ENVS2001/2014 LABORATORY #3
GUESS THAT WATER!

INTRODUCTION

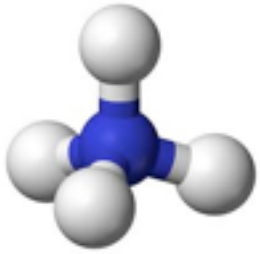
Water quality is an extremely important aspect of human and environmental health. In this lab you will use some common water quality analysis techniques to estimate the source of 4 volumes of water. The source names will be revealed to you at the end of the exercise, and you will match the source names with each unknown sample's dataset. We will discuss your data in the context of some common thresholds for acceptable water quality.

Unknown #	Salinity (‰)	pH	$[NH_4^+]$ (ug L^{-1})	$[PO_4^{2-}]$ (ug L^{-1})	TSS (mg L^{-1})	*Enterococcus* (CFU 100mL^{-1})	Predict the Water Source!
1							
2							
3							
4							

NAME:_____ **DATE:**_____

STATION #1: AMMONIUM CONCENTRATION (SPECTROPHOTOMETRY)

ammonium molecule



Ammonium (NH_4^+) is the ionic form of ammonium (NH_3). At pH < 9.0 ammonium is stable in seawater and pH >9.0 favors NH_3 which is volatile and removed as a gas. NH_4^+ is a highly bioavailable form of nitrogen preferred by primary producers. As the most reduced form of N it can also be respired as an energy source for bacteria. Even in low concentrations, NH_4^+ can be toxic to animal life.

NOTE: This procedure is to be conducted within a fume hood. Phenol vapors are corrosive to tissues.

1) Dispense 2mL of filtered water from each unknown bottle and each standard (see table below) into a 15 mL Falcon tube

2) In a separate tube, mix an **oxidizing reagent** with 4 mL **alkaline reagent** with 1 mL **sodium hypochlorite** (bleach)

3) add 0.15mL of **phenol** solution to each sample, vortex.

4) after 1 minute add 0.15mL **nitroprusside** solution, vortex.

5) after 1 minute, add 0.37mL **oxidizing reagent** to samples, vortex and immediately cover each tube with parafilm.

6) incubate tubes in the dark for 1 hour

SPECTROPHOTOMETER:

7) 10 minutes before measuring your samples, power on the spectrophotometer and allow to warm up

8) pour a volume of the blank, standard, and sample into the measuring cuvette of the spectrophotometer, ensure that the line mark on the cuvette lines up with the mark on the spectrophotometer.

9) rinse with DI between all standards & samples.

10) record the absorbance value at **640** nanometers

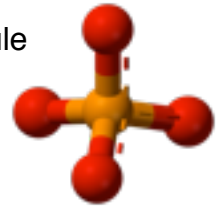
9) Calculate the equation of the line of best fit from the standard curve. (Absorbance = slope * concentration + intercept.

10) using the absorbance values from your unknowns, calculate their concentration based on the best fit line.

Concentration (ug L^{-1})	Absorbance (640 nm)	Unknown	Absorbance (640 nm)	Concentration (ug L^{-1})
0		1		
50		2		
100		3		
200		4		

STATION #2: PHOSPHATE DETERMINATION (SPECTROPHOTOMETRY)

phosphate molecule



Phosphate limits primary productivity in freshwaters, and is at times limiting or co-limiting in marine waters (*e.g.* Pearl River Delta). Phosphate is rapidly cycled in aquatic environments, and many organisms possess metabolic pathways and enzymes to transform inorganic and organic phosphate into usable forms. As such, phosphate determination can be designed for these various forms, such as total phosphate (TP = all forms of phosphate organic and inorganic), soluble reactive phosphate (SRP or orthophosphate = dissolved inorganic phosphate, directly taken up by plant cells).

1) create a combined reagent <u>in the following order</u> with mixing after each addition:
- **i.** 5mL H_2SO_4 (5N)
- **ii.** 0.5mL potassium antimonyl tartrate
- **iii.** 1.5mL ammonium molybdate
- **iv.** 3mL ascorbic acid

2) all solutions should be at room temperature before proceeding.

3) pipette 5mL of each standard and sample into a 15mL "Falcon" tube (see table to the right)

4) add 0.8mL combined reagent to each tube. Screw on cap and vortex.

5) wait 10 minutes but no more than 30 minutes to measure

6) pour 5 mL of each standard and sample into the cuvette and record the absorbance at **880 nm**. Make sure the mark on the cuvette is lined up with the mark on the spectrophotometer. Rinse cuvette with DI water between each sample.

9) calculate the equation of the line of best fit from the standard curve using the laptop. (Absorbance = slope * concentration + intercept.

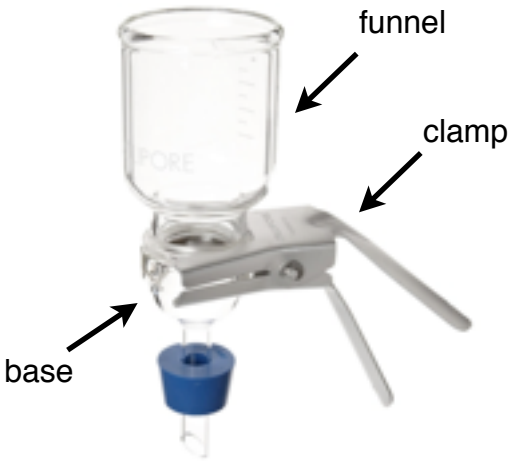
10) using the absorbance values from your unknowns, calculate their concentration based on the best fit line.

Concentration (ug L^{-1})	Absorbance (880)	Unknown	Absorbance (880)	Concentration (ug L^{-1})
0		1		
100		2		
200		3		
400		4		

STATION #3: pH, Total Suspended Solids, Salinity

TSS (mg L^{-1}) is a measure of how much particulate matter is suspended in the water column. This includes particulate organic and inorganic materials, plankton, and debris. It is one way to quantitatively assess water clarity, as there is a negative correlation between TSS and how far light can penetrate the water column. High TSS can inhibit primary productivity, especially in benthic habitats.

1) Obtain 1L of unknown water from the buckets
2) Using a balance, weigh a glass fiber filter before filtering each sample. This initial mass will be compared to the final mass after filtration.
3) Assemble the vacuum filtration rig by:
- **a)** connecting the vacuum tube to the vacuum line on the lab bench
- **b)** connect the other end of the tube to the side-arm flask
- **c)** insert the base of the filtration rig (with rubber stopper) on the top of the flask. Press down **gently** to create a seal.
- **d)** place a clean glass fiber filter disk (GF/F) on top of the base.
  - NOTE: the GF/F has been "ashed" or "muffled" in a high temperature furnace to burn off any organic contamination
- **e)** place the funnel on top of the GF/F and clamp together using the blue millepore clamp.



proper assembly of filter rig

4) fill the funnel with water and then open the vacuum valve slowly. Do not open the valve so much that the vacuum tube collapses.
5) If you do not observe water dripping through the filter disk, ask for assistance.
6) after filtering 1L of unknown water, rinse with a small volume of DI water
7) disassemble the vacuum rig by removing the clamp and funnel.
8) Remove the GF/F from the base and place on a clean square of aluminum foil labeled with sample ID & group number
9) Place in a drying oven at 103ºC until a constant weight is reached (1-2 hours).
10) Re-weigh using the same balance. Since you filtered 1L, **TSS = (final mass - initial mass)**

pH is a measure of free hydrogen ions (really, hydronium H_3O^+). The higher the concentration of H^+ the lower the pH. Note that pH is unitless and based on a logarithmic scale, so that the difference between pH 3.0 (*e.g.* orange juice) and 7.0 (*e.g.* deionized water) is 10,000x!!! Carbonated beverages (Coke) have a pH around 3.0… that's 10,000x more acidic than freshwater and can dissolve an iron nail.

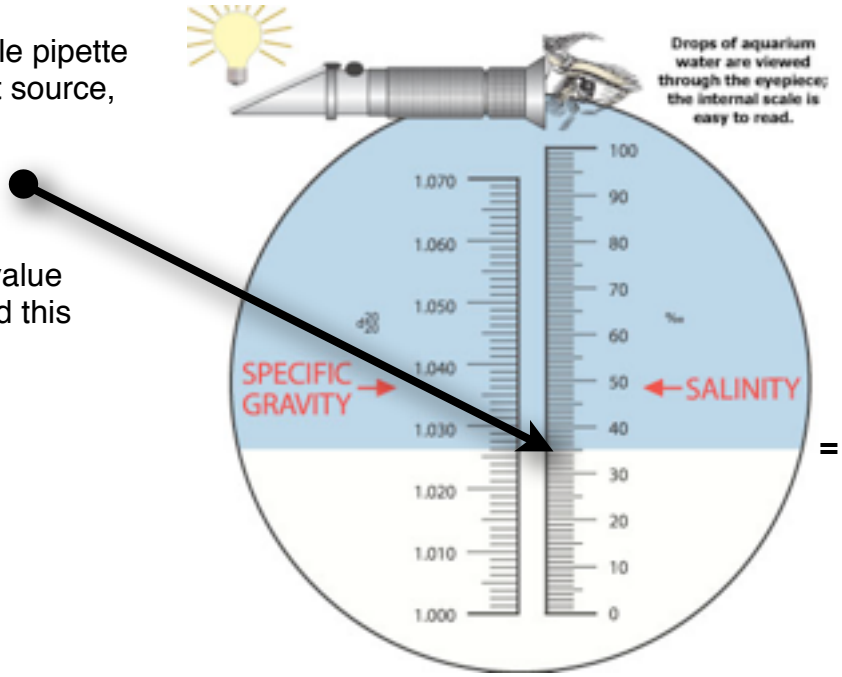
1) carefully remove the pH probe from the storage solution by first unscrewing the cap, removing the bottle, and then sliding the cap off the probe. GENTLY! **DO NOT YANK THE STORAGE BOTTLE OFF THE PROBE OR YOU MAY BREAK IT!**
2) open the blue vent on the side of the probe.
3) rinse the probe with DI water
4) Use the pH probe to measure the pH of two standards pH 10.0 and pH 7.0 **while stirring**. **rinse the probe between each measurement** with DI water.
5) if the standards do not match the pH reading, please inform your demonstrators.
6) if the readings match, Record pH for each unknown, and **rinse the probe between each measurement** with DI water.
7) rinse and store the pH probe in the provided storage solution bottle. First, slide on the cap and o-ring with the threads facing down. Then insert the probe into the bottle's solution. Bring the cap down and screw on gently.
8) replace the vent cap

Thermo pH probe and standards

SALINITY **is measured in parts per thousand (ppt or ‰), percent (rarely), specific gravity (mg L^{-1}) and "practical salinity units" (PSU). We will use the former (ppt or ‰) to quantify the salinity of our unknown samples. Salinity exerts a strong pressure on the evolution and biodiversity of aquatic organisms. To survive marine waters (>30 ppt) organisms must tolerate constant water loss from tissues, or maintain osmotic balance by storing other salts in their tissue and cells. Freshwater organisms have the opposite problem and must seek out salts and deal with eliminating excess water.**

1) open the refractometer lid and rinse with deionized water.
2) wipe clean with a kimwipe
3) apply a few drops of deionized water using the disposable pipette
4) with the flat end of the refractometer facing a strong light source, look through the eyepiece
5) record the salinity from the right hand scale at the line formed between the blue and white areas
6) repeat for each unknown sample
7) if the value for deionized water is positive, subtract this value from your unknown readings. if the value is negative, add this value to your unknown readings.

refractometer use and scale



= 35 ppt

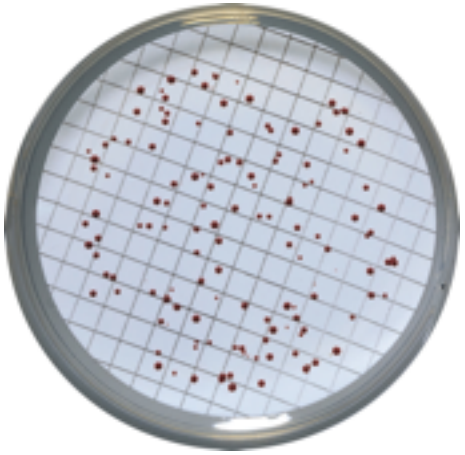
STATION #4: MICROBIAL INDICATORS (*Enterococcus*)

Enterococcus **Human and animal waste are loaded with microbes (x 10^{10} cells g^{-1}!!!) including bacteria which constitute up to 60% of the dry mass of feces. A minority of these microbes are extremely harmful to human and environmental health. *Enterococcus* are a genus of bacteria common to the mammalian gut. As such, they prefer to grow at high temperature (~37ºC). Moreover, *Enterococcus* survives well in water, including seawater, making it an ideal bioindicator of wastewater pollution.**

NOTE: Refer to STATION#3 instructions for proper use of the filter rig.

NOTE: In this station *be mindful of cross-contamination*. Forceps should be sterilized by dipping in ethanol after each use. The filter rig should be sprayed with ethanol between each site's series of dilutions (1 rinse after 3x filtering each unknown: 1, 5, and 100mL).

1) sterilize your filter rig by spraying with 70% ethanol in the spray bottle
2) rinse briefly with deionized water
3) unwrap and position a sterile membrane filter with the grid facing up on the filter rig base, reassemble the filter rig.
4) filter 100mL of deionized water through the membrane filter (this is your NEGATIVE CONTROL)
5) remove the filter using sterile forceps, place gently on the m-Enterococcus agar within a prepared plate.
6) repeat these steps with dilutions of your unknown waters. for EACH unknown you will prepare 3 plates TOTAL from 3 volumes of filtered water per unknown. 1 x 1mL, 1 x 5mL and 1 x 100mL. You DO NOT need to sterilize the rig between volumes from the same site but you DO need to sterilize between sets of unknowns.
7) label the bottom of each plate with your group number, unknown #, dilution volume (or control).
8) place in a 37ºC incubator for **24 hours.**
9) remove the plates from the incubator and count the number of colonies. You may remove the lid to count and use a dissecting microscope if necessary. At all times you should wear gloves to prevent contaminating yourself!
10) calculate *Enterococcus* concentration by normalizing to 100mL. Thus, 100mL filtered requires no correction, 5mL counts require multiplying by 20, and 1mL requires multiplying by 100.



Enterococcus colonies growing on a gridded membrane placed over selective media.

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