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MICROBIAL DEGRADATION OF SOIL APPLIED HERBICIDES

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ABSTRACT

The ability of some soil bacteria to degrade the herbicides, chloroxuron (N^1-4-(4-chlorophenoxy) phenyl)-N^1N^1-dimethyl-urea) and metobromuron, (N^1-bromo-phenyl)-N-methoxy-N-methylurea) in laboratory experiments was investigated. The R_f and maximum absorption range values of the original herbicides and the end products after incubation with soil micro-organisms indicated that new products were formed. The micro-organisms were gram positive bacilli (rod-shape). On agar, two different colonies were identified. It is concluded that chloroxuron and metobromuron are unlikely to persist in soils.

INTRODUCTION

During the past three decades, a large number of herbicides have been introduced as pre- or post- emergent weed killers in many countries of the world. In Nigeria, herbicides have been effectively used to control weeds in coffee and maize (Adenikunju and Folarin, 1976; Choudhary, 1976). As farmers continue to realise the usefulness of herbicides, larger quantities would be applied to soils. But, the fate of these compounds in the soil is becoming increasingly important since they could either be leached down in which case under-ground water is contaminated or if immobile, they would persist on the top soil. These herbicides could then accumulate to toxic levels in the soil and become harmful to micro-organisms, plants, wildlife and man. Microbiological degradation of non-volatile, immobile herbicides appear to be a major factor in controlling their rate of disappearance from soil (Audus, 1960). Earlier studies by Amakiri, (1974) on the phenylurea herbicides, chloroxuron and metobromuron, showed that they were immobile and moderately mobile respectively when applied to soils. The aim of this study is to investigate the role of soil micro-organisms in the degradation of the herbicides,

MATERIALS and METHODS

Herbicides

Pure re-crystallised compounds of chloroxuron and metobromuron supplied by CIBA[1] were used in this study.

Isolation of micro-organisms

One ml soil suspension (10g Teak plot soil in University of Ibadan/100 ml distilled water) was transfered into 250 ml of a liquid culture medium (LCM) in a conical flask. The LCM was made from 10 ml of MNa_2HPO_4 4 ml of M KH_2PO_4 and 10 ml of M $(NH_4)_2SO_4$; 1 ml 0.01% $FeSO_4$ + Ca(No3), and 0.02% herbicide per L. The flask was plugged with non-absorbent cotton wool and placed on a mechanical shaker at room temperature. The microbial cells were

[1] CIBA Agrochemicals, Basle, Switzerland.

harvested and re-introduced into the LCM to eliminate all the organic matter decomposing microbes. The micro-organisms were again harvested and multiplied in a fermenter containing mineral salt medium (replaced 0.02% herbicide in LCM with 10% glucose). After 3 days, the actively growing cells were harvested by centrifuging at $0^{o}C$, washed twice in 0.02 M phosphate buffer (pH 7.0) and resuspended in the buffer solution. One ml cell suspension was also used in inoculating mannitol-soil-extract agar (Amakiri, 1974) inorder to observe the colonial morphology of the microbes. The microbes were gram stained.

Degradation of herbicides

An amount of metobromuron was dissolved in the liquid culture in the fermenter to give 0.02% herbicide (Amakiri, 1974). An acetone solution of chloroxuron (solubility in water 3 ppm at $20^{o}C$) was also poured into another fermenter. After evaporating the solvent, the liquid culture was added to give 0.02% chloroxuron. Then one ml cell suspension was aseptically transfered into the fermenter. A third fermenter containing only the liquid culture was used as control.

Thin Layer Chromatography (TLC) and Ultra Violet (UV) absorption Spectrums

At 24 hours intervals, samples of microbial suspensions (50-100ml) were centrifuged to separate the organisms from the culture solution. The filtrate was acidified (1ml conc. HC1/ 500ml) and extracted twice with half its volume of diethylether. The combined either extracts were evaporated to 0.5 ml and applied to 6060 silica gel with fluorescent indicator (Eastman Chromatogram sheets). The sheets were developed in a benzene – acetone (4: 1, v/v) solvent system to a height of 16 cm. After location of the purple spots by means of a UV lamp at 254 nm, the region of interest was scapped and eluted with either and the resulting solution was filtered and evaporated to dryness. UV absorption spectra was obtained by dissolving the evaporated extract in carbon tetrachloride solution. The peak absorption of the metabolites was determined with a Perkin Elmer model 137 UV spectrophotometer. The control samples were also treated as for the metabolites.

RESULTS

Table 1 shows the morphological description of the bacterial colonies which degraded the herbicides. Two different colonies were identified. The first colony was oblong, with a diameter of 0.1 mm, effuse, smooth, entire, transparent and gummy to the touch. The second colony was circular with a diameter of 0.2 mm, smooth, entire, white and translucent. The bacteria were gram positive rod-shaped organisms (bacilli). One group stained darker at both poles while the other stained uniformly.

Table 1

Morphological description of bacterial colonies which degraded herbicides.

Bacterial colony	Colonial morphology	Gram Stain reaction	Form
1	Oblong in shape, diameter, 0.1mm, effuse, smooth, entire, white, translucent and gummy to touch.	+ve	bacilli
2	Circular, 0.2 mm diameter, smooth surface, entire edge white and translucent	+ve	bacilli

Table 2

R_f and UV absorption spectra value of metabolites from degradation of chloroxuron and metobromuron.

Type of herbicide and inocula used	R_f values in benzene: acetone (4 : 1)	Maximum absorption range (mm)
Chloroxuron	0.58	460 – 465
Chloroxuron + bacteria	0.60	292 – 296
metabromuron	0.71	502 – 507
metobromuron + bacteria	0.78	270 – 280

TLC of extracts from the fermenter after 24 and 48 hours of incubation did not reveal any new spots on the chromatogram sheets. After 72 hours, R_f values and maximum absorption spectra (Table 2) indicated the formation of new products. Chloroxuron and metobromuron had R_f values of 0.58 and 0.71 respectively while R_f for the metabolites were 0.60 and 0.78.

DISCUSSION

The results of this study showed that some soil bacteria degraded the phenylurea herbicides. The initial degradation occured after 72 hours of incubation. This probably showed an initial lag phase during which the bacteria are adapted enzymatically to cope with the foreign substrates to which they were exposed or the TLC was not sensitive enough to record the small quantities of metabolites. However, the UV absorption spectra confirmed the formation of metabolites. These metabolites were not identified and since the original substrate was not quantified, complete disappearance of the original compound cannot be inferred. Moreover, Geissbuhler *et al.* (1963) have observed a loss of chloroxuron by microbiological degradation in sandy loam which amounted to 35% during a period of no more than 8 weeks. The higher temperature and humid conditions found here would, however, hasten the degradation of the herbicide. Kearney *et al.*, (1967) in their review reported that high organic matter levels, warm temperatures and proper moisture for microbial activity favour the rapid decomposition of phenylureas from soil.

Various pathways of degradation of phenylureas have been proposed by several workers. For the dimethyl herbicide (chloroxuron), Geissbuhler *et al.*, (1963) proposed a stepwise demethylation and deamination – decarboxylation. They demonstrated the formation of aniline derivative and of carbon dioxide from the urea moiety. In this experiment, the formation of a lighter compound (R_f0.60) during the degradation of chloroxuron seem to confirm the removal of certain moiety, probably the methyl group, from the substrate (Rf0.58) . Similarly, Tweedy *et al.*, (1970) suggested a stepwise demethylation and demethoxylation to the *P* – bromophenylurea and hence to aniline for the methoxymethyl phenylurea (metabromuron). The aniline derivatives could form azo compounds which have been found to be carcinogenic. However, Tweedy et al., (1970) observed that azobenzene was not formed. Thus, from this experiment, chloroxuron and metobromuron are degraded by bacterial suspensions in agreement with the findings of Rosss and Tweedy, (1973) and Geissbuhler, et al., (1963).

It is therefore concluded that the health of the public would not be impaired by the use of chloroxuron and metobromuron at a rate up to 200 ppm. At this level, they are degraded by soil micro-organisms.

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