International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 30 No. 2 January 2022, pp. 501-508

Bacteriological Monitoring of Inlet Water Source at Mansoura Water Treatment Plant

Maali E. S. A. Alsulilai[1,2], Mohamed, A. A. Shehata[1], Yehia A. Osman[1*]

[1]Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt, 35516.

[2]Scholar from State of Kuwait

Abstract – Quality of drinking water is a global issue and has a major impact on human health. Advances in water treatment have significantly improved the quality and safety of drinking water but assurance of its quality remained a huge concern. The continuous monitoring of inlet water source for Mansoura Water Treatment Plant was our major concern This was achieved by total coliform testing and isolation and identification of bacteria. Bacteriological analyses involved total coliforms, faecal coliforms and pathogenic bacteria. The results showed that total coliforms varied according to the season, peaked in the summer followed by autumn and sping and the lowest number was isolated in the winter. The following bacteria were isolated in pure colonies Esherichia coli, Klebsiella, Pseudomonas, Sptreptococcus, Enterobacter, Acinetobacter, and comamonas. No Salmonella and or Shigella were isolated from the inlet water source. In conclusion our tests suggested that the River Nile water at the inlet of Mansoura Water Treatment Plant does contain normal flora and no pathogenic bacteria, however, the treatment process kept operating at the highest standard possible.

Keywords – Water, Purification, Treatment, Pathogenic Bacteria.

I. INTRODUCTION

Strict criteria and standards of drinking water all over the world must ensure safety and lack of pathogenic microbes as well as hazardous chemicals **[1]**. In Egypt, the River Nile is the main source of drinking water directly or after treatment, unfortunately contamination of its water is well documented and constitutes a serious environmental problem which affects the human health. For us as bacteriologists, fecal coliforms and fecal streptococci were established as indicators of microbial contaminations and existence of potential health risks for consumers. The presence of these indicator microbes warrants a decision of exclusion for drinking by humans **[2].**

The known water microbial pathogens are known to be members of the bacterial genera *Salmonella, Shigella, E. coli, and Cholera* **[3]**. This warrants a different test to distinguish between bacterial pathogens based on their ability to produce a Shiga-like toxin either directly or indirectly, Modern molecular biology tools such as polymerase chain reaction (PCR) considered a fast, reproducible and reliable technique to use for this purpose.

The main facility at Mansoura City Egypt for drinking water treatment plant is located on the River Nile, beside Mansoura University. Therefore, it was convenient to monitor the microbial population of the River Nile inlet water to the plant and test the quality of the treated water for possible disease-causing bacteria, amongst the fecal coliforms present.

It is acceptable that water of the Nile River, the longest river in the world, be contaminated with various types of microbes, including bacteria. This open water body passes through many African countries with diverse sanitation measures and is exposed to fecal contamination from humans, warm blooded animals, and birds. Therefore, it is extremely important to monitor bacterial population all year round, especially at the inlet sites of water treatment plants such as the one located at Mansoura City, Egypt. This is routinely done by detecting indicator organisms, total coliforms, or *E. coli.* Analysis is usually performed using culture,

Corresponding Author: Yehia A. Osman

biochemical and sometimes optical methods. When indicator organisms levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (where suspected) using specific culture methods or molecular biology **[1]**.

Moreover, it is to estimate not only the number of bacteria but to make sure no pathogenic bacteria exist in the inlet water. This is necessary to confirm the safety, draw inferences of the suitability of the drinking water source and proper measures to be taken during the treatment process. This water is intended for humans' usage; therefore, stringent measures should be taken.

II. Materials And Methods

2.1 Sampling techniques

Raw (untreated) and treated (potable) water samples were collected from the River Nile (Inlet untreated and plant treatment water (outlet) from November (2019), and continued through the months of March, July, and September, 2021. The samples were immediately transported to the laboratory for analysis bacteriological analysis and pH measurements

2.2 Bacteriological isolation and Purification

For bacteriological evaluation the 100µl from each water samples were inoculated into 5 ml Lauria-Bertani (LB) broth and incubated at 37ºC for 48 h. Next day a 100µl from each broth was spread on nutrient agar plates (NA) and incubated at 37ºC for 24 h. Different single colonies were isolated, preserved in 20% glycerol and kept at -20oC for further experiments.

2.3 Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Total cellular protein of any living organism including bacteria are usually analyzed by denatured gel electrophoresis as described by **Laemmli, (1970)**.Commonly known as sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS-PAGE). It is the standard method for separating and differentiating protein according to their molecular masses, where the anionic detergent (SDS) wraps around the polypeptide backbone, giving then a negative charge allowing them to migrate toward the anode as described below.

2.4 Differentiation between bacterial isolated by MALDI-TOF MS

Any bacterial sample was prepared for analysis by the MALDI instrument must be coating it with a matrix or an energy-absorbent organic molecule (-cyano, 4-hydroxycinnamic acid). This matrix absorbs the laser beam to ionize the bacteria automatically. The produced protonated ions travel through to desorption and ionization and separate apart according to their mass-to-charge ratio (m/z). The instrument determines the m/z ratio of an ion by calculating the time it takes to travel the length of the flight tube. Leading to a distinctive spectrum for each bacterial sampled or a peptide mass fingerprint (PMF). By comparing the PMF of unknown to the PMFs database identifies the unknown bacterium to the species and may be to the strain level **(Singhal *et al.*, 2015).**

III. Results

3.1 Samples Collection

Mansoura water treatment plant takes its water directly from the River Nile (Inlet water). Water samples used in this study were taken from the Inlet of the plant and the outlet, too. All samples were analyzed within 24 hours of collection. The pH of the two samples were measured and recorded.

3.2 Isolation of bacteria

The bacteria present in the collected water samples in this study were isolated and purified. Inlet: 10 bacteria isolates from water Inlet site: (N1,N2,N3,N4,N5,N6,N7,N8,N9,N10), Treated water (Outlet): Exit 1, Exit 2, Exit 3, Home Faust (Tank): 1.

3.3 Coliform test for water or Samples

I-At Zero Time

At zero time from adding water samples on MaCconky broth medium, there is no change in all tubes as shown in **Fig (1)**

Fig (1): the presumptive test of the first Nile water sample at time zero shows no change in medium color.

After 24 h

After 24 hours from inoculation with untreated Nile water all double strength tubes turned yellow completely, but slight change in all single strength tubes which inoculated with 1 ml and 0.1 ml from water sample as shown in **Fig (2)**.

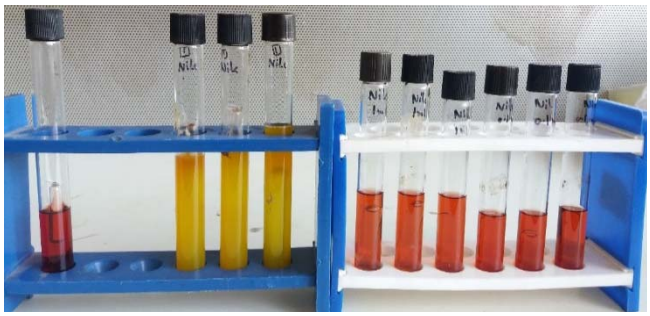

Fig (2): the presumptive test of the first Nile water sample After 24 h. The arrows show a change in the color of the nutrient medium in three test tubes that turned yellow with gas formation.

After 48 h

Yellow color was increased after 48 hours from inoculation with water sample in all double strength tubes but gas was formed only in one tube, also slight change in the rest of inoculated tubes as shown in **Fig (3).**

Fig (3): shown the presumptive test of the first Nile water sample After 48 h.

3.4 Confirmed Test by EMB

Eosin Methylene Blue, (EMB) medium is widely used to distinguish between different members of the Enterobacteriaceae member, depending on their abilities to ferment the lactose sugar (*E. coli)* or not. (., *Salmonella, or Shigella).* On this differential medium *E. coli produces* a characteristic green sheen because it ferments lactose, while the lactose non-fermenters, do not.

Bacteria isolated from first untreated Nile water (inlet) sample that gave green metallic sheen were N1, N2, N3, N5, N6, N10 as shown in **Fig (4).**

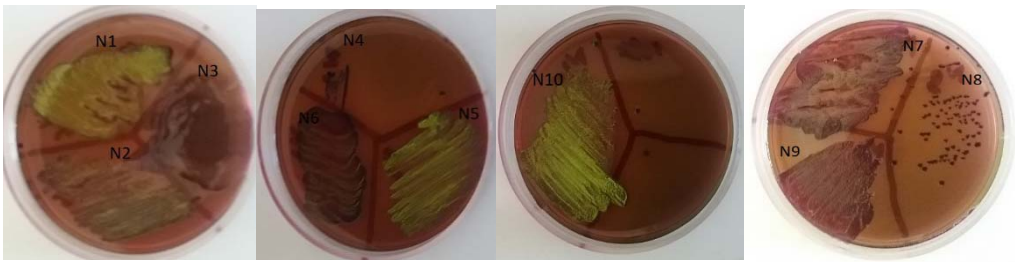

Fig (4): shown the green metallic sheen of some bacterial that isolated from untreated Nile water and the other dark purple colonies and colorless colonies.

3.5 Complete Test

Presence of faecal indicator *E.coli* is confirmed by the production of gas and color changes in media. Lactose fermentation occurred only in N2, N5, and N10 isolates and produced gad in Darham`s tubes as shown in **Fig (5).**

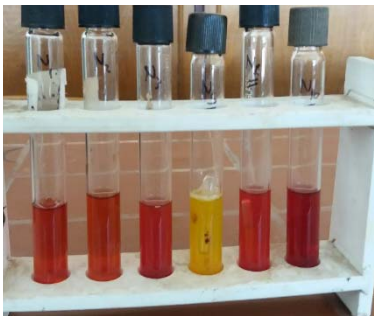

Fig (5): shown color change in maCconky medium due to lactose fermentation and gas production in Darham`s tubes inoculated with N2, N5, N10 isolates.

3.6 Comparison of Cellular protein of isolated bacteria

Significant differences between the total cellular protein patterns of all isolated bacteria from the four collected water samples. Each isolate showed distinctive proteins, which did not appear in the other isolate as shown in Fig (25, 26, 27, 28) Significant differences between the total cellular protein patterns of all isolated bacteria from the first water sample. Each isolate showed distinctive proteins, which did not appear in the other isolate as shown in **Fig (6)**

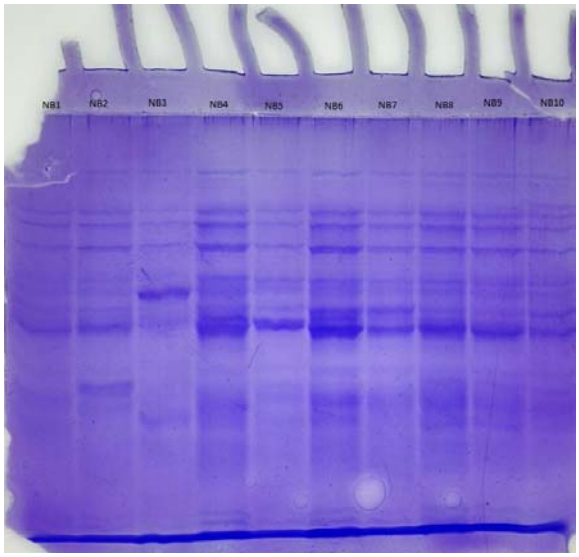

Fig (6): SDS-PAGDE protein profile, lane 1- NB1, lane 2- NB2, lane 3- NB3, >>>>>>>shows the differences between through the different appearance of protein bands in each isolate showing the differences in protein profile between isolated bacteria.

3.7 Identification of selected isolates by MALDI-TOF-MS

New approaches are required for rapid analysis of bacteria in clinical microbiology laboratories. Among recent development for bacterial identification, the use of protein profiles obtained by MALDI-TOF-MS directly from colonies was successfully proposed. The method analyzes the profiles of bacterial macromolecule that are obtained from whole bacteria. This new proteomic approach allows rapid and accurate identification of bacteria. So, we used MLDI-Tof-MS to illustrate the differences between these four different types of cells through clarify the protein contents of each type of cell. The intrinsic property of mass spectrometry is to detect the ions' mass-to-charge (m/z) ratio of a bioanalyte, providing spectra within minutes. The procedure provides a unique mass spectral fingerprint of the microorganisms. This method requires that the biopolymer molecules normally present in the condensed phase be converted into intact, isolated ionized molecules in the gas phase. Then, ions are separated according to their molecular weight after migration in an electric field. Each molecule detected is characterized by: the molecular mass (m), the charge (z), the ratio mass/charge (m/z), and the relative intensity of the signal.

Table (1): MALDI-TOF-MS identification of the final 10 mbacterial isolates Showing the identified bacterial isolates form the four water samples

Sample Name	Organism	Score
1	*Acinetobacter schindleri LT0105*	0.82
2	*Comamonas aquatica*	0.44
3	*Enterobacter cloacae LT6B45*	0.82
4	*Escherichia coli LT6BF3*	0.88
5	*Escherichia coli LT6BF3*	0.85
6	*Klebsiella variicola LT6E7B*	0.74
7	*Pseudomonas aeruginosa LT2992*	0.90
8	*Pseudomonas aeruginosa LT2992*	0.90
9	*Pseudomonas aeruginosa LT770C*	0.82
10	Streptococcus oralis LT7BE7	0.39

IV. Discussion

Drinking water quality is a worldwide concern and has a significant impact on human health. Advances in water treatment have significantly increased the quality and the safety of water; however, drinking water quality can remain susceptible to deterioration by microbial and chemical contaminants during transport, storage, and handling, as well as possibly in dispensing devices such as bottled water dispensers **[4]**. From (((BACTERIOLOGICAL ANALYSIS OF DRINKING WATER Pages with reference to book, From 92 To 96 Zumra Sami (Public Health Division, National Institute of Health, Islamabad.) Mubashir A. Khan, Abdul Ghafoor (PMRC Central Research Centre, National Institute of Health, Islamabad.)))

The consumption of drinking water contaminated with pathogenic microbes of faecal origin is a significant risk to human health. As a standard procedure, water intended for human consumption is distributed to consumers after treatment. Nevertheless, the quality of treated water can deteriorate during distribution due to contamination and inadequate storage conditions. In developing world, especially in remote rural areas and industrial areas, over 3 million deaths per year are attributed to water-bone diarrhoeal diseases, especially among infants and young children in poor communities **[5]**. It is therefore important to determine the quality, microbial diversity from water sources consumed by the people in the city, especially used by children, because they are vulnerable to different kinds of diseases since their immune systems are still developing. The study concluded that one of the main factors that contributed to this outbreak was the way water was usually stored. Cairncross and others pointed out that the

discussion is restricted to the water source and the distribution system, without taking into consideration the piping system inside the dwellings **[6]**. Regarding to storage water tanks emphasized that the major issue involving deterioration of drinking water quality is the inadequate maintenance of the internal distribution system, which is nowadays recognized as the major factor that compromises. Thus, water contamination problems have led investigators to assess the quality of the water which runs inside the buildings, dwellings and schools. The poor health status of population is reflected in high infant mortality rate of 12.6% and as low as 7% fertility rates reported in other study. The scanty hospital's data shows that many of the diseases treated are caused by water borne microbes indicating that a substantial proportion of morbidity in Pakistan is due to use of polluted water **[7].**

Several researchers have attempted to estimate the total burden of waterborne diseases which might account for one-third of the intestinal infections world-wide **[8]**. It has been estimated that water, sanitation and hygiene are responsible for 40% of all deaths and 5.7% of the total disease burden occurring worldwide **[9]**. In developing world, especially in remote rural areas and industrial areas, over 3 million deaths per year are attributed to water-bone diarrhoeal diseases, especially among infants and young children in poor communities. A survey in Pakistan on water revealed, bacterial causes of water contamination to be 68% giving rise to 100 million diarrheal cases seeking hospital admissions and 40% mortality associated with it **[10].**

Monitoring the quality of water is very essential for environmental safety. WHO (1985) specified that potable drinking water should be devoid of total coliform in any given sample. Also, according to USEPA standards water samples in which coliforms are detected should be considered unacceptable for drinking water as they are regarded as the principal indicators of water pollution **[11].** An acceptable pH for drinking water is between pH 6.5 to pH 8.5, recommended by WHO as a guideline value and in the absence of a distribution system acceptable range may. be broader. AU the water samples examined in this study were in acceptable pH range, which agree with our findings.

All untreated Nile water samples which collected in different seasons gave positive presumptive test so they are contaminated with coliform bacteria. In the other hands all treated water samples collected from water planet and bottled water samples gave negative presumptive test. This study has shown the presence of many pathogens in untreated Nile water and treated reservoirs, and that these pathogens can pose risks to human health. In many countries surface reservoirs serve as the main source of drinking water, and these surface water bodies are often vulnerable to pathogen contamination

One of the objectives of this study was to isolate environmental bacteria from untreated nile water, drinking water and different bottled water in Mansoura city. A motivation for this study was evaluate the occurrence of pathogenic microorganisms in drinking water and the associated diseases. In addition to this, resistance of microorganisms to antibiotics of clinical interest has previously been reported in the area. The study demonstrated the occurrence of total coliforms fecal coliforms, heterotrophic bacteria, Streptococcus, Comamonas, Acinetobacter and Pseudomonas in water samples analyzed which indicated the incidence of water contamination as some of these species are indicators of fecal contamination, our findings were in agree with **[12]**. These organisms may harbor potential pathogens and the presence of pathogenic organisms that can pose severe health risks to consumers in general and immunocompromised individuals in particular **[13]**. Reduction in the number of bacteria in the treated water could be due to the treatment process, when comparing drinking water to raw water. However, occurrence of bacteria in the water after treatment could also harbor potential pathogens and the health risk caused by these should be taken into consideration when water is distributed. This is of particular importance when the drinking water abstraction and purification facility are at a relatively short distance from the sewage treatment and effluent disposal facility. In Mansoura, the latter is the case. One of the waste water treatment plants in Mansoura is upstream from the drinking water purification plant. This may explain the larger number and diversity of isolates from this water. Results from this and other studies show that drinking water produced from the Mansoura water plant should be further analysed and that general microbiological tests that only include faecal coliforms and *E. coli* may be insufficient.

A further objective of this study was to characterise the isolates using their antibiotic resistance profiles. The results revealed that a large proportion of the environmental isolates were resistant to oxacillin followed by ampicillin and vancomicin. The trend was in accordance with earlier studies that showed resistance towards β-lactam, macrolides, and phenicols these results were in agreement with **[14]**. All the isolates from all the sites were resistant to oxacillin which was in accordance with the study conducted by **[15]** where most isolates were also resistant to ampicillin and vancomycin. A large percentage of isolates were also resistant to erythromycin and trimethoprim-sulphamethoxazole. All these results could be attributed to the overuse of these antibiotics in the clinical and veterinary setting. Most isolates were found to be susceptible to ciprofloxacin and kanamycin in line

with an earlier observation. This is not in agreement with yet another observation **[16]** where increased resistance against ciprofloxacin in E. coli and Pseudomonas was observed.

The impact of seasonal variation on the quality of drinking water of Mansoura City was evaluated. The bacteriological analysis was carried out by MPN method. A total of 16 samples were collected over a period of two years. The study was based on total number of contaminated samples in four sets of time period, i.e., November -Mach-July-and September. It was observed that in the summer months, i.e., September the percentage of contaminated sample with total coliform as well as faecal coliform bacteria was higher than in the winter months, i.e., March and September. The number of total and faecal coliform bacteria in drinking water was also higher in summer months than in winter months. Our results are in agreement with the results of Alkatib, as he reported that the bacteriological contamination was higher in winter months than in summer months in drinking water of Palestinian district Jenin **[17]**.

In the months of peak of summer, the cases of water-borne diseases were reported as higher than in winter months. This may be due to climate temperature, which favours the optimum growth of organism. **[18]**. reported that stream temperature change could be approximated by air temperature changes. Since the temperature ranges from 37-50°C, which supports mesophiles and pathogens come in the range means optimum temperature for their optimum growth, the contamination of drinking water in summer months has been reported as higher than in winter4. The source of microbial contamination in drinking water may be the human and animal activities in source water. In summer months, the consumption of water for different purposes increases and municipal has to supply more water to the consumer and, to clean the large amount of water urgently so it requires the treatment and thus the proper sedimentation cannot be achieved. On the other hand, due to human and animal activities contamination in source water increases in summer months, which enhance the fecal contamination in the source of drinking water.

The organisms that were identified in the MALDI -Tof device, (NB5, NB6, NB10, House tank tap, MB4, JB1, JB4, JB8, SB8, and SB9) most of them were coliform bacteria (E.coli , Enterobacter cloacae, Klebsiella variicola and Acinetobacter schindleri) also they were Multi-drug resistant, especially the Pseudomonas bacteria isolated from the tank tap were resistant to all the antibiotics that were tested, and this is an indication of the danger of using this water on human health. The dominant resistant heterotrophic bacteria in u water samples were *Pseudomonas* spp, followed by *E.coli* then Eterobacter , Klebsiella, Acinetobacter spp, Streptococcus spp, and Comamonas spp. The results indicate that resistant bacteria can regrow in drinking water distribution systems. Several of the identified bacteria were opportunistic pathogens; therefore, the results support the general medical advice to disinfect water at the point of use to protect the health of individuals who may be immunocompromised/suppressed.

REFERENCES

[1] American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C. 22nd Ed; 2012.

[2] Adingra AA, Kouadio AN, Blé MC, Kouassi AM.2012. Bacteriological analysis of surface water collected from the Grand-Lahou lagoon, Cte divoire. Afr. J. Microbiol. Res. 2012;6(13):3097-3105.

[3] Ali, J., A. Hussain and H. J. P. J. B. M. B. Abid (2011). The occurrence of coliforms bacteria in water reservoir at different schools of district Peshawar. 44(2): 64-67.

[4] Shams, M., M. Qasemi, M. Afsharnia, A. Mohammadzadeh and A. J. M. Zarei (2019). Chemical and microbial quality of bottled drinking water in Gonabad city, Iran: Effect of time and storage conditions on microbial quality of bottled waters. 6: 273-277.

[5] Kumar, D., S. Malik, M. Madan, A. Pandey and A. K. J. J. E. S. T. F. T. Asthana (2013). Bacteriological analysis of drinking water by MPN method in a tertiary care hospital and adjoining area Western UP, India. 4(3): 17-22.

[6] Cairncross, S., U. Blumenthal, P. Kolsky, L. Moraes, A. J. T. M. Tayeh and I. Health (1996). The public and domestic domains in the transmission of disease. 1(1): 27-34.

[7] Ali, J., A. Hussain and H. J. P. J. B. M. B. Abid (2011). The occurrence of coliforms bacteria in water reservoir at different schools of district Peshawar. 44(2): 64-67.

[8] Khadse, S., N. Gharami and T. Sapate COVID-19 OUTBREAKS AND IMPACT ON DRINKING WATER.

[9] Prüss, A., D. Kay, L. Fewtrell and J. J. E. h. p. Bartram (2002). Estimating the burden of disease from water, sanitation, and hygiene at a global level. 110(5): 537-542

[10] Faria, M., S. Javeria and S. J. B. Muhammad (2010). Bacteriological analysis of drinking water from services hospital Lahore and Services institute of Medical Sciences Lahore. 26: 66-69.

[11] Supply, U. S. E. P. A. O. o. W., C. f. D. C. C. f. P. Development and Training (1984). National Interim Primary Drinking Water Regulations: For Use with Homestudy Course 3014-G, Center for Professional Development and Training, US Department of Health ….

[12] Webster, L. F., B. C. Thompson, M. H. Fulton, D. E. Chestnut, R. F. Van Dolah, A. K. Leight, G. I. J. J. o. E. M. B. Scott and Ecology (2004). Identification of sources of Escherichia coli in South Carolina estuaries using antibiotic resistance analysis. 298(2): 179-195.

[13] Biyela, P., J. Lin, C. J. W. S. Bezuidenhout and Technology (2004). The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. 50(1): 45-50.

[14] Obi, C. L., P. Bessong, M. Momba, N. Potgieter, A. Samie and E. J. W. S. Igumbor (2004). Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities, South Africa. 30(4): 515-519.

[15] Lobova, T. I., Y. V. Barkhatov, V. S. Ol'ga and L. Y. J. M. R. Popova (2008). Multiple antibiotic resistance of heterotrophic bacteria in the littoral zone of Lake Shira as an indicator of human impact on the ecosystem. 163(2): 152-160.

[16] Oudhuis, G. J., A. Verbon, J. Hoogkamp-Korstanje, E. E. Stobberingh and S. S. S. G. J. I. j. o. a. agents (2008). Antimicrobial resistance in Escherichia coli and Pseudomonas aeruginosa from intensive care units in the Netherlands, 1998–2005. 31(1): 58-63.

[17] Al-Khatlb, I. A., F. Daoud, F. Rasmawl, S. Wa'rra, M. J. W. Kassabry and E. Journal (2005). Seasonal variation of bacteriological and chemical quality of drinking water: A case study in a Palestinian district. 19(3): 154-158.

[18] Ducharne, A. J. H. and E. S. Sciences (2008). Importance of stream temperature to climate change impact on water quality. 12(3): 797-810.