

# pH Dependence and Unsuitability of Fluorescein Dye as a Tracer for Pesticide Mobility Studies in Acid Soil

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**Abstract** The mobility of fluorescein and bromide used as tracers in packed soil columns was investigated. Five different soils were used in two application methods: soil surface application and soil incorporation, both of which simulate accepted methods of soil application of termiticides to prevent structural infestation. The breakthrough of bromide and fluorescein in column eluates were measured. The absorbance of fluorescein at 492 nm was pH dependent, and proper adjustments were made after measuring the eluate pH. Although high recoveries of bromide from the soil columns were observed, the breakthrough was different among the soil types, indicating that bromide behaves differently in different soils. Recovery of fluorescein, a weak acid, varied depending upon the pH of the soil used, and was only observed in the eluates of two of the five soils tested. Soil treated with bromide and fluorescein followed by soaking extraction showed high recovery of bromide but low recovery of fluorescein, except for in the most alkaline of the soils tested. If fluorescein is used as a conservative tracer in pesticide soil mobility studies, mobility can be underestimated in acidic soils because the active ingredient might travel more quickly than does the fluorescein.

**Keywords** Bromide · Fluorescein · Insecticide mobility · Soil · Tracer

## 1 Introduction

The mobility of insecticides in soil is important in product performance and environmental protection. Mobility of imidacloprid likely contributed to the loss of termiticidal activity in treated soil (Peterson 2007). Studies of pesticide deposition commonly use easily detected tracers in the application solution (for examples, see Barber and Parkin 2003; Zhu et al. 2005; Davis and Kamble 2008). Flury and Wai (2003) provide a review of tracers in soil hydrology. A perfect tracer moves with the soil water without sorption to soil particles, does not degrade during the course of the study, has a low background occurrence, is insensitive to changes in pH and other soil conditions, is easily detected at low levels with low interference from other substances and is non-toxic (Flury and Wai 2003).

Fluorescent dyes have been used in water tracer studies for at least 130 years (Flury and Wai 2003) and continue to be an important technique for describing water movement. More recent studies have used fluorescent dyes: to examine the role of macropores in soil water infiltration (Weiler and Naef 2003), to detect the deposition of a pesticide on the soil surface (Barber and Parkin 2003), to determine

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flow velocity in a brackish water sandfill (Chua et al. 2007), as an indicator of tetrachloroethylene movement in soil (Ghanem et al. 2003), as a tracer for gasoline storage tank leaks (Mushrush et al. 2001), to examine the distribution of a soil-injected termiticide solution (Davis and Kamble 2008), and to model urban runoff of rainwater (Ammann et al. 2003). Tracers in laboratory studies are useful to compare the effects of soil properties on solute movement, as well as to predict mobility in field applications. A tracer that is retained by the soil will underestimate solute mobility if the solute of interest is more mobile than is the tracer.

Fluorescein (color index number 45350, called uranine in Europe) is one such dye used in many studies (for example, Rahe et al. 1979; Mushrush et al. 2001; Ammann et al. 2003; Ghanem et al. 2003; Chua et al. 2007). The properties of fluorescein have been extensively studied (Feuerstein and Selleck 1963; Smart and Laidlaw 1977; Omoti and Wild 1979; Sabatini and Austin 1991; Kasnavia et al. 1999; Sabatini 2000; Smith and Pretorius 2002; Flury and Wai 2003) and its movement through sand has been modeled (Rahman et al. 2004). Although not always the most suitable dye available, fluorescein is often chosen due to ease of detection, lack of sorption to mineral components, low toxicity, and low cost (Smart and Laidlaw 1977). Although sometimes not recommended due to photodegradation and high background fluorescence (Feuerstein and Selleck 1963; Smart and Laidlaw 1977), sorption to the soil and low mobility are not noted as disadvantages of fluorescein (for example, Kissel et al. 1973). In fact, several studies report high recovery of fluorescein (Smart and Laidlaw 1977; Sabatini and Austin 1991; Chua et al. 2007) and Smith and Pretorius (2002) report that fluorescein can be used as a conservative tracer (near 100% recovery) when the UV absorbance of the molecule is corrected for solution pH. Similarly, Zhu et al. (2005) reported increased fluorescence detection at higher pH values. In only one study did fluorescein not perform as expected (Rahe et al. 1979).

Several inorganic ions may be used as tracers, with bromide being the most common (for example van Staden 1987; Ndongo et al. 2000; Ammann et al. 2003; Flury and Wai 2003; Clay et al. 2004; Petersen et al. 2004; Hamada et al. 2005), although chloride (Kissel et al. 1973) and chromium (Watson 1969) are sometimes used. It is believed that anions make ideal tracers due

to their repulsion by negatively charged soil particles, a phenomenon known as ion exclusion (Flury and Wai 2003). Bromide is suitable as a tracer due to low background levels in the environment, its invulnerability to degradation and to adsorption (although it will adsorb to soil at low pH), and low toxicity at the concentrations used (Flury and Wai 2003).

During the conduct of a previous study (Peterson 2009), the use of fluorescein dye was attempted as a tracer to determine the maximum depth of penetration of termiticide solutions applied to the soil surface. Preliminary tests of the method by using a readily available soil (used in the current study as G soil, see below) provided excellent results, with nearly 100% recovery of the fluorescein tracer. When conducting the experiment with a different soil, however, fluorescein was not detected at all. Examination of the other soil types used in that study revealed that very little fluorescein was recovered in most of the soils. In the current paper, soaking extractions and mobility studies of fluorescein and bromide in five soils by using two application methods in packed soil columns were conducted. As will be seen, fluorescein is unsuitable as a tracer in this type of study, especially in acidic soils.

## 2 Experimental

### 2.1 Soils

Five soils were collected and their properties are summarized in Table 1. U soil was a loamy sand collected from the USDA Forest Service Termiticide Testing Program site in Union County, SC in September, 2005. D soil was a silt loam collected from the John Starr Memorial Forest near Dorman Lake in Oktibbeha County, MS in July, 2005. P soil was a sandy loam soil collected from Parker Sand and Gravel Co., Lowndes County, MS in July 2005 and is of a type approved by local building authorities for use as construction fill. G soil was a sandy loam collected from the Mississippi Agriculture and Forestry Experiment Station greenhouses in July 2006. S was play sand purchased from a commercial retailer in July 2006. All soils were air-dried and clumps were broken apart with a hammer and then sieved (2.3 mm mesh size) to remove stones, roots, and other coarse materials.

**Table 1** Soils and soil properties

	Soil	Texture	% Silt	% Sand	% Clay	pH	OM <sup>a</sup>	CEC <sup>b</sup>	Field capacity <sup>c</sup>
	U	Loamy sand	19.75	77.75	2.50	5.2	1.41	4.10	16.6
<sup>a</sup> Percentage organic matter	D	Silt loam	50.00	42.50	7.50	5.3	2.43	15.20	35.9
<sup>b</sup> Cation exchange capacity	P	Sandy loam	40.00	55.00	5.00	5.1	0.52	6.00	21.2
<sup>c</sup> Maximum percentage soil moisture by weight	G	Sandy loam	14.75	75.25	10.00	7.8	1.49	NA <sup>d</sup>	20.0
<sup>d</sup> Data not available	S	Sand	5.25	94.75	0.00	6.5	0.13	NA <sup>d</sup>	7.9

The Mississippi State University Extension Service determined the texture, silt, sand, clay, pH, organic matter, and cation exchange capacity of each soil. The pH of each soil was measured a second time in the laboratory by using a 1:1 slurry (by weight) of the soil in water and measuring by using the same pH meter as used in the test. The water-holding capacity of each soil was estimated by placing 50 g oven-dried soil (100°C overnight) in a Buchner funnel fitted with filter paper to prevent loss of soil. Distilled water, enough to saturate the soil, was added and then a 34.5 kPa (5 psi) vacuum was applied until water was no longer observed dripping from the funnel. The soil was re-weighed and the water content was calculated (Cassel and Nielsen 1986).

## 2.2 pH Quenching of Fluorescein Absorbance

Quenching of fluorescein UV absorbance in response to pH has been reported in the literature (Diehl and Horchak-Morris 1987; Smith and Pretorius 2002), especially at pH values lower than about 7.0. To correct for the pH of the eluates collected in the current study, solutions of known concentrations of fluorescein (nine concentrations from 0.3 to 11.3 ppm) were prepared in solutions from pH 4.5 to 8.0 at 0.5 pH increments. Standard curves for each solution pH were constructed based on the linear relationship between the known fluorescein concentration and the UV absorbance.

## 2.3 Soil Soaking Extraction

The recovery of fluorescein and bromide was examined by soaking extraction from treated soil. The fluorescein solution used was Bright Dyes™ FLT Yellow/Green Liquid concentrate (Kingscote Chemicals, Miamisburg, OH, USA), which contained 7.5% disodium fluorescein salt that was 40% to 41% fluorescein and 59% to 60% sodium salt. Portions

(20±0.5 g) of oven-dried soil (100°C overnight) of each type were placed in jars and 2 mL of a mixture of 22.6 µg/mL fluorescein (200 µL/L of a 0.3 M stock disodium fluorescein solution) and 0.1 M sodium bromide (11.9 g/L NaBr) was added to the soil (0.14 µmol fluorescein and 0.2 mmol sodium bromide). Quenching of fluorescein UV absorbance by sodium bromide at 492 nm was not significant (data not shown). Distilled water (2 mL) was used for the control groups. The soils were thoroughly agitated and left to sit for several hours before extraction. For soil extraction, 20 mL water was added to each jar and the jars were shaken for 2 h at 200 rpm. The soil was allowed to settle and the supernatant was filtered by using Whatman GFA glass fiber filters. Fluorescein content was determined by measuring UV absorbance at 492 nm on a plate reader (Thermo Multiskan MCC/340). The response of the plate reader to fluorescein was linear from 0.03 to 34 µM. Bromide concentration was determined by using a bromide-specific electrode (Orion 9635 BNWP) and meter (Thermo 720A+). Response of the meter to bromide concentration was linear between 2.0 and 10,000 µM. The test had three replications in a completely randomized design. The results were analyzed by using the general linear model on SAS (SAS Institute 2001).

## 2.4 Soil Column Mobility

The mobility of fluorescein and bromide in the five different soils by using two application methods was examined in packed soil columns. Each column consisted of a plastic cone (21.5 cm tall, 4.1 cm ID at the top and 2.5 cm ID at the bottom) fitted with glass wool at the bottom to prevent loss of the soil.

For application to the soil surface, 140 g of each soil, which fills the column to a depth of approximately 15 cm, was added to the respective columns. To the top of each cone was added 5.5 mL of the

fluorescein/bromide mixture described in Section 2.2. Distilled water (5.5 mL) was used for the control groups. Two portions (30 mL each) of distilled water were added to saturate the soil and the eluate was collected.

For incorporation into the soil, 14 mL of the fluorescein/bromide mixture was added to 140 g soil (10% soil moisture by weight) in re-sealable plastic bags. The soil was mixed thoroughly and then was placed in the columns.

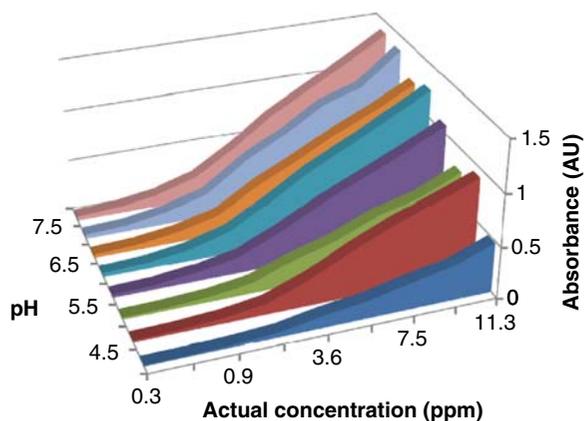
Each column for both test methods received distilled water (30 mL) daily and the eluate was collected in a beaker. The fluorescein content of each eluate was measured (in absorbance units, AU) by using the plate reader. Bromide ion concentration was measured by using the bromide-specific electrode and meter. Both instruments are described in Section 2.3. The pH of each collected eluate was measured and then was rounded to the nearest 0.5 pH unit. The fluorescein concentration was determined by fitting the measured AU to the standard curve corresponding to the pH of each eluate. Collection of eluates continued until both the bromide and the fluorescein were exhaustively removed from the soil or when it was apparent that the fluorescein would not elute in a reasonable number of washings (here, 11). A preliminary study determined that fluorescein applied to G soil did not significantly degrade or become significantly bound to the soil for at least 12 weeks (data not shown).

Both column tests each had three replicates in a completely randomized design. The data were analyzed by using mixed analysis of variance for repeated measures on SAS (SAS Institute 2001).

### 3 Results and Discussion

#### 3.1 pH Quenching of Fluorescein UV Absorbance

The results of the pH quenching tests are in agreement with the results of Diehl and Horchak-Morris (1987), where the UV absorbance of solutions of known fluorescein concentration increased with increasing pH, and there was not a significant increase in absorbance above pH 7.5 (Fig. 1). The standard curves for solutions at each pH were calculated separately.



**Fig. 1** Absorbance of fluorescein of known concentrations at different pH values

#### 3.2 Soil Soaking Extraction

Table 2 presents the percentage recovery and standard error of each tracer after a single soaking extraction of each soil type. When negative recovery values for fluorescein resulted from correction for background absorbance (i.e. less absorbance at 492 nm was measured in the treated eluate versus the control), the value was set to zero for statistical analysis, but were included in the values reported in Table 2. The general linear model detected significance due to soil for both tracers (bromide:  $F=3.74$ ,  $df=5$ ,  $P=0.0284$ ; fluorescein:  $F=288.75$ ,  $df=5$ ,  $P<0.0001$ ). The statistical analysis was conducted on the amount (in millimoles or micromoles) of each tracer recovered, and not on the percentage recovery, and therefore no transformations of the data were necessary.

The lowest single-washing recoveries of bromide were seen in G and D soils. As will be seen in Section 3.3.1, G and D soils had a greater affinity for bromide, requiring a greater number of elutions to remove the bromide from the column.

**Table 2** Percentage recovery (standard error) of bromide and fluorescein in a single soaking extraction

Soil	Bromide	Fluorescein
U	89.0 (1.6)	-8.1 (1.2)
D	85.2 (0.4)	8.6 (7.1)
P	90.0 (0.6)	-8.5 (0.6)
G	84.4 (0.3)	119.6 (0.9)
S	90.9 (2.6)	0.2 (0.6)

Except for G soil, very little fluorescein was recovered in a single extraction (Table 2). Extracts of G soil had the green color expected, but with 119% recovery it seems that there were interfering co-extractives in the soil.

### 3.3 Soil Column Mobility

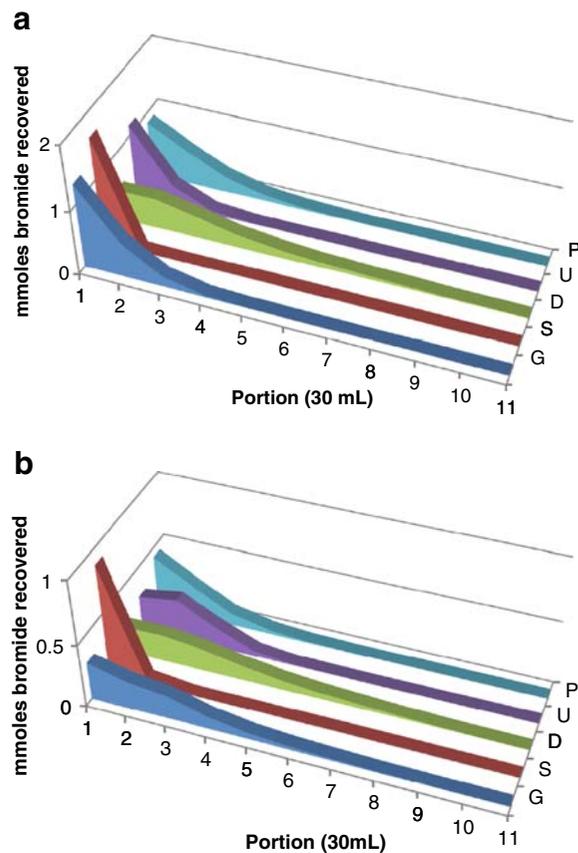
#### 3.3.1 Bromide Recovery from Soil Columns

When incorporated into the soil, bromide behaved differently in different soils (Fig. 2a), with a significant interaction between soil and elution number ( $F=56.78$ ,  $df=40$ ,  $100$ ,  $P<0.0001$ ). Beyond the fourth elution, there was no difference between the soils, except for D soil. The breakthrough curves for P, U, and G soils were all similar. D soil behaved differently than the others, with significant tailing of bromide out to the ninth elution. Of the applied dose, 99% was recovered by the second elution for S soil,

by the third for U soil, by the fourth for P and G soils, and by the eighth for D soil.

Similar patterns were observed for bromide recovery following surface application (Fig. 2b). There was a significant interaction between soil and elution number ( $F=56.26$ ,  $df=36$ ,  $90$ ,  $P<0.0001$ ). The long tailing of very low levels of bromide was responsible for the significant interaction; soils differed the most before the fifth elution, and did not differ much after this point. The data indicate that 99% of the applied bromide was recovered by the third elution for S, by the fifth for U and P soils, the seventh for G soil and the eighth for D soil.

Although bromide had very good tracer characteristics in this study (high recovery, low background interference and reliable detection by using a bromide-specific electrode), D and G soils appeared to retard the movement of bromide. This was consistent with the results of the soil soaking extraction, where less bromide was recovered from D and G soils following a single extraction. If bromide were retained in D and G soils relative to the other three (i.e., it does not act as a conservative tracer), it would require more elutions from the column and more extractions of the soil to recover 99% of the applied bromide. Others (Begin et al. 2003 and references therein) have noted bromide retention in different soil types, but the authors of that study did not pursue the reasons for this, nor could the reasons be deduced from the soil properties reported. Here, the higher field capacity and percentage soil organic matter of D soil relative to the other soils (Table 1) are potential factors, although other factors, such as porosity, bulk density, and tortuosity (which were not measured) might play a part. D soil was very spongy to the touch, even at low moisture, whereas the other soils were noticeably sandy.

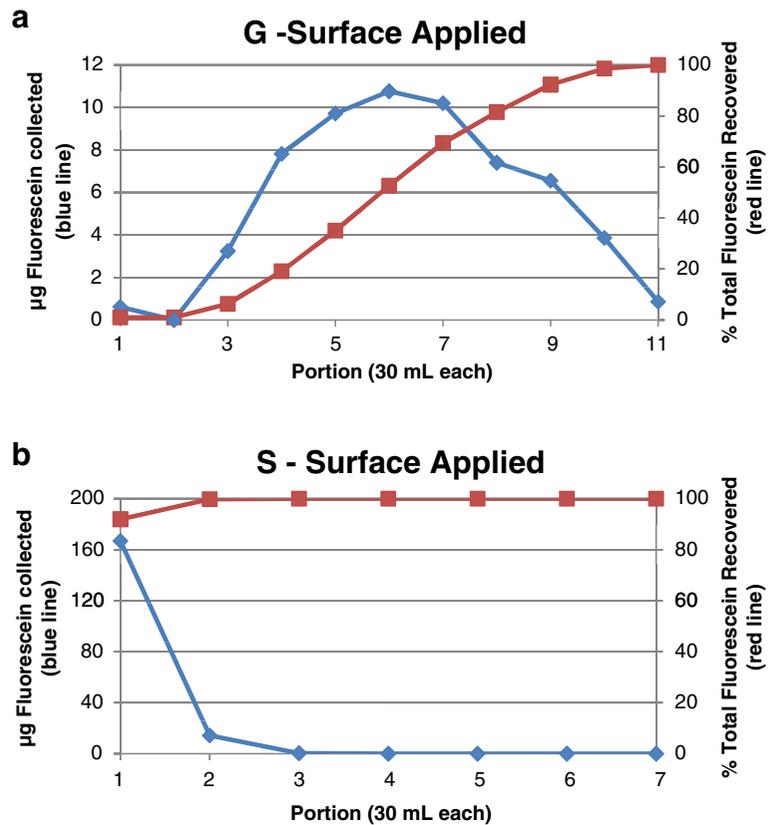


**Fig. 2** Recovery of bromide at each elution following **a** soil incorporation and **b** surface application

#### 3.3.2 Fluorescein Recovery from Soil Columns

In soil surface application, fluorescein was unsuitable as a tracer. No fluorescein was recovered in three of the five soils tested, and in the soils in which it was recovered (G and S soils) the breakthrough curves are clearly different (Fig. 3a and b). For all soils together, there was a significant elution number by soil interaction ( $F=772.45$ ,  $df=36$ ,  $90$ ,  $P<0.0001$ ). Only in S soil was a high amount of the applied fluorescein recovered, and this was in the first three elutions. This

**Fig. 3** Recovery of fluorescein in column eluate of G and S soils following surface application



is consistent with the work of Chua et al. (2007) who conclude that fluorescein does not interact with sand. The breakthrough of fluorescein in G soil was delayed relative to S soil (Fig. 3). The fluorescein did not begin to appear until the second elution, after which the eluate concentration increased to a maximum at the sixth elution, and then declined steadily to the 11th elution.

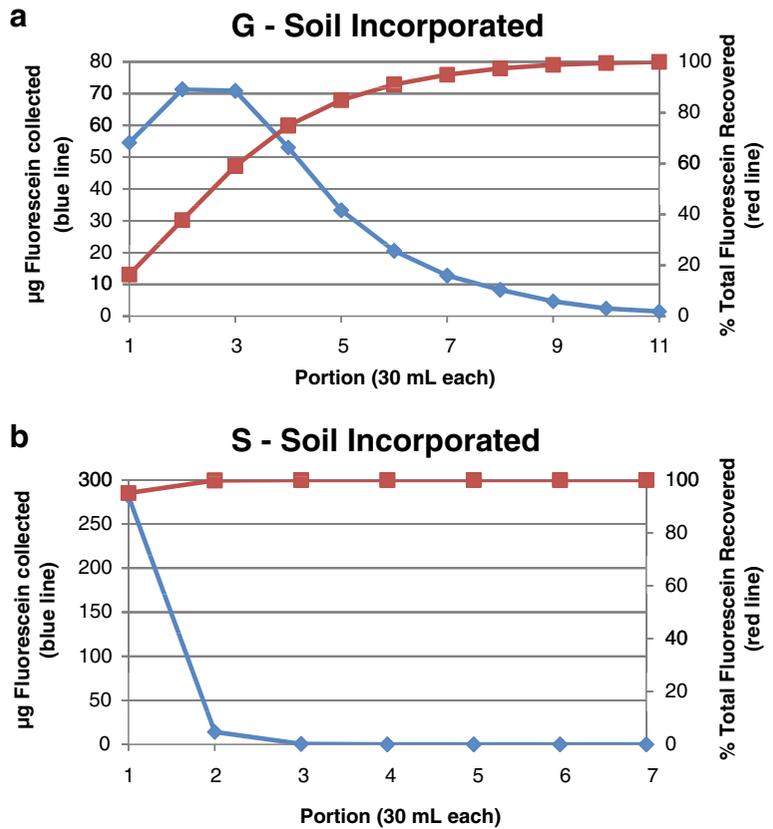
The breakthrough curves of fluorescein when incorporated into the soil (Fig. 4) were similar to those seen in the soil surface application. No fluorescein was recovered from U, D, or P soils. There was a significant interaction between soil and elution number ( $F=1930.44$ ,  $df=40$ ,  $100$ ,  $P<0.0001$ ). As with soil surface application, all of the fluorescein recovered from S soil was recovered in the first three elutions (Fig. 4b). Recovery from G soil began in the first elution, increased in the second and third elution, and then declined steadily to the tenth dilution (Fig. 4a).

Because so little fluorescein was recovered from either the soaking extractions or the soil columns, it is difficult to draw meaningful conclusions regarding how

soil characteristics affect fluorescein retention. Fluorescein is a weak acid, and as such has higher soil sorption in acidic soils than in alkaline soils. When the soil pH is below the pKa of the molecule, the protonated forms predominate and the proportion of the molecules bound to soil particles increases. This same trend was reported for the weakly acidic herbicide mesotrione (Dyson et al. 2002). Percentage fluorescein recovery in the soil columns in the current study was the highest in the soils with the highest pH values (G soil, pH 7.8 and S soil, pH 6.5) and fluorescein was not recovered in the more acidic soils (pH 5.0 to 5.3).

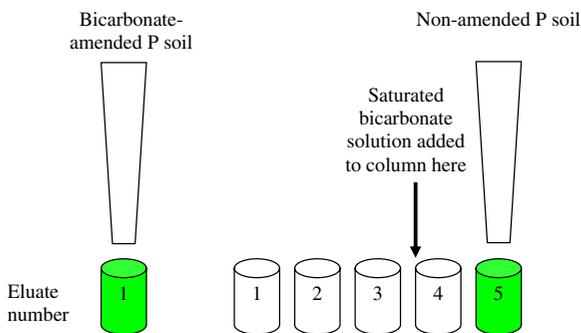
The effect of pH on fluorescein retention was illustrated by the following demonstration (Fig. 5). Two columns with P soil were constructed as described in Section 2.3: column A contained sodium bicarbonate-amended P soil (10% sodium bicarbonate by weight) and column B contained P soil with no added bicarbonate. The fluorescein/bromide mixture (30 mL) was applied to the soil surface of each column, and both columns were eluted several times with 30 mL distilled water. Fluorescein was visually

**Fig. 4** Recovery of fluorescein in column eluate of G and S soils following soil incorporation



observed in the first elution of the bicarbonate-amended column, while it was not observed in three elutions of non-amended soil. Following the third elution, 30 mL portions of a saturated sodium bicarbonate solution (pH=8.0) were added to the non-amended soil column, the eluates of which as yet contained no observable fluorescein. Fluorescein was observed in the second saturated bicarbonate eluate, the first eluate presumably containing the column void

volume. Clearly, fluorescein is retained by the soil, and increasing the pH of the application water with sodium bicarbonate released fluorescein from the soil.



**Fig. 5** First observation of fluorescein in eluate (*green*) of bicarbonate-amended and non-amended soil

The results reported here show that fluorescein, a weak acid, violates the criterion of pH insensitivity for a desirable tracer as reported by Flury and Wai (2003). Any turbidity in collected eluates would further limit the usefulness of fluorescein as suspended materials interfere with UV absorbance measurements; filtration or centrifugation of turbid samples would be necessary. Fluorescein should be used with caution, or not at all, in acidic soils. Amending the soil prior to application or increasing the pH of the applied water would allow release of the fluorescein, but at some point the researcher alters the test conditions to such a degree that he or she is no longer simulating relevant situations. If pH amendment affects the behavior of the tracer then the material being traced may be similarly affected. Also, adding steps and procedures adds to the time and cost of a study, and may not be worth the effort if other techniques are available.

Although bromide might not be a suitable tracer in other applications, it provided satisfactory results (simple and sensitive detection, high recovery, and ease of use) in this study without soil or elution water modification. Bromide behaved more or less as expected for a conservative tracer, although there were some differences due to soil. Analytical equipment used to detect bromide is no more expensive or complicated than that used to detect fluorescein (unless of course visual fluorescein detection is used, in which case the results are not quantitative). It can be argued, however, that fluorescein is sufficiently cheaper than bromide for treating large bodies of water, and would not increase salinity, which could be harmful to aquatic life.

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