Toxicological availability of nickel to the benthic oligochaete *Lumbriculus variegatus*

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1 **Toxicological availability of nickel to the benthic oligochaete *Lumbriculus***

2 *variegatus*

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13   **Abstract**

14

15   It is generally accepted that the bioavailability of metals in sediments is influenced by

16   the presence of acid volatile sulfides (AVS). The pore water hypothesis predicts that, if

17   the molar concentration of simultaneously extracted metals (SEM) in a sediment is

18   smaller than the molar concentration of AVS, the free metal ion activity in the pore

19   water is very small and that consequently no metal toxicity in short-term toxicity tests is

20   observed. In this study we examined (1) if this concept can be extended to predict the

21   absence of chronic Ni toxicity to the oligochaete deposit-feeding worm *Lumbriculus*

22   *variegatus* and (2) if the organic carbon normalized excess SEM; i.e. [SEM-AVS]/f_{OC}

23   predicts the magnitude of Ni toxicity to *L. variegatus*. A 28-day toxicity experiment was

24   performed in which biomass production of *L. variegatus* was determined in two natural

25   sediments with different [AVS] and f_{OC}, spiked at different Ni concentrations. The

26   absence of toxicity is predicted correctly by the [SEM-AVS] < 0 criterion when only the

27   0-1 cm surface layer of the sediment is considered, but not when the whole bulk

28   sediment is considered (0-3 cm). In both sediments, the same [SEM-AVS]/f_{OC} at the

29   surface corresponds with a similar decrease in *L. variegatus* biomass. Thus, [SEM-

30   AVS]/f_{OC} in the surface layer accurately predicts the magnitude of toxicity. This

31   measure is therefore a good estimator of toxicologically available Ni. On the other hand,

32   the free Ni^{2+} ion activity in the overlying water appeared to be an equally good predictor

33   of the magnitude of toxicity. Consequently, it was not possible to determine the relative

34   importance of the overlying water and pore water exposure route with the semi-static

35   laboratory experiments.

Introduction

It has been demonstrated that the simultaneously extracted metals – acid volatile sulfide (SEM-AVS) method is an effective tool in predicting the absence of metal toxicity in sediments in short-term toxicity tests when [SEM-AVS] < 0 (Di Toro et al., 1990; Di Toro et al., 1992; Casas and Crecelius, 1994; Pesch et al., 1995; Hansen et al., 1996). The underlying principle is that except for pyrite, all other iron and manganese mono sulfides that may be present in sediments have higher solubility products than other metal sulfides. Thus, Fe and Mn can be displaced by other divalent metals (Cu, Cd, Ni, Pb, Zn) on a mole-to-mole basis. Because these metal sulfides exhibit very low solubility, sediments with an excess of reactive sulfide will exhibit very low dissolved metal concentrations in pore waters and will not cause toxicity (Ankley et al., 1996).

Sediments with excess SEM do not always exhibit toxicity to invertebrate sediment organisms. Organic matter can bind non-sulfide bound trace metals, thus preventing them to enter the dissolved phase (Mahony et al., 1996). Based on this, [SEM-AVS]/f_{OC} has been proposed as a measure of bioavailable metal (Di Toro et al., 2005; Hansen et al., 2005). In a recently proposed biotic ligand model for sediments, it has been shown that [SEM-AVS]/f_{OC} determines the free metal activity in the pore water(Di Toro et al., 2005). If this value is greater than a critical threshold, sediments are predicted to be toxic.

The [SEM-AVS]/f_{OC} concept assumes that there is no metal toxicity caused by transformations of the sulfide and organic matter bound metal in the gut of sediment-ingesting organisms or via exposure to contaminated food (Meyer et al., 2005). Some authors (Lee B.G., Griscom S.B. et al., 2000; Lee B.G., Lee J.S. et al., 2000) have

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61 observed that benthic organisms can assimilate metals that are associated with sulfides

62 via dietborne exposure. They demonstrated significant bioaccumulation of metals at

63 concentrations where [SEM-AVS] < 0. However, these authors did not measure toxic

64 effects; they assume that dietary exposure as measured by accumulation is related to

65 chronic toxicity (Lee B.G., Griscom S.B. et al., 2000). However, they also note that

66 generic relationships between metal bioaccumulation and toxicity are not well

67 understood. Animal species differ in their abilities to detoxify bioaccumulated metals

68 (e.g. via metallothionein or granule induction) or develop tolerance (Adams et al., 2000;

69 Lee B.G., Lee J.S. et al., 2000). An increase in bioaccumulation may be a sign of active

70 metal uptake and not toxicity in the case of metal deficiency (Muyssen and Janssen,

71 2002; Bossuyt and Janssen, 2003). Thus, bioaccumulated metals may not always reflect

72 toxic effects (McGeer et al., 2003).

73

74 To our knowledge, the applicability of the SEM-AVS concept to predict the absence of

75 chronic, sub-lethal toxicity of nickel to a benthic organism exposed in single-species

76 laboratory toxicity tests has not been demonstrated. In this study, the hypothesis tested

77 is that Ni^{2+} in the pore water determines chronic toxicity to *L. variegatus*. In other

78 words, we examined (1) if [SEM-AVS] < 0 predicts the absence of chronic Ni toxicity

79 and (2) if [SEM-AVS]/f_{OC} predicts the presence and magnitude of Ni toxicity to *L.

80 variegatus*, independent of sediment characteristics. The vertical profiles of [SEM-

81 AVS] and pore water concentrations were considered, since vertical distributions of

82 AVS and SEM can affect metal toxicity in sediments and pore water metal

83 concentrations (Boothman et al., 2001). Two natural sediments with different

84 characteristics were chosen to test the hypothesis, so that at comparable [SEM],

85 different [AVS] accounted for differences in [SEM-AVS]. Finally, the applicability of

86   the mentioned sediment-biotic ligand model (sBLM) (Di Toro et al., 2005) will be

87   tested with the data on biomass of *Lumbriculus* in Ni spiked sediments. In this way, the

88   possible use of the sBLM for chronic single-species toxicity tests with Ni will be

89   evaluated.

90

91   **Materials and Methods**

92

93   Sediment sampling, spiking and analysis

94

95   Two natural sediments were sampled. Brakel sediments were taken by scoop sampling

96   from the banks of a small stream (near the source) in a nature reserve in Brakel,

97   Belgium (50°45' N, 3°46' E). Water depth at the sampling time was about 50 cm. Ijzer

98   sediments were taken by grab sampling with a Van Veen grab from the Ijzer river,

99   Belgium (about 50°58'N, 2°48'E). This river is situated in a low density farming zone

100   and has a water depth of about one meter. Plastic buckets were filled with 50-70%

101   sediment, after which overlying water was added to the top of the bucket, which was

102   subsequently closed with a plastic lid. After sampling, sediments were frozen at -20 °C

103   for two days to kill indigenous organisms and subsequently stored at 4 °C until use.

104   Brakel and Ijzer have a moderate and a high AVS content, respectively, and a low to

105   moderate organic carbon content. Sediment characteristics are provided in Table 1.

106

107   Before spiking, the sediments were cleaned by press sieving with a 0.5 cm sieve in

108   deaerated (<0.2 mg O_2/L) overlying water from the site of origin. Cleaned sediments

109   were stored at 4°C for 48h sedimentation, after which the overlying water was carefully

110   poured-off and spiking was started. Ni was added as a $NiCl_2$-solution (Merck) in

111 deaerated (< 0.2 mg/L O_2) deionised water, which was thoroughly manually mixed with

112 the sediments in airtight sealed plastic bags. Mean measured test concentrations of Ni in

113 the spiked sediments ranged from 127 to 1458 mg/kg dry sediment (Brakel) and from

114 514 to 3847 mg/kg dry sediment (Ijzer) (Table2). After spiking with Ni, sediments were

115 placed into glass test vessels and were stabilized/equilibrated for 70 days before test

116 initiation (Simpson et al., 2004). According to Simpson et al. (2004), equilibration of

117 Ni-spiked sediments occurs within 30-70 days. In natural sediments, AVS consists

118 largely of iron sulfides (Lee J.S. et al., 2000). When the spiked Ni^{2+} binds with AVS,

119 Fe^{2+} is released from the Fe(II)S phase. Fe(II) can be lost by diffusion to the oxic layers,

120 where it can be oxidized to solid Fe-hydroxide precipitates. After 43 days for the Brakel

121 sediments and after 58 days for the Ijzer sediments, precipitated Fe(OOH) was removed

122 by manually scraping it off the top layer. This was done to prevent toxicity due to

123 elevated dissolved Fe in the pore water and overlying water (Gonzalez, 1996) and to

124 prevent artefacts during the determination of SEM and AVS (Simpson et al., 1998).

125

126 At test initiation and termination, samples were taken for determination of dry wt,

127 %OC, total Ni concentration (Ni_T), AVS and SEM_{Ni}. Overlying water was sampled

128 about 1 cm above the sediment surface to assess dissolved Ni, pH, ammonia, hardness,

129 conductivity and dissolved organic carbon (DOC) (Table 3). Pore water was collected at

130 each cm depth by means of inert passive pore water samplers with polyether sulfon

131 membranes, so called mini-peepers (Doig and Liber, 2000). In each chamber, dissolved

132 Ni, DOC, pH and redox potential were measured. Sediment dry weight is defined as the

133 difference between wet and dry sediment (dried 72h at 60°C). Organic carbon content

134 was determined by loss on ignition (Egeler et al., 2005). AVS and SEM_{Ni} were

135 determined according to the modified diffusion method (Leonard et al., 1996) (5 g

136   sediment extracted for 1 hour in 60 mL of 1N HCl). Samples were taken with a core (2

137   cm diameter) and divided into 1 cm sediment layers. Cores were taken from a replicate

138   test vessel with overlying water present and subsections of 1 cm were immediately

139   (within 5 seconds) inserted into the diffusion systems, to prevent oxidation and loss of

140   AVS. Sediment destruction for total metal content was done by acid microwave

141   digestion. Ni was analyzed using flame AAS (Spectra AA 100-Varian) and/or a graphite

142   furnace AAS (Zeeman, SpectrAA300-Varian). The detection limit of the former is 8.7

143   μg Ni/L. For the lower nickel concentrations, the furnace was used with a detection

144   limit which ranged between 2 and 3.4 μg Ni/L.

145

146   Test designs

147

148   *Lumbriculus variegatus* is an ecologically relevant oligochaete, occurring throughout

149   Europe and the United States (Spencer, 1980). It is an epibenthic/benthic organism

150   subject to contaminant exposure via all routes of concern, including ingestion of

151   sediment particles (Phipps et al., 1993). The test was based on a proposed draft OECD

152   test guideline (Egeler et al., 2005). Organisms were from an in-house culture, with

153   parental organisms provided by Blades Biological LTD (United Kingdom). Biomass per

154   replicate of ten organisms was evaluated as a chronic endpoint integrating growth and

155   survival. *L. variegatus* reproduces parthenogenetically by fragmentation. Therefore

156   survival *sensu stricto* cannot be measured by counting the number of organisms at the

157   end of the test. Overlying water was a medium hard reconstituted water composed of

158   the following salts diluted in deionised water: 4 mg/L KCl, 123 mg/L $MgSO_4.7H_2O$, 96

159   mg/L $NaHCO_3$ and 6 g/L $CaSO_4$ (USEPA, 1985). Sixty to 70 % of the overlying water

160   was renewed twice a week. The temperature was 23+/- 2 °C and a 16:8 hours light:dark

161   regime was applied. Ten adult organisms with complete regeneration of tail or head were

162   added per replicate jar with 400 g wet sediment and 250 mL overlying water.

163   Organisms were fed ground Tetramin^{TM} fish flakes (200 µg per organism per day). Five

164   replicates per concentration were used for biological endpoints, whereas two replicates

165   were used to determine physico-chemical sediment properties. Tests were terminated

166   after 28 days.

167

168   Data treatment

169

170   Concentration - effect curves and EC_{50}s were obtained using the logistic model.

171   Parameter estimates were found by fitting the model to the observed data with a non-

172   linear least squares estimation according to the Levenberg-Marquardt algorithm

173   (Levenberg, 1944; Marquardt, 1963). Lowest observed effect concentrations (LOECs)

174   were based on significant differences ($p < 0.05$) of mean biomass between Ni treatments

175   and control treatments, determined using the Mann-Whitney U test, adjusted for ties.

176   All calculations are based on measured Ni concentrations. T-tests were performed on

177   the residuals of fitted data for comparison of relationships between biomass and

178   concentration after checking for normality with Kolmogorov-Smirnov goodness-of-fit

179   test and for homogeneity of variances with Levene's test. All significance levels were

180   set at $p = 0.05$. Statistics were performed using Statistica 6.0 software (Statsoft, Tulsa,

181   OK, USA). Speciation calculations were performed with the Windermere Humic

182   Aqueous Model (WHAM) VI version 6.0.8 (Natural Environment Research Council,

183   UK) (Tipping, 1998) or with the Biotic Ligand Model (BLM) version 2.1.2 (Hydroqual,

184   Mahwah, New Jersey), which incorporates WHAM V and with which BLM

185   calculations were performed.

186

Results and discussion

In comparison with AVS concentrations in different natural sediments reported in literature (van den Hoop et al., 1997; van den Berg et al., 1998; van den Berg et al., 2001), the sediments tested in this study can be regarded as having a medium and high AVS content. The increase in [AVS] at t_0 from the top cm to the 2-3 cm sediment section, i.e. from 3.5 µmol/g dry sediment to 13.5 µmol/g dry sediment in Brakel and from 42.1 µmol/g dry sediment to 118.2 µmol/g dry sediment in Ijzer, can be considered as representative for the AVS stratification observed *in situ* (van den Berg et al., 1998; van den Berg et al., 2001).

The LOECs expressed as [SEM-AVS] in the different sediment layers are given in Table 4. In the bulk (whole) core of the IJzer sediment [SEM-AVS]$_{LOEC,bulk}$ is < 0. This means that a significant toxic effect was observed, although the concept predicts that no toxicity is expected at [SEM-AVS] < 0. [SEM-AVS] < 0 measured on the bulk sediment basis does not predict the absence of chronic toxicity. However, when the surface layer of the sediments is considered (0-1 cm), [SEM-AVS]$_{LOEC,surface}$ > 0, confirming the concept. Following the SEM-AVS concept, this may suggest that the surface layer of the sediment contributes more to the observed toxicity than the deeper sediment layers when [SEM-AVS] < 0 in the deeper layers. Due to oxidation of AVS, more non-sulfide bound Ni will be present at the surface, resulting in the positive [SEM-AVS] values, as observed for other metals by other authors (DeWitt et al., 1996; Liber et al., 1996). Negative values for [SEM-AVS]$_{surface}$ were only found at lower Ni concentrations in both sediments, where no significant toxic effect was observed. The use of the SEM-AVS concept for risk assessment procedures or for determining sediment quality criteria

211 should preferentially consider surface layer-based analyses as an alternative to bulk

212 sample analyses (e.g. 10 cm, (van den Hoop et al., 1997)). Obviously, the potential

213 contribution to toxicity from nickel in the overlying water should not be disregarded

214 either in risk assessment (see also further).

215

216 In this study, no effect was observed at $[SEM-AVS]_{surface} < 0$. Lee et al.(Lee B.G.,

217 Griscom S.B. et al., 2000; Lee B.G., Lee J.S. et al., 2000) did observe accumulation of

218 metals in clams and marine polychaetes at $[SEM-AVS] < 0$, explaining this by dietary

219 metal uptake from ingested sediments being the dominant exposure route. These authors

220 assumed, but did not demonstrate, that dietary exposure as measured by

221 bioaccumulation is at least generally related to chronic toxicity (Lee B.G., Griscom S.B.

222 et al., 2000; Lee B.G., Lee J.S. et al., 2000). Possibly, bioaccumulation from the dietary

223 route at $[SEM-AVS] < 0$ in our study was small enough not to cause significant toxicity.

224

225 To investigate the utility of $[SEM-AVS]/f_{OC}$ as a measure of toxicologically available

226 Ni, the biomass data of both Brakel and Ijzer sediments were pooled and their

227 relationship with $[SEM-AVS]/f_{OC}$ was analyzed. Negative values of $[SEM-AVS]/f_{OC}$

228 were omitted, following the concept that no toxicity occurs when $[SEM-AVS] < 0$, as

229 demonstrated with the positive $[SEM-AVS]_{LOEC,surface}$. The effect on biomass of *L.*

230 *variegatus* as a function of $[SEM-AVS]/f_{OC}$ in the surface layer, $[SEM-AVS]/f_{OC, surface}$,

231 showed a similar trend in Brakel and Ijzer sediments. Using the positive values of

232 $[SEM-AVS]/f_{OC}$ in the surface layer (0–1 cm) of the sediments, one concentration-effect

233 curve can be fit to the pooled data of both sediments (Fig. 1a), with the mean of the

234 residuals of both sediments not significantly differing (t-test, $p = 0.14$). This indicates

235 that $[SEM-AVS]/f_{OC}$, or the equivalent (Ni^{2+}) in the pore water, is indeed a good

236 measure for the toxicologically available Ni. However, using the positive values of

237 [SEM-AVS]/f_{OC} in the deeper layers (1-2 cm and 2-3 cm), also one concentration-effect

238 curve can be fit to the pooled data of both sediments, although only one positive value

239 of [SEM-AVS]/f_{OC} remains for Ijzer (data not shown). This raises the question whether

240 all layers contribute equally to Ni toxicity when there is a positive [SEM-AVS]/f_{OC}. If

241 this is the case, the organisms would be exposed to an average [SEM-AVS]/f_{OC} over the

242 entire sediment depth. Those averages were calculated for both sediments, with negative

243 values set to zero, following the concept that this cannot elicit a toxic effect. One

244 concentration-effect curve can be fitted to the pooled data (Fig. 1b), also with the means

245 of the residuals for both sediments not significantly differing (t-test, p = 0.89). The fit is

246 similar (R^2 = 0.453) to the fit in Fig. 1a in which surface layer data only are represented

247 (R^2 = 0.402), indicating that *L. variegatus* may also be exposed to the deeper layers with

248 lower [SEM-AVS]/f_{OC}. During the test, the organisms were observed to be mainly

249 burrowed in the sediment, down to varying depths of maximum 3 cm.

250

251 The difference between the EC_{50}s for biomass in both sediments is reduced from a

252 factor 2.9 when expressed as total Ni in the bulk sediment (µg/ g dry sediment) to a

253 factor 1.6 when expressed as [SEM-AVS]/f_{OC} in the surface layer. According to a t-test

254 comparing the EC_{50}s in both sediments, the factor 2.9 difference is highly significant (p

255 = 0.00001), while the factor 1.6 difference is not significant (p = 0.51). Thus, the same

256 intensity of toxic effect (50% reduction of growth) is observed at not significantly

257 differing concentrations of [SEM-AVS]/fOC in two different sediments. This suggests

258 again that toxicity to *L. variegatus* relates to [SEM-AVS]/f_{OC} or free Ni^{2+} in the pore

259 water. These results support the use of [SEM-AVS]/f_{OC} as a predictor of sediment

260 toxicity.

261

262 The concentration of [SEM-AVS]/f_{OC} determines the free [Ni^{2+}] in the pore water (Di

263 Toro et al., 2005). Using the dissolved [Ni] in the pore water of the surface layer of the

264 sediments, one concentration-response curve can be fitted to the pooled data of the

265 Brakel and Ijzer sediments (Fig. 1c). However, this fit was not as good as the one

266 obtained with [SEM-AVS]/f_{OC}: there was a significant difference between the residuals

267 of both sediments (t-test, p = 0.02). This may be explained by different ratios of

268 dissolved [Ni] and free (Ni^{2+}) in the pore water in both sediments, due to different pore

269 water characteristics.

270

271 According to the SEM-AVS concept, due to the small solubility product constants of

272 metal sulfides, sediments with an excess of AVS are expected to have very low metal

273 activity in the pore water (Berry et al., 1996). However, in this study rather high Ni

274 concentrations were found in the pore water of sediment layers in which [SEM – AVS]

275 was smaller than zero . For instance, in the 2-3 cm horizon of Ijzer, [SEM-AVS] = -14.5

276 μmol/g, while [Ni]$_{PW}$ = 1221 μg/L. This is consistent with Gonzalez (1996)and Doig

277 and Liber (2006), who also observed high [Ni]$_{PW}$ at SEM/AVS ratios smaller than one.

278 One possible explanation could be that small colloidal NiS particles passed through the

279 0.45 μm peeper membrane and were measured as "dissolved" Ni in the pore water

280 (Leonard et al., 1999). Another possibility could be that nickel polysulfide complexes or

281 nickel bisulfide (NiHS$^+$) complexes, which are soluble and do not react with solid FeS

282 to form insoluble NiS, were present (Doig and Liber, 2006). A third possibility would

283 be that the system is not in equilibrium yet, even after the 70 days equilibration period

284 as recommended by Simpson et al. (2004). This last option seems less likely because of

285 the visible FeOOH precipitate that formed during the equilibration time as described

286   above, indicating the replacement of Fe by Ni in the FeS. This precipitate did not form

287   again after it was removed, suggesting that the formation of NiS has ended and

288   equilibrium is achieved.

289

290   The recently proposed sediment-biotic ligand model (sBLM) (Di Toro et al., 2005)

291   predicts the toxicity of sediments based on $[SEM-AVS]/f_{OC}$, assuming that organic

292   carbon and AVS are the only relevant metal-partitioning phases in sediments. The

293   LOECs expressed as $[SEM-AVS]/f_{OC, surface}$ are 122 µmol/g OC and 495 µmol/g OC for

294   Brakel and Ijzer, respectively. These results corroborate the observation that the onset of

295   toxicity occurs at $[SEM-AVS]/f_{OC} \approx 100$ µmol/g OC. This is an empirical observation

296   by Di Toro et al. (2005) based on a series of sediment toxicity data from acute tests with

297   different metals, mainly with marine amphipods and from chronic tests with Cd and Zn,

298   where effect/no effect was considered as a measure of toxicity in colonization or long

299   term single-species studies. This observation was also made by Burton et al. (2005)for

300   Zn-toxicity in colonization studies with sediments in the field.

301

302   Specifically for Ni, Di Toro et al. (2005) have, with the sBLM, calculated critical values

303   of $[SEM-AVS]/f_{OC}$ for a range of water types with varying pH, based on the acute

304   *Daphnia* critical gill-Ni accumulation. The chronic LOECs in our study, where the

305   average $pH_{(surface\ layer)}$ was 7.6 and 7.1 for Brakel and Ijzer respectively, are lower than

306   the critical acute Ni concentrations (LC_{50}) of 642 - 1057 µmol/g OC, calculated with the

307   sBLM, in a standard freshwater at pH 7.0 – 8.0 (Di Toro et al., 2005). Thus, our results

308   do not confirm the sBLM as opposed to the studies cited by Di Toro et al. (2005) in

309   which no chronic effects are observed under the lowest calculated (acute) critical value

310   of $[SEM-AVS]/f_{OC}$. This might be due to the fact that the *chronic* results from these

14

311 study with Ni and the freshwater organism *L. variegatus* are lower than the *acute* results

312 on which Di Toro et al. based their critical values, and/or due to the fact that the

313 *Daphnia magna* BLM is not applicable to *Lumbriculus variegatus* due to different

314 sensitivities of both organisms. However, Lumbriculidae are generally less sensitive to

315 metals than *Daphnia magna* (Von der Ohe and Liess, 2004), and as such one would

316 expect the LOEC to be higher than the sBLM-predicted critical $[SEM-AVS]/f_{OC}$. A

317 critical accumulation was calculated based on the 96 h LC50 for *L. variegatus* reported

318 by Schubauer-Berigan et al. (1993). Using this new parameter and the average measured

319 pore water chemistry, the sBLM calculated values of critical $[SEM-AVS]/f_{OC}$ are 2060

320 µmol/g OC and 1780 µmol/g OC at a pH of 7.6 (Brakel) and 7.1 (Ijzer), respectively,

321 which is indeed larger than the values based on *Daphnia magna* sensitivity (Di Toro et

322 al., 2005). Knowing that the acute to chronic ratio for Ni is about 30 for *Daphnia magna*

323 (Hunt et al., 2002), it is more likely that the difference between acute and chronic

324 sensitivity is the major cause of the LOECs being lower than the sBLM-predicted

325 critical $[SEM-AVS]/f_{OC}$. The sBLM as applied by Di Toro et al. (2005), does not work

326 for our chronic toxicity data of *L. variegatus* biomass. The acute *Daphnia magna* BLM

327 underestimates Ni toxicity in this case and further evaluation of the sBLM for chronic

328 endpoints will be necessary. It is possible, however, that elevated overlying water

329 concentrations at the LOECs in our experiments have a confounding effect on the

330 interpretation of $[SEM-AVS]/f_{OC}$, as explained below. This can be another explanation

331 for the observation of LOECs expressed as $[SEM-AVS]/f_{OC}$ below model predicted

332 critical values.

333

334 This discussion focused on the $[SEM-AVS]/f_{OC}$ or Ni^{2+} in the pore water of the

335 sediments as exposure route for Ni toxicity to *L. variegatus*. However, it should be

336 noted that the Ni concentrations in the overlying water of the tested sediments were

337 elevated in the higher treatments, e.g. mean overlying water concentrations at the LOEC

338 of 248 µg/L Ni for the Brakel treatment with 544 mg Ni/kg dry wt (mean) and 1135

339 µg/L Ni for the Ijzer treatment with 2234 mg Ni/kg dry wt (mean) (Table 2). Ni^{2+}

340 activity in the overlying water, $(Ni^{2+})_{OW}$, was calculated with two methods. Firstly,

341 speciation was calculated with WHAM VI, with log $K_{MA}(Ni)$=1.75 and assuming that

342 DOC consists of 40% active fulvic acid, as the latter was shown to yield a good fit

343 between observed and measured free Ni^{2+} in natural surface waters (Van Laer et al.,

344 2006). Secondly, speciation calculations were performed following Di Toro et al.

345 (2005), using the BLM software, with the default pK_{Ni-HA} = 2.7 and 1.4 for humic and

346 fulvic acids respectively and assuming that DOC consists of 84% humic acid and 16%

347 fulvic acid. To investigate the possibility of exposure to Ni via the overlying water, a

348 concentration-effect curve was fitted to the pooled data of $(Ni^{2+})_{OW}$ in the overlying

349 water of Brakel and Ijzer sediments (Fig. 2). Only concentrations with a positive [SEM-

350 AVS]/f_{OC}, i.e. concentrations where a significant effect was observed, were considered.

351 This yields a good fit with no significant difference between the residuals of both

352 sediments (t-test, p = 0.11 and 0.10 for WHAM VI and BLM speciation respectively).

353 The EC_{50} as (Ni^{2+}) in the overlying water is 9.6 µmol/L (95% confidence interval of 6.5

354 to 14.1 µmol/L) and 4.2 µmol/L (95% confidence interval of 2.3 to 7.7 µmol/L) for the

355 WHAM VI and BLM speciation calculations, respectively.

356

357 The Ni activity in the overlying water is an equally good predictor of toxicity as [SEM-

358 AVS]/f_{OC}. Thus, it cannot be excluded that Ni in the overlying water is an important part

359 of the toxicologically available Ni in our experiments.

360

361 The $(Ni^{2+})_{OW}$ as calculated with WHAM VI correlated significantly with [SEM-

362 AVS]/f_{OC} in the surface layer (R = 0.96, p = 0.008), but not significantly (R = 0.84, p =

363 0.08) with the averaged [SEM-AVS]/f_{OC} over depth as described above (with negative

364 [SEM-AVS]/f_{OC} set to zero). Similar results were found for $(Ni^{2+})_{OW}$ as calculated with

365 the BLM. This is not surprising, since the Ni in the overlying water originates from a

366 flux from the sediment via the pore water in the surface layer to the overlying water. So

367 although the results of this study do not allow to distinguish $(Ni^{2+})_{OW}$ from $(Ni^{2+})_{PW}$

368 (Ni^{2+} activity in the pore water) as the exposure route of Ni for *L. variegatus*, it can be

369 concluded that in these tests, either directly (via pore water) or indirectly (via overlying

370 water), $(Ni^{2+})_{PW}$ or the equivalent [SEM-AVS]/f_{OC} determines the toxicologically

371 available Ni for *L. variegatus*.

372

373 In historically contaminated sediments in the field, pore water concentrations are likely

374 to be higher than overlying water concentrations, due to higher dilution factors than

375 those occurring in the routinely used laboratory test designs: i.e. static renewal. Also,

376 the proportion of nickel in the solid phase will be higher in natural sediments, as nickel

377 does not ordinarily enter sediments in soluble forms, as was the case with this study.

378 Therefore [SEM-AVS]/f_{OC} is suggested as a measure of bioavailable Ni for risk

379 assessment procedures, provided that overlying water (Ni^{2+}) is measured and

380 sufficiently low. More research is needed to establish the relative importance of pore

381 water and overlying water as exposure routes for Ni to *L. variegatus*. Care should be

382 taken in laboratory experiments with high Ni concentrations in sediment, if the purpose

383 is to test the toxicity of nickel associated with sediment phases (i.e., particles and pore

384 water) and not of the overlying water.

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543

544 Table 1 – Sediment characteristics of Brakel and Ijzer sediments: [AVS] in the control
545   treatments at t_0 (test initiation) per horizontal layer under the surface, average
546   mass fraction of organic carbon in the tested sediments (f_{OC}) and particle size
547   distribution.
548
549

Sediment	[AVS] (mmol/kg dry wt)			f_{oc}	% sand	% silt	% clay
	0-1 cm	1-2 cm	2-3 cm				
Brakel	3.5	9.9	13.5	0.0221	62.9	24.5	12.7
Ijzer	42.1	104.3	118.0	0.0395	23.9	46.4	29.6

550 Table 2: Measured concentrations of total Ni in the sediment, $[Ni]_{OW}$ (dissolved Ni in the overlying water), [SEM-AVS] and $[Ni]_{PW}$
551 (dissolved Ni in the pore water) of Brakel and Ijzer sediments, at the beginning (t_0) and at the end (t_{end}) of the 28 day test period, for
552 the different tested concentrations, per horizontal layer when applicable and mean biomass per replicate of *L. variegatus* at the end
553 of the test period, expressed as % of the mean biomass per replicate in the control treatments
554
555

Brakel

Treat-ment	Total Ni (mg/kg dry wt)		[Ni]$_{OW}$ (µg/L)		[SEM-AVS] (µmol/g dry wt)						[Ni]$_{PW}$ (µg/L)						Biomass (% of control)
					0-1 cm		1-2 cm		2-3 cm		0-1 cm		1-2 cm		2-3 cm		
	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_{end}
control	8	5	7	3	-3.4	-1.5	-9.9	-5.9	-13.4	-10.3	11	32	12	17	28	17	100
C1	132	122	22	24	-2.2	-1.4	-8.3	-7.4	-12.2	-7.9	ND	128	ND	62	51	48	85.9
C2	201	179	21	35	-4.7	-3.4	-7.9	-8.8	-8.7	-5.7	ND	385	64	100	47	126	89.1
C3	547	540	263	233	1.8	3.6	0.9	0.9	0.9	1.0	351	1949	550	603	219	270	67.1
C4	1075	1068	941	967	12.6	14.1	7.1	10.8	9.4	9.6	1370	ND	1542	3343	3959	ND	39.9
C5	1544	1373	1986	2034	15.5	20.6	19.1	18.8	20.3	17.6	23000	8122	24177	7740	37474	10532	37.9

Ijzer

Treat-ment	Total Ni (mg/kg dry wt)		[Ni]$_{OW}$ (µg/L)		[SEM-AVS] (µmol/g dry wt)						[Ni]$_{PW}$ (µg/L)						Biomass (% of control)
					0-1 cm		1-2 cm		2-3 cm		0-1 cm		1-2 cm		2-3 cm		
	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_{end}
control	8	16	13	19	-42.0	-24.0	-104.2	-86.6	-118.1	-87.6	6	7	16	11	13	10	100
C1	460	567	36	46	-39.4	-22.7	-109.7	-66.9	-111.6	-83.0	664	205	62	254	94	63	105.9
C2	759	905	117	88	-47.1	-37.0	-109.2	-77.8	-104.2	-75.8	924	ND	146	ND	57	205	88.4
C3	1486	1484	346	209	-13.5	3.8	-51.5	-49.6	-60.2	-48.4	985	695	1441	608	2284	499	72.1
C4	2271	2197	1141	1128	11.9	27.1	-14.1	-16.0	-14.1	-14.5	3492	2171	4649	1124	4689	1221	54.1
C5	3664	4031	2734	2383	31.5	49.5	9.1	27.3	20.7	23.4	10718	3412	ND	4963	ND	4847	41.8

556

557 Table 3 – Overlying water characteristics per treatment for the Brakel and Ijzer sediment tests: pH, dissolved Ca and Mg and DOC, at the
558 beginning (t_0) and at the end (t_{end}) of the 28 day test period.
559

Brakel

Treatment	pH		[Ca] (mg/L)		[Mg] (mg/L)		[DOC] (mg/L)	
	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}
control	8.03	7.90	36.58	39.06	11.88	14.02	1.318	1.670
C1	7.94	7.82	33.53	32.96	9.86	13.01	1.067	4.844
C2	8.17	7.83	33.80	34.64	10.11	12.89	1.173	1.884
C3	7.82	7.65	27.88	26.65	8.21	11.58	1.013	2.012
C4	7.87	7.57	29.11	25.51	8.37	11.28	1.118	2.752
C5	7.69	7.51	30.06	24.66	8.35	11.15	1.369	1.726

Ijzer

| Treatment	pH		[Ca] (mg/L)		[Mg] (mg/L)		[DOC] (mg/L)	
	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}	t_0	t_{end}
control	7.70	7.99	36.29	48.29	15.952	12.877	1.067	2.403
C1	7.89	8.03	35.95	41.59	15.23	11.776	1.228	2.032
C2	7.63	7.77	30.85	23.13	12.631	7.899	1.226	2.547
C3	7.40	7.63	32.84	25.26	13.91	8.343	1.539	2.471
C4	7.43	7.42	33.33	26.33	13.586	8.37	1.345	1.934
C5	7.40	7.37	37.70	25.26	13.479	7.965	1.424	2.23

560
561

562   Table 4 - LOECs for biomass/replicate of *L. variegatus* in Brakel and Ijzer sediments expressed as [SEM-AVS] (µmol Ni/g dry sediment)
563   at different depths below the sediment surface at the beginning (t_0) and at the end (t_{end}) of the test, as well as the mean of the [SEM-
564   AVS]$_{LOEC}$ at t_0 and t_{end}
565

	Brakel			Ijzer		
	LOEC as [SEM-AVS] at t_0	LOEC as [SEM-AVS] at t_{end}	mean LOEC as [SEM-AVS]	LOEC as [SEM-AVS] at t_0	LOEC as [SEM-AVS] at t_{end}	mean LOEC as [SEM-AVS]
Total core, 0-3 cm	-0.4	1.5	0.6	-9.6	-7.4	-8.5
A-horizon, 0-1 cm	1.8	3.7	2.7	11.9	27.1	19.5
B-horizon, 1-2 cm	0.9	0.9	0.9	-14.1	-16.0	-15.0
C-horizon, 2-3 cm	0.9	1.0	0.9	-14.1	-14.5	-14.3
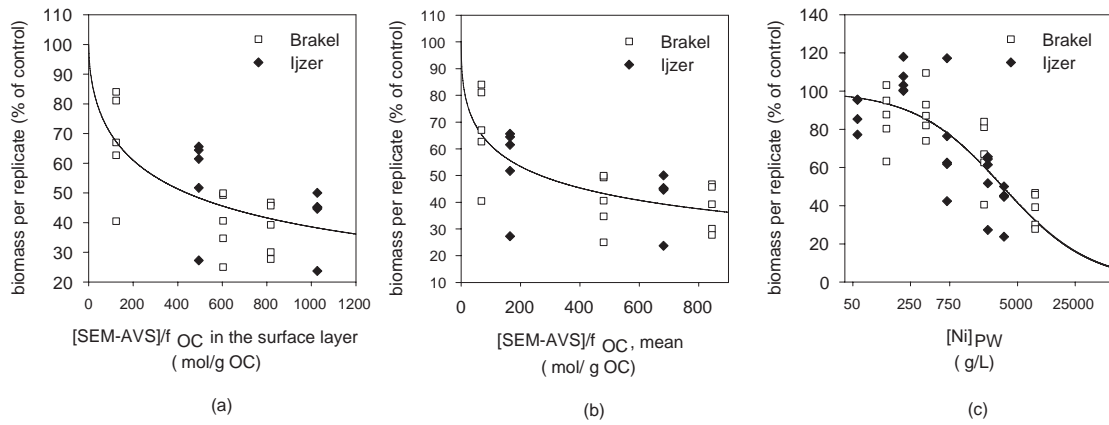
566
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569 Figure 1: (a) Concentration – effect curve of biomass per replicate as a function of mean
570   [SEM-AVS]/f_{OC} in the surface layer of Brakel and Ijzer; (b) Concentration –
571   effect curve of biomass per replicate as a function of mean [SEM-AVS]/f_{OC} as
572   mean of the three sediment layers (0-1, 1-2 and 2-3 cm depth) of Brakel and
573   Ijzer; (c) Concentration - effect curve of biomass per replicate as a function of
574   dissolved [Ni] in the pore water of the surface layer of Brakel and Ijzer at the
575   end of the test

27

576

(a)   (b)
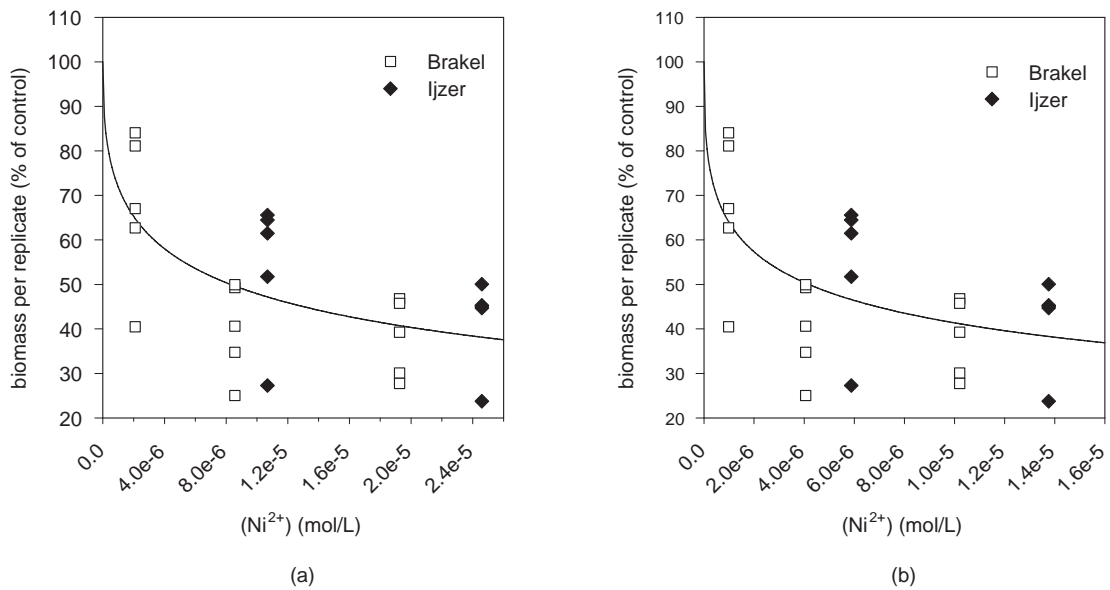
Figure 2: (a) Concentration-effect curve of biomass per replicate as a function of the
mean Ni^{2+} activity as calculated with WHAM VI (see text) in the overlying
water of those concentrations with positive [SEM-AVS] of Brakel and Ijzer
sediments; (b) Concentration-effect curve of biomass per replicate as a function
of the mean Ni^{2+} activity as calculated with the BLM speciation programme (see
text) in the overlying water of those concentrations with positive [SEM-AVS] of
Brakel and Ijzer sediments.