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IN REPLY REFER TO:

NATIONAL WATER QUALITY LABORATORY TECHNICAL MEMORANDUM 1999.08

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Subject: Method Change for the Determination of Phytoplankton Biomass

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SCOPE

The National Water Quality Laboratory (NWQL) historically has used USGS method B-6560-85 Biomass/Chlorophyll Ratio for Phytoplankton, (Britton and Greeson, 1989, p. 231-234) to determine chlorophyll and biomass in phytoplankton. In this method the chlorophyll and other organic materials are extracted before the biomass is determined. Biomass is determined by drying the extracted residue, weighing on a balance, ashing the residue, wetting, redrying, and reweighing. The dry weight and ash weight are reported to the data base. The customer then subtracts ash weight from dry weight to determine biomass.

Phytoplankton biomass is supposed to be the biomass from the algal community. When the samples are taken, the water is filtered through glass-fiber filters. The filters not only collect phytoplankton but also zooplankton, any plant material present, and organic carbon and inorganic salts contained in suspended sediments. This procedure can produce an inaccurate representation of the phytoplankton biomass if significant quantities of zooplankton and other carbonaceous materials are collected on the filter. This determination is more aptly titled "Plankton Biomass."

Method B-6560-85 has produced a significant low bias because the sample is extracted with diethyl ether before the biomass determination is started. The technique is also susceptible to analytical error because of the many steps in the procedure.

The Nineteenth Edition of Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1995) describes a more accurate method for the analysis of plankton biomass. The title of this method is "Determination of Biomass (Standing Crop)" and the method number is 10200I. In this method the plankton is collected on a filter, and the filter is dried

and weighed to determine "dry weight." The dried filter and plankton then are burned at 500oC, cooled and wetted, dried and reweighed to determine "ash weight." The Standard Methods 10200I (SM 10200I) method is procedurally the same as the USGS method for the analysis of periphyton biomass by method B-3520-85 (Britton, and Greeson, 1989, p. 139 and 140).

The two methods (USGS B-6560-85 and SM 10200I) were compared to determine bias and to describe its significance. The study concluded that the results for dry weight and ash weight produced by USGS method B-6560-85 were significantly lower than those produced by SM 10200I. The USGS method B-6560-85 results for biomass also were less than the SM method 10200I, but a paired T-test indicated that the bias was insignificant at the 0.05 significance level. An F-test also indicated that the difference in variability was insignificant at the 0.05 significance level.

The SM method 10200I will replace USGS method B-6560-85 because it is more accurate and less susceptible to analytical error than the USGS method. The labcode for dry weight by the SM method 10200I is 2190, the WATSTORE code is 81354, and the method code is B. The labcode for ash weight by the SM method 10200I is 2189, the WATSTORE code is 81353, and the method code is B.

The price of SM method 10200I is less than the price of USGS method B-6560-85 because it requires considerably less handling than the USGS method. The SM method 10200I will require that field personnel send a separate filter for phytoplankton chlorophyll determination and a separate filter for phytoplankton biomass determination.

The new method will produce less accurate results if small sample volumes are filtered. The NWQL recommends filtering sample volumes sufficient to produce 100 mg (milligrams) of wet sample on the filter to increase the accuracy of the method. One way to estimate when the filter contains an adequate amount of material is to continue passing sample through the filter until the filter flow-rate decreases by 50 percent or more.

BACKGROUND

Puerto Rico and Texas are the only districts to request phytoplankton biomass in the last 3 years. Puerto Rico always sends two filters for chlorophyll and biomass analysis. Only one filter was needed for the chlorophyll/biomass analysis by USGS method B-6560-85, so the other filter sample could be analyzed for biomass by SM method 10200I.

Sixty-four pairs of samples were analyzed, and the results were compared for dry weight, ash weight, and biomass (loss on ignition). Regression analyses were used to describe bias between the two methods, and T-tests were used to evaluate the significance of the biases. F-tests were used to evaluate the significance of the differences in variability between the two methods.

DISCUSSION

Dry Weight

The formula for the regression analysis used to compare dry weight for the two methods is

$$Y = 0.83X + 13.9$$

where Y = USGS method B-6560-85 results and

X = SM method 10200I results.

The slope of the regression line indicates that the USGS method will produce results that are about 17 percent less than the SM method 10200I. A paired T-test indicated that the bias is significant at the 0.05 significance level. An F-test demonstrated that the difference in variability between the two methods is insignificant at the 0.05 significance level. The correlation coefficient was 0.989, which is a strong indication of a predictable relation between the results from the two methods. The intercept from the regression equation suggests that the blank from the USGS method is about 14 mg/L (milligrams per liter) greater than the blank from the SM method 10200I. The mean dry weight from the analysis of five blanks was 14.2 mg/L, which supports the results of the regression analysis.

Ash Weight

The formula for the regression analysis used to compare ash weight for the two methods is

$Y = 0.83X + 13.4$

where Y = USGS method B-6560-85 results and

X = SM method 10200I results.

The slope of the regression line indicates that the USGS method will produce results that are about 17 percent less than the SM method 10200I. A paired T-test indicated that the bias is significant at the 0.05 significance level. An F-test demonstrated that the difference in variability between the two methods is insignificant at the 0.05 significance level. The correlation coefficient was 0.988, which is a strong indication of a predictable relation between the results from the two methods. The intercept from the regression equation suggests that the blank from the USGS method is about 13 mg/L greater than the blank from the SM method 10200I. The mean ash weight from the analysis of five blanks was 15 mg/L, which supports the results of the regression analysis.

Biomass

The ash weight is subtracted from the dry weight, and the loss on ignition is called biomass. The formula from the regression analysis used to compare biomass for the two methods is

$Y = 0.75X + 2.4$

where Y = USGS method B-6560-85 results and

X = SM method 10200I results.

The slope of the regression line indicates that the USGS method will produce results that are about 25 percent less than the SM method 10200I. However, a paired T-test indicated that the bias is insignificant at the 0.05 significance level. An F-test demonstrated that the difference in variability between the two methods is insignificant at the 0.05 significance level. The intercept from the regression equation suggests that the blank from the USGS method is about 2.5 mg/L greater than the blank from the SM method 10200I. The results from the analysis of four blanks showed that the mean USGS method blank was about 0.7 mg/L greater than the mean method blank from the SM method 10200I.

CONCLUSIONS

The USGS method produces results that are about 17 percent less than the results from the SM method 10200I for both dry weight and ash weight. These biases are statistically significant. The biomass results for the USGS method are biased low compared to the SM method 10200I, but this bias is not statistically significant.

The SM method 10200I will deliver more accurate results than the USGS method, with considerably less laboratory preparation. However, the SM method 10200I does require district personnel to filter two samples rather than one if chlorophyll determination is also needed. There is no statistically significant difference in the biomass of the two methods, and because of this result, the USGS

method will be replaced by the SM method 10200I, which requires less laboratory effort at a price savings for NWQL customers.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1995, Standard methods for the examination of water and wastewater (19th ed.): Washington, D.C., American Public Health Association, Inc., p. 10-25.

Britton, L.J., and Greeson, P.E., eds., 1989, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 363 p.

/Signed/
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