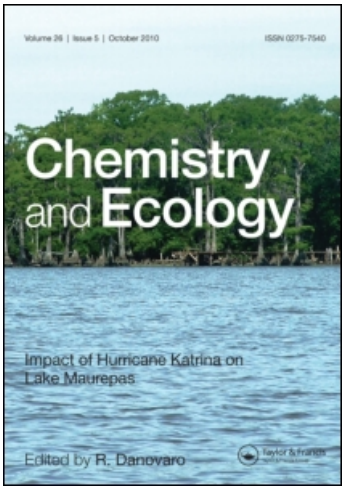

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EFFECTS OF CHEMICAL PRESERVATIVE AND TEMPERATURE STORAGE CONDITIONS ON CATIONS AND ANIONS IN NATURAL WATER

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The effect of storage on cation and anion concentrations in freshwater samples was investigated experimentally using chemical preservation treatments (mercuric chloride and/or acidification with hydrochloric acid) combined with three separate temperature levels (20°C, 4°C, or frozen). Two storage times were used, 21 days and approximately 90 days. Results were interpreted using analysis of variance and comparison of various means with results at day 0. Cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) were analysed at two different laboratories, yielding comparable results. Cation concentrations are generally resistant to change and are best untreated or preserved with mercuric chloride at any of the three temperatures. For anions, storage at 4°C with no chemical preservatives proved good or adequate. The main conclusions resulting from a large number of ANOVA tables and tables of means are presented, together with a summary table for all experimental treatments and chemical components studied: Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, PO_4^{3-}, SiO_4^{4-}, SO_4^{2-}, Cl^{-}, NH_4^{+}, total organic carbon. Particular storage treatments, such as removal of suspended matter by filtration and the use of iodised bottles for preserving phosphate, are important for some analyses. The biggest changes in ion concentrations occurred between days 21 and 90+; early analysis of stored samples is recommended.

INTRODUCTION

Concentrations of cations and anions in samples of natural waters may change during storage. While it is recommended generally to analyse water samples with minimum delay, it is not always practicable, as analytical equipment is seldom mobile, and samples may need to be transported before analysis is possible. Many ecological studies involve sampling at dispersed and remote sites, over a long period. For such studies it is convenient to store samples for short periods, so that batches may be accumulated.

Although the literature abounds with procedures for storage (Allen *et al.*, 1974; Wilson and Hunt, 1986), there is no universally accepted method for any cation or anion. Furthermore, workers may restrict their investigations to a single cation or anion for a particular method of storage, making cross-comparison of results from such studies impossible, if based upon different water samples and confounded with different 'batch effects' and 'laboratory effects'.

For a particular cation or anion, and storage method, the effect of storage time on the result of chemical analysis is important. If C_o denotes the true concentration value at time 0 (i.e. no storage time), and if C_t denotes the true concentration value at time t (after a storage duration of t days), then the null hypothesis, H_o is: C_t –

$C_o = 0$; the difference $(C_t - C_o)$, if H_o is rejected, can be estimated. Given the sample observations C_{o1}, C_{o2}, ..., C_{on1} at time 0, and C_{t1}, C_{t2}, ..., C_{tn2} at time t, the null hypothesis can be tested using Student's t-test (with $n_2 + n_2 - 2$ degrees of freedom). This procedure requires distributional assumptions such as that C_{o1} and C_{t1} values are normally distributed with constant variance σ^2 (Wilson and Hunt, 1986). Alternatively, an equivalent, distribution-free test such as Mann-Whitney U-test may be used (Siegel, 1956). Generalisations of the approach described by Wilson and Hunt (1986), in the form of designed experiments, and the use of powerful statistical analysis tools such as analysis of variance, are now well-established in agricultural, ecological and general biological research fields. The aim of this study was to test a variety of procedures for common cations and anions.

This paper reports collaboration between the Institute of Terrestrial Ecology's Monks Wood Experimental Station* (MW-KRB) and Merlewood Research Station** (M-APR) and a biometrician (MW-KHL). The statistical analyses and interpretation required numerous analyses of variance, and a detailed statistical examination of tables of means of the measured element concentrations in water samples subject to different treatment combinations. This led to a number of recommendations and comments for assessment of the studied chemical components (Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-}, SiO_4^{4-}, TOC, NO_3^-, SO_4^{2-}, Cl^- and NH_4^+). Cations were analysed at MW and M, anions were analysed at M.

If storage is unavoidable, acidification is recommended, for example for ammonium, while mercuric chloride is considered suitable for phosphate to prevent bacterial growth. More generally, storage at near zero temperature slows the rates of chemical change, and freezing is a practical and convenient alternative. Our experiments, therefore, looked at chemical preservatives (none, HCl, mercuric chloride and both HCl and mercuric chloride), and temperature (room, cold room and deep-freeze). Since the length of storage is also likely to be important, and since a delay of two to three weeks between the collection and chemical analysis of water samples is common, we analysed samples immediately upon collection, after storage for three weeks, and after three months (90+ days).

METHODS

Six samples from contrasting sites were used:

1 River Ecclesbourne, Derbyshire Site A
2 River Ecclesbourne, Derbyshire Site A Filtered
3 River Ecclesbourne, Derbyshire Site B
4 Loch Leven
5 River Eea
6 Elton Lower Hall, Sandbach

Filtration was made on site with 8 μm/0.45 μm filters (Sartorius membrane 50 mm). Samples 1–3 were analysed at MW, 1–6 at M. Sample 6 was included for ammonium analysis, but other samples were below detection limits. Samples were transported to laboratories in 25 litre acid-washed high density polyethylene containers.

* designated MW
** designated M

Sub-sample bottles (125 ml Xlon high-density polyethylene) were machine washed using detergent with citric acid and deionised water rinses. They were then washed with dilute hydrochloric acid and rinsed at least three times with deionised water. 100 ml of the sample water was added to each sub-sample bottle after rinsing twice with sample water.

Prior to analysis, frozen subsamples were left for 24 hours to equilibrate, followed by thorough mixing.

Experimental Units, Treatment Definition and Design

The twelve experimental treatments were: the combinations of four levels of the factor 'chemical preservative', and three levels of the factor 'temperature'. The four levels of the factor chemical preservative were (a) control: sub-samples given no chemical treatment, (b) hydrochloric acid: sub-sample treated with 1 ml of Aristar grade concentrated HCl, (c) mercuric chloride: sub-sample treated with 1 ml of Analar grade mercuric chloride solution (400 mgl^{-1} Hg) and (d) both: sub-sample given both treatments (b) and (c). The temperature levels were (i) 20°C (variable): sub-samples stored at room temperature, (ii) 4°C: sub-samples stored at about 4°C in a cold room and (iii) –12°C: sub-samples stored in a deep-freeze (–18°C at M).

A single replicate of the 4 × 3 factorial experiment to study the effects of chemical preservative and temperature levels would require 12 sub-samples. We intended having 4 replicates (48 sub-samples). However, it was not possible to carry out all 48 analyses in one batch. The chemical analyses could be done in batches of 24 sub-samples (experimental units), and hence our experiment may be thought of as two replicates of a three factor experiment: 4 chemical preservatives × 3 temperatures × 2 batches, each replicate requiring 24 sub-samples.

To assess the effects of storage time the water samples were analysed at different times after treatments. While the subsamples stored at room temperature (T_1 = 20°C) and in cold storage (T_2 = 4°C) could be analysed after 21 days and 90 days, those sub-samples stored in the deep-freeze (T_3 = –12°C) could be analysed only once. To provide material for the second storage time for the deep-freeze treatment, we used a further 16 sub-samples. In addition we also used 8 sub-samples for chemical analyses immediately on collection i.e. storage time = 0 day.

Thus, the full experimental set up of 4 chemical preservative levels × 3 temperature levels × 2 batches × 2 replicates, together with a provision for the initial records and for the final records for the deep-freeze treatments required, in all, 72 sub-samples.

Table I shows the full experimental set up. It will be seen that for ions such as NO_3^-, SO_4^{2-}, Cl^- and NH_4^+, analysed at M, fewer treatment combinations were used, avoiding the need for 2 batches. For both nitrate and sulphate, hydrochloric acid, could not be used as it interfered with the chemical analysis, while for chloride, only untreated samples were analysed since chloride is present in other treatments. No measurable ammonium could be detected in any of the five samples 1–5, but a further sample (Sample 6) was included with limited treatment combinations.

Chemical Analysis

At MW, samples 1–3 were analysed for cations. At M, samples 1–5 were analysed for cations as well as anions and other components, and an additional sample 6 for ammonium.

Table I A summary of the experimental treatments, number of batches and replicates used by two laboratories, Monks Wood (MW) and Merlewood (M).

Ion	TREATMENTS								
	Chemical preservative (C)				Temperature °C(T)			Batches(B)	Replicates
	None	HCl	Mercuric chloride	Both	20	4	−12		
Na^+	/	/	/	/	/	/	/	2	2
K^+	/	/	/	/	/	/	/	2	2
Ca^{2+}	/	/	/	/	/	/	/	2	2
Mg^{2+}	/	/	/	/	/	/	/	2	2
Phosphate	/	/	/	/	/	/	/	2	2
Silicate	/	/	/	/	/	/	/	2	2
Nitrate	/	*	/	*	/	/	/	1	2
Sulphate	/	*	/	*	/	/	/	1	4
Cl	*	*	*	*	/	/	/	1	4
TOC	/	/	/	/	/	/	/	1	2

Ammonium: using sample 6, at M. A (2 × 2) experiment: (none × HCl) × (Unfiltered, filtered), 1 batch, 4 replicates.

Symbols: / = treatment included; * = treatment not included

At MW cations were analysed with an Instrumentation Laboratories 251 atomic absorption spectrometer (AAS). Standard conditions with an air/acetylene flame were used and background corrections applied where appropriate (IL Handbook). Sodium and potassium were analysed in emission mode, calcium and magnesium by absorption; as magnesium concentrations were high, the burner was rotated through 90°. For calcium, solutions were treated with lanthanum (400 μgl^{-1}) to prevent phosphate and ionisation interferences. Standards of 1000 mgl^{-1} (Hopkin & Williams Ltd) were diluted and treated with preservative and lanthanum where appropriate.

At M, sodium and potassium were analysed on a dual channel Corning 150 filter flame photometer with an air/propane flame (Allen *et al.*, 1974). For calcium and magnesium, a Pye Unicam SP1900 AAS was used with an air/acetylene flame, a single slot burner and recommended conditions. Solutions of calcium and magnesium were diluted 5-fold prior to analysis to include lanthanum chloride (400 μgl^{-1} La) and 1% H_2SO_4 to reduce the interferences of refractory compounds and phosphate. An autosampler was used and 1000 mgl^{-1} standard stock solutions (BDH Ltd) were diluted with appropriate preservative for calibration solutions.

Phosphate was analysed (as PO_4^{3-} – P) using a Technicon Autoanalyser system. The module used the molybdenum blue reaction with stannous chloride reductant and detection at 700 nm using 2 cm cell (p453 Allen *et al.*, 1974). Silicate interference was avoided by use of low pH.

Silicate was analysed also using a Technicon Autoanalyser system (analysed as SiO_4^{4-} – Si) using the molybdenum blue reaction. Sodium sulphite was the reducing agent and tartaric acid was included to avoid phosphate interference. Detection was at 700 nm (p455 Allen *et al.*, 1974).

Nitrate analysis (NO_3^- – N) used a Technicon Autoanalyser module based on the diazotized sulphanilic acid method (Henriksen, 1965). The hydrazine reduction of nitrate to nitrite stage was replaced with a tube containing copper coated cadmium granules.

Chloride was analysed with an Autoanalyser method (p447 Allen *et al.*, 1974) using indirect mercuric thiocyanate colorimetry with detection at 485 nm.

Ammonium was determined using the indophenol blue method (as $NH_4^+ - N$) for Autoanalyser with the detector set to 625 mm (p450 Allen *et al.*, 1974).

Sulphate was determined (as $SO_4^{2-} - S$) by quantifying the excess lead remaining in solution after reaction of 25 ml sample/standard with 2 ml lead nitrate (0.2%) and 20 ml industrial methylated spirits. Samples were then diluted to 50 ml and stood overnight. Excess lead was determined by AAS using an air acetylene flame at 283 nm.

Total organic carbon (TOC) was analysed using a Carlo Erba TCM monitor. The sample was taken up and the volatile fraction stripped off and sent directly to the detector. The remaining fraction was acidified, the CO_2 fraction released being converted to methane to quantify the inorganic carbon. Residual carbon was transported to a combustion tube, the CO_2 again being methanised to give a peak for total organic carbon. Potassium hydrogen phthalate was used as a standard.

Statistical Analyses

For each sample, the data for a particular ion, and at a particular time point (see Table I), were statistically assessed separately. For the full factorial arrangement the standard analysis of variance used the factorial model:

$$X_{ijkl} = \mu + C_i + B_k + (CT)_{ij} + (CB)_{ik} + (TB)_{jk} + (CTB)_{ijk} + e_{ijkl}$$

where X_{ijkl} = the lth replicate (l = 1,2) of the observed ion value from the kth batch (k = 1,2) when the sub-sample was subject to ith chemical preservative treatment (i = 1,2,3,4) and jth temperature treatment (j = 1,2,3); μ = a constant; C_i = ith chemical treatment level effect; T_j = jth temperature treatment level effect; B_k = kth batch effect; $(CT)_{ij}$, $(CB)_{ik}$, $(TB)_{jk}$, $(CTB)_{ijk}$ = random error, assumed to be normally distributed with mean = 0 and variance = σ^2.

As usual, the analysis of variance provided the variance – ratio tests for testing the significance of the effects of the experimental treatments, batch effects and their interactions. The residual mean square provided an estimate of the experimental error used to compare various treatment means amongst themselves, as well as to compare the means of the initial eight observations with the corresponding experimental means after storage for three weeks or three months using the underlying normal distribution theory; similarly, comparisons could also be made between the two laboratories.

For the anions, NO_3^-, SO_4^{2-}, Cl^-, and TOC, there was one batch (see Table II), generally because of the restrictions upon the levels of the factor, chemical preservative and the factorial model above was modified accordingly. The underlying statistical theory is well described by Scheffé (1961). Kendall and Stuart (1966), and Winer (1971); a brief summary of statistical considerations is also given by Wilson and Hunt (1986).

Availability of statistical software such as GENSTAT, which we used on the Cambridge University's IBM 3081 main-frame computer, greatly facilitated the computations, even in the presence of a number of missing values in data at M. Most of these 'missing values' were unavoidable, and due to interference from chemical treatment.

RESULTS

The residual mean square in the ANOVA gave an estimate of experimental error, from which it is simple to calculate the standard error of any treatment mean. With 3 water samples analysed at MW, and 5 water samples at M, and since separate analyses were carried out for 11 components after storing for 21 days as well as for 90+ days, there were over a hundred separate ANOVA tables. While it is impossible to present the full details of all ANOVA tables here, a summary of all significant effects detected in these analyses is given in Table II. However, detailed comments about the various elements are based on a careful examination of the appropriate ANOVA tables and tables of means under different treatments. Table III gives mean concentrations for the four cations, measured at MW and M, for untreated water samples analysed at 0, 21 and after 90+ days storage.

Sodium

Day 0 sub-samples showed excellent agreement between the two laboratories for samples 1, 2 and 3 (Table III). At M, sodium concentrations were unchanged or showed an increase on storage; at MW, sodium concentrations decreased after day 0. However, the differences between the laboratory means were <5%.

Analysis of variance (ANOVA) showed many statistically significant effects particularly for C, T, B and CB effects. B, C, CB accounted for >70% of the sums of the squares (SS) of the MW data. Differences between the results were small and <5% from the grand mean. For M data, B and BC effects were less marked, whilst the chemical effects (C) were important but accounted for only 20% of the SS compared with 40% for the MW data. Treatment with mercuric chloride generally resulted in a higher analytical value at both laboratories. A few very low analytical

Table II A summary of significant ($P<0.05$) effects for cations and anions detected in 104 Analysis of Variance tables (one ANOVA for each water sample, at each of two storage times: 21 days and 90+ days in brackets).

Ion and laboratory	No. of samples	C	T	B	CT	CB	TB	CB
Na^+ (MW)	3	3 (3)	1 (2)	3 (3)	0 (2)	3 (3)	1 (1)	2 (2)
Na^+ (M)	5	4 (4)	1 (3)	2 (2)	1 (3)	1 (3)	1 (3)	1 (2)
K^+ (MW)	3	3 (3)	3 (3)	3 (3)	2 (2)	3 (3)	0 (0)	0 (1)
K^+ (M)	5	2 (4)	0 (2)	1 (3)	1 (2)	1 (2)	0 (1)	0 (1)
Ca^{2+} (MW)	3	2 (1)	2 (2)	3 (1)	2 (1)	1 (1)	0 (0)	0 (0)
Ca^{2+} (M)	5	5 (3)	1 (4)	3 (4)	0 (3)	1 (2)	1 (1)	0 (2)
Mg^{2+} (MW)	3	3 (3)	1 (3)	3 (2)	2 (3)	3 (3)	0 (1)	0 (1)
Mg^{2+} (M)	5	2 (1)	1 (1)	3 (1)	0 (1)	0 (1)	1 (1)	0 (1)
Phospate	5	3 (4)	2 (1)	3 (3)	1 (5)	1 (1)	0 (1)	0 (1)
Silicate	5	5 (5)	4 (5)	0 (1)	4 (5)	0 (1)	0 (3)	1 (3)
TOC	5	5 (3)	4 (3)	* *	2 (3)	* *	* *	* *
Nitrate	5	3 (5)	5 (5)	3 (3)	4 (5)	3 (3)	0 (2)	0 (3)
Total	52	40 (39)	25 (34)	27 (26)	19 (35)	17 (22)	4 (14)	4 (17)
Max. possible sig. results		52 (52)	52 (52)	47 (47)	52 (52)	47 (47)	47 (47)	47 (47)

CT etc. are combinations of C, T, B treatments
* = treatment combination not included (see Table I).

Table III Effect of storage on mean concentrations of cations ± standard errors (mgl^{-1}) in untreated samples at room temperature analysed at two laboratories. Monks Wood (MW) and Merlewood (M).

	Sample	Lab.	STORAGE TIME (Days) 0 (n = 8)	21 (n = 4)	90+ (n = 4)
Sodium	1	MW	17.44 ± 0.08	16.28 ± 0.07	16.68 ± 0.05
		M	17.70 ± 0.02	17.95 ± 0.03	17.70 ± 0.06
	2	MW	17.44 ± 0.01	16.55 ± 0.01	16.63 ± 0.06
		M	17.89 ± 0.02	18.00 ± 0.06	17.80 ± 0.00
	3	MW	23.38 ± 0.08	22.58 ± 0.13	21.68 ± 0.11
		M	23.45 ± 0.06	24.00 ± 0.04	23.85 ± 0.15
Potassium	1	MW	6.50 ± 0.01	6.51 ± 0.19	6.10 ± 0.04
		M	6.10 ± 0.01	5.97 ± 0.01	5.86 ± 0.02
	2	MW	6.61 ± 0.01	6.21 ± 0.06	6.44 ± 0.03
		M	6.10 ± 0.01	6.00 ± 0.03	6.24 ± 0.03
	3	MW	6.09 ± 0.02	5.69 ± 0.04	5.56 ± 0.05
		M	5.45 ± 0.01	5.41 ± 0.02	5.24 ± 0.05
Calcium	1	MW	50.24 ± 0.45	48.73 ± 0.81	45.60 ± 0.65
		M	50.10 ± 0.28	49.68 ± 0.54	43.45 ± 4.20
	2	MW	48.31 ± 0.48	47.08 ± 1.05	43.83 ± 0.75
		M	50.03 ± 0.26	49.98 ± 0.10	48.65 ± 0.56
	3	MW	54.41 ± 0.78	53.75 ± 0.95	45.93 ± 1.19
		M	56.29 ± 0.16	56.83 ± 0.40	53.30 ± 3.87
Magnesium	1	MW	12.20 ± 0.02	12.20 ± 0.04	12.73 ± 0.17
		M	12.95 ± 0.04	12.75 ± 0.07	13.15 ± 0.05
	2	MW	12.08 ± 0.03	12.43 ± 0.05	12.75 ± 0.10
		M	13.44 ± 0.07	12.78 ± 0.03	13.43 ± 0.13
	3	MW	9.41 ± 0.01	9.02 ± 0.15	9.31 ± 0.19
		M	10.07 ± 0.05	10.08 ± 0.03	10.20 ± 0.07

values were seen in the M data associated with acid treatment and acid + mercuric chloride treatment and storing at −18°C. Temperature effects (T) were often significant, particularly for MW; freezing gave lower values at both laboratories.

Potassium

Day 0 results (Table III) were not in such close agreement between the two laboratories; differences ranged from 3.2% to 5.9% from the mean. Untreated samples stored at room temperature showed similar differences (1.6–4.4%). M analytical values were always lower than the MW values. Generally potassium concentrations for these samples decreased with time but the rate of loss was generally quite small even for samples stored for 90+ days (mean decrease = 4.5%).

ANOVA showed (Table II) that C effects were the most important, accounting for 40% and 20% of the sum of squares of MW (6 of 6 significant results) and M (6 of 10 significant results) data. B effects were generally significant at both MW and M but were small (<3.5%). CB effects were significant for all MW data but not for M. C effects were similar for both laboratories, though less well defined for M. Acid treated and acid + mercuric chloride treated samples gave lower values than untreated and mercuric chloride treated samples. All treatments gave lower values than those at day 0. Mean values at each temperature (Table IV) showed that storage at 4°C gave the highest values (14 of 16) which were usually closest to those at day 0, whilst storage at either room temperature or deep-freeze conditions gave lower values. Acid treated, frozen samples yielded some low concentrations at M.

Table IV Mean potassium values (mgl^{-1}) at day 0, and subsequent means (averaged over chemical treatments and batches) for different samples analysed at Monks Wood and Merlewood, showing the effects of temperature and storage time.

Laboratory	Sample	Storage time (days)	Mean value (n = 8) unstored sample	Temperature °C 20	4	−12
Monks Wood	1	21	6.50	6.15	6.22	6.18
		91		6.15	6.22	6.20
	2	21	6.61	6.08	6.15	6.14
		91		6.25	6.29	6.23
	3	21	6.09	5.50	5.57	5.55
		91		5.62	5.70	5.66
Merlewood	1	21	6.10	5.96	6.00	5.92
		98		5.97	6.02	5.47
	2	21	6.10	6.05	5.94	5.91
		98		6.18	6.14	5.64
	3	21	5.45	5.45	5.46	5.41
		98		5.31	5.39	5.15
	4	21	1.74	1.63	1.65	1.60
		98		1.61	1.61	1.56
	5	21	3.17	3.20	3.22	3.14
		98		3.28	3.30	3.30

Notes: 1 Almost all means are smaller than the untreated mean values at day 0.
2 Of the three temperature means in each row, the mean at 4°C is often the highest i.e. T_2 is greater than T_1 or T_3.

Calcium

Calcium concentrations in untreated samples decreased with time, particularly for 90+ days. ANOVA (Table II) showed fewer significant effects in MW data compared with the other cations. Most of the significant effects occurred for B, C (11 of 16 cases) and to a lesser extent T (9 of 16 cases). These three effects accounted for about 40% of the sums of squares. Batch effects, though often significant, were generally small – <2% difference from mean for MW results, <4% in 8 of 10 cases for M values. C effects were significant in 8 of 10 cases for M data and 3 of 6 for MW data. With the M data, however, there were higher values for some samples.

Temperature effects showed different patterns for the two laboratories (Table V). Cold storage (4°C) generally give higher values though these were lower than those found at day 0 (Table IV). It is important to note that samples stored frozen gave the lowest values most often (10 of 16 cases). The high M mean value for frozen samples on day 90+ was due to a few very high values, especially the acid treated samples, often those with low potassium and sodium values.

Magnesium

Day 0 sample analyses and trends in time compared reasonably well for the two laboratories (Table III). Results differed by <6% from the mean. Untreated samples stored at room temperature were also within 6% of the mean. ANOVA results (Table II) showed that B, C, T, BC and CT effects in MW data yielded significant results more often than M data. However, these effects, even when significant, were generally

Table V General observed patterns of temperature on the concentrations of calcium and magnesium in water samples stored for 21 and 90+ days at (MW) and (M). The temperature mean values for each ion were obtained by averaging over chemical treatments and batches.

Laboratory	Storage time (days)	Observed pattern in the three* temperature means (highest first)
Calcium		
MW	21	$T_2 > T_1 > T_3$
	91	$T_2 > T_1 > T_3$
M	21	$T_1 > T_2 > T_3$
	98	$T_3 > T_2 > T_1$
Magnesium		
MW	21	$T_2 > T_3 > T_1$
	91	$T_2 > T_3 > T_1$
M	21	$T_2 > T_1 > T_3$
	91	$T_3 > T_1 > T_2$

*T_1 = mean at room temperature = up to 20°C (variable)
T_2 = mean at 4°C
T_3 = mean at −12°C

small e.g. B effects <3%, C effects <5%. MW results did not show this; acid + mercuric chloride treated samples gave low magnesium values but this was not the case for M data.

Temperature effects were inconsistent (Table V). While most MW data were higher than the values obtained at day 0, most M data at day 21 were lower. M data at day 90+ showed no clear pattern.

Phosphate

At day 0, phosphate concentrations varied by 2 orders of magnitude between the sites (0.015–1.5 $mgl^{-1}P$) (Table VIa). The lowest concentrations, for sample 4, were close to the limit of detection. Untreated samples stored at room temperature showed a pronounced decrease in phosphate concentration with time, both at day 21 and further by day 90+. The decrease was smaller for samples stored at 4°C (Table VI).

For uniodised bottles, ANOVA summary (Table II) showed significant effects for C, B and CT. B effects were generally small (<5% from mean) even when significant, except for concentrations close to the analytical detection limit. Chemical treatment effects showed some differences. Treatment with acid, mercuric chloride and acid/mercuric chloride all gave higher values than untreated samples; acid treatment generally gave the highest values. CT effects which were often significant, accounting for 25% of variation. The least change for all treatments occurred with samples stored at 4°C. Samples stored frozen and at room temperature appeared to decrease further. Mean concentrations well above detection limits showed a marked decreased concentration with time.

In addition to the samples stored, analysed and subjected to ANOVA, further untreated samples were stored at 4°C in iodised bottles. These samples showed even less decrease of phosphate concentrations with time than samples in clear bottles at 4°C (Table VI). Filtration may also affect storage. Sample 1 (unfiltered) values were consistently higher than for Sample 2 (filtered). It was also evident that there were fewer significant effects in the ANOVA for filtered samples.

Table VI Comparison of mean concentrations for phosphate (as P) ± standard errors (mgl^{-1}) in untreated samples at day 0 and samples kept at room temperature, in the cold room or in iodised bottles.

	Sample	Storage time (days) 0 (n = 8)	21 (n = 4)	90+ (n = 4)
(a) ordinary bottles room temperature	1	0.515 ± 0.004	0.440 ± 0.014	0.170±0.093
	2	0.446 ± 0.003	0.395 ± 0.005	0.313±0.031
	3	1.533 ± 0.005	1.435 ± 0.013	1.073±0.135
	4	0.015 ± 0.007	0.016 ± 0.011	0.004±0.001
	5	0.112 ± 0.004	0.106 ± 0.002	0.052±0.028
(b) ordinary bottles cold room	1	0.515 ± 0.004	0.485 ± 0.010	0.445±0.016
	2	0.446 ± 0.003	0.425 ± 0.005	0.413±0.010
	3	1.533 ± 0.005	1.415 ± 0.030	1.488±0.024
	4	0.015 ± 0.007	0.013 ± 0.001	0.034±0.008
	5	0.112 ± 0.004	0.116 ± 0.002	0.119±0.002
(c) iodised bottles cold room	1	0.515 ± 0.004	0.483 ± 0.003	0.485±0.005
	2	0.446 ± 0.003	0.440 ± 0.008	0.447±0.005
	3	1.533 ± 0.005	1.500 ± 0.027	1.530±0.009
	4	0.015 ± 0.007	0.019 ± 0.001	0.040±0.004
	5	0.112 ± 0.004	0.108 ± 0.001	0.124±0.004

Notes: (a) shows substantial decline, particularly for day 90+
(b) is much better
(c) is even better

Silicate

Day 0 values differed by a factor of 5 over the 5 sites (Table VII). The three Ecclesbourne samples (1, 2, 3) were very similar, close to 5 mgl^{-1} Si, and sites 4 and 5 were both about 1 mgl^{-1} Si. Untreated samples stored at room temperature showed very little change at day 21, but there was a marked decrease for some samples at day 90+. Silicon values from sample 4 decreased less, but the only results close to the day 0 value were those for the filtered sample i.e., Sample 2. The reason for this may be the uptake of silicate by micro-organisms. There is some suggestion of a similar but less marked effect for phosphate. Filtration presumably removes the micro-organisms; furthermore, it does not seem to affect the initial silicate concentrations.

ANOVA (Table II) showed that C, T and CT are nearly always significant, accounting for about 90% of the sums of the squares. In contrast, batch effects were seldom significant. C effects were clear. Acid treated and mercuric chloride treated samples were consistently lower than day 0. Mercuric chloride treated and untreated samples were much closer to day 0 values. Storing frozen samples is likely to produce low silicate values. CT interactions are probably significant due to acid treatment, with and without mercuric chloride, which together with freezing, gave very low values.

Total Organic Carbon

Day 0 values of TOC fell within a relatively narrow concentration range (2.9–5.4 mgl^{-1}) (Table VII). Some untreated samples stored at room temperature increased in TOC concentration with time after day 21, particularly the samples from sites 4 and 5 in which algal growth was noted.

ANOVA (Table II) showed C (8 of 10), T (7 of 10) and CT (5 of 10) effects reaching significance. With samples analysed in one batch, the underlying ANOVA model did not include any 'batch effect' terms.

Table VII Effect of storage on mean concentrations of anions, TOC and ammonium ± standard errors mgl^{-1} in untreated samples stored at room temperature.

	Sample	STORAGE TIMES (days) 0 (n = 8)	21 (n = 4)	98 (n = 4)
Silicate	1	4.79 ± 0.01	4.73 ± 0.01	0.68 ± 0.46
(SiO_4^{4-} – Si)	2	4.79 ± 0.09	4.75 ± 0.01	4.77 ± 0.01
	3	4.75 ± 0.01	4.76 ± 0.02	0.05 ± 0.03
	4	1.01 ± 0.01	1.07 ± 0.02	0.60 ± 0.27
	5	1.33 ± 0.01	1.37 ± 0.01	0.04 ± 0.02
TOC	1	4.46 ± 0.05	4.12 ± 0.08	4.40 ± 0.35
	2	4.44 ± 0.03	4.37 ± 0.53	4.23 ± 0.39
	3	4.80 ± 0.02	4.45 ± 0.10	4.99 ± 0.21
	4	5.33 ± 0.10	5.58 ± 0.23	9.15 ± 4.25
	5	2.97 ± 0.05	2.73 ± 0.08	26.55 ± 12.65
Nitrate	1	4.13 ± 0.05	3.15 ± 0.22	2.47 ± 0.95
(NO_3^- – N)	2	4.25 ± 0.04	2.96 ± 0.15	3.93 ± 0.18
	3	4.55 ± 0.06	3.10 ± 0.12	2.60 ± 0.69
	4	1.24 ± 0.03	1.45 ± 0.18	0.58 ± 0.12
	5	4.35 ± 0.17	3.02 ± 0.22	0.31 ± 0.30
Sulphate	1	15.23 ± 0.38	19.20 ± 0.95	23.02 ± 2.08
(SO_4^{2-} – S)	2	14.63 ± 0.85	16.12 ± 0.79	18.37 ± 0.25
	3	20.14 ± 1.25	24.12 ± 1.22	19.77 ± 0.20
	4	8.07 ± 0.27	8.49 ± 0.12	7.85 ± 0.03
	5	7.76 ± 0.17	7.25 ± 0.10	6.34 ± 0.26
Chloride	1	27.79 ± 0.06	26.90 ± 0.30	27.85 ± 0.22
	2	28.15 ± 0.08	26.92 ± 0.19	28.67 ± 0.17
	3	33.86 ± 0.09	33.50 ± 0.08	34.37 ± 0.22
	4	16.39 ± 0.09	16.90 ± 0.16	18.85 ± 0.12
	5	17.64 ± 0.11	17.70 ± 0.07	19.25 ± 0.19
Ammonium (NH_4^+ – N)	6	4.21 ± 0.02	4.03 ± 0.03	2.25 ± 0.05

All treatments gave higher TOC concentrations than those at day 0, but samples with added acid, with or without mercuric chloride, yielded higher values than those treated by mercuric chloride alone. Day 90+ values were generally higher than day 21 values for untreated samples (4 of 5 cases). All untreated day 21 mean values were closer to day 0 values than day 90+ values. Effects were different at day 21 and day 90+. For the former samples, storage at 4°C consistently gave the highest values and show the greatest change from day 0 values. For day 90+ samples, frozen samples gave the highest values, whilst cold room storage gave the lowest.

The use of acid or mercuric chloride and the freezing of samples should be avoided and samples should be analysed as soon as possible. Filtration of samples from sites1–3 did not markedly affect TOC values.

Nitrate

At day 0, nitrate did not vary much between sites (1.2–4.6 mgl^{-1} N) (Table VII). Untreated samples stored at room temperature showed lower concentrations of nitrate with time. All day 21 values were lower than day 0, and 4 of 5 samples at day 90+ decreased further.

ANOVA (Table II) showed temperature to have an important effect (significant in 10 of 10 cases); chemical treatment effects (8 of 10 cases) and CT effects (9 of 10 cases) appeared also important. Batch effects were significant, but relatively less

often (6 of 10). Temperature effects showed that storage at room temperature was likely to result in a decrease of nitrate concentrations; here, cold room storage is preferred. Although C effects were significant the differences were small compared with other factors. Changes with time were marked for mercuric chloride treated samples stored at low temperatures. The general pattern is a lower concentration at day 21 followed by an increase to day 90+.

Sulphate

For sulphate, only two levels of the chemical treatments were included: none and mercuric chloride. Here the four replicates could be analysed in one batch. Concentrations showed a threefold range between sites at day 0 (7.8–20 mgl^{-1} S) (Table VII). Values for untreated samples stored at room temperature increased with time for samples 1 and 2, and the filtered samples consistently gave lower values at all three time intervals, as shown by the means for Sample 2 (filtered) and Sample 1 (unfiltered) from the same site and date from River Ecclesbourne. ANOVA showed very few significant effects (only 1 of 10 cases for C, T and CT).

Chloride

Because of analytical interferences, chloride analyses were carried out only on 4 chemically untreated replicates stored at different temperatures in one batch, using a simple one-way classification ANOVA model. Concentrations at day 0 ranged from 16 mgl^{-1} (Sample 4) to 34 mgl^{-1} (Sample 3); samples stored at room temperature showed little change with time (Table VII). Filtration had no effect on the results, and ANOVA showed no significant temperature effect.

Ammonium

Only one water sample (Sample 6 from Site 6, see Table I) was analysed for ammonium. All other samples were below the limit of detection of the analytical method. All these samples were stored at 4°C. The underlying experiment was a simple 2 × 2 factorial: (none, HCl) × (unfiltered, filtered), with 4 replicates analysed in one batch.

Replicates which were untreated and unfiltered showed a marked decrease of ammonium concentration at day 90+ (Table VII). ANOVA showed that both filtration and treatment were highly significant at day 21 ($p<0.01$) and 90+ ($p<0.001$) and that filtration × treatment was highly significant at day 90+ ($p<0.001$). Acidified, filtered replicates showed less change in ammonium concentration (11% loss, 90+ days) than unfiltered or untreated samples (46% loss, 90+ days). All replicates stored well up to 21 days.

DISCUSSION

Cations

Cation analysis results (Table III) from the two laboratories generally agreed well for Samples 1, 2 and 3. Potassium values showed the biggest difference between laboratories, but even these were within 6% of the mean value. The only marked

changes with untreated samples stored at room temperature were with calcium concentrations at day 90+, though potassium concentrations showed some decrease. Cations, therefore, present no major preservation problems. ANOVA showed many statistically significant effects. However, an examination of treatment means showed that the magnitudes of differences were not large. It cannot be emphasised too strongly that the importance of any statistically significant results should be judged by the magnitudes of the difference (Wilson and Hunt, 1986). For example, batch effects were frequently highly significant statistically, especially for MW analyses. This is hardly surprising – batches are expected to be different and control of calibration/drift between batches will be variable. However, the magnitude of the batch effect differences were small; the batch means were seldom more than 3–4% different from the overall mean, indicating that the results are repeatable within tolerable limits.

There were few consistent trends in the data between both laboratories due to the small magnitude of changes measured. An exception was the decrease of potassium concentrations with time in samples treated with acid and acid + mercuric chloride. The reason for this is not clear, but it is an important observation as acid treatment is often recommended for preserving cations in water samples (Allen *et al.*, 1974).

At M, acid treated samples, stored frozen, were sometimes found to change their metal concentrations considerably; sodium and potassium concentrations increased. Calcium concentrations decreased, while magnesium concentrations varied from those at time 0. Often more than one metal was affected in the same sample, and the differences between the observed concentrations and the mean values was large (e.g. 100% increase for Na^+, 50% decrease for Ca^{2+}). We believe that all samples were completely thawed and were shaken to remix after freezing and thawing; nevertheless, failure to remix thoroughly to ensure complete dissolution could explain large concentration differences. Precipitation of calcium could explain a decrease in concentration but this seems unlikely, especially in acid treated samples, and was not consistent for all samples.

Adequate storage of cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) in fresh waters is achieved for periods up to 90+ days by storing at room temperature or at +4°C. Treatment with acid, mercuric chloride or both these reagents does not affect preservation except in the case of Mg^{2+} which decreases with acid treatment.

Phosphate

Other workers have recognised the problems of storing samples for phosphate analysis. Heron (1962) found iodised bottles prevented the decrease of phosphate levels on storage, attributed to microbiological action. Our results show that iodised bottles are an aid to preservation but results are only slightly better than those for bottles stored in cold room conditions. Hellwig (1967) found that treatment with mercuric chloride did not prevent deterioration of phosphate in polluted effluents, though values were within an order of magnitude of the original results. We found that mercuric chloride helped preserve samples at 4°C. Frozen stored samples deteriorated with time, in agreement with Philbert (1973). Chakrabarti *et al.* (1978) tried several methods of preservation and found sulphuric acid a useful preservative but it may hydrolyse inorganic polyphosphates and labile organic phosphates to orthophosphates. We conclude that using iodised bottles is the most effective preservative for phosphate; acidification is also a good preservative but samples must be stored at 4°C.

Silicate

For short term storage, even untreated water samples stored at room temperature seem suitable for silicate analysis, but beyond 21 days, deterioration takes place. This is perhaps caused by microbiological action, as filtered samples maintain their concentrations. Storage with acid treatment at low temperature is likely to result in loss of silicate possibly due to precipitation. Allen *et al.* (1974) recommended that freezing be avoided.

Total Organic Carbon

TOC values may increase considerably in some untreated samples in association with algal growth if stored at room temperature for 90+ days. Samples treated with acid, or mercuric chloride, or acid and mercuric chloride can also give high TOC values. This may be due to decomposition of micro-organisms killed by these treatments. Analysis of untreated samples within 21 days is recommended to avoid increase in TOC concentrations. Chemical treatments should be avoided.

Nitrate

Our results confirm that nitrate is susceptible to microbiological activity during storage (Allen *et al.*, 1974). For untreated samples stored at room temperature (Table VII), large decreases in nitrate concentrations occur. Although there are trends in the data, such as the decreasing nitrate values to day 21 followed by an increase to day 90+, the individual results are variable. Decreasing concentrations followed by an increase have been reported for unpreserved and chloroform treated samples (Chakrabarti *et al.*, 1978). Trends in our samples common to all chemical treatments and storage temperatures suggest that samples should be analysed as soon as possible.

Sulphate

Changes in sulphate concentrations occur with time but they are relatively small. The biggest changes were found with unfiltered Sample 1, suggesting that filtration is useful. Neither C nor T effects were significant and there were no consistent time effects with different treatments and temperatures.

Chloride

There were no problems associated with storage of samples for chloride analysis. Untreated samples were stable at all storage temperatures. The lack of chloride data from other investigations of water preservation methods indicates that difficulties were not usually encountered or that chloride is not considered important.

Ammonium

The decrease of ammonium concentration observed for untreated samples with time is consistent with observations of Klingman and Nelson (1976) and Degobbis (1973). Chakrabarti *et al.* (1978) stored samples successfully for up to one week using reduced temperature (4°C), or chloroform; use of sulphuric acid preserved samples for 30 days. Acid treatment helped to slow the rate of loss of ammonium in our experiment and the most marked decrease of ammonium occurred when the stored water was

unfiltered and unacidified. However, if samples are analysed before day 21, little change occurs even in untreated, unfiltered, samples.

CONCLUSIONS

The inferences and conclusions drawn from this multi-factor experimental study are based on the results of over a hundred separate ANOVA tables and a careful examination and cross-comparison of about five hundred tables of means (and their standard errors) under different treatment combinations. While results reaching statistical significance are obvious, the scientific and practical significance of the findings depends upon the magnitude of the observed differences under different combinations of factors (taking into account the standard errors of these differences) and also upon the consistencies in the trends for different samples.

Our conclusions are summarised in Table VIII. For each element we show which chemical preservative treatments and storage temperatures are good, adequate or not recommended; and in the last column, special comments are given which apply to the chemical analysis of particular elements. The main conclusions are as follows:

1 The chemical treatments used are not generally useful as they exclude the analysis of some of the variables, particularly anions (see Table I).

2 The cations Na^+, K^+, Mg^{2+} and Ca^{2+} change little under storage, whether the water sample is treated or not.

3 In contrast, phosphate and nitrate concentrations are likely to change at room temperature even if samples are treated chemically. Storage at 4°C appears quite effective and use of chemical treatments appears unnecessary.

4 ANOVA showed that the largest proportion of variance is due to the factor storage duration (levels of 21 days and 90+ days), and, as expected, more significant effects were noted for samples stored for 90+ days compared with 21 days.

Table VIII Conclusions, assessment and recommendations for various chemical elements in water samples stored before chemical analysis.

	TEMPERATURE												
	20°C				4°C				−12°C				Special
Element	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4	Comments
Na^+, K^+, Ca^{2+}	G	G	G	G	G	G	G	G	G	X	G	X	
Mg^{2+}	G	X	G	X	G	X	G	X	G	X	G	X	
PO_4^{3-}	X	X	X	X	G	G	A	A	X	X	X	X	a
SiO_4^{4-}	A	X	G	X	A	X	G	X	X	X	X	X	b,e
TOC	G	X	X	X	G	X	X	X	G	X	X	X	c,e
NO_3^-	X	X	X	X	A	–	A	–	A	–	A	–	d
SO_4^{2-}	G	G	–	–	G	G	–	–	G	G	–	–	b,e
Cl^-	G	–	–	–	G	–	–	–	G	–	–	–	e
NH_4^+	–	–	–	–	G	G	–	–	–	–	–	–	b,c

*C_1 =no chemical treatment; C_2 = HCl; C_3 = mercuric chloride; C_4 = both C_2 and C_3. G = Good; A = Adequate; X = not recommended; – = not analysed.

Comments: a = better results with iodised bottles
b = filtration recommended
c = analyse within 21 days
d = large variation between samples
e = possibility of large differences between samples from different sources

5 Obviously, filtration is recommended when samples contain suspended matter, as it accelerates changes in solution concentrations. Filtration procedures must, however, be checked to ensure against loss of trace fractions or contamination from filter papers.

6 Our results agree with literature that freezing affects phosphate and silicate values, and for phosphate iodised bottles are recommended.

7 Overall, our general advice is (i) to store the water samples at 4°C, and (ii) to analyse the samples within 21 days.

Analysts must be aware that these recommendations are effective in reducing the changes in solution concentrations between sampling and chemical analysis. It may be prudent to check that the procedures offered are applicable to the sample matrices under study and the sample handling operations employed.

Finally, our study also emphasises the fact that the results might also depend upon factors such as (a) which laboratory is used, (b) what instrumentation and analysts were involved, (c) was there any batching of the sample experimental material analysed. Obviously, care must always be taken to ensure that such effects are minimised.

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