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Assessing toxicity of metal contaminated soil from glassworks sites with a battery of biotests

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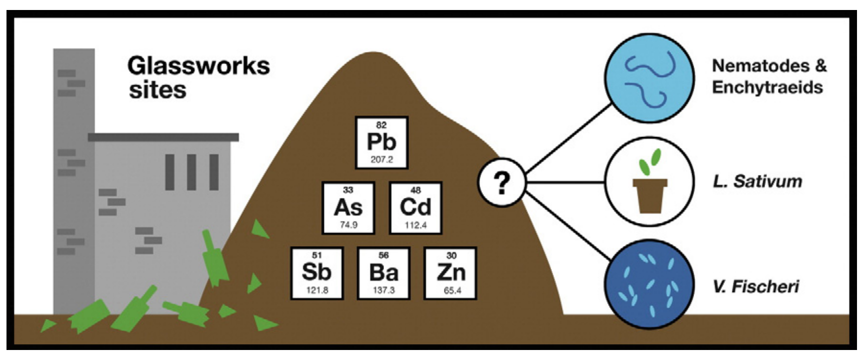
HIGHLIGHTS

- The toxicological properties of metal contaminated glassworks soils was addressed.
- The soils and landfills at glassworks sites contain high concentrations of several toxic metals.
- A battery of biotests was used to assess toxicity of glassworks soil environments.
- Toxicity of the glassworks soils was not detected by *ex situ* tests (phytotoxicity; the BioTox™).
- A decrease in enchytraeid abundance and biomass was observed implying *in situ* toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study addresses toxicological properties of metal contaminated soils, using glassworks sites in south-eastern Sweden as study objects. Soil from five selected glassworks sites as well as from nearby reference areas were analysed for total and water-soluble metal concentrations and general geochemical parameters. A battery of biotests was then applied to assess the toxicity of the glassworks soil environments: a test of phytotoxicity with garden cress (*Lepidium sativum*); the BioTox™ test for toxicity to bacteria using *Vibrio fischeri*; and analyses of abundancies and biomass of nematodes and enchytraeids. The glassworks- and reference areas were comparable with respect to pH and the content of organic matter and nutrients (C, N, P), but total metal concentrations (Pb, As, Ba, Cd and Zn) were significantly higher at the former sites. Higher metal concentrations in the water-soluble fraction were also observed, even though these concentrations were low compared to the total ones. Nevertheless, toxicity of the glassworks soils was not detected by the two *ex situ* tests; inhibition of light emission by *V. fischeri* could not be seen, nor was an effect seen on the growth of *L. sativum*. A decrease in enchytraeid and nematode abundance and biomass was, however, observed for the landfill soils as compared to reference soils, implying *in situ* toxicity to soil-inhabiting organisms. The confirmation of *in situ* bioavailability and negative effects motivates additional studies of the risk posed to humans of the glassworks villages.

1. Introduction

There are many sites that have been contaminated by metals released from past industrial activities, and metal contamination

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threatens the well-being of all components of the biosphere. Even though it is well known that high total concentrations may not always translate into high mobility, bioavailability and toxicity (Kördel et al., 2013; McLaughlin et al., 2000), risks at contaminated sites are often assessed based on analyses of total concentrations, sometimes complemented with weaker extractions that dissolve only the potentially bioavailable fraction. However, the potential for environmental hazards is better understood when chemical analyses are complemented with biotests, as organisms are only sensitive to the truly bioavailable fraction of metals (Garcia-Lorenzo et al., 2009; Römbke et al., 2005; Karjalainen et al., 2009). Biological tests could also integrate the effects of mixtures and their bioavailability and therefore provide a useful tool for site-specific assessment of actual ecological risks.

The long-lasting production of glass in south-eastern Sweden is one example of industrial activity, where the local soil environment has become severely contaminated over time, and where adverse health effects are now seen among local residents. Better understanding of soil toxicity properties is thus highly relevant at the Swedish glassworks sites. The main contamination occurred during the 1970s and earlier, when unsorted waste and crushed glass were thrown in a pile near the glassworks (Falk et al., 2005). A compilation of data from previous site investigations, available from the Kalmar County Administrative Boards (2016) and Kronberg County Administrative Boards (2016), reveals maximum total concentrations in glassworks soils of the region (or rather soil with a varying mix of glass waste) of 16,900 mg kg^{-1} Pb, 180 mg kg^{-1} Cd and 2600 mg kg^{-1} As. It has also been shown that soils of private gardens around the glassworks may contain metal concentrations of the same magnitude as the glassworks properties, that there is a positive correlation between metal contamination and metal concentration in homegrown vegetables, and that consumption of these vegetables is a risk factor (Augustsson et al., 2015; Uddh-Söderberg et al., 2015). Recent findings also imply that residents living near glassworks in the area are at an increased risk of developing cancer (Nyqvist et al., 2017).

When turning from soil contamination to toxicology, a battery of toxicity tests with species of varying sensitivities and exposure pathways is recommended (Karjalainen et al., 2009). The suitability of biotests, such as the Phytotoxkit (to test plants) and Microtox®/ BioTox™ (bacterial test), in the assessment of toxicity of bottom sediments, composts, sewage sludge, and for example mining activity contaminated soils, has been proven in several studies (Boularbah et al., 2006a, 2006b; Czerniawska-Kusza and Kusza, 2011; Mamindy-Pajany et al., 2011; Loureiro et al., 2005; Dubova and Zarina, 2004). Plants are essential primary food producers of ecosystems and thus it is important to identify the magnitude of the toxic effects on plants (Garcia-Lorenzo et al., 2009). Also, bacteria play a crucial role, being decomposers in the environment (Kahru et al., 2005). Other key organisms are enchytraeids and nematodes (Didden and Römbke, 2001). Especially enchytraeids are sensitive to environmental stresses and the presence and species composition of enchytraeid worms have therefore been suggested for use as indicators of metal toxicity (Kapusta and Sobczyk, 2015).

The aim of this study was to evaluate toxicity of glasswork contaminated soil. It was done by two *ex situ* tests: using a) a test of phytotoxicity with garden cress (*Lepidium sativum*), and b) the BioTox™ test for toxicity to bacteria using the bioluminescent bacterium *Vibrio fischeri* as test organism. A bioassay was also performed by measuring the abundancies of soil-inhabiting nematodes and enchytraeids. In contrast to standardized laboratory tests, the latter approach reflects the *in situ* situation of the soil animals and the effect of their exposure to the contaminant metals.

2. Materials and methods

2.1. Study region

Five typical glassworks sites in the glassworks region of south-eastern Sweden were selected for the present study: Johansfors, Bergdala, Kosta, Orrefors and Målerås (Fig. 1). Production at all these sites has included artistic and crystal glass. The major raw materials in the glass production were silica quartz, calcite, feldspar and oxides of several metals, such as As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn (Hermelin and Welander, 1986; Magnusson, 1971; Månsson and Carlsson, 2002). The volume of contaminated soil and landfill materials at the sites varies from a few thousand cubic metres to approximately 100,000 m^3, with high concentrations in particular of Pb and As (Fanger et al., 2004; Bergelin et al., 2006; Håkansson and Ländell, 2006; Werkelin and Gustavsson, 2006). Production is still running in Bergdala, Kosta, Målerås and Johansfors, but only on a small scale at the latter. The factory in Orrefors was closed down in 2013. However, most of the landfills were decommissioned in the late 1970s and have since been untouched while natural vegetation has been established and soils formed. The dominating tree species of the region are spruce and pine, and the natural soils typically show a podzolized profile. The quaternary deposits are dominated by sandy tills with a mineralogy that reflects the local granitic bedrock. One particularly relevant feature in this area is the relatively high geogenic concentrations of Pb (SGU, 2014). Mean January and July temperatures in the study area are −2.0 °C and 17.0 °C, respectively, and the mean annual precipitation is approximately 700 mm (based on data from 2006 to 2015; SMHI, 2016).

2.2. Sampling and chemical analyses of soils

Soil samples were collected in October 2015. At each of the five selected glassworks sites, samples were taken both from the main landfill and from a nearby (within a few hundred metres) reference area. The reference area of each glassworks site was selected on-site based on the criteria: 1) similar vegetation characteristics *i.e.* type of ground cover and dominating tree species, and 2) similar natural soil characteristics (based on assessment in the field, *e.g.* a rough estimate of soil grain size, colour, approximate content of organic matter (OM) *etc.*). At each study site, five samples (approximately 5 L each) were collected from the landfill area, and five were taken from the reference area. This gave in total 50 samples, each taken from a unique hand dug pit as a composite sample from 0 to 20 cm depth (after the upper vegetative layer of loose litter and mosses had been removed). Samples were homogenized thoroughly but gently by hand and stored at 4 °C until analysis. During the first week following sampling, a subset of the (unsieved) fresh material was used for determination of nematodes and enchytraeids (see Toxicity studies section). The soil moisture (dry matter = DM) was determined after drying 20 g of soil at 105 °C for 24 h (Standard ASTM D2216, ASTM, 2010). The remaining material was air dried and sifted through a sieve with a 2 mm mesh. In soil prepared this way, basic soil properties were determined. Soil OM content was measured as the ignition loss (4 h, 550 °C; Radojević and Bashkin, 2006). Electrical conductivity (EC) was measured using the WTW Cond330i meter, and pH was determined on a 1:2.5 (v/v) soil:distilled water suspension with a Mettler Delta 340 pH meter according to the ISO 10390 standard (ISO, 2005). Total nitrogen (tot-N) and carbon (tot-C) were analysed using a LECO CNS-analyser (Table 1).

The total concentrations of phosphorus (tot-P) and metal(loid)s (Ag, As, Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb, V, Sn, Mo, Sb, Zn) in the <2 mm soil fraction were determined with inductively coupled plasma sector field mass spectrometry (ICP-SFMS) at the commercial Swedish laboratory ALS Scandinavia. The ICP-SFMS analyses followed the protocols of SS EN ISO 17294-1 and the US Environmental Protection Agency's (EPA's) method 200.8. Before analysis, soil samples were dried at 50 °C and a 0.5 g sub-portion of the dried material was digested with 5.0 mL concentrated nitric acid (HNO_3) and 0.5 mL hydrogen peroxide (H_2O_2) in closed Teflon vessels in a high-pressure microwave oven. The received concentrations can be viewed as total, or pseudo-total. To get an efficient extraction of Sb, the addition of hydrochloric acid (HCl) is needed (Hjortenkrans et al., 2009). This element was therefore determined after an aqua regia extraction, where a 0.5 g