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Whole-cell bioreporters and risk assessment of environmental pollution: A proof-of-concept study using lead☆

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ABSTRACT

As the world burden of environmental contamination increases, it is of the utmost importance to develop streamlined approaches to environmental risk assessment in order to prioritize mitigation measures. Whole-cell biosensors or bioreporters and speciation modeling have both become of increasing interest to determine the bioavailability of pollutants, as bioavailability is increasingly in use as an indicator of risk. Herein, we examine whether bioreporter results are able to reflect expectations based on chemical reactivity and speciation modeling, with the hope to extend the research into a wider framework of risk assessment. We study a specific test case concerning the bioavailability of lead (Pb) in aqueous environments containing Pb-complexing ligands. Ligands studied include ethylene diamine tetra-acetic acid (EDTA), meso-2,3 dimercaptosuccinic acid (DMSA), leucine, methionine, cysteine, glutathione, and humic acid (HA), and we also performed experiments using natural water samples from Lake Tai (Taihu), the third largest lake in China. We find that EDTA, DMSA, cysteine, glutathione, and HA amendment significantly reduced Pb bioavailability with increasing ligand concentration according to a log-sigmoid trend. Increasing dissolved organic carbon in Taihu water also had the same effect, whereas leucine and methionine had no notable effect on bioavailability at the concentrations tested. We find that bioreporter results are in accord with the reduction of aqueous Pb^{2+} that we expect from the relative complexation affinities of the different ligands tested. For EDTA and HA, for which reasonably accurate ionization and complexation constants are known, speciation modeling is in agreement with bioreporter response to within the level of uncertainty recognised as reasonable by the United States Environmental Protection Agency for speciation-based risk assessment applications. These findings represent a first step toward using bioreporter technology to streamline the biological confirmation or validation of speciation modeling for use in environmental risk assessment.

1. Introduction

The traditional approach to assessing ecological or human health risk associated with soil and water contaminants typically relies upon measuring the total concentration of contaminant present. For heavy metals, total concentration is used as a predictor of the magnitude, frequency and duration of human exposure and used to define a level of acceptable carcinogenic or noncarcinogenic human health risk (EPA, 1992; EPA, 2007a). Similar considerations apply to ecological concentration-based acceptance criteria, though often such criteria are based on toxicity metrics such as Genus mean acute value (Stephen et al., 1985). Due to variations in bioavailability however, total concentration does not always relate to toxicity, and accordingly, the US EPA Framework for Metals Risk Assessment states that risk assessors should "explicitly incorporate factors that influence the bioavailability of a metal" (Fairbrother et al., 2007). With the ever-increasing amount of environmental contamination in the world, it is of crucial interest to develop risk assessment methods that enable a sort of environmental triage or ranking of contaminant risk, and bioavailability offers one avenue to this end.

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Many different methods have been and are being developed to utilize bioavailability in ecological and human health risk assessment (EPA, 2007a; Fairbrother et al., 2007). In human health risk assessment, methods include, but are not limited to, methods to assess human oral bioavailability (the definition for which is different from environmental bioavailability) (EPA, 2007a). On the front line of environmental monitoring, whole-cell bioreporters have become of increasing interest to determine the bioavailability of pollutants in environmental matrices such as water, soil, and other geomaterials (Al-Anizi et al., 2014; Ding, 2009; Deepthike et al., 2009; Kohlmeier et al., 2008; Selifonova et al., 1993; Van der Meer and Belkin, 2010; Wells, 2012). Whole-cell bioreporters are genetically engineered bacteria that produce dose-dependent signals in response to target chemicals or stress and enable highly sensitive and rapid assessment of the bioavailable fraction of contaminants in samples (Kessler et al., 2012; Magrisso et al., 2008; Van der Meer and Belkin, 2010). Genetic construction of bacterial bioreporter strains is achieved by a combination of a promoter gene (a sensing element) and a reporter gene (signaling element) within a host cell. The most common strategy for the selection of the sensing element is to use the promoter of a gene known to be induced by the stress condition of interest, in this case lead exposure (Kessler et al., 2012). Fusing the promoter to the reporter ensures that when the promoter senses the target, i.e. the promoter is "turned on", this then causes the reporter gene to be turned on. Reporter genes are chosen to produce reporter proteins that have a measurable signal, typically optical. Studies show that bioreporters specifically responsive to heavy metals may be constructed (Selifonova et al., 1993; Magrisso et al., 2008; Rasmussen et al., 2000; Yoon et al., 2016a). Recently, some work has assessed the bioavailability and toxicity of lead, arsenic, copper, zinc, cobalt and nickel by using whole-cell bioreporters (Jia et al., 2016; Magrisso et al., 2009; Yoon et al., 2016a, 2016b).

Lead (Pb) is ranked as the most prevalent heavy metal contaminant in the world today (Ojeba, 2016), and Pb and its compounds are found frequently in surface water. Also, Pb is incredibly toxic and of particular concern with reference to developmental neurotoxicity for children, accounting for most of the cases of pediatric heavy metal poisoning (Tiwari, 2012). The major sources of Pb in the environment are from mining, historical use of lead in vehicle fuel, smelters, and battery disposal (Adriano, 2001; Joumard et al., 1983; Sud et al., 2008). China is one of the places most affected by Pb poisoning. Recent studies have shown that nearly 30% of urban children aged 3–5 years had a blood lead level above the recommended 100 μg L^{-1} (Wang and Zhang, 2006; Ye et al., 2007). The risk of Pb introduction to the food chain increases as the Pb level rises in water and soil. Pb precipitates to insoluble species under alkaline conditions, however, the increasing occurrence of acid rain in China during the past decade (Larssen et al., 2011) has enhanced the release of Pb into water and soil solution. In addition to posing risks to human health, high levels of Pb in soils and water threaten ecosystems (Sekar et al., 2004), and it is therefore important to be able to rapidly evaluate potential biological effects of Pb in the environment and on a site-to-site basis.

The bioavailability of Pb depends on its speciation, which in natural waters is influenced by factors such as pH, ionic strength, and the types of organic and inorganic ligands present. Speciation modeling has been used to predict how water chemistry affects the speciation and the bioavailability of metals in aquatic systems (Niyogi and Wood, 2004), and this modeling has been demonstrated to be a cost-efficient and time saving approach to obtain optimized information for environmental risk assessment. Generally, metal toxicity is caused by free metal ions reacting with biological binding sites (Song et al., 2014a). Some cations, such as H^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+, as well as dissolved organic carbon (DOC), compete with metal ions for these binding sites and decrease the bioavailability, and hence the toxicity, of free metal ions (Celen et al., 2007; Song et al., 2014a), which in some environments may be accurately estimated through modeling. Even though bioavailability is highly influenced by speciation, it is important that models should be used in conjunction with biological techniques for validation. Many of these biological techniques are relatively unwieldy in terms of cultivating and maintaining test organisms for use, for example, the cultivation of daphnia magna and rainbow trout (De Schamphelaere and Janssen, 2002; Heijerick et al., 2005; Nys et al., 2014; Smith et al., 2017). In this study, we report a first case study to examine whether bioreporter results are able to reflect expectations based on chemical reactivity and speciation modeling, with the hope to extend the research into a wider framework of risk assessment.

Here we investigate bioavailability of Pb, as measured with a bioreporter in the presence of different ligands that complex Pb in aqueous environments (EDTA, DMSA, amino acids/peptides, HA, and DOC). Literature provides a rationale for ligand choice. Sillanpää and Oikari (1996) studied the effect of EDTA on heavy metal toxicity and found that complexation by EDTA noticeably reduced the toxicity of Zn and Pb. DMSA is a drug currently used for chelation therapy in lead poisoning (Besunder et al., 1996). Amino acids and HA are two subgroups of dissolved organic matter that occur in environmental settings and play an important role in binding heavy metals. Amino acids, peptides and proteins are known as complexation agents for metal ions due to the presence of metal-coordinating functional groups in their chemical structures. For amino acids, we follow the protocol of Ndu et al. (2012), who investigated the comparative effects of two thiol-containing amino acid compounds, cysteine and glutathione (a cysteine containing tri-peptide), to those of two non-thiol-containing amino acids, methionine and leucine, on the response of a mercury-sensitive bioreporter. Leucine with its α-amino group, α-carboxylic acid group and isobutyl side chain is classified as a non-polar amino acid, whereas methionine is also non-polar, however has an S-methyl thioether side chain. Cysteine in turn has an active thiolate instead of the S-methyl thioether side chain and is variously classified as polar to hydrophobic. Glutathione is a tripeptide incorporating glutamate, cysteine and glycine and having two carboxyl groups, a primary amine, two secondary amines, and a thiolate group (Sisombath, 2014; Wu et al., 2004). While the carboxylic acid and primary amine groups (e.g. as in leucine and methionine) are capable of complexing metals, the thiol groups in particular (cysteine, glutathione) are thought to be largely implicated in the complexation of metals by thiolate-containing amino acids and peptides (Simhadri et al., 2015; Sisombath, 2014).

DOC includes organic molecules that, operationally, pass filtration, typically with a 0.45 μm filter (Kolka et al., 2008). In terrestrial environments the source of DOC is typically from decomposition of dead organic matter (Camilleri and Ribi, 1986). Bivalent metals often complex strongly with HA and fulvic acid (FA) components of DOC, with HA complexing heavy metals more strongly (Fasurová and Pospíšilová, 2010). When comparing modeling results to experiment, the differences in metal binding properties in natural waters have been found to be relatively small between different HAs and FAs, suggesting that to some extent generalization within these groups is possible (Benedetti et al., 1996). This generalizability has led to the development of validated speciation models for metal-DOC binding (Di Toro et al., 2001; EPA, 2007b; Gustafsson, 2001; Tipping, 1994). Studies have shown that Pb complexes strongly with HA, which serves to reduce bioavailable Pb (Coles and Yong, 2006). As HA is a class of compound with no specific chemical formula or structure, we study bioreporter