Evaluation of metal dissolution from fresh hydrothermal core samples collected at Izena Hole during CK16-05 and rapid detection of their toxicity on marine phytoplankton community

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Environmental impact assessment is essential to reduce loads of seafloor metal-mining operations to marine environments. Accidental leakage of crushed hydrothermal ores from mining vessel is one of the possible concerns. Metals and metalloids could be released from mineral particulates and damage marine phytoplankton communities, the primary producers at the base of the marine food chain. Our previous study found that high amounts of metals can be released from fine particulates of oxidized hydrothermal ores into seawater, and they inhibit the growth of a marine phytoplankton species. In this study, metal leaching potential of deoxidized (fresh) ores was evaluated using whole round core (WRC) samples collected from Izena Hole, Okinawa Trough, by D/V Chikyu (CK16-05). Also, the toxicity of the leachates on marine phytoplankton was estimated onboard using delayed fluorescence (DF) -based bioassay method which is a useful tool to estimate rapidly and easily toxic metals (Yamagishi et al., 2016). Four whole round core samples (C9025A 6H-2, C9026A 7X-CC, C9027B 1X-CC, and C9028A 7S-CC) were taken from sulfide mineral rich sections and powdered manually with agate mortars. Approximately 3 g of each powdered sample was mixed with 30 mL of artificial seawater, and then the mixture was shaken at room temperature for 6 h. After shaking, the solid phase was separated by centrifugation and filtered. Dissolved metals and metalloids in the liquid phase is quantified using an inductively coupled plasma-mass spectrometry.

A newly developed bioassay technique was applied to evaluate the toxicity of the core-leachates onboard. A test organism for the bioassay was a marine Cyanophyceae of *Cyanobium sp.* (NIES-981), which had been cryopreserved and was resuscitated onboard just before the bioassay test. The inhibition effect of the leachates on the algal photosynthetic activity or growth was quantitatively determined by a custom made ultra-weak luminescence detector system (Type 7600, Hamamatsu Photonics) at 15 min, 1, 3, 6, 9, 12 and 24 h after the exposure to those leachates.

Zn (4–15 ppm), Pb (2–16 ppm), Cd (20–130 ppb), and Mn (130–160 ppb) were detected from the leachates of samples C9025A 6H-2, C9027B 1X-CC, and C9028A 7S-CC. These metal contents in the leachate from the core samples were 2–3 order of magnitude lower than in the leachates from the oxidized ore samples. On the other hand, the contents of these elements in the leachate from sample C9026A 7X-CC was significantly low.

On the bioassay experiment for the leachates from C9025A 6H-2, C9027B 1X-CC, and C9028A 7S-CC, the DF-intensities of *Cyanobium sp.* greatly decreased at 24 h comparing to the control, i.e., without the leachate. On the other hand, the leachate from C9026A 7X-CC which contained low amounts of metals did not cause the significant inhibition effect on *Cyanobium sp.*

Our results clearly show that non-oxidized fresh hydrothermal ores can release various toxic metals into seawater as same as the oxidized hydrothermal ores (e.g., weathered chimney ores), and such contaminated seawater leachates could inhibit the growth activity of natural marine phytoplankton. Therefore, appropriate management systems for controlling the leakage and disposal of mining ores and

wastes to the marine surface area should be devised to preserve the natural marine ecosystem.

Keywords: hydrothermal ore, metal contamination, marine phytoplankton