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SHORT COMMUNICATION

Effects of some inorganic substances on TTC assay of dehydrogenase activity in soils

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LENHARD (1956) proposed that dehydrogenase activity in soils be assayed by determining their ability to reduce 2,3,5-triphenyltetrazolium chloride (TTC) to 2,3,5-triphenyl formazan (TPF). This assay has received considerable attention during the past decade (for references, see Skujins, 1967; Klein, Loh and Goulding, 1971; Ross, 1971), presumably because it is believed to provide a good index of microbial activity. But this belief seems to be based entirely on Stevenson's (1959) report of a good correlation between oxygen uptake and TTC dehydrogenase activity for some Canadian soils, and no studies to evaluate the TTC method of assaying soil dehydrogenase activity have been reported. The purpose of this communication is to report experiments showing that the TTC method of assaying soil dehydrogenase activity is susceptible to interference by nitrate and other inorganic soil constituents.

Table 1 shows the effects of various inorganic substances on TTC assay of dehydrogenase activity in three Iowa soils previously described (Tabatabai and Bremner, 1971). The Clarion soil (pH 6·2) contained 2·45% organic C, 19% clay and 55% sand; the Harpster soil (pH 7·9) contained 3·98% organic C, 28% clay and 34% sand; and the Hayden soil (pH 7·2) contained 3·04% organic C, 12% clay and 58% sand. The method used for assay of dehydrogenase activity was the procedure described by Casida, Klein and Santoro (1964), which involves determination of the TPF produced on incubation of 6 g of $CaCO_3$-treated soil (10 mg $CaCO_3$/g of soil) with 1 ml of a 3% (w/v) solution of TTC and 2·5 ml of distilled water in a stoppered 16 × 25 mm test tube at 37°C for 24 h. The inorganic substances added were mixed with the soil before addition of TTC solution (MnO_2 and Fe_2O_3) or were dissolved in the distilled water added ($NaNO_3$, $NaNO_2$, Na_2SO_4, Na_3PO_4, NaCl and $FeCl_3$).

Comparison of the redox potentials of TTC and the inorganic substances listed in Table 1 provides an explanation of our finding that nitrate, nitrite, and Fe^{3+} reduce the value obtained in assay of dehydrogenase activity by the TTC method [for redox potential data, see Mattson, Jensen and Dutcher (1947) and Patrick and Mikkelsen (1971)]. But the finding that Fe_2O_3, MnO_2, sulfate, phosphate and chloride all increase the value obtained in assay of dehydrogenase activity with TTC cannot be explained from redox potential data, and we are unable to suggest an explanation of this finding.

Stevenson (1959) found that leaching of soils with water increased their dehydrogenase activity as assayed with TTC and that the TTC dehydrogenase activity of a leached soil was reduced to approximately that of the unleached soil when the leachate was concentrated and added to the leached soil. He suggested that leaching with water removed "some

TABLE 1. EFFECTS OF INORGANIC SUBSTANCES ON TTC ASSAY OF DEHYDROGENASE ACTIVITY IN SOILS

Substance added†	Dehydrogenase activity*		
	Clarion soil	Harpster soil	Hayden soil
—	98	137	313
$NaNO_3$	79	90	207
$NaNO_2$	64	119	101
Na_2SO_4	142	150	489
Na_3PO_4	202	230	519
NaCl	110	142	372
$FeCl_3$	67	96	246
Fe_2O_3	135	145	340
MnO_2	108	150	350

* Expressed as μg of TPF produced/g of soil/24 h.
† Amount added was equivalent to 10 μmoles of substance/g of soil.

inhibitory or antagonistic factor which interferes with TPF formation in soil, either through inhibition of respiratory enzymes in the soil or possibly by providing a more suitable hydrogen acceptor than TTC". His observations can be readily explained if his soils contained appreciable amounts of water-soluble nitrate, because Table 1 shows that nitrate reduces the value obtained when soil dehydrogenase activity is assayed with TTC.

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