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INFLUENCE OF FREEZING WATER SAMPLES
PRIOR TO CHEMICAL ANALYSIS

BY

ROGER RAY PATOCKA

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Civil Engineering, South Dakota
State University

1972

INFLUENCE OF FREEZING WATER SAMPLES

PRIOR TO CHEMICAL ANALYSIS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for the degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Date

Head, Civil Engineering
Department

Date

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INTRODUCTION

The need to collect and analyze samples of water resources periodically has been realized for many years by people concerned with the use and preservation of water resources. As water use increased, routine analysis of water quality also increased. In recent years, increased water usage and the resulting development of water quality standards by all states of the union, as required by the Federal Water Quality Act of 1965, indicate an increasing need for water quality analysis.

In order to obtain representative results of analyses, care must be taken to obtain samples which are representative of the existing field conditions. Care must also be taken to have a sample which is representative of the field conditions at the time the sample is being analyzed.

The time lapse between collection and analysis of water samples can become quite lengthy for the following reasons: time of transport to the testing laboratory, lack of convenience or manpower, number of samples to be tested, and laboratory testing capacity. During the time lapse between collection and analysis, the composition of the sample may change because of the chemical reactions that constantly occur in all substances (1-3). Freezing has been used as a method to preserve the chemical quality of the sample at the time of collection. Therefore, a simple, practical water sample preservation method such as freezing would be very beneficial to a water quality surveillance program.

A practical freezing process for preservation would involve the following: collection of representative samples, preservation by simply freezing in a convenient food storage freezer or locker, storage in the frozen state until the time the samples are to be analyzed, thawing the samples at room temperature, and, finally, analyses of the samples.

A rapid freezing technique such as the utilization of dry ice and acetone seems to be less practical and may be dangerous. If the more feasible freezing process (food storage freezer) preserved the chemical integrity of water samples, the process could easily be implemented within a water quality surveillance program.

A literature review revealed that adequate statistical evaluations of some of the freezing preservation processes were not made, and as a result, conflicting opinions on the effects of preservation by freezing prevailed. This research was undertaken to determine whether the freezing or thawing of water samples would significantly change the chemical quality of the samples. The decision to evaluate the data statistically was made in order to support the conclusions of this research project.

LITERATURE REVIEW

Previous Research

Research regarding the methods of water sample preservation by the utilization of a frozen storage process is somewhat limited, although the process is used and promoted by some researchers. Of the research that has been accomplished, fewer have been of a statistical nature.

The research performed by Fogarty and Reeder (2) was undertaken to determine whether the physical characteristics, specifically those governing the Biochemical Oxygen Demand (BOD) of raw water sources, would remain unchanged during frozen storage. Raw, semi-treated, and fully treated domestic sewage with BOD concentrations ranging from approximately 15 mg/l to 600 mg/l was utilized. Placing samples into dry ice or a mixture of dry ice and acetone were the two methods of freezing which were used. Samples were thawed at room temperature or in a warm-water bath. The "t-distribution" was used to determine percent significance of the data. Fogarty and Reeder determined that accurate BOD test results could be attained following their procedures of freezing and thawing and subsequent seeding of the samples to be tested for BOD.

Morgan and Clarke (3) researched the feasibility of utilizing freezing as a method of preservation of domestic waste samples. Grab samples taken from a primary clarifier inlet were mixed and transferred to 500 ml polyethylene bottles. A portion of the samples was tested immediately after collection and the remaining samples were placed in a deep freeze at minus 23°C for 2, 3, and 6 days. The samples were thawed in a water bath.

thawed-out samples after frozen storage. It was apparent that coalescence of solids was taking place during the frozen storage period. Although each of the individual 5-day BOD values were within ± 5 percent of the average of all the 5-day BOD values, an initial reduction in BOD was evident. A discernable reduction in the BOD values with time of storage was not indicated by regression lines calculated through the one-day and five-day BOD values. Destruction of a large percentage of microorganisms was also attributed to the initial freezing operation.

Agardy and Kiado (5) used grab samples of raw sewage which were mixed and split in order to constitute a statistical analysis of the research upon the effects of refrigerated storage on the characteristics of waste water. A portion of the split samples was analyzed following collection and the remaining samples were stored at 4°C or frozen in a conventional refrigerator freezing compartment held at minus 5°C . The samples were brought to room temperature by means of immersion in a warm water bath after being stored for one and seven days. Neither refrigerated storage nor frozen storage affected the total solids concentrations. However, large increases in suspended solids and volatile suspended solids concentrations as a result of frozen storage were noted. Both the rate constant and the first stage BOD were affected by both methods of storage. Agardy and Kiado suggested that if frozen storage is anticipated, a preliminary study should be performed in order to determine the effect of storage on the specific waste to be characterized.

Jenkins (6) described the development and application of analytical techniques for the determination of the various forms of

nitrogen and phosphorus in estuarine waters. Preservation techniques for short-term and long-term preservation were investigated. Concentration changes, expressed as percent of original concentration, were plotted for the preservation methods. The best preservation method for all forms of nitrogen seemed to be storage at 4°C in the presence of 40 mg Hg²⁺/liter. This method was not entirely satisfactory, but it was the best of the four methods which were studied. One other method was preservation by storage at minus 10°C.

For the phosphorus forms studied by Jenkins (6), the best preservation technique for long periods (one month) was storage at minus 10°C with 40 mg Hg²⁺/ liter. For storage periods of a few days, all of the methods were satisfactory with the exception of storage at 4°C with chloroform. Many of the results obtained from this study showed an irregular pattern of concentration change after preservation as compared to the original concentration.

McCarl (7) utilized the method of frozen storage in a food freezer at minus 15°C for periods of several months for samples of cropland runoff. High loadings of solids were characteristic of the runoff. Plastic bags inside one-half gallon paper cartons were used as freezing containers. The samples were thawed at room temperature.

McCarl (7) performed the following analyses on the thawed samples: pesticides, oxygen-demanding materials, solids, phosphorus, several nitrogen forms, pH and specific conductance. Of the above tests,

pH and specific conductance were performed before and after frozen storage. A regression line plotted by comparing specific conductance values before and after frozen storage resulted in a correlation coefficient of 0.988, although the regression line did not pass through the origin. Samples which initially exhibited low specific conductance values also had high concentrations of soil particles. McCarl theorized that ions adsorbed to soil particles probably dissolved in the solution in the time interval between analyses. This phenomenon would have resulted in higher specific conductance values after frozen storage. The samples which initially had high specific conductance values tended to have a lower value after frozen storage. McCarl attributed this phenomenon to the freeze-concentration action of the freezing process.

McCarl (7) also presumed that freezing changed the ionic characteristics of the runoff. The nitrate, ammonium and phosphate ions were thought to have been affected. Erratic changes in pH values of the samples were noted with approximately 75 percent of the samples exhibiting higher pH values after frozen storage. The increase in pH was attributed to the escape of carbon dioxide from the samples as a result of agitation after thawing.

Barth (8) stated that rapid freezing of samples for preservation of nitrogen forms was the most suitable method of preservation prior to analyses.

Logsdon and Edgerley (9) researched the method of sludge dewatering by freezing. This technique utilizes the phenomenon of migration of foreign particles to a specific location in the solution

(sludge) being frozen. The technique is a working example of the freeze-concentration process which portrays how particles are forced out of the vicinity of crystallizing ice as freezing occurs.

A review of the previous research in the area of sample preservation by freezing did not result in any uniformity of conclusions. Morgan and Clarke's (3) conclusion that there is no significant change in the composition of domestic waste as a result of freezing contradicts conclusions reached by Zanoni (4) and Agardy and Kiado (5). Jenkins' (6) storage method for nitrogen forms in estuarine waters does not agree with Barth's (8) suggested method of preservation for nitrogen forms. The lack of adequate statistical evaluation of data in most of the literature which was reviewed was evident. Although a limited amount of adequate research has been done, freezing is being used as a method of preservation. Conflicting conclusions in the literature concerning the validity of the preservation process are confusing.

Changes of State in the Preservation Method

In order to preserve water samples by the utilization of a freezing preservation method, the samples must experience two changes of state. The first change involves a change from the liquid state to the solid state during the freezing process, and the second involves a change from the solid state to the liquid state in the thawing process. In order to understand the changes of state encountered by the preservation method, a short review of the structure of water and ice seems necessary.

Water is commonly thought of as consisting of two molecules of hydrogen and one molecule of oxygen. But this assumption is not absolutely correct (10-139). The formula for water should be written as $(\text{H}_2\text{O})_x$, where x is 1, 2, 3, 4, ..., etc. The variable x indicates the degree of molecular association. As the temperature of liquid water decreases, the degree of molecular association increases (10-139).

The areas in the vicinity of the hydrogen atoms are both positively charged because the single electron of each hydrogen atom is part of the O-H bond. The areas of the nonbonding orbitals each have an unshared pair of electrons, thus giving these areas negative charges. This electron distribution gives the water molecule a polar characteristic (10-138).

The bond that unites two simple molecules of water results from the attraction of a positive hydrogen atom of one water molecule for some of the electrons of an oxygen atom of a second water molecule. This phenomenon is known as the hydrogen bond. Although the hydrogen bond is stronger than the van der Waals attraction between two molecules, it is not as strong as an electrovalent or a covalent bond (10-139). Large aggregations of simple water molecules can be formed by hydrogen bonding as thermal energy is transferred from a parcel of water. If enough thermal energy is removed and all the molecules form one large aggregation, the parcel is said to have changed state or to have frozen.

Theoretically, one mole of closely packed water molecules would occupy approximately nine cubic centimeters. However, the measured volume of a mole of water is approximately 18 cubic centimeters or twice

the theoretical value. It appears that about one-half of the space in water is occupied by water molecules and the other half is void. X-ray studies of ice show that each water molecule has four nearest neighbors, with the overall structure featuring repeating hexagonal groupings of six molecules arranged so that large empty holes penetrate the ice crystal (10-140).

X-ray studies also indicate that liquid water has a structure similar to that of solid ice I (10-140). Ice I is the form which usually fuses under normal conditions of atmospheric pressure and temperature (11-395). But, on melting, the volume occupied by water is only about 90 percent of the original volume of the ice. The decrease in volume indicates that the structure of ice partially collapses and that there is less empty space in water than in ice. As thermal energy is added to the ice, the hydrogen bonds dissociate, the ice structure collapses, the molecules become more closely packed, and the density increases. The associated water molecules dissociate to a greater extent as the water is heated, and the volume diminishes because of the closer packing of the molecules until maximum density at 4°C is reached. Above 4°C , the expansion resulting from the increased motion of the molecules becomes greater than the contraction resulting from the breaking up of hydrogen bonds (10-140).

Water in its natural state is not in a pure form. Water to be analyzed in a water quality surveillance program would usually contain varying amounts of dissolved gases, dissolved solids, inert solids, biota and other material. The presence of impurities in a water are also affected by the changes of state of the water (12-220).

Some dissolved gases evidently escape the freezing solution as freezing progresses. Alternate freezing and rapid thawing has been employed as a method to remove air from a solution (11-415). Crystals of ice formed from a dilute salt solution are themselves free of salt, but some of the solution is retained in the interstices between the crystals (11-403). As each small volume of the water crystallizes, it rejects the impurities it originally contained. Therefore, each minute crystal is surrounded by a layer of water more impure than that from which it was formed. As the crystal grows, this layer is continuously pushed out, becoming more impure, until a similar layer surrounding a neighboring crystal is met (11-402). The impure water is pushed out of the crystallization area by the strong attraction of the water molecules to form hydrogen bonds at the freezing temperature. The water molecules presumably force themselves into position to make a bond even if this action requires moving an impurity out of the way.

By the utilization of an ice cube tray or other type of liquid container, the thermal energy (heat of fusion) is removed from the outside perimeter of the impure water solution during the freezing process. Naturally, this is the water which first solidifies into ice. As the ice crystal layer grows, the impure layer of water is forced toward the center of the freezing solution because it has no other direction to go. Thus the impurities within the original water sample are concentrated within the ice and are subject to chemical change because of the increased pressure and concentration. In one instance brine was observed oozing out of sea-water ice at minus 30° C. The

brine was very rich in calcium and especially rich in magnesium chloride. It was theorized that it is probably quite impossible by any cold occurring in nature to solidify sea-water completely (11-423).

The above discussion of the changes of state encountered in the freezing preservation method indicates that impurities are freeze-concentrated as a solution is frozen. The susceptibility of impurities in water samples to experience quantitative chemical change as a result of a freezing method is probably dependent upon the type and quantity of impurities originally present in the sample. It seems as if the rate of the freezing process may have some influence upon amount of freeze-concentration experienced by the sample.

EXPERIMENTAL PROCEDURES

Statistical Design

With the assistance of Dr. William L. Tucker, Experiment Station Statistician, the experiment was designed in order to obtain unbiased estimates of treatment means and differences and of experimental error. For this research, water was categorized according to its source. The term source is used to differentiate between water taken from wells, sewerage, lakes, etc. A decision to perform separate analyses of variance for the two sources of water to be investigated (well water and waste water) was made. The sources were assumed to have different chemical characteristics.

Figure 1 is a schematic representation of the statistical design of the research project. For each of the two sources investigated, a minimum of five samples was collected. Each of the five samples was subsequently split into five portions. Each portion was then split in half, which is indicated in Figure 1 by the dotted line in the portions. Duplicate analyses were then performed on five half-portions. The remaining five half-portions were placed in a commercial deep freeze for subsequent duplicate analyses two weeks later. The analyses performed on the unfrozen half-portions and the analyses performed on the half-portions after frozen storage constituted the two methods of the statistical design. For each source of water investigated, a minimum of 100 identical analyses was required for each chemical determination.

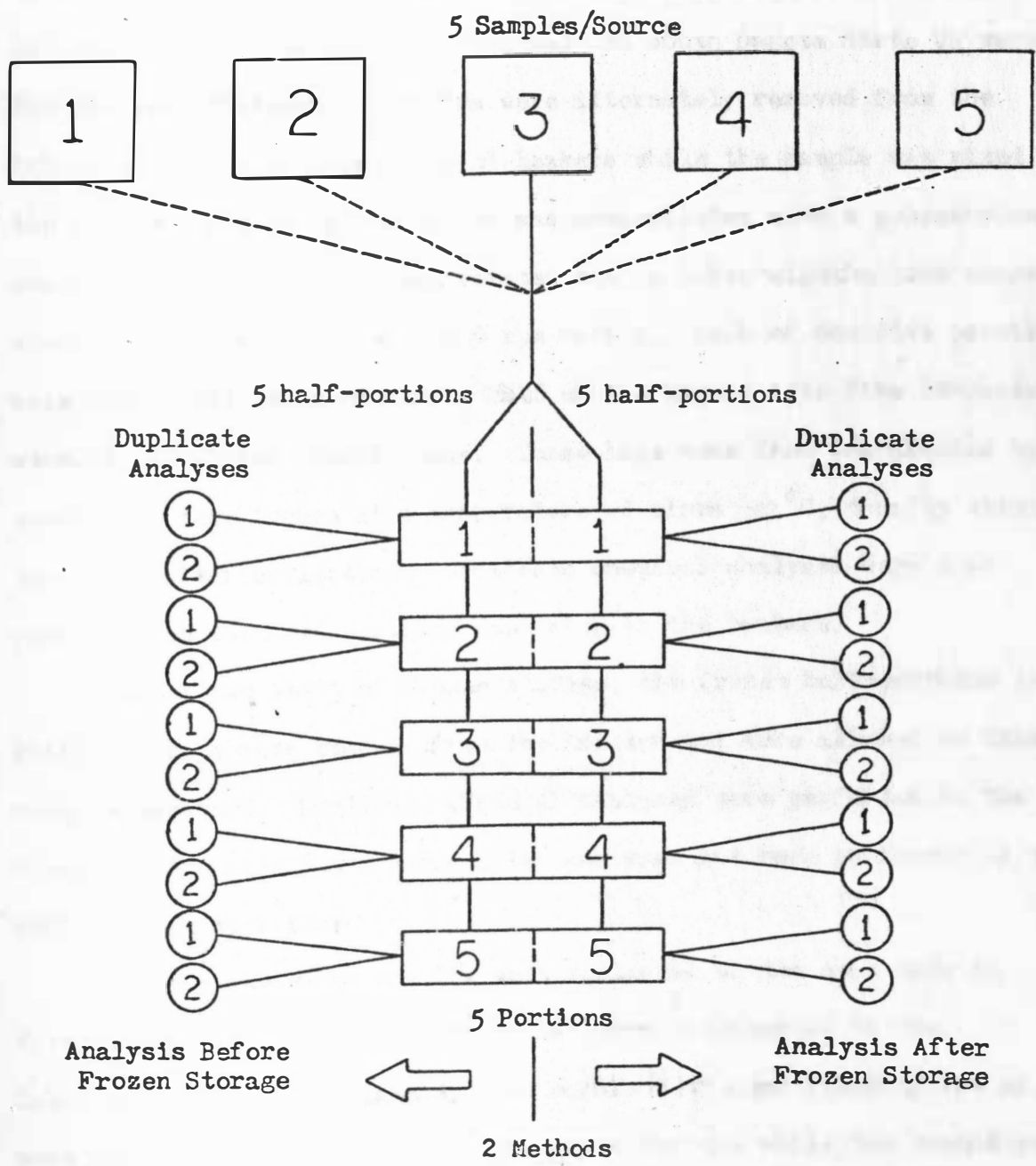


Figure 1. Statistical design for research

Sampling Methods

Two sources, a well-water source and a waste-water source, were investigated. Well-water samples were collected in 2-gallon polyethylene tubulated bottles and were transported to the Water Pollution Control Laboratory in Crothers Engineering Hall at South Dakota State University for chemical analyses. Portions were alternately removed from the tubulated bottle in five 1,000 ml beakers while the sample was mixed in the bottle. Mixing in the bottle was accomplished with a polyethylene-coated stirring rod which was rotated with a three-eighths inch chuck electric drill which had a 1,000 rpm rating. Each of the five portions were then split by transferring half of the liquid into five 18-ounce, sterile, Whirl-Pak plastic bags. These bags were then transferred to a commercial deep freeze at a temperature of minus 12°C , usually within two hours after collection. Duplicate chemical analyses were then performed on the half-portions remaining in the beakers.

After two weeks of frozen storage, the frozen half-portions in the Whirl-Pak bags were removed from the freezer and were allowed to thaw at room temperature. Duplicate chemical analyses were performed on the thawed half-portions two weeks after analyses had been performed on the unfrozen half-portions.

Five waste-water samples were collected on the same date in five 1,000 ml polyethylene bottles and were transported to the laboratory. From each sample, five portions of approximately 200 ml were poured from a bottle into five glass beakers while the sample was mixed manually by shaking. The five portions were then split by

transferring half of the liquid in each beaker into five corresponding 18-ounce Whirl-Pak bags. The bags were transferred to the freezer and duplicate chemical analyses were performed on the remaining half-portions. The thawing and testing procedures for the waste water were performed similar to the procedures for the well-water half-portions which were frozen.

Analytical Methods and Procedures

Of the tests commonly performed on well-water samples, the following were selected to be performed in this study: calcium and total hardness, phenolphthalein and total alkalinity, and specific conductance. The above chemical analyses were selected because they are tests which are normally performed on well water, and they would indicate if chemical concentration changes did occur as a result of the freezing preservation process. The relatively short time period needed to perform the analyses also prompted the selection of the analyses which were performed.

The analyses performed on the waste-water samples were specific conductance, total kjeldahl nitrogen, and total phosphorus. Nitrate and nitrite analysis was attempted, but because of the difficulty encountered in performing the analysis which was attributed to sulfide interference, the analysis was abandoned. Specific conductance analysis was performed in order to detect major changes in dissolved ion concentrations as a result of the freezing process. The nitrogen and phosphorus analyses were performed because the reviewed literature had

indicated that the freezing preservation process for these constituents was satisfactory. The time element was also a factor that limited the selection of additional analyses.

One or two well-water samples were all that could be analyzed in one day, whereas all five waste-water samples were prepared or analyzed in one day. The methods used for determining total kjeldahl nitrogen and total phosphorus made analyses of all five samples in one day more convenient than analyses of one or two samples in a day.

An Industrial Instruments conductivity bridge (model RC 16B2) was used for determining the specific conductance of the well water and waste water. Specific conductance for the waste water was determined with a pipet-type conductance cell at 25°C. An immersion-type conductance cell was used to measure specific conductance of the well water. The specific conductance analyses were performed in accordance with Standard Methods for Examination of Water and Wastewater (13-323).

The EDTA titrimetric method for determining calcium and total hardness of the well water was utilized (13-84, 179). Standard Titra-ver solution, Cal-ver II powder and Uni-ver I powder manufactured by the Hach Chemical Company, Ames, Iowa, were used for the hardness determinations. The indicator method for alkalinity was utilized with the use of phenolphthalein and brom-cresol green-methyl red indicators (13-52).

The total kjeldahl nitrogen and total phosphorus analyses were performed on a basic autoanalyzer system manufactured by the Technicon Corporation of Tarrytown, New York. This system consists of a sampler,

proportioning pump and manifold, heating bath, continuous digester (total kjeldahl nitrogen), colorimeter, and recorder.

The Technicon Autoanalyzer system uses the continuous flow analysis method. This method allows samples, standards, and reagents to be exposed to the same conditions as they continuously follow one another in tubing along the analytical train. Samples are segmented by air bubbles which promote mixing, separate samples, and aid in cleaning the system. The air bubbles are removed by a debubbler just prior to passing the sample through the flow cell in the colorimeter. All methods are photoelectric-colorimetric procedures which apply Beer's law. The absorbance of a monochromatic light is measured, and the absorbance (optical density) is directly proportional to the concentration of the substance being measured by the method of analysis. A graph of concentration against absorbance will be a straight line on semi-logarithmic paper. From the graph plotted from the results of standards of known concentrations, the concentrations in the unknown samples may be determined.

The automated methods used for total kjeldahl nitrogen and total phosphorus were supplied by the Technicon Corporation and are basically the same as those described in the manual of analytical procedures prepared by the Environmental Protection Agency entitled: Methods for Chemical Analysis of Water and Wastes (14). The total kjeldahl nitrogen was determined by the automated phenolate method (14-157). The total phosphorus in the waste water was determined by the automated single reagent method (14-246). A persulfate digestion method was utilized

in order to convert all the phosphorus to the orthophosphate form. The digestion procedure was performed on the same day as collection of the samples. The covered, digested samples were stored for several days in a 4°C incubator prior to analysis.

RESULTS AND DISCUSSION

Well-Water Samples

A total of six well-water samples was collected and analyzed. The results of the analyses are presented in Table 1. In Table 1, the three digits of the sample number refer to the sample, portion, and duplicate analysis, respectively. For example, sample number 3-4-2 refers to the third sample, the fourth portion of the third sample, and the second duplicate analysis of the fourth portion. The two digits in the columns beneath each of the five analyses refer to the two methods of analyses performed in this experiment. The columns headed by the number 1 refer to analyses performed before frozen storage of the portions, and the columns headed by the number 2 refer to analyses performed after frozen storage.

Samples 1, 2, and 3 were collected from the raw water sample tap at the water treatment plant at Brookings, South Dakota. The water quality of the three samples could be expected to differ because different wells were being pumped at the time the samples were obtained. The water quality of each of the seven wells in the Brookings well field presumably differs from the water quality of any of the other wells. Also, sample 3 was collected approximately five months after samples 1 and 2 were collected, and the water quality of the wells probably undergoes seasonal changes. Thus the water quality of the three Brookings well-water samples was expected to vary as is shown in Table 1.

Table 1

Results of Analyses of Well-Water Samples Before
and After Frozen Storage of Two Weeks

Sample Number	Specific Conductance ($\mu\text{mhos/cm}$)		Calcium Hardness (mg/l as CaCO_3)		Total Hardness (mg/l as CaCO_3)		Total Alkalinity (mg/l as CaCO_3)		Phenolphthalein Alkalinity (mg/l as CaCO_3)	
	1	2	1	2	1	2	1	2	1	2
1-1-1	(755)	1014	383	308	566	478	291	232	0	0
1-1-2	(762)	1035	386	316	550	478	292	234	0	0
1-2-1	(919)	1035	354	312	564	468	289	234	0	0
1-2-2	(932)	1030	364	308	568	454	292	233	0	0
1-3-1	(935)	984	380	264	568	446	291	201	0	0
1-3-2	(977)	981	390	268	570	460	292	200	0	0
1-4-1	(1034)	997	386	280	564	352	292	208	0	0
1-4-2	(1037)	997	406	280	572	332	294	209	0	0
1-5-1	(1051)	960	392	260	568	426	292	185	0	0
1-5-2	(1061)	960	334	256	570	432	294	185	0	0
2-1-1	1188	1106	396	244	596	556	291	256	0	0
2-1-2	1188	1103	404	240	600	552	292	254	0	0
2-2-1	1198	1137	404	252	588	572	294	276	0	0
2-2-2	1193	1144	400	248	588	592	294	276	0	0
2-3-1	1193	1075	396	328	612	548	294	230	0	0
2-3-2	1193	1081	392	324	608	548	296	229	0	0
2-4-1	1158	1020	392	288	616	512	296	196	0	6.0
2-4-2	1188	1009	400	288	620	500	296	194	0	6.0
2-5-1	1188	1060	400	308	612	532	294	220	0	0
2-5-2	1198	1058	392	304	620	532	296	220	0	0
3-1-1	1202	1061	420	292	636	524	306	263	0	0
3-1-2	1197	1072	424	292	640	528	308	205	0	0
3-2-1	1229	1040	424	276	640	508	308	181	0	0
3-2-2	1229	1035	424	280	640	508	308	181	0	0
3-3-1	1229	1035	424	276	640	512	308	185	0	0
3-3-2	1229	1040	424	272	640	500	308	185	0	0
3-4-1	1223	1077	424	300	644	532	308	207	0	0
3-4-2	1229	1077	420	296	640	532	310	207	0	0
3-5-1	1229	1046	424	280	636	512	308	183	0	0
3-5-2	1229	1046	424	284	640	508	308	191	0	0
4-1-1	685	553	216	(148)	320	276	226	158	0	19.5
4-1-2	687	559	220	(148)	324	276	230	158	0	17.6
4-2-1	693	629	216		316	300	228	195	0	0
4-2-2	691	630	216		316	308	228	194	0	0
4-3-1	686	569	216		318	284	228	162	0	15.6
4-3-2	691	566	220		290	284	229	162	0	17.6
4-4-1	694	567	220		316	272	230	162	0	19.5
4-4-2	693	565	216		324	284	230	160	0	19.5
4-5-1	690	548	220		328	264	230	152	0	21.4
4-5-2	690	548	220		320	364	230	152	0	17.6
5-1-1	698	641	228	172	340	296	230	195	0	9.8
5-1-2	698	646	232	184	348	312	230	197	0	9.8
5-2-1	698	577	232	144	340	260	230	160	0	9.8
5-2-2	701	577	228	140	340	260	230	158	0	7.9
5-3-1	698	612	232	164	336	272	228	179	0	9.8
5-3-2	698	609	228	160	340	272	230	177	0	7.9
5-4-1	698	632	228	168	344	284	230	191	0	9.8
5-4-2	701	629	228	168	340	284	230	189	0	9.8
5-5-1	698	609	232	156	340	276	230	173	0	0
5-5-2	698	611	228	156	344	272	230	173	0	0
6-1-1	650	546	208	144	304	256	220	164	0	0
6-1-2	661	554	204	144	312	260	220	162	0	0
6-2-1	661	575	208	152	312	264	222	171	0	0
6-2-2	661	575	208	152	308	254	222	171	0	0
6-3-1	661	573	204	152	308	260	224	171	0	0
6-3-2	657	569	204	152	312	260	222	171	0	0
6-4-1	661	577	204	152	308	264	222	173	0	0
6-4-2	661	577	204	152	308	260	222	173	0	0
6-5-1	661	575	204	152	304	264	222	173	0	0
6-5-2	661	577	208	156	308	256	222	173	0	0

(1) Before frozen storage - samples analysed on the same day as collection.

(2) After frozen storage - samples analysed after two weeks of frozen storage.

Note: Values within parentheses were considered unreliable and were not used in subsequent evaluations of data.

Samples 4, 5, and 6 were collected from the water distribution system at Volga, South Dakota. One well constituted the source of Volga's water supply at the times the samples were collected. Chlorination is the only water treatment which the city of Volga provides. Samples 4 and 5 were collected from the kitchen sink faucet at the home of the water superintendent. Sample 6 was collected from the sink faucet in the basement bathroom at the Volga City Hall. Samples 5 and 6 were collected approximately five months after sample 4 was collected. The slight difference in water quality among samples 4, 5, and 6 might be attributed to the different points of collection in the distribution system and to the time lapse between the periods of sample collection. The variations in water quality among all the well-water samples do not interfere with the analysis of the effects of frozen storage upon the samples because allowance for sample water quality variations was incorporated in the statistical design of the research project.

The specific conductance values for the unfrozen duplicates of sample 1 vary considerably from half-portion to half-portion as compared to the values for the other samples. Each of the five unfrozen half-portions was tested at a different temperature and a temperature correction factor was applied to the results (13-324). Because of the variation in specific conductance values for sample 1 attributed to temperature variations during testing, the values were not used in the subsequent interpretations of data.

In order to obtain a higher degree of precision for all specific conductance analyses, all of the sample portions for the remainder of

the tests after sample 1 were covered and warmed to a temperature of 25°C in a constant temperature bath and were then tested for conductivity. The values obtained by measuring specific conductance at a constant temperature were found to be more reproducible than were the values obtained by utilizing the temperature correction formula. These limited results would seem to indicate that values of specific conductance obtained by the use of the temperature correction factor given in Standard Methods (13) may not be as precise for some samples as the values obtained by measuring conductivity at the 25°C temperature.

Data are missing for the calcium hardness test for four of the frozen storage half-portions of sample 4 as shown in Table 1. A chemical was not added to the duplicates being tested while the analyses were being performed.

Because five blocks of sample data for each chemical determination were needed in order to perform the statistical analysis as originally designed, and because two blocks of data (specific conductance for sample 1 and calcium hardness for sample 4) were missing, a sixth sample was collected. This resulted in five blocks of data for specific conductance and calcium hardness and at least five or more blocks for the remaining water quality determinations for the samples from the well source as desired for the statistical design of the study.

In Table 1, a difference in water quality between the Brookings well-water samples (1, 2, and 3) and the Volga well-water samples (4, 5, and 6) can be noted. Specific conductance, calcium hardness, total hardness and total alkalinity values of the Brookings water samples are

all greater than the respective values for the Volga water samples. It is apparent that the Brookings raw well water is more mineralized than the Volga well water.

For all chemical determinations performed on the well water except phenolphthalein alkalinity, all of the portions exhibited decreases in concentrations of chemical constituents after frozen storage (see Table 1). Phenolphthalein alkalinity remained at zero or exhibited an increase in concentration after frozen storage. Also, greater ranges of values are exhibited by the portions within samples after frozen storage as compared to the values of portions within samples before frozen storage. For example, values for calcium hardness of sample 3 before frozen storage ranged from 420 mg/l to 424 mg/l. After frozen storage, the values ranged from 272 mg/l to 300 mg/l.

The arithmetical mean was calculated for the ten duplicate analyses for each method within each sample in Table 1. The results of these calculations and the ranges of values of each block of ten duplicate analyses are presented in Table 2. Table 2 portrays the average values of the chemical determinations performed on the samples before and after frozen storage, the percentage decrease in concentration of the chemical constituents, and the ranges of values exhibited by the portions within each sample and method.

The averages and the percentage changes in Table 2 indicate that the quality of the water changed as a result of the preservation process. Specific conductance experienced a reduction of approximately 10 percent to 17 percent. Calcium hardness concentrations were reduced by

Table 2

Average Values and Ranges of Chemical Analyses of Well Water Samples Before and After Frozen Storage

Well-Water Samples	Specific Conductance			Calcium Hardness			Total Hardness			Total Alkalinity			Phenolphthalein Alkalinity		
	(μmhos/cm at 25°C)			(mg/l as CaCO ₃)			(mg/l as CaCO ₃)			(mg/l as CaCO ₃)			(mg/l as CaCO ₃)		
	(1)	(2)	% Change	(1)	(2)	% Change	(1)	(2)	% Change	(1)	(2)	% Change	(1)	(2)	
1-Brookings	Ave.	999.3		377.6	285.2	-24.5	566.0	432.6	-23.6	291.9	212.1	-27.4	0	0	
	Min.	960		334	256		550	332		289	185		0	0	
	Max.	1035		406	316		572	478		294	234		0	0	
2-Brookings	Ave.	1190.5	1079.3	-9.4	397.6	282.4	-29.0	606.4	546.4	-9.9	294.1	235.1	-20.1	0	1.2
	Min.	1188	1009		392	240		588	512		291	194		0	0
	Max.	1198	1144		404	328		620	592		296	276		0	6.0
3-Brookings	Ave.	1222.5	1052.9	-13.9	423.2	284.8	-32.7	639.6	516.4	-19.3	308.0	193.8	-37.1	0	0
	Min.	1197	1035		420	272		636	500		306	181		0	0
	Max.	1229	1077		424	300		644	532		310	207		0	0
4-Volga	Ave.	690.0	573.4	-16.9	218.0	(148.0)	-32.2	317.2	281.2	-11.4	228.9	165.5	-27.7	0	14.8
	Min.	685	548		216			290	264		226	152		0	0
	Max.	694	630		220			328	308		230	195		0	21.4
5-Volga	Ave.	698.6	614.3	-12.1	229.6	161.2	-29.8	341.2	278.8	-18.3	229.8	179.2	-22.1	0	7.5
	Min.	698	577		228	140		336	260		228	158		0	0
	Max.	701	646		232	184		348	312		230	197		0	9.8
6-Volga City Hall	Ave.	659.5	569.8	-13.6	205.6	150.8	-26.7	308.4	260.8	-15.5	221.8	170.2	-23.3	0	0
	Min.	650	546		204	144		304	256		220	162		0	0
	Max.	661	577		208	156		312	264		224	173		0	0

(1) Averages and ranges of half-portions before frozen storage

(2) Averages and ranges of half-portions after frozen storage

Values in parentheses are unreliable data

Negative value in Percent Change Column indicates decrease in concentration after frozen storage

24 percent to 33 percent, and total hardness concentrations were reduced by 10 percent to 24 percent. Total alkalinity concentrations were reduced by 20 to 37 percent.

By reference to Table 2, it can be observed that the results of the several analyses performed on the half-portions of all the samples analyzed before frozen storage do not appear to comprise an excessive range of values within each sample. For example, the range of specific conductance of sample 2 is from 1,188 $\mu\text{mhos/cm}$ to 1,198 $\mu\text{mhos/cm}$. This relatively narrow range of values is probably due to analyses errors or inherent sample splitting procedure errors made by the analyst which resulted in portions with different values of specific conductance. However, the results of the analyses performed on the half-portions which were frozen do appear to encompass a comparatively larger range of values in each of the samples. For example, the range of specific conductance of sample 2 is from 1,009 $\mu\text{mhos/cm}$ to 1,144 $\mu\text{mhos/cm}$. Similar ranges of values are exhibited by the data obtained from the hardness and alkalinity determinations.

Although precautions were taken to split, freeze, store, thaw, and test the portions under identical conditions, the relatively larger range of values for the frozen half-portions was apparent. This observation seemed to indicate that any single frozen portion of any one sample did not experience the same degree of chemical change that any other of the portions of that sample experienced. Because it is common in chemical analyses of individual water samples to determine a concentration of a constituent such as calcium hardness or alkalinity on only a single portion, or perhaps duplicate portions, the variation

of results between portions after frozen storage appears to be an important consideration of freezing samples for preservation. This phenomenon of variance of values warranted a special statistical analysis which is presented in a following section.

In order to portray graphically the average values of concentrations of the chemical constituents in the well-water samples and the changes in concentrations as a result of the preservation methods, Figure 2 was drawn. The average water quality of the samples can be visually compared in Figure 2. The averages of phenolphthalein alkalinity were calculated for samples 2, 4, and 5 and are presented in Table 2, but a bar graph for phenolphthalein alkalinity was not drawn in Figure 2 because it would not accurately represent what occurred in the samples.

Upon examination of Figure 2, it can be observed that the water quality of the Brookings well source (samples 1, 2, and 3) differs considerably from the water quality of the Volga well source. Specific conductance, calcium hardness, and total hardness values for Brookings well water are approximately two times the respective values for the Volga well water. The total alkalinity of Volga water is slightly greater than two-thirds the total alkalinity of Brookings water.

Because specific conductance is a measure of a water's capacity to conduct an electrical current and this ability varies with the number and type of ions in the water, the decreases shown in Figure 2 indicate that the water, after experiencing frozen storage, could not conduct as much electrical current as the water which was not frozen.

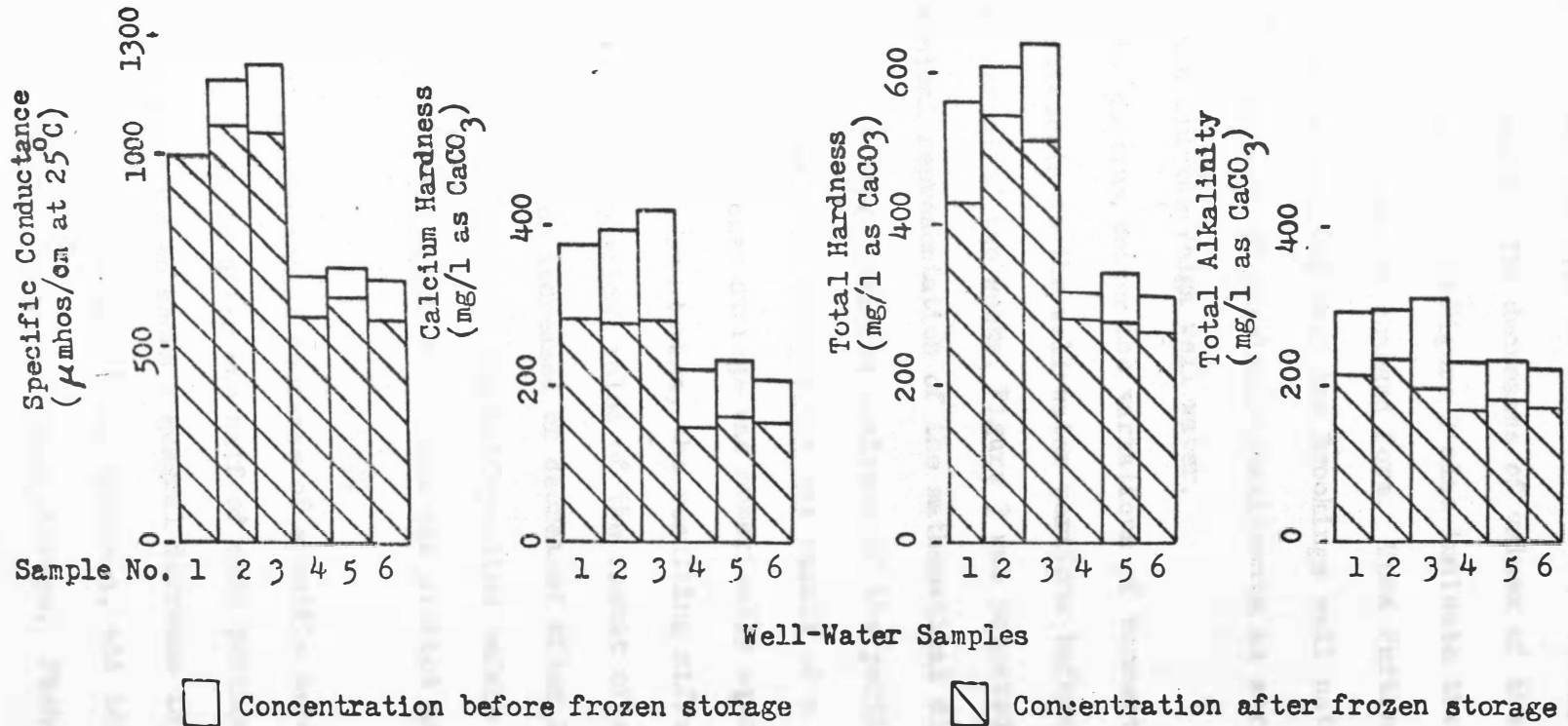


Figure 2. Concentrations of chemical constituents of well-water samples before and after frozen storage.

Consequently, the number and type of ions in the water samples that were frozen must have decreased. The decreases of values of the other chemical determinations shown in Figure 2 also indicate that the number of ions in solution decreased or changed form. Upon further examination of Figure 2, it can be observed that the Brookings well water experienced a larger numerical decrease of chemical constituents as a result of frozen storage than did the Volga well water.

In order to portray better the variations of concentrations of the chemical constituents in the well-water portions before and after a frozen storage period of two weeks, Figure 3 was prepared. This figure is a graphical representation of the mathematical differences of the arithmetic means of the duplicate analyses of the portions before and after frozen storage. For example, if the result of a chemical test on a half-portion after frozen storage was numerically equal to the result of a test before frozen storage, the resulting difference was plotted as zero. If the numerical value of the result of a chemical test of a water half-portion increased or decreased after frozen storage, relative to the result obtained on the half-portion before frozen storage, the respective increase or decrease was plotted as the difference.

In Figure 3, a substantial decrease of specific conductance values in all portions can be noted after one half of each portion underwent frozen storage. Figure 3 also shows a general decrease in the concentrations of calcium hardness, total hardness, and total alkalinity in the half-portions which underwent frozen storage. Phenolphthalein

Variation of Mean Concentrations of Portions After Frozen Storage
(mg/l as CaCO_3 , $\mu\text{mhos/cm}$ for specific conductance)

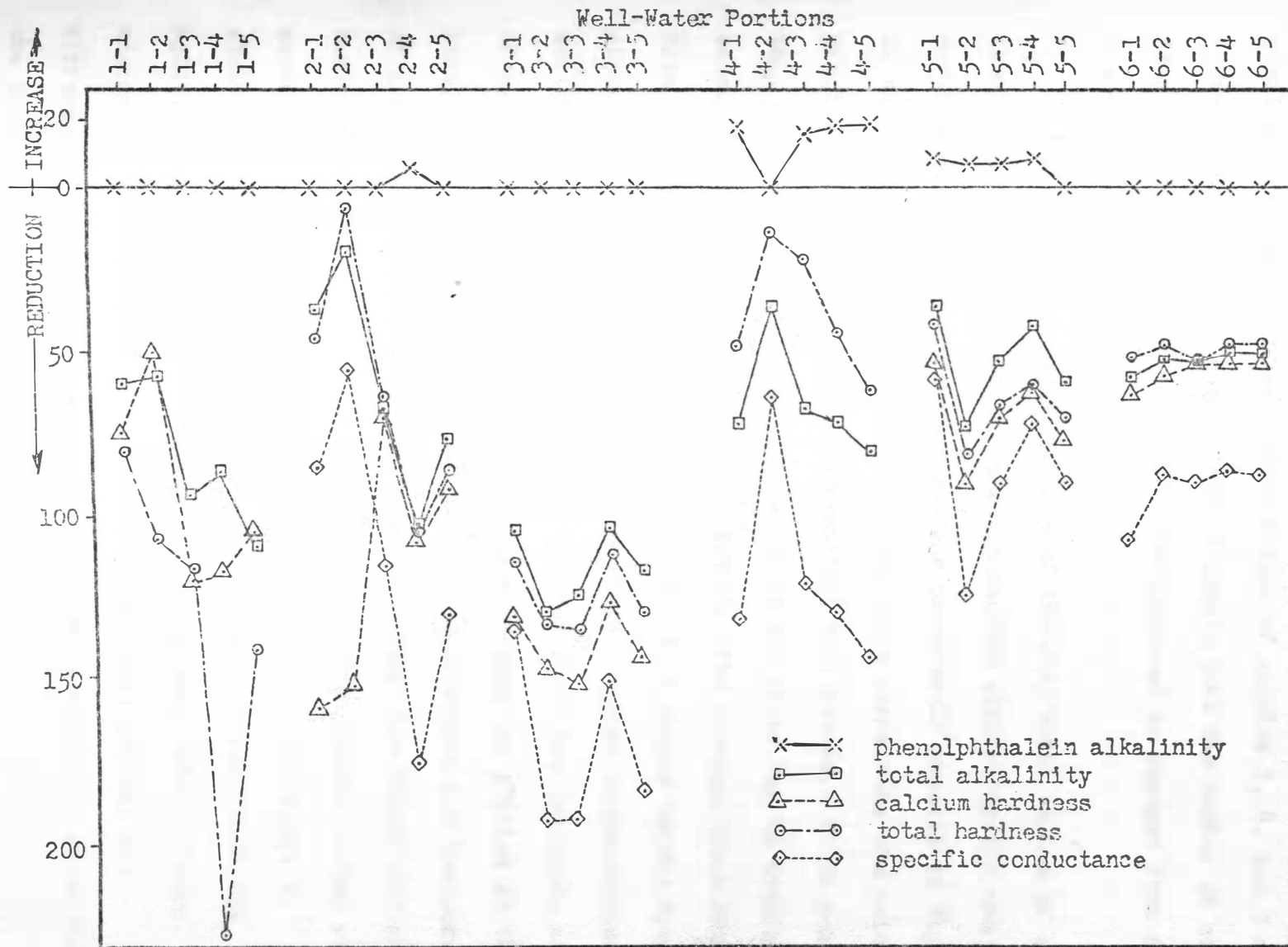


Figure 3. Change in concentration of selected chemical characteristics for six well-water samples after frozen storage of two weeks.

alkalinity appeared in some half-portions of samples 2, 4, and 5 after frozen storage. These changes also indicate that the number of conductive ions in individual portions of samples decreased or changed form during the preservation process.

The relatively large variance of the analytical values of all the chemical determinations except phenolphthalein alkalinity for the half-portions which underwent frozen storage prominently appears in Figure 3 as the random "zig-zag" character of the lines connecting the points which represent values of the mathematical differences. It is realized that not all of the "zig-zag" character of the lines can be attributed solely to the freezing preservation method. The average value obtained from the duplicate analyses of each portion of a sample before frozen storage was considered to be the value of the initial concentration of the constituent being quantified. Thus the average of the duplicate analyses of each portion of a sample before frozen storage was plotted on the zero line, when, in actuality, the values of concentrations for the portions of a sample were not numerically equal. However, the relatively small range of values for duplicate analyses of portions before frozen storage seems to justify the method of plotting the results in Figure 3, although the variance of values which occurred as a result of the splitting and testing of the half-portions analyzed before frozen storage is not shown in the figure. Figure 3 does portray the difference between halves of split, autonomous portions of water for the individual samples.

Any one line representing results of a chemical determination in Figure 3 seems to form a pattern which is similar to lines formed by the results of any other determination within a sample. The repetitious pattern formed by the lines in Figure 3 for all the chemical analyses performed on a sample seem to indicate that the random character is primarily the result of the preservation process rather than splitting and testing procedures. It seems rather unlikely that the splitting and testing procedures for each portion of a sample would result in such a similar repetitious pattern for each sample. The "zig-zag" character of the line also seems to indicate that each portion of a sample exhibited different chemical characteristics after frozen storage.

Some exceptions to the pattern of relationship among the chemical constituents of a sample may be noted. The total hardness in portion 1-4 and the calcium hardness in portions 2-1 and 2-2 would have been expected to experience a smaller decrease in concentration.

Only one of the portions (2-4) of the Brookings well water experienced an increase in phenolphthalein alkalinity, whereas four of five portions of Volga water samples 4 and 5 experienced an increase. The occurrence of phenolphthalein alkalinity in portion 2-4 and in the four portions of sample 4 corresponded with relatively larger decreases of other chemical constituents which were determined. However, this phenomenon does not appear in sample 5.

The portions which experienced the occurrence of phenolphthalein alkalinity presumably had a pH in excess of pH 8.3. The pH rise

possibly may have been caused by the loss of gaseous carbon dioxide during the freezing process as suggested by Zanoni (4). The zero values for phenolphthalein alkalinity obtained before frozen storage indicate that the alkalinity was primarily of the bicarbonate form. The occurrence of phenolphthalein alkalinity in some of the half-portions after frozen storage indicates that some of the bicarbonate alkalinity was converted to carbonate alkalinity.

After thawing, a flocculant, sometimes granular, white material was noticed in the bottom of the Whirl-Paks in which the Volga well-water samples were frozen. A larger quantity of rust-colored material was noticed in the thawed Brookings well-water samples. Presumably, dissolved iron in the Brookings well water added the red, rusty color to the precipitate which was observed in these samples. The flocculated material in the samples was thought to have been precipitated calcium carbonate which presumably had formed. The precipitate could have coagulated as a result of the increased dissolved solids concentration as the water froze toward the center of the ice block as suggested by Sawyer (12-220). An effort was made to transfer the precipitated material that had adhered to the walls of the freezing container to the labware in which the chemical determinations were performed in order to insure that representative half-portions were analyzed.

From the results of this section of the study, it was concluded that the water portions experienced a general decrease in dissolved chemical constituents after experiencing the freezing preservation process. The chemical changes which occurred in each portion of a single water sample was not uniform. This finding seemed to indicate

that each portion underwent a freezing preservation process peculiar to that portion. The relatively larger variation of values which occurred in the portions of an individual sample after frozen storage as compared to that which occurred before frozen storage supported this interpretation. Therefore, any attempt to predict the amount of reduction or increase in the values resulting from chemical analyses performed on the well-water samples as a result of the freezing preservation process seemed rather futile.

Waste-Water Samples

A total of five waste-water samples was collected and analyzed. The raw data of the various chemical analyses performed on the waste-water samples are presented in Table 3. In Table 3, the data are presented in the same manner as Table 1 for the well-water samples in that the three digits of the sample number refer to the sample, portion and duplicate analysis, respectively. In the case of waste-water samples the analyses included the following determinations: specific conductance, total phosphorus, and total kjeldahl nitrogen.

Sample 1 was collected from the final clarifier effluent at the waste-water treatment plant at Brookings, South Dakota. Samples 2 and 3 were collected within five minutes time from the effluent of the south stabilization pond of the treatment plant. Samples 4 and 5 were collected in the same manner from the north lagoon effluent which is the final discharge of the treatment processes. Both stabilization ponds had an ice cover and all five samples were collected on the same day, February 12, 1972.

Table 3

Results of Analyses of Waste-Water Samples Before
and After Frozen Storage of Two Weeks

Sample Number	Specific Conductance (μ mhos/cm)		Total Phosphorus (ng/l as P)		Total Kjeldahl Nitrogen (mg/l as N)	
	1	2	1	2	1	2
1-1-1	2878	2842	5.8	6.0	33.6	29.6
1-1-2	2904	2800	6.0	6.2	33.4	29.6
1-2-1	2878	2790	6.6	6.2	33.0	29.2
1-2-2	2878	2790	6.8	6.2	33.0	29.0
1-3-1	2852	2757	6.8	6.4	33.2	30.0
1-3-2	2878	2800	6.6	6.4	33.0	30.0
1-4-1	2891	2800	6.8	6.0	33.4	29.6
1-4-2	2891	2800	6.8	5.8	33.0	30.0
1-5-1	2878	2790	7.0	6.2	32.6	29.8
1-5-2	2891	2800	7.0	6.2	33.4	30.0
2-1-1	2800	2800	6.4	5.6	33.4	30.0
2-1-2	2826	2811	5.6	5.4	33.6	30.6
2-2-1	2787	2800	6.4	5.6	34.4	32.0
2-2-2	2775	2795	6.5	6.2	34.8	32.0
2-3-1	2749	2800	6.4	5.6	33.2	31.0
2-3-2	2787	2800	6.4	5.6	33.4	30.6
2-4-1	2800	2790	6.0	5.6	34.4	30.0
2-4-2	2813	2790	6.4	5.8	33.0	28.8
2-5-1	2813	2795	6.4	5.8	33.4	31.2
2-5-2	2826	2800	6.4	6.0	33.4	(34.4)
3-1-1	2217	2085	8.2	6.8	33.0	28.8
3-1-2	2204	2100	8.0	6.6	32.8	29.0
3-2-1	2217	2033	8.6	7.2	32.8	27.0
3-2-2	2204	2033	8.0	6.8	33.0	27.0
3-3-1	2204	2074	8.4	7.4	34.0	30.2
3-3-2	2204	2074	7.8	7.0	33.6	29.2
3-4-1	2204	2064	7.4	7.2	33.8	28.0
3-4-2	2204	2064	8.4	7.4	34.0	28.8
3-5-1	2204	2064	8.4	7.4	34.0	29.4
3-5-2	2204	2069	8.0	7.2	33.6	32.0
4-1-1	2282	2064	7.4	6.8	32.8	27.6
4-1-2	2282	2048	7.6	7.2	32.0	30.6
4-2-1	2256	2043	7.6	7.2	43.0	25.8
4-2-2	2269	2033	8.2	7.6	33.6	28.0
4-3-1	2269	2048	7.2	6.8	33.0	26.4
4-3-2	2269	2054	7.2	7.2	33.0	(43.0)
4-4-1	2282	2033	7.6	7.0	31.8	31.0
4-4-2	2269	2033	7.2	6.8	30.8	31.0
4-5-1	2282	2043	8.0	7.0	30.0	27.2
4-5-2	2282	2043	8.0	7.4	30.0	28.0
5-1-1	2437	2178	7.6	7.6	30.0	30.6
5-1-2	2450	2178	8.0	8.2	28.8	32.0
5-2-1	2411	2188	8.4	7.6	30.0	31.0
5-2-2	2437	2178	7.6	7.0	28.8	30.4
5-3-1	2411	2178	7.8	7.2	28.2	31.0
5-3-2	2437	2178	8.8	8.0	28.0	29.6
5-4-1	2424	2178	8.0	7.6	27.6	30.4
5-4-2	2437	2188	7.6	7.4	28.0	30.6
5-5-1	2437	2178	8.2	7.4	28.8	30.6
5-5-2	2430	2178	8.4	7.8	27.6	28.4

(1) Before frozen storage - samples analyzed on the same day as collection.

(2) After frozen storage - samples analyzed after two weeks of frozen storage.

Note: Values within parentheses were considered unreliable because of baseline drift of autoanalyzer and were not used in subsequent evaluations of data.

The values for the determinations, specific conductance for example, of portions analyzed before frozen storage for samples 2 and 3 (south lagoon) as shown in Table 3, were expected to differ from the values of samples 4 and 5 (north lagoon). However, the variation in quality between sample 2 and sample 3, which were collected within a period of five minutes from the same lagoon, was not expected. Likewise, the variation in quality between sample 4 and sample 5, which were collected in the same manner as samples 2 and 3, was also not expected. These results seemed to indicate that the water quality of both lagoon effluents changed considerably over a short time period. In addition to specific conductance, the values obtained from the total phosphorus and total kjeldahl nitrogen analyses performed before frozen storage also indicated that the water quality of the lagoon effluents changed quite rapidly.

In the review of the data, some questions arose as to the possibility that sample 5 had been the final clarifier effluent, samples 1 and 2 constituted samples from one lagoon and samples 3 and 4 constituted samples from the other lagoon. Although this possibility would not affect the results of this study because sample quality variation was expected, the laboratory research notebook was examined. It was decided that although there was a remote possibility that the samples could have become switched, the corresponding sample numbers and sources of collection are correct as recorded.

The indication that the water quality of the lagoon effluents changed quite rapidly may warrant future research of the lagoon effluent water quality as a function of time particularly when an ice cover prevails. As indicated by the above results, a single grab sample of a lagoon effluent may not be an accurate indication of the effluent quality.

The arithmetical mean was calculated for the duplicate analyses for each method within each sample in Table 3. The results of these calculations and the ranges of values of each block of duplicate analyses are presented in Table 4. The percentage change of the concentration of the chemical constituents experienced by the samples as a result of frozen storage is also presented in Table 4.

The averages and the percentage changes in Table 4 indicate that the quality of water changed as a result of the preservation process. Specific conductance experienced a small increase (0.1%) in sample 2 although approximately a 10 percent decrease was noted in samples 4 and 5. Total phosphorus concentrations were reduced from approximately 6 percent to 13 percent after frozen storage. Total kjeldahl nitrogen concentrations experienced approximately a 6 percent increase in sample 5, whereas the other four samples experienced a decrease of 9 percent to 13.5 percent.

The ranges of values in Table 4 for specific conductance and total phosphorus analyzed before frozen storage do not appear to be very different from the ranges obtained after frozen storage. The values of the total kjeldahl nitrogen analyses comprise a relatively larger range after frozen storage than the range before frozen storage.

Table 4

Average Values and Ranges of Chemical Analyses of Waste-Water Samples Before and After Frozen Storage

Waste Water Samples		Specific Conductance ($\mu\text{mho/cm at } 25^{\circ}\text{C}$)			Total Phosphorus (mg/l as P)			Total Kjeldahl Nitrogen (mg/l as N)		
		(1)	(2)	% Change	(1)	(2)	% Change	(1)	(2)	% Change
1-Final Clarifier	Ave.	2881.9	2797.9	-3.0	6.62	6.16	-7.0	33.16	29.68	-10.5
	Min.	2852	2767		5.8	5.8		32.6	29.2	
	Max.	2904	2842		7.0	6.4		33.6	30.0	
2-South Lagoon	Ave.	2797.6	2798.1	+0.1	6.28	5.72	-9.0	33.70	30.69	-9.0
	Min.	2749	2790		5.6	5.4		33.0	28.8	
	Max.	2826	2811		6.4	6.2		34.8	32.0	
3-South Lagoon	Ave.	2206.6	2066.0	-6.4	8.12	7.10	-12.6	33.46	28.94	-13.5
	Min.	2204	2033		7.4	6.6		32.8	27.0	
	Max.	2217	2100		8.6	7.4		34.0	32.0	
4-North Lagoon	Ave.	2274.2	2044.2	-10.2	7.60	7.10	-6.6	32.10	28.40	-11.6
	Min.	2256	2033		7.2	6.8		30.0	25.8	
	Max.	2282	2064		8.2	7.4		34.0	31.0	
5-North Lagoon	Ave.	2433.1	2180.0	-10.4	8.04	7.58	-5.8	28.58	30.26	+5.9
	Min.	2411	2178		7.6	7.0		27.6	28.4	
	Max.	2450	2188		8.8	8.2		30.0	32.0	

(1) Averages and Ranges of half-portions before frozen storage

(2) Averages and ranges of half-portions after frozen storage

+ value in % change column indicates concentration higher after frozen storage

- value indicates concentration lower after frozen storage

By a comparison of the waste-water specific conductance values in Table 4 with the well-water specific conductance values in Table 2, it can be observed that the waste water exhibits a substantially higher conductance than does the Brookings well water. This phenomenon was expected because domestic water usage usually results in the addition of conductive ions to the water. A large quantity of these ions probably originates from the dissociation of sodium chloride in the waste water. Presumably, the sodium chloride enters the water in human wastes and in discharges from home water softening units.

In order to portray graphically the average values of concentrations of the chemical constituents in the waste-water samples and the changes in concentrations as a result of the preservation methods, Figure 4 was drawn. Generally, the values for the determinations, specific conductance, total phosphorus, and total kjeldahl nitrogen, are shown to decrease as a result of the preservation process. This decrease would indicate that the ions in solution changed form or were absorbed on the plastic freezing containers.

In order to portray better the variations of concentrations of the specific chemical constituents in the waste-water portions before and after a frozen storage period of two weeks, Figure 5 was drawn. Figure 5 is a graphical representation similar to Figure 3.

A decrease in specific conductance was experienced by all half-portions except 2-2 and 2-3 as a result of the freezing preservation method (see Figure 5). The decrease in conductance in the majority of the half-portions which were frozen indicates that the number of conductive ions in solution decreased, possibly as a result of precipitation.

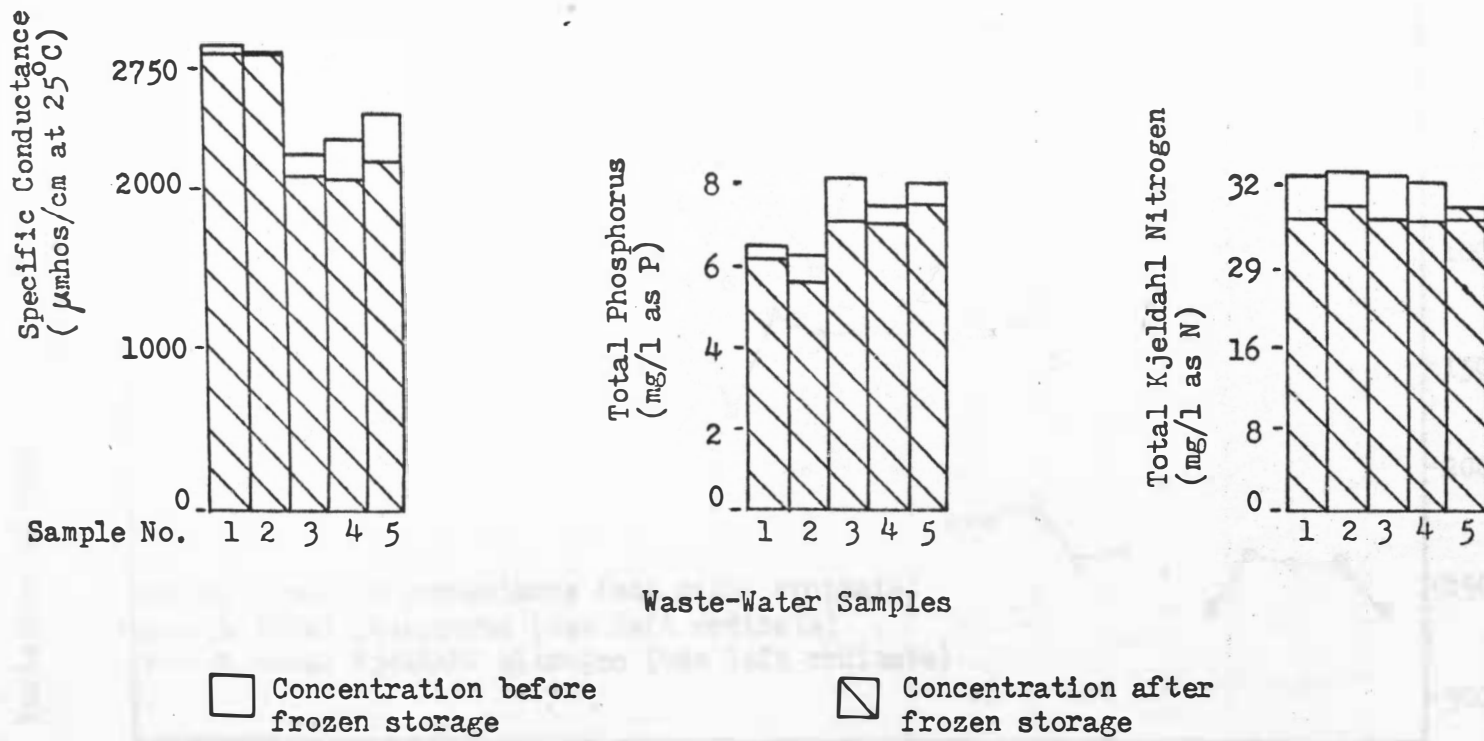


Figure 4. Concentrations of chemical constituents of waste-water samples before and after frozen storage.

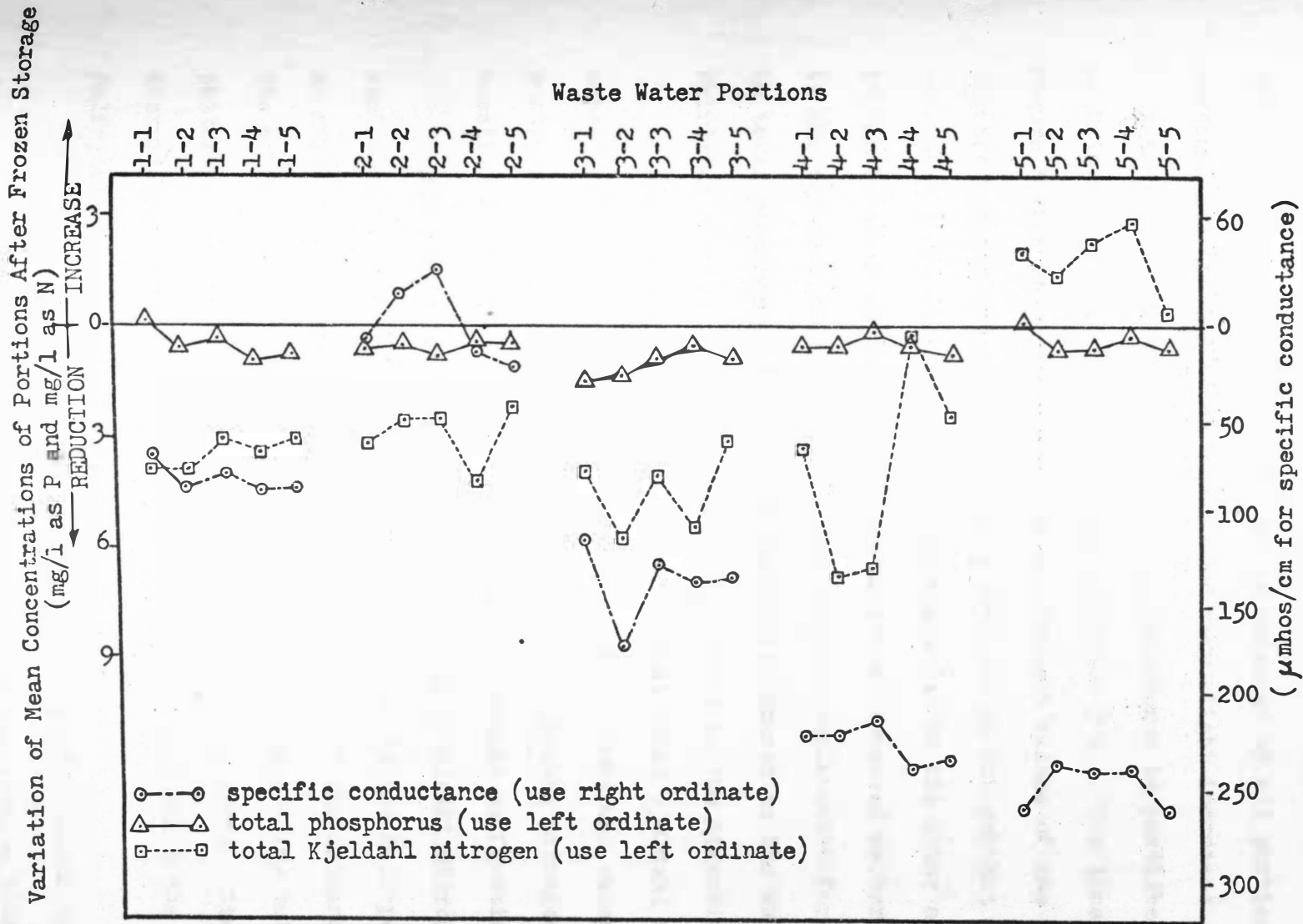


Figure 5. Change in concentration of selected chemical characteristics for five waste-water samples after frozen storage of two weeks.

Figure 5 indicates that a relatively small, general decrease in total phosphorus concentrations was experienced by all portions except portions 1-1 and 5-1 which experienced very slight increases after frozen storage. The decrease of total phosphorus in portions between methods did not exceed 1.4 mg/l as P (portion 3-1). The lines in Figure 5 connecting the points which represent values of the mathematical differences for the total phosphorus analyses do not exhibit a random "zig-zag" characteristic to the same degree as do the other analyses. It can be observed that the total phosphorus decreased rather uniformly in the portions of the samples. One possible explanation for the decrease in total phosphorus is that some phosphorus adhered to the walls of the Whirl-Pak storage bags and was not transferred to the digestion flasks.

From Figure 5, it can be observed that total kjeldahl nitrogen experienced a decrease in concentration for all portions except the portions of sample 5. The decrease of total kjeldahl nitrogen could possibly be attributed to a loss of gaseous ammonia as freezing occurred.

Difficulty was experienced in the total kjeldahl nitrogen analyses. After frozen storage, some substance in the half-portions had an affinity for the tygon tubing in one section of the autoanalyzer. The adhesion of this substance to the tubing disrupted the bubble pattern and caused the baseline of the recorder to drift. Similar disruptions in the bubble patterns were not noticed while the unfrozen half-portions were being analyzed.

The difficulties encountered with the total kjeldahl nitrogen determinations appeared to produce more irregular results than were

expected. The smaller differences in the total kjeldahl nitrogen concentration changes for portions 4-4 and 4-5 and the indicated increases in concentrations for all portions of sample 5 (see Figure 5) were attributed to a baseline drift, even though the autoanalyzer was recalibrated when the analyses were performed. Baseline drift was thought to have occurred during the time duplicate analyses 2-5-2 and 4-3-2 were analyzed.

From the results of the research, it was concluded that the portions of the waste-water samples experienced a general decrease in dissolved ions after experiencing the preservation process. Coalescence and precipitation of solids after frozen storage was also evident in the waste-water half-portions. The decrease of total phosphorus concentrations after frozen storage appeared to be relatively uniform for all samples which were analyzed. Although difficulty was encountered in the total kjeldahl nitrogen analyses, a general decrease in concentration was noted. Therefore, total phosphorus seemed to be the only chemical determination in which any attempt to predict the decrease in concentration would be feasible for the samples analyzed after frozen storage.

Statistical Evaluation

Analyses of variance were made on the method of preservation for all of the chemical analyses except phenolphthalein alkalinity. Before frozen storage, all values of phenolphthalein alkalinity were zero for all the well-water samples. Variance would not be exhibited for this distribution of phenolphthalein alkalinity values, and any change in

concentration would be highly significant. The term highly significant denotes significance at the one percent level. The term significant denotes significance at the five percent level.

Factorial analyses of variance were utilized for the statistical evaluation of the results (see Appendix A). Initially analyses of variance were performed on the data of each of the two sources, well water and waste water. For each of the four chemical analyses on the well water, the samples, methods, and the interaction between samples and methods were found to be highly significant (see Table 5).

For the three chemical analyses performed on waste water, the analyses of variance for specific conductance resulted in highly significant results for samples, methods, and the interaction between samples and methods (see Table 5). Highly significant results were obtained for the samples and methods of the total phosphorus analysis, and significant results were noted for the sample and method interaction. A significant difference was not noted between total kjeldahl nitrogen values for the samples; however, the method was highly significant, and the sample and method interaction was significant for total kjeldahl nitrogen.

For all of the chemical analyses performed on the water samples, the method of preservation resulted in highly significant results. The statistical evaluation indicates that the half-portions which were frozen do not have the same chemical characteristics as the half-portions which were not frozen. The statistical evaluation therefore supports the

Table 5

Summary of "F" Values for All Chemical Analyses^a

Well Water

Source of Variation	Specific Conductance	Calcium Hardness	Total Hardness	Total Alkalinity
Samples	186,526 ^{**}	3053 ^{**}	12,054 ^{**}	20,315 ^{**}
Methods	40,526 ^{**}	4579 ^{**}	5,384 ^{**}	149,868 ^{**}
Samples x Methods	687 ^{**}	118 ^{**}	253 ^{**}	2,936 ^{**}

Waste Water

Source of Variation	Specific Conductance	Total Phosphorus	Total Kjeldahl Nitrogen
Samples	22,428 ^{**}	146 ^{**}	1.50
Methods	4,708 ^{**}	104 ^{**}	20.98 ^{**}
Samples x Methods	515 ^{**}	3.29 [*]	3.17 [*]

^a See Appendix A for analyses of variance

* Denotes significance at the 5% level

** Denotes significance at the 1% level

conclusion that the freezing process does significantly change the chemical characteristics of the samples which were analyzed in this research.

The apparent phenomenon of the relatively large variance of values of the half-portions within samples after frozen storage warranted further statistical evaluation. The previous analyses of variance had shown that the method was highly significant; thus the data was split between methods in order to test the variance of the portions within the samples (see Appendix B). Table 6 shows the results of the analyses of variance after the data was split between methods.

Only three of the seven sources of variance of portions within samples before frozen storage in Table 6 (well water specific conductance, total hardness, and total kjeldahl nitrogen) exhibited highly significant results. However, five of the seven sources of variance of portions within samples after frozen storage (the four analyses on well water and specific conductance for waste water) resulted in highly significant values. After frozen storage, the variance of the portions within the samples is much more pronounced as is indicated by the comparatively higher F values. These results support the interpretation that each half-portion of any sample experienced a substantially different change in chemical concentration as a result of frozen storage.

The total phosphorus determinations before and after frozen storage for the portions within samples did not exhibit any significance. This statistical analysis supports the interpretation that the decrease of phosphorus concentrations within the portions of each sample was rather uniform.

Table 6

Summary of "F" Values for all Chemical Analyses Within Methods^b

Well Water

Source of Variation	Specific Conductance		Calcium Hardness		Total Hardness		Total Alkalinity	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Samples	144,297**	38,373**	1354**	5950**	7601**	4843**	13,607**	8825**
Port/Samp	14.00**	103.65**	1.24	104.52**	2.65**	40.06**	2.22*	843**

Waste Water

Source of Variation	Specific Conductance		Total Phosphorus		Total Kjeldahl Nitrogen	
	(1)	(2)	(1)	(2)	(1)	(2)
Samples	7026**	18,935**	64.99**	90.32**	227**	1.034
Port/Samp	2.37*	4.01**	1.52	1.35	6.86**	1.17

^b See Appendix B for analyses of variance

* Denotes significance at the 5% level

** Denotes significance at the 1% level

(1) Before frozen storage

(2) After frozen storage

Before frozen storage, the results of the statistical analysis of the portions within samples for total kjeldahl nitrogen determinations were highly significant. After frozen storage, significance was not noted. The difficulty experienced in the total kjeldahl nitrogen determinations after frozen storage resulted in a large error mean square (see Appendix B) which consequently decreased the value of F in the analysis. As a result, significance was not exhibited.

The statistical evaluation shows that the preservation process of freezing significantly changed the chemical characteristics of the water which was analyzed. Comparatively larger mean square values for portions within samples after frozen storage as compared to the mean square values before frozen storage were noted (see Appendix B). This comparison demonstrates that the values obtained from chemical determinations for half-portions analyzed after frozen storage constituted a larger range of values than did the values obtained from the unfrozen half-portions and also indicates that portions of the sample may experience different freezing processes and thus experience different changes in constituent concentrations.

SUMMARY AND CONCLUSIONS

There has been a need for a statistical study of the effects of freezing as a method of preservation for water samples because of conflicting recommendations in previous literature. The results of this research have, hopefully, partially fulfilled this need. A statistical analysis of the data was used to reinforce and verify the conclusions which were reached.

Results of this study have led to the following conclusions:

1. The method of freezing to preserve water samples used in this study was not satisfactory. The freezing preservation process resulted in unpredictable decreases in specific conductance, calcium and total hardness, total alkalinity, and total kjeldahl nitrogen in the samples which were analyzed. Phenolphthalein alkalinity increased in some portions of the samples and did not appear in other portions.
2. Total phosphorus reduction as a result of the freezing preservation process was rather uniform, but no general rule of thumb can be given for the amount of reduction which a sample will undergo.
3. If freezing is utilized as a preservation process, a preliminary study of the effect of the process upon samples from the source and the specific chemical analyses should be made. Agardy and Kiado (5) also recommended preliminary studies.

AREAS FOR FUTURE STUDY

The following possibilities for additional studies were realized during this investigation.

1. More research investigating the temperature of frozen storage and time of frozen storage could be made.
2. A study investigating the effect of the preservation process upon other major cations and anions in a water would be beneficial.
3. The accuracy resulting from the use of the temperature correction factor for specific conductance as presented in Standard Methods (13) may need to be examined for highly mineralized samples.

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APPENDIX A
Analyses of Variance

APPENDIX A

Analysis of Variance of Well Water

Specific Conductance

Source	df	Mean Square	F
Sample	4	1,486,619	186,526**
Method	1	322,992	40,526**
Sample x Method	4	5,476.9	687**
Error	50	7.97	

Calcium Hardness

Source	df	Mean Square	F
Sample	4	144,571	3,053**
Method	1	216,882	4,579**
Sample x Method	4	5,590.2	118**
Error	50	47.36	

Total Hardness

Source	df	Mean Square	F
Sample	5	390,559	12,054**
Method	1	174,456	5,384**
Sample x Method	4	8,211	253**
Error	60	32.4	

Total Alkalinity

Source	df	Mean Square	F
Sample	5	19,360	20,315**
Method	1	142,824	149,868**
Sample x Method	5	2,798	2,936
Error	60	0.953	

** denotes significance at the 1% level

APPENDIX A

Analysis of Variance of Waste Water

Specific Conductance

Source	df	Mean Square	F
Sample	4	2,382,755	22,428**
Method	1	500,132	4,708**
Sample x Method	4	54,697.32	515**
Error	50	106.24	

Total Phosphorus

Source	df	Mean Square	FF
Sample	4	12.59	146**
Method	1	9.0	104**
Sample x Method	4	0.284	3.29*
Error	50	0.0864	

Total Kjeldahl Nitrogen

Source	df	Mean Square	F
Sample	4	25.65	1.501
Method	1	328.7	20.98**
Sample x Method	4	54.20	3.17*
Error	50	17.10	

* denotes significance at the 5% level

** denotes significance at the 1% level

APPENDIX B

Analyses of Variance Within Methods

APPENDIX B

Analysis of Variance of Waste Water
Within Methods

Specific Conductance

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	4	935,534	7026**	4	1,501,919	18,935*
Port./Samp.	20	314.76	2.37*	20	318.12	4.01**
Error	25	133.16		25	79.32	

Total Phosphorus

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	4	7.019	64.99**	4	5.853	90.32**
Port./Samp.	20	0.164	1.52	20	0.087	1.35
Error	25	0.1080		25	0.0648	

Total Kjeldahl Nitrogen

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	4	44.684	227**	4	35.157	1.034
Port./Samp.	20	1.350	6.86**	20	39.765	1.17
Error	25	0.1968		25	33.998	

(1) Before frozen storage

(2) After frozen storage

* Denotes significance at the 5% level

** Denotes significance at the 1% level

APPENDIX B

Analysis of Variance of Well Water
Within Methods

Specific Conductance

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	4	793,633	144,297**	5	637,643	38,373**
Port./Samp.	20	77.001	14.00**	24	1722.2	103.65**
Error	25	5.5		30	16.617	

Calcium Hardness

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	5	98,595	1354**	4	49,500	5950**
Port./Samp.	24	90.203	1.24	20	869.6	104.52**
Error	30	72.800		25	8.32	

Total Hardness

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	5	232,061	7601**	5	166,108	4843**
Port./Samp.	24	80.79	2.65**	24	1,374	40.06**
Error	30	30.53		30	34.3	

Total Alkalinity

Source	(1)			(2)		
	df	M.S.	F	df	M.S.	F
Samples	5	14,818	13,607**	5	7207.31	8825**
Port./Samp.	24	2.410	2.22*	24	688.61	843**
Error	30	1.089		30	0.8167	

(1) Before frozen storage

(2) After frozen storage

* Denotes significance at the 5% level

** Denotes significance of the 1% level