J Soils Sediments (2016) 16:665–676
DOI 10.1007/s11368-015-1267-3

SEDIMENTS, SEC 1 • SEDIMENT QUALITY AND IMPACT ASSESSMENT • RESEARCH ARTICLE

Investigating speciation and toxicity of heavy metals in anoxic marine sediments—a case study from a mariculture bay in Southern China

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Received: 30 January 2014 / Accepted: 10 September 2015 / Published online: 6 October 2015

Abstract

Purpose The pollution of marine sediments by heavy metals is still a major concern, especially in zones affected by industry or mariculture. Toxicity of sediment heavy metal contents may be assessed using sequential extraction (SE) procedures, minding inherent constraints of such approaches. In this study, we investigated heavy metal speciation and toxicity in anoxic marine sediments in Zhelin Bay, a mariculture bay in Southern China, using an SE and acid volatile sulfur-simultaneously extracted metals (AVS-SEM) approach.

Materials and methods Speciation of Cd, Cu, Ni, Pb, and Zn were studied by a modified SE of five fractions, adapted to separate organic and sulfidic metal fractions in anoxic sediments: F1 weak acid soluble (readily available), F2 reducible fraction, F3 organic matter-bound fraction, F4 sulfide-bound fraction, and F5 residually bound fraction. Toxicity predictions based on the sum of non-residual (NR) metal fractions from sequential extraction were compared to predictions based on AVS-SEM.

Results and discussion Results showed that Cd, Ni, and Pb predominantly occurred in the weak acid soluble fraction (F1), residual fraction (F5), and sulfide-bound fraction (F4), respectively; Cu and Zn were mainly obtained in F4 and F5. Based on the distribution of indicator elements for metal fractions, the SEM from AVS extraction included different yields of non-residual and residual fractions besides the sulfidic fraction. Estimates for potential heavy metal toxicity based on NR metals of the SE procedure were thus based on a better-defined speciation compared to the simplistic approach of the AVS-SEM method.

Conclusions Based on the contents of NR metals and normalizing them by organic matter content, toxic effects are not expected for any of the sampling sites, irrespective of the presence or absence of mariculture. Using Pearson correlation analysis to identify predominant fractions influencing toxicity, we conclude that toxicity of heavy metals in anoxic sediments can be well predicted by their non-residual heavy metal contents.

Keywords Anoxic sediments · Chemical speciation · Heavy metals · Mariculture · Sequential extraction · Toxicity prediction

1 Introduction

The pollution of sediments by heavy metals is a concern in many marine environments (Villaescusa-Celaya et al. 2000; Morillo et al. 2004; Gargouri et al. 2011). Several heavy

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Responsible editor: Marc Babut

Pengran Guo is co-first author

Electronic supplementary material The online version of this article (doi:10.1007/s11368-015-1267-3) contains supplementary material, which is available to authorized users.

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metals are toxic and bioaccumulate, while as elements, they cannot be degraded (Bryan and Langston 1992). Due to rapid industrialization and economic development in the coastal regions of Southern China, there is a constant input of heavy metals into the estuarine and bay environment (Yu et al. 2008). In mariculture sediments, further inputs of heavy metals arise from copper-based antifoulants and fish feeds enriched with trace metals to fulfill micronutrient requirements (Petersen et al. 2005; Sapkota et al. 2008). Concentrations of heavy metals in mariculture sediments mostly exceeded those in non-mariculture sediments (Belias et al. 2003; Dean et al. 2007; Sutherland et al. 2007). Farm-raised fish had higher contents of heavy metals compared to wild-caught fish (Foran et al. 2004; Calvi et al. 2006). Moreover, heavy metals in sediments can be released back into the water column, depending on geochemical conditions and speciation (Payán et al. 2012). This can cause adverse effects on benthic invertebrates and on mariculture products and in consequence on human health.

To judge mobility and toxicity of heavy metals in the sediments, their physicochemical forms, i.e., the speciation, must be known (Lund 1990; Yuan et al. 2004; Zhou et al. 2004). The speciation can mostly be sufficiently determined by sequential extraction (SE) techniques (Usero et al. 1998), although these are operationally defined fractions only. Despite the pitfalls in interpretation of such fractions, SE remains one of the most widely used approaches (Ngiam and Lim 2001). For example, in a common SE protocol for coastal estuarine, bay, and marine sediments, the fractions represent metals that are (i) readily exchangeable, (ii) bound to carbonates, (iii) reductively dissolvable/ redox labile, (iv) bound to organic matter and sulfides, or (v) trapped in residual, refractory phases (Gleyzes et al. 2002; Gao et al. 2010; Zhang et al. 2012).

Many studies demonstrated that mariculture can cause eutrophication, favoring reducing and anoxic conditions, thereby also increasing sulfide contents in surface sediments (Wu et al. 1994; Cao et al. 2007). Hence, bioavailability of metals may be controlled by the dissolution equilibrium of metal sulfides (Machado et al. 2004; De Jonge et al. 2010). The solubility of metal sulfides is very low, and only metals dissolved in pore water (as free metals) cause toxicity to benthic organisms (McGrath et al. 2002). In addition, also sedimentary organic matter can bind metals, thereby reducing availability and toxicity to aquatic organisms, but metals bound to organic matter or sulfides show very distinct chemical behavior (Clark et al. 1998).

Due to an inverse relation of biological effects and the presence of sulfides and to account for interaction with organic matter, toxicity of metals can be predicted by normalizing the difference of metals co-extracted by the acid volatile sulfide (“simultaneously extracted metals” (SEM)) and “true acid volatile sulfide (AVS)” by the sedimentary organic carbon content. This ratio $(\sum SEM\text{-}AVS)/f_{OC}$ has been proposed to predict the toxicity of a number of divalent heavy metals, such as Cd, Cu, Ni, Pb, and Zn in sediments (Di Toro et al. 2005; Burton et al. 2007). However, in a large number of sediment data, this approach was found to successfully predict only a lack of toxicity but not the presence of toxicity (McGrath et al. 2002). As the SEM-AVS fraction does not only comprise free metals and a “truly” AVS-bound fraction (Fang et al. 2005; Poot et al. 2009), a more defined speciation might thus provide a better basis to predict toxicity.

Also, existing SE methods developed for sediments, e.g., those by Tessier et al. (1979) and the protocol proposed by the Community Bureau of Reference, hereafter termed BCR (Usero et al. 1998), were found to be unsuitable for anoxic sediments, as investigated by X-ray absorption spectroscopy (Peltier et al. 2005). As common protocols do not investigate the organic and sulfidic fractions separately, Wang et al. (2011) proposed a modified procedure, separating the organic and sulfidic fractions and maintaining reducing conditions in the preceding extraction steps to prevent the sulfidic fraction from being oxidized. As main difference, the authors propose to use sodium pyrophosphate to extract the organic-bound fraction, followed by a strong acidic extractant to extract sulfides, instead of using an acidic oxidizing solution (e.g., containing H_2O_2) to extract the organic and sulfidic fraction in one step. Nevertheless, many studies of heavy metal speciation in anoxic sediment have been carried out based on the Tessier method or the BCR scheme (Ngiam and Lim 2001; Yu et al. 2001; Fang et al. 2005; Hartley and Dickinson 2010). In these studies, the reported quantities of organic matter and/or sulfide-bound fractions and the derived speciation of heavy metals are thus probably biased (Peltier et al. 2005).

The aim of this study was therefore to investigate the speciation and toxicity of heavy metals in anoxic sediments from Zhelin Bay, taking special emphasis on the organic and sulfide-bound fraction by the use of a modified extraction procedure that separates these fractions (Wang et al. 2011). Specifically, the objectives of this study were (i) to investigate species distribution of heavy metals in surface sediments from a maricultural zone by SE, (ii) to elucidate speciation of AVS-SEM by comparison with the results of the modified SE and using electron microscopy with energy-dispersive X-ray spectroscopy (EDX), (iii) to predict the toxicity of heavy metals in the mariculture sediment by the toxicity index of $(\sum SEM\text{-}AVS)/f_{OC}$, and (iv) to compare these toxicity predictions with a prediction based on SE and obtained contents of non-residual (NR) bound metals.

2 Material and methods

2.1 Study area

Zhelin Bay in the eastern Guangdong province of China is a semiclosed estuarial bay covering an area of ~80 km^2, with an average water depth of 4.8 m and an average tidal range (irregular semidiurnal tide) of 1.69 m (Qiao et al. 2010). Mariculture activities in Zhelin Bay have been growing fast over the past three decades and have raised the loading of heavy metals in surface sediments, eventually posing an ecological risk (Qiao et al. 2010). Zhelin Bay has now become the largest cage mariculture base and a major culture fish base in South China (Qiao et al. 2010).

2.2 Sampling collection

For the present study, we sampled eight locations (Fig. 1) in November 2009. The chosen locations represent the inshore area (Z1, Z2, Z5), an area used for fish cages (Z6, Z7, Z8), a location for oyster farming (Z4), the shipping waterway (Z3, Z6), and the bay mouth outside regions (Z7). At each site, pH, temperature, dissolved oxygen, and salinity of bottom water were measured by an YSI water quality sensor (600R mode, YSI Inc., Ohio USA). The bulk surface sediments (10 cm) were collected using a grab sampler (KR-02 type, Keelrein Instrument Co. Ltd., Shanghai, China) and quickly placed in polypropylene bags under N_2 stream. The surface sediment was sampled manually in triplicates; thus, 24 sediment samples were collected and immediately transported to laboratory kept at 4~5 °C. Subsequently, the samples were frozen at −20 °C in the laboratory to preserve AVS concentrations of the sediment (De Lange et al. 2008).

2.3 Chemical analysis

Sediment subsamples were dried at 70 °C for >24 h to determine sediment moisture contents. Sediment organic carbon (OC) contents were estimated by wet oxidation using $K_2Cr_2O_7$-H_2SO_4 (Mingorance et al. 2007). Total digestion of the sediment samples was conducted following the EPA 3052 method: 0.2 g of sediment was digested by a mixture of HNO_3 and HF (3:1 *v*/*v*), using a CEM Mars microwave digestion system. The concentrations of Cu, Ni, Pb, Zn, and Cd in the final solutions were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES, Spectro Ciros Vision, SPECTRO GmbH, Kleve, Germany) or by graphite furnace atomic absorption spectrometry (GFAAS, Z-2000,

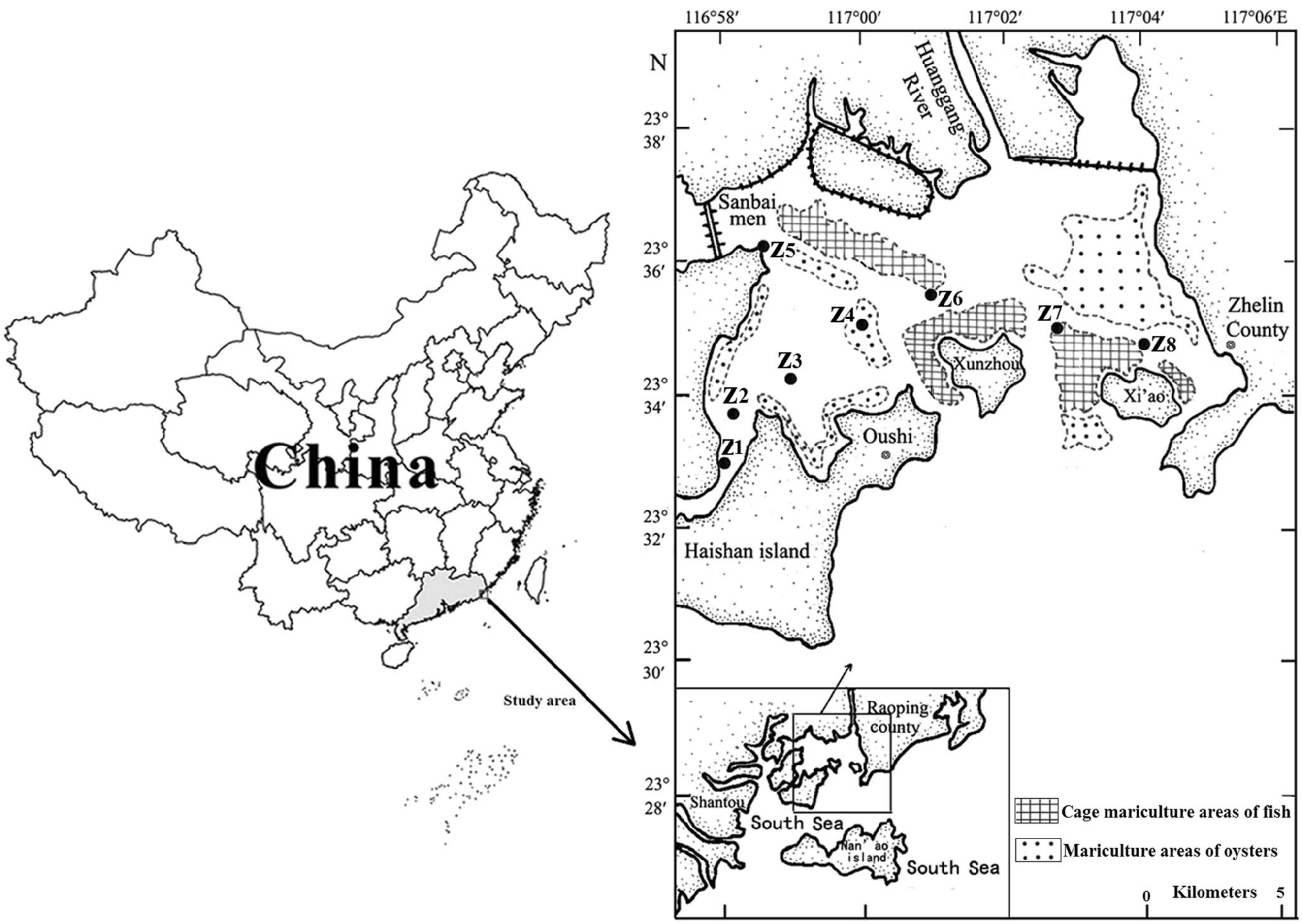


Fig. 1 Sampling locations in Zhelin Bay, a major mariculture zone in Guangdong Province, Southern China. Surface sediment samples were collected at sites Z1~Z8

Hitachi Ltd., Japan). Sediment moisture contents (SMCs) were determined by oven drying at 105±2 °C to constant weight and calculating weight loss. Analytical accuracy was assured by parallel analysis of blanks and certified reference materials.

The AVS analyses followed the procedure described by Allen et al. (1993). Briefly, sulfides in 5.0 g wet sediment samples were volatilized under N_2 stream by addition of 20 ml of 6 M HCl and trapped in 15 ml of 3 % alkaline zinc solution (75 ml of 20 % zinc acetate solution added to 425 ml of 2 M NaOH). The dissolved sulfide concentration in the trapping solution was determined using the methylene blue method. AVS analysis was validated using standard materials. AVS-SEM concentrations were analyzed in the acidified sediment suspension after filtration over a pre-rinsed 0.45-μm membrane filter (Fang et al. 2005) using ICP-OES for Ca, Cu, Fe, Ni, Pb, and Zn and GFAAS for Cd.

Five operationally defined fractions of heavy metals in 4.5 g of fresh, wet sediments were extracted by the modified SE procedure (Wang et al. 2011): F1: weak acid soluble fraction (1 M NaOAc, pH 5.0, solid sample dry weight/liquid extractant volume ratio (S/L) 1:15, for 5 h), F2: reducible fraction (0.2 M $NH_2OH{\cdot}HCl$ + 0.2 mM EDTA-Na_2, pH 2.0, S/L 1:15, for 5 h), F3: organic matter-bound fraction (0.1 M $Na_4P_2O_7$, pH 9.8, S/L 1:10, for 5 h), F4: sulfide-bound fraction (6 M HCl, S/L 1:10, for 1 h), and F5: residual fraction (estimated subtracting the sum of the fractions F1–F4 from the total metal content). The main benefits from the procedure following Wang et al. (2011) are thus—besides an improved selectivity as demonstrated in the original study—a separate evaluation of the organic- and sulfide-bound fractions. As the weak acid soluble fraction includes exchangeable and carbonate-bound metals, we considered this as the bioavailable fraction. Extractions were conducted on a rotary shaker at 20 °C in the dark and using sealed 100-ml centrifuge tubes. After each step, solid and liquid phase were separated by centrifugation at 4000 rpm for 10 min. The supernatant was then removed by pipette, and the residual sediment was washed by re-suspension in deionized water and centrifuged again. The washing solution was discarded. Handling of all samples was done under nitrogen atmosphere when centrifuge tubes were opened to prevent oxidation of sulfides. Concentrations of metals in all extracts were determined after acidification with HNO_3 (1 % vol) and using ICP-OES or GFAAS.

Ultra-pure, deionized water (Milli-Q) was used throughout the experiments and was boiled and purged with nitrogen to remove dissolved oxygen. All glassware and plastic wares were soaked in 2.7 M HNO_3 for at least 24 h and rinsed with deionized water prior to use. Standard solutions of metals were prepared by dilution of 1000 μg ml^{-1} stock solutions with deionized water. All chemicals used in the experiment were at least of analytical reagent grade (p.A.) or of superior purity. Concentrations of sediment components including AVS, OC, and metals are expressed as dry weight-normalized concentrations.

2.4 Scanning electron microscopy-energy dispersive X-ray analysis

Based on the selectivity of the SE for heavy metal fractions in the sediments, Ca and Mg, Fe and Mn, carbon (C), sulfur (S), and Al and Si could be taken as the indicator elements of F1: weak acid soluble fraction, F2: reducible fraction, F3: organic matter-bound fraction, F4: sulfide-bound fraction, and F5: residual fraction, respectively (Tessier et al. 1979). In addition, fresh wet sediments and the residual sediments of Z3 site samples after SE and the volatilization of AVS were freeze-dried and prepared for the determination of indicator elements (Al, O, C, Ca, Mg, Fe, Mn, and Si) by scanning electron microscopy-energy dispersive spectroscopy (Jeol, JSM-6330F, Tokyo, Japan).

2.5 Calculation of toxicity indices

Toxicity of sediment heavy metals to benthic organisms was predicted by calculating the ratio of AVS simultaneously extracted metals and the organic matter fraction $(\sum SEM\text{-}AVS)/f_{OC}$, using measured concentrations of OC, AVS, and SEM from all sediment samples (US EPA 2000; Landner and Reuther 2004). For comparison, and as all fractions F1–F4 of the applied SE procedure are non-residual fractions of metals and might thus potentially cause adverse effect on organisms (Peijnenburg et al. 2007), we calculated a sum of F1~F4, defined as "NR-bound metals" to judge sediment toxicity, and calculated a similar index using NR, i.e., $(\sum NR\text{-}AVS)/f_{OC}$.

2.6 Quality control and data analysis

All samples for total concentrations, AVS, SEM, and SE fractions were determined in triplicate. The relative standard deviation of all analysis was better than 12 %. Accuracy of the total analysis was assured using standard reference material (GBW). Recoveries based on analysis of the reference materials were within an error of 10 % of the certified values. Accuracy of the analysis of the SE fractions was assured by comparing the recovered sum of the fractions to the total content of heavy metals, which always ranged from 90 to 112 %.

All statistical analyses of the data were conducted using Origin 8.0 software (OriginLab). The concentrations of metals in Table 2 and Fig. 2 represent mean values of triplicate determination. Relations between AVS, SEM, sediment moisture contents, and extracted metals were estimated using Pearson correlation analysis. The level of significance is indicated as as $^*p<0.05$ and $^{**}p<0.01$.

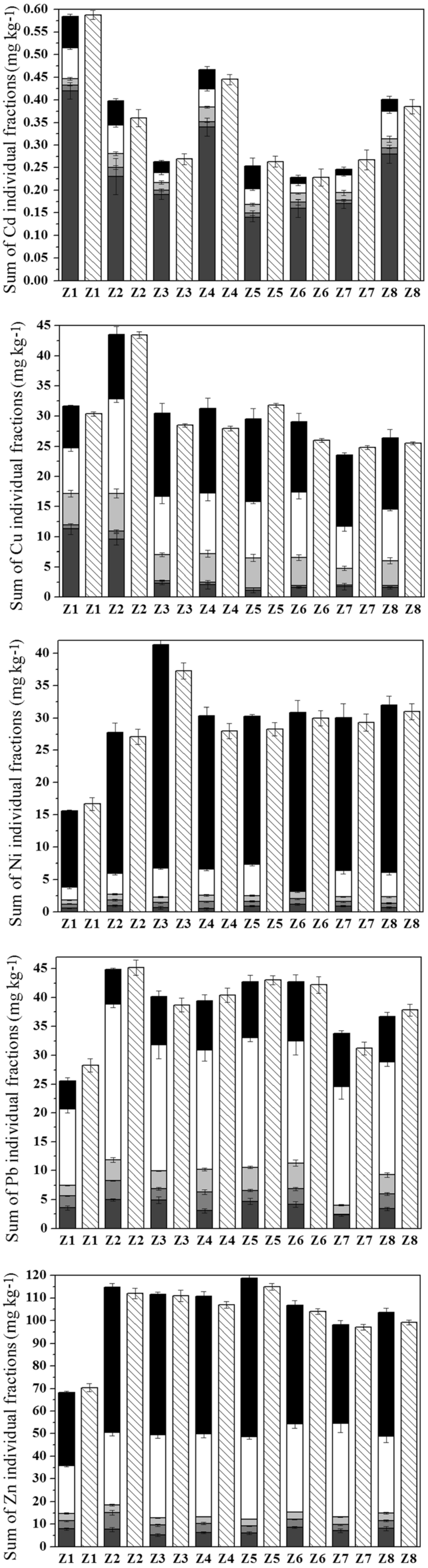


Fig. 2 Distribution of speciation and total content of heavy metals in ▶ mariculture sediments of Zhelin Bay. *Bars* represent average concentrations and standard deviations (n=6). ■ Weak acid soluble fraction (F1), ■ reducible fractions (F2), ■ organic matter-bound fraction (F3), □ sulfide-bound fraction (F4), and ■ residual fraction (F5) of the sequential extraction; ▨ total metal concentrations from total digestion (EPA 3052)

3 Results

3.1 Sediment characteristics

For all sampled sites, the overlying water column had a pH within a narrow range of 7.2–7.3, and dissolved oxygen concentrations (DO) ranged from 3.70 to 4.26 mg l^{-1}, indicative of notable oxygen depletion at all sampling sites. Contents of sediment OC ranged from 0.60 to 0.87 % and sediment water contents were 37.6–55.6 %. AVS concentrations in the sediments were variable among the various sites, ranging from 0.387 to 1.04 μmol g^{-1}, but the correlation of sediment AVS contents and water dissolved oxygen concentrations was not significant (Table 1). AVS and OC concentrations were elevated in sediment samples at the Z5 site situated inshore but notably lower at another inshore site Z2. AVS concentrations were positively correlated with OC contents of the sediment (n=24, r=0.727*). Ca and Fe contents released with the AVS extraction ranged from 0.23 to 1.36 % and from 0.75 to 1.15 %, respectively, indicating that sediment contents of calcium-bearing minerals were more variable among the sites compared to iron-bearing AVS extractable, less crystalline minerals. There was no specific difference between sites situated directly at mariculture sites of oysters and fish (Z4, Z6, Z7, and Z8), compared to sampling points several hundreds of meters away from such areas (Z1, Z2, Z3, Z5).

3.2 Total and SEM concentrations of heavy metals

Average concentrations of Cd, Cu, Ni, Pb, and Zn in sediments of Zhelin Bay were 0.35, 30.3, 28.5, 38.4, and 102 mg kg^{-1}, respectively (Table 2), and thus, heavy metal concentrations in the surface sediment as reported here generally exceeded results for Zhelin Bay from earlier studies (Qiao et al. 2010). Following the quality criteria for marine sediments of Hong Kong (Lau et al. 1993), Cd concentrations in surface sediments of the sampling sites of this study fell within a range of fairly clean sites, with the exception of sites Z1 (inshore) and Z4 (oyster farming) that fell into a moderately contaminated range; concentrations of Cu, Ni, Pb, and Zn at all sites exceeded those of a fairly clean status but were below a moderately contaminated status except of Z3 (waterway), which was in a range of high pollution by Ni. The sums of AVS simultaneously extracted metals $\sum SEM$ (SEM-Cd + SEM-Cu + SEM-Ni + SEM-Pb + SEM-Zn) at sites Z2, Z5

Table 1 Characterization of sediment and water in sampling sites from mariculture zones of Zhelin Bay

Site	Sediment					Water		
	AVS (μmol g^{-1})	OC (%)	SMC (%)	Ca (%)*	Fe (%)*	pH	DO (mg l^{-1})	Salinity (%)
Z1	0.766	0.81	37.6	1.14	0.75	7.2	3.70	21.3
Z2	0.387	0.60	50.7	0.23	1.15	7.3	4.10	24.4
Z3	0.812	0.71	52.6	0.48	1.12	7.3	4.26	24.8
Z4	0.840	0.68	52.2	0.31	1.10	7.3	4.19	25.6
Z5	1.04	0.87	51.4	0.62	1.06	7.2	4.42	26.7
Z6	0.875	0.71	55.6	0.64	1.09	7.2	4.11	26.4
Z7	0.666	0.74	52.0	0.56	0.93	7.3	4.17	27.4
Z8	0.810	0.81	53.5	1.36	1.08	7.2	4.03	27.5
RSD_{max} (%)	11.5	9.3						

RSD_{max} maximum value of relative standard deviation, *SMC* sediment moisture contents

*Concentrations of Ca and Fe in solutions during AVS extraction

(both inshore), and Z3 (waterway) sites were higher compared to all other sampling sites. The higher concentrations of Pb and Zn at sites Z2, Z3, Z4, and Z5 may indicate the enrichment of Pb and Zn in the northwest, inner areas of the bay but could not be attributed to specific human influence. The elevated concentration of Cd at site Z1 is presumably due to shore-based input. A clear pattern of elevated heavy metal concentrations specifically at mariculture sites (Z4, Z6, Z7, Z8) was thus not evident, as elevated Cd concentrations as observed at the Z4 oyster farming site also occurred elsewhere.

3.3 Speciation of heavy metals

Total recoveries as the sum of individual metal fractions from SE compared to total contents were reasonable and ranged from 92.5 to 110 % for Cd, 90.0–112 % for Cu, 93.4–110 % for Ni, 90.5–108 % for Pb, and 96.9–104 % for Zn, respectively (Fig. 2).

At all sites, most Cd was recovered from the weak acid soluble fraction (F1) that is considered as a bioavailable fraction, representing 55.5–72.9 % of the total concentration. However, these highest concentrations of Cd in F1 (0.0013 to 0.0038 μmol g^{-1}) were still much less than AVS concentrations (0.387 to 1.04 μmol g^{-1}) in the sediments, so the bioavailable Cd could be trapped by sulfide, as would be assumed in the SEM concept. The second large fraction of Cd was bound to sulfides (F4), representing 8.5–16.2 % of total concentration. Cu was predominantly present in sulfidic form (F4, 23.9–37 %) and in the residual fraction (F5, 21.9–50.2 %). A range of 11.8–16.9 % of the total Cu contents was recovered in the fraction associated with organic matter (F3), and a significant fraction of Cu (3.9–7.5 %) seemed to be bioavailable, as recovered from fraction F1 at most of the sampling sites. Exceptionally high recoveries of Cu from F1

Table 2 Total and SEM concentrations of heavy metals in mariculture sediments of Zhelin Bay

Site	Total, SEM (mg kg^{-1})					∑SEM[a] (μmol g^{-1})
	Cd	Cu	Ni	Pb	Zn	
Z1	0.588, 0.464	30.4, 11.9	16.7, 8.00	28.3, 18.5	70.4, 29.8	0.873
Z2	0.360, 0.284	43.1, 16.3	27.1, 9.70	45.2, 25.0	112, 53.0	1.356
Z3	0.269, 0.206	28.5, 17.8	37.3, 11.5	38.7, 25.8	111, 54.2	1.431
Z4	0.445, 0.350	28.0, 13.6	28.0, 9.30	40.4, 19.6	107, 36.0	1.021
Z5	0.263, 0.219	31.8, 23.0	28.3, 21.5	43.0, 23.6	115, 60.2	1.765
Z6	0.228, 0.176	26.0, 17.8	30.0, 13.2	42.2, 28.2	104, 36.8	1.210
Z7	0.267, 0.205	24.8, 10.0	29.3, 10.0	31.2, 17.5	97.0, 27.5	0.835
Z8	0.385, 0.304	25.5, 17.3	31.0, 11.3	37.8, 21.5	99.1, 32.1	1.062
RSD_{max} (%)	8.2, 9.2	2.3, 3.2	4.8, 9.4	7.2, 8.0	1.7, 3.8	

RSD_{max} maximum value of relative standard deviation

[a] ∑SEM=SEM-Cd+SEM-Cu+SEM-Ni+SEM-Pb+SEM-Zn

were found at the Z1 and Z2 sites (both inshore area, 22.0 and 35.6 % of total). Ni was mostly retained in the residual fraction F5, representing 69.2–83.7 % of total concentration, followed by F4 (sulfidic) accounting for 10.8–15.9 %. The predominant fraction of Pb (49.8–60.6 %) was recovered from the sulfidic fraction F4, followed by F5 (residual), F1 (weak acid soluble, 7.0–13.9 %), and F3 (organically bound). The predominant phases of Zn were recovered from F4 and F5, accounting for 44.5–59.4 % and 28.0–42.2 % of total concentrations, and a considerable amount of Zn could already be extracted in the weak acid extraction step F1, representing 4.6–11.1 % of the total concentration. Concentrations in the reducible fraction (F2) of each metal were lowest or very little compared to other fractions, representing only 2.0–5.6 % of total concentrations for Cd, 1.1–3.1 % for Cu, 2.2–3.9 % for Ni, 0.4–8.0 % for Pb, and 2.8–6.6 % for Zn, respectively.

We could find little difference in the speciation of metals related to the presence or absence of mariculture sites, though. Only the concentrations of sulfide-bound fractions of Pb and Zn were elevated at mariculture sites. The speciation of metals seemed thus to be mainly controlled by other local factors, such as the general sediment chemistry, which was also not significantly affected by mariculture, as outlined above.

3.4 Comparison of extraction schemes by scanning electron microscopy and EDX

Regarding the modified SE procedure and based on EDX of sulfur (S) distribution (Fig. 3a), as the indicator element for F4 (sulfidic fraction), the F1 step (weak acid soluble) had no obvious effect on sediment sulfides. However, extraction steps F2 (labile to reductive dissolution, i.e., bound to Fe/Mn (hydr)oxides) and F3 (bound to organic matter) obviously could cause a significant but small release of sediment sulfides during these steps. As depicted in Fig. 3b, after AVS extraction, sulfur was almost completely extracted from the sediment, similarly as observed for the F4 extraction step (Fig. 3a), demonstrating that sulfides were completely recovered by both extraction procedures of AVS and by the F4 step of the SE.

EDX mapping of the element distribution before and after AVS extraction (Fig. 3b) demonstrated that the indicator elements for F1 (Ca and Mg) and F4 (S) had almost completely been extracted during AVS extraction, and also, the indicator elements for F2 (Fe and Mn) were significantly affected by the AVS extraction. The dissolution of Fe and/or Mn (hydr)oxides indicated that this fraction is at least partly extracted by the AVS approach. No significant change was observed for Al and Si (indicative for F5) from EDX mapping (Fig. 3b).

Selected XRD patterns to illustrate the general sediment mineralogy, showing the effects of the four sequential extraction steps and of the single AVS-SEM extraction step, are provided as Electronic Supplementary Material.

3.5 Predicting toxicity of sediment heavy metals

Ratios of $(\sum SEM\text{-}AVS)/f_{OC}$ of less than 150 μmol g^{-1} OC would indicate that no adverse effects due to SEM metals can be expected. Ratios of $(\sum SEM\text{-}AVS)/f_{OC}$ exceeding 3400 μmol g^{-1} OC would suggest acute toxicity from these sediments; the range of uncertain effects is 150–3400 μmol g^{-1} OC (McGrath et al. 2002). Ratios obtained in our study were calculated to 13.2, 162.0, 87.2, 26.6, 83.3, 47.2, 22.8, and 31.1 μmol g^{-1} OC for sediments of Z1 to Z8; thus, no adverse effects of the heavy metals from the investigated sediments would be expected, except for the Z2 site, which was not a mariculture site but falling into the range of uncertain effects (Peijnenburg et al. 2007). Calculating the toxicity index using the contents of NR-bound metals, obtained ratios of $(\sum NR\text{-}AVS)/f_{OC}$ were 5.1, 146, 77.0, 21.1, 23.3, 34.5, 15.5, and 21.2 μmol g^{-1} OC for sediments of Z1 to Z8, and thus, notably lower, not exceeding the range, expected to cause no adverse effects.

4 Discussion

The previous study on the mineral phase selectivity of the modified SE procedure had demonstrated that this procedure had an equivalent recovery compared to that of BCR method. Furthermore, it had an improved selectivity for the non-residual fractions due to a defined extraction of the target fractions and a minor impact on other fractions and—in particular—could individually extract the sulfide-bound fraction due to the low redox potentials of the extractants used in the preceding steps (Wang et al. 2011). However, the slight changes of sulfur distribution obtained from EDX indicated that some part of the F4 fraction (sulfide-bound fraction) was released during the extraction steps of the F2 (0.2 M $NH_2OH \cdot HCl$ + 0.2 mM EDTA, pH 2.0; comparable to the second extraction step of the BCR method) and F3 fractions (0.1 mol l^{-1} $Na_4P_2O_7$, pH 9.8; possibly due to easier oxidation at high pH). Thus, also our improved method would not recover 100 % of sulfides in F4 due to small losses during steps F1–F3. Also for the BCR and Tessier methods (Tessier et al. 1979; Usero et al. 1998), some effect of the reducible fraction extraction step on sedimentary sulfides, leading to an overestimation of the reducible fraction, had been reported (Ngiam and Lim 2001).

Minding the critical role of AVS in controlling metal availability (Sibley et al. 1996; Di Toro et al. 1990) and metal immobilization by sedimentary organic matter, the index $(\sum SEM\text{-}AVS)/f_{OC}$ was proposed as an indicator for metal toxicity (McGrath et al. 2002). However, a critical review of many studies indicated a lack of selectivity of the AVS-SEM procedure, overestimating the availability and toxicity of metal (Landner and Reuther 2004). This is presumably due to a

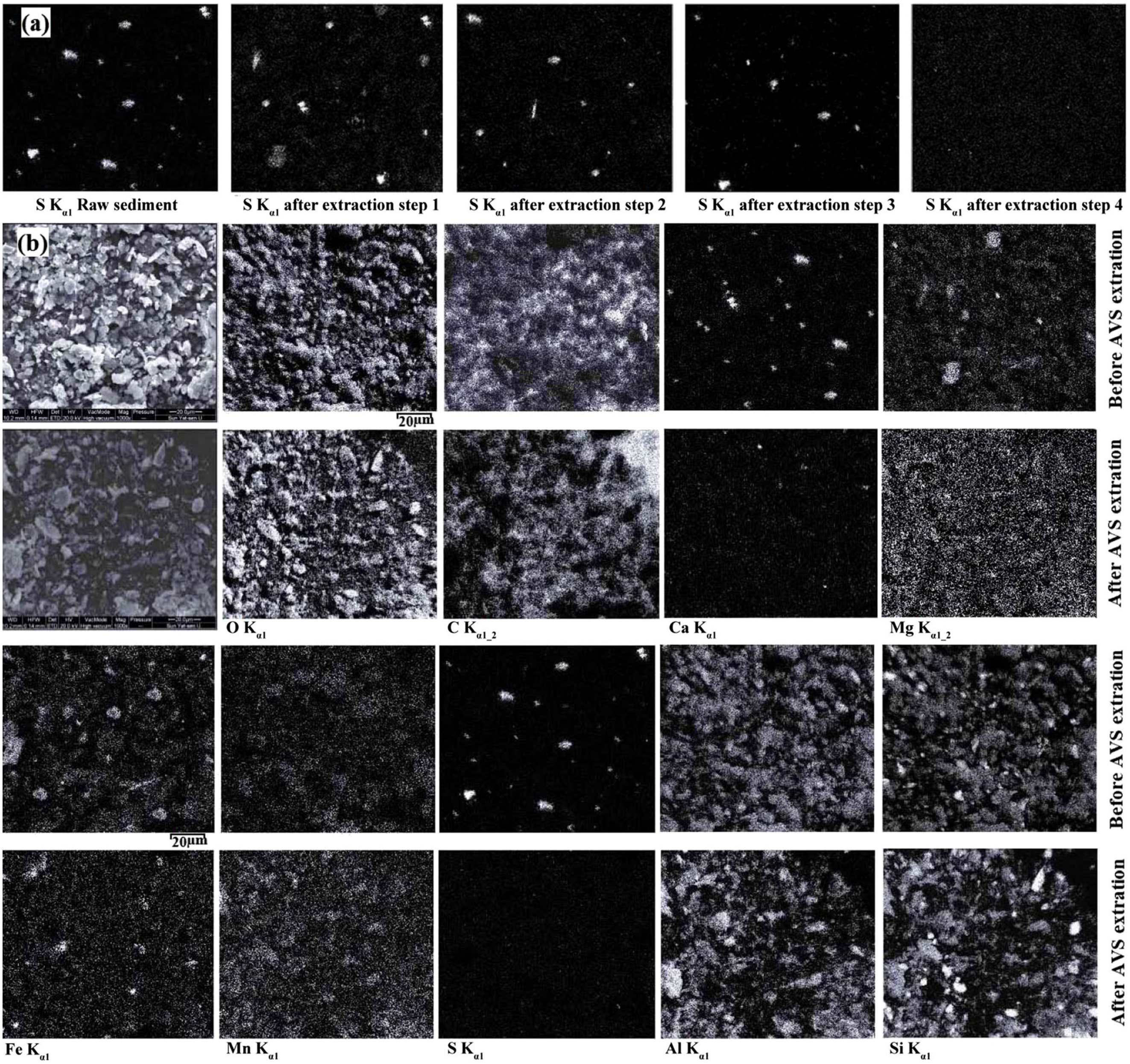
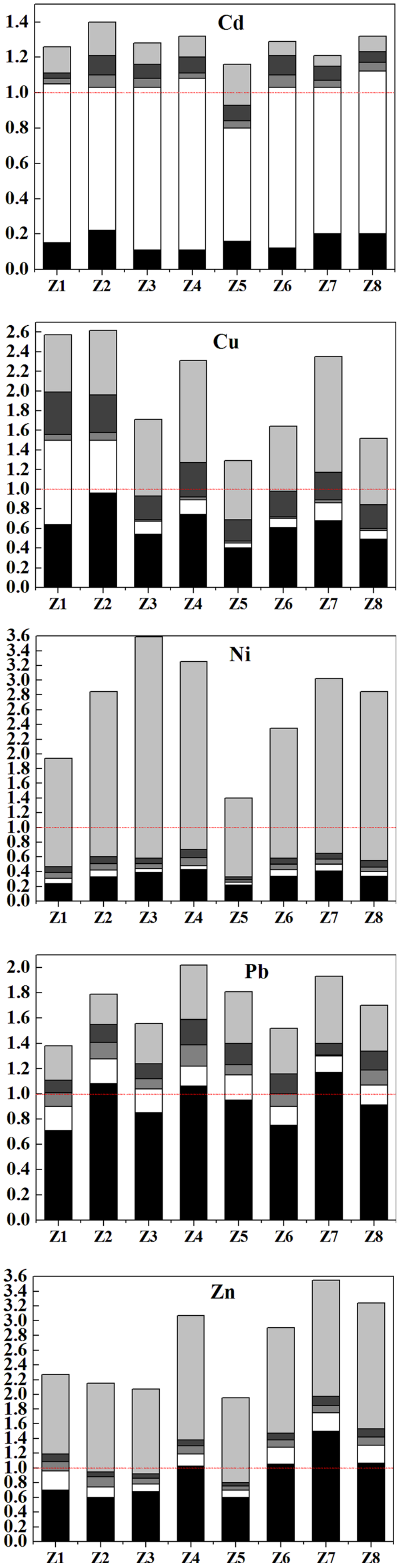


Fig. 3 Mapping of sulfur abundance in sediment samples after stepwise sequential extraction (**a**) and SEM image (*middle left*) and abundance of O, C, Ca, Mg, Fe, Mn, S, Al, and Si in sediment samples before and after AVS extraction (**b**). The element maps were characterized by scanning electron microscopy-energy-dispersive spectroscopy (HV 20.0 kV, Det ETD, see "Material andmethods" section)

simultaneous extraction of other metal fractions, like Fe/Mn oxides and carbonates (Fang et al. 2005; Machado et al. 2010). The SEM fraction of the AVS extraction was shown to represent a range of chemical fractions (O'day et al. 2000; Machado et al. 2010), and therefore, other binding forms of metals should be considered to assess the biotoxicity of metals in case the metal content exceeds AVS contents, i.e., SEM/AVS>1 (Chapman et al. 1998; Fang et al. 2005). In sediment samples of Zhelin Bay, the ratio of SEM/AVS always exceeded 1 for all the sampling sites (Tables 1 and 2). In order to elucidate the speciation of heavy metals extracted by the AVS-SEM approach, the individual fractions F1 to F5 of the modified extraction scheme were normalized by the quantity of the same element recovered from the AVS-SEM approach (Fig. 4). A ratio of 1 would thus indicate that similar amounts of a respective element would be recovered by AVS-SEM and the SE fraction.

As indicated by these ratios, the concentrations of heavy metals recovered from the AVS-SEM method were higher than the F4 fractions of the SE procedure at the studied sites, except for Pb and Zn at Z4, Z6, Z7, and Z8 sites, which had elevated Pb and Zn concentrations in the sulfidic fraction probably due to mariculture. This was not surprising, as 6 M HCl solution used in the AVS-SEM method undoubtedly also

Fig. 4 Ratios of individual fractions of heavy metals to their corresponding content of simultaneously extracted metals (AVS-SEM). Ratio values were calculated by averages of metal fraction concentrations and AVS-SEM contents, respectively (n=6). ☐ *F1/SEM*, ▨ *F2/SEM*, ▩ *F3/SEM*, ■ *F4/SEM*; ▒ *F5/SEM*



extracts the bioavailable F1 fraction defined in the SE (Hsieh and Yang 1989; Rao et al. 2008). Accordingly, the sums of Cd recovered from F1 plus F4 of the SE were 1.02~1.12 times the Cd extracted by the AVS-SEM method, with the exception of Z5 (0.80 of AVS-SEM), and were thus approximately consistent with the concentrations of $\mathrm{SEM}_{\mathrm{Cd}}$ at most of sites. The sums of Cu and Ni recovered from F1 plus F4 were mostly lower than their contents recovered from the AVS-SEM method ($\mathrm{SEM}_{\mathrm{Cu}}$ and $\mathrm{SEM}_{\mathrm{Ni}}$), and a part of the stable residual fraction must have been leached during AVS extraction, as sums of non-residual fractions were substantially lower than $\mathrm{SEM}_{\mathrm{Ni}}$ and $\mathrm{SEM}_{\mathrm{Cu}}$. For Cu, at the Z1 and Z2 sites, the sum of F1 plus F4 fractions were higher that concentrations of $\mathrm{SEM}_{\mathrm{Cu}}$, mostly due to a high contribution of the F1 fraction that likely resulted from the elevated release of Cu under low DO concentrations (Wen and Allen 1997) and low content of organic matter associated highly with Cu (Tack and Verloo 1995; Fangueiro et al. 2005).

Our results demonstrate again that the AVS-SEM method was not designed to extract defined geochemical metal fractions (Ankley et al. 1996; Cooper and Morse 1998), as are sequential extraction methods (Tessier et al. 1979; Rao et al. 2008), but to judge potential bioavailability only (Di Toro et al. 1992; Ankley et al. 1994; Leonard et al. 1996). AVS-SEM-based predictions thus represent a potential risk of mobilization only, limited by the less-defined selectivity of one single extraction step.

EDX mapping of the distribution of indicator elements before and after AVS extraction (Fig. 3b) suggested that the AVS-SEM method yielded an incomplete bulk extract of F1–F4. The NR concentrations of Cd at site Z5, Cu at sites Z3 and Z5, and Zn at sites Z2, Z3, and Z5 were lower than their corresponding concentrations from the AVS-SEM approach. At these sites, sulfur concentrations were low and DO concentrations were high. Moreover, a significant quantity of the residual fraction of these metals was extracted in the AVS extraction, also in the case of Ni (Fig. 4). In other words, this implied that SEM from AVS extraction corresponded to a large extent to metal contents of the weak acid soluble fraction and the reducible fraction besides the sulfide-bound fraction, as already reported earlier (e.g., Fang et al. 2005). As metals from the labile fraction (F1) and the potential labile fractions (F2 and F3) are particularly relevant for predicting sediment toxicity (Rauret 1998; van Griethuysen et al. 2006) and these fractions are only incompletely extracted by the AVS-SEM method, this may be inadequately considered in sediment toxicity prediction by $(\sum SEM\text{-}AVS)/f_{OC}$. While an immobilizing effect of organic matter on heavy metal ions is considered in the latter approach, an apparent release of metals of the residual fraction would overestimate sediment toxicity in our case.

Regarding the predominant fractions of metals recovered by AVS-SEM, there is thus a need for caution when judging heavy metal toxicity in mariculture zones by the $(\sum SEM\text{-}AVS)/f_{OC}$ ratio, and our revised SE protocol would have to be employed here. Therefore, we suggest that using the NR fractions from our proposed SE approach (Wang et al. 2011) to derive the toxicity index for heavy metals is superior to the index based on AVS-SEM. The content of metals that is not extracted by our sequential extraction procedure in the first four steps is presumably non-toxic and bound to a stable residual fraction (Gleyzes et al. 2002). In mariculture areas such as in our study here and elsewhere (Guo et al. 2009), the sediment environment is mostly anoxic and reducing, so fractions of metals bound to reductively dissolvable phases may be expected to be small (Gleyzes et al. 2002). Therefore, the reducible fraction of metals had no or little effect on prediction indexes when NR was used to assess toxicity risk. The AVS-SEM approach, however, partly included metals from the residual fraction and thereby presumably overestimated sediment toxicity.

Based on our findings of metal speciation, we evaluated the toxicity of sediment heavy metals using the better-defined NR fractions of our sequential extraction, because the NR fractions can not only individually be evaluated for possible biotoxicity effects but as a sum also provide a general index to judge heavy metal toxicity in anoxic surface sediments. To this end, the sum of fractions F1~F4, i.e., NR, was used to calculate the ratio of $(\sum NR\text{-}AVS)/f_{OC}$ instead of SEM. Under these conditions, the ratios for all heavy metals under study here were 5.1, 146, 70.0, 21.1, 23.3, 34.5, 15.5, and 21.2 μmol g^{-1} OC for sediments in Z1~Z8, respectively, and were thus always less than 150 μmol g^{-1} OC at all of sampling sites. These ratios indicate that sediment toxicity is not to be expected (Burton et al. 2007), although a classification based on concentrations of SEM from the AVS extraction would partially yield values that exceed the threshold of 150 μmol g^{-1} OC.

We also performed a Pearson correlation analysis to evaluate the effects of common major sediment properties and constituents on toxicity prediction of sediment heavy metals (Table 3). OC was positively correlated with AVS (r=0.727*), indicating that high deposition of OC in sediments probably favors the formation of sulfides. ∑SEM was positively correlated with SEM_{Fe} (r=0.573**), which may be due to a dissolution of sedimentary Fe oxides and associated heavy metals during AVS extraction by HCl solution. The toxicity index was correlated positively with ∑SEM and SEM_{Fe} and negatively with OC and AVS, which indicated that the toxicity prediction was primarily affected by the contents of Fe oxides. After replacing the SEM by NR, the toxicity prediction was affected by AVS, OC, and also by ∑NR (r=0.572*), rather than by ∑SEM. As this procedure seemed to better capture the critical sedimentary characteristics, i.e., the sulfide- and organic matter-bound element contents and weak acid extractable contents, we conclude that the toxicity prediction based on NR contents would be more suitable for anoxic surface sediments, such as sediments in mariculture zones.

It has to be noted, though, that for predicting sediment toxicity, all extraction procedures have their inherent limitations, as in the extraction protocols, equilibrium is always reached. Contrarily, in natural sediments, solid phases and pore water are mostly not in equilibrium, and therefore, a more recent, kinetic approach may also be promising (Fangueiro et al. 2005).

5 Conclusions

Based on our investigations at the eight sites in the mariculture zone of the Zhelin Bay, all sediments were predominantly anoxic and surface sediments at some sites were moderately contaminated by heavy metals. As the sum of metal individual fractions corresponded well with total contents, the sequential extraction procedure turned out to be reasonably accurate to estimate heavy metal fractions in anoxic sediments. Cd, Ni, and Pb were found dominantly in the weak acid soluble fraction (F1), bound to sulfides (F4), or remained in the residual

Table 3 Pearson correlation coefficient for sediment components, AVS and SEM in sediments (n=24)

Variables	AVS	OC	CaHCl	FeHCl	∑SEM	(∑SEM-AVS)/*f*OC	(∑NR-AVS)/*f*OC
AVS	1	0.727*	0.303	−0.061	0.302	−0.704*	−0.719*
OC		1	0.699**	−0.474**	0.120	−0.731*	−0.734*
CaHCl			1	−0.509**	−0.332	−0.564**	0.204
FeHCl				1	0.573*	0.577*	−0.562**
∑SEM					1	0.409*	0.572*
∑(SEM-AVS)/*f*OC						1	0.685*
∑(SEM*-AVS)/*f*OC							1

*p<0.05;**p<0.01

fraction (F5). Cu and Zn were mainly found in the sulfide-bound or residual fraction (F4 and F5). The proportions of heavy metals in the fraction susceptible to reductive dissolution or bound to organic matter (F2 or F3) were very low except for Cu, which had apparently high affinity to organic matter (F3). Compared to the AVS-SEM approach, the latter method seemed to extract labile phases only incompletely but included also metals from the residual fraction. In detail, AVS-SEM mostly comprised parts of weak acid soluble, reducible, and organic matter-bound, and sulfide-bound metal fraction. To avoid overestimation or underestimation of a risk of sediment toxicity, we suggest to use the sum of NR fractions based on the proposed sequential extraction scheme to predict sediment toxicity in marine surface sediments from mariculture zones. In our case study, the toxicity effects of sediment heavy metals can be assumed as negligible for all of the investigated sampling sites in Zhelin Bay as indicated by the ratio of $(\sum NR\text{-}AVS)/f_{OC}$, irrespective of the presence or absence of mariculture.

Acknowledgments This study was supported by the National Natural Science Foundation of China (No. 21307120), Science and Technology Planning project of Guangdong Province of China (No. 2012B061800043), and Science and Technology New Star Project of Pearl River of Guangzhou (No. 2012J2200054). The authors gratefully acknowledged Prof. Hong Du for his kind assistance in sample collections. We thank the reviewers for their valuable comments that substantially improved this study.

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