THE DISTRIBUTION AND BREAKDOWN OF PARAQUAT ~~DICHLORIDE~~ IN THE SOIL

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ABSTRACT

The microbial degredation of paraquat dichloride by Lipomyces starkeyi was investigated by both, spectrophotometric determination and C^{14}-labelled carbon dioxide emission. 90% of the paraquat, when used in liquid culture as the sole nitrogen source was decomposed in 72 hours. When incorporated as an unessential component of the medium, paraquat took longer to be broken down (7-11 days) depending on the time allowed for Lipomyces starkeyi establishment before addition of herbicide. The breakdown of paraquat, in liquid cultures containing a range of soils varying in organic/inorganic matter ratio, was investigated. Breakdown was seen to occur within the first 96 hours, after the commencement of incubation, in cultures containing high organic matter soils. Adsorption isotherms showed that the high organic matter soils had a greater total adsorption of paraquat in solution concentrations in excess of 250 ppm. A high organic matter soil was divided by ultra-sonic disintegration into its organic and inorganic components. Paraquat breakdown was seen to occur in cultures containing the former but not the latter. The passage of C^{14}-labelled paraquat from the organic components through a dialysis membrane was observed and demonstrated to be due largely to the presence of inorganic material outside the membrane.

A system was proposed, whereby the decomposition of paraquat in the soil by Lipomyces starkeyi occured during the stage when the herbicide was weakly adsorbed onto the organic soil components. After a period of time (<96 hours in one instance) readsorption onto the inorganic soil component rendered the paraquat unavailable to microbial degredation.

C O N T E N T S

GENERAL INTRODUCTION

1. History of paraquat.

Interest in the quaternary ammonium compounds dates back to 1947 (Brian 1964), when dodecyltrimethyl-ammonium bromide was subjected to field tests and found to be widely toxic to plants. In 1954 (Brian 1964) further quaternary compounds were evaluated and the bipyridilium quaternary ammonium salts were found to be unique compared with the conventional chemicals in this family. Applications of 5-10 lb/acre of the latter were required to kill a fixed number of species under greenhouse conditions, whereas as little as 1/16 lb/acre of what are now known as diquat and paraquat salts were equivalent in activity (Brian et al, 1958). From general observations of the effects of the treatment, and because the volume of application had so little effect on the activity it seemed likely that the new compounds were acting systemically. Proof of the uptake and translocation through the aerial parts of broad beans and oats was readily obtained (Brian et al, 1958).

2. Mode of action of paraquat.

To understand clearly the mode of action of the bipyridilium quaternary salts, it is necessary to recall that there is evidence that the salts themselves are not biologically active, but become so only upon reduction (Homer et al, 1960, Calderbank 1964). This process involves the addition to the herbicide molecule of one electron and the loss of one positive charge. The additional electron is then free to take up a number

of positions available to that electron. It is the correlation between the ease of reduction and the phytotoxic activity in paraquat that led to the conclusion that this compound is active by reduction to the free radical within the plant (Homer et al, 1960). The reduction is most likely by catalytic process connected through the normal photosynthetic mechanism (Boon 1967). The use of a quaternary salt in photosynthesis experiments was first reported by Horowitz (1952) when he obtained mass spectrographic evidence that benzyl viologen, a 4,4'-dipyridilium compound used by Michaelis and Hill (1933) is reduced in a photosynthetic reaction. The free radicle involved subsequently reacts with oxygen, regenerating paraquat and giving rise to a peroxide radical or hydrogen peroxide which is considered to be the actual phytotoxic agent. Kok (1963a) and Kok and Hocj (1963) have shown that paraquat can be reduced in chlorplast systems and Zweig and Avron (1965) have demonstrated that free radicals are formed under these conditions. A similar result has been observed with diquat (Zweig et al, 1965). Evidence has also been obtained suggesting that the reduced form of paraquat is reoxidised in these systems (Kok 1963b) and Davenport (1963) has observed the production of hydrogen peroxide. More recently, Slade (1966) with maize, tomato and broad beans, and using cation exchange chromatography, observed that paraquat was degraded in plants, not metabolically but only photochemically on the surface of the foliage. This is not a surprising conclusion in the light of the rapid appearance of toxic symptoms in and subsequent death of plants treated with paraquat, presumably resulting in the very rapid destruction of

enzyme systems potentially capable of degrading the herbicide. It is in agreement with this mode of action and the time factor involved, that paraquat acts essentially as a catalyst in the production of peroxide radicals or hydrogen peroxyde, without itself being degraded. Funderburk and Lawrence (1964) fed methyl-labelled paraquat-^{14}C through nutrient solution into alligator weed and found strong evidence that it was not metabolically degraded. Slade (1966) showed that photochemical degradation of paraquat can occur on the surface of plants during daylight; the amount of decomposition depending on the time of the year, since it is related to the quantity of ultra-violet light between the wavelengths 290mμ and 310mμ present in daylight. Analytical results obtained in Great Britain suggest that after exposure to strong sunlight for several weeks, degradation does not exceed 70% of the chemical applied. Presumably the paraquat which has remained undegraded, has moved into the plants soon after application, and is protected from the action of ultra-violet light. The photochemical breakdown products of paraquat-^{14}C dichloride, were isolated by Slade (1966) and identified as 4-carboxy-1-methyl-^{14}C) pyridilium chloride and methylamine-^{14}C hydrochloride.

Clearly then, paraquat is a very potent, translocatable, rapidly acting herbicide, and as such has found extensive usage in both agriculture and horticulture (Boon 1965; Springett 1965). A subsidiary effect of paraquat appeared with the discovery of its irreversible adsorption on to the clay fraction of the soil. (Webb *et al*, 1965; Coats *et al*, 1966). This means that

any herbicidal activity of paraquat is brought about by contact with the foliage of the crop. Once the herbicide reaches the soil, whether it be through the spraying or the ploughing in of dead crops, it is inactivated. There is thus no residual effect and the crop can be resown, with the minimum of delay, in a "clean" soil.

This character of immediate adsorption, which distinguishes the bipyridylium compounds from all other groups of herbicides, poses its own problems. Other herbicides are decomposed, both chemically and microbiologically, in the soil over a period of time. This decomposition removes the toxic compounds from the soil medium, and there is no problem of herbicide accumulation. With paraquat and its relatives, however, the irreversible adsorption on to the clay mineral fraction means that there is a build up of the highly toxic herbicide. There will become a theoretical point in time when all the available adsorption sites are exhausted and any further addition of herbicide will leach through the soil and eventually into the water systems, manifesting itself in acute symptoms of mammalian toxicity.

The system outlined here, emphasises the importance of microbial degredation of herbicides in the soil, and an understanding not only of the micro-organisms involved in paraquat breakdown, but also the dynamics of herbicide distribution, is of vital importance.

3. Herbicide adsorption in soil.

Adsorption is a key factor in the behaviour and utilisation of a new herbicide, the main characters influencing the phenomena being largely understood (Freed et al, 1962; Bailey and White, 1964).

The process of adsorption involves an interaction between the electrical charges on the herbicide and those on the soil components (namely sand, silt, clay and organic fractions), and is regulated to a large extent by the characteristics of the soil solution (Frissel 1961).

(i) The inorganic fraction. The mineral components of the soil include colloidal clays bearing negative charges. The clay minerals can be broadly subdivided into two groups, the montmorillonites and the kaolinites, which consist mainly of plates of aluminium and oxygen and plates of silicon and oxygen. These silica and alumina plates either simply alternate (1 : 1 type, e.g. kaolinites) or in the order silica-alumina-silica (2 : 1 type e.g. montmorillonites). The montmorillinitic clays have very high specific surface areas, high cation exchange capacities and expanding lattices. The kaolinitic type clays have low specific surfaces, low cation exchange capacities and non-expanding lattices. Due to these differences, the kaolinitic-type clays do not have as large an adsorption capacity as the montmorillonitic-types. This difference in adsorption has been demonstrated for monuron (Hill 1956, Yuen and Hilton 1962) and a variety of herbicides (Frissel 1961). Recently Bailey et al (1968) have shown that, regardless of chemical character, adsorption occurred to the greatest extent on highly acid montmorillonites. At pH below 5 the exposed OH groups of aluminium and silica tend to become positively charged, but this feature has not yet been directly related to herbicide activity.

For herbicides, such as paraquat and diquat,

which have a unit positive charge, inactivation occurs immediately contact is made with the soil (Webb et al, 1965: Coats et al, 1966). For these two herbicides the adsorption is apparently irreversible under normal conditions. In laboratory tests, once enough diquat and paraquat has been added to saturate the cation exchange capacity (C.E.C) of the soil, further additions show biological activity.

A special feature of adsorption by montmorillonitic type clays is that organic chemicals may be trapped within the expanding lattices (Dean 1960; Pinck et al, 1961) and it has been suggested (Coats et al, 1966) that interlammelar adsorption by montmorillonites is responsible for the unavailability of the paraquat ion to wheat. Organic cations, in general, interact with clay minerals and the identification of interlammelar adsorption by X-ray diffraction is well known (Gieseking 1939; Greene-Kelly 1955). Greenland (1965) suggests that the two main factors of importance in the adsorption of organic ions are the large number of possible points of contact between ion and adsorbent, which leads to a large change in the entropy of the system; and the occurence of specific adsorption sites which are dependant on the molecular characteristics of both the cation and the substrate. The adsorption of basic organic compounds by the montmorillonitic-type clays seems to be dependent upon surface acidity (3-4 pH) and not upon the pH of the suspension. The converse appears to hold true for the adsorption of acidic type compounds (Bailey et al 1968).

Such factors are not usually of importance for inorganic ions and it is possible, therefore, to explain why

organic cations are more strongly adsorbed than inorganic ones. Knight and Tomlinson (1967) propose that strong adsorption is primarily a function of the clay mineral fraction of the soil. Knight (personal comm) also suggests that there is a wide range of adsorption strengths in the soil which it is proposed, might be related to inorganic/organic matter ratios.

(ii) The organic fraction. Hartley (1964) has proposed that far too much emphasis is given to the clay colloids in relation to herbicide adsorption and that organic matter content is the most important factor involved. Soil organic matter has a high specific surface, and high cation exchange capacity which decreases with increasing acidity. This is due to the diminished ionization of functional groups on the organic matter as the hydrogen ion concentration increases. At low pH soil organic matter has positive charges, but the implication regarding herbicide adsorption is unexplained. Herbicides may possess unit negative or positive charges which may, or may not be related to pH. Knight and Tomlinson (1967) have shown, by treating with hydrogen peroxide to remove the organic matter, that the maximum paraquat adsorption capacity of some soils is significantly influenced by organic matter content.

As far back as 1862 Rautenberg concluded that soil humus played an important part in cation exchange. Heiden (1869) and others of this period came to the same conclusion. A comprehensive review of the literature on organic material and ion-exchange is given by Gehring (1931). Evidence presented by Sherburne and Freed (1954) investigating the mechanism of adsorption

of monuron suggests the overriding importance of soil organic matter. A number of investigations have correlated the phytotoxicity of soil incorporated herbicides with soil organic matter (Upchurch 1958; Jordan and Day 1962; Sheets and Drever 1962; Upchurch and Mason 1962; Harris and Sheets 1965). More extensive evidence is available through bioassay experiments. In these, herbicide is uniformly mixed into the contents of soil pans and the concentrations necessary to give the same response noted. All users of this system agree that the organic matter content of the soil is the most important factor involved in adsorption. Upchurch (1958), in an investigation concerning the adsorption of monuron in a wide range of soil types and using cotton and ryegrass as indicator plants, found that the best correlation was with organic matter content, and that the next best with cation exchange capacity, these two being themselves correlated. Clay content, base saturation, phosphorus content and pH did not appear to be significant. Upchurch and Mason (1962) endorse these findings. Considering the four soil properties, organic matter, total clay, cation exchange capacity, and pH (Sheets and Drever, 1962) found that organic matter was the best single factor in correlating simazine adsorption. By the inclusion of the other properties, the correlation was only minimaly increased. Most of this work, however, concerns the neutral herbicides; charged herbicides have been largely ignored. Frissel (1961) in a comprehensive publication suggests that there is no especially strong attraction between herbicides and clays. However, the enormous surface area available can accommodate the herbicide molecules at a very low density. It is worth noting

here that the organic matter of the soil also presents an enormous surface area.

Table 1 shows that organic matter has the highest cation exchange capacity of all the soil constituents and a surface area comparable to montmorillonite. Therefore it would appear that organic matter has a high potential adsorption capacity for both those herbicides which may act as cations and those that can be adsorbed by physical adsorption. Differences in the composition and amount of organic matter between soils would be expected, and these differences would account for the variation of herbicide adsorption (both total and type of) between these soils. The components which comprise the organic fraction have not been completely characterised, but partly decayed cellulose and the resulting lignin residues have an extensive and excessible internal surface. Moreover, in the case of lignin, the molecular constitution of this surface is more favourable to adsorption of organic molecules than the alumina-silicon surfaces.

It can be seen, therefore, that the adsorption of a chemical by the soil may be an important factor in the biological effectiveness of that chemical. This is evidenced by the fact that a herbicide which is strongly adsorbed by the soil colloids is less effective at a given rate of application as one goes from a light sandy soil to a heavy muck soil. The strength of the adsorption varies among the constituents of the soil - sand, silt, clay and organic matter. As a general rule, clay and organic matter are the portions of the soil that adsorb most strongly. Physical adsorption of certain herbicides on non-specific organic matter

sites is reversable (Yuen et al 1962; Harris and Warren 1964; Geissbuhler et al 1963). It is suggested that this may also apply to the bipyridylium compounds.

TABLE 1. Selected Physical Properties of Soil Components.

Soil constituent.	Cation exchange capacity meq. per 100 g.	Surface area sq. meters/g.
Organic matter	200-400[a]	500-800[b]
Montmorillonite	80-150[c]	600-800[d]
Kaolinite	3-15[c]	7-30[d]

a. Broadbent and Bradford (1952)

b. Bower and Gschwend (1952)

c. Grim (1953)

d. Diamond and Kinter (1958)

4. The breakdown of Paraquat by Lipomyces Starkeyi.

Lipomyces starkeyi Lod. and Rij. is a soil yeast that was first recorded in Britain at Aberystwyth by Brady and Jones (1964), and has been used extensively as a source of microbial polysaccharide in soil structure investigations for some years, (Jones, 1964; Griffiths and Jones, 1965; Griffiths and Burns, 1968). Baldwin et al, 1966, showed that the yeast, later identified as Lipomyces starkeyi, isolated from soils can utilise paraquat dichloride as its sole source of nitrogen. It decomposed all the 20 ppm. of herbicide in cultures, and utilised the paraquat in preference to nitrate nitrogen but not ammonia. The highest rate of breakdown was observed when sucrose was present. In an experiment when 1,1'-di(^{14}C)-methyl-4,4'-bipyridilium dichloride or 1,1'-dimethyl-2,3,2',3'(^{14}C)-4, 4'-bipyridilium dichloride was added to liquid cultures innoculated with this yeast, 95% of the paraquat was decomposed in two weeks and 82-84% of the radio activity was emitted as CO_2 during four weeks at 24°C.

These workers also found that Clostridium pasteurianum and Corynebacterium fasciens were effective in decomposing 20-30% of paraquat over a matter of weeks in cultures containing 10ppm. Bozarth et al (1966) have shown that a bacterium, Pseudomonas sp. goes profusely on a medium containing 1,000 ppm. paraquat producing reduced herbicide. Baldwin (person. comm.) has related how soil samples recovered from the field have lost considerable quantities of the original paraquat dosage, but has so far failed to reproduce any degradation in the laboratory in a paraquat/soil complex.

Due to paraquat's strong adsorption on to the soil components, it was hitherto believed that no significant microbial

decomposition of paraquat occurred in the soil.

The aims of the research described in this thesis are threefold. Firstly to investigate more thoroughly the breakdown of paraquat dichloride by the soil yeast Lipomyces starkeyi. Secondly to discover the nature of the soil adsorption of that herbicide, and thirdly, to discover where the breakdown of paraquat occurs in the soil. It is believed that decomposition of paraquat, after its irreversible adsorption on to the clay colloids, is unlikely and any degradation that occurs takes place elsewhere within the soil.

MATERIALS AND METHODS

1. Soils

The four soils used in the experiments were obtained from Rothamsted Experimental Station. They were of value in that within each pair of soils, the only significant difference in gross composition was the organic matter content. (See Table 2).

A more complex analysis, from X-ray investigations, showed that the clay types in Br2B and Br3 were similar, whilst those of 72 and 75 could fairly be compared with the data obtained from an adjacent plot (See Table 3).

2. Media used in Lipomyces starkeyi experiments.

The soil yeast, Lipomyces starkeyi, was grown on malt extract agar at a temperature of 30°C. After one week sufficient growth was observed to innoculate the two liquid media involved in the experiments.

(i) Sucrose medium.

Sucrose	3.0g.
Potassium dihydrogen-ortho phosphate	1.0g.
Potassium chloride	0.2g.
Magnesium sulphate	0.2g.
Ferrous sulphate	0.01g.

Made up to 1 litre with distilled water.

With paraquat dichloride at 20ppm.

(ii) Peptone/Dextrose medium

Peptone	10g.
Dextrose	50g.

Made up to 1 litre with distilled water.

TABLE 2

ANALYSIS OF ROTHAMSTED SOILS

SAMPLE NO.	SOIL TYPE	% CLAY	%D.M.	%N	%ORG.C.	%CARB.C.	pH.
Br2B	Silt-loam	18	98.7	0.274	2.88	0.07	7.6
Br3	Silt-loam	18	99.1	0.107	0.91	0.13	8.1
75	Sandy-loam	7	99.2	0.209	2.45	0	6.6
72	Sandy-loam	7	99.4	0.115	1.35	0	6.8

TABLE 3

X-RAY ANALYSIS OF CLAY FRACTION IN ROTHAMSTED SOILS

SOIL SAMPLE	KAOLIN	MICA	MONTMORILLONITE	VERMICULITE	MICA/CHLORITE	CHLORITE	QUARTZ	FELSPAR
Br2B	11-15	26-50	5-10	26-50	--	--	PRESENT	PRESENT
Br3	11-15	26-50	5-10	26-50	5-10	5	PRESENT	PRESENT
Butt Close (comparable with 72 & 75)	5-10	11-25	26-50	26-50	--	--	PRESENT	PRESENT

FIGURES ARE EXPRESSED IN PERCENTAGES

Supplied by G. Brown, Pedology Dept., Rothamsted Expt. Station.

3. Estimation of growth rates using a nephelometer.

A standard curve was produced by relating the number of Lipomyces starkeyi cells in culture, counted on a haemocytometer slide, to the relative turbulence of the solution. This was achieved with the use of an Eel nephelometer head, connected to a Univalgo 200 taut suspension mirror galvonometer. This method alllowed rapid and accurate measurements of cell numbers and consequently the growth rates of the cultured involved.

4. The measurement of paraquat dichloride concentrations in the soil.

(i) Ion-exchange.

Paraquat salts are extremely soluble in water, and are readily retained when dilute solutions of the salt are allowed to percolate through a cation exchange resin column. This affords a method of separating paraquat residues from soil constituents, which are generally not retained by the resin.

Aqueous solutions of paraquat have a broad adsorption band with a maximum at 256mμ but measurement at this wavelength is impracticable because of considerable interference by the trace amounts of soil constituents. Paraquat however, is reduced by alkaline sodium dithionite, resulting in a deep blue solution which is relatively stable in the presence of an excess of reducing agent (Homer and Tomlinson, 1959). Paraquat can then be determined, after reduction, at 399mμ. If adequate controls are used, the methods for correction of background adsorption proposed by Norton and Stubbs (1946) are unnecessary. The method of extraction followed was that used by Calderbank and Yuen (1965).

(a) Extraction Procedure.

2.0g of oven-dried soil were taken and 100 ml. of 12N sulphuric acid added. The mixture was then boiled for five hours in a flask with a reflux condenser and allowed to cool.

A Celite 545 filter was then prepared as follows:- A Whatman No.5 filter paper was placed in a Buchner funnel supported upon a 2-litre filter flask, moistened with water, and suction applied. This allowed the paper to be held firmly in the ~~flask~~ funnel, and eliminated the chance of floating when further additions were made. 150 ml. of an aqueous suspension of 10g of Celite 545 were then poured into the funnel and sucked dry. The filtrate was discarded. Any contents of the condenser were then washed down into the boiling flask with 50 ml. of distilled water and the resulting complex poured slowly into the Buchner funnel, where it was filtered under suction. The residue was washed with two 100 ml. portions of water, allowing the first to be sucked through before adding the secon.

The pale yellow filtrate was then transferred to a 1-litre separating funnel and allowed to percolate through the prepared resin column at approximately 5 ml. per minute. At the completion of this, the funnel was removed and the column washed, at a flow rate of 3 ml. per minute, with 25 ml. of water, 100 ml. of 2N hydrochloric acid, 25 ml. of water, 15 ml. of 2.5% ammonium chloride solution, and 25 ml. of water. The paraquat was then eluted from the column with 50 ml. of saturated ammonium chloride at a flow-rate of 1 ml. per minute.

(b) Spectrophotometric determination.

After the effluent was thoroughly shaken, 10 ml. were transferred by pipette to a boiling tube, and 2 ml. of 1% sodium dithionite solution added and mixed vigorously. Within two minutes of adding the sodium dithionite, the optical density of the solution at 399 mμ was measured. A reference solution of 10 ml. of saturated ammonium chloride and 2 ml. of sodium dithionite was prepared simultaneously.

The optical densities of 0.1, 0.2, 0.4, 0.5, 0.8 and 1.0 ppm of paraquat solution were measured similarly for each series of analyses and a standard curve relating concentration to adsorption was prepared. If the optical density at 399 mμ was much higher than 1.0 ppm. the accuracy of the determination diminishes, and the sample was diluted to come within the range of controls.

The concentration of paraquat is read off, using the previously constructed calibration curve. Then the amount of paraquat in the 2g sample = Concentration in ppm. in effluence X $\frac{100}{\%\ \text{recovery}^*}$.

*Before this method is applied, a series of recovery rates is established from the range of soils.

Apparatus

(a) Boiling flask - flasks of 500 ml. capacity fitted by means of B24 ground glass joints to a two-tier reflux condenser.

(b) Spectrophotometer - A Beckman DB-G Grating spectrophotometer was used.

Reagents

(a) Sulphuric acid 12N - add cautiously with stirring

335 ml. of concentrated sulphuric acid (sp.gr. 1.84), to 665 ml. of water.

(b) Hydrochloric acid 2N - dilute 175 ml. of hydrochloric acid (sp.gr. 1.18) to one litre with water.

(c) Ammonium chloride solutions - saturated and 2.5%.

(d) Cation exchange resin. Permutit Zeo-karb 225 (52 to 100 mesh) containing 8% divinylbenzene. The column is prepared by weighing out 3.5 gm. of the resin and washing into a 25 ml. burette, in which a glass-wool pad had previously been placed. The top of the resin was covered with glass beads. 20 ml. of 2N hydrochloric acid and 50 ml. of water were passed successively through the column at a flow-rate of 5 ml. per minute.

(d) Sodium dithionite solution. 1% w/v in N sodium hydroxide. This reagent was prepared immediately before use and was not used more than 30 minutes after preparation. As solid sodium dithionite is unstable in the presence of moisture, it was stored in small quantities in a desiccator.

(ii) C^{14}-labelled paraquat

10 g. of soil were taken and 5 ml. of labelled paraquat were added. The suspension was agitated for 24 hours at 20°C. when adsorption was known to be complete. A small quantity of the soil was extracted with a spatula, and placed on a previously weighed planchet. 500µl. of glue solution was then added by means of an Agla syringe. The sample was dried under an infra-red lamp, care being taken in avoiding over-drying which caused the sample/glue complex to contract. The sample was then counted for β-particle emission, using an IDL Betamat 6050 End Window Counter.

On completion of the counting procedure, the soil plus glue, plus planchet were weighed, and by extraction of the previously determined weights for both glue and planchet, the weight of the soil was arrived at. Allowing for a uniform distribution of labelled paraquat in the original soil sample, the weight of the individual samples should be related to herbicide content, and in turn, to radiation count.

5. The measurement of paraquat dichloride in culture.

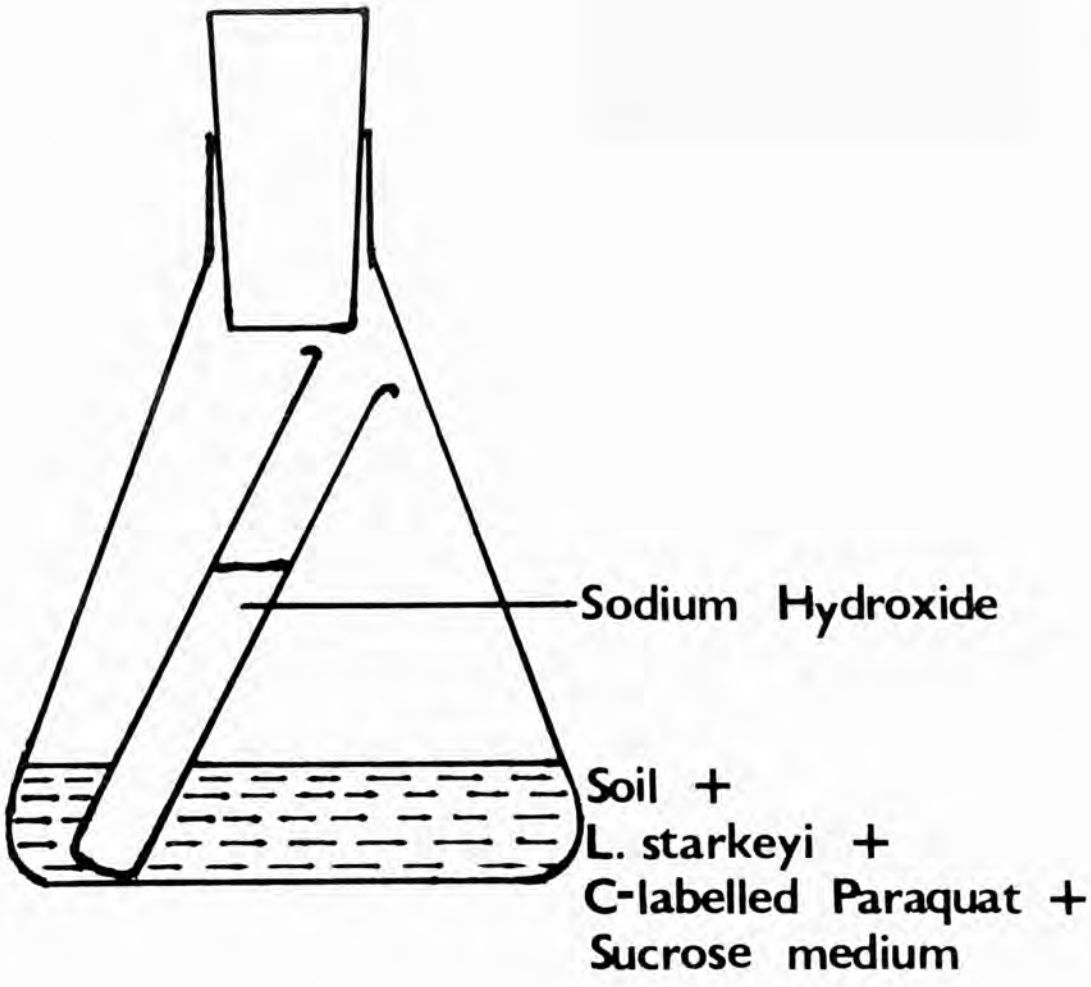
(i) Ion-exchange.

The method described above was found adequate although boiling under reflux in sulphuric acid was not necessary in cultures not containing soil. Sometimes, with concentrated cultures of Liposyces starkeyi, it was found helpful to apply air pressure to allow the suspension to pass through the resin column in a reasonable time.

(ii) C^{14} labelled paraquat.

Carbon^{14}-labelled paraquat dichloride was added to the sucrose medium and maintained at 30°C in an orbital shaker. Labelled carbon dioxide emitted during the growth of Liposyces starkeyi was collected in a small tube containing 2 ml. of 1N sodium hydroxide (see Fig. 1). The procedure used in estimating the carbon dioxide was a modification of that proposed by Devlin and Gallaway (1968). The 2 ml. of sodium hydroxide was then divided into two 1 ml. portions and 3.0mg. of sodium carbonate followed by one drop of 0.88 ammonia and 0.25 ml. of saturated barium chloride solution (35.7g. in 100 ml.) were added to each sample. The resulting white, floculent precipitate of barium carbonate was, in each instance, homogenized, placed on to a

Fig. 1. The apparatus used in the measurement of C^{14}-labelled carbon dioxide emission.


Sodium Hydroxide
Soil +
L. starkeyi +
C-labelled Paraquat +
Sucrose medium

stainless steel planchet and dried under an infra-red lamp. Application of too much precipitate at one time led to the formation of a crust when drying, and an eventual uneven distribution of the sample. Because of this, it was found best to apply the suspension in small quantities allowing each to dry before applying the next. The samples were then counted, using an IDL Betamat 6050 end window counter.

It was found that little or no enrichment of Lipomyces starkeyi occurred in the decomposition of paraquat. Because of this and other factors (page 32) in all soil/paraquat/Lipomyces starkeyi experiments, it was of advantage to establish each culture before addition of the soil sample. The paraquat used to establish the culture was seen to be broken down before the soil and more paraquat were added. Two different sequences concerning the addition of soil and paraquat were involved, in trying to discover the importance, if any, of the rate and strength of adsorption within the soil components.

6. Adsorption Isotherms.

Adsorption data was obtained by shaking suspensions of 1g. of oven-dried soil (<100 mesh) in 40 ml. of paraquat solution in conical flasks maintained at 20° ± 2° C, and using a wide range of herbicide concentrations.

The suspensions were shaken for 24 hours at which time preliminary investigations had shown that adsorption was complete. The supernatant was recovered by centrifugation at 9,000 rpm. for 30 minutes, and the unadsorbed paraquat estimated spectrophotometrically by the procedure outlined on page . Any

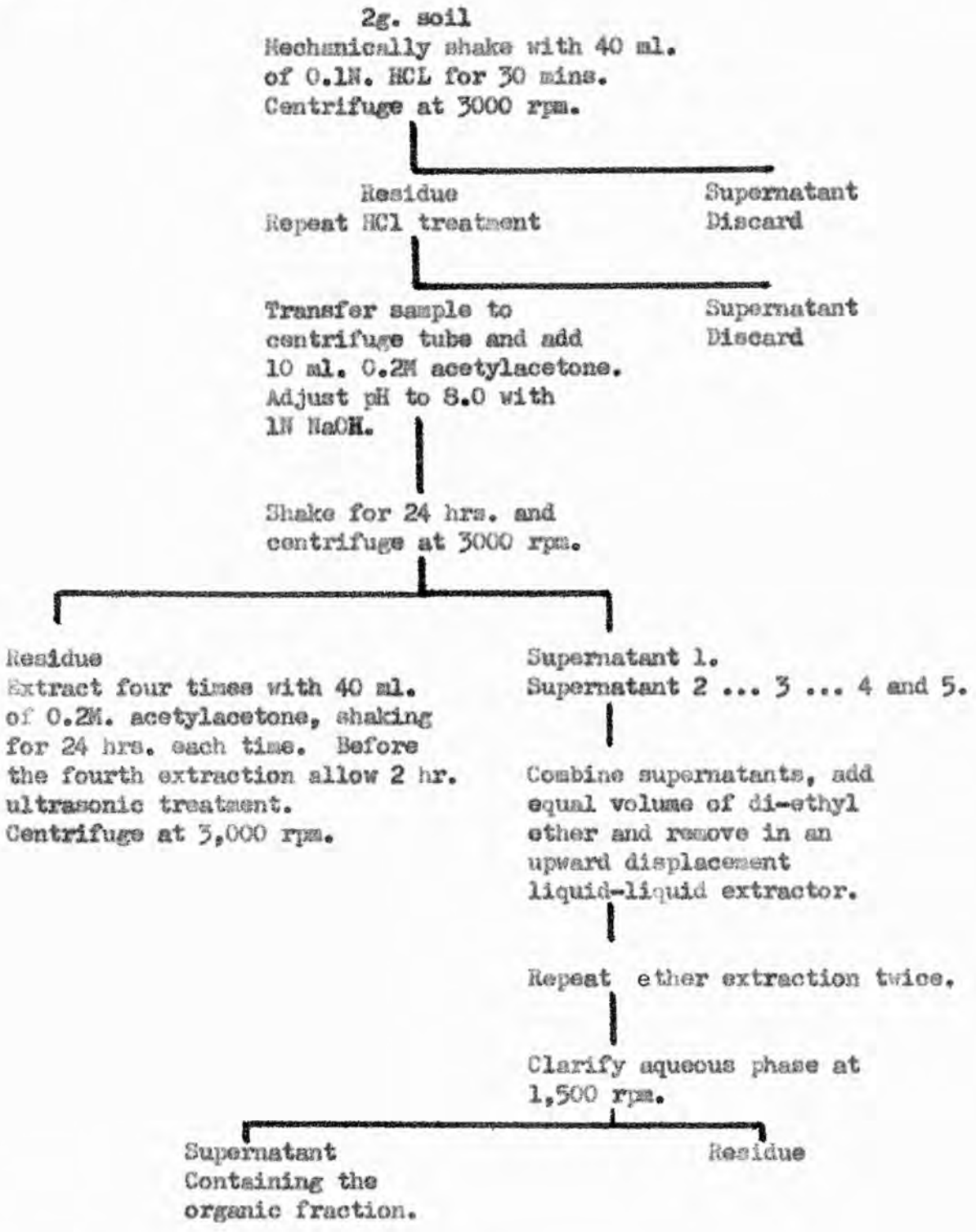
paraquat adsorbed on to the colloidal fraction which failed to sediment out during centrifugation, did not interfere with the analysis.

The quantity of paraquat adsorbed was found by difference between the known amount added and the amount recovered.

A number of controls was run in each instance to allow for any irrelevant adsorption produced by the soil components.

7. Extraction of soil organic matter. The extraction of organic matter from the soil in an unchanged form, so that herbicide distribution can be examined, is a difficult study at best. A method proposed by Halstead, Anderson and Scott (1966) was found to be the most rewarding, and involves the use of ultrasonic disintegration of the soil sample. The best extractant for organic matter was 0.2M aqueous acetylacetone (pH7.3) following an acid pre-treatment with 0.1N hydrochloric acid. Four successive extractions following the initial ultrasonic treatment were found to increase the % yield of organic material. Because the removal of excess acetylacetone and its complexes is an essential requirement of the method, the five extracts were combined, and extracted three times with di-ethyl ether. The aqueous phase often contained some colloidal clay which can be thrown down by centrifugation at a speed of 15,000 rpm. The final procedure used is outlined in Fig. 2.

FIG.2.



(After Halstead, Anderson and Scott 1966)

OBSERVATIONS AND EXPERIMENTAL RESULTS

1. The absorption spectrum of paraquat dichloride.

A solution of 2.0 ppm. paraquat dichloride, in saturated ammonium chloride, was reduced with sodium dithionite (Page 16). This resulted in a deep blue solution which had its absorption peak, in the visible range of the spectrum, at 399 mμ. This peak was seen to be well-defined, and was consequently suitable for use in quantitative measurements of the herbicide (Fig.3).

2. The standard curve for paraquat dichloride concentration.

The solvent used, in all cases, to elute paraquat dichloride from the exchange resin was saturated ammonium chloride. (377 mg in 1-litre at 20° C). Consequently, in deriving all standard curves for reduced paraquat measurements, this same solvent was used.

Two curves were prepared; the first to incorporate herbicide concentrations from zero to 1.0 ppm, the second for concentrations from 1.0 ppm to 5.0 ppm (Figs. 4 and 5, Appendix Table 1). This second curve was used essentially to determine what order of dilution was required to bring the sample into the range of concentrations calibrated on the first graph. This was especially important in determining soil adsorption isotherms (see Page 19). All final measurements were observed on or below the 1.0 ppm. mark, as, in excess of this level the accuracy of the method decreases. The relationship between concentration in ppm of paraquat dichloride and optical density at 399 mμ was seen to be represented by a straight line passing through zero.

WHEN REORDERING SPECIFY CHART NO. 93512
72
Fig.3
Absorption spectrum of reduced paraquat
E 399
100
90
80
70
60
50
40
30
20
10
0
400mμ
500mμ

Fig. 4. The standard curve for reduced paraquat dichloride at concentrations ranging from zero to 1.0ppm.

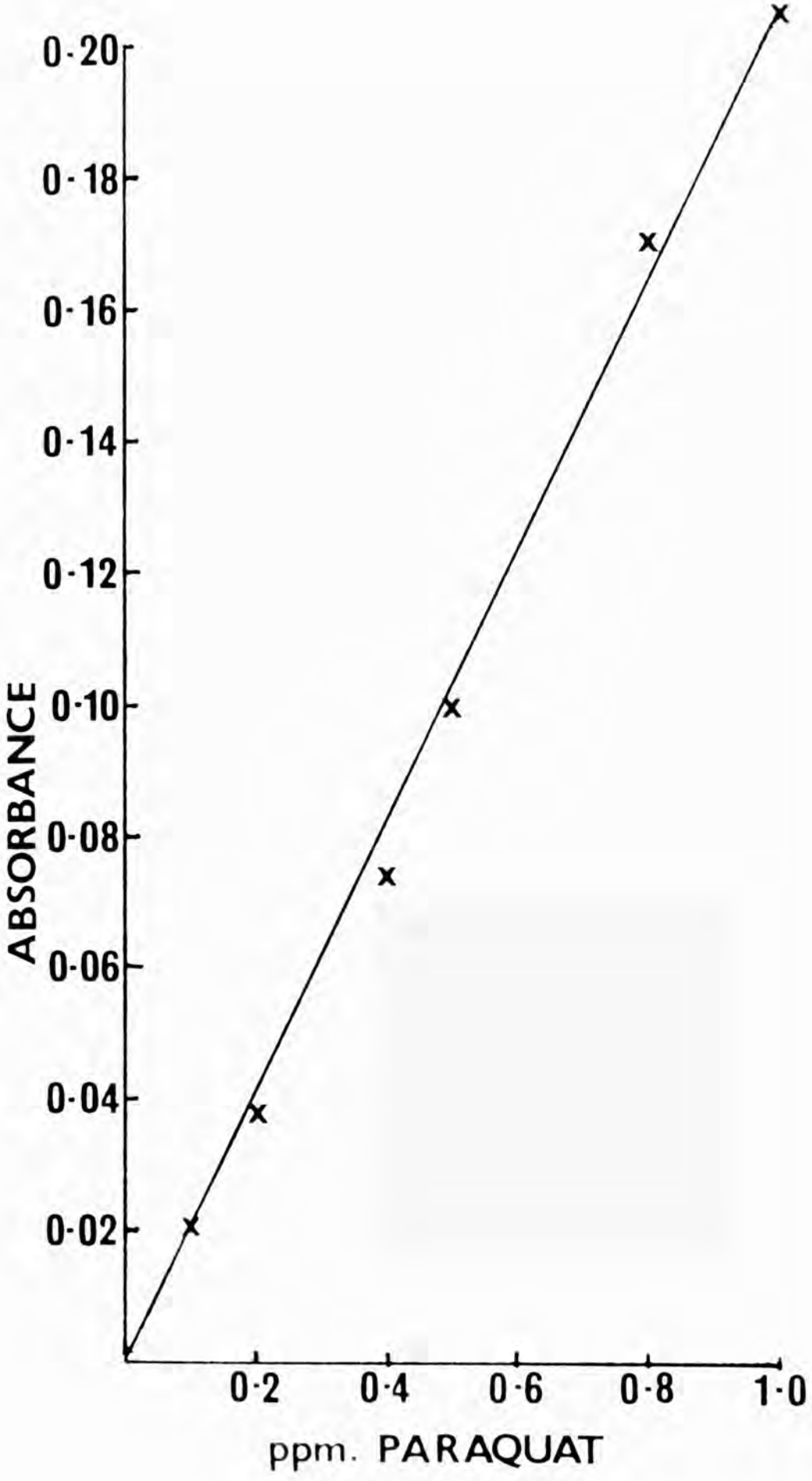
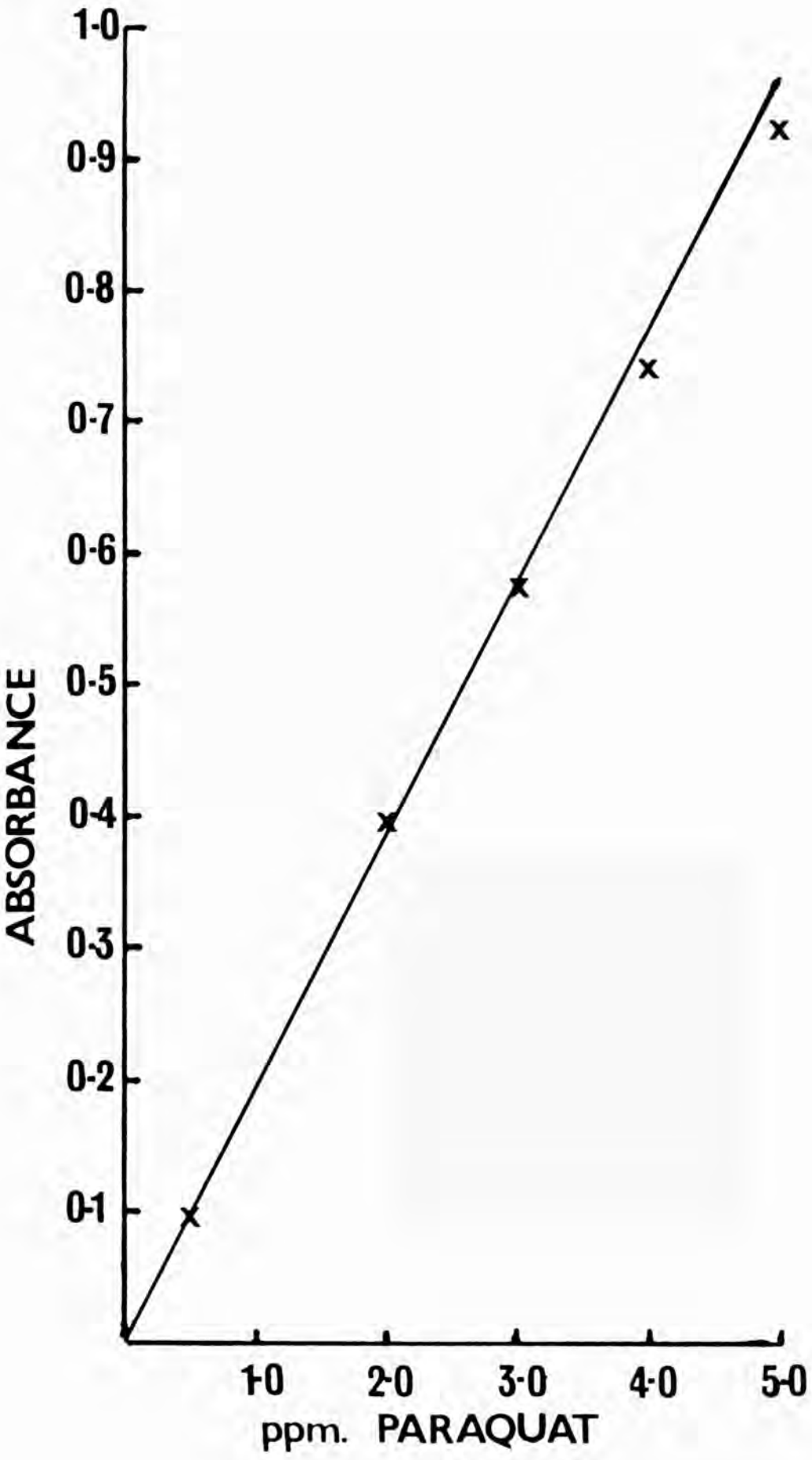

0·20
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
ABSORBANCE
0·2
0·4
0·6
0·8
1·0
ppm. PARAQUAT

Fig. 5. The standard curve for reduced paraquat dichloride at concentrations ranging from 1.0ppm.to 5.0ppm.


1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
ABSORBANCE
1·0
2·0
3·0
4·0
5·0
ppm. PARAQUAT

Consequently, any absorption reading less than that for 1.0 ppm. herbicide concentration can be expressed as a percentage of that concentration instead of reading off from the graph. Because of variation in temperature, time and reducing agent concentration, it is essential to run a series of controls concurrently with each estimation.

3. The estimation of comparative densities of Lipomyces starkeyi.

It was found impossible to ensure directly comparative growth rates of Lipomyces starkeyi between replicated cultures, especially at the earlier stages of development. Because of this, differing rates of microbial decomposition of paraquat dichloride were observed between replicates, which should have been strictly comparable. As the decomposition of the herbicide must be related to the growth rate of the yeast, a method was used, designed to supply information regarding the number of yeast cells present in each culture (page 14). This form of adjustment was used only in the case of the clear sucrose medium, with which the majority of the experimental work was performed. Decomposition in the peptone dextrose/medium was not subject to the same corrections.

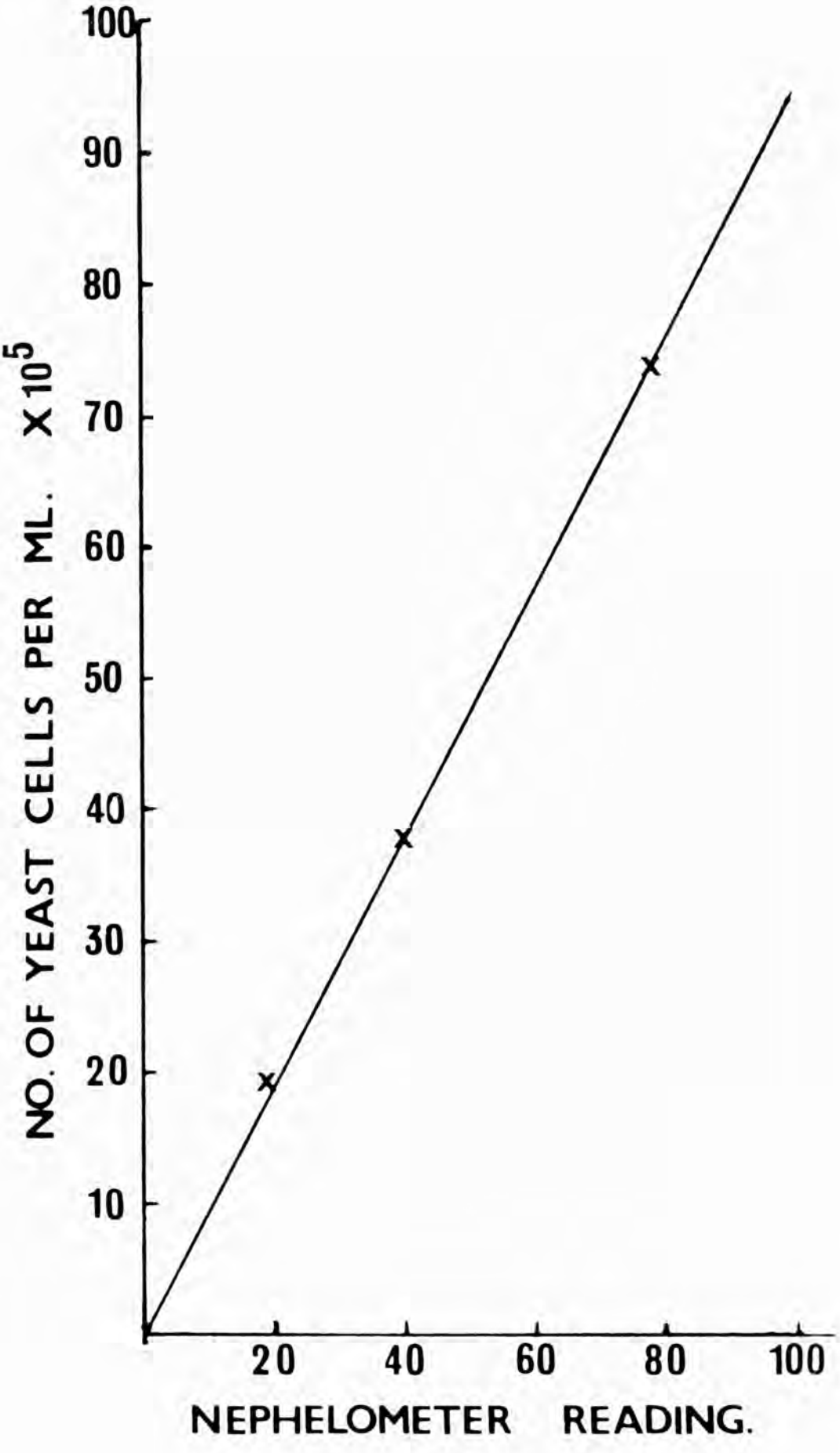
The volume of cell suspension counted, in each instance, on the haemocytometer slide is 0.02 mm^3. Consequently, the number of cells in each ml. of culture could be calculated as

$$\text{Haemocytometer count} \times 1000 \times \frac{100}{2} \times \text{dilution factor.}$$

Before all estimations were made, the samples were homogenized.

The results shown in Fig.6 and Appendix Table 2, indicated that the optical density of the culture was directly

Fig. 6. The relationship between the number of Lipomyces starkeyi cells in a sucrose medium to the turbidity of that medium, as measured with a Nephelometer.


NO. OF YEAST CELLS PER ML. X 10^5
100
90
80
70
60
50
40
30
20
10
20
40
60
80
100
NEPHELOMETER READING.

related to its yeast cell content.

4. The growth rate of Lipomyces starkeyi in sucrose medium.

Three flasks of the sucrose medium were inoculated with Lipomyces starkeyi and incubated in an orbital shaker at 30° C. Relative turbidity was measured, with a Nephelometer, every eight hours for twenty-four hours, and thereafter every twenty-four hours. Readings of < 10 cannot be regarded as indicative of growth, because of certain cloudiness in the medium at time zero. In view of this, a small lag phase in the growth rate was observed lasting approximately 16 hours. After this, a rapid rate of growth began rising steeply up to 72 hours, at which point it slowed down almost to a stop. (Fig.7, Table 4).

The growth curve coincides with the breakdown of paraquat (see page 26).

5. The microbial decomposition of paraquat dichloride by Lipomyces starkeyi.

A. Spectrophotometric method.

This experiment was designed to investigate the breakdown of paraquat dichloride in the two media. The first, a medium containing paraquat as its sole nitrogen source; the second a complete medium also containing paraquat. This would tell us whether the Lipomyces starkeyi utilised paraquat because it had no other nitrogen source, and whether this utilisation was still evident when an alternative source of nitrogen was available. Previous work (Baldwin, et al 1966) has demonstrated the breakdown in paraquat in a nitrogen starved medium.

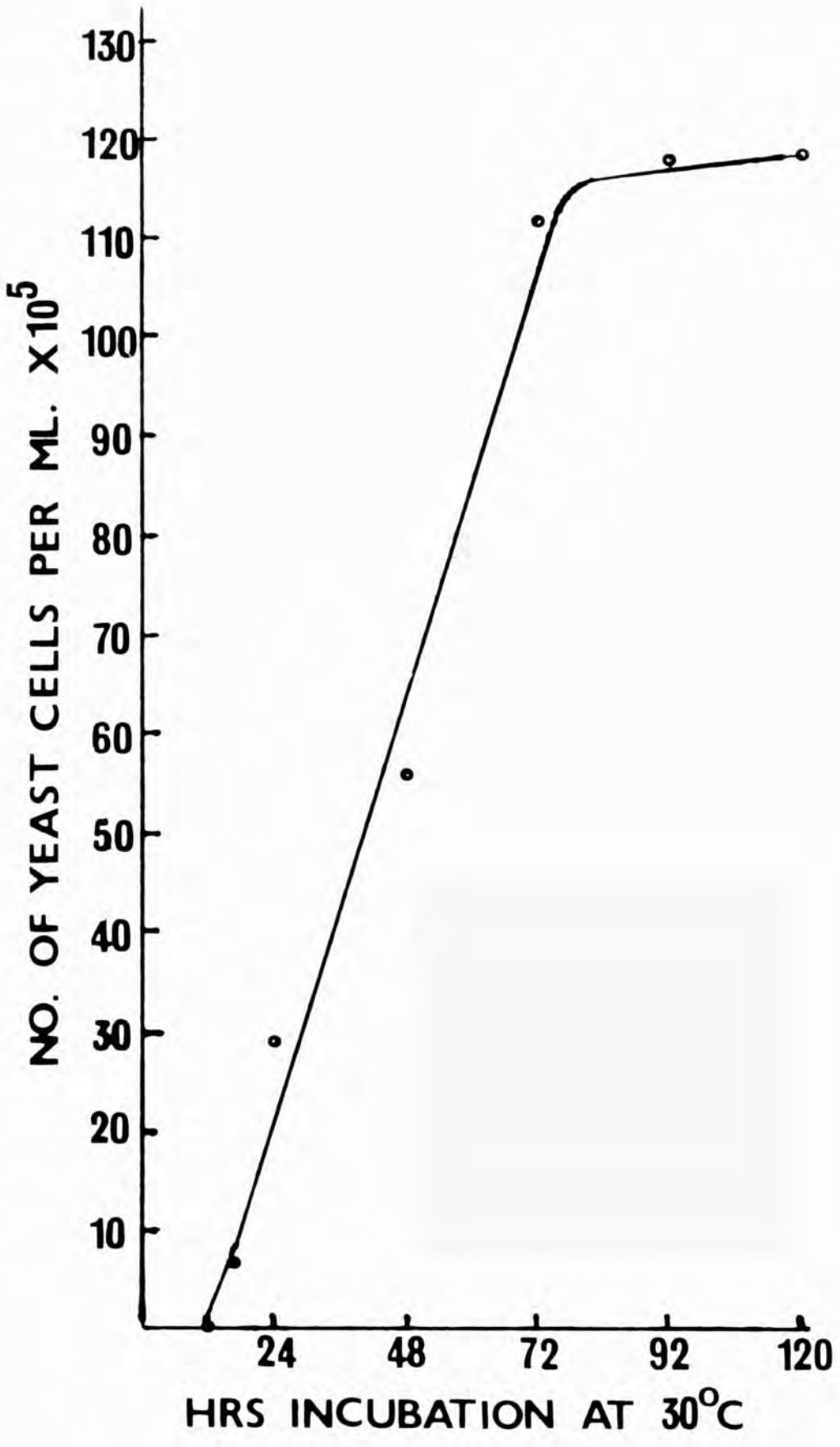
TABLE 4

THE GROWTH RATE OF LIPOMYCES STARKEYI IN A SUCROSE MEDIUM

Time in hours	0	8	16	24	48	72	96	120
Rep.1.	4	5	18	31	56	110	121	120
Rep.2.	6	3	12	32	64	116	120	117
Rep.3.	7	7	10	43	67	129	132	134
Total	17	15	40	106	187	355	373	371
Mean	6	5	13	35	62	118	124	124

Figures indicate Nephelometer readings.

Fig. 7. The growth rate of Lipomyces starkeyi in a sucrose medium, as measured with a Nephelometer.


NO. OF YEAST CELLS PER ML. X10^5
130
120
110
100
90
80
70
60
50
40
30
20
10
24
48
72
92
120
HRS INCUBATION AT 30°C

a) Sucrose medium (Page 13). This medium, containing the equivalent of 20 ppm. paraquat, was inoculated with a culture of Lipomyces starkeyi which had been grown on malt extract agar for seven days. The cultures were incubated in an orbital shaker, maintained at 30° C, and two replicates and a control (no Lipomyces starkeyi) were removed at certain fixed time intervals. These intervals were every four hours during the first twenty four, and subsequently every eight hours, for a total period of three days. Paraquat was extracted by the usual method (page 18) and spectrophotometric analysis made. It was observed, at this stage, that all cultures did not necessarily grow at the same rate, some taking longer to establish than others. Because of this it was decided that a direct comparison between the growth rate of replicate cultures might be used, and a factor evolved to enable a clearer interpretation of the results. This method, using a Nephelometer, which measures relative turbidity, is outlined on page 14. The absorbance reading for each replicate on the Nephelometer was recorded before the extraction procedure and a correction factor derived as follows:-

Let the reading on the nephelometer for Rep.1 = a.

" " " " " Rep.2 = b.

Where $a > b$.

Let the absorbance reading of reduced paraquat for Rep.1 = ax.

" " " " " " " Rep.2 = bx.

Then correction factor = $\frac{a}{b}$ = Y.

Then corrected reading for Rep.2. = $\frac{bx}{Y}$

A summary of these results is shown in Table 5, and expressed graphically in Fig.8. A full list of results is to be found in Appendix Table 3.

i) The use of the correction factor is of limited value, until the growth rate has begun to accelerate rapidly. Before this time, small differences in growth rate are magnified and only serve to distort the results. After 20 hours, some increase in correlation is observed, but rarely enough to justify the use of this method.

ii) The unadjusted readings show a period, where little decomposition occurs, lasting approximately 24 hours. Thereafter, rapid breakdown begins, the rate accelerating up to 72 hours when all paraquat detectable by this method has disappeared. This breakdown pattern closely follows the growth curve for Lipomyces starkeyi (Fig.7), as would be expected.

b) Peptone/dextrose medium. This complete medium also contained 20 ppm. of paraquat. However, the addition of herbicide at the commencement of the culture, as in the sucrose medium, seemed to have some kind of toxicity effect, and growth was slow and spasmodic. It was found best to allow the Lipomyces starkeyi to establish itself, for periods of either seven or fourteen days, prior to addition of the herbicide. Using this method, a series of results was achieves, seen in Table 6, and expressed graphically in Fig.9. The results are

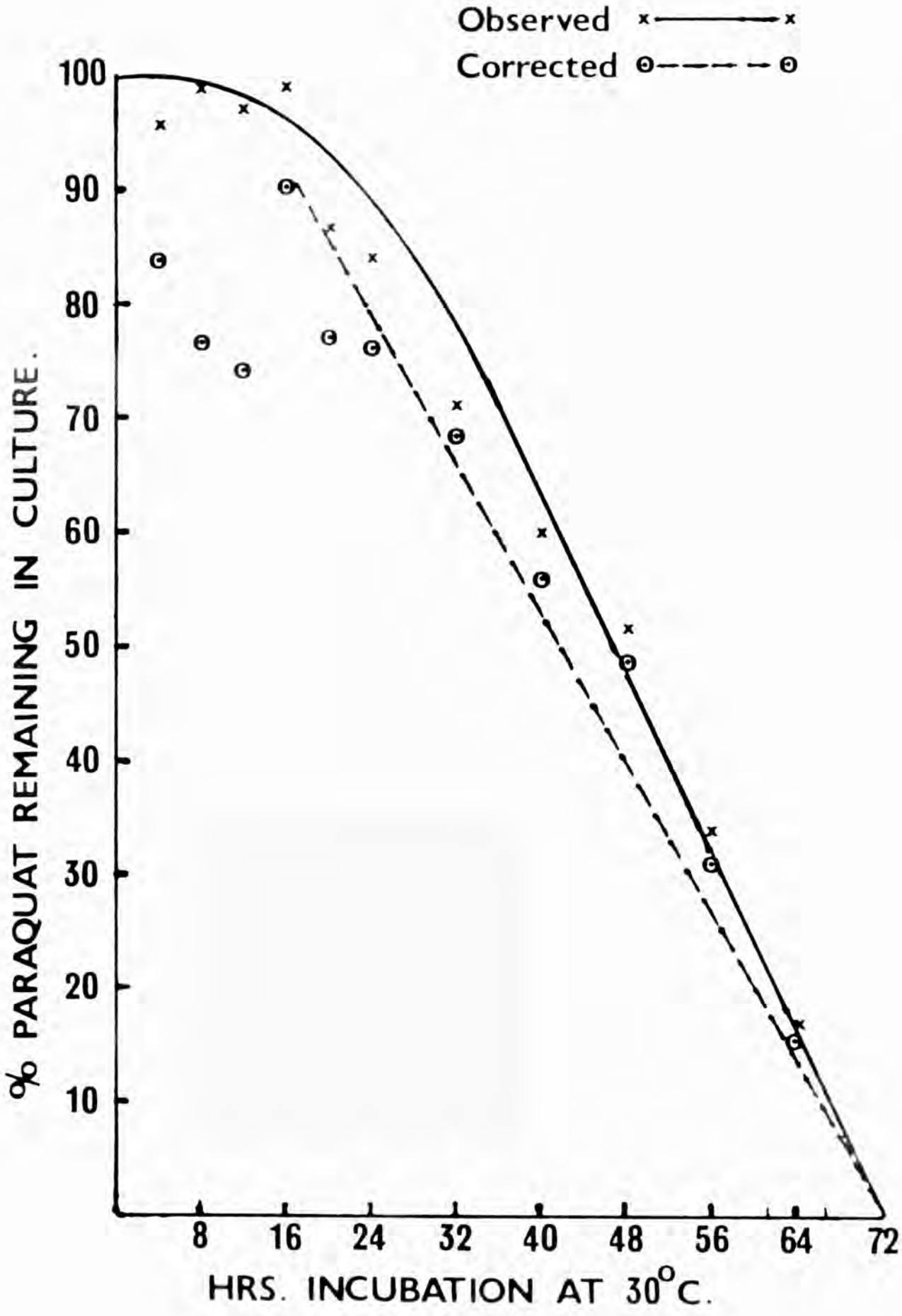
TABLE 5

THE MICROBIAL DECOMPOSITION OF PARAQUAT DICHLORIDE BY LIPOMYCES STARKEYI IN A SUCROSE MEDIUM AS MEASURED SPECTROPHOTOMETRICALLY.

Time in hours.	Rep.	% Paraquat remaining in culture			
		observed	Mean	Corrected	Mean
4	1	95.34	95.60	71.50	86.68
	2	95.85		95.85	
8	1	102.08	98.70	58.33	76.82
	2	95.31		95.31	
12	1	97.30	96.76	52.43	74.33
	2	96.22		96.22	
16	1	92.17	98.80	92.17	90.06
	2	105.42		87.15	
20	1	94.06	86.88	84.16	81.93
	2	79.70		79.70	
24	1	84.62	84.11	84.62	81.03
	2	83.59		77.44	
32	1	75.52	71.20	69.79	68.23
	2	66.67		66.67	
40	1	55.03	60.06	55.03	56.15
	2	65.08		58.20	
48	1	56.10	51.47	50.73	48.78
	2	46.83		46.83	
56	1	36.60	35.77	36.60	31.19
	2	30.93		25.77	
64	1	18.58	16.67	16.94	15.85
	2	14.75		14.75	
72	1	0	0	-	-
	2	0	0	-	-

See also Appendix Table 3

Fig. 8. The microbial decomposition of paraquat dichloride by Lipomyces starkeyi in a sucrose medium, as measured spectrophotometrically.


Observed
Corrected
100
90
80
70
60
50
40
30
20
10
% PARAQUAT REMAINING IN CULTURE.
8
16
24
32
40
48
56
64
72
HRS. INCUBATION AT 30°C.

found in Appendix Table 4.

i) Cultures grown for seven days, prior to addition of paraquat, showed on addition of herbicide, a gradual breakdown continuing for ten days, when approximately 10% of the herbicide remained undecomposed.

ii) Cultures grown for fourteen days, prior to addition of paraquat, showed a faster rate of breakdown which indicated that 50% of the paraquat had disappeared in sixty hours, as compared with 150 hours in (i). After 5 days approximately 10% of the herbicide remained undecomposed.

These differences were almost certainly accounted for by the greater establishment of the micro-organism in the fourteen-day cultures.

From these sets of results, it was seen that:-

i) Lipomyces starkeyi decomposed 20 ppm. of paraquat dichloride, when it was an essential component of the medium, in approximately 72 hours, under the conditions of the experiment.

ii) In an established culture, unessential paraquat dichloride was also decomposed but at a much slower rate.

It was observed during the course of these investigations, that Lipomyces starkeyi frequently lost its ability to decompose paraquat if kept in the presence of herbicide for long periods. "Rejuvenation" of the culture could be achieved by growing in a non-paraquat medium.

Because of a comparatively greater length of time involved in growing Lipomyces starkeyi in the peptone/dextrose

TABLE 6.

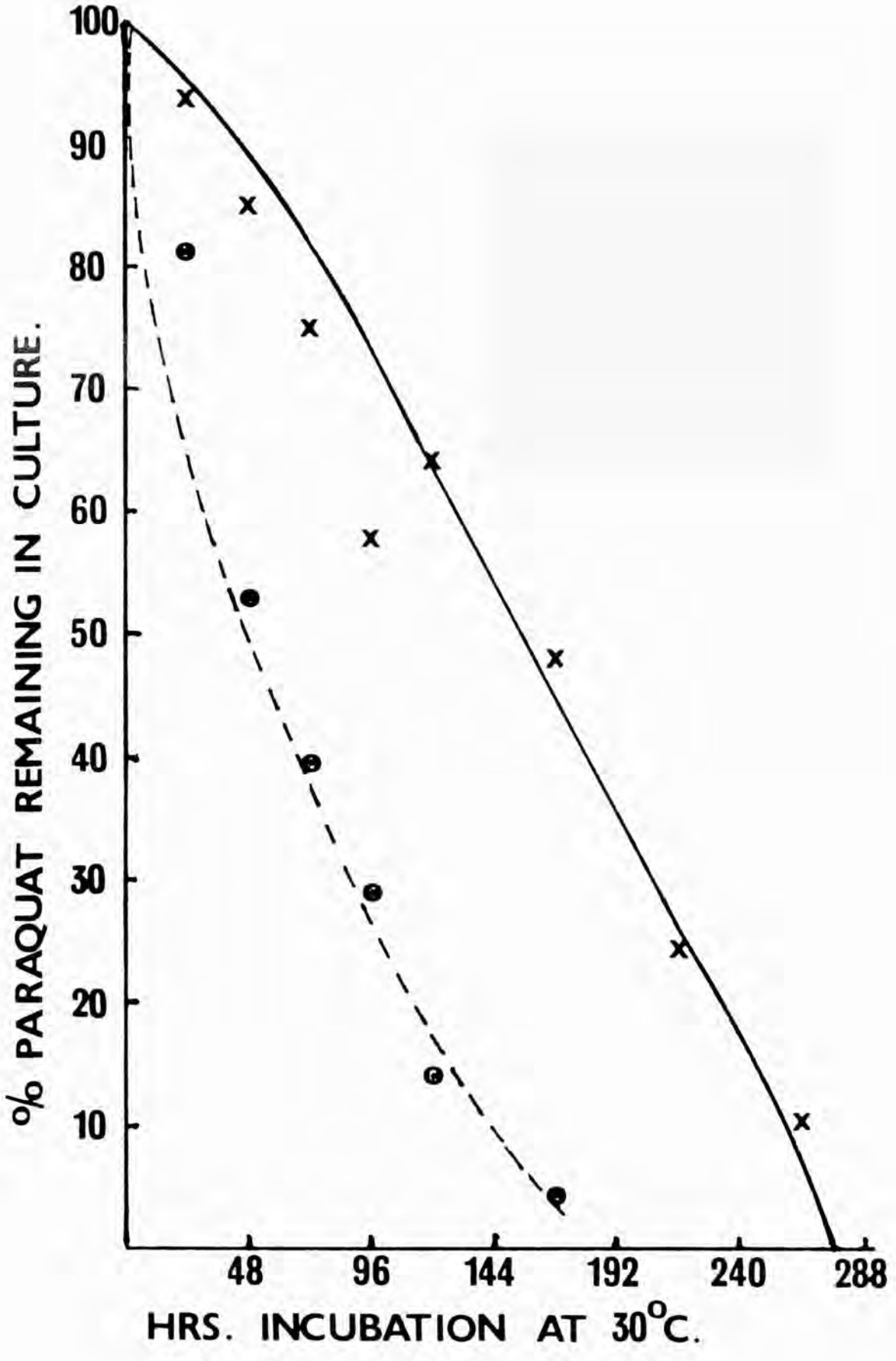
THE MICROBIAL DECOMPOSITION OF PARAQUAT DICHLORIDE BY LYPOMYCES STARKEYI IN A PEPTONE/DEXTROSE MEDIUM AS MEASURED SPECTROPHOTOMETRICALLY

Time in hours.	Rep.	% Paraquat remaining in culture			
		7-Day cultures	Mean	14 Day cultures	Mean
24	1	89.47	93.46	77.60	81.25
	2	97.44		84.90	
48	1	79.81	85.10	55.38	52.96
	2	90.38		50.54	
72	1	73.16	75.00	35.94	39.59
	2	76.84		43.23	
96	1	62.57	58.03	26.92	29.12
	2	53.48		31.32	
120	1	59.90	64.36	17.96	14.07
	2	68.81		10.18	
168	1	44.74	48.16	2.58	4.64
	2	51.58		6.70	
216	1	17.03	24.73		
	2	32.42			
264	1	6.63	10.46		
	2	14.29			

See also Appendix Table 4

Fig. 9. The microbial decomposition of paraquat dichloride by *Lipomyces starkeyi* in a peptone/dextrose medium as measured spectrophotometrically.

Broken line indicates 14 day cultures.
Continuous line indicates 7 day cultures.


100
90
80
70
60
50
40
30
20
10
% PARAQUAT REMAINING IN CULTURE.
48
96
144
192
240
288
HRS. INCUBATION AT 30°C.

medium, and the artificiality of the post-establishment herbicide addition, where possible, all experiments hereafter were conducted with the use of the sucrose medium.

B. Radioactive tracer method.

An estimation of paraquat dichloride decomposition was carried out, using C^{14}- labelled herbicide. Breakdown of herbicide resulted in C^{14}- labelled carbon dioxide being evolved, which was measured, after collection, in a caustic soda trap (see page 18).

The advantages of this method over that which measured the paraquat remaining undecomposed (see page 14) were that besides being much speedier, it eliminated the need for a different culture for each extraction. On the removal of the carbon dioxide trap for analysis, a fresh one was put in its place. However, despite this advantage there were certain limitations imposed by the method. It was necessary to know what total emission of labelled carbon dioxide was going to be before any accurate quantitative determination of paraquat decomposed was made.

The cultures were incubated at 30° C in an orbital shaker. Soda traps were removed and analysed every 24 hours. From the results, it can be seen that (Table 7, Fig.10, Appendix Table 5):-

i) The largest quantity of emission was at days 2 and 3, whilst some carbon dioxide was still being detected after 16 days. This shows up the limitations of the ion-exchange procedure (page 25) which indicated complete breakdown

TABLE 7

THE MICROBIAL DECOMPOSITION OF C^{14}-LABELLED PARAQUAT DICHLORIDE BY LIPOMYCES STARKEYI IN A SUCROSE MEDIUM. MEASURED BY THE EMISSION OF C^{14}-LABELLED CARBON DIOXIDE.

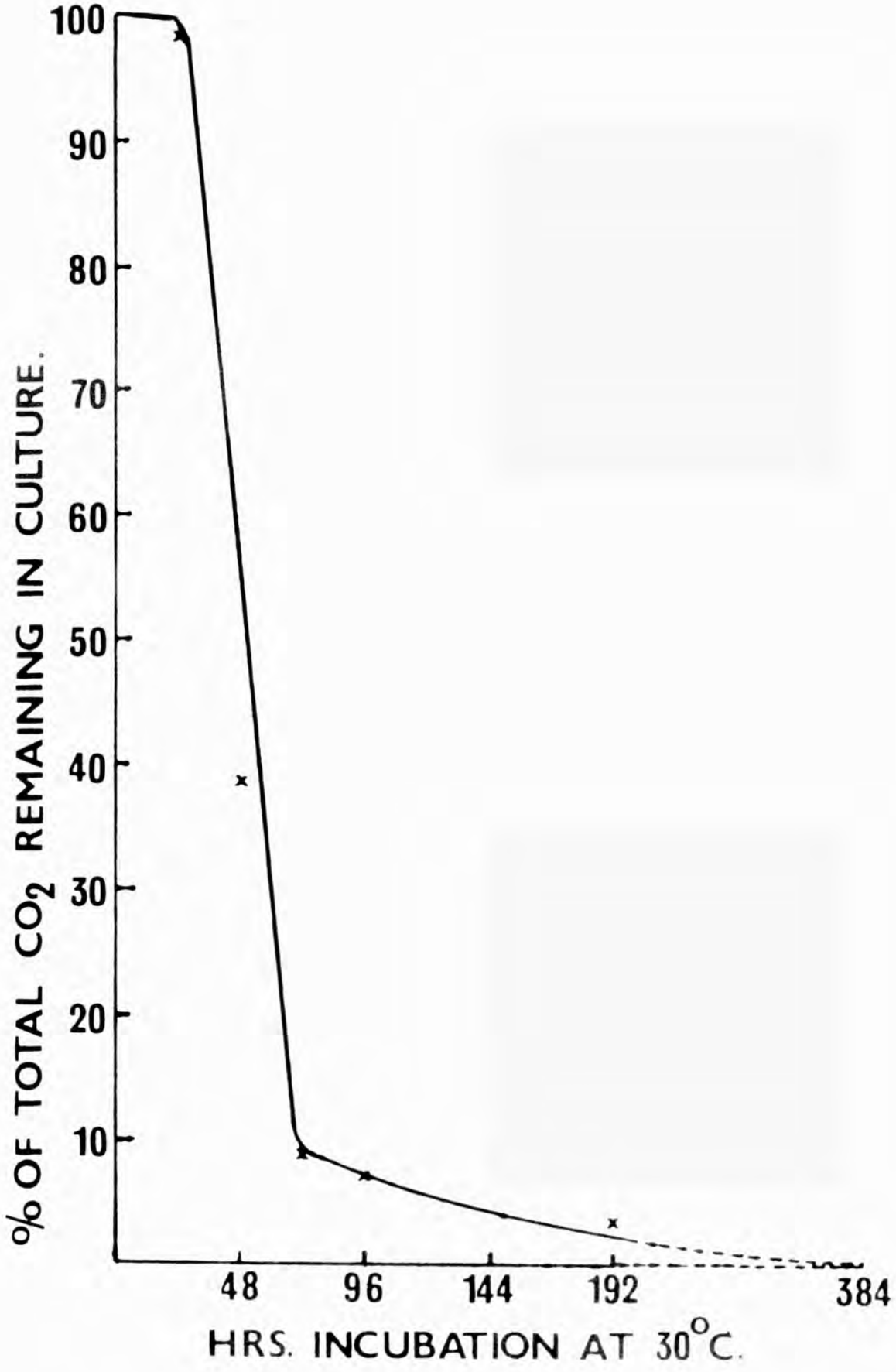
Replicates	Time in hours. 24	48	72	96	192	384
1	28	308	166	34	40	46
2	30	321	180	32	37	45
3	32	316	155	29	46	41
4	31	351	190	32	45	40
MEAN	30	324	173	32	42	43

Replicate Figures are counts per minute and each is the mean of six counts.

For purposes of comparison a background count of 23± cpm. may be used.

See also Appendix Table5.

Fig. 10. The microbial decomposition of C^{14}-labelled paraquat dichloride, as measured by labelled carbon dioxide emission.


100
90
80
70
60
50
40
30
20
10
% OF TOTAL CO_2 REMAINING IN CULTURE.
48
96
144
192
384
HRS. INCUBATION AT 30°C.

at 72 hours. However, by the use of tracer methods, approximately 91.5% of the paraquat was decomposed after 72 hours, which left only 8.5% of 1.0 ppm in the medium. Therefore, with the spectophotometric method of quantitative analysis, after 72 hours a detection level of 0.085 ppm. would be required. As can be deduced from the correlation curve on page 22 concentrations of paraquat below 0.1 ppm. produce unreliable absorbance readings. Therefore, the two methods of quantitative paraquat detection are not incompatible. The phase of rapid microbial breakdown was seen to be the same in both cases, that is, from 24 to 72 hours after innoculation.

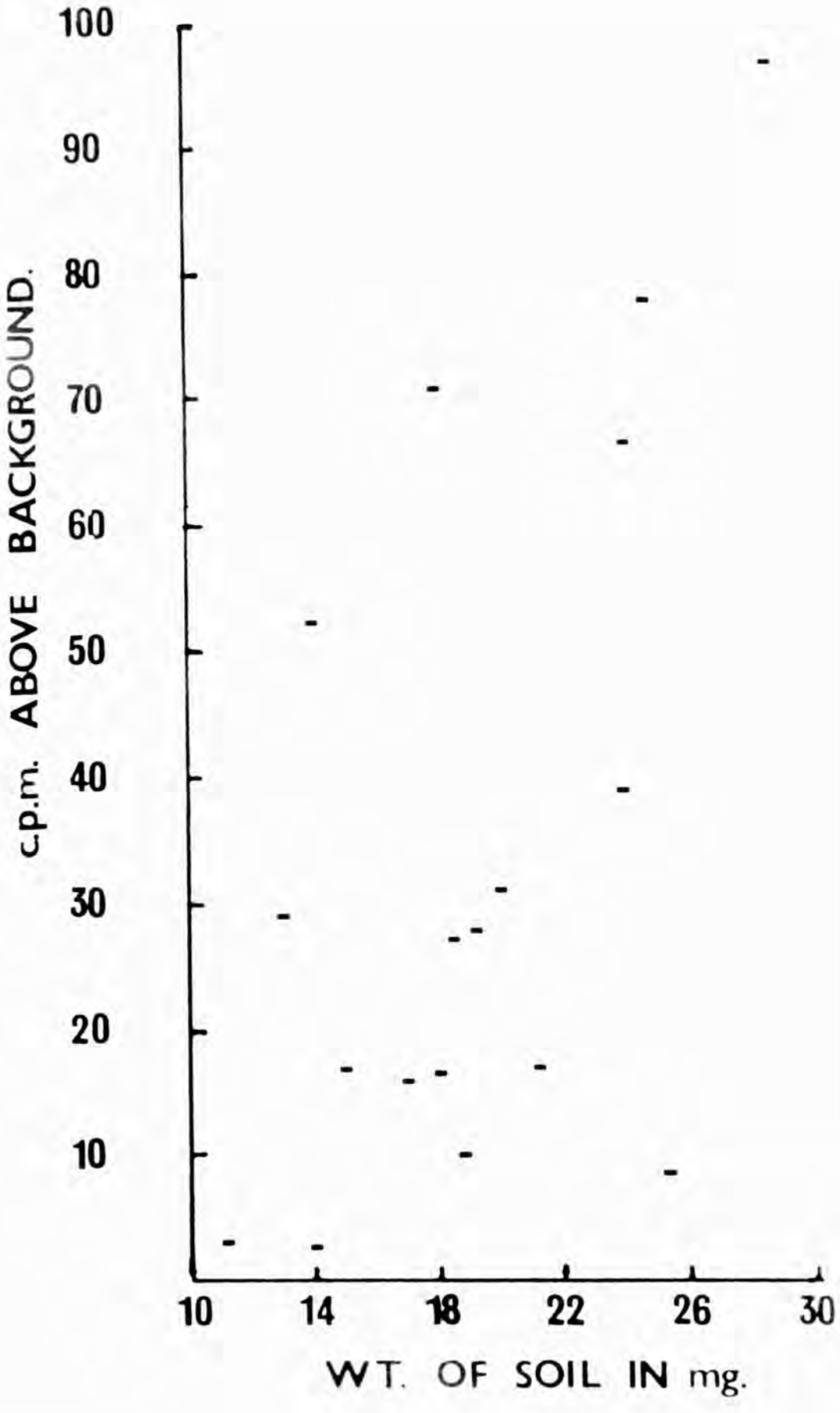
Another method, designed to show how much labelled paraquat remained in the soil, was investigated using the technique on page 17. Limited success was achieved (Fig.11, Appendix Table 6) ahtough a modification was used at a later date with far better results (page 37).

6. Adsorption isotherms.

The four soils used in this experiment are described on page 13 and the methods used in the determination of adsorption isotherms outlined on page 19. The results of each series of equilibrations were expressed in a graph showing the quantity of paraquat adsorbed in grams per 100 grams of soil, against the solution concentration (Fig.12). From the results (Table 8, Appendix Table 7) a number of conclusions can be reached.

i) The total amount of paraquat dichloride adsorbed on to the soil particles increased up to a certain level, dependent on the concentration of herbicide in solution, in equilibrium

Fig. 11. The estimation of C^{14}-labelled paraquat dichloride remaining in the soil.


100
90
80
70
60
50
40
30
20
10
c.p.m. ABOVE BACKGROUND.
10
14
18
22
26
30
WT. OF SOIL IN mg.

with the soil. The peak adsorption capacity (PAC) was in all instances reached when the paraquat concentration was in the region of 1,000 ppm. Higher concentrations than this did not significantly increase the PAC.

ii) Within comparable soils (75 and 72; Br2B and Br3) the PAC was significantly different in each pair. The higher PAC occurred in the soil with the high organic matter content. It is suggested that in soils 75 and Br2B the organic matter alone is responsible for the increase in PAC.

iii) There was a significant difference between the two pairs of soils, in that the PAC of 75 and 72 was higher than that of Br2B and Br3. This was, in all probability, accounted for by the difference in clay types between the two soil pairs (see Table 3). Soils 75 and 72 had 26 - 50% montmorillonite (high adsorption capacity) and 5 - 10% kaolinite (low adsorption capacity). Soils Br2B and Br3 in contrast, had only 5 - 10% montmorillonite, and as much as 11 - 25% kaolinite.

iv) The organic matter content of soil 75 caused as much as 42% of the increase in PAC due to the difference in clay types alone.

v) Organic matter seemed to have little effect at solution concentrations below 250 ppm. but a difference of the type shown above would not be expected until the concentration is reached at which the adsorption sites are approaching saturation. However, the limits imposed by the experiment do not necessarily preclude a range of adsorption strengths below 250 ppm.

TABLE 8.

THE PARAQUAT DICHLORIDE ADSORPTION ISOTHERMS FOR SOILS 75, 72, Br2B and Br3

ppm paraquat	Grams/100 grams adsorbed			
	75	72	Br2B	Br3
50	0.197	0.196	0.199	0.199
100	0.384	0.381	0.393	0.390
250	0.808	0.780	0.668	0.592
500	1.640	1.480	1.000	0.600
1000	1.920	1.540	1.120	0.640
2000	1.920	1.544	1.200	0.640

See also Appendix Table 7.

Fig. 12. The paraquat dichloride adsorption isotherms for soils Br2B,Br3,72 and 75.

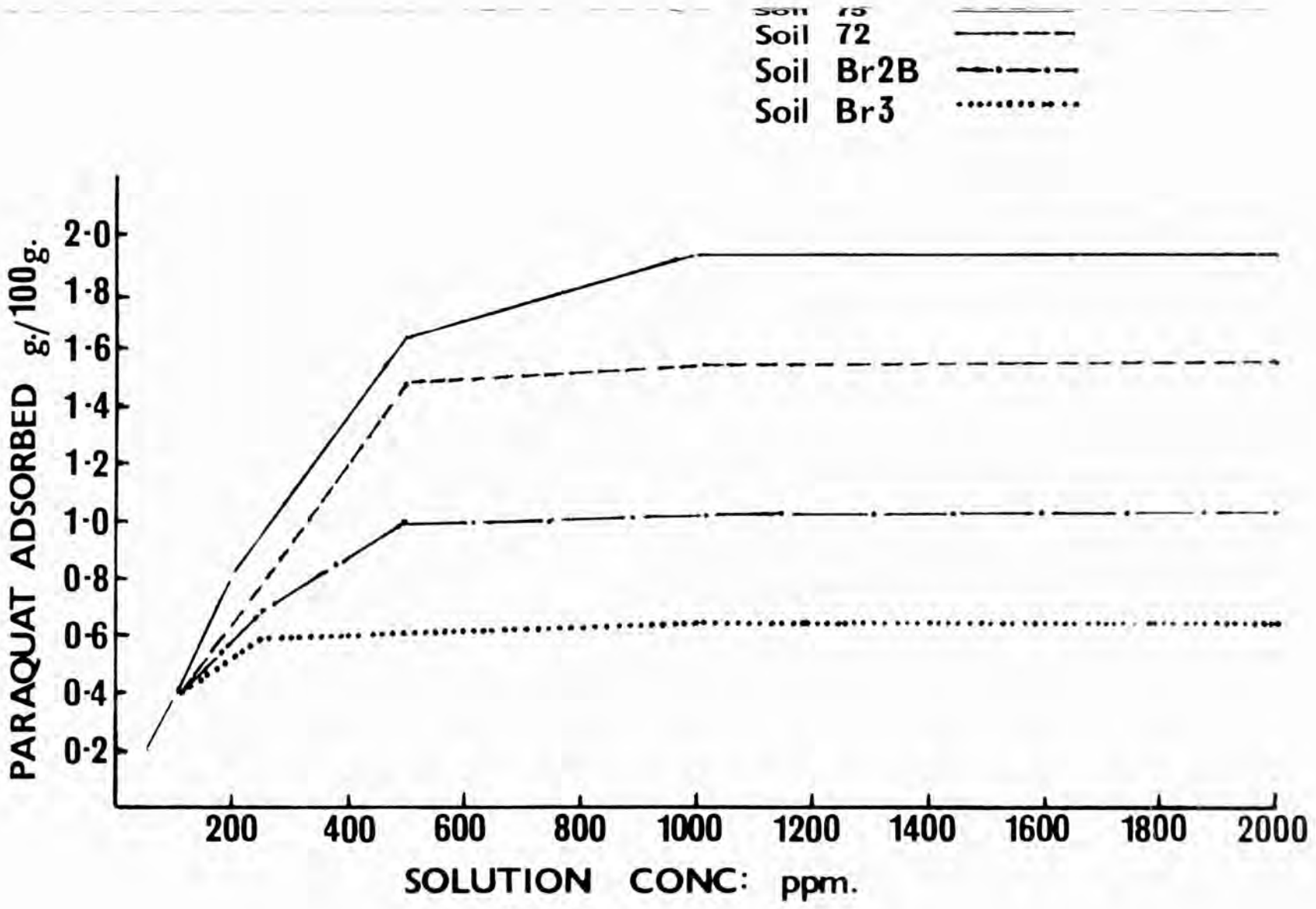
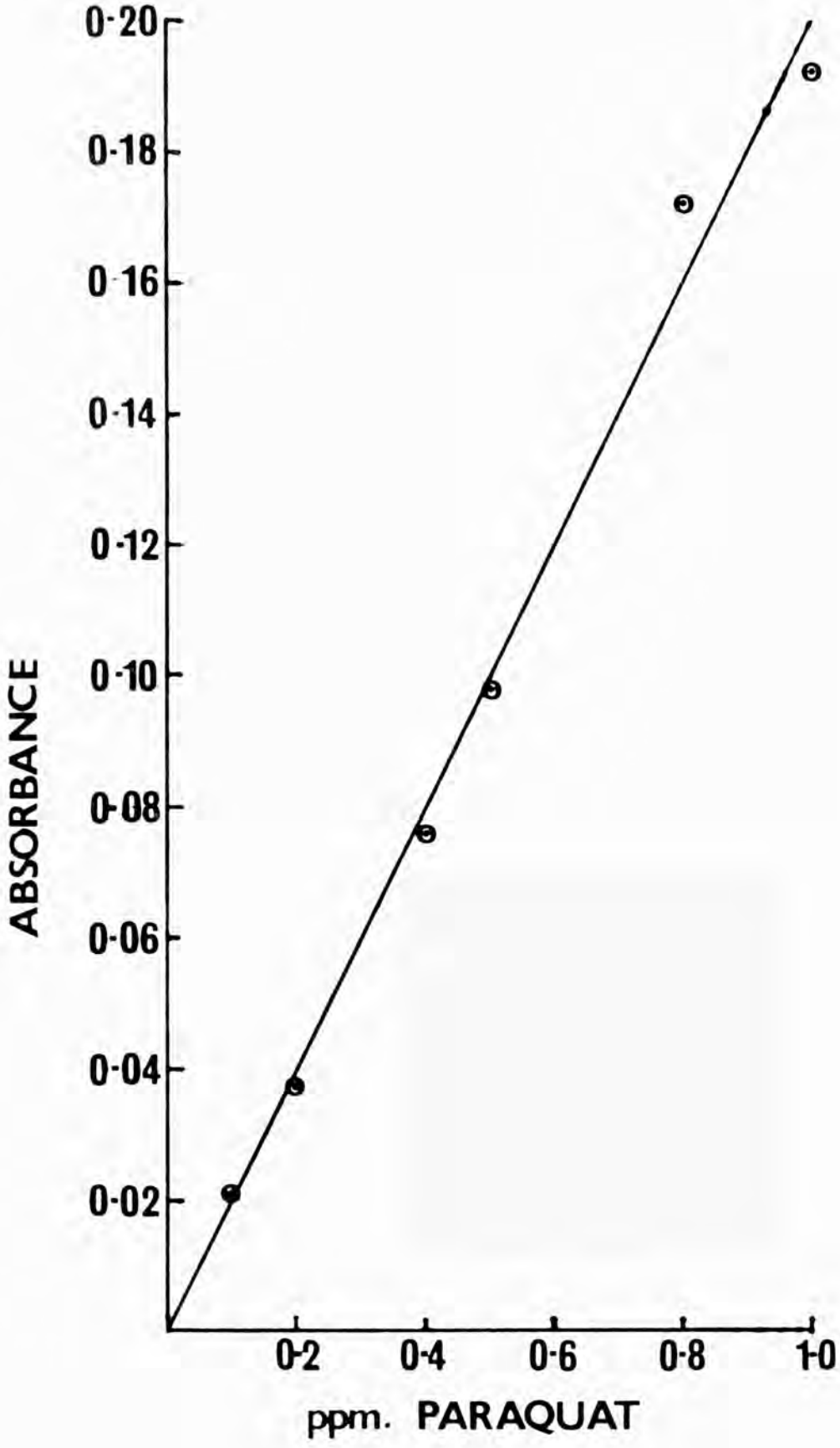

Soil 72
Soil Br2B
Soil Br3
PARAQUAT ADSORBED g./100g.
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
200
400
600
800
1000
1200
1400
1600
1800
2000
SOLUTION CONC: ppm.

Fig. 13. The standard curve for reduced paraquat dichloride. Used in adsorption isotherms experiments.


0·20
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
ABSORBANCE
0·2
0·4
0·6
0·8
1·0
ppm. PARAQUAT

7. The recovery rates of paraquat dichloride from the soil.

All four soils were subjected to extensive tests to ascertain the recovery rates of paraquat dichloride from the soil under ideal conditions.

In each case 2.0g of soil was incubated with 20 ml. of sucrose medium, containing 20 ppm. paraquat. The incubation process was carried out at 30° C in an orbital shaker, and the duration varied from 24 hours to 96 hours. This was to investigate if the length of adsorption time had any effect on the strength of that adsorption. However, under the conditions demanded for the extraction of the herbicide, it was unlikely that any form of adsorption would be resistant. The paraquat was extracted by the method described on page 14. After elution of the herbicide with 50 ml. of ammonium chloride, 1.25 ml. was extracted and made up to 10.0 ml. with the solvent. In the event of 100% recovery, this would give a final concentration of 1.0 ppm.

It will be seen from Appendix Table 8 that neither time nor soil type affected this drastic form of extraction. An average recovery rate of 90.3% was used in all experiments involving this procedure.

8. The breakdown of paraquat in the soil.

The purpose of these experiments was to determine if there was any difference between the four soils Br28, Br3, 72 and 75, as regards the breakdown of adsorbed paraquat dichloride. It had already been established (page 29) that at very high paraquat

levels total adsorption (PAC) was greater in soils with high organic matter content, as opposed to those with low organic matter content. However, at normal application rates (20 ppm) this surplus organic adsorption of herbicide may be inapplicable as not all the available adsorption sites are used. Therefore, as the total amount of paraquat adsorbed in this experiment is the same in all soils it might be unimportant compared with the strength of adsorption on to the respective soil components.

All cultures in these experiments were allowed to establish for 10 days in the sucrose medium containing unlabelled paraquat (20 ppm). This was for a number of reasons. It was decided initially to keep the soil unsterilised as any form of sterilisation might affect the adsorptive properties of that soil, especially those of an organic nature. Also, if unsterilised soil had been added at day 1 the competition for nutrients by other micro-organisms might well have prevented adequate growth of the yeast. After 10 days growth, cultures were selected that had approximately identical cell contents, thus ensuring an equality between replicates and treatments at the commencement of the experiment.

A. Spectrophotometric method

20 ml. of sucrose medium, containing 20 ppm. of paraquat dichloride and innoculated with Lipomyces starkeyi was allowed to grow for 10 days. After this time cultures were selected for the experiment and 2g. of soil added to each flask, together with a further 2 ml. of 200 ppm. paraquat. The cultures were agitated on an orbital shaker at 30° C and two replicates of each

soil extracted every 24 hours for 72 hours. This meant that each soil type was incubated in six flasks, so 24 cultures were used in all. As a control 20 ppm. paraquat was shaken in the sucrose medium alone and extracted at the same time intervals used for the treatments.

Extractions were carried out following the method outlined on page 14 and the reduced paraquat determined, after suitable dilutions (page 31) spectrophotometrically. All the soil extraction results were adjusted with the factor for recovery rate (page 31) so as to give a true representation of the loss of herbicide.

The results are expressed in Tables 9, 10 and 11 and illustrated in Fig.14.

i) After 24 hours cultures containing soil Br2B (high organic matter) lost some 10 - 20% more paraquat than the other three treatments. Whether all soils have lost some herbicide was doubtful and figures between 90 - 100% must be viewed as probable experimental errr.

ii) After 48 hours, cultures containing Br2B again contained significantly less paraquat than the other three, but the quantity had not increased on that lost after 24 hours.

iii) Br2B cultures incubated for 72 hours show no significant loss of paraquat.

The problems arising from this experiment were numerous. The need to have a different culture for every extraction immediately increases the error as, despite equality at the commencement of the experiment, the cultures were bound to

TABLE 9.

THE MICROBIAL DECOMPOSITION OF PARAQUAT DICHLORIDE BY *LIPOMYCES STARKEYI*, AS MEASURED SPECTROPHOTOMETRICALLY, IN SOILS Br2B, Br3, 72 and 75

Time in hours	Rep.	Br2B			Br3			72			75		
		O.D.	%PQ.	Cr%	O.D.	%PQ	Cr%	O.D.	%PQ	Cr%	O.D.	%PQ	Cr%
24	1	0.142	73.6	81.5	0.168	87.0	96.3	0.155	80.3	89.1	0.172	89.1	98.7
	2	0.136	70.5	78.1	0.158	81.9	90.7	0.162	83.9	92.9	0.163	84.5	93.6
	Mean	-	-	79.8	-	-	93.5	-	-	91.0	-	-	96.2
48	1	0.130	69.1	76.5	0.167	88.8	98.3	0.148	78.7	87.2	0.176	93.6	103.7
	2	0.126	67.0	74.2	0.151	80.3	88.9	0.163	86.7	96.0	0.158	84.0	93.0
	Mean	-	-	75.4	-	-	93.6	-	-	91.6	-	-	98.4
72	1	0.171	85.9	95.1	0.168	84.4	93.5	0.158	79.4	87.9	0.169	84.9	94.0
	2	0.138	69.3	76.7	0.155	77.9	86.3	0.178	89.4	99.0	0.157	78.9	87.4
	Mean	-	-	85.9	-	-	89.9	-	-	93.5	-	-	90.7

Cr% = percentage paraquat recorded adjusted for 90.3% recovery.
O.D. = optical density at 399mμ.

TABLE 10

ANALYSIS OF VARIANCE OF DATA PRESENTED IN TABLE 9.

	d.f.	SS			MS			VR			P		
		24	48	72	24	48	72	24	48	72	24	48	72
TOTAL	7	352	742	337	50.29	106.00	48.14						
REPLICATE	3	13	23	49	4.33	7.67	16.33	0.45	0.19	0.21			
TREATMENT	1	310	599	59	310	599.00	59.00	32.06	14.98	0.77	**	**	
ERROR	3	29	120	229	9.67	40.00	76.33						

TABLE 11.

A t TEST TO DETERMINE INDIVIDUAL DIFFERENCES BETWEEN TREATMENTS INDICATED IN TABLE 10.

24 Hours.

COMPARISON	SED	t	P
Br2B v 75	12.6	36.65	*
75 v 72	12.6	1.07	

48 Hours.

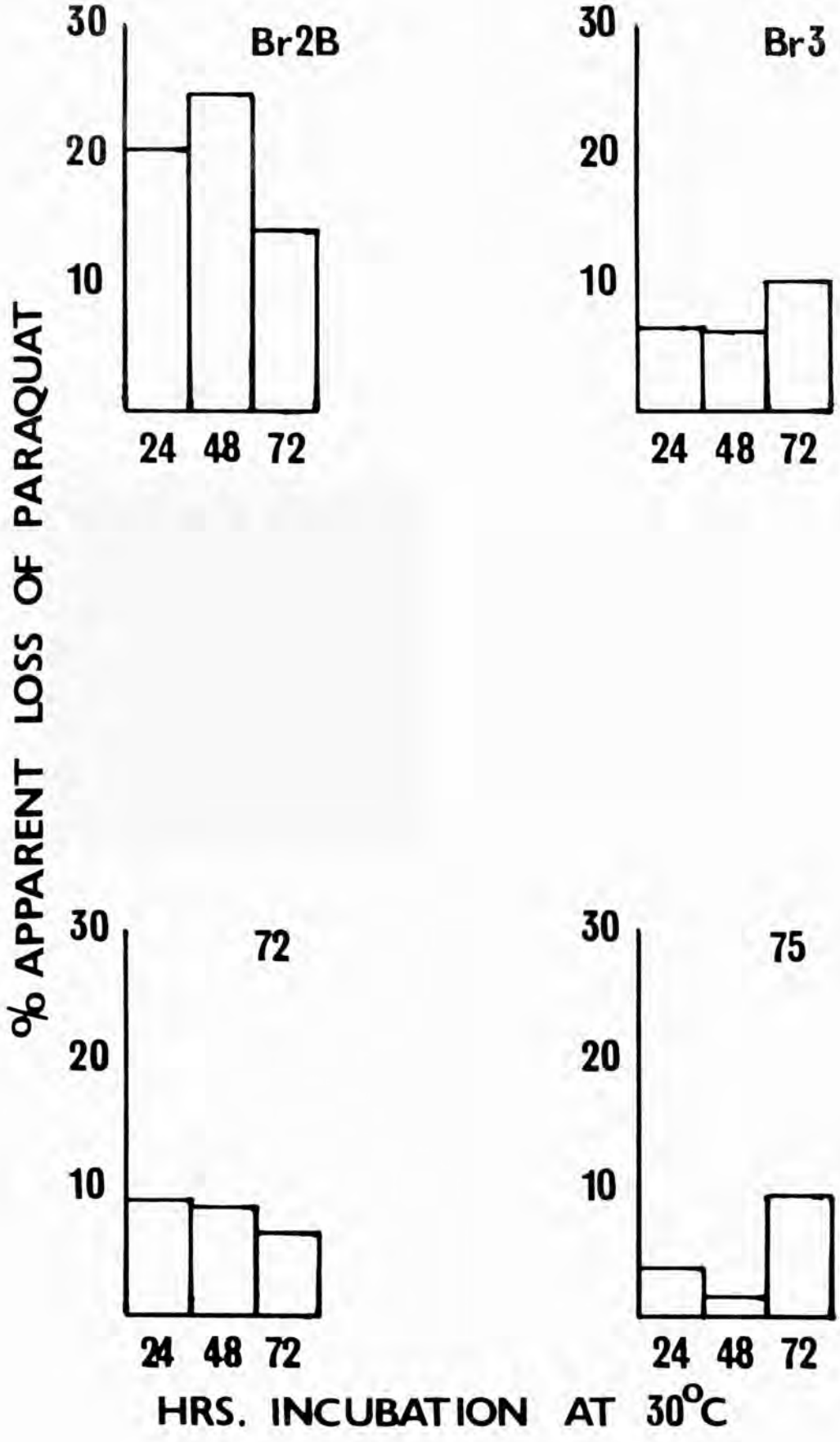
COMPARISON	SED	t	P
Br2B v 72	6.2	3.61	*
72 v 75	6.2	1.66	

t tests were made:

a. Comparing Br2B with the treatment that differs least from it

b. Comparing the two treatments, other than Br2B, that differ most from each other.

Fig. 14. The microbial decomposition of paraquat dichloride by Lipomyces starkeyi in cultures containing soils Br2B, Br3, 72 and 75. Measurements made spectrophotometrically.


Br2B
Br3
72
75
30
20
10
24 48 72
% APPARENT LOSS OF PARAQUAT
HRS. INCUBATION AT 30°C

have different growth rates depending on the contaminants added with the soil. Also, as each extraction process took about six hours to complete, it was impossible to treat all replicates at the same time. In these circumstances, cultures were placed in the refrigerator until they could be analysed. The results of cultures containing soil Br2B after 72 hours were rather suspect as one would expect a breakdown level of the same order as that at 24 and 48 hours. Due to the unsatisfactory and laborious nature of this method of estimating paraquat, it was decided to continue the work using labelled herbicide.

B. Radioactive tracer method.

Two methods were evolved in the addition of the soil and C^{14}- labelled paraquat to the ten day old cultures.

a) The soil and paraquat added separately but at the same moment in time.

b) The soil and paraquat allowed to equilibrate together for 24 hours before addition.

The purpose of having two methods of addition was to establish if there was any degree of variation between the strength of initial adsorption of paraquat and that after 24 hours, and whether this manifested itself in the availability of herbicide to breakdown.

The soda traps were removed and sampled after 48, 96 and 192 hours by the method described on page 18. The results in Tables 12, 13 and 14 and Fig.15 show that at 48 hours:-

i) Soils Br2B and Br3 - Application A. These cultures both appeared to indicate microbial decomposition of labelled paraquat.

However, cultures containing Br2B (the high organic matter soil) showed, on analysis, a significant increase in labelled carbon-dioxide emission; whereas cultures containing Br3 (the low organic matter soil) did not.

ii) Soils 72 and 75 - Application A. In this soil pair, neither cultures showed any significant paraquat breakdown although some small decomposition appeared to be occuring in cultures containing soil 75 (high organic matter).

iii) In all soils no significant breakdown of paraquat was observed when Application B was involved.

iv) Between 48 and 96 hours, labelled carbon dioxide was evolved in cultures containing soil Br2B - Application A. None of the others, regardless of method of application, was significantly above background level.

v) Between 96 and 192 hours no further emission of carbon dioxide was evident in any of the cultures.

vi) At 48 and between 48 and 96 hours, all breakdown of paraquat, shown by labelled carbon dioxide emission was in cultures containing high organic matter soils, notably Br2B.

From these series of results, it can be ~~proposed~~ concluded, that under the conditions of this experiment, the majority of decomposition of herbicide by Lipomyces starkeyi occured in under 96 hours. After this time any paraquat present had in some way become unavailable. With reference to application B, it was noted that no breakdown occurred at any time during the experiment. It was suggested that the paraquat had already become unavailable before it was added to the culture. This

TABLE 12.

THE MICROBIAL DECOMPOSITION OF C^{14}-LABELLED PARAQUAT DICHLORIDE BY *LIPOMYCES STARKEYI* IN SOILS Br2B, Br3, 72 AND 75, AS MEASURED BY C^{14}-LABELLED CARBON DIOXIDE EMISSION.

	Br2B						Br3						72						75					
Rep.	0-48		48-96		96-192		0-48		48-96		96-192		0-48		48-96		96-192		0-48		48-96		96-192	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	85	24	28	23	23	24	31	26	24	22	24	23	24	23	25	21	24	25	30	23	23	23	23	25
2	70	23	29	21	24	24	33	26	24	24	24	24	22	22	25	22	24	24	28	23	23	23	25	24
3	54	24	28	23	24	23	37	27	23	23	24	25	26	23	22	25	24	23	27	22	22	22	24	22
4	59	23	28	23	24	24	35	24	23	23	25	23	26	26	22	20	24	24	30	25	25	23	22	24
Mean	67	24	28	23	24	24	34	26	24	23	24	24	25	24	24	22	24	24	29	23	23	23	24	24

Figures denote counts per minute.

A and B denote methods of application (see page 34).

Br2B, Br3, 72 and 75 are soil types.

TABLE 13.

ANALYSIS OF VARIANCE OF DATA PRESENTED IN TABLE 12.

		Total	Rep.	Treat.	Error
d.f.		19	3	4	12
48	A	5691	64	5083	544
	B	43	4	20	19
SS. 96	A	96	5	79	12
	B	24	2	2	20
192	A	10	1	1	8
	B	13	3	1	9
48	A	299.5	21.3	1270.8	45.3
	B	2.26	1.33	5.00	1.58
M. 96	A	5.05	1.67	19.75	1.00
	B	1.26	0.67	0.50	1.67
192	A	0.53	0.33	0.25	0.67
	B	0.68	1.00	0.25	0.75
48	A		0.47	28.03	
	B		0.84	3.16	
VR. 96	A		1.67	19.75	
	B		0.40	0.30	
192	A		0.49	0.37	
	B		1.33	0.33	
48	A			***	
	B				
P. 96	A			***	
	B				
192	A				
	B				

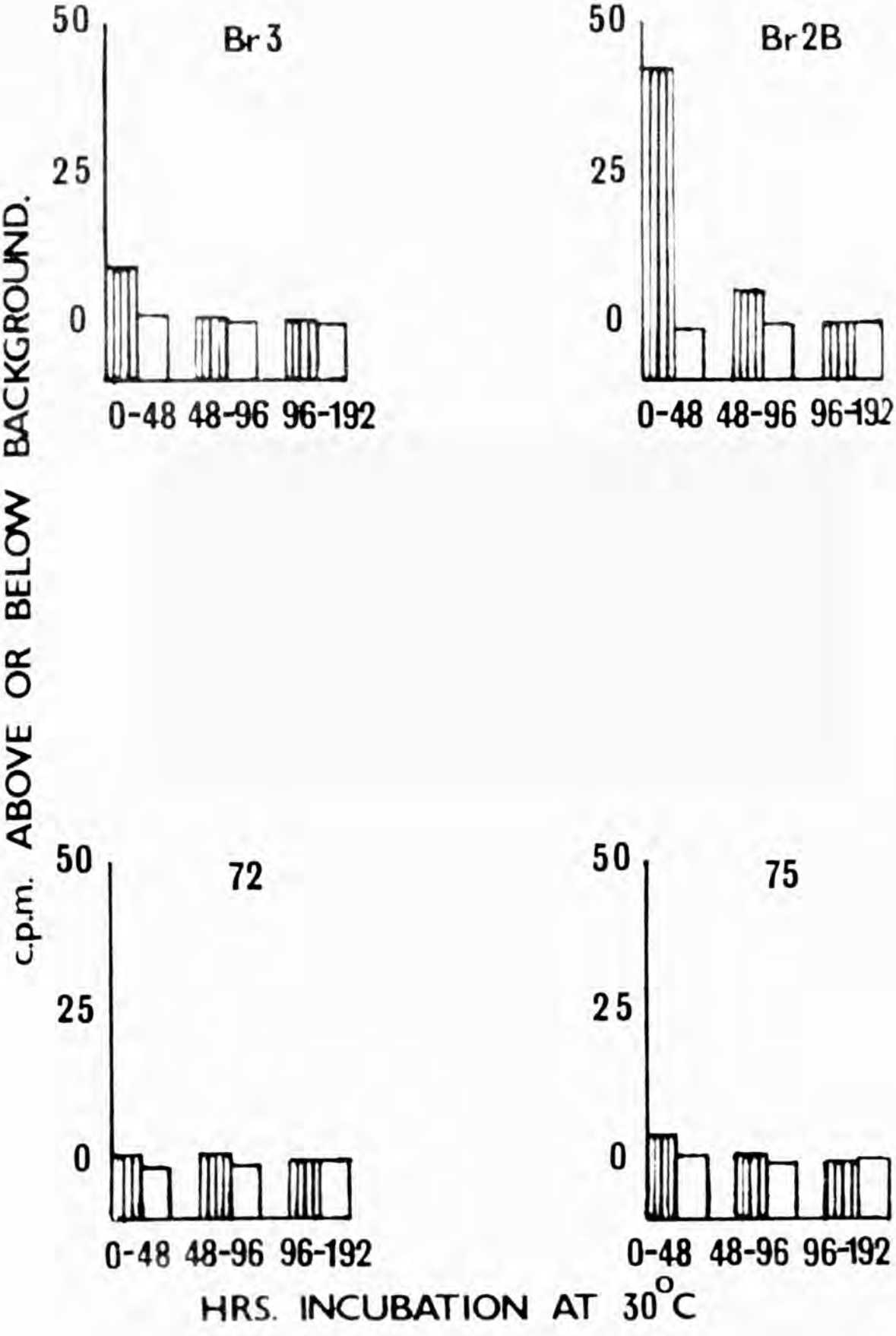
TABLE 14.

A t TEST TO DETERMINE UNDIVIDUAL DIFFERENCES BETWEEN TREATMENTS INDICATED IN TABLE 13.

SOIL	SED				t				P			
	48		96		48		96		48		96	
	A	B	A	B	A	B	A	B	A	B	A	B
Br2B	19.04	3.55	2.83	3.66	8.84	1.69	7.42	0	***		***	
Br3	"	"	"	"	1.89	0.85	0.71	0.55				
72	"	"	"	"	0.11	1.69	1.41	1.09				
75	"	"	"	"	0.79	1.97	0.35	0.27				

Fig. 15. The microbial decomposition of C^{14}-labelled paraquat dichloride by Lipomyces starkeyi in cultures containing soils Br2B, Br3, 72 and 75. Measurements made by C^{14}-labelled carbon dioxide emission.

Hatched columns indicate application A; empty columns indicate application B. (see text page 34).


Br 3
Br 2B
72
75
50
25
0
0-48 48-96 96-192
c.p.m. ABOVE OR BELOW BACKGROUND.
HRS. INCUBATION AT 30°C

must have been br ught about during the 24 hour period of equilibration.

A working hypothesis was forwarded at this point, suggesting that the type and strength of adsorption at times below 96 hours was different from that after this period had elapsed. It was already established that organic matter had a positive effect on PAC (page 29) and it was proposed that it also had an influence on the type of adsorption of herbicide at normal application rates (20 ppm).

With a view to examining this possibility an attempt was made to fractionate the soil into its organic and inorganic components. As soil Br2B had shown the greatest quantity of breakdown it was selected for use in the succeeding experiments.

9. The breakdown of paraquat in the separate soil components

(i) Organic matter

A sample of the organic matter residue from soil Br2B (extracted as shown on page 20) was shaken with 20ppm of C^{14}-labelled paraquat for 48 hours to allow for any adsorption. This was then incubated with Lipomyces starkeyi at 30°C in an orbital shaker and emission of radio-active CO_2 estimated as before (page 18), 90% breakdown of the herbicide was observed after approximately 96 hours.

(ii) Inorganic matter

A similar experiment was set up as above only this time using a portion of the inorganic material from Br2B and adding it separately with the paraquat. No significant

breakdown of paraquat was observed after 96 hours.

This experiment indicated that any organic matter adsorption was not strong enough to be resistant to microbial attack. Inorganic matter adsorption however took place almost immediately on addition of paraquat and rendered the herbicide unavailable for breakdown.

10. The determination of the strength of readsorption of inorganic material in Br2B

The organic matter residue from 2g. of soil Br2B (high organic matter) was extracted by the method shown on page 20 and taken up in 20ml. of distilled water. 10ml. of this solution was sampled and 10ml. of C^{14}-labelled 40ppm. paraquat dichloride added. This gave a herbicide concentration of 20ppm. The mixture was shaken gently for 48 hours to ensure complete adsorption. A 10ml. aliquot was then extracted by pipette and passed into a length of dialysis tubing which was then tied at both ends. 0.5g. of the inorganic fraction extracted from the 2g. of soil Br2B was placed in a 250ml. concical flask and 150ml. of distilled water added. The dialysis tubing containing in the organic fraction plus paraquat was placed into the inorganic suspension and the flask agigated in an orbital shaker. This served to keep the inorganic components in a permanent and mobile state of suspension, allowing for the occurrence if any, of maximum readsorption.

At intervals, after addition of the dialysis tubing, 10ml. aliquots were extracted from the inorganic suspension. From this three 2ml. sampled were taken and dried on a planchet,

1.0ml. at a time. Using this method, an even layer of soil was achieved which gave much more reliable results than the one described on page 29.

As a control, to discover if any paraquat had passed through the membrane by a process of diffusion along a concentration gradient, a second section of dialysis tubing, holding an equal quantity of the organic matter and labelled paraquat, was placed in a 250ml. conical flask containing 150ml. of distilled water. Each hour, corresponding with the treatment sampling, a 10ml. aliquot was drawn off and an equivalent portion of the inorganic soil sample added (0.033g.). This was shaken for 120 minutes, to allow for the adsorption of any herbicide present in the water, which was then detected by the same method as described above .

By subtracting the readings for the control from those of the treatments it is possible to arrive at an estimation of the real amount of diffusion due to the inorganic/organic matter relationship (Table 16).

At the end of the experiment(4 days) the dialysis tubing which had been in the inorganic soil suspension was split open and examined for any remaining paraquat.

The results summarised in Tables 15 and 16 and expressed graphically in Fig. 16 show that:-

(i) 30% of the paraquat passing through the membrane was not due to the readsorption effect of the inorganic fraction. It was brought about by a diffusion of paraquat from the dialysis tubing into the water along a concentration gradient. This

paraquat was in all probability not adsorbed onto the organic colloidal fraction and was free in solution within the tubing.

(ii) The larger fraction of paraquat (70%) was strongly enough adsorbed onto the organic matter to be resistent to diffusion of the type experienced in the control and it was only when the external medium contained inorganic material that the adsorbed herbicide began to pass out through the membrane. This was indicative of the comparative adsorption strengths of the inorganic soil components opposed to that of the organic.

(iii) 90% of the readsorption through the membrane had occured within 420 minutes after addition of the inorganic matter. Thereafter only small amounts of paraquat passed through the membrane.

(iv) There was found to be no labelled paraquat remaining in the dialysis tubing, which had received the inorganic matter treatment, after day 4. This showed that by this time all the herbicide had passed through the membrane to be adsorbed onto the inorganic matter.

TABLE 15.

THE R -ADSORPTION BY AN INORGANIC SOIL FRACTION, OF C^{14}-LABELLED PARAQUAT, DICHLORIDE, FROM AN ORGANIC SOIL FRACTION.

Counts per minute.

Time in minutes	Treatment Rep.1.	Rep.2.	Rep.3.	Mean	Control Rep.1.	Rep.2.	Rep. 3.	Mean
5	56	59	66	60				
15	70	70	98	79				
30	104	108	115	109				
60	177	174	176	176	48	46	42	45
120	337	343	345	342	94	93	93	93
180	414	403	413	410	142	142	138	141
240	422	475	473	457	142	152	151	148
300	501	502	520	508	156	166	174	165
420	515	576	555	549	159	165	170	165
1440	579	589	584	584	187	189	180	185
2880	611	598	600	603	179	185	184	183
5760	608	592	616	605	177	190	186	184

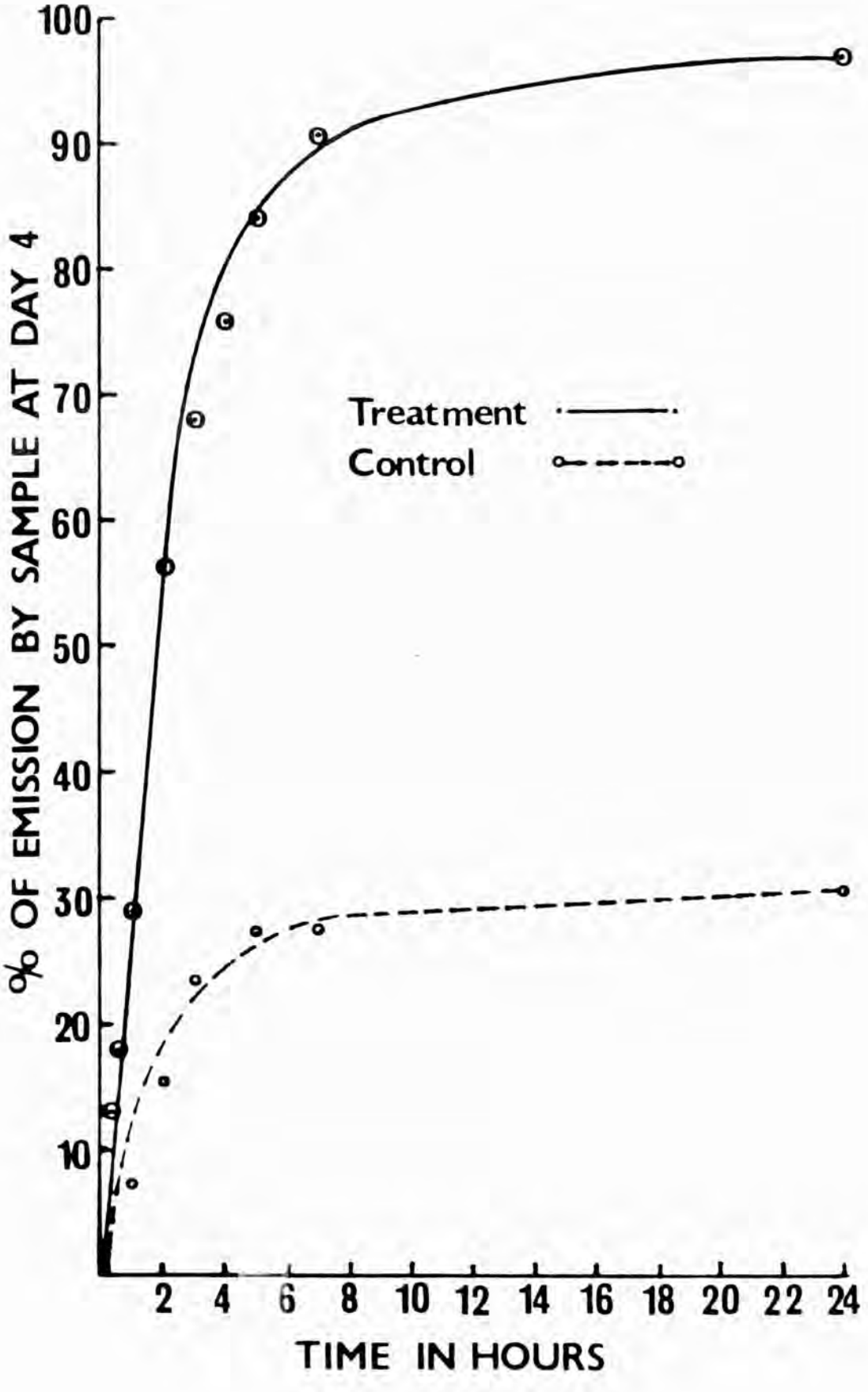
All readings converted from counts per 400 seconds.

TABLE 16.

THE DE-ACTIVATION OF PARAQUAT DICHLORIDE DIRECTLY DUE TO THE INORGANIC SOIL FRACTION (see Table 15)

Time in mins.	The effect of the inorganic material. Counts per minute	% of Total emission at day Four (605)
60	131	21.65
120	249	41.16
180	269	44.46
240	309	51.07
300	343	56.69
420	384	63.47
1440	399	65.95
2880	420	69.42
5760	421	69.59

Fig. 16. The readsorption, by an inorganic soil fraction, of C^{14}-labelled paraquat dichloride from an organic soil fraction.


100
90
80
70
60
50
40
30
20
10
% OF EMISSION BY SAMPLE AT DAY 4
Treatment
Control
2
4
6
8
10
12
14
16
18
20
22
24
TIME IN HOURS

CONCLUSION

Lipomyces starkeyi, a soil yeast reported by Brady and Jones (1964), was found to decompose the bipyridylium herbicide, paraquat in accordance with the work presented by Baldwin et at (1966). This decomposition was detected, firstly by spectrophotometric methods and secondly by the use of C^{14}-labelled paraquat and the emission of C^{14}-labelled carbon dioxide, resulting from its degredation.

Two nutrient media were used in these experiments. A complete peptone/dextrose medium and a sucrose medium. To the latter paraquat was obligatory as the sole nitrogen source, to the former it was not. Breakdown of 20ppm. herbicide was seen to occur in both media when incubated at 30°C in an orbital shaker. In the peptone/dextrose medium Lipomyces starkeyi, cultures were allowed to establish for 7 or 14 days before addition of paraquat. In the 7 day old cultures 50% of the added paraquat was decomposed in 150 hours. Cultures grown for 14 days prior to addition of paraquat showed a more raped rate of breakdown, 50% disappearing after 60 hours. This is most probably explained by the greater establishment of the microorganism in 14 day cultures compared with that of 7.

In the sucrose medium, where paraquat was necessary for growth, complete breakdown, as determined with the spectrophotometer, took place in 72 hours. Using the emission of C^{14}-labelled CO_2 as a guide to paraquat degredation only 90% had disappeared after 72 hours and the remaining 10% was gradually

decomposed over the next fortnight. The results of these two methods, despite appearances, are compatible(page 28) although the tracer method is far more sensitive to small amounts of breakdown. However, it is necessary to know the total emission of C^{14}-labelled CO_2 at the end of the experiment before any accurate estimation can be made of the percentage breakdown at intervals during the experiment.

In instances where paraquat was added at time zero (sucrose medium) a lag phase of approximately 16 hours was noticed. At the end of this time a period of rapid breakdown (page 26), directly correlated with growth rate(page 24), was observed which lasted approximately 56 hours.

Breakdown of paraquat in soil was then investigated (page 31). Two different methods of herbicide/soil application were evolved to try to discover if there was any difference in breakdown between soils which had weakly adsorbed herbicide and those which had strongly adsorbed herbicide. Method A involved the addition of paraquat and soil to the culture, separately, but at the same moment in time. Method B, involved the equilibration of the soil/paraquat complex for 24 hours before addition. In all experiments the sucrose medium was used and the cultures allowed to establish for 10 days prior to the addition of the soil and paraquat (page 32).

Initially, breakdown was investigated spectrophotometrically with method A, application. Extractions were carried out every 24 hours for 72 hours and reduced paraquat determined as before.

The results (page 33) showed that cultures containing the high organic matter soil, Br2B, had lost a significant quantity of herbicide compared to cultures containing the other soils. This loss was consistent (approximately 10-20%) in 24 hours and 48 hour samples but at 72 hours a greater error between replicated appeared and the loss was not statistically significant. Because of this apparent disparity between results, the phenomenon exhibited in this experiment was further investigated using C^{14}-labelled paraquat and measurement of its breakdown by labelled CO_2 emission.

The two methods of addition, of paraquat and soil, described above were used. The soda traps were removed and analysed for C^{14}-labelled CO_2 at the time intervals of 0-48, 48-96 and 96-192 hours. The results confirmed those of the earlier experiment in that with application A, cultures containing Br2B showed loss of paraquat (indicated by CO_2 emission) after 48 hours and between 48 and 96 hours. Between 96 and 192 hours no further emission was recorded. At 48 and 48-96 hours some breakdown also appeared to be occuring in cultures containing soil 75 (high organic matter) but statistical analysis failed to endorse these observations. In all treatments including soil Br2B no breakdown of paraquat was observed when application B was used.

It appears, from this set of results, that the 24 hour equilibration (application B) successfully removed most, if not all, paraquat from an available state for breakdown. Unequilibrated soils (application A) seemed to reach a point when after 96 hours no further paraquat is available for breakdown.

This is in close agreement with the results obtained from spectrophotometric analysis of application A, treatments. The disparity between the inactivation after 96 hours shown by application A and that after 24 hours shown by application B, might well be explained by an effect of the Liposyces starkeyi which, when included in the culture, retards the process of inactivation, possibly by some adsorptive effect of its own. It is suggested, at this juncture, that inactivation is synonomous with clay particle adsorption and that initially more reversable forms of adsorption are available to the herbicide, thus facilitating its decomposition by Liposyces starkeyi.

The next piece of work involved an investigation into the comparative adsorption properties of the four soils used throughout the course of these experiments. The adsorption isotherms shown on page 29 indicate the results of this investigation. It was seen that soils with high organic matter content (Br2B and 75) adsorb a greater total amount of paraquat (PAC) than those of low organic matter content (Br3 and 72). As the main difference within comparable soil pairs (Br2B and Br3; 72 and 75) is the organic matter/inorganic matter ratio, it is reasonable to propose that the differences discovered were due to this variation. The discrepancy between soil types, which shows total adsorption of 72 and 75 to be higher than that of Br2B and Br3 can be explained by the different proportions of high adsorptive clay particles (montmorillonites) and low adsorptive clay particles (kaolinites)(Table 3)

From a study of the adsorption isotherms, therefore, we can say that organic matter content significantly influences the total amount of herbicide adsorbed. No evidence is presented however, as to whether there is a range of adsorption strengths at the more normal (20ppm.) rates of paraquat application.

The two main soil components of the most active soil Br2B, the organic and inorganic fractions, were successfully separated by ultradisintigration using acetylacetone as the organic solvent (Halstead et al 1966). After allowing a portion of the organic fraction to equilibrate with C^{14}-labelled paraquat for 48 hours (application B) it was added to a Lipomyces starkeyi culture and 90% breakdown was observed after 96 hours. A similar experiment but this time by adding the inorganic matter and C^{14}-labelled paraquat separately but simultaneously (application A) showed no significant breakdown after 96 hours.

A length of dialysis tubing was then filled with a solution of organic matter, from Br2B, and C^{14}-labelled paraquat, which had been incubated for 48 hours to allow for any adsorption of herbicide on the organic colloid. This was then placed in a flask containing distilled water and the inorganic fraction from Br2B. The whole system was agigated to keep the inorganic material in a permanent state of mobility to allow the most favourable conditions for adsorption of any paraquat present. Samples of inorganic material were extracted at intervals and examined for C^{14}-labelled paraquat. A control was run simultaneously, having the same contents within the dialysis

tubing, but only distilled water without. This ensured the measurement of any passage of paraquat through the membrane independant of inorganic material. Samples were taken at intervals from the control and any paraquat present "salted out" by addition of an equivalent quantity of inorganic material.

From the results, expressed graphically in Fig.16, it can be seen that approximately 30% of carbon C^{14}-labelled paraquat passing through the dialysis membrane to be adsorbed onto the inorganic matter was by a diffusion processindependant of the external suspension. Movement of paraquat from the organic matter through the membrane dependant on inorganic material accounted for the remaining 70% and was itself 90% completed 420 minutes after the commencement of the experiment. The remaining 10% passed slowly out of the dialysis tubing over a period of four days. At this time no further paraquat remained in association with the organic matter .

From the results outlines here it is possible to propose a system in which at least some breakdown of paraquat occurs. When the herbicide reaches the soil it comes into contact with two strongly adsorptive fractions-the organic and the clay colloids. Adsorption occurs to a large extent on the first colloidal particle the paraquat molecule meets. So in a hypothetical medium consisting of an homogeneous mixture of 50% organic and 50% clay particles equal quantities of paraquat are adsorbed onto each component. This phenomenon we shall call 'initial adsorption'. Now the adsorptive strengths of the clay particles is for the most part

so retentive as to be irreversable. This is in contrast to the organic fraction. Because of this unequal system of adsorption strengths between soil particles, paraquat which has been adsorbed onto the organic matter, is gradually attracted onto the clay fraction. This process we shall call 're-adsorption'. Under the conditions of the experimental work described in this thesis and the soils used, the time taken for 're-adsorption' is anything approaching 96 hours.

It is proposed that at least a percentage of the degredation of paraquat caused by micro-organisms, particularly Lipomyces starkeyi, occurs during the time that the paraquat is reversibly adsorbed onto the organic matter. This, after all, is the site of microbial activity. Evidence produced by O'Toole (1965) which may well tie up with this hypothesis tells of the slowness of inactivation of paraquat in soils with high peat content.

Certainly the methods examined here - the separation of the soil components by ultradisintegration, adsorption isotherms and sites of microbial activity concerned in the breakdown of paraquat, indicate promising tools in the re-examination of the dynamics of, not only the bipyridyls but the whole range of artificial environment-controllers which find their way eventually into the soil.

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APPENDIX TABLE 1

The optical densities of reduced paraquat dichloride used in formulating standard curves (Figs. 4 and 5)

Conc. of PARAQUAT in ppm.	E_{399}
0.1	0.021
0.2	0.038
0.4	0.076
0.5	0.098
0.8	0.172
1.0	0.192
1.0	0.196
2.0	0.397
3.0	0.571
4.0	0.739
5.0	0.920

APPENDIX
TABLE 2

THE RELATIONSHIP OF THE NUMBER OF LIPOMYCES STARKEYI CELLS IN A SUCROSE MEDIUM TO THE TURBIDITY OF THAT MEDIUM, AS MEASURED WITH A NEPHELOMETER.

Haemocytometer reading		Nephelometer reading	No.of cells per ml.x 10^5
148			
147			
141		78.0	73.5
144			
156			
147	Mean		
79			
80			
72		39.2	38.0
75			
74			
76	Mean		
47			
19			
29			
56			
32		18.5	19.0
18			
43			
39			
61			
40			
38	Mean		

APPENDIX TABLE 3.

THE MICROBIAL DECOMPOSITION OF PARAQUAT DICHLORIDE BY LIPOMYCES STARKEYI IN A SUCROSE MEDIUM, AS MEASURED SPECTROPHOTOMETRICALLY

Time in hours	Rep.	Observed		Corrected		a/b=Y	O.D.Cr.
		O.D.	% Paraquat	O.D.	% Paraquat		
4	1	0.184	95.34	0.138	71.50	1.33	0.193
	2	0.185	95.85	0.185	95.85		
	Mean	-	95.60	-	83.68		
8	1	0.196	102.08	0.122	58.33	1.75	0.192
	2	0.183	95.31	0.183	95.31		
	Mean	-	98.70	-	76.82		
12	1	0.180	97.30	0.097	52.42	1.86	0.185
	2	0.178	96.22	0.178	96.22		
	Mean	-	96.76	-	74.33		
16	1	0.153	92.17	0.153	92.17	1.20	0.166
	2	0.175	105.42	0.146	87.75		
	Mean	-	98.80	-	90.06		
20	1	0.190	94.06	0.170	84.16	1.12	0.202
	2	0.161	79.70	0.161	79.70		
	Mean	-	86.88	-	81.93		
24	1	0.165	84.62	0.165	84.62	1.08	0.195
	2	0.163	83.59	0.151	77.44		
	Mean	-	84.11	-	87.03		
32	1	0.145	75.52	0.134	69.79	**1.08**	**0.192**
	2	0.128	66.67	0.128	66.67		
	Mean	-	71.20	-	68.23		
40	1	0.104	55.03	0.104	55.03	1.12	0.189
	2	0.123	65.08	0.110	58.20		
	Mean	-	60.06	-	56,15		
48	1	0.115	56.10	0.104	50.73	1.11	0.205
	2	0.096	46.83	0.096	46.83		
	Mean	-	51.47	-	48.78		
56	1	0.071	36.60	0.071	36.60	1.20	0.194
	2	0.060	30.93	0.050	25.77		
	Mean	-	33.77	-	31.19		
64	1	0.034	18.56	0.031	16.94	1.10	0.185
	2	0.027	14.75	0.027	14.75		
	Mean	-	16.67	-	15.85		
72	1	0	-	0	-	-	0.192
	2	0	-	0	-		
	Mean	-	-	-	-		

APPENDIX TABLE 4

THE MICROBIAL DECOMPOSITION OF PARAQUAT DICHLORIDE BY LIPOMYCES STARKEYI IN A PEPTONE/DEXTROSE MEDIUM, AS MEASURED SPECTROPHOTOMETRICALLY. (FIG.9. TABLE6.)

Time in hours	Rep	7-Day Cultures			14-Day Cultures		
		O.D.	% Paraquat	OD. Control	O.D.	% Paraquat	O.D. Control
24	1	0.170	89.47		0.149	77.60	
	2	0.190	97.44	0.195	0.163	84.90	0.192
	Mean	-	43.46		-	87.25	
48	1	0.166	79.81		0.103	55.38	
	2	0.188	90.38	0.208	0.094	50.54	0.186
	Mean	-	85.10		-	52.96	
72	1	0.139	73.16		0.067	35.94	
	2	0.146	76.84	0.190	0.083	43.23	0.192
	Mean	-	75.00			39.59	
96	1	0.117	62.57		0.149	26.92	
	2	0.100	53.48	0.187	0.057	31.32	0.182
	Mean	-	58.03		-	29.12	
120	1	0.121	59.90		0.030	17.96	
	2	0.139	68.81	0.202	0.017	10.18	0.167
	Mean	-	64.36		-	14.07	
168	1	0.085	44.75		0.005	2.58	
	2	0.098	51.58	0.190	0.013	6.70	0.194
	Mean	-	48.16		-	4.64	
216	1	0.031	17.03				
	2	0.059	32.42	0.182			
	Mean	-	24.73				
264	1	0.013	6.63				
	2	0.028	14.29	0.196			
	Mean	-	10.46				

O.D.= Optical density.

APPENDIX TABLE 5.

THE MICROBIAL DECOMPOSITION OF C^{14}-LABELLED PARAQUAT DICHLORIDE BY LIPOMYCES STARKEYI IN A SUCROSE MEDIUM. MEASURED BY THE EMISSION OF C^{14}-LABELLED CARBON DIOXIDE(TABLE 7,Fig.10)

			1	2	3	4	5	6	S.M.	R.M.	T.M.
24hrs.	R.1	A	177	168	200	194	214	181	189	195	202
		B	217	199	198	206	198	180	200		
	R.2	A	219	192	231	209	210	214	213	211	
		B	226	184	211	220	203	204	208		
48hrs.	R.1	A	2016	2068	2100	2034	2050	2062	2055	2099	2161
		B	1998	2073	2126	2180	2170	2310	2143		
	R.2	A	2066	2093	2122	2166	2053	2140	2107	2224	
		B	2252	2272	2385	2458	2459	2211	2340		
72hrs.	R.1	A	1054	1030	1106	1114	1189	1161	1109	1154	1151
		B	1119	1130	1172	1228	1246	1298	1199		
	R.2	A	905	1046	999	1075	1103	1067	1033	1149	
		B	1148	1121	1314	1354	1310	1336	1264		
96hrs.	R.1	A	200	217	234	229	215	263	226	220	211
		B	232	215	199	230	191	218	214		
	R.2	A	207	181	181	167	198	204	190	202	
		B	209	215	206	227	216	210	214		
192hrs.	R.1	A	265	230	264	279	273	275	264	254	279
		B	241	227	231	241	261	262	244		
	R.2	A	292	288	277	352	347	293	309	304	
		B	266	293	318	313	299	305	299		
384hrs.	R.1	A	338	327	329	278	259	310	307	305	289
		B	327	320	294	291	286	291	302		
	R.2	A	281	272	255	274	272	304	276	273	
		B	281	228	248	293	255	311	269		

R.=replicate
S.M.=sample mean
R.M.=replicate mean
T.M.=treatment mean

Figures expressed in counts per 400 seconds.
Converted to counts per minute in Table 7.

APPENDIX TABLE 6.

THE QUANTITY OF C^{14}-LABELLED PARAQUAT DICHLORIDE REMAINING IN SOIL SAMPLES (Fig.11).

cp 400secs.	cpm.	cpm.above B/G.	wt.soil (mg.)
260	39	16	16.1
333	50	27	18.6
360	54	31	19.6
167	25	2	14.0
173	26	3	11.4
673	101	78	24.2
220	33	10	18.8
207	31	8	25.4
340	51	28	19.1
627	94	71	17.9
267	40	17	18.4
507	76	53	13.9
413	62	39	23.6
600	90	67	23.5
800	120	97	27.9
267	40	17	21.1
267	40	17	14.8
347	52	29	13.1

APPENDIX TABLE 7.

THE PARAQUAT ADSORPTION ISOTHERMS FOR SOILS

Br2B.Br3.72 and 75. (FIG. [illegible]).

Ppm.PQ. applied	Observed O.D.		Corrected O.D.		ppm.unad.		ppm.ad.		%ad.		g/100g.ad		Dil.Fac.
	75	72	75	72	75	72	75	72	75	72	75	72	
50	0.142	0.176	0.178	0.220	0.85	1.05	49.15	48.95	98.3	97.9	0.1966	0.1958	0
100	0.135	0.160	0.168	0.200	4.10	4.80	95.90	95.20	95.9	95.2	0.3836	0.3808	5
250	0.080	0.091	0.100	0.114	48	55	202	195	80.8	78.0	0.808	[illegible]	100
500	0.029	0.043	0.036	0.054	90	130	410	370	82.0	74.0	1.640	1.480	500
1,000	0.097	0.102	0.109	0.128	520	615	480	385	48.0	38.5	1.920	1.540	1000
2,000	0.126	0.154	0.152	0.168	1520	1615	480	385	24.0	19.3	[illegible]	1.544	2000
	Br2B	Br3	Br2B	Br3	Br2B	Br3	Br2B	Br3	Br2B	Br3	Br2B	Br3	
50	0.14	0.022	0.018	0.026	0.09	0.14	49.91	49.86	99.8	99.7	0.1996	0.1994	0
100	0.064	0.087	0.080	0.104	1.95	2.50	98.15	97.50	98.2	97.5	0.3928	0.3900	5
250	0.122	0.171	0.153	0.214	73	102	167	148	66.8	59.2	0.668	0.592	100
500	0.083	0.117	0.104	0.146	250	350	250	150	50.0	30.0	1.000	0.600	500
1,000	0.120	0.140	0.150	0.175	720	840	280	160	28.0	16.0	1.12	0.64	1000
2,000	0.142	0.154	0.178	0.193	1700	1840	300	160	15.0	8.0	1.20	0.64	2000

PQ.= paraquat
O.D.=optical density
unad.=unadsorbed paraquat
ad.=adsorbed paraquat
Dil.Fac.=dilution factor

APPENDIX TABLE 8.

THE RECOVERY RATE OF PARAQUAT DICHLORIDE FROM THE FOUR ROTHAMSTED SOILS.

SOIL Br2B

Rep.	24 Hrs. O.D.	24 Hrs. %Rec.	48 Hrs. O.D.	48 Hrs. %Rec.	96 Hrs. O.D.	96 Hrs. %Rec.
1	0.177	90.8	0.168	86.2	0.171	87.7
2	0.185	94.9	0.175	89.7	0.184	94.4
3	0.171	87.7	0.180	92.3	0.172	88.2
Mean	-	91.1	-	89.4	-	90.1

SOIL Br3

1	0.168	89.8	0.176	94.1	0.160	85.6
2	0.173	92.5	0.170	90.9	0.168	89.8
3	0.169	90.4	0.162	86.6	0.158	84.5
Mean	-	90.9	-	90.5	-	86.6

SOIL 72

1	0.167	87.0	0.185	96.4	0.172	89.6
2	0.169	88.0	0.175	91.1	0.180	93.8
3	0.173	90.1	0.168	87.5	0.173	90.1.
Mean	-	88.4	-	91.7	-	91.2

SOIL 75

1	0.176	89.8	0.176	89.8	0.175	89.3
2	0.186	94.9	0.184	93.9	0.167	85.2
3	0.186	94.9	0.155	94.4	0.175	89.3
Mean	-	93.2	-	92.7	-	88.0

MEAN RECOVERY RATE= 90.3%

O.D.= optical density.

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SHORT COMMUNICATION

Effects of Gamma Irradiation on Soil Aggregate Stability

Introduction

In an investigation of the effects of soil irradiation on plant growth, Bowen and Cawse [1] noted that soils, which had received a dose of 2.5 megarads, dried out more slowly and that the rate of percolation of water through the soil appeared to be reduced. They offered no explanation for these observations which would seem to indicate that irradiation had in some way affected soil structure.

The nature of materials responsible for soil aggregation is still somewhat obscure. It is well known that extracellular polysaccharides of many soil micro-organisms are very effective aggregating agents but the extent to which they are important remains in doubt (Griffiths [5]). Metha *et al.*[9] working with a Swiss *braunerde* under forest, found that selective oxidation of polysaccharides by periodate produced no effect on aggregation. Greenland *et al.*[4] obtained similar results with soils under permanent grass in Australia but found that periodate treatment substantially reduced aggregates in soils from young leys, indicating that in these latter instances, polysaccharides were making a significant contribution to aggregation.

Gamma irradiation of polysaccharides produces degradation (Collinson and Swallow [2]) and it is also known (Geoghegan [3]) that reduction of the chain length of microbial polysaccharides reduces their capacity to aggregate soils. It seems possible therefore that irradiation may reduce aggregation in soils where this is due to microbial polysaccharides. In order to investigate this possibility a study was made of the effect of irradiation on natural and synthetic (polysaccharide amended) aggregates from a number of soils.

Materials and Methods

The four soils used were all from the N.A.A.S. Experimental Husbandry Farm, Kirton, Lincs. and some of their characteristics are summarized in Table 1. Natural aggregates (2 mm) were removed by dry sieving. Synthetic aggregates (2 mm), both with and without polysaccharides were prepared by the method described by Griffiths and Jones [6]. The polysaccharide used was isolated from cultures of the soil yeast *Lipomyces starkeyi*, (Jones and Griffiths [7]) and incorporated in the aggregates at a concentration of 0.5 per cent. The stability of aggregates was determined by the water drop method (McCalla [8]), details of the apparatus used being given by Griffiths

TABLE 1

Characteristics of Lincolnshire soils

Soil	Origin	Aggregate* stability	Mechanical analysis				
			Coarse sand $< 200\mu$ %	Fine 20–200μ sand %	silt 2–20μ %	clay $< 2\mu$ %	organic matter %
A	Permanent pasture	95.2	2.1	49.2	15.0	30.0	3.7
B	2 yr. ryegrass ley	42.5	8.6	67.7	7.5	12.5	3.7
C	1 yr. cocksfoot ley	14.5	4.9	73.4	6.8	5.8	9.3
D	Arable	11.6	5.1	68.1	17.5	6.3	3.1

* Determined by water-drop method. Each value represents the mean number of water drops to disintegrate 20 aggregates.

and Jones [6]. Samples of the natural and synthetic aggregates and of the purified polysaccharide were irradiated at a dose of 2.5 megarads by the A.E.R.A. at Wantage.

Results and Discussion

The effects of irradiation on the stability of natural and synthetic aggregates of the four soils studied are summarized in Table 2. Analysis of these data failed to reveal any significant effect of irradiation on either the natural aggregates or the synthetic aggregates which did not contain polysaccharide.

TABLE 2

Effect of gamma irradiation (2.5 megrads) on stability (water-drop method) of natural and synthetic aggregates of four Lincolnshire soils

Soil	Natural aggregates			Synthetic aggregates			Synthetic aggregates with 0.5% polysaccharide		
	Not irradiated	Irradiated	Change in stability after irradiation %	Not irradiated	Irradiated	Change in stability after irradiation %	Not irradiated	Irradiated	Change in stability after irradiation %
A	95.2	93.6	− 1.7	9.5	9.4	− 1.1	25.4	22.9	− 9.8
B	42.5	36.3	− 14.6	8.1	7.9	− 2.5	28.8	23.0	− 20.1
C	14.5	13.8	− 4.8	7.1	6.9	− 2.8	109.3	52.2	− 52.2
D	11.6	13.2	+ 5.2	7.7	7.5	− 2.6	106.3	54.0	− 49.2
Totals	163.8	156.9	—	32.4	31.7	—	269.8	152.1	—

LSD for $P = 0.05$ between sample means = 4.17

These latter aggregates were all uniformly unstable, a finding in accord with previous work (Mehta *et al.*[9]; Griffths and Jones [6]). It appears to make little difference whether the original sample was composed of stable aggregates or not and as Mehta *et al.*[9] put it, the aggregating substance 'can only be effective once, and new agent is necessary to form stable aggregates again'.

Synthetic aggregates containing 0.5 per cent polysaccharide were clearly more stable than those without and irradiation significantly reduced their stability. It can be seen, however, that aggregates from the four soils were not affected equally, those from soils C and D (originally the most stable) showing the greatest reduction in stability.

Inspection of Table 1 shows that soils whose polysaccharide treated aggregates responded least to irradiation had the highest clay content and vice versa. The correlation between percentage clay and percentage reduction in stability following irradiation was high ($r = -0.89$) and significant at the 5 per cent level. It would appear that clay in some way protects the polysaccharide from the damaging effects of irradiation.

Aqueous solutions of the irradiated polysaccharide were much less viscous than the untreated material. Using 0.5 per cent solutions the times taken for 10 ml to flow through the capillary of an Ostwald 200 Viscometer were 16 and 31 secs respectively for irradiated and non-irradiated samples. This reduction in viscosity is indicative of degradation of the polysaccharide and from the observations of Geoghegan [3] it would be expected that such material would be less effective in aggregation. This was found to be so. The stability of aggregates prepared from soil D with 0.5 per cent polysaccharide was reduced from 106 with untreated material to 19 with irradiated material.

It seems clear that aggregation dependent upon microbial polysaccharide is sensitive to the effects of gamma irradiation. From the work of Greenland *et al.*[4] it might have been expected that the natural aggregates from the grass leys (soils B and C) would also have shown reduced stability following irradiation. The differences found, though not statistically significant, do show a trend in this direction and with a larger body of data it might well be possible to demonstrate a differential effect of irradiation upon the aggregation in old grassland and young leys. Certainly gamma irradiation seems to offer promise as an analytical tool in assessing the extent to which aggregation in soils is dependent upon microbial polysaccharides.

Summary

Four soils, with a range of natural aggregate stability, were used to compare the effects of gamma irradiation on the stability of natural aggregates and of synthetic aggregates containing 0.5 per cent microbial polysaccharide. The stability of natural aggregates was not significantly affected by irradiation but that of the synthetic aggregates (prepared from the same soils) was significantly reduced, the magnitude of the reduction being negatively correlated with the clay content of the soil.

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