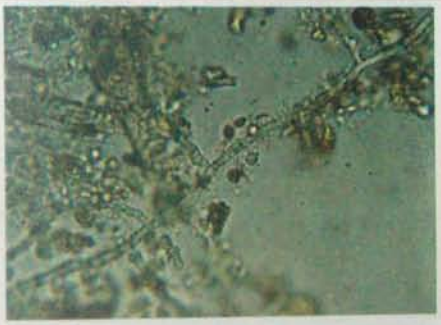
Compost Tea Quality:
Light Microscope Methods

Soil Foodweb Institute
Lismore, NSW

100 FLAQ Microscope



Example of bacteria, fungi and protozoa observed using light microscopy

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This publication is for serious students of compost tea, who want a relatively easy way to determine whether their tea contains the biology it needs to obtain all the possible benefits that good sets of bacteria, fungi, protozoa, and nematodes can give.

My thanks to Tom Jaszewski for his continuing interest and support of compost tea, to Jeff Lowenfels, for his work in educating the gardeners of America about the benefits of aerated compost tea, to Hendrikus Schraven, for his work in demonstrating that aerated compost tea works and works well.

This publication is dedicated to Dr. Donald Klein, my major professor at Colorado State University, who was the person most important in getting me started on light microscope methods. I thank him for the hours he spent training me.

Acknowledgements

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The rest of the wonderful Soil Foodweb Insitute team, that as always gives their best.

Last but not least, Thank you Elaine for your untiring guidance. The continued path of compost tea will benefit from this manual.

The key to freedom is through education.

Merline Olson
President Soil Foodweb Insitute

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Compost Tea Quality: Light Microscope Methods

Purpose of the Manual:

The user will be guided in the basic steps to use a light microscope to assess the organisms in the tea. With proper preparation of samples, compost and soil can be assessed as well.

Quantification of microorganisms is not possible using these basic microscope procedures, because a significant number of fungi, protozoa, nematodes and bacteria will not be observable using light microscopy.

The refractive index of water is not that different from these organisms, and quantification using these light microscopy methods would underestimate the actual number of organisms present, since they would not be seen. Given that many organisms are desired, however, in compost tea, if the number is over a threshold number, then it can be safely known that the tea will function as desired.

The benefits of compost tea derive from the actions of the organisms in the tea. For example, protection of leaf surfaces is strictly the result of high biomass of bacteria and fungi colonizing the leaf or root surfaces, and preventing disease organisms from reaching the root or leaf surface, or from obtaining any of the exudates released by plant surfaces. Competition for food is an important consideration in preventing disease growth.

A second important benefit that bacteria and fungi provide soil is preventing leaching and erosion. Work by Hendrikus Schraven, published on his website, elucidates the impact that bacteria and fungi can have in holding nutrients in soil, and preventing soil particles erosion, even in high rainfall events. If the tea becomes anaerobic, and the filamentous fungi are lost when anaerobic metabolism begins to dominate, then these benefits will be lost. Anaerobic compost teas cannot have the full set of benefits that can be seen with aerobic teas. Determination of the ENTIRE food web is an easy way to assess whether the beneficial organisms are still present.

Thirdly, protozoa and nematodes in compost and compost tea will consume bacteria and fungi, reducing pathogen populations, but also cycling nutrients into plant available forms. This is a critical function in soil and on leaf surfaces when foliar generation of plant nutrients into plant-absorbable forms is required.

The fourth benefit of using compost tea, and for which organism presence in high biomass levels is extremely important, is formation of soil structure. Micro- and macro-aggregates cannot be formed if bacteria and fungi are not present. Again, if aerobic species have been lost, or put to sleep, these functions of building soil structure will not occur. The larger size predators are also needed, to build the larger soil spaces and maintain pore structure in the soil.

In non-compacted, aerobic soil, roots of ryegrass grow to 4.5 foot depth in 4 months. In the picture above, Hendrikus Schraven, Soil Dynamics, Inc. in Issaquah, WA, holds sod planted in July, mowed twice and harvested in November. No root pruning occurred on these plants because soil structure was well-maintained by excellent levels of soil biology. Compacted soils, and the anaerobic metabolic waste products produced by facultative and true anaerobic bacteria are what cause root pruning to occur. The soil food web will build soil structure, and allow air and water to move into the soil to deeper levels. Roots then follow, reducing water and fertilizer inputs. Savings for growers can be significant.

Decomposition of toxic material is very important, as well, and is another benefit of getting aerobic organisms in soil. Pesticide residues, or the residues left from anaerobic conditions in soil have to be de-toxified and consumed. These functions are performed by the same organisms needed in the previous considerations. Thus, it is critical to maintain biomass, maintain active biomass, and maintain the greatest diversity possible within that community.

Diversity is somewhat maligned because non-environmentalists lack an understanding of what is meant by the concept. Especially in soil, all metabolic functions are required to make the soil operate correctly. But the metabolic capacity of any one individual species of bacteria, fungi, protozoa or nematode is narrow. Any one species can operate only in a narrow range of temperatures, moistures, food concentrations, salt concentrations, humidity, etc. Therefore, to have functional species operating in all conditions, a huge diversity is needed. If some species or set of species is absent, then sometime during the year, some important function will be lacking, and plant growth may be impacted.

Determination of active and total biomass is necessary to know that not only all the needed species are present, but that they are active when needed. Comparison of the total and active biomass in any sample needs to be made with known active and total biomass in soil that gave excellent plant responses. Thus, in any situation, comparison to optimal soil biology, based on plant response, is needed.

Needed Materials

Microscope Manual

Microscope:

- 4X, 10X, and 40X objective lenses (100X Lens optional)
- 10X W.F. eyepieces (W.F. means wide-field)
- Binocular microscopes are easier to see through, but a bit more expensive than monocular microscopes. If lots of samples are going to be done, the binocular microscope is worth the extra money.
- Resolution is the determinant of expense for the microscope. Inexpensive microscopes will not allow you to resolve individual bacteria, for example, even though the magnification would suggest adequate ability to see very small organisms.

Supplies

- Microscope slides
- Cover slips (18 X 18 mm)
- Transfer pipettes (approx 1 ml volume)
- Container for sample collection
- Cotton buds for cleaning microscope lenses
- Methylated spirits for cleaning microscope lenses
- Lint free tissue (camera lens cleaning tissues are suitable) for wiping clean microscope lenses. **Do not** use regular tissues on lenses. Tissue will leave lint particles on your lenses and have you see things that aren't really there.
- A very sturdy table to place your microscope on.

Always have methylated spirits, cotton buds and lint free tissues on hand when observing with your microscope.
Clean up mishaps immediately.

How to Take a Tea Sample

- Use a clean collection bottle, such as a plastic water bottle, to place the sub-samples of tea into.
- Wash your hands before working with tea, to make certain that the tea is not contaminated.
- While still mixing and/or aerating the compost tea in the tea brewer or holding tank or sprayer, use one of the following methods:

From brewing tea:

1. Using the pipette from your start-up kit to remove several approximately 1 to 2 ml amounts from the tea.

2. Place the 1 to 2 ml sample into a clean container larger enough to hold 100 ml or a half cup of tea.

3. Take the samples from various places in the tea brewer, so you have 25 to 50 ml of sample collected from several places.

From tea stream while pumping tea out of container:

1. Passage through a pump may kill organisms, so to determine what effect the pump has on the organisms in the tea, take several tea samples from the tea stream while the tea is being transferred.

From the sprayer:

- As tea is being sprayed from the sprayer, spray into a container. Repeat several times during the spraying event.

- Transport the container to the microscope. If there is some distance involved to get to the microscope, transfer the sample into a plastic, or non-breakable material container with a sealable top.

- Remember to leave at least ¾ of the bottle empty, so the organisms have something to breathe while they are being transported in the sealed bottle.

- Examples of different kinds of sealable jars. Note that the majority of testers over-fill the containers. Make sure ¾ of the container is filled with air space, so the tea will remain aerobic during travel to the lab. For greatest safety in mailing, plastic, light weight containers used to hold nothing more than water previously should be used.

Difference in tea color may be based on aeration being adequate (see picture below). Initial recipe is also important, as tea on the right contains an additional humic acid addition. All three plastic drinking water bottles were over-filled, and air-headspace may have limited ability of the organisms to survive during transport.

Assembly of 100FL & 100FLAQ Microscopes.

This is how your microscope will look fully assembled. Try and become familiar with the parts as they will be mentioned frequently when preparing a tea sample.

Steps for Setting up Compost Tea on The 100FL & 100FLAQ Microscopes.

Step 1.

Step 1. Using a clean pipette so there is no contamination; mix the sample to evenly distribute the organisms. Place the pipette about 1/3 into the tea sample, then draw the liquid up the pipette.

Step 2.

Step 2. Place a drop of tea on centre of slide. One or two drops are best.
TIP. Don't place too much liquid on the slide. You don't want the cover slip to be "swimming" and the microscope lens to make contact. If this happens, wipe clean with lens tissue immediately.
The 10X and 40X lenses are not designed for immersion in liquid.

Step 3.

Step 3. Carefully place one cover slip to the surface of the slide, just next to the drop of tea and lower the cover slip onto the tea.

Step 4.

Step 4. Place the slide on the microscope stage and clamp securely with the slide cam lever.

Turn the power switch ON.

Step 5.

Step 5.Use the stage controls to move the slide so the sample is directly over the light condenser in the centre position.

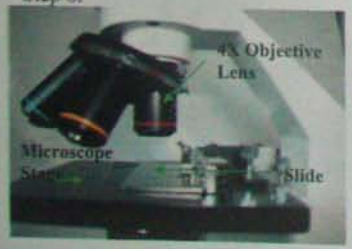
Step 6.

Step 6. Swing the 4X (RED) Objective Lens above the sample in the centre position.
You will feel the lens click into place.
Total magnification is 40X.
This is because the eyepiece is 10X magnification and the objective lens is 4X. Total magnification is 40X.

Step 7.

Step7. Turn the Coarse Focus (larger knob) towards you all the way until there is no more turning room.

Step 8.

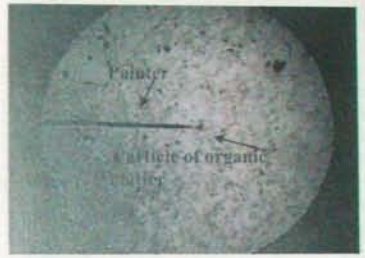


Step 8. Distance between 4X Objective Lens and stage position.

Step 9.

Step 9. With the 4X Objective lens locked into position and looking into the eyepiece, turn the Coarse Focus knob away from you until the sample comes into focus.
TIP: If you are having difficulty focusing or finding the sample, use the stage controls to move the slide until the edge of the cover slip comes into view. Use this line to find something to focus on.

Step 10.



Step 10. Example of magnification using 4X Objective Lens.
The sample may appear grainy and difficult to distinguish organisms. Don't try and look for organisms at this point. You are at the early stage of setting up your microscope.

Step 11.

Step 11. Place the Light Lever on the condenser all the way to the left.
Looking through the eyepiece at the sample, slowly move the Light Lever toward the right. You will see the intensity of light shift from dull to bright. Find where the light is comfortable for your eyes and you can see the sharpest image. Too much brightness will mask organisms.

Step 12.

Step 12. Swing the 10X (YELLOW) Objective Lens above the sample in the centre position.
You will feel the lens click into position.
Total magnification is 100X.

Step 13.

Step 13. With the 10X objective lens locked into position and looking into the eyepiece, slowly turn the fine focus (smaller knob) towards you until the sample comes into focus.

Step 14.

Step 14.
Example of magnification using the 10X Objective lens. You may see some movement of bacteria, strands of fungi, and the presence of protozoa in the sample.
Don't be discouraged if you don't see any organisms. The magnification is not high enough at this stage.

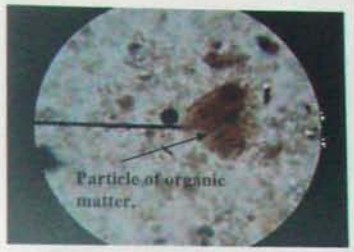
Step 15.

Step 15. For higher magnification, swing the 40X (Blue) objective lens above the sample in the centre position.
You will feel the lens click into place.
Total magnification is 400X

Step 16.

Step 16. With the 40X objective lens locked into position and looking through the eyepiece, slowly turn the fine focus until the sample image comes into focus.

Step 17. Sample image 40X Objective



Step 17. Example of magnification using the 40X Objective lens. Any organisms present will be easier to detect at this level.
Use the light lever to adjust the level of light and be aware of how the different organisms look under the light.
Remember. Don't use too much background light. This will mask organisms.
Adjust the light lever whilst looking into the eyepiece and observe the level of light intensity.
You are ready to observe.

TIP: When observing your sample, be certain there are organisms "floating". If this is not happening, your slide may have dried out, making it impossible to detect anything.
Be aware of the length of time you're looking into the sample. Every so often take the slide out from the stage and check if there is liquid present. If you notice the tea sample rapidly move in one direction, the sample is drying out and needs to be replaced.
Make a new slide and start again.
To place a new slide onto the microscope, make sure to rotate the 40X objective lens back and rotate the 10X (yellow) lens into the centre position. This will give more room to manoeuvre the new slide into place. Make this standard procedure when placing a new slide onto the microscope. This avoids the 40X objective lens making contact with liquids. If this happens, CLEAN the lens immediately using a cotton bud dipped in methylated spirits and wiped dry with lint free tissue.
The 40X lens is not designed for immersion in liquid

Problem: Sometimes you may find there is no "turning room" on the Fine Tuner, and you can't get the sample into focus.
Solution: Start back at the 4X Objective lens. Wind the Fine Tuner about half way, then use the Course Focus to find your sample. This will give the Fine Tuner more turning room.

100X Immersion Lens

Some microscope models have been provided with a 100X Lens. This is the only lens to use for immersion in liquid. To observe the sample under this magnification, place a small drop or two of tea onto the centre of a clean slide. **DO NOT** place a cover slip onto the sample.
Place the 4X (red) objective lens in the center position.
Place the slide onto the stage as you have previously done when setting up a sample.
Find the sample using the course focus tuner. Once you have located the sample, swing the 100X lens directly into the liquid and observe.
Total magnification is 1000X.
This will give you higher magnification, but the sample may seem a little distorted.

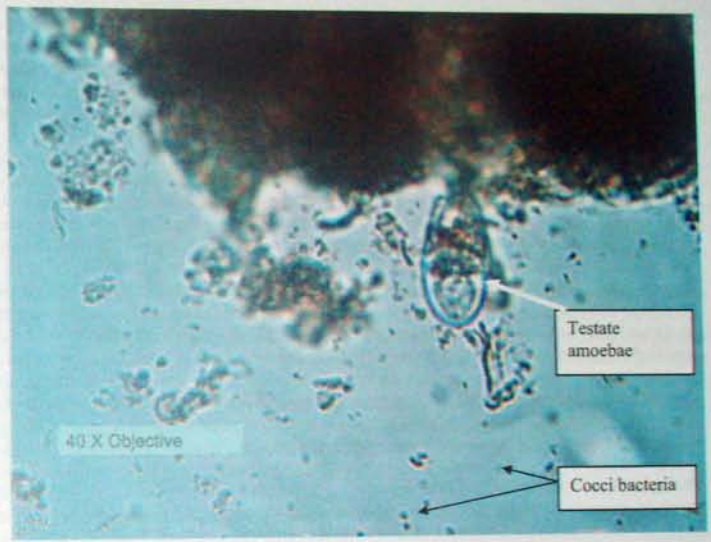
Blue Filter Disc

Some microscopes are issued with coloured filters. Blue is the preferred colour to use. The blue disc provided is a tool for observation. This doesn't change the magnification. This will help sharpen the image you see and takes the "golden" hue away. Place the filter in the slot at the base of the condenser. This is located below the light lever. This should pull out to the side. Gently place the filter on top and slide back under the condenser.

Observation of compost tea under 4X, 10X and 40X magnifications.

This picture is at low magnification (40X total). It is difficult to see anything. Use this magnification to find the sample.

Same picture at 100X total magnification. Need to go to a higher magnification for better definition.



Same picture at 400X total magnification. Much more definition. Now you can see the cocci bacteria more clearly and a testate amoeba is observed near the clump of organic matter.

Standard Curve of Compost Tea Quality

The focus in this manual is on the biological quality of the materials being examined. Chemistry can be assessed using these methods, to a limited degree. Look for crystals and mineral particles, salt particles, and so forth in your samples. The presence of salt crystals indicates a potential severe problem with high salt.

Make water solutions of compost in order to look at compost in a qualitative manner. Prepare a 1:1 or 1:2 dilution. This means add 1 part compost to one part water, or one part compost to two parts water. Another way of saying this is to add 1 teaspoon (one part) compost to one teaspoon (one part) of water. Mix the compost in the water and shake or stir vigorously for 3 to 5 minutes, then let settle for 10 seconds. Place a sample on the microscope slide, and follow the same directions are for looking at tea. While we have not assessed a compost standard curve as we have for compost tea (see below), compost quality can be qualitatively assessed using this approach, just as for tea. The "standard curve" remains to be assessed. Keep an eye on the SFI website for this series of pictures, just as we have developed this for compost tea.

Quantification will not be possible using these low magnification, bright field microscopes, but the following section will allow you to qualitatively assess "bad", "poor", "acceptable", "very good", and "excellent" levels of the desired organisms in the tea.

But again, these are QUALITATIVE measures. The results are expressed in categories, not precisely quantified biomass or numbers of individuals. Because many fungi and bacteria are invisible unless the shadowing method is done just exactly correct, some bacteria and fungi will not be quantified using these light microscope methods. Using the iris diaphragm properly will allow visualization of some of these bacteria, fungi, and protozoa. To be truly quantitative, higher resolution microscopes and different shadowing techniques, called differential interference contrast microscopy, and epi-fluorescence microscopy are required.

A "field" is what you see when you look through the microscope, when you move the slide and look at another area of the sample, that is a second field.

Be consistent in the way you observe your sample. Choose the pattern you will use to decide where to look. Decide before you start looking at a sample how you will choose which set of fields to look at – a series of 15 fields all in-a-row, or 10 fields in a diagonal pattern across the slide, separate each field by a tenth of a turn of the stage controls going up or down, and left or right. Start at the same point of the microscope coverslip, and proceed from there in your pre-set pattern of observation.

Remember, you want to test ACTIVITY of your tea once you know you are making tea with the bacterial and fungal biomass you need.

Definitions of the Levels of Biological Tea Quality

Bad: None to very few organisms observed. When examining 10 to 20 fields, only bacteria are observed, and less than 25 bacteria observed in each field. See picture below of "bad" tea.

Poor: More than 25 but less than 500 bacteria seen in each field. If patchy clumps of hundreds of bacteria are seen, then this puts the tea into the poor category. The key is that nothing other than bacteria, but high numbers of bacteria, are observed in poor, but not bad, tea.

Acceptable: Somewhere in the first 10 fields, at least one fungal hypha should be seen, preferably a thick hyphal strand, that goes across the full field of view. If only bits of hyphae are seen, then the sample is still in the poor category. More than 500 bacteria per field should be observed, with a range of bacterial shapes, from rods to cocci to picket fences observed (see pictures of bacteria below to get an idea of the different sizes and shapes you might see; the more sizes and shapes the better). Protozoa should be observed skimming across the water sample. Remember to focus up and down in the water drop because flagellates and ciliates like to hang-out at the top of the water drop, while amoebae like to ooze across the solid slide surface.

Very good: In the first 5 fields, a fungal hypha should be seen, and preferably more than one in that volume of liquid. Again, remember to focus up and down through the sample. Bacteria should be so numerous that you would not want to consider counting them. Thousands per field should be present, around the organic matter, zipping around in the liquid. The smallest critters zipping back and forth are mobile bacteria. Not all bacteria are mobile, but if you see them zipping back and forth, then you know you have active organisms. Unfortunately, you can't tell if they are aerobic, or facultative anaerobes, or true anaerobes, since all three categories of bacteria have motile representatives. Lots of protozoa should be seen, preferably more than one kind of protozoan – such as several species of flagellate or amoebae.

Excellent: Each field of view should contain a strand of fungal hypha. Thick strands are better than the skinny ones. Just like very good tea, the bacteria should be everywhere, and too numerous to count easily. Protozoa should be dense, with several individuals observable per field. In EXCELLENT tea, nematodes will be found as well. This general requires compost with great levels of nematodes. Nematodes will not reproduce in compost tea. The brew time is too short. If nematode eggs were laid in the compost, they might wake up during an extended holding period for the tea.

Reasons for poor quality tea:
There are many reasons for a lack of biomass of bacteria, fungi or protozoa in compost tea. If fungal filaments are encased in a layer of bacteria, they have been attacked by anaerobic bacteria, and it indicates that the sample has dropped below 6 micrograms oxygen per liter. Generally, the anaerobic bacteria consume the "innards" of the fungi hyphae, leaving the outer wall remaining behind. Sometimes even that wall will disintegrate, leaving no sign that fungi were ever present in the tea.

Protozoa and nematodes also cannot tolerate reduced oxygen conditions, and will generally move into a dormant condition if the reduction in oxygen occurs slowly enough. If the drop in oxygen is too fast, or lasts for too long, the organisms may be killed.

Poor compost quality (if there are no fungi in the compost, there will be no fungi in the compost tea), lack of food, high levels of consumers are additional reasons.

High salt levels in a component of the tea production can be visually assessed in some cases. High salt levels will show up as salt crystals in the tea. Bad smells, no active organisms, too low total biomass all suggest a problem during the tea brewing process.

A problem in many poorly designed compost tea makers is the inability of the fungi to escape the compost container. Larger opening size containers must be used. In general, 400 micrometers opening size or larger is needed.

If fungi are extracted into the tea, they can hang-up on "chunky" materials during filtration. If particulate materials escape the compost bag and are filtered, the fungi can be caught and held in that layer of particulate material instead of going out onto your plant surfaces. Use of screens that are washed, or sludge removed every few minutes are required if compost is put free into the tea, or if too much particulate material comes out of the tea maker.

Beware of tea makers where high amounts of compost have to be used. Compost is an inoculum for the tea and should not be viewed as the source of ALL the organisms in the final tea. If more than 7 to 10 kg, or 14 to 20 pounds, of compost for each 2000 litres of tea are used, suspect that the brewing cycle kills some of the organisms, either through lack of decent aeration during the brew, or inability to get the organisms out of the tea maker. Any tea maker using more than 2 to 3 kg per basket, or container (3 to 7 pounds), in a 400 to 2000 litre brewer, should be carefully questioned. Is this really an extractor masquerading as a brewer?

Compost extract is where the organisms are extracted from the compost, but no growth period is allowed.

Compost Tea includes extraction, but compost amounts used are minimal to achieve the same or higher biomass of organisms than an extract, because the brewing period allows the organisms to grow. The regulations under consideration suggest that compost or tea needs to be tested for human pathogens so you know that the compost and the compost tea are below significant risk levels for pathogens. Testing is especially necessary if the tea will be sold commercially, to protect the seller from claims that the tea might have been the source of a problem.

With the microscope set-up in this manual, pictures of each tea can be taken, to document that the biomass of beneficial organisms is present in the tea when sold. In conjunction with an *E. coli* test, any legal concerns about the quality of the compost tea should be alleviated.

The "standard curve" is useful as a general comparison with respect to quality. You don't need to quantify the organisms in every batch of tea, use a relative level to establish that the organisms are present. Then confirm that your reading is reflecting what you are seeing by sending samples every 10^{th} or 20^{th} brew to the SFI lab to confirm what you are seeing. Lots of critters? Excellent tea. Can't find any? Poor tea, don't sell it to anyone.

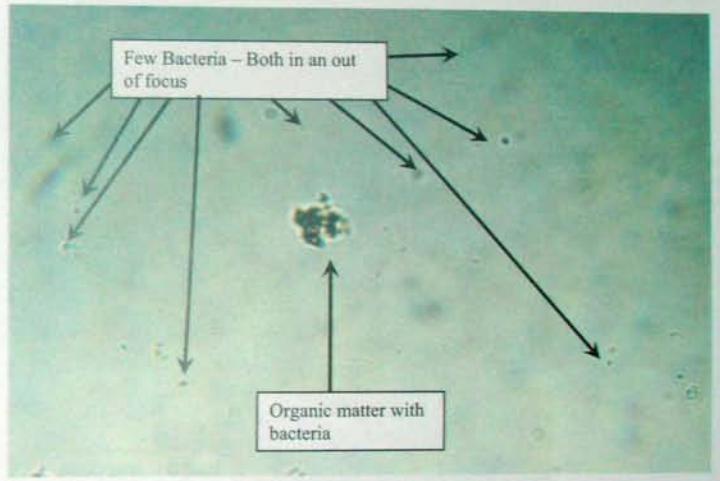
Can poor tea be "fixed"? Sure. Add more critters, add more foods, and test again.

Did you get the organisms in the tea? Check with your light microscope. When you think your tea is in great shape, send a sample into an SFI lab and confirm activity is what it needs to be.

Note: All the following pictures are 400X total magnification unless noted.

Bad Tea

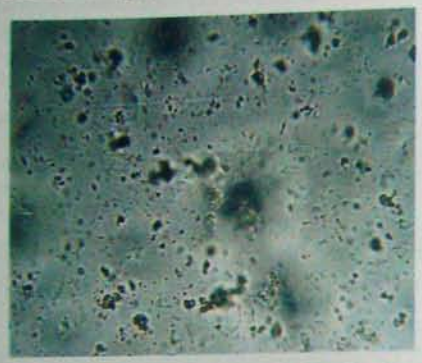
Throughout this tea, shown in this next picture, there were only a few bacteria per field, no fungi in any field observed, no protozoa and no nematodes in any field of view. At least 20 fields should be examined to make certain no fungal, protozoan or nematode biomass is present. Confirmation with SFI testing also showed only bacteria present, most of which were anaerobic (did not stain with FDA).



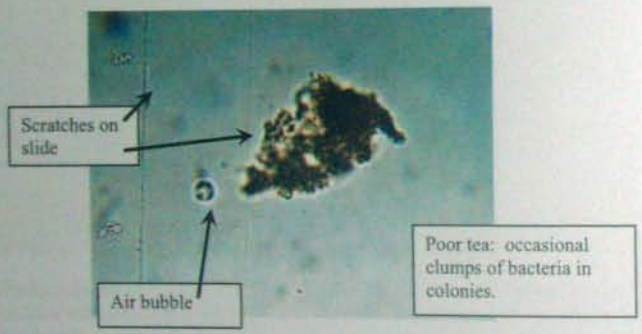
Fungi, protozoa and nematodes may have been present in the compost used for this brew, but during the brewing, anaerobic conditions most likely developed, and the aerobic organisms were lost.

Poor tea

While higher bacterial biomass can be seen than in the previous bad tea, the tea has only bacteria present. No fungi, no protozoa, and no nematodes were found in 20 fields of view. Confirmation with SFI testing showed no active fungi, protozoa or nematodes.

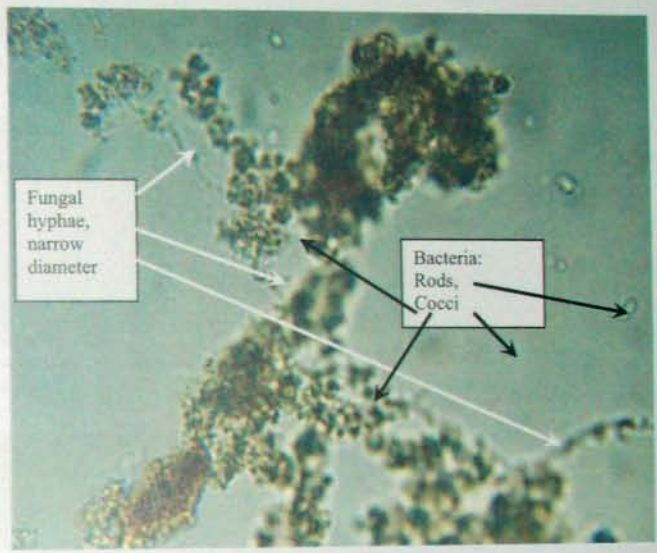


In this next sample, there is a good level of bacteria in the clump of organic matter, but only bacteria were observed in the sample. Again, confirmatory testing with SFI methods revealed no aerobic activity present.



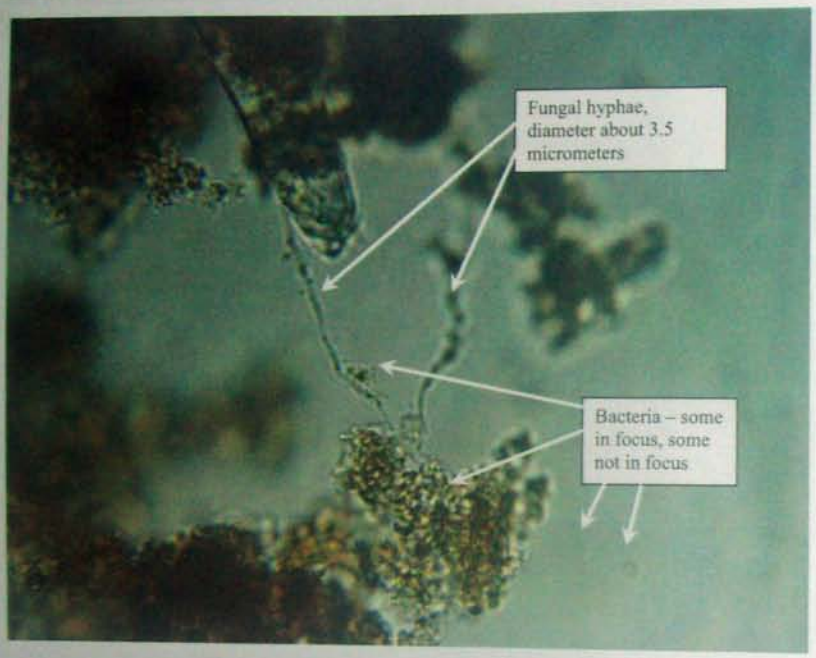
Acceptable tea

Ten fields had to be examined in order to find this single strand of fungal hypha. The diameter of the fungus was narrow, about 2 micrometers, indicating that it most likely was not the most beneficial of fungi. Bacteria are in good numbers (hundreds in this field alone), with rods and cocci of many different sizes and shapes. No protozoa or nematodes were observed anywhere in this sample.



Very Good to Excellent Tea

In the first field observed, and for the most part, in each field observed, fungi were visible, many bacteria were seen, and protozoa were present. The protozoa observed were flagellates and amoebae, not ciliates.



Examples of Organisms

In this section, examples of different organisms and what they look like, as well as other materials that are not organisms are shown. Training sessions are needed to help people identify what is being seen in the microscope. When in doubt, take a picture of the material in question, and send the picture to SFI. If we can't identify it in that fashion, send a sample of the tea into SFI to have a confirmatory sample performed, to figure out whether the material is a problem or not.

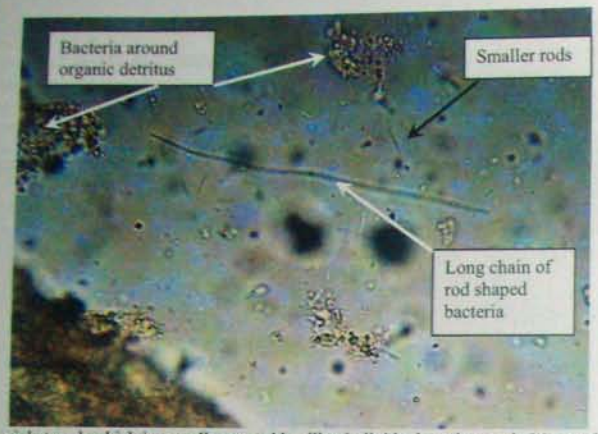
Bacteria

Bacteria can be round (called cocci), rounded rods (coccobacilli), rod shaped (rods) or spirillum (corkscrew shape) in shape. They are generally 1 to 3 µm long, and about half that width wide, if they are rod-shaped. They are typically 1 to 3 µm in diameter if cocci (round). Spirilla are long rods, 3 to 5 µm in length, and 1 to 2 µm in width, while bacilli can be short (2 to 3 µm) to very long (5 to 10 µm). Some bacteria will join together, and make two-bacteria "V"-shapes, or "picket-fence" structures (corynebacteria are famous for this). Yet other bacteria can form one-two-three or more bacteria together in long chains. Be careful not to mistake these for narrow-diameter hyphae! Look for the slight indents where the individual bacteria connect in order to differentiate this chain of bacteria from a narrow hypha.

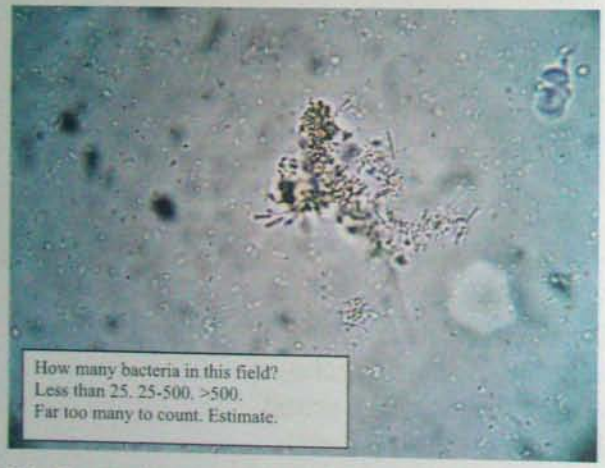
Some bacteria have flagella (short to long whip-like structures used for motility), and move rapidly around, generally in a back and forth motion, searching for food. Other bacteria are not motile, and at best can exhibit "Brownian motion" which is the result of vibration in the water film. The bacteria do not actually move anyplace.

Bacteria Criteria

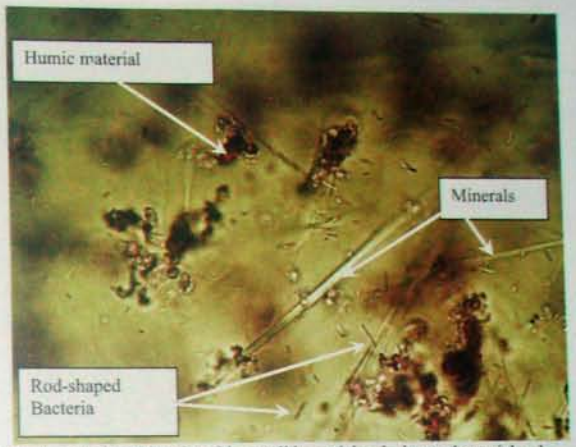
- Rod shape
  - Single rods, long, short, fat, thin, motile or not motile
  - Double rods (two in chain), motile or not
  - Linked rods, in chains
- Round (cocci)
- Coccobacilli (round rods)
  - Single, double, in chains
  - Motile or not, big, small
- Comma shaped (vibrio)
- Spirilla (wave shapes, highly mobile)
- Estimate < 25, 25-500, > 500



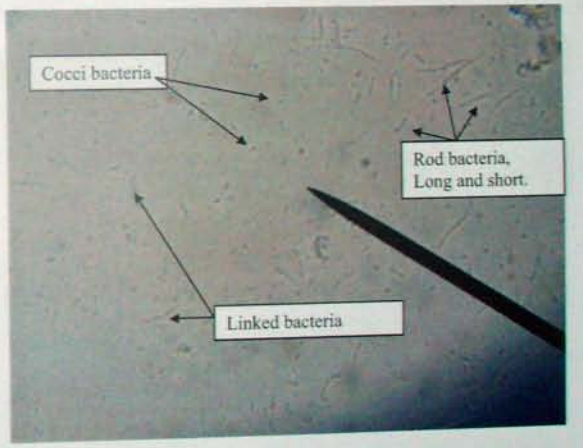
Long bacterial strand, which is actually several bacillus individuals end-to-end. Many other smaller individual rods, cocci, organic matter debris, and a large piece of organic matter in the lower left corner.



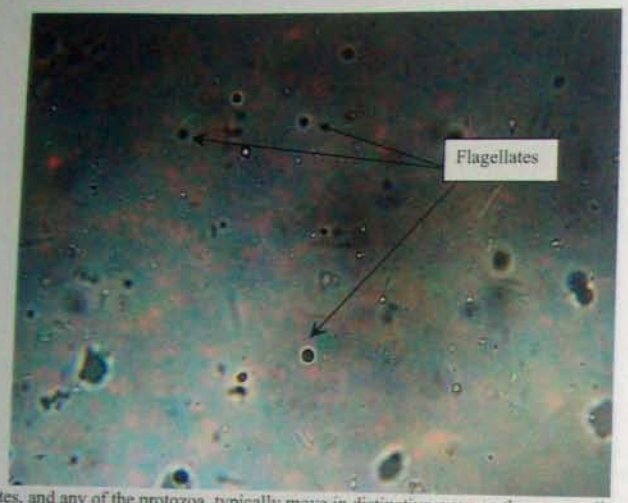
This picture contains many cocci bacteria. Note the rod bacteria surrounding the cluster of organic matter.



Mineral crystals, complexed humic acids, small bacterial rods, longer bacterial rods, many bacterial cocci.

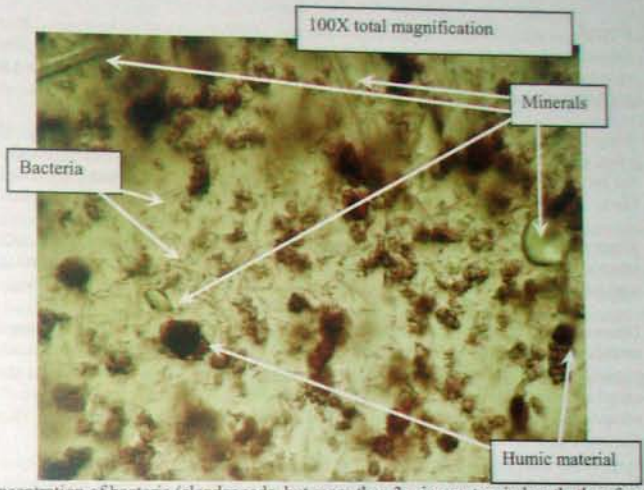


Many cocci bacteria and rod bacteria. Note the various lengths of rod bacteria. Some short, some long and some linked. Linked bacteria is observed as sharp angles.

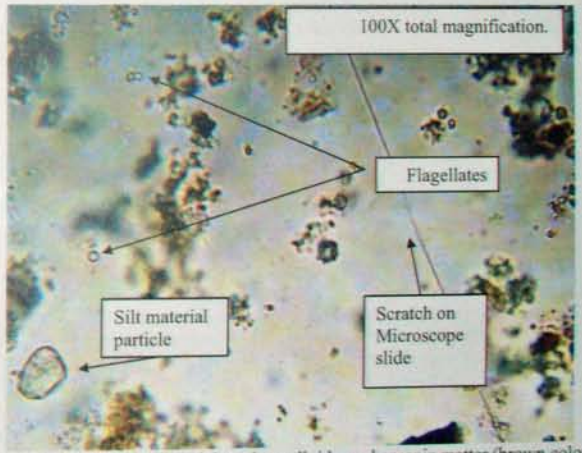


Flagellates, and any of the protozoa, typically move in distinctive ways so they are easily differentiated by movement from bacteria. Even the most motile of bacteria cannot move far in a short period of time. Many, many rods of many sizes, and cocci, from big to small in this picture.

The next picture is just left of the previous picture. Most of the bacteria are not in the same position, showing how many of the bacteria are actually moving. Find the four long bacterial rods, many shorter bacterial rods, many cocci of different sizes. A flagellate cyst (lower left), and three flagellates can also be seen.



High concentration of bacteria (slender rods, but more than 3 micrometers in length, therefore most likely Bacillus species) in a tea containing globules of complexed humic acid. Various soil minerals are present as well.



More bacteria, mostly around soil particles, clay colloids, and organic matter (brown colors). Mineral particle, protozoa, scratch on the microscope slide.

Fungi

Filamentous fungi always appear as long strands, thread-like, of various lengths. They can branch, or remain one single strand. Chains of bacteria cannot branch, so the branching is a good indication of fungal material.

Fungi can be clear (hyaline), tan, gold, reddish, light brown, dark brown, or black. The darker the color, the more melanin or humic acid materials the fungus has put into the cell wall. In general, the beneficial fungi will be dark colored – from tan to gold, to red to light brown, dark brown or black. This is not a perfect correlation, as some beneficial fungi are hyaline, or clear.

Many strands have a grainy or minutely rough appearance. This is because fungi will complex mineral elements on the cell wall, most often calcium for example, but could be other mineral materials as well. Calcium oxalate is a common crystal on the surface of many fungi.

Diameter of the hypha is another good indication of a beneficial fungus. The extremely slender strands are probably not fungi at all, but bacteria of one kind or another. Actinobacteria have extremely slender hyphae – from 1 to 2 micrometers diameter. The actinobacteria used to be called actinomycetes, but these hypha-forming bacteria are not fungi at all. Their cell-wall composition clearly puts them in the bacterial kingdom, not the fungal kingdom. Thus very, very slender strands are not fungi at all.

The "lower" fungi generally have slender hyphae, although not as slender as actinobacteria. Oomycetes, hyphomycetes, and many ascomycetes, which are all large-in-number groups of genera of fungi ranging from true pathogens to typical soil fungi, have hyphal diameters in the 2 to 2.5 micrometer range.

The truly beneficial fungi have wider diameter hyphae than any other group of fungi. Hyphal diameters in the 3 micrometer or larger category are almost always beneficial fungi. Dark color and large diameter means a beneficial fungus. The only exception to the large diameter beneficial rule are Rhizoctonia and related species. They can have hyphal diameters in the 3 micrometer range, but are always clear, or hyaline. They do not generally grow well in soil, generally existing as spores, until a plant root, or other plant material is present. They do not compete well with other true-soil fungi, so presence of the true soil fungi generally suppresses the disease fungi.

Look carefully at the strands of hyphae to see septa, or cross-walls. This is another good clue that a strand that you are looking at is a fungus. Most of the "lower" fungi (oomycetes for example, which is the group of fungi that contain most, but not all, of the pathogenic fungi) do not have regular septa. They make cross-walls at random. The beneficial fungi make cross-walls at regular distances along the hypha, another distinguishing characteristic of beneficial fungi. Unfortunately, not all beneficial fungi make regular cross-walls, so if there are no cross-walls observed, you cannot conclude "bad fungus". If you see regular distances between cross-walls, then you can conclude that this is a higher fungus, and most likely a beneficial one.

Fungal hyphae are ALWAYS even in diameter along their whole length. There cannot be any fraying, tattiness, splintered, rough or torn bits in hyphae. Organic material can be attached to the surface of the fungus, so be careful not to dismiss a hyphae because of organic material on its surface. Fungi do not bend at a sharp angle, although they can be broken, which will produce a sharp break. But hyphae do not fray at the break. Hyphae can branch, and the branched hyphae can be a smaller diameter than the main hyphal strand. In general it is only the higher fungi which will become smaller diameter when they branch.

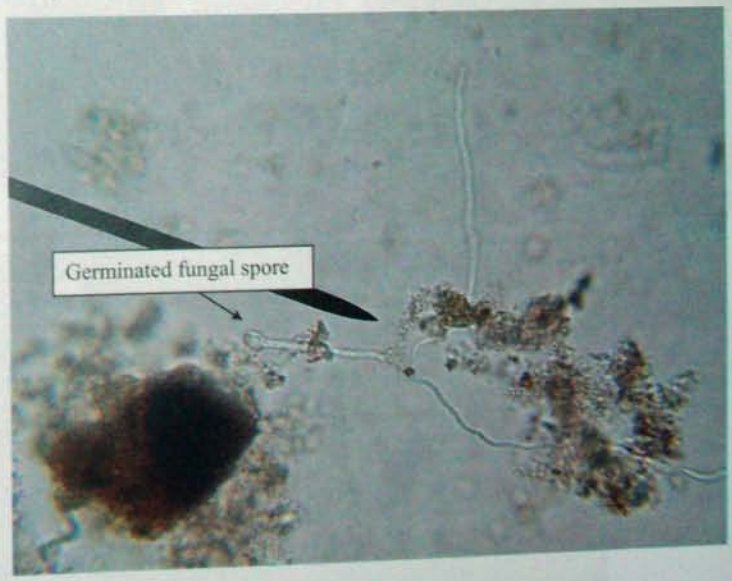
Hyphae will sometimes be cytoplasm-filled, and sometimes empty. The vacuoles inside the cell appear clear, of a less-dense nature than the cytoplasm-filled areas. The cytoplasm-filled area can grow and produce more fungal strands, while the empty areas cannot. But many fungi move cytoplasm along their hyphae, so what may be empty today can be filled tomorrow. Or in a few hours.

Fungi stream their cytoplasm along their "super highway" transportation system, which we know as hyphae. Fungi grow at the tips, so cytoplasm tends to be concentrated there.

Fungi produce spores, and these can be produced on the hyphae, on growing tips, between strands of fungi that meet and are of different mating types (chlamydospore production), or in fruiting bodies we call mushrooms or truffles. Higher fungi produce mushrooms, or truffles, so most of the fungi you encounter in agricultural soil which is disturbed by plowing will not be these higher fungi. Most of the fungi you encounter in healthy forest soils will most likely be the mushroom or truffle forming fungi.

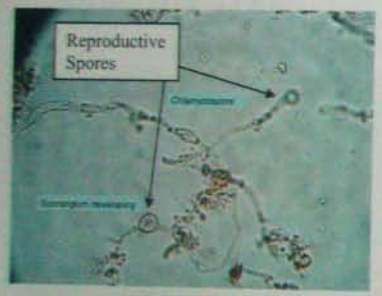
In the following pictures, magnifications are 400 X total magnification unless otherwise noted.

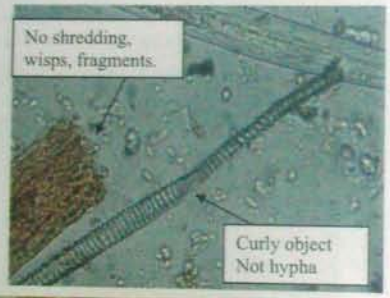
Fungal spore which germinated and is growing out to find food (humus particle). Many small cocci present, a few larger cocci, and organic material and humic acid present.

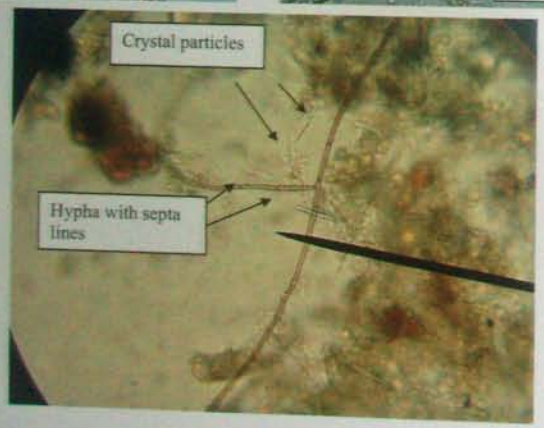


Fungal Criteria

- Spores
  - Dormant stage, single, double, triple
  - Germinating spores, VAM very tender, Trichoderma not
- Hyphae
  - Strands or threads, parallel lines, may branch
  - Septa, no structures inside hyphae,
  - No shredding, wisps, fragments, clear breaks
  - Not crystalline, rarely curled
  - Diameters
- Reproductive structures
  - Sporangia, conidiophores, chylamydospores



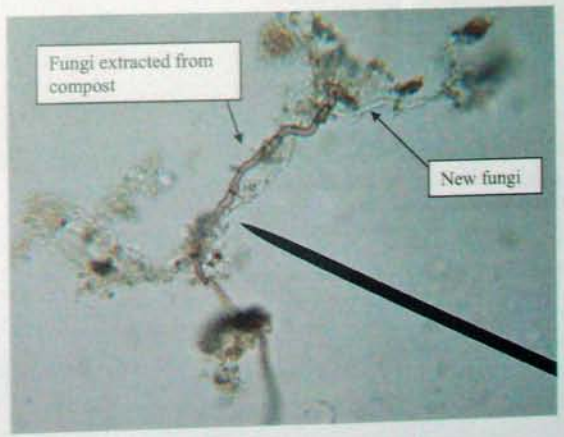




Fungal appearance

- When fungal growth occurs in the brewer, many active hyphae occur, branching common, hyphae soft, puffy with rounded growing tips
- When extracted from compost, usually no growing tips, cells walls thick, breaks sharp, look like fungi in healthy soil
- Anaerobic bacteria may attack hyphae, enmesh hyphae so completely covered, indicates low oxygen

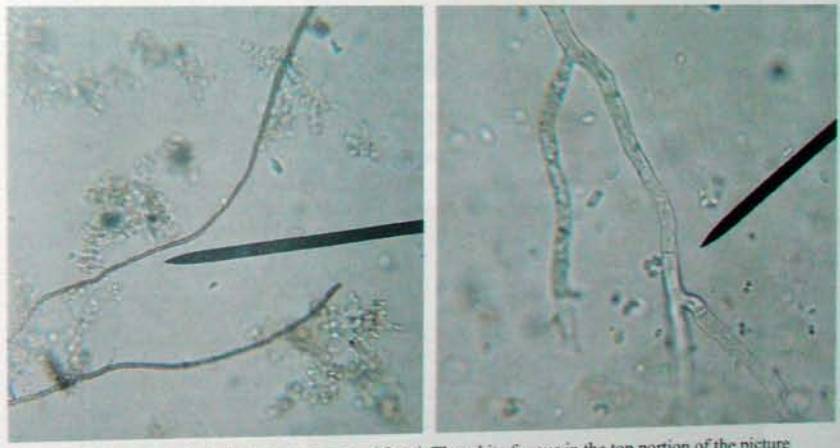
New fungi growing in compost tea. Branching common, hypha soft, puffy with rounded growing tips.



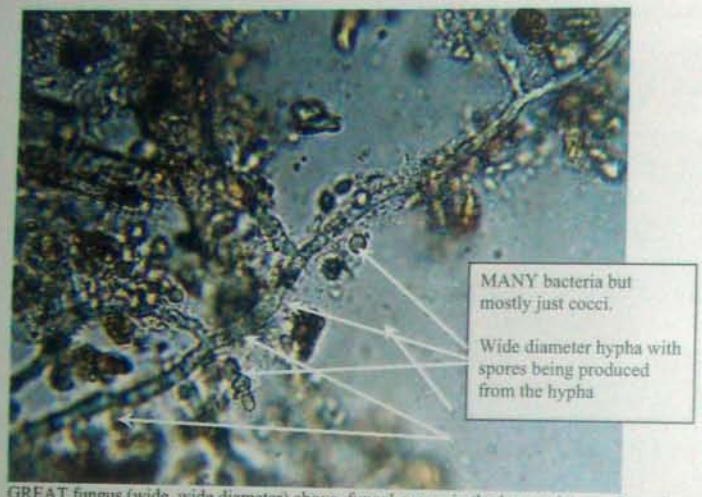
The following is a DIC microscope picture of a tea that dropped below 6 mg oxygen per litre. The fungi are being attacked by the anaerobic bacteria.

Fungal Categories

- Presence/absence in each field
- Diameter: Actinobacteria 1 -1.5 µm
  Oomycetes 1.5 – 2.5 µm
  Ascomycetes 2.5 µm
  Basidiomycetes > 3 µm
- Most beneficial hyphae are generally wide diameter, colored (tan, honey, golden, red, brown, dark brown)
- Hyphae stay uniform along whole strand

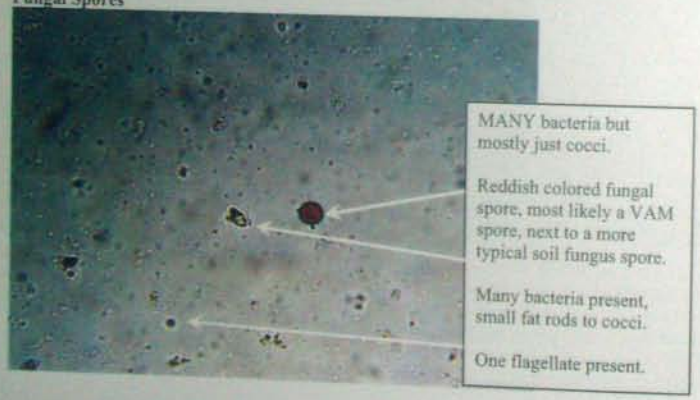


The picture on the left show two types of fungi. The white fungus in the top portion of the picture is a new strand of fungus. Thus having reproduced during the brewing cycle. The two dark brown strands have been extracted from compost during the brewing cycle. Diameter is approx 2.5µm.
The picture to the right is also a new strand of fungi. Note the much wider diameter.
The black pointer shown is approx 6µm. This is seen at 400X total magnification
In both pictures the hyphae is uniform long the whole strand.



GREAT fungus (wide, wide diameter) above, fungal spores in the lower picture. Fungal spores are fine in a tea to be used for soil drenching; they may germinate and grow if conditions are correct. But spores are not typically of much good in foliar sprays, as they do not germinate and grow rapidly enough on a leaf surface to provide protection.

Fungal Spores



Protozoa

Unicellular microorganisms motile through flagella, streaming or cilia. All protozoa consume bacteria, and some amoebae have been shown to attack fungi.

When conditions move beyond the species ability to tolerate, they will encyst and form a dormant stage. Too hot, too cold, too wet, too dry, lack of preferred bacterial species for consumption, high salts, toxic chemical presence are all things which can make protozoa "go to sleep".

Flagellates are the smallest (3 to 15 micrometers diameter usually) of the three types major groups of protozoa, but always larger than single-cells of bacteria. Two flagella per individual is the norm, and their movement is rolling, or looping when observed. Cysts are small and circular, about the size of the flagellate when active (called trophic stage), with a single thick wall around the cyst.

Amoebae move by putting out a pseudopod and drawing the rest of the body along. They can be very small, the size of a flagellate, to immense in size, 100 micrometers across the body. They can have shells, called tests, around their bodies that they build, or they can be "naked", without a shell. Look for the motion of the "fake foot" moving out from the amoebae body. Some amoebae look like stars, with pointed spike-like pseudopods. Other amoebae "bleb" the false foot suddenly. Others smoothly ooze. Some look like snowflakes, with thin filigreed threads making up the amoebal body. Amoeba cysts are double-walled and circular. The outer wall of the double wall is not always completely symmetrical but the inner wall is always very close to perfectly round.

There are "amoeboid flagellates" which bend and look amoebae-like, but have flagella. They are flagellates. There are some amoebae which have flagella, but the flagellum is used as a fishing rod to catch bacteria. Do the best you can to differentiate these amoebae from flagellates, but they are all strict aerobes, they all eat bacteria.

Ciliates numbers increase rapidly when oxygen drops below true aerobe levels and facultatively anaerobic bacteria start to rule. Is this because the anaerobes are the preferred food or is it because strict aerobes can't compete with ciliates for food when oxygen becomes limiting? We don't know.

Ciliates are the largest (10 to 200 micrometers) and fastest-moving of the protozoa. They are fun to watch. They can scare you as they come zipping out of organic matter moving at fast speeds. They plow their way through the sample, pushing soil particles, bacteria and other things out of the way. The cilia, which are hair like strands in their bodies, are used like oars to motor them through the soil or compost suspension. Most soil or compost ciliates are kidney bean shape (Colpoda), but hairbrush and vase shapes can be often seen. Vorticella are the vase-shape ciliates on stalks that set up vortexes with their cilia, then suddenly contract the stalk and shoot off into the suspension, looking for a better place to capture bacteria.

Ciliate cysts are large, rather clear, and single-walled. They can be mistaken for yeast.

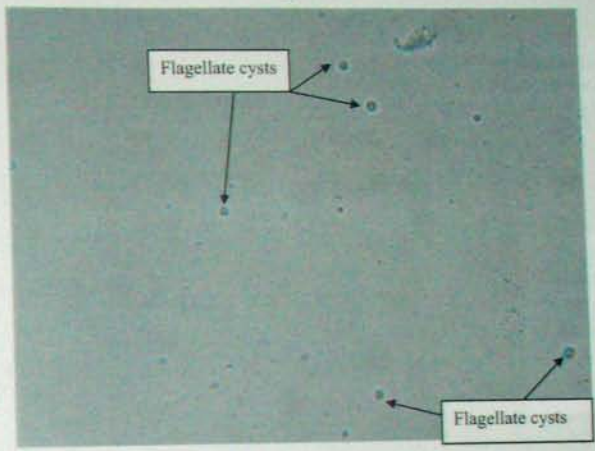
Protozoa

- Presence/absence
- What kinds? Flagellates, amoebae indicate aerobic conditions
- Ciliates – a few are Ok.
  - Lots of ciliate indicate anaerobic conditions. Bad news if making ACT

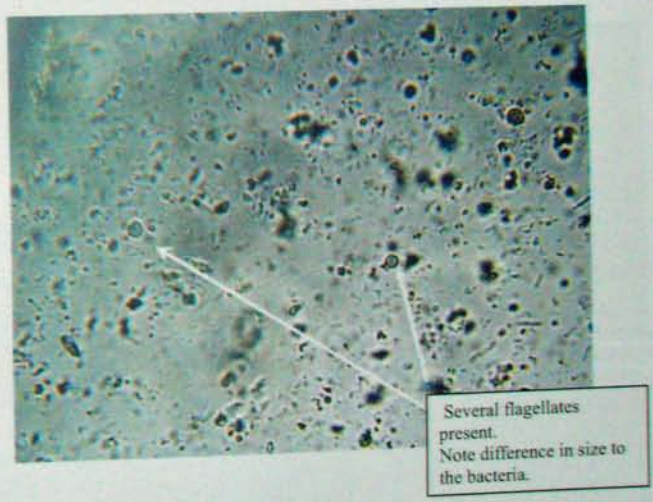
Protozoa Criteria

- want to see mix, high numbers reduce bacterial numbers
- Flagellates
  - Round, pear, teardrop, banana
  - Rolling, bumbling motion, one to several flagella
  - Cysts – single layer outer membrane, small, round
- Amoebae
  - Very slow oozing movement
  - Testate amoebae
  - Cyst – double outer membrane
- Ciliates
  - Very fast, many cilia all over cell, larger than other protozoa, stalked

Flagellates

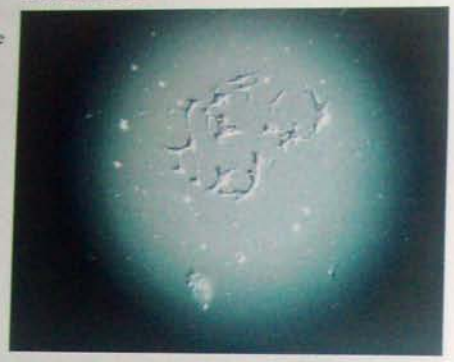


Flagellates cysts are much larger in size compared to the cocci bacteria in the background of this picture.

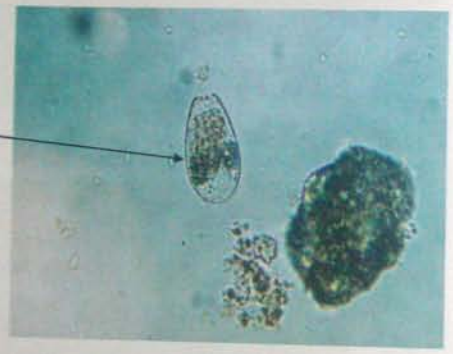


Amoebae

Amoebae are very slow moving for the most part. They like to bend, stretch and curve their bodies. They are the hardest protozoa to see in the microscope. The Iris diaphragm has to be set correctly in order to see these. This is a DIC picture taken on the SFI microscopes.

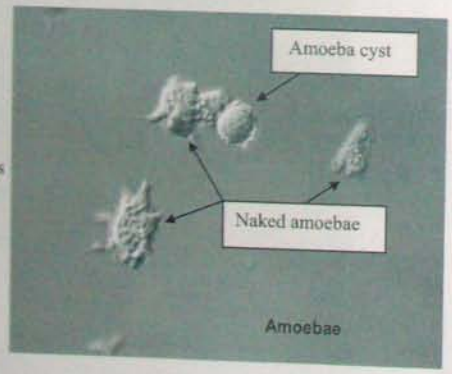


Testate amoebae have a hard shell like covering.
This type of amoebae does not change shape like the naked amoebae.

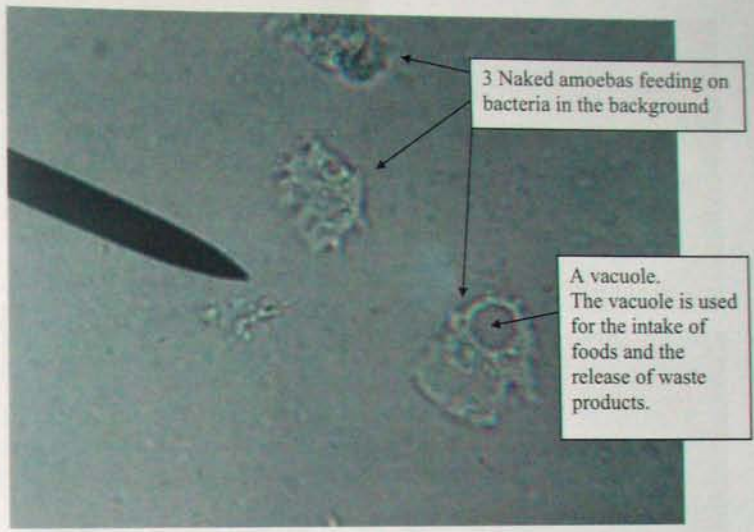


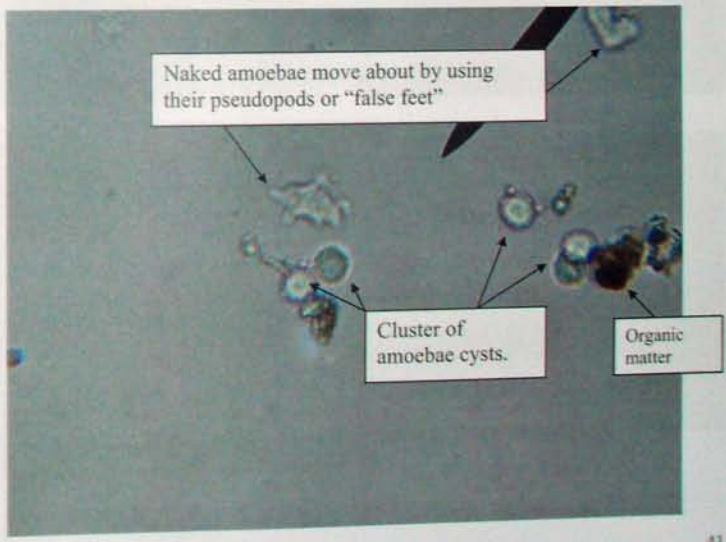
Naked amoebae and amoebae cyst.

Amoebae cysts are double walled and circular. The outer wall is not always symmetrical but the inner wall is always very close to perfectly round.



Amoebae

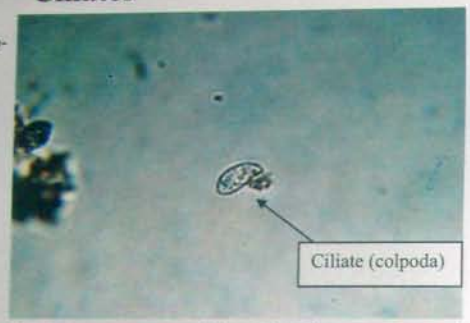




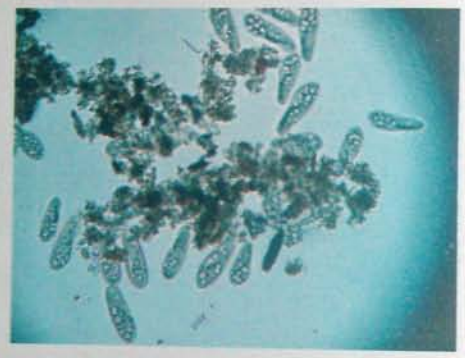
Ciliates

Ciliates are very large, 10 to 100 micrometers in length, and VERY mobile. These are the race car drivers of the soil.

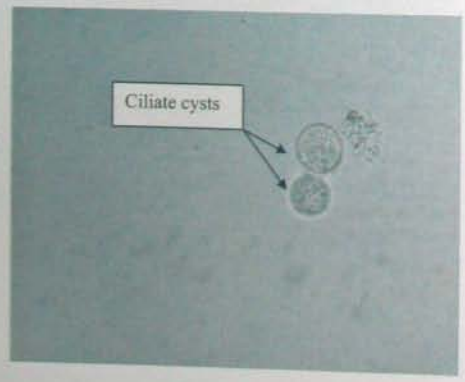
Note the bacteria INSIDE the ciliate.



Lots and lots of ciliates in this sample denote anaerobic conditions. There is fungal hypha in the middle of the thick clumps of organic matter and bacteria. But they are very hard to see as the anaerobic bacteria are consuming the fungal strands.

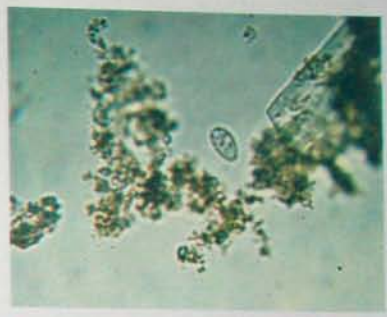


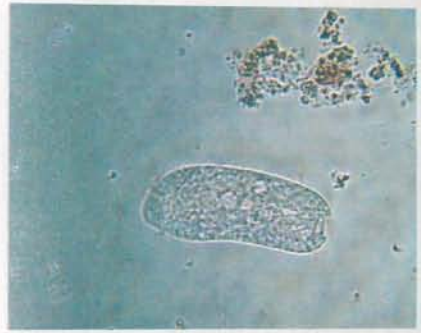
Ciliate cysts are very large in comparison to the other protozoan cysts. They are single walled and have a grainy appearance on the inside. This is the bacteria they have consumed. Sometimes you may see the ciliate rolling and spinning about within the cyst.



Ciliates

More ciliates, which come in all sizes and shapes.





Nematodes

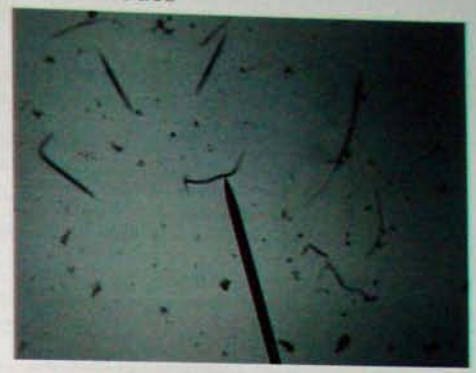
Nematodes are round worms that move like snakes. They need surfaces to attach to, but need a high percentage of water. In soil they live on the films surrounding soil particles and organic matter. There are four major groups of nematodes based on what they eat:

1. Bacterial-feeders
2. Fungal feeders
3. Predatory nematodes
4. Root-feeders

There is a group of nematodes that have the equipment to both attack roots and consume fungi. Do they eat fungi? Yes, they have been observed doing that. Do they eat roots? Yes, some have been observed doing that too. Do they switch from one food to the other? Why wouldn't they? When fungal hyphal biomass is low, it would be unlikely that they would chose to starve to death, given that they can attack roots of plants. But the actual event of switching has not been observed. Does that make it impossible that this happens? Is it important that switching occurs? Will plant production ever be harmed because switchers reach high numbers when fungal biomass is low? Anecdotal evidence suggests that this is a potential real-world scenario, based on work done in Idaho. Replicated trials are required, where fungal biomass, nematode numbers and plant production is controlled and monitored.

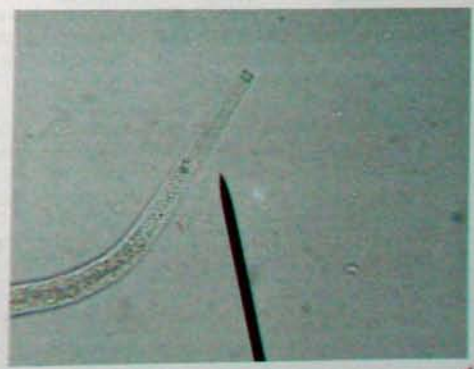
Bacterial-feeding nematode, tail in organic matter

Nematodes



Nematodes are one of the larger organisms to observe under the microscope. This image of lots of nematodes is taken at 40X total magnification.

Same image at 100X total magnification.



Same nematode at 400X total magnification.

Qualitative Assessment

To place compost tea into a category, you are required to look at 20 fields. A "field" is the whole image you see when you look into the microscope. Once you move to another location on the sample, this becomes another field.

The category of the compost tea is based on the presence or absence of organisms of the food web in 20 fields. On page 47 is the QA Data sheet for you to use as a guide to document and assess the tea.

There are 20 squares for each organism to be documented on the QA sheet. You do not count the organisms. This is a presence/ absence assay. You either see the organism in the field, or you don't.

Set the sample ready for analysis.

Begin at the top left corner of your sample. Not at the very edge of the cover slip but in a little from the edge.

This is field #1. Observe what you see and document.

Use the fine focus, moving up and down through the sample. Repeat this process several times while looking through the field. You can easily miss seeing organisms as not everything sit flat on the bottom of the slide.

In square #1 on the bacteria section of your sheet, place a tick if there is a positive sighting, don't count the bacteria, observe if there are say <25. 25- 500. or > 500.

Next, go to square #1(you are still observing the same field) of the fungi section and observe if you see true fungi. A tick for yes. A negative for no. It can range from a small piece of fungi to a long strand. Actinobacteria is also a positive, but is not classed as fungi. Note what is actinobacteria and what is true fungi.

Continue the same process with protozoa and nematodes. Once you have completed the first field, move the sample a little so you don't see the previous field and begin the same process for field #2

Move in one line down the slide for about 7 fields. THEN STOP.

Move to the centre of your sample and repeat another 7 fields, going down the sample. STOP.

Move to an area to the bottom right side of the slide and complete the remaining 6 fields.

You have completed 20 fields. This will give you a true representation of your tea sample.

DO NOT RANDOMLY GO LOOKING FOR ORGANISMS IF PLACING THE TEA INTO A CATEGORY.

Begin with the bacteria section of the sheet and count how many squares have a positive sighting. Each square is valued at 5%. If all 20 squares have a positive sighting of >500 bacteria per field you have an excellent rating for bacteria alone.

Repeat the same process for fungi and count the number of squares with positive reads. Note the percentage in the fungi section. Do the same for protozoa and nematodes.

The overall rating of the tea is based on the lowest percentage of any organism given. Nematode percentage is not an overall indicator as nematodes cannot reproduce during a brewing cycle. If you see nematodes this is a bonus.

Eg: You have an excellent rating (100%) for bacteria, 35% fungi, 10% protozoa and no nematodes, this tea would rate as an adequate tea. The lowest percentage is the deciding factor.

Eg: Bacteria is 100%, Fungi is 0%, and protozoa are 40%. This would rate a poor tea.

Use the Qualitative categories chart to place the tea in the category.

THE LOWEST PERCENTAGE IS THE DECIDING FACTOR.

Qualitative Assessment

Sample Date: **Identifier:**

Bacteria	Observed	1	2	3	4	5	6	7	8	9	10
Rating		11	12	13	14	15	16	17	18	19	20
Fungi	Observed	1	2	3	4	5	6	7	8	9	10
Rating		11	12	13	14	15	16	17	18	19	20
Protozoa	Observed	1	2	3	4	5	6	7	8	9	10
Rating		11	12	13	14	15	16	17	18	19	20
Nematodes	Observed	1	2	3	4	5	6	7	8	9	10
Rating		11	12	13	14	15	16	17	18	19	20

Rating: Excellent Very Good Good Acceptable Poor Bad

Notes: _____

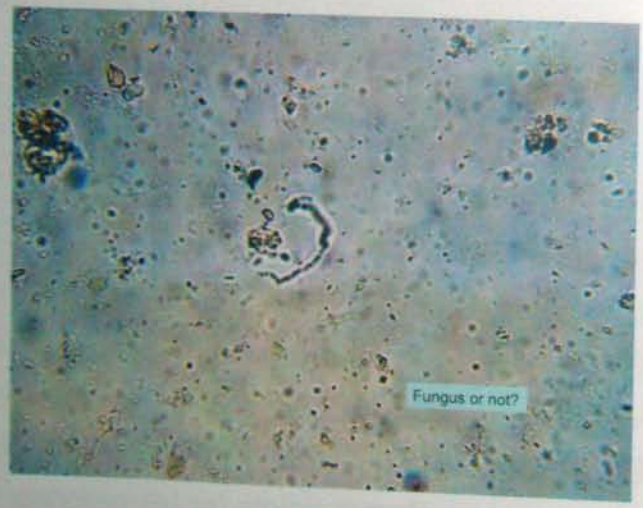
Qualitative Categories

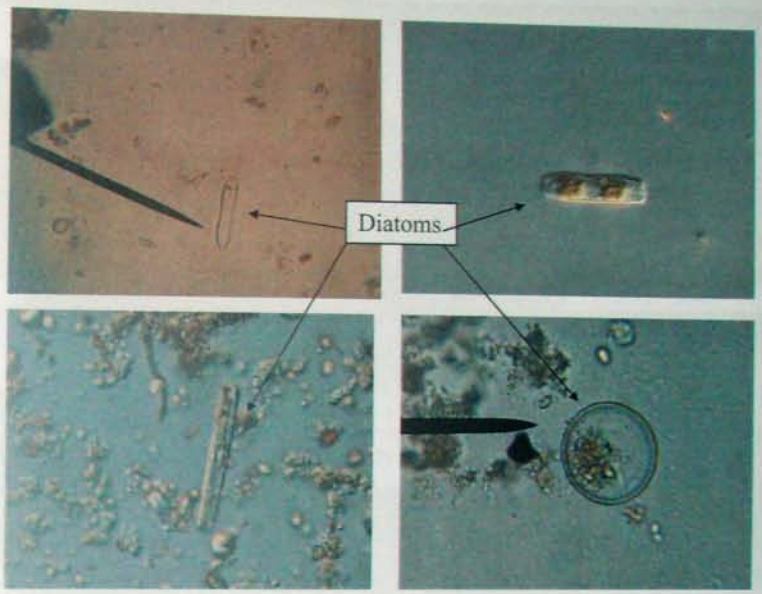
	B/field	F/field	P/field	N
Bad	< 25	0%	0%	0%
Poor	25 – 500	0%	0%	0%
Adequate	>500	5%	5%	0%
Good	> 500	20%	20%	Maybe
Very Good	>500	50%	50%	Maybe
Excellent	> 500	100%	100%	Maybe

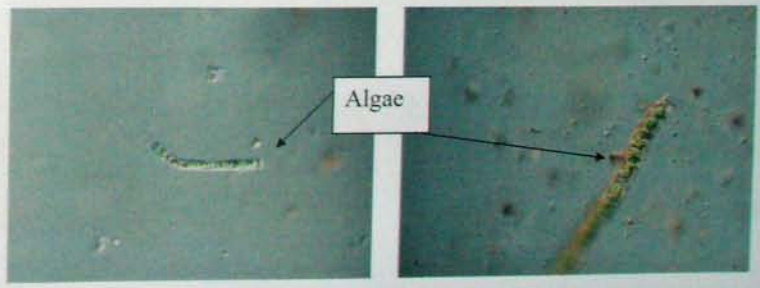
Bonus points for Nematodes

Other Materials

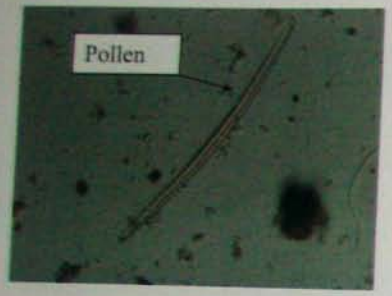
- Fibers – often torn, textured, twisted, flattened
- Roots – cells, stele and cortex
- Crystals – calcium carbonate, silica,
- Diatoms – photosynthetic, square cells, filigree
- Alga, algae – chlorophyll, symmetrical looking plant cells, filaments
- Organic Matter – brown, chunky, humics
- Insect larvae – segmented, accordion movement
- Yeasts – filaments, budding, larger than bacteria, often anaerobic

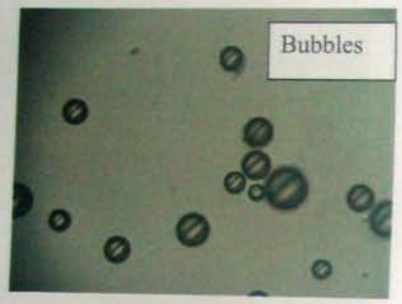


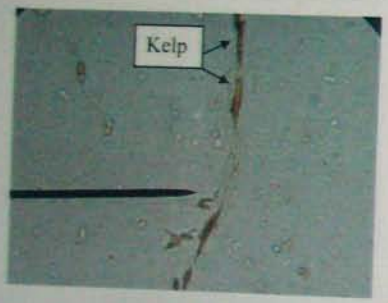


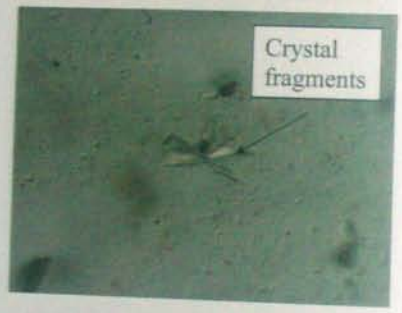


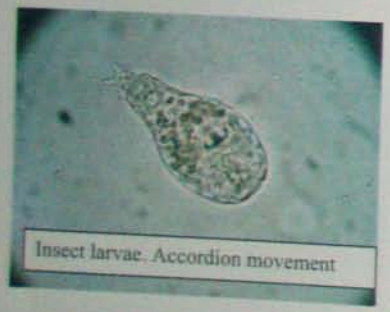
Other Materials

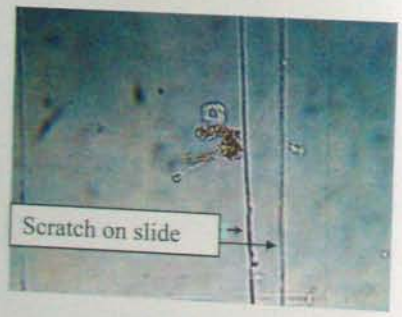








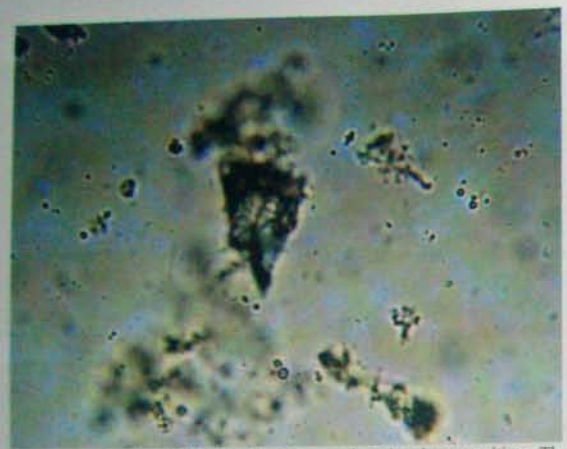




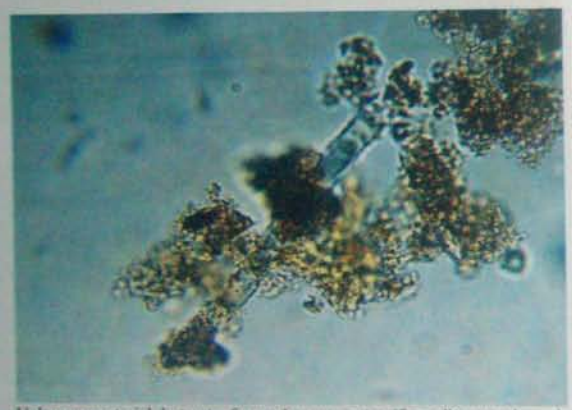
Non-organism materials

This may look like fungal hyphae, but diameter does not remain constant and there is a metallic quality to it. This is a thread from a newspaper which was treated with strong acid, denaturing the organic matter and making it more difficult to decompose.

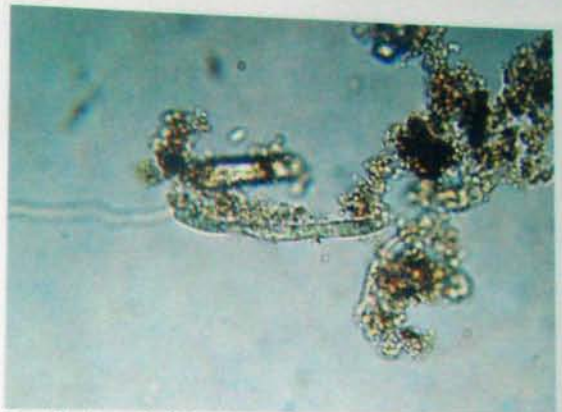
Plant root in the process of being decomposed. Not uniform diameter, very frayed and becoming amorphous.



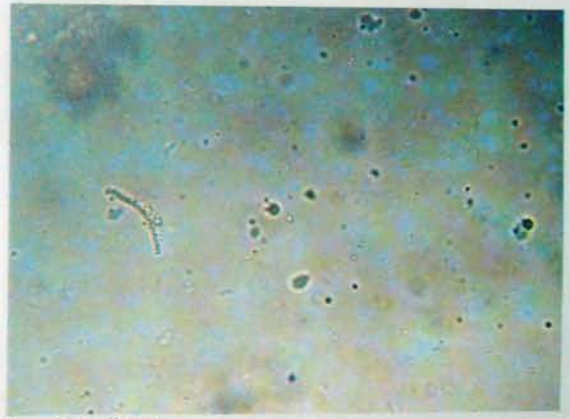
Many bacteria, plant debris in mid-picture, undergoing decomposition. The strand to the right of the plant material is too narrow diameter, and not consistent diameter to be a fungus.



Unknown material, but not a fungus because not uniform diameter through whole length.

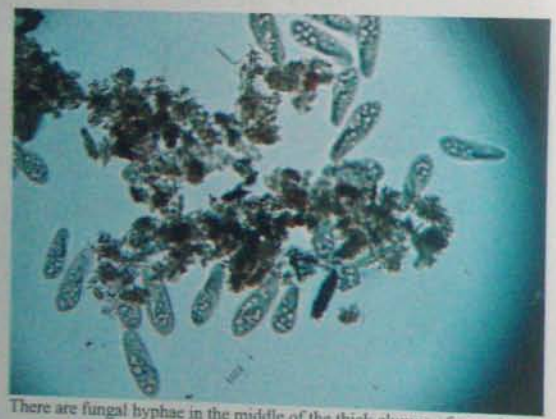


Fungal hyphae to the left and below right of the non-fungal material. Probably a shed cuticle from a nematode, in center field.



Not uniform diameter.

More algae, more decomposing organic matter, more bacteria, but no fungal hyphae.



There are fungal hyphae in the middle of the thick clumps of organic matter and bacteria, but they are very hard to see as the anaerobic bacteria are consuming the fungal strands. The ciliates are having a grand time eating the anaerobic bacteria. Beneficial aerobic organisms do not survive for long in anaerobic conditions, not because of the lack of oxygen, but because of what anaerobic organisms do, and the metabolites they make.

Center field is an insect larva. The "accordion tail" is characteristic, with the hold fast attached to the slide, and the head of the larval insect in the organic debris. Two ciliates of the group Mastigophora are also in the field. Insect larvae are quite common in compacted soils, when lack-of-oxygen restricts the growth of the beneficial microbes.

Another nematode – a bacterial feeder – in a tea with many ciliates, plant debris, and organic matter. Organic matter is just another name for plant debris that has been so decomposed by microbes that the original plant from which it came cannot be determined.

Cleaning Up

When you take a slide from the microscope stage, always dial back in the 4 X lenses, or the empty objective position, so you don't accidentally scratch a lens.

Take the coverslip off the slide. You can clean coverslips, but you will have to be very careful to not break the slip, and cut your hands.

Wash the coverslip and the slide in warm water with a little soap. Rinse in clean water. Dry, and store.

Microscope slides can be easily washed with water, dried and stored.

Pipettes need to be washed thoroughly to ensure no cross contamination. Rinse any residue from inside the pipette. Draw water up, shake and empty. Do this several times. Have a vessel containing a weak solution of bleach and draw this liquid into the pipette. Leave in the bleach solution for approx 20 minutes then rinse the inside of the pipette thoroughly (at least 3 times with clean water)

Use the cotton swabs to dab the microscope lenses, GENTLY. No wiping motions. If oil or other viscous material gets on the lens, or on other glass parts of the microscope (condenser, eyepieces, bottom light), put a drop of xylene or dilute alcohol on a swab, and clean the glass with the swab, gently wiping the oil from the lens. Then remove any remaining liquid with a clean swab.

ALWAYS replace the dust cover on the microscope. Dust and dirt will destroy the mechanisms in the microscope. KEEP DUST OUT!!!!

In a year, call a microscope service to clean and align the microscope to maintain maximum performance.

Reading materials

Andrews, J.H., And S. Hirano. 1991. Microbial Ecology of Leaves. Springer Verlag.
What's happening on the leaf surface?

Darbyshire, J. 1994. Soil Protozoa. CAB International.
THE textbook on soil protozoa and what they do in soil.

Dindal, D.L. 1990. Soil Biology Guide. John Wiley and Sons.
Taxonomic guide to ALL the organism groups in the Foodweb. Good pictures.

Hall, G.S. (ed). 1996. Methods for the Examination of Organismal Diversity in Soils and Sediments.
CAB International.
Excellent compendium of classic methods for examining microbes in soil.

Kilham, K. 1994. Soil Ecology. Cambridge University Press.
Pictures, drawings, descriptions of processes of all the organisms in the soil food web.

Paul, E.A., and Clark, F. E. 1989. Soil Microbiology and Biochemistry. Academic Press.
THE CLASSIC Soil food web book.

Richards, B.N. 1987. The Microbiology of Terrestrial Ecosystems. Longman Scientific & Technical.
Interrelationships between soil organisms; descriptions of organism groups.

Soil Science Society. 1994. Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. SSSA
The standard testing procedures in soil science

Stolp, H. 1988. Microbial Ecology: Organisms, habitats, activities. Cambridge Studies in Ecology.
Basic text, descriptions of functions, metabolism of bacteria and fungi, classic nutrient cycles.

Journals to look for:

Applied Soil Ecology
Biology and Fertility of Soils
Soil Biology and Biochemistry
Agriculture and the Environment