Original article

Evaluation of toxic chemical parameters and ecotoxicity levels in bottled mineral waters

G. SIGNORILE, A. NEVE, F. LUGOLI[*], M.C. PICCINNI[**], R. ARENA[**], R. DI MARINO[**]
University of Bari, Department of Internal and Public Medicine, Section of Hygiene; [*] University of Lecce, Department of Biological and Environmental Sciences and Technologies; [**] Apulian Water Works, Bari

Key words

Mineral waters • Heavy metals • Ecotoxicity

Summary

Heavy metals, phthalates, characterizing elements, CO_2 concentrations and pH and hardness levels were measured in forty samples of bottled mineral waters. In some samples arsenic, manganese, mercury and selenium were present in higher concentrations than permitted by Italian law. No significant release of phthalates from containers in PET was observed. In the same samples, toxicity tests with Daphnia magna were carried out. Toxicity levels, expressed as % of immobile organisms, ranged from 0 to 100%; generally the highest toxicity values were found in the hardwaters.

Introduction

Since the 1980s the consumption in Italy of bottled mineral water has grown rapidly, reaching a world record at the beginning of the 21st century of about 172 litres per person per year, corresponding to a production of more than 10 billion litres a year, bottled by around 190 factories under more than 270 brands [1].
This is due partly to the greater attention paid to health issues by the general population and the positive image that the producers of mineral waters are able to transmit via advertising and sponsorship of their products, partly to the frequent warnings, sometimes groundless, concerning pollution in the public water distribution networks and, in a few cases, to the unpleasant taste of drinking water. This is because drinking water can sometimes be subjected to treatment that produces secondary substances that give it a funny taste.
This growing consumption of mineral waters has prompted the scientific world to dedicate more attention to the quality of these waters, and particularly to the microbiological aspects [2-7]. Indeed, there are many risk factors deriving from microbial contamination to which the waters are exposed, especially during the production cycle [8, 9].
Less attention has been paid to the potential toxic risk represented by these bottled waters, for example from heavy metals that can contaminate the sources of supply as a result of human activities [10, 11].
Studies of toxicity have concerned themselves mainly with the migration into the water of substances released by poly(ethylene terephthalate) (PET) containers [12-16].
Monarca et al. [17] evaluated the effects produced by sunlight and temperature on PET containers destined for the bottling of mineral waters, with the possible release of substances harmful to health. The research presented here belongs to the same line of studies; our aim was to evaluate potential toxicity in samples of mineral waters selected from among the best-selling commercial brands. To this end, the concentration of various heavy metals and phthalates was measured, along with the characterizing elements; the levels of CO_2, pH and hardness were also measured.
An eco-toxicological test was carried out on the same samples using *Daphnia magna* in order to evaluate the levels of toxicity in terms of the percentage of immobile organisms [18, 19] and to determine whether these correlated with any of the chemical parameters examined.

Materials and methods

40 samples of mineral waters produced by the same number of companies, on sale in retail stores in the city and province of Bari, were analysed.
The bottles examined were selected on the basis of the commercial brand, the type of container (glass or coloured or clear plastic) and the level of CO_2.
For all the samples the concentrations of the following parameters were determined:
- CO_2, by means of direct phenolphthalein titration with sodium carbonate;
- total hardness, by means of titration with EDTA in the presence of Eriochrome Black-T as an indicator;
- dissolved cations and anions, analysed by ionic chromatography coupled with conductometric detection. The method is based on the chromatographic separation of cations and anions by means of ionic exchange columns. The individual analytes are eluted and determined by a conductometric detector

after electrochemical suppression of the electrical conductivity of the eluent. A DX100 ionic chromatograph (made by Dionex) was used;

– heavy metals, by mass spectrometry with Inductively Coupled Plasma Source Mass Spectrometry (ICP-MS). The technique is based on the capacity of the plasma to transform the metals present in the sample into ions, which are subsequently separated in the analyzer based on their charge-to-mass ratio by means of a system generally made up of a quadrupole fuelled by a radiofrequency generator. The quantitative analysis was carried out with an ICP-MS Elan 6000 mass spectrometer (made by Perkin Elmer);

– phthalates, by means of gas chromatography coupled with mass spectrophotometry of the active substances, after liquid-liquid extraction. In the proposed method, the analytes are extracted from the water with dichloromethane, concentrated in the Rotary Vacuum Evaporator (rotavapor) and solubilized in hexane. Subsequently the sample, injected automatically by the autosampler, reaches the column, where it is subjected to a thermal cycle for the separation of the various analytes. These then reach the ion trap (mass spectrophotometer), where they are subjected to bombardment with electrons (energy = 70 eV). For each analyte characteristic mass fragments are obtained (mass spectra), which are identified for comparison with those present in a standard library

Tab. I. Concentration of characterizing elements in mineral waters from 40 companies.

Sample n.	Li mg/l	Na mg/l	K mg/l	Mg mg/l	Ca mg/l	F^- mg/l	Br^- mg/l	NO_3^- mg/l	PO_4^{3-} mg/l	SO_4^- mg/l	Cl^- mg/l
1	0.00	9.80	0.80	1.40	5.80	0.01	0.00	0.60	0.00	4.50	12.00
2	0.00	1.31	0.25	4.33	54.60	0.01	0.00	0.81	0.00	1.52	3.15
3	0.00	0.52	21.37	12.32	51.04	0.25	0.00	0.03	0.00	77.68	74.01
4	0.00	19.08	1.99	39.62	94.61	0.17	0.19	22.95	0.00	5.28	33.46
5	0.00	4.20	0.56	31.60	43.21	0.13	0.00	6.33	0.00	5.86	2.34
6	0.00	4.00	1.30	4.70	74.70	0.10	0.00	1.80	0.00	3.20	4.50
7	0.04	30.10	18.91	6.27	20.53	0.80	0.00	6.20	0.00	13.55	17.74
8	0.00	125.76	47.36	50.19	133.13	2.28	0.00	2.06	0.00	117.46	39.68
9	0.09	222.33	22.09	14.34	41.78	0.69	0.24	24.78	0.00	277.31	77.43
10	0.00	0.97	1.10	1.70	18.00	0.24	0.00	1.04	0.00	12.56	0.52
11	0.00	121.90	1.40	5.79	44.45	0.13	0.00	0.75	0.00	5.30	11.51
12	0.00	17.13	2.10	14.02	59.25	0.14	0.00	4.01	0.00	33.44	24.05
13	0.00	4.66	0.55	4.84	33.11	0.07	0.00	4.28	0.00	21.09	7.89
14	0.00	3.31	0.27	3.31	59.07	0.15	0.00	0.94	0.00	7.73	7.38
15	0.00	12.62	1.12	5.77	131.37	0.15	0.00	3.68	0.00	20.65	18.54
16	0.00	1.67	0.69	10.42	32.10	0.29	0.00	3.62	0.00	18.11	2.87
17	0.15	84.57	9.99	29.13	156.38	3.51	0.34	6.35	0.00	115.49	87.36
18	0.00	22.86	7.67	14.95	300.05	2.82	0.00	1.18	0.00	55.86	24.39
19	0.02	5.67	1.56	7.80	16.20	0.11	0.00	0.45	0.00	1.08	5.40
20	0.01	11.30	16.21	4.01	13.49	0.80	0.00	3.73	0.00	6.63	7.90
21	0.03	41.52	27.90	8.55	34.28	0.39	0.00	0.40	0.00	10.97	17.44
22	0.00	2.00	0.59	19.03	85.08	0.12	0.00	3.76	0.00	16.78	4.19
23	0.00	2.59	0.97	26.53	78.52	0.52	0.00	3.18	0.00	80.13	2.56
24	0.00	5.41	0.79	22.28	78.38	0.07	0.00	3.31	0.00	11.62	6.42
25	0.00	4.75	2.20	15.32	300.39	0.47	0.00	3.85	0.00	6.10	8.24
26	0.08	45.76	41.82	19.25	344.30	1.17	0.00	4.86	0.00	3.28	17.69
27	0.13	333.79	75.08	48.38	224.57	0.60	0.28	19.58	0.00	257.05	68.68
28	0.00	2.52	0.00	0.50	0.60	0.00	0.00	0.12	0.00	0.73	5.77
29	0.00	0.87	0.00	1.97	2.20	0.03	0.00	0.33	0.00	2.06	0.30
30	0.00	0.98	0.26	0.27	9.89	0.00	0.00	1.15	0.00	6.79	0.43
31	0.04	1.13	0.00	1.50	12.00	0.00	0.00	0.20	0.00	7.91	0.19
32	0.00	15.32	0.25	2.50	87.41	0.25	0.00	0.63	0.00	18.82	16.25
33	0.00	16.87	1.52	2.97	81.04	0.29	0.00	5.23	0.00	28.56	18.42
34	0.00	5.60	2.02	38.90	133.00	0.30	0.00	6.09	0.00	244.90	5.70
35	0.06	40.27	28.67	16.59	263.86	0.67	0.00	6.33	0.00	5.26	23.71
36	0.11	35.15	2.82	44.60	151.77	1.32	0.00	1.14	0.00	485.47	69.87
37	0.00	0.52	0.07	1.31	5.45	0.05	0.00	1.94	0.00	1.21	0.53
38	0.04	52.04	19.34	12.59	46.50	0.00	0.00	27.52	0.00	23.56	15.63
39	0.00	30.51	2.64	26.59	63.88	0.29	0.00	23.28	0.00	11.80	56.78
40	0.00	0.52	0.14	19.27	51.20	0.01	0.00	1.84	0.00	8.92	0.84

of known compounds. The calibration solution used was the certified blend Supelco Mix 4 n. 4-8805, containing the following phthalates: dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, benzyl butyl phthalate, bis (2-ethyl) phthalate and di-n-octyl phthalate.
For all the analytical procedures reference was made to the Standard Methods for the Examination of Water and Wastewater [20].
In exactly the same samples (after treatment to extract the CO_2 by means of agitation for 30 minutes or insufflation of N_2) an eco-toxicological test with *Daphnia magna* was carried out, evaluating the effects of acute toxicity, expressed as a percentage of immobile organisms, on neonates of this cladoceran crustacean that were less than 24 hours old [18, 19].
The test should be considered valid if the immobilization of the Daphnias in the control (ISO standard water) is ≤ 10%. The sample is defined as toxic if the percentage of immobilization is ≥ 50%. Finally the ecotoxicity curves as a function of hardness, pH and phthalates were determined; for the preparation of the solutions to be tested, ISO standard water (pH 7.5-8.5; total alkalinity 110-120 mg $CaCO_3$/litre; hardness approximately 15 °F) was used.

Results

The results obtained in the course of the chemical and eco-toxicological tests carried out on the samples of mineral water bottled by 40 different companies are shown in Tables I-V.
Table I shows the concentrations of the characterizing elements, while Table II shows the values for CO_2, pH and total hardness.
The concentrations of the heavy metals examined here are shown in Table III. Their evaluation was conducted with reference to the maximum permitted values as specified in Ministerial Decree 542/92 and in the Decree of the Ministry of Health of 29/12/2003, which regulate mineral waters, as well as in Legislative Decree 31/01 relating to waters destined for human consumption [21-23].
Regarding silver and molybdenum, in most cases the concentrations detected were extremely low; however, it was not possible to make reference to maximum permitted values, since there is no legislation currently in force concerning these metals.
The concentrations of aluminium and vanadium were below their respective maximum permitted values, as set down in Legislative Decree 31/01, which are values that do not provide for mineral waters.
Regarding cadmium, chromium, copper, lead, nickel and antimony, the maximum permitted concentration as set down in the above-mentioned laws was not exceeded in any of the samples.
In some cases, concentrations above legal limits for mineral waters were observed for arsenic (10 µg/litre), manganese (500 µg/litre), mercury (1 µg/litre) and selenium (10 µg/litre).

Tab. II. Concentration of carbon dioxide, hardness and pH in mineral waters from 40 companies.

Sample n.	CO_2 mg/l	pH	Hardness °F
1	19.00	6.00	2.00
2	13.46	7.47	16.38
3	23.76	6.94	17.60
4	75.64	7.31	41.83
5	19.80	7.40	23.84
6	10.00	7.75	24.43
7	266.51	6.25	12.69
8	1432.93	6.64	53.83
9	876.94	6.38	26.17
10	8.71	7.43	5.05
11	14.40	6.51	15.40
12	12.27	7.77	16.20
13	4.55	7.64	11.12
14	8.31	7.51	16.11
15	40.79	7.21	35.34
16	6.14	7.72	12.93
17	851.40	5.80	53.62
18	572.81	6.28	82.10
19	8.04	7.19	7.55
20	9.50	7.28	5.20
21	7.05	6.96	12.70
22	27.72	7.25	28.39
23	30.49	7.46	25.67
24	31.09	7.17	28.76
25	1229.58	5.89	81.56
26	1114.34	6.01	82.04
27	1336.10	6.10	75.84
28	6.40	5.94	0.42
29	3.37	6.93	1.47
30	7.33	7.35	3.56
31	7.50	7.34	4.16
32	25.94	7.43	24.20
33	18.46	7.54	21.83
34	34.05	7.30	47.97
35	1460.45	6.04	74.58
36	1539.65	5.33	52.34
37	8.32	6.09	1.88
38	1440.09	5.40	18.69
39	36.23	7.28	29.01
40	23.70	7.53	22.43

In two samples, a concentration of boron was detected that conformed to the legislation concerning mineral waters (5 mg/litre), but not the legislation concerning waters destined for human consumption (1 mg/litre).
Table IV shows the concentrations of phthalates in five samples, in PET containers, one of which was of clear plastic and the other four in various colours.
Three of the six phthalates examined (diethyl phthalate, benzyl butyl phthalate and di-n-octyl phthalate) were absent in all the samples; in two samples dimethyl phthalate was found, but in very low concentrations. In contrast, di-n-butyl phthalate and bis (2-ethyl) phthalate were detected in all the samples, with a range of concentrations of about 0.4-3.5 µg/litre and 0.3-10 µg/litre respectively.

Tab. III. Concentration of heavy metals in mineral waters from 40 companies.

Sample n.	Ag µg/l	Al µg/l	As µg/l	B µg/l	Cd µg/l	Cr µg/l	Cu µg/l	Hg µg/l	Mn µg/l	Mo µg/l	Ni µg/l	Pb µg/l	Sb µg/l	Se µg/l	V µg/l	Zn µg/l
1	1.7	11.0	0.2	11.1	0.1	1.9	13.2	1.0	1.7	2.0	2.8	2.1	3.2	7.1	0.6	59.6
2	2.9	4.0	0.2	4.6	0.0	1.7	4.8	0.4	0.1	0.7	0.9	0.0	0.9	1.6	1.0	12.4
3	0.4	1.7	0.2	68.9	0.0	1.9	2.2	0.2	0.3	0.1	0.5	0.0	0.4	0.0	0.2	6.8
4	0.1	1.2	1.0	33.3	0.0	4.3	1.2	0.1	0.0	1.7	0.4	0.0	0.4	2.3	4.7	5.4
5	2.5	2.5	1.8	29.2	0.0	9.2	1.7	1.1	1.6	0.7	0.3	0.0	1.1	43.9	5.4	5.8
6	0.1	2.0	0.4	9.3	0.0	5.6	2.1	0.5	5.3	0.1	0.8	0.2	0.3	1.7	0.5	8.3
7	0.0	5.9	21.0	93.7	0.0	3.7	1.8	0.1	6.2	2.8	0.1	0.6	0.4	0.2	57.5	2.0
8	0.1	27.2	2.1	320.7	0.1	3.1	6.4	0.3	645.2	2.2	2.2	0.8	0.2	0.6	22.3	23.2
9	0.6	6.7	48.3	1226.1	0.0	14.0	4.1	0.1	139.5	1.7	8.3	0.6	0.3	1.7	23.7	12.8
10	0.4	1.7	8.3	17.3	0.0	1.0	0.5	0.1	0.5	1.9	0.0	0.7	0.2	0.7	0.8	0.0
11	0.1	1.4	0.4	21.4	0.0	2.8	1.3	0.2	0.7	0.2	0.2	0.0	0.1	5.7	0.8	1.1
12	0.0	1.2	0.4	9.7	0.0	16.5	0.9	0.1	0.4	0.1	0.1	0.6	0.1	0.9	0.3	1.9
13	6.8	12.0	0.7	16.8	0.2	2.3	25.5	1.3	1.0	3.4	5.4	6.7	3.2	2.2	0.8	230.1
14	2.6	7.5	0.3	8.3	0.1	1.4	10.9	0.2	0.3	0.4	1.7	3.7	0.9	0.0	0.3	92.9
15	1.9	6.7	0.8	53.2	0.1	2.2	12.4	0.9	0.5	0.2	2.2	2.4	0.8	3.9	0.6	130.4
16	6.6	7.6	2.0	4.5	0.1	2.4	11.2	0.2	0.3	0.9	1.0	2.2	0.6	0.5	1.0	113.7
17	0.8	5.1	1.0	753.1	0.0	7.8	3.4	0.3	0.9	0.6	0.5	0.5	0.6	0.9	0.3	31.3
18	0.0	6.5	0.6	59.2	0.0	5.8	3.9	0.1	50.0	0.0	1.5	0.8	0.4	0.0	0.1	42.9
19	0.9	4.6	2.4	16.1	0.0	3.6	6.4	0.1	2.2	0.0	0.4	0.8	0.3	0.0	7.7	38.3
20	0.7	4.3	10.4	48.1	0.0	1.3	3.4	0.2	0.1	0.7	0.2	0.7	0.4	0.5	18.7	39.7
21	0.0	18.3	7.2	75.7	0.1	1.6	2.6	0.1	25.9	2.2	0.1	0.4	0.3	1.0	48.8	26.7
22	2.9	4.6	0.6	51.6	0.0	2.1	3.7	0.1	0.3	0.6	1.4	0.7	0.3	1.1	1.3	46.5
23	3.3	2.3	0.6	10.6	0.0	0.9	2.2	0.1	0.4	2.4	0.5	0.4	0.5	2.4	0.1	27.2
24	2.6	4.8	0.6	8.8	0.0	1.1	5.7	0.1	1.3	0.6	0.7	0.9	0.4	0.9	0.2	50.8
25	1.1	3.3	2.0	7.9	0.0	2.9	4.7	0.8	2.2	1.6	2.5	0.8	0.4	0.0	15.3	45.5
26	0.0	10.1	11.9	454.4	0.0	3.6	3.3	0.1	44.2	0.4	1.8	0.7	0.6	0.0	11.4	36.3
27	2.2	6.1	7.0	1433.3	0.0	6.9	6.1	0.1	10.4	0.8	9.0	0.5	0.4	1.4	4.6	45.9
28	0.0	0.9	1.4	5.6	0.0	10.0	0.8	0.2	6.3	0.8	9.3	0.0	0.2	1.9	3.2	0.0
29	0.3	2.3	0.6	0.0	0.0	4.6	2.0	0.2	0.2	0.4	4.3	0.0	0.2	0.7	1.4	0.0
30	0.0	4.4	6.4	0.0	0.0	2.4	0.0	0.1	0.2	1.1	1.6	0.0	0.7	1.2	1.0	0.0
31	0.1	1.9	7.4	0.0	0.0	1.8	2.6	0.1	0.9	1.1	1.2	0.1	0.8	1.2	0.8	7.7
32	0.0	1.5	0.6	115.9	0.0	2.5	0.4	0.0	0.0	0.4	1.0	0.0	0.2	1.3	1.8	0.0
33	1.4	2.0	0.5	77.3	0.0	1.6	1.5	0.2	1.7	1.0	0.0	0.6	1.3	0.0	0.9	5.1
34	0.8	2.3	0.3	25.6	0.0	1.8	0.0	0.3	1.8	2.4	0.8	0.0	1.1	1.4	0.3	17.5
35	1.2	30.0	17.8	376.7	0.1	4.9	4.2	0.3	133.9	0.7	1.3	0.7	0.7	1.3	8.6	19.8
36	2.8	7.2	3.0	257.3	0.1	1.7	6.3	0.0	2.5	47.8	3.3	0.9	0.4	0.4	1.8	40.0
37	3.1	4.8	0.1	5.3	0.0	0.5	1.0	0.1	1.9	0.5	0.2	0.3	0.3	0.7	0.1	9.5
38	8.2	96.1	9.7	722.6	0.0	0.6	2.1	0.0	41.2	4.1	0.1	0.9	0.3	0.4	30.3	25.3
39	3.3	9.5	0.7	177.4	0.0	2.1	0.6	0.1	0.2	0.5	0.8	1.0	0.7	0.2	5.9	23.2
40	2.7	9.0	0.1	0.0	0.0	0.2	2.3	0.0	0.4	0.9	0.2	0.9	0.1	0.4	0.1	16.4

Tab. IV. Concentration of Phthalates in mineral waters from 5 companies.

Sample n°	Colour of container	Dimethyl Phthalate µg/l	Diethyl Phthalate µg/l	Di-n-butyl Phthalate µg/l	Benzyl Butyl Phthalate µg/l	Bis (2-ethyl) Phthalate µg/l	Di-n-octyl Phthalate µg/l
8	clear	0.01	0.00	0.45	0.00	0.42	0.00
16	turquoise	0.00	0.00	0.83	0.00	0.63	0.00
18	green	0.00	0.00	3.34	0.00	0.54	0.00
24	blue	0.05	0.00	3.48	0.00	9.87	0.00
25	pink	0.00	0.00	0.92	0.00	0.37	0.00

Tab. V. Levels of ecotoxicity with Daphnia Magna in mineral waters from 40 companies.

Sample n.	Same sample	Treated sample (after shaking for 30 mins)	Treated sample (after insufflation of N_2)
1	10%	10%	10%
2	10%	10%	10%
3	20%	20%	20%
4	95%	95%	95%
5	0%	0%	0%
6	20%	20%	20%
7	0%	0%	0%
8	100%	100%	100%
9	0%	0%	0%
10	0%	0%	0%
11	5%	5%	5%
12	60%	60%	60%
13	0%	0%	0%
14	6%	6%	6%
15	60%	60%	60%
16	5%	5%	5%
17	100%	100%	100%
18	100%	100%	100%
19	0%	0%	0%
20	6%	6%	6%
21	0%	0%	0%
22	75%	75%	75%
23	0%	0%	0%
24	90%	90%	90%
25	100%	100%	100%
26	100%	100%	100%
27	100%	100%	100%
28	15%	15%	15%
29	10%	10%	10%
30	5%	5%	5%
31	0%	0%	0%
32	0%	0%	0%
33	5%	5%	5%
34	0%	0%	0%
35	100%	100%	100%
36	5%	5%	5%
37	30%	30%	30%
38	10%	10%	10%
39	70%	70%	70%
40	20%	20%	20%

Table V shows the values for ecotoxicity, as measured in the same samples after treatment to extract the CO_2, expressed as a percentage of immobile *Daphnia magna* organisms in a range between 0 and 100%.
The ecotoxicity value in the ISO standard solution used for the test with *Daphnia magna* was always 0 with pH levels between 5.3 and 9.0, and with a blend of phthalates in a range of concentrations of 0.5-13.5 μg/litre; in contrast, ecotoxicity levels between 0 and 30% were observed when the hardness ranged between about 15 and 83 °F (Fig. 1).

Conclusions

A look at the results shows that 40% of the samples examined had a toxicity level above 50%, the threshold value at which a sample of water is considered toxic according to Legislative Decree 152/99.
In the mineral waters produced by eight companies the concentration of one or two heavy metals was above legal limits.
Regarding the release of phthalates from PET containers in the samples of mineral water examined, some of the compounds tested were never present, others had trace levels or just a few μg/litre.
The concentration variations of Phthalates observed in the examined samples can be attributed to the kind of container selected in base to the color, but very more probably to a different time of maintenance of the mineral waters in the bottles in PET considered.

Fig. 1. Pattern of ecotoxicity values in relation to hardness.

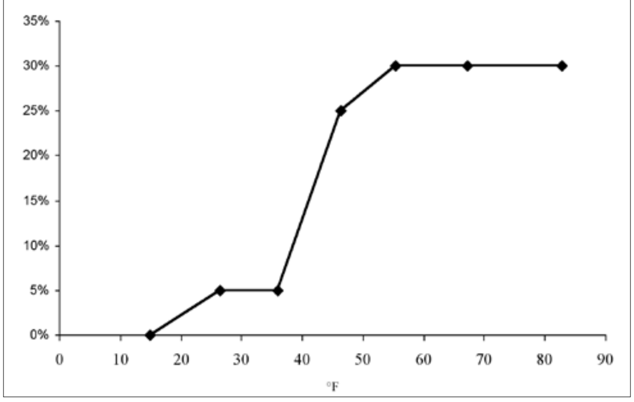
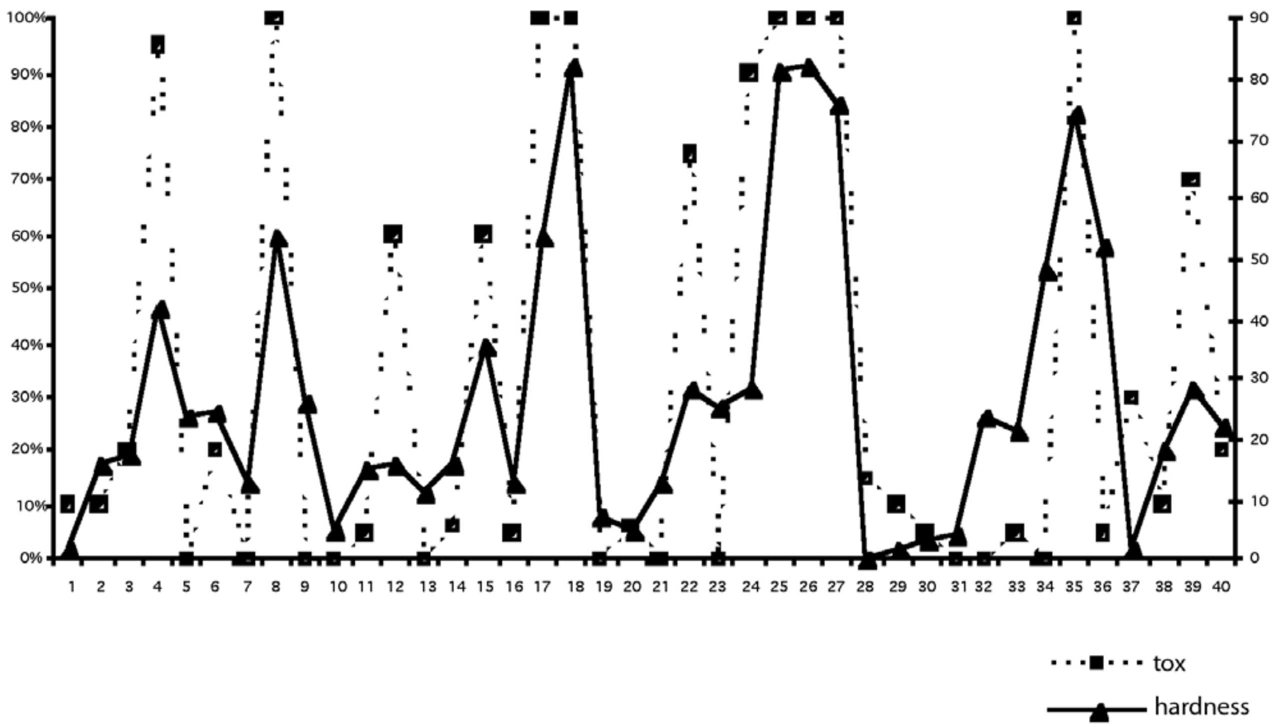


Fig. 2. Comparison of levels of hardness and ecotoxicity in mineral waters from 40 companies.



The toxicity values do not seem to correlate significantly with the quantity of heavy metals detected, given that in many samples in which the concentrations were higher the levels of toxicity were lower, and vice versa.

The quantity of carbon dioxide present in the various samples examined does not seem to influence the viability of the Daphnias either: the biological test performed on the same sample after elimination of CO_2 yielded similar results.

Similarly, there was little correlation of toxicity levels with pH values and the concentration of phthalates: at variable pH values and at levels of phthalates detected in the samples the percentage of immobile organisms observed was always zero.

Furthermore, any correlation with the characterizing elements (except for calcium and magnesium) can also be excluded, given the great variability of composition of the samples that were and were not found to be toxic.

The only parameter for which a certain correlation with toxicity was found is hardness: in general, the highest toxicity values were observed in the hardest mineral waters, while in the soft water samples the toxicity levels detected were almost always low (Fig. 2).

This consideration is also supported by the toxicity curve; moving progressively from hardness values typical of ISO standard water (used for the control) to values of about 82 °F, an increase in toxicity was observed, although not in an absolutely clear manner, from 0% to a maximum of 30%.

This result contrasts with what has been argued by other authors [24] concerning the better survival of *Daphnia magna* in hard waters and the difficulty of distinguishing between signs of stress resulting from toxic substances present and those resulting from the lack of hardness of the waters. Consider that in our experiment the ISO standard water used for the control had a low hardness value (about 15 °F) and zero toxicity.

A correlation with hardness, a parameter certainly not dangerous for human health, may be only partially relevant to the problem of toxicity, which was found in a fair percentage of commercial mineral waters. It is thus necessary to look for other risk factors that may account for levels of toxicity of up to 100%, linked for example to other substances released by PET containers (stabilizers, colours, plasticizers and lubricants).

For a better toxicological evaluation of the mineral waters it would in any case be advisable to carry out toxicity tests with other types of organisms, with varying sensitivity to a range of stressors [24-26].

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Correspondence: Prof. Giuseppe Signorile, Department of Internal and Public Medicine, Section of Hygiene, University of Bari, piazza G. Cesare 11, 70124 Bari, Italy - Tel. +39 80 5478477 - Fax +39 80 5478472 - E-mail: g.signorile@igiene.uniba.it