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Effect of Amphetamine Precursors and By-Products on Soil Enzymes of Two Urban Soils

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Amphetamines, a class of drugs, which stimulate the central nervous system, are known to produce a feeling of euphoria, increased confidence and alertness (Moore 1999). Some amphetamine derivatives, such as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) are also hallucinogens. The stimulant and hallucinogenic properties of amphetamines and their derivatives have made these compounds popular as recreational drugs worldwide. The illicit use of amphetamines has become a major problem also in Australia in recent decades. In 1998, almost 4% of the Australian population have been reported using amphetamines, compared to 2% in 1993 (Miller 2001). The dramatic increase in the use of this class of drugs has created a significant market for the local production of many amphetamines.

Chemicals associated with clandestine drugs laboratories, such drugs, their precursors, reagents and by-products, are often disposed of covertly into soil, sewerage systems, or public waste management facilities. There are two significant issues relating to such dumps of material; they might contain valuable evidence as to drug manufacture; and they might be a source of pollution. Chemicals buried in soil could be subjected to metabolism by soil microorganisms. An investigation of microbiological and forensic science literature indicated that there is no information relating to the effects of drugs and related compounds on microbial activities in the soil, therefore at present the environmental impact of clandestine drug manufacture cannot be reliably assessed. This study was therefore aimed at determining the effect of amphetamine and its precursors on two important biological activities in the soil; viz., dehydrogenase activity and potential nitrification.

MATERIALS AND METHODS

Two soils were used in this study, both from suburban areas south of Adelaide, South Australia: soil collected from Flinders University campus (campus soil) and soil from a residential garden in Daw Park (garden soil). Table 1 details the characteristics of these two soils. Soil samples were collected from a depth of 0-10 cm.

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Table 1. Characteristics of the soils used in the study.

Soil	pH	Conductivity (mS cm-1)	Total carbon (%)	Total nitrogen (%)	Clay (%)	Silt (%)	Sand (%)
Campus Soil	8.5	258	4.98	0.29	25	22.5	52.5
Garden Soil	7.5	141	3.01	0.21	12.5	10	77.5

The effects of 6 compounds – [safrole (S), phenylacetic acid (PA), phenyl-2-propanone (P2P), 3,4-methylenedioxybenzaldehyde (MDB), methamphetamine (MA) and ψ-ephedrine (Eph)] - on microbial activity in the two soils were examined. Each of the compounds studied is either an amphetamine or a precursor known to be used in the illicit synthesis of a drug in this class (Lukaszweski 1978; Soine 1986; Forbes and Kirkbride 1992). Two microbial parameters were used to determine the toxicity of the compounds: dehydrogenase activity and potential nitrification.

After allowing the soils to equilibrate at 70% water holding capacity for 7 days, the soils were spiked with the various compounds at concentrations of 10, 50, 100, 500 and 1000 μg g^{-1} from stock solutions in 5 μL of solvent (water or acetone) per gram of soil. Of the 6 compounds studied, only methamphetamine and ψ-ephedrine are water-soluble. Hence the stock solutions for these 2 compounds were prepared in water, whereas acetone was used as the solvent for the remaining 4 compounds. Appropriate controls were maintained: (i) water (5 μL g^{-1}) alone for methamphetamine and ψ-ephedrine and (ii) acetone (5 μL g^{-1}) alone for the remaining four compounds for valid comparison.

After spiking, the soil samples for the dehydrogenase study were incubated for 10 days at room temperature (21 ± 1°C). The dehydrogenase activity in the soil was measured by incubating the soil at 37°C for 24 h with 2,3,5-triphenyltetrazolium chloride (TTC) for the production of triphenyltetrazolium formazan (TPF) (Casida et al. 1964). After 24 h the TPF was extracted with methanol and the optical density of the supernatant at 485 nm was measured. The samples for the potential nitrification study were incubated for 14 days at room temperature after spiking. A solution of ammonium sulfate was then added to the samples along with sodium chlorate to inhibit the further oxidation of nitrite to nitrate. The samples were shaken for 5 h and extracted with potassium chloride solution. A color reagent (sulfanilamide/N-(1-naphthyl)-ethylenediamine hydrochloride in phosphoric acid) was then added and the optical density of the supernatant measured at 520 nm (Kandeler 1996). Duplicate samples were maintained for all treatments for every sampling for the assay of dehydrogenase and potential nitrification. Variations between duplicate estimations for all measurements of dehydrogenase activity and potential nitrification never exceeded 15%, as indicated by error bars in Figs.

RESULTS AND DISCUSSION

The results on the effect of amphetamine and its precursors on the dehydrogenase activity in the campus and garden soils are shown in Figs. 1 and 2, respectively. In both soils, controls receiving only water or acetone showed a very similar dehydrogenase activity. Evidently, acetone was not deleterious to the dehydrogenase activity.

In the campus soil samples, all the test drug compounds were not toxic to dehydrogenase activity up to 1000 $\mu g\ g^{-1}$. In fact, all the tested compounds could even stimulate the dehydrogenase activity in varying degrees. Methamphetamine and ψ-ephedrine were the most effective in stimulating dehydrogenase activity. Interestingly, both compounds exerted almost similar degrees of stimulation, several-fold over that in the control, at all concentrations from 10 to 1000 $\mu g\ g^{-1}$. Furthermore, the stimulatory effect of methamphetamine and ψ-ephedrine at 10 $\mu g\ g^{-1}$ was as high as that at 1000 $\mu g\ g^{-1}$. The same trend was noticed also in the garden soil samples.

The results on the potential nitrification study for the campus and garden soils are shown in Figs. 3 and 4, respectively. In both soils the addition of acetone (used for dissolving the test chemical), instead of water, even at the low level (at 5 $\mu L\ g^{-1}$) used had a deleterious effect on the potential soil nitrification, although overall microbial activity, in terms of dehydrogenase activity, was not adversely affected. Nitrifiers, generally present in low populations in soil, are known to be very sensitive to a wide range of chemicals.

In the campus soil, safrole stimulated the potential nitrification over that in the acetone control at all concentrations used (10-1000 $\mu g\ g^{-1}$) whilst MDB was inhibitory at 1000 $\mu g\ g^{-1}$. Interestingly, the remaining five compounds studied were non-toxic even at 1000$\mu g\ g^{-1}$. In the garden soil, MDB was toxic at 1000 $\mu g\ g^{-1}$ as in the campus soil. Interestingly, ψ-ephedrine at 1000 $\mu g\ g^{-1}$ was toxic to nitrification in garden soil, in contrast to its stimulatory effect in campus soil. More pronounced toxicity of ψ-ephedrine to potential nitrification in garden soil than in campus soil might be due to its increased bioavailablity in the former soil with low clay content. Clay is known to decrease the availability of organic pollutants in solution due to sorption and formation of complexes. Generally, amphetamine and its precursors were less toxic to potential nitrification in campus soil with high clay content than in garden soil with relatively low clay content.

Clandestine drug laboratories can be involved in large-scale operations, for example it is reported (Bureau of Justice Assistance 2000) that in Mexico some laboratories are capable of producing 20-100 lbs of methamphetamine in each batch. In 1999, 12 tonnes of methamphetamine were seized worldwide and obviously much more than this escaped detection. It has been estimated (Lukas 1997) that for every pound of methylamphetamine produced, 5-6 pounds of waste chemical are produced. Together, these figures indicate the magnitude of the

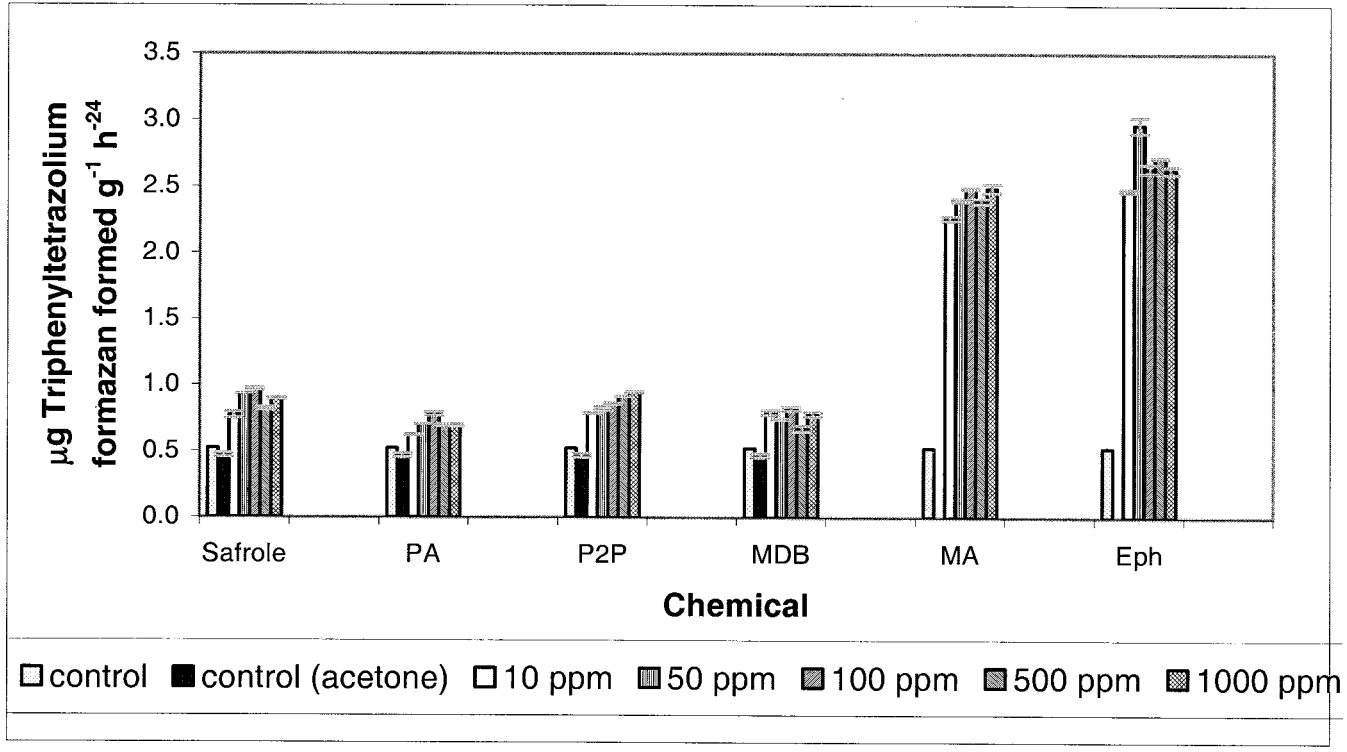

Figure 1. Effect of compounds on dehydrogenase activity (mean ± SD) in campus soil.

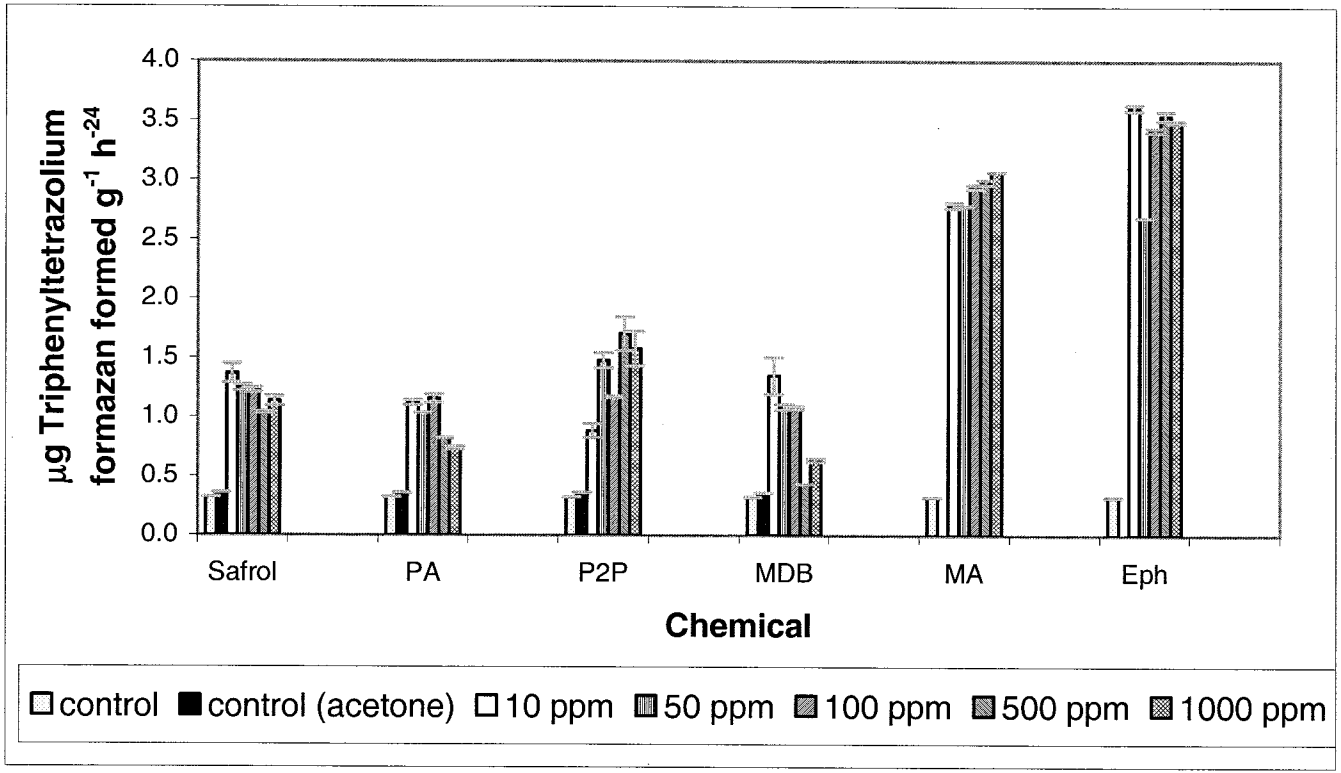

Figure 2. Effect of compounds on dehydrogenase activity (mean ± SD) in garden soil.

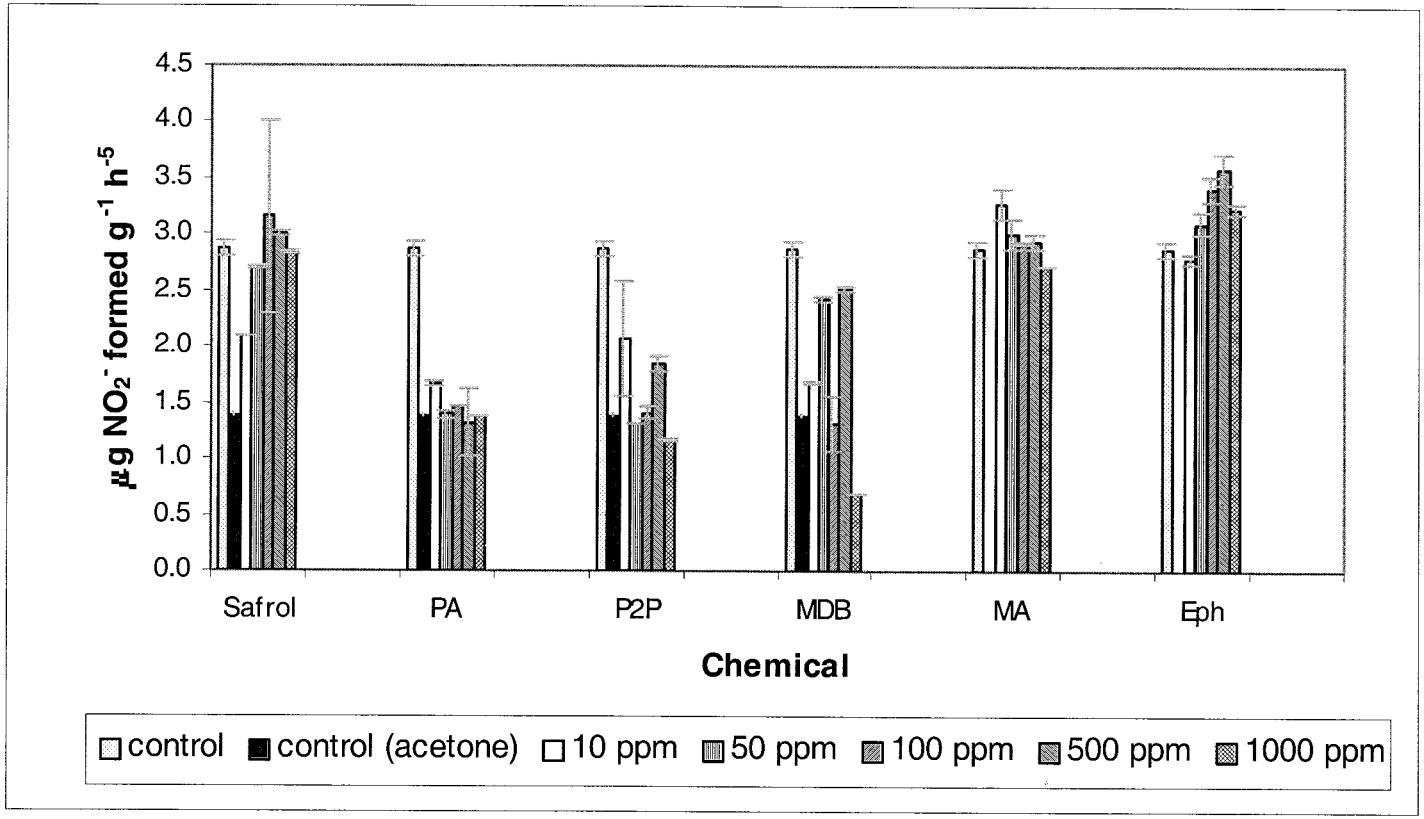

Figure 3. Effect of compounds on potential nitrification (mean ± SD) in campus soil.

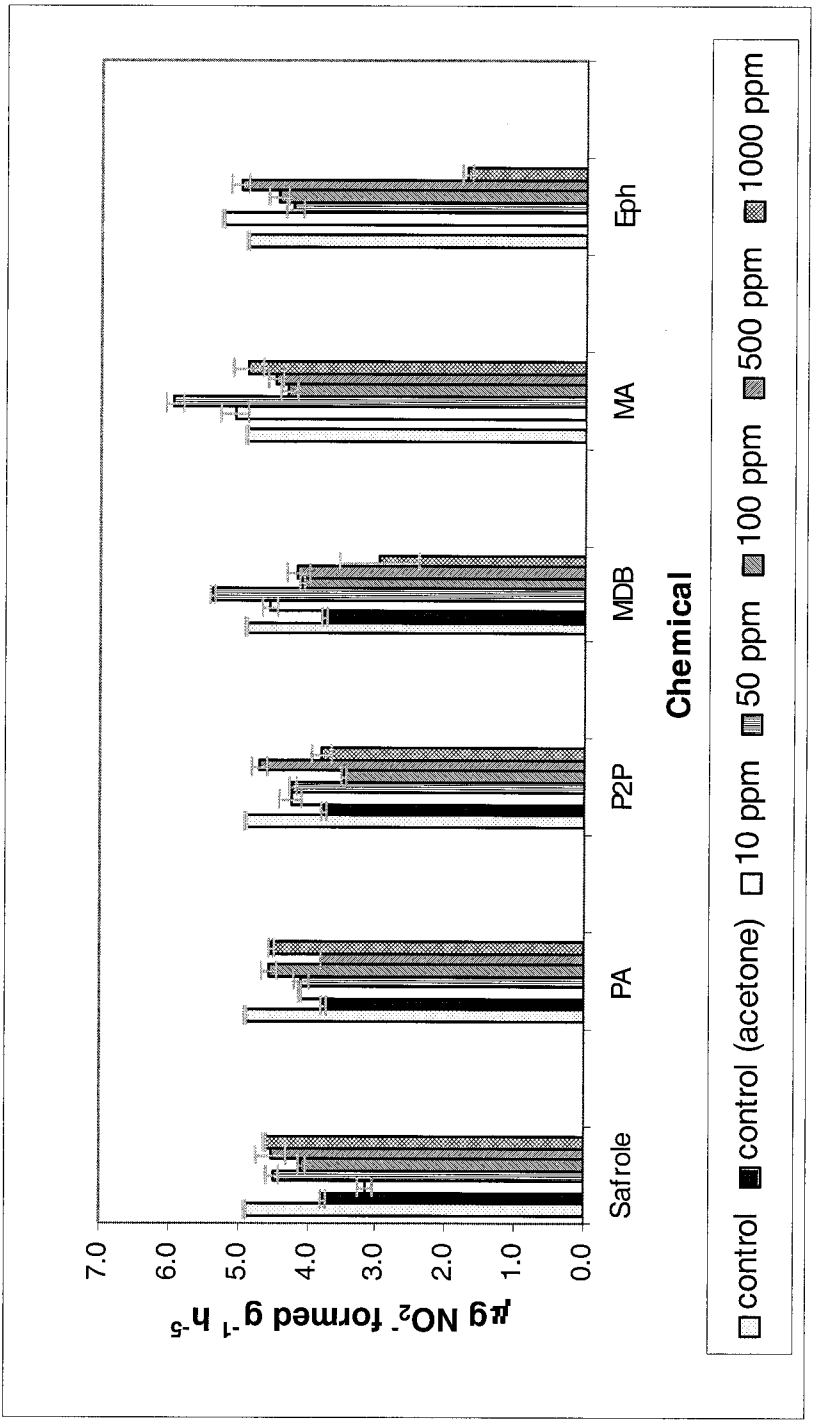

Figure 4. Effect of compounds on potential nitrification (mean ± SD) in garden soil.

problem, but the levels of the pollutants typically present in soil associated with clandestine laboratories are not known. Evidently, some laboratories handle large quantities of chemicals, and clandestine disposal of wastes from such laboratories is likely to lead to high concentrations of the drugs and related products in places.

Data presented in this study indicate that amphetamine precursors and by-products do not have deleterious effects on important microbial activities at concentrations upto 1000 $\mu g\ g^{-1}$. ψ-Ephedrine and MDB exhibited some toxicity at 1000 $\mu g\ g^{-1}$, depending on the soil type. What is particularly interesting is the stimulation of dehydrogenase activity even at high concentrations by methamphetamine and ψ-ephedrine and potential nitrification by safrole. The chemicals tested in this study appeared to exhibit either stimulatory or inhibitory effects depending on the chemical used, its concentration and the enzyme.

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