# Comparison of Fluoride Level and Bacterial Count in Tap Water, Reverse Osmosis Purified Water and Non-reverse Osmosis Purified Water

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**Water is one of the most important elements for all forms of life. With the general population concerned with polluted water, tendencies toward purchasing bottled water and water filtration systems are high which is the basic need for life and questions are being raised as to whether fluoride and bacterial content in public water supplies is affected by these filters. The objective of this study was to determine and compare fluoride level by spectrophtometric method and bacterial count by CFU (colony forming units) in tap water, reverse osmosis purified water and non-reverse osmosis purified water. Results of this study showed that the mean fluoride level was 0.06±0.04 in reverse osmosis purified water, 0.18±0.06 in non-reverse osmosis purified water, 0.20±0.06 in tap water. Mean bacterial count was 3.8±3.70 in reverse osmosis purified water, 8.66±6.63 in non-reverse osmosis purified water, and 21.6±5.31 in tap water. Considering the beneficial effects of fluoride on dental caries prevention, this paper highlights that when drinking water is subjected to water purification systems these system reduced fluoride content significantly below the optimal level along with reducing the bacterial count and playing a major role in initiation of dental caries.**

**Key words:** Water purifiers, Tap water, Reverse osmosis (RO), Colony forming units (CFU), Point of entry (POE), Point-of-use (POU), Parts per million (ppm).

Water is one of the most important elements for all forms of life on earth and is essential for the composition and renewal of cells. Notwithstanding, human beings increasingly continue to pollute the reserves which still remain, provoking illnesses that can jeopardize the population[1]. Domestic water treatment systems include water conditioners, softeners and water filters. These systems fall into two basic categories: point of entry [POE] and point-of-use [POU]. Point-of-entry water treatment systems treat all the water entering and being used at home. Point-of-use water treatment systems, on the other hand, treat part of the water in the house water distribution system, usually at one faucet. Reverse osmosis water purification, distillation and activated carbon filtration are examples of POU water treatment systems[2]. In a Reverse osmosis [RO] separation process, fed water flows across a membrane surface under hydraulic pressure, water molecules permeate through the membrane while particles, or

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even ions and organic molecules are rejected by the mechanism of size exclusion or competitive diffusion[3]. There are several basic types of water purification systems, e.g., reverse osmosis water purification, distillation, filtration, oxidation, disinfection, cation exchange softening, anion exchange, activated carbon, etc. These systems can be used alone or in combination[4]. With the general population concerned with polluted water, tendencies toward purchasing bottled water and water filtration systems are high and questions are being raised as to whether fluoride and bacterial content in public water supplies is affected by these filters. In homes, when employing a domestic purification system, the fluoride levels of drinking water need to be monitored, as the children in these houses may need fluoride supplementation[2].

The purpose of this study was to determine and compare fluoride levels and bacterial counts in reverse osmosis purified water and non-reverse osmosis purified water and to compare these values with those of municipal tap water.

## MATERIALS AND METHODS

### Fluoride Analysis

After obtaining the ethical clearance the present comparative study was conducted. The inclusion criteria were domestic reverse osmosis purified water and non-reverse osmosis purified water which are connected to municipal water. Exclusion criteria were Water purifiers which are exceeded the manufacturer's water usage guidelines, water purifiers which are not working effectively. A total of 35 samples were selected for the study. Sample size of 15 from RO purifiers, 15 from non- RO purifiers and 5 from Municipal tap water was selected to achieve 80 % power to detect difference of 0.75 [marginal error] between the three experimental group. The study was scheduled over a period of 45 days.15 separate samples from [RO] water purifying system and non-RO water purifying system were collected in 200ml sterile container from residence [domestic] area in which all purifiers were connected to Municipal tap water, 5 water samples from municipal water processing plant were collected on unannounced visit. The water samples were collected directly from purification plant because the quality of tap water collected outside the water plant e.g. at a residence or business could be altered by residential / commercial plumbing or filter systems. To avoid the possible reaction of fluoride with glass, water samples were collected and stored throughout the experiment period in plastic containers [polyethylene cans] that had been previously rinsed twice with deionized water to remove any fluoride residue. All water samples were transported to the Department of Biotechnology and Civil Engineering for fluoride and bacterial analysis. At the laboratory, technicians measured 200-mL samples of water from sterile containers and all the samples were given a code by investigator. The lab technicians were blinded to the type of water contained in the sample in order to avoid any potential bias and human errors. Laboratory test used were Spectrophtometric Method to determine the concentration of fluoride in different samples of water. This method relies on the fact that when fluoride reacts with certain zirconium dyes, a colourless complex anion and a dye are formed. The complex, which is proportional to the fluoride concentration, tends to bleach the dye which therefore becomes progressively lighter as the fluoride concentration increases. In the case of the fluoride ion reaction with Zr-SPADNS [sodium 2-[parasulphophenylazo-] 1,8-dihydroxy-3,6-naphthalene disulphonate], the resulting coloured complex is measured in a spectrophotometer at 570 nm. Jenway Version 6505, UV/vis spectrophotometer was used for fluoride level analysis which is expressed as ppm (*parts per million*).

### Bacterial Analysis

The bacterial count was derived from 100mL of each water sample. Water was cultured quantitatively and levels of bacteria were calculated as CFU per millimetre. After incubating in Nutrient agar at 37$^{o}$ C for 2 days CFU were counted and colony forming units (CFUs) per milliliter were calculated. Samples with confluent colonies were re-filtered at 10 mL and 0.1 mL per sample and CFUs per milliliter were calculated. The SPSS software version 17 was used for the analysis of the data. ANOVA test was used to compare the difference in fluoride content of drinking water between various groups.

## RESULTS AND DISCUSSION

Mean fluoride level was 0.06±0.04 in reverse osmosis purified water, 0.18±0.06 in non-reverse osmosis purified water, 0.20±0.06 in tap water. Variation within the groups, the difference in mean fluoride among the groups was found to be statistically significant [p<0.001]. Distribution of samples based on mean bacterial levels. Mean bacterial count was 3.8±3.70 in reverse osmosis purified water, 8.66±6.63 in non- reverse osmosis purified water, and 21.6±5.31 in tap water. Variation within the groups, the difference in mean bacterial count among the groups was found to be statistically significant [p<0.007].

The use of water purification systems has increased dramatically since its inception many years ago and is gaining popularity as people are becoming increasingly concerned about the pollutants present in public water supplies which are hazardous to their life. The purpose of using these systems is to remove or modify impurities in the water. Most domestic water users seek to improve the taste and quality of their drinking water by using a relatively simple filter to remove unwanted impurities such as inorganic salts, heavy metals, suspended and colloidal matter. Maintenance of the microbiological quality of water has been used as an important means of preventing waterborne disease throughout the twentieth century. The commonest microbiological tests done on water are for coliforms and Escherichia coli [or faecal coliform][5]. Diseases related to contamination of drinking-water constitute a major burden on human health. Interventions to improve the quality of drinking-water provide significant benefits to health. The potential health consequences of microbial contamination are such that its control must always be of paramount importance and must never be compromised at any cost[6]. In present study bacterial count in [CFU] was less in RO water purified water [3.8 ± 3.7] compared to non- RO purified water [8.6± 6.6] and municipal tap water [21.6±5.31]. Similar finding by Park et al [2010] showed more than 99% of bacterial cells in the tap

**Table 1**. Mean fluoride level in three groups

| Purifying System | Mean | Standard Deviation |
|---|---|---|
| RO | 0.06 | 0.04 |
| Non RO | 0.18 | 0.20 |
| Tap Water | 0.20 | 0.06 |

RO- Reverse osmosis

**Table 2.** Variation within the groups

| Source of Variation | df | Sum of Squares [SS] | Mean SS | F | P-Value |
|---|---|---|---|---|---|
| Between Groups | 2 | 0.125 | 0.063 | 22.362 | <0.001* |
| Within Groups | 32 | 0.090 | 0.003 | - | - |
| Total | 34 | 0.215 | - | - | - |

df- degree of freedom, SS- Sum of Squares, F- Fischer value, P- Probability

**Table 3.** Mean Bacterial count [CFU] in three groups

| Purifying System | Mean | Standard Deviation |
|---|---|---|
| RO | 3.8 | 3.70 |
| Non RO | 8.6 | 6.63 |
| Tap Water | 21.6 | 5.31 |

RO- Reverse Osmosis

**Table 4.** Variation within the groups

| Source of Variation | df | Sum of Squares [SS] | Mean SS | F | P-Value |
|---|---|---|---|---|---|
| Between Groups | 2 | 271.276 | 135.638 | 5.877 | 0.007* |
| Within Groups | 32 | 518.267 | 703.696 | - | - |
| Total | 34 | 789.543 | - | - | - |

df- degree of freedom, SS- Sum of Squares, F- Fischer value, P- Probability water and was retained by RO membranes, leaving <50 cells/ml in the permeate water. For microbial analysis, WHO [1993 and 1996] recommended that CFUs/ml should be 0 after 2 days at $37^o$ c [if water is disinfected] or 10 [if water is not disinfected]. In a study conducted by A. Kuchewar et al [2012] showed 98% to 99% efficiency in removal of microbiological load by branded water purifiers and 95% efficiency in removal by local water purifier[7]. The primary issue for membrane technology [RO] is membrane fouling, water contains both organics [dissolved and suspended] and high concentration of multivalent ion species that adhere to membrane surface and/or pore entrance. As a result, membrane performance can be seriously deteriorated[3]. RO purified water had significant effect on fluoride level in drinking water [mean-0.06±0.04, $p<0.001$]. Fluoride levels in RO, non-RO purified water and tap water were below optimum level. [0.7-1.2]. A study by Prabhakar et al [2008] showed that the systems based on reverse osmosis, and Reviva [R] showed maximum reduction in fluoride levels, the former proving to be more effective than the latter; followed by distillation and the activated carbon system, with the least reduction being brought about by candle filter. Some manufacturers claim to remove specific elements such as chlorine and there in, the possibility exist that the fluoride content would be affected. Job son et al [2002] and Glass R G et al [1991] showed strong evidence that systems such as those based on RO and distillation removed a substantial amount of fluoride, but tests of those based on activated carbon have given contradictory results[4]. The variations in fluoride reductions by these systems may be attributed to differences in the type of membrane and differing pressure lines; regular maintenance of equipment may also influence its efficacy in fluoride reduction. Most of the studies [*Ong YS et al, Buzalaf MA et al]* had used findings of the effect of Water filtration systems on fluoride levels to advice against unnecessary prescription of fluoride supplements[8,9]. A study by Robinson et al, in which water was passed through softeners and a conditioner, tested for fluoride concentration using a specific ion metering device, revealed no alteration in levels when compared with controls. Similar experiments of filtered water demonstrated that highly significant amounts of fluoride ion were removed. In one filter tested, 90% of the fluoride content was lost in the filtration process[2]. At one of the working meetings for preparation of guidelines for drinking water quality, the World Health Organization [WHO] considered the issue of the desired or optimum mineral composition of desalinated drinking water by focusing on the possible adverse health effects of removing some substances that are naturally present in drinking water [WHO 1979][6]. Excessive consumption of fluoride above the optimal level causes dental and skeletal fluorosis. But, in drinking water, to prevent dental caries, the fluoride content needs to be reduced to the optimal level. The importance of the fluoride ion in cariostasis is well documented. In some studies, fluoride supplementation did not affect the likelihood of developing caries, which may have been due to low compliance or over-reporting of their use. According to American Dental Association [ADA] and AAPD [American Academy of Pediatric Dentistry] guidelines, children receiving water fluoridated at less than 0.7 ppm should receive fluoride supplements. Accurate prescription of fluoride is possible only when the water fluoride concentration is known. One should also take into account the carry-over or diffusion effect of fluoride[4].

## CONCLUSION

Bacterial count was high in tap water followed by non-RO purifiers and RO purifiers. Systems based on reverse osmosis showed maximum reduction in bacterial count. Fluoride concentration was below the optimal level in RO, non-RO and municipal tap water. Considering the beneficial effects of fluoride on dental caries prevention, when drinking water is subjected to water purification systems these system reduced fluoride significantly below the optimal level.

**Recommendations**

Regular surveillance as well as monitoring of water purifiers, pipelines, and water treatment plants to meet required microbial standards is recommended. Monitoring or regulating the optimal range of fluoride in water purifiers as well as tap water should be recommended.

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