ANALYSIS OF WATER QUALITY IN JABALPUR CITY

THESIS

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1. INTRODUCTION

Water plays a significant role in the sound health of every individual and is essential for plant and animal life also. About 75% of the earth's crust is covered with water and the body of humans and animals comprises approximately 70% water (Pant, 2004). On earth, water is distributed unevenly viz. oceans (97.00%), surface water (2.26%) and subsurface water (0.61%). Fresh water is approximately 3%, which is available for humans and animal use. Water contributes in a number of ways to the health, progress and enjoyment of living beings. It is having important functions like universal solvent, thermoregulation of body, maintenance of blood and plasma volumes, cellular osmotic pressure and assist in secretary and excretory functions of body. Thus, water is an essential element for life on earth.

Water, the "Elixir of Life" is facing a severe threat due to pollution. Now days, there is a growing concern among public health agencies from both developed and developing countries that zoonotic pathogens in livestock exert a pose to an unacceptable waterborne public health risk. With growing urbanization and industrialization, water is getting contaminated with various impurities (microorganisms and chemicals) resulting in various diseases. Poor sanitary practices lead to the growth of pathogens such as *Campylobacter jejuni*, enterotoxigenic *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Vibrio cholera,* etc. causing mild to severe fatal form of diarrhoea. Also, water, on contamination by pathogens, heavy metals, pesticides, antibiotics and toxins may become dangerous to human and animal health and cause diseases such as, reproductive disorders, cardiovascular diseases, teeth decay, neurological diseases, etc.

Hardness and pH of water are also essential parameters in assessing the suitability of water for domestic and industrial uses. Too soft or too hard water is harmful. Soft water causes dental caries. Hardness of water prevents lather formation with soap therefore hard water is not suitable for bathing and washing. Hard water has high boiling point and so is not good for cooking too. Very hard water has been found to be responsible for

development of goiter, renal calculi, dyspepsia and gastric disturbances. Low pH water has a tendency to cause corrosion, while high pH water may contribute to scale formation in for e.g. boiling or cooling system.

Other important contaminants of water are the heavy metals. Iron, mercury, cadmium, nickel, cobalt, copper, arsenic, selenium, lead, etc. are important group of environmentally hazardous substances when present above permissible limits (Nicolau *et al*., 2006). Water bodies contaminated with heavy metals may lead to bioaccumulation in the food chain of the environment (Aycicek *et al.,* 2008). According to WHO (2011), permissible limit for arsenic, lead and selenium is 0.01 ppm. In general, the heavy metals are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects. Heavy metals can directly influence behavior by impairing mental and neurological functions, influencing neurotransmitter production and utilization and alternating numerous metabolic body processes. Systems in which toxic metal elements can impair and dysfunction include the blood and cardiovascular eliminative pathways (colon, liver, kidneys and skin), endocrine (hormonal), energy production pathways, enzymatic and immune systems (Teresa *et al.,* 1997).

Jabalpur is the third largest city of Madhya Pradesh state. Jabalpur is an important trade, commerce, industrial, educational and administrative centre of regional and national importance. Good water resources are located around Jabalpur like Narmada river and many ponds, which are sources of animals and human consumption. River Narmada and ponds around Jabalpur receive a large amount of domestic wastes, sewage, agricultural and industrial effluents. An immediate attention is therefore required to determine the physical, chemical and bacteriological quality of water resources of Jabalpur District.

OBJECTIVES

1. Analysis of physical and bacteriological quality of water.
2. To observe the presence of heavy metals particularly Lead, Arsenic and Selenium.

2. REVIEW OF LITERATURE

Water plays a vital role in human life. The most common and widespread health risk associated with drinking water is contamination; whether directly or indirectly. Before water can be described as potable, it has to comply with certain physical, chemical and microbial standards which are designated to ensure that the water is potable and safe for drinking. Potable water released into the distribution system becomes altered during its passage through pipes, open reservoirs, stand pipes and storage tanks. Water bodies receive domestic raw sewage from surrounding habitation and also the activities like cattle washing, cloth washing, bathing, religious activities like idol immersion (Bajpai *et al.,* 2002) pave the way for high concentration of hazardous chemicals in the river and pond water (Miller *et al.,* 2003). Transient negative pressure and pipeline leak events provide a potential portal for the entry of pollutant to enter the distribution system (LeChevallier *et al.,* 2003). Contamination in drinking water is usually manmade due to improper handling, storage and serving which leads to serious water borne diseases.

In recent times, many water bodies in India have received elevated inputs of heavy metals as a result of an increase in atmospheric deposition. Runoff from surroundings during rainy season is rich in heavy metals like copper, cadmium, lead, etc. It has also been realized now that ground water quantity and quality are declining and deteriorating rapidly. This has implications on per capita availability of fresh water. The available water per capita per year is 2384 cubic meter in the year of 2000 as against 6008 cubic meter in 1947. This shows a drastic decline in per capita availability of water (Lodhia, 2009).

Heavy metal pollution is posing a serious problem in India, threatening the animal and human health and environment. Environmental pollution in most of the developing countries is often attributed to negative effects of technological developments, rapid urbanization and industrialization, poor planning of waste disposal, etc.

2.1 Sampling

Water sampling is the process of collecting a representative portion of water for the purpose of analyzing it for constituents or contaminants.

Batley and Gardner (1977) described various methods for the collection, preservation and storage of natural water samples for the analysis of trace levels of heavy metals, particularly zinc, copper, lead, cadmium and mercury. Carefully cleaned high density polythene or teflon containers are recommended for both sampling and storage, with a storage temperature of 4°C.

Cenci and Martin (2004) studied the concentration and fate of trace metals in Mekong river delta. The samples were collected in acid cleaned polypropylene bottles and then filtered through a 0.45 µm membrane and transferred to an acid cleaned 250 ml polypropylene bottle. High purity nitric acid was added to set pH of approximately 1.

Khadse (2010) presented a seminar in a training programme on quality assurance and quality control in water quality monitoring assessment at National Environmental Engineering Research Institute, Nagpur for collection and preservation of samples and field analysis.

Pandey *et al.* (2010) investigated the mid stream water quality of Ganga river as influenced by aerially driven heavy metals at Varanasi, India. Twelve sampling stations were selected along a 20 kms long stretch of the river. Mid stream sub surface water samples were collected at fortnightly intervals from all the sites. They were acid digested and further checked for presence and concentration of Zn, Ni, Cr, Pb, Cu and Cd.

2.2 Physical quality of water based on pH and total hardness

The pH of water is a measure of the acid - base equilibrium. In most of the natural waters it is controlled by the carbon dioxide - bicarbonate - carbonate equilibrium system. An increased carbon dioxide concentration lowers the pH, whereas a decrease causes it to rise. Temperature also affects the equilibria and the pH. In pure water, a decrease in pH of about 0.45 occurs as the temperature is raised by 25°C. In water with a buffering capacity

imparted by bicarbonate, carbonate and hydroxyl ions, this temperature effect is modified (Eaton *et al.,* 2005). The pH of most drinking water lies within the range 6.5 - 8.5. Natural water can be of lower pH, as a result of acid rain or higher pH in limestone areas (WHO, 2007).

Rokade and Ganeshwade (2005) studied the high fluctuations in the physico - chemical parameters indicating the intensity of pollution. The pH was found to be 6.6 to 8.4, chlorides from 132.5 to 820.4 mg / l, hardness ranged from 74 to 281 mg / l, CO_2 from 2.1 to 5.09, biological oxygen demand from 4.437 to 112.432 mg / l, sulphates 0.192 to 5.12 mg / l and nitrates 0.5 to 1.012. The minimum pH value of 6.3 was found during winter season and maximum of 8.93 in summer. The pH shows general decline from upstream to downstream.

Ochieng *et al.* (2007) analyzed heavy metals, pH, salinity, electrical conductivity and temperature in water and surface sediment in five Rift valley lakes in Kenya for assessment of increase in anthropogenic activities. The study revealed higher concentration of Ag, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sn and Zn. pH of Rift valley lake water samples were alkaline indicating presence of carbonates and bicarbonates derived from carbonatite volcanic rocks.

Gaur *et al.* (2011) analyzed ground water samples to check fitness for drinking. The study was conducted in five regions of Uttarakhand, including Haridwar, Vikas nagar, Mussoorie, Dehradun and Dakpathar. Ten samples of ground water were collected from each of five regions during pre - monsoon and post - monsoon seasons. Parameters like pH, total hardness and heavy metals particularly Mn, Al, Ba, Cd, Cr, Co, Cu, Fe and Pb by inductively coupled plasma mass spectroscopy. Total hardness was below maximum permissible limit but pH and metals concentration of all samples were above permissible limit given by BIS.

Kumar and Kumar (2013) carried out experimental work on physico - chemical properties of ground water of Uttar Pradesh, India. The study performed with evaluation of granite mines situated in Jhansi (Goramachia) for their status about physicochemical contamination of ground water. Six different sites were selected from mines and urban areas. Three

samples have been taken at various distances on the site. The physico - chemical parameters such as pH, dissolved oxygen, electrical conductivity, total dissolved solids, alkalinity, turbidity, Ca and Mg, total hardness, NO_3 (nitrate), F (fluoride), Fe^{+3} (iron) and Cl^- (chloride) have been tested. It has been found that parameters are not in limit when compared with WHO standards.

2.3 Most probable number count

Coliforms are bacteria that are always present in the digestive tracts of animals and humans and are found in their wastes, also in plants and soil material. They are called as the indicator organisms. Water pollution caused by faecal contamination is a serious problem due to its potential for contracting diseases from pathogens (disease causing organisms). Frequently, concentrations of pathogens from fecal contamination are small, the presence of pathogens is determined with indirect evidence by testing for an "indicator" organism such as coliform bacteria. Coliforms are relatively easy to identify because these are usually present in larger numbers than more dangerous pathogens. As a result, testing for coliform bacteria can be a reasonable indication of other pathogenic bacteria presence.

September *et al.* (2007) studied the presence of biofilms in the drinking water distribution system. Ninety five biofilms samples from different parts of South Africa were tested for the presence of *E. coli. Aeromonas, Pseudomans, Salmonella, Shigella and Vibrio* spp. Members of these genera were quantified by the three tube most probable number approach using enrichment broths and plating on selective agars. The 16S rRNA identity of the putative pathogenic isolates revealed that high numbers of *Aeromonas, Pseudomonas, Klebsiella* and *Enterobacter* were present.

Omezuruike *et al.* (2008) analyzed the microbiological and physico - chemical status of different water samples used for domestic purposes. Samples of tap, well, stream and waste water were collected from Lagos State, Nigeria. Total viable count was made by pour plate technique while most probable number (MPN) count were by the multiple tube fermentation technique. MPN count ranges from 9.3 to 44 MPN / 100 ml. The faecal coliform counts on EMB agar plate ranged between 5 and 48 cells, also exceeding standard limit for water.

Tambekar *et al.* (2008) studied the presence of thermotolerant coliform (*E. coli*) and faecally associated *S. typhi* from surface water (13), shallow ground water (42), deep ground water (129) and public water supply (76) in the villages of Akola and Buldhana district of Vidharbha . A total of 260 samples were analysed and recorded 99.00% samples contaminated with coliform (by MPN technique) of which 75 contain *E. coli*, 50 samples contaminated with faecally associated *S. typhi*.

Shafi *et al.* (2013) estimated the coliform bacteria as a tool for assessing water quality of Manasbal Lake of Kashmir, Himalaya. In the present study, total coliforms were enumerated using a multiple tube fermentation technique with lactose broth as the presumptive medium and EMB agar medium as the confirmatory medium and brilliant green bile broth for completed test. All the samples obtained from the lake were positive with respect to the coliform occurrence, though the count was variable ranging between 4 and 460 MPN / 100 ml.

2.4 Prevalence of *E. coli* in water

E. coli found in all mammal faeces, but it does not multiply appreciably in the environment. *E. coli* O157:H7 is a zoonotic pathogen with its ability to cause human illness ranging from diarrhoeal disease to fatal hemolytic uremic syndrome. It is also associated with waterborne outbreaks resulting in high morbidity and mortality worldwide. *E. coli* survives in water for between 4 to 12 weeks, depending upon environmental conditions like temperature, microflora, etc. In distribution system, the longitivity of *E. coli* is higher. Therefore, it is important to investigate the prevalence of *E. coli* O157:H7 in water sources especially used for drinking and to develop the diagnostic methods for its early detection.

Johnson *et al.* (2003) studied the prevalence of *E. coli* O157:H7 and *Salmonella* spp in the Oldman river in Canada. They conducted a 2 year study to estimate the prevalence of *E. coli* O157:H7 and *Salmonella* spp in surface water within the basin. Prevalence of *E. coli* O157:H7 and *Salmonella* spp in water samples were 0.90% (n = 1483) and 6.20% (n = 1429), respectively.

Raju *et al.* (2011) collected 277 drinking water samples from bore wells, taps of consumer point and stored household water samples from Mysore city and analyzed for microbial parameters like heterotrophic plate count and coliform count. Out of 227 samples from consumer points, 80 samples were contaminated with enteric bacteria. Nearly 325 isolates of coliform were identified of which there were 79 *E. coli*, 26 *Salmonella* spp, 92 *Klebsiella* spp and 98 *Citrobacter* isolates.

Sapkota *et al.* (2012) studied the microbiological quality of potable water in Dehradun city of Uttarakhand state of India. The bacterial isolates were *E. coli, P. aerugenosa, Klebsiella* spp, *S. aureus, Proteus* spp.

Chouhan (2015) investigated the bacterial flora of Jaju Sagar Dam, a potential and sole municipal drinking water resource in Neemuch, (M.P.) and found elevated standard plate count levels than the standard ($P<0.05$) throughout the year indicated deteriorated quality of dam water. Identification study resulted in repeated occurrence of *E. coli and* other bacteria.

2.5 Molecular characterization of *E. coli* isolates

Osek (2001) did the multiplex polymerase chain reaction assay for identification of enterotoxigenic *E. coli* strains to differentiate them from other Gram's negative enteric bacteria. The test amplified heat labile and heat stable toxin sequences and the *E. coli* specific universal stress protein (*usp*A). The specificity of the method was validated by single PCR tests performed with the reference *E. coli* and non - *E. coli* strains and with bacteria isolated from pig faeces. The multiplex PCR allowed the rapid and specific identification of enterotoxin positive *E. coli* and it can be used as a method for direct determination of ETEC and to differentiate them from other *E. coli* and Gram's negative enteric isolates.

Jakee *et al.* (2009) conducted the work on molecular detection of virulence genes of *E. coli* from water. Fifty water samples were studied for the occurrence of coliforms. All *E. coli* isolates were serotyped and screened for virulence genes. *E. coli* strains isolated from water sources were characterized by PCR and showed that 8 isolates carried *stx*1 gene (verocytotoxin 1) and 4 possessed *stx*2 genes (verocytotoxin 2).

Tonu *et al*. (2011) detected the pathogenic *E. coli* through pathological study of the colibacillosis affected birds and isolated *E. coli* were further confirmed by PCR using specific primers *ECO-r* and *ECO-f.*

Nazemi *et al.* (2012) detected stx1, stx2, LT and ST toxic genes and O:157 and H7 antigens genes among uropathogenic *E. coli.* A total of 100 urinary samples were collected from patients with urinary tract infection who were referring to the health centers in Tehran, Iran, during years 2010 - 2011. *E. coli* isolation was performed, DNA was extracted from samples and the presence of stx1, stx2, LT and ST toxic genes and O:157 and H7 genes was investigated by PCR method. Out of 100 samples, 10 (10%) were carrying stx1, 7 (7%) H7, 6 (6%) stx2, 2 (2%) LT and 1 (1%) O:157 genes.

Nema (2013) carried out PCR for characterization of *stx* gene of *E. coli* from milk and noted the prevalence to be 12.60%.

2.6 Antimicrobial susceptibility of *E. coli* isolates

Reinthaler *et al.* (2003) evaluated the resistance patterns of *E. coli* in wastewater treatment plants without an evaluation of basic antibiotic resistance mechanisms. A total of 767 *E. coli* isolates were tested regarding their resistance to 24 different antibiotics. Among the antimicrobial agents tested, the highest resistance rate in the penicillin group were found for ampicillin (18.00%) and piperacillin (12.00%) in the cephalosporin group for cefalothin (35.00%) and cefuroxime - axetil (11.00%) in the group of quinolones for nalidixic acid (15.00%) and for trimethoprim / sulfamethoxazole (13.00%) and for tetracycline (57.00%).

Sayah *et al.* (2004) determined the patterns of antimicrobial resistance in 1,286 *E. coli* strains isolated from human septage, wildlife, domestic animals, farm environments, and surface water in Michigan by the disk diffusion method. The test was conducted for neomycin, gentamicin, streptomycin, chloramphenicol, ofloxacin, trimethoprim - sulfamethoxazole, tetracycline, ampicillin, nalidixic acid, nitrofurantoin, cephalothin, and sulfisoxazole. *E. coli* isolates showed resistance to the large number of antimicrobial agents. The agents to which resistance was demonstrated most frequently were tetracycline, cephalothin, sulfisoxazole and streptomycin.

Nema (2013) recorded the overall antibiogram profile of *E. coli* isolates displayed sensitivity against chloramphenicol (91.50%), netilin (90.10%), streptomycin (92.90%), gentamycin (84.50%), amikacin (71.80%), kanamycin (70.40%), norfloxacin (81.60%), ciprofloxacin (81.60%) and ofloxacin (80.20%). Multidrug resistance was found against nitrofurantoin (100%), cefotaxime (76%), ampicillin (67.60%), nalidixic acid (42.20%) and tetracycline (30.90%).

Rani (2016) assessed the bacteriological quality of water in and around Jabalpur city of Madhya Pradesh, India. A total of 135 samples were collected from branded drinking water, household purifier, tap water / public place water, Narmada river water and panipuri water was collected. *E. coli* and *Salmonella* isolates exhibited sensitivity to streptomycin and netillin. Isolates were resistant to tetracycline and nalidixic acid.

2.7 Quantitative analysis of heavy metals in atomic absorption spectrophotometer

Heavy metal toxicity is one of the major current environmental health problems and is potentially dangerous because of bioaccumulation through the food chain (Aycicek *et al.,* 2008). In general, the hazardous effects of these elements depends upon the dietary concentration of the element, absorption of the element by the system, homeostatic control of the body for the element and also the species of the animal involved (Underwood, 1977).

Lead

Specific properties

Under strongly oxidizing conditions Pb may occur but the most stable ionic form is the divalent. Pb (+II) forms stable salts as lead sulfate ($PbSO_4$) and lead carbonate ($PbCO_3$). Lead also has a high preference of forming complexes with organic matter (Drever, 1997). Through human activities such as mining, smelting, refining, manufacturing and recycling lead finds its way into the air, water and surface soil. Lead containing manufactured products (gasoline, paint, printing inks, lead water piper, lead glazed pottery, lead soldered cans, battery casings, etc.) also contributes to

the lead burden. Lead in contaminated soil and dust can find its way into the food and water supply.

Toxicity

Lead toxicity symptoms are often non - specific but characterized by fatigue, depression, sleep disturbance, anorexia, intermittent abdominal pain, nausea, constipation, diarrhea and myalgia (ATSDR, 2003).

Selenium

Specific properties

Selenium is widely distributed in nature and found in combination with sulfides and other minerals (ATSDR, 2003). It has semiconducting properties and it is used in photocopying machines, light meters and rectifiers. Selenium is used in agriculture as a component of fertilizers, pesticides and animal feeds, and selenium sulfide is an active ingredient of antidandruff shampoo. Although selenium has long been known to protect vitamin E deficient rats from liver necrosis (Rotruck *et al.,* 1973).

Toxicity

Selenium toxicity seen mostly in cattle grazing on milk vetch (legume) grown in the seleniferous soils (Harr *et al.,* 1972). Acute infection in livestock is known as “blind staggers” and is characterized by signs of CNS impairment (ataxia, impaired vision, disorientation) and respiratory distress. Chronic exposure to moderately toxic selenium levels is known as “alkali disease” and results in skin lesions with alopecia, hoof necrosis and loss, growth retardation, anaemia and cardiac atrophy. In humans, chronic sublethal selenium toxicity has been observed in individuals living in seleniferous areas and is characterized be hair or nail loss, thickened or brittle nails, garlicky breath, tooth decay (Hadjimarkos, 2013).

Arsenic

Specific properties

Arsenic is a group VA element of the periodic table, the 52nd most abundant element in the Earth’s crust. Arsenic is refined from the minerals arsenopyrite and loellingite, or it can be prepared from the reduction

of arsenic trioxide. The main use of arsenic is in the production of herbicides and other agricultural chemicals. Arsenic is also used in semiconductor industry.

Toxicity

Arsenic exerts its toxic effects particularly on keratinized tissues. Mee's line (horizontal white lines on the fingernails) appears in exposed individuals after the exposed nail bed grows to the exterior. At one time, inorganic arsenic was widely used as a "criminal poison".

Singh and Chandel (2006) analyzed the heavy metals of industrial effluents in Jaipur, Rajasthan. The results exhibited that As, Cd, Cr and Pb were not found in any studied wastewater samples, while some of the following heavy metals ranged from : Cu (0.0 - 1.0 mg / L), Fe (0.1 - 0.4 mg / L), Mn (0.0 - 0.4 mg / L), Ni (0.01 - 0.07 mg / L) and Zn (0.68 - 60.84 mg / L).

Dixit and Tiwari (2007) estimated the level of water pollution in the Shahpura lake of Bhopal. Water temperature, concentration of heavy metals like Pb, Cu, Cd, Mn and Cr were studied every month for a period of six months. It was found that the concentration of the heavy metals in the lake water substantially increased after the religious activities like idol immersion around August and September.

Kar *et al.* (2007) analyzed the water samples for pH, EC, Fe, Mn, Zn, Cu, Cd, Cr, Pb and Ni. A total of 96 surface water samples collected from river Ganga in West Bengal during 2004 - 05. The pH was found in alkaline range (7.21 - 8.32). All in all, the dominance of various heavy metals in the surface water of river Ganga followed the sequence: Fe >Mn >Ni >Cr > Pb >Zn >Cu >Cd.

Singh *et al.* (2013) studied the concentration of lead in Narmada river, ponds and other water resources in Jabalpur. Total 192 water samples were analyzed to find out concentration of lead. In each target area, 12 water samples were analyzed from three different locations, out of which, 5 were found below detection limit; whereas remaining all 11 target were showed concentration more than maximum permissible limit stated by WHO.

3. MATERIAL AND METHODS

Location of work

Proposed work was conducted in the Department of Veterinary Public Health and Epidemiology and Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.).

Study period

The study was conducted for a period of six months.

3.1 Biologicals, chemicals and equipments

3.1.1 Biologicals

All isolates from samples were maintained on nutrient agar (NA) (Hi - Media) slants by sub culturing at regular interval.

3.1.2 Media, chemicals, buffers, reagents, primer and metal standards

Chemicals, reagents, media, etc used in the study were of analytical and / or molecular grade from various reputed national and international companies like Hi - media, SRL, etc. The oligonucleotide primer used in the polymerase chain reaction was synthesized from integrated DNA technology. Preparation, procedure and composition of various media, buffers, reagents and metal standards are listed in appendices. Metal standards of lead, arsenic and selenium (Merck) were used throughout the course of experiment.

3.1.3 Glasswares and plasticwares

During course of study, glasswares and plasticwares of standard brand were used. Glasswares used were procured from Borosil and plasticwares were from Axygen, Tarsons and other reputed suppliers. Glassware and other materials of routine use were cleaned and sterilized properly following the standard procedures.

3.1.4 Scientific instruments

The instruments / equipments used in the study were from national and international enterprises viz. microscope (Labovision), incubator (Sector), hot air oven (Unilab), thermocycler (Bio - Rad), electrophoresis assembly (GeNei), UV transilluminator (GeNei), gel documentation system (Bio - Rad), micropipette (Nichipet EX), Atomic Absorption Spectrophotometer (Lab India AA8000), etc.

3.2 Collection of samples

Total of 117 samples were collected from different sources of water in Jabalpur city consisting of 20 samples each of different banks of river Narmada and public taps, 21 from tube wells, 35 samples of ponds, 21 samples of hand pumps.

Approximately 100 ml of water samples were collected from ponds, different banks of Narmada river, hand pumps, tube wells and public taps of Jabalpur city, in sterile bottles and brought to laboratory under sterile conditions on ice for bacteriological examination, for the detection of pH, hardness and metal analysis 200 ml of water was collected in polypropylene bottle. Samples were stored in refrigerator at 4ºC till further analysis.

Table 01: Samples from different water sources

S.No.	Source of water sample	Total no.
1.	Different banks of river Narmada	20
2.	Ponds	35
3.	Hand pumps	21
4.	Tube wells	21
5.	Public taps	20
Total		**117**

Table 02: Samples from different banks of river Narmada

S.No	Sample	Sample source	Number of samples	Total samples
1	Different banks of river Narmada	Bheda ghat	07	20
		Gwari ghat	07	
		Tilwara ghat	02	
		Lamhaita ghat	03	
		Jilhari ghat	01	

Table 03: Samples from ponds

S.No.	Sources	Collection Period			Total samples
		Pre Ganesha	Post Ganesha	Random	
1	Hanuman tal	01	01	01	03
2	Gulawwa tal	01	01	01	03
3	Dev tal	01	01	01	03
4	Mahanadda tal	01	01	01	03
5	Balasagar tal	01	01	01	03
6	Shani kund	01	01	01	03
7	Lal baba tal	01	01	01	03
8	Imrati tal	01	01	01	03
9	Supa tal	00	00	03	03
10	Bhairav nagar	00	00	03	03
11	Kal bhairav tal	00	00	03	03
12	Prakash colony	00	00	01	01
13	Rani Durgawati fort	00	00	01	01
Total		**08**	**08**	**19**	**35**

Table 04: Samples from hand pumps

S. No	Sample	Sample source	Number of samples
1	Hand pumps	Different places in Jabalpur city	21

Table 05: Samples from tube wells

S. No	Sample	Sample source	Number of samples	Total samples
1	Tube wells	Public Place	05	21
2		Hotels	07	
3		Residence	05	
4		Hospital	01	
5		College	03	

Table 06: Samples from public taps

S. No.	Sample	Sample source	Number of samples	Total samples
1	Public Taps	Different regions of Jabalpur	20	20

3.3 Determination of physical quality of water

3.3.1 Physical quality of water by pH determination

To determine the pH, the commercially available strips were dipped in the water sample and then immediately held it up against the colour indicator chart provided with the strips and the reading was noted.

3.3.2 Physical quality of water by total hardness determination

The water samples were collected and 100 ml volume was transferred in a conical flask. Two ml of ammonia buffer solution and 8 - 10 drops of colour indicator *i.e.,* Eriochrome Black T was added to it and mixed thoroughly. The solution was titrated against N/50 EDTA till the colour of water turns blue.

The hardness was calculated as per the formula given below (IS, 2002).

$$\text{Total Hardness} = \frac{\text{Volume of N/50 EDTA}}{\text{Volume of water in ml}} \times 100$$

3.4. Determination of bacteriological quality of water

3.4.1 Bacteriological quality of water by coliform count

3 Bacteriological quality of water by coliform count

In this method, three tube set in triplicate were used. The first , second and third set of tube had taken 10 ml double strength MacConkey lactose broth ,5ml single strength MacConkey lactose broth and 5ml single strength MacConkey lactose broth respectively. The water sample of amount 10ml, 1ml and 0.1ml were added in respective first, second and third set of tube followed by incubation at 37°C for 24-48 hrs. and then observing production of gas and change in colour of the medium. The count was made by using McCrady table according to method described by Cruickshank *et al*. (1975).

3.4.2 Isolation of *E. coli*

Isolation of *E. coli* from the samples of water was done as per the method described by Agarwal *et al*. (2003). The samples showing positive results in MPN count were streaked (0.1 ml) on eosin methylene blue agar (EMB, Hi - Media) plates and incubated at 37°C for 24 hrs. Colonies showing green metallic sheen were picked up and considered as presumptive *E. coli*. These presumptive *E. coli* were inoculated onto nutrient agar slants in duplicate and after incubation the slants were stored at 4°C for further characterization. The organisms were subjected to biochemical tests for confirmation.

3.4.3 Morphological and biochemical identification of *E. coli* isolates

On the basis of colony morphology, presumptive isolates of *E. coli* were characterized by Gram's staining, motility and biochemical tests like catalase test, oxidase test, IMViC according to method described by Agarwal *et al*. (2003).

3.4.4 Microscopic examinations

3.4.4.1 Gram's staining

The colonies showing typical green metallic sheen were selected for Gram's staining. Gram negative reaction with short rods was presumptively considered positive for *E. coli*.

3.4.4.2 Motility

The motility of bacteria was examined after incubation of culture in brain heart infusion broth by hanging drop method as described by Cruickshank *et al*. (1975).

3.4.5 Biochemical identification

Colonies showing characteristic morphology from solid media were picked up and tested for biochemical reactions as per the method described by Cruickshank *et al*. (1975) and Agarwal *et al*. (2003) with certain modifications as and when required.

3.4.5.1 Catalase test

A drop of 3% (v/v) hydrogen peroxide was taken on a clean, grease free glass slide. The suspected colony was picked up and added with the help of inoculation loop. A positive test was indicated by appearance of gas bubbles within 30 seconds.

3.4.5.2 Oxidase test

In this, colony of isolates smeared on the oxidase disc. The positive reaction was indicated by the development of violet colour within 30 seconds.

3.4.5.3 Indole test

In this test, the microorganism was incubated in tryptone broth (Hi - media) at 37°C for 24 hrs followed by addition of Kovac's reagent (Hi - media, Appendix). Appearance of red coloured ring was taken as positive.

3.4.5.4 Methyl red (MR) test

Presumptive isolates were inoculated in tubes containing 5 ml of glucose phosphate broth (Hi - media). After 48 hrs incubation at 37°C, 5 drops of MR solution was added. Appearance of red colour immediately was taken as positive for MR test. In case of negative result (yellowish - orange colour), incubation of the broth was continued for an additional three days and retesting of the broth culture was done.

3.4.5.5 Voges - Proskauer (VP) test

In this test glucose phosphate broth (Hi - media) was used to test presumptive isolates after 72 hrs incubation at 37°C Barritt's reagent, solution A and B (Hi - media) was added. Appearance of pink burgundy colour was taken as positive for VP test and in case of negative reaction copper / yellow colour was observed.

3.4.5.6 Citrate utilization test

For citrate utilization, commercially available Simmon's citrate medium (Hi - media) was used. Slants were prepared and cultures to be tested were inoculated and incubated at 37°C for 24 hrs. Change in colour from green to blue was taken as positive while no colour change was taken as negative for citrate utilization.

Table 07: Biochemical tests for identification of *E. coli*

S.No.	Biochemical test	Reaction
1.	Catalase	+
2.	Oxidase	-
3.	Indole production	+
4.	MR test	+
5.	VP test	-
6.	Citrate utilization	-

3.5 Molecular characterization of *E. coli* isolates

The molecular characterization of *E. coli* isolates was done by polymerase chain reaction. The standard PCR protocol was employed to amplify the strain specific gene of *E. coli*. Primer used to detect virulent gene of *E. coli* is listed in table 08.

Table 08: Details of primer used for PCR reaction

Pathogen	Gene	Primer	Reference
E. coli	*usp*A (F)	CCGATACGCTGCCAATCAGT	Osek (2001)
	*usp*A (R)	ACGCAGACCGTAAGGGCCAGAT	

3.5.1 Preparation of DNA template

Template DNA incorporated in PCR reactions were prepared by boiling and snap chilling method. Overnight grown brain heart infusion (BHI, Hi-Media) broth cultures measuring 1.5 ml were taken into sterilized microcentrifuge tubes. The tubes were centrifuged at 10,000 rpm for 5 min. The supernatant was removed and the pellet remained as sediment was washed twice with sterile distilled water and after final washing the crude lysate was resuspended in 1 ml sterile distilled water. Further, tubes were incubated in boiling water bath for 20 min followed by immediate chilling on crushed ice for at least 20 min. Finally, tubes were centrifuged at 10,000 rpm for 2 min and 5 µl of clear supernatant was used as template DNA in PCR assay (Sambrook and Russel, 2001).

3.5.2 Standardization of PCR protocol

PCR reaction for amplification of *usp*A gene was setup in 25 µl of reaction volume. The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture used in the PCR assay, by adjusting the annealing temperatures and cycling conditions as per need.

3.5.3 Amplification of strain specific genes of *E. coli*

To investigate the virulence potential of *E. coli*, isolates were subjected to PCR methodology as described by Osek (2001), with necessary modifications. In the present study, PCR was optimized with individual primer pair targeting the gene.

Following initial optimization trials, reaction mixture was standardized in 25 µl volume containing 5 µl of purified DNA template, 2.5 µl of 10X Taq DNA polymerase buffer (20 mM Tris - HCl, pH 8.0, 1mM DTT, 0.1 Mm EDTA, 100 mM KCl, 0.5% Nonidet P40, 0.5% Tween 20 and 50% glycerol and 20 mM $MgCl_2$), 0.2 mM dNTP mix, 10 pmol of each forward and reverse primer, 1 unit Taq DNA polymerase and volume make up was done by autoclaved milli Q water. The reaction mixture was properly mixed and the amplification cycles were carried out in thermocycler (Bio - Rad) with preheated lid (105°C).

The standardized amplification reaction started with initial denaturation at 94°C for 5 min, followed by 35 cycles each having denaturation at 94°C for 1 min, annealing at 52°C for *usp*A gene and extension at 72°C for 1 min, with final extension for 10 min at 72°C. On completion of PCR, amplified products were analyzed by agarose gel electrophoresis.

3.5.4 Analysis of PCR products

To analyze amplified products agarose gel electrophoresis was used. Agarose gel was dissolved in 1X TBE buffer by boiling and after brief cooling ethidium bromide (10 mg / ml) was added at the rate of 0.5 µg / ml. The gel casting tray, with its open ends sealed with adhesive tape was placed on a plane surface and then 12 well gel comb was placed properly. The molten agarose was poured into the tray and left undisturbed for gel formation for about 30 min. After solidification, gel comb was removed carefully and after removing adhesive tape, the tray with solidified gel was submerged in electrophoresis tank which was filled with electrophoresis buffer. The electrophoresis buffer should be at least 2 - 3 mm above the upper surface of gel, with orientation of wells towards cathode end.

PCR products were subjected to electrophoresis on 1% agarose gel in 1X TBE buffer at 70 V and 200 mA (5V / cm) for 1 hrs along with Gene ruler ^{TM} 100 bp DNA ladder plus (Hi - Media). For this 10 µl PCR product was mixed with 2 µl of 6X loading dye (Hi - Media) and was loaded into individual wells. Following agarose gel electrophoresis, PCR amplicons

were visualized as a single compact band of expected size under UV transillumination and documented by gel documentation system (Bio - Rad) and data were recorded photographically.

3.6 Antimicrobial sensitivity test

The *in-vitro* antibiogram of *E. coli* isolates against 16 different antibiotics was determined by disc diffusion method (CLSI, 2013) on Muller - Hinton agar (Hi - Media) with certain modifications. A loopful of the growth from each isolate was inoculated in brain heart infusion (BHI) broth and incubated at 37°C for 12 hrs. A sterile cotton swab was dipped into the broth culture and lawn was prepared on the surface of agar plate then plate was kept at room temperature for 30 min to allow the inoculums to be absorbed on the surface. Antibiotic sensitivity octadisc (Hi - Media) was placed with the help of sterile forceps on the agar. The plates were incubated overnight at 37°C. Antibiotics used in the present study are given in table 09. The results were interpreted according to the instructions of the manufacturer.

Table 09: Antibiotic disc, their concentration and interpretation

S. No.	Antibiotic disc used	Concentration (mcg)	Interpretation (mm)		
			R	I	S
1.	Tetracycline (TE)	25	11	12 – 14	15
2.	Ampicillin (AMP)	10	13	14 – 16	17
3.	Chloramphenicol (C)	30	12	13 – 17	18
4.	Amikacin (AK)	10	14	15 – 16	17
5.	Co - trimoxazole (COT)	25	10	11 – 15	16
6.	Kanamycin (K)	30	13	14 – 17	18
7.	Gentamycin (GEN)	30	12	13 – 14	15
8.	Streptomycin (S)	10	11	12 – 14	15
9.	Nitrofurantoin (NIT)	300	14	15 – 16	17
10.	Norfloxacin (NX)	10	12	13 – 16	17
11.	Netillin (NET)	30	12	13 – 14	15
12.	Ofloxacin (OF)	05	12	13 – 15	16
13.	Ceftazidime (CAZ)	30	17	18 – 20	21
14.	Ciprofloxacin (CIP)	05	15	16 – 20	21
15.	Cefotaxime (CTX)	30	22	23 – 25	26
16.	Nalidixic acid (NA)	30	13	14 – 18	19

R= Resistant, I=Intermediate and S=Sensitive

3.7 Concentrations of heavy metals in different water resources

Samples (200 ml) collected in bottles were stored in refrigerator at 4ºC till further analysis.

The materials like nitric acid (concentrated trace metal grade) and water (HPLC grade) were used throughout the course of experiment. Before use, glasswares were cleaned by rinsing once with deionized water and then once more with HPLC grade water. The stock solutions for Pb, As and Se were prepared in 1 liter volumetric flasks and labeled and then distributed into separate transfer beakers.

3.7.1 Sample analysis

Lead, arsenic and selenium concentrations were determined directly in the acidified filtrates by Atomic Absorption Spectrophotometer (Lab India AA8000). Around 15 to 20 ml sample was taken and measured by a detector to calculate the concentration of that element in the original sample as per standard method for the examination of water and waste water as described by Eaton *et al*., 2005.

3.7.2 Standardization of instrument

3.7.2.1 Standards

Lead: Lead standard solution (Merck, HC 43285076)

Arsenic: Arsenic standard solution (Merck, HC 42357873)

Selenium: Selenium standard solution (Merck, HC 398599)

3.7.2.2 Standardization

In all cases, standards and blanks were treated in the same way as the real samples to minimize matrix interferences during analysis. The instrument was warmed up and calibrated. Three different concentrations of standards were used to find out calibration curve.

Table 10: Operating condition of Atomic Absorption Spectrophotometer

Element	**Pb**	**As**	**Se**
Wavelength	283.3	193.62	195.81
Slit (nm)	0.7	0.7	0.7
Mode	AA	AA	AA
Flame	Air - Ac	Air – Ac	Air - Ac
Calibration equation	Lin. Cal. Int.	Lin. Cal. Int.	Lin. Cal. Int.
Sample volume (µl)	20	20	20
Lamp	EDL	EDL	EDL
Lamp current (mA)	440	440	440
Standards	01, 03, 05	02, 04, 06	01, 03, 05
Spiked concentration (µg/L)	25	25	25

3.7.3 Statistical analysis

Data gathered from the study were analyzed, using statistical methods of one way ANOVA to find out the level of significance in different sources (Snedecor and Cochran, 1994).

4. RESULT

4.1 Physical quality of water from different water resources

4.1.1 Physical quality of water by pH determination

Out of 117 water samples tested, 111 (94.87%) samples had pH in the range of 6.5 - 8.5, remaining 6 (5.12%) had pH >8.5. All the samples from different banks of river Narmada were in range of 6.5-8.5. Out of 35 samples of ponds, 32 (91.42%), 3 (8.58%) had pH in range of 6.5-8.5 and >8.5, respectively. Nineteen (90.47%) samples of hand pumps had pH range of 6.5-8.5 and 2 (9.52%) samples had >8.5. Among 21 samples of tube well, 20 (95.23%) had a range of 6.5-8.5 and 1 (4.76%) showed pH >8.5. All the 20 (100%) samples of public taps were in range of 6.5-8.5, as shown in table 11.

Table 11: pH of different water resources

S. No.	Samples tested	Number of samples	6.5 - 8.5		>8.5	
			Number of positive samples	Percentage of positive samples	Number of positive samples	Percentage of positive samples
1	Different banks of river Narmada	20	20	100.00	00	00.00
2	Ponds	35	32	91.42	03	08.58
3	Hand pumps	21	19	90.47	02	09.52
4	Tube wells	21	20	95.23	01	04.76
5	Public taps	20	20	100.00	00	00.00
	Total	**117**	**111**	**94.87**	**06**	**05.12**

4.1.2 Physical quality of water by total hardness determination

Out of 117 samples tested, none of the samples were soft or moderately hard, 72 (61.53%) were hard (3 - 6 mEQ/L) and 45 (38.46%) were very hard (>6 mEQ/L). All the samples from different banks of river Narmada 20 (100.00%) and ponds 35 (100.00%) were hard (3 - 6 mEQ/L). Two (9.52%) samples of hand pumps were hard and rest 19 (90.47%) samples were very hard. Among 21 samples of tube wells, 2 (9.52%) and 19 (90.47%) were hard and very hard, respectively. Thirteen (65%) samples of public taps were hard and remaining 7 (35%) were very hard, as depicted in table 12.

Table 12: Total hardness of different water sources

S. No.	Sample source	Number of samples	Soft (<1mEQ/L)		Moderately Hard (1 - 3 mEQ/L)		Hard (3 - 6 mEQ/L)		Very Hard (>6 mEQ/L)	
			Number of positive samples	Percentage of positive samples	Number of positive samples	Percentage of positive samples	Number of positive samples	Percentage of positive samples	Number of positive samples	Percentage of positive samples
1	Different banks of river Narmada	20	00	00.00	00	00.00	20	100.00	00	00.00
2	Ponds	20	00	00.00	00	00.00	35	10.00	00	00.00
3	Hand pumps	21	00	00.00	00	00.00	02	09.52	19	90.47
4	Tube wells	21	00	00.00	00	00.00	02	09.52	19	90.47
5	Public taps	20	00	00.00	00	00.00	13	65.00	07	35.00
	Total	**117**	**00**	**00.00**	**00**	**00.00**	**72**	**61.53**	**45**	**38.46**

4.2 Determination of Bacteriological quality of water from different water sources

4.2.1 Bacteriological quality of water by coliform count

Out of 117 samples tested, 40 (34.18%) were satisfactory result *i.e.,* most probable number (MPN) count between 1-3. Forty (34.18%) were suspicious and rest 37 (31.62%) were unsatisfactory as per recommendations given by CPCB (2011). Eleven (55%) samples from different banks of river Narmada were satisfactory, whereas 5 (25%) and 4 (20%) were suspicious and unsatisfactory, respectively. Five (14.28%), 14 (40%) and 16 (45.71%) samples of ponds were satisfactory, suspicious and unsatisfactory respectively. 10 (47.61%), 7 (33.33%) and 4 (19.04%) samples from hand pumps were suspicious and unsatisfactory, respectively. From all the 21 samples of tube well, 7 (33.33%) samples were satisfactory, 10 (47.61%) suspicious and 4 (19.04%) samples were unsatisfactory. In case of public tap water samples, 7 (35.00%) were satisfactory, 4 (20.00%) were suspicious and 9 (45.00%) samples were found to be unsatisfactory as shown in table 13.

4.2.2 Prevalence of *E. coli* from different water sources

The identification of *E. coli* was done by various morphological and biochemical tests as described in material and methods viz. Gram's staining, motility, catalase test, oxidase test and IMViC pattern.

Out of 117 samples examined, 18 isolates were obtained showing an overall prevalence of 15.38%. The highest prevalence of *E. coli* was observed in ponds water (34.28%) followed by water from different banks of river Narmada (15%), public taps (10%) and hand pumps (4.76%). *E. coli* wasn't obtained from tube wells as depicted in table 14.

Table 13: Categorization of different water resources by most probable number method

S. No.	Sample source	Grading (Coliform/ 100ml) Number of samples	I		II		III		IV	
			Excellent 01	Percentage	Satisfactory 01 – 03	Percentage	Suspicious 04 – 10	Percentage	Unsatisfactory >10	Percentage
1	Different banks of river Narmada	20	00	00.00	11	55.00	05	25.00	04	20.00
2	Ponds	35	00	00.00	05	14.28	14	40.00	16	45.71
3	Hand pumps	21	00	00.00	10	47.61	07	33.33	04	19.04
4	Tube wells	21	00	00.00	07	33.33	10	47.61	04	19.04
5	Public taps	20	00	00.00	07	35.00	04	20.00	09	45.00
	Total	**117**	**00**	**00.00**	**40**	**34.18**	**40**	**34.18**	**37**	**31.62**

Table 14: Prevalence of *E. coli* from different water sources (source wise)

S. No	Samples tested	Sample source	Number of samples	Number of samples positive	Prevalence of *E. coli*
1.	Different banks of river Narmada	Bheda ghat	07	00	03
		Gwari ghat	07	02	
		Tilwara ghat	02	00	
		Lamhaita ghat	03	01	
		Jilhari ghat	01	00	
		Hanuman tal	03	02	
2.	Ponds	Gulawwa tal	03	01	12
		Supa tal	03	01	
		Dev tal	03	00	
		Bhairav nagar tal	03	01	
		Mahanadda	03	00	
		Balasagar tal	03	01	
		Kal bhairav tal	03	02	
		Shani kund	03	00	
		Lal baba	03	01	
		Imrati tal	03	02	
		Prakash colony	01	01	
		Rani Durgawati fort	01	00	
		Gwari ghat	07	02	
		Tilwara ghat	02	00	
		Lamhaita ghat	03	01	
		Jilhari ghat	01	00	
3.	Hand pumps	Various places in Jabalpur	21	01	01
4.	Tube wells	Public place	05	00	00
		Hotels	07	00	
		Home	05	00	
		Hospital	01	00	
		College	03	00	
5.	Public Taps	Various places of Jabalpur	20	02	02
Total					**18**

Table 15: Prevalence of *E. coli* from different water sources

S. No.	Samples Type	Number of samples	Number of samples positive for *E. coli*	Percentage of prevalence of *E. coli*
1.	Different banks of river Narmada	20	03	15.00
2.	Ponds	35	12	34.28
3.	Hand pumps	21	01	04.76
4.	Tube wells	21	00	00.00
5.	Public taps	20	02	10.00
Total		**117**	**18**	**15.38**

4.3 Molecular characterization of *E. coli* isolates

PCR screening of all the 18 isolates revealed that 15 (83.33%) isolates were positive for *usp*A gene of *E. coli* as shown in table 16.

All the isolates obtained from different banks of river Narmada (03 / 03) and hand pumps (01 / 01) had *usp*A gene. Most of the isolates from ponds (10 / 12) were having *usp*A gene. One isolate from public taps revealed the *usp*A gene.

4.4 Antimicrobial sensitivity test of *E. coli* isolates

In the present study all the 18 isolates of *E .coli* were subjected to antibiogram assay against 16 commonly used antimicrobial agents. The overall antibiogram assay revealed that all the *E. coli* isolates were resistant to multiple drugs. Tetracycline (38.88%), ampicillin (83.33%), co - trimoxazole (44.44%), streptomycin (11.11%), nitrofurantoin (72.22%), netillin (5.55%), cefotaxime (72.22%) and nalidixic acid (5.55%) showed resistance for different isolates. Among 16 antibiotics tested, 9 different antibiotics were having intermediate resistance to various isolates including tetracycline (5.55%), ampicillin (16.66%), kanamycin (44.44%), streptomycin (44.44%), nitrofurantoin (22.22%), norfloxacin (5.55%), ceftazidime (33.33%), cefotaxime (11.11%) and nalidixic acid (5.55%). All the isolates were sensitive against chloramphenicol**,** ciprofloxacin, amikacin, gentamicin and ofloxacin while most of the isolates were sensitive to norfloxacin (94.44%), netillin (94.44%) and nalidixic acid (88.88%) as shown in table 16.

Table 16: Molecular characterization of *E. coli* isolates

S.No.	Isolates from various sources	Number of *E. coli* isolates	*E. coli* positive for *usp*A	
			Number of positive samples	Percentage of positive samples
1	Different banks of river Narmada	03	03	100.00
2	Ponds	12	10	83.33
3	Hand pumps	01	01	100.00
4	Tube wells	00	00	00.00
5	Public taps	02	01	50.00
Total		**18**	**15**	**83.33**

Table 17: Isolate wise molecular profile of *E. coli* isolates

S. No.	Isolates	*usp*A
1.	E1	+
2.	E2	+
3.	E3	+
4	E4	+
5.	E5	+
6.	E6	-
7.	E7	+
8.	E8	-
9.	E9	-
10.	E10	+
11.	E11	+
12.	E12	+
13.	E13	+
14.	E14	+
15.	E15	+
16.	E16	+
17.	E17	+
18.	E18	+

E= Isolates of *E. coli*

Table 18: Antimicrobial pattern of *E. coli* isolates (source wise)

S. No.	Antimicrobial agent	Pattern of antibiogram	Different banks of river Narmada	Ponds	Hand Pumps	Tube Wells	Public Taps
1.	Tetracycline	R	00	06	00	00	01
		I	00	00	00	00	01
		S	03	06	01	00	00
2.	Ampicillin	R	02	10	01	00	02
		I	01	02	00	00	00
		S	00	00	00	00	00
3.	Chloramphenicol	R	00	00	00	00	00
		I	00	00	00	00	00
		S	03	12	01	00	02
4.	Amikacin	R	00	00	00	00	00
		I	00	00	00	00	00
		S	03	12	01	00	02
5.	Co - trimoxazole	R	00	07	00	00	01
		I	00	00	00	00	00
		S	03	05	01	00	01
6.	Kanamycin	R	00	00	00	00	00
		I	00	05	01	00	02
		S	03	07	00	00	00
7.	Gentamicin	R	00	00	00	00	00
		I	00	00	00	00	00
		S	03	12	01	00	02
8.	Streptomycin	R	00	02	00	00	00
		I	01	04	01	00	02
		S	02	06	00	00	00
9.	Nitrofurantoin	R	02	08	01	00	02
		I	01	03	00	00	00
		S	00	01	00	00	00
10.	Norfloxacin	R	00	00	00	00	00
		I	00	00	01	00	00
		S	03	12	00	00	02
11	Netillin	R	01	00	00	00	00
		I	00	00	00	00	00
		S	02	12	01	00	02
12.	Ofloxacin	R	00	00	00	00	00
		I	00	00	00	00	00
		S	03	12	01	00	02
13.	Ceftazidime	R	00	00	00	00	00
		I	00	06	00	00	00
		S	03	06	01	00	02
14.	Ciprofloxacin	R	00	00	00	00	00
		I	00	00	00	00	00
		S	03	12	01	00	02
15.	Cefotaxime	R	02	10	00	00	01
		I	00	01	00	00	01
		S	01	01	01	00	00
16.	Nalidixic acid	R	00	01	00	00	00
		I	01	00	00	00	00
		S	02	11	01	00	02

Table 19: Antimicrobial pattern of *E. coli* isolates

S. No.	Antimicrobial agents	Pattern of antibiogram of *E. coli* isolates					
		Resistant	%	Intermediate	%	Sensitive	%
1.	Tetracycline	07	38.88	01	05.55	10	55.55
2.	Ampicillin	15	83.33	03	16.66	00	00.00
3.	Chloramphenicol	00	00.00	00	00.00	18	100.00
4.	Amikacin	00	00.00	00	00.00	18	100.00
5.	Co - trimoxazole	08	44.44	00	00.00	10	55.55
6.	Kanamycin	00	00.00	08	44.44	10	55.55
7.	Gentamicin	00	00.00	00	00.00	18	100.00
8.	Streptomycin	02	11.11	08	44.44	08	44.44
9.	Nitrofurantoin	13	72.22	04	22.22	01	05.55
10.	Norfloxacin	00	00.00	01	05.55	17	94.44
11.	Netillin	01	05.55	00	00.00	17	94.44
12.	Ofloxacin	00	00.00	00	00.00	18	100.00
13.	Ceftazidime	00	00.00	06	33.33	12	66.66
14.	Ciprofloxacin	00	00.00	00	00.00	18	100.00
15.	Cefotaxime	13	72.22	02	11.11	03	16.66
16.	Nalidixic acid	01	05.55	01	05.55	16	88.88

Table 20: Multiple drug resistance exhibited by different isolates

S. No.	Source	Number of antimicrobial agents / Number of isolates	02	03	04	05
1	Different banks of river Narmada	03	00	01	01	01
2	Ponds	12	04	03	03	02
3	Hand pumps	01	01	00	00	00
4	Tube wells	00	00	01	01	00
5	Public taps	02	00	00	00	00
	Total	**18**	**05**	**05**	**05**	**03**

4.5 Determination of heavy metals particularly lead, arsenic and selenium from different water resources

The concentrations of lead, arsenic and selenium were analyzed by Atomic Absorption Spectrophotometer in 117 water samples collected from various sources. The overall concentrations of lead, arsenic and selenium were ranging from 0.097 - 0.450 ppm, 0 - 0.345 ppm and 1.365 - 2.047 ppm, respectively. The concentration of lead in water samples from different banks of river Narmada, ponds, hand pumps, tube wells and public taps water was found in range of 0.097 - 0.354 ppm, 0.105 - 0.418 ppm, 0.101 - 0.450 ppm, 0.105 - 0.418 ppm and 0.109 - 0.450 ppm, respectively. The concentration of arsenic in water samples from different banks of river Narmada, ponds, hand pumps, tube wells and public taps was found in range of 0 - 0.240 ppm, 0 - 0.101 ppm, 0 - 0.152 ppm, 0 - 0.110 ppm and 0 - 0.345 ppm, respectively and the concentration of selenium in water samples from different banks of river Narmada, ponds, hand pumps, tube wells and public taps was found in range of 1.365 - 2.137 ppm, 1.206 - 1.950 ppm, 1.450 - 2.047 ppm, 1.587 - 1.915 ppm and 1.541 - 1.990 ppm, respectively, as shown in table 21.

Table 21: Concentrations of heavy metals in different water resources

S.No.	Source	Pb	As	Se
1	Different banks of river Narmada	0.097 - 0.354	0 - 0.240	1.365 - 2.137
2	Ponds	0.105 - 0.418	0 - 0.101	1.206 - 1.950
3	Hand pumps	0.101 - 0.450	0 - 0.152	1.450 - 2.047
4	Tube wells	0.105 - 0.418	0 - 0.110	1.587 - 1.915
5	Public taps	0.109 - 0.450	0 - 0.345	1.541 - 1.990
Total		**0.097 - 0.450**	**0 - 0.345**	**1.365 - 2.047**

Table 22: Concentrations of heavy metals in different water resources (statistical analysis)

S. No.	Source	Pb	As	Se
1.	Different banks of river Narmada	0.03±0.01	0.01±0.02	1.80^{a} ±0.04
2.	Ponds	0.01±0.00	0.01±0.02	1.69^{b}±0.03
3.	Hand pumps	0.01±0.01	0.01±0.02	1.81^{a}±0.03
4.	Tube wells	0.01±0.01	0.01±0.02	1.77^{ab}±0.02
5.	Public taps	0.02±0.02	0.01±0.02	1.79^{a}±0.03

Values with different superscript shows significant difference (P<0.05)

Table 23: Concentrations of lead in different ponds after religious activity

S. No.	Source	Pb concentration (ppm)	
		Pre Ganesha Festival	Post Ganesha Festival
1	Hanuman tal	0.023	0.322
2	Gulawwa tal	0.257	0.354
3	Dev tal	0.193	0.289
4	Mahanadda tal	0.105	0.249
5	Balasagar tal	0.322	0.418
6	Shani kund	0.129	0.418
7	Lal baba tal	0.322	0.386
8	Imrati tal	0.193	0.418

5. DISCUSSION

Water is one of the prime elements responsible for life on earth. It is an excellent solvent and helps in osmoregulation and thermoregulation functions of body. It is an important constituent of various hormones, enzymes, vitamins, etc. It contributes in progress, heath, recreation and in development of civilization by having important role in agriculture and industries beside in biological system. But unplanned urbanization, industrialization, population explosion, etc had created a lot of pressure on this essential element of life. Due to various anthropogenic activities, water bodies gets contaminated by various microorganism (viz. pathogenic bacteria, viruses and parasites, etc), wide spectrum of chemicals like heavy metals, pesticides, toxins, drug residues, antibiotics. Physical changes such as elevated temperature, discoloration, change in pH and hardness may also make water unfit for life process as well as for other domestic uses. Therefore, there is need for regular monitoring for physico - chemical and biological parameters before it is used for drinking, domestic, agricultural or industrial purposes. So, the present study was done to observe the water quality in Jabalpur.

5.1 Determination of physical quality of water

5.1.1 Physical quality of water by pH determination

Out of 117 water samples analyzed, 111 (94.87%) samples had pH between 6.5 - 8.5 and 6 (5.12%) samples had pH >8.5. All the samples from different banks of river Narmada were in the pH range of 6.5 - 8.5 but 3 (8.58%) samples from ponds, 2 (9.52%) from hand pumps, 1 (4.76%) from tube wells and 10 (50.00%) samples from public taps had pH >8.5. Similar results for pH are also reported by Rokade and Ganeshwade (2005) where they didn't get any sample exceeding pH >8.5. pH of water may vary according to temperature, composition of water and material used for manufacturing distribution system in public supply. Health based guideline for pH proposed by WHO (2011) is between 6.5 - 8.5 with no relaxation. The optimum pH is necessary for all stages of water treatment *i.e.*, clarification

and disinfection. For chlorination, pH should be less than 8.0. Further, if pH of water is not optimum, it may leads to corrosion of pipes in house hold as well as industrial supply, so this may alters taste, odour and appearance of water. The lower pH of water may also cause gastric disorder like acidity in humans and animals.

5.2 Physical quality of water by total hardness determination

Among 117 water samples from different sources, 45 (38.46%) samples had total hardness >6 mEQ/L *i.e.,* very hard, 72 (61.53%) were between 3 - 6 mEQ/L *i.e.*, hard. No water sample was found soft and moderately hard. Source wise, all the samples from different banks of river Narmada and ponds were hard, while water samples from hand pumps and tube wells were very hard. Among 20 public tap water samples 13 (65.00%) and 7 (35.00%) were hard and very hard, respectively. Ramya *et* al. (2015) estimated total hardness of ground water from a town and two different villages of Andhra Pradesh and revealed that out of 120 samples tested, 39 (32.50%) samples were moderately hard, 76 (63.33%) samples were hard water and 5 (4.16%) samples had very hard water. In our study, comparatively higher percentage (38.46%) of very hard water was observed and it may be attributed to ground water resources like hand pumps and tube wells, which were contributing 84.44%. The results revealed that surface water resources (different banks of river Narmada and ponds) were having hard water. The hardness of water is also depending upon geological formation of crust because the minerals in soil are getting dissolved in water during flow of stream, percolation, etc.

Although most of the people have a misconcept that hard water is harmful for health but some degree of hardness is needed for the taste of drinking water as demineralization of water may give it a flat taste. Hard water can be a source of calcium and magnesium supplementation in the diet and is important for the group with marginal intake of calcium and magnesium and in the person with low intake of dairy products (main sources of dietary calcium and magnesium). Recommended dietary intakes for calcium and magnesium are about 1000 mg and 200 – 400 mg / per day, respectively and taste threshold for the calcium ion is in the range 100 - 300 mg / l per day. Various

studies revealed that dietary calcium reduces the incidence of kidney stones while increased risk of kidney stones is associated with calcium supplements when ingested as a bolus and not with food or the supplements were taken by those who exceeded the upper intake level of 2500 mg / day. Similarly adequate calcium intake has been associated with lowered risk of elevated blood pressure and a negative association (*i.e.,* protective effect) between cardiovascular mortality.

Hard water can pose serious problems in industrial settings because it may leads to breakdowns of costly boilers, cooling towers, and other equipment. In domestic use also hard water have low leather formation ability when soap is agitated in water. It may form lime scale in kettles and water heaters.

The acceptable limit given by BIS (2009) is 4 mEQ/L and permissible limit in the absence of alternate source is 12 mEQ/L. In this study out of 117 samples 37 (31.62%), 57 (48.71%) and 23 (19.65%) had total hardness <4 mEQ/L, 4 - 12 mEQ/L and >12 mEQ/L, respectively. 23.80% samples from hand pumps, 66.66% of tube wells and 20.0% of public taps had total hardness >12 mEQ/L.

Very hard water (>6 mEQ/L) may be harmful for health. It may aggravate eczema. Hard water may consume more soap and results in soap salt residues on the skin and on clothes which are not easily rinsed off and lead to contact irritation (Thomas & Sach, 2000). Excessively hard water can also have corrosion tendencies which can be associated with health risks due to leaching effect on lead, copper and other metals. It may also reduce lifespan of the distribution pipes and system (WHO, 2011).

5.2 Determination of bacteriological quality of water

5.2.1 Bacteriological quality of water by coliform count

Total coliform count is used to assess contamination level of drinking and swimming waters with fecal and sewage material. It also indicates presence of intestinal origin pathogens. Enumeration of coliforms as a water quality monitoring method involves inoculating a series of tubes containing MacConkey lactose broth with appropriate decimal dilutions; coliform bacteria present in the water sample multiply and are detected by

formation of acid and gas. The present study was designed to detect the coliforms bacteria in water samples and to determine the water supply system being operated correctly and safe water for drinking or food preparation. During present study, the coliform bacteria have been found in all kinds of samples tested. Out of 117 samples tested, 40 (34.18%) showed satisfactory result *i.e.,* MPN count <3, 38 (32.47%) were suspicious and rest 39 (33.33%) were unsatisfactory as per recommendations given by CPCB (2011). As per FAO, recommended MPN values for drinking water is 2 / 100 ml and permissible limit for drinking water by WHO and BIS is 10 / 100 ml. The present study displayed range of MPN index from <3 to >2400. Similar study have conducted by Shafi *et al.* (2013) to assess water quality of Manasbal Lake of Kashmir and also got variable range between 4 and 460 MPN / 100 ml. None of the sample was found to be fit for drinking purpose and 5.00% samples were unfit for even domestic and recreational use also. Our results also showed similarity, wherein, 7 (5.98%) samples were found unfit for bathing and swimming, 4 (57.14%) of which are from ponds. Tambekar *et al.* (2008) examined 260 samples and recorded 99.00% samples contaminated with coliform (by MPN technique) of which 75 contains *E. coli.* In a study conducted in Nigeria by Omezuruike *et al.* (2008), the microbiological and physicochemical status of different water samples used for domestic purposes was assessed. They got MPN count from 9.3 to 44 MPN / 100 ml. All the water samples were found to harbor coliforms organisms in numbers greater than the required WHO / FAO standards for water. Bacteriological analysis of drinking water in western Uttar Pradesh, by Kumar and Kumar (2013) revealed that MPN was very high (≥ 180) in 58 (50.00%), 32 (28.00%) and 26 (22.00%) of municipal tap water, government hand pumps and water cooler, respectively. Presence of coliform in all the category indicates that consumption of such type of water may lead to different types of diseases especially of intestinal pathogens. Coliform presence in surface water (river and ponds) indicates contamination of water resources by surface runoff, direct disposal of untreated domestic and municipal wastes, sewage and animal excreta. The water contamination from hand pumps, tube wells and public taps indicates cross contamination of water distribution lines and system with nearby sewer line.

5.2.2 Prevalence of *E. coli* in different water samples and molecular characterization of isolates

In this study, all samples showed positive reaction in MPN count test were further subjected for selective plating on EMB. Isolates showing characteristic colonies with green metallic sheen were characterized on the basis of Gram's staining and IMViC pattern. Of the total samples examined, 18 *E. coli* isolates were procured with an overall prevalence of 15.38%. Category wise prevalence in case of different banks of river Narmada - 15.00% (3 / 20), ponds - 34.28% (12 / 35), hand pumps - 4.76% (1 / 21) and in public taps - 10.00% (2 / 20). None of the water samples from tube well had *E. coli* which may be due to water coming from deeper stratum of earth that provides some degree of filtration. Isolates were further subjected for molecular identification using universal stress protein (*usp*A) gene which is a marker to differentiate pathogenic *E. coli* from other Gram negative enterobacteria (Nachin *et al.*, 2005). *usp*A gene was detected in 15 out of 18 isolates *i.e.,* 83.33%, so our study indicates better association between this gene and isolates than Pandey *et al*. (2015) wherein he reported only 31.48% association, Rani (2016) reported 100.00% association.

Similar results with prevalence ranging from 11.82% to 23.64 % were also reported by Mupidwar *et al.* (2015) in their study conducted in Salburdi river and Kamptee river Maharashtra. One study conducted by Rani (2016) in Narmada river revealed 10.0% prevalence of faecal coliform organism which is in accordance to our findings. Nontongana *et al.* (2014) have also reported various *E. coli* pathotypes from the Kat river in South Africa.

Borah *et al.* (2010) have found over 78.00% of tested ponds samples contaminated with *E. coli a*nd Gogoi and Sharma (2013) also reported three ponds of Dibrugarh district having faecal coliforms *i.e.*, *E. coli*. Our study disclosed that ponds water was contaminated with coliform organism although with lower percentage comparatively. Improperly treated septic - sewage discharges, animal manure, storm water runoff washed into rivers or ponds may be responsible for this.

Hand pumps and tube wells are generally used to access to shallow / deep groundwater and considered microbiologically safe but in many cases they may contain significant levels of faecal indicator bacteria such as faecal coliforms and *E. coli*. Islam *et al*. (2001), Leber *et al*. (2011), Van Geen *et al*. (2011), Ferguson *et al.* (2011) reported in their study that 41.00%, tube well water samples were contaminated with total coliforms and 13.00% with *E. coli* . In our study 4.76% prevalence of *E. coli* was noticed in case of water samples from hand pumps but none of the water samples of tube well was found contaminated with *E. coli* organism. Coliform bacteria are found in shallow wells compared to deeper wells because bacteria are naturally filtered out by soil and rock as is noticed in tube well because surface water infiltrates into the ground. Though sometimes, deeper wells / tube wells may also contain coliform bacteria, if surface water get entry into the well or if they are improperly constructed or poorly maintained. The contamination may be due to improperly treated septic and sewage discharges or leaching of animal manure.

Presence of *E. coli* in public tap water indicates contamination either through faulty distribution system or it indicates improper / insufficient treatment of drinking water. As such presence of coliform bacteria in drinking water does not always cause illness because most of these bacteria are harmless to humans. *E. coli* is the only member of the total coliform group of bacteria that is found only in the intestines of mammals, including humans, so total coliforms and *E. coli* are used as indicators to measure the degree of pollution and sanitary quality of drinking water. The presence of *E. coli* in water indicates recent fecal contamination and may indicate the possible presence of disease causing pathogens, such as bacteria, viruses, parasites, etc (Park, 2011).

5.2.3 Antibiogram assay of *E coli* isolates

Various antibiotics are used in prevention and control bacterial infections. Now these days, antibiotic resistance is one of the biggest threats to global health, food security and development today. Such pathogens are specially creating risk in immunocompromised host, children, elderly patients, etc. Antibiotic resistance leads to longer

hospital stays, higher medical costs and increased mortality. They can cause severe and often deadly infections such as bloodstream infections and pneumonia. (WHO, 2011) Some degree of antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. The most critical group is multidrug resistant bacteria as these bacteria show resistant to more than one antibiotic.

In the present study all the 18 isolates of *E. coli* were subjected to antibiogram assay against 16 commonly used antimicrobial agents. The overall antibiogram assay revealed that all the *E. coli* isolates were resistant to multiple drugs. Tetracycline (38.88%), ampicillin (83.33%), co - trimoxazole (44.44%), streptomycin (11.11%), nitrofurantoin (72.22%), netillin (5.55%), cefotaxime (72.22%) and nalidixic acid (5.55%) showed resistance for different isolates. Among 16 antibiotics tested, 9 different antibiotics were having intermediate resistance to various isolates including tetracycline (5.55%), ampicillin (16.66%), kanamycin (44.44%), streptomycin (44.44%), nitrofurantoin (22.22%), norfloxacin (5.55%), ceftazidime (33.33%), cefotaxime (11.11%) and nalidixic acid (5.55%). All the isolates were sensitive against chloramphenicol**,** ciprofloxacin, amikacin, gentamicin and ofloxacin while most of the isolates were sensitive to norfloxacin (94.44%), netillin (94.44%) and nalidixic acid (88.88%). Scaria *et al*. (2010) have also found all the strains were susceptible to amikacin, ciprofloxacin and nalidixic acid. Antibiotic resistance pattern of *E. coli* was also studied by various workers like Hassan and Elmalt (2008), Roopnarine *et al*. (2009), Molaabaszadeh *et al*. (2013), etc examined the antibiotic susceptibility and resistance pattern of *E. c*oli isolates against 10 different antibiotics. The results showed the highest sensitivity for ciprofloxacin (100.00%), ceftizoxim (80.85%), and ceftriaxone (74.47%) and the most resistance antibiotics were amoxicillin (95.74%), oxacilin (82.98%), kanamycin (61.70%).

Similar results were also observed by Mujeeb *et al*. (2017) where they examined antimicrobial susceptibility of 89.68% *E. coli* isolates were sensitive to ceftizoxim followed by 64.85% susceptible to ceftriaxon. The antibiotic resistance is mainly mediated by R conjugate plasmid particularly in Gram negative bacteria. Besides this, it is also attributed to indiscriminate use of antibiotics.

5.3 Presence of heavy metals in water samples particularly lead, arsenic and selenium

Heavy metals are naturally occurring elements with high atomic weight and density at least 5 times greater than that of water. They are dangerous because of bioaccumulation tendency. Various heavy metals like lead (Pb), arsenic (As), selenium (Se), mercury (Hg), chromium (Cr) specially hexavalent chromium, nickel (Ni), barium (Ba), cadmium (Cd), cobalt (Co), vanadium (V), etc are having wide spread application viz. industrial, domestic, agricultural, medical, technological, etc. This may be reason for their distribution in the environment from where they can enter into various water resources as industrial and consumer waste. When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues (Trueby, 2003) and when animals graze on such contaminated plants and drink from polluted waters, waters also accumulate such metals in their tissues / meat and milk, if lactating (Verma and Dwivedi, 2013). Marine lives that breed in heavy metal polluted water also become contaminated. Human being top consumer in ecosystem is more prone to heavy metal toxicity. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency.

In present study, all the water samples analyzed had lead, arsenic and selenium ranging from 0.097 - 0.450 ppm, 0 - 0.345 ppm and 1.365 - 2.047 ppm, respectively. From various water sources, the concentration of lead in different banks of river Narmada, ponds, hand pumps, tube wells and public tap water was found in range of 0.097 - 0.354 ppm, 0.105 - 0.418 ppm, 0.101 - 0.450 ppm, 0.105 - 0.418 ppm and 0.109 - 0.450

ppm, respectively. While the concentration of arsenic in water from different banks of river Narmada, ponds, hand pumps, tube wells and public tap water was found in range of 0 - 0.240 ppm, 0 - 0.101 ppm, 0 - 0.152 ppm, 0 - 0.110 ppm and 0 - 0.345 ppm, respectively.

The concentration of selenium in different banks of river Narmada, ponds, hand pumps, tube wells and public tap water was found in range of 1.365 - 2.137 ppm, 1.206 - 1.950 ppm, 1.450 - 2.047 ppm, 1.587 - 1.915 ppm and 1.541 - 1.990 ppm, respectively. All the samples analyzed had lead and selenium concentration significantly higher than the maximum permissible limits (0.01 ppm with no relaxation given by WHO, 2011). In arsenic, it was more than maximum permissible limit (0.01 ppm given by WHO) in 16 (13.67%) samples and higher than acceptable limit in 10 (8.54%) samples, at least one from each source. Statistically source wise, there was no significant variation in case of lead and arsenic but concentration of selenium was significantly high in different banks of river Narmada, hand pumps, and public tap waters in comparison to ponds and tube wells.

These heavy metals may be neurotoxic, carcinogenic, mutagenic or teratogenic. Rate of absorption and effect is also influenced by factors such as age and physiological status. Contamination with high levels of these heavy metals is of concern because they may cause a number of human health effects. Generalized signs associated with lead, arsenic, mercury, zinc, copper and aluminium poisoning are gastrointestinal (GI) disorders, diarrhea, ataxia, paralysis, vomiting and convulsion, depression, (McCluggage, 1991). Effects may be acute, chronic or sub - chronic depending upon the dose, duration and route of exposure.

In the human body, the greatest percentage of lead is taken into the kidney, then liver, heart, brain and skeleton. Adults absorb 35 to 50% of lead through drinking water and the absorption rate for children may be greater than 50.00%. The nervous system is the most vulnerable target of lead poisoning. Headache, poor attention spam, irritability, loss of memory and dullness are the early symptoms of the effects of lead exposure on the central nervous system. Exposure to lead is of special concern among women

particularly during pregnancy. Lead absorbed by the pregnant mother is readily transferred to the developing fetus and leads to reduced birth weight and preterm delivery, and with neuro - developmental abnormalities in offspring in animals also (Tchounwou *et al.,* 2014).

Similarly arsenic may leads to cardio - vascular diseases, teratogensis, neurologic and neuro - behavioural disorders, diabetes, hearing loss, portal fibrosis, hematologic disorders (anemia, leukopenia and eosinophilia) and carcinoma. Arsenic exposure affects virtually all organ systems including the cardiovascular, dermatologic, nervous, hepatobilliary, renal, gastrointestinal, and respiratory systems and cancers of the bladder, kidney, skin, and liver in many areas of arsenic pollution (NRC, 1974).

Selenium is an essential trace mineral and small amount of selenium is good for our health by playing important role in antioxidant enzymes. It also prevent certain cancers and cardiovascular. Lack of selenium may cause skeletal and cardiac myopathies, encephalopathies, transudative diathesis and infertility (Diplock, 1976; NRC, 1983), in animals white muscle disease in cattle. Thus, poisoning with regards to selenium is more complex and toxicity of selenium depends on whether it is in the biologically active oxidized form, which occurs in alkaline soils. Alkaline and oxidizing conditions favour the formation of selenates. Because selenites and selenates are soluble in water, selenium in alkaline soils is available for uptake by plants, (NRC, 1983).These conditions can leads to increased uptake of metal by plants. The syndromes “blind staggers” and “alkali disease” have been described in livestock consuming selenium accumulator plants (Shamberger, 1983). It is known that selenium accumulates in living tissues and is bioaccumulated in aquatic habitats, resulting in higher concentrations in organisms than the surrounding water. A water concentration of 2 µg / l be considered highly hazardous to sensitive fish and aquatic birds. Selenium poisoning can be passed from parents to offspring through the egg, and selenium poisoning may persist for many generations. Physiological changes may occur in fish with high tissue concentrations of selenium. Fish affected by selenium may experience swelling of the gill lamellae, which impedes oxygen diffusion across the gills and blood flow within the gills.

Respiratory capacity is further reduced due to selenium binding to hemoglobin. Other problems include degeneration of liver tissue, swelling around the heart, damaged egg follicles in ovaries, cataracts, and accumulation of fluid in the body cavity and head. Selenium often causes a malformed fish fetus which may have problems in feeding or respiration, distortion of the fins or spine is also common. Adult fish may appear healthy despite their inability to produce viable offspring. Adverse effects may results from exposure to excessive levels of selenium, selenite, selenate, selenocysteine and selenomethionine are highly toxic and kill laboratory animals in single doses of 1.5–6 mg / kg of body weight (Högberg & Alexander, 1986, IPCS, 1987). Selenate, selenite, selenocysteine and selenomethionine are each teratogenic in avian species (Hoffman *et al.,* 1988) and fish (Birge *et al.,* 1983). Teratogenicity has also been observed in sheep and pigs (Wahlström and Olson, 1959). In human selenium excess may cause gastrointestinal disturbances, discoloration of the skin and nail, loss of hair, dermatitis and decayed teeth fatigue, depression and loss of feeling in the arms and legs (Smith and Westfall, 1937). Ingestion of extremely high levels of selenium compounds can be fatal. Dermal contact with selenium compounds mainly occurs in the occupational setting and can cause rashes, swelling, redness and pain. Contact with the eyes can cause irritation and burning. Long term expose may leads to growth retardation, apparently caused by reduced secretion of growth hormone from the anterior pituitary gland as a result of local selenium accumulation (Thorlacius, 1990).

Similar study have been conducted by Singh *et al.* (2013). They studied the concentration of lead in Narmada river, ponds and other water resources in Jabalpur) by using Atomic Absorption Spectrophotometer. Out of 16 target areas (192 samples), 5 were found below detection limit, whereas remaining all 11 target were showed concentration more than maximum permissible limit stated by WHO (2011). Singh and Chandel (2006) did the analytical study of heavy metals of industrial effluents in Jaipur, Rajasthan. The results exhibited that As, Cd, Cr and Pb were not found in any studied wastewater samples, while some of the following heavy metals ranged from : Cu (0.0 - 1.0 mg/L), Fe (0.1 - 0.4 mg/L), Mn (0.0 - 0.4 mg/L), Ni

(0.01 - 0.07 mg/L) and Zn (0.68 - 60.84 mg/L). Cu, Fe, Mn and Zn were found above the standard limit recommended by IS: 3307 (1977). However, Ni was found below the regulated safety values for all studied samples. Khan *et al*. (2014) detected heavy metal traces Arsenic and lead were reported below recommended values. Dixit and Tiwari (2007) estimated the level of water pollution in the Shahpura lake of Bhopal and found that the concentration of the heavy metals in the lake water substantially increased after the religious activities like idol immersion around August and September. Our study also stated similar findings wherein, the concentration of Pb in ponds has been increased after Ganesha festival.

Kar *et al.* (2007) surface water samples (96) collected from river Ganga in West Bengal during 2004 - 05. The heavy metals in the surface water of river Ganga followed the sequence: Fe >Mn >Ni >Cr >Pb >Zn >Cu > Cd. The presence of heavy metals above permissible limit in water in different parts of our country is of great concern because it directly affects the health of animals, humans and water life.

The results of present study indicated wide spread pollution of different water sources in Jabalpur with various physical, chemical and biological contaminants. This is an alarming situation regarding environment, public and animal health. Therefore, there is an immediate need for preventive and corrective measures to preserve wholesome water.

6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

Water is one of the important elements responsible for life on earth. It plays a significant role in the sound health of human and is essential for plant and animal life. It is an excellent solvent and helps in osmoregulation and thermoregulation function of body. Due to human activity, water is getting contaminated by various microorganisms (viz. bacteria, viruses and parasites etc), wide spectrum of chemicals like heavy metals, pesticides, toxins, drug residues, antibiotics. In developing countries due lack of recording and surveillance system, there is no data available regarding quality of water to be used for drinking and domestic as well as industrial use. So, the present study was done to observe the physical, chemical and bacteriological quality of water in Jabalpur on the basis of most probable number count, isolation and characterization of *E. coli* isolates and the presence of heavy metals particularly lead, arsenic and selenium.

During the present study, a total of 117 samples comprising of 20 samples from different banks of river Narmada, 35 from ponds, 21 from hand pumps, 21 of tube wells, and 20 from public taps water samples were collected from different areas of Jabalpur. The samples were analyzed for the coliform count by most probable number method in MacConkey lactose broth and presence of *E. coli* by using selective / differential plating on EMB. Of the samples tested, 40 (34.18%) showed satisfactory result *i.e.*, most probable number (MPN) count between1-3; 40 (34.18%) were suspicious and 37 (31.62%) were unsatisfactory as per recommendations given by CPCB (2011). Samples from different banks of river Narmada (55.00%), ponds (14.28%), hand pump (47.61%), tube well (33.33%) and public tap water (35.00%) were satisfactory. The overall prevalence of *E. coli* was 15.38% with highest in pond water (34.28%), followed by water from different banks of river Narmada (15.00%), public taps (10.00%) and hand pumps (4.76%). *E. coli* wasn't obtained from tube wells. The molecular characterization of isolates revealed 83.33% samples were positive for *usp*A gene by polymerase chain

reaction. The antimicrobial susceptibility by disc diffusion technique displayed that all the 18 isolates were resistant to multiple drugs. The highest resistance was observed in ampicillin followed by nitrofurantoin and cefotaxime, co - trimoxazole, tetracycline, streptomycin, netillin and nalidixic acid. The field survey of target areas for heavy metal in water bodies in Jabalpur revealed the overall concentrations of lead, arsenic and selenium were ranging from 0.097 - 0.450 ppm, 0 - 0.345 ppm and 1.365 - 2.047 ppm, respectively. The concentration of lead in water samples from different banks of river Narmada, ponds, hand pumps, tube wells and public tap water was found in range of 0.097 - 0.354 ppm, 0.105 - 0.418 ppm, 0.101 - 0.450 ppm, 0.105 - 0.418 ppm and 0.109 - 0.450 ppm, respectively. The concentration of arsenic in water samples from different banks of river Narmada, ponds, hand pumps, tube wells and public taps was found in range of 0 - 0.240 ppm, 0 - 0.101 ppm, 0 - 0.152 ppm, 0 - 0.110 ppm and 0 - 0.345 ppm, respectively and the concentration of selenium in water samples from different banks of river Narmada, ponds, hand pumps, tube wells and public tap water was found in range of 1.365 - 2.137 ppm, 1.206 - 1.950 ppm, 1.450 - 2.047 ppm, 1.587 - 1.915 ppm and 1.541 - 1.990 ppm, respectively.

The overall result indicated that the different sources of water in Jabalpur are contaminated with coliform bacteria particularly *E. coli*. This shows that the water is polluted with sewage or faecal material. Further, the study showed higher concentration (above recommended level) of lead, arsenic and selenium in water in Jabalpur city. Therefore, it is suggested that the water should be consumed after proper treatment to prevent from various water borne diseases.

6.2 Conclusions

- Physical examination of water revealed that most of the samples (94.87%) had pH between 6.5 - 8.5 and hardness between 3 -6 mEQ/L (61.53%) viz. hard.
- The overall prevalence of *E. coli* was observed 15.38% with sample wise prevalence of 00.00%, 4.76%, 10.00%, 15.00%, 34.00%, and 28.00% in tube wells, hand pumps, public taps, river Narmada and ponds, respectively.
- Characterization of *E. coli* with PCR revealed the presence of 83.33% *uspA* genes.
- Overall antibiogram profile of isolates exhibited sensitivity to tetracycline, chloramphenicol, amikacin, co - trimoxazole, kanamycin, gentamicin, streptomycin, nitrofurantoin, norfloxacin, netillin, ofloxacin, ceftazidime, cefotaxime and nalidixic acid. Antimicrobial resistance was found against ampicillin, nitrofurantoin, netillin, cefotaxime, nalidixic acid, tetracycline, co - trimoxazole and streptomycin.
- All the samples analyzed had lead and selenium concentration significantly higher than the maximum permissible limit while, arsenic had 13.67% samples more than maximum permissible limit.

6.3 Suggestions for further work

- Investigation on large scale is needed to assess the water quality of various water sources of Madhya Pradesh based on different physical, chemical and microbiological parameters.
- Epidemiological study is required for various water borne diseases for different water sources to observe their potential public health significance.
- Pathogenic isolates should be further characterized by advanced molecular techniques like RFLP, real time PCR, whole genome sequencing, etc and comparative study is needed for the phenotypic and genotypic characteristics of pathogenic strains.
- To understand the molecular mechanism of drug resistance study should be extended to find out the plasmid mediated transferable drug resistance.
- More extensive study is needed to find out seasonal variations in the level of heavy metal toxicants in water bodies either due to change climatic conditions and religious activities like idol immersion.
- Along with lead, arsenic and selenium, study of other toxic metals is also needed in the body tissues/ milk of animals/ humans, as well as in plants, and aquatic flora and fauna in association with water and soil of a particular area.
- Use of advanced technologies like graphite furnace atomic absorption spectrophotometry, electrochemical sensors, high resolution surface plasmon resonance spectroscopy combined with anodic stripping voltammetry for detection of heavy metals.

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APPENDIX - I

Chemicals used for determination of total hardness by titration

1) **Ammonia buffer solution**

Ammonium Chloride	13.5 g
Conc. Ammonium hydroxide	114 ml

Add distilled water to make a volume of 200 ml

2) **Eriochrome black - T indicator**

Eriochrome black – T dye	0.50 g
Ethyl alcohol (80%)	100 ml

3) **N/50 EDTA solution**

EDTA (0.5 M), pH 8.0	3.723 g
Distilled water	1000 ml

APPENDIX - II

Media used for isolation of *E. coli*

1) Brain Heart Infusion Broth (BHI broth) (Dehydrated, HiMedia)

Ingredients	Gram's/liter
Protease peptone	10.00
Calf brain, infusion	200.00
Beef heart infusion (solids)	250.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50
Final pH (at 25°C)	7.4±0.2

Suspend 37.0 gm in 1000 ml distilled water, distributed in test tube and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

2) MacConkey Broth (Double Strength) w/ Neutral Red (Dehydrated, HiMedia)

Ingredients	Gram's/liter
Peptic digest of animal tissue	40.00
Lactose	20.00
Bile salts	10.00
Sodium chloride	10.00
Neutral Red	14.00
Sodium chloride	5.00

Suspend 80.14 gm in 1000 ml distilled water, distributed in test tubes and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

3) Eosin Methylene Blue (EMB) Agar (Dehydrated, HiMedia)

Ingredients	Gram's/liter
Pancreatic digest of gelatin	10.00
Lactose	10.00
Dibasic potassium phosphate	2.00
Eosin - y	0.40
Methylene blue	0.065
Agar	15.00
Final PH (at 25°C)	7.2

Suspend 35.96 gm in 1000 ml distilled water and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

4) Mueller Hinton (MH) Agar No. 2 (Dehydrated, HiMedia)

Ingredients	Gram's/liter
Casein acid hydrolysate	17.50
Beef heart infusion	2.00
Starch, soluble	1.5
Agar	17.00
Final pH (at 25°C)	7.3 ± 0.2

Suspend 38.00 gm in 1000 ml distilled water and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

5) Nutrient agar (Dehydrated, HiMedia)

Ingredients	Gram's/liter
Peptic digest of animal tissues	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.00
Final pH (at 25°C)	7.4 ± 0.2

Suspend 28.00 gm in 1000 ml distilled water and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

APPENDIX - III

Media and reagents used for biochemical tests

1) MR - VP Medium

(Glucose phosphate broth)

Ingredient	**Gram's / litre**
Dextrose	5.00
Monopotassium phosphate	5.00
Pancreatic digest of casein	3.50
Peptic digest of animal tissue	3.50

Suspend 17.0 gm in 1000 ml distilled water and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

2) Methyl red indicator

Ingredient	**Gram's / litre**
Methyl red	0.1 g
Alcohol	300 ml
Distilled water	200 ml

3) Simmon's citrate agar

Ingredient	**Gram's / litre**
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromo thymol blue	0.08
Agar	15.00
Final pH (at 25°C)	6.8 ± 0.2

Suspend 24.28 gm in 1000 ml distilled water and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

4) Tryptone water

Ingredients	**Gram's/liter**
Casein enzymic hydrolysate	10.00
Sodium chloride	5.00
Final pH (at 25°C)	7.5 ± 0.2

Suspend 15.0 gm in 1000 ml distilled water and sterilized by autoclaving at 15 psi pressure, 121°C for 20 minutes.

APPENDIX - IV

Chemicals used for Agrose gel electrophoresis

1) Tris - Acetate EDTA (TAE) Buffer 10x stock solution)

Tris base	48.4 g
Glacial acetic acid	11.4 ml
EDTA (0.5 M), pH 8.0	3.7g
Distilled water	800 ml

2) Ethidium bromide

Ethidium bromide	10 mg
Distilled water	1 ml

3) Gel loading dye (6X)

Bromophenol blue	0.25%
Xylene cynol	0.25%
Sucrose	40%

Stored at 4°C temperature.

APPENDIX - V

Chemicals used for metal standards preparation

1) Lead blank solution

HCL	2.5 ml
Potassium ferricyanide	4 g
Distilled water	500 ml
Distilled water	800 ml

2) Lead standard preparation

Put 1 µg/ml of 0.25 ml, 0.5 ml, 0.75 ml and 1 ml into four different 100 ml volumetric flasks. Dissolve them with 0.50% HCL lead blank. The validity for the solution is not more than 3 days.

3) Arsenic blank solution (10% HCL)

HCL	50 ml
Distilled water	500 ml

4) Arsenic standard preparation

Put 0.2 ml, 0.4 ml, 0.6 ml and 0.8 ml into four 100 ml volumetric flasks. Dissolve them to 100 ml with 10% HCL. They are the 2, 4, 6 and 8 ng/ml standards of arsenic. The validity for the solution is not more than 3 days.

5) Selenium blank solution (20% HCL)

HCL 100 ml

500 ml Distilled water

5) Selenium standard mother liquid

Selenium standard	2 ml
Selenium blank solution	100 ml

6) Selenium standard preparation

Put 1 ml, 2 ml, 3 ml and 4 ml Selenium standard mother liquid into four 100 ml volumetric flasks, dilute to 100 ml with the prepared blank solution for 10, 20, 30 and 40 ng/ml, respectively.