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Study of Photosynthetic Microorganisms in Two Different Pond Ecosystem using Winogradsky Columns

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Abstract
To study the Photosynthetic microorganisms, Winogradsky Columns were set up. Water sources from two different Pond ecosystems were used for the study. The ponds selected were Singanallur and Ukkadam. The Columns were enriched with Carbon, Sulfur, Carbon and sulfur. A control was also maintained. Two sets of columns were used. One set was incubated in the light and the other in the dark. Microscopic observations were made periodically and the colour changes in the Winogradsky columns were observed. Anaerobic bacteria were cultured in Robertson's cooked meat media and thioglycollate media. Naked eye and Microscopic observations shows the presence of algae, Gram positive spore forming bacteria and gram negative bacteria. Different gradients were observed over a period of time, indicating the presence of diverse groups of phototrophic microorganisms in the selected natural ecosystems. One of the ponds showed presence of rich organic matter when compared to the other.

Keywords
Winogradsky Column, Photosynthetic bacteria, Pond ecosystems, algae, sulfur bacteria.

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INTRODUCTION
The Winogradsky column is a method for culturing diversity of microorganisms from a natural environment. Invented in the 1880s by Sergei Winogradsky [1], the device is a column of pond mud and water mixed with a carbon source such as newspaper, blackened marshmallows or egg-shells, and a sulfur source such as gypsum or egg yolk [2]. Incubating the column in sunlight for months results in an aerobic/anaerobic gradient as well as a sulfide gradient. The oxygen and sulfur gradients promote the growth of different types of microorganisms such as Clostridium, Desulfovibrio, Chlorobium, Chromatium, Rhodomicrobium, and Beggiatoa, as well as many other species of bacteria, cyanobacteria, and algae [3]. The Winogradsky column is an example to study the diversity of microbes as a mixed culture method [4]. Its purpose is to determine and study the growth of microorganisms, while monitoring their activity over a period of time. The column is compiled with mud from a freshwater source; in this particular experiment the water and organic material was obtained from Ukkadam and Singanallur located in Coimbatore. A source of carbon is then added as well as ($CaCO_3$) and

a source of sulfur ($CaSO_4$). These materials will help to promote the growth of different microorganisms. In conducting the following experiment numerous biofilms were formed. Biofilms are microorganisms that are held together by a matrix of extracellular polymeric substances. Adhesion to the liquid surface is believed to occur through proteins and polysaccharides by their hydrophobic regions. Biofilms can include a variety of microorganisms including but not limited to; fungi, algae, protozoa, and bacteria. Biofilms that loosely adhere to the surface of static or dynamic liquid mediums (i.e. Lakes, ponds, rivers, and streams) are called surface microlayer biofilms [5]. Individual (planktonic) bacterial cells have the ability to adhere to surfaces. Other planktonic bacteria can then attach to the adhered bacteria. This process of continued adhesion eventually leads to multi layers of bacteria on the surface. A large amount of extra cellular polymeric substances (EPS) accompany the bacterial cells, creating a matrix throughout the biofilm. Surface microlayer biofilms are made up of phototropic aerobic organisms and are considered to be generally weaker in composition than biofilms that are in a more mature stage of development [6].

Periphyton communities by contrast are sessile organisms that live attached to surfaces projecting from the bottom of freshwater aquatic environments. Periphyton communities are known to exhibit rapid response to environmental conditions and nutritional environments. An excess of periphyton communities in agricultural and waste management facilities has been known to be problematic in controlling aquatic environments necessary for these industries to function effectively. In this study, we have analyzed the diversity of microbial population in two different pond/ lake systems, in the city.

MATERIALS AND METHODS:

SAMPLE COLLECTION:

Water samples from Ukkadam and Singanallur were collected in buckets as required. Ukkadam pond is called the large lake in Coimbatore. It receives water from Siruvani river, that is seasonal. It is rich in fauna and flora. Singanallur lake is an attractive tourist spot in the City. This lake also is rich in fauna and flora. More than 100 species of birds are present here. Both the water bodies are contaminated by domestic, hospital and industrial wastes.

PREPARATION OF WINOGRADSKY COLUMN:

Plastic bottles (1lt) were used. Top of the bottles are cut and three fourth was filled with the water and sediment. Enough mud was put into the large mixing bowl to fill three-quarters of the bottle. Water is added and stirred until mud is the consistency of a milkshake. The column is a rough mixture of ingredients. A bottle is filled one third full of pond mud, omitting any sticks, debris, and air bubbles. Supplementation of ~0.25% w/w calcium carbonate and ~0.50% w/w calcium sulfate or sodium sulfate is required (ground eggshell and egg yolk respectively are rich in these minerals), mixed in with some shredded newspaper (for cellulose). This is followed by water from the pond to saturate the mud (or sand) and occupy half the remaining volume. After preparation, the column is covered tightly with polythene cover to prevent evaporation of water and incubated for seven weeks in strong natural light. Another set was incubated in the dark. Three sets of columns from each pond water are used for the study. A control is maintained without any enrichment (fig.1).

Carbon Enrichment (shredded newspaper, Calcium carbonate, Egg shell),

Sulfur Enrichment (Egg Yolk, iron bits, calcium sulfate)

Carbon and Sulfur together (shredded newspaper, Calcium carbonate, Egg shell, Egg Yolk, iron bits, calcium sulfate)

Figure 1. Prepared Columns

OBSERVATION OF MICROORGANISMS:

Periodically samples from the surface were observed under the microscope. Algal masses, bacteria of different shapes were seen.

CULTURING OF MICROBES:

Samples were taken from the columns and inoculated into Robertson's Cooked meat media and Thioglycollate media and incubated anaerobically. Colour changes in the meat pieces were observed. Microscopic observation was also made after Gram Staining.

BIOFILM FORMATION:

Glass Slides were inserted into the winogradsky columns and left for 2 weeks and the adhering biofilms were observed as wet films.

RESULTS:

WEEK 1

Colour Change: No colour change was observed in both Pond systems

WEEK 1

WEEK 2

WEEK 2

Black Colour was observed at the bottom of all the columns except control.

WEEK 3

WEEK 4

WEEK 3

Control: Slight change in colour (Green)

Carbon Source/ Sulfur/ Carbon and Sulfur: Black colour at the bottom; Purple colour started to appear in the middle of the column

WEEK 4

Control: Dark green colour

Carbon Source/ Sulfur/ Carbon and Sulfur: Black colour at the bottom; Intense Purple colour in the middle of the column

WEEK 5

WEEK 6

WEEK 7

In these columns, a clear differentiation of colour gradients were observed (fig. 2). In the control bottle, green and pale yellow colour was seen. This was due to the growth of autotrophic algal forms and cyanobacteria. In the bottle enriched with Carbon (first bottle), algal growth is predominant. In the mid zone purple sulfur oxidizing bacteria is the predominant however in the sulfur enriched bottle and the carbon and sulfur enriched bottles, the mid zone is completely occupied by purple and green sulfur bacteria. In the last bottle (carbon and sulfur enriched), there is a thick bottom layer that may be due to anaerobic sulfur oxidizing *Desulfovibrio* and *Clostridium* Sp.

The following changes were observed.

Intensification of colours

Upper layer: Algal growth

Middle layer: Yellow colouration in carbon enriched column, Purple colour in Sulfur enriched column, Black and purple colour in Carbon and sulfur enriched column.

Bottom layer: Black anoxic zone- Clostridia/ Desulfovibrio sp.

Figure. 2. Week 7
Colour gradients in the bottles incubated under sunlight.

A set of columns were incubated in the dark. These columns did not show any colour change except that black colouration throughout the column (figure. 3). This shows the absence of photosynthetic organisms and the growth of anaerobic organisms. This may be due the presence of methanogenic bacteria.

Figure 3. WEEK 7
Columns incubated under dark, without any colour gradients.

MICROSCOPIC OBSERVATION

In simple staining, Large Bacilli were observed, wet mount showed the presence of algal masses. Cocci, bacilli and irregular shaped organisms were observed (figure 4).

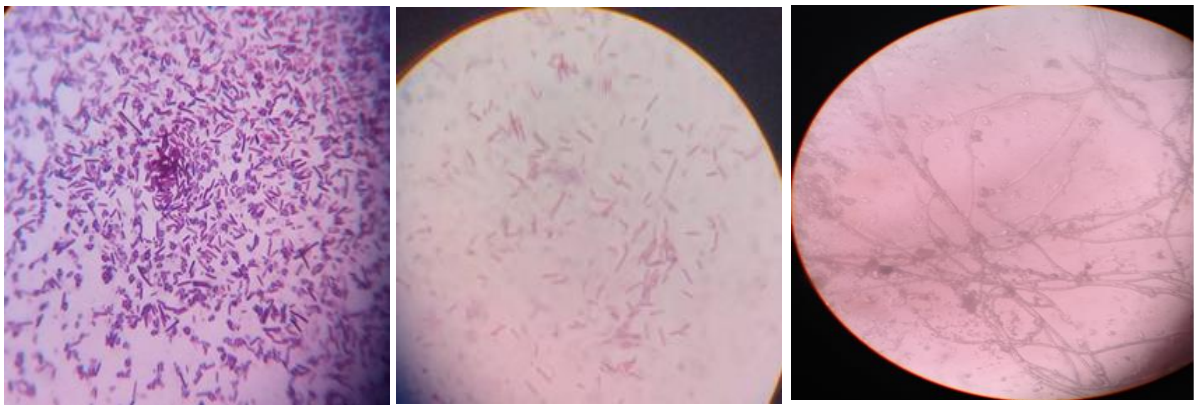
Figure 4. Large bacilli, algae, Cocci, and Irregular shapes were seen

CULTURING IN ROBERTSONS COOKED MEDIA

Meat Pieces turned to black in colour indicating the presence of proteolytic Clostridium Sp. Samples taken from the broth showed the presence of spore forming bacilli. Samples from thioglycollate broth showed the presence of large gram negative bacilli. Filamentous bacteria were seen in the biofilms formed on the surface of the slides inserted into the columns (fig. 5).

Figure 5.

Gram positive spore forming bacilli **Gram negative bacilli** **Filamentous organisms**



DISCUSSION:

The Winogradsky Column explains that a combination of microbial metabolism and physical parameters (such as light availability and diffusion) lead to ecosystem stratification. The metabolic requirements of one group of organisms can be provided by the byproducts of another group. Microbes play a role in elemental cycling (Carbon, Phosphorous) [7].

Colour gradients were observed as columns are incubated. Oxygen gradients also develop. When the column is prepared, oxygen is evenly distributed throughout the column. It is consumed by respiration throughout the column, but it is only produced in the photosynthetic layer at the top. Any oxygen that diffuses down into the sediment from the topmost layer reacts with chemical compounds in the anoxic layer.

Over time, an oxygen gradient develops from high at the top to completely anoxic (no oxygen) at the bottom of the column. The overlying air has the highest oxygen concentration, and the concentration decreases as you move down through the water and sediment to the bottom of the column.

Winogradsky columns form sulfide concentration gradients as well. When the column is prepared, sulfur will be distributed throughout the column by mixing. Sulfur is converted to sulfide by anaerobic respiration, which will only occur in the bottom of the column. Any sulfide that diffuses upward will react with oxygen (either abiotically or through microbial metabolism).

Sulfur reduction is a form of anaerobic respiration. *Desulfovibrio* are an example of bacteria that reduce sulfur as a way of respiring in the absence of oxygen and release sulfide. *Desulfovibrio* would be found in the bottom parts of the columns where there is no oxygen.

Purple sulfur bacteria and green sulfur bacteria are two types of bacteria that use sulfide to support photosynthesis. In general, green sulfur bacteria tolerate higher levels of sulfide than purple sulfur bacteria do.

Purple sulfur bacteria will be concentrated in a layer above the green sulfur bacteria because that is where there is less sulfide. In addition, both the green and purple sulfur bacteria will be layered above the *Desulfovibrio* bacteria [8].

Since *Desulfovibrio* can reduce sulfur, they produce sulfide that supports the metabolism of green and purple sulfur bacteria. However, *Desulfovibrio* do not require light, so they will be found lower in the column. Photosynthetic cyanobacteria and algae will most likely be sampled from the water at the top of each column because they require only water, carbon dioxide, and light, which is most intense at the top of the column.

CONCLUSION:

Different layers form in the column based on the availability of oxygen and other nutrients. Different groups of organisms occupy each of these layers, but they all came from the original sample. This illustrates the point that there is a rich diversity of organisms in very common environments such as Singanallur and Ukkadam ponds. Furthermore, the gradients themselves are a product of microbial metabolism. This illustrates that microbes don't just adapt to their environment; their metabolisms actually create chemical niches with the environment.

Gradients may also form in control column because there are natural forms of carbon, sulfur, and other nutrients in the sediment. All the same microbial processes will occur but maybe to a lesser extent (depending on the sediment source).

In the samples collected for our study, Singanallur pond showed a good stratification of colour gradients than the Ukkadam Pond. This may be due to the rich organic content from the collected source. The rich purple colour could be used for the extraction of pigments from purple bacteria. The algal diversity could be used for the production of biofuels, as the ecosystem is contaminated with textile, oil and other pollutants, these organisms will serve as a major source of microbial enzymes that could be explored. These organisms enriched from the sediment will also have greater potential for biodegradation of pollutants, hydrocarbons and pesticides which can be further studied.

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