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

Hagner M[a,b], Romantschuk M[a,c], Penttinen O-P[a], Egfors A[d], Marchand C[d], Augustsson A[d]*

[a] Department of Enviromental Sciences, University of Helsinki, Lahti, Finland.
[b] Natural Resources Institute Finland (Luke), Jokioinen, Finland
[c] Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia.
[d] Department Biology and Environmental Science, Linnaeus University, Kalmar, Sweden.

*Corresponding author at: Department of Biology and Environmental Science, Linnaeus University, SE-391 82 Kalmar, Sweden. E-mail: anna.augustsson@lnu.se

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 analyzed 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.

Keywords: metal contamination, glassworks sites, enchytraeids, biotests, bioavailability

Introduction

There are many sites that have been contaminated by metals released from past industrial activities, and metal contamination 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 severily 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 and Kronoberg 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, b; 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 standardised laboratory tests, the latter approach reflects the *in situ* situation of the soil animals and the effect of their exposure to the contaminant metals.

Materials and methods

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).

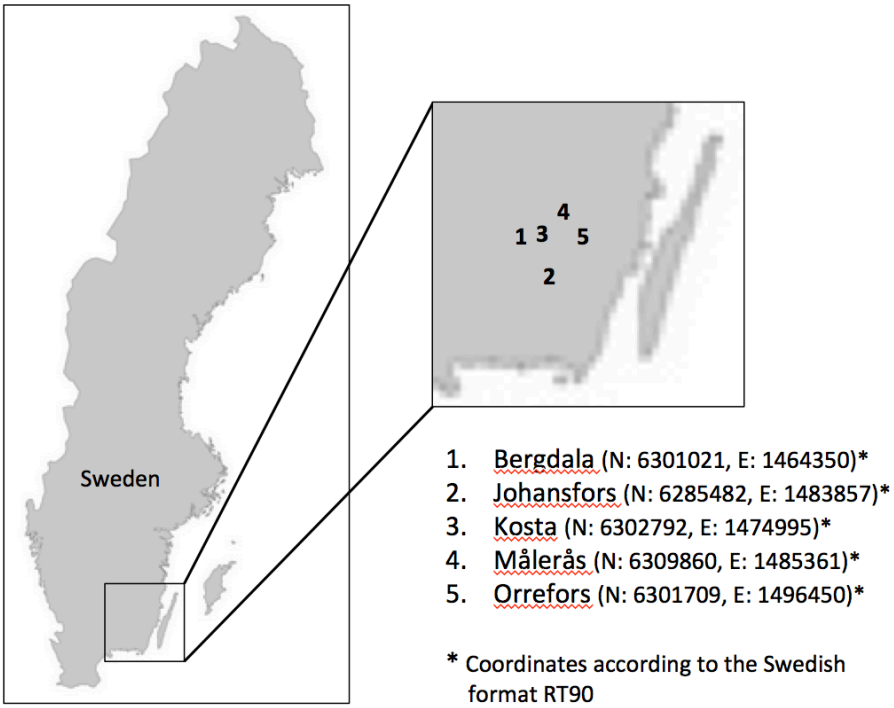


Fig. 1. Location of the five glassworks sites in southeastern Sweden.

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 0–20 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*). 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 sub-portion of the material was digested using 7.5 mL concentrated HCl and 2.5 mL concentrated HNO_3. All chemicals used were of analytical grade quality.

Extracts of water-soluble metals (referred to as H_2O-metals throughout the paper) were obtained after a multi-step procedure (Loureiro et al. 2005). Firstly, 10 mL of the air-dried and sieved (< 2 mm) soil was mixed with 40 mL of distilled water (18.2 MΩ/cm^2 Milli-Q^{TM} water) and shaken mechanically (150 rpm) for 24 h in the dark (Loureiro et al. 2005). The suspension was then centrifuged (20 min, 4600 g) and filtered through glass microfibre using vacuum filtration apparatus (Whatman GF/C Ø 47 mm, 1.2 µm porosity). The collected elutriate was divided in half and water extractable metals (Cd-112, Co, Cr-52, Cu, Mn, Ni, Zn-64, P, V, As, Pb-sum) were determined from one half. The other half was processed further to get elutriates for the BioTox™ test. The concentrations of soluble metals were analysed with inductively coupled plasma mass spectrometry (ICP-MS) in the Almalab of the University of Helsinki. Prior to the ICP-MS analyses, 0.1 mL concentrated HNO_3 was added to 5 mL elutriates.

Toxicity studies

Phytotoxicity to garden cress (*L. sativum*) was evaluated by the biomass production (root and shoot weight) following the methodology of OECD (2006), with some minor modifications. Seeds were rinsed and imbibited in distilled water for 8 h prior to planting in 300 mL pots with moist sample soil (sieved to < 2 mm). Fifteen seeds were planted per pot, evenly distributed over the surface of the soil. Each pot was then placed on a Petri dish, to which irrigation water (distilled water) was added daily. The temperature in the cultivation room was 20°C and the relative humidity 35%. Samples were illuminated for 16 h/day with warm fluorescent light of a photon flux density of approximately 100 μmol m^{-2} s^{-1}. Plants were harvested after 21 days. The soil was then carefully removed, and root and shoot parts were separated and rinsed in distilled water prior to drying at 70°C for 24 h. Three pots were planted for each landfill and reference soil.

To assess toxicity to bacteria, the BioTox™ test was performed using freeze-dried *V. fischeri* (strain NRRL B-11177, Aboatox Oy, Finland), which were exposed to elutriates obtained from water extraction of the sample soils. The acidity of the elutriates was adjusted to pH 6.5–7.0 with a phosphate buffer and the salinity was adjusted to 2% with sodium chloride (NaCl). Elutriates were stored (max 2 days) at 4°C up to the testing time. Luminescence (RLU) was measured before and after 15 min incubation of the bacterial suspension with the sample elutriates, according to the ISO 11348-3 (1998) standard. The 2% NaCl with phosphate buffer addition was used as a control liquid. Toxicity results were expressed as RLU inhibition percent (INH%). Each analysis was performed on duplicate samples.

Luminescence inhibition of *V. fischeri* was calculated as follows:

$$KF = \frac{IC15}{IC0}$$

$$INH\% = 100 - \frac{IT15}{KF \times IT0} \times 100$$

where:

KF = correction factor
IC15 = luminescence intensity of control solution after contact time (15 min) in RLU

IC0 = initial luminescence of control sample in RLU
IT15 = luminescence intensity of test sample after contact time (15 min) in RLU
IT0 = initial luminescence intensity of test sample in RLU

In our study, nematodes and enchytraeids were extracted from non-sieved soil samples, as sieving may hurt soil organisms. Glass pieces larger than approximately 4 mm were, however, gently removed by hand. Nematodes were extracted from 5 to 10 g and enchytraeids from 30 to 50 g of fresh soil samples using the wet funnel methods by Sohlenius (1979) and O'Connor (1955), respectively. The number of nematodes and enchytraeids were counted under a binocular-microscope. In addition, enchytraeids were classified into size classes according to length (0–2, 2–4, 4–6, 6–8, 8–10, 10–12 and > 12 mm), and the total biomass of enchytraeids per sample was calculated according to Abrahamsen (1973). All the nematodes and enchytraeids data were expressed per g of soil OM. The presence of glass remains, which is a distinctive feature of the landfill soil samples, might inevitably dilute all natural soil components – both minerogenic and organic. By presenting the nematodes and enchytraeids data per g soil OM, these dilution effects are reduced when interpreting toxicity effects.

Statistical analyses

The normality of data was analysed using Kolmogorov–Smirnov and Shapiro–Wilk tests and the homogeneity of variances with Levene's test. The data were not always normally distributed and the variances were sometimes heterogeneous, and thus the data that required so were log transformed to meet the requirements of parametric tests.

The main aim of the study was to explore whether the biological responses (number of nematodes, number and biomass of enchytraeids, luminescence inhibition of *V. fischeri*, and shoot and root growth of *L. sativum*) differ between glasswork landfills (Johansfors, Kosta, Målerås, Orrefors, Bergdala) and reference sites (Johansfors ref., Kosta ref., Målerås ref., Orrefors ref., Bergdala ref.). Mean values per site based on five replicate soil samples collected from each sampling site were used as tested values. In all analyses concerning soil parameters (total and H_2O extractable metal concentrations, nutrient concentrations, pH, EC) and biota (plants, number of nematodes per g OM, number and biomass of enchytraeids per g OM), comparisons between landfill and reference sites were performed using a paired sample *t*-test. As the luminescence inhibition of *V. fischeri* data was not normally distributed even after transformation, paired comparisons were performed using the

non-parametric Wilcoxon's test. In addition, the differences in measured parameters between single glasswork landfills and their nearby reference areas were analyzed using independent *t*-test.

Multiple regression analysis (with stepwise correction) was used to explore whether the abundances of soil fauna within the sites are influenced by measured soil variables. Response variables were the root and shoot biomass of *L. sativum*, the number of nematodes, and the biomass of enchytraeids. Soil metal and nutrient concentrations, moisture, OM, pH and EC were used as predictor variables. In the regression analyses the five samples per site were used as a replicates despite the fact that they do not represent independent sample units. This makes 25 (5*5) tested values for both glassworks and reference sites (altogether 50 samples). Thus, *p*-values should be considered with caution. All tests were run using IBM SPSS Statistics 23 (SPSS Inc.).

Results

Soil parameters

Each landfill site was largely contaminated with various metals, as expected. Average concentrations for each sampling site are presented in Table 1, and a more detailed compilation is provided in Supplementary material I. Pb together with As, Ba, Cd and Zn were the major contaminants, with mean concentrations at all glassworks sites exceeding the guideline values of Swedish legislation for less sensitive land use (Swedish EPA 2009). Also concentrations of Cu and Sb were found in elevated mean concentrations, exceeding the guideline values for sensitive land use (Swedish EPA 2009). Overall, however, a huge variation in total metal concentrations was observed for the landfill samples, which can be explained by the large heterogeneity of the deposited waste materials: for example, the concentration of Pb varied from 161 to 38 000 mg kg^{-1}, As from 64 to 7790 mg kg^{-1}, Ba from 30 to 3560 mg kg^{-1}, Cd from 0.2 to 62.8 mg kg^{-1}, and Zn from 45 to 1050 mg kg^{-1} (see Supplementary material I). Nevertheless, considering the whole data set, positive correlations between all the contaminant metals (Pb, As, Ba, Cd, Zn and Sb) were observed, implying a common source (the glass waste). Pearson's correlations (two-tailed) were found in the range 0.53–0.88, with all p-values < 0.001. As expected, since elevated concentrations are due to disposal of glass waste materials, significantly higher concentrations at the glassworks sites than at the reference areas were also found for all six metals (all t-test, $p > 0.05$).

However, only a small part of the analysed total metals were found to be easily water-soluble (Table 2). Of the potential contaminant metals, water-soluble concentrations were determined for As, Cd, Pb and Zn (but not Ba and Sb). Pb and As were significantly higher in water extracts from landfill soils than from reference areas (*t*-test, $p = 0.003$ and < 0.001, respectively), even though only 0.02% and 0.38% of the total concentrations were water extractable. At the reference areas, the average concentration of water extractable Pb was 40 µg kg^{-1} and the corresponding value for As was 201 µg kg^{-1} (Table 2 and Supplementary material II). The water extractable concentrations at the landfill sites were 447 µg kg^{-1} (Pb) and 989 µg kg^{-1} (As), i.e. concentrations were on average only 11 and 5 times (respectively) higher in water extracts derived from the landfill soils. The average concentration of Cd in the water extracts was ca 20 times higher in landfill sites (ca 30 µg kg^{-1}) compared to reference sites (ca 1.5 µg kg^{-1}), but due to the high variation the difference was not statistically significant (paired *t*-test, $p = 0.113$). The concentrations of Zn-64 did not vary between landfill and reference sites (paired *t*-test, $p > 0.05$), but this was also the metal with the least obvious contamination impact.

Comparing the other investigated soil geochemical parameters between the landfill and reference areas, significant differences were observed for pH in Johansfors (independent *t*-test, $p < 0.05$). In addition, soil OM and nutrient contents varied between sites but no clear trend was shown (paired *t*-test, $p > 0.05$) (Table 1). The EC was significantly different, being ca 50% higher in landfill sites (paired *t*-test, $p = 0.003$) (Table 1), but overall, the conductivity of all soils was quite low.

In summary, although both the basic soil geochemistry and metal concentrations were highly variable, significant differences were seen only for the metal concentrations and EC.

Table 1. Soil metal and nutrient (N, C, P) concentrations (mg kg^{-1}; mean ± standard error; n = 5) and characteristics at landfill and reference sites. Electric conductivity (EC in **ms cm^{-1}** and the dry- and organic matter content (DM and OM) in **g g^{-1}**. The first, and lowest, of the two generic guideline values (mg kg^{-1}) of the Swedish EPA (2009) corresponds to the maximum permissible metal concentration in areas with sensitive land use. Concentrations above this level may, among other negative effects, result in negative impacts on soil ecosystem functions. The higher value represents the corresponding figure for land used for industrial purposes (where some reduction in soil functions is accepted). Values in bold highlight metal concentrations that exceed the generic guideline values for sensitive land use.

	Guideline	Bergdala	Bergdala ref.	Johansfors	Johansfors ref.	Kosta	Kosta ref.	Målerås	Målerås ref.	Orrefors	
As	**10/25**	**180±82**	3.0±0.53	**2200±1400**	11±1.2	**380±99**	2.5±0.50	**500 ±190**	8.4 ± 2.3	**170 ± 37**	2.7 ± 0.49
Ba	**200/300**	180±57	34±4.8	**1300±640**	51±9.1	**470±88**	41±5.7	**1100 ±530**	91 ± 15	**220 ± 50**	120 ± 21
Cd	**0,5/15**	**13±6.0**	0.23±0.03	**4.4±1.8**	0.18±0.02	**6.5±4.0**	0.16±0.02	**5.5±2.2**	0.38 ± 0.08	**19 ± 11**	0.30 ± 0.30
Cu	**80/200**	24±2.7	27±19	62±9.1	8.4±0.48	**140±75**	8.4±1.2	42±14	21 ± 2.2	48 ± 16	16 ± 1.7
Pb	**50/400**	**800±380**	19±2.4	**12000 ±6900**	**62±13**	**7900±2100**	30±5.5	**1200±670**	**100 ± 26**	**7000 ± 2200**	45 ± 12
Sb	**12/30**	**28±7.2**	0.58±0.15	6.9±2.9	1.0±0.21	**31±5.7**	0.41±0.05	**17±5.9**	1.4 ± 0.34	3.4 ± 1.0	0.41 ± 0.03
Zn	**250/500**	**330±100**	26±7.8	**430±180**	20±3.7	**350±42**	36±4.8	**340±85**	170 ± 41	**420 ± 170**	72 ± 7.6
Tot-P	**-**	510±43	360± 65	3800±1700	280±49	1100±1200	680±100	3100±1300	680 ± 74	420 ± 80	1800 ± 240
Tot-N	**-**	3500±830	2300±280	1700±920	2100±730	3300±430	2200±370	1900±370	2800 ± 360	700 ± 290	2900 ± 200
Tot-C	**-**	73000±19000	35000±5900	42000±25000	57000±23000	82000±1400	32000±4200	68000±14000	47000±5600	14100 ± 4900	40000 ± 1900
C:N	**-**	21±0.56	15±1.1	24±1.3	27±1.5	25±2.6	14±1.1	36±12	17 ± 0.31	20 ± 5.2	14 ± 0.64
EC	**-**	0.12±0.01	0.04±0.01	0.09±0.02	0.04±0.01	0.10±0.02	0.06±0.01	0.14±0.01	0.07±0.01	0.06±0.01	0.05±0.01
DM	**-**	0.85±0.02	0.86±0.02	0.91±0.04	0.82±0.02	0.79±0.02	0.88±0.02	0.78±0.03	0.82±0.02	0.90±0.02	0.86±0.01
OM	**-**	0.13±0.03	0.06±0.01	0.10±0.07	0.09±0.02	0.11±0.02	0.06±0.01	0.11±0.01	0.07±0.02	0.02±0.01	0.06±0.003
pH	**-**	5.9±0.05	5.9±0.09	7.1±0.04	5.3±0.50	6.2±0.10	6.0±0.11	6.7±0.09	6.7±0.12	5.9±0.08	5.9±0.10

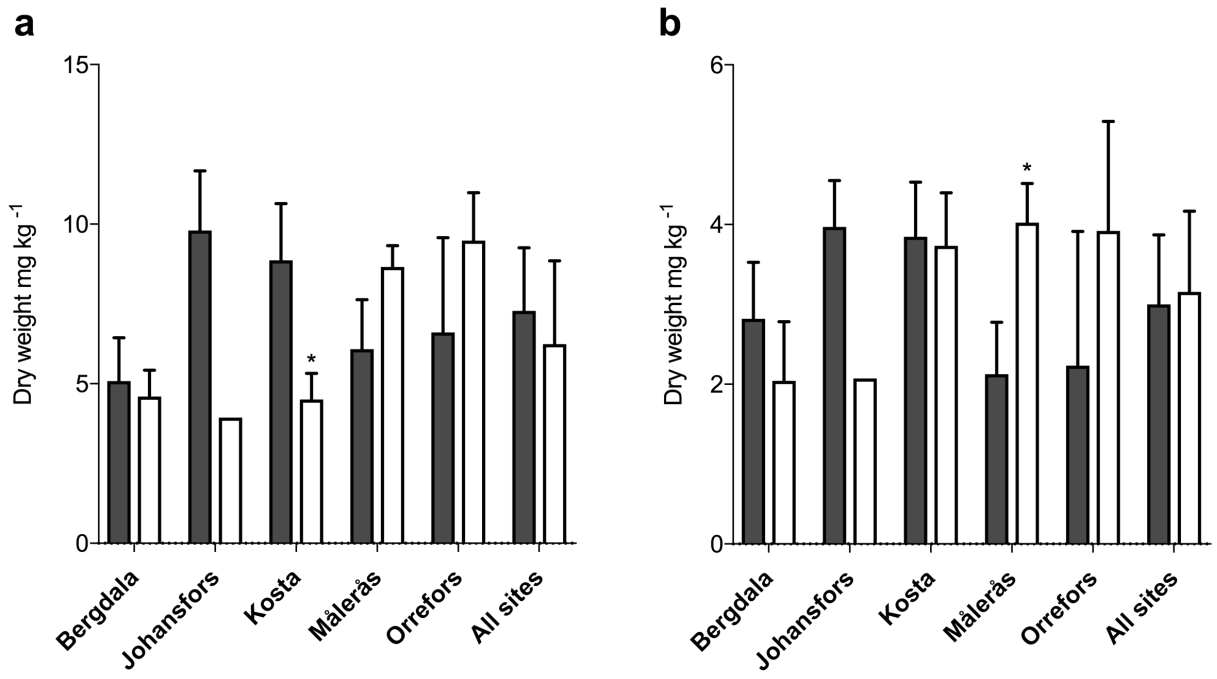
Table 2. Concentrations of H_2O extractable metals (mean± standard error), expressed per kg dry weight of the soil (µg kg^{-1}).

	Bargdala	**Bergdala ref.**	**Johansfors**	**Johansfors ref.**	**Kosta**	**Kosta ref.**	**Målerås**	**Målerås ref.**	**Orrefors**	**Orrefors ref.**
	H_2O extractable metals in soils (µg kg^{-1})									
As	794 ± 222	208 ± 14	1072 ± 333	226 ± 28	1482 ± 498	228 ± 21	1214 ± 301	205 ± 24	382 ± 36	139 ± 5.6
Cd-112	29 ± 8.3	1.2 ± 0.18	1.5 ± 0.60	0.83 ± 0.12	9.9 ± 6.2	0.89 ± 0.12	0.6 ± 0.18	0.70 ± 0.18	111 ± 91	3.8 ± 2.0
Pb-sum	152 ± 41	13 ± 4.1	1006 ± 376	68 ± 31	544 ± 147	36 ± 11	20 ± 6.4	53 ± 29	512 ± 369	27 ± 10
Zn-64	462 ± 169	195 ± 50	530 ± 325	651 ± 107	106 ± 34	262 ± 63	356 ± 70	465 ± 55	511 ± 246	224 ± 72

Plant and microbe toxicity

In the plant toxicity test with *L. sativum,* the results varied greatly between sampling sites. In Bergdala and Orrefors, for example, there was a lack of difference between biomass production of plants growing on soils from the contaminated glassworks areas and those from reference areas (Fig. 2; paired *t*-test, $p > 0.05$). In Kosta, the plants cultivated in the landfill soil samples had a higher *root* biomass than those grown in the reference soils (independent *t*-test, $p > 0.05$) (Fig 2a), whereas in Malerås, the *shoot* biomass of plants growing in soil from the landfill was reduced (independent *t*-test, $p < 0.05$) (Fig 2b). When analysing all sites together, no difference between landfill and reference sites were observed. The same results applied both to the shoot biomass and the root biomass (paired *t*-test, $p = 0.933$, $p = 0.384$).

However, regression analysis showed a positive correlation between *shoot* weight and soil H_2O-Pb ($R^2 = 0.300$, $F = 13.047$, $p = 0.028$) and P concentrations ($R^2 = 0.519$, $F = 9.018$, $p = 0.030$).



306

307 **Fig. 2.** Biomass production of a) roots and b) shoots of *L. sativum* at the soils from the glassworks
308 landfill areas (grey) and those from reference areas (white). Each column represents mean values of
309 five samples (with ± standard error bars, n = 5). An asterisk denotes statistical significance between
310 the glassworks soil samples and reference samples ($p<0.05$).

311

312

313

314 **Table 3.** Results of linear regression analysis (Stepwise model).

Variable	Predictors in model	β	*p*-value	R^2	R
Number of enchytraeids	H_2O-Pb	-0.450	0.005	0.202	0.450
Biomass of enchytraeids	H_2O-Pb	-0.408	<0.001	0.286	0.535
	H_2O-As	-0.343	<0.001	0.388	0.623
Number of nematodes	Tot-Pb	-0.279	0.050	0.078	0.279
	Tot-As	0.755	0.004	0.206	0.453
Shoot weight of *L. sativum*	H_2O-Pb	0.548	0.028	0.300	0.548
	Tot-P	0.468	0.030	0.519	0.720

315

316

317

318 In the BioTox™ tests, the elutriates from both the metal contaminated landfill and reference soils
319 exposed very low toxicity to *V. fischeri* with no differences between reference and landfill sites
320 (Wilcoxon, all sites, $p = 0.345$). In addition, high variation between sampling sites was observed
321 (Fig. 3). Out of the tested landfill sites, only the landfill soil from Kosta reduced light emission of

V. fisheri in comparison to its reference soil (independent *t*-test, $p = 0.038$). Both landfill and reference soils from Målerås were biostimulative, increasing the light emission of *V. fisheri*; the soils from the landfill more so than reference soils (independent $p = 0.008$). No statistically significant correlation between H_2O extracted metals and INH% was shown in the regression analysis (Stepwise model).

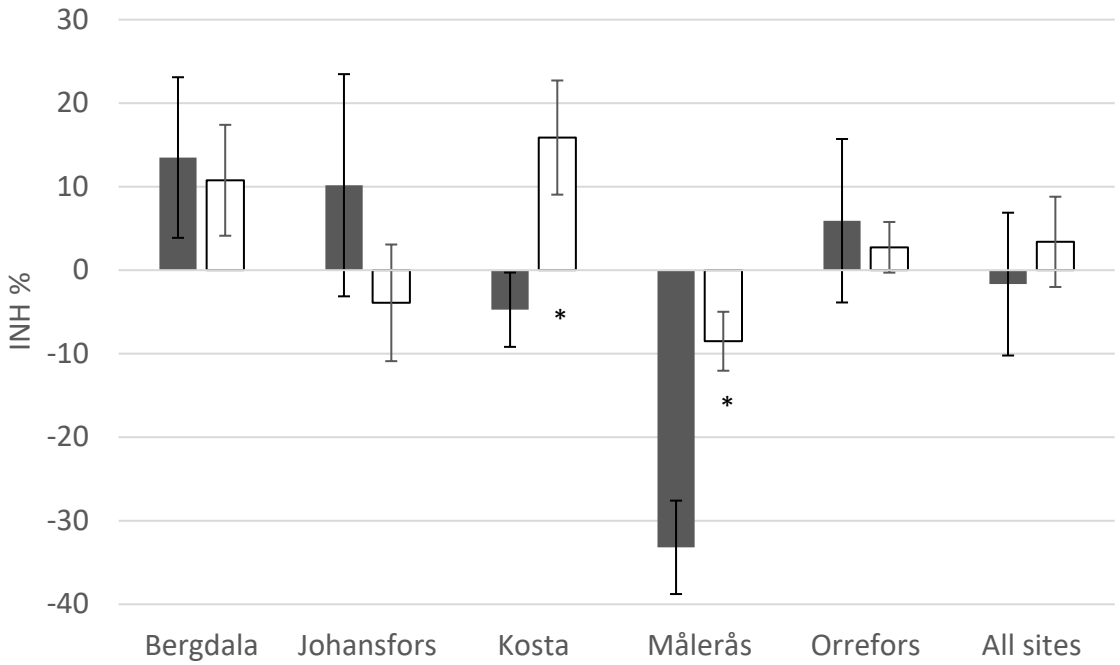


Fig. 3. The luminescence inhibition percent (INH%) of water elutriates from glassworks landfill soils (grey) and from reference areas (white) for *V. fischeri*. Mean ± standard error, n = 5. Negative values indicate increased light emission/biostimulation. An asterisk denotes statistical significance (with $p < 0.05$).

Soil organisms

In the soil samples from the contaminated landfill sites, 13 out of 25 samples were totally lacking enchytraeids. In comparison, only one sample from the reference areas was without enchytraeids. The number and biomass of enchytraeids were significantly reduced in landfill sites of Bergdala, Johansfors and Kosta (*t*-test, $p < 0.05$). (Fig. 4). Consequently, the total biomass of enchytraeids (expressed per g OM) was significantly lower in landfill sites (paired *t*-test including all sites,

$p = 0.016$), being on average 17.7 µg g^{-1} OM in the landfill soils and 44.1 µg g^{-1} OM in the reference soils. Also average biomass of enchytraeid individuals was reduced in glasswork landfill sites (average weight 17.8 µg) compared to reference sites (average 28.1 µg), with the exception of Bergdala (paired *t*-test including Bergdala $p = 0.337$, excluding Bergdala $p = 0.011$).

According to the regression analysis (stepwise; Table 3), none of the following analysed parameters could alone explain the difference in number and biomass of enchytraeids: 1) total concentrations of different metals (neither of As, Ba, Cd, Pb, Zn, Sb or any other of the metals listed in Supplementary material I), 2) total concentrations of nutrients (N, C or P), or 3) soil characteristics (pH, dry or OM content, EC). The multiple regression analysis showed that 20.2% and 28.6% of the observed variation in number and biomass (respectively) of enchytraeids could be explained by H_2O-Pb (number: $R^2 = 0.202$, $F = 8.870$, $p = 0.005$ and biomass: $R^2 = 0.286$, $F = 18.425$, $p > 0.001$). In addition, ca 10% of reduced enchytraeid biomass can be explained by H_2O-As (increase in the coefficient of determination by 10%; $R^2 = 0.388$, $F = 14.242$, $p < 0.001$).

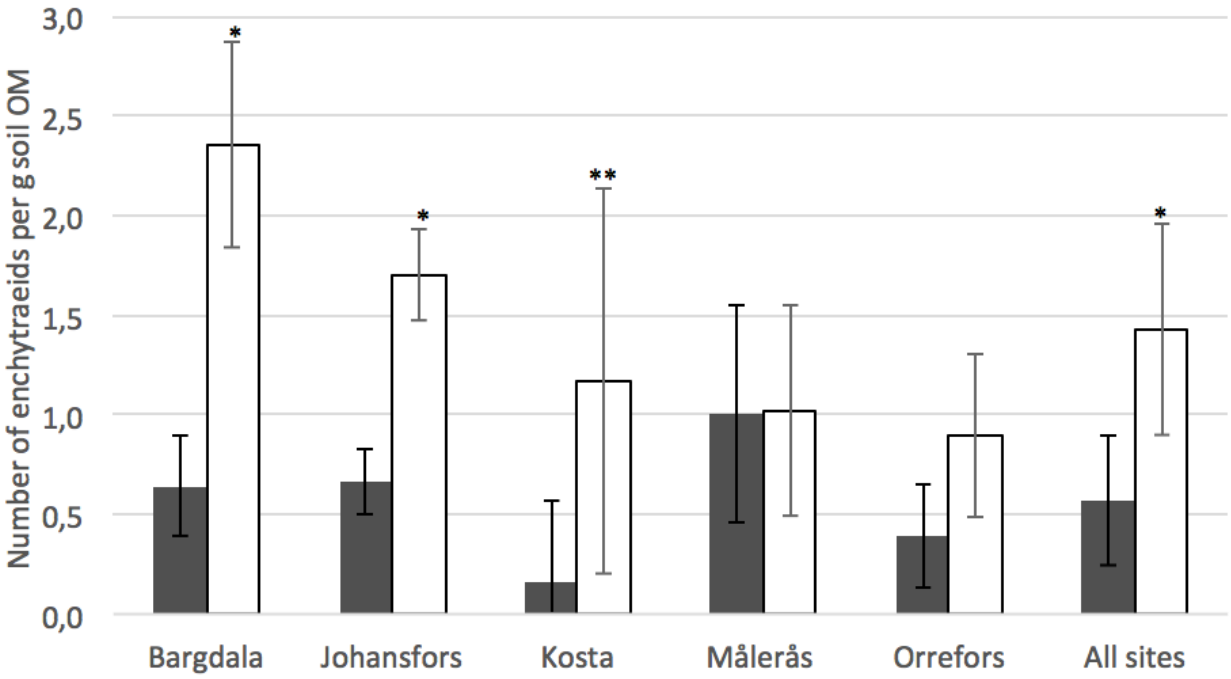


Fig. 4. Number of enchytraeids per g soil OM from the glassworks landfill areas (grey) and those from reference areas (white). Mean ± standard error, n = 5. Asterisks denote statistical significance (* <0.05, ** <0.01).

Also the number of nematodes tended to be reduced in the landfill soils, but the difference was statistically significant only in Målerås (independent *t*-test, $p = 0.038$; Fig. 5). When analyzing all sites together, no statistically significant difference between landfill and reference soils was observed (paired *t*-test, $p = 0.087$). The average number of individuals in landfill soils was 54.9 g^{-1} OM, compared to 74.4 individuals per g OM in reference sites (Fig. 5). According to the regression analysis (Table 3), 27.9% of the observed variation in the numbers of nematodes could be explained by tot-Pb ($R^2 = 0.078$, $F = 4.060$, $p = 0.050$). In addition, 22% of the reduction in nematode numbers could be explained by tot-As (increase in the coefficient of determination $R^2 = 0.276$, $F = 6.084$, $p = 0.004$).

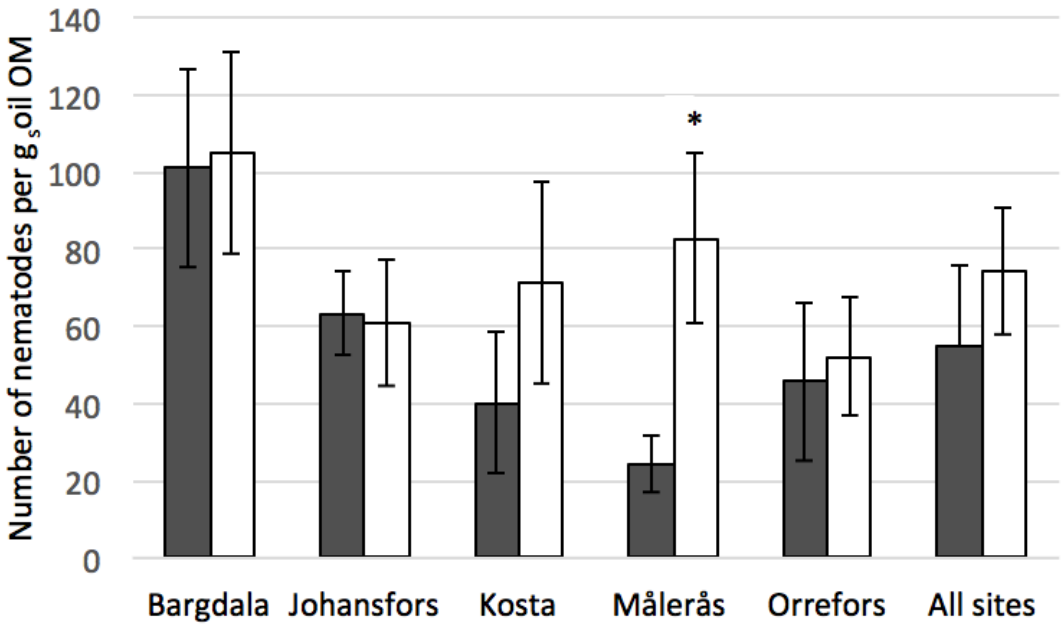


Fig. 5. Number of nematodes per g soil OM from the glassworks landfill areas (grey) and those from reference areas (white). Mean ± standard error, n = 5. An asterisk denotes statistical significance (with $p < 0.05$).

Discussion

The assessment of potential risks to ecological functions and humans living around contaminated sites is a task that is as important as it is complex and challenging. In Sweden alone, there are approximately 85,000 potentially contaminated sites. Since remediation of all these sites is not feasible in the near future, addressing the national goal of a non-toxic environment requires understanding of actual risks for humans and ecosystems around contaminated sites. One way of achieving this, in Sweden as well as elsewhere, may be to include relevant biotests in generic risk assessment methodologies. Several studies have shown that biotests give a better indication of metal bioavailability and ecological risks than chemical analyses alone (Kahru et al. 2005; Zhang et al. 2013). The tests used and evaluated in the present study have all been proven useful in other studies:

Firstly, the biomass production (root and shoot weight) of garden cress (*L. sativum*) has been found a suitable indicator for the evaluatiton of potential phytotoxicity to vascular plants due to its well-documented sensitivity to metal contaminants (Baderna et al. 2015; Gill et al. 2012; Montvydiene and Marciulioniene 2004; Vasile et al. 2013). Secondly, the measurement of luminescence inhibition in *V. fischeri* by the BioTox™ method is a well established and standardized approach. Briefly, the metabolic pathway responsible for light emission by the bacterium is intrinsically linked to cellular respiration, and any disruption of normal cellular metabolism causes a decrease in light production (Parvez et al. 2006). Finally, enchytraeid worms (mostly omnivorous) and nematodes (covering several trophic positions) are often selected as test biota for toxicity tests (Kapusta and Sobczyk 2015). They are present in a wide range of ecosystems, occur abundantly, play a key role in the functioning of soil ecosystems, are easy to use, collect and culture, come into contact with a variety of stress factors (the soil solution, the solid phase, and the gaseous phase in soil), and are sensitive to environmental stresses (Didden and Römbke 2001; Römbke and Moser 2002). The indicator capacity of enchytraeids and also nematodes has been proved in several laboratory (Didden and Römbke 2001) and field experiments (Hui et al. 2009; Höss and Williams 2009; Selonen et al. 2014).

When applied to the five glassworks sites of this study and their reference sites, we saw that the different tests were not consistent, and that there was a large variability between sites. For example, the number of enchytraeids and nematodes was consistently lower at the landfill areas (Fig 4+5), indicating *in-situ* toxicity to soil inhabiting organisms. But the *L. Sativum* biomass production (Fig

2) and luminescence inhibition in *V. fischeri* (Fig. 3) were higher at some of the glassworks sites compared to the corresponding reference sites and lower at others, resulting in no overall statistical difference. Similar site specific inconsistencies between different tests were observed by Karjalainen et al. (2009) regarding CCA-contaminated soil samples. Thus, our results clearly demonstrate that risk assessments of contaminated sites are often complicated due to several influencing factors in the field.

Chosing relevant biotests for assessing soil toxicity

Most previous studies have focused on the impact of single pollutants (Kapusta and Sobczyk 2015). In reality, many contaminated sites are affected by mixtures of pollutants. Considering landfill sites, the composition of the waste material may also be quite different even in two adjacent soil volumes – something which was clearly manifested by the large variability in, for example, total metal concentrations at single landfill sites of our study. In addition, different subsamples - even from the same site - may differ substantially when it comes to basic geochemical properties (i.e. redox pontial, pH, average grain size, content of sorbants such as organic particles or Al/Fe/Mn hydroxides). Large variabilities between subsamples regarding leachability/bioavailability and toxicity are therefore to expect. From such a perspective, the consistent decline in enchytraeid and nematode abundance and size constitutes a relatively robust indication of the toxic properties of the glassworks soils, despite the low number of samples analyzed. The lack of measurable effects using the acute toxicity tests with bacteria and plants, however, indicated that these tests were not appropriate for the studied environment. As concluded by Waara et al. (2009), the detectability of toxicity in challenging environments depends on site specific conditions and the test organism chosen.

Toxicity not implied by *V. fisheri* or *L. sativum*

One possible explanation for the lacking inhibition in both *V. fisheri* luminescence and in *L. sativum* growth is obviously that the glassworks soils don't leach metal contaminants to such a degree that the soil pore water becomes toxic enough. In favor of this hypothesis are the results which showed a low extractability when the highly polluted soils were shaken with destilled water.

However, one must also consider the possibility of these tests not being suitable for the evaluation of toxicity of glassworks contaminated soils. Regarding the evaluated plant growth, the positive

connection observed between soil metal contamination (in particular of Pb) and shoot growth of *L. sativum* was contrary to what would be expected if soil contamination was limiting plant growth. However, the increase in shoot weight at moderately elevated bioavailable concentrations is probably due to the hormesis effect. Hormesis, which is a dose–response relationship that is characterized by low-dose stimulation and high-dose inhibition, has previously been observed in plants growing on metal contaminated soils (Wang et al. 2010; Calabrese and Blain 2009). Baderna et al. (2015), for example, investigated the phytotoxicity of different metals (As, Cd, Cr, Pb, Hg, Ni and Zn) alone and in mixtures using *L. sativum* and two others plants, and found a clear hormesis dose–effect relationship. As they highlighted, the biostimulation observed at moderately elevated bioavailable concentrations may be a potential alert flag because it could be the initial adaptive response to low doses of one or several toxicants, where the initial stimulation could turn into strong toxicity at longer exposure times or higher toxicant doses.

Regarding the lacking effect on *V. fisheri* luminescence, the first important note to make is that water extracts produced in batch leaching tests may not adequately mirror the true pore water composition of the site under investigation. Dissolved metal concentrations may be significantly higher in the field (Petänen et al. 2001; Petänen and Romantschuk 2003), for example due to longer contact times and lower L/S ratios. In addition, while the leachability was low with distilled water in our study, a sequential extraction of samples from the fine fraction (< 2 mm) of another glassworks landfill of the region showed that 15–35% of the total Pb concentrations and 40–60% of the total Cd were dissolved in the first step (using 1 M CH_3COONa) (Augustsson et al. 2016). This step (at least theoretically) targets metals that are relatively easily assessible; associated with pore water of the fresh soil and including ions that are weakly sorbed by ion exchange and associated with carbonate complexes (Hall et al. 1996; Kersten and Förstner 1989). Beside the uncertainties in the representativety of the water extracts, it has also been found that bacteria may develop resistance in metal-contaminated enviroments. Their suitability as proxies for metal contamination is thus indistinct. For example, tests made at a heavily Pb contaminated shooting range in southern Finland showed that the frequency of Pb resistant bacteria increased in metal polluted soils, but that neither the bacterial numbers nor the community profile was significantly altered (Hui et al. 2012). Also plants appeared to be uneffected by the high Pb levels at these sites (Hui et al. 2009, 2011, 2012). When compared to laboratory experiments of bacterial luminescence inhibition, however, plant growth experiments (direct exposure) have been suggested much more sensitive in assessing soil toxicity (Alvarenga et al. 2008). In tests using Hg- and As-sensing bacteria, the conclusion made by Petänen and Romantschuk (2003) was that some soil bacteria mobilize heavy metals,

489 which means that soil animals ingesting these bacteria, like enchytraeids and nematodes, could
490 become exposed. While soil microbes become resistant to heavy metals and plants are able to close
491 them out from the root tissue, soil fauna that ingest detritus including bacterial and fungal cells
492 become exposed to heavy metals in their food. That is another possible explaintion for the response
493 seen in enchytraeids (and nematodes), and the lack of such in our indicator bacteria.
494
495 1.2. Toxicity implied by enchytraeids and nematodes
496
497 It has, reasonably, been argued that the most relevant risk assessment should be based on the
498 evaluation of effects in soil organisms that are exposed to contaminants in their real habitats
499 (Markert et al. 2003). *In situ* risks are always affected by a cascade of factors (such as soil texture,
500 geochemistry, and hydrology) that may mitigate or amplify the negative effects from contaminants.
501
502 The observed low *in situ* frequency and decreased biomass of enchytraeids (and nematodes) in the
503 metal contaminated soils compared to reference soils, imply that the decomposer community did
504 suffer from the heavy metal contamination. In our case, where we didn't reach the same conclusion
505 from the tests with *V. fisheri* and *L. sativum*, it may be that enchytraeids and nematodes are more
506 sensitive in general, or simply that they give a better reflection of the true field conditions. While
507 the hormesis effect may complicate the interpretation of *L. sativum* experiments and soil bacteria
508 may develop tolerance against high metal concentrations, previous studies agree on enchytraeids
509 being sensitive to metal contamination (Salminen et al. 2001a, b; Hui et al. 2009), in particular to
510 Pb (Didden and Römbke 2001). For example, Karjalainen et al. (2009) and Selonen (2015)
511 observed reduced numbers of enchytraeids and nematodes extracted from metal contaminated soils
512 compared to similar pristine forest soil in southern Finland. Also Haimi and Mätäsniemi (2002)
513 showed that close to a metal emission source in central Finland the numbers of enchytraeids and
514 nematodes were clearly decreased. Even though much of the Pb is relatively immobile, high tot-Pb
515 concentrations may still be harmful to soil organisms (Selonen et al. 2012). Such an effect is
516 suggested in our study too, since the multiple regression analysis showed a connection between soil
517 H_2O-Pb and also H_2O-As and the numbers and biomass of enchytraeids. Also, reduced numbers of
518 nematodes seems to be dependent on the concentrations of Pb and As in the soil. As the metals at
519 the glassworks sites occur as complex mixtures with variable/covariable metal concentrations, we
520 cannot determine the effect of each metal separately. Furthermore, since the concentrations of
521 single metals had quite low explanatory power on the observed changes, the effect seen is probably
522 a result of multiple stressors having an additive effect. One possible explanation for the larger

impact seen on enchytraeids than nematodes may be that the summer of 2015 was unusually dry in south-eastern Sweden. Enchytraeids are more sensitive to changes in soil moisture than nematodes are (Lindberg et al. 2002), and the dry conditions may have increased the enchytraeids' sensitivity also for other environmental stress factors.

To sum up, the enchytraeids and nematodes were in this study found to be the most reliable bioindicator species. We suggest therefore – in line with Kapusta and Sobczyk (2015) – that enchytraeid and nematode worm density and biomass are to be used as a proxies of soil quality in metal polluted soils together with chemical analyses of the total and 'bioavailable' fraction.

Conclusions

In understanding health and environmental risks at contaminated sites, the issues of bioavailability and toxicity are of key relevance. Despite high total concentrations of several toxic metals (primarily Sb, As, Ba, Cd, Pb and Zn) at five examined glassworks sites in south-eastern Sweden, the toxicity of the glassworks soils was not revealed by indirect tests; inhibition of light emission by *V. fischeri* could not be seen, nor was an effect seen on the growth of *L. sativum*. That the soils can be toxic to organisms was, however, shown by the decrease in enchytraeid abundance and biomass in these soils compared to reference soils collected nearby. Direct observation of soil fauna is therefore the better proxy for *in situ* toxicity. In this case they confirm the results from previous chemical leaching tests of glassworks soils, which have indicated that a significant fraction of the metals may be available for biological uptake. The confirmation of *in situ* bioavailability to soil-inhabiting organisms also motivates additional studies of the risk posed to humans of the glassworks villages, who consume local foodstuffs and/or drink water from private wells.

Acknowledgement

We thank Juha Mikola (Department of Enviromental Sciences, University of Helsinki, Lahti, Finland) for advice on the statistical analyses and Jukka Pellinen (Almalab) for participation in chemical analyses. The work was supported by the Faculty of Health and Life Sciences at Linnaeus University, Kalmar, Sweden.

Supplementary material I. Concentrations (minimum, median and maximum values) of total metals (mg kg^{-1}) in the studied glass landfill sites.

		Bergdala	Johansfors	Kosta	Målerås	Orrefors
As	min	76.4	96.6	228	64.1	73.9
	max	503	7790	764	1180	241
	median	96	473	300	331	228
Ba	min	67.3	30.2	188	337	94.1
	max	393	3560	668	3110	384
	median	143	1350	435	416	197
Cd	min	3.3	0.2	1.7	1.6	1.3
	max	36.7	8.2	22.4	13.9	62.8
	median	9.9	5.2	2.3	3.8	10.0
Co	min	1.4	3.2	3.9	0.7	1.2
	max	3.7	7.9	6.2	8.6	5.7
	median	2.2	5.4	4.9	2.9	2.8
Cr	min	4.4	3.5	8.0	3.6	2.9
	max	39.9	18.0	31.1	12.9	7.1
	median	6.2	14.2	12.7	7.3	5.8
Cu	min	16.4	31.4	39.3	15.6	17.3
	max	32.1	79.8	430	89.4	108
	median	24.0	68.2	54.5	26.7	30.8
Hg	min	< 0.1	< 0.1	0.4	< 0.1	< 0.1
	max	< 0.1	< 0.1	0.9	–	< 0.1
	median	< 0.1	< 0.1	0.5	2.0	< 0.1
Ni	min	2.8	2.7	7.1	1.4	1.8
	max	6.9	19.8	15.0	22.9	7.4
	median	4.2	8.5	11.1	7.2	4.7
Pb	min	161	981	4520	539	1530
	max	2030	38 000	16 000	4210	13 000
	median	263	4480	6240	1290	6480
V	min	6.3	7.1	10.4	1.9	2.4
	max	19.2	14.4	21.4	18.3	10.3
	median	8.7	11.4	16.6	6.6	7.0
Zn	min	142	45.0	197	101	76.9
	max	710	1050	441	589	1040
	median	301	422	354	275	419

Ag	min	0.1	0.1	0.5	0.1	0.1
	max	0.1	3.0	1.7	0.4	0.6
	median	0.1	0.9	1.3	0.2	0.2
Mo	min	0.4	0.4	1.8	0.5	0.3
	max	0.9	2.1	5.0	1.5	1.1
	median	0.6	0.9	2.4	1.0	0.8
Sb	min	15.8	1.1	16.6	2.6	0.4
	max	55.7	15.2	51.4	38.6	6.4
	median	23.2	3.7	30.2	15.4	3.8
Sn	min	1.1	1.7	3.5	1.7	1.1
	max	2.3	17.2	22.6	5.7	2.7
	median	1.6	6.0	6.0	3.9	2.0

556

Supplementary material II. Concentrations (minimum, median and maximum values) of H_2O extractable metals (mg kg^{-1}) in the studied glass landfill soils.

		Bargdala	Johansfors	Kosta	Målerås	Orrefors
As	min	0.316	0.328	0.573	0.613	0.272
	max	1.559	1.922	3.416	2.219	0.497
	median	0.565	1.019	1.084	1.091	0.383
Cd-112	min	0.012	0.001	0.002	0.000	0.003
	max	0.059	0.003	0.034	0.001	0.475
	median	0.026	0.001	0.003	0.000	0.030
Zn-64	min	0.126	0.158	0.030	0.356	0.050
	max	0.936	1.504	0.183	0.356	1.275
	median	0.249	0.229	0.106	0.356	0.209
Pb-sum	min	0.060	0.298	0.226	0.011	0.063
	max	0.304	1.922	0.908	0.045	1.971
	median	0.143	0.902	0.435	0.014	0.101

560 References

561

562 Abrahamsen, G. 1973: Studies on body-volume, body-surface area, density and live weight of
563 Enchytraeidae (Oligochaeta). – Pedobiologia 13: 6-15.
564 Alvarenga, P., Palma, P., Goncalves, A.P., Frnandes, R.M., de Varennes, A., Vallini, G., Duarte, E.,
565 Cunha-Queda, A.C. 2008. Evaluation of tests to assess the quality of mine-contaminated soils.
566 Environ Geochem Health 30: 95-99 doi: 10.1007/s10653-008-9147-z.
567 Augustsson, A., Åström, M., Bergbäck, B., Elert, M., Höglund, L.O., Kleja, D.B. 2016. High metal
568 reactivity and environmental risks at a site contaminated by glass waste. Chemosphere 154:
569 434-443
570 Augustsson, A.L.M., Uddh-Söderberg, T.E., Hogmalm, K.J., Filipsson, M.E.M. 2015. Metal uptake
571 by homegrown vegetables – the relative importance in human health risk assessments at
572 contaminated sites. Environ Res 138: 181-190
573 Baderna, D., Lomazzi, E., Pogliaghi, A., Ciaccia, G., Lodi, M., Benfenati, E. 2015. Acute
574 phytotoxicity of seven metals alone and in mixture: Are Italian soil threshold concentrations
575 suitable for plant protection? Environ Res 140: 102-111
576 Bergelin, A., Jansson, M., Tapper, M., Bylin, S. 2006. Miljötekniska undersökningar Etapp 1 - 13
577 Orrefors. Geosigma AB, Uppsala. In Swedish.
578 Boularbah, A., Schwartz, C., Bitton, G., Morel, J.L. 2006a. Heavy metals contamination from
579 mining sites in south Morocco: 1. Use of biotest to assess metal toxicity of tailings and soils.
580 Chemosphere 63:802–810. doi: 10.1016/j.chemosphere.2005.07.079
581 Boularbah, A., Schwartz, C.H., Bittonm G., Aboudrarm W., Ouhammoum A., Morelm J.L. 2006b.
582 Heavy metal contamination from mining sites in south Morocco: 2. Assessment of metal
583 accumulation and toxicity in plants. Chemosphere 63:811–817. doi:
584 10.1016/j.chemosphere.2005.07.076
585 Calabrese, E.J., Blain, R.B. 2009. Hormesis and plant biology. Environ Poll 157: 42-48
586 Czerniawska-Kusza, I., Kusza, G. 2011. The potential of the Phytotoxkit microbiotest for hazard
587 evaluation of sediments in eutrophic freshwater ecosystems. Environ Monit Assess 179:113–
588 121. doi: 10.1007/s10661-010-1722-y.
589 Didden, W., Römbke, J. 2001. Enchytraeids as indicator organisms for chemical stress in terrestrial
590 ecosystems. Ecotox Environ Safety 50: 25−43.
591 Dubova, L., Zarina, D. 2004. Application of toxkit microbiotest for toxicity assessment in soil and
592 compost. Environ Toxicol.19 :274-279 doi: 10.1002/tox.20034

593 Falk, T., Fredriksson, H., Holmér, G., Johansson, L, G., Lang, M, Sundberg, P. 2005. Boken om
594 glas. 1. Uppl. Växjö: Glafo cop. In Swedish
595 Fanger, G., Höglund, L.O., Jones, C., Svensson, H. 2004. Undersökning och fördjupad
596 riskbedömning av fem glasbruk i Kalmar och Kronobergs län samt förslag på generell metodik
597 för riskbedömningar vid glasbruk. Kemakta AR 2003-07, Stockholm. In Swedish.
598 Garcia-Lorenzo, M.L., Martinez-Sanchez, M.J., Perez-Sirvent, C., Molina, J. 2009.
599 Ecotoxicological evaluation for the screening of areas polluted by mining activities.
600 Ecotoxicology 18: 1077–1086. doi: 10.1007/s10646-009-0362-x.
601 Gill, S.S, Khan, N.A., Tuteja, N. 2012. Cadmium at high dose perturbs growth, photosynthesis and
602 nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant
603 machinery in garden cress (*Lepidium sativum* L.), Plant Sci 182: 112-120
604 Haimi, J., Mätäsniemi, L. 2002. Soil decomposer animal community in heavy-metal contaminated
605 coniferous forest with and without liming. Eur J Soil Biol 38: 131–136
606 Hall, G.E.M., Vaive, J.E., Beer, R., Hoashi, M. 1996. Selective leaches revised, with emphasis on
607 the amorphous Fe oxyhydroxide phase extraction. J Geochem Explor 56: 59-78
608 Hermelin, C., Welander, E. 1986. Glasboken – Historia, teknik och form, Centraltryckeriet AB,
609 Borås. In Swedish.
610 Hjortenkrans, D. S. T., Månsson, N. S., Bergbäck, B. G., Häggerud, A.V. 2009. Problems with Sb
611 analysis of environmentally relevant samples. Environ Chem 6: 153-159
612 Hui, N., Selonen, S., Hanzel, J., Tuomela, M., Rainio, A., Kontio, H., Hakala K., Lankinen, P.,
613 Steffen, K., Fingerroos, T., Strömmer, R., Setälä, H., Hatakka, A., Romantschuk, M. 2009.
614 Influence of lead on organisms within the detritus food web of a contaminated pine forest soil.
615 Boreal Environ Res 14 : 70-85
616 Hui, N., Jumpponen, A., Niskanen, T., Liimatainen, K., Jones, K., Koivula, T., Romantschuk, M.,
617 Strömmer, R. 2011. EcM fungal community structure, but not diversity, altered in a Pb-
618 contaminated shooting range in a boreal coniferous forest site int Southern Finland. FEMS
619 Microbiol Ecol 76: 121–132
620 Hui, N., Liu, X., Kurola, J., Mikola, J., Romantschuk, M. 2012. Lead (Pb) contamination alters
621 richness and diversity of the fungal, but not the bacterial community in pine forest soil. Boreal
622 Environ Res 17: 46-58
623 Håkansson, K., Ländell, M. 2006. Miljötekniska undersökningar Etapp 1 - 16/17 Kosta Glasbruk
624 och glasbruksdeponi. Geo Innova, Linköping. In Swedish.

625 Höss, S., Williams, P.C. 2009. Ecotoxicity testing with nematodes. In book: Wilson, M.S., Kakouli-
626 Suarte, T. (Ed.) Nematodes as environmental indicators. CAB International, London, Uk. pp-
627 208-224
628 Kahru, A., Ivask, A., Kasemets, K., Llumaa, L., Kurvet, I., Ois, F.M., Dubourguier, H. 2005.
629 Biotests and biosensors in ecotoxicolgical risk assessment of field soils polluted with zinc, lead,
630 and cadmium. Environ Toxicol Chem 24: 2973–2982. doi: 10.1897/05-002R1.1
631 Kalmar County Administrative Board, 2016. Prioritized contaminated sites in the county.
632 http://www.lansstyrelsen.se/kalmar/sv/miljo-och-klimat/verksamheter-med-
633 miljopaverkan/ebh/lanets-mest-fororenade-omraden-prioriteringslista/Pages/default.aspx .
634 Assessed April 13/2016.
635 Kapusta P., Sobczyk Ł. 2015. Effects of heavy metal pollution from mining and smelting on
636 enchytraeid communities under different land management and soil conditions. Sci Total
637 Environ **536:** 517–526
638 Karjalainen, A.M., Kilpi-Koski, J., Väisänen, A.O., Penttinen, S., van Gestel, C.A., Penttinen, O.P.
639 2009. Ecological risks of an old wood impregnation mill: application of the triad approach.
640 Integr Environ Assess Manag 5: 379-389
641 Kersten, M., Förstner, U. 1989. Speciation of trace elements in Sediments, In: Batley, G. Ed. , Trace
642 Element Speciation: Analytical Methods and Problems, CRC Press, Boca Raton, FL, pp 245–
643 317
644 Kronoberg County Administrative Board. 2016. Prioritized contaminated sites in the county.
645 http://www.lansstyrelsen.se/kronoberg/sv/miljo-och-klimat/verksamheter-med-
646 miljopaverkan/fororenade-omraden/prioriterade/Pages/index.aspx?keyword=prioriteringslista
647 Assessed April 13/2016
648 Kördel, W., Bernhardt, C., Derz, K., Hund-Rinke, K., Harmsen, J., Peijnenburg, W., Comans, R.,
649 Terytze, K. 2013. Incorporating availability/bioavailability in risk assessment and decision
650 making of polluted sites, using Germany as an example. J. Hazard Mater 261: 854–862
651 Lindberg, N., Engtsson, J.B., Persson, T. 2002. Effects of experimental irrigation and drought on
652 the composition and diversity of soil fauna in a coniferous stand. J Appl Ecol 39: 924–936
653 Loureiro, S., Ferreira, A., Soares, A., Nogueira, A. 2005. Evaluation of the toxicity of two soils
654 from Jales Mine (Portugal) using aquatic bioassays. Chemosphere 61:168–177 doi:
655 10.1016/j.chemosphere.2005.02.070.
656 Magnusson, J. 1971. Glas – Glasets egenskaper och tillverkning, Svenska Reproduktions AB,
657 Stockholm. In Swedish.

658 Mamindy-Pajany, Y., Hamer, B., Roméo, M., Géret, F., Galgani, F., Durmisi, E., Hurel, C.,
659 Marmier, N. 2011.The toxicity of composted sediments from Mediterranean ports evaluated by
660 several bioassays. Chemosphere 82 :362–369 doi: 10.1016/j.chemosphere.2010.10.005
661 Markert, B.A., Breure, A.M., Zechmeister, H.G. 2003. Bioindicators & Biomonitors, Elsevier
662 Science, Oxford. p. 997
663 McLaughlin, M.J., Zarcinas, B.A, Stevens, D.P., Cook. N. 2000. Soil testing for heavy metals.
664 Commun Soil Sci Plan 31: 1661–1700
665 Montvydiene, D., Marciulioniene, D. 2004. Assessment of toxic interactions of heavy metals in a
666 multicomponent mixture using Lepidium sativum and Spirodela polyrhiza. Environ Toxicol 19:
667 351–358
668 Månsson, N., Carlsson, E. 2002. Antimony emissions of concern for the crystal glass industry.
669 Glasteknisk tidskrift **57**: 50–53.
670 Nyqvist, F., Helmfrid, I., Augustsson, A., Wingren, G. 2017. Increased cancer incidence in the local
671 population around metal-contaminated glassworks sites. Accepted for publication in Journal of
672 Occupational and Environmental Medicine.
673 O'Connor, F. B. 1955. Extraction of enchytraeid worms from a coniferous forest soil. *Nature* 175:
674 815−816
675 OECD Guidelines for the testing of chemicals 2006. Test No. 208: Terrestrial Plant Test: Seedling
676 Emergence and Seedling Growth Test, Organization for Economic Cooperation and
677 Development Publishing, Paris
678 Parvez, S., Venkataraman, C., Mukherji, S. 2006. A review on advantages of implementing
679 luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals.
680 Environ Int 32: 265-268
681 Petänen, T., Virta, M., Karp, M., Romantschuk, M. 2001. Construction and use of broad host range
682 mercury and arsenite biosensor plasmids in the soil bacterium Pseudomonas fluorescens OS8.
683 Microb Ecol 41:360-369
684 Petänen, T., Romantschuk, M. 2003. Measurement of bioavailability of mercury and arsenite using
685 bacterial biosensors. Chemosphere 50: 409-413.
686 Radojević, M., Bashkin, V.N. 2006. Practical environmental analysis.2nd ed. RSC Publishing,
687 USA, p. 457
688 Römbke, J., Jänsch, S., Didden, W. 2005. The use of earthworms in ecological soil classification
689 and assessment concepts. Ecotoxicol Environ Safe 62:249–265. doi:
690 10.1016/j.ecoenv.2005.03.027.

691 Römbke, J., Moser, T. 2002. Validating the enchyt1raeid reproduction test: organisation and results
692 of an international ringtest. Chemosphere 46: 1117−1140.
693 Salminen, J., Anh, B., van Gestel, C. 2001b. Indirect effects of zinc on soil microbes via a keystone
694 enchytraeid species. Environ Toxicol Chem 20: 1167-1174
695 Salminen, J., van Gestel, C. A., Oksanen, J. 2001a. Pollution-induced community tolerance and
696 functional redundancy in a decomposer food web in metal-stressed soil. Environ Toxicol
697 Chem. 20: 2287-2295
698 Selonen, S. 2015. The fate and effects of lead (Pb) at the active and abandoned shooting ranges in a
699 boreal forest ecosystem. Doctoral Thesis. Reports from the department of Environmental
700 Sciences, Lahti. University of Helsinki. Unigrafia, Helsinki. ISBN 978-951-51-0907-1
701 Selonen, S., Liiri, M., Setälä, H. 2014. Can the soil fauna of boreal forests recover from lead-
702 derived stress in a shooting range area? Ecotoxicology 23:437-448
703 Selonen, S., Liiri, M.m, Strömmer, R., Setälä, H. 2012. The fate of lead at abandoned and active
704 shooting ranges in a boreal pine forest. Environ Tox Chem 31: 2771-2779
705 SGU (Swedish Geological Survey). 2014. Geokemisk atlas över Sverige. In swedish.
706 SMHI (Swedish Meteorological and Hydrological Institute). 2016. Meteorological data online.
707 http://opendata-download-metobs.smhi.se/ Assessed May 24, 2016
708 Sohlenius, B. 1979. A carbon budget for nematodes, rotifers and tardigrades in a Swedish
709 coniferous forest soil. *Holarctic Ecol* 2: 30−40
710 Swedish EPA (Environmental Protection Agency). 2009. Report 5976, Riktvärden för förorenad
711 mark. In Swedish.
712 Uddh-Söderberg, T.E., Gunnarsson, S.J., Hogmalm, K.J., Lindegård, MIBG., Augustsson, ALM.
713 2015. An assessment of health risks associated with arsenic exposure via consumption of
714 homegrown vegetables near contaminated glassworks sites. Sci Total Environ 536: 189-197
715 Vasile, L.P., Sobariu D.L., Diaconu M., Statescu F., Gavrilescu M. 2013. Effects on heavy metals
716 on *Lepidium sativum* germination and growth. Environ Eng Manage J 12: 727-733
717 Waara, S., Farm, C. 2008. An assessment of the potential toxicity of runoff from an urban
718 roadscape during rain events. Environ Sci Poll Reserch 15: 205-210
719 Wang, C-R., Tian, Y., Wang, X-R., Yu, H-X., Lu, X-V., Wang, C., Wang, H. 2010. Hormesis
720 effects and implicative application in assessment of lead-contaminated soils in roots of
721 Viciafaba seedlings. Chemosphere 80: 965-971
722 Werkelin, D., Gustavsson, S. 2006. Miljötekniska undersökningar Etapp 1 - Objekt 03 - Johansfors
723 Glasbruk, Emaboda. Kalmar län. PentaCon, Visby. In Swedish.

724 Zhang, X., Wang, X., Wei, D., Li, B., Ma, Y., Huang, Z. 2013. The influence of soil solution
725 properties on phytotoxicity of soil soluble copper in a wide range of soils. Geoderma 211-2012:
726 1-7
727