Installation of new monitoring technology
新しい監視技術の導入
- Seawater quality monitoring system using bioassay and others –
－バイオアッセイ等による海洋汚染モニタリングシステムー

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Hydrothermal fields, seafloor massive sulfide (SMS) deposits, are one of the main mining targets.

**Surface environment**

- **Transport ship**
- **Mother ship**

**Deep sea environment**

- **Lifting**
- **Returning water**
- **Seafloor disturbance**

Photos from JOGMEC news (2015)
Processes of deep sea mining and EIA (Environmental Impact Assessment)

Exploration → Exploitation → Closing

EIA

Guidelines

Technical protocols & methods

Onboard bioassay

**Targets:** Ocean surface environment

- Test organisms
- Easy, quick, compact, reliable methods for monitoring water quality

Leaching tests for SMS and those effects on the phytoplankton
Hydrothermal fields, seafloor massive sulfide (SMS) deposits, are one of the main mining targets.
EIA of sea surface is required?!

ISBA/19/LTC/8 (2013, Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area)

Activities requiring environmental impact assessment:

✓ 22. The impact assessment should address not only areas directly affected by mining but also the wider region impacted by near-bottom plumes, the discharge plume and material released by transporting the minerals to the ocean surface, depending on the technology used.

✓ 25. A discharge plume in surface water may interfere with primary productivity by increasing nutrient levels and decreasing light penetration into the ocean.

Annex I Explanatory commentary

✓ 37. If there is potential for surface discharge, the plankton community in the upper 200 m of the water column should be characterized. Depending on plume modelling studies, it may be necessary to study plankton communities, especially gelatinous plankton, over a wide depth range.

✓ 38. A combination of monitoring and shipboard and laboratory experimentation may be necessary to resolve, prior to test mining, potential ecotoxicological impacts, including possible impacts on phytoplankton and zooplankton if the discharge plume occurs at the sea surface or in mid-water.
3.3 Ecotoxicology

✓ Knowledge on the ecotoxicological limits of deep-water species to certain chemicals (or mixtures) is helpful to assess their tolerances and define the limits of ecotoxicological impact from a mining site.

✓ Rapid assessment of ore and plume toxicity on board the survey/support vessel is recommended with an approved assay during both the exploration phase and the exploitation phase.

MIDAS (2016, report on the implications of MIDAS results for policy makers with recommendations for future regulations to be adopted by the EU and the ISA)

政策立案や将来の規制を目標としたEUのMIDASの報告書 (2016)
Useful tool for water quality management, “Bioassay” “Toxic or Non Toxic”

**Example of the international certified test organisms**

- **Fish embryo toxicity**
  - OECD TG203
- **Growth inhibition test, green alga**
  - OECD TG201
- **Reproduction test, Daphnia**
  - OECD TG202

**General bioassay steps for waste water on land (example of WET system in US)**

1. Take the Sample
2. Run the tests
3. Record the biological response (data)
4. Analyze the data
5. Make a Decision

- **NOEC**: Non-observed effect concentration
- **LC50**: Lethal concentration for 50%
- **IC25**: Inhibition concentration for 25%, etc...

- ✓ Bioassay detect not only target toxicants but also unexpected toxic compounds.
- ✓ But cannot identify the chemical composition and concentrations of the contaminants.
- ✓ Need large space and long time.
Bioassay example for seafloor mining

Bioavailability and Chronic Toxicity of Metal Sulfide Minerals to Benthic Marine Invertebrates: Implications for Deep Sea Exploration, Mining and Tailings Disposal

EC_{50} (Median Effect Concentration) = 15-20 ppb

- Response of the invertebrates are known to be very sensitive to toxicants well, but...
- Are the test organisms such as amphipod suitable for onboard use?
- Need long periods to identify the toxic effects, and large space to keep the organisms.
Leachates from SMS affect on phytoplankton

Various pico-phytoplankton, dominates in this region

Leaching test from SMS

Primary producers in food chain

FCM analysis of microbial community before and after leachate exposure

0 hours
Before addition

Addition of leachate (0.6%) from Ore

24 hours
Only 5% cells remained

熱水域コア試料を用いた洋上溶出試験（好気／嫌気、異なる温度条件）
**Chimney minerals (Okinawa Trough)**

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**Hydrothermal deposits (Okinawa Trough)**

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- **High content in minerals and high release**
- **High content in minerals but low release**
- **Low content in minerals but high release**
- **Low content in minerals and low release**

### Various metals can be released randomly

**Leachate ≠ ore chemical ratio**

- Yellow iron pyrite: FeS₂
- ZnS: Sphalerite
- PbS: Galena
Effects of metals on pelagic phytoplankton strains
様々な外洋性植物プランクトンに対する重金属等の影響調査

NIES collection
Covering various pelagic species

Detection of vulnerable groups, useful strains for ecotoxicological approach.
Useful data for model-based studies for prediction/evaluation.

Prochlorococcus sp. NIES-2882
EC$_{50}$ (median effect concentration) = ____ ppb
To overcome drawbacks in existing analytical and ecotoxicological technologies, we are newly developing an onboard bioassay system for monitoring mining sites.

Selection of a high sensitivity test organism from oceanic pico-phytoplankton

We performed screening proper strains from NIES culture collection, and chose a strain *Cyanobium* sp. (NIES-981) originally isolated from Okinawa.

- *Cyanobium* is closely related to genera *Synechococcus* / *Prochlorococcus* known as major primary producer in marine environment.
- Response of *Cyanobium* might be a proxy of that of oceanic picoplankton.

- Easy handling, good growth rate, stably culturable & cryopreservable
- For Cu, lower EC$_{50}$ than the other marine algae previously reported.

<table>
<thead>
<tr>
<th>Strain number</th>
<th>NIES-981</th>
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<tbody>
<tr>
<td>Species</td>
<td><em>Cyanobium</em> sp.</td>
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<tr>
<td>Origin</td>
<td>Irionote Isl., Okinawa, Japan</td>
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<td>Habitat</td>
<td>Marine</td>
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<td>Conditions</td>
<td>Axenic, Unialgal, Clone</td>
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<td>Others</td>
<td>Full genome sequence</td>
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<th>CuSO$<em>4$ EC$</em>{50}$ (mg/L)</th>
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<tr>
<td><em>Cyanobium</em></td>
<td>1.53</td>
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<td><em>Prorocentrum minimum</em></td>
<td>13.5</td>
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<td><em>Tetraselmis suecica</em></td>
<td>40</td>
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<td><em>Dunaliella salina</em></td>
<td>220</td>
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<tr>
<td><em>Heterocapsa triquetra</em></td>
<td>7</td>
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</table>

- Available from NIES microbial culture collection with minimum costs (http://mcc.nies.go.jp).
General bioassay protocol with algae: required space & long incubation time

国際標準的なバイオアッセイはスペース等が必要,,,洋上では不向き！?

Standards method of algal inhibition test needs experts, space and long testing time!

Protocol of the OECD TG201

- Pre-culture: -72h ~ -48h
- Exposing: 0h, 24h, 48h, 72h

Inhibition testing at NIES using Pseudokirchneriella subcapitata

1 week incubation, cell counting

Incubator and bigger sample volume (200-500ml)

Need expert maintenance!
Medium, inoculation, keep axenic state.

Culture management at laboratory
To overcome the drawback of time and space in algal bioassay protocol, "Delayed Fluorescence" measurement with smaller test sample is adopted.

簡便なバイオアッセイ法の開発 範遅延発光計測による毒性評価結果は標準試験法と同等の結果

5-10ml sample volume
Compact & easy operation

DF decay curves

Analysis time (sec) vs. Fluor Intensity

Concentration vs. Inhibition (%)

Ex.) DF decay curves

15 min – 24h incubation

Rapid evaluation
Highly corresponding to the standard method

Delayed fluorescence (DF): delayed light emission, very weak fluorescence that is emitted by photosynthetically active cells, and measured as a delay of milliseconds to minutes after cells are transferred from light to dark conditions, used as a sensitive index of photosynthetic activity, corresponding to growth inhibition test.
## Comparison of instruments for chemical analysis with DF bioassay system

<table>
<thead>
<tr>
<th>Methods</th>
<th>Performance</th>
<th>Initial cost</th>
<th>Pros and cons</th>
</tr>
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<tbody>
<tr>
<td>ICP-MS (Inductively Coupled Plasma Mass Spectrometry)</td>
<td>Multi-elements, High sens &amp; high throughput</td>
<td>&gt; 200,000 USD</td>
<td>Ideal analytical techs for multiple elemental analysis. But need experts and large space for operation &amp; maintenance. Need sample pretreatment such as desalination. Lab use on land.</td>
</tr>
<tr>
<td>ASV Anodic stripping Voltammetry</td>
<td>Specific elements but high sens analysis of Cu, Pb, Zn, Cd, and Fe.</td>
<td>&gt; 50,000 USD</td>
<td>One of promised technology. Submersible version also available. This tech has long history but has not seemed to become popular yet. System stability problem? Mercury electrode inside is critical?</td>
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<tr>
<td>Others: ICP-AES (Atomic Emission Spectrometry), AAS (Atomic Absorption Spectrometry), etc.</td>
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<tr>
<td>Luminometer for DF (Hamamatsu Photonics) Plus other devices (PC, incubator, shaker)</td>
<td>Detecting wide spectrum of toxicants</td>
<td>&gt; 30,000 USD</td>
<td>Easy maintenance, compact system, rapid measurement, high sensitivity &amp; reliability</td>
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<td>Starter test kit: 200 USD / 1 set</td>
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Demonstration of our onboard bioassay during Chikyu cruise (2016)

C9028A 7S-CC (Zn-Pb rich ore)

onboard

Off site Lab.

Solid: C9028A 7S-CC
Dotted line: ZnCl₂

Metal compositions of C9028A 7S-CC (ICP-MS analysis)

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To overcome drawbacks in existing analytical and ecotoxicological technologies, we are newly developing an “onboard bioassay test kits and protocol”.

Cyanobium does not need “transfer culture” on board!

1. Prepare concentrated cryopreserved Cyanobium in advance
2. Keep in deep freezer on vessel
3. Defrost & preculture for a few hours in fresh media prior to “onboard bioassay”
4. Add SAMPLE water, taken from the site, to be evaluated
4’. Add artificial seawater for control
5. Incubation for a few hours to a day
6. Compare DF signals between “sample” and “control”, and verify “NO pollution”

- Responses of the cryopreserved and the transfer cultured Cyanobium are almost compatible each other (Yamaghishi et al. in prep).

“Cryopreservable” is essential for the development of test kits.
Recent research developments & plans in NIES team

Development of test organisms (e.g. NIES-981 *Cyanobium* sp.)

- Quality control for preservation
- Test kits

Protocol, standardization

Systems design & integration for onboard bioassay, program development

Side event during ISA meeting (2017)

Alert system for sudden accidents

Dynamic change of Fv/Fm
Planning alert system operation

- Measurements by 3 different instruments (PAM, Phytoflash, FRRF) after the dark adaptation of surface seawater from pump.
- Artificial events for checking the accuracy and usefulness (e.g. adding toxicants, leachates).

Kaimei cruise (KM17-12C, 13-29 Nov. 2017)

Alert system for sudden accidents

Dynamic change of Fv/Fm
Workflow of surface water quality monitoring during seabed mining

Alert system by realtime monitoring and onboard bioassay

海底鉱物資源開発時の海洋汚染モニタリングワークフロー

**At mining site (on site)**

- Normal operation
  - exploitation & lifting
  - Realtime monitoring
  - Periodical sampling
  - Onboard bioassay

**At land base (off site)**

- Precise chemical analysis
Workflow of surface water quality monitoring during seabed mining
Alert system by realtime monitoring and onboard bioassay

At mining site (on site)
- Normal operation
  - exploitation & lifting
  - OK
- Realtime monitoring
  - Alert
  - OK
- Periodical sampling
  - Onboard bioassay
    - Identification of toxicants/contaminants
    - Source identification and removal procedure
    - Improvement of process etc.
  - OK

At land base (off site)
- Precise chemical analysis
  - Identification of toxicants/contaminants
  - Source identification and removal procedure
  - Improvement of process etc.
Thank you very much for your attention.

National Institute for Environmental Studies

Poster 1: Ecotoxicological research

Our protocol is available

Poster 2: Toxic effects of heavy metals on microalgal strains

Demo of bioassay

Poster 3: Ecological model team

Dr. Yamagishi

Dr. Yamaguchi

Dr. Yoshida

Dr. Suzuki