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**Marine technology — Marine  
environment impact assessment  
(MEIA) — On-board bioassay to  
monitor seawater quality using  
delayed fluorescence of microalga**



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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organisations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 8, *Ships and marine technology*, Subcommittee SC 13, *Marine technology*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

Mining of offshore mineral resources has attracted much interest. These resources can be utilized as potential mineral resources. However, deep-sea mining of the seafloor can pose potential hazards to deep-sea environments and ecosystems (see ISO 10253 and References [3], [19], [21]). One concern is the toxicity of heavy metals released from excavated minerals. Such heavy metals can be released into the seawater of the deep marine ecosystem<sup>[2],[18]</sup>. Further, there is a risk of unexpected leakage of the recovered minerals and mining wastewater from the mining plant, which can result in heavy metal contamination of the surface seawater<sup>[8]</sup>.

Considering the above, an appropriate scheme for the monitoring and evaluation of the quality of deep and surface seawater can ideally be introduced at each deep-sea mining site. The International Seabed Authority (ISA) states that environmental impact assessments should address not only areas directly affected by mining, but also the wider region impacted by discharged plume and materials released during mineral transport to the surface<sup>[8]</sup>.

An on-board or onsite method for heavy metal evaluation is essential, as it would allow prompt action in case of an unexpected pollution incident. Rapid evaluation of the nature and extent of pollution provides an opportunity to prevent wider spread of the toxic contaminants and, consequently, minimize idle periods of a mining plant.

Although many chemical analytical methods are available at land-based laboratories, few methods have been developed for on-board application. Deep-sea mineral deposits are inhomogeneous and can be the source of release of various types of metal elements. Therefore, evaluation of mining contaminants requires simultaneous analysis of multiple elements. Special instruments are needed to perform such analyses, such as inductively coupled plasma mass spectrometry. Further, these instruments have to be operated by expert staff, require considerable laboratory space and are expensive to install. Such instrument types are difficult to install at every mining site as standard equipment for environmental monitoring.

Bioassays constitute an alternative approach to specialist equipment, and are commonly used to assess ecological risks of chemical contaminations (see ISO 10253). Bioassays do not provide quantitative information about the contaminating substances, but can be used to detect a wide spectrum of toxicants, including unknown toxicants. This feature is advantageous for the monitoring of water quality during deep-sea mining activities.

General bioassay test protocols that use a variety of aquatic organisms have been published by organisations, such as ISO (see ISO 10253), the Organization for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (US-EPA). These authorized protocols are accepted in various water quality management fields. However, similarly to chemical analyses, they require a considerable amount of time and space, and are thus not suitable for on-board monitoring. It should also be noted that most protocols have been developed for inland freshwater quality assessments.

This document was developed to address the shortcomings of the currently available bioassays for monitoring seawater quality on-board. It describes a bioassay specifically for on-board determinations.



# Marine technology — Marine environment impact assessment (MEIA) — On-board bioassay to monitor seawater quality using delayed fluorescence of microalga

## 1 Scope

This document specifies a bioassay for the determination of the presence of unknown toxic contaminants in test seawater (see [Figure A.1](#)). It is based on the inhibition of photosynthetic activity of the marine cyanobacterium *Cyanobium* sp. (NIES-981) by such toxic contaminants. The inhibition is determined based on delayed fluorescence (DF) intensity.

The method is rapid and requires less laboratory space than standard bioassays. Hence, it can be used on-board to generate basic data for seawater quality management at deep-sea mining sites where time and space are extremely limited.

## 2 Normative references

There are no normative references in this document.

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

### 3.1

#### **delayed fluorescence**

##### **DF**

#### **delayed light emission**

weak fluorescence signal from photosynthetically active cells that originates upon repopulation of the excited energy states of chlorophyll by stored energy after charge separation

### 3.2

#### **DF decay curve**

time-course change of the *DF* ([3.1](#)) intensity of *test algae* ([3.7](#)) that had been left in darkness after exposure to light

Note 1 to entry: See [Annex E](#), [Table E.1](#) and [Figure E.1](#).

### 3.3

#### **effective concentration**

##### **EC<sub>x</sub>**

concentration of test substance that results in an x % reduction in specific growth rate relative to the controls

### 3.4

#### **no observed effect concentration**

##### **NOEC**

tested concentration below the *LOEC* ([3.5](#)) that has no statistically significant effect