Role of an organic carbon-rich soil and Fe(III) reduction in reducing the toxicity and environmental mobility of chromium(VI) at a COPR disposal site

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HIGHLIGHTS

- A Fe(II)-containing organic rich soil is found beneath a COPR waste tip.
- Cr(VI) is removed from COPR leachate by reaction with Fe(II), producing $Cr(OH)_3$.
- The soil contains alkaliphilic fermentative and Fe(III)-reducing bacteria.
- The bacterial community maintains a redox barrier reducing the spread of Cr(VI).
- Organic matter addition to OM poor soils is a low cost, long-term treatment.

GRAPHICAL ABSTRACT
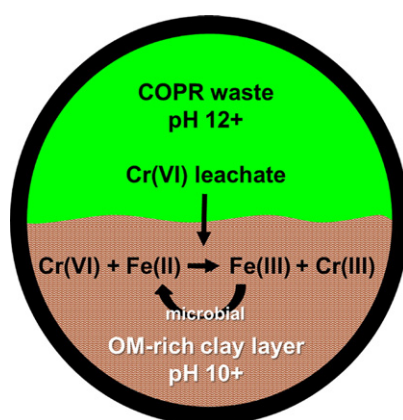
ABSTRACT

Cr(VI) is an important contaminant found at sites where chromium ore processing residue (COPR) is deposited. No low cost treatment exists for Cr(VI) leaching from such sites. This study investigated the mechanism of interaction of alkaline Cr(VI)-containing leachate with an Fe(II)-containing organic matter rich soil beneath the waste. The soil currently contains 0.8% Cr, shown to be present as $Cr(III)(OH)_3$ in EXAFS analysis. Lab tests confirmed that the reaction of Cr(VI) in site leachate with Fe(II) present in the soil was stoichiometrically correct for a reductive mechanism of Cr accumulation. However, the amount of Fe(II) present in the soil was insufficient to maintain long term Cr(VI) reduction at historic infiltration rates. The soil contains a population of bacteria dominated by a *Mangroviflexus*-like species, that is closely related to known fermentative bacteria, and a community capable of sustaining Fe(III) reduction in alkaline culture. It is therefore likely that in situ fermentative metabolism supported by organic matter in the soil produces more labile organic substrates (lactate was detected) that support microbial Fe(III) reduction. It is therefore suggested that addition of solid phase organic matter to soils adjacent to COPR may reduce the long term spread of Cr(VI) in the environment.

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1. Introduction

Chromium chemicals are produced for a range of industries including; leather tanning, chromium plating, pigments, refractories and magnetic media (Darrie, 2001). Chromium has two environmentally stable oxidation states; Cr(III) and Cr(VI). Cr(III) is an essential trace element for mammals (Vincent, 2000; Cefalu and Hu, 2004), which has low solubility at pH 4–12 (Fendorf, 1995), whereas Cr(VI) compounds are toxic and carcinogenic and easily mobilised in water as the chromate (CrO_4^{2-}) anion (James, 1994; Farmer et al., 2006; Tinjum et al., 2008). Hexavalent chromium is, therefore, an important environmental contaminant associated with a wide range of industrial processes and wastes, including the production of chromate chemicals from chromite ore ($FeCr_2O_4$) by the high-lime process. In this process, chromate is extracted from chromite by heating to 1150 °C with an alkali-carbonate to oxidise Cr(III) to Cr(VI), which is extracted with water upon cooling (Darrie, 2001; Burke, 1991) (lime (CaO) or limestone is added to the kiln to increase air penetration (Farmer et al., 2002)). The high-lime process is notoriously inefficient and produces large volumes (up to 4 tonnes per tonne of product) of caustic waste with pH > 12 (Deakin et al., 2001a; Higgins et al., 1998; Walawska and Kowalski, 2000). This waste, known as Chromium Ore Processing Residue (COPR), typically contains 2–7% chromium as a mixture of Cr(III) and Cr(VI) compounds (Geelhoed et al., 2002). COPR mineral phases typically include portlandite, calcite, ettringite, hydrogarnet and brownmillerite (Chrysochoou et al., 2010). When water comes into contact with high-lime COPR, portlandite and other soluble phases dissolve. As a result COPR pore water has a characteristically high pH of ~12, and contains up to 1.6 mmol·L^{-1} Cr(VI) as chromate (Farmer et al., 2002; Geelhoed et al., 2002; Fuller et al., 2013).

The high lime method was superseded by lime-free processing in more developed countries in the 1960s, but continued in China, Russia, India and Pakistan (40% of world production in 2001 (Darrie, 2001)). Prior to last few decades the hazards arising from COPR were not fully recognised, and as a result vast quantities of COPR have been used as a fill material for roads and other construction projects (Burke, 1991; Graham et al., 2006; Watts et al., 2015), or simply dumped in unlined tips (Stewart et al., 2010; Stewart et al., 2007; Breeze, 1973; Deakin et al., 2001b; Jeyasingh and Philip, 2005), in and around urban areas at, or near, the production facility (e.g. Glasgow and Bolton, UK; Baltimore, Maryland and Hudson County, NJ, USA; Cracow, Poland; Hubei and Hunan Provinces, China, and; several sites in India (Burke, 1991; Higgins et al., 1998; Walawska and Kowalski, 2000; Geelhoed et al., 2002; Breeze, 1973; Jeyasingh and Philip, 2005; Wang et al., 2013; Brose and James, 2013; Sreeram et al., 2003)). Unless the waste is properly capped, rainwater will readily infiltrate COPR, therefore, there are numerous sites around the world where COPR leachate is contaminating the surrounding area with Cr(VI).

Recently, at a legacy site in the North of England where COPR had been dumped, Cr(VI)-containing water was found to be leaching from the waste into adjacent soils (Whittleston et al., 2011a). However, chromium was found to be accumulating in the original topsoil layer directly beneath the waste exclusively in its reduced Cr(III) form associated with Fe(III) oxy-hydroxides in the soil. It was found that Cr immobilisation by reduction from Cr(VI) to Cr(III) was intrinsically linked to the microbially mediated generation of Fe(II), suggesting that the soil organic matter (SOM) was providing an electron donor for microbial metabolism including iron reduction.

SOM is a complex mixture of organic compounds (polysaccharides, lignin, protein, humics) that derive from plant and microbial biomass (Fierer et al., 2009). The in-situ biodegradation of SOM is controlled by the prevailing physical, chemical and biological conditions (Dungait et al., 2012), and SOM generally becomes more stable lower in the soil profile (Dungait et al., 2012; VandenBygaart et al., 2015). Consequently, the buried soil horizons at the COPR sites could be expected to be relatively stable. However the presence of an alkaline leachate promotes the process of alkaline hydrolysis, where cellulosic materials degrade under anoxic conditions to generation of a range of soluble low molecular weight products dominated by saccharinic acids (Humphreys et al., 2010; Knill and Kennedy, 2002). These degradation products are then potentially available to support a wide range of microbial processes, including iron reduction (Bassil et al., 2015; Rout et al., 2014; Rout et al., 2015).

There are currently no effective low cost treatments for COPR tips that offer long term environment protection. This paper reports a field investigation of COPR tip where initial investigations suggest a naturally occurring organic-rich soil beneath the waste has accumulated high concentrations of chromium. The aim of the work was to use chemical analysis, characterise of the soluble organic fraction, microbial community analysis and X-ray absorption spectroscopy, to better under the mechanism controlling Cr accumulation in alkaline affected soils. This new understanding is then used to provide an assessment of the potential for organic matter addition to enhance natural Cr attenuation processes occurring at COPR sites.

2. Material and methods

2.1. Field sampling

COPR waste, which was dumped into a gravel extraction site next to the former production site in the north of England (Fig. 1), now forms a domed hill covered with gravel and topsoil (the site was redeveloped as a secondary school and recreational area). Springs containing alkaline leachate occur at the edge of the waste which flow in all directions away from the site (only the spring that was sampled is shown in Fig. 1).

Three boreholes (BH1, BH2 and BH3; Fig. 1.) where advanced at the site in March 2013 using a Van Walt Cobra percussion drill fitted with a 75 mm diameter core sampler. Solid material recovered from the sampler was stored in 150 mL plastic containers. Selected containers (from below the level of water strike) were stored in 2 L glass jars containing anaerogen sachets to prevent sample oxidation. Approximately 1 g sub-samples of each horizon was transferred to 1.5 mL tubes and stored at −80 °C or −20 °C for synchrotron and microbial community analysis, respectively. Sub-samples from each depth were suspended (1:1 ratio w/w) in deionised water for pH measurement in the field (ASTM, D4972-01, 2006). Leachate was collected from a spring located at the edge of the COPR and stored in 1 L polypropylene bottles. The pH of soil suspensions and the leachate were determined using a Myron Ultimeter2 portable multimeter.

2.2. Sample characterisation

X-ray powder diffraction (XRD) analysis of selected solid samples (dried at 65 °C; ground to <75 μm) was performed on a Bruker D8 XRD using Cu K-alpha radiation. X-ray fluorescence (XRF) analysis was performed on powered samples on an Olympus Innovex X-5000 XRF. Total organic carbon content was determined using a LECO SC-144DR elemental analyser after pre-treated with 20% HCl to remove carbonates (Schumacher, 2002). Extractable Fe(II) and Fe(total) in solids were determined using field moist samples after extraction by 0.5 N HCl and reaction with Ferrozine (Lovley and Phillips, 1986). Sulphate concentrations were determined by ion chromatography using a Dionex DX500 and AS9 column.

2.3. Characterisation of soluble organic fraction

Acid soluble organic substances were determined in the BH2-180 cm sample after extraction with 2 M HCl (1 g soil in 5 mL of 2 M HCl for 3 days at 4 °C). The extractant was then separated by centrifugation at 8000 g for 10 min, pH neutralised by drop-wise addition of 2 M NaOH, evaporated to dryness; and finally the resulting solid dissolved in ultra-pure water at 1 g·L^{-1}. Total organic carbon in the extractant was determined using a Shimadzu total organic carbon analyser
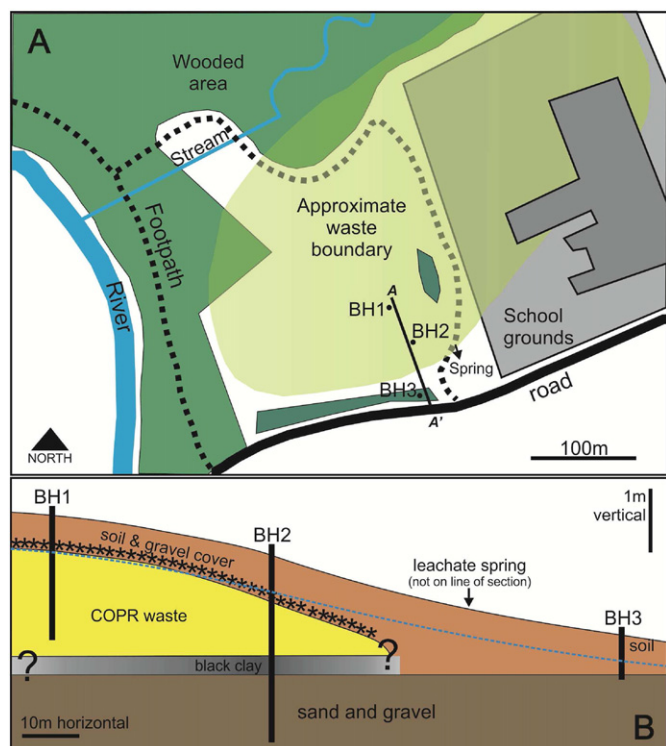
Fig. 1. A. Map of the sampling site showing the approximate boundary of COPR waste, and the location of boreholes and the sampled leachate spring. B. Approximate ground model along the line of section A–A′ showing the relationship of soil and waste horizons found in this part of the site. The blue dashed line joins the observed height of water strike in the boreholes. Question marks indicate areas of uncertainty over the position and structure of inferred boundaries.

5050A. Alkaline cellulose degradation products; α, β and xylo-isosaccharinic acids, α and β metasaccharinic acids, were determined by high performance anion exchange chromatography (Rout et al., 2014) that was also capable of detecting a wide range of sugars ranging from glycerol (C-3) to maltose (C-12). Glucose/lactate, ethanol and formic acid were measured by enzymatic methods using a Picotrace glucose/lactate sensor and QuantiChrom™ ethanol assay kit a commercial formic assay kit. Remaining volatile fatty acids were detected using gas chromatography (Rout et al., 2015) capable of determining C2–C8 volatile fatty acids.

2.4. Microcosm experiments

To investigate the interaction of COPR leachate with sediments found beneath the waste, microcosm experiments were established where 10 g sediment was incubated with 100 mL site leachate in 120 mL glass serum bottles. Three sediments recovered from 180 ± 10, 235 ± 20 and 295 ± 30 cm below ground level (bgl) from BH2 were used, providing a range of initial pH values between 8 and 10. Triplicate microcosms were purged with N_2 gas, sealed with butyl rubber stoppers and aluminium crimps, and stored at 21 °C in the dark. Samples (3 mL) of sediment/water suspension were periodically removed over a 30 day period using sterile syringes and needles. After sampling the solid and aqueous phase were separated by centrifugation (5mins; 16,000 g), and pH was measured using an Orion bench top metre and calibrated electrodes (pH calibrated at 7 and 10). Aqueous chromium (VI) concentrations were determined using a standard UV/VIS spectroscopy method based on the reaction with diphenycarbazide using a Jenway 6715 UV/Visible spectrophotometer (USEPA, 1992). Solid Fe phases were analysed using the Ferrozine method previously described (Lovley and Phillips, 1986) (the solids content of the suspension was

100 ± 1 g·L^{-1} and 1.5 mL suspension was used in each measurement, therefore Fe(II) concentrations (μmol·g^{-1}) were calculated on the basis that 0.15 g was used). To test for the presence of a viable Fe(III)-reducing bacteria, 0.1 g samples of the 180 ± 10 cm soil was added to triplicate 100 mL serum bottle containing a defined Fe(III)-citrate growth media at pH 9.2 (see (Fuller et al., 2014) for details of the media composition). After 14 days the media was scored for growth with formation of a black Fe(II) phosphate precipitate indicating a positive result.

2.5. X-ray absorption spectroscopy (XAS)

Cr K-edge XANES spectra were collected on beamlime I20, Diamond Light Source, UK, from 13 soil and COPR samples. EXAFS spectra were collected from soil sample BH2-180 cm. Spectra were compared to standard laboratory chemicals, natural chromite ore, and precipitated Cr-hydroxide. All spectra were summed and normalised using Athena v0.8 (Ravel & Newville, 2005). Background subtracted EXAFS spectra were prepared using PySpline v1.1 (Tenderholt et al., 2007), and were modelled using DLexcurv v1.0 (Tomic et al., 2005) (see Supplementary material for details).

2.6. DNA extraction and sequencing of the 16S rRNA gene

Microbial DNA was extracted from sample BH2-180 cm, and a 16S rRNA gene fragment (~1.5 kb) was amplified by polymerase chain reaction (PCR) using broad specificity primers. The PCR product was ligated into a standard cloning vector, and transformed into *Escherichia coli* competent cells to isolate plasmids containing the insert, which were sent for sequencing (see SI for details). The quality of each gene sequence was evaluated using UCHIME implemented within USEARCH 6.0.310 (Edgar et al., 2011), and non-chimeric sequences were grouped into operational taxonomic units (OTUs) using MOTHUR 1.30.2 (Schloss et al., 2009) and classified using the Ribosomal Database Project (RDP) naïve Bayesian Classifier (Wang et al., 2007). Sequences were submitted to the GenBank database (GenBank Numbers: *accession request #Hx2000047540 submitted 07-May-2015*). Phylogenetic trees were constructed using the MEGA5.2.2 (Tamura et al., 2011).

3. Results

3.1. Sample recovery and characterisation

The upper parts of BH1 consisted of a soil layer over a gravel capillary barrier. Waste material was encountered at 75 cm bgl. From 75 to 135 cm the waste primarily consisted of furnace clinker mixed with gravel and some pale white/green COPR. COPR was then recovered from 135 to 200 cm, where the borehole terminated. BH2 consisted of a similar soil and gravel cover to 90 cm bgl. COPR was recovered from 90 to 170 cm. immediately below the COPR was a black clay layer (170-190 cm), then interlayered brown sand and gravel to 320 cm, where the borehole terminated. BH3 consisted of a dark brown clay soil layer to 40 cm bgl, which then graded into brown sands and gravels to 75 cm, where the borehole terminated (see Fig. 1B).

All soil samples had essentially the same bulk mineralogy dominated by quartz, feldspars (albite, orthoclase) and clays (muscovite, kaolinite, chlorite). The COPR was dominated by portlandite and calcite with ettringite and quartz components. The leachate had a pH of 11.2 and contained 384 ± 17 μmol·L^{-1} Cr(VI) and 135 ± 4 μmol·L^{-1} sulphate.

A detailed geochemical characterisation was produced using solid samples recovered from BH2 (Fig. 2). Surface soil pH was 9.0, but increased to 9.7 with depth. The COPR horizon pH was 12.5 ± 1. The black clay horizon immediately below the COPR had a pH value of 10.4, below this layer the pH decreased to pH 7.9 in the deepest sample. In addition to high pH, the COPR horizon was characterised by high Ca (>20%) and Cr (>2%) content, low TOC (<0.5%). The black clay horizon also contained a high Cr content (~0.8%), was rich in organic matter

(TOC = 4.8%), and had an acid extractable Fe(II) content of 75 ± 14 $\mu mol \cdot g^{-1}$ (equivalent to 66% of total extractable Fe). Acid extractable organic carbon in the black clay was 2740 $mg \cdot kg^{-1}$ and lactate was detected at 0.48 $mg \cdot kg^{-1}$. None of the other organic substrates considered were detectable in the extract. Soils found below the black clay horizon contained <1% total organic carbon, <7 $\mu mol \cdot g^{-1}$ extractable Fe(II) (<45% of total extractable Fe) and Cr concentrations where <0.1% in all samples.

XANES analysis (Fig. 3) showed that between 50 and 70% of the Cr present in the COPR waste horizons was present in the Cr(VI) oxidation state (based on analysis of the pre-edge peak present at 5995 eV (Parsons et al., 2007); shown in Fig. 2). Most of the soil samples analysed, however, contained predominately Cr(III), although the sand from below the black clay occasionally contains up to 20% Cr(VI) as part of a moderate overall Cr concentrations. Similarly, in the soil cover up to 20% of the Cr was Cr(VI).

The EXAFS spectra collected from the black clay sample BH2-180 cm (Fig. 4) could be well-fitted with a model using three shells of 6 O backscatters @ 1.98 Å and two additional shells of Cr backscatters at 3.00 and 3.94 Å (Table 1), consistent with Cr K-edge EXAFS analysis of $Cr(III)(OH)_3$ presented by Fendorf et al. (1997).

3.2. Microcosm tests

The experimental pH in the BH2-180 cm black clay, −235 cm sand and −295 cm sand microcosms equilibrated to pH 10.2 ± 0.2, 9.2 ± 0.2 and 7.9 ± 0.4 respectively within 1 day then remained constant throughout the tests. The concentration of extractable Fe(II) in the black clay was initially 75 ± 14 $\mu mol \cdot g^{-1}$ and reduced to around 57 ± 12 $\mu mol \cdot g^{-1}$ at the first sampling point, but recovered to 70 ± 2 $\mu mol \cdot g^{-1}$ by 30 days. The two deeper aquifer sands initially contained <7 $\mu mol \cdot g^{-1}$ extractable Fe(II), which was consumed by 3 days

(Fig. 5). Aqueous Cr(VI) (from the site leachate) was removed from solution rapidly in the microcosms containing the black clay, with ~60% removed after 1 day, but very little Cr(VI) removal was observed during 30 days incubation in tests using either of the aquifer sand samples. Sulphate removal did not occur in any microcosm system, indicating that sulphate reduction did not occur in these tests.

3.3. Fe(III) citrate media tests

When the BH2-180 cm black clay samples were incubated in alkaline Fe(III)-citrate media at pH 9.2, the orange Fe(III) colour of the media was removed within two weeks and black precipitate formed (identified as the Fe(II)-phosphate mineral, vivianite, in similar previous tests (Fuller et al., 2014)) indicating a positive score for both microbial growth and Fe(III)-reduction.

3.4. DNA characterisation of microbial communities

59 rRNA gene sequences were obtained from BH2-180 cm. These were assigned to just 3 different bacterial phyla with a confidence threshold of 100% (see Fig. 6 and SI Table S1). The population was dominated Bacteriodetes sequences (70%), with Proteobacteria and Firmicutes sequences representing 21% and 9% of the population, respectively. MOTHUR analysis indicated that the sequences could be grouped into 12 OTUs based on >98% nearest neighbour similarity (97% identity is commonly used to group at "species-level" (Zaura et al., 2009; Fierer et al., 2008)). The most abundant OTU contained 68% of the sequences, each of which was classified as belonging to the genus *Mangroviflexus*, within the class *Bacteroidia*, with a >95% confidence value (an 80% confidence value results in 98.7% of sequences being correctly classified to genus level (Claesson et al., 2009)). A phylogenetic tree (Fig. 7) compares the characteristic member of the
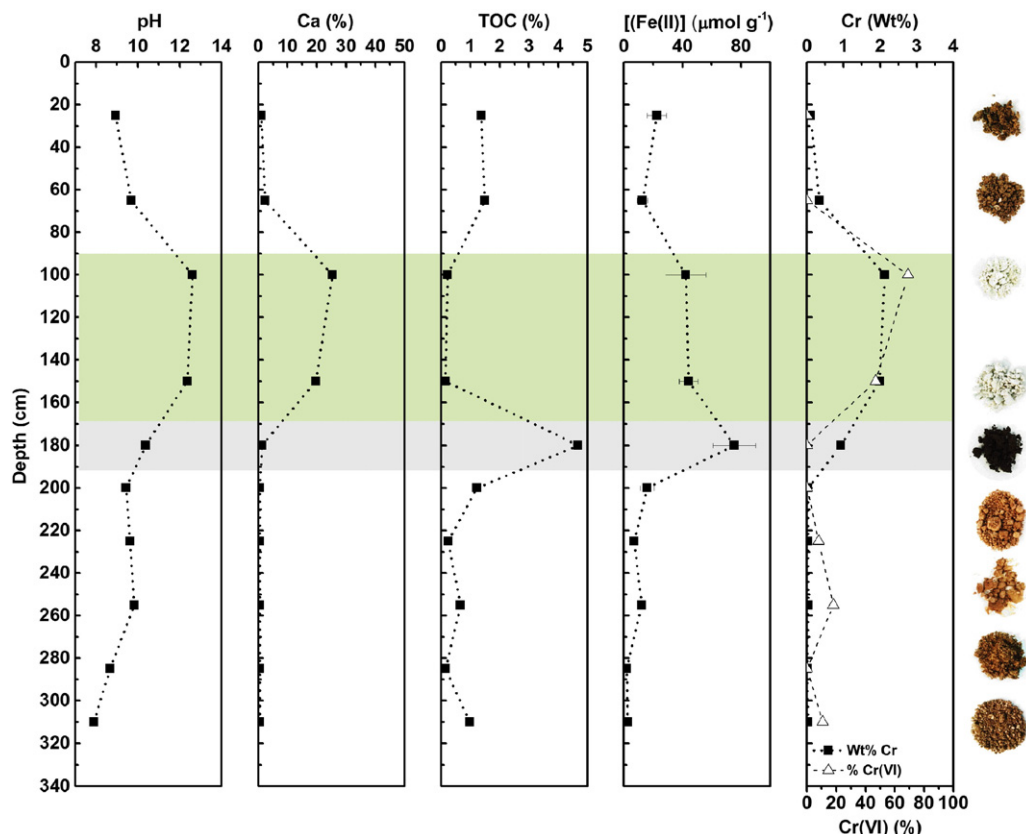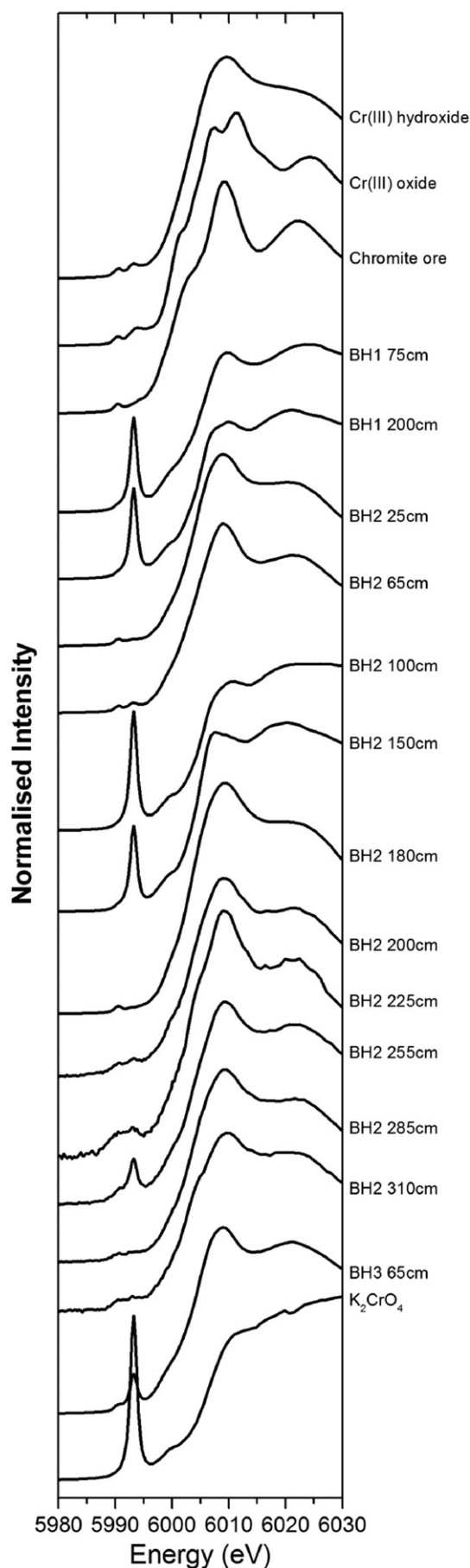

Fig. 2. Geochemical (and colour) variation in solid samples recovered from BH2. COPR waste and black organic-rich clay horizons marked by green and grey bands respectively.

Fig. 3. Cr K-edge XANES spectra collected from samples recovered from BH1-3 and several Cr(III) and Cr(VI) containing standards.

Mangroviflexus-like OTU with other members of the order *Bacteroidales*. The next most abundant OTU contained 10% of the sequences were classified as belonging to the genus *Pseudomonas* (confidence 100%), within the class γ-*proteobacteria*.

4. Discussion

4.1. The black clay horizon acts as an in situ reactive zone

The COPR present on this site contained around 2% (*w/w*) Cr, with 50–70% present in the toxic Cr(VI) oxidation state. Water entering the COPR (primarily as rainwater infiltration) buffers with portlandite to produce a high pH (pH 12+) leachate containing high concentrations of Cr(VI) due to slow dissolution of a range of sparingly soluble Cr(VI)-containing minerals (Chrysochoou et al., 2010; Deakin et al., 2001b; Hillier et al., 2003). The black clay horizon found directly beneath the COPR is acting (to a certain extent) as an aquitard (resulting in springs at the base of the waste), but it also has elevated pH and Cr concentrations due to long-term interaction with COPR leachate.

The accumulated Cr was present as Cr(III)-hydroxide. Although, dissimilatory Cr(VI)-reduction using organic matter as an electron donor is possible, it is quite a fragile process under alkaline conditions (Fuller et al., 2015). The black clay was also relatively rich in solid phase Fe(II), which in known to readily react with aqueous Cr(VI) (Stewart et al., 2007; Whittleston et al., 2011b), Therefore, the redox reaction of Fe(II) and Cr(VI) is proposed as the likely mechanism for Cr(VI) reduction (Eq. (1)).

$$3Fe(II)_{(s)} + Cr(VI)_{(aq)} + 6OH^- \rightarrow 3Fe(III)OH_{3(s)} + Cr(III)OH_{3(s)}. \qquad (1)$$

To test if this reaction mechanism was feasible, Cr(VI)-containing site water was reacted with sediments recovered from below the COPR. Cr(VI) was only removed in experiments using the black clay. Stoichiometrically, in order to remove all the Cr(VI) present in these experiments by reaction with soil associated Fe(II), ~12 μmol·g^{-1} Fe(II) are required. Dissolved oxygen present in the leachate (calculated at 10 mg·L^{-1} at 15 °C) could have consumed an additional ~6 μmol·g^{-1} Fe(II). The observed initial removal of Fe(II) in the black clay microcosms (~18 μmol·g^{-1}), was therefore, broadly consistent with the total amount of oxidants present (notwithstanding the relatively large analytical errors associated with the Fe(II) measurements). Microcosms tests using the aquifer sand materials contained <7 μmol·g^{-1} Fe(II), therefore this Fe(II) could be oxidised by the dissolved oxygen present alone. The aquifer sands also contained low organic carbon content, therefore, no Fe(III)-reduction was supported and consequentially no appreciable Cr(VI) removal occurred.

4.2. The source of Fe(II) and energy for microbial processes within the black clay

The total Cr concentration of black clay layer was ~0.8% (~150 μmol·g^{-1}). Uncontaminated soil Cr concentrations are <0.01% (Banks et al., 2006; Madrid et al., 2006; Lado et al., 2008), therefore, this excess Cr can be attributed almost completely to infiltration of Cr(VI) leached from the COPR and in situ reduction and storage in this soil. The waste was deposited in its present location in the 1970s (~40 years prior to sampling), therefore, ~4 μmol·g^{-1}·yr^{-1} of Cr have accumulated in the black clay, implying an Fe(II) utilisation rate of ~12 μmol·g^{-1}·yr^{-1}. An implication of this calculation is that the (0.5 N HCl extractable) Fe(III) currently present in the black clay (~40 μmol·g^{-1}), even if originally present as Fe(II), can only account for a few years of Cr(III) accumulation. Indeed, the Fe(II) currently remaining in the black clay will be
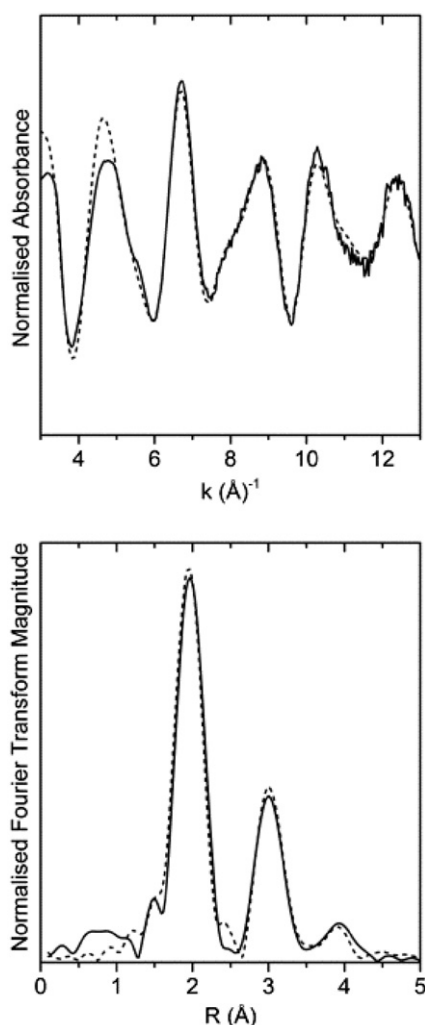
Fig. 4. Cr K-edge EXAFS spectra (top) and corresponding Fourier transformation (bottom) collected from the BH2-180 cm sample. Dotted line represents a Cr(OH)$_3$ model fit using the parameters listed in Table 1.

completely depleted in substantially less than 10 years, if future Cr(VI) infiltration continues at historical rates.

Therefore, it is very likely that the Fe(II) present in the black clay is replenished over time by on-going microbial Fe(III) reduction occurring in this soil despite the high pH. At pH 10.4, microbial Fe(III) reduction is energetically marginal, and predicted respiration and growth rates are slow (Williamson et al., 2013; Burke et al., 2012; Rizoulis et al., 2012). However, the rate of Fe(II) reduction required to maintain current concentrations is also relatively slow, and the black clay contains a viable population of alkaliphilic anaerobes capable of sustaining Fe(III) reduction at pH 9.2 in Fe(III)-citrate containing media. Therefore, it is conceivable that in situ Fe(III) reduction will maintain Fe(II) at favourable concentrations as long as sufficient labile organic matter is present to support dissimilatory Fe(III) reduction.

Table 1
Black Clay Cr K-edge EXAFS Fits, where N is the Occupancy, r is interatomic distance, $2\sigma^2$ is the Debye–Waller Factor and R is the Least Squares Residual goodness of fit parameter. Uncertainties in the last digit shown in parentheses.

Shell	N	r (Å)	$2\sigma^2$ (Å^2)	R (%)
O	6[a]	1.98(2)	0.007(2)	21.3
Cr	3[a]	3.00(5)	0.013(3)	
Cr	1[a]	3.94(5)	0.014(4)	

[a] Values fixed during fitting.

The black clay contained ~4.8% (4,000 µmol·g^{-1}) total organic carbon. According to equation 2 below (a generalised equation for dissimilatory Fe(III)-reduction using carbohydrate as an electron donor; (Lovley, 1997)), this amount of organic carbon might support the production of up to 16,000 µmol·g^{-1} Fe(II) over time.

$$CH_2O + 4Fe(III) + 2H_2O \rightarrow HCO_3^- + 4Fe(II) + 5\,H^+. \qquad (2)$$
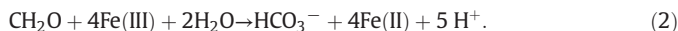
That amount of Fe(II) would treat Cr(VI) at historic infiltration rates for over 1000 years. However, this assumes that all of the organic matter present both is available for dissimilatory metabolism, and, that dissimilatory Fe(III) reduction is the dominant metabolism occurring in the soil — neither of which are realistic assumptions. Indeed, the lack of detectable soluble organic matter fractions (except lactate) suggests that most of the organics present are cellulosic and therefore sparingly soluble. Therefore, either the cellulosic materials are being degraded directly by microbes or that alkaline degradation products (expected to form at the pH present; (Knill & Kennedy, 2002)) are being rapidly utilised by microbes to ensure there is no residual to detect.

The microbial community in the black clay was dominated by a *Mangroviflexus*-like specie from the family Marinilabiliaceae, in the order Bacteroidales. It is interesting to note that type species from the family Marinilabiliaceae are obligately anaerobic, fermentative bacteria (Zhao & Chen, 2012; Zhao et al., 2012; Zhilina et al., 2004; Denger et al., 2002; Gao et al., 2013). Also, the most closely related type species, *Mangroviflexus xiamenensis*, *Alkalitalea saponilacus*, and *Alkaliflexus imshenetskii*, were all isolated from anaerobic cellulose-degrading environments (Zhao & Chen, 2012; Zhao et al., 2012; Zhilina et al., 2004). *A. saponilacus*, and *A. imshenetskii* are alkaliphiles that can degrade xylan (Zhao & Chen, 2012; Zhao et al., 2012), which is a group hemicelluloses that common in plant cell walls (Erlandson et al., 2001), whereas *M. xiamenensis*, is a halo-tolerant bacterium that can degrade mono and disaccharides derived from the breakdown of cellulose and hemicelluloses (Zhao et al., 2012). Therefore, the black clay layer contains a population of bacteria that could produce labile organic matter as a by-product of fermentation that could potentially support other metabolic processes such as dissimilatory iron reduction. Extractable lactate was present in the black clay at low concentrations. Lactate is a potent electron donor source for dissimilatory metal reduction (Lovley, 1995), so its presence in the soil was probably a result of on-going fermentative respiration, possibly coupled to on-going dissimilatory Fe(III)-reduction.

4.3. Implications for management of COPR contaminated sites

It is clear that the organic rich clay horizon, that fortuitously is present at this site, is acting as an in situ redox barrier, effectively preventing the downwards spread of Cr(VI) that has leached from the COPR. The continued existence of the barrier depends on the continual production of Fe(II) over time to maintain a supply of reductant in the soil to react with Cr(VI) in the infiltrating COPR leachate. The supply of Fe(II), in turn, depends on electron donor availability, both in terms of total organic carbon present, and the availability of the organic carbon source for bioreduction. In the black clay layer alkaline hydrolysis reactions are predicted at high pH, which, coupled to the actions of alkaliphilic fermentative anaerobes, could maintain a long term supply of labile organic matter to support Fe(III)-reduction. As the soil naturally contains relatively high concentrations of organic matter, it is expected that these beneficial circumstances will be maintained for many decades to come, possibly even until all the available Cr(VI) has been leached form the COPR.

However, the sandy soils found beneath, and adjacent to, the COPR contains relatively low amounts of organic carbon (< 1%), and also low concentrations of protective Fe(II). Although total Cr concentrations in these soils are relatively low, some of that Cr occurs as Cr(VI). Therefore, the natural barrier provided by the black clay horizon is probably not
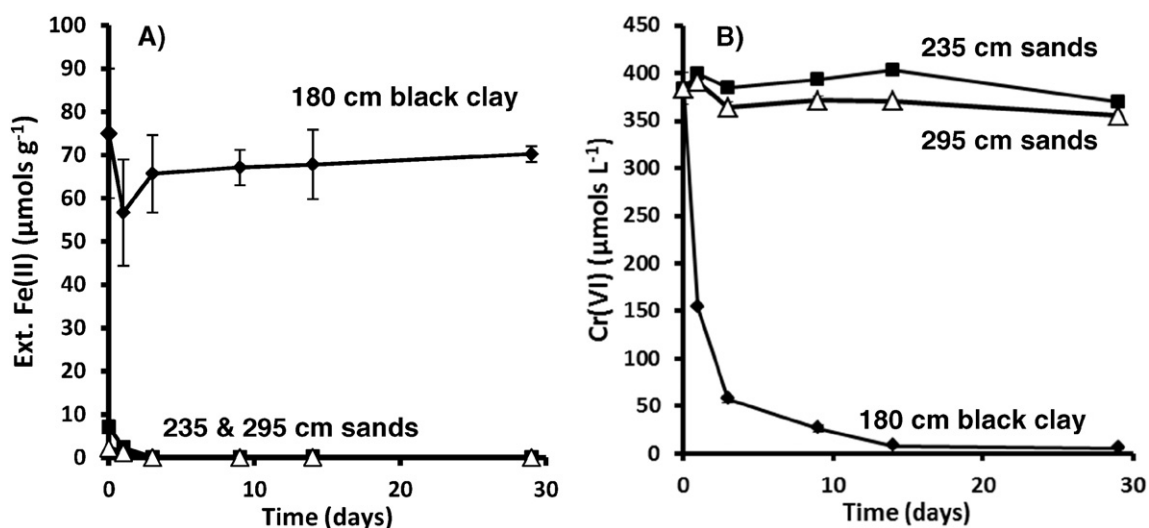
Fig. 5. Results from microcosm tests using sediments recovered from below the COPR waste in BH2 mixed with Cr(VI)-containing site leachate. A) Variation in 0.5 N HCl extractable Fe(II) and, B) variation in aqueous Cr(VI) concentrations. Error bars are 1σ of triplicate microcosms and were not shown are less than the size of the symbol used.

continuous across the whole site and other soils present do not contain sufficient organic matter to support Fe(III) bioreduction processes at sufficient rates to counteract the effects of Cr(VI) infiltration.

Addition of labile organic electron donors (e.g. acetate, lactate etc.) to organic matter poor soils and sediments has been proposed as a way to promote in situ dissimilatory Fe(III)-reduction and Cr(VI) removal (Whittleston et al., 2011b; Lovley, 1995; Marsh & McInerney, 2001). Indeed, several recent trials using alkaline effected soils have shown this to be possible in laboratory tests (Stewart et al., 2007; Whittleston et al., 2011b), especially if implemented with soils at pH < 10 (or in conjunction with bicarbonate or acid addition designed to control soil pH (Whittleston et al., 2013)). However, addition of labile organic matter would be unlikely to produce a long-lived effect. Infiltration of oxygenated Cr(VI)-containing waters would over time consume any Fe(II) produced, and the subsurface geochemistry would return to the initial pre-treatment conditions. This would result in the need for extended periods of planned groundwater monitoring, and potentially additional 'top-up' treatment phases, applied as required.

Alternatively, an organic matter source containing mixture of labile and refractory organic carbon could be used, preferably as an immobile solid phase (e.g. sewage sludge, injected as a water suspension using in situ jet grouting methods). In this situation, much like the natural black clay, conditions might be established where in situ populations of alkali-tolerant fermentative bacteria develop and use the refractory part of the added organic matter slowly over time. This would in turn produce a long lived source of labile metabolic by-products to support Fe(III) reduction for decades (or even centuries) to come. Emplacing and evenly distributing solid phase organic matter additions in the correct locations, would likely add significant costs and technical challenge in comparison to addition of a dissolved electron donor addition dispersed by groundwater. However, if achieved, the benefits of reduced future monitoring and no requirement for future interventions may be cost effective when calculated over the long term.

5. Conclusions

Cr(VI) leaching from COPR waste enters an organic-carbon rich and Fe(II)-containing soil horizon beneath the waste. Cr(VI) in leachate is reduced to Cr(III) by reaction with Fe(II) in the soil and accumulates as Cr(OH)$_3$. Despite a pH of 10.4 the soil contains a viable community of alkaliphilic microbes dominated by a *Mangroviflexus*-like specie of fermentative bacteria. Members of this microbial community are also
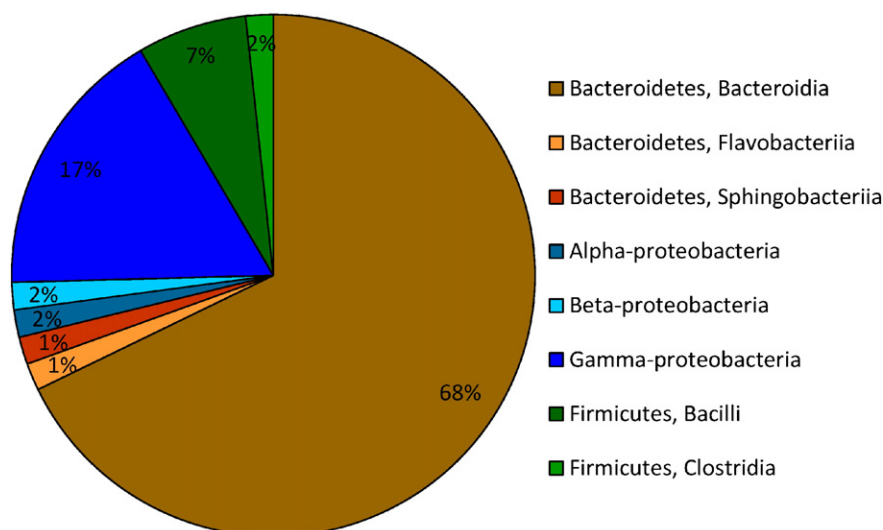

Fig. 6. Phylogenetic affiliation of the 16S rRNA gene sequences obtained from sample BH2-180 cm (assignments to both phylum and class were made with 100% confidence).
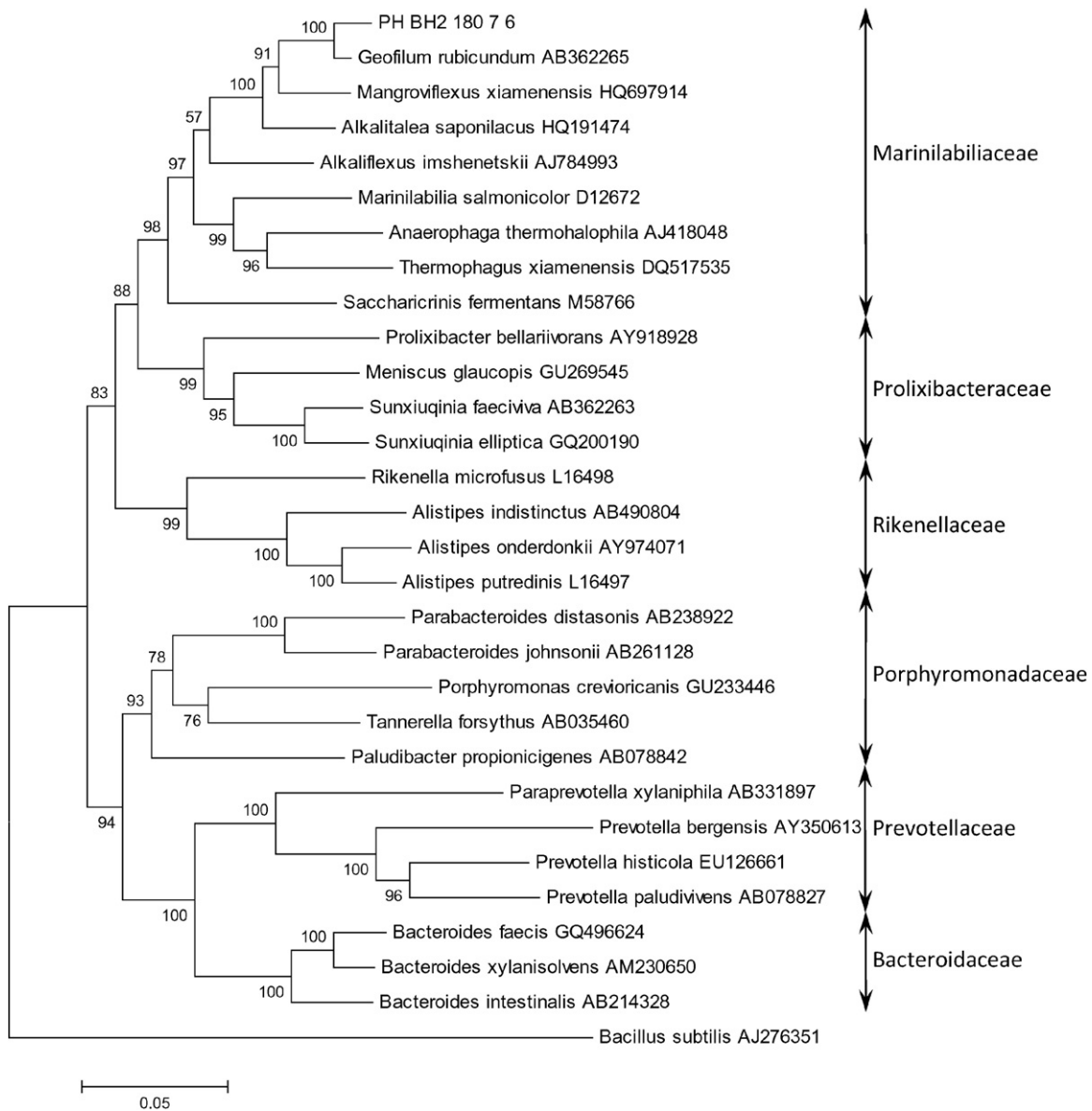
Fig. 7. Phylogenetic tree showing the relationship between characteristic sequence from the *Mangroviflexus*-like OTU and other members of the order *Bacteroidales*. *Bacillus subtilis* (*Firmicutes*) is included as an out-group. The scale bar corresponds to 0.05 nucleotide substitutions per site. Bootstrap values (%) based on 2000 replications are shown at branch points.

capable of Fe(III) reduction at pH > 9. The organic carbon and Fe(II)-rich soil, therefore, act as a potent in-situ reactive zone, effectively trapping and preventing the spread of Cr(VI) at the site. It is predicted that addition of organic matter to soils below or adjacent to COPR waste could be a viable strategy to prevent the spread of Cr(VI) at COPR disposal sites.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx. doi.org/10.1016/j.scitotenv.2015.09.150.

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