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Assessment of Nutrients and Particulate Organic Matter in Freshwater Fishponds at Krishna-Godavari Delta, India.

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Abstract
Pollution in freshwater ecosystems leads to the eutrophication, which results in high phytoplankton biomass, structural losses to aquatic habitats, low oxygen availability and at times logged toxic waters. Altogether nine freshwater fishponds were taken for the study, which are located at Krishna- Godavari delta. The main aim of this study is to understand the water quality variations on a time scale along with the particulate organic matter and some account on bacterial abundance. Multivariate Euclidean cluster analysis isolated the entire year January 2016 to December 2016 into three significant seasons such as pre-monsoon (March-June), monsoon (July to October) and post-monsoon (November to February). Water temperature (24.12±0.88°C), dissolved oxygen (3.42±0.08 mg/litre), total alkalinity (175.42±47.97 mg/litre) and dissolved nutrients were observed with respect to the seasons and type of fish culture. The total bacterial abundance of water varied between 1.33±0.22 CFU/Ml*10^5 and 7.01±05 CFU/Ml*10^5. Whereas, heterotrophic bacteria are positively linear correlated to the total bacterial count, which ranged from 0.52±0.12 CFU/Ml*10^5 to 5.71±0.5 CFU/Ml*10^5 and constituted 39.12 to 81.45% of total bacterial communities during the observed seasons. This study can be considered as a model to assess the freshwater ponds to understand the water quality variations due to the accumulation of organic deposits influences the bacterial growth.

Keywords
Bacterial growth, freshwater fishponds, Krishna-Godavari Delta, nutrients, particulate organic matter

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1. INTRODUCTION:
Industrialization, urbanization, agriculture, and climate change are belonging to a wide group of components which were induced by the human habitations or anthropogenic activities mostly. The species composition and habitations follow biogeochemical patterns with respect to the changes in water quality. Pollution in freshwater ecosystems leads to the eutrophication, which results in high phytoplankton biomass, structural losses to aquatic habitats, low oxygen availability and at times logged toxic waters (Rabotyagov *et al.*, 2014). In spite of all the above issues, it was noteworthy that the aquaculture industry became an important cash crop

in many regions of world, but the impacts of environmental situations have also increased (Cao *et al*., 2007). The common impacts include pathogens, introduction of genetically modified organisms, additives and drugs, antimicrobial resistance, spread of diseases, escapes, over exploitation of wild species, and nutrient enrichment (Pelletier *et al*., 2007). The aquaculture ponds have also been recognized as an N_2O source to the atmosphere and also a CO_2 sinks as well (Boyd *et al*., 2010; Hu *et al*., 2012). The high usage of organic feed in aquaculture farms may be the reason for the enrichment of organic matter and causing the disease outbreaks due to the bacterial invasion. The presence of bacterial infections and disease outbreaks are the major restrictions for the aquaculture production affecting both economic development and socio-economic status of local people in many countries. The microbial populations in aquatic environments are playing important role to convert the organic matter in the form of mineralization and to get rid of undesirable waste compounds. To understand such conditions, it was highly appreciated to study not just for living organisms but also the influence of external factors which are responsible for the above said reasons.

The fundamental support for all the living organisms is water, which not only gives the physical support but also supports the life functions such as feeding, swimming, breeding, digestion and excretion (Bronmark and Hansson, 2005).Hence the studies on the evaluation of water quality such as dissolved and non-dissolved components; physical and chemical components which must be present for the optimum growth of aquatic organisms has to be identified whether they have the affects may be in direct and indirect relation to the organism (Moses, 1983). However, the extent of aquaculture either from small ponds to large-scale commercial system depends on the physical, chemical and biological characteristics of the water. In fish rearing ponds all these factors are inter-related and require careful and constant monitoring to avoid contamination and/or degradation of the aquaculture environment (Gorlach-Lira *et al*., 2013).The excessive accumulation of organic carbon in the sediment enhances anaerobic process and results the release of toxic metabolic substances within the pond and oxygen depletion (Bunting and Pretty, 2007), causing stress to fish, leading to disease outbreaks or direct mortality and lowering yield per crop. Commercial floating pellet feeds and organic matter contains about 45 to 50% carbon (Boyd *et al*., 2002) hence its monitoring is important. Fertilizers, manures, and feedscan cause phytoplankton blooms that increase the concentration of suspended organic particles (Boyd *et al*., 2002). Hence, the present study aimed to understand the water quality variations on a time scale along with the particulate organic matter and some account on bacterial abundance.

2. MATERIALS AND METHODS:

Water samples were collected from nine fish culture systems for twelve months during January to December, 2016 in Gudivada (16.4410° N, 80.9926° E), Krishna district Andhra Pradesh, India wherein culture of Indian major carps (*Labeo rohita* and *Catla catla*), Monoculture of striped catfish (*Pangassiandon hypophthalmus*) and mixed culture of *Labeo rohita* and *Pangassiandon hypophthalmus* was being carried out. Physico-chemical parameters such as dissolved oxygen (DO) was measured using the modified Winkler method (Carrit and Carpenter, 1966) of iodometric titration while nitrogen ($NH4^+$, NO^{2-}, NO^{3-}) and phosphorus (PO_4^{3-}) were analyzed on filtered samples following the standard spectrophotometric procedures (Grasshoff *et al*., 1999, Lu *et al*., 2015) within 8 h of sampling at regional laboratory, Vijayawada (16.4682° N, 80.7195° E). Acetone extraction method was followed for the determination of Chlorophyll-a (Aminot and Rey, 2000; Parsons and Strickland, 1963).

Particulate organic carbon (POC) was measured by filtering known volume of water through filter papers which were oven dried at 60°C and were treated with HCl (1N) fumes or a combination of H_2SO_4 and $FeSO_4$ (3 mL of 2N H_2SO_4 and 5% $FeSO_4$) were added to the sample to remove inorganic carbonates (Nelson and Sommers,1996) and analysed through Elemental Analyzer using L-Cystine as standard for calibration.

Heterotrophic bacterial analysis was carried out in terms of abundance following Standard manual (APHA, 1998; Cochran, 1997; Sorokin, 1985) methods for enumerating total and heterotrophic bacterial colonies. Counting was done in triplicate for each sample and expressed in number L^{-1}.

2.1 Data Analysis:

Statistical analysis of the data was performed in Primer 6, Graph pad prism 5 (Motulsky, 1999). Microsoft excel was used for the data analysis, table preparations and linear regression graphs (Moffet, 2010; Rendulić, 2011). Multivariate Euclidean cluster analysis (Jackson *et al*., 2010; Ozbay *et al*., 2009; Tryfos, 1996) was performed on the monthly water quality data (Bharati and Singh, 2013) of experimental ponds together categorized into three significant seasons (Fig.1) *viz.,* pre-monsoon (March-

June), monsoon (July to October) and post-monsoon (November to February) during the sampling period mentioned elsewhere.

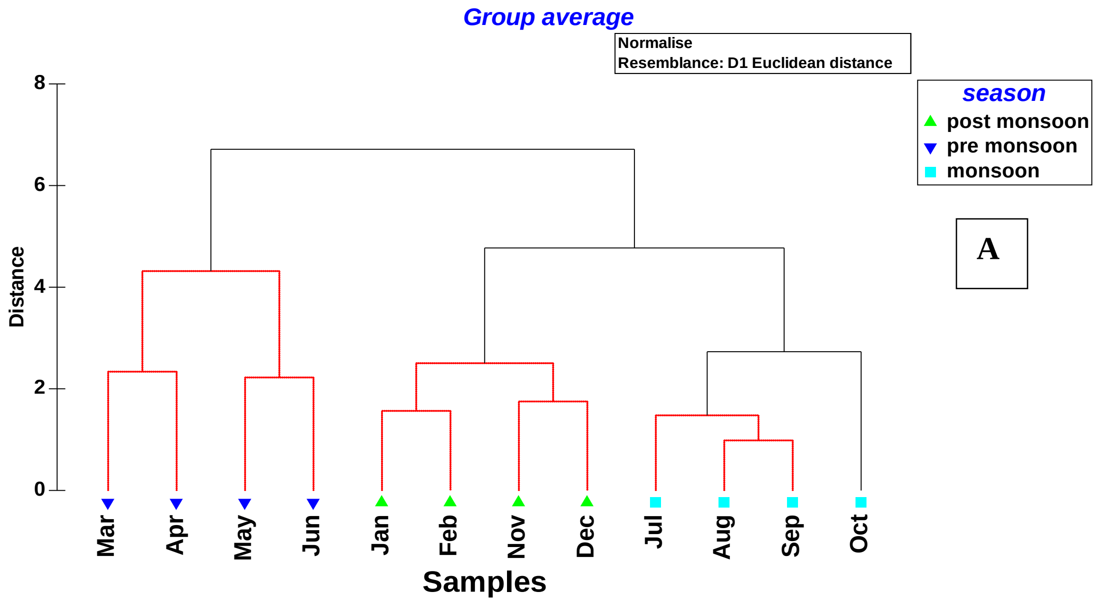
3. RESULTS:

Multivariate Euclidean cluster analysis was performed on the mean monthly water quality data of all the ponds together isolated the entire year January 2016 to December 2016 into three significant seasons such as pre-monsoon (March-June), monsoon (July to October) and post-monsoon (November to February) (Fig.1). Water quality measurements that were recorded in the present study and presented as values exhibited significant seasonal variations for each designated parameter (Table-1). Water temperature was found consistent throughout the year in three culture systems with mean±SD of 24.12±0.88°C and a gross difference of 7.30±0.05°C between minimum (mean±SD 24.92±0.52) and maximum (mean±SD 32.22±0.11) ranges through the year (Fig.2). Mean (±SD) dissolved oxygen of 3.42±0.08 mg/litre has been recorded in experimental ponds extending over different seasons. Highest DO of 6.61 mg/litre was recorded in Pangassius monoculture during monsoon season followed by carp culture systems (6.31 mg/litre) whereas DO was found to be consistent in mixed culture systems (1.62 to 5.62 mg/litre) irrespective of seasonal variations. Lowest DO of 1.32 mg/litre was recorded in striped catfish ponds during pre-monsoon months followed by carp culture systems (1.61 mg/litre). Potential hydrogen (pH) in experimental ponds did not exhibit significant variation with respect to culture system as also seasonal variation. pH in experimental ponds was found to be consistent with mean ± SD value of 7.78±0.05 throughout the year.

Total alkalinity as a measure of Na_2CO_3 reflected consistent values of (mean±SD 154.19±0.15) mg/litre during various seasons in carp culture ponds unlike *pangassius* monoculture and mixed culture systems which recorded highest alkalinity levels of (means±SD) 249.55±40.71 and 250.04±40.91mg/litre respectively during post-monsoon months. Similar trend could be noticed with regard to Total Hardness ($CaCO_3$) wherein *pangassius* monoculture and mixed culture systems recorded highest water hardness levels of (means±SD) 287.62±17.92 and 288.34±18.64 mg/litre respectively during post-monsoon months while total hardness in carp culture systems was consistent with mean±SD 165.39±0.17 mg/litre. Electrical conductivity as a measure to the capacity of water to conduct electrical current ranged from 0.62 to 1.46 mS in different experimental ponds. Although conductivity was found largely independent of culture system and seasonal variation, it was high during monsoon months in culture systems comprising IMC (mean±SD 1.07±0.27 mg/litre), *pangassius* (mean±SD 1.06±0.21 mg/litre) and mixed culture systems ((mean±SD 1.11±0.22 mg/litre).

Ammoniacal nitrogen (NH_3-N) as a function of fish excretion as also *insitu* decomposition was a consistent parameter during the sampling period through various seasons (mean±SD 0.02±0.01 mg/l). Similar trend could be recorded in nitrite nitrogen (NO_2-N) trend which exhibited mean values (±SD) of 0.02±0.01 mg/l, 0.02±0.01 mg/l and 0.02±0.01mg/l during pre-monsoon, monsoon and post-monsoon respectively. Nitrate nitrogen (NO_3-N) in experimental ponds ranged from 0.31 mg/l during post-monsoon months to 0.58 mg/l in monsoon season. Ammoniacal (NH_3-N), Nitrite (NO_2-N) and Nitrate (NO_3-N) forms of nitrogen followed similar trend in the present study. Phosphate in experimental ponds did not vary significantly during study period. Total dissolved solids (TDS) were high in all types of culture systems during monsoon and post-monsoon sampling with mean±SD (mg/litre) values of 628±169.3, 622.9±134.2 and 720.4±137.1 and 622.1±152.9, 636.2±104.2 and 636.3±111.3 in IMC, *Pangassius* and mixed culture systems respectively, while corresponding pre-monsoon values for TDS (mean±SD 512.2±158.8, 552.5±101.8 and 579.6±128.4 mg/litre) exhibited comparatively low trend. *Pangassianodon hypophthalmus* and mixed cropping systems were characterized by relatively high suspended particulate matter throughout the year ranging from 22.19 to 63.23 mg/litre in comparison to IMC culture systems wherein SPM ranged from 16.2 to 32.9 mg/litre in Krishna-Godavari delta in the present study.

Chlorophyll-a as proportion to phytoplankton biomass in experimental ponds ranged from 3.84 to 12.64 during the present study wherein this pigment was recorded abundantly during large part of the year comprising post and pre monsoon seasons (mean±SD 12.08±0.84 &11.25± 0.47). Chlorophyll *a* was positively related to SPM (R^2=0.563; $P<0.05$). Particulate organi carbon ranged from 4.59 mg/l during monsoon in IMC and pre- monsoon of Pangassius culture systems to 15.69 mg/l during post-monsoon in Pangassius culture system. Particulate organic carbon was identified in positive relation to chlorophyll *a* (R^2=0.510; $P<0.05$) and suspended particle matter (R^2=0.579; $P<0.05$). Heterotrophic bacterial abundance was taken as a total quantity for milliliter. The detailed analysis at species level was not reported in this study since this study was designed to estimate the total and heterotrophic bacterial counts in the presence of fish and changes in the water quality conditions with respect to environmental variations. Figure 3 shows the total bacterial abundance of water varied between 1.33±0.22 CFU/Ml*10^5 and 7.01±05 CFU/Ml*10^5. Heterotrophic bacteria are positively linear correlated to the total bacterial count, which ranged from 0.52±0.12 CFU/Ml*10^5 to 5.71±0.5 CFU/Ml*10^5. Heterotrophic bacteria constituted 39.12 to 81.45% of total bacterial communities during various seasons.



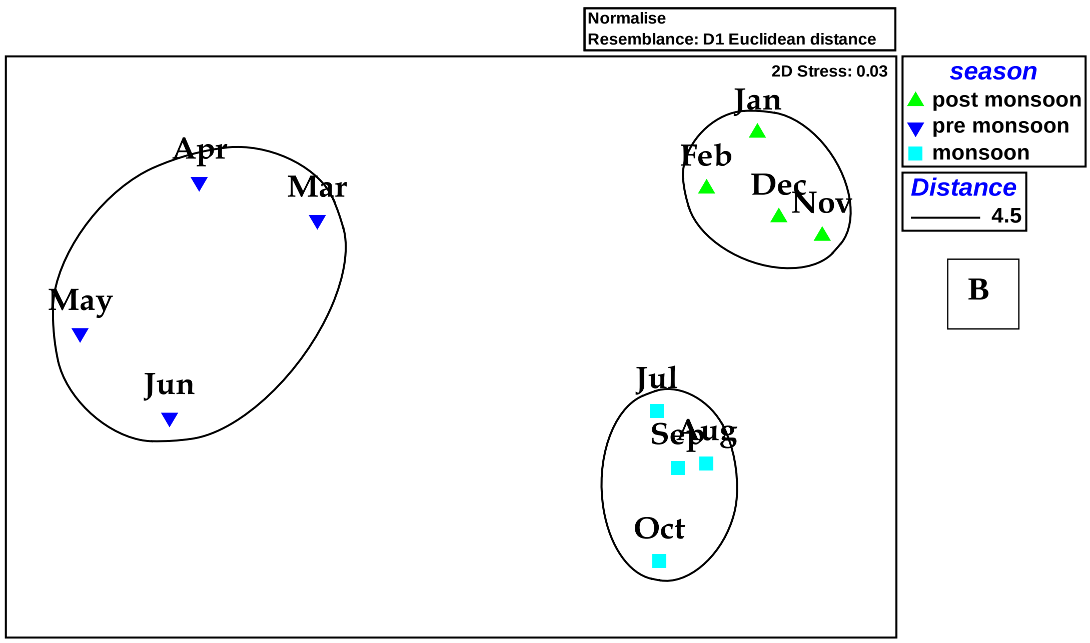


Figure 1: Normalized Euclidean distance-based classification showing three distinct regions (A) Dendrogram and (B) MDS

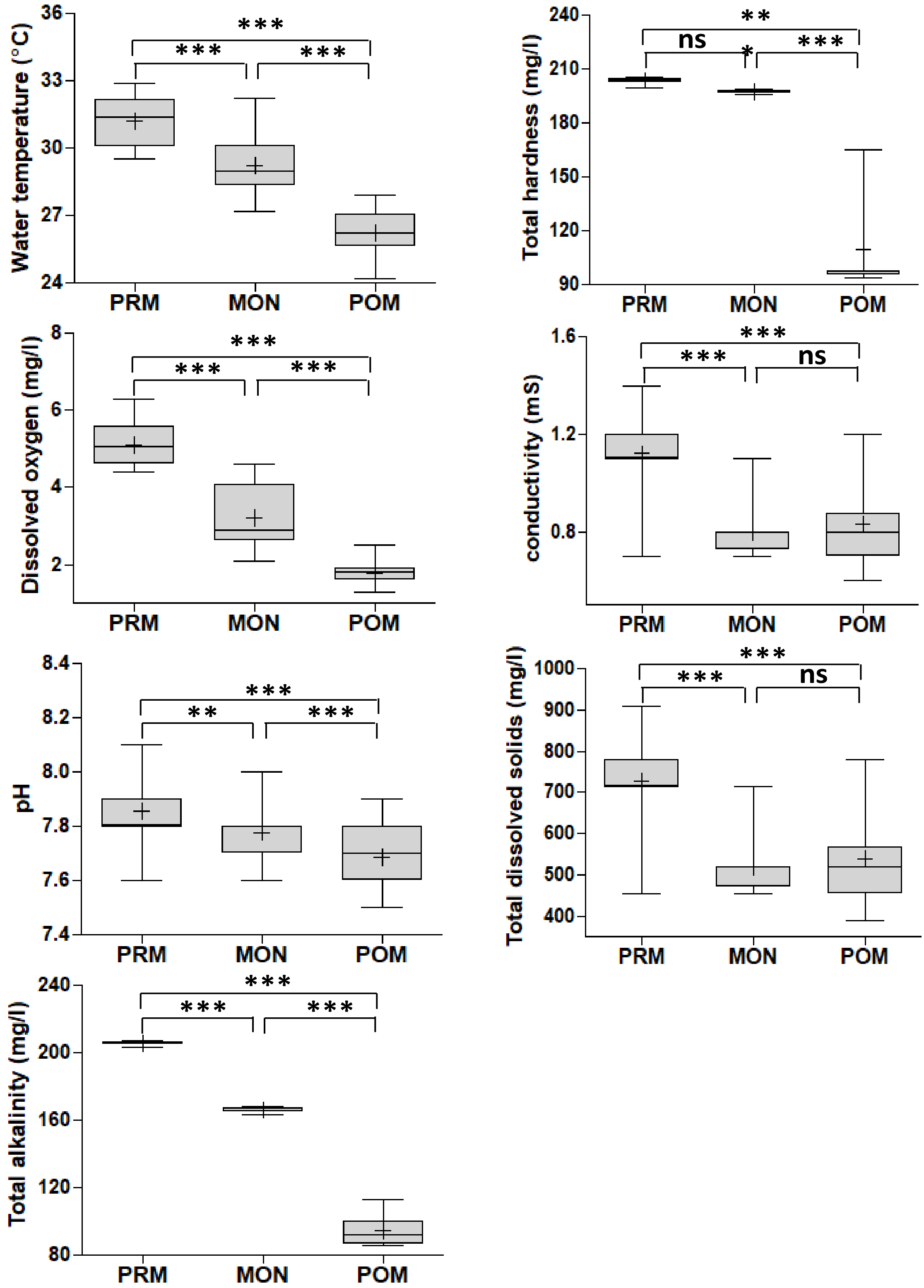


Figure 2: Box plots represents the seasonal variations in the water quality variables
note: * denotes the significant level at P<0.0001, ** = P<0.001, * = P<0.01; ns= not significant**

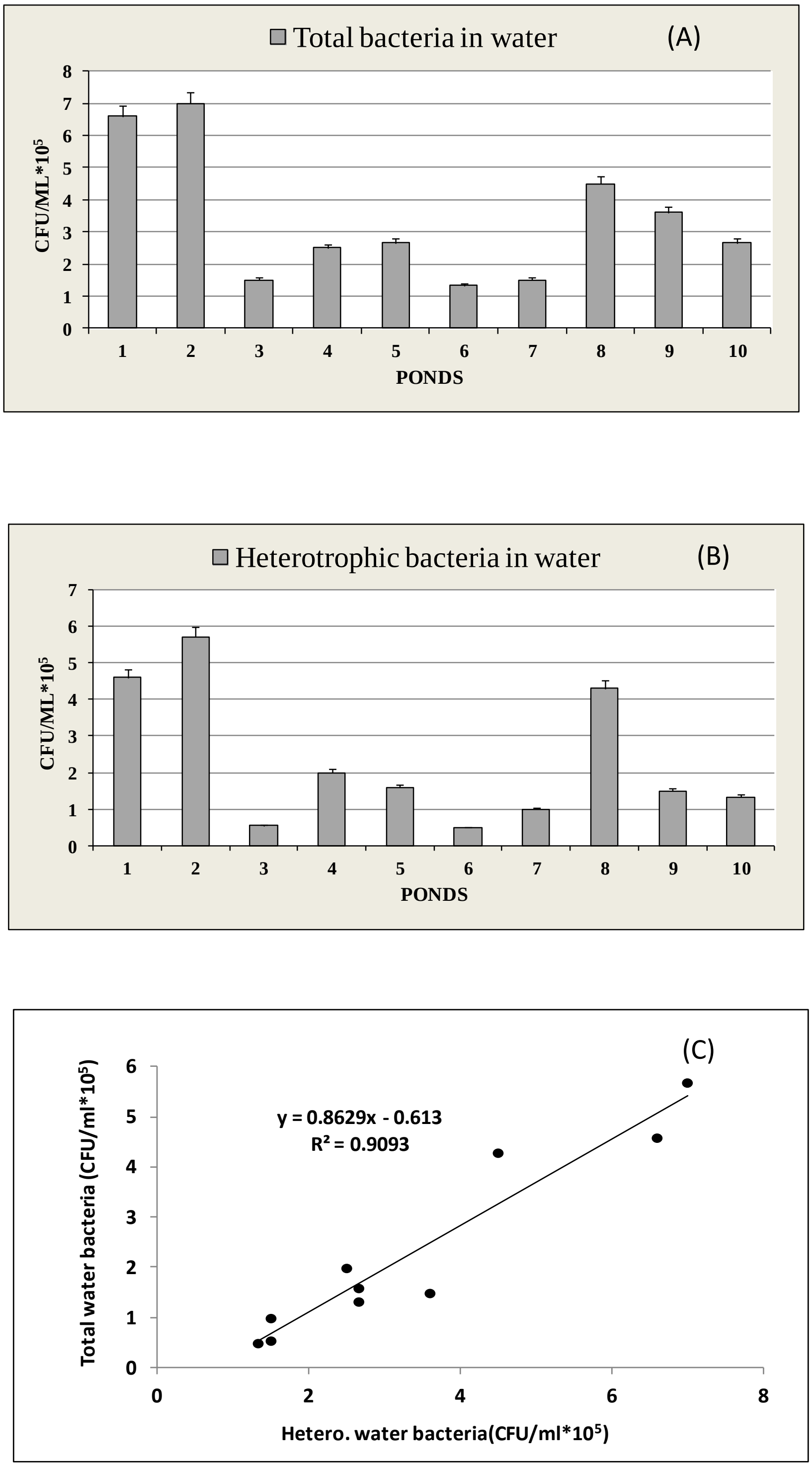


Figure 3: Bacterial count of water in ponds 1 to 10 for total bacteria (A) and heterobacteria (B). (C)Linear regression analysis in between total and heterobacteria in water

Table-1. Seasonal variation of water quality parameters in different fish culture systems in Krishna-Godavari Delta in Andhra Pradesh showing minimum-maximum and mean±SD values.

Parameter	IMC			*Pangassius*			Mixed Culture		
	1	2	3	1	2	3	1	2	3
Water temperature	25.19-32.21	25.2-32.12	25.15-32.15	25.62-32.19	25.36-32.09	24.14-32.18	25.15-32.50	24.12-32.29	24.40-32.26
(°C)	(28.93±2.11)	(29.29±2.27)	(29.21±2.39)	(28.83±2.50)	(28.84±2.52)	(29.18±2.56)	(29.00±2.25)	(28.70±2.55)	(28.27±2.62)
Dissolved oxygen	1.61-5.73	1.42-6.31	1.82-5.91	1.32-5.81	1.51-6.61	1.34-5.16	1.62-5.62	1.62-5.62	1.62-5.62
(mg/l)	(3.46±1.55)	(3.46±1.67)	(3.46±1.57)	(3.27±1.61)	(3.27±1.5)	(3.45±1.56)	(3.47±1.51)	(3.43±1.49)	(3.47±1.53)
pH	7.60-7.90	7.70-8.00	7.50-8.00	7.70-8.10	7.60-7.90	7.50-7.80	7.60-8.00	7.60-8.10	7.60-7.80
	(7.78±0.11)	(7.83±0.09)	(7.77±0.15)	(7.87±0.13)	(7.75±0.1)	(7.70±0.11)	(7.79±0.12)	(7.80±0.17)	(7.72±0.09)
Total alkalinity	85.63-206.93	85.64-207.24	86.24-206.91	86.94-207.22	85.42-207.22	108.12-287.32	85.63-206.93	85.63-207.23	108.26-287.24
(mg/l as $NaCO_3$)	(153.98±49.7)	(154.30±49.7)	(154.30±49.7)	(153.95±50.6)	(153.47±50.9))	(249.55±40.71)	(153.82±49.7)	(154.45±49.9)	(250.04±40.91)
Total hardness	94.21-204.66	95.3-205.65	94.25-205.81	94.21-205.82	94.61-205.81	159.32-303.72	94.24-204.62	95.33-205.83	159.33-305.82
(mg/l as $CaCO_3$)	(165.32±51.5)	(165.22±51.6)	(165.63±51.8)	(165.7±51.9)	(165.7±51.7)	(287.62±17.92)	(165.4±51.6)	(166.1±51.5)	(288.34s±18.64)
Conductivity	0.71-1.40	0.86-1.41	0.72-1.42	0.74-1.46	0.84-1.42	0.72-1.42	0.71-1.41	0.82-1.42	0.62-1.42
(mS)	(0.99±0.25)	(1.07±0.27)	(0.95±0.24)	(0.95±0.16)	(1.06±0.21)	(0.93±0.17)	(0.92±0.2)	(1.11±0.22)	(0.83±0.18)
TDS	455.60-710.12	455.23-945.22	455.01-915.32	455.63-715.21	455.23-780.23	455.22-880.21	455.31-780.11	520.32-910.23	390.21-880.22
(mg/l)	(512.2±158.8)	(628.3±169.3)	(622.1±152.9)	(552.5±101.8)	(622.9±134.2)	(636.2±104.2)	(579.6±128.4)	(720.4±137.1)	(636.3±111.3)
Ammonia-N	0.01-0.03	0.01-0.02	0.01-0.03	0.01-0.02	0.01-0.02	0.01-0.02	0.01-0.02	0.01-0.02	0.01-0.02
(mg/l)	(0.02±e0.05)	(0.02±0.01)	(0.02±0.05)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)
Nitrite-N	0.01-0.03	0.01-0.03	0.01-0.03	0.01-0.03	0.01-0.03	0.01-0.03	0.01-0.03	0.01-0.03	0.01-0.03
(mg/l)	(0.03±0.01)	(0.03±0.01)	(0.02±0.01)	(0.03±0.01)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)	(0.02±0.01)
Nitrate-N	0.32-0.55	0.33-0.58	0.31-0.55	0.32-0.55	0.32-0.57	0.31-0.49	0.32-0.49	0.32-0.58	0.31-0.55
(mg/l)	(0.41±0.07)	(0.43±0.08)	(0.41±0.07)	(0.41±0.07)	(0.42±0.07)	(0.41±0.06)	(0.41±0.06)	(0.42±0.08)	(0.42±0.07)
Phosphate	0.06-0.09	0.05-0.09	0.06-0.09	0.06-0.09	0.06-0.09	0.05-0.09	0.06-0.09	0.05-0.09	0.05-0.09
(mg/l)	(0.07±0.02)	(0.07±0.02)	(0.07±0.02)	(0.07±0.01)	(0.07±0.01)	(0.07±0.02)	(0.07±0.02)	(0.07±0.02)	(0.07±0.02)
Chlorophyll-a	4.63-6.32	4.14-6.01	3.84-5.98	8.06-12.46	8.75-12.32	7.23-12.43	10.45-11.98	10.32.5-12.21	10.12-12.64
(µg/l)	(5.42±0.47)	(4.93±0.65)	(4.69±0.59)	(9.84±0.5)	(10.8±1.06)	(11.4±0.74)	(11.25±0.47)	(10.04±1.33)	(12.08±0.84)
POC	6.59-9.65	4.59-9.12	5.01-11.79	4.59-11.15	9.55-13.21	11.43-15.69	10.46-15.32	9.89-15.12	11.48-15.12
(mg/l)	(8.03±0.97)	(7.55±1.16)	(8.11±1.77)	(7.58±1.83)	(11.1±1.2)	(12.94±1.11)	(13.05±1.38)	(12.1±1.82)	(12.89±1.13)
SPM	16.2-32.9	21.3-32.6	18.4-32.6	22.19-52.6	24.1-52.13	39.21-52.21	32.3-54.21	35.2-52.21	29.2-63.23
(mg/l)	(25.8±6.89)	(27.91±4.66)	(25.65±5.7)	(48.05±4.83)	(43.83±6.3)	(46.2±4.3)	(49.27±4.55)	(44.29±6.31)	(46.26±10.03)

[1]pre-monsoon sampling, [2]monsoon sampling, [3]post-monsoon sampling

4. DISCUSSION:

The evaluation of total nine fish culture ponds at Krishna-Godavari delta on a seasonal basis was identified a significant variation temporally than spatial. Hence, we have not given much importance to the spatial observation or the changes in between the individual ponds. We have projected the data in various ways to show the seasonal changes in fish culture ponds with respect to the inflow, evaporation and accumulation of dissolved nutrients such a way that to aware the farmers to follow these changes or observations while practice. The allochthonous material allowed through the human activities in natural ponds may affect the physical and chemical variables via seasonal patterns (Davies *et al*., 2009). However, the culture fishponds may also be affected due to environmental factors such as availability and intensity of sun light, rainfall, organic deposits through fish feed and metabolic waste from the cultured fish as well as aquatic organism. Hence, the water temperature was observed high during summer months (32.50 °C June 2016) lowest was recorded in the month of January 2016 (24.20 °C) with an average of 26.23±0.92°C. Several reports (Garg *et al*., 2010) noticed the similar trend that temperature generally increases during summer and decreases during monsoon followed by post monsoon periods. The variations in dissolved oxygen (1.3 to 6.6 mg/l) suggested that the low light availability and photosynthetic activity during the post monsoon period along with high turbulence because of shallow water conditions which could be justified by the Chlorophyll-a ranged from 3.84 to 12.64 µg/l. The dissolved oxygen concentration and carbon dioxide contents in freshwater bodies always available in the form of dissolved CO_2 in water which depends on temperature, depth, rate of respiration, organic matter decomposition, chemical properties and geographical specifications. (Sakhare and Joshi, 2002) while pH also has similar trend like dissolved oxygen.

The nutrient components such as ammonia, nitrite, nitrate and phosphorous were recorded high during the monsoon period due to the accumulation of nutrients from the nearby fresh water sources i.e., from river Krishna input. In spite of this the ammonia levels were noticed high during the premonsoon and monsoon periods indicative of contamination of coastal environments. Aquaculture ponds general recognized as sources for the high amounts of nitrogen, phosphorous suspended solids (Alongi *et al*., 2000; Wahab *et al*., 2003) because of local sewage, organic deposits and domestic materials. There are some reports (Biao *et al*., 2004, Costanzo *et al*., 2004) concluded the incursion of high nutrient loads from the shrimp or aquaculture ponds into the nearby coastal waters has made the aquaculture ponds as victims for coastal nutrient enrichment sources.

The Particulate organic carbon followed similar trend along with high chlorophyll a level's (Positive correlation R^2=0.510; $P<0.05$) and suspended particle matter (R^2=0.579; $P<0.05$) suggested that the biological species density may also affects the suspended particle matter such a way that during summer months phytoplankton growth will be high and increases the particulate organic matter. Accumulation of high chemical components into the aquatic ecosystems can interact with temperature dissolved oxygen, which is very essential for the biological production. However, the bacterial invasion occurs when the condition in these water bodies facilitates the bacterial growth, hence we also analysed the bacterial abundance to understand the seasonal variations with respect to the water quality. The total bacterial abundance in water samples varies between 1.33±0.22 CFU/Ml*10^5 (pond 6) and 7.0±05 CFU/Ml*10^5 (pond 2) while, the heterotrophic bacteria are linearly significant (positive) to the total bacterial count, which ranged from 0.5±0.12 CFU/Ml*10^5 to 5.7±0.5 CFU/Ml*10^5. The non-consumed organic material accumulation and gets degraded later which leads to the formation of algal blooms (Mercante *et al*., 2004). Simoes *et al*., 2008 reported high levels of turbidity and oxygen depletion. Which results high level of thermo tolerant coliforms, these are indicators for the eutrophication process. Numberg (1996) studied 30 fishponds from and noticed 21 hypereutrophic and nine eutrophics based on the total phosphorous concentrations. These situations are quite common in shallow water bodies (Brasil, 2011; Rangel *et al*., 2012). The freshwater aquaculture ponds, which are nitrogen limiting and with high concentration of total phosphorous might favour cyanobacterial dominance which in turn results the high chlorophyll 'a' as its biomass (Moss *et al*., 2011; Lurling *et al*., 2013).The present study however, indicated that water quality assessment in fish ponds should be monitored more frequently in during the culture period due to the high organic and inorganic deposits. Water management within these fishponds may be optimized to avoid risks associated with harmful bacteria and impairment to the water uses.

5. CONCLUSION

The findings in this study highlights the environmental controlled freshwater system and ecological relations in between the water quality and fish (major carp fishes). In general the physicochemical and biological observations during pre-monsoon strongly suggests the change in environmental due to the increase water temperature leads to the increase in total alkalinity, total hardness and total dissolved oxygen which can be interlinked with increase in rate of photosynthesis and evaporation obviously. However, the consistency in fish growth was observed along with the period of study. Overall this study indicates the importance of significant variations in the water quality with respect to the seasonal variations.

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