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Alkaline Phosphatase Activity as Indicator of Fecal Contamination of Mumbai Coastal Water

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Abstract*: Mumbai coast is facing adverse impact of sewage discharge up to 5km from the shore. Microbiological quality of Mumbai coastal water was estimated by determination of faecal coliforms [FC], which is the standard used for assessment of level of fecal pollution of marine water. Determination of levels of faecal pollution of the coastal ecosystem can also be carried out by assessment of microbial metabolic activities in terms of alkaline phosphatase [APase] activity. High organic matter- allochthonous or autochthonous, or contaminants i.e. hydrocarbons support viability of bacterial and stimulates synthesis of enzymes to degrade new organic compounds. However AP activity is not only affected by various variables like pH, buffer ions, metal ion content, incubation time etc. but is also negatively correlated with availability of phosphates. Correlation between FC count and AP activity was studied to check use of AP activity as marker of ecological quality.*

Keywords: Alkaline Phosphatase, Fecal Coliforms, Mumbai Coast, Sewage Contamination.

Introduction

Pollution due to anthropogenic activities is the greatest problem in all ecosystems. It has brought about rapid degradation of habitats and numerous long-term including adverse shifts in fish-harvests, diseases, community and species diversity and economic losses. It is a hazard to human health when marine organisms are eaten raw or when people go swimming. Molluscs and crustaceans together called shellfish are important components of global catch. Though smaller than finfish, shellfish catch value is generally higher than that of finfish. Gastrointestinal pathogens like *Vibrio cholarae* [VC] from fecal pollution can concentrate in shellfish (as they are filter feeders) rendering it unfit for human consumption, as they are generally eaten raw. Pet faeces that go in coastal waters are also health hazard to marine animals and humans. *Toxoplasma gondii*, a protozoan parasite carried by cats, has been linked to lethal brain infections in sea utter. *Giardia intestinalis* and other human parasites that are also carried by pets have been found in oysters and other edible bivalves.

Saprophytic bacteria use up oxygen for decomposition of organic matter thus creating anoxic conditions. This results in decrease in number of species. The resident flora are replaced by hardier forms such as certain species of polychaete worms. Fishes around sludge disposal sites show abnormalities like tumours, finerosion etc. due to high concentration of toxic substances.

In marine ecosystems, phosphorus is often present in limiting concentrations for growth of algal and bacterial populations. In seawater inorganic phosphorusis derived chiefly from weathering of rocks, bird guano or from fertilizers and other human activities. Dissolved Organic Phosphorus (DOP) occurs primarily as P-esters, resulting from excretion, decomposition, death and autolysis of marine organisms.

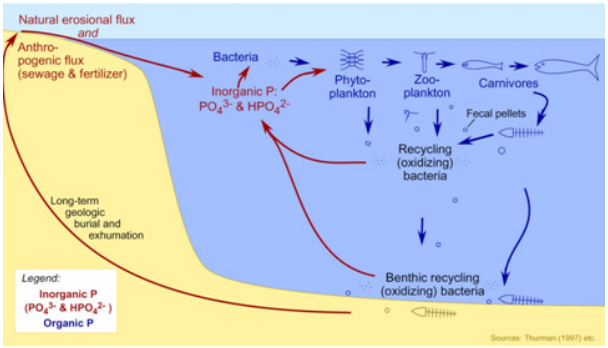

Fig. 1 Phosphorus cycle in marine environment- Phyto planktons can only use phosphorus in inorganic form [orthophosphate]. The recycling of organic phosphorus back to its inorganic form, occurring in the benthic zone, makes upwelling critical for biological productivity. Faster recycling of P in surface water, makes upwelling less critical. Vaulot*et al*., [1996]observed that P- limitation may be encountered more frequently than previously thought. Average phosphorus content in waters of world oceans is 70-72µg/l [varies from <1in surface water -300 µg/l in stagnant basins].

Eutrophication, due to excessive amounts of nutrients & hence excessive algal growth,is a problem of not only coastal waters but also sea beds and coral reefs. There is an accelerated growth of sea weeds at the bottom due to reduced penetration of sunlight. Phytoplankton blooms may also be toxic. Toxins can accumulate in shellfish causing poisoning in humans. Remains of phytoplankton blooms and faeces of fishes that feed on plankton, fall to the bottom. Decay bacteria then breakdown this organic matter and use up oxygen at the bottom. The vertical distribution of dissolved nutrients is usually opposite that of dissolved oxygen. Seasonal anoxic zones have become a common feature.

Microbes play a key role in the biogeochemical cycles in the marine ecosystems, due to the spectrum of hydrolytic enzymes they elaborate. They are capable of hydrolysing organic polymers. The use of enzymatic biomarkers to assess the P status of oceans has been extensively employed in microbiological oceanography. Alkaline Phosphatase and 5'nucleotidase activities contribute significantly to phosphate regeneration in marine environment [Ammerman & Azam 1985, James *et al*., 1991].

The determination of enzyme activity is a simple tool for characterization of the ecological status. Leucine amino-peptidase, chitinase and alkaline phosphatase are useful in assessment of ecological damages due to industrial discharges. Significant correlations have been demonstrated between Leucine amino-peptidase activity, Alkaline Phosphatase activity and Particulate Organic Carbon. Microbial enzymatic activity can be used to detect the presence of organic contamination [Cappello *et al*.,]. Alkaline phosphatase inhibition assays can be used as early warning signals of toxicity due to heavy metals and phenolic compounds in aquatic environments.

Alkaline phosphomonoesterases, also called as APase [EC3.1.3.1], can potentially hydrolyse a broad spectrum of DOP compounds optimally at sea water pH. A Pase is a relatively non-specific enzyme that releases Pi from a variety of phosphomonoesters, including di, tri and polyphosphate organic derivatives [ex. Nucleotides]. It is located on cell surface and its extracellular phosphatase activity is inducible, i.e. biosynthesis of enzyme is switched on when inorganic phosphate supply is low or organic P- sources are available [Alba *et al*., 1993]. It is present in almost all forms of life, from algae, bacteria, and protozoa to higher plants and animals, but also as a free enzyme in natural waters and sediments.

Bacterial A Pases are important for utilization of organophosphates in ocean. Common heterotrophic marine bacteria, like Proteobacteria, hydrolyzes DOP and release extracellular A Pase that could provide P for organisms that cannot use organophosphates. This increases marine biological productivity and diversity. PhoA, PhoD & PhoX prokaryotic

A Pase gene families have been recognized. In heavily polluted coastal areas occurrence of high enzymatic activity rates was reported. Enzymatic assays and culture methods could be combined to assess the pathogen viability.

According to the Environment Status Report of Greater Mumbai prepared by Municipal Corporation of Greater Mumbai (MCGM) 2012 – 13, 2228 MLD of domestic & 279 MLD of industrial waste is generated while the treatment capacity is only 109 MLD. Mumbai flushes rest of the sewage generated directly into the sea. We could recycle this water; sludge can be used as landfills, fertilizer, converted into fossil fuel, made into construction blocks or used as compost.

Materials and Methods

Two strategic marine locations were identified and selected for studying the pollution levels.

Mahim Bay - It is highly polluted due to polluted Mithi River that drains into it. The water smells foul due to the dumping of untreated effluents from small scale tannery and textile dyeing industries. Mushrooming of slums around the waters has caused concern for the mangrove ecosystem. In addition to sewage outlets open defecation is a major problem.

Bhayander Creek- It is widely used during Ganesh visarjan. It is famous for fishing and sand business. Main pollution source is Industrial & domestic effluent through local nalla and drains.

Sampling

Water samples [500ml] were collected from the shores during low tide and from fishing area that was approximately 5km from shore of Mahim bay and Bhayender creek. Samples were collected from at least five locations. The quality of water varies spatially as well as temporally. Hence both pre-monsoon and post monsoon samples were collected in April and September end of 2015 respectively. Grab sampling was done and the samples were analysed for microbes within 4hrs. & refrigerated [5^{o}C] for physicochemical analysis which was carried out within 72 hrs.

Physicochemical Parameters Studied

Nitrite levels were estimated using AQUA Check Nitrite Testing Kit 5-100mg/L (WT007A- Hi-Media).

Phosphate levels were estimated using AQUA Check Phosphate Testing Kit 0.1-10mg / L (WT00813- Hi-Media).

Determination of Alkaline Phosphatase Activity

A Pase activity can be determined by measuring the hydrolysis rate of an artificial substrate under controlled reaction conditions. The microbial cells were harvested by centrifugation (6000 g, 10 min) of 10ml of sample. Cold osmotic shock was given by using modified standard (e.g. Neu & Heppel 1965) procedure which is more suitable for the marine bacteria. Pelleted cells were suspended in 2ml of cold shock medium i.e. salt medium with 20% sucrose [salt medium (200 mM NaCl, 10 mM KCl, 10 mM $CaC1_2$, 50 mM Tris-HC1, 19 mM NH_4Cl 50 mM MgCl and 10 mM EDTA in deionized H_2O pH 8.0]. torelease Alkaline phosphatase. The suspension was incubated on an ice bath for 10 min and centrifuged for 15 min at 6000 g. The shockable proteins come in supernatant. A further modification of procedure was done by adding 0.1ml of 0.01% lysoszyme to salt medium and incubating at 37^{o}C for 15mins before placing on ice. 0.5 ml aliquot of this mixture was incubated with 4.5 ml of 0.1 mm old m–3 p-Nitrophenol substrate and incubated at room temperature for 24 hours. The absorbance is then measured at 405nm. Measurements were run against a blank containing autoclaved seawater sample.

Calculations

A Pase activity [µmolpNPP L^{-1} h^{-1}] =

A^{o} * TV* 1000/ t* Epsilon* SV* path length= A^{o} * 4.433

Where TVis total volume in lit, t is time in hrs, epsilon is molar extinction coefficient 18800 $mM^{-1}cm^{-1}$and SV is sample volume in lit.

Microbiological Parameters Studied

Determination of MPN

Total coliform count was estimated using Mac Conkey Broth with Neutral Red (Hi media GM007), by multiple tube fermentation technique. Most Probable Number (MPN) index of coliforms was obtained.

The water sample was initially diluted 1:10 as all tubes showed positive result with undiluted sample.

Faecal Coliform Count

Appropriately diluted samples were filtered through a 0.45-mm porosity cellulose membrane and incubated (44.5°C) on plates of a medium selective for faecal Coliforms (M-FC Agar Base, Hi-media, M1122) as suggested by APHA (1992). An estimation of the abundance of bacteria present in the sample in terms of colony-forming units (CFU/ 100 ml) was obtained.

Results and Discussion

Nitrite Levels

High levels of nitrites in the range 10- 55 mg/L were noted in samples. <1mg/L levels were generally obtained by most researches in coastal water samples. However the higher values obtained are in accordance with that obtained by Ingole & Kadam in a study of Mumbai beaches. As the organic load is found to be high in coastal water sample, the ammonium ions produced during decomposition of proteins and amino acids, might be deliterious to the nitrite oxidizing populations [Stojanovic & Alexander, 1958].

Phosphate Levels

High levels of Pi were noted in samples. Lot of variations in phosphate levels in sea water is observed in various reports. Values ranging from >1µmol L^{-1} to as high as 50 mg L^{-1} have been reported. Values in the range 0.5- 2.2mg L^{-1} were obtained in samples.

Alkaline Phosphatase Activity

A Pase is released to the environment through either cell lysis or excretion. Depending on its own lifetime and the prevailing environmental conditions, the enzyme may remain active outside the cell [Hong Li *et al.,* 1998], and is detectable in the aqueous phase but boiling seawater can be run as control as it destroys A Pase activity.

A Pase activity in the range 0.07 – 0.89 µmol pNPP L^{-1} h^{-1} was obtained, which is much higher than reported by most researchers from sea water across globe however Ivančić I, *et al*., 2010 reported activity in range of 0.05-4.64 µmol l^{-1} h^{-1}, in Adriatic water.

Total Coliform [TC] Count & Faecal Coliform [FC] Count

The pre-monsoon and post-monsoon TC counts obtained from coastal water of were found to be far above the permissible limits. Average pre-monsoon counts ranged from 1700- >24000 /100ml. Average post-monsoon counts ranged from 2400 to >24000 /100ml. According to Primary Water Quality Criteria for Class SW-II Waters, For Bathing, Contact Water Sports and Commercial Fishing, National Institute of Ocean Technology, Department of Ocean Development, Govt. of India, average FC counts should not exceed 100/100ml and not more than 200/100 ml in 20% of samples in the year. Also, waters designated for shell-fishing should not have average FC levels above 14 cfu/100 ml. However the FC levels 650-35000 cfu/100ml pre-monsoon & 350-25000

Table 1 Observations at Mahim and Bhayander

AREA	*MAHIM (premonsoon)*					*MAHIM (post-monsoon)*				
SAMPLE	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
MPN of coliforms/100ml	16,000 [4000-46000]	3300 [580-4000]	9200 [2200-26,000]	>24,000	>24,000	16,000 [4000-46000]	16,000 [4000-46000]	>24,000	>24,000	>24,000
Fecal Coliform count [cfu /100ml]	5,000	800	23,000	10,000	35,000	2,000	7500	12,000	15,000	25,000
APase activity [µmolpNPP L^{-1} h^{-1}]	0.53	0.37	0.42	0.89	0.79	0.22	0.17	0.33	0.31	0.35
Nitrite levels mg/L	12	14	28	35	30	24	20	25	47	55
Phosphate levels mg/L	0.5	0.7	0.5	1.3	1.0	0.8	0.6	0.6	1.5	2.2
AREA	*BHAYANDER (premonsoon)*					*BHAYANDER (post-monsoon)*				
SAMPLE	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
MPN of coliforms/100ml	16,000 [4000-46000]	1700 [580-4000]	5400 [1500-17,000]	9200 [2200-26,000]	16,000 [4000-46000]	16,000 [4000-46000]	2400 [700-7,100]	5400 [1500-17,000]	5400 [1500-17,000]	>24,000
Fecal Coliform count [cfu / 100ml]	2000	650	950	5000	3000	450	350	2000	5000	8000
APase activity [µmolpNPP L^{-1} h^{-1}]	0.20	0.23	0.09	0.44	0.53	0.1	0.18	0.07	0.28	0.22
Nitrite levels mg/L	10	14	28	35	30	24	20	25	37	50
Phosphate levels mg/L	0.5	0.6	0.8	0.9	1.0	0.5	0.6	0.7	0.9	1.4

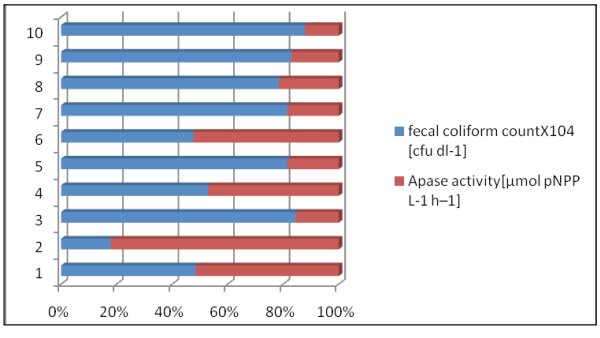

Fig. 2 Percentage bar diagram for observations at Mahim.

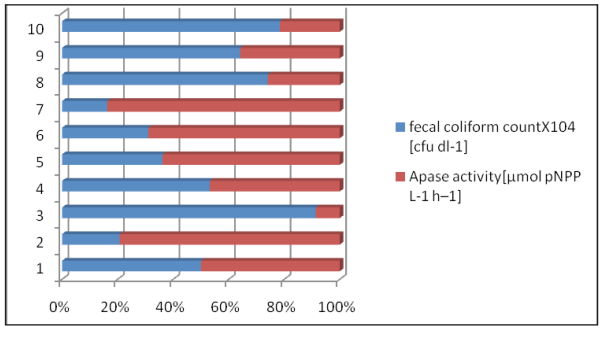

Fig. 3 Percentage bar diagram for observations at Bhayander.

cfu/100ml post-monsoon was obtained. Higher levels were obtained from Mahim samples due to sewage outlets in these regions.

Vibrio cholarae [VC] & V. parahaemolyticus [VP] count

Pre-monsoon levels of VC ranged from 30,000 to 1,72,000 cfu/100ml and post-monsoon 10,000 to 1,20,000 cfu/100ml. VP levels ranged from 150 to 6500 cfu/100ml in pre-monsoon samples. An average count of 80 to 5700 cfu/100ml was obtained post-monsoon. VC survives better in sea water hence the counts obtained are much higher as compared to TC, FC or VP. Positive correlation was obtained between FC & VC showing that both are arising from enteric source. TC of 470000, VC of 82000 and FC of 92000 CFU /100ml was reported by Sudhanandh V. *et al*, 2012, in a study on potentially pathogenic enteric bacteria in coastal sea waters along the Southern Kerala coast. The counts of TC were 5.7 times the VC count. However, in the current study TC counts were lower (approximately 5 times) than VC count. This could be because of poor survival of coliforms in sea water. Also the variation in counts observed of VC is greater than TC, FC & VP. Hence VC could be a better indicator of faecal contamination than coliforms and the standards for marine water could be given in terms of VC rather than FC.

Interestingly, the counts of FC from Mahim station were noted to be higher near fishing area [around five km from the shore] as compared to shores. This could be due to the fact that partially treated or untreated sewage is discarded by pipes around 3km from the shores into the sea. *Vibrio cholerae* and *V. parahaemolyticus* were detected in shell fish samples [collected during low tide from shore]. This is of grave concern as they are eaten raw. However mere presence of VC in shell fish does not indicate that the person consuming it will suffer from cholera. This is so because VC serogroups O 1 and O139 only cause epidemic cholera. Even among these strains may lack cholera toxin. The other serogroups however can cause mild diarrheal illness. Approximately 2.5 million of worldwide cases infectious hepatitis annually is a result of people eating oysters and other shellfish that concentrate the viruses during filtering of water for food. *Vibrio parahaemolyticus* [VP] lives in brackish saltwater and cause gastrointestinal illness in humans. It is present in high concentrations during summer. VP infections are also associated with exposure of wounds to seawater. Swallowing of contaminated water during swimming in sewage polluted water can cause gastrointestinal disorders or ear, throat or eye infections just by contact with water.

Conclusion

Assimilation of phosphorus by phytoplankton is restricted to uptake of inorganic phosphate. During long periods of phosphorus limitation phytoplankton produce extracellular A Pase, to grow and survive under conditions of phosphorus deficiency. However in coastal waters due to high organic load bacterial A Pase is produced, which can be linked to survival of the pathogens. However as there are many factors that can affect activity of A Pase like availability of phosphates etc, it use in indicating pathogen viability, as suggested by some researchers, is questionable. Nirite levels observe were high enough to have delitarious effect on survival of fishes. It effect on ecology needs to be investigated.

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