



Combined Chemical and Ecotoxicological Measurements for River Sediment Management in an On-Land Deposit Scenario

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Abstract

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

Sediment can be both a sink and source of contaminants for the overlying water column and biota, especially when dredged from a river (Perrodin et al. 2006; Volatier et al. 2009; Bedaa et al. 2020; Heise et al. 2020). Several management options can be considered after dredging river sediments, such as capping, on-land deposits, or discharge into water by resuspension. In the context of the European Water Framework Directive (2000/60/EC) and, more recently, the Waste Framework Directive (2008/98/EG), the reuse of dredged sediments requires a demonstration of environmental acceptability based on the assessment of physical and chemical characteristics, especially by applying HP14 and NF EN 12920+A1 methodologies (Lecomte et al. 2019). In the case of an on-land sediment deposit, an environmental

risk assessment (ERA) is usually performed to assess the risks to organisms and ecosystems surrounding the sediment deposit location (e.g. watercourses, wetlands, terrestrial ecosystems) and groundwater quality (Perrodin et al. 2006; Pesce et al. 2020). Therefore, assessing the contaminant content and potential toxicity of sediments to organisms growing on the surface or living in such deposits is necessary. The sediment matrix approach is generally used to test seed germination, plant growth, earthworm avoidance, and reproduction. Although it does not enable the determination of the substance(s) responsible for the observed effect, it has the advantage of characterising hazards and exposure according to the contaminant load.

In risk evaluation, the first step is to calculate a contamination index based on a comparison of contaminant concentrations with quality thresholds from the literature (e.g. PEC, probable effect concentration threshold) before determining a risk quotient (MacDonald et al. 2000a, b). The risk quotient (RQ) was used to assess the effect of mixtures of

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contaminants and compare samples with various compounds (MacDonald et al. 2000a). The RQ can be defined as the ratio between the measured concentration of each pollutant in the environment (dredged sediments) and the corresponding predicted no effect concentration for sediment-dwelling organisms ($PNEC_{sed}$) (ECB 2003). The use of $PNEC_{sed}$ is valuable for considering a wide range of micropollutants that are not included in the PEC (MacDonald et al. 2000a). Then, all RQ are added to the Risk Quotient mixture (RQ_{mix}) to estimate the global risk based on a concentration addition model (Backhaus et al. 2012). As an example, the RQ_{mix} approach was recently used to assess the risk from the effects of a cocktail of contaminants in dated sediment from backwater sites along the Rhône, highlighting a high risk of mixture dominated by metals before 2005, and the time span since 2005 has proven to be mainly driven by POPs (Liber et al. 2019; Dendievel et al. 2020b).

Another approach to qualify the risk is to implement bioassays to evaluate the effects of exposure on fauna or plants. Ecotoxicological tools can provide added value for monitoring the true state of river quality, considering contaminants potentially present at concentrations high enough to cause biological effects (Martinez-Haro et al. 2022). Bioassays performed on sediments dredged from canals have previously shown the importance of phytotoxicity tests in determining the risks related to their deposits (Bedell et al. 2003; Perrodin et al. 2006; Lecomte et al. 2019). Studies on seed germination have demonstrated a significant reduction in the growth of some 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 toxicity of anthropogenic matrices such as manure and wastewater treatment plant sludge (Fuentes et al. 2004; Oleszczuk 2008; Czerniawska-Kusza and Kusza 2011; Oleszczuk et al. 2012). Acute ecotoxicity tests based on earthworm avoidance bioassays (ISO 11268-1 1993) and chronic toxicity tests are usually combined to determine their effects on reproduction (ISO 11268-2 1998) and to assess soil toxicity (Heupel 2002; Greenslade and Vaughan 2003; Da Luz et al. 2004; Lecomte et al. 2019). The avoidance test can be used to distinguish soils or matrices subjected to different anthropogenic stresses (Sousa et al. 2008; Alvarenga et al. 2012) or in various environmental conditions (Garcia et al. 2008; Buch et al. 2013). Earthworm reproduction tests have been used to assess chlorpyrifos-contaminated soils (Zhou et al. 2007), petroleum hydrocarbons, lead-contaminated soils (Whitfield Aslund et al. 2013; Luo et al. 2014), urban soils (Hankard et al. 2005) and other anthroposols (Coehlo et al. 2018). Another frequently used bioassay is the standardised ostracod toxicity test (using *Heterocypris incongruens*). Indeed, another study, performed on 33 types of sediments, highlighted the potential of this test to provide

a reliable and sensitive alternative for sediment toxicity assessment (Belgis et al. 2003). This ostracod test was also implemented to study the effects of contaminants on ecological functions supported by sediment communities and showed high toxicity of Cu-spiked and Cu-plus-As-spiked sediments and low toxicity of As-spiked sediments (Pesce et al. 2020). This test has also been used successfully in wetland and river contexts for the treatment of landfill leachate (Buitrago et al. 2013), urban sediment toxicity assessment (Gonzalez-Merchan et al. 2014), and assessment of hospital effluents (Mubedi et al. 2013; Wang et al. 2009; Perrodin et al. 2013).

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

In Western Europe, the Rhône River, which flows from Switzerland to France, is a notable case of an engineered river that flows managed from Switzerland to France. This river corridor presents major urban-industrial conurbations (mainly Lyon and Geneva) and a complex land-use history (Thorel et al. 2018). Sedimentary quality 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 RhôneEco Program (Lamouroux et al. 2015; Olivier 2016) and the Rhône sediment observatory (Piégay et al. 2022). Spatiotemporal trends of sediment contamination have been extensively evaluated by dated sediment core studies on Metal Trace Elements (MTE) and 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; Vauclin et al. 2021). In a synthesis work combining dated sediment cores and bed sediments, Dendievel et al. (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, high concentrations 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).

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

Materials and Methods

Sediment Sampling

Sediment surface samples were taken from four sites to represent the sediment diversity found along the 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 2011; Lécrivain et al. 2018). Three other samples were collected from the Rhône River backwater areas. These areas are permanently

connected to the main channel of the river and continually supplied with water and sediment (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 site was carefully selected because it is located downstream of the Lyon urban area, and its industrial corridor which extends south of the city. To the south, the sediment at the GEC site was collected in February 2011 (44° 23' 23" N; 4° 39' 21" E). This corresponds to a secondary channel located in the Pierrelatte floodplain, downstream of the confluence with the Isere and Drôme Rivers. Finally, the TRS site is the most downstream site (43° 43' 30" N, 4° 37' 07" E), located near the Rhône River delta, and the sediment was collected in February 2012. The samples were collected using a UWITEC® corer in order to obtain samples from short sediment cores. The sampling depth reached was approximately 20–30 cm to fit with the most recent sediments (age-depth models are available in Mourier et al. 2014). To obtain sufficient material for the tests, we repeated the operation several times over a limited area at the same sampling depth. These sediments were stored in containers in a cold chamber at 5 °C in the dark. After storage, the decanted water was separated from the sediments. Each sediment samples in different storage containers was homogenised before the experiments.

Analytical Methods

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

All measurements and most of the treatments were performed in four replicates (exceptions are mentioned in the tables). All reagents were of analytical grade or ultrapure water. The glassware and plastic containers used for the tests were cleaned by soaking them in HNO₃ (5% v/v) for 12 h and then rinsing them several times with deionised water. Most of the physical and chemical characteristics of the sediments were obtained using several protocols and standards (ISO 13878 for nitrogen determination, NFX 31–130 for cation exchange capacity [CEC], ISO 10390 for pH, NFX 31-102 standards for water content [see details in SI-1]).

For metal quantification, 1 g of dry matter from each sediment sample was dissolved in a mixture of 2 mL HNO₃ and 6 mL HCl and heated in a CEM-type microwave oven (CEM/Xpress). After filtration through ash-free filter paper, 25 mL ultrapure water was added. Then, metal concentrations were measured by Atomic Absorption Spectrometry (AAS) with a PerkinElmer PinAAcle 900 T. Flame absorption was used for Cr, Pb, and Zn (standard FD T90-112; 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 Material (BCR-280 R, lake sediment from EU Joint Research Centre) and control samples, such as Surface Water Level 2 (Spectrapure standards), were used to assess the efficiency of mineralisation and the calibration of the metal studied. PCBs were analysed by the EUROFINs laboratory, Saverne, France (<http://www.eurofins.fr>) and extracted in accordance with the certified method: QMA 504-192 (DIN ENISO/IEC 17025:2000; for details, see the references in Sup. Materials 1, and in Mourier et al. 2014). The sample extract was analysed by high-resolution gas chromatography–high-resolution mass spectrometry (HRGC/HRMS) with a VG-AutoSpec in selected ion monitoring (SIM) mode. For the seven indicator PCBs (PCB 28, 52, 101, 118, 138, 153 et 180), the quantification limits ranged from 0.042 µg/kg dry weight (DW; PCBs 118 and 180) to 0.146 µg/kg DW (PCB 153).

Sediment Ecotoxicity Assessment Protocols

Sediment ecotoxicity was assessed using acute and chronic ecotoxicity bioassays (SI-2). Acute tests comprised plant bioassays (germination and early root growth) based on species that are known to be tolerant or which are potential bioaccumulators of POPs and MTE. These plant species include zucchini (*Cucurbita pepo*), rapeseed (*Brassica napus*), ray grass (*Lolium perenne*), and black mustard (*Brassica nigra*). These choices were based on key literature, showing that *Lolium perenne* (Tato et al. 2011), *Cucurbita pepo* (White 2001; Whitfield 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 phytotoxicity 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 BER, and four for TRS and LDB, depending on the available quantity of sediment.

Germination and root inhibition growth tests were performed on microplates (Phytotoxkit® supplied by R-Biopharm, France). The microplates used were made of transparent plastic and were flat and shallow with two compartments, one containing a solid matrix (ISO substrate or sediment) humidified at 70% retention capacity (Phytotoxkit 2004). The other compartment was empty and allowed for the emergence and growth of seedlings. The cells were then incubated vertically in the dark for 48 h at 19 °C in a climate culture room. The analysed sediments were compared with

the control (100% ISO substrate). The reference soil ISO substrate (OECD 2010) is a mixture of 10% peat, 70% silica (industrial sand smaller than 2 mm, with more than 50% of the particles comprising between 50 µm and 200 µm), 20% clay (kaolin content: less than 30%), and ≤ 1% CaCO₃.

An additional acute ecotoxicity bioassay was performed on the earthworm *Eisenia fetida* to test its avoidance (ISO 11268-1). It involved studying the behaviour of *E. fetida* placed in a container with two compartments: one with the sediment to be tested and the other with the ISO substrate (OECD 2010). This test was valid if the number of dead/missing worms was less than 10% per treatment (SI-2).

The chronic tests were based on the growth and reproduction of *Eisenia fetida* (ISO 11268-2). This test was performed on 10 adult earthworms placed in the sediment for 4 weeks. At the end of the experiment, adult earthworms were weighed and compared with that of the control. The test was then continued for four more weeks to allow reproduction, after which the juveniles were recovered, counted, and compared with the controls (see SI-2).

The Ostracodtoxkit® test was used (supplied by R-Biopharm, France) in order to place the ostracod *Heterocypris incongruens* in direct contact with the sediment to be tested for 6 days (the control was performed with washed, sieved, and dried sand supplied in the Ostracodtoxkit®). This test allowed to assess the growth and mortality of ostracods (SI-2).

Data Analysis

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 ΣPCBi; 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 Addition model, which assumes a similar mode and site of toxic action of all pollutants on sediment-dwelling organisms (Backhaus and Faust 2012).

Mixture risk quotients (RQ_{Mix}) were calculated based on (1):

$$RQ_{\text{Mix}} = S(\text{MEC}_{i,x}/\text{PNEC}_{\text{sed}i}) \quad (1)$$

In the aforementioned equation, RQ_{Mix} corresponds to the sum of all RQs. The RQ for each pollutant is based on

Table 1 Physical and chemical parameters of the sediments studied (average values; $n=3$, except for GEC where $n=1$ for nitrogen, phosphorus, potassium, CEC and the C/N ratio measurements—these values are mentioned in italics)

Parameters/sediments	BER	TRS	GEC	LDB
pH	7.47 ± 0.08	7.83 ± 0.13	7.54 ± 0.20	7.34 ± 0.04
Conductivity (µS/cm)	307 ± 27	347 ± 50	795 ± 85	644 ± 14
Water content (%)	50.5 ± 1.6	61.1 ± 4.3	39.2 ± 0.1	55.3 ± 0.3
Grain-size (% Clay/Silt/Sand)	4/40/56	10/50/40	8/44/48	5/40/55
Cation exchange capacity (CEC: meq/kg DW)	76.5 ± 0.40	81.55 ± 0.93	<i>51</i>	110 ± 1.41
Total Organic Carbon (TOC: g/kg DW)	17.9 ± 0.1	17.2 ± 0.3	12.8 ± 1.8	68.2 ± 6.8
Phosphorus (g/kg DW)	0.16 ± 0.01	0.24 ± 0.01	<i>0.13</i>	0.19 ± 0.01
Potassium (g/kg DW)	0.09 ± 0.01	0.09 ± 0.01	<i>0.09</i>	0.16 ± 0.01
Total nitrogen (g/kg DW)	1.65 ± 0.02	1.48 ± 0.07	<i>1.20</i>	3.50 ± 0.01
Ratio C/N	9.67 ± 0.58	12.50 ± 0.71	<i>12</i>	12 ± 0.15
Trace element (mg/kg DW)				
Cd	1.24 ± 0.48	0.90 ± 0.09	1.02 ± 0.08	1.19 ± 0.15
Cr	70.17 ± 0.28	68.76 ± 1.93	54.25 ± 3.10	47.67 ± 4.03
Cu	20.21 ± 0.86	22.01 ± 1.26	32.55 ± 7.67	83.81 ± 4.43
Ni	29.47 ± 0.66	23.53 ± 0.47	27.47 ± 11.33	34.09 ± 3.47
Pb	25.03 ± 0.89	29.27 ± 3.50	60.53 ± 6.49	108.54 ± 7.36
Zn	105.18 ± 1.40	123.74 ± 6.08	120 ± 10	260 ± 10
PCB (µg/kg DW)				
PCB 28	1.41 ± 0.03	0.92 ± 0.04	9.06 ± 7.6	7.21 ± 5.51
PCB 52	1.69 ± 0.13	2.03 ± 0.18	10.70 ± 2.54	67.32 ± 7.47
PCB 101	2.81 ± 0.35	3.10 ± 0.13	14.91 ± 2.81	156.75 ± 62.58
PCB 118	1.83 ± 0.30	1.99 ± 0.09	8.66 ± 0.53	66.17 ± 14.33
PCB 138	3.98 ± 0.47	5.33 ± 0.28	16.74 ± 0.90	245.78 ± 56.89
PCB 153	5.95 ± 0.70	7.44 ± 0.20	34.05 ± 1.62	379.57 ± 58.60
PCB 180	4.51 ± 0.73	6.21 ± 0.27	24.97 ± 8.02	279.562 ± 33.15
Total 7 PCBs indicator (ΣPCBi)	22.19 ± 2.42	27.02 ± 0.86	122.12 ± 1.58	1204.84 ± 233.57

Table 2 Ecotoxicological risk mixture estimation on the sediment (All values in mg kg⁻¹ DW)

		Metal Elements						RQ _{mix} metal	POPs	RQ _{mix} Total
		Cd	Cr	Cu	Ni	Pb	Zn		Σ7PCBis	
Sediment Quality Guidelines	PEC ¹	0.50	111	149	48.6	128	459		0.68	
	TEC ¹	0.99	43.4	31.6	22.7	35.8	121		0.04*	
	PNEC _{sed} ²	2.5	ND	0.8	4	53.4	37		ND	
	S1 values for dredged sediment ³	2	150	100	50	100	300		0.68	
Local Geo-chemical Background	Geneva lake ⁴	0.45	26	22.7	56	73	304		-	
	Upper Rhône ⁵	0.17	36.1	10.2	33.9	11.4	53.2		-	
	Middle to Lower Rhône ⁵	0.14	29.3	18.4	32.7	17.7	64.6		-	
Risk Quotient	LDB	0.48	1.1	104.76	7.93	2.03	7.03	123.32	34.35	157.68
	BER	0.5	1.62	25.26	6.85	0.47	2.84	37.54	0.63	38.17
	GEC	0.41	1.25	40.69	6.39	1.13	3.24	53.11	3.4	56.51
	TRS	0.36	1.58	27.51	5.47	0.55	3.34	38.82	0.77	39.59

Color scale for RQ (risk quotient) and RQ_{mix} (risk quotient mixture): white (> 1)=negligible risk, yellow (1–10)=low risk, orange (10–100)=medium risk, red (> 100)=high risk. Nota Bene: PNEC_{sed} values are not defined (ND) for Cr and Σ7PCBis, thus we used the TEC (Threshold Effect Concentrations) as the reference value for the RQ estimate. References: ¹PEC (Probable Effect Concentrations) and TEC values follow MacDonald et al. (2000a), while the PCBs TEC* comes from MacDonald et al. (2000b); ²PNEC_{sed}=Predicted No Effect Concentration for sediment-dwelling organisms (available online at <https://substances.ineris.fr>); ³S1 threshold (JORF 2006); ⁴Gascon Diez et al. (2017); ⁵Median values after Dendievel et al. (2020b)

$MEC_{i,x}$, which is the Measured Environmental Concentration of pollutant i at site x ($\text{mg kg}^{-1} \text{ dw}$), and on $PNEC_{\text{sed } i}$ according to Equilibrium Partitioning ($\text{mg kg}^{-1} \text{ dw}$). RQ and RQ_{Mix} values range from < 1 (negligible risk) $>$ to > 100 (high risk) according to Gosset et al. (2020) and Perrodin et al. (2012).

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).

Post hoc comparisons between normally distributed populations (e.g. germination and root inhibition (RI) at 7 days on microplate assays) were performed using the t test. A nonparametric 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).

Results/Discussion

Sediment Characterisation

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

Trace Metal Elements (TME) and ΣPCBi 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 ($PEC = 0.99 \text{ mg/kg DW}$) 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).

The sediment concentrations in the sum of the seven PCB indicators (ΣPCBi) were highest for LDB site (1204 $\mu\text{g/kg DW}$ on average). This value largely exceeds the regulatory threshold S1 (680 $\mu\text{g/kg}$) and PEC for total PCBs (MacDonald et al. 2000a). GEC sediment also contained 122.1 $\mu\text{g/kg DW}$ ΣPCBi on average. This concentration was between the TEC (35 $\mu\text{g/kg}$; MacDonald et al. 2000b) and the PEC/S1. Finally, BER and GEC sediment contained between 22 and 27 $\mu\text{g/kg DW}$ of ΣPCBi on average. Even if these levels are lower than the aforementioned thresholds (TEC, PEC, S1), they remain within the ranges of the sediment benchmarks (10 $\mu\text{g/kg}$ and 60 $\mu\text{g/kg}$) calculated from biota-to-sediment accumulation factor models in fish (Babut et al. 2012; Lopes et al. 2012).

In the studied samples, PCB congener concentrations were in the order of PCB 153 $>$ PCB 180 $>$ PCB 138 $>$ PCB 101 (Table 1). This assemblage might be considered a marker related to river contamination by industries using 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.

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 “Risk Quotient Assessment”). 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.

LBD sediment presents the most important concentrations of trace elements and $\Sigma PCBi$ (Table 1). Consequently, these concentrations lead to a high RQ_{MIX} value (> 100), especially driven by Cu and $\Sigma PCBis$. 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.

The Risk Quotient mixture (RQ_{MIX}) is based on the analysed metals and PCBs to assess the relative contribution of each pollutant to the effect of a cocktail of contaminants on sediment-dwelling organisms. According to data in the literature on core sediments from the Rhône River, the cumulative ecotoxicity risk is mainly driven by (1) dichlorodiphenyltrichloroethane (DDT) metabolites (DDE and DDD), (2) lindane isomers (HCHs), and (3) PCBs that remain a major cause of concern for the Rhône (Dendievel et al. 2020a). The Risk Quotient mixture could also fluctuate, such as in English coastal rivers (Manuel Nicolaus et al. 2015), or negligible, such as in the Garonne Estuary (metals: Larrose et al. 2010), due to sorption processes and organic matter degradation, reducing the estimation of the impact of industrial inputs in sediments, while high metal concentrations have been found in fauna (Lanceleur et al. 2011). Other studies have been shown that in such a global approach by risk ratio, or RQ, this is mainly due to the acetochlor ($C_{14}H_{20}ClNO_2$) in four sediments of Argentina’s rural streams (Fernández San Juan et al. 2022) or to the tributyltin concentrations at almost all the study sites of the Odra River estuary (Kucharski et al. 2022). Such results cited also underly the weight of POP contamination in such an evaluation. At the scale of the French Rhône River (Fig. 1), the data presented in this work are consistent with other RQ_{MIX} values published for

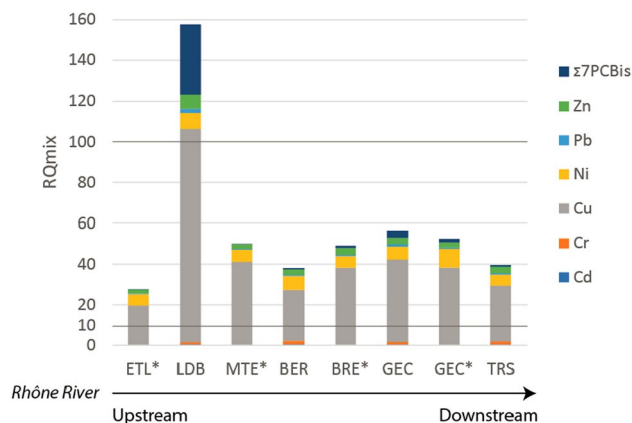


Fig. 1 Changes in RQ_{mix} values for sediments collected along the French sections of the Rhône River in the downstream direction. LDB, BER, GEC and TRS data come from this study; while ETL*, MTE*, BRE*, and GEC* come from Dendievel et al. (2020b), referring to the period 2005–2011

sediment cores (Dendievel et al. 2020b). Thus, the BER, GEC, and TRS RQ_{MIX} values are in line with the known RQ_{MIX} values in the Middle and Lower Rhône River sections (Fig. 1). LDB sediment is an exception and offers strong RQ_{MIX} without link between ETL and MTE. Notably, LDB sediments are from the Aix-les-Bains marina, located in Bourget Lake. The latter lake is a major sediment deposition zone, only temporarily connected to the Rhône River by a short channel (“canal de Savières”) during floods, which explains no relationship between the LDB peak and the other upstream sites.

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).

Acute Toxicity Tests of Sediments

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

Table 3 Germination and root inhibition (RI) after 7 days on microplate assays (n=number of microplates with 10 seeds/species)

Sediment (n)	BER (n=3)		TRS (n=4)		GEC (n=2)		LDB (n=4)	
	Germination inhibition (%)	Root inhibition (%)	Germination inhibition (%)	Root inhibition (%)	Germination inhibition (%)	Root inhibition (%)	Germination inhibition (%)	Root inhibition (%)
<i>Brassica nigra</i>	10.4 ^{a1} (± 0.64)	− 11.9 ^{a2} (± 18.9)	− 6.3 ^{a1} (± 12.5)	− 7.3 ^{a1} (± 27.4)	ND	ND	1.31 ^{a1} (± 1.6)	− 167.7 ^{a3} (± 55.64)
<i>Lolium perenne</i>	− 0.8 ^{b2} (± 11.0)	49.8 ^{b2} (± 11.37)	− 3.8 ^{b2} (± 24.7)	29.1 ^{b1} (± 1.1)	ND	ND	4.28 ^{a2} (± 2.55)	36.46 ^{a2} (± 12.93)
<i>Brassica napus</i>	ND	ND	− 2.8 ^{a3} (± 5.5)	− 13.5 ^{c1} (± 14.6)	ND	ND	0.98 ^{b3} (± 0.7)	− 8.45 ^{b1} (± 6.5)
<i>Cucurbita pepo ssp. pepo</i>	3.3 ^{c4} (± 5.0)	11.1 ^{a1} (± 1.3)	5 ^{a4} (± 10)	14.6 ^{b1} (± 19.3)	18 ⁴ (± 2.88)	58.49 ² (± 12.23)	18.12 ^{b4} (± 1.15)	47.33 ^{b3} (± 12.67)

Superscripted letters in each cell refer to significance levels according to a Fisher Test ($p < 0.05$): values with a small 'a' in superscript are similar between them, as well as values with a small 'b' in superscript, while 'a' and 'b' superscript groups significantly differ between them; the superscripted numbers on the same line indicate a significant difference for the same species ($p < 0.05$) according to tested sediments. ND=Non-determined

of 60% of the seeds (n=5; Whitfield Ashlund et al. 2010). Three sediments (from BER, TRS, LDB) were also tested with *Brassica nigra* and *Lolium perenne*, and germination inhibition seemed effective for LDB (both plants: 1 to 4%) and BER (*B. nigra*, ca. 10%) sediments. By contrast, an inhibition of the germination associated with a notable standard error on TRS suggested a lack of effect or a weak stimulation of the seeds. Chigbo and Batty (2013) reported that the combined effect of Cr and Benzo(a)pyrene (BaP) significantly reduced the germination rate of *L. perenne* in comparison of the control and assays with single contaminant effects. These variations in *L. perenne* germination (e.g. inhibition or stimulation) have also been detected using microplate assays and sediments (Bedell et al. 2003, 2013). For *Brassica napus*, the two sediments tested (from TRS and LDB) had no significant effect on seed germination (Table 3). This result supports that of Wierzbicka and Obidzinska (1998), who showed that seed coat morphology is an important defence against some metallic ions, especially for Brassicaceae (Cruciferae). However, even if *B. napus* has high emergence variability (Fernandez et al. 2005), the sensitivity of germination of *B. napus* to dredged canal sediment was observed during a three-year phytoremediation trial (King et al. 2006). Regarding other plants that could be used in these tests, alfalfa (*Medicago sativa*), a dicotyledonous species, seems to be the most relevant species for screening sediment toxicity before reuse (Lecomte et al. 2019). Such variability and sensitivity according to the species tested, which was also observed in our data, may result from the different protection provided to embryos by seed coverage or the permeability of this coverage to contaminants or water.

Two physiological phenomena are active during germination: oxygen use increases when respiration begins and imbibition. Imbibition can occur when the seed rehydrates

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

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

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

Earthworm (*Eisenia fetida*) Avoidance Test

The earthworm avoidance test requires a large quantity of sediment; thus, it was only conducted on BER, LDB, and TRS sediments (Table 4). We observed that from 60

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

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

Table 4 Avoidance test: distribution of earthworms in the sediments and the ISO soil (n=4)

Sediment	Percentage of earthworms (control; ISO Soil)	Percentage of earthworms between the two compartments	Percentage of earthworms in the tested sediment
BER	77.5 ^a (± 13.4)	0 ^b	22.5 ^c (± 13.4)
TRS	66 ^a (± 11.4)	8 ^b (± 8.4)	26 ^b (± 11.4)
LDB	27.5 ^a (± 17.1)	3.25 ^a (± 4.7)	62.5 ^b (± 20.6)

Superscripted letters in each cell refer to significance levels according to a Mann–Whitney test ($p < 0.05$): a values are similar between them, as well as b or c values, while a, b and c groups significantly differ between each other

Chronic Toxicity Tests on Sediments

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 (Ostracodtookit®) 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 ($\times 1.5$ between T0 and T + 6) validated the experiment.

Regarding the tests performed on the Rhône sediments, almost all ostracods in contact with the LDB sediment died (90%), and a mortality of 50% was reported for ostracods in contact with the GEC sediment (Fig. 2A). Thus, LDB and GEC sediment had a major effect on *Heterocypris incongruens* survival, even if the variability associated with this test was greater for LDB and GEC sediment than for the other sediments. Ostracod growth was inhibited from 35 to 110% in the four sediments tested (Fig. 2B). Their growth was considerably inhibited by LDB sediment and to a lesser extent by GEC sediment. However, TRS and BER sediment showed low effect on ostracod 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. High correlations ($r^2 > 0.8$) were observed among physicochemical parameters (e.g. PCBi levels and several TMEs, including Cu, Pb, and Zn) and growth inhibition or mortality.

Our results agree with those of another study, which examined 33 sediments and concluded that the ostracod microbioassay is a reliable, sensitive alternative to whole sediment assays for toxicity assessment (Belgis et al. 2003). Pesce et al. (2020) recently highlighted the promising prospects of the ostracod test for investigating the ecotoxicological effects of metal contamination on natural sediment communities, using the degradation and decomposition of particulate organic matter as a functional descriptor. Lecomte et al. (2019) demonstrated up to a 40.6% increase in ostracod growth inhibition in dam sediments but found no effects on mortality. In contrast with results of Lecomte et al. (2019), our study of river sediments showed that the responses of *H. incongruens* revealed a potential toxicity gradient (with mortality) in the sediments tested. In a similar context (reservoir sediments), Palma et al. (2014) showed that *H. incongruens* is sensitive to contaminants found in sediments with higher mortality but lower growth

inhibition for similar sediment characteristics and similar MTE contents.

Finally, the sediments were classified according to the inhibition of the growth of *Heterocypris incongruens*: LDB > GEC > TRS > BER. In the case of an on-land deposit scenario, LDB sediment, and to a lesser extent GEC sediment, presented the greatest risk of toxicity for *Heterocypris incongruens*.

Chronic Earthworm (*Eisenia fetida*) Reproduction Test in TRS and LDB Sediments

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

After 8 weeks, the growth inhibition affected 50% of the juveniles from TRS and LDB sediment compared to the control (Table 5). In other contexts (chlorpyrifos-contaminated soils), Zhou et al. (2007) also showed a significant effect of exposure to chlorpyrifos-contaminated soils on earthworm reproduction. The number of juveniles was significantly and positively correlated with pH, Ca, silt content, Zn, Cd, and CEC but significantly and negatively correlated with sand content (Luo et al. 2014). In our sediment samples, those from LDB and TRS presented small differences in pH, silt, and sand values (Table 1). However, the high CEC, Zn,

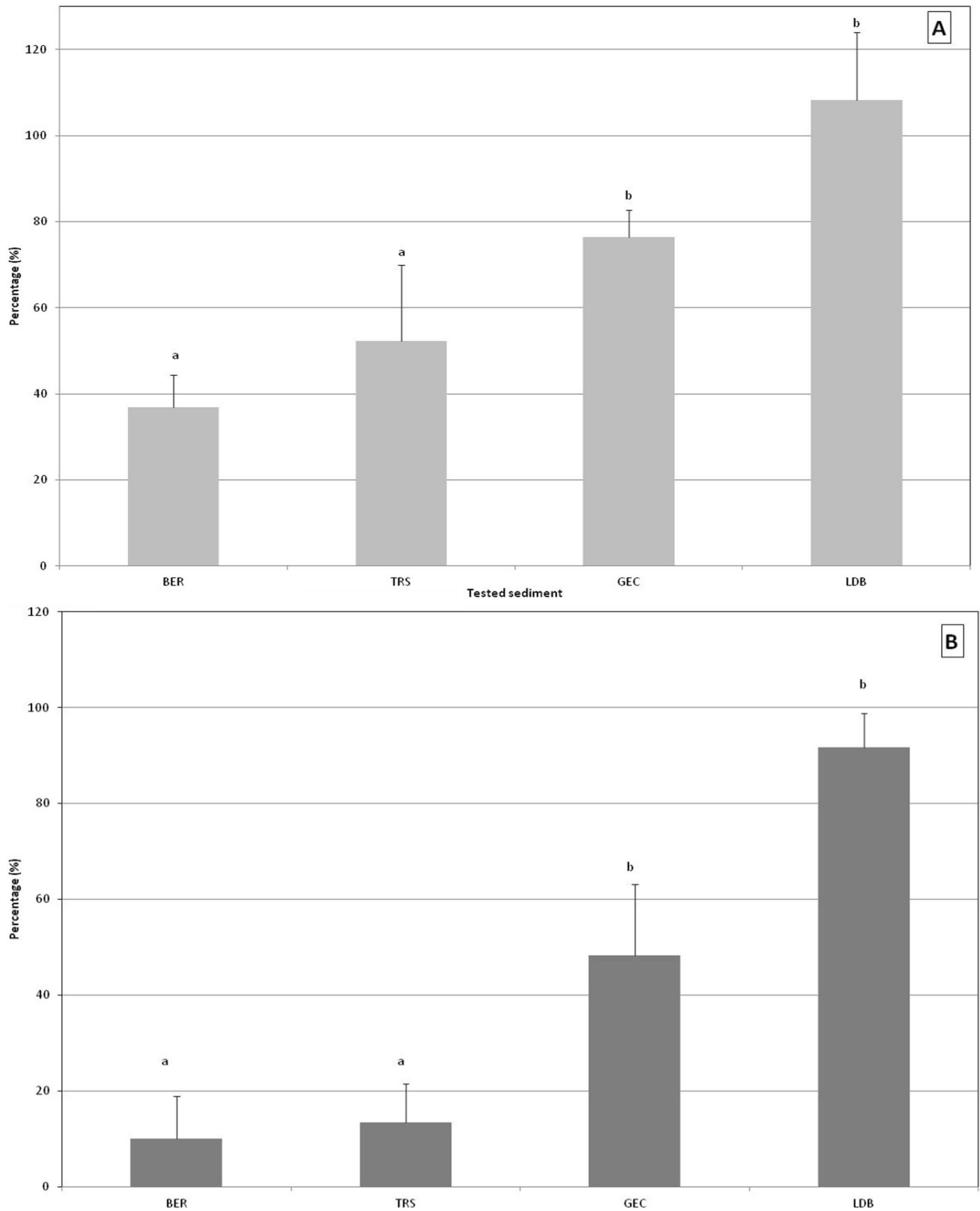


Fig. 2 Percentage of growth inhibition (**A**) and mortality (**B**) of *Heterocypris incongruens* according to the tested sediments in comparison with control (ISO soil). Histograms with the same letter are

not significantly different at the $p < 0.05$ level (Mann–Whitney test). Standard errors of the means are represented by bars on the histograms

Table 5 Earthworm growth and reproduction test for TRS and LDB sediments

	T = 4 weeks			T = 8 weeks	
	Growth		Death	Reproduction	
	Biomass loss (%)	Inhibition/control (%)	Inhibition/control (%)	Number of young (n)	Inhibition/control (%)
Control (ISO soil)	0.79 ^a (± 2.83)	–	–	89.67 ^a (± 11.83)	–
TRS	0.62 ^a (± 4.26)	21.91	0	44.25 ^b (± 14.34)	50.65
LDB	3.38 ^b (± 2.88)	36.32	0	55.40 ^b (± 9.96)	69.19

Ten adult worms were deposited at T=0 in each replicate (6 replicates for control and 5 for each sediment). At T=4 weeks, the adults were removed and then at T=8 weeks the juvenile resulting from reproduction were recovered. Superscripted letters in each cell refer to the significance according to a Mann–Whitney test ($p < 0.05$): a values are similar between them, as well as b values, while a and b groups significantly differ between each other

and Cd contents in LDB sediment can explain the greater reduction in the number of juveniles than in TRS sediment (Tables 1 and 5). Moreover, discolouration of the juveniles was observed during the experiments with LDB sediment but not with TRS sediment (data not shown). The CEC and pollutant content can explain the effect of our sediments on earthworm reproduction. Similarly, Hankard et al. (2005) showed that earthworms could survive in all the urban soils they tested, but differences occurred in the reproduction rate, possibly affecting the fitness of worms inhabiting the sediment deposit.

Risk Evaluation Discussion

In this study, hazard characterisation (via chemical analyses) and ecotoxicological tests were conducted on a panel of sediments with different levels of pollutants (Table 6). Based on RQ_{mix} 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 on-land 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. Thus, the results obtained for the different acute and chronic toxicity test are summarized in Table 6:

- 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 pol-

lutant 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.

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

Moreover, the choice of scenario for the management of the sediment can be linked to other possibilities of the sediment valorisation. Some studies have suggested the valorisation of sediments as a plant substrate (Ferrans et al. 2022), metal recovery in a cost-integrated approach, or a circular management approach of dredged sediments (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

Table 6 Synthesis of toxicity effects obtained according to the multiple bioassay approach on the sediments tested

Biological support	Parameters measured	BER	GEC	TRS	LDB
Acute tests					
Earthworms	Avoidance	YES	<i>N.D.</i>	YES	NO
Plant	Germination inhibition	YES (<i>Brassica nigra</i>)	YES (<i>Cucurbita pepo</i>)	NO	YES (<i>Lolium perenne</i> and <i>Cucurbita pepo</i>)
	Root inhibition	YES (<i>Lolium perenne</i>)	YES (<i>Cucurbita pepo</i>)	YES (<i>Lolium perenne</i>)	YES (<i>Lolium perenne</i> and <i>Cucurbita pepo</i>)
Chronic tests					
Ostracods	Effect on growth	NO	YES	YES	YES
	Death	NO	YES	NO	YES
Earthworms	Effect on growth	<i>N.D.</i>	<i>N.D.</i>	YES	YES
	Impact on reproduction	<i>N.D.</i>	<i>N.D.</i>	YES	YES

N.D. = not determined; Orange cell with “YES” = Toxicity effect observed (with name of species in italics); Green cell with “NO” = no effect observed

sediments along large and heterogeneous rivers (Beyer et al. 2014; Kortenkamp et al. 2019).

Notably, the risk of dredged sediment is also linked to deposit site specificities. Thus, a collaborative effort among stakeholders, scientists, and representatives of the government must be realised for site determination linked to risk evaluation. For example, a collaborative approach among the French Government, the “Grand Port Maritime de Rouen”, and a scientific committee has made it possible to define an integrative protocol to survey all the compartments that might be affected by dumping in the management of port dredged sediment in the Bay of Seine (France) (Marmin et al. 2014). All these monitoring procedures (i.e. sediment and water quality, including chemical contamination; bioaccumulation and ecotoxicology on target species; microbiology, invertebrates, and fish surveys; impact on the Natura 2000 areas) were conducted by partners under the responsibility of the “Grand Port Maritime de Rouen” from 2012 to 2013 (Marmin et al. 2014).

Conclusion

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

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

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Declarations

Conflict of interest The authors have not disclosed any conflict of interest.

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