

acid-loving plants has been observed when ordinary garbage was composted.

Quick release of ammonia and calcium damage are part of the reason why "reinforced" composts cause burns on lawns, flowers and shrubs; they are too "rich". Undecomposed poultry manure is known to cause such burns.

While it is relatively easy to get composting underway, because it is in the nature of the materials involved to get started with some kind of breakdown (decay will always be the result), it is more difficult to direct this decay into definite channels of fermentation and to correct an undesirable trend. The knowledge of all the factors mentioned so far is of help, but the home gardener, farmer or even the manager of an organic composting plant will ask: How do I get to know about all this without a laboratory? Our advice in the past has been (a) to use as many different materials as possible for composting, for then you will have the best chance of getting a little from every process; (b) in order to brake violent, one-sided action, to use soil as addition, which acts as a kind of buffer and also favors the development of the phase 2 organisms; (3) to control your moisture; (d) in case anything goes wrong, to turn your pile. Excessive heat is as undesirable as a cold, wet, "jame-duck". Excess of anything is undesirable.

We could, of course, give composting classes, showing the different types of fermentation and behavior of source materials and explaining why and how the pile looks and smells as it does and has its particular structure. We could show, for comparison, controlled experiments with most or even all of the contributing factors known. In this way the layman could learn by comparison and orient himself by the examples. I have on occasion given such instructions and demonstrations. Usually, people in the audience begin to tell how *they* make it, assuming that their method is gospel. To me it is a puzzle why in this field we have on the one hand so much dilettantism, and from the side of science, on the other hand, so much prejudice.

The plant manager in charge of large-scale composting, in order to make the operation efficient and economical, needs some basic information; knowing the temperature, moisture, organic matter, nitrogen, structure and degree of aeration are all musts. It is important for him to know when to turn a pile, when to interrupt fermentation, when and how much water to add. It must be realized that some of the tests we have mentioned above, especially isolation of micro-organisms and fermentation tests, take time and the compost is probably stockpiled or sold by the time the results come in.

Temperature can be read in a few minutes; a moisture test with suitable equipment takes up to 1 hour; a micro-Kjeldahl test for nitrogen about 1 hour, a macro-test longer; organic matter by

combustion, several hours. (These need analytical equipment, exact scales and some experience.) All these tests indicate details. We have been looking out for a simple method to check the overall pattern. We have tried the sensitive crystallization method developed by this author which, in the checking of other biological materials, has performed quite satisfactorily. With regard to compost it may give some information, but the method is too cumbersome for practical surveys. For the last three years, we have been using a method of circular filter paper chromatography which has performed quite satisfactorily and is rather simple, requiring very little equipment. This method is based on the property of filter paper to separate fractions in a solution by way of capillarity. It then needs a reagent to "fix" the fractions and make them visible.

Chromatographic Determination of Humus Extracts from Compost Fractions

Principle:

The test described is a qualitative one in order to separate different fractions of humus extracts by means of the capillarity of suitable filter papers. The filter paper is prepared with a photo-reactive substance (for instance, silver nitrate) which also reacts with the extraction substances.

The precipitation of this reaction occurs at various distances from the point of application of the substance to be tested. The distance, the pattern, the color and the shape of the reaction area are significant for an interpretation of the substances contained in the extracts.

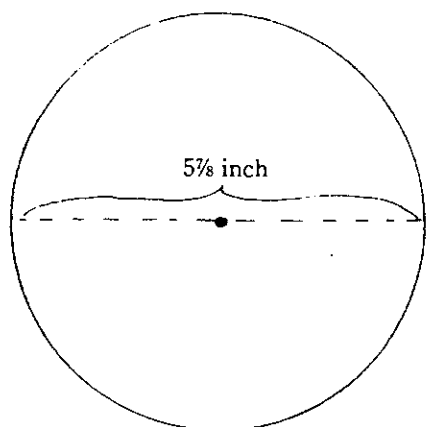
In using this method, no attempt is made to identify the chemical nature of the reacting substance, since the pattern obtained can itself be used as a diagnostic means. However, identification is possible. Of the different possible techniques for chromatography, the circular method of chromatography was selected since it gives easily obtainable results with simple equipment and is easy to interpret.

Method:

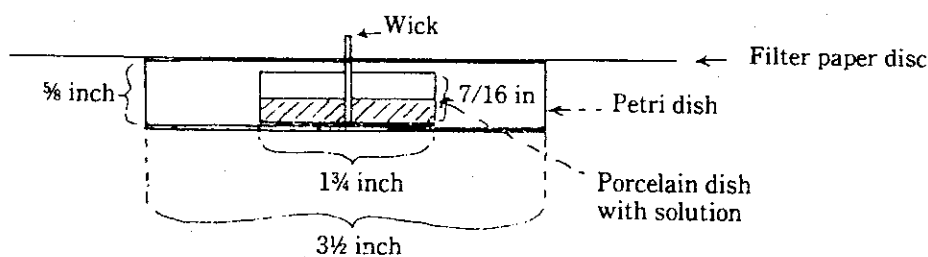
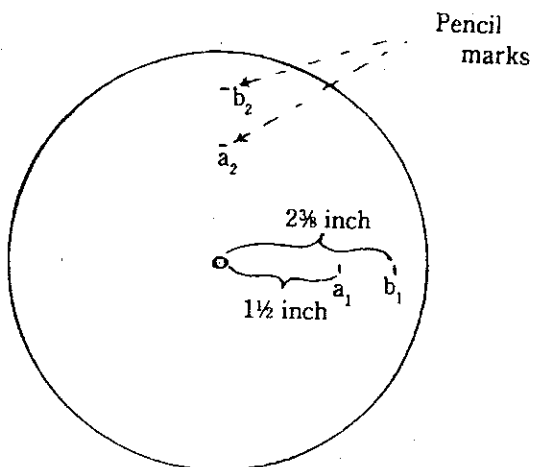
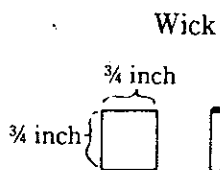
1. Preparatory Steps:

(a) Use circular filter paper discs of Whatman No. 1, or No. 4, paper, 5 7/8 inch (15 cm.) in diameter. Punch a hole in the center, about 1/16 inch diameter.

(b) Prepare a wick of the same filter paper by cutting strips 3/4 inch square and rolling them tightly into a cylinder. Place this wick into the center hole, protruding on either side of the disc but so that it touches the bottom of the porcelain dish underneath (cf. e). It is important that



Filter Paper Disc
(Whatman #1)



- the wick touch evenly on the circumference of the center hole in the filter paper disc in order to make a good, homogeneous contact.
- (c) Make pencil marks at $1\frac{1}{2}$ inch and $2\frac{3}{8}$ inch distances from the center to indicate how far the solution should penetrate or be absorbed (see sketch). Depending on the run of the fiber in the filter paper, the solutions sometimes spread more quickly in one direction than in the other — hence the double pencil marks in two different directions at a right angle (see sketch).
- (d) Have a 0.5% solution of nitrate of silver, AgNO_3 , tested purity, in aqua dest., available. The solution should be kept in a green or brown bottle, not exposed to direct sunlight (no cork stopper); it can be made once a week. In any case, it should not be too old and not show any dark rings or deposits.
- (e) Place the filter paper disc with the wick on a petri dish of $3\frac{3}{4}$ inch diameter, center over center; the height of the petri dish is $\frac{5}{8}$ inch. Inside the petri dish, center over center, place a porcelain dish of $1\frac{3}{4}$ inch diameter and $7/16$ inch height (see sketch).
- (f) Pour 3 to 5 cc of the 0.5% AgNO_3 solution into the porcelain dish; remove the filter paper disc before pouring and replace it afterward. This amount of AgNO_3 solution can be used for the preparation of 3 filter paper discs in succession.
- (g) The solution of 0.5% AgNO_3 will, by capillarity, spread over the disc, radiating in all directions. When it has reached the first pencil mark at $1\frac{1}{2}$ inch distance (a_1 , or a_2 — see sketch), remove the disc immediately from the petri dish and take out the wick at once, very carefully in order not to tear the hole. Then place the disc on another petri dish for drying. Protect from direct light and dust (no dark-room is necessary). As soon as the disc is dry, put it into a dark box to keep it in a dry atmosphere. The discs will last for about 3-5 hours before the AgNO_3 reacts with the paper and causes discoloration. No disc showing even a slight discoloration should be used. It is, therefore, best to prepare the disc immediately prior to use. Needless to say, all filter paper should be handled with care; no fingerprints or smudges should be retained by the filter paper; these will cause undesirable reactions.
- 2. Testing Procedure:**
- Have the petri dishes and the porcelain dishes ready in a box (cf. apparatus). Pour the humus extract, or whatever you want to test, into the porcelain dish. Use 5 ml of the extract to be tested (see No. 6). Put the prepared filter paper with a *new* wick over the solution on the petri dish, so that the wick just touches the bottom of the porcelain dish. Let spread until the solution reaches the first $2\frac{3}{8}$ inch pencil mark (b_1 , or b_2). This will take about 20-60 minutes. Don't let it run

over the mark. Remove the disc and wick and place disc again on a petri dish for drying.

3. *Developing Phase:*

The development of the pattern should take place in diffuse daylight (not direct sun).

In order to obtain comparable results, the developing should be done in the same intensity of light. Unless you use measured artificial light (for instance, fluorescent lights), you need a certain judgment as to the intensity of development — that is, when to evaluate the pattern. Just do not expose to direct sunlight.

4. *Evaluation:*

Keep discs in a dark (any folder) and dry place. Put a dry paper in between each sheet in order to avoid reactions between discs. Fingerprints will still show on finished discs.

5. *Apparatus:*

The test is carried out in a box, the top of which consists of a glass plate, subdivided into three removable sections for observation purposes. The size of the box is arbitrary. We use one 36" long, 17" wide and 4" high. Twelve discs can be processed in it. The box is painted with aluminum paint. It should be aired and cleaned after each use. It is wise not to crowd too many discs into one box; you should have several boxes if you want to run more tests at a time. The atmosphere in the box should be moist, close to the saturation or dew point. Therefore, we place shallow, small dishes (crystallization dishes or beakers) with distilled water at equal distances here and there in the box. This water will evaporate and maintain an even moisture. The temperature is not too essential but should be between 65° and 85°F.

Needed glassware, etc.:

- Petri dishes, 90mm (3½") diam., 15 mm (¾") high
- Porcelain dishes, 40mm (1½") diam., 12 mm (½") high
- Filter paper, Whatman No. 1 or No. 4, circular,
 - 15 cm. diam.
- 5 cc graduated cylinders or test tubes marked at the 5 cc level

Standards for Preparation and Extraction of Samples

Many different methods of extraction and degrees of concentration of samples have been tried out in the Biochemical Research Laboratory. In the following we report only those standards which

have given us the most satisfactory results and which can be used for comparison with our test materials and for our way of interpretation. It must be realized that any change of extraction, or of concentration, changes the observable pattern, so that chromatograms deriving from the differences in the preparatory steps cannot be compared. Only those which have been prepared completely alike can be compared. For all other cases, new standards of interpretation must be developed. Concentrations and extraction times may also need occasional variation.

Preparation of Soil and Compost Extracts

Put 5 grams of compost or soil into a 125 cc Erlenmeyer flask. Add 50 cc of a 1% sodium hydroxide solution (prepared from sodium hydroxide pellets). Mix thoroughly by twirling the flask, let stand 15 minutes, then repeat the twirling. After an hour, mix thoroughly once more by twirling the flask. Then allow the flask to stand undisturbed for five more hours. After the sixth hour, carefully pour the supernatant liquid into a small beaker. Use 5 cc of this extract for each porcelain dish.

Extracts of Grains, such as Wheat, Rye, Oats, or Barley

Grains are ground, as received, in a laboratory grinder, such as the Wiley Multicut Mill (for materials with a low oil content). This mill contains shearing plates and shreds. A nut mill can also be used. It is important that the material pass through the mill in the shortest possible time, without heating up. The sample should be processed immediately after grinding. Ground samples should not be stored for any length of time. We usually use 12 grams of sample in order to obtain a good average sampling and have enough material on hand.

Weigh 2.25 grams of the ground material, place in an Erlenmeyer flask of 125 cc capacity, add 50 cc of the 0.1% sodium hydroxide solution. Mix thoroughly by twirling the flask, repeat after 15 minutes and then let stand for 14 hours. Cover the flask with an inverted glass beaker. The extraction should be started in the late afternoon to be ready for the next morning. Carefully decant the supernatant liquid into a glass beaker of about 50 cc capacity and measure 5 cc with a small measuring cylinder for each flat-bottomed, capsule-form, porcelain crucible (10 cc capacity).

We also run a test with 1.5 grams of ground sample in 50 cc of the 0.1% NaOH solution, proceeding in the same way as described for the 2.5 gram sample. This method gives additional information if needed. It does not always show such drastic differences as the other.

Small Seeds

Grind the seeds cautiously in a small nut mill; avoid heating up. From the ground material weigh 1 gram into the flask and add 50 cc of the 0.1% NaOH solution. Mix by twirling the flask at the start, after 15

minutes and again after 30 minutes. Total extraction time: 4 hours.

Flours and Dough

Flour and dough are used as received from the outside, or as freshly prepared in the laboratory. Again we use 2.25 gram and 1.5 gram samples and proceed exactly as described above.

Breads of All Kinds

The measured amount of bread is mortared for a few minutes in a porcelain or glass mortar with a small amount of the 50 cc of the 0.1% NaOH solution (to be used for the extraction) to make a homogeneous paste. 2.5 grams of bread are used for our standard determination and 1.5 grams for supplementary information. Proceed as with the grains. In the case of bread samples, however, the extraction time is 4 hours.

Fresh Green Leaves (All Kinds), Vegetables, Fruit, Nuts

The incoming material should be as fresh as possible. It is first cut as finely as possible with clean (not rusty) scissors and then mortared. Use 2.5 grams of the finely-cut material to 50 cc of the 0.1% NaOH solution and continue as described previously. The time interval between cutting, mortaring and adding to the extracting solution should be kept as short as possible. The extraction time for all of these products is 4 hours. Incoming material can be kept in a refrigerator at about 40°F. Special storage and research on keeping quality may demand different handling of the source material, but the method of extraction, etc. remains the same.

Roots, Tubers, and Bulbs

These are finely grated prior to mortaring; then one proceeds as with leaves, vegetables, etc. Use 2.5 grams of material to 50 cc of the 0.1% NaOH solution. Extraction time is 4 hours.

Green Herbs

Fresh green material is finely cut, mortared and processed as with fresh green leaves; that is, no tea or infusion is brewed. Our standard is 2.5 grams of material; because of grade variations in strength, 1.5 grams may be needed for added information.

Dried Herbs and Teas

Two paths of investigation are open: (a) to proceed as with any other dried or dehydrated material, or (b) to test the material in the form of a tea. In the latter case, an infusion of the dry herb or tea is made by putting 2.5 grams in 50 cc of distilled water, bringing it up to the boiling point without losing fragrance. Then immediately add 50 cc of the 0.1% NaOH solution, let stand for 2 hours, then proceed as usual. A cold extraction might be used, as well.

In the case of very strong teas (black) containing lots of extractives, a direct chromatogram of the tea alone could be tried. In that case, 5

grams may be used in 100 cc of water.

Juices, Pressed Extracts, Soft Drinks

The juices are not filtered but used as they are received. 5cc are added to 5cc of the 0.1% NaOH solution. Proceed as usual but here the extraction time is only 1 hour.

Enzyme Preparations

Of the pure enzyme preparation in dry powder form, 0.1 gram is used. 10 cc of the 0.1% NaOH solution are used for all enzymes which are active in alkaline media, while 10 cc of the 0.1% HCL solution are used for enzymes active in acid solution. Extraction time is 1 hour.

Yeast Preparations

0.1 gram of the dried yeast powder or yeast cake is extracted in 10 cc of the 0.1% NaOH solution. Extraction time is 1 hour.

Vitamin Preparations, Single, Pure, or in Mixture (i.e., Complex)

The concentration depends somewhat on the strength of the preparation. To begin with, we use 0.1 gram in 10 cc of 0.1% NaOH solution. Extraction time is 1 hour. It might be necessary to modify this concentration. The spread is between 0.5 gram and 0.01 gram. For pure crystalline vitamins, one can either reduce the amount of substance or increase the amount of the NaOH solution. Then, too, if different vitamin preparations are to be compared, it will be necessary to calculate the actual formula to have the same amount of strength of each vitamin, especially if comparison with pure vitamins is desired.

Dried Foods, such as Macaroni, Egg Noodles, etc.

Use 2.5 grams in 50 cc of 0.1% NaOH solution. Extraction time is 4 hours.

Sugars, Honey, Molasses, Maple Syrup

In general, 2.5 grams in 50 cc of 0.1% NaOH solution are used. Extraction time is 1 hour.

Milk

For fluid milk, 2.5 grams in 50 cc of 0.1% NaOH solution are used. Extraction time is 1 hour.

Dried, Dehydrated Milk Powder

0.1 gram in 10 cc of 0.1% NaOH solution is used. Extraction time is 1 hour.