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Determination of nitrate and nitrite exposure and their health risk assessment in 21 brands of bottled waters in Isfahan's market in 2013

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ABSTRACT

Aims: The purpose of this study was to determine of nitrate and nitrite exposure and their health risk assessment in 21 brands of bottled waters in Isfahan's market in 2013.

Materials and Methods: The samples bought from shops in Isfahan city and were analyzed for nitrate and nitrite. The measured concentration of nitrate and nitrite were compared with the labeling data and codex guideline values. Statistical analysis on data was performed with the Kolmogorov-Simonov non-parametric test, the paired *t*-test and the student's *t*-test at $P < 0.05$ of confidence level. The health risk was assessed through chronic daily intake (CDI) and hazard index (HI).

Results: The mean concentrations ± standard deviations based on statistical *t*-test for nitrate and nitrite were 1.42 ± 0.55 and 0.02 ± 0.015 mg/l, respectively. Based on results nitrate concentrations have a statistically significant difference with labeling values. There was no labeling value for nitrite on the samples. The amounts of CDI for nitrate and nitrite were 1.5E-04 and 3.3E-04 mg/kg/day, respectively and their related HI was same and below 1.

Conclusion: According to the results of the present study, nitrate and nitrite levels in the studied bottled waters were not found in concentrations considered hazardous in terms of public health. The label values not provide reliable data for consumer. The health risk of bottled water consumers from nitrate and nitrite exposure in Isfahan were in the acceptable range.

Key words: Bottled water, brand, health risk assessment, nitrate, nitrite

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INTRODUCTION

Consumption of bottled water is growing repetition and is something a necessity rather than a select[1] because people living in developed countries have proper drinking bottled water.[2] Water quality can have a main effect on both individuals and communities health.[3] It is very important to human healthiness to ensure the safety of bottled drinking water.[4] Drinking water is important for survival and their biological and chemical

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pollution is a serious matter that may have serious health effects.[5] Increasing pollution of tap waters led more and more to consumption of bottled waters.[3] Natural bottled waters are defined as microbiologically healthful water originating in an underground water table or deposit and emerging from a spring tapped at one or more natural or bore exits with a unique and constant chemical composition.[6] It differs from treated water in its original purity and its content of minerals, trace minerals and other constituents, which must remain constant, when it earn of spring.[3] In measuring the quality of drinking water, consumers rely principally upon their senses with bottled waters being perceived as pure, safe and of good taste, thus, their consumption is increasing.[7,8] Polluted waters can contain toxic compounds, insecticides such as Dichloro Diphenyl Trichloroethane (DDT) and heavy metals,[3] usually in trace concentrations, whose effects on human health are inadequately understood or unknown.[5] Bottled drinking water has been occasionally related to diarrhea conditions known as traveller's disease.[7] Pollution of bottled water with nitrate and nitrite affect the health of children more than adults, as they are more susceptible to serious health conditions due to the change in their body size and chemical conditions of the body.[9] Nitrite relates with hemoglobin, causing blood to be less efficient in transporting oxygen and resulting in a disorder known as methemoglobinemia that may occur after a single dose and therefore an acute reference dose (ARD) would be suitable. An ARD for nitrite has not yet been well-known. These are called as exogenous nitrites or nitrates. The human body is able to convert a number of nitrate in food into nitrite that is identified as endogenous nitrite.[10] Exposure to nitrate and nitrite at levels below health-based risk values has been reported to have contrary health effects on infants and children. Infants of ages 0-3 months are at top risk for methemoglobinemia because their normal intestinal flora contribute to the generation of methemoglobin; older children and adults can feel this syndrome, but at upper concentrations of nitrates.[11] Nitrate is used in food products, because it is a reservoir for *in situ* production of nitrite. Nitrates can an allowable additive for cheese[12] and the other food products such as sausage and etc. where it fulfills the functions of preservative, antimicrobial agent and color protective.[7] Nitrate is a final product of biochemical oxidation of naturally nitrogen. However, nitrite is an important indicator of fecal pollution of natural water and is readily oxidized to nitrate by dissolved oxygen.[3] Health effect that was meaningfully associated with nitrate or nitrite exposure during fertility include increased occurrence of intrauterine growth retardation, cardiac defects, central nervous system weaknesses, sudden infant death syndrome and miscarriage.[13] Human activities increase their levels through different point and non-point sources, which finally cause them to be present in our drinking water. Such activities include the unnecessary use of fertilizers that leach into the ground and surface waters and the release of untreated industrial and sewage wastewater.[9] Nitrite is quickly oxidized to nitrate in water and is often discovered in well-oxygenated or chlorinated water.[13] According to the World Health Organization (WHO), the maximum acceptable levels of nitrate and nitrite in bottled water are 50 mg/l and 3 mg/l, respectively.[14] The WHO has also set a temporary guideline level for nitrite in drinking water of 0.2 mg/L for long term exposure.[13] The hazard index (HI) is the mark of a taught awareness to rest content with the degree of accuracy which the nature of the subject licenses and not to pursue accuracy where only an estimate of the truth is probable. Its index number can range from 1 through 80 with the hazard increasing number. HI of 1 through 20 is of minor concern. An index number greater than 20 should receive careful care. The first step in HI determination is to conclude whether the soil, crop and water resources or a mixture of them contributed to the larger index number. This estimation will help the researchers focus on the section of their management system that requires the greatest attention.[15,16] For estimating the non-carcinogenic effects of nitrate and nitrite a reference dose (RfD) was useful. RfD has been resultant from the so called no-observed-adverse-effect-level, that is from highest concentration (or dose) of a toxic material, that creates no health effects.[17] Many studies such as Azlan *et al*.,[2] Cemek *et al*.,[3] Bertoldi *et al*.,[6] Abouleish,[9] and Samadi *et al*.,[18] have been reported concentrations of nitrate and nitrite in bottled drinking water. These studies show that nitrate and nitrite in bottled waters are not higher than standards. Some studies such as Thomson,[10] Moore *et al*.,[19] and Schuddeboom[20] have been surveyed risk assessment of nitrate and nitrite in bottled waters. The aim of this study was to measure the concentrations of nitrate and nitrite in bottled waters of Isfahan's market in 2013 and their health risk assessment.

MATERIALS AND METHODS

Water samples

In the present study, 21 brands of more consumption-bottled mineral waters were purchased from shops in the city of Isfahan, Center of Iran at 2013. The samples were analyzed for the nitrates (NO_3^-) and nitrites (NO_2^-).

Chemical analysis

Chemical analysis was carried out according to the Hach analytical methods. Determination of nitrate (as mg/l NO_3^-) was as cadmium reduction method and for nitrite (as mg/l NO_2^-) was as diazotization method. A DR-5000 (HACH LANGE, USA) was used for all determinations.[21] A comparative analysis also was carried out based on Equation 1.[22] This equation show the nitrate and nitrite concentrations in water samples versus their guidance values should be lower than or equal to one, simultaneously.

$$\frac{\text{Nitrate concentration}}{\text{Guidance}} + \frac{\text{Nitrate concentration}}{\text{Guidance}} \leq 1 \quad (1)$$

Estimation of nitrate and nitrite exposure

After concentration computation of nitrate and nitrite in the bottled water, chronic daily intake (CDI) has been earned

based on Iranian average body weight (60 kg)[23] using the following Equation 2.

$$CDI = \frac{C \times DI}{BW} \quad (2)$$

Where, CDI (mg/kg/day), C is nitrate and nitrite levels in bottled water (mg/l), DI is an average daily intake rate of bottled water (l/day) and BW is adults body weight (kg).

For non-carcinogenic health effects posed by nitrate and nitrite in drinking water, the HI was calculated using Equation (3). It was noticed that a HI value more than 1 (HI >1) shows a significant risk level, while a HI <1 shows no health risk on consumers.

$$HI = \frac{CDI}{RfD} \quad (3)$$

Where, CDI (mg/kg/day) and RfD (mg/kg/day). RfDs for nitrate and nitrite in this study were 1.6 mg/kg/day[24] and 0.1 mg/kg/day,[25] respectively.

Statistical analysis

The normality assumption of the data was tested using Kolmogorov-Smirnov test. To compare nitrate and nitrite mean concentrations between bottled waters and codex regulations we used Student's *t*-test. In addition, comparison of ions concentrations between bottled mineral waters contents and their labels was done using paired sample *t*-test. All the statistical analysis was performed with the SPSS statistics package program (SPSS software, IBM SPSS product version 20 at $P < 0.05$). In all data analysis, a value of $P < 0.05$ was considered to be statistically significant.

RESULTS

In this study, the amounts of NO_3^- and NO_2^- in 21 samples of bottled mineral waters in Isfahan, Iran were surveyed. Table 1 shows the percent of equal, greater and lower than label and not specified label of studied bottled mineral water in respect to nitrate and nitrite water contents.

Table 2 shows ranges, mean and standard deviations of obtained results comparing to water quality guidelines. Furthermore, results of statistical *t*-test to compare the nitrate and nitrite mean concentrations between bottled waters and codex regulations such as Table 2. According to the results, there were meaningful differences between the amount of each ions with water guidelines ($P < 0.001$). Paired *t*-test also showed that there was significant different between the mean concentration of nitrate in water (1.42 ± 0.55 mg/l) and the mean concentration inserted on bottle's labels (5.44 ± 2.96 mg/l) ($P < 0.001$). There was no nitrite concentration inserted on the studied bottle's labels.

The results of Equation 1 showed that the ranges, mean and standard deviations of calculations were 0.01 ± 0.07 and 0.03 ± 0.01 mg/l, respectively. Estimation of nitrate and nitrite exposure is summarized in Table 3.

DISCUSSION

As can be seen in Table 1, about 95% of studied samples had no specified label regarding to nitrite contents. However, about 57% of these bottled mineral waters had the nitrate contents lower than their label insertion and about 23% from those samples had no specified label respecting to nitrate contents. On the other hand, there was no rational relationship between the real contents of studied samples and their label insertions.

The results of Samadi *et al*., showed that, nitrate had a statistically significant difference with industrialist's labeling values. Their results suggested an extremely important for the health supervisory agencies such as a ministry of health and institute of standards and industrial research of Iran to have more effective controls on bottled water industries and to improve periodical the proposed standard values.[18]

As shown in Table 2, the mean and standard deviation for NO_3^- and NO_2^- was 1.418 ± 0.547 and 0.0163 ± 0.023 mg/l, respectively. On the other word, all real contents of studied samples were lower than bottled mineral water quality guidelines ($P < 0.001$). However, concentration of studied ions in this study was less than its guideline value. Based on the guideline for drinking water quality presented by WHO, the simultaneous concentrations of nitrate and nitrite should

Table 1: Comparison of label and real contents of bottled mineral water

Parameter	% equal to label	% greater than the label	% lower than the label	% not indicated label
NO_2^-	0.00	4.76	0.00	95.24
NO_3^-	19.05	0.00	57.14	23.81

Table 2: Ranges, mean and SD of results comparing to water quality guidelines

Parameter	Ranges (mg/l)	Mean ± SD (mg/l)	Bottled mineral water quality guidelines (mg/l)[22]	*P*
NO_3^-	0.42-2.66	1.42 ± 0.55	50	<0.001
NO_2^-	0.00-0.051	0.02 ± 0.015	3	<0.001

SD: Standard deviation

Table 3: Estimation of nitrate and nitrite exposure for Iranian population

Parameter	Measured concentration (mg/l)	DI* (l/day)[26]	CDI (mg/kg/day)	HI value
NO_3^-	0.142	0.1	0.0002	1.5E-04
NO_2^-	0.02	0.1	0.00003	3.3E-04

*DI: Daily intake of bottled water in Iranian community. In this study, DI was assumed 0.1 l/day. CDI: Chronic daily intake, HI: Hazard index

be less than or equal to one [Equation 1].[27] The ranges, mean and standard deviation of Equation 1 calculations show that there were no samples with simultaneous nitrate and nitrite concentrations greater than one, which means all of the studied samples had nitrate and nitrite concentrations in the acceptable levels.

In an article in 2011 Cidu *et al*., stated that in 20% of the bottled water samples, one or more components have been found at concentrations exceeding the Italian regulations (Cl^-, SO_4^{2-}, NO_3^-, F^- and As).[8] A study by Cemek *et al*., indicated that nitrate and nitrite levels in the mineral waters were not found in concentrations considered to be hazardous terms of public health.[3] Furthermore, Bertoldi *et al*., based on a survey of the chemical composition of 571 European bottled mineral waters, reported that according to European legislation, 9% of samples had boron, nitrate or nitrite levels above the legal limit existing in individual European countries.[6] Abouleish described that all bottled water samples demonstrated nitrate, nitrite and other anions levels below the permissible levels accepted by United States Environmental Protection Agency (US-EPA), US-Food and Drug Administration/Code of Federal Regulations and other international organizations.[9] Therefore, in present research the mean concentrations of nitrate and nitrite had values less than codex guideline values. Chiarenzelli and Pominville showed that exception of one sample of mineral water, all bottled waters tested meet US-EPA primary standards for drinking water supplies.[4]

As inorganic nitrogen species such as ammonium, nitrite and nitrate continue to enter aquatic systems from manmade sources, it results is hastened eutrophication in freshwater and nautical ecosystems. Elevated nitrate and nitrite concentrations cause blue baby syndrome in human infants and aquatic animals.[28] Inoue-Choi *et al*., appraised the interaction of dietary and water nitrate intake with total folate (folic acid) intake on breast cancer risk in the Iowa women's health study.[12] Joint FAO/WHO Expert Committee on Food Additives (JECFA) endorsed that calculations of the intake of nitrite should include sources, such as vegetables and drinking water. The foods should be analyzed as 'ready to consume' to account for losses of chemicals finished time and during bottled water storage and preparation. Nitrate-contaminated drinking water often rises because of fertilizers applied to crops, which are converted to nitrate in the soil and then leak into groundwater and into private suburban wells. Another certain concern about nitrate or nitrite detection is proximity of animal food lots to some groundwater sources of drinking water. This may lead to groundwater pollution with nitrates from run-off from these food lots.[25] Base on groundwater include of nitrate and nitrite and some bottled waters provided of these sources, then it may bottled waters contained of these ions.[11] Nitrite has been concerned in a variety of long term health effects and the toxicity of nitrite has been estimated several times as new information has been available. The toxic effects of nitrate are also due to its endogenous adaptation to nitrite. JECFA determined that the range of nitrate adaptation is 5-7% for normal individuals and 20% for individuals with a high rate of change.[10] Exposure to higher levels of nitrate or nitrite has been related with increased occurrence of cancer in adults and possible increased incidence of brain tumors, leukemia and nasopharyngeal (nose and throat) tumors in children in some studies but not others.[25] Based on a review study, theoretic infant diet, bottled water was the major contributor sodium nitrate exposure for infant age 9 months.[13] Effects on systems in humans have been related with childhood exposure to nitrates or nitrites. Exposure of children to nitrates or nitrites was related with increased occurrence of childhood diabetes. Some studies have reported relations between children's exposure to nitrates or nitrites and recurrent respiratory tract infections, increased risk for thyroid complaints during adulthood, or recurrent diarrhea.[19,25] Lee had surveyed the nitrate risk assessment process consists of both exposure assessment and human health risk assessment. Based on this study, doubts related to the nitrate risk assessment or risk management process and their influence on results are characterized with an application of fuzzy-set theory and combined into the trade-off analysis. In the present study, HI values for all studied compounds were less than 1. This designate the risk of adverse health effect of nitrate and nitrite pollution in bottled water of Isfahan, center of Iran was acceptable. Pawełczyk study with purpose the determined values communicated by HI indicate that the water pollutants and their concentrations do not exceed unity.[17] The results of Jamaludin *et al*.'s cross-sectional study that have done on concentrated agricultural area of Bachok Kelantan from nitrate exposure in drinking well water, showed that the health risk of residents from nitrate exposure in this area were in the acceptable range.[24]

CONCLUSION

In this study, the concentrations of nitrate and nitrite were measured, in 21 brands of bottled waters in Isfahan, Iran. The sample quality contents were compared with labels insertion on the bottles and then by water quality guidelines. Results showed that there were differences between concentration of these ions in water contents and label insertion on the bottles. In about 95% and 23% of studied samples no specified label concerning to nitrite and nitrate contents was presented, respectively. Approximately 57% of samples had the nitrate contents lower than their label insertion. The concentrations of nitrate and nitrite in all of the studied samples were less than water quality guidelines values. It is recommended that all of the marketed bottled waters monitor regarding to nitrate and nitrite concentrations, periodically. The health risk of nitrate and nitrite in Isfahan's market was considered equally low.

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