An excerpt from: Myers, D.N., Stoeckel, D.M., Bushon, R.N., Francy, D.S., and Brady, A.M.G., 2007, Fecal indicator bacteria: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.1 (version 2.0), available from http://pubs.water.usgs.gov/twri9A/.

PROCESSING BED SEDIMENTS 7.1.3.B

Standard methods for processing bed sediments for analysis of fecal indicator bacteria are not documented by the American Public Health Association or by the U.S. Environmental Protection Agency. The following method is recommended for general use. Samples are processed in a laboratory environment to elute fecal indicator bacteria from bed sediments. Once eluted, the supernatant is analyzed for fecal indicator bacteria by use of membrane-filtration or enzyme substrate methods. The proportional dry weight of the bed sediment is also determined.

To process bed sediments:

1. Prepare for processing by labeling the following items with site identifiers and date and time of sample collection: sterile jar for compositing (if done in the laboratory), a 500-mL sterile bottle for eluting, a 500-mL sterile bottle for collection of supernatant, and a dish for proportional dry-weight analysis.
2. Samples collected at deep-water sites with a sampler are composited in the field. If the sample was collected from a wading site, prepare a composite in the laboratory, as follows:
   a. Measure the tare weight of a clean, sterile, wide-mouthed jar.
   b. Using a sterile spatula, remove 50 g of bed sediment from each of the three replicate sample jars and place into the wide-mouthed composite jar.
   c. Mix the 150 g of sediment thoroughly.
3. Prepare an aliquot of composite bed sediment for proportional dry weight of sediment.
   a. Weigh a clean, dry, heat-tolerant glass or metal dish and record as "tare weight."
   b. Add approximately 25 g of composited sediment and record as "weight before drying."
   c. Place in an oven at 105°C. If an oven is not available, dry in a desiccator until a constant weight is obtained.
4. Elute bacteria from the sediment as soon as possible after compositing the sample.
   a. Place 20 g of the sediment composite into a bottle containing 200 mL of phosphate buffered water with magnesium chloride (U.S. Environmental Protection Agency, 2000). NOTE: If preparing a sample for split replicate analysis, increase the amount of sediment and buffered water appropriately. For example, place 30 g of the sediment composite into a bottle containing 300 mL of buffered water.
   b. Label the lid of this bottle with the time the bottle should be removed from the shaker (the bottle will be shaken for 45 minutes).
   c. Place the bottle on a wrist-action shaker.

d. After 45 minutes, remove the bottle from the shaker and let it stand for 30 seconds undisturbed. Pour off the supernatant into a new, labeled sterile bottle.

5. Analyze the supernatant using the membrane-filtration method or by the enzyme substrate MPN method. Autoclave the sediment and supernatant and discard.

   TECHNICAL NOTE: Supernatants commonly carry high concentrations of suspended sediments. In cases when the sediments in the supernatant clog membrane filters, the enzyme substrate in MPN format is recommended.

6. Remove the dish for proportional dry weight of sediment after 24 hours or until a constant weight is obtained.
   a. Record the constant weight obtained as "weight after drying."
   b. Use the following equation to calculate the proportional dry weight:

      Proportional dry weight = (Wdry - Wtare) / (Wwet - Wtare)

      where,

      Wtare = Tare weight of empty dish,

      Wwet = Weight of dish with wet bed sediment before drying, and

      Wdry = Weight of dish with bed sediment after drying.

For calculations based on colony count or enzyme substrate MPN tests for sediment samples: Densities of bacteria in sediment are reported as colony-forming units per gram of dry-weight sediment (CFU/gDW) or most-probable number per gram of dry-weight sediment (MPN/gDW).

Measure and calculate the sediment dilution factor.

- Sediment dilution factors are site specific and are determined by performing several displacement experiments of representative sediments.
- Example: based on beach sediments from Lake Erie (Francy and Darner, 1998), 20 g of dry or wet sediment displaced approximately 10 mL of water, so the total volume of the sediment/buffer mixture was 210 mL. The dilution factor for the sediment samples

To calculate CFU/gDW for membrane filtration results:

CFU/gDW = (density x dilution factor) / (proportional DW),

where density is the result in CFU/100 mL, dilution factor is the site specific dilution factor in mL/g, and DW is proportional dry weight of sediment (see section 7.1.3.B).

To calculate MPN/gDW for enzyme substrate MPN results:

MPN/gDW = (density x dilution factor) / (proportional DW),

where density is the result in MPN/100 mL, dilution factor is the site specific dilution factor in mL/g, and DW is the proportional dry weight of sediment.

Example 1: Calculation of results in terms of CFU per gram dry weight (Membrane filtration method)

Calculate proportional dry weight

Tare weight of empty dish (Wtare)	1.86 g
Weight of dish with wet bed sediment before drying (Wwet)	27.4 g
Weight of dish with bed sediment before drying (Wdry)	13.6 g

Proportional dry weight 0.46
(13.6 g - 1.86 g) / (27.4 g - 1.86 g)

Calculate density in supernatant

	Sample volume	Colony count
	3	7 (do not use)
	10	21
	30	101 (do not use)
Sum	10	21

Density in supernatant = (21 CFU / 10 mL) X 100 = 210 CFU/100 mL

Calculate density in sediment

Sediment dilution factor (calculated from site specific displacement experiments)
= 10.5 mL/g

Density in supernatant 210 CFU/100 mL
Proportional dry weight 0.46

Density in sediment 48 CFU/g $_{DW}$
(210 CFU/100 mL x 10.5 mL/g) / 0.46

[CFU, colony-forming unit]

Example 2: Calculation of results in terms of MPN per gram dry weight (Enzyme substrate method)

Calculate proportional dry weight

Tare weight of empty dish (Wtare)	1.86 g
Weight of dish with wet bed sediment before drying (Wwet)	27.4 g
Weight of dish with bed sediment before drying (Wdry)	13.6 g

Proportional dry weight 0.46
(13.6 g - 1.86 g) / (27.4 g - 1.86 g)

Calculate density in supernatant

Positive large wells 46
Positive small wells 10

Density in supernatant 150 MPN/100 mL (from MPN table)

Calculate density in sediment

Sediment dilution factor (calculated from site specific displacement experiments)
= 10.5 mL/g

Density in supernatant 150 MPN/100 mL
Proportional dry weight 0.46

Density in sediment 34 MPN/g $_{DW}$
(150 MPN/100 mL x 10.5 mL/g) / 0.46

[MPN, most probable number]