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Variation of bacterial population in four soil depths in the Sundarbans mangrove forest, Bangladesh

Afruza Begum[1]
[1*] Research Assistant, Environmental Systems Engineering, University of Regina, SK, Canada
*Corresponding author: *afruza.ju@gmail.com*

Abstract

Large diversity of microorganisms is usually found in soil profiles. Various factors such as soil depth, porosity, and soil pH, etc. influence the microbial population. Soil profiles are sometimes many meters deep, but most of the research in soil microbiology considers only the surface soil. To expand our knowledge on microbial communities inhabiting in the deeper soil horizons, the bacteria population from five distinct zones of the Sundarbans mangrove forest was studied. The present study revealed that the highest number of bacteria were isolated from surface soil. A decreasing trend of the total bacterial population was found with increasing depth of soil. A similar type of decreasing profile was found for the pH of the soil which indicated a correlation between bacterial population and soil pH. The aim of this study was to determine the effect of various factors in the growth of bacteria that can be used in future research prospects to ascertain their role in maintaining ecological balance in the Sundarbans Mangrove forest.

Keywords: Sundarbans; Mangrove Forest; pH; Productivity; Bacterial population; Soil depth.

1. Introduction

Mangrove is a distinctive ecological niche to different microbes where organisms play notable role in different environmental activities, biodegradation and nutrient recycling (Alongi D.M, 1994; Amir & Ahmed, 1993; Asutin, 1988; Sudakshina Das et al., 2016).

The fundamental part of the mangrove ecosystem is formed by microorganisms. They play significant role in conservation, and recovery of mangrove forest and are necessary for productivity of the ecosystem(Holguin G, 2001). Diverse range of microbes such as bacteria, fungi, algae, protozoa, and actinomycetes, etc. are present in the marine environment (Asutin 1988; Amir and Ahmed 1993). Microorganisms actively engage in sulphur, carbon, nitrogen and phosphorous cycles in mangrove forest (Rojas et al.,2001; Toledo et al.,1995; Vazquez et al., 2000).

The distribution of microbial population in mangrove ecosystems is quite variable. Some studies explored the impact of soil salinity on the microbial community and found that high salinity had a significant negative impact on microbial population (Siddikee et al., 2011; Yuan et al., 2007). In order to define the distribution of microbial population and the major factors involved in controlling the distribution in mangrove estuaries much research remains to be done (Afruza Begum, Rasheda Yasmin Shilpi, 2020; Subhajit Das et al., 2011; K. Kathiresan, 2002; Khan A.S. and Ali M.S, 2007; Ozcelik et al., 2008).

Halophilic microorganisms can survive and grow in saline and hyper saline environments (Amir & Ahmed, 1993; Subhajit Das et al., 2011; Gilmour D, 1990). These microorganisms are the target of studies related to the origin of life in earth and adaptation mechanisms to saline environments (DasSarma S. and Arora 2002). Mangrove consists of intertidal zones of estuaries, deltas, creeks, brackish waters and lagoons of tropical and subtropical latitudes (Subhajit Das et al. 2011) and are usually considered as highly productive areas. The high productivity demands for more nutrients for the growth of plants which appears to be fulfilled by a highly efficient system of nutrient uptake and recycling (Alongi et al.,1993; J. Hernotand G. Robertson, 1994; R. K. Jain et al.,2005; S. K. Singh and J. P. N. Rai, 2004). Soil microbes, plants and soil organic matters are closely related to each other for better management of nutrients within the whole ecosystem(Alongi D.M, 1994).

Mangrove provides a vibrant habitat for different microbes (Bhattarai1 A, 2015).Microorganisms are intimately involved in biogeochemical cycling and in some instances are the only biological agents to regenerate elements used by plants and other organisms (Capone, 2002; Subhajit et al., 2011).They live in every part of the biosphere, including soil, ocean, atmosphere, and inside rocks within the earth's crust. Bacteria, protozoa, and actinomycetes can tolerate more soil disturbance than fungal populations (Janusauskaite D and Kadziene G, 2013; Silva et al.,2013). Soils occupies about 8 to 15 tons of bacteria, protozoa, nematodes, fungus, earthworms, and arthropods (Brady NC 2012). Soil bacteria are essential in soil formation, pollutant degradation, and the maintenance of groundwater quality (Afruza Begum, Rasheda Yasmin Shilpi, 2020; Blume et al., 2002; Dodds et al. 1996; Fierer et al., 2003; Fritze H, 2000; Ghiorse W. and Wilson J, 1988; Hiebert FK, 1992; Konopka & Turco, 1991; Madsen E, 1995; Mandal SN, 2013; Richter & Markewitz, 1995; Van et al.,1992). Salinity, soil depth, pH, water temperature, and many other factors affect the dispersion of microorganisms (Alavandi 1990). In the marine ecosystem salt-tolerant bacteria are the governing species (Zaharan H.H, 1992).In the present study, an attempt has been taken to explore the vertical distribution of bacterial population through four depths of sediments collected from Mrighamari, Kolisgong, Sutarkhali, Sarbotkhali, and Kalaboghi areas of Sundarban, Bangladesh.

2. Materials and Methods

2.1. Site description

Sundarban is a distinctive forest which is located between 21°56´59´´N and longitude 89°10´59.988″ in Bangladesh. Samples were collected from Mrighamari, Kholisgonj, Sutarkhali, Sarbotkhai and Kalaboghi areas of Khulna range in the Sundarbans National forest (Figure 1) (Afruza Begum, Rasheda Yasmin Shilpi 2020).

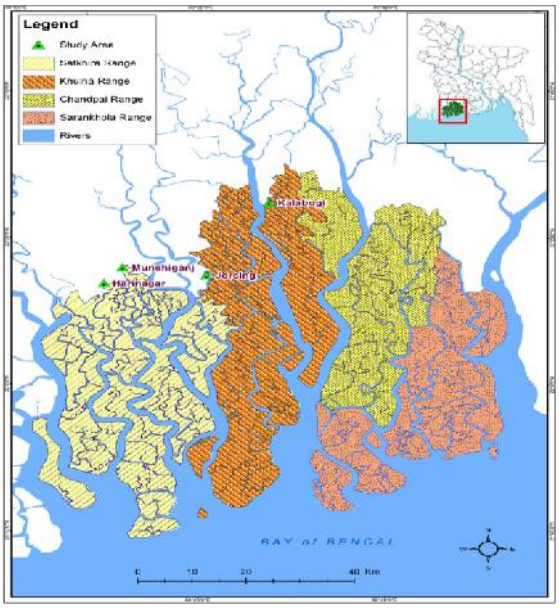

Figure 1. The map represents the zones of the present study.

2.2. Collection of soil sediments:

Soil sediments were collected from four depths of soil within the range of 0 to 100 cm. These soil segments were indicated as a top, middle, lower middle and bottom segments for (0- 25) cm, (25-50) cm, (50-75) cm and (75-100) cm respectively and were stored in sterilized polythene bags in the cooler and transported to the laboratory (Afruza Begum, Rasheda Yasmin Shilpi 2020).

2.3. Preparation of serial dilution of soils samples

Each of the ten test tubes was filled with 9ml of distilled water and autoclaved. Then 1 gm of soil sample was taken in a test tube and the content of the test tube was mixed well using a vortex mixer. This tube was marked as 10^{-1} dilution. 1ml of the sample from this test tube was transferred into another test tube and was vortexed to obtain 10^{-2} dilution. In this way by transferring 1ml of the diluted sample, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6},10^{-7}, 10^{-8}, 10^{-9}dilution samples were prepared.

2.4 Spread Plate Procedure

There are several methods to determine the number of micro-organisms that are present in a population. In this experiment bacterial colonies were accomplished by spread plate technique. The method is somewhat more time consuming but provides statistically accurate and repeatable results. In spread plate technique, 0.1 ml of 10^{-9}dilution solution was spread over the surface of an agar plate and distinct colonies were formed after 24 hours (Figure -02).From the plate count data, the concentration of bacteria in the original sample was calculated by using the following equation:

$$\frac{\text{number of CFU}}{\text{Volume plated(ml)} \times \text{total dilution used}} = \frac{\text{number of CFU}}{\text{ml}}$$

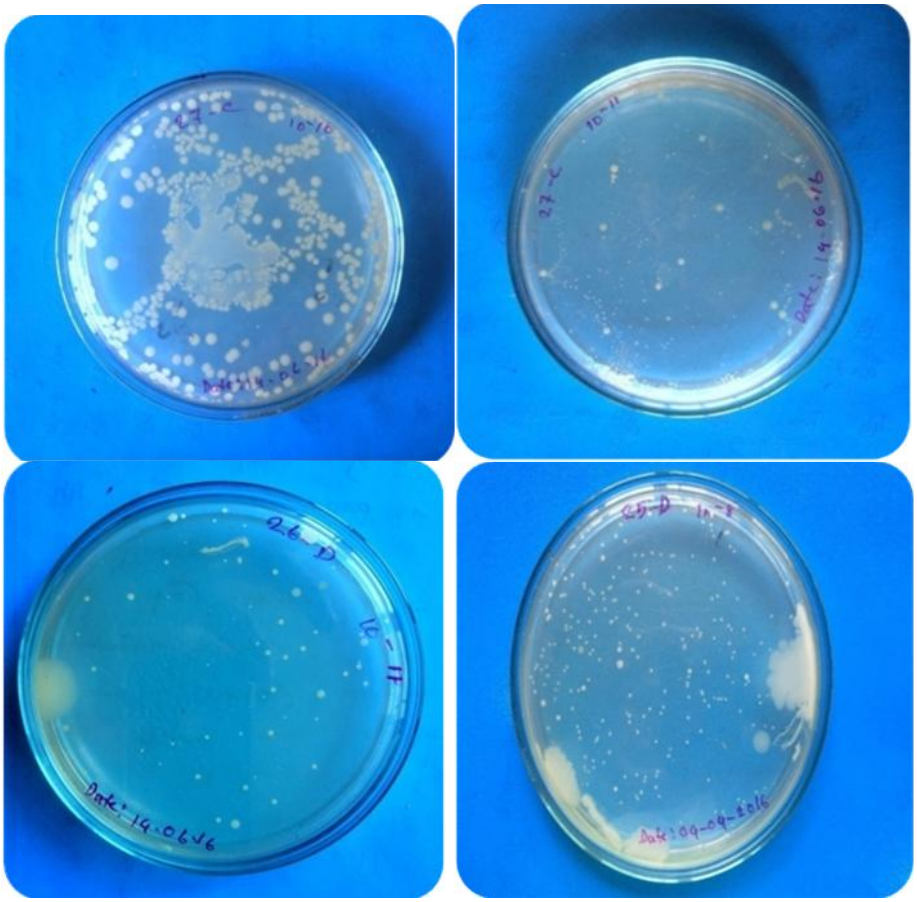

Figure 02: Photographic representation of bacterial population on Nutrient Agar media

3. Results and Discussion

The relative number and morphology of bacterial species at top, middle, lower middle and bottom soil profiles in exploration areas have been covered in the following section (Table -01).

Table 01: Bacterial load and colony characteristics in four depths of mangrove (Sundarbans) soil in 10 $^{-9}$ dilution.

Location	Depth (in cm)	Colony code number	Avg bacterial C.F.U (× 10^{10}) gm–1 wt. of sediment ±stdv	Color	Size	Form	Margin	Elevation
Mrighamari	0-25	4A	427.5 ± 17.6	Orange Cream	Pinpoint Small Moderate Large	Circular Irregular	Entire	Raised Flat
	25-50	24B	311.5 ± 4.95	Off-white Cream	Small Large Moderate Pinpoint	Circular Irregular	Entire Undulate	Flat
	50-75	24C	95 ± 1.41	Off white	Small Moderate Large	Circular Irregular Rhizoid	Lobate, Undulate Entire	Flat
	75-100	24D	72 ± 4.24	Off-white	Small Moderate Pinpoint	Circular	Entire	Raised
Kholisgonj	0-25	25A	512 ± 4.24	Off-white Cream Orange	Pinpoint Small Moderate	Circular Irregular	Entire Undulate	Raised
	25-50	25B	380 ± 7.0	Orange off-white Yellow	Pinpoint Moderate Large	Circular Irregular	Entire Undulate	Raised
	50-75	25C	52 ± 2.82	Off-white Yellow	Large Moderate Pinpoint	Circular Rhizoid	Entire Filiform	Raised
	75-100	25D	42 ± 4.24	Off-white	Pinpoint Small Moderate	Circular	Entire	Raised
Sutarkhali	0-25	26A	190 ± 2.82	Off-white	Small Moderate	Circular	Entire	Raised
	25-50	26B	118 ± 7.0	Off-white Cloudy – white White	Large Moderate Small Pinpoint	Circular Irregular	Entire Lobate	Flat

	50-75	26C	66 ± 5.65	Off-white	Large Moderate Small	Circular, Filamentous	Entire Filiform	Raised
	75-100	26D	98 ± 7	Off-white	Moderate Small Pinpoint	Circular Curled	Entire curled	Raised
Sarbotkhali	0-25	27A	250 ± 49.49	Off-white	Moderate Small	Circular Rhizoid	Entire	Raised
	25-50	27B	210 ± 4.24	Cream	Moderate Small	Circular	Entire	Flat
	50-75	27C	90 ± 5.65	Cream	Moderate Pinpoint	Circular Irregular	Entire Undulate	Flat
	75-100	27D	112 ± 14.14	Off-white	Moderate Small Pinpoint	Circular	Entire	Raised
Kalaboghi	0-25	28A	267.5 ± 10.6	Off-white Cloudy-White	Small Moderate	Circular	Entire	Flat Raised
	25-50	28B	162.5 ± 17.67	Off-white	Moderate Small	Circular	Entire	Raised
	50-75	28C	75 ± 00	Off-white	Moderate Small Pinpoint	Circular	Entire	Raised
	75-100	28D	28 ± 4.2	Off-white	Moderate Small Pinpoint	Circular	Entire	Raised

3.1. Interpretation of result in Mrighamari station

The bacterial community in soil sediments depends on soil depth, porosity, soil organic matter, and pH, etc. In this experiment, bacterial load was determined by the depth of soil. According to Brattarai et al., (2015) lower number of microorganisms is found in compact soil, soil with low organic matter, and on deeper soil strata.

In Mrighamari station, total bacterial load declined with increasing depth of soil (427.5 ($\times 10^{10}$)gm-1 ± 17.6 in 0-25cm soil segment, 311.5 ($\times 10^{10}$)gm-1 ± 4.95 in 25-50cm soil segment and 95 ($\times 10^{10}$)gm-1 ± 1.4 in 50-75cm soil segment and 72($\times 10^{10}$)gm-1 ± 4.2 in 75-100cm soil segment) which is supported by Bhattarai et al., 2015; Das et al., 2011 (Table -01, Figure-3).

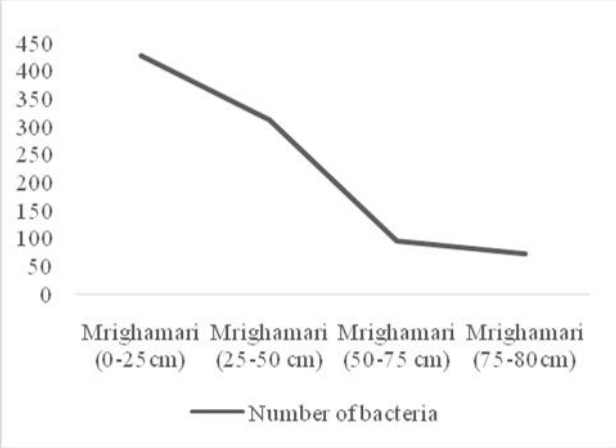

Figure 3: Total bacterial CFU (× 10^{10} gm^{-1}dry weight of soil) in four soil segments in Mrighamari area.

3.2. Interpretation of result in Kholishgonj station

The bacterial growth profile found in Kholisgonj station was like Mrighamari station. In Kholisgonj, total bacterial colony in four soil depths were 512 (×10^{10})gm^{-1} ± 4.2 in 0-25cm soil segment, 380 (×10^{10}) gm^{-1} ± 7 in 25-50cm soil segment and 52(×10^{10})gm^{-1} ± 2.8 in 50-75cm soil segment and 42(×10^{10})gm^{-1} ± 4.2 in 75-100cm soil segment)(Table -01, Figure-04).

Microbial population in the soil is limited by soil porosity, more the pore space higher is the count of microbes (Collins H 2010; Grubinger V 2004; Magdoff F 2010). The bacterial population is found to be more in O_2 rich soil compared to CO_2(McNabb DH 2009). A major contributor to poor aeration is soil compaction(Day SD 1994). Compact soil can cause problems by the reduction in total pore space, soil oxygen content, water infiltration and percolation rates, etc. (Brady NC 2012). Soil compaction increases with an increase in depth(Swer H, Dkhar MS 2011). Thus, bacterial load decreased in lower parts of the soil segment in Kholisgonj station was supported by previous experiments.

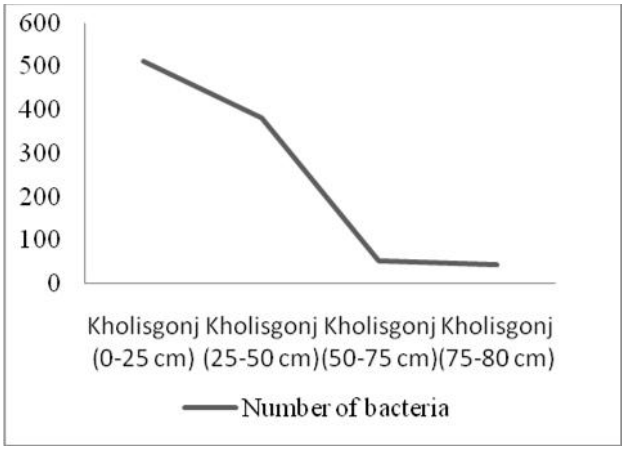

Figure 4: Total bacterial CFU (× 10^{10} gm^{-1}dry weight of soil) in four soil segments in Kholisgonj area.

3.3. Interpretation of result in Kalaboghi station

In Kalaboghi station, bacterial load in four soil profiles were 267.5 (×10^{10})gm^{-1} ± 10.6 in 0-25cm soil segment, 162.5 (×10^{10})gm^{-1} ± 17.6 in 25-50cm soil segment and 75 (×10^{10}) gm^{-1} ± 00 in 50-75cm soil segment and 28 (×10^{10})gm^{-1} ± 4.2 in 75-100cm soil segment) which was similar to the decreasing trend of Mrighamari and Kholisgonj stations supported by Das *et. al.* 2011; Fierer*et al.*, 2003 (Table -01), Figure-05).

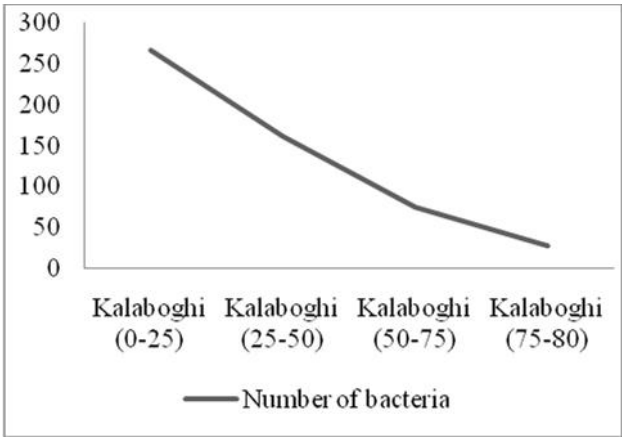

Figure 5: Number of bacterial CFU (× 10^{10} gm^{-1}dry weight of soil) of four soil segments in Kalaboghi area.

3.4 Interpretation of result in Sutarkhali and Sarbotkhali stations

In Sutakhali and Sarbotkhali stations, bacterial load decreased gradually up to 75cm depths and then slightly increased in 75-100cm soil segment. In Sutarkhali station, total CFU were counted as 190 (×10^{10}) gm^{-1} ± 2.8 in 0-25cm soil segment, 118 (×10^{10})gm^{-1} ± 7 in 25-50cm soil segment and 66 (×10^{10})gm^{-1} ± 5.6 in 50-75cm soil segment and 98(×10^{10})gm^{-1} ± 7 in 75-100cm soil segment) (Figure-06).

In Sarbotkhali station, 250 (×10^{10})gm^{-1} ± 4.9 in 0-25cm soil segment, 210 (×10^{10})gm^{-1} ± 4.2 in 25-50cm soil segment and 90 (×10^{10})gm^{-1} ±5.6in 50-75cm soil segment and 112(×10^{10})gm^{-1} ± 14 in 75-100cm soil segment (Figure -07).

Usually, bacterial load decreased along with increasing soil depth which has been explained by (Fierer, Schimel, and Holden 2003). Soil salinity also reduces bacterial diversity and control bacterial abundance, composition, and functions (Moradi1, A., Tahmourespour, A., Mehran Hoodaji, M. and Khorsandi 2011). As bacterial load slightly increased in the 75-100cm soil segment in Sutarkhali and Sarbotkhali which was the bottom segment, it might be stated that soil salinity reduced to a small degree in the 75-100cm segment due to environmental effect.

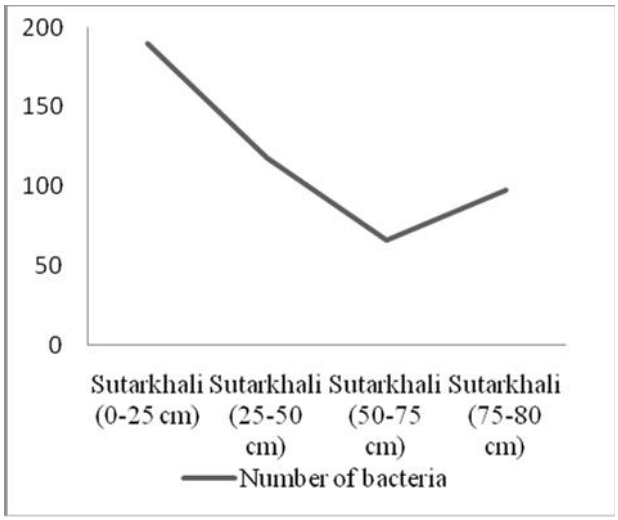

Figure 6: Total bacterial CFU (× 10^{10} gm^{-1}dry weight of soil) in four soil segments in Sutarkhali area.

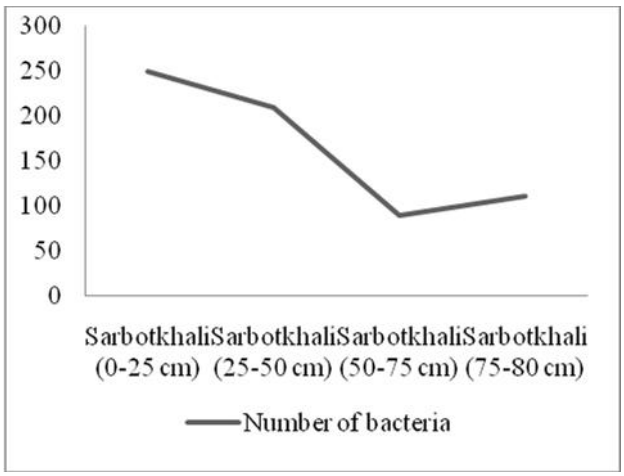

Figure 7: Total bacterial CFU (× 10^{10} gm^{-1}dry weight of soil) in four soil segments in Sarbotkhali area

3.5 The overall result of the bacterial population in relation to depth in five sampling areas

Overall, a reducing trend of cultivable bacterial load was detected with rising depth of soil in all the five zones of Sundarbans, Bangladesh. A huge number of bacteria were remarked even at 10^{-9} dilution (Kathiresan K. and Bingham B. L. 2001) and most of the colonies were off-white, small, circular, and pinpoint due to similar ecological conditions (Figure-02). Some bacterial species such as *Salmonella*, *Enterobacter*, *Klebsiella*, *Staphylococcus*, *Bacillus*, *Pseudomonas* sp. have been recognized in previous work from all these locations based on some biochemical tests and selective media tests which has been described in detail in (Afruza Begum, Rasheda Yasmin Shilpi 2020) (Figure -08).

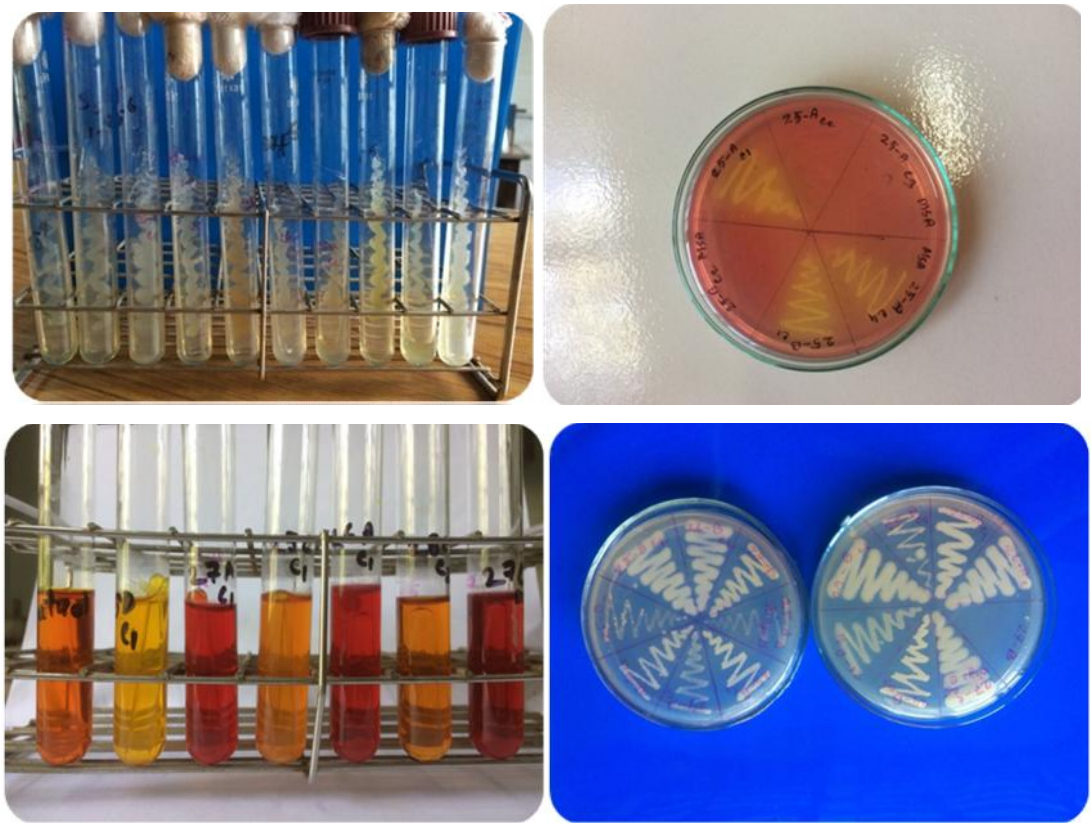

Figure 8: Photographic representation of selective media tests and biochemical tests

3.6 Result of soil pH

Soil pH is an indicator of the soil's acidity which is a primary factor controlling nutrient availability, bacterial processes, and plant growth. A pH of 7.0 is neutral, less than 7.0 is acidic, and greater than 7.0 is alkaline. Results of pH showed that all the 20 soil samples are slightly acidic (Table- 02).

A decreasing trend in soil pH was observed with gradual extension in-depth in all stations. The uppermost layers are slightly acidic, and the acidity of soil increased with increasing depth. In Mrighamari, pH values in all depths were 6.7, 6.3, 6.1, and 6.0 respectively. In Kholishgonj, the values were 6.8, 6.5, 6.0, and 5.8. In Sutarkhali, 6.5, 6.2, 6.0, and 5.9, in Sarbotkhali, 6.2, 6.0, 5.9, 5.9 and in Kalaboghi, values were 6.5, 6.3, 6.2, 5.7 respectively in top, middle, lower middle and bottom sediments. The reduction of pH with depth denoted the generation of organic acids and carbon dioxide by roots of mangrove plants. The surface soils are slightly acidic because of the impact of alkaline delta water in the mangrove ecosystem(Clarke PJ 1985).

Table 02: Soil pH change with depth in five studied zones in Sundarban mangrove forest

Location	Depth (in cm)	Colony code number	pH of soil
Mrighamari	0-25	24A	6.7
	25-50	24B	6.3
	50-75	24C	6.1
	75-100	24D	6.0
Kholisgonj	0-25	25A	6.8
	25-50	25B	6.5
	50-75	25C	6.0
	75-100	25D	5.8
Sutarkhali	0-25	26A	6.5
	25-50	26B	6.2
	50-75	26C	6.0
	75-100	26D	5.9
Sarbotkhali	0-25	27A	6.2
	25-50	27B	6.0
	50-75	27C	5.9
	75-100	27D	5.9
Kalaboghi	0-25	28A	6.5
	25-50	28B	6.3
	50-75	28C	6.2
	75-100	28D	5.7

4. Conclusion

The most remarkable finding of this research was the bacterial growth pattern in four depths of soil in Sundarbans National forest. This growth pattern may be responsible for healthy and fertile soil and management of nutrients of the forest. However, sea level rising due to global warming may cause fluctuation of water as well as soil salinity which may ultimately hamper the activity of bacteria and may become a risk to maintain the ecological balance of the Sundarbans National forest.

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