

# SUSPENSION COUNTING OF $^{14}\text{C}$ IN SOIL, SOIL EXTRACTS AND PLANT MATERIALS BY LIQUID SCINTILLATION

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## ABSTRACT

Standard  $^{14}\text{C}$ -labelled soils or minerals were prepared by addition of standard  $^{14}\text{C}$ -labelled benzoic acid. It was shown that addition of labelled benzoic acid as an internal standard to suspensions of these samples, stabilized by CAB-O-SIL in toluene-PPO-POPOP scintillant, yielded efficiencies which allowed 100% recoveries of activity in the solid samples, provided a) the solid samples were ground to  $<53\ \mu\text{m}$  diameter and b) the weights of sample used were such that the optical density of the gel remained below about 0.9 at 450 nm (1 cm cell). The method was applied successfully to soil samples containing labelled microbial tissue and to freeze-dried coloured extracts of soil.

## INTRODUCTION

The determination of  $^{14}\text{C}$  in soil has generally involved oxidation of the organic materials to carbon dioxide using either wet (1,2) or dry (3,4) combustion. The  $^{14}\text{CO}_2$  evolved has been trapped in alkaline solutions and counted as  $\text{Ba}^{14}\text{CO}_2$  on planchets, or in suspension. Alternatively, aliquots of the alkaline solutions have been rendered miscible with scintillation fluors and the  $^{14}\text{C}$  counted by liquid scintillation.

These methods require quantitative conversion of organic materials to  $^{14}\text{CO}_2$ , which is difficult to achieve. The trapping of  $\text{CO}_2$  and subsequent counting as  $\text{Ba}^{14}\text{CO}_2$  or as  $^{14}\text{CO}_2$  in alkaline solution render the procedure tedious and

subject to error at several stages.

The requirement for a reliable routine method for the determination of  $^{14}\text{C}$  in soils and soil extracts led to a study of liquid scintillation counting of suspensions of soil particles. Suspensions of samples for counting by liquid scintillation have been stabilized by gelling agents such as aluminium stearate (5), Thixcin - a castor oil derivate (6) and CAB-O-SIL - a finely divided silica (7). While this system of counting has been applied to  $\text{Ba}^{14}\text{CO}_3$  in particular, it has also been tried on  $^{14}\text{C}$ -tagged, coloured biological materials such as liver and bacteria with success (8). Cheshire *et al.* (9) recently described application of the method to counting of  $^{14}\text{C}$  in soils. However, the data presented in this present paper indicate that counting of  $^{14}\text{C}$  in suspensions of soil particles is more complicated than suggested by these workers.

Direct counting of soil particles tagged with  $^{14}\text{C}$  has several advantages. The necessity for quantitative combustion to  $\text{CO}_2$  is eliminated. The method is simple, rapid and can be applied to small samples with low activity. The main problems anticipated were colour quenching, self-absorption and the effects of large quantities of heavy metals, e.g. iron.

#### MATERIALS

*$^{14}\text{C}$ -Labelled materials and reagents.* Benzoic acid- $^{14}\text{C}$  (specific activity  $5.32 \times 10^6$  dpm/g) was obtained from Packard Instrument Company Inc. and used as a primary standard. Benzoic acid- $^{14}\text{C}$  (specific activity 452  $\mu\text{Ci}/\text{mg}$ ) from The Radiochemical Centre, Amersham, England, was dissolved in water diluted with unlabelled benzoic acid and the mixture recrystallized twice to ensure a uniformly labelled product, which was dried *in vacuo* over  $\text{P}_2\text{O}_5$  to a constant weight. The specific activity of this secondary standard was established by counting equal weights of the two standards in 10 ml of toluene-PPO-POPOP scintillant (0.5% PPO plus 0.03% POPOP w/v in toluene). The vials were shaken vigorously and counted in a Packard Tri-Carb scintillation counter Model 3375 operating at  $4^\circ\text{C}$ , after allowing one hour for the equilibration of the vials with the temperature of the counter. Vials were counted for 5 min to give a standard deviation of 0.1% for the diluted secondary standard and 0.5% for the primary standard. The settings used were

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20-1000 with 6% gain in the Red Channel and 20-100 with 6% gain in the Green Channel. Counts in the Red Channel were used to calculate the specific activity of the secondary standard. The efficiency of counting based on the primary standard was 96%. The secondary standard contained 20,000 dpm/mg.

PPO, POPOP, CAB-O-SIL and Instagel were obtained from Packard Instrument Company Inc. Triton X-100 (Rohm and Haas technical grade) was from Robert Bryce and Co., Australia; this product yielded results similar to those obtained by scintillation grade Triton X-100 provided it was centrifuged or filtered before use.

*Mineral and soil samples.* All samples were ground in a Siebtechnik mill to pass through a 300-mesh sieve (<53  $\mu\text{m}$ ) and the colour was compared with a Munsell Colour Chart.

- (a) Acid-washed sand (greyish white N 8/0)
- (b) Red-brown earth (dull brown 7.5 YR 5/4)
- (c) Ground-water rendzina (grey N 5/0)
- (d) Goethite,  $\alpha\text{-FeOOH}$  - yellow iron oxide (yellow 2.5 Y 7/8)
- (e) Haematite,  $\alpha\text{-Fe}_2\text{O}_3$  - red iron oxide (red 7.5 R 4/8)

Samples (4.9 g <53  $\mu\text{m}$ ) were mixed with 100 mg of the benzoic acid- $^{14}\text{C}$  secondary standard in the mill for 2 min, to ensure uniform distribution of  $^{14}\text{C}$  in the sample. The samples then contained 400 dpm/mg and are referred to as "standard" samples.

*Soils containing a range of  $^{14}\text{C}$ -labelled compounds.* To ensure that  $^{14}\text{C}$  present in compounds other than benzoic acid could be counted in soil suspensions, a sample of the red-brown earth was incubated with [ $\text{G-}^{14}\text{C}$ ]glucose for 10 days. The  $^{14}\text{CO}_2$  released was trapped in 1N NaOH; 0.1 ml aliquots plus 0.9 ml  $\text{H}_2\text{O}$  were mixed with 10 ml of Triton X-100 scintillant [4 g PPO, 0.1 g POPOP in 1 litre 2:1 v/v toluene/Triton X-100; (10)] and the samples counted at 4°C after 24 hr equilibration at this temperature to eliminate chemiluminescence. After incubation, the soil was dried at 70°C overnight and passed through sieves with a range of mesh sizes. It was assumed that the  $^{14}\text{C}$  in the incubated soil sample was present in microbial tissue, i.e. a wide variety of biological compounds.

A further sample of the red-brown earth was incubated

with [G- $^{14}\text{C}$ ]glucose for 14 days and then freeze dried. The incubated soil was subjected to a series of extraction and fractionation procedures (11). The various products were freeze dried, ground to  $<53\ \mu\text{m}$  and counted for  $^{14}\text{C}$  in the same manner as for standard samples.

*$^{14}\text{C}$ -Labelled plant material.*  $^{14}\text{C}$ -Labelled wheat straw (specific activity  $171\ \mu\text{Ci/g C}$ ) was obtained from Landwirtschaftlich-Chemische, Bundesversuchsanstalt, Vienna. This was diluted with unlabelled wheat straw (3 parts labelled to 7 parts unlabelled) and ground in the Siebtechnik mill. A sample of the diluted straw ( $<53\ \mu\text{m}$ ) yielded  $43,000\ \text{cpm/mg}$ . 100 mg of this material was mixed with 4.9 g of  $<53\ \mu\text{m}$  acid-washed sand to give  $860\ \text{cpm/mg}$ , and was counted as for standard samples.

#### METHODS

*Counting of  $^{14}\text{C}$ -tagged solid samples.* Duplicate samples (5 to 100 mg) were weighed into scintillation vials. 500  $\pm$  10 mg of CAB-O-SIL were added, along with 10 ml of toluene-PPO-POPOP scintillant (0.5% w/v PPO and 0.03% w/v POPOP in toluene). Caps with Polyseal cones were screwed tightly onto the vials, which were shaken for 2 min on a vortex mixer using maximum speed. This treatment yielded a transparent or an opaque gel (depending on the colour of sample) containing a good dispersion of the samples. Vials prepared in this way were either stored at  $4^\circ\text{C}$  or allowed to equilibrate with the temperature of the counter ( $4^\circ\text{C}$ ) for one hour before counting. Window settings were 20-1000 with 20% gain in the wider channel (Red) and 20-100 with 20% gain in the narrow (Green) channel. The background varied from 40-52 cpm during the period of study, which was more than 18 months.

#### RESULTS AND DISCUSSION

*Stability of suspensions.* The results obtained from counting suspensions formed using a range of weights and particle sizes of the red-brown earth showed no significant difference between the counts obtained 1 hr after placing in the spectrometer and those obtained after 3 weeks' storage at  $4^\circ\text{C}$ . The 500 mg of CAB-O-SIL formed a gel which was rigid enough to hold even the largest particles, as found by Ott *et al.* (7) and Lloyd-Jones (12). However,

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evaporation of the scintillant did occur in occasional vials (6,13).

*Effect of sample size on recovery of activity.* The recoveries of activity have been expressed as a percentage of the expected dpm based on the weight of "standard" sample added. The linear part of the curves (Fig. 1) was reproducible provided the particle size of the samples was the same. Below a critical weight of sample per vial the recoveries of activity could be used as per cent efficiencies. This was established by addition of standard benzoic acid to vials already containing the "standard" soil samples, i.e. using the  $^{14}\text{C}$ -benzoic acid as an internal standard. When sample weights exceeded the critical value, aggregation of particles occurred resulting in self-absorption of  $^{14}\text{C}$   $\beta$ -particles (Table II), and the gels became obviously coloured and optically dense.

The best recoveries of activities, or alternatively the highest efficiencies, were obtained for the standard acid-washed sand. For both this sample and the plant material mixed with acid-washed sand there was a linear relationship between recovery of activity and weight of sample from 5 to 100 mg. The linear relationship existed up to 45 and 25 mg sample per vial for the rendzina and red-brown earth respectively. The most difficult sample was the haematite, where even with 10 mg of sample per vial it was not possible to obtain meaningful counts. With more than 25 mg of this sample the activity was lost, such that counts obtained were below background.

It will be shown later how the maximum weight of a particular sample which can be counted with close to 100% recovery of activity using an internal standard is dependent on the optical density in the 400-500 nm region.

*Incubated soil samples.* After 10 days' incubation of soil to which  $[\text{G-}^{14}\text{C}]$ glucose was added, release of  $^{14}\text{CO}_2$  accounted for 70% of the activity of the original labelled glucose incorporated into soil. The activity obtained using up to about 25 mg of  $<53 \mu\text{m}$  sample accounted for 30% of the  $^{14}\text{C}$  added before incubation (Fig. 3). The recovery of 100% of the activity initially added to the soil indicates that the counting of the  $^{14}\text{CO}_2$  and  $^{14}\text{C}$  in the soil was probably reliable. The efficiency *versus* weight curve

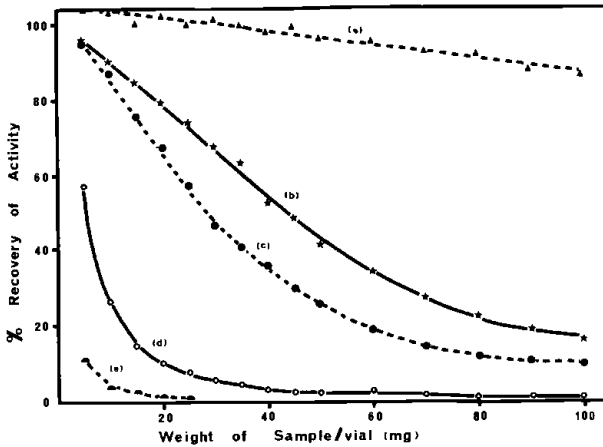


Fig. 1. Effect of weight of sample on recovery of activity. "Standard" samples - (a) Acid-washed sand; (b) Rendzina; (c) Red-brown earth; (d) Goethite,  $\alpha$ -FeOOH; (e) Haematite,  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>.

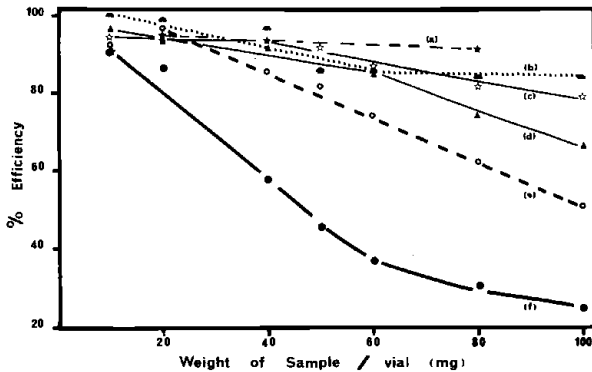


Fig. 2. Effect of particle size on efficiency of counting of <sup>14</sup>C in microbial tissue (red-brown earth). (a) 422-500  $\mu$ m; (b) 353-422  $\mu$ m; (c) 250-353  $\mu$ m; (d) 124-250  $\mu$ m; (e) 53-124  $\mu$ m; (f) <53  $\mu$ m.

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(Fig. 2) is similar to the corresponding curve in Fig. 1. It is offset due to a difference in the particle-size distribution within the  $<53 \mu\text{m}$  range caused by the second grinding of the "standard" samples after addition of the labelled benzoic acid. Close to full recoveries of activity were obtained only for  $<53 \mu\text{m}$  samples with less than about 25 mg per vial (Fig. 3). With 100 mg per vial the recovery was 70%, the rest of the activity having been lost through either self-absorption, colour quenching or other factors. Thus, the chemistry of the  $^{14}\text{C}$ -labelled compounds in the soil is not important, as the method works equally well with  $^{14}\text{C}$ -labelled benzoic acid, microbial tissue or plant materials.

Attempts were made to determine  $^{14}\text{C}$  in the samples to which  $^{14}\text{C}$ -benzoic acid was added and also in soils after incubation with  $[\text{C-}^{14}\text{C}]$ glucose. The samples were combusted in oxygen in a conventional tube furnace, in a Fisher Carbon Induction furnace and in a Beckman Oxidiser. Recoveries varied from 70 to 90% and were never quantitative. It was originally considered that the combustion procedure could be used as a basis against which the suspension counting could be compared. The results indicate that the suspension counting is more rapid and quantitative than the combustion methods examined.

*Effect of particle size.* The average density of soil particles is  $2.6 \text{ g/cm}^3$ . It was therefore expected that variations in particle size would be reflected by changes in counting efficiency due to self-absorption of the  $^{14}\text{C}$   $\beta$ -particles (6). Using the weight of material which yields a layer of infinite thickness ( $\sim 30 \text{ mg/cm}$ ), it can be calculated that the critical diameter of soil particles is in the range  $60\text{-}120 \mu\text{m}$  to cover the density range  $2.5\text{-}5.0 \text{ g/cm}^3$ . The  $300 \mu\text{m}$  diameter particles used by Cheshire *et al.* (9) were beyond this critical size range.

Even though there was a decrease in counting efficiency with reduction in particle size (Fig. 2), there was an increase in recovery of activity (Fig. 3). The lower efficiencies with increasing weight per vial obtained for the  $<53 \mu\text{m}$  particles were probably due to changes in the optical properties as a result of increase in colour intensity, in addition to self-absorption due to the formation of microaggregates within the gel.

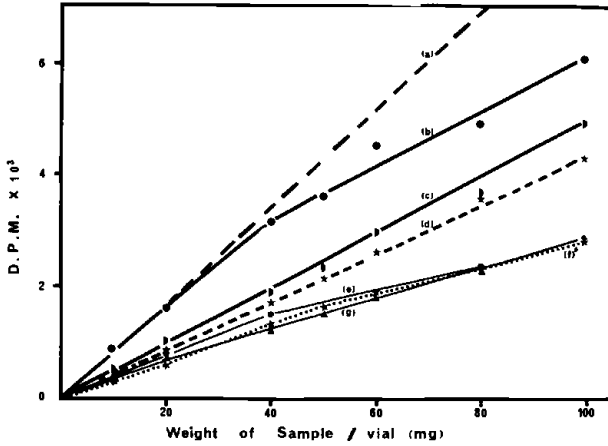


Fig. 3. Effect of particle size on recovery of activity in microbial tissue (red-brown earth). (a) 100% recovery of activity remaining in soil; (b) <53 μm; (c) 53-124 μm; (d) 124-250 μm; (e) 422-500 μm; (f) 353-422 μm; (g) 250-353 μm.

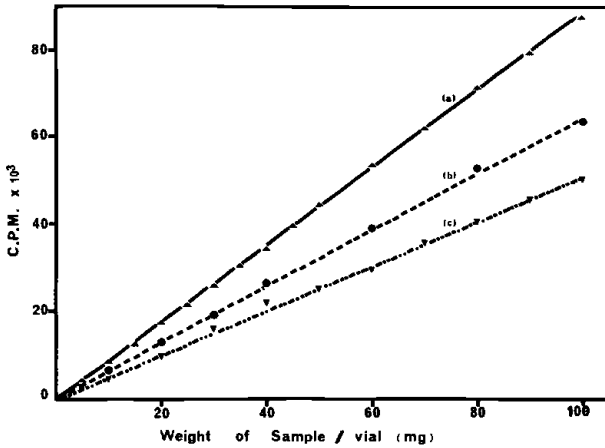


Fig. 4. Effect of particle size on counts (<sup>14</sup>C/wheat straw/acid-washed sand mixture). (a) <53 μm; (b) 53-124 μm; (c) 124-250 μm.

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High efficiencies were obtained for the larger particles because they were scattered sparingly in the suspension, so that when particles  $>53 \mu\text{m}$  were present the gel was not significantly coloured even with increasing weight of the sample. However, the efficiencies given using  $^{14}\text{C}$ -benzoic acid as an internal standard were too high and resulted in poor recoveries of activity (Fig. 3). Only particles  $<53 \mu\text{m}$  yielded quantitative recoveries of activity and then only when the critical weight of about 25 mg sample per vial was not exceeded.

It was thought that the result of the effect of size might have been affected by segregation during sieving. The  $^{14}\text{C}$  might have been concentrated in the clay fraction ( $<2 \mu\text{m}$ ) resulting in high counts for the  $<53 \mu\text{m}$  samples. That this was not the case was shown when part of the 53-124  $\mu\text{m}$  and 124-250  $\mu\text{m}$  size fractions were ground to  $<53 \mu\text{m}$  and recounted. The recovery of activity increased and matched that of the original  $<53 \mu\text{m}$  fraction, i.e. the 60% recovery given by the larger particle-size fractions is real and not due to segregation of particles differing in activity during the sieving process.

The importance of particle size was also demonstrated with the plant material (Fig. 4). The activities for the same weight of sample per vial for 53-124  $\mu\text{m}$  and 124-250  $\mu\text{m}$  size fractions were 71 and 56% respectively of those given by the  $<53 \mu\text{m}$  fractions.

*Self-absorption.* The ratio of suspension counting efficiency to homogeneous internal standard efficiency has been denoted by a factor  $f$ , and this deviates from unity by an amount that depends only on self-absorption of the  $^{14}\text{C}$   $\beta$ -particles (8). The results in Table I explain why there were low recoveries from the larger particle sizes of the incubated soil (Fig. 3) and to some extent why quantitative recoveries of activities for the smallest particles could be obtained for  $<25 \text{ mg}$  of the red-brown earth and  $<45 \text{ mg}$  of the rendzina (Fig. 1 or 2). The table also shows that self-absorption increased with increasing weight of sample, while it decreased with particle size.

The low values of  $f$  obtained for particles  $>53 \mu\text{m}$  are evidence of the expected self-absorption of the weak  $\beta$ -spectrum of  $^{14}\text{C}$  by the particles of the suspension because of their relatively large size compared with the range of

TABLE I.  $f^*$  VALUES FOR RED-BROWN EARTH AND RENDZINA

Weight in mg	Particle diameter ( $\mu\text{m}$ )						
	Red-brown earth						Rendzina
	422- 500	353- 422	250- 353	124- 250	53- 124	<53	<53
10	0.64	0.46	0.42	0.59	0.65	0.98	1.01
20	0.47	0.36	0.43	0.55	0.60	0.98	1.02
40	0.44	0.38	0.37	0.51	0.57	0.92	1.20
50		0.38	0.35	0.52	0.55	0.84	0.84
60		0.36	0.35	0.51	0.57	0.89	0.93
80	0.31	0.33	0.33	0.53	0.51	0.71	0.93
100		0.33	0.33	0.50	0.57	0.71	0.83

$$*f \text{ value} = \frac{\% \text{ suspension counting efficiency}}{\% \text{ homogeneous counting efficiency}}$$

$^{14}\text{C}$   $\beta$ -particles (8). Self-absorption in the rendzina was not as strong as in the red-brown earth. This could be part of the reason why efficiencies for this sample were higher than for the red-brown earth. The rendzina contains more clay than the red-brown earth, which would tend to make the density of particles lower.

It is quite clear that self-absorption is a significant factor unless particles of  $<53 \mu\text{m}$  diameter are used. This is close to the predicted critical size of particles as calculated from a layer of infinite thickness.

*Effect of colour.* Colour quenching is one of the serious problems in liquid scintillation counting. With soils and soil extracts it is a major problem and is mainly responsible for the low recoveries of activity and low efficiencies shown in Figs. 1 and 2.

This effect can be overcome in suspension counting up to a point by the use of internal standards to determine efficiencies. The cpm measured and corrected for these efficiencies will give 100% recoveries of activity until the critical amount of sample is exceeded when recoveries are not quantitative. The critical weights of sample for the rendzina, red-brown earth, goethite and haematite samples were 45, 25, 10 and  $<5 \text{ mg}$  respectively.

Fig. 5 shows that these weights were really the weights of sample responsible for increasing the optical density of

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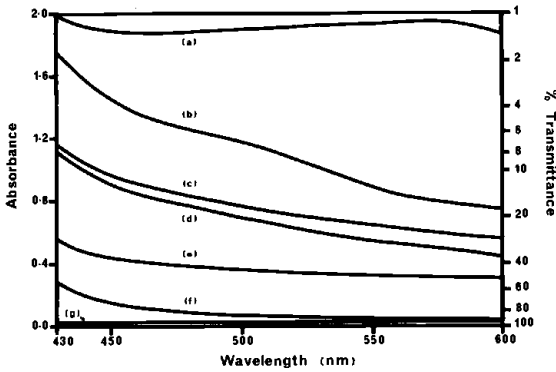


Fig. 5. Visible spectra for suspensions of  $^{14}\text{C}$ -labelled samples in CAB-O-SIL-toluene-PPO-POPOP scintillant determined on a Unicam SP800 spectrophotometer in 1 cm cells. (a) 5 mg Haematite; (b) 5 mg Goethite; (c) 45 mg Rendzina; (d) 25 mg Red-brown earth; (e) 100 mg Acid-washed sand; (f) CAB-O-SIL alone; (g) Toluene-PPO-POPOP.

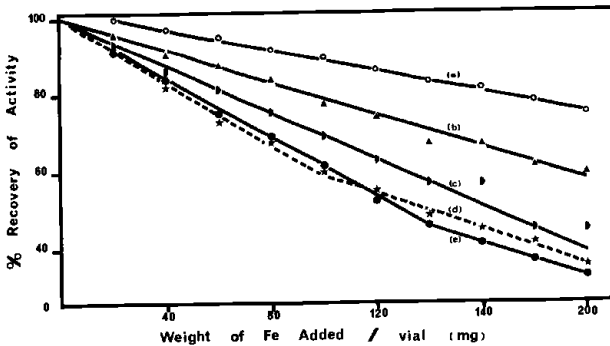


Fig. 6. Effect of iron in  $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$  on the recovery of activity. (a) Unground; (b)  $<250 \mu\text{m}$ ; (c)  $<45 \mu\text{m}$ ; (d)  $<250 \mu\text{m}$ ; (e)  $<45 \mu\text{m}$ . a, b, and c with toluene-PPO-POPOP scintillant only; d and e with Triton X-100/scintillant mixture.

the CAB-O-SIL scintillant mixture to a critical level. The spectra show that with 25 mg of the red-brown earth and 45 mg of the rendzina in the vial a similar OD is obtained, e.g.  $\sim$ OD 0.9 at 450 nm. 100 mg of acid-washed sand gave an OD of 0.42 at 450 nm. Quantitative recoveries of activity in the acid-washed sand were obtained using sample weights from 5 to 100 mg. On the other hand, 5 mg of the goethite or haematite both yielded OD's well above 0.9, which appears to be the maximum if quantitative recoveries of activities are expected. When the critical OD is exceeded, counting is non-coincident so that many events are not recorded. In addition the "bialkali" (K-Cs) used in the Tri-Carb spectrometer shows maximum quantum efficiency (25%) with photons of wavelength near to 400 nm, but this falls rapidly as the wavelength increases. Thus, increased absorbance in the 400 to 500 nm region eliminates the most efficient portion of the quantum efficiency curve. This means that problems of non-coincidence are compounded with quantum efficiency percentages of less than 10%.

To eliminate self-absorption it is necessary to reduce particles to  $<53 \mu\text{m}$ . This leads to lower counting efficiencies due to the increased colour or OD at 450 nm given to the gel by the dispersion of the finer particles. However, quantitative recoveries can be obtained using an internal standard until the optical density exceeds 0.9 at 450 nm.

*Effect of iron.* Fig. 6 shows the effect of iron on counting efficiency. The use of toluene-PPO-POPOP scintillant (0.5% w/v PPO and 0.03% w/v POPOP in toluene) gave a yellow colour with the basic ferric sulphate [ $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ ]. It was therefore thought that reduction in efficiency of counting might have been due to colour quenching. That this was not the case is shown in the different curves in Fig. 6.

Grinding to  $<250 \mu\text{m}$  reduced recoveries by about 15% of that obtained for the unground sample, while a second grinding to  $<45 \mu\text{m}$  again reduced the recovery by a further 13%. Thus, while the colour was kept constant with respect to the weight of sample used, the effect of iron was increased due to decrease in particle size. On the other hand, reduction in particle size has the effect of light scattering due to increase in total surface area (14).

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This may account for the more drastic effect of Triton X-100 scintillant on counting efficiency than the toluene-PPO-POPOP scintillant. Though it maintained the greyish-white colour of the ferric sulphate, Triton X-100 dispersed the ferric sulphate better in the scintillant. The better dispersion increased the opacity of the gel and as the amount of the ferric sulphate was increased, scattering of the light emitted increased (14,15), resulting in lower counts.

With soil samples, however, this factor will not be of great importance unless samples very rich in iron oxides are studied, since the amount of iron required to reduce counts by 10% was about 20 mg of elemental iron per vial. Heavy metals such as iron are more likely to influence  $^{14}\text{C}$  counting through colour quenching.

*Examination of soil extracts and fractions.* Various freeze-dried extracts and fractions of the red-brown earth incubated with  $[\text{G-}^{14}\text{C}]$ glucose were counted in suspension. The results (Table II) establish confidence in the procedure and its applicability to recalcitrant coloured materials. The 97.6% recovery of activity does not include some  $^{14}\text{C}$  known to be present in the heavy liquid (1,2-dibromo-3-

TABLE II. DISTRIBUTION OF  $^{14}\text{C}$  IN SOIL FRACTIONS AND EXTRACTS DETERMINED BY SUSPENSION COUNTING AND BY HOMOGENEOUS COUNTING USING INSTAGEL OR TRITON X-100

Fraction	% $^{14}\text{C}$ in sample	
	Suspension	Instagel/Triton X-100
Soil after incubation	100	
Light fraction	17.5	
Acid extract >G-10	33.0	12.3
<G-10	8.1	
Fulvic acid >G-10	20.7	6.3
<G-10	2.0	
Humic acid	3.4	
Acetylation extract		
Chloroform layer	2.8	
Aqueous layer	2.5	
Final residue	<u>7.6</u>	
Total recovery	97.6	

chloro-propane) used to float off the light fraction. Various attempts to count  $^{14}\text{C}$  in this organic liquid indicated that at least a further 1% of the  $^{14}\text{C}$  was present in compounds dissolved in the heavy liquid.

Attempts were also made to count  $^{14}\text{C}$  in materials behaving as high molecular weight compounds on Sephadex G-10 from both the acid extract and fulvic acid extract. Aqueous aliquots of these samples were mixed with scintillant using either Instagel or Triton X-100. The percentage of  $^{14}\text{C}$  in these fractions, determined using either of these emulsifiers (Table II), was about a third of the figure obtained by suspension counting.

The low recovery using the Instagel and the Triton X-100 could be explained by the fact that even though the system appeared to be homogeneous, it was virtually heterogeneous, due to the formation of microphases. The internal standards added to the system presumably existed in a different phase from the sample and were counted at considerably higher efficiencies than the samples. Suspension counting of the freeze-dried fractions must have yielded reliable quantitative results; otherwise the total recovery of  $^{14}\text{C}$  in the various fractions would not have been close to 100%.

#### CONCLUSIONS

Liquid scintillation counting of  $^{14}\text{C}$  in insoluble solid samples, or freeze-dried coloured extracts, in suspension is possible providing the samples are ground finely enough to minimize effects of  $\beta$ -particle absorption. The weight of finely ground sample which can be counted is limited by the colour it confers on the gel. The optical density (1 cm) of the gel at 450 nm must be less than 0.9; otherwise quantitative recoveries of activity will not be obtained.

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