

## Total Nitrite and Nitrate Levels in Public Water Supplies of Istanbul City

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Water quality has a major importance for public health. However, nitrite and nitrate levels in water are still an important contamination issue in the world. The aim of this study was to determine the levels of total nitrite and nitrate in public water (tap water) and wells from different districts of Istanbul city and to evaluate the seasonal variation from November 2013 to March 2014. The average levels in tap waters were highest in March 2014 compared with November, December 2013 and February 2014. Two of the tap water and 3 of the well water samples exceed the maximum acceptable concentration according to the U.S. Environmental Protection Agency (10 ppm). However, these levels were still lower than the maximum allowable limits reported in official documents of Turkey. Also, in order to determine the annual variation, tap water samples were collected between December 2012 and February 2013 and evaluated for total nitrite and nitrate levels. Results showed that total nitrite and nitrate levels during December 2013 and February 2014 were decreased in the ratio of 2, 10 and 18% compared to the same months during December 2012 and February 2013. In conclusion, our results suggested that due to seasonal and annual variations, nitrite and nitrate levels of waters should be routinely controlled to prevent various health hazards.

**Key words:** Nitrite, Nitrate, Tap water, Safety

### İstanbul Şebeke Sularında Toplam Nitrit ve Nitrat Düzeyleri

Suyun kalitesi halk sağlığı için çok önemlidir. Ancak sularda nitrit ve nitrat düzeyleri hala önemli bir kontaminasyon problemidir. Bu çalışmanın amacı İstanbul'un farklı bölgelerinden toplanan musluk suyu ve çeşme sularında toplam nitrit ve nitrat düzeylerini tespit etmektir. Bu amaçla çeşitli bölgelerden musluk suyu örnekleri Kasım 2013'den Mart 2014'e kadar toplanmış ve değerlendirilmiştir. Su örneklerinde ortalama toplam nitrit ve nitrat düzeyleri Kasım, Aralık 2013 ve Şubat 2014 ile karşılaştırıldığında Mart 2014'te en yüksektir. ABD Çevre Koruma Ajansına göre, musluk sularından iki tanesi ve kuyu sularından üç tanesi toplam nitrat ve nitrit için maksimum kabul edilebilir konsantrasyonu (10 ppm) aşmaktadır. Ancak tüm örneklerde bu düzeyler Türkiye'nin resmi dökümanlarında bildirilen maksimum kabul edilebilir düzeylerin altındadır. Ayrıca, yıllar arası değişimi saptamak için, bir önceki yıla ait su örnekleri de değerlendirilmiştir. Sonuçlar Aralık 2013-Şubat 2014 döneminde toplam nitrit ve nitrat düzeylerinin Aralık 2012-Şubat 2013 dönemine göre %2, 10 ve 18 oranında azalma göstermiştir. Sonuçlarımıza göre, mevsimsel değişim göz önüne alınarak, halk sağlığının korunması için sularda nitrit/nitrat düzeyleri rutin olarak kontrol edilmelidir.

**Anahtar kelimeler:** Nitrit, Nitrat, Musluk suyu, Güvenlik

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## INTRODUCTION

Water quality has a major importance for public health. However, nitrite and nitrate levels in drinking water are still an important contamination issue in the world. The most significant sources of nitrate contamination in groundwater are from intensive fertilizer use in agricultural activities and another major source of contamination is industrial sources (1).

Water with high nitrate concentration is not suitable for human consumption, especially when its concentration exceeds the threshold limit value (50 ppm) recommended by World Health Organization (WHO) (1). The same acceptable limit is also applied in Turkish standards for drinking water quality in public water systems (2). However, some studies reported that nitrate levels in drinking and tap waters exceeded the acceptable limits and consequently population may have been exposed to nitrate levels above 50 ppm (3-5).

High intake of nitrate and its subsequent reduction to nitrite leads to the formation of methemoglobin (MetHb) which is the most detectable sign of nitrite and nitrate toxicity in humans especially in infants (1). Furthermore, pregnant women are more vulnerable to the effects of nitrate due to a natural increase in MetHb levels in blood during the later stages of pregnancy beginning around the 30<sup>th</sup> week of pregnancy (6). Although WHO and Turkish standard recommended the threshold limit value of 50 ppm, the U.S. Environmental Protection Agency (EPA) set the maximum contaminant level (MCL) for total nitrate and nitrite in public drinking water at 10 ppm to protect infants from methemoglobinemia. However, there is no sufficient data on whether chronic exposure to levels below the MCL will produce adverse effects or not (7). In addition, high dietary intake of nitrate leads to the formation of N-nitroso compounds has been also indicated as a risk factor for cancer development in human (8).

The aim of this study was to determine the levels of nitrite/nitrate in tap and well water samples from the most populated city of Turkey, Istanbul, and to evaluate the seasonal and annual variation.

## MATERIAL AND METHODS

### *Chemicals and reagents*

All chemicals used in the study were analytical grade. Ultra-high pure distilled water (Millipore Simplicity UV, France) was used throughout the procedure. A standard calibration solution in the range of 0.5 – 30 ppm of sodium nitrate (Fluka, Germany) was prepared. Nitrate reductase (Sigma-Aldrich, USA) at 1 U/mL concentration was prepared in pH 7.5 phosphate buffer. Disodium hydrogen phosphate (7.2%, v/v) (Fluka, Switzerland) and sodium monobasic phosphate (0.86%, v/v) (Riedel-de Haën, Germany) solutions were dissolved in distilled water and pH was adjusted to 7.5 with phosphoric acid using pH-meter (Mettler, Switzerland). 10.86 M NADPH (Calbiochem, Germany) solution was prepared in distilled water. To prepare fresh Griess reagent, 1:1 mixture of sulfanilamide (1%, v/v) (Sigma-Aldrich, USA) solution in phosphoric acid (5%, v/v) (Merck, Germany) and N-(1-naphthyl) ethylenediaminedihydrochloride (0.1 %, v/v) (Sigma-Aldrich, USA) in distilled water were stirred with sonicator (Bandelin Sonorex, Germany).

### *Sample collection*

16 well and 144 tap water samples (totally 160 water samples) were collected from different districts in Istanbul (Figure 1) for 4 months, from November 2013 to March 2014. In order to determine the year-to-year differences in nitrite/nitrate levels, 72 tap water samples were collected from November 2012 to February 2013. Water samples were stored at 4°C until they were analyzed.

### *Determination of total nitrite and nitrate levels*

Total nitrite and nitrate (NO<sub>x</sub>) levels were measured by using the Griess reaction according to the method by Tracey et al (9). The assay was adapted to standard microtiter 96 well plate. The reaction mixture consisted of sample or standard, reduced NADPH, nitrate reductase and phosphate buffer (pH 7.6). After one hour incubation period, Griess reagent was added into each well and ten minutes later, the absorbance was measured at

540 nm (Microplate photometer, Multiskan Ascent, Finland) to assess the total level of nitrite/nitrate.

*Statistical analysis*

All of the results are expressed as the mean ± standard deviation (SD). The differences among groups were evaluated with nonparametric Friedman-test and Wilcoxon signed-ranks test. p<0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

NOx levels in tap water samples varied from 0.30 to 10.89 ppm (Figure 2). The NOx level of Yeşilköy area in November and Şirinevler in December 2013 were under the detection limit (0.5 ppm). The highest level was obtained from Gaziosmanpaşa (10.36 ppm) and Eyüp (10.89 ppm) districts in March 2014. These results indicated that even the highest detected nitrate levels were still quite below the reference value setting for drinking water in Turkey (2). However, according to EPA standards, both the samples collected from Gaziosmanpaşa and Eyüp seem to exceed the maximum acceptable concentration of 10 ppm (10). It should be considered that natural levels of nitrate in surface waters seldom exceed 0.1 ppm, but waters influenced by human activity normally contain up to 5 ppm, according to Sandru et al. (11). In our study, 31% of the collected samples exceed 5 ppm nitrate level, indicating water pollution.

The mean NOx level of tap waters given in Table 1 showed that, there were statistically significant differences between the months.

statistically increased significantly in December and February in both 2013 and 2014. Furthermore the mean NOx levels from November 2012 to February 2013 were significantly higher than levels during November 2013 and February 2014. The mean NOx levels decreased in the ratio of 23%, 26% and 17% in November, December 2012 and February 2013, respectively (Figure 3). This annual variation may be due to the increased rainfall during 2014 (12), resulting in decreased nitrate concentration (5). In addition, according to Istanbul annual amount of water data, the amount of clean water given to the city was increased by 52x10<sup>6</sup> m<sup>3</sup> from 2012 to 2014 (13), proving evidence of decreasing nitrate levels.

In case of well water, the NOx levels were shown in Figure 4. The Emirgan well water was found higher than the others for each month and the maximum values were detected as 16.31 ppm and 17.93 ppm for November and March, respectively. Both values exceed the MCL for nitrate in drinking water (7). In similar studies conducted in Turkey, the nitrate levels in 83 well waters in Şanlıurfa region were found to be between 0.63-46.61 ppm (14) and between 2.08 and 12.52 ppm in 9 well waters in Kayseri (15). The higher amount of NOx levels in well water compared to tap water samples found in present study supports previous findings that nitrate levels above the MCL are more likely to be found in well waters compared to public water system (7). It is plausible that samples taken from the inside tap would show a lower proportion exceeding the MCL.

However, water is not the only source of nitrite and additional exposure to nitrates by

**Table 1.** The mean nitrite/nitrate levels by 4 months.

	NOx levels (ppm)		
	Min	Max	Mean ± SD
November 2013	0.30	6.21	1.25 ± 1.09
December 2013	0.30	6.51	3.37 ± 1.67 <sup>a</sup>
February 2014	1.23	7.58	3.40 ± 1.46 <sup>a</sup>
March 2014	4.52	10.89	7.78 ± 1.40 <sup>a, b, c</sup>

<sup>a</sup> p<0.05 vs November, <sup>b</sup> p<0.05 vs December, <sup>c</sup> p<0.05 vs February

The mean levels were significantly lower in November 2013 compared to the other months. On the other hand, average levels

other sources can increase the risk of exceeding the limits. For instance, nitrate can be easily transferred to plants via water and

consequently nitrate levels of vegetables tend to increase (16). Additionally, nitrate and nitrite are used in combination with salts as food preservatives, flavoring agent and color fixing agent in various foods such as meat products, cheeses and canned vegetables (2, 17). Convenience foods such as bouillons and ready-made soups are other sources of nitrite and nitrate (18, 19). It is therefore very important to assess the total exposure.

the limits reported in settled value (Turkish Official Newspaper, (17.02.2005, No: 25730) and 2011 WHO Guidelines for Drinking-water Quality).

Considering that the nitrate levels above the 10 ppm in groundwater cause blue babies syndrome in infants of ages 0-3 months and susceptibility of pregnant women to nitrate effects due to a natural increase in MetHb levels around the 30<sup>th</sup> week, the high nitrate levels of Emirgan district's well and 2 tap



Figure 1. The map of samples collected districts in Istanbul.

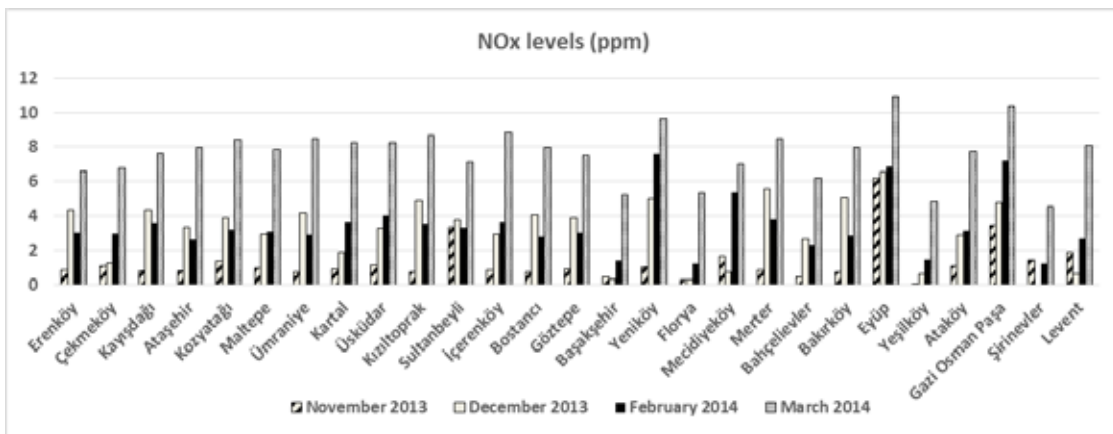


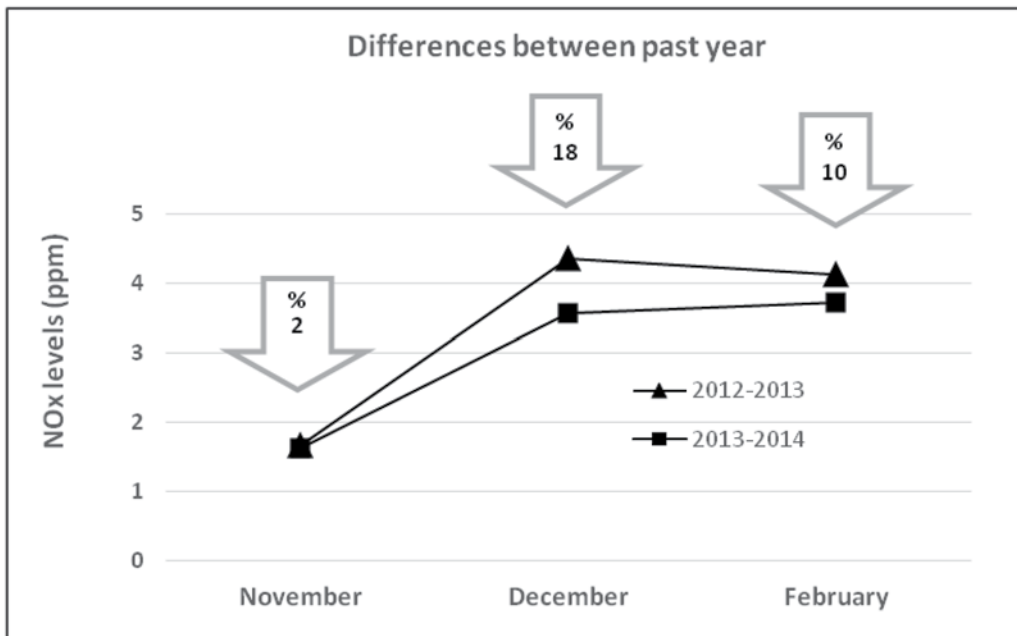
Figure 2. The Nitrite/Nitrate levels of tap water in 27 districts of Istanbul between November 2013 and March 2014.

## CONCLUSION

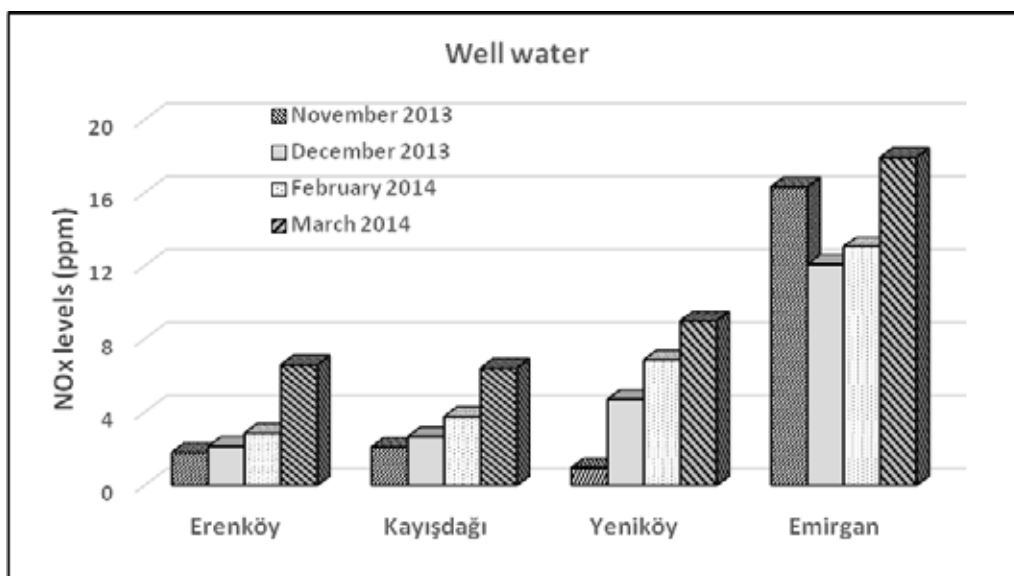
This study demonstrated the effect of seasonal and annual variations, and district changes on nitrate levels in tap and well water. Total nitrite and nitrate levels of all samples analyzed in Istanbul were lower than

water samples may be alarming.

Nitrate and nitrite contamination of waters is one of the most hazardous threats for public health. Methemoglobinemia, carcinogenicity and pregnancy toxicity are some of the important severe toxicities of these substances.



**Figure 3.** The comparison of mean nitrite/nitrate levels with previous year  
\*p<0.05 vs 2013-2014



**Figure 4.** Nitrite/Nitrate levels of 4 well water in Istanbul

However, it should be considered that such health hazards may develop over time, and the seasonal and annual variations of nitrite and nitrate levels in tap/drinking water should be routinely monitored to prevent possible toxicological effects of high nitrite levels.

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