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ORIGINAL ARTICLE

An optimized sequential extraction scheme for molybdenum association in environmental samples

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Abstract By comparing three sequential extraction procedures, a new optimized extraction scheme for the molybdenum association in environmental samples was proposed. Five operational steps were described as exchangeable ($KH_2PO_4 + K_2HPO_4$: including water-soluble), associated with organic matter (NaOH), Fe–Mn oxides and/or carbonates (HCl), sulfides (H_2O_2) and residue ($HNO_3 + HF + H_2O_2$). An optimized extraction scheme was compared with Tessier's procedure and the Commission of European Communities Bureau of Reference (BCR) was applied to black shales. Results showed Tessier's procedure gave the lowest concentration values for exchangeable molybdenum and the highest values for the residual molybdenum, which could not present the efficiency of the extraction reagents. BCR's procedure showed the highest values in oxidizable molybdenum and presented four fractions of molybdenum, which did not demonstrate the fractions of molybdenum in the black shales in detail. The optimized extraction scheme demonstrated a certain improvement on extraction efficiency over Tessier's procedure for the lowest residual molybdenum, and revealed more featured fraction information of molybdenum in black shales than BCR's. Therefore, after a comparison with other two extraction procedures, the optimized extraction scheme proved suitable for the molybdenum in black shales and it also showed an accurate determination of the molybdenum in the fractions and source of bioavailable Mo.

Keywords Molybdenum · Sequential extraction procedure · Black shales

1 Introduction

Molybdenum is an essential element for humans and plants (Neunhäuserer et al. 2001). In the human body, a molybdenum deficiency causes Keshan disease and Kaschin-Beck disease; while over abundance can lead to gout syndrome (Shi and Liao 2001). In plants, molybdenum takes part in processes of nitration, reduction and biological nitrogen fixation (Bortels 1930). Meanwhile, molybdenum also has influence on some other plant metabolic activities. Plants' nutrition can be affected by interactions between molybdenum and other elements. For example, plants would stop growing due to a lack of nitrogen without molybdenum intake, which alters their metabolic processes (Arnon 1958). Therefore, investigating the speciation of molybdenum in rocks, soils and sediments have important implications on human and plant health. Study of molybdenum's different fractions in rocks, soils and sediments can provide information on the release, migration, fixation and enrichment of molybdenum in the environment.

Molybdenum (Mo) is a relatively special transitional element. The average content of molybdenum in the continental crust is approximately 1.2 mg/kg (Anbar and Rouxel 2007; Johnson et al. 2004), while molybdenum is

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the most abundant transitional element in the ocean, where its concentration reaches up to 10.3 ppm (Wang et al. 2011). Different concentrations are closely related to the geochemical behavior of molybdenum. Molybdenum generally exists as Mo^{+6}(mainly $MoO_4{}^{2-}$) or Mo^{+4} (MoS_2) in the surface environment (Anbar and Rouxel 2007; Johnson et al. 2004). $MoO_4{}^{2-}$ is the main species being transported from crust to river and is further delivered to the ocean during weathering processes, owing to its chemical inertness (Bullen and Eisenhauser 2006; Morford and Emerson 1999). It can be accumulated in iron and manganese oxides, and associated with organisms or organic/inorganic compounds (Wang et al. 2011). The major mechanisms for molybdenum precipitation in the ocean are the absorption of $MoO_4{}^{2-}$ to iron/manganese oxides or their formation as molybdenum sulfides (Anbar and Rouxel 2007; Johnson et al. 2004). Molybdenum in the ocean and sediments has been developed as a potential proxy for indicating redox conditions of a paleo-environment (Erickson and Helz 2000). Thus, in order to better understand the geochemical behavior of molybdenum, it is crucial to know the processes of molybdenum speciation in nature.

Chemical sequential extraction is an alternative approach to elements' fractions and speciation in order to know elements' existing state in the environment. The sequential extraction procedure (SEP) provides a useful tool for revealing the information of molybdenum's fractions in the environment. Tessier et al. (1979) first proposed SEP as a means for investigating the existing state of heavy metals in soils and sediments. This procedure included five fractions, which were exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to organic matter, and residual, respectively. Although Tessier's procedure was popularized in 1980s, many previous studies have pointed out that limited choices of reagents, redistribution during the extracting processes and lack of quality control all occurred in Tessier's procedure (Davidson et al. 1999). In 1989, according to Tessier's extraction procedure, the Commission of European Communities Bureau of Reference (BCR) proposed a three-stage sequential extraction procedure to be applied to sediments, which included exchangeable, water and acid soluble, reducible and oxidizable fractionations (Rauret et al. 1989). Meanwhile, a standard reference BCR 601 was developed for well controlling the determination and data (Quevauviller et al. 1993). However, both Tessier and BCR did not explain the extraction procedures and chemical reagents about molybdenum in detail. Davidson et al. (1994) applied BCR to sediment in freshwater, but molybdenum could not be determined as the higher detection limit for atomic absorption spectroscopy (AAS) in 1994. Žemberyová et al. (2010) applied a modified BCR to the certified reference materials of soil and sewage sludge, and then they used electrothermal atomic absorption spectroscopy (ET-AAS) to determine the concentrations of molybdenum in each fraction. They found the molybdenum in the soils was almost present in the mineral lattice. The content of molybdenum in the fractions of sludge was predominant in the fractions of molybdenum bound to organic matter and sulfides. In addition, they suggested that BCR was also applicable to environmental matrices. Aydin et al. (2012) also used a seven-step sequential extraction process (i.e., water-soluble, exchangeable, reducible, oxidizable, sulfides and residual) to investigate the speciation of molybdenum from asphaltite bottom ash.

In past work, sequential extraction procedures were mainly applied to sediments, soils and bottom ash. No SEP was proposed for black shales, especially black shales abundant in molybdenum and other heavy elements. Black shales are a variety of shales that contains the abundant organic matter, pyrite. Black shales occur in thin beds in many areas at various depths. Black shales contain relatively high organic carbon content and sulfides because they were deposited under anaerobic conditions. The weathering processes of black shales were always related to acid production, acid neutralization with carbonate rocks, organic matter degradation and the development or formation of clay minerals (Kulp and Pratt 2004). During the weathering processes, organic carbon is released and heavy metals are dissolved, which are important sources of environmental contamination. Change of Eh–pH, degradation and formation of organic matter, and the development of secondary mineral in the weathering process influence the transportation of molybdenum (Anbar and Rouxel 2007; Fox and Doner 2003; Kulp and Pratt 2004). For a better understanding of the behavior of molybdenum in black shales, based on Tessier and BCR's extraction procedures, we improved and proposed a sequential extraction procedure especially for black shales. This new procedure includes five steps: exchangeable (including water-soluble), bound to organic matter, bound to Fe–Mn oxidizes and carbonates, bound to sulfides and residual. Compared with Tessier's and BCR's procedures, our scheme can extract more effectively than Tessier's and can also reveal more precise sample fraction information about molybdenum than BCR. By applying the sequential extraction procedure to molybdenum in black shales, we present detailed distribution information of each fraction in selected samples. By comparing the molybdenum content in the fractions of samples, we can obtain information about the mobility of molybdenum, which is a useful clue to a better understanding the transformation of molybdenum from one fraction to another in a sedimentary environment.

2 Experimental

2.1 Sample preparation

All selected samples are black shales with a relatively high molybdenum concentration, which were collected from the weathering profiles of the Se-rich shales of the Permian Maokou Formation in Yutangba and Shadi, China (Zhu et al. 2014). All rock samples were trench-cut, generally 1 kg in weight and sealed in polyester plastic bags within cotton cloth bags for transport. The wet samples were freeze-dried (Instrument model: FD-3-85-MP) as soon as they were taken back to the laboratory. Dried samples were ground to pass through a 200 mesh sieve using an agate mortar and pestle.

2.2 Apparatus

Molybdenum concentrations were determined with inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC-e from PerkinElmer Company). A HY-4 speed control reciprocating oscillator and tabletop centrifuge (Thermo Scientific Legend Mech 1.6R type) were used for the experiment. A W201B numerical control thermostat water bath was used for water bathing; a Vortex-2 vortex mixer for shaking and blending residues and extraction reagents; a numerical control ultrasonic cleaner (KQ-100DE, Kunshan, China) was used in our improved extraction procedure. The freeze-drying apparatus (EYELA FDU-1100, Tokyo Rikakikai Co. LTD, Japan) was for the samples' preparation. A 50 mL custom glass conical centrifuge tube was used for the extraction procedures and a 30 mL high-pressure airtight digestion bomb for samples' digestion.

2.3 Reagents

The molybdenum standard stock solution (1000 μg mL^{-1}) was obtained from the Alfa Aesar Company in China, and kept at 4 °C. KH_2PO_4, $K_2HPO_4·3H_2O$, NaOH, NH_4Ac, CH_3COOH, CH_3COONa, $NH_2OH·HCl$, $MgCl_2$ and H_2O_2 (30 %), all guaranteed reagents, were obtained from the Chemreagent company of Tianjin, China. The trace level HCl, HF and HNO_3 were bought from Fisher Scientific Company. The black shale standard SGR-1b was obtained from U.S. Geological Survey, which was collected from the Mahogany zone of the Green River Formation.

2.4 Sequential extraction procedures

The sequential extraction procedures of Tessier's, BCR's and our own in this study are listed in Table 1. The optimized five-step extraction scheme for molybdenum in black shales is described in detail as follows:

Table 1 Fractions and reagents of three extraction procedures

Extraction Step	Tessier		BCR		Our scheme	
	Fractions	Reagents	Fractions	Reagents	Fractions	Reagents
F1	Exchangeable	1.0 M $MgCl_2$ (pH 7.0)	Exchangeable, water and acid soluble	0.11 M CH_3COOH	Exchangeable, water-soluble	0.1 M $KH_2PO_4 + K_2HPO_4$, pH 7.0
F2	Bound to carbonates	1.0 M CH_3COONa adjusted to pH 5 with CH_3COOH	Reducible	0.5 M $NH_2OH·HCl$ at pH 1.5	Bound to organic matter	0.1 M NaOH
F3	Bound to iron and manganese oxides	0.04 M $NH_2OH·HCl$ in 25 % CH_3COOH (96 °C)	Oxidizable	H_2O_2 (85 °C) then 1.0 M CH_3COONH_4	Bound to Fe–Mn oxides, carbonates	1 M HCl
F4	Bound to organic matter	HNO_3/H_2O_2 (85 °C), 3.2 M CH_3COONH_4 in 20 % HNO_3	Residual	Aqua regia	Bound to sulfides	H_2O_2 (85 °C) then 1.0 M CH_3COONH_4
F5	Residual	$HClO_4$ and HF			Residual	HNO_3, HF and H_2O_2

Step 1 Exchangeable: A 10 mL volume of 0.1 mol L^{-1} $KH_2PO_4 + K_2HPO_4$ buffered solution (pH 7.0) was added to 0.5 g of sample in a glass conical centrifuge tube, and mixed by vortex mixer. The tube was shaken for 2 h at room temperature in a reciprocating oscillator at 160 motions min^{-1}. The extract was separated from the solid residue by centrifugation at 3000 rpm for 20 min and decanted into a polyethylene Corning tube (50 mL). The residue was then washed with 10 mL MQ water and the above procedures were repeated as described. A 20 mL extract was blended and stored in a refrigerator at 4 °C for analysis.

Step 2 Bound to organic matter: To the residue from step 1, 10 mL of 0.1 mol L^{-1} NaOH was added and mixed by vortex mixer. Then the tube was shaken for 30 min at room temperature. The tubes were bathed in ultrasonic cleaner for 2 h and shaken occasionally (temperature controlled under 40 °C). Centrifugation was performed at 3000 rpm for 20 min after cooling. Extract was decanted into a polyethylene Corning tube. The residue was washed with 10 mL MQ water, shaken for 2 h and centrifuged 20 min at 3000 rpm. The 20 mL extract was blended and stored in a refrigerator at 4 °C for analysis.

Step 3 Bound Fe–Mn oxidizes and carbonates: to the residue from step 2 was added to 10 mL of 1 mol L^{-1} HCl, still mixed by the vortex mixer and shaken for 30 min at room temperature. The rest of this step was repeated exactly the same as Step 2.

Step 4 Bound to sulfides: 10 mL 30 % of H_2O_2 (pH 2–3, adjusted by HNO_3) was added to the residue of step 3, then mixed by vortex mixer. The tube was water bathed in an ultrasonic cleaner for 1 h and shaken occasionally (temperature controlled under 40 °C) with the tube cap loosened. Then we removed the cap and placed the tube in a water bath kettle at 85 °C water for 1 h (until the solution was less than about 3 mL), shaken occasionally. The tubes were taken out for cooling, then added with 10 mL 30 % of H_2O_2 (pH 2). Previous description was repeated. After cooled, we added 1 mol L^{-1} CH_3COONH_4 (pH 2, adjusted by HNO_3) to 20 mL, shaken for 2 h, and centrifuged at 3000 rpm for 20 min. We then decanted the extract and stored it in a refrigerator at 4 °C for analysis.

Step 5 Residual: the residue was heated in an oven at 110 °C for 3–5 h, grinded after it was dried and weighed for analysis.

2.5 Determination of molybdenum

The total rocks, extracting solution and residue after extraction were digested using a mixed acid solution (HF, HNO_3) in high-pressure digestion vessels. Typical masses of powder were 50–100 mg; these were weighed into 30 ml PTFE bomb liner vessels. The volumes of solution were taken by approximate estimation from the total molybdenum; these were also added into vessels but dried first. To each sample, 0.5 mL concentrated HF (38 %) and 0.5 mL HNO_3 (15.6 mol L^{-1}) was added; the solution was allowed to decompose carbonate minerals at room temperature for about 2 h. Then, 2.2–2.7 mL of HNO_3 was added. The bombs were sealed and placed in a pre-heated oven for 18 h at 180 ± 5 °C. After cooling, 1–2 mL of 30 % H_2O_2 was added, liner covered on a hot plate at 90 °C for about 1 h. Then caps were removed and the digestion solution was heated at 100 °C to nearly dry. Then we added 0.5 mL HNO_3 twice to remove the fluorine and evaporated it to near dryness. 1 mL Rhodium solution was added as an internal standard, 2 ml HNO_3 and 2 mL MQ water were added at the same time. Then the bombs were sealed and put back in the oven for 4–6 h at 135–145 °C. After cooling, 1 mL solution was transferred to tubes and MQ water was added to total volume of 10 mL. The molybdenum content of the samples was measured by using ICP-MS (ELAN DRC-e), with Rh as an internal standard, completed at the State Key Laboratory of Deposits, Institute of Geochemistry, Chinese Academy of Sciences. Duplicates and reagent blanks were treated exactly as the samples were. The measured molybdenum in standard USGS SGR-1b was 34.7 ± 0.24 % ($n = 3$), which is in agreement with the standard sample reference range (34.1–35.9). The relative percentage difference was less than 10 % for duplicate samples.

3 Results

In this experiment, three carbonaceous shale samples (CP0-1, SDI-5 and Si-2,) were selected for the sequential extracting procedures' comparison. Three different sequential extracting procedures were applied to those three samples (two repeated extraction procedures). Each scheme was required for a good agreement between the total molybdenum and the additional values of molybdenum in each fraction. Table 2 gives data on each fraction molybdenum and total molybdenum in the three sequential extraction procedures. In the table, the percentage recovery ranged from 91.5 % to 102.2 %, and the precision (RSD) in the extracts was below 10 %. The results of duplicate samples were presented in the consistence of molybdenum extraction. Higher recovery also supported the reliability of the experimental results.

From the data in Table 2, among all three procedures, fractions of molybdenum in the samples bound to organic matter and sulfides basically were higher than molybdenum in other fractions (except residual). In F4 of Tessier's procedure, the percentages of molybdenum were CP0-1 (33.8 %), SDI-5 (66.3 %), Si-2 (50.7 %); In F3 of BCR

Table 2 Mo concentration (mg/kg) [a] and percentage (%) [b] of each fraction obtained by three sequential extraction procedures and total content

Sample	Procedure	F1	F2	F3	F4	F5	Sum[c]	Total	Rec.[d]
CP0-1	TESSIER	4.68 (0.8)	30.7 (5.5)	56.8 (10.2)	187 (33.8)	275 (49.6)	554	606	91.5 %
	BCR	13.7 (2.5)	101 (18.2)	331 (59.6)	109 (19.6)		555	606	91.6 %
	Our's	61.5 (10.0)	169 (27.3)	61.8 (10.0)	242 (39.2)	85 (13.8)	618	606	102.2 %
SDI-5	TESSIER	19 (2.8)	40.3 (6.0)	106 (15.7)	449 (66.3)	62.2 (9.2)	677	664	102.0 %
	BCR	27.1 (4.3)	62.1 (9.9)	494 (78.7)	45.1 (7.2)		628	664	94.6 %
	Our's	400 (59.0)	189 (27.9)	7.3 (1.1)	66.5 (9.8)	14.2 (2.1)	679	664	102.2 %
Si-2	TESSIER	4.62 (4.4)	11.3 (10.8)	16.1 (15.3)	53.2 (50.7)	19.4 (18.5)	105	108	96.4 %
	BCR	5.55 (5.0)	28.9 (26.3)	61.2 (55.6)	14.5 (13.2)		110	108	101.7 %
	Our's	17.6 (16.9)	48.4 (46.6)	0.35 (0.3)	31.8 (30.6)	7.3 (7.0)	104	108	95.9 %

[a] *Mean values* of 2 replicate analysis (2 repeated extraction procedures), [b] *In brackets,* percentage = (Mo concentration of each fraction/summary) × 100 %, [c] *Summary of in each fraction* of Mo in samples obtained in each fraction (F1–F5), [d] *Recovery* = Sum/Total × 100 %

procedure, CP0-1 (59.6 %), SDI-5 (78.7 %), Si-2 (55.6 %); in the summary of F2 and F4 in optimized extraction scheme, CP0-1 (66.5 %), SDI-5 (37.7 %), Si-2 (77.2 %), reflecting the accordance of extracting results in three different schemes. Differences among the three procedures were also listed, and Tessier's procedure showed largest proportion of residual molybdenum (49.6 % in CP0-1, 9.2 % in SDI-5 and 18.5 % in Si-2) compared to the other two. There were four fractions in BCR; information from all four fractions was not as specific as in the other two procedures, and detailed speciation in each fraction needed to be analyzed further. The optimized extraction scheme gave five detailed fractions: residual molybdenum had a lower proportion (13.8 % in CP0-1, 2.1 % in SDI-5 and 7 % in Si-2) than the other two procedures (Tessier's procedure, 49.6 % in CP0-1, 9.2 % in SDI-5 and 18.5 % in Si-2; BCR's procedure, 19.6 % in CP0-1, 7.2 % in SDI-5 and 13.2 % in Si-2). The analysis of data showed that our procedure was more clear and effective relative to the other two for black shales.

4 Discussion

4.1 Comparisons between optimized extraction procedure and conventional schemes

As stated above, Tessier's procedure was primarily for soil and stream sediment, BCR's procedure was for sediments; the optimized extraction procedure was aimed at molybdenum in black shales. Three extraction procedures represented the content of molybdenum in different aiming fractions in different samples. The distribution of molybdenum in the resulting fractions varied due to the different choices of extraction reagents in the three procedures. As shown in Table 1, each fraction in each step of the optimized extraction scheme was not identical with Tessier, and BCR's procedures. Discussion is conducted mainly based on five steps of the optimized extraction scheme.

(a) Exchangeable molybdenum, including exchangeable and water-soluble molybdenum that can be extracted. Exchangeable molybdenum refers to molybdenum adsorbed on clay minerals and other ingredients such as iron hydroxide, manganese hydroxide and humus by relatively weak electrostatic interaction, which can be easily exchanged by anionic groups such as SO_4^{2-}, PO_4^{2-} and Cl^-, and is relatively easily used by organisms (Tessier et al. 1979). Water-soluble molybdenum is the free oxide ion group of molybdenum, which generally refers to molybdenum extracted by MQ water, and is also the most easily bioavailable molybdenum. Since the concentration of water-soluble molybdenum is often lower than the detection limit of the instrument, water-solubility and exchangeability are generally are calculated together, and named exchangeable molybdenum in optimized extraction scheme. Tessier's procedure uses neutral salt solution ($MgCl_2$) as the extraction reagent to extract water-soluble and exchangeable molybdenum. In Tessier's procedure, due to the strong exchange capacity of Mg^{2+} and weak complex force of Cl^-, some slight dissolution phenomena of carbonates (2 % to 3 %) can be observed (Rauret et al. 1989). When the composition of ions in solution changes, fractions in subsequent extraction steps will be affected and represent inaccurate results. In the data, the percentage of exchangeable molybdenum in the three samples extracted by Tessier's procedure is the lowest, only 0.8 % in CP0-1, 2.8 % in SDI-5 and 4.4 % in Si-2. These low extraction percentages brought questions about the effective extraction of Tessier's, because ineffective extraction may lead to uncorrected distribution of molybdenum in the rest fractions. BCR scheme uses acetic acid in the first step; the goal fractions are water-soluble, exchangeable and carbonate-bound (Rauret et al. 1989). In this step, the acidic solution can be neutralized with alkaline matter, also many carbonates (which molybdenum bound to), and can change the proportion of extraction fractions. In addition, due to the combination of the three associations in one step, it is inconvenient to discuss specific fractions individually. In our optimized extraction scheme, 0.1 mol L^{-1} $KH_2PO_4 + K_2HPO_4$ buffered solution (pH 7.0) was selected as an extraction reagent. Phosphate ions can efficiently exchange molybdenum oxyanions in the form of anions, and because pH is neutral, does not affect the molybdenum bound to weak acids (Zhu et al. 2007), which can avoid causing early release carbonate-bound and other acid soluble molybdenum. Of the first extraction step in all three procedures, our optimized extraction scheme results in the highest percentage of molybdenum (10 % in CP0-1, 59 % in SDI-5 and 16.9 % in Si-2), which shows that the reagent in this procedure could extract effectively.

(b) Molybdenum bound to organic matter refers to molybdenum in different forms to enter or be adsorbed on organic particles and complexed with organic matter. Mo^{+6} can be easily enriched in sediment abundant in organic matter such as black shales; dissolved Mo^{+6} can also be regenerated in the re-mineralization process of organic matter (Wang et al. 2011). Previous studies have demonstrated that molybdenum and organic matter (TOC) had a strong positive correlation in black shales (Wilde et al. 2004). Molybdenum bound to organic matter (F4) in Tessier's procedure and the oxidizable molybdenum (F3) in BCR's procedure both refer to molybdenum bound to organic matter and sulfides, which do not differentiate those two factions of molybdenum separately. For the molybdenum in black shales, the combination of those two fractions cannot display comprehensive information. In the optimized extraction scheme, we separate the molybdenum bound to organic matter and the molybdenum bound to sulfides, which is supportive to discussing the relationship between molybdenum and organic matter. The oxidative degradation of organic matter results in relatively low molecular weight of dissolved organic matter (DOC), fulvic acid, and humic acid and particulate organic matter such as humin and so on. In terms of the International Humic Substances Society recommended humic acid extraction scheme (International Humic Substances Society http://www.humicsubstances.org/), the choice is 0.1 mol L^{-1} NaOH. Studies have shown that this concentration of NaOH can effectively extract Selenium bound to the organic matter in sediment and black shales, but cannot destroy other mineral ingredients in the sample (Zhu et al. 2007). Since 0.1 mol L^{-1} NaOH can extract the Selenium bound to organic matter, both the molybdenum and Selenium bound to organic matter refers to different forms to be entered or adsorbed on organic particles and complexed with organic matter. This indicates that our selected reagent will be equally effective to molybdenum for the same study object. MoO_4^{2-} and SeO_3^{2-} are anionic groups, easy to combine with low molecular weight dissolved organic matter and fulvic acid or humic acid (Tribovillard et al. 2004). The percentages of molybdenum bound to organic matter extracted by the optimized extraction scheme in CP0-1 (27.3 %), SDI-5 (27.9 %) and Si-2 (46.6 %) were lower than the percentages of molybdenum bound to organic matter extracted by Tessier (33.8 % in CP0-1, 66.3 % in SDI-5 and 50.7 % in Si-2) and oxidizable molybdenum by BCR (59.6 % in CP0-1, 78.7 % in SDI-5 and 55.6 % in Si-2). These results explained that our optimized extraction scheme is basically in accordance with the other two conventional procedures, indicating that our extraction procedure is suitable for molybdenum bound to organic matter. However, our optimized extraction procedure can give more detailed information on the individual molybdenum bound to organic matter.

(c) Molybdenum bound to Fe–Mn oxides and carbonates. The molybdenum bound to Fe–Mn oxides refers to the molybdenum adsorbed to or precipitated with iron and manganese oxides in the form of element and molybdenum oxygen anion group (MoO_4^{2-}). The molybdenum bound to carbonates refers to the molybdenum sequestered carbonate combined with the non-crystalline form of FeOOH and molybdenum precipitated with carbonates (Filgueiras et al. 2002; Koschinsky and Halbach 1995; Kuhn et al. 2003). Both Tessier and BCR's procedures presently are bound to Fe–Mn oxides by using hydroxylamine hydrochloride, which can release adsorbed metal elements via reducing Fe(III) and Mn(IV) under anoxic conditions and their subsequent dissolution. Unlike previous researchers, molybdenum bound to Fe–Mn oxides and carbonates in this study was extracted in one step, because the test samples are black shales, which average content of manganese oxide is low (0.06 %) (Wang and Li 1996). The combined extraction of the two fractions could guarantee a certain amount of molybdenum, which may prevent the low concentration of single fraction from being determined. Previous research has shown that different concentrations of HCl can destroy the Fe–Mn oxide in soil and sediments to different degrees (Berner et al. 1979; Cary et al. 1967). We used 1 mol L^{-1} of HCl to extract the molybdenum bound to Fe–Mn oxides and carbonates, which can dissolve the Fe–Mn oxides and carbonate rock, then release the molybdenum. The content of molybdenum of this fraction in three samples extracted by the optimized extraction scheme was relatively low and the proportion in three samples both are less than 10 %. The percentage of molybdenum in Si-2 was only 0.3 %, and this result was consistent with the low concentration of Fe–Mn oxides and carbonates in black shales. The data proved that the optimized extraction scheme was suitable for black shales. In addition, in the operation process, we needed to pay attention to the production of CO_2 after HCl added, to avoid breaking caused by excessive gas pressure.

(d) Molybdenum bound to sulfides, mainly refers to molybdenum associated with sulfides or existing in sulfides. Reactions illustrated that molybdenum precipitation in diagenesis process of black shales are simplified as: $MoO_4^{2-} + 2SO_4^{2-} + 6H^+ = MoS_2 + 4.5O_2 + 3H_2O$ and $MoO_4^{2-} + 4SO_4^{2-} + 8H^+ = MoS_4^{2-} + 8O_2 + 4H_2O$ (Fox and Doner 2003), and the second reaction could produce Fe-Mo-S (Bostick et al. 2003; Vorlicek et al. 2004) or an organic-S-Mo combination (Orberger et al. 2007; Tribovillard et al. 2004) through the S^{2-} bridge. From the reaction, we can see that the molybdenum bound to sulfides is closely related to the pH and sulfur fugacity of the environment, and information of molybdenum bound to sulfides can reveal pH and sulfur information of paleo-environment. Previous studies generally extracted organic matter and sulfides together (F4 in Tessier and F3 in BCR). In this study, the optimized extraction scheme extracted the molybdenum bound to organic matter in the second step. We selected acidified H_2O_2 in this step as an extraction reagent (Žemberyová et al. 2010), which is same as F3 in BCR's procedure. Tessier and BCR both chose H_2O_2 and NH_4Ac as a reagent to extract the fraction bound to organic matter and oxidizable fraction. Acidified H_2O_2 offers oxidizing condition, which can leach metals associated with sulfides in the oxidation process (Filgueiras et al. 2002), but it cannot oxidize MoS_2 for the low temperature condition in experiment and diluted acid. We chose the parameters of reagents in BCR to extract the molybdenum bound to sulfides, because it was more effective to extract the molybdenum bound to sulfides after being compared with Tessier's. After oxidation, molybdenum is easily adsorbed on the solid phase, and then added to the acidified NH_4Ac prevents adsorption. In the optimized extraction scheme, we acidified H_2O_2 for the water bath and 1 mol L^{-1} NH_4Ac to extract the molybdenum bound to sulfides. Compared with results of F4 in Tessier, F3 in BCR and sum of F2 and F4 in optimized extraction scheme, Tessier was the lowest (33.8 % in CP0-1, 66.3 % in SDI-5 and 50.7 % in Si-2), indicating that its reagents are not effective as other two. Although both Tessier and BCR defined their factions bound to organic matter and sulfides, sum of F2 and F4 in our optimized extraction scheme was higher (66 % in CP0-1, 37.7 % in SDI-5 and 77.2 % in Si-2), proving that our scheme was more effective to extract the molybdenum bound to organic matter and sulfides, separately.

(e) Residual molybdenum, referring to the molybdenum occurring in lattices of quartz, clay minerals, or kerogen (Žemberyová et al. 2010). We assume that this fraction of molybdenum is difficult to be released in natural conditions, with minimal impact on the environment, or can barely be used by the plants. We use a $HNO_3 + HF + H_2O_2$ mixed digestion solution by high-pressure digestion bomb to extract the residual molybdenum. This scheme is relatively safer than that in Tessier, aqua regia in BCR, which also can effectively extract molybdenum at the same time. The residual molybdenum in Tessier took the biggest proportion among three schemes (49.6 % in CP0-1, 9.2 % in SDI-5 and 18.5 % in Si-2), reflecting its inadequate extraction in the previous steps. The percentages of residual molybdenum in BCR's procedure were 19.6 % in CP0-1, 7.2 % in SDI-5 and 13.2 % in Si-2. In the optimized extraction scheme, the residual molybdenum were 13.8 % in CP0-1, 2.1 % in SDI-5 and 7 % in Si-2, which were the lowest of the three extraction procedures. Two reasons could be supposed: one is that the molybdenum in the previous four fractions have been extracted effectively and successfully; the other is that the molybdenum in the lattices of quartz, clay minerals, or kerogen is inherently low.

4.2 Extraction efficiency of three extraction procedures

Results acquired by the three procedures show different content and percentages of molybdenum in the different aiming fractions. In Tessier's procedure, molybdenum in F1 in the three samples not only took the lowest proportion in five fractions, but also was lower than the molybdenum in the F1 extracted by the other two schemes. The percentage of molybdenum in F1 extracted was only 0.8 % in CP0-1, 2.8 % in SDI-5 and 4.4 % in Si-2. And the residual molybdenum had the largest percentage compared with the residual molybdenum extracted by the other two procedures, which had a certain influence on the quality control results of each fraction. In the BCR scheme, the percentage of molybdenum in F1 extracted was also lower (2.5 % in CP0-1, 4.3 % in SDI-5 and 5.0 % in Si-2), compared with the F1 in our optimized extraction scheme. The content of F1 (exchangeable, water and acid soluble molybdenum) in BCR was less than the sum of molybdenum in F1 and F2 (exchangeable molybdenum, including water soluble and molybdenum bound to carbonates) by Tessier's procedure, indicating that the first step of BCR's procedure was not effective, and the rest of the steps might be impacted after all. The sum of the molybdenum bound to organic matter and sulfides in our method, compared with the molybdenum bound to organic matter in Tessier and oxidizable molybdenum in BCR separately, our method extracted the most molybdenum, indicating 0.1 mol L^{-1} NaOH was effective to extract the molybdenum bound to organic matter. It is a better choice to extract the molybdenum bound to organic matter and sulfides separately, for the molybdenum in those two fractions takes a relatively large proportion. The content of residual molybdenum by the three schemes ranked Tessier (275 mg/kg in CP0-1, 62.2 mg/kg in SDI-5, 19.4 mg/kg in Si-2) > BCR (109 mg/kg in CP0-1, 45.1 mg/kg in SDI-5, 14.5 mg/kg in Si-2) > optimized extraction scheme (85 mg/kg in CP0-1, 14.2 mg/kg in SDI-5, 7.3 mg/kg in Si-2), it was clear that the optimized extraction scheme has the most effective extracting reagents. Tessier has the same five steps as the optimized extraction scheme, but the molybdenum could not be extracted effectively, as the optimized extraction scheme or the BCR. BCR has four steps to extract, but information of the four fractions provided is not detailed enough. Our five-step method can be more effective to extract the different fractions of molybdenum, and the proportion of residual molybdenum was the lowest, and was more efficient to extract the bioavailable molybdenum in the samples.

In addition, because Tessier and BCR primarily study soil and stream sediments, both run a longer period, and almost every step is required to precede overnight. The continuous duration of Tessier is 50 h and BCR is 60. Our method uses an ultrasonic cleaner, greatly reducing the time required for the whole experiment, and also improves the extraction efficiency of the extraction reagents. Under normal circumstances, the time required to complete the entire process is only 2 days, which is much shorter than traditional methods. Ultrasonic extraction is a simple and fast extraction tool, which can effectively elute organic, inorganic substances from the sediment, soil particles and biological samples into the solution phase (Ken and Lo 2002; Luque-Garcıa and De Castro 2003; Pérez-Cid and Boia 2001). Ultrasonic extraction, mainly through acoustic cavitations, increases the efficiency of substances dissolving, thus improving the chemical sequential extraction efficiency (Pumure et al. 2010). From the extraction efficiency of three programs, the residual proportion of our method is the minimum, showing the effectiveness of the extraction reagents with ultrasonic. The adaptability of our method is the best for molybdenum.

5 Conclusions

This study proposed an optimized sequential extraction scheme for molybdenum in black shales and applied it to carbonaceous rock samples. The optimized sequential extraction procedure includes the following five steps, which are exchangeable (including water-soluble) (F1, $KH_2PO_4 + K_2HPO_4$), bound to organic matter (F2, NaOH), bound to Fe–Mn oxides and carbonates (F3, HCl), bound to sulfides (F4, H_2O_2) and residue (F5, $HNO_3 + HF + H_2O_2$), respectively.

The optimized extraction procedure is sufficiently repeatable and reproducible for the molybdenum study in black shales. In comparison with Tessier's extraction procedure, it is simple and convenient in operation, and also provides more comprehensive information of Mo fractions than BCR's.

The data from the three extraction procedures showed that the optimized extraction scheme is more efficient for capably delivering a higher yield in the bioavailable Mo than Tessier's and BCR'S procedures. The data acquired by the optimized extraction scheme revealed the distribution of molybdenum in black shales, which is compatible with the features of the relationship between molybdenum and black shales. The optimized extraction scheme is suitable for the Mo study in black shales, and may provide a practical/promising procedure for other environmental samples, such as soil and sediment samples.

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