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Potential microbial toxicity and non-target impact of different concentrations of glyphosate-containing herbicide (GCH) in a model pervious paving system.

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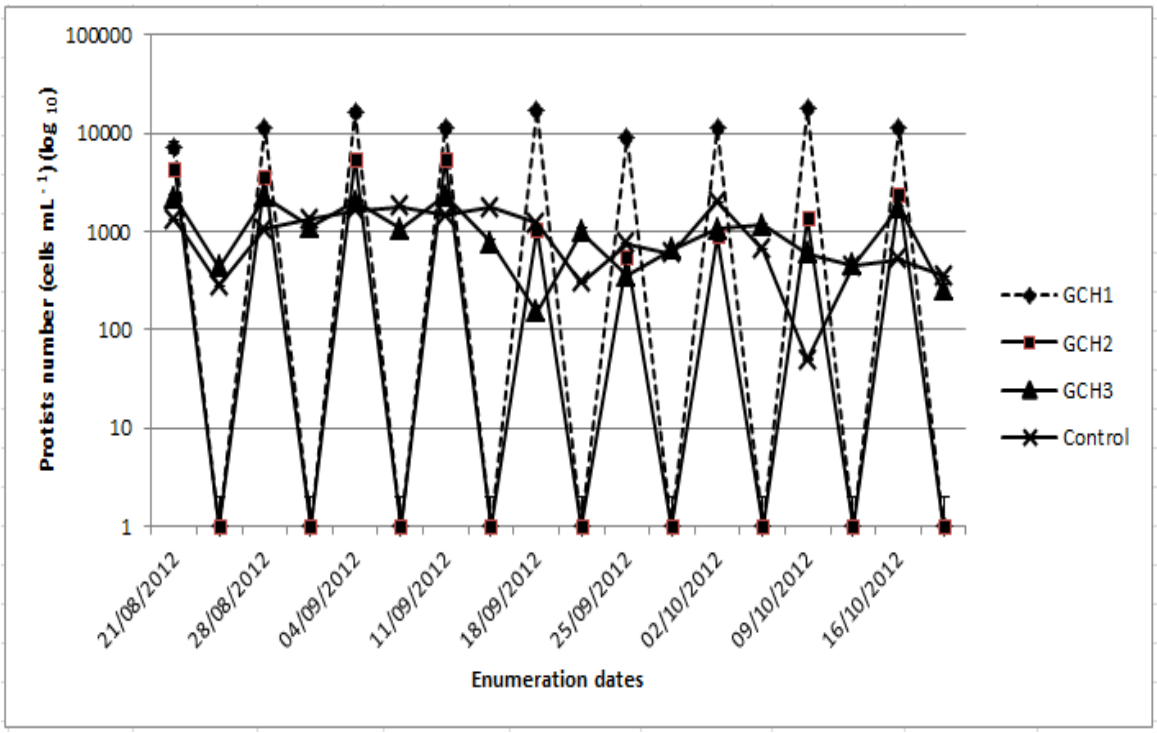
Abstract

Pervious Pavement Systems are Sustainable Drainage devices that meet the three-fold SUDS functions of stormwater quantity reduction, quality improvement and amenity benefits. This paper reports on a study to determine the impact of different concentrations of glyphosate-containing herbicides on non-target microorganisms and on the pollutant retention performance of PPS. The experiment was conducted using 0.0484 m^2 test rigs based on a four-layered design. Previous studies have shown that PPS can trap up to 98.7% of applied hydrocarbons, but results of this study show that application of glyphosate-containing herbicides affected this capability as 15%, 9% and 5% of added hydrocarbons were released by high (7,200 mg L^{-1}), medium (720 mg L^{-1}) and low (72 mg L^{-1}) glyphosate-containing herbicides concentrations respectively. The concentrations of nutrients released also indicate a potential for eutrophication if these effluents were to infiltrate into aquifers or be released into surface waters. The effect of glyphosate-containing herbicides application on the bacterial and fungal communities was slightly different; fungi exhibited a "top-down" trend as doses of 7,200 mg L^{-1} glyphosate-containing herbicides yielded the highest fungal growth whilst those with a concentration of 720 mg L^{-1} glyphosate-containing herbicides applied yielded the highest bacterial growth. In the case of protists, doses of glyphosate-containing herbicides above 72 mg L^{-1} were fatal, but they survived at the lower concentration, especially the ciliates *Colpoda cucullus* and *Colpoda steinii* thus indicating potential for their use as biomarkers of herbicide-polluted environments. Data also showed that at the lowest concentration of glyphosate-containing herbicides (72 mg L^{-1}), biodegradation processes may not be affected as all trophic levels required for optimum biodegradation of contaminants were present.

Key words: Glyphosate-containing herbicide; pervious paving; biofilm;

microbiology; Eutrophication; heavy metals.

Graphical Abstract



1. **Introduction**

Sustainable drainage systems (SUDS) are environmentally-friendly and cost-effective solutions which mimic natural drainage processes for storm peak and floodwater management through infiltration, storage and slow release into the environment (Charlesworth et al., 2003). These processes facilitate the filtration and treatment of contaminants found in floodwater as well as enhancement of groundwater recharge (Ellis et al., 2004). It is now established that SUDS is a better alternative to conventional drainage, offering more benefits to stormwater management (Pratt, et al., 1989; Fernandez-Barrera et al., 2010) including mitigation of climate change (Charlesworth, 2010).

In terms of SUDS devices, herbicides may well become part of a general maintenance strategy for the removal of nuisance plants particularly growing in association with PPS. Glyphosate-containing herbicides (GCH) are broad-spectrum, non-selective, post-emergence and are intensively used worldwide (Schuster and Gratzfeld-Husgen, 1992). However, due to the high solubility of glyphosate in water and its potential increase in usage as PPS becomes more accepted, its toxicological profile and ecological impacts have become a source of concern as pesticides have frequently been detected in groundwater, in excess of the EU maximum allowable concentration limit of 0.1 μg L^{-1} for individual pesticides (Ramwell et al. 2004). The half-life of glyphosate has been reported by Lund-Hoie and Friestad (1986) as 4 days, and the usual flow of water through a PPS is a matter of hours. Thus there is a likelihood that the complete breakdown of glyphosate is unlikely to occur before it is washed through the PPS and into the environment. This is therefore of environmental concern due to possible risk of GCH leaching down to aquifers and surface waters.

Geotextiles are often incorporated in the PPS structure, primarily to act as a barrier to the ingress of finer material near the surface into the coarser aggregate bedding layers below. However, one of the other functions of the geotextile, well-documented by Coupe et al., 2003; Newman et al., 2013, is that it acts as an *in-situ* bioreactor for the breakdown of contaminants carried in stormwater from the surface layers. This is carried out by trapping particulate-associated pollutants and also biodegradation of hydrocarbons facilitated by a biofilm, a microecosystem of microorganisms such as bacteria, fungi and protists, growing on the geotextile.

There are many studies of the toxicity of GCH on non-target organisms in the environment. Coupe and Smith (2006) demonstrated that GCH can significantly increase protozoan mortality in a PPS environment. Relyea (2005) found that the commercial GCH product, Roundup® caused high rates of mortality in several species of amphibians, Tsui and Chu (2003) attributed the toxicity of glyphosate to its acidity.

Previous work has focussed on the soil (Crisanto et al., 1994), aquatic systems (Tsui et al., 2005) and terrestrial environments (Mann and Bidwell, 1999; Relyea, 2005) but there are very few studies examining the impact of glyphosate application on PPS. No guideline currently exists for the concentration of commercially-available GCH being applied in amenity areas, landscapes in front gardens, footpaths, car parks etc. to ensure environmental conservation. The commercial formulation containing glyphosate as active ingredient is readily available in shops and supermarkets at concentrations ranging from 3600 mg L^{-1} to 8400 mg L^{-1}, although 7200 mg L^{-1} is most common.

The present study is the second part of an on-going research investigation which has already been reported in Mbanaso et al. 2012. The main aim of the present study was therefore to investigate the impacts of different concentrations of GCH to non-target organisms and the performance characteristics of the PPS. The objectives of the study include:

1. To determine whether there will be any apparent change in the effects of different concentrations of GCH on the bacterial and fungal community resident in the PPS.
2. To determine whether the observed negative effects of the GCH on the protists community (Mbanaso et al. 2012) is dependent on the concentration of GCH.
3. To determine whether the different concentrations of GCH will alter the long term and verified patterns of oil retention and biodegradation.

2. Methodology

Experiment was set-up using the 0.0484 m^2 test rigs (60 cm x 22 cm x 22 cm) previously described (Brownstein 1998; Bond 1999; Coupe 2004). The four test models were dosed with three concentrations of GCH and used oil as contaminants as follows:

Test model 1 (GCH1) = Oil + GCH (7200 mg L^{-1})

Test model 2 (GCH2) = Oil + GCH (720 mg L^{-1})

Test model 3 (GCH3) = Oil + GCH (72 mg L^{-1})

Test model 4 (Control) = Oil only

The rigs were set up 90 days prior to dosing with the contaminants to give enough time for the establishment of a biofilm (Davit, 2010).

2.1. Contaminants addition

The derived average oil loading based on a literature is 9.27 g m^2 $year^{-1}$, equivalent to 17.8 mg m^2 $week^{-1}$ (Bond, 1999) which equates to an oil application of 0.14 g $week^{-1}$ for the rig size. However, 1.4 g $week^{-1}$ was used in order to simulate a worse-case scenario. Prior to a rainfall event, used engine oil was added to the centre of the treated rig surfaces using a calibrated pipette.

About 10 ml of the proprietary GCH was added using a trigger gun for deploying the contents over the test area of the rig on weekly basis. This commercially-available product had no specified recommended application rate on the can. After application, the rigs were then left for 1 h for the contaminants to infiltrate into the rigs followed by a rainfall event.

2.2. Rainfall Simulation

Previous researchers (Brownstein, 1998; Bond, 1999; Coupe, 2004; Newman et al., 2006; Nnadi et al., 2012) consider gentle rainfall regime of 13 mm to represent a typical 'rain event' and this regime was adopted for this study. De-ionised water was applied to the rig surfaces by placing a plastic bowl, with 2 mm diameter holes drilled at 5 mm spaced intervals, over the rig surface and filling with water.

Effluent samples from the drainage point on each rig were collected after about 2 h to allow a representative sample to drain through. A separate sample was taken 1 h before the scheduled contamination and rainfall addition, to determine the extent of protist growth in the days following the previous contamination event.

2.3. Microbial Activity (gas monitoring)

Carbon dioxide evolution was carried out using infra-red gas analysis (IRGA model: ADC-225-MK3, UK) (Coupe et al., 2006). Equipment calibration was achieved with 3% ppm CO_2 at a standard volume of 0.2 ml.

2.4. Analysis

On collection of effluent samples, the following analyses were carried out: physical examination of effluent, microbiological examination, microscopic examinations for eukaryotes, metal concentration, hydrocarbon analysis and pH of effluent (Charlesworth et al. 2013). For statistical analysis of data, the means and standard error (SE) were determined using Microsoft Office Excel (2007 version). General Linear Model (univariate) on SPSS was used for the analysis of variance (ANOVA) while multiple comparisons of data sets were done by post-hoc tests (Tukey's honestly significant difference).

2.4.1. Physical examination

Effluent samples were examined for colour differences, particulates and sediments as well as other physical properties

2.4.2. Microbiological examination

This was done to determine the impact of GCH on the autochthonous microbes. Bacterial and fungal culturing and enumeration were carried out by direct plate count method (Page et al., 1982). Nutrient Agar (NA) was used for bacteria while, Rose-Bengal Chloramphenicol base Agar (RBC) was used for fungi due to its selectivity. Enumeration of both was done using an automatic colony counting and zone measuring instrument, PROTOCOL-2 scientific counter manufactured by Synbiosis (Synoptics Group) and supplied by Scientific Laboratory supplies, UK.

2.4.3. Microscopic examination for Eukaryotes

Haemocytometer and a light microscope were used for enumeration and identification of protists (Coupe et al., 2003). Using a pipette, effluent samples were dropped onto a glass slide or counting chamber and observed at 100 – 1000 x magnification using a Leica CME microscope. Identification was done as previously described (Lee et al. 2000; Patterson 1992).

2.4.4. Heavy metal concentration in effluent

The metal content of the effluent samples was determined by the use of an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Optima 5300DV© (Newman et al., 2011) supplied by Perkin Elmer, USA. Equipment calibration was accomplished by the use of standard concentrations which were made up using the 1000ppm analytical grade standards supplied by Fisher Scientific, UK.

2.4.5. Total hydrocarbon content

The was determined by infra-red spectroscopy using a Horiba OCMA 310 analyser (Coupe 2004; Nnadi 2009) supplied by Q Instrument Services Ltd, UK. The equipment uses S-316 trichlorotrifluoroethylene solvent to extract the oil components from samples which are then measured using infra-red spectroscopy. It can measure hydrocarbon concentrations in the range 0 – 200 mg L-1. Calibration of the equipment was achieved by combining the standard and S-316 solvent in a 2:1 ratio.

2.4.6. Potential of hydrogen (pH)

The pH, was determined using Fisherbrand Hydrus 300 pH meter (Mercer and Frostick, 2012), manufactured by Orion Research Inc, U.S.A. Prior to the

measurement of the samples, the equipment was calibrated using pH 4.00 and pH 7.00 buffers.

3. Results

3.1. Physical examination

Effluent from the control rig was clear without any oil sheen or sediments. However, the effluent from rigs which had GCH applied had levels of cloudiness related to the GCH concentration applied. Thus, those with the highest concentration of glyphosate (i.e. GCH1) were the cloudiest. In terms of observable oil sheen, it was very visible in the GCH1 sample as a thin layer over the effluent surface, with relatively less for GCH2 and 3. When the samples were agitated, there was build-up of foam in GCH1 with less so in GCH2 and less again in GCH3. The presence of foam in the effluent is thought to be due to the presence of surfactants such as polyoxyethylene alkylamine, (Benachour et al., 2009) incorporated in the GCH formulation. According to US EPA (1993) glyphosate binds tightly to most soils and therefore the potential for it to move through the soil to contaminate groundwater is low. Furthermore, once glyphosate is in contact with surface water, it is removed through mechanisms such as adsorption and degradation by microorganisms. Since glyphosate is strongly adsorbed to soil particles, which prevents it from excessive leaching (Tu et al. 2001), the degree of adsorption of glyphosate to PPS components is likely to be lower than in soils due to the lower available surface area.

3.2. *Microbiological examination via plate count for bacterial and fungal populations*

There was a progressive increase in the population of bacteria particularly in the models containing high (i.e. GCH1) and medium (GCH2) concentrations of GCH shortly after the contaminants were added (Fig. 1).

The first 5 weeks of monitoring established bacterial growth in the rigs before addition of any contaminant. During this period, growth in all the models progressed at a similar pace. The control rigs fluctuated after GCH addition, but were always lower in numbers than any of the other rigs in the same week. Weekly additions of the different GCH concentrations showed different trends in different models. For example, GCH2 significantly ($p < 0.05$) maintained greater numbers than those in the other rigs throughout the rest of the experiment. It was initially thought that since bacteria utilize GCH as nutrient source (Mbanaso et al., 2012), those from GCH1 would experience highest growth; however, it may be that the medium GCH concentration in GCH2 rigs provided more favourable condition. Amoros et al., (2007) observed an increase in *Aeromonas* counts when an aquatic system was treated with different concentrations (50 mg L^{-1} and 100 mg L^{-1}) of GCH. However, Moneke et al., (2010) recorded high cell densities for *Acetobacter* sp. and *P. fluorescens* at GCH concentrations of 7.2 mg L^{-1}, 25 mg L^{-1} and 50 mg L^{-1} but low densities at 100 mg L^{-1} and 250 mg L^{-1}. This suggests that although these microbes can tolerate high concentrations of GCH, it is likely that there is a favourable concentration at which optimum growth can be achieved, thus enhancing biodegradation rates.

Initially, fungal numbers in all the models were very similar in terms of trend with time as they all progressively increased. However, the highest growth in all the models was recorded in the week before the first contaminants were applied as is

shown in Fig 2. Shortly after contaminant addition, fungal counts reduced in all rigs, the control included, and numbers never recovered to pre-treatment conditions.

Unlike that displayed by bacteria (Fig 1), fungi exhibited a different trend (Fig 2) whereby the high concentration dose (GCH1) yielded the highest growth followed by GCH2 and then GCH3. Fungal growth in GCH1 was significantly ($p<0.05$) higher than that of GCH2 and GCH3. This may indicate that the toxicity of GCH for fungi in PPS is more pronounced than that for bacteria. Bacterial growth was significantly ($p < 0.05$) higher than fungal growth.

These findings are consistent with other studies based on the *soil* microbial community (Stratton and Stewart 1992; Haney *et al.* 2000; Busse et al., 2001) which found that microbial activity was stimulated in the presence of GCH. Wardle and Parkinson (1990a) found that the presence of glyphosate in soils temporarily increased populations and activities of soil bacteria whilst that of fungi was unaffected. Similarly, Ratcliff et al., (2006) reported that 100-times the field application rate of glyphosate to forest soils resulted in a substantial growth of bacteria with minimal change to the fungal community, thus culturable bacteria made up a greater proportion of the total microbial population and also accounted for greater carbon utilization. This change in community structure tends to support the proposal that the presence of labile substrates favours bacterial dominance while composite substrates (e.g. lignin) favour fungi (Wardle and Parkinson, 1990b; Bittman et al., 2005; Ratcliff et al., 2006). It is not known whether this is a short-lived response by the microbes in the PPS which eventually reverts to the pre-treatment population and activity levels once the GCH biodegrades, is adsorbed and dissipates and thus is a subject of further investigation in later phases of this study.

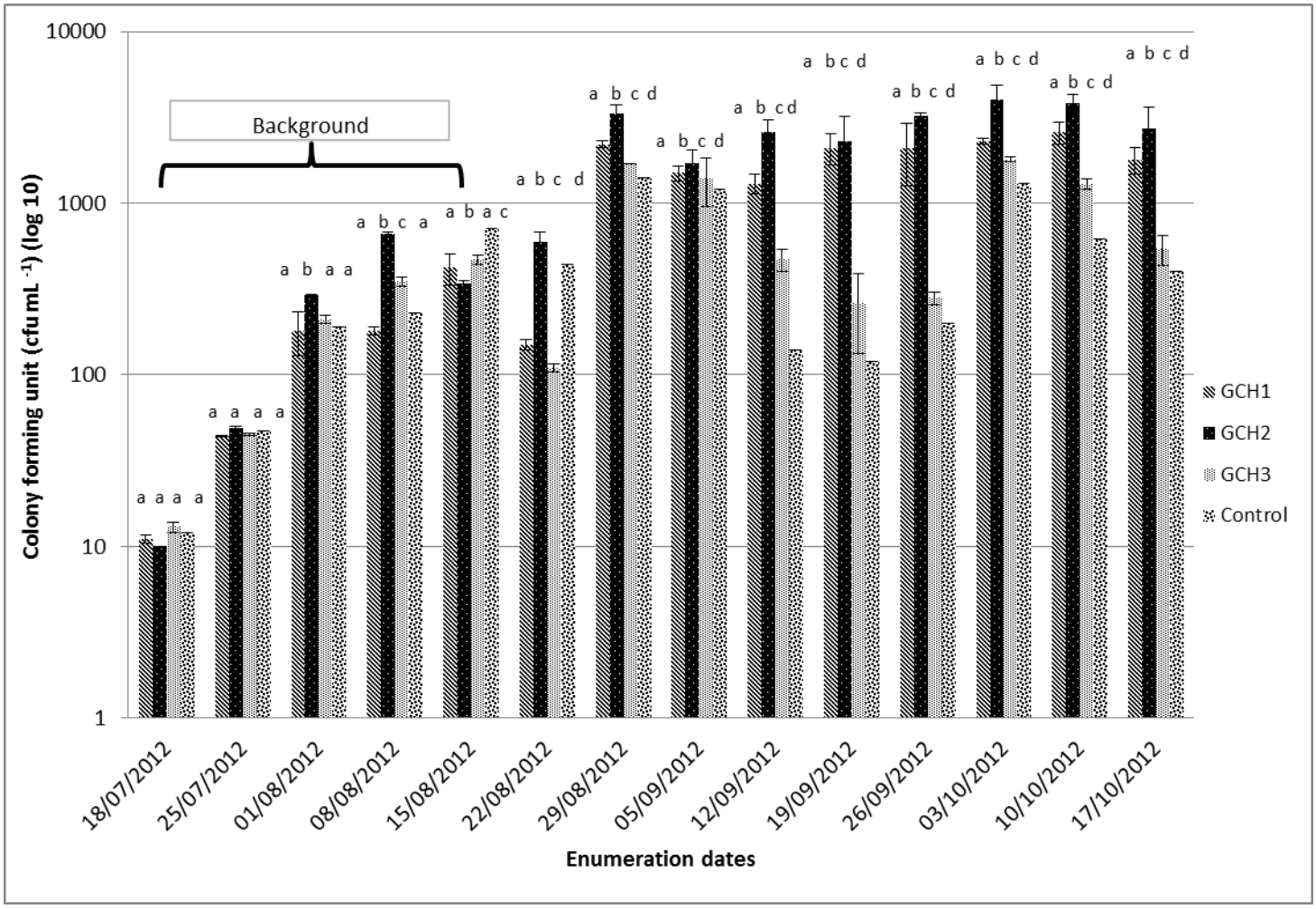


Fig.1: Effluent bacterial count: 18/07/12 to 15/08/12 were to establish background; contaminants were added on 21/08/12 with bacteria enumeration the following day, followed by weekly additions. The values shown above represent the mean ± S.E. (where n = 3), and for each date, dissimilar letters above bars indicate a significant difference ($p < 0.05$) based on post-hoc test (Tukey's HSD).

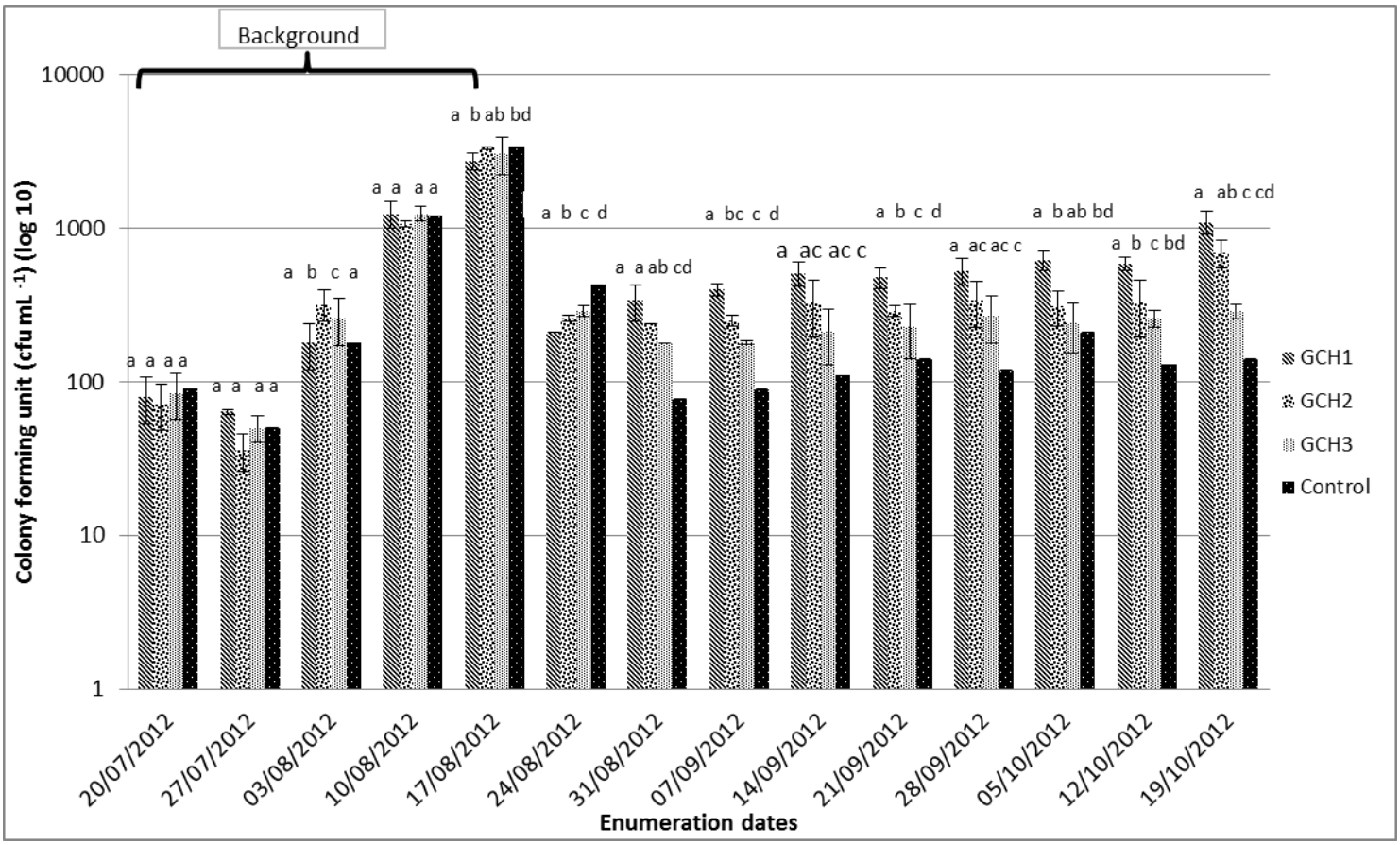


Fig.2: Effluent fungal count: 20/07/12 to 17/08/12 were to establish background; contaminants were added on 21/08/12 with fungal enumeration 3 days later, followed by further weekly additions. The values shown above represent the mean ± S.E. (where n = 3), and for each date, dissimilar letters above bars indicate a significant difference ($p < 0.05$) based on post-hoc test (Tukey's HSD).

3.3. Microscopic examination of protists

The different concentrations of GCH had varying degrees of impact on the protist community as shown in figure 3.

Application of the two highest concentrations of GCH to the models had an almost instantaneous effect on the protists as their population was immediately reduced by almost four orders of magnitude resulting in very few observable living organisms.

However, the protist community recovered after 7 days with growth in GCH1 averaging 25 % more than that of the GCH2 models and 30 % more than GCH3. The GCH3 and control models maintained similar growth trends throughout the duration of the experiment, neither of which exhibited the major fluctuations shown by the other rigs.

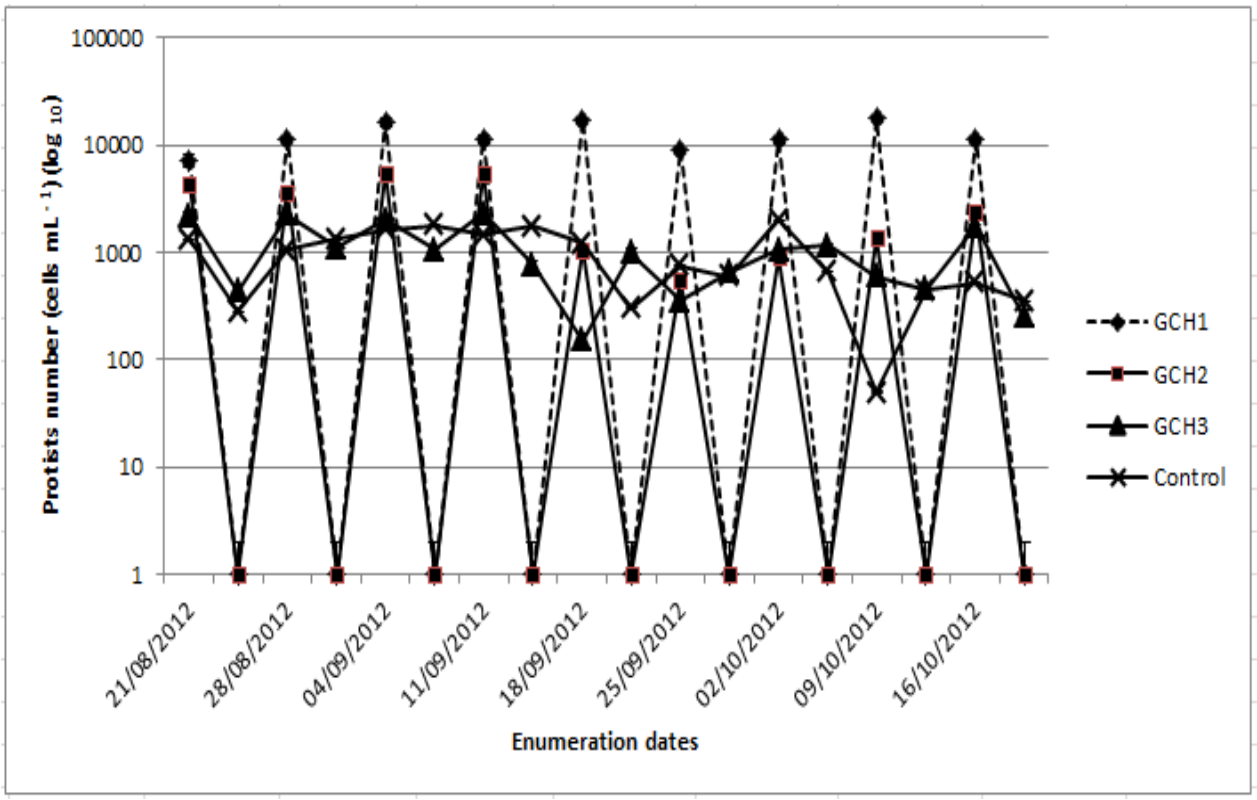


Fig. 3: Mean number of protists in effluent from test rigs. Peak heights indicate protist growth level prior to contaminant addition. Standard deviation was low, error bars are therefore not visible (n=3).

Figure 4 shows that, in terms of taxonomic richness, the control rigs averaged nearly 35 taxa (cumulatively) in the final weeks of the experiment. In comparison with this, rigs which had GCH applied had much lower diversity of species, with the GCH1 rigs being approximately half that of the control. Although the numbers of individual protists were highest in GCH1 7 days after application of the contaminants, only few

of the original taxa were able to survive, thus the high numbers represented few taxa. The lowest concentration GCH rig, GCH3, contained the most taxa recorded of the 3 concentrations, but was still approximately 75% of the amount found in the control.

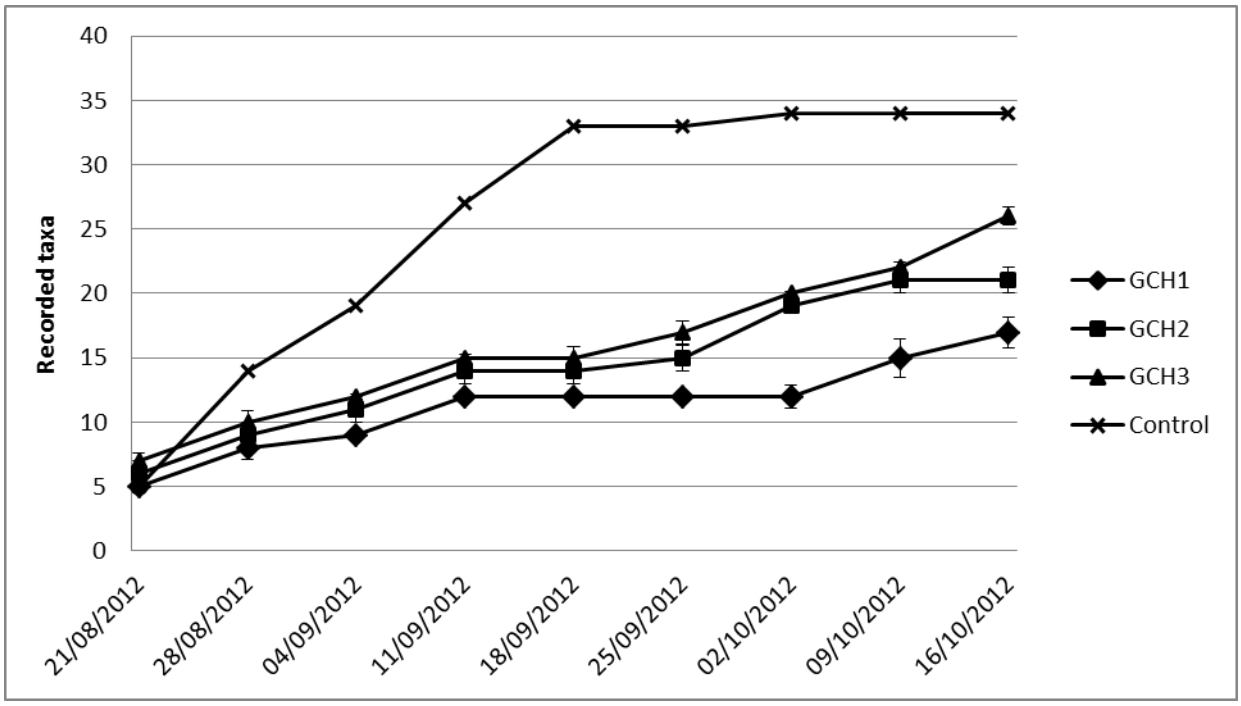


Fig.4: Cumulative taxonomic richness of protist at each sampling interval found in effluent from test rigs. Each data point is the mean of three replications.

The ciliates *Colpoda cucullus* and *Colpoda steinii* were found in relatively high numbers in the GCH3 models after the addition of contaminants and on many occasions, flagellates such as *Bodo*. There are a few other studies of the impacts of GCH on these protists: for example, Coupe et al., 2006 demonstrated lethal effects of low concentrations of GCH (at 100 mg L^{-1}) on *Colpoda cucullus*, and Pesce *et al.* (2009) found no significant effect at a concentration of 0.01 mg L^{-1}. Bonnet et al. (2007) found that the ciliates *T. pyriformis* were more sensitive during toxicity assessments than the bacteria *Vibrio fischeri*, and that using population growth was a

better measure than enzymatic criteria. Liu et al. (2007) reported that the taxonomic composition and population distribution of protozoa in municipal waste treatment plants (MWTPs) indicate that the ciliates are the predominant inhabitants in terms of diversity and abundance. In a similar study, Salvado *et al.* (1995), described ciliates as the most representative groups of protists in an activated sludge ecosystem. Population growth of ciliates may therefore have the potential to provide a biomarker of GCH and hydrocarbon polluted sites, since at lower concentrations of glyphosate, i.e. 72 mg L^{-1}, biodegradation processes overall may not be affected as all trophic levels required for optimum biodegradation (as suggested by Newman et al., 2002) were present.

3.4. CO_2 evolution

Results shows that CO_2 evolution, here used as a proxy for microbial activity and hence biodegradation rates (Heinonen-Tanski, 1989), steadily increased in GCH1, GCH2 and GCH3 models from 800, 543 and 457 ppm respectively to 1714, 914 and 886 ppm, more so in the GCH rigs in comparison with the control. The higher the concentration of applied GCH, the higher the microbial activity and hence higher biodegradation rates, with GCH1 in particular exhibiting almost double the rate of the control at any given sampling point throughout the study.

According to Carlisle and Trevor (1988), glyphosate may stimulate or inhibit microbes in the soil depending on the herbicide concentration and soil type. In a study of the PPS environment, Coupe et al., (2006) observed that at a concentration of 2400 mg GCH L^{-1}, degradation proceeded at a rapid rate with peak CO_2 evolution reached after 16 days. This was also corroborated by Wardle and Parkinson (1990a) who noted that the breakdown of glyphosate in soil is related to the evolution of CO_2.

3.5. Effluent nutrients concentration

The analysis of effluent from all the test models showed increasing concentration of nutrients i.e. those required by microorganisms for growth and metabolism, which ultimately affects biodegradation rates. Macronutrients such as phosphorous (P) and potassium (K) which are required in relatively large quantities, recorded an initial increase after the addition of contaminants especially in GCH1 and GCH2 models before subsequent decline in concentration levels. Phosphorus is given in Figure 5 as an example, but K exhibited similar trends with time. Maximum concentrations of up to 198 mg L^{-1} and 54 mg L^{-1} were recorded by GCH1 and GCH2 respectively before decreasing to 66 mg L^{-1} and 36 mg L^{-1} respectively. The GCH3 and Control models recorded lower concentrations with maximum recorded values of 6 mg L^{-1} and 1 mg L^{-1} respectively. Micronutrients including trace elements such as iron (Fe) and copper (Cu) which are required in relatively smaller amounts by microorganisms recorded similar trend (figure 6). Although the reason for the decline is not clearly understood, it is thought that it may be due to the increasing microbial population placing increasing demand for nutrients over time.

In biodegradation studies (Newman et al. 2011), the absence of nitrogen or phosphorous (or both) are the most common limiting nutrients which inhibit growth of biota required to breakdown contaminants. Nutrients in the PPS may have been obtained from 3 different sources:

1. Glyphosate itself can act as a source of P, N and K once adsorbed onto the surfaces of mineral or organic systems (Dick and Quinn, 1995).

2. The applied oil could be a source of carbon requirements; Newman et al. (2006) noted that nutrients such as P are present in waste oil. However, it is

not possible to distinguish between GCH and oil in terms of their importance as nutrient sources.

3. Coupe, 2004 demonstrated that the materials used in the construction of PPS can contain nutrients required for microbial growth and metabolism and Newman et al. (2010) commented that nutrients available in PPS structures may be sufficient to maintain long-term biodegrading activity without additional inputs of nutrients. However, such nutrients would have been present from the start of the experiment (Mbanaso et al. 2012), and would thus be represented in the background values recorded in the first 5 weeks.

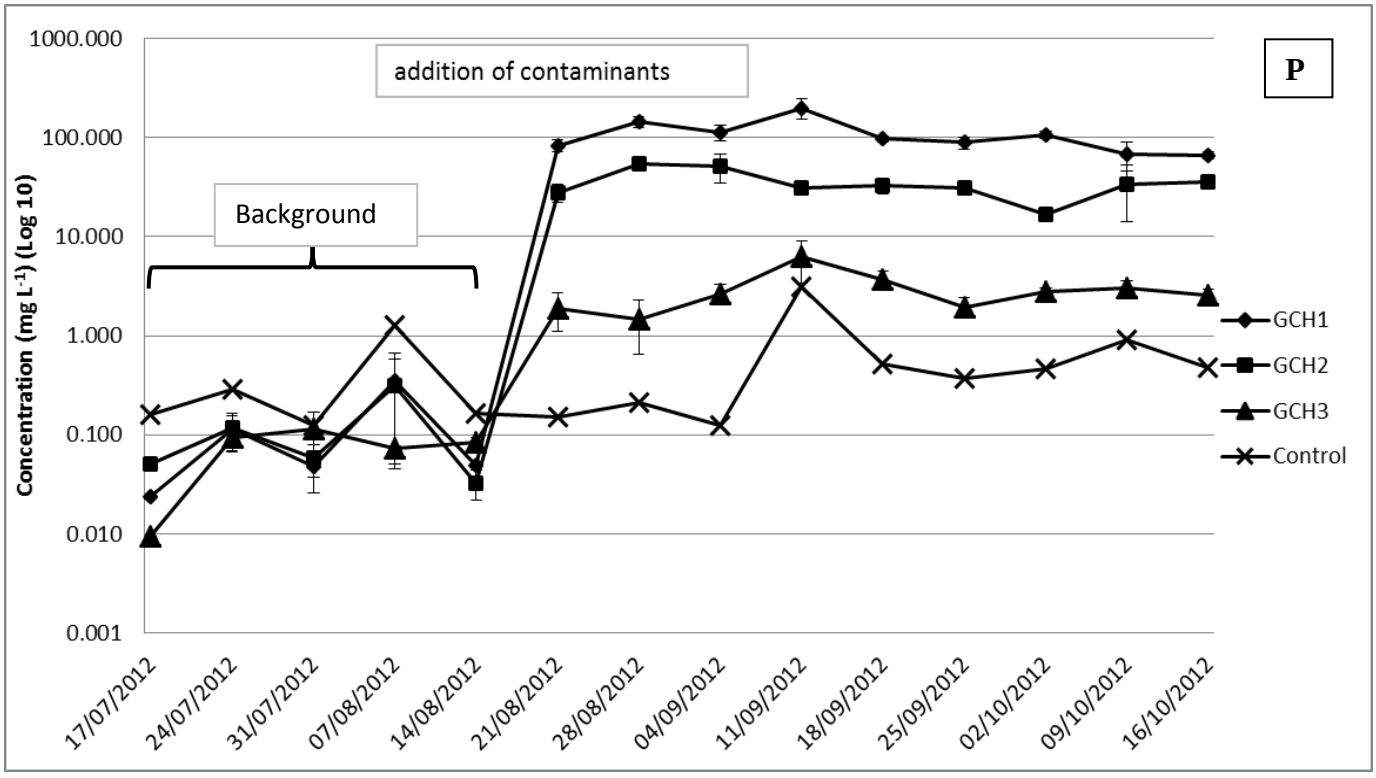


Fig. 5: Mean Effluent P concentration: 17/07/12 to 14/08/12 represent background studies. Contaminants were added on 21/08/12 followed by weekly contaminant addition.

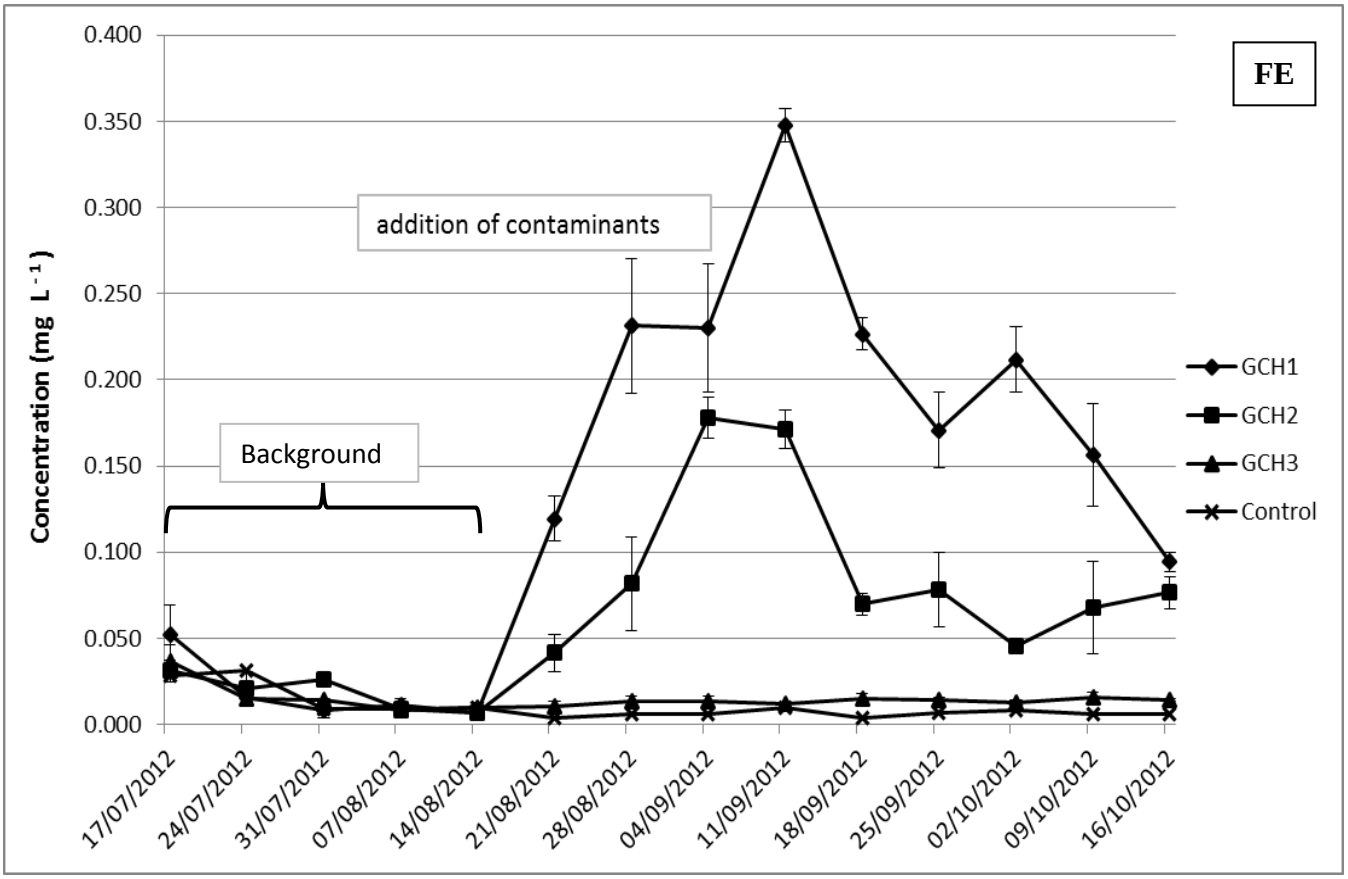


Fig. 6: Mean Effluent Fe concentration: 17/07/12 to 14/08/12 represent background studies. Contaminants were added on 21/08/12 followed by weekly contaminant addition.

The concentration of the nutrients released also indicates the potential for eutrophication to occur if they were to infiltrate into aquifers or be released into surface waters. Khan and Ansari (2005) reported that moderately eutrophic water bodies contain between 0.01 mg L^{-1} – 0.03 mg L^{-1} of phosphorus and 0.5 mg L^{-1} – 1.1 mg L^{-1} of nitrogen and as Fig 5 illustrates, P concentrations in the effluent from GCH rigs is higher than these quoted figures. Furthermore, Mbanaso et al. (2012) observed that the control rigs, to which no contaminants were added were not vulnerable to eutrophication occurrences thus, should be further investigated.

The summary of the other elements analysed is presented in Table 1.

Table 1: Elemental analysis of rig effluents (maximum values recorded) (where nrp represents no regulatory parameter while ngv represents no guideline value)

Rigs	Maximum concentration (mg L^{-1})									
	Al	Ca	Cd	K	Mg	Na	Zn	S	Cu	Pb
GCH1	0.050	98.1	0.003	566.8	11.7	36.110	2.077	692.8	0.145	0.110
GCH2	0.013	108.9	0.001	174.9	10.1	32.970	1.093	254.7	0.090	0.062
GCH3	0.008	37.9	0.001	14.0	3.4	14.900	0.143	22.1	0.024	0.021
Control	0.010	33.7	0.001	14.6	3.8	14.270	0.101	22.9	0.021	0.015
WHO limits	0.2	ngv	0.003	ngv	ngv	200	3	(as SO_4) 0.5	2	0.025
EU limits	0.2	nrp	0.005	nrp	nrp	200	3	(as SO_4) 0.5	2	0.025
US EPA limits	0.2	ngv	0.005	ngv	ngv	100	5	ngv	1.3	0.015

3.6. Total hydrocarbon analysis

Results shows that the GCH1 models released significantly ($p < 0.05$) higher concentrations of hydrocarbons from the rigs than GCH2 and that released from GCH2 was similarly significantly different from GCH3 ($p < 0.05$). The control model was below limits of detection throughout the experimental period.

PPS is known to retain very high percentages of applied hydrocarbons (up to 98.7% see Newman et al., 2002) mainly due to the incorporation of a geotextile where biodegradation by microorganisms takes place. The present study showed that the 3 concentrations of GCH negatively impacted hydrocarbon retention as oil was released through the rigs.

3.7. Effluent pH

The pH chart has already been presented in Charlesworth et al. 2013 (‘Accepted Article’, doi: [10.1002/clen.201300157]) where it was found that the background for all models was fairly similar, fluctuating around 7.9 due to alkaline sub-base of PPS. Addition of the GCH lead to the pH of Control and GCH models reducing and, GCH1

and GCH2 also reducing but, at a steeper rate with the difference in pH between GCH1 and GCH2 being significant ($p < 0.05$). However, only the last 3 samples from the GCH1 models fell below 7 whilst it remained above neutral in the other rigs. The reduction in pH reflects addition of the acidic (pH 4.6) GCH (Amoros et al., 2007) which may have been responsible for the release of metals in models to which GCH was added relative to the control (section 3.5). Adsorption of glyphosate and desorption of metals (Morillo et al., 1994) may have increased metal mobility (Ramstedt et al., 2005) and bioavailability (Wang et al., 2007). However, most effluent samples were neutral or above, conditions in the GCH1 rigs only becoming slightly acidic in the last 3 weeks of the experiments, thus only low concentrations of metals were found.

4. Conclusions

GCH is the most-preferred herbicide for weed control by many Local Authorities in the UK (Tuffnell and Britt 2011) and most European cities (Rask 2012; Kempenaar et al. 2006) due to its cost-effectiveness. However, impacts on the immediate environment need to be constantly and comprehensively assessed in the light of emerging organic pollutants owing to urban creep and development trends associated with the adoption of SUDS to ensure sustainability. This study demonstrated that although the use of GCH stimulated growth of microbial consortia necessary for biodegradation of organic contaminants, the concentration at which these commercial formulations are made available is potentially toxic, particularly to protists and thus should be further investigated. It is further indicated that the population growth of ciliates may provide a biomarker of GCH and hydrocarbon polluted sites.

GCH concentration determines species richness as the trend showed that as the concentration of GCH increased, species richness decreased. Data also showed that at low concentrations of glyphosate < 72 mg L^{-1}, biodegradation processes may not be affected as all trophic levels required for optimum biodegradation of contaminants were present.

It is therefore necessary to evaluate the efficacy of different concentrations of GCH in order to ascertain the difference in effectiveness in terms of its primary function – weed control. This has become imperative since this study has shown that the low concentration dose of 72 mg L^{-1} exerted the least toxic effect on soil microbes and minimal contaminant wash through. These studies are currently on-going and it is expected that data generated will give more insight into the concentrations of GCH that may have may have minimal impact on PPS while retaining its primary functions as a broad-spectrum weed control herbicide.

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