

## Original Research Article

### Observation on *Pseudomonas aeruginosa* in Kshipra River with Relation to Anthropogenic Activities

Shivi Bhasin\*, Arvind N. Shukla and Sharad Shrivastava

School of Studies in Zoology and Biotechnology, Vikram University, Ujjain (M.P.), India

\*Corresponding author

#### ABSTRACT

*P.aeruginosa* is a Gram negative rod shaped, monoflagellated bacterium, commonly found in soils, water, humans, animals, plants and sewage. The microbe is responsible for causing skin, ear, nose, gastrointestines and urinary track infections. It is known to affect those patients more who suffer with cystic fibrosis, skin infections, cancer and AIDS. The paper provides a brief description of *P.aeruginosa* contamination, its occurrence and its role in contribution of natural, fresh water and drinking water as a route of *Pseudomonas* infection. Anthropogenic activities like mass bathing influence abundance, occurrence and distribution of *P.aeruginosa*. Isolation of *P.aeruginosa* was performed by membrane filtration technique and species identification was done by applying different biochemical tests. The study shows that most of the sites of Kshipra river are heavily contaminated with *P. aeruginosa* ( $20-160 \times 10^3$  CFU/100ml.) that constantly increased WHO limits for portability. The count of *P.aeruginosa* was found to be high during summer and onset of rain. Mass baths causes a sharp increase (49.10-51.16%) in *P.aeruginosa* count which returned back to normal stage after long time. The most impaired segment were Ramghat, Mangalnath and Triveni study sites which get polluted due to various anthropogenic activities and human interferences. Variations in the occurrence of *P.aeruginosa* 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. The population exposed to health risk could be reduced by minimizing discharge of untreated sewage, domestic waste and human activities.

#### Keywords

*Pseudomonas aeruginosa*, mass bath, anthropogenic activities, bacteria, water quality, Kshipra river, water born diseases.

#### Introduction

Surface water plays a key role in transmission of pathogenic agents discharged through human and animal faeces. These agents may find their way into water via domestic waste water/surface

runoff from agriculture land and pastures during rainfall or by direct deposition of faecal matter with access to steam channels (Eyles *et al.*, 2003, Collins *et al.*, 2005). They can be transferred to humans by

various roots like recreation, irrigation of crops, vegetables and drinking water (Davies-Colley *et al.*, 2004). Thus, quality of water needs evaluation to generate base line data for welfare of society. Microbial investigation of water is required frequently to know the status of water body in terms of microbial pollution.

One of the important microbe for assessment of water quality is *Pseudomonas* which is a genus of heterogeneous and ecologically important group of bacteria including Gram negative, motile, aerobic rods and wide spread in nature. They are abundantly found in natural habitat like soil, fresh and marine water (Franzetti and Scarpellini *et al.*, 2007). *P. aeruginosa* is a gram- negative, rod shaped, asporogenous and monoflagellated bacterium which has incredible nutritional versatility. It is about 1-2  $\mu\text{m}$  long and 0.5-1.0  $\mu\text{m}$  wide. *P. aeruginosa* is an obligate respirer and is a ubiquitous microorganism which is found in environment with soil, water, human, animal, plants, sewage and at hospitals (Laderberg *et al.*, 2000). *P. aeruginosa* is catalase, oxidase, arginine dihydrolase, urease and gelatine positive whereas it is found to be indole, phenyleactate and cystine arylamidase negative. The pathogen grows best at temperature between 37-42 °C. It is a potent agent of infection and is one of the most frequently identified pathogen associated with waterborne outbreaks of dermatitis (Tripathy *et al.*, 2006).

The main objective of this study was to evaluate the occurrence and distribution of *P. aeruginosa* in Kshipra river and its health impact on the population residing near to the river. The effect of physicochemical parameters and anthropogenic activities like mass bathing on abundance and distribution of *P. aeruginosa* was studied. Such a study is important as it shall provide a framework

for practical measures to guide local authorities for river water management, control of anthropogenic activities on the banks of the river and would enlighten them to mitigate and control the impact of pollution on river ecosystem and population.

## Materials and Methods

### Study area

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 22°49' and 23°50'N, longitude of 75°45' and 75°35'. 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.

### Water sampling

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. Every month qualitative and quantitative analysis of *P. aeruginosa* was performed at the base laboratory.

### Isolation and Identification of *P. aeruginosa*

Isolation of *P. aeruginosa* was performed by membrane filtration technique (APHA, 2005). After filtration, the membrane was dried and was cultured on P.A. agar at 30°C 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 (Palleroni, 1984), Gram staining, cytochrome, oxidase, catalase production and growth on MacConkey agar at 37°C. Isolated strains were biochemically identified by conventional test followed by use of API 20 NE identification system.

### Analysis of physicochemical parameters

Sampling and analysis of various physicochemical parameters were done by using standard methods given in APHA (2005).

### Water borne diseases

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.

### Results and Discussion

#### Spatial distribution of *P.aeruginosa* in river Kshipra

The microbiological survey was carried out on Kshipra river. The result revealed the presence of *Psuedomonas aeruginosa* in all collected water samples, (100% occurrence) higher than the level prescribed by WHO that is <1 recommended for portable water. The evaluated samples contained *P.*

*aeruginosa* count ranging from 21–40 x 10<sup>3</sup> CFU/100 ml. in Kshipra village, 24–84 x 10<sup>3</sup> CFU/100 ml. at Triveni, 28–108 x 10<sup>3</sup> CFU/100 ml. at Ramghat, 29–90 x 10<sup>3</sup> CFU/100 ml. at Mangalnath and 20–50 x 10<sup>3</sup> CFU/100 ml. at Mahidpur study site respectively. The distribution trends of *P. aeruginosa* at different study sites was maximum at Ramghat (27.10%), followed by Mangalnath (24.24%), Triveni (20.21%), Mahidpur (15.30%) and Kshipra village (13.09%).

#### Temporal variation in *P. aeruginosa* count

In Kshipra river the overall count of *Psuedomonas* varied in different months throughout the year. In Kshipra village study site minimum count of 22 x 10<sup>3</sup> CFU/100 ml. was observed in December whereas maximum count of 40 x 10<sup>3</sup> CFU/100 ml. was observed in the month of June. At Triveni study site 24 x 10<sup>3</sup> CFU/100 ml were reported in the month of February and maximum count of 84 x 10<sup>3</sup> CFU/100 ml were noticed in the month of June. Remarked increase in colonies at Ramghat were recorded which were 30 x 10<sup>3</sup> CFU/100 ml in December and January, and maximum count 108 x 10<sup>3</sup> CFU/100 ml was recorded in the month of May. At Mangalnath study site 28 x 10<sup>3</sup> CFU/100 ml count were observed in the month of December and 90 x 10<sup>3</sup> CFU/100 ml. were found during the month of June and from 20-50 x 10<sup>3</sup> CFU/100 ml. (Fig.2).

#### Increased *Psuedomonas* count during Mass Baths

Various anthropogenic activities like mass bath tend to increase the count of *Psuedomonas*. In the present study a higher count of *P. aeruginosa* was observed during and immediately after mass bath indicating

the increased load of pollution due to performance of worship rituals in the rivers. During mass bath in March  $66 \times 10^3$  CFU/100 ml. colonies were reported before dip which increased up to  $129 \times 10^3$  CFU/100 ml. during and after dip showing an increase of 51.16%. During mass bath in July,  $54 \times 10^3$  CFU/100ml. colonies were observed before dip which increased up to  $107 \times 10^3$  CFU/100ml. registering an increase of 50.46%. In November mass bath  $29 \times 10^3$  CFU/100ml were recorded before dip which were found to increase up to  $63 \times 10^3$  CFU/100ml an increase by 49.15% after mass bath. These increased values recovered to normal stage after longer period.

### Correlation between physicochemical parameters and *P. aeruginosa* count

*P. aeruginosa* shows positive correlation with BOD, COD, turbidity, air temperature, water temperature, calcium, chloride and hardness. However, a negative correlation with dissolved oxygen and transparency was observed. Like *P. aeruginosa* count higher values of BOD, and COD were observed in summer which were accompanied by lower DO values (Table 2).

### Incidence of water born diseases

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, gastroenteritis and urinary tract infections. About 27-31% cases of urinary tract infections and approximately 17-21% cases of skin infections and 15-20 % cases of gasterointestines 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 *P. aeruginosa* was higher. More than 60%

people living near to the river use the river water for drinking, bathing and other domestic use.

Total coliform and heterotrophic bacteria are used to evaluate hygienic status of a water body and presence of any coliform group in water indicates contact with sewage (Markosova and Jezek, 1994). Similarly, *P. aeruginosa* is also regarded as an organism of faecal origin (Wheater *et al.*, 1979). Higher count of *P. aeruginosa* indicate faecal pollution of a water body and certify the fact that untreated waste, sewage, industrial effluents and agricultural runoff has entered in the water body. Fruits and vegetables washed or irrigated by these contaminated water and products consumed by inhabitants lead to various gasterointestinal, skin and urinary tract infections in consumer.

### Spatial distribution of *P. aeruginosa* in Kshipra river system

In the present study 100 % occurrence of *P. aeruginosa* was detected from collected water samples. The study site wise spatial distribution of *P. aeruginosa* shows higher count in Ramghat followed by Mangalnath, Triveni, Mahidpur and Kshipra village study sites respectively (Fig.1). The pattern of these spatial distribution is in accordance with anthropogenic activities and pollution status on these study sites. *P. aeruginosa* is observed by different researchers in various water bodies around the world. Janamet *et al.*, (2010) isolated *P. aeruginosa* from Ganga river, India. Warghane *et al.*, (2011) reported *P. aeruginosa* from Godavari river, India and pointed out the fact that the pathogens affects immunocompromised patients quickly. Bilgrami *et al.*, (1986) noticed *P. aeruginosa* in Ganga river and stated that the main source of contamination of most of the sites was sewage discharge,

decomposition of plant material, cattle washing and performance of worship rituals by bathers. Kumbar *et al.*, (2014) observed *P. aeruginosa* within a range of  $10^2$ - $10^5$  CFU/100ml in Lohagaon lake and Lakundi lake, Bijapur district, Karnataka. He also reported the effect of *P. aeruginosa* on health of bathers (Hallet *et al.*, 2011). He pointed out that *P. aeruginosa* may alter the taste, colour and turbidity of water. Bhawsar and Singh *et al.*, (2014) isolated *P. aeruginosa* from Kosi Dam, Betul district, India and identified its role in breaking aromatic hydrocarbons and making rhamnolipids, quinolones, hydrogen cyanides, phenazines and lectins. *P. aeruginosa* was found to produce two pigments – pyoverdin and pyocyanin. They also isolated a strain *Rsan-ver* of *P. aeruginosa* which they found to be useful for bio surfactant production. Tambekar *et al.*, (2014) studied microbial parameters of Wardha river and reported the presence of *P. aeruginosa* in water of this particular river due to heavy discharge of waste effluents and domestic sewage in the river. Kumar *et al.* (2010) reported the presence of *P. aeruginosa* in major north Indian rivers like Ganga, Yamuna and Devprayag. Nasreen *et al.* (2015) isolated *P. aeruginosa* from different lakes, ponds and rivers of Dhaka city in Bangladesh. She reported 54.1% *P. aeruginosa* from ponds, 69.9% from lakes and 60% from rivers. She also focused on 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 (Sivaraj *et al.*, (2011). Z. Sabae and A. Rabeh *et al.*, (2007) reported 12 % *P. aeruginosa* in Dameitta branch of river Nile, Egypt. They reported that count of *P. aeruginosa* showed significant increase by addition of human waste and untreated waste (Eyles *et al.*, 2003). Suzuki *et al.* (2013) reported 2-46 CFU/100ml of *P.*

*aeruginosa* in Kyotake and Yae rivers of Miyazaki city, Japan. Aoi *et al.*, (2000) isolated *P. aeruginosa* from Ushubetsu river, Japan. Florez *et al.*, (2006) isolated *P. aeruginosa* from water samples of rivers in Columbia. Williamset *et al.*, (2008) reported the presence of 76.5% of *P. aeruginosa* in spa baths of England. Neiwolak and Opeka *et al.*, (2000) isolated *P. aeruginosa* from 0-1060 CFU/100ml from Czarna river, Poland. *P. aeruginosa* organism of faecal contamination (Bahkrowf *et al.*, 1988 and Devecente 1986). Their high count was found and it was attributed to high recreational activities which were high in summer season.

#### Temporal variation in *P. aeruginosa* count in Kshipra river

*P. aeruginosa* was isolated from non-polluted water (Foster *et al.*, 1971). According to this, *P. aeruginosa* is a penetrating species found in soil, water and sewage which gets to water during rainfall. Bilgrami *et al.*, (1986) reported higher count of *Psuedomonas* during rainy season in Ganga river India, but antagonistic findings were observed by Abo *et al.*, (2004) who noticed higher counts of *Psuedomonas* in summer and least in winter in Nile river. Marsalek *et al.* (1994) also found higher *Psuedomonas* counts during summer in water of river St Clair in Sarnia. The above finding is well supported by the present study in which higher counts of *Psuedomonas aeruginosa* were observed in summer, followed by monsoon and least 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 (Castillo *et al.*, 2004). *P. aeruginosa* is not able to use trace amount of organic carbon for its growth and is not able to grow below 15°C. (Vanderet *et al.*, 1988, Kersters *et al.*, 1995). 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.

### **Impact of anthropogenic on occurrence of *P. aeruginosa***

Environmental status of Kshipra river is influenced by various factors like urbanisation, industrialisation, accumulation of effluents, intense agricultural operations and faecal contamination. The count of *P. aeruginosa* is known to increase with these activities. So, a higher count of *P. aeruginosa* is reported at different studied sites of Kshipra river. Lack of proper sanitation facilities in urban cities has been cited as the main cause of high bacterial pathogens in rivers transversing major cities in the world. Million litters of untreated human sewage are discharged in the river Kshipra which carries high bacterial load. The occurrence of *P. aeruginosa* is correlated with proximity of water contamination by sewage discharge and anthropogenic activities. About 12-15 lake population of Dewas and Ujjain district depend on water of river Kshipra for irrigation, domestic and drinking purpose. Crops irrigated by this water are consumed by inhabitants of these and neighbouring cities which gives rise to outbreak of water born diseases. High values of *P. aeruginosa*

are obtained from Ramghat, followed by Mangalnath and Triveni. These increased values are evident because of high anthropogenic activities and human interventions viewed at these sites (Table1). Ramghat is the main bathing centre of Ujjain city where many festive mass baths, accompanied by various worship rituals like flower dumping, ashes dumping etc. are performed. Triveni also witnesses mass baths on different occasions which are participated by pilgrims across the world. The site is demarcated by presence of a Shani temple and local crimmatorium. At this site oil leakage is observed as a result of which oil directly enters into the river increasing pollution load of the river. This study site is also the meeting point of river Khan which receives 1500 m<sup>3</sup> of textile effluents having all poisonous textile dyes with oil and grease resulting in higher BOD and COD. At Sawer about 4000 m<sup>3</sup> sludge is added into Khan river. Water from here is drawn for irrigation of 600 acres for growing vegetables (Rao *et al.*, 1992). Similarly at Mangalnath also different worship rituals are observed which increase nutrient concentration, organic pollution and microbial load of the river. Presence of brick making activity of 100 brick kilns have damaged the flood plains. These pollutants enter the river and contribute to the increased pollution at Ramghat and Mangalnath sites. Kshipra village is near to the origin point of Kshipra river because of which a lower count of *Psuedomonas* is observed over here. However, this site is marked by comparatively less anthropogenic activities. Similarly, lower counts of *P. aeruginosa* are observed at downstream of Mahidpur which are attributed to self-purification capacity of the river and also because of the distance of 150 km from the origin point which reduces the number and concentration of bacteria (Fig. 1 and 2).

### Impact of mass bath on occurrence of *P. aeruginosa*

One of the biggest source of water contamination of our holy rivers are mass baths which leads to increased nutrient concentration, organic and inorganic substances in the water body along with different pathogenic microorganisms, which remains constant for a longer period of time (Table 2). Mass bath serve as a hazard for water quality because when million of pilgrims enter in Kshipra or any other river ecosystem the settled silt, clay and sand mixes with the surface water which enhances total hardness, calcium, chloride, total alkalinity, and other metals present in the soil of the river. Increased values of all physicochemical and microbiological parameters were observed during and after mass baths which returned to normal position after a long period due to self purification capacity of river. Mass bath is ruining ecology of river Kshipra from a very long time. The Ujjain city hosts Mahakumbh (mass bath) which greatly influences river ecosystem of Ujjain city especially at Ramghat. In Mahakumbh of 1992 a major drop in DO values from 8.1 mg/ litre to 1.07 mg/litre were observed (Rao *et al.*, 1992). The massive mass bath had its influence on the water quality of the particular site for about an year. The accumulation of soap films, hair, dead skin, body oil, dirt and faeces in the river create ideal conditions for bacterial survival and multiplication. Shedding of skin from infected patients is the predominant source of *P. aeruginosa* in river during mass baths. The warm and moist environment promote the growth of this bacteria (Pollock 1990). *Pseudomonas* is found more with high swimming activities (Hoadley 1977). In the present study, high cases of skin, urinary tract and eye infections are reported in people who live on the banks of river Kshipra and use water of

river for bathing regularly. The count of patients with skin problem and gastrointestinal disorders was found to increase in March after a mass bath. These patients were mostly out of those who took bath in holy river.

### Implications for public health

Fresh water quality criteria for domestic supply requires that *Pseudomonas* should not increase 1 CFU/100 ml.(WHO, 2001). 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 *P. aeruginosa* count which is of great consents with the increased number of HIV patients as it causes opportunistic infections in diseased patients (Baron and Hollander, 1993). *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 (Botzenhardt *et al.*, 1993). 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 (Fick, R. *et al.*, 1993). *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 (Hoadley *et al.*, 1977).

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 (Mintz et al., 2001). In developed countries, industrial effluents, agricultural

runoff and mixing of pesticides and fertilizers 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 (Berg 2008, Risebro 'et al.' 2007). In a resource constrained country like India, surface water is used for drinking, bathing, recreational and holy activities.

**Table.1** Anthropogenic activities observed at Kshipra river

S.No.	Activities	Study sites				
		Kshipra village	Triveni	Ramghat	Mangalnath	Mahidpur
1.	Mass bathing	-	+	+	-	-
2.	Flower dumping	-	+	+	+	-
3.	Coconut dumping	-	+	+	+	-
4.	Other rituals	-	+	+	+	-
5.	Oil leakage	-	+	-	-	-
6.	Ashes of dead bodies	-	+	+	-	-
7.	Presence of crimnetorium	+	+	-	-	-
8.	Domestic waste water disposal	+	-	+	+	-
9.	Tributary with industrial and domestic waste (Khan river)	-	+	-	-	-
10.	Agricultural runoff	+	+	-	-	+
11.	Boating	-	-	+	-	-

**Table.2** Occurrence of *P. aeruginosa* during mass bath (Shanivari Amavasya i.e. first day of full moon falling on Saturday) at Triveni study site of Kshipra river

1.	Mass Bath date 1/3/2014 →	Pre	During	After Dip									
		Dip	Dip	2 Mar.	3 Mar.	4 Mar.	5 Mar.	6 Mar.	7 Mar.	8 Mar.	9 Mar.	10 Mar.	
		<i>P. aeruginosa</i> count X 10 <sup>3</sup> CFU/100 ml	66	108	129	127	125	82	86	76	70	74	70

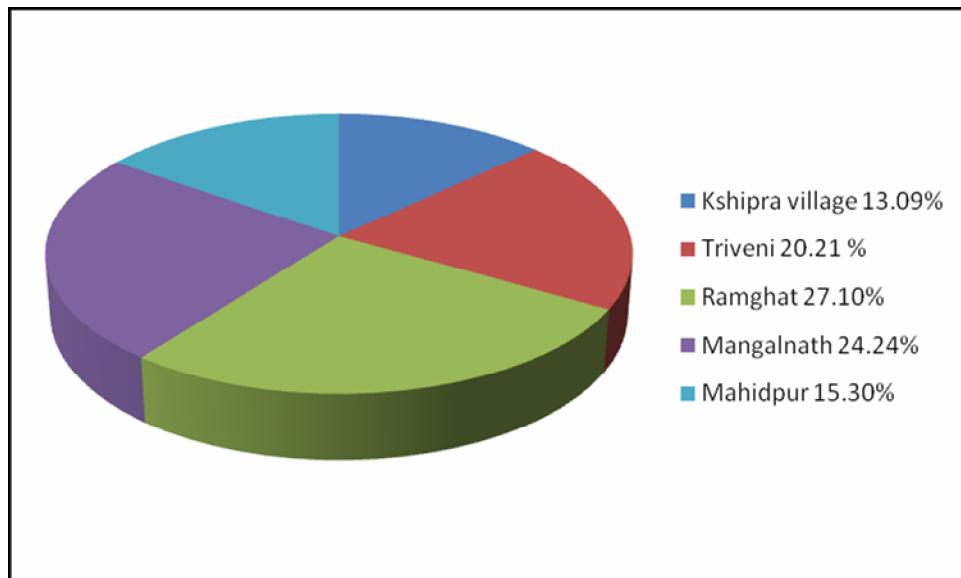
  

2.	Mass Bath date 26/7/2014 →	Pre	During	After Dip									
		Dip	Dip	25 Jul.	27 Jul.	28 Jul.	29 Jul.	30 Jul.	31 Jul.	1 Aug.	2 Aug.	3 Aug.	4 Aug.
		<i>P. aeruginosa</i> count X 10 <sup>3</sup> CFU/100 ml	54	86	107	112	110	113	102	80	61	52	49

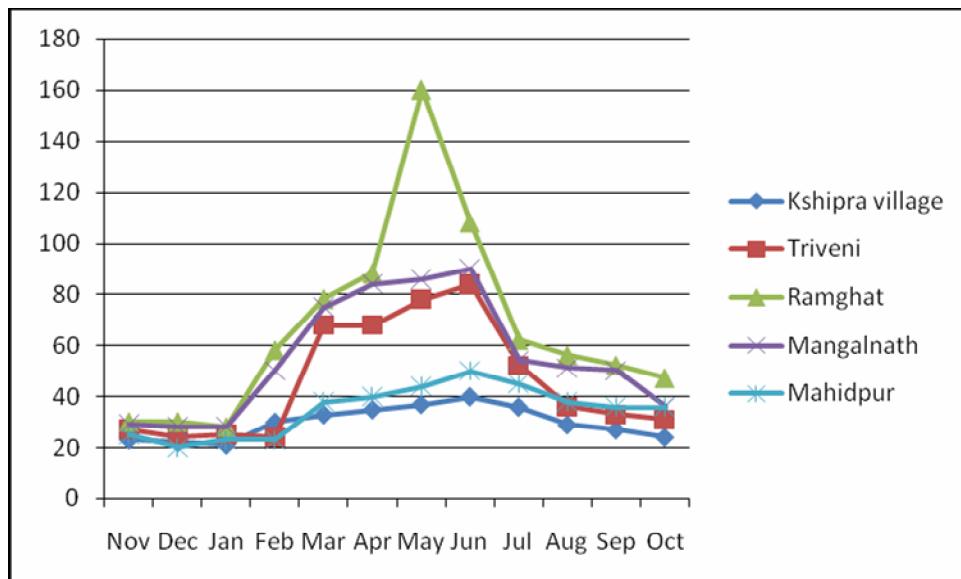
  

3.	Mass Bath date 21/11/2014 →	Pre	During	After Dip									
		Dip	Dip	21 Nov.	22 Nov.	23 Nov.	24 Nov.	25 Nov.	26 Nov.	27 Nov.	28 Nov.	29 Nov.	30 Nov.
		<i>P. aeruginosa</i> count X 10 <sup>3</sup> CFU/100 ml	29	45	59	63	52	44	41	38	30	28	25

**Fig.1** Percentage Composition of *P. aeruginosa* in different study sites of Kshipra river



**Fig.2** Monthly Variations on *P.aeruginosa* count (count x103 CFU/100ml) at different study sites of Kshipra river



However, factors like sewage and waste discharge, industrial effluents, 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, Godhavari, Kshipra etc. The river water gets flooded with many worship rituals and this water if used without proper treatment can lead to various health hazards.

River Kshipra hosts the Mahakumbh mela which is a religious festival organized in every twelve years attracting millions of tourists and devotees from all around the world to take bath in this sacred river, this gives rise to massive mass baths further depleting water quality. River Khipra enjoys status of Goddess in Hindu mythology so dumping of body ashes and statues of Lord Ganesha is an evident act observed at the banks of this river. These activities certify that in holy rivers of India mode and nature

of pollution is different from water bodies across the world.

The high levels of *P.aeruginosa* 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 *P.aeruginosa* is observed at Ramghat which is a site with highest human interventions. Maximum counts were reported in summer due to high nutrient concentration.

The count of *P. aeruginosa* was known to increase for during and after mass baths. 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 *P. aeruginosa* 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, maintain 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.

### Acknowledgements

Authors are thankful to Prof. M.S. Parihar, Prof. and Head S.S. in Zoology and Biotechnology, Vikram University, Ujjain (M.P.) for providing necessary research and laboratory facilities to complete this work.

### References

- Abo, M.H. 2004. Environmental Studies on river Nile at Damietta branch region, Egypt. *Journal of Egyptian Academic Society for Environmental Development* 5(2):85-105.
- Aoi, Yoh., Nakata, Hidehiko. and Kida, Hiroshi. 2000. Isolation of *Pseudomonas aeruginosa* from Ushubetsu river in Hokkaido, Japan. 200. *Jpn. J.Vet. Res.* 48(1):29-34.
- Bahkrouf, A.,Gauthier, MJ., Jeddi, M., Boudal, A. 1988. Starvation survival of *Pseudomonas aeruginosa* in sea water before and after adaptation to salinity. *Letters in Applied Microbiology* 7:59.
- Baron, AD., Hollander, H. 1993. *Pseudomonas aeruginosa* bronchopulmonary infections in late immunodeficiency virus disease, *Am.Rev.Resp.Dis.* 148:992-996.
- Berg, R. 2008. The Ala Mosa *Salmonella* outbreak: A gumshoe investigation. *Journal of Environmental Health.* 11(2):54-58.
- Bhawsar Niharika Amrute and Singh, Madhulika. 2014. Isolation and Characterization of *Pseudomonas aeruginosa* from waste Soyabean oil as biosulfactant which enhances biodegradation of industrial waste with special reference to Kosmi Dam, Betul district (M.P.). *Int. Journ. of ADV. Research.* 6: 778-783.
- Bilgrami, K.S., Bhowmick BN. and Singh A.K. 1986. Impact of Abiotic factors on bacterial population of river Ganga. *Proc. Indian natn. Sci. Acad.* 52(4): 509-514.
- Botzenhardt, K. and Doring, G. 1993. Ecology and epidemiology of *Pseudomonas aeruginosa*. “*Pseudomonas aeruginosa* as an opportunistic pathogen”. PP. 1-7.
- Castillo, MA., Allan, JD., Sinsabaugh, RL., Kling, GW. 2004. Seasonal and interannual variations of bacterial production in lowland rivers of Orinoco basin. *Freshw. Biol.* 49: 1400-1414.
- Collins, R., Elliott, S., Adams, R. 2005. Overland flow delivery of faecal bacteria to a headwater postoral stream. *J. Appl. Microbiol.* 99:126-132.
- Devicente, A., Borrego, J., Arrabal, F., Romero, P. 1986. Comparative study of selective media for enumeration of *Pseudomonas aeruginosa* from water

- by membrane filtration technique. *Appl. Environ. Microbiol.* 51:832.
- Eyles R., Niyogi, D., Townsend, C., Benwell, G., Weinsteen, P. 2003. Spatial and temporal patterns of *Campylobacter* contamination underlying public health risk in Taieri river, New Zealand. *J. Environ. Qual.* 32:1820-1828.
- Fick, R. 1993. *Pseudomonas aeruginosa*- the Microbial Hyena and its role in disease: An introduction *Pseudomonas aeruginosa*. The opportunist. PP. 1-6.
- Florez-Vives, Martha and Garnica, Diana. 2006. Comparison of Virulence between clinical and environmental *Pseudomonas aeruginosa* isolates. *International Microbiology* 9:247-252.
- Foster, DH., Hanos, N.B., Lord, S.M. 1971. A critical examination of bathing water quality standards. *Jour. Water Pollut. Control Fed.* 43:2229.
- Franzetti, L. and Scarpellin, M. 2007. Charcterization of *Pseudomonas* species isolated from floods. *Annals of Microbiology*. 57(1):39-47.
- Hall, Nancy., Cathy, Lord., John, Kempf., Carrie, Leveck., Cindy, Rieflin. and Karen. owens state Hygienic Laboratory, University of Iowa Research Park, Iowa City, IA. 2011. PP. 522-542.
- Hoadley, A.W. 1977. Potential health hazards associated with *Pseudomonas aeruginosa* in water. (In): Bacterial indicators/ Health Hazards associated with water. Ed. By A.W. Hoadley B.J. Dutka. ASTM, Philadelphia PP80-114.
- Janam, Ram., Gulati, K., Anil. and Nath, Gopal. 2011. Antibiogram and Geno typing of *Pseudomonas aeruginosa* isolated from human, animal, plant, water and soil sources in North India. *Southeast Asian Trop Med Public Health* 42(6):1477-1488.
- Kersters, I., Van Vooren, L., Hwys, G., Janssen, P., Kersters, K., Verstraete, W. 1995. Influence of temperature and process technology on the occurrence of *Aeromonas* species and hygienic indicator organisms in drinking water production plants. *Microb. E.col.* 30:203.
- Kumar, Ashok, Bisht, B.S., Joshi, V.D., Singh, A.K., and Jalwar, Amitabh. 2010. Physical, chemical and bacteriological study of water from river of Uttarakhand. *J Hum E col.* 32(3):169-173.
- Kumbar, M.I., Pujari, K.G., Yadawe, M.S., Hiremath, S.C., Piyar, A.S., Hiremath, D.M., Pujari, U.S. 2014. Physicochemical and Bacteriological study of lake water. *Int. interdisciplinary research Journ.* 1:163-167.
- Lederberg, Joshua. 2000. *Pseudomonas*. Encyclopedia of Microbiology, Second Edition. Volume 3. San Diego, PP. 876-891.
- Markosova, R., Jezek, J., 1994. Indicator bacteria and limnological parameters in fish ponds. *Water Res.* 12:2477-2485.
- Marsalek, J., Dutka, B.J., Tsanis, I.K. 1994. Urban impacts on microbiological pollution of St. Clair river in Sarnia, Ontario. *Wat. Sci. Tech.* 30:177.
- Mintz, E., Bartram, Iochery., P. and Wegelin M. 2001. Not just a drop in the bucket expanding access to point of use water treatment system. *American Journal of public health.* 91:1565-1570.
- Nasreen, Marufa., Sarker, Animesh., Malek, M.A. and Ansaruzzaman Md. 2015. Prevalence and resistance pattern of *Pseudomonas aeruginosa* isolated from surface water. *Advances in Microbiology* 5: 74-81.
- Niewolak, S. and Opieka A. 2000. Potentially pathogenic

- Microorganisms in water and bottom sediments in Czarna Haneza river. *Polish Journal of Environmental Studies*. 9(3):183-194.
- Pellerony, NJ., 1984. Pseudomonadaceae in Kreig N.R., Hoet, J.G. (ed). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, PP. 140-218.
- Pollock, M. 1990. Principles and practice of infectious diseases, 3<sup>ed</sup> edn, Churchill Living stone, Melbourne.
- Rao, K.S., Pandya, S.S., Shrivastava, S., Dhanekar, S. and Chouhan, A. 1992. Long term changes in water quality of River Kshipra. *Proc. Chemical industries in western region of M.P.* 42-45.
- Ravies-Colley, RJ., Nagels, JW., Smith, AR., Yough, RG., Phillips, CJ. 2004. Water Quality impact of a dairy Cow herd crossing a stream. New Zealand *J. Mar. Freshw. Res.* 38:569-576.
- Risebro, H.L., Doria, M.F., Andersson, Y., Medema, G., Osborn, K., Schlosser, O. 2007. Fault tree analysis of the water-borne outbreak. *Journal of water and health*. 5(1):1-18.
- Sabae, Z. Shawky and Rabeh, A. Saleh. 2007. Evaluation of the microbial quality of river Nile water at Damietta branch, Egypt. *Egyptian Journal of Aquatic Research*. 33(1):301-311.
- Sivaraj, S., Murugesan, P., Muthuvelu, S., Purusothan, S. and Silambarasan, A. 2011. Comparative study of *Pseudomonas aeruginosa* isolate recovered from clinical and environmental samples against antibiotics International *Journal of Pharmacy and Pharmaceutical Science* 4:103-110.
- Suzuki, Yoshihiro, Shota, Kaju, Masateru, Nishiyama and Alusi iguchi. 2013. Susceptibility of *Pseudomonas aeruginosa* isolates collected from river water in Japan to anti *pseudomonas* agent. *J. Scetoenv.* 0:02-11.
- Tambekar, Pratiksha, Batra, R.J. and Weginivar, R.G. 2014. Physicochemical and microbiological analysis of certain water sources and industrial waste water samples in Chandrapur district, Maharashtra, India. *Journal of Chemical and Pharmaceutical Research* 6(5):710-714.
- Tripathy. S., Kumar, N., Mohanty, S., Samanta, M., Mandal, R.N. and Maiti, NK. 2006. Characterization of *Pseudomonas aeruginosa* isolated from freshwater culture system. *Microbiol. Res.* 162:391-396.
- Vander, Kooij D., Hijnon W.A.M. 1988. Nutritional versatility and growth kinetics of and *Aeromonas hydrophila* strain isolated from drinking water. *Appl. Environ. Microbiol.* 54:2842.
- Warghane, J. Ashish., Wagh, G.N., Nag, B.B.S.P. Jishani, M.L., Thaware, R.R. and Kitey H.S. 2011. Isolation and characterization of *Pseudomonas* species from Godavari river sample. *Asiatic Jour. of biotic resources* 2(07):862-866.
- Wheater, D.W.F., Mara D.D., oragui J.I. 1979. Indicator systems to distinguish sewage from storm water run-off and human from animal fecal material. Chapter 21.PP.21-27(in). A. James and L. Evison (Ed.) Biological indicators of water quality. John Wiley and sons, Chichester, England.
- WHO (2001). Guidelines for drinking water quality. Addendum. Microbiological agents in drinking water. World Health Organization, Geneva, Switzerland. 188 P.
- Williams, Chris. 2008. Spa baths in motel accommodations and *Pseudomonas* risk. *Env. Health* 8(2):22-26.