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Research Article

Isolation and Identification of Micro-Organisms from Water Sample of Different Site of Bilaspur (C.G.)

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Abstract
The aim of present work to isolation and screening of the micro organisms from water samples from sites of Bilaspur. These organisms are too small to be clearly perceived by unaided human eye and main key of biodiversity on earth is due to evolution. The structural and function diversity of any cell represents its evolutionary event which occurred through Darwinian Theory of natural selection. Natural selection and survival of fittest theory is involved on the micro-organisms. Water is one of the most important requirements for survival of life on the earth. Now a days, the demand of the water increase due to the increase of human population in cities. The main problem how before the world is that of safe drinking water, which is fast assuming alarming properties problem related to storage, misuse and pollution of water are wide spread both in the rural areas. The problem is becoming increasingly complex with growing population, industrialization urbanization.
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Key words: Isolation, Identification, Water Sample, Micro organism

Introduction
Water has curious and unusual properties, and plays an important role in living systems. Thus, "no life without water" is common saying. It is a master solvent and all metabolic reaction of the living organism depends on the presence of water (Saha et al. 1971). Nearly three or fourths of the earth's surface is covered by the water mainly oceans to a lesser degree by rivers, lakes, and streams (Chona 1991). This water is in continuous circulation and the process is known as the water cycle or hydrological cycle. Water is lost from the earth by the way of evaporation, transpiration, exhalation, and is returned to the earth by the way of precipitation (Verma, 2004). Microorganism get into natural water from air, soil, sewage, organic waste, dead plants and animals, etc. thus almost any types of organisms may be found in water (Deshmukh et. al. 1964).

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The microorganisms have become a significant attraction as natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, antitumorals, antioxidant and anti-inflammatory (Okami, 1982; Nunez et al., 2006; Uzair et al., 2009; Shankar et al., 2010). Water is one of the most important requirements for survival of life on the earth. Now a days, the demand of the water increase due to the increase of human population in cities.

Material and Methods
Water Sample
From different place of Water collected at Mama-Bhanja pond and sewage water.
Laboratory media
Dehydrated chemically defined media was used and prepared as per manufacture instructions.
Nutrient Agar Media (NAM), Potato Dextrose Agar Media (PDA), and MacConkey Agar Media.
Other Requirements
Petri plates, Test tube, Test tube stand, Sprit lamp, Inoculation loop, Spreader, Conical flask, Distilled water, Thread, Tape, Alcohol , Culture Tube, Cover slip, Cotton.
Methods
To identify the bacterial unknowns in a mixed culture by morphological and biochemical methods. The identification of bacteria is a careful and systematic process that uses many different techniques.
Isolation
Sample preparation:
Water Sample Take from Different region of Bilaspur (C.G) of some Sewage and some Pond Water collected.
Isolation from sample (Water) by serial dilution Agar plating Method:
Water sample was performed by serial Dilution plate technique.
Principle
Micro-organism are abundant and ubiquitous in environmental. Soil is the principle habitat for different micro-organism. The quality and quantity of microbes depends on the Water nature. The method is based upon the principle that when material containing micro-organism is cultured, each viable micro-organism will developed into a colony, hence the no. of colonies appearing on the plates represent the no. of living organism present in the sample. Therefore, we can say that as the dilution increases, the no. of colonies decreases and the isolation of micro-organism from soil by serial dilution are made easy.

Procedure:

- Normal saline solution was prepared and 10ml was taken into the test tube labelled as Stock and 9ml each into the remaining Ten test tubes and was sterilized by autoclaving at 121^{0}C, 15 lbs pressure for 15-20 minutes.
- 1gm of sample was weighed and added into the first tube, it was the stock solution.
- 1ml of suspension from stock solution was transferred into the test tube labelled as 10^{-1}. Then again 1ml was transferred from 10^{-1} to the tube labelled as 10^{-2}. Like this, up to 10^{-10} Serial dilution was done and 1ml from the last tube discarded to make the volume of all the tubes equal. This procedure was repeated for the sample.
- Required amount of Constituent of the NAM media was dissolved in distil water, and was autoclaved at 121^{0}C, 15 lbs pressure for 15-20 minutes.
- After Sterilization, the medium was poured into the sterile petri plates and was allowed to Solidify.
- After Solidification 0.1 ml of the suspension from the dilution 10-6 and 10-7 & 10-8 was spread on Petri plates using spread plate technique.
- The inoculated plates were labelled and incubated at 28^{0}C for 5-7 days.
- Culture thus obtained are then purified and maintained in respective media for further studies.

Results and Discussion

Isolation and Identification of Bacterial isolates from Water Samples, Obtained during present investigation were identified on the basis of their Morphological chareateristics, Gram's Staning and biochemical features.

Isolation of some bacterial and fungi flora

After the nine Month and Continued Study from August 09 to April 10, altogether Six strains of bacteria were observed and isolate from different Water Samples, Obtained during present investigation. e.g: *E. coli, Staphylococcus, Streptococcus, Psuedomonas, Shigella, & Salmonella typhi* spp. etc. Some fungi species are also isolated from Water samples eg; *Aspergllus spp.*(Table 1 & 2)

Identification of bacterial and fungal strains

The cultural, Morphological, Gram staning and biochemical Charecterisyics of all the Bacterial and fungal strains Obtained from all water samples.

Table 1: Showing the Monthly prevalence of bacterial and some fungal in water samples

S/No.	Bacterial and fungal flora found in water	Month(Year 2013-2014)			
		Jan	Feb	Mar	April
1.	*E. coli*	+	+	+	+
2.	*Shigella*	-	+	+	+
3.	*Staphylococcus aureus*	+	+	+	+
4.	*Streptococcus*	+	+	-	-
5.	*Pseudomonas aeruginosa*	-	+	+	-
6.	*Salmonella*	+	-	+	+
7.	*Aspergillus*	+	+	+	+

Table 2: Represents the observation of different Biochemical tests

S/No.	Name of Sp.	Biochemical Test								
		Indole	M.R.	V.P.	Citrate	Urease	TSI	Sugar	Catalase	oxidase
1.	*Shigella sp.*	+ve	+ve	-ve	-ve	-ve	A/A no gas	A/A no gas	+ve	-ve
2.	*Salmonella sp.*	-ve	+ve	-ve	+ve	-ve	A/A no gas /$_{H2S}$+ve	A/A no gas	+ve	-ve

Much of ill health which affects humanity, especially in developing countries can be traced to lack of safe and

whole some water and food consumption. Since water is vital for our life, we expect it to be clean and safe. The water intended for human consumption must be free of pathogenic and chemical agents, pleasant to taste and usable for domestic purposes. For ensuring, safe and portable pond water local administration and public health specialist should take some action to preventing water quality problems in pond water with some proper management technique. For examples- Test the pond water periodically to determine bacteria levels and to monitor the presence of any other non-visible problems, strictly limit polluting activities in areas that drain into pond and especially near the pond, use diversion ditches and land grading to divert contaminated surface water away from the pond.

The result showed that variation of climatic conditions favours the growth and proliferation of different physiological types of bacteria. The bacteria numbers were generally higher during the dry season than rainy season. The bacteria population being smaller during the cooler, wetter season and the drier months supported large active population. In this study bacterial number were higher during the drier season than the wetter seasons. The reason adduced was that during wetter season, lower temperature inhibited bacterial activity, also saturation of the soil by rain limited activity by reducing aeration (Marshall and Devinny 1988). Cohen et al., (1993) reported on constitutive expression of mar-A leading to differential expression of over 60 chromosomal genes in E. coli has been reported by different investigators. According to Barbosa and Levy (2000), such kind of expression possibly conferred multiple antibiotic resistance on E. coli. Another gene namely emr harboured on E. coli chromosome has been identified that codes for protein products of membrane translocase family and accounts for multi-drug resistance (Lomovskaya et al., 1992). Many species of bacteria can survive in the pond water. The present investigation revealed that of the six bacterial isolates only two species of bacteria occur in all the season while the other bacteria occurred in some seasons and not in other season. This means that different seasons encouraged the growth and proliferation at certain bacteria and not the other. The response of different bacterial types to seasonal changes may be due to their physiological potentials which could be altered at the different seasons. The variation of climatic conditions such as distinct wet and dry seasons selectively favours the growth and proliferation of different physiological types of micro-organisms. Multi-drug resistance by Shigella sp. was not uncommon (Lien et al., 1994).

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