



STUDY OF DIFFERENCES & PATHOGENICITY OF MICROBIAL FLORA IN VARIOUS CATCHMENT AREA AT THE BANK OF ALAKNANDA THE HEADSTREAM OF RIVER GANGA IN SRINAGAR GARHWAL, UTTARAKHAND DURING SUMMER MONTH.

Microbiology

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ABSTRACT

Background: Water pollution including pollution of major rivers have great impact on health of society leading to increased toll of diarrhoeal death. Household water treatment and safe storage (HWTS) interventions can lead to dramatic improvements in drinking water quality and reduction in diarrhoeal diseases.

Materials & Methods: A cross sectional study was done to evaluate the MPN (Most Probable Number) count & parasitological estimation of water samples taken from Alaknanda River from Srinagar during the summer month (May-June) of 2016. Both quantitative & qualitative estimation of bacteriological indicator was done using Multiple Tube Method & Medias from HiMedia Laboratories Pvt. Ltd. respectively. Quantitative analysis of MPN count was done by using McCardy's Tables.

Results: MPN count of all the 8 samples was found >150/ml after 48 hour inoculation & qualitative results were also evaluated.

KEYWORDS

Alaknanda River, Hwts, Mpn Count, Mccardy's Tables.

Introduction:-

Water is a most important element responsible for life on the only living planet, the Earth. The universal solvent exists in the form of glaciers, oceans, seas, rivers, lakes & ground water and only 1-2% of this is fit for drinking. Increasing human population, devastating effects of industrialization & continuous deforestation has put a threat on the purity of this precious molecule without which all the creatures including human beings on the planet will be just a heap of sand!

Srinagar is a city (Census 2011:-1, 50,000) rest on the south bank (30.22 degrees North 78.78 degrees East) of Alaknanda River in Pauri Garhwal district of Uttarakhand. Positioned in middle Himalaya at an elevation of 560m above mean sea level, Srinagar is gradually sprawling on the either side of NH-58 restricted only by steep hill on one side & Alaknanda river on other. It exhibits sharp variation in seasonal temperature, unusually roaring upto 45°C in summer & falling to 02°C in winter. In the recent past 2013, Himalayan Tsunami has caused morphological changes to the river.

Considerable amount of solid & liquid waste from Srinagar town is reaching the river making it dumping ground & waste disposal channel. [1] Srinagar Hydro Electric Project (330MW) & diversion of river via canal is one of the major changes that have put a threat for the future integrity of river. [2] Apart from those, biomedical infectious waste from health institutions can also contaminate the water resources. The highest risk of water borne disease is due to escape of human feces in water supply. [4] Preventing the transmission of waterborne diseases is one of the most significant challenges what public health professionals face. Household water treatment and safe storage (HWTS) interventions can lead to dramatic improvements in drinking water quality and reduction in diarrhoeal diseases- from polluted rivers, lakes and, in some cases, unsafe wells or piped water supplies.[3] Our aim was to find out qualitative & quantitative estimation of microbiological pollution indicators in the river with in the Srinagar city.

Review of Literature:

Citation	Design	Objectives	Study population	Sample size	Measurable outcomes & results	Authors conclusion
[5]	Cross sectional study	Assessment of bacterial diversity on the basis of phenotypic & genomic properties in the Gangetic river system of Uttarakhand, India	Total stretch of 440km including Alaknanda (200km).	16 sites	31 species were isolated from Alaknanda. Diversity & evenness of species was calculated using Shannon Diversity Index & Evenness formula.	Data provided here give an insight into the bacterial diversity, ecosystems stability and their role as pollution indicators. Further functional analyses are required for understanding functional diversity.
[6]	Cross sectional study	Water quality, Biodiversity & Ecology of water at Hydropower project sites.		Srinagar: Under Construction Hydropower Project (1-2 sample) was collected as per pre-defined protocol of study.	Temperature (°C), pH, Dissolved Oxygen (mg/l), Conductivity (µs/cm), Total Dissolved Solids (mg/l), Turbidity (NTU), BOD (mg/l), Fecal Coliform (MPN/100 ml) , Total Phosphates (mg/l), Nitrate- Nitrogen (mg/l)	Hydropower plants operations may also affect water quality through increase in silting, change of water temperatures, and lowering the dissolved oxygen.
[7]	Field Study (2004-05)	Water quality assessment of sacred Himalayan rivers of Uttaranchal in view of ecological sustainability.	60 locations were selected on 19 river systems & tributaries in Uttaranchal.	19 river systems were selected.	Total coliforms ranged from 900 to 11,90,000 MPN/100ml. Fecal coliform were in the range of 80-7700MPN/ml.	Continuous use of clean water river stretches for domestic purposes and run offs from the places of open defecation on the banks & hydrological conditions of river are responsible for increasing coliform count.

[8]	Microbial biodiversity of tributaries of river Ganga in Uttarakhand. (2012-13)	Water samples were taken in different seasons such as summer, post monsoon and winter from different spots.	Samples were taken from Alaknanda & Bhagirathi at the confluence near Devprayag.	MPN count for coliforms was highest in the month of June (summer) & lowest value was observed in the month of October (winter).	Bacteriological analysis showed six bacteria in river water samples and all samples had <i>E. coli</i> as indicator of fecal pollution. <i>E. coli</i> , <i>Enterobacter aerogenes</i> , <i>Staphylococcus</i> were found to be dominating in river Alaknanda.
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Over a century ago in 1896, British physician E Hanbury Hankin reported in the French journal *Annales de l'Institut Pasteur* that cholera microbes died within three hours in the Ganga water, but continued to thrive in distilled water even after 48 hours^[5]. But presently, *E. coli* is the highest among all pathogens posing threat to human health.

In an earlier study done in 2011 by Alternate Hydro Energy Centre (AHEC), IIT Roorkee, sampling and analysis were done as per APHA (20th Edition). Water samples were collected from Alaknanda and Bhagirathi rivers during four visits organized from the end of October, 2010 to middle February 2011. They were taken 0.50 m below the surface. The samples were stored in polyethylene bottles. It was found that faecal coliform count of Alaknanda river with in Srinagar town was 43 & 150MPN/ml^[5]. As per author's conclusion, water quality of the study area conforms to the standards prescribed for the designated best use "Source without Conventional Treatment but after Disinfection" i.e., it satisfies standards prescribed for CPCB class 'A'. In some places the Fecal Coliforms exceed the value of 50/100ml, but is lower than 100mg/100ml. Disinfection will be necessary before it is used for drinking. Study was conducted in winter months which can be a deciding factor for results obtained. In 2010 study, on Alaknanda, Bhagirathi and Lower Ganga, 31 different bacterial species were obtained from Alaknanda stretch Shannon Diversity Index of various organisms in Alaknanda stretch were as follows-*E. coli*(0.268), *S. faecalis* (0.25), *Enterobacter* sp. (0.225) *Pseudomonas* sp. (0.197), *Bacillus* sp. (0.184), *Proteus* sp. (0.161)^[6]. In 2006 study, the coliform count varies from 1.1X10³ MPN /100ml (Rudraprayag) to 5.1x10⁵ MPN /100ml (Badrinath) and faecal coliform count varies from 1200 MPN /100ml (Badrinath) to (6500 MPN /100ml (Kalisaur, Near Dhari Devi Temple) in Alaknanda river zone^[7]. In study of year 2012-2013, *E. coli*, *Enterobacter aerogenes*, *Staphylococcus* spp. were found to be dominating in river Alaknanda than the river Bhagirathi at Devprayag & concentration of *E. coli* was increased from winter to post monsoon to summer^[8]. *Salmonella* species was isolated during summer season. Maximum bacteriological concentration of all the species was higher during summer season and minimum during post monsoon season.

Objectives:

1. Primary Objectives:-

- Microbiological aspects
- Bacteriological & other Biological indicators.

2. Secondary Objectives:- To find out effect of human activities including washing, bathing, holistic rituals on the banks of river & disposal of human & animal excreta on qualitative aspects.

Materials and Methods:-

a) Type of Study:- Cross Sectional Study

b) Sample size:- 200ml of water sample was collected separately (100ml for bacteriological test & 100ml for parasitological test respectively) in the sterile containers supplied by HiMedia Laboratories Pvt. Ltd. from 8 different sites mentioned below.

c) Site of sample collection:- Samples were collected from take off point (preferably centre) of Alaknanda river from 8 different sites with a distance of 1 km range of city. [Numbering as per site from where sample was taken]

1. Dam Colony Site
2. Srikot Temple Site
3. Saraswati Vidya Mandir Site
4. Chauras Bridge/ Bhagirathi Gas Station Site
5. Srinagar Shiv Temple Site
6. Project Canal Opening Site
7. Pauri Diversion Road Route Site
8. NIT Srinagar Site

Note:- Site 2,3,4 & 5,6,7 are highly populated sites (River bank) of

Village Srikot & Town Srinagar respectively. Medical College is situated between site 1&2. Base Teaching Hospital is situated between site 2&3.

d) Method used for taking sample:- As described in Annexure A4.2 by WHO(2011) mentioned method was followed for this study.

Water was collected from all the above mentioned sites at 2:00 to 4:00 pm and transported immediately to the Microbiology Laboratory for further processing.

e) Site of Microbiological study & analysis:- Microbiology & Immunology Departmental Laboratory, HNB Base Hospital, Srinagar, Pauri Garhwal, Uttarakhand.

f) Analysis of specimen & further processing for bacteriological & parasitological study:-

Acceptability & Bacteriological Analysis

After collection & transport of water samples from the various aforementioned sites to the laboratory, water was separately processed for physical, bacteriological (quantitative as well as qualitative) analysis. Physical properties of water samples were noted down and were further processed according to multiple tube method for quantitative analysis for both total coliform counts & fecal streptococci count.

Method of Processing(According to WHO Booklet)

1. Three rows of five tubes were arranged for each sample & tubes in the first row (F1) hold 10 ml of double-strength MacConkey broth while the tubes in the second and third rows (F2, F3) contain 10 ml of single-strength presumptive medium.
2. 10 ml of sample was added to each of the five tubes in row F1 & 1 ml of sample to each of the five tubes in row F2 respectively.
3. A 1:10 dilution of the sample was prepared by adding 1 ml of sample to 9 ml of dilution water. Recap the bottle containing the diluted sample and shake it vigorously.
4. 1 ml of the 1:10 dilution was added to each of the five tubes in row F3.
5. After gently shaking, the tubes were incubated at 37°C for 24 hours.
6. Recording is noted down both after 24 as well as 48 hours and further confirmatory tests are carried out on each sample & after that MPN table (McCardy's Tables) mentioned on (WHO, 2011 Annexure 5, Water Analysis) is used for calculations.



Fig 1: Multiple Tube Method analysis for various samples in Double Strength & Single Strength preparation (1ml,0.1ml,0.01 ml) after 24-48hours indicating via colour change & gas production

Similar test was performed by Brilliant green lactose broth, but results were better in MacConkey broth.

Qualitative assessment was done by using both the protocols mentioned by HiMedia Labs as well as subculture plating method on appropriate growth media.

Comment on subculture plating of various sites:- On further subculture of various samples from above mentioned site also showed the significant presence of *E.coli*, *Klebsiella*, *Citrobacter* from all the sites & *Proteus mirabilis* with characteristic swarming, fishy smell & biochemical reaction (indole negative) especially from Site 6th(mentioned above).



Fig 2: Growth pattern of Enterobacteriaceae family including E.coli & Klebsiella on MacConkey agar of various samples.

Vibrio sp. was not found even on successive sampling & inoculation onto *Vibrio* specific HiMedia kits probably indicates the presence of vibriophage in river water. Repeated sampling, analysis & even on successive subculture did not reveal the presence of most fecal streptococci except enterococci even after providing requisite culture conditions. Enterococci were further tested on growth in MHA with 6.5% NaCl, 40% bile broth, 600C for 30 min. Bile esculin test, failing to reduce nitrate to nitrite & production of acid but no gas in mannitol [9]

Apart from these certain H₂S producing small centrally spore producing proteolytic & non saccharolytic gram positive bacilli spp(probably *Clostridium bifermantans* were also seen in Robertson Cooked Meat broth could not perform anaerobic culture with limited available sources. Isolates obtained in our study were similar to study conducted by Kistemann T et al 2002 [10]



Fig 3: Qualitative Assessment Results for Samples [Table given below]

Biochemical Test	1	2	3	4	5	6	7	8
1.Citrate	+	+	+	+	+	+	-	+
2.Lysine	+?	+?	+?	+	+	+	+	+?
3.Ornithine	+	+	+?	+?	+	+	+	+
4.Urease	+	+	+	+	+	+	+	+
5.PPD	+	-	-	-	-	-	-	-
6.Nitrate	+?	+?	+	+	+?	+	+	+?
7.H ₂ S+	+	-	-	-	-	-	-	-
8.Glucose	Y	R	R	Y	R	O	Y	R
9.Adonitol	R	R	Y	R	Y	Y	R	Y
10.Lactose	R	Y	Y	Y	Y	Y	Y	Y
11.Arabinose	R	Y	Y	Y	Y	Y	Y	Y
12.Sorbitol	R	R	Y	R	Y	Y	R	Y

On prolonged incubation H₂S production is seen more intensely in some of the sample sites including water from site 1, 2, 4 and 7.

Abbreviation used:-

Positive:- (+); Negative:- (-); Y:- Yellow; R:- Red; O:- Orange

Parasitological Analysis & Evaluation:-

Floation method using saturated salt, saturated sucrose solution & ZnSO₄ floation technique and sedimentation process like formalin

ether sedimentation method were used to concentrate all the samples of water. [11] After forming appropriate saturated solution the samples were left out for floatation for 10- 30 min [12]. Then with application of cover slip on the meniscus formed by solution at brim of test tube, slides were prepared, similarly supernatant were discarded in sedimentation method.

Detailed microscopic examination of all the water samples did not reveal presence of any cysts, trophozoites or eggs. Absence of finding of protozoans including *Giardia* & *Entamoeba* sp. does not rule out the presence of these parasites in water samples. Only motile & non motile bacteria along with dust & silica crystals were visible.

It leaves lacunae in area of research for demonstrating presence of pathogenic parasites. It may be detected in future using various methods and will entirely depend on the condition of water status at that time.

Ethical Considerations:-

As the study did not involve any human and animal participant during the entire phase of study, but Institutional Ethics Committee permission for "Exemption from Review" was taken prior to study.

Results:-

MPN count of all the 8 samples was found >150/ml after 48 hour inoculation & qualitative results were also evaluated. MPN count for site 1-8 after 1st 24 hours were 2400, 5400, 3500, 2400, 1300, 3500, 16000, 16000/100ml and after 48 hours were 9200, 16000, 16000, 9200, 16000, 9200, 16000, 16000/100ml respectively.

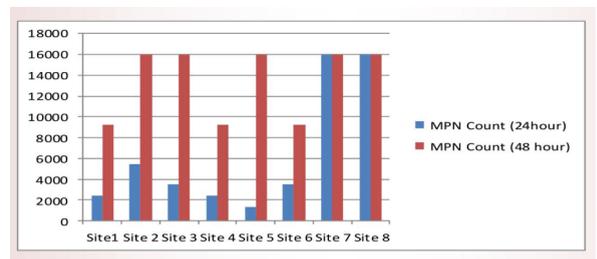


Fig 4: MPN Count after 24 & 48 hours of culture

Discussion:-

Detailed analysis of bacteria from all the above mentioned sites shows MPN count ranging from 1300MPN/100ml in water samples of first site on 24 hours incubation at 37°C to 16000MPN/100ml of most of the samples on 48 hours incubation at 37°C & on further confirmation both thermotolerant as well as non thermotolerant coliforms were found too high as per standard guidelines laid down by NRCP (National River Conservation Plan, 2010) [13].

The Central Pollution Control Board has classified water in five classes on the basis of water quality as given in the Table [14]

S.No.	Designated-Best use	Class of water	Criteria
1.	Drinking Water Source without conventional treatment but after disinfection	A	1. Total coliforms organism MPN/100ml shall be 50 or less 2. pH between 6.5 and 8.5 3. Dissolved Oxygen 6mg/l or more 4. Biochemical oxygen Demand 5 days 20°C 2mg/l or less
2.	Outdoor bathing (Organized)	B	1.Total Coliforms Organism MPN/100ml shall be 500 or less 2. pH between 6.5 and 8.5 3.Dissolved Oxygen 5mg/l or more 4.Biochemical Oxygen Demand 5 days 20°C 3mg/l or less
3.	Drinking water source after conventional treatment and disinfection	C	1. Total Coliforms Organism MPN/100ml shall be 5000 or less 2. pH between 6 to 9 3. Dissolved Oxygen 4mg/l or more 4. Biochemical Oxygen Demand 5 days 20°C 3mg/l or less

4.	Propagation of Wild life and Fisheries	D	1. pH between 6.5 to 8. 2. Dissolved Oxygen 4mg/l or more 3. Free Ammonia (as N) 1.2 mg/l or less
5.	Irrigation, Industrial Cooling, Controlled Waste disposal	E	1. pH between 6.0 to 8.5 2. Electrical Conductivity at 25°C micro mhos/cm Max.2250 3. Sodium absorption Ratio Max. 26 4. Boron Max. 2mg/l

* Below-E Not Meeting A, B, C, D & E Criteria.

Water quality standards of rivers for bathing purposes of faecal coliforms as per NRCP (desirable & maximum permissible) is 500 & 2500 MPN/100 ml respectively. Although, presence of coliforms in low numbers are not considered as specific indicators of faecal pollution in the water bodies.^[15] The presence of huge amount of coliforms with respect to standard values & presence of faecal streptococci on qualitative basis questions the water quality.^[16] The diversion of river within city as Project canal under Srinagar Hydro Electric Project (SHEP) created a major portion of river with decreased flow thus contributing to form potential source of infectious diseases & source of breeding places for mosquitoes as evident in recent year in increasing reports of Dengue & Chikungunya (as per hospital records) fever from such hilly areas.

Limitations:-

In the study, even after application of traditional methods of parasitological evaluation on repeated samples we were unable to find out parasites, trophozoites, cysts & eggs. It must be taken into consideration in future study to evaluate same by advance methods of detection including PCR.

Conclusions:-

Study was done during summer season which can be further correlated with the effect of seasonal & site variation from river, treatment plant & household- This study may help us to find out the root cause of diarrheal illness. Purpose of the study was to find out pathological correlation of microbes in river water caused by effluents in the Srinagar city with increasing population. In our study it was observed that total coliform count & enterococci counts were varying even within a short range proportionally with the human population & sewage disposal. Thermotolerant *Escherichia coli* after confirming by standard test were isolated indicating recent fecal pollution thus making water unfit for drinking. Apart from these spore producing H₂S reducing Gram positive bacilli assumed to be sulphite producing *Clostridium* were detected which survive physical & chemical disinfection probably indicates presence of other chlorine resistant pathogenic organism in river like viruses & protozoa.

Summary:-

Large amount of stagnant water in dams increases the survival of bacteria & can potentially degrade the quality of water & exacerbate public health risk. Also increasing construction near the banks of river & decreasing vegetation can affect quality of water.

Appropriate measures for disposal of water into the river through proper sewage treatment plant & increase in green vegetation should be undertaken to rejuvenate the quality of river water. Environmental & nature preservation programmes started by government should be implemented to preserve the natural beauty of these rivers. Adequate treatment of biomedical waste effluent should be done as in western countries before its drainage to river remains utmost important to preserve purity of river Ganga.

Conflicts of Interest:-

No conflicts of interest.

Acknowledgement:-

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