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ORIGINAL PAPER

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# Evalution of sequential extractions on dry and wet sediments

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**Abstract** A five-step sequential extraction procedure was applied on dried and wet Ballastplaat Scheldt estuary sediments. When wet (fresh) sediments were used, all sample handling up to the 3rd extraction step, inclusive, was carried out under inert atmosphere. The repeatability of the procedure was very good on dry samples. For Fe as for Mn, RSD values are lower than 4%, except for Mn in the fifth extraction step where a spread of 10% is observed. The observed RSDs for Pb are of the same order of magnitude as those for Mn. On wet samples the spread of the results is higher than on dried ones. The highest RSDs observed for Fe amount to 20%, for Mn to 15% but for Pb an RSD of up to 44% was found. Better homogenization of the solid sediment part of lyophilized sediments and different porosities of wet sediment sub-samples may be the explanation. These results also indicated that drying/oxidizing of the sediment sample causes a shift from less available/mobile metal fractions to more available/mobile fractions. The Mn and Fe oxyhydroxide spikes added to a wet sediment sample were recovered between 100±10%. The results obtained after changing the sequence of the extraction steps (multiple rotations and inversions were tested) corroborated the progressive increase in the aggressive nature of the extraction solutions in our standard scheme. Although there is also no need to change the ratio volume of extractant to amount of sediment, increasing the number of extraction repetitions in steps 1 to 3 resulted, for some of those extraction steps, in a partially modified analyte distribution. Finally the method was applied to sandy and muddy sediment cores of the Scheldt estuary and revealed clear differences between metal distributions in both types of sediment.

**Keywords** Sequential extractions · Dry sediments · Wet sediments · Metal analysis

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## Introduction

The mobility and bioavailability of trace metals in soils and bottom sediments strongly depend on their specific chemical and mineralogical forms and their binding characteristics. This implies that these forms and characteristics should be studied rather than their total concentrations. Two different approaches for metal speciation in soils and sediments were developed:

1. speciation of chemical compounds using hyphenated techniques; and
2. assessment of the metal distribution over various sedimentary phases using leaching and extraction techniques.

The first type of speciation mainly focuses on the separation and quantification of organometallic compounds such as methylmercury [1, 2], tributyltin [3], arsenobetaine [4], and their inorganic homologues in all kinds of environmental matrices (water, biota, suspended and bottom sediments, soils, etc.). The second type of speciation deals only with soils and sediments. Since the early 1980s, single and sequential extraction schemes have been designed for the speciation of metals in this kind of substrate [5, 6]. The speciation in this case aims at understanding the distribution of a metal over the various sedimentary substrates such as carbonates, iron and manganese oxyhydroxides, organic matter, sulfides, silicates, etc. Under particular conditions some of these substrates will dissolve (for example the oxyhydroxides under reducing conditions and the carbonates under acidic conditions) or release adsorbed metals (for example when the electrolytic strength of the solution is increased). It is thus possible, by carefully selecting the composition of the extraction solutions, also called extractants, to destroy selectively specific soil or sediment substrates such as, for example, reduced or oxidized forms. However, the metals extracted with a selective extraction scheme should only be related to the extractant used, e.g. EDTA-extractable element, and not as, e.g., “bioavailable”, “mobile”, etc., forms which are interpretations of data rather than results of actual measure-

ments. Nevertheless, information obtained from sequential extraction procedures can be very interesting, although a prerequisite is that the analytical procedures are well-defined and accepted. BCR has developed a 3-step sequential extraction procedure and certified a sediment reference material (CRM 601) following that extraction procedure [7, 8]. It is clear that for intercomparison reasons stable, which means in this case dried, samples have been used. However, drying of a sample involves a number of drawbacks when studying the natural metal distribution in a soil or sediment, especially when one tries to make the link to mobility and bioavailability.

This article describes a rather classic sequential extraction procedure applied to non-treated fresh sediment samples under conditions which do not disturb the natural trace metal distribution. The repeatability of the method and the recovery of spikes have been assessed, but also the effect of drying/oxidizing the sample and changing the sequence of the extraction steps. Finally the method has been applied on sandy and muddy Scheldt bottom sediments.

## Sequential extraction procedures

To get a better insight in the speciation of metals in bottom and suspended sediments, different sequential extraction schemes were developed during the '70s and '80s [5, 9, 10, 11]. The idea behind the development of sequential extraction methods was that the successive application of selective reagents (single or mixtures), could stepwise liberate the metals associated with a specific bottom fraction, like carbonates, oxyhydroxides, sulfides, organic material or present in a very labile (exchangeable-adsorbed) or non-labile form (crystalline). Most of the time, extraction liquids that are appropriate to dissolve specific mineral phases (e.g. acids for carbonates) are selected. Table 1 gives an overview of such extraction liquids.

**Table 1** Specificity of extraction liquids

| Attacked sediment fraction | Ref. |
|---|---|
| Exchangeable and adsorbed fraction: | |
| $BaCl_2$–triethamine, pH 8.1 | [12] |
| $MgCl_2$ | [13] |
| Ammonium acetate, pH 7 | [14] |
| Carbonate fraction: | |
| $CO_2$ treatment | [15] |
| Acidic cation exchanger | [16] |
| NaOAc/HOAc–buffer, pH 5 | [5] |
| Reducible fraction: | |
| Hydroxylamine,0.01 mol $L^{-1}$ $HNO_3$ | [17] |
| Ammonium oxalate buffer | [18] |
| Ascorbic acid–oxalate buffer | [19] |
| Hydroxylamine–acetic acid | [20] |
| Citrate–dithionite buffer | [21] |
| Oxidisable fraction: | |
| $H_2O$–$NH_4OAc$, pH 2.5 | [14] |
| Organic solvents | [22] |
| 0.1 mol $L^{-1}$ $NaOH/H_2SO_4$ | [23] |

The protocol we used, distinguishes five specific extraction liquids:

1. 1 mol $L^{-1}$ sodium acetate (NaOAc) buffered at pH 5 with acetic acid
2. 1 mol $L^{-1}$ hydroxylamine ($NH_2OH.HCl$) buffered at pH 5 with sodium citrate
3. 0.01 mol $L^{-1}$ $HNO_3$
4. extraction with $H_2O_2$ at 80 °C followed by an addition of 1 mol $L^{-1}$ ammonium acetate ($NH_4OAc$).
5. aqua regia/hydrogen fluoride (8/2)

Although it is more rational to express the extracted fractions in relation to the extracting reagent, it is also worthwhile to examine which kind of sediment fraction can be brought into solution by the extraction liquids used in our protocol.

### Exchangeable and carbonate fraction

Some of the extraction protocols [5, 10] tried to distinguish between absorbed metals and metals associated with the carbonate fraction. At pH 7 $MgCl_2$ and $NH_4OAc$ attack carbonates, manganese oxyhydroxides, and sulfate compounds [9]. Ammonium salts can also attack the organic matrix [24]. Some other less frequently used solutions can bring structural cations of clay minerals into solution. Regarding all the problems resulting from a differentiation approach between exchangeable and carbonate associated metals, we decided to extract both fractions together.

A 1 mol $L^{-1}$ NaOAc-solution, buffered at pH 5 with acetic acid [12], was selected because of its extraction efficiency regarding adsorbed and carbonate associated metals and its minimal influence on other phases reported in the literature. According to Robbins et al. [9], this extractant liberated 99.9% of the total Ca amount associated with the carbonate fraction, even in carbonate-rich sediments.

Because the adsorption of trace metals on iron oxyhydroxides increases abruptly at a pH above 5 [25], this adsorbed fraction will be liberated. Not all metals adsorbed on manganese oxyhydroxides will be liberated, since the adsorption isotherm starts just below pH 5. However, in general, a pH of 5 is enough to bring the major part of the absorbed metal fraction into solution [5].

The solution is buffered to prevent acidification of the extractant due to the dissolution of carbonates. Extractants having lower pH values may attack other sediment fractions.

### Reducible fraction

This extraction step is specifically focused on the destruction of oxidized compounds (especially oxyhydroxides) in the sediments.

Most of those oxyhydroxides are only partially crystallized [9]. The proposed extraction liquid ($NH_2OH.HCl$ buffered at pH 5 with citrate) is generally considered to be

the most efficient extractant of oxyhydroxides. The solution is buffered at pH 5 to avoid deterioration of the aluminosilicate fraction. In addition, the chelating activity of sodium citrate limits the re-adsorption of liberated metals.

Although several researchers recommended the use of acidified hydroxylamine [17, 20], dissolution of silicates cannot be avoided [9]. Other reducing solutions (see Table 1) are $Na_2S_2O_4$–Na citrate–$NaHCO_3$ and ammonium oxalate but drawbacks are dissolution of silicates and precipitation. Recently [19] a distinction was made between metals bound by Fe (hydr)oxides of low crystallinity and metals bound by crystalline Fe (hydr)oxides by dissolution of the former substrate with ammonium oxalate buffer and the second one with ascorbic acid–oxalate buffer.

### Acid-soluble fraction

Cold acid extractions are in general applied to assess the total bioavailable amount of metals. The low pH of the extractant will liberate eventually re-adsorbed metals during previous steps and destroy all remaining carbonates. However, amorphous sulfides will also partially be destroyed [26].

### Oxidisable fraction

The treatment of the residue with warm (80 °C) hydrogen peroxide results in oxidation of organic material and sulfides and no distinction is made between metals associated with each of these fractions. Campanella et al. [27] developed a procedure to distinguish between the metal fraction bound to organic matter by using HCl and NaOH, which solubilize humic substances, from that bound in the sulfide form (soluble in $HNO_3$ solution).

It should be noticed that metals, which are liberated by a peroxide treatment, show the tendency to re-adsorb onto iron and manganese oxyhydroxides. To avoid this, all reducible oxides need to be removed before applying the peroxide treatment [6]. The extraction with ammonium acetate, performed after the peroxide treatment, aims at isolation of all metals liberated during the oxidation [5]. A possible alternative is the use of surfactants, but that protocol is not very suitable for routine analyses [6]. Organic solvents were also tested for the extraction of the organic phases [22].

### Residual fraction

The last extraction step corresponds to a total digestion method common for bulk analyses.

From this literature overview it can be concluded that two major problems do not allow the relating of specific sediment substrates to specific sequential extraction solutions:

1. incomplete destruction of the selected sediment substrate; and
2. partial destruction of unselected sediment substrates.

In fact, some sediment substrates are extracted during several extraction steps. In addition, few studies have dealt with repeatability, except the BCR studies [7, 8], the influence of oxidation and/or drying of the sediment sample, or other processes modifying the metal distribution in the soil or sediment. The applicability and the analytical limitations of extraction schemes were discussed by Quevauviller et al. [28].

Various investigators have shown that the sample treatment before and during the extraction manipulations can have a very strong influence on the results. Storage procedures (freezer, refrigerator, drying, ...) and/or exposure to atmospheric oxygen may significantly influence the initial, natural metal distribution in oxic [29, 30] and anoxic sediments [14, 24, 31].

Therefore, most extraction schemes do not satisfy the expected criteria of specificity, nor those of efficiency [9, 24, 32]). One must be aware that:

– the sequentially obtained fractions do not belong to specific mineralogical phases but result from operationally defined procedures [5];
– redistribution of the liberated metals on the residual material occurs [33];
– differences in mineralogical composition result in differences in extraction efficiencies [34]; and
– the sample preparation has a large influence on the speciation results [24, 29].

In this context it is clear that some precautions have to be taken when applying a sequential extraction method and when reporting the results:

– The anoxic or suboxic character of the Scheldt sediments necessitates carrying out the sample preparations (e.g. weighing of the sediments) and the first extraction steps under inert atmosphere. Taking into account the negative influence of most storage procedures, samples have preferable to be manipulated immediately after their collection and extractions have to be applied on wet material.
– Re-adsorption can be limited by keeping the ratio of the volume of extraction liquid to the volume of sediment as large as possible and by repeating the extractions with the same reagent several times.
– Keeping in mind that the resulting sequentially obtained fractions are operationally defined, it is necessary to relate the results to the used extraction liquids, instead of associating them with a well defined sediment structure.
– The specific characteristics of the analysed metals have to be taken into account when interpreting the results. In this context the reader can be referred to a study of Wallmann et al. [35] in which binding forms of a number of trace metals in sulfide-rich sediments were studied by using both thermodynamic equilibrium calculations and sequential extraction results. It was illustrated that in the extraction steps prior to the peroxide treatment, sulfides are already attacked. This is mainly due to the dissolution of amorphous iron and zinc sulfides. As a consequence of this process the sulfide ion ($S^{2-}$) is

present in the corresponding extraction liquids, which can lead to re-precipitation of trace metals which were liberated from other phases, in the form of metal sulfides.

The aim of our work was, in relation to the points just mentioned above, to better understand the effect of the operational parameters on the results. Therefore, we examined:

1. the reproducibility of the complete extraction scheme on dry and wet sediments;
2. the recovery of a spike;
3. the influence of reversing one or more steps of the extraction sequence; and
4. the extraction efficiency, by increasing the number of extractions in a given extraction step.

## Materials and methods

### Sampling

For each series of experiments, about 1 kg of surface sediment is sampled at station B at the Ballastplaat, an intertidal mudflat in the Scheldt estuary (Fig. 1). The plastic container is completely filled with the muddy sediment and then capped taking care that all air is pressed out. This sample was immediately transferred to the laboratory and deep-frozen (dried samples) or stored in a glove box under $N_2$ atmosphere (wet samples, see Ref. [36] for more details). In this glove box necessary instruments such as a centrifuge and a weighing balance, but also chemical products and laboratory material were present allowing all sample handling to be performed in an oxygen-free environment.

During the last decade, many analyses and experiments were carried out on Ballastplaat sediments [37]. The seasonal variability in trace metal content of the Ballastplaat sediments at station B is summarized in Table 2.

### Sequential extraction procedure

Generally wet sediment was used for the evaluation of the sequential extraction scheme although some tests were also performed on dried samples. In the oxygen-free glove box the sample is homogenized manually and subdivided in small amounts of ±2 g. A weighing balance is available inside the glove box. A few sub-samples were taken for the determination of the dry weight, showing an RSD of about 5%.

The first two extraction steps were performed in the glove box, immediately after homogenization was finished. It is necessary to work in an inert atmosphere in order to avoid oxidation, inducing changes in metal speciation of those suboxic and anoxic sediments. Thomson et al. [29] illustrated already the importance of the fast reaction of oxygen, while Kersten and Förstner [24] described the influence of manipulations under aerobic circumstances on the metal speciation in suboxic and anoxic sediments.

Because the authors mentioned above already showed that drying of the sediments has a negative influence on in-situ metal speciation, most extractions were performed on wet sediment.

The sequential extraction scheme we tested is a five step procedure based on literature schemes [5, 9, 40]. It is shown in Table 3,

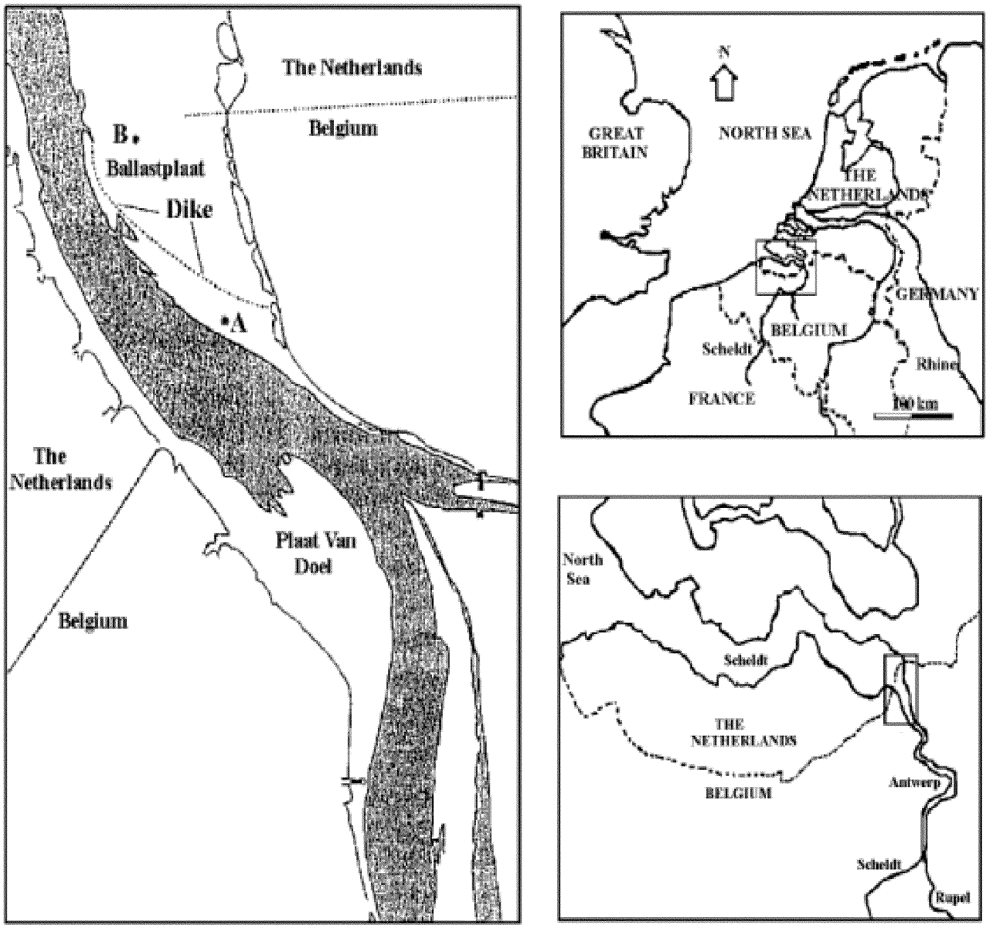



**Fig. 1** Sampling site Ballastplaat on the Scheldt estuary

**Table 2** Temporal variability of sediment composition at station B, Ballastplaat [38, 39]

| Period (month-year) | Al (mg $kg^{-1}$) | Fe (mg $kg^{-1}$) | Mn (mg $kg^{-1}$) | Cd (mg $kg^{-1}$) | Pb (mg $kg^{-1}$) | Ignition loss (%) |
|---|---|---|---|---|---|---|
| Jun-90 | 41.0 | 36.6 | 936 | 5.6 | 104 | 14.0 |
| Nov-90 | 42.3 | 34.1 | 814 | 5.7 | 92 | 9.2 |
| Apr-91 | 38.8 | 31.7 | 685 | 5.6 | 98 | 10.3 |
| Jul-91 | 39.9 | 34.7 | 970 | 5.2 | 98 | 10.5 |
| Dec-91 | 35.7 | 32.6 | 814 | 7.0 | 107 | 9.8 |
| Mar-92 | 37.0 | 28.2 | 683 | 3.9 | 88 | 8.9 |
| Jul-93 | | | 709 | 4.1 | 100 | |
| Sep-93 | | | 718 | 4.9 | 102 | |
| Nov-93 | | | 774 | 6.4 | 105 | |
| Jan-94 | | | 746 | 5.7 | 112 | |
| Mar-94 | | | 809 | 4.9 | 104 | |
| May-94 | | | 831 | 6.7 | 121 | |
| Average | 39.1 | 33.0 | 791 | 5.5 | 103 | 10.5 |
| STD | 2.5 | 2.9 | 92 | 0.9 | 8.7 | 1.8 |
| RSD (%) | 6.3 | 8.8 | 11.6 | 17.3 | 8.4 | 17.7 |

**Table 3** Sequential extraction schemes: our scheme and the modified BCR scheme

| | Our scheme (five steps) | Modified BCR scheme (three steps) |
|---|---|---|
| Step 1 | Solution A: acetic acid, 0.516 mol $L^{-1}$, buffered at pH 5 with 1 mol $L^{-1}$ Na acetate | Solution A: acetic acid, 0.11 mol $L^{-1}$ |
| | 2 g wet sediment+20 mL A (total 40 mL) | 1 g dry sediment+40 mL A |
| | 2×2 h shaking (total 4 h); centrifugation; washing of residue with 20 mL $H_2O$ and discard the water | 16 h shaking; centrifugation; washing of residue with 20 mL $H_2O$ and discard the water |
| | pH 5 | Initial pH 2.83 |
| Step 2 | Residue from step 1 | Residue from step 1 |
| | Solution B: Hydroxylammonium chloride, 1 mol $L^{-1}$, buffered at pH 5 with Na citrate | Solution B: Hydroxylammonium chloride, 0.5 mol $L^{-1}$,+$HNO_3$ to pH 2 |
| | Residue+20 mL B (total 40 mL) | Residue+40 mL B |
| | 2×2 h shaking (total 4 h); | Rest idem step 1 |
| | Rest idem step 1 | Initial pH 2 |
| | pH 5 | Steps 1 and 2 under ambient air |
| | Steps 1 and 2 under inert atmosphere | |
| Step 3 | Solution: $HNO_3$, pH 2 | |
| | Residue+3×33 mL $HNO_3$, pH 2 | |
| Step 4 | Solution C: hydrogen peroxide (30%), pH 2 ($HNO_3$) | Solution C: Hydrogen peroxide, 8.8 mol $L^{-1}$ (pH 2–3) |
| | Solution D: Ammonium acetate, 1.0 mol $L^{-1}$, in 6% $HNO_3$ | Solution D: Ammonium acetate, 1.0 mol $L^{-1}$ (pH 2) |
| | Residue+2×100 mL C: reduce volume slowly ~1 mL; +50 mL D, 16 h. | Residue+10 mL C (1 h, *T*=room temp; 1 h *T*=85 °C) |
| | Centrifugation and decantation (see Step 1) | Reduce volume <3 mL+10 mL C (1 h, *T*=85 °C) |
| | | Reduce volume <1 mL+50 mL D, shake 16 h |
| | | Centrifugation and decantation (see Step 1) |
| Step 5 | Residual fractions | Residual fractions |
| | Dry residue: 0.25 g+6 mL HCl+2 mL $HNO_3$+2 mL HF; 16 h at 60 °C | Residue digested in aqua regia according ISO standard 11466, reflux method |
| | To dryness and redissolution in 4% $HNO_3$ | |

All extraction solutions are acidified to pH 2 with $HNO_3$

together with the modified BCR sequential extraction (3-step) procedure [8].

### Analyses

#### *Reagents and labware*

All chemicals were of analytical-reagent grade purchased from Merck. Solutions were prepared in distilled, deionized water obtained with a Milli-Q apparatus (Millipore). Calibration standards for AAS were supplied by Aldrich (AA/ICP and ICP/DCP standard solutions).

All laboratory materials such as, for example, glass and PTFE bottles were cleaned by soaking in 20% nitric acid for at least 24 h and then rinsed thoroughly with distilled, deionized water before use.

#### *Analysis of total sediment (lyophilized sample)*

To 0.25 g sediment powder in a PTFE (Teflon) container, 2 mL $HNO_3$ (conc.) 6 mL HCl (conc.) and 2 mL HF (conc.) were added. The Teflon container was closed and heated at 60° C for 12 h. After cooling down, the solvent was evaporated and the dry residue re-dissolved in 25 mL $HNO_3$ (4% *v/v*).

Analyses of Al, Fe, and Mn were performed with FAAS (Varian 10 Q A.B) and deuterium background correction. Lead and Cd were measured with GFAAS (Perkin Elmer 3030) and Zeeman background correction. Appropriate dilutions were made with a 4% (*v/v*) $HNO_3$ solution.

Reference materials included in each run (10 samples) were either NBS 1646 or MESS-1. Agreement with the certified values was generally very satisfactory.

Separation of extraction solution and residual sediment substrate was performed via centrifugation (Labofuge Ae, Heraus) at 3800 rpm for 20 min.

#### *Analysis of extraction solutions*

After each extraction step, extraction solutions were acidified with $HNO_3$ to pH 2 and stored in polyethylene tubes prior to analyses. Calibration curves were established with standard solutions prepared in a similar matrix as the extraction solution. Signals of calibration standards and samples were corrected for blanks. Detection limits expressed as 3 times the standard deviation of the blank were respectively 0.05 mg $L^{-1}$ for Al, 0.01 mg $L^{-1}$ for Fe, 0.003 mg $L^{-1}$ for Mn, 0.006 µg $L^{-1}$ for Pb and 0.003 µg $L^{-1}$ for Cd.

Analyses of Al, Fe, Mn, Pb, and Cd were carried out as described above.

## Results and discussion

### Testing and optimizing the sequential extraction procedure

#### *Reproducibility*

The reproducibility of the procedure has been assessed on dried and fresh (wet) sediment samples. Both samples were collected at low tide at exactly the same location at the Ballastplaat (a marker indicates the location of station B) with a time delay of 3 days. Within these 3 days, the first sample was deep-frozen, lyophilized, and ground. In this way, the sequential extraction procedure on four sub-samples of the dried and wet sediment samples and the processing of the analyses afterwards occurred in parallel.

**Table 4** Sequential extraction of lyophilised Scheldt sediment (*n*=4). Repartition of Fe, Mn, Pb over the various extraction steps (mg $kg^{-1}$)

| | Fe | Mn | Pb |
|---|---|---|---|
| F1 | 730±10 (1.4%) | 261±7 (2.1%) | 36.9±0.3 (0.9%) |
| F2 | 2,800±100 (3.6%) | 29.1±0.9 (3.1%) | 13.5±0.2 (1.6%) |
| F3 | 2,590±90 (3.5%) | 26.2±0.4 (1.5%) | 1.54±0.04 (2.5%) |
| F4 | 5,650±60 (1.1%) | 28.9±0.2 (0.7%) | 6.4±0.5 (7.8%) |
| F5 | 10,800±200 (1.9%) | 60±6 (10%) | 9.7±0.9 (9.3%) |
| $\Sigma_1^5$ | 22,600±300 (1.3%) | 405±9 (2.2%) | 67.9±1.3 (1.9%) |
| Bulk analyses | 21,400±700 (3.2%) | 385±17 (4.4%) | 70±9 (12%) |

**Table 5** Sequential extraction of a wet Scheldt sediment (n=4). Repartition of Fe, Mn, Pb over the various extraction steps (in %)

| | Fe | Mn | Pb |
|---|---|---|---|
| F1 | 10.0±1.7 | 46.4±5.9 | 14.4±5.3 |
| F2 | 10.6±2.2 | 19.5±2.9 | 21.1±9.3 |
| F3 | 5.5±0.3 | 10.4±1.5 | 11.1±2.0 |
| F4 | 4.6±0.2 | 10.4±1.5 | 36.5±4.2 |
| F5 | 69±2 | 13.1±0.3 | 16.7±1.8 |

The resulting extraction liquids were analysed for Fe, Mn, and Pb.

Table 4 gives an overview of the average analytical results and the relative standard deviations (RSD) obtained on the dried sediment samples. For Fe, as for Mn, RSD values are lower than 4%, except for Mn in the fifth extraction step where a spread of 10% is observed. The observed RSDs for Pb are of the same order of magnitude as those for Mn. In general the reproducibility of the measurements appears acceptable and comparable for example with results obtained on the Yamasha river by Tessier and Campbell [41], although for some extraction steps they observed RSDs up to 23%. The sum of the concentrations obtained in each of the five extraction steps compares very well with the total concentration obtained from bulk analysis. Their ratios vary between 97 and 105%.

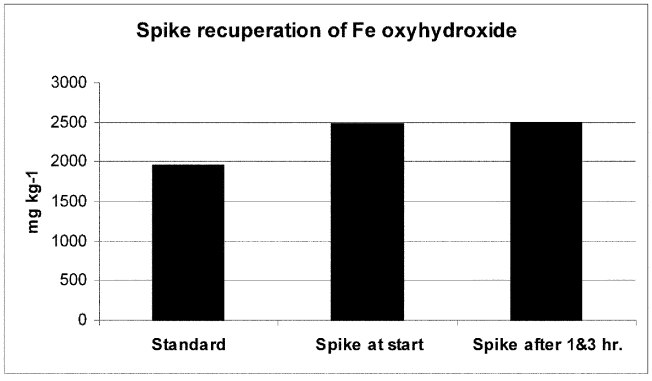
Table 5 gives a summary of the distribution of Fe, Mn, and Pb, including the RSDs obtained on the wet sediment samples. It can be noticed that the spread on the results is larger than for lyophilized (dried) samples. The RSDs vary between 2.9 and 20% for Fe, between 2.3 and 15% for Mn, and between 10.8 and 44% for Pb. These RSD values are, however, not surprising and are similar to literature data. As an illustration, results reported by Kersten and Förstner [24] for Elbe sediments showed RSDs up to 13.6% for Fe, 54% for Mn and 58% for Pb. The application of a complex protocol such as a sequential extraction technique on wet sediment samples unavoidably induces a decline in reproducibility of the analyses compared to those performed on lyophilized and homogenized samples. The manual homogenization of wet sediment is less efficient than the mechanical homogenization of a dried sample while the porosity of wet sediment samples (% of water in wet sediment) can also vary slightly; these effects induce

more variability between sub-samples of wet sediment than between those of lyophilized sediment sub-samples.

The dried and wet Scheldt sediment samples used to test the reproducibility of the extraction method were both collected at the same station but at an interval of 3 days. The question may thus be raised to what extent both samples are identical. During 2 previous studies in 1990–1992 and 1993–1994, respectively, station B was sampled seasonally [38, 39] and several heavy metals were determined. As can be observed from Table 2, those sediments were fairly rich in organic matter and heavy metals, and their composition between June 1990 and March 1992 ($N$=6) or May 1994 ($N$=12) was relatively constant. The RSDs varied between 6.3 and 17.7%. Although this natural variability over a period of almost 2 to 4 years is relatively low, it was much lower over the 3-day period – the largest difference between metal concentrations (RSD) observed for the two samples was 3.9% for Cd. In fact, changes in the composition of the sediments are mainly induced by changes in water-column characteristics. The average residence time of a watermass in the Scheldt estuary, having a length of 90 km, amounts to 3 months. This means that over short time periods the estuarine system can be considered as being in quasi steady-state. Therefore, we can assume that the initial samples used for testing the reproducibility of the sequential extractions are sufficiently comparable, so that at least major effects on the metal distribution in the samples due to drying and exposure to oxygen, can be inferred. Comparing Tables 4 and 5 shows that drying/oxidation of the samples causes a shift from less available to more available/mobile fractions. For Fe the residual fraction (step 5) decreases, favouring the oxidisable one (step 4), for Mn steps 2, 3 and 4 decrease while the carbonate and exchangeable one (step 1) increase, for Pb steps 3 and 4 decrease and the carbonate and exchangeable one (step 1) also increases. In 1999 Davidson et al. [31] reported changes in speciation occurring when a sediment is air-dried, oven-dried or frozen and Bordas and Bourg [30] suggested that the main agent of the modifications is contact with atmospheric oxygen, favouring the formation of Fe and Mn oxides. In addition, oxidizing conditions seem to favour metal mobilization in reduced sediments [42].

*Spike recovery*

Six sub-samples of 2 g wet sediment were submitted to the first extraction step. Then to test the reduction efficiency of hydroxylamine in the second extraction step, three parallel experiments were performed on these residues. In the first experiment, the standard procedure as described in Table 3 was applied. In the second experiment, 50 µg $g^{-1}$ manganese(IV) oxide ($MnO_2$) and 500 µg $g^{-1}$ iron(III) oxide ($Fe_2O_3$) were added to the residue, the tube was shaken and then the extraction started. In the third experiment the extraction was stopped after 1 h. Then 25 µg $g^{-1}$ $MnO_2$ and 250 µg $g^{-1}$ $Fe_2O_3$ were added to the residue and the extraction continued. During the repetition (extraction step 2 involves 2×2 h of extraction) the previous procedure was repeated.



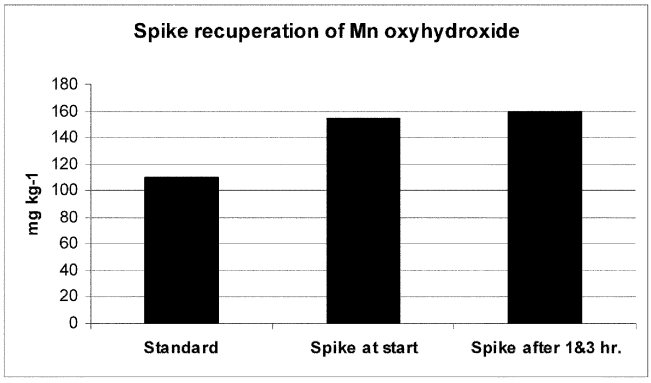




**Fig. 2** Recovery of Fe and Mn oxyhydroxide spikes

The results (Fig. 2) indicate that recovery of the spikes is 100±10%.

*Order of extraction*

Based on literature data and preliminary tests, a five-step extraction scheme was adopted. In order to test the specificity and efficiency of the various extraction steps, the sequence of the standard extraction scheme was modified. One or more of the steps were reversed except the last, which represents a classical total digestion.

Experiments were performed on wet Scheldt sediment, showing high total concentrations of Fe and Mn (see Tables 2 and 4), while their distributions over the various bottom fractions were significantly different.

Table 6 presents an overview of the various extraction sequences tested. The distributions of Fe and Mn over the various bottom fractions, expressed as a percentage of

**Table 6** Experimental variants of the standard sequential extraction sequence

| Standard procedure | 1-2-3-4-5 |
|---|---|
| Variant a | 2-1-3-4-5 |
| Variant b | 4-3-1-2-5 |
| Variant c | 2-4-3-1-5 |
| Variant d | 3-1-2-4-5 |

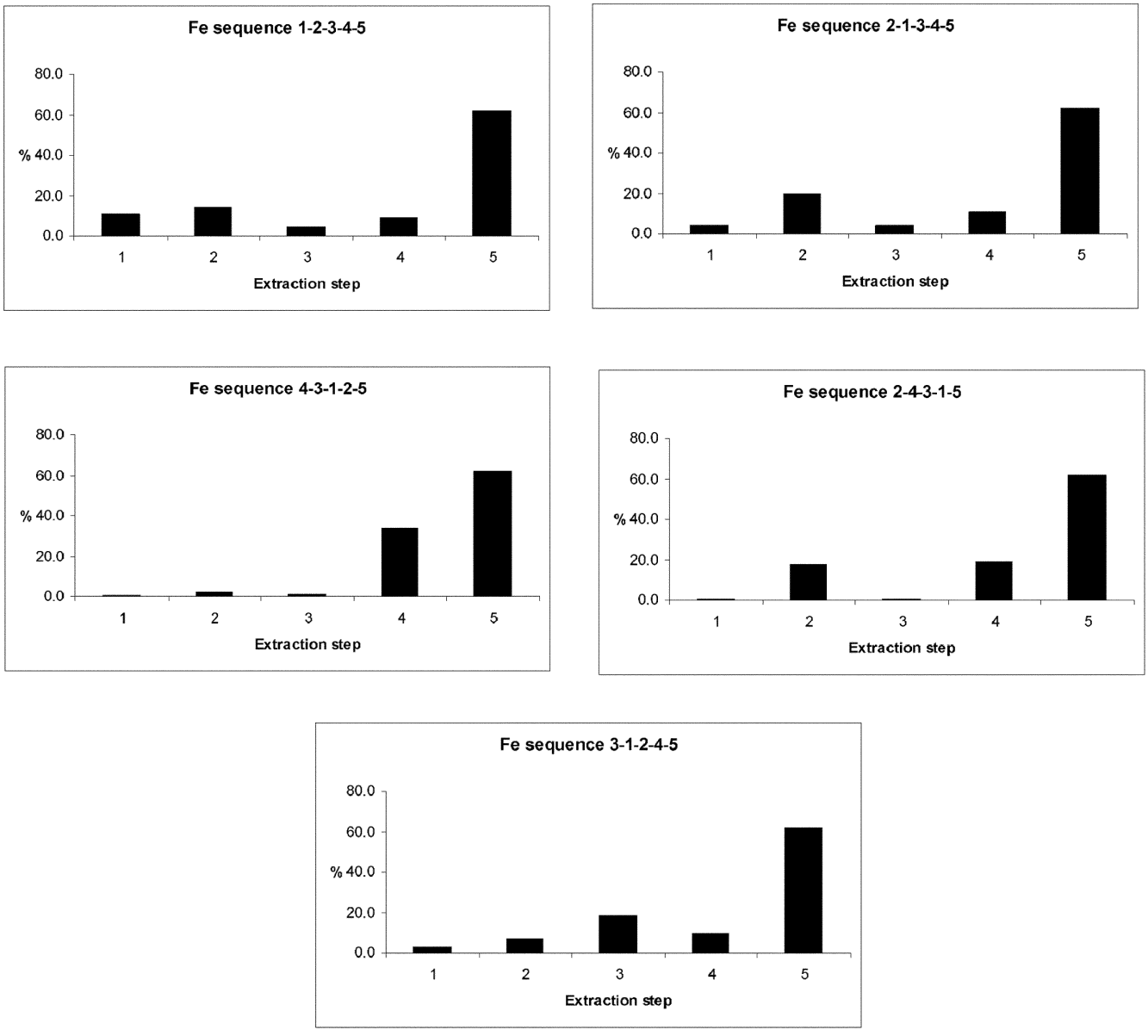



**Fig. 3** Results for Fe when modifying the sequence of the extraction steps

their total content (defined as the sum of the concentrations in the five extraction steps), are shown in Figs. 3 and 4.

Based on these results, following conclusions can be drawn.

- When the fourth step (acid/oxidation) is performed in first place, the Mn and Fe contents in the last two steps (4 and 5) amount to more than 95% of the total burden of those metals. This implies that steps 1 to 3 of the standard scheme should be performed before the acid-oxidation step. The extraction liquid ($H_2O_2$ at pH 2) apparently destroys all sediment fractions related to steps 1 to 3.
- When the fourth step is performed second, the next extraction steps (with exception of the last) do not produce any more extractable metals.

Since the first three extraction steps are of similar aggressiveness and by far less aggressive than the last two, it is much more difficult to find out if any of these initial steps liberates metals that do not belong to the intended fraction. Results show us, though, that each of these steps, independently from the order of performance, still keeps a minimum of specificity. By changing the sequence, the first applied step will always increase in importance, but not to the extent that the next steps are eliminated. In experiments 1, 2, and 5 steps 1 to 3 have been rotated. For Fe, step 1 varies from 2.7 to 10.6%, step 2 from 6.8 to 19.5% and step 3 from 3.8 to 18.8%. For Mn, step 1 varies from 13.5 to 54.2%, step 2 from 8.0 to 57.7% and step 3 from 6.4 to 47.5%. The second extraction step seems to have a larger impact than the other two, though it is possible that this can be attributed to the nature of the studied sediment. The reducible Fe and Mn compounds (associated with the second extraction step) are apparently only

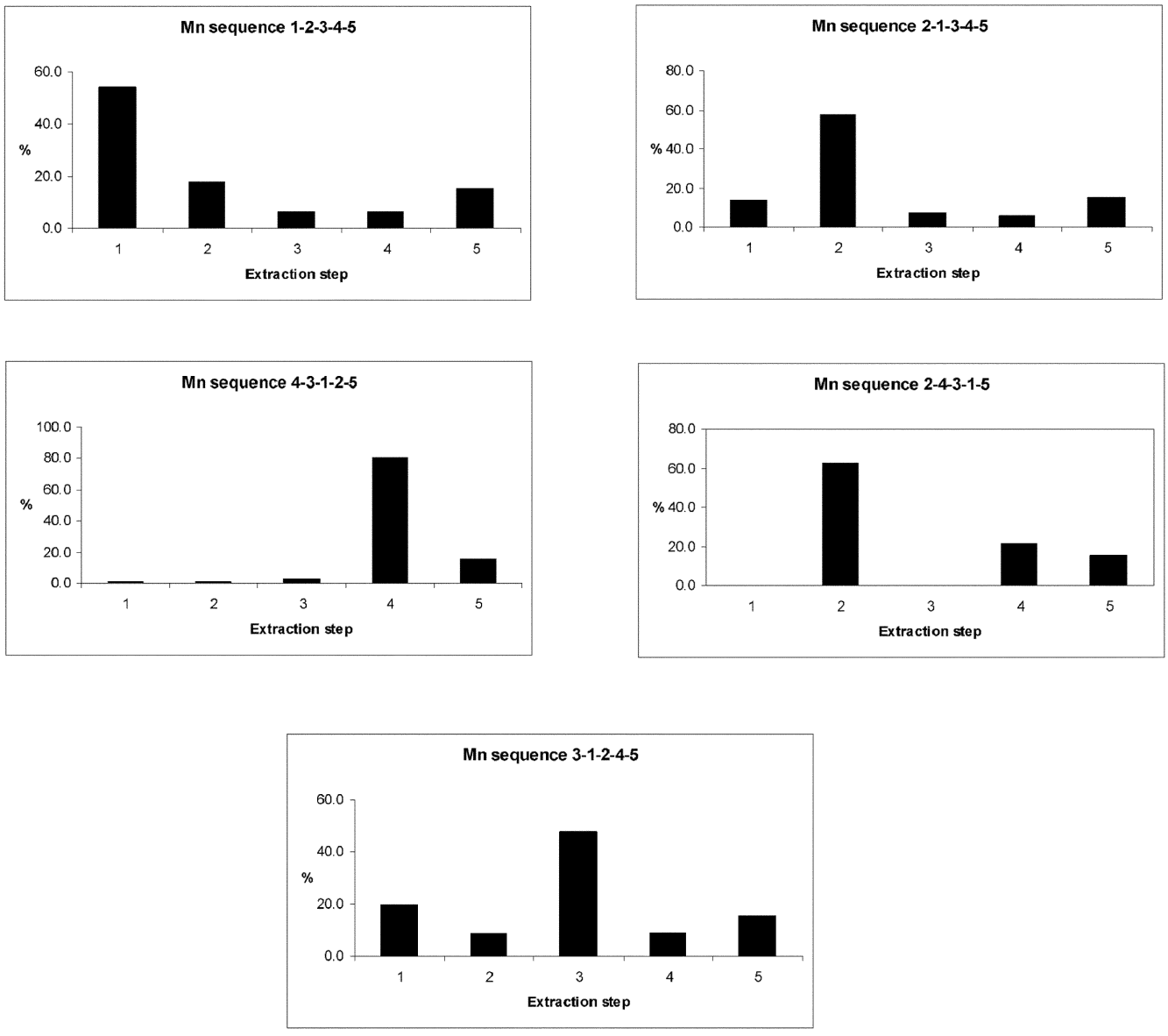



**Fig. 4** Results for Mn when modifying the sequence of the extraction steps

weakly affected by extraction steps one and three. The fourth extraction step has been three times in its normal position, but with changing sequences of the previous steps. Under these special circumstances, averages and STDs of the 4th step yield 9.8±0.9% for Fe and 7.1±1.6% for Mn.

Taking all this information into account and knowing that:

– the first extraction step is carried out at almost neutral pH and without oxidant/reductant, the second step at the same pH but with a reductant and the third step at low pH, but without oxidant/reductant, and
– redox differences in sediments more frequently appear than significant pH variations,

the proposed extraction scheme sequence seems scientifically sound (electrolytic solution at pH 5, then adding a reductant, then lowering the pH).

*Extraction volumes and repetitions*

Halving the extraction volume has only a small influence on the extraction efficiency. For both Fe and Mn, halving the extraction volume while keeping all other parameters constant, has only a small effect on the results of steps 1 to 3. Table 7 shows us those results for step 3, performing steps 1 and 2 according to the standard procedure.

In order to evaluate the efficiency of the extraction frequencies related to a given extraction step, the number of extraction repetitions was increased from two to seven (steps one and two), respectively from three to seven (step three). This can also provide information about re-adsorption of analyte on matrix compounds, which is, according

**Table 7** Influence of extractant volume on the extraction efficiency for step 3. Ratio of step 3:sum of steps 1 and 2

| Element | Procedure | Ratio of step 3 to sum of steps 1 and 2 |
|---|---|---|
| Fe | Standard (3×33 mL) | 26.8% |
| | Variant (3×16.5 mL) | 23.3% |
| Mn | Standard (3×33 mL) | 9.2% |
| | Variant (3×16.5 mL) | 6.9% |

to Calmano et al. [43], the most important risk of artefact formation.

Results for Fe and Mn are presented in Table 8. Because steps 1 to 3 deal with the more mobile fractions of the sediment and steps 4 and 5 with the less mobile, we presented the results each time as 100% for fractions 1 to 3 and for fractions 3 to 5, to better appreciate shifts in the analyte distribution. It appears that for the standard procedure and for the exhaustive extractions in steps 1 and 2, the first two extraction steps together amounted to 80% (step 3 to 20%) for Fe and to more than 90% (step 3 to maximum 8%) for Mn. Exhaustive extraction of step 1 or step 2 had thus almost no effect on step 3. However, increase of metal content in the first step, due to the increase of the extraction frequency, results in a proportional concentration decrease in the second extraction step.

Increase of the extraction frequency in step 3 has for both metals an influence on extraction step 4 or 5. While the third step increases with about 10% for Mn, the fourth step (oxidisable compounds including organic material) showed the reverse behaviour. For Fe a good agreement existed between the increase in step 3 and the decrease in step 5.

These results show that increasing the number of extraction repetitions in steps 1 to 3, resulted for some of those extraction steps in a partially modified analyte distribution.

## Application of the extraction method on Scheldt sediments

The method has been applied on two sediment cores (a sandy and a muddy core) sampled on the Ballastplaat in the Scheldt estuary (Fig. 1). Four depth sections (0–1 cm, 3–5 cm, 7.5–10 cm, and 12–15 cm) of wet sediment were analysed according to the standard procedure. Depth-averaged values and standard deviations for Al, Fe, Mn, Cd, and Pb were calculated because differences between the sandy

**Table 8** Results of exhaustive extractions (seven extraction repetitions) applied to steps 1 to 3 (see text for more explanation)

| | Standard procedure $\Sigma_1^3$=100% | Standard procedure $\Sigma_3^5$=100% | Step 1 – Extra repetitions $\Sigma_1^3$=100% | Step 2 – Extra repetitions $\Sigma_1^3$=100% | Step 3 – Extra repetitions $\Sigma_3^5$=100% |
|---|---|---|---|---|---|
| Fe | | | | | |
| F1 | 41.3% | | 52.3% | 36.7% | |
| F2 | 37.5% | | 26.9% | 41.2% | |
| F3 | 21.3% | 5.7% | 20.8% | 21.9% | 22.3% |
| F4 | | 10.1% | | | 14.6% |
| F5 | | 84.2% | | | 63.1% |
| Mn | | | | | |
| F1 | 69.3% | | 92.9% | 73.5% | |
| F2 | 22.5% | | 3.4% | 20.0% | |
| F3 | 8.1% | 33.6% | 3.7% | 6.5% | 42.8% |
| F4 | | 23.9% | | | 9.3% |
| F5 | | 42.5% | | | 47.9% |

**Table 9** Sequential extraction results (%) on a sandy and a muddy sediment core of the Scheldt estuary

| Extraction step | Al | Fe | Mn | Cd | Pb |
|---|---|---|---|---|---|
| Sandy station | | | | | |
| 1 | 0.5±0.1 | 5.0±2.4 | 30.3±3.6 | 13.4±8.3 | 13.4±3.3 |
| 2 | 0.6±0.1 | 5.1±2.9 | 11.9±9.2 | 18.8±6.7 | 11.9±5.0 |
| 3 | 0.5±0.2 | 6.5±0.9 | 7.4±1.7 | 10.1±2.9 | 0.9±0.5 |
| 4 | 2.0±0.2 | 2.7±0.4 | 2.4±0.5 | 54±12 | 13.7±3.3 |
| 5 | 96.4±0.2 | 80.8±3.3 | 48±14 | 4.0±2.1 | 60.2±2.2 |
| Muddy station | | | | | |
| 1 | 0.4±0.1 | 9.8±3.2 | 43.8±1.1 | 2.7±3.5 | 5.9±4.5 |
| 2 | 0.5±0.1 | 6.4±1.7 | 12.1±2.7 | 3.2±0.8 | 13.6±1.4 |
| 3 | 1.0±0.2 | 8.7±0.9 | 13.6±6.2 | 11.0±3.4 | 1.1±0.3 |
| 4 | 1.1±0.1 | 2.2±0.3 | 3.0±0.6 | 81.2±7.9 | 18.5±2.8 |
| 5 | 97.0±0.3 | 72.2±2.1 | 27.5±5.1 | 2.0±0.4 | 61.0±0.5 |

and the muddy core are much larger than depth variations (Table 9). Al belongs almost entirely to the last extraction step, when the crystalline silicate structures are destroyed. Fe is also mainly present in the last fraction with a slightly higher percentage in the sandy core. 70% of Mn is associated with fractions 1 to 3 (the more mobile ones) in the muddy core while in the sandy core about half of the Mn is in the refractory, last fraction. Mn carbonates are, as a result of the high carbonate concentrations in the Scheldt estuary, also well represented in the bottom sediments (Ca concentrations in the sediments at station B, Ballastplaat, were around 40 mg $g^{-1}$ [38]). Cd is strongly associated with the oxidisable compounds, which include organic matter but also non-crystallised sulfides, especially in reduced environments, but in the sandy station still 40% is also present in the more mobile first 3 fractions. About 60% of Pb is non-mobile, the remaining burden spread equally over fractions 1, 2, and 4 in the sandy station, fractions 2 and 4 in the muddy station. As has been reported in literature [44], Fe and Pb behaviour shows many similarities.

## Conclusions

The information produced by a sequential extraction experiment on a soil or sediment sample is only useful and usable if the analytical protocol satisfies some general rules. BCR did a lot of work to certify a 3-step extraction procedure – in fact the improved BCR procedure [8] recommended a pseudo-total aqua regia leach in addition to the three extraction steps as an internal check – and the trace metal extractable contents in a sediment reference material (CRM 601) following that extraction method. However, they used dried sediment material and that does not reflect natural conditions.

Our aim was therefore to evaluate a sequential extraction protocol applied on fresh soil or sediment material, allowing semi-quantitative assessment of the natural trace metal distribution in such samples. A 5-step sequential extraction scheme was applied on sandy and muddy Scheldt sediments. The tests of reproducibility, spike recovery and specificity of the extraction solutions yielded satisfactory results with regard to the fixed aims, although the results of the exhaustive extraction experiments indicated that increasing the number of extraction repetitions led to an increase of the amount of analyte in some extraction steps. Therefore, the possibility of uncompleted liberation and/or partial re-adsorption of liberated analytes in some extraction steps should be further studied.

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