THE EFFECT OF POLYMER CONTAMINATED OIL PRODUCED WASTEWATER ON THE GROWTH AND HEALTH OF FIVE EMERGENT MACROPHYTES IN A PILOT SCALE SURFACE FLOW CONSTRUCTED WETLAND

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ENG460 – Engineering Thesis

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School of Engineering and Information Technology
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I, Marco Warmt-Murray, declare that the work in this document is original apart from where indicated by form of reference. The completion of this document is in accordance with the Murdoch University policy.

Signed__________________________________________

Marco Warmt-Murray

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Executive Summary

A Water Treatment Plant (WTP) located in the south east Arabian Peninsula currently receives oil produced wastewater (PW) from local oilfields. Hydrocarbons are biologically treated/degraded within a series of vegetated surface flow constructed wetlands (SF CW) and then water is evaporated in large evaporation ponds. Over the next 5 years there are plans to increase the efficiency of oil production by injecting partially hydrolyzed polyacrylamide (HPAM) into oil fields located in the Middle East, raising oil production up to 12%. The broad aim of this report is to determine if the WTP will be able to receive HPAM contaminated PW, either with or without design modifications. The aim of this study was to evaluate the response of the five main wetland plant species used at the WTP, to the addition of HPAM contaminated PW, and to identify the effects of these responses on the treatment performance of PW in a SF CW system. Four SF CWs or trial wetlands (TW) (Length = 40 m x Width = 40 m) were designed to mimic the treatment wetland at the WTP. Each TW had a surface area of 1600 m² and was planted with Phragmites australis, Schoenoplectus littoralis, Typha domingensis, Cyperus laevigatus and Juncus rigidus. Each TW received 30 m³ per day of PW contaminated with a different HPAM concentration (0 ppm (control), 250 ppm, 500 ppm and 1000 ppm). This report is based on data collected between 1st March 2015 and 31st of May 2015. No major effects to plant growth and health were observed when exposed to HPAM and no clear trends occur between treatments. Oil in Water (OiW) measurements at the outlets of all four TWs were less than 1 mg/l and an OiW removal efficiency of 97.5%, 97.8%, 96.9% and 96.8% was recorded for 0 ppm, 250 ppm, 500 ppm and 1000 ppm, respectively. This suggests the removal of hydrocarbons from polymer contaminated PW had little to no difference when compared to the control (0 ppm). On average the polymer contaminated TWs showed a 20 to 26% lower water loss compared to the control wetland. This implies a larger surface area would be required to achieve the same evaporation rates as the control TW. However, a long term study should be conducted in order to observe a full seasonal growth cycle to further substantiate the encouraging results obtained.
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<th>Definition</th>
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<tbody>
<tr>
<td>AGB</td>
<td>Above Ground Biomass</td>
</tr>
<tr>
<td>AGDB</td>
<td>Above Ground Dry Biomass</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BT</td>
<td>Buffer Tank</td>
</tr>
<tr>
<td>C</td>
<td><em>Cyperus laevigatus</em></td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CW</td>
<td>Constructed Wetland</td>
</tr>
<tr>
<td>EOR</td>
<td>Enhanced Oil Recovery</td>
</tr>
<tr>
<td>HDPE</td>
<td>High Density Polyethylene</td>
</tr>
<tr>
<td>HF</td>
<td>Horizontal Flow</td>
</tr>
<tr>
<td>HPAM</td>
<td>Hydrolyzed Polyacrylamide</td>
</tr>
<tr>
<td>H₂S</td>
<td>Hydrogen Sulphide Gas</td>
</tr>
<tr>
<td>HSSF</td>
<td>Horizontal Subsurface Flow</td>
</tr>
<tr>
<td>IGF</td>
<td>Induced Gas Floatation</td>
</tr>
<tr>
<td>J</td>
<td><em>Juncus rigidus</em></td>
</tr>
<tr>
<td>LLC</td>
<td>Limited Liability Company</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>Ammonium-Nitrogen</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>Nitrite-Nitrogen</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>Nitrate-Nitrogen</td>
</tr>
<tr>
<td>NORM</td>
<td>Natural Occurring Radioactive Material</td>
</tr>
<tr>
<td>WTP</td>
<td>Wastewater Treatment Plant</td>
</tr>
<tr>
<td>P</td>
<td><em>Phragmites australis</em></td>
</tr>
<tr>
<td>PPM</td>
<td>parts per million</td>
</tr>
<tr>
<td>PPT</td>
<td>parts per thousand</td>
</tr>
<tr>
<td>PW</td>
<td>Produced Water</td>
</tr>
<tr>
<td>S</td>
<td><em>Schoenoplectus littoralis</em></td>
</tr>
<tr>
<td>SF</td>
<td>Surface Flow</td>
</tr>
<tr>
<td>T</td>
<td><em>Typha domingensis</em></td>
</tr>
<tr>
<td>TN</td>
<td>Total Nitrogen = (NO₂-N + NO₃-N + N + NH₄-N)</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>TP</td>
<td>Total Phosphorus</td>
</tr>
<tr>
<td>TSS</td>
<td>Total Suspended Solids</td>
</tr>
<tr>
<td>TW</td>
<td>Trial Wetland</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile Organic Compounds</td>
</tr>
<tr>
<td>VSSF</td>
<td>Vertical Subsurface Flow</td>
</tr>
</tbody>
</table>
1 Introduction

Constructive wetlands (CWs) are engineered systems that are designed and constructed to mimic the basic treatment processes that occur in natural wetland systems (Kadlec & Wallace, 2009). CWs are becoming more popular as a treatment system due to their ability to treat a wide variety of wastewater effluent with lower external energy requirements, cost, and ease of operation and maintenance in comparison to conventional treatment systems (Garcia et al., 2010; Vymazal, 2011; Stefanakis et al., 2011). Wastewater effluent in CWs is treated by a number of physical, chemical and biological treatment processes, such as sedimentation, filtration, sorption, volatilization, UV degradation, microbiological degradation and plant uptake (Kadlec & Wallace, 2009; Jia et al., 2010). Wetland plants are an integral component of wetland systems, providing key treatment functions (Brix, 2003; Wu et al., 2015; Tanner C. C., 1996). Emergent macrophytes are a commonly used vegetation type in CW designs, often in conjunction with surface flow (SF) CWs for their influence on physical, ecological and chemical treatment processes (Vymazal, 2013). A range of such influences associated with emergent macrophytes include evaporative evaporation, flow restriction, sediment stabilization, leaf litter, provision for biofilm development (Chappell and Goulder, 1994), oxygenation and nutrient and pollutant uptake (Brix, 1994). Types of wastewater effluents treated using a SF CW planted with emergent macrophytes include tertiary sewage (Bachand & Horne, 2000) stormwater runoff (Lai & Lam, 2009), chemical industrial wastewater (Domingos, 2011), mining wastewater (Schaller et al., 2014), slaughterhouse waste (Wu et al., 2015) and oil produced water (Breuer et al., 2012; Knight et al., 1999).

Globally the oil and gas industry has adopted the use of wetland technology for their capability, versatility and success to treat a number of different applications. Some examples for the treatment of petroleum wastewater include: a SF CW in Mandan, North Dakota (Litchfield, 1990), a subsurface flow wetland (SSF) CW implemented by Mobil Oil AG in Bremen, Germany (Vymazal et al., 1998), a SSF CW used for the Gulf Strachan Gas plant near Calgary, Alberta (Moore et al., 2000) and the SF CW by BAUER Nimr LLC’s NWTP in Nimr, Oman, the largest of its kind (Breuer et al., 2012).

A water treatment plant (WTP), operated in the South East of the Arabian Peninsula, and currently receives oil produced water. Since the WTP’s commissioning period in 2009, a stilling basin and a series of hydrocyclones, separate the bulk of oil from the received produced water. The remaining oil in water (OiW) is biologically treated/degraded within a series of vegetated SF CWs. Water is finally evaporated in large ponds with the intent of recovering crystallized salt in a salt works facility.

Over the next 5 years it is planned to increase the efficiency of oil production by injecting partially hydrolyzed polyacrylamide (HPAM), a water-soluble polymer used in enhanced oil recovery (EOR). This follows the successful employment of HPAM EOR in the Daqing oil fields, China raising oil production up to 12% (Deng et al., 2002; Li F., 2012). However, many major implications arise from contaminating produced water with HPAM, such as an increase in the difficulty of oil-water separation, increased cost in water treatment and/or disposal, and possible harm caused by the wastewater to...
the environment (Lucas et al., 2009; Li F., 2012). The future contamination of HPAM in the produced water received by the WTP raises concern, regarding the impact of polymer on the wetlands ecosystem and treatment efficiency. A pilot scale, short-term study lead by Johnston (2013), investigated the potential impacts of HPAM on plant growth and health, evaporation, evapotranspiration and the infiltration on the mineral sealing layer used at the WTP.

The study carried out by Johnston (2013) entailed 27 mesocosms (A = 1.2 m², H= 0.6 m), each filled with soil and planted/unplanted with *Phragmites australis* the most commonly used reed within the WTP surface flow wetlands. Well water containing 20 ppm OIW was mixed with one of three HPAM concentrations (0 ppm, 250 ppm and 500 ppm) and then applied with weekly batch-loading to selected mesocosms. The study identified that the application of polymer increased growth rate up to 47% on some of the mesocosms. A statistical one way ANOVA test determined a variability between replicates suggesting there was no significance in the observation (increased growth rate). Results for mean above ground dry biomass were 337 ± 75 g/m², 1001 ± 661 g/m² and 1006 ± 622 g/m² for 0 ppm, 250 ppm and 500 ppm, respectively. The outcome of the study determined a larger pilot scale wetland and a longer trial should be undertaken to obtain a better understanding on the long term effects of HPAM on the wetland system.

The WTP is currently undergoing a yearlong feasibility study on the potential effects of HPAM contaminated produced water on a larger scale pilot project. The broad aim of their project is to determine if the WTP will be able to treat polymer-contaminated produced water, either with or without design modifications and to identify the impact of polymer on the health of the five main wetland plant species (*Phragmites australis*, *Schoenoplectus littoralis*, *Typha domingensis*, *Cyperus laevigatus*, *Juncus rigidus*) used at the WTP.

Currently the use of polymer injection during EOR in oil fields around the world is raising concerns of the potentially harmful effects associated with polymer on the environment (Li F., 2012). CWs are being progressively adopted for treating wastewater streams produced from the oil and gas industry. It is important to determine the effects that polymer may pose on treatment processes within CWs, for the feasibility of current and future designs to tolerate polymer contaminated produced water.
2 Project Aims and Objectives

The broad aim of this project was to study the response of five wetland plant species to the addition of HPAM contaminated produced water and identify the effects of these responses on the treatment performance of produced water in a SF CW system.

Objectives:

1. To identify the plant species capable of growing and surviving in the presence of polymer contaminated produced water.
2. To determine the removal of hydrocarbons from polymer contaminated produced water in a SFCW planted with the 5 wetland plant species.
3. To evaluate potential design modifications to the WTP.

3 Literature Review

3.1 Petroleum Industry

The petroleum industry is comprised of upstream (exploration, extraction), midstream (refining and processing) and downstream (marketing and sales) processes (Lucas et al., 2009). The main process covered in this report will be linked to upstream processing and more specifically, to do with the hydrolysed polyacrylamide (HPAM) contaminated produced water extracted during the enhanced oil recovery (EOR) process.

Petroleum, also known as “crude oil” is the accumulation of hydrocarbons in underground rock formations, formed over millions of years of decomposing organic material (such as animal and plant biomass) under vast amounts of pressure and high temperatures from the Earth’s core (OPEC, 2013). A variety of products produced from petrochemical products and applications, produced from petroleum are; fuels (aviation, diesel, petrol, and kerosene), motor oils, plastics, paint, pharmaceuticals, monomers and more specifically polymers (Speigh, 2014).

3.2 Produced Water

Produced Water is water brought to the surface from underground rock formations during the production of oil. The produced water may include water from natural reservoirs stored in these underground formations and from water injected during the drilling and oil extraction phase or enhanced oil recovery operation. (Veil C. C., 2009). The contaminants in the produced water vary significantly between locations (countries, regions and even oil wells), however the same major constituents are generally present and of concern; salt content (salinity, total dissolved solids or electrical conductivity), oil content (Oil in water), natural inorganic and organic compounds (calcium, magnesium, sulfates, and boron) and naturally occurring radioactive material (NORM) (NPC, 2011). Produced water is the largest waste stream by volume and is typically generated for the life span of
the oil well. The water to oil ratio increases as the age of the well increases. Due to the contamination of the water with organic and inorganic components, adequate treatment must be ensured before the water can be re-injected or disposed of without having a negative impact on the environment (Lucas et al., 2009).

The petroleum industry has adopted three main oil treatment classifications:

- Primary separation: Bulk removal of oil and solids via mechanical processes, e.g. hydrocyclones, washing tanks or degasification.
- Secondary separation: Removal of remaining oil and solids, often by induced gas flotation (IGF) or chemical additives.
- Tertiary separation: Final polishing and removal of oil and greases, via activated charcoal filtration, centrifuges, membrane filtration or bioremediation (Lucas et al., 2009).

Once produced water has been treated to a sufficient level, disposal is required. The commonly used disposal methods are: deep well disposal, shallow well disposal, reuse in drilling operations, reuse for EOR or through evaporation (Arthur et al., 2005).

Table 1 Operational energy requirements for treating/disposing of 45,000 m$^3$/day produced water (Breuer et al., 2012). A comparison between deep well disposal and a WTP treatment wetland

<table>
<thead>
<tr>
<th>Disposal Options</th>
<th>Power required (kWh/m$^3$)</th>
<th>Total power used (MWh)</th>
<th>CO$_2$ emissions (t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep well disposal</td>
<td>Up to 5.5</td>
<td>3,630,000</td>
<td>1,960,000</td>
</tr>
<tr>
<td>WTP Treatment wetland</td>
<td>0.1</td>
<td>66,000</td>
<td>35,700</td>
</tr>
</tbody>
</table>

The large cost and energy demands associated with current treatment and disposal methods of produced water along with high risk operations has directed the petroleum industry to explore new avenues of treatment and disposal. A German based company has adopted the use of a treatment wetland and evaporation ponds to treat and dispose PW since 2012 (Breuer et al., 2012). Primary and secondary treatment consists of a vortex oil separator, hydrocyclones and a skimming buffer pond. Tertiary treatment uses bioremediation through the SFCW system and final disposal is via evaporation ponds (Breuer et al., 2012). Comparison of a WTP with the traditional disposal methods (see Table 1) employed in oil fields (located in the Middle East) indicates 98% less power requirement and carbon emissions. The SFCW design will be the focal treatment and disposal method considered for the pilot scale wetland.

3.3 Polymer

3.3.1 Hydrolysed Polyacrylamide

One of the main forms of polymer used in EOR is a synthetic, water soluble polyacrylamide generally in its partially hydrolysed form, HPAM (Pancharoen, 2009). HPAM is a straight chain polymer with a
chemical structure containing the basic monomer molecule Acrylamide as seen in Figure 1 (Littman, 1988).

![Chemical Structure of HPAM](image)

*Figure 1 Chemical Structure of HPAM (Littman, 1988)*

The hydrolysis of HPAM allows for a unique shear-thickening property which disperses in water and swells resulting in an increase in viscosity (Husdal, 2011). The increased viscosity comes from the repulsion between the polymer molecules and the other molecules within the HPAM chain. This causes the molecule to lengthen and snag on other molecules, lowering the mobility of the polymer solution. This feature increases efficiency of EOR or polymer flooding, allowing for a greater sweep efficiency for oil recovery.

### 3.3.2 Polymer Assisted Enhanced Oil Recovery

Polymer flooding or polymer assisted EOR is the process in which the polymer is injected under high pressure via a pump into an oil reservoir through the injection well (see Figure 2). A mixture of HPAM and flood water is released at varying depths to maximize vertical coverage and improve the volumetric sweep. The polymer solution reduces the mobilization of water and forces the water to travel through more flow channels, enhancing the sweep of oil locked in the reservoir rock (Shah & Schechter, 1977). A surfactant may also be used in advance to facilitate the release of oil from the pores of the reservoir rock, a term known as Micellar polymer flooding (Sheng, 2013).

![HPAM Enhanced Oil Recovery process](image)

*Figure 2 HPAM Enhanced Oil Recovery process (modified from Johnston, 2013)*
3.3.3 Biodegradation of Hydrolysed Polyacrylamide

The degradation of HPAM in contaminated PW is important. Possible effects of not degrading the HPAM is an increased difficulty to separate oil from water resulting in decreased oil production. However the degradation of HPAM can also produce toxic acrylamide a neurotoxin endangering human health and local ecosystems (Bao et al., 2012). Literature covering the biodegradation of HPAM is limited, with the majority of available studies undertaken in China (not available in the English language). The available literature does not explore the use of CWs for the treatment and degradation of HPAM. A study by Bao et al. (2012) investigates two strains of bacteria, Bacillus cereus and Bacillus sp. on the biodegradation of HPAM in produced water. The two naturally occurring aerobic bacteria were found to utilize nitrogen from the amide group of HPAM. The two strains of bacteria partially utilized the carbon backbone of the polymer chain resulting in a nontoxic monomer byproduct. Fang et al. (2007) reports using strict anaerobic bacteria (Clostridium bifermentans-H5) whose carbon uptake was only from HPAM. This resulting in a decrease in viscosity of solution containing HPAM. The biodegradation of HPAM using the anaerobic bacteria achieved a 52.5% HPAM removal efficiency. The encouraging results from the two studies suggest natural occurring microorganisms have the potential to degrade HPAM without endangering human health or the environment, whilst maintaining the beneficial use of polymer in EOR.

3.4 Constructed Wetlands

3.4.1 Constructed Wetlands Used for Produced Water Treatment

CWs are nowadays largely used for sewage water treatment in Europe and North America (Wu et al., 2015). CW technology for hydrocarbon removal was first documented in 1966 by Dr K. Seidel’s paper “Purification of Water by Higher Plants”. Since then, it has been identified that “hydrocarbons naturally degrade in natural wetland environments” (Wemple & Hendricks, 2000). This discovery initiated the research and development of CW technologies with a focus toward a cost-effective, long-term solution to natural hydrocarbon degradation. A paper by Wallace (2012) reports on two case studies by BP in the US, focusing primarily on the treatment performance of two low cost full scale treatment wetlands (the first located in Casper, Wyoming and the second in Wellsville, New York). The outcome of the study verified a high removal efficiency of the hydrocarbon “benzene”, the Casper and Wellsville CW successfully removed 100% and 87% of the benzene, respectively. Breuer and Headley (2012) report a removal efficiency of ~100% for OiW at a WTP, located in the south east Arabian peninsula.

3.4.2 General Distinction

The two main distinctions of CWs can be determined by the hydrological behaviour; subsurface flow (SSF) and surface flow (SF) CWs. SF CWs can be further categorized (see Figure 3) into SF CWs with floating plants, submerged plants or emergent plants. SSF CWs are divided depending on flow path direction; horizontal flow (HSSF) or vertical flow (VSSF) (see Figure 3). This study is concerned with SF CWs supporting the growth of emergent wetland plants.
3.4.3 Surface Flow Constructed Wetlands

Surface flow CWs (as shown in Figure 4) are designed to mimic the behaviour of natural wetlands (Farooqie et al., 2008). Typically water flow into the wetland system is regulated through the inlet manifold, after which it flows via gravity horizontally above the soil substrate through the emergent/floating vegetation to the outlet zone (Kadlec and Knight, 2009). The movement of water through the wetland is slowed by vegetation allowing particulate matter to settle and plant uptake of organic material, dissolved nutrients and pollutants (Langergraber and Haberl, 2001; Vymazal, 2011). The water depth is considered when trying to achieve a desired retention time, typically ranging from 0.1m to 0.6m depending on the type of waste treatment (Tousignant et al., 1999). The basin of a reed bed is water tight usually consisting of a HDPE liner with a mixture of coarse and fine substrate ranging between 0.4m to 1.0m in depth (Headley et al., 2005).

SF CWs are also largely used throughout the USA for pollutant diffusion and polishing such as the stormwater treatment areas of Everglades, Florida and the wastewater reclamation wetlands in
Alcarts, California (Abetew et al., 2007; US EPA, 1999). The Middle East has adopted a SF CW system for hydrocarbon removal (Breuer et al., 2012).

3.4.4 Horizontal Subsurface Flow Constructed Wetlands

In HSSF CWs, wastewater enters the system through an inlet pipe which distributes the influent across the width of CW’s entry point. The influent flows through a permeable substrate media remaining below the surface, toward the collection pipe before being discharged through an outlet pipe (see Figure 5). The focus of HSSF CWs is to optimize contact time for microbial treatment via maximizing total surface area through substrate media. In addition the substrate supports plant growth and coupled with below surface flow has the ability to inhibit pathogen exposure to the atmosphere, ultimately lowering the risk of disease via human and animal contact (Kadlec and Wallace, 2009).

![Figure 5 Profile view of a HSSF CW (Source from Fonder & Headley, 2010).](image)

HSSF systems are commonly used in Europe and USA as secondary treatment for wastewater and throughout the Middle East and USA for hydrocarbon removal. (Wallace et al., 2011; US EPA, 1999; Knight et al., 1999; Salmon, et al., 1998).

3.4.5 Vertical Subsurface Flow Constructed Wetlands

A VSSF system receives wastewater through an inlet pipe, distributing influent across the surface bed via a perforated piping system (see Figure 6), vertical infiltration occurs from the top to bottom travelling through a substrate media (see Figure 7) for collection and removal via a collection pipe system (see Figure 6). There are three general vertical flow classifications, depending on hydraulic application; Fill-and-Drain (mixed flow direction, alternating between up and down flow), Down flow (free draining without surface flooding) and Up flow (surface flooding) (Fonder & Headley, 2010).
The VSSF system originated in France in the early 1990’s, a two stage vertical down-flow system for raw sewage treatment has been named “The French system” and has been implemented in 2300+ municipals in France (Esser, 2015). USA, Australia and New Zealand have also widely adopted vertical flow systems for decentralised wastewater and industrial wastewater treatment (Fonder & Headley, 2010; Nelson, 2008; Domingos, 2011).

3.5 Plants in Constructed Wetlands

3.5.1 Role of Plants in Constructed Wetlands

Wetland plants are an important component of wetland design. It is stated that plants play a vital role in the treatment processes that take place in CWs (Brix, 2003; Oren Shelef, 2013). It has been documented that vegetation plays an important role in hydrocarbon degradation in surface flow wetlands (Knight et al., 1999). Vegetation reduces velocity and mixing within the water column, increasing the rate of sedimentation. Plant roots aid in the oxygenation of root zones promoting aerobic conditions, the increasing of surface area for biofilm development and nutrient uptake and
directly degrading of pollutants (Wallace S. D., 2005; Kadlec & Wallace, 2009; Tanner, 1996; Stottmeister, et al., 2003). Commonly used plant species in CWs include cattail (Typha spp.), club rush (Schoenoplectus spp.), green rush (Juncus spp.), and the most universally found species Phragmites australis, otherwise known as “common reed” (Brix, 2003).

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![Figure 8 Sketches of emergent aquatic macrophytes, showing Scirpus (Schoenoplectus) lacustris (left), Phragmites australis (middle) and Typha latifolia (right) (From Brix and Schierup, 1989)](image)

3.5.2 Description of Plants Used in the Study

The Large wetlands of the WTP are currently trialing five wetland plant species endemic to the middle east; Phragmites australis, Typha domingensis, Schoenoplectus litoralis, Juncus rigidus and Cyperus laevigatus. Propagules and germinated seeds from the five species were collected from local ephemeral riverbeds (Naughton, 2015). Headley and Damuschun (2012) believe some species may have been introduced through migratory birds, such as the unidentified Cyperus sp. (Prigent et al., 2014). This study relates to the five aforementioned wetland plant species in identifying the effects of HPAM PW on plant health and growth.

3.5.2.1 Phragmites australis

The wide distribution of Phragmites australis (Cav.) Trin. ex Steud, (Family Poaceae) or “Common Reed” has made it the most commonly used perennial emergent macrophyte in CW designs throughout Europe, Australia, Middle East and some parts of USA (Kumari & Tripathi, 2015; Asaeda et al., 2002; Tanner, 1996; Brix, 2003; US EPA, 1999). P. australis has high potential productivity due to its fast-and-robust growth, high tolerance to harsh climates (cold and hot), and extensive rhizome and root system (Asaeda et al., 2002). Brix (1999) describes the plant as having strong hollow aerial stems growing up to 4 m in height. The grass-like leaves measure between 10-60 cm in length and 1-6 cm wide for matured plants. During flowering large feathery flowers are produced measuring up to 50cm long. Where growth occurs in natural environments, P. australis provides benefits such as improved bank stabilization along rivers and lakes and habitat for wildlife (Brix, 1999; Naughton, 2015).
Some recent case studies identify the use of *P. australis* for a wide range of treatment wetlands under different influent applications. At Heathrow airport, London a SF CW is planted with *P. australis* to treat de-icing water (Worrall, 2000). The first built primary treatment CW in Masaya, Niguagra is planted with *P. australis* and services over 100 homes with final effluent reused for horticulture (WSP-LAC, 2008). Some other reported influent treatment applications using *P. australis* are heavy metal and hydrocarbon removal (Schaller et al., 2014; Kumari & Tripathi, 2015).

The WTP was originally designed as a monoculture system with *P. australis* as the key treatment plant species, however the local genotype proved susceptible to a large decline from pest attacks (African army worm, aphids and aquatic reed borer (*Schoenobius gigantellus*)). Water loss through high evapotranspiration rates associated with *P. australis* (Headley et al., 2012; Shelef et al., 2013) contributes to an increase in salinity and boron levels (~ 8 mg/L), this is identified with salinity levels recorded as: 6 ppt at the inflow (upper treatment cells) and increasing up to 15 ppt at the outflow (lower treatment cells) (Breuer et al., 2012). The increase in salinity and boron correlates to the lack of uniform growth down the wetland cells and it is caused by additional stress to the plants (Naughton, 2015).

3.5.2.2 Typha domingensis

*Typha domingensis* (Family Typhaceae) is commonly referred to as “Cattail”. The name is given for its sausage-shaped flower heads. *T. domingensis* is a rhizomatous aquatic perennial, generally found growing in either deep or shallow water bodies (often ephemeral) to a height of 4 m (Vymazal J., 2013). The fleshy base of the stem grows to 4 cm in diameter with green flat paneled leaf blades fanning from the stem. *T. domingensis* can be found in the local wadis, and is widespread globally throughout warm temperate to tropical regions. Typha is known as a colonizing, moderately salt tolerant macrophyte thriving in disturbed environments and preferring rich organic soils. However it is also known to grow rapidly in swamps, lakes and river beds.

3.5.2.3 Schoenoplectus litoralis

*S. litoralis* (Family Cyperaceae) “Clubrush” is the common name for the medium-sized moderately salt tolerant perennial sedge. *S. litoralis* is found to be preeminent in moderately deep to shallow fresh or brackish waterbodies (Wilson, 2015) and is known to respond well to high nutrient levels, hence the inclusion in CW systems (Romanowski, 2010). “Clubrush” sedges grow to a height of 1.5 m and shoots measure between 0.3-1 cm in diameter.

3.5.2.4 Juncus rigidus

J. rigidus (Family Juncaceae) is native to some Middle Eastern countries, often found in sandy saline environments with little moisture, such as wadis, pools and marsh areas. J. rigidus has the common name “Sea Rush” due to its salt tolerant nature as a perennial rush. Sea rush forms dense clumps growing to a height of around 0.5-1 m with rigid sharp pointed culms.
3.5.2.5 Cyperus laevigatus

Cyperus laevigatus (Family Cyperaceae) is a marshy sedge with a flattened stem, growing to a height of 1 to 2.5 meters tall. The species is a perennial rhizomatous herb found across the tropics and subtropics worldwide. The species is endemic to the Middle East and tends to grow in terrestrial fresh and brackish waters. Smooth linear culms grow to approximately 2.5 mm in diameter.

3.6 Hydrocarbon Treatment Processes in Constructed Wetlands

The design of the CW governs the hydrocarbon removal efficiency of the biological chemical and physical treatment processes. Salmon et al., (1998) reports microbial degradation as a key treatment process, however volatilisation, sorption and sedimentation also play a role in hydrocarbon removal, (Figure 9 represents the percentage of hydrocarbon removal by each process). Wallace (2003) also suggests that microbiological communities growing as biofilms in the plant root zones (rhizosphere) induce degradation of volatile organic compounds (VOC).

A list of key wetland treatment processes are outlined by Kadlec and Wallace (2009).

- **Microbiological degradation** of VOC by bacteria and microorganisms.
- **Volatilization** of inorganic and organic compounds which are then released into the atmosphere.
- **Sedimentation**, settling and removal of suspended solids.
- **Sorption** of contaminants onto substrate and emergent plant material.
- **UV degradation** from the sun’s rays, converting or degrading waterborne microorganisms such as pathogens, bacteria and viruses.
- **Plant uptake** of nutrients and trace metals through contact with the root zones and emergent plant material in the water column. Removal of nutrients and trace metals occurs when harvesting the AGB.

![Major hydrocarbon removal processes](image-url)

*Figure 9 Major hydrocarbon removal processes in surface flow constructed wetland systems (Salmon, et al. 1998)*
4 Materials and Methods

4.1 Site Description

The study site is located in the south east of the Arabian Peninsula. The specific project site remains within the boundary of the WTP’s ~850 ha site, situated west of the large treatment wetlands. The region has an arid climate with an annual average rainfall of 34 mm, all though highly variable. The mean monthly temperatures ranging from 17°C (January) to 35°C (June), and extreme temperatures ranging from a maximum of 50°C to a minimum of 6°C.

4.2 General Project Description of Trial Wetlands

Four surface flow constructed trial wetlands (SF CWs), referred to as Trial Wetlands (TWs), were constructed during January 2014. Each TW was designed to simulate the larger treatment wetland used by WTP i.e.; gravity flow, hydraulic retention time, plant species, as well as soil depth and composition. Each wetland receives PW from the same source as the larger wetland. A 15,000 ppm HPAM mother solution was injected onto the TW feedwater using a polymer dosing system to achieve the following concentrations of HPAM contaminated produced water;

- Trial Wetland 1 (TW1) = 500 ppm
- Trial Wetland 2 (TW2) = 1000 ppm
- Trial Wetland 3 (TW3) = 0 ppm (Control)
- Trial Wetland 4 (TW4) = 250 ppm

The above polymer concentrations were selected for the experiment by the operating company, taking into account a worst case scenario of 1000 ppm and the predicted values ranging between 250 ppm and 500 ppm.

Full operation of the large scale experiment begun on 1st March 2015.

4.3 Experimental System Set-Up

An oil and water separator receives PW from oil pipelines from the nearby oil fields. From there the PW enters the WTP’s stilling basin, and gravity feeds the PW into a series of hydrocyclone-oil separators to further remove oil from the PW. A secondary treatment process for H₂S gas includes activated carbon filtration before finally entering the buffer pond. A series of skimmers remove any further oil accumulation from the surface.

Figure 10A depicts an aerial view of the WTP polymer project site. PW is pumped from the closest point (upper left of picture) of the buffer pond to a 4 m³ buffer tank (BT) located inside the polymer dosing skid area (see Figure 10B). 30 m³ of PW from the BT is gravity fed to TW3 (control). The skid system draws PW from the BT via a dilution pump. Manual valves control the flow rate (30m³) for PW into TW2, TW3 and TW4. The injection of the polymer (HPAM; 15,000 ppm) mother solution is
controlled by three dosing pumps, each assigned to a desired polymer concentration; TW1 (500 ppm), TW2 (1000 ppm) and TW4 (250 ppm). Static mixers in the polymer dosing unit homogenize the produced water with the injected mother solution (HPAM) before entering the TW systems via underground pipelines.

Figure 10 Aerial view of Water Treatment Plant (A), Overview of the polymer dosing skid area (B), and Top view of Treatment Wetland 4 (C)

4.4 Trial Wetland Set-Up

4.4.1 Trial Wetland Design

Each TW was designed with an area of 1,600 m² (Length = 40 m; Width = 40 m) and marked by a concrete block containment wall of 0.7 m height. An impermeable bottom layer consists of; a 5 cm sand layer, 200 g/m² of geotextile material and a 1.5 mm HDPE liner. The substrate depth used for plant growth is 0.3 m and a water depth is maintained at 0.1 m with 0.3 m of freeboard remaining.

Each TW is divided into six tracks; five planted with the wetland plant species to be monitored (*Phragmites australis, Typha domingensis, Schoenoplectus littoralis, Juncus rigidus and Cyperus*
spp.) and the sixth planted with a mixture of different plant species which will not be monitored for this report (Figure 10C).

An inlet manifold pipe has twelve elbows (two per track) to distribute the contaminated PW evenly along the width of the TW. Water travels by gravity flow through the wetland from the inlet zone to the outlet zone. A collection pipe spanning the width of the outlet zone collects the effluent. A stand pipe incorporated in the outlet manifold enables the adjustment of the water level in the TW. The use of a tipping bucket measures the quantity of outflow from each TW. Each tipping bucket counts ~5 L per tip.

Each outlet manhole collects water through another collection pipe and travels by gravity to a collecting pond. A diesel pump is used to empty the collecting pond every two days into reed bed track terrace 1.1 (RBTT1.1 as shown in Figure 10A).

4.4.2 Substrate Establishment

The substrate is built up to a height of 0.3 m. The soil structure was developed by sieving (0-20 mm) local sands and mixing with a course gravel (size fraction ~ 18 mm – 24 mm).

4.4.3 Plant Establishment

The WTP established a nursery which supplied the five plant species trialed in this study. The original plant species were sourced by seed and propagules from local wadis under official license. The planting density for each species and track was 4.5 plants/m². Approximately 5000 plants (with similar maturity) per species were acquired from the nursery. Each track was planted with approximately 1250 plants (see layout in Figure 11), roughly 50 cm apart.

![Figure 11 Planting arrangement in each TW bed](image)

- Planting density ~ 4.5/m²
- Plants per track ~ 1250
- Total number each species ~ 5000

Figure 11 Planting arrangement in each TW bed
Originally it was planned to feed well water to the TWs for a period of two months, to allow the plants to establish under less stressful conditions. However due to the delay in commissioning, the trial system was fed well water for the duration of one year until 1st of March 2015 when full operation of the research project begun.

4.5 Sampling and Analysis

4.5.1 Oil in Water Measurement

A water sampling campaign was initiated on the 9th March 2015 to monitor the inlet and outlet physico-chemical parameters (see section 4.5.2 Water quality for parameters monitored) of each wetland. Using two 400 ml sample bottles for each sampling point (total of 8 samples bottles) a grab sample was taken every second week. Using the same method monthly inlet and outlet samples were taken for each TW and sent for external lab analysis for OiW measurements. This data was then used to infer removal efficiency of OiW, comparing the results across the different polymer concentrations.

4.5.2 Water Quality

General monitoring of the physical parameters such as water temperature, pH, conductivity, dissolved oxygen (DO) and oxidation-redox potential (ORP) were measured in-situ. DO, pH and ORP readings were taken using a Hach HQ30d flexi meter with the respective probes; Hach IntelliCAL pH PHC101 probe and Hach ORP redox probe. Conductivity and water temperature were recorded with the WTW multiline P4 universal meter using the same WTW TetraCon 325 probe. Chemical parameters were analyzed in the WTP laboratory; Boron, Chemical Oxygen Demand (COD), Total Nitrogen (TN), Ammonia (NH4-N), Total Phosphorus (TP) and Total Dissolved Solids (TDS) using a HACH Lange DR3900 spectrophotometer and HACH procedures. For Acrylamide concentration, Oil in Water (OiW), polymer concentration and Total Organic Content (TOC) analysis, a separate sample was sent to an external laboratory within the same day. The water quality testing was mainly used to ensure no major variations occurred between the treatments, for the scope and objectives of this study these will not be included in the results.

4.5.3 Plant Growth and Health

4.5.3.1 General Remarks

Five wetland plant species are planted in each TW; *Cyperus laevigatus* (C), *Juncus rigidus* (J), *Schoenoplectus littoralis* (S), *Typha domingensis* (T) and *Phragmites australis* (P). The aim of this study is to monitor the plant growth and health according to the polymer concentration applied. It is also expected that salinity increases over the length of the TW due to evapo(transpi)ration. Therefore, plant growth over the length of the TW is a function of the polymer concentration and also the increase of physical and chemical parameters such as salinity. It was therefore decided to conduct the vegetation monitoring in the middle of each TW. It is hypothesized that the salinity is the same in each sampling point for each TW.
The analysis of plant growth refers to a quantitative set of methods that interprets plant form and function into primary data to derive the performance of plant growth. Primary data can include weights, areas, volumes and dimensions of particular or whole components of a plant (Hunt, 2003). A study by Li et al. (2013) determined plant growth by measuring the relative growth rate of plants based on total dry biomass (g DW/m²), whereas Vymazal & Kropfelov (2005) analysed plant growth using biomass, stem count and stem length data. For the purpose of this study a quantitative method (described below) provided data for above ground dry biomass (g/m²) and shoot density (shoots/m²), the two parameters used to quantify the performance of plant growth. A qualitative method was used to quantify plant health by measuring the percentage of necrotic plant material of each wetland plant species, this method was built upon from the previous small scale mesocosm study undertaken by Johnston (2013).

4.5.3.2 Plant Growth: Quantitative Method

4.5.3.2.1 General Methods

The quantitative method monitored the dry weight (g/m²) of above ground biomass (AGB) for alive and dead/necrosis plant material and shoot density. Initially each TW was evaluated and a 2.5 m wide transect area was selected on the basis of location, average height, health and density of vegetation within each track and across all TWs. Transect areas were selected in an area before each central
intermediate walkway for C, J, S and T and the outlet zone for P. For Phragmites the outlet zone was selected due to this being the most representative zone across all four TWs. Each identified transect was marked with 6 lanes across the width of the track representing the line of future quadrat sampling. A baseline quadrat sampling campaign (T=0) was completed between 02/03/2015 and the 8th March 2015; this consisted of three quadrats of 0.25m² (Length= 0.5 m and Width= 0.5 m) being harvested (except for J in which a clump was harvested) from lanes 1, 3 and 6 (Figure 12). This process was again reproduced after 6 and 12 weeks from T=0 (see Figure 12 for sample locations in cut transects for each sampling week).

4.5.3.2.2 Above Ground Dry Biomass (AGDB)

To achieve the dry weight of the standing, above ground biomass, the harvested alive and Dead AGB was manually separated. Each individual alive and dead shoot was counted, any segment of alive shoot affected by necrosis was then removed and added to the dead plant material. Each AGB sample was dried in an air-conditioned porta-cabin and weighed frequently until a constant weight was achieved.

4.5.3.2.3 Shoot Density

Shoot density was calculated by using the average of the total number of alive and dead shoots harvested from the three quadrats per species. Shoot density (shoots per m²) was estimated for each plant species in each TW by using the average density of the sampled 0.25m² and multiplying it by four.

*Figure 13 Mobile steel quadrat set-up before harvesting of Typha (A), Cut transect of a treatment wetland (B), Alive and dead shoot separation (C)*
4.5.3.3 Plant Health: Qualitative Sampling

4.5.3.3.1 General Methods

The qualitative method was used to study the change in percentage of necrosis within the one year old established standing biomass and the re-growth of the new shoots from the transect using the quantitative method. Thirty six healthy plants per wetland species was randomly selected for each polymer concentration (18 new shoots and 18 one year old established (old shoots)). Random selection involved measuring every 45 cm and at this point the shoot was to be selected. Eighteen healthy old shoots were selected on the opposite side of the walkway in the same mirrored area as the cut transect (see dotted area in Figure 12) another eighteen healthy new shoots were selected from the cut transect outlined in Figure 12 by the quadrats marked with a star. Individual shoots were selected for Cyperus, Phragmites and Schoenoplectus and for Juncus and Typha individual clumps were identified and then the tallest healthy individual shoot was selected. A total of seven hundred and twenty new and old shoots were selected and tagged across the four wetlands. Monitoring of total shoot height and length of necrosis begun on the 19th March 2015 and was repeated every two weeks for the duration of the study.

4.5.3.3.2 Percentage of Necrosis

The percentage of necrosis was determined using the length of necrosis divided by the total length of the shoot/stalk/leaf. Statistical analysis was conducted using one-way ANOVA test to verify if there is any significant difference between each polymer treatment on necrosis of each wetland plant species. Total shoot height and length of necrosis was measured differently across the plant species due to the individual rhizome systems of each plant species. Individual healthy shoots were selected for Cyperus and Schoenoplectus, then for Juncus and Typha the tallest stalk per clump was selected. For C, J, S and T the total height of the shoot/stalk was measured using a graduated ruler from the base of the shoot/stalk to the tip. The length of necrosis was measured by the browning and dried proportion of the shoot/stalk starting from the tip, running down the center until green pigment/color was identified. For Phragmites the total plant height was measured in the same way as the other species, however the first, third and fifth fully developed leaves were measured for total leaf length and length of necrosis was analyzed measuring the browning and dried proportion of each leaf starting from the tip of the leaf, and running down the center until the leaf had green pigment/color.
5 Results

5.1 Plant Growth and Health

5.1.1 Plant growth: Quantitative method

5.1.1.1 General remarks

Plant growth is determined by above ground biomass and shoot density derived from the quantitative method. In the below chapters above ground biomass is presented for each wetland plant species and a summary is provided for the above ground biomass as well as shoot density.

5.1.1.2 Cyperus

Figure 14 compares the average above ground dry biomass of Cyperus, among the four polymer concentrations, over 12 weeks (2 samples).

![Figure 14: Average above ground dry biomass over 12 weeks for Cyperus](image)

The AGDB steadily increases over the duration of the sample period for all polymer concentrations. The fastest rate and heaviest AGDB (460.8 g/m²) were recorded in the absence of HPAM (shown as the blue line of 0 ppm). 1000 ppm represented the lowest AGDB (276.0 g/m²).
5.1.1.3 Juncus

Figure 15 compares the average above ground dry biomass of Juncus, among the four polymer concentrations, over 12 weeks (2 samples).

![Juncus](image)

*Figure 15 Average above ground dry biomass over 12 weeks for Juncus*

All polymer concentrations gradually increased at a similar rate however 250 ppm continued at a faster rate than the other three concentrations. 250 ppm had a final AGDB of 322.7 g/m² as opposed to the 0 ppm the lowest of the four measurements at 113.3 g/m².

5.1.1.4 Schoenoplectus

Figure 16 compares the average above ground dry biomass of Schoenoplectus, among the four polymer concentrations, over 12 weeks (2 samples).

![Schoenoplectus](image)

*Figure 16 Average above ground dry biomass over 12 weeks for Schoenoplectus*

The 1000 ppm increased at a linear rate from 0 g/m² to 313.2 g/m², with a final value higher than the other three polymer concentrations. The lowest rate of increase was seen in the 0 ppm, resulting with a final value of 80.8 g/m² in week 12.
5.1.1.5 Typha

Figure 17 compares the average above ground dry biomass of Typha, among the four polymer concentrations, over 12 weeks (2 samples).

![Figure 17 Average above ground dry biomass over 12 weeks for Typha](image)

Typha indicated a similar increasing trend for all polymer concentrations however 1000 ppm recorded the highest final value with 456.7 g/m², more than double the AGDB of 250 ppm and triple that of 0 ppm and 500 ppm.

5.1.1.6 Phragmites

Figure 18 compares the average above ground dry biomass of Phragmites, among the four polymer concentrations, over 12 weeks (2 samples).

![Figure 18 Average above ground dry biomass over 12 weeks for Phragmites](image)

Between the start and week 6 both 250 and 1000 ppm increased at the same rate for AGDB, however after week 6; 250 ppm continued with a stronger rate of increase ending 47.7 g/m² higher than 1000 ppm which recorded 541.4 g/m². Overall the final values of AGDB among the four wetlands where similar, measuring within a range of 85.7 g/m² (14.5%) of the highest value.
5.1.1.7 Summary

Figure 19(A-B) shows a summary of quantitative method results, at the end of week 12. Figure 20A compares the average total above ground dry biomass in grams per square meter of each wetland plant species, across the four polymer concentrations. Figure 20B compares the average number of shoots per meter square of each plant species, across the different polymer concentrations.

![Figure 19](image1.png)

*Figure 19 summarizes the quantitative method results over 12 weeks A) Average total above ground dry biomass (g/m$^2$) for each plant species, among the four polymer concentrations. B) Average shoot density (shoots/m$^2$) for each plant species, among the four polymer concentrations.*

In both Figure 19A and Figure 19B, the results indicate a link between the shoot density and the total weight of AGDB for Juncus. For Typha, 1000 ppm has a lower shoot density than 0 ppm and 250 ppm, however the AGDB is more than double in 1000 ppm than the other three concentrations. The measured values for AGDB in Typha for 1000 ppm is 456.7 g/m$^2$, 147.3 g/m$^2$ for 250 ppm and 147.2 g/m$^2$ for 0 ppm.

Standard deviation is included to identify whether or not a significant difference occurs between TWs. Cyperus shows no significant difference between 0 ppm and the three other polymer concentrations (250 ppm, 500 ppm and 1000 ppm). A significant difference occurs between the 1000 ppm and the three other concentrations for Juncus. Typha is significantly different between 1000 ppm and the
other three concentrations, whilst Phragmites shows no significant difference between polymer concentrations.

5.1.2 Plant health: Qualitative method

5.1.2.1 General remarks

Plant health is determined by the percentage of necrosis using the qualitative method. In the below sections the percentage of necrosis is presented for each wetland plant species.

5.1.2.2 Cyperus

Figure 20 compares the percentage of necrosis in Cyperus between one year old established plants and new plant shoots, across the four polymer concentrations over 13 weeks (6 samples).

![Graph A: Percentage of necrosis for one year old established plant shoots](image)

![Graph B: Percentage of necrosis for new plant shoots](image)

*Figure 20 Percentage of necrosis of Cyperus over 13 weeks A) One year old established plant shoots B) New plant shoots*

The percentage of necrosis steadily increases for all polymer concentrations for both new and old shoots. 500 ppm has the lowest necrosis in both old and new shoots, whereas 250 ppm has the highest.
5.1.2.3 Juncus

Figure 21 compares the percentage of necrosis in Juncus between one year old established plants and new plant shoots, across the four polymer concentrations over 13 weeks (6 samples).

The percentage of necrosis over the duration of the 13 week sampling campaign illustrates very little to no change, however in week 11 necrosis in old shoots increased rapidly for 0 ppm and 500 ppm. Across all treatments the total percentage of necrosis is very low in both old and new shoots, ranging from 0% to 6% and 0% to 3%, respectively.
5.1.2.4 Schoenoplectus

Figure 22 compares the percentage of necrosis in Schoenoplectus between one year old established plants and new plant shoots, across the four polymer concentrations over 13 weeks (6 samples).

Necrosis steadily increased between week 5 and 11 for each polymer concentration, except in the 1000 ppm new shoots (Figure 22B). The 1000 ppm new shoots has minimal change in percentage of necrosis except between week 9 and 11, identified by the rapid increase from 8% to 60% before continuing the earlier trend. Figure 22 (A-B) suggests that after week 11 the percentage of necrosis tends to level for all polymer concentrations meaning very little to no variation although at a high percentage (>55% for all species), however 250 ppm in the old shoots (Figure 22B) decreased by 10%.
5.1.2.5 Typha

Figure 23 compares the percentage of necrosis in Typha between one year old established plants and new plant shoots, across the four polymer concentrations over 13 weeks (6 samples).

![Graph A](image1.png)

**Figure 23 Percentage of necrosis of Typha over 13 weeks A) One year old established plant shoots B) New plant shoots**

Figure 23A identifies old Typha has a lower percentage across all HPAM concentrations at week 13. Figure 23A shows a steady increase in percentage of necrosis for all polymer concentrations, ranging from 0% to 10% for week 3 and 20% to 40% in week 13. 500 ppm has the lowest percentage of necrosis at the end of 13 weeks and 0 ppm the highest (Figure 23A). Figure 23B also shows a steady increase for all polymer concentrations, however at week 9 the 0 ppm rapidly increased from 25% to 66%, then stabilizing after week 11.
5.1.2.6 Phragmites

Figure 24 compares the percentage of necrosis in Phragmites between one year old established plants and new plant shoots, across the four polymer concentrations over 13 weeks (6 samples).

Figure 24 Percentage of necrosis of Phragmites over 13 weeks A) One year old established plant shoots B) New plant shoots

Figure 24A shows a steady increase in percentage of necrosis for all polymer concentrations, fluctuating between 10% and 25%. The 0 ppm records a final value of 29% whilst the other TWs receiving HPAM record between 20% and 25%. Values in Figure 23B maintain a steady percentage of necrosis for 0 ppm, 500 ppm and 1000 ppm after week 3. 250 ppm steadily increases after week 3.
5.1.2.7 Statistical Analysis

Table 2 summarizes the statistical analysis using a one way ANOVA test (with an alpha of 0.05) for both old and new shoots percentage of necrosis. The analysis is used to identify the significant differences between TWs on each plant species. If the F value is greater than the $F_{crit}$ and the P-value is less than 0.05 (the alpha) it is considered that a difference occurs between TWs. Correspondingly the opposite suggests no differences between groups.

Table 2  Summary of statistical analysis using a one way ANOVA test for qualitative sampling analysis: percentage of necrosis for each plant species among polymer concentrations.

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<th>P-value</th>
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<tr>
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<td>2.739502</td>
<td>0.134766</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>New</th>
<th>ANOVA: One way</th>
<th>F</th>
<th>$F_{crit}$</th>
<th>P-value</th>
</tr>
</thead>
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<td>1.07E-08</td>
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<td>0.686742</td>
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</tbody>
</table>

P-values in red colour indicate statistically significant effects at the 0.05 significance level.

Phragmites has no significant difference for either old or new shoots. Both Juncus and Schoenoplectus show no significant difference for old shoots. Cyperus new indicates no significant difference. All other plant species old and new, show some significant difference occurring between groups.

5.2 Hydrocarbon Removal

Hydrocarbon removal is presented by the OiW treatment efficiency from inlet to outlet of each trial wetland.

Figure 25 shows the OiW concentration at the inlet and outlet for each polymer concentration.
The OiW concentrations are less than 1mg/l at the outlet of each TW. Figure 25 indicates there is no significant difference between OiW concentrations of 0 ppm and polymer contaminated trial wetlands (250 ppm, 500 ppm and 1000 ppm) for both, inlet and outlet.

Table 3 shows the treatment efficiency of hydrocarbons in each trial wetland, derived from the OiW removal from inlet to outlet (6 sampling campaigns).

Table 3 Oil in Water removal efficiency for each Trial Wetland

<table>
<thead>
<tr>
<th>Trial Wetland</th>
<th>Polymer concentration (ppm)</th>
<th>OiW removal efficiency (%)</th>
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</thead>
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<tr>
<td>TW3</td>
<td>0</td>
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<tr>
<td>TW4</td>
<td>250</td>
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<td>TW1</td>
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</tr>
<tr>
<td>TW2</td>
<td>1000</td>
<td>96.8</td>
</tr>
</tbody>
</table>

5.3 Water Balance

The water balance is represented by the volume of water loss, calculated from total inflow and outflow per wetland.

Figure 26 illustrates a timeline of the water loss for each trial wetland (0 ppm, 250 ppm, 500 ppm and 1000 ppm) over the three month sampling period. The Class-A pan represents the evaporation measured for the large treatment wetland at the WTP and has been included for comparison.
The overall water loss of the four polymer treatments follows a similar, steady trend to that of the Class-A pan, reflecting the change in seasonal weather conditions (an increase in temperature due to the approaching warmer summer months). 0 ppm had the greatest water loss over the three months, the polymer contaminated TWs; 250 ppm, 500 ppm and 1000 ppm had on average 23%, 25% and 34% less water loss over the same time period, respectively.

Figure 26 Water loss of each trial wetland system and WTP Class-A Pan.
6 Discussion

6.1 Plant Growth and Health

The above results provide an overall indication that there were no major effects on wetland plants after receiving three months of polymer contaminated water. The observed effects on AGDB show no clear trend when comparing the different polymer concentrations (250 ppm, 500 ppm and 1000 ppm) to the control (0 ppm). The same applies to shoot density, where likewise a random pattern was also observed between polymer concentrations. These random effects could be partly due to natural variability that occurs when conducting a large pilot scale field study. Another factor contributing to the random effects could be the high variability in some samples (also experienced in Johnston’s (2013) study), outlined in Figure 19A-B by the differing standard deviations. The preceding small scale study conducted by Johnston (2013) observed an increased growth rate for *P. australis* in some mesocosms of up to 47%. However Johnston (2013) reported these observations were not statistically significant (statistical analysis was completed using a one way ANOVA test) (F=1.607, p=0.276) due to the variability amongst replicates. The variability and random positive/negative effects observed in this study, don’t detract from the overall conclusion that polymer contamination has no major effects on plant growth of the five wetland plant species.

Results from the qualitative method also suggest, overall no major effects occur to the health of the five wetland plant species receiving the different polymer concentrations, neither for new shoots or one year old established shoots. There was no clear correlation between plant necrosis and the application of different polymer concentrations. A one way ANOVA test was used to test the hypothesis; there is no significant difference on wetland plants across treatments. However, results from the ANOVA test identified a significant difference for old shoots in Cyperus (F(3,68)=5.614, p=0.001) and Typha (F(3,68)=6.095, p=0.001) and for new shoots in Juncus (F(3,68)=17.98, p=1.069E-08), Schoenoplectus (F(3,68)=3.861, p=0.013) and Typha (F(3,68)=3.332, p=0.024). By evaluating the qualitative results for each plant species and comparing with the ANOVA results, the plant species that show a significant difference don’t indicate any clear trends between treatments, suggesting the observed differences are random. Natural variability may play a role in the random positive/negative effects observed by the contamination of polymer. To help identify the random effects on plant health a leaf analysis should be considered to detect the macro and micro nutrients within samples across each treatment. The results could then be evaluated to suggest a reason for the random effects observed for plant health. Overall the observed random effects do not suggest any major effects to plant health are related to polymer contamination.

6.2 Hydrocarbon Removal

The WTP treatment wetland receives PW containing ~ 22.0 mg/l of OiW at the inlet and measures less than 0.01 mg/l (lower than the detection limit) at the outlet, reporting a 100% OiW removal efficiency (Breuer et al., 2012). The OiW concentrations observed during this study were less than 1 mg/l at the outlet of each TW. All four TWs were designed as a large pilot scale trial wetland to
replicate the design of the WTP. The results of the 0 ppm TW achieved a similar OiW removal efficiency measuring 97.5%. Analyzing the three replicate trial wetlands contaminated with polymer 250 ppm, 500 ppm and 1000 ppm, the OiW removal efficiency achieved was 97.8%, 96.9% and 96.8%, respectively. The removal efficiencies observed across all four treatments, are consistent with range of OiW removal efficiencies in other treatment wetlands, such as of the Casper and Wellsville CWs reporting 100% and 87% removal efficiencies (measured by the removal of benzene, a constituent of crude oil), respectively. This suggests the removal of hydrocarbons from polymer contaminated PW has little to no difference when compared to the control (0 ppm).

6.3 Water Balance

Water loss followed the same evaporation loss trend that occurred in the class-A pan, which was related to the rising temperatures due to seasonal change. Water loss could be associated with a number of causes including; evaporation (as highlighted above), evapotranspiration through plant biomass and/or a leakage in the wetland system. As the system was lined with a HDPE liner and there was no evidence of water leaking. The possibility of a leakage may be ruled out. However to prove this, a tracer experiment should be conducted. A tracer test would derive information on the internal hydrodynamics by injecting a tracer substance into the inlet of the TW. The recovered concentration of tracer at a point further down the TW system, may help determine if a loss of water occurs from the system. The observed increase in water loss was most probably due to an increase in the rate of evaporation and/or evapotranspiration. This is a common response observed when temperature rises during the hotter summer months and can be seen in the results of the 27 mesocosm pilot scale by Johnston (2013). Another possibility could include the increase in plant biomass (as seen in the results section 5.1.1 Plant Growth) resulting in an increased plant surface area and therefore an increased evapotranspiration. However this could have an adverse effect on the total volume lost through evaporation. An increase in AGDB is known to reduce the rate of evaporation through the shading effect prohibiting direct sunlight on the water surface of the wetland system (Kadlec & Wallace, 2009).

The results for water loss suggest a relationship, identifying the total water loss decreased with the increase in polymer concentration. On average the polymer contaminated TWs indicated a 20 to 26% lower water loss compared to the control wetland. This suggests that polymer has an effect on water loss.
7 Conclusion

The aim of this study was to investigate the response of five emergent macrophytes (*Phragmites australis*, *Schoenoplectus littoralis*, *Typha domingensis*, *Cyperus laevigatus* and *Juncus rigidus*) to the addition of HPAM contaminated produced water and to evaluate the effects of these responses on the treatment performance of PW in a SF CW system. The results observed for plant growth and health (section 6.1 Plant Growth and Health) indicate no major effects when exposed to HPAM and no clear trends occur between treatments. The results (see 6.2 Hydrocarbon Removal) show the OiW measurements at the outlets of all four TWs were less than 1 mg/l and an OiW removal efficiency of 97.5%, 97.8%, 96.9% and 96.8% was recorded for 0 ppm, 250 ppm, 500 ppm and 1000 ppm, respectively. This suggests the removal of hydrocarbons from polymer contaminated PW had little to no difference when compared to the control (0 ppm). On average the polymer contaminated TWs indicated a 20 to 26% lower water loss compared to the control wetland. These results suggest three main findings of the study:

1. There are no major effects on wetland plants after receiving three months of polymer contaminated PW.
2. The removal of hydrocarbons from polymer contaminated PW shows little to no difference when compared to the control.
3. Polymer has an effect on water loss which is most likely associated with evaporation.

Therefore it can be concluded from the main findings in point 1 and 2, that over a three month period, the presence of HPAM (up to 1000 ppm) does not affect the treatment performance of PW in SF CWs. This could be attributed to the growth and survival of the five wetland plant species amongst the different treatments. However, this should be verified by allowing a full seasonal growing cycle, to monitor the natural growth cycle of the five plant species. This study began in March 2015 during the period of natural plant senescence, which could attribute to the random effects seen in the results for plant growth and health. A long term study should allow for the full seasonal growth cycle to identify the growth and survival of the five plant species in the presence of HPAM. A series of leaf analysis at the end of each season should also be conducted to determine the random effects observed.

In concluding the third finding, HPAM decreased water loss, which was associated with the increase in polymer concentration. Therefore the current evaporation ponds at the WTP would need to be designed to accommodate these effects. Such modifications could include a larger evaporation pond area to achieve the same evaporation as the current design. An investigation into the direct effects of polymer concentration on evaporation should be undertaken to determine current/potential design considerations.

The results suggest that a SF CW system with a healthy vegetation is able to survive and remove hydrocarbons in HPAM contaminated PW. However, the trending data in regard to percentage of necrosis should reach a steady state to suggest the plant/s can survive in HPAM contaminated PW. The short duration of data collected has limited the opportunity for a steady state to occur and for a
full seasonal plant growth cycle. Further research is suggested, to collect a sufficient amount of data for an improved conclusion on the outcome of the objectives.
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