Methods of Analyzing Feathers for Elemental Content

by W. J. Pannekoek, John P. Kelsall and Roland Burton

FISHERIES AND MARINE SERVICE SERVICE DES PÉCHES ET DES SCIENCES DE LA MER

TECHNICAL REPORT No. RAPPORT TECHNIQUE N°

498



•

Environment Canada

Fisheries and Marine Service

Environnement Canada

Service des pêches et des sciences de la mer

Technical Reports

6

4

ŧ,

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

Rapports Techniques

Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique prioritaire. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable. Department of the Environment Fisheries and Marine Service Research and Development Directorate Direction de la Recherche et Développement

TECHNICAL REPORT NO. 498

(Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. The series name was changed with

report number 457)

RAPPORT TECHNIQUE NO. 498

(Les numéros 1-456 dans cette série furent utilisés comme Rapports Techniques de l'office des recherches sur les pêcheries du Canada. Le nom de la série fut changé avec le rapport numéro 457)

METHODS OF ANALYZING FEATHERS FOR ELEMENTAL CONTENT¹

by

W. J. Pannekoek², John P. Kelsall³, and Roland Burton⁴

¹The project was conducted at the Pacific Biological Station in 1972 and 1973, and the methodology is published in this Technical Report Series because of its possible applicability to parallel problems in fisheries. In fact, in subsequent work the senior author applied some of the described methodology to salmon tails with only minor modifications.

²Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.

³Canadian Wildlife Service, Department of the Environment, 10025 Jasper Avenue, Edmonton, Alberta.

44684 West 4th Avenue, Vancouver, B.C.

This is the 231st Technical Report from the Pacific Biological Station Nanaimo, British Columbia

November 1974

Pannekoek, W. J., John P. Kelsall, and Roland Burton. 1974. Methods of analyzing feathers for elemental content. Fish. Mar. Ser. Res. Dev. Tech. Rep. 498: 16 p.

ABSTRACT

Methods of analyzing waterfowl flight feathers, by atomic absorptionflame emission spectroscopy, were developed for a Canadian Wildlife Service study of their chemical composition. Some of the techniques are innovative or new and would be applicable to other biological materials of a keratinous nature. The report describes the methods in a "cook book" manner for use by minimally trained technicians. An original computer program for calculation of metal concentrations is included.

RÉSUMÉ

Des méthodes d'analyse des rémiges d'oiseaux aquatiques, fondées sur la spectroscopie de l'absorption atomique et l'émission de flammes, ont été mises au point pour les besoins d'une étude du Service canadien de la faune sur leur composition chimique. Certaines des techniques sont innovatrices ou nouvelles et pourraient s'appliquer à l'étude d'autres matières biologiques kératiniques. Le rapport fait état des méthodes à la manière d'un livre de cuisine afin de les mettre à la portée de techniciens possédant un minimum de formation. Un programme informatique original pour le calcul des concentrations métalliques y est aussi inclus.

INTRODUCTION

In 1968 the Canadian Wildlife Service commenced a study of the chemical content of primary feathers of waterfowl. Preliminary work, where wet chemical analyses were done, has been reported (Kelsall 1970a, 1970b; Kelsall and Calaprice 1972). Non-destructive X-ray fluorescent spectroscopy is now used as an analytical tool, but wet chemical techniques are used to complete some work and to complement the X-ray program.

As part of the wet chemistry program procedures for multi-element analyses of feathers using flame emission-atomic absorption spectroscopy were developed as the primary tools. This report describes our methods in a "cook book" manner for use by minimally trained technicians. The work was carried out at Pacific Biological Station, Nanaimo, B.C., where the authors were fortunate in being able to work under ideal laboratory conditions while on temporary transfer from Canadian Wildlife Service, Edmonton, Alberta.

While Hanson and Jones (1968) briefly described their methods of analyzing feathers, a number of procedures reported here seem innovative or new. Also new is a computer program (attached) for the calculation of metal concentrations. While it was developed for a NOVA computer, it would be readily adaptable to other computers which use the BASIC language. It is thought that our procedures might be useful to others, particularly where keratinous materials (hair, nails, feathers, hoofs) are being examined. Of particular interest are laundering techniques, the use of multi-element standards and the significant reduction in the quantity of feather material required for a multi-element analysis. The latter is of great practical importance should the technique ever be used in waterfowl management. With only one or two primary flight feathers needed per bird to do a diagnostic analysis, it is possible to use wet chemistry as a tool for working with wild, living waterfowl.

ANALYTICAL METHODS

In the following discussion a "sample" is any mass of feathers from which quantitative analysis is desired. Most of our individual samples included the clipped primary and secondary flight feathers from a single bird. Since we wanted results from primary feathers only, they were separated, cut into pieces, mixed and then subsampled to provide weights of material suitable for analysis.

Preparation of standard solutions

Use commercially available atomic absorption reference solutions. They provide standard concentrations of 1000 ppm. 1) Using the appropriate sized pipettes, add the following amounts of reference solutions to a 500 ml volumetric flask:

Ca - 150 ml	Mg - 30 ml	K - 20 ml
Cu - 5 mì	Mn - 5 ml	Na - 100 ml
Fe - 20 ml	Ni – 5 ml	Zn - 20 ml

- 2) Make the solution up to 500 ml with distilled water.
- Label eight 400 ml graduated beakers and eight 500 ml volumetric flasks successively
 - 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128
- 4) Using a pipette, add 250 ml of the prepared standard solution to the beaker labelled 1:1.
- 5) Similarly, add 125 ml to the beaker labelled 1:2 62.5 ml to the beaker labelled 1:4 31.2 ml to the beaker labelled 1:8 15.6 ml to the beaker labelled 1:16 7.8 ml to the beaker labelled 1:32 3.9 ml to the beaker labelled 1:64 2.0 ml to the beaker labelled 1:128
- 6) Using a graduated cylinder add 200 ml of a 1:1 nitricperchloric acid solution to each of the beakers excepting that labelled 1:1.
- 7) Add some clean glass boiling stones to each beaker and boil the solutions, preferably on a hot plate, until the volume of each is reduced to 125-150 ml. At this point add 200 ml of the acid solution to beaker 1:1.
- 8) Remove each beaker from heat as its volume becomes sufficiently reduced.
- 9) Weigh eight 10.0 g portions of LaCl $_3$ $^{6H}_2$ 0 and put one in each 500 ml volumetric flask.
- 10) Transfer each of the reduced solutions to the appropriately labelled 500 ml volumetric flask.
- Rinse each beaker with distilled water and add this to the flask.
- 12) Make the solutions up to volume with distilled water.
- Transfer each solution into four, labelled 4-oz polyethylene bottles.

When a mixture of several metals is to be analyzed by atomic absorption or flame emission, they are usually separated by various chemical techniques to enable analysis on an individual basis. This is a time-consuming process, not suitable for handling large numbers of samples, each containing several metals. Analysis of the mixture itself is a more efficient procedure but the metals react with each other in the flame, enhancing or depressing the results.

The method employed with the feather material accurately analyzes the mixture of metals because the reaction problems are circumvented in three ways. First, the standard solutions resemble the subsample solutions, providing a relative measure of metals in feathers. The lanthanum chloride additive is a deionizing agent which, when present in the flame with the alkali metals, preferentially interacts with them, freeing the metals of interest. By forming metal perchlorates in solution, and later aspirating them into the flame, one substantially reduces almost all types of interferences and interactions. This effect is also partially due to the low pH of the resulting solutions.

Initial sample preparation

- Write the sample number from the original feather container on a strip of masking tape placed on a graduated 100 ml beaker opposite the lip.
- Repeat for each sample. LIMIT A TEST SERIES TO 25 OR LESS SUBSAMPLES.
- Weigh each beaker to ±0.005 g and record the weight with the sample number.
- 4) If necessary, remove and discard all but the primary feathers from each sample.
- 5) Count and note the number of primary feathers in each sample.
- 6) With pointed scissors, Cut each into about 1/4-inch lengths at the shaft.
- 7) Spread each sample on a clean, flat surface (e.g., a large sheet of paper) and take from the mass enough feather pieces to make up a 0.75 to 1 g subsample and put the pieces into the marked beaker.
- 8) Store the remaining material in a clean, labelled plastic bag.

Placement of the masking tape opposite the beaker lip avoids perchloric acid burns on the sample number and, more important, significantly decreases the chances of a perchlorate explosion from residual perchloric acid oxidation on the masking tape. The explosion hazard is also the reason for limiting the size of a subsample set to 25. The feathers in a sample must be sorted unless only primaries are present. There are usually 10 primaries on each wing. In mixed samples, primaries are recognized by their unbalanced shape. The vanes on one side of the shaft are broader than those on the other. All other feathers are more symmetrical. If large numbers of samples are to be analyzed a mechanical feather cutting technique might save 25% in time.

Subsamples for analysis are taken as described to ensure a random selection. The previous analytical results for this project (Kelsall 1970a, 1970b; Kelsall and Calaprice 1972) were obtained using samples containing approximately 4 g of feather material. Our recent work has been done using approximately 0.75 g feather subsamples. Should colorimetric analysis for S and P prove unnecessary, as they were in this work, the subsample size could readily be reduced to 0.25 g.

As a large primary feather from a mallard or black duck weighs about 0.3 g and a large primary feather from a lesser scaup about 0.1 g, it is quite feasible to remove only one or two feathers from a live bird for analysis. Should single feather analysis be considered, it should be noted that mineralization varies between primary feathers. Our studies suggest that primaries 4 and 5 from the distal end of the wing are the most highly mineralized.

Feather laundering

- Using a graduated cylinder or volumetric dispenser, add 50 ml of l:l (V/V) acetone-carbon tetrachloride solution to each subsample. Use analytical grade solvents.
- Place the subsamples in an ultrasonic bath for approximately 2 minutes.
- 3) Decant the solvent, being careful not to lose any feather pieces.
- Repeat the above procedure with 25 ml of acetone-carbon tetrachloride.
- 5) Wash again with 25 ml acetone, then 50 ml of distilled water and finally 25 ml of acetone. Exact quantities of washes may have to vary dependent on the size of the ultrasonic bath used.
- 6) Place the subsample in a drying oven at 120⁰C for 1 hour (or until their weights remain constant).
- 7) Reweigh the subsamples to determine their washed weight.
- 8) Place subsamples in individual graduated beakers.

It is important that the laundering technique be followed closely and consistently since it has a pronounced effect on the feathers. The initial acetone-carbon tetrachloride wash removes most of the surface oils from the feathers, the remaining oil being dissolved in the second wash. Much of the dirt trapped in the oils is also removed. The next acetone wash serves to remove the residual mixed solvent as well as any semi-polar materials from the feather surfaces. The water wash removes any polar impurities and blood remaining after removal of the oils exposes the feather surface. The final acetone wash simply removes residual water to enable the feathers to dry faster

The laundering procedure results in weight losses ranging from 5% to 12% of the original subsample weight. In one subsample, when the first acetone wash was accidentally missed, the final weight reduction was only about 3%.

Although not essential, recovery of the used wash solvents is advisable. Several liters of solvent are needed for each 25 subsamples. For large numbers, the solvent becomes expensive unless recovered. Recovery is accomplished through standard distillation techniques.

A study on cleaning feathers before analysis showed that washing the feathers only with distilled water was not adequate. As all waterfowl feathers have an oily coating to make them waterproof, washing with water has little effect upon contaminants.

By washing the feathers first with organic solvents, oils and greases with their incorporated contaminants are readily removed. Washing with water is then effective as the water is able to wet the feather surface. Table 1 indicates the magnitude of elemental contamination in feather oils from captive waterfowl with which we worked. It is evident that results based on washing feathers with water alone should be looked upon with some suspicion.

Table 1. Values, in parts per million, for elements in primary wing feathers contrasted with values from the oil residues removed from feathers by solvent.

Element	Calcium	Copper	Iron	Magnesium	Manganese	Zinc	Sodium	Potassium
Clean Feathers	822	15	96	236	14	132	318	71
Oil residue from 2 feathers	1,000	20	600	900	30	1	11,000	900

¹Mean values of 271 samples from mixed mallards, black ducks and lesser scaup.

²Single approximate determinations from the residues from 50 of the above mixed samples.

Subsample digestion*

- 1) Using a graduated cylinder or volumetric dispenser, add 20 ml of 1:1 (V/V) nitric acid-perchloric acid solution to each subsample.
- 2) Cover each beaker with a ribbed watchglass and place on a hot plate at 150 - 200°C. CAUTION: EXPLOSION HAZARD. Do the digestion only in a fume hood suitable for perchloric acid. A plexiglass safety shield should be installed in the fume hood. <u>DO NOT</u> leave the lab until the digestion has been completed.
- Remove the subsamples from the hot plate one by one as digestion is completed. Completed digestion is indicated by:
 - i) solution turning a light yellow color;
 - ii) volume of the solution decreasing to ~ 12-15 ml as indicated by the beaker graduations;
 - iii) white fumes rising from the solution;
 - iv) the solution beginning to bubble vigorously.
- 4) Allow the subsamples to come to room temperature.
- 5) Wash off the beaker covers into their beakers using a wash bottle filled with distilled water.
- 6) Add distilled water to each solution to make it up to 40 ml as indicated by the beaker graduations.

Heating a mixture containing perchloric acid and an organic material is dangerous. The presence of nitric acid helps to stabilize the mixture, considerably reducing the risk of an explosion. When the acid solution is reduced in volume by boiling off the nitric acid, it is important that no pieces of feather material remain undigested on the beaker walls. When only hot perchloric acid remains, sudden contact with the feathers could initiate an explosion. It is also important that the acid solutions be cooled to near room temperature. If warm, the acid will react with the filter papers in the next step.

Final preparation

 Filter each solution through Whatman #40 ashless filter paper, contained in a 3.5 cm funnel, into a labelled 50 ml volumetric flask containing 1.0 g LaCl₃·6H₂O.

^{*} Preparation of a blank solution (see below) normally proceeds concurrently with this step.

- Rinse the beaker walls with 1-2 ml of distilled water and add this through the filter paper.
- 3) Wash each of the filter papers further with distilled water.
- 4) Add distilled water to make each solution up to 50 ml.
- 5) Transfer each solution from the volumetric flasks to a similarly labelled 2-oz polyethylene bottle.
- Place each filter paper in a clean, weighed and labelled 50 ml beaker.
- 7) Add 1 ml of 50% nitric acid to each filter paper. Put a 2-in watchglass cover on each beaker.
- 8) Place the beakers into a furnace at 550° C for 4 hours. Remove the covers after the first hour.

It is important that the filter papers be well washed with distilled water in order to remove as much perchlorate as possible. This is done not only to reduce the loss of analytical material but, more important, to reduce the rate of combustion of the filter papers when they are ashed in the oven. The addition of nitric acid to the filter papers prevents explosions and helps speed up the oxidation rate. The bottle used for final storage of the sample solution should be of polyethylene. Perchlorate solutions tend to be unstable at these concentrations in glass or other types of plastic containers. Also, it is imperative that the 50 ml beakers used to contain the filter papers to be preweighed. Any markings put on them are quickly burned off in the furnace so the only method of identifying individual samples is by their weights.

Preparation of a blank solution

- Put 40 ml of 1:1 (V/V) nitric acid-perchloric acid solution into a 100 ml graduated beaker.
- Place the beaker on a hot plate and heat as with the subsamples until the volume of the solution has been reduced to 20-25 ml as indicated by the beaker graduations.
- 3) Remove the beaker from the heat and allow it to cool to room temperature.
- 4) Add distilled water to the beaker to make the volume of the solution up to 80 ml as indicated by the beaker graduations.

- 5) Pour the contents of the beaker into a 100 ml volumetric flask containing 2.0 g LaCl₂·6H₂O.
- Rinse out the beaker with distilled water and add this to the volumetric flask.
- 7) Make the solution up to 100 ml with distilled water and transfer it to a labelled 4-oz polyethylene bottle.

The purpose of the blank solution is to permit compensation for extraneous solvent and matrix effects which cannot be controlled. First, and most important, is the purity of the acids used. They are certain to contain small amounts of some of the metals of interest. Also the lanthanum chloride added may contain some impurities. The actual analytical method may have pronounced effects on the results and so is carefully followed in preparing the blank. Some other possible contributing effects were examined. Contamination from the solvent residue during the laundering was tested and the results indicated no measurable effect. It was also found that there were no measurable amounts of impurities introduced by the filter papers.

Some problems were encountered in using the blank as prepared. For both copper and iron, the level of the blank proved to be higher than those of the more dilute standard solutions. This phenomenon proved worse for some sets of subsamples than for others, which suggests external contamination at some point. The exact cause of this problem was not found so the blank level was not used in determining the concentrations of these elements. This is possible because the analysis involves relative rather than absolute standardization techniques.

Analysis

- 1) Silicon
 - i) Weigh the 50 ml beakers used to ash the sample filter papers, to 5 decimal places, on an analytical balance and note the weight.
 <u>If identification marks have disappeared</u>, use earlier weights to identify the individual samples.
 - ii) Remove the contents of each beaker, brushing out any remains with a clean, fine-haired brush.
 - iii) Reweigh the empty beakers.
 - iv) Take the difference between the two weights to obtain the weight of insoluble silicate recovered. The silicate analysis described should not be considered wholly adequate. Although it serves well enough for the purposes of this study, a more refined, colorimetric analytical procedure should be used for silicon. The present

gravimetric determination is used because it is quickly carried out.

2) Sodium and potassium

Analysis performed by the process of flame emission spectroscopy. Instrument: Jarrell-Ash Model 82-500 with chart recorder output. Burner: Triflame - 10 cm slot. Flame: Air (20 SCFH*) - acetylene (21 SCFH) Analytical lines: Sodium 5890 Å Potassium 7665 Å

3) <u>Ca, Cu, Fe, Mg, Mn, Ni, Zn</u>

Analysis performed	by the Proc	cess of atomic absorption spectroscopy.				
Instrument:	Jarrell-Ast	Model 82-500 with chart recorder				
	output.					
Burner:	Triflame - 10 cm slot.					
Flame:	Air (20 SCF	FH) - ącetylene (20 SCFH)				
Analytical lines:	Calcium	4227 Å				
	Copper	3247 Å				
	Magnesium	2852 Å				
	Manganese	2795 Å				
	Iron	2483 Å				
	Nickel	2320 Å				
	Zinc	2129 Å				

4) Procedure

The procedures for obtaining results by atomic absorption and emission spectroscopy are the same except for differences in spectrometer operation. The atomic absorption work was done using the CONCENTRATION mode on the spectrometer.

The running procedure to be followed after the spectrometer is warmed up and operating properly is as follows:

- Aspirate several of the subsamples and adjust the spectrometer output so that the sample readings are about 20 to 30% emission/absorption.
- ii) Find the standard solution giving a reading of 55 to 75%.
 If either of the above emission/absorption ranges cannot be attained, use the highest permitted scale expansion. If the signals are too strong, rotate the burner head to decrease the signal levels.

^{*}Standard cubic feet per hour as measured by the flow meters on the spectrometer.

- iii) Run the standards, blank, and subsamples in the following order:
 - a) blank;
 - b) standards run four consecutive standards, starting with the least concentrated one and ending with that standard used to establish the 55 - 75% reading;
 - c) subsample solutions;
 - d) blank;
 - e) standards (same set of 4).

Repeat the entire procedure for each element of interest after appropriately adjusting the spectrometer.

Calculations

1) Silicon

The determination of silicon present in feather subsamples assumes that the recovered silicates are in the form of SiO₂. Complete oxidation is assumed because two strong oxidising procedures have been used, the digestion of perchloric acid and the high (> 400° C) temperature achieved by the oven.

SiO₂ is $\frac{28.09}{60.08} = 46.75\%$ Si by weight (Si = 28.09 amu*) Therefore 46.75% of the weight of recovered solid represents its silicon content.

Concentration of Si in ppm is found by:

$$\frac{\text{wt solid (g) X 0.4675}}{\text{sample wt (g)}} \times 10^6 \frac{\text{ug}}{\text{g}} = \text{amount Si (ug/g)} = \text{ppm Si}$$

2) Ca, Cu, Fe, Mg, Mn, Ni, Na, K, Zn

The measurement technique for all of the above elements is the same. The recorder output is handled as follows:

- Using a straight-edge and pencil, draw in a base line for each peak. It should be a line connecting the points of average noise level on either side of the peak base.
- ii) Draw a straight line through the average noise level at the peak maximum. This line should be parallel to the horizontal grid line on the chart paper.
- iii) Using the chart paper grid lines, measure the height of each peak to the nearest 1/10 of a unit.

*Atomic mass unit

- iv) Subtract the average of the two blank values from all of the other peak heights.
- v) Obtain averaged values from the two heights for each of the standard peaks.
- vi) Record these corrected peak heights for further computer processing. This will yield directly the concentration of a given element in each feather sample.

3) Program for the calculation of metal concentrations

The program, written in BASIC for the NOVA computer, fits a curve to the set of standard concentrations and then uses the curve to obtain concentrations for the unknowns. This is considerably faster than a graphical interpolation, probably more accurate, and decreases the chance of blunders. The curve fitted is of the form:

$$\log Y = A \log X^2 + B \log X + C$$

where Y is concentration, X is absorbance, and A, B, C are curve fit parameters calculated by the program. This form of curve fit tends to weight heavily the lower concentrations of standards. It may not be suitable if the unknowns are higher in concentration than the standards.

The program, followed by operating instructions, is in Appendix I. A sample calculation is in Appendix II. The program can readily be modified for computers other than the NOVA.

ACKNOWLEDGEMENTS

We are grateful to the Pacific Biological Station, Fisheries Research Board of Canada, Nanaimo, B.C. for provision of laboratory facilities and major equipment. Throughout our period of work there, April 1972 to July 1973, many of the Station staff assisted us in ways too numerous for individual recognition.

LITERATURE CITED

- Hanson, H. C., and R. L. Jones. 1968. Use of feather minerals as biological tracers to determine the breeding and moulting grounds of wild geese. III. Natural Hist. Survey, Biol. Note No. 60. 8 p.
- Kelsall, J. P. 1970a. Chemical elements in waterfowl flight feathers. Canadian Wildl. Service Progress Notes No. 17. 11 p.

______. 1970b. Comparative analysis of feather parts from wild mallards. Canadian Wildl. Service Progress Notes No. 18. 6 p.

Kelsall, J. P., and J. R. Calaprice. 1972. Chemical content of waterfowl plumage as a potential diagnostic tool. J. Wildl. Mgmt. 36(4): 1088 - 1097. APPENDIX I. PROGRAM FOR CALCULATING METAL CONCENTRATIONS FROM SPECTROMETRIC DATA.

.

```
0202 REM AS OF 22 MARCH '73
2225 DIM N[50], A[50], W[50], X[50], Y[50]
0010 DIM S[10],C[10]
0140 PRINT
Ø141 GOSUB
           1900
Ø180 PRINT
0190 PRINT
0200 PRINT "ELEMENT ATOMIC NUMBER =";
0210 INPUT EL
2215 IF E1=0 GOTO 0140
Ø220 PRINT
Ø230 GOSUB 0600
Ø235 GOSUB
           1440
Ø237 GOSUB
            Ø25Ø
Ø24Ø GOTO Ø13Ø
0250 PRINT
0260 PRINT
0290 PRINT "NO.", "ABS. ", "CONC., PPM."
0310 FOR Q=1 TO 50
0312 IF N[Q]=0 GOTO 0550
      PRINT NEQL,
0350
      INPUT ALQ]
0440
0445
      PRINT TAB 28;
0450
      IF ALQ]<0 GOTO 0555
0451
      LET ALQ]=LOGALQ]
0460 LET X[Q]=D+A[Q]*S+A[Q]*A[Q]*F
0461
      LET X[Q]=EXPX[Q]
2500
     LET Y[Q]=(X[Q]*50)/W[Q]
0505 LET YEQ]=INT(10*YEQ]+.5)/10
2510
     PRINT YEQI
0520 NEXT Q
2550 RETURN
0555 PRINT " ERROR, ABOVE LINE DELETED"
0556 LET Q=Q-1
Ø557 GOTO Ø35Ø
0600 PRINT "CONC OF STD ";
0602 INPUT A[1]
0603 LET A[0]=2*A[1]
0604 PRINT
0605 PRINT "ABSORBANCES, STDS"
9606 LET K=0
0612 LET K=K+1
0613 LET A[K]=A[K-1]/2
0614 INPUT CEKI
0616 IF C[K]>0 GOTO 0612
0617 IF C[K]<0 GOTO 0600
0627 LET J=K-1
0630 FOR K=1 TO J
0631 LET C[K]=LOGC[K]
```

```
0632
      LET A[K]=LOGA[K]
0633 NEXT K
0650 RETURN
1440 FOR K=1 TO 7
     LET S[K]=Ø
1450
1460 NEXT X
1470 FOR K=1 TO 7
      FOR L=1 TO J
1480
1490
         IF K>4 GOTO
                     1520
         LET SEKJ=SEKJ+CELJ+K
1500
         GOTO 1530
1510
         LET S[K]=S[K]+C[L]+(K-5)*A[L]
1520
1530
       NEXT L
1540 NEXT K
1550 LET T1=J*S[2]-S[1]*2
1560 LET T2=J*S[3]-S[1]*S[2]
1570 LET T3=J*S[6]-S[1]*S[5]
1580 LET T4=S[1]*S[3]-S[2]+2
1590 LET T5=S[1]*S[4]-S[2]*S[3]
1600 LET T6=S[1]*S[7]-S[2]*S[6]
1610 LFT F=(T3*T4-T1*T6)/(T2*T4-T1*T5)
1620 LET B=(T3-T2*F)/T1
1630 LET D=(SE5)-SE1)*B-SE2)*F)/J
1640 RETURN
1900 PRINT "#"; TAB 6; "WEIGHT"
2000 FOR I=1 TO 50
       INPUT NEI]
2005
2010
       IF N[1]>0 GOTO 2017
       IF V[1]=0 GOTO 2030
2012
       PRINT "ERROR, ABOVE LINE AND THIS ONE LELETED"
2013
2014
       LET I = I - I
       PRINT
2015
2216
       GOTO 2005
2117
       PRINT TAB 6;
       INPUT WEID
2020
2021
       IF WUIJ<=0 COTO
                         2013
2922
       TUIRS
2025 NEXT I
2030 RETURN
         PROGRAM FITS AA DATA TO STANIARE CURVES IN LOG-LOG
5000 REM
         SPACE. DATA STRINGS ARE TERMINATED BY TYPING ZERO.
5001 REM
5002 REM
          :TYPING -1 WILL USUALLY DELETE THE LINE JUST TYPEL,
          :IN CASE IT IS IN ERROR.
5003 BE1
5004 P.EM
          :IT IS ASSUMED THAT THE STANLARD DILUTIONS ARE SERIAL
5005 REM
          : IE. 20, 10, 5, 2. 5.
          : TO USE PROG, FIRST TYPE SAMPLE NUMBERS AND WEIGHTS
5006 REM
5007 REM
          : FOR A SET OF DATA; TERMINATE WITH ZERO.
5008 REM
          : NEXT, TYPE ELEMENT NUMBER, CONCENTRATION OF HIGHEST
5209 REM
          : STANLARD, AND THEN ABSORBANCES FOR EACH STD;
5010 REM
          : TERMINATE WITH ZERO
5011 REM
         : NOW PROGRAM WILL LIST SAMPLE NUMBERS AND YOU TYPE
5212 REM
          : ABSORBANCES, AFTER LAST SAMPLE, PROGRAM ASKS FOR ANOTHER
         -: ELEMENT. ELEMENT #0 CALLS FOR MODE SAMPLE NUMBERS AND
5013 REM
5014 REM
         : WEIGHTS.
```

APPENDIX II. SAMPLE PRINTOUT OF PROCESSED DATA FOR ELEMENT 19 (POTASSIUM).

Ť.

.

4

Ŧ

κ.

RIN WEIGHT # ? 72 ? 1.026 ? 73 ? .983 ? 74 ? 1.017 ? 75 ? . 969 ? 76 ? 1.032 ? 77 ? .962 ? 82 ? 1.053 ? 83 ? 1.008 ? 84 ? . 994 ? 85 ? .955 ? 86 ? .805 ? 87 ? .961 ? 2 ELEMENT ATO'IIC NUMBER =? 19 CONC OF STE ? 10 ABSORBANCES, STDS ? 74.4? 36? 19.2? 11.8? 7? Ø N0• ABS. CONC., PPM 72 ? 29.7 197.6 ? 19.2 73 123.9 ? 17.9 74 109.3 75 ? 14 84.2 76 ? 15.2 33 77 ? 14.9 92 82 ? 29.9 194 83 ? 25.9 172.4 84 ? 19.6 125.7 85 ? 19.2 127.5 86 ? 14 101.3 87 ? 15.8 99.3