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DETERMINATION OF SOLUBLE HUMIC SUBSTANCES (HUMIC ACID, FULVIC ACID & SALTS) IN WATER BY UV-VISIBLE SPECTROPHOTOMETRY

Authored by:

Mu-Hao Sung Wang, Lawrence K. Wang, Betty C. Wu
LENOX INSTITUTE OF WATER TECHNOLOGY
Auburndale, MA 02466, USA
Lenox.Institute@gmail.com

Geng Yuan Hu
ZHEJIANG UNIVERSITY
Hang Zhou, 310027, China

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ABSTRACT

An analytical procedure has been developed for quantitative determination of humic substances which are common agricultural and gardening products. The residual of humic substances in surface water become the undesirable trihalomethane (THM) precursors to people and chronic toxic substances to aquatic plants and animals. Removal and analysis of humic substances are important tasks of environmental researchers. The principle of humic substances is similar to that of tannin and lignin analysis except that the two analytical methods use different analytical instruments. The principle is that soluble humic substances, such as humic acid, fulvic acid and their salts, reduce the tungstophosphoric and molybdophosphoric acids to produce a grayish color up to at least 20 mg/L at UV wavelength 265-400 nm. The grayish color substance can be quantitatively measured with a UV-Visible Spectrophotometer. This publication is one of the authors' memoirs.

KEYWORDS

Memoir, Lenox Institute of Water Technology, Zhejiang University, Call for Further Research, Environmental Chemistry, Water Quality, Analytical Method, Humic Acids, Fulvic Acids, Humic Substances, Tannin, Lignin, Trihalomethane (THM) Precursor , UV-Visible Spectrophotometer

ACRONYM

HS:	**Humic substances**
LIWT:	Lenox Institute of Water Technology
NSF:	National Science Foundation
APHA:	American Public Health Association
AWWA:	American Water Works Association
MSDS:	Material Safety Data Sheet
WEF:	Water Environment Federation
WPCF:	Water Pollution Control Federation
THM:	Trihalomethane
UV:	Ultraviolet

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and Geng Yuan Hu

1. . INTRODUCTION

1.1 Environmental Engineering Significance

Soluble humic substances (including humic acid and fulvic acid) are natural products resulting from biological decomposition of organic substances by soil microorganisms over a prolonged period of time. They are also very common agricultural farming and gardening products. The soluble humic substances may also enter the water supply through either the natural organic degradation process or man-made non-point sources of water pollution. [1]

In natural water systems, humic and fulvic acids are water pollutants, known as the trihalomethane precursors. or aquatic chronic toxic substances. Many environmental engineers and chemists have devoted themselves to removing them from water.

Humic substances all react with tungstophosphoric and molybdophosphoric acids to form a color complex. However, the reaction is not specific for a specific humic substance (humic acid, fulvic acid, and their salts) inasmuch as other reducing materials respond similarly.

The nature of the substance suspected in the water sample will dictate the choice of humic acid, fulvic acid, or humic substances in general for use in the preparation of the standard solution. This is necessary because it is impossible to distinguish among hydroxylated aromatic compounds.

Unless humic acid, fulvic acid or humic substances is definitely known to be present, the results of this determination logically may be reported in the more general terms of "humic acid-like" or simply as "hydroxylated aromatic compounds. [2]

1.2 Acknowledgment

This research was supported by the National Science Foundation, Division of Civil and Mechanical Engineering, Washington, D.C., USA, under a research grant No. CME-8014203. Professor Lawrence K. Wang served as the Project Manager.

The analytical method for determination of humic substances was jointly developed and experimentally evaluated by both the Lenox Institute of Water Technology (formerly Lenox Institute for Research, Massachusetts, USA. and Professor Geng-Yuan Hu of Chemistry Department, Zhejiang University, China. The evaluation results were fully documented in a government report [2].

2. GENERAL DISCUSSION

2.1 Principle

Soluble humic substances reduces the same tungstophosphoric and molybdophosphoric acids to produce a grayish color up to at least 20 mg/L (in terms of humic acid) at UV wavelength 265-400 nm. The grayish color substance can be quantitatively measured with a UV-Visible Spectrophotometer.

2.2 Interference

Such reducing substances as 2 mg ferrous iron/L and 125 mg sodium sulfite/L individually produce a color equivalent to 1 mg humic acid/L.

2.3 Minimum Detestable Concentration

Approximately 0.2 mg/L for soluble humic substances.

3. APPARATUS

An UV/Visible Spectrophotometer is needed. It is for use at 265-400 nm for soluble humic substances determination, and at 600-700 nm for tannin and lignin determinations. A light path of 1 cm or longer yields satisfactory results.

4. REAGENTS

4.1 Tungstate-Molybdate Reagent

Transfer 100 g sodium tungstate, $Na_2WO_4*2H_2O$, and 25 g sodium molybdate, $Na_2MoO_4 *2H_2O$, together with 700 mL distilled water, to a 2,000-ml flat-bottom boiling flask. Add 50 mL 85% H_3PO_4 and 100 mL concentrated hydrochloric acid HCl. Connect to a reflux condenser and boil gently for 10 hr. Add 150 g Li_2SO_4, 50 mL distilled water, and a few drops of liquid bromine. Boil without condenser for 15 min to remove excess bromine. Cool to 25 °C, dilute to 1 L and filter. Store finished reagent, which should have no greenish tint, in a tightly stoppered bottle to protect against reduction by dust and organic materials.

4.2 Carbonate-Tartrate Reagent

Dissolve 200 g sodium carbonate Na_2CO_3 and 12 g sodium tartrate, $Na_2C_4H_4O_6*2H_2O$, and 750 mL hot distilled water, cool to 25°C, and dilute to 1 L.

4.3 Stock Solution

Weigh 1.000 g soluble humic substances, humic acid, or fulvic acid being used for agricultural farming, gardening, or other applications, or known to be a contaminant of the water sample. Dissolve it (i.e. reagent grade humic acid, fulvic acid, or their salts) in distilled water and dilute it to 1,000 mL.

4.4 Standard Solution

Dilute 10.00 mL or 50.00 mL stock solution to 1,000 mL with distilled water: 1.00 mL = 10.0 or 50.0 ug, respectively, active ingredient.

5. PROCEDURE

Bring 50 mL clear sample and standards to a temperature above 20°C and maintain within a ±2°C range. Add in rapid succession 1 mL tungstate-molybdate reagent and 10 mL carbonate-tartrate reagent. Allow 30 min for color development. Compare visually against simultaneously prepared standards or make UV-visible spectrophometric readings against a reagent blank prepared at the same time. Use the following guide for the instrumental measurements in the suggested wavelength regions assuming the humic acid analysis in 61-mL final volume:

1. 50-1000 ug humic acid uses 1 cm light path at 265-400 nm wavelength
2. 10-500 ug humic acid uses 5 cm light path at 265-300 nm wavelength

6. TECHNICAL DISCUSSIONS

6.1. Presence in the Environment and Applications of Humic Acid , Fulvic Acids, and Their Salts

Soluble humic substances (including humic acid and fulvic acid) are natural products resulting from biological decomposition of organic substances by soil microorganisms over a prolonged period of time.

Figures 1 and 2 show that the humic substances are wildly used for agricultural farming and gardening applications, and can be purchased in many stores, such as Home Depot, Walmart, Lows, or from online, such as Amazon, eBay. Their common presence in the natural water causing water pollution is obvious, and they should be removed, and monitored.

Inexpensive regular commercial products (Examples: the products shown in Figures 1 and 2 or equivalent) can be used for treatability research.

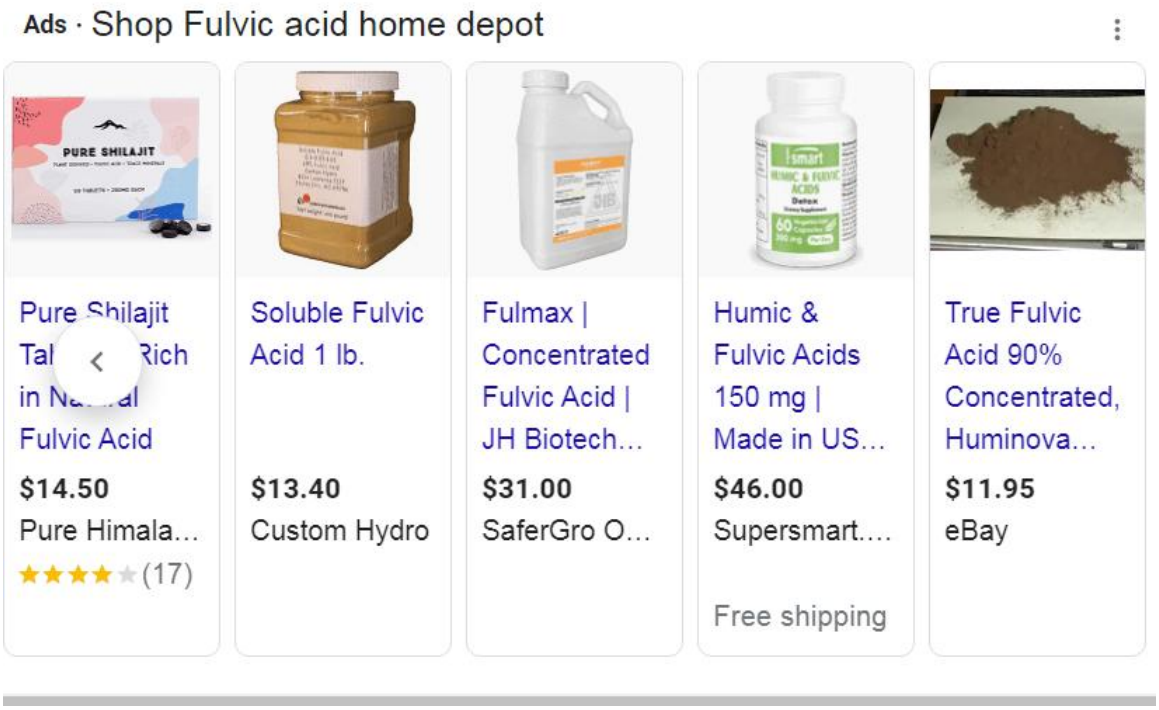

Figure 1. Availability of Fulvic Acid and Related Products.

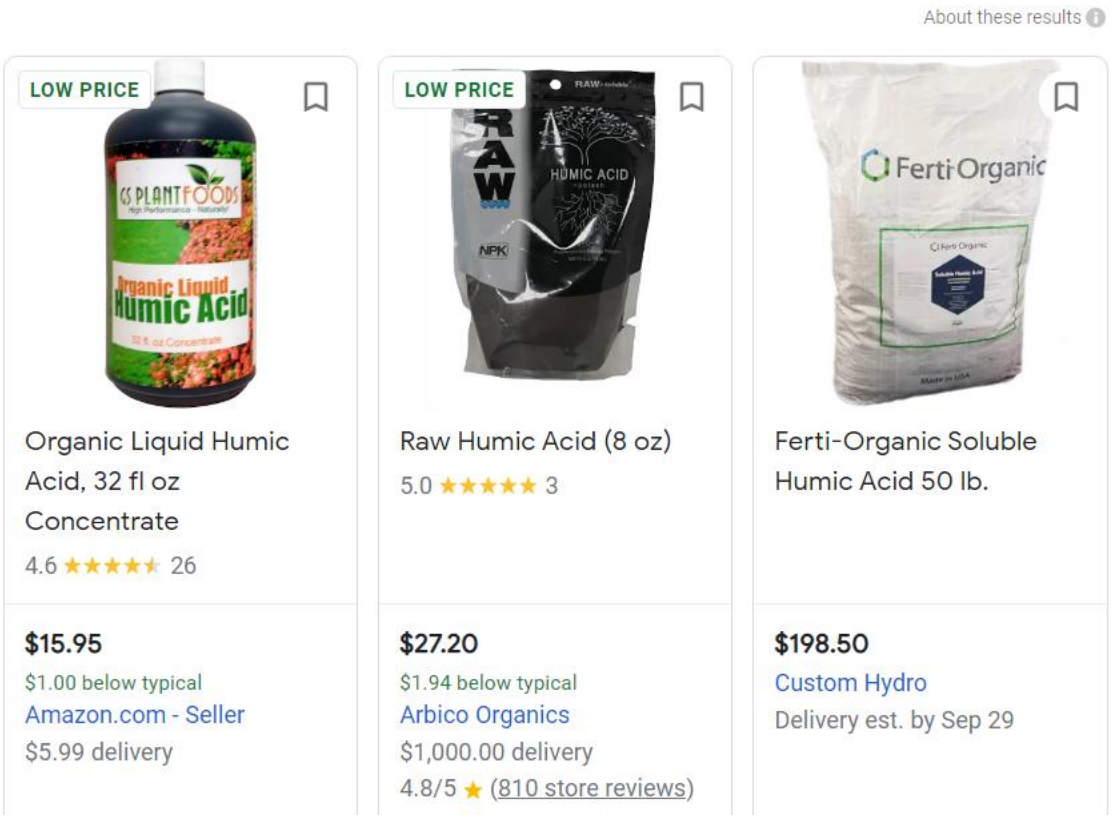

Figure 2. Availability of Humic Acid and Related Products.

6.2. Chemical Formula, Reagent Grade and Material Safety Data Sheets of Humic Substances

Figure 3 and Figure 4 are the molecular structures of fulvic acids and humic acid (sodium salt), respectively.. Figure 5 and Figure 6 are the Material Safety Data Sheets (MSDS) of fulvic acid and humic acid , respectively. The reagent grade of both chemicals can be purchased from Sigma-Aldrich, Fisher Scientific, etc.

Only the reagent grade chemicals (Examples: the products shown in Figures 5 and 6 or equivalent) should be used for preparation of the standard solutions in this analytical method.

6.3 Selected Reagent Grade Chemicals for This Research

Figure 7 shows the selected chemical (humic acid, sodium salt, with a chemical formula of $C_9H_8Na_2O_4$ for preparation of the Standard Solutions in this research.

Humic acid sodium salt is a complex mixture of multiple acids, which functions as a dibasic or tribasic acid. This acid mixture is usually found in dead organic matter via biodegradation It is known that humic acid sodium salt is important in regulating the bioavailability of metal ions as a result of its chelating properties to metal ions.

Figure 8 shows another chemical (fulvic acid) with a chemical formula of $C_{14}H_{12}O_8$ for preparation of the Standard Solutions of fulvic acid.

Any chemical suppliers' reagent grade chemicals can be used.

6.4 Recommendations

The newly developed analytical method for humic substances determination is very similar to the existing analytical methods [3-7] for tannin and lignin determinations in terms of chemistry. The major difference is that the current Standard Methods [6] for tannin and lignin determination is the colorimetry using Nessler Tubes, photometer, or regular spectrophotometer at the wavelength range of 600-700 nm; while the new humic substances determination method requires the UV-Visible Spectrophotometry to be controlled at the wavelength range of 265-400 nm.

It is recommended that the two analytical methods be combined together.

The authors invite the researchers around the world to continuously study this proposed new method.

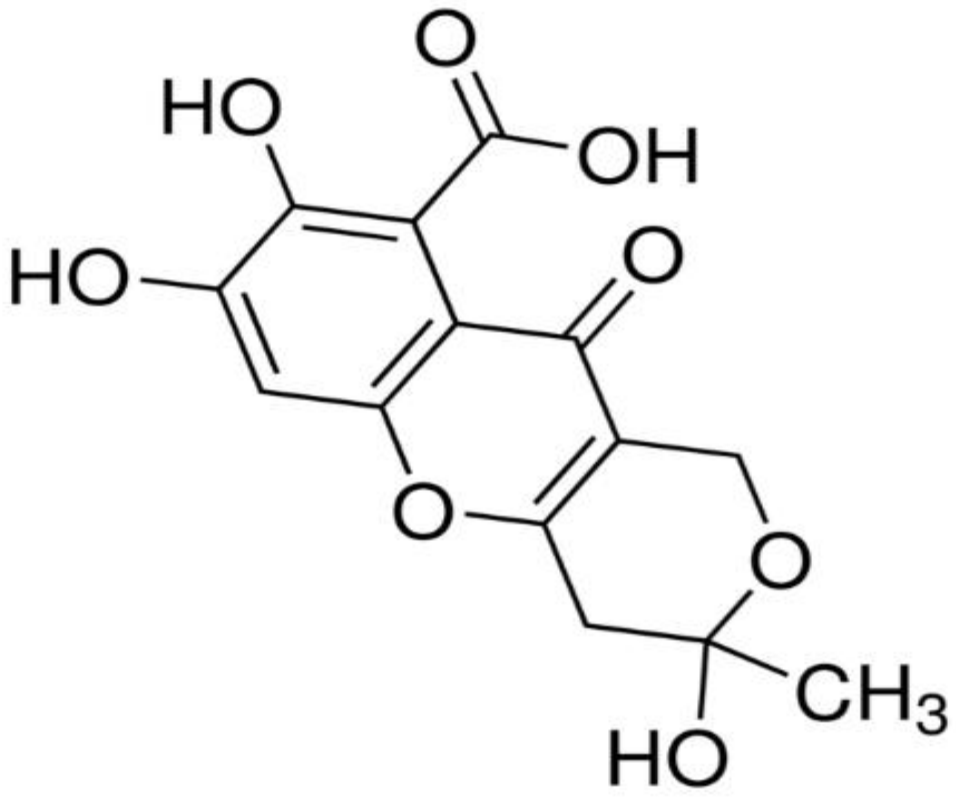

Figure 3. Molecular Structure of Fulvic Acid

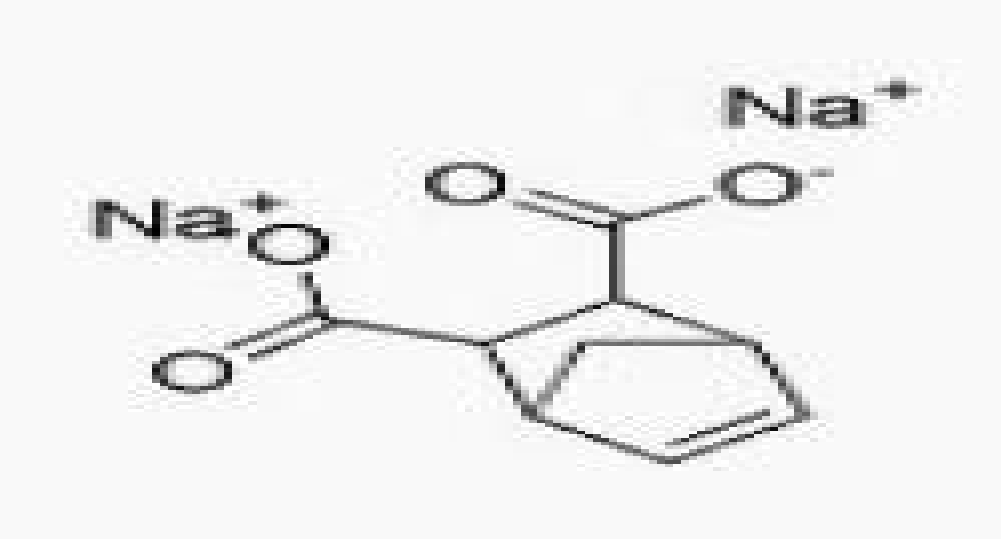

Figure 4. Molecular Structure of Humic Acid - Sodium Salt

(Source: Santa Cruz Biotechnology, Inc.)

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Fulvic acid

Product Number : CDS025195
Brand : Aldrich

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Inc.
3050 SPRUCE ST
ST. LOUIS MO 63103
UNITED STATES

Telephone : +1 314 771-5765
Fax : +1 800 325-5052

1.4 Emergency telephone

Emergency Phone # : 800-424-9300 CHEMTREC (USA) +1-703-527-3887 CHEMTREC (International) 24 Hours/day; 7 Days/week

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Short-term (acute) aquatic hazard (Category 2), H401
Long-term (chronic) aquatic hazard (Category 2), H411

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Figure 5. Material Safety Data Sheet of Fulvic Acid

(Source: Sigma-Alrich, Inc.)

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Humic acid

Product Number : 53680
Brand : Aldrich
CAS-No. : 1415-93-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Inc.
3050 SPRUCE ST
ST. LOUIS MO 63103
UNITED STATES

Telephone : +1 314 771-5765
Fax : +1 800 325-5052

1.4 Emergency telephone

Emergency Phone # : 800-424-9300 CHEMTREC (USA) +1-703-527-3887 CHEMTREC (International) 24 Hours/day; 7 Days/week

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

Figure 6. Material Safety Data Sheet of Humic Acid

(Source: Sigma-Alrich, Inc.)

Figure 7. Recommended Humic Acid Sodium Salt For Preparation Of Standard Solutions (Source: Santa Cruz Biotechnology, Inc.)

Fulvic acid (CAS 479-66-3)

Alternate Names:	Fulvic acid is known as a colloidal poly-electrolyte.
Application:	Fulvic acid is a naturally occurring electrolyte that may enhance nutrient availability and adsorption, and prolong its residence.
CAS Number:	479-66-3
Purity:	>95%
Molecular Weight:	308.24
Molecular Formula:	$C_{14}H_{12}O_8$

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Figure 8. Recommended Fulvic Acid For Preparation Of Standard Solutions (Source: Santa Cruz Biotechnology, Inc.)

GLOSSARY

Humic substances (HS): They are organic compounds, including humic acid, fulvic acid, their salts, etc. that are important components of humus, the major organic fraction of soil, peat, and coal.

Spectrophotometry: It is a quantitative analytical method to determine how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution in a cell. Each chemical compound absorbs or transmits light over a certain range of wavelength.

Ultraviolet-visible (UV-Vis) spectrophotometry: It is an analytical technique used to measure light absorbance of chemical substance across the ultraviolet and visible ranges of the electromagnetic spectrum across a wavelength range of 190 – 840nm.

UV-Vis spectrophotometer: It is an instrument used to determine the concentration of specific analytes in a microvolume by controlling the analysis wavelengths (190-840 nm) and the pathlength. The amount of light absorbed by the instrument is directly proportional to the concentration of the sample and the distance the light travels through the sample (the pathlength).

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APPENDIX:

INTRODUCTION OF THE EDITORS OF ENVIRONMENTAL SCIENCE, TECHNOLOGY, ENGINEERING AND MATHEMATICS (STEM) SERIES

1. Editor Lawrence K. Wang

Editor Lawrence K. Wang has served the society as a professor, inventor, chief engineer, chief editor and public servant (UN, USEPA, New York State) for 50+ years, with experience in entire field of environmental science, technology, engineering and mathematics (STEM). He is a licensed NY-MA-NJ-PA-OH Professional Engineer, a certified NY-MA-RI Laboratory Director, a licensed MA-NY Water Operator, and an OSHA Instructor. He has special passion, and expertise in developing various innovative technologies, educational programs, licensing courses, international projects, academic publications, and humanitarian organizations, all for his dream goal of promoting world peace. He is a retired Acting President/Professor of the Lenox Institute of Water Technology, USA, a Senior Advisor of the United Nations Industrial Development Organization (UNIDO), Vienna, Austria, and a former professor/visiting professor of Rensselaer Polytechnic Institute, Stevens Institute of Technology, University of Illinois, National Cheng-Kung University, Zhejiang University, and Tongji University. Dr. Wang is the author of 750+ papers and 50+ books, and is credited with 29 invention patents. He holds a BSCE degree from National Cheng- Kung University, Taiwan, ROC, a MSCE degree from the University of Missouri, a MS degree from the University of Rhode Island and a PhD degree from Rutgers University, USA. Currently he is the book series editor of CRC Press, Springer Nature Switzerland, Lenox Institute Press, World Scientific Singapore, and John Wiley. Dr. Wang has

been a Delegate of the People to People Internatonal Foundation, a Diplomate of the American Academy of Environmental Engineers, a member of ASCE, AIChE, ASPE, WEF, AWWA, CIE and OCEESA, and a recipient of many US and international engineering and science awards.

2. Editor Mu-Hao Sung Wang

Editor Mu-Hao Sung Wang has been an engineer of the New York State Department of Environmental Conservation, an editor of CRC Press, Springer Nature Switzerland, and Lenox Institute Press, and a university professor of the Stevens Institute of Technology, National Cheng-Kung University, and the Lenox Institute of Water Technology. Totally she has been a government official, and an educator in the USA and Taiwan for over 50 years. Dr. Wang is a licensed Professional Engineer, and a Diplomate of the American Academy of Environmental Engineers (AAEE). Her publications have been in the areas of water quality, modeling, environmental sustainability, solid and hazardous waste management, NPDES, flotation technology, industrial waste treatment, and analytical methods. Dr. Wang is the author of over 50 publications and an inventor of 14 US and foreign patents. She holds a BSCE degree from National Cheng-Kung University, Taiwan, ROC, a MS degree from the University of Rhode Island, RI, USA, and a PhD degree from Rutgers University, NJ, USA. She is the Co-Series Editor of the Handbook of Environmental Engineering series (Springer Nature Switzerland), Coeditor of the Advances in Industrial and Hazardous Wastes Treatment series (CRC Press of Taylor & Francis Group) and the Coeditor of the Environmental Science, Technology, Engineering and Mathematics series (Lenox Institute Press). She is a member of AWWA, NYWWA, NEWWA, WEF, NEWEA, CIE and OCEESA.

3. Editor Yuriy I. Pankivskyi

Editor Yuriy I. Pankivskyi has 25 years of professional experience of scientific research and environmental education. He has expertise in strategic environmental assessment, environmental impact assessment, drinking water treatment, waste waters treatment, water and air pollution control, solid waste management. He works as environmental consulting engineer for industrial enterprises, state administrations of cities and towns of Western Ukraine, communities, private firms and institutions and as researcher, educator for state universities. He is the Associate Professor and Deputy Head of Department of Ecology of Ukrainian National University of Forestry. His research and publications have been in areas of water and air quality control, waste water treatment, environmental sustainability and education, analytical methods, investigations of multifunctional material for optoelectronics and environment testing. Dr. Pankivskyi is author of over 70 scientific publications. He earned his Specialist degree from Lviv State Ivan Franko University (Ukraine), ME degree from Lenox Institute of Water Technology (MA, USA), and his PhD degree from Lviv National Ivan Franko University (Ukraine). He is a member of National Ecological Center of Ukraine (Lviv Department).