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SHORT COMMUNICATION

Effect of mercuric chloride on carbon mineralization in soils

Summary

The short and long-term effects of mercuric chloride amendment of five surface soils from southeastern Montana on carbon mineralization was studied. Short-term radiorespirometric studies utilizing a glucose substrate indicated Hg levels greater than 40 μg/g soil were required for significant inhibition in all soils tested. Under chronic exposure conditions, levels from 0.1 to greater than 100 μg Hg/g soil proved necessary for inhibition.

Introduction

The fungicidal and bactericidal properties of mercurial compounds has long been recognized and utilized in agriculture and medicine. However, little attention has focused upon the quantitative assessment of the impact of inorganic mercuric compounds on soil micro-organisms *in situ*.

Mercuric ion toxicity presumably is a consequence of the displacement of the normal suite of catalytic metal ions from their binding sites on a protein, particularly the sulfhydryl groups, and the resultant impairment of enzyme function[6]. Two common soil fungi, *Aspergillus niger* and *Penicillium notatum*, were found to grow normally in liquid medium containing up to 10 ppm Hg as $HgCl_2$[5]. Oxygen-consumption by baker's yeast (*Saccharomyces cerevisiae*) grown in liquid culture was severely limited at 100 ppm $HgCl_2$[8]. Tonomura *et al.*[12] reported on a mercury-resistant, soil-isolated Pseudomonas whose growth was uninhibited at $HgCl_2$ levels in solution culture of below 450 ppm. Van Faassen[13] showed 100 ppm $HgCl_2$ amendment of two soils (one of which had a history of treatment with mercurial fungicides) to result in little effect in number of microbes plated, but to reduce carbon and nitrogen mineralization and dehydrogenese activity. Peterson[9] showed that moistening of a silty clay loam to 87 per cent of its water holding capacity with either 0.001 or 0.01 *M* $HgCl_2$ reduced its oxygen consumption in 50 hours by 24 or 80 per cent, respectively.

This study was designed to assess the short- and long-term effects of mercuric chloride amendments to a variety of soils on CO_2-evolution from glucose and native soil carbon substrates.

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Materials and methods

Surface soils (0–20 cm depth) collected from uncultivated sites in southeastern Montana, and ground to pass a 2 mm sieve were used in this study. Selected physical and chemical characteristics of the soils are presented in Table 1. Short term radiorespirometric studies utilized uniformly-labelled ^{14}C-glucose. Five grams of soil was transferred to each reaction flask containing 10 ml of water, or $HgCl_2$ solution (1×10^{-4} *M* to 1×10^{-3} *M*) spiked with 0.5 μCi (0.43 μg) of ^{14}C-glucose. The samples were maintained at 25° C for 6 hours and continually flushed with CO_2-free air. Respired $^{14}CO_2$ was trapped in 0.5 *N* NaOH, precipitated as $BaCO_3$, transferred to a weighed glass fiber filter disc and assayed with a windowless gas flow proportional counter. Correction was made for self-absorption by the $BaCO_3$ filter cake. Each treatment was replicated four times.

Long-term studies utilized no added carbon substrate. Bulk soil samples were moistened to 50% of the $\frac{1}{3}$-bar moisture holding capacity with distilled water and incubated for one week at 25°C. The soils were amended to 0, 0.1, 1.0, 10, or 100 μg Hg (as $HgCl_2$)/g soil with sufficient water to bring them to the $\frac{1}{3}$-bar moisture level. Twenty-five gram samples (oven-dry basis) of treated soil were placed in 1 quart mason jars containing a flask with 10 ml of dilute standardized NaOH. The jars were sealed, maintained at 25°C, and, at weekly intervals for a month, opened for reaeration and sampling of the NaOH traps. Carbonates were precipitated as $BaCO_3$ and the remaining alkali titrated with HCl as outlined by Stozky[11]. Each treatment was replicated three times.

TABLE 1

Chemical and physical properties of selected eastern Montana soils

Soil	pH	Organic carbon %	Total nitrogen %	Sand %	Silt %	Clay %
Arvada	8.1	1.6	0.10	35	25	40
Campspass	6.6	6.7	0.27	19	56	25
Heldt	8.3	1.7	0.12	27	48	25
Bainville	7.5	1.9	0.08	29	42	29
Terry	8.3	0.9	0.10	74	14	12

Results and discussion

Table 2 shows the results of the short-term radiorespirometry trials. In all of the soils, amendments greater than 40 μg Hg/g soil were required to yield significant reductions in CO_2-evolution from glucose. The 200 μg Hg/g soil amendment reduced CO_2-production in the Arvada and Bainville soils, and 400 μg Hg/g soil was sufficient to depress glucose metabolism in all soils examined.

Several patterns of effect are seen in Table 3, showing the results of the month-long examination of CO_2-evolution from the $HgCl_2$ amended soils. The

TABLE 2

Short-term effects of mercuric chloride amendments on the evolution of CO_2 from glucose in soil-water suspensions

$HgCl_2$ concentration		ng CO_2/g soil/6 hours*				
M	μg Hg/g soil	Arvada	Campspass	Heldt	Bainville	Terry
0	0	18.9 a	18.0 a	9.9 a	19.2 ab	11.2 a
1×10^{-4}	40	16.3 a	15.2 a	9.7 a	21.2 a	12.8 a
5×10^{-4}	200	12.6 b	10.6 ab	6.2 ab	16.1 b	11.0 a
1×10^{-3}	400	4.8 c	5.9 b	3.9 b	8.4 c	3.5 b

* Means (4 replicates) in same column followed by same letter are not significantly different at the 5% level by Duncan's multiple range test

Terry soil shows essentially no inhibition of CO_2-production during the monitoring period. In the Arvada soil, inhibition of CO_2-production is evident during the first week at 1 ppm Hg and above; however, in succeeding weeks only the 100 ppm Hg amendment continues to show any depression. In contrast, the Campspass soil shows depressed CO_2-production at the 0.1 ppm Hg amendment and above for all but week 2. The Heldt soil shows no treatment effect during the first week, but marked inhibition at all levels of amendment for the succeeding 3 weeks; here the 1 ppm Hg treatment shows greater CO_2-production than the 0.1 ppm Hg treatment. The Bainville soil is unique in exhibiting increased CO_2 production with respect to the control at the 1 ppm Hg amendment during the first week; in succeeding weeks inhibition is seen only at progressively higher levels.

It is clear that exposure time and/or substrate type are important considerations in assessing the respiratory impact of mercuric chloride in these soils. All of the soils except the Terry showed suppressed CO_2-production throughout the month-long monitoring period at 100 ppm Hg, while only the Arvada and Bainville soils showed any such effect at 200 ppm Hg in the short-term exposure studies. Jeffries and Butler[7] observed such an acute *vs* chronic exposure dichotomy with the growth of the photosynthetic bacterium *Rhodopseudomonas capsulata*, where exposure in liquid medium for 20–80 minutes to levels of methylmercury acetate that were bacteriostatic for chronic (300 hour) exposures, stimulated growth.

The reasons for the differences in response of the soils studied are probably multifaceted. Differences in the binding ability of the soils will perhaps mitigate the toxic properties of the added mercury. As mercury tends to bind with the organic fraction of soils[4], the apparently greater sensitivity observed in the long term study of the Campspass *vs* the Terry soil, is surprising. However, the Campspass soil's much greater CO_2-production probably reflects a larger, and perhaps more varied microflora. As pointed out earlier, micro-organisms vary widely in their sensitivity to mercurials. Strains of many environmentally-common procaryotic and eucaryotic genera have demonstrated mercury-

TABLE 3

Long-term effects of mercuric chloride amendments on CO_2-evolution from moist soils

Soil	μg Hg/g soil	mg CO_2/g soil/week*			
		Week 1	Week 2	Week 3	Week 4
Arvada	0	0.65 a	0.41 a	0.47 a	0.31 a
	0.1	0.62 ab	0.35 a	0.33 a	0.25 ab
	1.0	0.56 bc	0.34 a	0.35 a	0.34 a
	10	0.52 c	0.32 a	0.32 a	0.25 ab
	100	0.43 d	0.26 b	0.22 b	0.17 b
Campspass	0	1.60 a	1.24 a	1.28 a	0.95 a
	0.1	1.45 b	1.22 a	1.13 b	0.85 b
	1.0	1.48 b	1.28 a	1.12 b	0.84 b
	10	1.40 b	1.24 a	1.00 c	0.80 b
	100	1.26 c	0.98 b	0.85 d	0.68 c
Heldt	0	0.33 a	0.47 a	0.33 a	0.45 a
	0.1	0.32 a	0.16 c	0.07 b	0.06 c
	0.1	0.34 a	0.28 b	0.30 a	0.18 b
	10	0.34 a	0.17 c	0.08 b	0.06 c
	100	0.36 a	0.18 c	0.10 b	0.06 c
Bainville	0	0.40 b	0.36 ab	0.43 ab	0.44 a
	0.1	0.36 c	0.39 a	0.48 a	0.42 a
	1.0	0.46 a	0.44 a	0.52 a	0.42 a
	10	0.36 c	0.30 bc	0.44 ab	0.31 a
	100	0.32 c	0.23 c	0.38 b	0.40 a
Terry	0	0.19 ab	0.21 a	0.16 a	0.16 a
	0.1	0.20 a	0.18 a	0.21 a	0.16 a
	1.0	0.22 a	0.22 a	0.19 a	0.20 a
	10	0.21 a	0.23 a	0.20 a	0.19 a
	100	0.18 b	0.18 a	0.16 a	0.17 a

* Means (3 replicates) in same column (for each soil) followed by same letter are not significantly different at the 5% level by Duncan's multiple range test

tolerance, and the existence of such an array suggests resistance to mercurials may be common and widespread[2]. A possible genetic link exists between resistance to antibiotics and to mercury[10]. Zajic[14] reports work which showed gram-negative bacteria to be more resistant than gram positive bacteria to mercury penetration, suggesting that differences in the cell wall which influence the staining properties may also influence the uptake and toxicity of mercury. Thus, the nature of the indigenous microflora probably influences the tolerances observed in this study. Also the ability of the soils to lose by volatilization, presumbably of Hg^0, mercury added as a mercuric salt by either biotic[3] or abiotic[1] mechanisms, may influence the response observed to mercuric chloride amendment.

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E. R. LANDA and S. C. FANG

Departments of Soil Science and
Agricultural Chemistry, Oregon State University,
Corvallis, Oregon 97331, U.S.A.

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References

1 Alberts, J. J., *et al.*, Science **184**, 895–897 (1974).
2 Brunker, R. L., Appl. Microbiol. **32**, 498–504 (1976).
3 Brunker, R. L. and Bott, T. L., Appl. Microbiol. **27**, 870–873 (1974).
4 D'Itri, F. M., The Environmental Mercury Problem, CRC Press, Cleveland (1972).
5 Hardcastle, J. E. and Mavichakana, N., Bull. Environ. Contam. Toxicol. **11**, 456–460 (1974).
6 Hughes, M. N., The Inorganic Chemistry of Biological Processes, John Wiley & Sons, London (1972).
7 Jeffries, T. W. and Butler, R. G., Appl. Micriobol. **30**, 156–158 (1975).
8 Kokke, R., *In* Isotope Tracer Studies of Chemical Residues in Food and the Agricultural Environment, 81–85, I.A.E.A., Vienna (1974).
9 Peterson, G. H., Soil Science **94**, 71–74 (1962).
10 Richmond, M. H. and John, M., Nature **202**, 1360–1361 (1964).
11 Stozky, G., *In* Methods of Soil Analysis 2, ed. C. A. Black *et al.*, Agronomy 9, 1550–1572, Am. Soc. Agron., Madison, Wisc. (1965).
12 Tonomura, K., *et al.*, Nature **217**, 644–646 (1968).
13 Van Faassen, H. G., Plant and Soil **38**, 485–487 (1973).
14 Zajic, J. E., Microbial Biogeochemistry, Academic Press, New York (1969).