

Detection of endocrine disrupting compounds in abiotic and biotic matrices from the Phongola floodplain using tissue culture based bio-assays

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- My caring, understanding and very patient parents, family members; and friends and
- to my Heavenly Father, through whom anything is possible, and without this would not be possible .

Abstract

Detection of endocrine disrupting compounds in abiotic and biotic matrices from the Phongolo floodplain using tissue culture based bio-assays

Increasing rates of urbanization, industrialisation, population growth and agricultural demand in South Africa have considerably decreased water quality leading to different forms of water pollution, which might be the case for the Phongolo floodplain. Some compounds commonly produced synthetically or naturally pose a risk to biological and ecosystem health. Biological responses due to these compounds could be mutagenic, carcinogenic and in some cases endocrine disruptive. These endocrine disrupting compounds (EDCs) are described as an exogenous compound, or mixture of compounds/chemicals that interfere with endocrine systems in organisms. The present study screened/assessed agonistic/anti-agonistic interaction of chemicals/compounds with the ability to bind to the oestrogen receptor (ER) and the aryl hydrocarbon receptor (AhR) in the aquatic environment of the Phongolo floodplain. Three matrices, sediment, water and fish bile, were collected from sites in the protected environment of the Ndumo game reserve in KwaZulu-Natal, South Africa; the Nyamiti Pan, the Phongola River, and the Ushutu River. One site was selected from outside the borders of Ndumo, the Nomaneni Pan, in order to determine the direct influences of communities on water sources. The water samples were extracted using solid phase extraction and applicable clean-up methods. Sediment samples were extracted using accelerated solvent extraction in a dichloromethane:acetone solution. Clean-up steps involved solid phase extraction and size exclusion chromatography. Bile was liquid-liquid extracted after enzymatic hydrolysis followed by a solid phase extraction as clean-up step. These extracts were screened with the MVLN reporter gene bio-assay for both activation and inhibition of the oestrogen receptor. The sediment samples were additionally analysed using the H4IIE-*luc* reporter gene bio-assay to determine if these target compounds bind to the AhR. The results obtained, indicated an activation of the oestrogen receptor for sediment, water and fish bile for different seasons and at different levels. Inhibition of the ER also occurred at different sites and at different events. Some samples from the Phongolo River elicited both

activation and inhibition of the same receptor. Samples from the second sampling event (wet season) caused the highest percentage of activation of the ER. There was less evidence of ligands capable of binding to the AhR and very little response by the extracts containing the more persistent AhR-ligands. The aquatic habitat most affected by contamination of these compounds was the Phongolo River. Results obtained from the Nomaneni Pan were irregular probably due to being directly impaired on by surrounding rural communities. Although, this study was successful in determining the presence of EDCs, and specifically those capable of binding to the ER, in the aquatic environment of the Phongolo floodplain the identity of the compounds remain unknown. Instrumental analysis would be beneficial in determining the specific compounds responsible for disrupting the endocrine system and their concentration levels in the environment.

Keywords: Endocrine disrupting compounds (EDCs), oestrogen receptors (ER), aryl hydrocarbon receptors (AhR), MVLN Bio-assay, H4IIE-luc, Phongolo floodplain, KwaZulu-Natal, South Africa, solid phase extraction (SPE)

List of abbreviations and acronyms

AhR	Aryl hydrocarbon receptor
AIP	Aryl hydrocarbon receptor-interacting protein
AhRR	Aryl hydrocarbon receptor repressor
Arnt	AhR nuclear translocator
CCK-8	Cell Counting Kit 8
DAWF	Department of Water Affairs and Forestry
DCM	Dichloromethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulphoxide
E₂	17 β -oestradiol
EC	Effects concentration
EDCs	Endocrine disrupting compounds
ER	Oestrogen receptor
ERE	Oestrogen response element
HAH	Halogenated hydrocarbons
HDPE	high density polyethylene
HLB	Hydrophilic-lipophilic-balanced
Hsp90	Heat shock proteins
ICI	Faslodex
LOD	Level of detection
LOQ	Level of quantification
MAPK	Mitogen-activated protein kinase
MeOH	Methanol
MtBe	Methyl <i>tert</i> -butyl ether
MVLN	MCF-7-derived cell
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide

NMDRCs	nonmonotonic dose-response curves
NGR	Ndumo Game Reserve
P23	Co-chaperone protein
PAH	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenyl ethers
PBS	Phosphate buffered saline
PCB	Polychlorinated biphenyls
POPs	Persistent organic pollutants
PI3-kinase	Phosphoinositide kinase
REP	Relative potency
RLU	Relative light units
SASRI	South African Sugarcane Research Institute
SC	Solvent control
SPE	Solid phase extraction
SPSS	Statistical Package for the Social Sciences
TBT	Tributyltin
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TCDF	2,3,7,8-tetrachlorodibenzofuran
USEPA	United States Environmental Protection Agency
UV	Ultra violet
WWF	World Wildlife Fund
xl-compounds	Xenobiotic-like compounds
XRE	Xenobiotic response element

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1. Introduction

1.1. Introduction

The natural aquatic environment can be exposed to many forms of pollution and contaminants from various origins: untreated sewage, agricultural-, industrial- and urban runoff, combustion processes, pollutants transported via air and natural sources (Mills *et al.*, 1993). Pollutants from these different sources and origins can place pressure on the natural aquatic environment affecting human and ecosystem health (Benotti *et al.*, 2009). Pollutants not only cause acute toxicity but can cause chronic effects such as creating cancers and disrupting the endocrine system (Sakai and Takigami, 2003).

1.2 Phongolo floodplain

A common problem in rural areas of developing countries, such as South Africa, is the poor quality of drinking water. Rural communities, such as the uPhongolo district in north-eastern KwaZulu-Natal, South Africa, often do not have access to treated drinking water and thus have no alternative but to use water directly from untreated sources such as streams, rivers, wells and ponds (Humphries *et al.*, 2005). South Africa has an estimated population of 54 million citizens (Statistics South Africa, 2014) and has the largest economy and industrial base of any country in Africa (World Bank, 2009). The province in which the study took place, KwaZulu-Natal, has 19.8% of the total population and the uPhongolo district, had a population of 127 238 in 2011. The municipality is largely composed of traditional areas, with tribal land patterns and high density rural settlements. Most of the population lives in rural areas and use the Pongolapoort Dam and its tributaries as main source of food and water supply.

1.3. Endocrine disrupting compounds (EDCs)

There are countless effects pollutants may exert on biota. This study focussed on those compounds that are known to disrupt normal functioning of the endocrine system and

interfere with natural hormonal processes in biota (Damstra, 2002). Such compounds are referred to as endocrine disrupting compounds (EDCs), many of which have the ability to bio-accumulate in food webs, posing significant health threats to humans, animals and the environment (Klánová *et al.*, 2007).

The endocrine system is a complex physiological process by which the body can react to in response to a range of internal and external signals and stressors. Hormones are the chemical messengers of the body that are secreted from the endocrine glands directly into the bloodstream and are involved in regulating growth, development and other functions of the body (Hinson *et al.*, 2007). Endocrine disrupting compounds may interact with these physiological systems and cause alterations in development, growth and reproduction in humans and wildlife (Hinson *et al.*, 2007). Detrimental effects implicated by the interactions with EDCs may include cancer, allergies, hypersensitivity, damage to the central and peripheral nervous systems, reproductive disorders and disruption of the immune system (Khim *et al.*, 1999). The presence of EDCs can be determined by using instrumental as well as biological analysis.

1.4. Methods of detection

Targeted instrumental chemical analyses are well suited for quantifying specific congeners and isomers but is time consuming and expensive and does not provide an indication of the toxic effects the pollutant mixture has on organisms. On the other hand, biological assays immediately show detrimental effects but cannot be used to identify the different compounds in the mixture responsible for these effects. Biological screening assays are invaluable to assist in learning if pollutants with a very specific mechanism of action are present in the environment (Sakai and Takigami, 2003).

In this project reporter gene bio-assays were used to determine if compounds found in the Phongolo floodplain can interfere with the endocrine system of humans and wildlife. As the endocrine system involves a variety of glands and hormones, this study focussed primarily on detecting compounds that affect hormone disruption via the oestrogen receptor (ER) and secondary those that activate the aryl hydrocarbon receptor (AhR). Compounds

capable of binding to the ER are referred to as (anti)-oestrogenic compounds, i.e. compounds mimicking oestradiol and those xenobiotics capable of activating the AhR are commonly referred to as the dioxin-like compounds. Screening tests that were used to detect these specific responses are the genetically modified breast cancer cells (MVLN-assay) and rat hepatoma cells (H4IIE-*luc*-assay). These assays not only detect the effect, but also semi-quantify the toxic response in terms of their respective positive control chemicals.

- Examples of compounds that mimic oestradiol and act as ligands of the ER in the MVLN cells (breast cancer cells) include certain non-planar polychlorinated biphenyls (PCBs), and a selection of pharmaceuticals and pesticides, personal care products and plasticisers such as bisphenol A (BPA) to name but a few (Porta and Zumeta, 2002).
- Examples of dioxin-like compounds that are ligands of the AhR in the H4IIE-*luc* cells (rat hepatoma cells) include the polycyclic aromatic hydrocarbons (PAHs), planar polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) and -furans (PCDFs) (Nordic Council of Ministers, 1998).

1.5. Oestrogen receptor (ER)

Oestrogen receptors are found in both male and female vertebrates and play an important role in the function of their reproductive systems. This receptor is activated by the steroidal hormone oestrogen (17 β -estradiol). Once activated, the ER is able to regulate the activity of different genes. Some exogenous pollutants have the ability to bind to the ER inducing a response which could potentially have negative effects on wildlife and human health (Diamanti-Kandarakis *et al.*, 2009).

1.6. Aryl hydrocarbon receptor (AhR)

The AhR is a ligand-dependent transcription factor that mediates a variety of cellular events in a variety of tissues (Denison and Nagy, 2003). Aryl hydrocarbon receptor expression is found in many vertebrates including fish, animals and humans. When the AhR binds to xenobiotic ligands it regulates the expression of certain genes involved in a variety of

responses such as cell proliferation, apoptosis, immune function, development, and reproductive functions (Bonnesen *et al.*, 2001). Compounds that cause these mechanisms of response could potentially have adverse effects on human and wildlife health.

1.7. Hypothesis

Compounds found in the aquatic environment of the Phongolo floodplain are endocrine disruptive.

1.7.1. Aim and objectives

The aim was to determine whether oestrogen active compounds as well as AhR-ligands are found in the abiotic as well as biotic compartments of the Phongolo floodplain.

1.7.2. Objectives:

The objectives were:

1. to semi-quantify activation as well as inhibition of the oestrogen receptor by extracts from water, sediment and fish bile using the MVLN reporter gene bio-assay;
2. to semi-quantify the AhR-ligands in the sediment using the H4IIE-*luc* bio-assay;
3. to distinguish between the more toxic persistent and less toxic non-persistent AhR-ligands by differentiating extraction techniques and quantifying them using the H4IIE-*luc* bio-assay;
4. to determine if bio-accumulation of oestrogen receptor ligands takes place in biota by comparing the quantities of these extracts in sediment, water and fish tissue to one another.

2. Literature review

Increasing rates of urbanization, industrialisation, population growth and agricultural demand in South Africa have considerably decreased water quality leading to different forms of water pollution (Falconer *et al.*, 2006). Water pollution also occurs as a result of poor sanitation and lack of the necessary infrastructure able to handle the large quantities of water to be treated. Groundwater may also become polluted through seepage from wastewater or by rain containing soluble pollutants entering the ground (Kifr, 1981). There is an extremely wide range of water-borne pollutants, organic and inorganic, ranging from a wide spectrum of chemicals used in the industrial and agricultural areas that might find their way to the freshwater systems of South Africa.

Air pollution also contributes to the declining water quality as airborne pollutants from human activities and natural sources can deposit back onto land and water bodies. Some of these pollutants can travel great distances from the original source (Reddy and Lee, 2012).

There are many forms of pollution and indefinite number of effects that could be caused by these pollutants. This study specifically investigated a group of pollutants that are called endocrine disrupting compounds.

2.1. Endocrine disrupting compounds

Over the past two decades, an increasing concern and public debate developed over the potential adverse effects in wildlife and humans that are caused by exposure to a group of chemicals that have the potential to alter the normal functioning of the endocrine system (Niehs, 2006, Sharpe and Skakkebeak, 1993). These chemicals are known as endocrine disrupting chemicals (EDCs) since they act like or mimic or suppress the action of natural occurring hormones (McLachlan and Arnold, 1996).

The World Health Organisation (WHO) defined endocrine disrupting compounds as exogenous chemicals that interfere with the synthesis, secretion, transport and elimination

of naturally produced hormones in the body that control the functioning and development of endocrine system in organisms (WHO, 2013).

According to the WHO (2013) there are close to 800 chemicals suspected of having ED capabilities. Compounds that may have ED effects include both naturally occurring chemicals (e.g. phytoestrogens) and man-made pollutants (Hester and Harrison, 1999). This chapter is not intended to be a comprehensive description of all EDCs, but it identifies and describes major classes of chemicals with known or potential endocrine disrupting properties, with examples from each class (Table 1).

Table 1: Endocrine disrupting compounds can be grouped in multiple ways. In this table known or potential EDCs are grouped into 11 categories with examples of individual EDCs (adapted from WHO, 2013.)

Classification		Examples of EDCs
1.	Persistent and bio-accumulative halogenated chemicals	
1.1	Persistent Organic Pollutants (POPs) as described by the Stockholm Convention	dibenzo- <i>p</i> -dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), perfluorooctane sulfonic acid (PFOS), polybrominated biphenyls (PBBs), chlordane, mirex, toxaphene, dichlorodiphenyltrichloroethane (DDE), dichlorodiphenyltrichloroethane (DDT), lindane, endosulfan and hexabromocyclododecane (HBCD) (classified as a POP by the Stockholm Convention in 2013)
1.2	Other persistent and bio-accumulative chemicals	perfluorinated carboxylic acid (PFCAs) e.g. perfluorooctanoic acid (PFOA), octachlorostyrene, PCB methyl sulfones
2.	Less persistent and less bio-accumulative chemicals	
2.1	Plasticizers and other additives in materials and goods	Phthalate esters, triphenyl phosphate, bis(2-ethylhexyl) adipate, <i>n</i> -butylbenzene, triclocarban, butylated hydroxyanisole

Table 1 continued

	Classification	Examples of EDCs
2.2	Polycyclic aromatic chemicals (PACs)	polycyclic aromatic hydrocarbons(PAHs) such as benzo(<i>a</i>)pyrene, benzo(<i>a</i>)anthracene, pyrene, anthracene
2.3	Halogenated phenolic chemicals (HPCs)	2,4-dichlorophenol, pentachlorophenol, hydroxy-PCBs, hydroxy-polybrominated diphenyl ethers (OH-PBDEs), tetrabromobisphenol A, 2,4,6-tribromophenol, triclosan
2.4	Non-halogenated phenolic chemicals (Non-HPCs)	bisphenol A, bisphenol F, bisphenol S, nonylphenol, octylphenol, resorcinol
3.	Pesticides, pharmaceuticals and personal care product ingredients	
3.1	Current-use pesticides	2,4-dichlorophenoxyacetic acid (2,4-D,) atrazine, carbaryl, malathion, mancozeb, vinclozolin, prochloraz, procymidone, chlorpyrifos, fenitrothion, linuron
3.2	Pharmaceuticals, growth promoters, and personal care product ingredients	diethylstilbestrol (DES), ethinylestradiol, tamoxifen, levonorgestrel, selective serotonin reuptake inhibitors (ssris), flutamide, 4-methylbenzylidene camphor, octyl-methoxycinnamate, parabens, cyclic methyl siloxanes (d4, d5, d6), galaxolide, 3-benzylidene camphor
4.	Other chemicals	
4.1	Metals and organometallic chemicals	arsenic, cadmium, lead, mercury, methylmercury, tributyltin, triphenyltin
4.2	Natural hormones	17 β -estradiol, estrone, testosterone
4.3	Phytoestrogens	isoflavones (e.g. genistein, daidzein), coumestans (e.g. coumestrol), mycotoxins (e.g. zearalenone), prenylflavonoids (e.g. 8-prenylnaringenin)

These chemicals are grouped into eleven broad classes based on their physical-chemical characteristics or origin/application areas (Table 1). The classes include chemicals that are currently produced for commercial purposes, and those that are no longer manufactured or are being phased out of production.

2.2. Health effects of endocrine disrupting compounds

The exposure to EDCs in the environment may come from air, water, food, soil or sediment and depends on the properties and persistence of the EDC. While some are rapidly degraded by ultra violet (UV), bacteria and chemical processes, others resist breakdown and can remain in the environment for long periods (EEA, 2012). Another concern is that many EDCs such as some of the POPs listed in table 1 are also known carcinogens (Collins *et al.*, 1998, Khim *et al.*, 1999,). These compounds can bio-accumulate and magnify in the food chain, and have large impacts on top predator species, including humans. However, because an extensive array of these compounds occur and accumulate simultaneously in biota it is very difficult to say conclusively which effect is due to which particular chemical, or family of chemicals. Indeed, several families of chemicals act synergistically (Solomon and Schetter, 2000).

2.2.1. Effects on wildlife

It is evident that many wildlife species may be exposed to and affected by biologically active concentrations of EDCs. Effects can be observed in a broad number of species representing many major taxa including mammals, birds, reptiles, fish and molluscs (CSTEE, 1999). The following examples of ED effects on wildlife were selected from literature as they might apply to the similar types of wildlife found in the study area.

- EDCs have detrimental impacts on the immunological and reproductive function in birds resulting in population declines (Tyler *et al.*, 1998, CSTEE, 1999).
- Bio-concentration of DDE in birds of prey (Priskorn, 2012) can lead to increased egg-shell thinning.

- EDCs have adversely affected a variety of fish species. In the vicinity of certain sources (e.g. effluents of water-treatment plants) and in the most contaminated areas this exposure is causally linked with effects on reproductive organs, which could have implications for fish populations (Villeneuve *et al.*, 1977),
- Distorted sex-organ development and function in reptiles (Tauber and Hughes, 1950).
- Masculinisation (imposex) in female marine snails by tributyltin (TBT). TBT is used for various industrial purposes such as slime control in paper mills, disinfection of circulating industrial cooling waters, antifouling agents, and the preservation of wood. Due to its widespread use as an antifouling agent in boat paints commonly known as bottom paint, TBT is a common contaminant of marine and freshwater ecosystems exceeding acute and chronic toxicity levels. Bottom paint improves ship performance and durability as it reduces the rate of bio-fouling (Antizar-Ladislao, 2008).

2.2.2. Human health effects

Human health problems caused by disruption of the endocrine system include: cancerous tumours, birth defects, impaired behavioural/mental, immune and thyroid function in developing children, development of osteoporosis, and sexual development problems (Colborn *et al.*, 1993). Examples of the latter include feminising of males or masculine effects on females, reductions in male fertility, abnormalities in male reproductive organs, female reproductive diseases, earlier puberty, decline in the numbers of males born, and precocious puberty.

2.3. Endocrine disruption mediated via cellular receptors

Environmental chemicals/compounds can have effects on the hormonal receptors directly and influence the receptor function, but they can also impact hormone delivering to the receptor (Bergman *et al.*, 2103). Other compounds can interrupt hormone function at different levels of the endocrine system not, even interacting with the hormonal receptor (Hilscherova *et al.*, 2000). In this dissertation the focus of endocrine disrupting effects is

mainly on detecting compounds capable of interfering with the ER as suitable *in vitro* screening techniques exist in the ecotoxicology laboratories at the NWU. As this project formed part of a much larger investigation on human influences in the Phongolo floodplain, this study contributed to the overall project. Others investigated the androgen effects.

Supplementary to detecting ER ligands in the Phongolo aquatic environments, sediments only were screened for ligands binding to the AhR as well. The AhR is capable of being activated by a number of xenobiotics such as PCDD/Fs, dioxin-like PCBs and PAHs of which many have endocrine effects (cf Table 1). Detrimental consequences mediated by AhR when activated by xenobiotics include impairment in development, fertilisation and reproduction, endocrine, nervous and immune systems, as well as cause cancer (Tian *et al.*, 2015). The endocrine effect induced by, for example PCDD/Fs might not be directly affected by the AhR, but by an indirect method where a PCDD/F induces a protein that inhibits oestrogen signalling (Rogers and Denison, 2002).

The general principal for the signalling pathways of both the AhR and the ER are similar but as these receptors are of importance for this study they will be discussed separately.

2.3.1. Oestrogen receptor

The hormone oestrogen plays a central role in the control of sexual behaviour, reproductive functions, growth, differentiation, and function in a broad range of target tissues. Oestrogen regulates and controls the biological physiology via diffusion through the plasma membrane of target cells and by signalling hormone-specific oestrogen receptors (ERs). Most of their hormonal actions are mediated by two specific ER subtypes, namely, the ER α and ER β (Nilsson and Gustafsson, 2002) which are members of a large superfamily of nuclear receptors. Two distinct types of signalling can be mediated when ligands bind to the target receptors. These pathways can be referred to as genomic (nuclear) and non-genomic (extra-nuclear) pathways (Hall *et al.*, 2001). The bio-assay used in this study to detect ER activity uses the genomic pathways and therefore this is explained further.

2.3.1.1. Genomic pathways

Genomic pathways are initiated when an oestradiol binds to the oestrogen receptor (ER) with a high affinity and specificity (cf 1 in Fig. 1). Once bound, the oestradiol/ER complex can exert its effects at both nuclear and cell membranous sites. The ER is equipped with a heat shock protein (hsp 90) complex within the cytoplasm of the cell. Once the oestradiol binds to the ER the pathway of transcriptional control is activated. This action leads to conformational changes causing dissociation of the heat shock proteins (hsp90) from the ER (cf 2 in Fig. 1). Receptor dimerisation and preferential nuclear localisation then follows (MacGregor and Jordan, 1998, Beato, 1989) (cf 3 in Fig. 1). The oestrogen–ER dimer complex binds to specific DNA sequences, the oestrogen response elements (EREs) (cf 4 in Fig. 1). Transcription takes place via two regions, androgen receptor AF1 and AF2 (cf 5 in Fig. 1), which recruit other proteins such as transcriptional co-activators and co-repressors to the transcriptional complex (Beato, 1989; Tsai and O'Malley, 1994; Horwitz *et al.*, 1996) (cf 6 Fig. 1). AF1 activity is regulated by growth factors that act via the mitogen-activated protein kinase (MAPK) pathway (Kato *et al.*, 1995), while the AF2 domain is activated by oestrogen (Kumar *et al.*, 1987).

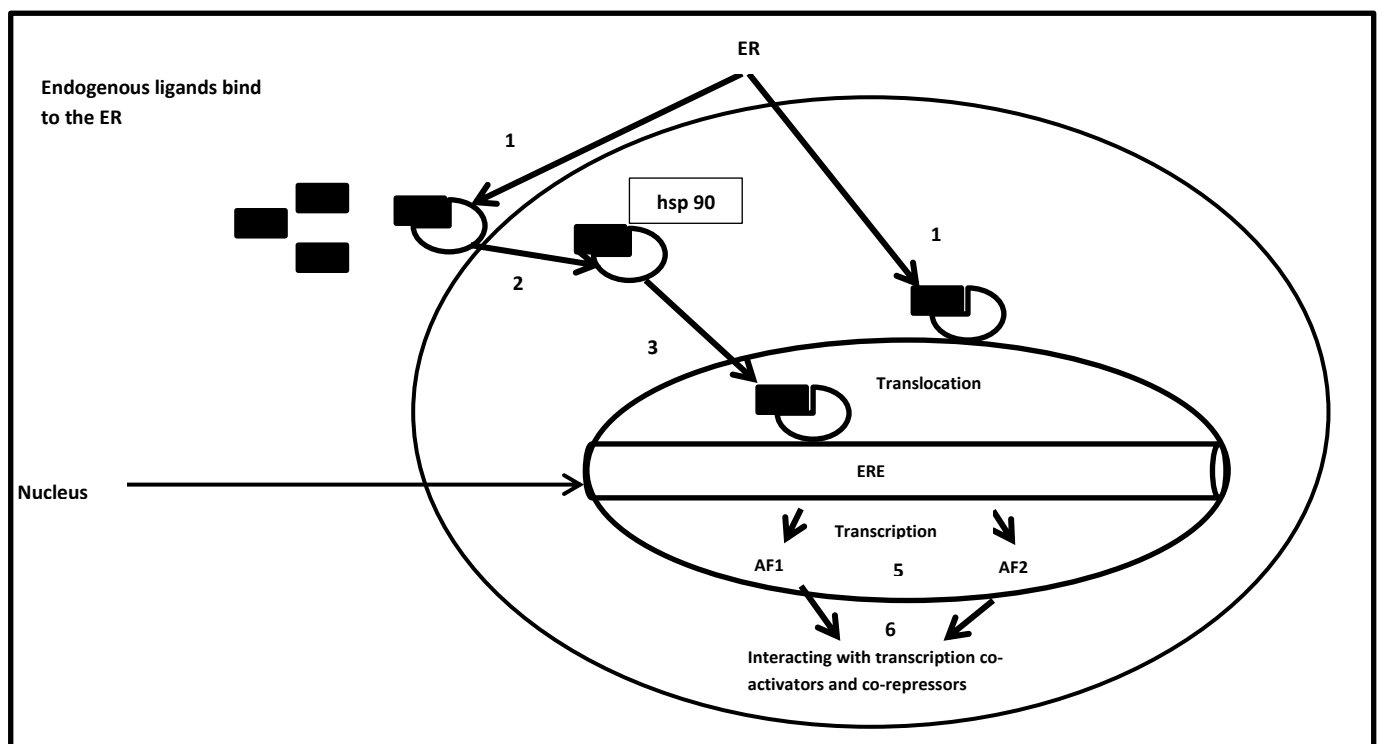


Figure 1 Activation of the oestrogen receptor (ER) and the ER pathway by oestradiol (Huertero *et al.*, 2011) hsp90 = heat shock protein; ERE = oestrogen response elements; MAPK = mitogen-activated protein kinase.

In this study the ability of oestrogen-like and/or oestrogen mimicking compounds to bind to the ER was detected by the reporter gene bio-assay, using MVLN cells. These cells were derived from MCF-7 human breast cancer cells that were genetically engineered. They had been stably transfected with a plasmid containing a firefly luciferase gene downstream of the oestrogen response element (ERE) in front of the tyrosin-kinase-promoter. Exposure to oestradiol or oestrogen-like and/or oestrogen mimicking compounds capable of binding to the ER leads to induction of the luciferase gene. Luciferin is added to the cells, and when in the presence of luciferase, a catalytic oxidation occurs, light is produced (Whyte *et al.*, 2004). When the substrate, luciferin, together with adenosine triphosphate and oxygen react with luciferase and magnesium ions they form oxyluciferase, inorganic phosphorus (PPi), adenosine monophosphate (AMP), CO₂ and light (Alam and Cook, 1990). The volume of light produced is directly proportional to the amount of ER ligands present within the sample extract to which the cells were exposed (Hilscherova *et al.*, 2000).

The response elicited by a sample extract is reported in relation to the response caused by a known positive control and expressed as relevant potency (REP). In this way it is possible to semi-quantify the effect these pollutant mixtures might have on biota (Behnisch *et al.*, 2001).

2.3.1.2. Oestrogen ligands

2.3.1.2.1. Natural oestrogen ligands.

Endogenous ligands that bind to the ER have aromatic properties such as oestrone and 17 β -oestradiol. Oestrogen ligands are produced through enzymatic aromatisation of androgens. These actions occur mostly in the ovaries and extra gonadal tissues such as breast, bone, vasculature, and brain (Simpson and Davis, 2001). In men the production of ER-ligands are less important as oestrogens occur at relatively low levels in their blood. However, there are ERs present in many tissues and cell types of the male physiology, such as in the prostate, bladder, brain, heart and lymphatic tissues (Prins *et al.*, 2001). The presence of these receptors in male vertebrates are functional and can be activated leading to strong biological effects.

The ligand binding pocket in the ER can be rather large thus accommodating non-aromatic structures as well. A total of 60 non-aromatic steroids have the ability to bind to the ER. A natural occurring ligand that is ubiquitous and best studied is 17 β -oestradiol. This compound is a steroid hormone, also the primary female sex hormone and has a high affinity for the ER. Oestradiol is essential for the development and maintenance of female reproductive tissues, however, they are present in male tissues at lower concentrations as previously mentioned (Heldring *et al.*, 2007). These ligands are primarily produced in the follicles of the ovaries, but also in other endocrine (i.e., hormone-producing) and non-endocrine tissues (e.g., including fat, liver, adrenal, breast, and neural tissues).

2.3.1.2.2. Man-made oestrogen ligands

A major mechanism of endocrine disruption is binding of an exogenous chemical to the ER. It has been demonstrated that a number of the environmental contaminants are able to function in a manner similar to oestradiol (Colborn *et al.*, 1993, Daston *et al.*, 1997). According to Blair *et al.*, (2000), more than 188 structural diverse chemicals and chemical groups have ER-binding affinities. They include bisphenol A (Perez *et al.*, 1998), alkylphenols (Soto *et al.*, 1991, White *et al.*, 1994), PCBs (Bergeron *et al.*, 1994), pharmaceuticals and industrial wastes to name but a few (cf chapter 2.3).

Natural compounds with these capabilities include phytoestrogens which are plant-derived oestrogens and myco-estrogens originating from fungi. These natural occurring exogenous ligands can also be called dietary oestrogens.

2.3.2. Aryl hydrocarbon receptor (AhR) and signalling pathways

The AhR is a ligand-activated transcription factor which belongs to the basic helix-loop-helix Per-Arnt-Sim superfamily of transcription factors and is best known to respond to environmental stressors (Tian *et al.*, 2015). The AhR plays key roles in gene expression, cell differentiation, host defence as many studies has shown that the AhR is crucial for

controlling bacterial infections (Tian *et al.*, 2015) and many physiological and developmental processes (Han, 2004).

The AhR regulates gene expression in a wide range of species and tissues (Hankinson, 1995). It has a multi-protein complex with a chaperone 90-kDa heat shock protein (hsp90) (cf Fig. 2), an aryl hydrocarbon receptor-interacting protein (AIP) and a co-chaperone protein (p23) within the cytoplasm of animal cells (Perdew, 1988).

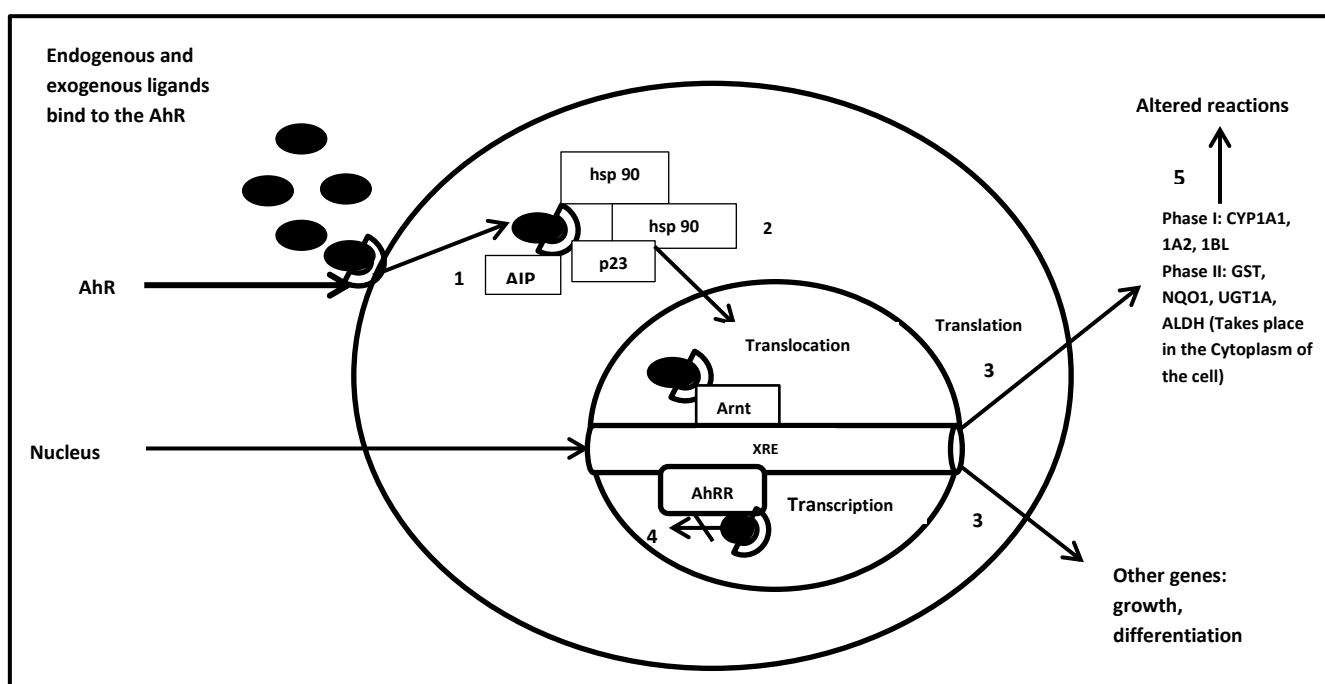


Figure 2: Activation of the AhR and the AhR pathway (Huertero *et al.*, 2011) hsp90 = heat shock protein; AIP = aryl hydrocarbon receptor-interacting protein; p23 = co-chaperone protein, Arnt = arylhydrocarbon receptor nuclear translocator, XRE = xenobiotic responsive element, AhRR = aryl hydrocarbon receptor repressor.

When AhR ligands enter the cytoplasm of a cell they bind to the AhR and cause activation of the signalling pathway (cf 1 in Fig. 2). The AhR is already bound to two hsp90 as well as proteins p23 and AIP (Fig. 2). Upon binding with the ligand the ligand-AhR complex undergoes a conformation change exposing its nuclear localization sequence. The ligand-bound AhR is translocated (cf 2 in Fig. 2) to the nucleus, where the proteins dissociate and where it forms a heterodimer with the AhR-nuclear-translocator (Arnt) resulting in AhR:Arnt complex. This complex interacts with the xenobiotic responsive element (XRE) on the deoxyribonucleic acid (DNA) strand (Denison *et al.*, 2002). The interaction stimulates transcription of several genes as Phase I, the expression of cytochromes P450 (*CYP1A1*,

CYP1B1 and *CYP1A2*) and Phase II (*GST*, *NQO1*, *UGT1A* and *ALDH*) of the body's detoxification processes. Other genes may also participate in different cellular responses (cf 3 in Fig. 2). A mechanism of negative feedback regulation of the AhR function is performed by the aryl hydrocarbon receptor repressor (AhRR): a ligand-activated AhR/Arnt heterodimer trans-activates the expression of target genes including the *AHRR* gene. AhRR suppresses AhR transcriptional activity by competing with AhR for dimerising with Arnt and binding to the XRE sequence of target genes (Pavek, 2008) (cf 4 in Fig. 2).

The ability of a compound to bind to the AhR and eliciting a response is the basis of the reporter gene bio-assay, using H4IIE-*luc* rat hepatoma cells, which had been stably transfected with a luciferase reporter gene under control of XRE (Houtman *et al.*, 2004, Whyte *et al.*, 2004). When the AhR is ligand bound, the cytochrome P450 response is induced, and because the luciferase gene has been inserted downstream of the P450 gene, luciferase is also produced together with the already mentioned CYP enzymes (Hilscherova *et al.*, 2000, Allan *et al.*, 2006). Luciferase production can then be measured by adding the luciferin (cf chapter 2.3.1.1) substrate to the cells and the volume of light emitted is directly proportional to the amount of ER ligands present within the sample to which the cells were exposed (Demirpence *et al.*, 1993).

2.3.2.1. AhR ligands

Although most of the action of the AhR had been discovered because of its role in detoxifying xenobiotics, it has been found that it does have endogenous functions and natural occurring ligands (Tian *et al.*, 2015).

2.3.2.1.1. Natural AhR ligands.

During the last four decades most of the experimental focus was invested into the understanding of the AhR and the activation of the response induced by a wide variety of exogenous compounds. However, the most pervasive and best studied natural AhR ligands are the endogenous tryptophan and its metabolites. These metabolites are primarily known for inhibition of cancer cell proliferation and mediate inflammatory responses (Öberg *et al.*,

2005). Other natural AhR ligands include indoles (produced by a variety of bacteria), the plant polyphenols flavonoids (e.g. genistein, Table 1), tetrapyrroles (such as bilirubin and biliverdin), arachidonic acid metabolites (e.g. prostaglandin G) and indigoids (colourings from plants) (Nguyen and Bradfield, 2008, Tian *et al.*, 2015).

2.3.2.1.2. Man-made AhR ligands

Exogenous AhRs are hydrophobic molecules that commonly fall into one of two structural classes, halogenated aromatic hydrocarbons (HAHs) or PAHs. These chemicals enter the environment through contamination of commercial products, industrial accidents or as products of waste incineration (Bjeldanes *et al.*, 1991).

2.3.2.1.2.1. Halogenated aromatic hydrocarbons

HAHs include a wide range of chemicals and a discussion and identification of each HAH is not the purpose of this study. Only a few compounds; polychlorinated dibenzo-*p*-dioxins (PCDDs),-dibenzofurans (PCDFs),-biphenyls (PCBs) are described, as these toxic industrial compounds or by-products are found in almost every matrix of the environment, including tissues of wild mammals, fish, and birds (Kimbrough and Jensen, 1989). The dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), is considered one of the most potent AhR agonists and in this study 2,3,7,8-TCDD was used as a positive control in the H4IIE-*luc* bioassay (Poland and Knutson 1982).

Many HAHs can also be classified as persistent organic pollutants due to their long half-life, and are resistant to biological and chemical degradation (Jaikanlaya *et al.*, 2009). Some of these persistent organic pollutants as mentioned previously are endocrine disruptive and their presence in the environment have led to establishment of the Stockholm Convention (SC) on Persistent Organic Pollutants in May 1995 by the United Nations Environmental Program (UNEP). The aim of the SC is to minimise and ultimately ban the use of the chemicals on the list. Initially the list contained twelve pollutants and pollutant classes: aldrin, chlordane, DDT, dieldrin, chlorinated dioxins, endrin, chlorinated furans, hexachlorobenzene (HCB), heptachlor, mirex, PCBs and toxaphene, (UNEP, 2005).

According to the UNEP, Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment, bio-accumulate through the food web, and pose a risk of causing adverse effects to human health and the environment.

2.3.2.1.2.2. Polycyclic aromatic hydrocarbons (PAH)

Another group of AhR ligands that is less persistent than the HAHs are PAHs. They degrade slowly under natural conditions. These compounds are hydrocarbons containing only carbon and hydrogen that consists of multiple aromatic or benzene rings. Polycyclic aromatic hydrocarbons are created from the incomplete combustion of products like coal, oil, gas, and garbage (Choi *et al.*, 2010). These compounds have low water solubility, are semi-volatile and lipophilic (Ahrens and Depree, 2010). These compounds are pervasive environmental toxins, primarily found in freshwater and marine environments (Neff *et al.*, 2005) and are major contributors to detrimental effects on aquatic life through exposure to contaminated sources. The United States Environmental Protection Agency (USEPA) has identified 16 of the PAHs to be monitored because of their harmful effects. The USEPA priority PAHs are naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene (Achten and Hofmann, 2009). These priority PAHs are known to be carcinogenic, mutagenic, and teratogenic (Achten and Hofmann, 2009).

2.3.2.1.3. Naturally occurring dietary AhR-ligands

Some studies have documented that a variety of naturally occurring dietary chemicals can act as AhR ligands. Examples include: extracts of vegetables and flavonoids. The latter are the largest group of naturally occurring dietary AhR-ligands, and include: flavones, flavanols, flavanones, and isoflavones (Wattenberg and Loub, 1978).

2.4. Methods of detection

2.4.1. Instrumental analysis vs. biological analysis

Instruments such as high-resolution gas chromatography and mass spectrometry (HRGC/MS) are used for the detection and quantification of compounds. Instrumental assessments are usually conducted to compare toxicant levels to published guidelines. These techniques are accurate and reliable in identifying the specific chemicals present in a heterogeneous sample (Song, *et al.*, 2007). However, the limitation of this method is that environmental samples are complex mixtures and the biological impacts of these mixtures cannot be determined by measuring the concentrations of the respective constituents. Also instrumental analyses are considered costly and time consuming. Generally, determination of the levels targeted chemicals by instrumental analysis can only be performed for those chemicals for which analytical techniques and standards are available (Hilscherova *et al.*, 2000).

In vitro cell bio-assays offer a rapid, sensitive and somewhat inexpensive solution to the limitations of the instrumental analysis. Bio-assays enable an estimation of total biological activity of targeted ligands (Khim *et al.*, 1999). They also integrate possible interactions among chemicals (Hilscherova *et al.*, 2000). However, unlike instrumental analytical methods, bio-assays generally cannot identify the chemical(s) causing the response, nor can they precisely quantify the concentrations of chemical(s) present.

In this study, bio-assays were used as screening tools, to estimate the presence and potential effects of complex mixtures in an aquatic environment.

3. Materials and methods

This study forms a part of a larger project funded by the Water Research Commission (WRC) that took place in the northern Kwa-Zulu Natal area, on the Phongolo floodplain. The overarching aim of the project was to determine the ecological health of the Phongolo floodplain, the impact of major stressors on the Lower Phongolo River and lastly to provide all necessary helpful information to local conservation authorities for conservation efforts. One of these aspects was also to determine how human impact possibly pollutes the floodplain. The experimental section conducted for this dissertation fits into the toxicological division of the overarching study.

3.1. Site selection

The Phongolo River in South Africa is situated in the northern KwaZulu-Natal and is a tributary of the Maputo River, and is dammed at Jozini. The Phongolo floodplain is classified as the largest subsistence fishery at a natural inland wetland in South Africa. This area also has very extensive grazing lands; both have been reduced due to the effects of extensive cultivation (Provincial Planning Commission, 2011). The Phongolo floodplain is known to be affected by various sources of pollution. One such a source is the agricultural activities that include sugar cane and tomato cultivation. The herbicides used on sugar cane include ametryn, atrazine, hexazinone, paraquat and glyphosate, 2-methyl-4-chlorophenoxyacetic acid (MPCA) and acetochlor. These are only a few of the most commonly used herbicides in this specific region (LeBaron *et al.*, 2008). Sugar cane cultivation in South Africa requires the annual burning of the excess bio-mass resulting in a large amount of emissions released into the air. Tomato crops enquires fertilisation as well as controlled weed and pest removal by using various herbicides and pesticides (Trench *et al.*, 1992) such as metribuzin, rimsulfuron, sethoxydim, trifluralin and flurochloridane (Van Zyl, 2012).

The spraying of DDT can also contribute as a source of pollution in this area. DDT is a known POP and ED which is used in the Phongolo floodplain for malaria vector control (Coetzee *et al.*, 2013). During the early 2000s, South Africa reintroduced DDT to control malaria in the

KwaZulu Natal Province, as this province was the worst affected by a malaria epidemic that struck SA in the late 1990s (SA Dept of Health, 2003).

eDumbe Coal, a coal mine in the uPhongolo district is another possible source of pollution. These activities can cause atmospheric pollution. In addition coal mining and the burning procedures deposits a large amount of waste into the air and the surroundings such as mercury, uranium, thorium, arsenic, and other heavy metals (WWF-SA, 2011). Other pollution sources can include transport emissions and rural runoff into the natural water sources.

Due to lack of piped water the rural communities use the pans and river streams as direct source of water. Most of the sites reported in this study were selected in the Ndumo game reserve because it would be an indication as to how expected pollution would influence the wildlife in the protected area; an area where there is no industrial and little human activities. Any pollution detected would have to come from outside the game reserve. The Ndumo game reserve (NGR) was initially established in 1924 as part of a hippo protection plan. This reserve is regarded as one of the unspoilt and most biodiverse reserves in South Africa and is located in the far northeast district of KwaZulu-Natal, situated on the border with Mozambique (Footprint Travel Guide, 2015). The Phongolo River connects here with the Ushutu River in the NGR, located alongside the Tembe Elephant Park (Wesołowska and Haddad 2009). The closest urban settlement to NGR is Mkuze 110 km away. NGR is mostly surrounded by rural communities.

NGR hosts a diverse range of environmental habitats from sand forests to floodplains and grassland. Because of its diverse landscape Ndumo is popular for its birdlife and has recorded in excess of 430 bird species including residents and seasonal migrants. Despite being only 102 km² the park has an abundance of floodplains, rivers and pans that could provide the perfect habitat for fresh water aquatic species. This area is generally rich in wildlife, crocodiles and hippos (Wesołowska and Haddad, 2009).

The NGR receives a high annual rainfall of 638 mm, and is seen as a pristine environment (Wesołowska and Haddad, 2009). Any toxicological effects found in this area would indicate the long range transport of persistent pollutants via the water and/or the air.

Table 2 indicates the six sites where sampling occurred, one of which were sampled outside the NGR and their positions are plotted on the map in Figure 3. The site codes have their origin from the overarching project where there were many more sites. These were the only ones selected for screening for endocrine disruption.

Table 2: A list of 6 aquatic sites in the Pongola floodplain, South Africa and their coordinates

Site name	Site code	Coordinates	Date of collection
Phongolo River pumping station	8	26°54'08.0"S 32°19'34.0"E	1. September 2012 2. April 2013 3. September 2013
Pan west of the river pumping station	8.1	26°53'57.4"S 32°19'31.5"E	1. April 2013
Pan east of the river pumping station	8.2	26°54'25.6"S 32°19'38.5"E	1. April 2013
Nyamiti Pan	9	26°53'12.2"S 32°17'54.0"E	1. September 2012 2. April 2013 3. September 2013
Nyamiti outflow	90	26°88'47.0"S, 32°31'22.9"E	1. September 2013
Ushutu River	10	26°50'21.9"S 32°12'31.7"E	1. April 2013
Nomaneni Pan	Ref site	26°59'12.4"S 32°16'27.4"E	1. September 2013

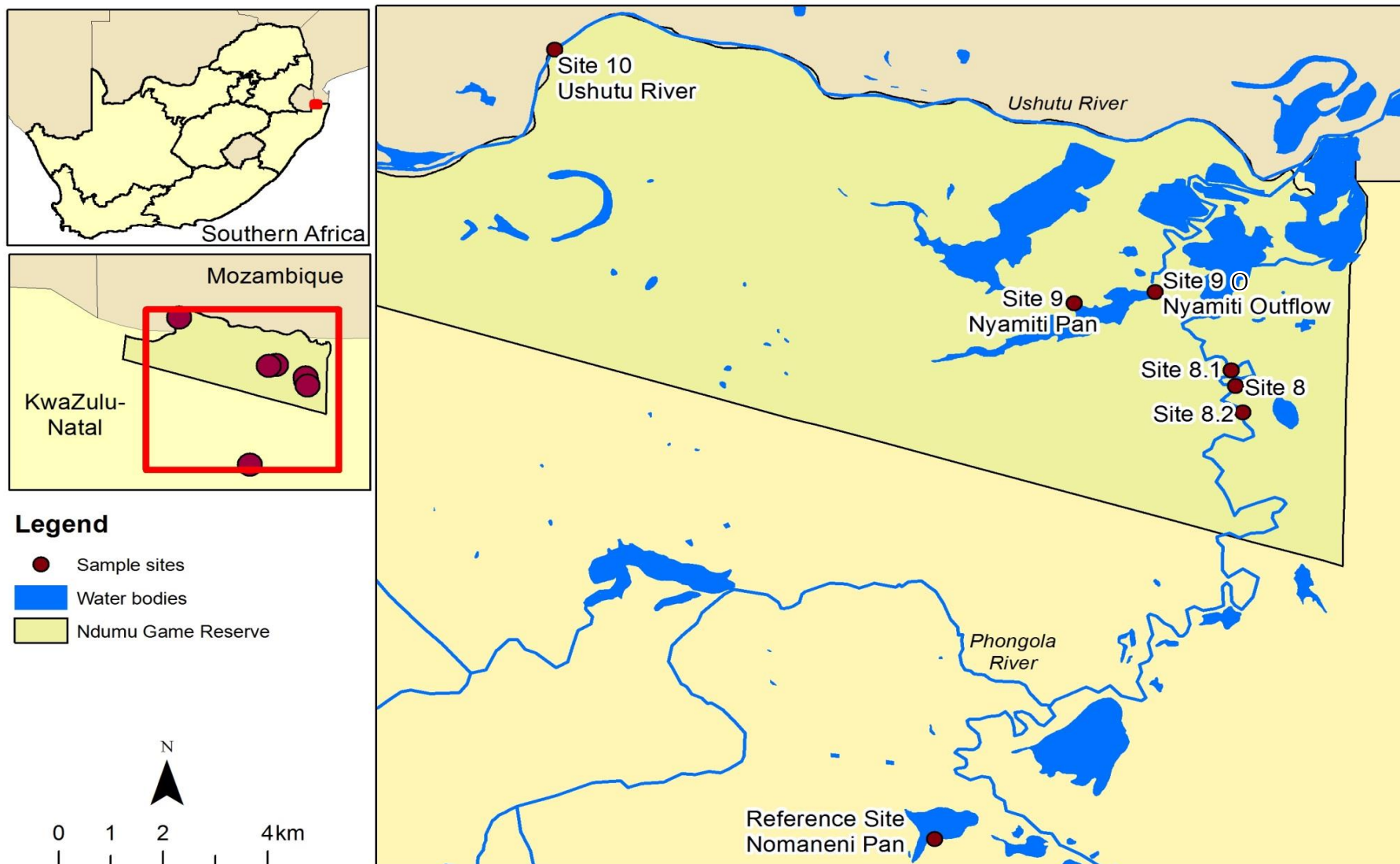


Figure 3 Locations where samples were taken for this study. The coordinates are listed in Table 2.

Factors that influenced site selection were the availability of water at the time of sampling during wet and dry seasons and safety consideration due to wildlife dangers, such as crocodiles and hippos and physical access to the sites.

Results from the samples collected in the river compared to those collected from the pans would indicate whether the observed pollution was carried into the game reserve via the water or whether it was transported to the pans via air pollution.

Most of these pans were dry during the low flow seasons and no water was collected, only sediments. The Nomaneni Pan (Table 2) (Fig. 5) was selected as it is central to a large number of rural communities using the pan for food, water, and hygiene. The pan is the only site outside of the NGR. It was expected that this pan would have a different pollutant pattern than those inside the game reserve.

3.2. Sample matrices

Both biotic and abiotic matrices were sampled: water, sediment and fish bile. Water was collected to determine the levels of these persistent pollutants in flowing and stationary water at that specific point in time. Sediment samples were collected mainly because sediment is considered an environmental sink (Jarvie *et al.*, 2005) for many contaminants, since many pollutants originally introduced into the water column have affinities for sediment particles. Sediments are also an important biological habitat, uptake of toxicants into the food web are influenced by the toxicant concentrations in sediment. Sediment usually provides an indication of the pollutants to which the river was exposed to over time, while the pollutants found in water samples is an indication of compounds flowing in the water at the time of collection. Fish bile was collected to determine the environmental effects on the fish and if the pollutants can bio-accumulate. Mixtures and compounds, natural and exogenous, are often conjugated by a series of enzymes in the liver of which its metabolites are excreted in the bile as inactive conjugates (Truman, 2006). Two fish species were sampled: *Oreochromis mossambicus* and *Clarias gariepinus*.

Both these fish species are used by the communities as food source.

3.2.1. *Oreochromis mossambicus*

Oreochromis mossambicus commonly known as the Mozambique tilapia or Bloukurper (Skelton 1993), belongs to the order Perciformes and the Cichlidae family. This omnivore thrives in stationary water and is tolerant to a wide variety of pH, salinity and temperature ranges (Skelton, 1993). They primarily feed on plant materials such as algae, diatoms, rich organic matter, as well as smaller fish (Kotze *et al.*, 1999). The bile of only male individuals was sampled to limit the effect the endogenous oestradiol in the females would have on the (anti)oestrogenic assay. Although male fish are known to have oestrogen, their basal concentrations are much lower than those in females (Houtman *et al.*, 2004). Positive results on the male bile would indicate that the fish were exposed to environmental pollutants mimicking oestradiol.

3.2.2. *Clarias gariepinus*

Clarias gariepinus is a species of air breathing catfish of the family Clariidae, with a scaleless, bony, elongated body with long dorsal and anal fins, and a helmet like head (Skelton, 2001). This fish species can tolerate turbid waters low in dissolved oxygen, and is often the last or only fish species found in remnant pools of drying rivers. They primarily consume fruits and seeds, all types of aquatic invertebrates and small vertebrates, small mammals and even plankton (Skelton, 2001). *Clarias gariepinus* was used as they are benthic invertebrates (bottom dwellers) that come in constant contact with sediment and are omnivores. They are higher on the food web than *O. mossambicus* and if bio-accumulation of the pollutants took place, they would probably have higher assay responses than the *O. mossambicus*.

3.3. Sampling of water, sediment and fish bile.

All equipment that were used for the sampling and extraction purposes were made of glass, stainless steel or high density polyethylene (HDPE) and were pre-cleaned: washed with warm water and phosphate-free soap (Liquinox® critical cleaning liquid detergent), rinsed with tap water, followed with de-ionised water (18 MΩ), acetone and hexane (both analytical grade; Burdick and Jackson™). The acetone removed all polar contaminants and the hexane all non-polar contaminants (US EPA, 2004b).

Water samples were collected in 1 l (Schott®) laboratory glass bottles that was pre-cleaned as mentioned above and rinsed with methanol (MeOH) (analytical grade; Burdick and Jackson™). Samples were immediately acidified to a pH of 2–3 using hydrogen chloride (HCl) (Arizia *et al.*, 2000). The acidification is to prevent microbial degradation. The water was stored at 4°C to prevent the breakdown of target compounds until transported to the laboratory where these samples were immediately extracted.

Sediment samples were collected from the upper 0 to 5 cm sediment layer in equal volumes from five different locations at a particular site to obtain a composite sample, by means of a brass grab sampler or a metal spade. The composite sample was vigorously stirred before it was deposited in pre-cleaned HDPE bottles. The samples were covered to protect them from UV breakdown and transported at -20°C to prevent microbial degradation. Sampling equipment was cleaned between consecutive sampling sites to prevent cross-contamination.

Fish sampling were done at the specific sampling sites using fyke nets, seinnets, electro fishing and rods. In the Nyamiti and Nomaneni Pans, seinnets were dragged over bays of interest (stretches where fish would be expected). Fish sampled were placed in aerated tanks to be transported to the field laboratory where selected species were dissected. In the river stretches and deep waters fyke nets were used and left for overnight to ensure capture. Twenty species of fish per site were the minimum requirement for sampling to be able to get statistically valid data. Other fish species that were caught during sampling were tagged, documented and released as they formed part of the larger study.

At the field laboratory, all tools used for sampling were pre-cleaned as mentioned in section 3.3 before the fish was processed. Almost all tissue was harvested for the various projects, but the bile was dissected out and collected in 4 mL glass amber vials for this study. The bile was kept frozen until extraction could commence.

All three matrices, water, sediment and fish bile was used in the oestrogenic mediated bio-assays (MVLN bio-assay), as the fundamental aim was to determine the presence of EDCs in the various matrices of the study area. Only sediment samples were given to the AhR-mediated bio-assay (H4IIE-*luc* bio-assay) as it is a supplementary investigation to detect other pollutants with ED effects, not detected by the MVLN assay (cf. section 2)

3.4. Extractions

The extraction methods used differed slightly from each other to accommodate for the differences between the matrices (water vs sediment vs bile) as well as the physico-chemical nature of the target compounds expected to elicit a response via the two receptors. Figure 4 summarises which matrices were sampled for the different assays.

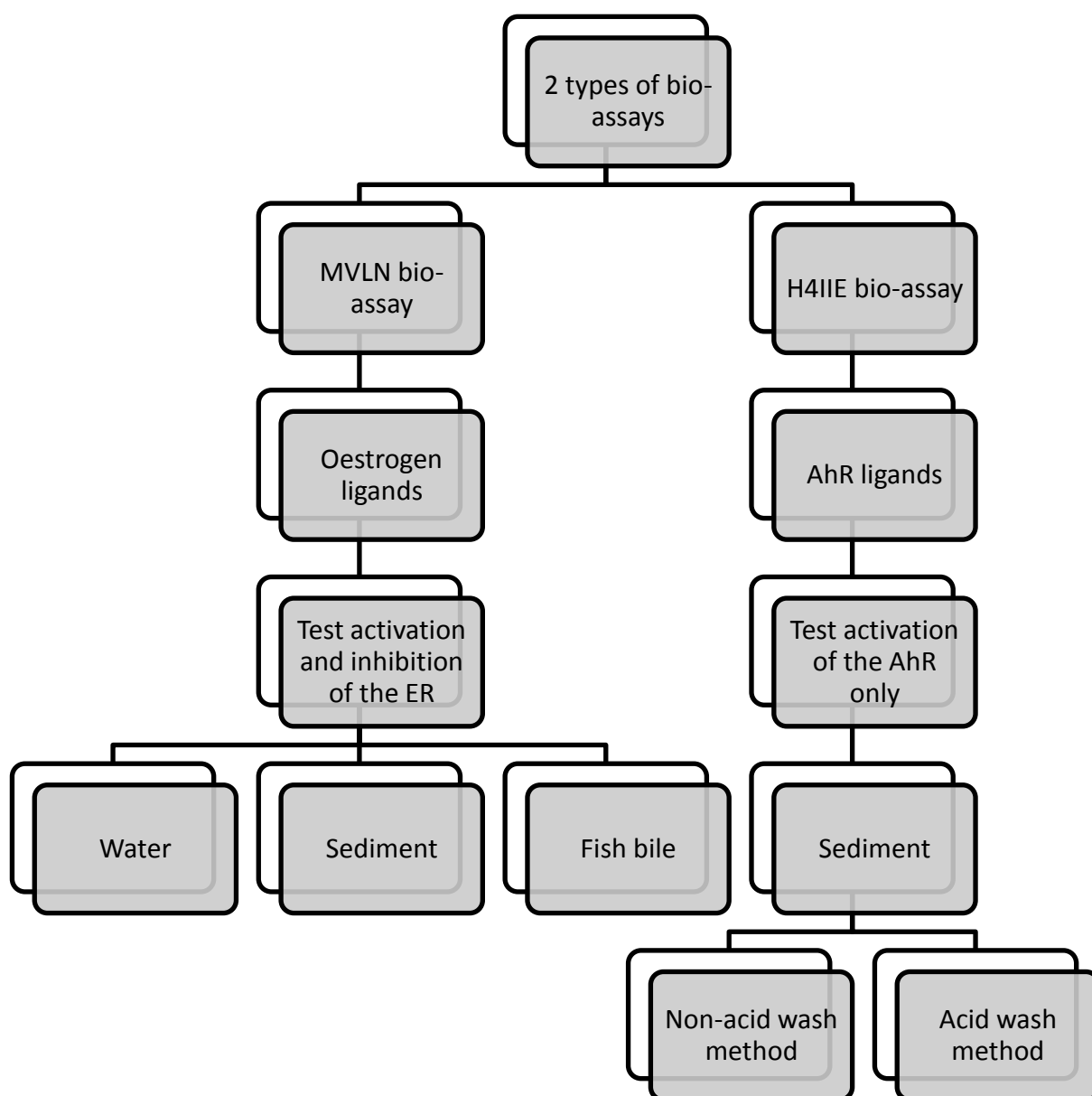


Figure 4: A flow diagram to indicate which matrices were screened by which extraction method in order to identify target compounds.

3.4.1 Water extraction

Water samples were pre-filtered through MN-GF paper filters under gentle vacuum before commencing with solid phase extraction (SPE) through Oasis hydrophilic-lipophilic-balanced (HLB) reverse phase cartridges on a SPE vacuum manifold (Winterberg, *et al.*, 2010). These cartridges were primed and conditioned with 10% methanol (MeOH) in methyl tert-butyl

ether (MtBe), followed by 100% MeOH, equilibrated with 100% water and 5% MeOH in water (H₂O). After conditioning the entire sample was allowed to pass through cartridges, washed with 5% MeOH in H₂O and allowed to dry completely. It is important to allow the cartridge to dry completely for effective elution therefore we left it overnight (Winterberg, *et al.*, 2010).

The target compounds were eluted from the cartridge with 10% MeOH in MtBe, under gravitation only. The solvent was evaporated to dryness under gentle stream of nitrogen gas (N₂), and the extract reconstituted to 1 mL MeOH. The extracts were stored at -80°C until the assays were conducted.

All solvents used were of analytical grade.

3.4.2 Sediment extraction and clean-up

For all extraction purposes the samples were thawed, and air dried at room temperature while protected from UV degradation. The dried sediment was ground and sieved through a copper sieve with mesh of 0.5 mm to obtain a homogenous sample (Mai *et al.*, 2007). Forty grams (40 g) of sediment was mixed with an equal volume of anhydrous sodium sulphate (Merck, univAR) to remove any possible traces of moisture (Hilscherova *et al.*, 2003).

3.4.2.1. Sediment extraction and clean-up

Sediment was extracted using high pressure liquid extraction in an accelerated solvent extractor (ASE) apparatus (Dionex ASE 100). This instrument operates on the principle of pressurised fluid extraction by using elevated temperatures and pressure to achieve rapid, effective extraction (Bernsmann and First, 2004). A known mass of sediment was placed into a 60 mL stainless steel extraction cylinder, between two 30 mm cellulose filters. The ASE parameters were: 10 342 kPa, 100°C, 5 min static and heat time, 60% flush volume and 100 s nitrogen purge (McCant *et al.*, 1999). The extract was evaporated to dryness under a gentle stream of N₂-gas at 40°C inside a TurboVap® II (Caliper). The target compounds were resuspended in 2 mL DCM for gel permeation chromatography (GPC).

A GPC system equipped with a Waters 2487 dual λ absorbance detector, Waters 717plus autosampler, Waters 1515 isocratic high pressure liquid chromatography (HPLC) pump and a Waters fraction collector III, running on Breeze™ software were used. Two Envirogel columns (19 x 300 mm and 19 x 150 mm) in series were used to separate the molecules based on the size or hydrodynamic volume of the compounds of interest. A reference mixture containing corn oil (Sigma-Aldrich), bis(2-ethylhexyl)phthalate, methoxychlor, elemental sulphur (PESTANAL®, Riedel-de-Haën) and perylene (OEKANAL®, Riedel-de-Haën) was prepared and used as calibration mixture to establish the retention time for the various sized molecules. The reconstituted sample was passed through a 1 μ m glass fibre filter (Sigma-Aldrich) into a recovery vial before injection into the GPC. The recovery vials were weighed before and after filling with the sample as well as after injection, in order to determine the mass fraction lost to the GPC process. The system was set to a flow rate of 5 mL per minute for 30 minutes, with DCM as the mobile phase.

The fraction of the extract containing all the molecules bigger than sulphur was collected. Sulphur was specifically removed as it is cytotoxic (Schipper *et al.*, 2013). The solvent was evaporated from the extract and resuspended to 1 mL MeOH to be used in the MVLN bio-assay and hexane for the H4IIE-*luc* bio-assay.

Duplicate extractions were performed to be able to distinguish between persistent AhR-ligands and an extract containing most AhR-ligands. The description above would have collected all AhR ligands. To target the persistent AhR ligands an acid wash step was introduced. The acid destroys the less persistent compounds.

To prepare the persistent AhR-ligand extract, the extract that was created by the ASE was evaporated as well, as previously described, but then resuspended in 10 mL hexane and transferred to pre-cleaned separation funnels. A 98% sulfuric acid (H₂SO₄, Merck) was added and shaken gently, venting often to prevent gas build-up. The phases were allowed to separate ($\pm$ 1 h) before tapping off the acid phase (Khim *et al.*, 1999a). This step was repeated until the acid phase was clear and as transparent as the organic phase (the acid phase originally appears black but becomes clearer with consecutive washes). However, this step was not repeated more than 6 times. The extracts was washed with 5% sodium

chloride (NaCl) in water once and allowed separation for 1 h before tapping off the bottom layer, followed by 10% potassium hydroxide (KOH) in water and allow separation for 15 min only, because KOH may disintegrate target compounds. An additional sodium chloride wash step was included to remove traces of KOH. The samples were evaporated to dryness and resuspended into 2 mL DCM for gel permeation chromatography (Khim *et al.*, 1999).

3.4.3. Bile extraction

Fish bile was extracted to determine (anti)oestrogenic activity. Bile extractions took place in three steps: enzymatic hydrolysis of 100 µL bile mixed with 700 µL sodium acetate buffer (0.1 M, pH = 5) and 600 µL β-glucuronidase (40U) and sulfatase to return the metabolised target compounds into their native equivalent. The substrate was incubated at 37°C for 3 hours. The mixture was extracted by liquid-liquid extraction with DCM as follows: 100 µL HCl (1 N), 500 µL isopropanol and 2 mL ethyl acetate were added to the bile mixture. The mixture was vortexed for 1 min. and centrifuged at 1 356 g for 5 min. The top layer consisting of ethyl acetate was removed and this entire step was repeated twice. Solid phase extraction followed as clean up step using 6 mL Strata-X Phenomenex cartridge with 200 mg polymeric reverse phase sorbent. The solvent was evaporated and the compounds of interest were reconstituted to 2 mL DCM and size exclusion clean-up followed on the GPC (Houtman *et al.*, 2004). Lastly the extracts were given to the MVLN cells for biological analysis.

3.5. Cell maintenance

MVLN breast cancer cells and the H4IIE-*luc* rat hepatoma cells were received as a gift from Prof John Giesy in 2003, then based at the Michigan State University in the United States of America.

MVLN cells were maintained at 37°C in humidified 5% CO₂/95% air (Demirpence *et al.*, 1993). Its culture media was F-12Ham Medium (Sigma-Aldrich) (N6013) supplemented with 10% foetal bovine serum (FBS) (Bonefeld-Jorgensen, 2005).

The H4IIE-*luc* cells were maintained under the same conditions as the MVLN cell line, however their culture media was Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) without phenol red and sodium bicarbonate, supplemented with 10% foetal bovine serum (FBS, Sigma-Aldrich) and sodium bicarbonate.

3.6. Bio-assay, inhibition and activation

Activation of the receptor was tested for both the H4IIE-*luc* cell line and the MVLN cell line. The bio-assay for both cell lines was performed over a period of five days, during which the cells were grown individually on their supplemented media. On the first day, 96-micro well plates (400 μl , Nunc) were seeded with a 0.25 mL cell suspension (H4IIE-*luc* 50 000 cells/mL and MVLN 25 000 cells/mL) in hormone stripped media to prevent false positives due to hormones in the FBS. The micro well plates are opaque white, had optical clear bottoms and lids, and are tissue culture treated. The outer wells of each plate were not seeded, but contained 0.25 mL phosphate buffered saline (PBS) acting as a buffer area to prevent a hydrostatic pressure from having an effect on cells response.

The method of the bio-assay for both the MVLN cell line and the H4IIE-*luc* cell line are similar and the methodology will be discussed in detail below. Cells were seeded into the 96-well plates and incubated for 24 hours. On day two, the cells were dosed in triplicate with 2.5 μl of the reference compounds 17 β -oestradiol (E_2) for ER-mediated activity and, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for AhR-mediated activity at six different concentrations. The E_2 concentrations were: 0.33 $\mu\text{g/mL}$, 0.033 $\mu\text{g/mL}$, 0.3×10^{-3} $\mu\text{g/mL}$, 3.3×10^{-4} $\mu\text{g/mL}$, 3.3×10^{-5} $\mu\text{g/mL}$ and 3.3×10^{-6} $\mu\text{g/mL}$ and the TCDD concentrations 120 pg/mL, 30 pg/mL, 7.5 pg/mL, 1.9 pg/mL, 0.5 pg/mL, and 0.1 pg/mL respectively. A solvent control (SC) row (containing methanol-MVLN/hexane-H4IIE) and blank row (containing cells only) separated the wells dosed with the reference compound from the wells dosed with extracts, to prevent cross communication of cells (Giesy *et al.*, 1997, Hilscherova *et al.*, 2001). The plates were incubated for 72 hours.

TCDD/E₂ per well were used as a reference compound to convert the data into relevant potency (REP) values. Sample extracts were serially diluted to create 5 more dilutions apart from the original extract and dosed in triplicate.

Figure 5 indicates a schematic plate layout that was used in both bio-assays. The cells were seeded into the inner wells as previously explained and each plate contained a solvent control (SC) specific for the assay: (hexane for the H4IIE-*luc* assay and methanol for the MVLN assay) and blank controls (BC) (Fig. 6). The outer wells contain PBS.

	1	2	3	4	5	6	7	8	9	10	11	12					
A																	
B		Sample 1			Sample 2			Blank	Reference compound								
C								Blank									
D								Blank									
E								Solvent control									
F								Solvent control									
G								Solvent control									
H																	

Figure 5: 96-well plate layout used for the bio-assay. Grey wells indicate the outer PBS filled wells.

On the fifth and final day of the assay, the plates were inspected microscopically for confluency and viability. The culture medium was removed and the cells were washed with PBS containing Ca²⁺ and Mg²⁺. After adding 25 µl of cellLytic (Sigma-Aldrich) reagent to each well, the plates were frozen at -80°C for 10 minutes to ensure rupturing of the cell membranes. After reaching room temperature the plates were placed in a plate reader (Berthold LB 940 Multimode Microplate Reader). Luciferase assay reagent (LAR) (20 mM

tricine (Life Technologies), 1.07 mM $\text{Mg}(\text{CO}_3)_4$ $\text{Mg}(\text{OH})_2 \cdot 5 \text{H}_2\text{O}$ (Sigma), 2.67 mM $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ (Sigma), 0.1 mM EDTA-disodium salt (J.T. Baker, Phillipsburg, NJ, USA), 33.3 mM dithiothreitol (DTT, Sigma), 270 mM coenzyme A (Sigma), 530 mM ATP (Sigma), and 470 mM beetle luciferin (Promega, Madison, WI, USA) was injected into each well by the instrument and the volume of bio-luminescence quantified as RLUs. .

To calculate activation of the cellular receptor and consequent successful induction of the luciferase gene (for both cell lines) the mean RLUs, standard deviation, coefficient of variation (CV) and the percentage maxima induction relative to the positive reference compound for the respective luminescence assay were determined in Microsoft Excel XP. The CV had to be less than, or as close as possible to 20 to have accepted variation. When this value was greater than 20, one of the RLUs was dropped from the calculations (Whyte *et al.*, 2004).

Dose-response curves were plotted for the reference compound series with the concentration plotted on the x-axis in log scale and % E_2 or TCDD-max (depending on the assay) on the y-axis. Three data points lying on the linear part of the sigmoid curve were used to calculate the slope, y-intercept and correlation coefficient (R^2), using the equation $y = mx + c$ (Schramm *et al.*, 2001) (cf appendix 8.1). The effect concentration (EC) at which both the reference compound and samples elicited a 20, 50 and 80% response were calculated from the dose response curves. The relevant potency (REP) values were determined by relating the amount of unknown sample required to give the response equal to 20, 50 and 80% max respectively, to the equivalent amount of reference compound required to cause the same magnitude of response. These values obtained from this calculation were then back calculated to the mass of sediment or the volume of bile/water initially extracted.

In some instances the responses were not high enough to reach EC 50 and 80 and these values had to be extrapolated (Nieuwoudt *et al.*, 2009).

Inhibition of only the ER-mediated activity was determined. Inhibition of the AhR was not investigated as it is the activation of the AhR that indicates the presence of xenobiotics and

not so much its inhibition. For detecting inhibition of the ER the MVLN cells were excited with a background of 33 $\mu\text{g}/\text{mL}$ 17 β -oestradiol already on the first day of the assay. Sample responses were compared to that of the SC wells and expressed as percentage fold inhibition. Fold inhibition above 1.0 indicated inhibition by the sample. Statistical significant differences between the mean of SC and the mean of the sample were determined with the non-parametric Mann-Whitney U test using IBM SPSS Statistics Version 22. Differences between the means were regarded as statistical significant when $p < 0.05$.

A level of detection (LOD) indicates the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified (Badday *et al.*, 2015). Limit of detection was calculated by determining the X-intercept, standard deviation and average of each individual curve that had a linear gradient below 20% to the reference compound, and comparing it to the X-intercept of the reference compound. A confidence calculation in Microsoft Excel of 0.05 was then calculated in order to determine the 95% intercept confidence. All values below that calculated answer were used to determine whether these samples were <LOD

3.7. MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) viability assay

Cell viability and proliferation greatly affect the reliability of results produced by *in vitro* assays. For this reason a viability assay was run concurrently with the luminescent assays. Doing this prevented false negatives, i.e. ensuring that a low or no response by the extract in the luminescent assays was due to absence of ligands and not dead or dying cells due to toxicity in the extract. The MTT bio-assay is a reliable approach to examine cell proliferation, therefore determining if the samples were toxic to any extent, influencing the response of cells. This bio-assay functions on the principle of tetrazolium salt reduction (Oh *et al.*, 2004). The yellow tetrazolium salt—MTT in short—is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to produce reducing agents such as nicotinamide-adenine dinucleotide (NADH) and nicotinamide-adenine dinucleotide phosphate (NADPH). The resulting blue formazan is solubilized in and quantified by determining optical density of the solution (Oh *et al.*, 2004).

Cell culturing, seeding, and dosing were performed with the same method used in the luminescence bio-assays, using both cell lines. On day five, the final day of the bio-assay, a fresh solution of 0.5 mg/mL MTT was prepared (Kangarloo *et al.*, 2004). The cells were washed with PBS and 100 µL of the MTT solution was added to each well. Six wells of each plate that was not exposed to sample, only nutrient medium acted as the negative control and were killed with 100% MeOH. The 96-well plates were incubated for 30 minutes at 37°C and 5% CO₂. The MTT solution was removed and the blue formazan crystals solubilised with 20 µL dimethyl sulphoxide (DMSO) (Saarchem, uniLAB) for 30 minutes (Kangarloo *et al.*, 2004). Viability was expressed as a percentage of the absorbance of the control cells (Vistica *et al.*, 1991). These calculations were performed in Microsoft Excel XP. If the cells were less than 80% viable, it was assumed that it indicated toxicity (Gracia *et al.*, 2007).

4. Results

In this section, bio-luminescence as well as the viability results of the MVLN reporter gene assay and the H4IIE-*luc* assay are presented and compared to one another and will be further discussed in Chapter 5.

4.1. MVLN reporter gene bio-assay

The oestrogen assay results are presented first as this was the most extensive investigation of the study. The sediment findings are summarised in table 3, the water results in table 4 and bile in table 5 and 6, the results of the H4IIE-*luc* assay are tabulated in table 7. A “no response” indicates that the cells showed no detectible increase or decrease in RLUs. Values followed by an * are values that have been extrapolated and have not been quantified by the assay. This often happens when the maximum response from a sample only reaches 20%. Values highlighted in red had high percentages of E₂-max but these samples created no linear gradient in their dose-response curves and no response could be calculated as three points above 20% in a straight line section of the dose-response curve is necessary.

Sediment samples for the first sampling event in September 2012 elicited low ER responses for both sampling sites only providing a REP 20 (Table 3). During the second sampling season (April 2013) the Phongolo River and its pans (sites 8, 8.1 and 8.2) had high responses with the lowest of the three sites, at the Phongolo River, providing a REP 50 (Table 3). The pans west (8.1) and east (8.2) of the river had an activation responses of 99.2% and 118.9% respectively (Table 3). These extracts had the highest responses for the ER of all the samples during the entire study period.

Sediment from the Nyamiti Pan, the Ushutu River and the Nomaneni Pan for the second sampling event caused no activation of the ER.

Table 3: The %E₂-max and relative potencies (REP) of the sediment samples as well as the inhibition results obtained from the MVLN bio-assay. REP values are presented as the mean $\pm$ standard deviation.

Sampling season	Site	%E ₂ -max	REP 20	REP 50	REP 80	FI	
(E ₂ eq µg/g)							
September 2012	9	Nyamiti Pan	30.9	2.2±0.5x10 ⁻⁴	0.6±1.0x10 ^{-5*}	5.5±5.4x10 ^{-7*}	-
	8	Phongolo River	23.9	1.1±0.5x10 ⁻⁴	3.5±2.2x10 ^{-7*}	0.8±1.4x10 ^{-7*}	2.43
April 2013	10	Ushutu River	1.3	No response	-	-	-
	9	Nyamiti Pan	4.6	No response	-	-	-
	8	Phongolo River	55.8	1.7±1.6x10 ⁻⁹	0.7±3.8x10 ⁻⁶	2.23±3.86*	1.12
	8.1	Pan west of Phongolo River	99.1	0.7±1.1x10 ⁻⁵	2.9±2.4x10 ⁻⁴	0.019±0.018*	-
	8.2	Pan east of Phongolo River	118.9	0.5±0.1x10 ⁻⁹	2.5±8.6x10 ⁻⁷	1.6±0.8x10 ⁻³	-
	Ref	Nomaneni Pan	21.1	<LOD	-	-	1.09
September 2013	9	Nyamiti Pan	13.9	No response	-	-	1.01
	90	Nyamiti outflow	22.1	<LOD	-	-	-
	8	Phongolo River	18.3	No response	-	-	-
	Ref	Nomaneni Pan	7.3	No response	-	-	1.06

*= extrapolated values, - = no response could be quantified, **red bold** = obvious response, but no quantification was possible, FI = fold inhibition. Only statistical significant inhibition is indicated in the table, with FI >1: LOD = 3.32x10⁻¹¹ E₂-eq µg/g

During the third sampling event (September 2013), there were no quantifiable activation results for any of the samples (Table 3). However, the outflow of the Nyamiti Pan (site 90) did show some response but it could not be calculated as it was only the undiluted extract that elicited a response (cf chapter 3.6).

Many of samples showed statistical significant inhibition (Table 3). Sediment from the Phongolo River (September 2012 and April 2013) apparently contained ligands capable of both activation as well as causing inhibition (Table 3). The Nomaneni Pan had two other

occasions at which its sample caused inhibition, once with evidence of also activating the ER (April 2013) and once without activating the ER (September 2013).

It seems as if the sediment samples from the Phongolo River either elicited both an activation and inhibition response (September 2012 and April 2013) or no had no response (September 2013) (Table 3).

Water samples of September 2012 (Table 4) had activation of the ER for both the sampling sites, providing a REP 20. Compared to the sediment samples of the same season for these sites, the sediment elicited a higher response (Table 3) when comparing the REP 20 values.

Table 4: The %E₂-max and relative potencies (REP) of the H₂O samples as well as the inhibition results obtained from the MVLN bio-assay. REP values are presented as the mean $\pm$ standard deviation.

Sampling season		Site	%E ₂ -max	REP 20	REP 50 (E ₂ eq μ g/m ℓ)	REP 80	FI [#]
September 2012	9	Nyamiti Pan	35.8	2.1 $\pm$ 0.5 $\times 10^{-6}$	0.8 $\pm$ 4.0 $\times 10^{-7}$ *	8.1 $\pm$ 3.5 $\times 10^{-8}$ *	-
	8	Phongolo River	31.6	2.2 $\pm$ 0.2 $\times 10^{-6}$	0.4 $\pm$ 1.2 $\times 10^{-7}$ *	6.3 $\pm$ 3.5 $\times 10^{-9}$ *	1.8
	10	Ushutu River	10.3	No response	-	-	-
April 2013	9	Nyamiti Pan	18.1	No response	-	-	-
	8	Phongolo River	23.5	0.29 $\pm$ 0.5	5.4 $\pm$ 3.1 $\times 10^3$	3.4 $\pm$ 6.0 $\times 10^3$	1.66
	8.1	Pan west of Phongolo River	12.1	No response	-	-	-
	8.2	Pan east of Phongolo River	8.0	No response	-	-	-
	Ref	Nomaneni Pan	20.0	-	<LOD	-	-
September 2013	9	Nyamiti Pan	33.7	-	<LOD	-	1.63
	90	Nyamiti outflow	24.0	-	<LOD	-	-
	8	Phongolo River	8.3	No response	-	-	-
	Ref	Nomaneni Pan	49.1	2.8 $\pm$ 3.1 $\times 10^{-7}$	0.06 $\pm$ 3.7 $\times 10^{-8}$ *	1.1 $\pm$ 0.7 $\times 10^{-8}$ *	-

*= extrapolated values, - = no response could be quantified, **red bold** = obvious response, but no quantification was possible, FI = fold inhibition. Only statistical significant inhibition is indicated in the table, with FI >1; LOD = 4.0 $\times 10^{-13}$ E₂-eq μ g/m ℓ

During the second sampling event (April 2013) responses were considerably lower compared to 2012 with no activation of the oestrogen receptor (Table 4). The Phongolo River was the exception with some activation of only 23.5%. The water from the Nomaneni Pan showed a similar pattern as for the sediment samples: biological activity was detected but could not be quantified (Table 3 and 4).

Throughout the third sampling season (September 2013) obvious activity for the Nyamiti Pan (Site 9) and its outflow (Site 9O) were detected but could not be calculated. The Nomaneni Pan (Table 4) had a higher response and could be quantified for this season.

Again, as for the sediment samples, there were water samples that caused both activation as well as inhibition of the ER: water of the Phongolo River (September 2012 and April 2013) and the Nyamiti Pan from September 2013.

Table 5 represents the responses of *O. mossambicus* bile samples for the second and third sampling events. No bile was collected for the first sampling season of September 2012. Bile collected in April 2013 elicited little activation response. There was a response from one fish (8) caught in the Nyamiti Pan and the single fish from the Phongolo River showed quantifiable response, as high as REP 50 (Table 5). Bile contents of four fish from the Nyamiti Pan caused inhibition of the ER (3, 4, 12 and 13). As for the only fish caught during April 2013 from the Phongolo River caused both activation as well as inhibition of the ER, and two fish (9 and 11) from September 2013 had the same pattern of response.

During the third season (September 2013) the majority of fish bile samples caused activation of the ER. Bile extracts of 6 of the 24 fish caused no quantifiable activation responses (Table 5). Another 5 showed response that could not be quantified (cf. bold red in Table 5) but the other 13 bile samples contained ligands capable of activating the ER.

As for the sediment and the water, there were examples of bile that elicited both activation as well as inhibition of the ER especially by fish collected from the Phongolo River. Examples include fish numbers 4 (April 2013), and, 9 and 11 (September 2013) (Table 5). Fish no 19

and 21 (Table 5) had the highest anti-oestrogenic activity recorded for all the matrices during the entire study event.

Table 5: The results obtained from the MVLN bio-assay represent the %E₂-max and relative potencies (REP) as well as the inhibition results of the bile samples for the species *Oreochromis mossambicus*. REP values are represented as the mean ± standard deviation.

Sampling season	Fish number	%E ₂ -max	REP 20	REP 50	REP 80	FI
(E ₂ eq µg/ml)						
April 2013	Nyamiti Pan					
	3	0.02	No response	-	-	1.66
	4	2.7	No response	-	-	1.47
	8	22.4	<LOD	-	-	-
	12	0.03	No response	-	-	1.79
	13	0.13	No response	-	-	1.85
	15	12.8	No response	-	-	-
September 2013	Phongolo River					
	4	68.5	4060.2±7032.5	169.3±97.7	2.3±4.1*	1.57
	Nyamiti Pan					
	2	0.3	No response	-	-	3.75
	3	0.6	No response	-	-	2.65
	6	46.6	8.7±2.5x10 ⁻³	1.9±4.3x10 ⁻³ *	2.2±1.2x10 ⁻³ *	-
	7	50.0	0.9x±1.2x10 ⁻²	4.9±4.4x10 ⁻³ *	2.0±2.0x10 ⁻³ *	-
	8	50.9	4.2±3.0x10 ⁻³	8.5±7.4x10 ⁻³	2.0±2.9x10 ⁻² *	-
	9	46.7	1.1±0.4x10 ⁻³	3.2±4.9x10 ⁻³ *	2.4±1.9x10 ⁻² *	-
	10	52.3	1.3±0.8x10 ⁻³	0.012±0.014	0.17±0.20*	-
	11	32.3	<LOD	-	-	-
	12	39.6	6.5±3.1x10 ⁻³	0.2±1.7x10 ⁻² *	4.7±0.8x10 ⁻² *	-
	14	52.8	4.8±2.9x10 ⁻³	0.1±1.1x10 ⁻²	3.0±0.8x10 ⁻² *	-
	17	12.9	No Response	-	-	-

Table 5 continued

Sampling season	Fish number	%E ₂ -max	REP 20	REP 50	REP 80	FI
(E ₂ eq µg/mL)						
Phongolo River						
	1	0.2	No response	-	-	-
	2	39.2	<LOD	-	-	-
	5	33.0	<LOD	-	-	-
	7	30.9	<LOD	-	-	-
	8	36.2	<LOD	-	-	1.22
	9	33.6	4.2±1.8x10 ⁻⁶	3.7±2.5x10 ⁻⁶ *	2.5±4.2x10 ⁻⁶ *	1.02
	10	34.8	1.6±2.2x10 ⁻⁵	0.8±1.0x10 ⁻⁶ *	2.6±4.1x10 ⁻⁷ *	-
	11	44.0	4.0±5.5x10 ⁻⁵	2.0±2.6x10 ⁻⁶ *	0.6±1.0x10 ⁻⁶ *	1.03
	15	58.1	1.2±0.5x10 ⁻⁵	0.3±1.4x10 ⁻⁴ *	1.6±0.31x10 ⁻³ *	-
	16	22.1	<LOD	-	-	1.04
	17	19.8	<LOD	-	-	-
	19	22.5	No response	-	-	3.05
	21	19.4	No response	-	-	4.50

*= extrapolated values, - = no response could be quantified, **red bold** = obvious response, but no quantification was possible, FI = fold inhibition. Only statistical significant inhibition is indicated in the table, with FI >1 LOD = 9.6x10⁻¹³ E₂-eq µg/mL

Table 6 contains the responses of *C. gariepinus* bile samples for the last two sampling events. A similar pattern as for *O. mossambicus* emerged for the April 2013 season: little activation response for the Nyamiti Pan, and somewhat more for the *C. gariepinus* collected from the Phongola River (Table 6) where two of three fish had quantifiable responses (fish numbers 1 and 13) and one (fish no 2) showed some activation, however, unquantifiable response. There was indication of inhibition for only one sample (fish no 3) from April 2013 (Table 6).

No fish were caught from the Phongola River in September 2013. Of the five individuals collected from the Nyamiti Pan, three showed activation response, of which two were quantifiable (fish no 3 and 5) (Table 6). No inhibition can be observed for the *C. gariepinus* samples (Table 6).

Table 6: The results obtained from the MVLN bio-assay represent the %E₂-max and relative potencies (REP) as well as the inhibition results of the bile samples for the species *Clarias gariepinus* REP values are represented as the mean $\pm$ standard deviation.

Sampling season	Fish number	%E ₂ -max	REP 20	REP 50	REP 80	FI [#]
(E ₂ eq μ g/mL)						
April 2013	Nyamiti Pan					
	1	0.02	No response	-	-	-
	3	33.6	<LOD	-	-	1.08
	4	9.0	No response	-	-	-
	5	46.9	<LOD	-	-	-
	6	19.6	No response	-	-	-
	8	0.2	No response	-	-	-
	Phongolo River					
	1	31.5	0.35 $\pm$ 0.61	2.1 $\pm$ 1.3 $\times 10^{-5}$ *	3.8 $\pm$ 6.5 $\times 10^{-6}$ *	-
	2	38.9	<LOD	-	-	-
	13	54.8	2.2 $\pm$ 1.4 $\times 10^{-5}$ *	0.8 $\pm$ 1.5 $\times 10^{-3}$ *	1.9 $\pm$ 1.9 $\times 10^{-9}$ *	-
September 2013	Nyamiti Pan					
	3	29.01	4.7 $\pm$ 4. $\times 10^{-5}$ *	0.1 $\pm$ 6.7 $\times 10^{-7}$ *	4.1 $\pm$ 4.7 $\times 10^{-3}$ *	-
	4	14.0	No response	-	-	-
	5	47.5	1.2 $\pm$ 1.8 $\times 10^{-3}$	2.9 $\pm$ 5.9 $\times 10^{-3}$ *	1.1 $\pm$ 1.5 $\times 10^{-1}$ *	-
	6	28.6	<LOD	-	-	-
	8	19.6	No response	-	-	-

*= extrapolated values, - = no response could be quantified, **red bold** = obvious response, but no quantification was possible, FI = fold inhibition. Only statistical significant inhibition is indicated in the table, with FI >1; LOD = 9.6 $\times 10^{-13}$ E₂-eq μ g/mL

4.2. H4IIE-*luc* reporter gene bio-assay

Only extracts from sediment were investigated for AhR-ligands, and to be able to distinguish between all AhR-ligands and persistent AhR-ligands, two separate extracts were prepared for each sediment sample (cf chapter 3.4). The basic distinction between the two extracts is that one was treated with acid, which would contain the persistent AhR-ligands only (further referred to as the “acid washed samples”) and the extract not treated with acid, which would contain all the AhR-ligands extracted using the particular conditions of the method in section 3.4 and Figure 4 and are further referred to as the “non-acid washed

samples". As already mentioned previously a "no response" indicates that the cells showed no detectible increase or decrease in RLUs. Values followed by an * are values that have been extrapolated and have not been quantified by the assay. Values highlighted in red had high percentages of TCDD-max from the undiluted extract but their dose-response curves did not meet the requirements to be used for calculating REPs. .

Table 7 contains the responses of the persistent as well as all AhR ligands in the sediment samples obtained from the H4IIE-*luc* bio-assay, for only the third sampling event. Samples screened for ligands binding to the AhR were only collected during the last sampling season as this data is only supplementary to detecting ER ligands in the Phongolo floodplain aquatic environments (cf chapter 2.3). Those extracts containing only the persistent ligands ("acid washed", Table 7) did not elicit any response high enough to reach REP 20, however the non-acid washed extracts (Table 7) caused quantifiable activation for two of the four sites.

Table 7. The results obtained from the H4IIE-*luc* bio-assay represent the %TCDD-max and relative potencies (REP) of the acid washed and non-acid washed extractions. REP values are represented as the mean $\pm$ standard deviation.

Acid wash						
Sampling season	Site		%TCDD-max	REP 20	REP 50	REP 80
(TCDDeq µg/g)						
September 2013	9	Nyamiti Pan	6.66	No response	-	-
	90	Nyamiti outflow	19.25	<LOD	-	-
	8	Phongolo River	9.12	No response	-	-
	Ref	Nomaneni Pan	7.20	No response	-	-
Non-acid wash						
September 2013	9	Nyamiti Pan	6.72	No response	-	-
	90	Nyamiti outflow	32.30	$6.8 \pm 7.4 \times 10^{-11}$	$1.3 \pm 0.7 \times 10^{-6*}$	$0.011 \pm 0.020^*$
	8	Phongolo River	21.03	$1.2 \pm 0.1 \times 10^{-12}$	$1.2 \pm 0.9 \times 10^{-12*}$	$0.014 \pm 0.021^*$
	Ref	Nomaneni Pan	11.20	No response	-	-
* = extrapolated values, - = no response could be quantified, red bold = obvious response, but no quantification was possible. LOD = 1.3×10^{-16} TCDD-eq µg/g						

The lack of response of the acid washed extract could be an indication of no or undetectable AhR-ligands. The higher responses by the non-acid washed extracts (Table 7) were probably due to the fact that these extracts contained more AhR ligands than its acid washed equivalent.

For all assay results (Tables 3–7) a “no response” could mean one of two things: little or no receptor ligands present or unresponsive cells due to cytotoxicity and should the results contained in tables 3–7 be interpreted in conjunction with the results from the MTT viability that was run simultaneous with the luminescence assays.

4.3. Viability assay

The MTT assay was used to determine the viability of the MVLN and H4IIE-*luc* bio-assays. This assay measures cell viability in terms of reductive activity as enzymatic conversion of the tetrazolium compound to water insoluble formazan crystals as previously discussed in section 3.7. Figures 8–12 indicates the results obtained for the viability assays. Viability percentages below 80% were accepted as cytotoxic (Gracia *et al.*, 2007). None of the sediment extracts were really cytotoxic to the MVLN cells (Fig. 6) aiding in the interpretation of the luminescence assay.

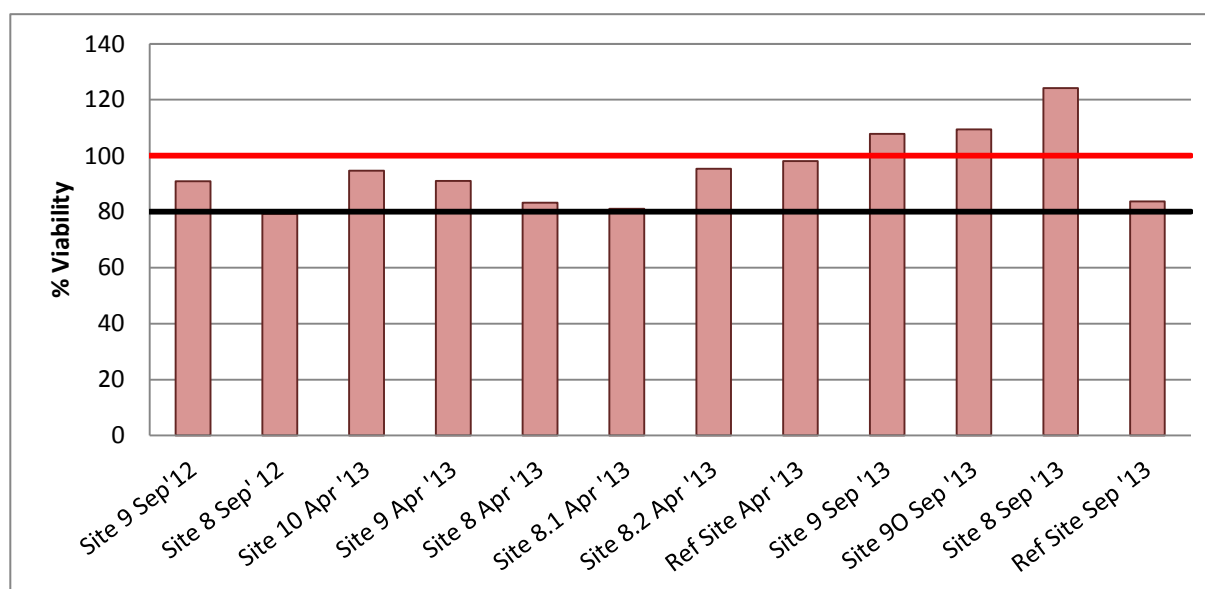


Figure 6: The viability results of the sediment samples during the 3 sampling seasons for the MVLN assay. The red line indicates 100% viability, i.e. those wells that received solvent only but no environmental extract. The black line indicates viability <80% deemed cytotoxic (Gracia *et al.*, 2007).

All the water samples seemed to have been non-toxic to the MVLN cells, except for those collected in September 2012 and site 10 of April 2013 (Fig. 7). In five instances the viability exceeded the 100% viability. This is a phenomenon observed many times and can be caused by bacteria that also metabolised the tetrazolium salt (Prinsloo *et al.*, 2013); extract ingredients capable of metabolising the tetrazolium salt such as caffeic acid (Wang *et al.*, 2015); or compounds that enhance cellular growth. At least these extracts were not cytotoxic to the cells, but rather beneficial and the luminescence results for these extracts should be interpreted accordingly. Only two of the bile samples from *O. mossambicus*: fish 10 and 11 of the September 2013 catch (Fig. 8) negatively influenced their cellular growth. There was also no cytotoxicity detected for the bile extracts from *C. gariepinus* (Fig. 9).

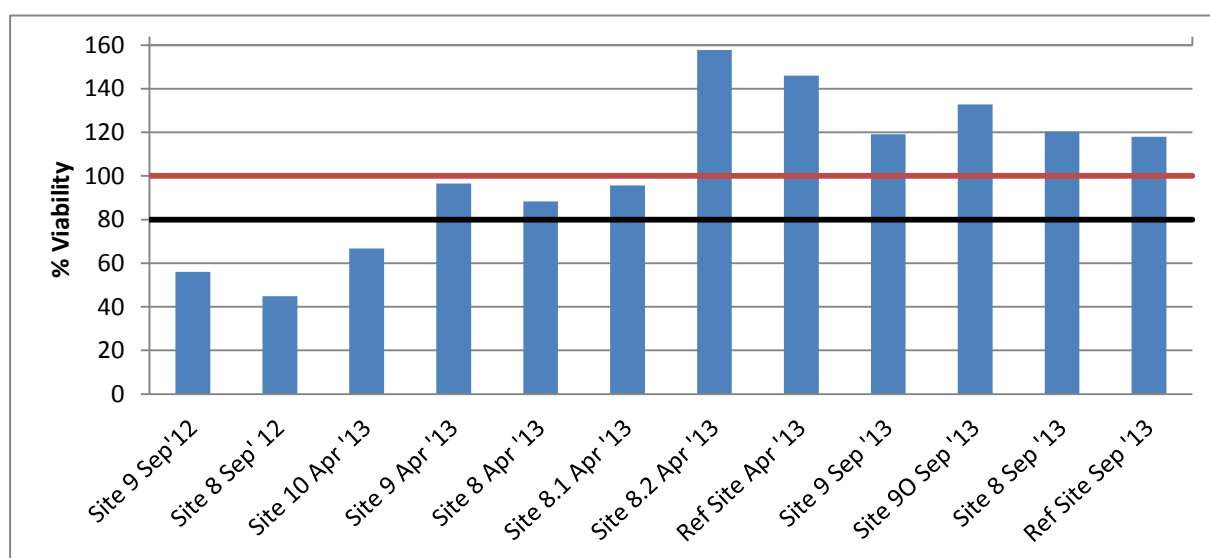


Figure 7: The viability results of the water samples during the 3 sampling seasons for the MVLN assay. The red line indicates 100% viability, i.e. those wells that received solvent only but no environmental extract. The black line indicates viability <80% deemed cytotoxic (Gracia *et al.* 2007).

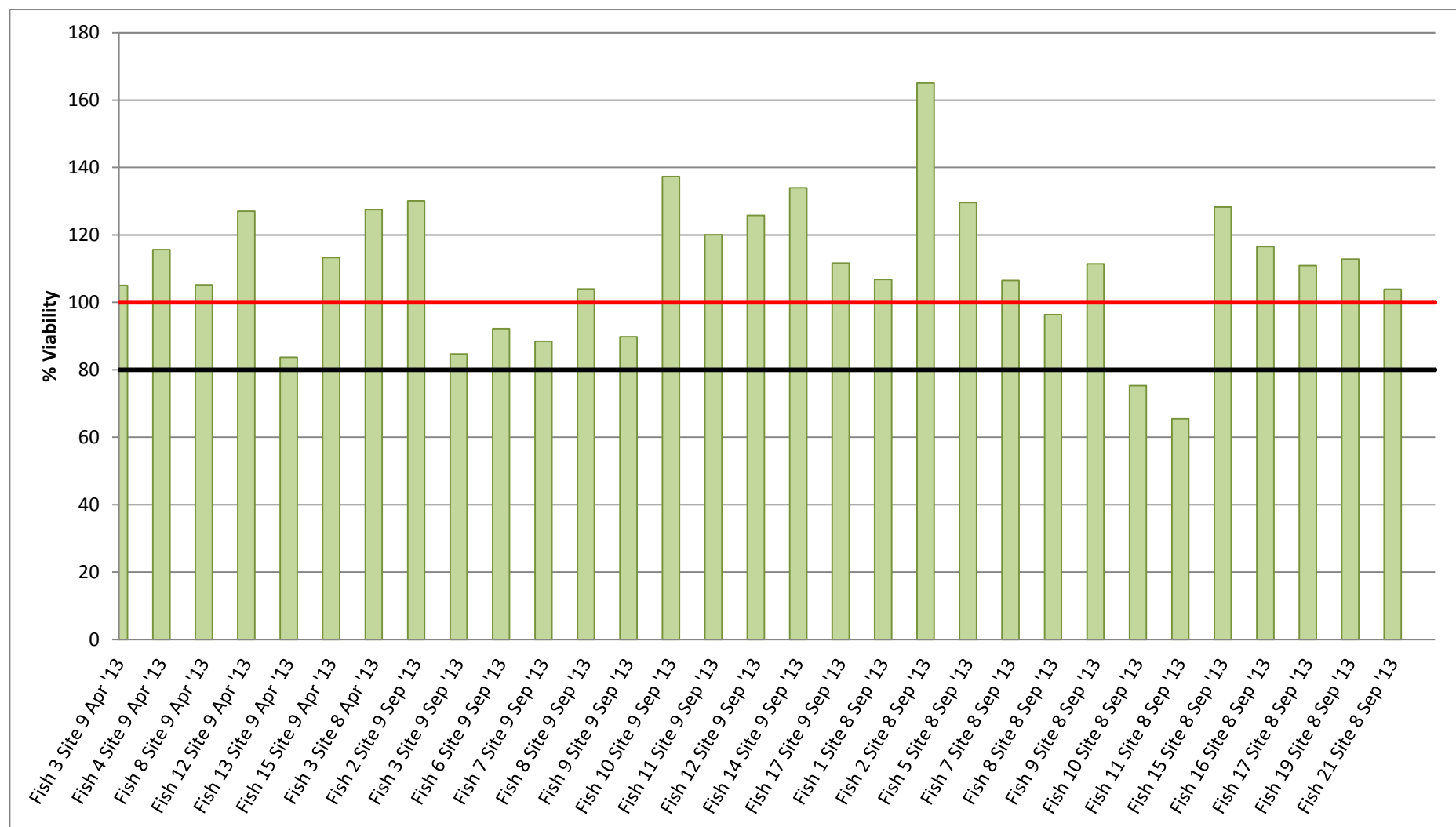


Figure 8: The viability results of the bile samples from *Oreochromis. mossambicus* caught during the last two sampling seasons for the MVLN assay. The red line indicates 100% viability, i.e. those wells that received solvent only but no environmental extract. The black line indicates viability <80% deemed cytotoxic (Gracia *et al.* 2007).

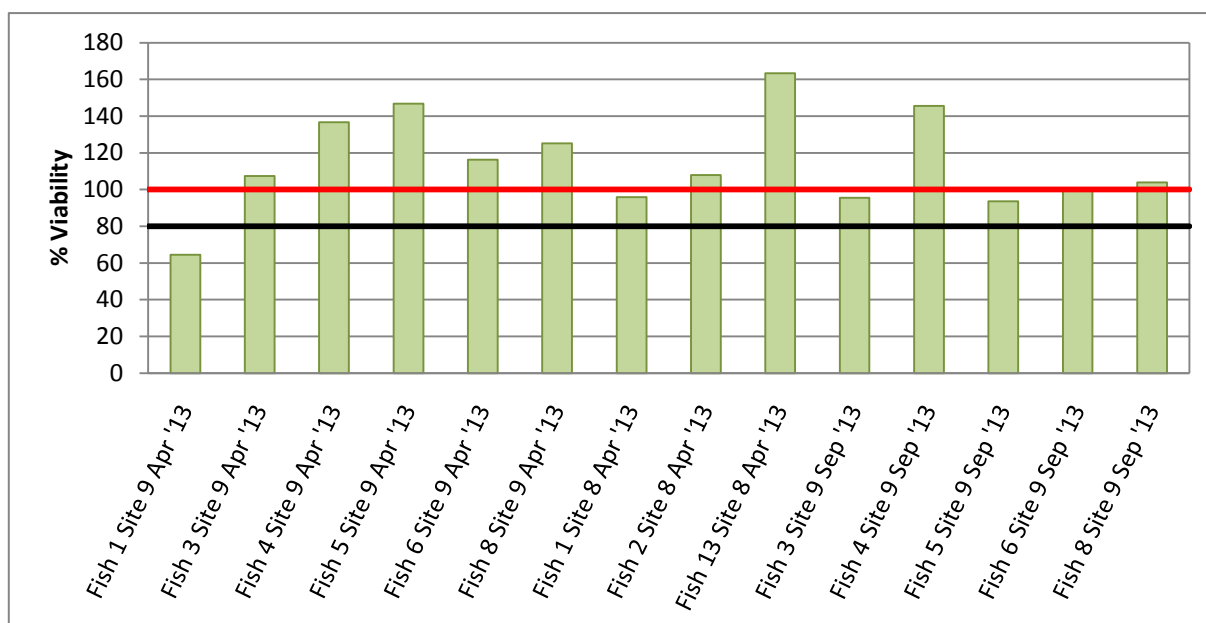


Figure 9: The viability results of the sediment samples from *Clarias gariepinus* caught during the last two sampling seasons for the MVLN assay. The red line indicates 100% viability, i.e. those wells that received solvent only but no environmental extract. The black line indicates viability <80% deemed cytotoxic (Gracia *et al.* 2007).

When the sediment extracts that were prepared for the H4IIE assay were tested for their influence on viability, only two were cytotoxic: in both cases it was sediment collected at the Nomaneni Pan, the reference site outside of NGR (Fig. 10).

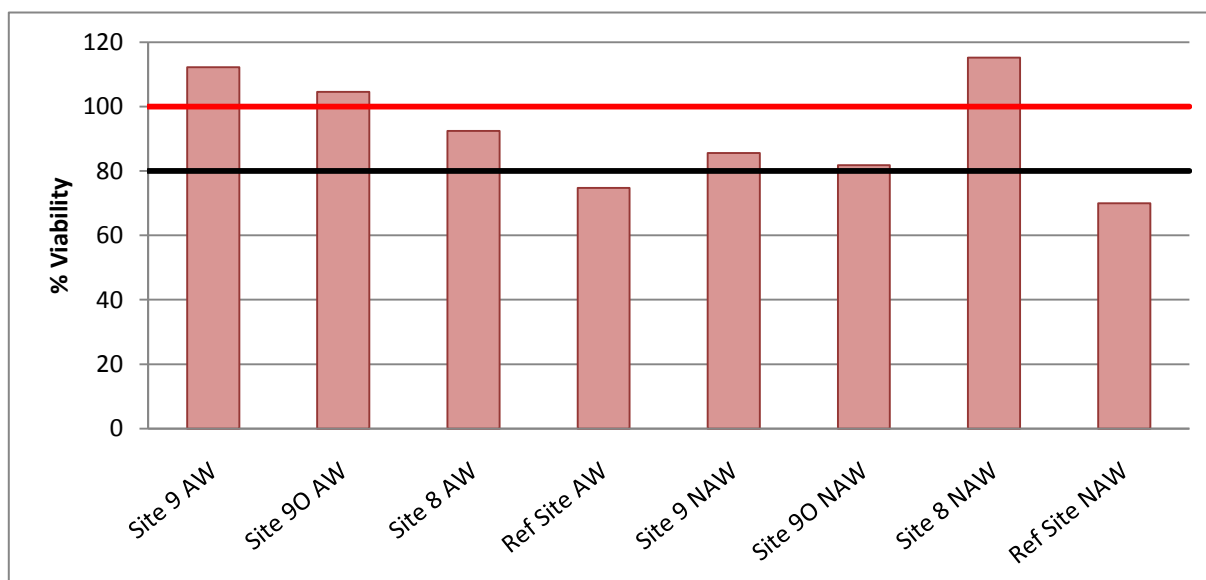


Figure 10: The viability results of the sediment samples during the third sampling season that were prepared for the H4IIE cells. The red line indicates 100% viability, i.e. those wells that received solvent only but no environmental extract. The black line indicates viability <80% deemed cytotoxic (Gracia *et al.* 2007). NAW = non-acid washed extracts; AW = acid washed extracts.

5. Discussion

In this chapter the results of the biological analysis gathered from the three matrices will be discussed and phenomena will be interpreted and compared to similar studies.

5.1. Oestrogen activity

5.1.1. ER responses induced by sediment samples

Sediment is a useful matrix because it serves as a trap for pollutants (Saha *et al.*, 2001, Praveena *et al.*, 2007, Varol, 2011) and is therefore an indicator of recent and accumulated pollution. Responses of sediment samples to the ER and AhR can change through time due to erosion, deposition, transport and flooding (Lane, 1955) (cf section 3.2).

The sediment samples collected for this study elicited oestrogenic activity for all of the sites during at least one of the three sampling events. The Ushutu River is the only exception never eliciting any responses. Target ligands that bind to the ER receptor either elicited an activation, or inhibition, or a simultaneous response, activating and inhibiting the ER as interpreted by the MVLN luminescence assay (cf Table 3). The results from the sediment samples suggest that the Phongolo River and the pans west and east of the Phongolo River are the most affected by pollution and this is noticed in the entire study (cf Table 3–7). This specific site was the only site where the ER elicited a simultaneous activation and inhibition response. These sites were also capable of super-induction of the ER (cf Table 3) and can be observed for samples from the pan west of the river (cf Table 3). Super-induction is the ability of extracts to elicit a %E₂-max that exceeds the maximum induction caused by the E₂ standard (Larsson *et al.*, 2012). This effect can be made possible by chemicals/compounds in the extract capable of enhancing induction of the ER response (Sotoca *et al.*, 2010). Compounds such as isoflavone, genistein (cf Table 1) can cause super-induction in luciferase reporter gene assays (Joung *et al.*, 2003). Apparently genistein can directly bind to and stabilize the firefly luciferase reporter enzyme thereby increasing its half-life, leading to super-induction (Auld, 2008).

The sediment results furthermore suggested that compounds mimicking the oestrogen hormone were absent during the last sampling event. A possible explanation for this observation could be that due to the high rainfall averages recorded from the previous wet season the pollutants were washed away (Przybysz *et al.*, 2014). According to the National State of Water Resources Report (2012/13), Kwa-Zulu Natal recorded the highest average rainfall for the period October 2012–April 2013.

It seemed that the seasons in which the sampling occurred could have possibly contributed to the variation noticed in the results. The dry seasons, September 2012 and 2013 had lower responses than the wet season, April 2013. During the dry seasons no floods and intrusion of the sediment layers takes place, therefore pollutants found in the top heterogeneous layer of sediment are composed of materials that have accumulated for a shorter period of time (Zhao *et al.*, 2011). However in the wet seasons flooding could possibly intrude the bottom sediments reintroducing captured pollutants to the water system and upper sediment layers (Wampler, 2012).

Confirming the assumption that seasonal changes could affect pollutant concentration levels in abiotic matrices such as sediment and water, Wampler (2012) conducted a study specifically focussing on movement of water and found that water levels and water movement have profound impacts on the concentration levels of pollutants in aquatic environments.

In addition to the luminescence assay, a viability assay can be used as a benchmark to measure the validity, toxicity and reliability of the luminescence assay. According to the MTT-assay results, no cytotoxicity was recorded for any of the sediment samples used in the MVLN bio-assay therefore the luminescence results were not influenced by cells battling to survive toxic compounds in the extracts.

5.1.1.1. AhR responses induced by sediment samples

Sediment samples were also collected for the H4IIE-*luc* bio-assay (Table 7; cf section 2.3). Responses in both the acid washed and non-acid washed samples for this event had a

similar pattern to the results seen in the MVLN assay, i.e. little to no responses related to receptor binding. All of the acid washed extracts had very low responses proving that little congeners were available to elicit any response. The non-acid washed sediment samples had a greater activation of the AhR than the acid washed samples. This occurrence was expected and previously observed (Nieuwoudt *et al.*, 2009). This phenomenon was expected considering the non-acid washed extract also contained all the AhR agonists, including the persistent compounds.

Samples from Nomaneni Pan were cytotoxic to the cells of the H4IIE-*luc* assay (Fig. 10) for the acid washed and non-acid washed extracts. Unidentified compounds present in the sample extracts reduced cell viability. Studies have shown that increased concentration of persistent and bio-accumulative environmental pollutants (cf Table 1) can significantly decrease cell viability (Addae *et al.*, 2013).

5.1.2. ER responses induced by water samples

Water can be an useful matrix as it represents recent and current pollution. In catchments, contaminant levels are generally expected to be highest in dams, lakes and harbours, where water circulation is minimal (Barra *et al.*, 2009) and samples from rivers are representative of pollutants passing through the sampling area at that specific point in time. Responses of the ER and AhR to water samples can change through time due to fluctuations in water levels, flow rate, turbulence, currents and floods to name but a few (Fawell and Nieuwenhuijsen, 2003).

Results of the water samples (Table 4) reflect a similar outcome as the sediment for all the sampling seasons. ER activity was noticed during the first and second sampling season, however during the third season ligands binding to the ER appear to be absent. Almost all the sites elicited some kind of oestrogenic activity except for the Ushutu River, again eliciting no activity whatsoever.

Seasonal changes appear to have an effect on the EDC levels found in freshwater aquatic systems. The results from the water samples compared to the sediment results (Table 3)

had opposite outcomes. During the first dry season the samples from the water had increased responses of the ER compared to the sediment samples from the same season. It is expected that during dry seasons lower water levels lead to concentrated pollutants (Schlusener and Bester, 2008, Phillips *et al.*, 2012) and according to the annual report of the South African cane growers association (sugar cane cultivation) during the period August to September 2012 the Phongolo district had an abnormal low rainfall (South African Cane Growers' Association Report of the Board of Directors 2012/13). On the contrary during the wet seasons, increased rainfall causes flooding of the Phongolo floodplain diluting pollutants in the water systems leading to a lower response to the ER compared to the sediment for the same season (Przybysz *et al.*, 2014).

The MTT (Fig 6.) analysis showed that all the samples from the second and third sampling events had a viability of 80% or more, thus no cytotoxicity were observed and it was therefore unlikely that cell viability affected the credibility of the luminescence assay's results (Gracia *et al.*, 2007). However all the samples from the first sampling session elicited cytotoxic effects whilst inducing responses to the ER affecting the credibility of the luminescence bio-assay. As previously discussed (cf section 3.7) viability analysis can be used to aid in the interpretation and discussion of the results obtained from luminescence assays. Viability assays as observed in the results section (Fig. 6–10) can have conflicting outcomes. In some instances the viability of the samples increases when compared to the control, or cells appear to be cytotoxic although ER and AhR responses are observed (cf section 5.1.1–5.1.3). Thus the toxicity observed in the viability assay could be an indication that although ER activity can be noticed the total response of the ER to these samples is not measured as some cells were killed by these samples.

5.1.3. ER responses induced by bile samples

The use of bile in this study can be used as an aid in determining the bio-accumulative and bio-physical effects of EDCs on organisms (Gibson *et al.*, 2005, Tyler *et al.*, 2005). Biotic matrices serve as an indicator of the effects of pollution on an organism over a period of time.

The results obtained for *O. mossambicus* (Table 5) have certain similarities to the results obtained for *C. gariepinus* (Table 6) as well as to the abiotic matrices. Both sampling seasons and both sampling sites, the Nyamiti Pan and the Phongolo River elicited some kind of ER activity. Some samples specifically from the Phongolo River were capable of simultaneous activation and inhibition of the same receptor.

These results from the Phongolo River for both fish species correlate with the water and sediment results. It seems that increased concentrations in the water and sediment samples contribute to elevated active responses in the bile samples (Table 4). Similar result patterns can also be observed in other studies conducted by Yang *et al.* (2014) and Houtman *et al.* (2004). Yang and co-authors (2014) conducted their study on wild carp in the Pearl River Delta, South China with the aim to investigate the distribution and bio-concentration of EDCs in this area. Houtman and his group focussed on the identification of compounds responsible for estrogenic activity in fish bile (male breams) collected at three locations in The Netherlands, by developing a bio-assay-directed fractionation method for oestrogenic chemicals. Houtman *et al.* (2004) presumed that there is a correlation between high responses of the ER to the bile samples, and similar responses in water samples and that these responses suggest a common cause of events (i.e., exposure to oestrogen-active pollutants).

The third sampling season was the only season that did not reflect similar responses as seen in the abiotic matrices. Two factors could contribute to this conflicting outcome: sample size and flood levels. During wet seasons fish species such as *C. gariepinus* and *O. mossambicus* can travel between sites, especially between pans and rivers, whereas during drying seasons they might become trapped in the pans, indicating that increased water leads to increased fish distribution (Amoros and Bornette, 2002). Górski *et al.* (2013) also confirmed that the distribution and movement of fish species can be influenced by the size and shape of water bodies as well as flood extent. As a result of no floods during the third sampling season more fish were sampled as they were possibly marooned in river stretches and/or pans leading to bigger catches (Meyer and Edwards, 1990; Sparks *et al.*, 1990; Bayley, 1995). The other factors as referred to are the size, age and of the captured fish which might have had an effect on the responses reported (Zhao *et al.*, 2012, El-Moselhy *et*

al., 2014). The sex of the fish that could have had an effect have on the outcome of the results are controlled and not of concern as only males were collected. The, age, however were not controlled and might have an impact on the results observed as much older fish would have been exposed to these pollutants for a longer period of time. Higher responses are expected to be seen in older species of fish collected.

Conclusively it appears that seasonal changes within the Phongolo floodplain could cause variation in the responses of ER and AhR to the sediment and water samples. The advantage of the bile samples is that they represent evidence of long term exposure, and are less dependent on seasonal changes as the sediment and specifically the water samples are.

Supportive to the luminescence bio-assay, the MTT analysis indicated cytotoxicity for only two *O. mossambicus* samples from the Phongolo River during September 2013.

5.1.4. Non-monotonic dose response curves

A very small number of sample responses could not be quantified (cf appendix 8.2). These samples elicited a response of which all of the data points were above the 20% E₂-max and did not display the typical linear dose-response relationship but rather a U-shaped curve (cf appendix 8.2; *C. gariepinus* no 2, site 8 April'13; water site 9 Sep'13; *O. mossambicus* no 2,5,7,8 site 8 Sep' 13). These occurrences are possible examples of the non-monotonic dose-response curves (NMDRCs). Non-monotonic can be defined as the slope of a curve that changes constantly from positive to negative or vice versa in the range of examination and are often U-shaped or inverted U shaped (Kohn and Melnick, 2002). This phenomenon has been described forendocrine disrupting compounds. Mechanisms that cause these occurrences may include cytotoxicity, cell- and tissue-specific receptors and co-factors, receptor selectivity, receptor down-regulation and desensitisation, receptor competition, and endocrine negative feedback loops (Fenner-Crisp, 2000, Vandenberg, 2012). According to Vandenberg (2013) these occurrences demonstrate that the environmental samples react to a response of other unexamined pathways and needs further investigation.

5.1.5. (Anti)-and-oestrogenic responses

EDC-research mostly focuses on exposure of EDCs with just one mechanism of action (activation of the ER). There are fewer instances where studies examined the effects of simultaneous exposure to EDCs that have different mechanisms of action even although these types of studies are more encompassing and would better reflect the real natural exposure situations. In this study, the information obtained indicates that both activation and inhibition of the ER took place, and sometimes even simultaneously in one extract. This situation was true for at least one sample from all three matrices specifically for the Phongolo River (cf Table3–7)

According to other studies (Hoffman and Kloas, 2012, Sun *et al.*, 2009) experimental results with co-exposure to oestrogenic and anti-oestrogenic actions can have distinct, divergent outcomes. Oestrogenic effects could possibly be neutralised or reinforced by anti-oestrogenic exposure, as it was also shown in this and several other studies.

Hofmann and Kloas (2012) studied the combined effects of activation and inhibition of the ER on the calling behaviour of male African clawed toad, *Xenopus laevis*. That study demonstrated that the sole exposure of compounds causing activation of the ER resulted in significant alterations in male behaviour. However, exposure to anti-oestrogenic compounds did not have any outcome. Co-exposure neutralised all oestrogenic affects as well. Sun *et al.* (2009) also confirmed that anti-oestrogenic exposure (inhibition) itself does not exhibit any effects and EDCs that typically could cause such effects are pharmaceuticals and specifically a chemical called TBT (cf Table 1, section 2.2.1) (Santos *et al.*, 2006, Elias *et al.*, 2007, Antizar-Ladislao, 2008).

To conclude there is need to further investigate the combined effects of EDCs with various mechanism of action. Thus, further studies should focus on the question whether simultaneous exposure to EDCs with different actions always leads to an eradication of some EDC effects, or whether exposure compounds can also act synergistically and result in additional effects.

Most of these occurrences were noticed in the Phongolo River.

5.2. Increased viability observation during the MTT analysis.

During the execution of the viability assay using MTT, the solvent control cells represent the 100% viable cells. However, often the cells receiving sample extracts would respond with seemingly higher viability than that of the solvent control. These occurrences could be due to a chemical compound that increases the formation of purple/blue formazan crystals, which indicates living cells (Wang *et al.*, 2015). One such a compound is caffeic acid, an organic compound produced by fresh water plants known as *Salvinia molesta* (Kariba weed). *S. molesta* grows in slow-moving waters such as lakes, ponds, streams, pans, and rivers, and prefers nutrient rich waters (Choudhary *et al.*, 2008). They are widely distributed in South Africa (Western and Eastern Cape, KwaZulu-Natal, Gauteng, Mpumalanga and Limpopo) as a category 1 invasive species according to the Conservation of Agriculture Resources Act 1983 (SA Act 43 of 1983) and are native to Brazil. Caffeic acid is hydrophobic (Paracatu *et al.*, 2014) and binds to particles in the water. It could have been co-extracted, skewing the viability results. However, its influence might have been little, as an extract is diluted one hundred times during the dosing process, and after the exposure period, the cells are washed repeatedly before the MTT assays commences, which would further decrease the presence of any caffeic acid.

Another explanation for the increased viability seen in the samples may be due to bacterial microbes, which can also metabolise the MTT, thereby masking their detrimental effect on the cells (Prinsloo *et al.*, 2013), but the bacterial effect on the cells would have been visible during the microscopic inspection of the cells at the end of the exposure leading to repeating assay, or commenting on the infection, that would have led to invalid luminescence results.

5.3. Site overview

In contrast to the preceding section where the focus was on the separate matrices, this section attempts to present a more specific site overview.

Phongolo River

Comparing pans to rivers, the Phongolo River seemed to have the most evidence of both ER and AhR activity. This was observed for all three matrices and throughout all seasons. From this observation it can be deducted that the pollutants mainly enter the NGR via the river system and not as much through the air. If there were substantial air pollution, the pans would have shown that, also in the dry seasons. Since all sites, (except the Nomaneni Pan) are within the borders of the protected environment of the game reserve, the pollution in its aquatic environment is likely transported over long distances from outside its borders.

It is interesting to note that responses from the Phongolo River matrices sometimes had simultaneous activation as well as inhibition effect on the ER. What the resultant effect of these mixtures are on the aquatic fauna are, needs further investigation.

Nyamiti Pan

Samples from the Nyamiti Pan also showed some ER activity, but not as regular as those from the Phongolo River. Most activity was observed for samples collected from the dry season, and more in the water than in the sediment indicating concentration of the ligands. In the first wet season, the response from the sediment was higher than in the water, indicating a possible dilution effect in the water, but the sediment, as was expected, trapped previous pollutants. In the following dry season, no responses were elicited by either sediment or water, which could be a result of the cleaning effect by the previous exceptionally wet season.

During dry seasons, water flows from the Nyamiti Pan toward the river, but during wet seasons water is pushed from the river into the pan, in this way, transporting pollutants

from the river into the pan. It seems that there are no direct sources of pollution in these waters, as was expected as this pan is within the NGR.

Nomaneni Pan

The Nomeneni Pan never had any repetitive pattern for any of the samples, some instances elicited activation, some instances elicited inhibition and sometimes no response could be quantified. This site is located in a rural environment surrounded by small scale farming, and vegetable gardens which could have led to varying degrees of and volumes of point source pollution. There were no restriction to access to the pan and cattle and small children have unhindered access to the shoreline. This situation could explain the absence of a clear pollution pattern.

Ushutu River

The Ushutu River had the lowest results recorded for this particular study. According to the Department of Water Affairs and Sanitation only a small quantity of water from the Ushutu River is available for domestic use. More importantly the area around the upper Ushutu River is not densely populated and is isolated from larger populations thus limiting human settlements (DWAF, 1996). This situation might explain low activation of the oestrogenic receptor.

6. Conclusion

This study forms a part of a larger project funded by the Water Research Commission (WRC) that took place in the northern Kwa-Zulu Natal area, on the Phongolo floodplain. The Phongolo River in South Africa is situated in the northern KwaZulu-Natal and is a tributary of the Maputo River, and is dammed at Jozini. The overarching aim of the project was to determine the ecological health of the Phongolo floodplain, the impact of major stressors on the Lower Phongolo River and lastly to provide all necessary helpful information to local conservation authorities for conservation efforts. One of these aspects was also to determine how human impact possibly pollutes the floodplain. The experimental section conducted for this dissertation fits into the toxicological division of the overarching study specifically investigating a group of pollutants that are called endocrine disrupting compounds (cf section 1 and 2 and 3).

EDCs are known as exogenous chemicals that interfere with the synthesis, secretion, transport and elimination of naturally produced hormones in the body that control the functioning and development of endocrine system in organisms (WHO, 2013). After all EDCs act like or mimic or suppress the action of natural occurring hormones (McLachlan and Arnold, 1996) and were therefore the main focus of this dissertation (cf section 2).

The aim and hypothesis that were stated at the end of the introductory chapter have been met:

- It has been established that compounds found in the aquatic environment of the Phongolo area are endocrine disruptive and
- these ED compounds from the sediment, water and fish bile caused active responses of the ER and AhR to a limited extent (cf section1).

EDCs are chemically diverse compounds and occur in a wide range of materials and goods. They vary in nature from artificial (anthropogenic) to naturally occurring compounds. Since the start of this century extensive research has led to better understanding of EDCs. Scientific reviews published by, for example, the Endocrine

Society (Diamanti-Kandarakis *et al.*, 2009), the European Commission (Kortenkamp *et al.*, 2011) and the European Environment Agency (2012) on EDCs it has found that at low concentrations these compounds play a very important role in adverse health effects to wildlife and humans (cf section 3).

This study has shown that EDCs are wide spread contaminants of sediment, water and fish bile particularly in the Phongolo River catchments. Samples from the Phongolo River elicited oestrogenic activity for all of the sites during at least one of the three sampling events. These EDCs were also relatively wide spread in the Nyamiti pan and the Nomaneni pan however they were almost absent in the Ushutu River (cf section 4). From this study it is clear that the pollutants find their way to the protected environment of the NGR from the Phongolo River, especially during floods. It is also evident that flooding during wet seasons can play a crucial role in the distribution of target contaminants in the different matrices. It was expected and proven that during the dry seasons pollutants are concentrated in the water and higher responses are observed. Nevertheless during the wet seasons the pollutants are diluted and sediment intruded expecting higher responses for sediment (cf section 5).

The MVLN assay determined target compounds binding to the ER, mimicking the oestrogen hormone. This mechanism of action is most commonly investigated. Oestrogen active compounds were found in all the matrices collected during the entire study. In the dry seasons the concentrations of these compounds were higher in the water samples than in the sediment and vice versa during the wet seasons. The fish tissue also had activated ER responses but varied between sites and sampling events due to different influencing factors.

The H4IIE-*luc* assay was additionally used for determination of target compounds binding to the AhR. The AhR is a ligand-dependent transcription factor that mediates a variety of cellular events in a variety of tissues (Denison and Nagy, 2003). AhR expression is found in many vertebrates including fish, animals and humans (cf section 2). When the AhR binds to xenobiotic ligands it regulates the expression of certain genes involved in a variety of responses such as cell proliferation, apoptosis, immune function, development, and reproductive functions (Bonnesen *et al.*, 2001). The responses elicited from the non-acid

washed (all the AhR-ligands extracted) extract were higher than those elicited from the acid washed (persistent AhR-ligands only) extract. However, the overall evidence of AhR ligands was small.

Recommendations for future studies

Despite substantial research efforts and many advances in our understanding on EDC mechanisms and actions, there are many uncertainties and knowledge gaps that still exist that are too important to ignore. These knowledge gaps hamper progress towards better protection of human and wildlife health. This study identified some of these gaps as the simultaneous activation and inhibition of the target receptors and the typical non-monotonic dose response curves associated with *in vitro* EDC research.

For future studies it is recommended: that

- Instrumental analysis to be done together with biological analysis. This would identify compounds present in the extract that potentially cause these biological responses. This analysis can also detect the compounds responsible for super induction and increased cell viability during the MTT-assay. Although, instrumental analysis could be time consuming and expensive it could be used to identify more specific compounds of interest. Compounds known to be released in the environment from known activities such as from the agricultural and industrial indices. These analysis can target these known compounds.
- It would be beneficial to control for the age of the fish from which bile is collected as age plays a role in bio-accumulation. Comparison between various age groups can in return be helpful to evaluate bio-accumulation.
- To evaluate EDCs using more mechanisms of action such as the regulation of the adrenal steroidogenesis by use of the H295R cell line (Rehman *et al.*, 2003). To provide a more comprehensive view of their effects on the biological system.
- To have a better understanding of the simultaneous effect of inhibition and activation of the same receptor. Can this occurrence have different effects on the

health of biota or does the inhibition of the receptor counter-act the total ED effects?

- To use a variety of viability tests such as the Cell Counting Kit 8 (CCK 8) assay to evaluate the luminescence assay credibility. As the MTT assay can be negatively influenced by natural occurring compounds such as caffeic acid (cf section 5.3)

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8. Appendix A

8.1 Formula sheet:

The formula sheet indicates the basic formulas used in Microsoft Excel 2010 to convert the data acquired from the Berthold LB 940 Multimode Microplate Reader into more understandable and interpretable values.

Mathematical Formulas	As retrieved from Microsoft Excel
$Concentration = \frac{mass}{volume}$	$Concentration = \frac{mass}{volume}$
$log = log(x)$ $log = log(E^2(\mu g / well))$	$log = log(x)$ $log = log(E_2(\mu g / well))$
$mean = \frac{\sum x}{n}$	$= AVERAGE(number1, [number2], ...)$
Standard deviation $= \sigma \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \mu)^2}$	$= STDEV((number1, [number2], ...))$
$CV = 100 \times \frac{SD}{mean}$	$= AVERAGE / STDEV * 100$
<i>Max Function returns the largest value in a set of values</i>	$= MAX(number1, [number2] ...)$ $* 100 / AVERAGE$
$y = mx + c$ <i>Y-intercept of a line is the point where a line's graph intersects the Y-axis</i>	$= INTERCEPT(known\ y's, known\ x's)$

$$Slope = \frac{\Delta y}{\Delta x} = SLOPE(known\ y's, known\ x's)$$

$$RSQ = r^2 = RSQ(known\ y's, known\ x's)$$

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2} \sqrt{n(\sum y^2) - (\sum y)^2}}$$

$$EC = x = \frac{y - c}{m} = 10^{((x - INTERCEPT)/SLOPE)}$$

$$REP = \frac{EC\ standard}{ECsample} = EC\ standard/sample$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}} = Ttest(array1, array2, tails, type)$$

8.2. Back calculations for oestrogen activity retrieved from Microsoft Excel 2010.

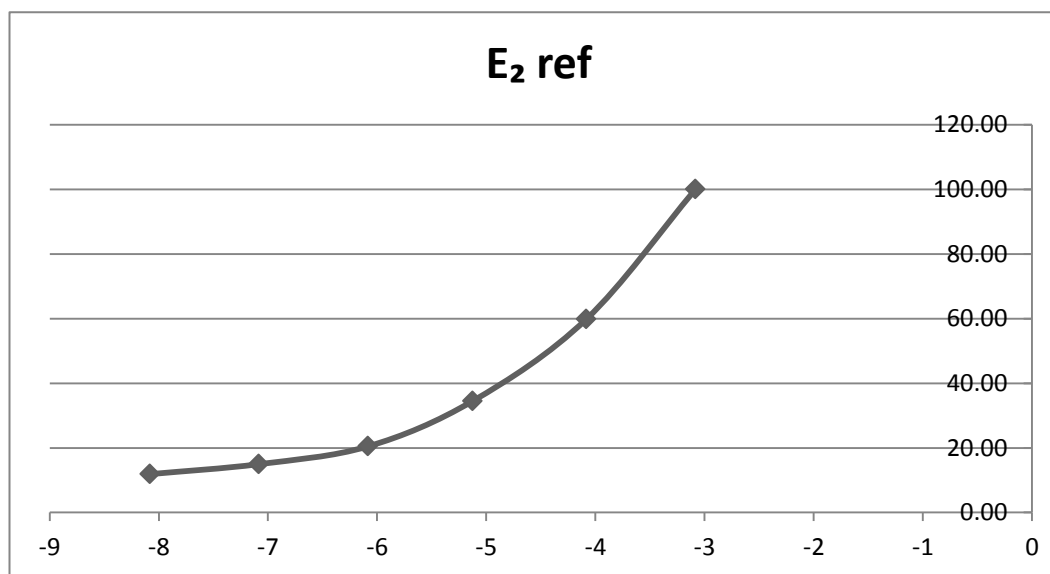
8.2.1 1st Sampling event

8.2.1.1 Reference linear curve as for 1st sampling event, September 2012. Water and sediment samples were compared to the figure indicated below.

E ₂ (µg/)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.0835461	3249998	3373905	3106401	3243434.667	133872.721	4.13	100.00
8.25E-05	8.25E-11	3.03E-13	-4.0835461	2035739	1973696	1811018	1940151	116055.298	5.98	59.82
7.50E-06	7.50E-12	2.75E-14	-5.1249387	1170318	1202671	982490	1118493	118887.725	10.63	34.48
8.25E-07	8.25E-13	3.03E-15	-6.0835461	660429	661214	669387	663676.6667	4960.84532	0.75	20.46
8.25E-08	8.25E-14	3.03E-16	-7.0835461	451092	529845	475805	485580.6667	40276.3143	8.29	14.97
8.25E-09	8.25E-15	3.03E-17	-8.0835461	383554	386767	165154	385160.5	2271.93409	0.59	11.88

E₂
 intercept 174.64
 slope 26.33
 r2 0.96

	µg/well
EC20	1.34E-06
EC50	1.84E-05
EC80	2.54E-04



8.2.1.2. Activation calculations of September 2012 retrieved from Microsoft Excel 2010.

Sediment 9 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	975749	974916	1062886	1004517	50550.7526	5.03	30.97
0.83	-7.92E-02	683945	880747	843062	802584.667	104458.45	13.02	24.74
0.28	-5.56E-01	609205	625114	659191	631170	25537.3531	4.05	19.46
0.09	-1.03E+00	483412	473021	506248	487560.333	16997.4964	3.49	15.03
0.03	-1.51E+00	363218	363672	385464	370784.667	12714.7021	3.43	11.43
0.01	-1.99E+00	340892	340819	1369168	340855.5	51.618795	0.02	10.51

y=mx+c

intercept 26.01

slope 12.06

r² 1.00

μℓ/well

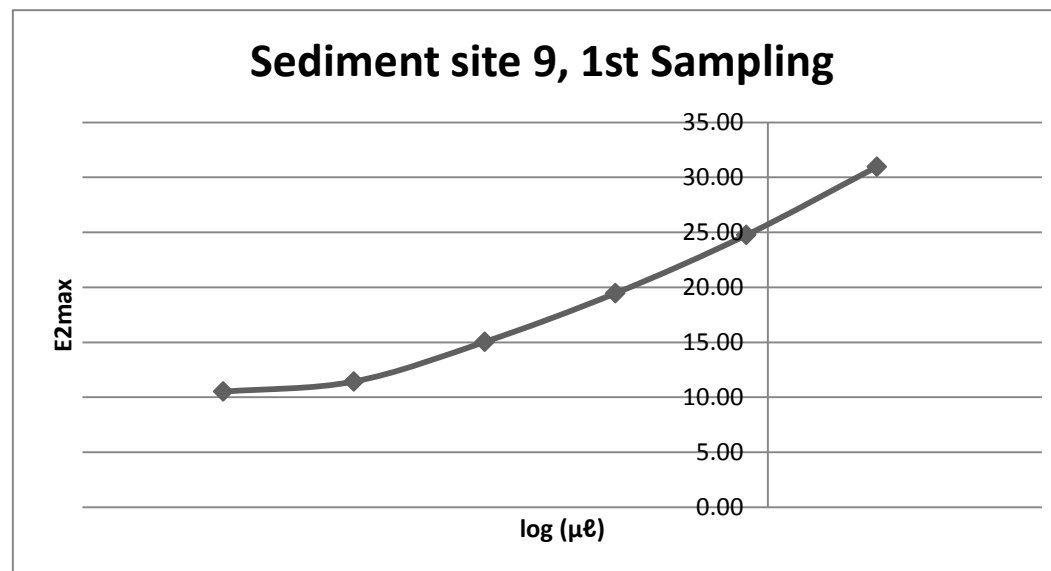
EC20 3.17E-01

EC50 9.74E+01

EC80 2.99E+04

	μg/μℓ	μg/mℓ
REP20	4.22E-06	4.22E-03
REP50	1.89E-07	1.89E-04
REP80	8.51E-09	8.51E-06

	E ₂ eq μg/g
REP20	2.10E-04
REP50	9.45E-06
REP80	4.24E-07



Mass sediment extracted: 20.05 g
concentrated to 1 ml

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
975749	30.08	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
683945	21.09	intercept	24.26	EC20	0.437184836	REP 20	3.06E-06	3.06E-03	1.53E-04	Mean	STEDV
609205	18.78	slope	11.84	EC50	149.2121809	REP 50	1.24E-07	1.24E-04	6.17E-06	2.15E-04	5.38E-05
483412	14.90	r ²	0.90	EC80	50926.45741	REP 80	4.99E-09	4.99E-06	2.49E-07		
363218	11.20									REP50	
340892	10.51									Mean	STEDV
RLU	E ₂ max									5.92E-06	1.02E-05
974916	30.06	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
880747	27.15	intercept	26.39	EC20	0.272014671	REP 20	4.92E-06	4.92E-03	2.45E-04	REP80	
625114	19.27	slope	11.30	EC50	122.7357292	REP 50	1.50E-07	1.50E-04	7.50E-06	Mean	STEDV
473021	14.58	r ²	0.93	EC80	55379.58362	REP 80	4.59E-09	4.59E-06	2.29E-07	5.51E-07	5.41E-07
363672	11.21										
340819	10.51										
RLU	E ₂ max										
1062886	32.77	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
843062	25.99	intercept	27.40	EC20	0.271039384	REP 20	4.94E-06	4.94E-03	2.46E-04		
659191	20.32	slope	13.04	EC50	54.08207971	REP 50	3.41E-07	3.41E-04	1.70E-05		
506248	15.61	r ²	1.00	EC80	10791.31492	REP 80	2.36E-08	2.36E-05	1.18E-06		
385464	11.88										
1369168	42.21										

Sediment 8 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	846655	766291	719955	777633.6667	64107.0555	8.24	23.98
0.83	-7.92E-02	773355	611948	687004	690769	80769.34029	11.69	21.30
0.28	-5.56E-01	586199	569157	529966	561774	28834.33871	5.13	17.32
0.09	-1.03E+00	496028	517496	509970	507831.3333	10892.62032	2.14	15.66
0.03	-1.51E+00	436550	425350	428814	430238	5734.181023	1.33	13.26
0.01	-1.99E+00	446617	469161	9177	457889	15941.01528	3.48	14.12

y=mx+c

intercept 26.01

slope 12.06

r2 1.00

μℓ/well

EC20 3.17E-01

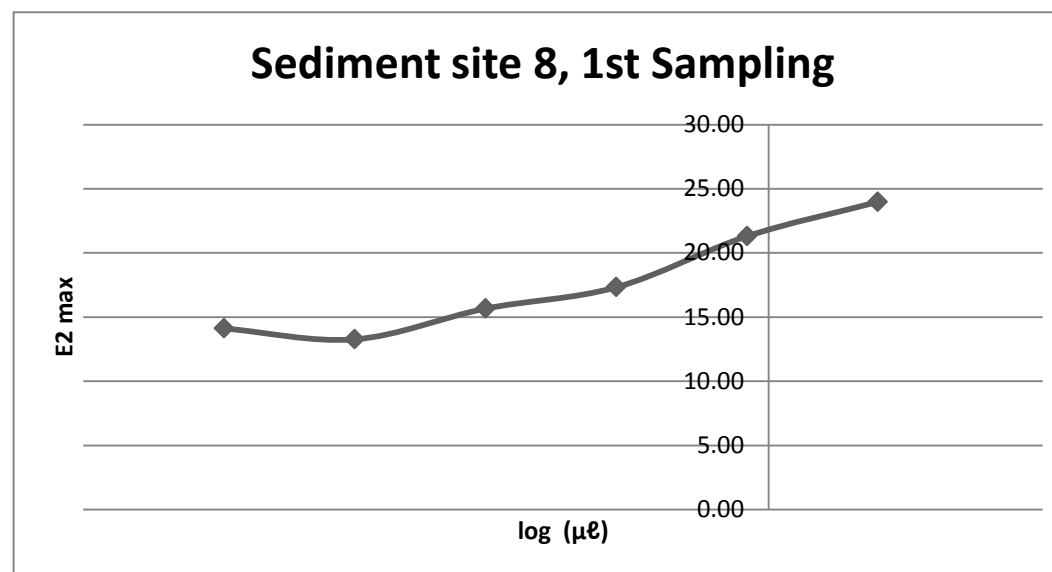
EC50 9.74E+01

EC80 2.99E+04

	μg/μℓ	μg/mℓ
REP20	4.22E-06	4.22E-03
REP50	1.89E-07	1.89E-04
REP80	8.51E-09	8.51E-06

	E ₂ eq μg/g
REP20	2.10E-04
REP50	9.45E-06
REP80	4.24E-07

Mass sediment extracted: 20.05 g
concentrated to 1 ml



Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
846655	26.10	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
773355	23.84	intercept	23.34	EC20	0.400971788	REP 20	3.34E-06	3.34E-03	1.66E-04	Mean	STEDV
586199	18.07	slope	8.42	EC50	1472.455361	REP 50	1.25E-08	1.25E-05	6.22E-07	1.08E-04	5.03E-05
496028	15.29	r ²	0.94	EC80	50926.45741	REP 80	4.99E-09	4.99E-06	2.48E-07		
436550	13.46									REP50	
446617	13.77									Mean	STEDV
RLU	E ₂ max									3.49E-07	2.19E-07
766291	23.63	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
611948	18.87	intercept	20.52	EC20	0.8292097	REP 20	1.61E-06	1.61E-03	8.01E-05	REP80	
569157	17.55	slope	6.37	EC50	42529.85981	REP 50	4.34E-10	4.34E-07	2.15E-08	Mean	STEDV
517496	15.96	r ²	0.90	EC80	2181340829	REP 80	1.17E-13	1.17E-10	5.79E-12	8.26E-08	1.43E-07
425350	13.11										
469161	14.46										
RLU	E ₂ max										
719955	22.20	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
687004	21.18	intercept	20.39	EC20	0.863205046	REP 20	1.55E-06	1.55E-03	7.69E-05		
529966	16.34	slope	6.14	EC50	66569.56276	REP 50	2.77E-10	2.77E-07	1.38E-08		
509970	15.72	r ²	0.88	EC80	5133782182	REP 80	4.95E-14	4.95E-11	2.46E-12		
428814	13.22										
9177	0.28										

H ₂ O 8 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	1013937	1001725	1065945	1027202.333	34103.20955	3.32	31.67
0.83	-7.92E-02	846404	758814	804179	803132.3333	43804.37944	5.45	24.76
0.28	-5.56E-01	615630	647627	678507	647254.6667	31440.15357	4.86	19.96
0.09	-1.03E+00	484585	483258	510335	492726	15264.26851	3.10	15.19
0.03	-1.51E+00	418860	389858	380511	396409.6667	19996.36723	5.04	12.22
0.01	-1.99E+00	347803	354390	334329	345507.3333	10225.62929	2.96	10.65

y=mx+c

intercept 26.43

slope 12.28

r2 0.99

μℓ/well

EC20 2.99E-01

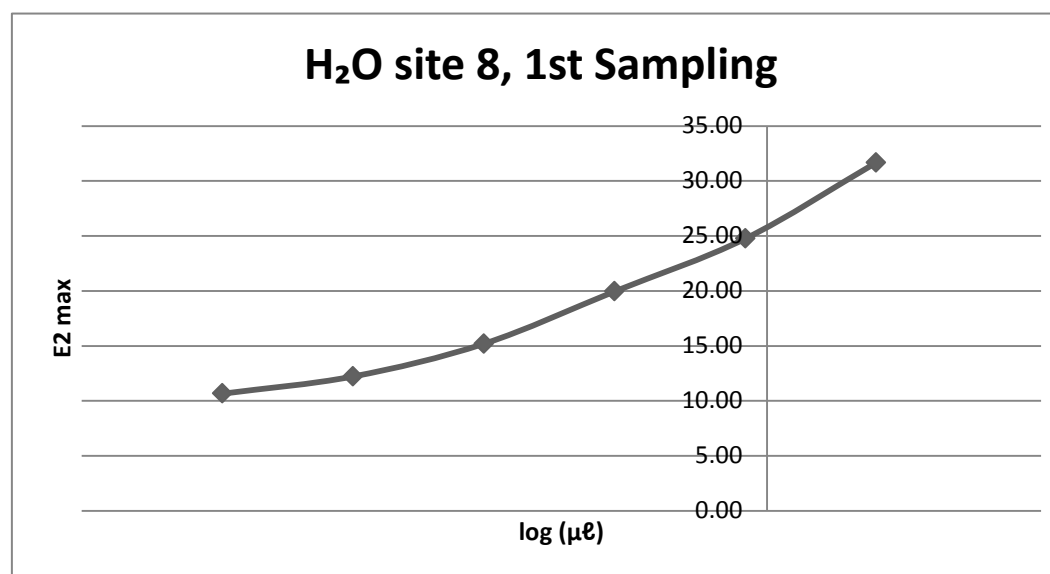
EC50 8.31E+01

EC80 2.31E+04

	μg/μℓ	μg/mℓ
REP20	4.47E-06	4.47E-03
REP50	2.22E-07	2.22E-04
REP80	1.10E-08	1.10E-05

	E ₂ eq μg/mℓ
REP20	2.24E-06
REP50	1.11E-07
REP80	5.51E-09

2ℓ water used in extraction
concentrated to 1mℓ



Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
1013937	31.26	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
846404	26.10	intercept	26.46	EC20	0.314515432	REP 20	4.25E-06	4.25E-03	2.13E-06	Mean	STEDV
615630	18.98	slope	12.87	EC50	67.41800704	REP 50	2.74E-07	2.74E-04	1.37E-07	2.24E-06	2.32E-07
484585	14.94	r ²	0.99	EC80	14451.39797	REP 80	1.76E-08	1.76E-05	8.80E-09		
418860	12.91									REP50	
347803	10.72									Mean	STEDV
RLU	E ₂ max									3.99E-08	1.15E-07
1001725	30.88	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
758814	23.40	intercept	25.66	EC20	0.320417846	REP 20	4.18E-06	4.18E-03	2.09E-06	REP80	
647627	19.97	slope	11.44	EC50	134.243056	REP 50	1.37E-07	1.37E-04	6.87E-08	Mean	STEDV
483258	14.90	r ²	0.96	EC80	56242.80397	REP 80	4.52E-09	4.52E-06	2.26E-09	6.25E-09	3.50E-09
389858	12.02										
354390	10.93										
RLU	E ₂ max										
1065945	32.86	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
804179	24.79	intercept	27.18	EC20	0.266758078	REP 20	5.02E-06	5.02E-03	2.51E-06		
678507	20.92	slope	12.52	EC50	66.47342351	REP 50	2.77E-07	2.77E-04	1.39E-07		
510335	15.73	r ²	0.96	EC80	16564.50691	REP 80	1.54E-08	1.54E-05	7.68E-09		
380511	11.73										
334329	10.31										

H ₂ O 9 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	1218305	1144075	1127130	1163170	48494.16898	4.17	35.86
0.83	-7.92E-02	782451	776600	697005	752018.6667	47732.96754	6.35	23.19
0.28	-5.56E-01	722464	677778	580559	660267	72555.0384	10.99	20.36
0.09	-1.03E+00	521113	594542	446843	520832.6667	73849.89905	14.18	16.06
0.03	-1.51E+00	480693	342438	390352	404494.3333	70204.09853	17.36	12.47
0.01	-1.99E+00	306953	280816	258246	282005	24375.25903	8.64	8.69

y=mx+c

intercept 27.75

slope 16.25

r² 0.88

μℓ/well

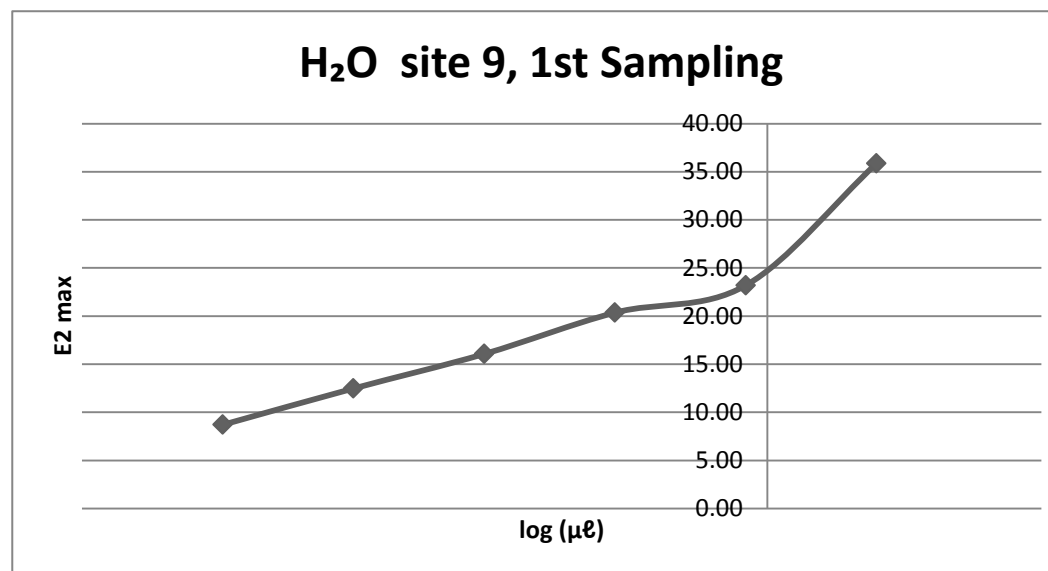
EC20 2.99E-01

EC50 8.31E+01

EC80 2.31E+04

	μg/μℓ	μg/mℓ
REP20	4.47E-06	4.47E-03
REP50	2.22E-07	2.22E-04
REP80	1.10E-08	1.10E-05

	E ₂ eq μg/mℓ
REP20	2.24E-06
REP50	1.11E-07
REP80	5.51E-09



2ℓ water used in extraction
concentrated to 1mℓ

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
1218305	37.56	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/ml	REP20	
782451	24.12	intercept	29.26	EC20	0.264407003	REP 20	5.06E-06	5.06E-03	2.53E-06	Mean	STEDV
722464	22.27	slope	16.02	EC50	19.71801076	REP 50	9.36E-07	9.36E-04	4.68E-07	2.08E-06	5.40E-07
521113	16.07	r ²	0.84	EC80	1470.460103	REP 80	1.73E-07	1.73E-04	8.65E-08		
480693	14.82									REP50	
306953	9.46									Mean	STEDV
RLU	E ₂ max									7.73E-08	3.97E-07
1144075	35.27	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/ml		
776600	23.94	intercept	27.90	EC20	0.299084935	REP 20	4.47E-06	4.47E-03	2.24E-06	REP80	
677778	20.90	slope	15.07	EC50	29.31093519	REP 50	6.29E-07	6.29E-04	3.15E-07	Mean	STEDV
594542	18.33	r ²	0.90	EC80	2872.531582	REP 80	8.85E-08	8.85E-05	4.43E-08	8.12E-08	3.46E-08
342438	10.56										
280816	8.66										
RLU	E ₂ max										
1127130	34.75	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/ml		
697005	21.49	intercept	26.11	EC20	0.450727378	REP 20	2.97E-06	2.97E-03	1.48E-06		
580559	17.90	slope	17.66	EC50	22.52680434	REP 50	8.19E-07	8.19E-04	4.09E-07		
446843	13.78	r ²	0.90	EC80	1125.862193	REP 80	2.26E-07	2.26E-04	1.13E-07		
390352	12.04										
258246	7.96										

8.2.1.3. Inhibition calculations of September 2012 retrieved from Microsoft Excel 2010

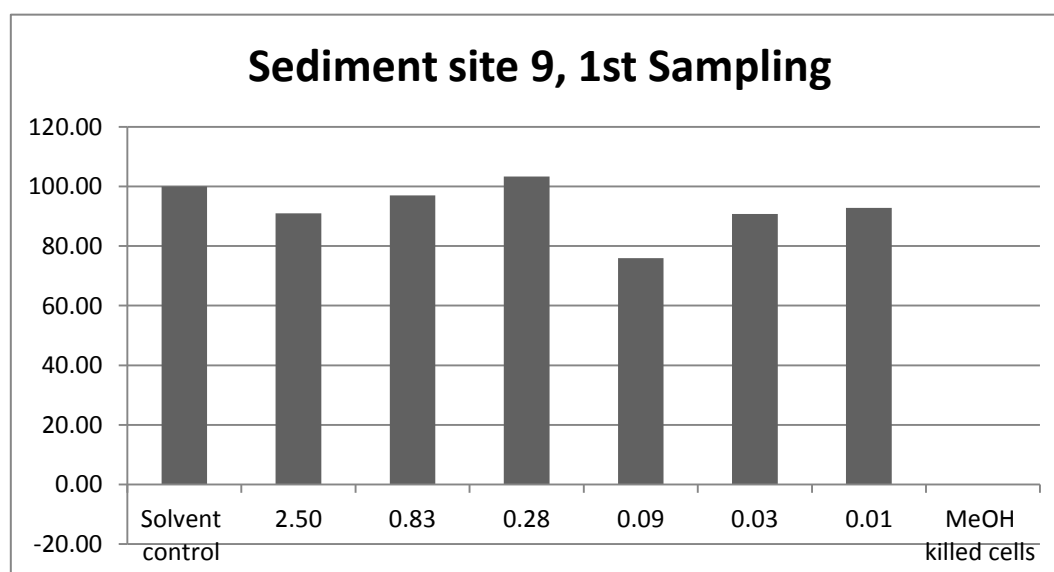
Solvent control	mean	stdev	cv						
557002	736066.5	253235	34.4039						
915131									
1429900									
Sediment8 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	Fold inhibition	
2.5	3.98E-01	358460	248309	486568	303385	77889	25.67	2.43	
0.83	-7.92E-02	1223775	1209776	1091881	1175144	72447	6.16	0.63	
0.28	-5.56E-01	1304714	1549300	1693042	1515685	196334	12.95		
0.09	-1.03E+00	1440395	1802246	1736376	1659672	192735	11.61		
0.03	-1.51E+00	1527694	1588984	1475524	1530734	56791	3.71		
0.01	-1.99E+00	1283714	1382439	1420416	1362190	70565	5.18		
Sediment 9 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	Fold inhibition	
2.5	3.98E-01	1249193	1633314	2103673	1662060	427965	25.75	0.44	
0.83	-7.92E-02	2102993	1934708	2553575	2197092	319984	14.56	0.34	
0.28	-5.56E-01	2418653	2410723	2517759	2449045	59640	2.44		
0.09	-1.03E+00	2015322	2118562	2395251	2176378	196452	9.03		
0.03	-1.51E+00	2492657	2507708	2379495	2459953	70084	2.85		
0.01	-1.99E+00	1799811	1687167	1878062	1788347	95962	5.37		

Solvent control	mean	stdev	cv						
1615842	1562450	75508	4.833						
1509058									
1457341									
H2O 8	log	RLU	RLU	RLU	mean	stdev	cv	Fold inhibition	
2.5	3.98E-01	2E+06	2E+06	2E+06	2E+06	3E+05	14.15	1.81	
0.83	-7.92E-02	2E+06	2E+06	2E+06	2E+06	86352	5.148	0.93	
0.28	-5.56E-01	2E+06	2E+06	2E+06	2E+06	1E+05	7.654		
0.09	-1.03E+00	2E+06	2E+06	2E+06	2E+06	1E+05	8.621		
0.03	-1.51E+00	1E+06	2E+06	2E+06	2E+06	3E+05	14.01		

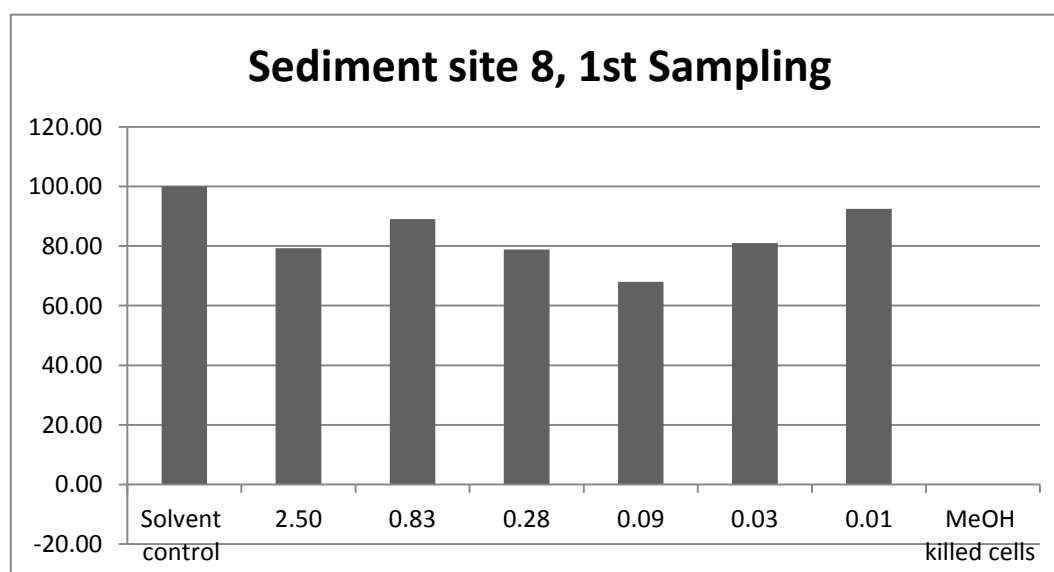
0.01	-1.99E+00	2E+06	1E+06	1E+06	2E+06	1E+05	8.395	
H2O 9	log	RLU	RLU	RLU	mean	stdev	cv	Fold inhibition
2.5	3.98E-01	3E+06	2E+06	2E+06	2E+06	1E+05	6.098	0.65
0.83	-7.92E-02	2E+06	2E+06	2E+06	2E+06	2E+05	10.68	0.84
0.28	-5.56E-01	2E+06	2E+06	2E+06	2E+06	2E+05	8.076	
0.09	-1.03E+00	2E+06	2E+06	2E+06	2E+06	51754	2.617	
0.03	-1.51E+00	2E+06	2E+06	2E+06	2E+06	2E+05	9.998	
0.01	-1.99E+00	2E+06	2E+06	2E+06	2E+06	2E+05	10.39	

8.2.1.4. MTT calculations of September 2012 retrieved from Microsoft Excel 2010.

Absorbance reading from plate reader				Site 9 Sediment			
Raw data	1	2	3				
Solvent control	0.271	0.286	0.309				
2.50	0.254	0.26	0.289				
0.83	0.282	0.277	0.286				
0.28	0.298	0.299	0.292				
0.09	0.213	0.259	0.227				
0.03	0.266	0.245	0.291				
0.01	0.261	0.278	0.277				
MeOH killed cells	0.042	0.068	0.062				
MeOH killed cells subtracted							
Solvent control	0.214	0.229	0.252				
2.50	0.197	0.203	0.232				
0.83	0.225	0.220	0.229				
0.28	0.241	0.242	0.235				
0.09	0.156	0.202	0.170				
0.03	0.209	0.188	0.234				
0.01	0.204	0.221	0.220				
MeOH killed cells	-0.015	0.011	0.005				
% of live cells	1	2	3	Average	s.d.	CV	Ttest
Solvent control	92.36	98.85	108.7	100.00	8.27	8.27	1.00
2.50	85.01	87.61	100.1	90.92	8.09	8.90	0.25
0.83	97.12	94.96	98.85	96.97	1.95	2.01	0.57
0.28	104.03	104.47	101.4	103.31	1.64	1.58	0.53
0.09	67.29	87.18	73.34	75.94	10.19	13.42	0.03
0.03	90.20	81.12	101.0	90.78	9.95	10.97	0.28
0.01	88.04	95.39	94.96	92.80	4.12	4.44	0.25
MeOH killed cells	-6.63	4.44	1.85	-0.11	5.79	2.0	0.00



Absorbance reading from plate reader		Site 8 Sediment		
Raw data	1	2	3	
Solvent control	0.271	0.286	0.309	
2.50	0.246	0.228	0.248	
0.83	0.246	0.265	0.279	
0.28	0.229	0.215	0.275	
0.09	0.228	0.212	0.204	
0.03	0.232	0.262	0.24	
0.01	0.254	0.274	0.286	
MeOH killed cells	0.042	0.068	0.062	
MeOH killed cells subtracted				
Solvent control	0.214	0.229	0.252	
2.50	0.189	0.171	0.191	
0.83	0.189	0.208	0.222	
0.28	0.172	0.158	0.218	
0.09	0.171	0.155	0.147	
0.03	0.175	0.205	0.183	
0.01	0.197	0.217	0.229	
MeOH killed cells	-0.015	0.011	0.005	
% of live cells	1	2	3	Average s.d. CV Ttest
Solvent control	92.36	98.85	108.79	100.00 8.27 8.3 1.00
2.50	81.56	73.78	82.42	79.25 4.76 6.0 0.02
0.83	81.56	89.77	95.82	89.05 7.16 8.0 0.16
0.28	74.21	68.16	94.09	78.82 13.57 17.2 0.08
0.09	73.78	66.86	63.40	68.01 5.28 7.8 0.00
0.03	75.50	88.47	78.96	80.98 6.72 8.3 0.04
0.01	85.01	93.66	98.85	92.51 6.99 7.6 0.30
MeOH killed cells	-6.63	4.61	2.02	0.00 5.88 -3.06E+17 0.00



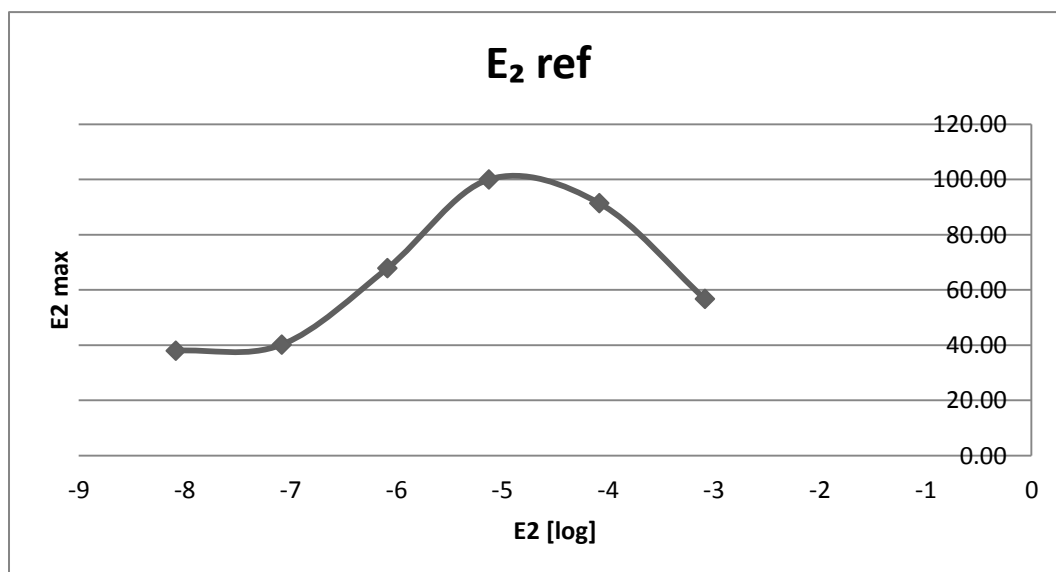
8.2.2. 2nd Sampling event

8.2.2.1. Reference linear curve as for 2nd sampling event April 2013.

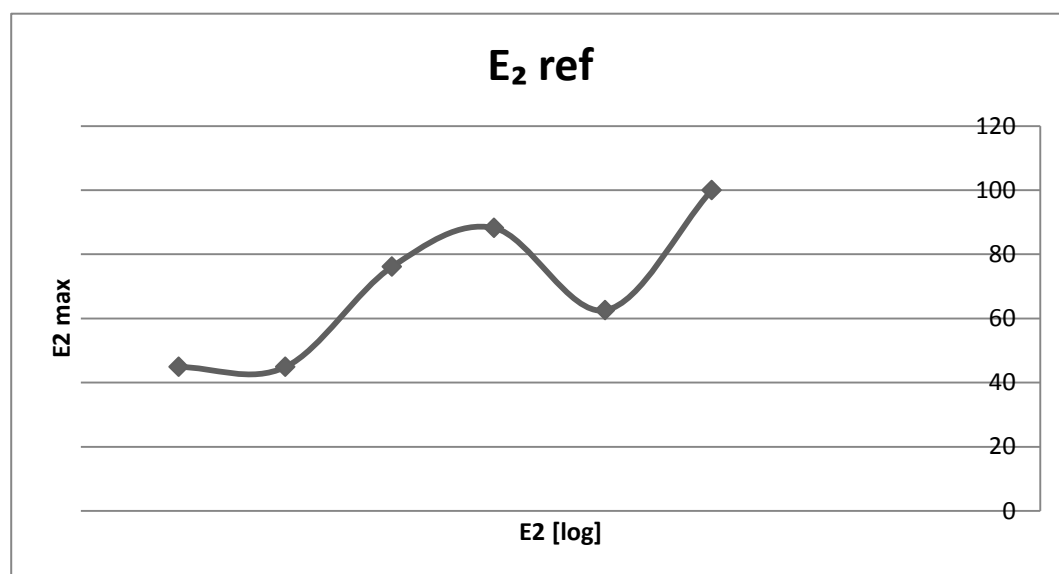
Sediment samples from the 2nd sampling season April 2013 were compared to the reference compound linear curve as indicated below.

During these tests the more samples that were involved the standards were dosed

E ₂ (µg)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	2161977	642472	512034	577253	92233.59432	15.98	56.66
8.25E-05	8.25E-11	3.03E-13	-4.083546051	890878	971485	658964	931181.5	56997.75631	6.12	91.41
7.50E-06	7.50E-12	2.75E-14	-5.124938737	1164670	935466	956077	1018737.667	126800.5827	12.45	100.00
8.25E-07	8.25E-13	3.03E-15	-6.083546051	730677	708594	634760	691343.6667	50231.44655	7.27	67.86
8.25E-08	8.25E-14	3.03E-16	-7.083546051	383603	360491	483711	409268.3333	65496.74878	16.00	40.17
8.25E-09	8.25E-15	3.03E-17	-8.083546051	387568	386225	394600	386896.5	949.6444071	0.25	37.98



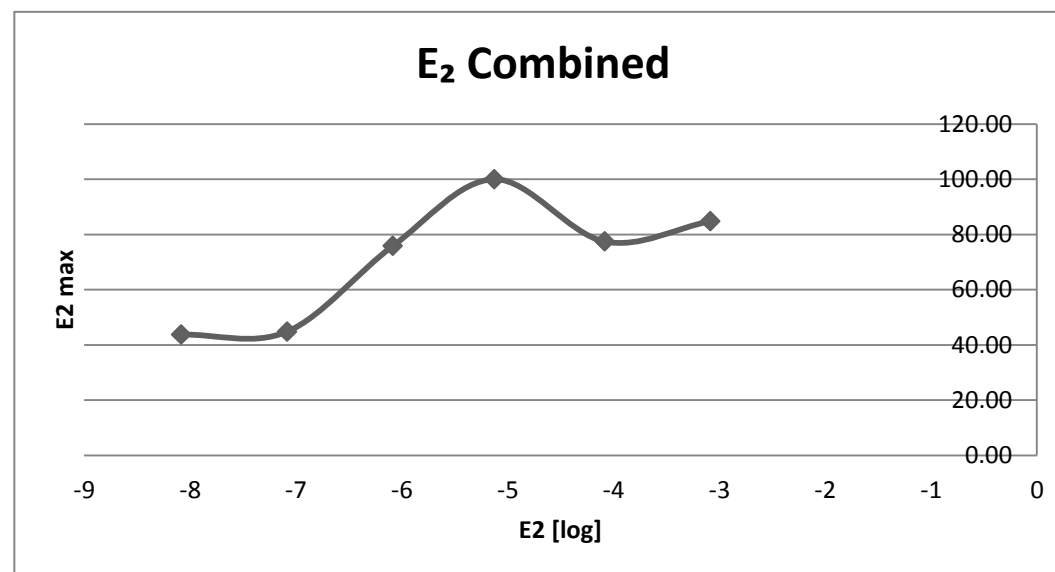
E ₂ (µg)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	1069214	878683	723475	890457.3333	173169.975	19.44730741	100
8.25E-05	8.25E-11	3.03E-13	-4.083546051	605677	566956	498123	556918.6667	54475.01055	9.781502006	62.54299289
7.50E-06	7.50E-12	2.75E-14	-5.124938737	916733	790592	649075	785466.6667	133902.5878	17.04752009	88.20935459
8.25E-07	8.25E-13	3.03E-15	-6.083546051	817409	606324	608517	677416.6667	121241.8754	17.8976812	76.07514041
8.25E-08	8.25E-14	3.03E-16	-7.083546051	371157	417104	409910	399390.3333	24713.94834	6.187918503	44.85227067
8.25E-09	8.25E-15	3.03E-17	-8.083546051	437472	405816	355474	399587.3333	41352.33025	10.34875903	44.87439413



E ₂ (µg/well)	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.08355	2161977	642472	512034	1069214	878683	723475	765175.6	215753.9	28.20	84.82
8.25E-05	8.25E-11	3.03E-13	-4.08355	890878	971485	658964	605677	566956	498123	698680.5	189295.4	27.09	77.45
7.50E-06	7.50E-12	2.75E-14	-5.12494	1164670	935466	956077	916733	790592	649075	902102	172996.8	19.18	100.00
8.25E-07	8.25E-13	3.03E-15	-6.08355	730677	708594	634760	817409	606324	608517	684380	83350.4	12.18	75.87
8.25E-08	8.25E-14	3.03E-16	-7.08355	383603	360491	483711	371157	417104	409910	404329.3	44603.9	11.03	44.82
8.25E-09	8.25E-15	3.03E-17	-8.08355	387568	386225	394600	437472	405816	355474	394525.83	26885.7	6.81	43.73

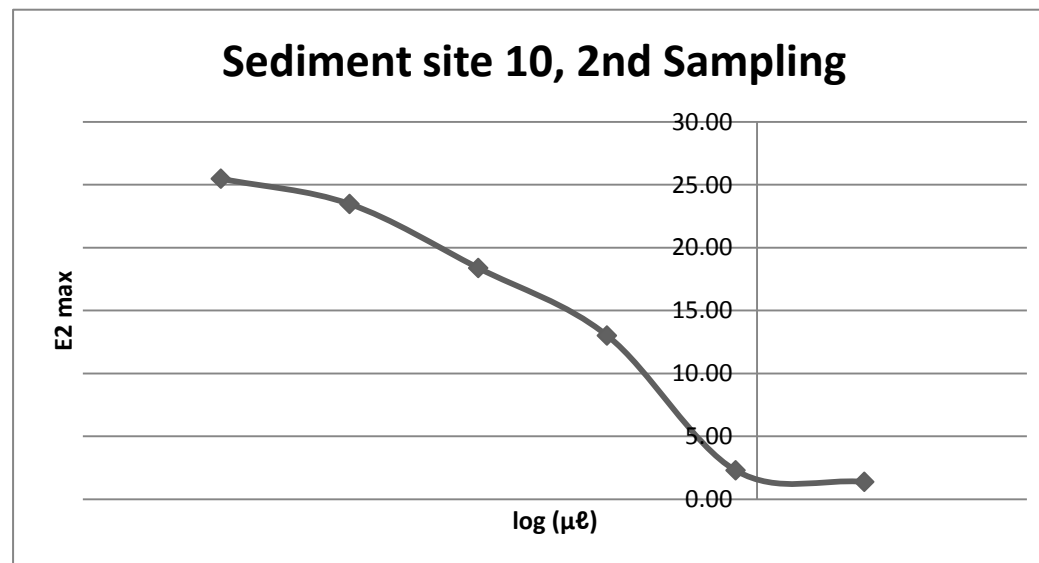
intercept 117.63
 slope 8.06
 r2 0.40

	µg/well
EC20	7.72E-13
EC50	4.07E-09
EC80	2.14E-05



8.2.2.2. Activation calculations of April 2013 retrieved from Microsoft Excel 2010

Sediment 10 ($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	11993	12794	17350	12393.5	566.3925	4.570077313	1.37
0.83	-7.92E-02	22488	18628	92397	20558	2729.432	13.27673984	2.28
0.28	-5.56E-01	58045	93805	140942	117373.5	33330.89	28.39728929	13.01
0.09	-1.03E+00	137058	178943	181241	165747.3	24872.25	15.00612094	18.37
0.03	-1.51E+00	193428	248309	193209	211648.7	31748.97	15.00078848	23.46
0.01	-1.99E+00	214872	192076	282434	229794	46990.87	20.44912715	25.47



Mass sediment extracted
concentrated to 1 ml

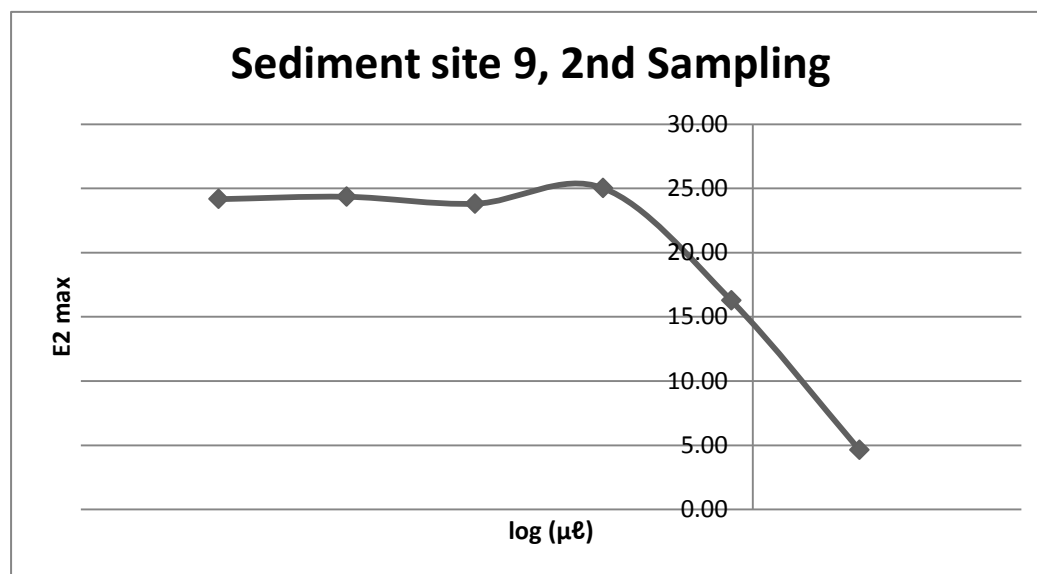
20.15 g

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration indicates that sample could be cytotoxic

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

Sediment 9 (μg)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	35185	48246	71656	41715.5	9235.521669	22.13930474	4.62
0.83	-7.92E-02	126271	167063	236138	146667	28844.29982	19.66652336	16.26
0.28	-5.56E-01	190377	219226	267455	225686	20399.32353	9.038807693	25.02
0.09	-1.03E+00	192198	208568	243559	214775	11575.33801	5.389518337	23.81
0.03	-1.51E+00	223353	219452	216175	219660	2758.423553	1.255769623	24.35
0.01	-1.99E+00	215358	216361	222188	217969	709.2281015	0.325380261	24.16



Mass sediment extracted
concentrated to 1ml

20.1

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration indicates that sample could be cytotoxic

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

Sediment 8 pump (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	233209	503185	503707	503446	369.1097398	0.07331665	55.81
0.83	-7.92E-02	360953	215714	432625	396789	50679.75722	12.77247031	43.98
0.28	-5.56E-01	338659	341904	443833	374798.6667	59807.49861	15.95723356	41.55
0.09	-1.03E+00	292638	294508	416028	334391.3333	70705.60963	21.14457002	37.07
0.03	-1.51E+00	262244	294694	363502	306813.3333	51705.45737	16.85241538	34.01
0.01	-1.99E+00	258190	267528	347560	291092.6667	49124.52912	16.87590748	32.27

y=mx+c

intercept 48.30

slope 14.94

r2 0.87

μℓ/well

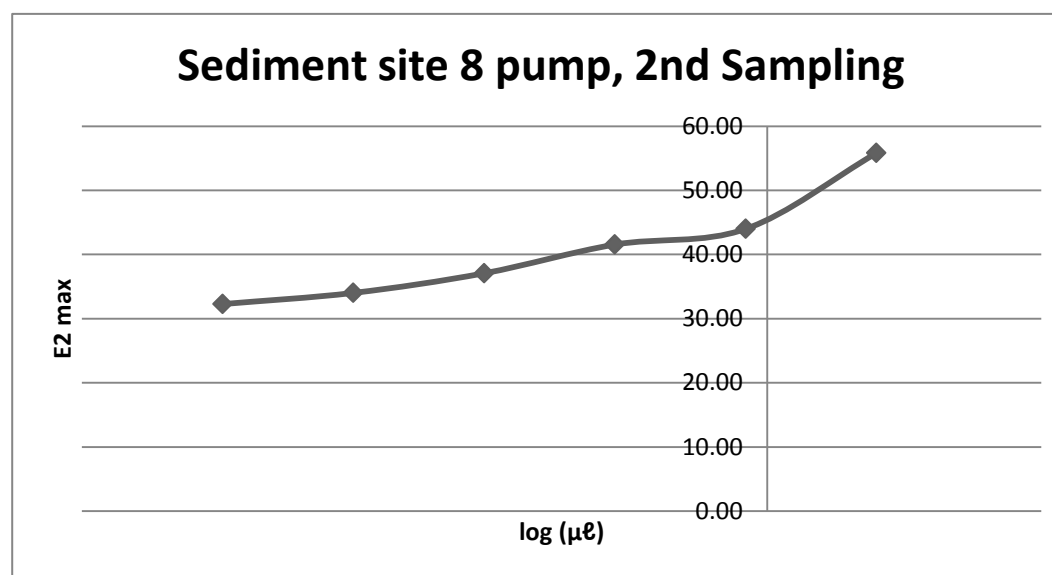
EC20 1.28E-02

EC50 1.30E+00

EC80 1.32E+02

	μg/μℓ	μg/mℓ
REP20	6.04E-11	6.04E-08
REP50	3.13E-09	3.13E-06
REP80	1.62E-07	1.62E-04

	E ₂ eq μg/g
REP20	3.01E-09
REP50	1.56E-07
REP80	8.08E-06



Mass sediment extracted 20.01g
concentrated to 1ml

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
233209	25.85	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
360953	40.01	intercept	33.50	EC20	12.64517492	REP 20	6.10E-14	6.10E-11	3.04E-12	Mean	STEDV
338659	37.54	slope	-12.25	EC50	0.044969986	REP 50	9.04E-08	9.04E-05	4.51E-06	1.74E-09	1.60E-09
292638	32.44	r2	0.60	EC80	0.000159927	REP 80	1.34E-01	1.34E+02	6.68E+00		
262244	29.07									REP50	
258190	28.62									Mean	STEDV
RLU	E ₂ max									7.08E-07	3.81E-06
974916	125.69	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
880747	113.55	intercept	110.35	EC20	0.012251804	REP 20	6.30E-11	6.30E-08	3.14E-09	REP80	
625114	80.59	slope	47.26	EC50	0.052843342	REP 50	7.70E-08	7.70E-05	3.84E-06	Mean	STEDV
473021	60.98	r2	0.93	EC80	0.227918994	REP 80	9.40E-05	9.40E-02	4.69E-03	2.23	3.86
363672	46.89										
340819	43.94										
RLU	E ₂ max										
1062886	137.03	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
843062	108.69	intercept	114.55	EC20	0.018466413	REP 20	4.18E-11	4.18E-08	2.08E-09		
659191	84.98	slope	54.54	EC50	0.065528274	REP 50	6.21E-08	6.21E-05	3.10E-06		
506248	65.27	r2	1.00	EC80	0.232527817	REP 80	9.22E-05	9.22E-02	4.60E-03		
385464	49.69										
1369168	176.52										

Sediment 8.1 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	948284	938145	762755	883061.3333	104311.6015	11.81249791	99.17
0.83	-7.92E-02	711539	927730	775662	804977	111036.7689	13.79378154	90.40
0.28	-5.56E-01	716103	771357	664596	717352	53391.45794	7.44285343	80.56
0.09	-1.03E+00	464945	521866	533211	506674	36580.86026	7.219802132	56.90
0.03	-1.51E+00	428296	509841	400281	446139.3333	56917.80748	12.7578546	50.10
0.01	-1.99E+00	327605	340698	332962	334151.5	9258.149086	2.77064418	37.53

y=mx+c

intercept 91.59

slope 19.50

r2 1.00

μℓ/well

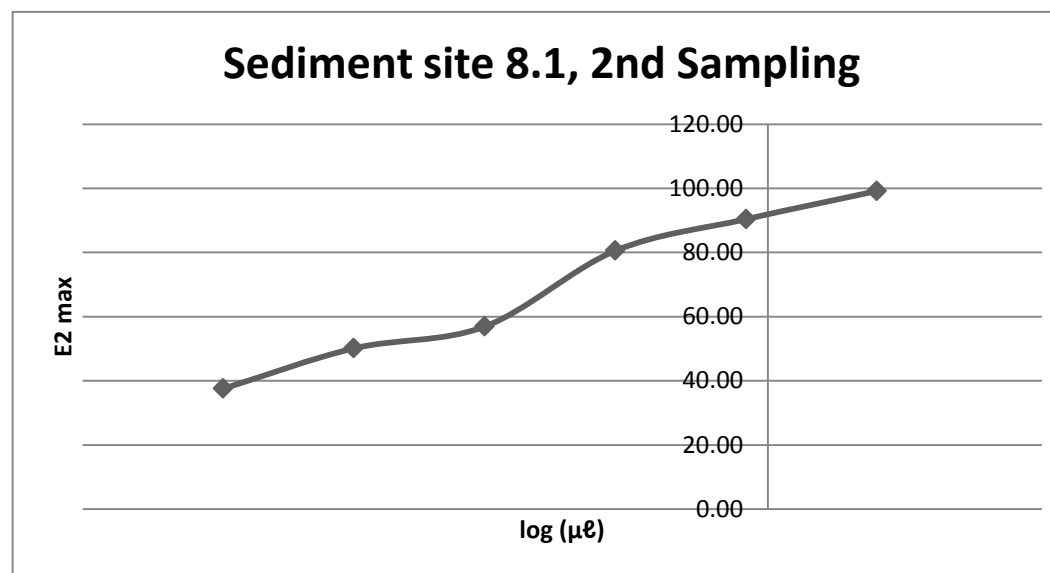
EC20 2.13E-04

EC50 7.37E-03

EC80 2.55E-01

	μg/μℓ	μg/mℓ
REP20	3.62E-09	3.62E-06
REP50	5.52E-07	5.52E-04
REP80	8.42E-05	8.42E-02

	E ₂ eq μg/g
REP20	1.79E-07
REP50	2.74E-05
REP80	4.18E-03



Mass sediment extracted
concentrated to 1ml

20.15g

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
948284	105.12	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
711539	78.88	intercept	89.93	EC20	0.002554962	REP 20	3.02E-10	3.02E-07	1.50E-08	Mean	STEDV
716103	79.38	slope	26.97	EC50	0.033086552	REP 50	1.23E-07	1.23E-04	6.10E-06	6.76E-06	1.12E-05
464945	51.54	r ²	0.74	EC80	0.428468143	REP 80	5.00E-05	5.00E-02	2.48E-03		
428296	47.48									REP50	
327605	36.32									Mean	STEDV
RLU	E ₂ max									2.90E-04	2.43E-04
938145	120.95	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
927730	119.60	intercept	115.12	EC20	6.01063E-05	REP 20	1.28E-08	1.28E-05	6.37E-07	REP80	
771357	99.44	slope	22.53	EC50	0.001289009	REP 50	3.15E-06	3.15E-03	1.57E-04	Mean	STEDV
521866	67.28	r ²	0.80	EC80	0.027643447	REP 80	7.75E-04	7.75E-01	3.85E-02	0.019	0.018
509841	65.73										
340698	43.92										
RLU	E ₂ max										
762755	98.34	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
775662	100.00	intercept	95.72	EC20	1.95045E-06	REP 20	3.96E-07	3.96E-04	1.96E-05		
664596	85.68	slope	13.26	EC50	0.000356689	REP 50	1.14E-05	1.14E-02	5.66E-04		
533211	68.74	r ²	0.65	EC80	0.065229713	REP 80	3.29E-04	3.29E-01	1.63E-02		
400281	51.61										
332962	42.93										

Sediment 8.2 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	828164	1159281	986334	1072807.5	122291.9965	11.39924884	118.92
0.83	-7.92E-02	629662	634348	686057	650022.3333	31294.76874	4.814414387	72.06
0.28	-5.56E-01	418998	504330	500494	474607.3333	48197.27367	10.15518941	52.61
0.09	-1.03E+00	396914	407183	465034	423043.6667	36725.3885	8.681228769	46.90
0.03	-1.51E+00	319302	350263	397618	355727.6667	39442.94487	11.08796098	39.43
0.01	-1.99E+00	296660	322312	375559	331510.3333	40245.74577	12.14011804	36.75

y=mx+c

intercept 86.70

slope 69.49

r2 0.95

μℓ/well

EC20 1.10E-01

EC50 2.96E-01

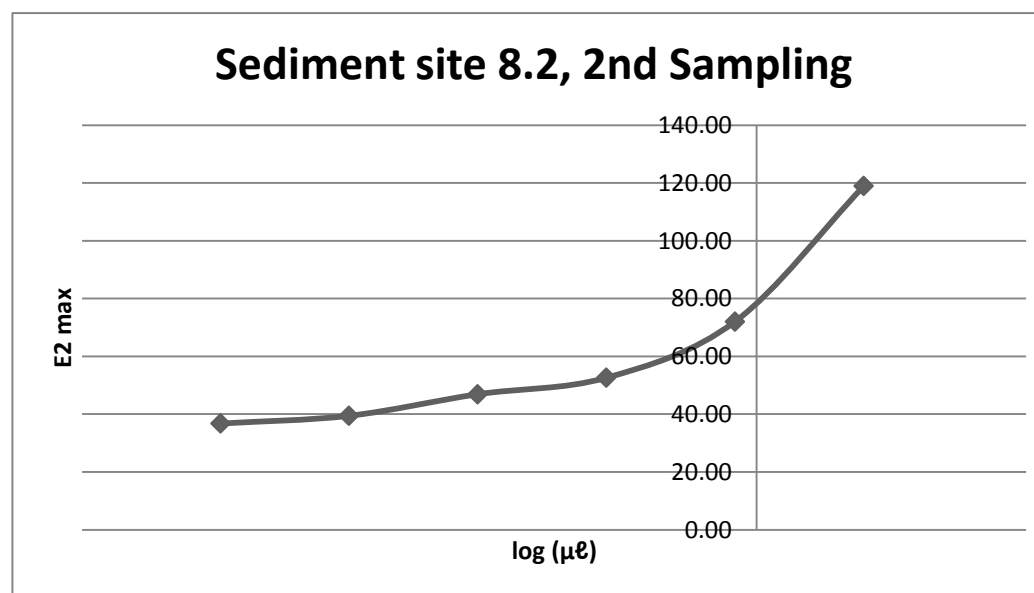
EC80 8.01E-01

	μg/μℓ	μg/mℓ
REP20	7.04E-12	7.04E-09
REP50	1.37E-08	1.37E-05
REP80	2.68E-05	2.68E-02

	E ₂ eq μg/g
REP20	3.49E-10
REP50	6.81E-07
REP80	1.33E-03

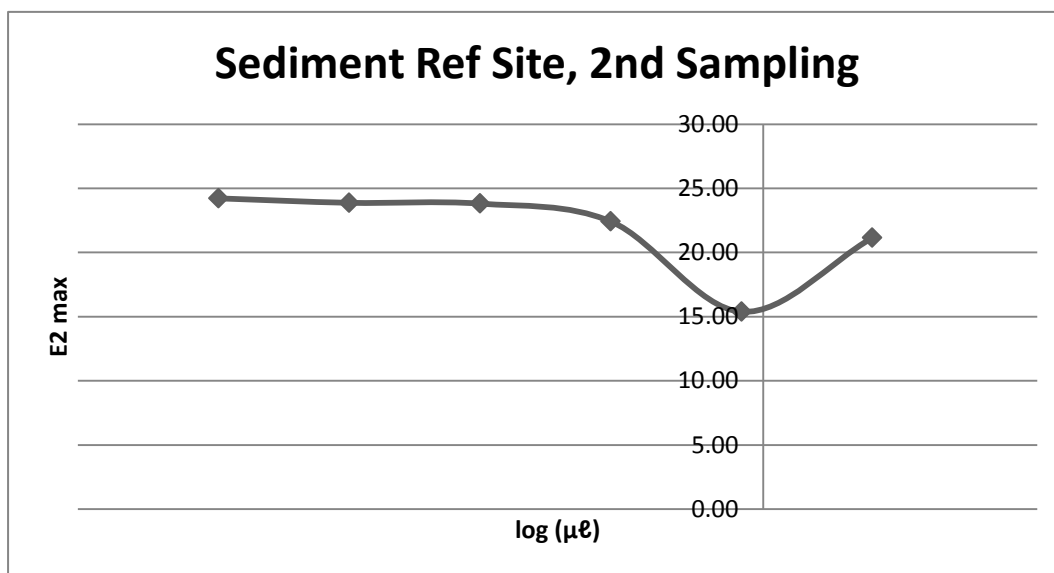
Mass sediment extracted
concentrated to 1ml

20.15g



Standard deviation and Mean of each individual RLU											
RLU	E2max										
828164	91.80	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
629662	69.80	intercept	73.11	EC20	0.076307639	REP 20	1.01E-11	1.01E-08	5.02E-10	Mean	STEDV
418998	46.45	slope	47.53	EC50	0.3263798	REP 50	1.25E-08	1.25E-05	6.18E-07	4.87E-10	1.24E-10
396914	44.00	r2	1.00	EC80	1.395977843	REP 80	1.54E-05	1.54E-02	7.62E-04		
319302	35.40									REP50	
296660	32.89									Mean	STEDV
RLU	E2max									2.45E-07	8.63E-07
1159281	149.46	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
634348	81.78	intercept	105.76	EC20	0.107355488	REP 20	7.19E-12	7.19E-09	3.57E-10	REP80	
504330	65.02	slope	88.49	EC50	0.234346577	REP 50	1.74E-08	1.74E-05	8.61E-07	Mean	STEDV
407183	52.49	r2	0.89	EC80	0.51155576	REP 80	4.19E-05	4.19E-02	2.08E-03	1.63E-03	7.50E-04
350263	45.16										
322312	41.55										
RLU	E2max										
986334	127.16	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
686057	88.45	intercept	98.58	EC20	0.063521627	REP 20	1.21E-11	1.21E-08	6.03E-10		
500494	64.52	slope	65.64	EC50	0.181956229	REP 50	2.24E-08	2.24E-05	1.11E-06		
465034	59.95	r2	0.98	EC80	0.521209403	REP 80	4.11E-05	4.11E-02	2.04E-03		
397618	51.26										
375559	48.42										

Sediment Ref ($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	181977	218303	171967	190749	24381.6983	12.78208447	21.14
0.83	-7.92E-02	129224	159829	127177	138743.3333	18289.38371	13.18217118	15.38
0.28	-5.56E-01	183628	218635	204498	202253.6667	17611.08419	8.707423939	22.42
0.09	-1.03E+00	212202	235912	196203	214772.3333	19978.89212	9.302358367	23.81
0.03	-1.51E+00	211126	240840	194148	215371.3333	23633.72288	10.97347661	23.87
0.01	-1.99E+00	216240	249191	190053	218494.6667	29633.40011	13.56252789	24.22



Mass sediment extracted
concentrated to 1ml

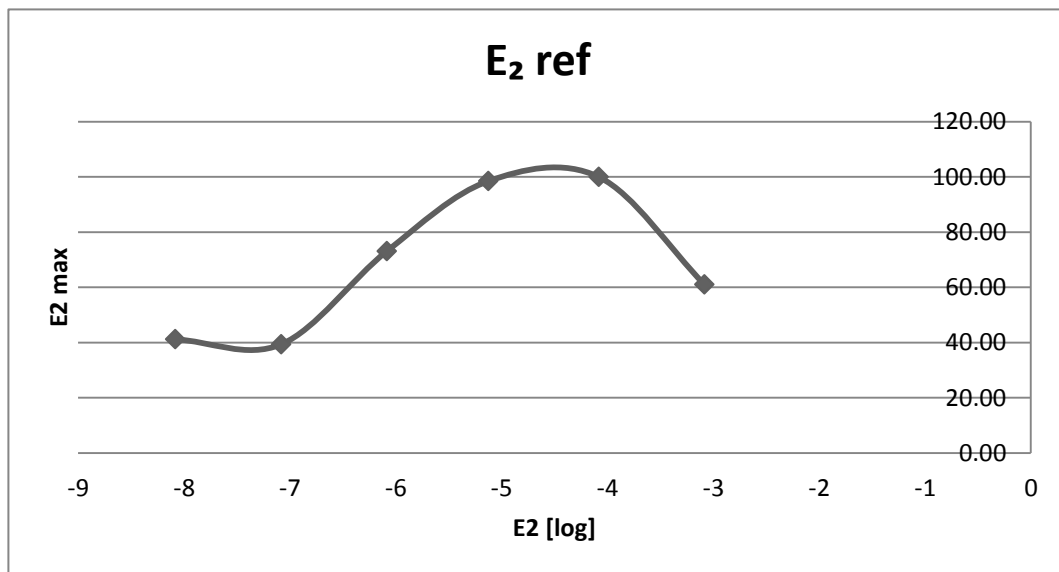
20.1

No linear gradient thus a response occurred which cannot be quantified by the cells

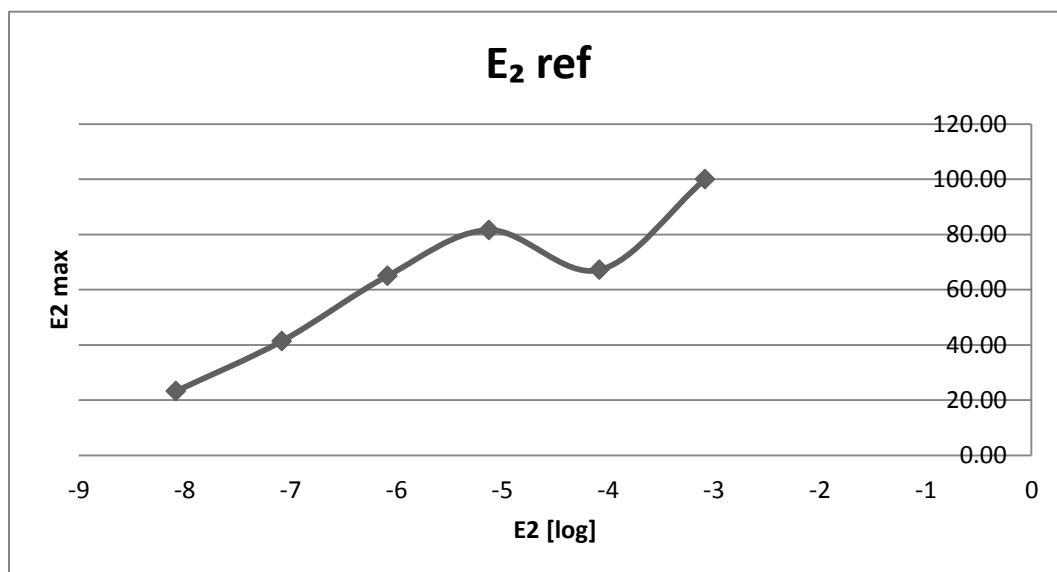
Reference linear curve as for 2nd sampling event April 2013.

Water samples from the 2nd sampling event April 2013 were compared to the reference compound linear curve as indicated below.

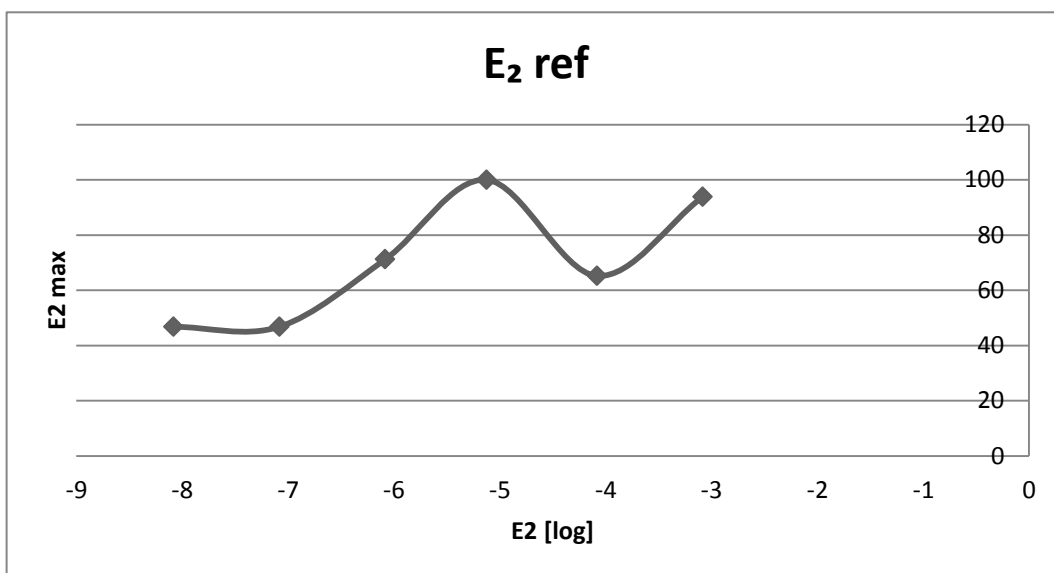
E ₂ (µg/)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	2161977	642472	512034	577253	92233.59432	15.97801905	61.04
8.25E-05	8.25E-11	3.03E-13	-4.083546051	1164670	935466	956077	945771.5	14574.17787	1.540982982	100.00
7.50E-06	7.50E-12	2.75E-14	-5.124938737	890878	971485	658964	931181.5	56997.75631	6.12101468	98.46
8.25E-07	8.25E-13	3.03E-15	-6.083546051	730677	708594	634760	691343.6667	50231.44655	7.265770842	73.10
8.25E-08	8.25E-14	3.03E-16	-7.083546051	383603	360491	483711	372047	16342.65193	4.392631019	39.34
8.25E-09	8.25E-15	3.03E-17	-8.083546051	387568	386225	394600	389464.3333	4498.023603	1.154925681	41.18



$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2 \text{ max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	1722303	1908367	2202713	1944461	242230.3132	12.4574529	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	1297576	1313461	2085757	1305518.5	11232.39122	0.86037779	67.14
7.50E-06	7.50E-12	2.75E-14	-5.124938737	1535050	1637441	2229377	1586245.5	72401.37043	4.564323141	81.58
8.25E-07	8.25E-13	3.03E-15	-6.083546051	1187676	1166046	1436859	1263527	150499.0063	11.91102416	64.98
8.25E-08	8.25E-14	3.03E-16	-7.083546051	812093	533227	795730	803911.5	11570.38826	1.439261444	41.34
8.25E-09	8.25E-15	3.03E-17	-8.083546051	507697	419686	427058	451480.3333	48824.39763	10.81429113	23.22



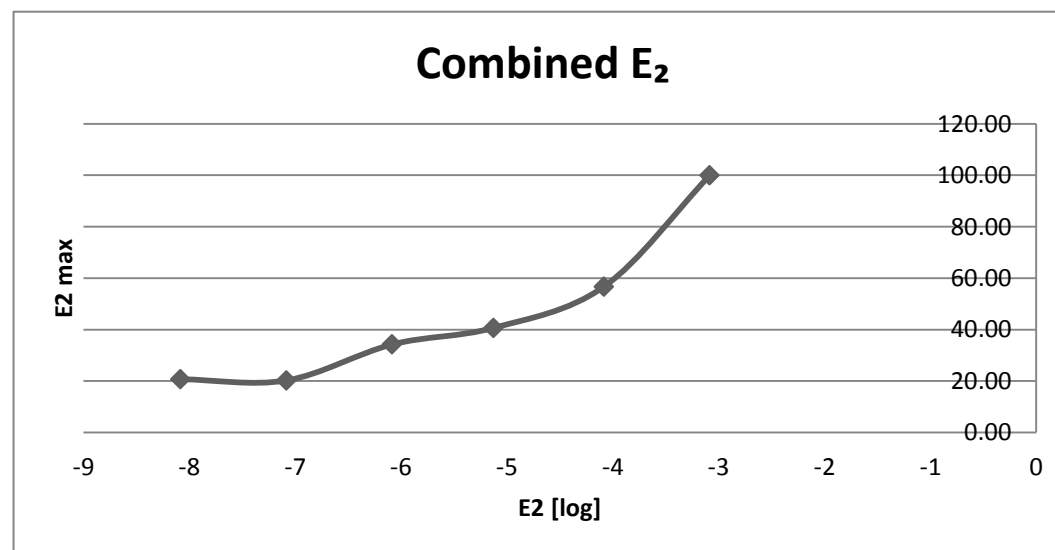
E ₂ (µg)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	1069214	878683	723475	801079	109748.6293	13.70010065	93.84024717
8.25E-05	8.25E-11	3.03E-13	-4.083546051	605677	566956	498123	556918.6667	54475.01055	9.781502006	65.23874092
7.50E-06	7.50E-12	2.75E-14	-5.124938737	916733	790592	649075	853662.5	89195.15649	10.44852696	100
8.25E-07	8.25E-13	3.03E-15	-6.083546051	817409	606324	608517	607420.5	1550.685171	0.255290227	71.1546425
8.25E-08	8.25E-14	3.03E-16	-7.083546051	371157	417104	409910	399390.3333	24713.94834	6.187918503	46.78550754
8.25E-09	8.25E-15	3.03E-17	-8.083546051	437472	405816	355474	399587.3333	41352.33025	10.34875903	46.80858458



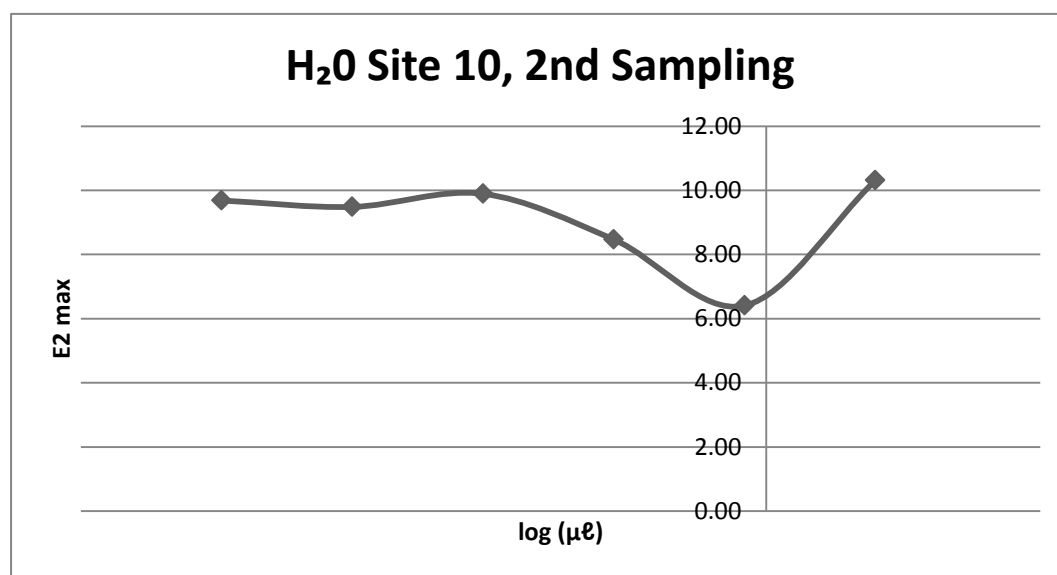
E ₂ (µg/)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.08	2161977	642472	512034	1069214	878683	723475	1722303	1908367	2202713	1998840	225710	11.2	100.0
8.25E-05	8.25E-11	3.03E-13	-4.08	1164670	935466	956077	605677	566956	498123	1297576	1313461	2085757	1133450	180952	15.9	56.7
7.50E-06	7.50E-12	2.75E-14	-5.12	890878	971485	658964	916733	790592	649075	1535050	1637441	2229377	812954	136413	16.7	40.6
8.25E-07	8.25E-13	3.03E-15	-6.08	730677	708594	634760	817409	606324	608517	1187676	1166046	1436859	684380	83350	12.1	34.2
8.25E-08	8.25E-14	3.03E-16	-7.08	383603	360491	483711	371157	417104	409910	812093	533227	795730	404329	44603	11.0	20.2
8.25E-09	8.25E-15	3.03E-17	-8.08	387568	386225	394600	437472	405816	355474	507697	419686	427058	413510	43112	10.4	20.6

intercept 143.24
 slope 18.24
 r2 0.88

	µg/well
EC20	1.75E-07
EC50	7.73E-06
EC80	3.41E-04



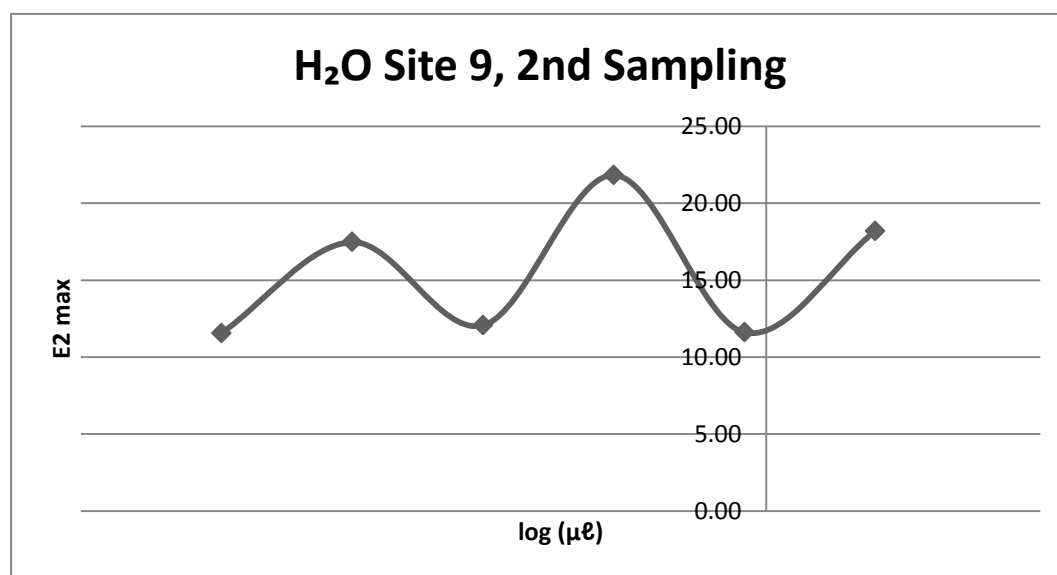
H ₂ O 10 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	213497	198704	294305	206100.5	10460.23061	5.08	10.31
0.83	-7.92E-02	110936	145198	262114	128067	24226.89254	18.92	6.41
0.28	-5.56E-01	167096	171417	250429	169256.5	3055.408402	1.81	8.47
0.09	-1.03E+00	189115	177875	226217	197735.6667	25297.71059	12.79	9.89
0.03	-1.51E+00	194731	184437	247613	189584	7278.957206	3.84	9.48
0.01	-1.99E+00	187626	199505	224858	193565.5	8399.721454	4.34	9.68



2ℓ water used in extraction
concentrated to 1mℓ

No linear gradient thus a response occurred which cannot be quantified by the cells

H ₂ O 9 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	512455	323769	403493	363631	56373.38102	15.50	18.19
0.83	-7.92E-02	264413	199497	2598	231955	45902.54381	19.79	11.60
0.28	-5.56E-01	583059	466507	405961	436234	42812.48717	9.81	21.82
0.09	-1.03E+00	322053	241924	240427	241175.5	1058.538851	0.44	12.07
0.03	-1.51E+00	340237	358056	263603	349146.5	12599.93573	3.61	17.47
0.01	-1.99E+00	297833	242111	219752	230931.5	15810.20052	6.85	11.55



2ℓ water used in extraction
concentrated to 1mℓ

No linear gradient thus a response occurred which cannot be quantified by the cells

H ₂ O 8 pump (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	372152	315029	301354	329511.6667	37555.29159	11.40	16.49
0.83	-7.92E-02	241811	499993	228928	470739	152916.1813	32.48	23.55
0.28	-5.56E-01	403364	444942	317522	388609.3333	64978.75992	16.72	19.44
0.09	-1.03E+00	207638	217607	207306	210850.3333	5853.799137	2.78	10.55
0.03	-1.51E+00	251894	224769	194027	223563.3333	28952.33404	12.95	11.18
0.01	-1.99E+00	235483	251538	237805	243510.5	11352.59937	4.66	12.18

y=mx+c

intercept 19.58

slope -3.10

r2 0.17

μℓ/well

EC20 7.32E-01

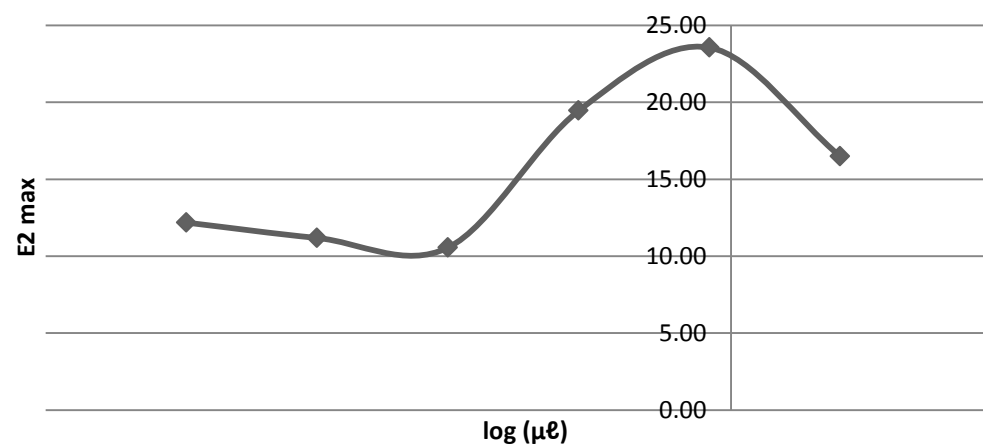
EC50 1.52E-10

EC80 3.16E-20

	μg/μℓ	μg/mℓ
REP20	2.39E-07	2.39E-04
REP50	5.08E+04	5.08E+07
REP80	1.08E+16	1.08E+19

	E ₂ eq μg/mℓ
REP20	1.20E-07
REP50	2.54E+04
REP80	5.40E+15

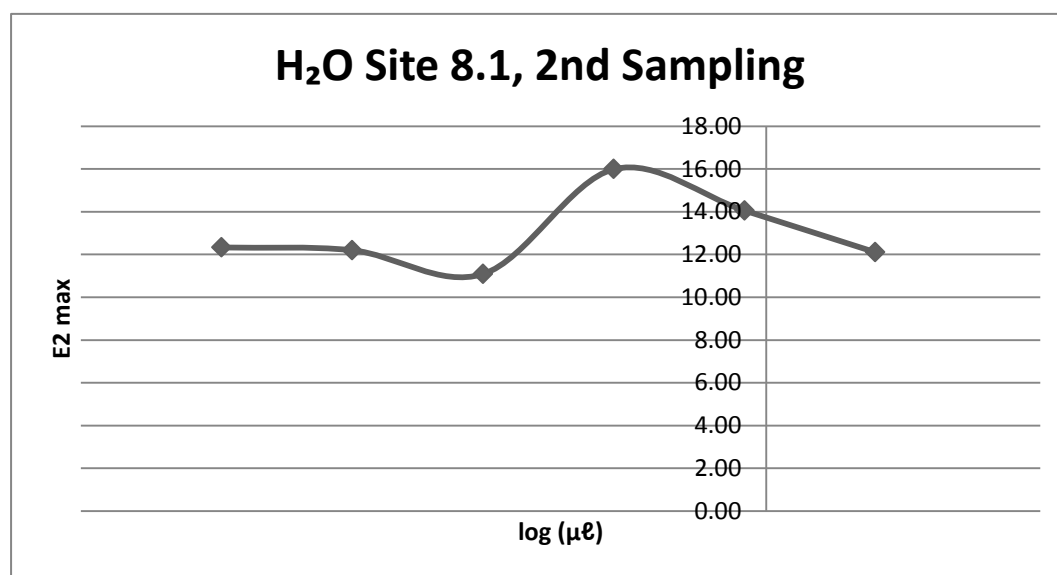
H₂O Site 8 pump, 2nd Sampling



2ℓ water used in extraction
concentrated to 1mℓ

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
372152	18.62	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/ml	REP20	
241811	12.10	intercept	16.84	EC20	0.01164917	REP 20	1.50E-05	1.50E-02	7.51E-06	Mean	STEDV
403364	20.18	slope	-1.64	EC50	5.40969E-21	REP 50	1.43E+15	1.43E+1	7.14E+4	0.29	0.50
207638	10.39	r2	0.03	EC80	2.51217E-39	REP 80	1.36E+35	1.36E+3	6.79E+3		
251894	12.60									REP50	
235483	11.78									Mean	STEDV
RLU	E ₂ max									5.43E+3	3.13E+3
315029	15.76	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/ml		
499993	25.01	intercept	20.47	EC20	1.173112225	REP 20	1.49E-07	1.49E-04	7.46E-08	REP80	
444942	22.26	slope	-6.81	EC50	4.62115E-05	REP 50	1.67E-01	1.67E+02	8.36E-02	Mean	STEDV
217607	10.89	r2	0.47	EC80	1.82037E-09	REP 80	1.87E+05	1.87E+08	9.37E+04	3.41E+3	5.90E+3
224769	11.24										
251538	12.58										
RLU	E ₂ max										
301354	15.08	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/ml		
228928	11.45	intercept	14.07	EC20	1.01296E-07	REP 20	1.73E+00	1.73E+03	8.64E-01		
317522	15.89	slope	-0.85	EC50	4.11021E-43	REP 50	1.88E+37	1.88E+4	9.40E+3		
207306	10.37	r2	0.03	EC80	1.66778E-78	REP 80	2.04E+74	2.04E+7	1.02E+7		
194027	9.71										
237805	11.90										

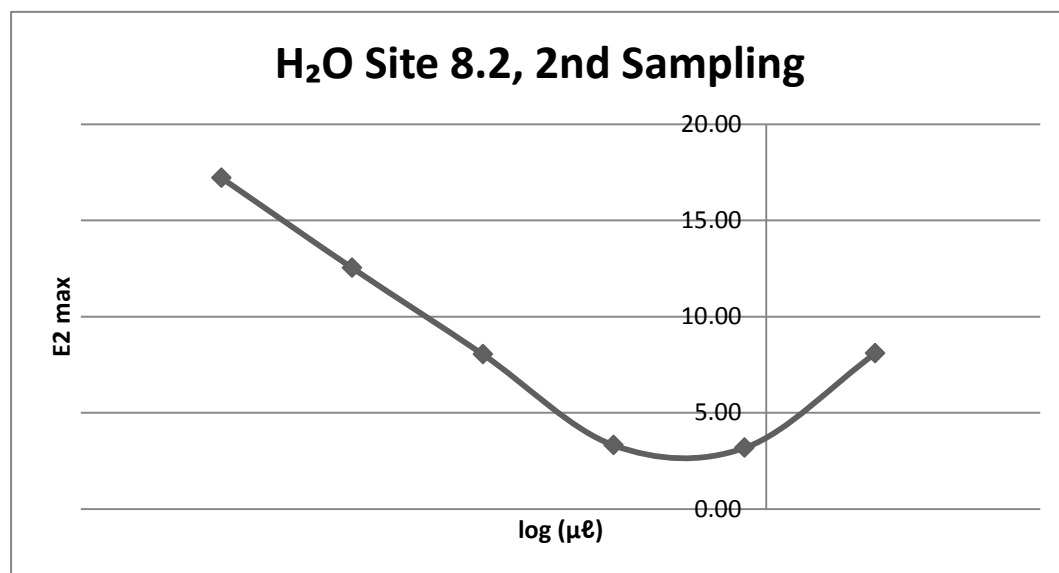
H ₂ O 8.1 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	259347	244465	222131	241981	18731.93412	7.74	12.11
0.83	-7.92E-02	243430	318436	440976	280933	53037.25123	18.88	14.05
0.28	-5.56E-01	302406	319512	336903	319607	17248.69621	5.40	15.99
0.09	-1.03E+00	205914	210171	248689	221591.3333	23563.59833	10.63	11.09
0.03	-1.51E+00	208690	263749	259072	243837	30527.89362	12.52	12.20
0.01	-1.99E+00	209531	250818	278971	246440	29194.31767	11.85	12.33



2ℓ water used in extraction
concentrated to 1mℓ

No Response

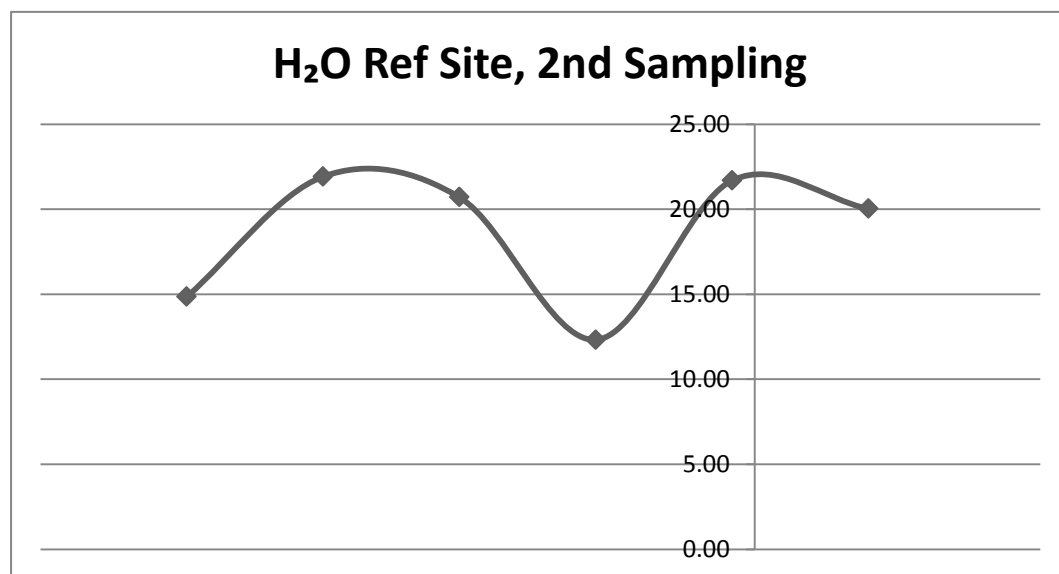
H ₂ O 8.2 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	140481	145991	198647	161706.3333	32109.96209	19.86	8.09
0.83	-7.92E-02	18515	53222	73817	63519.5	14562.86416	22.93	3.18
0.28	-5.56E-01	76301	56047	118389	66174	14321.74075	21.64	3.31
0.09	-1.03E+00	158211	162945	227059	160578	3347.443502	2.08	8.03
0.03	-1.51E+00	272278	233193	245809	250426.6667	19947.46702	7.97	12.53
0.01	-1.99E+00	324303	408794	363639	343971	27814.75234	8.09	17.21



2ℓ water used in extraction
concentrated to 1mℓ

No Response

H ₂ O Ref (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	295980	377671	422866	400268.5	31957.69098	7.98	20.03
0.83	-7.92E-02	263183	392998	473993	433495.5	57272.11374	13.21	21.69
0.28	-5.56E-01	234107	258497	437521	246302	17246.33439	7.00	12.32
0.09	-1.03E+00	311898	405662	421960	413811	11524.42632	2.78	20.70
0.03	-1.51E+00	301192	383813	492370	438091.5	76761.39085	17.52	21.92
0.01	-1.99E+00	261354	332266	746570	296810	50142.35607	16.89	14.85



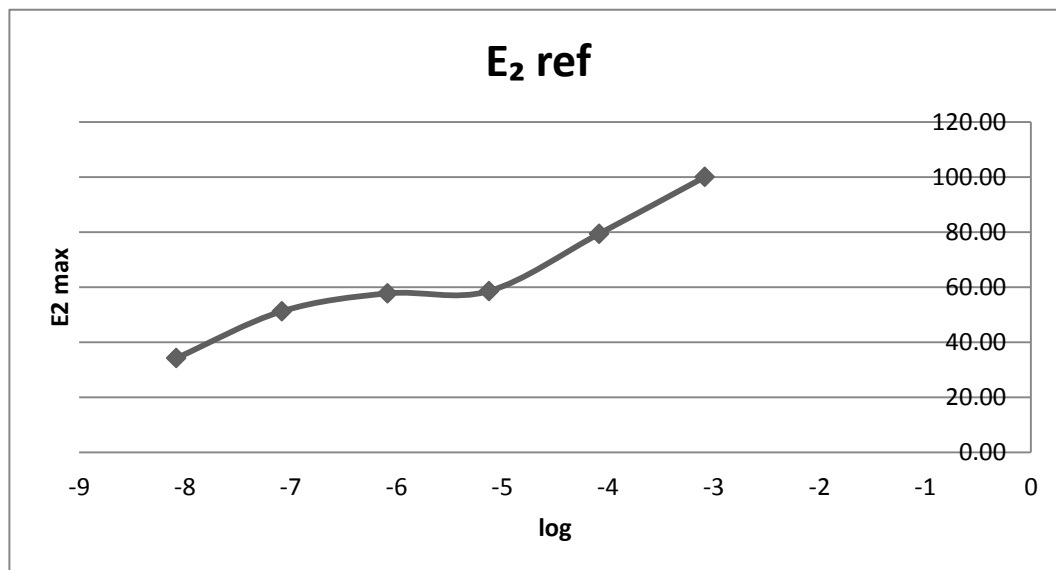
2ℓ water used in extraction
concentrated to 1mℓ

No linear gradient thus a response occurred which cannot be quantified by the cells

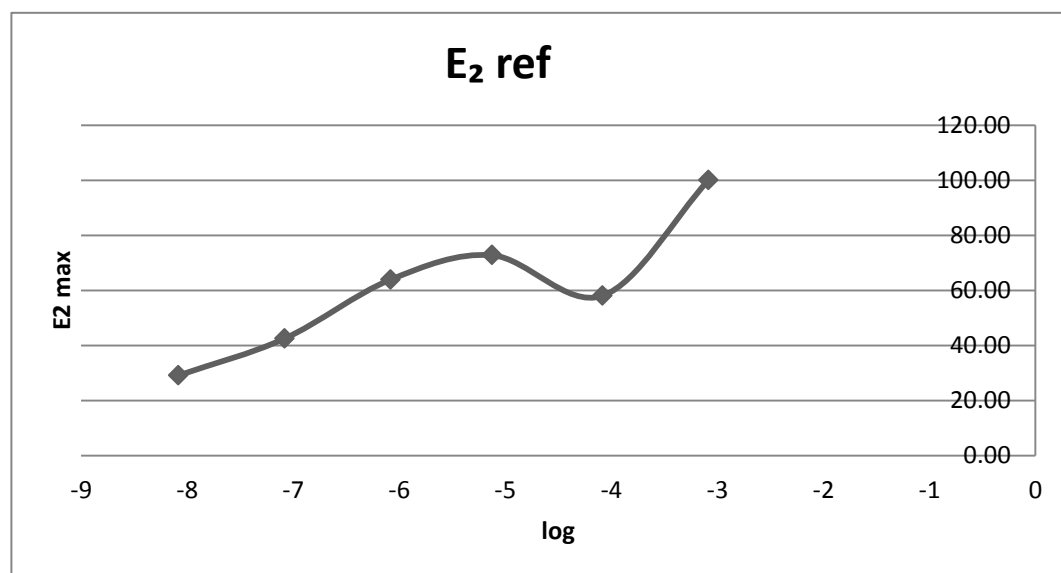
Reference linear curve as for 2nd sampling season April 2013.

Bile samples from the 2nd sampling season April 2013 were compared to the reference compound linear curve as indicated below

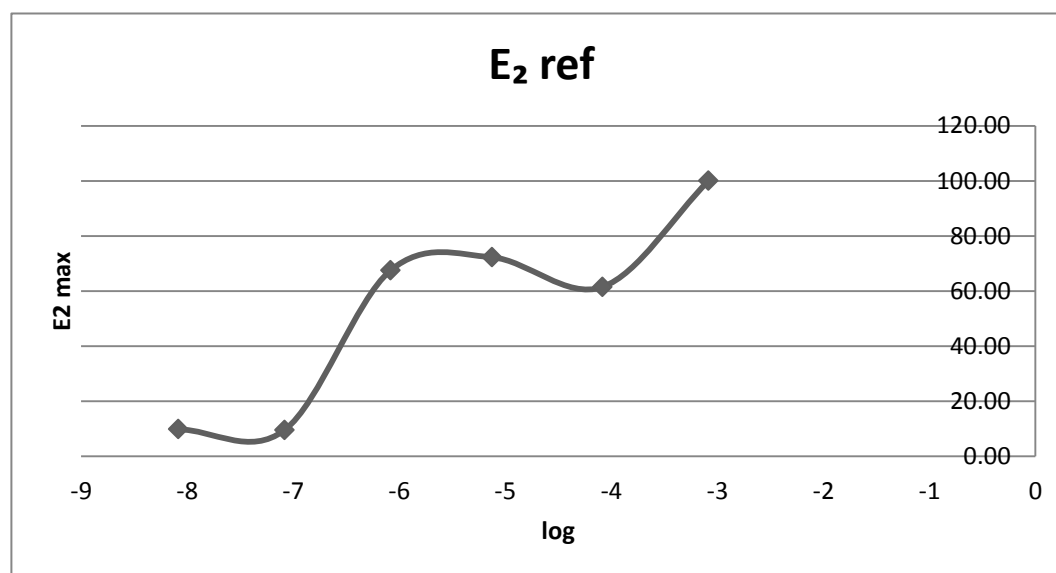
$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	5989717	6917795	6267822	6391778	476294.0384	7.45	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	5289515	5430279	4503431	5074408.333	499464.6757	9.84	79.39
7.50E-06	7.50E-12	2.75E-14	-5.124938737	3812300	3749027	3672427	3744584.667	70042.23566	1.87	58.58
8.25E-07	8.25E-13	3.03E-15	-6.083546051	4006869	3685560	3382855	3691761.333	312053.2173	8.45	57.76
8.25E-08	8.25E-14	3.03E-16	-7.083546051	3784188	3282601	2758841	3275210	512713.4558	15.65	51.24
8.25E-09	8.25E-15	3.03E-17	-8.083546051	2268252	2041686	2254382	2188106.667	126993.5154	5.80	34.23



$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	6707066	5218158	5555320	5826848	780709.5165	13.40	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	6280964	3856888	2910595	3383741.5	669130.1973	19.77	58.07
7.50E-06	7.50E-12	2.75E-14	-5.124938737	4036762	3235836	5451117	4241238.333	1121706.47	26.45	72.79
8.25E-07	8.25E-13	3.03E-15	-6.083546051	3985797	3935876	3248970	3723547.667	411753.5644	11.06	63.90
8.25E-08	8.25E-14	3.03E-16	-7.083546051	2368498	2889789	2168062	2475449.667	372560.6795	15.05	42.48
8.25E-09	8.25E-15	3.03E-17	-8.083546051	2011543	1915861	1163270	1696891.333	464599.3509	27.38	29.12

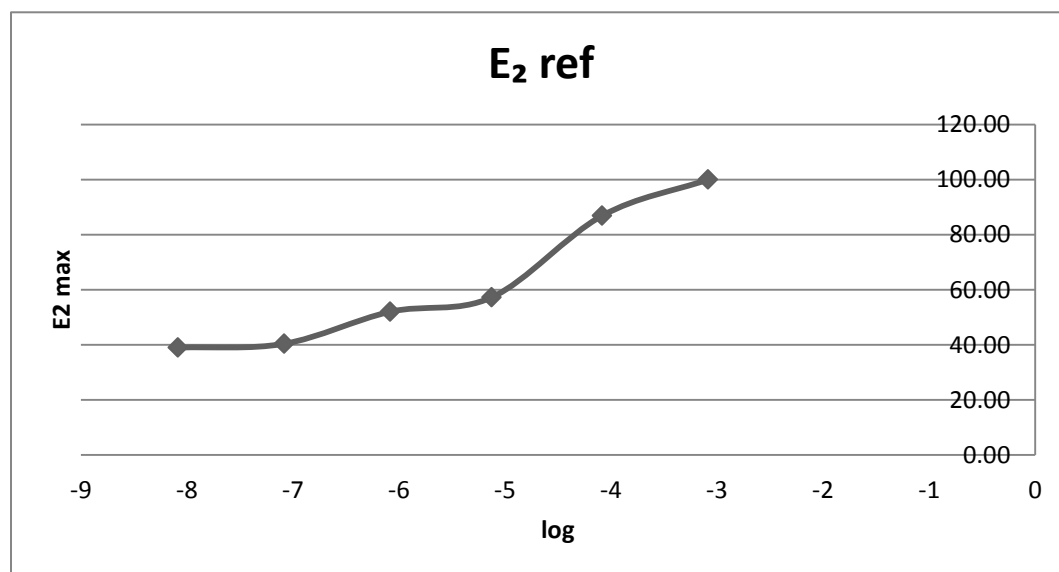


E ₂ (µg/) _{well}	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	4758003	4525822	3554612	4279479	638397.3136	14.92	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	2317121	4709911	2946443	2631782	444997.8537	16.91	61.50
7.50E-06	7.50E-12	2.75E-14	-5.124938737	2511180	3511432	3256034	3092882	519701.7868	16.80	72.27
8.25E-07	8.25E-13	3.03E-15	-6.083546051	1122825	2997262	2775884	2886573	156537.885	5.42	67.45
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1999235	439528	372921	406224.5	47098.26137	11.59	9.49
8.25E-09	8.25E-15	3.03E-17	-8.083546051	484181	817142	355264	419722.5	91158.08491	21.72	9.81



Reference will not be used as the standard curve deviates.
Error could be caused by dosing, standards, bacteria or cells.

$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	3482859	5026729	4399082	4302890	776416.9755	18.04	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	3436685	4384322	3395164	3738723.667	559489.8608	14.96	86.89
7.50E-06	7.50E-12	2.75E-14	-5.124938737	2112526	2879148	2400422	2464032	387249.2696	15.72	57.26
8.25E-07	8.25E-13	3.03E-15	-6.083546051	1928258	2684280	2102515	2238351	395892.511	17.69	52.02
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1555815	2062014	1594843	1737557.333	281664.5036	16.21	40.38
8.25E-09	8.25E-15	3.03E-17	-8.083546051	1605703	1686852	1738884	1677146.333	67118.88456	4.00	38.98



E ₂ (µg/) _{well}	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	3.03E-12	-3.0835	5989717	6917795	6267822	5989717	6917795	6267822	6707066	5218158	5555320	6203468	587870	9.48	100.00
8.25E-05	3.03E-13	-4.0835	5289515	5430279	4503431	5289515	5430279	4503431	6280964	3856888	2910595	4832766	1005768	20.81	77.90
7.50E-06	2.75E-14	-5.1249	3812300	3749027	3672427	3812300	3749027	3672427	4036762	3235836	5451117	3910135	615366	15.74	63.03
8.25E-07	3.03E-15	-6.0835	4006869	3685560	3382855	4006869	3685560	3382855	3985797	3935876	3248970	3702356	302202	8.16	59.68
8.25E-08	3.03E-16	-7.0835	3784188	3282601	2758841	3784188	3282601	2758841	2368498	2889789	2168062	300862	571000	18.98	48.50
8.25E-09	3.03E-17	-8.0835	2268252	2041686	2254382	2268252	2041686	2254382	2011543	1915861	1163270	2024368	349785.56	17.28	32.63

Combined E₂

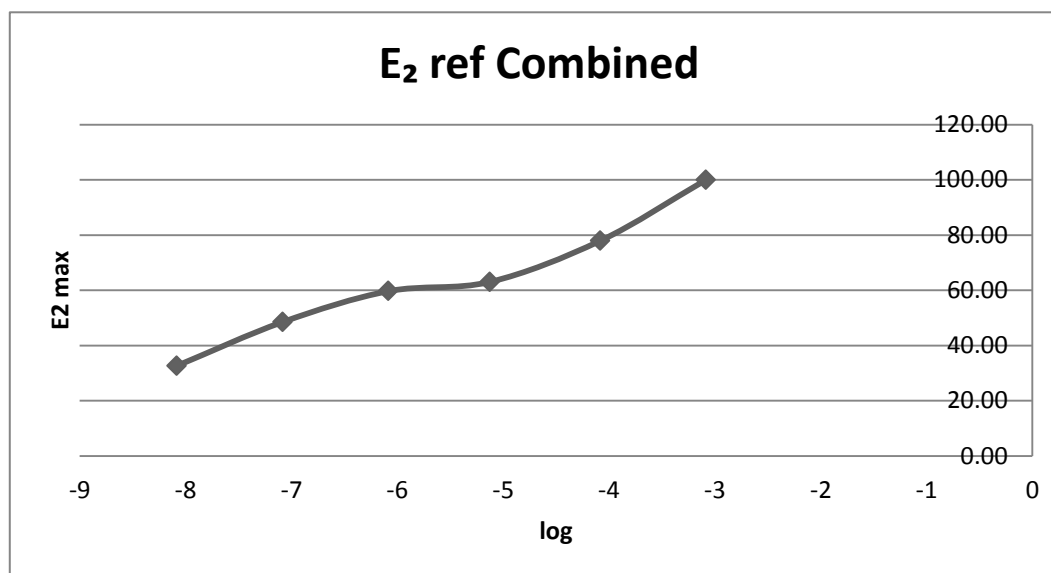
y=mx+c

intercept 154.40

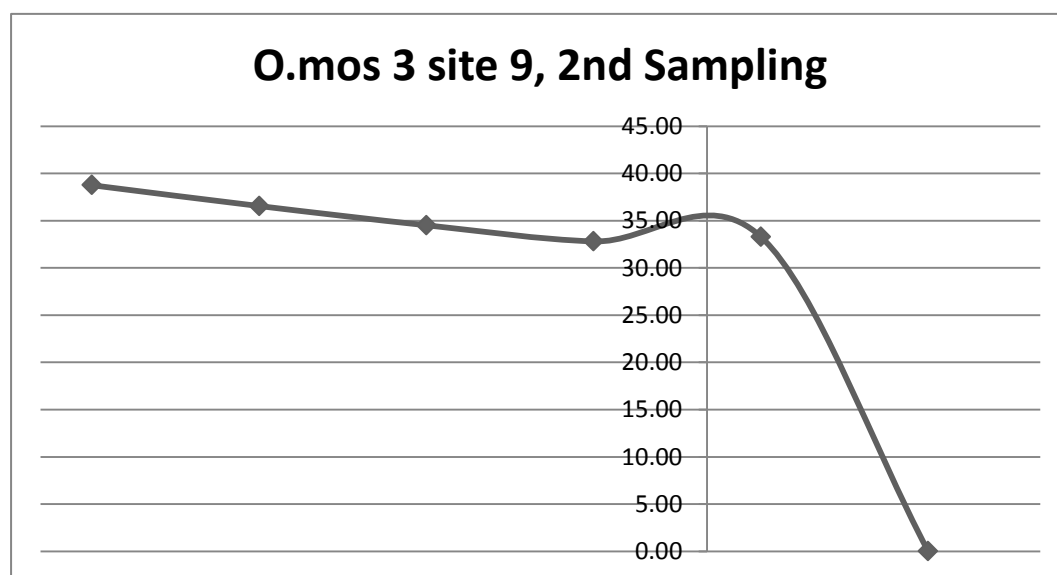
slope 18.08

r2 0.98

	µg/well	M/ℓ
EC20	3.69E-08	3.39E-14
EC50	1.68E-06	1.55E-12
EC80	7.68E-05	7.05E-11



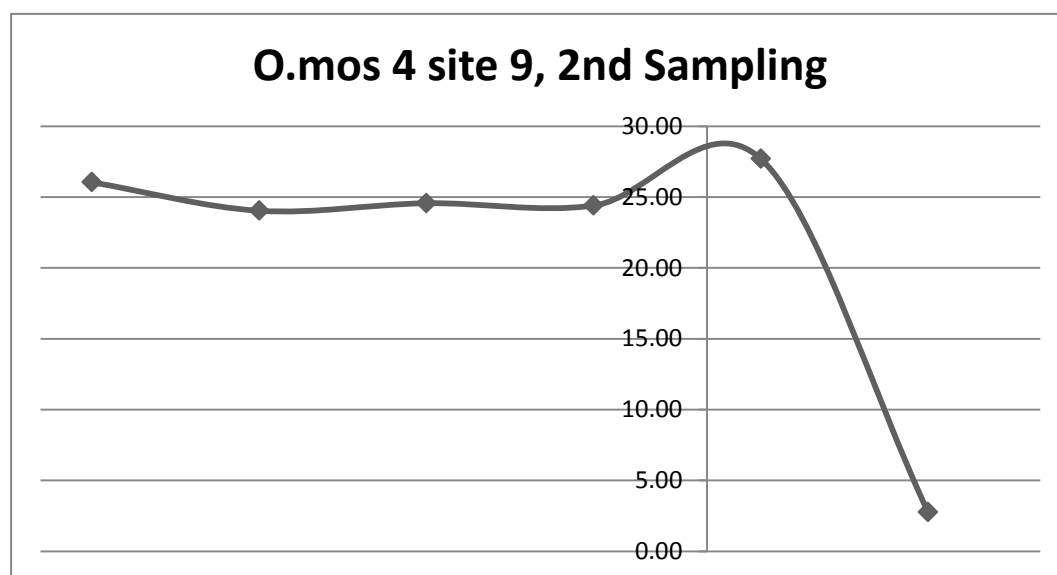
O.mos 3 site 8, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1012	494	987	999.5	17.67766953	1.77	0.02
1.25	9.69E-02	2439483	1894473	1861425	2065127	324622.6326	15.72	33.29
0.63	-2.04E-01	2398925	1941877	1764626	2035142.667	327273.0798	16.08	32.81
0.31	-5.05E-01	2467190	2092781	1863949	2141306.667	304534.0396	14.22	34.52
0.16	-8.06E-01	2868863	2020663	1912753	2267426.333	523646.5316	23.09	36.55
0.08	-1.11E+00	2830490	2323101	2058753	2404114.667	392194.9897	16.31	38.75



250μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.25
250μℓ bile used in extraction

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

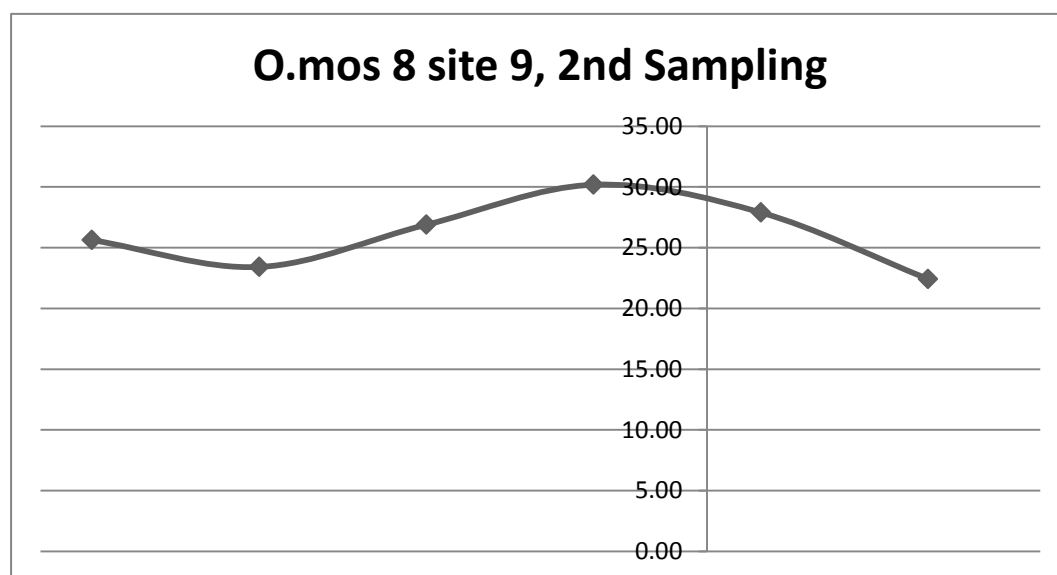
O.mos 4 site 9, 2nd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	939	2735	510278	171317.3333	293549.9218	171.35	2.76
1.25	9.69E-02	1298798	1919543	1934651	1717664	362827.2418	21.12	27.69
0.63	-2.04E-01	1358106	1670246	1512473	1513608.333	156073.0971	10.31	24.40
0.31	-5.05E-01	1155947	1857298	1558517	1523920.667	351953.102	23.10	24.57
0.16	-8.06E-01	1179471	1712585	1580795	1490950.333	277680.9002	18.62	24.03
0.08	-1.11E+00	1344001	1887133	1615155	1615429.667	271566.1042	16.81	26.04



150 $\mu\ell$ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.15
150 $\mu\ell$ bile used in extraction

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

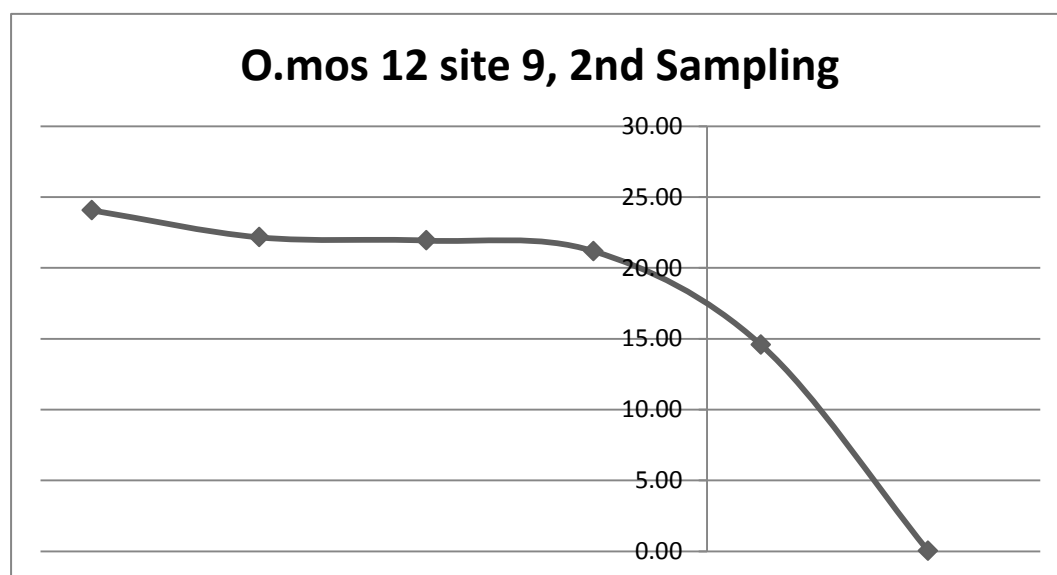
O.mos 8 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1158568	1324483	1689206	1390752.333	271455.1347	19.52	22.42
1.25	9.69E-02	1693673	1713313	1784565	1730517	47825.95663	2.76	27.90
0.63	-2.04E-01	1358041	2448554	1810614	1872403	547875.9542	29.26	30.18
0.31	-5.05E-01	1373222	1851431	1780066	1668239.667	257972.4957	15.46	26.89
0.16	-8.06E-01	1335391	1410098	1611918	1452469	143049.8922	9.85	23.41
0.08	-1.11E+00	1530284	1588070	1650882	1589745.333	60316.45263	3.79	25.63



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1
100μℓ bile used in extraction

No linear gradient thus a response occurred which cannot be quantified by the cells

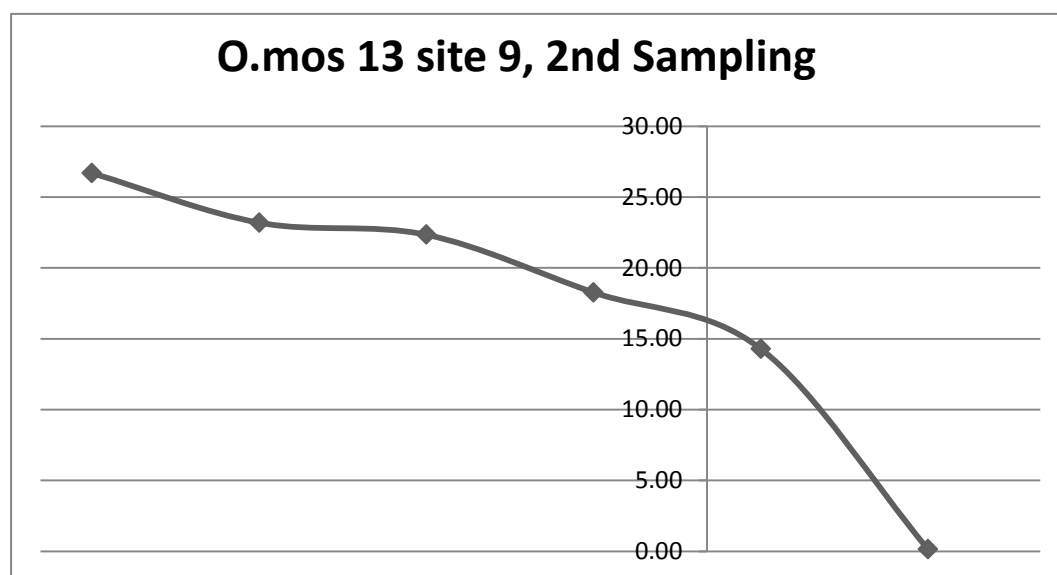
O.mos 12 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1449	1974	550	1711.5	371.2310601	21.69	0.03
1.25	9.69E-02	863883	929470	919436	904263	35328.15575	3.91	14.58
0.63	-2.04E-01	1544040	1264228	1133159	1313809	209879.7411	15.97	21.18
0.31	-5.05E-01	1442864	1523907	1114571	1360447.333	216756.4773	15.93	21.93
0.16	-8.06E-01	1470150	1328189	1325413	1374584	82774.2219	6.02	22.16
0.08	-1.11E+00	2210401	1650712	1334566	1492639	223548.9804	14.98	24.06



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

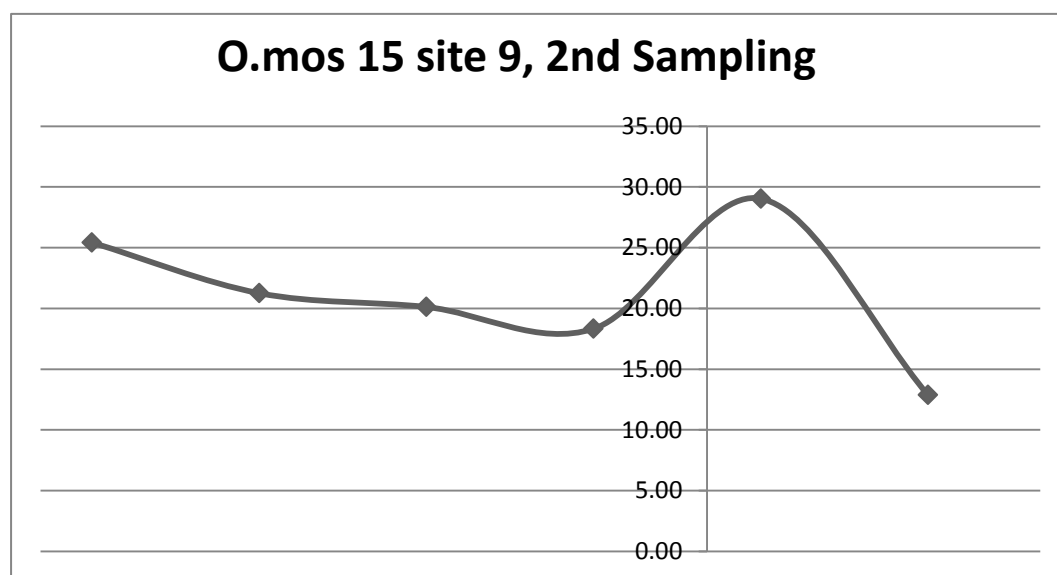
O.mos 13 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	4111	12397	101695	8254	5859.086789	70.98	0.13
1.25	9.69E-02	571018	1126677	960204	885966.3333	285171.2721	32.19	14.28
0.63	-2.04E-01	699627	1203221	1496693	1133180.333	403122.5909	35.57	18.27
0.31	-5.05E-01	1329654	1247000	1580552	1385735.333	173703.9756	12.54	22.34
0.16	-8.06E-01	1215740	1230549	1869768	1438685.667	373401.6741	25.95	23.19
0.08	-1.11E+00	1344657	1575462	2046210	1655443	357549.8192	21.60	26.69



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

O.mos 15 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	513984	3366	1080997	797490.5	400938.7373	50.28	12.86
1.25	9.69E-02	1370503	1756655	2276045	1801067.667	454401.7409	25.23	29.03
0.63	-2.04E-01	1036538	1099115	1275387	1137013.333	123852.4281	10.89	18.33
0.31	-5.05E-01	1149222	1184463	1410543	1248076	141799.6001	11.36	20.12
0.16	-8.06E-01	1110088	1183954	1661215	1318419	299158.6029	22.69	21.25
0.08	-1.11E+00	1241894	1273818	2215483	1577065	553116.5722	35.07	25.42



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

O.mos 4 site 8, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	5139846	3366586	1080997	4253216	1253884.171	29.48	68.56
1.25	9.69E-02	1370503	1756655	2276045	1563579	273050.6978	17.46	25.20
0.63	-2.04E-01	1036538	1099115	1275387	1067826.5	44248.62105	4.14	17.21
0.31	-5.05E-01	1149222	1184463	1410543	1248076	141799.6001	11.36	20.12
0.16	-8.06E-01	1110088	1183954	1661215	1318419	299158.6029	22.69	21.25
0.08	-1.11E+00	1241894	1273818	2215483	1257856	22573.67688	1.79	20.28

y=mx+c

intercept 28.73

slope 85.29

r2 0.86

μℓ/well

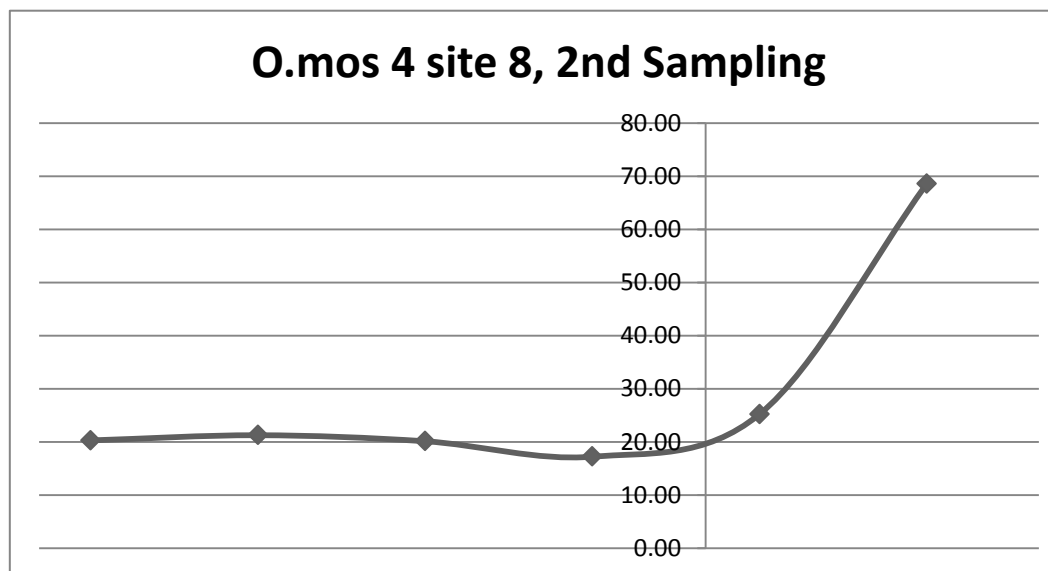
EC20 7.90E-01

EC50 1.78E+00

EC80 3.99E+00

	μg/μℓ	μg/mℓ
REP20	4.67E-08	4.67E-05
REP50	9.48E-07	9.48E-04
REP80	1.92E-05	1.92E-02

	E ₂ eq μg/mℓ
REP20	4.67E-04
REP50	9.48E-03
REP80	1.92E-01

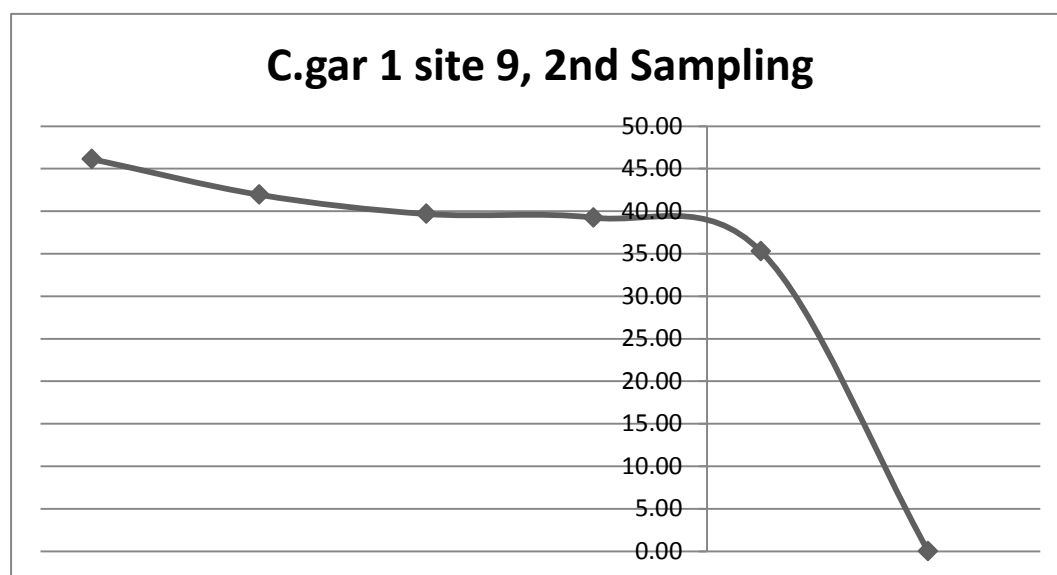


100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
5139846	82.85	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1370503	22.09	intercept	20.08	EC20	0.952879849	REP 20	3.88E-08	3.88E-05	3.88E-04	Mean	STEDV
1036538	16.71	slope	3.58	EC50	228382169.6	REP 50	7.37E-15	7.37E-12	7.37E-11	4060.24	7032.53
1149222	18.53	r ²	0.36	EC80	5.47377E+16	REP 80	1.40E-21	1.40E-18	1.40E-17		
1110088	17.89									REP50	
1241894	20.02									Mean	STEDV
RLU	E ₂ max									169.30	97.74
3366586	54.27	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1756655	28.32	intercept	24.15	EC20	0.334868144	REP 20	1.10E-07	1.10E-04	1.10E-03	REP80	
1099115	17.72	slope	8.74	EC50	903.7787462	REP 50	1.86E-09	1.86E-06	1.86E-05	Mean	STEDV
1184463	19.09	r ²	0.48	EC80	2439216.861	REP 80	3.15E-11	3.15E-08	3.15E-07	2.35	4.08
1183954	19.09										
1273818	20.53										
RLU	E ₂ max										
1080997	17.43	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2276045	36.69	intercept	29.94	EC20	0.082097044	REP 20	4.50E-07	4.50E-04	1.22E+04		
1275387	20.56	slope	9.15	EC50	155.5238128	REP 50	1.08E-08	1.08E-05	2.93E+02		
1410543	22.74	r ²	0.25	EC80	294622.7434	REP 80	2.61E-10	2.61E-07	7.06E+00		
1661215	26.78										
2215483	35.71										

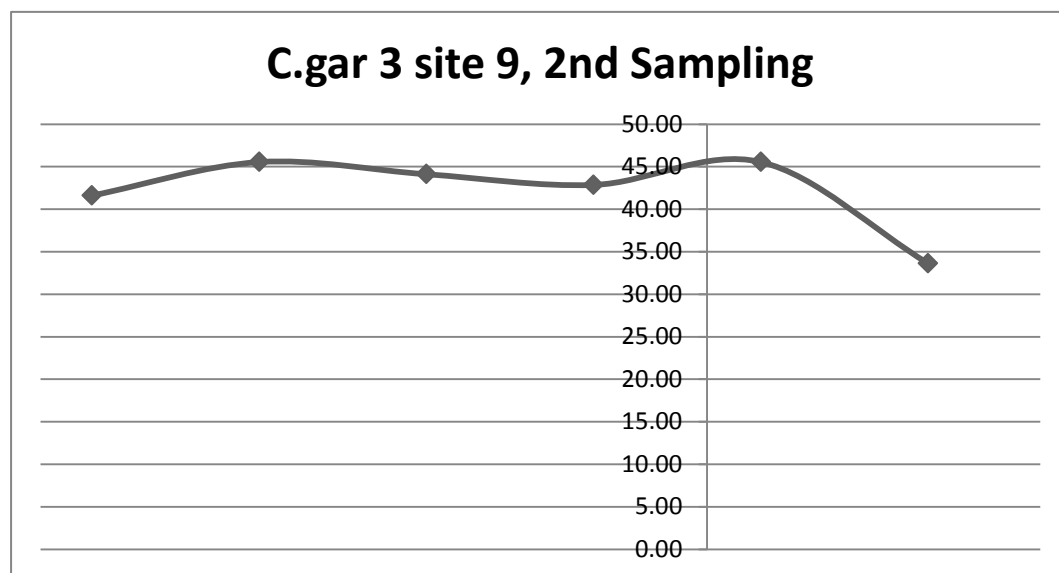
C.gar 1 site 9, 2nd sampling(μl)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	833	1117	401826	975	200.8183259	20.60	0.02
1.25	9.69E-02	2070155	1872826	2224643	2055874.667	176342.6957	8.58	35.28
0.63	-2.04E-01	2257489	2114977	2487842	2286769.333	188149.0911	8.23	39.25
0.31	-5.05E-01	2286694	2180427	2468445	2311855.333	145648.2461	6.30	39.68
0.16	-8.06E-01	2328814	2431302	2571313	2443809.667	121732.3808	4.98	41.94
0.08	-1.11E+00	2835993	2658798	2569322	2688037.667	135718.7317	5.05	46.13



300 μl bile used in extraction
concentrated to 1m ℓ
Concentration factor 0.33

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

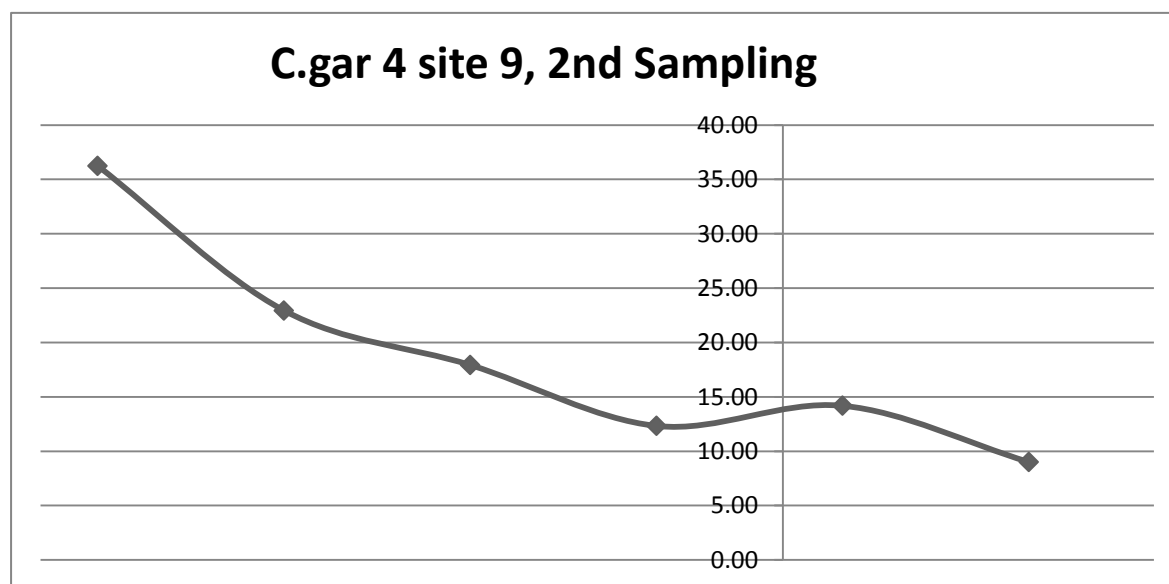
C.gar 3 site 9, 2nd sampling(μl)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1566699	2351504	2670944	1959101.5	554940.9374	28.33	33.62
1.25	9.69E-02	2780124	2308397	2873937	2654152.667	303084.8982	11.42	45.55
0.63	-2.04E-01	2448133	2452487	2591843	2497487.667	81743.10996	3.27	42.86
0.31	-5.05E-01	2471641	2689249	2551220	2570703.333	110104.5441	4.28	44.12
0.16	-8.06E-01	2749107	2545758	2667327	2654064	102321.2311	3.86	45.55
0.08	-1.11E+00	2393754	2380701	2499236	2424563.667	64996.64012	2.68	41.61



500 μl bile used in extraction
concentrated to 1m l
Concentration factor 0.5

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

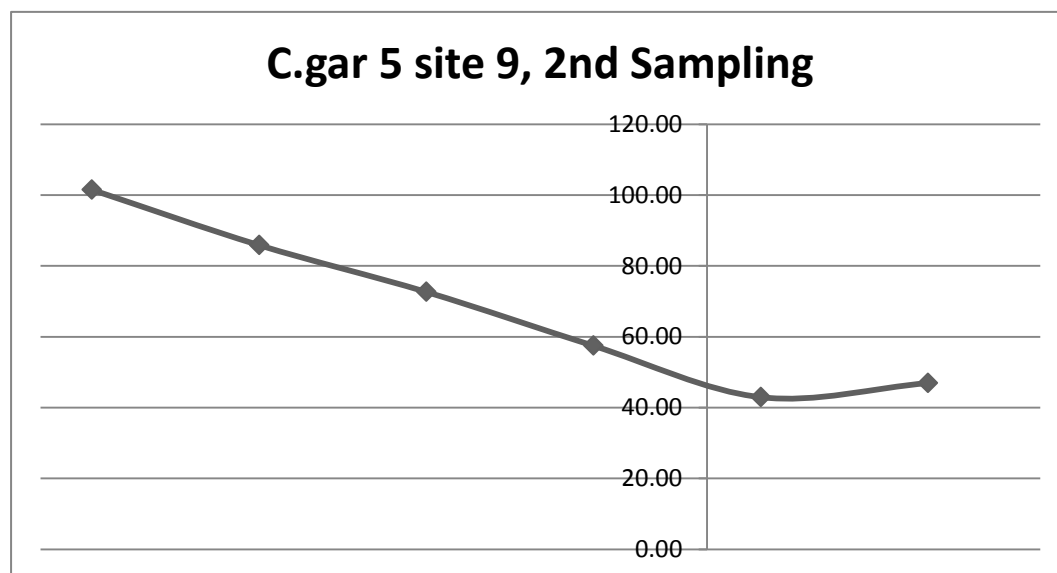
C.gar 4 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	486	1048984	1056186	524735	741400.0459	141.29	9.01
1.25	9.69E-02	881030	708302	889260	826197.3333	102183.2444	12.37	14.18
0.63	-2.04E-01	532572	536982	1084743	718099	317530.6742	44.22	12.32
0.31	-5.05E-01	757139	911085	1465578	1044600.667	372614.1156	35.67	17.93
0.16	-8.06E-01	1055514	1235671	1719026	1336737	343107.5572	25.67	22.94
0.08	-1.11E+00	1574418	1832268	2929134	2111940	719358.1729	34.06	36.24



500μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.5

No linear gradient thus a response occurred which cannot be quantified by the cells
Strongest concentration could indicate cytotoxicity

C.gar 5 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2881746	2588007	2341365	2734876.5	207704.8388	7.59	46.94
1.25	9.69E-02	2465434	2499495	2540643	2501857.333	37660.10999	1.51	42.94
0.63	-2.04E-01	3212150	3064799	3764273	3347074	368740.1978	11.02	57.44
0.31	-5.05E-01	4411091	4116349	4170558	4232666	156879.7769	3.71	72.64
0.16	-8.06E-01	5105458	4793326	5108622	5002468.667	181129.7711	3.62	85.85
0.08	-1.11E+00	5817361	6075478	5848897	5913912	140805.927	2.38	101.49



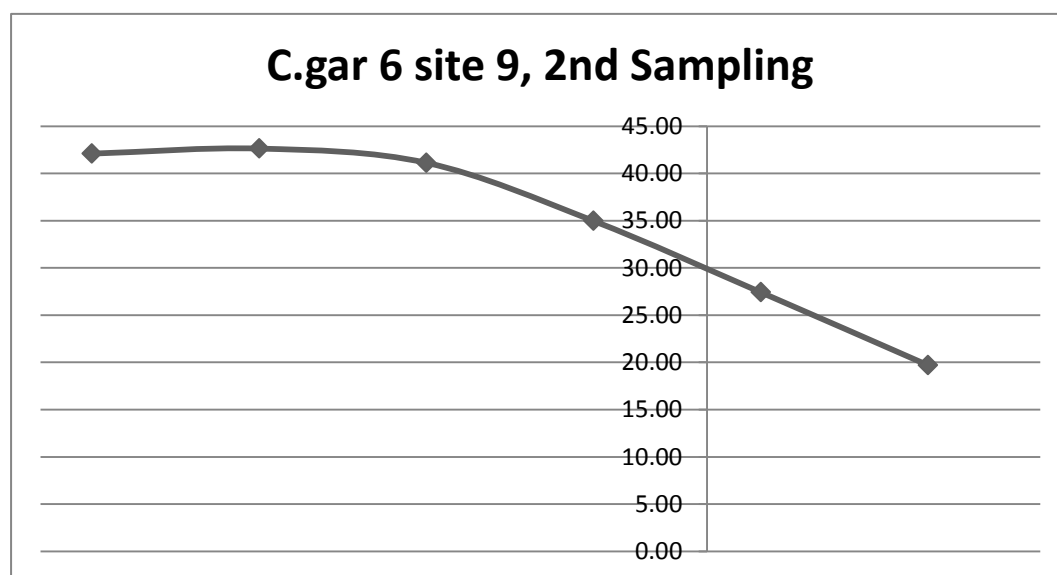
500μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.5

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration could indicate cytotoxicity

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

C.gar 6 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1342051	953140	917639	1147595.5	275001.6054	23.96	19.69
1.25	9.69E-02	1622381	1592861	1577291	1597511	22901.8318	1.43	27.42
0.63	-2.04E-01	2081492	1827923	2203652	2037689	191656.196	9.41	34.97
0.31	-5.05E-01	2209001	2482202	2497512	2396238.333	162332.878	6.77	41.12
0.16	-8.06E-01	2497035	2396287	2559118	2484146.667	82177.0375	3.31	42.63
0.08	-1.11E+00	2268721	2527680	2562816	2453072.333	160616.6094	6.55	42.10



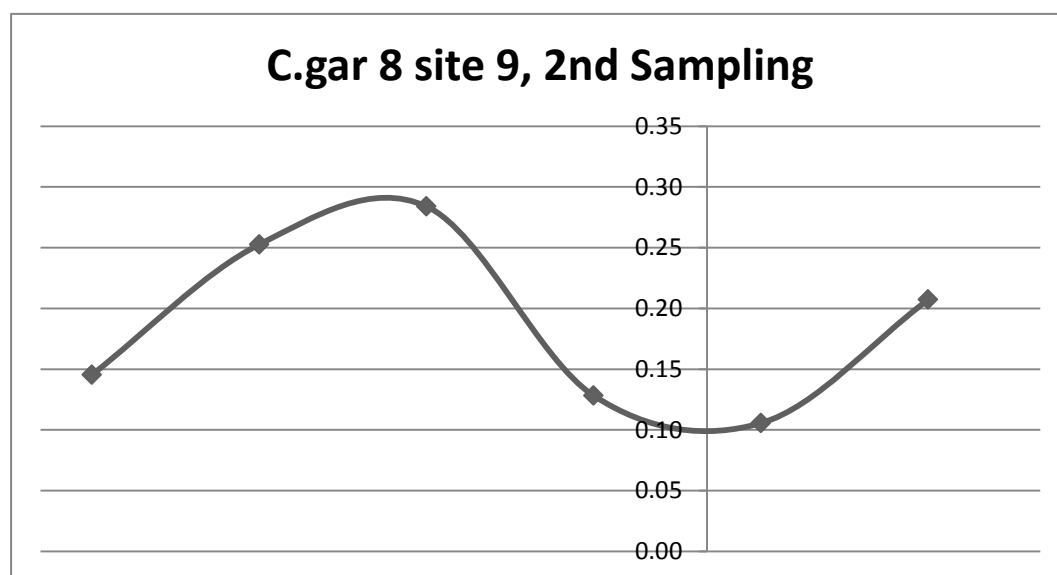
500μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.5

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration could indicate cytotoxicity

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

C.gar 8 site 9, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	15246	8893	4475	12069.5	4492.249381	37.22	0.21
1.25	9.69E-02	2937	6369	9144	6150	3109.28979	50.56	0.11
0.63	-2.04E-01	5778	8060	8570	7469.333333	1486.768756	19.90	0.13
0.31	-5.05E-01	11111	14040	24495	16548.66667	7035.831176	42.52	0.28
0.16	-8.06E-01	8521	15035	20603	14719.66667	6047.169365	41.08	0.25
0.08	-1.11E+00	5899	8788	10722	8469.66667	2427.207106	28.66	0.15



300μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.33

No Response

C.gar 1 site 8, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1955634	1846543	1703869	1835348.667	126255.2519	6.88	31.50
1.25	9.69E-02	1971737	1647621	1484878	1701412	247846.7777	14.57	29.20
0.63	-2.04E-01	2155455	1496086	1620665	1757402	350306.4728	19.93	30.16
0.31	-5.05E-01	1823585	1579573	1559157	1654105	147128.5359	8.89	28.39
0.16	-8.06E-01	2337853	1475920	1452971	1755581.333	504392.5897	28.73	30.13
0.08	-1.11E+00	1559505	1255586	1328278	1381123	158701.4025	11.49	23.70

y=mx+c

intercept 29.96

slope 2.78

r2 0.65

μℓ/well

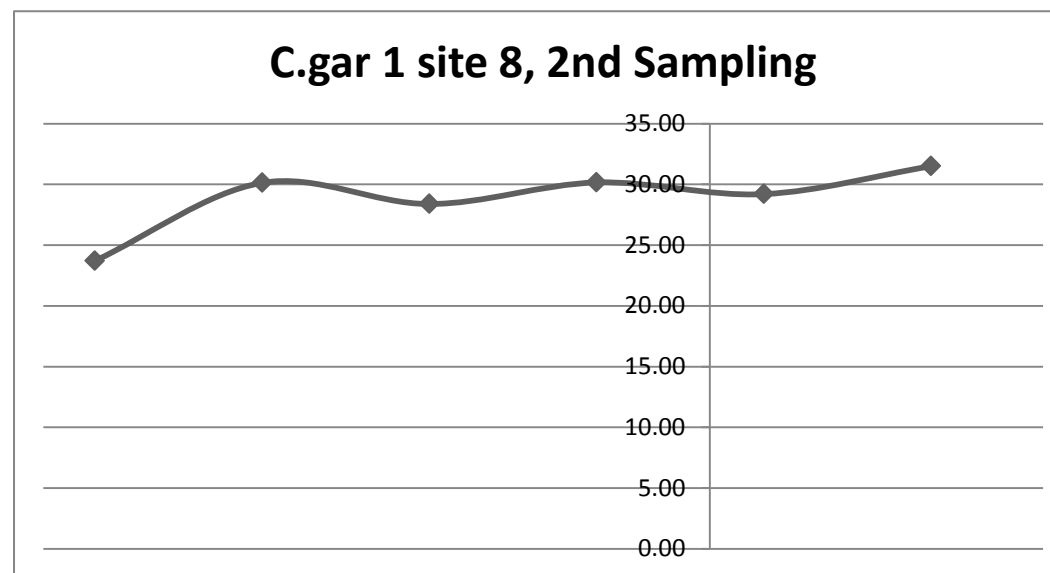
EC20 2.62E-04

EC50 1.61E+07

EC80 9.90E+17

	μg/μℓ	μg/mℓ
REP20	8.24E-06	8.24E-03
REP50	4.08E-14	4.08E-11
REP80	2.02E-22	2.02E-19

	E ₂ eq μg/mℓ
REP20	8.24E-02
REP50	4.08E-10
REP80	2.02E-18



100μℓ bile used in extraction

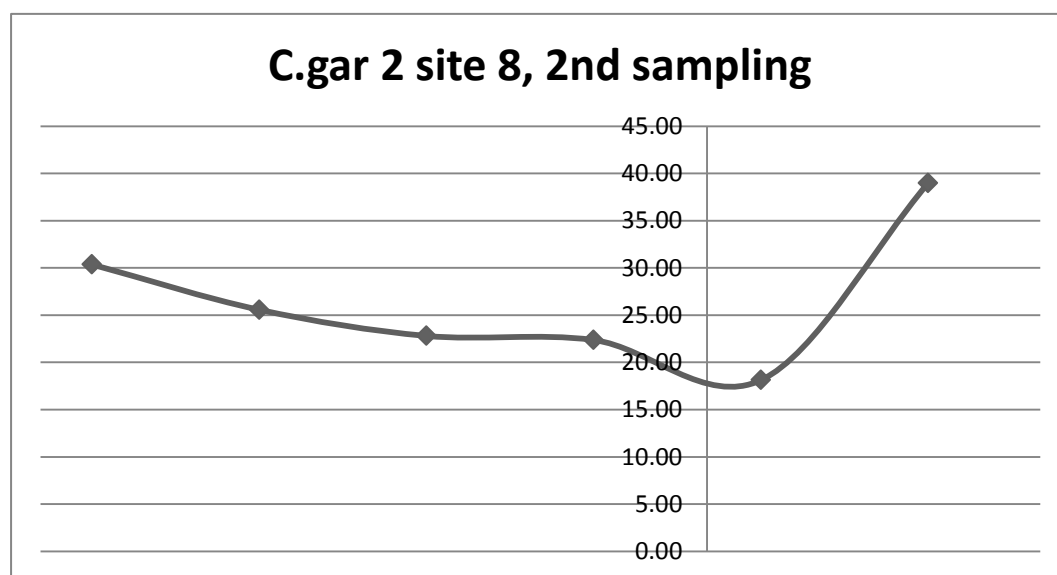
concentrated to 1mℓ

Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
1955634	33.56	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1971737	33.84	intercept	32.91	EC20	2.05657E-05	REP 20	1.05E-04	1.05E-01	1.05E+00	Mean	STEDV
2155455		slope	2.75	EC50	1605154.296	REP 50	4.09E-13	4.09E-10	4.09E-09	0.35	0.61
1823585	31.30	r2	0.82	EC80	1.25283E+17	REP 80	1.60E-21	1.60E-18	1.60E-17		
2337853	40.12									REP50	
1559505	26.76									Mean	STEDV
RLU	E ₂ max									2.12E-05	1.29E-05
1846543	31.69	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1647621	28.28	intercept	27.58	EC20	0.174301399	REP 20	1.24E-08	1.24E-05	1.24E-04	REP80	
1496086	25.68	slope	9.99	EC50	175.5225669	REP 50	3.74E-09	3.74E-06	3.74E-05	Mean	STEDV
1579573	27.11	r2	0.99	EC80	176752.2906	REP 80	1.13E-09	1.13E-06	1.13E-05	3.77E-06	6.53E-06
1475920	25.33										
1255586	21.55										
RLU	E ₂ max										
1703869	29.24	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1484878	25.48	intercept	29.25	EC20	0.022373349	REP 20	9.64E-08	9.64E-05	9.64E-04		
1620665	27.81	slope	5.61	EC50	5023.587793	REP 50	1.31E-10	1.31E-07	1.31E-06		
1559157	26.76	r2	0.07	EC80	1127968565	REP 80	1.77E-13	1.77E-10	1.77E-09		
1452971	24.94										
1328278	22.80										

C.gar 2 site 8, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1239715	1016958	4557536	2271403	1982979.637	87.30	38.98
1.25	9.69E-02	1126678	960204	1081069	1055983.667	86025.30808	8.15	18.12
0.63	-2.04E-01	1203211	1496693	1210464	1303456	167387.4402	12.84	22.37
0.31	-5.05E-01	1247000	1580552	1158366	1328639.333	222618.4858	16.76	22.80
0.16	-8.06E-01	1230549	1869768	1369799	1490038.667	336144.9324	22.56	25.57
0.08	-1.11E+00	1575462	2046210	1685937	1769203	246172.3785	13.91	30.36



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

C.gar 13 site 8, 2nd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	5139846	3366586	1080997	3195809.667	2034806.433	63.67	54.85
1.25	9.69E-02	1370503	1756655	2276045	1801067.667	454401.7409	25.23	30.91
0.63	-2.04E-01	1036538	1099115	1275387	1137013.333	123852.4281	10.89	19.51
0.31	-5.05E-01	1149222	1184463	1410543	1248076	141799.6001	11.36	21.42
0.16	-8.06E-01	1110088	1183954	1661215	1318419	299158.6029	22.69	22.63
0.08	-1.11E+00	1241894	1273818	2215483	1577065	553116.5722	35.07	27.07

y=mx+c

intercept 33.66

slope 37.10

r2 0.79

μℓ/well

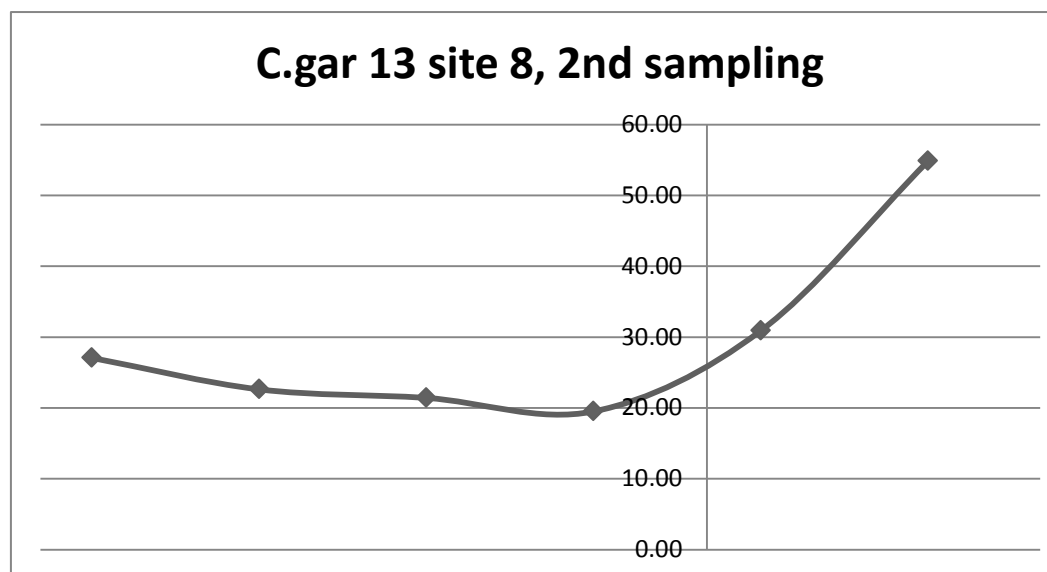
EC20 4.28E-01

EC50 2.76E+00

EC80 1.77E+01

	μg/μℓ	μg/mℓ
REP20	5.04E-09	5.04E-06
REP50	2.38E-07	2.38E-04
REP80	1.13E-05	1.13E-02

	E ₂ eq μg/mℓ
REP20	2.52E-05
REP50	1.19E-03
REP80	5.63E-02



200μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor

0.2

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
5139846	88.21	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1370503	23.52	intercept	31.84	EC20	0.792122781	REP 20	2.72E-09	2.72E-06	1.36E-05	Mean	STEDV
1036538	17.79	slope	116.97	EC50	1.429804601	REP 50	4.59E-07	4.59E-04	2.30E-03	2.21E-05	1.35E-05
1149222	19.72	r ²	0.81	EC80	2.58083879	REP 80	7.75E-05	7.75E-02	3.87E-01		
1110088	19.05									REP50	
1241894	21.31									Mean	STEDV
RLU	E ₂ max									8.00E-04	1.52E-03
3366586	57.78	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1756655	30.15	intercept	29.33	EC20	0.71716407	REP 20	3.01E-09	3.01E-06	1.50E-05	REP80	
1099115	18.86	slope	64.64	EC50	2.088155176	REP 50	3.15E-07	3.15E-04	1.57E-03	Mean	STEDV
1184463	20.33	r ²	0.94	EC80	6.080048096	REP 80	3.29E-05	3.29E-02	1.64E-01	0.19	0.19
1183954	20.32										
1273818	21.86										
RLU	E ₂ max										
1080997	18.55	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2276045	39.06	intercept	33.42	EC20	0.2857492	REP 20	7.55E-09	7.55E-06	3.77E-05		
1275387	21.89	slope	24.67	EC50	4.698568849	REP 50	1.40E-07	1.40E-04	6.99E-04		
1410543	24.21	r ²	0.64	EC80	77.25848132	REP 80	2.59E-06	2.59E-03	1.29E-02		
1661215	28.51										
2215483	38.02										

8.2.2.3. Inhibition calculations for April 2013 retrieved from Microsoft Excel 2010

Fold Inhibition		Plate aa			Fold Inhibition		Plate bb	
Solvent control	mean	stdev	cv		Solvent control	mean	stdev	cv
1317014	1016860	277476	27.29		1129647	1174882	97823	8.33
963862					1107863			
769706					1287137			
Sediment 10($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	3.98E-01	1208295	1101931	968054	1034992	94665	9.15	0.98
0.83	-8.09E-02	1133054	872396	835989	854192	25743	3.01	1.19
Sediment 9 ($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	3.98E-01	1293910	1240405	1444069	1326128	105585	7.96	0.77
0.83	-8.09E-02	956765	887577	957461	922519	49415	5.36	1.10
Sediment 8 pump ($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	3.98E-01	1531805	682779	919946	1044843	167702	16.05	1.12
0.83	-8.09E-02	1394861	1254874	1435208	1361647	127515	9.36	0.86
Sediment 8.1($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	3.98E-01	1107563	998359	960617	979488	26687	2.72	1.20
0.83	-8.09E-02	1203860	1311309	1278568	1294938	23151	1.79	0.91
Sediment 8.2 ($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	1481374	1595191	1925758	1760474	233746	13.28	0.67
0.83	9.69E-02	1293781	1423564	1920773	1672168	351579	21.03	0.61
Ref Sediment ($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	1567443	1917002	1535527	1726264	269743	15.63	0.96
0.83	9.69E-02	1323957	1718662	1316730	1517696	284208	18.73	1.09

Fold Inhibition		Plate cc			Fold Inhibition		Plate dd	
Solvent control	mean	stdev	cv		Solvent control	mean	stdev	cv
1669963	1650097	285780	17.32		1111828	1298588	205529	15.83
1925426					1265151			
1354901					1518785			
H2O 10 ($\mu\ell$)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	0.3979	1928962	1694790	1896723	1840158	142788	7.76	0.90
0.83	-0.0792	1340611	1206482	1460796	1335963	179827	13.46	1.24

H2O 9 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	0.3979	1305329	732935	532588	856950	141667	16.53	0.52
0.83	-0.0792	1493189	582776	433354	836439	105657	12.63	0.55
H2O 8 pump(μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	0.3979	650815	753643	809908	781775.	39785.4	5.09	1.66
0.83	-0.0792	517011	589889	631305	610597	29285.5	4.80	2.13
H2O 8.1 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	0.3979	981552	1219211	1744177	1481694	371207	25.05	0.88
0.83	-0.0792	897538	1117363	1355177	1236270	168160	13.60	1.05
H2O 8.2(μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	0.3979	1583652	1722101	1804852	1763476.5	58513.8	3.32	0.95
0.83	-0.0792	1438251	1553500	1596672	1575086	30527.2	1.94	1.06
H2O Ref(μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.50	0.3979	1657096	1758775	1748530	1753652.5	7244.31	0.41	0.95
0.83	-0.0792	1513395	1739257	1269399	1504328	332240	22.09	1.11

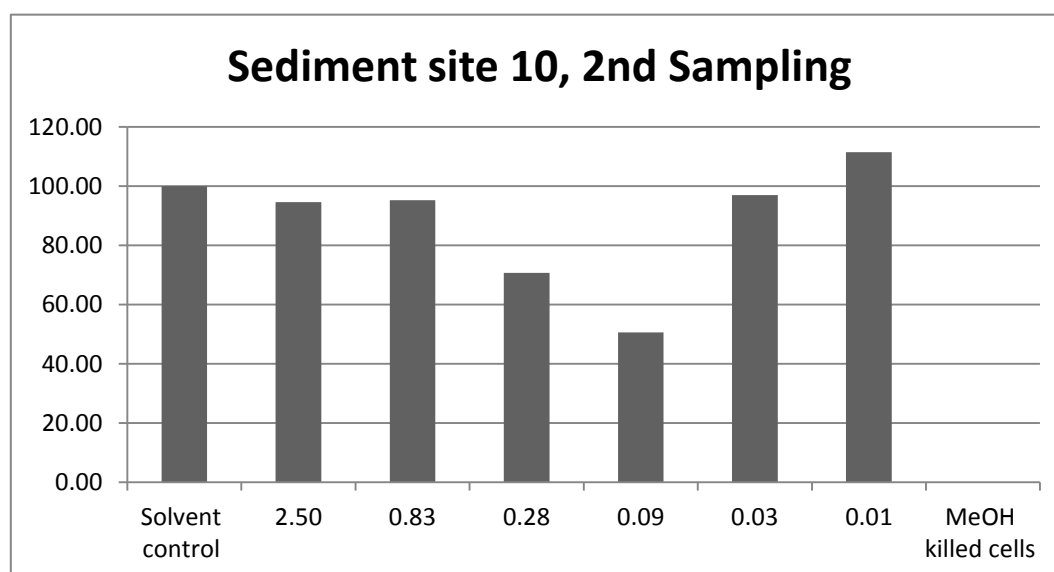
Site 9	Fold Inhibition		Plate D		Fold Inhibition			Plate C
Solvent control	mean	stdev	cv		Solvent control	mean	stdev	cv
3645520	3174168	408267	12.8		1287137	1627508	321255	19.73
2945771					1669963			
2931213					1925426			
O.mos 3	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2395332	2404212	2562034	2453859	93787	3.82	1.66
1.25	9.69E-02	1938900	2648656	1640312	1789606	211133	11.80	0.91
O.mos 4	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4969411	3783912	3176213	3480062	429708	12.35	1.47
1.25	9.69E-02	3108214	5610315	4462016	5036165	811970	16.12	0.32
O.mos 8	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3460298	3709408	3721684	3630463	147495	4.06	0.87
1.25	9.69E-02	3735975	3778604	3726790	3747123	27647	0.74	0.85
O.mos 12	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3857722	4028475	4163105	4016434	153047	3.81	1.79
1.25	9.69E-02	3515187	3790128	4208049	3837788	348881	9.09	0.83
O.mos 13	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3726725	3687268	3740984	3718325	27825	0.75	1.85
1.25	9.69E-02	3590669	3751156	3662417	3668080	80393	2.19	0.87

O.mos 15	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3445165	4901454	4862150	4881802	27792	0.57	0.65
1.25	9.69E-02	3160830	3060486	4302081	3110658	70953	2.28	1.02
Site 8	Fold Inhibition		Plate C					
Solvent control	mean	stdev	cv					
1287137	1627509	321255.3	19.739					
1669963								
1925426								
O.mos 3	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2906786	2813178	8369607	2859982	66190	2.31	1.57
1.25	9.69E-02	2647828	1323885	2661501	2654665	9668	0.36	0.61
Site 9	Fold Inhibition		Plate E					
Solvent control	mean	stdev	cv					
2905496	2651992	265422.8	10.008					
2674407								
2376072								
C.gar 1	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	6472365	5993201	6883025	6449530	445351	6.91	0.41
1.25	9.69E-02	2316247	3968739	3129772	3549256	593239	16.71	0.75
C.gar 3	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2468760	4764364	2432370	2450565	25731	1.05	1.08
1.25	9.69E-02	2704300	2886990	2046801	2795645	129181	4.62	0.95
C.gar 4	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3708566	4136555	2614906	3922561	302633	7.72	0.68
1.25	9.69E-02	2655342	3022963	2077907	2585404	476394	18.43	1.03
C.gar 5	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	1202331	3855383	3466585	3660984	274921	7.51	0.72
1.25	9.69E-02	3258745	3478894	3820109	3519249	282849	8.04	0.75
C.gar 6	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3360068	2763333	3875347	3617708	364357	10.07	0.73
1.25	9.69E-02	1829590	2712951	3972040	2271271	624630	27.50	1.17
C.gar 8	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2861442	3084035	2867156	2937544	126896	4.32	0.90
1.25	9.69E-02	2516771	2465936	920431	2491354	35945	1.44	1.06
Site 8	Fold Inhibition		Plate E					
Solvent control	mean	stdev	cv					
2905496	2651992	265422.8	10.008					

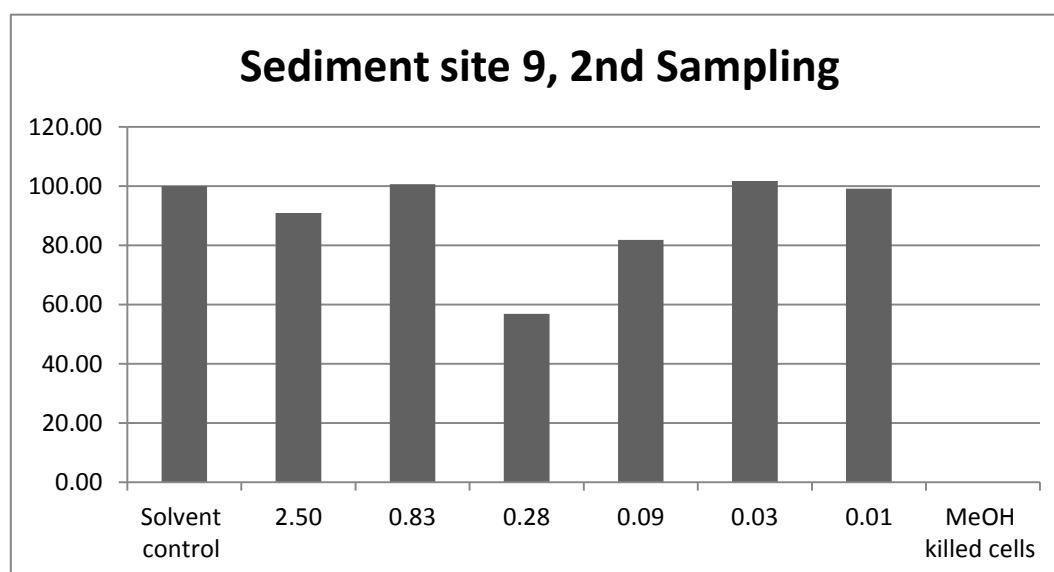
2674407								
2376072								
C.gar 1	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	9559856	7283256	6314785	6799021	684812	10.	0.39
1.25	9.69E-02	2995838	3776978	3696436	3489751	429632	12.	0.76
C.gar 2	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4319568	4452693	3143941	4386131	94133	2.15	0.60
1.25	9.69E-02	4710624	2784591	2654646	2719619	91884	3.38	0.98
C.gar 13	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4678295	4074326	3215104	4376311	427070	9.76	0.61
1.25	9.69E-02	3714344	3688417	3239170	3547310	267172	7.53	0.75

8.2.2.4.MTT calculations for April 2013 retrieved from Microsoft Excel 2010

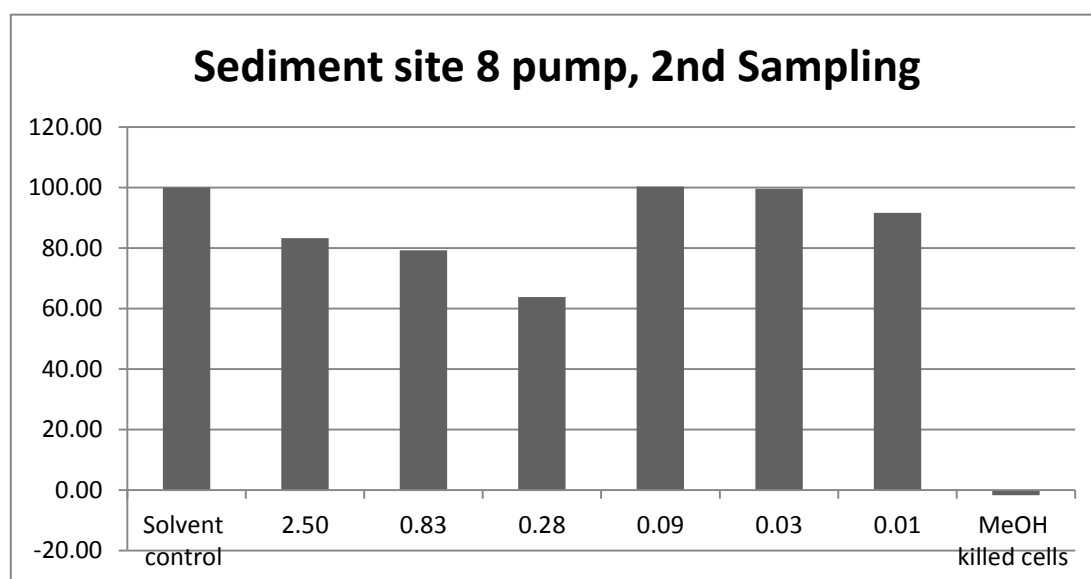
Absorbance reading from plate reader				Site 10 Sediment			
Raw data	1	2	3				
Solvent control	0.161	0.199	0.219				
2.50	0.181	0.202	0.171				
0.83	0.187	0.2	0.17				
0.28	0.157	0.16	0.126				
0.09	0.125	0.119	0.106				
0.03	0.193	0.195	0.177				
0.01	0.202	0.216	0.214				
MeOH killed cells	0.013	0.012	0.091				
MeOH killed cells subtracted							
Solvent control	0.122	0.160	0.180				
2.50	0.142	0.163	0.132				
0.83	0.148	0.161	0.131				
0.28	0.118	0.121	0.087				
0.09	0.086	0.080	0.067				
0.03	0.154	0.156	0.138				
0.01	0.163	0.177	0.175				
MeOH killed cells	-0.026	0.027	0.052				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	79.27	103.89	116.8	100.00	19.09	19.1	1.00
2.50	92.22	105.83	85.75	94.60	10.25	10.8	0.69
0.83	96.11	104.54	85.10	95.25	9.75	10.2	0.72
0.28	76.67	78.62	56.59	70.63	12.20	17.3	0.09
0.09	55.94	52.05	43.63	50.54	6.29	12.5	0.01
0.03	100.00	101.30	89.63	96.98	6.39	6.6	0.81
0.01	105.83	114.90	113.6	111.45	4.91	4.4	0.37
MeOH killed cells	-16.63	-17.28	33.91	0.00	29.37	0.0	0.01



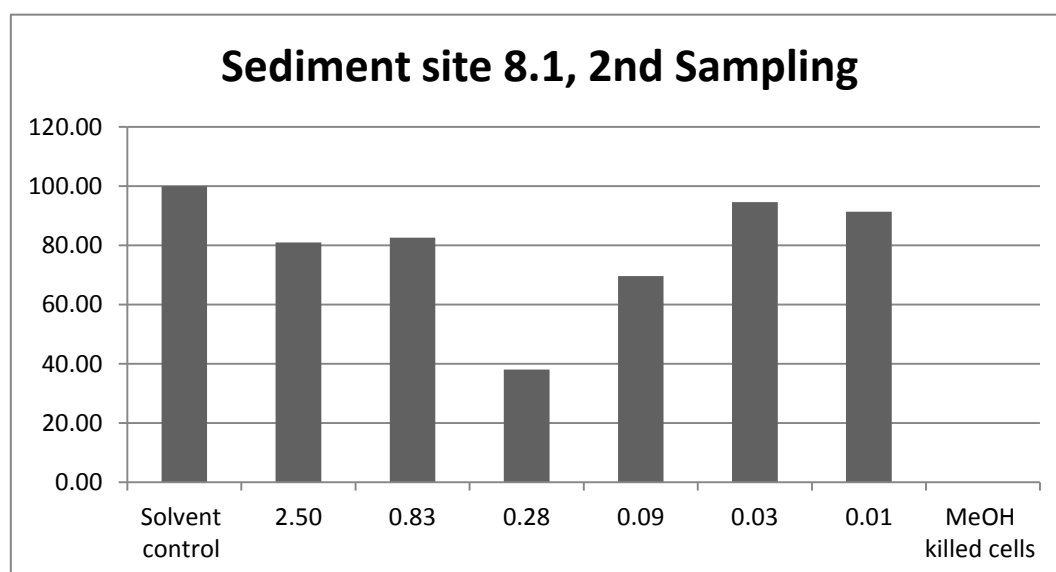
Absorbance reading from plate reader				Site 9 Sediment			
Raw data	1	2	3				
Solvent control	0.161	0.199	0.219				
2.50	0.225	0.131	0.181				
0.83	0.199	0.16	0.184				
0.28	0.132	0.126	0.121				
0.09	0.192	0.174	0.129				
0.03	0.209	0.185	0.193				
0.01	0.148	0.209	0.218				
MeOH killed cells	0.013	0.012	0.091				
MeOH killed cells subtracted							
Solvent control	0.122	0.160	0.180				
2.50	0.186	0.092	0.142				
0.83	0.160	0.121	0.145				
0.28	0.093	0.087	0.082				
0.09	0.153	0.135	0.090				
0.03	0.170	0.146	0.154				
0.01	0.109	0.170	0.179				
MeOH killed cells	-0.026	0.027	0.052				
% of live cells	1	2	3	Average	stdev	CV	Ttest
Solvent control	79.27	103.8	116.	100.00	19.09	19.0	1.00
2.50	120.7	59.83	92.2	90.93	30.47	33.5	0.02
0.83	103.8	103.8	94.1	100.65	5.61	5.58	0.16
0.28	60.48	56.59	53.3	56.80	3.57	6.28	0.08
0.09	99.35	87.69	58.5	81.86	21.03	25.6	0.00
0.03	110.3	94.82	100.	101.73	7.92	7.78	0.04
0.01	70.84	110.3	116.	99.14	24.68	24.8	0.30
MeOH killed cells	0	1	0	0.00	0	0.00	0.00



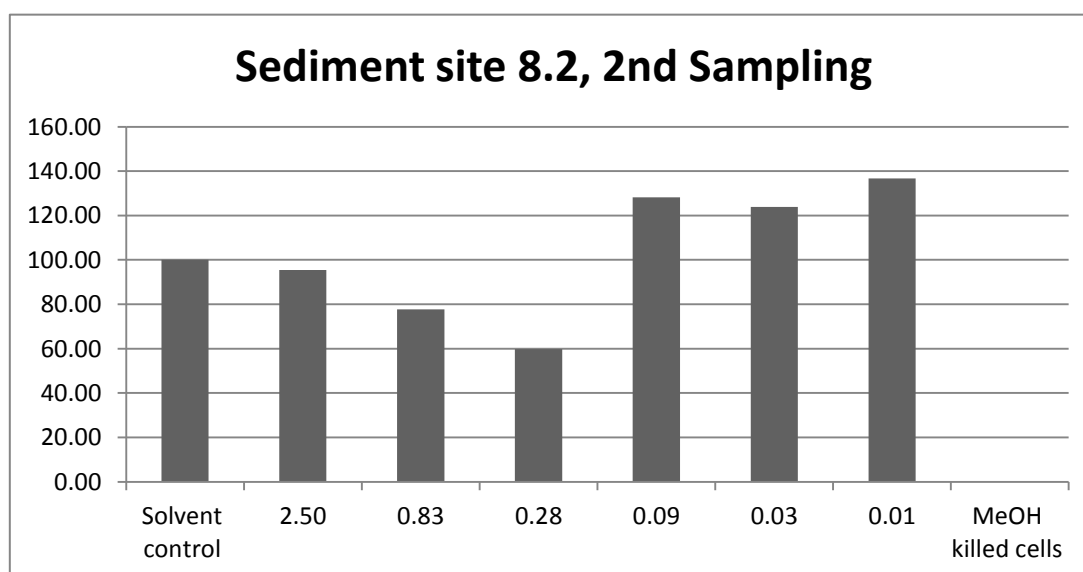
Absorbance reading from plate reader				Site 8 Pump Sediment			
Raw data	1	2	3				
Solvent control	0.161	0.199	0.219				
2.50	0.164	0.148	0.154				
0.83	0.142	0.154	0.13				
0.28	0.089	0.112	0.092				
0.09	0.175	0.239	0.167				
0.03	0.202	0.205	0.169				
0.01	0.202	0.157	0.165				
MeOH killed cells	0.013	0.012	0.091				
MeOH killed cells subtracted							
Solvent control	0.122	0.160	0.180				
2.50	0.125	0.109	0.115				
0.83	0.103	0.115	0.091				
0.28	0.050	0.073	0.053				
0.09	0.136	0.200	0.128				
0.03	0.163	0.166	0.130				
0.01	0.163	0.118	0.126				
MeOH killed cells	0.026	0.027	0.052				
% of live cells	1	2	3	Average	stdev	CV	Ttest
Solvent control	79.27	103.1	116.1	100.00	19.09	19.0	1.00
2.50	81.21	93.71	74.7	83.22	9.65	11.6	0.02
0.83	66.95	111.1	59.18	79.25	28.30	35.1	0.16
0.28	32.61	124.2	34.56	63.82	52.38	82.7	0.08
0.09	88.34	129.2	83.15	100.25	25.25	25.1	0.00
0.03	105.8	108.4	84.45	99.59	13.18	13.2	0.04
0.01	105.8	87.01	81.86	91.57	12.62	13.7	0.30
MeOH killed cells	16.63	17.28	29.02	-1.63	26.55	0.00	0.00



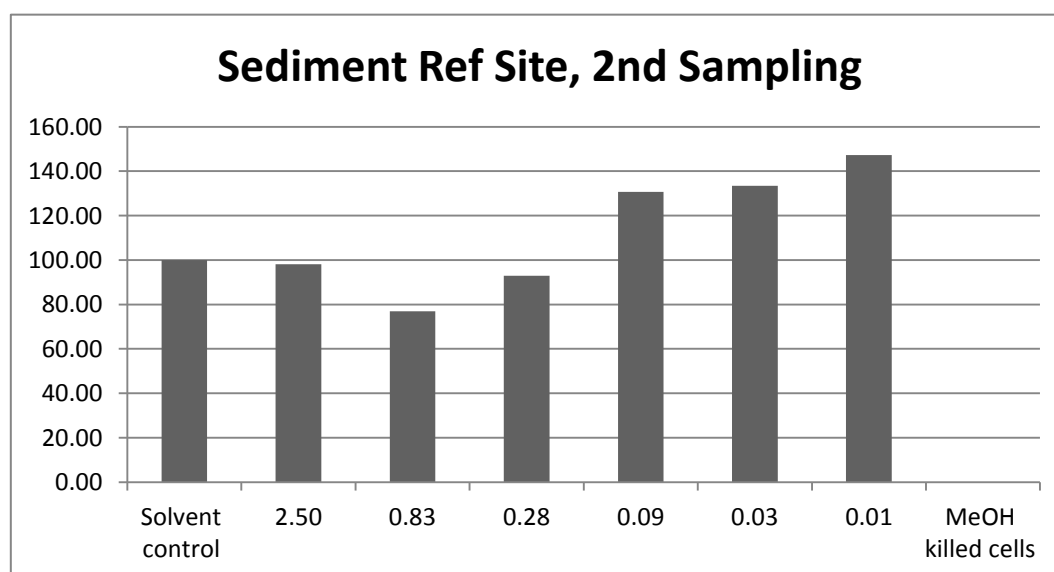
Absorbance reading from plate reader				Site 8.1 Sediment			
Raw data	1	2	3				
Solvent control	0.202	0.222	0.183				
2.50	0.225	0.131	0.181				
0.83	0.199	0.16	0.184				
0.28	0.132	0.126	0.121				
0.09	0.192	0.174	0.129				
0.03	0.209	0.185	0.193				
0.01	0.148	0.209	0.218				
MeOH killed cells	0.161	0.019	0.059				
MeOH killed cells subtracted							
Solvent control	0.122	0.142	0.103				
2.50	0.145	0.051	0.101				
0.83	0.119	0.080	0.104				
0.28	0.052	0.046	0.041				
0.09	0.112	0.094	0.049				
0.03	0.129	0.105	0.113				
0.01	0.068	0.129	0.138				
MeOH killed cells	0.081	0.061	-0.021				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	99.73	116.0	84.24	100.00	15.90	15.9	1.00
2.50	118.4	41.85	82.61	80.98	38.34	47.3	0.47
0.83	97.28	65.49	85.05	82.61	16.04	19.4	0.25
0.28	42.66	37.77	33.70	38.04	4.49	11.8	0.00
0.09	91.58	76.90	40.22	69.57	26.45	38.0	0.16
0.03	105.4	85.87	92.39	94.57	9.96	10.5	0.64
0.01	55.71	105.4	112.77	91.30	31.05	34.0	0.69
MeOH killed cells	66.30	49.46	-16.85	0.00	59.69	0.00	0.05



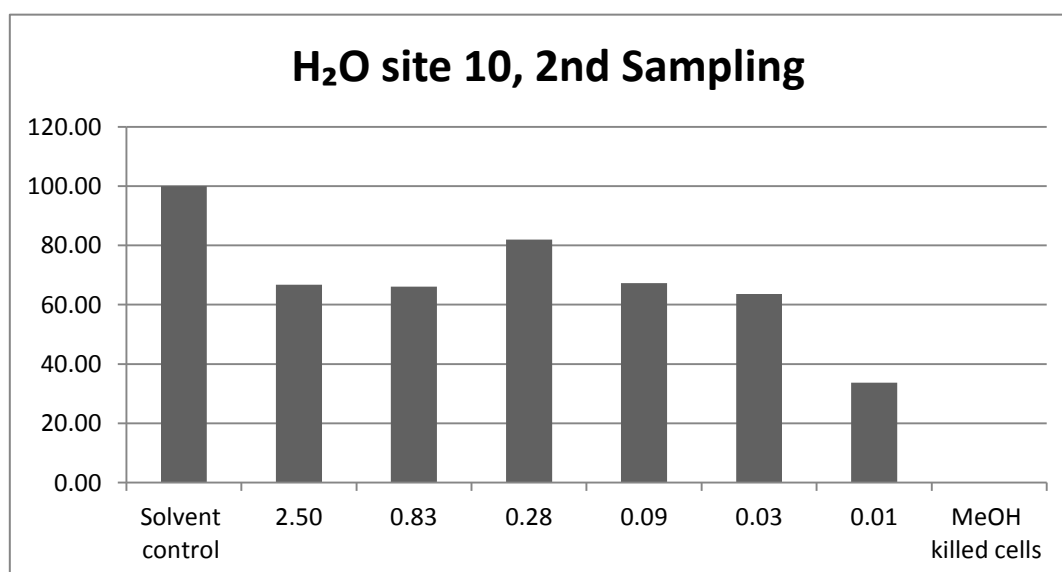
Absorbance reading from plate reader				Site 8.2 Sediment			
Raw data	1	2	3				
Solvent control	0.202	0.222	0.183				
2.50	0.182	0.181	0.227				
0.83	0.183	0.158	0.184				
0.28	0.128	0.167	0.164				
0.09	0.255	0.245	0.211				
0.03	0.228	0.231	0.236				
0.01	0.247	0.247	0.248				
MeOH killed cells	0.161	0.019	0.059				
MeOH killed cells subtracted							
Solvent control	0.122	0.142	0.103				
2.50	0.102	0.101	0.147				
0.83	0.103	0.078	0.104				
0.28	0.048	0.087	0.084				
0.09	0.175	0.165	0.131				
0.03	0.148	0.151	0.156				
0.01	0.167	0.167	0.168				
MeOH killed cells	0.081	0.061	-0.021				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	99.73	116.0	84.24	100.00	15.90	15.9	1.00
2.50	83.42	82.61	120.11	95.38	21.42	22.4	0.78
0.83	84.24	63.86	85.05	77.72	12.01	15.4	0.12
0.28	39.40	71.20	68.75	59.78	17.69	29.5	0.04
0.09	142.9	134.7	107.07	128.26	18.80	14.6	0.12
0.03	120.9	123.3	127.45	123.91	3.29	2.66	0.06
0.01	136.4	136.4	137.23	136.68	0.47	0.34	0.02
MeOH killed cells	66.30	49.46	-16.85	0.00	59.69	0.00	0.05



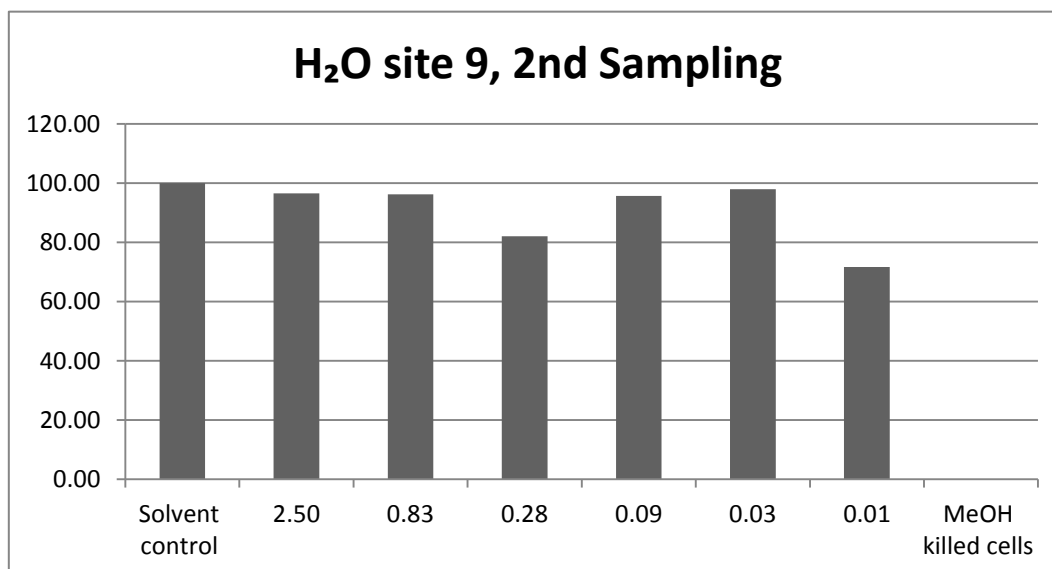
Absorbance reading from plate reader				Ref Site Sediment			
Raw data	1	2	3				
Solvent control	0.202	0.222	0.183				
2.50	0.191	0.214	0.195				
0.83	0.216	0.155	0.151				
0.28	0.215	0.185	0.181				
0.09	0.241	0.243	0.236				
0.03	0.256	0.252	0.222				
0.01	0.265	0.269	0.247				
MeOH killed cells	0.161	0.019	0.059				
MeOH killed cells subtracted							
Solvent control	0.122	0.142	0.103				
2.50	0.111	0.134	0.115				
0.83	0.136	0.075	0.071				
0.28	0.135	0.105	0.101				
0.09	0.161	0.163	0.156				
0.03	0.176	0.172	0.142				
0.01	0.185	0.189	0.167				
MeOH killed cells	0.081	0.061	-0.021				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	99.73	116.0	84.24	100.00	15.90	15.9	1.00
2.50	90.76	109.5	94.02	98.10	10.02	10.2	0.87
0.83	111.1	61.41	58.15	76.90	29.70	38.6	0.30
0.28	110.3	85.87	82.61	92.93	15.15	16.3	0.61
0.09	131.5	133.1	127.45	130.71	2.94	2.25	0.03
0.03	143.7	140.4	116.03	133.42	15.15	11.3	0.06
0.01	151.0	154.3	136.41	147.28	9.55	6.49	0.01
MeOH killed cells	66.30	49.46	-16.85	0.00	59.69	0.00	0.05



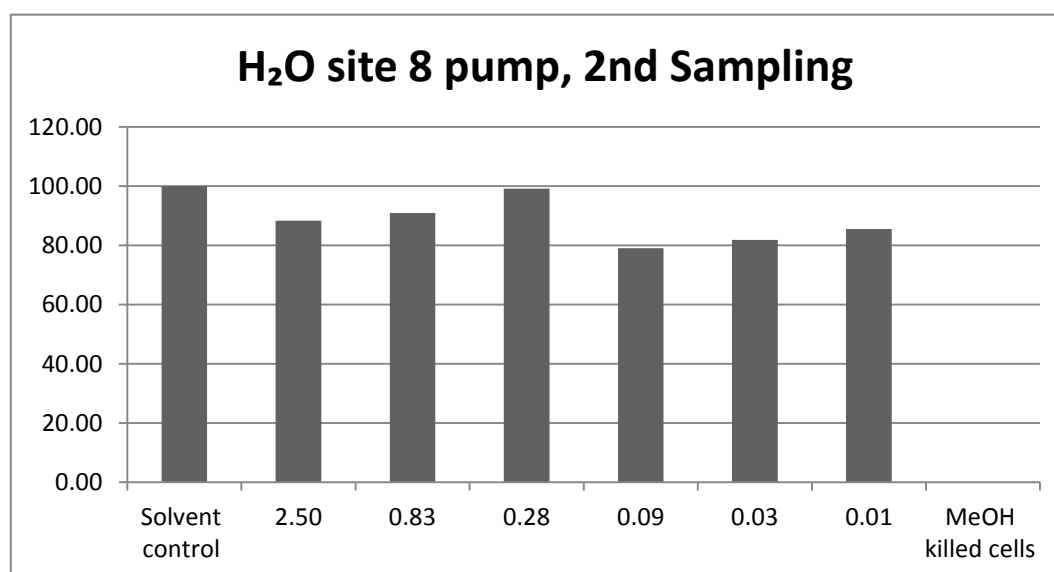
Absorbance reading from plate reader				Site 10 H ₂ O			
Raw data	1	2	3				
Solvent control	0.23	0.272	0.281				
2.50	0.191	0.204	0.198				
0.83	0.197	0.196	0.196				
0.28	0.208	0.228	0.244				
0.09	0.181	0.208	0.207				
0.03	0.182	0.204	0.189				
0.01	0.147	0.128	0.129				
MeOH killed cells	0.026	0.028	0.157				
MeOH killed cells subtracted							
Solvent control	0.160	0.202	0.211				
2.50	0.121	0.134	0.128				
0.83	0.127	0.126	0.126				
0.28	0.138	0.158	0.174				
0.09	0.111	0.138	0.137				
0.03	0.112	0.134	0.119				
0.01	0.077	0.058	0.059				
MeOH killed cells	-0.044	0.042	0.087				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	83.74	105.77	110.4	100.00	14.28	14.3	1.00
2.50	63.29	70.10	66.96	66.78	3.41	5.1	0.02
0.83	66.43	65.91	65.91	66.08	0.30	0.5	0.01
0.28	72.20	82.69	91.08	81.99	9.46	11.5	0.14
0.09	58.04	72.20	71.68	67.31	8.03	11.9	0.03
0.03	58.57	70.10	62.24	63.64	5.90	9.3	0.02
0.01	40.21	30.24	30.77	33.74	5.61	16.6	0.00
MeOH killed cells	-23.25	-22.20	45.45	0.00	39.37	0.0	0.01



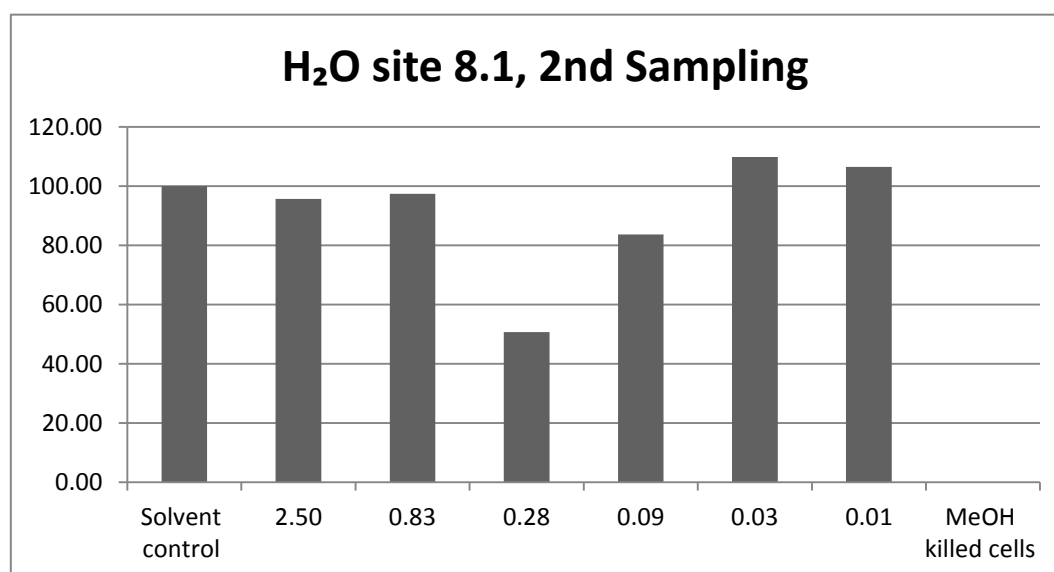
Absorbance reading from plate reader				Site 9 H ₂ O			
Raw data	1	2	3				
Solvent control	0.23	0.272	0.281				
2.50	0.258	0.241	0.264				
0.83	0.256	0.244	0.249				
0.28	0.261	0.17	0.249				
0.09	0.249	0.266	0.243				
0.03	0.262	0.233	0.276				
0.01	0.225	0.196	0.2				
MeOH killed cells	0.026	0.028	0.157				
MeOH killed cells subtracted							
Solvent control	0.160	0.202	0.211				
2.50	0.188	0.171	0.194				
0.83	0.186	0.174	0.179				
0.28	0.191	0.100	0.179				
0.09	0.179	0.196	0.173				
0.03	0.192	0.163	0.206				
0.01	0.155	0.126	0.130				
MeOH killed cells	-0.044	-0.042	0.087				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	83.74	105.77	110.49	100.00	14.28	14.28	1.00
2.50	98.43	89.51	101.57	96.50	6.26	6.48	0.72
0.83	97.38	97.38	93.71	96.15	2.12	2.20	0.67
0.28	100.00	52.27	93.71	81.99	25.93	31.62	0.35
0.09	93.71	102.62	90.56	95.63	6.26	6.54	0.65
0.03	100.52	85.31	107.87	97.90	11.50	11.75	0.85
0.01	81.12	65.91	68.01	71.68	8.24	11.50	0.04
MeOH killed cells	-23.25	-22.20	45.45	0.00	39.37	0.00	0.01



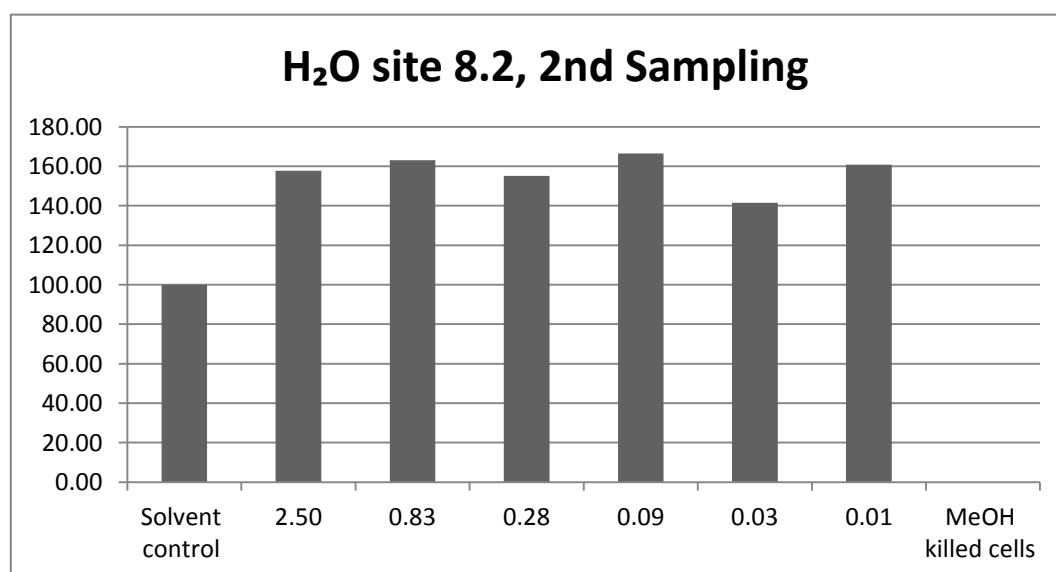
Absorbance reading from plate reader				Site 8 pump H ₂ O			
Raw data	1	2	3				
Solvent control	0.23	0.272	0.281				
2.50	0.247	0.215	0.254				
0.83	0.247	0.216	0.268				
0.28	0.251	0.27	0.257				
0.09	0.218	0.207	0.238				
0.03	0.224	0.216	0.239				
0.01	0.232	0.225	0.243				
MeOH killed cells	0.026	0.028	0.157				
MeOH killed cells subtracted							
Solvent control	0.160	0.202	0.211				
2.50	0.177	0.145	0.184				
0.83	0.177	0.146	0.198				
0.28	0.181	0.200	0.187				
0.09	0.148	0.137	0.168				
0.03	0.154	0.146	0.169				
0.01	0.162	0.155	0.173				
MeOH killed cells	0.044	0.042	0.087				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	83.74	105.7	110.49	100.00	14.28	14.2	1.00
2.50	92.66	75.87	96.33	88.29	10.91	12.3	0.32
0.83	92.66	76.40	103.67	90.91	13.72	15.0	0.47
0.28	94.76	104.7	97.90	99.13	5.09	5.14	0.93
0.09	77.45	71.68	87.94	79.02	8.24	10.4	0.09
0.03	80.59	76.40	88.46	81.82	6.12	7.48	0.11
0.01	84.79	81.12	90.56	85.49	4.76	5.57	0.17
MeOH killed cells	23.25	22.20	45.45	0.00	39.37	0.00	0.01



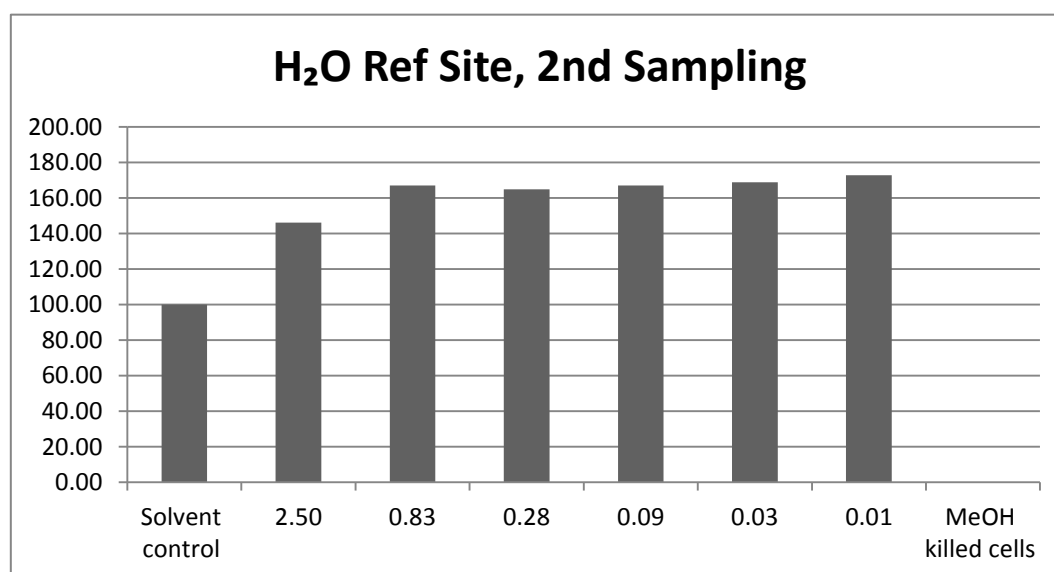
Absorbance reading from plate reader				Site 8.1 H ₂ O			
Raw data	1	2	3				
Solvent control	0.26	0.023	0.269				
		4					
2.50	0.225	0.131	0.181				
0.83	0.199	0.16	0.184				
0.28	0.132	0.126	0.121				
0.09	0.192	0.174	0.129				
0.03	0.209	0.185	0.193				
0.01	0.148	0.209	0.218				
MeOH killed cells	0.018	0.012	0.171				
MeOH killed cells subtracted							
Solvent control	0.193	0.044	0.202				
2.50	0.158	0.064	0.114				
0.83	0.132	0.093	0.117				
0.28	0.065	0.059	0.054				
0.09	0.125	0.107	0.062				
0.03	0.142	0.118	0.126				
0.01	0.081	0.142	0.151				
MeOH killed cells	0.049	0.055	0.104				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	164.7	37.22	172.45	100.00	118.90	118.	1.00
2.50	134.8	54.64	97.32	95.62	40.15	41.9	0.95
0.83	112.6	79.40	99.89	97.32	16.79	17.2	0.97
0.28	55.49	50.37	46.10	50.65	4.70	9.28	0.51
0.09	106.7	91.35	52.93	83.67	27.70	33.1	0.83
0.03	121.2	100.7	107.57	109.85	10.43	9.50	0.89
0.01	69.15	121.2	128.91	106.43	32.51	30.5	0.93
MeOH killed cells	41.83	46.96	88.79	0.00	76.94	0.00	0.29



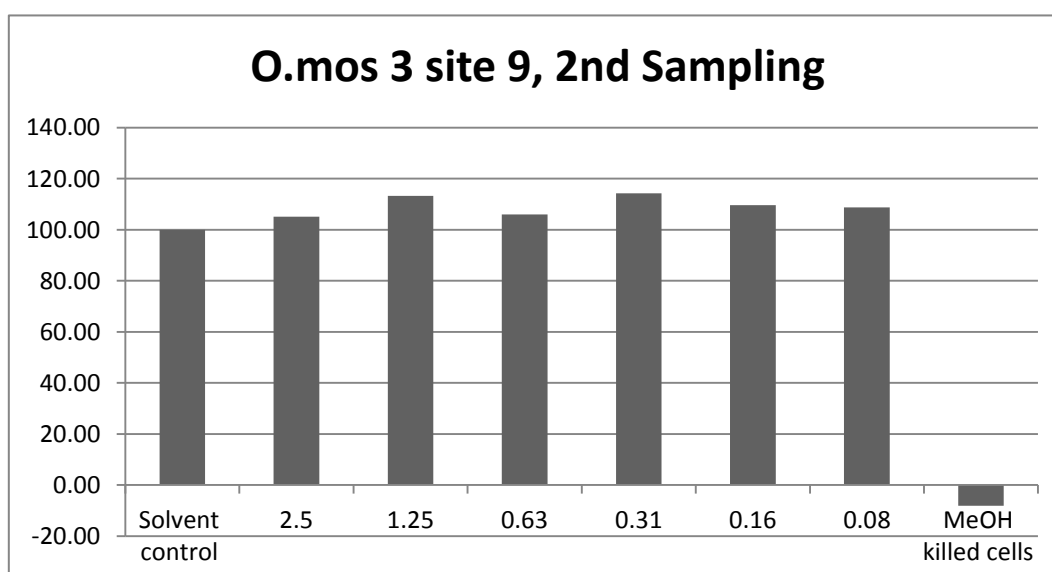
Absorbance reading from plate reader				Site 8.2 H ₂ O			
Raw data	1	2	3				
Solvent control	0.26	0.023	0.269				
2.50	0.245	0.255	0.255				
0.83	0.272	0.252	0.25				
0.28	0.259	0.246	0.241				
0.09	0.278	0.259	0.249				
0.03	0.243	0.246	0.209				
0.01	0.25	0.25	0.266				
MeOH killed cells	0.018	0.012	0.171				
MeOH killed cells subtracted							
Solvent control	0.193	0.044	0.202				
2.50	0.178	0.188	0.188				
0.83	0.205	0.185	0.183				
0.28	0.192	0.179	0.174				
0.09	0.211	0.192	0.182				
0.03	0.176	0.179	0.142				
0.01	0.183	0.183	0.199				
MeOH killed cells	0.049	0.055	0.104				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	164.7	37.22	172.45	100.00	118.90	118	1.00
2.50	151.9	160.5	160.50	157.66	4.93	3.13	0.45
0.83	175.0	157.	156.23	163.06	10.39	6.37	0.41
0.28	163.9	152.8	148.55	155.09	7.93	5.11	0.47
0.09	180.1	163.9	155.38	166.48	12.58	7.55	0.39
0.03	150.2	152.8	121.23	141.43	17.54	12.4	0.58
0.01	156.2	156.2	169.89	160.79	7.89	4.90	0.43
MeOH killed cells	41.83	46.96	88.79	0.00	76.94	0.00	0.29



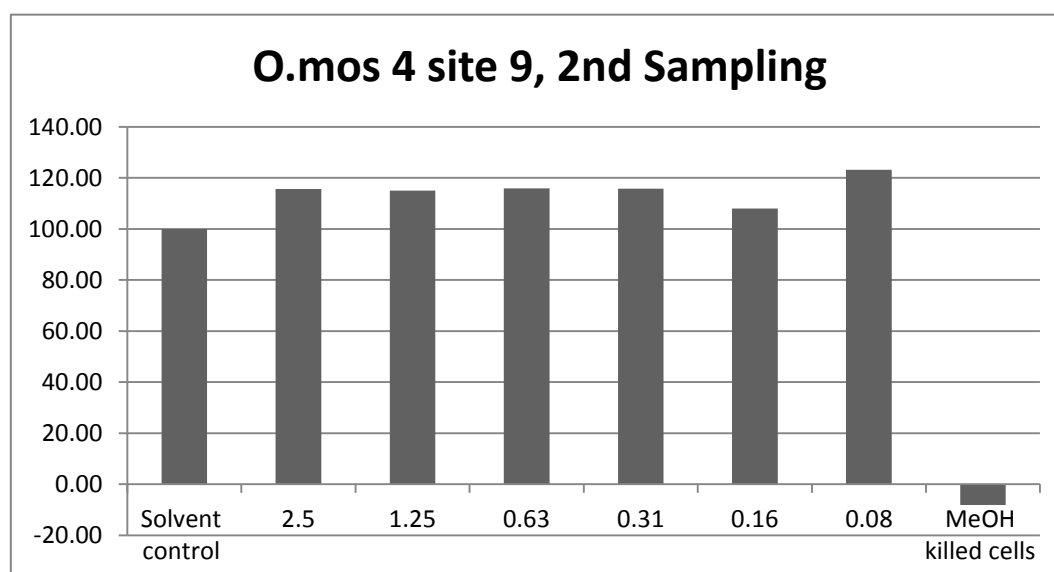
Absorbance reading from plate reader				Ref Site H ₂ O			
Raw data	1	2	3				
Solvent control	0.26	0.023	0.269				
2.50	0.225	0.243	0.246				
0.83	0.253	0.277	0.258				
0.28	0.267	0.224	0.289				
0.09	0.278	0.253	0.257				
0.03	0.263	0.27	0.261				
0.01	0.279	0.277	0.252				
MeOH killed cells	0.018	0.012	0.171				
MeOH killed cells subtracted							
Solvent control	0.193	0.044	0.202				
2.50	0.158	0.176	0.179				
0.83	0.186	0.210	0.191				
0.28	0.200	0.157	0.222				
0.09	0.211	0.186	0.190				
0.03	0.196	0.203	0.194				
0.01	0.212	0.210	0.185				
MeOH killed cells	0.049	0.055	0.104				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	164.7	37.22	172.45	100.00	118.90	118	1.00
2.50	134.8	150.2	152.82	145.99	9.70	6.64	0.54
0.83	158.7	179.2	163.06	167.05	10.81	6.47	0.39
0.28	170.7	134.0	189.53	164.77	28.22	17.1	0.41
0.09	180.1	158.7	162.21	167.05	11.46	6.86	0.39
0.03	167.3	173.3	165.62	168.75	4.03	2.39	0.37
0.01	180.9	179.2	157.94	172.74	12.84	7.44	0.35
MeOH killed cells	41.83	46.96	88.79	0.00	76.94	0.00	0.29



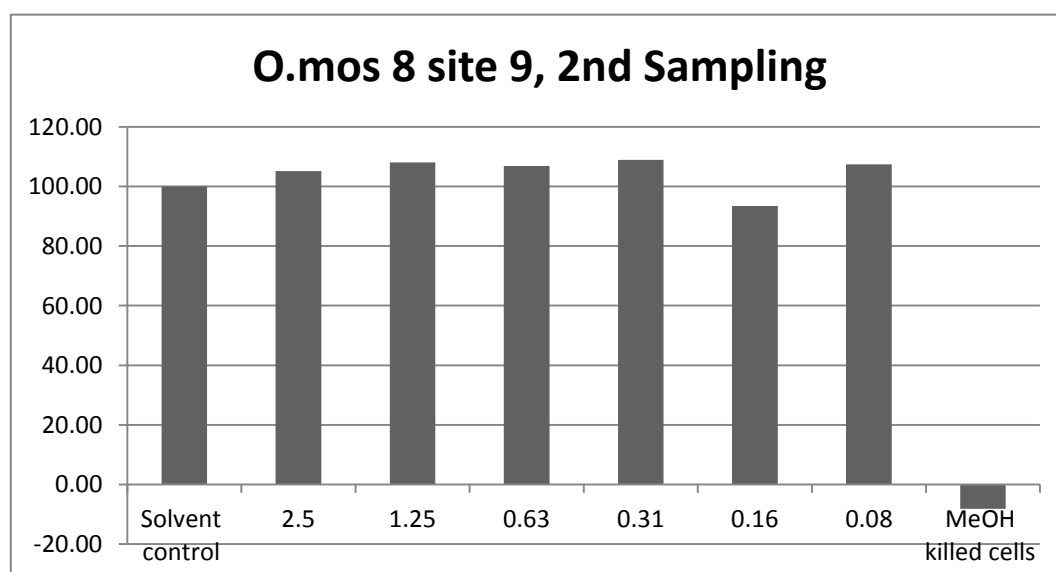
Absorbance reading from plate reader				O.mos 3 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.385	0.397	0.437				
2.5	0.414	0.435	0.409				
1.25	0.442	0.468	0.411				
0.63	0.432	0.409	0.424				
0.31	0.452	0.429	0.448				
0.16	0.438	0.415	0.44				
0.08	0.397	0.438	0.451				
MeOH killed cells	0.03	0.178	0.176	0.191	0.191	0.186	0.189
MeOH killed cells subtracted							
Solvent control	0.236	0.248	0.288				
2.5	0.265	0.286	0.260				
1.25	0.293	0.319	0.262				
0.63	0.283	0.260	0.275				
0.31	0.303	0.280	0.299				
0.16	0.289	0.266	0.291				
0.08	0.248	0.289	0.302				
MeOH killed cells	-0.119	0.029	0.027				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	91.71	96.37	111.9	100.00	10.58	10.6	1.00
2.5	102.98	111.14	101.0	105.05	5.36	5.1	0.50
1.25	113.86	123.96	101.8	113.21	11.09	9.8	0.21
0.63	109.97	101.04	106.8	105.96	4.54	4.3	0.42
0.31	117.75	108.81	116.1	114.25	4.78	4.2	0.10
0.16	112.31	103.37	113.0	109.59	5.40	4.9	0.23
0.08	96.37	112.31	117.3	108.68	10.95	10.1	0.38
MeOH killed cells	-46.24	11.27	10.49	-8.16	32.98	-404.2	0.01



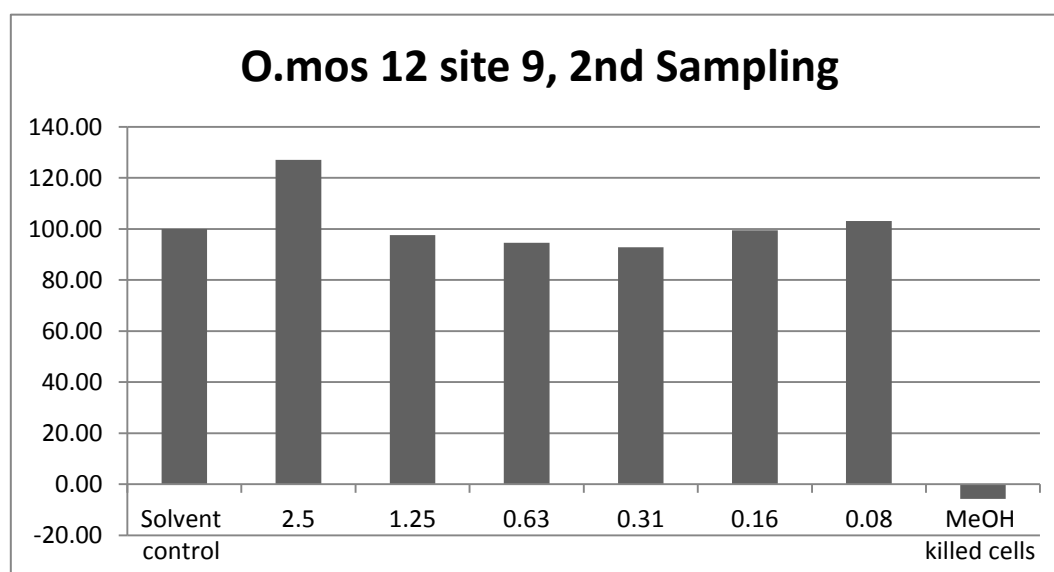
Absorbance reading from plate reader				O.mos 4 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.385	0.397	0.437				
2.5	0.425	0.473	0.442				
1.25	0.439	0.447	0.449				
0.63	0.438	0.441	0.463				
0.31	0.436	0.465	0.44				
0.16	0.418	0.423	0.44				
0.08	0.475	0.454	0.469				
MeOH killed cells	0.03	0.178	0.176	0.191	0.191	0.1	0.18
MeOH killed cells subtracted							
Solvent control	0.236	0.248	0.288				
2.5	0.276	0.324	0.293				
1.25	0.290	0.298	0.300				
0.63	0.289	0.292	0.314				
0.31	0.287	0.316	0.291				
0.16	0.269	0.274	0.291				
0.08	0.326	0.305	0.320				
MeOH killed cells	-0.119	0.029	0.027				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	91.71	96.37	111.9	100.00	10.58	10.6	1.00
2.5	107.25	125.9	113.8	115.67	9.46	8.2	0.13
1.25	112.69	115.8	116.5	115.03	2.06	1.8	0.07
0.63	112.31	113.4	122.0	115.93	5.30	4.6	0.08
0.31	111.53	122.8	113.0	115.80	6.11	5.3	0.09
0.16	104.53	106.4	113.0	108.03	4.48	4.1	0.29
0.08	126.68	118.5	124.35	123.19	4.20	3.4	0.02
MeOH killed cells	-46.24	11.27	10.49	-8.16	32.98	1.00	0.01



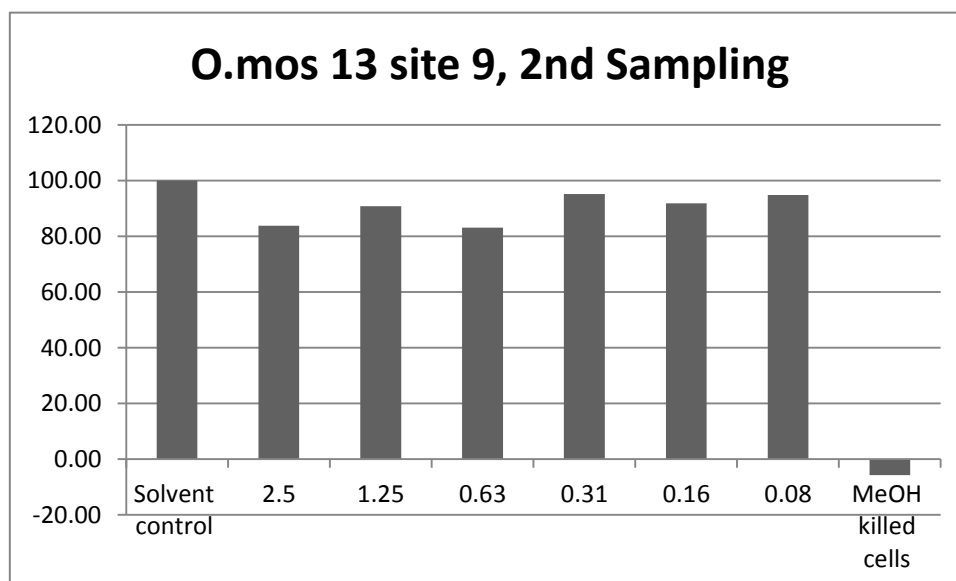
Absorbance reading from plate reader				O.mos 8 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.385	0.397	0.437				
2.5	0.403	0.422	0.434				
1.25	0.423	0.434	0.424				
0.63	0.414	0.423	0.435				
0.31	0.428	0.431	0.429				
0.16	0.414	0.368	0.386				
0.08	0.43	0.422	0.424				
MeOH killed cells	0.03	0.178	0.176	0.191	0.191	0.18	0.18
MeOH killed cells subtracted							
Solvent control	0.236	0.248	0.288				
2.5	0.254	0.273	0.285				
1.25	0.274	0.285	0.275				
0.63	0.265	0.274	0.286				
0.31	0.279	0.282	0.280				
0.16	0.265	0.219	0.237				
0.08	0.281	0.273	0.275				
MeOH killed cells	0.119	0.029	0.027				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	91.71	96.37	111.92	100.00	10.58	10.6	1.00
2.5	98.70	106.9	110.75	105.18	6.07	5.8	0.50
1.25	106.4	110.7	106.87	108.03	2.36	2.2	0.27
0.63	102.9	106.4	111.14	106.87	4.09	3.8	0.35
0.31	108.4	109.5	108.81	108.94	0.59	0.5	0.22
0.16	102.9	85.10	92.10	93.39	9.01	9.6	0.46
0.08	109.2	106.0	106.87	107.38	1.62	1.5	0.30
MeOH killed cells	-46.24	11.27	10.49	-8.16	32.98	-4	0.01



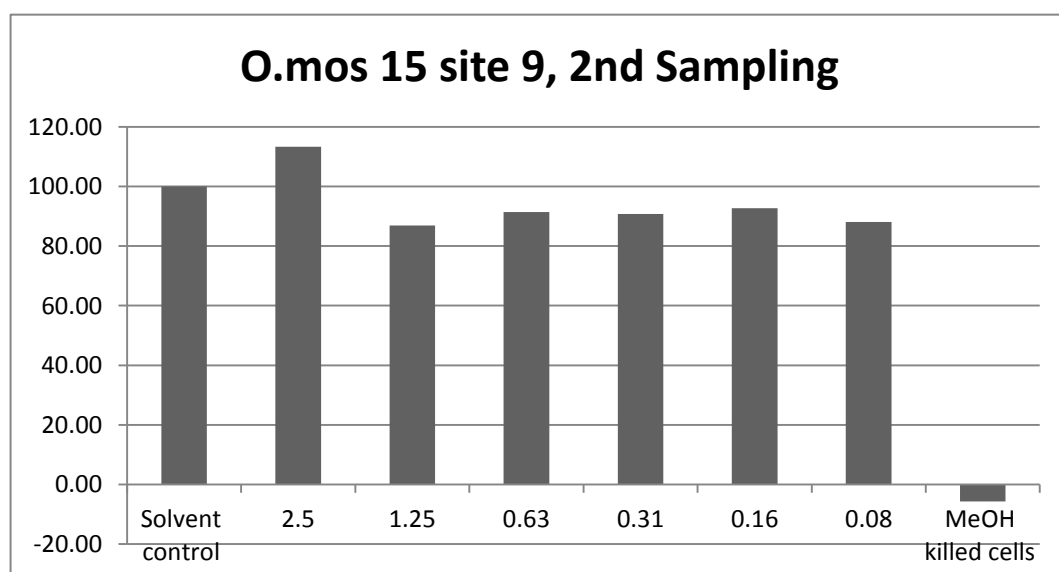
Absorbance reading from plate reader				O.mos 12 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.292	0.331	0.325				
2.5	0.282	0.287	0.534				
1.25	0.315	0.304	0.315				
0.63	0.311	0.305	0.301				
0.31	0.327	0.294	0.286				
0.16	0.328	0.317	0.3				
0.08	0.32	0.331	0.315				
MeOH killed cells	0.025	0.16	0.158	0.163	0.147	0.15	0.16
MeOH killed cells subtracted							
Solvent control	0.167	0.206	0.200				
2.5	0.157	0.162	0.409				
1.25	0.190	0.179	0.190				
0.63	0.186	0.180	0.176				
0.31	0.202	0.169	0.161				
0.16	0.203	0.192	0.175				
0.08	0.195	0.206	0.190				
MeOH killed cells	0.100	0.035	0.033				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	87.42	107.8	104.72	100.00	11.01	11.0	1.00
2.5	82.18	84.80	214.29	127.09	75.53	59.4	0.57
1.25	99.48	93.71	99.48	97.55	3.33	3.4	0.73
0.63	97.38	94.23	92.14	94.58	2.64	2.8	0.45
0.31	105.7	88.47	84.27	92.84	11.39	12.3	0.48
0.16	106.2	100.5	91.61	99.48	7.40	7.4	0.95
0.08	102.1	107.8	99.48	103.15	4.29	4.2	0.67
MeOH killed cells	52.56	18.22	17.17	-5.72	40.56	-7	0.01



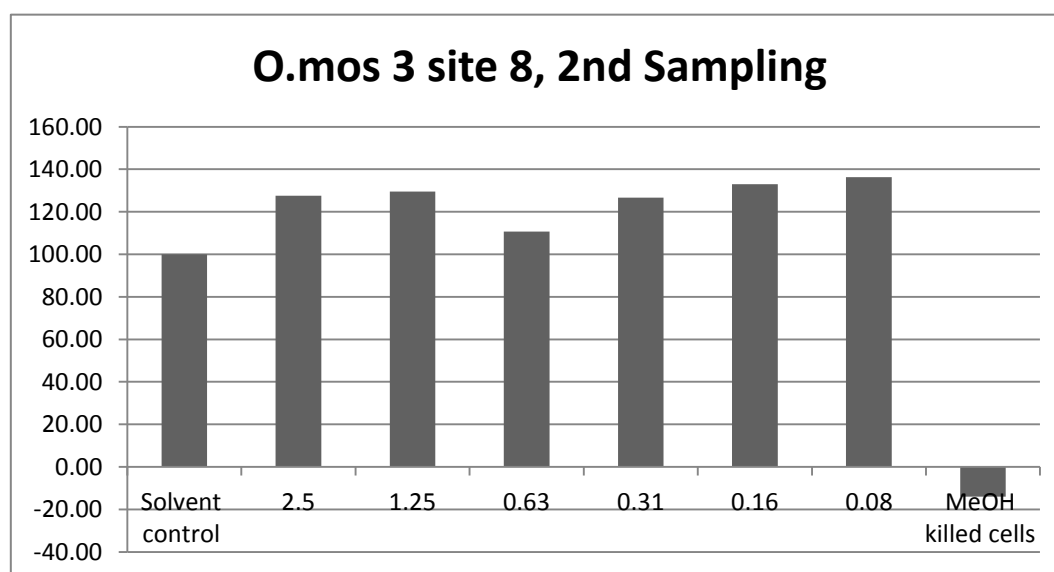
Absorbance reading from plate reader				O.mos 13 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.292	0.331	0.325				
2.5	0.284	0.287	0.284				
1.25	0.279	0.315	0.301				
0.63	0.275	0.294	0.282				
0.31	0.299	0.315	0.306				
0.16	0.301	0.285	0.315				
0.08	0.31	0.292	0.316				
MeOH killed cells	0.025	0.16	0.158	0.163	0.147	0.15	0.16
MeOH killed cells subtracted							
Solvent control	0.167	0.206	0.200				
2.5	0.159	0.162	0.159				
1.25	0.154	0.190	0.176				
0.63	0.150	0.169	0.157				
0.31	0.174	0.190	0.181				
0.16	0.176	0.160	0.190				
0.08	0.185	0.167	0.191				
MeOH killed cells	0.100	0.035	0.033				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	87.42	107.8	104.72	100.00	11.01	11.0	1.00
2.5	83.22	84.80	83.22	83.75	0.91	1.1	0.06
1.25	80.60	99.48	92.14	90.74	9.51	10.5	0.33
0.63	78.51	88.47	82.18	83.05	5.04	6.1	0.07
0.31	91.09	99.48	94.76	95.11	4.20	4.4	0.51
0.16	92.14	83.75	99.48	91.79	7.87	8.6	0.35
0.08	96.85	87.42	100.00	94.76	6.55	6.9	0.52
MeOH killed cells	52.56	18.22	17.17	-5.72	40.56	-7	0.01



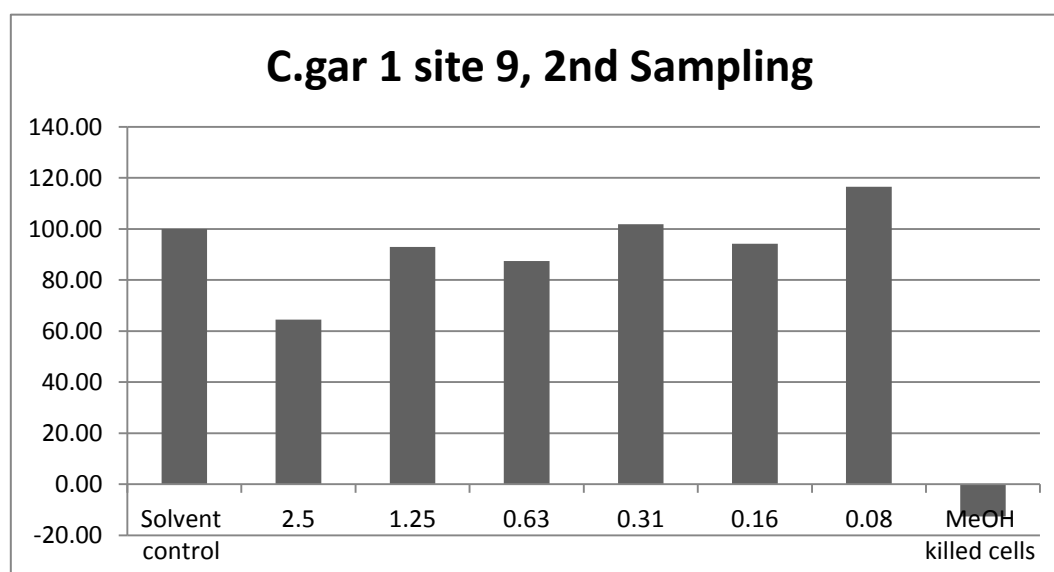
Absorbance reading from plate reader				O.mos 15 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.292	0.331	0.325				
2.5	0.276	0.475	0.273				
1.25	0.276	0.298	0.299				
0.63	0.292	0.315	0.292				
0.31	0.298	0.299	0.298				
0.16	0.298	0.306	0.302				
0.08	0.282	0.309	0.289				
MeOH killed cells	0.025	0.16	0.158	0.163	0.147	0.15	0.16
MeOH killed cells subtracted							
Solvent control	0.167	0.206	0.200				
2.5	0.151	0.350	0.148				
1.25	0.151	0.173	0.174				
0.63	0.167	0.190	0.167				
0.31	0.173	0.174	0.173				
0.16	0.173	0.181	0.177				
0.08	0.157	0.184	0.164				
MeOH killed cells	0.100	0.035	0.033				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	87.42	107.8	104.72	100.00	11.01	11.0	1.00
2.5	79.03	183.3	77.46	113.28	60.69	53.6	0.73
1.25	79.03	90.56	91.09	86.89	6.82	7.8	0.15
0.63	87.42	99.48	87.42	91.44	6.96	7.6	0.32
0.31	90.56	91.09	90.56	90.74	0.30	0.3	0.22
0.16	90.56	94.76	92.66	92.66	2.10	2.3	0.32
0.08	82.18	96.33	85.85	88.12	7.35	8.3	0.19
MeOH killed cells	52.56	18.22	17.17	-5.72	40.56	-7	0.01



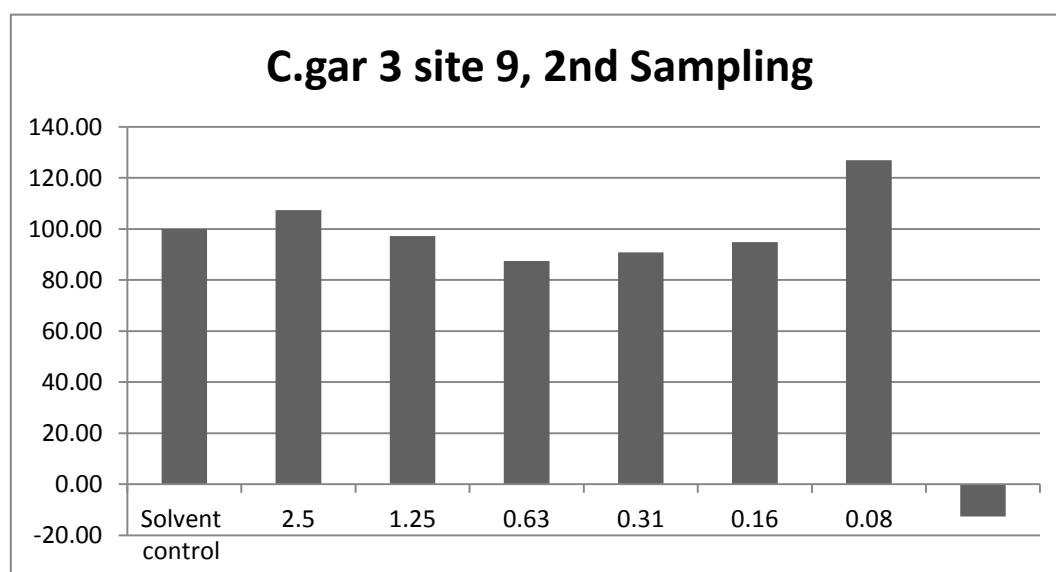
Absorbance reading from plate reader				O.mos 3 Site 8 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.291	0.312	0.295				
2.5	0.31	0.349	0.363				
1.25	0.35	0.349	0.332				
0.63	0.346	0.3	0.3				
0.31	0.344	0.311	0.363				
0.16	0.34	0.35	0.357				
0.08	0.371	0.352	0.339				
MeOH killed cells	0.03	0.178	0.176	0.191	0.191	0.18	0.18
MeOH killed cells subtracted							
Solvent control	0.142	0.163	0.146				
2.5	0.161	0.200	0.214				
1.25	0.201	0.200	0.183				
0.63	0.197	0.151	0.151				
0.31	0.195	0.162	0.214				
0.16	0.191	0.201	0.208				
0.08	0.222	0.203	0.190				
MeOH killed cells	0.119	0.029	0.027				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	94.46	108.4	97.12	100.00	7.42	7.4	1.00
2.5	107.1	133.0	142.35	127.49	18.27	14.3	0.07
1.25	133.7	133.0	121.73	129.49	6.73	5.2	0.01
0.63	131.0	100.4	100.44	110.64	17.67	16.0	0.39
0.31	129.7	107.7	142.35	126.61	17.50	13.8	0.07
0.16	127.0	133.7	138.36	133.04	5.68	4.3	0.00
0.08	147.6	135.0	126.39	136.36	10.71	7.9	0.01
MeOH killed cells	79.16	19.29	17.96	-13.97	56.46	-4	0.03



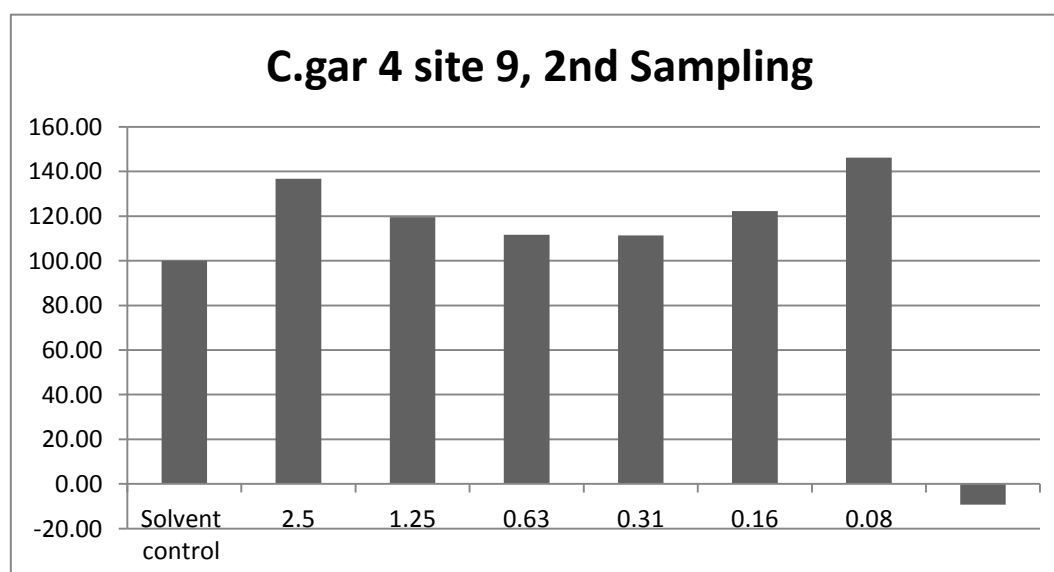
Absorbance reading from plate reader				C.gar 1 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.275	0.278	0.316				
2.5	0.297	0.167	0.289				
1.25	0.282	0.278	0.286				
0.63	0.274	0.275	0.279				
0.31	0.292	0.299	0.284				
0.16	0.284	0.283	0.283				
0.08	0.315	0.301	0.307				
MeOH killed cells	0.02	0.227	0.254	0.238	0.243	0.242	0.20
MeOH killed cells subtracted							
Solvent control	0.094	0.097	0.135				
2.5	0.116	-0.014	0.108				
1.25	0.101	0.097	0.105				
0.63	0.093	0.094	0.098				
0.31	0.111	0.118	0.103				
0.16	0.103	0.102	0.102				
0.08	0.134	0.120	0.126				
MeOH killed cells	-0.161	0.046	0.073				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	86.53	89.29	124.1	100.00	20.98	21.0	1.00
2.5	106.73	-12.62	99.39	64.50	66.89	103.7	0.43
1.25	92.96	89.29	96.63	92.96	3.67	4.0	0.60
0.63	85.62	86.53	90.21	87.45	2.43	2.8	0.36
0.31	102.14	108.57	94.80	101.84	6.89	6.8	0.89
0.16	94.80	93.88	93.88	94.19	0.53	0.6	0.66
0.08	123.26	110.41	115.9	116.53	6.45	5.5	0.26
MeOH killed cells	-147.59	42.46	67.25	-12.62	117.54	-931.1	0.18



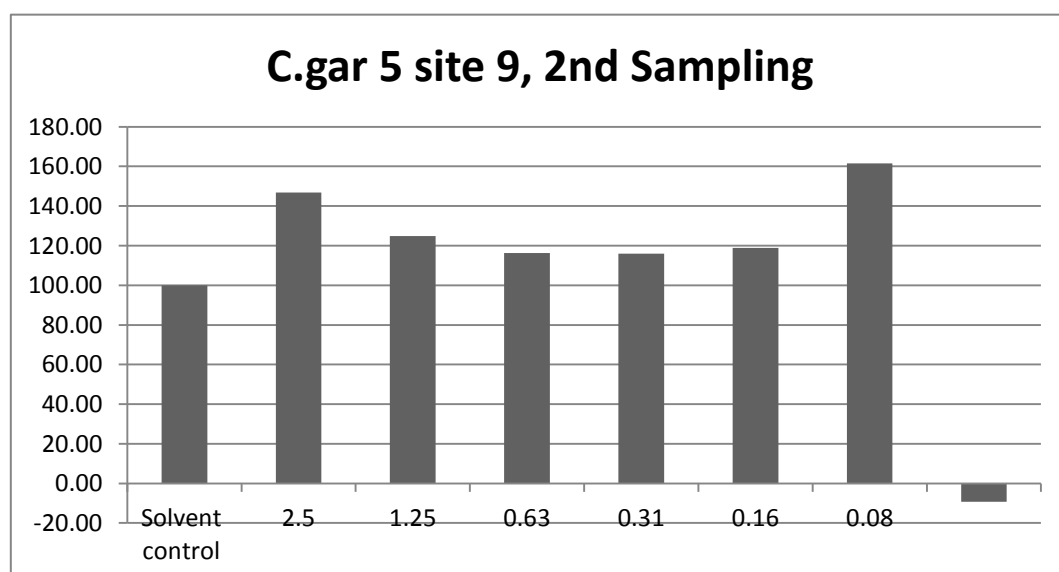
Absorbance reading from plate reader				C.gar 3 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.275	0.278	0.316				
2.5	0.292	0.297	0.304				
1.25	0.274	0.282	0.304				
0.63	0.264	0.275	0.289				
0.31	0.276	0.278	0.285				
0.16	0.274	0.286	0.292				
0.08	0.305	0.315	0.337				
MeOH killed cells	0.02	0.227	0.254	0.238	0.243	0.24	0.20
MeOH killed cells subtracted							
Solvent control	0.094	0.097	0.135				
2.5	0.111	0.116	0.123				
1.25	0.093	0.101	0.123				
0.63	0.083	0.094	0.108				
0.31	0.095	0.097	0.104				
0.16	0.093	0.105	0.111				
0.08	0.124	0.134	0.156				
MeOH killed cells	0.161	0.046	0.073				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	86.53	89.29	124.18	100.00	20.98	21.0	1.00
2.5	102.1	106.7	113.16	107.35	5.53	5.2	0.59
1.25	85.62	92.96	113.16	97.25	14.26	14.7	0.86
0.63	76.43	86.53	99.39	87.45	11.50	13.2	0.42
0.31	87.45	89.29	95.72	90.82	4.34	4.8	0.50
0.16	85.62	96.63	102.14	94.80	8.41	8.9	0.71
0.08	114.0	123.2	143.46	126.93	15.03	11.8	0.15
MeOH killed cells	147.5	42.46	67.25	-12.62	117.54	-9	0.18



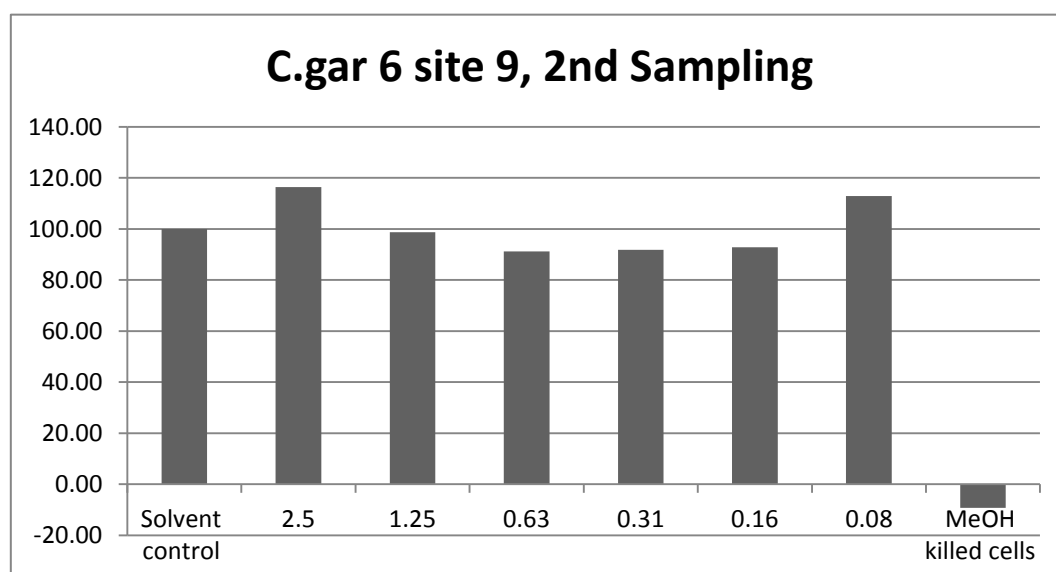
Absorbance reading from plate reader				C.gar 4 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.294	0.293	0.284				
2.5	0.356	0.32	0.312				
1.25	0.329	0.311	0.293				
0.63	0.322	0.294	0.292				
0.31	0.329	0.293	0.285				
0.16	0.351	0.298	0.293				
0.08	0.353	0.331	0.334				
MeOH killed cells	0.017	0.251	0.255	0.252	0.253	0.19	0.2
MeOH killed cells subtracted							
Solvent control	0.110	0.109	0.100				
2.5	0.172	0.136	0.128				
1.25	0.145	0.127	0.109				
0.63	0.138	0.110	0.108				
0.31	0.145	0.109	0.101				
0.16	0.167	0.114	0.109				
0.08	0.169	0.147	0.150				
MeOH killed cells	0.167	0.067	0.071				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	103.4	102.5	94.04	100.00	5.19	5.2	1.00
2.5	161.8	127.9	120.40	136.72	22.07	16.1	0.05
1.25	136.4	119.4	102.51	119.46	16.95	14.2	0.13
0.63	129.8	103.4	101.57	111.61	15.79	14.1	0.29
0.31	136.4	102.5	94.98	111.30	22.07	19.8	0.44
0.16	157.1	107.2	102.51	122.28	30.26	24.7	0.28
0.08	159.0	138.2	141.11	146.14	11.23	7.7	0.00
MeOH killed cells	-1	62.97	66.73	-9.22	128.30	-1	0.21



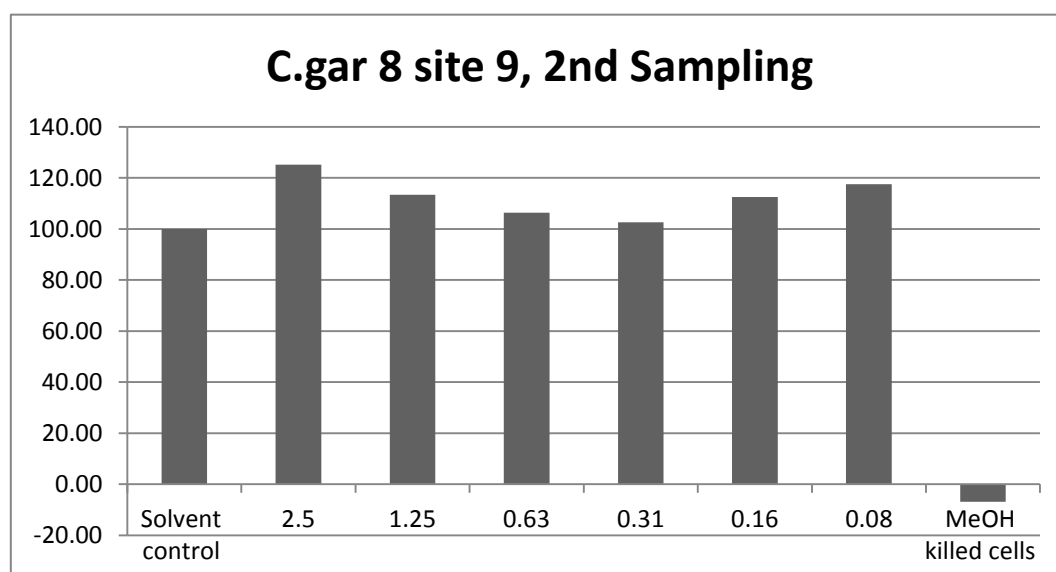
Absorbance reading from plate reader				C.gar 5 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.294	0.293	0.284				
2.5	0.347	0.344	0.329				
1.25	0.324	0.311	0.315				
0.63	0.305	0.311	0.307				
0.31	0.309	0.304	0.309				
0.16	0.303	0.309	0.319				
0.08	0.348	0.366	0.353				
MeOH killed cells	0.017	0.251	0.255	0.252	0.253	0.1	0.23
MeOH killed cells subtracted							
Solvent control	0.110	0.109	0.100				
2.5	0.163	0.160	0.145				
1.25	0.140	0.127	0.131				
0.63	0.121	0.127	0.123				
0.31	0.125	0.120	0.125				
0.16	0.119	0.125	0.135				
0.08	0.164	0.182	0.169				
MeOH killed cells	0.167	0.067	0.071				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	103.4	102.5	94.04	100.00	5.19	5.2	1.00
2.5	153.3	150.5	136.41	146.76	9.08	6.2	0.00
1.25	131.7	119.4	123.22	124.79	6.27	5.0	0.01
0.63	113.8	119.4	115.69	116.32	2.88	2.5	0.01
0.31	117.5	112.8	117.58	116.01	2.72	2.3	0.01
0.16	111.9	117.5	126.99	118.83	7.61	6.4	0.02
0.08	154.3	171.2	159.00	161.51	8.75	5.4	0.00
MeOH killed cells	-1	62.97	66.73	-9.22	128.30	-1	0.21



Absorbance reading from plate reader				C.gar 6 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.294	0.293	0.284				
2.5	0.279	0.312	0.332				
1.25	0.275	0.292	0.3				
0.63	0.271	0.281	0.291				
0.31	0.271	0.276	0.298				
0.16	0.276	0.272	0.3				
0.08	0.307	0.296	0.309				
MeOH killed cells	0.017	0.251	0.255	0.252	0.253	0.19	0.23
MeOH killed cells subtracted							
Solvent control	0.110	0.109	0.100				
2.5	0.095	0.128	0.148				
1.25	0.091	0.108	0.116				
0.63	0.087	0.097	0.107				
0.31	0.087	0.092	0.114				
0.16	0.092	0.088	0.116				
0.08	0.123	0.112	0.125				
MeOH killed cells	0.167	0.067	0.071				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	103.4	102.5	94.04	100.00	5.19	5.2	1.00
2.5	89.33	120.4	139.23	116.32	25.20	21.7	0.33
1.25	85.56	101.5	109.10	98.74	12.02	12.2	0.88
0.63	81.80	91.21	100.63	91.21	9.42	10.3	0.23
0.31	81.80	86.50	107.22	91.84	13.52	14.7	0.38
0.16	86.50	82.74	109.10	92.78	14.26	15.4	0.46
0.08	115.6	105.3	117.58	112.87	6.59	5.8	0.06
MeOH killed cells	-16	62.97	66.73	-9.22	128.30	-1	0.21



Absorbance reading from plate reader				C.gar 8 Site 9 2nd Sampling			
Raw data	1	2	3				
Solvent control	0.319	0.305	0.278				
2.5	0.334	0.307	0.34				
1.25	0.314	0.304	0.326				
0.63	0.345	0.298	0.279				
0.31	0.321	0.304	0.285				
0.16	0.344	0.315	0.282				
0.08	0.334	0.317	0.306				
MeOH killed cells	0.024	0.184	0.359	0.237	0.241	0.28	0.21
MeOH killed cells subtracted							
Solvent control	0.123	0.109	0.082				
2.5	0.138	0.111	0.144				
1.25	0.118	0.108	0.130				
0.63	0.149	0.102	0.083				
0.31	0.125	0.108	0.089				
0.16	0.148	0.119	0.086				
0.08	0.138	0.121	0.110				
MeOH killed cells	0.172	0.012	0.163				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	117.5	104.1	78.29	100.00	19.96	20.0	1.00
2.5	131.9	106.0	137.67	125.22	16.83	13.4	0.17
1.25	112.7	103.1	124.26	113.41	10.55	9.3	0.36
0.63	142.4	97.45	79.25	106.38	32.54	30.6	0.79
0.31	119.4	103.1	85.00	102.55	17.25	16.8	0.87
0.16	141.5	113.7	82.12	112.45	29.71	26.4	0.58
0.08	131.9	115.6	105.11	117.56	13.51	11.5	0.28
MeOH killed cells	-1	11.53	155.87	-6.88	160.47	-2	0.32

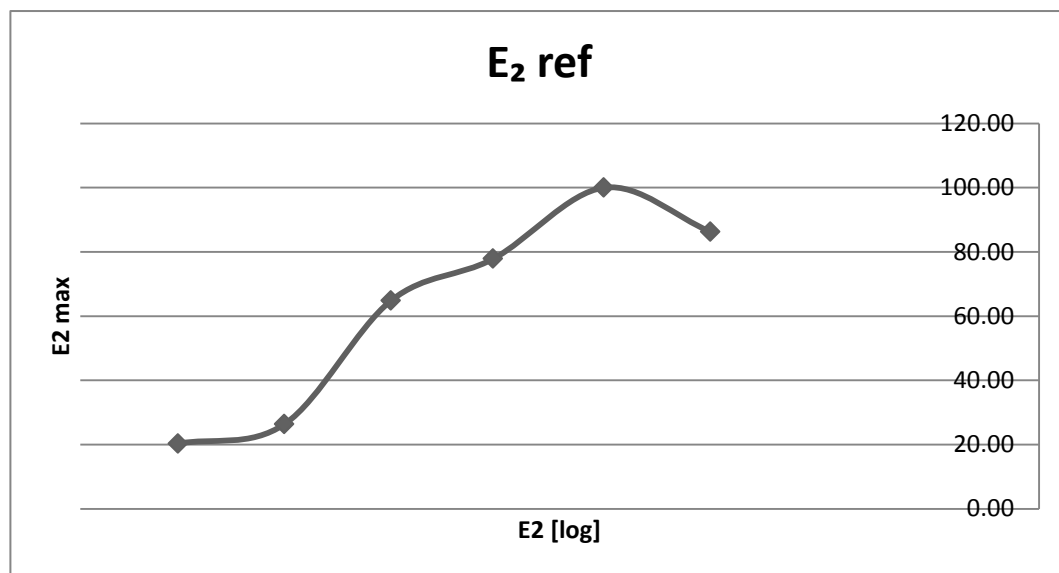


8.2.3. 3rd Sampling event

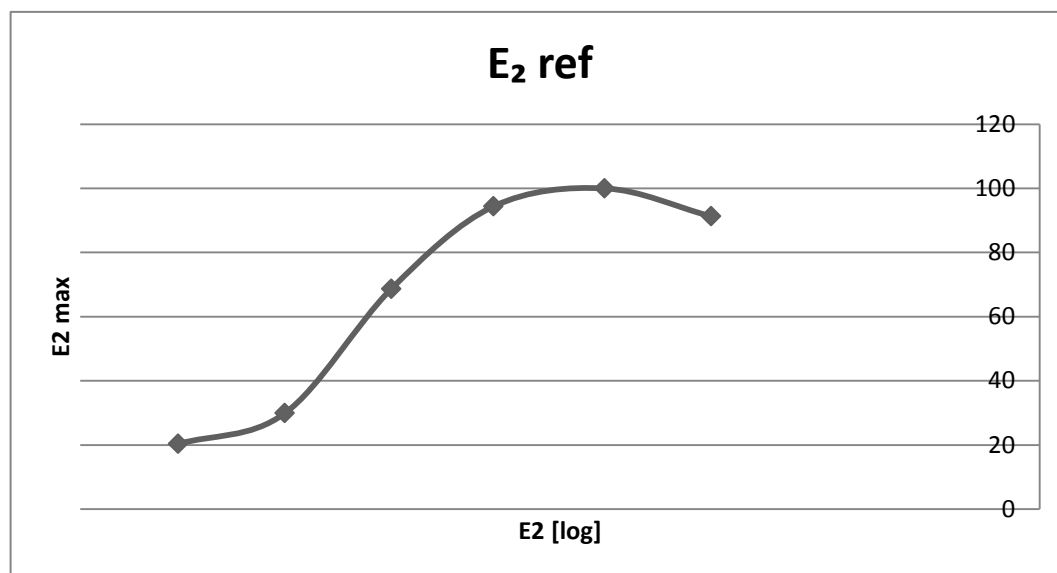
8.2.3.1. Reference linear curve as for 3rd sampling event September 2013.

Sediment samples from the 3rd sampling event September 2013 were compared to the reference compound linear curve as indicated below.

E ₂ (µg)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	4646104	5572953	5821844	5346967	619591.3097	11.59	86.25
8.25E-05	8.25E-11	3.03E-13	-4.083546051	3916568	6030526	6367801	6199163.5	238489.4396	3.85	100.00
7.50E-06	7.50E-12	2.75E-14	-5.124938737	4290889	4504564	5676573	4824008.667	746031.9754	15.46	77.82
8.25E-07	8.25E-13	3.03E-15	-6.083546051	3989859	3742521	4320078	4017486	289767.9423	7.21	64.81
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1484878	1576668	1841493	1634346.333	185171.9664	11.33	26.36
8.25E-09	8.25E-15	3.03E-17	-8.083546051	1187102	1215974	1368852	1257309.333	97671.50537	7.77	20.28



$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/l}$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	4646104	5572953	5821844	5346967	619591.3097	11.59	86.25
8.25E-05	8.25E-11	3.03E-13	-4.083546051	3916568	6030526	6367801	6199163.5	238489.4396	3.85	100.00
7.50E-06	7.50E-12	2.75E-14	-5.124938737	4290889	4504564	5676573	4824008.667	746031.9754	15.46	77.82
8.25E-07	8.25E-13	3.03E-15	-6.083546051	3989859	3742521	4320078	4017486	289767.9423	7.21	64.81
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1484878	1576668	1841493	1634346.333	185171.9664	11.33	26.36
8.25E-09	8.25E-15	3.03E-17	-8.083546051	1187102	1215974	1368852	1257309.333	97671.50537	7.77	20.28



E ₂ (µg/) _{well}	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.08355	4646104	5572953	5821844	5175342	5401973	538010	533305	400051	7.50	94.67
8.25E-05	8.25E-11	3.03E-13	-4.08355	3916568	6030526	6367801	6448610	5228751	580706	563321	949002	16.85	100.00
7.50E-06	7.50E-12	2.75E-14	-5.12494	4290889	4504564	5676573	5237474	6006428	525576	516194	660815	12.80	91.63
8.25E-07	8.25E-13	3.03E-15	-6.08355	3989859	3742521	4320078	3928569	3797354	427776	400935	241513	6.02	71.17
8.25E-08	8.25E-14	3.03E-16	-7.08355	1484878	1576668	1841493	1644635	1778868	179459	168685	140503	8.33	29.94
8.25E-09	8.25E-15	3.03E-17	-8.08355	1187102	1215974	1368852	1100426	1158269	128793	121975	95893	7.86	21.65

Combined E₂

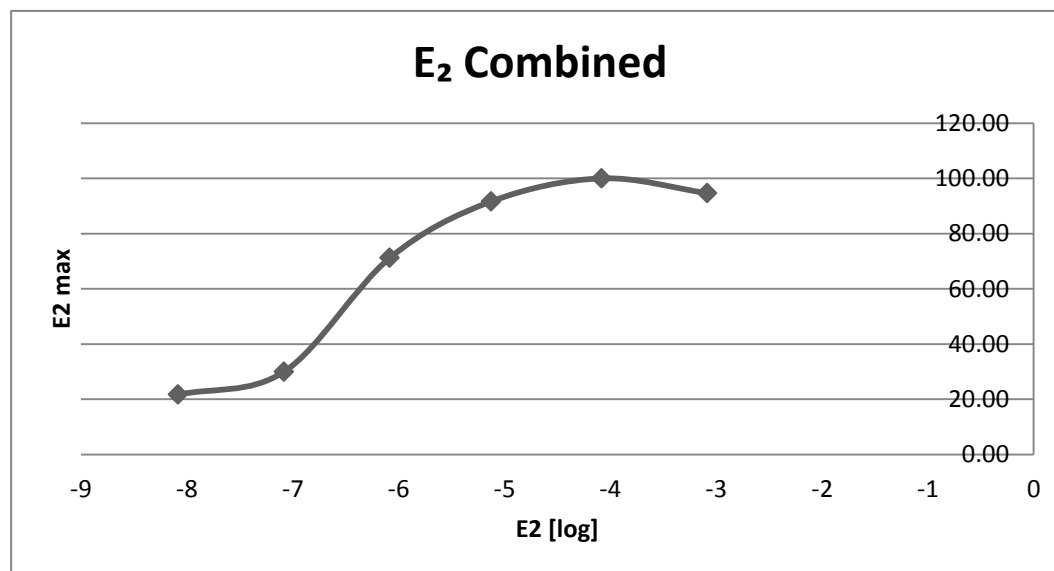
y=mx+c

intercept 196.28

slope 21.90

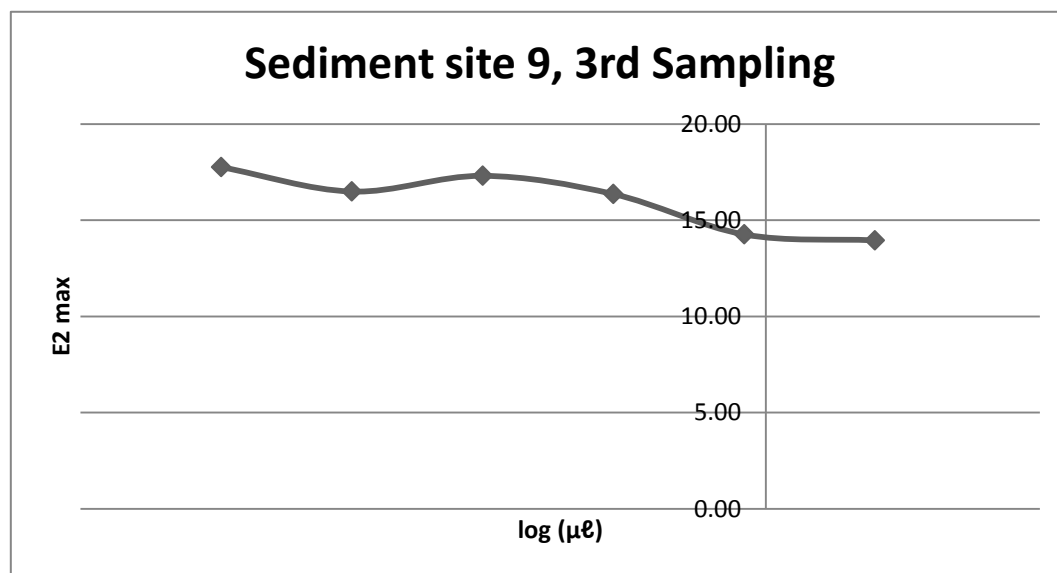
r2 0.94

	µg/well
EC20	8.91E-09
EC50	2.09E-07
EC80	4.90E-06



8.2.3.2 Activation calculations for September 2013 retrieved from Microsoft Excel 2010

Sediment 9 (μL)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	889292	687797	780833	785974	100845.8287	12.83068253	13.95
0.83	-7.92E-02	804065	855831	750066	803320.6667	52886.4286	6.583476661	14.26
0.28	-5.56E-01	924615	1012674	827622	921637	92561.93623	10.04320966	16.36
0.09	-1.03E+00	927326	1024918	972747	974997	48834.8901	5.008722088	17.31
0.03	-1.51E+00	840909	956652	989344	928968.3333	77993.75844	8.395739192	16.49
0.01	-1.99E+00	907071	1067127	1027507	1000568.333	83359.1603	8.331181142	17.76

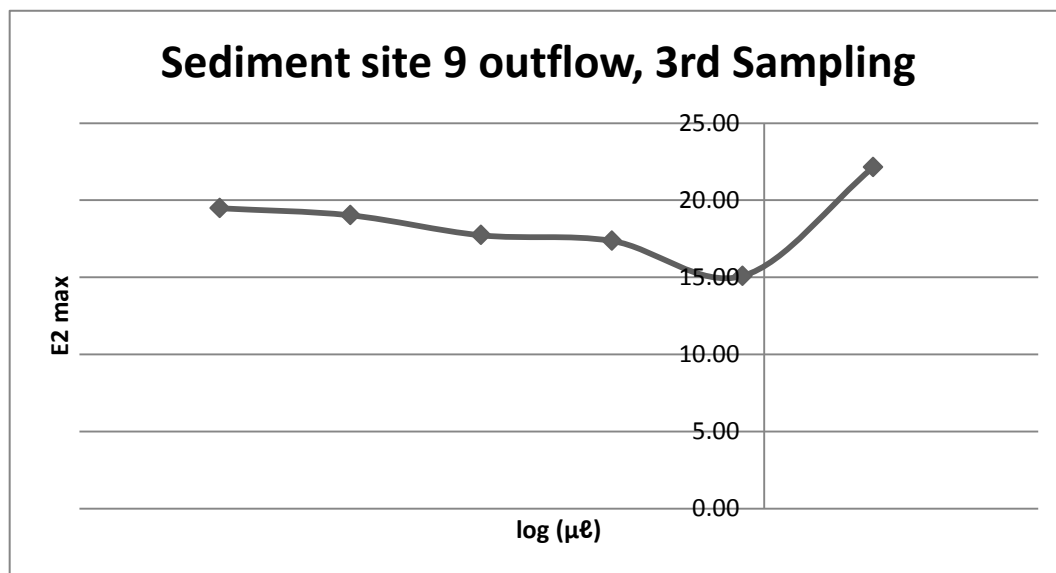


Mass sediment extracted: =20.2g
concentrated to 1 ml

No Response

Sediment 9O (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	1352021	1227668	1161668	1247119	96655.68893	7.750318048	22.14
0.83	-7.92E-02	1197613	842260	857441	849850.5	10734.58805	1.263114871	15.09
0.28	-5.56E-01	1028770	869288	1036279	978112.3333	94319.3931	9.643002126	17.36
0.09	-1.03E+00	1066552	992792	936179	998507.6667	65374.16475	6.54718706	17.73
0.03	-1.51E+00	1048871	1136145	1029328	1071448	56874.93797	5.308231288	19.02
0.01	-1.99E+00	1077201	1184714	1030121	1097345.333	79240.73786	7.221130436	19.48

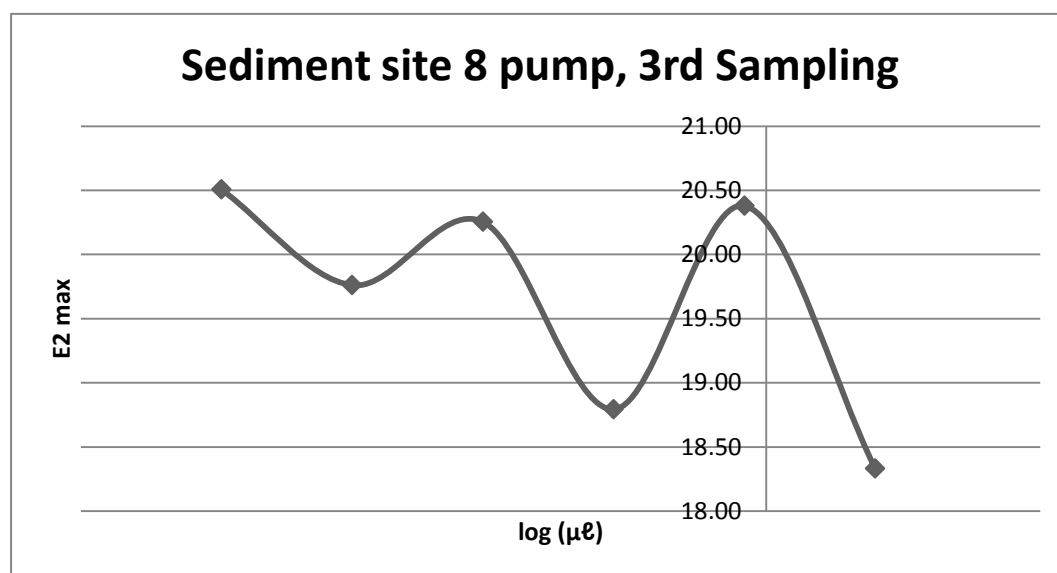
Mass sediment extracted:=20.14g
concentrated to 1ml



No Response

Sediment 8 pump	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	936446	1062166	1098945	1032519	85209.68083	8.252601727	18.33
0.83	-7.92E-02	1047673	1217237	1179115	1148008.333	88959.01336	7.748986725	20.38
0.28	-5.56E-01	994426	1069166	1112305	1058632.333	59641.28822	5.633805651	18.79
0.09	-1.03E+00	1048280	1112378	1262181	1140946.333	109774.8683	9.621387532	20.25
0.03	-1.51E+00	1046613	1050546	1242331	1113163.333	111879.7645	10.05061532	19.76
0.01	-1.99E+00	1124986	1114312	1226284	1155194	61796.63942	5.349459868	20.51

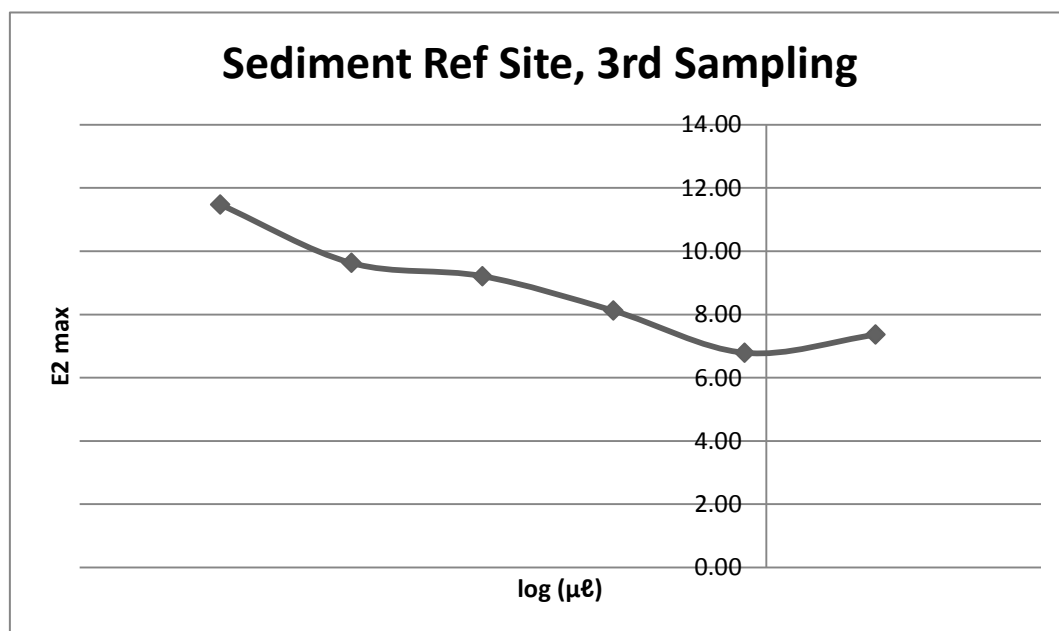
20.14g



Mass sediment extracted: =
concentrated to 1 ml

No linear gradient thus a response occurred which cannot be quantified by the cells

Sediment Ref (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	186380	142212	914993	414528.3333	433977.3784	104.6918494	7.36
0.83	-7.92E-02	162192	125623	859432	382415.6667	413512.708	108.1317383	6.79
0.28	-5.56E-01	212331	155993	1003489	457271	473876.6403	103.6314659	8.12
0.09	-1.03E+00	252914	221403	1082105	518807.3333	488084.4514	94.07817124	9.21
0.03	-1.51E+00	302163	298044	1026746	542317.6667	419532.2981	77.35914278	9.63
0.01	-1.99E+00	361932	373803	1202711	646148.6667	482033.6641	74.60104601	11.47



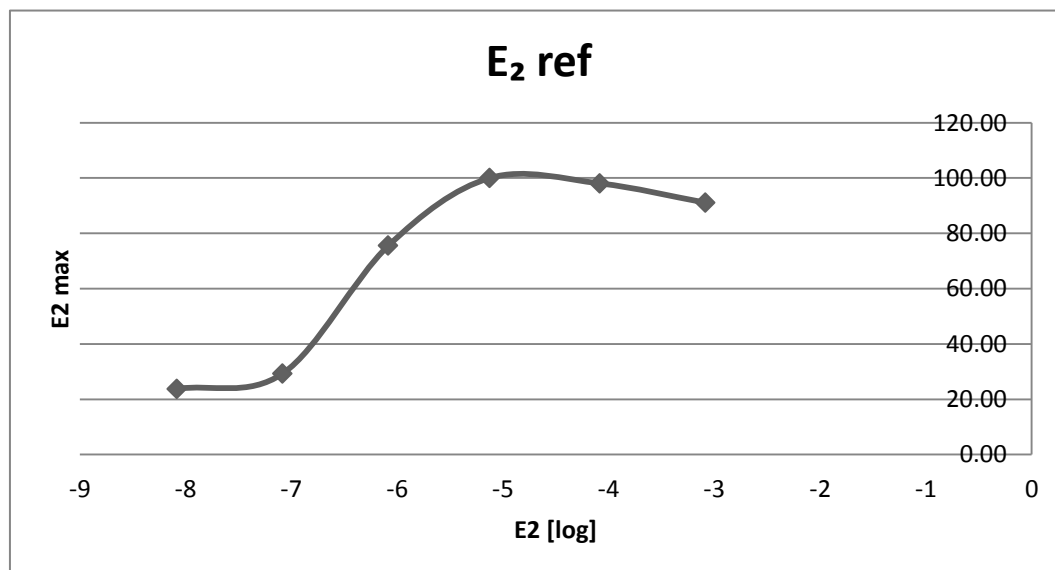
Mass sediment extracted: = 20.1g
concentrated to 1 ml

No Response

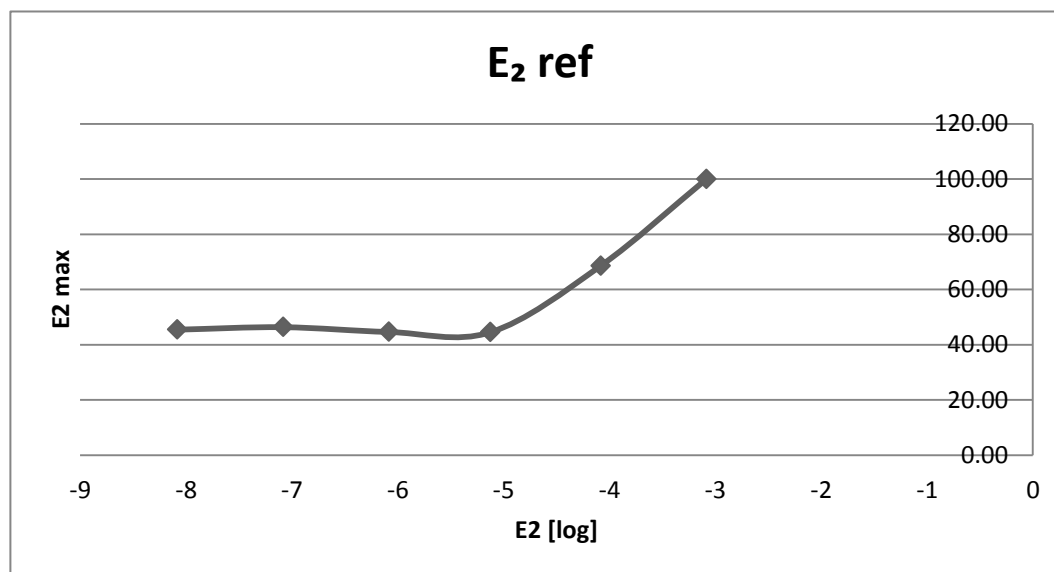
Reference linear curve as for 3rd sampling event, September 2013.

Water samples from the 3rd sampling event September 2013 were compared to the reference compound linear curve as indicated below. During these tests where more samples were involved, re standards were dosed.

E ₂ (µg/)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	5714768	6451312	7595274	6.59E+06	9.48E+05	1.44E+01	91.09
8.25E-05	8.25E-11	3.03E-13	-4.083546051	6239637	6941643	8090323	7.09E+06	9.34E+05	1.32E+01	98.05
7.50E-06	7.50E-12	2.75E-14	-5.124938737	6711744	7117608	7864421	7.23E+06	5.85E+05	8.09E+00	100.00
8.25E-07	8.25E-13	3.03E-15	-6.083546051	5012341	5397538	5978186	5.46E+06	4.86E+05	8.90E+00	75.54
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1960675	2098413	2299269	2.12E+06	1.70E+05	8.03E+00	29.31
8.25E-09	8.25E-15	3.03E-17	-8.083546051	1850112	1613310	1694887	1.72E+06	1.20E+05	7.00E+00	23.78



$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	1248950	1118569	1111496	1159671.667	77398.14232	6.67	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	934277	729585	720732	794864.6667	120815.7395	15.20	68.54
7.50E-06	7.50E-12	2.75E-14	-5.124938737	604091	384121	559924	516045.3333	116364.5275	22.55	44.50
8.25E-07	8.25E-13	3.03E-15	-6.083546051	504209	529416	518451	517358.6667	12638.95195	2.44	44.61
8.25E-08	8.25E-14	3.03E-16	-7.083546051	519754	627720	464929	537467.6667	82828.4849	15.41	46.35
8.25E-09	8.25E-15	3.03E-17	-8.083546051	442271	412273	726591	527045	173461.594	32.91	45.45



E ₂ (µg/) _{well}	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.08354605	5714768	6451312	7595274	1248950	1118569	1111496	3873394	3131917.22	80.86	98.24
8.25E-05	8.25E-11	3.03E-13	-4.08354605	6239637	6941643	8090323	934277	729585	720732	3942699	3524966.484	89.40	100.00
7.50E-06	7.50E-12	2.75E-14	-5.12493873	6711744	7117608	7864421	604091	384121	559924	3873651	3679073.305	94.98	98.25
8.25E-07	8.25E-13	3.03E-15	-6.08354605	5012341	5397538	5978186	504209	529416	518451	2990023	2733148.557	91.41	75.84
8.25E-08	8.25E-14	3.03E-16	-7.08354605	1960675	2098413	2299269	519754	627720	464929	1328460	943257.8391	71.00	33.69
8.25E-09	8.25E-15	3.03E-17	-8.08354605	1850112	1613310	1694887	442271	412273	726591	1123240	741984.5199	66.06	28.49

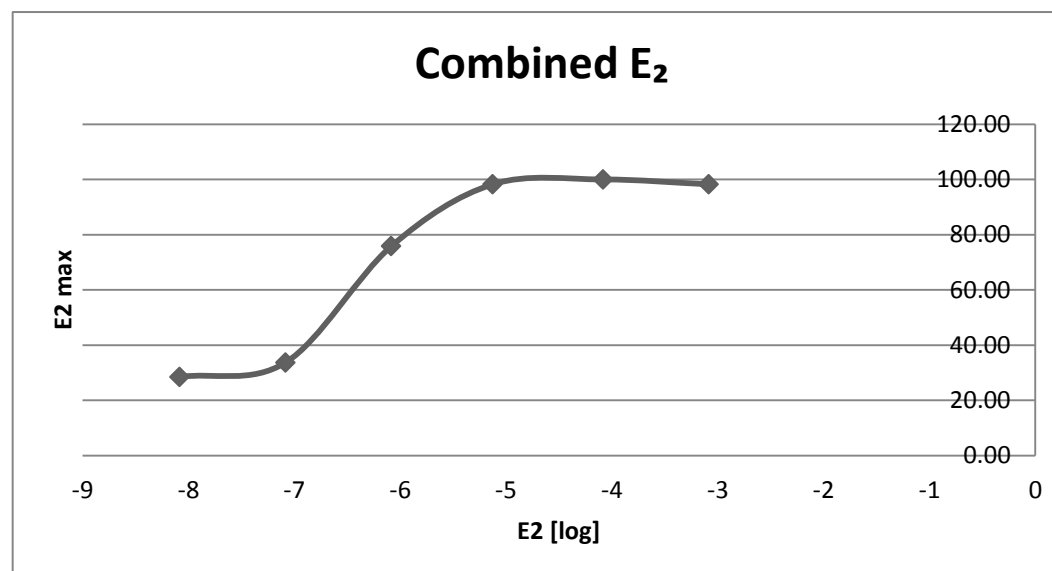
Combined E₂

intercept 270.63

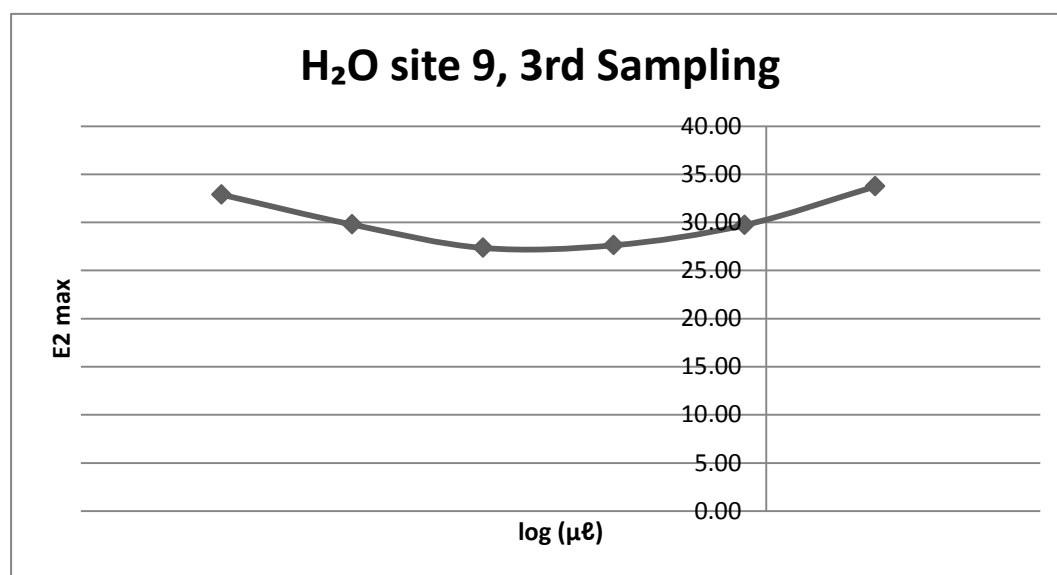
slope 33.03

r² 0.97

	µg/well
EC20	2.58E-08
EC50	2.09E-07
EC80	1.69E-06



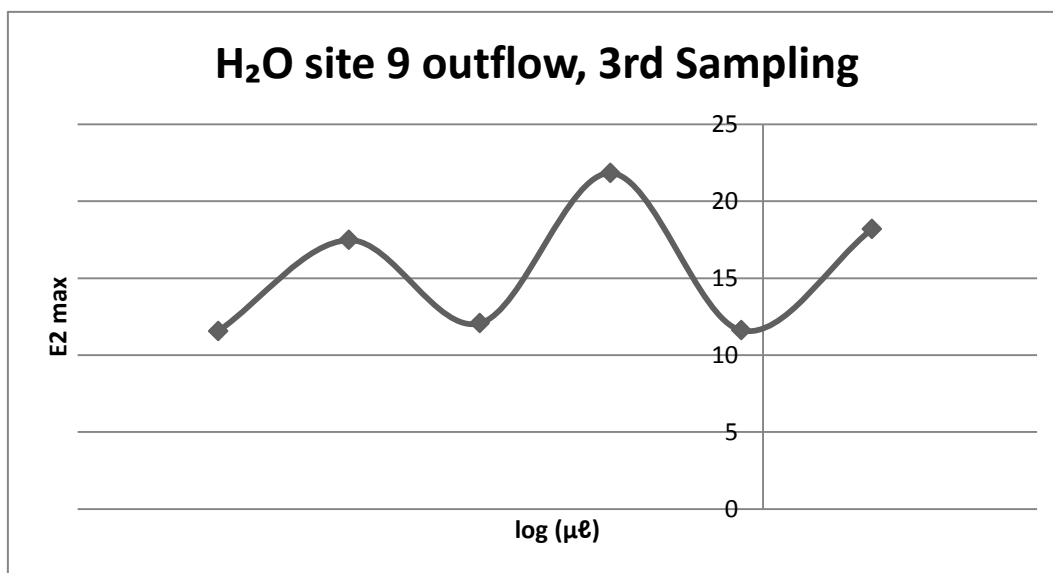
H ₂ O 9 (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	954192	1326660	1334032	1330346	5212.791191	0.39	33.74
0.83	-7.92E-02	816924	1181057	1162922	1171989.5	12823.38148	1.09	29.73
0.28	-5.56E-01	873893	1106042	1072314	1089178	23849.29752	2.19	27.63
0.09	-1.03E+00	981527	1119143	1134187	1078285.667	84132.39665	7.80	27.35
0.03	-1.51E+00	1074183	1215270	1233276	1174243	87120.93152	7.42	29.78
0.01	-1.99E+00	1247922	1347546	1294995	1296821	49837.09513	3.84	32.89



2ℓ water used in extraction
concentrated to 1mℓ

No linear gradient thus a response occurred which cannot be quantified by the cells

H ₂ O 9 Outflow (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	471613	898129	994515	946322	68155.19421	7.20	24.00
0.83	-7.92E-02	373746	690791	738527	714659	33754.44931	4.72	18.13
0.28	-5.56E-01	338189	500414	607692	554053	75857.00127	13.69	14.05
0.09	-1.03E+00	356575	415235	390967	387592.3333	29475.24693	7.60	9.83
0.03	-1.51E+00	350999	411124	812004	381061.5	42514.79522	11.16	9.66
0.01	-1.99E+00	541967	551281	402150	498466	83542.00465	16.76	12.64

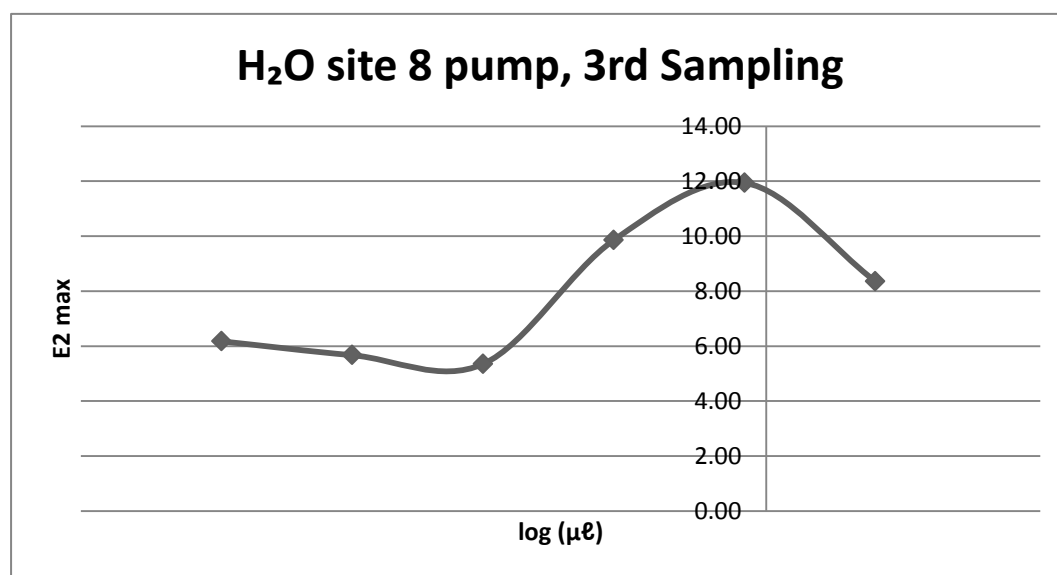


2ℓ water used in extraction
concentrated to 1mℓ

No linear gradient thus a response occurred which cannot be quantified by the cells

H ₂ O 8 pump (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	372152	315029	301354	329511.6667	37555.29159	11.40	8.36
0.83	-7.92E-02	241811	499993	228928	470739	152916.1813	32.48	11.94
0.28	-5.56E-01	403364	444942	317522	388609.3333	64978.75992	16.72	9.86
0.09	-1.03E+00	207638	217607	207306	210850.3333	5853.799137	2.78	5.35
0.03	-1.51E+00	251894	224769	194027	223563.3333	28952.33404	12.95	5.67
0.01	-1.99E+00	235483	251538	237805	243510.5	11352.59937	4.66	6.18

20.1g



Mass sediment extracted: =
concentrated to 1 ml

No Response

H ₂ O Ref (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.50	3.98E-01	1938098	1964746	1915845	1939563	24483.39476	1.26	49.19
0.83	-7.92E-02	1692354	1464259	1458643	1538418.667	133341.4789	8.67	39.02
0.28	-5.56E-01	1410292	1160122	1001070	1190494.667	206294.7781	17.33	30.19
0.09	-1.03E+00	1367169	1203634	1221396	1264066.333	89730.1068	7.10	32.06
0.03	-1.51E+00	1223743	1265013	1260498	1249751.333	22636.72587	1.81	31.70
0.01	-1.99E+00	1378887	1370689	1293287	1347621	47232.82194	3.50	34.18

y=mx+c

intercept 41.05

slope 19.91

r² 1.00

μℓ/well

EC₂₀ 8.77E-02

EC₅₀ 2.82E+00

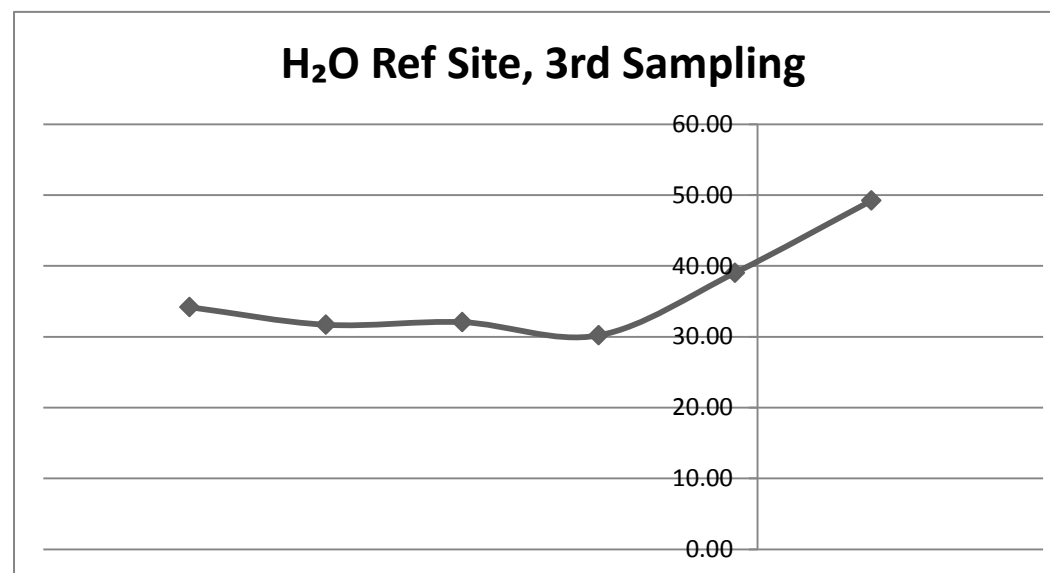
EC₈₀ 9.05E+01

	μg/μℓ	μg/mℓ
REP20	2.94E-07	2.94E-04
REP50	7.41E-08	7.41E-05
REP80	1.87E-08	1.87E-05

	E ₂ eq μg/mℓ
REP20	1.47E-07
REP50	3.70E-08
REP80	9.34E-09

2ℓ water used in extraction
concentrated to 1mℓ
Concentration factor

2000

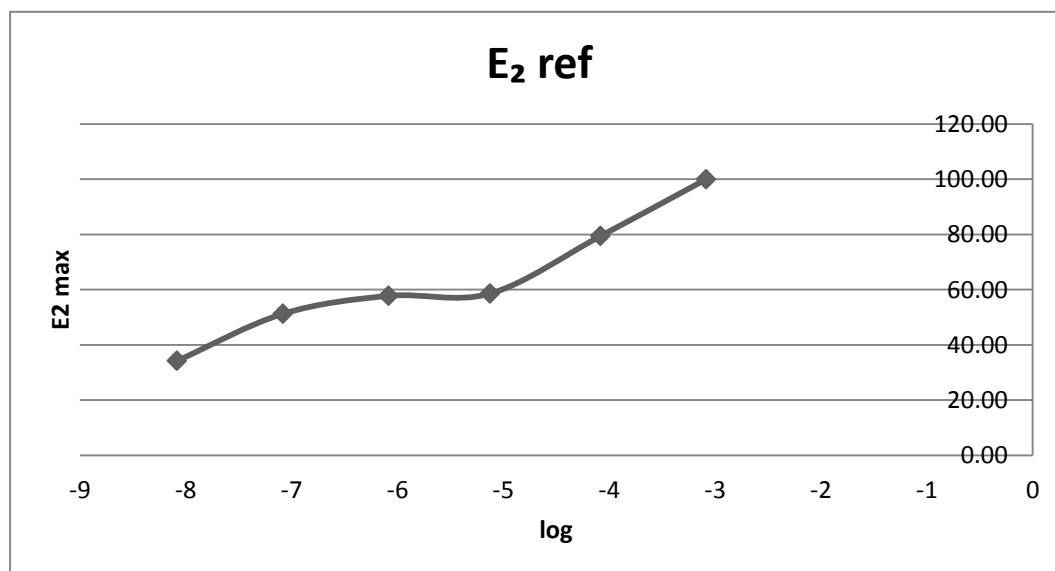


Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
975749	30.08	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
683945	21.09	intercept	24.26	EC20	0.437184836	REP 20	3.06E-06	3.06E-03	1.53E-04	Mean	STEDV
609205	18.78	slope	11.84	EC50	149.2121809	REP 50	1.24E-07	1.24E-04	6.17E-06	2.15E-04	5.38E-05
483412	14.90	r ²	0.90	EC80	50926.45741	REP 80	4.99E-09	4.99E-06	2.49E-07		
363218	11.20									REP50	
340892	10.51									Mean	STEDV
RLU	E ₂ max									5.92E-06	1.02E-05
974916	30.06	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
880747	27.15	intercept	26.39	EC20	0.272014671	REP 20	4.92E-06	4.92E-03	2.45E-04	REP80	
625114	19.27	slope	11.30	EC50	122.7357292	REP 50	1.50E-07	1.50E-04	7.50E-06	Mean	STEDV
473021	14.58	r ²	0.93	EC80	55379.58362	REP 80	4.59E-09	4.59E-06	2.29E-07	5.51E-07	5.41E-07
363672	11.21										
340819	10.51										
RLU	E ₂ max										
1062886	32.77	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
843062	25.99	intercept	27.40	EC20	0.271039384	REP 20	4.94E-06	4.94E-03	2.46E-04		
659191	20.32	slope	13.04	EC50	54.08207971	REP 50	3.41E-07	3.41E-04	1.70E-05		
506248	15.61	r ²	1.00	EC80	10791.31492	REP 80	2.36E-08	2.36E-05	1.18E-06		
385464	11.88										
1369168	42.21										

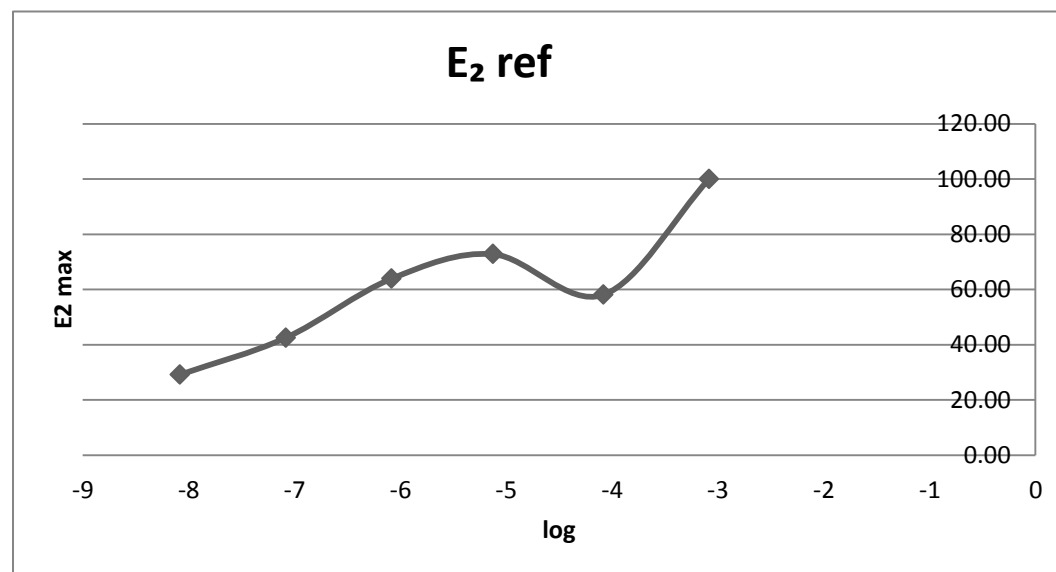
Reference linear curve as for 3rd sampling event, September 2013.

Bile samples from the 3rd sampling event, September 2013 were compared to the reference compound linear curve as indicated below. During these tests where more samples were involved, re standards were dosed.

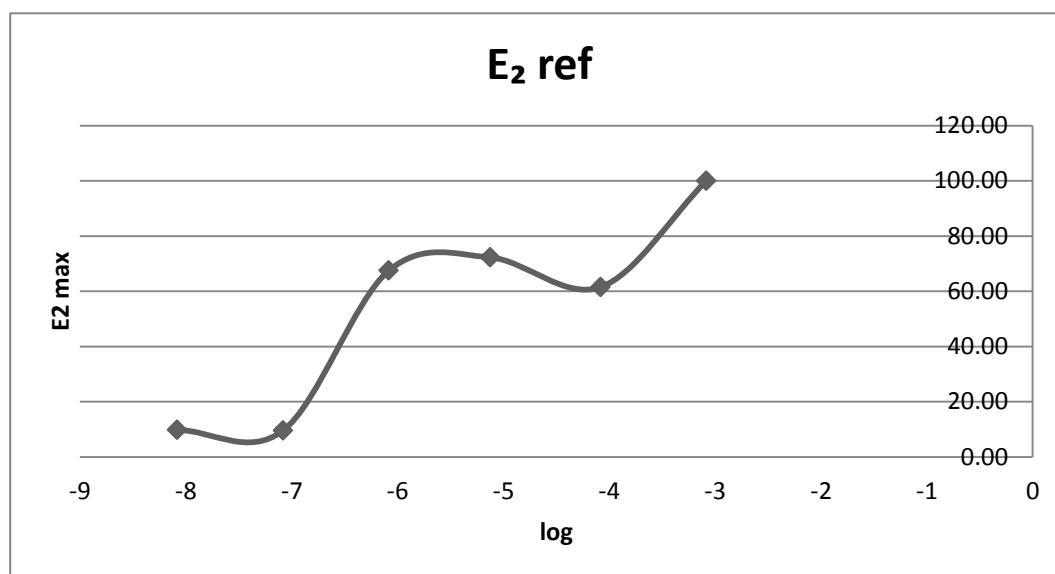
E ₂ (µg/)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	5989717	6917795	6267822	6391778	476294.0384	7.45	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	5289515	5430279	4503431	5074408.333	499464.6757	9.84	79.39
7.50E-06	7.50E-12	2.75E-14	-5.124938737	3812300	3749027	3672427	3744584.667	70042.23566	1.87	58.58
8.25E-07	8.25E-13	3.03E-15	-6.083546051	4006869	3685560	3382855	3691761.333	312053.2173	8.45	57.76
8.25E-08	8.25E-14	3.03E-16	-7.083546051	3784188	3282601	2758841	3275210	512713.4558	15.65	51.24
8.25E-09	8.25E-15	3.03E-17	-8.083546051	2268252	2041686	2254382	2188106.667	126993.5154	5.80	34.23



$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	6707066	5218158	5555320	5826848	780709.5165	13.40	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	6280964	3856888	2910595	3383741.5	669130.1973	19.77	58.07
7.50E-06	7.50E-12	2.75E-14	-5.124938737	4036762	3235836	5451117	4241238.333	1121706.47	26.45	72.79
8.25E-07	8.25E-13	3.03E-15	-6.083546051	3985797	3935876	3248970	3723547.667	411753.5644	11.06	63.90
8.25E-08	8.25E-14	3.03E-16	-7.083546051	2368498	2889789	2168062	2475449.667	372560.6795	15.05	42.48
8.25E-09	8.25E-15	3.03E-17	-8.083546051	2011543	1915861	1163270	1696891.333	464599.3509	27.38	29.12

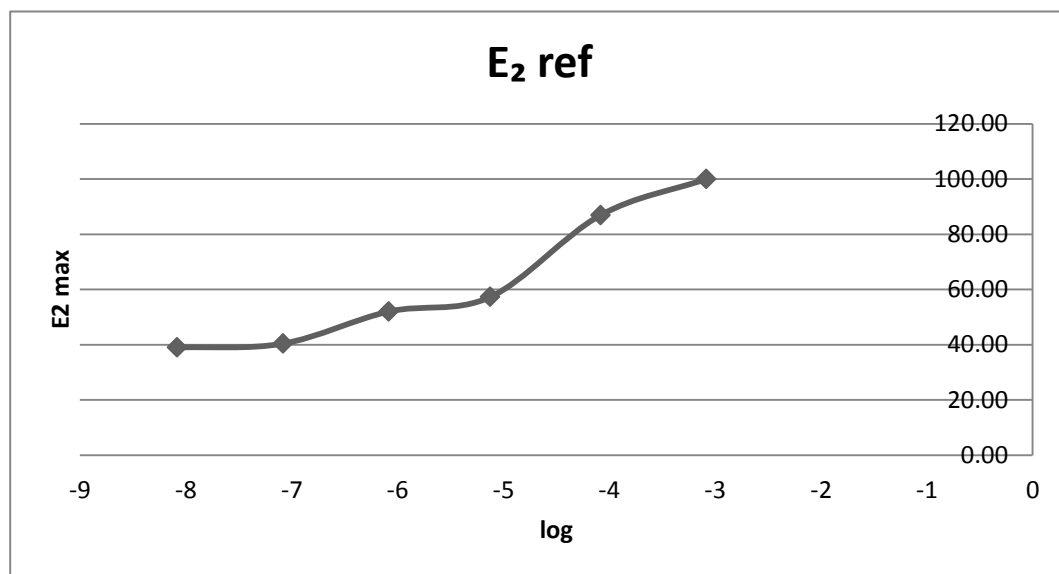


E ₂ (µg)/well	E ₂ g/ℓ	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	8.25E-10	3.03E-12	-3.083546051	4758003	4525822	3554612	4279479	638397.3136	14.92	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	2317121	4709911	2946443	2631782	444997.8537	16.91	61.50
7.50E-06	7.50E-12	2.75E-14	-5.124938737	2511180	3511432	3256034	3092882	519701.7868	16.80	72.27
8.25E-07	8.25E-13	3.03E-15	-6.083546051	1122825	2997262	2775884	2886573	156537.885	5.42	67.45
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1999235	439528	372921	406224.5	47098.26137	11.59	9.49
8.25E-09	8.25E-15	3.03E-17	-8.083546051	484181	817142	355264	419722.5	91158.08491	21.72	9.81



Reference will not be used as the standard curve deviates.
Error could be caused by dosing, standards, bacteria or cells.

$E_2(\mu\text{g/})\text{well}$	$E_2 \text{ g/}\ell$	$E_2 \text{ Mol per well}$	$\log E_2$	RLU	RLU	RLU	mean	stdev	cv	$E_2\text{max}$
8.25E-04	8.25E-10	3.03E-12	-3.083546051	3482859	5026729	4399082	4302890	776416.9755	18.04	100.00
8.25E-05	8.25E-11	3.03E-13	-4.083546051	3436685	4384322	3395164	3738723.667	559489.8608	14.96	86.89
7.50E-06	7.50E-12	2.75E-14	-5.124938737	2112526	2879148	2400422	2464032	387249.2696	15.72	57.26
8.25E-07	8.25E-13	3.03E-15	-6.083546051	1928258	2684280	2102515	2238351	395892.511	17.69	52.02
8.25E-08	8.25E-14	3.03E-16	-7.083546051	1555815	2062014	1594843	1737557.333	281664.5036	16.21	40.38
8.25E-09	8.25E-15	3.03E-17	-8.083546051	1605703	1686852	1738884	1677146.333	67118.88456	4.00	38.98



E ₂ (µg/) _{well}	E ₂ Mol per well	log E ₂	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
8.25E-04	3.03E-12	-3.0835	5989717	6917795	6267822	5989717	6917795	6267822	6707066	5218158	5555320	6203468	587870	9.48	100.00
8.25E-05	3.03E-13	-4.0835	5289515	5430279	4503431	5289515	5430279	4503431	6280964	3856888	2910595	4832766	1005768	20.81	77.90
7.50E-06	2.75E-14	-5.1249	3812300	3749027	3672427	3812300	3749027	3672427	4036762	3235836	5451117	3910135	615366	15.74	63.03
8.25E-07	3.03E-15	-6.0835	4006869	3685560	3382855	4006869	3685560	3382855	3985797	3935876	3248970	3702356	302202	8.16	59.68
8.25E-08	3.03E-16	-7.0835	3784188	3282601	2758841	3784188	3282601	2758841	2368498	2889789	2168062	300862	571000	18.98	48.50
8.25E-09	3.03E-17	-8.0835	2268252	2041686	2254382	2268252	2041686	2254382	2011543	1915861	1163270	2024368	349785.56	17.28	32.63

Combined

E₂

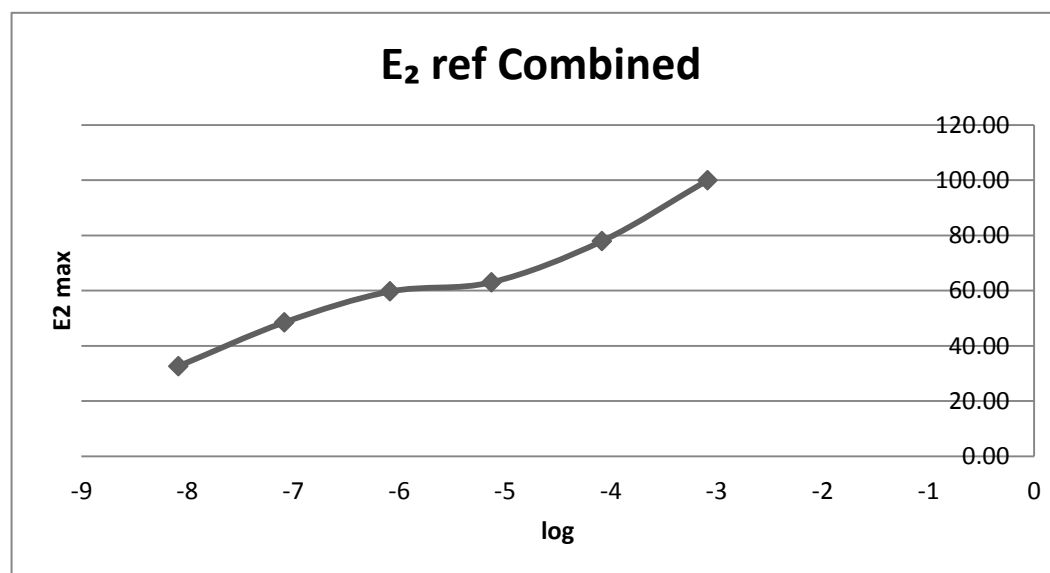
y=mx+c

intercept 154.40

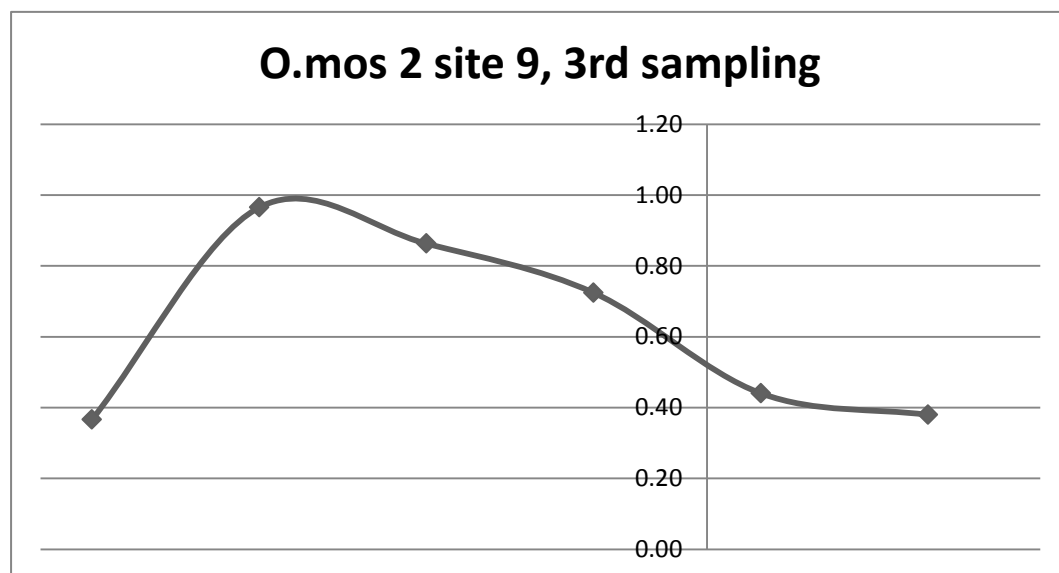
slope 18.08

r2 0.98

	µg/well	M/ℓ
EC20	3.69E-08	3.39E-14
EC50	1.68E-06	1.55E-12
EC80	7.68E-05	7.05E-11



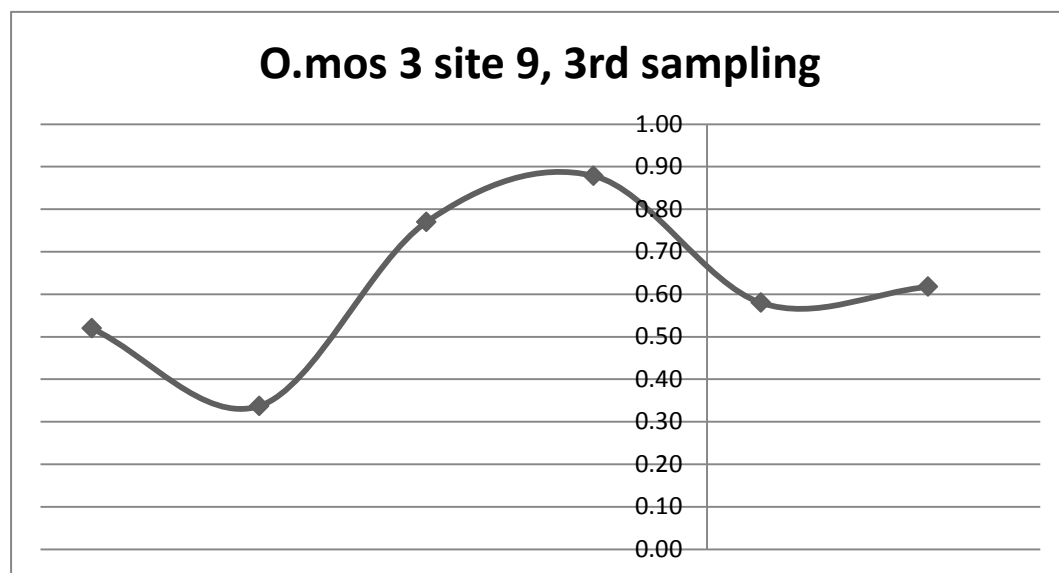
O.mos 2 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	5381	14364	32725	23544.5	12983.18761	55.14	0.38
1.25	9.69E-02	27149	24001	30807	27319	3406.183201	12.47	0.44
0.63	-2.04E-01	49128	58159	27489	44925.33333	15760.99712	35.08	0.72
0.31	-5.05E-01	64624	35670	60271	53521.66667	15612.4519	29.17	0.86
0.16	-8.06E-01	68840	57495	53263	59866	8054.623703	13.45	0.97
0.08	-1.11E+00	18175	22893	27036	22701.33333	4433.608275	19.53	0.37



500μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.5

No Response

O.mos 3 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	33227	45697	35986	38303.33333	6550.019109	17.10	0.62
1.25	9.69E-02	81917	44175	27781	35978	11592.30857	32.22	0.58
0.63	-2.04E-01	45243	63192	55019	54484.66667	8986.422221	16.49	0.88
0.31	-5.05E-01	46020	49492	99680	47756	2455.074744	5.14	0.77
0.16	-8.06E-01	68670	13506	28307	20906.5	10465.88747	50.06	0.34
0.08	-1.11E+00	30985	33477	6781	32231	1762.110099	5.47	0.52



250μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.25

No Response

O.mos 6 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	3087814	3035036	2563455	2895435	288711.6548	9.97	46.67
1.25	9.69E-02	2615488	2615763	2542877	2591376	42001.59112	1.62	41.77
0.63	-2.04E-01	2417132	2478560	2106375	2334022.333	199526.5172	8.55	37.62
0.31	-5.05E-01	2170911	2133169	1820850	2041643.333	192141.5798	9.41	32.91
0.16	-8.06E-01	2038352	2028439	1861279	1976023.333	99495.04217	5.04	31.85
0.08	-1.11E+00	1961347	2006591	1708231	1892056.333	160796.6721	8.50	30.50

y=mx+c

intercept 40.55

slope 15.09

r2 1.00

μℓ/well

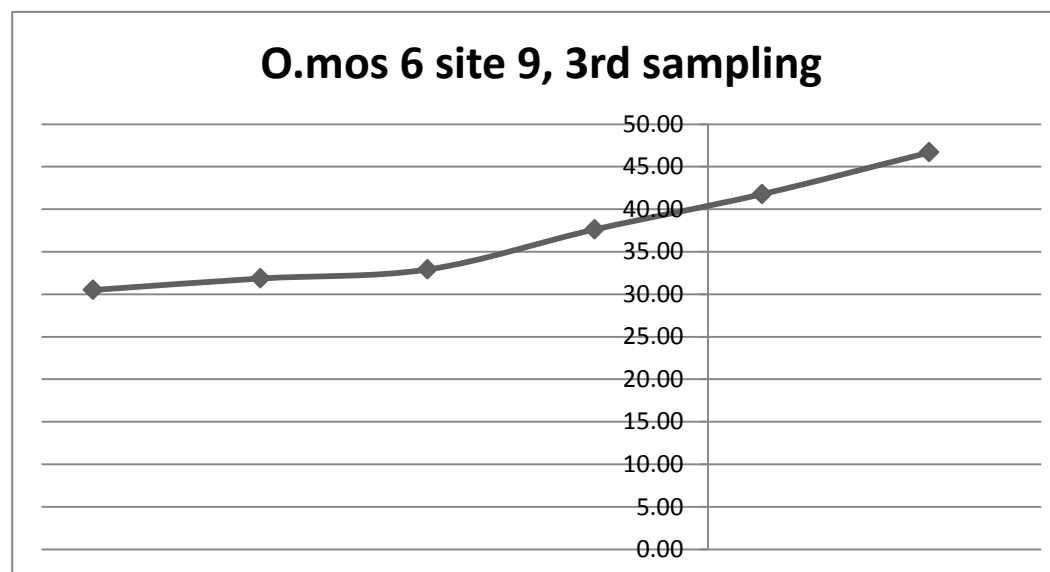
EC20 4.35E-02

EC50 4.22E+00

EC80 4.10E+02

	μg/μℓ	μg/mℓ
REP20	8.50E-07	8.50E-04
REP50	3.99E-07	3.99E-04
REP80	1.87E-07	1.87E-04

	E ₂ eq μg/mℓ
REP20	8.50E-03
REP50	3.99E-03
REP80	1.87E-03



100μℓ bile used in extraction

concentrated to 1mℓ

Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
3087814	49.78	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
2615488	42.16	intercept	42.32	EC20	0.038600373	REP 20	9.57E-07	9.57E-04	9.57E-03	Mean	STEDV
2417132	38.96	slope	15.79	EC50	3.063902214	REP 50	5.50E-07	5.50E-04	5.50E-03	8.68E-03	2.46E-03
2170911	35.00	r ²	0.96	EC80	243.1970506	REP 80	3.16E-07	3.16E-04	3.16E-03		
2038352	32.86									REP50	
1961347	31.62									Mean	STEDV
RLU	E ₂ max									1.86E-03	4.26E-03
3035036	48.92	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2615763	42.17	intercept	42.17	EC20	0.034945576	REP 20	1.06E-06	1.06E-03	1.06E-02	REP80	
2478560	39.95	slope	15.22	EC50	3.266508236	REP 50	5.16E-07	5.16E-04	5.16E-03	Mean	STEDV
2133169	34.39	r ²	0.97	EC80	305.3341022	REP 80	2.52E-07	2.52E-04	2.52E-03	2.15E-03	1.24E-03
2028439	32.70										
2006591	32.35										
RLU	E ₂ max										
2563455	41.32	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2542877	40.99	intercept	37.17	EC20	0.062595543	REP 20	5.90E-07	5.90E-04	5.90E-03		
2106375	33.95	slope	14.27	EC50	7.92966523	REP 50	2.12E-07	2.12E-04	2.12E-03		
1820850	29.35	r ²	0.91	EC80	1004.537821	REP 80	7.65E-08	7.65E-05	7.65E-04		
1861279	30.00										
1708231	27.54										

O.mos 7 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2738757	3167740	3403701	3103399.333	337108.9169	10.86	50.03
1.25	9.69E-02	2581752	2616047	2997319	2731706	230665.8537	8.44	44.04
0.63	-2.04E-01	2295183	2262822	2181892	2246632.333	58354.87683	2.60	36.22
0.31	-5.05E-01	1857120	2116944	2763567	2245877	466775.4597	20.78	36.20
0.16	-8.06E-01	1429827	1813494	1802076	1681799	218288.8208	12.98	27.11
0.08	-1.11E+00	2230720	1677222	1748660	1885534	301066.2351	15.97	30.39

y=mx+c

intercept 41.20

slope 22.94

r2 0.99

μℓ/well

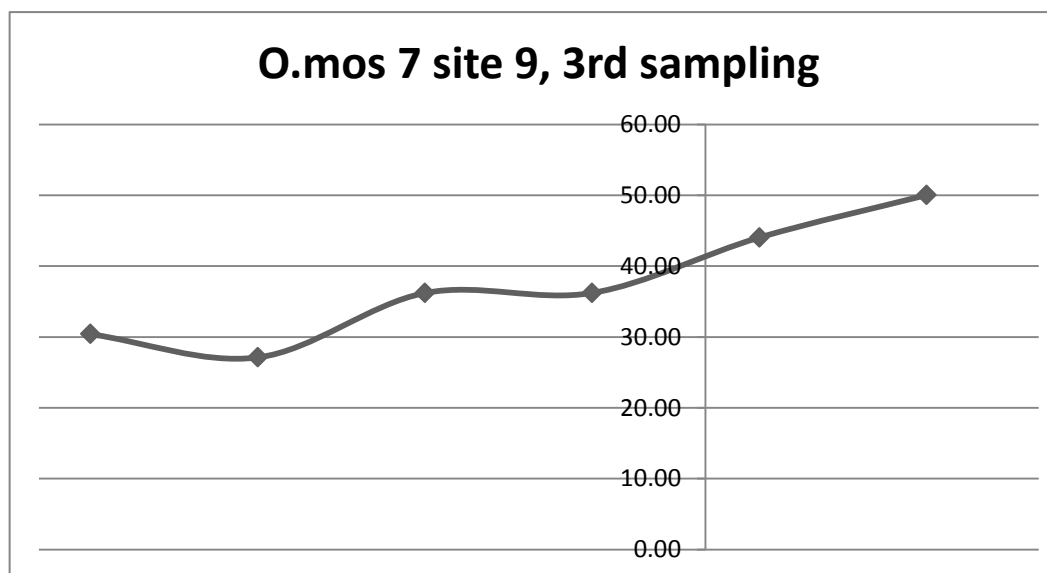
EC20 1.19E-01

EC50 2.42E+00

EC80 4.91E+01

	μg/μℓ	μg/mℓ
REP20	3.10E-07	3.10E-04
REP50	6.96E-07	6.96E-04
REP80	1.56E-06	1.56E-03

	E ₂ eq μg/mℓ
REP20	3.10E-03
REP50	6.96E-03
REP80	1.56E-02



100μℓ bile used in extraction

concentrated to 1mℓ

Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
2738757	44.15	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
2581752	41.62	intercept	39.02	EC20	0.061456778	REP 20	6.01E-07	6.01E-04	6.01E-03	Mean	STEDV
2295183	37.00	slope	15.70	EC50	5.007820768	REP 50	3.36E-07	3.36E-04	3.36E-03	1.18E-02	1.04E-02
1857120	29.94	r ²	0.96	EC80	408.063515	REP 80	1.88E-07	1.88E-04	1.88E-03		
1429827	23.05									REP50	
2230720	35.96									Mean	STEDV
RLU	E ₂ max									3.19E-03	6.46E-03
3167740	51.06	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2616047	42.17	intercept	41.97	EC20	0.067593777	REP 20	5.46E-07	5.46E-04	5.46E-03	REP80	
2262822	36.48	slope	18.77	EC50	2.679156431	REP 50	6.29E-07	6.29E-04	6.29E-03	Mean	STEDV
2116944	34.13	r ²	0.94	EC80	106.1914201	REP 80	7.23E-07	7.23E-04	7.23E-03	4.36E-03	2.70E-03
1813494	29.23										
1677222	27.04										
RLU	E ₂ max										
3403701	54.87	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2997319	48.32	intercept	46.51	EC20	0.015500946	REP 20	2.38E-06	2.38E-03	2.38E-02		
2181892	35.17	slope	14.65	EC50	1.73024159	REP 50	9.73E-07	9.73E-04	9.73E-03		
2763567	44.55	r ²	0.48	EC80	193.1324657	REP 80	3.98E-07	3.98E-04	3.98E-03		
1802076	29.05										
1748660	28.19										

O.mos 8 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	3915573	2408838	1114442	3162205.5	1065422.536	33.69	50.97
1.25	9.69E-02	2532923	2041071	627858	2286997	347791.8845	15.21	36.87
0.63	-2.04E-01	2432103	3148157	1798370	2790130	506326.6391	18.15	44.98
0.31	-5.05E-01	1649538	1871799	516436	1760668.5	157162.2603	8.93	28.38
0.16	-8.06E-01	1540528	1639723	365905	1590125.5	70141.45716	4.41	25.63
0.08	-1.11E+00	1521520	1625869	383117	1573694.5	73785.88551	4.69	25.37

y=mx+c

intercept 43.31

slope 9.96

r2 0.18

μℓ/well

EC20 4.58E-03

EC50 4.70E+00

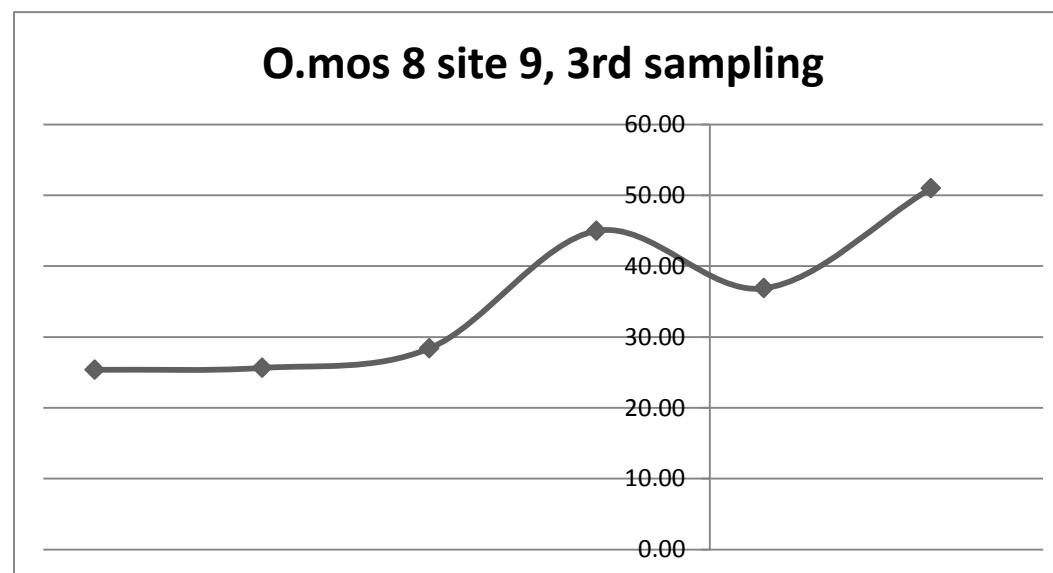
EC80 4.82E+03

	μg/μℓ	μg/mℓ
REP20	8.07E-06	8.07E-03
REP50	3.59E-07	3.59E-04
REP80	1.59E-08	1.59E-05

	E ₂ eq μg/mℓ
REP20	8.07E-02
REP50	3.59E-03
REP80	1.59E-04

100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor

0.1



Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
3915573	63.12	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
2532923	40.83	intercept	38.76	EC20	0.058420635	REP 20	6.32E-07	6.32E-04	6.32E-03	Mean	STEDV
2432103	39.21	slope	15.21	EC50	5.478686937	REP 50	3.07E-07	3.07E-04	3.07E-03	4.22E-03	3.00E-03
1649538	26.59	r ²	0.71	EC80	513.7912422	REP 80	1.49E-07	1.49E-04	1.49E-03		
1540528	24.83									REP50	
1521520	24.53									Mean	STEDV
RLU	E ₂ max									8.51E-03	7.40E-03
2408838	38.83	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2041071	32.90	intercept	50.24	EC20	0.066566244	REP 20	5.55E-07	5.55E-04	5.55E-03	REP80	
3148157	50.75	slope	25.70	EC50	0.978713484	REP 50	1.72E-06	1.72E-03	1.72E-02	Mean	STEDV
1871799	30.17	r ²	0.73	EC80	14.38987722	REP 80	5.34E-06	5.34E-03	5.34E-02	1.98E-02	2.91E-02
1639723	26.43										
1625869	26.21										
RLU	E ₂ max										
1114442	17.96	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
627858	10.12	intercept	27.78	EC20	0.467094198	REP 20	7.91E-08	7.91E-05	7.91E-04		
1798370	28.99	slope	23.54	EC50	8.784694192	REP 50	1.92E-07	1.92E-04	1.92E-03		
516436	8.32	r ²	0.67	EC80	165.214752	REP 80	4.65E-07	4.65E-04	4.65E-03		
365905	5.90										
383117	6.18										

O.mos 9 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	3074623	3370345	2255709	2900225.667	577420.2763	19.91	46.75
1.25	9.69E-02	2464043	2043847	1491878	2253945	297123.441	13.18	36.33
0.63	-2.04E-01	2058874	2089034	1146349	2073954	21326.34052	1.03	33.43
0.31	-5.05E-01	1191706	1421557	1041369	1218210.667	191474.8074	15.72	19.64
0.16	-8.06E-01	972391	1234797	690807	965998.3333	272051.3365	28.16	15.57
0.08	-1.11E+00	1147871	1109683	644754	967436	280102.3664	28.95	15.60

y=mx+c

intercept 36.70

slope 22.12

r² 0.90

μℓ/well

EC₂₀ 1.76E-01

EC₅₀ 3.99E+00

EC₈₀ 9.07E+01

	μg/μℓ	μg/mℓ
REP20	2.10E-07	2.10E-04
REP50	4.22E-07	4.22E-04
REP80	8.47E-07	8.47E-04

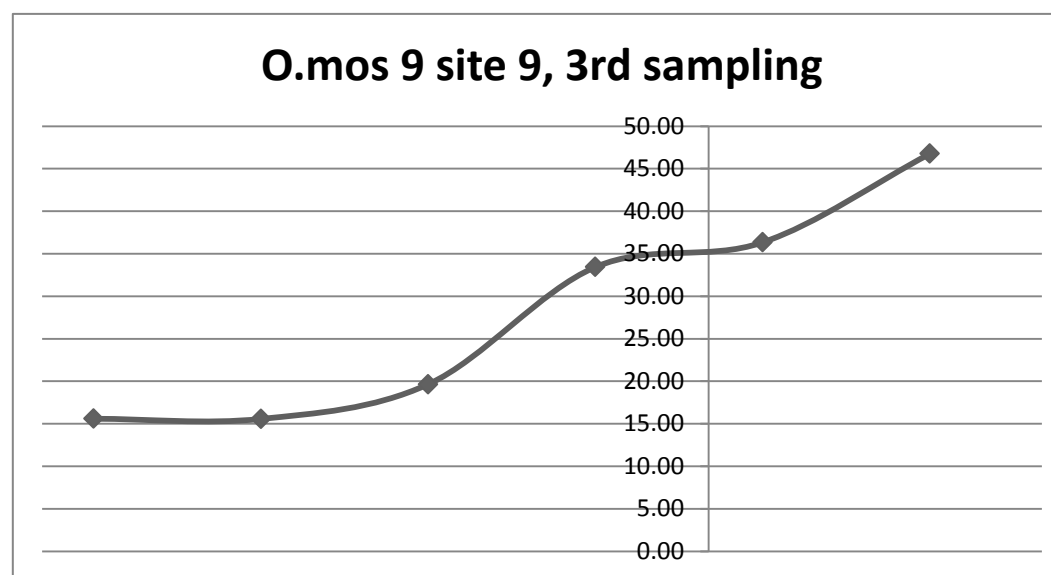
	E ₂ eq μg/mℓ
REP20	2.10E-03
REP50	4.22E-03
REP80	8.47E-03

100μℓ bile used in extraction

concentrated to 1mℓ

Concentration factor

0.1



Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
3074623	49.56	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
2464043	39.72	intercept	37.16	EC20	0.295609661	REP 20	1.25E-07	1.25E-04	1.25E-03	Mean	STEDV
2058874	33.19	slope	32.42	EC50	2.489529896	REP 50	6.77E-07	6.77E-04	6.77E-03	1.08E-03	3.91E-04
1191706	19.21	r ²	0.98	EC80	20.96602352	REP 80	3.66E-06	3.66E-03	3.66E-02		
972391	15.67									REP50	
1147871	18.50									Mean	STEDV
RLU	E ₂ max									3.24E-03	4.88E-03
3370345	54.33	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2043847	32.95	intercept	37.63	EC20	0.270652244	REP 20	1.36E-07	1.36E-04	1.36E-03	REP80	
2089034	33.68	slope	31.07	EC50	2.501080667	REP 50	6.73E-07	6.73E-04	6.73E-03	Mean	STEDV
1421557	22.92	r ²	0.84	EC80	23.11233192	REP 80	3.32E-06	3.32E-03	3.32E-02	2.40E-02	1.91E-02
1234797	19.90										
1109683	17.89										
RLU	E ₂ max										
2255709	36.36	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1491878	24.05	intercept	25.06	EC20	0.579287486	REP 20	6.38E-08	6.38E-05	6.38E-04		
1146349	18.48	slope	21.36	EC50	14.70576445	REP 50	1.15E-07	1.15E-04	1.15E-03		
1041369	16.79	r ²	0.88	EC80	373.3198341	REP 80	2.06E-07	2.06E-04	2.06E-03		
690807	11.14										
644754	10.39										

O.mos 10 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2660262	4425414	2655213	3246963	1020571.625	31.43	52.34
1.25	9.69E-02	4476929	5052414	2237493	3922278.667	1487169.393	37.92	63.23
0.63	-2.04E-01	4678951	3248080	876847	3963515.5	1011778.587	25.53	63.89
0.31	-5.05E-01	2973714	3011318	1380683	2455238.333	930782.138	37.91	39.58
0.16	-8.06E-01	2579454	2194718	2577544	2450572	221578.1217	9.04	39.50
0.08	-1.11E+00	831805	1051768	2266107	1383226.667	772466.3051	55.85	22.30

y=mx+c

intercept 61.68

slope -19.19

r2 0.79

μℓ/well

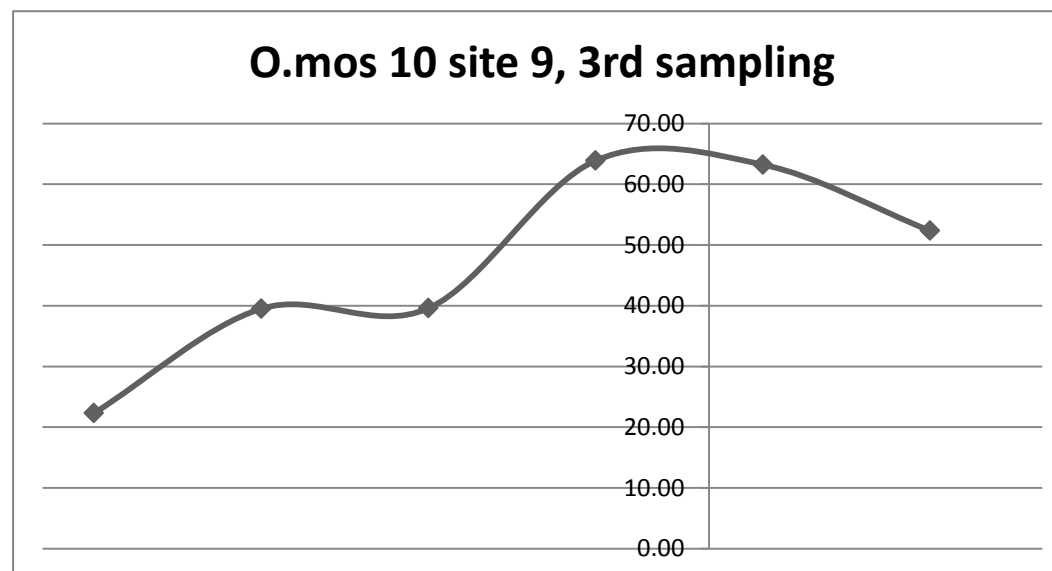
EC20 1.49E+02

EC50 4.06E+00

EC80 1.11E-01

	μg/μℓ	μg/mℓ
REP20	2.48E-10	2.48E-07
REP50	4.15E-07	4.15E-04
REP80	6.92E-04	6.92E-01

	E ₂ eq μg/mℓ
REP20	9.93E-07
REP50	1.66E-03
REP80	2.77E+00



250μℓ bile used in extraction

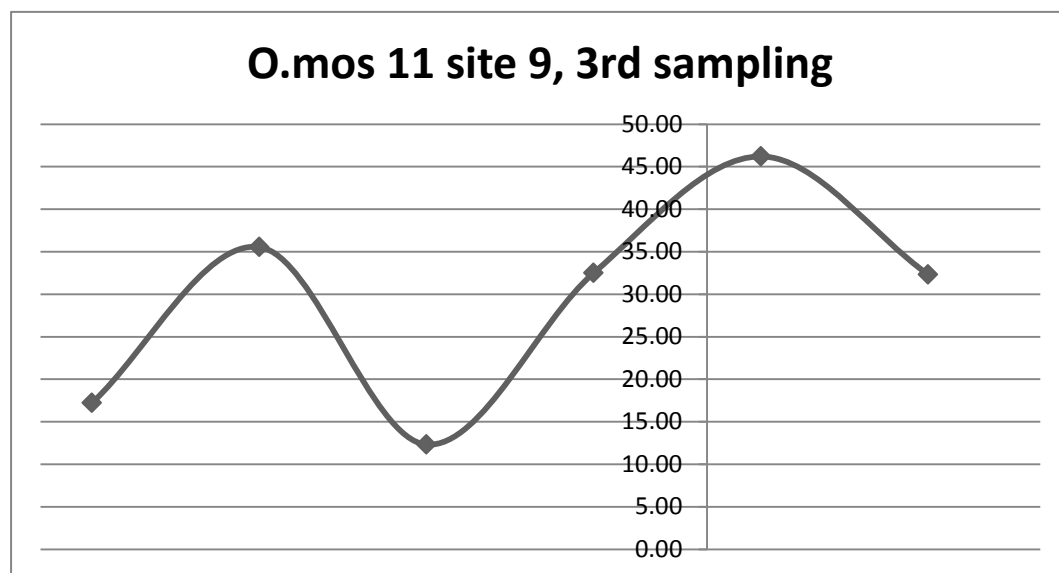
concentrated to 1mℓ

Concentration factor

0.25

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
2660262	42.88	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
4476929	72.17	intercept	86.49	EC20	0.091126061	REP 20	4.05E-07	4.05E-04	1.62E-03	Mean	STEDV
4678951	75.42	slope	63.92	EC50	0.268543855	REP 50	6.27E-06	6.27E-03	2.51E-02	1.31E-03	8.35E-04
2973714	47.94	r ²	0.95	EC80	0.791385045	REP 80	9.71E-05	9.71E-02	3.88E-01		
2579454	41.58									REP50	
831805	13.41									Mean	STEDV
RLU	E ₂ max									0.012	0.014
4425414	71.34	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
5052414	81.44	intercept	64.31	EC20	0.076321913	REP 20	4.84E-07	4.84E-04	1.94E-03	REP80	
3248080	52.36	slope	39.66	EC50	0.435661813	REP 50	3.87E-06	3.87E-03	1.55E-02	Mean	STEDV
3011318	48.54	r ²	0.93	EC80	2.486850883	REP 80	3.09E-05	3.09E-02	1.24E-01	0.17	0.20
2194718	35.38										
1051768	16.95										
RLU	E ₂ max										
2655213	42.80	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2237493	36.07	intercept	28.84	EC20	0.411943185	REP 20	8.97E-08	8.97E-05	3.59E-04		
876847	14.13	slope	22.94	EC50	8.366544223	REP 50	2.01E-07	2.01E-04	8.05E-04		
1380683	22.26	r ²	0.39	EC80	169.9240691	REP 80	4.52E-07	4.52E-04	1.81E-03		
2577544	41.55										
2266107	36.53										

O.mos 11 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2314118	3715089	1695753	2004935.5	437250.0847	21.81	32.32
1.25	9.69E-02	2426713	3472161	2700246	2866373.333	542161.5058	18.91	46.21
0.63	-2.04E-01	1514196	2660877	1874502	2016525	586384.8807	29.08	32.51
0.31	-5.05E-01	697718	911101	684883	764567.3333	127064.0425	16.62	12.32
0.16	-8.06E-01	1859911	2564718	2191991	2205540	352598.7923	15.99	35.55
0.08	-1.11E+00	1546954	1149602	505471	1067342.333	525591.7612	49.24	17.21



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

O.mos 12 site 9 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	3041292	3281670	3509943	2458226.25	234351.554	9.53	39.63
1.25	9.69E-02	3202165	3458080	3280869	2485278.5	131079.2034	5.27	40.06
0.63	-2.04E-01	2652502	2845792	2725202	2055874	97628.77154	4.75	33.14
0.31	-5.05E-01	1908748	2201240	2361514	1617875.5	229578.0099	14.19	26.08
0.16	-8.06E-01	1745018	2307119	1818471	1781744.5	51939.1144	2.92	28.72
0.08	-1.11E+00	1556972	1653277	2332569	1070083	68097.91856	6.36	17.25

$y=mx+c$

intercept 35.57

slope 15.80

r² 0.87

$\mu\ell/\text{well}$

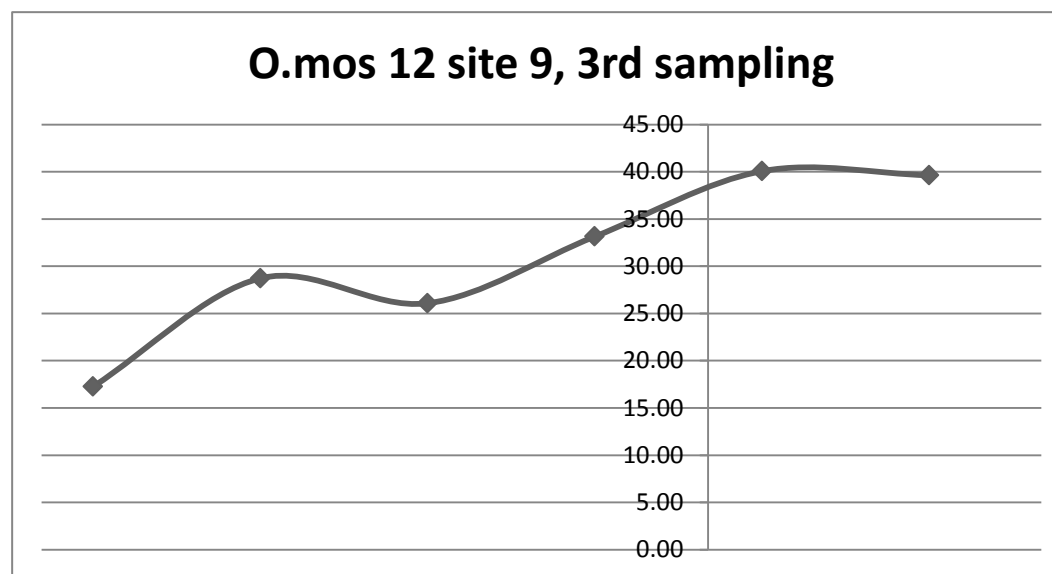
EC₂₀ 1.03E-01

EC₅₀ 8.19E+00

EC₈₀ 6.48E+02

	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	3.57E-07	3.57E-04
REP50	2.06E-07	2.06E-04
REP80	1.18E-07	1.18E-04

	E ₂ eq $\mu\text{g}/\text{m}\ell$
REP20	3.57E-03
REP50	2.06E-03
REP80	1.18E-03



100 $\mu\ell$ bile used in extraction

concentrated to 1m ℓ

Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
3041292	49.03	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
3202165	51.62	intercept	48.03	EC20	0.094750888	REP 20	3.90E-07	3.90E-04	3.90E-03	Mean	STEDV
2652502	42.76	slope	27.39	EC50	1.179799925	REP 50	1.43E-06	1.43E-03	1.43E-02	6.48E-03	3.10E-03
1908748	30.77	r ²	0.95	EC80	14.69039389	REP 80	5.23E-06	5.23E-03	5.23E-02		
1745018	28.13									REP50	
1556972	25.10									Mean	STEDV
RLU	E ₂ max									2.57E-03	1.69E-02
3281670	52.90	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
3458080	55.74	intercept	51.35	EC20	0.03723888	REP 20	9.92E-07	9.92E-04	9.92E-03	REP80	
2845792	45.87	slope	21.94	EC50	0.867489322	REP 50	1.94E-06	1.94E-03	1.94E-02	Mean	STEDV
2201240	35.48	r ²	0.84	EC80	20.20838785	REP 80	3.80E-06	3.80E-03	3.80E-02	4.72E-02	7.94E-03
2307119	37.19										
1653277	26.65										
RLU	E ₂ max										
3509943	56.58	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
3280869	52.89	intercept	50.07	EC20	0.065759488	REP 20	5.62E-07	5.62E-04	5.62E-03		
2725202	43.93	slope	25.44	EC50	0.993502362	REP 50	1.70E-06	1.70E-03	1.70E-02		
2361514	38.07	r ²	0.99	EC80	15.00995472	REP 80	5.12E-06	5.12E-03	5.12E-02		
1818471	29.31										
2332569	37.60										

O.mos 14 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	3041292	3281670	3509943	3277635	234351.554	7.15	52.84
1.25	9.69E-02	3202165	3458080	3280869	3313704.667	131079.2034	3.96	53.42
0.63	-2.04E-01	2652502	2845792	2725202	2741165.333	97628.77154	3.56	44.19
0.31	-5.05E-01	1908748	2201240	2361514	2157167.333	229578.0099	10.64	34.77
0.16	-8.06E-01	1745018	2307119	1818471	1956869.333	305540.4352	15.61	31.54
0.08	-1.11E+00	1556972	1653277	2332569	1847606	422741.6431	22.88	29.78

y=mx+c

intercept 47.43

slope 21.07

r2 0.87

μℓ/well

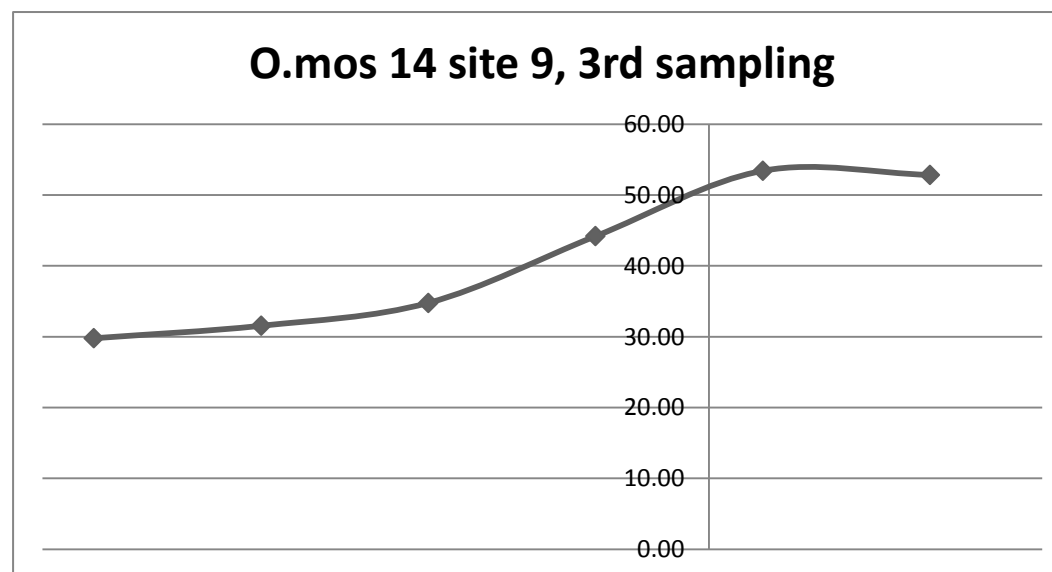
EC20 4.99E-02

EC50 1.32E+00

EC80 3.52E+01

	μg/μℓ	μg/mℓ
REP20	7.41E-07	7.41E-04
REP50	1.27E-06	1.27E-03
REP80	2.18E-06	2.18E-03

	E ₂ eq μg/mℓ
REP20	4.94E-03
REP50	8.48E-03
REP80	1.46E-02



150μℓ bile used in extraction

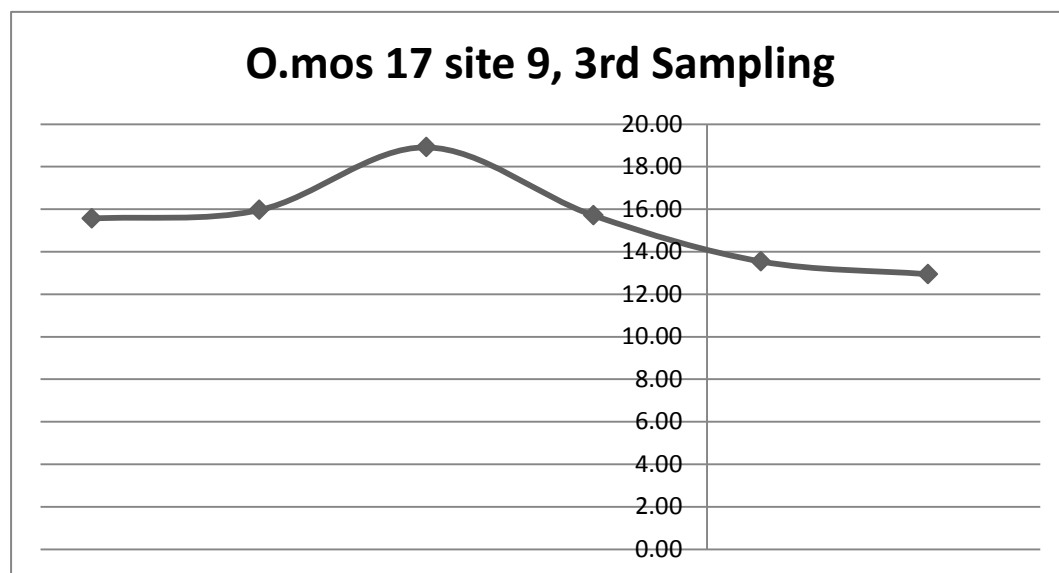
concentrated to 1mℓ

Concentration factor

0.15

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
3041292	49.03	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
3202165	51.62	intercept	48.03	EC20	0.094750888	REP 20	3.90E-07	3.90E-04	2.60E-03	Mean	STEDV
2652502	42.76	slope	27.39	EC50	1.179799925	REP 50	1.43E-06	1.43E-03	9.52E-03	4.83E-03	2.93E-03
1908748	30.77	r ²	0.95	EC80	14.69039389	REP 80	5.23E-06	5.23E-03	3.49E-02		
1745018	28.13									REP50	
1556972	25.10									Mean	STEDV
RLU	E ₂ max									1.77E-03	1.13E-02
3281670	52.90	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
3458080	55.74	intercept	51.35	EC20	0.030239062	REP 20	1.22E-06	1.22E-03	8.14E-03	REP80	
2845792	45.87	slope	20.64	EC50	0.859720833	REP 50	1.96E-06	1.96E-03	1.31E-02	Mean	STEDV
2201240	35.48	r ²	0.84	EC80	24.44255426	REP 80	3.14E-06	3.14E-03	2.09E-02	3.00E-02	7.82E-03
2307119	37.19										
1653277	26.65										
RLU	E ₂ max										
3509943	56.58	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
3280869	52.89	intercept	50.07	EC20	0.065759488	REP 20	5.62E-07	5.62E-04	3.74E-03		
2725202	43.93	slope	25.44	EC50	0.993502362	REP 50	1.70E-06	1.70E-03	1.13E-02		
2361514	38.07	r ²	0.99	EC80	15.00995472	REP 80	5.12E-06	5.12E-03	3.41E-02		
1818471	29.31										
2332569	37.60										

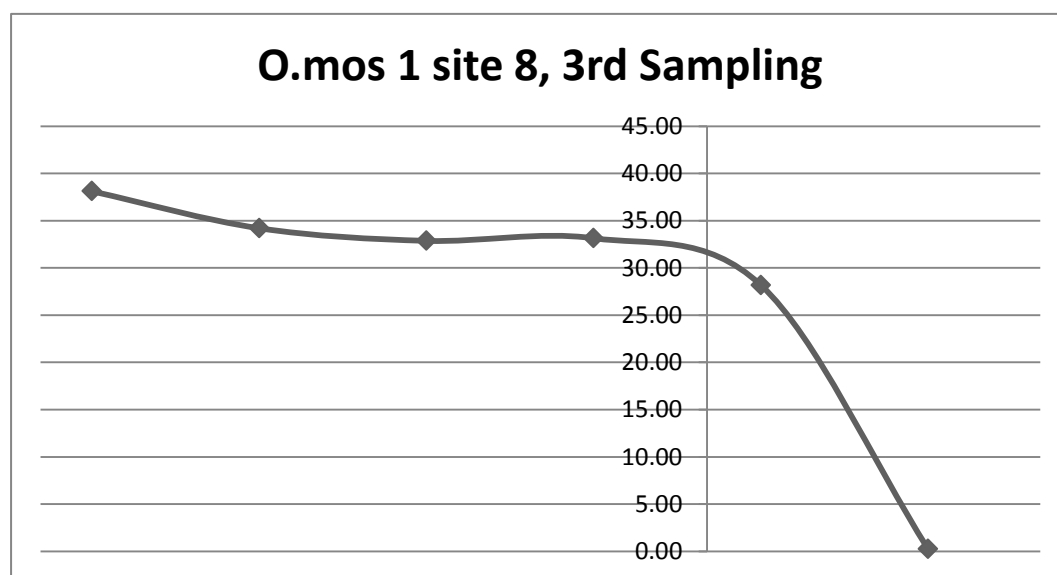
O.mos 17 site 9 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	772481	850442	785696	802873	41722.49246	5.20	12.94
1.25	9.69E-02	912226	830948	777426	840200	67874.5875	8.08	13.54
0.63	-2.04E-01	1065613	1045019	811907	974179.6667	140908.9847	14.46	15.70
0.31	-5.05E-01	1180417	1163456	1175433	1173102	8717.456682	0.74	18.91
0.16	-8.06E-01	912566	856058	1201667	990097	185390.634	18.72	15.96
0.08	-1.11E+00	1048312	902305	945493	965370	75005.54885	7.77	15.56



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No Response

O.mos 1 site 8 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	12899	10504	803248	11701.5	1693.52	14.47	0.24
1.25	9.69E-02	1369589	210235	1400056	1384822.5	21543.42	1.56	28.18
0.63	-2.04E-01	1587625	172445	1669906	1628765.5	58181.45	3.57	33.14
0.31	-5.05E-01	1642798	290639	1587665	1615231.5	38984.92	2.41	32.87
0.16	-8.06E-01	1618416	325379	1743157	1680786.5	88205.21	5.25	34.20
0.08	-1.11E+00	1837957	508142	1907922	1872939.5	49472.73	2.64	38.11



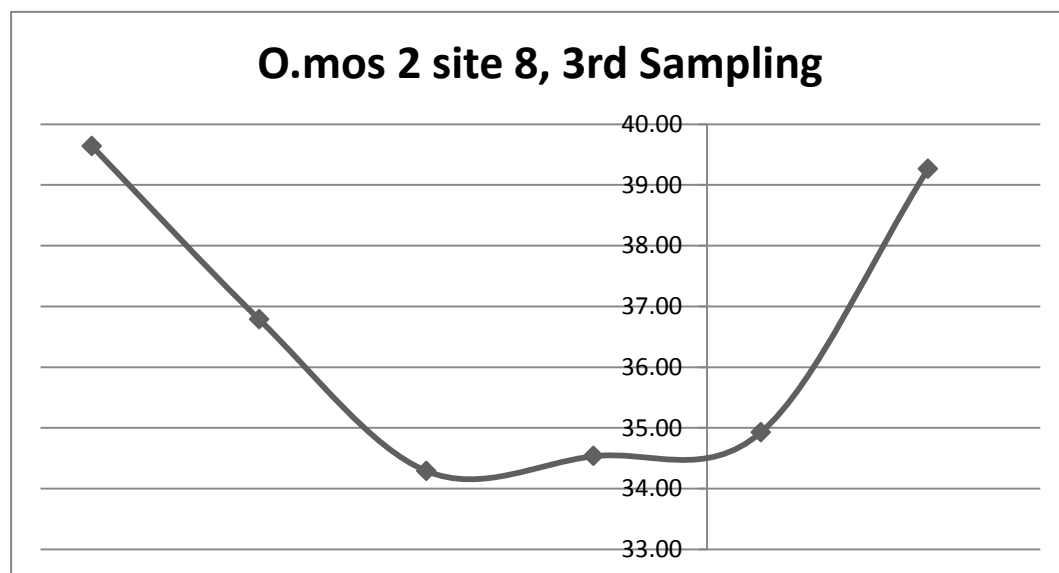
100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration could indicate cytotoxicity

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

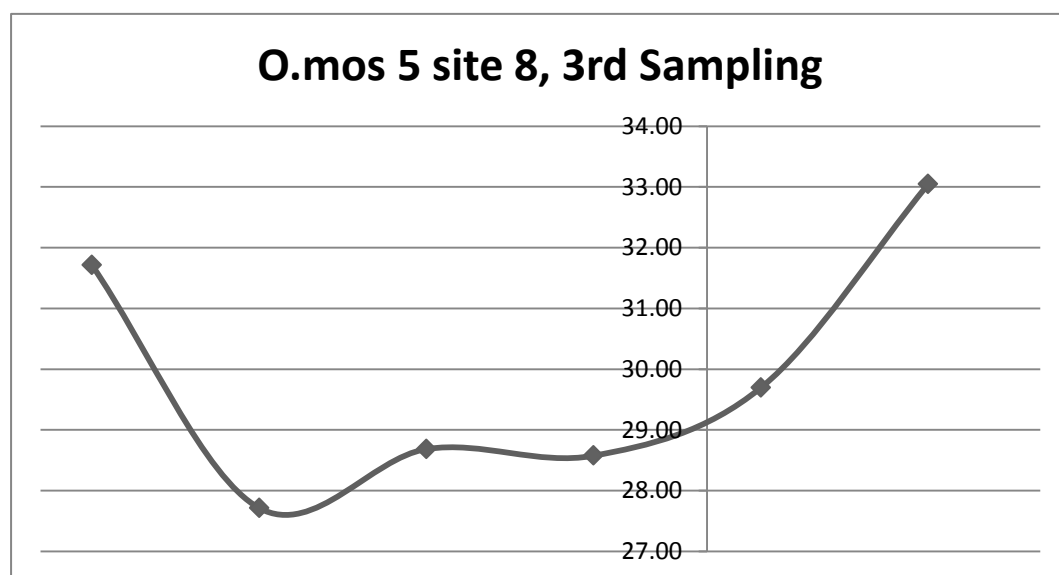
O.mos 2 site 8 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1131039	1654798	2204485	1929641.5	388687.4052	20.14	39.26
1.25	9.69E-02	1507294	1485631	2156256	1716393.667	381085.9163	22.20	34.93
0.63	-2.04E-01	1521358	1536207	2033756	1697107	291641.1067	17.18	34.53
0.31	-5.05E-01	1518234	1451765	2085400	1685133	348230.937	20.66	34.29
0.16	-8.06E-01	1594358	1801825	2027315	1807832.667	216541.0123	11.98	36.79
0.08	-1.11E+00	1993902	1862525	1987525	1947984	74078.31696	3.80	39.64



100 $\mu\ell$ bile used in extraction
concentrated to 1m ℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

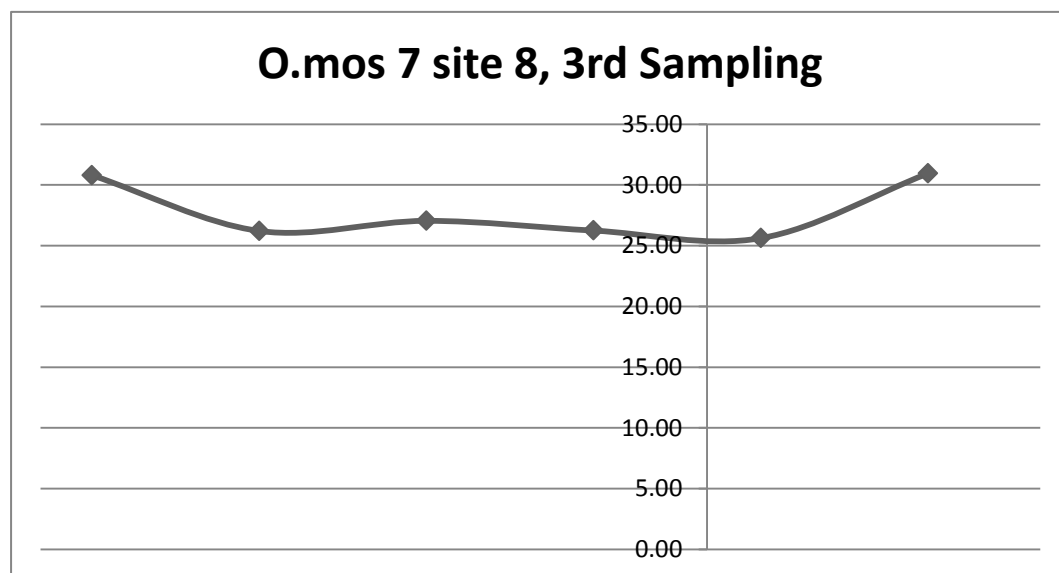
O.mos 5 site 8 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1919955	1380376	1571627	1623986	273573.5239	16.85	33.05
1.25	9.69E-02	1610340	1305507	1462042	1459296.333	152435.0468	10.45	29.69
0.63	-2.04E-01	1580641	1327153	1305045	1404279.667	153132.8862	10.90	28.57
0.31	-5.05E-01	1650582	1321238	1257050	1409623.333	211129.8987	14.98	28.68
0.16	-8.06E-01	1497672	1293360	1294793	1361941.667	117548.1004	8.63	27.71
0.08	-1.11E+00	1805038	1450810	1419518	1558455.333	214119.2564	13.74	31.71



100 $\mu\ell$ bile used in extraction
concentrated to 1m ℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

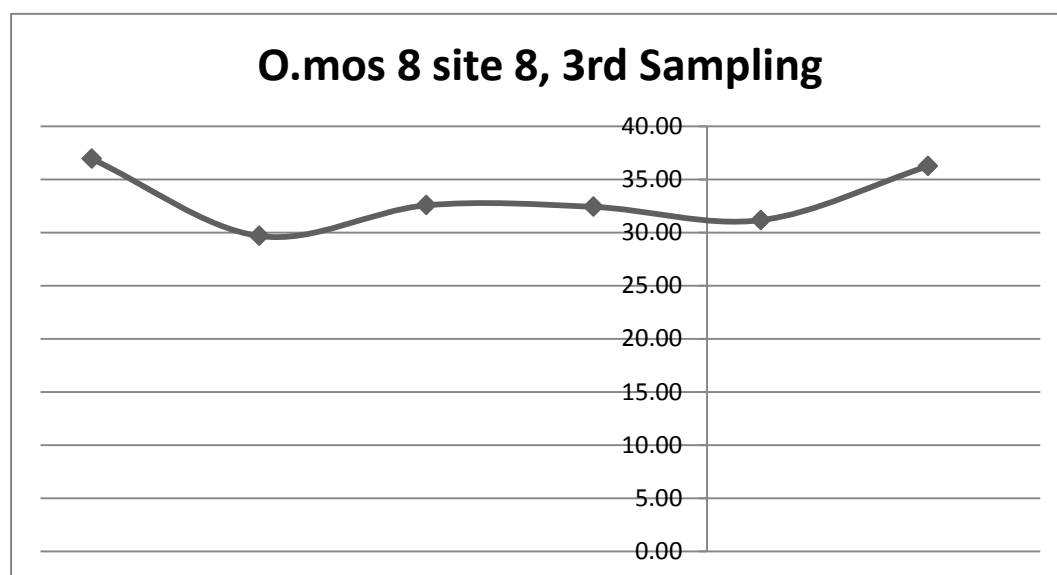
O.mos 7 site 8 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1462706	1369896	1730825	1521142.333	187426.0909	12.32	30.95
1.25	9.69E-02	1180191	1170812	1426234	1259079	144836.4144	11.50	25.62
0.63	-2.04E-01	1230006	1220482	1418530	1289672.667	111695.2815	8.66	26.24
0.31	-5.05E-01	1273316	1247032	1466946	1329098	120101.0637	9.04	27.04
0.16	-8.06E-01	1274465	1181016	1409022	1288167.667	114618.9614	8.90	26.21
0.08	-1.11E+00	1343289	1389674	1807878	1513613.667	255893.5628	16.91	30.80



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

O.mos 8 site 8 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1263249	2028189	2342708	2185448.5	222398.5177	10.18	36.25
1.25	9.69E-02	1166814	1784597	1973396	1878996.5	133501.0532	7.10	31.16
0.63	-2.04E-01	1298758	1725888	2182766	1954327	323061.532	16.53	32.41
0.31	-5.05E-01	993876	1783861	2141949	1962905	253206.4531	12.90	32.55
0.16	-8.06E-01	1056315	1932191	2379261	1789255.667	672955.728	37.61	29.67
0.08	-1.11E+00	182414	2060468	2391642	2226055	234175.3812	10.52	36.92



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

O.mos 9 site 8 3rd sampling (µℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2063932	1386307	1504737	1651658.667	361916.278	21.91	33.61
1.25	9.69E-02	1527468	1236860	1298693	1354340.333	153087.3009	11.30	27.56
0.63	-2.04E-01	1317337	1088207	1052552	1152698.667	143691.1805	12.47	23.46
0.31	-5.05E-01	1209954	928240	847334	995176	190351.3519	19.13	20.25
0.16	-8.06E-01	1044994	935361	857466	945940.3333	94210.55894	9.96	19.25
0.08	-1.11E+00	1003125	838773	847164	896354	92561.53116	10.33	18.24

y=mx+c

intercept 26.57

slope 16.86

r2 0.99

µℓ/well

EC20 4.08E-01

EC50 2.45E+01

EC80 1.47E+03

	µg/µℓ	µg/mℓ
REP20	6.44E-10	6.44E-07
REP50	8.86E-10	8.86E-07
REP80	1.22E-09	1.22E-06

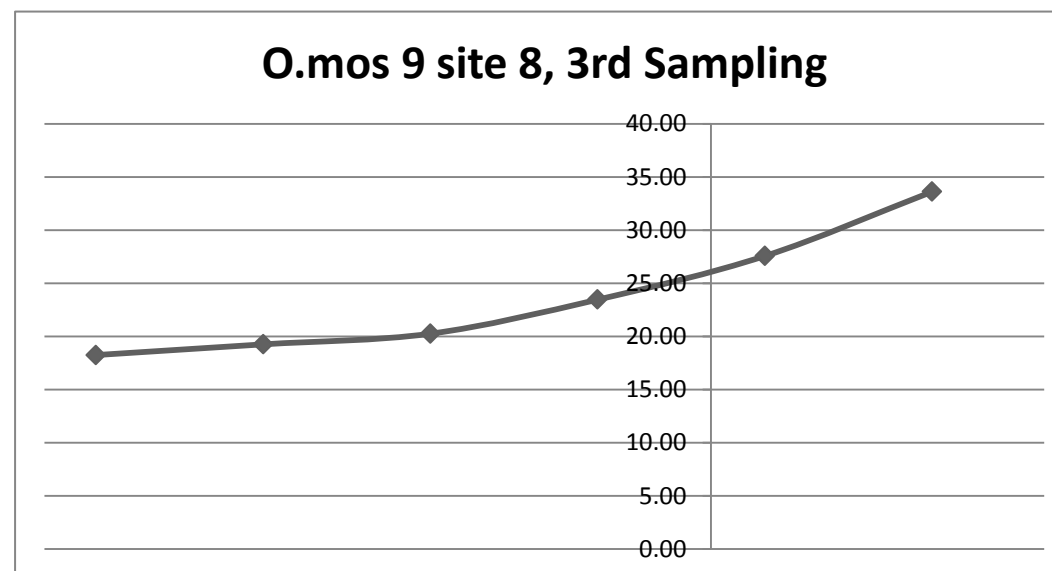
	E ₂ eq µg/mℓ
REP20	2.57E-06
REP50	3.54E-06
REP80	4.87E-06

250µℓ bile used in extraction

concentrated to 1mℓ

Concentration factor

0.25



Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
2063932	42.00	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1527468	31.08	intercept	32.40	EC20	0.166323188	REP 20	1.58E-09	1.58E-06	6.31E-06	Mean	STEDV
1317337	26.81	slope	15.92	EC50	12.74150044	REP 50	1.70E-09	1.70E-06	6.81E-06	4.23E-06	1.84E-06
1209954	24.62	r ²	0.89	EC80	976.08659	REP 80	1.84E-09	1.84E-06	7.35E-06		
1044994	21.26									REP50	
1003125	20.41									Mean	STEDV
RLU	E ₂ max									3.73E-06	2.51E-06
1386307	28.21	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1236860	25.17	intercept	24.36	EC20	0.293331447	REP 20	8.94E-10	8.94E-07	3.58E-06	REP80	
1088207	22.14	slope	8.18	EC50	1360.595752	REP 50	1.59E-11	1.59E-08	6.38E-08	Mean	STEDV
928240	18.89	r ²	0.94	EC80	6311020.575	REP 80	2.84E-13	2.84E-10	1.14E-09	2.50E-06	4.20E-06
935361	19.03										
838773	17.07										
RLU	E ₂ max										
1504737	30.62	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1298693	26.43	intercept	25.04	EC20	0.374106836	REP 20	7.01E-10	7.01E-07	2.81E-06		
1052552	21.42	slope	11.80	EC50	130.3334399	REP 50	1.66E-10	1.66E-07	6.66E-07		
847334	17.24	r ²	0.93	EC80	45406.29553	REP 80	3.95E-11	3.95E-08	1.58E-07		
857466	17.45										
847164	17.24										

O.mos 10 site 8 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1916630	1791451	1426105	1711395.333	254873.2552	14.89	34.82
1.25	9.69E-02	1634697	1282695	1136719	1351370.333	255993.6487	18.94	27.50
0.63	-2.04E-01	1512885	1074490	967301	1184892	289062.1498	24.40	24.11
0.31	-5.05E-01	1524716	985525	899464	1136568.333	338888.7462	29.82	23.13
0.16	-8.06E-01	1637271	966362	847812	1150481.667	425718.7063	37.00	23.41
0.08	-1.11E+00	1902517	1028462	808492	1246490.333	578683.8003	46.43	25.36

y=mx+c

intercept 27.09

slope 17.79

r2 0.96

$\mu\ell$ /well

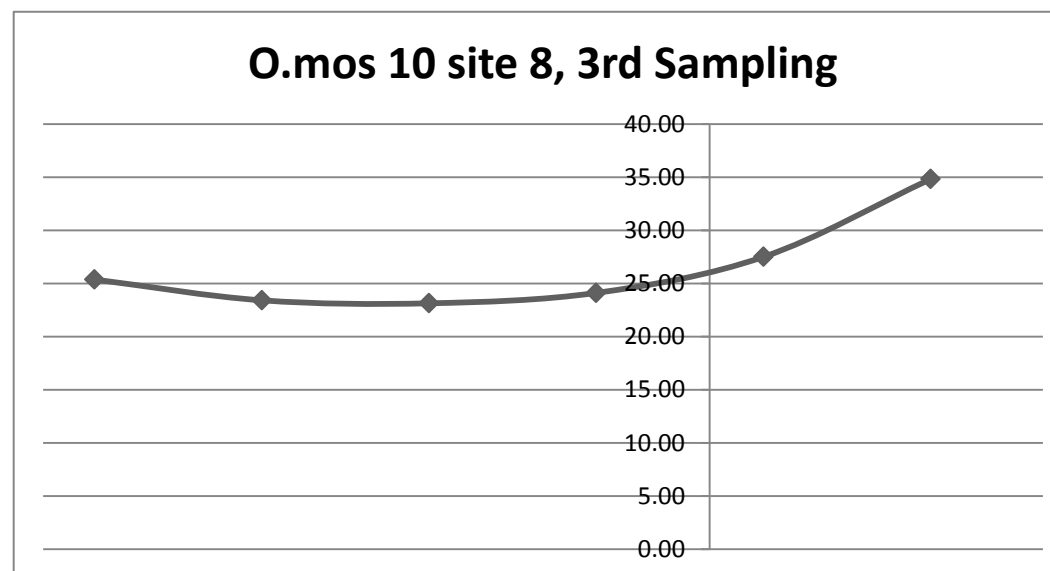
EC20 4.00E-01

EC50 1.94E+01

EC80 9.41E+02

	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	6.56E-10	6.56E-07
REP50	1.12E-09	1.12E-06
REP80	1.91E-09	1.91E-06

	E ₂ eq $\mu\text{g}/\text{m}\ell$
REP20	2.63E-06
REP50	4.47E-06
REP80	7.63E-06



250 $\mu\ell$ bile used in extraction

concentrated to 1m ℓ

Concentration factor

0.25

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
1916630	39.00	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1634697	33.26	intercept	33.99	EC20	0.025417446	REP 20	1.03E-08	1.03E-05	4.13E-05	Mean	STEDV
1512885	30.78	slope	8.77	EC50	66.92007113	REP 50	3.24E-10	3.24E-07	1.30E-06	1.59E-05	2.20E-05
1524716	31.03	r ²	0.80	EC80	176189.8462	REP 80	1.02E-11	1.02E-08	4.07E-08		
1637271	33.32									REP50	
1902517	38.71									Mean	STEDV
RLU	E ₂ max									8.15E-07	1.04E-06
1791451	36.45	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1282695	26.10	intercept	27.51	EC20	0.268646412	REP 20	9.77E-10	9.77E-07	3.91E-06	REP80	
1074490	21.86	slope	13.16	EC50	51.08261928	REP 50	4.25E-10	4.25E-07	1.70E-06	Mean	STEDV
985525	20.05	r ²	0.81	EC80	9713.265758	REP 80	1.85E-10	1.85E-07	7.39E-07	2.62E-07	4.13E-07
966362	19.66										
1028462	20.93										
RLU	E ₂ max										
1426105	29.02	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1136719	23.13	intercept	23.40	EC20	0.435615568	REP 20	6.02E-10	6.02E-07	2.41E-06		
967301	19.68	slope	9.42	EC50	665.6467468	REP 50	3.26E-11	3.26E-08	1.30E-07		
899464	18.30	r ²	0.89	EC80	1017148.201	REP 80	1.76E-12	1.76E-09	7.06E-09		
847812	17.25										
808492	16.45										

O.mos 11 site 8 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2001921	2190275	2300030	2164075.333	150771.5519	6.97	44.03
1.25	9.69E-02	1628976	1973364	1931721	1844687	187967.98	10.19	37.54
0.63	-2.04E-01	1524918	2074168	1860899	1819995	276900.2411	15.21	37.03
0.31	-5.05E-01	1364855	1961557	1841186	1722532.667	315550.7556	18.32	35.05
0.16	-8.06E-01	1458999	1891819	1765475	1705431	222569.651	13.05	34.70
0.08	-1.11E+00	2040375	3036809	2134828	2404004	550056.332	22.88	48.92

$y=mx+c$

intercept 38.41

slope 11.63

r² 0.80

$\mu\ell$ /well

EC20 2.61E-02

EC50 9.93E+00

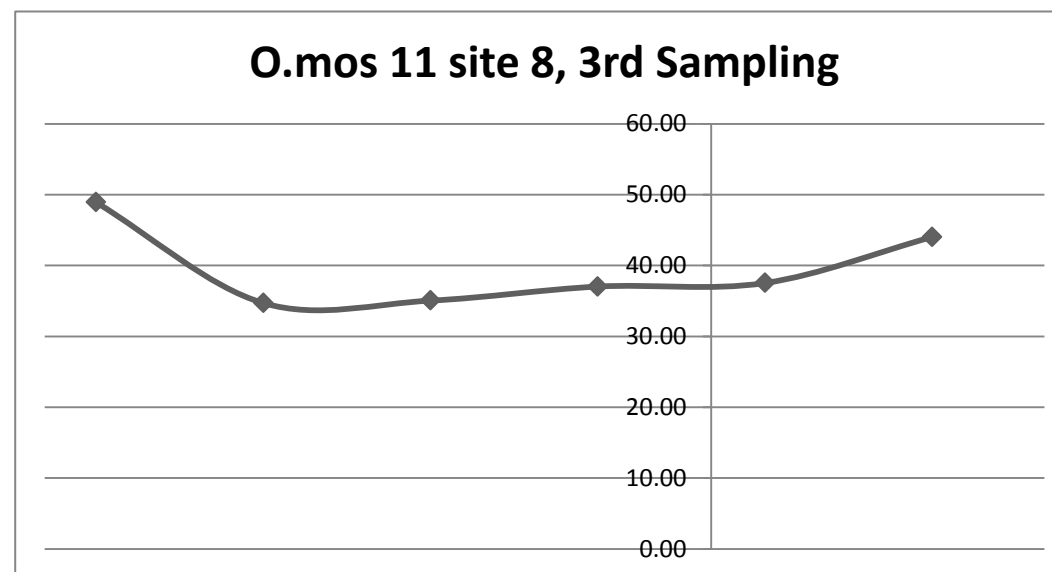
EC80 3.77E+03

	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	1.00E-08	1.00E-05
REP50	2.19E-09	2.19E-06
REP80	4.76E-10	4.76E-07

	E ₂ eq $\mu\text{g}/\text{m}\ell$
REP20	4.02E-05
REP50	8.74E-06
REP80	1.90E-06

100 $\mu\ell$ bile used in extraction
concentrated to 1m ℓ
Concentration factor

0.1



Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
1916630	39.00	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1634697	33.26	intercept	33.99	EC20	0.025417446	REP 20	1.03E-08	1.03E-05	1.03E-04	Mean	STEDV
1512885	30.78	slope	8.77	EC50	66.92007113	REP 50	3.24E-10	3.24E-07	3.24E-06	3.97E-05	5.51E-05
1524716	31.03	r ²	0.80	EC80	176189.8462	REP 80	1.02E-11	1.02E-08	1.02E-07		
1637271	33.32									REP50	
1902517	38.71									Mean	STEDV
RLU	E ₂ max									2.04E-06	2.61E-06
1791451	36.45	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1282695	26.10	intercept	27.51	EC20	0.268646412	REP 20	9.77E-10	9.77E-07	9.77E-06	REP80	
1074490	21.86	slope	13.16	EC50	51.08261928	REP 50	4.25E-10	4.25E-07	4.25E-06	Mean	STEDV
985525	20.05	r ²	0.81	EC80	9713.265758	REP 80	1.85E-10	1.85E-07	1.85E-06	6.56E-07	1.03E-06
966362	19.66										
1028462	20.93										
RLU	E ₂ max										
1426105	29.02	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1136719	23.13	intercept	23.40	EC20	0.435615568	REP 20	6.02E-10	6.02E-07	6.02E-06		
967301	19.68	slope	9.42	EC50	665.6467468	REP 50	3.26E-11	3.26E-08	3.26E-07		
899464	18.30	r ²	0.89	EC80	1017148.201	REP 80	1.76E-12	1.76E-09	1.76E-08		
847812	17.25										
808492	16.45										

O.mos 15 site 8 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	2827795	2902802	2842636	2857744.333	39720.38286	1.39	58.15
1.25	9.69E-02	2022524	2645421	1749930	2139291.667	459022.9284	21.46	43.53
0.63	-2.04E-01	1659646	1923184	1822606	1801812	132993.8427	7.38	36.66
0.31	-5.05E-01	1626047	1855809	2211008	1897621.333	294713.4987	15.53	38.61
0.16	-8.06E-01	2170029	1860478	1957455	1995987.333	158331.9643	7.93	40.61
0.08	-1.11E+00	1119248	1815728	2205683	1713553	550377.1891	32.12	34.87

y=mx+c

intercept 42.66

slope 35.69

r2 0.96

μℓ/well

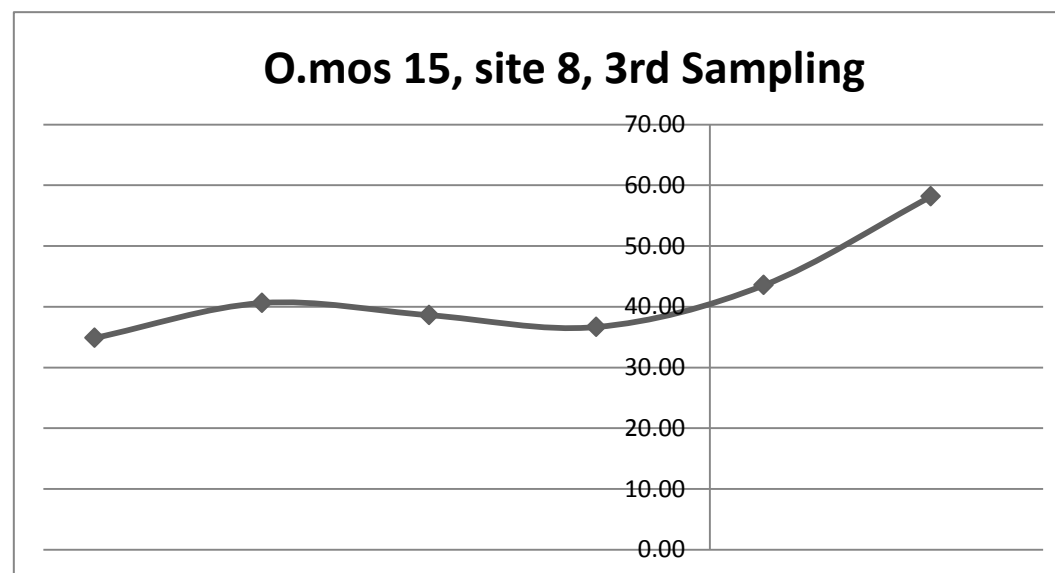
EC20 2.32E-01

EC50 1.61E+00

EC80 1.11E+01

	μg/μℓ	μg/mℓ
REP20	1.13E-09	1.13E-06
REP50	1.35E-08	1.35E-05
REP80	1.61E-07	1.61E-04

	E ₂ eq μg/mℓ
REP20	1.13E-05
REP50	1.35E-04
REP80	1.61E-03



100μℓ bile used in extraction

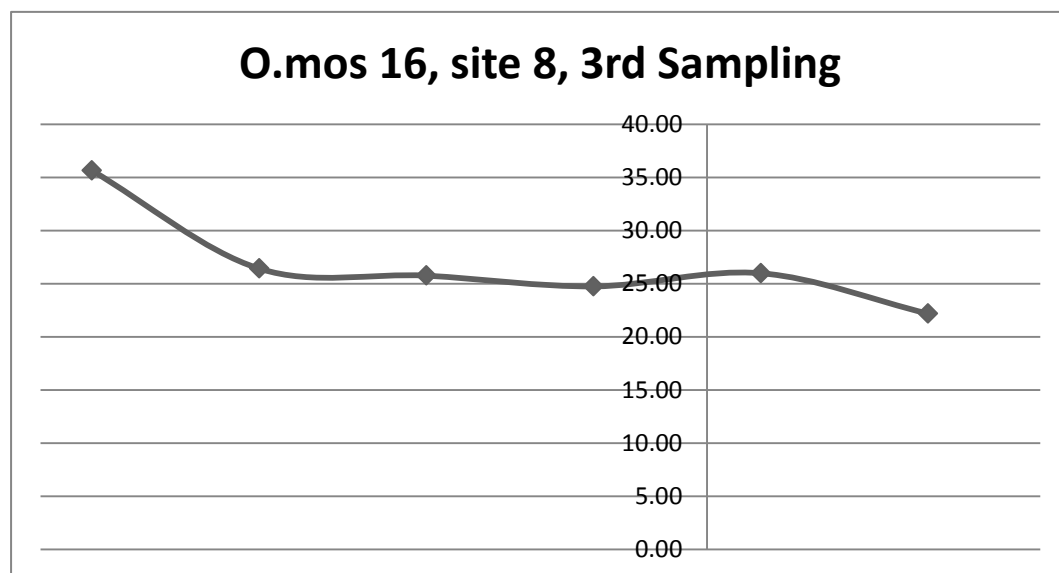
concentrated to 1mℓ

Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
2827795	57.54	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
2022524	41.15	intercept	40.33	EC20	0.305550512	REP 20	8.59E-10	8.59E-07	8.59E-06	Mean	STEDV
1659646	33.77	slope	39.48	EC50	1.757718592	REP 50	1.23E-08	1.23E-05	1.23E-04	1.21E-05	4.90E-06
1626047	33.09	r ²	0.95	EC80	10.1115021	REP 80	1.77E-07	1.77E-04	1.77E-03		
2170029	44.16									REP50	
1119248	22.77									Mean	STEDV
RLU	E ₂ max									3.73E-05	1.39E-04
2902802	59.07	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2645421	53.83	intercept	47.47	EC20	0.14803746	REP 20	1.77E-09	1.77E-06	1.77E-05	REP80	
1923184	39.13	slope	33.11	EC50	1.192550993	REP 50	1.82E-08	1.82E-05	1.82E-04	Mean	STEDV
1855809	37.76	r ²	0.93	EC80	9.606878342	REP 80	1.87E-07	1.87E-04	1.87E-03	1.63E-03	3.30E-04
1860478	37.86										
1815728	36.95										
RLU	E ₂ max										
2842636	57.84	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1749930	35.61	intercept	40.17	EC20	0.259949796	REP 20	1.01E-09	1.01E-06	1.01E-05		
1822606	37.09	slope	34.47	EC50	1.927952506	REP 50	1.13E-08	1.13E-05	1.13E-04		
2211008	44.99	r ²	0.70	EC80	14.2989182	REP 80	1.25E-07	1.25E-04	1.25E-03		
1957455	39.83										
2205683	44.88										

O.mos 16 site 8 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1052690	1121401	1094964	1089685	34658.35052	3.18	22.17
1.25	9.69E-02	1147676	1289363	1393177	1276738.667	123236.4215	9.65	25.98
0.63	-2.04E-01	1329152	1153276	1165155	1215861	98292.50058	8.08	24.74
0.31	-5.05E-01	1088895	1163367	1544291	1265517.667	244279.447	19.30	25.75
0.16	-8.06E-01	1052310	1416985	1425206	1298167	212958.0817	16.40	26.42
0.08	-1.11E+00	1150549	1980202	2123337	1751362.667	525218.7198	29.99	35.64



100 $\mu\ell$ bile used in extraction
concentrated to 1m ℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

O.mos 17 site 8 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	835447	1130877	953682	973335.3333	148692.3379	15.28	19.81
1.25	9.69E-02	927884	766906	956077	883622.3333	102057.5238	11.55	17.98
0.63	-2.04E-01	702063	828318	911368	813916.3333	105393.0821	12.95	16.56
0.31	-5.05E-01	735265	817466	985080	845937	127317.8383	15.05	17.21
0.16	-8.06E-01	750592	890814	984287	875231	117624.2351	13.44	17.81
0.08	-1.11E+00	1009292	820921	1225402	1018538.333	202398.9645	19.87	20.73

y=mx+c

intercept 17.59

slope 5.39

r2 0.99

μℓ/well

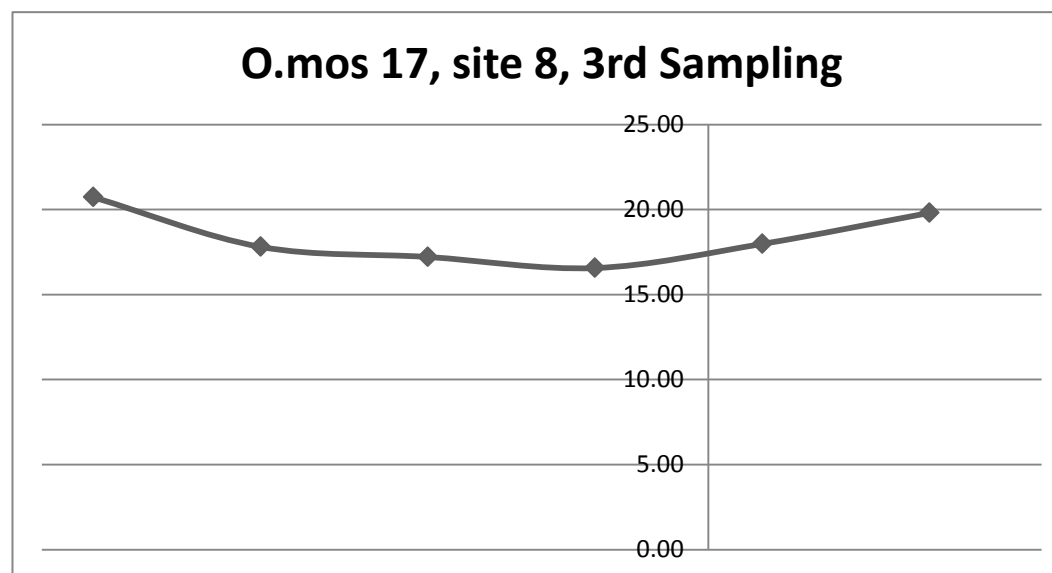
EC20 2.80E+00

EC50 1.03E+06

EC80 3.82E+11

	μg/μℓ	μg/mℓ
REP20	9.38E-11	9.38E-08
REP50	2.10E-14	2.10E-11
REP80	4.69E-18	4.69E-15

	E ₂ eq μg/mℓ
REP20	9.38E-07
REP50	2.10E-10
REP80	4.69E-14



100μℓ bile used in extraction

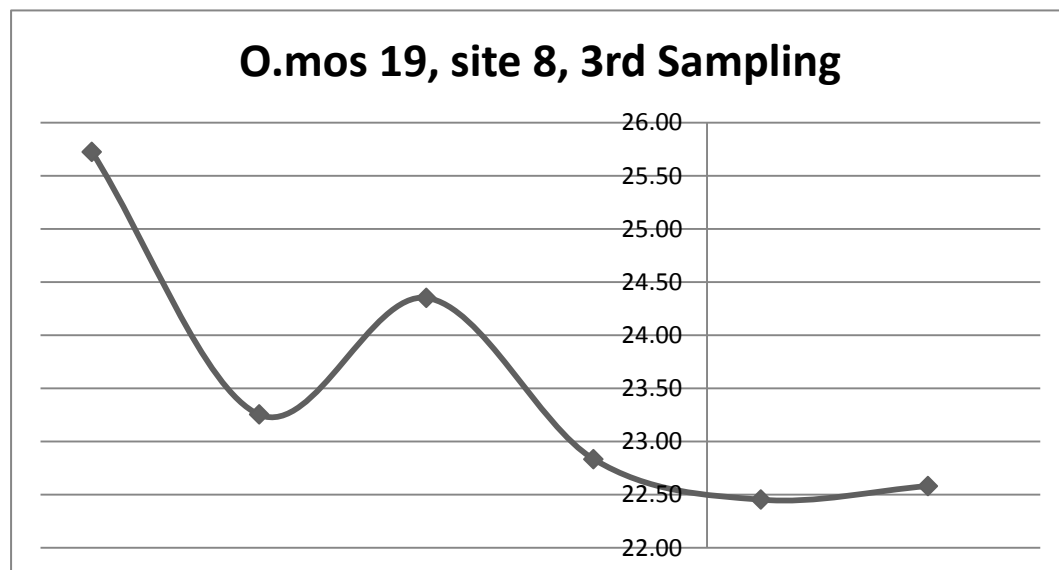
concentrated to 1mℓ

Concentration factor

0.1

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
2827795	57.54	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
2022524	41.15	intercept	40.33	EC20	0.305550512	REP 20	8.59E-10	8.59E-07	8.59E-06	Mean	STEDV
1659646	33.77	slope	39.48	EC50	1.757718592	REP 50	1.23E-08	1.23E-05	1.23E-04	1.21E-05	4.90E-06
1626047	33.09	r ²	0.95	EC80	10.1115021	REP 80	1.77E-07	1.77E-04	1.77E-03		
2170029	44.16									REP50	
1119248	22.77									Mean	STEDV
RLU	E ₂ max									3.73E-05	1.39E-04
2902802	59.07	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2645421	53.83	intercept	47.47	EC20	0.14803746	REP 20	1.77E-09	1.77E-06	1.77E-05	REP80	
1923184	39.13	slope	33.11	EC50	1.192550993	REP 50	1.82E-08	1.82E-05	1.82E-04	Mean	STEDV
1855809	37.76	r ²	0.93	EC80	9.606878342	REP 80	1.87E-07	1.87E-04	1.87E-03	1.63E-03	3.30E-04
1860478	37.86										
1815728	36.95										
RLU	E ₂ max										
2842636	57.84	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1749930	35.61	intercept	40.17	EC20	0.259949796	REP 20	1.01E-09	1.01E-06	1.01E-05		
1822606	37.09	slope	34.47	EC50	1.927952506	REP 50	1.13E-08	1.13E-05	1.13E-04		
2211008	44.99	r ²	0.70	EC80	14.2989182	REP 80	1.25E-07	1.25E-04	1.25E-03		
1957455	39.83										
2205683	44.88										

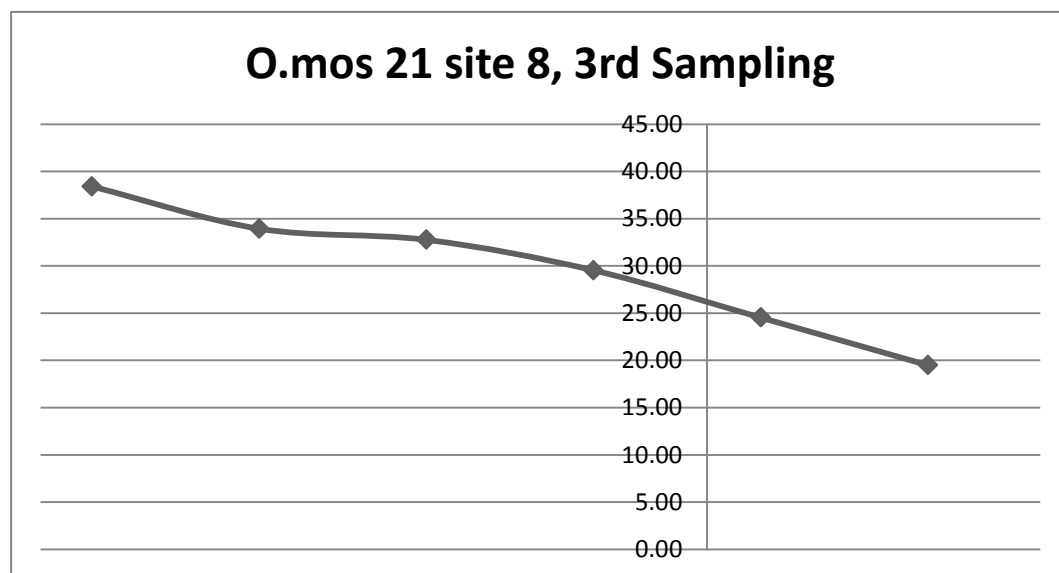
O.mos 19 site 8 3rd sampling (μℓ)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1061527	1092131	1175416	1109691.333	58940.22447	5.31	22.58
1.25	9.69E-02	1048191	1160689	1101251	1103377	56279.12498	5.10	22.45
0.63	-2.04E-01	1020774	1204726	1140830	1122110	93393.86155	8.32	22.83
0.31	-5.05E-01	1040471	1339421	1210140	1196677.333	149929.0104	12.53	24.35
0.16	-8.06E-01	1098961	1171451	1157727	1142713	38506.68996	3.37	23.25
0.08	-1.11E+00	1217682	1309002	1265936	1264206.667	45684.55478	3.61	25.72



100μℓ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

O.mos 21 site 8 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	4321229	1021956	893015	957485.5	91175.05547	9.52	19.48
1.25	9.69E-02	1190751	7581	1219535	1205143	20353.36159	1.69	24.52
0.63	-2.04E-01	1274044	1601220	1478672	1451312	165295.0724	11.39	29.53
0.31	-5.05E-01	1532290	1675668	1622389	1610115.667	72472.67592	4.50	32.76
0.16	-8.06E-01	1545416	1769991	1687904	1667770.333	113633.2084	6.81	33.94
0.08	-1.11E+00	2002973	1944491	1715635	1887699.667	151854.2828	8.04	38.41



100 $\mu\ell$ bile used in extraction
concentrated to 1mℓ
Concentration factor 0.1

No linear gradient thus a response occurred which cannot be quantified by the cells

C.gar 3 site 9, 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1659961	1721308	1004024	1690634.5	43378.87971	2.57	29.01
1.25	9.69E-02	1172997	863033	1024019	1098508	105343.354	9.59	18.85
0.63	-2.04E-01	1657040	1363859	857077	1510449.5	207310.2732	13.73	25.92
0.31	-5.05E-01	1438955	1457632	888815	1448293.5	13206.63335	0.91	24.86
0.16	-8.06E-01	1273235	1451061	1489904	1404733.333	115525.1203	8.22	24.11
0.08	-1.11E+00	1259753	1049138	793133	1154445.5	148927.2947	12.90	19.81

$y=mx+c$

intercept 27.83

slope 6.34

r² 0.84

$\mu\ell/\text{well}$

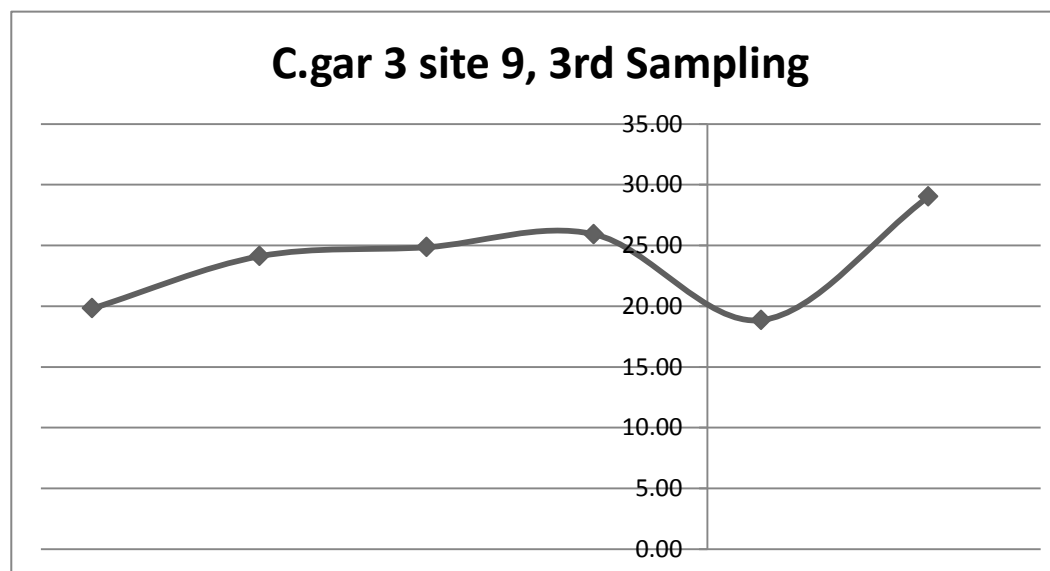
EC20 5.81E-02

EC50 3.15E+03

EC80 1.71E+08

	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	3.71E-08	3.71E-05
REP50	2.08E-10	2.08E-07
REP80	1.17E-12	1.17E-09

	E ₂ eq $\mu\text{g}/\text{m}\ell$
REP20	1.86E-04
REP50	1.04E-06
REP80	5.85E-09



200 $\mu\ell$ bile used in extraction

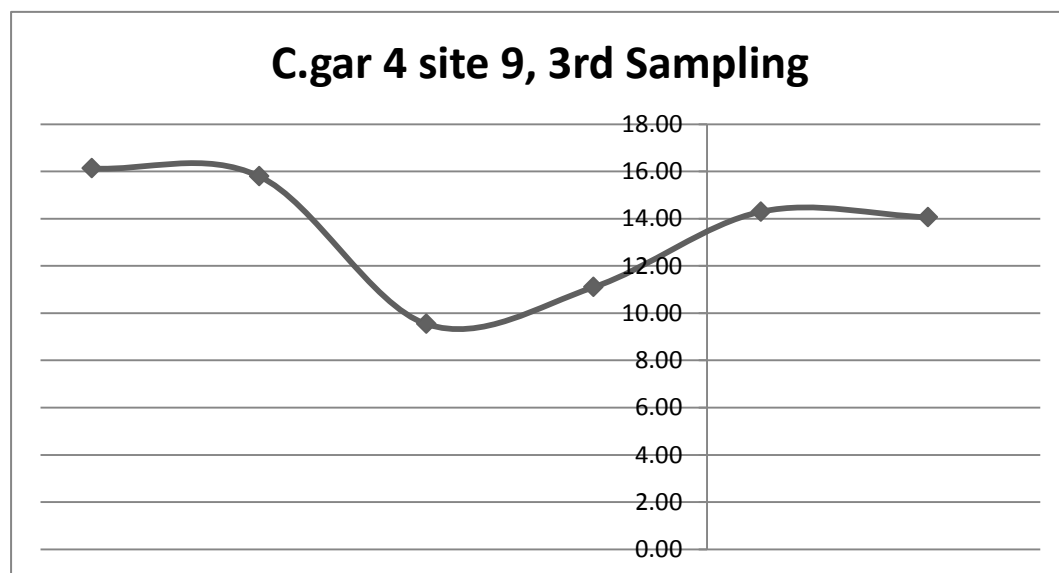
concentrated to 1m ℓ

Concentration factor

0.2

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
1659961	28.49	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
1172997	20.13	intercept	30.52	EC20	0.109221027	REP 20	1.98E-08	1.98E-05	9.88E-05	Mean	STEDV
1657040	28.44	slope	10.94	EC50	60.31304754	REP 50	1.09E-08	1.09E-05	5.45E-05	4.70E-05	4.93E-05
1438955	24.70	r ²	0.99	EC80	33305.52567	REP 80	6.00E-09	6.00E-06	3.00E-05		
1273235	21.85									REP50	
1259753	21.62									Mean	STEDV
RLU	E ₂ max									1.16E+08	6.71E+07
1721308	29.54	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
863033	14.81	intercept	23.19	EC20	19.13181765	REP 20	1.13E-10	1.13E-07	5.64E-07	REP80	
1363859	23.41	slope	-2.49	EC50	1.63245E-11	REP 50	4.02E+04	4.02E+07	2.01E+08	Mean	STEDV
1457632	25.02	r ²	0.69	EC80	1.39291E-23	REP 80	1.44E+19	1.44E+22	7.18E+22	4.10E+03	4.73E+03
1451061	24.90										
1049138	18.01										
RLU	E ₂ max										
1004024	17.23	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
1024019	17.57	intercept	9.40	EC20	0.258403117	REP 20	8.35E-09	8.35E-06	4.17E-05		
857077	14.71	slope	-18.04	EC50	0.005613463	REP 50	1.17E-04	1.17E-01	5.85E-01		
888815	15.25	r ²	0.79	EC80	0.000121945	REP 80	1.64E+00	1.64E+03	8.20E+03		
1489904	25.57										
793133	13.61										

C.gar 4 site 9, 3rd sampling(μl)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	26000	1612541	973103	819270.5	1121853.9	136.93	14.06
1.25	9.69E-02	913852	1149376	751895	832873.5	114520.893	13.75	14.29
0.63	-2.04E-01	535906	757705	393443	646805.5	156835.577	24.25	11.10
0.31	-5.05E-01	367459	744183	408883	555821	266384.095	47.93	9.54
0.16	-8.06E-01	863810	963474	933767	920350.3333	51168.67628	5.56	15.79
0.08	-1.11E+00	748148	1131055	738955	939601.5	270756.1363	28.82	16.13



250 μl bile used in extraction
concentrated to 1m l
Concentration factor 0.25

No linear gradient thus a response occurred which cannot be quantified by the cells

C.gar 5 site 9, 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	923	3246502	2938318	1623712.5	2294970.92	141.34	27.87
1.25	9.69E-02	3185875	2996000	2354013	2769944	588215.2612	21.24	47.54
0.63	-2.04E-01	2752408	2446596	3269589	2599502	216241.739	8.32	44.61
0.31	-5.05E-01	2831380	2465532	2434951	2648456	258693.6017	9.77	45.45
0.16	-8.06E-01	2505361	2725882	2091712	2440985	321948.9078	13.19	41.89
0.08	-1.11E+00	2331865	2010353	2043491	2171109	227343.3154	10.47	37.26

$y=mx+c$

intercept 52.50

slope 13.61

r² 0.99

$\mu\ell$ /well

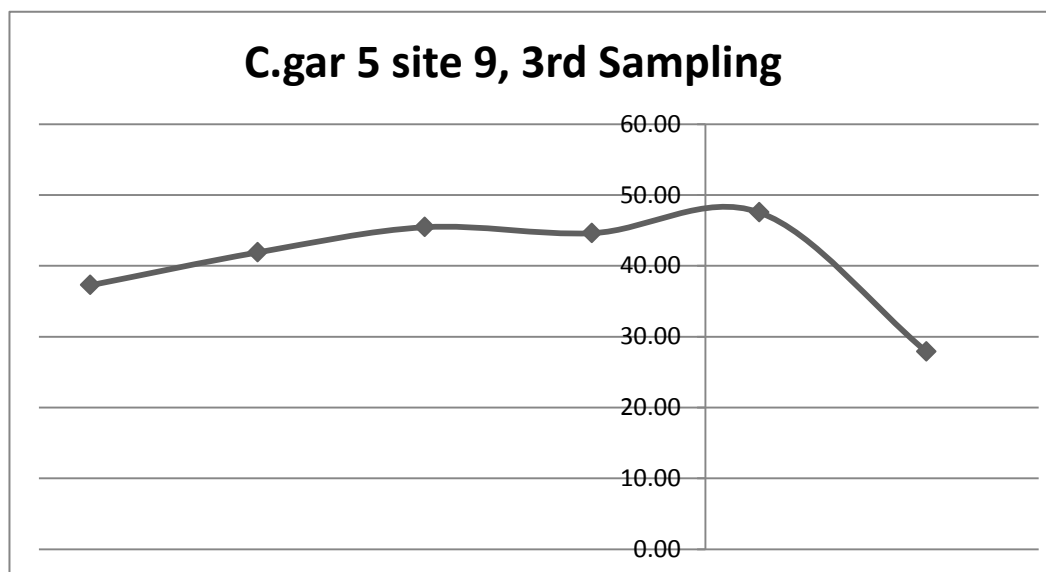
EC20 4.08E-03

EC50 6.55E-01

EC80 1.05E+02

	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	5.28E-07	5.28E-04
REP50	1.00E-06	1.00E-03
REP80	1.91E-06	1.91E-03

	E ₂ eq $\mu\text{g}/\text{m}\ell$
REP20	2.64E-03
REP50	5.02E-03



200 $\mu\ell$ bile used in extraction

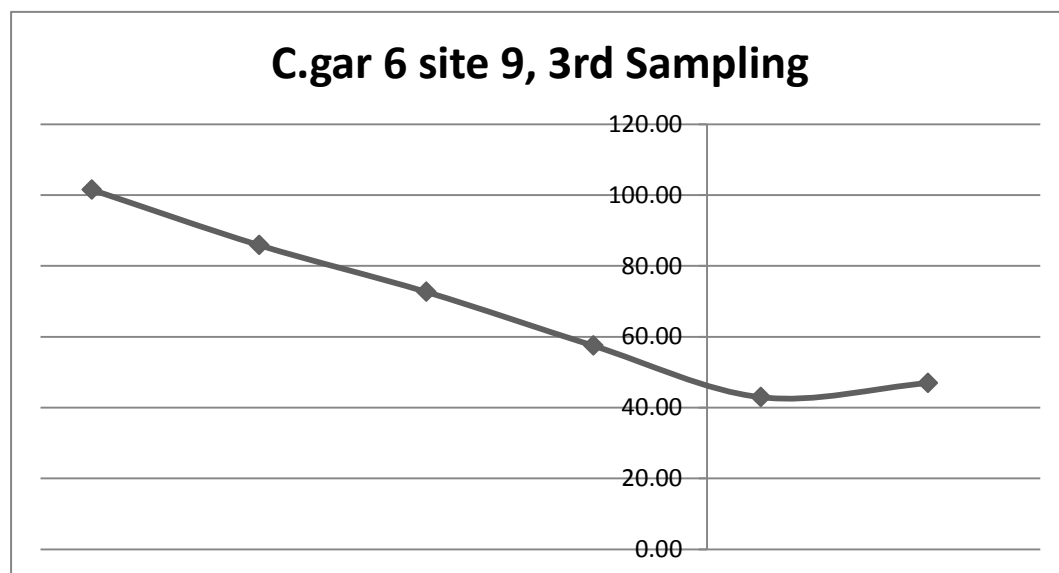
concentrated to 1m ℓ

Concentration factor

0.2

Standard deviation and Mean of each individual RLU											
RLU	E ₂ max										
923	0.02	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ	REP20	
3185875	54.68	intercept	55.35	EC20	0.003292038	REP 20	6.55E-07	6.55E-04	3.28E-03	Mean	STEDV
2752408	47.24	slope	14.24	EC50	0.421090859	REP 50	1.56E-06	1.56E-03	7.80E-03	1.21E-03	1.79E-03
2831380	48.59	r ²	0.97	EC80	53.86253025	REP 80	3.71E-06	3.71E-03	1.86E-02		
2505361	43.00									REP50	
2331865	40.02									Mean	STEDV
RLU	E ₂ max									2.89E-03	5.87E-03
3246502	55.72	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2996000	51.42	intercept	47.50	EC20	0.062237527	REP 20	3.47E-08	3.47E-05	1.73E-04	REP80	
2446596	41.99	slope	22.80	EC50	1.287505064	REP 50	5.10E-07	5.10E-04	2.55E-03	Mean	STEDV
2465532	42.31	r ²	0.96	EC80	26.63456212	REP 80	7.51E-06	7.51E-03	3.75E-02	0.11	0.15
2725882	46.78										
2010353	34.50										
RLU	E ₂ max										
2938318	50.43	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/mℓ		
2354013	40.40	intercept	61.56	EC20	0.057835127	REP 20	3.73E-08	3.73E-05	1.86E-04		
3269589	56.11	slope	33.58	EC50	0.45257664	REP 50	1.45E-06	1.45E-03	7.26E-03		
2434951	41.79	r ²	0.95	EC80	3.541543438	REP 80	5.65E-05	5.65E-02	2.82E-01		
2091712	35.90										
2043491	35.07										

C.gar 6 site 9, 3rd sampling($\mu\ell$)	log	RLU	RLU	RLU	mean	mean	stdev	cv
2.5	3.98E-01	2777874	1358737	1977370	2037993.667	1668053.5	437439.5894	26.22
1.25	9.69E-02	1908181	1658367	1631768	1732772	1695569.5	52612.28005	3.10
0.63	-2.04E-01	3193991	1653358	1497348	2114899	1575353	110315.7289	7.00
0.31	-5.05E-01	1488099	572596	1369257	1143317.333	970926.5	563324.3954	58.02
0.16	-8.06E-01	1450203	588020	1016583	1018268.667	874290.5556	247919.0061	28.36
0.08	-1.11E+00	1092164	349114	1166086	869121.3333	757600	577686.4412	76.25



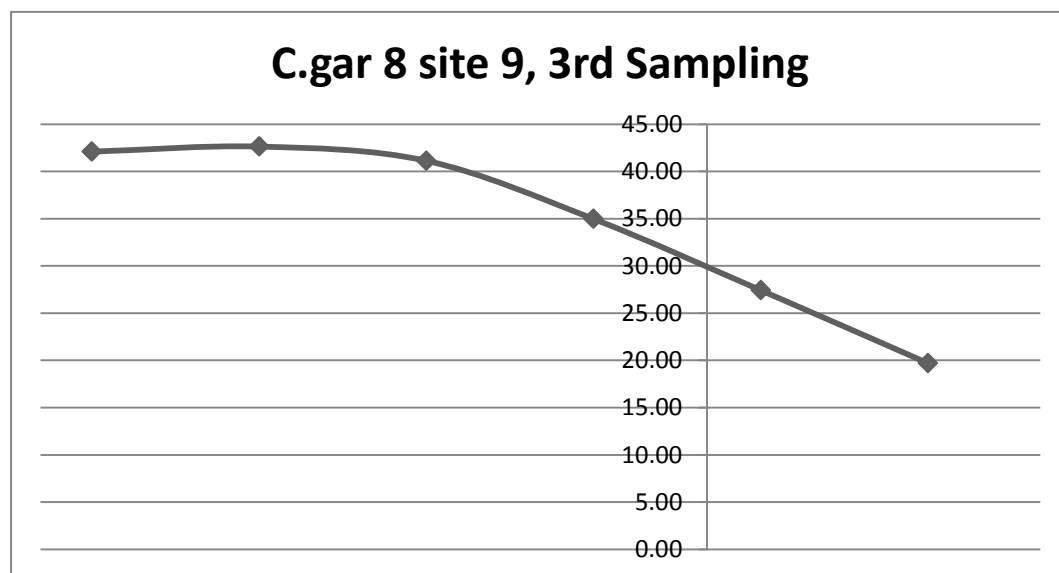
500 $\mu\ell$ bile used in extraction
concentrated to 1m ℓ
Concentration factor 0.5

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration could indicate cytotoxicity

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

C.gar 8 site 9, 3rd sampling(μl)	log	RLU	RLU	RLU	mean	stdev	cv	E ₂ max
2.5	3.98E-01	1342051	953140	917639	1147595.5	275001.6054	23.96	19.69
1.25	9.69E-02	1622381	1592861	1577291	1599836	31883.44476	1.99	27.46
0.63	-2.04E-01	2081492	1827923	2203652	1954707.5	179300.3594	9.17	33.55
0.31	-5.05E-01	2209001	2482202	2497512	2345601.5	193182.2797	8.24	40.26
0.16	-8.06E-01	2497035	2396287	2559118	2484146.667	82177.0375	3.31	42.63
0.08	-1.11E+00	2268721	2527680	2562816	2398200.5	183111.6649	7.64	41.16



500 μl bile used in extraction
concentrated to 1m ℓ
Concentration factor 0.5

No linear gradient thus a response occurred which cannot be quantified by the cells

Strongest concentration could indicate cytotoxicity

Can indicate that the concentration may be too strong and that activation actually took place at lower concentrations

8.2.3.3. Inhibition calculations for September 2013 retrieved from Microsoft Excel 2010

FoldInhibition			Plate A		FoldInhibition			Plate B	
Solvent control	mean	stdev	cv	Fold inhibition	Solvent control	mean	stdev	cv	
1614677	1453124	154740	10	1	1317014	866784	137289	15.	
1438453					963862				
1306243					769706				
Sediment Site 9 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1249193	1633314	2103673	1441254	271615	18.85	1.01	
0.83	9.69E-02	2102993	1934708	2553575	2197092	319984	14.56	0.66	
Sediment Site 90 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1208295	1101931	968054	1092760	120383	11.02	0.79	
0.83	9.69E-02	1133054	872396	835989	947146	162027	17.11	0.92	
Sediment Site 8 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1293910	1240405	1444069	1326128	105585	7.96	0.65	
0.83	9.69E-02	956765	887577	957461	933934	40148.1	4.30	0.93	
Sediment Ref Site (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1358460	1248309	1486568	1364446	119242	8.74	1.06	
0.83	9.69E-02	1123775	1109776	1191881	1141811	43923.5	3.85	1.27	

Fold Inhibition			Plate A		Fold Inhibition			Plate B	
Solvent control	mean	stdev	cv	Fold inhibition	Solvent control	mean	stdev	cv	
1615842	1527413.	80829	5.29	1	2E+06	2E+06	285781	17.32	
1509058					2E+06				
1457341					1E+06				
H ₂ O Site 9 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	2579915	2308486	2346083	2411494	147062	6.10	1.63	
1.25	9.69E-02	1891843	2047561	1652217	1863873	199150	10.68	0.82	
H ₂ O Site 90 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1567443	1917002	1535527	1673324	211633	12.65	0.99	
1.25	9.69E-02	1323957	1718662	1316730	1453116	229997.	15.83	1.14	
H ₂ O Site 8 (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1928962	1694790	1896723	1840158.	126920.	6.90	0.90	
0.83	9.69E-02	1340611	1206482	1460796	1335963	127220	9.52	1.24	
H ₂ O Ref Site (μℓ)	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI	
2.5	3.98E-01	1614847	2069224	2104061	1929377	272947	14.15	0.79	

0.83	9.69E-02	1594552	1766891	1671015	1677486	86351	5.15	0.91

Fold Inhibition		Plate B		Fold Inhibition			Plate C	
Solvent control	mean	stdev	cv	Solvent control	mean	stdev	cv	
2839678	3127090	283226	9.06	1287137	1627509	321255	19.74	
3135656				1669963				
3405935				1925426				
O.mos 2	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	872889	866804	759340	833011	63873	7.67	3.75
1.25	9.69E-02	1659629	679041	805336	742188.5	89304	12.03	4.21
O.mos 3	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	693097	981899	1374404	1178152	277543	23.56	2.65
1.25	9.69E-02	3870354	2799610	3561005	3410323	551046	16.16	0.92
O.mos 6	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	5535061	8497622	2259500	7016342	2094847	29.86	0.45
1.25	9.69E-02	4733781	2715342	2848450	2781896	94122	3.38	1.12
O.mos 7	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4155671	3293282	3198850	3246066	66774	2.06	0.96
1.25	9.69E-02	4514801	3459402	3050270	3254836	289300	8.89	0.96
O.mos 8	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	5596971	5350082	3202970	5473527	174577	3.19	0.57
1.25	9.69E-02	4221086	3887890	3485400	3864792	368386	9.53	0.81
O.mos 9	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3301496	4524770	4500234	4108833	699282	17.02	0.40
1.25	9.69E-02	3204288	3619099	4089855	3637747	443078	12.18	0.45
O.mos 10	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	5286440	5761104	6020888	5689477	372426	6.55	0.29
1.25	9.69E-02	2835329	6218378	4966491	4673399	1710463	36.60	0.35
O.mos 11	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4221086	3887890	3485400	4054488	235605	5.81	0.40
1.25	9.69E-02	3918713	4159318	4466102	4181378	274360	6.56	0.39
O.mos 12	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2004899	1305685	37119	1655292	494419	29.87	0.98
1.25	9.69E-02	224203	780657	375887	578272	286216	49.49	2.81

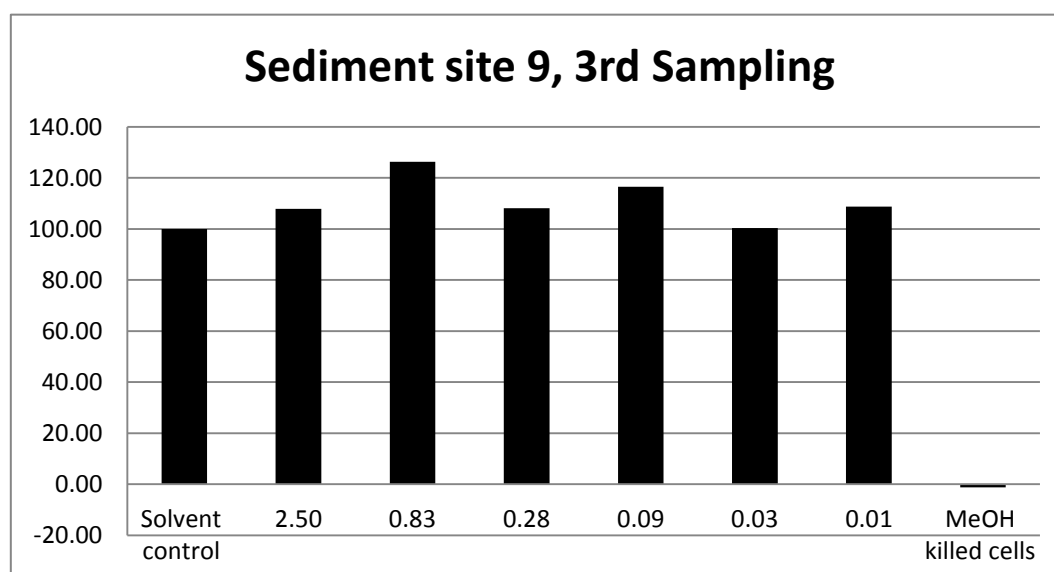
O.mos 14	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3857232	3737124	3225624	3606660	335406	9.30	0.45
1.25	9.69E-02	4691802	4449951	4133280	4425011	280095	6.33	0.37
O.mos 17	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4010413	1187044	4796253	4403333	555672	12.62	0.37
1.25	9.69E-02	1520127	1200328	1476029	1398828	173314	12.39	1.16
Fold Inhibition			Plate A	Fold Inhibition			Plate B	
Solvent control	mean	stdev	cv	Solvent control	mean	stdev	cv	
4561962	4176021	352723	8	2839678	3127090	283226	9	
4095746				3135656				
3870354				3405935				
O.mos 1	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	5296377	5239360	458254	5267869	40317	0.77	0.79
1.25	9.69E-02	4547080	4776947	4681751	4668593	115497	2.47	0.89
O.mos 2	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4716523	3811904	485051	4264214	639662	15.00	0.98
1.25	9.69E-02	4761046	4541335	4784789	4695723	134230	2.86	0.89
O.mos 5	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	5256183	3696121	4656268	4536191	786932	17.35	0.92
1.25	9.69E-02	3594377	4314154	4469864	4126132	467048	11.32	1.01
O.mos 7	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4200410	4573878	5652693	4808994	754149	15.68	0.87
1.25	9.69E-02	4528306	3786195	3950329	4088277	389813	9.53	1.02
O.mos 8	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2799610	3561005	3882719	3414445	556229	16.29	1.22
1.25	9.69E-02	4361097	3232348	4292079	3961841	632702	15.97	1.05
O.mos 9	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3301496	4524770	4500234	4108833	699282	17.02	1.02
1.25	9.69E-02	3204288	3619099	4089855	3637747	443078	12.18	1.15
O.mos 10	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	5286440	5761104	6020888	5689477	372426	6.55	0.73
1.25	9.69E-02	2835329	6218378	4966491	4673399	1710463	36.60	0.89
O.mos 11	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	4221086	3887890	3485400	4054488	235605	5.81	1.03
1.25	9.69E-02	3918713	4159318	4466102	4181378	274360	6.56	1.00

O.mos 15	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3839150	4603920	4367619	4270230	391576	9.17	0.98
1.25	9.69E-02	3996422	4397998	4622645	4339022	317250	7.31	0.96
O.mos 16	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2944873	1773843	3057711	3001292	79789	2.66	1.04
1.25	9.69E-02	3580621	3091585	3679807	3450671	314907	9.13	0.91
O.mos 17	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3618800	1834469	3111807	3365304	358498	10.65	0.93
1.25	9.69E-02	2102855	1732403	2370489	2236672	189246	8.46	1.40
O.mos 19	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	971331	668982	1428492	1022935	382376	37.38	3.06
1.25	9.69E-02	4716523	3811904	485051	4264214	639662	15.00	0.73
O.mos 21	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	658001	732571	482643	695286	52729	7.58	4.50
1.25	9.69E-02	835924	1086232	341345	961078	176994	18.42	3.25

Fold Inhibition			Plate E					
Solvent control	mean	stdev	cv					
2905496	2651992	265422.8	10.008					
2674407								
2376072								
C.gar 3	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2468760	4764364	2432370	2450565	25731	1.05	0.39
1.25	9.69E-02	2704300	2886990	2046801	2795645	129181	4.62	0.95
C.gar 4	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3708566	4136555	2614906	3922561	302633	7.72	0.68
1.25	9.69E-02	2655342	3022963	2077907	2585404	476394	18.43	1.03
C.gar 5	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	1202331	3855383	3466585	3660984	274921	7.51	0.72
1.25	9.69E-02	3258745	3478894	3820109	3519249	282849	8.04	0.75
C.gar 6	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	3360068	2763333	3875347	3617708	364357	10.07	0.73
1.25	9.69E-02	1829590	2712951	3972040	2271271	624630	27.50	1.17
C.gar 8	ICI log	RLU	RLU	RLU	mean	stdev	cv	FI
2.5	3.98E-01	2861442	3084035	2867156	2937544	126896	4.32	0.90
1.25	9.69E-02	2516771	2465936	920431	2491354	35945.	1.44	1.06

8.2.3.4.MTT calculations for September 2013 retrieved from Microsoft Excel 2010

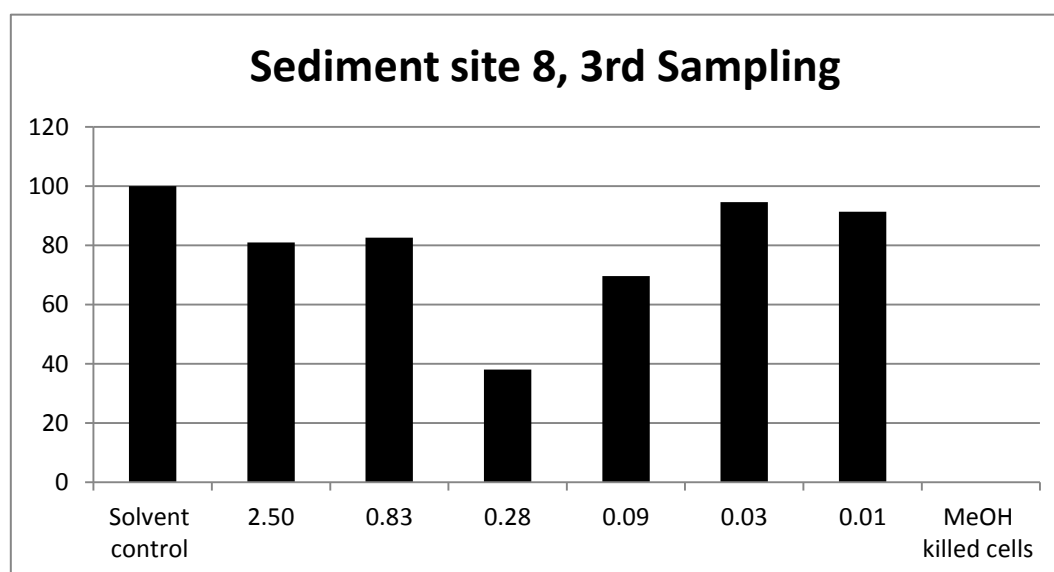
Absorbance reading from plate reader				Site 9 Sediment			
Raw data	1	2	3				
Solvent control	0.267	0.256	0.256				
2.50	0.182	0.299	0.35				
0.83	0.315	0.331	0.323				
0.28	0.246	0.299	0.288				
0.09	0.286	0.3	0.302				
0.03	0.268	0.245	0.268				
0.01	0.284	0.284	0.269				
MeOH killed cells	0.034	0.035	0.039	0.04	0.039	0.051	0.033
MeOH killed cells substracted							
Solvent control	0.228	0.217	0.217				
2.50	0.143	0.260	0.311				
0.83	0.276	0.292	0.284				
0.28	0.207	0.260	0.249				
0.09	0.247	0.261	0.263				
0.03	0.229	0.206	0.229				
0.01	0.245	0.245	0.230				
MeOH killed cells	-0.005	0.004	0.000				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	103.32	98.34	98.34	100.00	2.87	2.87	1.00
2.50	64.84	117.80	140.8	107.85	38.99	36.15	0.75
0.83	125.05	125.05	128.6	126.25	2.09	1.66	0.00
0.28	93.81	117.80	112.8	108.15	12.66	11.71	0.34
0.09	111.92	118.26	119.1	116.45	3.95	3.39	0.00
0.03	103.77	93.36	103.7	100.30	6.01	5.99	0.94
0.01	111.01	111.01	104.2	108.75	3.92	3.60	0.04
MeOH killed cells	-2.15	-1.70	0.12	-1.24	1.20	0.00	0.00



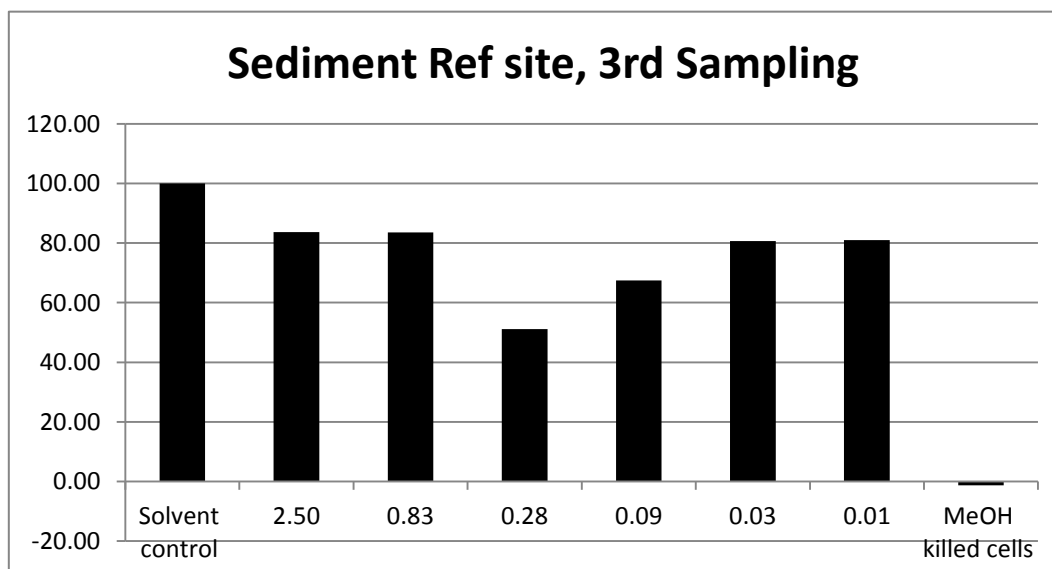
Absorbance reading from plate reader				Site 9 Outflow Sediment			
Raw data	1	2	3				
Solvent control	0.203	0.213	0.23				
2.50	0.298	0.262	0.222				
0.83	0.27	0.247	0.233				
0.28	0.315	0.244	0.22				
0.09	0.11	0.224	0.204				
0.03	0.296	0.25	0.212				
0.01	0.284	0.264	0.25				
MeOH killed cells	0.031	0.079	0.054	0.041	0.055	0.03	0.05
MeOH killed cells subtracted							
Solvent control	0.151	0.158	0.178				
2.50	0.246	0.207	0.170				
0.83	0.218	0.192	0.181				
0.28	0.263	0.189	0.168				
0.09	0.058	0.169	0.152				
0.03	0.244	0.195	0.160				
0.01	0.232	0.209	0.198				
MeOH killed cells	0.021	0.027	0.002				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	93.04	97.32	109.6	100.00	8.62	8.62	1.00
2.50	151.4	99.67	77.12	109.41	38.10	34.83	0.70
0.83	134.2	97.45	82.10	104.59	26.78	25.61	0.79
0.28	161.8	91.45	76.22	109.85	45.70	41.60	0.73
0.09	35.88	133.6	68.97	79.50	49.73	62.55	0.52
0.03	150.2	97.65	72.60	106.82	39.61	37.08	0.79
0.01	142.8	98.11	89.80	110.25	28.52	25.87	0.58
MeOH killed cells	-1	16.83	1.33	1.83	14.76	0.00	0.00



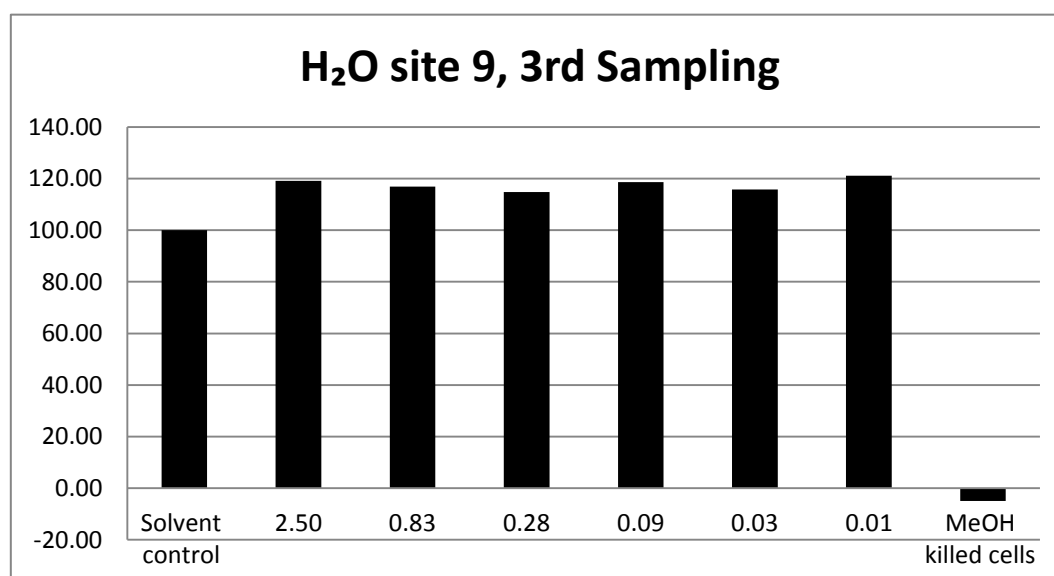
Absorbance reading from plate reader				Site 8 Sediment			
Raw data	1	2	3				
Solvent control	0.203	0.213	0.23				
2.50	0.207	0.238	0.319				
0.83	0.219	0.206	0.344				
0.28	0.19	0.205	0.369				
0.09	0.188	0.212	0.294				
0.03	0.188	0.203	0.26				
0.01	0.209	0.215	0.299				
MeOH killed cells	0.031	0.079	0.054	0.041	0.055	0.03	0.05
MeOH killed cells subtracted							
Solvent control	0.151	0.158	0.178				
2.50	0.155	0.183	0.267				
0.83	0.167	0.151	0.292				
0.28	0.138	0.150	0.317				
0.09	0.136	0.157	0.242				
0.03	0.136	0.148	0.208				
0.01	0.157	0.160	0.247				
MeOH killed cells	-0.021	0.027	0.002				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	93.04	97.32	109.64	100.00	8.62	8.62	1.00
2.50	95.50	112.6	164.34	124.18	35.83	28.8	0.32
0.83	102.8	93.02	179.71	125.20	47.46	37.9	0.42
0.28	85.05	92.40	195.07	124.18	61.51	49.5	0.54
0.09	83.82	96.70	148.98	109.83	34.50	31.4	0.66
0.03	83.82	91.17	128.08	101.02	23.72	23.4	0.95
0.01	96.73	98.55	152.05	115.78	31.43	27.1	0.45
MeOH killed cells	-12.68	16.83	1.46	1.87	14.76	0.00	0.00



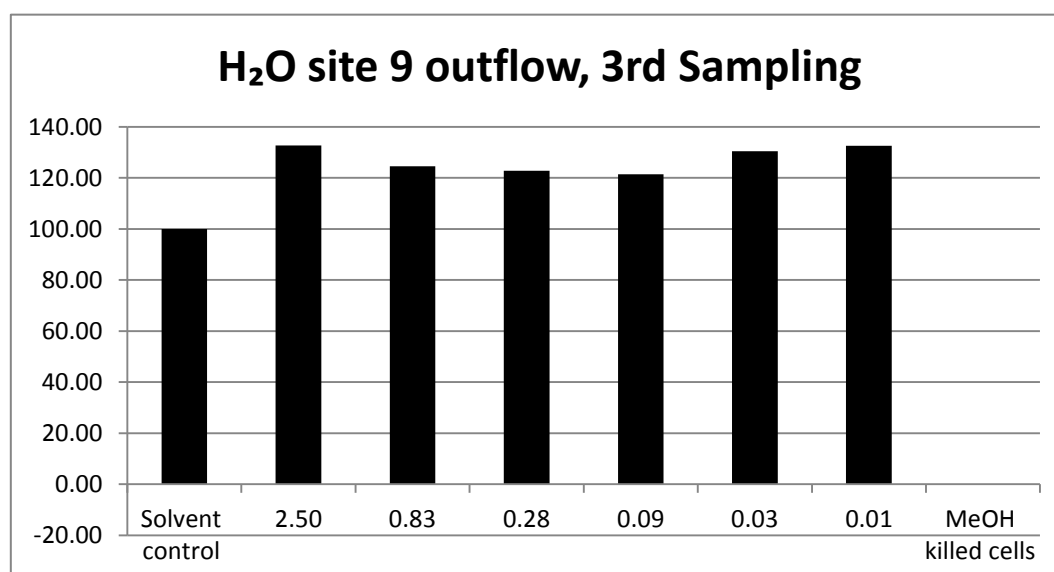
Absorbance reading from plate reader				Site Ref Sediment			
Raw data	1	2	3				
Solvent control	0.267	0.256	0.256				
2.50	0.135	0.244	0.292				
0.83	0.163	0.24	0.267				
0.28	0.131	0.16	0.164				
0.09	0.138	0.187	0.238				
0.03	0.133	0.25	0.268				
0.01	0.136	0.209	0.308				
MeOH killed cells	0.034	0.035	0.039	0.04	0.039	0.05	0.03
MeOH killed cells subtracted							
Solvent control	0.228	0.217	0.217				
2.50	0.096	0.205	0.253				
0.83	0.124	0.201	0.228				
0.28	0.092	0.121	0.125				
0.09	0.099	0.148	0.199				
0.03	0.094	0.211	0.229				
0.01	0.097	0.170	0.269				
MeOH killed cells	-0.005	-0.004	0.000				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	103.3	98.34	98.34	100.00	2.87	2.9	1.00
2.50	43.57	92.91	114.64	83.70	36.42	43.5	0.48
0.83	56.24	91.10	103.32	83.55	24.43	29.2	0.31
0.28	41.76	54.88	56.70	51.11	8.15	15.9	0.00
0.09	44.93	67.11	90.19	67.41	22.63	33.6	0.07
0.03	42.66	95.62	103.77	80.69	33.18	41.1	0.37
0.01	44.02	77.07	121.88	80.99	39.08	48.2	0.45
MeOH killed cells	-2.15	-1.70	0.11	-1.24	1.20	-96.2	0.00



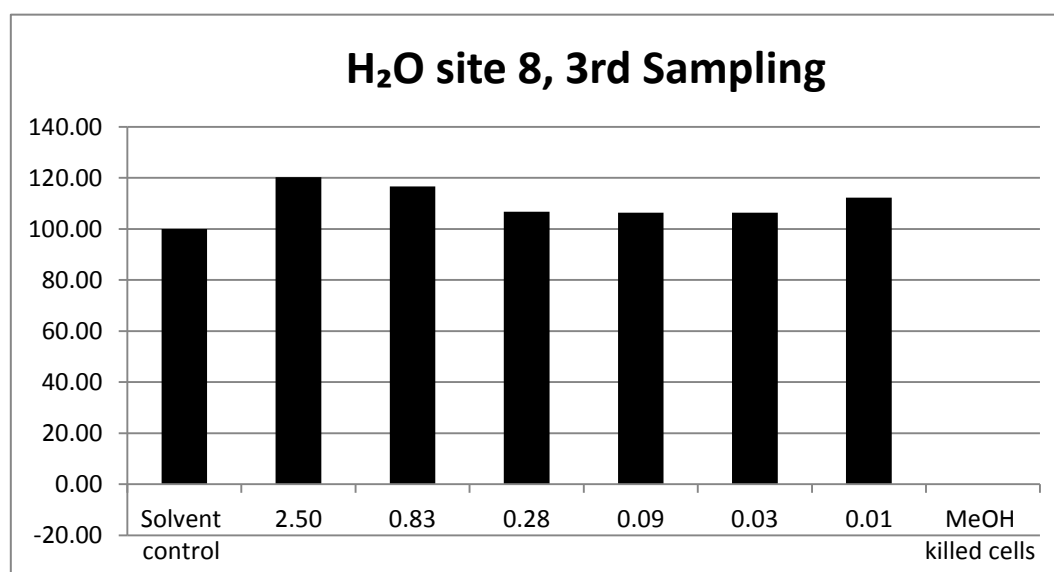
Absorbance reading from plate reader				Site 9 H ₂ O			
Raw data	1	2	3				
Solvent control	0.192	0.2	0.187				
2.50	0.208	0.228	0.221				
0.83	0.204	0.235	0.24				
0.28	0.178	0.241	0.22				
0.09	0.18	0.228	0.247				
0.03	0.176	0.226	0.241				
0.01	0.193	0.223	0.249				
MeOH killed cells	0.035	0.065	0.052	0.055	0.067	0.062	0.06
MeOH killed cells substracted							
Solvent control	0.135	0.143	0.130				
2.50	0.151	0.171	0.164				
0.83	0.147	0.178	0.183				
0.28	0.121	0.184	0.163				
0.09	0.123	0.171	0.190				
0.03	0.119	0.169	0.184				
0.01	0.136	0.166	0.192				
MeOH killed cells	-0.022	0.008	0.005				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	99.26	105.16	95.58	100.00	4.83	4.83	1.00
2.50	111.05	125.78	120.6	119.15	7.48	6.27	0.02
0.83	108.10	108.10	134.6	116.94	15.31	13.09	0.14
0.28	88.95	135.36	119.8	114.73	23.63	20.60	0.35
0.09	90.42	125.78	139.7	118.66	25.44	21.44	0.28
0.03	87.48	124.31	135.3	115.72	25.07	21.67	0.35
0.01	100.00	122.10	141.2	121.12	20.64	17.04	0.16
MeOH killed cells	-16.39	5.71	-4.05	-4.91	11.07	1.00	0.00



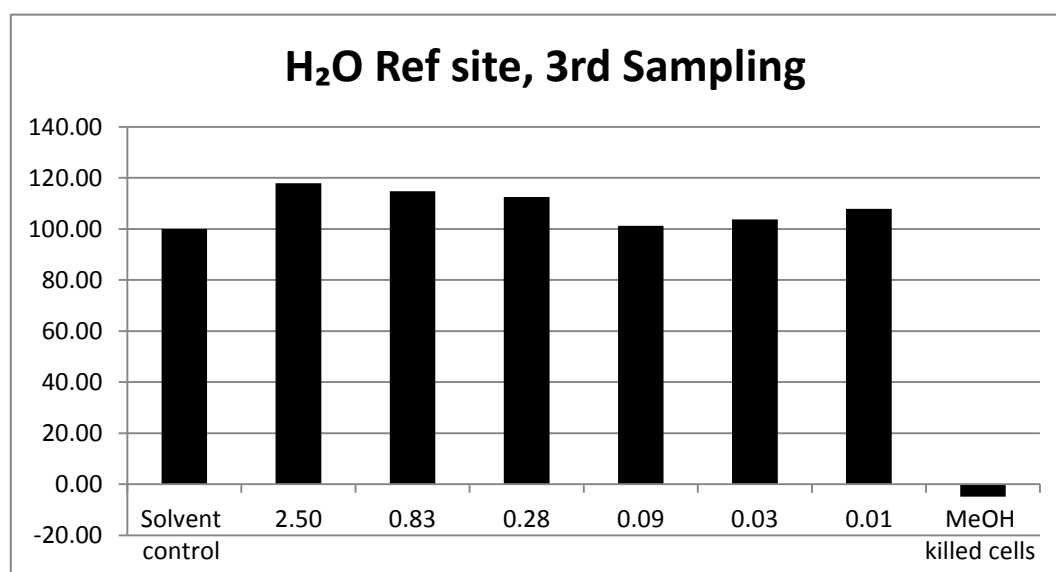
Absorbance reading from plate reader				Site 9 Outflow H ₂ O			
Raw data	1	2	3				
Solvent control	0.253	0.265	0.261				
2.50	0.281	0.297	0.284				
0.83	0.32	0.268	0.236				
0.28	0.298	0.276	0.235				
0.09	0.274	0.249	0.253				
0.03	0.294	0.29	0.269				
0.01	0.271	0.298	0.288				
MeOH killed cells	0.032	0.033	0.033	0.035	0.035	0.033	0.03
MeOH killed cells subtracted							
Solvent control	0.220	0.232	0.228				
2.50	0.248	0.264	0.251				
0.83	0.287	0.235	0.203				
0.28	0.265	0.243	0.202				
0.09	0.241	0.216	0.220				
0.03	0.261	0.257	0.236				
0.01	0.238	0.265	0.255				
MeOH killed cells	0.001	0.000	0.000				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	97.01	102.4	100.5	100.00	2.76	2.76	1.00
2.50	109.3	103.8	184.9	132.71	45.28	34.12	0.28
0.83	126.5	97.33	149.5	124.48	26.16	21.02	0.18
0.28	116.8	102.7	148.8	122.81	23.59	19.21	0.17
0.09	106.2	95.82	162.0	121.38	35.61	29.34	0.36
0.03	115.0	102.3	173.8	130.43	38.14	29.24	0.24
0.01	104.9	104.9	187.8	132.59	47.86	36.09	0.30
MeOH killed cells	-0.44	0.00	0.00	-0.15	0.25	1.00	0.00



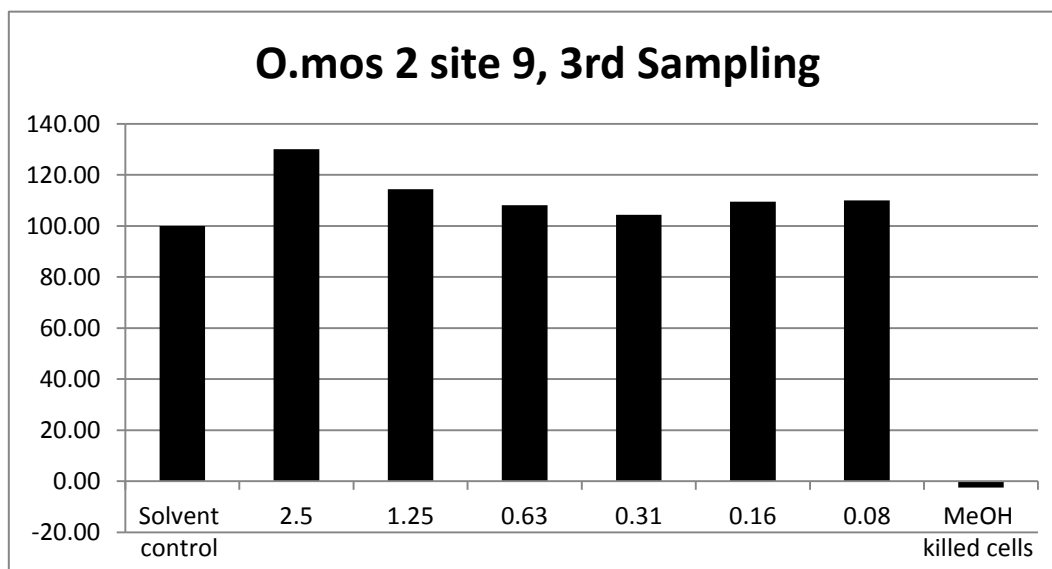
Absorbance reading from plate reader				Site 8 H ₂ O			
Raw data	1	2	3				
Solvent control	0.253	0.265	0.261				
2.50	0.281	0.29	0.346				
0.83	0.279	0.293	0.32				
0.28	0.269	0.286	0.27				
0.09	0.24	0.287	0.295				
0.03	0.263	0.301	0.258				
0.01	0.178	0.344	0.34				
MeOH killed cells	0.032	0.033	0.033	0.035	0.035	0.03	0.03
						3	
MeOH killed cells subtracted							
Solvent control	0.220	0.232	0.228				
2.50	0.248	0.257	0.313				
0.83	0.246	0.260	0.287				
0.28	0.236	0.253	0.237				
0.09	0.207	0.254	0.262				
0.03	0.230	0.268	0.225				
0.01	0.145	0.311	0.307				
MeOH killed cells	0.001	0.000	0.000				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	97.06	102.3	100.59	100.00	2.70	2.70	1.00
2.50	109.4	113.3	138.09	120.29	15.54	12.9	0.09
						2	
0.83	108.5	114.7	126.62	116.62	9.19	7.88	0.04
0.28	104.1	111.6	104.56	106.76	4.21	3.94	0.08
0.09	91.32	112.0	115.59	106.32	13.11	12.3	0.46
0.03	101.4	118.2	99.26	106.32	10.37	9.76	0.36
0.01	63.97	137.3	135.59	112.30	41.87	37.2	0.64
MeOH killed cells	-0.44	0.00	0.00	-0.15	0.25	1.00	0.00



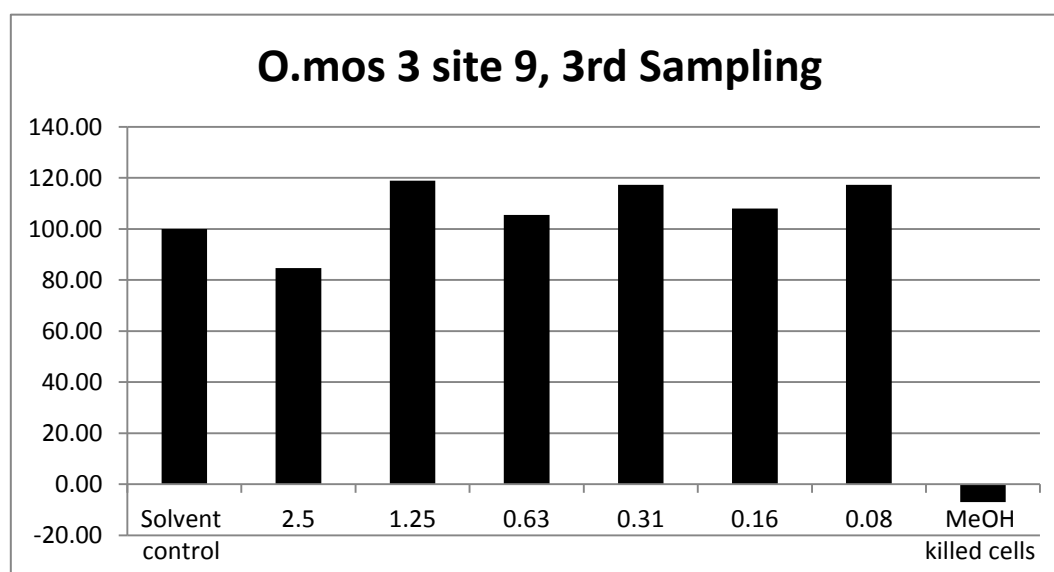
Absorbance reading from plate reader				Site Ref H ₂ O			
Raw data	1	2	3				
Solvent control	0.192	0.2	0.187				
2.50	0.239	0.225	0.188				
0.83	0.232	0.223	0.184				
0.28	0.219	0.221	0.19				
0.09	0.189	0.206	0.189				
0.03	0.219	0.194	0.181				
0.01	0.216	0.204	0.191				
MeOH killed cells	0.035	0.065	0.052	0.055	0.067	0.06	0.06
MeOH killed cells subtracted							
Solvent control	0.135	0.143	0.130				
2.50	0.182	0.168	0.131				
0.83	0.175	0.166	0.127				
0.28	0.162	0.164	0.133				
0.09	0.132	0.149	0.132				
0.03	0.162	0.137	0.124				
0.01	0.159	0.147	0.134				
MeOH killed cells	0.022	0.008	-0.005				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	99.26	105.1	95.58	100.00	4.83	4.8	1.00
2.50	133.8	123.5	96.32	117.93	19.41	16.5	0.20
0.83	128.7	122.1	93.37	114.73	18.80	16.4	0.26
0.28	119.1	120.6	97.79	112.52	12.78	11.4	0.19
0.09	97.05	109.5	97.05	101.23	7.23	7.1	0.82
0.03	119.1	100.7	91.16	103.68	14.23	13.7	0.69
0.01	116.9	108.1	98.53	107.86	9.21	8.5	0.26
MeOH killed cells	-6.39	5.71	-3.87	-4.85	11.08	1.00	0.00



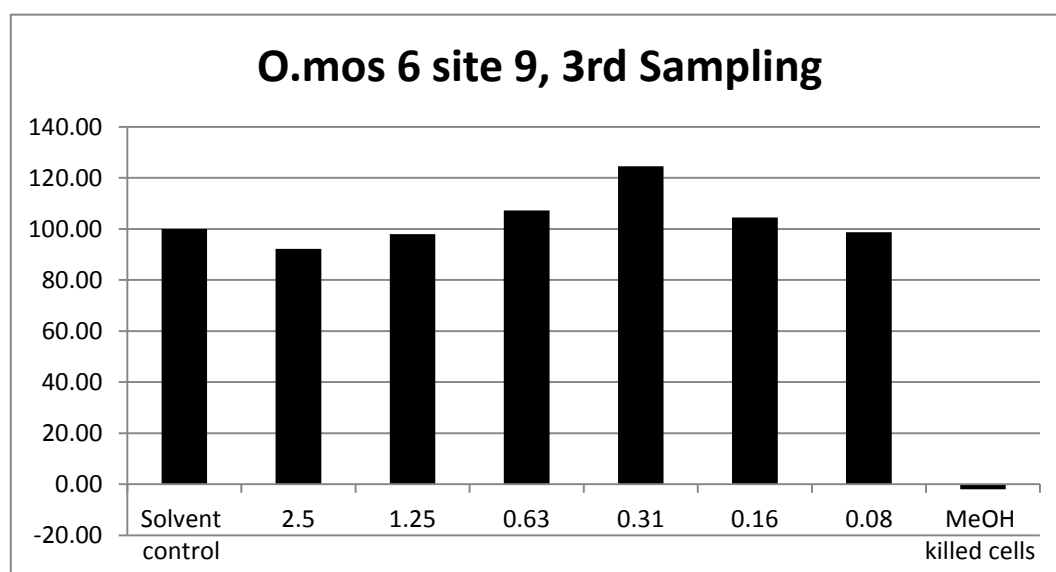
Absorbance reading from plate reader				O.mos 2 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.319	0.305	0.278				
2.5	0.342	0.373	0.353				
1.25	0.331	0.33	0.32				
0.63	0.309	0.308	0.33				
0.31	0.306	0.311	0.309				
0.16	0.315	0.322	0.317				
0.08	0.312	0.314	0.331				
MeOH killed cells	0.049	0.148	0.14	0.147	0.139	0.151	0.142
MeOH killed cells subtracted							
Solvent control	0.202	0.188	0.161				
2.5	0.225	0.256	0.236				
1.25	0.214	0.213	0.203				
0.63	0.192	0.191	0.213				
0.31	0.189	0.194	0.192				
0.16	0.198	0.205	0.200				
0.08	0.195	0.197	0.214				
MeOH killed cells	-0.068	0.031	0.023				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	109.98	102.36	87.67	100.00	11.34	11.3	1.00
2.5	122.49	139.36	128.4	130.11	8.55	6.6	0.02
1.25	116.50	115.96	110.5	114.33	3.31	2.9	0.10
0.63	104.53	103.99	115.9	108.16	6.76	6.2	0.34
0.31	102.90	105.62	104.5	104.35	1.37	1.3	0.55
0.16	107.80	111.61	108.8	109.43	1.96	1.8	0.23
0.08	106.17	107.25	116.5	109.98	5.68	5.2	0.24
MeOH killed cells	-36.93	16.93	12.58	-2.47	29.92	1.00	0.01



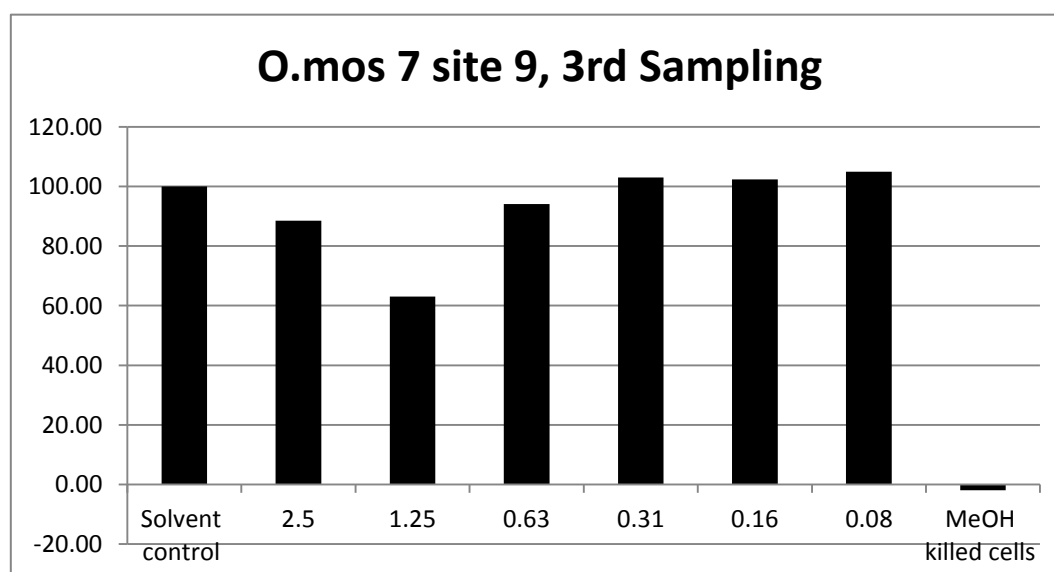
Absorbance reading from plate reader				O.mos 3 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.319	0.305	0.278				
2.5	0.318	0.359	0.177				
1.25	0.3	0.328	0.333				
0.63	0.291	0.311	0.317				
0.31	0.323	0.31	0.323				
0.16	0.291	0.313	0.323				
0.08	0.293	0.322	0.341				
MeOH killed cells	0.024	0.184	0.359	0.237	0.241	0.289	0.21
MeOH killed cells subtracted							
Solvent control	0.123	0.109	0.082				
2.5	0.122	0.163	-				
			0.019				
1.25	0.104	0.132	0.137				
0.63	0.095	0.115	0.121				
0.31	0.127	0.114	0.127				
0.16	0.095	0.117	0.127				
0.08	0.097	0.126	0.145				
MeOH killed cells	0.172	0.012	0.163				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	117.5	104.1	78.29	100.00	19.96	20.0	1.00
2.5	116.6	155.8	18.44	84.68	91.43	108.0	0.79
1.25	99.36	126.1	130.9	118.83	17.03	14.3	0.28
0.63	90.74	109.9	115.6	105.43	13.04	12.4	0.71
0.31	121.3	108.9	121.3	117.24	7.19	6.1	0.23
0.16	90.74	111.8	121.3	107.98	15.68	14.5	0.61
0.08	92.66	120.4	138.6	117.24	23.15	19.7	0.38
MeOH killed cells	-164	-11.7	155.8	-6.94	160.47	1.0	0.32



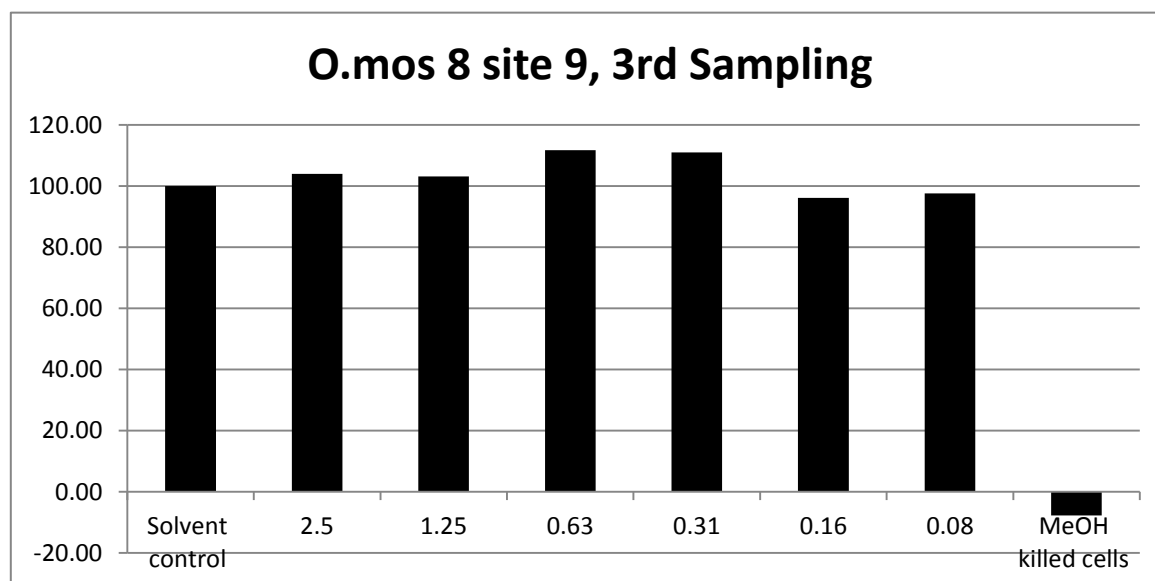
Absorbance reading from plate reader				O.mos 6 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.367	0.339	0.338				
2.5	0.332	0.306	0.352				
1.25	0.371	0.336	0.323				
0.63	0.372	0.384	0.338				
0.31	0.396	0.43	0.388				
0.16	0.334	0.383	0.358				
0.08	0.333	0.346	0.356				
MeOH killed cells	0.049	0.148	0.14	0.147	0.139	0.15	0.14
MeOH killed cells subtracted							
Solvent control	0.250	0.222	0.221				
2.5	0.215	0.189	0.235				
1.25	0.254	0.219	0.206				
0.63	0.255	0.267	0.221				
0.31	0.279	0.313	0.271				
0.16	0.217	0.266	0.241				
0.08	0.216	0.229	0.239				
MeOH killed cells	0.068	0.031	0.023				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	108.2	96.11	95.67	100.00	7.12	7.1	1.00
2.5	93.08	81.83	101.73	92.21	9.98	10.8	0.33
1.25	109.9	94.81	89.18	97.98	10.74	11.0	0.80
0.63	110.3	115.5	95.67	107.21	10.32	9.6	0.38
0.31	120.7	135.4	117.31	124.52	9.65	7.7	0.02
0.16	93.94	115.1	104.33	104.47	10.60	10.1	0.58
0.08	93.51	99.13	103.46	98.70	4.99	5.1	0.81
MeOH killed cells	29.37	13.47	10.01	-1.97	23.79	1.00	0.00



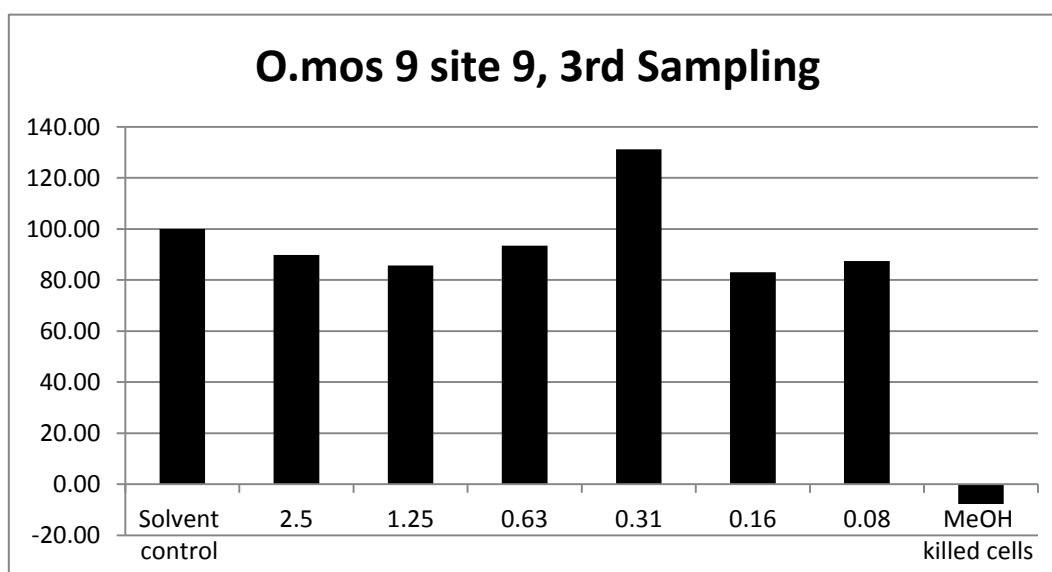
Absorbance reading from plate reader				O.mos 7 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.367	0.339	0.338				
2.5	0.323	0.321	0.32				
1.25	0.336	0.115	0.337				
0.63	0.345	0.345	0.313				
0.31	0.365	0.342	0.358				
0.16	0.355	0.344	0.361				
0.08	0.361	0.353	0.364				
MeOH killed cells	0.049	0.148	0.14	0.147	0.139	0.15	0.14
MeOH killed cells subtracted							
Solvent control	0.250	0.222	0.221				
2.5	0.206	0.204	0.203				
1.25	0.219	0.002	0.220				
0.63	0.228	0.228	0.196				
0.31	0.248	0.225	0.241				
0.16	0.238	0.227	0.244				
0.08	0.244	0.236	0.247				
MeOH killed cells	0.068	0.031	0.023				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	108.2	96.11	95.67	100.00	7.12	7.1	1.00
2.5	89.18	88.32	87.89	88.46	0.66	0.7	0.05
1.25	94.81	-0.81	95.24	63.08	55.33	87.7	0.32
0.63	98.70	98.70	84.86	94.09	7.99	8.5	0.39
0.31	107.3	97.40	104.33	103.03	5.10	5.0	0.58
0.16	103.0	98.27	105.62	102.31	3.73	3.6	0.65
0.08	105.6	102.1	106.92	104.90	2.46	2.3	0.32
MeOH killed cells	29.37	13.47	10.01	-1.97	23.79	1.0	0.00



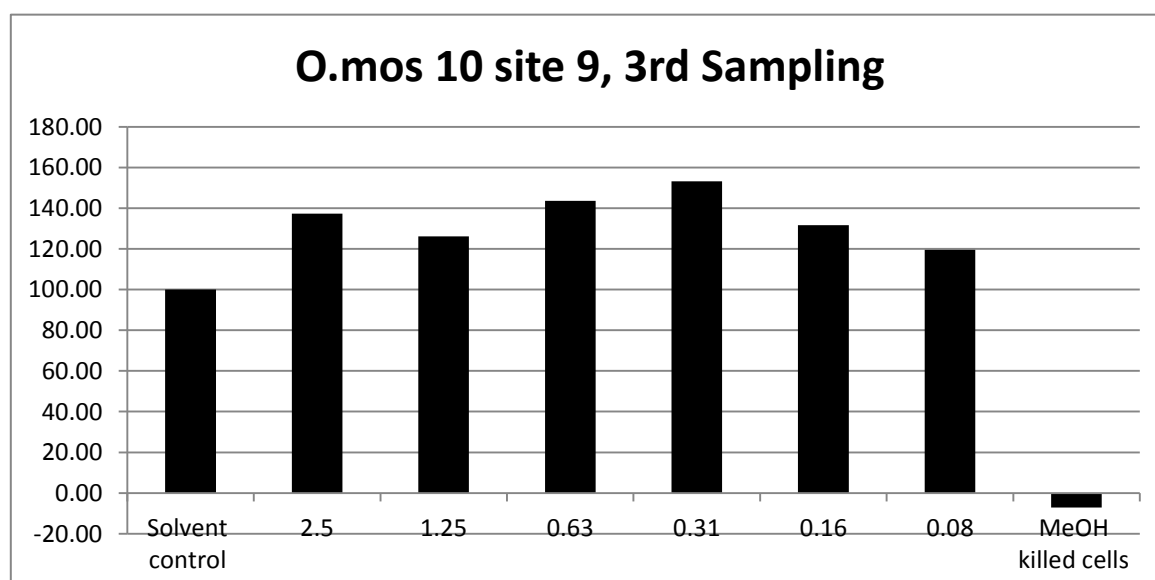
Absorbance reading from plate reader				O.mos 8 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.388	0.343	0.445				
2.5	0.42	0.361	0.426				
1.25	0.412	0.393	0.395				
0.63	0.436	0.405	0.427				
0.31	0.421	0.414	0.427				
0.16	0.397	0.378	0.37				
0.08	0.391	0.38	0.386				
MeOH killed cells	0.021	0.158	0.151	0.156	0.165	0.164	0.181
MeOH killed cells subtracted							
Solvent control	0.258	0.213	0.315				
2.5	0.290	0.231	0.296				
1.25	0.282	0.263	0.265				
0.63	0.306	0.275	0.297				
0.31	0.291	0.284	0.297				
0.16	0.267	0.248	0.240				
0.08	0.261	0.250	0.256				
MeOH killed cells	-0.109	0.028	0.021				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	98.47	81.27	120.2	100.00	19.54	19.5	1.00
2.5	110.70	88.15	113.0	103.95	13.73	13.2	0.79
1.25	107.64	100.38	101.1	103.06	3.99	3.9	0.80
0.63	116.82	104.97	113.3	111.72	6.10	5.5	0.38
0.31	111.08	108.41	113.3	110.96	2.49	2.2	0.39
0.16	101.91	94.65	91.59	96.05	5.30	5.5	0.75
0.08	99.62	95.41	97.71	97.58	2.11	2.2	0.84
MeOH killed cells	-41.81	10.56	7.88	-7.79	29.49	-378.7	0.01



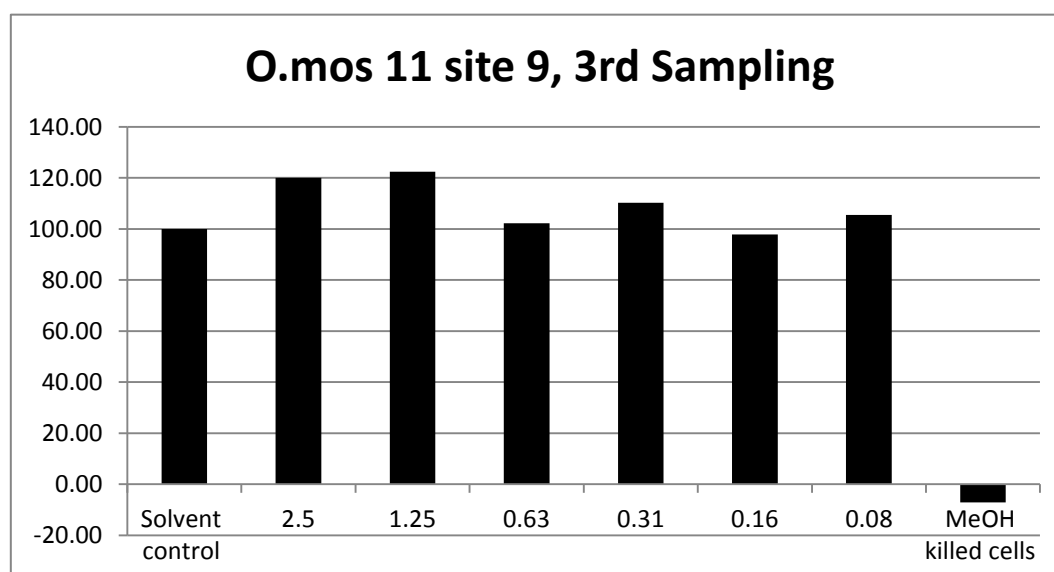
Absorbance reading from plate reader				O.mos 9 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.388	0.343	0.445				
2.5	0.401	0.343	0.352				
1.25	0.376	0.373	0.314				
0.63	0.391	0.372	0.361				
0.31	0.412	0.651	0.358				
0.16	0.342	0.351	0.35				
0.08	0.394	0.335	0.348				
MeOH killed cells	0.021	0.158	0.151	0.156	0.165	0.164	0.181
MeOH killed cells subtracted							
Solvent control	0.258	0.213	0.315				
2.5	0.271	0.213	0.222				
1.25	0.246	0.243	0.184				
0.63	0.261	0.242	0.231				
0.31	0.282	0.521	0.228				
0.16	0.212	0.221	0.220				
0.08	0.264	0.205	0.218				
MeOH killed cells	0.109	0.028	0.021				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	98.47	81.27	120.2	100.00	19.54	19.5	1.00
2.5	103.4	81.27	84.71	89.81	11.93	13.3	0.48
1.25	93.88	92.74	70.19	85.60	13.36	15.6	0.35
0.63	99.62	92.36	88.15	93.37	5.80	6.2	0.60
0.31	107.6	199.0	87.00	131.22	59.60	45.4	0.44
0.16	80.89	84.33	83.95	83.05	1.89	2.3	0.21
0.08	100.7	78.21	83.18	87.39	11.85	13.6	0.39
MeOH killed cells	-41.8	10.56	7.88	-7.79	29.49	1.00	0.01



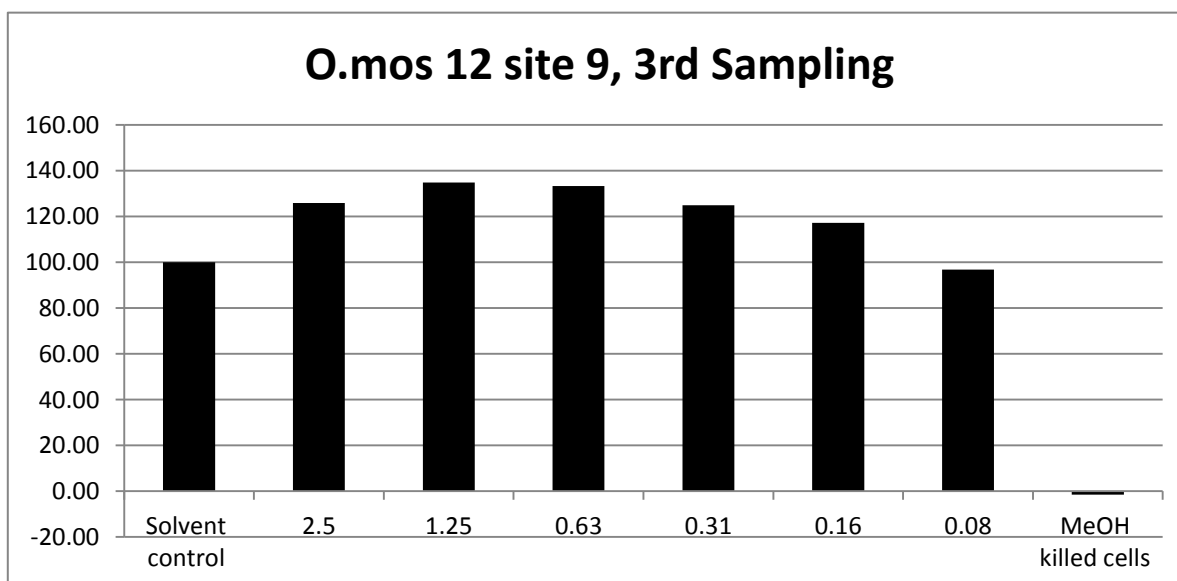
Absorbance reading from plate reader				O.mos 10 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.291	0.312	0.295				
2.5	0.376	0.391	0.321				
1.25	0.379	0.322	0.33				
0.63	0.374	0.359	0.387				
0.31	0.368	0.407	0.394				
0.16	0.359	0.382	0.318				
0.08	0.343	0.317	0.337				
MeOH killed cells	0.046	0.151	0.156	0.153	0.16	0.17	0.16
MeOH killed cells subtracted							
Solvent control	0.161	0.182	0.165				
2.5	0.246	0.261	0.191				
1.25	0.249	0.192	0.200				
0.63	0.244	0.229	0.257				
0.31	0.238	0.277	0.264				
0.16	0.229	0.252	0.188				
0.08	0.213	0.187	0.207				
MeOH killed cells	0.084	0.021	0.026				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	95.09	107.4	97.44	100.00	6.58	6.6	1.00
2.5	145.2	154.0	112.78	137.35	21.73	15.8	0.05
1.25	146.9	113.3	118.08	126.14	18.20	14.4	0.08
0.63	144.0	135.1	151.70	143.64	8.26	5.8	0.00
0.31	140.4	163.4	155.82	153.27	11.71	7.6	0.00
0.16	135.1	148.7	111.01	131.65	19.12	14.5	0.05
0.08	125.7	110.4	122.21	119.46	8.03	6.7	0.03
MeOH killed cells	49.39	12.53	15.48	-7.13	36.63	1.00	0.01



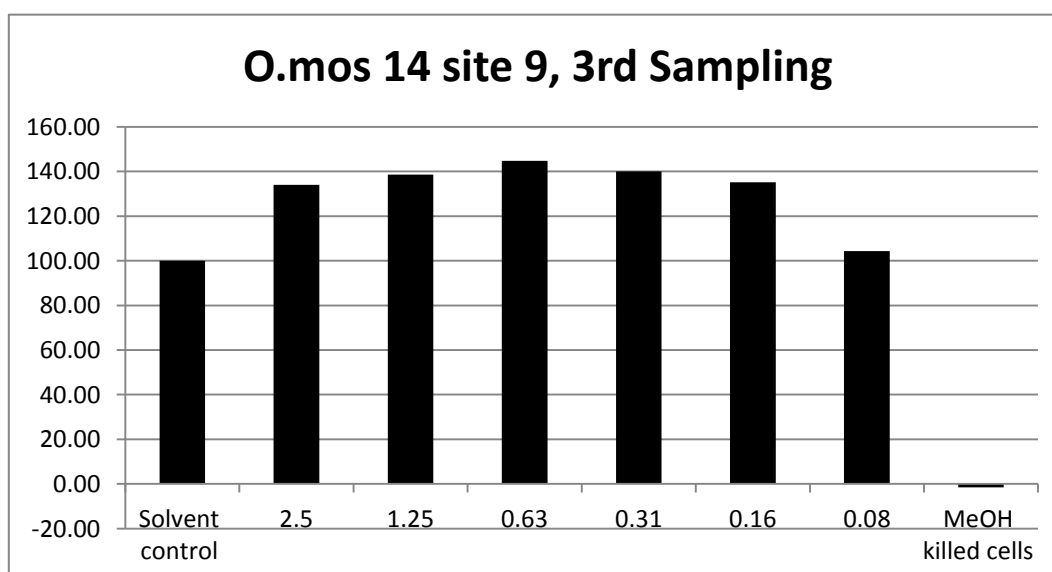
Absorbance reading from plate reader				O.mos 11 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.291	0.312	0.295				
2.5	0.311	0.378	0.311				
1.25	0.334	0.346	0.332				
0.63	0.305	0.292	0.312				
0.31	0.344	0.312	0.294				
0.16	0.28	0.318	0.289				
0.08	0.334	0.301	0.291				
MeOH killed cells	0.046	0.151	0.156	0.153	0.16	0.17	0.16
MeOH killed cells subtracted							
Solvent control	0.161	0.182	0.165				
2.5	0.181	0.248	0.181				
1.25	0.204	0.216	0.202				
0.63	0.175	0.162	0.182				
0.31	0.214	0.182	0.164				
0.16	0.150	0.188	0.159				
0.08	0.204	0.171	0.161				
MeOH killed cells	0.084	0.021	0.026				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	95.09	107.4	97.44	100.00	6.58	6.6	1.00
2.5	106.8	146.3	106.88	120.05	22.81	19.0	0.22
1.25	120.4	127.5	119.26	122.41	4.46	3.6	0.01
0.63	103.3	95.68	107.47	102.16	5.98	5.9	0.70
0.31	126.3	107.4	96.86	110.22	14.93	13.5	0.34
0.16	88.60	111.0	93.91	97.84	11.71	12.0	0.79
0.08	120.4	100.9	95.09	105.50	13.27	12.6	0.55
MeOH killed cells	-49.39	12.53	15.48	-7.13	36.63	1.00	0.01



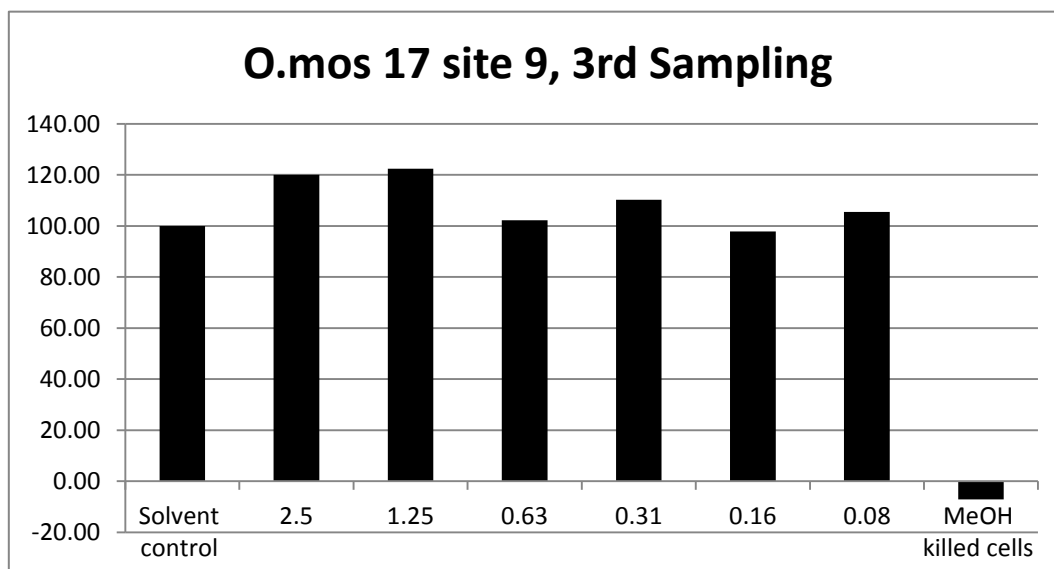
Absorbance reading from plate reader				O.mos 12 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.286	0.309	0.293				
2.5	0.354	0.333	0.339				
1.25	0.354	0.358	0.362				
0.63	0.37	0.353	0.343				
0.31	0.322	0.353	0.346				
0.16	0.315	0.324	0.341				
0.08	0.283	0.293	0.295				
MeOH killed cells	0.08	0.137	0.128	0.132	0.144	0.137	0.139
MeOH killed cells subtracted							
Solvent control	0.168	0.191	0.175				
2.5	0.236	0.215	0.221				
1.25	0.236	0.240	0.244				
0.63	0.252	0.235	0.225				
0.31	0.204	0.235	0.228				
0.16	0.197	0.206	0.223				
0.08	0.165	0.175	0.177				
MeOH killed cells	0.038	0.019	0.010				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	94.39	107.2	98.32	100.00	6.61	6.6	1.00
2.5	132.5	120.7	124.1	125.81	6.07	4.8	0.01
1.25	132.5	134.	137.0	134.78	2.24	1.7	0.00
0.63	141.5	131.9	126.3	133.29	7.66	5.7	0.00
0.31	114.5	131.9	128.0	124.87	9.12	7.3	0.02
0.16	110.6	115.7	125.2	117.20	7.41	6.3	0.04
0.08	92.71	98.32	99.44	96.82	3.61	3.7	0.51
MeOH killed cells	-21.1	10.80	5.75	-1.54	17.19	1.0	0.00



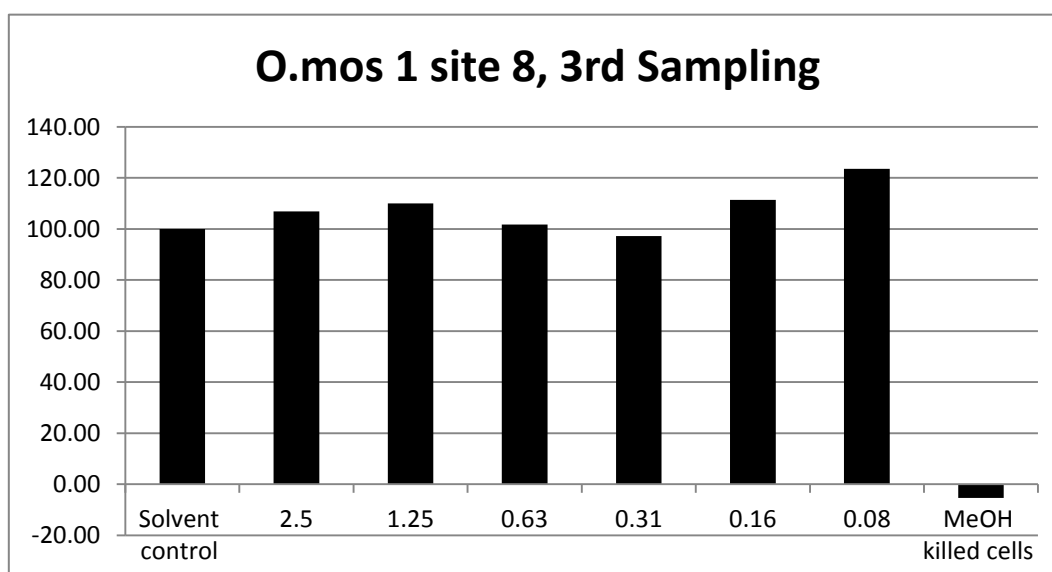
Absorbance reading from plate reader				O.mos 14 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.286	0.309	0.293				
2.5	0.347	0.39	0.333				
1.25	0.362	0.364	0.368				
0.63	0.392	0.378	0.357				
0.31	0.375	0.366	0.361				
0.16	0.359	0.375	0.342				
0.08	0.303	0.302	0.306				
MeOH killed cells	0.08	0.137	0.128	0.132	0.144	0.1	0.1
						37	
MeOH killed cells subtracted							
Solvent control	0.168	0.191	0.175				
2.5	0.229	0.272	0.215				
1.25	0.244	0.246	0.250				
0.63	0.274	0.260	0.239				
0.31	0.257	0.248	0.243				
0.16	0.241	0.257	0.224				
0.08	0.185	0.184	0.188				
MeOH killed cells	-0.038	0.019	0.010				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	94.39	107.2	98.32	100.00	6.61	6.6	1.00
2.5	128.61	152.7	120.7	134.03	16.66	12	0.03
1.25	137.03	138.1	140.3	138.52	1.71	1.2	0.00
0.63	153.86	146.0	134.2	144.69	9.88	6.8	0.00
0.31	144.32	139.2	136.4	140.02	3.98	2.8	0.00
0.16	135.34	144.3	125.8	135.16	9.26	6.8	0.01
0.08	103.93	103.3	105.6	104.30	1.17	1.1	0.33
MeOH killed cells	-21.18	10.80	5.75	-1.54	17.19	1.0	0.00



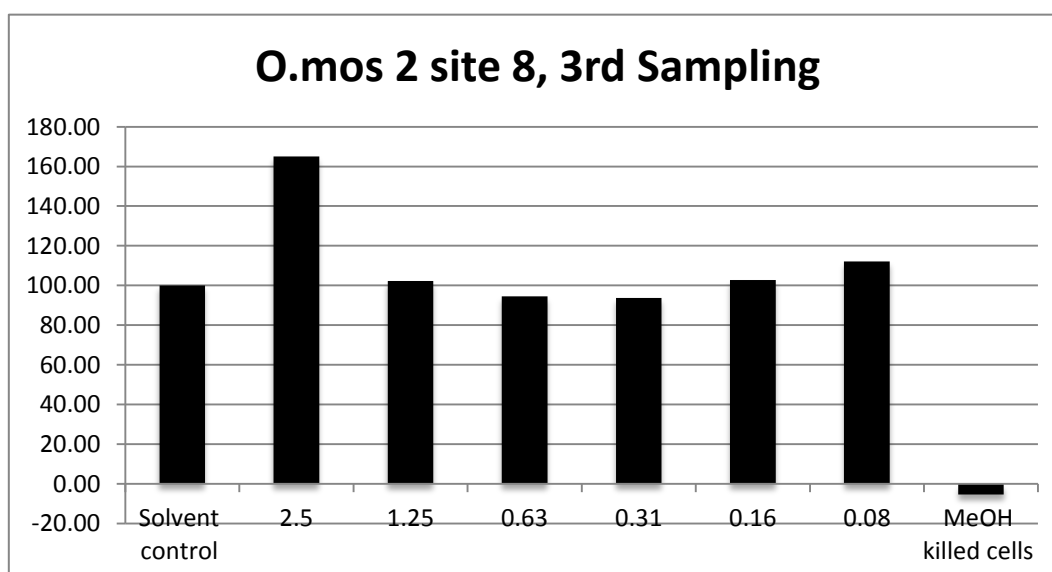
Absorbance reading from plate reader				O.mos 17 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.359	0.342	0.343				
2.5	0.352	0.35	0.401				
1.25	0.378	0.385	0.357				
0.63	0.391	0.391	0.392				
0.31	0.365	0.376	0.389				
0.16	0.372	0.378	0.362				
0.08	0.354	0.361	0.349				
MeOH killed cells	0.039	0.176	0.41	0.271	0.168	0.17	0.16
MeOH killed cells subtracted							
Solvent control	0.180	0.163	0.164				
2.5	0.173	0.171	0.222				
1.25	0.199	0.206	0.178				
0.63	0.212	0.212	0.213				
0.31	0.186	0.197	0.210				
0.16	0.193	0.199	0.183				
0.08	0.175	0.182	0.170				
MeOH killed cells	0.169	0.032	0.202				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	106.5	96.45	97.04	100.00	5.65	5.6	1.00
2.5	102.3	101.1	131.38	111.65	17.10	15.3	0.33
1.25	117.7	121.9	105.33	115.00	8.63	7.5	0.07
0.63	125.4	125.4	126.05	125.66	0.34	0.3	0.00
0.31	110.0	116.5	124.28	116.98	7.11	6.1	0.03
0.16	114.2	117.7	108.29	113.42	4.79	4.2	0.03
0.08	103.5	107.7	100.59	103.95	3.57	3.4	0.36
MeOH killed cells	100.2	19.15	119.42	0.00	1.00	1.0	0.19



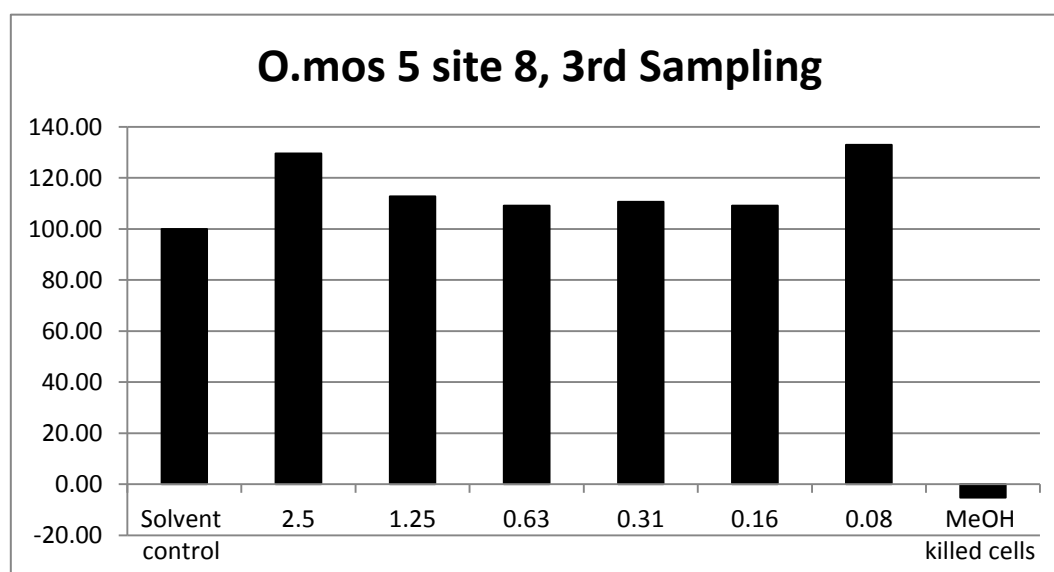
Absorbance reading from plate reader				O.mos 1 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.206	0.199	0.198				
2.5	0.215	0.2	0.212				
1.25	0.204	0.214	0.22				
0.63	0.205	0.191	0.213				
0.31	0.194	0.191	0.208				
0.16	0.208	0.208	0.227				
0.08	0.228	0.229	0.229				
MeOH killed cells	0.008	0.113	0.111	0.109	0.11	0.107	0.107
MeOH killed cells subtracted							
Solvent control	0.122	0.115	0.114				
2.5	0.131	0.116	0.128				
1.25	0.120	0.130	0.136				
0.63	0.121	0.107	0.129				
0.31	0.110	0.107	0.124				
0.16	0.124	0.124	0.143				
0.08	0.144	0.145	0.145				
MeOH killed cells	-0.076	0.029	0.027				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	104.26	98.30	97.44	100.00	3.71	3.7	1.00
2.5	111.93	99.15	109.3	106.82	6.76	6.3	0.20
1.25	102.56	111.08	116.1	109.94	6.89	6.3	0.09
0.63	103.41	91.48	110.2	101.70	9.49	9.3	0.79
0.31	94.04	91.48	105.9	97.16	7.73	8.0	0.60
0.16	105.96	105.96	122.1	111.36	9.35	8.4	0.12
0.08	123.00	123.86	123.8	123.57	0.49	0.4	0.00
MeOH killed cells	-64.43	25.03	23.32	-5.36	51.16	-954.5	0.02



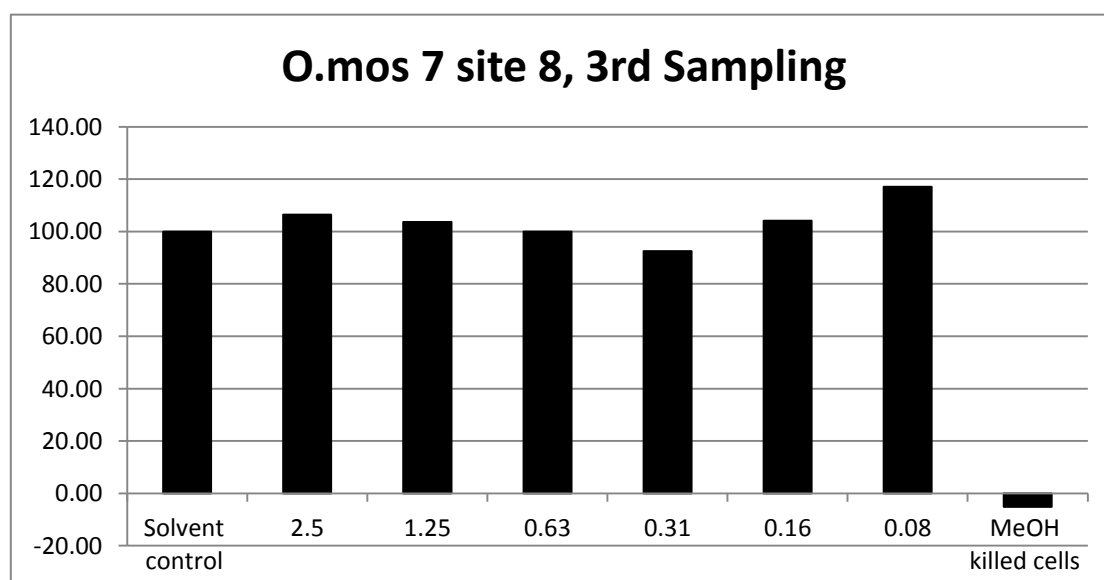
Absorbance reading from plate reader				O.mos 2 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.206	0.199	0.198				
2.5	0.404	0.21	0.218				
1.25	0.194	0.202	0.215				
0.63	0.192	0.191	0.201				
0.31	0.187	0.198	0.196				
0.16	0.205	0.197	0.211				
0.08	0.224	0.211	0.211				
MeOH killed cells	0.008	0.113	0.111	0.109	0.11	0.107	0.107
MeOH killed cells subtracted							
Solvent control	0.122	0.115	0.114				
2.5	0.320	0.126	0.134				
1.25	0.110	0.118	0.131				
0.63	0.108	0.107	0.117				
0.31	0.103	0.114	0.112				
0.16	0.121	0.113	0.127				
0.08	0.140	0.127	0.127				
MeOH killed cells	0.076	0.029	0.027				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	104.2	98.30	97.44	100.00	3.71	3.7	1.00
2.5	272.9	107.6	114.4	165.03	93.52	56.7	0.30
1.25	94.04	100.8	111.9	102.27	9.03	8.8	0.71
0.63	92.33	91.48	100.0	94.60	4.69	5.0	0.19
0.31	88.07	97.44	95.74	93.75	4.99	5.3	0.16
0.16	103.4	96.59	108.5	102.84	5.98	5.8	0.52
0.08	119.6	108.5	108.5	112.21	6.39	5.7	0.05
MeOH killed cells	-64.4	25.03	23.32	-5.36	51.16	1.0	0.02



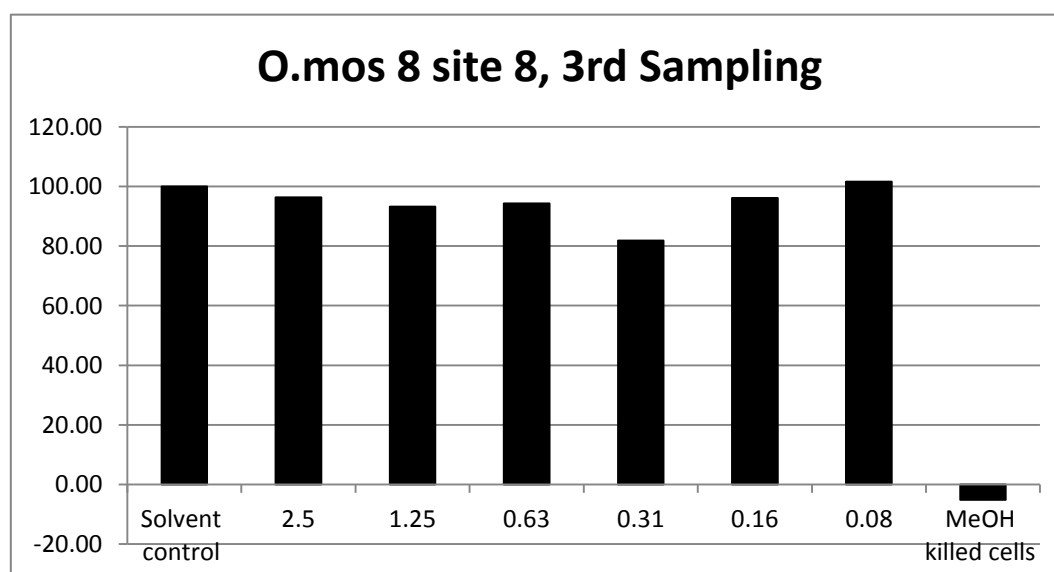
Absorbance reading from plate reader				O.mos 5 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.22	0.229	0.227				
2.5	0.278	0.264	0.248				
1.25	0.255	0.254	0.216				
0.63	0.246	0.231	0.234				
0.31	0.254	0.232	0.231				
0.16	0.259	0.239	0.213				
0.08	0.3	0.275	0.228				
MeOH killed cells	0.016	0.128	0.127	0.122	0.124	0.12	0.126
						1	
MeOH killed cells subtracted							
Solvent control	0.123	0.132	0.130				
2.5	0.181	0.167	0.151				
1.25	0.158	0.157	0.119				
0.63	0.149	0.134	0.137				
0.31	0.157	0.135	0.134				
0.16	0.162	0.142	0.116				
0.08	0.203	0.178	0.131				
MeOH killed cells	0.081	0.031	0.030				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	95.84	102.8	101.30	100.00	3.68	3.7	1.00
2.5	141.0	130.1	117.66	129.61	11.70	9.0	0.01
1.25	123.1	122.3	92.73	112.73	17.32	15.4	0.28
0.63	116.1	104.4	106.75	109.09	6.18	5.7	0.09
0.31	122.3	105.1	104.42	110.65	10.13	9.2	0.16
0.16	126.2	110.6	90.39	109.09	17.97	16.5	0.44
0.08	158.1	138.7	102.08	132.99	28.49	21.4	0.12
MeOH killed cells	63.12	24.16	23.38	-5.19	50.16	1.00	0.02



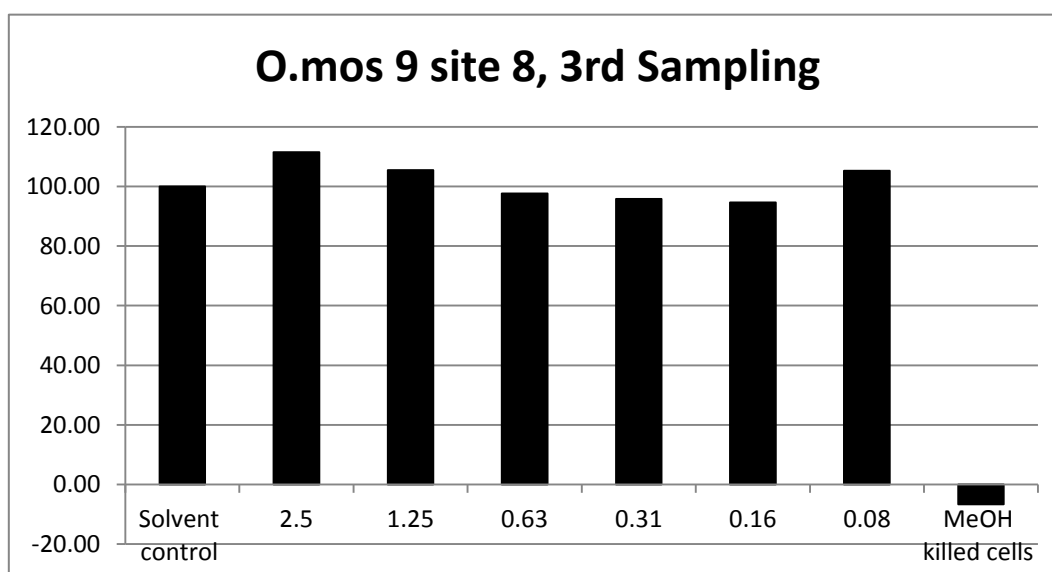
Absorbance reading from plate reader				O.mos 7 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.22	0.229	0.227				
2.5	0.233	0.237	0.231				
1.25	0.241	0.241	0.208				
0.63	0.233	0.225	0.218				
0.31	0.222	0.229	0.196				
0.16	0.238	0.236	0.218				
0.08	0.257	0.257	0.228				
MeOH killed cells	0.016	0.128	0.127	0.122	0.124	0.12	0.126
MeOH killed cells subtracted							
Solvent control	0.123	0.132	0.130				
2.5	0.136	0.140	0.134				
1.25	0.144	0.144	0.111				
0.63	0.136	0.128	0.121				
0.31	0.125	0.132	0.099				
0.16	0.141	0.139	0.121				
0.08	0.160	0.160	0.131				
MeOH killed cells	0.081	0.031	0.030				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	95.84	102.8	101.30	100.00	3.68	3.7	1.00
2.5	105.9	109.0	104.42	106.49	2.38	2.2	0.06
1.25	112.2	112.2	86.49	103.64	14.85	14.3	0.70
0.63	105.9	99.74	94.29	100.00	5.85	5.8	1.00
0.31	97.40	102.8	77.14	92.47	13.55	14.7	0.41
0.16	109.8	108.3	94.29	104.16	8.58	8.2	0.48
0.08	124.6	124.6	102.08	117.14	13.05	11.1	0.09
MeOH killed cells	63.12	24.16	23.38	-5.19	50.16	1.00	0.02



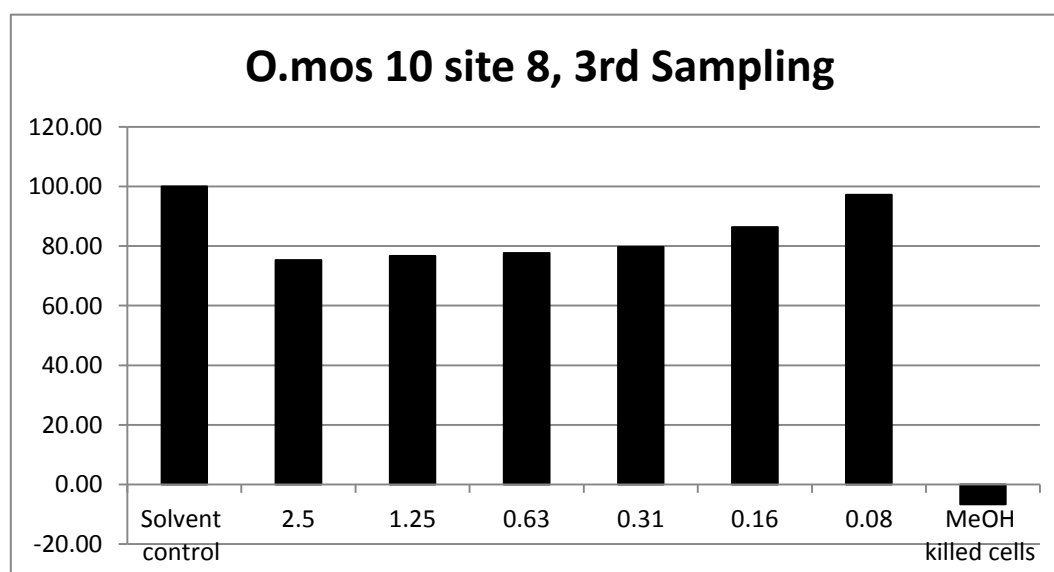
Absorbance reading from plate reader				O.mos 8 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.22	0.229	0.227				
2.5	0.212	0.214	0.236				
1.25	0.208	0.218	0.224				
0.63	0.212	0.217	0.225				
0.31	0.185	0.219	0.202				
0.16	0.227	0.227	0.207				
0.08	0.222	0.227	0.233				
MeOH killed cells	0.016	0.128	0.127	0.122	0.124	0.121	0.126
MeOH killed cells subtracted							
Solvent control	0.123	0.132	0.130				
2.5	0.115	0.117	0.139				
1.25	0.111	0.121	0.127				
0.63	0.115	0.120	0.128				
0.31	0.088	0.122	0.105				
0.16	0.130	0.130	0.110				
0.08	0.125	0.130	0.136				
MeOH killed cells	-0.081	0.031	0.030				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	95.84	102.86	101.3	100.00	3.68	3.7	1.00
2.5	89.61	91.17	108.3	96.36	10.38	10.8	0.60
1.25	86.49	94.29	98.96	93.25	6.30	6.8	0.18
0.63	89.61	93.51	99.74	94.29	5.11	5.4	0.19
0.31	68.57	95.06	81.82	81.82	13.25	16.2	0.08
0.16	101.30	101.30	85.71	96.10	9.00	9.4	0.53
0.08	97.40	101.30	105.9	101.56	4.29	4.2	0.66
MeOH killed cells	-63.12	24.16	23.38	-5.19	50.16	-965.6	0.02



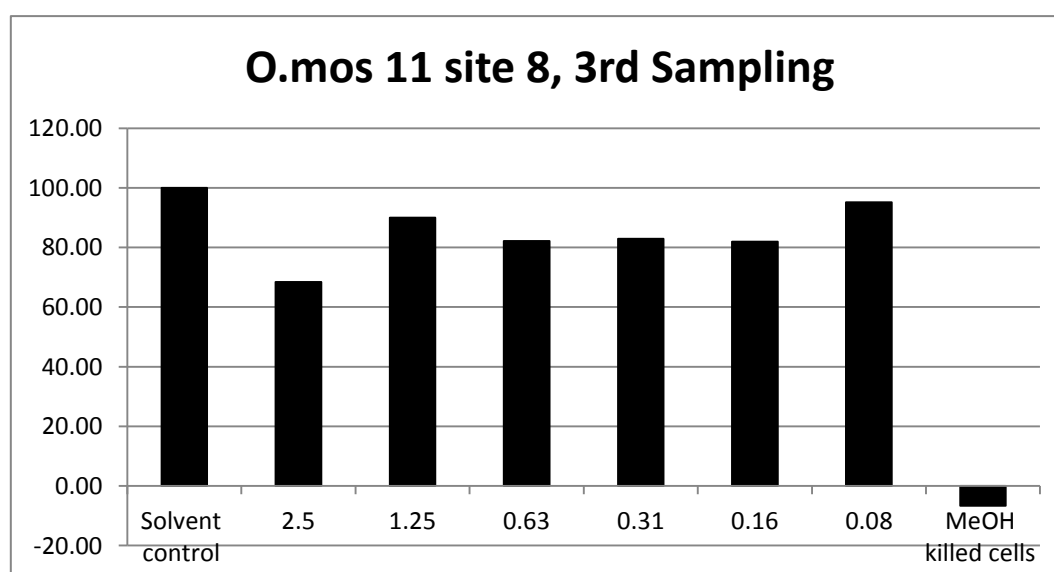
Absorbance reading from plate reader				O.mos 9 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.258	0.301	0.221				
2.5	0.276	0.298	0.263				
1.25	0.301	0.256	0.25				
0.63	0.271	0.25	0.247				
0.31	0.274	0.252	0.233				
0.16	0.258	0.257	0.238				
0.08	0.281	0.277	0.248				
MeOH killed cells	0.012	0.119	0.118	0.117	0.119	0.115	0.123
MeOH killed cells subtracted							
Solvent control	0.164	0.207	0.127				
2.5	0.182	0.204	0.169				
1.25	0.207	0.162	0.156				
0.63	0.177	0.156	0.153				
0.31	0.180	0.158	0.139				
0.16	0.164	0.163	0.144				
0.08	0.187	0.183	0.154				
MeOH killed cells	-0.08	0.025	0.024				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	98.79	124.7	76.49	100.00	24.14	24.1	1.00
2.5	109.6	122.9	101.8	111.45	10.67	9.6	0.49
1.25	124.7	97.59	93.97	105.43	16.80	15.9	0.77
0.63	106.6	93.97	92.16	97.59	7.88	8.1	0.88
0.31	108.4	95.18	83.72	95.78	12.37	12.9	0.80
0.16	98.79	98.19	86.74	94.57	6.79	7.2	0.73
0.08	112.6	110.2	92.77	105.22	10.86	10.3	0.75
MeOH killed cells	-49.5	15.00	14.39	-6.71	37.07	-552.7	0.01



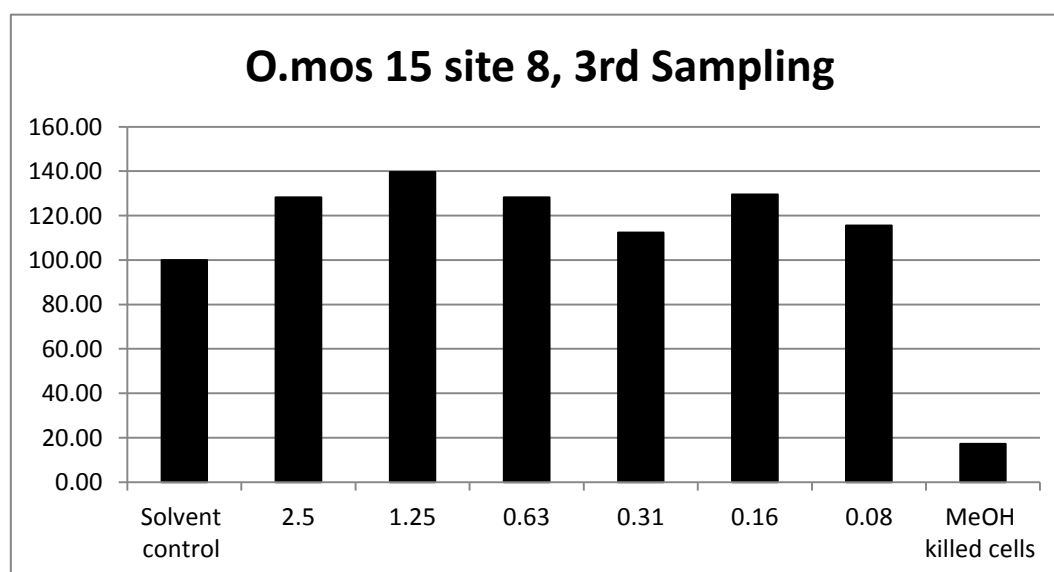
Absorbance reading from plate reader				O.mos 10 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.258	0.301	0.221				
2.5	0.232	0.221	0.204				
1.25	0.235	0.23	0.199				
0.63	0.223	0.23	0.216				
0.31	0.228	0.233	0.218				
0.16	0.244	0.243	0.225				
0.08	0.262	0.255	0.249				
MeOH killed cells	0.012	0.119	0.118	0.117	0.119	0.11	0.12
MeOH killed cells subtracted							
Solvent control	0.164	0.207	0.127				
2.5	0.138	0.127	0.110				
1.25	0.141	0.136	0.105				
0.63	0.129	0.136	0.122				
0.31	0.134	0.139	0.124				
0.16	0.150	0.149	0.131				
0.08	0.168	0.161	0.155				
MeOH killed cells	0.082	0.025	0.024				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	98.79	124.7	76.49	100.00	24.14	24.1	1.00
2.5	83.12	76.49	66.24	75.28	8.50	11.3	0.17
1.25	84.93	81.91	63.23	76.69	11.76	15.3	0.21
0.63	77.69	81.91	73.47	77.69	4.22	5.4	0.19
0.31	80.71	83.72	74.68	79.70	4.60	5.8	0.23
0.16	90.35	89.75	78.90	86.34	6.45	7.5	0.40
0.08	101.2	96.99	93.37	97.19	3.92	4.0	0.85
MeOH killed cells	49.51	15.00	14.39	-6.71	37.07	1.00	0.01



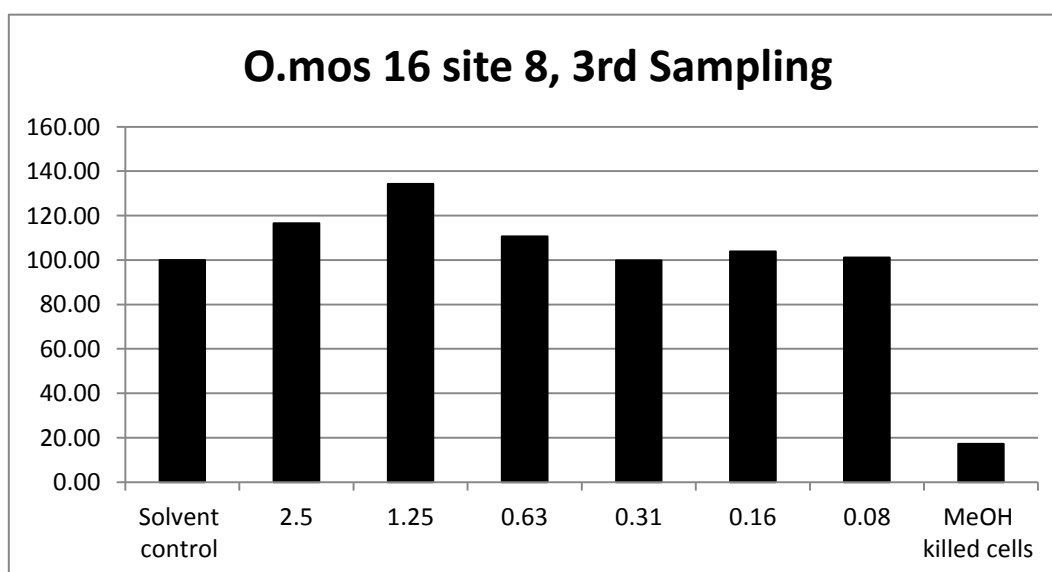
Absorbance reading from plate reader				O.mos 11 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.258	0.301	0.221				
2.5	0.234	0.129	0.26				
1.25	0.216	0.254	0.26				
0.63	0.224	0.231	0.236				
0.31	0.225	0.225	0.245				
0.16	0.215	0.239	0.236				
0.08	0.247	0.252	0.257				
MeOH killed cells	0.012	0.119	0.118	0.117	0.119	0.11	0.12
MeOH killed cells subtracted							
Solvent control	0.164	0.207	0.127				
2.5	0.140	0.035	0.166				
1.25	0.122	0.160	0.166				
0.63	0.130	0.137	0.142				
0.31	0.131	0.131	0.151				
0.16	0.121	0.145	0.142				
0.08	0.153	0.158	0.163				
MeOH killed cells	0.082	0.025	0.024				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	98.79	124.7	76.49	100.00	24.14	24.1	1.00
2.5	84.33	21.02	100.00	68.45	41.81	61.1	0.32
1.25	73.47	96.38	100.00	89.95	14.38	16.0	0.57
0.63	78.30	82.52	85.53	82.12	3.63	4.4	0.27
0.31	78.90	78.90	90.96	82.92	6.96	8.4	0.30
0.16	72.87	87.34	85.53	81.91	7.88	9.6	0.28
0.08	92.16	95.18	98.19	95.18	3.01	3.2	0.75
MeOH killed cells	49.51	15.00	14.39	-6.71	37.07	1.00	0.01



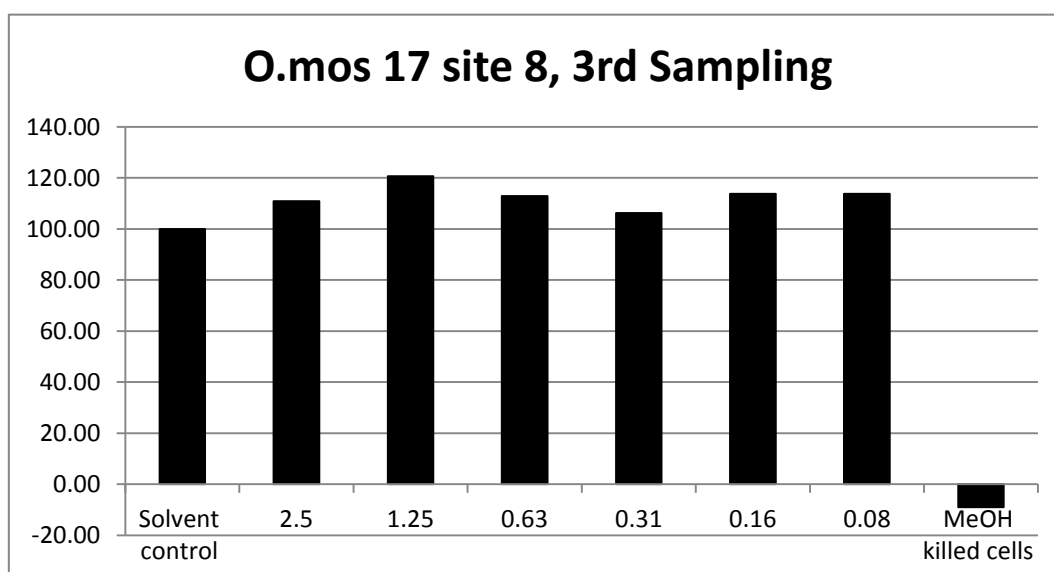
Absorbance reading from plate reader				O.mos 15 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.359	0.342	0.343				
2.5	0.389	0.411	0.387				
1.25	0.424	0.418	0.403				
0.63	0.382	0.408	0.397				
0.31	0.365	0.358	0.384				
0.16	0.38	0.387	0.427				
0.08	0.364	0.387	0.372				
MeOH killed cells	0.039	0.176	0.41	0.271	0.168	0.17	0.166
MeOH killed cells subtracted							
Solvent control	0.180	0.163	0.164				
2.5	0.210	0.232	0.208				
1.25	0.245	0.239	0.224				
0.63	0.203	0.229	0.218				
0.31	0.186	0.179	0.205				
0.16	0.201	0.208	0.248				
0.08	0.185	0.208	0.193				
MeOH killed cells	0.140	0.003	0.231				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	106.5	96.45	97.04	100.00	5.65	5.6	1.00
2.5	124.2	137.3	123.0	128.23	7.89	6.1	0.01
1.25	145.0	141.4	132.5	139.67	6.41	4.6	0.00
0.63	120.1	135.5	129.0	128.23	7.73	6.0	0.01
0.31	110.0	105.9	121.3	112.44	7.97	7.1	0.09
0.16	118.9	123.0	146.7	129.61	15.02	11.6	0.03
0.08	109.4	123.0	114.2	115.59	6.91	6.0	0.04
MeOH killed cells	-82.8	-1.85	136.7	17.30	111.09	642.3	0.27



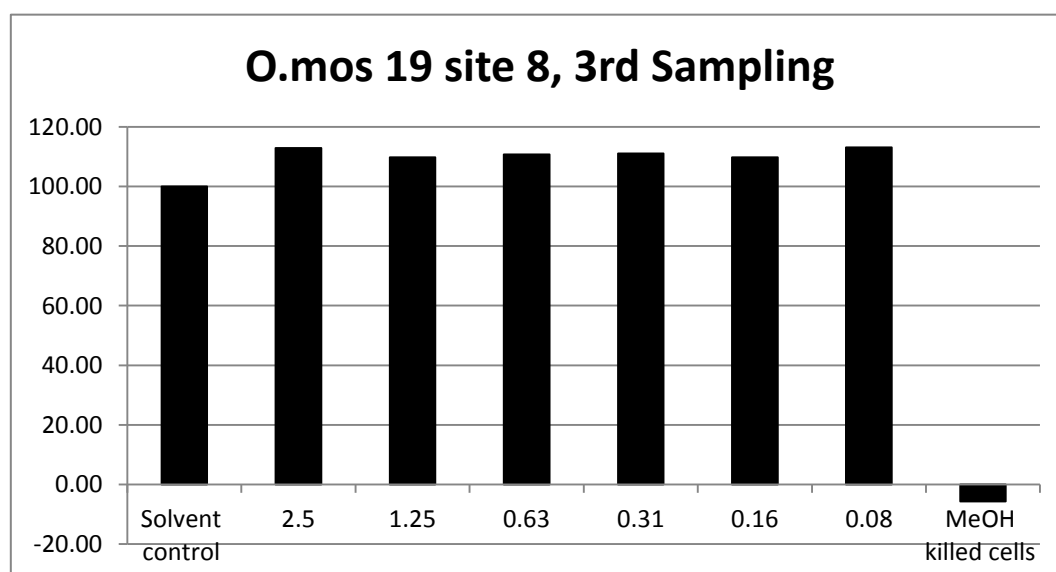
Absorbance reading from plate reader				O.mos 16 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.359	0.342	0.343				
2.5	0.379	0.392	0.357				
1.25	0.427	0.385	0.406				
0.63	0.364	0.371	0.363				
0.31	0.351	0.355	0.337				
0.16	0.353	0.353	0.358				
0.08	0.371	0.351	0.328				
MeOH killed cells	0.039	0.176	0.41	0.271	0.168	0.1	0.166
MeOH killed cells subtracted							
Solvent control	0.180	0.163	0.164				
2.5	0.200	0.213	0.178				
1.25	0.248	0.206	0.227				
0.63	0.185	0.192	0.184				
0.31	0.172	0.176	0.158				
0.16	0.174	0.174	0.179				
0.08	0.192	0.172	0.149				
MeOH killed cells	-0.140	-0.03	0.231				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	106.51	96.45	97.04	100.00	5.65	5.6	1.00
2.5	118.36	126.0	105.3	116.58	10.48	9.0	0.07
1.25	146.78	121.9	134.3	134.34	12.44	9.3	0.01
0.63	109.47	113.6	108.8	110.66	2.58	2.3	0.04
0.31	101.78	104.1	93.49	99.80	5.60	5.6	0.97
0.16	102.96	102.9	105.9	103.95	1.71	1.6	0.31
0.08	113.62	101.7	88.16	101.18	12.74	12.6	0.89
MeOH killed cells	-82.98	-1.85	136.7	17.30	111.09	1.0	0.27



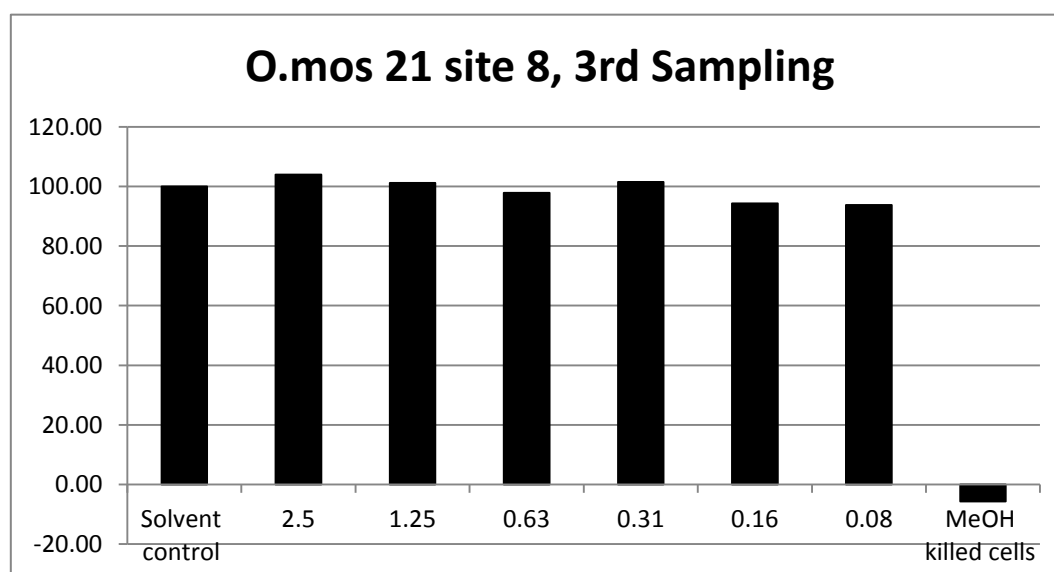
Absorbance reading from plate reader				O.mos 17 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.343	0.341	0.331				
2.5	0.363	0.348	0.371				
1.25	0.375	0.424	0.343				
0.63	0.366	0.368	0.36				
0.31	0.361	0.351	0.341				
0.16	0.356	0.383	0.36				
0.08	0.368	0.385	0.346				
MeOH killed cells	0.037	0.163	0.167	0.151	0.159	0.15	0.17
MeOH killed cells subtracted							
Solvent control	0.209	0.207	0.197				
2.5	0.229	0.214	0.237				
1.25	0.241	0.290	0.209				
0.63	0.232	0.234	0.226				
0.31	0.227	0.217	0.207				
0.16	0.222	0.249	0.226				
0.08	0.234	0.251	0.212				
MeOH killed cells	0.097	0.029	0.033				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	102.2	101.3	96.41	100.00	3.14	3.1	1.00
2.5	112.0	104.7	115.98	110.92	5.71	5.1	0.04
1.25	117.9	141.9	102.28	120.71	19.95	16.5	0.15
0.63	113.5	114.5	110.60	112.88	2.04	1.8	0.00
0.31	111.0	106.2	101.30	106.20	4.89	4.6	0.14
0.16	108.6	121.8	110.60	113.69	7.13	6.3	0.04
0.08	114.5	122.8	103.75	113.69	9.56	8.4	0.08
MeOH killed cells	57.36	14.24	16.20	-8.97	41.92	1.0	0.01



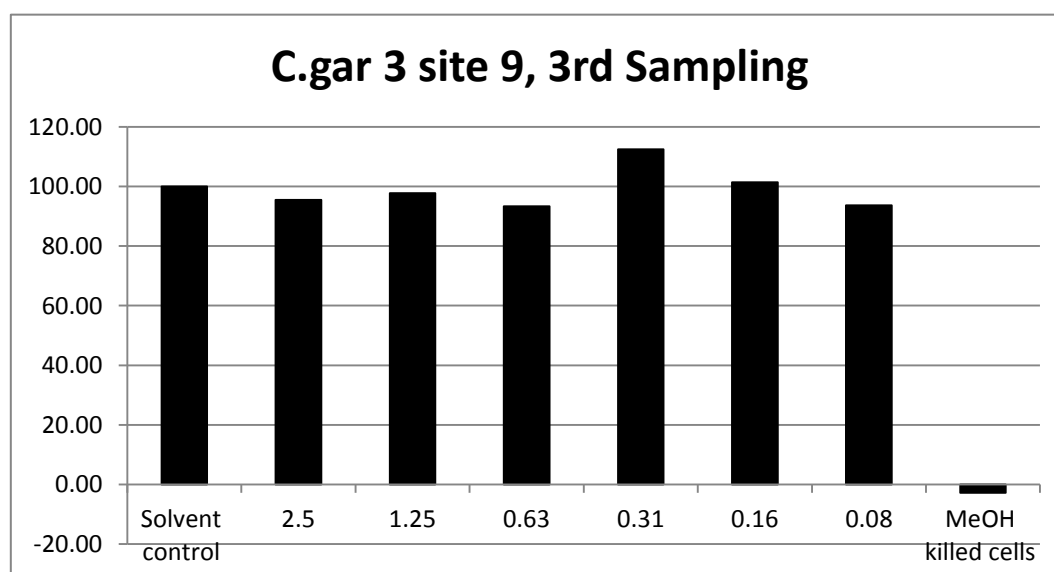
Absorbance reading from plate reader				O.mos 19 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.343	0.341	0.331				
2.5	0.353	0.389	0.352				
1.25	0.372	0.345	0.358				
0.63	0.356	0.377	0.348				
0.31	0.365	0.359	0.359				
0.16	0.361	0.357	0.357				
0.08	0.371	0.358	0.366				
MeOH killed cells	0.037	0.163	0.167	0.151	0.159	0.15	0.17
MeOH killed cells subtracted							
Solvent control	0.209	0.207	0.197				
2.5	0.219	0.255	0.218				
1.25	0.238	0.211	0.224				
0.63	0.222	0.243	0.214				
0.31	0.231	0.225	0.225				
0.16	0.227	0.223	0.223				
0.08	0.237	0.224	0.232				
MeOH killed cells	0.097	0.029	0.033				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	102.2	101.3	96.41	100.00	3.14	3.1	1.00
2.5	107.1	124.7	106.68	112.88	10.31	9.1	0.11
1.25	116.4	103.2	109.62	109.78	6.60	6.0	0.08
0.63	108.6	118.9	104.73	110.76	7.33	6.6	0.08
0.31	113.0	110.1	110.11	111.09	1.69	1.5	0.01
0.16	11.09	109.1	109.13	109.78	1.13	1.0	0.01
0.08	115.9	109.6	113.53	113.04	3.21	2.8	0.01
MeOH killed cells	47.38	14.24	16.20	-5.64	36.16	1.00	0.01



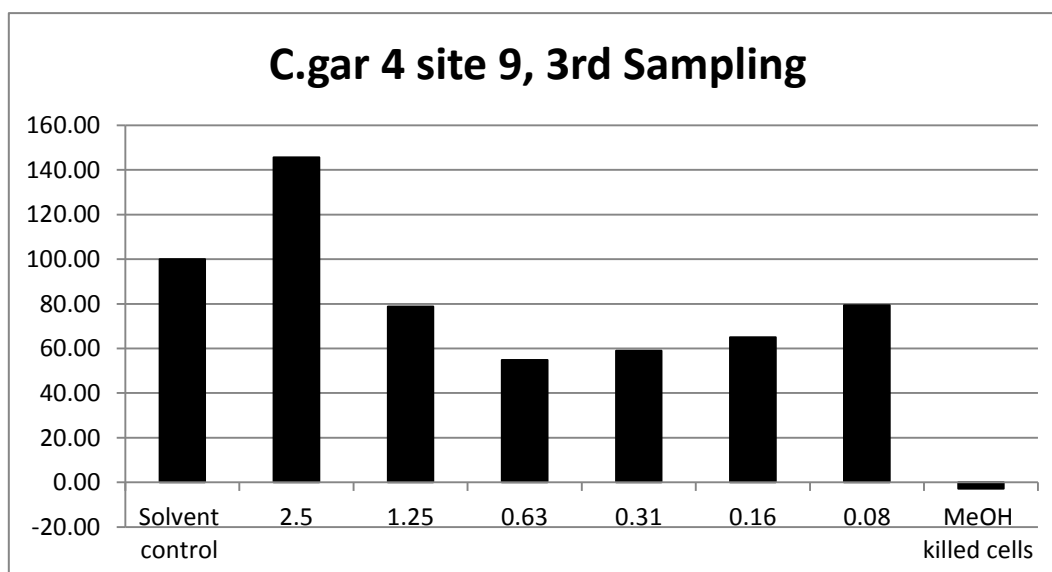
Absorbance reading from plate reader				O.mos 21 Site 8 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.343	0.341	0.331				
2.5	0.346	0.364	0.329				
1.25	0.35	0.343	0.329				
0.63	0.335	0.342	0.325				
0.31	0.353	0.328	0.343				
0.16	0.329	0.348	0.303				
0.08	0.329	0.334	0.314				
MeOH killed cells	0.037	0.163	0.167	0.151	0.159	0.15	0.17
MeOH killed cells subtracted							
Solvent control	0.209	0.207	0.197				
2.5	0.212	0.230	0.195				
1.25	0.216	0.209	0.195				
0.63	0.201	0.208	0.191				
0.31	0.219	0.194	0.209				
0.16	0.195	0.214	0.169				
0.08	0.195	0.200	0.180				
MeOH killed cells	0.097	0.029	0.033				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	102.	101.3	96.41	100.00	3.14	3.1	1.00
2.5	103.7	112.5	95.44	103.91	8.56	8.2	0.50
1.25	105.7	102.2	95.44	101.14	5.23	5.2	0.76
0.63	98.37	101.7	93.48	97.88	4.18	4.3	0.52
0.31	107.1	94.95	102.28	101.47	6.15	6.1	0.73
0.16	95.44	104.7	82.72	94.29	11.05	11.7	0.44
0.08	95.44	97.88	88.10	93.80	5.09	5.4	0.15
MeOH killed cells	47.38	14.24	16.20	-5.64	36.16	1.00	0.01



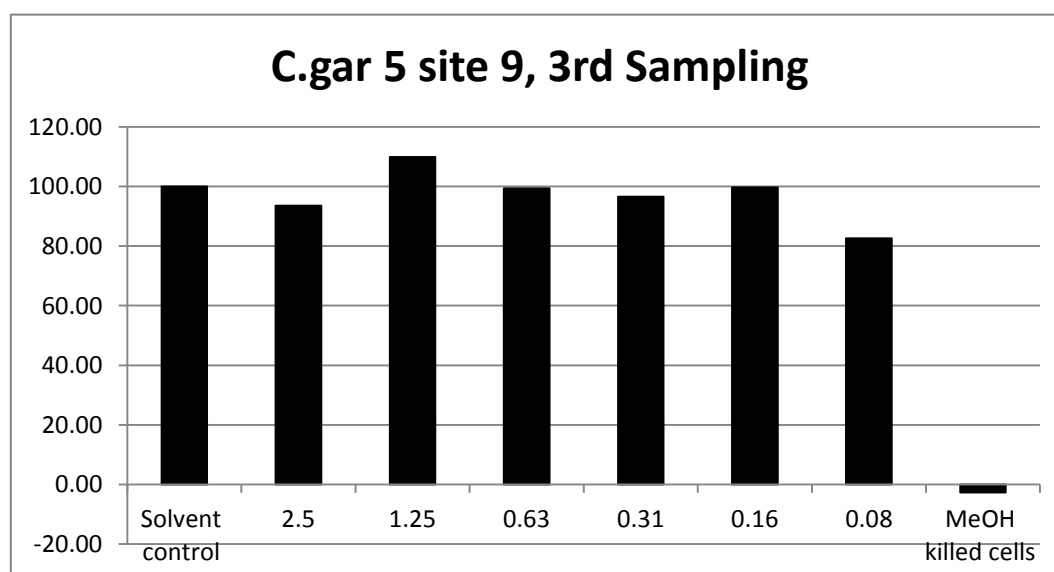
Absorbance reading from plate reader				C.gar 3 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.31	0.326	0.316				
2.5	0.309	0.285	0.332				
1.25	0.321	0.291	0.327				
0.63	0.319	0.287	0.308				
0.31	0.344	0.349	0.33				
0.16	0.33	0.321	0.309				
0.08	0.31	0.309	0.297				
MeOH killed cells	0.037	0.158	0.171	0.157	0.157	0.136	0.156
MeOH killed cells subtracted							
Solvent control	0.183	0.199	0.189				
2.5	0.182	0.158	0.205				
1.25	0.194	0.164	0.200				
0.63	0.192	0.160	0.181				
0.31	0.217	0.222	0.203				
0.16	0.203	0.194	0.182				
0.08	0.183	0.182	0.170				
MeOH killed cells	0.090	0.031	0.044				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	96.14	104.5	99.30	100.00	4.26	4.3	1.00
2.5	95.61	82.98	107.7	95.44	12.37	13.0	0.58
1.25	101.9	86.14	105.0	97.72	10.15	10.4	0.74
0.63	100.8	84.03	95.09	93.33	8.56	9.2	0.29
0.31	114.0	116.6	106.6	112.46	5.18	4.6	0.03
0.16	106.6	101.9	95.61	101.40	5.55	5.5	0.75
0.08	96.14	95.61	89.30	93.68	3.81	4.1	0.13
MeOH killed cells	-47.5	16.12	22.97	-2.83	38.90	1.00	0.01



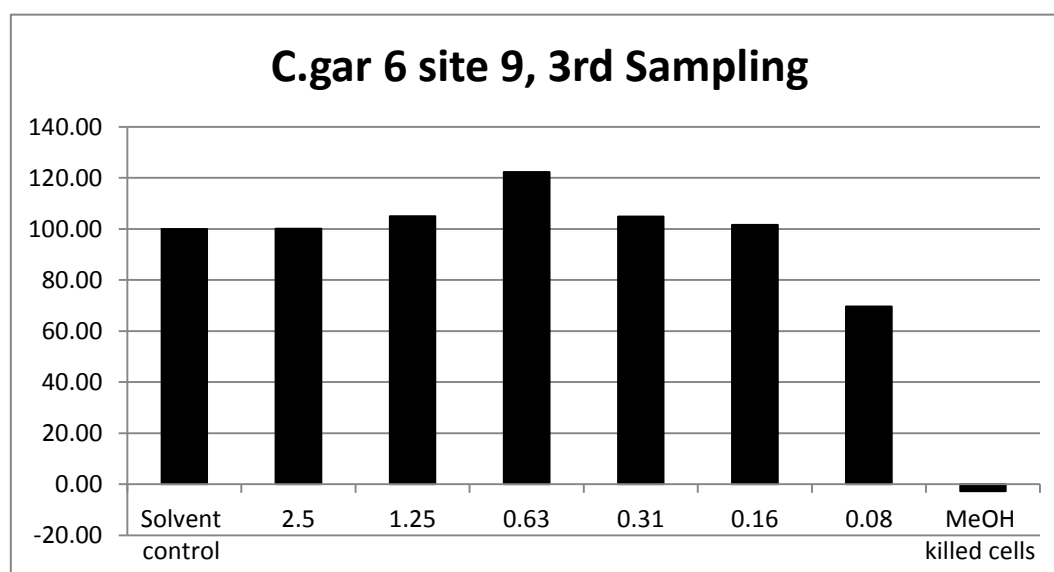
Absorbance reading from plate reader				C.gar 4 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.31	0.326	0.316				
2.5	0.291	0.282	0.639				
1.25	0.293	0.263	0.275				
0.63	0.25	0.221	0.224				
0.31	0.257	0.232	0.229				
0.16	0.254	0.26	0.239				
0.08	0.272	0.266	0.296				
MeOH killed cells	0.037	0.158	0.171	0.157	0.157	0.1	0.156
MeOH killed cells subtracted							
Solvent control	0.183	0.199	0.189				
2.5	0.164	0.155	0.512				
1.25	0.166	0.136	0.148				
0.63	0.123	0.094	0.097				
0.31	0.130	0.105	0.102				
0.16	0.127	0.133	0.112				
0.08	0.145	0.139	0.169				
MeOH killed cells	-0.090	0.031	0.044				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	96.14	104.5	99.30	100.00	4.26	4.3	1.00
2.5	86.14	81.40	269.3	145.62	107.16	73	0.50
1.25	87.19	71.40	77.71	78.77	7.95	10.	0.02
0.63	64.55	49.29	50.87	54.90	8.40	15	0.00
0.31	68.24	55.08	53.50	58.94	8.09	13	0.00
0.16	66.66	69.82	58.76	65.08	5.69	8.7	0.00
0.08	76.14	72.98	88.77	79.29	8.36	10	0.02
MeOH killed cells	-47.58	16.12	22.97	-2.83	38.90	1.0	0.01



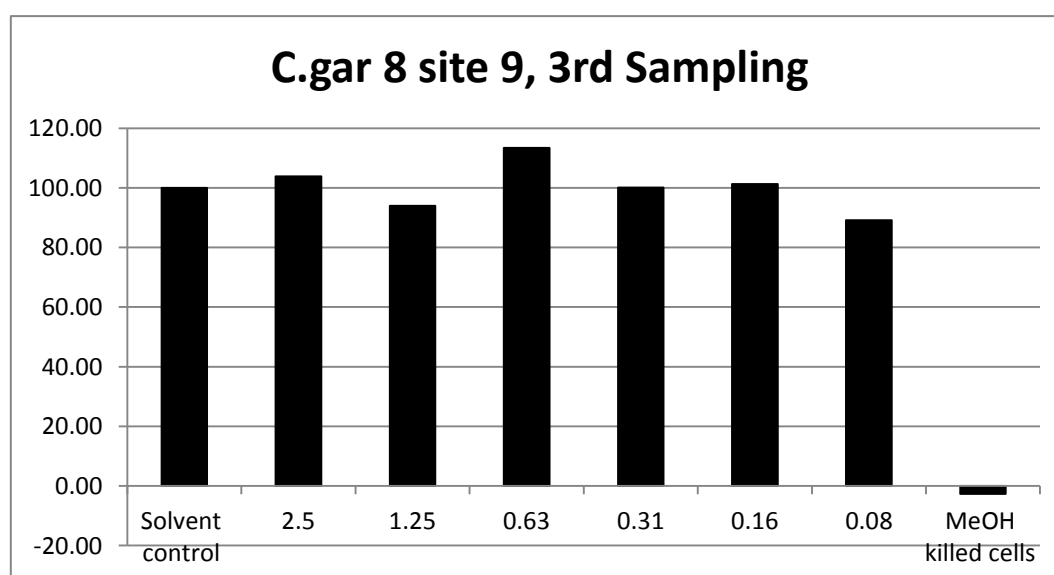
Absorbance reading from plate reader				C.gar 5 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.404	0.504	0.491				
2.5	0.364	0.462	0.512				
1.25	0.493	0.493	0.506				
0.63	0.46	0.444	0.489				
0.31	0.458	0.45	0.458				
0.16	0.438	0.495	0.463				
0.08	0.39	0.4	0.445				
MeOH killed cells	0.084	0.169	0.176	0.181	0.167	0.18	0.17
MeOH killed cells subtracted							
Solvent control	0.253	0.353	0.340				
2.5	0.213	0.311	0.361				
1.25	0.342	0.342	0.355				
0.63	0.309	0.293	0.338				
0.31	0.307	0.299	0.307				
0.16	0.287	0.344	0.312				
0.08	0.239	0.249	0.294				
MeOH killed cells	0.068	0.018	0.025				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	80.20	111.9	107.83	100.00	17.27	17.3	1.00
2.5	67.50	98.62	114.51	93.54	23.91	25.6	0.72
1.25	108.4	108.4	112.60	109.85	2.38	2.2	0.38
0.63	97.99	92.91	107.20	99.36	7.25	7.3	0.96
0.31	97.35	94.81	97.35	96.51	1.47	1.5	0.74
0.16	91.0	109.1	98.94	99.68	9.08	9.1	0.98
0.08	75.75	78.93	93.22	82.64	9.31	11.3	0.20
MeOH killed cells	21.44	5.56	7.78	-2.70	16.27	1.00	0.00



Absorbance reading from plate reader				C.gar 6 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.404	0.504	0.491				
2.5	0.508	0.463	0.429				
1.25	0.46	0.506	0.48				
0.63	0.532	0.532	0.545				
0.31	0.5	0.514	0.431				
0.16	0.478	0.454	0.482				
0.08	0.476	0.187	0.449				
MeOH killed cells	0.084	0.169	0.176	0.181	0.167	0.18	0.17
MeOH killed cells subtracted							
Solvent control	0.253	0.353	0.340				
2.5	0.357	0.312	0.278				
1.25	0.309	0.355	0.329				
0.63	0.381	0.381	0.394				
0.31	0.349	0.363	0.280				
0.16	0.327	0.303	0.331				
0.08	0.325	0.036	0.298				
MeOH killed cells	0.068	0.018	0.025				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	80.20	111.9	107.83	100.00	17.27	17.3	1.00
2.5	113.2	98.94	88.14	100.11	12.59	12.6	0.99
1.25	97.99	112.6	104.34	104.98	7.33	7.0	0.67
0.63	120.8	120.8	124.99	122.23	2.38	2.0	0.09
0.31	110.6	115.1	88.78	104.87	14.11	13.5	0.72
0.16	103.7	96.08	104.98	101.59	4.81	4.7	0.89
0.08	103.0	11.28	94.49	69.61	50.70	72.8	0.38
MeOH killed cells	21.44	5.56	7.78	-2.70	16.27	1.00	0.00



Absorbance reading from plate reader				C.gar 8 Site 9 3rd Sampling			
Raw data	1	2	3				
Solvent control	0.404	0.504	0.491				
2.5	0.523	0.46	0.452				
1.25	0.432	0.476	0.434				
0.63	0.509	0.514	0.503				
0.31	0.514	0.478	0.408				
0.16	0.451	0.486	0.474				
0.08	0.436	0.456	0.404				
MeOH killed cells	0.084	0.169	0.176	0.181	0.167	0.18	0.17
MeOH killed cells subtracted							
Solvent control	0.253	0.353	0.340				
2.5	0.372	0.309	0.301				
1.25	0.281	0.325	0.283				
0.63	0.358	0.363	0.352				
0.31	0.363	0.327	0.257				
0.16	0.300	0.335	0.323				
0.08	0.285	0.305	0.253				
MeOH killed cells	0.068	0.018	0.025				
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	80.20	111.9	107.83	100.00	17.27	17.3	1.00
2.5	118.0	97.99	95.45	103.81	12.35	11.9	0.77
1.25	89.09	103.0	89.73	93.97	7.89	8.4	0.61
0.63	113.5	115.1	111.65	113.45	1.75	1.5	0.25
0.31	115.1	103.7	81.47	100.11	17.12	17.1	0.99
0.16	95.13	106.2	102.44	101.27	5.65	5.6	0.91
0.08	90.37	96.72	80.20	89.09	8.33	9.4	0.38
MeOH killed cells	21.44	5.56	7.78	-2.70	16.27	1.00	0.00



8.3. Back calculations for AhR activity retrieved from Microsoft Excel 2010.

8.3.1. Acid wash

8.3.1.1. Reference linear curve as for 2nd sampling event April 2013.

TCDD pg/well	TCDD µg/ml	TCDD µg/well	TCDD g/l	TCDDMol per well	log TCDD	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
120	1.20E-04	4.80E-04	3.00E-11	3.73E-13	-3.31875	14186	12697	12883	13255.	811	6.12	100.00
30	3.00E-05	1.20E-04	7.50E-12	9.32E-14	-3.9208	11224	10997	10479	10900	38	3.50	82.23
7.5	7.50E-06	3.00E-05	1.88E-12	2.33E-14	-4.5228	8335	6725	8675	7911	1041	13.17	59.69
1.875	1.88E-06	7.50E-06	4.69E-13	5.82E-15	-5.1249	4443	4410	4095	4316	192	4.45	32.56
0.4668	4.67E-07	1.87E-06	1.17E-13	1.45E-15	-5.7286	2638	2104	2800	2514	364	14.49	18.97
0.1172	1.17E-07	4.68E-07	2.93E-14	3.64E-16	-6.3297	1254	1643	1440	1445.	194.	13.46	10.91

TCDD

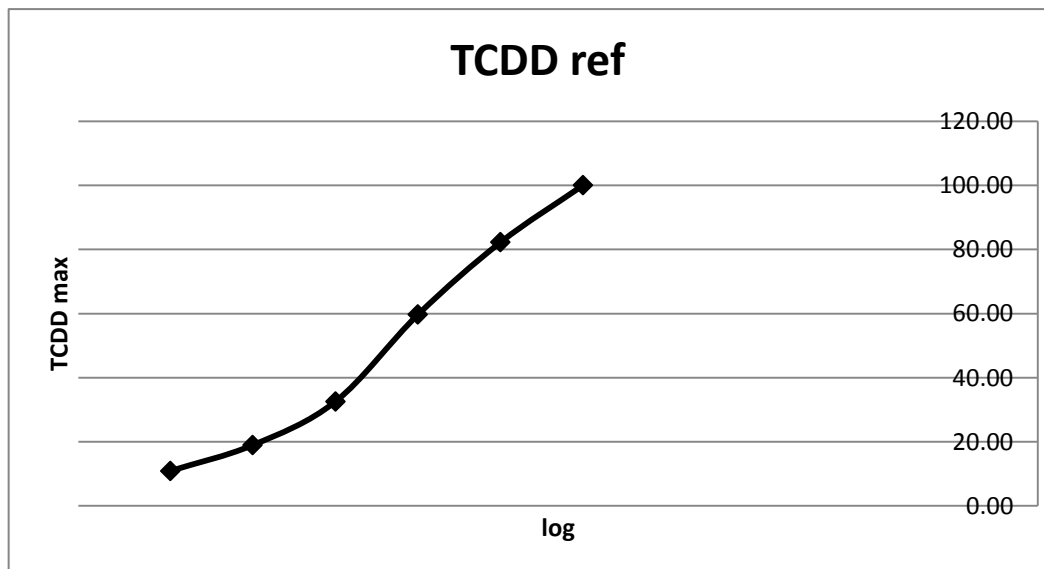
y=mx+c

intercept 226.30

slope 37.35

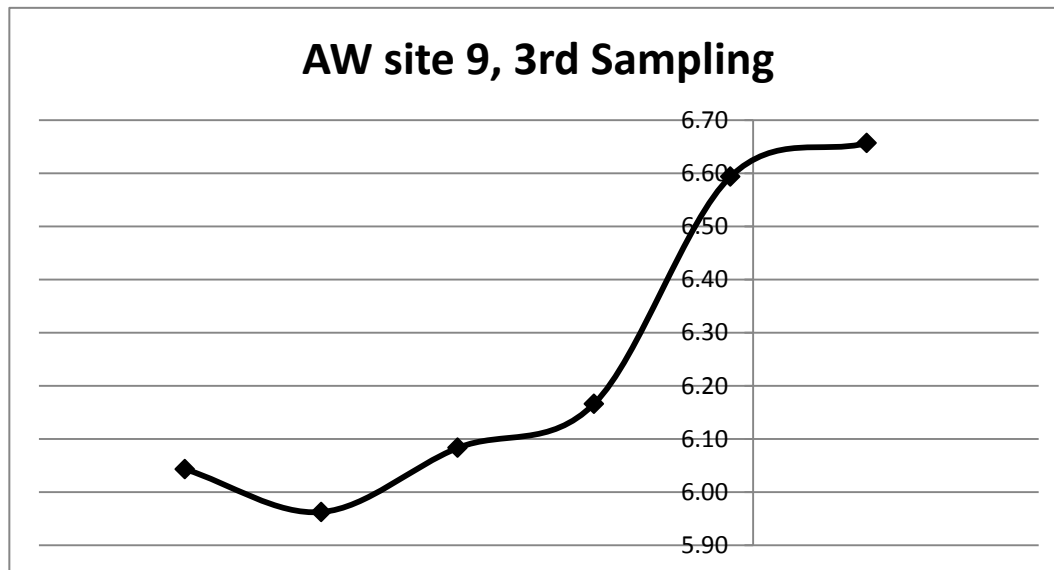
r2 0.71

	pg/well	µg/well
EC20	2.99E-06	2.99E-12
EC50	1.90E-05	1.90E-11
EC80	1.21E-04	1.21E-10



8.3.1.2.Activation calculations for April 2013 retrieved from Microsoft Excel 2010

AW Site 9, 3rd sampling (µℓ/well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	866	923	858	882.3333333	35.44479275	4.02	6.66
0.83	-7.92E-02	850	809	963	874	79.75587753	9.13	6.59
0.28	-5.56E-01	728	882	842	817.3333333	79.90828076	9.78	6.17
0.09	-1.03E+00	817	777	825	806.3333333	25.71640203	3.19	6.08
0.03	-1.51E+00	712	809	850	790.3333333	70.86842268	8.97	5.96
0.01	-1.99E+00	833	793	777	801	28.8444102	3.60	6.04



Mass sediment extracted
concentrated to 1 ml

40.04g

No Response

AW Site 90, 3rd sampling (μℓ/well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	2533	2622	2500	2552	63.10573138	2.47	19.25
0.83	-7.92E-02	1764	1465	1716	1648	160.5750084	9.74	12.44
0.28	-5.56E-01	1222	1149	1206	1192	38.37099599	3.22	9.00
0.09	-1.03E+00	1125	979	1230	1111	126.0568655	11.34	8.38
0.03	-1.51E+00	931	1020	923	958	53.8423625	5.62	7.23
0.01	-1.99E+00	963	1012	979	985	24.98666311	2.54	7.43

intercept 14.41

slope 10.75

r2 0.97

μℓ/well

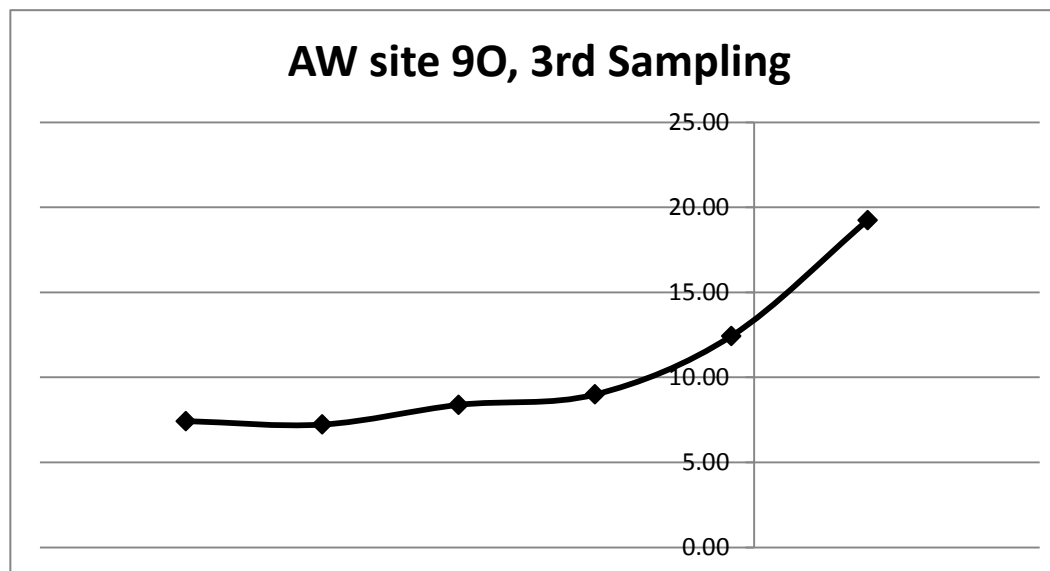
EC20 3.31E+00

EC50 2.05E+03

EC80 1.27E+06

	μg/μℓ	μg/mℓ
REP20	9.04E-13	9.04E-10
REP50	9.29E-15	9.29E-12
REP80	9.54E-17	9.54E-14

	TCDDeq μg/g
REP20	2.26E-11
REP50	2.32E-13
REP80	2.38E-15



Mass sediment extracted
40.06g
concentrated to 1 ml

AWSite 8, 3rd sampling($\mu\ell$ /well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	0.397940009	971	1109	1546	1208.666667	300.1771699	24.84	9.12
0.83	-0.079181246	1133	914	1052	1033	110.7293999	10.72	7.79
0.28	-0.556302501	939	1012	939	963.3333333	42.14656965	4.38	7.27
0.09	-1.033423755	801	793	761	785	21.16601049	2.70	5.92
0.03	-1.51054501	704	793	890	795.6666667	93.02866942	11.69	6.00
0.01	-1.987666265	672	720	785	725.6666667	56.71272638	7.82	5.47

intercept 8.21

slope 1.94

r2 0.94

$\mu\ell$ /well

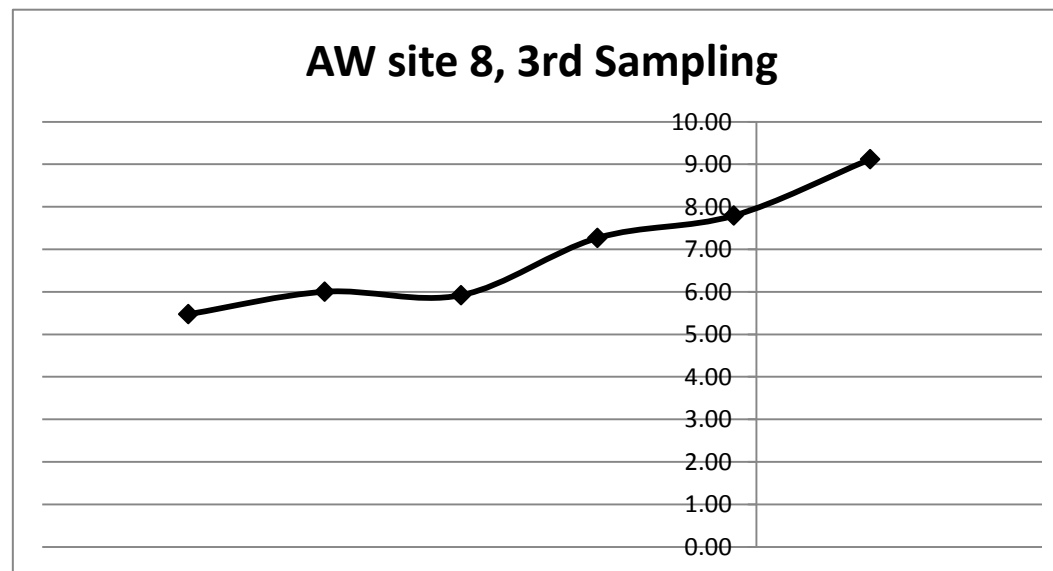
EC20 1.19E+06

EC50 3.50E+21

EC80 1.03E+37

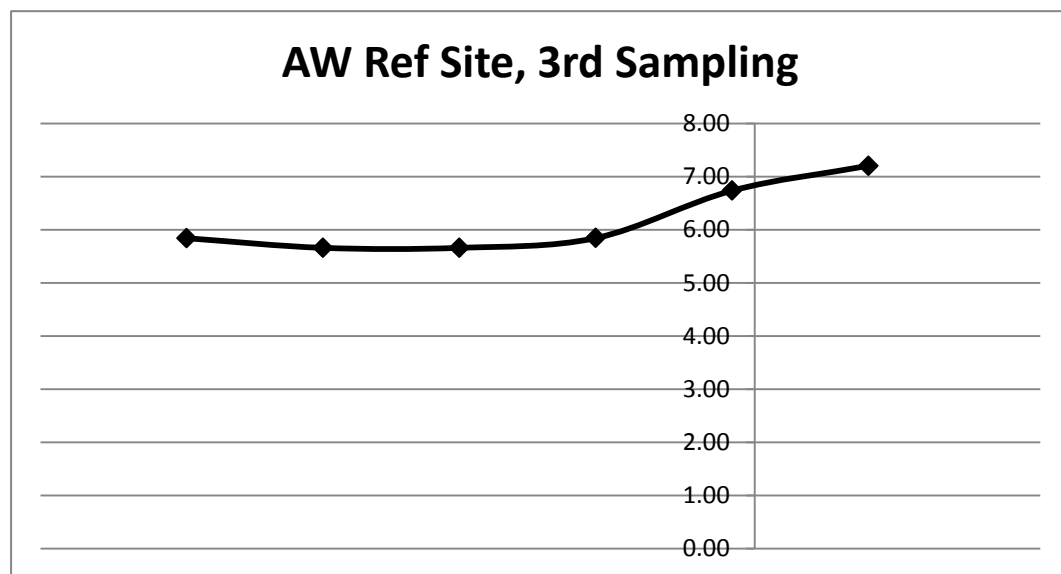
	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	2.51E-18	2.51E-15
REP50	5.44E-33	5.44E-30
REP80	1.18E-47	1.18E-44

	TCDDeq $\mu\text{g}/\text{g}$
REP20	6.26E-17
REP50	1.36E-31
REP80	2.94E-46



Mass sediment extracted
40.24g
concentrated to 1 ml

AW REF Site, 3rd sampling($\mu\text{g}/\text{well}$)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	858	1003	1003	954	83.71578903	8.77	7.20
0.83	-7.92E-02	874	801	1003	892	102.285548	11.46	6.73
0.28	-5.56E-01	785	761	777	774	12.22020185	1.58	5.84
0.09	-1.03E+00	696	793	761	750	49.42671343	6.59	5.66
0.03	-1.51E+00	672	785	793	750	67.66830868	9.02	5.66
0.01	-1.99E+00	801	728	793	774	40.03748244	5.17	5.84



Mass sediment extracted
concentrated to 1 ml

40.26g

No Response

8.3.2. Non Acid wash

Reference linear curve as for 2nd sampling season April 2013.

TCDD pg/well	TCDD µg/mℓ	TCDD µg/well	TCDD g/ℓ	TCDD Mol per well	log TCDD	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
120	1.20E-04	4.80E-04	3.00E-11	3.73E-13	-3.318758	14186	12697	12883	13255.	811	6.12	100.00
30	3.00E-05	1.20E-04	7.50E-12	9.32E-14	-3.92088	11224	10997	10479	10900	38	3.50	82.23
7.5	7.50E-06	3.00E-05	1.88E-12	2.33E-14	-4.52287	8335	6725	8675	7911	1041	13.17	59.69
1.875	1.88E-06	7.50E-06	4.69E-13	5.82E-15	-5.12493	4443	4410	4095	4316	192	4.45	32.56
0.4668	4.67E-07	1.87E-06	1.17E-13	1.45E-15	-5.72862	2638	2104	2800	2514	364	14.49	18.97
0.1172	1.17E-07	4.68E-07	2.93E-14	3.64E-16	-6.32975	1254	1643	1440	1445.	194.	13.46	10.91

TCDD

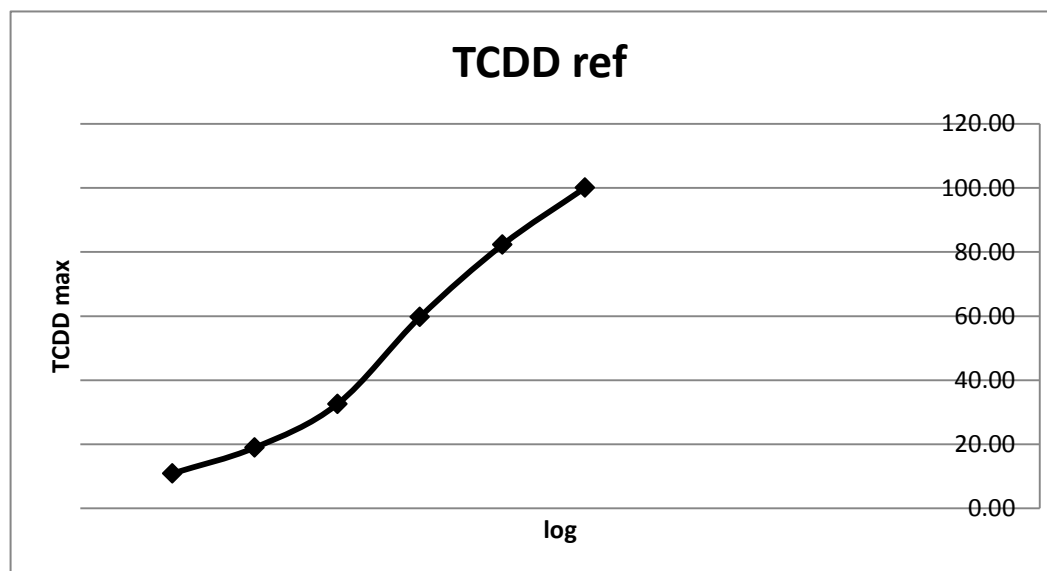
$y=mx+c$

intercept 226.30

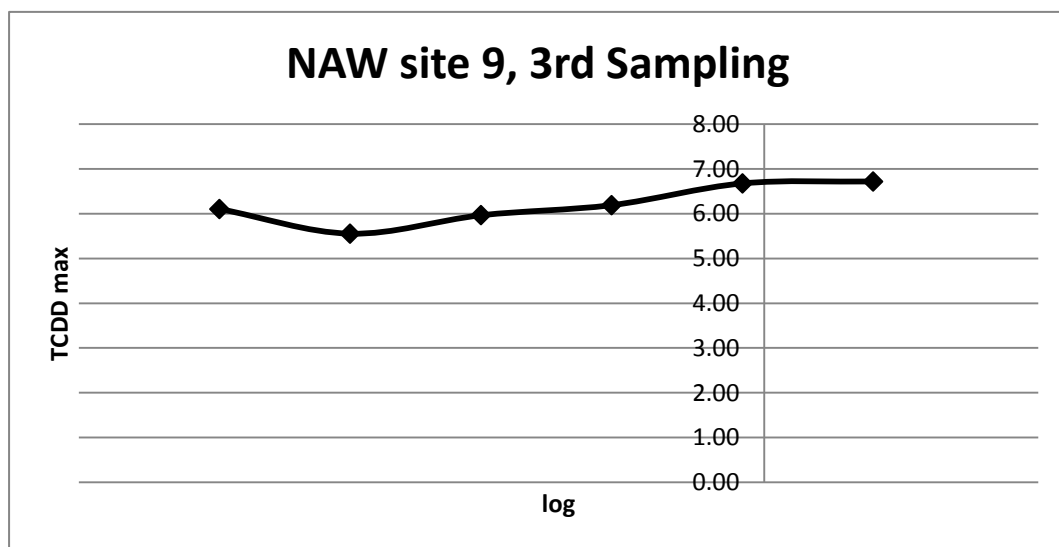
slope 37.35

r2 0.71

	pg/well	µg/well
EC20	2.99E-06	2.99E-12
EC50	1.90E-05	1.90E-11
EC80	1.21E-04	1.21E-10



NAW 9 (μℓ/well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	858	858	955	890.3333333	56.00297611	6.29	6.72
0.83	-7.92E-02	874	890	890	884.6666667	9.237604307	1.04	6.67
0.28	-5.56E-01	761	753	947	820.3333333	109.7694554	13.38	6.19
0.09	-1.03E+00	777	817	777	790.3333333	23.09401077	2.92	5.96
0.03	-1.51E+00	744	720	744	736	13.85640646	1.88	5.55
0.01	-1.99E+00	801	809	817	809	8	0.99	6.10



Mass sediment extracted
concentrated to 1 ml

40.04g

No Response

NAW 90($\mu\ell$ /well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	947	3504	5058	4281	1098.843938	25.67	32.30
0.83	-7.92E-02	1999	1764	3706	1881.5	166.1700936	8.83	14.19
0.28	-5.56E-01	2072	2015	1788	1958.333333	150.2409176	7.67	14.77
0.09	-1.03E+00	947	1287	1465	1233	263.1881456	21.35	9.30
0.03	-1.51E+00	1214	1092	1003	1103	105.9292217	9.60	8.32
0.01	-1.99E+00	1125	1133	1068	1108.666667	35.44479275	3.20	8.36

$y=mx+c$

intercept 21.88

slope 18.36

r2 0.73

$\mu\ell$ /well

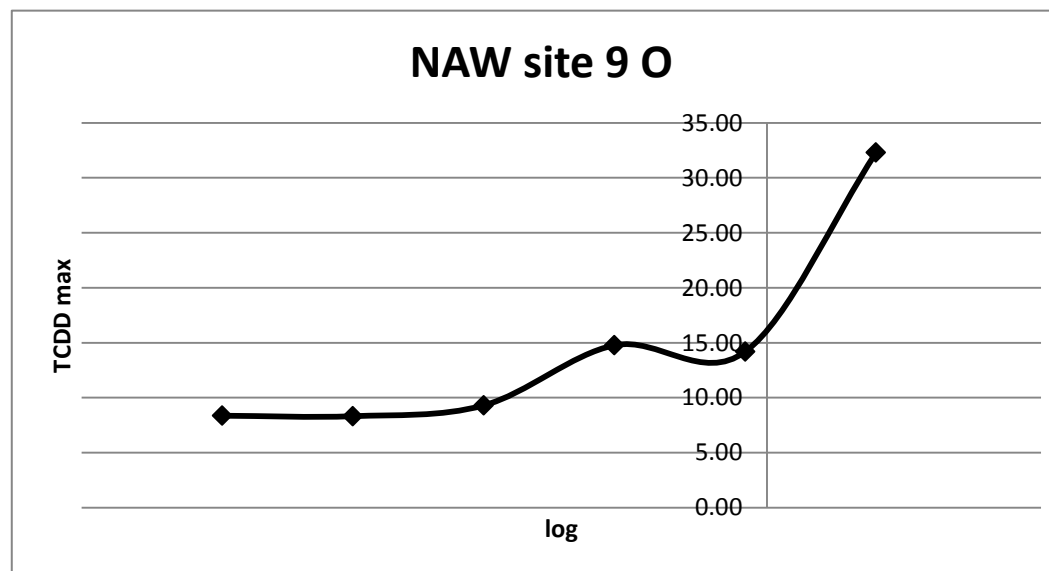
EC20 7.90E-01

EC50 3.40E+01

EC80 1.46E+03

	$\mu\text{g}/\mu\ell$	$\mu\text{g}/\text{m}\ell$
REP20	9.47E-13	9.47E-10
REP50	1.40E-13	1.40E-10
REP80	2.07E-14	2.07E-11

	TCDDeq $\mu\text{g}/\text{g}$
REP20	2.37E-11
REP50	3.49E-12
REP80	5.16E-13



Mass sediment extracted
concentrated to 1 ml

40.05

Standard deviation and Mean of each individual RLU											
RLU	TCDDmax										
947	7.14	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g	REP20	
1999	15.08	intercept	11.91	EC20	1.23E-01	REP 20	6.07E-12	6.07E-09	1.52E-10	Mean	STEDV
2072	15.63	slope	-8.89	EC50	5.22289E-05	REP 50	9.11E-08	9.11E-05	2.28E-06	6.83E-11	7.35E-11
947	7.14	r2	0.80	EC80	2.21257E-08	REP 80	1.37E-03	1.37E+00	3.41E-02		
1214	9.16									REP50	
1125	8.49									Mean	STEDV
RLU	TCDDmax									1.31E-06	7.58E-07
3504	26.43	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
1764	13.69	intercept	15.81	EC20	1.44E+00	REP 20	5.22E-13	5.22E-10	1.30E-11	REP80	
2015	15.64	slope	26.71	EC50	1.91E+01	REP 50	2.50E-13	2.50E-10	6.23E-12	Mean	STEDV
1287	9.99	r2	1.00	EC80	2.53E+02	REP 80	1.19E-13	1.19E-10	2.98E-12	0.01	0.02
1092	8.48										
1133	8.79										
RLU	TCDDmax										
5058	38.16	y=mx+c			μℓ/well		μg/μℓ	μg/mℓ	E ₂ eq μg/g		
3706	27.96	intercept	28.58	EC20	4.66E-01	REP 20	1.61E-12	1.61E-09	4.02E-11		
1788	13.49	slope	25.85	EC50	6.74E+00	REP 50	7.06E-13	7.06E-10	1.76E-11		
1465	11.05	r2	0.99	EC80	9.75E+01	REP 80	3.10E-13	3.10E-10	7.75E-12		
1003	7.57										
1068	8.06										

NAW 8 (μℓ/well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	3423	2897	2679	2788	154.1492783	5.53	21.03
0.83	-7.92E-02	1748	1894	2743	1821	103.2375901	5.67	13.74
0.28	-5.56E-01	987	1287	1554	1420.5	188.7975106	13.29	10.72
0.09	-1.03E+00	1149	1368	1246	1254.333333	109.7375657	8.75	9.46
0.03	-1.51E+00	1028	906	1028	987.3333333	70.43673284	7.13	7.45
0.01	-1.99E+00	1012	1109	1109	1076.666667	56.00297611	5.20	8.12

y=mx+c

intercept 16.02

slope 10.81

r2 0.95

μℓ/well

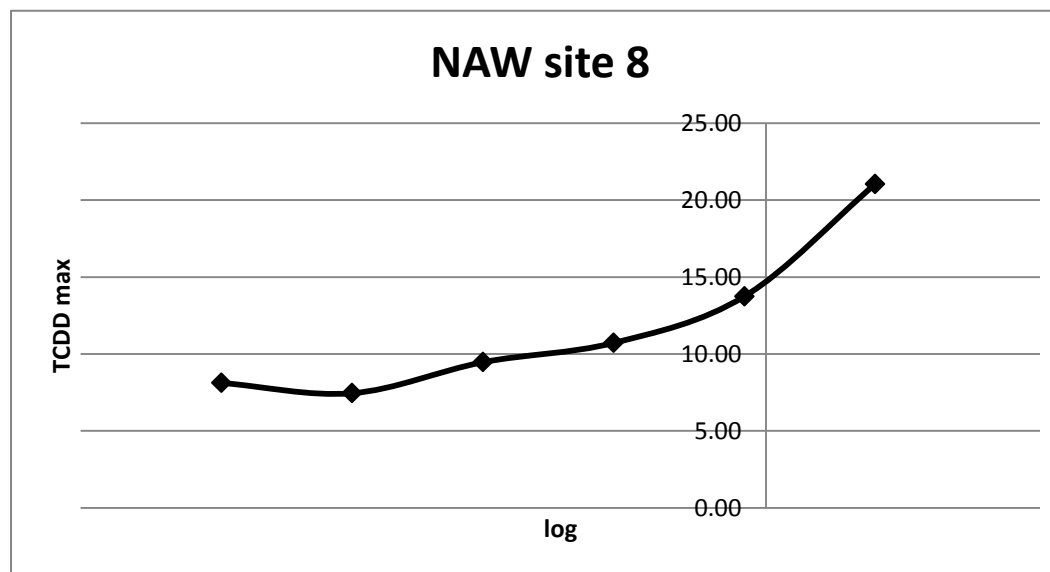
EC20 2.33E+00

EC50 1.39E+03

EC80 8.28E+05

	μg/μℓ	μg/mℓ
REP20	3.21E-13	3.21E-10
REP50	3.42E-15	3.42E-12
REP80	3.65E-17	3.65E-14

	TCDDeq μg/g
REP20	8.01E-12
REP50	8.55E-14
REP80	9.13E-16



Mass sediment extracted
concentrated to 1 ml

40.02

Standard deviation and Mean of each individual RLU											
RLU	TCDDmax										
3423	25.82	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/g	REP20	
1748	13.19	intercept	17.01	EC20	1.43E+00	REP 20	5.24E-13	5.24E-10	1.31E-11	Mean	STEDV
987	7.45	slope	19.26	EC50	5.16E+01	REP 50	9.22E-14	9.22E-11	2.30E-12	1.16E-11	1.58E-12
1149	8.67	r2	0.96	EC80	1.87E+03	REP 80	1.62E-14	1.62E-11	4.05E-13		
1028	7.76									REP50	
1012	7.63									Mean	STEDV
RLU	TCDDmax									1.18E-12	9.88E-13
2897	21.86	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/g		
1894	14.70	intercept	15.89	EC20	1.88E+00	REP 20	3.98E-13	3.98E-10	9.94E-12	REP80	
1287	9.99	slope	14.99	EC50	1.88E+02	REP 50	2.53E-14	2.53E-11	6.31E-13	Mean	STEDV
1368	10.62	r2	1.00	EC80	1.89E+04	REP 80	1.60E-15	1.60E-12	4.00E-14	0.014	2.10E-02
906	7.03										
1109	8.61										
RLU	TCDDmax										
2679	20.21	y=mx+c			µl/well		µg/µl	µg/ml	E ₂ eq µg/g		
2743	20.69	intercept	18.25	EC20	1.57E+00	REP 20	4.76E-13	4.76E-10	1.19E-11		
1554	11.72	slope	8.89	EC50	3.72E+03	REP 50	1.28E-15	1.28E-12	3.20E-14		
1246	9.40	r2	0.71	EC80	8.77E+06	REP 80	3.45E-18	3.45E-15	8.61E-17		
1028	7.76										
1109	8.37										

NAW REF Site, 3rd sampling (μℓ/well)	log	RLU	RLU	RLU	mean	stdev	cv	TCDDmax
2.50	3.98E-01	9152	1602	1368	1485	165.4629868	11.14	11.20
0.83	-7.92E-02	4256	1133	882	1007.5	177.4838021	17.62	7.60
0.28	-5.56E-01	2339	1020	947	983.5	51.61879503	5.25	7.42
0.09	-1.03E+00	1238	939	712	825.5	160.5132393	19.44	6.23
0.03	-1.51E+00	906	761	761	809.3	83.71578903	10.34	6.11
0.01	-1.99E+00	753	753	753	753	0	0.00	5.68

intercept 9.06

slope 3.96

r2 0.79

μℓ/well

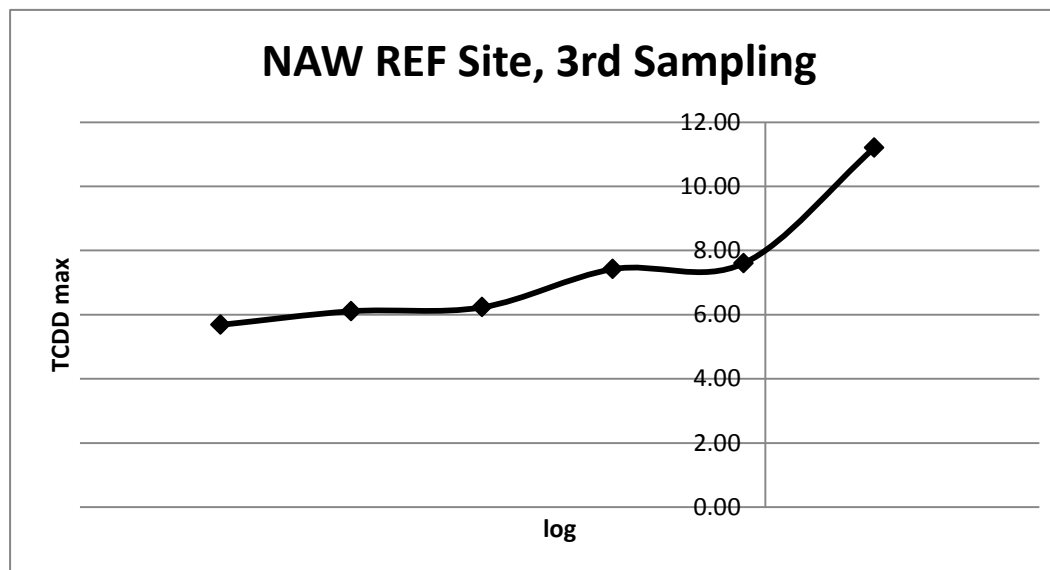
EC20 5.76E+02

EC50 2.12E+10

EC80 7.83E+17

	μg/μℓ	μg/mℓ
REP20	1.30E-15	1.30E-12
REP50	2.24E-22	2.24E-19
REP80	3.86E-29	3.86E-26

	TCDDeq μg/g
REP20	3.23E-14
REP50	5.57E-21
REP80	9.60E-28



Mass sediment extracted
40.27g
concentrated to 1 ml

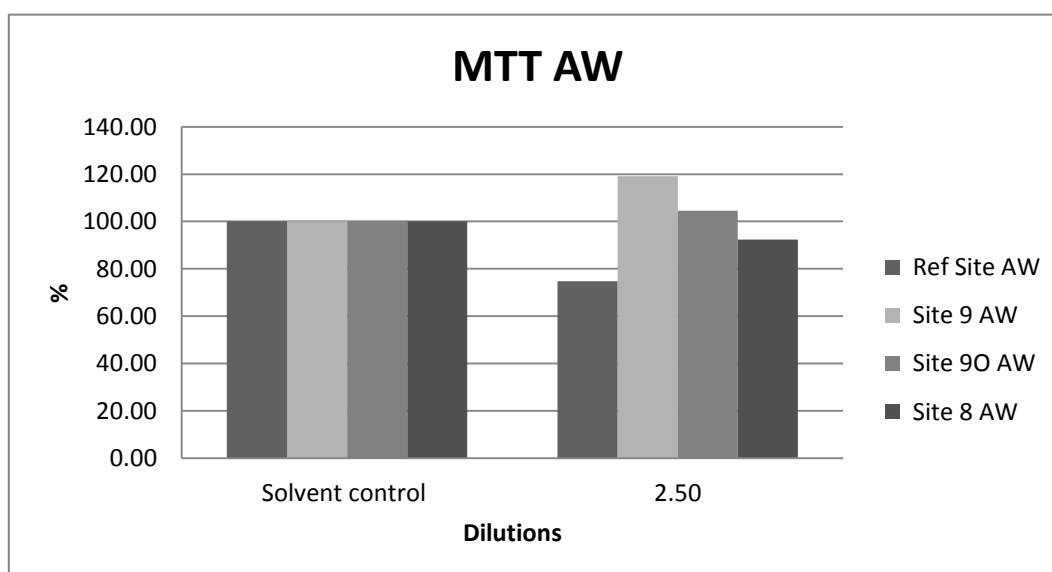
8.3.3.MTT calculations for April 2013 retrieved for Microsoft Excel 2010

Absorbance reading from plate reader				Site 9 AW			
Raw data	1	2	3				
Solvent control	0.862	0.565	0.564				
2.50	0.753	0.785	0.815				
0.83	0.555	0.551	0.551				
0.28	0.559	0.553	0.506				
0.09	0.523	0.472	0.508				
0.03	0.463	0.477	0.497				
0.01	0.664	0.57	0.686				
MeOH killed cells	0.034	0.029	0.034	0.037	0.027	0.03	
MeOH killed cells subtracted							
Solvent control	0.829	0.532	0.531				
2.50	0.720	0.752	0.782				
0.83	0.522	0.518	0.518				
0.28	0.526	0.520	0.473				
0.09	0.490	0.439	0.475				
0.03	0.430	0.444	0.464				
0.01	0.631	0.537	0.653				
MeOH killed cells	0.001	-0.004	0.001	0.004	-0.006	0.02	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	131.4	84.36	84.20	100.00	27.22	27.2	1
2.50	114.1	119.2	123.98	119.12	4.91	4.1	0.30
0.83	82.78	82.14	82.14	82.36	0.37	0.4	0.32
0.28	83.41	82.46	75.01	80.30	4.60	5.7	0.28
0.09	77.71	69.62	75.33	74.22	4.15	5.6	0.18
0.03	68.20	70.42	73.59	70.73	2.71	3.8	0.14
0.01	100.0	85.16	103.54	96.25	9.76	10.1	0.83
MeOH killed cells	0.21	-0.58	0.21	-0.05	0.46	1.00	0.00

Absorbance reading from plate reader				Site 90 AW			
Raw data	1	2	3				
Solvent control	0.862	0.565	0.564				
2.50	0.494	0.623	0.96				
0.83	0.561	0.37	0.631				
0.28	0.376	0.496	0.381				
0.09	0.312	0.394	0.192				
0.03	0.382	0.484	0.337				
0.01	0.601	0.783	0.224				
MeOH killed cells	0.034	0.029	0.034	0.037	0.027	0.035	
MeOH killed cells subtracted							
Solvent control	0.829	0.532	0.531				
2.50	0.461	0.590	0.927				
0.83	0.528	0.337	0.598				
0.28	0.343	0.463	0.348				
0.09	0.279	0.361	0.159				
0.03	0.349	0.451	0.304				
0.01	0.568	0.750	0.191				
MeOH killed cells	0.001	0.004	0.001	0.004	-0.006	0.002	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	131.4	84.36	84.20	100.00	27.22	27.2	1.000
2.50	73.11	93.56	146.9	104.54	38.13	36.5	0.875
0.83	83.73	53.46	94.82	77.34	21.41	27.7	0.320
0.28	54.41	73.43	55.20	61.01	10.76	17.6	0.082
0.09	44.27	57.26	25.25	42.26	16.10	38.1	0.034
0.03	55.36	71.53	48.23	58.37	11.94	20.4	0.072
0.01	90.07	118.9	30.32	79.77	45.18	56.6	0.543
MeOH killed cells	0.21	-0.58	0.21	-0.05	0.46	-866.0	0.003

Absorbance reading from plate reader				Site 8 AW			
Raw data	1	2	3				
Solvent control	0.797	0.767	0.889				
2.50	0.769	0.73	0.774				
0.83	0.832	0.598	0.534				
0.28	0.85	0.708	0.744				
0.09	0.784	0.798	0.746				
0.03	0.764	0.734	0.831				
0.01	1.013	0.861	0.956				
MeOH killed cells	0.024	0.025	0.035	0.032	0.033	0.031	
MeOH killed cells subtracted							
Solvent control	0.767	0.737	0.859				
2.50	0.739	0.700	0.744				
0.83	0.802	0.568	0.504				
0.28	0.820	0.678	0.714				
0.09	0.754	0.768	0.716				
0.03	0.734	0.704	0.801				
0.01	0.983	0.831	0.926				
MeOH killed cells	-0.006	0.005	0.005	0.002	0.003	0.001	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	97.38	93.57	109.0	100.00	8.07	8.1	1.00
2.50	93.82	88.87	94.46	92.38	3.06	3.3	0.20
0.83	101.82	72.11	63.99	79.31	19.92	25.1	0.17
0.28	104.10	86.08	90.65	93.61	9.37	10.0	0.42
0.09	95.73	97.50	90.90	94.71	3.42	3.6	0.35
0.03	93.19	89.38	101.6	94.75	6.30	6.7	0.42
0.01	124.80	105.5	117.5	115.95	9.75	8.4	0.09
MeOH killed cells	-0.76	-0.63	0.63	-0.25	0.77	-304.1	0.00

Absorbance reading from plate reader				Ref Site AW			
Raw data	1	2	3				
Solvent control	0.862	0.565	0.564				
2.50	0.17	0.703	0.639				
0.83	0.673	0.57	0.553				
0.28	0.665	0.616	0.463				
0.09	0.294	0.635	0.475				
0.03	0.645	0.537	0.545				
0.01	0.792	0.714	0.607				
MeOH killed cells	0.034	0.029	0.034	0.027	0.037	0.035	
MeOH killed cells subtracted							
Solvent control	0.829	0.532	0.531				
2.50	0.137	0.670	0.606				
0.83	0.640	0.537	0.520				
0.28	0.632	0.583	0.430				
0.09	0.261	0.602	0.442				
0.03	0.612	0.504	0.512				
0.01	0.759	0.681	0.574				
MeOH killed cells	0.001	-0.004	0.001	-0.006	0.004	0.02	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	131.	84.36	84.20	100.00	27.22	27.2	1.00
2.50	21.76	106.2	96.09	74.70	46.12	61.7	0.45
0.83	101.4	85.16	82.46	89.70	10.29	11.5	0.57
0.28	100.2	92.45	68.20	86.95	16.70	19.2	0.51
0.09	41.42	95.46	70.10	68.99	27.04	39.2	0.23
0.03	97.04	79.93	81.19	86.05	9.54	11.1	0.44
0.01	120.3	107.9	91.02	106.44	14.72	13.8	0.73
MeOH killed cells	0.21	-0.58	0.21	-0.05	0.46	1.00	0.31



Absorbance reading from plate reader				Site 9 NAW			
Raw data	1	2	3				
Solvent control	0.614	0.55	0.53				
2.50	0.214	0.677	0.575				
0.83	0.462	0.618	0.547				
0.28	0.403	0.567	0.545				
0.09	0.556	0.582	0.542				
0.03	0.512	0.543	0.512				
0.01	0.65	0.648	0.612				
MeOH killed cells	0.032	0.033	0.038	0.042	0.038	0.049	
MeOH killed cells subtracted							
Solvent control	0.575	0.511	0.491				
2.50	0.175	0.638	0.536				
0.83	0.423	0.579	0.508				
0.28	0.364	0.528	0.506				
0.09	0.517	0.543	0.503				
0.03	0.473	0.504	0.473				
0.01	0.611	0.609	0.573				
MeOH killed cells	-0.007	-0.006	-0.001	0.003	-0.001	0.01	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	109.3	97.21	93.41	100.00	8.34	8.3	1.00
2.50	33.33	121.3	101.96	85.55	46.25	54.1	0.62
0.83	80.48	110.1	96.64	95.75	14.85	15.5	0.69
0.28	69.26	100.4	96.26	88.66	16.92	19.1	0.36
0.09	98.35	103.3	95.69	99.11	3.86	3.9	0.88
0.03	89.99	95.88	89.99	91.95	3.40	3.7	0.20
0.01	116.2	115.8	109.00	113.69	4.07	3.6	0.06
MeOH killed cells	-1.27	-1.08	-0.13	-0.82	0.61	-74.2	0.00

Absorbance reading from plate reader				Site 90 NAW			
Raw data	1	2	3				
Solvent control	0.614	0.55	0.53				
2.50	0.393	0.578	0.436				
0.83	0.501	0.346	0.401				
0.28	0.394	0.476	0.391				
0.09	0.419	0.355	0.351				
0.03	0.556	0.332	0.579				
0.01	0.58	0.489	0.577				
MeOH killed cells	0.032	0.033	0.038	0.042	0.038	0.049	
MeOH killed cells subtracted							
Solvent control	0.575	0.511	0.491				
2.50	0.354	0.539	0.397				
0.83	0.462	0.307	0.362				
0.28	0.355	0.437	0.352				
0.09	0.380	0.316	0.312				
0.03	0.517	0.293	0.540				
0.01	0.541	0.450	0.538				
MeOH killed cells	-0.007	-0.006	-0.001	0.003	-0.001	0.01	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	109.3	97.21	93.41	100.00	8.34	8.3	1.00
2.50	67.36	102.5	75.54	81.81	18.41	22.5	0.19
0.83	87.90	58.43	68.88	71.74	14.94	20.8	0.0
0.28	67.55	83.14	66.98	72.56	9.17	12.6	0.
0.09	72.31	60.14	59.38	63.94	7.25	11.3	0.00
0.03	98.35	55.77	102.72	85.61	25.94	30.3	0.4
0.01	102.9	85.61	102.34	96.96	9.83	10.1	0.70
MeOH killed cells	-1.27	-1.08	-0.13	-0.82	0.61	1.00	0.

Absorbance reading from plate reader				Site 8 NAW			
Raw data	1	2	3				
Solvent control	0.614	0.55	0.53				
2.50	0.446	0.473	0.951				
0.83	0.348	0.462	0.202				
0.28	0.324	0.507	0.377				
0.09	0.368	0.381	0.193				
0.03	0.465	0.568	0.184				
0.01	0.502	0.403	0.221				
MeOH killed cells	0.032	0.033	0.038	0.042	0.038	0.049	
MeOH killed cells subtracted							
Solvent control	0.575	0.511	0.491				
2.50	0.407	0.434	0.912				
0.83	0.309	0.423	0.163				
0.28	0.285	0.468	0.338				
0.09	0.329	0.342	0.154				
0.03	0.426	0.529	0.145				
0.01	0.463	0.364	0.182				
MeOH killed cells	0.007	0.006	0.001	0.003	-0.001	0.010	
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	109.3	97.21	93.41	100.00	8.34	8.3	1.00
2.50	77.44	82.57	173.4	111.15	54.01	48.6	0.74
0.83	58.81	80.48	31.05	56.78	24.78	43.6	0.05
0.28	54.25	89.04	64.32	69.20	17.90	25.9	0.05
0.09	62.61	65.08	29.34	52.34	19.96	38.1	0.02
0.03	81.05	100.6	27.63	69.77	37.79	54.2	0.25
0.01	88.09	69.26	34.66	64.01	27.10	42.3	0.09
MeOH killed cells	-1.27	-1.08	-0.13	-0.82	0.61	-74.2	0.00

Absorbance reading from plate reader				Ref Site NAW			
Raw data	1	2	3				
Solvent control	0.797	0.767	0.889				
2.50	0.82	0.628	0.296				
0.83	0.633	0.788	0.736				
0.28	0.597	0.681	1.271				
0.09	0.602	0.778	0.327				
0.03	0.687	0.779	0.809				
0.01	0.72	0.708	0.344				
MeOH killed cells	0.024	0.025	0.035	0.032	0.033		0.3
MeOH killed cells subtracted							
Solvent control	0.767	0.737	0.859				
2.50	0.790	0.598	0.266				
0.83	0.603	0.758	0.706				
0.28	0.567	0.651	1.241				
0.09	0.572	0.748	0.297				
0.03	0.657	0.749	0.779				
0.01	0.690	0.678	0.314				
MeOH killed cells	-0.006	0.005	0.005	0.002	0.003		0.001
% of live cells	1	2	3	Average	stdev	CV	ttest
Solvent control	97.38	93.57	109.0	100.00	8.07	8.1	1.000
2.50	100.30	75.92	33.77	70.00	33.66	48	0.208
0.83	76.56	96.23	89.63	87.47	10.02	11	0.167
0.28	71.98	82.65	157.5	104.06	46.63	44	0.889
0.09	72.62	94.96	37.71	68.43	28.86	42	0.142
0.03	83.41	95.09	98.90	92.47	8.07	8.7	0.317
0.01	87.60	86.08	39.86	71.18	27.13	38	0.153
MeOH killed cells	-0.76	-0.63	0.63	-0.25	0.77	1.0	0.000

