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Illicit Drug Laboratories and the Environment

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Executive Summary

The illicit manufacture of amphetamine-type substances in clandestine laboratories is a significant problem in Australia and overseas. Disposal of chemical waste from clandestine laboratories is more likely to involve practices that maintain secrecy rather than practices that protect the environment; inevitably some clandestine laboratories will dispose of waste by burial and that waste that is disposed of in domestic waste will end up in landfill. It has been estimated that approximately 5–6 pounds of waste is produced for each pound of methamphetamine produced (Lukas, 1997). Although the waste itself represents a direct environmental threat, soil is an active material that digests some chemicals and converts them into other chemicals, which themselves could also be a threat. Surprisingly, there has only been one investigation into what happens to drugs, their precursors, and manufacturing by-products when they are buried; therefore it is not yet known whether these chemicals represent an environmental threat or not.

A critical task of the forensic clandestine laboratory investigator is to analyse residues of manufacture in order to gather evidence of illicit drug manufacture and evidence of the particular synthetic route used. The chemical make-up of residues that have been buried has not been investigated.

The experimental aims of this project are fourfold:

- to measure the toxicity of certain illicit drugs, their precursors, and manufacturing by-products towards soil;
- to examine whether these materials are altered by soil, or migrate through soil;
- to identify any altered products and measure whether they are toxic towards soil;
- to develop analytical methodology and data relating to breakdown products.

Each of these aims was achieved using three soils of varying physico-chemical properties representative of common Australian environments: urban, agricultural, and bushland. The first stage of the project focussed on the examination of the toxicological aspects of fifteen ATS and ATS-related compounds on a number of soil microbial functions that probe general soil health. The ATS chosen were:

- methamphetamine, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (ecstasy or MDMA).

Key precursors to these drugs that were examined were:

- benzaldehyde, 3,4-methylenedioxyphenyl-2-propanone, N-methylformamide, nitroethane, 2-nitro-1-phenylpropene, piperonal, pseudoephedrine, and safrole.

Key intermediates and by-products to these drugs that were examined were:

- 4-methyl-5-phenylpyrimidine, N-formylmethamphetamine, 1-benzyl-3-methylnaphthalene, 3,4-dimethyl-1-phenylaziridine, 1,3-diphenylacetone, and 1-(1,4-cyclohexadienyl)-methyl-2-aminopropane. These are associated with the Leuckart reaction, the hydriodic acid/red P method, the conversion of phenylacetic acid into phenyl-2-propanone, and the Nazi method, respectively.

Earlier work by us has investigated the fate and toxicological impacts of another key precursor, phenyl-2-propanone. Significantly, there was in general no apparent adverse response on the microbial functions assessed up to 1000 mg of the test compounds per kilogram of soil. However, some compounds did inhibit microbial activity measured in terms of dehydrogenase enzyme activity which is linked to microbial respiration.

The next stage of the project involved the investigation of the environmental behaviour (e.g., sorption, degradation, and metabolism pattern) of selected compounds including parent drug (e.g., methamphetamine and 3,4-methylenedioxymethamphetamine), precursors (e.g., pseudoephedrine), and the by-products (e.g., 1-(1,4-cyclohexadienyl)-2-methylaminopropane, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene). The degradation study was carried under non-sterile and sterile conditions; the former indicates whether soil microbes are responsible for degradation of compounds (i.e. biotic processes), while the latter indicates whether non-biological (or abiotic) processes are responsible.

It was found that the relative proportion of biotic versus abiotic processes on the degradation of target compounds varied significantly for different soils. The resistance to degradation (which is equivalent to chemical persistence) under biotic conditions was found to rise in the following order (associated half-life in brackets): 1-(1,4-cyclohexadienyl)-2-methylaminopropane (1 to 8) < pseudoephedrine (4 to 30) < MDMA (15 to 59) < N-formylmethylamphetamine (35 to 44) < methamphetamine (131 to 502) < 1-benzyl-3-methylnaphthalene (150 to 10034). However, for abiotic degradation the following order was found: 1-(1,4-cyclohexadienyl)-2-methylaminopropane (3 to 6) < MDMA (75 to 107) < N-formylmethylamphetamine (188 to 301) < pseudoephedrine (143 to 502). Methamphetamine and 1-benzyl-3-methylnaphthalene showed no measureable changes under abiotic conditions within a one year period, therefore it would appear that the limited degradation exhibited by these compounds in soil takes place purely through biotic processes.

Interestingly, 1-(1,4-cyclohexadienyl)-2-methylaminopropane was found to be highly susceptible to degradation in all the three test soils, with conversion into methamphetamine within a few weeks. A substantial amount of methamphetamine was also recorded from the soils spiked with N-formylmethylamphetamine after one year incubation.

Batch sorption studies revealed a higher sorption potential of 1-(1,4-cyclohexadienyl)-2-methylaminopropane and MDMA in all the test soils, especially that from bush land. On the other hand, maximum desorption potential was highest for pseudoephedrine in any soil and in the bush land soil in particular.

Implications for forensic and law enforcement bodies

This work has contributed new and relevant information relating to the issue of clandestine laboratories and their potential harm to the environment. Such information is critical for the development of evidence-based guidelines, priority pollutant lists and thresholds, and policy and legislation relating to the remediation of clandestine laboratory sites and prosecution under environmental laws. In particular:

- 1-(1,4-cyclohexadienyl)-2-methylaminopropane does not represent an environmental threat as it is not persistent in the environment.
- Although 1-benzyl-3-methylnaphthalene, methamphetamine, MDMA, and

N-formylmethamphetamine all persist in the environment to a greater or lesser degree, they are not an environmental threat because they are not toxic to soil.

- Of the compounds studied, pseudoephedrine is most susceptible to migrate from its initial interment location to the surrounding environment, including groundwater.
- Our previous study has indicated that the popular precursor (and by-product) phenyl-2-propanone very quickly degrades to a number of compounds in soil; therefore in itself phenyl-2-propanone is not a threat to the environment.
- With the exception of N-formylmethamphetamine and 1-(1,4-cyclohexadienyl)-2-methylaminopropane, most compounds degraded very slowly in soil. It was not possible to identify degradation products of them and measure their toxicity.

The results obtained are of direct use in forensic clandestine drug laboratory investigations where interred residues are collected.

- Given that methamphetamine arises from degradation of 1-(1,4-cyclohexadienyl)-2-methylaminopropane and N-formylmethamphetamine, the origin of methamphetamine in clandestine laboratory residues found in soil must be deduced with caution.
- From the above it also follows that the absence of N-formylmethamphetamine in interred residues is not a reliable basis upon which to rule out the involvement of the Leuckardt reaction in the production of methylamphetamine.
- A route specific by-product for the popular hydriodic acid-red phosphorus method (1-benzyl-3-methylnaphthalene) is very persistent in soil and therefore is a reliable forensic marker.
- Of the compounds studied, pseudoephedrine is the most susceptible to migrate from its initial interment location to the surrounding environment, including groundwater.
- Our previous study has indicated that the popular precursor (and by-product) phenyl-2-propanone very quickly degrades to a number of compounds in soil.
- Given its similarity with N-formylmethamphetamine, it can reasonably be expected that N-formylamphetamine will degrade to amphetamine in soil. Therefore, until additional work is conducted to confirm or refute this expectation, caution should be exercised when amphetamine or amphetamine and N-formylamphetamine mixtures are detected in interred residues.
- In addition to knowledge, a comprehensive laboratory method including the extraction, clean-up, and quantitative liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry analysis of compounds in soil has been developed.

Future directions and recommendations

Although the soils used in this study are representative of many found across Australia, the behaviour of other types of soil upon exposure to ATS-related chemicals should be investigated.

Of necessity the range of chemicals investigated in this study was restricted, especially in the degradation study. A study involving additional precursors, reagents, and manufacturing by-products is warranted.

With the exception of N-formylmethamphetamine and 1-(1,4-cyclohexadienyl)-2-methylaminopropane, which degrade quickly, most compounds studied did not degrade in soil or did so only slowly. A degradation study involving larger quantities of slow degraders should be conducted in order to identify degradation products and examine their toxicity.

It is probable that the effects of two chemicals acting together will be greater than the effect of each chemical individually, or the sum of the individual effects. Thus, it is important to determine the degradation patterns employing appropriate mixtures of target compounds, and a parallel toxicity test.

As waste might remain *in situ* for long periods, or clandestine laboratories might operate in the same location for a long time, degradation studies over a period longer than 12 month should be conducted with selected compounds.

Microorganisms can adapt to the presence of chemicals and utilise them as a source of energy. Attempts should be made to isolate and characterise tolerant microbial communities from degradation experiments and investigate whether it is possible to exploit their capability for safe and efficient remediation of contaminated sites. This tactic has been used successfully in relation to oil spills at sea.

It is possible that clandestine drug laboratories might involve levels of contamination much greater than were modelled in this study. Measurement of the concentrations of compounds at real clandestine laboratory sites should be undertaken in order to identify a realistic 'upper concentration limit' for microbial function assays.

In order to minimise uncontrolled variance in this study, the soil samples were sieved to remove plant matter, stones, etc. It is possible that the materials removed for this study have an affect upon the fate of chemicals in the soil; therefore tests involving 'real life' soils should be conducted in order to highlight any effects.

Chapter one: Introduction

The abuse of illicit drugs has gained worldwide concern due to their significant adverse impacts on human health and social welfare (Sloan, 2008; Rieckermann & Christakos, 2008). Illicit drugs are those whose non-medical use is prohibited by the law, and mainly belong to the class of opiates, cocaine, cannabis, amphetamine-type substances (ATS), etc. (Hall et al., 2008; UNODC, 2007). ATS comprise two groups of compounds: (1) the amphetamines group (e.g., amphetamine, methamphetamine) and (2) the ecstasy group (e.g., 3,4-methylenedioxymethamphetamine (MDMA) and analogous compounds) (UNODC, 2008a). Amphetamine group substances account for more than three-quarters of ATS (UNODC, 2008b) and currently demand the most attention of all the synthetic illicit drugs (EMCDDA, 2007). Methamphetamine continues to be the most widely manufactured ATS and accounted for 68% of the amphetamine groups as per 2006 estimates (UNODC, 2008a, b). In comparison to the plant-based drugs (e.g. heroin and cocaine), methamphetamine is relatively easy to manufacture in clandestine laboratories from commonly available chemicals (Sasaki & Makino, 2006). Methamphetamine manufacture is typically located throughout East and South-East Asia, North America and Oceania due to easy availability of precursors and high demand (UNODC, 2008a). In Australia, the ATS market is second only to the cannabis market and may continue to grow (ACC, *Organized Crime in Australia*, 2009). In 2006–07 ATS seizures accounted for 46% by weight of all drugs seized in Australia, a period when 356 clandestine laboratories were detected across Australia (ACC, *Illicit Drug Data Report*, *2006–2007*, 2008).

ATS are generally manufactured domestically in clandestine laboratories using legally-produced precursors through a variety of manufacturing processes (UNODC, 2007). In 2007, global ATS production increased slightly although there has been a decline in ecstasy production (UNODC, 2008a). In 2006–2007 about 4.9% (208 million) of the world population aged 15 to 64 (i.e., 4272 million) were reported to use drugs, while problem drug use remained about 0.6% (26 million) (UNODC, 2008a). As of 2006–2007 the proportion of drug users had been almost stable for four years (UNODC, 2008a), with an increase at the global level from 200 million in 2005–2006 to 208 million in 2006–2007 and an associated increase in problem drug users from 25 to 26 million. The extent of drug use (i.e., the number of abusers in million) of the world population aged 15 to 64 in 2006–2007 followed the descending order of cannabis (165.6) > amphetamines (24.7) > opiates (16.5) > cocaine (16) > ecstasy (9) (UNODC, 2008a).

Illicit drugs are manufactured through a variety of synthetic routes, employing many different precursors, most commonly in small clandestine labs (in Australia) but also in industrialised mega- and super-laboratories. The synthetic pathways can be specific to geographical regions. The clandestine manufacture of methamphetamine frequently involves pseudoephedrine and/or ephedrine as the precursor. This is a relatively simple procedure, which produces the more pharmacologically potent (+)-methamphetamine (Freeman & Alder, 2002; Skinner, 1990). In South-East Asia it is common for ephedrine or pseudoephedrine to be converted into chlorephedrine, which is then reduced to methamphetamine (using the so-called Emde method). In Australia and the USA the reduction of pseudoephedrine or ephedrine with hydroiodic acid and red phosphorous (the Nagai method) or its variants are favoured (Lee et al., 2006; Windahl et al., 1995). Variations of the hydroiodic/red phosphorus pathway include the use of iodine, water, and red phosphorus, which is known as the 'Moscow' method, or iodine and hypophosphorous acid (Lee et al., 2006). Phenyl-2-propanone is another key precursor, as it can be converted into either methamphetamine or amphetamine using a wide variety of reagents. One favoured process,

called the Leuckart reaction, uses phenyl-2-propanone (P2P) as a precursor and formamide or N-methylformamide for amphetamine or methamphetamine synthesis, respectively.

All synthetic methods produce reaction by-products as well as the target drug. In some instances the by-products are characteristic of the synthetic procedure that was used, in which case the by-products are called 'route-specific'. Forensic chemists are often able to detect route-specific by-products in either residues from clandestine laboratories or drugs themselves, and thereby infer the route of manufacture. A route-specific by-product in the Leuckart synthesis of amphetamine is 4-methyl-5-phenylpyrimidine (Sinnema, 1981; Kirkbride, 2001). N-formylamphetamine and N-formylmethamphetamine are also by-products in the Leuckart method, but they are not route-specific (Ko et al, 2007). 1-(1',4'-cyclohexadienyl)-2-methylaminopropane is a route-specific by-product in the clandestine manufacture of methamphetamine by reduction of ephedrine or pseudoephedrine in the presence of ammonia and excess lithium (Person et al., 2005; Zvilichovsky & Gbara-Haj-Yahia, 2004). The synthetic route is similar to the Birch reduction and is commonly referred to as the Nazi method in the forensic community (Person et al., 2005). Route-specific by-products for the Nagai method and its variants are 1-benzyl-3-methylnaphthalene and 1,3-dimethyl-2-phenylnaphthalene, while phenyl-2-propanone and 3,4-dimethyl-1-phenylaziridine are also present, which are not route-specific (Ko et al, 2007).

The above influenced the choice of N-methylformamide, pseudoephedrine, 4-methyl-5-phenylpyrimidine, N-formylmethamphetamine, 1-benzyl-3-methylnaphthalene, 3,4-dimethyl-1-phenylaziridine, 1,3-diphenylacetone, and 1-(1,4-cyclohexadienyl)-methyl-2-aminopropane as priorities to be investigated during the course of this research. Less common methods for drug manufacture in Australia involve the use of nitroethane in the conversion of benzaldehyde into amphetamine or methamphetamine and in the conversion of piperonal into MDMA, the use of safrole in the production of MDMA or MDA, and the conversion of phenylacetic acid into phenyl-2-propanone under conditions that lead to its contamination with dibenzyl ketone. As a consequence, benzaldehyde, piperonal, nitroethane, dibenzyl ketone, and safrole were also included in this study. In addition to their precursors and route-specific by-products, methamphetamine, MDMA and MDA were also included for investigation.

The chemicals associated with clandestine drug laboratories (e.g., drugs, their precursors, by-products, etc.) are often illegally buried or disposed of into the sink, toilet, soil, sewerage system, and public waste management facilities (Janusz et al., 2003; Scott et al., 2003). These chemicals once released may undergo diverse processes such as sorption, degradation, leaching, surface runoff, etc., become exposed throughout the environmental compartments (i.e., soil, sediments, ground water, surface water, etc.), and eventually may have potential implications for humans and wildlife. Thus, the environmental impact of these potentially toxic chemicals is increasingly being recognised as a critical issue of concern. The illicit drugs, similar to the licit pharmaceuticals, may have potent pharmacological and biological activities. Their presence in surface waters even at low environmental concentrations together with the residues of many therapeutic pharmaceuticals and several other organic compounds may lead to unexpected pharmacological interactions causing toxic affects to aquatic organisms, or may cause a wide variety of environmental and human health problems (Zuccato et al., 2008; Castiglioni et al., 2007; Al-Rifai et al., 2007; Pomati et al., 2006). Thus, a systematic investigation of the environmental behavior of chemicals associated with the manufacture of illicit drugs and the potential risks associated with their release into the environment is warranted. Although the impacts of inorganic materials such as iodine upon the environment are known, the work described here is the first study to deal with the fate of precursors, by-products, and illicit drugs in the environment.

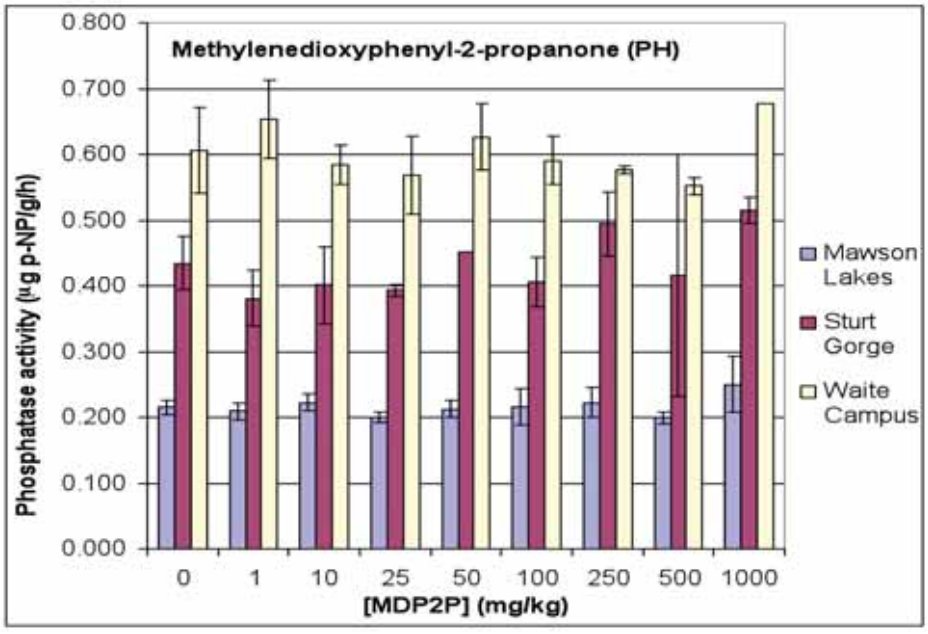
Chapter two: Project results and discussion

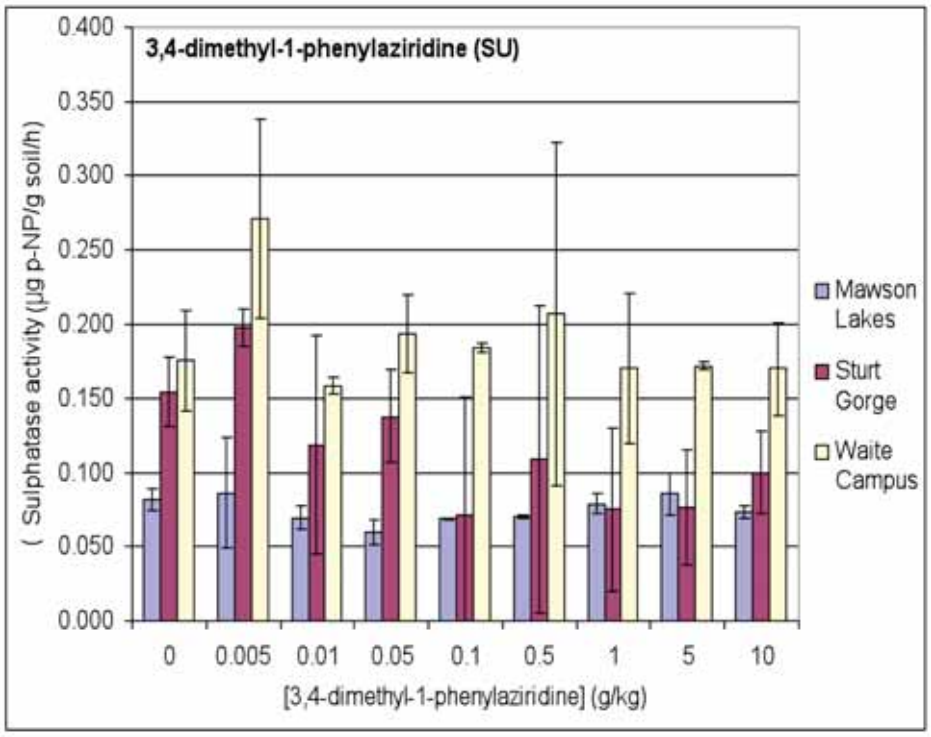
Toxicity study

Toxicity testing is a process to measure the hazard of chemicals on living organisms. The toxicity of contaminated soils has become a major focus in ecological risk assessment. Soil toxicity tests can be used as a valuable tool for identifying hot spots at hazardous waste sites, to set site-specific soil quality guidelines, and for guiding on-site contamination mapping and remediation. The soil toxicity can be measured by several methods, e.g., survival, growth, and reproduction of earthworms, seed germination tests, growth and activities of soil microbes, etc. In the present study, the toxicity of the test compounds were determined by measuring the potential nitrification rate, dehydrogenase enzyme activity, the activities of enzymes involved in key nutrient cycles such as nitrate reductase (nitrogen cycle), sulphatase (sulphur cycle), and phosphatase (phosphorous cycle). Dehydrogenase activity in soil is a measure of total microbial activity, commonly used to study the effects of pollutants on microbial activity in soils (Megharaj et al., 1999, 2000).

In general, there was not found to be any adverse affects on the microbial function of the soils at levels up to 10 g/kg spiking concentrations. There was no change in the level of sulphatase and phosphatase activity for any of the test compounds (Figure 1).

Figure 1: Examples of phosphatase activity (following exposure to MDP-2-P) and sulphatase activity (following exposure to 3,4-dimethyl-1-phenylaziridine) after the 10-day exposure period. Error bars represent the standard error of the mean.





Similarly, there was no affect for 15 of the compounds on the potential nitrification rate of all three soils (Figure 2). The one exception to this was for nitroethane, where a substantial increase in the potential nitrification rate was found at concentrations as low as 50 mg/kg for the Waite Campus soil. The nitrate reductase activity was also found to be unaffected following application of 15 of the compounds, while nitroethane was found to substantially increase the nitrate reductase activity. This was especially marked in the Mawson Lakes soil (Figure 3). The basis for nitroethane stimulating the potential nitrification rate and nitrate reductase activity or the potential consequences of these effects on the terrestrial environment are not clear.

Figure 2: The potential nitrification rate of the 3 test soils following 10 days of exposure to nitroethane. Error bars represent the standard error of the mean.

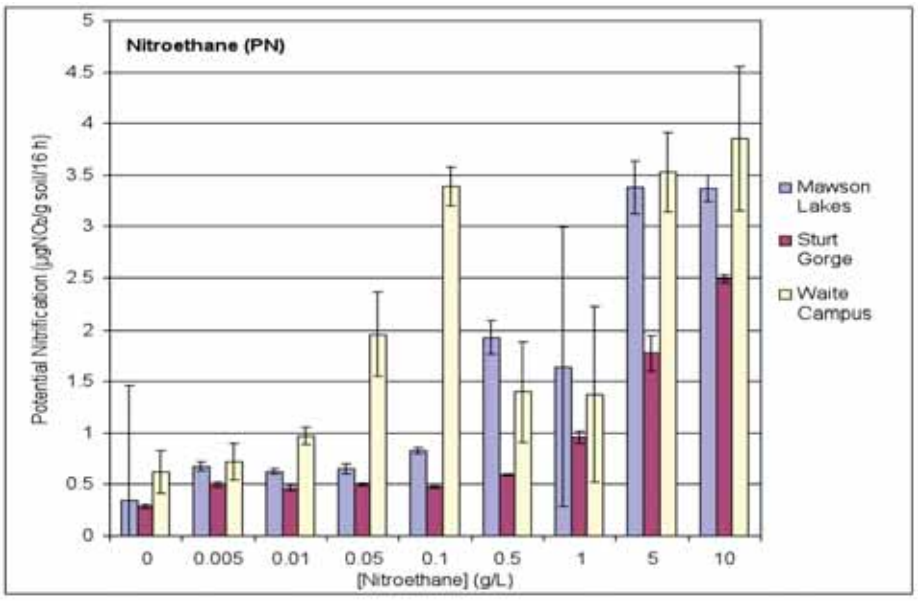
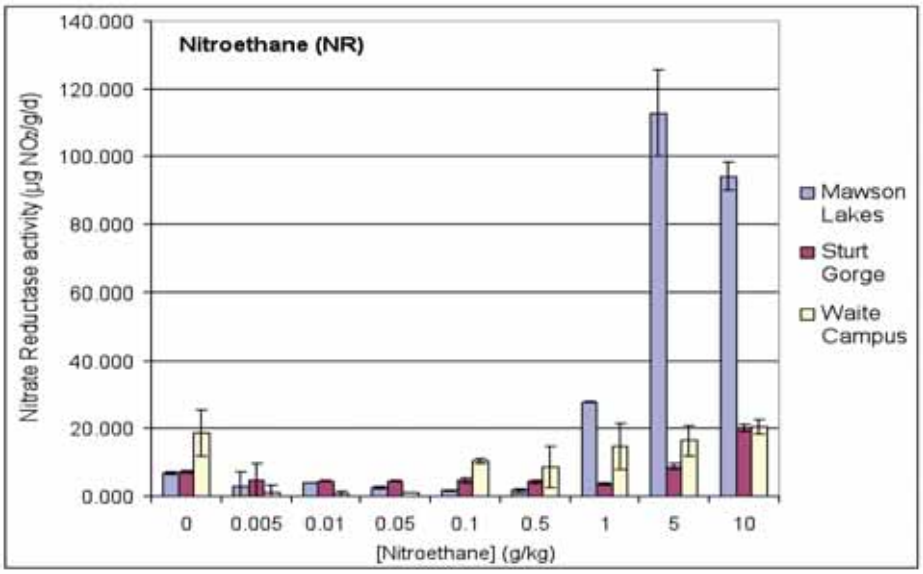
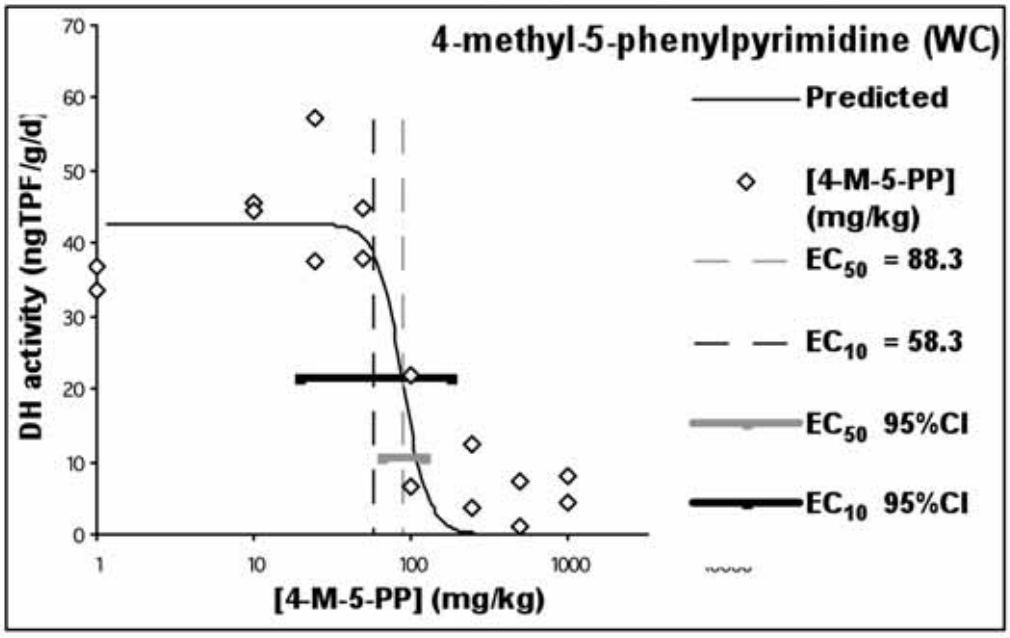


Figure 3: The nitrate reductase activity of the 3 test soils following 10 days exposure to nitroethane. Error bars represent the standard error of the mean.



The dehydrogenase activity of the 3 test soils was found to be affected following 10 days of exposure to 6 of the 16 test compounds. The compounds were benzaldehyde, 4-methyl-5-phenylpyrimidine, nitroethane, 2-nitro-1-phenylpropene, piperonal and safrole, and for each compound an effective concentration (EC) was determined (Table 1). EC values were determined from a sigmoidal logistic curve of the data (Figure 4), using a model developed by CSIRO.

Figure 4: Sigmoidal logistic curve used to predict the EC_{10} and EC_{50} values for dehydrogenase activity following 10 days of exposure of 4-methyl-5-phenylpyrimidine in Waite Campus soil. Dashed vertical lines are the estimated EC values, while the solid horizontal lines represent the 95% confidence intervals for the estimates.



In this case, the EC is defined as the concentration at which the extent of the dehydrogenase activity is reduced, relative to control samples. That is, an EC_{50} is the concentration where there is a 50% reduction in the dehydrogenase activity, relative to the unspiked soil samples. The EC_{50} is an important measure for determining when a significant alteration to a function of interest (in this case, dehydrogenase activity) has occurred. The EC_{10} can also be used as an arbitrary measure of the concentration where an effect on the function of interest is becoming statistically significant (Thiele-Bruhn & Beck, 2005).

In general, the EC_{50} values were close to or greater than 1 g/kg (one part per thousand). Two notable exceptions were found for 4-methyl-5-phenylpyrimidine with Mawson Lakes and Waite Campus soils and for 2-nitro-1-phenylpropene in Mawson Lakes soil, where the EC_{50} was calculated to be around 0.1 g/kg (Table 1). Also, for both of these compounds, the EC_{10} values determined are within an order of magnitude of the EC_{50}. This indicates that the difference in concentration between the effect becoming statistically relevant and where a significant reduction in the dehydrogenase activity occurred was relatively small.

Table 1: EC_{10} (mg/kg) and EC_{50} (mg/kg) values for the dehydrogenase activity of the 3 test soils

Compound	Soil	EC^{a}_{10} (mg/kg)	EC_{50} (mg/kg)
Benzaldehyde	Mawson Lakes	383	1660
	Sturt Gorge	2200	3280
	Waite Campus	1170	1960
4-methyl-5-phenylpyrimidine	Mawson Lakes	26	127
	Sturt Gorge	57	440
	Waite Campus	58	88
Nitroethane	Mawson Lakes	331	696
	Sturt Gorge	375	1660
	Waite Campus	283	603
2-nitro-1-phenylpropene	Mawson Lakes	6	67
	Sturt Gorge	135	1690
	Waite Campus	283	604
Piperonal	Mawson Lakes	2160	3590
	Sturt Gorge	86	734
	Waite Campus	339	648
Safrole	Mawson Lakes	5190	8090
	Sturt Gorge	3350	8390
	Waite Campus	1010	8490

[a] Effective concentration

Degradation study

Organic compounds in soil are subject to a variety of degradation or transformation or metabolism processes. The overall degradation of a compound in soil results from a combination of mechanisms such as microbial degradation or biotic processes, and a variety of abiotic processes such as chemical hydrolysis, photolysis, etc. The degree to which each mechanism will contribute to the overall degradation of the compound is in turn dependent on the physicochemical

properties of the compound (e.g., water solubility, sorptive affinity), characteristics of the soil (e.g., pH, organic matter content, microbial biomass, and redox status), and environmental conditions (e.g., temperature, moisture). The role of microbes in a degradation process is indicated if degradation occurs very rapidly in soils that have not been sterilised, or if sterilisation of a soil is seen to inhibit or stop its ability for degradation. The metabolism of organic compounds may largely depend on the dominating factor responsible for the degradation leading to the production of a range of metabolites. The metabolite produced due to the degradation of the parent compound may be more, less, or similar in terms of persistence and toxicity compared to the parent compound. In addition, the toxicity level of the metabolite will also influence the persistence of the metabolites in soil. Thus, the overall metabolism/transformation pattern of the compound is important for devising remediation/management strategy for a contaminated site.

Development of analytical technique

For this study it was important to develop robust, reliable analytical procedures for the recovery of parent drugs, precursors, manufacturing by-products, and their metabolites from soils; a technique for soil extraction, sample clean-up, and analytical methods for HPLC-MS and GC-MS has been developed for the 6 test compounds (i.e., methamphetamine, MDMA (3,4-methylenedioxymethamphetamine), pseudoephedrine, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, and N-formylmethylamphetamine). Eleven solvent combinations were assessed in order to develop a sample extraction technique with optimum recovering efficiency of the target compounds from the soil matrix (Appendix 6). Solvent type [7] and [10] produced reliable results throughout the target compounds from the test soils (Table 2), with solvent type [10] the better overall with the exception of the extraction of pseudoephedrine in Sturt Gorge and Waite Campus soils.

Table 2: Recovery efficiency of the proposed methods for the extraction of target compounds from test soils

Target compound	Test soil	Solvent type [7][a]	Solvent type [10][b]
Methamphetamine	Mawson Lakes	31.4	73.3
	Sturt Gorge	44.3	51.4
	Waite Campus	72.0	85.2
MDMA	Mawson Lakes	43.0	76.8
	Sturt Gorge	54.4	53.6
	Waite Campus	83.6	94.8
Pseudoephedrine	Mawson Lakes	43.0	57.3
	Sturt Gorge	56.9	33.8
	Waite Campus	82.0	63.3
1-(1,4-cyclohexadienyl)-2-methylaminopropane	Mawson Lakes	32.6	76.9
	Sturt Gorge	43.2	48.3
	Waite Campus	65.5	80.9
N-formylmethylamphetamine	Mawson Lakes	72.4	77.5
	Sturt Gorge	73.8	88.3
	Waite Campus	80.4	93.0

[a] Methanol:Acetic acid (99:1)

[b] Chloroform:Acetonitrile:Methanol:Acetic acid (80:10:9:1)

A subset of the compounds used in the toxicity study was selected for the degradation study as there were too many to tackle in the project time frame. Methamphetamine, MDMA, pseudoephedrine, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene were selected and studied both under non-sterile soil conditions (where both biotic and abiotic degradation can take place) and sterile conditions (where only abiotic degradation can take place) in all the three soils. Most of the compounds showed substantial degradation in the sterile soils, indicating the possible role of the abiotic factors other than photolysis as the incubation was performed under dark throughout the experimental period. The degradation pattern of the 6 compounds both under the non-sterile and sterile soil conditions is presented in Figures 5 and 6.

The resistance to degradation in non-sterile soils was found in the following descending order: 1-benzyl-3-methylnaphthalene > methamphetamine > N-formylmethylamphetamine > MDMA > pseudoephedrine > 1-(1,4-cyclohexadienyl)-2-methylaminopropane (Figure 5). The degradation of methamphetamine and 1-benzyl-3-methylnaphthalene showed a fairly steady pattern throughout the incubation period when compared in terms of the residual concentration.

No changes in concentrations of methamphetamine and 1-benzyl-3-methylnaphthalene were apparent in the sterile soils within a one-year period (data not shown). MDMA, pseudoephedrine, and N-formylmethylamphetamine were somewhat less stable while 1-(1,4-cyclohexadienyl)-2-methylaminopropane was very unstable (Figure 6).

The half-life ($t_{½}$), degradation rate constant (k), regression equation, and correlation coefficient (r^2) values for the degradation of the 6 compounds in all the soils both under the non-sterile and sterile conditions is presented in Tables 3 and 4. In general, the half-life (days) values for the non-sterile degradation were recorded in the following ascending order: 1-(1,4-cyclohexadienyl)-2-methylaminopropane (0.8 to 8.3) < pseudoephedrine (3.7 to 30.1) < MDMA (15.4 to 59.0) < N-formylmethylamphetamine (35.0 to 43.6) < methamphetamine (130.9 to 501.7) < 1-benzyl-3-methylnaphthalene (150.5 to 10034.3). However, the same for the sterile degradation were found to follow the order: 1-(1,4-cyclohexadienyl)-2-methylaminopropane (2.60 to 5.6) < MDMA (75.3 to 107.5) < N-formylmethylamphetamine (188.1 to 301.0) < pseudoephedrine (143.3 to 501.7).

Interestingly, 1-(1,4-cyclohexadienyl)-2-methylaminopropane showed the fastest degradation both under the non-sterile and sterile conditions as indicated by the highest degradation rate constant (k) values (non-sterile: 0.0364 to 0.3834 day^{-1} and sterile: 0.0534 to 0.1157 day^{-1}) (Tables 3 and 4). In addition, almost a parallel degradation pattern of 1-(1,4-cyclohexadienyl)-2-methylaminopropane both under non-sterile and sterile conditions indicated the dominant role of the soil abiotic factors compared to biotic components.

Figure 5: Degradation patterns of the 6 compounds in 3 experimental soils under non-sterile condition

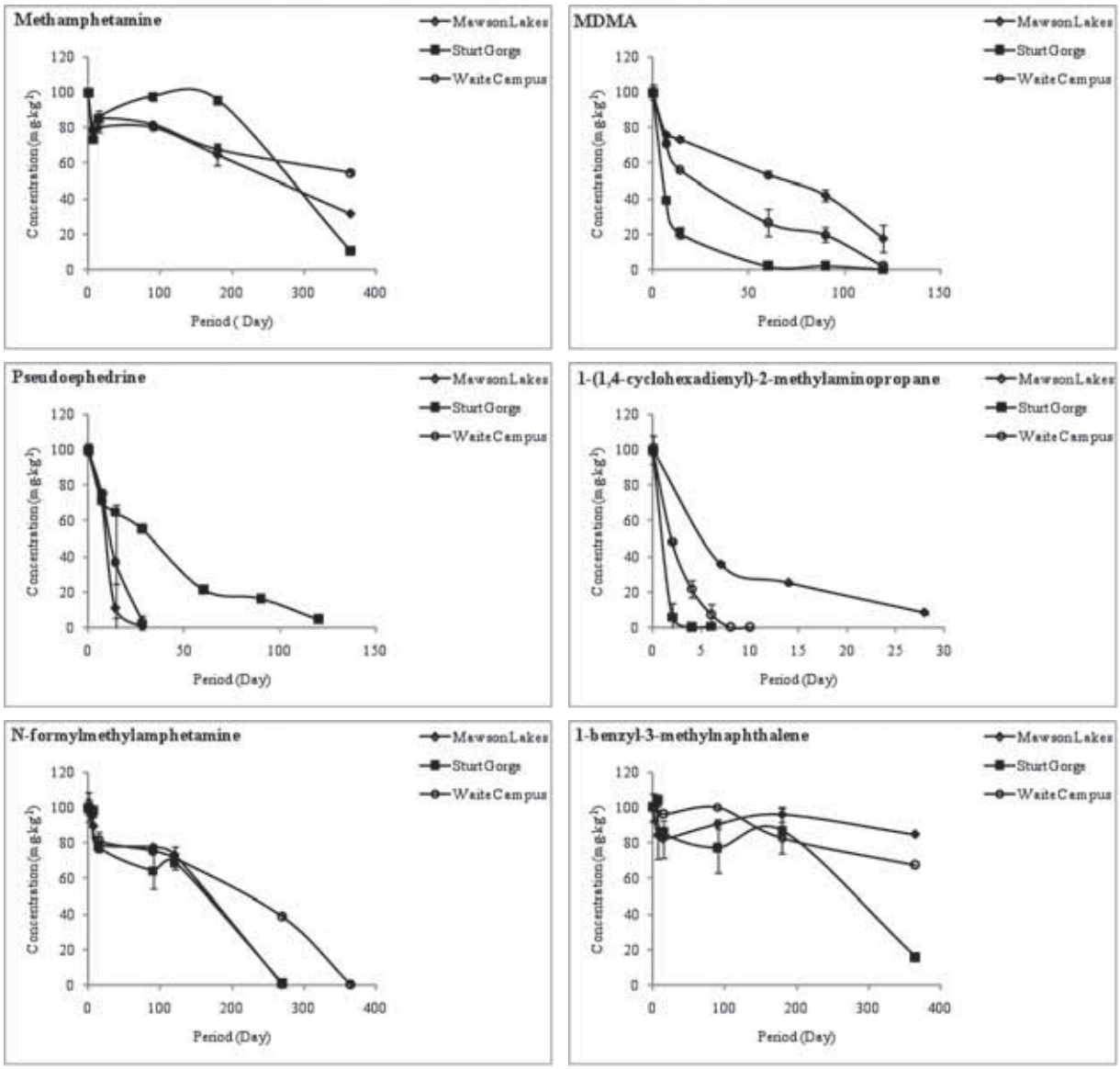


Figure 6: Degradation patterns of the 4 compounds in 3 experimental soils under sterile condition

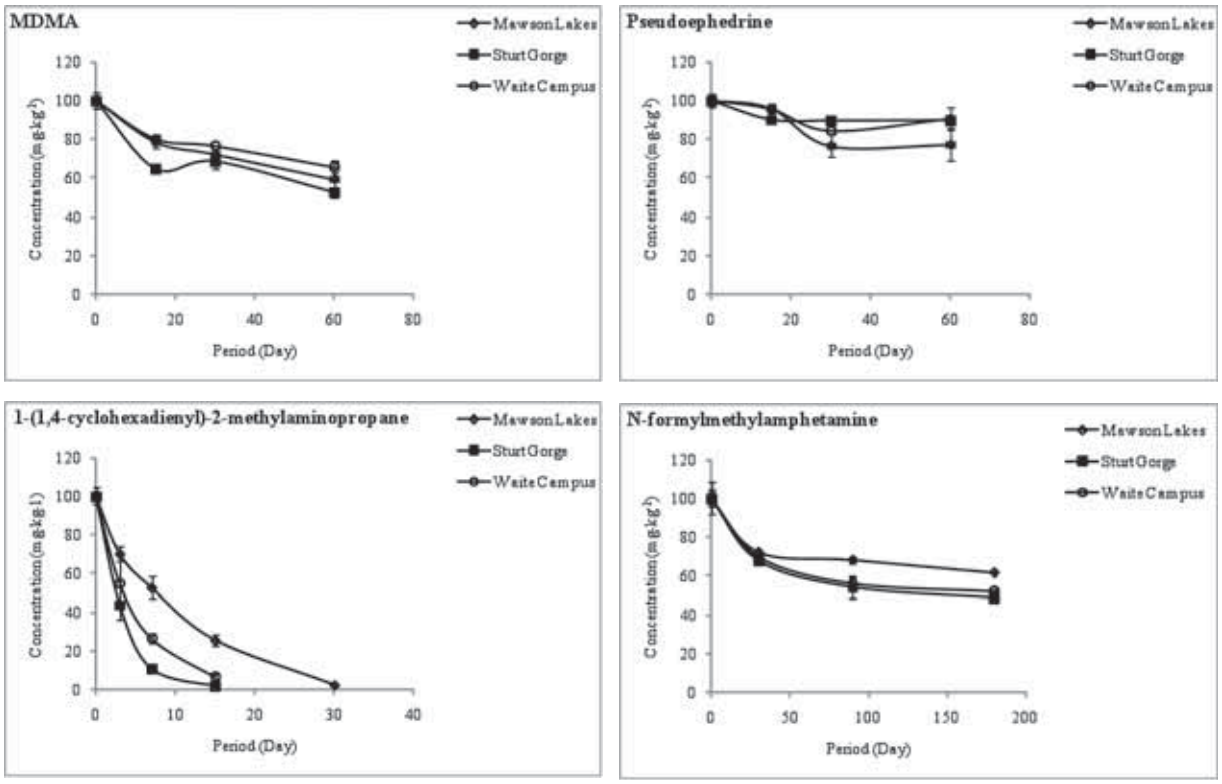


Table 3: Regression equation, degradation rate constant (k), half-life ($t_{½}$), and correlation coefficient (r^2) values for the degradation of the 6 compounds in the experimental soils under non-sterile condition

Soil	Compound	Regression equation	k (Day^{-1})	$t_{½}$ (Day)	r^2
Mawson Lakes	Methamphetamine	y = -0.0011x + 1.9563	0.0011	273.7	0.8873
Sturt Gorge		y = -0.0023x + 2.0450	0.0023	130.9	0.7095
Waite campus		y = -0.0006x + 1.9489	0.0006	501.7	0.8688
Mawson Lakes	MDMA	y = -0.0051x + 2.9735	0.0051	59.0	0.9078
Sturt Gorge		y = -0.0195x + 2.7630	0.0195	15.4	0.9420
Waite campus		y = -0.0113x + 2.9967	0.0113	26.6	0.8844
Mawson Lakes	Pseudoephedrine	y = -0.0814x + 3.1800	0.0814	3.7	0.9680
Sturt Gorge		y = -0.0100x + 2.9782	0.0100	30.1	0.9713
Waite campus		y = -0.0553x + 3.1646	0.0553	5.4	0.9409
Mawson Lakes	1-(1,4-cyclohexadienyl)-2-	y = -0.0364x + 2.9165	0.0364	8.3	0.9666
Sturt Gorge	methylaminopropane	y = -0.3834x + 2.6694	0.3834	0.8	0.7668
Waite campus		y = -0.2930x + 3.2280	0.2930	1.0	0.8834
Mawson Lakes	N-	y = -0.0086x + 3.2364	0.0086	35.0	0.8066
Sturt Gorge	formylmethylamphetamine	y = -0.0069x + 3.1672	0.0069	43.6	0.8404
Waite campus		y = -0.0052x + 3.1817	0.0052	57.9	0.7036
Mawson Lakes	1-benzyl-3-	y = -0.00003x + 1.9542	0.0000		0.0134
Sturt Gorge	methylnaphthalene	y = -0.0020x + 2.0461	0.0020	150.5	0.8213
Waite campus		y = -0.0005x + 2.0108	0.0005	602.1	0.9326

Table 4: Regression equation, degradation rate constant (k), half-life ($t_{½}$), and correlation coefficient (r^2) values for the degradation of the 6 compounds in the experimental soils under sterile condition

Soil	Compound	Regression equation	k (Day^{-1})	$t_{½}$ (Day)	r^2
Mawson Lakes	MDMA	y = -0.0036x + 1.9753	0.0036	83.62	0.9424
Sturt Gorge		y = -0.0040x + 1.9471	0.0040	75.26	0.7819
Waite campus		y = -0.0028x + 1.9751	0.0028	107.51	0.9071
Mawson Lakes	Pseudoephedrine	y = -0.0021x + 1.9921	0.0021	143.35	0.7313
Sturt Gorge		y = -0.0006x + 1.9816	0.0006	501.72	0.4839
Waite campus		y = -0.0008x + 1.9861	0.0008	376.29	0.3762
Mawson Lakes	1-(1,4-	y = -0.0534x + 2.0577	0.0534	5.64	0.9783
Sturt Gorge	cyclohexadienyl)-2-	y = -0.1157x + 1.9607	0.1157	2.60	0.9890
Waite campus	methylaminopropane	y = -0.0777x + 1.9839	0.0777	3.87	0.9991
Mawson Lakes	N-	y = -0.0010x + 1.9426	0.0010	301.03	0.7085
Sturt Gorge	formylmethylamphetamine	y = -0.0016x + 1.9290	0.0016	188.14	0.7898
Waite campus		y = -0.0014x + 1.9324	0.0014	215.02	0.7679

Metabolism/transformation patterns in soil

When the chromatograms were examined for the degradation of 1-(1,4-cyclohexadienyl)-2-methylaminopropane, both under non-sterile and sterile conditions, a peak at the same retention time as methylamphetamine was detected in each case after day zero. In full scan positive ionisation mode of ES-MS analysis, the most abundant ion detected from the target peak was 150.2 m/z, the $(M+H)^+$ ion, followed by 119.2 $[(M+H - CH_3NH)^+]$ (Appendix 7–9); this and the fragmentation pattern of the target peak confirmed the identification of it as methylamphetamine (Jones-Lepp et al., 2004; Castiglioni et al., 2006). To confirm further, we analyzed the soil extracts spiked with a known amount of methamphetamine standard. The spiked methamphetamine, 150.2 m/z ion, co-eluted at the same retention time with the original 150.2 m/z ion in the unspiked sample extract.

In Figure 7, data are plotted to show the formation of methamphetamine from 1-(1,4-cyclohexadienyl)-2-methylaminopropane at different period of incubation both in non-sterile and sterile soils. The results revealed that the maximum formation of methamphetamine occurred within the first four weeks of incubation in all the soils irrespective of experimental conditions. The data are re-displayed in Figures 8 and 9 in order to highlight the differences in methylamphetamine production caused by different types of soil, and the differences in methylamphetamine production caused by sterile and non-sterile soils.

Similar to the case for 1-(1,4-cyclohexadienyl)-2-methylaminopropane, N-formylmethylamphetamine was also found to produce methamphetamine, but at a lower rate (Appendix 10). Even after 1 year some N-formylmethamphetamine was still present in the incubation mixture.

Figure 7: A plot for the appearance of methamphetamine and the decline of 1-(1,4-cyclohexadienyl)-2-methylaminopropane at different periods of incubation both in non-sterile and sterile soils

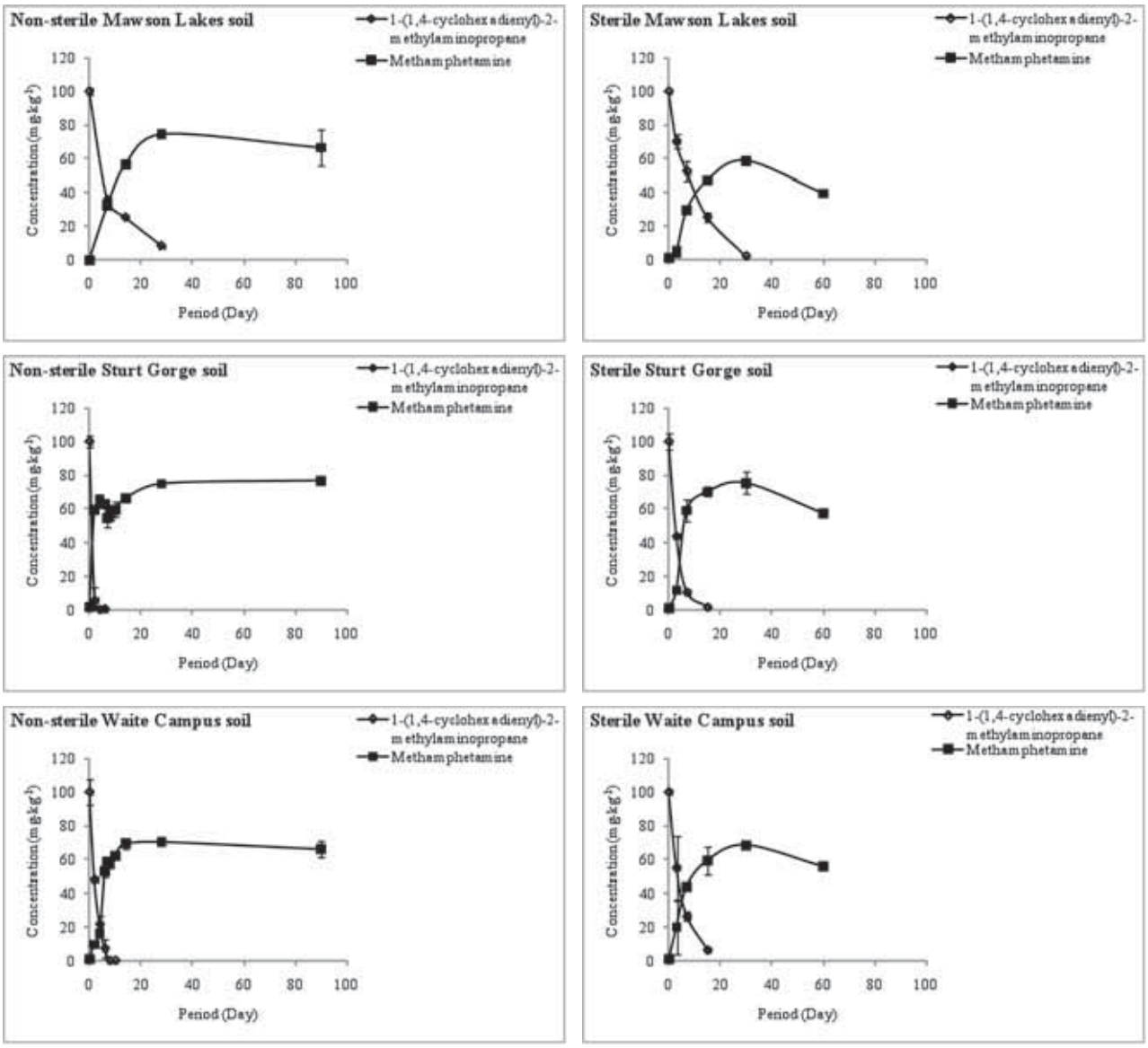


Figure 8: A plot to compare the potential of biotic-abiotic factors in the formation of methamphetamine from 1-(1,4-cyclohexadienyl)-2-methylaminopropane in 3 soils at different periods of incubation

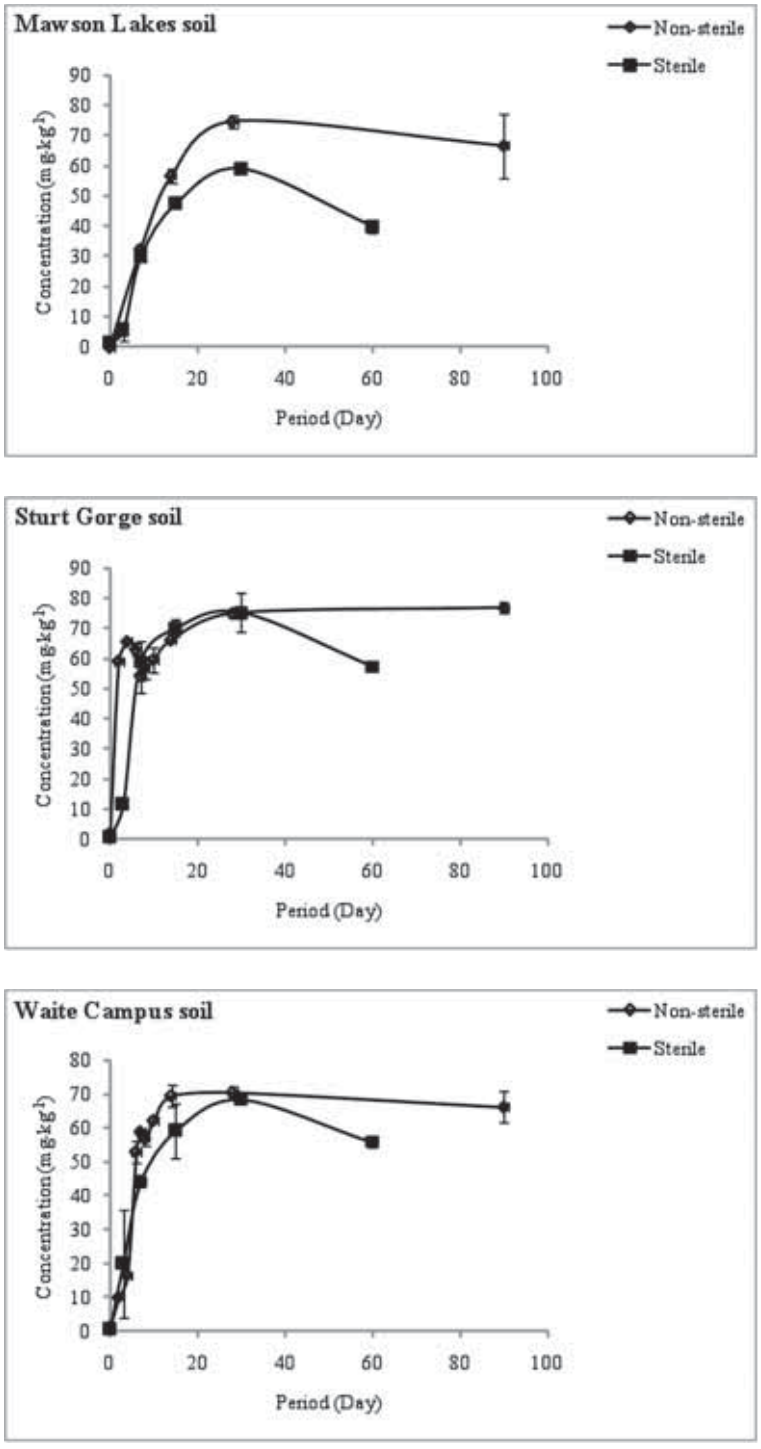
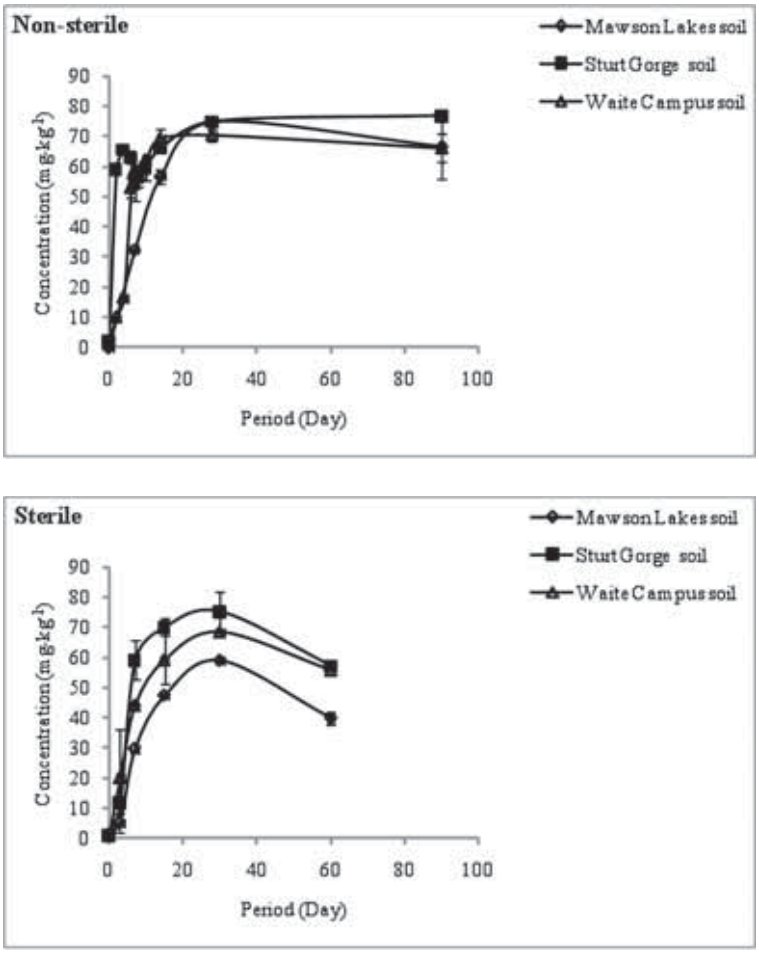


Figure 9: A plot to compare the potential of methamphetamine formation from 1-(1,4-cyclohexadienyl)-2-methylaminopropane among the soils at different periods of incubation under non-sterile and sterile conditions



Adsorption desorption pattern

Batch sorption studies of the compounds will give an indication of the strength of association that the test compounds have with soil, which has important implications for migration of these compounds within soil. Also, the extent of sorption gives an indication of the potential availability of the test compounds to degrade microorganisms within the soil. To begin with, the batch sorption of methamphetamine, MDMA, pseudoephedrine, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane was conducted in all the three soils varying initial analyte concentration. To check the best fit pattern, the data were discussed employing both the Langmuir and Freundlich equations with different exploratory approaches. A summary of the sorption parameters are presented in Table 5. The Langmuir isotherm principally assumes monolayer sorption with no interaction between the sorbate, while the Freundlich isotherm is an empirical relationship describing the sorption of solute to solid surface (Voudrias et al., 2002).

In Figure 10, the adsorption isotherms (amount sorbed vs. equilibrium concentration) have been plotted for all the target compounds. The adsorption of all the target compounds on different experimental soils revealed the differences induced by the initial concentrations, and increased

mostly with increase in the concentration level with few exceptions. Thus, the present study seems to be best represented by Freundlich isotherms. The results when discussed in terms of the respective r^2 values (i.e., regression coefficient of each equation fit), show experimental data fits better in Freundlich equation ($r^2 > 0.99$) irrespective of the target compounds and experimental soils. 1/n value in Freundlich equation measures the sorption intensity and $1/n < 1$ indicates a normal Langmuir isotherm, while $1/n > 1$ indicates cooperative sorption (Voudrias et al., 2002). The 1/n values in the present study mostly recorded below unity with exceptions for methamphetamine, pseudoephedrine, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane in Mawson Lakes soils, thus conforming towards the Langmuir equation. Thus, it is reasonable to assume that both the Langmuir and Freundlich equations can be employed to describe the sorption pattern in this study.

The equilibrium constants (i.e., K_L and K_F values in Langmuir and Freundlich equations, respectively) values ($\mu g\text{-}g^{-1}$) represent the amount of compound adsorbed per unit soil when equilibrium concentration is 1. The K values in the present study when compared between the target compounds showed the maximum values for 1-(1,4-cyclohexadienyl)-2-methylaminopropane and MDMA followed by methamphetamine and pseudoephedrine. On the other hand, Sturt Gorge soil recorded highest K values among the test soils for all the target compounds. Thus, relatively higher sorption potential is likely for 1-(1,4-cyclohexadienyl)-2-methylaminopropane and MDMA in Sturt Gorge soil. For better understanding of the sorption pattern, the results were analyzed with different exploratory approaches. The adsorption coefficient (K_d) and organic carbon normalised adsorption coefficient (K_{OC}) were calculated for all the treatment cases. The results of K_d and K_{OC} were compared between the soils as a function of the initial concentration levels of the target compounds and plotted in Figures 11 and 12. In general, all these values were found to differ with target compound as well as the experimental soils. The K_d values were relatively stable for the Mawson Lakes and Waite Campus soils for most of the experimental conditions, although few exceptions recorded in the lower concentration range for all the compounds. In contrast, the K_d values for the Sturt Gorge soil declined with increase in the initial concentration of the compounds. Mix patterns were also discernible for different compounds when compared in terms of test soils. The K_{OC} values followed nearly a similar pattern with K_d, although 1-(1,4-cyclohexadienyl)-2-methylaminopropane recorded dissimilar patterns for the different soils. In summary, the result of the present study indicates higher sorption potential of 1-(1,4-cyclohexadienyl)-2-methylaminopropane and MDMA in all the test soils, especially in Sturt Gorge soil.

The sorption results can be extrapolated to get an idea of the theoretical values of maximum amount of the analyte sorbed in each soil. It was apparent from the results that the maximum amount of MDMA, pseudoephedrine, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane was sorbed in the following descending order: Mawson Lakes soil > Sturt Gorge soil > Waite Campus soil. In contrast a reverse pattern was discernible for methamphetamine. Although Sturt Gorge soil showed moderate sorption potential irrespective of the target compounds, the increase in the analyte concentration might have influenced the interaction between the analyte and test soils. To get an idea on the leaching potential of the target compounds, investigation on the desorption behavior of the compounds is important. In general, percent desorption was recorded in the following descending order: pseudoephedrine > methamphetamine > MDMA > 1-(1,4-cyclohexadienyl)-2-methylaminopropane irrespective of the test soils (Figure 13). Thus, pseudoephedrine can be considered most prone to leaching in the test soils. When we compared between the test soils, Sturt Gorge soil recorded the least percent desorption throughout the target compounds, indicating least leaching potential through this soil.

In order to more fully describe the sorption kinetics, a detailed experiment was performed with 1-(1,4-cyclohexadienyl)-2-methylaminopropane at a single concentration level (100 $\mu g/mL^{-1}$) for 48 hours (Figure 14). The adsorption of 1-(1,4-cyclohexadienyl)-2-methylaminopropane over time showed a stable pattern for the Mawson Lakes soil. In contrast, the adsorption pattern for the Sturt Gorge and Waite Campus soils was found to increase moderately over time.

Figure 10: A plot for the sorbed amount (µg/g-1) of 4 compounds in 3 experimental soils as a function of equilibrium concentration (µg/mL^{-1})

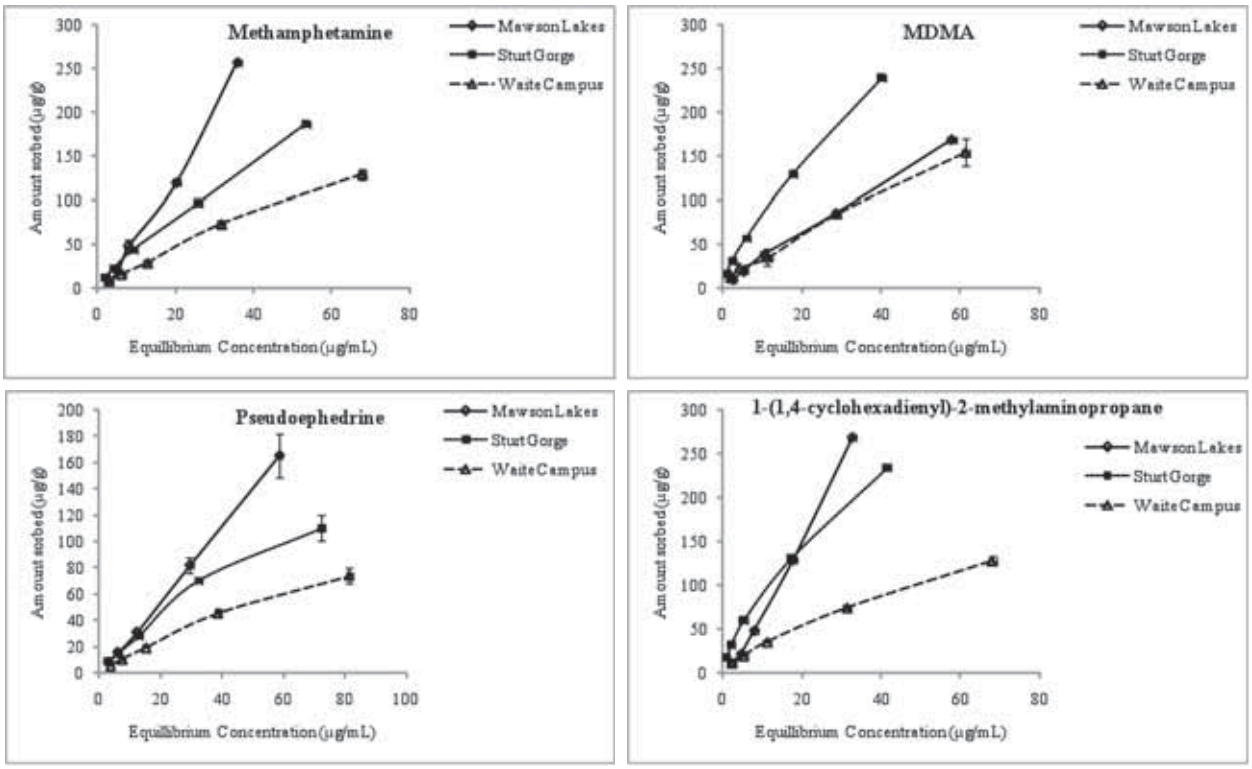


Figure 11: Adsorption coefficient (K_d) values derived for each of the 4 compounds are compared between the soils as a function of the initial concentration levels of adsorbate

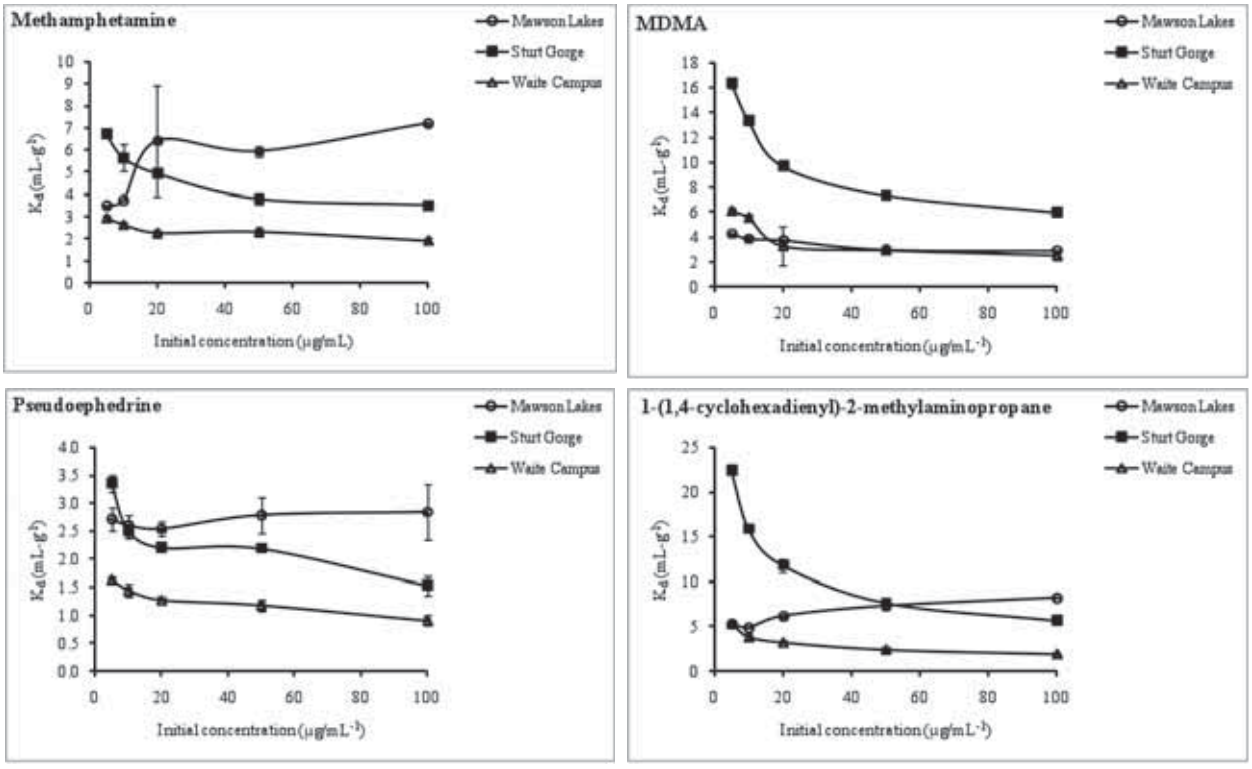


Figure 12: Organic carbon normalised adsorption coefficient (K_{OC}) values derived for each of the 4 compounds are compared between the soils as a function of the initial concentration levels of adsorbate

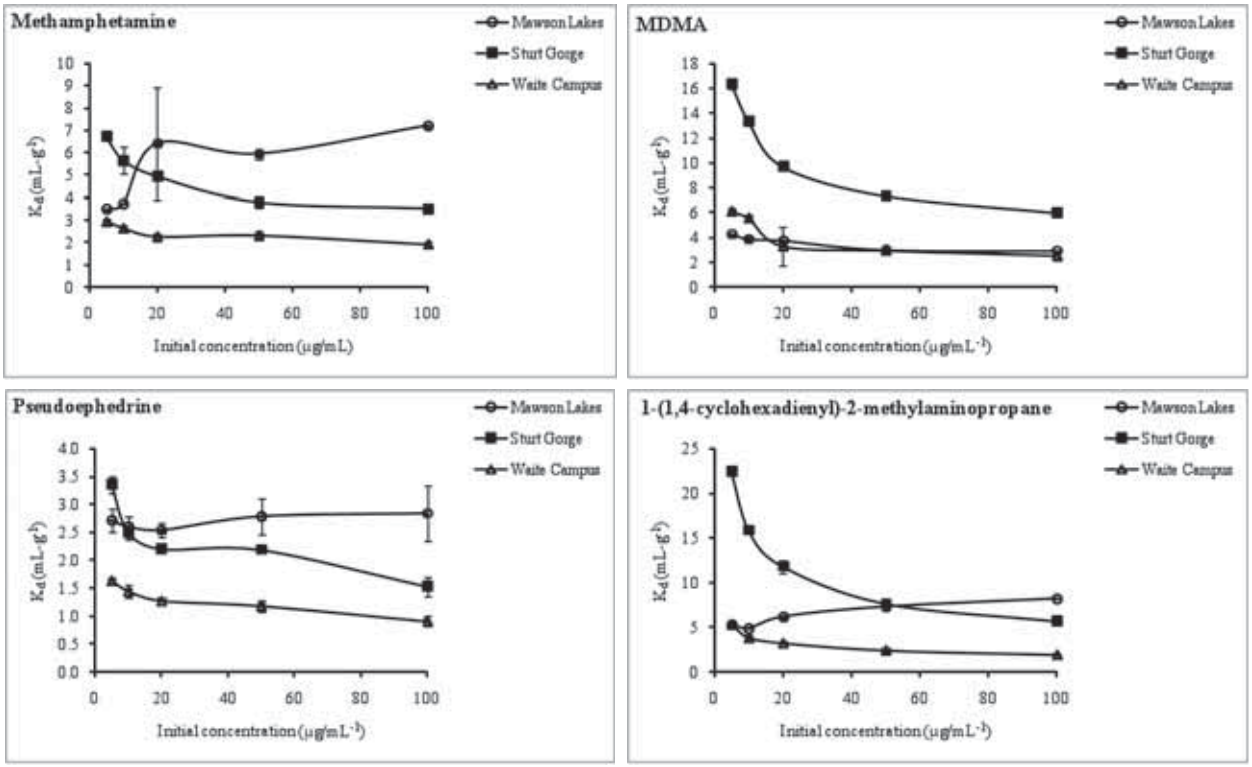
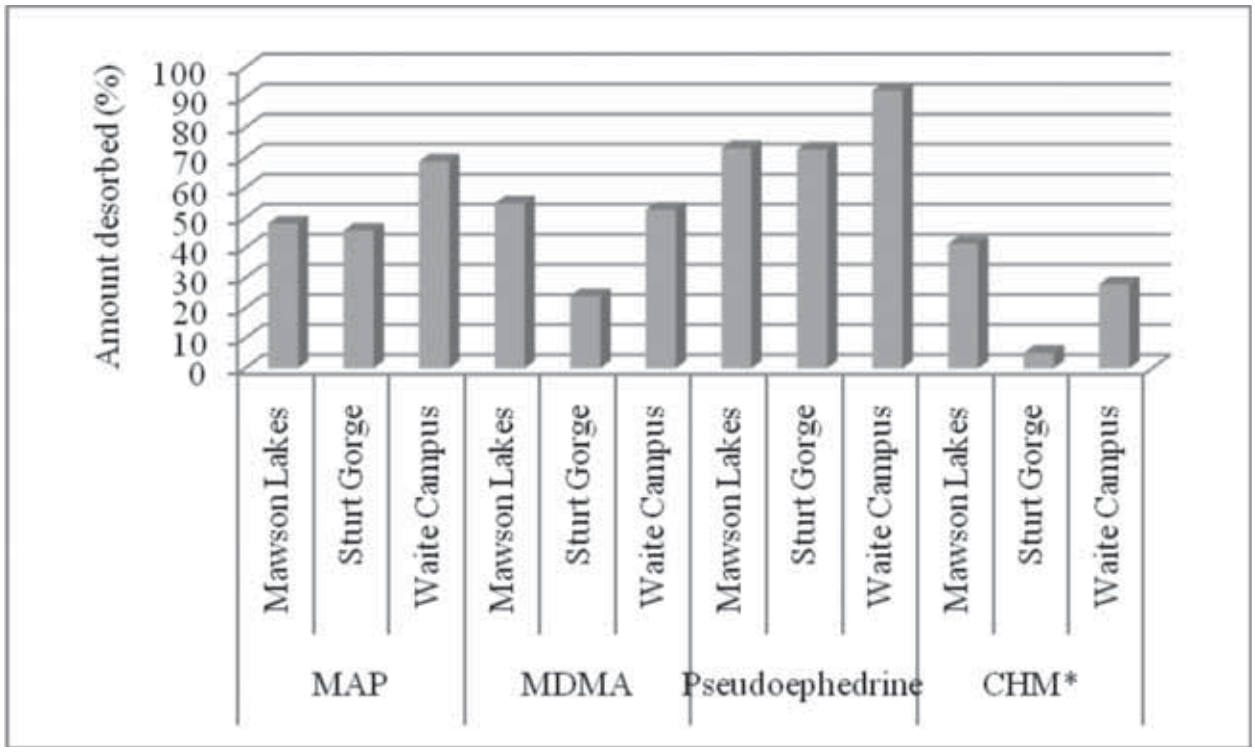


Figure 13: Desorption pattern of the of the target compounds in 3 experimental soils



*1-(1,4-cyclohexadienyl)-2-methylaminopropane

Figure 14: Adsorption patterns of 1-(1,4-cyclohexadienyl)-2-methylaminopropane compared between the experimental soils as a function of time

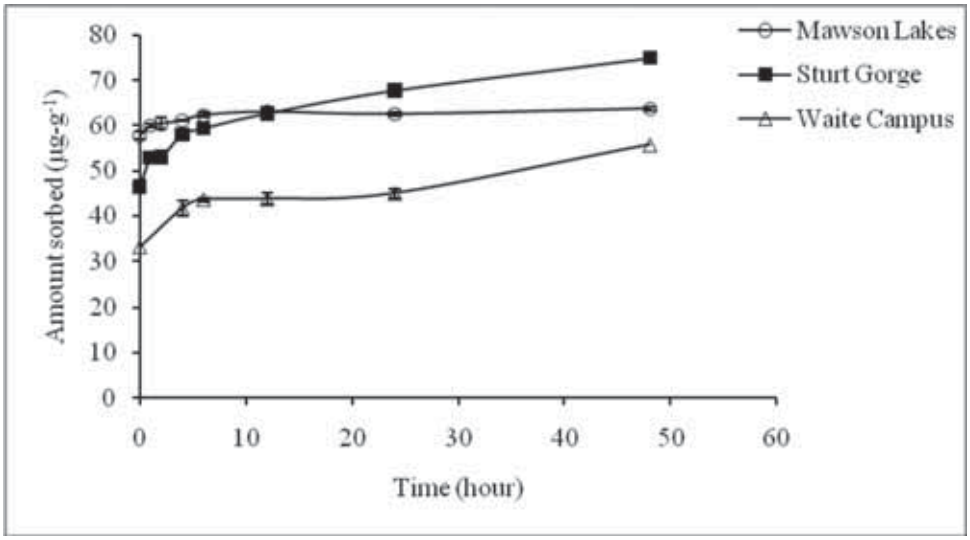


Table 5: Summary of the sorption parameters for the 5 illicit drugs in 3 experimental soils

Soil	Compound	Langmuir parameters		Freundlich parameters		
		K_L	r^2	K_F	n	r^2
Mawson Lakes	Methamphetamine	0.0150	0.6158	2.70	0.78	0.9890
Sturt Gorge		0.0147	0.8464	7.52	1.25	0.9994
Waite campus		0.0065	0.8315	3.27	1.14	0.9980
Mawson Lakes	MDMA	0.0076	0.8302	4.78	1.14	0.9991
Sturt Gorge		0.0334	0.8908	16.31	1.38	0.9995
Waite campus		0.0177	0.7343	7.31	1.38	0.9873
Mawson Lakes	Pseudoephedrine	0.0013	0.5168	2.55	0.98	0.9991
Sturt Gorge		0.0124	0.8851	3.92	1.28	0.9926
Waite campus		0.0087	0.9554	2.05	1.21	0.9979
Mawson Lakes	1-(1,4-	0.0128	0.8099	4.20	0.84	0.9958
Sturt Gorge	cyclohexadienyl)-2-	0.0502	0.9261	20.33	1.52	0.9999
Waite campus	methylaminopropane	0.0202	0.9176	6.39	1.41	0.9990

Chapter three: Summary

The data on toxicological studies suggest that potential for the disruption of normal soil microbial function in the selected soils is only likely to occur at relatively high concentrations of the test compounds. This was only seen for 6 compounds, where dehydrogenase activity was reduced. Since dehydrogenase activity is an indicator of active microbial biomass generally used to study the effects of pollutants on microorganisms in soil, this observed effect is worth further consideration. Longer-term effect on dehydrogenase activity should be assessed to determine whether adaptation to ATS and related compounds occurs. A long-term decrease in soil respiration could potentially lead to a decrease in the ability of a terrestrial environment to degrade such compounds. This has important implications for remediation strategies for clandestine laboratory clean-up sites. The results of the present study involves test soils with widely different physico-chemical properties; thus, they may provide an immediate idea on the fate and impact of these target compounds on soils within other climatic zones of Australia.

The degradation study revealed that MDMA, pseudoephedrine, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane were less persistent in all three test soils than methamphetamine, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene over a one-year incubation period. In general, resistance to degradation in non-sterile soils was recorded in the following descending order: 1-benzyl-3-methylnaphthalene > methamphetamine > N-formylmethylamphetamine > MDMA > pseudoephedrine > 1-(1,4-cyclohexadienyl)-2-methylaminopropane. In sterile soils, methamphetamine and 1-benzyl-3-methylnaphthalene showed no measurable changes in concentration level over one-year period of incubation. 1-(1,4-cyclohexadienyl)-2-methylaminopropane was found most susceptible to degradation amongst the target compounds. It transformed into methamphetamine mostly within a few weeks in all the test soils. Similarly, the soils spiked with N-formylmethylamphetamine also showed a production of methamphetamine, but the transformation was incomplete at one year. These results indicate that residues of drug manufacture entered in soil can be used in a forensic context to form evidence of drug manufacture under certain circumstances, but caution is required. In the case that residues from the production of methylamphetamine from pseudoephedrine using the HI/P method are encountered, methylamphetamine and the key by-product 1-benzyl-3-methylnaphthalene will persist, making the forensic interpretation relatively straightforward. However, as indicated in our previous work, any phenyl-2-propanone by-products present will degrade quickly, and as indicated in this work, pseudoephedrine precursor will suffer the effects of leaching. In relation to interred residues from the Leuckart synthesis of methamphetamine, the key intermediate N-formylmethamphetamine will be susceptible to degradation; its absence in residues therefore should not be relied upon to exclude the possibility of the Leuckart synthesis. Moreover, the results of this work show that the discovery of methylamphetamine in interred residues, especially if it is present at low levels or with N-formylmethamphetamine, cannot be used as proof of the manufacture of methylamphetamine prior to the interment of the residue; the possibility exists that the methylamphetamine could have arisen as a result of decomposition of N-formylmethamphetamine in the soil. The situation is likely to be similar for the Leuckart synthesis of amphetamine. It is reasonable to expect that N-formylamphetamine will degrade to amphetamine in soil analogous to the way in which N-formylmethamphetamine degrades to methylamphetamine. Therefore, until additional work is conducted to confirm or refute this expectation, caution should be exercised when amphetamine or amphetamine and N-formylamphetamine mixtures are detected in interred residues. In relation to the Nazi method, the key manufacturing by-product 1-(1,4-cyclohexadienyl)-2-methylaminopropane also decomposes to methylamphetamine. Similarly, its absence in interred residues cannot be used to exclude the involvement of the Nazi method, nor can the presence of methylamphetamine or mixtures of 1-(1,4-cyclohexadienyl)-2-methylaminopropane and methylamphetamine be used to infer the manufacture of methylamphetamine prior to interment of the residue.

The current LC-MS and GC-MS methods selected for the compounds used in the degradation assays are suitably sensitive and selective. These methods have been optimised for soil extractions to assess how the soil matrix can affect the analytical sensitivity, along with a suitable soil extraction technique for the range of compounds selected.

Batch sorption studies revealed higher sorption potential of 1-(1,4-cyclohexadienyl)-2-methylaminopropane and MDMA in all the test soils, especially in Sturt Gorge soil. On the other hand, maximum desorption potential as measured by percent desorption was highest for pseudoephedrine amongst the target analytes and Sturt Gorge soil within the test soils. To sum up, pseudoephedrine is found most labile in the present work and may lead to potential contamination of the surrounding environment.

Chapter four: Future Directions and Recommendations

Although the soils used in this study are representative of many found across Australia, the behaviour of other types of soil upon exposure to ATS-related chemicals should be investigated.

Of necessity the range of chemicals investigated in this study was restricted, especially in the degradation study. A study involving additional precursors, reagents, and manufacturing by-products is warranted.

The results of the present study were achieved using each compound in isolation. It is probable that the effects of two chemicals on microorganisms will be greater than the effect of each chemical individually, or the sum of the individual effects. As clandestine laboratory waste might contain mixtures of drug, unused precursor, reagents, and by-products, it is important to determine toxicity and degradation patterns employing appropriate combinations of compounds.

As waste might remain *in situ* for long periods, or clandestine laboratories might operate in the same location for a long time, degradation studies over a period longer than 12 months should be conducted with selected compounds.

Although some compounds studied did show some degradation, products of degradation were not detected. This could be because products were only present in trace amounts under the small-scale conditions used, or it could be that products are rapidly degraded. A study involving larger quantities of pseudoephedrine, MDMA, or methamphetamine could be informative.

As N-formylamphetamine is chemically very similar to N-formylmethamphetamine, it is likely to undergo an analogous degradation, which would produce amphetamine. As both amphetamine and N-formylamphetamine are of high significance to clandestine laboratory investigation (especially in Europe), a study of the degradation of N-formylamphetamine is also warranted.

The fate of any chemicals in soil depends on the soil physico-chemical properties, moisture level, temperature, soil microbial biomass and their activities, nature of plant cover, etc. The nature and content of organic matter and clay in a soil determines the sorption-desorption pattern of chemicals and their potential availability in the soil solution to undergo chemical (abiotic) and/or microbial (biotic) factors to act on the compound. Thus, a detailed characterisation of the influence of soil organic matter and clay upon the behavior of illicit drugs in soil is warranted.

Microorganisms can adapt to the presence of chemicals and utilise them as a source of energy. Attempts should be made to isolate and characterise tolerant microbial communities from degradation experiments and investigate whether it is possible to exploit their capability as a safe and efficient remediation of contaminated sites. This tactic has been used successfully in relation to oil spills at sea.

In order to minimise uncontrolled variance in this study, the soil samples were sieved to remove plant matter, stones, etc. It is possible that the materials removed for this study have an effect upon the fate of chemicals in the soil; therefore tests involving 'real life' soils should be conducted in order to highlight any effects.

It is possible that clandestine drug laboratories might involve levels of contamination much greater than were modelled in this study. Measurement of the concentrations of compounds at real clandestine laboratory sites should be undertaken in order to identify a realistic 'upper concentration limit' for microbial function assays.

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Appendix 1

Experimental Methods

Target compounds

The first phase of the project dealt with a total of 15 compounds focusing on the toxicological impacts on a number of soil microbial functions (Appendix 1A). However, in the interests of achieving a successful conclusion to this project in the required time frame, for the degradation and adsorption-desorption study the Project Steering Committee reduced the list of compounds to be investigated. The compounds selected for the second phase of this project were parent drug (methamphetamine, MDMA (3,4-methylenedioxymethamphetamine)), precursor (pseudoephedrine), and by-product (1-(1,4-cyclohexadienyl)-2-methylaminopropane, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene). The basic information (e.g., IUPAC nomenclature, molecular formula, molecular weight, chemical structure, etc.) of the target compounds investigated in this study are briefly summarised in Appendix 1B.

The method of synthesis for amphetamine sulphate, 1-benzyl-3-methylnaphthalene, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane are described in Appendix 2.

Test soils

In the present study, the soils were collected from the Mawson Lakes (ML), Sturt Gorge (SG), and Waite Campus (WC), which are originally urban impacted backyard, bush, and agricultural land, respectively. The basic physico-chemical properties of the soils are summarised in Appendix 3. The physico-chemical properties of the soils were measured following standard analytical procedures. The dissolved organic carbon of the test soils were extracted with 0.01 M $CaCl_2$ for 1 h at 25^0C. The three soils exhibited a broad range of characteristics in terms of organic carbon, clay content, soil texture, pH, surface area, etc. The pH (in 1:2.5 H_2O) of the soils ranged between 5.64 and 5.98 (slightly acidic) for WC and SG soils, respectively to 8.91 (alkaline) for ML soil. The cation exchange capacity of the soils ranged between 6.30 to 19.24 meq/100g soil. The organic carbon content varied between 1.11% (ML) to 2.88% (SG). The soils contained a moderate level of clay (15–20%). The ML and SG soils were sandy loam while WC soil was loam in texture. The B.E.T. surface area (m^2/g) was recorded in the descending order of 26.67 > 9.36 > 4.59 for the ML, SG, and WC soils, respectively.

For the purpose of the present study, we collected surface soils (0–15 cm) from the three sites mentioned above. The soils were stored in polyethylene buckets and brought to the laboratory. The soils were screened physically to remove any plant parts or other artifacts, passed through 2 mm sieve, and kept in a cold room maintained at 4^0C temperature. Although plant material and stones are common in soil under natural conditions, they were removed in order to eliminate uncontrolled additional variances in the degradation study.

To study the sorption behavior, we used air dried soils. However, for the degradation study the soils were pre-incubated in the dark at 25^0C for 7 days to stabilise the microbial activities prior to commencement of the experiment. Here, soils were incubated at 50% of the maximum water-holding capacity of each soil by adding requisite amounts of sterile Milli-Q water.

The soils throughout Australia are quite variable in respect of their genesis and basic properties. Those used in this study are representative of the most common soil types in Australia, with the soil

from Mawson Lakes representative of soils influenced by urban activities, the soil from Sturt Gorge representative of dry bush lands, and soil from Waite Campus typical of agricultural soils. Other soils from around Australia that exhibit similar physico-chemical properties (e.g., organic carbon, clay content, soil texture, pH, and surface area) to the ones studied can be reasonably expected to yield similar results to those described in this work.

Experimental design to study the adsorption-desorption pattern in soils

In this section, the sorption-desorption pattern of methamphetamine, MDMA, pseudoephedrine, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane was investigated in all the three soils (ML, SG, and WC). N-formylmethylamphetamine and 1-benzyl-3-methylnaphthalene, being insoluble in water, were not used in this study. Sorption experiments were run at 25 ± 1^{0}C temperature and in the dark to avoid any chances of photodegradation. For this purpose, we used 50 mL PP tubes fitted with a screw cap. The background solution was 0.01 M $CaCl_2$ in Milli-Q water. Stock solutions (100µg/mL^{-1}) for all the target compounds were prepared in 0.01M $CaCl_2$ solution. Adsorption isotherms were developed at five different initial concentration levels (e.g., 5, 10, 20, 50, and 100µg/mL^{-1}) for all the compounds. To 5 g of soil, requisite amount of the stock solution was added to maintain the desired concentration level. The background solution was maintained at 20 mL volume. Blank sample (without soil) was also maintained as the reference for the initial concentration. All the experiments were conducted in duplicate. The vials were shaken in an end-over-end shaker at 10 rpm. Samples were taken at the end of the 24 h period, by which time it was judged that adequate equilibration had been achieved. The supernatants were then centrifuged at 3000 rpm for 15 min. and passed through 0.45 µm filter for direct analysis in HPLC-MS. The amount sorbed was determined by the mass balance calculation of the initial and final concentrations of the compound in solution. The detailed calculation of sorption isotherms are described in Appendix 4.

Desorption experiment was conducted only at the 20µg/mL concentration level for all the three soils. For this purpose, the aliquot from the sorption study was discarded and replaced by the background solution (20 mL). The vials were shaken under the same experimental conditions for 24 h and preceded likewise the sorption experiment. The amount desorbed was also estimated on the basis of the mass balance calculation.

To gain a better knowledge on the sorption kinetics, our experimental plan was extended further. At this stage, an experiment was designed with 1-(1,4-cyclohexadienyl)-2-methylaminopropane to study the adsorption isotherm as a function of time at a single concentration of 100µg/mL^{-1}. 1-(1,4-cyclohexadienyl)-2-methylaminopropane was chosen for this purpose due to its relatively fast degradation rate in all the three soils compared to the other compounds. Here a similar experimental procedure was followed as in the previous sorption experiments. However, the samples were withdrawn at different time intervals, such as 0, 1, 2, 4, 6, 12, 24, and 48 h. The sorption experiments were conducted with non-sterile soils.

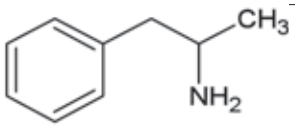
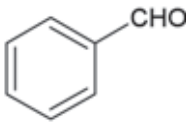
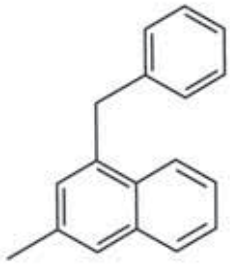
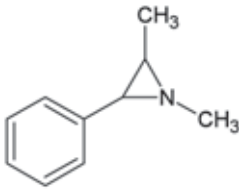
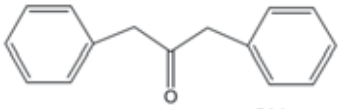
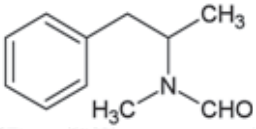
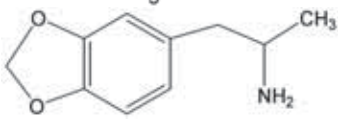
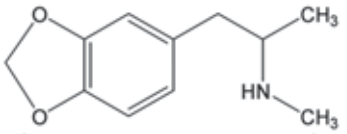
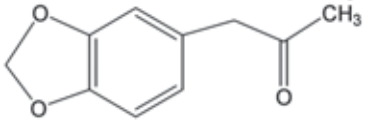
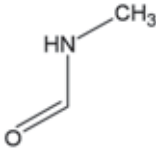
Experimental design to study the degradation pattern in soil

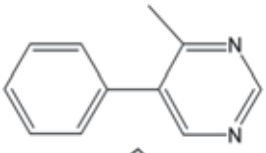
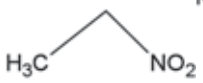
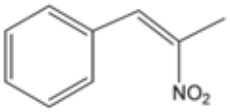
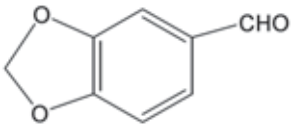
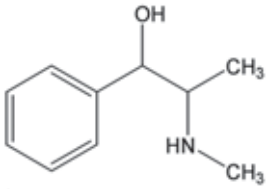
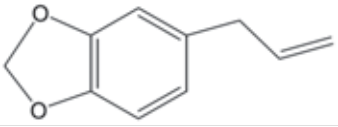
In this section, the biotic-abiotic degradation patterns of methamphetamine, MDMA, pseudoephedrine, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene were investigated at 50% of maximum water-holding capacity of all the three soils (ML, SG, and WC) for a one year period. The stock solutions (2g/L) for the methamphetamine, MDMA, pseudoephedrine, and 1-(1,4-cyclohexadienyl)-2-methylaminopropane were prepared in water, while the same for N-formylmethylamphetamine and 1-benzyl-3-methylnaphthalene were prepared in acetone and hexane, respectively, at 20g/L concentration level. In the present study, soils were spiked at a single application rate (100µg/g)

and a single compound. The degradation patterns were investigated at 50% of maximum water-holding capacity of soils and at 25 ± 2^{0}C temperature.

The incubation temperature and moisture level considered in the present work represents the optimum conditions for the soil microbial activities. To avoid any chance of photodegradation, the soils were incubated in the dark. Five grams of soil in individual amber colored screw cap vials of 40 mL capacity were spiked with the test compounds. The pre-incubated soils were used for both the non-sterile and sterile degradation studies. For abiotic degradation, the soils (in individual vials) were autoclaved for three consecutive days at 121^{0}C for 20 min. The soils for both the non-sterile and sterile degradations were spiked with requisite amount of the freshly prepared stock solution. In the case of the sterile degradation, the stock solutions were passed through sterile 0.45 µm filter and soils were spiked aseptically within a laminar air flow. The soils were vortexed for homogenisation. The vials containing soils spiked with N-formylmethylamphetamine or 1-benzyl-3-methylnaphthalene were kept open for a few minutes for the complete removal of the solvents (acetone or hexane) at room temperature. Three different types of control soils were also maintained, which received only the background solvents of the respective stock solutions without compound. The moisture content of the soils (both in non-sterile and sterile) were maintained by aseptic addition of sterile Milli-Q water. All the experiments were conducted in duplicate. The sample extraction procedure, determination by HPLC-MS and GC-MS, and calculation of half-life values are described in Appendix 5.

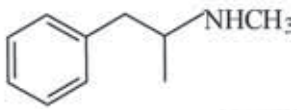
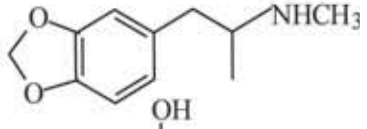
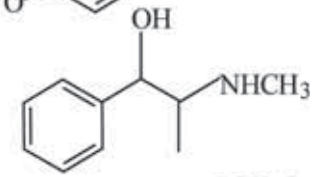
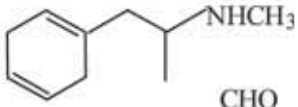
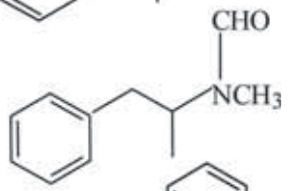
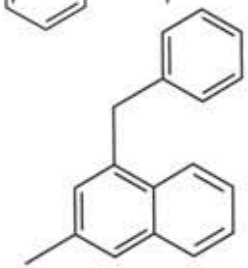
Appendix 2A A list of the target compounds used in the first phase of the project to assess their effect on soil microbial functions in soils

Compound	Use of compound	Structure
Amphetamine	ATS [a]	
Benzaldehyde	Precursor	
1-benzyl-3-methylnaphthalene	By-product	
3,4-dimethyl-1-phenylaziridine	By-product	
1,3-diphenylacetone	By-product	
N-Formylmethamphetamine	Intermediate	
MDA [b]	ATS	
MDMA [c]	ATS	
MDP-2-P [d]	Precursor	
N-methylformamide	Precursor	

4-methyl-5-phenylpyrimidine	By-product	
Nitroethane	Precursor	
2-nitro-1-phenylpropene	Precursor	
Piperonal	Precursor	
Pseudoephedrine	Precursor	
Safrole	Precursor	

[a] Amphetamine-type stimulant
[b] Methylenedioxyamphetamine
[c] Methylenedioxymethamphetamine
[d] Methylenedioxyphenyl-2-propanone

Appendix 2B A list of the target compounds used in the second phase of the project to study their sorption and degradation patterns in soils

Target compound Full name	IUPAC nomenclature	Molecular formula	Molecular weight	Molecular structure
Methamphetamine	N-methyl-1-phenyl-propan-2-amine	$C_{10}H_{15}N$	149.24	
3,4-methylenedioxy-methamphetamine	1-(benzo[1,3]dioxol-5-yl-N-methylpropan-2-amine	$C_{11}H_{15}NO_2$	193.25	
Pseudoephedrine	(1*S*,2*S*)-2-methylamino-1-phenylpropan-1-ol	$C_{10}H_{15}NO$	165.23	
1-(1,4-cyclohexadienyl)-2-methylaminopropane	1-(1′,4′-cyclohexadienyl)-2-methylaminopropane	$C_{10}H_{17}N$	151.25	
N-Formylmethylamphetamine	N,N-dimethyl-1-phenyl-propan-2-amine	$C_{11}H_{15}NO$	177.25	
1-benzyl-3-methylnaphthalene	1-benzyl-3-methylnaphthalene	$C_{18}H_{16}$	232.32	

Appendix 3

Synthesis of compounds

Amphetamine sulphate and 1-benzyl-3-methylnaphthalene were synthesised as detailed below.

Amphetamine sulphate was synthesised from the reductive amination of phenyl-2-propanone (P-2-P). P-2-P was mixed with hydroxylamine hydrochloride to form an oxime. This oxime was reduced with sodium metal to yield amphetamine, which was precipitated as the sulphate salt through the addition of sulphuric acid in diethylether. The purity of the product was determined using GC-MS.

1-benzyl-3-methylnaphthalene is a by-product of the hydroiodic acid/red phosphorus reduction of pseudoephedrine, where P-2-P, a by-product of the reaction, reacts further with acid and rearranges to form a mixture of 1-benzyl-3-methylnaphthalene and 1,3-dimethyl-2-phenylnaphthalene (Cantrell et al., 1988). P-2-P was refluxed with hydroiodic acid and yielded equal quantities of 1-benzyl-3-methylnaphthalene and 1,3-dimethyl-2-phenylnaphthalene. Purity of the products was assessed using GC-MS. The respective naphthalenes were separated using a silica column. Purity of the separated naphthalenes was verified using GC-MS, TLC and ^{1}H-NMR.

Synthesis of 1-(1,4-cyclohexadienyl)-methyl-2-aminopropane:

1-(1,4-cyclohexadienyl)-methyl-2-aminopropane is a by-product of the Birch reduction of pseudoephedrine, where pseudoephedrine is reduced by lithium metal in the presence of ammonia. The presence of protons allows reduction of the aromatic ring of methamphetamine into the cyclohexadienyl moiety.

The Birch reduction process was undertaken by dissolving pseudoephedrine in liquid ammonia and adding lithium metal (Zvilichovsky & Gbara-Haj-Yahia, 2004). Tertiary butyl alcohol was also added to the mixture as a proton source, further reducing methamphetamine to 1-(1,4-cyclohexadienyl)-methyl-2-aminopropane. The reaction was allowed to take place overnight before the 1-(1,4-cyclohexadienyl)-methyl-2-aminopropane was extracted and precipitated as a hydrochloride salt. ^{1}H-NMR and LC-MS were used to assess the purity of the product.

Appendix 4

Basic physico-chemical properties of the experimental soils

Soil	Short name	pH (1:2.5 H_2O)	Electrical conductivity (µS/cm^{-1})	Cation Exchange capacity (meq/100g^{-1})	Organic carbon (%)	Dissolved Organic carbon (µg/mL^{-1})	Particle size distribution			Textural class	B.E.T. Surface area (m2/g^{-1})
							Sand (%)	Silt (%)	Clay (%)		
Mawson Lakes	ML	8.91	159	19.24	1.11	8.71	55.0	25.0	20.0	Sandy loam	26.67
Sturt Gorge	SG	5.98	36	6.30	2.88	5.84	60.0	25.0	15.0	Sandy loam	9.36
Waite Campus	WC	5.64	965	17.42	2.26	3.90	42.5	42.5	15.0	Loam	4.59

Appendix 5

Calculation of adsorption isotherm

The adsorption isotherms were obtained by plotting the amount sorbed (X) versus equilibrium concentration (C). There are different types of adsorption isotherms. Langmuir and Freundlich isotherms are the most commonly used models for describing the adsorption phenomenon, since they can be applied to a wide range of adsorbate concentrations. The general form of the Langmuir equation can be expressed as:

$C_s = C_m K_L C_e / (1 + KC)$ ..... Equation 1

K_L = Langmuir equilibrium constant
C_e = Sorbent concentration in solution
C_s = Amount adsorbed

C_m = Maximum amount adsorbed as C_e increases

Equation 1 when fitted to the linear regression equation would convert to

$C_e/C_s = C_e/C_m + 1/K_L C_m$ ..... Equation 2

The plot of C_e/C_s versus C_e gives a straight line with the slope = $1/C_m$ and an intercept = $1/K_L C_m$.

The Freundlich equation used for describing the adsorption phenomenon can be expressed as:

$C_s = K_F C_e^{1/n}$ ..... Equation 3

K_F = Freundlich equilibrium constant
C_e = Sorbent concentration in solution
C_s = Amount adsorbed
n = Freundlich exponent

Equation 1 when fitted to the logarithmic form would convert to

$\log C_s = \log K_F + (1/n)\log C_e$ ..... Equation 4

The plot of $\log C_s$ versus $\log C_e$ gives a straight line with the slope = 1/n and an intercept = $\log K_F$.

Calculation of adsorption coefficients

The soil adsorption coefficient K_d (mL/g^{-1}) can be calculated as

$K_d = C_s/C_e$ ..... Equation 5

The organic carbon (OC) normalised adsorption coefficient (K_{OC}) can be calculated as

$K_{OC} = K_d(100/OC)$ ..... Equation 6

Appendix 6

Extraction of target compounds from the test soils

In this study, different solvent combinations were used for the extractions of mainly two different groups of compounds, such as (1) water soluble (e.g., methamphetamine, MDMA, pseudoephedrine, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, and n-formylmethylamphetamine) and (2) water insoluble (e.g., 1-benzyl-3-methylnaphthalene). In early phase of the project, for the water soluble compounds, soils were first extracted with 20 mL of solvent type (7) (see Appendix 6) followed by the next extraction with 15 mL of same solvent type. In each of the extraction steps, the vials were shaken vigorously for 1 min. followed by sonication for 15 min at 30^0C. Subsequently the soils were allowed to settle and the aliquots were pipetted out and filtered through 0.22 µm Teflon filter. The aliquots from both the extraction steps were combined in a 40 mL capacity amber-colored screw cap vial. The aliquots were diluted as and when required for the direct analysis in HPLC-MS. In a later phase of the project, the soils spiked with methamphetamine and N-formylmethylamphetamine were extracted twice with 20mL of solvent type (10) (see Appendix 9), where soils were shaken with the extracting solvent for one hour and 30 min., respectively in two steps in an end-to-end shaker. The remaining steps were as with the previous procedure. However, for the water insoluble compounds (1-benzyl-3-methylnaphthalene), a total of three extraction steps were employed. To begin with, 10 mL of acetone was used followed by subsequent extractions with 10 mL of ethyl acetate. The extraction procedures were then followed as described for the water soluble compounds. The aliquots were combined and analyzed by GC-MS.

Determination of target compounds

The determination of methamphetamine, MDMA, pseudoephedrine, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, and N-formylmethylamphetamine was performed using HPLC (Agilent 1100 series) equipped with (1) auto-sampler, (2) binary pump system, (3) mass selective detector (Agilent 1100) with Electro Spray (ES), and (4) Chemstation software for data integration. Chromatographic separation of the target compounds was achieved using a ZORBAX Eclipse XDB-C18 150 x 4.6 mm, 5µm column operated at 25 ± 0.8^0C temperature. The mobile phase consisted of two combinations of solvent A (20% methanol + 0.1% acetic acid + 10 mM ammonium acetate) and solvent B (90% methanol + 0.1% acetic acid + 10 mM ammonium acetate) maintaining the flow-rate of 0.8 mL/min^{-1}. The timetable for the operation of mobile phase for the total run time (26 min) was 0–8 min (100% A), 8–12 min (90% A + 10% B), 12–25 min (100% B), and 25–26 min (100% A). The mass spectra were collected over the mass range of 100–350 m/z. Propranolol was used as the internal standard during the analysis.

The determination of 1-benzyl-3-methylnaphthalene was performed in GC (Agilent 6890N system) equipped with (1) auto-sampler, (2) electron impact mass selective (EIMS) detector (Agilent 5973), and (3) Chemstation software for data integration. The GC system was run with Helium as the carrier gas with constant flow mode at 1 mL/min^{-1} (27 cm/Sec^{-1} at 13.99 psi). The GC inlet was operated in splitless mode at 250^0C temperature. The oven temperature was started at 90^0C for 2.50 min and ramped at 45^0C min^{-1} to 300^0C, and held for 9 min. The separation was achieved on a DB-5 column (30 m x 0.25 mm x 0.50 µm). The mass spectra were collected after a 4 min. solvent delay over the mass range of 50 – 550 m/z. Phenanthrene was used as the internal standard during the analysis.

Calculation of degradation pattern

Theoretically, the compound residues should fall logarithmically since the amount lost per unit time is proportional to the total amount present in a particular time. It was established when log residues were plotted against time lapsed that a straight line was obtained following the general equation ($y = mx + c$). The experimental data (log of concentration versus time elapsed) are then subjected to simple regression analysis. Thus, the regression equation can be expressed as:

$\log C_t = -k t + \log C_0$ ..... Equation 7

$\log C_t$ = log of concentration at any time t (in days)
$\log C_0$ = log of initial concentration
k = slope (rate of degradation with time t)
t = time elapsed in days.

Half-life ($t_{½}$) is the time required to reduce the concentration to half of its initial concentration. Thus, Equation 5 can be expressed as

$\log (½C_0) = -k t + \log C_0$..... Equation 8

Equation 6 can be simplified to get the half-life value as

Half-life ($t_{½}$) = $\log 2/k$ ..... Equation 9

Appendix 7

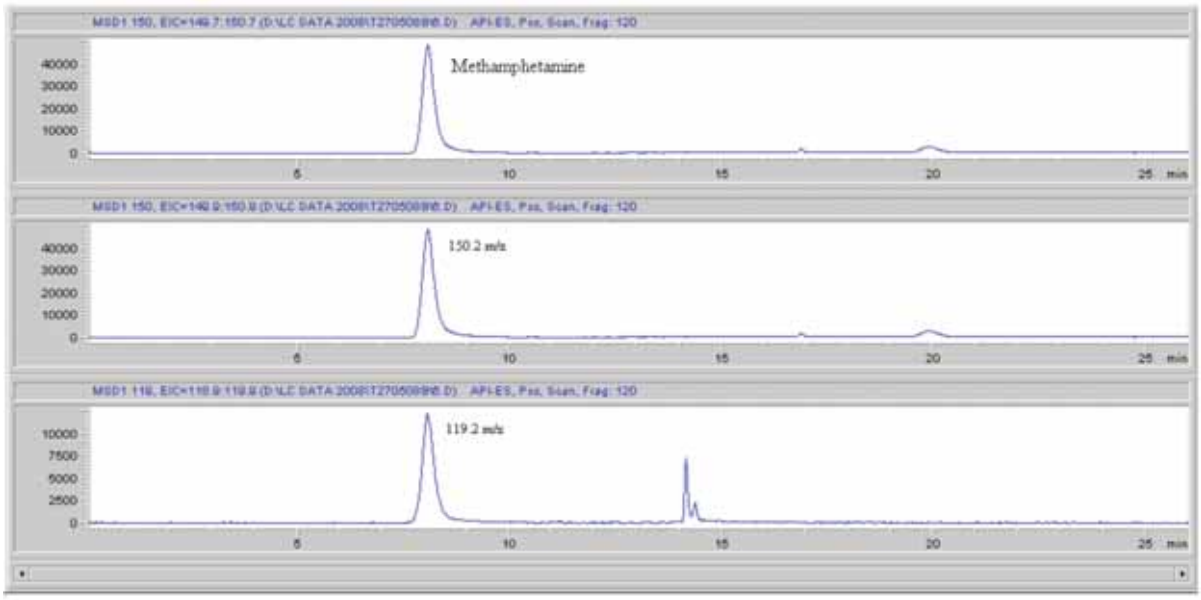
A list of solvent combinations employed to investigate the recovery efficiency of the target compounds from test soils

Sr. No.	Extracting solvent
1	Methanol
2	Acetonitrile
3	Acetone
4	Ethyl acetate
5	Acetonitrile:Methanol (70:30)
6	Chloroform:Methanol (2:1)
7	Methanol:Acetic acid (99:1)
8	Acetonitrile:Acetic acid (99:1)
9	Acetonitrile:Methanol:Acetic acid (70:29:1)
10	Chloroform:Acetonitrile:Methanol:Acetic acid (80:10:9:1)
11	Chloroform:Acetonitrile:Methanol:Hydrochloric acid (80:10:9.5:0.5)

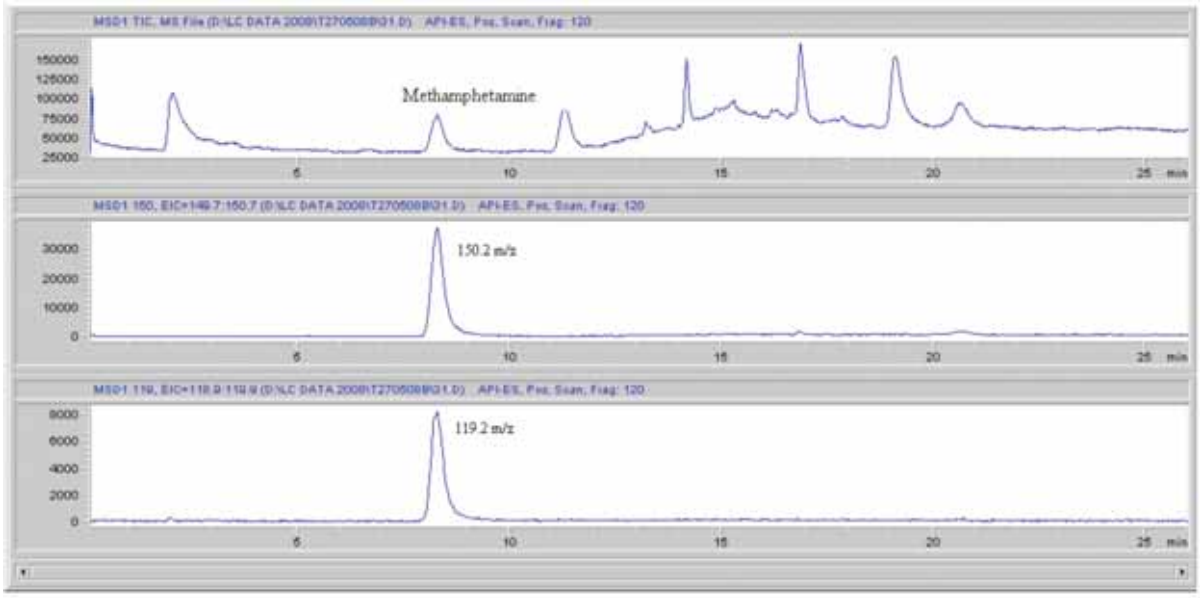
Appendix 8

Ion chromatogram of methamphetamine from standard solution and soil sample extract for 1-(1,4-cyclohexadienyl)-2-methylaminopropane degradation

[A] Methamphetamine standard



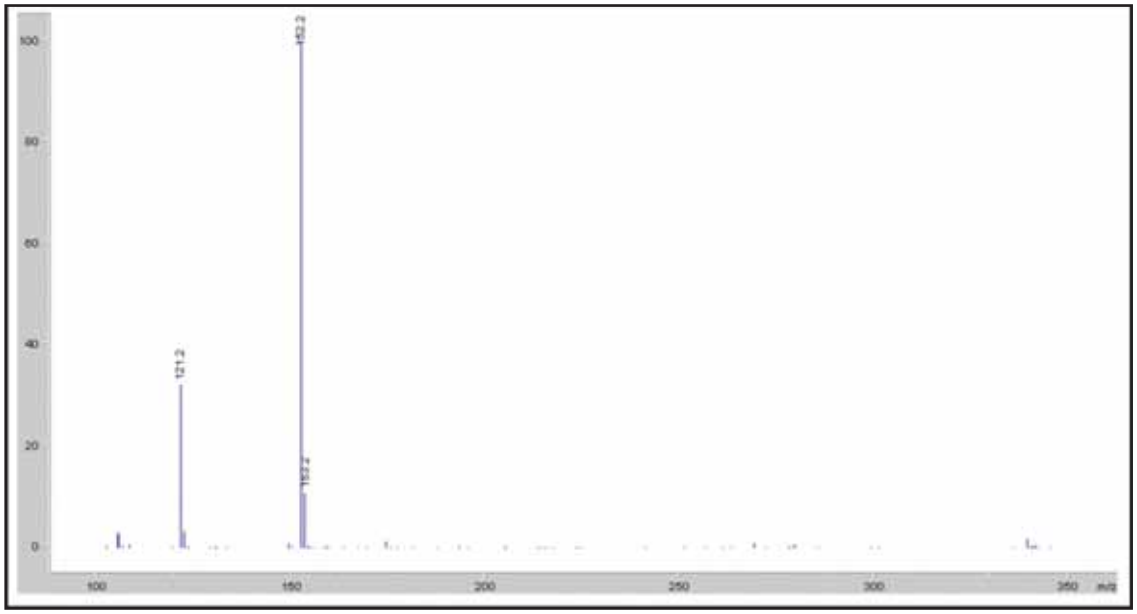
[B] Sample



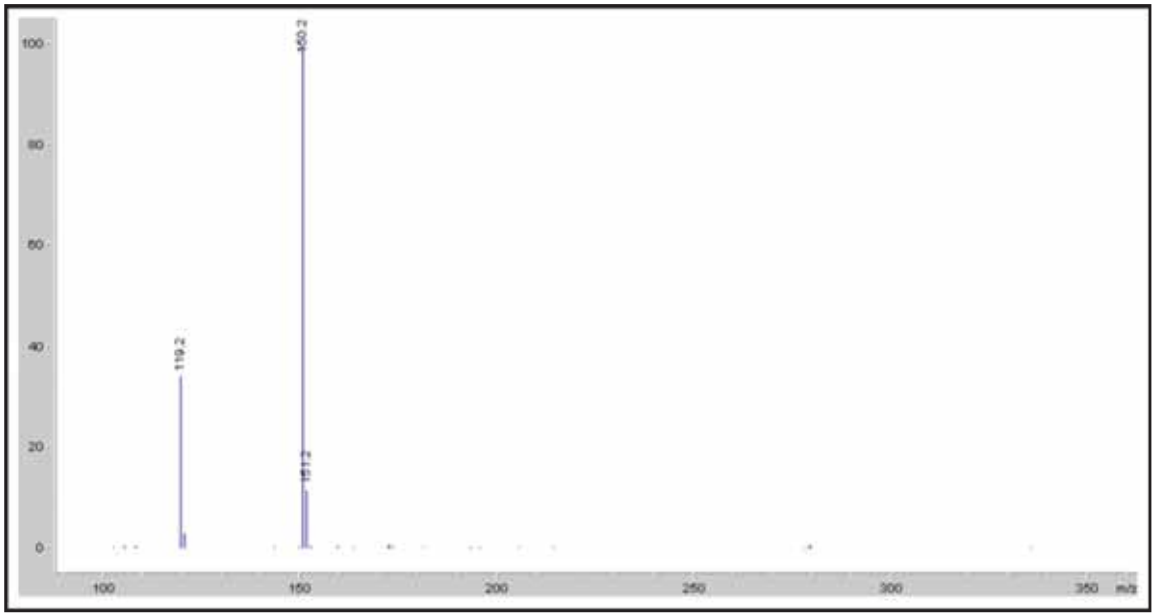
Appendix 9

Mass spectra of pure compounds

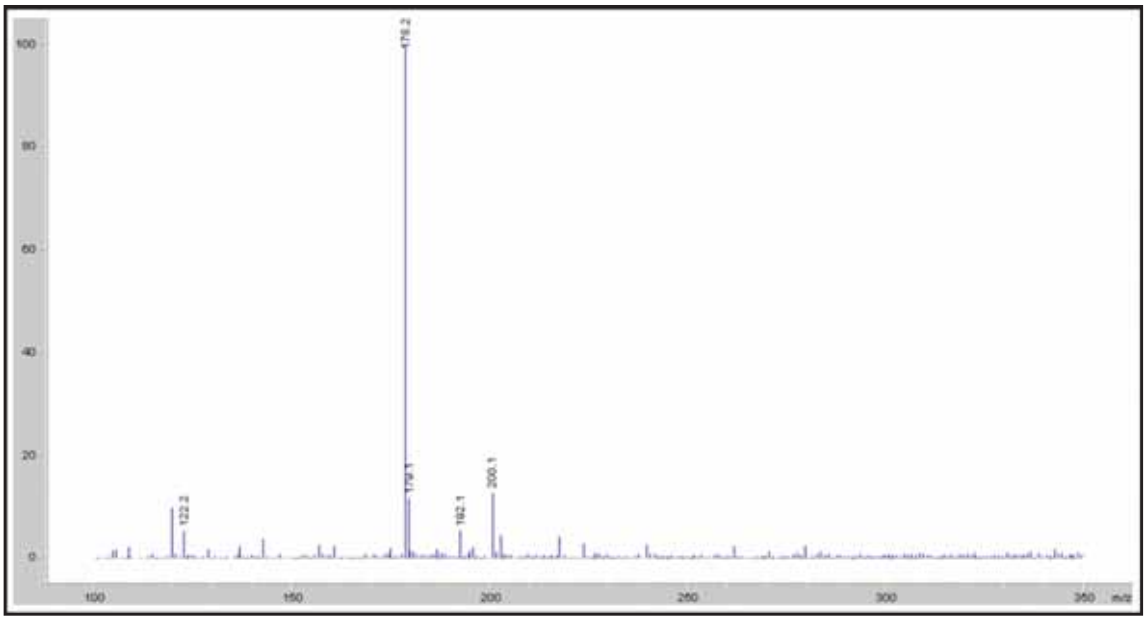
[A] 1-(1,4-cyclohexadienyl)-2-methylaminopropane



[B] Methamphetamine



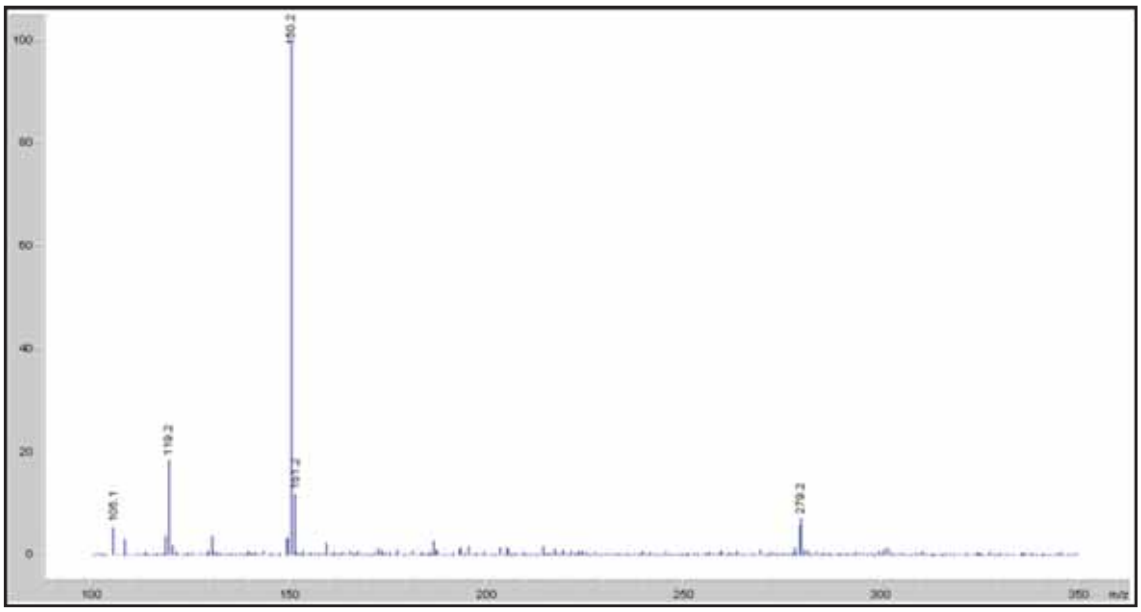
[C] N-formylmethylamphetamine



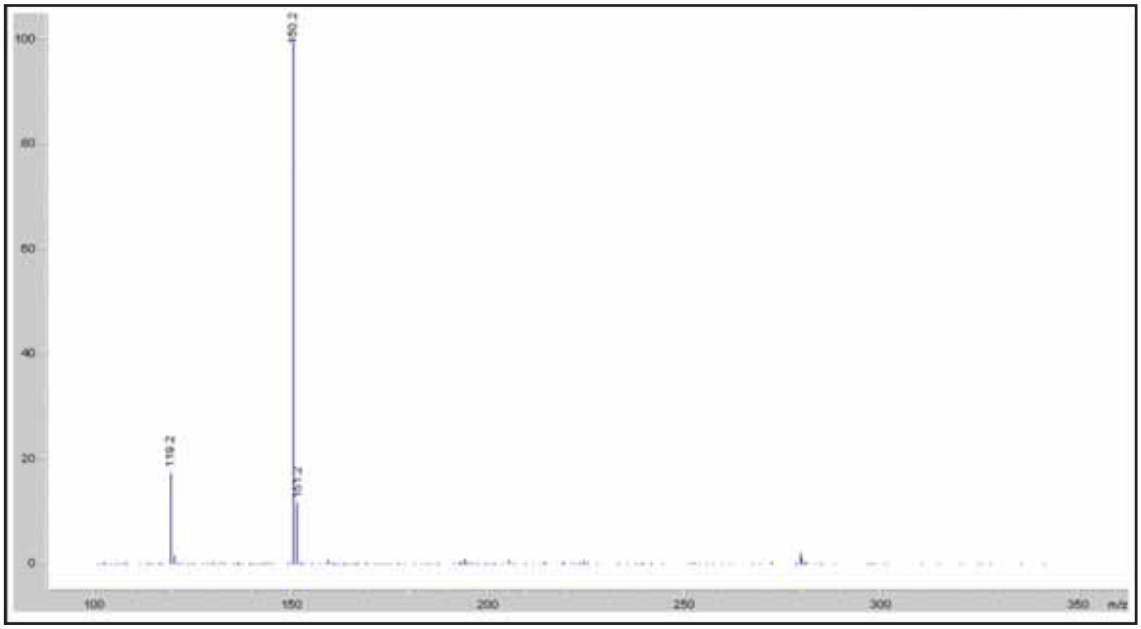
Appendix 10

Mass spectra of methamphetamine from the transformation of 1-(1,4-cyclohexadienyl)-2-methylaminopropane in non-sterile and sterile soils

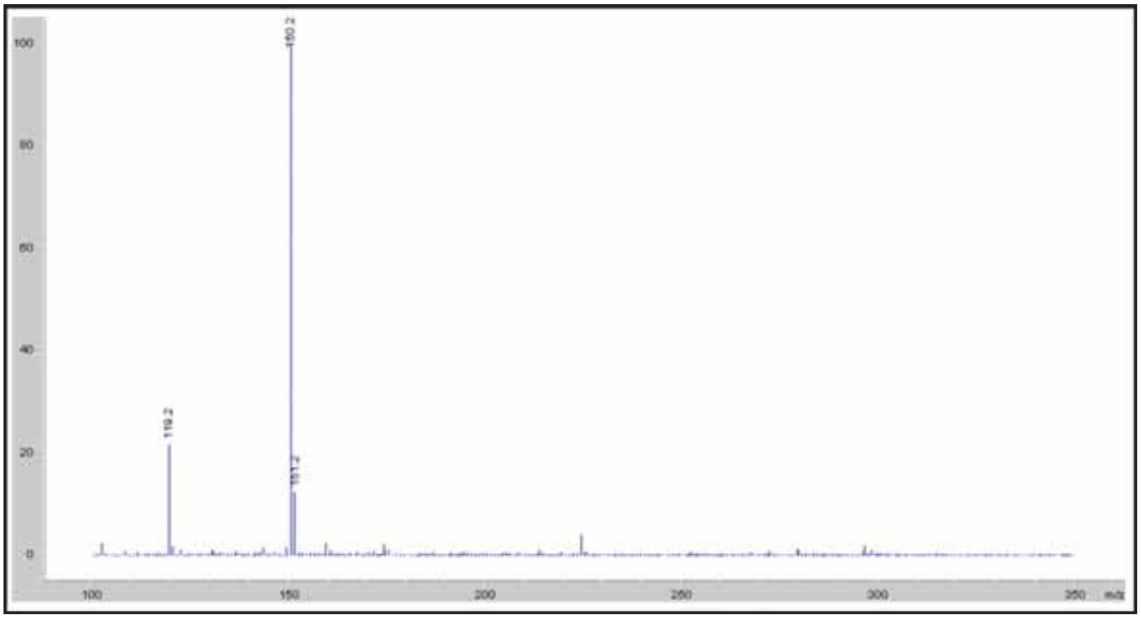
[A] Mawson Lakes soil – Non-sterile



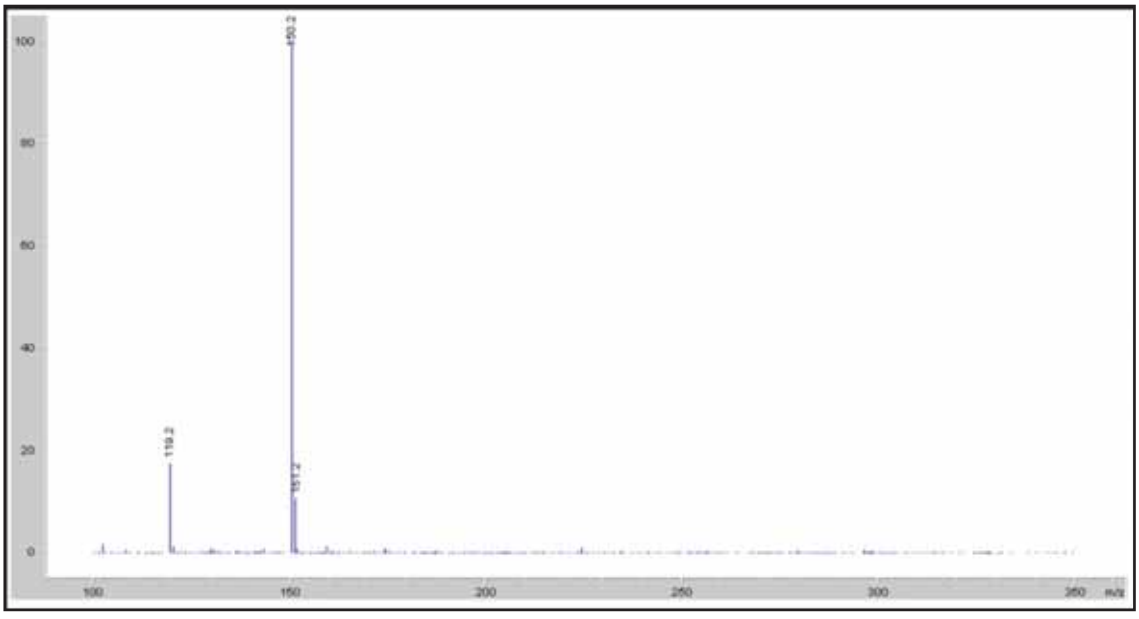
B] Mawson Lakes soil – Sterile



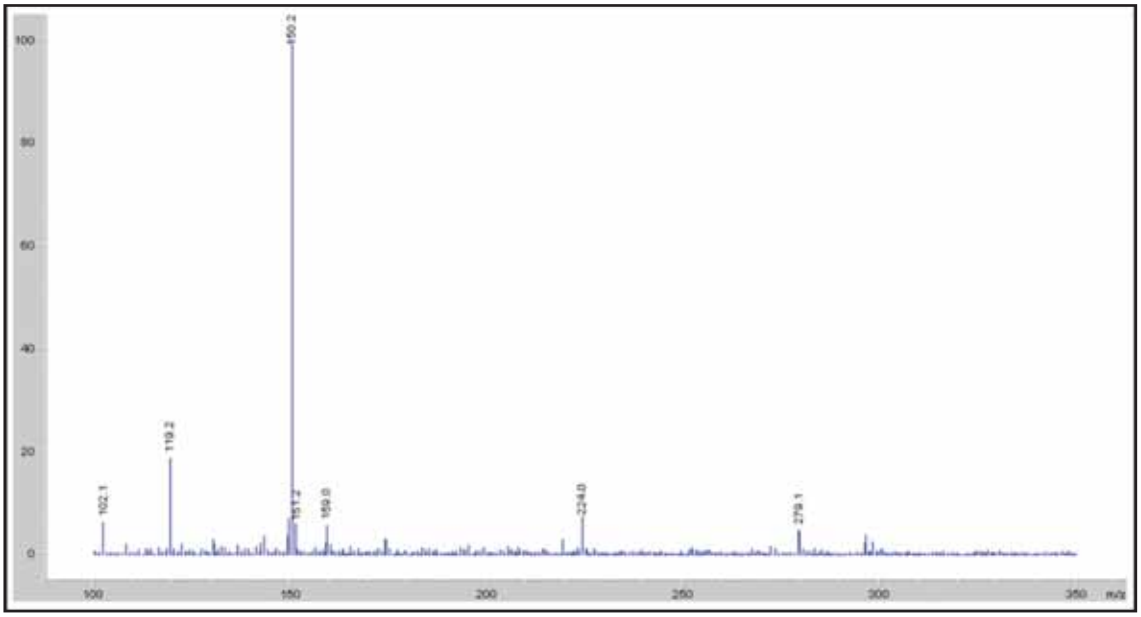
[C] Sturt Gorge soil – Non-sterile



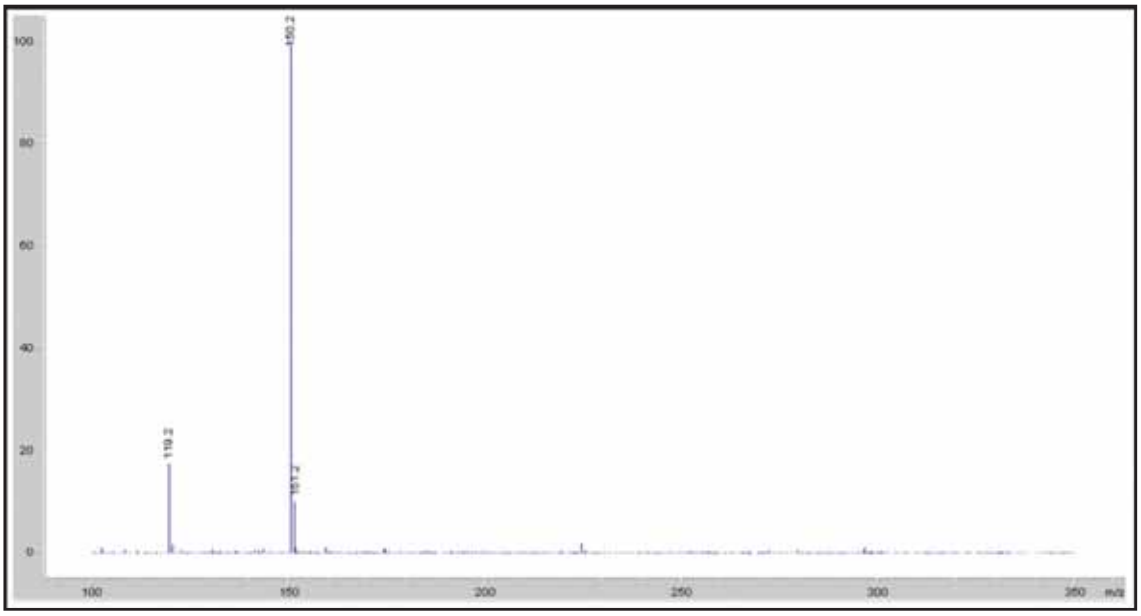
[D] Sturt Gorge soil – Sterile



[E] Waite Campus soil – Non-sterile



[F] Waite Campus soil – Sterile

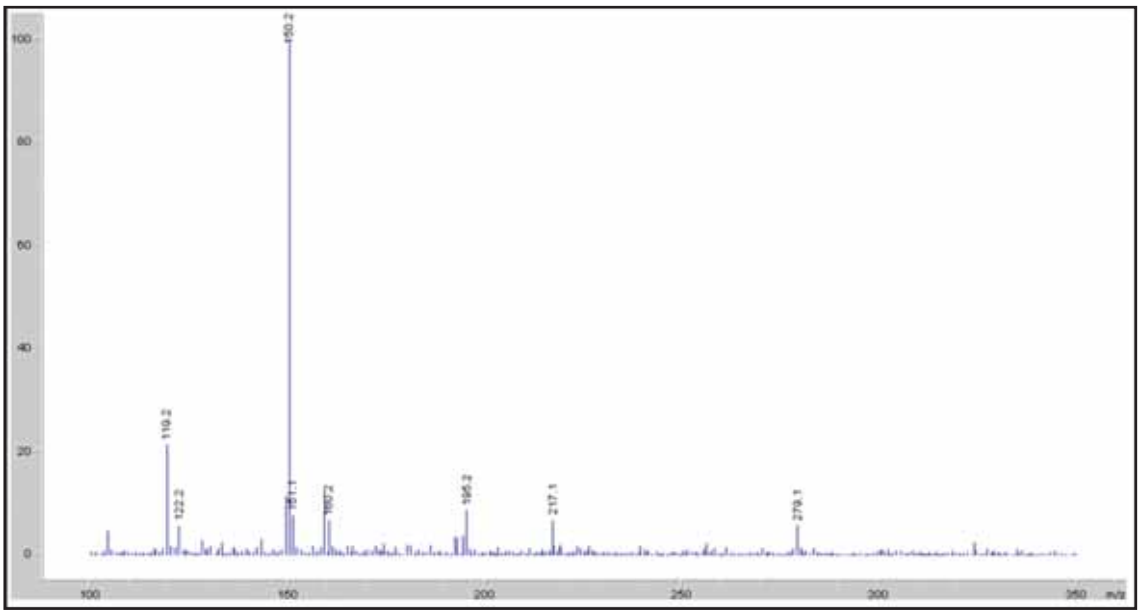


Appendix 10: Mass spectra of methamphetamine from the transformation of 1-(1,4-cyclohexadienyl)-2-methylaminopropane in non-sterile and sterile soils

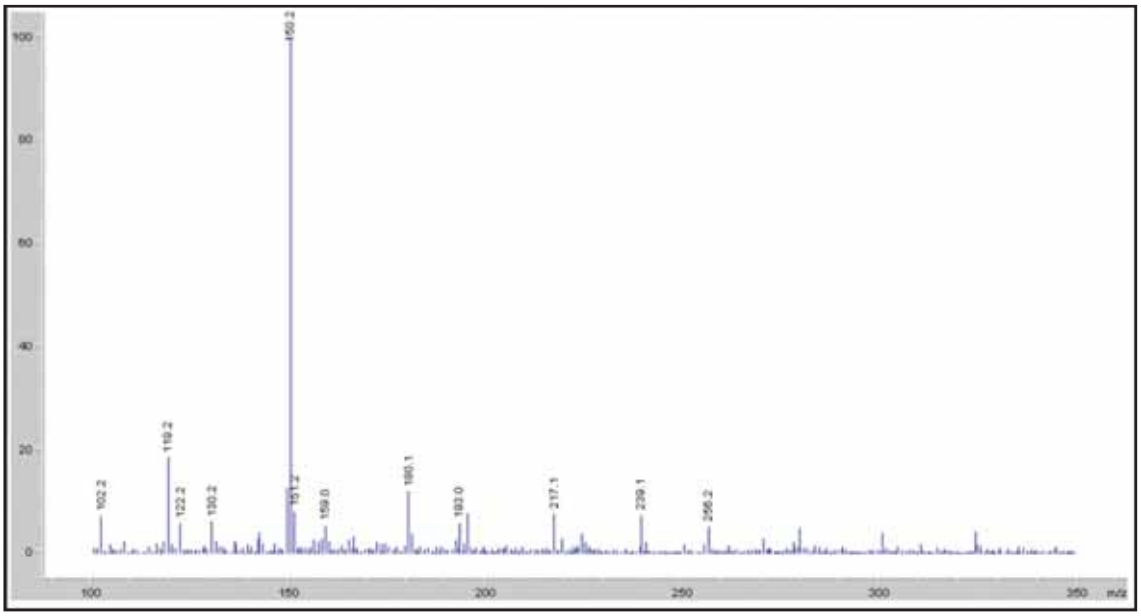
Appendix 11

Mass spectra of methamphetamine for the transformation of N-formylmethylamphetamine in non-sterile soils.

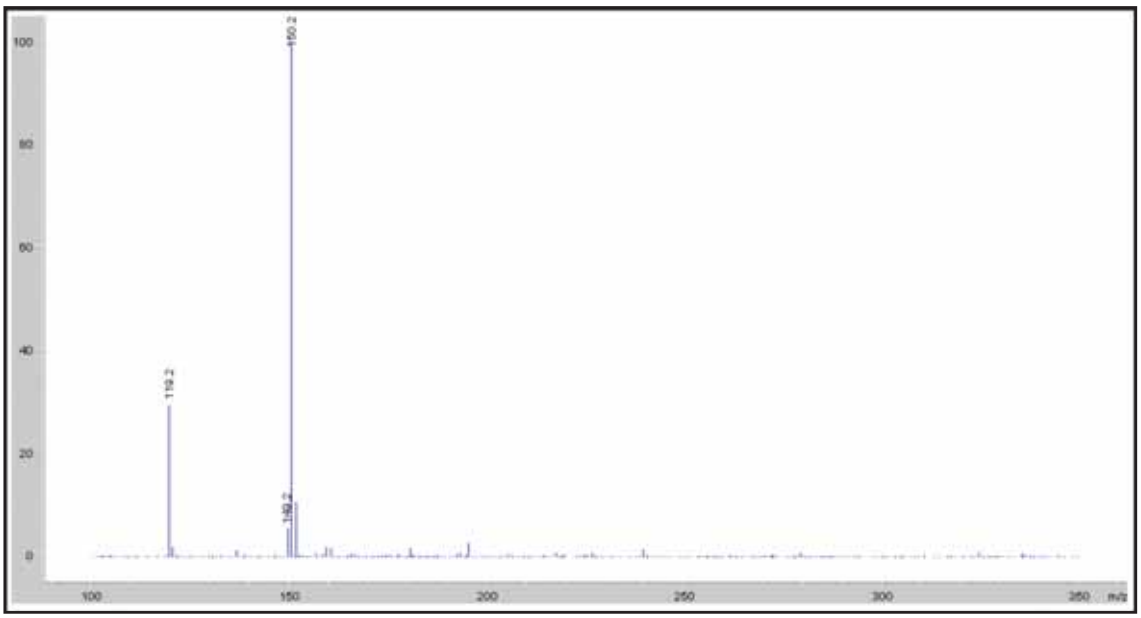
[A] Mawson Lakes soil



[B] Sturt Gorge soil



[C] Waite Campus soil



Appendix 11: Mass spectra of methamphetamine for the transformation of N-formylmethylamphetamine in non-sterile soils.