

Abstract

The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings. The factors such as water, soil, temperature, climate, pH, availability of nutrients, oxygen and the medium affect the growth of microbes. Microorganisms have vital roles in ecosystems such as decomposition, oxygen production and evolution. Microbial communities are indispensable to the water bodies as they carry out essential biogeochemical cycles (nutrient cycling). The effects of water from different sources in growth of microbes were examined.

The diversity of microbes in each sample with different concentration were observed. Getting water samples from different sources, prepared the medium and then kept them for incubation. Observed the microbes under microscope and then differentiated between them by the shape, color and response to gram staining.

The natural sources were found to have more diversity than artificial sources. Among natural sources, muddy water and among artificial, filter water had the most diverse colonies of microorganisms.

Introduction

Microbes - bacteria, archaea, fungi, algae, protozoa and viruses - have been around for at least 3,500 million years and were the only life forms on Earth for most of that time. These microscopic organisms play a key role in maintaining life on earth, fixing gases and breaking down dead plant and animal matter into simpler substances that are used at the beginning of the food chain. Biotechnologists can also exploit the activities of microbes to benefit humans, such as in the production of medicines, enzymes and food. They are also used to breakdown sewage and other toxic wastes into safe matter. This process is called bioremediation.

Microbes-

- Generate Oxygen in the Atmosphere.
- Recycle nutrients stored in organic matter to an inorganic form.
- Fix nitrogen from the Atmosphere into a Useable Form.
- Allow Herbivores to Consume Poor Quality Food.
- Give Plant Roots Access to Nutrients in the Soil.

Hypothesis

Natural Sources

More diversity due to lack of treatment

- a) Rain water- Less Diversity as the microbes participating in the Bio precipitation cycle are less.
- b) Muddy water-High Diversity due to availability of nutrients and energy sources. The microbial community should be diverse in muddy water because it contains energy sources such as sugars, starch, protein, fats and other compounds that provide the nutrients.
- c) Well water -Less diverse due to percolation through soil. Soil filter the water due to which the microbes are less diverse.
- d) Stagnant water -More diverse due less disturbance of medium and availability of nutrients. Stagnant water will be enriched with microbial communities because it provides a better incubator than running water for many kinds of bacteria and parasites. Stagnant water is often contaminated with human and animal feces which provides nutrition for the microbes.
- e) River water -More diverse as the sources of nutrition are qualitatively and quantitatively better.

2)Artificial sources

Microbes are less abundant as they are killed off due to various purification and filtration techniques.

- a) Distilled water-Least diverse due to filtration and chemical treatment.
The microbial community should be the least diverse in distilled water because the process of filtration filters out ions, micro-organisms, mineral and other chemicals from water.
- b) Tap water-Less diverse due to chlorine treatment. Tap water is treated with a large number of chemicals in order to kill bacteria and other microorganisms. The relative diversity may be more when compared to distilled water.
- c) Filtered water -Less diverse due to UV treatment, reverse osmosis and chemical treatment. Filtered water goes through many filtration techniques such as reverse osmosis and chemical filtration and UV which kills many microbes.

NULL HYPOTHESIS

The growth and diversity of the micro-organisms is same in water from all sources. Water does not have much effect in growth of microbial communities.

Procedure

1. Collection of samples

Samples of water were collected from well, river, rain, stagnant and muddy water. The artificial sources were tap water, distilled water and filter water.

2. Preparation of medium

The liquid medium chosen was nutrient broth and the solid medium was nutrient agar. It was autoclaved. This medium was responsible for the growth of microbes.

3. Serial dilution and plating

Transferred the medium into the plates i.e. plating. Then serial dilution was carried out on each of these samples. A serial dilution is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. The next step was spreading using an L-rod. Care was taken during plating so as to not contaminate it.

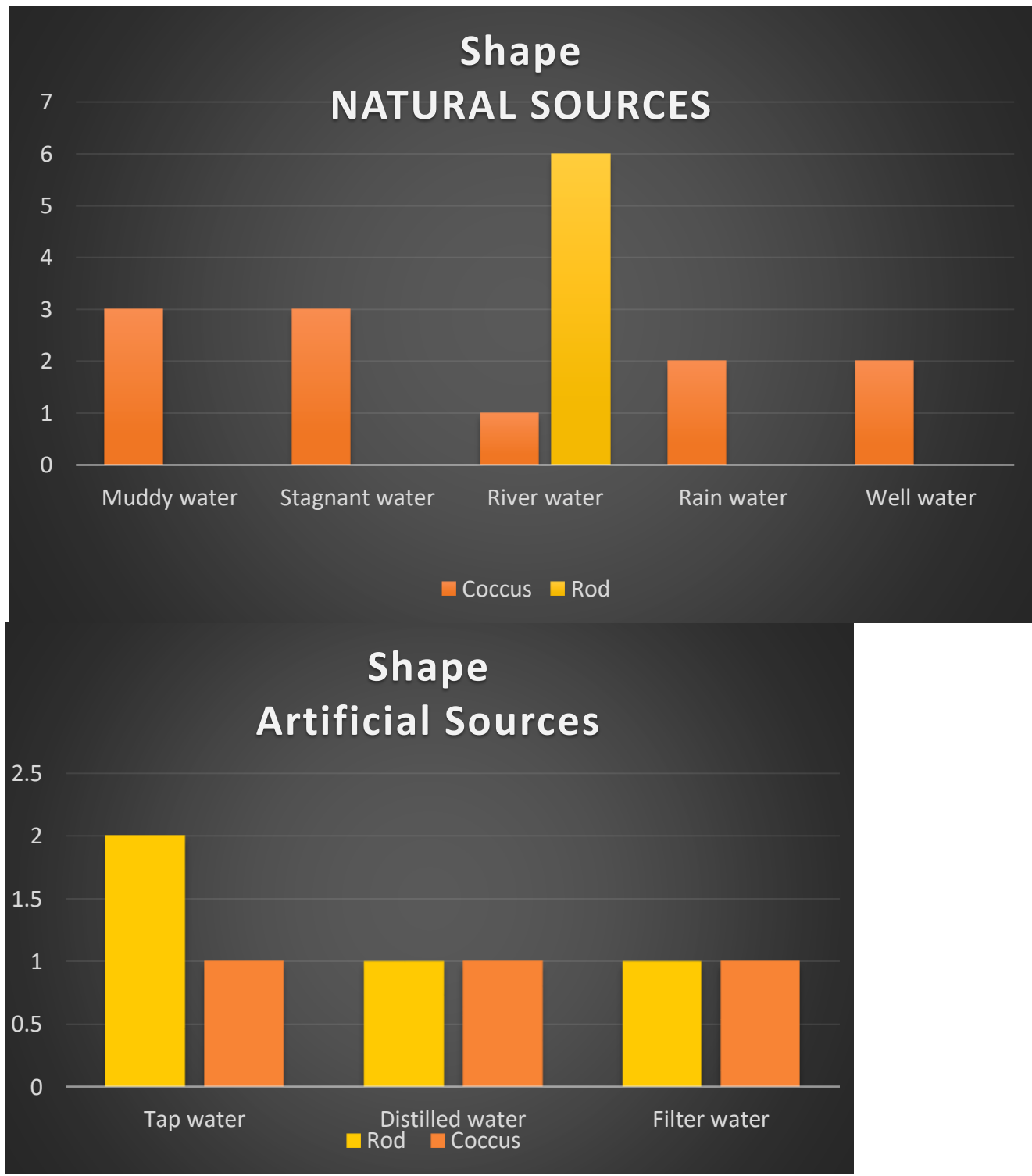
4. Incubation

The plates were incubated for approximately 24-48 hours to aid in the microbial growth.

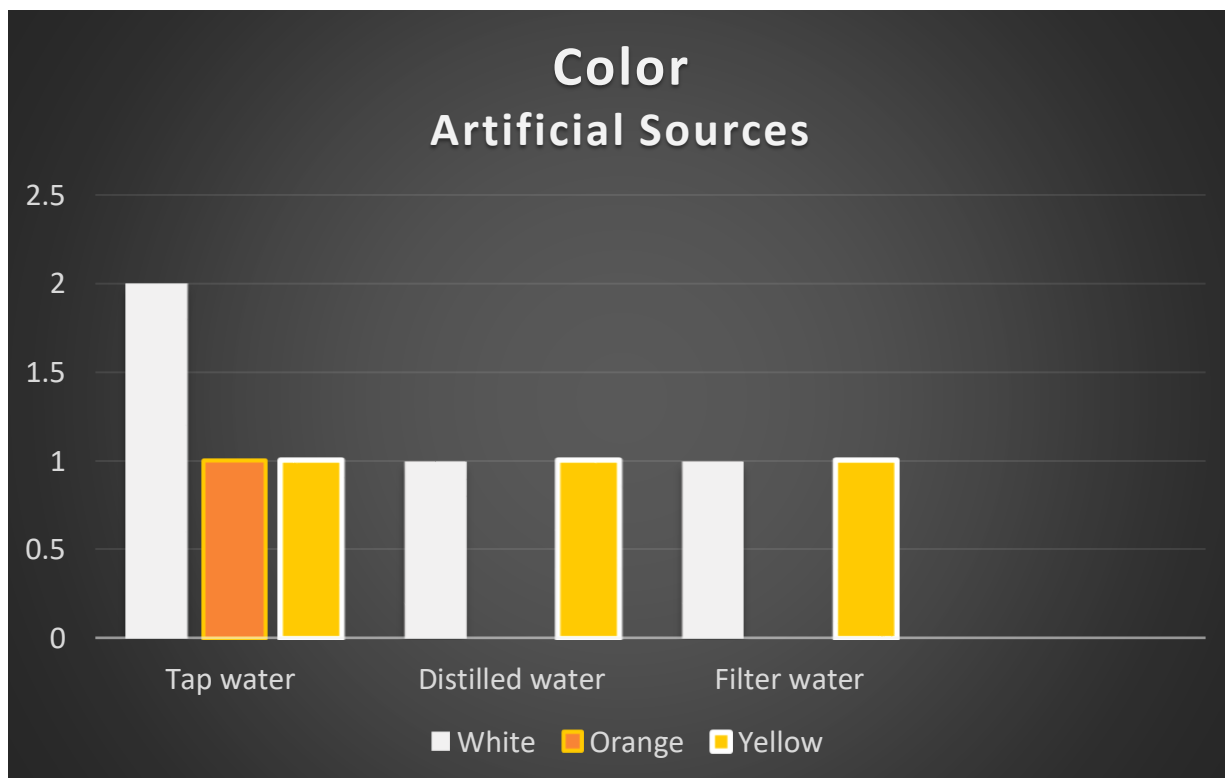
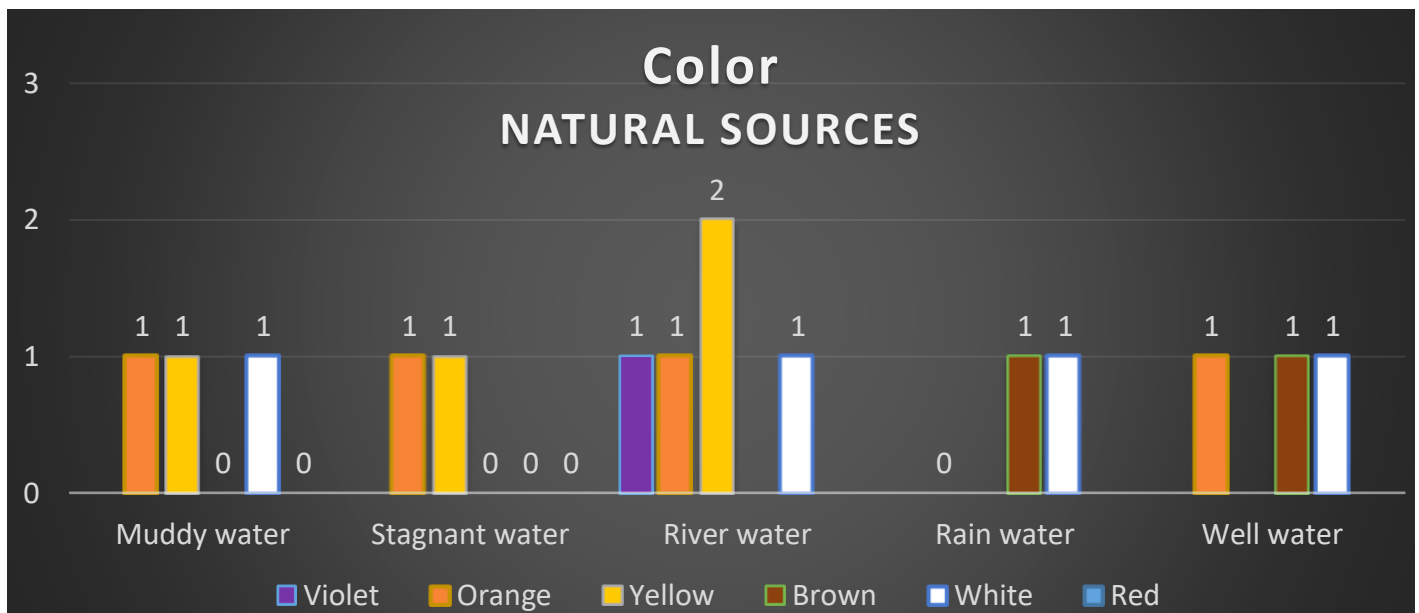
5. Gram staining

Each sample was carefully observed and stained. Stains used were crystal violet, gram's iodine, gram's decolorizer and safranin. These stains were used to identify the type of colonies. (gram positive/gram negative).

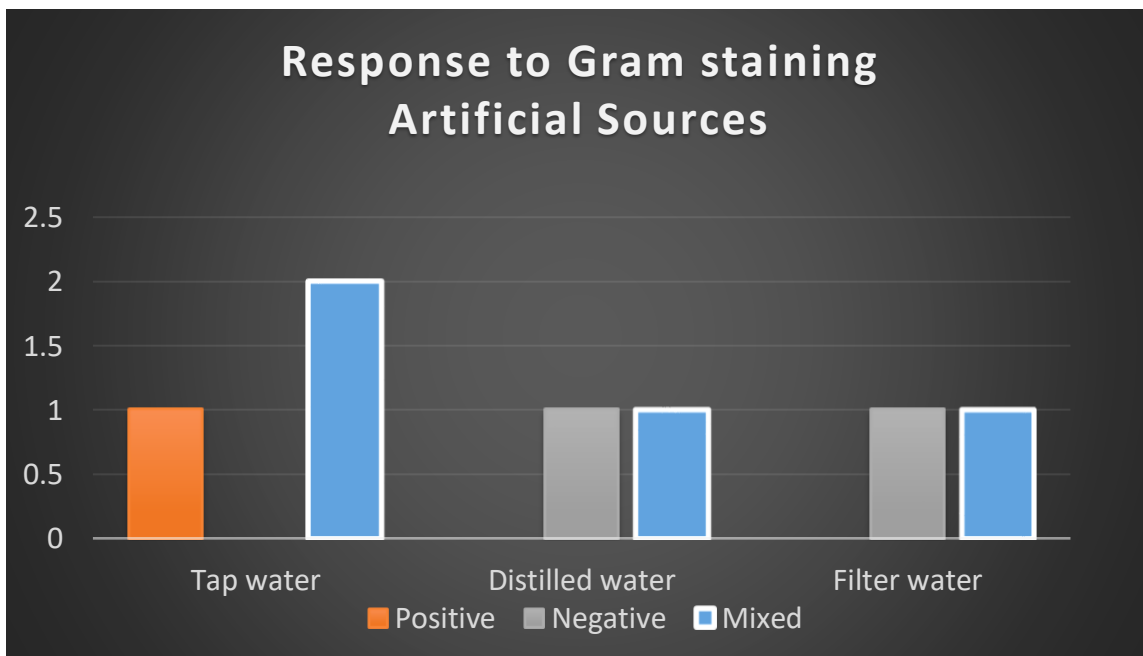
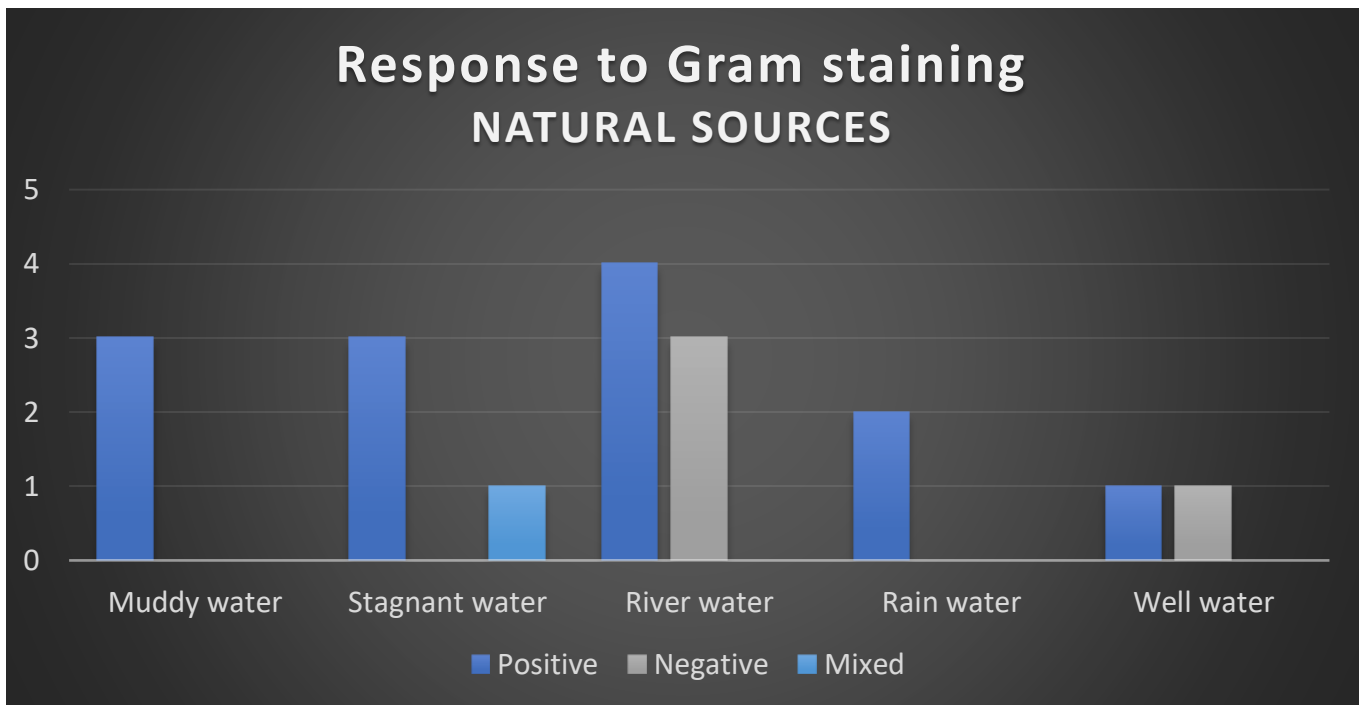
Results



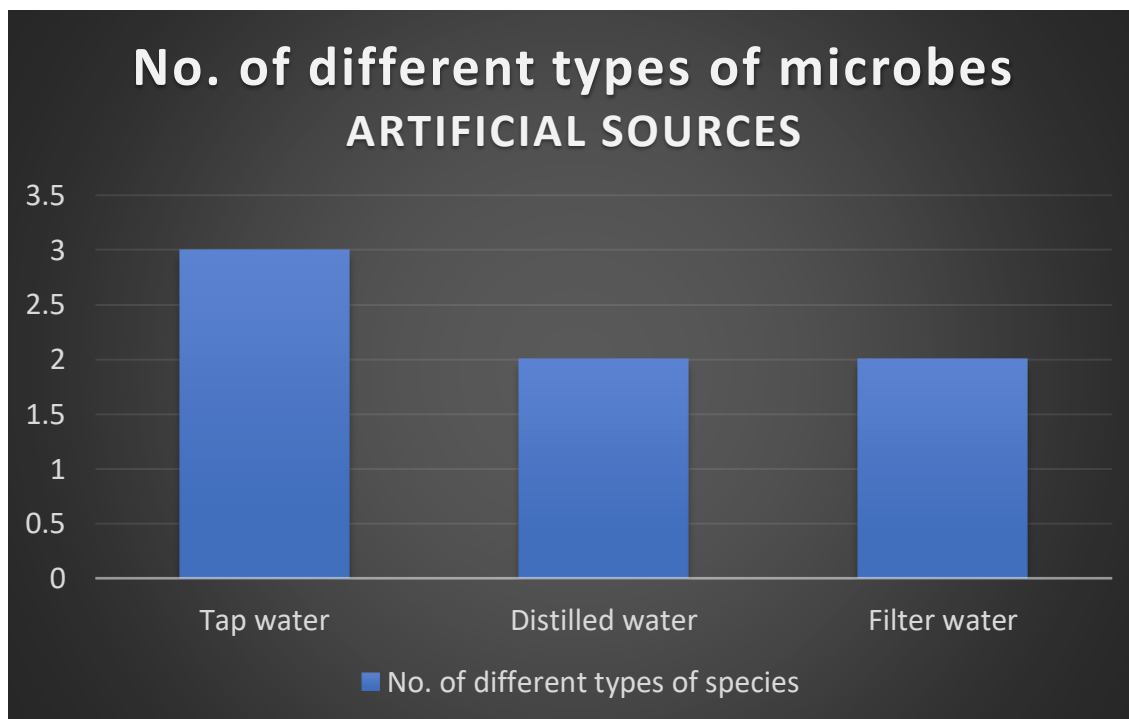
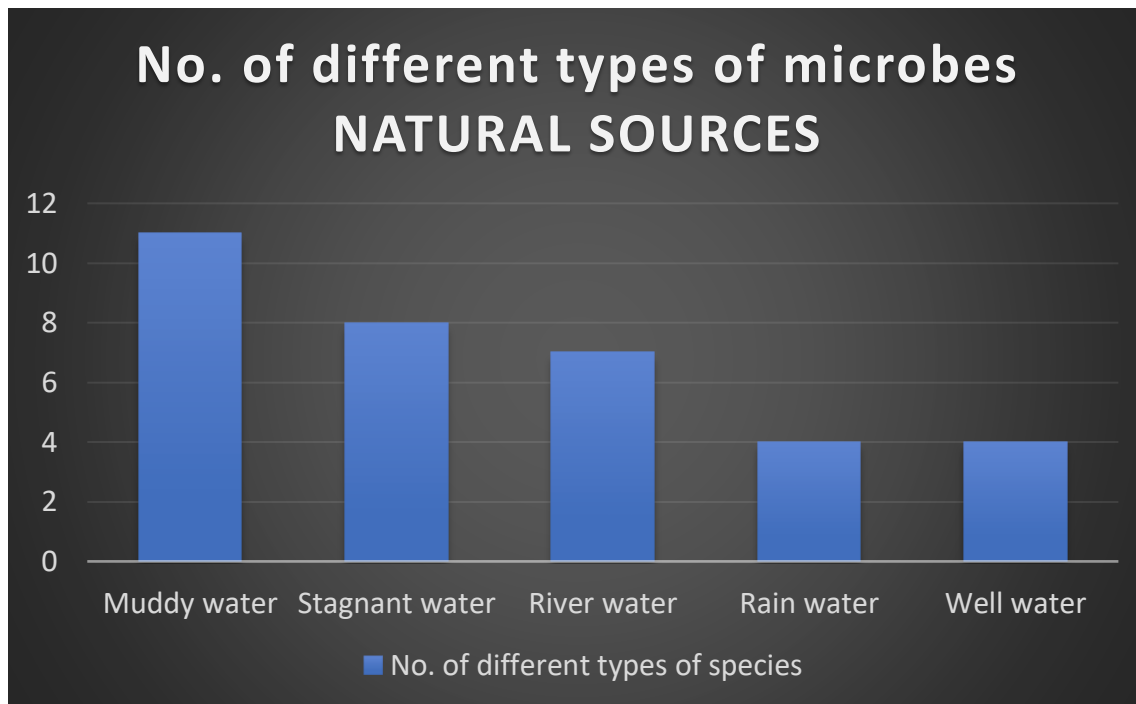
Above graph indicates that the number of colonies having rod or coccus shape were more in natural sources of highest concentration (10^{-2}) than artificial sources with same concentration.



The above graphs show that the microorganisms with various colors are found in natural sources. Also, the number of colonies having color are more in natural sources than artificial sources.



The gram staining responses have been recorded in the above graphs. Most of the colonies are gram positive, but some are gram negative. Also, it is observed that all the artificial sources have mixed colonies i.e. gram positive and negative.



The above graphs show the number of different types of microbes, the diversity is determined by these graphs. A microbe is differentiated on the basis of color, shape and response to gram staining. Two species having even a single different criterion is observed as different species.

As variations are seen by different sources of water, the null hypothesis i.e. no effect of water on microbial communities can be rejected. The above graph depicts the diversity of microbes in different water sources which are treated (artificial) and not treated (natural). Hence the effect of water on microbial community was shown.

Natural sources were found to have greater microbial diversity than artificial sources. The diversity was most in muddy water due to availability of nutrients and energy sources. Considering only artificial sources, the diversity was found the most in tap water.

Discussion

The data collected from the graphs gives the general information of the different types of microbes survive and grow in different sources of water.

Comparing amongst the natural sources, the microorganism, the colony number and the diversity, were not so different from one another; same in the case of artificial sources.

Most diversity of microorganisms were found in higher concentrations i.e. 10^{-2} , the observation recorded and graph plotted were with respect to 10^{-2} concentration. This was done as method of serial dilution was used, the microorganisms present in higher concentration were also present in lower concentrations. Any different microbes found in lower concentration would be the result of contamination.

A colony of fungi was found in the sample of well water in 10^{-4} concentration, which is most likely the result of contamination.

Also, a large number of colonies of same type were found in distilled water with different concentrations, which was highly unlikely. Therefore, the medium must be contaminated in this replicate for this to happen.

The results showed that the microbial community structure was significantly dependent on the level of water pollution, both in absolute microbial counts and in relative abundance of phylogenetic groups. (Mlejnková H, et al. Water Sci Technol. 2010.)

The results indicated that thermal and oxygen stratification shaped the phylogenetic composition of microbial communities in the reservoir. (Yu Z, et al. Sci Rep. 2014.)

According to above researches, the microbial growth depended on pollution of water and oxygen stratification. Our results may vary due to the above stated factors. The oxygen level, thermal and oxygen stratification caused the diversity in microbial community.

Most bacteria, including potential pathogens, could be effectively removed by chlorine disinfection. However, some bacteria presented great resistance to chlorine. qPCRs showed that *Mycobacterium* spp. could not be effectively removed by chlorine. (Front. Microbiol., 12 December 2017)

The microbes found in tap water may be *Mycobacterium* spp., according to above research.

References

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(Yu Z, et al. Sci Rep. 2014.) - <https://www.ncbi.nlm.nih.gov/m/pubmed/20489251/>

(Front. Microbiol., 12 December 2017)

<https://www.frontiersin.org/articles/10.3389/fmicb.2017.02465/full>

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