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Influence of Microbial-Nutrients Relationship on the Mangrove Sediments of Divar Island, Goa, India

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Abstract: Samples were collected from the mangrove ecosystem in three seasons. The samples include a 30cm sediment core and its overlying water. In the Mangrove sediments of Divar Island, microbial actions are complex in both the mineralization and decomposition processes that synchronize depth integrated nutrient profiles. Productivity (Primary by C^{14} Radio Isotope method and Bacterial by 3H-thymadine incorporation assay) in this ecosystem is significantly varied with the seasons. Total viable counts in terms of Colony Forming Units (CFU) showed more non-significant profiles with the depth whereas Microscopic direct counts showed a very significant profile with the depth. The decreasing trend of Organic matter and an increasing trend of Acid Volatile Sulphides suggesting that remineralization with the depth and thus resulting in nutrient supply.
Keywords: Mangrove, Nutrients, Primary Productivity, Bacterial Productivity, Radio Isotopes

I. INTRODUCTION

Mangrove routes a unique ecological niche to different microbes, which play innumerable functions in nutrient recycling (Sahoo, K. and Dhal, N.K., 2009). The breakdown of complex organic matter in to inorganic nutrients in this system incorporates multi-step process. An enzymatic hydrolysis is the first step to convert polymeric material to soluble monomeric and oligomeric compounds. In aerobic conditions, the soluble compounds are directly mineralized to CO_2 and H_2O, whereas under anaerobic conditions, various physiological clusters are involved in degradation after the preliminary depolymerisation. Fermentative bacteria modify the organic matter accessible in the mangrove ecosystem in to array of byproducts by hydrolysis, mostly short chain fatty acids, carbon dioxide and hydrogen (Das, S., et al. 2012) Further conversion across the action of secondary fermenters, sulphate reducers, acetogens and methanogens produce the end products as CO_2, CH_4 and H_2S, which may escape into the atmosphere (Alavandi, S.V., 1990).
Salinity and the soil properties of coastal sediment determine the degrees of inhibition over microbial activity and the biochemical processes that are fundamental in maintaining ecological quality and production (Ashaduzzaman, S.M., et al. 2011). Microorganisms play a major role in Carbon, Sulphur, Nitrogen and Phosphorous cycles in mangrove forest both in oxic and anoxic condition (Das, S., et al. 2011). Microbial activity is accountable for major nutrient transformations within a mangrove ecosystem (Alongi, D.M., et al., 1993, Holguin, G., et al., 1999) and also accountable for most of the carbon flux in tropical mangrove sediments. They route most of the energy flow and nutrients, and act as a carbon sink (Ghosh, A., et al., 2010). While microbial communities are believed to be accountable for key nutrient transformations within near-stream sediments, there is comparatively less information on factors that regulate microbial activities in these areas. Mangrove ecosystems are rich in bacterial flora. Productiveness of the mangrove waters results from the microbial decomposition of organic matter and recycling in to inorganic nutrients (Alongi, D.M., 1988). The mangrove environment is described by a particular cycling of nutrients and enzymatic relations in which organic matter decomposition is based on a set of distinct final electron acceptors, due to the ready use of oxygen that is released from plant roots, lacking its accessibility in oxidation processes (Kristensen, E., et al., 1994). Data to date recommend that high productivity in mangroves is attained when nutrients limit growth through highly efficient nutrient cycling and nutrient retention mechanisms (Ball, M.C., 2010). Owing to their estuarine and marine existence, mangroves are generally not limited by the relatively large quantities of sulfur, boron, potassium, magnesium, and sodium in seawater but are frequently limited by nitrogen and phosphorus. Iron and copper have been found to be limiting for mangroves in mesocosm studies (Alongi, D.M., 2010, 2017). The serious necessity for nitrogen and phosphorus has been repeatedly confirmed in field and laboratory studies (Reef, R., et al., 2010). A vital function in mangrove nutrition is the interactive consequences between different nutrients and environmental aspects such as soil type and texture, salinity and frequency of tidal flood (Lovelock, C.E., et al., 2006) The competence of microbial metabolic means is also an effective nutrient management strategy. an enormous fraction of plant root breathing goes towards the

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uptake and assimilation of nitrogen especially ammonium. Ammonium is favorably taken up when equated with nitrite & nitrate as the uptake of ammonium involves the less energy investment for mangroves (Ball, M.C., 2010, Reef, R., et al., 2010) in spite of the fact that, mangrove ecosystems are rich in organic matter or and highly productive, normally, they are nutrient-poor, especially of nitrogen and phosphorus (Sengupta and Chaudhuri, 1991; Holguin, et al., 1992; Alongi, et al., 1993; Vazquez, et al., 2000). This anomaly may describe by a very systemised nutrient recycling in which scarce essential nutrients are held and new nutrients are regenerated from decomposing (Alongi, D.M., et al. 1993). The concentration of Dissolved Organic Carbon (DOC) in pore-waters is greater than that in over lying waters at sediment, yet no net flux of DOC takes place between them. Simultaneously, a very active and productive microbial community thrives in the sediments (Alongi, D.M., et al. 1989). Present study was planned to understand the heterotrophic bacterial population and its relationship with nutrients variation & in relation to the physicochemical parameters of mangrove sediments of Divar Island during three seasons. Sediment core from surface to 25cm depth is our main focus in this study.

II. MATERIAL AND METHODS

A. Site Description and Sampling Procedure

Samples were collected from mangrove island of Divar (15°30′35″ N; 73°52′63″ E), Mondovi estuary (Fig. 1). Water samples were collected close to the bottom (just above the sediments) and the sediment samples were collected using a core size of 30 cm long and width of 5cm dia. Samplings were done at monthly intervals over a period of ten months from November 2011 to August 2012 except April 2012. Two liters of water samples were collected and pre-filtered through 220 µm nylon mesh to remove the debris. These samples were kept in ice box and transported to the laboratory for analysis. Sediment samples collected (25cm) were sub sampled carefully by 10 sections at every 2.5cm interval in the lab. Data were looked on the seasonal basis, pre-monsoon (February, March, and May) monsoon (June, July, and August) and post-monsoon (November, December, and January).

Temperature was measured immediately after sample collection using the centigrade thermometer with a graduation of 0 to 50°C with ±0.1°C accuracy. pH was measured using a portable bench top pH meter (Lab India), calibrated with standard buffers before measuring the samples. Dissolved Oxygen (DO) was measured by Winkler's method [20]. Chlorinity was estimated by Mohr□Knudsen's method (Mohr, F., 1856) and from the knowledge of chlorinity, salinity (S) was calculated using the Knudsen relation: S (× 10 –3) = 1.80655 × Cl (× 10–3). The organic matter was determined by the modified Wakly-Black method (oxidation with potassium dichromate in sulphuric acid solution) to obtain organic carbon (Walkley, A. and Black, I.A., 1934).

Primary Productivity (PP) is measured using one ampoule of $NaH^{14}CO_3$ (Board of Radiation and Isotope Technology, Mumbai; Specific activity 185 kBq) and Bacterial Productivity (BP) rates were derived from determination of methyl-^{3}H-thymidine incorporation rates (TdR; specific activity 18000 mCi/mmole, Bhabha Atomic Research Centre, Mumbai) by the water column bacteria following the method described in the JGOFS 1996 protocols (Knap, A.H., et al., 1996, Ducklow, H.W., 1993). Total viable count done by spread plate method in Nutrient agar plates (Hi-Media, India). Acridine orange direct counts for Total Bacterial Count (TBC) were made following Hobbie, et al., (1977) and the mean cell number per field was calculated and used for estimating total abundance by using the relationship detailed in Parsons et al. (1984). Nitrate, Nitrite, Silicate and Phosphate were estimated using the methods suggested by Grasshoff, P., (1983). Acid Volatile Sulphide (AVS) is measured by method suggested by Simpson, et al., (2005).

B. RESULTS AND DISCUSSION

A. Environmental Factors

Significant variations were recorded in environmental factors during the present investigation. Overlying water samples were examined for Salinity, Temperature, pH and Dissolved Oxygen (DO) from November 2011 to August 2012 (Table No.1). Salinity varied seasonally with the high peak during Pre monsoon (26.3‰) followed by Post monsoon (19‰) and monsoon (6‰). Salinity values were ranging from 3‰ to 29‰. Temperature recorded notably, ranged between 25□C to 31□C. The lowest was recorded during the Post monsoon (26□c) followed by Monsoon (27.3□C) and Pre monsoon (29□c). pH does not show much variation during the study period; more or less it maintained constant value ranging 7.5 to 7.8. Whereas DO indicated a variation ranging from 2.64 ml/L to 3.77ml/L with a peak in Post monsoon (3.12 ml/L) followed by Monsoon (2.90 ml/L) and Pre monsoon (2.72 ml/L).

B. Seasonal Differences in Productivity in Overlying Water

The seasonal rates of PP are highly fluctuated and ranged from 241.9 mgC/m /day to 1673.1 mgC/m /day. The highest PP recorded in Pre monsoon (1286.962 mgC/m /day) followed by Post monsoon (1274.271 mgC/m /day) and Monsoon (422.486 mgC/m /day). The prime reason for low PP in Monsoon may be due to the high turbidity in overlying water caused by monsoon rainfall land runoff (Fig No. 2A). Similarly, the seasonal rates of BP show high fluctuations, in the range of 0.0035 mgC/m /day - 0.0861 mgC/m

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/day. The highest BP recorded in Monsoon is (0.086 mgC/m /day) followed by Pre-monsoon (0.040 mgC/m /day) and Post monsoon (0.026 mgC/m /day). This may be due to the temperature fall (Table No.1) and low levels of Nutrients (Fig No.2B).

1) *Seasonal variations in bacterial abundance vertical profile:* The dynamics of Bacterial population are taken in terms of Total Viable Counts (TVC) and Acridine Orange Direct Counts (AODC). In the TVC, there is no substantial variation in the vertical profile of bacterial population dynamics excluding overlying water (OW); where it is two folds lower to the populations in sediment but has a slight hike in the mid depths (Fig No.3). This describes the uniform proliferation of the bacterial abundance. It is depicting the descending vertical profile i.e. high at the surface and low at bottom (Fig No.4). The average Bacterial load (avg. of TVC and AODC) in seasonal variations is as noted; Monsoon (2.95x1010), <Post monsoon (2.65x1010) < Pre monsoon (2.53x1010) (Table no.2). The probable reasons for these variations are allochthonous sources of nutrients due to the continues rainfall during the monsoon and post monsoon. Alongi, et. al., (1993) and others have also recorded similar kind of results in their investigations conducted in Australian mangrove ecosystem.
2) *Seasonal differences in nutrient vertical profile:* The nutrient vertical profile of core sediment showed a clear picture of seasonal fluctuations; this may be due to the internal biogeochemical processes and external nutrient sources. The distribution of Nitrite, Nitrate, phosphorus, and Silicate are shown in Fig No. 5. The vertical profile of these nutrient concentrations has significant variations along the depth with a notable seasonal fluctuation. Silicate and Phosphate (Fig No. 5 C&D) has more or less constant vertical profile trend in all the seasons but there is only hike in concentration. Phosphate recorded a peak in the monsoon (6.536mg/L) followed by post-monsoon (0.816mg/L) and pre-monsoon (0.751mg/L) with the range of 0.29mg/L to 8.36g/L; on other hand, Silicate recorded a peak in the monsoon (12.081mg/L) followed by pre-monsoon (0.788mg/L) and post-monsoon (0.419mg/L) in the range of 0.172mg/L to 15.971mg/L. Nitrite and Nitrate values increased with depth in pre-monsoon, monsoon decreasing and in post monsoon it has a maximum in the middle. The highest concentration of Nitrite was observed (8.140mg/L) in monsoon and lowest observed (1.597mg/L) during Post monsoon with the range of 0.076μg/L to 17.023μg/L. Similarly, Nitrate recorded its highest in the Monsoon (6.291mg/L) and lowest during Pre monsoon (0.447mg/L) with the range of 0.073mg/L to 6.299mg/L.
3) *Seasonal fluctuations of organic matter (om) vertical profile:* Organic Matter % instability is significantly noticed in both seasonally and vertically. In vertical profile, surface sediments have highest concentration, followed by the middle and bottom. High levels of organic matter were observed during the monsoon (3.639%) followed by post monsoon (3.188%) and pre-monsoon (1.955%) with a range of 1.03% to 8.47% (Fig No. 6). Presence of such high level of organic matter in surface sediments is may be the recent accumulation from the adjacent non-point sources brought in by the monsoon rains and/or may be due to the presence of fine clay which gives a more surface area and consequently leads to increase in sorption of more organic matter (Naidu, A.S., et al., 1982).
4) *Seasonal fluctuations of Acid Volatile Sulphide (AVS) vertical profile:* Sulphide concentrations in the deep core sediments are high compared to the surface and are may be largely controlled by the sulphate reducers (Fig No.7). However, studies conducted have shown that AVS concentrations are varied seasonally with depth. In this study, AVS concentrations were measured monthly for 10 months in several segments of sediment cores. AVS concentrations showed two orders of magnitude in relation to Organic matter (Table No.4). AVS is observed at the surface sediments low and high concentrations at bottom (Fig No. 11). The ascending vertical profile is could be due to the carbon mineralization at bottom by the sulphur-reducing bacteria. The highest AVS observed during Monsoon (110.94mmol/kg) followed by Post monsoon (104.64mmol/kg) and Pre monsoon (103.92mmol/kg) (Table No.4). The correlation between AVS and OM shows a fascinating trend. A positive correlation is observed with seasons (Table No.4) and whereas negative correlation is observed with vertical profile (Fig No.7)

III. CONCLUSION

In the present study depth profiles of overlying-water and sediment biogeochemical characteristics regarding microbial-nutrients relationship, thus microbiological study of this sample will be an effective clue for determination of Productivity, Nutrients, remineralisation and other Hydrographic parameters along with Bacterial load. Variation in seasons and as well as with depth is clearly observed. Low levels of carbon and high levels of AVS at lower depths may be due to high mineralization by the anaerobic bacteria and resulting in nutrient supply. The correlation between AVS and organic matter is likely related to changes in primary productivity and sediment microbial population. In order to understand the ecosystem response to temporal and seasonal changes, chemical and biochemical tracers like carbon stable isotopes, molecular microbial diversity and molecular organic molecules (lignin, lipid, phenol, etc.) of sediment, particulate matter, biological systems, rate of CO_2 emission, etc. are to be investigated for better understanding and sustainable management of the mangroves which are of highly sensitive and critical ecosystem.

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APPENDIX -I: TABLES

Table No.01: Physicochemical seasonal variation data

Season	Post- Monsoon	Pre-Monsoon	Monsoon
Salinity	19	26.3	6
Temperature	26	29.0	27.3
pH	7.6	7.6	7.6
DO	3.1	2.7	2.9

Table No.02: Seasonal variations in bacterial population (an average between AODC and TVC

Season	AVS mmol/kg	OM%
Post monsoon	104.64	3.19
Pre monsoon	103.92	1.96
Monsoon	110.94	3.64

Table No. 03: Seasonal correlation between AVS and OM

Season	TVC No. X 10^9(Cells/L)	AODC No X 10^{11}(Cells/L)	avg. No X 10^{10}(Cells/L)
Post monsoon	1.98	3.31	2.65
Pre monsoon	1.93	3.13	2.53
Monsoon	2.08	3.83	2.95

APPENDIX -II: FIGURES

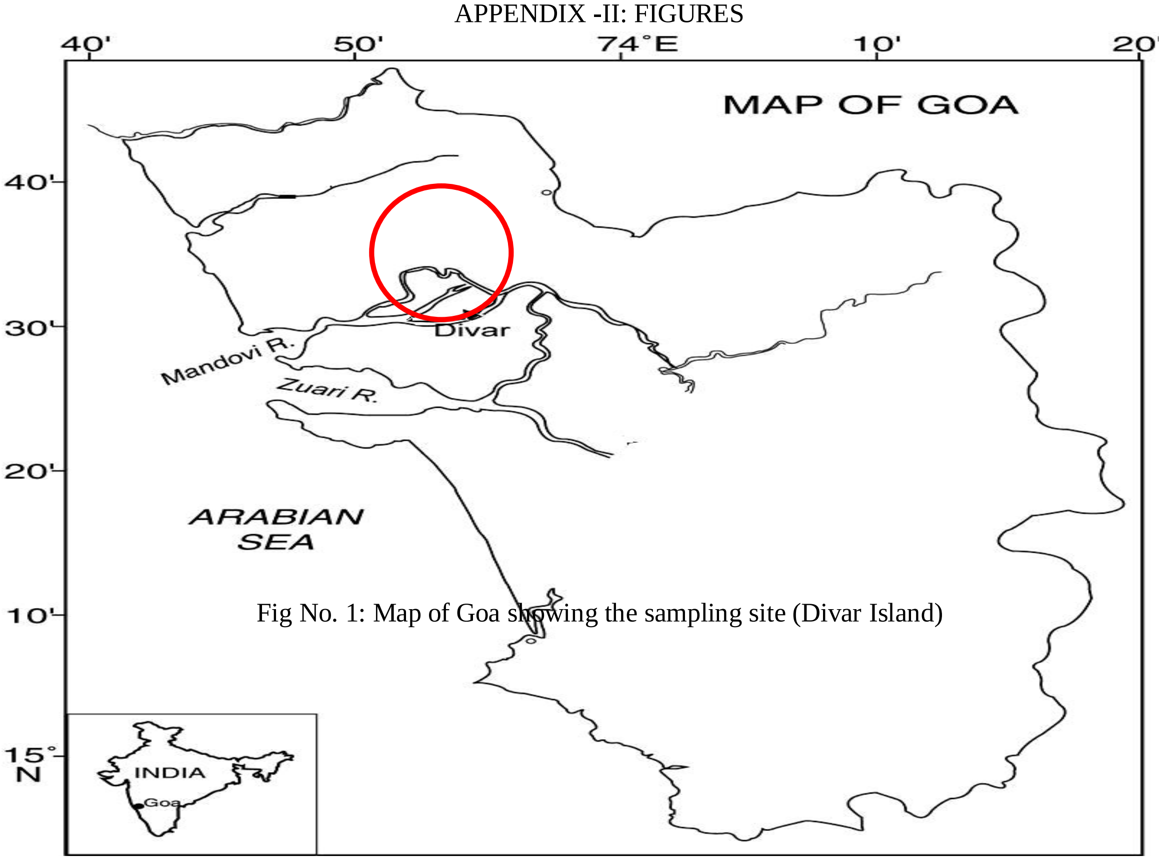

Fig No. 1: Map of Goa showing the sampling site (Divar Island)

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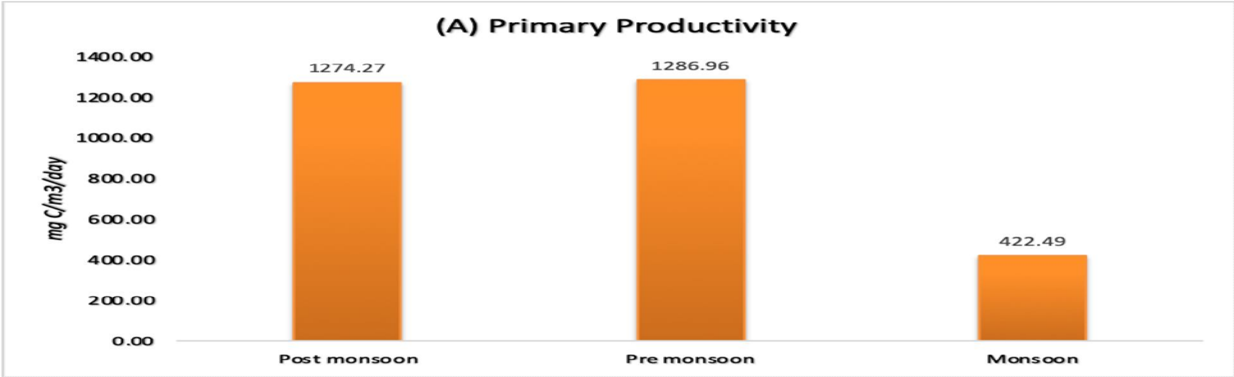

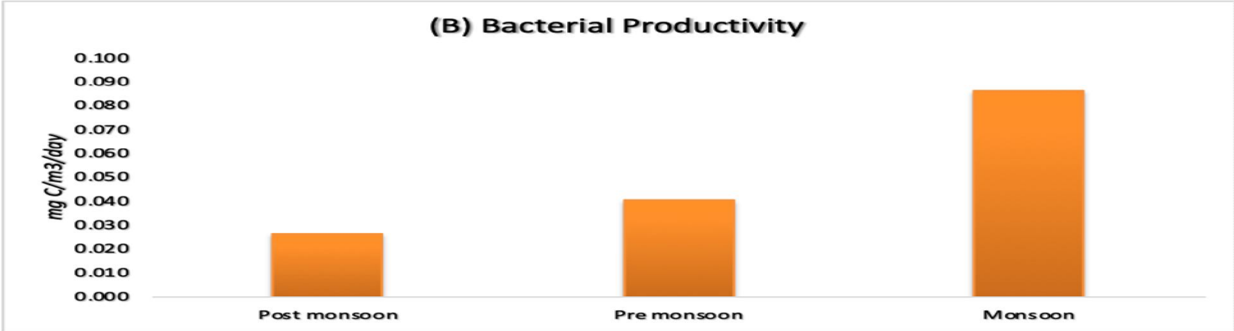

Fig No.2: seasonal variations in Productivity (A) Primary and (B) Bacterial

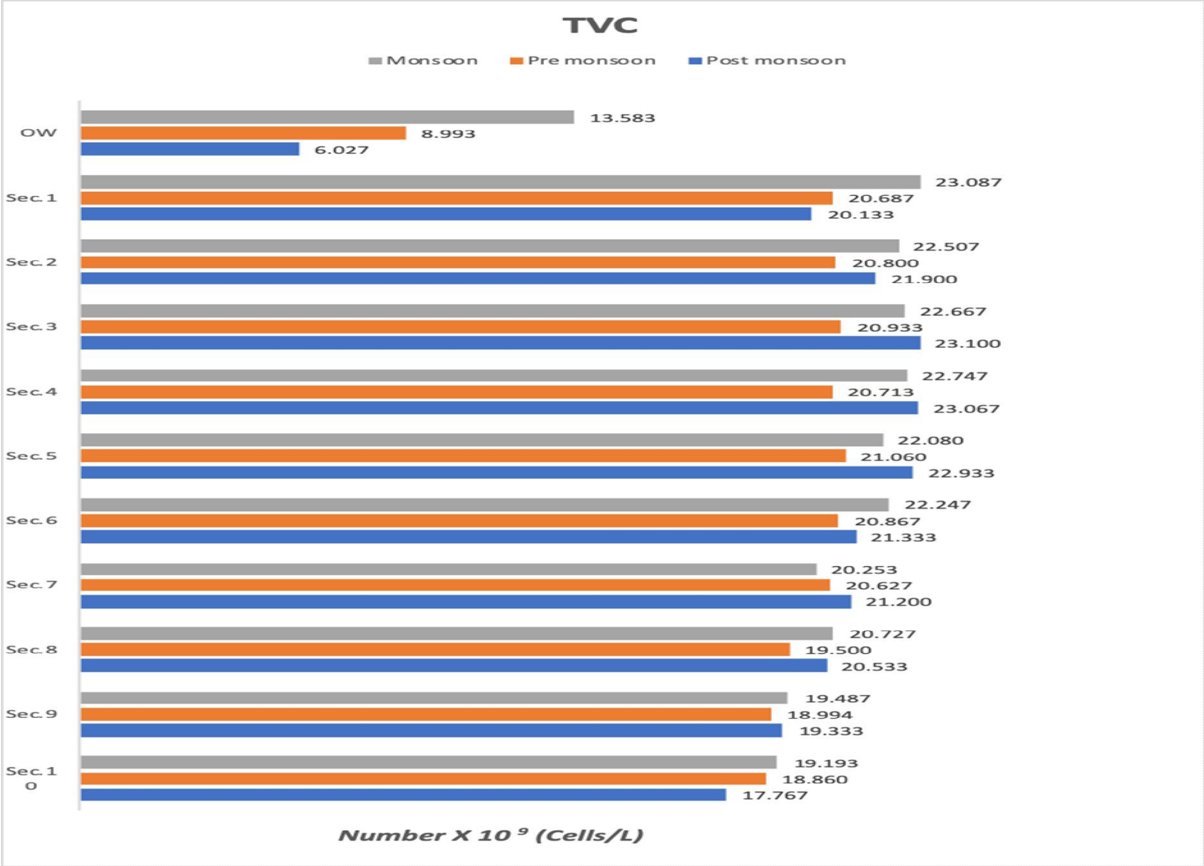

Fig No.3: TVC Seasonal vertical profile variation

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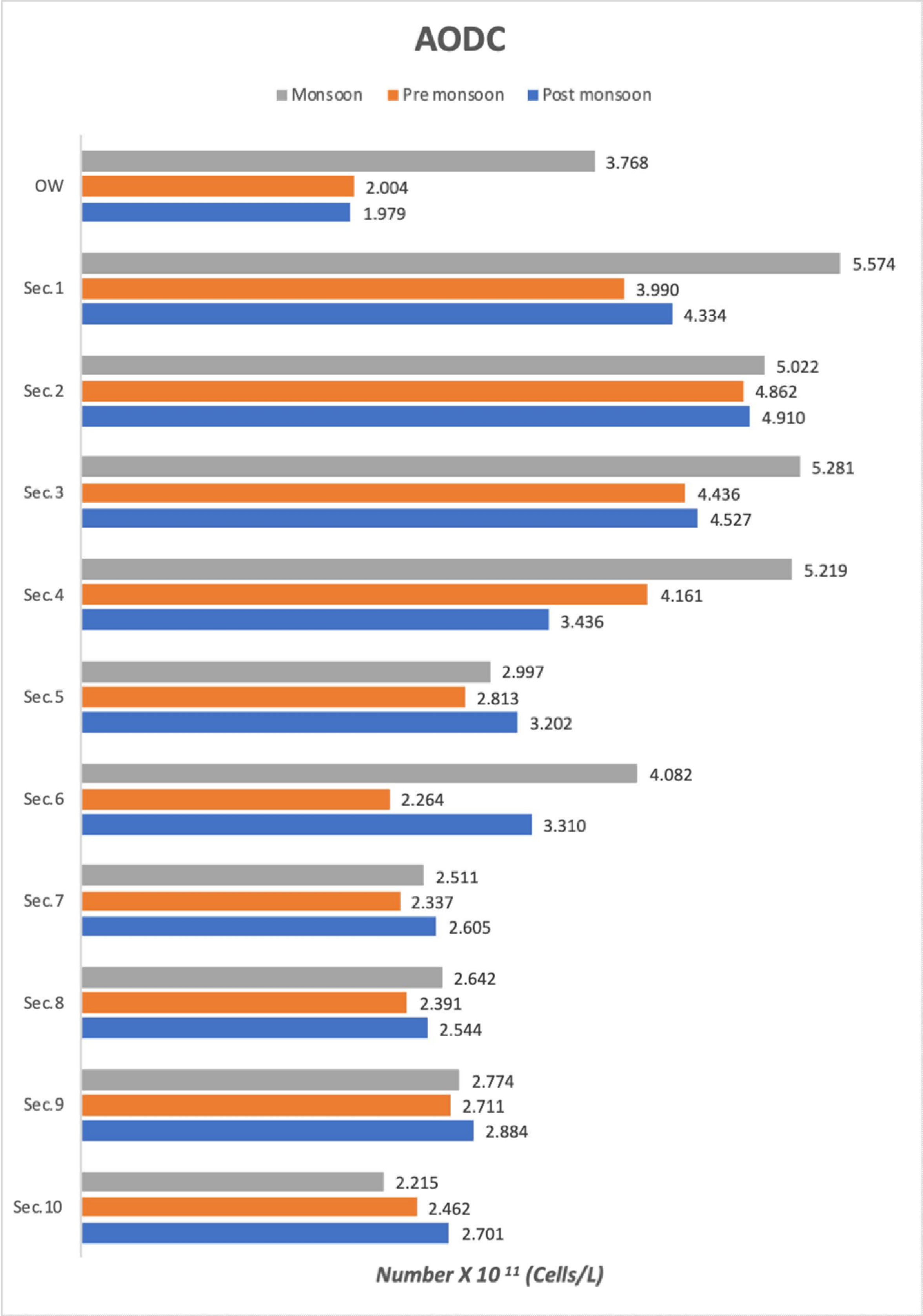

Fig No.4: AODC Seasonal vertical profile variation

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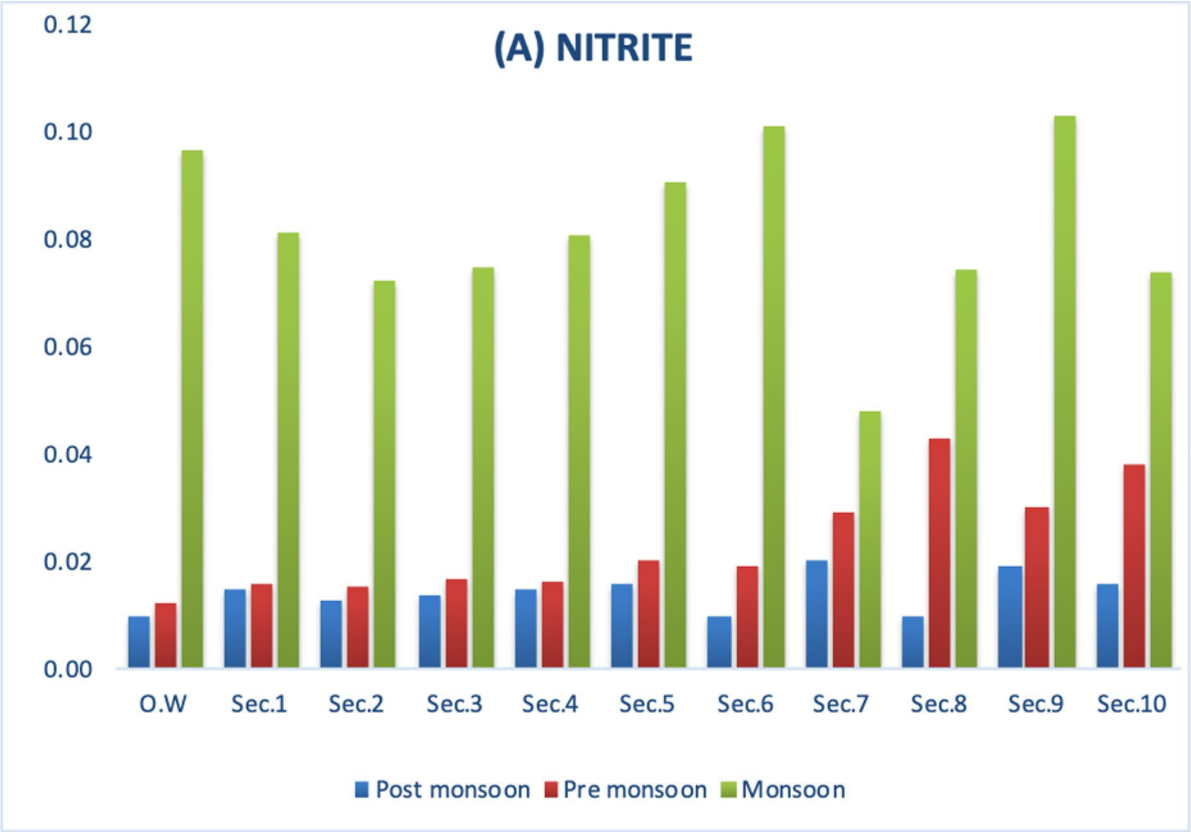

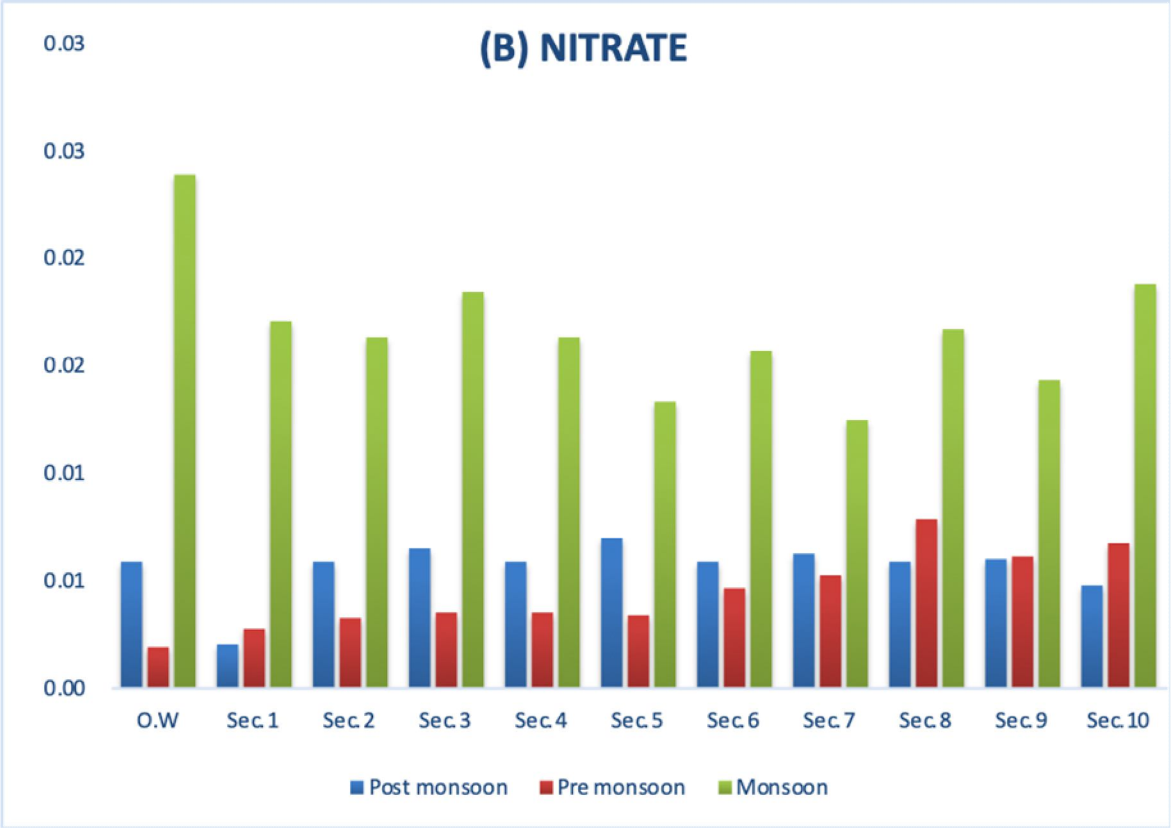

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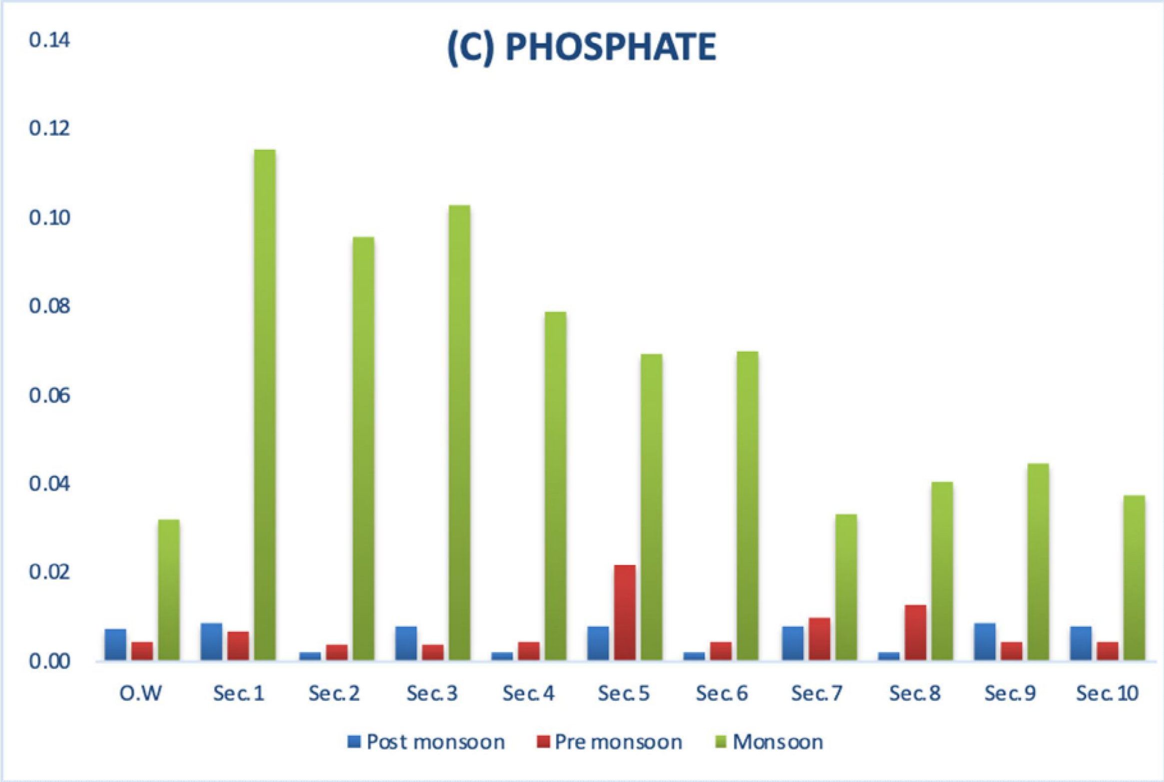

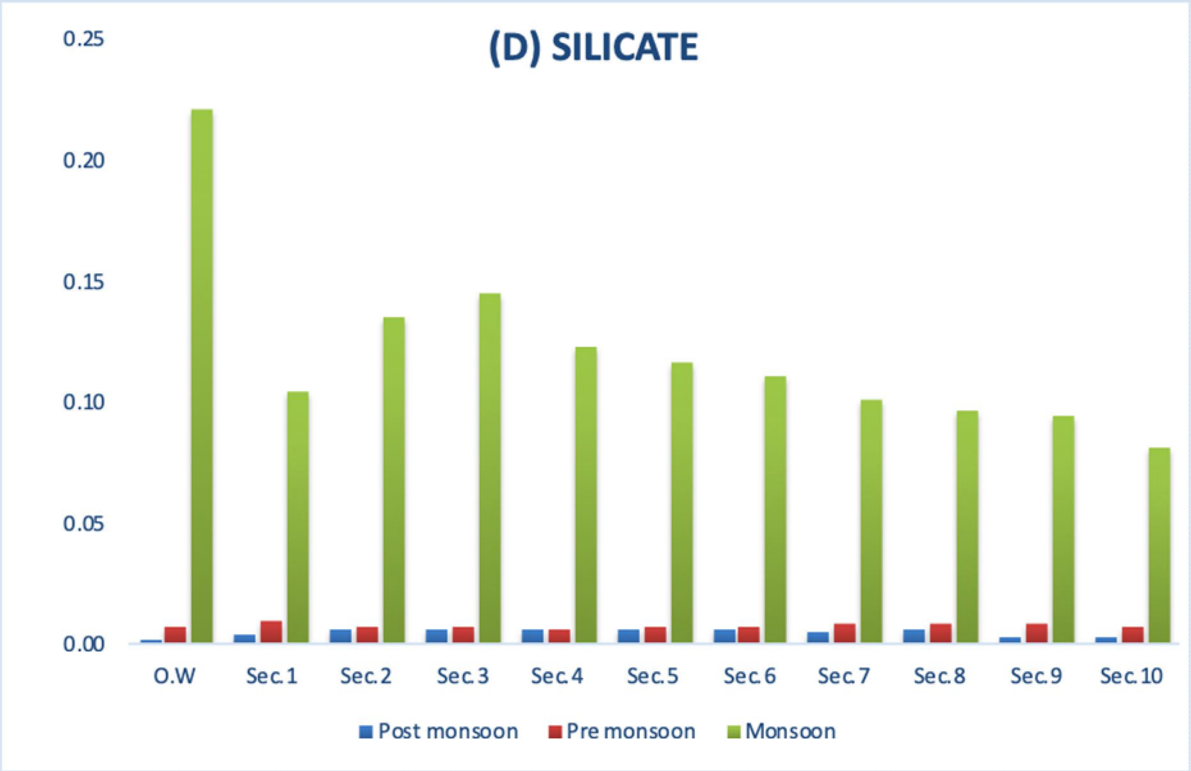

Fig No.5: Seasonal differences in Nutrients (µmol/L for water and mg/L for Sediment): (A) Nitrite, (B) Nitrate, (C) Phosphate, (D) Silicate.

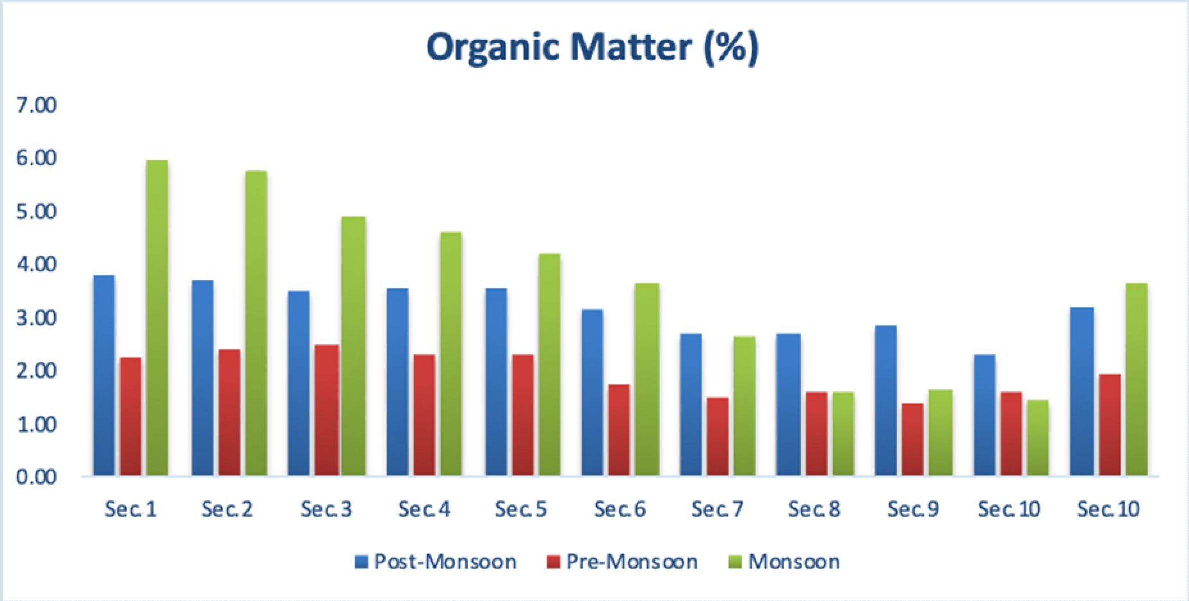

Fig No.6: Seasonal variation of OM vertical profile

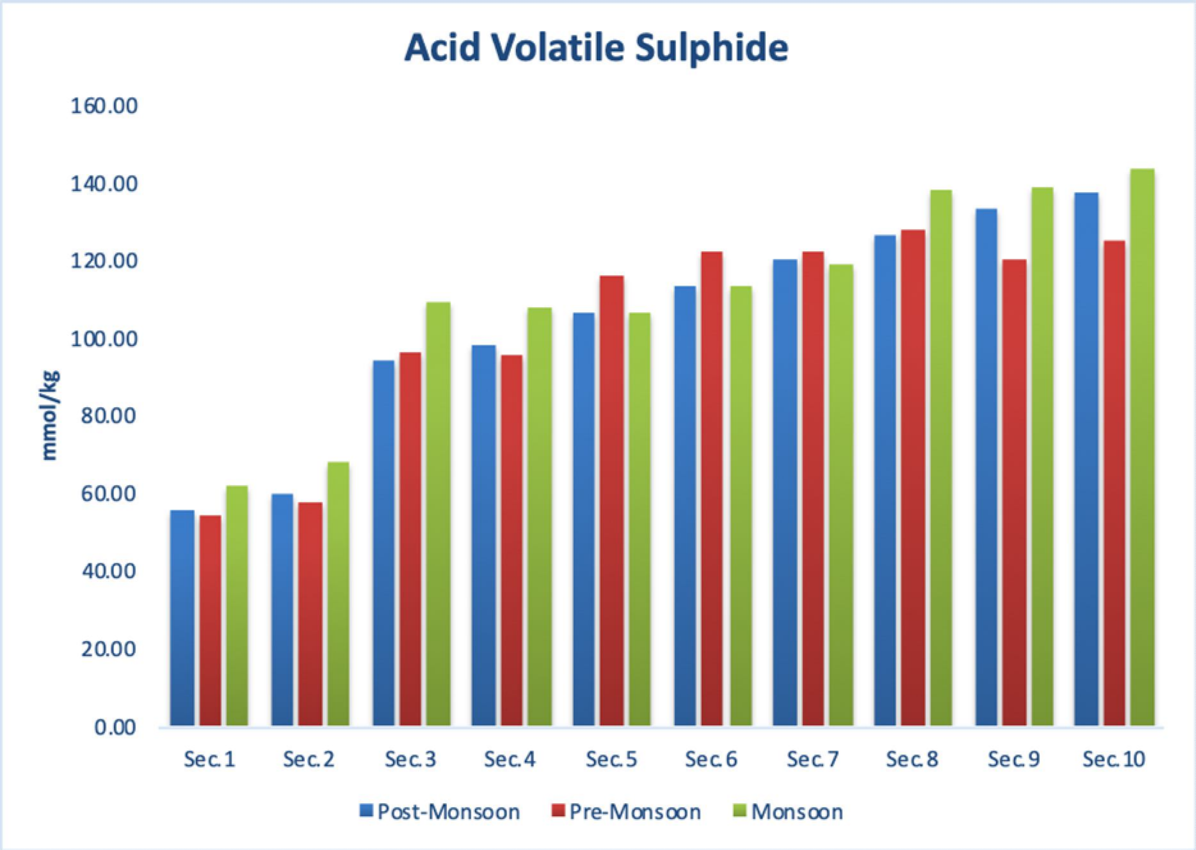

Fig No.7: Seasonal variation of AVS vertical profile

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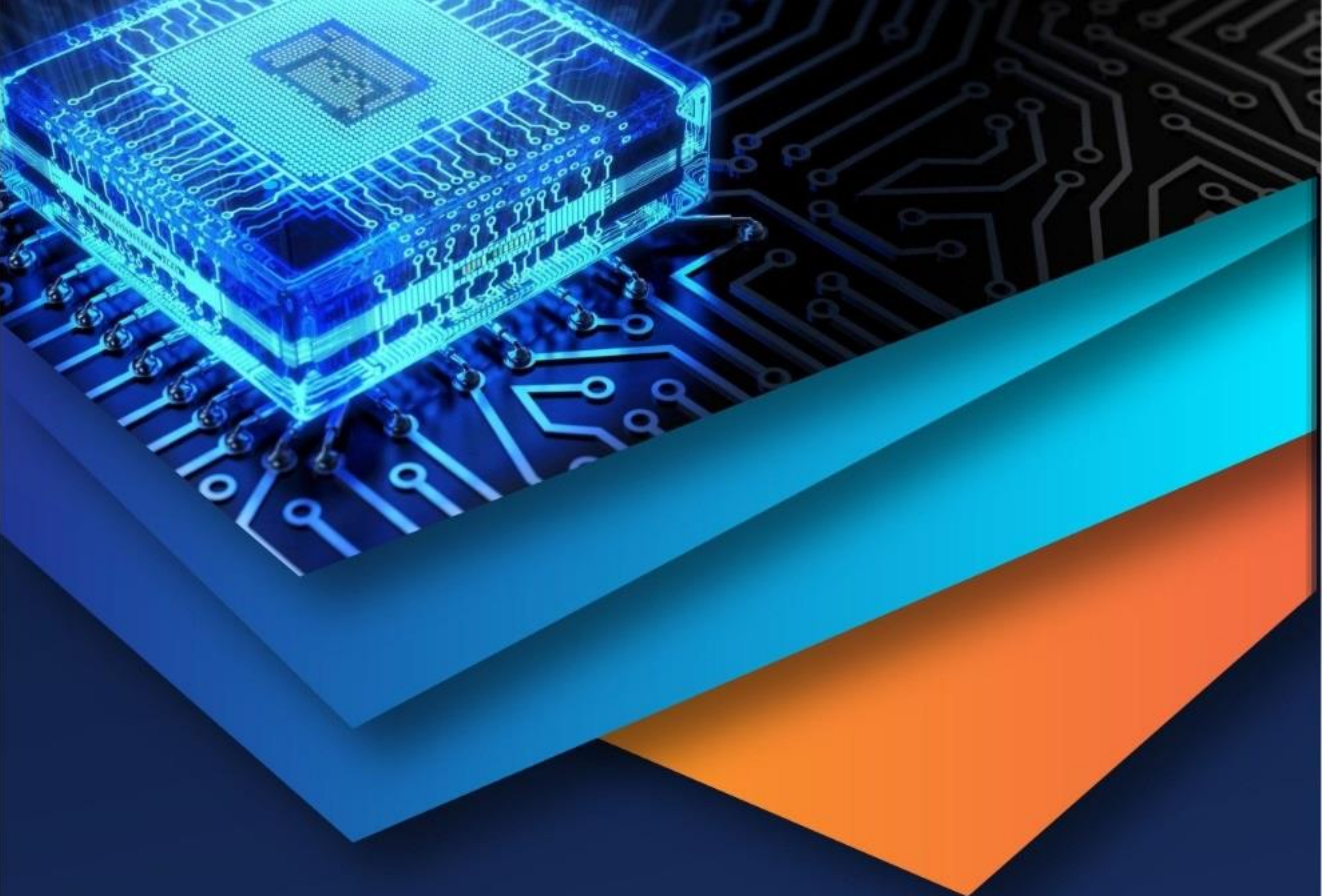

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