Survey of Microbial quality of drinking water of distribution system using heterotrophic bacteria plate count index in Zahedan-Iran 2011-2012

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Abstract
Today, heterotrophic bacteria have been considered as HPC index as a supplement to coliform index in water quality control. In the present study, the HPC index in Zahedan drinking water was evaluated and determined in accordance with the standard. 80 water samples were prepared according to the national standard of Iran No. 4208 based on the population of different areas of Zahedan freshwater distribution network, so that the entire distribution network is covered. Samples were taken separately in two seasons, summer and winter. 4 areas in areas with new distribution network and made of polyethylene and 4 areas in areas with old distribution network and cast iron were selected and 10 samples were taken from each area. Samples were tested from 8 am to 10 am under standard conditions for HPC, TOC, temperature, turbidity, free residual chlorine, PH and Do. HPC test was performed by plate diffusion method. In only two points, the maximum HPC level in summer was 300 CFU / ml, which was lower than the standard and within the allowable range. There was a direct relationship between temperature, turbidity, pH and TOC of water with the growth of heterotrophic bacteria. There was also an indirect relationship between the amount of HPC and the amount of chlorine remaining in water and dissolved oxygen. Network age and material had no effect on HPC.

Keywords: Heterotrophic, Plate culture, Drinking water, Zahedan

1- Introduction
Water is the most important substance of life and development. This substance is the beginning of life and the main component of all living beings. Fifty-six verses are mentioned in the Holy Quran about the origin and importance of water and the dependence of life on this valuable substance. Water is of special importance in human civilization. In ancient Iran, water was considered one of the four basic elements (water, soil, wind and fire) [5]. According to the theory of water evolution, methane and ammonia are the main constituents of the Earth's atmosphere. A high percentage of the mass of each living cell is water [1]. Water forms a large part of the vast cellular network of the human body and would not be vital without water. Water is one of the most abundant substances on Earth and is the only substance that exists naturally in solid forms (ice), liquid and gas (water vapor). Drinking and meeting individual needs has been the first human attitude to

water [16]. The history of water use and water engineering goes back to ancient times and the creation of the first human group life. The first cities in ancient civilization were formed along rivers and lakes, and water supply and irrigation methods were created, in which access to water resources was of special importance for human life from both quantitative and qualitative perspectives [6]. Humans initially paid more attention to the quantity of water, although in that era the available water was of relatively high quality and there was no pollution from the growth of industry and so on. Due to the scarcity of water resources, the value of this vital substance was known in Iran [9]. Herodotus and Xenophon have written about the fact that Iranians do not throw everything dirty in the water and take care to keep the water and soil clean [18].

Construction of irrigation canals six thousand years ago in the hills of Kashan Sialak; The construction of Sakami and Lakourian dams in Balochistan with a history of four thousand years ago and the design of aqueducts and its construction three thousand years ago have shown the importance of this valuable material and the ability of Iranians in engineering and design of water affairs [20]. In the new era, the presentation of the physical properties of water by Leonardo da Vinci, the identification of water constituents by Priestley and Love Vazie and the determination of the relationship between cholera and well water led to new knowledge and various engineering systems in the field of water and wastewater] 8 [. If the earth is viewed from above, it looks like a blue sphere, because a large part of its surface is covered by oceans and seas. Of the 510 million square kilometers of land, 361 million square kilometers are covered with water [10].

The source of fresh water on earth is only 35 million cubic kilometers, approximately 2.5 percent of the total volume of groundwater, plus 68.9 percent of fresh water and about 24 million cubic kilometers in the form of snow and ice in the mountains and the north and south poles. Land is stored that is difficult to access for human consumption. Also, most of the remaining freshwater resources, 8 million cubic kilometers (30.8% of freshwater resources) are groundwater resources [15]. Currently, 4 million people die each year from water-related or quality-related diseases, and more than 25 countries are in critical water shortages. 1.5 billion people do not have access to safe drinking water, up from 2.3 billion by 2005. (4 out of every 10 inhabitants of the land) [13]. 1.7 billion people will be on the verge of a very critical water shortage. By 2025, 1.8 billion of the world's 8 billion people will experience water shortages, with about 22 percent of them living in countries and regions affected by clean water shortages. (450 million households) [19].

According to studies, water is one of the problems of sustainable development today. In recent decades, due to the severity of pollution of water resources, including rivers, lakes and dams, which has occurred mostly through the entry of municipal and domestic, industrial and agricultural wastewater [2], the concentration of water pollutants is alarming. Increased. Among these, organic pollutants are of special importance due to their number and diversity in water resources, increase in concentration and special properties, as well as the impossibility of complete elimination in conventional drinking water treatment plants [4].

Increased water consumption and the resulting shortage, which is exacerbated by pollution; It has caused the supply of sanitary water to become one of the main concerns of the international community, including our country [20]. Our country, Iran, in terms of water resources potential, is among the countries with water stress, which if not properly managed water resources will undoubtedly be among the water-scarce countries in the not too long term. One of the important activities is related to its water treatment [16]. The type of water source (surface or groundwater), its microbial, physical and chemical quality and the community covered are among the factors that determine the type and method of water treatment. The reason that the water produced by some treatment plants is not of very good quality can be due to several problems, including changes in water quality indicators in surface reservoirs, including the growth of algae, aquatic plants, water layering, and aquatic animal activity or increasing indicators. Such as turbidity, TDS, TOC, electrical conductivity, etc. [12].

In the study of microbial quality of water and its compliance with existing standards, the common index used includes determining the presence of coliform and Escherichia coli bacteria in water during three possible stages, confirmatory and complementary [17]. However, today the plate count of heterotrophic bacteria or HPC is one of the indicators that is considered as a complement to the coliform index in water quality control and in evaluating water quality in water storage and distribution systems, HPC bacteria analysis can be useful. Heterotrophic bacteria are a diverse group that are ubiquitous. In all systems dependent on water, wildlife, plant life, and dust particles, there are heterotrophic bacteria, the predominant genera of which are Flavobacterium, Cytophagus, Acromobacter. , Acinetobacter, Alkaligens, Moragella, Vibrioeromonas, Pseudomonas and peripheral coliforms (Klebsiella, Aerobacter, Citrobacter) [13]. Studies on genera and species of heterotrophic bacteria have shown that Pseudomonas species (which may be resistant to antibiotics) often make up the majority of this bacterial population [20].

Regarding the determination of heterotrophic bacteria in water, Robert Koch first proposed in 1883 at a congress in Berlin (Germany) heterotrophic bacteria, the method of identification, colony formation and colony count in determining water quality. Bacteria, such as Legionella and Aero Monas hydrophilic, are naturally present with pathogenicity [7], and the absence of Escherichia coli in water does not necessarily mean the absence of these microorganisms. Numerous studies on heterotrophic bacteria have shown that these bacteria have pathogenic factors. Considering individual immunity, opportunistic pathogens with heterotrophic flora can be a major risk to children's health [2]. For example, some invasive Pseudomonas species are a serious secondary pathogen in causing infections in children with burns. These bacteria are also important in people with defective immune systems, so it seems that HPC testing in water should be considered as an additional tool to ensure the health of consumers.

The US Environmental Protection Agency (EPA) in 2000, in the latest microbial standard, set the maximum allowable number of heterotrophic bacteria in water distribution networks 500 CFU / mL [16]. According to the World Health Organization, drinking water for human consumption and all household uses, including personal hygiene, must be of good quality and HPC can be used as a tool for the following:

- Identify the effectiveness of the water treatment process and in fact as an indirect indicator of the removal of pathogens.
- As a measure of the regrowth of organisms that may or may not be important for improvement.
- As a measure to determine possible interference with coliform formation in lactose culture method.

Considering the above cases and considering that the determination of heterotrophic bacteria in drinking water of Zahedan is not a common test and until now there was no published information about the presence of heterotrophic bacteria in Zahedan drinking water network, so in the present study HPC index in drinking water Zahedan was examined and the amount was determined.

2- Method

This is a practical research that was done to map the city of Zahedan and was divided into eight districts. 4 areas of the distribution network had old cast iron pipes and 4 areas had new polyethylene pipes. To study HPC changes according to standard 4208 of the Iranian Institute of Standards and Industrial Research, based on the population of Zahedan, sampling was performed in two time intervals of summer and winter in the distribution network. 5 samples were taken from each area in summer and 5 samples in winter, which made a total of 10 samples from each area (point). According to the division of the city into eight districts and the number of 10 samples from each area (point), we will finally have 80 samples. Zones 1, 2, 3 and 4 are related to the points of the distribution network whose pipe life is over 25 years and is made of ductile iron and old.

At the sampling site, temperature, turbidity, free chlorine and pH and dissolved oxygen (DO) were measured. A WTW portable digital device made in Germany was used to measure water turbidity on site. A digital portable diameter was used to measure dissolved oxygen and on-site temperature. A portable digital Colorimeter Palin test device made in England was used to measure the residual chlorine. The WTW digital portable device was used to measure the pH of drinking water. Samples were taken from public places such as shops, parks and residential areas in sterile glass bottles containing thiosulfate in accordance with the conditions of prevention of secondary contamination. During sampling, faucets without hoses and without apparent contamination after sterilization by alcohol and flame cotton were selected for sampling. Sampling time was daily from 8-10 am. After running the water for one minute, two-thirds of the jars were filled with water under sterile conditions and transported to the laboratory within two hours at a cold box temperature of 4-8 ° C. For sampling HPC and Toc test separately, 125 cc dark glassware that had been thoroughly washed and sterilized was used. All sampling and testing conditions were performed according to the instructions of the standard method book for water and wastewater testing.

The heterotrophic plate count, or HPC standard, counts almost all types of bacteria in a sample of water that can grow in a general culture medium called a standard plate count or nutrient agar. If the number of colonies formed is less than 50 colonies per milliliter of the sample, then the water disinfection is well done, and conversely, if it was more than this amount, disinfection was not appropriate, plus if the number of colonies is more than 500 Colony per milliliter, in this case, will interfere with the growth of total coliform bacteria. This test is a complementary method used to control the process. In this method, only 0.1 and 1 ml of the sample are used. This test is used to determine the quality of source water, the level of disinfection required to control the process and perform adequate disinfection of water in distribution systems.

3- Results

The results of HPC and TOC tests, temperature, turbidity, free residual chlorine, pH and dissolved oxygen were entered and analyzed in the form of recording the measured parameters after the end of the tests.

Check the results of temperature parameter measurements

Temperature is a physical parameter that is directly related to the amount of HPC. Rising temperature along with other interfering factors, especially in the warm season, caused an increase in HPC. In the study, the average temperature measured in degrees Celsius in 8 areas and urban water distribution points in summer and winter were investigated. The average temperature in summer in regions 1 to 8 is 27-28-29-28-27-28-27 degrees Celsius, respectively, and the average temperature in winter in regions 1 to 8 is 16, 16, and 16, respectively. 17, 16, 18, 16, 17 degrees Celsius. The highest water temperature is related to zone 3 and the lowest is related to zones 1, 2, 3, 5 and 7. Also, the average temperature during the project showed that the highest temperature is related to summer and the lowest is related to winter.

Check the turbidity measurement results

Turbidity is one of the parameters that is directly related to the growth of heterotrophic bacteria. Increased turbidity in water increases heterotrophic bacteria. In the study, the average turbidity measured in terms of NTU was investigated in 8 sampling areas of the distribution network. The average turbidity changes in 8 sampling areas of the distribution network in summer are 0.19, 0.24, 0.3, 0.41, 0.12, 0.06, 0.06 and 0.08, respectively. The average turbidity changes in 8 sampling areas of the distribution network in winter are 0.15, 0.1, 0.02, 0.23, 0.05, 0.04, 0.07 and 0.06, respectively. . The highest average turbidity changes are in area 4 and the lowest is in sampling area 3. The highest average turbidity changes in summer are related to the old distribution network and ductile iron and the lowest is related to the winter season and the old distribution network. And is made of ductile iron. The amount of turbidity in the urban water distribution network has always been in accordance with Iranian standard 1053 in the desired range (less than NTU 1). This indicates the high efficiency of Zahedan treatment plant in removing turbidity, which is very effective in reducing HPC.

Investigation of residual chlorine parameter measurement results

Residual chlorine is one of the parameters that is indirectly related to the amount of heterotrophic bacteria. The increase in residual chlorine and its contact time in water reduced the population of heterotrophic bacteria in the water distribution network. In the study, the mean residual chlorine measured in ppm in 8 water sampling areas in summer and winter was investigated. The average changes of residual chlorine in summer in 8 areas are 0.9, 0.5, 0.7, 0.8, 0.7, 0.8, 0.8 and 0.9, respectively. The average changes in the amount of chlorine remaining in the water in winter in 8 areas are equal to 1.2, 0.8, 0.8, 0.9, 0.9, 0.7, 1.1 and 0.8. The highest average amount of residual chlorine changes is in zone 1 in winter and the lowest is in zone 2 in summer.

Investigation of PH parameter measurement results

PH is a physical parameter that is directly related to the amount of HPC. Increasing the pH along with other interfering factors increases the HPC. In the study, the average pH measured in 8 sampling areas in summer and winter was examined. The average pH in 8 sampling areas in summer is 8, respectively. / 7, 8/7, 8/7, 8/7, 7/7, 7/7, 7/7 and 7/5. The average pH in 8 sampling areas in winter is 7.9, 7.8, 7.8, 7.7, 7.8, 7.8, 7.7 and 7.8, respectively. Accordingly, the highest mean value of pH changes is related to region 1 in winter and the lowest is related to region 8 of sampling.

Evaluation of dissolved oxygen measurement results

The DO level is indirectly related to the HPC value. As the dissolved oxygen increases, the amount of heterotrophic bacteria decreases. In the present study, the amount of dissolved oxygen was measured in 8 sampling areas in summer and winter. The amount of dissolved oxygen in summer in 8 sampling areas is 4.5, 4.4, 4.3, 4.3, 4.3, 4.5, 4.4 and 4.5, respectively. The amount of dissolved oxygen in winter in 8 sampling areas is equal to 5.7, 5.6, 5.7, 5.8, 5.7, 5.6, 5.7 and 5.6. The highest amount of dissolved oxygen is related to region 4 in winter and the lowest amount of dissolved oxygen is related to regions 3, 4 and 5 of sampling.

Evaluation of TOC measurement results

TOC is a chemical parameter that is directly related to the amount of heterotrophic bacteria. Increased TOC along with other interfering factors increases the growth of heterotrophic bacteria. As mentioned, heterotrophic bacteria are optional aerobic and anaerobic bacteria that derive their carbon and energy from organic compounds. In the study, the average TOC measured in mg / l in 8 sampling areas of the distribution network in summer and winter was investigated. The mean TOC in summer in 8 sampling areas was 0.8, 0.88, 0.94, 0.96, 1.02, 0.78, 0.93 and 0.89, respectively. The average TOC in winter in 8 sampling areas is 0.83, 0.81, 0.83, 0.85, 0.85, 0.81, 0.84 and 0.81, respectively. The highest TOC rate is in Zone 5 in summer and the lowest is in Zone 6 in summer. Also, the amount of TOC is higher in summer than in winter. Significant low TOC in the municipal water distribution network indicates the appropriate efficiency of the treatment plant in the removal of organic matter, which is very effective in reducing the amount of HPC.

Evaluation of HPC measurement results

In this study, the average HPC in terms of CFU / ml in 8 areas of the distribution network in 2 summer and winter seasons was investigated. The average amount of HPC in summer in 8 areas is 65, 20, 13, 32, 67, 20, 16 and 17, respectively. The average amount of HPC in winter in 8 areas is zero, zero, zero, 16, zero, zero, one and zero, respectively. As two brothers and his colleagues showed in 2006, at higher temperatures, turbidity and pH, the growth of heterotrophic bacteria increases. However, with increasing DO and residual chlorine, the amount of HPC decreases and in the residual chlorine levels below 0.2 ppm, there is a possibility of growth of heterotrophic bacteria along with other interfering factors. Allen also found in his results that there is a possibility of Escherichia coli when the HPC level is between 1000-500 CFU per milliliter of drinking water. For this reason, HPC was introduced as a useful tool for monitoring water treatment processes and water quality during storage and distribution. The results of this study also show that there is a direct relationship with increasing temperature, turbidity, pH and TOC of water and the growth of heterotrophic bacteria. This is true of Figures 4-8, 4-9, 4-11, and 4-13 during project implementation. The results also show that the amount of HPC is indirectly related to the amount of residual chlorine and dissolved oxygen, and with increasing residual chlorine and dissolved oxygen, HPC decreases. Also, considering that the sampling areas 1, 2, 3 and 4 were related to the old distribution network of Zahedan and were made of ductile iron, but compared to the 5, 6, 7 and 8 sampling areas which were the new distribution network and were made of polyethylene, there was not much difference in their HPC. This may be due to the lack of wear of old tubes and the lack of growth of heterotrophic bacteria. Also, considering that the amount of residual chlorine has always been in the allowable range of ppm (0.8-0.2) and even higher, this is one of the reasons why the amount of HPC is in the allowable range and below 500 CFU per milliliter. The results of this study show that the level of HPC in 8 areas in summer and winter has been reported from a maximum of 300 to a minimum of zero, which is low compared to the microbial standard of water, i.e. the maximum allowable 500 CFU / ml, and the main reason is It is possible to timely wash the network and tanks, the new construction of the Zahedan water treatment plant and its operation on 2010/01/03 and as a result, reduce the amount of heterotrophic bacteria.

4- Conclusions and suggestions

Looking at the correlation column between the amount of residual chlorine and HPC shows that with the increase of free residual chlorine in the distribution network, the number of colonies decreases, the main reason being the sensitivity of heterotrophic bacteria to chlorine. There is a direct relationship between turbidity and HPC, so that with increasing turbidity, the amount of HPC also increases, which may be able to act as a shield against chlorine disinfection and reduce its effect on HPC. Therefore, turbidity removal and water purification is a safe process for drinking water purification to reduce HPC values by reducing turbidity. Therefore, HPC is a control tool for the water supply network, which is probably stuck in the wall of pipes and at the knees and joints of the microbial mass distribution network and formed as a biofilm. And prevents the effects of chlorine and other disinfectants and provides a suitable substrate for the survival and production of various microorganisms [4[

Temperature, turbidity, PH and TOC were directly related to the increase in HPC. The quality of drinking water has been rarely studied in recent years, and perhaps one of the reasons is the problem of drinking water supply in the regions, so more attention has been paid to water supply than to its quality, but in recent years good studies on quality Drinking water has been provided in many rural and urban areas of the country [13[

Heterotrophic bacteria are widely used as an indicator of drinking water quality. Some heterotrophic bacteria may be potentially pathogenic and cause secondary infections in patients with weakened immune systems. Among heterotrophic bacteria, Acinetobacter, Aero monas, Flavobacterium, Klebsiella, Legionella, Moraxella, Mycobacterium, Ceratia, Pseudomonas and Xanthomonas have been associated with opportunistic infections [5.[

In drinking water piping networks, measuring the HPC index can indicate the condition of the distribution network. Microorganisms grow normally in water as well as on water-contact surfaces as biofilms. The growth of microorganisms in water after treatment is known as regrowth. This growth indicates high levels of HPC in water samples. High levels of HPC are especially seen in stagnant parts of water piping systems, household connections, bottled water, and in equipment such as hardeners, carbon filters, and water coolers [20.[

Due to the fact that HPC is directly related to temperature, measuring HPC in hot seasons, especially in July and August, from the drinking water distribution network is necessary. Considering that the source of drinking water supply in Zahedan is surface water sources and winds of 120 Sistine fasting is more in summer and turbidity is also directly related to HPC. Measurement of TOC and turbidity is also necessary in summer. Accurate counting of this type of bacteria to control the process and guide the distribution of safe water in water supply networks is an important factor to eliminate defects in facilities from supply sources to consumption points.

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