Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 35, 240-255, 2014

Cheap & Easy Diversity for Introductory Biology

Mary Ann McLean

St. Mary's University College, 14500 Bannister Rd. SE, Calgary AB T2X 1Z4 CAN
(**maryann.mclean@stmu.ca**)

Diversity is an important component in our Introductory Biology sequence. However, bringing home the idea of biodiversity can be a challenge: students relate biodiversity to programs on the Discovery Channel and have little concept of their local biodiversity. Creating Winogradsky columns during the first week of labs allows students to investigate their local diversity of microbes and invertebrates over the next eight months. Each student constructs a Winogradsky column from soil and water and includes sources of carbon, sulfur, and other minerals. Column communities and diversity differ significantly depending on the source of the original soil and water, the nutrients supplied and the proportion of masking on the column. During the year, the students sample their columns for bacteria, cyanobacteria, protists, algae, fungi, moss, plant seedlings and small invertebrates observing a wide diversity of organisms in their own and in other students' columns. In addition to promoting discussions of food webs and ecological communities,the value to each student of a personal Winogradsky column using the student's own garden or local soil was tremendous in increasing knowledge and enthusiasm about biodiversity.

Keywords: Diversity, microbes, invertebrates, Winogradsky columns, bacteria, protists, ecological community

Introduction

Diversity is an important component in many Introductory Biology courses. However, bringing home the idea of biodiversity to first year students can be a challenge: many students relate biodiversity to programs on the Discovery Channel and nature videos about Madagascar and the Amazon and may have little concept of their local biodiversity.

The Winogradsky column was developed in the late 19th century by the Russian microbiologist Sergei Winogradsky to grow aquatic and sediment microbes in semi-natural communities (Atlas & Bartha, 1998, Barton & Northrup, 2011). Essentially, the method is to fill a glass or plastic column 1/3 with sediments or soil and fill the rest of the column with water. These columns have been used extensively to examine the relationships between bacteria, especially those involved in sulfur transformations. Since soils and sediments contain a wide diversity of other organisms (e.g. fungi, cyanobacteria, algae, protists, rotifers, nematodes, mites, crustaceans, insects, and earthworms), these columns can also be used over several months in Introductory Biology labs to investigate the diversity of other phyla. Soil has been called the "poor man's tropical rainforest" in recognition of the diversity of soil micro-arthropods, largely mites and collembola (Giller, 1996). However, when other groups are included (e.g. fungi, cyanobacteria, algae, protists, rotifers, nematodes, crustaceans, insects, and earthworms), biodiversity in these columns becomes very high and they are a very useful way to introduce students to the biodiversity of local ecosystems.

The creation of Winogradsky columns during the first week of labs in the fall allows students to investigate their local diversity of microbes and invertebrates over the next 8 months. Each student constructs a Winogradsky column from soil and water and includes sources of carbon, sulfur, and other minerals. Columns are maintained on the lab windowsill throughout the school year. Column communities and diversity differ significantly depending on the source of the original soil and water, the nutrients supplied and the proportion of masking on the column. During the year, the students sample their columns for bacteria, cyanobacteria, protists, algae, fungi, moss, plant seedlings and small invertebrates (e.g. nematodes, mites, insect larvae, rotifers, crustaceans, earthworms). Not only does each student observe a wide diversity of organisms in their own column, but they also observe a different suite of organisms in other students' columns. These columns also permit discussions of food webs and ecological communities. For each student, the value of having his or her own Winogradsky column using his or her own garden or local soil can be tremendous in terms of knowledge and enthusiasm.

The columns are set up during the first lab in the fall; this takes about 20 minutes. They are first sampled about 4-6 weeks later. The method is both easy and cheap and the col-

umns, if watered occasionally, can continue to develop for years. If windowsill space is scarce, groups of 2 to 4 students could share a column, or artificial lights could be used.

This method is appropriate for use by senior high school students, first year undergraduate students, and upper level undergraduate students in biology, microbiology, microbial ecology, and invertebrate zoology.

This is a very flexible, open-ended inquiry activity which can be used to explore diversity in general or to answer specific questions about diversity. For example, each student could detail the location and type of soil/sediments and water for comparisons of invertebrate diversity between different environments. Microbes present in columns with and without a particular nutrient (e.g. sulfur) could be compared. If facilities permitted, studies of aerobic (near the surface) and anaerobic (at the bottom) microbes could be conducted.

The use of these columns is limited only by the taxonomic expertise available. The key provided as an Appendix will give a good start to anyone. If more detail is desired, there are many keys and guidebooks available (see list of references).

Student Outline

Background

The Winogradsky column was developed in the late 19th century by the Russian microbiologist Sergei Winogradsky in order to grow aquatic and sediment microbes in semi-natural communities (Atlas & Bartha, 1998, Barton & Northrup, 2011). Essentially, the method is to fill a glass or plastic column 1/3 with sediments or soil and fill the rest of the column with water. These columns have been used extensively to examine the relationships between bacteria especially those involved in sulfur transformations. Soils and sediments also contain a wide diversity of other organisms and we will use these columns over several months to investigate the diversity of many phyla.

Soil has been described as the "poor man's tropical rainforest" (Giller 1996) due to its high biodiversity. Soil is teeming with species: microbes such as bacteria, fungi, protists, viruses, cyanobacteria; plants such as algae, mosses, liverworts; and invertebrates such as rotifers, nematodes, earthworms, crustaceans, and insects. Many of these are crucial to decomposition of organic matter and nutrient cycling. Although we think of bacteria as "germs"and causes of disease, only a small proportion known bacterial species actually cause disease or can even live at human body temperature. The vast majority of prokaryotes participate in nutrient cycling or are food for other species (Atlas & Bartha, 1998).

We will construct Winogradsky columns to observe some of these species. As we sample our columns through the year, think about the total diversity you observe in this sub-sample of your local ecosystem.

Materials

- Several pairs of scissors and utility knives
- Black construction paper
- 1 roll plastic film
- Elastics
- Tape

Procedure

Bring the Following from Home:

- A clean 2 L pop bottle (clear colorless is best)
- 1 L of soil, sediment, compost or mud (potting soil does NOT work well; it is usually sterile)
- 1-2 L of water from a stream, pond, well, lake etc (tap water does NOT work well due to the chlorine)
- 5-10 g of a C source (e.g. shredded newspaper, straw, grass clippings (not treated with herbicides))
- 5-10 g of a S source (e.g. egg yolks or shells, broccoli, onions, gypsum)
- 5-10 g of an Fe source (e.g. iron filings, rusty nails)
- 5-10 g of a Mg source (Epsom salts)
- 5-10 g of a N source (plant food with only N)

Constructing a Winogradsky Column

1. Use scissors or a utility knife to cut the top off the pop bottle at the shoulder to produce a cylindrical container.
2. Fill the bottle 1/4 full of soil/sediments.
3. Add a layer of the C source.
4. Fill the bottle 2/3 full with alternating layers of soil/sediments and the S, or other nutrient sources.
5. Add water to the bottle until it saturates the soil/sediments and fills half of the remaining height of the bottle. This step may have to be repeated since it takes time to soak into dry soil/sediments.
6. When you are done, you should have a column 2/3 full with soil and a layer of water on top.
7. Since the availability of light will determine what photosynthesizers can grow, you can modify this by covering part of the outside of your column with black paper.
8. Cover with plastic film and secure with an elastic band. This will reduce the smell as microbial communities develop.
9. Label your column with your name so it is easy to find it again.
10. Since the communities of organisms will be developing over the next 8 months, it is important to add water periodically so the column does not dry out. DO NOT use tap water.

Looking for Organisms in your Winogradsky Column

Most invertebrates will be found in areas where there is organic matter, since this can be a food source or the food of their food source. If the water is not too deep, aerobic invertebrates may be found at the water/soil interface or growing around the edges of the top of the column.

Green photosynthesizers such as plants, moss, algae, and cyanobacteria are often found on the surface of the column or around the edges of the column where they have been exposed to light and have turned green.

Sampling your Winogradsky Column for Bacteria or Fungi

To sample the water at the top of your column use a sterile swab, soaking it in the water and rolling it around the edges of the column. Streak the swab over the surface of a petri plate. Incubate for 1 week and observe your plate.

To sample the soil at the top of your column, moisten a sterile swab with sterile water and roll the swab around on the soil surface. Streak the swab over the surface of a petri plate. Incubate for 1 week and observe your plate.

Sampling your Winogradsky Column for Protists

These are likely found in the water at the top of your column, near the edges where there is some organic matter. Use a pipet to collect some water in these locations and make a wet mount slide. Sometimes these organisms move very fast. It can be helpful to add a small bit of shredded cotton ball to the slide to confine the organisms to one area for better viewing. Or use one of the compounds (e.g. Detain or Protoslo) which slow down the movement of these species.

Sampling your Winogradsky Column for Invertebrates

Many of the invertebrate groups are easily crushed if you use ordinary microscope slides and a cover slip. Therefore it is much better to use cavity slides.

For other organisms, you will have to take a sample of water or soil or both from your column. You can either sample using a pipet or a petri plate. If your column is particularly rich, you may find that simply taking a pipet full of water and organic material from the edges of your column and placing it on a cavity slide will be sufficient. View with a compound scope. The other method uses a glass petri plate. Using a spoon, get a few spoonfuls of soil/sediment and place it at one side of the petri plate. Fill the rest of the plate a couple of mm deep in water from your column or distilled water. Wait for 5-15 minutes while the fine sediments settle and view the water/sediment interface for organisms under a dissecting scope. This method is good for many invertebrates. Organisms you want to look at can be picked up with a pipet (for bigger organisms cut the tip off the pipet so you can suck them up) and placed on a cavity slide.

Cleanup

Remove all slides from your microscope. Wash live animals from cavity slides into the fish tank. Wash all petri dishes, probes, spoons and cavity slides and place in the drying rack. Throw out disposable pipettes in the garbage and used cover slips in the glass bucket.

Materials

Column Construction

Per Class of 25

- scissors, utility knives
- 1 box plastic film
- elastics
- 25 sheets black construction paper
- tape
- a few extra 2 L pop bottles (in case some student forgets or can't get this)
- extra"live" soil/sediment (in case some student forgets or can't get this)
- extra"live" water (in case some student forgets or can't get this)

Diversity Studies

Per Class of 25

- compound microscopes - enough for 1 per pair
- 1 ml & 3 ml disposable plastic pipettes
  - plastic are best so you can cut the tips off to collect larger organisms without damage
- 10 long handled spoons for sampling
- fine & blunt probes (enough for 2 each)

Per Student

- 4 glass petri dishes
- 2 cavity slides
- 2 cover slips
- dissecting microscope

Bacterial Studies

Per Class of 25

- 25 or more petri dishes containing agar: the type of agar will determine the types of bacteria isolated
  - Check a *Handbook of Microbiological Media* (any edition) for different types of agar
  - Use of nutrient agar will encourage isolation of a wide variety of common bacteria
  - Use of *Beggiatoa* agar will encourage isolation of *Beggiatoa*, a common white S oxidizer
  - There are media to isolate sulfate reducers, hydrogen sulfide producers, iron oxidizers etc.
- incubator set to 28°C
- sterile swabs
- tubes of sterile water (1 per pair)
- 1 ml & 3 ml disposable plastic pipettes
- bacterial spreaders

Per Student

- 1 compound microscope

Fungal studies

Per Class of 25

- 25 or more petri dishes containing agar: the type of agar will determine the types of fungi isolated
  - Check a Handbook of Microbiological Media for different types of agar:
  - Use of oatmeal agar will encourage the isolation of fungi & actinomycetes
  - Use of malt extract agar will encourage the isolation of yeasts & fungi
- incubator set to 28°C
- sterile swabs
- tubes of sterile water (1 per pair)
- microscope slides & coverslips

Per Student

- 1 compound microscope

Notes for the Instructor

Safety

While most organisms living in soil do not also live at human body temperature or become pathogenic, it is possible that some species of bacteria or fungi may pose a hazard to students. Make sure students don't touch their microbes and that they wash their hands carefully after the lab.

It may be necessary to contact your institution's safety personnel for advice on precautions to take when having students work with Winogradsky columns.

Organization

Students generally enjoy searching for organisms in their columns, particularly if they have had good luck in the past. The key is patience. Students who are not willing to spend the time to carefully scan water or soil/sediment samples often see very little and are consequently very frustrated. Sometimes you can find a few things for them so they catch the excitement and are then willing to take more time.

When a student finds something interesting, announce it and encourage the rest of the students to come and look. Students find it amazing to watch rotifers or *Vorticella* swirl the water around and suck food particles in.

When the students will be sampling their columns as part of a lab on diversity of invertebrates, for example, encourage them to look at the other slides and samples in the lab first since they can spend hours looking at samples from their Winogradsky column.

Since most of the fascination with these columns is from observations of live animals, it is important to treat the animals respectfully. This includes using cavity slides, keeping the microscope light low while observing organisms and turning it off when no one is actually observing them. This also includes not just washing them down the drain. We have

a large "fish tank"in the lab into which I ask students to wash their cavity slides, explaining that I want to increase the diversity of the tank. Please consult your institution's animal care policies to make sure live animals are handled properly during this study.

To get the most diversity in these columns, it is helpful to place them in a window or under lights so that some species are photosynthesizing and producing oxygen. The addition of nutrient sources is critical if you want to investigate microbial diversity.

Expected Results

Encourage students to bring "real"soil/sediments. Students who bring potting soil are usually very disappointed since it is often sterilized. Garden soil that has been treated with pesticides is also not satisfying, but samples from a compost heap are generally very rich in diversity. Encourage students to bring "live"water, too, since often tap water is chlorinated to kill microorganisms, protists etc.

Note that as the communities develop, particularly if they have incorporated sources of S, the columns will smell for a couple of weeks. Following that, they tend to smell only when students open them. Students usually remark on the smell and I explain that this is the smell of decomposition and nutrient cycling.

Since each student will bring soil/sediments and water from different environments and add different proportions of water and soil/sediments, the columns will differ significantly from each other. Columns can be covered with green or brown scums, moss, green slime, dark or clear water or soil with a few plants.

Depending on the nutrients added, microbial communities will differ between columns.

Greens indicate photosynthesis, with grass green indicating eukaryotes, and dark greens indicating cyanobacteria. To complicate matters, cyanobacteria can also be black or brown-black if dry, or brown, yellow-brown, red or orange. Purple sulfur bacteria are purplish pink, sulfide oxidizers are white and sulfate reducers are black. More details on the typical or diagnostic bacterial colors are given in Dyer (2003).

Linking with Other Labs

If desired, the project can focus on microbial activities, and to culture microbes with different nutrient requirements, ask students to bring some of the following from home: rusty nail or iron filings as a source of Fe, Epsom salts as a source of Mg, etc.

Winogradsky columns are useful throughout Introductory Biology. In the fall we sample the columns for bacteria and animal-like and plant-like protists, and in the winter semester we sample for fungi, plants including moss and algae, and many invertebrate groups including nematodes, earthworms, insect larvae, mites, collembola, rotifers, and crustaceans.

Level of Identification Possible

Note that identification of many of these groups to species or even genus is very difficult and challenging even for experts. Also know that many of these groups are not well studied, so little may be known. It partly depends on what taxonomic work on these groups has been done in your area. Check with local universities, colleges, museums, extension agents, etc. to see what is available. If you are lucky, there is information for your area about invertebrates or at least some groups of them. For example, in Alberta, there is a guide to the aquatic invertebrates of Alberta (Clifford 1991) which covers many of the invertebrates we see.

However, since many of these groups are transported dry in air as cysts or some inert stage they are widely distributed across the continent or northern hemisphere. This means guides from other areas may also be useful.

For the purposes of an introductory biology course, students might only identify organisms to the level of phylum for very diverse and difficult groups such as nematodes, rotifers and flatworms, but other groups can be identified to class or possibly genus (see the key). For a class in invertebrate zoology, it is appropriate to expect more detailed identifications using some of the resources listed.

Organisms may occur in more than one place in the keys, since it is not clear where some organisms belong. For example, euglenoids are motile, photosynthetic organisms and are keyed out under algae as well as under protists; and insect larvae can look like segmented worms, so they are keyed out under segmented worms as well as insects.

The key presented is by no means complete, and may require modification to be useful in different parts of the continent, but it does give students somewhere to begin when thinking about diversity of many different groups.

Reference Books for Identification

The following list is intended for reference or if you would like to explore a particular group further. Note that guides to soil and aquatic organisms are both potentially useful since Winogradsky columns contain both.

Algae

John, D. M., B.A. Whitton, and A. J. Brook. 2011. *The Freshwater Algal Flora of the British Isles*. Second edition. Cambridge University Press, 896 pages.
This enormous book is a valuable resource but probably too detailed for the beginner.

Needham, J. G., 1988. *A Guide to the Study of Fresh-water Biology*. Fifth edition. McGraw-Hill, 108 pages.
This is available in several editions from the 1930s on; earlier editions are co-authored by P. R. Needham. This is a small gem covering all freshwater aquatic life to genus.

Bacteria

Dyer, B. B. 2003. *A Field Guide to Bacteria*. Cornell University Press, 355 pages.
An excellent field guide to bacteria, focusing on the colors, smells, typical environments, major nutrients etc.

Invertebrates

Clifford, H. F. 1991. *The Aquatic Invertebrates of Alberta*. The University of Alberta Press, 550 pages.
This is an excellent introduction to the aquatic invertebrates of Alberta. Lots of illustrations, drawings, keys, information about each family etc. Some keys to species level.

Dindal, D. L. 1990. *Soil Biology Guide*. John Wiley & Sons, Inc., 1349 pages.
An older reference book which is available secondhand, containing lots of details, illustrations and keys on soil organisms in N America.

Eisenbeis, G., and W. Wichard. 1987. *Atlas on the Biology of Soil Arthropods*. Springer-Verlag, 437 pages.
Recently reprinted in 2011, this atlas has amazing electron micrographs of soil arthropods. Less of an identification guide than a celebration of the form, function and interactions of these organisms, this would be a great book for further explorations.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. *An Introduction to the Aquatic Insects of North America*. Fourth edition. Kendall Hunt Pub., 1158 pages.
This huge text is an excellent resource but probably is too daunting for the beginner.

Needham, J. G., 1988. *A Guide to the Study of Fresh-water Biology*. Fifth edition. McGraw-Hill, 108 pages.
This is available in several editions from the 1930s on; earlier editions are co-authored by P.R. Needham. This is a small gem covering all freshwater aquatic life to genus.

Thorpe, J. H., and D. C. Rogers. 2010. *Field Guide to Freshwater Invertebrates of North America*. Academic Press, 304 pages.
This is a comprehensive guide to freshwater invertebrates that can be identified using a magnifying glass and includes ecological information.

http://soilbugs.massey.ac.nz/index.php
This website is devoted to New Zealand soil invertebrates. Includes lots of great information about different groups, although in N America the descriptions of the species are not so useful.

Protists

Needham, J. G., 1988. *A Guide to the Study of Fresh-water Biology*. Fifth edition. McGraw-Hill, 108 pages.
This is available in several editions from the 1930s on; earlier editions are co-authored by P. R. Needham. This is a small gem covering all freshwater aquatic life to genus.

http://www.microscopy-uk.org.uk/index.html?http://www.microscopy-uk.org.uk/pond/
This is very useful and has info on different types of protists at a simple enough but not trivial level.

http://www.microscopy-uk.org.uk/
This small page on the web has beautiful photos of algae.

Acknowledgements

I am very grateful to the post-secondary mentors and teachers who introduced me to the fascinating organisms that live in soil. Many thanks also to Wim van Egmond for allowing the use of his drawings in the key.

Literature Cited

Atlas, R. M., and R. Bartha. 1998. *Microbial Ecology: Fundamentals and applications*. Benjamin Cummings Publishing Company, Inc., 694 pages.

Barton, L. L., and D. E. Northrup. 2011. *Microbial Ecology*. Wiley-Blackwell, 440 pages.

Clifford, H. F. 1991. *The Aquatic Invertebrates of Alberta*. The University of Alberta Press, 550 pages.

Dyer, B. B. 2003. *A Field Guide to Bacteria*. Cornell University Press, 355 pages.

Giller,P. S. 1996. The diversity of soil communities, the ”poor man’s tropical rainforest“. *Biodiversity and Conservation*, 5: 135-168

Usher, M. B., P. Davis, J. Harris, and B. Longstaff. 1979. A profusion of species? Approaches towards understanding the dynamics of the populations of microarthropods in decomposer communities. In: Anderson, R. M., B. D. Turner, and L. R. Taylor, eds. *Population Dynamics*, pp. 359-84, Blackwell Scientific Publications, Oxford.

About the Author

Mary Ann McLean currently teaches at a small liberal arts university in Calgary, Alberta, Canada, which is conveniently adjacent to a provincial park. She received her B. Sc. from the University of Guelph, and her M.Sc. and Ph.D in soil ecology from the University of Calgary. She has spent countless hours looking at microbes and soil invertebrates through a microscope, and yes, fungi are beautiful! She did a postdoc with the soil ecology group at the University of Jyvaskyla in Finland and at Fordham University in NY. She is fascinated by the diversity of organisms all around us and passionate about introducing students to their local ecology.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/.**

Papers published in *Tested Studies for Laboratory Teaching: Peer-Reviewed Proceedings of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Citing This Article

McLean, M. A. 2014. Cheap & Easy Diversity for Introductory Biology. Pages 240-255 in *Tested Studies for Laboratory Teaching,* Volume 35 (K. McMahon, Editor). Proceedings of the 35th Conference of the Association for Biology Laboratory Education (ABLE), 477 pages. http://www.ableweb.org/volumes/vol-35/?art=14

APPENDIX

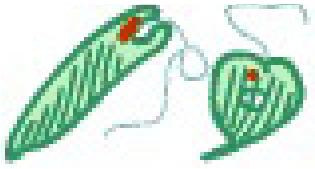
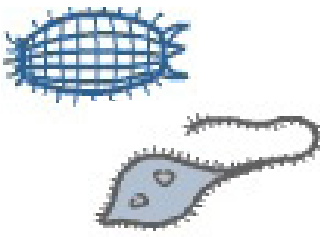
Keys to Organisms in Winogradsky Columns

General Key to Groups

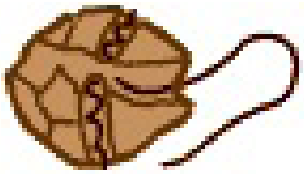
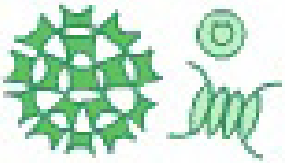
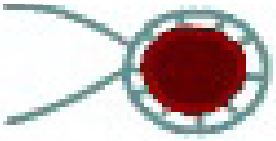
1	unicellular, colourless or pigmented	2	
1	multicellular	5	
2	cells usually colourless	3	
2	cells green, blue-green, brown, yellow-brown	4	
3	cells are tiny, colourless dots or strands, only just visible at 1000x	Bacteria	
3	cells larger, usually colourless, may be green, motile	Protists (see key)	
4	cells are larger, green, brown or yellow-brown, usually non-motile	Algae (see key)	
4	cells are larger, blue green under the microscope, macroscopically may look purple, orange, orange-brown, some motile	Cyanobacteria	
5	green, non-motile or motile	6	
5	not green, various shapes	7	
6	green, non-motile	Plants (see key)	
6	green, motile or non-motile	Algae (see key)	
7	not usually green, motile or non-motile, various shapes	Protists (see key)	
7	not green, non-motile, fuzzy, fine filamentous, various colours	Fungi (see key)	
7	not green, motile or non-motile, various colours, may be worm-like, may have an exoskeleton and/or appendages	Animals (see key)	

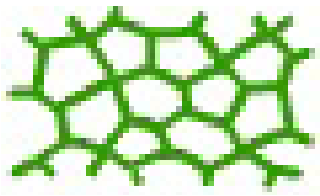
Protists

NOTE: although cilia and flagella are hard to see using a standard student lab microscope, often the waves created by their movement are visible and can give an idea of the size of the flagella or cilia

1	cells can change shape, moving & engulfing food with pseudopods, cytoplasmic streaming often visible	Amoebae	
1	may change shape slightly (i.e. elongate) but no pseudopods	2	
2	one or more flagella (long whip-like cilia)	3 Flagellates	
2	many cilia, cells various, cilia arrangement various	4 Ciliates	
3	green flagellates (see algae)	Phytoflagellates	
3	flagellates not green	Zooflagellates	
4	often bell-shaped or cylindrical, attached to substrate	5	
4	various shapes, free-living	6	
5	ring of cilia at wide end of bell, bell < 0.25 mm, some stalked, often colonial, attached to animals or plants	Ciliates in part (Peritrichs)	
5	bell shaped, no cilia, have tentacles with knobs on top on bell edge	Ciliates in part (Suctoria)	
6	up to 2 mm long, trumpet shaped with ring of cilia around opening, may be green due to symbiotic green algae	*Stentor*	
6	not as above	7	
7	oval, may elongate, cilia around edge, spirals through water	*Paramecium* & relatives	
7	not as above, may have long neck, may be barrel-shaped etc	Ciliates in part	

Algae

1	microscopically seen as individual cells or colonies	2	
1	microscopically seen as filaments	7	
2	cells brown, yellow brown or green	3	
2	cells green	4	
3	cells yellow-brown, symmetrical, with glass shells in 2 parts, many shapes, may glide slowly, single-celled or colonial	Diatoms	
3	cells larger, brown or green, motile, usually grooved around the circumference, 2 flagella, 1 in the groove	Dinoflagellates	
4	cells blue-green under the microscope, up to 50 µm dia., solitary or colonial, cells may have brown, purple or orange sheath, may be motile	Cyanobacteria	
4	cells green, non-motile	5	
4	cells green, motile	6	
5	cells green composed of 2 identical “half cells”with distinct constriction between, non-motile, usually solitary	Desmids	
5	cells green, non-motile, not attached to surfaces, many shapes	Chlorophyta (Green algae)	
6	cells green, 1 flagellum, < 400 µm dia, flexible oval body, red eye spot	Euglenoids	
6	cells oval, green, 2 anterior flagella, < 50 µm diameter	*Chlamydomonas* & relatives	
6	cells green, spherical colonies, up to 1 mm diameter, each cell with 2 flagella	Chlorophyta (Green algae) *Volvox* & relatives	

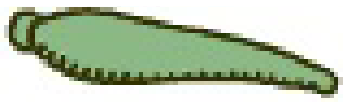
7	filaments joined to form a sac-like net, green, up to 10 s of cm	*Hydrodictyon* (water net)	
7	not as above	8	
8	non-branching filaments	9	
8	branching filaments	Chlorophyta (Green algae)	
9	non-branching filaments, with prominent, distinctive chloroplasts in spirals, stars etc	Chlorophyta (Green algae) *Spirogyra* & relatives	
9	non-branching filaments composed of disk-like cells, filaments oscillate slowly	*Oscillatoria* (Cyanobacteria)	

Plants

1	microscopic, green, single cells, filaments or colonies, may appear as green scum	Chlorophyta (Green algae) (see Algae key)	
1	macroscopic, green	2	
2	green with tiny leaves up to 5 mm	3	
2	green with larger leaves usually > 5 mm	Anthophyta (flowering plants)	
3	no vein of vascular tissue in center of leaves	Bryophyta (mosses)	
3	1 vein of vascular tissue in center of leaves	Lycophyta (club mosses)	

Animals

NOTE: since Winogradsky columns are not ideal environments for most insects and they are infrequently seen, they are have not been keyed out below the level of class

1	multicellular, worm-like	2	
1	multicellular, not worm-like, no legs	7	
1	multicellular, with legs or prolegs or head capsule	9	
2	worms segmented, various colours	3	
2	worm-like not segmented, various colours	5	
3	worms cylindrical, even width	4	
3	worms not cylindrical, flexible body, one end larger than other, sucker on each end, black, brown, green, red, may be patterned	Hirudinea (leeches)	
4	pink, brown, grey, green or white up to 10 cm	Oligochaeta (earthworms)	
4	worms cylindrical, hair-like, up to 30 cm long	Nematomorpha (horsehair worms)	
4	“worms”with chitin head capsule	Insect larvae	
5	tiny, clear or white unsegmented worms, up to 10 mm long, usually < 3 mm, move sideways like snakes	Nematoda	
5	not as above	6	
6	flat, ribbon-like, triangular or truncate head with 2 eyespots, black, brown or grey, usually < 2 cm	Platyhelminthes	
6	wormlike body with long tentacles, various colours, tentacles can be retracted	Cnidaria in part (Hydra)	
7	microscopic, clear, with crown of rotating cilia, may swim or move like an inchworm, usually < 1 mm	Rotifera	

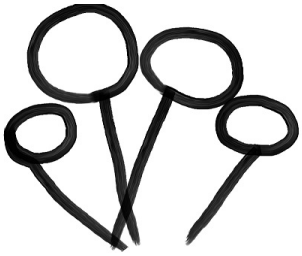
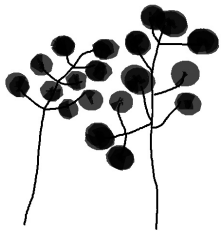
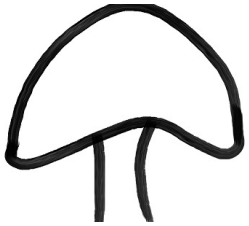
7	microscopic, clear, with long antennae, 1 or more eyespots, may have egg sacs	Copepoda (copepods)	
7	soft flexible body, often leaving slime trail, may have shell	8	
8	with spiral shell	Gastropoda in part (Snails)	
8	with 2 part shell, 1-10 mm or more	Bivalvia (Clams)	
8	no shell	Gastropoda in part (Slugs)	
9	no legs, or rudimentary prolegs, or head capsule	Insect larvae	
9	1 pair of branched legs in front, prominent eye	Cladocerans e.g. *Daphnia*	
9	3 or more pairs of legs	10	
10	3 pairs of stubby legs, rounded, cartoon-like body & antenna	Collembola	
10	3 pairs of jointed legs, shapes various	Insects	
10	5 or more pairs of jointed legs, legs may be cylindrical or leaf -like	Crustacea in part (see key)	
10	4 pairs of legs, body shapes various	11	
11	body with 2 distinct sections, head/thorax & abdomen, jointed legs	Arachnida (Spiders)	
11	body with 1 section, jointed legs, brown, red, black, clear	Arachnida (Mites)	
11	microscopic, 4 pairs of unjointed legs, clear or brown, round body, featureless head, lumbering gait	Tardigrada (water bears)	

Crustacea

1	microscopic, may or may not have bivalve-like carapace covering all or part of the body, legs always enclosed	2	
1	carapace absent or if present, not bivalve-like or enclosing legs	3	
2	carapace seed-like, covering entire body, carapace can open to let legs emerge, < 2 mm	Ostracoda (seed shrimp)	
2	carapace not covering head, usually < 2 mm	Cladocera (water fleas)	
3	10 or more pairs of leaf-like appendages	4	
3	< 10 pairs of appendages, which are usually cylindrical	5	
4	no carapace	Anostraca (fairy shrimp)	
4	horseshoe-shaped carapace	Notostraca (tadpole shrimp)	
5	long antennae, body < 5 mm, usually 5 pairs thoracic appendages, often egg sacs	Copepoda (copepods)	
5	mature body length usually much > 5 mm, 8 pairs thoracic appendages	6 Malacostraca	
6	stalked eyes, < 30 mm	Peracarida (in part)	
6	eyes absent or present, but not stalked	7	
7	eyes absent, body dorso-ventrally flattened	Isopoda	
7	eyes present or absent, body laterally compressed	Amphipoda (scuds)	

Fungi

NOTE: Zygomycete sporing structures are often visible with the naked eye, although details can be seen using a dissecting scope. To see ascomycete sporing structures, it is necessary to use a dissecting scope or to make a slide and use a compound scope. Basidiomycetes are usually easy to see with the naked eye, although they are not common in Winogradsky columns.

1	sporing structures are large spherical sporangia on stalks, sporangia black, brown, pink or red, stalks clear, brown or black	Zygomycota	
1	sporing structures smaller, not enclosed in sporangia	2	
2	spores in tiny brushes, clusters etc, various colours	Ascomycota	
2	mushrooms/toadstools with a cap, these are not common in Winogradsky columns	Basidiomycota	

Colored illustrations are by Wimvan Egmond and used with permission. (**http://www.microscopy-uk.org.uk/index.html**).