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Observation on *Pseudomonas* Diversity from a Tropical- River Kshipra M.P. India

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Abstract : *Pseudomonas* genus is a heterogeneous and ecologically significant group of Gram negative rod shaped, bacteria, commonly found in soils, water, sewage, humans, animals, and plants. Some species of the bacteria like *P.aeruginosa* are pathogenic and are responsible for causing skin, ear, nose, gastrointestites and urinary track infections, while some other species like *P. fluorescence* and *P.putida* are non-pathogenic and are known to play important role in the process of bioremediation. The paper provides a brief description of seasonal variation, distribution of *Pseudomonas* diversity and its role in occurrence of water born diseases. Isolation of *Pseudomonas* was performed by membrane filtration technique and species were identified by applying different biochemical tests. Total four *Pseudomonas* species were isolated which include *Pseudomonas aeruginosa*, *Pseudomonas anguilliseptica*, *Pseudomonas putida*, and *Pseudomonas fluorescens*. The highest count among *Pseudomonas* species was of *P.aeruginosa* (36.22% in surface and 34.74% in bottom) whereas lowest count was of *P.anguilliseptica* (12.4% in surface and 12.8% in bottom). The pattern of occurrence of *Pseudomonas* diversity were comparatively higher in bottom water and lower in surface water. As far as seasonal variation is concerned highest count was reported in summer, and minimum in winter. High *Pseudomonas* counts were reported at Ramghat (3711x10^3CFU/100 ml) followed by Triveni (2884 x10^3CFU/100 ml) and Mangalnath (2586 x10^3CFU/100ml) whereas, comparatively lower counts were observed at Mahidpur (2103 x10^3CFU/100 ml) and Kshipra village (1460 x10^3CFU/100 ml). Values of Shannon-Weaver index ranged between 0.680-1.012 which indicates eutrophication in the river. Variations in the occurrence of *Pseudomonas* in ambient water are affected by physicochemical parameters like DO, BOD, COD that influence survival, abundance and distribution of *Pseudomonas* in the river system. The incidence of waterborne diseases showed a seasonal pattern similar to the seasonality of causative agents in water samples. Occurrence of *Pseudomonas* in higher densities has made water unfit for consumption at all levels without proper treatment. The population exposed to heath risk could be reduced by minimizing discharge of untreated sewage, domestic waste and human activities. Hence, water needs conventional treatment including disinfection to conserve this holy river.

Keywords: *Pseudomonas*, Anthropogenic activities, Bacteria, Water quality, Kshipra river, water born diseases.

1. Introduction

Water is an essential resource for living system, industrial process, agricultural production and domestic use. The use of water increases with growing population, putting increasing strain on these water resources. The quality of water needs evaluation to generate base line data for welfare of the society. It is thus essential to isolate and identify microorganisms present in different water samples. In order to alleviate microbial water pollution a systematic study on types and concentration of microorganisms at different sites of a water body is required. One such important microorganism for assessing water quality of a particular water body is Pseudomonas. The genus Pseudomonas is the most heterogeneous and ecologically significant group of bacteria which includes Gram negative motile aerobic rods that are wide spread throughout nature. They are abundantly found in natural habitat like soil, fresh water and marine water [1]. Certain members of this genus are pathogenic like *P.aeruginosa* which is an agent of infection and is one the most frequently identified associated with waterborne outbreaks of dermatitis whereas some other strains of the genus

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exhibit activities like bioremediation and bio control [2]. So, the present work is planned to access presence of different species of Pseudomonas, its abundance, seasonal variation in surface and bottom water of river Kshipra in relation to physicochemical parameters. This would in turn help to access the risk of water born infection due to *Pseudomonas* species in humans and fishes. The main objective of this study was to evaluate the occurrence and distribution of *Pseudomonas* in Kshipra river and its health impact on the population residing near to the river. The effect of physicochemical parameters on abundance and distribution of *Pseudomonas* were studied. Such a study is important as it shall provide a framework for practical measures for river water management, control of anthropogenic activities on the banks of the river and would enlighten concerning authorities to mitigate and control the impact of pollution on river ecosystem and population.

2. Materials and Methods

River Kshipra originates from a hill of Vindhya range, one mile south of Kshipra village lying 12 km south-east of Indore city (M.P.). It flows approximately between latitude 22º49' and 23º50'N, longitude of 75º45' and 75º35'. River flows across Malwa plateau to join river Chambal which later joins Gangetic system. In the present study, five study sites with high anthropogenic activities were selected on the banks of river Kshipra, they include Kshipra village, Triveni, Ramghat, Mangalnath and Mahidpur. Sampling was carried out monthly from November 2013 to October 2014 for isolation of microorganisms. Bacterial samples were collected aseptically using 500 ml sterile bottles and were kept in ice bucket, they were then transported to the base laboratory within 24 hours after which qualitative and quantitative analysis of *Pseudomonas* was performed. Isolation of *Pseudomonas* was done by membrane filtration technique [3], after which the membrane was dried and cultured on P.A. agar at 30ºC for 24 hrs. Positive cultures were then incubated on nutrient agar for isolation and identification of single colony. Species were confirmed by applying different biochemical tests like colony characters, including pigment production which were determined on Pseudomonas agar. All strains were identified according to Bergy Manual of Systemic Bacteriology [4], Gram staining, cytochrome, oxidase, catalase production and growth on Maconkey agar at 37ºC. Isolated strains were biochemically identified by conventional test followed by use of API 20 NE identification system. Sampling and analysis of various physicochemical parameters were done by using standard methods given in APHA [3].The species diversity indices (H') was calculated according to Shannon-Wiener [5] and species Evenness (E) after Pielou [6]. Data on water born diseases was obtained by survey from major hospitals of Ujjain and Dewas city. A structural interview involving 500 households randomly selected from all the four quarters in the city of Ujjain and Dewas. Respondents were required to furnish information on their sources of water for domestic use and the occurrence of water born diseases in their respective families. Local fishermen were interviewed and their caught fishes were also examined for interpreting their diseased condition.

3. Results and Discussion

The results of the present study reveal the presence of *Pseudomonas* in all collected samples (100%). Total four *Pseudomonas* species were isolated which include *P. aeruginosa*, *P.anguilliseptica*, *P. putida*, and *P.fluorescens*. Quantification of *Pseudomonas* shows colony count ranging between $1785x10^3$CFU/100ml in surface and $1926x10^3$CFU/100ml in bottom at Ramghat study site. The count of *Pseudomonas* varied from $1785x10^3$CFU/100ml in surface and $1501x10^3$CFU/100ml in bottom at Triveni study site. In Mangalnath study site $1248x10^3$CFU/100ml at surface and $1388x10^3$CFU/100ml were observed at bottom. Colony count of $1005x10^3$CFU/100ml in surface and $1098x10^3$CFU/100ml in bottom were noticed at Mahidpur and $657x10^3$CFU/100ml in surface and $803x10^3$CFU/100ml in bottom were recorded at Kshipra village. The overall count of *Pseudomonas* is found to vary in different months throughout the year. In Kshipra river system minimum count of $652x10^3$ CFU/100 ml was obtained in the month of December at Kshipra village study site and maximum count of $1925x10^3$ CFU/100 ml were obtained in the month of June at Ramghat study site (Fig1 A). The highest count among *Pseudomonas* species was of *P.aeruginosa with* $2369x10^3$ CFU/100 ml at surface and $2494x10^3$ CFU/100 ml at bottom of the river. The second highest reported species was *P.putida* with $1819x10^3$ CFU/100 ml at surface and $2023x10^3$ CFU/100 ml at bottom,. *P.putida* was followed by *P.fluorescence* with $1540x10^3$ CFU/100 ml at surface and $1740x10^3$ CFU/100 ml at bottom of the river. *P.anguilliseptica* was the least found species of *Pseudomonas* in Kshipra river with $811x10^3$ CFU/100 ml at surface and $921x10^3$ CFU/100 ml at bottom of the river (Fig.1A, 1B).

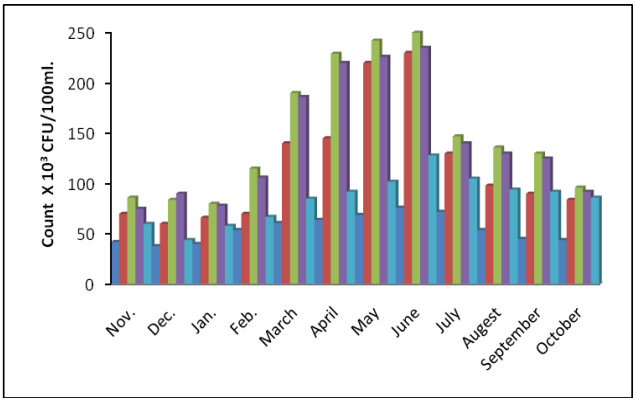

Fig.1 A : Monthly Variation in total count of *Pseudomonas* at various sites in surface water of river Kshipra

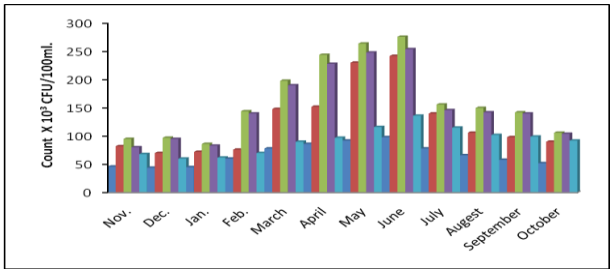

Figure1 B : Monthly Variation in total count of *Pseudomonas* at various sites in Bottom water of river Kshipra

Pseudomonas count in a river system is used to evaluate hygenic status of a water body and presence of some indicator bacteria like Faecal coliform and species of *Pseudomonas* like *P.aeruginosa* indicate contact of river with sewage [7],[8]. In the present study 100% occurrence of *Pseudomonas* genus is reported from collected water samples. The *Pseudomonas* count was found to increase the level prescribed by WHO < 1 recommended for Potable water. *Pseudomonas* species were reported by different researchers in India and across the globe. Presence of *Pseudomonas* from all studied sites of river Ganga India was reported, and it was found that *Pseudomonas* is a major cause of community acquired and ventilator associated Pneumonia [9]. *Pseudomonas* was also recorded from river Godavari India, and pointed out that species of this genus can live in harsh conditions as a result of their hardy cell wall that contains porines [10]. *Pseudomonas* was isolated from Pavana river India [11]. Higher count of *Pseudomonas* in the downstream and its urban tributaries of the river Mezam river South Africa was reported [12].

Pseudomonas count in Kshipra river system were found to show marked variations with changing seasons. Since Kshipra river is situated in tropical climatic conditions and aquatic flora and fauna of the river is found to be widely affected by different physicochemical parameters like temperature, DO, BOD and COD. In the present study also higher count were observed in summer and with onset of rain, whereas low count were observed in winter season. Higher count of *Pseudomonas* during rainy season in Ganga river India, was observed[13]. *Pseudomonas* is not able to grow in water temperature less than 15^0 C because it is not able to use the organic matter [14]. Finding of the present study well support the above stated fact as higher counts of *Pseudomonas* were observed in summer, followed by monsoon and least were observed in winter. Higher counts are observed in summer due to increased nutrient concentration, organic matter and reduction in water volume. In summer the reduced water flow, and other water resources pressurise use of river water as a chief source of irrigation for bank vegetation. This makes river accessible to both humans and animals, due to which there is a rise in faecal matter and the river is compounded by minimum dilution and low river flow [15]. Lower counts in winter are attributed to the fact that in winter season higher values of dissolved oxygen and lower values of BOD and COD are observed. On the other hand, regular rainfall flushes, faecal matter deposition from land and increased volume of water in river channels, there is maximum dilution resulting in lower counts in monsoon.

Kshipra river was mapped for distribution and occurrence of *Pseudomonas* at surface and bottom respectively. The results revealed that out of total *Pseudomonas* 43.80% *Pseudomonas* was present in surface and 56.19% was present in the bottom of the river. Higher count of *Pseudomonas* in bottom is due to sediment absorption and possibly extended survival of pathogen in aquatic system [16] which was due to the fact that high amount of organic matter and phytoplanktons get absorbed to clay which is advantageous for proliferation and survival of bacteria. However, twice count was found in bottom of Czarna Haneza river Poland [17], but in the case of Kshipra river due to less depth a lower difference between surface and bottom count was observed. The clay-argillaceous-sandy character of bottom, higher organic matter, presence of sandy and gravely deposits enhance long survival of bacteria in bottom of the river High counts at bottom are also attributed to dipping and swimming activities.

P. aeruginosa has also been isolated from non-polluted water but, is regarded as an organism of faecal origin [18]. The present study registers occurrence of four species of *Pseudomonas* in the river. The count the *P. aureginosa*(36.22% at surface, and 34.74% in bottom) was found to dominate the entire sanario (Fig.2A, 2B). The colonies count ranged between 22 x 10^3 to 46 x 10^3 C.F.U/ 100ml at Kshipra village, 24 x 10^3 C.F.U/100 ml to 88 x 10^3 C.F.U./100 ml at Triveni, 28 x 10^3 to 113 x 10^3 C.F.U/ 100 ml at Ramghat, 28 X10^3 to 94 X 10^3 C.F.U/100ml and 20 x 10^3 to 52 x 10^3 at Mahidpur (Fig.3A, 3B). High count of *P.aureginosa* was observed at Ramghat due to the fact that *P. aureginosa* is an indicator of faecal origin and Ramghat is centre for mass bathing, performance of worship rituals and various anthropogenic activities [19], which tends to deplete water quality and increase pollution load at this particular study site. Occurrence of *P. aeruginosa* was reported from Ganga river [20]. Density of *P. aeruginosa* ranged between within a range of 10^2-10^5 CFU/100ml in Lohagaon lake and Lakundi lake, Bijapur, Karnataka [21]. The impact of *P. aeruginosa* on health of bathers was documented [22]. *P. aeruginosa* was recorded from Kosi Dam, Betul, India and its role in breaking aromatic hydrocarbons and making rhamnolipids, quinolones, hydrogen cyanides, phenazines and lectins was analysed [23]. Microbial parameters of Wardha river were studied and the presence of *P. aeruginosa* in water of this particular river due to

heavy discharge of waste effluents was reported [24]. The presence of *P. aeruginosa* in major north Indian rivers like Ganga, Yamuna and Devprayag [9]. Occurrence of *P. aeruginosa* from different lakes, ponds and rivers of Dhaka city in Bangladesh which show distribution

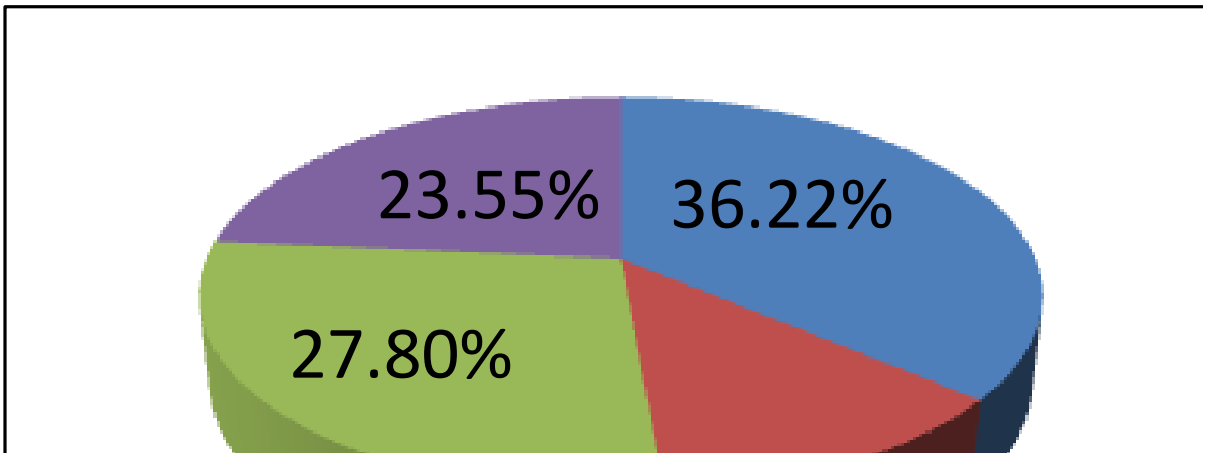

Figure 2 A : Species Wise Composition of *Pseudomonas* in Surface Water of Kshipra River

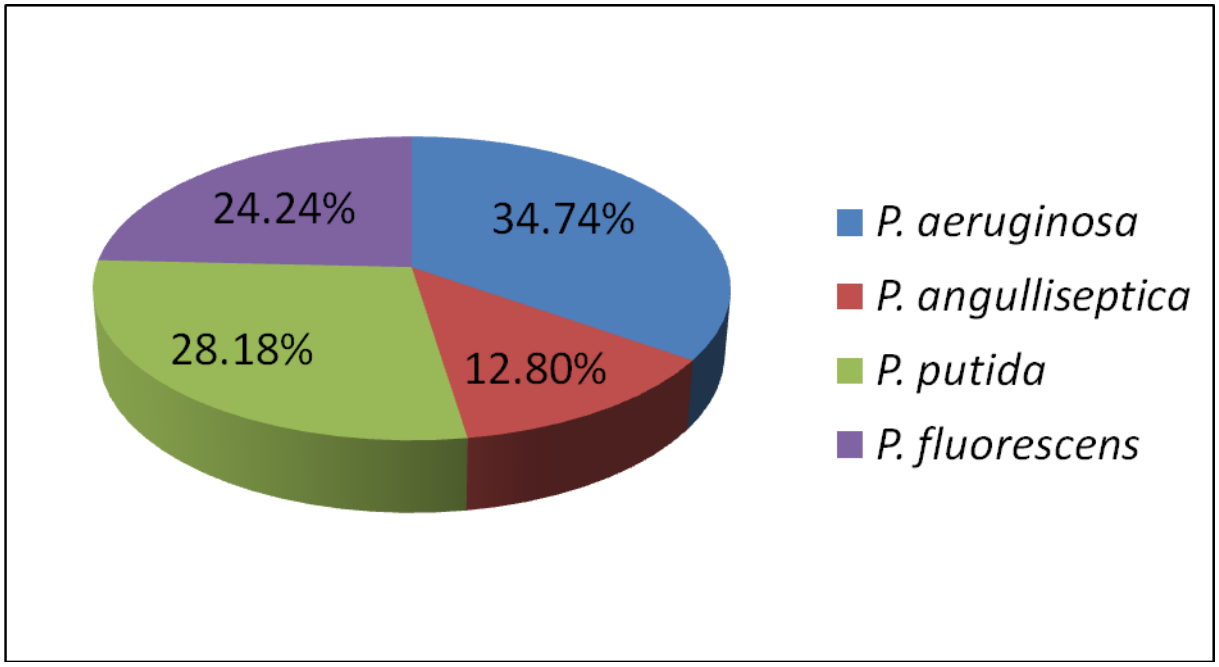

Figure 2 B : Percentage composition of *Pseudomonas* species in bottom water of river Kshipra

pattern as 54.1% from ponds, 69.9% from lakes and 60% from rivers. The average percentage of *P. aeruginosa* in various fresh water bodies of Bangladesh which was found to be 61.5 % and was much higher than 45.54% which was reported in India [25],[26]. River Nile Egypt is represented by 12 % *P. aeruginosa* count which shows significant increase by addition of human untreated waste [27], [28]. 2-46 CFU/100ml of *P. aeruginosa* in Kyotake and Yae rivers of Miyazaki city, Japan were reported [29]. *P. aeruginosa* is pathogenic in nature and is responsible for causing Pnuemonia, septic shock, urinary tract infection, gastro intestinal infection.

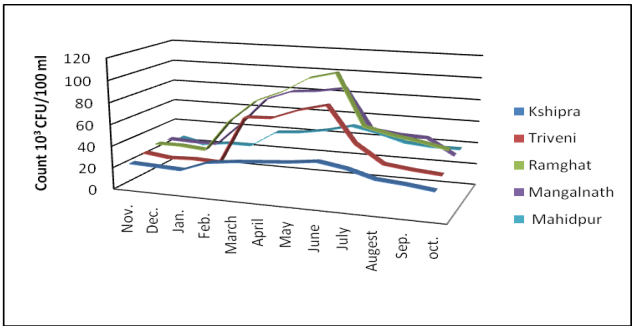

Figure 3 A : Occurrence of *P.auregenosa* in Surface water of river Kshipra

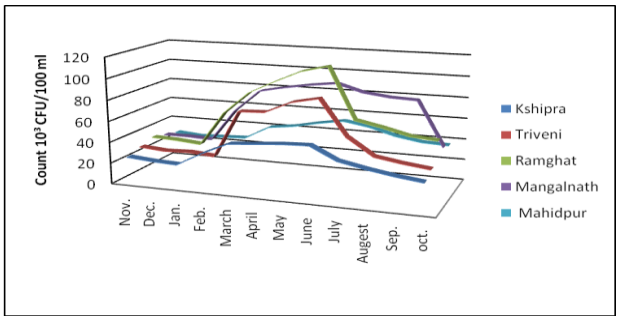

Figure 3 B : Occurrence of *P.aerugenosa* in Bottom water of river Kshipra

P.anguilliseptica, is a potent fish pathogen which is Gram negative and was contributed by 12.40% at surface and 12.8% at bottom of the total *Pseudomonas* (Fig.2A, 2B) . However, it was absent at Kshipra village study site. The density of *P.anguilliseptica* ranged between 4-39 X 10^3 C.F.U/100 ml at Triveni, 10-42X 10^3 at Ramghat, 13-34 X 10^3 C.F.U/100 ml at Mangalnath, 4-12X 10^3 C.F.U /100 ml at Mahidpur (Fig.4A, 4B). The higher counts of *P.anguilliseptica* were observed at Triveni and Ramghat respectively. A minimum count was reported in Madhipur due to less pollution and self-purification capacity of the river after a long distance. *P. anguilliseptica* was also noticed from *Cyprinus carpeo* and *Labeo rohita* at West Godavari South India [30]. This is a highly pathogenic strain in fishes and is responsible for causing Red Spot disease as well as motility in fishes. However, its count was least in Kshipra river.

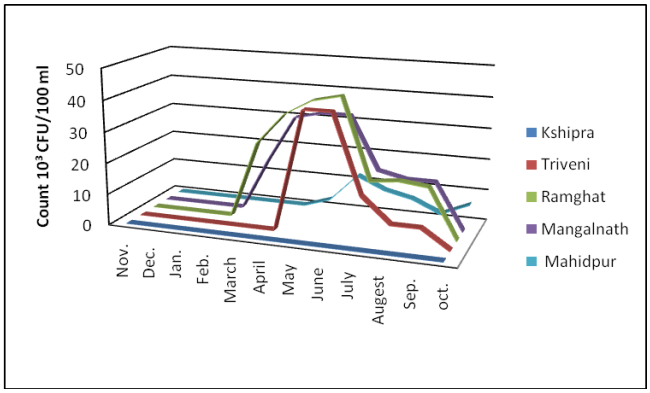

Figure 4.A : Occurrence of *P.anguilliseptica* in Bottom water of river Kshipra

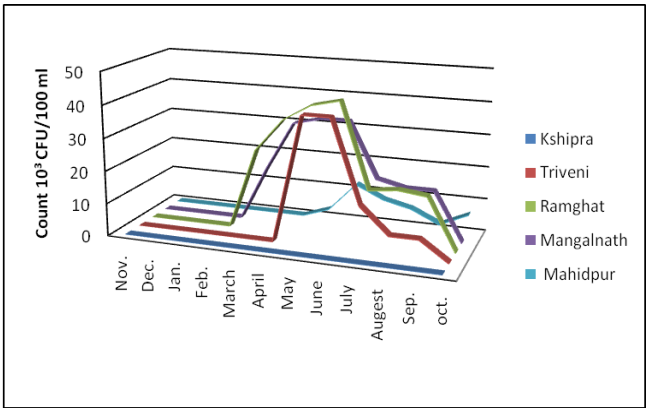

Figure 4.B : Occurrence of *P.anguilliseptica* in Bottom water of river Kshipra

P.putida contributed as the second highest species (27.81% at surface,28.18% at bottom) occurred throughout the study period (Fig.2A, 2B) . It is Gram negative, rod shaped, non-pathogenic in nature and capable of converting styrene oil into biodegradable plastic. In the present study, it was reported within a range of 16-40 x 10^3 CFU/100ml at Kshipra village ,19-65 X 10^3CFU/100ml at Triveni, 20-54 x 10^3 CFU/100ml to at Ramghat 24-66 x 10^3 CFU/100ml at Mangalnath and 15-38X 10^3 CFU/100 ml at Mahidpur (Fig.5A, 5B) . *P. putida* is known to be pathogenic in fishes as at causes haemorrhages in fishes and detachment of scales from fish body. *P.putida* is able to tolerate environmental stress due diverse control of protein. It also carries certain important lipids that has developed as an adaptation mechanism to respond to physical and chemical stress. The bacteria is able to change its fatty acid saturation and so cell can response to the environment in a better way and can also tolerate toxins. It is found to be highest at Mangalnath due to enchrichment of organic matter. Presence of *Eichhornia* and algal bloom provide another evidence of organic pollution in this study site.

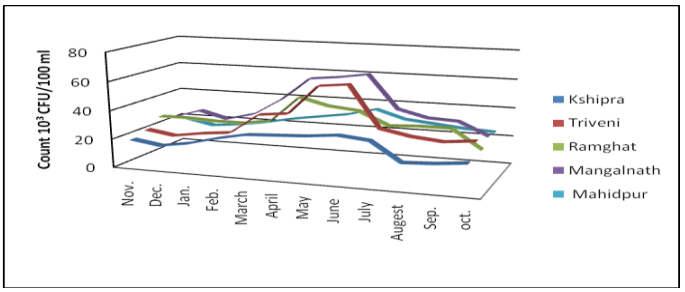

Figure 5.A : Occurrence of *P.putida* in Surface water of river Kshipra

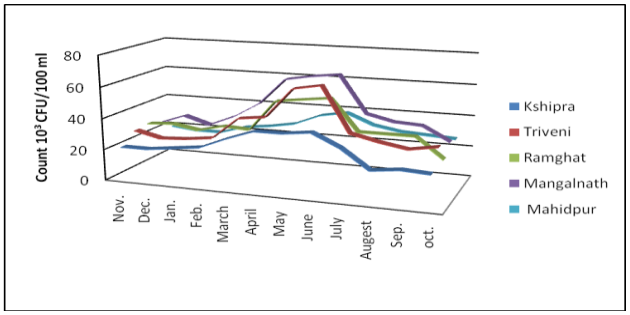

Figure 5.B : Occurrence of *P.putida* in Bottom water of river Kshipra

P.fluoresence is also a gram negative, rod shaped bacterium with multiple flagella, versatile metabolism and found in soil. It was found to be the third highest species (23.55% at surface and 24.24%) at river Kshipra (Fig.2A, 2B). It was found within the range of 2-12 x 10^3 C.F.U/100ml at Kshipra village, 17-50x 10^3 C.F.U/100ml at Triveni, between 25-67x 10^3 C.F.U/100ml at Ramghat 22-61 x 10^3 C.F.U/100ml at Mangalnath and between 9-34 x 10^3 C.F.U/100ml at Mahidpur. *P. fluorescence* is found to have significant role in bioremediation of heavy metals, pesticides and phenolics.

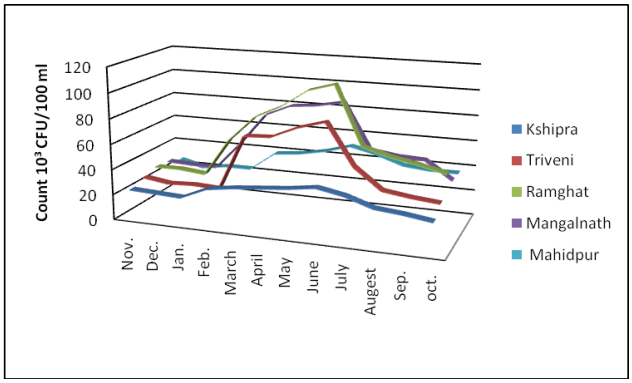

Figure 6.A : Occurrence of *P.fluorescens* in Surface water of river Kshipra

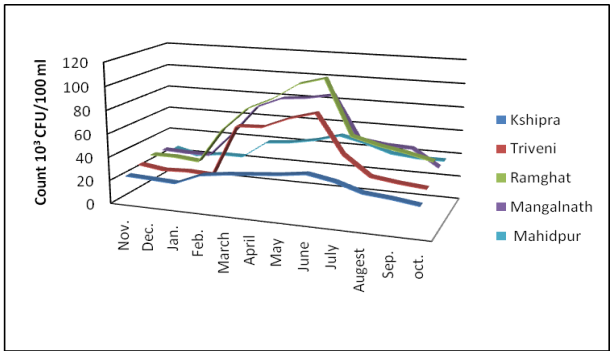

Figure 6.B : Occurrence of *P.fluorescens* in Bottom water of river Kshipra

In the present study values of Shannon-Weivner Index ranged from 0.680-1.012 at Kshipra village, 1.045-1.383 at Triveni, 1.033-1.315 at Ramghat, 1.087-1.323 at Mangalnath and 0.841-1.230 at Mahidpur (Fig.6A, 6B) . Eveness ranged between 0.724-1.00 at Kshipra village, 0.351-0.997 at Triveni, 0.854-1.00 at Ramghat, 0.726-1.00 at Mangalnath and 0.795 -1.00 at Mahidpur. Maximum values of diversity indices were reported in summer and minimum in winter (Table). Values of Shannon-Weaner Index and Eveness for microbial diversity were observed to be high during summer and low during winter [31]. This index was useful for community structure but could not stand alone for assessing water quality [32]. However, an another concept came forward which stated diversity index to be a suitable indicator for water quality [33]. Diversity was noticed to be within 1-2.5 in eutrophic lakes and up to 4.5 in oligotrophic lakes [34]. Similarly, in the present study values of diversity indices indicate eutrophic status of the river Kshipra. In the present study various physiochemical parameters were analysed and it was found that *Pseudomonas* count is positively correlated with air temperature, water temperature, calcium, chloride, hardness C.O.D., B.O.D. and turbidity. A negative correlation between *Pseudomonas* species and transparency and dissolved oxygen was found (Table 2).

Different physicochemical parameters were reported to be higher in summer and low in winter season except dissolved oxygen and Transparency which was found to be maximum in winter and minimum in summer season. Atmospheric temperature ranged between 15.8-38^{0}C, water temperature 18.3-30.8^{0}C, transparency 38.9-47.3 cm, turbidity 14-35.7 NTU, pH 7.9-8.7, DO 4.1-7.8 mg/litre, BOD 6.1-53.4mg/litre and COD 25.6-153.9mg/litre.

Household interview is conducted in Ujjain and Dewas city showed that in most of the households, inhabitants residing nearby river constantly suffered from skin diseases, gastrointestinitis and urinary tract infections. About 27-31% cases of urinary tract infections, 17-21% cases of skin infections and 15-20 % cases of gasterointestrites were reported from people residing near to the river area during the study period. However, the rate of incidences increased during summer and early monsoon season when occurrence of *Pseudomonas* was higher. More than 60% people living near to the river use the river water for drinking, bathing and other domestic use. 8-10% cases of Red spot disease in fishes have been reported. Similarly, desclatation in fishes have also been reported.

Fresh water quality criteria for domestic supply requires that *Pseudomonas* should not increase 1 CFU/100 ml [35]. Most of the portion of river Kshipra was highly polluted and was unacceptable for public health supply, or require fairly expensive treatment before use. At some places alongside river water is used extensively for

drinking, bathing, washing of fruits and vegetables, cleaning utensils and irrigation for crops cultivated nearby river. This leads to an increased count of pathogens like *P. aeruginosa* count which is of great consents with the increased number of HIV patients as it causes opportunistic infections in diseased patients [36]. *P. aeruginosa* is an opportunistic human pathogen, because it seldom affects healthy individuals. It often colonizes immunocompromised patients like those with cystic fibrosis, cancer or AIDS [37]. It is such a potent pathogen that it attacks two-third of critically ill hospitalized patients, this provokes more diseases. *P. aeruginosa* is responsible for 40-60% mortality rates. It causes about 90 % deaths via cystic fibrosis and is linked to worst visual diseases [38]. *P. aeruginosa* is also known to cause cardiac, CNS, ear, eye, bone, joint, urinary tract, respiratory, gastrointestinal, skin, soft tissue infection. High values of *P. aeruginosa* is known to cause skin, ear and eye problems in swimmers [39]. *P.anguliseptica* is known to cause Red spot disease,

Table 1 : General characteristic features of *Pseudomonas* species

S.No.	Organism	Source	Nature	Features	Disease Caused/Significance	Symptoms	Biochemical Test
1.	*P.aeruginosa*	Soil, Water, faecal contamination	Pathogenic	Gram negative Cocobacilus with unipolar motility	Pneumonia, Septic shock, urinary track infection, skin infection	Gastric pain, fever, tissue infection	Catalase, MR-VP,Indole -, Oxidase, Urease and H2S –ve
2.	*P.anguilliseptica*	Mostly water	Pathogenic to fish	Gram-bacterium	Red Spot disease in fishes	No particular symptoms	Catalase +, MR-VP +, Oxidase+, Urease+,Indole+ and H2S +ve
3.	*P.putida*	Found mostly in soil and water	Non-Pathogenic	Gram negative Rod shaped, Saprophytic bacterium	Not pathogenic, capable of converting oil into biodegradable plastic.	----------	Catalase +, Oxidase+, Urease-,Indole- and H2S-ve
4.	*P.fluoresence*	Found in soil and water	Less Pathogenic	Gram negative Rod shaped bacterium, multiple flagella and versatile metabolism	Affects immunocompromised patients	No particular symptom	Catalase+, MR-VP-,Indole -, Oxidase+, Urease+ and H2S +ve

Table 2. Correlation between physicochemical parameters and *Pseudomonas* species in river Kshipra

S.No.	Parameters	*P.aureginosa*	*P.anguilliseptica*	*P.putida*	*P.fluorescens*	Total Pseudomonas
1.	Atmospheric Temperature	0.790	0.806	0.622	0.809	0.802
2.	Water Temperature	0.878	0.866	0.712	0.852	0.875
3.	Transparency	-0.636	-0.635	-0.502	-0.599	-0.630
4.	Turbidity	0.694	0.680	0.604	0.634	0.686
5.	pH	0.798	0.773	0.731	0.735	0.793
6.	Carbonate	0.676	0.623	0.560	0.666	0.667
7.	Free.co2	-0.164	-0.110	-0.198	-0.198	-0.157
8.	Bicarbonate	0.874	0.845	0.732	0.817	0.863
9.	Total Alkalinity	0.914	0.876	0.764	0.862	0.902
10.	Dissolved Oxygen	-0.641	-0.584	-0.585	-0.585	-0.611
11.	Chloride	0.767	0.730	0.612	0.679	0.741
12.	Calcium	0.854	0.823	0.720	0.786	0.840
13.	Total Hardness	0.577	0.548	0.493	0.484	0.555
14.	B.O.D.	0.818	0.806	0.681	0.762	0.810
15.	C.O.D.	0.813	0.822	0.714	0.771	0.818
16.	*P.aureginosa*	1.00	0.946	0.778	0.960	0.980
17.	*P.anguilliseptica*		1.00	0.864	0.990	0.988
18.	*P.putida*			1.00	0.830	0.862
19.	*P.fluorescens*				1.00	0.990
20.	Total *Pseudomonas*					1.00

and *P.fluorescens* causes decolouration in fishes some cases of these disease have been reported in Kshipra river but their number is low due to low count of the respective pathogens.

In developing countries, the main source of river pollution is mainly via faecal contamination, discharge of untreated waste and sewage in the water body, lack of proper sanitation facilities and agricultural run off. However, in such countries lack of water supply, self-sustaining decentralised approaches including point of chemical and solar disinfection, safe water storage and behaviour changes are indicated as reliable options to directly target most affected population and reduce water-born disease burden through improved drinking water quality [40]. In developed countries, industrial effluents, agricultural runoff and mixing of pesticides and fertilizes with the river or tap water contributes as a major source of water contamination. In such industrialised countries, the success of applied control strategies is confirmed by small number of water- born outbreak caused by various water born microbes. Nevertheless, outbreaks caused by microbial contamination of drinking water still result in substantial human and economic cost in these countries [41]. In a resource constrained country like India, surface water is used for drinking, bathing, recreational and holy activities. However, factors like sewage, waste discharge, industrial effluents, and agricultural runoff contribute to increase the level of pollution in Indian river, but another factor which is a very important reason for pollution of Indian river system is the occurrence of religious festivals conducted on the banks of major Indian holy rivers like Ganga, Yamuna, Godavari, Kshipra etc. Development of Sewage treatment plants should be encouraged, use of soaps and dumping of flowers, coconut and ashes of dead bodies in the river should be restricted. Irrigation of fruits and crops with contaminated river water should be prohibited [42]. The concept of Bioremediations can be applied to reduce pollution level of effluent in the river. By using bio manipulation techniques beneficial Pseudomonas species like *P.putida* and *P.fluorescens* should be introduced in higher densities which can create competition against *P.aeruginosa* and can also aid in the bioremediation of organic waste.

4. Conclusion

The high levels of *Pseudomonas* are recorded in the study as well as the presence of pathogens in the river shows that it receives faecal contaminates on continuous basis. The river is highly impacted by religious rituals, domestic, drinking and irrigation activities. However, highest count of *Pseudomonas* is observed at Ramghat which is a site with highest human interventions. Maximum counts were reported in summer due to high nutrient concentration. The implications of these finding are that people who are dependent on the river water for domestic or agricultural uses may be exposed to public health risk. This risk can be reduced by minimising the discharge of both liquid and solid waste into water channels. Another essential requirement for reducing *Pseudomonas* count in the river is that performance of worship rituals on banks of the river should be minimized, rather awareness among people should be evoked and some eco-friendly methods should be introduced in a way that emotions of the pilgrims do not get hurt and at the same time water quality is also safe guarded. Certain other steps like maintain water volume, ma intain at least minimum flow rates, preventing addition of factory, industrial effluents and domestic waste discharge, providing river water use for irrigation, industrial and religious purpose, construction of research and development wing, creation of river protection force and conduction of regular water monitoring programs. These would aid to maintain water quality status of this pure and holy river.

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