Original Article

Efficacy of Derelict Water Bodies to be a Fish Culture Pond: A Potential Survey Based on Influence of Physicochemical Parameters on the Bacterial Population and Enzyme Activity

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Abstract

Industrial effluents, agricultural run offs and sewage from households are regularly discharged into the derelict water bodies as well as fish culture ponds, which generates a potential risk for both human and fish. In the present study, one month survey (August, 2019) was conducted in eight different derelict ponds randomly selected from Kalyani subdivision of Nadia district, West Bengal, India to see its potential efficacy to be a fish culture pond by analyzing its physicochemical and microbiological parameters along with enzymatic assay. Different physicochemical parameters like dissolved oxygen, pH, temperature, conductivity, phosphate, nitrate, nitrite, ammonium-N, chemical oxygen demand, hardness, organic carbon and total alkalinity of the water were statistically analyzed with the abundance of heterotrophic and phosphate solubilizing bacteria in the studied ponds. High and low bacterial enzyme activity on the other hand clearly reflected the optimum and unfavouranle nutrient enrichment conditions in water. It was found that the sample data provide strong enough evidence to conclude that the bacteria count have a pressure over water quality parameter studied in different ponds particularly on phosphates and hardness of the water as the P value is less than significance level of 0.05 causing rejection of null hypothesis. The data provides additional information regarding alteration of bacterial enzyme activity in the studied ponds. Thus, the ponds warrant for adoption of proper measures to reduce the pollution level at the point source to be a fish culture pond.

Keywords: Water pollution; Physicochemical parameters; Bacterial load; Bacterial enzyme.

Introduction

In view of the harmful effects caused by the effluents of various industries as well as domestic sewage to the aquatic environment, efforts are now being made to assess the utility of water for human as well as aquatic organisms. A major portion of Nadia district, West Bengal, India located in the basin of river Ganges is marked with a number of derelict wetlands in the form of ponds and ox bow lakes are facing high degree of inorganic and organic pollution from pesticides, jute retting practice, chemical fertilizers from the adjoining agricultural fields and cause a great alteration of the ecological homeostasis of the aquatic eco systems. Inorganic contaminants, in particular heavy metals are known to a prominent environmental concern because they are not biodegradable and can accumulate in living

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organisms (Fu and Wang, 2011). As water is vital for our existence and its importance in our daily life makes it imperative for the quality test thorough microbiological and physicochemical examination (Chandra et al., 2006; Shittu et al., 2008). Total coliform and faecal coliform counts are known

to widely used bacteriological procedures for assessment of the quality of drinking and surface water (Mcdaniels et al. 1985). On the other hand, the efficacy of total heterotrophic bacteria and phosphate solubilizing bacteria has been evaluated in earlier studies (Hasan et al., 2012). There are reports that the fluctuating physical and chemical characteristics of water and their interactions bear an effect on the biological features of aquatic ecosystems of rivers (Downing 1971), its catchments (Venter et al., 1997) and watersheds (Guissani et al., 2008), thus prevention of aquatic pollution requires effective monitoring of physicochemical and microbiological parameters (Bonde 1977; Ramteke et al., 1994). Considering the importance of aquatic ecosystem, an assessment of fish health, water quality status and bacteriological studies of the derelict water bodies is of immense importance in order to evaluate its restoration capacity back to its resilient status. Since the affected aquatic nutrient cycle involves changed microbial degradation pathways, it is necessary to employ the microbial signature status of stress levels through assay of nutrient mineralization enzymes such as urease, L-asparaginase, nitrate reductase, nitrite reductase, acid and alkaline phosphatase (Luo et al., 2017). These enzymes catalyze the release of Nitrogen and Phosphorus from organic matter and thus serve as sensitive indicators to pollution induced environmental stress (Brzezinska et al, 2006; Dodds

et al., 2010), whose quantitative determination along with bacterial biomass give a clear assessment of alteration of biogeochemical cycling metabolic pathways (Zhou et al., 2005). Meaningfully, bacterial population with their functional attributes has been found to act as an ecological signature of biological integrity to measure the performance and reclamation ability of wastewater ecosystem (Lahiri et. al., 2015)

In this backdrop, the present paper aims to evaluate the influence of physicochemical parameters on the abundance of bacterial load and alteration of bacterial enzyme activity in ponds of Nadia district, West Bengal, India for a better understanding of the ecosystem responses to pollutants and to formulate sustainable prevention measures.

Materials and Methods

Site selection: For the present study, the authors randomly selected eight different derelict ponds based on their eutrophication status, run-off distance from agricultural fields, domestic usage and discharge etc. from both the municipality and block areas from Kalyani Subdivision of Nadia district, West Bengal, India. Fishes are available in all the ponds. The study was conducted during August, 2019 in the following ponds.

Table 1: Details of selected ponds indicating their physical stress status obtained from field survey.

Name of the ponds	Major sources of pollution	Cultivable species of fish
Unused pond at Chakdaha Municipality (P-1)	Agricultural waste, jute retting waste and religious waste.	Rohu, catla, mrigal, grass carp, etc.
Unused pond at Chakdaha Municipality (P-2)	Agricultural field runoffs, religious wastes and dumping of household wastes.	No fish present.
Fish culture pond at Chakdaha Municipality (P-3)	Washing clothes, cleaning household utensils, bathing and runoffs.	Rohu, catla, mrigal, grass carp.
Fish culture pond at Chakdaha Municipality (P-4)	Washing clothes, cleaning household utensils, bathing and runoffs.	Rohu, catla, mrigal, bata, tilapia, etc.
Unused ponds at Dighra under Chakdaha Block (P-5)	Runoffs from surrounding agriculture fields.	Magur, tilapia and other hardy fishes
Fish culture pond at Dighra under Chakdaha Block (P-6)	Household wastewater, bathing (both human and animals), washing clothes and utensils.	Bata, catla, rohu, mrigal etc.
Unused fish pond at Kalyani Municipality (P-7)	Dumping of municipal wastes, idol immersion.	Mrigal, tilapia etc.
Used fish pond at Kalyani Municipality (P-8)	Bathing, washing clothes, runoffs.	Rohu, catla, mrigal

Examination Procedures

Water samples were collected (Rodina, 1972), at regular intervals for physicochemical, bacteriological and enzyme activity study. The surface and bottom samples were pooled separately

into single sterilized containers. Two subsamples were taken from each of pooled surface and bottom water samples; one in a properly sterilized 125 ml glass stoppered bottle for enumeration of microbial populations and the other in a 500 ml

plastic sampling bottle for the examination of the physicochemical characteristics of water.

Water quality analysis

Different physicochemical parameters, viz., dissolved oxygen (DO), pH, temperature, conductivity, phosphate, nitrate, nitrite, ammonium nitrogen, chemical oxygen demand (COD), hardness, organic carbon and total alkalinity was measured during study time in all the selected eight ponds following the standard protocols of APHA (APHA, 2017).

Examination of bacterial population

All the routine procedures (Rodina, 1972) were followed for culture of heterotrophic bacteria (HB) and phosphate solubilizing bacteria (PSB) of water samples using conventional spread plate technique (Buck and Cleverdon, 1960).

Qualitative and quantitative assay of microbial enzyme activity

Urease (URE): Bacterial isolate was grown in modified urea broth base (HiMedia) supplemented with 5 ml of 40 % urea solution in 100 ml broth. At every 24h, broth was withdrawn and from its cell free supernatant urease assay was performed till 7 days. The urease activity was measured by phenolhypochlorite assay (Weatherburn, 1967). Controls used for the enzyme reactions were reaction mixture without substrate and reaction mixture without incubation. One unit of urease activity was defined as the amount of enzyme liberating 1 μg NH3 from urea per minute, under the above assay conditions.

L-asparaginase (LAS): In the sample tube, 0.1 ml of enzyme was taken along with 1.0 ml of Tris buffer solution and 0.1ml of asparagine with 0.90ml deionized water. The reaction mixture was incubated at 37°C for 30 minutes. The sample and control tubes were allowed to centrifuge for few minutes to clarify the enzyme.Each tube (sample and control) containing 0.2 ml of supernatant was mixed with 4.30 ml of distilled water and then added 0.5ml of Nessler's reagent. The contents in the tube were mixed by inversion for 1 minute and the absorbance was noted at 436nm.

Enzyme activity (U/ml)=Amount of NH4 liberated* total reaction volume/ (Incubation time × ml of enzyme taken for test).

Statistical analysis

All the data were subjected to statistical analysis (Gomez and Gomez 1984). One way analysis of variance (ANOVA) with the help of MS Excel and computer software SPSS (version 7.5) were used at 1% and 5% levels of probability between all the physicochemical and bacteriological parameters.

Results

A summarize data of physicochemical parameters for all the eight ponds is tabulated in Table 2 and Fig. 1. It was found that among the physicochemical parameters studied, the DO ranges between 4.31 and 7.91 mg/l, pH ranges between 7.2 and 8.6, temperature ranges between 35.7 and 37.10C, conductivity ranges between 463.7 and 1048.8 μSiemens/cm, phosphate ranges between 0.060 and 0.469 mg/l, nitrate ranges between 0.107 and 0.618 mg/l, nitrite ranges between 0.0022 and 0.0101 mg/l, ammonia-N ranges between 0.071 and 0.683 mg/l, chemical oxygen demand ranges between 139.2 and 288.4 mg/l, hardness ranges between 31.72 and 57.34 mg/l, organic carbon ranges between 4.24 and 6.92 mg/l and total alkalinity ranges between 11.25 and 55.73 mg/l in the eight different studied pond (see table 2). On the other hand, the density of heterotrophic bacteria varied from 77 to 158 cfu/100 ml and the density of phosphate solubilizing bacteria ranges between 33 and 62 cfu/100ml in all the studied ponds (Table-3; Figure 2). Maximum urease activity with high concentration recorded on 3rd, 4th, and 6th day in P-1, P-3 and P-4 were shown by HB isolates, whereas comparatively lower activity occurred maximally 3rd day in P-2 for PSB isolates.

Maximum L-asparaginase enzyme activity with very concentration was shown by HB isolates in P-4 and P-8 whereas comparatively lower enzyme activity was shown by HB in P-2.

A critical analysis of the data showed that the amount on nitrate and nitrite is very low in all the studied ponds when compared with its standard range. However, the amount of Ammonium-N is considered within the standard value of fish culture ponds except for P-3 and P-4, whereas the hardness in not in the standard range for P-3, P-4, P-6 and P-8. The amount of total alkalinity is found to be lowered in all the ponds except for P-3. The amount of COD in all the ponds clearly indicated

the pollution load in all the studied ponds (Table-2). The trend of the density of both the heterotrophic as well as phosphate solubilizing bacteria is different for different ponds indicated differential bacterial load in the culture ponds (Table-3).

Table 2: Water quality regime of the eight ponds under study (August, 2019).

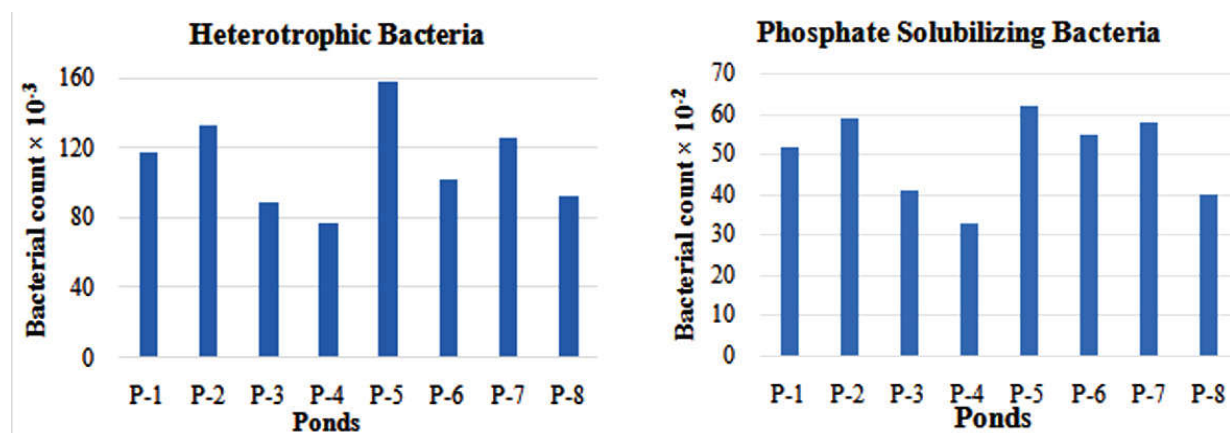| Studied Ponds	Water Quality Parameters											
	DO (mg/Lt)	pH	Tempe-rature (˚C)	Conduc-tivity (μS/cm)	Phos-phate (mg/Lt)	Nitrate (mg/Lt)	Nitrite (mg/Lt)	Ammo-nium -N (mg/Lt)	COD (mg/Lt)	Hard-ness (mg/Lt)	Organic Carbon (mg/Lt)	Total alka-linity (mg/Lt)
*Standard value for aquac-ulture	>4.0 mg/l	7.5-8.5	Species depend-ent	30–5000 μSiemens/cm	< 0.5 mg/l	< 100 mg/l	< 1 ppm	0 – 0.5 ppm	2-3 mg/l	40 – 400 ppm	< 10 ppm	50 – 300 ppm
P-1	4.72	8.4	36.8	937.1	0.379	0.141	0.0026	0.343	270.2	57.34	6.05	16.73
P-2	4.31	8.5	36.6	989.9	0.469	0.317	0.0053	0.388	281.5	50.43	4.78	14.18
P-3	7.58	8.6	36.9	463.7	0.076	0.286	0.0119	0.521	146.7	32.66	4.24	55.73
P-4	7.79	8.2	36.9	447.2	0.084	0.618	0.0106	0.683	157.1	31.72	4.95	47.54
P-5	5.29	7.2	37.1	1048.8	0.317	0.372	0.0051	0.418	288.4	40.28	5.33	11.25
P-6	7.74	8.0	36.7	517.2	0.060	0.214	0.0101	0.133	139.2	34.67	5.67	49.36
P-7	6.72	7.4	36.4	661.3	0.327	0.221	0.0022	0.071	211.6	49.33	6.92	16.34
P-8	7.91	7.7	35.7	567.8	0.125	0.107	0.0061	0.116	182.4	37.86	4.28	41.86
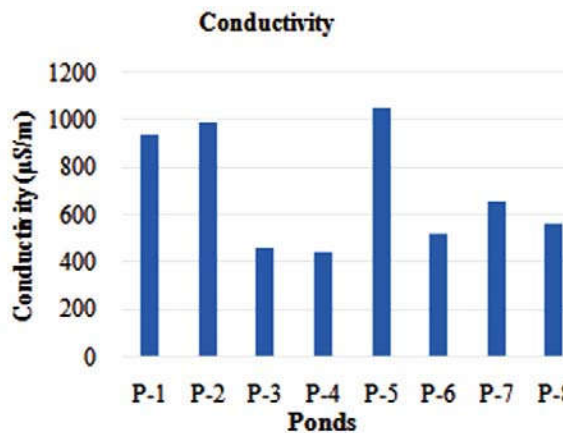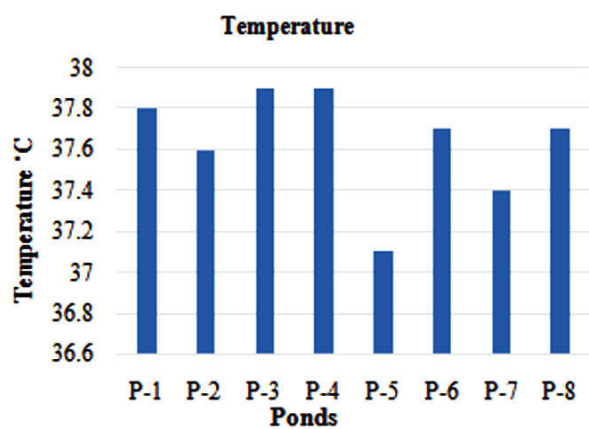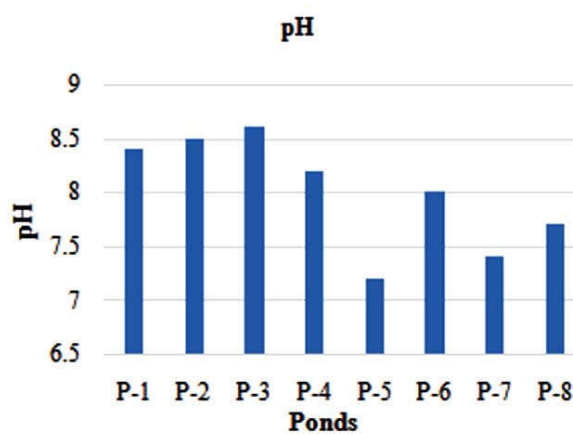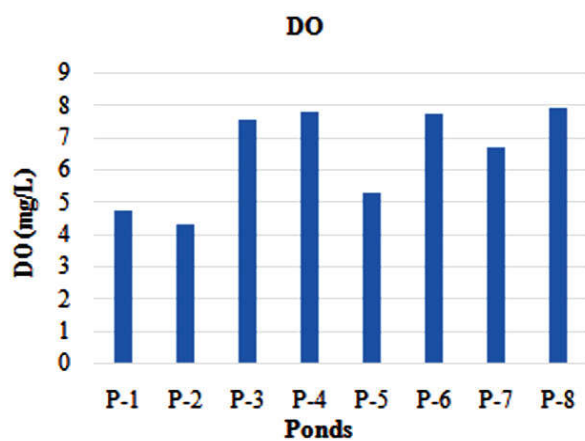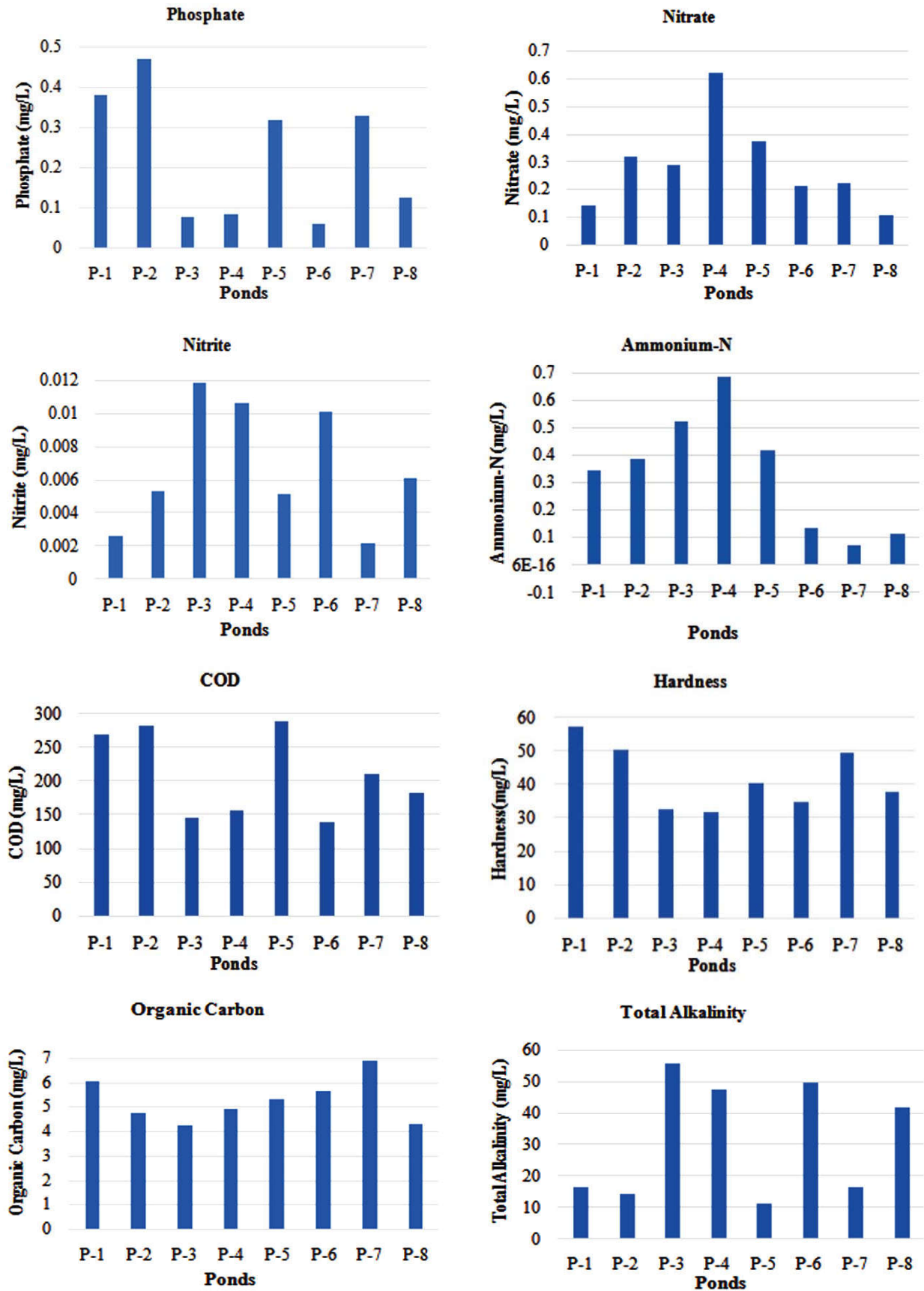
Fig. 1: Graph of all water parameters in the studied ponds.

Table 3: Bacterial counts of heterotrophic and phosphate solubilizing bacteria in eight ponds under study.

Sampling Points	Bacteria count (CFU)	
	Heterotrophic bacteria ($\times 10^3$)	Phosphate solubilizing bacteria ($\times 10^2$)
P-1	117	52
P-2	133	59
P-3	89	41
P-4	77	33
P-5	158	62
P-6	102	55
P-7	126	58
P-8	92	40

Fig. 2: Graph of bacterial counts of heterotrophic and phosphate solubilizing bacteria in eight ponds under study.
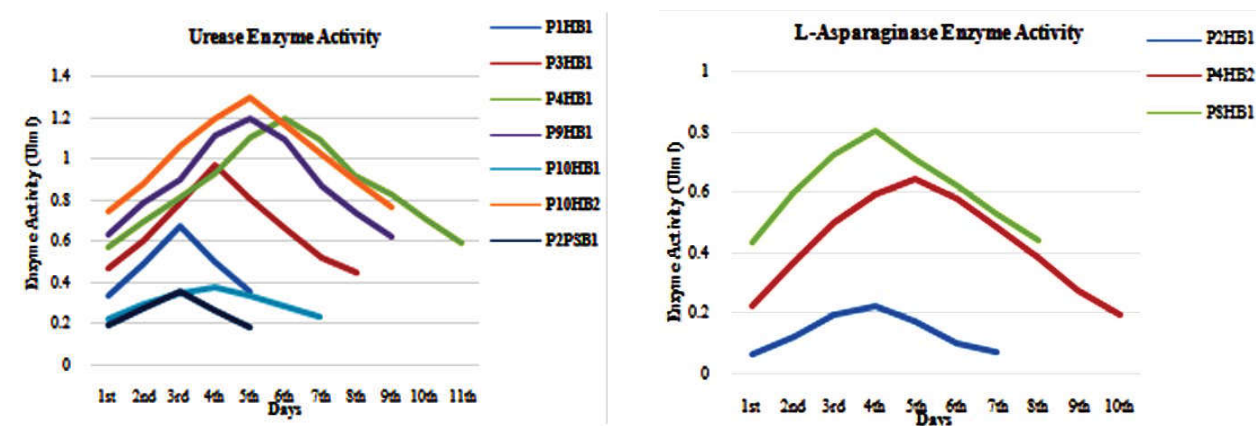
Fig. 3: Graph of Urease and L-asparaginase enzyme activity of the selected bacterial isolates.

Discussion

In the present study, it is evident from the data that different physicochemical and bacteriological parameters of water varied remarkably in eight different ponds under study. It is also found that the studied ponds harbors both the heterotrophic as well as phosphate solubilizing bacteria, the amount was higher than the standard limit of heterotrophic count for drinking water (EPA 2002). In aquaculture, total heterotrophic bacteria (THB) in general and particularly species of Bacillus, Pseudomonas and Lactobacillus provide beneficial effects (Jaganmohan and Prasad, 2010). However, the beneficial effect of using such microbial products in aquaculture is still debatable and controversial as their efficacy is yet unclear. Thus, it can be said that the water of the studied ponds was

Efficacy of Derelict Water Bodies to be a Fish Culture Pond: A Potential Survey Based on Influence of
Physicochemical Parameters on the Bacterial Population and Enzyme Activity

53

polluted in nature and the primary sources of these bacteria in water were mainly animal and human wastes. These sources of bacterial contamination include surface runoff, pasture and other land areas where animal wastes were deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil/ plant bacteria (Shittu et al., 2008). As the study was carried out during monsoon time (August, 2019), thus irregular pattern of the occurrence of bacterial count was due to the mixing of some domestic sewerage during the overflowing of runoff water in monsoon time (Chandra et al., 2006). However, optimum level of total alkalinity and low ammonium nitrogen in the ponds indicated higher carbon source, buffering capacity and decay of organic matter respectively (Fast and Lester, 1992). This abundance of the microbes may have a correlation with the physicochemical properties of the water bodies because there are reports that the industrial effluents cause contamination to water during the mixing process (Jain et al., 1996). The slight variations in the physicochemical properties in the studied ponds were noted during monsoon seem to be related with the run off of organic matter into ponds from adjacent river or possibly due to the precipitation factors (Chatterjee et al., 2010; Jain et al., 1996). Nutrient concentration, COD and DO values favored balanced nutrient cycling with biological integrity resulting in high enzyme activity in P-3 and P-4, which are also been used for fish culture. This indicates regulated discharge of waste in these ponds. However, high concentration of phosphate and COD with high population of PSB and low DO in P-2 did not support optimum bacterial metabolism due to its nutrient enrichment condition because of very high dumping of households and other wastes. No fish has been found due to this in P-2 pond.

In ANOVA one way factor analysis between the physicochemical and bacteriological parameters, the experiment indicates that the sample mean is different. The data further support the notion that the population means are not equal and it might be the result of random sampling error. The sample data provide strong enough evidence to conclude that the bacterial count (both HB and PSB) have a pressure over water quality parameter studied in different ponds particularly on phosphates and hardness of the water as the P value is less than significance level of 0.05 causing rejection of null hypothesis.

The present findings seem to be influenced by the domestic and agricultural refusals of the study ponds. Moreover, high COD values in all the ponds indicate the presence of contaminants and bacterial load in the water. Although the nitrite, nitrate, alkalinity and hardness levels are obeying the standard value of aquaculture and the optimum level of DO, pH and temperature amply speaks for suitable fish culture, but the altered bacterial metabolism in the nutrient cycle as indicated by the enzyme activity responses reflected lack of biological integrity of the aquatic ecosystem influenced by the physicochemical water quality parameters. Therefore, the entire study can be applied for as a molecular biomarker of nutrient enrichment status to call for science and technology intervention before making them suitable for any fish culture.

Acknowledgements

Conclusion

In search of suitable fish culture ponds for increased production of fish throughout the globe, the study of physicochemical parameters and their influence on the the bacterial counts and bacterial enzymatic assays with their feddback mechanism is of immense importance. Although there are other valid parameters to study, the present results depict that physicochemical environment induced bacterial growth and activity varied remarkably among different derelict bodies with different magnitude of nutrient status, although they are randomly selected from same geographical location. The result depicts that the water of the studied ponds was polluted in nature and the primary sources of the bacteria in water were mainly animal and human wastes and/or due to varied anthropogenic origin of the ponds. The altered bacterial metabolism in the nutrient cycle as indicated by the enzyme activity responses reflected lack of biological integrity of the aquatic ecosystem influenced by the physicochemical water quality parameters in the studied ponds. Therefore, the entire study can be applied as a molecular biomarker of nutrient enrichment status for searching the efficacy of derelict water bodies to be a fish culture pond.

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