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Toxicity of biomining effluents to *Daphnia magna*: Acute toxicity and transcriptomic biomarkers

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HIGHLIGHTS

- Biomining affected waters show sub-lethal toxicity to *Daphnia magna*.
- Water samples up-regulated *monooxygenase*, *vtg-sod* and *catalase*.
- Transcriptomic biomarkers are more sensitive than acute toxicity tests.

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ABSTRACT

Increasing metal consumption is driving the introduction of new techniques such as biomining to exploit low grade ores. The biomining impacts notably aquatic ecosystems, yet, the applicability of ecotoxicological tests to study the complex mixture effects of mining waters is insufficiently understood. The aim of the present work was to test if transcriptomic biomarkers are suitable and sensitive for the ecotoxicity assessment of biomining affected waters. The study site had been affected by a multimetal biomine, and the studied water samples formed a concentration gradient of contamination downstream from the biomining site. Cadmium and nickel were used as positive controls in the toxicity tests. Selected transcriptomic biomarkers, previously shown to be differentially regulated by metals, were used to evaluate the ecotoxicity of the water samples. Parallel samples were used to compare the transcriptomic biomarkers with the conventional acute *D. magna* toxicity test. In the acute test, one sample was acutely toxic to *D. magna,* when pH was adjusted according to the standard, whereas, in the native pH, three samples caused total immobility. Monooxygenase was up-regulated by the highest concentration of Cd in control samples and three of the water samples. Vtg-SOD was up-regulated by one of the water samples, and catalase by the second highest concentration of Cd. The results show that transcriptomic biomarkers in *D. magna* can be used as sensitive bioindicators for metal mixture toxicity assessment in complex environmental water samples.

1. Introduction

Mineral mining is increasing due to global economic growth and consequent demand for the metals (Pokhrel and Dubey, 2013). While the demand for most metals has steadily increased in the last decade, number, grade and quality of new ore deposits have declined (Brierley, 2008; Brierley and Brierley, 2013). The biohydrometallurgy i.e. biomining is particularly suitable technically and economically for processing lower grade and complex polymetallic mineral assemblages (Brierley, 2008; Brierley and Brierley, 2013). Biomining simplifies the enrichment process of sulphide ores as microbes are used to oxidize insoluble metal sulphides to soluble metal sulphates, which are then extracted from leachates (Morin et al., 2008). Biomining also has lower process temperatures, lower energy costs and smaller carbon footprints than conventional mining (Morin et al., 2008). Thus, biomining has been perceived as a more environmentally benign approach than conventional mining processes (Morin et al., 2008, Johnson, 2014, 2015). The contamination from conventional mining include CO_2 emissions, SO_2 emissions and acid mine drainage (AMD) (Dold, 2008). Although biomining consumes less energy, it is likely to cause similar releases of salts, heavy metals and acid mine drainage

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(AMD) to the environment as processing with other techniques such as floatation (Dold and Weibel, 2013). As the utilisation of biomining is likely to increase (Brierley, 2008; Brierley and Brierley, 2013), and novel approaches such as *in situ* biomining are developed (Johnson, 2015; Morin et al., 2008) the environmental impacts of biomining, particularly, on water resources, should be studied.

Biochemical biomarkers have been proposed as sensitive tools for risk assessment (Jemec et al., 2010). Biomarkers can be used for understanding the mechanism of toxic action, for screening of unknown pollutants in the environment (i.e. stressor identification), and for detecting early signs of chronic toxicity (Snell et al., 2003). Biochemical biomarkers are generally considered to be more sensitive to stressors than whole-organism responses are (Jemec et al., 2010). Effect based tools such as toxicogenomics can be used for monitoring water quality, and for identifying either priority groups of pollutants or potentially affected biological targets (Brack et al., 2015). Toxicogenomics in *D. magna* have been shown as a useful tool for environmental monitoring of a copper mining contaminated site (Poynton et al., 2008).

The aim of the present study was to test if transcriptomic biomarkers are suitable and sensitive for the ecotoxicity assessment of biomining affected waters. The study site was polluted by a multimetal mine that uses bioleaching. The studied water samples formed a concentration gradient of contamination downstream from the biomining site. The water flea *Daphnia magna* was selected as a test species as it is a widely studied species, which is known to be sensitive to metal contamination and metal mixture contamination (Okamoto et al., 2015; Yim et al., 2006). *D. magna* represents also an important trophic level in an aquatic food chain. Selected transcriptomic biomarkers were studied and compared to the conventional acute toxicity test with *D. magna*. First, preliminary testing was conducted with a set of known biomarker genes that had shown differential expression upon metal exposure (Poynton et al., 2007; Kim et al., 2010). Monooxygenase (*mox*), vitellogenin superoxide dismutase (*vtg-sod*) and catalase (*cat*) showed promising results and were selected for this study. Secondly, the observed changes in the expression of biomarker genes were compared against the background variables including physico-chemical water characteristics, to reveal the underlying causes.

2. Materials and methods

2.1. Study site

The water samples for this study were collected from two watersheds that receive effluents from the Talvivaara multimetal mine in Sotkamo, Finland. The mine uses bioheapleaching to recover metals from low-grade ore. The Talvivaara deposit is hosted by metamorphosed black shales (black schists) and contains 300 million metric tons (Mt) of low-grade ore averaging 0.26 percent Ni, 0.14 percent Cu, and 0.53 percent Zn (Loukola-Ruskeeniemi and Heino, 1996). The main products of Talvivaara are a mixed nickel cobalt sulphide, copper sulphide and zinc sulphide (Riekkola-Vanhanen, 2010). In Talvivaara, an environmental accident happened in November 2012 affecting waterways of Oulujärvi and Vuoksi. The wall of a storage pond ruptured and acidic waste water containing raffinate from enrichment process leaked to the nearby waterways (Onnettomuustutkintakeskus, 2014). According to the report of Finnish Environmental institute, the main contaminants were Ni, Zn, Cd, Al, U and salting due to sulphate (Kauppi et al., 2013). The accident also caused fluctuating pH, as the waste water was acidic and the accident treatment included occasional neutralisation of waters with lime addition (Kauppi et al., 2013).

2.2. Water samples and chemical analyses

As a part of a larger monitoring survey of the effects of the spill, eight water samples were collected by a consulting company and Kainuu Centre for Economic Development, Transport and the Environment between February and March within 11 days from the rivers and lakes affected the most three months after the accident (see the map in Fig. 1). Seven of the samples were from the watershed of Oulujärvi and one (River Lumijoki) from the watershed of Vuoksi (Fig. 1). River water samples were taken 0.5–1.0 m below water surface into 10–20 l plastic containers, rinsed with the water at the site and stored at 4 °C until exposures. The lake water samples were taken 1 m above the lake bottom with a Limnos sampler. The lake water sampling was designed to study concurrent effects of metals and salts in the lakes hypolimnion. Representative one litre water samples for the chemical analyses were taken at the same sampling site as the samples for the exposures. To measure the total and dissolved concentrations of elements two parallel 100 ml sub-samples were taken. Both of the 100 ml sub-samples were preserved with 0.5 ml of HNO_3 (100441, Suprapur 65%, Merck, Germany), and the sample representing dissolved concentration was filtered (GD/XP, 0.45 μm, Whatman) in the field.

Concentration of Al, As, Ba, Ca, K, Mg, Na, S, Sr and Ti were analysed with ICP-OES following standard SFS-EN ISO 11885:2009. Cd, Co, Cr,Cu, Ni, Pb, Sb, Se, Zn, U and V were analysed with ICP-MS according to SFS-EN ISO 17294–1:2006 and 17294–2:2005. Fe and Mn were measured with IRIS Intrepid II XSP (Thermo Scientific). For the total concentration analyses the samples were microwave digested with HNO_3 (ISO 15587–2:2002). Analyses were performed in accredited laboratories (EN ISO/IEC 17025) of the Finnish Environmental institute (FINAS T003, K054) and Nablabs Ltd. (FINAS T111, T142). The measurements were done according to standards (standard number given in brackets): Oxygen concentration at field (SFS-EN 25813:1996), pH (SFS-EN ISO 10523-2012), conductivity with temperature compensation to 25 °C (SFS-EN 27888-1994), solids content (SFS-EN 872:2005), total hardness (SFS 3003:1987), fluoride (SFS-EN-ISO 10304–1:2009), and sulphate (SFS-EN10304-2009).

2.3. Acute toxicity assays

The 24-h acute toxicity assays were performed according to the ISO standard (6341:2012). The *Daphnia magna* neonates originated from dormant eggs (MicroBioTests Inc., Belgium), which were cultivated in the laboratory for several generations prior to deployment in the toxicity assays. To preserve the environmental relevance of the samples they were pre-treated as little as possible prior to the acute toxicity testing (e.g. samples were not diluted). The samples were vacuum filtrated (Whatman 25-mm GD/XP syringe filter, pore size 0.45 μm) to remove the particulate material. Samples were tested both in original and in adjusted pH. The toxicity assay was replicated five times for each of the samples. Each replicate contained five *Daphnia magna* neonates (<24 h) in 10 ml volume, which contained 9 ml of the sample and 1 ml of the ISO test water (ISO 6341:2012). ISO test water addition was due to neonate transfer, which was done with an automated pipette using 200 μl of ISO test water per neonate. Therefore, the final sample concentration in the test flask was 90%. The sample concentrations were corrected according to the dilution factor for the calculations of the effective concentrations (EC-values). Mobility of the neonates was used as the end point. The assay was carried out at 20 °C (±0.2), with a light rhythm of 8:16 h (dark: light), and light intensity < 1000 lux.

Each sample was tested both in native pH and with pH adjusted to 6.5 (±0.2) with 0.1 M NaOH which was assumed to be close to the