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Methods for the Determination of Chemical Substances in
Marine and Estuarine Environmental Matrices - 2nd Edition

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Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268**

DISCLAIMER

This manual has been reviewed by the National Exposure Research Laboratory - Cincinnati, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The National Exposure Research Laboratory - Cincinnati (NERL-Cincinnati) conducts research to:

- Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in marine and estuarine waters, drinking waters, surface waters, ground waters, wastewaters, sediments, sludges, and solid wastes.
- Investigate methods for the identification and measurement of viruses, bacteria, and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.
- Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in marine and estuarine waters, drinking waters, surface waters, ground waters, wastewaters, sediments, and solid wastes.
- Develop methods and models to detect and quantify responses in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on chemical and biological indicators.

This NERL-Cincinnati publication, "Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices - 2nd Edition" was prepared as the continuation of an initiative to gather together under a single cover a compendium of standardized laboratory analytical methods for the determination of nutrients, metals, chlorophyll and organics in marine matrices. It is the goal of this initiative that the methods that appear in this manual will be multilaboratory validated. We are pleased to provide this manual and believe that it will be of considerable value to many public and private laboratories involved in marine studies for regulatory or other reasons.

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ABSTRACT

This manual contains eleven methods for determination of nutrients, metals, and chlorophyll. Since Revision 1.0 appeared in 1992, four new methods have been added, one deleted and four have been multilaboratory validated. Methods 440.0, 445.0, 446.0 and 447.0 have been multilaboratory validated, and Method 353.4 has been replaced with an improved method.

The metals methods, Methods 200.10, 200.12 and 200.13 have not changed since the 1992 manual. Method 365.5 has remained the same and Method 440.0, that appeared in 1992, now contains multilaboratory validation data. Two new chlorophyll methods, Methods 446.0 and 447.0, have been added and all three chlorophyll methods have been multilaboratory validated. Since the chlorophyll methods validation study was also a comparison study of the methods, that data has been added to the methods. Anyone interested in obtaining a copy of the full chlorophyll study final report should contact the Chemical Exposure Research Branch office of NERL-Cincinnati.

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Method Number	Title	Revision	Multilab Validation Status
200.10	Determination of Trace Elements in Marine Waters by On-line Chelation Preconcentration and Inductively Coupled Plasma - Mass Spectrometry	1.6	No
200.12	Determination of Trace Elements in Marine Waters by Stabilized Temperature Graphite Furnace Atomic Absorption	1.0	No
200.13	Determination of Trace Elements in Marine Water by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption	1.0	No
349.0	Determination of Ammonia in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis	1.0	No
353.4	Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis	1.0	No
365.5	Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis	1.4	Yes
366.0	Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis	1.0	No

440.0	Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis	1.4	Yes
445.0	<i>In Vitro</i> Determination of Chlorophyll <i>a</i> and Pheophytin <i>a</i> in Marine and Freshwater Phytoplankton by Fluorescence	1.2	Yes
446.0	<i>In Vitro</i> Determination of Chlorophylls <i>a</i> , <i>b</i> , c_1+c_2 and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry	1.2	Yes
447.0	Determination of Chlorophylls <i>a</i> and <i>b</i> and Identification of Other Pigments of Interest in Marine and Freshwater Algae Using High Performance Liquid Chromatography with Visible Wavelength Detection	1.0	Yes

ACKNOWLEDGMENTS

This manual is dedicated to the memory of Dr. Barbara Metzger, late Director of the Environmental Services Division of USEPA Region 2. She was the impetus and driving force for this work.

This manual was prepared by the Chemical Exposure Research Branch of the Microbiological and Chemical Exposure Assessment Research Division, NERL-Cincinnati. The metals and chlorophyll methods were authored by in-house scientists and the nutrient methods were authored under contract by Carl Zimmermann and Carolyn Keefe at the Chesapeake Biological Laboratory, University of Maryland and under an interagency agreement by Dr. Jia-Zhong Zhang, National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Ocean Chemistry Division. Dr. Zhang deserves recognition for the outstanding efforts he put into making these methods both informative and practical.

Special thanks go out to Dr. Margo Hunt of USEPA Region 2 for staying so involved in the chlorophyll methods study. The need to standardize analytical methods for use in the marine environment was identified and championed by the USEPA regions. The staff at Regions 2 and 3 were instrumental in identifying resources for this project. They provided insight from the regional perspective and served as technical advisors. Their input has been valuable.

Diane Shirmann and Helen Brock put a tremendous effort into preparing this manuscript and we are extremely thankful for their hard work.

INTRODUCTION

Since the first edition of this manual was published in 1992, the Environmental Monitoring Systems Laboratory (EMSL) has been reorganized and its name changed to the National Exposure Research Laboratory (NERL). The principal aim of this manual is to bring together under one cover a suite of analytical methods specifically adapted or developed for the examination of coastal and estuarine environmental samples. Many of the methods presented here are adaptations of analytical techniques which, for many years, have been used routinely by the marine community. Hallmarks of the methods which appear in this manual, however, are the integrated quality control/quality assurance requirements, the use of standardized terminology and the Environmental Monitoring Management Council (EMMC) format. The mandatory demonstration of laboratory capability and the continuing checks on method performance ensure the quality and comparability of data reported by different laboratories and programs. Another distinction of this manual is the multilaboratory validation data for many of the methods.

Multilaboratory validation studies test the ruggedness of methods, provide single-analyst and multilaboratory precision and accuracy statements, and method detection limits that are "typical" of what most laboratories can achieve. Methods that reach this level of evaluation have been thoroughly investigated to the fullest extent possible by a single laboratory and have usually been informally adopted as standard methods by the analytical community. When a method does not perform as expected in a multilaboratory study, it must be returned to the development phase. For example, although widely accepted and routinely used in the marine community, Method 353.4 (Determination of Nitrite + Nitrate in Estuarine and coastal Waters by Automated Colorimetric Analysis) failed the ruggedness test in 1992 when 50% of the participating laboratories in the multilaboratory study returned unacceptable data. Review of the data suggested that the cadmium reduction column chemistry and maintenance required further investigation. The method was subsequently reevaluated by Dr. Jia-Zhong Zhang, under an Interagency Agreement between the U.S. EPA and NOAA. The new nitrite/nitrate method is improved in technical detail and QA/QC requirements.

We are pleased to present this 2nd Edition manual to the public and to research and monitoring labs in the hope that it contributes to better protection and preservation of our estuarine and coastal ecosystems.

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