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# EVALUATING THE DRINKING WATERS MICROBIAL FLORA OF REVERSE OSMOSIS TREATMENT SYSTEMS IN KASHAN CITY DURING SUMMER AND AUTUMN (2015)

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**Abstract**

According to the various difficulties of Distillation desalination system, currently the membrane technology such as Reverse Osmosis (RO) is more useful. High concentration of dissolve solids in supplying water resources in Kashan caused a bad taste and reduced the consumer's desire for using such water, and often they used treated water in a way that at the conducting time of this study, 20 centers treated the water by RO method in the city. Therefore, this study evaluated the microbial flora of produced drinking water of RO treatment system in Kashan city during summer and autumn 2015. In this study the census method have used and the sampling have done from all the RO water treatment centers of the city. Three input, output and after storage, samples have taken. The evaluation of the temperature, residual chlorine, TDS, the storage time, and RO membrane operation time in samples have done. The HPC test has done on the samples in the laboratory and the positive plates have identified in terms of bacterial spices. The tests have repeated in four months of the year from July to October. The results showed 95% residual chlorine in the input samples and the HPC test for these samples showed no contamination. The sample contamination after treatment and the tank were 8 and 15% respectively. In addition, the most contamination level have related to September, which the samples temperature in this month reach its highest level. The Pearson coefficient results showed that there was a significant relation between the Heterotroph colonies number and the residual chlorine and temperature parameters, which was consistent with Karami et al. in Kermanshah and Dobaradaran et al. in Esfahan studies. In addition, there was a significant relation between the Heterotroph colonies number and storage time and RO membrane operation time.

**Keywords:** Water Treatment, RO, Microbial Flora, HPC, KashanCity

## Introduction

The drinking water quality in terms of the physical, chemical and microbial has an important role in consumer's health and hygiene(1-5). Due to the growth of population, increasing the demand of water in drinking and industrial consumption and the quality reduction of water resources particularly in low water region like Middle East, today using the desalination system as an advanced method for water treatment have a great increased. The distillation and membrane technology are among the most common methods for water distillation (6 and 7). Due to the various difficulties of distillation desalination system, currently the membrane technology such as RO has more application (7-10). In Iran in terms of production capacity the RO, MSF and MED process were in the ranking of one to third (11). The overall principle of RO was the osmosis phenomenon, which in it a semi-permeable membrane separated two solutions that contain different amount of chemical material and the membrane passed the dissolved component under the osmosis pressure effect. In the RO system the pressure applied to the more concentrated side and caused the pure water to driven from concentrated side to the diluted side (12). RO work continuously and could reduced the TDS of raw water to 99%, which in practice the TDS reduction to 95% is natural. The remove of bacteria, viruses and other microorganism with RO is 100%. But due to the incomplete sealing of the RO system a small amount of these impurities have entered the treated water. For treatment of the usual water with the TDS of about 500 ppm or more, the RO is a quite reassuring method (13). During past years, some studies have done about drinking water and produced water with desalination devices quality in some part of the country, which mostly include RO process. Dehghani et al. (2012) evaluated the chemical and Microbial quality of the desalination device in Qeshm city. The results showed that the output waters microbial quality of RO unit in terms of MPN and EC have no contamination (14). Karami et al. (2013) evaluated the HPC indicator of drinking water distribution network of Kermanshah. The results showed that all the samples have the HPC level of lower than the standard and the standard and they find a significant relation between the Heterotroph colonies number and some chemical parameters. In this way the colonies number, have an invers relation with residual chlorine and a direct relation with turbidity (15). Dobaradaran (2005) evaluated the HPC level and *Aeromonas* bacteria in drinking water network of Esfahan. The results showed that all the samples were positive in terms of HPC with the maximum number of 21 cfu/100ml. In addition, the increased of the temperature and turbidity and the reduction of PH caused the increase of the heterotroph bacteria number and the positive cases of *Aeromonas, and* positive cases of *Aeromonas* (16). Jafari Poor et al. (2008) have done a research on the domestic *Aeromonas* desalination unit in Qom city. The results showed that the output TDS level of these devices were 21±235, which was one-fifth of the input level and the pH level reported 0.2±0.7 which in comparison with the input pH that was in the range of 6.5-9 were closed to the neutral and stable form (17). Miran Zadeh and Rabbani (2008) evaluated the chemical quality of input and output desalination unit in Kashan city. The results showed that the average concentration of the output waters quality parameters from desalination device for total hardness was 118 g/l and for TDS, Nitrate, Fluorine, Sulfate, and chloride were 245.18, 2.46, 0.2, 24, and 68 mg/l respectively. In addition, the pH have evaluated in the range of 6.8-7 (18). Kashan in terms of climate have two type of mountainous and desert. The north and the east part of the city includes a wide plant plains with hot and dry desert climate and the west and south part of it includes mountains and foothills with temper and mountainous climate (19). High concentration of dissolved solids in water supplying resources of Kashan caused a bad taste in it and reduced the consumer's desire for using it (20). According to the development of new technologies in drinking water treatment system in the beginning of 2001, the use of desalination device with the RO system became common in Kashan city, In a way that at the time of conducting this study 20 desalination device was active desalination in Kashan by private section. Although there were several studies in relation with RO system in the country, but still there is a need for conducting more studies about microbial quality of produced water by active RO system. Therefore, the aim of this study is to evaluate the microbial flora of drinking water, whichproduced by RO water treatment system in Kashan during summer and autumn of 2015.

**Material and Methods**

This is a cross-sectionalstudy**,** which evaluated the input and output waters quality of RO device in desalination center of Kashan city. After identifying water treatment centers based on the timetable, we referred to the water treatment center and after identifying the devices and equipment's position, first the microbial sampling have done from the input position based on the A9060 method of water and wastewater standard method test, edition 20 (21). For evaluating the presence of *Escherichia coli*, *Staphylococcus aureus, Pseudomonas, Citrobacter, Klebsiella, Enterobacter, and catarrhalis*, HPC test have done. Also at the sampling, second the evaluation of residual chlorine, TDS, temperature, storage time and membrane operation time have done for measuring the effect of these parameters on bacterial growth in RO system. The term of storage, means the time that the treated water remains in the storage tank after the treatment process until it have purchased by a customer. This time calculated based on the day and the way of calculating it is by asking the water seller, Because the declared time have recorded on the form at the sampling time. In addition, the membrane operation time means a time, which the treatment system have, worked until the membrane have replaced and calculated based on the month. The replacing membrane time have asked from the operator and the interval time of replacing, until the sampling have determined based on the month. All these activities repeated for the faucets after treatment and storage. For collecting the microbial samples a 25 ml container and for chemical sample a four lit container have used. The bacterial determination test HPC have done based on the instruction of B9215 water and wastewater standard method test edition 20, and *Escherichia coli, Staphylococcus aureus, Pseudomonas, Citrobacter, Klebsiella, Enterobacter, and catarrhalis*based on the practical technique of standard method book in determination laboratory (22). The residual chlorine parameter have measured by DPD Chlorimetry kit of Amur Company and TDS and temperature by TDS meter model C-275 Aquawin. All the mentioned parameter have evaluated for each sample by three time repeating. All of the sampling process have done in four consecutive month in two season of summer and autumn by the mentioned method.

The results of the evaluated parameters have analyzed by excel and SPSS software and the Pearson correlation coefficient have used for determining the relation between variables.

**Results**

- **Microbial contamination**

The results of the HPC have presented in table1 based on the cfu/ml of each sample.

As it have seen in table1 from 80 samples of the after treatment stage in water treatment centers, 8.8% of the samples were positive. In addition, the counted colonies in this stage is lower than 100 number and from 80 samples of water treatment center in the stage of after storage tank 15% of the samples were positive and 70% of them have the colonies number of more than 100 based on the ml.

**Table-1: Number of counted heterotrophic colonies separately sampling steps**

| Code of treatment centers | Number of colonies | Location of sampling | Time of sampling |
|---|---|---|---|
| B | 26 | After treatment | September |
| B | 22 | After treatment | October |
| E | 25 | After treatment | August |
| E | 32 | After treatment | September |
| E | 29 | After treatment | October |
| E | 25 | After treatment | November |
| O | 12 | After treatment | September |
| B | 190 | After reservoir tank | September |
| B | 208 | After reservoir tank | October |

| | | | |
|---|---|---|---|
| E | 125 | After reservoir tank | August |
| E | 180 | After reservoir tank | September |
| E | 217 | After reservoir tank | October |
| E | 200 | After reservoir tank | November |
| I | 45 | After reservoir tank | September |
| J | 68 | After reservoir tank | September |
| N | 110 | After reservoir tank | September |
| O | 36 | After reservoir tank | August |
| O | 125 | After reservoir tank | September |
| Q | 185 | After reservoir tank | September |

**Figure-1** showed the samples number with HPC contamination by separation of sampling place and revealed that from 19 positive samples, more than 50% of them have related to the samples of after storage.

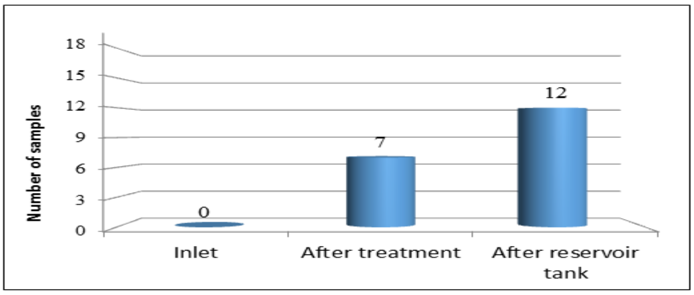


**Figure-1.The number of positive samples HPC by sampling place**

Figure2 showed the positive samples of each month. According to the figure, 53% of the positive samples have related to September month.

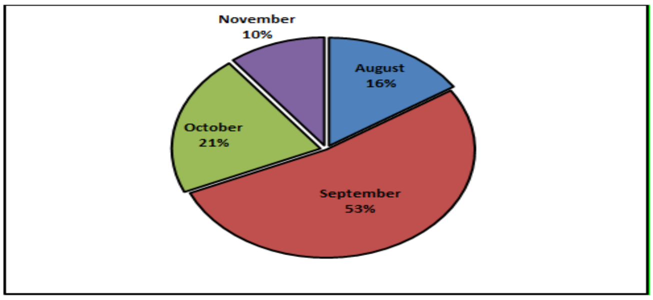


**Figure-2.The number of positive samples HPC by sampling month.**

The identified bacteria include five spices of *scherichia coli, Pseudomonas, catarrhalis, Enterobacter and Citrobacter*which the sampling date and their frequency have presented in table2.

**Table-2: The frequency of detected bacteria by sampling month.**

| Month / Type of bacteria | **August** | **September** | **October** | **November** |
|---|---|---|---|---|
| Escherichia coli | 1 | 3 | 1 | - |
| Pseudomonas | 2 | 7 | 4 | 2 |
| Moraxella catarrhalis | - | 2 | - | - |
| Enterobacter | - | 1 | - | - |
| Citrobacter | - | 1 | - | - |

As it have observed from 19 positive plate, 63% of the samples have *Pseudomonas* contamination and the rest of them have related to *E. coli Citrobacter*, *Enterobactercatarrhalis* with 21%, 8%, 4% and 4% contamination respectively.

- **Residual chlorine**

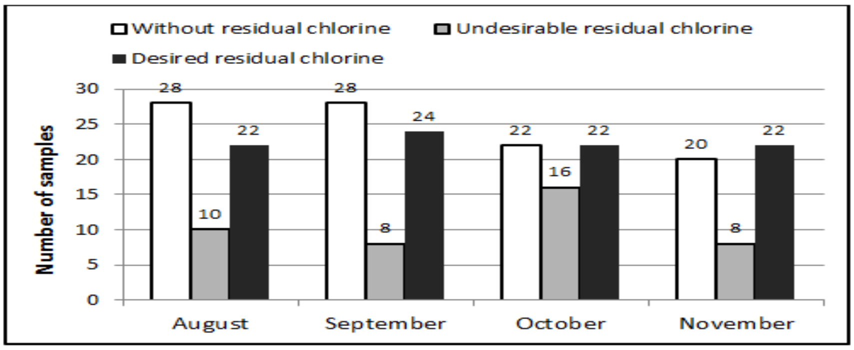


**Figure-3. The frequency of samples with chlorine and withoutchlorine by sampling month.**

As it have seen in figure3 classifying free residual chlorine have done based on the national standard number 1053, the Iran industrial research and standard institute (23), and the Chlorimetry guideline and recording the results of the health ministry which the desirable free residual chlorine amount have determined 0.5-0.8 in three desirable, undesirable and zero category. The desirable free residual chlorine means the amount that was in the desirable range and the zero residual chlorine means the samples have no residual chlorine and the undesirable residual chlorine means the samples have more or less chlorine than the mentioned range. In the august and September month from 60 samples, 46% of them do not have residual chlorine and this number for September and October were 36 and 33% respectively. In addition, the number of samples without chlorine has reduced during the time respectively. In a way that the without chlorine samples in autumn were less than summer.

In figure-4, the evaluated chlorine of the input water of all the water treatment centers have showed separately.

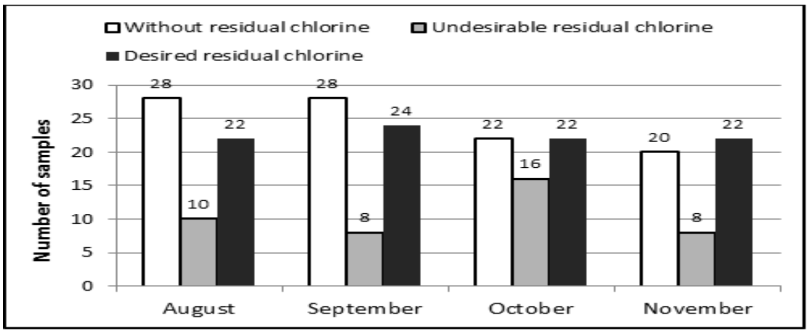


**Figure-4. The frequency of samples with chlorine and without chlorine in inlet of treated water centers by desirability and sampling time.**

The presented results in the above figure showed the fact that 95% of the collected samples from the inputs have chlorine and the existed chlorine were in the standard range.

Figure5, showed the desirability frequency's figure and lack of the residual chlorine in the samples of after storage in the water treatment centers.

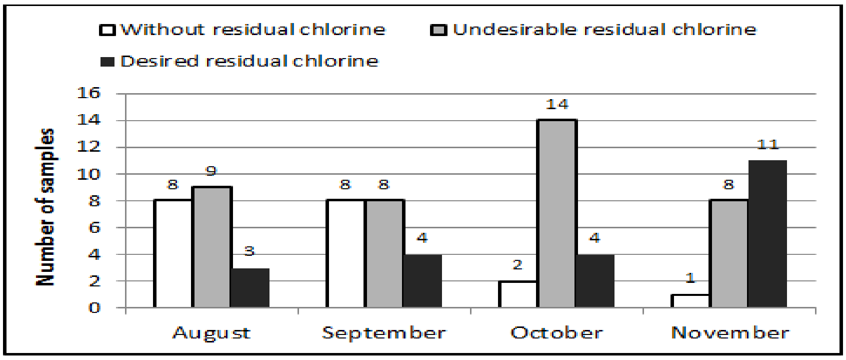


**Figure-5. The frequency of samples with chlorine and without chlorine after storage in treated water centers by desirability and sampling time.**

The network water chlorine by passing through activated carbon filter, which placed before RO membrane, reach to zero, however, after treatment would added to the storage tank manually or automatically by chlorinator or operator. The number of samples with chlorine during the sampling time has increased after the tank, in a way that the number of the samples without chlorine become one in November.

- **Temperature:** The evaluated temperature in different times and places have presented averagely in figure 6 and 7.

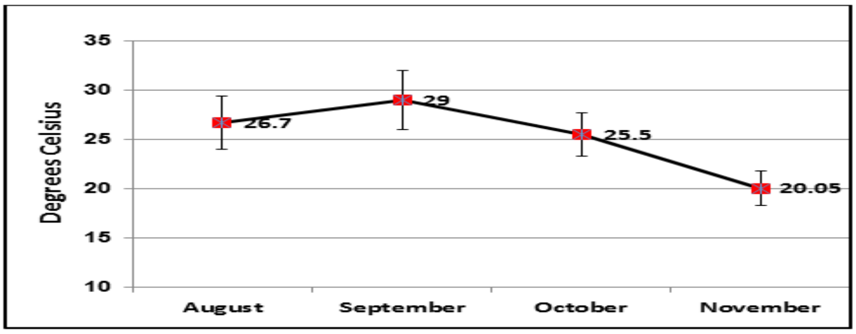


**Figure-6. The average of measured temperature by each sampling month.**

As it has seen in figure6, the average of evaluated temperature in all places during the sampling time have reduced in September month.

Also in figure 7, it has seen that the average of the temperature from input to after storage increased respectively.

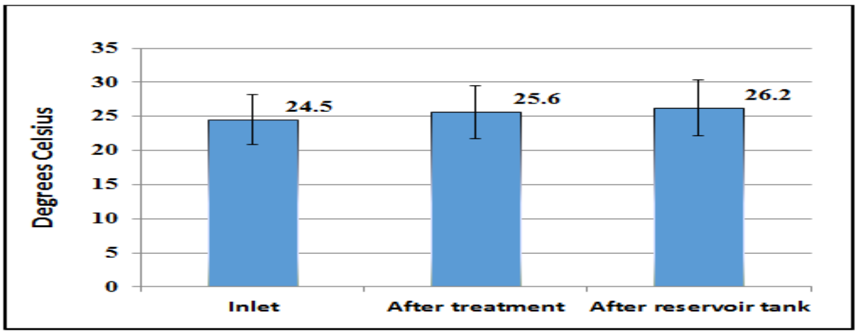


**Figure-7. The average of measured temperature by each sampling place.**

- **TDS**

The results of the TDS evaluation have presented averagely which this information have presented in the below figures by the separation of sampling places and times.As it has seen in figure 8, the dissolved solids amount have reduced after September during the sampling time.

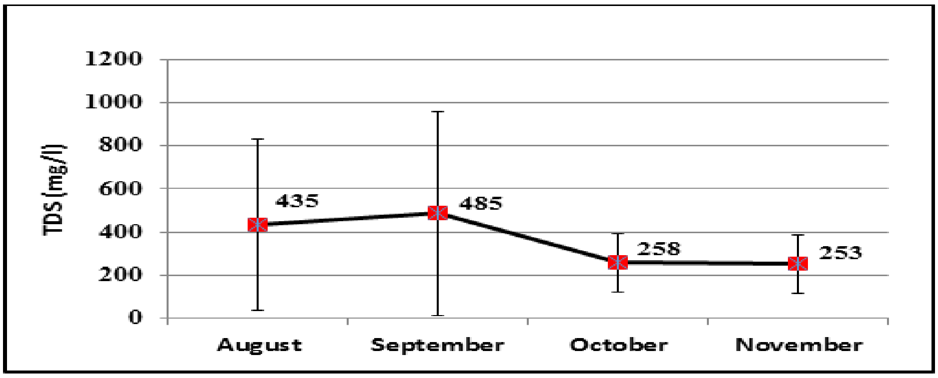


**Figure-8. The average of measured TDS by each sampling month.**

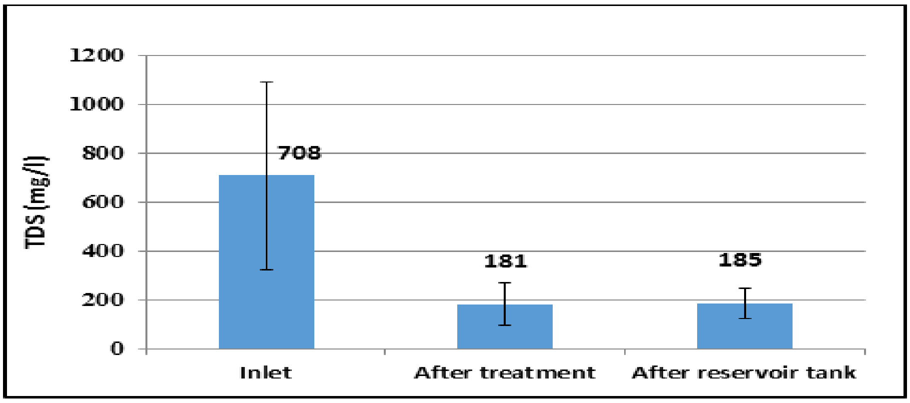


**Figure-9. The average of measured TDS by each sampling place**

As it has seen in figure9, the samples TDS of water treatment center reduced to one-fourth of its initial amount after the treatment.

- **Storage time**

The storage time have applied only for the samples of after tank storage, which its results have presented in figure 10, as it seen in figure the storage time of water in water, treatment centers during the sampling have increased.

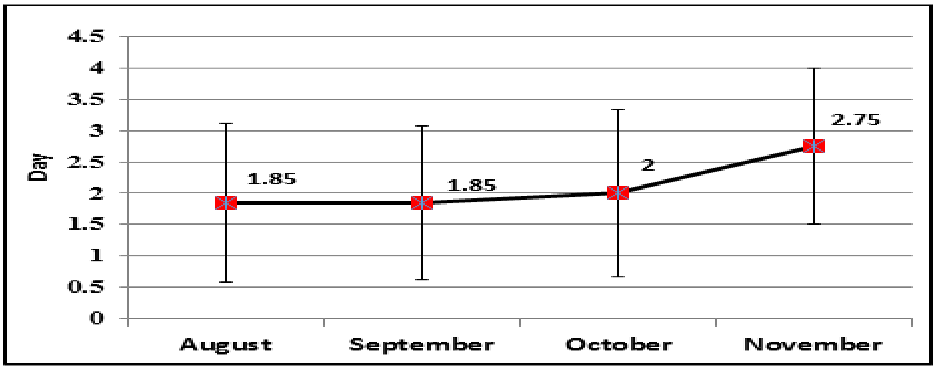


**Figure-10. The average of storage time of treated water in treated water centers by each sampling month.**

- **RO operation time**

The RO operation time have applied for the collected samples after the treatment and the samples of the after the storage tank, which this time was based on the month. The average operation of the membrane have presented in figure 11 by the sampling month separation.

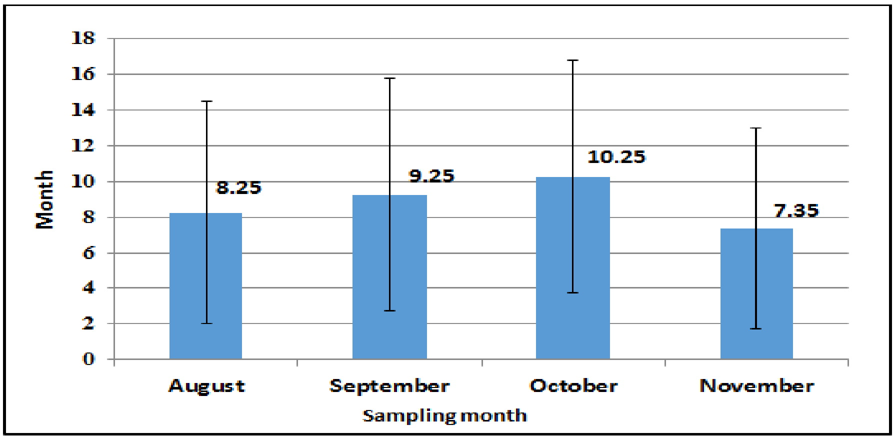


**Figure-11. The working time average of membrane by each sampling month.**

**Discussion and Conclusion**

- **Microbial contamination**

The common indicators, which used in the evaluation of water microbial quality, include the determination of *coliform* and *E.coli*bacteria presence. In addition, HPC is also among the used indicators as a supplement for *coliform* indicator (20). According to the fact that the HPC standard for drinking water was 500 cfu/ml (24), and the counted colonies in all positive samples were less than 500. Therefore, all the samples were in the standard range in terms of HPC, which this result is consistent with Sartory DP (2004) in England (25) and BeateHambsch et al. (2004) in Germany (26) studies.

According to the criteria of treated drinking water in desalination system, which have provided by health and energy ministry,it have declared that the standard number was less than 100 colonies per ml. Therefore, it couldhave said that the produced water of all the water treatment centers were in the standard range after the treatment stage in terms of the heterotroph bacteria number. In addition, it has observed that from 80 collected samples from water treatment center after the tank, 15% of the samples were positive. This number which was more than the positive samples number after the treatment stage showed the increasing effect of the storage on the positive heterotroph cases. The Pearson correlation test showed that there was a direct and significant relation between the heterotroph colonies number and storage time with the decision criterion of less than 0.05. Also from 12 positive cases, 75% of the samples were higher than the standard limit of 100 colonies/ml.

The figure1 showed that from 19 positive samples more than 50% of them have related to the samples of after storage, which its reason could have been not observing health principles in water storage and unprincipled operation of tanks. According to the fact that the input water of treatment process have not shown any positive heterotroph case, the incidence of some positive case after the treatment process showed the technical defects in water treatment path such as improper membrane coupling and fitting and numerous leaks in pipelines.

In addition, the lack of positive samples in the input, which is from residual chloride, were in the municipal network, which presented in figure4 and showed that there was no problem with input resources of water.

- **Residual chloride**

According to the Pearson correlation coefficient it have observed that there was an invers significant relation between the residual chloride and heterotroph colonies number and decision criteria of less than 0.05, which these results are consistent with Karami et al. study in Kermanshah (2013) (15) and Dobaradaran et al. Esfahan (2005) (16) and Mosaferi et al. in Tabriz (2010) (27). The increase of the residual chlorine could have because of the monthly continuous inspection of the water treatment centers, which due to the evaluation and declaring the Chlorimetry results to the operators continuously caused a kind of motivation in adding residual chloride.

- **Temperature**

As it has seen in figure7 the water, temperature increased after the treatment and storage. The reason of the temperature increasing after the treatment is the fact that water passed through various stage of treatment and transportation from under high-pressurepump, which this fact, after storage and the time increasing of remaining in the environment temperature would cause the increase of water temperature. The Pearson correlation coefficient was 0.155 between two temperatures and heterotroph colonies number and the decision criteria was 0.016 and less than 0.05.

Therefore, the correlation coefficient is significant. It means there was a significant relation between temperatures and HPC, which these results are consistent with Wolfgang Uhl et al. (2003) in Germany (28) and Karami et al. (2013) (15) and Dobaradaran et al. (2005) Esfahan studies (16).

- **TDS**

As it have seen in figure 9 the TDS amount after treatment stage reduced significantly, After the RO operation. The total dissolved solid have removed and TDS reach to zero. However, for treating it for drinking water some amount of input water would have added to it, which this fact caused the TDS increasing in water. It have seen that some amount of TDS have reduced during the time which it is because of the seasons air temperature become cold and water consumption reduced which this reduction in consuming cause the loss using of water from underground Aquifer which have higher TDS. As a result, the Zayandeh Rood water percentage, which have lower TDS become higher in consumed water, and the TDS of the consumed water reduced in cold seasons of the year. The Pearson correlation coefficient showed that there was not a significant relation between heterotroph colonies number and TDS level of the water.

- **The storage time**

As it have seen in the related figure the storage, time in autumn season is higher due to the possibility of the air coldness and less consumption of water for drinking. Therefore, the requirement for the consumption becomes less and the storage time of the water in the tank have increased. The Pearson coefficient between two storage time and heterotroph colonies number variables have done and revealed that there was a direct and significant relation between these two parameters, which the results are consistent with Wolfgang Uhl et al. (2003) in Germany (28) study.

- **The membrane operating time**

The results showed that the membrane operating time have increased during the membrane operating time and reduced in October in a way that in this month it become less than other months and it is because of the membrane replacing in water treatment centers in cold months of the year. The membrane replacing have done simultaneously and required a lot of time two or three days to prevent the lack of selling and economic losses. The replacing have done in cold months of the year in order to storage meet the buying demand, Which this fact caused the reduction of membrane operation time to one month and in fact, the average have reduced. The results of the Pearson coefficient test showed that there was a direct and significant relation between RO membrane operation time and the heterotroph colonies number with the decision criteria of less than 0.05.

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