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1 Combined chemical and ecotoxicological measurements for river sediment management in

2 an on-land deposit scenario

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9 ABSTRACT

10 Sediment management along engineered river systems includes dredging operations and sediment deposition in the 11 sea (capping) or on land. Thus, determining the ecotoxicological risk gradient associated with river sediments is 12 critical. In this study, we investigated sediment samples along the Rhône River (France) and conducted 13 Environmental Risk Assessment (ERA) tests with the idea to evaluate them in the future for deposit on soil. Based 14 on an on-land deposit scenario, the capacity of the sediment samples from four sites (LDB, BER, GEC, and TRS) to 15 support vegetation was evaluated by characterising the physical and chemical parameters (pH, conductivity, total 16 organic carbon, grain size, C/N, potassium, nitrogen, and selected pollutants, including polychlorinated biphenyls 17 (PCBs) and metal trace elements. All tested sediments were contaminated by metallic elements and PCBs as follows: 18 LDB > GEC > TRS > BER, but only LDB had levels higher than the French regulatory threshold S1. Sediment 19 ecotoxicity was then assessed using acute (plant germination and earthworm avoidance) and chronic (ostracod test 20 and earthworm reproduction) bioassays. Two of the tested plant species, Lolium perenne (ray grass) and Cucurbita 21 pepo (zucchini), were highly sensitive to sediment phytotoxicity. Acute tests also showed significant inhibition of 22 germination and root growth, with avoidance by *Eisenia fetida* at the least contaminated sites (TRS and BER). 23 Chronic bioassays revealed that LDB and TRS sediment were significantly toxic to E. fetida and Heterocypris 24 incongruens (Ostracoda), and GEC sediment was toxic for the latter organism. In this on-land and spatialised 25 deposit scenario, river sediment from the LDB site (Lake Bourget marina) presented the highest potential toxicity 26 and required the greatest attention. However, low contamination levels can also lead to potential toxicity (as 27 demonstrated for GEC and TRS site), underlining the importance of a multiple test approach for this scenario.

28 KEY WORDS:

Cucurbita pepo; Dredged sediments; Environmental Risk Assessment (ERA); *Eisenia fetida; Heterocypris incongruens; Lolium perenne;* Metals; PCBs; Rhône River;

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32 Introduction

33 Sediment can be both a sink and source of contaminants for the overlying water column and biota, 34 especially when dredged from a river (Perrodin et al., 2006; Volatier et al., 2009; Bedaa et al., 2020; Heise et al., 35 2020). Several management options can be considered after dredging river sediments, such as capping, on-land 36 deposits, or discharge into water by resuspension. In the context of the European Water Framework Directive 37 (2000/60/EC) and, more recently, the Waste Framework Directive (2008/98/EG), the reuse of dredged sediments 38 requires a demonstration of environmental acceptability based on the assessment of physical and chemical 39 characteristics, especially by applying HP14 and NF EN 12920+A1 methodologies (Lecomte et al., 2019). In the 40 case of an on-land sediment deposit, an environmental risk assessment (ERA) is usually performed to assess the risks 41 to organisms and ecosystems surrounding the sediment deposit location (e.g. watercourses, wetlands, terrestrial 42 ecosystems) and groundwater quality (Perrodin et al., 2006; Pesce et al., 2020). Therefore, assessing the contaminant 43 content and potential toxicity of sediments to organisms growing on the surface or living in such deposits is 44 necessary. The sediment matrix approach is generally used to test seed germination, plant growth, earthworm 45 avoidance, and reproduction. Although it does not enable the determination of the substance(s) responsible for the 46 observed effect, it has the advantage of characterising hazards and exposure according to the contaminant load.

47 In risk evaluation, the first step is to calculate a contamination index based on a comparison of contaminant 48 concentrations with quality thresholds from the literature (e.g. PEC, probable effect concentration threshold) before 49 determining a risk quotient (MacDonald et al., 2000a,b). The risk quotient (RQ) was used to assess the effect of 50 mixtures of contaminants and compare samples with various compounds (MacDonald et al., 2000a). The RQ can be 51 defined as the ratio between the measured concentration of each pollutant in the environment (dredged sediments) 52 and the corresponding predicted no effect concentration for sediment-dwelling organisms (PNEC_{sed}) (ECB, 2003). 53 The use of PNEC_{sed} is valuable for considering a wide range of micropollutants that are not included in the PEC 54 (MacDonald et al., 2000a). Then, all RQ are added to the Risk Quotient mixture (RQ_{mix}) to estimate the global risk 55 based on a concentration addition model (Backhaus et al., 2012). As an example, the RQ_{mix} approach was recently 56 used to assess the risk from the effects of a cocktail of contaminants in dated sediment from backwater sites along 57 the Rhône, highlighting a high risk of mixture dominated by metals before 2005, and the time span since 2005 has 58 proven to be mainly driven by POPs (Liber et al., 2019; Dendievel et al. 2020b).

59 Another approach to qualify the risk is to implement bioassays to evaluate the effects of exposure on fauna or plants. 60 Ecotoxicological tools can provide added value for monitoring the true state of river quality, considering 61 contaminants potentially present at concentrations high enough to cause biological effects (Martinez-Haro et al., 62 2022). Bioassays performed on sediments dredged from canals have previously shown the importance of 63 phytotoxicity tests in determining the risks related to their deposits (Bedell et al., 2003; Perrodin et al., 2006; 64 Lecomte et al., 2019). Studies on seed germination have demonstrated a significant reduction in the growth of some 65 plant species in contact with metal-contaminated soils and sediments (Adam and Duncan, 2002; Chen et al., 2002; Bedell et al., 2003; Czerniawska-Kusza et al., 2006). Seed germination studies have also been used to evaluate the 66 67 toxicity of anthropogenic matrices such as manure and wastewater treatment plant sludge (Fuentes et al., 2004;

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68 Oleszczuk, 2008; Czerniawska-Kusza and Kusza, 2011; Oleszczuk et al., 2012). Acute ecotoxicity tests based on 69 earthworm avoidance bioassays (ISO 11268-1, 1993) and chronic toxicity tests are usually combined to determine 70 their effects on reproduction (ISO 11268-2, 1998) and to assess soil toxicity (Heupel, 2002; Greenslade and 71 Vaughan, 2003; Da Luz et al., 2004, Lecomte et al., 2019). The avoidance test can be used to distinguish soils or 72 matrices subjected to different anthropogenic stresses (Sousa et al., 2008; Alvarenga et al., 2012) or in various 73 environmental conditions (Garcia et al., 2008; Buch et al., 2013). Earthworm reproduction tests have been used to 74 assess chlorpyrifos-contaminated soils (Zhou et al., 2007), petroleum hydrocarbons, lead-contaminated soils 75 (Whitfield Aslund et al., 2013; Luo et al., 2014), urban soils (Hankard et al., 2005) and other anthroposols (Coehlo et 76 al., 2018). Another frequently used bioassay is the standardised ostracod toxicity test (using Heterocypris 77 incongruens). Indeed, another study, performed on 33 types of sediments, highlighted the potential of this test to 78 provide a reliable and sensitive alternative for sediment toxicity assessment (Belgis et al., 2003). This ostracod test 79 was also implemented to study the effects of contaminants on ecological functions supported by sediment 80 communities and showed high toxicity of Cu-spiked and Cu-plus-As-spiked sediments and low toxicity of As-spiked 81 sediments (Pesce et al., 2020). This test has also been used successfully in wetland and river contexts for the 82 treatment of landfill leachate (Buitrago et al., 2013), urban sediment toxicity assessment (Gonzalez-Merchan et al., 83 2014), and assessment of hospital effluents (Mubedi et al., 2013; Wang et al., 2009; Perrodin et al., 2013).

84 Because chemical analyses alone do not necessarily reflect the bioavailability and toxic action of measured 85 contaminants, multidisciplinary approaches are required to assess the chemical, biological, and toxicological impact 86 of complex mixtures of contaminants (Todaro et al., 2019). In this respect, ecotoxicological bioassays are essential complementary tools for investigating the link between contaminant contents and ecological responses and are even 87 88 more powerful in detecting the effects of pollutant mixtures that are not necessarily targeted (Heise et al., 2020). 89 However, to our knowledge, only a few studies have combined chemical and ecotoxicological approaches to assess 90 the toxicity of sediments in on-land deposit scenarios. Todaro et al. (2019) highlighted an unexpected toxic effect not 91 revealed by conventional approaches in the context of sediments from coastal areas, and the level of contamination 92 was not observed to be proportional to the ecotoxicological assessment. Another study on contaminated sediments 93 dredged from an urban river (New Jersey, USA) showed by calculating the Hazard Quotient that 7 years after 94 dredging, the risk associated with metals (especially Cu, Pb, and Hg) remained high, but without attention to the 95 effects of contaminants on biota and ecological functions (Soetan et al., 2022). Some studies have used chemical 96 characterisation as a first step to screen the level of contamination in sediments before implementing ecotoxicity tests 97 on the most contaminated samples (Ingersoll et al., 2000). The complementary application of chemical analyses and 98 ecotoxicological testing appears to be the best method to perform risk assessment from contaminated sediment or 99 dredged material analyses (How et al., 2023). Such a combined approach can reduce the probability of false-negative 100 results and is an opportunity for decision-making in sediment management in Europe (Heise et al., 2020).

101 In Western Europe, the Rhône River, which flows from Switzerland to France, is a notable case of an engineered 102 river that flows managed from Switzerland to France. This river corridor presents major urban-industrial 103 conurbations (mainly Lyon and Geneva) and a complex land-use history (Thorel et al., 2018). Sedimentary quality 104 issues related to Rhône River management make it a satisfactory candidate for assessing the chemical and ecological risks of an on-land deposit scenario. Since 1998, numerous monitoring and restoration works have been conducted,
including dredging and restoration operations in the framework of the RhônEco Program (Lamouroux et al., 2015;

- 107 Olivier, 2016) and the Rhône sediment observatory (Piégay et al., 2022). Spatiotemporal trends of sediment
- 108 contamination have been extensively evaluated by dated sediment core studies on Metal Trace Elements (MTE) and
- 109 Persistent Organic Pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides, and
- brominated flame retardants (Desmet et al., 2012; Mourier et al., 2014; Dendievel et al., 2020a; Liber et al., 2019;
- 111 Vauclin et al., 2021). In a synthesis work combining dated sediment cores and bed sediments, Dendievel et al.
- 112 (2020b) highlighted a combined toxicity risk mainly related to metals in the Upper Rhône River, particularly due to
- copper release and leaching (vineyards and mine tailings). Downstream of the « Grand Lyon » urban area, highconcentrations of POPs, their metabolites, and MTE produced a major increase in the mixture risk along the Middle
- and Lower Rhône River (Dendievel et al., 2020b).

116 This rich scientific literature has led us to develop an approach consistent with these previous studies. Thus, in this 117 study, our objective is in line with the concern of the combined analysis. We aim to characterise the potential toxicity 118 and, therefore, the risk to ecosystems, in the context of on-land deposits after dredging. Contaminant measurements 119 and bioassays were conducted on a panel of representative sediments extracted from the Rhône River in France. 120 First, the evaluation of contaminant levels (MTE, PCB) in sediments was interpreted with ecotoxic reference values 121 and calculations to estimate potential ecotoxicological risk. Acute (plant germination and earthworm avoidance) and 122 chronic bioassays (ostracod and earthworm growth and reproduction tests) were performed to evaluate the effects of 123 contaminants on ecological functions. Finally, all the results are discussed to define the potential risk and to provide 124 recommendations in the framework of a land-based sediment deposit scenario.

125 Materials and methods

126 Sediment sampling

127 Sediment surface samples were taken from four sites to represent the sediment diversity found along the 128 Rhône River. In the north, sediment was sampled from the marina of the Aix-les-Bains at Lake Bourget (LDB site; 45°41'38" N, 5°53'26" E) in May 2010. This site is a known hotspot for PCB contamination (Touzé and Bataillard, 129 130 2011; Lécrivain et al., 2018). Three other samples were collected from the Rhône River backwater areas. These areas 131 are permanently connected to the main channel of the river and continually supplied with water and sediment 132 (Desmet et al., 2012, Mourier et al., 2014). The BRE site (45°28'48''N, 4°46'83''E) was sampled in May 2008. This 133 site was carefully selected because it is located downstream of the Lyon urban area, and its industrial corridor which 134 extends south of the city. To the south, the sediment at the GEC site was collected in February 2011 (44°23'23" N; 135 4°39'21" E). This corresponds to a secondary channel located in the Pierrelatte floodplain, downstream of the 136 confluence with the Isere and Drôme Rivers. Finally, the TRS site is the most downstream site (43°43'30" N, 137 4°37'07" E), located near the Rhône River delta, and the sediment was collected in February 2012. The samples 138 were collected using a UWITEC[®] corer in order to obtain samples from short sediment cores. The sampling depth 139 reached was approximately 20-30 cm to fit with the most recent sediments (age-depth models are available in 140 Mourier et al., 2014). To obtain sufficient material for the tests, we repeated the operation several times over a 141 limited area at the same sampling depth. These sediments were stored in containers in a cold chamber at 5°C in the 142 dark. After storage, the decanted water was separated from the sediments. Each sediment samples in different storage

143 containers was homogenised before the experiments.

Analytical methods 144

145 The physical and chemical characteristics (pH, conductivity, TOC – total organic carbon, granulometry), 146 agronomic properties (potassium, nitrogen, and C/N ratio), and pollutant contents (PCBs and trace elements) were 147 acquired for each sediment sample (see SI-1 for analysis protocol details).

148 All measurements and most of the treatments were performed in four replicates (exceptions are mentioned in the 149 tables). All reagents were of analytical grade or ultrapure water. The glassware and plastic containers used for the 150 tests were cleaned by soaking them in HNO₃ (5% v/v) for 12 hours and then rinsing them several times with 151 deionised water. Most of the physical and chemical characteristics of the sediments were obtained using several 152 protocols and standards (ISO 13878 for nitrogen determination, NFX 31-130 for cation exchange capacity [CEC],

153 ISO 10390 for pH, NFX 31-102 standards for water content [see details in SI-1]).

- 154 For metal quantification, 1 g of dry matter from each sediment sample was dissolved in a mixture of 2 mL HNO₃ and 155 6 mL HCl and heated in a CEM-type microwave oven (CEM/Xpress). After filtration through ash-free filter paper, 156 25 mL ultrapure water was added. Then, metal concentrations were measured by Atomic Absorption Spectrometry 157 (AAS) with a Perkin Elmer PinAAcle 900 T. Flame absorption was used for Cr, Pb, and Zn (standard FD T90-112; 158 AFNOR, 1998), and a graphite furnace was employed for Cd and Cu (NF EN ISO 15586; AFNOR, 2004). The detection and quantification limits (see SI-1) were calculated as described by Baffi et al. (2002). Certified Reference 159 160 Material (BCR-280 R, lake sediment from EU Joint Research Centre) and control samples, such as Surface Water 161 Level 2 (Spectrapure standards), were used to assess the efficiency of mineralisation and the calibration of the metal 162 studied. PCBs were analysed by the EUROFINS laboratory, Saverne, France (www.eurofins.fr) and extracted in 163 accordance with the certified method: QMA 504-192 (DIN ENISO/IEC 17025:2000; for details, see the references in 164 Sup. Materials 1, and in Mourier et al., 2014). The sample extract was analysed by high resolution gas 165 chromatography-high resolution mass spectrometry (HRGC/HRMS) with a VG-AutoSpec in selected ion 166 monitoring (SIM) mode. For the seven indicator PCBs (PCB 28, 52, 101, 118, 138, 153 et 180), the quantification 167 limits ranged from 0.042 µg/kg dry weight (DW; PCBs 118 and 180) to 0.146 µg/kg DW (PCB 153).
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Sediment ecotoxicity assessment protocols

169 Sediment ecotoxicity was assessed using acute and chronic ecotoxicity bioassays (SI-2). Acute tests 170 comprised plant bioassays (germination and early root growth) based on species that are known to be tolerant or 171 which are potential bioaccumulators of POPs and MTE. These plant species include zucchini (Cucurbita pepo), 172 rapeseed (Brassica napus), ray grass (Lolium perenne), and black mustard (Brassica nigra). These choices were 173 based on key literature showing that Lolium perenne (Tato et al., 2011), Curcubitta peto (White, 2001; Whitfield 174 Aslund et al., 2007, 2008), and Brassica napus (Javorska et al., 2009) potentially accumulate pollutants, especially PCBs. Bedell et al. (2003; 2013) also highlighted a significant response of these plants to phytoxicity tests conducted on canal and port sediments. Moreover, some of these species or families are recommended in the international standard for this test (ISO 11269-1) (SI-2). During the germination tests on microplates, the seeds were deposited on blotting paper in contact with the sediment matrix. Therefore, the effect measured on germination was clearly linked

- to the availability of water or soluble compounds via root suction. Two replicates were performed for GEC, three for
- 180 BER, and four for TRS and LDB, depending on the available quantity of sediment.
- 181 Germination and root inhibition growth tests were performed on microplates (Phytotoxkit ® supplied by R-182 Biopharm, France). The microplates used were made of transparent plastic and were flat and shallow with two 183 compartments, one containing a solid matrix (ISO substrate or sediment) humidified at 70% retention capacity 184 (Phytotoxkit, 2004). The other compartment was empty and allowed for the emergence and growth of seedlings. The 185 cells were then incubated vertically in the dark for 48 h at 19°C in a climate culture room. The analysed sediments 186 were compared with the control (100% ISO substrate). The reference soil ISO substrate (OECD, 2010) is a mixture 187 of 10% peat, 70% silica (industrial sand smaller than 2 mm, with more than 50% of the particles comprising between 188 50 μ m and 200 μ m)], 20% clay (kaolin content: less than 30%), and \leq 1% CaCO₃.
- 189 An additional acute ecotoxicity bioassay was performed on the earthworm *Eisenia fetida* to test its avoidance (ISO
- 190 11268-1). It involved studying the behaviour of *E. fetida* placed in a container with two compartments: one with the 191 sediment to be tested and the other with the ISO substrate (OECD, 2010). This test was valid if the number of 192 dead/missing worms was less than 10% per treatment (SI-2).
- 193 The chronic tests were based on the growth and reproduction of *Eisenia fetida* (ISO 11268-2). This test was 194 performed on 10 adult earthworms placed in the sediment for 4 weeks. At the end of the experiment, adult 195 earthworms were weighed and compared with that of the control. The test was then continued for four more weeks to 196 allow reproduction, after which the juveniles were recovered, counted, and compared with the controls (see SI-2).
- 197 The Ostracodtoxkit® test was used (supplied by R-Biopharm, France) in order to place the ostracod *Heterocypris*198 *incongruens* in direct contact with the sediment to be tested for 6 days (the control was performed with washed,
 199 sieved, and dried sand supplied in the Ostracodtoxkit®). This test allowed to assess the growth and mortality of
- 200 ostracods (SI-2).

201 Data analysis

202 Risk quotient assessment

The RQ assessment is based on the measured environmental concentration (MEC) of each pollutant, divided by the corresponding $PNEC_{sed}$ (Predicted No Effect Concentration for sediment-dwelling organisms), currently used in ecotoxicological studies (ECB, 2003). $PNEC_{sed}$ values are available for a large variety of pollutants (https://substances.ineris.fr/fr/; see Table 2). However, the $PNEC_{sed}$ values are not defined for Cr and $\Sigma PCBi$; thus, we used the threshold effect concentration (TEC) as the reference value for the RQ estimate (Table 2). Finally, individual RQs (for each pollutant) were added to assess the risk of mixtures according to the Concentration

- 209 Addition model, which assumes a similar mode and site of toxic action of all pollutants on sediment-dwelling
- 210 organisms (Backhaus and Faust, 2012).
- 211 Mixture risk quotients (RQ_{Mix}) were calculated based on (1):
- 212 (1) $RQ_{Mix} = \Sigma (MEC_{i,x}/PNEC_{sed i})$
- 213 In the aforementioned equation, RQ_{Mix} corresponds to the sum of all RQs. The RQ for each pollutant is based on
- 214 MEC_{i,x}, which is the Measured Environmental Concentration of pollutant i at site \times (mg kg⁻¹ dw), and on PNEC_{sed i}
- according to Equilibrium Partitioning (mg kg⁻¹ dw). RQ and RQ_{Mix} values range from < 1 (negligible risk) > to > 100
- 216 (high risk) according to Gosset et al. (2020) and Perrodin et al. (2012).

217 Statistical tests

- The significance level of the differences between the bioassay data for different sediments and groups of stations was assessed using the *t* test and Mann–Whitney U-test. Statistical significance was set than 0.05. Statistical analyses were performed using STATISTICA© (version 10).
- 221 Post hoc comparisons between normally distributed populations (*e.g.* germination and root inhibition (RI) at 7 days
- 222 on microplate assays) were performed using the *t* test. A non-parametric test (Mann-Whitney U-test) was applied
- when a normal distribution could not be achieved. The Mann-Whitney test was used to analyse (i) the differences in
- the distribution of earthworms in the two compartments at the end of the avoidance test and (ii) between the control
- and the sediment in the growth and death of ostracods and earthworms (reproduction test).

226 **Results/Discussion**

227 Sediment characterisation

228 The sediments tested were slightly alkaline (pH 7.5; Table 1). The conductivity measurements showed two 229 groups: (i) TRS and BER sediments between 307 and 347 µs/cm, and (ii) LDB and GEC sediments between 644 and 230 795 µs/cm (Table 1). These groups can be linked to local physical and geochemical settings or to potential release in 231 nearby areas. Overall, the sediment grain size was silty to silty-sandy, with the highest percentage of clays for TRS 232 and GEC sediments (10 to 8%). The Total Organic Carbon content (TOC) was between 12.8 and 17.9 mg/kg DW for 233 most of the sediment samples, except for that of LDB, which was three times higher than the others (68.2 mg/kg 234 DW). LBD sediment also had much higher potassium and nitrogen contents than the other sediments (Table 1). 235 Phosphorous contents ranged from 0.13 to 0.24 g/kg DW. The C/N ratio was approximately 12, except for that of 236 BER sediment, which is approximately 9.7 (Table 1). These values are not observed to be limiting factors for plant 237 growth and development (Doucet, 1992). Only BER and GEC sediments had nutrient contents (especially, 238 potassium) that were slightly lower than those of the other sediments (Table 1).

239 Trace Metal Elements (TME) and $\sum PCBi$ contents

Concerning TME, Cd presents the most severe concentrations for all sediments: between 0.9 and 1.4 mg/kg
 DW (Table 1). Such concentrations often reach the Probable Effect Concentration threshold (PEC = 0.99 mg/kg

- 242 DW) defined for sediment-dwelling organisms (MacDonald et al., 2000a) but not that of the French regulatory
- threshold S1, defining the contaminant levels to be considered in sediments extracted from rivers and canal dredged
- sediments (JORF, 2006; see also Table 2). Because of the variability of the measures, the contents of Cr and Ni were
- also high for the different samples (47.7–70.2 mg/kg DW for Cr, and 23.5–34.1 mg/kg DW for Ni). Even if these
- values are higher than the local geochemical background along the Rhône River, they are much lower than the
- French threshold S1 (Table 2). For the other elements, the concentrations of Cu, Pb, and Zn were generally higher at
- LDB and GEC site than at the other sites (two to three times higher). Cu and Zn were lower than the French
- threshold S1 (JORF, 2006). Pb values in the LDB sediment slightly exceeded the regulatory threshold S1 (Tables 1 and 2).
- 251 The sediment concentrations in the sum of the seven PCB indicators (Σ PCBi) were highest for LDB site (1204 µg/kg 252 DW on average). This value largely exceeds the regulatory threshold S1 (680 μ g/kg) and PEC for total PCBs 253 (MacDonald et al., 2000a). GEC sediment also contained 122.1 µg/kg DW ∑PCBi on average. This concentration 254 was between the TEC (35 µg/kg; MacDonald et al., 2000b) and the PEC/S1. Finally, BER and GEC sediment 255 contained between 22 and 27 μ g/kg DW of Σ PCBi on average. Even if these levels are lower than the 256 aforementioned thresholds (TEC, PEC, S1), they remain within the ranges of the sediment benchmarks (10 µg/kg 257 and 60 µg/kg) calculated from biota-to-sediment accumulation factor models in fish (Babut et al., 2012; Lopes et al., 258 2012).
- In the studied samples, PCB congener concentrations were in the order of PCB 153 > PCB 180 > PCB 138 > PCB
- 260 101 (Table 1). This assemblage might be considered a marker related to river contamination by industries using
- 261 PCBs, as demonstrated by Lasserre et al. (2009). Mourier et al. (2014) highlighted the same congener distribution in
- historical sediment cores extracted along the Rhône River (1960–2011).
- In summary, the sediment samples present decreasing concentrations of TME and PCB in the following order: LDB
 > GEC > TRS > BER. Except for the high levels of contaminants measured in the LDB sediment, the "hazard status"
 of the other sediments is complicated to define because some contaminants have high concentrations individually.
 They all present a potential risk of pollutant transfer to plants, and an ecotoxicological risk for organisms likely to
 colonise and reproduce in such deposits. Based on their physicochemical characteristics and agronomic values, the
 sediment samples are not expected to be a limiting factor for plant growth.

269 Potential environmental risk

- To evaluate the ecotoxicological risk and potential hazard of the sediment samples, we calculated the risk
 quotient mixture (RQ_{mix}) based on MEC and PNEC_{sed} data (see section 2.4.1). In our study, the mixture quotient was
 based on the concentrations of metals and PCBs to assess the relative contribution of each pollutant to the cocktail
- effect on sediment-dwelling organisms.
- 274 LBD sediment presents the most important concentrations of trace elements and ΣPCBi (Table 1). Consequently,
- these concentrations lead to a high RQ_{Mix} value (> 100), especially driven by Cu and ΣPCB is. Individual RQ for Cr,
- Ni, Pb, and Zn suggested a low-level risk for the mixture. RQ values derived from Cd appear relatively low owing to

the high $PNEC_{sed}$ limit (2.5 mg/kg), which underestimates the risk. This result is somewhat surprising because Cd values are problematic with respect to toxicology thresholds for most sites (Table 2: values > PEC). The other tested sediments (from BRE, GEC, and TRS) presented a moderate ecotoxicological risk (38.17 < RQ_{Mix} < 56.51). The sediments tested can be classified according to a decreasing gradient of the RQ_{Mix} total in the following order: LDB > GEC > TRS > BER.

282 The Risk Quotient mixture (RQ_{Mix}) is based on the analised metals and PCBs to assess the relative contribution of 283 each pollutant to the effect of a cocktail of contaminants on sediment-dwelling organisms. According to data in the 284 literature on core sediments from the Rhône River, the cumulative ecotoxicity risk is mainly driven by (1) 285 dichlorodiphenyltrichloroethane (DDT) metabolites (DDE and DDD), (2) lindane isomers (HCHs), and (3) PCBs 286 that remain a major cause of concern for the Rhône (Dendievel et al., 2020a). The Risk Quotient mixture could also 287 fluctuate, such as in English coastal rivers (Manuel Nicolaus et al., 2015), or negligible, such as in the Garonne 288 Estuary (metals: Larrose et al., 2010), due to sorption processes and organic matter degradation, reducing the 289 estimation of the impact of industrial inputs in sediments, while high metal concentrations have been found in fauna 290 (Lanceleur et al., 2011). Other studies have been shown that in such a global approach by risk ratio, or RQ, this is 291 mainly due to the acetochlor ($C_{14}H_{20}CINO_2$) in four sediments of Argentina's rural streams (Fernández San Juan et 292 al., 2022) or to the tributyltin concentrations at almost all the study sites of the Odra River estuary (Kucharski et al., 293 2022). Such results cited also underly the weight of POP contamination in such an evaluation. At the scale of the 294 French Rhône River (Figure 2), the data presented in this work are consistent with other RQ_{Mix} values published for 295 sediment cores (Dendievel et al., 2020b). Thus, the BER, GEC, and TRS RQ_{Mix} values are in line with the known 296 RQ_{Mix} values in the Middle and Lower Rhône River sections (Figure 2). LDB sediment is an exception and offers 297 strong RQ_{Mix} without link between ETL and MTE. Notably, LDB sediments are from the Aix-les-Bains marina, 298 located in Bourget Lake. The latter lake is a major sediment deposition zone, only temporarily connected to the 299 Rhône River by a short channel ("canal de Savières") during floods, which explains no relationship between the 300 LBD peak and the other upstream sites.

301 Ecotoxicity evaluation

Our ecotoxicological approach used acute (germination, avoidance) and chronic exposure tests (e.g.
 reproduction, growth) on different components of an ecosystem, such as primary producers (plants), decomposers
 (earthworms), and target organisms of the deposit (plants) or the sediment (ostracods).

305 Acute toxicity tests of sediments

306 Early germination and growth on sediment in microplates

Germination tests using the French HP14 methodology were performed on four sediments. The germination
inhibition of *Cucurbita pepo* ssp. *pepo* was more notable for GEC and LDB (ca. 18%) than for BER and TRS (3.3 to
5%; Table 3). This result is much less than that in a study analysing the germination of *Cucurbita pepo* ssp. *pepo* in
soil amended with DDT (~1500 ng/g), which showed the development of 60% of the seeds (n=5; Whitfield Ashlund

311 et al., 2010). Three sediments (from BER, TRS, LDB) were also tested with Brassica nigra and Lolium perenne, and 312 germination inhibition seemed effective for LDB (both plants: 1 to 4%) and BER (B. nigra, ca. 10%) sediments. By 313 contrast, an inhibition of the germination associated with a notable standard error on TRS suggested a lack of effect 314 or a weak stimulation of the seeds. Chigbo and Batty (2013) reported that he combined effect of Cr and 315 Benzo(a)pyrene (BaP) significantly reduced the germination rate of L. perenne in comparison of the control and 316 assays with single contaminant effects. These variations in L. perenne germination (e.g. inhibition or stimulation) 317 have also been detected using microplate assays and sediments (Bedell et al., 2003; 2013). For Brassica napus, the 318 two sediments tested (from TRS and LDB) had no significant effect on seed germination (Table 3). This result 319 supports that of Wierzbicka and Obidzinska (1998), who showed that seed coat morphology is an important defence 320 against some metallic ions, especially for Brassicaceae (Crucifereae). However, even if B. napus has high emergence 321 variability (Fernandez et al., 2005), the sensitivity of germination of B. napus to dredged canal sediment was 322 observed during a three-year phytoremediation trial (King et al., 2006). Regarding other plants that could be used in 323 these tests, alfalfa (Medicago sativa), a dicotyledonous species, seems to be the most relevant species for screening 324 sediment toxicity before reuse (Lecomte et al., 2019). Such variability and sensitivity according to the species tested, 325 which was also observed in our data, may result from the different protection provided to embryos by seed coverage 326 or the permeability of this coverage to contaminants or water.

327 Two physiological phenomena are active during germination: oxygen use increases when respiration begins and 328 imbibition. Imbibition can occur when the seed rehydrates but can also be linked to sediment properties such as 329 texture (and clay content). This case was not observed in our study because BER sediment has greater inhibition of 330 root growth and germination than TRS sediment, despite a lower clay content for the former than for the latter (4% 331 vs 10%; Table 1). Another hypothesis suggests that differences in seed germination are related to seed respiration 332 and that levels of metals (e.g. As, Hg, or Cd) and PCBs are potential inhibitors of seed respiration (Sethy and Ghosh, 333 2013). Beyond contaminants, these variations can be attributed to several factors related to the experimental design, 334 such as the use of microplates (blotting paper) and seed quality.

335 Germination tests performed on seeds with decant water collected from the sediments did not show a significant 336 inhibitory effect on germination or early root growth. However, a correlation was observed between the root growth 337 of black mustard and the concentrations of pollutants in the decanted water of sediment BER (Fisher test = 0.0103 at 338 P < 0.05). Thus, according to our results, the sediment matrix is more relevant for toxicity tests than the decant 339 water. Such conclusions have also been well demonstrated in other studies, highlighting that sediment induces a 340 higher toxic response than pore water does (Palma et al., 2014). In our case, we found correlations between *Curcubita pepo* germination and RI with sediment conductivity (r^2 of 0.77 and 0.92, respectively), suggesting an 341 342 influence of water quality on seed imbibition and, therefore, germination.

Notably, in the case of germination inhibition, root growth inhibition generally occurs for a given type of sediment and plant species and *vice versa* (Table 3). Regarding the inhibition of early root growth (see SI-2 for details), the radicles of *Cucurbita pepo* were inhibited in all tested sediments (from TRS, GEC, BER, and LDB), and

346 considerable inhibition was observed for Lolium perenne (from TRS, BER, and LDB; Table 3). The radicle growth 347 of Brassica napus and B. nigra was neither affected nor slightly stimulated (Table 3). However, for Lolium perenne, 348 opposing effects were observed in our results: germination inhibition and increased root growth (Table 4). This 349 contradiction was also highlighted by Chigbo and Batty (2013), who demonstrated that root and shoot elongation of 350 L. perenne was significantly inhibited (p > 0.05) at a high concentration of Cr, whereas increasing concentrations of 351 BaP accelerated shoot elongation. In our case, RI was more pronounced for Lolium perenne in BER sediment than in 352 TRS sediment, despite similar SPCBi contents. BER sediment had lower metal contents than TRS sediment did 353 (Table 1). Consequently, we concluded that there was no correlation between germination inhibition and early root 354 growth. A similar absence of proven correlations between pollutant content and the effect observed in this type of 355 acute test was observed by Mamindy-Pajany et al. (2011) in some bioassays with Poaceae (Sorghum saccharatum) 356 and Brassicaceae (Lepidium sativum) on seaport sediments. Nonetheless, in the case of the most contaminated 357 samples (e.g. LBD and GEC sediments), Cucurbita pepo and Lolium perenne were the species associated with the 358 most marked inhibition.

Finally, acute germination and early root growth tests allowed us to classify the sediments from most to least inhibition regarding the plants tested: LDB > BER \ge GEC > TRS. This order is not only related to the levels of TMEs and PCBs in the sediment but also to the availability of nutrients, such as nitrogen and phosphorus, which depend on the sediment type. The presence of other POPs, which were not measured in this study, is also a potential factor affecting plants. Because of the experimental system used (blotting paper), The TMEs were undoubtedly the only elements that had an effect in both experiments.

365 Earthworm (*Eisenia fetida*) avoidance test

366 The earthworm avoidance test requires a large quantity of sediment; thus, it was only conducted on BER, 367 LDB, and TRS sediments (Table 4). We observed that from 60 to 62.5% of the earthworms preferred LDB sediment 368 to the ISO reference soil. By contrast, earthworms avoided TRS and BER sediments (Table 4). These differences can 369 be explained by the organic matter and clay content (Table 1). LDB sediment had a higher organic matter content 370 than TRS and BER sediments did (Table 1), which may explain the earthworms' preference for LDB sediment, 371 despite the samples being contaminated by Pb and PCBs (Table 1). Davies et al. (2003 a,b) demonstrated the effect 372 of Pb on worms, especially through accumulation due to uptake regulation at low contamination levels. Moreover, 373 earthworms can sense chemicals via many chemoreceptors, as shown by the contamination avoidance results 374 obtained for pesticides (Zhou et al., 2007; Garcia et al., 2008) and 2,4,6-trinitrotoluene (TNT) (Schaefer, 2004). 375 Edwards and Bohlen (1996) observed that most organophosphates (OP) herbicides were not toxic to E. fetida worms 376 because they were unable to transform OP into toxic metabolites. Although toxic, substances such as cadmium salts 377 may not be perceived as repulsive if the body is forced to remain in contact with them (Greenslade and Vaughan, 378 2003). An avoidance test performed on four fluviatile sediments showed 47.2% of earthworms' avoidance and 45% 379 for dam sediment avoidance (Lecomte et al., 2019).

380 TRS sediment was richer in clay (10%) than BER and LDB sediment. This clay content may be one of the factors 381 explaining sediment colonisation by earthworms. Hund-Rinke et al. (2005) found different avoidance factors (EC50) 382 according to the soil type in the presence of pentachlorophenol (PCP): 8 mg/kg for sandy soil and 24 mg/kg for silty 383 soil. This result clearly illustrates the key role of the sediment characteristics with respect to the real conditions of 384 exposure of earthworms to contaminants, such as through the adsorption of PCP, which is positively correlated with 385 organic matter. Finally, the sediments were graded from least to most colonisable by earthworms in the following 386 order: BER - TRS - LDB. The avoidance test remains a satisfactory compromise for the rapid, easy evaluation of 387 potential colonisation by earthworms.

388 Chronic toxicity tests on sediments

389 Monitoring mortality and growth of *Heterocypris incongruens*: ostracod test

In this section, we aim to determine the growth inhibition and mortality of the benthic ostracod *Heterocypris incongruens*. To achieve this objective, we use a chronic sediment toxicity test (Ostracodtoxkit®) in the presence of the sediment samples and compare them to a control (sand provided in the kit). The maximum ostracod mortality rate was approximately 8% in the six control replicates. This low percentage (<20%) and the correct growth of the ostracod length in the controls (x1.5 between T0 and T+6) validated the experiment.

395 Regarding the tests performed on the Rhône sediments, almost all ostracods in contact with the LDB sediment died 396 (90%), and a mortality of 50% was reported for ostracods in contact with the GEC sediment (Figure 1-A). Thus, 397 LDB and GEC sediment had a major effect on *Heterocypris incongruens* survival, even if the variability associated 398 with this test was greater for LDB and GEC sediment than for the other sediments. Ostracod growth was inhibited 399 from 35 to 110% in the four sediments tested (Figure 1-B). Their growth was considerably inhibited by LDB 400 sediment and to a lesser extent by GEC sediment. However, TRS and BER sediment showed low effect on ostracod 401 survival but a significant effect (40 to 50% on average) on the growth of *H. incongruens*. A strong relationship can be assumed between the pollutant concentrations in the sediments (Table 1), and the effects observed on ostracods. 402 High correlations ($r^2 > 0.8$) were observed among physicochemical parameters (e.g. PCBi levels and several TMEs, 403 404 including Cu, Pb, and Zn) and growth inhibition or mortality.

405 Our results agree with those of another study, which examined 33 sediments and concluded that the ostracod 406 microbiotest is a reliable, sensitive alternative to whole sediment assays for toxicity assessment (Belgis et al., 2003). 407 Pesce et al. (2020) recently highlighted the promising prospects of the ostracod test for investigating the 408 ecotoxicological effects of metal contamination on natural sediment communities, using the degradation and 409 decomposition of particulate organic matter as a functional descriptor. Lecomte et al. (2019) demonstrated up to a 410 40.6% increase in ostracod growth inhibition in dam sediments but found no effects on mortality. In contrast with 411 results of Lecomte et al. (2019), our study of river sediments showed that the responses of H. incongruens revealed a 412 potential toxicity gradient (with mortality) in the sediments tested. In a similar context (reservoir sediments), Palma

- 413 et al. (2014) showed that *H. incongruens* is sensitive to contaminants found in sediments with higher mortality but
- 414 lower growth inhibition for similar sediment characteristics and similar MTE contents.
- 415 Finally, the sediments were classified according to the inhibition of the growth of *Heterocypris incongruens*: LDB >
- 416 GEC > TRS > BER. In the case of an on-land deposit scenario, LDB sediment, and to a lesser extent GEC sediment,
- 417 presented the greatest risk of toxicity for *Heterocypris incongruens*.

418 Chronic earthworm (*Eisenia fetida*) reproduction test in TRS and LDB sediments

419 Chronic reproductive tests of Eisenia fetida were performed after 4 and 8 weeks of exposure to TRS and 420 LDB sediments. After 4 weeks, the growth of 10 adult earthworms on the TRS sediment presented a loss of biomass 421 of approximately 20% (Table 5). This loss was most significant for the LDB sediment (36% of biomass loss). 422 However, this inhibition did not increase the mortality rate (Table 5). Lecomte et al. (2019) also showed a small 423 effect on biomass intake but a greater effect on earthworm reproduction with undiluted sediment. Spurgeon and 424 Hopkin (1996) demonstrated a major link among metals, especially Zn, and the survival and growth of Eisenia fetida 425 in artificial soil. They found a significant worm mortality gradient in soils containing from 1200 to 2000 mg.kg⁻¹ Zn 426 (all worms died in the latter case). These results were obtained for soil that was approximately 10 times more 427 polluted than our sediments. In another study (Schaefer, 2004), biomass loss reached from 10 to 20% in soils 428 contaminated with TNT. However, the author did not draw a conclusion regarding the significance of toxicity 429 because all the individuals in all the variants had lost weight by the end of the test (Schaefer, 2004). However, in 430 other soils (Luo et al., 2014), the weight loss of earthworms was significantly and positively correlated with water-431 extractable Pb but negatively correlated with CEC. Luo et al. (2014) also observed that a low organic matter content 432 in soils could have an adverse effect on earthworm growth. In our study, CEC, Pb concentration, and TOC in the 433 LDB sediment were higher than those in the TRS sediment, which might explain the higher earthworm biomass loss 434 in LDB than in TRS (Table 1).

435 After 8 weeks, the growth inhibition affected 50% of the juveniles from TRS and LDB sediment compared to the 436 control (Table 5). In other contexts (chlorpyrifos-contaminated soils), Zhou et al. (2007) also showed a significant 437 effect of exposure to chlorpyrifos-contaminated soils on earthworm reproduction. The number of juveniles was 438 significantly and positively correlated with pH, Ca, silt content, Zn, Cd, and CEC but significantly and negatively 439 correlated with sand content (Luo et al., 2014). In our sediment samples, those from LDB and TRS presented small 440 differences in pH, silt, and sand values (Table 1). However, the high CEC, Zn, and Cd contents in LDB sediment can 441 explain the greater reduction in the number of juveniles than in TRS sediment (Tables 1 and 5). Moreover, 442 discoloration of the juveniles was observed during the experiments with LDB sediment but not with TRS sediment 443 (data not shown). The CEC and pollutant content can explain the effect of our sediments on earthworm reproduction. 444 Similarly, Hankard et al. (2005) showed that earthworms could survive in all the urban soils they tested, but 445 differences occurred in the reproduction rate, possibly affecting the fitness of worms inhabiting the sediment deposit.

- 446 **Risk evaluation discussion**
- 447

In this study, hazard characterisation (via chemical analyses) and ecotoxicological tests were conducted on a panel of sediments with different levels of pollutants. Based on RQmix and a comparison of concentrations with ecotoxicological thresholds, we highlighted a decreasing gradient of potential danger in the following order: LDB > GEC > TRS > BER. These chemical analyses showed that LDB sediment was problematic for management in an onland deposit scenario, and the hazard was considered moderate for the other samples. The mortality and growth of *Heterocypris incongruens* were well related to pollutant loads (PCBi and TMEs, especially copper, lead, and zinc), but other ecotoxicological tests have provided less marked results.

- The acute ecotoxicity bioassays showed the effect of contaminated sediments on germination and early root growth, with the greatest sensitivity for the species *Lolium perenne* and *Cucurbita pepo*. LDB, GEC, and BER sediment were the most ecotoxic owing to their pollutant loads. Moreover, LDB sediment was twice as rich in organic matter as the samples from other sites.
- The chronic tests showed significant toxicity for *Eisenia fetida* and *Heterocypris incongruens* living in LDB sediment. Tests on earthworms (*E. fetida*) also demonstrated a significant effect of TRS and LDB sediment on reproduction and growth. LDB sediment appeared to be toxic to ostracods (*H. incongruens*), but the variability observed did not permit a definitive conclusion on its toxicity.
- In their study of seaport sediments, Mamindy-Pajany et al. (2011) showed that the relationship between chemical
 data and toxicity tests was unclear. Our results indicate satisfactory complementarity of the chemistry and ostracod
 test results for all tested sediments and observed results for the other bioassays.

466 The combination of ecotoxic results and RQ_{mix} values at different concentrations in the sediments demonstrates that 467 LDB (marina) sediment is still not available for an on-land deposit scenario. TRS and GES sediment are not directly 468 reusable, especially based on the results of chronic tests. However, BER sediment did not show a combined effect 469 and is suitable for such a scenario.

- 470 Moreover, the choice of scenario for the management of the sediment can be linked to other possibilities of the
- 471 sediment valorisation. Some studies have suggested the valorisation of sediments as a plant substrate (Ferrans et al.,
- 472 2022), metal recovery in a cost-integrated approach, or a circular management approach of dredged sediments
- 473 (Crocetti et al., 2022; Svensson et al., 2022). The latter case points to the critical and current need to consider metals
- and POPs together, and to integrate physical and socio-environmental parameters in order to assess over time the
- toxicity of sediments along large and heterogeneous rivers (Beyer et al., 2014; Kortenkamp et al., 2019).

476 Notably, the risk of dredged sediment is also linked to deposit site specificities. Thus, a collaborative effort among 477 stakeholders, scientists, and representatives of the government must be realised for site determination linked to risk 478 evaluation. For example, a collaborative approach among the French Government, the "Grand Port Maritime de 479 Rouen", and a scientific committee has made it possible to define an integrative protocol to survey all the 480 compartments that might be affected by dumping in the management of port dredged sediment in the Bay of Seine 481 (France) (Marmin et al., 2014). All these monitoring procedures (i.e. sediment and water quality, including chemical

- 482 contamination; bioaccumulation and ecotoxicology on target species; microbiology, invertebrates, and fish surveys;
- 483 impact on the Natura 2000 areas) were conducted by partners under the responsibility of the "Grand Port Maritime484 de Rouen" from 2012 to 2013 (Marmin et al., 2014).

Conclusion

485

486 The ecotoxicological risks induced by a scenario of land-based deposition of dredged sediments from the 487 Rhône River were assessed by characterising sediments and the associated pollution (physical and chemical 488 contents) and combining multiple tests on various organisms likely to colonise the deposit (e.g. plants, ostracods, 489 worms). The potential danger of the selected sediments was measured by simulating the risks from exposure to the 490 selected of pollutants based on the RQ_{mix} calculations. According to this approach, one site presented problematic 491 pollution for management in an on-land deposit scenario: the LBD sediment extracted from Bourget Lake Marina. 492 Various complex responses were found for the other tested sediments and sites, which confirms the need to perform 493 complementary ecotoxicological tests.

Bioassays involving earthworms are essential because they show that sediments can have different effects depending on the exposure time. The LDB sediment had the greatest inhibitory effect on earthworm reproduction and growth. In addition, some tests are sometimes difficult to interpret because earthworms seem to prefer sediments with agronomic characteristics favourable to their development (high organic matter and low clay content), even though this matrix may have notable toxic effects. However, our series of bioassays showed that all the sediments tested were potentially ecotoxic to organisms that sought to colonise the deposit. Because it is the most toxic, LDB sediment is complicated to manage; thus, the on-land deposit scenario should be avoided in this case.

The response to toxicity bioassays obtained on the other sediments also showed the limitations of these tests and the need for a complementary assessment, particularly over time, of the dispersion, or mobility of contaminants for definitive decision-making. Then, TRS sediment is not available directly for such deposits based on chronic test results, and on the other hand, BER and GEC sediment can be used for such deposit scenarios with attention to germination in the case of the revegetating approach. Further investigations before management should be performed by a temporal simulation of ageing (by leaching in columns) or by mimicking several redox conditions.

507 Finally, an integrative approach among the physicochemical interrelations and ecotoxicological tests provided 508 relevant perspectives for management and modalities for land deposit scenarios of dredged sediments. Therefore, to 509 manage sediments dredged from the Rhône River and to deposit them on land, determining the cumulative risks 510 linked to the sediments is necessary. In addition, the content and release of pollutants from runoff water should also 511 be studied because such runoff water could be a vector of the transport and diffusion of the pollution and ecotoxic, 512 particularly for local aquatic and wetland ecosystems.

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- 518

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