

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

BIOLOGICAL MONITORING OF POLLUTANT IMPACT
ON SOIL VITALITY AS DETERMINED
BY LIQUID SCINTILLATION

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In the soil are many types of microorganisms. Some are pathogenic to plants and some to humans, but most exist in a symbiotic relationship from which agriculture and the environment benefit. While one group may have a clearly defined niche in the ecological plan, e.g., nitrogen fixing bacteria, others appear to be only curiosities that are observed and classified by the scientist. One of the curiosities is a group of soil bacteria that are capable of oxidizing elemental hydrogen. These bacteria are facultative autotrophs that can either exist on organic nutrients or derive their entire supply of energy from the oxidation of hydrogen. The metabolic ability of these organisms has long been used to classify and separate these bacteria, but only recently was there an important environmental spin-off from this reaction.

While studying the environmental fate of elemental tritium (T_2 or HT) we realized (McFarlane et al., 1978) that these bacteria were sufficiently active to account for changing large quantities of HT into tritiated water. This biologically available molecule is, therefore, a possible pollutant of water and food. Likewise, large amounts of hydrogen are continuously being safely oxidized to form water, resulting in a stable environmental H_2 concentration (Ehalt et al., 1972). Since these hydrogen-oxidizing bacteria live in the same soil as those known to account for soil vitality, discovering a quick and easy assay of their activity is thought to represent a fruitful step useful in ascertaining pollutant impact on soils.

The use of hydrogen-oxidizing microbes for a biological monitoring technique has some advantages over other organisms that collect or concentrate a pollutant. It also yields information different from what is learned by chemical analyses of the soil, air, or water. The basis of this technique is to determine the biological activity of a specific group of bacteria. If their metabolism has been altered by a pollutant, the rate of activity will be changed. This change is not dependent on the concentration of the pollutant, but on its availability and toxicity.

The difference between chemical concentration and availability can best be understood by an example. Plants and other organisms often grow in soils heavily contaminated with mercury (a highly toxic element). However, in soil much of the mercury becomes bound to soil particles and, therefore, is biologically unavailable and in this form is nontoxic. When we applied our hydrogen oxidation test to organisms in soil or in solution, we obtained drastically different results.

The results of a variety of mercury concentrations in solution, pH 7.2, on the ability of *Alcaligenes paradoxus* to oxidize tritium are shown in Figure 1. It is evident that this organism is very sensitive to somewhere between 0.1 and 1.0 ppm mercury in an uncomplexed solution. In soil amended with mercury (Figure 2), 1.0 ppm was sufficient to completely inhibit the oxidation reaction, but only after approximately 20-percent conversion of the tritium to tritiated water had taken place. For the same concentration of mercury in solution, only about 5-percent conversion took place. It is noteworthy that 100.0 ppm mercury in the soil produced the same effect as 1.0 ppm in solution. This suggests that the clay loam soil offered some degree of protection not available in solution.

Thus the toxicity of mercury as determined by the solution tests reveals the wrong picture if extended to its effect in soils. The important point is that this technique indicates the impact of a pollutant in soil and not only its concentration.

The test is conducted in the following manner. One hundred grams of a fine sandy loam soil is put into a 1-liter round bottom flask. To this is added 10 ml of water containing the desired concentration of the toxic chemical. Other types of soil will work equally as well if they are porous enough to allow aeration, and the amount of water added is about 40 percent of the soil water-holding capacity. The flasks are closed with a rubber stopper, and the soil is incubated for 16 to 24 hours. They are next opened and flushed with room air by sucking air from inside the flask. We use a vacuum pump and a polyethylene tube to draw 13 liters of air from the flask in 30 seconds. The flasks are then stoppered and immediately 5 ml of

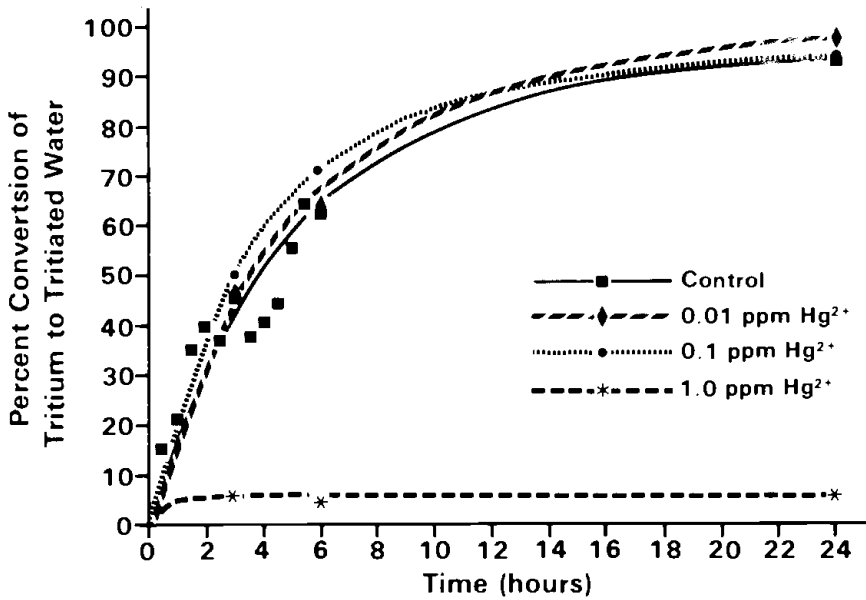


Figure 1. Tritium oxidation by *Alcaligenes paradoxus* in 0.025M potassium phosphate solution, pH 7.2, and amended with either 0.01, 0.1, or 1.0 ppm mercury [as Hg(NO₃)₂].

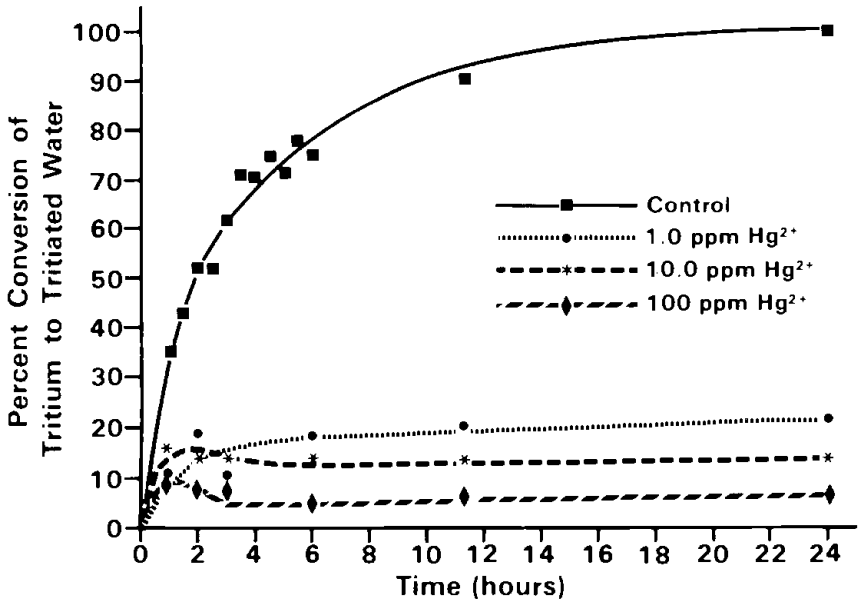


Figure 2. Tritium oxidation by *Alcaligenes paradoxus* in sterilized clay loam soil, and amended with either 1.0, 10.0, or 100.0 ppm mercury [as Hg(NO₃)₂].

nitrogen containing 0.2 μCi of elemental tritium is injected through the rubber stopper. Remember that hydrogen in air is 0.5 ppm (v:v) (Ehalt et al., 1974) and that tritium is only a tracer. It is, therefore, important that these two operations are performed quickly and that the time of injection is recorded accurately.

When investigating a gaseous pollutant, all procedures are similar except that during the 16- to 24-hour incubation the flasks are continually purged with moist air containing the toxic vapor, and prior to tritium injection the soil is weighed and water is added to restore the original water content.

After tritium is injected, the soils are allowed to incubate for three hours and then the reaction is stopped by removing any remaining gaseous tritium by changing the flask air as described before. To aid in the recovery of biologically produced tritiated water, an additional 10 ml of distilled water is added to each flask. Water is then extracted from the soil by distillation. The temperature of the distillation process is regulated so that the soil organic matter is not burned. Distillation is stopped after 15 ml of water are recovered. We recognize that some fractionation of $\text{HTO}/\text{H}_2\text{O}$ occurs during distillation (McFarlane et al., 1978) and that more precise ways of obtaining a water sample exist. However, the variation resulting from our impatience is minimized by first diluting (addition of 10 ml) and then removing the same amount of water from each replicate (15 ml). Variations of less than 10 percent between replicates still allows us to understand the insult of the toxicant being studied.

Eight ml of the extracted water is added to 12 ml of scintillation cocktail (Moghissi et al., 1973) and the tritium activity is then determined. Because of the nature of this conference, it should be stated that the liquid scintillation technique used is common. Nevertheless, this biological assay of soil vitality is possible only because of liquid scintillation counting of tritium and is being reported because we feel that it constitutes a new and exciting technique in soil analysis.

The results of insult by several toxic chemicals will demonstrate the type of information obtained by this test (Table I). In a previous test the $\text{HT} \rightarrow \text{HTO}$ reaction curve was determined to follow a function known as the exponential growth model (Figures 1 and 2). Three hours of incubation with tritium was chosen for this soil because it represented the point on that curve where maximum differences between control and any treatment exist. More intensive studies could be done by evaluating the reaction curve for each treatment, and calculating the maximum $\text{HT} \rightarrow \text{HTO}$ rates which occur at time zero.

TABLE I. The Effect of Toxic Chemicals on the Activity of Hydrogen-Oxidizing Bacteria

Chemical	Percent of HT → HTO ^a
Control	100
CdCl ₂ (213 ppm Cd)	82
AgNO ₃ (160 ppm Ag)	85
Hg(NO ₃) ₂ (160 ppm Hg)	84
NaF (2850 ppm F)	88
NO ₂ (6 ppm vapor)	85
O ₃ (.159 ppm vapor)	86
MSMA (50 mg/l)	74
2-4-D (10 mg/l)	100

^aTreatment incubation was 16 hours. HT → HTO reaction stopped after 3 hours.

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