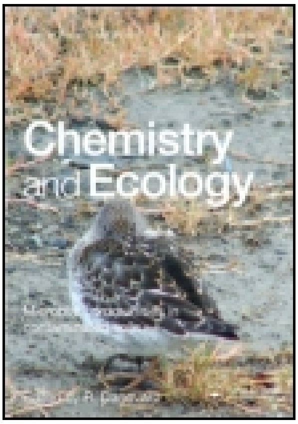

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Speciation of heavy metals in sediments from the Scheldt estuary, Belgium

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Speciation of heavy metals in sediments from the Scheldt estuary, Belgium

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A simple three-step sequential extraction procedure was applied to study the speciation of heavy metals in sediment from Scheldt estuary, and their relationship to sediment grain size and organic matter content. The sedimentary metal content was fractionated into carbonate and exchangeable, metals bound to organic matter and residual fractions. Sedimentary total metal content was also determined using an industrial microwave (ETHOS 900) HF/HNO_3 extraction method. The extracts were analysed for metals using inductively coupled plasma atomic emission spectrometry. The bioavailable fraction (exchangeable and metals bound to organic matter) comprised less than the other forms. Residue metals were the dominant form of metals in almost all studied sites. The average total metal content for the studied sites decreased in the order Fe $>$ Cr $>$ Cu $>$ Co $>$ Zn $>$ Pb $>$ Cd. Based on average values for the studied sites, the highest bioavailable metals in sediments were Cd (38%) from Westkapelle, Zn (17%) from Yerseke, Co (12%) from Domburg, Cr (9%) from Vlissingen, Fe and Pb each (2%) from Yerseke, and Cu (1%) from Domburg. Metal recovery was good, with $<$10% difference between the total metal recovered through the extractant steps and the total metal determined using HF/HNO_3 extract.

Keywords: heavy metals; Scheldt estuary; sediment; sequential extraction; speciation; bioavailable

1. Introduction

Total metal concentration has been exhausted in most studies to determine trace metals in sediment [1]. It is a good tool that gives some indication of the level of contamination in sediments, but consequently, the total metal concentration in sediments is not a reliable indicator of bioavailable metal, i.e. it does not give sufficient information about the mobility and bioavailability of metals [2–4]. Metals in sediments are associated with a number of physico-chemical forms, which in turn affect their bioavailability to organisms. Ray et al. [5] recommended that metals associated with an EDTA extract are a good measure of available metal. Alternatively, Rule and Alden [6] suggested that the sum of amounts of metals found in exchangeable and easily reducible fractions can be used to indicate Cd bioavailability to blue mussel *Mytilus edulis*. Fractionation of metals from sediments can therefore differentiate metal species and define fractions which are related to their specific nature [7].

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Depending on the toxic metals, there are quite large differences between their total and ecologically effective amounts. Because determination of the total metal gives little information about speciation, and hence the bioavailable fraction of metals to organisms, methods to determine different forms of metals in sediments have been proposed. In particular, a sequential extraction procedure has been widely used to achieve this goal (i.e. to speciate potentially toxic metals in a variety of samples) [8–10]. In a sequential extraction procedure, a series of reagents is used to extract operationally defined discrete phases from sediment in an outlined sequence [11]. The phases are usually adsorptive or exchangeable, carbonate, reducible (Fe–Mn oxide), organic and residue [12]. Many investigators have performed studies on the quantitative distribution of metals among various chemical phases of sediment with the aim of assessing the toxic potential of pollutants in the environment [13–16]. Fractionation is performed by a sequence of selective chemical leaching techniques that include the successive removal of these phases and their associated metals [12]. Tessier and others developed the first sequential extraction procedure in 1979 [17]. Since then, several attempts to modify the sequential extraction procedure for the assessment of metal speciation among different phases have been made, with different degrees of success, aimed at improving the selectivity of the extracting agent towards a particular phase and reducing the operation time to facilitate its routine application [18,19].

In our study, a simple sequential extraction procedure with few steps, but still relevant for metal bioavailability and accumulation, was used with the aim of studying the speciation of heavy metals in the sedimentary phase, and their relationship to the organic matter content and sediment grain size.

2. Materials and methods

2.1. *The Scheldt estuary*

Baeyens et al. [20] extensively described the Scheldt estuary. The River Scheldt (Figure 1) starts in the northern part of France (St Quentin) and empties into the North Sea near Vlissingen (The

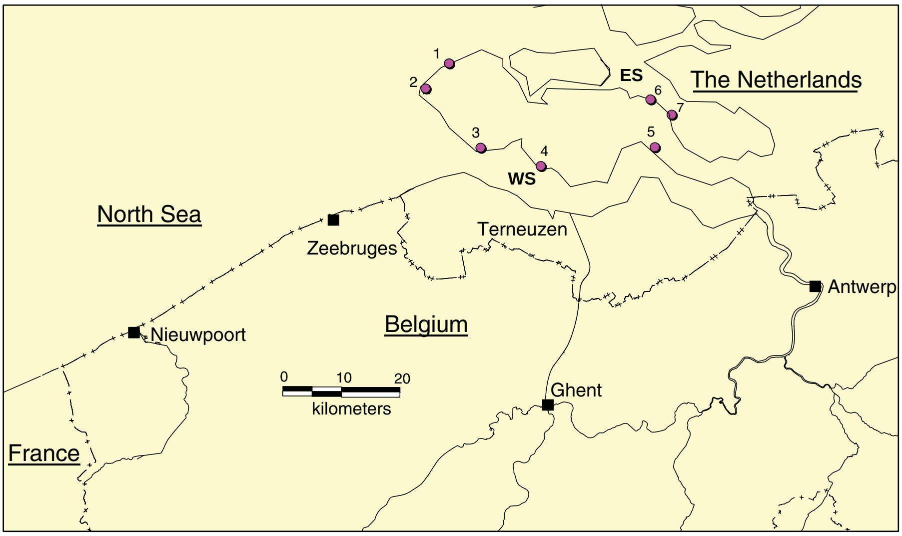

Figure 1. Map showing location of the sampling sites. Stations with code numbers 1, 2, 3, 4, 5, 6 and 7 are Domburg, Westkapelle, Vlissingen, Borsele, Hansweert, Wemeldinge and Yerseke, respectively.

Netherlands). It has a total length of 355 km and its mean depth is ∼10 m [20]. Together with all its branches, the River Scheldt is fed by rains, which causes their discharge to vary considerably with maximum values (400 $m^3 \cdot s^{-1}$) occurring in winter and spring. The minimum discharges (20 $m^3 \cdot s^{-1}$) occur during summer and autumn [20].

2.2. *Sampling campaign*

The locations of the sampling sites are presented in Figure 1. In total, seven sampling sites in the Scheldt estuary were selected and sampled along the salinity gradient. All sediment samples were collected during the winter season (30 January 2002 to 13 February 2002) at low tide. Surface (0–5 cm) sediment samples were collected at the edge of the water using a hand corer. Samples were put into clean 20 cm^3 polyethylene bottles and tightly closed. Five replicates of the sediment samples were collected per site to study metal speciation on different sedimentary phases and for total metal analysis. The samples were put immediately into plastic bags, stored in an icebox, transferred to the laboratory and then stored in the freezer at −20 °C until all the moisture content had turned to ice. Samples were then taken into the freeze drier for lyophilisation. Thereafter, samples were analysed for heavy metals.

2.3. *Sequential extraction procedure*

The sequential extraction method was carried out in three steps, at each of which a specific extraction solution was added. All water used was of Milli-Q (Millipore; resistively 18 $M \cdot cm^{-1}$) quality. We applied a combined simple three-step sequential extraction procedure, partly adopted from Community Bureau of Reference (BCR) or Standards, Measurement and Testing (SMT) for fractions 1 and 3 (F_1 and F_3), and partly from Whitworth et al. [21] for fraction 2 (F_2) (Figure 2). The two fractions (F_1, carbonate and exchangeable metals and F_2, metals bound to organic matter) are considered to be the most labile or leachable and hence relevant for bioavailability and accumulation by aquatic organisms [4].

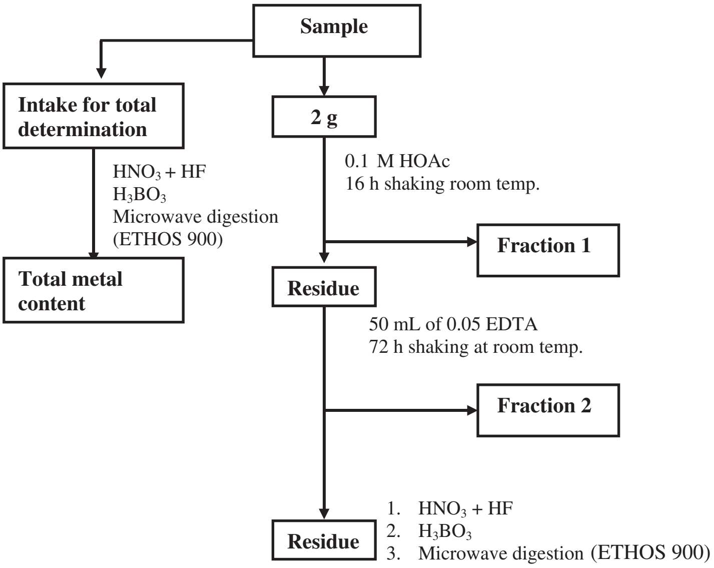

Figure 2. Schematic presentation of the sequential extraction protocol followed in this study.

In the first step, ∼2 g of dry homogenised sediment sample was transferred into a pre-cleaned polycarbonate tube and 50 mL of 0.1 M acetic acid was added. The mixture was shaken for 16 h (overnight) in a horizontal end-over-end shaker at 140–150 rpm at room temperature. Care was taken to avoid any delay between the addition of the extracting solution and the starting of shaking. The solution was then centrifuged at 5000 rpm for 25 min and the supernatant fluid decanted into a pre-cleaned 60 mL HDPE bottle and stored at 4 °C until measurement with inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectrometry (ICP-AES).

Fifty millilitres of 0.05 M EDTA solution was added to the washed residue of step 1; the mixture was then shaken in an end-over-end shaker at 140–150 rpm for 72 h at room temperature. The solution was then centrifuged at 5000 rpm for 10 min and the supernatant fluid decanted into a pre-cleaned 60 mL HDPE bottle and stored at 4 °C until measurement with ICP-MS. The residue sediment from this extraction step was washed with 20 mL of milli-Q water, frozen, lyophilised and used for the third step, which is residue total metal extraction.

Total metal extraction from the sediment samples was performed using an industrial microwave (ETHOS 900) HF/HNO_3 extraction and the HF acid was neutralised by H_3BO_3 according to Van Ryssen et al.'s [22] total digestion protocol for soil or sediment samples. Approximately 0.2 g of dried and homogenised sediment samples were weighed in the digestion vessel. Four millilitres of sub-boiled (purified) HNO_3 and 4 mL of sub-boiled (purified) HF was added. The digestion pumps were closed tightly according to the instruction manual, and placed in the rotor inside the industrial microwave (ETHOS 900) and the digestion program was started as described by Van Ryssen et al. [22]. Blanks were analysed alongside samples and received the same treatment. The method was checked by analysing reference sediment samples (CRM 320). The values from the experiment and their standard deviations were in good agreement with values for the certified material (see Table 3).

Carbon content in sediment samples was analysed using a C:N analyser (Carlo Erba Strumentazione NA 1500 nitrogen analyser). Both total and organic carbon were analysed. The difference between total carbon and total organic carbon was recorded to represent inorganic carbon. To measure the organic carbon, sediment samples (∼10 mg) were spiked with a few drops of 5 M HCl and placed in an oven at 50 °C for at least 1 h to dry. Decalcified samples were then analysed in the C:N analyser and the valued recorded. The quality of this analysis was controlled by analysing blanks (empty silver capsules) and a standard reference material (acetanilide). Sediment samples were analysed for grain sizes using a grain size analyser (Malvern Instruments Ltd, UK) and various size fractions were composed as recorded in Table 1.

All chemicals used were of analytical reagent grade from Merck Co. All materials in contact with the samples during sampling campaigns, all glass and plastic ware were subjected to decontamination processes by filling and storing them in 5–15% nitric acid (HNO_3, Pro analysis) at

Table 1. Mean ($n = 5$) percentage volume of the sediment grain size fractions (μm) used in the study.

Site code	Grain size fractions in (%)						
	<2 μm	2–16 μm	16–63 μm	63–125 μm	125–250 μm	250–500 μm	500–1000 μm
1	0.32 (0.02)	0.05 (0.02)	0.03 (0.01)	0.07 (0.01)	7.53 (0.77)	73.93 (9.95)	18.08 (10.70)
2	0.33 (0.22)	0.08 (0.15)	0.00 (0.00)	0.74 (0.14)	33.67 (3.19)	64.61 (2.61)	0.58 (0.41)
3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.30 (0.11)	18.79 (3.31)	72.69 (0.91)	8.22 (2.58)
4	1.96 (1.51)	4.57 (4.43)	13.60 (2.69)	18.48 (6.29)	37.34 (2.37)	23.25 (13.00)	0.80 (0.82)
5	6.57 (0.81)	20.02 (2.67)	40.18 (1.69)	20.55 (2.23)	10.80 (2.27)	1.90 (0.86)	0.00 (0.00)
6	0.67 (0.06)	0.49 (0.13)	1.30 (0.50)	16.96 (5.70)	49.33 (7.51)	25.02 (9.57)	6.22 (3.60)
7	1.10 (0.09)	1.07 (0.06)	2.57 (0.37)	21.14 (2.14)	60.71 (1.45)	13.41 (1.39)	0.00 (0.00)

Note: Numbers in parentheses represent percentage standard deviations.

room temperature for at least 24 h prior to use, rinsing at least three times with Milli-Q-water (Millipore; resistively 18 M·cm^{-1}) quality.

2.4. *Statistical analysis*

Correlation analysis was made to test for a relationship between heavy metals and both carbon content and sediment grain size. The strength and significance of the parameters estimated was evaluated using correlation coefficients (r) and significance levels (p). All statistical analyses were performed using SPSS software version 11.

3. Results

3.1. *Grain size distribution and the carbon content of the sediment*

The percentage distribution of different size fractions in sediments from sites 1–5 (west Scheldt), 6 and 7 (eastern Scheldt) which were used in the speciation study are given in Table 1. The average content of the $< 63\,\mu$m fraction was 24.74% by volume with a minimum of 0.30% and a maximum of 87.32% by volume. In the $< 63\,\mu$m sediment fraction, the average proportion of clay-size sediment ($< 4\,\mu$m) ranged from 0.00 to 26.59% by volume. Sediments with a clay content $>$ 5% were found mainly at site 5. The distribution of organic carbon (C_{org}) was similar to grain size distribution for all sites. The inorganic carbon content in the sediment was inverse to that of organic carbon. The carbon content distribution in the sediment is shown in Table 2.

Organic carbon in marine sediments may either be terrigenous or derived from biological productivity in the marine environment. Higher primary and secondary productivities in marine environments may induce a large flux of organic matter to the sea bottom. The organic carbon from the sediment in our study area showed a landward increase (see Table 2) suggesting that it did not originate from a marine environment [23].

3.2. *Bottom sediment metals*

3.2.1. *Analytical quality assurance*

The accuracy of the digestion method was appraised using standard reference material CRM 320 (river sediment of known elemental concentration) and subjecting this material to the same analytical procedure as the samples. Mean experimental and certified values, both with their standard deviations, are here presented in Table 3. A good agreement was obtained for most metals.

Table 2. Mean ($n = 5$) and percentage concentrations of the carbon content distribution in sediment sample from western and eastern Scheldt.

Station	TC content (mg·g^{-1})	OC content (mg·g^{-1})	% OC content	IC content (mg·g^{-1})	% IC content
1	2.61 (1.47)	0.22 (0.12)	8.43	2.39 (1.47)	91.57
2	3.95 (1.71)	0.37 (0.08)	9.37	3.58 (1.66)	90.63
3	2.60 (0.74)	0.18 (0.08)	6.92	2.43 (0.73)	93.08
4	13.71 (3.84)	4.25 (1.69)	30.99	9.46 (2.21)	69.01
5	51.38 (7.36)	37.35 (5.93)	72.69	14.03 (6.71)	27.31
6	21.19 (4.20)	5.66 (3.60)	26.71	15.54 (4.28)	73.29
7	6.44 (0.34)	2.29 (0.72)	35.56	4.15 (0.73)	64.44

Notes: Numbers in parentheses represent standard deviations. TC, total carbon; OC, organic carbon; IC, inorganic carbon.

Table 3. Mean experimental values for the CRM 320 river sediment reference standard material calculated from six replicates with standard deviations and certified values.

Metal	Reference standard materials Experimental value (SD)	Reference standard materials Certified value (SD)
Fe	39,200 (39)	[45,000]*
Cr	69.85 (8.77)	75.2 (10.42)
Zn	154.92 (9.63)	142.00 (8.23)
Cu	52.65 (2.45)	44.1 (1.86)
Pb	51.83 (7.71)	42.3 (4.61)
Cd	0.88 (0.18)	0.533 (0.06)
Co	13.57 (0.48)	[19.00]*

Notes: (Concentrations are in $\mu g \cdot g^{-1}$ dry wt). Numbers in parentheses represent standard deviations *Noncertified, indicative values.

Table 4. Correlation between heavy metal fractions, gain size and organic carbon content of sediment.

	Cd	Co	Cr	Cu	Pb	Fe	Zn
			F_1				
TC	0.99	0.89	ns	ns	-ve	-ve	0.83
OC	0.78	0.90	ns	ns	-ve	-ve	0.88
IC	ns	ns	ns	ns	-ve	-ve	ns
			F_2				
TC	0.96	0.94	0.98	0.94	0.94	0.95	0.96
OC	0.99	0.99	0.99	0.99	0.99	0.99	0.99
IC	ns	ns	ns	ns	ns	ns	ns
			F_3				
TC	0.95	0.95	0.93	0.96	0.96	0.91	ns
OC	0.99	0.98	0.97	0.99	0.88	0.92	ns
IC	ns	ns	ns	ns	0.87	ns	ns
			Total metal				
TC	0.93	0.95	0.94	0.95	0.96	0.92	Ns
OC	0.99	0.99	0.97	0.99	0.99	0.94	Ns
IC	ns	ns	ns	ns	ns	ns	ns
			Grain size				
<2 μm	0.98	0.92	0.99	0.89	0.85	0.97	0.70
2–16 μm	0.74	0.80	0.92	0.64	0.76	0.86	0.60
16-63 μm	0.68	0.67	0.82	0.72	0.64	0.72	0.58
63–125μm	0.52	0.56	0.68	0.50	0.48	0.70	0.46
125–250 μm	ns	ns	ns	ns	ns	ns	ns
250–500 μm	ns	ns	ns	ns	ns	ns	ns
500–100 μm	ns	ns	ns	ns	ns	ns	ns

Notes: TC, total carbon; OC, organic carbon; IC, inorganic carbon. ns, correlations not significant at $p < 0.05$.

3.2.2. *Sequential extraction results for the bottom sediment*

All metals extractable by acetic acid (F_1) correlated significantly with organic carbon, except Cr and Cu ($p < 0.05$) (Table 4). Although most of these correlations were positive, Pb and Fe showed significant negative correlation with organic carbon with all forms of carbon (total, organic and inorganic). All metals (except Zn) from F_1 showed a significant positive correlation with inorganic carbon. Metals from F_2 and F_3 correlated significantly with organic carbon indicating that organic matter is a good scavenger of heavy metals and can act as a vehicle for metal settling in a marine environment.

Table 5. Total metal concentration and their standard deviation (in parentheses) in bottom sediment obtained from Scheldt estuary.

	Total concentration ($\mu g \cdot g^{-1}$)						
Fraction	Cd	Co	Cr	Cu	Pb	Fe	Zn
1	0.04 (0.01)	0.59 (0.18)	115.53 (6.04)	5.41 (1.89)	3.77 (6.04)	3782.18 (6.04)	2.08 (0.72)
2	0.01 (0.01)	0.93 (0.34)	186.9 (6.04)	3.35 (0.99)	5.39 (6.04)	4422.7(6.04)	8.23 (3.04)
3	0.04 (0.01)	0.67 (0.28)	58.29 (6.04)	3.06 (1.21)	4.51 (6.04)	3850.1 (6.04)	6.91 (2.02)
4	0.08 (0.01)	3.85 (0.98)	42.64 (6.04)	6.56 (1.84)	10.73 (6.04)	19290.78 (6.04)	45.92 (8.08)
5	11.08 (3.15)	14.13 (4.56)	239.89 (6.04)	42.1 (16.01)	62.93 (6.04)	39380.86 (6.04)	144.97 (52.04)
6	0.17 (0.04)	1.63 (1.50)	81.92 (6.04)	7.15 (2.86)	10.01 (6.04)	7149.01 (6.04)	13.94 (5.08)
7	0.11 (0.04)	1.56 (0.84)	48.31 (6.04)	6.26 (2.64)	7.62 (6.04)	5891.86 (6.04)	23.27 (9.02)

3.2.3. *Total metals in sediment*

Sediments act as sinks for heavy metals and the degree of scavenging activity of sediments is a function of their physical, chemical and biological properties. It is common that heavy metals associate more with sediments of smaller grain size. In this study, a significant correlation ($p < 0.05$) was found for all metals between heavy metal concentrations in the bottom sediment and sediment grain sizes of 63–125 µm. Cadmium showed a significant correlation ($p < 0.05$) with sediment grain sizes $>$ 125 µm. The levels of total metals in the sediment at all sites are presented in Table 5. Iron was the predominant metal at all sites, followed by Cr, Zn, Pb, Cu, Co and Cd.

3.2.4. *F_1–carbonate and exchangeable metals*

Metals in this fraction are the most mobile and readily available for biological uptake in the environment. Metal associated with the carbonate and exchangeable fraction in contaminated sediments may reach up to 50% of the total metals [24]. In this study, metals were concentrated $<$ 50%. The trend shows a high concentration of Cd followed by Co and Zn. The respective percentages of metals associated with the F_1 fraction varied between 2 and 38% (Cd), 2 and 11% (Co) and 1 and 17% (Zn) of total metal content. Other metals were present in this fraction in small concentrations (Table 6). The concentration of Cu, for example, was low, probably due to the poor adsorption behaviour of Cu on carbonate minerals. Cu has high affinity for dissolved organic matter and less affinity for adsorption on $CaCO_3$. Carbonates are also regarded as inefficient metal-carrier phases. The absence and/or presence of metals at low concentrations at most sampling stations may indicate the poor availability of these elements to benthic organisms.

3.2.5. *F_2–metals bound to organic matter*

Heavy metals have high affinity for organic matter, the decomposition product of which can be appreciated for the behaviour of heavy metals in aquatic environments. Organic matter plays a key role in the scavenging, distribution and dispersion of heavy metals in the environment through cation exchange and chelation mechanisms. In this study, the heavy metal concentration in the organically bound fraction was appreciable for most metals at sampling sites 4–6; Pb being more important at these sites followed by Cd, Cu and Zn. Toxic metals such as Cd, Cu and Pb were partitioned in this fraction at between 6 and 72% (Cd), 1 and 47% (Cu) and 16 and 83% (Pb) (Table 6). These results show a behaviour similar to the results of metal burdens in the tissue of *M. edulis*, as studied by Shilla et al. [25]. F_1 and F_2 metal fractions are considered the most labile, can easily be leached and are therefore available for uptake by organisms.

Table 6. Percentages of metal concentration in the three sedimentary fractions obtained by sequential extraction on bottom sediment samples.

Fraction	Site	Cd	Co	Cr	Cu	Pb	Fe	Zn
F_1	1	14.39	11.67	1.64	1.22	1.66	0.72	1.13
	2	38.34	4.46	0.50	0.31	1.15	1.44	1.61
	3	20.83	9.11	8.88	1.03	1.68	0.98	4.26
	4	22.54	5.32	0.02	0.58	0.69	0.32	16.79
	5	4.14	2.40	0.01	0.15	0.02	0.01	6.67
	6	2.19	5.67	0.22	0.49	0.15	0.04	3.04
	7	23.40	6.46	0.17	0.65	1.75	1.85	17.12
F_2	1	14.45	8.29	3.76	1.55	16.03	3.81	0.78
	2	24.62	4.52	1.49	1.16	21.04	5.86	0.38
	3	6.16	5.01	2.34	2.29	35.23	7.50	2.66
	4	50.31	6.20	1.25	20.03	56.01	7.15	17.21
	5	72.78	24.04	3.08	47.13	83.48	16.23	42.50
	6	62.55	10.62	7.42	10.20	25.85	8.45	17.35
	7	32.59	8.33	1.07	12.39	33.15	9.30	9.35
F_3	1	71.16	80.04	94.60	97.24	82.31	95.47	98.09
	2	37.03	91.02	98.01	98.53	77.81	92.71	98.01
	3	73.00	85.88	88.77	96.69	63.10	91.53	93.07
	4	27.15	88.48	98.73	79.39	43.30	92.53	65.99
	5	23.09	73.56	96.92	52.72	16.50	83.77	50.82
	6	35.27	83.71	92.36	89.31	74.01	91.51	79.61
	7	44.01	85.21	98.77	86.96	65.10	88.85	73.53

3.2.6. *F_3–residual fraction (metals associated with the crystal lattice of silicates)*

This fraction represents the part of metals that is largely unavailable to organisms. Residual metal fractions are not soluble under experimental conditions because they are held within the mineral matrix and their major input to the marine environment is not via anthropogenic sources. This fraction includes major transition metals whose concentrations are controlled by the mineralogy and weathering of the rocks in aquatic environments. In other words, this fraction is important because it gives a better insight into mechanism by which heavy metals are associated with mineralogical phases of the sediments. The results of this study indicate that at almost all of the sampling stations $>$ 50% of the total metals are located in the residue fractions. Fe, Zn, Cu, Cr and Pb constituted 83–95, 50–98, 52–98, 88–98 and 16–82% of the total metal concentration respectively. The percentage of other metals varied between 73–91% (Co) and 23–71% (Cd).

4. Discussion

4.1. ***Grain size distribution and the carbon content of the sediment***

As expected, the concentration of heavy metals showed a dependent relationship with the grain size of the sediment. Heavy metals were highly concentrated in sediments with smaller grain size, possibly due to the surface properties of clay minerals. Finer grains also have a larger surface area to volume ratio which enhances metal binding [26]. A high carbon content in the sediment also indicated a relationship with the total metals in the sediment, meaning that carbon, and in particular organic carbon, has a high affinity to metals, sequestering and storing them in a matrix. The correlation coefficients for the various metals to the total carbon content of the sediment at $p = 0.05$ are shown in Table 4. Both total metal concentrations in the sediment and the sequentially extracted fractions F_1 and F_2 correlated well with grain sizes up to 125 μm (Table 4). The sediment grain size and its carbon content are vital parameters in the partitioning of metals in the sediment. Because the sediment particles are crucial as far as the selective behaviours of filter feeders are concern, metals bound to the active sites of sediments, together with those sequestered into the matrix of organics, can be taken up, assimilated and incorporated into the tissues of biota, which may be of public concern.

4.2. *Total metals in sediment*

Sediment is an important pool for heavy metals in marine systems. When the marine environment is in a dynamic state, there is an interaction between the sedimentary phase, the dissolved phase and the particulate phase through processes like resuspension, settling, adsorption and desorption. Hence sediment may play a key role as an indirect metal uptake route for organisms. Total metal concentrations of Co, Cu, Fe, Pb and Cr in sediment showed a landward increase at all sites. These metals showed a significant correlation ($p < 0.05$) with sediment grain size as well as sediment carbon content, which also showed a landward increase.

4.3. *Metal speciation in sediments*

Sequential extraction can be regarded as a procedure in which the natural sediment is transferred to a well-defined artificial environment and the shift in equilibria is registered. Therefore, sequential extraction can be used as an effective tool to reduce the complexity of the natural system and to gain a complete understanding of trace metal speciation and mobility in sediments. The exchangeable/mobile fraction is the most important because this portion is a candidate to resupply elements to the water body. Although the mobile fraction in this study correlated well with organic carbon and sediment grain size, its concentration was lowest compared with the F_2 and F_3 fractions. Both acetic acid-extracted (F_1) and EDTA-extracted (F_2) metals showed a similar trend at all sites. Only Cd, Co and Zn were extracted in $F_1 > 10\%$ in relation to their total content. Metals in F_2 were extracted up to 72% (Cd), 24% (Co), 47% (Cu), 83% (Pb), 16% (Fe) and 42% (Zn) for samples from site 5. Organic matter, as stated earlier, is a scavenger of metals and metals, in turn, preferentially associate with organic matter. Most metals were retained in the residual, the per cent extraction of which was 23–71 (Cd), 73–91 (Co), 88–98 (Cr), 52–98 (Cu), 16–82 (Pb), 83–95 (Fe) and 50–98 (Zn). The increase in metal concentrations in F_1, F_2, and F_3 was reflected by the increase in total metal concentrations in the sediment. Bhupendra [27] reported a similar trend in his study of metal mobility in sediments from Nepal.

The lower concentration of some metals extracted from the sediment into F_1 and F_2 may indicate lower exposure doses for marine organisms because the bioavailability of such metals to organisms is limited under normal conditions. Unless a special condition prevails in such marine sediments, the contribution from sediment alone may not be significant for the metals accumulated in the tissues of biota. Increased solubility of these metals, for example, may result in an increased leaching rate and exchangeable or biologically available pools of metals in the sediment. The solubility of Pb, for example, starts to increase at lower pH values (4 or lower), as reported by Bhupendra [27]. Although the amount of Pb extracted by EDTA was appreciable, the pH of the extracting solution (7) may still have had an effect. In general, the lower the pH of the sediment in the environment the higher the mobility (bioavailability) of Pb; the toxicity may be higher also. The availability of the nonessential element Cd and the essential element Zn (F_2 fraction) was better, probably because of the higher pH of the extracting solution, which was close to the pH at which Cd and Zn solubilises (6.5 for Cd and 6.0 for Zn).

A simple sequential extraction procedure, as presented in this study, has elucidated the speciation of heavy metals in the sedimentary phase and their relationship with organic matter content and sediment grain size. As mentioned earlier, the role of sediment in metal speciation in the marine environment is not insignificant. Animals such as filter feeders are secondarily or indirectly exposed to metals present in sediment and therefore for them, it is not a direct uptake route. In dynamic situations, the sediment compartment may interact with the organism's main metal uptake routes, namely, dissolved and particulate phases, and be exposed thereto.

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