.

Compared to other industrial effluent discharges to the marine environment in South Africa, the concentrations of most parameters in the PetroSA effluent sample were low. The pH was relatively high (8.3) compared to effluent from other wastewater treatment facilities. Metal concentrations were low, with most metals actually at concentrations too low to accurately measure in the laboratory. Some BTEX constituents and most Total Petroleum Hydrocarbons were represented in the PetroSA effluent, but at relatively low concentrations. The effluent needed to be diluted 14 times by the receiving water to render it non-toxic to sea urchin gametes. This number of dilutions is low compared to other industrial effluent discharges to the marine environment in South Africa.

The *in situ* water quality data provide no clear evidence for an effluent signal in the receiving environment with the possible exception of turbidity, which was elevated in the upper 3 m of the water column at the diffuser section after effluent discharge. The turbidity of surface water and the turbidity through most of the water column at sites situated to the east of the diffuser section were also generally higher compared to sites situated to the west of the diffuser section. The strongest effluent signals were provided by faecal indicator bacteria and zinc in a surface water sample collected above the diffuser section of the outfall. The values/concentrations of all physical, chemical and biological parameters at all sites measured *in situ* and in water samples were compliant with the South African Water Quality Guidelines for Coastal Marine Waters (Natural Environment) with the exception of turbidity. With regards to the turbidity non-compliance, this is somewhat spurious and in the professional opinion of the scientists that prepared this report, of little ecological significance. BTEX and Total Petroleum Hydrocarbons were either at concentrations too low to accurately measure in the laboratory or at such low concentrations that it is the professional opinion of the scientists that prepared this report that these chemicals are also of little ecological concern.

There is thus no evidence that that the concentrations of chemicals in effluent discharged through the PetroSA outfall are posing a significant risk to marine organisms beyond the zone of initial dilution.

From a textural perspective, the sediment at all but one site is classified as sand. At the latter site, situated furthest west (2000 m) from the outfall, the sediment is classified as muddy-sand. The sediment at this site is anomalous in the context of the broader study area, since mud contributed 49.65% of bulk sediment weight at this site compared to between 3.05 - 9.06% at other sites. At all other sites the dominant grain size class was fine-grained sand (between 71.38 - 83.33% of bulk weight). The grain size composition of sediment was similar to that measured for the 2011 survey. The low mud fraction of sediment at the majority of sites theoretically implies there is a low probability for the accumulation of particle reactive contaminants in sediment from Vleesbaai.

The total organic content of the sediment at all sites fell within the baseline range for the Mossel Bay area. There was thus no evidence that particulate organic matter in effluent discharged through the PetroSA outfall is accumulating in sediment. This was the same conclusion reached for the 2011 survey.

Metal concentrations in sediment collected from the receiving environment were compared to baseline metal concentration models and a baseline cadmium concentration for the Mossel Bay area. The beryllium and cadmium concentrations at single sites were very marginally higher than the highest concentrations expected for uncontaminated sediment. There was thus no evidence that metals in effluent discharged through the PetroSA outfall is accumulating in sediment. This was the same conclusion reached for the 2011 survey.

BTEX and Total Petroleum Hydrocarbons were either at concentrations too low to accurately measure in the laboratory or at such low concentrations that it is the professional opinion of the scientists that prepared this report that these chemicals are of little ecological concern. There is thus no evidence that hydrocarbons in effluent discharged through the PetroSA outfall are accumulating in sediment to concentrations that pose an ecological risk. This was the same conclusion reached for the 2011 survey.

The benthic macrofauna community sampled in Vleesbaai was characteristic of marine environments in the southern Cape region. Univariate analyses of the macrobenthic communities sampled did not provide evidence of any marked impact that might be caused by effluent discharge from the PetroSA outfall. Multivariate analyses indicated significant differences amongst most sites sampled, but also provided no spatial trends that could be interpreted as arising from a pollution impact. There was no significant difference between benthic communities in close proximity to the PetroSA outfall and those some distance away. Similarly meiofauna data gave no indication of a pollution effect. Total abundance of meiofauna has increased in the 2011 and 2012 surveys compared to previous surveys in 2000 and 2002. Furthermore there has been a reduction in Nematode/Copepod ratio to levels that are approaching those reported in 1989. It is not known if the nature and/or volume of the PetroSA effluent, or other potential pollution sources in Vleesbaai have changed over the years, but previous indications of a pollution impact in 2000 and 2002 have not persisted to recent times.

Based on the weight of evidence presented by the various components of the 2012 survey of the PetroSA outfall monitoring programme, there is no evidence that the discharge of effluent is significantly adversely impacting the receiving environment in Vleesbaai. This does not mean there is no impact, but rather that the impact is so small that it is unlikely to pose significant ecological and human health risks.

# Table of Contents

1. Introduction and background ..... 7
2. Brief Description of the Marine Receiving Environment ..... 9
3. Outfall Design and Nature of the Effluent ..... 9
4. Monitoring Programme Components ..... 9
4.1. Water quality ..... 9
4.2. Sediment quality ..... 10
4.3. Benthic invertebrates ..... 11
5. Materials and Methods ..... 12
5.1. Survey sampling design ..... 12
5.2. Field procedures ..... 14
5.2.1. Water quality ..... 14
5.2.2. Sediment quality and benthic invertebrates ..... 14
5.3. Laboratory procedures ..... 16
5.3.1. Effluent and water samples ..... 16
5.3.2. Sediment ..... 17
5.3.3. Benthic invertebrates ..... 18
5.4. Data analysis ..... 18
5.4.1. Sediment quality ..... 18
5.4.2. Benthic invertebrates ..... 20
6. Results and Discussion ..... 21
6.1. Effluent quality and toxicity ..... 21
6.2. Water quality ..... 22
6.3. Sediment quality ..... 34
6.3.1. Sediment grain size ..... 34
6.3.2. Total organic content ..... 35
6.3.3. Metals ..... 36
6.3.4. Hydrocarbons ..... 37
6.4. Benthic invertebrates ..... 41
6.4.1. Macrofauna ..... 41
6.4.2. Meiofauna ..... 44
7. Conclusions ..... 46
8. Recommendations for future monitoring ..... 46
9. Acknowledgements ..... 46
10. References ..... 47
11. Appendices ..... 49
12. Glossary of Terms ..... 55

# Photographic plates

**Plate 1.** Yellow Springs Instrument 6600 multiparameter water quality sonde that was used to measure water quality through the water column.
**Plate 2.** A National Institute of Oceanography bottle was used to collect water samples.
**Plate 3.** Water samples were transferred to containers for analysis in the laboratory.
**Plate 4.** A van Veen grab was used to collect sediment from the seabed.

**Plate 5.** Sediment samples were transferred to containers for analysis in the laboratory.
**Plate 6.** Benthic macrofauna were isolated by passing sediment through a sieve.

## Tables

**Table 1.** Details of sites and the monitoring performed at each site for the November 2012 survey of the PetroSA outfall monitoring programme.
**Table 2.** Physical, chemical and biological parameters analysed in effluent and water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.
**Table 3.** Physical and chemical parameters analysed in sediment samples collected for the 2012 survey of the PetroSA outfall monitoring programme.
**Table 4.** Results for physical, chemical and microbiological parameters analysed in an effluent sample collected for the 2012 survey of the PetroSA outfall monitoring programme.
**Table 5.** Average abundance of meiofauna per 100 ml of sediment collected for the 2012 survey of the PetroSA pipeline monitoring programme.

## Figures

**Figure 1.** Positions of sites where *in situ* measurements were made and water and sediment samples were collected for the 2012 survey of the PetroSA outfall monitoring programme. Aerial view reproduced from Google Earth©.
**Figure 2.** Baseline model for chromium in sediment from the Mossel Bay area. Open symbols are chromium concentrations used to define the baseline model while numbered solid symbols represent four hypothetical scenarios: 1. concentration falls within the baseline model upper and lower prediction limits (dashed lines) and is not enriched; 2, 3 and 4. concentrations exceed baseline model upper prediction limit and reflect various levels of enrichment that can broadly be defined from low (2) through to high (4). Situations 3 and 4 would be interpreted as reflecting enrichment through contamination with a high level of confidence.
**Figure 3.** *In situ* temperature profiles for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 4.** *In situ* surface and bottom water temperature for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 5.** *In situ* salinity profiles for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 6.** *In situ* surface and bottom water salinity for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 7.** *In situ* pH profiles for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 8.** *In situ* surface and bottom water pH for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 9.** *In situ* dissolved oxygen profiles for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 10.** *In situ* surface and bottom water dissolved oxygen concentration and saturation for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 11.** *In situ* turbidity profiles for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 12.** *In situ* surface and bottom water turbidity for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 13.** Faecal indicator bacteria colony forming unit counts in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 14.** pH of discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.
**Figure 15.** Total suspended solids in discrete surface water samples collected for the 2012 survey of

the PetroSA outfall monitoring programme.

**Figure 16.** Turbidity of discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

**Figure 17.** Ammonia concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

**Figure 18.** Fluoride concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

**Figure 19.** Metal concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

**Figure 20.** Total Petroleum Hydrocarbon concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

**Figure 21.** Ternary plot illustrating the proportional contribution of gravel, sand and mud to bulk sediment in the study area.

**Figure 22.** Baseline model for total organic content in sediment from the Mossel Bay area, with the total organic content in sediment collected for the 2012 survey of the PetroSA monitoring programme superimposed.

**Figure 23.** Aluminium normalised baseline models for metals in sediment in the Mossel Bay area, with metal concentrations in sediment collected for the 2012 survey of the PetroSA monitoring programme superimposed.

**Figure 24.** Total Petroleum Hydrocarbon concentrations in sediment collected for the 2012 survey of the PetroSA outfall monitoring programme.

**Figure 25.** Indices of macrobenthic community structure for the 2012 survey of the PetroSA outfalls monitoring programme.

**Figure 26.** Dendrogram (top) and MDS ordination (bottom) of macrobenthic samples (abundance) taken from sampling sites at the PetroSA outfall in Vleesbaai in 2012. Test and Control stations indicated.

# 1. Introduction and background

Wastewater from the PetroSA refinery near Mossel Bay is discharged through a deepwater outfall into the marine environment of Vleesbaai, situated just west of Mossel Bay (Figure 1). The managed discharge of wastewater (effluent) to the marine environment is recognised as an acceptable disposal option from multiple perspectives, including human and environmental health, social acceptability, and economic prudence. The PetroSA effluent nevertheless contains a range of contaminants, including ammonia, metals and oils, which have the potential to impair the ecological functioning of the Vleesbaai marine environment and to pose a risk to the health of human users of this environment. Whether the discharge of effluent impairs the ecological functioning of a receiving environment or poses a human health risk depends on the assimilative capacity of the receiving environment, that is, its capacity to receive effluent or toxic materials without deleterious effects to aquatic life or humans that live in or use the water. The assimilative capacity differs depending on the nature of the effluent discharged and the characteristics of the receiving environment in terms of its ability to dilute, disperse and degrade contaminants. Not surprisingly, a voluminous and high-energy marine environment has a higher assimilative capacity compared to smaller volume sheltered waters, such as estuaries. Of importance in the context of assimilative capacity is the volume of effluent discharged. While the concentrations of contaminants in an effluent might be low and elicit no acute toxic effects to aquatic life, the persistent introduction of a large volume of effluent may introduce such a high load of contaminants over time that this overwhelms the assimilative capacity of the receiving environment in the long-term and leads to chronic toxic effects.

Prior to the development and first use of the outfall in 1991/1992, PetroSA commissioned CSIR to conduct environmental baseline studies in Vleesbaai. These were performed in 1986 and 1989 (CSIR 1989) and focussed on beach and subtidal meiofaunal communities, surf-zone nutrient and sulphate concentrations, and metal concentrations in intertidal mussels and oysters. In 2000 and 2002, PetroSA commissioned the Centre of Marine Studies (CMS) to determine whether effluent discharge through the outfall was adversely affecting the receiving environment (CMS 2001, CMS 2003), through a comparison with findings from the earlier baseline studies. The study performed in 2000 (CMS 2001) analysed meiofaunal communities (retained on a 150 µm sieve) at four shoreline sites and three subtidal sites. A modified sampling design was employed in 2002 (CMS 2003), where 10 subtidal sites were assessed for meiofaunal communities (retained on a 63 µm sieve) and sediment grain size composition. The latter studies (CMS 2001, CMS 2003) detected considerably fewer meiofauna than the baseline surveys conducted in 1986 and 1989. However, the different sampling protocol and data analysis methods used in each of these studies precludes any meaningful interpretation or comparisons over time of the findings (CMS 2001, CMS 2003). In 2011, PetroSA expressed the intention to develop and implement a standardised, long-term environmental monitoring program to assess the impact of effluent discharge through the outfall on the Vleesbaai receiving environment.

To ensure that the integrity of a receiving environment is not unacceptably compromised by the discharged effluent, the South African government issues effluent discharge licenses[1] that stipulate the conditions under which a discharge is authorised. One such condition is that the effluent discharger must implement and report on an environmental monitoring programme designed to identify the impact, or lack thereof, of the discharge on the ecology of the receiving environment and human health. In fulfilment of this condition, PetroSA commissioned the South African Environmental Observation Network (SAEON) with expertise provided by the Council for Scientific and Industrial Research (CSIR), in 2011 to design, initiate

[1] In the past, the Department of Water Affairs was the government agency mandated with the control of effluent discharges to all surface waters of South Africa in terms of the National Water Act (Act 36 of 1998). With the promulgation of the National Environmental Management: Integrated Coastal Management Act (Act 24 of 2008), the responsibility of regulating effluent discharges to the marine environment transferred to the Department of Environmental Affairs. Therefore, in future licensing of marine effluent discharges will become the responsibility of Department of Environmental Affairs under the Integrated Coastal Management Act. However, the issuing of effluent discharge licenses for freshwater receiving waters remains the responsibility of the Department of Water Affairs under the National Water Act.

and report on such a monitoring programme for the Vleesbaai receiving environment. This report discusses the findings of the second annual survey conducted in November 2012 to assess the impact of effluent discharge through the PetroSA outfall on the Vleesbaai receiving environment. This report provides PetroSA with strategic information for managing the effluent discharge and to inform the public on the ecological status of the Vleesbaai receiving environment. Furthermore, this report demonstrates PetroSAs compliance with conditions of authorisation for effluent discharge in terms of environmental impact monitoring and reporting. Although this report deals with technical issues, where possible these are discussed and presented in a simplified manner for the benefit of non-specialist audiences. A glossary of terms has also been included for this purpose, and wherever possible the use of acronyms is avoided.


**Figure 1.**Aerial view of the study area showing the position of Vleesbaai relative to Mossel Bay and the positions of sites in Vleesbaai where *in situ* water quality measurements were made and water and sediment samples were collected for the 2012 survey of the PetroSA outfall monitoring programme. The red letters denote the position of the outfall diffuser section. Aerial view reproduced from Google Earth©.

# 2. Brief Description of the Marine Receiving Environment

Vleesbaai (the receiving environment) is situated to the immediate west of Mossel Bay (Figure 1), in the warm-temperate Agulhas marine ecoregion of the South African coastline (Sink et al. 2012). Although the Agulhas Current is the dominant oceanographic feature of this coastline, the core of this current is situated some distance offshore and the local oceanography is undoubtedly influenced more by localised wind driven flows. Upwelling is a significant feature of this coastline, wherein cold nutrient rich water is welled up from deeper areas to replace surface water advected offshore by strong winds.

Recent oceanographic monitoring performed in Mossel Bay as a specialist study for an Environmental Impact Assessment (EIA) for a seawater desalination plant (Aurecon 2011) provides some perspective on the oceanography of the area, although not in particular to Vleesbaai. The average current speeds experienced in Mossel Bay are lower than those along other parts of the South African coastline, ranging from 0.0565 $m.s^{-1}$ at a depth of 1.1 m to 0.0390 $m.s^{-1}$ at 7.1 m depth. The general current direction is north to south. The bay often experiences current reversals (bottom current is different to the surface current). The average temperature recorded during the EIA study period (31 July - 7 September 2010) was 15.39$^{o}$C, with a mean salinity of 35.10 (Aurecon 2011).

# 3. Outfall Design and Nature of the Effluent

The PetroSA outfall extends about 1.3 km off the Vleesbaai shoreline. Effluent is discharged at a depth of about 22 m through two diffusers. A condition of the license authorising the discharge of effluent is that PetroSA must monitor various physical, chemical and biological characteristics of the effluent on a daily basis prior to discharge, and must record the volume of effluent discharged each month. The primary purpose of the physical, chemical and biological analysis of the effluent is to determine whether the parameter values/concentrations are compliant with stipulated license limits. Effluent samples are collected by PetroSA staff and analysed in-house. Data resulting from this monitoring for the period October, November and December 2010 was shared with SAEON and CSIR scientists and is summarised in the previous report in this series (Weerts et al. 2012).

# 4. Monitoring Programme Components

The impact of effluent discharge on a receiving environment can be evaluated by measuring for the presence of, and spatial trends for certain physical and chemical parameters that provide a tracer (or indicator) of the effluent in water, sediment and/or biological tissue. An indicator could, for example, be the concentration of ammonia in the water column or a metal in sediment. The impact can also be evaluated by comparing the structure and composition of benthic invertebrate communities near an outfall diffuser section with communities situated beyond the influence of the discharge.

Various physical, chemical and biological indicators of environmental condition of the marine environment in the vicinity of the PetroSA outfall were analysed for the 2011 survey (Weerts et al. 2012) and for the 2012 survey (this report). Results are used in a weight of evidence approach to reach a conclusion on the impact of the discharge on the Vleesbaai receiving environment. A brief rationale and frame of reference for the components of the monitoring programme and associated indicators is provided below.

## 4.1. Water quality

Water quality monitoring in the vicinity of effluent outfalls serves three main purposes. First, the measurements can be used to track the dispersion of effluent in the receiving environment. Second, the measurements can be used to determine whether the outfall is meeting design specifications and license conditions. These typically require that water quality at the margin of the zone of initial dilution must be compliant with water quality guidelines (or other defined targets) for the protection of ecological and human health. Third, measurements can be used to assess the potential risk posed by contaminants to

aquatic organisms and humans in receiving environments inside and outside the zone of initial dilution. This is achieved by comparing water quality indicator values and concentrations to water quality guidelines that are in place to protect ecological and human health.

PetroSA conducts water quality sampling at and surrounding the outfall diffuser on a quarterly basis each year with samples being analysed in-house. PetroSA also regularly monitors the effluent prior to discharge to ensure various parameter concentrations are within the specified license limits (see Weerts et al. 2012 for details).

The physical, chemical and biological characteristics of the water column in marine ecosystems are naturally highly variable, due to variability of currents and other forms of water column mixing. Periodic large-scale events influencing the marine environment contribute further to challenges in interpreting any anomalous measurements being as a result of effluent discharge or simply as a result of the large-scale event. The nature of the effluent discharged from the PetroSA outfall is also variable in its composition (Weerts et al. 2012), further adding to the complexity of assessing the impact thereof on the receiving environment. It is, therefore, emphasised that for effective water column monitoring, sampling should occur as frequently as is feasible (i.e. monthly to bi-monthly).

During the 2012 survey of the PetroSA outfall monitoring programme a suite of physical, chemical and biological parameters were measured in effluent prior to discharge and in receiving environment water samples, to determine whether parameters present at high values/concentrations in the effluent were also present at high values/concentrations in the receiving environment. The main purpose of monitoring the receiving environment was to track the dispersal of the effluent and to determine whether water quality beyond the margin of the zone of initial dilution was compliant with the South African Water Quality Guidelines for Coastal Marine Waters (Natural Environment) (DWAF 1995). The zone of initial dilution is a three-dimensional zone of intense mixing and dilution around the diffuser section of an outfall within which water quality can reasonably be expected to be compromised on a regular basis.

## 4.2. Sediment quality

A major focus of the 2012 survey of the PetroSA outfall monitoring programme was on the benthic environment. This is because sediment is the predominant sink for many contaminants that are introduced to surface waters. Many contaminants have a low water solubility and are particle reactive. Once introduced in solution to estuarine and marine waters they rapidly adsorb onto fine-grained suspended sediment and organic matter. In this way the contaminants are ‘scavenged’ from the water column through flocculation, coagulation and sedimentation. Consequently, the concentrations of particle-reactive contaminants in sediment and at the sediment water interface usually exceed concentrations in the overlying water column by several orders of magnitude. With continued input and limited sediment redistribution, contaminants can accumulate in sediment to such high concentrations that they directly and indirectly adversely affect benthic and epibenthic organisms (Chapman 1989, Hornberger et al. 2000, Fleeger et al. 2003, Thompson and Lowe 2004).

In addition to environmental concerns, there are pragmatic reasons for monitoring contaminant concentrations in sediment rather than in the water column. The typically higher concentrations of contaminants in sediment compared to the water column make detection and measurement in the laboratory easier. The typically low and highly variable concentrations of contaminants in the water column, due to differences in flow (e.g. currents) and variable anthropogenic inputs, mean that the water column only provides a snapshot of contamination problems. In contrast, the analysis of contaminant concentrations in sediment provides a more realistic, spatially and temporally integrated measure of possible contamination.

The primary focus of the sediment quality component of the 2012 survey of the PetroSA outfall monitoring programme was to determine whether sediment samples collected at various distances from the outfall were contaminated by particulate organic matter, metals and hydrocarbons. If these contaminants were detected, analysis was conducted to determine the likelihood that the contaminants were derived from the PetroSA effluent and whether their concentrations pose an ecological health risk. The grain size composition of the sediment was also analysed since this provides important information for interpreting chemical concentrations in sediment.

## 4.3. Benthic invertebrates

An important concern in any situation where effluent is discharged to a receiving environment is whether the ecology of the receiving environment is being unacceptably compromised. Effluent discharge can impact the ecology of the receiving environment in numerous ways, including changes in water column primary productivity, changes in benthic invertebrate community structure and composition, and the accumulation of contaminants in the tissue of fish and shellfish, which not only affects the health of these organisms but may also affect the health of animals and humans that consume them. Chemical analysis of sediment only provides an estimate of the ecological effects of contaminants in the sediment, by, for example, comparison of the concentrations to sediment quality guidelines. Elevated contaminant concentrations in sediment do not necessarily mean the contaminants are exerting an adverse ecological effect. This is because the contaminants can only exert an adverse effect if they are in a bio-available form (i.e. a form that can cross biological membranes). The monitoring of biological communities is important in this context since it provides a direct measure of contaminant effects (or contaminant bioavailability). During the 2012 survey of the PetroSA outfall monitoring programme, the ecological impact of effluent discharge was evaluated by comparing the structure and composition of benthic macrofaunal and meiofaunal communities in sediment collected at various pre-determined distances from the outfall.

Marine benthos refers to invertebrate fauna that live in or on the surface of the sediments. In contrast to the pelagic groups (e.g. fish and plankton) which can move in and out of an area avoiding temporarily contaminated waters, the benthos, by virtue of their relatively sedentary nature, have to adapt to the prevailing conditions or perish (Warwick 1993). The difference between benthic macrofauna and meiofauna is largely a human artefact. Simply put, macrofauna are larger than meiofauna. Meiofauna are benthic invertebrates retained on sieve mesh sizes of 150 µm or less, while macrofauna are usually regarded as invertebrates retained on sieve mesh sizes of 500 - 1000 µm. Internationally, both meiofauna and macrofauna are used for monitoring the impact of effluent discharges through marine outfalls. They often complement one another as biomonitoring indicator assemblages. However, macrofauna have emerged as the most widely used indicator group for a variety of reasons (Ballesteros et al. 2007, Ranasinghe et al. 2009). First, they are generally sessile or have limited mobility. This means that they are compelled to respond to local conditions *in situ* and cannot simply move away when presented with a stress. Second, they comprise a range of taxonomic groups with varying sensitivities to pollution. Third, most macrobenthic taxa have life spans that extend over months to years and are therefore particularly useful in integrating the effects of an impact over the relatively long inter-survey time frames (e.g. one year). This renders them appropriate for measuring the 'long-term' impact of a disturbance. Response to pollution is often species specific, but inevitably the overall species response is reflected at the community level. Chronic exposure to contamination may cause sensitive species to die and allow more tolerant, opportunistic species to proliferate. The net effect is a skewing of the community structure that can be interpreted to reflect the general state of the environment. Pollution impacts are reflected by shifts in the abundance of macrofauna species, reductions in diversity, or a relative proliferation of pollution tolerant and opportunistic species.

While many of these attributes are also true of the meiofauna, they have much shorter generation times and can, therefore, recover from the effects of pollution events more rapidly than macrofauna. Most

importantly in South Africa, very real practical challenges are posed by the lack of appropriate taxonomic resolution and skills available when identifying meiofauna. Most assessments that have used meiofauna as indicator organisms have worked at very coarse taxonomic resolution. Advances in statistical approaches now make it possible to analyse multi-species community arrays and detect subtle changes at the community level. These techniques are now standard practice in marine pollution assessment studies, but cannot be fully exploited on datasets which have very poor taxonomic resolution (e.g. meiofauna).

The structure of marine benthic invertebrate communities is influenced by many factors. These include abiotic factors, such as sediment conditions, salinity and temperature, and biotic factors such as food availability, competition and predation. A major challenge in environmental monitoring is to distinguish between naturally occurring and anthropogenically induced changes to benthic invertebrate communities. This is best achieved through comparison of communities from impacted sites to those from reference sites. While benthic invertebrate community data have limitations, with appropriate replication and analysis they remain the most ecologically relevant source of evidence regarding possible impacts on the benthos (McPherson et al. 2008).

# 5. Materials and Methods

## 5.1. Survey sampling design

The sampling design for the 2012 survey of the PetroSA outfall monitoring programme sought to provide answers to three key questions that are usually of concern to wastewater facility managers and regulatory authorities in situations where effluent is discharged to a receiving environment, namely:

1. Is there a measureable impact attributable to effluent discharge?
2. What is the spatial extent of the effluent discharge impact?
3. What is the magnitude of the effluent discharge impact?

To address these questions, eleven sampling sites were identified in the Vleesbaai receiving environment (Figure 1, Table 1). Site specifics and details on the monitoring performed at each site are presented in Table 2. One station (station DB/DA) was situated at the outfall diffuser section (Figure 1). The remaining stations were positioned at distances between 100 - 2000 m to the west and east of the diffuser section, in water of a comparable depth to the diffuser section. There are no detailed oceanographic data available for Vleesbaai. An *a priori* assumption was thus made that the predominant current flows are parallel to the shoreline. Thus, if the discharge of effluent is significantly adversely impacting the receiving environment then a gradient in the values/concentrations of physical, chemical and biological parameters that provide a tracer of the effluent should be evident in water and sediment samples taken at increasing distances from the diffuser. The highest concentrations should be recorded nearest the outfall diffuser section and the lowest concentrations at the most westward and/or eastward sites. Similarly for benthic invertebrate communities, if the discharge of effluent is significantly adversely impacting the communities then the structure and composition of communities at sites near the diffuser section of the outfall should be different to communities at increasing distances from the diffuser section.

An important objective for the water quality monitoring component of the 2012 survey of the PetroSA outfall monitoring programme was to track dispersal of the effluent and to determine whether water quality beyond the zone of initial dilution was compliant with the South African Water Quality Guidelines for Coastal Marine Waters (Natural Environment) (DWAF 1995). There is no clear guidance in South Africa on how the spatial extent of the zone of initial dilution should be defined for marine outfalls. The most commonly adopted approach is to consider the margin of the zone of initial dilution to be a distance from the diffuser that is two or three times the average water column depth at the diffuser section. This would make the margin of zone of initial dilution to be between 44-66 m in any direction from the diffuser section of the PetroSA outfall. All water quality monitoring sites, apart from site DB/DA, were thus situated beyond

the calculated zone of initial dilution. The *a priori* expectation was thus that water quality at all sites other than DB/DA would be compliant with the South African Water Quality Guidelines for Coastal Marine Waters (Natural Environment) (DWAF 1995).

**Table 1.** Details of sites and the monitoring performed at each site for the November 2012 survey of the PetroSA outfall monitoring programme.

| Station | Latitude | Longitude | Distance from diffuser | Monitoring performed | | | |
|---|---|---|---|---|---|---|---|
| | | | | *In situ* measurements | Water quality | Sediment quality | Benthic invertebrates |
| W5 | -34.241183 | 21.963783 | 2000 m | ✓ | | ✓ | |
| W4 | -34.235183 | 21.972100 | 1000 m | ✓ | | ✓ | ✓ |
| W3 | -34.233050 | 21.976933 | 500 m | ✓ | ✓ | ✓ | ✓ |
| W2 | -34.231867 | 21.979850 | 200 m | ✓ | ✓ | | |
| W1 | -34.231567 | 21.980867 | 100 m | ✓ | | ✓ | ✓ |
| DB/DA | -34.231167 | 21.981833 | - | ✓ | ✓ | | |
| E1 | -34.230733 | 21.982750 | 100 m | ✓ | | ✓ | ✓ |
| E2 | -34.230302 | 21.983736 | 200 m | ✓ | ✓ | | |
| E3 | -34.229360 | 21.986787 | 500 m | ✓ | ✓ | ✓ | ✓ |
| E4 | -34.228038 | 21.991915 | 1000 m | ✓ | | ✓ | ✓ |
| E5 | -34.225788 | 22.002248 | 2000 m | ✓ | | ✓ | |

To provide perspective on the values/concentrations of various physical, chemical and biological parameters measured in water and sediment samples collected from the receiving environment, the same suite of parameters measured in water samples collected from the receiving environment were also measured in the effluent sample prior to discharge. The effluent sample monitoring thus served to identify potential indicators (or tracers) of the effluent in the receiving environment. Various physical, chemical and biological parameters were also measured *in situ* at the diffuser section prior to and shortly after effluent discharge commenced. This was possible because effluent is not continuously discharged through the PetroSA outfall, but done so according to demand.

Investigation of potential impacts to benthic fauna relied on sampling macrofauna and meiofauna from six of the eleven pre-identified sites (Table 1). Significant adverse impact would be expected to result in a gradient in community 'health' with increasing distance from the outfall. 'Control' and 'Test' sites were also designated according to their proximity to the source of potential impact.

**Table 2.** Physical, chemical and biological parameters analysed in effluent and water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

| Chemical Class | Parameter | Chemical Class | Parameter | Chemical Class | Parameter |
|---|---|---|---|---|---|
| *Conventional* | pH | *Metals* | Iron | *BTEX* | Benzene |
| | Conductivity | | Cadmium | | Toluene |
| | Fluoride | | Cobalt | | Ethylbenzene |
| | Turbidity | | Copper | | o-Xylene |
| | Suspended solids | | Chromium | | m'p-Xylene |
| | | | Manganese | | |
| *Nutrients* | Ammonia | | Nickel | *Total petroleum* | C6-C8 |
| | | | Lead | *hydrocarbons* | C8-C10 |
| *Microbiological* | Faecal coliforms | | Zinc | | C10-C12 |
| | *E. coli* | | | | C12-C16 |
| | | | | | C16-C21 |
| | | | | | C21-C30 |
| | | | | | C30-C35 |
| | | | | | C35-C40 |

## 5.2. Field procedures

### 5.2.1. Water quality

A Yellow Springs Instrument 6600 multiparameter water quality sonde (Plate 1) was used to measure profiles of temperature, salinity, pH, turbidity, dissolved oxygen concentration and saturation through the water column at each of the eleven sampling sites (Table 1). Probes of the sonde were calibrated prior to fieldwork. The sondes probes were held below the water surface for approximately two minutes to equilibrate, and the sonde was then slowly lowered through the water column to the seabed. The sonde was programmed to log at three second intervals, providing a near-continuous profile of measurements through the water column. An initial profile was measured at the outfall diffuser section before effluent was discharged. In graphs, tables and appendices in this report, surface and bottom water measurements taken from this profile are denoted as DB (Diffuser Before). Once effluent discharge had occurred for about one hour, a second profile was measured at the outfall diffuser section (denoted DA, Diffuser After). Thereafter profiles were made measured at all other sampling sites.

In addition to making multiparameter measurements, discrete water samples were also taken in near-surface waters at the diffuser section before effluent discharge (site DB), near-surface and middle waters after effluent discharge (surface, DA Top, and middle ,DA Middle) and in near-surface waters at sites E3, E2, W2 and W3. Water samples were collected using a National Institute of Oceanography bottle (90 cm in length, 5 litre capacity, Plate 2). The bottle was lowered vertically into the water column to the required depth and remotely triggered. On retrieval the bottle was inverted several times to ensure the water sample was well-mixed. Aliquots (subsamples) of water were then transferred to pre-cleaned, sterilised high-density polyethylene or glass containers (Plate 3). Samples were kept cool on ice in the field and until delivery to the laboratory, where they were held at 4°C until analysis.

### 5.2.2. Sediment quality and benthic invertebrates

At each of the eight sediment sampling sites (see Table 1) a van Veen grab that samples an area of 0.084 $m^2$ was deployed to collect sediment from the seabed (Plate 4). One grab sample from each site was processed for grain size composition, total organic content, and metal and hydrocarbon analysis. Subsamples of the sediment were transferred to pre-cleaned high-density polyethylene containers for grain size composition, total organic content and metal analysis (Plate 5), and to glass containers for hydrocarbon analysis. Samples were kept cool on ice in the field and until delivery to the laboratory, where the samples were frozen (-4°C) until further analysis.

At all sediment sampling sites, except those at the extreme ends of the sample transect (W5 and E5), five additional sediment grabs were taken and processed for benthic macrofauna. The sediment was washed through a 500 µm mesh size sieve in the field (Plate 6). All macrofauna and material retained by the sieve was transferred to high-density polyethylene containers and immediately preserved in a buffered formaldehyde/seawater solution. Phloxine dye was added to the formaldehyde to stain fauna in the sample a red-brown colour and thus aid sample processing in the laboratory.

An additional sediment grab was collected at each site using a smaller Ponar grab that samples an area of 0.023 $m^2$. The sediment was transferred to a high-density polyethylene container and preserved with buffered formaldehyde/seawater solution, pending analysis for meiofauna in the laboratory.

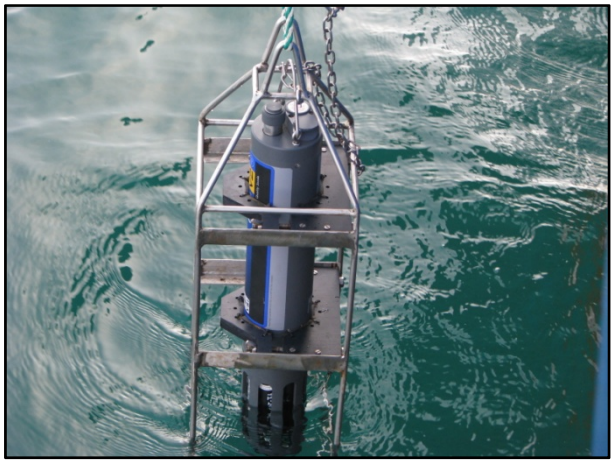

**Plate 1.** Yellow Springs Instrument 6600 multiparameter water quality sonde that was used to measure water quality through the water column.

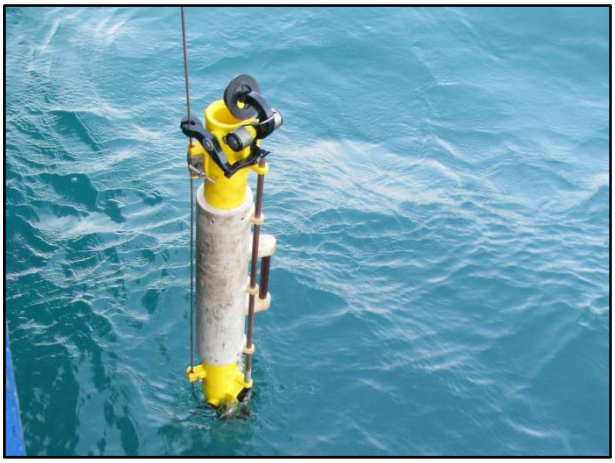

**Plate 2.** A National Institute of Oceanography bottle was used to collect water samples.

**Plate 3.** Water samples were transferred to containers for analysis in the laboratory.

**Plate 4.** A van Veen grab was used to collect sediment from the seabed.

**Plate 5.** Sediment samples were transferred to containers for analysis in the laboratory.

**Plate 6.** Benthic macrofauna were isolated by passing sediment through a sieve.

## 5.3. Laboratory procedures

### 5.3.1. Effluent and water samples

The effluent sample collected from the sump prior to discharge and water samples collected from the receiving environment was analysed for the suite of physical, chemical and biological parameters listed in Table 2. A summary of the methods used for the analyses is provided below. Analyses were performed at SANAS accredited CSIR laboratories in Durban and Stellenbosch.

#### pH

pH was measured with a probe following the standard procedure. pH was not corrected for temperature.

#### Conductivity

Conductivity was measured with a probe following the standard procedure.

#### Fluoride

Fluoride was determined potentiometrically using an ion analyser coupled to a fluoride ion specific electrode.

#### Total suspended solids

Total suspended solids concentrations were determined gravimetrically. Water samples were vigorously agitated and a 500 ml aliquot was then vacuum filtered through pre-dried and pre-weighed 0.45 µm pore size membrane filters. The filters were dried at 105$^{o}$C for 2 hrs and weighed. The total suspended solids concentration was determined as the difference in the dry weight of filters before and after filtration.

#### Nutrients

Water samples were vacuum filtered through 0.45µm pore size membrane filters. The (dissolved) concentration of ammonia in the filtrate was measured colourimetrically using a four-channel flow injection auto-analyser.

#### Faecal indicator bacteria

Faecal coliforms and *Escherichia coli* were detected and enumerated using the Escherichia Partition Method with NA-MUG agar as per Method 9222G in Standard Methods for Water and Wastewater (1992). It should be noted that the samples were only analysed about 18 hrs after collection, due to the need to transport the samples to the CSIR laboratory in Stellenbosch.

#### Metals

Water samples were vacuum filtered through 0.45 µm pore size membrane filters. Dissolved metals were then concentrated by adding a chelating agent to filtered samples. The metal-chelate complex was extracted using an organic solvent, the latter then removed by heating. The metal-chelate complex was then dissolved in dilute nitric acid and the concentrations in solution then detected and quantified using inductively coupled plasma mass emission spectroscopy or optical emission spectroscopy. In the case of mercury the samples were analysed using a direct mercury analyser.

#### Hydrocarbons

Water samples were analysed for BTEX (Benzene, Toluene, Ethylbenzene and Xylenes), gasoline range organics (C6 - C10) and extractable hydrocarbons – fuel oil (>C10 - C16), fuel oil (>C16 - C21) and lube oil (>C21 - C32) range organics. BTEX and gasoline range organics were analysed by purge and trap-gas chromatography/mass spectrometry or Headspace-gas chromatography. Extractible hydrocarbons,

including diesel and lube range organics were analysed using capillary column gas chromatography. Quantitation was accomplished by comparing responses of major ions to an internal standard.

### Toxicity

The toxicity of effluent was tested using the sea urchin fertilisation test. Effluent dilutions were prepared by adding relevant amounts of clean seawater to the effluent, with four replicates prepared for each dilution. Adult sea urchins (*Echinometra mathaei*) were induced to spawn by injecting 1 - 2 ml of 0.5 molar potassium chloride into their coelomic cavity. Gametes from males and females were collected separately. Sperm was activated by exposure to seawater and 100 µl aliquots of the sperm suspension were then transferred to each replicate of the control (seawater) and effluent dilution treatments. After ten minutes of sperm exposure, 1 ml of egg suspension was added and left for a further ten minutes. The test was then terminated by adding 100 µl of formalin to each test vial. Fertilisation success was determined by microscopic examination of an aliquot of the egg suspension from each replicate. The fertilisation success data was used to construct a dose-response curve, which was used to calculate the Minimum Acceptable Toxicant Dilution. This represents the number of times that the effluent needed to be diluted so that fertilisation success was not statistically different to fertilisation success in the control treatment.

## 5.3.2. Sediment

Sediment samples were analysed for the suite of physical and chemical parameters listed in Table 3. A summary of the methods followed is provided below. Analyses were performed at SANAS accredited CSIR laboratories in Durban, Stellenbosch and Pretoria.

### Grain size composition

The grain size composition of sediment was determined by wet and dry sieving sediment into seven grain size classes according to the Wentworth Scale, namely mud (<0.063 mm), very fine grained sand (0.063 - 0.125 mm), fine grained sand (0.125 - 0.250 mm), medium grained sand (0.25 - 0.50 mm), coarse grained sand (0.5 - 1.0 mm), very coarse grained sand (1.0 - 2.0 mm) and gravel (>2.0 mm).

### Total organic content

Aliquots of sediment were oven dried, weighed, and organic matter then degraded using hydrogen peroxide. The sediment was washed in distilled water, re-dried and re-weighed, and the difference in dry weight before and after degradation used to determine the total organic content.

### Metals

Sediment samples were freeze dried and ball milled to a fine consistency. Approximately 1 g of the sediment was weighed into a high-pressure digestion vessel and digested in concentrated nitric and perchloric acids and hydrogen peroxide, with microwave assistance. Digestates were diluted to volume with deionised water and metals in solution then detected and quantified using inductively coupled plasma mass emission spectroscopy or optical emission spectroscopy. Mercury was analysed using a direct mercury analyser.

### Hydrocarbons

Hydrocarbons were extracted from approximately 20 g of freeze dried and ball milled sediment using solvents. Sediment samples were analysed for BTEX (Benzene, Toluene, Ethylbenzene and Xylenes), gasoline range organics (C6 - C10) and extractable hydrocarbons – fuel oil (>C10 - C16), fuel oil (>C16 - C21) and lube oil (>C21 - C32) range organics. BTEX and gasoline range organics were analysed by purge and trap-gas chromatography/mass spectrometry or Headspace-gas chromatography. Extractible hydrocarbons, including diesel and lube range organics were analysed using capillary column gas chromatography. Quantitation was accomplished by comparing responses of major ions to an internal standard.

**Table 3.** Physical and chemical parameters analysed in sediment samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

| Chemical Class | Parameter | Chemical Class | Parameter |
|---|---|---|---|
| *Conventional* | Sediment grain size | *BTEX* | Benzene |
| | | | Toluene |
| *Organic indicators* | Total organic content | | Ethylbenzene |
| | | | o-Xylene |
| *Metals* | Aluminium | | m'p-Xylene |
| | Iron | | |
| | Arsenic | *Total petroleum* | C6-C8 |
| | Barium | *hydrocarbons* | C8-C10 |
| | Beryllium | | C10-C12 |
| | Cadmium | | C12-C16 |
| | Cobalt | | C16-C21 |
| | Copper | | C21-C30 |
| | Chromium | | C30-C35 |
| | Manganese | | C35-C40 |
| | Mercury | | |
| | Nickel | | |
| | Lead | | |
| | Vanadium | | |
| | Zinc | | |

### 5.3.3. Benthic invertebrates

In the laboratory, macrobenthic samples were washed gently and all matter retained by sieves was decanted into sorting trays. Organisms were individually removed from the matter with fine forceps and the aid of magnifying glasses. The composite fauna for each sample was preserved in 70% ethanol and subsequently identified to the lowest level of taxonomic resolution practicable and enumerated under stereomicroscope. In addition to establishing abundance of organisms (per $m^{-2}$), biomass of macrofauna was determined. Different taxa in each sample were laid on blotting paper to remove excess water and wet mass measured to the nearest 0.0001 mg. Biomass less than 0.0001 mg was recorded as 0.0001 mg.

Meiofauna were extracted from the sediment using a modified Oostenbrink separator (Fricke 1979) and a 45 µm mesh sieve. Subsamples were then counted and expressed as meiofauna per 100ml sediment for each site sampled. Counts were made of each meiofaunal group distinguishable at 63x magnification under a stereomicroscope.

## 5.4. Data analysis

### 5.4.1. Sediment quality

Metal concentrations cannot be directly compared between sediment samples unless the samples have an identical grain size composition. This is because grain size is one of the most important variables that control natural and anthropogenic concentrations of metals in sediment. Metal concentrations are naturally higher in mud dominated sediment compared to sand dominated sediment. High metal concentrations in sediment thus does not automatically imply that the sediment is metal contaminated, but may simply reflect the mineralogy of the geological material that gave rise to the sediment or the grain size composition of the sediment. Also, despite input and transport dissimilarities, naturally occurring and anthropogenically introduced metals accumulate in the same areas. Therefore, to meaningfully interpret metal concentrations in sediment the factors that influence the natural variation of metal concentrations in sediment must be compensated for before naturally occurring concentrations can be differentiated from anthropogenically enhanced concentrations (i.e. concentrations indicative of contamination). Metal concentrations in sediment samples collected from the Vleesbaai receiving environment were interpreted using baseline metal concentration models and a baseline cadmium concentration defined for the Mossel

Bay area by the CSIR Coastal Systems research group. The baseline models compensate for the natural factors that influence metal concentrations in sediment. The procedure that was used to define the baseline models and baseline cadmium concentration are provided in Weerts et al. (2011).

The way in which the baseline models are used to interpret metal concentrations in sediment can be explained using a theoretical example, based on the baseline model for chromium in sediment from the Mossel Bay area (Figure 2). Chromium concentrations derived from samples are superimposed on the baseline model of aluminium normalised chromium concentrations. In Figure 2, four hypothetical chromium concentrations are superimposed on the baseline model. Chromium concentrations that fall within the baseline model prediction limits (i.e. within the dashed lines), such as hypothetical concentration 1, fall within the baseline concentration range and are not considered to be enriched with chromium. Chromium concentrations that exceed the baseline model upper prediction limit (i.e. above the upper dashed line), such as hypothetical concentrations 2, 3, and 4, are interpreted to be enriched with chromium. In rare circumstances, metal concentrations fall below a baseline model lower prediction limit (i.e. below the lower dashed line) and are interpreted as metal depleted, unreliable, or possibly reflecting enrichment/contamination of the sediment by the normaliser.

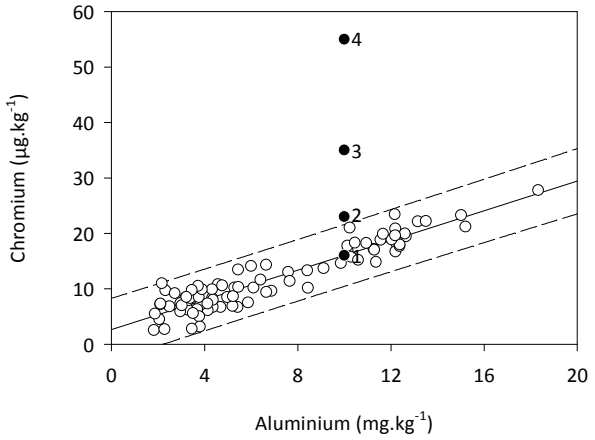


**Figure 2.**Baseline model for chromium in sediment from the Mossel Bay area. Open symbols are chromium concentrations used to define the baseline model while numbered solid symbols represent four hypothetical scenarios: 1. concentration falls within the baseline model upper and lower prediction limits (dashed lines) and is not enriched; 2, 3 and 4. concentrations exceed baseline model upper prediction limit and reflect various levels of enrichment that can broadly be defined from low (2) through to high (4). Situations 3 and 4 would be interpreted as reflecting enrichment through contamination with a high level of confidence.

A metal concentration that exceeds a baseline model upper prediction limit does not necessarily imply that the concentration was enhanced through an anthropogenic contribution (i.e. contamination), but rather that the concentration is atypical of the data used to define the baseline model (Horowitz et al. 1991). Several reasons, in addition to an anthropogenic contribution, can lead to a metal concentration exceeding a baseline model upper prediction limit, including analytical errors, poor baseline model assumptions, the probability that concentrations in some samples will naturally exceed the baseline model upper prediction limit, and natural enrichment not captured by the data set used to define the baseline model (Schropp et al. 1990, Rae and Allen 1993). Interpretation of metal enrichment, and ultimately whether this reflects contamination, thus requires consideration of additional factors, including (a) possible (bio)geo-chemical processes leading to natural enrichment, (b) the difference between a metal concentration and baseline model upper prediction limit (i.e. the magnitude of enrichment), (c) the number of metals in a sediment sample at concentrations that exceed baseline model upper prediction limits, and (d) the position of metal enriched sediment relative to known or suspected anthropogenic sources of metals. The greater the exceedance of a baseline model upper prediction limit by a metal concentration, the greater the number of metals enriched in sediment at a particular location. The nearer the location is to known or suspected

anthropogenic sources of metals the greater the likelihood the metal concentration/s were enhanced through an anthropogenic contribution and thus reflect contamination.

In Figure 2, hypothetical chromium concentration 2 is interpreted as enriched, but whether this reflects contamination should only be concluded after considering the abovementioned additional factors. This is because the concentration only marginally exceeds the baseline model upper prediction limit. Hypothetical chromium concentrations 3 and 4 exceed the baseline model upper prediction limit substantially and the sediment at these stations would be interpreted as contaminated by chromium with a high level of confidence. The magnitude of exceedance of the baseline model upper prediction limit by hypothetical chromium concentrations 3 and 4 is in fact sufficient that this interpretation would be made even if no other metals in the sediment were enriched.

Cadmium concentrations in sediment from the Mossel Bay area are weakly correlated to aluminium concentrations and consequently a baseline model could not be defined for this metal. Instead, a baseline cadmium concentration was defined using cumulative frequency and probability plots. Cadmium concentrations in sediment are interpreted by directly comparing the concentrations to the baseline cadmium concentration. Cadmium concentrations that exceed the baseline concentration are interpreted as enriched while those that fall below are considered to occur within the baseline concentration range.

### 5.4.2. Benthic invertebrates

Investigating for impact on benthic invertebrate communities requires testing for differences amongst communities sampled before and after the potential impact or sampled from 'Control' and 'Test' sites, preferably both (Underwood 1994). Control sites are typically sites far enough away from the potential source of impact (in this case the diffuser section of the outfall) to be unaffected by it, while Test sites are those in closer proximity and potentially affected. Differences, if detected, must be investigated and explainable in the light of expected disturbance effects and species biologies (e.g. reductions in species diversity, shifts in the abundance of taxa, decreases in abundance of sensitive species or a relative proliferation of pollution tolerant and opportunistic species). For this survey, sites closest in proximity to the outfall (100 m, W1 and E1) were treated as Test sites and those further away (≥500 m, W3, W4, E3, E4) were treated as Control sites.

PRIMER v6 software (Clarke and Warwick 2001), which has achieved international acceptance as a tool for analysing and interpreting ecological community data, was used to delineate biological trends in survey results. Where appropriate, data were square root transformed prior to analysis. This down-weighs the abundance of dominant or large species (Field et al. 1982). Differences in macrobenthos sampled at increasing distances from the diffuser section of the PetroSA outfall were then investigated in the following manner.

A range of univariate community parameters and diversity indices was calculated for benthic communities at each site sampled. These are key ecological measures that can provide an indication of the status of an ecosystem (Magurran 1988). In this study, five univariate indices are reported on; total number of taxa, total number of individuals, species richness (Margalef), species evenness (spread of numbers among species), and diversity (Shannon-Weiner). Average indices were compared amongst sites using one-way Analysis of Variance (ANOVA), followed where appropriate by a Tukey multiple comparison test (Zar 1996). In cases where the prerequisites of ANOVA (variance homogeneity and normality) were not met, the Kruskal-Wallis ANOVA test (one-way ANOVA on ranks) was used. Hierarchical clustering and non-metric multi-dimensional scaling (MDS) were used to analyse full species arrays sampled. These multivariate routines investigate the relative similarity of biological communities. They rely on a similarity measure between each species array. For analyses performed in this study the Bray-Curtis similarity coefficient (Bray and Curtis 1957) was used. Outputs of ordination (a cluster diagram) and non-metric multidimensional

scaling (an ordination plot) are typically complimentary to each other and provide a visual aid to interpreting community differences (or similarities). Statistical confirmation of differences was achieved using Analysis of similarities (ANOSIM). This routine tests for statistical differences between pre-defined groups and is analogous to the univariate ANOVA. The ANOSIM routine results in a test statistic (R) and level of significance (Clarke and Green 1988). In order to identify species that contributed most to differences amongst groups confirmed by ANOSIM the Similarity of percentages routine (SIMPER) was used. Lastly a routine (BEST) that links biological patterns with physical and chemical parameters by cross correlating data sets was used to define which of the measured physical and chemical parameters were most likely to be 'drivers' of the biological trends detected.

Previous meiofaunal analysis performed in Vleesbaai relied on the use of the Nematode/Copepod ratio (N/C). To allow comparisons with previous studies, the same analysis was applied to meiofauna for this study. The N/C ratio is calculated as the number of nematodes divided by the number of harpacticoid copepods. Where there were no copepods in samples the number of nematodes was divided by 1.

# 6. Results and Discussion[2]

## 6.1. Effluent quality and toxicity

Results from the physical, chemical and biological analysis of the once-off PetroSA Vleesbaai effluent sample are presented in Table 4. Compared to other industrial effluent discharges to the marine environment in South Africa, such as in Durban and Richards Bay, the concentrations of most parameters in the PetroSA effluent sample were low. The pH was relatively high (8.3) compared to effluent from other wastewater treatment facilities, for which the pH is often <7 (e.g. Southern Works wastewater facility in Durban ~6.8). Metal concentrations were low to modest in concentration, with most metals actually at concentrations below the method detection limit. For comparative purposes, metal concentrations in effluent from the Southern Works wastewater facility in Durban often exceed the method detection limit, with nickel and zinc concentrations up to ~26 and ~15 $\mu g.l^{-1}$ respectively. Some BTEX constituents and most Total Petroleum Hydrocarbons were detected in the PetroSA effluent, but at relatively low concentrations. For comparative purposes, BTEX and Total Petroleum Hydrocarbon concentrations in effluent from the Southern Works wastewater facility in Durban commonly exceed 300 and 600 $\mu g.l^{-1}$ respectively. The theoretical minimum initial dilution for the PetroSA outfall is not known. If this is established then a mass balance model could be used to calculate the likely concentrations of various contaminants in the receiving water beyond the zone of initial dilution.

It is important to note that the results from the analysis of a single effluent sample may not be representative of the quality of effluent discharged at all times. In terms of developing the current monitoring program for Vleesbaai, the reason for analysing the single effluent sample was to provide some perspective for water quality monitoring in the vicinity of the outfall shortly after effluent discharge. The results from the once-off effluent analysis provide an indication of what can be expected to be detected in the receiving environment, post-discharge. However, it is noted that the results from the 2012 sample are considerably different to the values/concentrations of most parameters when compared with summary effluent data provided by PetroSA for the period October to December 2010 (see Weerts et al. 2012).

The Minimum Acceptable Toxicant Dilution of the effluent sample was 14. In other words, the effluent needed to be diluted 14 times by the receiving water to render it non-toxic. The Minimum Acceptable Toxicant Dilution for the effluent sample is low compared to other industrial effluent discharges to the marine environment in South Africa. In many other cases the Minimum Acceptable Toxicant Dilution of effluent exceeds 100, and often 200 (e.g. Durban and Richards Bay). Once again it is important to note that the results of this once-off toxicity test may not be representative of the quality of effluent discharged at all

[2]Raw data are provided as appendices to this report.

times. A more comprehensive approach would be to determine the variability in the Minimum Acceptable Toxicant Dilution, which would require testing of a composite effluent sample collected each month for at least one year.

**Table 4.** Results for physical, chemical and microbiological parameters analysed in an effluent sample collected for the 2012 survey of the PetroSA outfall monitoring programme.

| Chemical Class | Parameter | Result | Chemical Class | Parameter | Result |
|---|---|---|---|---|---|
| *Conventional* | Fluoride (mg.l$^{-1}$) | 0.8 | *BTEX* | Benzene (µg.l$^{-1}$) | <0.20 |
| | Conductivity (mSm) | 285 | | Toluene (µg.l$^{-1}$) | <0.20 |
| | pH | 8.3 | | Ethylbenzene (µg.l$^{-1}$) | 0.79 |
| | Turbidity (NTU) | 4.6 | | o-Xylene (µg.l$^{-1}$) | 1.00 |
| | Suspended solids (mg.l$^{-1}$) | 8.0 | | m'p-Xylene (µg.l$^{-1}$) | 0.73 |
| | | | | BTEX (sum) (µg.l$^{-1}$) | 2.50 |
| *Nutrients* | Ammonia (µg.l$^{-1}$) | 484 | | | |
| | | | *Total petroleum* | C6-C8 (µg.l$^{-1}$) | <30 |
| *Microbiological* | Faecal coliforms (cfu.100ml$^{-1}$) | 2 | *hydrocarbons* | C8-C10 (µg.l$^{-1}$) | 33 |
| | *E. coli* (cfu.100ml$^{-1}$) | 2 | | C10-C12(µg.l$^{-1}$) | <60 |
| | | | | C12-C16(µg.l$^{-1}$) | 4.6 |
| *Metals* | Iron (µg.l$^{-1}$) | <5.0 | | C16-C21(µg.l$^{-1}$) | 25 |
| | Cadmium (µg.l$^{-1}$) | <0.1 | | C21-C30(µg.l$^{-1}$) | 52 |
| | Cobalt (µg.l$^{-1}$) | <0.2 | | C30-C35(µg.l$^{-1}$) | 37 |
| | Copper (µg.l$^{-1}$) | 6.0 | | C35-C40(µg.l$^{-1}$) | <5 |
| | Chromium (µg.l$^{-1}$) | <0.5 | | C10-C40(µg.l$^{-1}$) | <8 |
| | Manganese (µg.l$^{-1}$) | 1.1 | | C10-C40(µg.l$^{-1}$) | 120 |
| | Nickel (µg.l$^{-1}$) | <0.5 | | | |
| | Lead (µg.l$^{-1}$) | <1.0 | | | |
| | Zinc (µg.l$^{-1}$) | 8.0 | | | |

## 6.2. Water quality

Results from analyses of water quality *in situ* and for water samples are portrayed in Figures 3 - 20 (and listed in Appendix 1). Figures 3 - 12 compare the *in situ* profiles of temperature, salinity, pH, dissolved oxygen and turbidity through the water column at each site and the values/concentrations for the parameters in surface and bottom water as taken from the profiles. Figures 13–20 portray the values/concentrations of physical, chemical and biological parameters measured in water samples collected before (DB) and after effluent discharge (DA Top and DA Mid). Also included in Figures 13 - 20 are the values/concentrations of parameters measured in the single effluent sample (Sump) collected prior to water quality monitoring in the receiving environment.

The identification of an effluent discharge impact/presence of effluent in a receiving environment requires that the values/concentrations of the parameters (or indicators) be higher, or in the case of some parameters (e.g. salinity) possibly lower, in the immediate vicinity of the outfall and then decrease (or increase) with increasing distance from the outfall, as the effluent is diluted and dispersed in the receiving environment. Depending on the prevailing oceanographic conditions at the time of monitoring, the likelihood is that the decreasing/increasing values/concentrations will be most evident on one side of the outfall diffuser (i.e. 'downstream' in terms of current flow).

The water quality data collected during the 2012 Vleesbaai environmental monitoring survey provide no clear evidence of an effluent signal in the receiving environment with the possible exception of turbidity, which was elevated in the upper 3 m of the water column at the diffuser section after effluent discharge (Figure 11). The turbidity of surface water and the turbidity through most of the water column at sites situated to the east of the diffuser section were also generally higher compared to sites situated to the west of the diffuser section (Figures 11 and 12). If this *in situ* turbidity trend provides an effluent signal,

which seems plausible considering the turbidity of the effluent sample was somewhat higher compared to discrete water samples collected from the receiving environment (Figure 16), then the implication is that the effluent was dispersing in an easterly direction at the time of monitoring. However, this does not agree with the wind direction, which was about 20 knots from the east (i.e. blowing westwards) at the time of monitoring. This said, it is not uncommon for the direction of effluent dispersion (i.e. currents) at depth to be opposite to wind-driven surface water movement. As a further complicating factor, a similar trend was not evident for turbidity measurements in water samples, which in fact showed little difference between sites (Figure 16).

The salinity and pH of the water column measured *in situ* at stations DA, E1 and E2 was generally slightly lower compared to other sites sampled after effluent discharge (Figures 5 and 7). However, the salinity and pH of the water column at the diffuser section before effluent discharge (DB) was even lower (Figures 5 - 8). This implies the lower salinity and pH recorded at sites DA, E1 and E2 did not represent an effluent discharge signal. The reason for the lower salinity and pH prior to effluent discharge is undetermined. The reason for the low pH in the vicinity of the diffuser after effluent discharge (8.1) is also interesting from the perspective that the pH of the effluent was somewhat higher (8.3).

There was also no conclusive evidence for an effluent signal based on the values/concentrations of the majority of physical and chemical parameters measured in water samples (Figures 13 - 20). Although the values/concentrations for some parameters were highest in the surface water sample (DA Top) collected at the diffuser section after the discharge of effluent (e.g. fluoride- Figure 18, manganese, lead and zinc- Figure 19), these values/concentrations were often similarly high at sites distant from the diffuser section. More importantly, this was often the case for sites situated to the west of the diffuser section. If the turbidity trend reflects effluent dispersal in an easterly direction, as previously identified, then the higher concentrations for some parameters at sites to the west of the diffusers imply these did not provide an effluent signal. The strongest effluent signals were provided by faecal indicator bacteria (i.e. faecal coliforms and *E. coli*) (Figure 13) and zinc (Figure 19), for which measurements were considerably higher in the surface water sample collected above the diffuser section (DA Top) compared to other sites. Faecal indicator bacteria are usually one of the most useful and reliable tracers of effluent discharged through deepwater outfalls. This is because the effluent is discharged some distance offshore and faecal indicator bacteria are found only in the alimentary tracts (faeces) of humans, other mammals and birds, and populations of marine mammals and birds are usually not resident in the vicinity of deepwater outfall discharges. There was, however, no clear evidence from measurements of faecal indicator bacteria counts and zinc concentrations in the direction of effluent dispersion, since the counts and concentrations at other sites, albeit far lower compared to DA Top, were essentially identical (and at several sites below the method detection limit) (Figures 13 and 19).

The values/concentrations of all physical, chemical and biological parameters at all sites measured *in situ*, with the exception of turbidity, were compliant with the South African Water Quality Guidelines for Coastal Marine Waters (Natural Environment) (DWAF 1995). With regards to the turbidity non-compliance for *in situ* measurements, if the data for stations situated 1000 m and 2000 m from the outfall are taken as representing the ambient environment then the turbidity at numerous stations exceeded the threshold of 10% above ambient. However, the use of this guideline is problematic in that a 10% change from ambient when the ambient turbidity is only 1.4 NTU is so small (i.e. 0.14 NTU) that it is essentially ecologically meaningless and even difficult to accurately measure in the laboratory. It is the professional opinion of the scientists that prepared this report that little significance should, therefore, be placed on this instance of turbidity non-compliance.

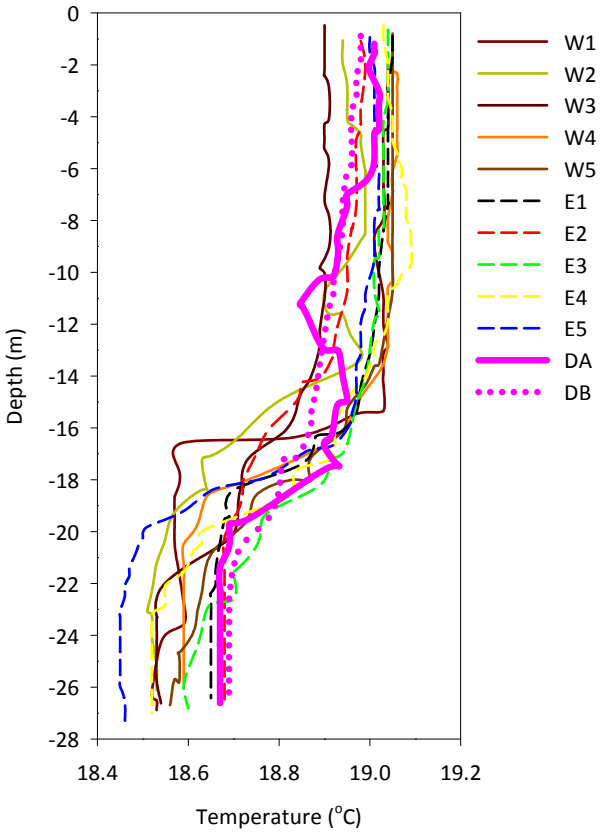


**Figure 3.** *In situ* temperature profiles for the 2012 survey of the PetroSA outfall monitoring programme.

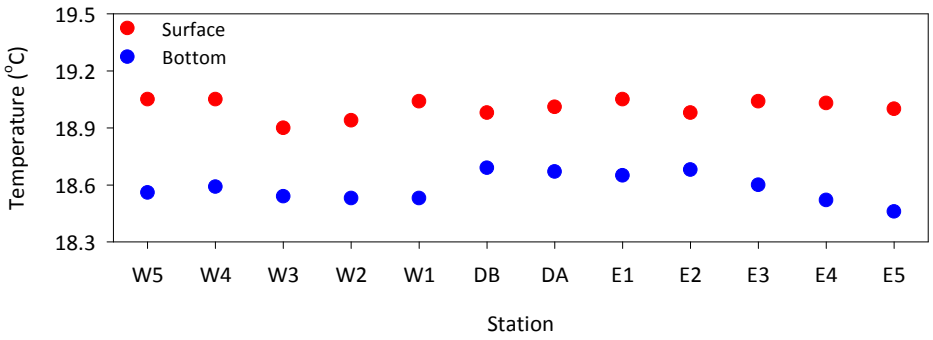


**Figure 4.** *In situ* surface and bottom water temperature for the 2012 survey of the PetroSA outfall monitoring programme.

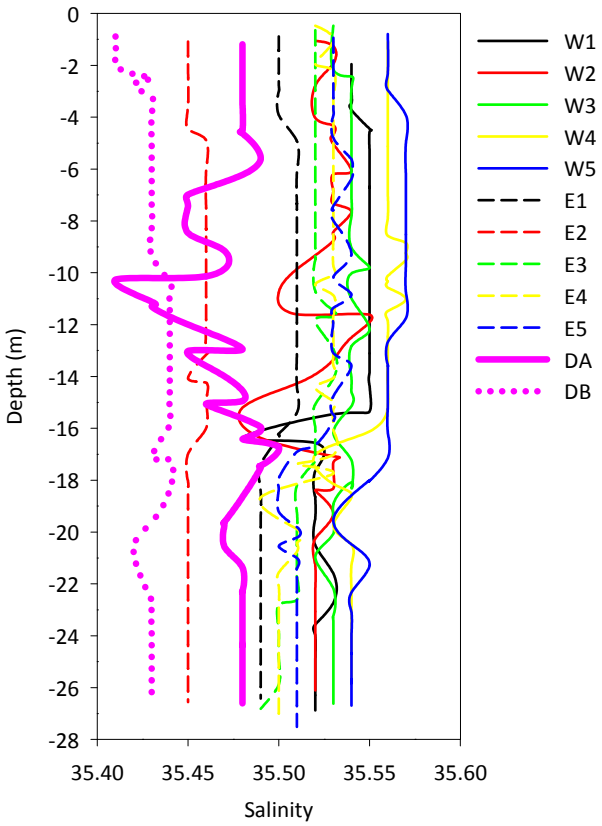


**Figure 5.** *In situ* salinity profiles for the 2012 survey of the PetroSA outfall monitoring programme.

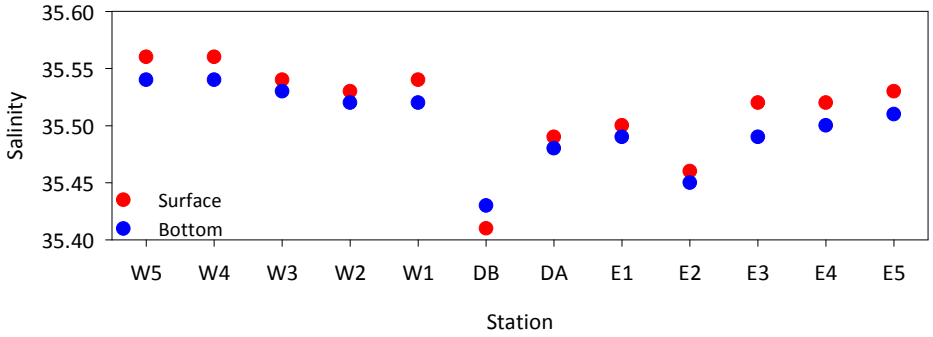


**Figure 6.** *In situ* surface and bottom water salinity for the 2012 survey of the PetroSA outfall monitoring programme.

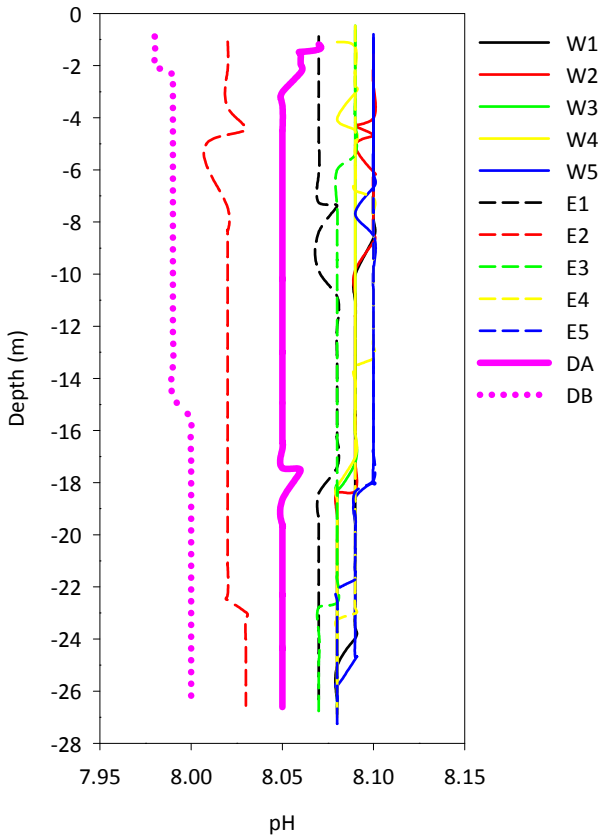


**Figure 7.** *In situ* pH profiles for the 2012 survey of the PetroSA outfall monitoring programme.

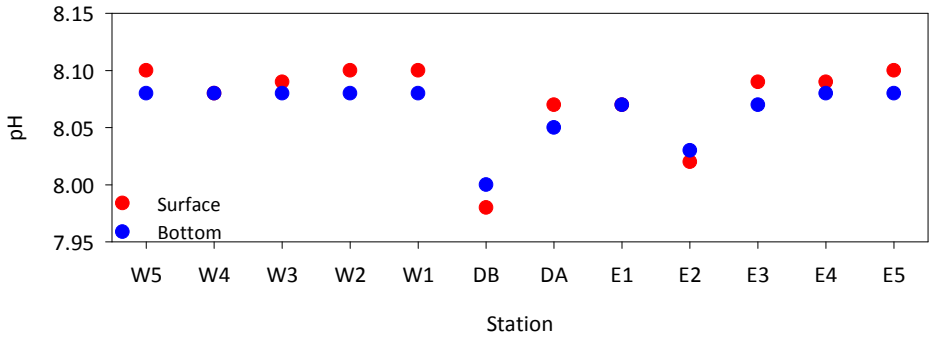


**Figure 8.** *In situ* surface and bottom water pH for the 2012 survey of the PetroSA outfall monitoring programme.

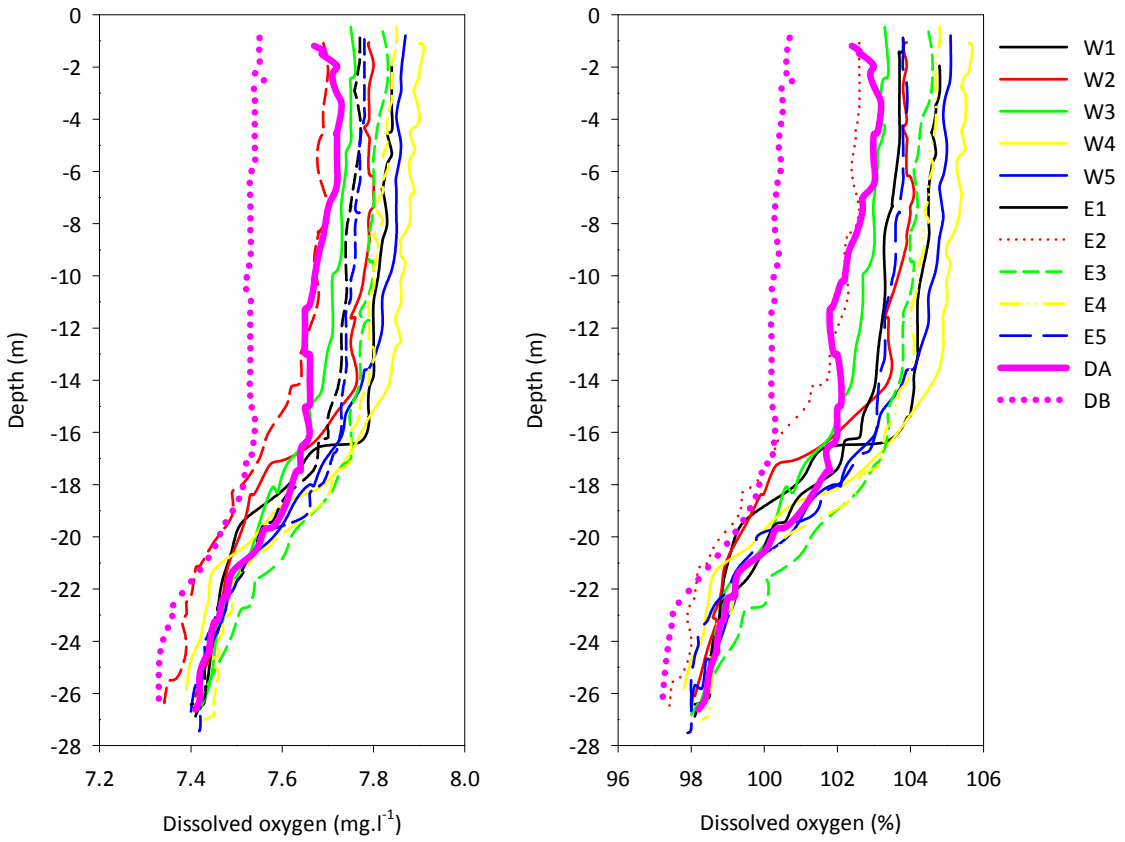


**Figure 9.** *In situ* dissolved oxygen profiles for the 2012 survey of the PetroSA outfall monitoring programme.

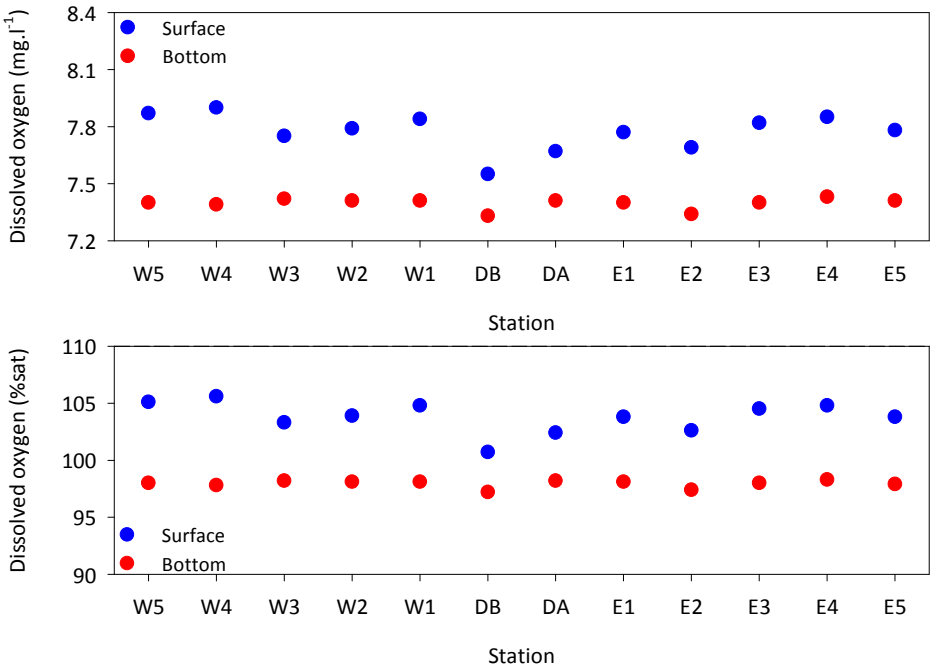


**Figure 10.** *In situ* surface and bottom water dissolved oxygen concentration and saturation for the 2012 survey of the PetroSA outfall monitoring programme.

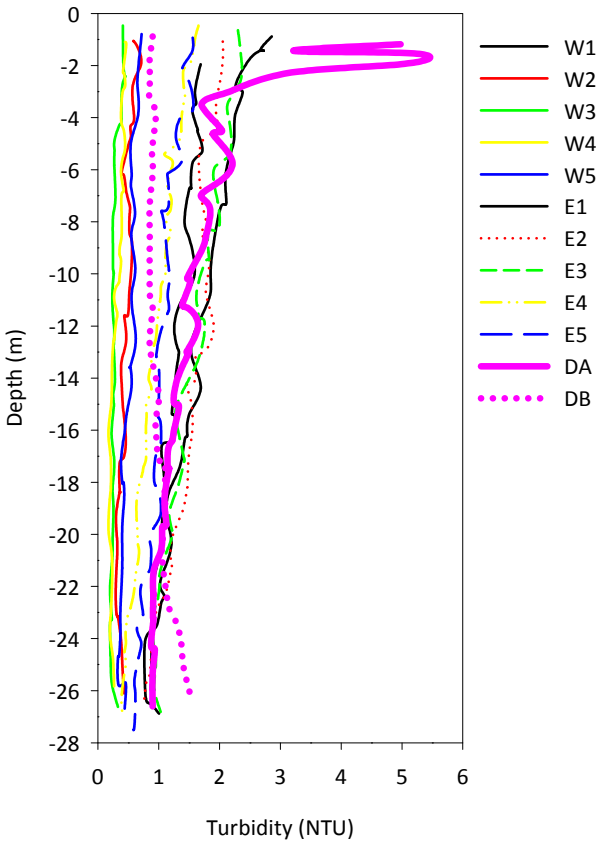


**Figure 11.** *In situ* turbidity profiles for the 2012 survey of the PetroSA outfall monitoring programme.

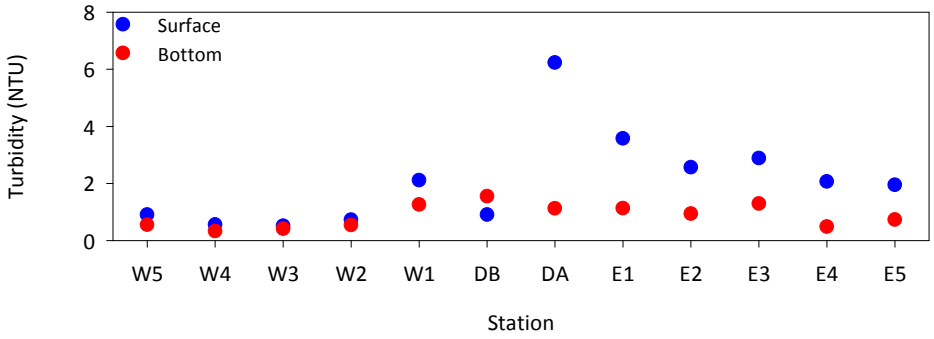


**Figure 12.** *In situ* surface and bottom water turbidity for the 2012 survey of the PetroSA outfall monitoring programme.

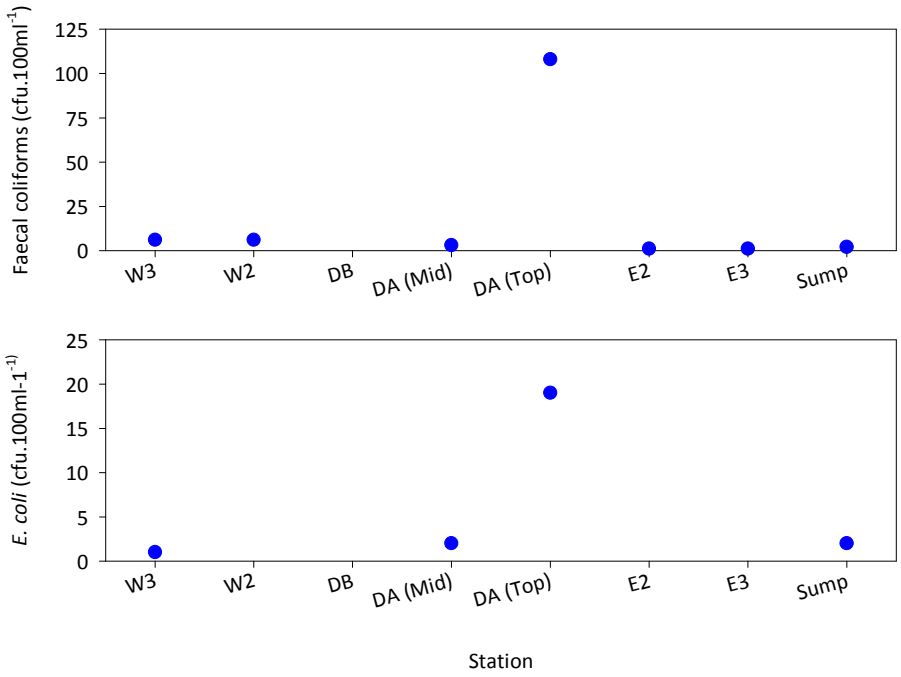


**Figure 13.** Faecal indicator bacteria colony forming unit counts in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. Absent data points reflect the colony forming unit count was below the method detection limit.

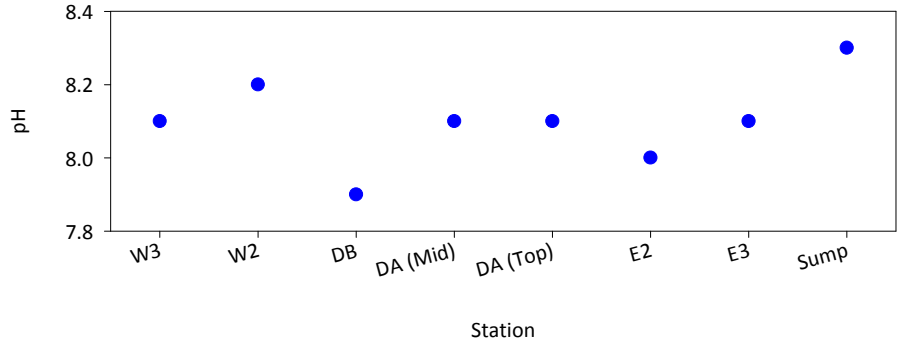


**Figure 14.** pH of discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

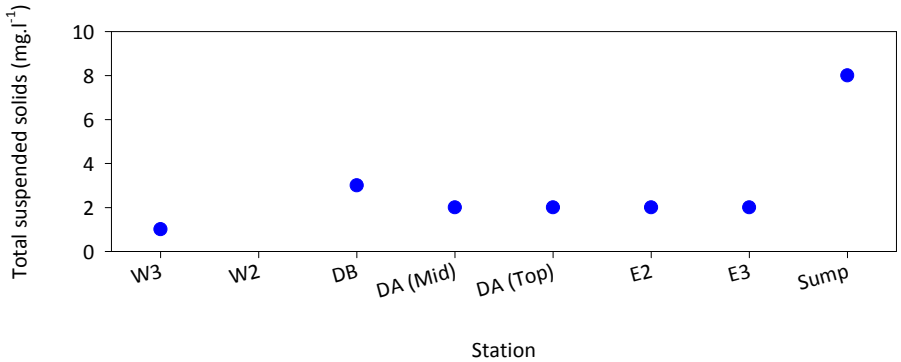


**Figure 15.** Total suspended solids in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. Absent data points reflect the concentration was below the method detection limit.

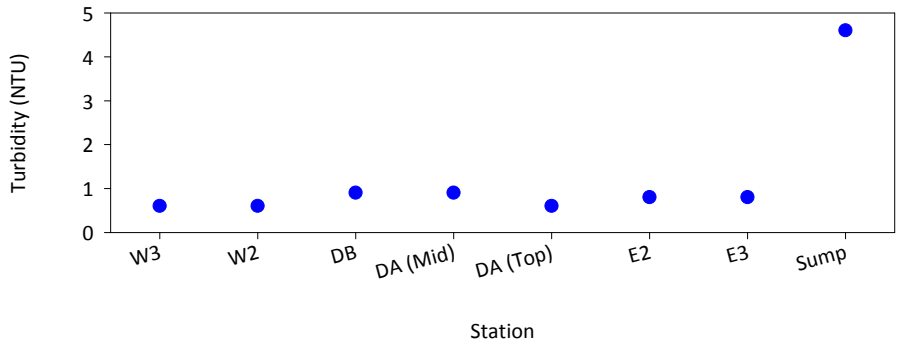


**Figure 16.** Turbidity of discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

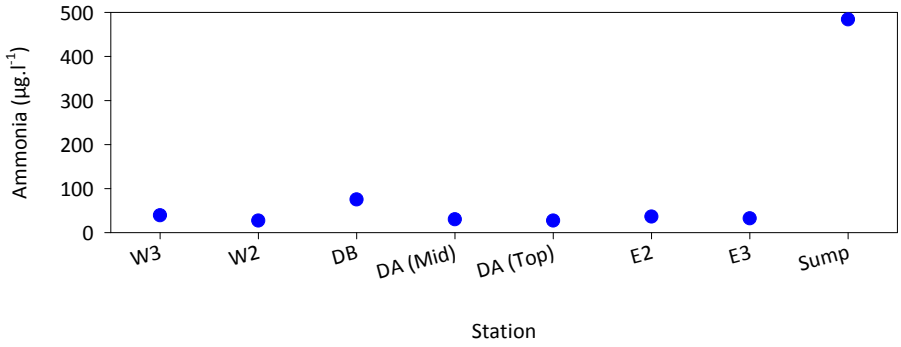


**Figure 17.** Ammonia concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

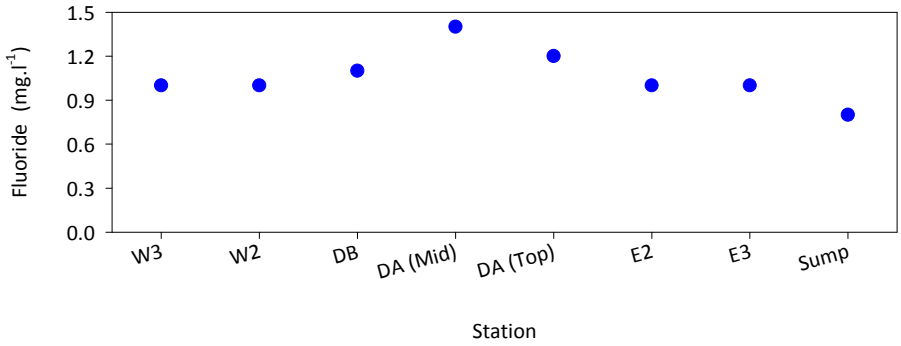


**Figure 18.** Fluoride concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

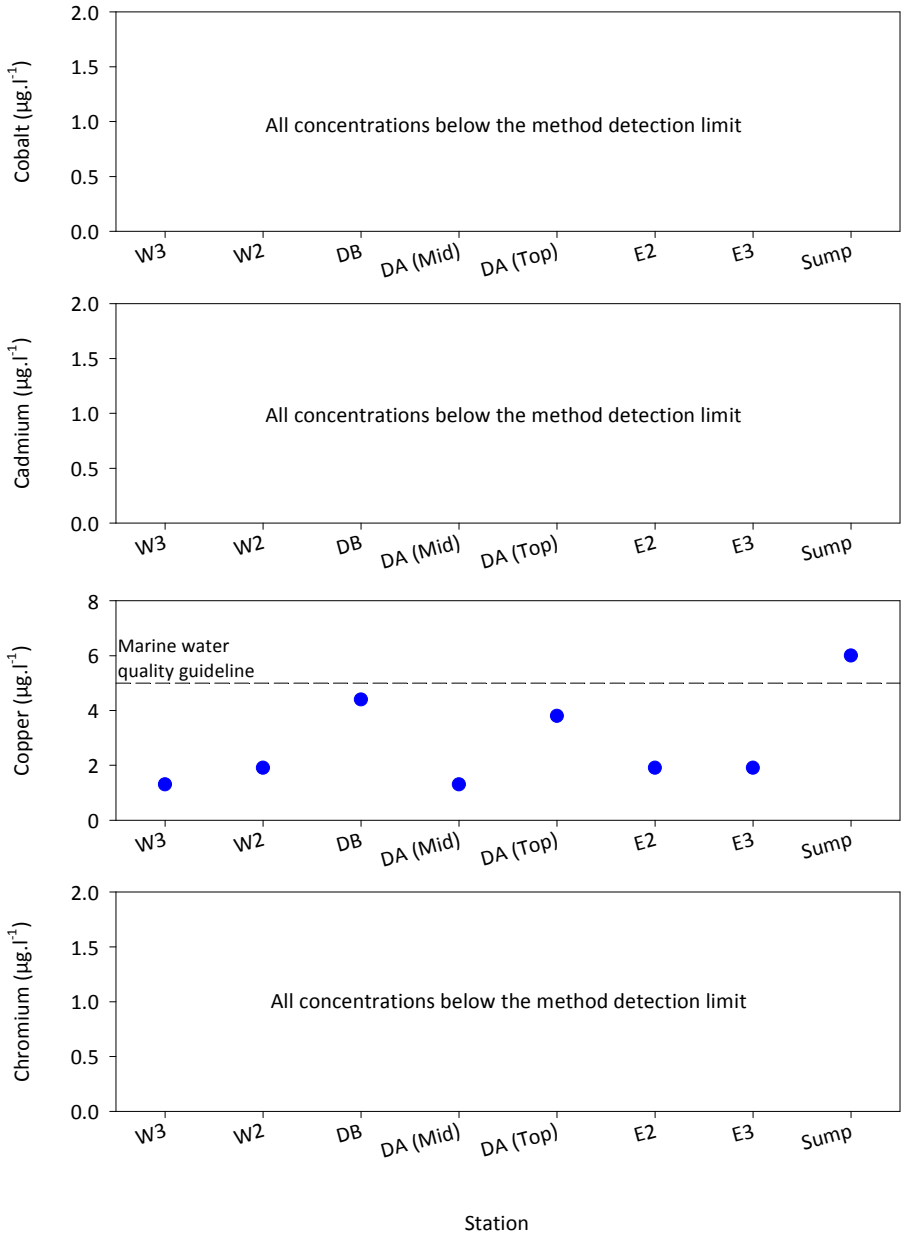


**Figure 19.** Metal concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme.

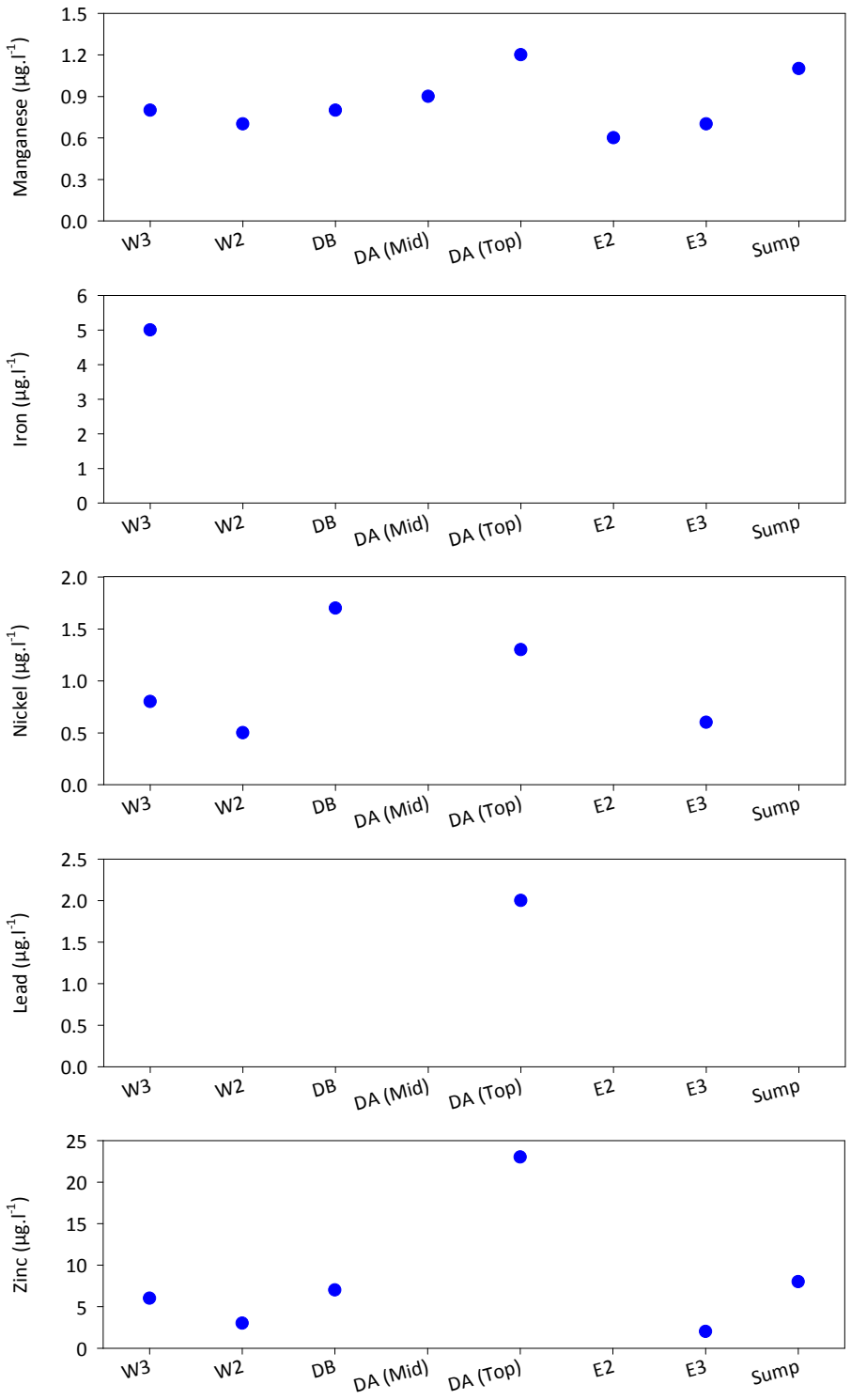


**Figure 19 continued.** Metal concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. Absent data points reflect the concentration was below the method detection limit.

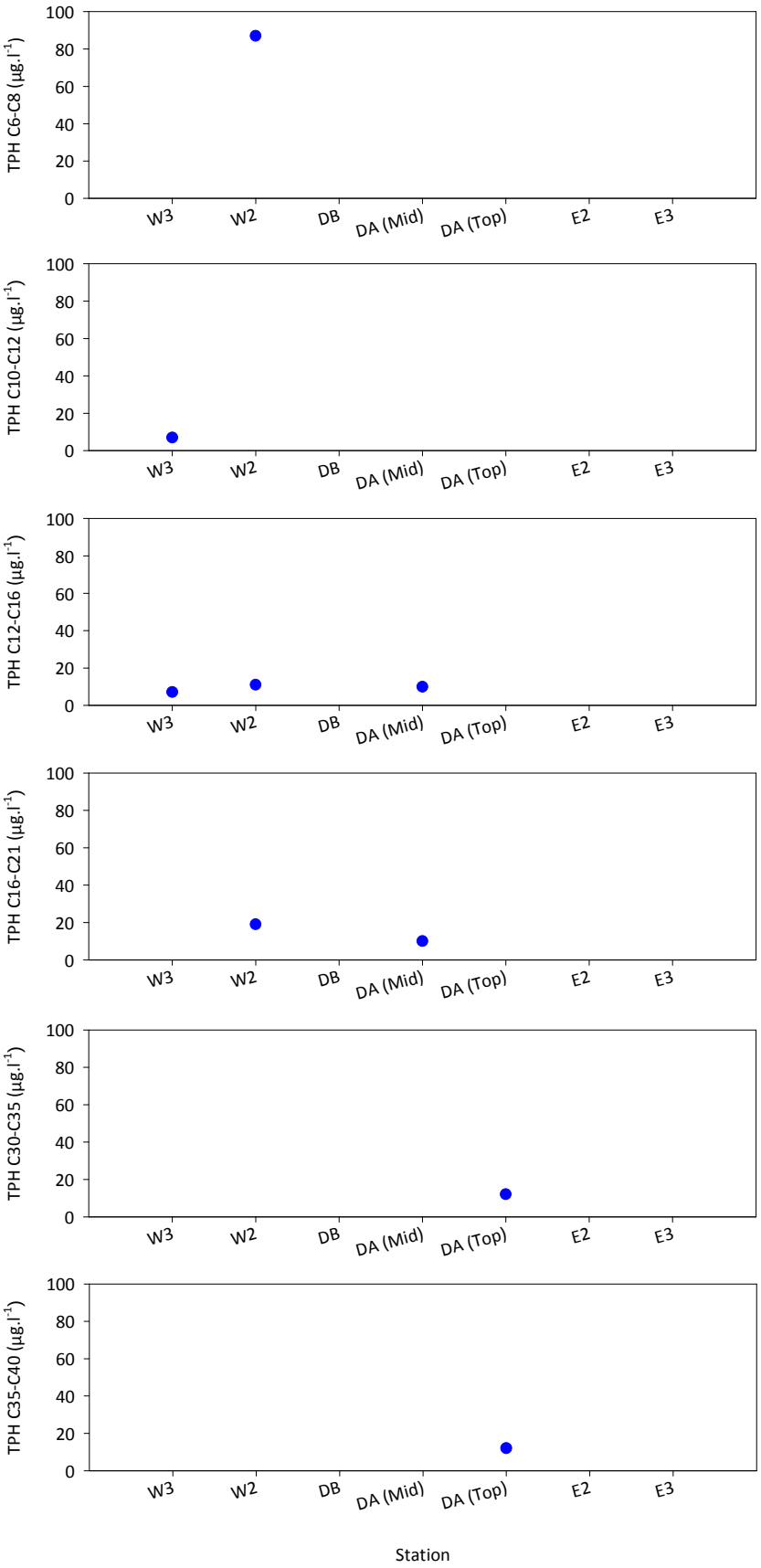


**Figure 20.** Total Petroleum Hydrocarbon concentrations in discrete surface water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. Absent data points reflect the concentration was below the method detection limit.

Very few countries have derived water quality guidelines for BTEX compounds and Total Petroleum Hydrocarbons. Those that are available generally have a very low associated confidence and are very rarely used for assessing water quality. Perhaps the most widely used guidelines for BTEX are those derived for Canadian waters, where the marine water quality guidelines for benzene, ethylbenzene and toluene are 110, 25 and 215 $\mu g.l^{-1}$ respectively. All BTEX concentrations in the Vleesbaai receiving environment were below the method detection limits. Since the method detection limits are well below the Canadian marine water quality guidelines for BTEX constituents, this implies that the BTEX can be considered to pose little ecological risk in Vleesbaai. Total Petroleum Hydrocarbon measurements represent a complex mixture of hydrocarbons that can differ from one sample to another. This explains why it is not possible to relate measurements of these compounds to a toxic effect between samples. Total Petroleum Hydrocarbon measurements are useful for identifying instances of gross hydrocarbon contamination, which if evident indicate that a more detailed assessment of toxic hydrocarbons is required. As stated above, it is not possible to evaluate the possible ecological implications of Total Petroleum Hydrocarbon concentrations in water samples. However, based on the professional opinion of the scientists that prepared this report these chemicals were so sporadically detected and at such low concentrations that they are of little ecological concern.

In conclusion, while there was some evidence for an effluent signal in the receiving environment this was not pronounced and was typically restricted to the water column near the diffuser section. There were no instances of exceedance of the South African Water Quality Guidelines for Coastal Marine Waters (Natural Systems) within or beyond the zone of initial dilution with the exception of turbidity. However, this non-compliance was considered not ecologically significant.

## 6.3. Sediment quality

### 6.3.1. Sediment grain size

Grain size is one of the most important variables that control natural and anthropogenic concentrations of metals in sediment. Alumino-silicates, which predominate in clay, are the major natural metal-bearing phases of sediment. The natural concentrations of most metals are usually strongly positively correlated to the silt and clay (i.e. mud) fraction of sediment, meaning that mud dominated sediment naturally has a higher metal content than sand dominated sediment. Mud also sequesters metals and other particle-reactive contaminants that are anthropogenically introduced to surface waters because of the large surface area provided for adsorption and surface electric charges that render the grains reactive (Förstner and Wittmann 1979, Schropp et al. 1990). Anthropogenically derived contaminants are also preferentially transported with (i.e. adsorbed onto) fine-grained suspended particulate material, which is ultimately deposited and accumulates in depositional areas. These are areas where the sediment is dominated by mud and form where currents are so weak that fine-grained material settles from the water column. In contrast, sand dominated areas are characterised by strong currents, which prevent the settlement and accumulation of fine-grained material. Sand is also dominated by metal deficient quartz. This, coupled with a smaller surface area to volume ratio compared to mud and the absence of surface electric charges, mean that natural and anthropogenically introduced metals and other contaminant concentrations are usually lower in sand dominated sediment.

The grain size composition of sediment thus provides important information for identifying depositional areas in Vleesbaai, where the accumulation of anthropogenically introduced contaminants is theoretically highest. The grain size composition of sediment also provides important information for understanding factors that influence the composition and structure of benthic macrofaunal and meiofaunal communities (discussed in a subsequent section of this report).

From a textural perspective, the sediment at all but one site is classified as sand (Figure 21). At site W5, situated furthest west (2000 m) from the outfall, the sediment is classified as muddy-sand. The sediment at

site W5 is anomalous in the context of the broader study area, since mud contributed 49.65% of bulk sediment weight at this site compared to between 3.05 - 9.06% at other sites. At all sites other than W5, the dominant grain size class was fine-grained sand (between 71.38 - 83.33% of bulk weight). This is similar to the grain size of sediment in 2011, which was also dominated by fine-grained sand at all but one station (Weerts et al. 2012). The low mud fraction of sediment at the majority of sites theoretically implies there is a low probability for the accumulation of particle reactive contaminants in sediment from Vleesbaai.

The sediment at all sites other than W5 was very well- to well-sorted (see Appendix 4 for sorting coefficients). In other words, the sediment was comprised predominantly of grains of roughly the same size. The sediment at site W5 was moderately sorted. Well-sorted sediment is characteristic of high-energy environments. The implication is that the currents that are efficiently sorting sediment over most of the study area are also likely to disperse effluent discharged through the PetroSA outfall efficiently.

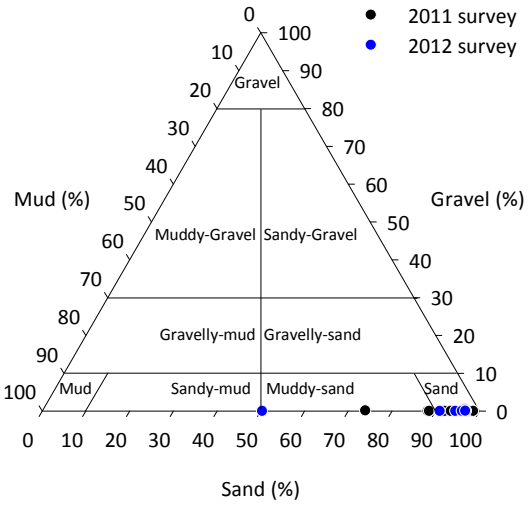


**Figure 21.** Ternary plot illustrating the proportional contribution of gravel, sand and mud to bulk sediment in the study area.

### 6.3.2. Total organic content

Particulate organic matter in sediment provides an additional binding site for contaminants (Wangersky 1986, Stumm and Morgan 1996). Anthropogenic contaminants commonly transported and introduced to aquatic ecosystems also bind to fine-grained particulate matter, including particulate organic matter. Understanding the total organic content of sediment thus assists in identifying areas where the accumulation of contaminants that are partial to adsorption onto particulate organic matter (e.g. hydrocarbons) are theoretically most likely to occur. Some effluents are rich in particulate organic matter and the excessive deposition and accumulation of this matter in sediment near outfalls leads to a host of problems. The most significant is the development of hypoxia and in extreme situations anoxia in sediment and bottom waters when oxygen-consuming bacteria break down the matter and consume oxygen at a rate greater than the rate of re-ventilation. This can lead to the substantial disturbance of benthic macrofaunal communities and have ripple-like impacts through ecosystems (Pearson and Rosenberg 1978, Diaz and Rosenberg 1995).

The total organic content of sediment at all sites other than W5 was very low (≤0.82% of bulk sediment weight, Figure 22). At site W5 the total organic content was relatively high (3.15% of bulk sediment weight, Figure 22). The total organic content cannot be directly compared between sediment samples unless the samples have a similar grain size composition. This is because there is usually a strong positive relationship between the mud fraction and total organic content of sediment in coastal ecosystems in the absence of a significant anthropogenic contribution of particulate organic matter. This is due to the fact that these fine-grained materials are similarly deposited on or winnowed from sediment, depending on prevailing

hydrodynamic conditions. Organic matter is also consumed by bottom-dwelling organisms, preventing its excessive accumulation. The relationship between the mud fraction and total organic content of sediment is beneficial since it permits the definition of a baseline model that can be used to identify sediment that is enriched with organic matter in the same way that baseline models are used to identify metal enriched sediment.

Figure 22 compares the total organic content of sediment collected for the 2012 survey of the PetroSA outfall monitoring programme to the baseline model for total organic content in sediment from the Mossel Bay area. It is evident that the total organic content of sediment at all sites was within the baseline model prediction limits. This indicates that there was no evidence that particulate organic matter in effluent discharged through the PetroSA outfall was accumulating excessively in Vleesbaai sediment. Data from the 2011 survey indicated evidence for very marginal enrichment of sediment with particulate organic matter at a single site near the outfall (Figure 22).

In conclusion, the combined evidence from the 2011 and 2012 surveys of the PetroSA outfall monitoring programme indicates that any particulate organic matter in the discharged effluent is not accumulating excessively in sediment near the outfall, and certainly not to a degree that will result in adverse effects to bottom-dwelling organisms.

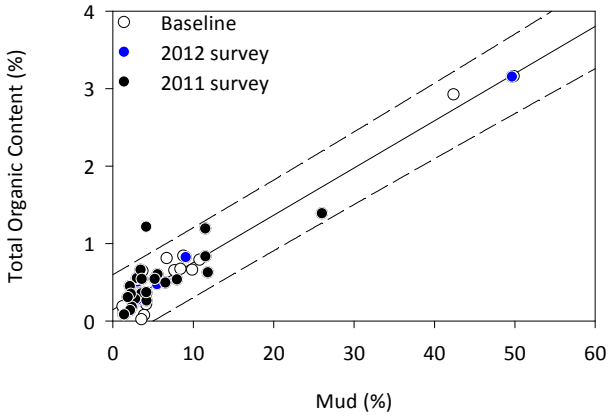


**Figure 22.** Baseline model for total organic content in sediment from the Mossel Bay area, with the total organic content in sediment collected for the 2012 survey of the PetroSA monitoring programme superimposed.

### 6.3.3. Metals

Figure 23 compares the concentrations of metals in sediment collected for the 2011 and 2012 surveys of the PetroSA outfall monitoring programme to baseline models and the baseline cadmium concentration for the Mossel Bay area. The beryllium concentration at one site exceeded the baseline model upper prediction limit and the cadmium concentration at one site exceeded the baseline cadmium concentration. In both these cases, the exceedance was minimal (Figure 23) making it is impossible to conclude that this reflects enrichment due to an anthropogenic contribution. It is highly unlikely that these two instances of elevated metal concentrations reflect enrichment due to anthropogenic input at Vleesbaai.

In the 2011 survey there was also little evidence that metals had accumulated in sediment near the outfall, although in the 2011 survey two manganese concentrations and two arsenic concentrations exceeded baseline model upper prediction limits, and three cadmium concentrations exceeded the baseline cadmium concentration (Figure 23). In all cases the magnitude of exceedance was minimal and it was impossible to conclude that this reflected contamination. Importantly, only one of the sites where metal concentrations exceeded a baseline model upper prediction limit or the baseline cadmium concentration was situated in the vicinity of the outfall. The other sites were situated in the broader Mossel Bay area, and served as sites from which to gain background environmental information.

In conclusion, the combined evidence from the 2011 and 2012 surveys of the PetroSA outfall monitoring programme indicates that metals in the discharged effluent are not accumulating in sediment near the outfall. The almost complete lack of metal enrichment/contamination of sediment implies that there is essentially no risk that metals in sediment within Vleesbaai are adversely affecting bottom-dwelling organisms through toxic effects.

### 6.3.4. Hydrocarbons

BTEX at all sites and most Total Petroleum Hydrocarbons were present in sediment at concentrations below the method detection limit (Figure 24, see also Appendix 6). When this was not the case, the concentrations were typically only slightly higher than the method detection limit and showed no trend with regard to proximity to the outfall diffuser section. The 2012 Vleesbaai environmental monitoring sediment samples thus did not yield any conclusive evidence that hydrocarbons in effluent discharged through the PetroSA outfall are accumulating in the sediment. However, considering that concentrations of BTEX and some Total Petroleum Hydrocarbons were present at fairly high concentrations in the effluent sample analysed (Table 4) and, more importantly, there are no easily identifiable sources of hydrocarbons in Vleesbaai other than the PetroSA effluent discharge, the hydrocarbons that were detected in the sediment samples may indeed have been derived from this effluent.

In the 2011 survey, C10-C12 and C12-C16 Total Petroleum Hydrocarbons were detected at all but a few sites, albeit at low concentrations. Other Total Petroleum Hydrocarbons were only sporadically detected. This is obviously different to the findings for the 2012 survey. BTEX was not measured in the 2011 survey.

Very few countries have derived sediment quality guidelines for BTEX and Total Petroleum Hydrocarbons. Those that are available have a very low associated confidence and are very rarely used for assessing sediment quality. Total Petroleum Hydrocarbon measurements represent a complex mixture of hydrocarbons that can differ from one sample to another and explains why it is not possible to relate measurements of these compounds to a toxic effect across different samples. However, Total Petroleum Hydrocarbon measurements are useful for identifying instances of gross hydrocarbon contamination, which if evident, indicates that a more detailed assessment of toxic hydrocarbons is required. Based on the professional experience and opinion of the scientists that prepared this report these chemicals were so sporadically detected and at such low concentrations that they are of little ecological concern. To provide a point for comparison, the C10-C40 Total Petroleum Hydrocarbon concentrations measured at three sites in the Port of Mossel Bay in 2011 were 42, 130 and 170 $\mu g.kg^{-1}$, compared to <38 $\mu g.kg^{-1}$ at all sites for the 2011 and 2012 surveys of the PetroSA outfall monitoring programme. The only hydrocarbons for which high confidence sediment quality guidelines are available are for Polycyclic Aromatic Hydrocarbons. Polycyclic Aromatic Hydrocarbons were not analysed in the 2012 survey because all but three isomer concentrations in the 24 sediment samples collected for the 2011 survey were at concentrations below the method detection limit. Clearly, therefore, Polycyclic Aromatic Hydrocarbons are not important contaminants of sediment in the Vleesbaai/Mossel Bay area. Should Total Petroleum Hydrocarbon concentrations in sediment collected for future surveys point to gross contamination, then the analysis of Polycyclic Aromatic Hydrocarbons will become necessary.

In conclusion, regardless of the actual source of the hydrocarbons in sediment from Vleesbaai the concentrations were so low that these are of little ecological concern.

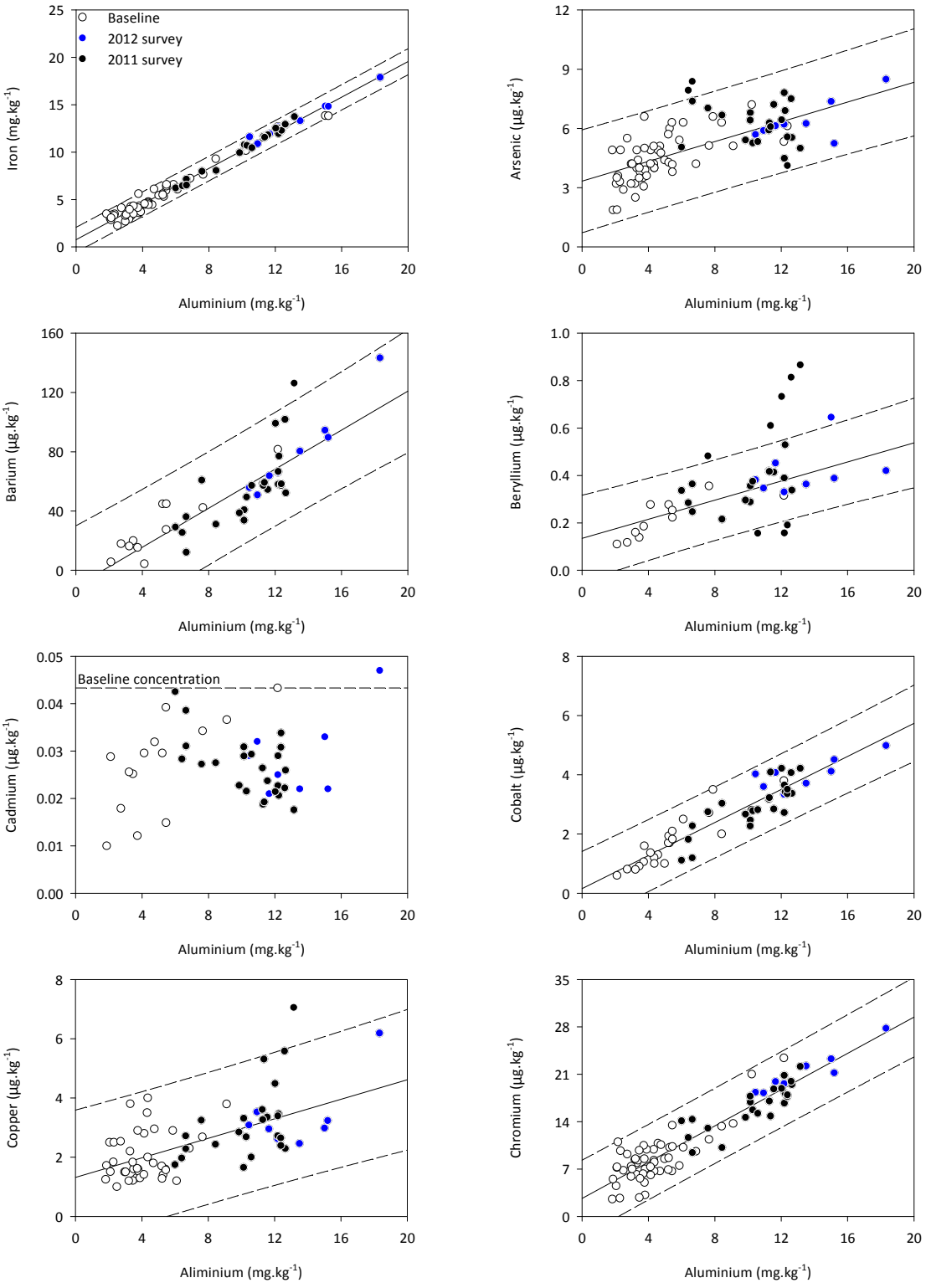


**Figure 23.** Aluminium normalised baseline models for metals in sediment in the Mossel Bay area, with metal concentrations in sediment collected for the 2012 survey of the PetroSA monitoring programme superimposed.

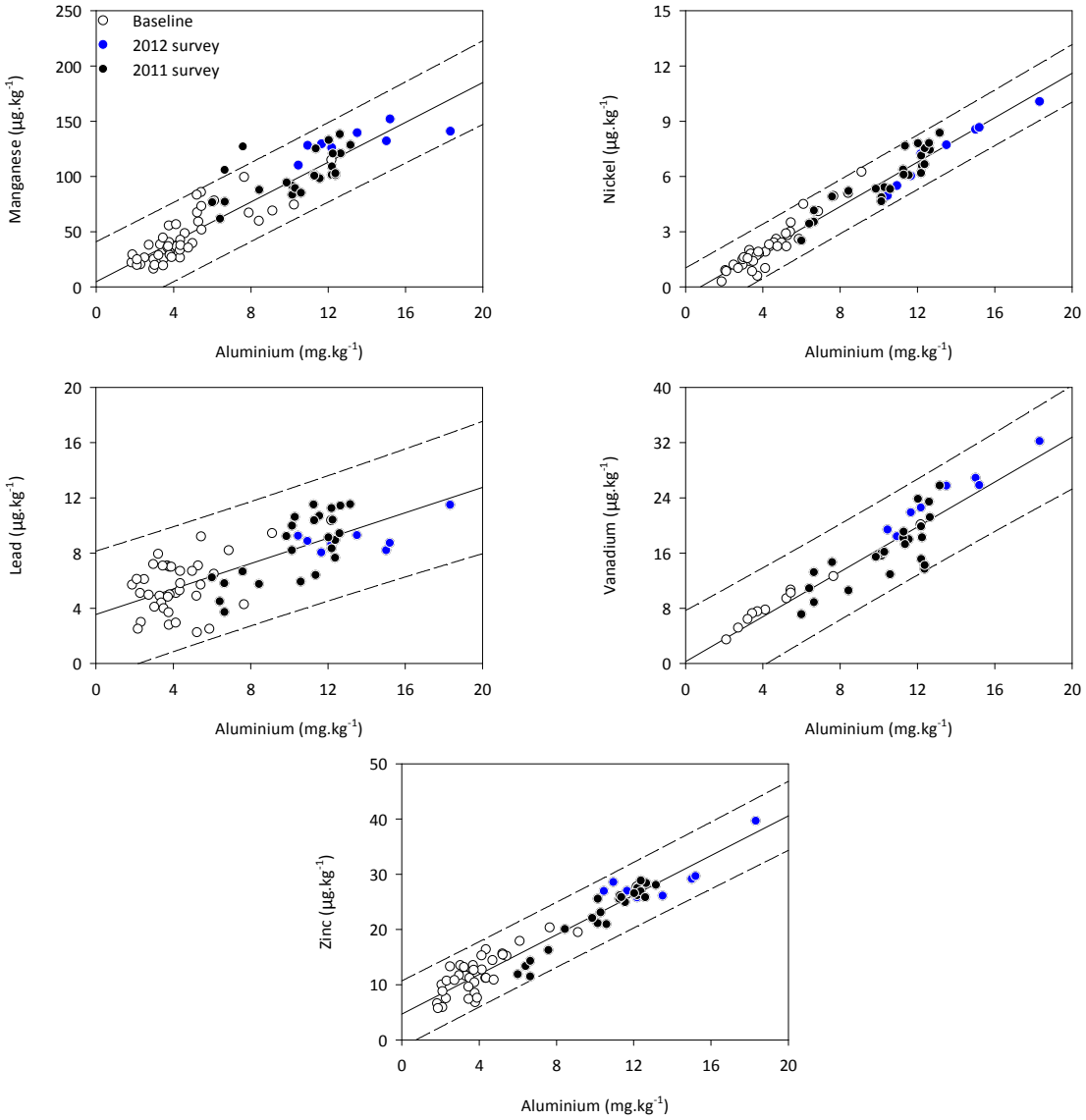


**Figure 23 continued.** Aluminium normalised baseline models for metals in sediment in the Mossel Bay area, with metal concentrations in sediment collected for the 2012 survey of the PetroSA monitoring programme superimposed.

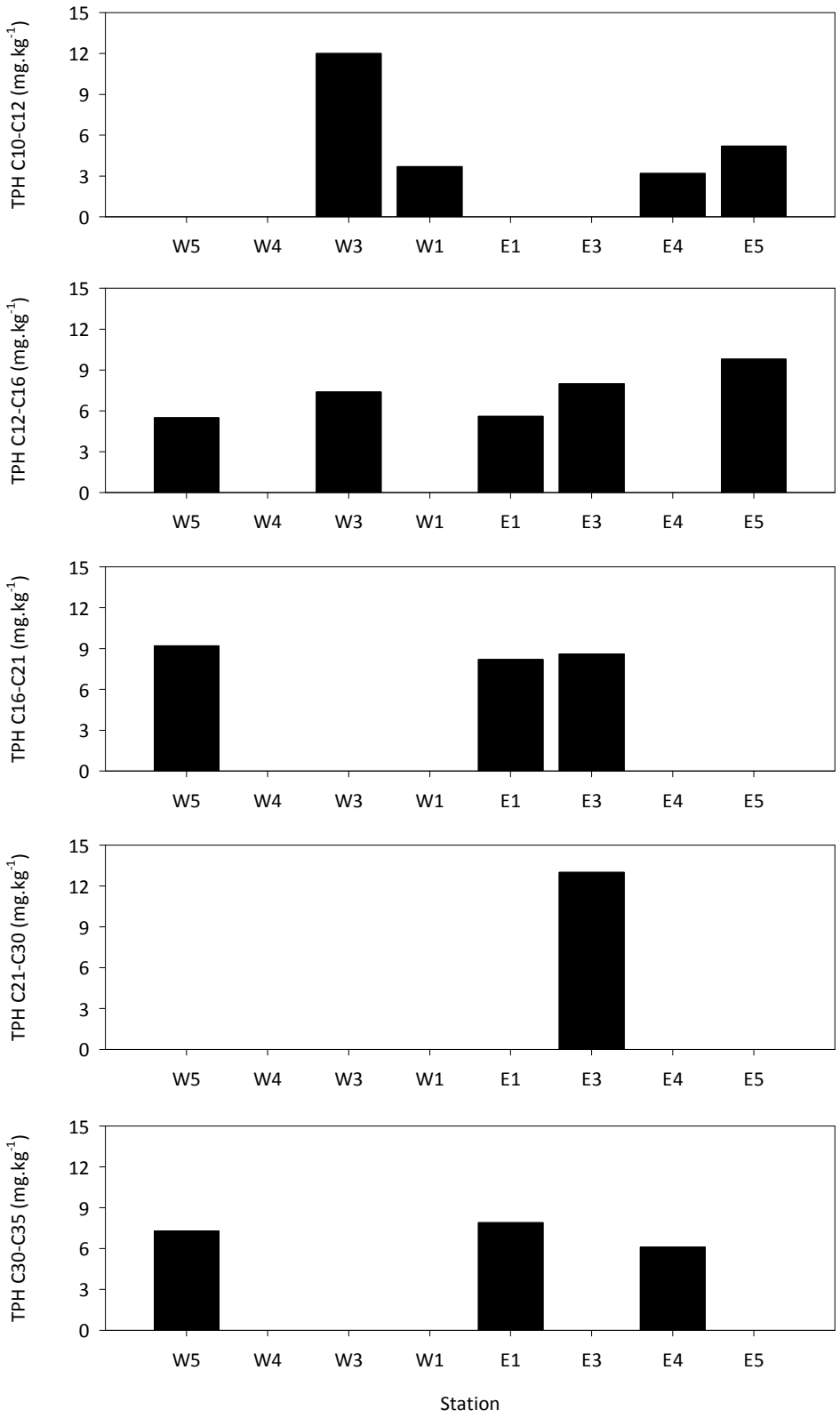


**Figure 24.** Total Petroleum Hydrocarbon concentrations in sediment collected for the 2012 survey of the PetroSA outfall monitoring programme.

## 6.4. Benthic invertebrates

### 6.4.1. Macrofauna

A good array of macrofauna species was sampled from the vicinity of the PetroSA outfall in Vleesbaai in 2012. As was the case in 2011, the benthic community was generally dominated, in terms of abundance, by bristle worms (polychaetes) and small crustaceans (amphipods and isopods) (Appendix 7). Echinoderms (starfish and urchins) and hermit crabs were the main contributors to benthic biomass sampled (Appendix 8), as was also the case in 2011. These are features typical of benthic communities in shallow marine waters of the southern Cape and elsewhere on the South African coast.

Univariate measures of community structure essentially condense the data for each sample into a single index/measure. These measures provide insight into community composition and are based on the number of different taxa and the number of individual animals sampled. Conventional wisdom in assessing marine macrobenthic communities is that a 'healthy' community is characterised by high diversity and an even spread of numbers amongst species. A superabundance of one species in combination with reduced diversity often indicates that the community is stressed.

Figure 25 presents the average univariate index values for sites samples. The highest numbers of taxa, abundance of organisms, species richness and diversity were recorded at site W4. In several cases these differences were significant. With the exception of benthic abundance however, these differences were not markedly consistent across the sampling transect. Typically the only consistent statistical difference noted was that the benthic community at site W3 was more abundant and diverse than that at site E3. Univariate indices at Test sites W1 and E1, in closest proximity to the outfall, did not differ significantly from those at Control sites W3, W4, E3, E4, except for Pielou's evenness (which was higher at Test sites).

In terms of biomass, marked differences were noted with highest biomass sampled at sites E3 and E4 (Figure 25). Although these differences were marked, they were not significant due to the high variability amongst samples from the same site. Biomass at these sites was chiefly attributable to the occurrence of a large bodied sea star *Astropecten cingulatus* (previously called *Astropecten antares*) in a single grab from each site.

Univariate analyses of the benthic communities sampled in Vleesbaai did not provide evidence of any marked impact that might be caused by effluent discharge from the PetroSA outfall. Multivariate analyses however, are typically regarded as a more powerful analytical tool for developing an understanding of ecological impact because they take cognisance of full species arrays and allow a combined analysis of biological community characteristics and univariate physico-chemical measures.

Outputs of ordination (a cluster diagram) and non-metric multidimensional scaling (an ordination plot) on abundance data are given in Figure 26. For the purpose of presentation and interpretation, the ordination plot is forced into two dimensions. This process results in a 2D stress value. The higher this value, the greater the difficulty in presenting the scatter in two dimensions, and the less useful the plot is for interpretation. At a stress value here of 0.18 the ordination plot is still deemed useful. These diagrams give little indication of strong separation of Control and Test sites. Replicate grabs from site W4 returned a distinct cluster of similar communities. ANOSIM conducted on individual sites indicated that there were in fact significant differences amongst all sites with the exception of sites W1 and E1. ANOSIM conducted on Test vs. Control sites indicated no significant differences in benthic communities. Analysis of biomass data returned the same results.

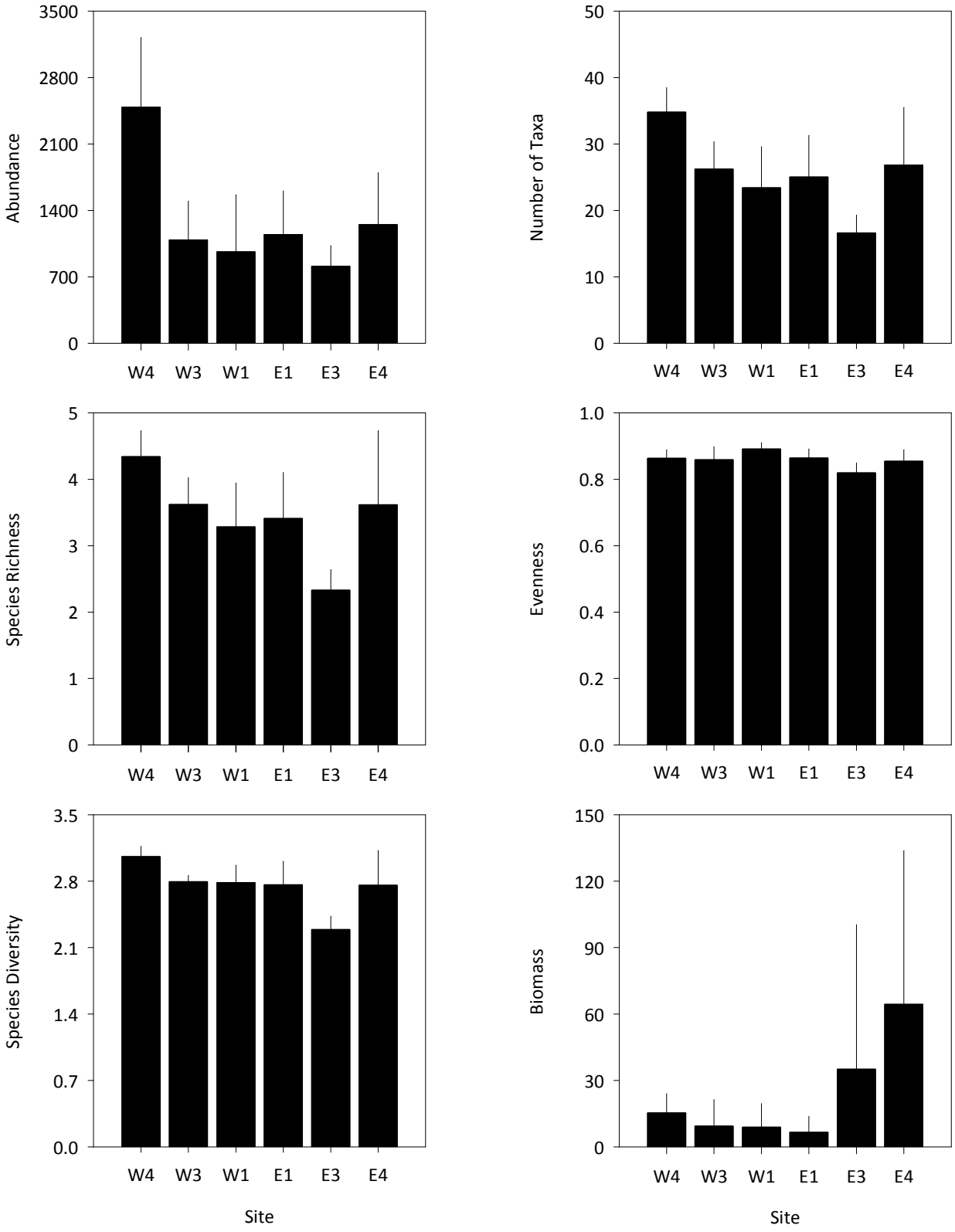


**Figure 25.** Indices of macrobenthic community structure for the 2012 survey of the PetroSA outfalls monitoring programme.

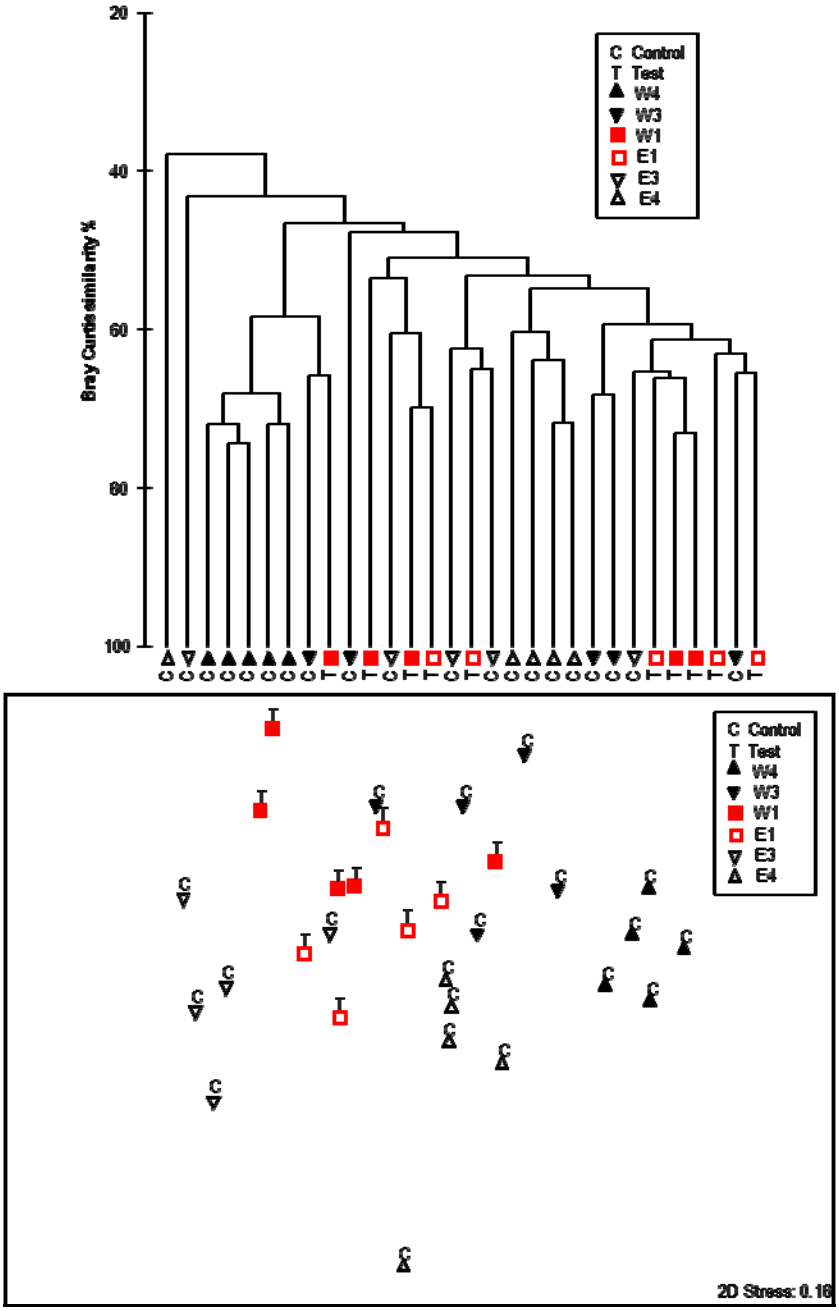


**Figure 26.** Dendrogram (top) and MDS ordination (bottom) of macrobenthic samples (abundance) taken from sampling sites at the PetroSA outfall in Vleesbaai in 2012. Test and Control stations indicated.

The greatest differences amongst sites invariably involved site W4. This site was also identified as supporting a distinct invertebrate community by univariate analyses above (Figure 25). High similarity amongst grab samples taken at site W4 were the result of a similar species array sampled in similar abundances. SIMPER analyses indicated that dissimilarities between this site and others were driven mainly by a relative abundance of *Prionospio* spp. polychaetes and Corophiidae amphipods. The genus *Prionospio* (and the wider Spionid family) includes many taxa that are tolerant of pollution (Nicolaidou et al. 1993, Dean 2008) as are some species of Corophiidae (Lowe and Thompson, 1997). Their relative abundance at a Control site rather than in close proximity to the outfall suggests no pollution impact at the sites monitored. This is strengthened by the paucity of pollution tolerant and strongly opportunistic species at test sites.

BEST analyses were performed incorporating a range of measured factors potentially influencing the benthic communities in Vleesbaai. These included sediment characteristics (granulometry and total organic content) and a suite of trace metal and hydrocarbon concentrations. BEST analyses using abundance data indicated a set of four parameters combined (% fine sand, % very fine sand, manganese and nickel) to give the highest Weighted Spearman correlation ($\rho = 0.854$). This represents a strong correlation. However, in the light of very low levels of metals in sediments in Vleesbaai and the high improbability of metal enrichment, it is most likely that the inclusion of manganese and nickel is a spurious artefact of sediment granulometry rather than a pollution impact. Sediment granulometry, and the percentage of fine sand especially, appears to be the most important driver of benthic communities over the sites surveyed.

In the above the influence of sediment granulometry at site W4 plays a major role. Sediments to the west of the outfall became increasingly muddy. This is most marked at site W5, but of all the sites analysed for benthic communities, site W4 sediments were finer that those sampled elsewhere (Appendix 4). This had a marked impact on the differences noted in benthic assemblages and is typical of soft sediment marine benthos.

### 6.4.2. Meiofauna

Numbers of meiofauna per 100 ml sediment and Nematode/Copepod ratios from the six sites sampled in 2012 are given in Table 5. As was the case in previous studies of meiofauna in 1989 (CSIR 1989), 2000 (CMS 2001), 2002 (CMS 2003) and 2011 (Weerts et al. 2012) there was variability in total numbers of meiofauna. This reflects the natural patchy nature of meiofauna distribution in marine sediments (Armenteros et al. 2008) and differences in sediment grain size.

There has been higher variability in meiofaunal abundance on temporal scales. The pre-outfall survey conducted by the CSIR in 1989 (CSIR 1989) returned numbers of meiofauna ranging from 661 to 2624 individuals per 100 ml sediment. After the installation of the Vleesbaai outfall, two subsequent studies by the University of Cape Towns' Centre for Marine studies (CMS 2001, CMS 2003) revealed lower numbers of meiofauna in proximity to the diffuser section of the outfall (ranges were 344 to 613 and 134 to 430 respectively). The most recent surveys produced numbers per 100 ml of sediment ranging from 1188 to 12268 individuals in 2011 (Weerts et al. 2012) and 857 to 2241 (this survey, 2012).

These differences are marked, but are likely to be influenced to a large extent by differences in sampling and sample processing techniques. The initial survey (CSIR 1989) used a Shipek grab from which cores were removed for meiofauna analysis. The surface area of sediment sampled and analysed is not clear in the report, as neither the cores nor the mesh sizes used were described. The subsequent survey (CMS 2001) used a Ponar grab from which 100 ml sediment was removed for meiofauna analysis after mixing the sediment. A 150 µm mesh was used to retain the meiofauna. The 2002 survey (CMS 2003) employed divers to collect samples with 4 cm diameter cores to a depth of 15 cm. They then also mixed the sediment and removed 100 ml of sediment for meiofauna analysis using a 63 µm mesh. In the most recent surveys (2011

and 2012) a Ponar grab with a bite area of 225 $cm^2$was used. Slightly different volumes were collected each time the grab was deployed so the volume of sediment for each sample was measured prior to separation. The meiofauna from the whole sample were extracted using a modified Oostenbrink separator (Fricke 1979) and a 45 µm mesh. Sub samples were then counted and abundances converted to be expressed as meiofauna per 100 ml sediment.

**Table 5.**Average abundance of meiofauna per 100 ml of sediment collected for the 2012 survey of the PetroSA pipeline monitoring programme.

| Taxa | W4 | W3 | W1 | E1 | E2 | E3 |
|---|---|---|---|---|---|---|
| Turbellaria | 0.4 | 25.6 | 50.9 | 204.0 | 81.5 | 64.0 |
| Nematoda | 1843.2 | 1171.2 | 734.5 | 1273.0 | 640.0 | 1856.0 |
| Rotifera | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Gastrotricha | 19.2 | 76.8 | 14.5 | 408.0 | 110.5 | 12.8 |
| Kinorhyncha | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Annelida | 0.4 | 1.6 | 2.9 | 24.0 | 1.5 | 25.6 |
| Tardigrada | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Acarina | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ostracoda | 38.4 | 1.6 | 2.9 | 8.0 | 1.5 | 0.0 |
| Copepod nauplii | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Harpacticoida | 6.4 | 102.4 | 36.4 | 228.0 | 154.2 | 76.8 |
| Amphipoda | 0.0 | 0.0 | 1.5 | 0.0 | 5.8 | 0.0 |
| Isopoda | 19.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sarcomastigophora | 115.2 | 51.2 | 13.1 | 96.0 | 29.1 | 70.4 |
| | | | | | | |
| Total abundance | 2042.4 | 1430.4 | 856.7 | 2241.0 | 1024.0 | 2105.6 |
| Number of Taxa | 8 | 7 | 8 | 7 | 8 | 6 |
| Nematode/Copepod ratio | 288.0 | 11.4 | 20.2 | 5.6 | 4.2 | 24.2 |

The lack of information on sample processing methods in the initial CSIR reports, and especially the mesh size used to retain meiofauna, is unfortunate. Despite their best efforts the scientists who prepared this report have not been able to find this information. Differences in mesh size used to sieve meiofauna would undoubtedly have played a major role in differences in abundances of fauna reported. CMS (2003) reported that the 1989 baseline survey used 500 µm meshes. This is unlikely for several reasons. The fauna recorded included high numbers of nematodes and harpacticoid copepods, taxa that typically pass through 500 µm meshes. This survey also reported higher abundance of meiofauna than both the 2000 and 2002 survey. Larger sieve size would more likely have resulted in far reduced abundances.

Sampling technique might have contributed to differences over different surveys. The depth of sediment analysed plays an important role in the abundance of meiofauna sampled (when expressed per unit volume of sediment). The majority of the meiofauna are found in the top few centimetres of sublittoral sediment. Mazzola et al. (2000), for example, found approximately 60% of meiofauna in the upper 1cm of sublittoral sediment sampled, and approximately 97 % in the upper 5cm. Using a 15 cm core and mixing the sediment before extracting 100 ml for meiofauna analysis (as per CMS 2003), probably resulted in a dilution of meiofauna by approximately 66 % compared to the recent surveys. The CMS survey of 2002 used cores with a surface area of 12.6 $cm^2$ (diameter of 4 cm). Therefore broader shallower cores would collect many times more meiofauna per 100 ml sediment than narrow deep cores.

Differences however, might also have been real. A concern noted from surveys in 2000 and 2002 was the complete lack of harpacticoid copepods in meiofauna at most sites in the vicinity of the Vleesbaai outfall. Along with a high Nematode/Copepod ratio, this was regarded (justifiably so) as evidence of pollution impact (although not necessarily from the PetroSA outfall). The 2011 and 2012 surveys have seen an increased total abundance of meiofauna compared to both the 2000 and 2002 surveys, and a reduction in Nematode/Copepod ratio to levels that are approaching those reported in 1989. In both 2011 and 2012,

Nematode/Copepod ratios in close proximity to the diffuser section of the outfall were generally lower than those further away. This is at least partly caused by sediment granulometry, with the finest sediments in both surveys reported further away from the outfall. Harpacticoid copepod abundance and the (related) Nematode/Copepod ratio are directly influenced by granulometry. Nematodes have a broad preference for muddy sediments while copepods prefer sands (McLachlan et al. 1981, Warwick 1981, Vinx and Heip 1991). Overall, however, the spatial distribution of meiofauna in the area sampled in 2012 gives no indication of a persistent pollution impact from the PetroSA outfall. It is not known if the nature and/or volume of the PetroSA effluent, or other potential pollution sources in Vleesbaai have changed over the years, but previous indications of a pollution impact in 2000 (CMS 2001) and 2002 (CMS 2003) have not persisted to recent times.

# 7. Conclusions

The primary purpose of this study was to determine whether the discharge of effluent through the PetroSA outfall is adversely impacting on the ecology of the Vleesbaai receiving environment. The analysis of a wide suite of physical, chemical and biological parameters in water and sediment collected from Vleesbaai in November 2012 provided little evidence that the water or sediment was impaired by effluent discharge with the exception of water quality (turbidity) in the immediate vicinity of the discharge. Impairment of water quality in the immediate vicinity of effluent discharges is to be expected. Importantly, however, the values/concentrations of all parameters in water sampled were lower than or within target guidelines defined for South African coastal marine waters, with the exception of turbidity.

There was no evidence that particulate organic matter or metals in effluent discharged are accumulating in sediment in Vleesbaai. In fact, all metal concentrations were within the expected concentration range for uncontaminated sediment in the Mossel Bay area. Some petroleum hydrocarbons were detected in sediment, but at such low concentrations that they are of little ecological concern.

The lack of any significant water or sediment quality impairment is reflected in the status of benthic macrofauna and meiofauna communities, which showed no aberrations typical for effluent discharge or pollution.

The above conclusions on the impact of effluent discharge through the PetroSA outfall were similar to those concluded for the 2011 survey of the monitoring programme. Thus, based on the findings of the 2011 and 2012 surveys, there is no evidence that the assimilative capacity of the Vleesbaai receiving environment has been exceeded.

# 8. Recommendations for future monitoring

No modifications to the sampling design are deemed necessary for sampling in 2013. Whilst not a critical component, the sampling design would be strengthened by the toxicity testing (using the sea urchin fertilisation test) of effluent at regular intervals (monthly). The toxicity testing of receiving water samples collected during the annual survey is also recommended.

# 9. Acknowledgements

The South African Environmental Observation Network (SAEON) and the CSIR acknowledge the cooperation and assistance of the captain and crew of the tender vessel *Pacific Askari*, who competently assisted with the field component of this survey. Colin Barclay (PetroSA) is thanked for his assistance in coordinating the use of the vessel for environmental sampling. The parataxonomy team from the SAEON Elwandle Node in Grahamstown are thanked for their macrofauna sorting expertise.

# 10. References


Armenteros M, Williams JP, Creagh B and Capetillo N (2008) Spatial and temporal variations of meiofaunal communities from the western sector of the Gulf of Batabano, Cuba: III. Vertical distribution. *Revista de Biologia Tropical* 56: 1127-1134.

Aurecon (2011) Section 24G Environmental Impact Assessment: 15 Mℓ/day Emergency Desalination Plant, Mossel Bay: Draft Environmental Impact Report. Available at http://aurecon.webfoundryza.com/assets/files/MosselBayDesal.

Ballesteros E, Torras X, Pinedo S, García M, Mangialajo L and de Torres M (2007) A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. *Marine Pollution Bulletin* 55:172-80.

Bray JRand Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecological monographs* 27: 325-349.

Chapman PM (1989) Current approaches to developing sediment quality criteria. *Environmental Toxicology and Chemistry* 8: 589-599.

Clarke KR and Green RH (1988) Statistical design and analysis for a 'biological effects' study.*Marine Ecology Progress Series* 46: 213-226.

Clarke KR and Warwick RM (2001) *Change in marine communities: An approach to statistical analysis and interpretation.*Second edition. PRIMER-E: Plymouth: Plymouth Marine Laboratory.

CMS (2001) *Beach and sub-tidal meiofauna from around the outfall of the Mossgas (Pty) Ltd outfall in Mossel Bay: possible pollution effects*. Final Report compiled by Centre for Marine Studies by AC Brown for Mossgas (Pty) Ltd.

CMS (2003) *Biomonitoring of subtidal Meiofauna around the outfall of the Petroleum Oil and Gas Corporation of South Africa Manufacturing site outfall in Mossel Bay*. Final Report compiled by Centre for Marine Studies by N Steffani, for PetroSA.

CSIR (1989) Biological Assessment for the new Effluent Pipeline in Mossel Bay. CSIR Report EMA-C 89118.

Dean HK (2008) The use of polychaetes (Annelida) as indicator species of marine pollution: a review. *Revista de Biologia Tropical* (San Jose) 56: 11-38.

Diaz RJ and Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology* 33: 245-303.

DWAF (Department of Water Affairs and Forestry) (1995) *South African Water Quality Guidelines for Coastal Marine Waters. Volume 2: Recreational Use.* Pretoria, South Africa.

Field JG, Clarke KR and Warwick RM (1982) A practical strategy for analyzing multispecies distribution patterns. *Marine Ecological Progress Series* 8: 37-52

Fleeger JW, Carman KR and Nisbet RM (2003) Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment* 317:207-233.

Förstner U and Wittmann GTW (1979) *Metal Pollution in the Aquatic Environment*. Springer-Verlag, New York.

Fricke AM (1979) Meiofauna extraction by a modified Oostenbrink apparatus. *Helgolander Wissenschaft Meeresuntersuchungen*32: 436-443.

Hornberger MI, Luoma SN, Cain DJ, Parchaso F, Brown CL, Bouse RM, Wellise C and Thompson JK (2000) Linkage of bioaccumulation and biological effects to changes in pollutant loads in South San Francisco Bay. *Environmental Toxicology and Chemistry* 34: 2401-2409.

Horowitz AJ (1991) *A Primer on Sediment-Trace Element Chemistry*. Lewis Publishers Inc, Michigan.

Lowe S and Thompson B (1997) Identifying benthic indicators for San Francisco Bay, Regional Monitoring Program, Annual Report. San Francisco Estuary Institute, Oakland, CA.

McLachlan A, Wooldridge T and Dye A (1981) The ecology of sandy beaches in South Africa. *South African Journal of Zoolog*y 16: 219-131.

Magurran AE (1988) *Ecological Diversity and its Measurement*. Chapman and Hall, London.

Mazzola A, Mirto S, La Rosa T, Fabiano M and Danovaro R (2000) Fish Farming effects on benthic

community structure in coastal sediments: analysis of meiofaunal recovery. *Journal of Marine Science* 57: 1454-1461.

McPherson C, Chapman PM, de Bruyn AMH and Cooper L (2008) The importance of benthos in weight of evidence sediment assessments - A case study. *Science of the Total Environment*394:252-264.

Nicolaidou A, Zenetos A, Pancucci MA and Simboura N (1993), Comparing Ecological Effects of two Different Types of Pollution Using Multivariate Techniques. *Marine Ecology*: 14: 113–128.

Pearson TH and Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanographic and Marine Biology Annual Review* 16: 229-311.

Rae JE and Allen JRL (1993) The significance of organic matter degradation in the interpretation of historical pollution trends in depth profiles of estuarine sediment. *Estuaries* 16: 678-682.

Ranasinghe JA, Weisberg SB, Smith RW, Montagne DE, Thompson B, Oakden JM, Huff DD, Cadien DB, Velarde RG and Ritter KJ (2009) Calibration and evaluation of five indicators of benthic community condition in two California bay and estuary habitats. *Marine Pollution Bulletin* 59: 5-13.

Schropp S, Lewis FG, Windom HL, Ryan JD, Calder FDand Burney LC (1990) Interpretation of metal concentrations in estuarine sediments of Florida using aluminum as a reference element. *Estuaries* 13: 227-235.

Sink K, Holness S, Harris L, Majiedt P, Atkinson L, Robinson T, Kirkman S, Hutchings L, Leslie R, Lamberth S, Kerwath S, von der Heyden S, Lombard A, Attwood C, Branch G, Fairweather T, Taljaard S, Weerts S, Cowley P, Awad A, Halpern B, Grantham H and Wolf T (2012) National Biodiversity Assessment 2011: Technical Report. Volume 4: Marine and Coastal Component. South African National Biodiversity Institute, Pretoria.

Stumm W and Morgan JJ (1996) *Aquatic chemistry: An introduction emphasizing chemical equilibria in natural water*. Wiley, New York.

Thompson B and Lowe S (2004) Assessment of macrobenthos response to sediment contamination in the San Francisco estuary, California, USA. *Environmental Toxicology and Chemistry* 23: 2178-2187.

Underwood AJ (1994) On Beyond BACI: Sampling designs that might reliably detect environmental disturbances. *Ecological Applications*4: 3-15

Vinx M and Heip C (1991) The use of meiobenthos in pollution monitoring studies: A review. *International Council for the Exploration of the Sea* 16: 50-67.

Wangersky PJ (1986) Biological control of trace metal residence time and speciation: A review and synthesis. *Marine Chemistry* 18: 269-297.

Warwick RM (1981) The Nematode/Copepod ratio and its use in pollution ecology. *Marine Pollution Bulletin* 12: 329-333.

Warwick RM (1993) Environmental impact studies on marine communities: pragmatical considerations. *Australian Journal of Ecology* 18: 63-80.

WeertsSP, Newman BK, Blair AG and Atkinson LJ (2012) *Assessment of the inshore subtidal environment in the vicinity of the PetroSA Vleesbaai pipeline: November 2011 survey.* CSIR Report CSIR/NRE/CO/ER/2012/0040/C.

Zar JH (1996) *Biostatistical Analysis*. Prentice-Hall, New Jersey.

# 11. Appendices

**Appendix 1.** Results for physical, chemical and biological parameters analysed in discrete water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. < = value/concentration below the method detection limit.

| Site | Fluoride ($mg/l^{-1}$) | Conductivity (mSm) | pH | Turbidity (NTU) | Suspended solids ($mg/l^{-1}$) | Ammonia ($\mu g.l^{-1}$) | *E. coli* (cfu/100 ml) | Faecal coliforms (cfu/100 ml) |
|---|---|---|---|---|---|---|---|---|
| W3 | 1.0 | 5000 | 8.1 | 0.6 | 1 | 39 | 1 | 6 |
| W2 | 1.0 | 4950 | 8.2 | 0.6 | <1 | 27 | <1 | 6 |
| DA1 | 1.4 | 5400 | 8.1 | 0.9 | 2 | 30 | 2 | 3 |
| DA2 | 1.2 | 5000 | 8.1 | 0.6 | 2 | 27 | 19 | 108 |
| DB | 1.1 | 5000 | 7.9 | 0.9 | 3 | 75 | <1 | <1 |
| E2 | 1.0 | 5000 | 8.0 | 0.8 | 2 | 36 | <1 | 1 |
| E3 | 1.0 | 4900 | 8.1 | 0.8 | 2 | 32 | <1 | 1 |

**Appendix 2.** Metal concentrations ($\mu g.l^{-1}$) in discrete water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. < = concentration below the method detection limit.

| Site | Cadmium | Lead | Cobalt | Chromium | Copper | Iron | Manganese | Nickel | Zinc |
|---|---|---|---|---|---|---|---|---|---|
| W3 | <0.1 | <1 | <0.2 | <0.5 | 1.3 | 5 | 0.8 | 0.8 | 6 |
| W2 | <0.1 | <1 | <0.2 | <0.5 | 1.9 | <5 | 0.7 | 0.5 | 3 |
| DA1 | <0.1 | <1 | <0.2 | <0.5 | 4.4 | <5 | 0.9 | <0.5 | <2 |
| DA2 | <0.1 | 2 | <0.2 | <0.5 | 1.3 | <5 | 1.2 | 1.3 | 23 |
| DB | <0.1 | <1 | <0.2 | <0.5 | 3.8 | <5 | 0.8 | 1.7 | 7 |
| E2 | <0.1 | <1 | <0.2 | <0.5 | 1.9 | <5 | 0.6 | <0.5 | <2 |
| E3 | <0.1 | <1 | <0.2 | <0.5 | 1.9 | <5 | 0.7 | 0.6 | 2 |

**Appendix 3.** Hydrocarbon concentrations ($\mu g.l^{-1}$) in discrete water samples collected for the 2012 survey of the PetroSA outfall monitoring programme. TPH- Total Petroleum Hydrocarbons, < = concentration below the method detection limit.

| Site | W3 | W2 | DA1 | DA2 | DB | E2 | E3 |
|---|---|---|---|---|---|---|---|
| Benzene | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 |
| Toluene | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 |
| Ethylbenzene | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 |
| o-Xylene | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 |
| m,p-Xylene | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 | <0.20 |
| Xylenes (sum) | <0.40 | <0.40 | <0.40 | <0.40 | <0.40 | <0.40 | <0.40 |
| BTEX (sum) | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| TPH (C6-C8) | <30 | 87 | <30 | <30 | <30 | <30 | <30 |
| TPH (C8-C10) | <30 | <30 | <30 | <30 | <30 | <30 | <30 |
| TPH (C6-C10) | <60 | 110 | <60 | <60 | <60 | <60 | <60 |
| TPH (C10-C12) | 6.9 | <4.0 | <4.0 | <4.0 | <4.0 | <4.0 | <4.0 |
| TPH (C12-C16) | 7.1 | 11 | 9.9 | <5.0 | <5.0 | <5.0 | <5.0 |
| TPH (C16-C21) | <6.0 | 19 | 10 | <6.0 | <6.0 | <6.0 | <6.0 |
| TPH (C21-C30) | <10 | <10 | <10 | <10 | <10 | <10 | <10 |
| TPH (C30-C35) | <5.0 | <5.0 | <5.0 | 12 | <5.0 | <5.0 | <5.0 |
| TPH (C35-C40) | <8.0 | <8.0 | <8.0 | 9.2 | <8.0 | <8.0 | <8.0 |
| TPH (C10-C40) | <38 | <38 | <38 | <38 | <38 | <38 | <38 |

**Appendix 4.** Grain size composition and statistics, and total organic content of sediment collected for the 2012 survey of the PetroSA outfall monitoring programme. VCS = very coarse-grained sand, CS = coarse-grained sand, MS = medium-grained sand, FS = fine-grained sand, VFS = very fine-grained sand, TOC = total organic content.

| Site | Gravel (%) | VCS (%) | CS (%) | MS (%) | FS (%) | VFS (%) | Mud (%) | Mean (mm) | Median (mm) | Sorting coefficient | TOC (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| W5 | 0.04 | 0.07 | 0.88 | 5.09 | 20.84 | 23.44 | 49.65 | 0.08 | 0.07 | 0.99 | 3.15 |
| W4 | 0.00 | 0.00 | 0.25 | 3.19 | 71.38 | 16.11 | 9.06 | 0.18 | 0.17 | 0.50 | 0.82 |
| W3 | 0.07 | 0.29 | 0.73 | 9.37 | 77.38 | 8.97 | 3.18 | 0.18 | 0.20 | 0.35 | 0.27 |
| W1 | 0.00 | 0.09 | 0.34 | 17.23 | 71.83 | 7.46 | 3.05 | 0.19 | 0.20 | 0.38 | 0.28 |
| E1 | 0.00 | 0.05 | 0.38 | 4.81 | 80.66 | 8.59 | 5.50 | 0.18 | 0.18 | 0.54 | 0.47 |
| E3 | 0.00 | 0.03 | 0.16 | 1.59 | 81.70 | 13.25 | 3.27 | 0.17 | 0.17 | 0.28 | 0.34 |
| E4 | 0.00 | 0.00 | 0.40 | 4.23 | 82.30 | 9.97 | 3.09 | 0.18 | 0.18 | 0.30 | 0.51 |
| E5 | 0.00 | 0.12 | 0.35 | 3.50 | 83.33 | 8.73 | 3.97 | 0.17 | 0.18 | 0.29 | 0.30 |

**Appendix 5.** Metal concentrations ($mg.g^{-1}$ for aluminium and iron, $\mu g.g^{-1}$ for all other metals; dry weight) in sediment collected for the 2012 survey of the PetroSA outfall monitoring programme. Al = aluminium, Fe = iron, As = arsenic, Ba = barium, Be = beryllium, Cd = cadmium, Co = cobalt, Cu = copper, Cr = chromium, Hg = mercury, Mn = manganese, Ni = nickel, Pb = lead, V = vanadium, Zn = zinc. < = concentration below the method detection limit.

| Site | Al | Fe | As | Ba | Be | Cd | Co | Cu | Cr | Mn | Hg | Ni | Pb | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W5 | 18.32 | 17.89 | 8.49 | 143.3 | 0.42 | 0.05 | 4.98 | 6.18 | 27.77 | 140.9 | <0.03 | 10.07 | 11.49 | 32.20 | 39.65 |
| W4 | 10.94 | 10.88 | 5.89 | 50.78 | 0.35 | 0.03 | 3.60 | 3.53 | 18.22 | 128.0 | <0.03 | 5.49 | 8.88 | 18.42 | 28.55 |
| W3 | 12.18 | 12.66 | 6.20 | 65.92 | 0.33 | 0.03 | 3.34 | 2.62 | 19.60 | 125.9 | <0.03 | 7.23 | 8.66 | 22.57 | 25.76 |
| W1 | 13.50 | 13.30 | 6.25 | 80.31 | 0.36 | 0.02 | 3.71 | 2.46 | 22.20 | 139.5 | <0.03 | 7.71 | 9.29 | 25.75 | 26.09 |
| E1 | 15.01 | 14.85 | 7.37 | 94.48 | 0.65 | 0.03 | 4.11 | 2.98 | 23.26 | 132.1 | 0.10 | 8.55 | 8.20 | 26.88 | 29.14 |
| E3 | 15.20 | 14.82 | 5.24 | 89.68 | 0.39 | 0.02 | 4.51 | 3.23 | 21.20 | 151.9 | <0.03 | 8.66 | 8.73 | 25.83 | 29.66 |
| E4 | 11.66 | 11.93 | 6.13 | 63.77 | 0.45 | 0.02 | 4.07 | 2.95 | 19.89 | 129.4 | <0.03 | 6.03 | 8.04 | 21.88 | 26.98 |
| E5 | 10.45 | 11.61 | 5.69 | 55.68 | 0.38 | 0.03 | 4.03 | 3.08 | 18.31 | 110.1 | <0.03 | 4.96 | 9.25 | 19.40 | 26.94 |

**Appendix 6.** Hydrocarbon concentrations ($mg.kg^{1}$)in sediment collected for the 2012 survey of the PetroSA outfall monitoring programme. TPH- Total Petroleum Hydrocarbons, < = concentration below the method detection limit.

| Site | W5 | W4 | W3 | W1 | E1 | E3 | E4 | E5 |
|---|---|---|---|---|---|---|---|---|
| Benzene | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 |
| Toluene | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 |
| Ethylbenzene | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 |
| o-Xylene | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 |
| m,p-Xylene | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 | <0.050 |
| Xylenes (sum) | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| BTEX (sum) | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 |
| TPH (C6-C8) | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 |
| TPH (C8-C10) | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 | <0.60 |
| TPH (C6-C10) | <1.2 | <1.2 | <1.2 | <1.2 | <1.2 | <1.2 | <1.2 | <1.2 |
| TPH (C10-C12) | <3.0 | <3.0 | 12 | 3.7 | <3.0 | <3.0 | 3.2 | 5.2 |
| TPH (C12-C16) | 5.5 | <5.0 | 7.4 | <5.0 | 5.6 | 8 | <5.0 | 10 |
| TPH (C16-C21) | 9.2 | <6.0 | <6.0 | <6.0 | 8.2 | 8.6 | <6.0 | <6.0 |
| TPH (C21-C30) | <12 | <12 | <12 | <12 | <12 | 13 | <12 | <12 |
| TPH (C30-C35) | 7.3 | <6.0 | <6.0 | <6.0 | 7.9 | <6.0 | 6.1 | <6.0 |
| TPH (C35-C40) | <6.0 | <6.0 | <6.0 | <6.0 | <6.0 | <6.0 | <6.0 | <6.0 |
| TPH (C10-C40) | <38 | <38 | <38 | <38 | <38 | <38 | <38 | <38 |

**Appendix 7.** Benthic macrofauna abundance (individuals.m$^{-2}$) in sediment collected for the 2012 survey of the PetroSA outfall monitoring programme.

| Site | W4 | W3 | W1 | E1 | E3 | E4 |
|---|---|---|---|---|---|---|
| Oligochaeta | 0.0 | 0.0 | 4.8 | 0.0 | 0.0 | 0.0 |
| *Lumbrineris* spp. | 2.4 | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Arabella* spp. | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 |
| *Diopatraneapolitana capensis* | 57.1 | 9.5 | 14.3 | 7.1 | 0.0 | 2.4 |
| *Onuphisonuphiseremita* | 2.4 | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 |
| Paraonidae | 2.4 | 0.0 | 14.3 | 0.0 | 0.0 | 0.0 |
| *Glycera* spp. | 109.5 | 4.8 | 2.4 | 0.0 | 0.0 | 26.2 |
| *Glycinde* spp. | 7.1 | 0.0 | 0.0 | 0.0 | 0.0 | 4.8 |
| *Gyptis capensis* | 0.0 | 0.0 | 4.8 | 0.0 | 4.8 | 9.5 |
| *Nephtyshombergi* | 0.0 | 0.0 | 0.0 | 0.0 | 50.0 | 0.0 |
| *Nephtyssphaerocirrata* | 383.3 | 161.9 | 100.0 | 166.7 | 109.5 | 188.1 |
| *Nereis* spp. | 47.6 | 11.9 | 26.2 | 14.3 | 0.0 | 21.4 |
| Phyllodocidae spp. | 14.3 | 7.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Sigambraparva* | 0.0 | 0.0 | 0.0 | 2.4 | 4.8 | 2.4 |
| *Harmothoelunulata* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 9.5 |
| *Sigalioncapense* | 0.0 | 0.0 | 7.1 | 4.8 | 4.8 | 0.0 |
| *Sthenelais boa* | 0.0 | 4.8 | 4.8 | 0.0 | 4.8 | 0.0 |
| Syllidae | 26.2 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| *Branchiostoma capensis* | 0.0 | 2.4 | 2.4 | 2.4 | 2.4 | 0.0 |
| *Capitellacapitata* | 0.0 | 0.0 | 0.0 | 4.8 | 0.0 | 0.0 |
| Maldanidae | 4.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Mediomastus capensis* | 4.8 | 9.5 | 54.8 | 26.2 | 16.7 | 26.2 |
| *Magelonacincta* | 47.6 | 33.3 | 88.1 | 95.2 | 9.5 | 26.2 |
| *Poecilochaetus* sp. | 40.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Prionospio* spp. | 209.5 | 4.8 | 0.0 | 9.5 | 2.4 | 104.8 |
| *Scolelepisgilchristi* | 21.4 | 19.0 | 11.9 | 4.8 | 0.0 | 2.4 |
| *Spiophanessoederstromi* | 81.0 | 9.5 | 19.0 | 14.3 | 2.4 | 14.3 |
| *Scolariciadubia* | 4.8 | 0.0 | 7.1 | 2.4 | 4.8 | 9.5 |
| *Sabellides capensis* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 |
| *Sabellidesluderitzi* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.8 |
| Cirratulidae | 28.6 | 14.3 | 35.7 | 76.2 | 61.9 | 23.8 |
| *Pherusaswakopiana* | 2.4 | 9.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Pectinaria capensis* | 7.1 | 4.8 | 0.0 | 4.8 | 2.4 | 2.4 |
| Orbiniidae | 7.1 | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 |
| Terebellidae spp. | 0.0 | 7.1 | 2.4 | 0.0 | 0.0 | 0.0 |
| *Ampeliscabrachyceras* | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Ampeliscabrevicornis* | 11.9 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| *Ampelisca* spp. | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Caprellidae spp. | 0.0 | 4.8 | 4.8 | 2.4 | 0.0 | 2.4 |
| Corophiidae A | 59.5 | 28.6 | 50.0 | 0.0 | 0.0 | 23.8 |
| Corophiidae B | 109.5 | 4.8 | 0.0 | 0.0 | 0.0 | 2.4 |
| Unciollelasp | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 | 2.4 |
| *Hippomedonlongimanus* | 23.8 | 21.4 | 9.5 | 28.6 | 19.0 | 4.8 |
| Lysianassidae spp. | 0.0 | 0.0 | 7.1 | 0.0 | 0.0 | 9.5 |
| *Megaluropusnamaquaeensis* | 11.9 | 19.0 | 21.4 | 19.0 | 0.0 | 4.8 |
| *Perioculodeslongimanus* | 26.2 | 64.3 | 14.3 | 59.5 | 23.8 | 33.3 |
| *Photislongidactylus* | 0.0 | 0.0 | 7.1 | 11.9 | 0.0 | 0.0 |
| *Heterophoxus opus* | 2.4 | 35.7 | 57.1 | 23.8 | 38.1 | 4.8 |
| Synopiidae | 2.4 | 2.4 | 0.0 | 2.4 | 0.0 | 0.0 |
| *Urothoegrimaldi* | 9.5 | 47.6 | 50.0 | 50.0 | 35.7 | 57.1 |
| Urothoe spp. | 11.9 | 7.1 | 40.5 | 78.6 | 221.4 | 114.3 |
| *Bodotriaelevata* | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| *Bodotria vertebrata* | 2.4 | 7.1 | 0.0 | 4.8 | 0.0 | 2.4 |
| *Iphinoecrassipes* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 |

| Site | W4 | W3 | W1 | E1 | E3 | E4 |
|---|---|---|---|---|---|---|
| Bodotriidae | 0.0 | 16.7 | 0.0 | 11.9 | 2.4 | 4.8 |
| *Diastylisalgoae* | 33.3 | 28.6 | 7.1 | 7.1 | 4.8 | 26.2 |
| *Afrophilapunctata* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 |
| Dromidae spp. | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 |
| *Philyrapunctata* | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| Paguridae | 16.7 | 2.4 | 0.0 | 0.0 | 0.0 | 11.9 |
| Pinnotheridae spp. | 4.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Brachyura spp. | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| Anthuridae | 7.1 | 0.0 | 2.4 | 11.9 | 7.1 | 26.2 |
| *Arcturinascutula* | 0.0 | 0.0 | 0.0 | 0.0 | 9.5 | 0.0 |
| *Microarcturusquadriconus* | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Synidoteahirtipes* | 90.5 | 7.1 | 9.5 | 4.8 | 0.0 | 23.8 |
| Mysidacea | 14.3 | 7.1 | 23.8 | 7.1 | 0.0 | 9.5 |
| *Pterygosquillaarmata capensis* | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| Tanaidacea | 2.4 | 11.9 | 7.1 | 4.8 | 0.0 | 0.0 |
| Ostracoda sp1 | 161.9 | 128.6 | 90.5 | 40.5 | 0.0 | 9.5 |
| Ostracoda sp2 | 16.7 | 33.3 | 4.8 | 23.8 | 14.3 | 14.3 |
| Anemone | 0.0 | 0.0 | 0.0 | 4.8 | 0.0 | 0.0 |
| Virgularia spp. | 4.8 | 11.9 | 2.4 | 11.9 | 2.4 | 28.6 |
| *Astropectencingulatus* | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 | 2.4 |
| *Echinocardiumcordatum* | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 2.4 |
| Holothuroidea | 71.4 | 11.9 | 2.4 | 9.5 | 4.8 | 102.4 |
| *Amphioplus integer* | 73.8 | 107.1 | 19.0 | 81.0 | 40.5 | 19.0 |
| *Solencylindraceus* | 4.8 | 0.0 | 0.0 | 0.0 | 2.4 | 4.8 |
| *Pernaperna* | 66.7 | 2.4 | 2.4 | 0.0 | 0.0 | 0.0 |
| *Nucula nucleus* | 9.5 | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 |
| *Tellina* spp. | 171.4 | 33.3 | 7.1 | 21.4 | 11.9 | 42.9 |
| *Macomacrawfordi* | 109.5 | 64.3 | 81.0 | 123.8 | 14.3 | 138.1 |
| *Macomadispar* | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Aliculastrumcylindricum* | 7.1 | 7.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Haminoeasp | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 |
| Cypraea spp. | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| *Nassariusspeciosus* | 47.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Bulliaannulata* | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| *Bullia* spp. | 0.0 | 2.4 | 2.4 | 4.8 | 0.0 | 0.0 |
| *Ancillamarmorata* | 0.0 | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| Nematoda spp. | 14.3 | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 |
| Nemertea | 102.4 | 28.6 | 19.0 | 23.8 | 57.1 | 11.9 |
| Platyhelminthes | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.1 |

**Appendix 8.** Benthic macrofauna biomass (g.m$^2$) in sediment collected for the 2012 survey of the PetroSA outfall monitoring programme.

| *Site* | W4 | W3 | W1 | E1 | E3 | E4 |
|---|---|---|---|---|---|---|
| *Astropectencingulatus* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 29.4767 | 30.7569 |
| *Holothuroidea* | 3.4224 | 0.6719 | 0.2914 | 0.2752 | 0.0143 | 10.3293 |
| *Amphioplus integer* | 0.3826 | 0.6460 | 0.1705 | 0.2136 | 3.0948 | 5.9107 |
| *Echinocardiumcordatum* | 0.0000 | 0.0000 | 0.0000 | 0.0021 | 0.0000 | 10.3607 |
| *Diopatraneapolitana capensis* | 4.0748 | 0.0412 | 0.1290 | 0.4981 | 0.0000 | 0.0036 |
| *Ancillamarmorata* | 0.0000 | 4.7443 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Bulliasp.* | 0.0000 | 0.1421 | 3.9988 | 0.0033 | 0.0000 | 0.0000 |
| *Paguridae* | 0.1079 | 0.0002 | 0.0000 | 0.0000 | 0.0000 | 3.6374 |
| *Bulliaannulata* | 0.0000 | 0.0000 | 0.0000 | 3.5367 | 0.0000 | 0.0000 |
| *Synidoteahirtipes* | 1.6624 | 0.0071 | 0.0052 | 0.0140 | 0.0000 | 0.0250 |
| *Macomacrawfordi* | 0.0571 | 0.1267 | 1.1945 | 0.1479 | 0.0031 | 0.0521 |
| *Afrophilapunctata* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 1.5555 |
| *Magelonacincta* | 0.0731 | 0.0888 | 0.6574 | 0.5690 | 0.0338 | 0.1174 |
| *Glyceraspp.* | 1.2200 | 0.0026 | 0.0200 | 0.0000 | 0.0000 | 0.0219 |
| *Tellinaspp.* | 0.8000 | 0.3793 | 0.0052 | 0.0133 | 0.0064 | 0.0210 |
| Terebellidaespp. | 0.0000 | 0.0131 | 0.8467 | 0.0000 | 0.0000 | 0.0000 |
| Cirratulidae | 0.0681 | 0.0967 | 0.1714 | 0.2731 | 0.1286 | 0.0488 |
| *Sthenelais boa* | 0.0000 | 0.1460 | 0.3326 | 0.0000 | 0.2919 | 0.0000 |
| *Nephtyssphaerocirrata* | 0.2755 | 0.0971 | 0.0855 | 0.0981 | 0.0781 | 0.1338 |
| *Nucula nucleus* | 0.5255 | 0.0000 | 0.0000 | 0.0000 | 0.1879 | 0.0000 |
| *Branchiostoma capensis* | 0.0000 | 0.0374 | 0.0595 | 0.0074 | 0.5902 | 0.0000 |
| *Pherusaswakopiana* | 0.0031 | 0.6567 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Pinnotheridae spp.* | 0.6088 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Nemertea | 0.1690 | 0.0419 | 0.0200 | 0.0957 | 0.2424 | 0.0081 |
| Brachyura spp. | 0.0000 | 0.5679 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Nephtyshombergi* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.5529 | 0.0000 |
| *Pernaperna* | 0.5124 | 0.0010 | 0.0110 | 0.0000 | 0.0000 | 0.0000 |
| *Urothoegrimaldi* | 0.0195 | 0.0307 | 0.0612 | 0.1255 | 0.0824 | 0.1290 |
| *Virgulariaspp.* | 0.0076 | 0.0660 | 0.0429 | 0.1026 | 0.0010 | 0.1738 |
| *Nassariusspeciosus* | 0.3855 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Scolelepisgilchristi* | 0.1521 | 0.0760 | 0.0681 | 0.0098 | 0.0000 | 0.0024 |
| *Scolariciadubia* | 0.0067 | 0.0000 | 0.1750 | 0.0071 | 0.0274 | 0.0905 |
| *Sigalioncapense* | 0.0276 | 0.0848 | 0.0126 | 0.0000 | 0.0000 | 0.1724 |
| *Hippomedonlongimanus* | 0.0000 | 0.0350 | 0.0276 | 0.0548 | 0.0979 | 0.0069 |
| *Nereisspp.* | 0.0469 | 0.0448 | 0.0298 | 0.0221 | 0.0000 | 0.0760 |
| *Pectinaria capensis* | 0.0133 | 0.1807 | 0.0000 | 0.0069 | 0.0124 | 0.0002 |
| *Harmothoelunulata* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.2010 |
| *Heterophoxus opus* | 0.0002 | 0.0157 | 0.0726 | 0.0381 | 0.0398 | 0.0060 |
| *Macomadispar* | 0.0000 | 0.0819 | 0.0000 | 0.0862 | 0.0000 | 0.0000 |
| *Solencylindraceus* | 0.0781 | 0.0000 | 0.0000 | 0.0000 | 0.0102 | 0.0633 |
| *Sabellidesluderitzi* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.1471 |
| *Spiophanessoederstromi* | 0.0560 | 0.0079 | 0.0381 | 0.0174 | 0.0002 | 0.0164 |
| *Diastylisalgoae* | 0.0336 | 0.0307 | 0.0079 | 0.0088 | 0.0110 | 0.0331 |
| *Mediomastus capensis* | 0.0048 | 0.0021 | 0.0486 | 0.0143 | 0.0071 | 0.0257 |
| *Urothoespp.* | 0.0005 | 0.0002 | 0.0088 | 0.0193 | 0.0333 | 0.0243 |
| *Onuphisonuphiseremita* | 0.0267 | 0.0000 | 0.0474 | 0.0000 | 0.0000 | 0.0000 |
| Anemone | 0.0000 | 0.0000 | 0.0000 | 0.0702 | 0.0000 | 0.0000 |
| *Phyllodocidaespp.* | 0.0281 | 0.0417 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Mysidacea | 0.0200 | 0.0021 | 0.0295 | 0.0005 | 0.0000 | 0.0079 |

| *Site* | W4 | W3 | W1 | E1 | E3 | E4 |
|---|---|---|---|---|---|---|
| Corophiidae A | 0.0224 | 0.0100 | 0.0164 | 0.0000 | 0.0000 | 0.0107 |
| *Ampelisca*spp. | 0.0579 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Haminoeasp* | 0.0557 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0019 |
| *Sipunculida* | 0.0000 | 0.0000 | 0.0000 | 0.0531 | 0.0000 | 0.0000 |
| *Perioculodeslongimanus* | 0.0124 | 0.0145 | 0.0014 | 0.0124 | 0.0062 | 0.0057 |
| *Prionospio* spp. | 0.0260 | 0.0005 | 0.0000 | 0.0012 | 0.0002 | 0.0181 |
| Ostracoda sp1 | 0.0176 | 0.0102 | 0.0095 | 0.0031 | 0.0000 | 0.0014 |
| Corophiidae B | 0.0345 | 0.0005 | 0.0000 | 0.0000 | 0.0000 | 0.0050 |
| Cypraea sp. | 0.0000 | 0.0000 | 0.0000 | 0.0369 | 0.0000 | 0.0000 |
| Orbinidae spp. | 0.0179 | 0.0000 | 0.0186 | 0.0000 | 0.0000 | 0.0000 |
| *Glycinde*spp. | 0.0195 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0136 |
| Oligochaeta | 0.0290 | 0.0000 | 0.0002 | 0.0000 | 0.0000 | 0.0000 |
| Ostracoda sp2 | 0.0093 | 0.0079 | 0.0043 | 0.0029 | 0.0019 | 0.0021 |
| Lysianassidaespp. | 0.0000 | 0.0000 | 0.0157 | 0.0000 | 0.0000 | 0.0124 |
| *Ampeliscabrevicornis* | 0.0233 | 0.0000 | 0.0000 | 0.0026 | 0.0000 | 0.0000 |
| *Atyscylindrica* | 0.0181 | 0.0031 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Dromidae spp. | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0193 |
| *Poecilochaetusserpens* | 0.0183 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Anthuridae* | 0.0031 | 0.0000 | 0.0002 | 0.0024 | 0.0026 | 0.0088 |
| *Gyptis capensis* | 0.0000 | 0.0000 | 0.0024 | 0.0000 | 0.0036 | 0.0105 |
| Caprellidae spp. | 0.0000 | 0.0045 | 0.0076 | 0.0002 | 0.0000 | 0.0002 |
| *Megaluropusnamaquaeensis* | 0.0017 | 0.0033 | 0.0033 | 0.0036 | 0.0000 | 0.0005 |
| Bodotriidae | 0.0000 | 0.0095 | 0.0000 | 0.0005 | 0.0002 | 0.0019 |
| Paraonidae | 0.0031 | 0.0000 | 0.0076 | 0.0000 | 0.0000 | 0.0000 |
| *Philyrapunctata* | 0.0000 | 0.0000 | 0.0000 | 0.0088 | 0.0000 | 0.0000 |
| *Bodotria vertebrata vertebrata* | 0.0024 | 0.0012 | 0.0000 | 0.0029 | 0.0000 | 0.0007 |
| *Arcturinascutula* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0055 | 0.0000 |
| Maldanidae | 0.0055 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Arabella*spp. | 0.0000 | 0.0000 | 0.0052 | 0.0000 | 0.0000 | 0.0000 |
| *Photislongidactylus* | 0.0000 | 0.0000 | 0.0019 | 0.0033 | 0.0000 | 0.0000 |
| *Microarcturusquadriconus* | 0.0038 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Lumbrineris*spp. | 0.0031 | 0.0005 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Ampeliscabrachyceras* | 0.0000 | 0.0033 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *Sigambraparva* | 0.0000 | 0.0002 | 0.0007 | 0.0017 | 0.0000 | 0.0002 |
| *Platyhelminthes* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0014 |
| *Pterygosquillaarmata capensis* | 0.0000 | 0.0000 | 0.0000 | 0.0014 | 0.0000 | 0.0000 |
| *Sabellides capensis* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0014 |
| Tanaidacea | 0.0002 | 0.0005 | 0.0005 | 0.0002 | 0.0000 | 0.0000 |
| *Bodotriaelevata* | 0.0000 | 0.0000 | 0.0000 | 0.0012 | 0.0000 | 0.0000 |
| Syllidae | 0.0010 | 0.0000 | 0.0000 | 0.0002 | 0.0000 | 0.0000 |
| Nematoda | 0.0007 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Synopiidae | 0.0002 | 0.0002 | 0.0000 | 0.0002 | 0.0000 | 0.0000 |
| *Unciollela*sp | 0.0000 | 0.0000 | 0.0002 | 0.0000 | 0.0000 | 0.0002 |
| *Capitellacapitata* | 0.0000 | 0.0000 | 0.0000 | 0.0002 | 0.0000 | 0.0000 |

# 12. Glossary of Terms[3]

| | |
|---|---|
| Abiotic factors | The non-living factors that affect the ability of living organisms to survive in an environment (e.g. temperature, salinity). |
| Adsorption | Bonding of metals and nutrients onto the surfaces of suspended particles by way of physical, chemical and biological processes. |
| Aliquot | A sub-sample of the original sample. |
| Analysis of Variance (ANOVA) | A statistical procedure used to compare the average condition between three or more treatments. |
| Anthropogenic | Made and/or introduced into the environment by humans, especially pertaining to contaminants/pollutants. |
| Assimilative capacity | The amount of contaminant load that can be discharged to a specific water body without exceeding water quality standards or criteria. Assimilative capacity is used to define the ability of a water body to naturally absorb and use a discharged substance without impairing water quality or harming aquatic life. |
| Benthic | Pertaining to the environment inhabited by organisms living on or in the ocean bottom. |
| Benthos | Living organisms (e.g. algae and animals) associated with the sea bottom. |
| Biota | The living organisms within a habitat or region. |
| Biotic | Relating to life or living things. |
| Community | Any group of organisms belonging to a number of different species that co-occur in the same habitat or area. An association of interacting assemblages in a given water body. |
| Concentration | The quantifiable amount of a substance in water, food or sediment. |
| Contaminants | Biological or chemical substances or entities, not normally present in a system, capable of producing an adverse effect in a biological system, seriously injuring structure or function. |
| Control site | A geographic location that is far enough from a known pollution source (e.g. outfall) to be considered representative of an undisturbed environment. Information collected within control sites is used as a reference and compared to impacted sites. |
| Crustacea | A group (Phylum) of marine invertebrates characterised by jointed legs and an exoskeleton (e.g. crabs, shrimps, and crayfish). |
| Dendrogram | A tree-like diagram used to represent hierarchal relationships from a multivariate analysis where results from several monitoring parameters are compared among sites. |
| Diversity | A measurement of community structure that describes the abundances of different species within a community, taking into account their relative rarity or commonness. |
| Ecosystem | An interrelating complex of plant and animal communities and their associated non-living environment. |
| Effluent | The discharge to a body of water from a defined or point source, generally consisting of a mixture of waste and water from industrial or municipal facilities. |
| Grab | A mechanical device designed to collect bottom sediment samples. The device consists of a pair of hinged jaws and a release mechanism that allows the opened jaws to close and entrap a 0.25 $m^2$ sediment |

[3] This glossary of terms was compiled from numerous sources, which are available from the CSIR on request.

| | |
|---|---|
| | sample once they touch bottom. |
| Guideline | A numerical concentration limit or narrative statement recommended to support and maintain a designated water use. |
| Habitat | A place where the physical and biological elements of ecosystems provide an environment and elements of the food, cover and space resources needed for plant and animal survival. |
| Heavy metal | An imprecise term with no sound terminological or scientific basis, used loosely to refer to metals that are toxic. |
| Impact | A change in the chemical, physical or biological quality or condition of a waterbody caused by external sources. |
| Indicator | Characteristics for the environment, both abiotic and biotic, that can provide quantitative information on environmental conditions. |
| Infauna | Those animals that live within the sediments of the sea floor. |
| Invertebrate | An animal without a backbone (e.g. a starfish, crab, or worm). |
| Macrofauna | Epifaunal or infaunal benthic invertebrates that are visible with the naked eye. These animals inhabit soft-bottom marine habitats and are retained on a 1 mm mesh screen. |
| Meiofauna | Small interstitial (i.e. occurring between sediment particles) animals that pass through a 1 mm mesh sieve but are retained by a 0.045 mm mesh. |
| Multivariate analysis | Statistical methods (e.g. ordination or discriminant analysis) for analysing physical and biological community data using multiple variables. |
| Normalise | Perform a data calculation in order to express results in terms of a reference parameter or characteristic. |
| Ordination | A two-dimensional scatter plot, generated through multivariate community analysis, which depicts the relative taxonomic similarities amongst a group of faunal samples. |
| Physico-chemical | Measurement of both physical properties (e.g. temperature, salinity) and chemical determinants (e.g. metals and nutrients) to characterise the state of an environment. |
| Population | An aggregate of interbreeding individuals of a biological species within a specified location. |
| Pollution | The Paris Convention defines pollution as the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as hazards to human health, harm to living resources and to marine ecosystems, damage to amenities or interference with other legitimate uses of the sea. |
| Polychaeta | A taxonomic group (Class) of, mainly marine, invertebrates characterised by having wormlike features, segments, and bristles or hairs. They are very variable in form and lifestyle and are good environmental indicators. |
| Receiving environment | A river, stream, lake or other body of surface water into which effluent or treated effluent is discharged. |
| Replicate | Taking more than one sample or performing more than one analysis. |
| Sediment | Mud, sand, silt, clay, shell debris, and other particles that settle on the bottom of rivers, lakes, estuaries, and oceans. |
| Significance | Used in terms of statistics; statistical significance is a mathematical tool used to determine whether the outcome of an experiment is the |

| | |
|---|---|
| | result of a relationship between specific factors or due to chance. |
| Species | A category of biological classification ranking immediately below the genus, comprising related organisms potentially capable of interbreeding. A species is identified by a two part name; the name of the genus followed by a Latin or Latinised un-capitalised noun agreeing grammatically with the genus name. |
| Species richness | The number of species per unit area. A metric used to evaluate the health of macrofauna and meiofauna communities. |
| Site | A sampling location within a study area or site, where physical, chemical, or biological sampling and/or testing occurs. |
| Ternary plot | A diagram that depicts the ratios of three variables as positions in an equilateral triangle. It is used in sediment granulometry to show the relative proportions of the fine, coarse and silt/clay of size fractions in a sediment sample. |
| Trace metal | A metal found in low concentration, in mass fractions of ppm (µg) or less, in some specified source (e.g. sediment, tissue). |
| Taxon (taxa) | Any group of organisms considered to be sufficiently distinct from other such groups to be treated as a separate unit (e.g. species, genera, families). |
| Toxic | Poisonous, carcinogenic, or otherwise directly harmful to life. |
| Toxicity | A measure of the impact on a chosen biological process or condition. |
| Weight of evidence approach | Use of multiple lines of evidence to evaluate an issue or risk; evidence can be scientific in nature or inclusive of other disciplines; e.g., socio-economic, political and legal. |
| Zone of Initial Dilution | An area in the immediate vicinity of a marine outfall discharge where there is rapid mixing of the effluent with sea water as a result of jetting and buoyant rise. An allocated impact area, or mixing zone, in a water body where numeric water quality criteria can be exceeded as long as acutely toxic conditions are prevented. |