Supporting Information for:

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

Weixuan Ding[1], Douglas I. Stewart[2]*, Paul Humphreys[3], Simon Rout[3] and Ian T. Burke[1]*

[1]School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, UK.

[2]School of Civil Engineering, University of Leeds, Leeds, LS2 9JT, UK.

[3] Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, UK

*Corresponding Authors E-mail: D.I.Stewart@leeds.ac.uk / I.T.Burke@leeds.ac.uk

This section consists of 6 pages and 1 Table.

MATERIALS AND METHODS

X-ray Absorption Spectroscopy (XAS).

Frozen samples were transported on ice to the Diamond Light Source in September 2013. During beamtime all samples were stored at -20°C and defrosted as needed prior to mounting. Sample tubes were defrosted and solids were transferred to Teflon sample holders and sealed using Kapton tape. Samples were mounted in a liquid nitrogen cryostat at 80 K for analysis. Cr K-edge XAS data was collected from samples on beamline I20. Here the x-ray source is derived from a wiggler insertion device and the energy of the collimated beam is then selected by a unique 4 crystal monochromator that yields a beam with very high energy stability. The monochromated beam is then focused to give a final spot size of 400 x 350 µm. Fluorescence spectra were gathered using a 64 element solid state Ge detector, and, Cr K-edge XAS data was energy calibrated using an in-line Cr^0-foil. Multiple scans were averaged to improve the signal to noise ratio using Athena version 0.8 [1]. For the XANES spectra, absorption was also normalised in Athena over the full data range and plotted from 5980 eV to 6030 eV. For EXAFS analysis of the BH2 1.80 m sample the data was background subtracted using PySpline v1.1 [2]. Cr is only expected to occur in the Cr(III) or Cr(VI) oxidation states, and Cr(VI) spectra all exhibit a large pre-edge peak at 5993 eV that is absent in Cr(III) spectra. Therefore, the normalised height of the Cr pre-edge peak can be calibrated to give the Cr(III):Cr(VI) ratio in the sample [3]; 100% Cr(VI) has a normalised pre-edge peak height of ~1.00 and 100% Cr(III) has a normalised pre-edge peak height of ~0.05.

EXAFS Data Analysis and Fitting.

Background subtracted EXAFS spectra were analysed in DLexcurv v1.0 [4] using full curved wave theory [5]. Phaseshifts were derived from ab initio calculations using Hedin-Lundqvist potentials and von-Barth ground states [6]. Fourier transforms of the EXAFS spectra were used to obtain an approximate radial distribution function around the central Cr atom (the absorber atom); the peaks of the Fourier transform were related to "shells" of surrounding backscattering ions that were characterised by atom type, number of atoms, absorber-scatterer distance, and the Debye-

Waller factor (± 25%), $2\sigma^2$. Atomic distances calculated by DLexcurv have an error of approximately ± 0.02 and ± 0.05 Å in the first and outer shells respectively. The data was fitted by defining a theoretical model and comparing the calculated EXAFS spectrum with experimental data and with published spectra for Cr-substituted compounds [7]. Shells of backscatterers were added around the Cr and by refining an energy correction (E_f; the Fermi Energy; which for final fits was~-18 eV), the absorber-scatterer distance, and the Debye-Waller factor for each shell; goodness of fit was determined by calculating a least squares residual (the R factor [8]). The amplitude factor (or AFAC in DLexcurv V1.0) was retained as the default of 1 throughout. Shells or groups of shells were only included if the overall fit (R-factor) was reduced overall by >5%. For shells of scatterers around the central Cr, the number of atoms in the shell was chosen as an integer to give the best fit and not further refined.

DNA Extraction and Sequencing of the 16S rRNA Gene

Bacterial DNA was extracted sample BH2 180cm using a FastDNA spin kit for soils (MP Biomedicals, USA). A 1.5 kb fragment of the 16s rRNA gene was amplified by Polymerase Chain Reaction (PCR) using broad specificity primers (8f: AGAGTTTGATCCTGGCTCAG; and 1525r: AAGGAGGTGWTCCARCC). The PCR product was isolated using an agarose-TBE gel and extracted using a QIAquick gel extraction kit (QIAGEN N.V., The Netherlands). The PCR product was ligated into a standard cloning vector (pGEM-T Easy; Promega Corp., USA), and transformed into E. coli competent cells (E.coli XL-1 blue; Agilent Technologies UK Ltd) to isolate plasmids containing the insert, which were sent for sequencing.

The quality of gene sequences was evaluated using uchime_ref (using the gold_f database and the self-checking function) and uchime_denovo implemented within USEARCH 6.0.310 [9], and putative chimeras were excluded from subsequent analyses. Sequences were classified using the Ribosomal Database Project (RDP) naïve Bayesian Classifier [10]. Sequences were grouped into operational taxonomic units (OTUs) using MOTHUR 1.30.2 using a >98% nearest neighbour sequence similarity cut-off [11], and selected sequences were aligned with sequences from closely related type

species obtained from the European Nucleotide Archive using MUSCLE and neighbour-joining phylogenetic trees were constructed using the MEGA5.2.2 integrated phylogenetics package [12].

REFERENCES

[1] B. Ravel, M. Newville, ATHENA, ARTEMIS, HEPHAESTUS: Data analysis for X-ray absorption spectroscopy using IFEFFIT, Journal of Synchrotron Radiation, 12 (2005) 537-541.

[2] A. Tenderholt, B. Hedman, K.O. Hodgson, PySpline: A modern, cross-platform program for the processing of raw averaged XAS edge and EXAFS data, in: AIP Conference Proceedings, 2007, pp. 105-107.

[3] M.L. Peterson, G.E. Brown, G.A. Parks, Quantitative determination of chromium valence in environmental samples using XAFS spectroscopy, in: Materials Research Society Symposium - Proceedings, 1997, pp. 75-80.

[4] S. Tomic, B.G. Searle, A. Wander, N.M. Harrison, A.J. Dent, J.F.W. Mosselmans, J.E. Inglesfield, New Tools for the Analysis of EXAFS: The DL_EXCURV Package, in, CCLRC Technical Report DL-TR-2005-01, Daresbury, UK., 2005.

[5] S.J. Gurman, N. Binsted, I. Ross, A rapid, exact, curved-wave theory for EXAFS calculations. II. the multiple-scattering contributions, Journal of Physics C: Solid State Physics, 19 (1986) 1845-1861.

[6] N. Binsted, CLRC Daresbury Laboratory EXCURV98 program, CLRC Daresbury Laboratory, Warrington, UK., 1998.

[7] S. Fendorf, M.J. Eick, P. Grossl, D.L. Sparks, Arsenate and chromate retention mechanisms on goethite. 1. Surface structure, Environmental Science and Technology, 31 (1997) 315-320.

[8] N. Binsted, R.W. Strange, S.S. Hasnain, Constrained and restrained refinement in EXAFS data analysis with curved wave theory, Biochemistry, 31 (1992) 12117-12125.

[9] R.C. Edgar, B.J. Haas, J.C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection, Bioinformatics, 27 (2011) 2194-2200.

[10] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, Applied and Environmental Microbiology, 73 (2007) 5261-5267.

[11] P.D. Schloss, S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, C.F. Weber, Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities, Applied and Environmental Microbiology, 75 (2009) 7537-7541.

[12] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Molecular Biology and Evolution, 28 (2011) 2731-2739.

Table S1. Assignment of the 16S rRNA gene sequences obtained from sample B2 180cm.

Sequence ID	Accession number	OTU	Sequence Length	RDP classification based on a 95% confidence threshold				
				Phylum	Class	Order	Family	Genus
PH_BH2_180_1_1	LN851746	3	1423	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_1_2	LN851747	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_1_4	LN851748	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_1_5	LN851749	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_1_6	LN851750	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_1_7	LN851751	3	1510	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_1_8	LN851752	10	1530	Proteobacteria	γ–proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
PH_BH2_180_2_1	LN851753	4	1527	Firmicutes	Clostridia	Clostridiales	-	-
PH_BH2_180_2_2	LN851754	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_2_3	LN851755	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_2_4	LN851756	11	1533	Proteobacteria	γ–proteobacteria	-	-	-
PH_BH2_180_2_5	LN851757	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_2_6	LN851758	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_2_7	LN851759	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_2_8	LN851760	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_3_3	LN851761	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_3_4	LN851762	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_3_5	LN851763	5	1552	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	Anaerobacillus
PH_BH2_180_3_7	LN851764	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_4_1	LN851765	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_4_2	LN851766	6	1551	Firmicutes	Bacilli	Bacillales	Paenibacillaceae_1	-
PH_BH2_180_4_3	LN851767	2	1509	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	-
PH_BH2_180_4_4	LN851768	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_4_5	LN851769	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_4_6	LN851770	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_4_7	LN851771	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_4_8	LN851772	10	1530	Proteobacteria	γ–proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
PH_BH2_180_5_1	LN851773	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_5_2	LN851774	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_5_3	LN851775	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_5_4	LN851776	8	1459	Proteobacteria	α-proteobacteria	Rhodobacterales	Rhodobacteraceae	-
PH_BH2_180_5_5	LN851777	10	1530	Proteobacteria	γ–proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
PH_BH2_180_5_6	LN851778	10	1530	Proteobacteria	γ–proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
PH_BH2_180_5_7	LN851779	10	1530	Proteobacteria	γ–proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
PH_BH2_180_5_8	LN851780	1	1513	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Cyclobacteriaceae	Algoriphagus
PH_BH2_180_6_1	LN851781	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_6_2	LN851782	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_6_3	LN851783	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_6_4	LN851784	9	1537	Proteobacteria	β–proteobacteria	Rhodocyclales	Rhodocyclaceae	Azoarcus
PH_BH2_180_6_5	LN851785	5	1551	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	Anaerobacillus
PH_BH2_180_6_6	LN851786	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_6_7	LN851787	3	1540	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_6_8	LN851788	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_7_1	LN851789	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_7_2	LN851790	12	1531	Proteobacteria	γ–proteobacteria	-	-	-
PH_BH2_180_7_3	LN851791	12	1530	Proteobacteria	γ–proteobacteria	-	-	-
PH_BH2_180_7_4	LN851792	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_7_5	LN851793	10	1530	Proteobacteria	γ–proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
PH_BH2_180_7_6	LN851794	3	1511	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	Mangroviflexus
PH_BH2_180_7_7	LN851795	7	1549	Firmicutes	Bacilli	Bacillales	Paenibacillaceae_1	Paenibacillus

PH_BH2_180_7_8	LN851796	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_1	LN851797	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_2	LN851798	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_3	LN851799	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_4	LN851800	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_5	LN851801	**3**	**1512**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_6	LN851802	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_7	LN851803	**3**	**1511**	**Bacteroidetes**	**Bacteroidia**	**Bacteroidales**	**Marinilabiliaceae**	**Mangroviflexus**
PH_BH2_180_8_8	LN851804	**12**	**1532**	**Proteobacteria**	**γ–proteobacteria**	-	-	-