

1 Water Testing and Laboratory Processing Water

Testing

Storage: Water samples collected from the field were stored in 1L sterilized bottles. Bottles were stored at 4 deg C in a refrigerator (Celfrost refrigerator; capable of -22 deg C – 20 deg C). Samples were tested within 28 days. Typically, water samples can be stored up to 6 months if kept in the refrigerator. To prepare a water sample for testing, sample was left at room temperature for approximately 30 minutes to reach 25 deg C. All other reagents used were stored at room temperature

Equipment:

- Fluoride and nitrate analytic test kits were purchased from Merck Germany (Merck Spectroquant® Fluoride (F-) kit 1.14598.0001/1.14598.0002; HC563464; Merck Spectroquant® Nitrate (NO₃-) kit 1.09713.0001/1.09713.0002; HC570450)
- Fluoride and nitrate content were analyzed with a UV-Visible Spectrophotometer (Thermo Scientific Evolution 201 UV-Visible Spectrophotometer)
- Two 10mm silicate optical glass cuvettes (Thermo Scientific) were used to hold samples.
- Calibration was performed in accordance to ISO 8466-1. TDS content and pH level were analyzed with an ion electrode (Hach sensION+ MM150)

2.1 Fluoride Contents

Preparation: Sample water was prepared in a buffered, weakly acidic solution which allows fluoride ions to react with alizarin complexone and lanthanum(III) to form a violet complex, which can then be measured photometrically.

Methodology: Procedure is as indicated by Merck Spectroquant® Fluoride Test and is analogous to EPA 340.3 and APHA 4500-F- E.

- Prepare blank solution (control) Pipette 2.0 mL of Reagent F-1 into standard test tube Add 5.0 mL of distilled water (room temperature)
- Add 1 level microspoon (provided in reagent bottle cap) of Reagent F-2
Place stopper on test tube and shake 2-3 times until reagent is completely dissolved
- Leave for 15 minutes to allow reaction to reach completion
- Prepare sample solution (for fluoride content 0.1-2.0mg/L)
- Pipette 2.0 mL of Reagent F-1 into standard test tube
- Add 5.0 mL of sample water (room temperature)
- Add 1 level microspoon (provided in reagent bottle cap) of Reagent F-2
- Place stopper on test tube and shake 2-3 times until reagent is completely dissolved
- Leave for 15 minutes to allow reaction to reach completion
- In case of higher fluoride concentrations, between 1.0-20.0 mg/L, 5.0 mL of distilled water is added to the sample solution prior to step 2b where sample water is added. If fluoride concentration exceeds 20 mg/L, samples are diluted by 1:10 ratio
- Analyze solutions in UV-Visual spectrophotometer
- Pipette and fill first optical glass to the line with blank solution for calibration
- Run spectrophotometer at 620 nm (as indicated by Thermo Scientific for F-) and record displayed measurement
- Pipette and fill second optical glass to the line with sample solution (~1 mL)
- Run spectrophotometer again at 620 nm and record displayed measurement
- Repeat procedure for 2 additional trials of sample water and calculate average for total 3 trials of sample water
- Between each trial, clean optical glass
 1. Internally: cleanse first with distilled water, then acetone, and then again with distilled water
 2. Externally: clean outer surface with dry, clean tissue paper
- Thoroughly clean optical glasses between testing different water samples
 1. Cleanse first with distilled water, then acetone, and then again with distilled water
 2. Dry optical glasses in air dried oven at 65 deg C for approximately 15-30 minutes

2.2 Nitrate Content

Preparation: Sample water is prepared in a sulfuric and phosphoric solution which allows nitrate ions to react with 2,6-dimethylphenol (DMP) to form 4-nitro-2,6-dimethylphenol that can be measured photometrically.

Procedure: Methodology is as indicated by Merck Spectroquant RNitrate Test and is analogous to DIN 38405-9.

- Prepare blank solution (control)}
- Pipette 4.0 mL of Reagent NO₃-1 into standard test tube
- Add 0.5 mL of distilled water (room temperature)

- Add 0.5 mL of Reagent NO3-2
- Place stopper on test tube and shake 2-3 times until reagent is completely dissolved
- Leave for 10 minutes to allow reaction to reach completion
- Prepare sample solution
- Pipette 4.0 mL of Reagent NO3-1 into standard test tube
- Add 0.5 mL of sample water (room temperature)
- Add 0.5 mL of Reagent NO3-2
- Place stopper on test tube and shake 2-3 times until reagent is completely dissolved
- Leave for 10 minutes to allow reaction to reach completion
- Analyze solutions in UV-Visual spectrophotometer
- Pipette and fill first optical glass to the line with blank solution for calibration
- Run spectrophotometer at 220 nm (as indicated by Thermo Scientific for NO3-) and record displayed measurement
- Pipette and fill second optical glass to the line with sample solution (~1 mL)
- Run spectrophotometer again at 220 nm and record displayed measurement
- Repeat procedure for 2 additional trials of sample water and calculate average for total 3 trials of sample water
- Between each trial, clean optical glass
 1. Internally: cleanse first with distilled water, then acetone, and then again with distilled water
 2. Externally: clean outer surface with dry, clean tissue paper
- Thoroughly clean optical glasses between testing different water samples
 1. Cleanse first with distilled water, then acetone, and then again with distilled water
 2. Dry optical glasses in air dried oven at 65 deg C for approximately 15-30 minutes

2.3 TDS Content

Procedure: TDS content can be simply measured using an ion electrode.

- For calibration:
 1. Place electrode in provided control solution of 147 μ Semens/cm at 25 deg C
 2. Then calibrate electrode with the provided control solution of 1413 μ Semens/cm 25 deg C
 3. Then calibrate electrode with the provided control solution of 12.18 μ Semens/cm 25 deg C
- To analyze sample
 1. Fill a glass beaker with approximately 50 mL of sample water
 2. Using the calibrated electrode, measure TDS content of sample water and record
 3. Repeat and take measurements for a total of 3 times and then calculate the average
 4. Between readings, clean electrode with distilled water and wipe with clean, dry tissue paper

2.4 pH level

Procedure: pH level can be simply measured using an ion electrode.

- For calibration

1. Place electrode in provided control solution of pH 4.01 at 25 deg C
2. Then calibrate electrode with the provided control solution of pH 9.21 at 25 deg C
3. Then calibrate electrode with the provided control solution of pH 7 at 25 deg C

- To analyze sample

1. Fill a glass beaker with approximately 50 mL of sample water
2. Using the calibrated electrode, measure pH level of sample water and record
3. Repeat and take measurements for a total of 3 times and then calculate the average
4. Between readings, clean electrode with distilled water and wipe with clean, dry tissue paper

2.5 Disposal

- Solutions mixed from fluoride and nitrate testing can be safely disposed by washing down drain
- Leftover water samples can be stored up to 6 months in refrigerator in case of need for further/repetitive testing
- After water samples are no longer needed, water can be safely disposed by washing down drain
- Acids used for electrode calibration are neutralized in dilute solution of sodium hydroxide pellets (40 g) and distilled water (~1 L) until approximate pH 6-7 is reached
- Solution can then be safely disposed by washing down drain