Middle-East Journal of Scientific Research 2 (3-4): 151-156, 2007
ISSN 1990-9233

Mineral and Microbial Contents of Bottled and Tap Water in Riyadh, Saudi Arabia

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Abstract: Water is essential to health however its purity, potability and the mineral content is important for consumption by humans. This study aim to determine the clinically important levels of minerals in bottled water and to determine the microbiological content of commercially available bottled waters and tap water from 5 regions of Riyadh, Saudi Arabia. Commercially available bottled mineral waters were brought from all 5 regions of Riyadh (i.e. Northern, Southern, Eastern, Western and Central area of Riyadh). Similarly, tap water was obtained from all 5 different areas of Riyadh. Samples were examined microbiologically and mineral contents were determined by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS). Of the thirty bottled samples tested, 2 bottled samples showed contamination with *Bacillus cereus* and *Pseudomonas spp.* Tap water obtained from different areas of Riyadh showed contamination with *Staphylococcus, Corynebacterium, Acinetobacter iwoffi, Flavobacterium* and *Bacillus cereus.* No fungal growths were recorded. Mineral contents of 9 branded bottled water showed differences in levels. Traces of aluminum were recorded in all bottled and Tap water samples. Purity including the absence of bacterial contamination of bottled water in contrast to tap water is not guaranteed thus individuals and consumers should check the mineral content of their drinking water and choose the water that is most appropriate for their individual dietary needs.

Key words:

INTRODUCTION

Water is the most important resource for humans. It forms 50 to 60% in weight of our body and play an active role in all the vital processes of our body: It allows digestion, food elaboration and waste elimination. Every day we drink water or we eat watery food to replenish our metabolic reserve. But what is the water we drink made of? Can we rely on drinking tap water or are preferable to buy bottled water?

Epidemiological studies have reported the occurrence of disease including problems with reproduction [1], cancer [2], congenital malformations of the central nervous system [3-6] cardiovascular disease [7-10] and even death [11, 12] due to exposure to trace elements and mineral contents of water.

Calcium (Ca) and Magnesium (Mg) are essential elements for our body. They can be provided to our organisms by food, but even diets rich in calcium and magnesium intake may not be able to compensate their absence in drinking water. *Calcium* is important for fetal growth, pregnancy and lactation. [13-15]. It is essential in our body for teeth and bones formation, blood coagulation, right functioning of our nervous system. Drinking calcium poor water is considered dangerous for the risk of coronary diseases. An excess in calcium can alter the water taste or cause scaling problems in pipes and household appliances. The World Health Organization (WHO) recommends a minimum calcium daily intake of about 700 mg.

Magnesium plays an important role as a cofactor and activator of more than 300 enzymatic reactions including glycolysis, ATP metabolism, transport of elements such as sodium (Na), potassium (K) and calcium through membranes, synthesis of proteins and nucleic acids, neuromuscular excitability and muscle contraction. Magnesium deficiency increases risk to humans of developing various pathological conditions such as vasoconstrictions, hypertension, cardiac arrhythmia, atherosclerotic vascular disease, acute myocardial infarction, preeclampsia in pregnant women, possibly diabetes mellitus of type II and osteoporosis. The daily recommended intake for Mg is 150-500 mg.

The recommended daily intake of Sodium (Na) is 20 mg. Numerous studies have shown that a high Na+ intake is associated with hypertension [16-19] and that dietary Na+ restriction, achieved by not adding salt and avoiding Na+ rich foods, may effectively reduce blood

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pressure. [20]. However, drinking certain waters may unnecessarily increase Na+ intake to a level may be detrimental for health, especially for individuals on a Na+ restricted diet [21, 22].

Chlorinated water in which the anion Cl- is dominant (hypertonic) increases internal peristalsis. If water has low concentration (hypotonic) it stimulates gastric secretion. It can have spasmodic, coleretic action and protect liver from toxic compounds. The recommended daily intake is 7-15 g [23, 20].

Fluorine (F) is useful for the good health of bones and teeth, sometimes it is even essential. Fluorine values useful for our body are close to toxic values, so a dispense not aimed and personalized can cause high risk of overdosing and chronicle poisoning, with consequent skeleton deformation, spots on tooth enamel, osteosclerosis, neurological disorders, damages on the thyroids and even tumours. The maximum admissible content in water is 1.7 mg L^{-1} [23,20].

Other inorganic toxic substances are contained in water. The sulphate (SO_4) content of water should be lower than 200 mg L^{-1}. Nitrates (NO_4) are the main source of nitrogen essential for nucleic acids and amino acids. Nitrate water content of about 10 mg L^{-1} is considered normal and natural. Excessive nitrate presence can be transformed to nitrites (NO_3) which are toxic to the human body. Aluminum (Al) is not important for human nutrition. It can have toxic effects even in small qualities. [24, 25]. The WHO recommends an Aluminum concentration in water lower than 20 mg L^{-1}. Bicarbonates (HCO_3) in water should have a concentration lower than 600 mg L^{-1}.

Microbiological pollution in drinking water includes bacteria, virus and fungi. They can be responsible of serious diseases as typhoid, cholera, hepatitis etc. and their presence can be easily detected. Bacterial re-growth is encouraged by the lack of a residual disinfectant and by the possibly great variability of nutrients in aggressive water, such the low-mineral water, particularly if it has a high temperature [26, 27].

Because drinking water may be an important source of mineral intake, the shift in consumption from tap water to bottled water may have implications for health and disease. Thus the objectives of this study were 1) to determine the clinically important levels of minerals in bottled water and to determine the microbiological content of commercially available bottled waters and tap water in Riyadh, Saudi Arabia.

METHODS

In this study, we conducted several steps for both microbial and mineral content of bottled and tap water in Riyadh, Saudi Arabia. Commercially available bottled mineral waters were brought from all 5 regions of Riyadh (i.e. Northern, Southern, Eastern, Western and Central Riyadh). Similarly, tap water was obtained from all these 5 different areas of Riyadh.

Phase 1: Microbial study of commercially available bottled water and tap water

A. Commercially available bottled mineral water

a. Two bottles of each different sized commercial preparations (1.5, 2.5 and 19 liter bottles) were randomly brought from 5 different randomly selected grocery stores in Riyadh were collected. A total of 30 samples of bottled mineral water were taken. All samples were stored in the grocery stores where they are sold at room temperature (25-30 degrees centigrade)

b. In the laboratory, each bottle was adequately shaken then, two samples of one milliliter each sample was taken for microbiological analysis, properly labeled and recorded.

c. One ml. sample was inoculated in Blood Agar Plate (BAP) and another one ml. sample was inoculated on Sabouraud's Dextrose Agar Plate (SDAP).

d. All samples were incubated for 24 to 48 h at 25 to 30 degrees and observed for growth.

e. Fungal identification was done microscopically.

f. Samples were cultured quantitatively and levels of bacteria were determined as Colony-forming Units (CFUs) per milliliter. Bacterial identification was conducted using the API system of bacterial identification after the required 24 to 48 hours of incubation.

B. Tap water

a. Two one-ml. samples were taken from 5 randomly selected households for each 5 region of Riyadh City. Samples were obtained after opening the faucet and allowing tap water to run for approximately 5 minutes. A total of 30 samples of tap water were taken from all 5 areas of Riyadh. All samples were stored placed in sterilized tubes prior to analysis in the laboratory and stored at 25-30 degrees centigrade.

b. In the laboratory, each bottle was adequately shaken then, two samples of one milliliter each sample was taken for microbiological analysis, properly labeled and recorded.

c. One ml. sample was inoculated in Blood Agar Plate (BAP) and another one ml. sample was inoculated on Sabouraud's Dextrose Agar Plate (SDAP).
d. All samples were incubated for 24 to 48 hours at 25 to 30 degrees and observed for growth.
e. Fungal identification was done microscopically.
f. Samples were cultured quantitatively and levels of bacteria were determined as Colony-forming Units (CFUs) per milliliter. Bacterial identification was conducted using the API system of bacterial identification after the required 24 to 48 h of incubation.

Phase 2: Mineral content of commercially available bottled mineral water and tap water in Riyadh, Saudi Arabia

Collection of samples: Nine (9) different commercially available mineral water products sold in Riyadh were obtained from grocery stores. For uniformity, we bought similar 19 liter sizes for all 9 of the different commercially available bottled mineral waters.

Measurements of mineral content in commercially available bottled mineral waters were done by ICP-MS and pH of water was also determined.

ICP-MS are by far the most common type of plasma sources used in today's commercial ICP Optical Emission Spectrometry (OES) and ICP-MS instrumentation. In ICP, the first step that takes place is the desolvation of the droplet with the water molecules stripped away; it then becomes a solid particle. As the sample moves further into the plasma, the solid particle changes first into a gaseous form and then into a ground-state atom. The final process of conversion of an atom to an ion is achieved mainly by collisions of energetic argon electrons with the ground state atom. The ion then emerges from the plasma and is directed into the interface on the mass spectrometer. The measurement determines the parts per milliliter (ppm) of anions and cations in the sample.

RESULTS

Microbial analysis of commercially available bottled mineral water in Riyadh, Saudi Arabia revealed bacterial contamination with Bacillus cereus and Pseudomonas species in two of the 30 (6.7%) bottled samples. Microbial analysis of tap water samples obtained from Northern Riyadh revealed that 2 samples contaminated with *Staphylococcus* spp, 4 samples contaminated with *Corynebacterium* spp, 1 sample contaminated with Acinetobacter iwoffi, 2 samples contaminated with *Pseudomonas* spp and 1 sample was contaminated with Flavobacterium. Tap water microbial analysis obtained from Southern Riyadh revealed one sample contaminated with Bacillus cereus. Microbial analysis of tap water samples obtained from Western and Eastern Riyadh showed no bacterial contamination. Furthermore, fungal growth was not seen in all tap water samples from all areas of Riyadh (Table 1).

Mineral content analysis by ICP of all sampled commercially available bottled mineral water showed diverse results as to mineral contents as well as the presence of some inorganic toxic substances such as phosphates, sulphates, nitrates and nitrites and aluminum. (Table 2).

Seven of 9 (77.8%) of the mineral water samples had neutral pH (pH 7.0-7.4) and two (22.2%) were slightly alkaline (pH >7.4). Sodium content was in the range of 13 to 20 ppm in 7 samples. One sample had Na content of 5 ppm and another had 22.4 ppm, which was above the recommended limit of 20 ppm. Eight samples had acceptable calcium levels of 20-50 ppm, whereas one sample had 76 ppm. Chloride levels in 3 samples

Table 1: Microbiological analysis of tap and bottled water in Riyadh, Saudi Arabia

Sample	Bacterial content	Fungal content
Tap water from Northern Riyadh	*Staphylococcus spp* (+) in 2 samples	
	Corynebacterium spp (+) in 4 samples	
	Acinetobacter iwoffi (+) in 1 sample	
	Pseudomonas spp. (+) in 2 samples	
	Flavobacterium spp (+) in 1 sample	No growth
Tap water from Southern Riyadh	*Bacillus cereus* (+) in 1 sample	No growth
Eastern Riyadh	No growth	No growth
Western Riyadh	No growth	No growth
Central Riyadh	No growth	No growth
Bottled Mineral Water (n = 9)	2 samples positive for *Bacillus spp* and *Pseudomonas spp.*	No growth

Table 2: Average composition (ppm) of anions and cations from 9 commercially available bottled mineral water in Riyadh, Saudi Arabia

Sample	Na^+	K^+	Ca^+	Mg^+	Al^{+3}	Cl^-	SO_4^-	NO_3^-	HCO_3^-	Fe^+	F^-	pH
Allowable range	10-20	<10	20-50	10-30	≤0.2	≤30	<25	1-5	<600	≤0.2	≤1.7	7.2-7.4
A	22.4	0.5	8.4	1	0.006	32	21	2.5	7	0.02	0.85	7.0
B	14	0.2	17	2.5	0.011	34	14	1	28	0.02	0.9	7.0
C	20	2.5	17	7	0.010	27	12	2	80	0	1	7.0-7.6
D	20	2.5	17	7	0.005	27	12	2	80	0	1	7.0-7.6
E	18	0.2	36	4.7	0.019	86	22	0.5	42	0.02	0.9	7.0
F	5	1	76	24	0.008	4.8	10	3.8	357	0.02	0.9	7.0
G	15.84	1.05	10	4.45	0.012	17	33	3.08	20	0.02	0.8	7.0
H	13	0.9	15	4	0.012	14	50	4	12	0.02	0.9	7.0
I	20	0.8	13	4	0.005	30	20	5	30	0	0.8	7.15

Table 3: Average composition (ppm) of anions and cations from tap water in 5 regions of Riyadh, Saudi Arabia

Sample	Na^+	K^+	Ca^+	Mg^+	Al^{+3}	Cl^-	SO_4^-	NO_3^-	HCO_3^-	Fe^+	F^-	pH
Allowable range	10-20	<10	20-50	10-30	= 0.2	= 30	<25	1-5	<600	=0.2	=1.7	7.2-7.4
Northern Riyadh	68	5.4	56	3.6	0.00761	149	139	1.2	80.5	0	0.21	7.7
Southern Riyadh	62	6.1	60	3.5	0.0036	106	146	1.2	73	0	0.1	7.8
Eastern Riyadh	57	4.6	52	3.4	0.00784	135	116	1.5	78	0	<0.1	7.8
Western Riyadh	11.5	0.7	9.6	1.5	0.00988	28.5	21	0.7	36.6	0	<0.1	7.9
Central Riyadh	26	6.6	39.5	3.1	0.00419	106	102	1.1	73	0	0.18	7.7

were above the acceptable limit of up to 30 ppm. Two samples had 33 and 50 ppm. of sulphate, this was above the acceptable limit. Traces of aluminum contents were seen in all bottled samples (range: 0.004 to 0.019 ppm). Other anions and cations were in the acceptable ranges (Table 2).

When tap water from the 5 regions of Riyadh were analyzed according to the mineral and inorganic toxic substances, all samples contained traces of aluminum although they were all below the toxic range. Sulphate and chloride content was high in all samples except the sample from the western area which was within the allowable range. All of the samples had alkaline pH and were above the allowable range of 7.2-7.4. All samples also had high sodium and calcium contents except samples from the western and central Riyadh (Table 3).

Comparing bottled mineral water and tap water contents analyzed in this study, tap water showed higher levels of mineral and toxic substances content especially sodium, potassium, calcium, magnesium, aluminum, chloride, bicarbonates and pH. Nitrate, fluoride and iron were higher in bottled mineral water compared to tap water.

DISCUSSION

Every country has its own legal drinking water standards. These prescribe which substances can be in drinking water and what are the maximum concentrations of these substances are. The standards are called maximum contaminant levels. They are formulated for any contaminant (whether it is a substance or a foreign bacterial, viral or fungal element) that may have adverse effects on human health and each company that prepares drinking water has to follow them up.

In our study, the presence of two different species of bacteria, *Bacillus cereus* and *Pseudomonas spp* in supposedly bacteria-free commercially available bottled mineral water is of a concern. Whether the species of bacteria present in two samples are pathogenic or not, the fact that these are present, the hazard of contamination and health risk to consumers should not be taken for granted. Pseudomonas usually cause infections of the urinary tract however, it can cause bacteremia especially if without a urinary focus and is due to Pseudomonas species other than aeruginosa. *Bacillus cereus* can also cause serious diseases and affect vital organs of humans especially in immunocompromised patients. This is similar to the study conducted by Shy and Stroba [2] that purity including the absence of bacterial contamination of bottled water in contrast to tap water is not guaranteed. In their report, they reported CFU levels of upto 4900 CFUs mL^{-1}, which is substantially above the acceptable level of 1000 CFUs mL^{-1}. Furthermore, they found out that their findings on the bacterial content on tap water were lesser compared to bottled water, suggesting that quality of bottled water as compared to tap water is not really determined by labeling. In short, the use of bottled water

is only based on assumption of purity and this can be misguiding. One explanation to growth of bottled mineral waters is brought about by the mineral contents of the water itself. In a report by Botzenhart and Kufferath, salts used such as potassium phosphate and especially magnesium sulfate showed a growth promoting effect on *Enterobacteriaceae, Pseudomonas* and *Alkaligenes.*

Tap water samples obtained from Northern Riyadh is obviously contaminated. In fact, with strict governmental controls, bottled water should often offer less warranties of purity, since local officials monitor and control tap water on a regular basis. Most countries have the technology to treat water; Saudi Arabia is one of the most highly industrialized nations. However, tap water is less often if not at all used as potable drinking water in Saudi Arabia. Most of the rest of water is used for bathing, watering and washing.

Several debates have gone in publications regarding the benefit of drinking bottled water. In tests people can not distinguish between bottled water and tap water- and some reports say that there is no health benefit to drinking bottled water. Minerals are important parts of drinking water and are of both direct and indirect health significance. Sufficient evidence is now available to confirm that a certain minimum amount of minerals in water is desirable, since their deficiency have many negative health effects: diseases and possible aggression from toxic elements and bacteria. In our study results, our samples were supposedly "within the required mineral contents" as stated in their company's levels. However, the variability of minerals and elemental contents is great from bottle to bottle. In fact, no two brands of bottled water were identical in their mineral content. The issue of safety and potability of these bottled water is not guaranteed by the labeling. Take into consideration the presence of Aluminum in bottled water. Aluminum is known to be toxic and hazardous to health. In our study, we found out that all of our samples have traces of aluminum in the range of 0.006-0.019 ppm. Although our results were in the acceptable ranges according to the European Union standards, some reports suggested that the presence of aluminum can have toxic effects even in small quantities. These effects occur in nervous system. The hazard of use especially in kidney patients is a concern, but health effects originating from aluminum intake through water are still on debate.

The results of our study have several implications for the consumption of water in Saudi Arabia. Because of the variations in the mineral content of bottled mineral water, Saudis do not equally consume Ca^{2+}, Mg^{2+} and Na^{+} when drinking the same quantity of bottled water. If Saudis prefer to drink commercially available bottled waters, they should be selective when deciding which water to drink. Individuals should choose to drink bottled water with an optimal mineral profile, i.e., high levels of Ca^{2+} and Mg^{2+} and little Na^{+}. In the bottled mineral water we examined, only one brand (Brand F, see Table 2) was optimal with a high Ca^{2+}content of 76 ppm, high Mg^{2+} content of 24 ppm and have the lowest Na^{+} content of 5 ppm.

Furthermore, the differences in the mineral and tap water content for mineral and toxic substances as our study showed should be further investigated since the issue of potability and safety of both drinking water and water used for other purposes may advertently harm consumers.

The results of our study suggest that drinking water may be an important dietary source of Ca^{2+}, Mg^{2+} and Na^{+}. This is because minerals are highly bioavailable in water and because drinking water sources available in the market may contain clinically important levels of these minerals.

Physicians should therefore encourage their patients to check the mineral content of their drinking water and to choose the water that is most appropriate for their individual dietary needs.

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