THE EFFECT OF GLYPHOSATE CONTAINING HERBICIDES ON THE SURVIVAL OF MICROORGANISMS ISOLATED FROM PERMEABLE PAVEMENTS

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SUMMARY

Spraying herbicides for landscaping purposes is a widespread activity, practised at intervals throughout the year in the UK. If herbicides are to be are sprayed onto pervious pavements, the extent of attenuation and also the discharge into receiving soils and waters is currently unknown. Whilst preparing an experiment to test the physical attenuation of herbicides by a permeable pavement, research was initiated to perform toxicological testing of microbes known to be present in these pavements, with commonly used herbicides. This would give an early indication as to the effects of herbicides on an oil degrading biofilm, and to the possibility of herbicide biodegradation.

In a survey performed previously, glyphosate containing herbicides were the type most commonly applied in the UK, and these were used as the standard contaminant in subsequent tests. A ciliate *Colpoda cucullus*, isolated from pavement effluent, was shown to be very sensitive to glyphosate with an LD_{50} of around 70 mg/l. The sensitivity of nematode worms, also from pavement effluent was much less however, with an LD_{50} of around 1000 mg/l. Separate mixed cultures were set up and it was found that considerable microbial respiration was observed in glyphosate containing mixed cultures with as much as 1500 ppm CO_2 evolved as a direct result of glyphosate metabolism. From the mixture of pavement-derived organisms added to these cultures, it appeared that fungi were primarily responsible for glyphosate degradation. These results demonstrate that the effect of glyphosate on pavement microbes is highly variable and that if the contaminant can be immobilised by the pavement, biodegradation will occur.

1. INTRODUCTION

1.1 Herbicides and pervious paving systems (PPS)

Research into the ability of pervious pavement systems or PPS (meant to mean permeable pavements within this context and used interchangeably) to improve water quality is ongoing. After demonstrating that particulates, clean and used lubricating oils and metals are attenuated by PPS and hydrocarbons are actively degraded, research has now begun to investigate the effects on and fate of, herbicides within the PPS environment.

This paper will focus on the likely effects of a particular glyphosate containing herbicide on the oil degrading microbial community of the PPS, the toxicity of the compound to certain key PPS species and the effect of the herbicide on a more complex mixed microbial community in laboratory benchtop experiments.

1.2 Glyphosate containing herbicides (GCH)

Herbicides containing glyphosate (the active ingredient responsible for weed control) represent a relatively new and supposedly environmentally benign generation of weed control chemicals. Glyphosate, or N-(phosphonomethyl) glycine is a component of herbicides that works on contact by inhibiting the synthesis of amino acids, thereby effectively suppressing normal plant cellular function. Glyphosate is advertised as safe to non-target organisms in terrestrial ecosystems as it tends to be bound quite securely to particles in soil and organic matter. It also has a relatively short half life (50 days) when compared to other herbicides (Schuette, 1989).

Despite the fact that these compounds probably represent the best choice currently, for weed killing with minimal export of the product to non-target ecosystems, concern has still been expressed as to the environmental effect of using large quantities of the compound (Relyea, 2005). As GCH are now very extensively used in agriculture, forestry, gardens and parks in a number of formulations, analysis of the effect of GCH on non-target organisms is required to determine any potential problems.

The reason for the concern over the effect of GCH on PPS is that PPS are specifically designed for the promotion of infiltration to reduce flooding. They also cleanse the water of particles and chemicals by the use of an appropriate geotextile. If GCH are not immobilised and treated effectively in a PPS and end up in groundwater, or if the PPS biofilm known to degrade hydrocarbons is killed by GCH, water quality will suffer.

As UK local authorities routinely spray GCH, around and probably onto PPS in the course of routine landscaping, the pathway for delivery of the contaminant to infiltration systems is in place. This threat is currently unquantified in terms of the amount of actual product getting onto PPS in the field. Research into the efficiency of PPS in filtering out GCH is ongoing and is not featured in this paper. The research here is focused on the effect of the compound on common PPS organisms both singly and in larger communities composed of representative PPS fauna.

2. METHODOLOGY

2.1 Single species experiments

Two of the commonest PPS eukaryotes, identified throughout PPS research as present and found in large numbers have been the ciliated protist *Colpoda cucullu*s and the nematode worm, a metazoan or small animal, *Caenorhabditis*. These organisms are also ubiquitous in soils and are numerically very important in soil processes (Griffiths, 1995). They are also the closest things available to ‘typical’ PPS organisms and therefore may be considered as a general marker as to how GCH would affect the PPS eukaryotic community. These organisms were also originally isolated from oil degrading PPS and were cultured in the laboratory on oil based agar plates with an oil degrading *Pseudomonas* bacterium as their food source.

In these experiments, GCH was applied to culture vessels to achieve the manufacturers recommended dose of 2400 mg/l in the vessels. In a separate experiment, glyphosate was applied solely in a pure analytical form in order to differentiate the effects of the compound itself from the formulation in its ready to use state. The compound was given 30 minutes to work on the organisms and the remaining density of organisms assessed by direct counting under a light microscope or with a haemocytometer. All experiments were replicated threefold with a control culture untreated with GCH.

2.2Mixed culture experiments

A mixed microbial culture containing bacteria, protists, fungi and metazoa was treated with 2400 mg/l glyphosate and incubated for 80 days. Some of the cultures also contained oil to see whether oil and GCH were more toxic in combination than alone. During this time, counts were made of the total number of organisms and the numbers of different types of eukaryote present, in order to examine the effect of the compound both quantitatively and qualitatively. During the course of the experiment, CO_2 readings to monitor microbial activity via respiration were also taken using an infra-red gas analyser (IRGA) in order to examine the rate of microbial breakdown of the GCH and oil. This was important to determine whether or not GCH inhibits the action of microbes in oil biodegradation processes.

3. RESULTS

3.1 Single species experiments

The survival of *Colpoda cucullus* with increasing GCH concentrations decreased much more rapidly than with *Caenorhabditis*. Live nematodes were still observed even up to 32 grams per litre of GCH, this is more than ten times the maximum concentration tolerated by *Colpoda*. There was slightly higher survival of Colpoda with pure glyphosate, indicating that something in the GCH formulation other than the glyphosate was also toxic to these organisms. This was probably a detergent or some dispersing agents that are often included in herbicide formulations.

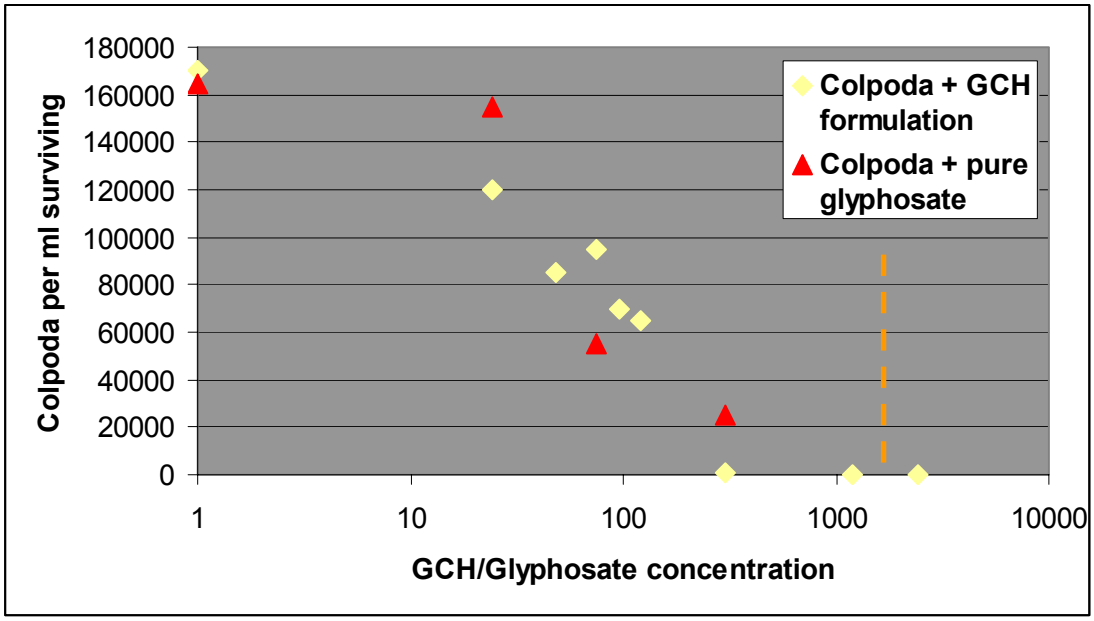


Figure 1. Survival of Colpoda with increasing GCH and glyphosate concentrations. The dashed line shows the recommended applied concentration.

As can be seen in Figures 1 and 2, at the recommended concentration for GCH of 2400 mg/l there were no surviving *Colpoda*. This suggests that as the manufacturers recommend the compound should be applied when rainfall is not forecast, the GCH concentration at the geotextile surface may well be at this concentration. Locally, *Colpda* may disappear from the biofilm as may other species. With *Caenorhabditi*s however, 2400 mg/l had a strong effect but 20 % of the population survived.

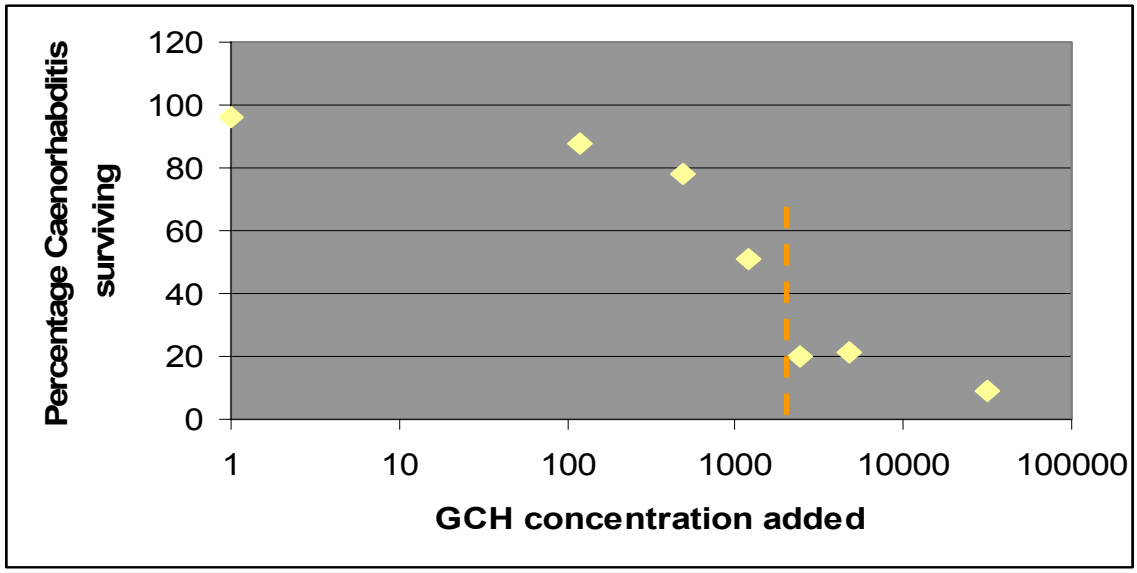


Figure 2. Percentage survival of *Caenorhabditis* with increasing GCH concentrations. The dashed line is the recommended applied concentration.

Table 1. GCH LC_{50} results for a number of tested organisms. Results from the current study are displayed in red.

Organism	LC_{50}
Colpoda	ca 70 ppm
Colpoda **(Glyphosate)**	ca 100 ppm
Caenorhahditis	ca 1000 ppm
Tetrahymena/Euplotes (Tsui and Chiu, 2003)	23-30 ppm
Daphnia magna (Schuette, 1989)	930 ppm
Tadpoles (Relyea, 2005)	0.4-11.5 ppm
Rainbow trout (Schuette, 1989)	38 ppm
8 day mallard duck (Schuette, 1989)	>4640 ppm

3.2 LC_{50} results

LC_{50} is the concentration of a compound required to kill 50 % of a given population of organisms. This is a standard ecotoxicological technique when screening organisms within an environment for

susceptibility to a certain chemical. *Colpoda* have similar LC_{50} to other protists (*Tetrahymena* and *Euplotes*) and the LC_{50} for *Caenorhabditis* was similar to an organism of similar size, a water flea, *Daphnia magna.*

3.3 Mixed culture experiments

The microbial communities tested in culture were originally extracted from an oil degrading PPS rig. This ensured that the organisms tested in this experiment were representative of the kind of assemblage found in a field situation. As can be seen in Figure 3, the cultures monitored for CO_2 evolution demonstrate that glyphosate is degraded quickly when it is added alone as the contaminant and also where glyphosate is added in combination with oil. In fact, where oil and GCH were added together the highest CO_2 evolution was recorded. This shows that GCH is not recalcitrant to complex mixed microbial communities despite its toxicity in single species tests. Also there was no obvious inhibition of biodegradation by the combination of oil and glyphosate. The bacteria and fungi are seen to metabolise oil and glyphosate within the first few days and a peak CO_2 reading was seen after about 15 days. The levels of CO_2 indicate that decomposer organisms have a different response to GCH than eukaryotes do and that the contaminant will be degraded efficiently within PPS.

Counts of the number of eukaryotes per millilitre of culture (Figure 4) revealed a clear inhibition of growth in the cultures with GCH present. The single species experiments had shown that very common soil species such as *Colpoda* are killed by GCH at the recommended application concentration. As *Colpoda* is often one of the numerically dominant species in such cultures (Coupe et al., 2003) its absence may partly account for the lower counts compared to oil only cultures. *Colpoda* was recorded in high numbers in oil only cultures. From being an order of magnitude higher in number, towards the end of the experiment the counts for all treatments were much more similar at around 5.0×10^1 organisms per ml.

The number of different recorded taxa increased much more quickly in cultures with no GCH (Figure 5) yet the final total from these cultures surpassed the GCH free flasks at the end of the experiment. As shown in table 2, there was a real qualitative difference between the glyphosate containing cultures and those without. Without glyphosate, the composition of taxa was much more inclusive of ciliated protists and flagellates. These are eukaryotes that are quick to reproduce and generally more common than the kind of organisms that became dominant in the GCH cultures. These were, with the exception of nematodes, testate amoebae. Testate amoebae are relatively slow growing compared to *Colpoda* and generally exist in relatively low numbers in most environments. The shift in dominant species between the GCH containing and GCH free cultures displays some level of tolerance of GCH by testate amoebae when compared to *Colpoda*. It is not possible to state whether the low numbers of testates in the GCH cultures early in the experimental period was due to some inhibition from the herbicide or due to the slow growth rates of these particular kinds of protist.

Taken together, the results from the mixed community work show that glyphosate in culture is degraded by PPS decomposers but does have identifiable secondary effects on the predator organisms, on their numbers and community structure.

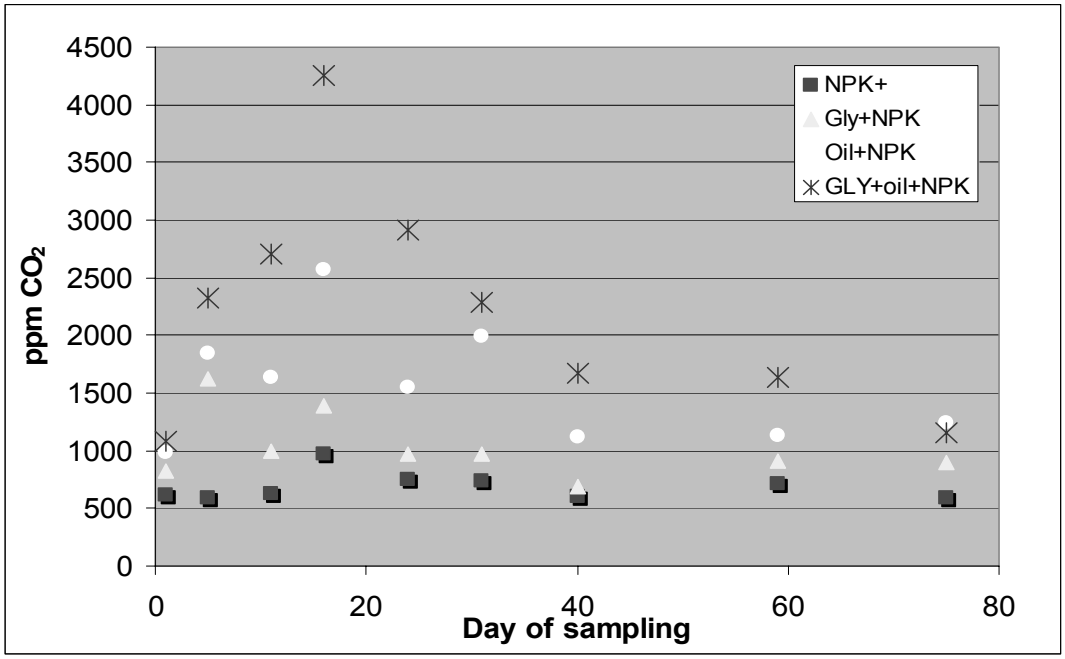


Figure 3. CO_2 concentrations from culture vessels containing inorganic nutrients and/or oil and glyphosate.

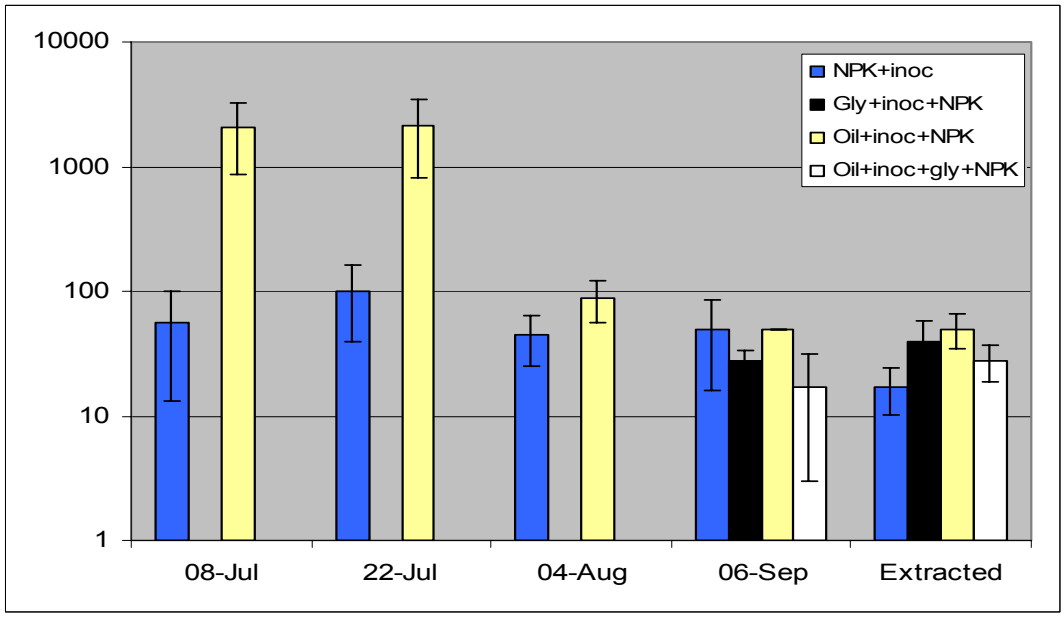


Figure 4. Counts of eukaryotic organisms containing inorganic nutrients and/or glyphosate and oil.

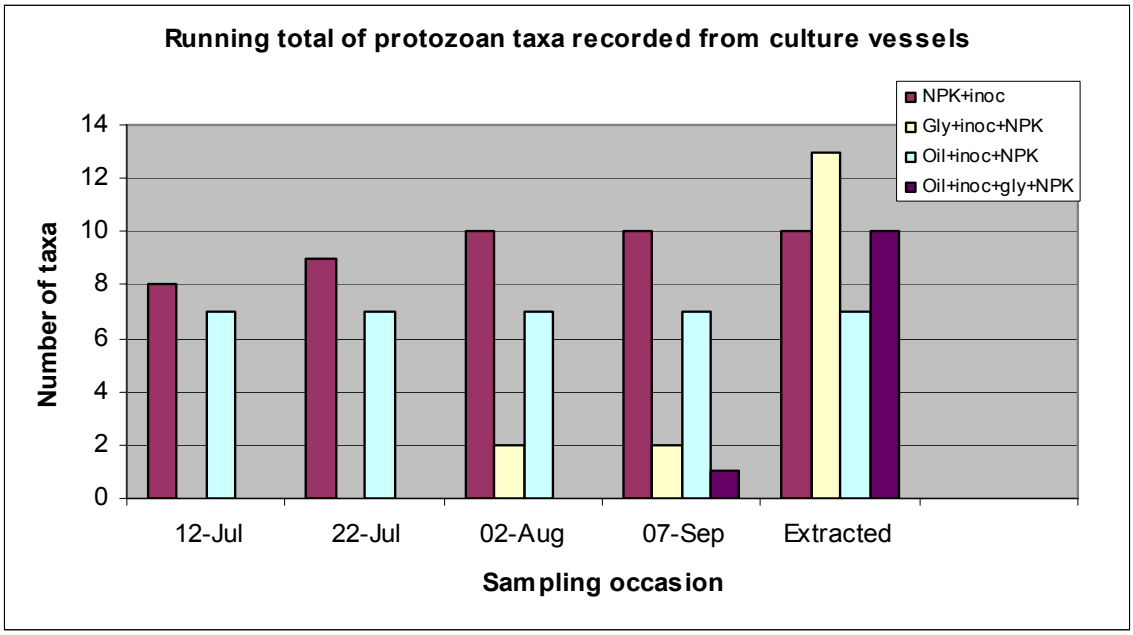


Figure 5. Accumulation of eukaryotic diversity in cultures containing inorganic nutrients and/or glyphosate and oil.

Table 2. Species recorded from cultures containing inorganic nutrients and/or glyphosate and oil. Testate amoebae are highlighted in bold.

No oil or Roundup	Roundup only	Oil only	Oil and Roundup
Cercomonas sp.	***Arcella arenaria***	*Colpoda cucullus*	***Arcella arenaria***
Colpoda cucullus	***Arcella conica***	*Colpoda steinii*	***Centropyxis aerophila***
Colpoda steinii	***Assulina muscorum***	*Ceratiomyxa sp.*	***Cryptodifflugia sp.***
Corythion dubium	***Centropyxis aerophila***	***Euglypha rotunda***	***Cyclopyxis eurystoma***
Euglypha rotunda	***Corythion dubium***	*Leptopharynx sp.*	***Euglypha compressa***
Heteromita globosa	***Cryptodifflugia sp.***	*Spongomonas sp.*	***Euglypha rotunda***
Pseudoglaucoma sp.	***Cyclopyxis eurystoma***	***Trinema lineare***	***Euglypha strigosa***
Trinema enchelys	***Difflugia sp.***		Nematode
Trinema lineare	***Euglypha rotunda***		***Trinema enchelys***
Spongomonas sp.	***Euglypha strigosa***		***Trinema lineare***
	Nematode		
	Trinema enchelys		
	Trinema lineare		
10	**13**	**7**	**10**

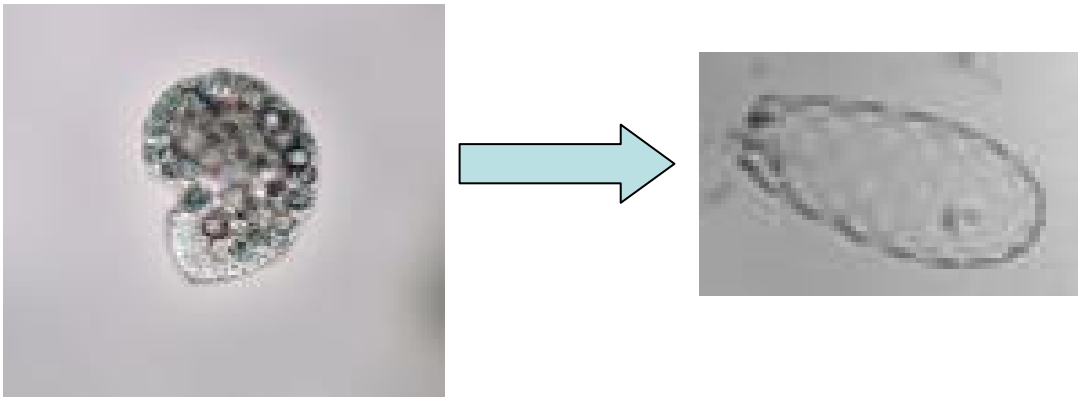


Figure 6. Summary of changes in eukaryotic community structure from common ciliated protists to testate amoebae.

4. DISCUSSION

In one sense, the view that glyphosate is a relatively benign herbicide ingredient is upheld by the experiments in this study. GCH are degraded by mixed microbial communities and the turnover of the contaminating material, inferred from CO_2 data is rapid with degradation proceeding at a rapid rate. Indeed, the 50 day half-life for the compound projected (Schuette, 1989) was longer than the peak CO_2 evolved in the GCH only culture, this being reached after only 16 days. There were no observed additive effects when glyphosate and oil were added together, in other words oil degradation was not suppressed by glyphosate judging by the respiration data. GCH do act in this study as an extra carbon source utilisable by a diverse set of decomposers growing formerly in an oil rich environment. As long as GCH is held for a sufficient time in the structure before it is released it will be degraded at the recommended application rate.

However not all the results were in this vein. It was clear that very common soil and PPS organisms would be killed where GCH is applied in the field. Whether this would disrupt the normal operation of the oil degrading biofilm over time is not clear. It may be that if the maintenance role of the eukaryotic community on the decomposers is affected negatively the performance of the system may still suffer despite these being secondary effects.

Without a fairly detailed examination of the qualitative and quantitative nature of the eukaryotic community structure it would be difficult to discern any significant effect of GCH on the community as a whole except for stimulation via carbon substrate. In this study, the effects of GCH on eukaryotes were significant enough to completely alter the density of the eukaryotic community and also change the dominant community structure from a fairly ordinary PPS assemblage to one dominated by testate amoebae both numerically and in the species composition.

It may well be that a compensation effect occurs in the PPS under contamination by GCH and that at the applied concentration, organisms such as *Colpoda* would be effectively replaced in the maintenance role by the new dominant community. It can be seen from the single species toxicity tests that nematodes like *Caenorhabditis* would form part of this community as would testate amoebae. Nematodes are extremely resistant to changes in environmental conditions. They have been observed by the author to be present in alkaline PPS effluent even up to a pH of 10. They are not affected by used engine oil with its associated metals and organics and it would seem that they are also resistant to GCH up to concentrations much higher than those expected to be applied to or spilled onto PPS.

Testate amoebae are defined morphologically as having a shell which is thought to protect these organisms from predators. It could also be the case that it offers some protection from chemicals and prevents the migration of the glyphosate from the medium into the cell. As GCH work by contact, a shielding of the organism by the protective shell may allow survival and even increases in population as observed from counts of organisms in effluent.

5. CONCLUSIONS

It is clear that the effect of glyphosate on PPS microbes is highly variable dependent upon the organism affected. The short term operation of the PPS should not be affected biologically, and as long as GCH can be retained in the PPS water quality and certainly oil biodegradation should be unaffected. Whether the long term effects of GCH on the eukaryotic community lead to a decrease in PPS performance is unknown but should be investigated further.

6. RFERENCES

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