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SOIL WATER DYE TRACING, WITH SPECIAL REFERENCE TO THE USE OF RHODAMINE WT, LISSAMINE FF AND AMINO G ACID

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ABSTRACT

Three fluorescent dyes (Rhodamine WT, Lissamine FF and Amino G Acid) are compared for use in soil water tracing. Severe limitations are evident, but practical applications are possible. Background fluorescence, adsorption, desorption, pH and other non-adsorptive effects are reviewed in the contexts of soil column work and field tracing of soil water. Lissamine FF and Amino G Acid are to be preferred for soil column work because of their lower adsorption; Rhodamine WT exhibits higher adsorption but is useful in field situations where organic fluorescence backgrounds are high. Semi-quantitative work may be undertaken in soil columns once a priming and flushing procedure has been adopted.

KEY WORDS Dye tracing Rhodamine WT Lissamine FF Amino G Acid

INTRODUCTION

Fluorescent dyes are readily detectable and relatively cheap to use and they have been widely used in water tracing (e.g. Smart and Laidlaw, 1977; White, 1977). Their use for tracing soil water has not been extensively reported (McLaughlin, 1982) but Corey (1966) evaluated a range of non-fluorescent dyes for use as visual tracers in acid soils. Anderson and Bouma (1973) and Omoti and Wild (1979) report on the use of non-fluorescent and fluorescent dyes, respectively, for pathway staining. White (1977) reviews the use of tracer dyes for ground-water studies and Reynolds (1968) and Mosley (1979) used fluorescent dyes to demonstrate the existence of rapid flow systems in soils.

Their potential for soil water dye tracing is considerable but they are known to suffer from adsorption, pH effects, photo-decomposition and background fluorescent problems. In addition, in soils, their behaviour with respect to high solid: solution ratios is unknown and the effects of soil aggregates also have to be allowed for. This paper therefore discusses work on dye use in soils, bearing in mind these problems. In the present paper the dyes Rhodamine WT, Lissamine FF and Amino G Acid are tested. Dye nomenclature follows that of Smart and Laidlaw (1977) and the choice of dyes for tracing of soil water is based on their findings. Alternative names and excitation and emission spectra of the dyes are shown in Table I and chemical structures are shown in Figure 1. It is known from the work of Smart and Laidlaw that Amino G Acid and Lissamine FF are less adsorbed than Rhodamine WT and, as the present paper shows, these are therefore more suitable as soil water tracers. The dyes have been evaluated for use as tracers of field and laboratory column soil water in terms of behaviour during transport through adsorptive, chemically reactive, structured, porous soil media. Adsorptive mechanisms are reviewed and background interference, adsorption characteristics and non-adsorptive effects are compared empirically for a range of soils. Applications in soil column breakthrough analysis and in field tracing of soil drainage waters are also considered.

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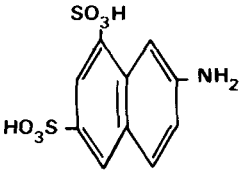
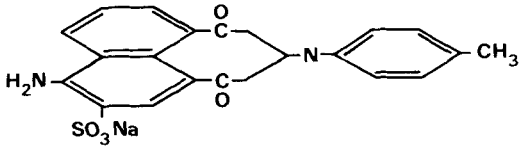
AMINO G ACID



LISSAMINE F.F.



RHODAMINE W.T.

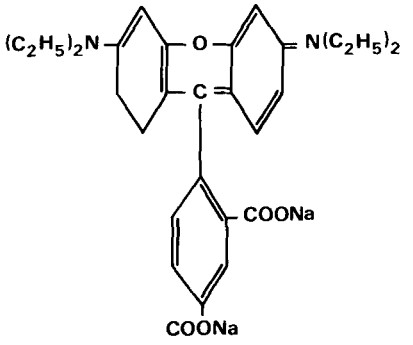


Figure 1. Structure of dyes (from Smart and Laidlaw, 1977)

Table I. Excitation and emission peaks for fluorescent dyes used (From Smart and Laidlaw, 1977)

Dye	Maximum excitation (nm)	Maximum emission (nm)
Rhodamine WT*	590	580
Lissamine FF†	420	515
Amino G Acid‡	355	445

*Atomic wt. 566.
†Cl acid Yellow 7; Lissamine Yellow FF, Brilliant Sulpho flavine FF, Brilliant acid yellow 8G.
‡7 amino 1·3 Naphthalene disulphonic acid.

REVIEW

Adsorption

If soil water dye traces are to be interpreted in terms of media characteristics, and not erroneously in terms of dye behaviour, then the chemical and physical interactions between the tracer and the media should be investigated. The dyes tested vary in their adsorption characteristics and lag behind wetting fronts in displacement systems to varying degrees. White (1977) presents examples of porous media traces where maximum dye effluent concentrations lag behind tritium peaks, although times of first arrival are similar. However, the advantages of using dyestuffs as tracers are that they are relatively easy to handle, simple to detect and rapid to analyse quantitatively using fluorometric techniques. They are therefore worth considering for routine use as a viable alternative to isotopes and chemical tracers, once their limitations have been specified.

Smart and Laidlaw (1977) report that in the presence of suspended sediment Rhodamine WT is the most adsorbed of the three dyes, suffering high adsorption losses for both organic and inorganic substrates. With humus they report 89% loss for Rhodamine WT, 61% loss for Amino G Acid and 32% for Lissamine FF (from initial concentrations of $100\,\mu g\,l^{-1}$ and $20\,g\,l^{-1}$ sediment). For kaolinite they report 33, 3 and 4% losses for the same dyes respectively.

The presentation of adsorption isotherms (amount of dye adsorbed per soil weight graphed against amount of dye in solution) is a standard method for the description and prediction of adsorption of absorbates on solid surfaces. However, the application of adsorption isotherms may be limited under certain conditions. The isotherms apply to an equilibrium situation, referring specifically to a thermodynamic equilibrium between the adsorbate and the adsorbent surface, and are independent of the initial concentration of the dye. In operational terms, an equilibrium situation is one where the rate of sorption between the soil solution and the solid phase is much greater than the rate of change of concentration of solute in the soil solution because of any other cause. When fast flow occurs, however, the adsorption reaction rate may be slow in comparison to flow rate (such as may be the case in macropore flow in soil columns). Reaction kinetics may better describe this situation (Travis and Etnier, 1981) and adsorption rate may be concentration dependent.

Moreover, while empirically derived adsorption isotherms are a guide to the relative behaviour of different dyes in different soils, they do not provide proof of the operation of specific adsorption mechanisms. Nor do they necessarily discriminate between adsorptive and non-adsorptive mechanisms (Travis and Etnier, 1981). In addition, soil pH can have a marked effect upon (1) dye fluorescence itself (Smart and Laidlaw, 1977) and (2) the specific adsorption mechanisms and capacity. Furthermore, if after a dye application, non-dyed flow is maintained, desorption may occur and desorption equilibria will act to influence the shape of the tail of a dye trace. Finally, there are a multiplicity of adsorption mechanisms and sites which need to be considered because they can influence dye sorption and behaviour in structured soils.

Adsorption mechanisms

The dyes tested are anionic in character and may be expected to be largely repelled by negatively charged clay surfaces; thus minimal adsorption would be expected. However, Rhodamine WT has a functional sodium attached which may be adsorbed onto clays. In addition, although anionic, the dyes are complex organic molecules which may, in fact, be adsorbed by a wide range of mechanisms. These can be grouped into specific and non-specific bonding. *Specific bonding* is often irreversible and is dominated by normal covalent bonding between atoms. *Non-specific bonding* can be divided into two main types: those which are due to the electrostatic attraction experienced between surfaces of opposite charge and those which arise from a variety of weaker inter-molecular forces.

In distinguishing between specific and non-specific adsorption, the occurrence of desorption of the adsorbed solute is important. In cases where adsorption is irreversible (minimal desorption) it is likely that the strong ion to ion specific adsorption forces are operative. In soils, partial desorption of dyes occurs (Omoti and Wild, 1979) and thus the adsorption is partly reversible and partly irreversible. This

precludes the operation of the variety of ion exchange (Giles *et al.*, 1964) and hydrolytic mechanisms which are often completely reversible. Some relatively strong adsorption force must operate to prevent complete desorption of the dyes. A complicating factor is that, in structured soils, desorption can occur because sorbed dye may be released from sites which are not surface-adsorption sites and such desorption is difficult to evaluate precisely.

Three adsorption mechanisms can be considered: firstly, hydrogen bonding between a strongly electro-negative atom and a hydrogen atom; secondly, the formation of electron donor/acceptor complexes, in which electrons of the dye's aromatic ring structure may form either the donor or the acceptor (Loughlin, 1969); thirdly, Van der Waals forces, which cover a variety of intermolecular attractions.

Of these three mechanisms, Van der Waals forces and hydrogen bonding appear to be the most important. Allingham *et al.* (1958) suggested that an acid dye particle is likely to be adsorbed by the soil particle through hydrogen bonding if a free phenolic group exists in the dye. It is, in fact, unlikely that hydrogen bonding alone is responsible for all the bonding dyes with free phenolic groups as hydrogen bonding is generally non reversible and desorption is, moreover, evident even for unstructured soil material, as shown below. Hydrogen bonding is, however, likely to be increased where soil organic matter is involved (as in organic soil horizons) due to bonding with the oxygen of organic acid groups. It may also be significant for undissociated dye molecules where a bond may be established between the acid proton and oxygen held in surface functional groups of clay materials.

The bonding of electron donor/acceptor complexes with the surfaces of soil particles by mixing between electron poor and electron rich species can occur but this is generally reversible, (Smart, 1972). In addition, the presence of a substituent in a dye's molecular structure can result in electron deficiency and this can reduce the ability of a dye to be adsorbed in this manner.

Non-specific Van der Waals forces exhibit a rapid distance decay function and are most effective for flat orientation on basal planes where no surface functional groups are present and where approach of the whole molecular area is possible (Snoeijink and Weber, 1967). When Van der Waals forces are operative, it is generally found that large molecules are more strongly adsorbed than smaller ones and that the bond strength is also additive, being augmented when the adsorbed molecule experiences forces from both sides of a pore (Loughlin, 1969); this is important in cases where dye penetrates microporous sites.

From the evidence of the occurrence of desorption, it would appear that Van der Waals forces or electron/donor acceptor complex formation are responsible for a considerable proportion of dye adsorption in soils. No information is available to separate these two effects, but it is likely that electron donor/acceptor formation will be supressed by the occurrence of oxygen-containing functional groups on the edges of clays and mineral particles and Van der Waals forces are therefore likely to dominate. Some irreversible dye loss will also occur partly as a result of ion to ion specific adsorption forces and partly as a result of hydrogen bonding.

The amount of irreversible bonding that occurs can also be affected by pH. As pH is raised, hydrogen held by organic and inorganic colloids becomes ionized and is replaceable. Greater irreversible adsorption is therefore likely to occur in alkaline soils or with dyes that can effect a change in soil solution pH. (In many proprietry solutions Rhodamine WT has a higher pH than equivalent concentrations of Amino G Acid or Lissamine FF and can cause in increase in soil solution pH of acid soils, the amount depending on dye: solution concentration ratio).

The consideration of adsorption mechanisms, although by no means comprehensive, does allow some criteria for the choice of an adsorption resistant dye to be suggested. Corey (1968) suggested that in acid soils the presence of more than one sulphonic acids group is a useful indicator of resistance to adsorption. This correlates well with the adsorption values reported by Smart and Laidlaw (1977). An anionic dye of small molecular size is least likely to be adsorbed, as is one with sulphonic acid functional groups giving a high negative surface charge. The dye that most closely corresponds to this is Amino G Acid, followed by Lissamine FF. The greater adsorption of Rhodamine WT, already reported by Smart and Laidlaw (1977, is most likely to be associated with an increase in the effectiveness of non-specific adsorption.

High dye concentrations

If applications of high dye concentrations are made, micellization of dye may occur and simple monolayer adsorption models may not apply (Giles *et al.*, 1964; Easton *et al.*, 1964). Rhodamine WT is known to form dye micelles (Smart, 1972), the size of which is dependent upon the initial dye concentration in solution. At low concentrations the majority of micelles present are small, whereas at high concentrations, much larger micelles are present. The importance of this is that the large and small micelles can occupy the same adsorption sites within the soil, thus giving a false impression of the relationship between adsorption and dye solution concentration. If micellization is occurring in association with high dye concentrations, then this presents an additional difficulty in quantifying and interpreting adsorption losses and also desorption patterns.

Soil aggregates

There are a multiplicity of adsorption and absorption sites in structured soils which have to be considered in predicting the behaviour of dyes in soils. These are illustrated in Figure 2. Dye may be adsorbed onto ped surfaces or diffuse into the ped and be held either by particle surface adsorption or in high tension pore or film water. The presence of soil plasma and microporosity within the peds and the skeletal grains can result in additional sorption losses. Not only are there a large number of sorption sites, several adsorption mechanisms are possible depending on which properties of the dyes are dominant, as discussed above. The fluorescent dyes tested can therefore be both adsorbed and desorbed in structured soils at several scales and by several possible mechanisms.

This paper therefore not only presents adsorption isotherms for the dyes and dispersed soils considered, it also discusses adsorption mechanisms and presents empirical data for intact, aggregated soil columns in order to discuss the utility and interpretations of dye tracing for field applications. This treatment thus extends that of Smart and Laidlaw (1977) to cover soil bodies, where substrate to solution ratios are greater than were considered in their paper.

In the context of dye application to aggregated soil material *in situ* the sorption mechanisms involved cannot be clearly identified and this therefore precludes precise quantitative predictions of dye adsorption. Quantification of adsorption may thus have to be made empirically for any soil under consideration.

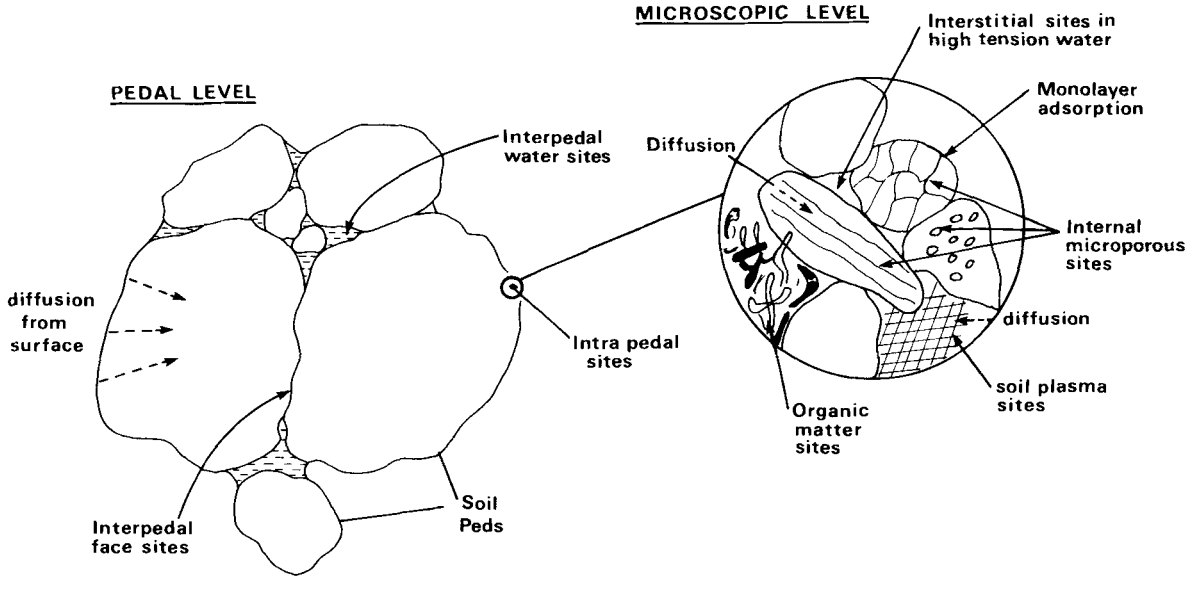


Figure 2. Sorption sites in structured soils

In addition, the surface chemistry of clays differs in aqueous suspensions and in air dry environments (Mortland, 1970), such as might be encountered in the field. With high water content, polarization forces are distributed among a large number of water molecules; as water content decreases, these polarisation forces become more concentrated on the fewer remaining water molecules, causing an increase in hydrolysis and proton donation. Work on aqueous suspensions may therefore be an unreliable guide to the behaviour of field soils as the surface acidity of clays increases as water content decreases.

The aggregation of field soils also affects the diffusion of large molecules (Barraclough and Nye, 1979), which can become dependent upon the presence of large inter-aggregate pores. This provides a further reason why adsorption data on dispersed material may be an unreliable guide to dye movement in structured soils.

pH

pH can affect dye behaviour in soil for two reasons. Firstly, by altering the chemical structure of the dye and influencing fluorescence and secondly by influencing the adsorption mechanism and capacity. These latter effects will be most marked in base-poor mineral soils and acid humus soils where the exchange complex is dominated by H^+.

Loss of fluorescence is apparent for Amino G Acid below pH 6·5, Rhodamine WT below 5·5 and Lissamine FF below 3·8 (Smart and Laidlaw, 1977). At these pH levels the dyes become unstable and begin to protonate, changing to a colourless lactone form which is undetectable by fluorometry. Without pH data this can be interpreted as adsorptive loss or non-arrival of dye.

The greater resistance to fluorescence loss with decreasing pH of Amino G Acid and Lissamine FF compared to Rhodamine WT can be explained in part by the presence of a carboxyl acid functional group in the latter. This is liable to protonation at higher pH values than the sulphonic acid groups on Amino G Acid and Lissamine FF.

It is advisable to compare the pH of the dye in solution with the pH of the soil prior to a dye trace. A dye with a markedly different pH to that of the soil solution could effect changes in adsorption, dye stability and soil structure during a trace, thus rendering interpretation of dye recovery extremely difficult. High concentrations of dye are likely to affect soil pH, especially the Rhodamine WT marketed in an alkaline solution. The actual degree of effect will depend upon the type and buffer capacity of the solution in which the dye is made up, Rhodamine WT being marketed in solutions of both pH 12 and pH 7·0.

The ability of an adsorbing surface to protonate compounds is dependent upon the nature of the metal cations saturating the exchange sites on the surface (Mortland, 1970), protonation increasing where the cations are H^+, Al^{3+} and Fe^{3+}, as is the case with acid, ferritic soils. Protonation of the organic molecule at the surface occurs from exchangeable H^+ from the surface, water associated with metal cations on the surface or proton transfer from cationic species already at the surface. Many compounds become cationic or neutral after adsorption at the clay surface through protonation.

Photochemical decay

A detailed examination of photochemical decay is given by Smart and Laidlaw (1977). For the fluorescent dyes discussed in this paper Amino G Acid is subject to significant photochemical decay over a period of days. Although field soil water traces may take a number of weeks, the dye is only subject to incident light prior to entry into the soil and after collection, prior to analysis. Thus dye solutions of Amino G Acid should be shielded from light before use and during collection and analysis; a covering of heavy duty black polythene is recommended for water sampling apparatus.

Background fluorescence

Background fluorescence is that occurring naturally in soil-water suspensions, filtrates, soil drainage water and runoff water. It is largely due to naturally occurring organic material and the background fluorescence of any aqueous medium, at the relevant wavelengths for the dye in use, has to be evaluated before any quantitative dye detection work can be undertaken.

The occurrence of natural fluorescence in runoff waters has been well documented, largely in relation to the presence of colloidal and dissolved organic matter. Total organic carbon concentration has been found to correlate linearly with fluorescence in natural waters between 400–600 nm emission wavelengths (Smart *et al.*, 1976). Fulvic acid extracts have a peak emission of 520 nm (Seal *et al.*, 1964) and Smart and Laidlaw (1977) report fluorescence of several organic substances in the range 400–600 nm, with peak emissions at 450–500 nm. The organic matter emission spectra reported overlap with those of Lissamine FF (peak 515 nm) and Amino G Acid (peak 445 nm) but there is little overlap with Rhodamine WT (peak 580 nm) making the latter most useful for dye work in waters with high background fluorescence.

It is possible to overcome high background fluorescence at dye wavelengths by the use of large enough quantities of dye to make detection unambiguous. However, this may lead to structural collapse in soils or undesirable run-off water discolouration. In addition, one of the main advantages of using fluorescent dyes is their detectability at low concentrations which are acceptable on environmental grounds and are below the visible threshold. Thus large dosages are unwarrented in these contexts. Large dosages will also lead to increased diffusion and dispersion of the dyes and also necessitate the dilution of samples for analysis. Therefore, dosage calculations are necessary to estimate the minimum concentration detectable above background levels (see Trudgill, *et al.*, 1983, p. 267).

For many practical purposes, initial dye detection in a soil outflow can be taken as the time when the detection point carries a fluorescence at least two times greater than the maximum background recorded prior to the trace. Adequate monitoring of an effluent site prior to tracing is thus a prerequisite of dye tracing. This is especially so since background fluorescence can vary in field soil drainage waters with flow conditions and in relation to source area. Increased fluorescence commonly occurs during high flow conditions due to the entrainment of decomposing organic material in surface or near surface runoff. Periods of high flow are likely to correspond to expected tracer arrival times and it is therefore important to assess background fluorescence variations at a range of flow conditions and especially at high flow. In laboratory leaching columns background fluorescence assessment is also necessary and can be measured by leaching the columns with deionised water prior to tracing and analysing the effluent.

EXPERIMENTAL WORK: MATERIALS AND METHODS

A Turner II filter fluorometer was used for fluorescence determinations, with filter combinations as specified by Smart and Laidlaw (1977).

Sources of dye used were: Lissamine FF, Amino G Acid (in powder form) from L. B. Holliday Ltd, Rhodamine WT from Dupont Ltd, in liquid form. The solution of Rhodamine WT was of alkaline pH, around 12. The dyes may be marketed with unknown impurities.

Five widely differing soil materials were used. The British soils examined are described using the terminology of Hodgson (1974) and Avery (1973). The principle soil investigated was a brown calcareous earth (1) with subsidiary work on soils (2)–(5) (described below) to gain a range of acidities, particle size distributions and exchange capacities.

1. *Brown calcareous earth* (Aberford Series, Reeve, 1976), from Whitwell Wood, 20 km south east of Sheffield, UK, on glacial drift overlying Lower Magnesian Limestone (Permian). A profile description is given in Table II.
2. *Brown earth* (Nordrach Series, Johnson, 1971), from Derbyshire on silt loam (loess) over Carboniferous Limestone. A profile description and analytical data are given in Table III.
3. *Ferralitic soil*, Acid Red Soil from the Bulls Head Catchment Canberra, Australia. This is an acid, strongly leached soil. Data are given in Table IV.
4. *Clay soil.* Coal measure clays, East Sheffield, slightly acid with a high CEC (35 meq 100 g), Table V.
5. *Washed quartz sand.* Table VI. CEC 0·5 meq 100g.

Elution: soil samples of 10 g were shaken for 2 h in 30 ml distilled water and centrifuged at 4000 rpm for 20 min. During batch adsorption tests, dye solution were shaken with soil sample replicates (see also Smettem and Trudgill, 1983, p. 48).

Table II. Properties of soil (Aberford series) (Reeve, 1976)

Horizon	Ap	Bw
Depth (cm)	0–20	20–31
Sand		
200 μm–2 mm (%)	7	7
60–200 μm (%)	13	15
Silt 2–60 μm (%)	56	56
Clay 2 μm (%)	24	22
Loss on ignition (%)	9·6	7·6
$CaCO_3$ equivalent (%)	4·2	10·8
<2 μm $CaCO_3$ (%)		0·8
Organic carbon (%)	2·4	1·8
pH in water (1 : 2·5)	7·2	7·8
pH in 0·01 m $CaCl_2$ (1 : 2·5)	7·2	7·3
CEC (meq 100g)	31·1	23·7

Table IIIa. Profile characteristics of soil (2), brown earth

cm	
0–20 A	Silt loam, strong fine and medium crumb and subangular blocky structure, abundant fine fibrous roots.
20–35 Eb	Silt loam, moderate medium and fine angular blocky structure, abundant fine fibrous roots.
35–75 Bt	Silty clay loam, moderate medium angular blocky structure, common fine fibrous roots.
75+ R	Crystalline Carboniferous Limestone

Table IIIb. Analytical data, soil 2, brown earth

Horizon Depth, cm	A 5–15	Eb 20–30	Bt 40–60
Silt, 2–50 μm %	63·4	60·3	51·8
Clay, < 2 μm %	26·8	29·2	39·5
$CaCO_3$ %	0·0	0·0	0·0
Organic carbon %	5·3	3·4	0·0
pH (water, 1 : 2·5)	31·6	28·8	18·4
CEC (meq 100g)	5·9	6·0	6·0

Table IV. Analytical data of profile of soil 3, acid red earth (Data from Talsma, Mansell and Hallam, 1980)

Depth, cm	0–10	20–40	80–100	140–160
Sand, 20–2000 μm %	45	41	43	41
Silt, 2–20 μm %	26	21	21	21
Clay, 2 μm %	24	36	36	37
Organic matter %	6·7	2·4	0·7	0·4
pH (water, 1 : 1)	4·7	4·8	5·1	5·1
CEC (meq 100g)	11·5	7·5	5·5	5·5

Table V. Characteristics of soil 4

	%
Sand, 200 μm–2 mm	1
60–200 μm	3
Silt, 2–60 μm	26
Clay*, < 2 μm	70
$CaCO_3$	0·0
pH (1 : 2·5 water)	6·8

*Some swelling and shrinkage occurs, indicating the presence of montmorillonitic clay.

Table VI. Characteristics of washed sand (5)

Coarse sand	> 600 μm	6·5%
	600–212 μm	52·3%
Fine sand	212–63 μm	41·1%

Infiltration: Using narrow gauge (10 cm) tube with constant head bottle. *Breakthrough curves:* Intact soil columns were collected by excavation round a column of soil and lowering a plastic tube, 19·5 cm dia., over the column. The column was placed on a fraction collector. Input was by use of a constant head device. *Field soil drainage waters* were sampled from throughflow troughs (Atkinson, 1978) and from ceramic suction cups (Soil Moisture Equipment Corp).

RESULTS: BACKGROUND FLUORESCENCE

Examples of fluorescence values for field soil drainage waters, soil suction cup samples, soil column leachates, centrifuged supernatants from soil extracts and stream water are given in Table VII. Background fluorescence at the Rhodamine WT wavelength is minimal. Soil water fluorescences are generally higher than stream water values. Background fluorescence at the Amino G Acid and Lissamine FF wavelengths is always higher than that at the Rhodamine WT wavelengths. In all cases the higher values are from organic soils or 'A' horizons of mineral soils where organic carbon contents are in the range of 1–2%. The table also presents dye concentration data of the standard fluorometer working range for each of the dyes without the use of a density filter or of sample dilution. It is important to note that Rhodamine WT is more fluorescent than either Lissamine FF or Amino G Acid and that the calibration curve relating fluorescence reading to concentration in $\mu g\ l^{-1}$ is much steeper for the former. Background fluorescence leaves most of the working range free for detection of Rhodamine WT (covering up 0·69% of the range) but may restrict the use of the other two dyes (covering up to 38% of the range of Lissamine FF and 80% of Amino G Acid).

RESULTS: ADSORPTION

The earlier work on adsorption by Smart and Laidlaw (1977) employed a 1 : 50 substrate (sediment) to solution ratio, which is far more dilute than the ratios which occur in soil columns or field soils. They also report that adsorptive losses are mainly irreversible and while this may be the case with dilute single grain dispersed material it is not necessarily the case with microporous grains in heterogeneous porous media, such as soils, where non-specific sorption sites also exist. It is therefore necesary to test and extend their findings to cover soil materials.

Table VII. Ranges of values recorded for background fluorescence at dye wavelengths in soil waters. Fluorescence converted to $\mu g\ l^{-1}$ equivalent dye concentration

Soil (1)	Rhodamine WT	Lissamine FF	Amino G Acid
Soil drainage waters (throughflow troughs)	0·01–0·08	10–30	275–300
Suction cups	0·01–0·1	40–100	200–800
Streams	0·02–0·05	1–25	20–100
Leaching columns	0·02–0·08	1–38	NDA
Extracts	0·18	58	380
Sand extract	0	0	0
column	0	0	NDA
Clay extract	0·03	11	170
column	0	5	NDA
Working Range of Fluorometer (without density filter or dilution)	0–26	0–260	0–1000
Backgrounds as % of working range	0·39–0·69%	0·39–38%	2–80%

NDA—No data available.

An attempt was made to control for the effects of structure on adsorption by batch equilibriations using both soil structures and dispersed material. However, the structured material also dispersed and the attempt was unsuccessful. Results are available for dispersed material at high solid: solution ratios under equilibrium conditions and for structurally intact soil columns where equilibrium is unknown but most likely incomplete, both in terms of low solid – solution contact time and incomplete surface area contact due to structural effects on water flow.

Adsorption isotherms for disaggregated soil in batch equilibriations are presented for soil 1 in Figure 3, for soil 2 in Figure 4 and for soil 3 in Figure 5. Data for adsorption on clay and sand are shown in Table VIII. Data for high concentrations of dye, as might be expected near to a bulk field application, are presented for soil 3 in Table IX. Data for structurally intact saturated soil columns are presented in Table X (the data are selected for breakthrough occurrence close to one pore volume, ie for non-macroporous soils).

For all the soils tested Amino G Acid is the least absorbed dye. This is in contrast to the data of Smart and Laidlaw (1977) who found that Lissamine FF dye was the least adsorbed by humus (see Introduction: *Adsorption*). In soils Lissamine FF was adsorbed more than Amino G Acid but less than Rhodamine WT; the latter often suffered up to 90–99% losses. The order of resistance to adsorption holds for both dispersed soils at high solid : solution ratios and for soil columns. Amino G Acid and Lissamine FF were absorbed more on the calcareous soils and the clay; Rhodamine WT losses were greatest on the clay substrate. Despite the probable lack of equilibriation in the soil columns, the losses for the structured soil columns are similar to or a small amount less than the batch adsorption losses. The precise effects of structure cannot be assessed from these data, but it does suggest that adsorption isotherms may be of use in the prediction of adsorption losses in intact soils by indicating the maximum levels of adsorption, with lower levels being experienced under non-equilibrium conditions in structured soils.

The data presented for soil 3 cover a very wide range of initial concentrations of dye. High and variable backgrounds for Amino G Acid precluded the plotting of reliable data for this soil. They demonstrate that isotherms should be attempted for such a range as might be encountered during field use, especially

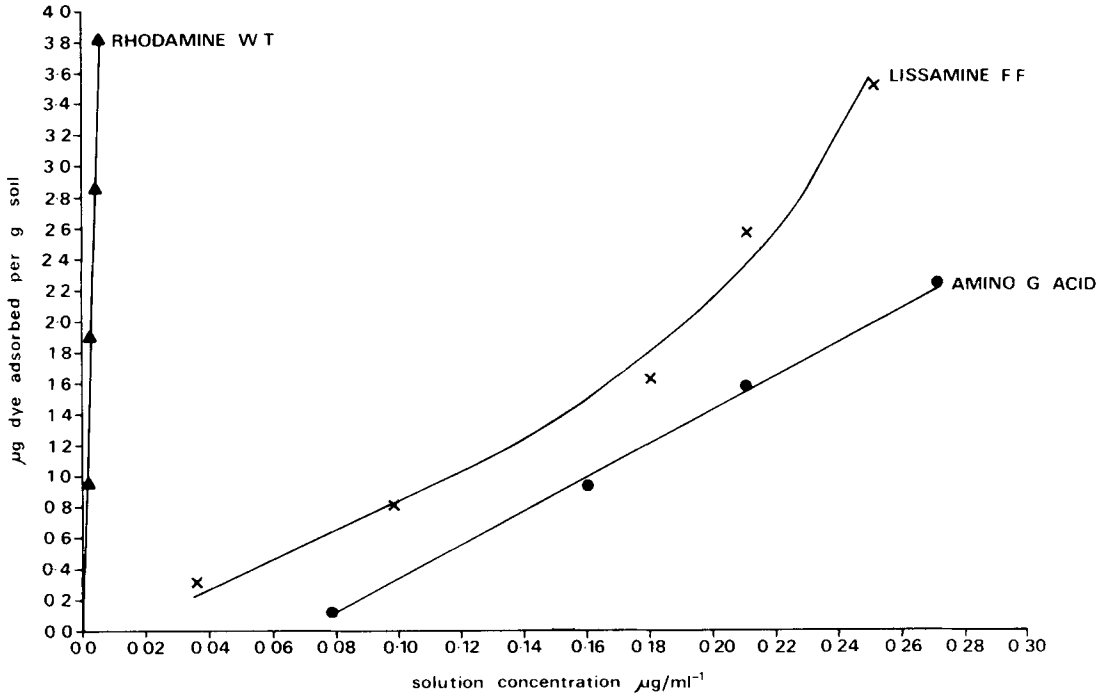


Figure 3. Adsorption isotherms for soil 1, brown calcareous earth, 1 : 2 soil water ratio

where very high concentrations will be found close to the emplacement point. The high concentration data are not consistent with the low concentration data, indicating a probable change in the relative importance of adsorption mechanisms at high and low concentrations of dye. At high concentrations specific adsorption may become suppressed, with probable increases of non-specific adsorption and micellisation.

While the data for adsorption show that the relative order of resistance to adsorption is the same for all the soils tested, there are differences in the amounts adsorbed for each soil. Values for Rhodamine WT losses are in the region of 90–99%, with a decrease to 60% for sand. The high values are in the most acid and organic soils and part of this loss may be a pH effect. This will lead to a loss in fluorescence, rather than adsorptive loss. Lissamine FF showed least adsorption for sand (14–18% loss) and clay (35–36%) with a wide range of intermediate values for soil 3 (4–54%) and higher values for soil 1 (80–87%) and soil 2 (68–72%), the greatest loss being in the most alkaline soil. Amino G Acid lost little in soil 3 (0·5–3%) with higher losses in soil 1 (62–80%) and soil 2 (64–66%).

For resistance to adsorption, Amino G Acid and Lissamine FF are preferable to Rhodamine WT for dye tracing in soils. Both Amino G Acid and Lissamine FF survived better in the acid soils tested, with low organic matter and low clay contents; in those soils tested with higher pH and greater cation exchange capacity survival was less but, Amino G Acid survived slightly better than Lissamine FF.

RESULTS: DESORPTION

Results are presented in Table XI for a one-step desorption on soil 1. The difference between the pre-desorption solution concentration and the post-desorption concentration yields the amount of desorption that has occurred, from which a new substrate loading in μg dye/g soil can be calculated.

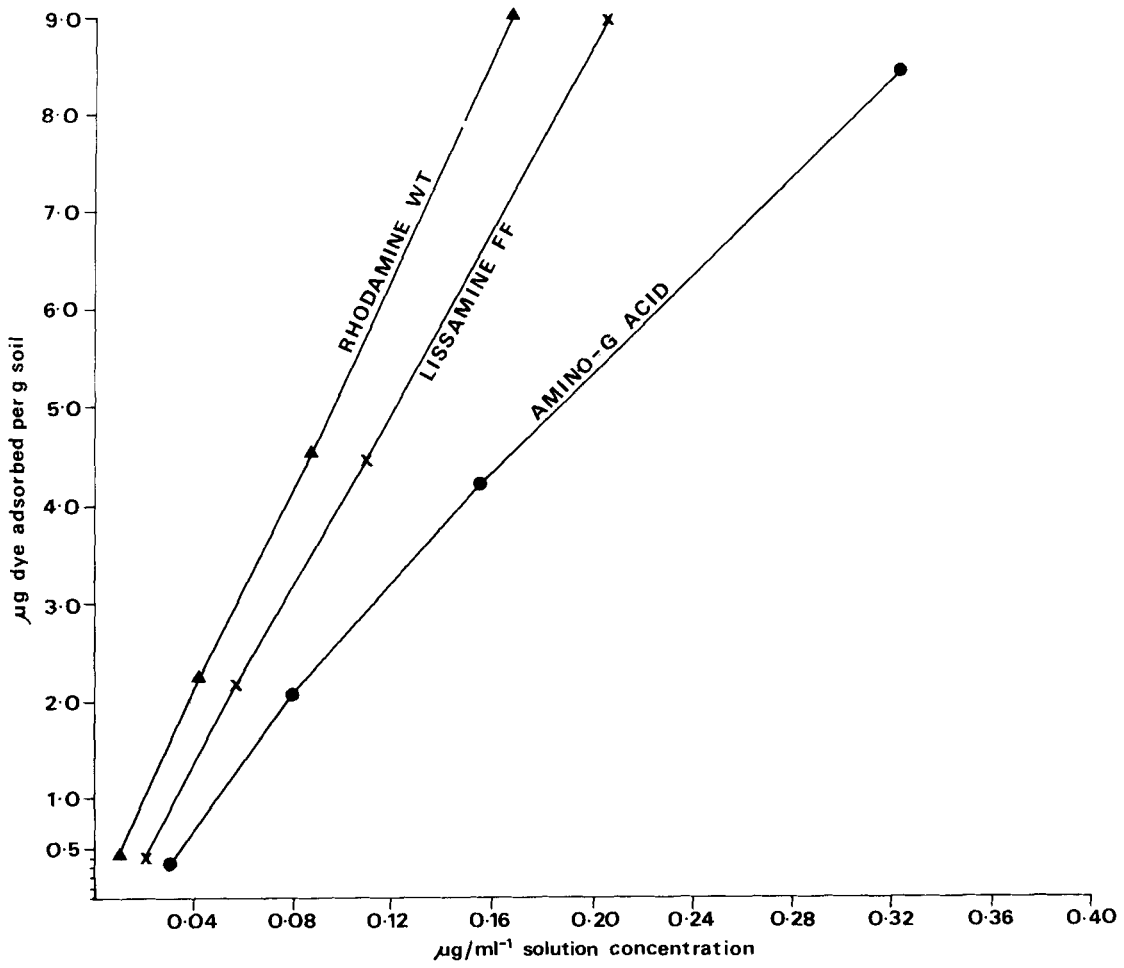


Figure 4. Adsorption isotherms for soil 2, acid brown earth, 1 : 5 soil water ratio

The occurrence of partial desorption from the soil material indicates that only some of the adsorption is irreversible. The occurrence of the irreversible proportion implicates a hydrogen bonding mechanism, especially in organic rich soils or mineral soils where the exchange complex is dominated by H^+. The reversible dye desorption implicates the occurrence of the much weaker Van der Waals forces. The greater the amount of desorption the more a weaker, reversible mechanism is implicated. Generally, Lissamine FF is more desorbed than Amino G Acid which is desorbed more than Rhodamine WT, but at high concentrations, Rhodamine WT desorption is greater than Amino G Acid. This pattern implicates the occurrence of weaker, non-specific bonding at higher concentrations of Rhodamine WT and possible micellisation. At low concentrations, background interference is high and, although background values have been subtracted from the data presented, background variability makes it difficult to present consistent and reliably interpretable data for low concentrations.

RESULTS: NON-ADSORPTIVE EFFECTS

pH

From the data presented for soil 3 (Table XII) it appears that decreasing final solution acidity occurs as relatively greater proportions of dye are adsorbed at low concentrations; at high concentrations a more

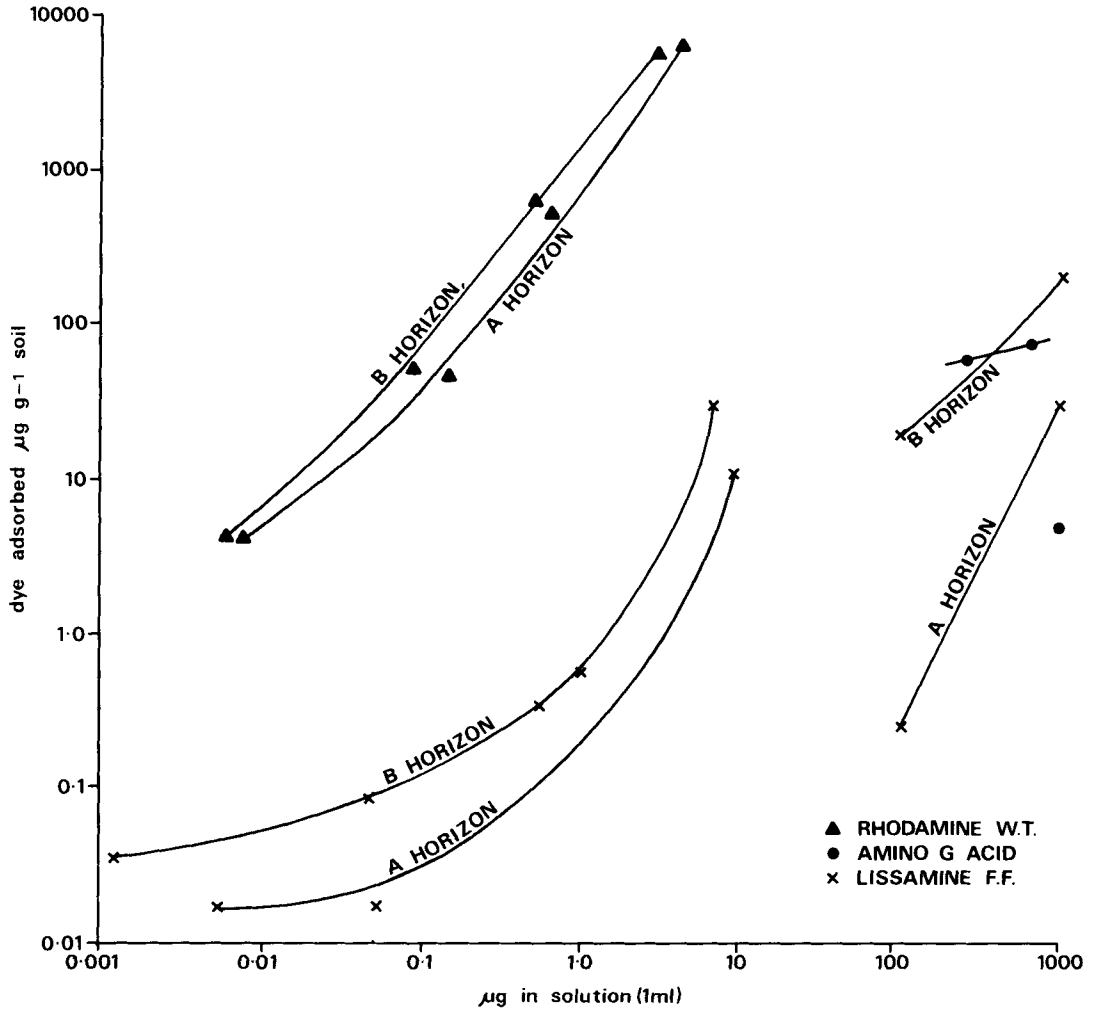


Figure 5. Adsorption isotherms for soil 3, red ferralitic soil, 1 : 5 soil water ratio

acid final solution pH occurs as there are greater amounts of unadsorbed acid dye remaining in solution. Increasing acidity of aqueous suspensions of Rhodamine WT originally buffered in an alkaline solution implicates the importance of dilution of the buffer and dissociation of functional groups.

Breakdown of soil structure

Rhodamine WT causes a significant reduction of the infiltration rate of water into laboratory soil columns (Figure 6). This effect is especially marked if the soil is acid or has a high clay content. The probable cause is a combination of sodium ion dissociation from the dye functional groups and swelling and exfoliation of clay particles caused by the strong adsorption of large dye molecules. A similar effect has been observed by Laidlaw (personal communication) during field infiltration tests using Rhodamine WT. Structural breakdown is minimal using low concentrations (50–150 µg l^{-1}) of Lissamine FF.

RESULTS: BREAKTHROUGH CURVES

Dye pulses are known to lag with respect to chloride and tritium (White, 1977; Omoti and Wild, 1979), though the chloride arrival may be influenced by anion exclusion phenomena. The dye lag with respect to

Table VIII. Adsorption on clay and sand

	Soil	Soil : solution ratio	Initial dyee concentration (mg l^{-1})	Per cent dye adsorbed
Lissamine FF	4	1 : 2	200	36
	5	1 : 2	200	18
Rhodamine WT	4	1 : 2	50	95
	5	1 : 2	200	60

Table IX. Adsorption data for high dye concentrations, soil 3, 1 : 5 soil water ratio, 'A' and 'B' horizons

Dye	Soil	Initial concentration (mg l^{-1})	% Loss
Amino G Acid	3A	1000	3
	3B	1000	0·5
	3B	820	2
	3B	400	3
Lissamine FF	3A	1100	6
	3A	100	10
	3A	11·5	20
	3B	1100	4
	3B	100	4
	3B	11·5	50
Rhodamine WT	3A	1120	96
	3A	110	98
	3A	11	88
	3B	1120	97
	3B	110	95
	3B	11	92

Table X. Column adsorption data

Dye	Soil	Initial concentration (μg l^{-1})	% Loss	Batch*
Lissamine FF	1	200	40	81
	4	200	35	36
	5	200	24	28
Rhodamine WT	1	50	90	95
	4	50	99	95

*% Loss for equivalent concentration in batch adsorption tests.

Table XI. Desorption of the dyes

Initial solution concentration	$\mu g\,g^{-1}$ Adsorbed prior to desorption	$\mu g\,g^{-1}$ Adsorbed after desorption	% Desorption
Lissamine FF			
2000	3·50	3·12	10·8
1500	2·60	2·41	7
1000	1·63	1·38	15
500	0·80	0·70	12
200	0·334	0·30	10
Amino G Acid			
2000	3·0	2·9	3·0
1500	2·44	2·3	6·0
1000	1·6	1·42	11·0
500	0·7	0·5	28·0
Rhodamine WT			
2000	3·8	3·6	5·3
1500	2·85	2·8	2·0
1000	1·9	1·84	3·0
500	0·95	0·93	3·0

Table XII. Initial pH of dye solutions and pH of final solution in batch equilibriations of soil 3(A) (1 : 5 soil : water ratio)

	pH 1	Final pH
Amino G Acid		
1000 mg l^{-1}	2·0	3·4
820	3·2	6·0
400	5·4	6·1
Lissamine FF		
1100 mg l^{-1}	4·8	5·6
100	5·2	6·7
11·5	5·3	7·0
Rhodamine WT		
1120 mg l^{-1}	10·6	5·4
110	6·2	6·4
11	6·8	6·6

tritium is thought to be principally due to adsorption phenomena, rather than to diffusion or dispersion phenomena, dye breaking through later than other tracers in relation to initial adsorption losses in the column. Adsorption losses can thus be minimized by an initial primary trace and flushing.

Figure 7 shows breakthrough curves for a saturated soil column (soil 2) using 100 $\mu g\ l^{-1}$ Rhodamine WT. The solid line shows the dye breakthrough subsequent to water input and the pecked line water breakthrough subsequent to dye input. If dye dispersion is marked then the former can be expected to be dissimilar to the latter, with dye dispersing ahead of the water due to greater density or diffusion. In fact, the effect is present but negligible in terms of approximately 5% of a pore volume.

Lissamine shows a slight lag behind chloride during rapid breakthrough in soil 2, as shown in Figure 8. In an open conduit system (Figure 9) diffusion and dispersion for Lissamine FF and Rhodamine WT are

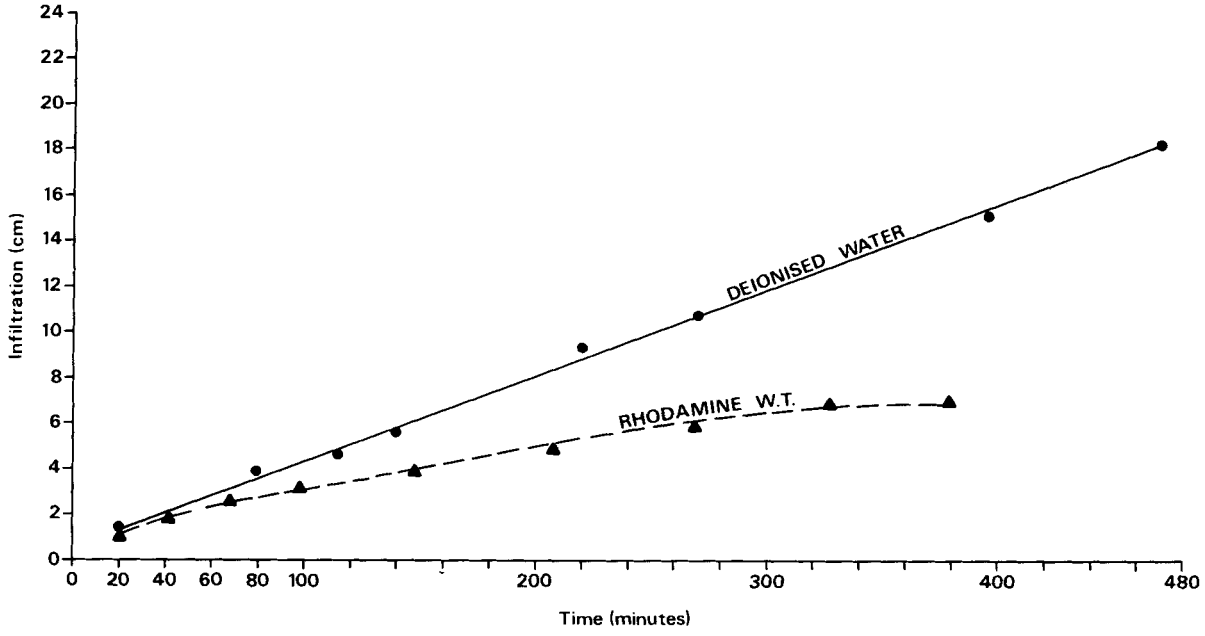


Figure 6. Reduction of infiltration with 50 μg l^{-1} Rhodamine WT in soil 4, clay, in a column

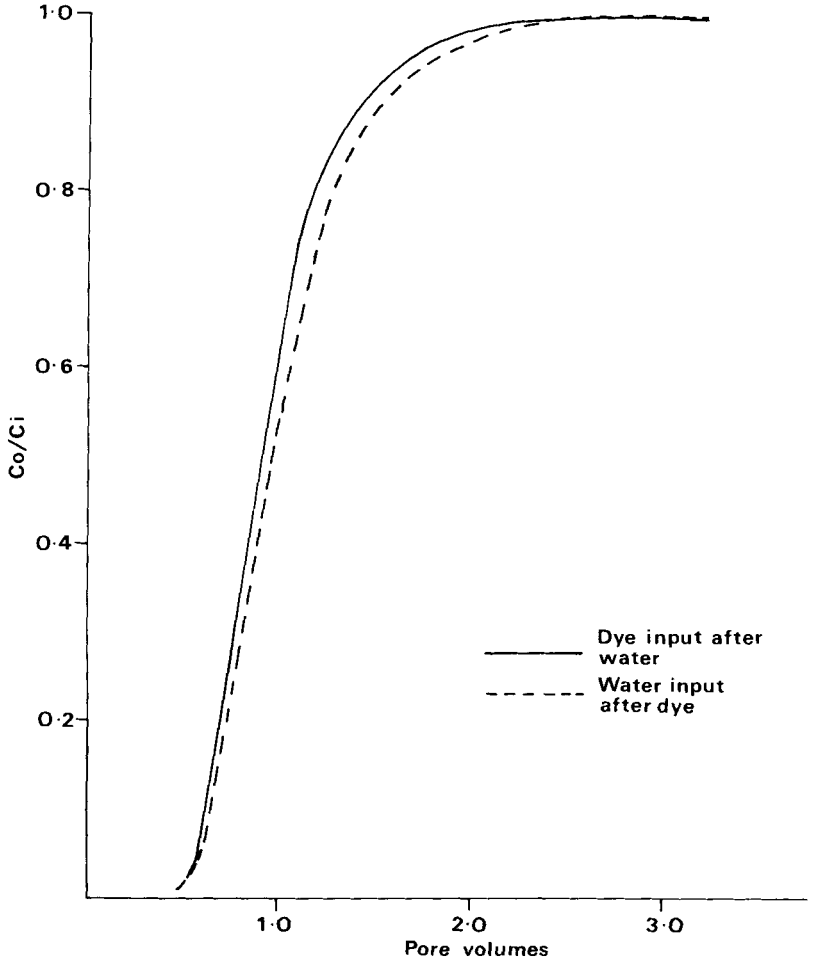


Figure 7. Dispersion of Rhodamine WT in column of soil 2

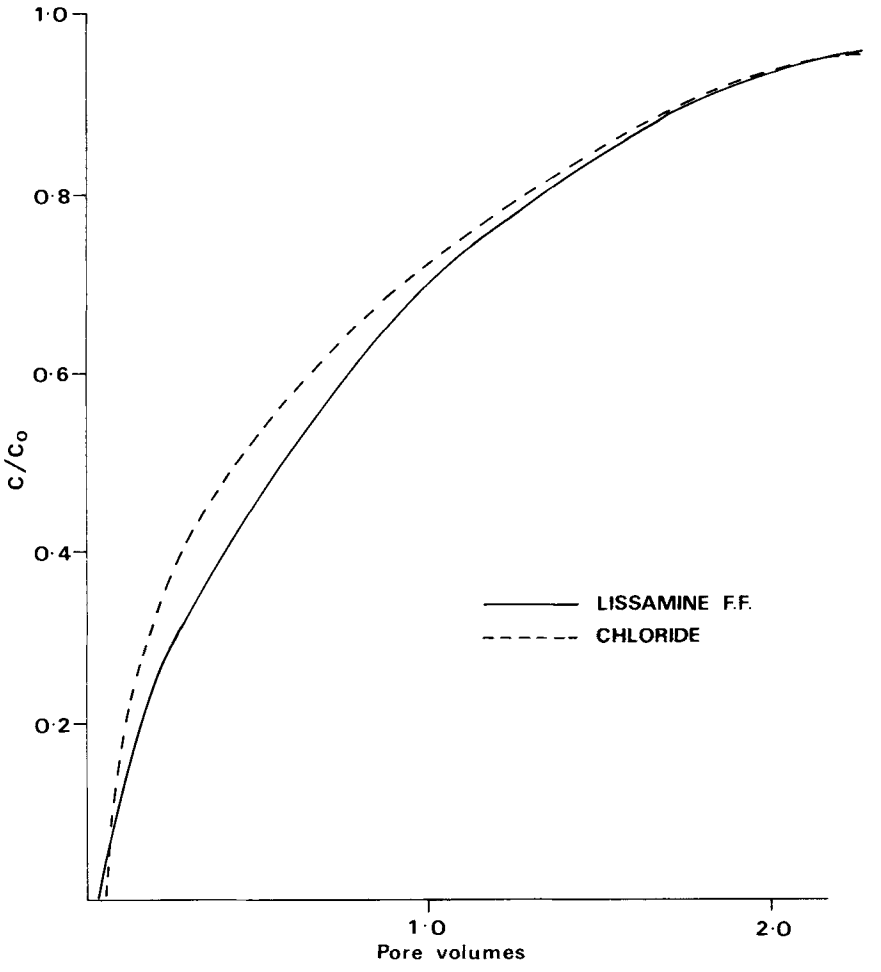


Figure 8. Breakthrough of chloride and Lissamine FF in soil 2 column

similar, with identical first times of arrival and peak concentrations. However, in a soil column (soil 2) with dye, followed by water input, Rhodamine WT exhibits a lag relative to Lissamine FF in relation to the former's lower resistance to adsorption, (Figure 10).

A priming and flushing routine was also used, involving passing at least 1 pore volume of dye solution through the column and then flushing with a dye free solution of an equivalent amount to a stable fluorescence reading. This procedure occupies the irreversible dye adsorption sites, minimizing subsequent dye adsorptive losses; subsequent dye traces can then be interpreted in terms of column characteristics, especially with a dye experiencing relatively low adsorption (Figure 11).

Data for breakthrough of slug injection of the three dyes in soil 1 are shown in Figure 12. The data are for breakthrough curves of inputs of 50 $\mu g\ l^{-1}$ Rhodamine WT, 100 $\mu g\ l^{-1}$ Amino G Acid and 150 $\mu g\ l^{-1}$ Lissamine FF after an initial adsorption priming run and washing. The soil hydraulic characteristics are strongly influenced by the existence of macropores and breakthrough is almost instantaneous (one pore volume = 3252 ml). The lag of Rhodamine WT is evident, even under the rapid flow conditions occurring.

APPLICATIONS AND CHOICE OF DYES

Rhodamine WT is highly adsorbed, loses fluorescence below pH 5·5, has a deleterious effect on soil structure and lags markedly in breakthrough curves. The background values for this dye are extremely

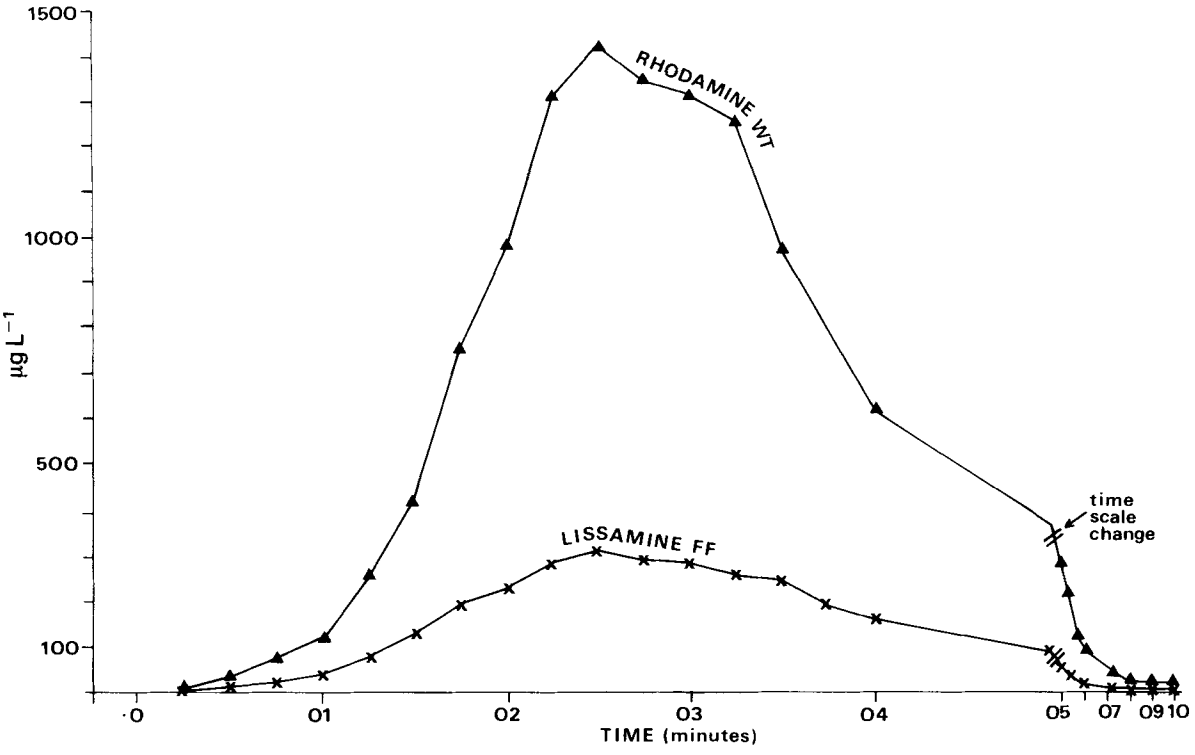


Figure 9. Dispersion of Rhodamine WT and Lissamine FF in an open conduit

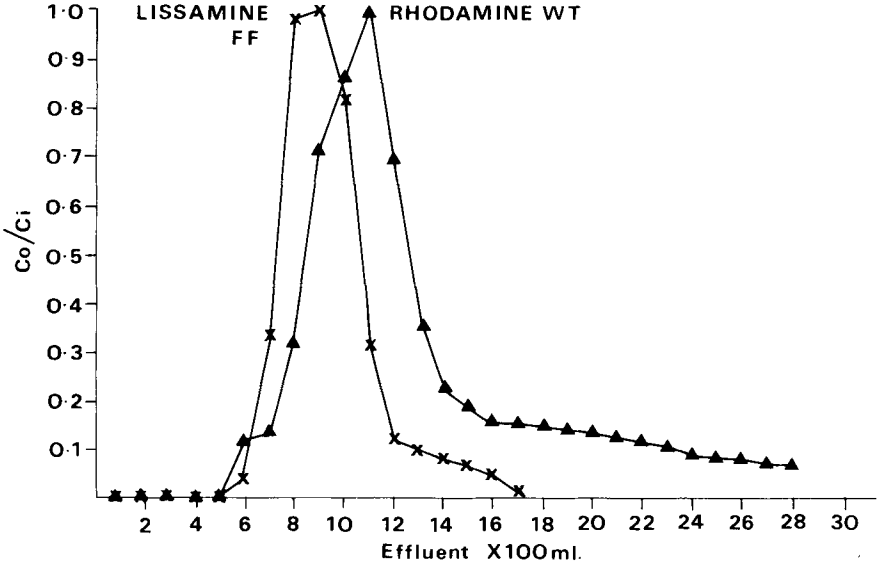


Figure 10. Breakthrough of Rhodamine WT and Lissamine FF in soil 2

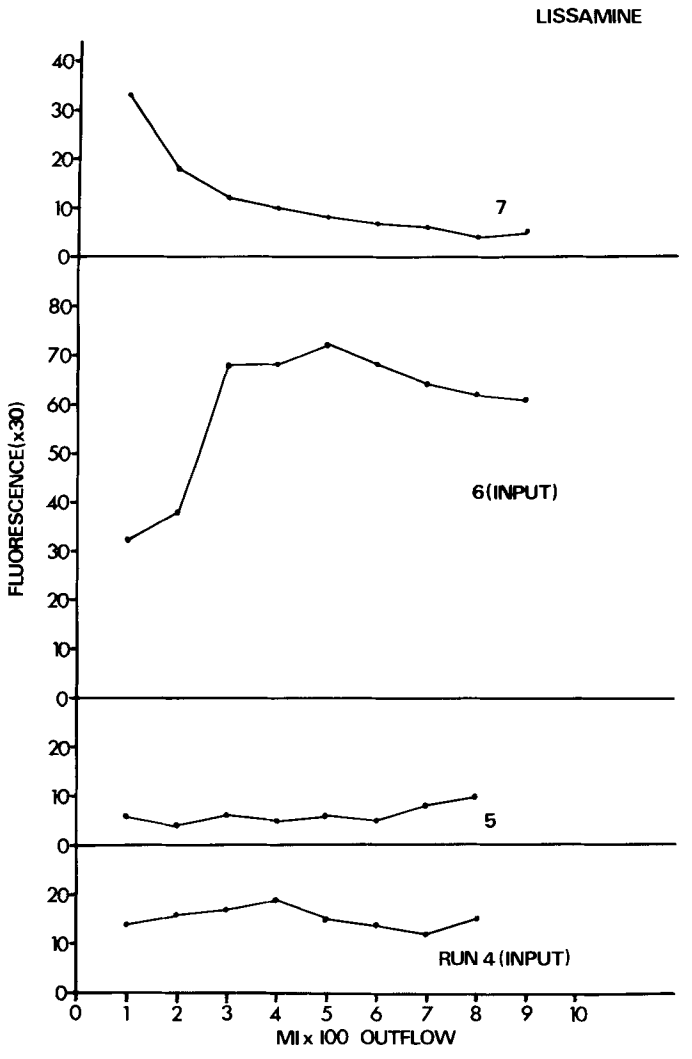


Figure 11. Priming and flushing of columns of soil 1, using 100 μg l^{-1} Lissamine FF; run 4 dye input, run 5 flushing, run 6 breakthrough curve, run 7 flushing. 1 pore volume = 3250 ml. (Fluorescence reading is direct reading from fluorometer, × 30 is 10 × more excitation light than × 3, providing a more sensitive scale for low concentrations)

low. It can be recommended for field tracing of soil water when simple identification of the presence of soil water in streams or groundwater is required as it is the most readily detected dye in terms of background fluorescence. This use of the dye should, however, be limited to traces over the order of around 1 m of soil only, as lag effects in response to rainfall events will become significant over larger distances, if timing of dye response is of interest. In allowing for adsorption, and with travel times of the order of a few days to a few hours, dosage should be of the order of 100–250 ml of dye solution (at approximately 20% w/v, 100 ml = 23·8 g l^{-1}). Using this dosage range field traces have been carried out successfully on soil 1, (Trudgill *et al.*, 1981, 1983) with detection of output concentrations in the range of 0·06–10 μg l^{-1}. The dye is not recommended for quantitative column work, because of its high adsorption and structural effects, but it may be of use if it is merely necessary to establish whether or not rapid flow paths exist in soils with high background fluorescence at the Lissamine FF and Amino G Acid wavelengths. It survives better in soils with low clay or low organic content.

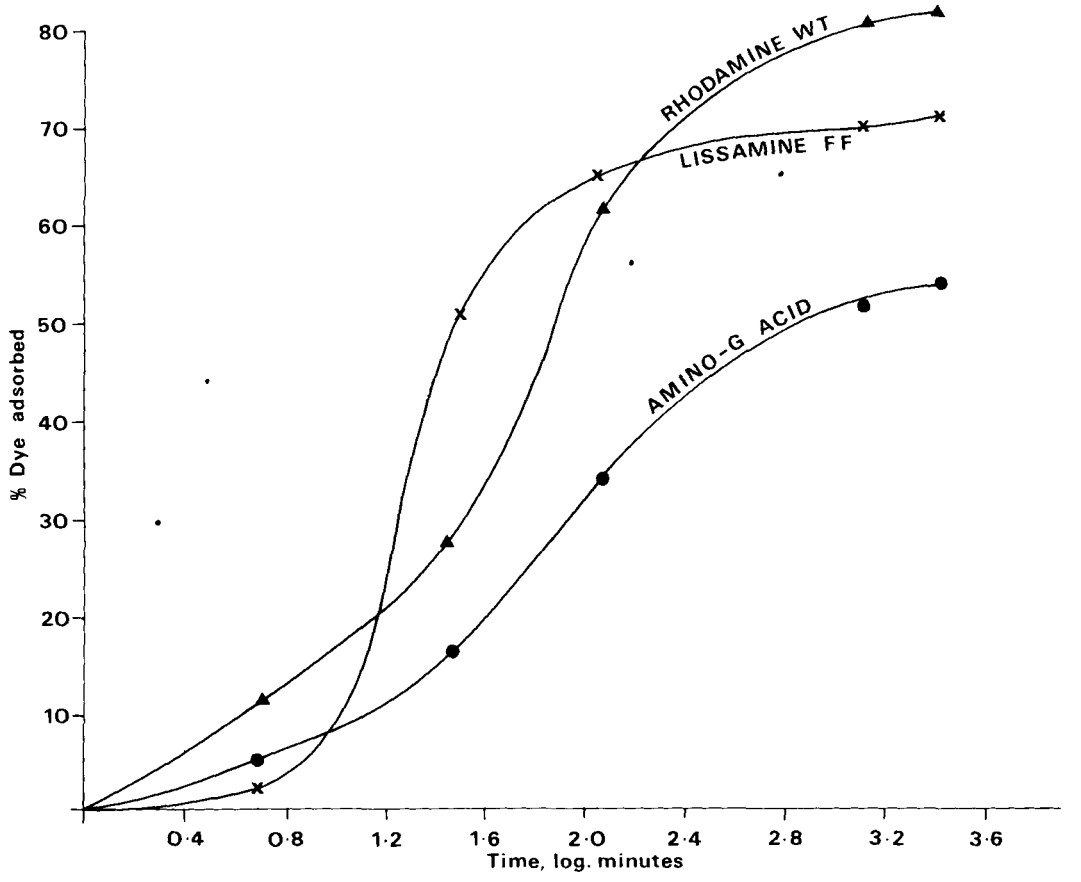


Figure 12. Breakthrough of simultaneously applied Rhodamine WT, Lissamine FF and Amino G Acid in soil 1, intact structure in soil column. Macropores present, 1 pore volume = 3250 ml

Lissamine FF and Amino G Acid are less adsorbed than Rhodamine WT but have much higher background values, especially in field situations. They are more suited to column work (Walker and Trudgill, 1983). Lissamine FF loses fluorescence below pH 3·8 and is more adsorbed in alkaline soils and is more suited for work in moderately acid soils. Amino G Acid loses fluorescence below pH 6·5 and is also more adsorbed in alkaline soils; it also has a high rate of photochemical decay.

In soil column work quantitative dye recovery analysis cannot be undertaken with accuracy because of adsorption, even if a priming and flushing routine is followed. However, if such a routine is followed, comparative analysis of breakthrough curves of soils of similar chemical characteristics (pH, CEC) but differing structure can be affected (Walker and Trudgill, 1983).

Continued leaching of a column with Rhodamine WT will result in structural collapse by clay dispersion (Figure 6); with Lissamine FF and Amino G Acid, cation loss and structural deterioration may be experienced if leaching is repeated more than about 5–10 times, the effect being most marked where the supply of cations is lowest; values for K_{sat} or first times of arrival can be used to monitor for this effect. When using Amino G Acid in column experiments in brightly lit laboratories it is advisable to use controls of standard concentrations stored with the samples under replicate conditions during collection and analysis to enable a photochemical decay correction factor to be applied. Concentrations for any dye used in columns should be kept as low as possible consistent with detection above background levels; concentrations which are frequently appropriate are 5 $\mu g\ l^{-1}$ Rhodamine WT, 100 $\mu g\ l^{-1}$ for Amino G Acid and 150–200 $\mu g\ l^{-1}$ for Lissamine FF.

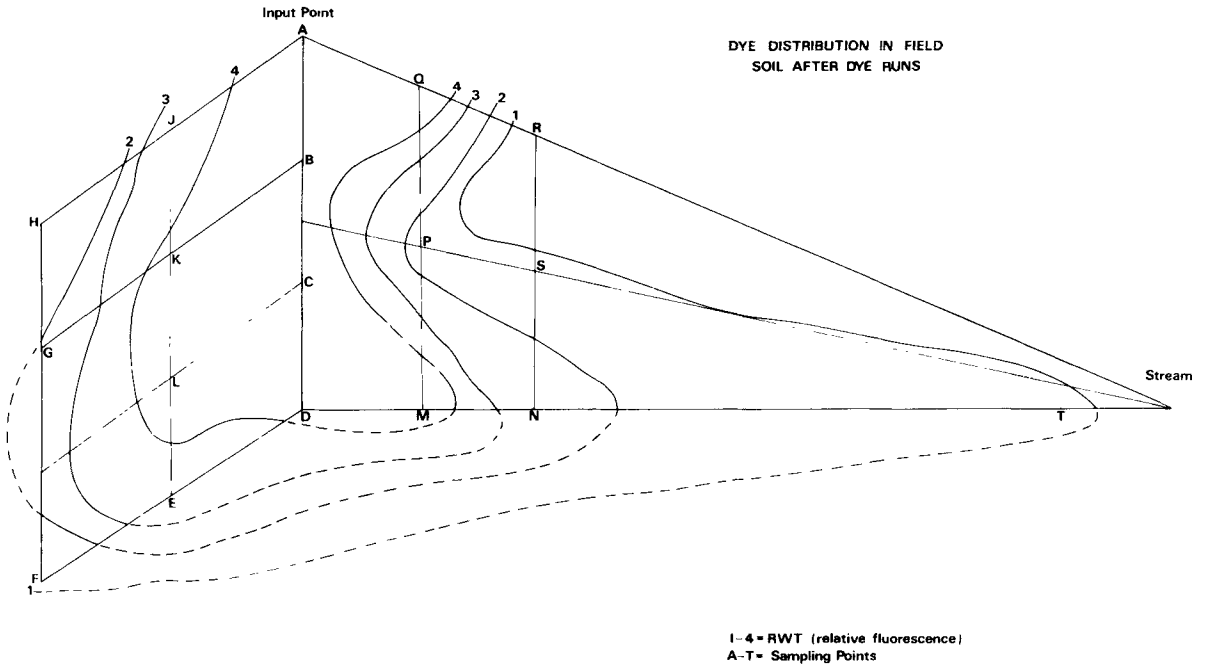


Figure 13. Section of stream bank subsequent to dye tracing. A–D–T is onthogonal to the stream and H–A–D–F is parallel to the stream. H–F and A–D is 30 cm, D–T is 60 cm

The adsorptive properties of the dyes can be utilised in pathway staining, at both large and small scales. Figure 13 shows data for relative Rhodamine dye concentration for an excavation orthogonal to a stream bank and for a face parallel to the stream subsequent to surface dye emplacement and detection at the stream bank. An adequate demonstration of overall flow routes was obtained from a soil sampling grid and elution of samples. Both vertical and horizontal movement appears to be involved. With lateral transfer (PM–SN–T) subsequent to vertical movement (A–B–C) leaving top soil (RS–T) dye free. The use of micro-samples in relation to soil structural pathways can also be undertaken, as described in detail by Smettem and Trudgill (1983).

Fluorescent dyes thus have many limitations as soil water tracers, but they can have applications. They are highly useful for the identification of the presence of soil water in groundwater and streamwater systems. Thus, for the identification of the existence of flow routes, that is, the specification of links between systems, fluorescent dyes have many field applications. If establishment of *linkage* is more important than establishing travel times then any one of the tree dyes may be used, the choice being based on an assessment of background fluorescence levels, Rhodamine WT being useful where backgrounds are high. In establishing *pathways* for pollutants, adsorbed dyes may be of use as they may mimic the behaviour of other sorbed reactive solutes. If *travel times* are of interest, this will be better replicated with the least adsorbed dyes and time of first arrival should be used as a more reliable index of water travel speed rather than time to peak concentration. Lissamine FF is the most useful dye for this work. Lissamine FF can also be recommended with caution for dye column work. Amino G Acid can be used in either case but may represent greater operational difficulties in respect of its photodecomposition.

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