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Concern for the propriety of carbon cycling in the food chain is currently promoting a rapid increase of interest in the practical significance of organic matter cycling in soils (Lupin 1977; Flaig et al. 1977). Additionally, the primary role that organic matter and humus play in maintenance of soil productivity and the losses attendant upon their removal are becoming widely recognized (Eckholm 1976; Aina 1979).

Concomitant with this renewed interest in the fate of organic materials is the need for laboratory techniques that enable rapid evaluation of the condition and quality of humifying organic matter, particularly in view of composting and soil recycling.

There is a wide array of laboratory methods for evaluating organic matter and humus include carbon:nitrogen and photometric analysis as well as more sophisticated techniques such as infrared spectrometry and pyrolysis— gas chromatography (Schnitzer and Khan 1972). Despite their usefulness and adaptability, many modern methods are expensive and time consuming, require a fairly high level of technical sophistication, and may give results that are difficult to translate into practice. It may also be apparent that strictly analyzing organic content per se, as commonly practiced with soils, will not provide information regarding its condition or quality, information that may be indispensable to interpreting the fate of added organic material (Brinton 1979). For example, amending an acid, podzol soil with organic matter may involve entirely different pathways of decomposition and fixation— implicating soil structure and productivity—than may be the case in a soil of calcareous or alkaline nature (Martin et al, 1979; Pauli 1967; Khantulev et al. 1968).

THE CIRCULAR CHROMATOGRAPHIC METHOD

The objective of this paper is to report on the technique and use of a paper chromatographic method, which is a simple and fairly rapid qualitative tool for evaluating humic extracts of compost and soil. Developed by Swiss biochemist Pfeiffer in the late 50's and used in the early natural farming movement in the states in the early 60's, it has been largely overlooked recently. The circular technique of paper chromatography is one in which the substances to be separated (in this case soluble humus compounds) are allowed to travel radially from the centre of a filter-paper disk. The material is applied to the centre of the disk from which a small sector is cut out and folded down to allow it to dip into the eluting solvent.

Though the method has been applied to qualitative analysis of plant material and food products (Pfeiffer 1960; Sabarth 1962; Smith 1973), our discussion will be confined to its use in investigating humification in composts and soils as reported by Pfeiffer and others (Koepf 1964; Smith 1973; Stickelberger 1975; Hertelendy 1973; Brinton 1979, 1983; Hassold-Piezunka 2003).

To date, efforts in evaluating the chromatograms have largely revolved around drawing inferences based upon knowledge of other features of the material being examined. For example, Pfeiffer (1959) and Sabarth (1962) observed that chromatograms performed on organic material during a composting process would change in accordance with the characteristic breakdown and subsequent stabilization of the organic material. This suggested that the chromatograms could be applied as a complement to analytical data to give a characteristic pictorial representation of the degree of ripening of the humifying material. Similar relationships were drawn with soils, particularly by comparing acid soils with near-neutral soils of varying organic content (Koepf 1964). These efforts led to establishing various chromatogram patterns as typical of a certain type of humus condition, such as "acid, carbonaceous," "stable, matured," and "poor, mineralized" (Pfeiffer 1959; Koepf 1964). Notwithstanding this work, there is an evident paucity of information proving a link between the appearance of the chromatograms and the humus condition—the latter not having been strictly defined—nor is there information that relates to the nature of the chemical reactions that occur at the time of performing the paper chromatography.

Our laboratory has made use of this particular chromatographic method for a number of years, chiefly in conjunction with soil and compost testing. As an additional basis for this paper we draw on preliminary results from a research project undertaken more recently in which we explored more closely some of the problematic areas related to methodology and interpretation. Using 20 randomly selected soils primarily from the northeastern United States and Canada, we have attempted to draw correlations between the appearance of the chromatograms of these soils and some of their chemical and biological features. In particular, we looked at such aspects as mineral content, pH, mobility of humus, and the humic—fulvic acid ratio of the humus. Humus mobility, defined as the solubility of soil organic matter, is highly indicative of past soil-forming processes that may or may not lead to the complexing of newly formed humus molecules with inorganic soil constituents (Springer 1949; Kononova 1966). The humic : fulvic (Ha:FA) acid ratio of humus is similarly characteristic of soil type and is indicative of a soil's tendency to retain organic matter in a more mobile, less decomposed state (fulvic acid type) or to humify it further to a darker and more stable form (humic acid type) (Kononova 1966; Schnitzer and Kahn 1972; Freytag 1962). Our results, though preliminary, do give positive indications that the appearance of the chromatograms relates to these important soil features; and we use these findings to underscore our recommendations for the method.

Experimental Method

As originally proposed by Pfeiffer (1959), the method utilizes circular (15 cm diameter) Whatman #1 (or #4 for composts) chromatographic paper. Alternatively, square 15 x 15 cm sheets may be prepared. Paper wicks, rolled from 20 x 20 mm squares of the same grade of paper, are pushed through a small pre-punctured hole in the center of the disks. Prior to this step, pencil dots indicating radial distances of 35 mm and 50 mm have been placed on the sheets, as shown-in Fig. 15.1, and serve to gauge when to stop the moving liquid front. The liquid is eluted into the paper via the wick from a watch glass placed inside a 100 mm petri dish on which the paper sheet rests.

Reagents used include 0.25 N sodium hydroxide for extracting organic matter and 0.5% silver nitrate for sensitizing the filter paper prior to eluting the humic extract. The main reaction pathway involves the formation of silver hydroxide and almost immediately silver oxide, the oxide form being insoluble and causing a yellow-brown precipitate to form. The weakly soluble silver hydroxide causes a partial blocking of the paper's capillary pores, resulting in a streaking effect that is enhanced by the presence of larger organic molecules in the extracts; consequently, a fairly dramatic ray-like appearance is produced. This effect does not occur when extracting solutions lacking hydroxides are used.

Deposition of Reagent and Humic Extract. The two-step procedure involves (1) prior sensitization and preconditioning of the paper with the silver nitrate solution and (2) elution of the humic extract with the sodium hydroxide solution. Sensitization is accomplished by wicking approximately 1 ml of the silver nitrate solution into the paper to the nearer radius of 35 mm, at which point the paper is removed, the wick taken out and discarded, and .the paper allowed to dry for 2-4 hours in subdued light at a temperature not exceeding 29°C. The nature of the drying period is very important in influencing the final chromatograph. This effect is due in part to the fact that partial reduction of the silver occurs at this time, possibly causing a swifter and more complete reaction in response to eluting the humic extracts later on. We standardize this 'preconditioning" phase by allowing the partial silver reduction to reach the point where the first faint signs of yellow silver oxide appear, at which point the humic extract, prepared in advance, is promptly chromatographed. This is accomplished by placing a fresh wick into the disk and eluting the humic extract. This part of the procedure should be carried out at approximately 21°C and at a relative humidity of not less than 70%, preferably done inside a temperature- and humidity-controlled chamber. The-necessity of high and constant humidity during this period cannot be underestimated. At completion, the paper is removed and hung to dry at room temperature and diffused daylight. After two or more days of further reduction of incompletely reacted silver (including free silver ions and silver hydroxide formed by reaction with the extracting reagent) the chromatogram has stabilized and may be used for interpretation.

Humus Extraction. The mobility and extractability of organic matter with NaOH is a feature sharply dependent on the pH of the sample (Kononova 1966). The relationship we have observed between pH and extractability of organic matter (using an 8 hr extraction period) is shown in Fig. 15.2. The generally low recovery of soil organic matter is partly explained by the fact that a relatively large percentage of the humus may be present as alkali-insoluble mineral-bound humins (Kononova 1966; Najmr 1962; Ricardo 1967). Additionally, under near-neutral conditions a portion may be bound by prominent cations (e.g., calcium) as humates, which, unlike humins, may be recovered if prior to extraction the sample is decalcified with dilute mineral acid. For the chromatographic method, Pfeiffer's original recommendations called for a standard 1:10 sample:solution extracting ration with no mention of differences in extraction efficiency (Pfeiffer 1969). It is therefore apparent that the amount of soluble organic matter applied to the chromatograms would differ widely between samples of varying origin, which clearly could bias the results. This implies that the extractability of the humus exerts a first-order effect on the appearance of the chromatograms. This trait of extractability is nonetheless a significant clue to the condition in which humus may exist. For example, compare a soil high in acidity and low in biological activity with an intensively managed agricultural one (Fig. 15.3). However, does a link exist

between the appearance of the chromatogram and the quality of the humus, defined by its degree of humification, as originally purported (Pfeiffer 1959; Koepf 1964).

We investigated this point by adjusting the soil:solution ratio to eliminate any differences in humus extractability (determined in advance), and then ranking the subsequent chromatograms by appearance and statistically analyzing the connection to the humic:fulvic acid ratios by a rank-order correlation procedure (Goodman 1964; Norman 1975). These results gave evidence (> 95% significance) of a correlation between the appearance of the chromatograms and the soil humic:fulvic acid ratios. The results suggest to us that in reading the chromatograms one must distinguish between the first-order effect (humus extractability) and the second-order effect (humus quality), as will be shown later. However, in order to avoid a bias associated with samples of either very high or very low organic content; we apply a correction factor in determining the actual soil:solution ratio for extracting the humus, as follows:

$$r = 0.1 \; / \; \frac{\sqrt{OM\%}}{5}$$

where r is the dilution ratio. This empirically formulated calculation, which assumes that a normal sample of 5% organic matter is ideal for the 1:10 extraction ratio, has worked excellently in our uses.

Six to eight hours extraction time is generally adequate. Periodic shaking, e.g, at 15 min and 1 hr, is advisable; and centrifugation before chromatographing is usually required, unless the solids are allowed to settle for many hours, as originally recommended (Pfeiffer 1959).

The use of the silver nitrate substrate in conjunction with humic extracts means that the chromatograph is a qualitative oxidation–reduction system, responding with varying degrees of coloration to the sample's reducing abilities.

Interpretation of Paper Chromatograms

Chromatographs of varying soil and compost conditions are shown, in Fig. 2 and 3. In distinguishing between them, it is helpful at first to recognize the three general zones of the chromatogram: center, usually light-colored or even blank; middle, the area in which the dark-colored spikes occur; and outer, extending beyond the spikes and varying in color from yellow to diffuse dark brown. Each of these zones
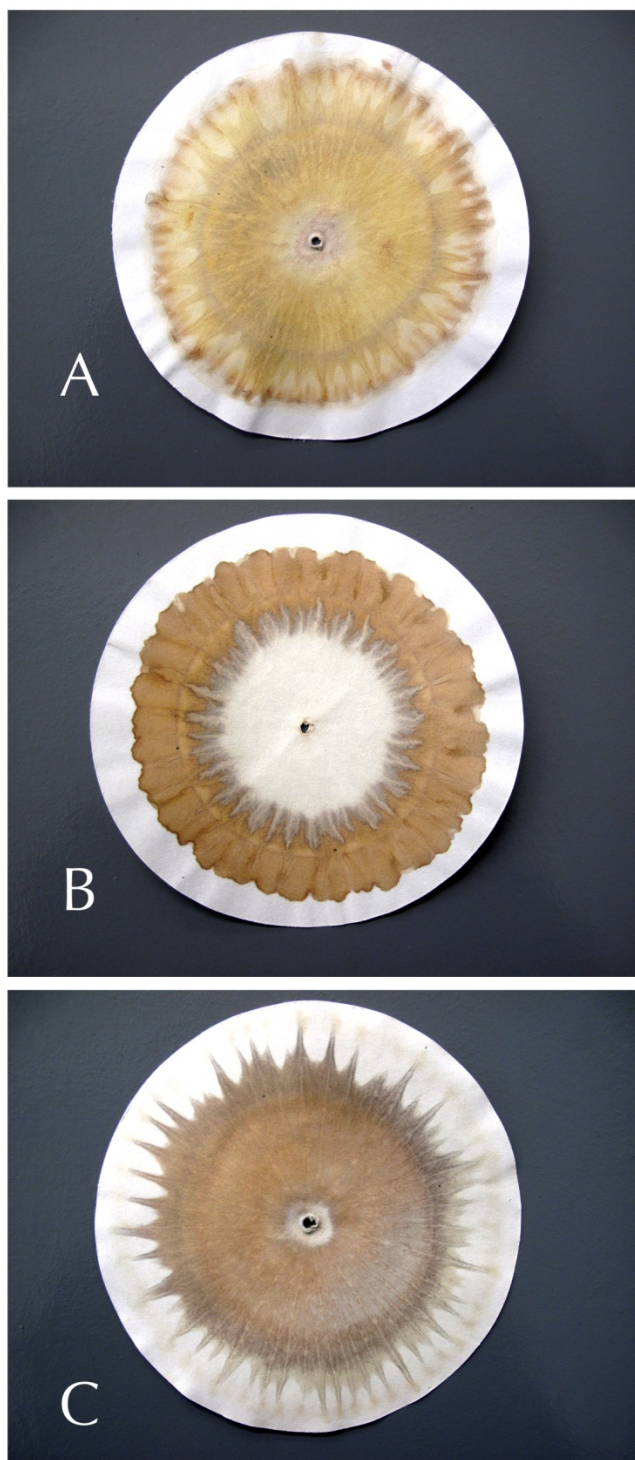
FIGURE 1 CIRCULAR CHROMATOGRAMS OBTAINED FROM (A) COMPOST TREATED GARDEN SOIL (PA) (B) ACID-FOREST SOIL AND (C) MID-WEST FIELD SOIL, LOW OM

This outer zone is of primary importance in evaluating the chromatograms. As conventional applications of humus chromatography show, three main fractions of humic materials are generally distinguished: the least mobile, most condensed compounds, which travel only a short distance in paper before stopping; intermediary compounds; and finally, highly mobile, fulvic-acid compounds, which migrate the furthest when chromatographed (Kononova 1966; Pospisil 1962). Of course, with this particular use of chromatography, the laws that govern the retention factors, or Rf, defined as the distance traveled by the compost or soil humic extract divided by the distance traveled by the solvent in paper will not be strictly obeyed, but nevertheless the general relationships will hold. Therefore, we may expect that, where the relative concentration of fulvic acid compounds is high in the extract, a thick moving front will be established, leading to a dark outer zone as may be typically observed in the chromatogram of the acid forest humus in Fig. 15.3. Where the proportion of these mobile compounds is lower in relation to humic acid types, the outer zone becomes considerably lighter (see garden soil, Fig. 15.3). Finally in an intensively cultivated agricultural soil, receiving virtually no fresh organic amendments, the more mobile compounds may be absent and consequently the outer zone may disappear altogether (see field soil, Fig. 15.3). Judging by the intensity of color between the three chromatograms in Fig. 15.3, it is evident that the extractability has been the highest in the acid forest soil and lowest in the field soil. The levels of organic matter are not identical, of course. The type of gradation in soil humus condition of which these chromatograms are suggestive may be represented as in Fig. 15.5.

When the method described is applied to evaluation of composting materials, the basis for interpretation is similar. Here, fresh, semi-decomposed hen manure of an anaerobic nature gives strong coloration and a thick outer zone. After a period of composting, the changes observed on the chromatograph point to a loss of mobile compounds associated with fresh materials and a decrease in extractability suggesting that otherwise extractable humic compounds have been complexed by inorganic constituents and thereby rendered more stable and insoluble. Such stabilization of organic materials is a fundamental goal of composting.
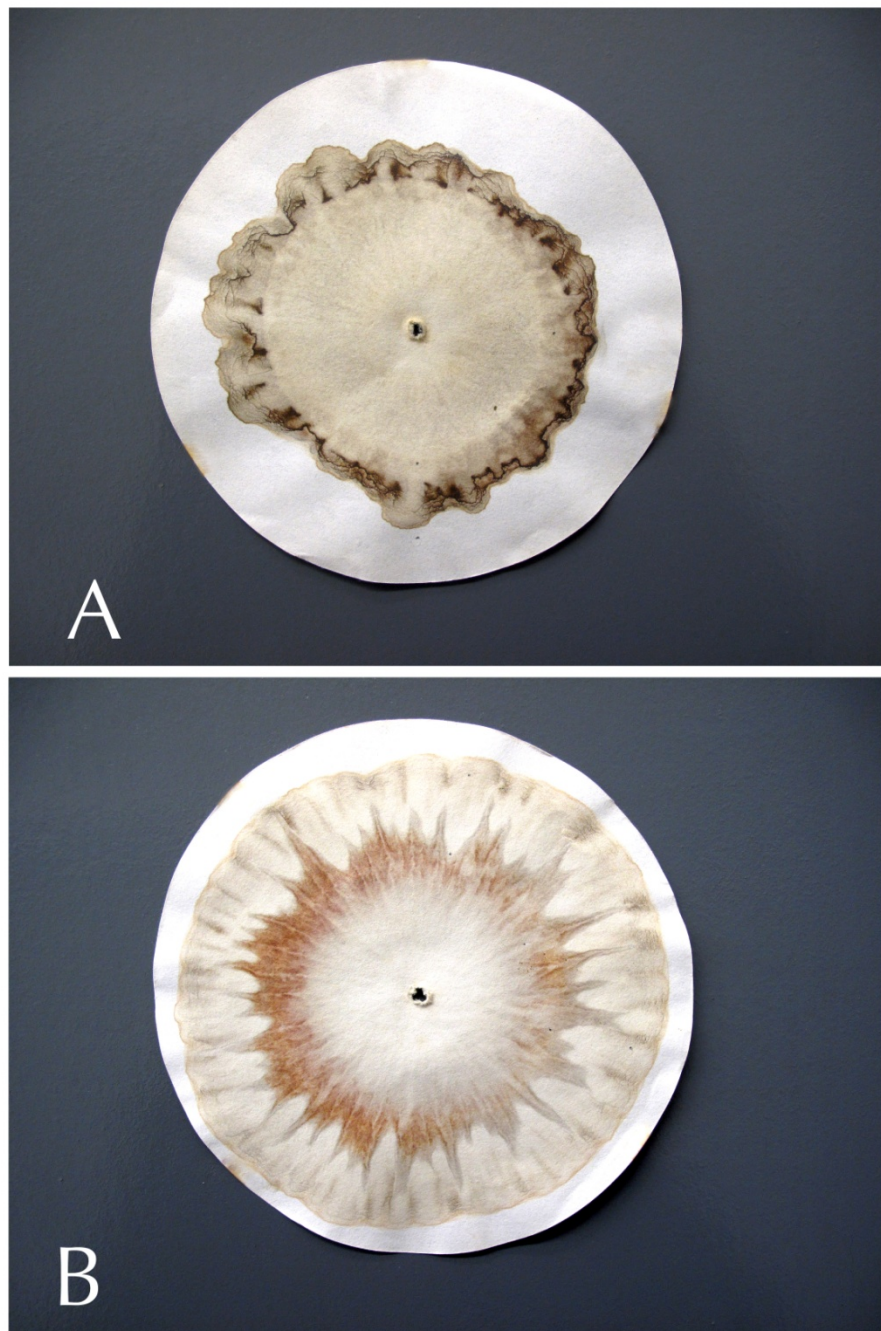
Figure 2 CIRCULAR CHROMATOGRAMS OBTAINED FROM (A) UNCOMPOSTED MANURE AND (B) SAME MANURE, COMPOSTED FOR 65 DAYS

CONCLUSIONS

The use of the Pfeiffer circular chromatography as a complementary tool in soil and compost analysis provides a strictly qualitative means for interpreting the condition of humus-like compounds that may be present. The appearance of the chromatograms relates not so much to the absolute quantity of organic matter in a sample being tested as to its qualities—qualities that are first and foremost defined by the extracting solution. In view of this feature, chromatograms must be interpreted with care; the solutions of humus obtained by the sodium hydroxide extraction are generally not representative of the entire sample, and sodium hydroxide may arbitrarily select for some compounds over others (Waksman and Stevens 1930). However, similar research with sodium hydroxide extracts of soils has shown that qualities of the extracted compounds are characteristic of soil type, most particularly when humic:fulvic ratios are investigated (Kononova 1966; Tokudome and Kanno 1968; Harris 1979). It may also be apparent that those humus-related compounds of most immediate agricultural concern are the ones amenable to nondestructive extraction; these include the most physiologically active forms and those which are precursors to the stable, structure-conferring forms important in soils.

Organic materials applied to varying soils may not share common fates, particularly in view of moisture conditions, biological activity, acidity, and the soil's current use (Webber and Beauchamp 1977). Soil conditions of high acidity and low biological activity may select for mobility in organic materials, leading to ultimate loss as carbon dioxide or leaching of soluble organo–mineral products, contributing to dark-colored water leachate and soil podzolization (Kononova 1966; Franco 1968; Khantulev et al. 1968). However, under near-neutral soil conditions, humic-like compounds may be readily coagulated in polymer and organo–mineral (humate) forms, preserving them in the arable layer and conferring favorable structural effects on the soil (Alexandrova et al. 1968; Pauli 1967; Martin and Focht 1977). Soil management therefore has a significant potential impact of seqestration of humus-carbon,

From these points it may appear obvious that the efficiency of retention of added organic materials will vary appreciably depending on soil type, bringing into question generalized principles for organic waste application. For example, traditional sheet composting of organic material is thought by many to be equally effective in carbon retention in soils compared to prior composting and subsequent soil application, since the dissolved organic matter will react rapidly with soil minerals. A composting environment may affect stabilization of organic material very differently. If high pH values in compost are associated with elevated levels of ammonium, potassium and sodium then solubility of organic matter may be reinforced. However, at moderate pH and in presence of appreciable calcium, magnesium, and other multi-valent minerals, significant polymerization and complexation of the compost humus may take place (Springer 1949), whereas the same materials applied as sheet compost to an acid soil may never enjoy such a form of humification.

The fairly simple, qualitative chromatographic technique described in this paper can provide a means of distinguishing these features, whether from a soil or a composting point of view. As a non-quantitative technique, the method is challenging and may require collaborative data—for example, organic matter content and pH—to assist interpretation. Used for monitoring short-term changes in composts, the method provides clues to the progress of humification and may suggest probable effects the compost will have on a soil. Used in conjunction with soils, the method can reveal humus effects related to past

and present soil-forming processes and agricultural management, contributing an important framework for understanding efficient soil utilization of organic materials

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