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Isolation and Identification of microflora of Khan River at different sampling stations of Ujjain District at Triveni.

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Abstract: Water is the resource that sustains all life on earth and is a key element of sustainable development. Water pollution occurs when unwanted pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Water pollution affects plants and organisms living in water bodies and also to the natural biological communities. The direct effect of water pollution results in increased amount of microbial population. In these microbes some species are useful and some are harmful for human being.

Present study deals with the isolate and identify microflora species at different levels of Khan River of Ujjain District.Micro floral study of the river also shows that there are number of microbial population present in it, which are all pathogenic and not only harmful for the human health but also for aquatic flora and fauna. The results indicated that therewere different types of bacteria distributed throughout different sampling stations as *Pseudomonas* (37.79)%, *Salmonella* (15.74)%,*Klebshilla* (11.81)%, *Enterobactor* (7.08)%, *Streptococcus* (7.34)%, *Actenomycetes* (5.24)%,and finally *E.Coli* (5.24)%. We concluded that *Pseudomonas* were the most dominant followed by *salmonella* bacterial strain in the sample.

Keywords: Microflora, *Staphylococcus*,sewage, fresh water.

Introduction: An aquatic ecosystem is an ecosystem in a body of the water. Communities of organisms that are dependent on each other and on their environment live in aquatic ecosystems. Aquatic ecosystem is the most diverse ecosystem in the world. The first life originated in the water and first organisms were also aquatic where water was the principle external as well as internal medium for organisms. Water pollution is of grave consequence because both terrestrial and aquatic life may be affected; it may cause disease due to presence of some hazardous substances may distort the water quality, impose physiological stress on biotic community, add odours and significantly hinder economic activities (Asonye, et. al., 2007, Niti, 2013). Water pollution is a major global problem which requires

ongoing evaluation and revision of water resource policy at all levels (International down to individual aquifers and wells).

The sewage most often contains the organic matter that encourages the growth of microorganisms. These organisms besides spreading diseases also consume the oxygen present in water (Sinha, 2014a &2014b). This is called oxygen depletion. The aquatic organisms like the fish cannot then survive in such waters. This creates an imbalance in the aquatic ecosystems (Okoh, 2007).

Water pollution by effluent has become a question of considerable public and scientific concern in the light of evidence of their extreme toxicity to human health and to biological ecosystems (Katsuro, et. al., 2010, WHO, 2010).Water contaminated by effluent from various sources is associated with heavy disease burden (Sarang, 2013, Ashbolt, 2004) and his could influence the current shorter life expectancy in the developing countries compared with developed nation.

The most polluting of them are the city sewage and industrial waste discharged into the rivers. The facilities to treat waste water are not adequate in any city in India. Presently only about 10% of the waste water generated is treated; the rest is discharged as it is into our water bodies. Due to this, pollutants enter groundwater, rivers, and other water bodies. Such water, which ultimately ends up in our households, is often highly contaminated and carries disease-causing microbes. Sample collected from Khan River, which comes from Indore and through Sanwer it reaches Ujjain and joins Kshipra at Triveni. There are several industries in Indore, which throw their effluents in the river and it receives untreated sewage, storm drain, septic tanks, run off from farms from the villages which are situated at the bank of this river. Due to these industries and organic wastes river get contaminated.

Materials and Methods:

Sampling sites and procedures In present study micro flora of the river Khan were analysed , which were selected for sample collection.Water samples (500 ml) were collected at all sites using sterile screw-capped bottles and analyzed in the laboratory within 18 h of collection.

Bacteriological analysisTotal bacterial (TB) counts were determined by microscope as previously described by Hobbie (Hobbie et al.1977).For the study of microflora different types of media such as Nutrient agar, Chocolate agar, MacConkey agar plates were prepared and were inoculated by water samples (Fujioka,1999, 2001). These plates were incubated for 24 hrs. After then observed colonies were transferred into different media and broth for the identification of particular microbe with the help of biochemical test (Byamukama, 2005). Plates were incubated at 30°C under aerobic conditions for 48 h, and colonies were counted.

Isolation and identificationA range of 12 to 23 colonies were isolated from different media plates from sampling site and date. Colonies were randomly selected, providing that all different morphological characteristics(shape, size, surface texture, colour, opacity) were taken into account, and were purified on TSA plates.The strains obtained were identified using standard tests such as Gram stain, respiration/fermentation, and presence or absence of oxidase.

Table1. Number of strains of the different genera isolated from the Khan River of Ujjain District.

Pseudomonas sp.	**45**
Klebshilla	14
Salmonella sp.	06
Enterobactor sp.	22
Staphylococcus sp.	14
Streptococcus sp.	21
Shigella	7
Citrobactersp.	7
E.Coli sp.	8
Actenomycetes	9
Other Gram negative genera	4

Including genera with 4 isolates and strains not identified to the genus level.

Results and Discussion

Bacterial colonies were isolated from differen media plates and identified to the species level (Table 1). Strains of the genus *Pseudomonas* were dominant (37.79%). Among them, *Salmonella* was predominant (15.74%) and strains of *Klebshilla*(11.81%), *Enterobactor* (7.08%), *Streptococcus*(7.34%),*Actenomycetes* (5.24%)*E.Coli*(5.24%)were also commonly isolated (Table1). According to the sampling, *Pseudomonas*represented more than 35% of all isolates downstream from the inflow, isolates at this site.

Micro floral study also shows that the numbers of coliform bacteria are much more than the permissible limits. The presence of above mentioned microbes are all pathogenic. They can cause many diseases ranging from typhoid, dysentery to respiratory and skin disorders. Protozoan population indicates that river is highly contaminated.These pollutants are harmful for the human health, aquatic flora and fauna therefore it must be removed or detoxify through bioremediation for the healthy environment.

In spite of the considerable self-purification capacity of the river, unabated disposal of municipal sewage and industrial effluents affects the quality of river water, and thus the bacterial population. In our case, while total bacterial numbers re-attained normal levels within the 38 km zone downstream from the sewage input, the bacterial community structure did not. Indeed, a stable population of *Salmonella* spp., (which can represent up to 15.74% of the whole community for some stations) was present in the water downstream from the discharge. Among all the major Gram negative bacterial groups, *Pseudomonas* spp. were the least affected by the polluted effluent, and their occurrence rates were similar both upstream and downstream from the discharge. Consequently, our data cause us to seriously question the proposed monitoring of *Pseudomonas* spp. as an indicator of water quality in rivers receiving urban and industrial wastewaters.

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