CYTOTOXIC AND CHEMICAL ANALYSIS OF SPRING WATER FROM NORTH LEBANON

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ABSTRACT

The cytotoxicity and chemical characteristics of spring water samples from Dannia region in north Lebanon were studied by two colorimetric techniques using MTT and Crystal Violet. Based on the results, the samples were divided into 3 groups: water samples with a level of cytotoxicity near 0, 10% and over 15% respectively.

The rate of mineralization and other classical parameters studied were the following: conductivity, pH, Ca, Mg, K, Na, Fe, Ba, Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn, Si, V, NH_4, HCO_3, Cl, SO_4, NO_3, NO_2, and F. All the samples were shown to have a satisfactory ionic balance and confirm the calcareous bicarbonate character of the majority of the water sources in north Lebanon (Dannia).

Cytotoxic and chemical analyses, in addition to routine bacteriological analyses, could be a useful tool in order to ensure the correct sanitary quality of water to the consumer.

Keywords: cytotoxicity, spring water quality, Lebanon

INTRODUCTION

Indispensable for life, water is particularly vulnerable. Contaminants are often found in trace amounts that are at the limit of the detection capacities of analytical equipment (Bontoux, 1993; Montiel & Husson, 1991; Gaujoux, 1993).

Relatively simple cytotoxicity techniques could provide a rapid, reproducible and sensitive alternative to the classical sanitary surveillance techniques now used by most laboratories. The two cytotoxic tests proposed are based on the incorporation of two dyes: MTT in the mitochondria and Crystal Violet in the cellular nuclei respectively.

The study zone is considered rich in water resources with an average annual rainfall between 500 and 1500mm. However, less than 10% of these resources are available for human consumption. There are still some villages that do not have access to piped water, and there are occasional reports of faecal contamination of drinking water.

MATERIALS AND METHODS

A) Water samples

The water samples are taken from Dannia, 60 Km, east of the city of Tripoli in north Lebanon, at various altitudes ranging from 630 m to 2100 m (Figure 1). The names of the springs and their respective altitudes are given in Table 1.

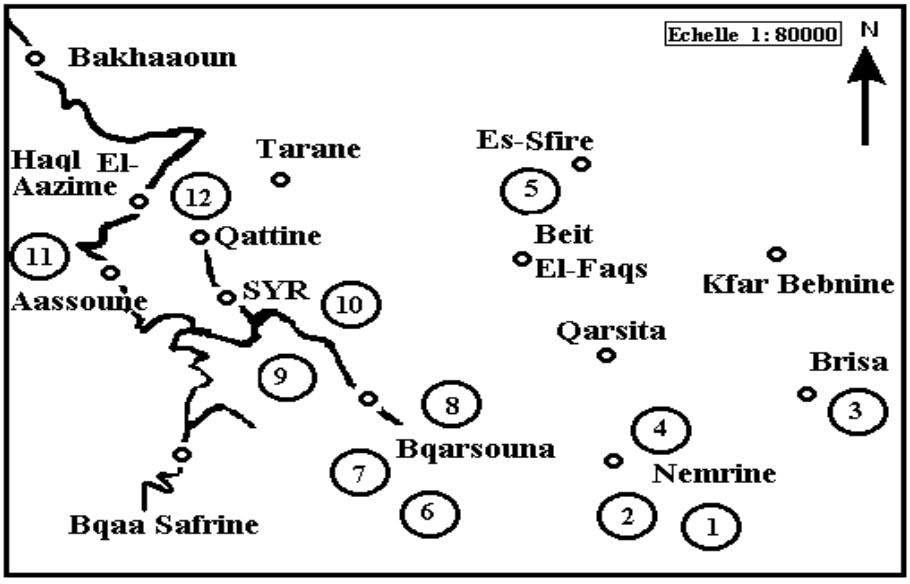

Figure 1 . Sampling stations in the Dannia region.

The French water samples were collected from the thermal spring at La Chaldette, which emerges on a basaltic plateau at an altitude of 1000 m in the center of France in Lozère. Samples from the Paris region were obtained from the Loing spring which emerges on land of a chalky nature. The treated tap water was obtained from the laboratory in Paris. Volvic natural mineral water was used as reference for the chemical analyses.

B) Cells and culture media used

Neuroblasts from a sub-clone of human origins SK-N-SH are maintained in flasks of cultures in RPMI 1640 -1X, (GBCO) medium to which 10% of fetal calf serum, 1% of glutamate 200 mM and 0.5% of gentamycine (50 mg/ml) (ATGC Biotechnology) were added. 96 well plates were used during the experiments with 20000 cells per well (Gillies *et al*., 1986). When the cells join, a 10 X culture medium (GIBCO) restored with the water to be studied, filtered to 0.22µ replaced the normal culture medium. In practice, the replacement medium is prepared using the following constituants as follows: 10 ml of 10X RPMI medium, 90 ml of the water to be tested, 10 ml of calf serum (GIBCO), 1 ml of glutamine (GIBCO), 0.1 ml of gentamicine (GIBCO), 2.8 ml of $NaHCO_3$ (7.5%), (GBCO). The microplates with the new medium are left in the drying oven for 24 hours at 37°C with 5% CO_2. The cells and the culture media are used to run the cytotoxicity tests.

C) Cytotoxic tests

1 – MTT test

This test utilizes the property of succinate deshydrogenase to reduce Thiazolyl yellow (MTT) [3-(4,5-dimethylithiazolyl-2)-2,5-biphenyl monotertazolium Bromide] to Formazan, which concentrates in the mitochondria as a blue precipitate (Figure 2). The speed of the reduction reaction increases when the culture cells are in a good physiological state. The Formazan is then dissolved in the Dimethyl sulfoxide (DMSO). The intensity of the blue color of Formazan is measured at 540 nm (Scudiero *et al*., 1988; Martin & Clynes, 1993; Husson *et al*., 1993).

After the dehydrogenase has been added to the well at 37°C in monolayer cultures and on microplates for 24 hours, the culture medium is eliminated from each well and replaced by a 5% MTT solution. The microplate is put back in the drying oven for 4 hours at 37°C. The MTT is reduced to Formazan blue, then dissolved in the DMSO. The variation in the intensity of the color, which is related to the toxic effect of the product to be tested, is indicated using a Multiscan microplate reader (Kodak) at 540 nm.

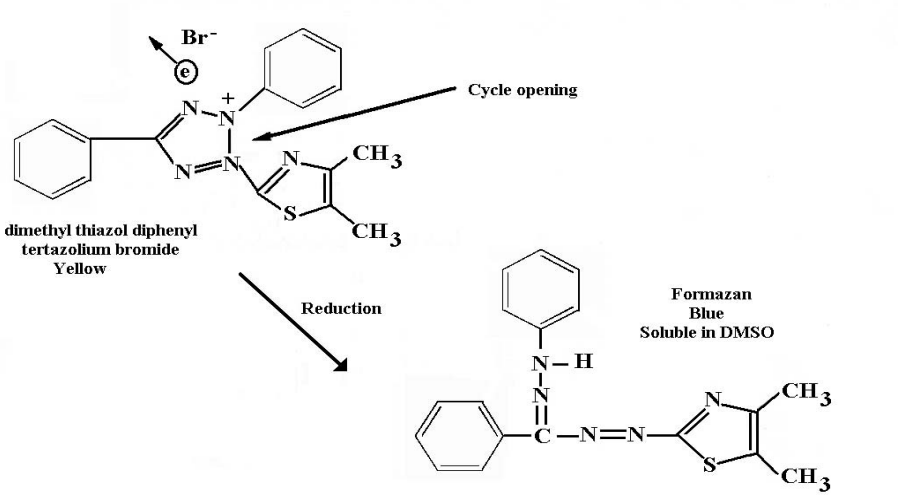

Figure 2: Reduction of the MTT in the mitochondria.

2 – Crystal Violet test

This test utilizes the property of this non-vital dye (Methylviolet 10B: $C_{25}H_{30}ClN_3$) to concentrate in cell nuclei preferentially. Extraction is done using 33% chilled acetic acid and the intensity of the color is measured at 540 nm (Gillies & Deam, 1979; Kueng *et al*., 1989). The dye is then added to the cells cultivated on microplates, are placed in a drying oven at 37°C for 24 hours. To the supernatant produced on the surface of each well, 100 µl of 1N Hydrochloric acid is added per well to fix the cells. The microplate is washed with running water once. Then 100 µl of Crystal Violet at 0.25% is added to each well. After 20 minutes of contact, the microplate is washed with tap water 4 times, dried, and then 100 µl of 33% chilled acetic acid is deposited in each well. The microplates are shaken moderately. The absorbance is read using a spectrophotometer (Titrek C380). It is then possible to correlate the intensity of the color with cell viability .

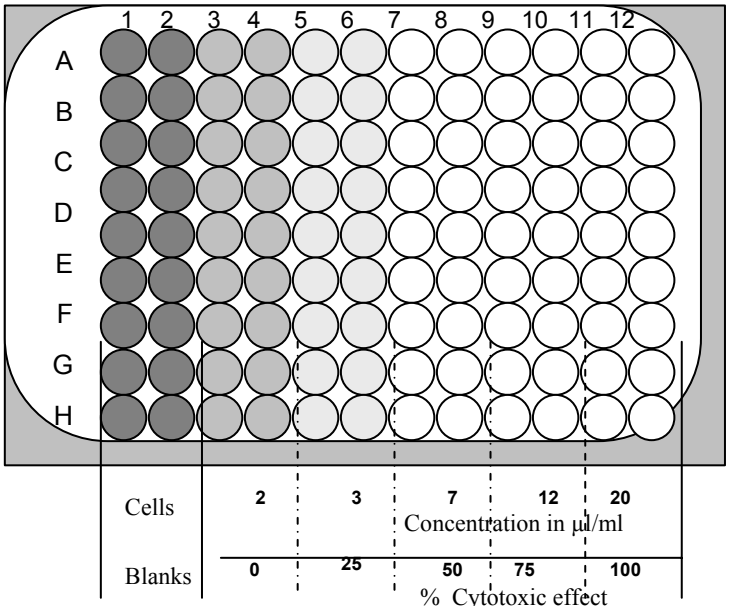

Figure 3. Cytotoxcity test on microplates.

D) Chemical analysis

Chemical analyses of these same water samples were run in accordance with the French decree 89.3 concerning drinking water guidelines (Ministère de la Santé, 1995) and using the AFNOR standardized techniques (Rodier, 1996): the complexometry with EDTA for the Calcium ions, the Atomic Emission Spectrophotometer ICP (VARIAN) for the Magnesium and trace elements (Barium, Cadmium, Chromium, Cobalt, Copper, Lead, Manganese, Nickel, Vanadium, Zinc and Silica), the Flame Emission Photometry (Varian) for the Sodium and Potassium ions, a colorimetric method with Nessler reagent for the ammonia ion. The bicarbonates are obtained based on the Alkalinity results, the chlorides are determined by ionic chromatography with a conductivity detector (Dionex). The nitrates are reduced to nitrites and measured by spectrophotometry. The fluoride ions are measured using a specific electrode (Radiometer). The iron is determined by Atomic Absorption Spectrophotometry (Varian). The total hydrocarbons, after extraction with carbon tetrachloride, are determined by an Infrared Spectrophotometer (Varian) at 3420 nm.

RESULTS

A) Cytotoxic tests

The results of the cytotoxic analyses using the two colorimetric methods (MTT and Crystal Violet), are given in Table 1.

Using the MTT method the examined water samples could be divided into 3 major groups:

Water with a level of cytotoxicity near 0, (samples N° 1,2,4,9,11 and 12).
Samples N° 8 and 10 have a cytotoxicity level around 10%.
Finally, samples N° 3, 5, 6, 7 have a level of cytotoxicity around 15% or more.

Considering the variability of the live material used and the actual status of cytotoxicity analyses as proposed by Fauris (1992) conducted on various water samples from the Paris region, a ceiling value of 30% cytotoxicity may be considered alarming as regards the sanitary aspects of drinking water. It is worth mentioning that the same cytotoxicity test applied to water samples from France and Poland give an acceptable ceiling value of 10% (Husson *et al*., 1996). Fauris *et al*. (1985) using another technique: RNA synthesis technique, came up also with cytotoxicity values under the ceiling of 30% (100% corresponding to a cytotoxic effect = 0).

Table 2 indicates similar test results, which makes it possible to foresee general usage of these two tests in routine sanitary testing of water in Lebanon.

B) Chemical analysis

The result of routine analyses of water samples from Lebanon are shown in Tables 3A and 3B. Samples N° 5 and 12 show relatively higher values. However, they fall within an average scale of mineralization. All of these samples have a satisfactory and well-balanced ionic content.

The concentrations of the chemical species are in conformity with the international standards and are in harmony with the geological nature of the crossed strata and the type of aquifer from which they originate. The slightly high magnesium content in the source Nabaa Al-Zahlan (middle Jurassic aquifer) is due

TABLE 1

Results of the Cytotoxicity of the Water Samples Studied

N°	Water Source	Village	Altitude	MTT Method	Crystal Violet Method
	Lebanese Samples				
1	Nabaa Al-Sanaoubar	Nemrine	1650	0 %	9 %
2	Nabaa Al-Soukkar	"	1680	3 %	11%
3	Nabaa Brisa	Kfar Bebnine	2100	14 %	14 %
4	Ain Al-Arousse	Nemrine	1500	4 %	7 %
5	Ain Fraydesse	Es-Sfiré	1000	15 %	20 %
6	Nabaa Al-Kassam	Bqarsouna	1100	17 %	23 %
7	Ain Beit Hamdane	"	1070	13 %	12 %
8	Ain Al-Haour	"	1100	9 %	
9	Ain Al-Bahsa	Syr	950	2 %	9 %
10	Nabaa Syr	"	900	9 %	
11	Ain Al-Biret	Aasoune	820	2 %	9 %
12	Nabaa Al-Zahlane	Haql Al-Azimet	630	2 %	4 %
	Other origin				
13	La Chaldette	Lozere (F)	1000	15%	10%
14	Local drinking water	Paris (F)	150	16%	14%
15	Polish spring water	Braczewo (PL)	200	7%	10%

TABLE 2

Comparison of Three Cytotoxic Analysis Methods Applied to Various Waters Samples

Water Source	Localization	MTT	Crystal Violet
La Chaldette	Lozere (F)	15%	10%
Drinking water	Paris (F)	16%	14%
Spring Water	Braczewo (PL)	7%	10%

to the presence of magnesium in the Jurassic calcareous strata. It should be noted that abnormal high nitrate levels in sample N°5 (Ain Fraydesse) might be due to contamination caused by animal husbandry (cows, sheep, goats) farms in the neighbourhood of the source. It is also noted that the concentration of ammonium in the source Ain Fraydesse is very low whereas that of the nitrates is higher . This could be due to the incomplete degradation of the organic matter of vegetable and animal origin, thus oxidizing ammonia into nitrates .

Moreover, the absence of hydrocarbons is clearly evident in samples 5, 6 and 12. No concentration of hydrocarbons was detected in water samples N° 5, 6 and 12. Iron content never exceeded 20 µg/l.

Table 3A compares the samples from Lebanon and France. The water from la Chaldette represents highly mineralized water with 770 mg/l of total mineralization, mainly due to a high sodium and bicarbonate content. A relatively high level of fluoride is noted. The water from Loing has an average mineralization, but with a concentration of 40 mg/l of nitrates, which must be monitored considering the European standard of 50 mg/l for adult and 25 mg/l for infant and pregnant women (WHO, 1993). The water from Volvic has very low mineralization with very low anion and cation content (Hartemann, 1992). Table 3B shows the results of trace elements analysis, which are within accepted norms for drinking water.

The chemical analyses confirm the calcareous bicarbonate character of the majority of the water sources in Dannia. A great number of the studied springs belong to the middle cretaceous basin (Nabaa Al-Soukkar, Nabaa Brisa, Nabaa Al-Arouse, Nabaa Al-Kassam, Nabaa Syr, *etc*). It was clear that Nabaa Al-Arousse constitutes part of Nabaa Al-Soukkar and it is fed from it, and Nabaa Syr is related to Nabaa Al-Kassam.

CONCLUSION

The rapid development of economical activity in Lebanon requires increased conservation of the drinking water resources and especially the establishment of a protected zone around the existing water springs.

In addition, chemical and cytotoxic analysis must be continued over time, especially according to seasons, in order to ensure constant correct sanitary quality of drinking water springs for the population of Northern Lebanon. In order to obtain a set of analysis over an entire year, as Fauris cited above. Likewise, bacteriological follow-up would be necessary (Haslay, 1993).

TABLE 3A
Results of the Chemical Analyses of Waters from Lebanon and from France

Water Source	Ain Fraydesse		Nabaa Al-Kassam		Nabaa Al-Zahlane		La Chaldette		Loing Source		Volvic Source	
Altitude	1000 m		1100 m		630 m		1 000 m		20 m		1 000 m	
pH à 20 °C	7.85		8.1		8.1		7.79		7.2		7	
Conductivity (µS/cm)	558		168		432		806		510		128	
Alkalinity (TAC °F)	19.9		9.2		20.6		41					
	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l
Calcium (Ca)	102	5,09	25,2	1,26	65,3	3,26	8,2	0,41	108	5,4	9,4	0,52
Magnesium (Mg)	13	1,07	7,3	0,60	21,7	1,78	1,1	0,09	7	0,29	5,6	0,50
Sodium (Na)	5	0,22	1,5	0,07	4,7	0,20	199	8,65	8,5	0,37	8,2	0,35
Potassium (K)	2,4	0,06	0,4	0,01	1,7	0,04	8,2	0,21	2,6	0,07	5,1	0,13
Ammonia (NH_4)	<0,10	0	<0,10	0	<0,10	0	<0,10	0	>0,1	0	<0,1	0
CATIONS Total	6.44		1.94		5.28		9.36		6.13		1.5	
Bicarbonates (HCO_3)	242,8	3,98	112,2	1,84	251,3	4,12	501	8,22	273	4,48	63,4	1,04
Chlorides (Cl)	15	0,42	2	0,06	7	0,20	16,4	0,46	25	0,7	8,7	0,21
Sulfates (SO_4)	42	0,87	3	0,06	36	0,75	15,5	0,32	17	0,35	5,8	0,14
Nitrates (NO_3)	75	1,21	3	0,05			3	0,05	40	0,65	3	0,03
Nitrites (NO_2)	0,10	0	<0,05	0	<0,05	0	<0,1	0	<0,1	0	<0,1	0
Fluorides (F)	0,10	0	0,10	0	0,10	0	7,2	0,37	<1	0		
ANIONS Total	6.48		2.01		5.18		9.42		6.18		1.42	
Iron (Fe) µg /l	<20		20		<20							
Total Hydrocarbons (mg/l)	0.01		0.01		0.01							

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TABLE 3B

Results of the trace elements analyses of springs in Dannia

Num.	Resource	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	Si
		µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	mg/l
1	Nabaa Al-Sanaoubar	5.7	1.46	2.94	0.01	10.18	2.0	0.01	0.01	0.04	0.99	10.5	1.4
2	Nabaa Al-Soukkar	5.8	1.46	2.93	0.01	10.2	2.0	0.01	0.01	0.02	1	13.3	1.4
3	Nabaa Brisa	5.9	2.47	1.87	0.01	0.1	2.6	0.02	0.01	0.01	2.48	7.2	1.0
4	Ain Al-Arousse	7.2	1.47	2.93	0.01	10.2	3.1	0.02	0.01	0.02	0.98	6.3	1.5
5	Ain Fraydesse	5.3	1.45	3.21	0.01	8.36	2.9	0.02	0.05	0.04	0.5	7.2	4.7
6	Nabaa Al-Kassam	5.5	1.46	2.95	0.01	6.48	2.7	0.02	0.04	0.03	0.4	7.2	1.4
7	Ain Beit Hamdane	7.9	1.38	3.25	0.01	5.23	0.05	0.01	0.02	0.02	0.35	7.1	2.1
8	Ain Al-Haour	7.4	1.45	3.33	0.01	5.69	2.7	0.02	0.03	0.03	0.26	6.4	2.1
9	Ain Al-Bahsa	11.2	1.46	3.55	0.01	4.26	2.8	0.02	0.02	0.04	0.48	7.1	2.4
10	Nabaa Syr	6.0	1.47	2.95	0.01	6.35	0.05	0.01	0.02	0.02	0.65	19.2	1.4
11	Ain Al-Biret	8.8	1.43	3.65	0.01	4.28	2.7	0.02	0.03	0.01	0.25	6.4	3.6
12	Nabaa Al-Zahlane	9.9	1.44	3.85	0.01	9.25	2.3	0.02	0.02	0.03	0.15	6.2	2.8

The Cytotoxicity analysis of samples from Northern Lebanon thus appears to be a good means of sanitary testing, providing rapid and highly sensitive results, which may be reproduced. But at present, when a very great number of chemical products or biologically degraded products in very low concentrations may react among themselves in the environment, it appears necessary to add other tests, environmental biomarkers, such as mutagenesis test and an anti-radical test.

ACKNOWLEGMENT

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