Bull. Environm. Contam. Toxicol. 25, 424-426 (1980)

A Rapid Assay for the Examination of Protein Degradation in Soils

Jason A. Caplan and Jed W. Fahey
Borriston Laboratories, Inc., 5050 Beech Place, Temple Hills, MD 20031

The recently proposed pesticide registration guidelines (FEDERAL REGISTER 1978) require soil microbial function studies of the toxic effects of pesticides on protein, starch and cellulose degradation, nitrogen fixation and nitrification. Of primary concern to laboratories performing microbial function tests on soils is that these assays be sensitive, rapid, and adaptable to different soil types.

Commonly used methods for measuring proteins in biological fluids such as salting-out and density gradient centrifugation are too prone to interference to be used with a soil matrix. The Kjeldahl and Lowry methods (KJELDAHL 1883, LOWRY et al. 1951) are routinely used to measure soil protein. These acid digestion techniques are used to measure soil protein content in subsamples taken before and after a suitable incubation. They are, however, very time consuming methods and are subject to numerous interferences. We have explored an alternative method for measuring protein degradation based on the *in-vitro* radiometric methods of CAPLAN et al. (1980) and ISBISTER et al. (1980) for monitoring starch and cellulose degradation. Although convenient, this method would be specific for the radio-labeled protein(s) used and is prohibitively expensive for routine use.

We have, therefore, developed a rapid, inexpensive method for monitoring protein degradation in soil by measuring the enzymatic release of a substrate-bound dye. Azocoll® (Calbiochem-Behring Corp.) is an inexpensive, insoluble protein containing an adsorbed red azo dye which is released into solution by the activity of numerous proteolytic enzymes. It has been used for activity measurements of numerous enzyme preparations including collagenase, protease and trypsin (John Snow, pers. comm.). We have shown that the relative proteolytic activity of a biological matrix, such as soil, can be quantitatively determined by spectrophotometric measurement of the red azo dye released from Azocoll.

The sensitivity of this technique was examined by measuring the effects of an inhibitor on protein degradation. Mercuric chloride, a common antimicrobial agent, was applied to soils at varying increments to measure inhibition of protein degradation.

0007-4861/80/0025-0424 $01.00

MATERIALS AND METHODS

The soil used for the experiment was a clay-loam soil obtained from Prince Georges County, Maryland. The soil was sifted (2mm mesh) and aliquots (0.5 g) were added to sterile 13 ml pyrex centrifuge tubes. Five ml of sterile 10% dextrose in 0.1 M phosphate buffer (pH 7) and 12.5 mg Azocoll were added to each tube. Tubes were then vortexed and incubated at 37°C for 5 hours. After incubation, the tubes were vortexed again, centrifuged for 30 minutes at 2600 x g and the absorbance of the supernatant was read (A_{520}) with a Bausch and Lomb Spectronic 70 Spectrophotometer. Sterile soil and Azocoll-buffer solution was used as a background.

Mercuric chloride from a freshly prepared stock solution was added to each soil-Azocoll-buffer solution for a final concentration of 0, 50, 100, 250, 500 and 1000 ppm. Protein degradation was monitored in triplicate as outlined above.

RESULTS AND DISCUSSION

The optimum incubation time for measurement of released azo dye was 5 h at 37°C, as determined by a pilot study. The ability of the released-azo dye technique to measure protein degradation by soil microbes is illustrated in Fig. 1. Complete inhibition of degradation was observed upon adding 1000 ppm $HgC\ell_2$, with decreasing inhibition at lower levels of $HgC\ell_2$. The coefficient of correlation (R^2) of $HgC\ell_2$ concentration vs. absorbance was 0.995, using a natural log conversion. Since the azo dye released in the autoclaved soil tubes did not result in a significant increase in absorbance (A_{520} = 0.104), dye release was determined to be biological rather than physico-chemical.

The results illustrate the potential of the released azo dye method as a functional test for monitoring microbial protein degradation in soil. Advantages of this technique over the Lowry or Kjeldahl methods, or the use of a radiolabeled protein substrate are its sensitivity, rapidity of response, ease of execution and low operating costs. Futhermore, the Lowry and Kjeldahl methods require measurement of small differences in protein content, when the soil being tested may already have a low endogenous protein content.

ACKNOWLEDGEMENTS

This work was supported by Borriston Laboratories, Inc. We wish to thank L.T. Mulligan, G. Milligan and B. Betz for their assistance on this project.

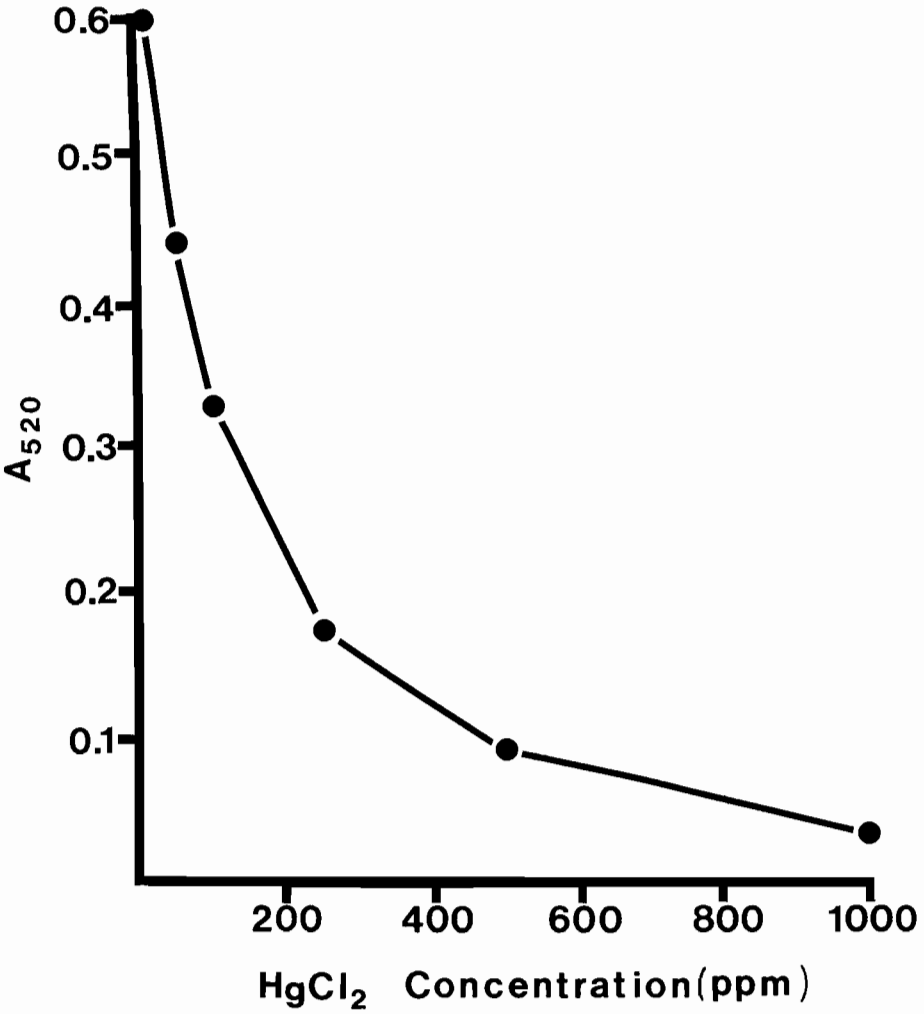

FIGURE *1*. Mercuric chloride inhibition of protein degradation in a clay-loam soil as measured by released azo dye content. (Data are corrected for background.)

REFERENCES

CAPLAN, J.A., J.W. FAHEY and L.T. MULLIGAN: Appl. Environm. Microbiol. (submitted).
FEDERAL REGISTER: 43(132), 29697 (1978).
ISBISTER, J.D., R.S. SHIPPEN and J.A. CAPLAN: Bull. Environm. Contam. Toxicol. 24, 570 (1980).
KJELDAHL, J.: Z. Anal. Chem. 22, 366 (1883).
LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR and R.J. RANDALL: J. Biol. Chem. 193, 265 (1951).