Microbially-mediated chromate reduction in highly alkaline groundwater systems

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Abstract:
Chromium ore processing residue (COPR) has been deposited at a site in the North of England, probably at the end of the nineteenth century. The site covers an area of approximately 2.2 ha, and is situated between a canal and a river that are about 150m apart. It is in a glacial valley underlain by millstone grit and in-filled with alluvial deposits (silt, clay and sand). The original surface deposit is a thin layer of sandy clay that was probably deposited during over-bank flow of the river. COPR has been tipped onto the hillside between the river and canal (which is ~7m above the river), possibly to support the canal bank. At some time in the past top-soil has been placed over the COPR, and the site is now covered with grass. Ground level on the tip is about 1.5m higher than the canal towpath. Currently the site is a cause for environmental concern because groundwater emerging from the waste is alkaline, visibly yellow and has an elevated Cr(VI) concentration.

This paper reports an investigation into the possible fate of any Cr(VI) that migrates downwards from the waste into the underlying soils. Sandy clay from immediately beneath the waste (assumed to be the topsoil layer prior to waste tipping) contains 30-70% acid extractable iron as reduced Fe(II), and between about 3,000 and 600 mg.kg^{-1} of Cr decreasing with depth. DNA fragments from soil bacteria were extracted from this soil, and microcosm experiments with this soil where the pH was reduced showed that it contains a viable bacterial population capable of iron-reduction. This sandy clay layer, despite a pH value of 10.5, appears to be acting as a natural reactive zone beneath the waste as it is accumulating chromium. It is thought that the mechanism of Cr(VI) reduction is most likely to be an abiotic reaction with the Fe(II) present in the soil, and that Fe(II) in the soil is being replenished by microbial iron reduction (albeit probably at a slow rate).

Background:
Poorly controlled landfilling of chromite ore processing residue (COPR) is a globally widespread problem [1]. Chromite ore is processed by roasting it with an alkali-carbonate at 1150°C to oxidise the insoluble Cr(III) to soluble Cr(VI) which is then extracted with water upon cooling. Traditionally, limestone was added to the reaction mixture to improve air penetration, and this "high-lime" process was the only commercial method of chromium smelting in the UK up to the 1960s [2]. COPR from the high-lime process is highly alkaline and typically contains 2-6% total chromium by weight [1, 3, 4]. Much of that chromium is unreacted insoluble chromite ore (i.e. Cr(III)) but, as a result of oxidation during ore processing, up to 30% can be chromate (Cr(VI)) [5]. As a result, the pore water in abandoned waste piles can have a pH>12 and contain up to about 640 μmol.L^{-1} of chromate [6, 7].

This paper describes the investigation of a site in the North of England where COPR has been deposited against a valley side close to a river, probably at the end of the nineteenth century. The site is causing environmental concern because the groundwater which is emerging from near the base of the waste pile is visibly yellow and has an elevated Cr(VI) concentration. This paper reports an investigation into the possible fate of any Cr(VI) that migrates downwards from the waste into the underlying soils.

Methods:
Site Description. The site (approx. 2.2 ha) is situated between a canal and a river that are about 150m apart (see Fig. 1). The canal is approximately 7m above the river. Soil and/or waste have been tipped onto the hillside between the river and canal, possibly to support the canal bank. Geological maps indicate that this is a glacial valley within the millstone grit series in-filled with alluvial deposits (silt, clay and sand). Historical OS maps show that this landform (called the "old tip") first appeared in the late 19th century, and has not been substantially altered since. Ground level on the tip is currently about 1.5m higher than the canal towpath.

Site Sampling. Soil and waste samples were taken from boreholes 1, 8 and 10 in March 2007 using cable-percussion drilling. Sediments were transferred directly into sterile polythene containers, transported back to the laboratory, and stored in the dark at 4°C until use. Experiments were started within 6 months of sample collection

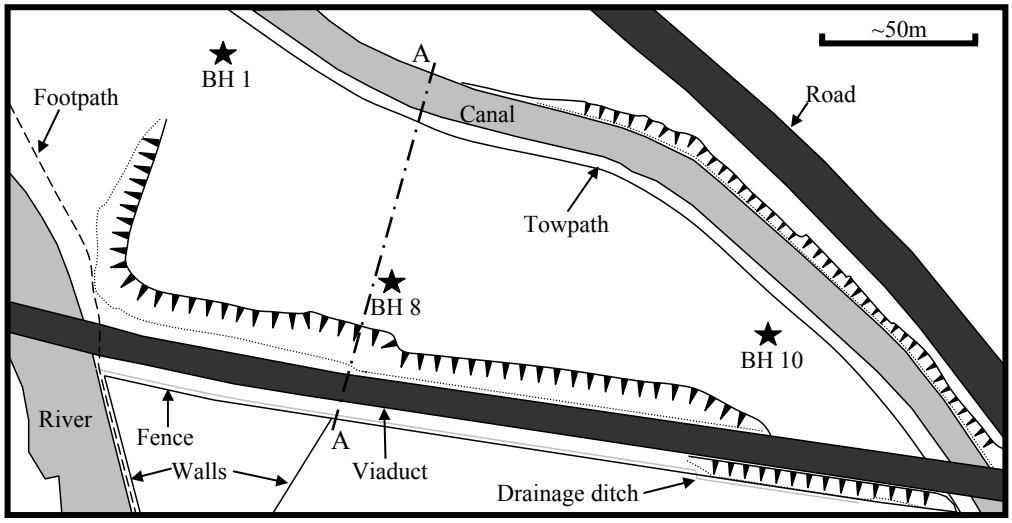

Fig. 1: Sketch map of the site showing the sampling location and the position of cross-section AA.

and all sediment manipulation was kept to a minimum prior to use. In addition, water samples were taken from the site drainage ditch close to where it enters the river (at the base of the viaduct) and from the canal for use in microcosm and leaching experiments.

Sequential Leaching Tests. Sequential leaching tests were performed in order to assess the potential release of Cr(VI) from the waste materials found at the site. Samples from both visibly weathered material from 2-3 m below surface in BH10 and unweathered material from 4-7 m below surface in BH1 were used. In triplicate tests, waste material was suspended in both canal water or deionised water in either 1:1 or 1:10 ratios (w/w) and shaken on an orbital shaker at 150 rpm for a minimum of 24 hours. After shaking the mixture was centrifuged at 8000 rpm and the supernatant removed analysed for Cr(VI), pH, TDS, Ca and sulfate as described below. Fresh water was then added to the leached samples and the cycle was repeated 10-20 times per sample.

Microcosm Incubation Experiments. Microcosm experiments were performed to assess the behaviour of the Cr(VI) contaminated site leachate when mixed with the soils found directly below the waste. Microcosms were made up using 10 g of the grey clay soil from 7 m below surface in BH1 and 100 ml of site leachate in 120 ml glass serum bottles and sealed with butyl rubber stoppers and aluminium crimps. Once equilibrated the microcosms had a pH of 10-11, and an initial Cr(VI) concentration of between 100-250 $\mu mol.L^{-1}$. pH amendment was achieved by addition of 30 $mmol.L^{-1}$ HCl or 20 $mmol.L^{-1}$ $NaHCO_3$ solution, which reduced the pH to 4-5 and 8.5-9.5 respectively (the *HCl* and *HCO_3^- amended* microcosms). For each reduced pH system three repeat microcosms were amended to produce a final concentration of 20 $mmol.L^{-1}$ sodium acetate. Sterile control microcosms were established by heat treatment for 20 minutes at 120°C. Two further microcosms were run without pH adjustment (called the *pH-unamended* microcosms), one with 20 $mmol.L^{-1}$ sodium acetate, and one without.

All microcosm experiments and controls were incubated anaerobically at 21 °C in the dark. Microcosm experiments and controls were periodically sub-sampled for geochemical analysis to produce a progressive time series. During sampling soil microcosms were shaken and 3 ml soil slurry samples withdrawn using aseptic technique with sterile syringes and needles [8]. Samples were centrifuged (5 min, 16,000g) and then porewaters and sediments analysed for a range of redox indicators and Cr(VI).

Freshwater Growth Media Incubations. Sediment was taken from the HCO_3^- amended microcosm experiments from a single bottle after 101 days incubation. Sediment samples (0.1g) were added to 100ml of freshwater growth media at pH 7.3 in 120 ml glass serum bottles and sealed with butyl rubber stoppers and aluminium crimps. Each bottle also contained either 20 mM sodium acetate or 20 mM sodium lactate as the only electron donors, and either 10 mM Fe(III) citrate or 250 μM potassium chromate as the only electron acceptor. Triplicate incubations were run for each electron donor / acceptor pairing.

Geochemical Methods. Cr(VI) and total aqueous Fe were determined by standardised UV-vis spectroscopy methods [9, 10]. Sulfate was determined by ion chromatography and Ca by Atomic Absorption Spectroscopy. Fe(II) in solids was determined after extraction by 0.5 N HCl and reaction with Ferrozine™ [11]. Standards were used regularly to check method quality and calibration linear regressions or quadratic fits normally produced r-squared values of 0.99 or better. Eh, pH and TDS readings were taken using Orion bench-top meters and calibrated electrodes.

Table 1: Major elements in fused waste samples measured by XRF (corrected for loss on ignition).

	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	MgO %	CaO %	SO_3 %	Cr_2O_3 %	Mn_3O_4 %	LOI %
Weathered waste	4.14	5.33	5.91	4.11	26.77	12.00	3.70	0.05	37.00
Unweathered waste	3.61	4.27	7.04	5.85	40.29	5.12	4.93	0.07	28.40

16S rRNA Gene Sequencing. Microbial DNA was extracted from soil samples (0.25g) using a FastDNA spin kit and FastPREP instrument (Qbiogene, Inc.). A fragment of the 16S rRNA gene of approximately ~500 bp was amplified by polymerase chain reaction (PCR) using broad-specificity bacterial primers. After ligation of the PCR product into a standard cloning vector and transformation of the resulting plasmid into competent *E. coli* cells, plasmid DNA was extracted from colonies that were grown-up from single cells and sent for automated DNA sequencing. This protocol is described in detail by [12]. Sequences were analysed against the EMBL release nucleotide database in July 2007 using the NCBI-BLAST2 program and matched to known 16S rRNA gene sequences. Default settings were used for the BLAST parameters (match/mismatch scores 2, -3, open gap penalty 5, gap extension penalty 2).

Phylogenetic tree building. Gene sequences were aligned using the ClustalX software package and a phylogenetic tree constructed from the distance matrix by neighbour joining. Default alignment parameters were used for the alignment (gap opening penalty 15.0, gap extension penalty 6.66). Bootstrap analysis was performed with 1000 replicates. Phylograms were drawn using the TreeView software package.

Results:

The ground investigation revealed that a typical vertical profile through the waste tip consists of about 0.5m of topsoil overlying up to 8m of alkaline chromium containing waste. Under the waste there is 6 to 8m of brown slightly sandy slightly gravelly clay (the alluvium) with occasional gravel layers overlying siltstone/sandstone bedrock (millstone grit series). The bedrock elevation is fairly constant across the tip. Immediately under the waste there is a soft, dark brown clay that is 1-2m thick and in places contains decayed vegetation. Borehole logs and historical maps indicate that this was probably the original surface deposit, which may have been deposited from the river during over-bank flow. The upper horizons of the waste material are a grey to brown slightly clayey sand-sized material, whereas deeper horizons are a greenish yellow slightly clayey sand-sized material (these will henceforth be referred to as weathered and unweathered waste). The chemical compositions of the weathered and unweathered waste (measured by XRF) are reported in Table 1. Groundwater was encountered during drilling, rising to about 6m below ground level within 20 minutes. A cross-section through the site is shown in Fig. 2.

In borehole 1, which is close to the edge of the waste tip, there was 1.5m of building rubble over the waste which is only 4m thick in this location. The clay from immediately below the chromium containing waste was slightly grey in colour and friable in texture, but quickly becoming soft and dark brown with depth.

The canal water sample had a pH of 6.8, a TDS of 220 $mg.L^{-1}$, and no aqueous Cr(VI) was detected. The water sample taken from the site drainage ditch just before the water enters the river in March 2007 had a pH of 12.0, a Cr(VI) concentration of 225 $\mu mol.L^{-1}$ and a TDS of 406 $mg.L^{-1}$ Analysis of a ditch water sample taken from the same location on a subsequent site visit suggests that the ditch water composition varies seasonally with rainfall.

The major crystalline phase in the weathered waste detected by XRD was calcite (calcium carbonate) with a minor quantity of quartz (see Table 2). In sequential 1:1 leaching tests with canal water, the pH was consistently about 11, and the aqueous Cr(VI) concentration in the first step was about 2000 $\mu mol.L^{-1}$ dropping to 1500 $\mu mol.L^{-1}$ in the second step and decreasing steadily to about 150 $\mu mol.L^{-1}$ after 20 steps. In the same step in these tests the

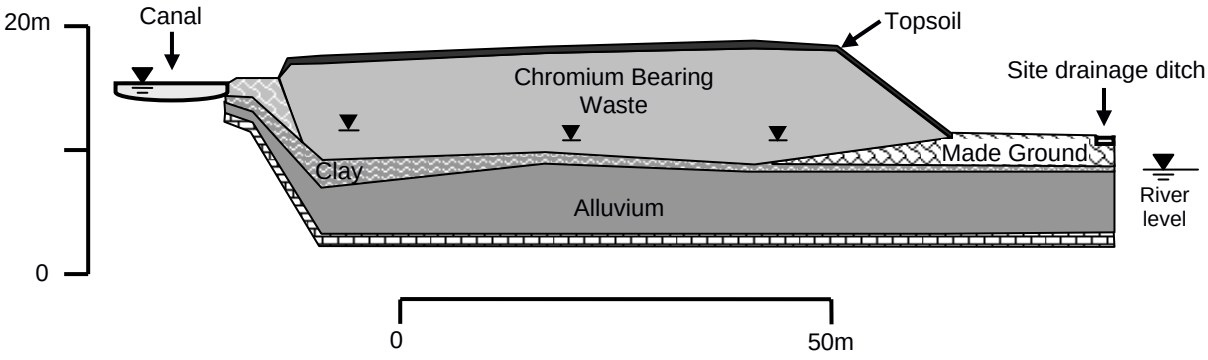

Fig. 2: Sketch diagram showing cross-section AA through the site

Table 2: Leaching properties of the chromium waste

	Predominant mineral phase (XRD analysis)	pH	Sequential 1:1 leaching tests with canal water					
			Step 1			Step 20		
			Cr(VI) $mmol.L^{-1}$	Ca^{2+} $mg.L^{-1}$	TDS $mg.L^{-1}$	Cr(VI) $mmol.L^{-1}$	Ca^{2+} $mg.L^{-1}$	TDS $mg.L^{-1}$
Weathered Waste	Calcite	11	2,000	450	870	150	75	250
Unweathered Waste	Portlandite	13	3,400	700	4,500	500	670	4,000

aqueous calcium concentrations were about 450, 90 and 75 $mg.L^{-1}$, and the total dissolved solids were about 870, 680 and 250 $mg.L^{-1}$. The major crystalline phase in the unweathered waste detected by XRD was portlandite (calcium hydroxide). In sequential 1:1 leaching tests, the pH was consistently about 13, and the aqueous Cr(VI) concentration in the first step was almost 3,400 $\mu mol.L^{-1}$, dropping to about 2,600 $\mu mol.L^{-1}$ in the second step and decreasing steadily to just under 500 $\mu mol.L^{-1}$ after 20 steps. In the same steps in these tests the aqueous calcium concentrations were about 700, 690 and 670 $mg.L^{-1}$, and total dissolved solids were about 4,500, 4,700 and 4,000 $mg.L^{-1}$. The chemical compositions, mineralogy and leaching behaviours reported in Tables 1 and 2 are consistent with the waste being chromium ore processing residue (COPR) from the "high lime" process [13].

XRD and XRF analysis of the grey friable clay recovered from immediately below the waste in BH1 indicates the major mineral is quartz with some kaolinite and muscovite, and the XRF analysis indicates that there is 3020 $mg.kg^{-1}$ of chromium in the solid phase. The soil pH was 10.5 and around 70% of the acid extractable iron was Fe(II). A rough estimate of the total acid extractable iron is approximately 3,500 $mg.kg^{-1}$. XRD and XRF of the dark brown clay recovered from just below the waste in BH8 indicates that the major mineral is also quartz with some kaolinite and muscovite. XRF analysis indicates the brown clay has a higher clay content than the grey clay and that there is 630 $mg.kg^{-1}$ of chromium in the solid phase. The soil pH was 10.6.

Microbiological community analysis on the grey clay sample recovered from immediately beneath the waste in BH1 is shown in Fig. 3. A total of 32 clones were sequenced from this sample but just 8 were assigned to a phylum by the Blast analysis conducted in July 2007. This indicates that bacterial DNA can be recovered from the grey clay, but provide little information on the bacterial species present in this chromium contaminated highly alkaline environment. The failure to match 16S rRNA gene sequences to sequences in the database is probably an indication that such environments have not been widely studied.

Initial ClustalX analysis and neighbour joining tree construction indicated that many of the initially unassigned sequences fell into three distinct clades. Clade A contained 15 sequences (including sequence C01-7-30), clade B contained 4 sequences (including C01-7-40), and clade C contained 2 sequences (including C01-7-28). Four sequences (including C01-7-7) that were assigned to the phylum firmicutes by Blast analysis were found to form a fourth clade (clade D). The phylogenetic tree shown in Fig. 4 has been constructed by further ClustalX analysis and

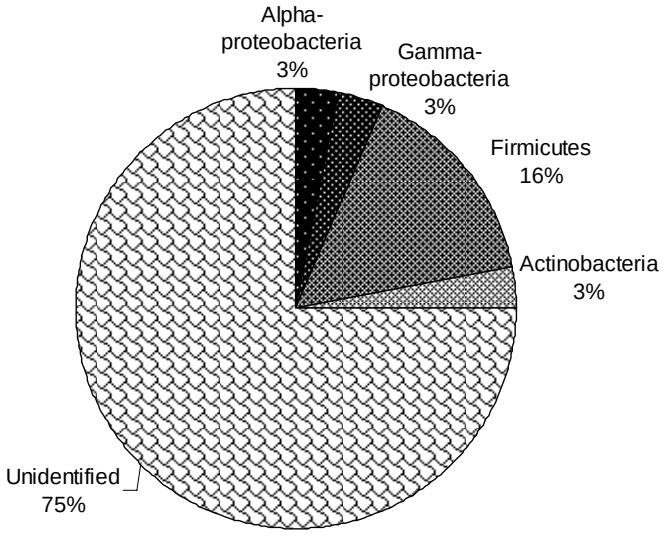

Fig. 3: Microbial community of the grey clay recovered from BH1 (32 clones). Each 16S rRNA gene sequence has been assigned to a phylum (class in the case of proteobacteria) based >95% homology to a known sequence in the database

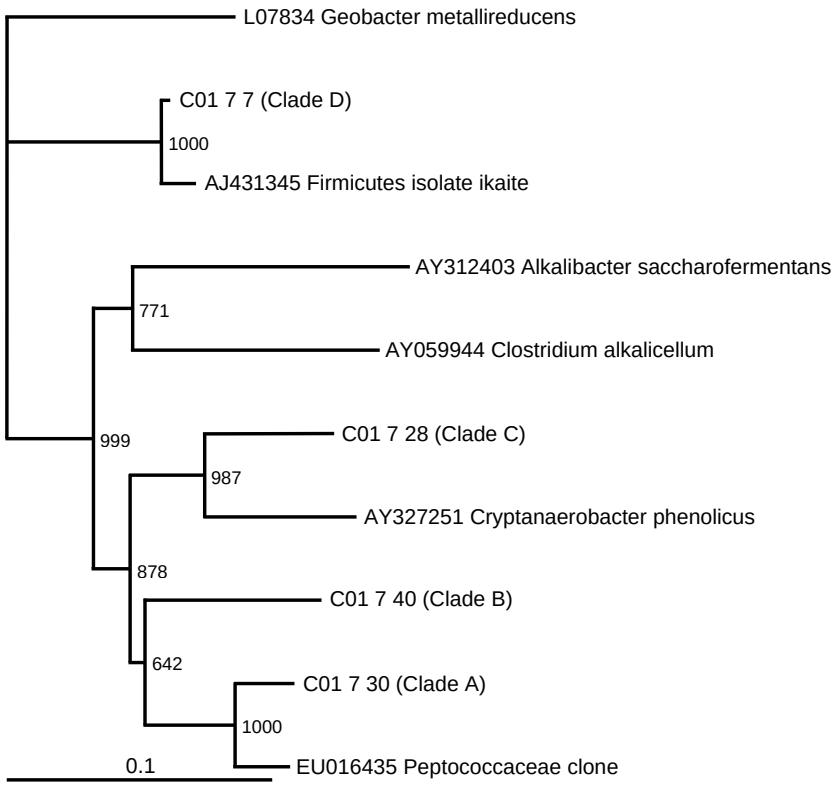

Fig. 4: Phylogenetic tree showing the relationship between representatives of four clades of bacterial sequences from the grey clay recovered from BH1 to 16S rDNA sequences of previously described bacteria. *Geobacter metallireducens* was included as an out-group. The scale bar corresponds to 0.1 nucleotide substitutions per site. Bootstrap values (from 1000 replications) are shown at branch points.

neighbour joining tree construction where, for clarity of presentation, only the representative member of each clade was included in the analysis. The bacteria *Alkalibacter saccharofermentans*, *Clostridium alkalicellum*, *Cryptanaerobacter phenolicus*, and the *Peptococcaceae* clone, whose 16S rDNA sequences are used for comparison in the ClustalX analysis, are all members of the order Clostridiales within the class Clostridia of the phylum Firmicutes (the δ–proteobacterium, *Geobacter metallireducens*, was included as an out-group). *C. phenolicus* is a member of the family *Peptococcaceae*. The phylogentic tree presented in Fig. 4 strongly suggests that sequences in clade A, B and C belong to phylum Firmicutes, probably within the order Clostridiales. Thus it appears that members of the phylum Firmicutes may represent over 80% of the 16S rRNA gene sequence recovered from the grey clay.

The results of the microcosm tests are presented in Table 3. These show that when Cr(VI) contaminated water is incubated with the grey clay from immediately below the waste Cr(VI) is removed from solution in all experiments. In all tests some Cr(VI) removal occurred rapidly on day 0, with the proportion removed at once increasing with decreasing pH. The soil was found to contain approx 30-70% of HCl extractable Fe as Fe(II), which is able to react abiotically with aqueous Cr(VI) reducing it to insoluble Cr(III) [12]. The amount of Cr(VI) that was removed immediately correlates with the solubility of iron oxyhydoxide phases at the various pH values (natural variations in the acid extractable Fe content probably mask any correlation with the proportion of Fe(II) in the solid phase).

In the microcosms amended with HCl the pH was immediately lowered to pH 4-5. This would have

Table 3: Results of pH-amended and pH-unamended microcosms containing contaminated ditch water and soil from below the waste.

Microcosm system	**Time (d)**	**Cr(VI) (C/Co)**	**% Fe(II)$_{(s)}$**	**Fe$_{(aq)}$ (µmol.L^{-1})**
HCl amended @ pH 4-5	0	n.d.	28±7	360±98
HCO_3^- amended @ pH 8.5-9.5	0	0.48	54±3	18±1
	14	0.01	96±2	18±2
pH-unamended (no acetate) @ pH 10-11	0	0.82	40	3.7
	29	n.d	47	2.7
pH-unamended (with acetate) @ pH 10-11	0	0.89	36	1.8
	101	n.d.	54	0.4

Table 4: Microbially significant half-reaction reduction potentials: Standard Reduction Potential, E^0, and redox potential, Eh, at pH 7, 9 and 10.5 (at 25°C and atmospheric pressure).

Transformation	Reaction	E0 (V)	Eh @ pH 7 (V)	Eh @ pH 9 (V)	Eh @ pH 10.5 (V)	Assumptions
Mn reduction [+] Mn(IV) to Mn(II)	$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	1.230	0.544	0.308	0.131	$[Mn^{2+}]$=18 μmol.L^{-1}
Fe reduction[+] Fe(III) to Fe(II)	$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	0.975	0.014	-0.342	-	$[Fe^{2+}]$=18 μmol.L^{-1}
Fe reduction[+] Fe(III) to Fe(II)	$Fe(OH)_3 + HCO_3^- + 2H^+ + e^- = FeCO_3 + 3H_2O$	1.078	-	-0.088	-0.266	$[HCO_3^-]$ = 20 mmol.L^{-1}
Sulfate reduction [+] S(VI) to S(–II)	$SO_4^{2-} + 10H^+ + 8e^- = H_2S + 4H_2O$	0.301	–0.217	-0.365	-0.476	$[SO_4^{2-}]=[H_2S]$
Cr reduction[*] Cr(VI) to Cr(III)	$CrO_4^{2-} + 8H^+ + 3e^- = Cr^{3+} + 4H_2O$	1.507	0.404	0.089	-0.150	$[CrO_4^{2-}]=[Cr^{3+}]$
Bicarbonate reduction to acetate[×] C(VI) to C(0)	$2HCO_3^- + 9H^+ + 8e^- = CH_3COO^- + 4H_2O$	0.187	-0.292	-0.425	-0.525	$[HCO_3^-]= [CH3COO^-]$ = 20 mmol.L^{-1}

[+] after [14]
[*] calculated using thermodynamic data from [15]
[×] calculated using thermodynamic data from [16]

caused the release of acid extractable Fe(II) to solution and thus Cr(VI) removal is probably an instantaneously abiotic reduction by Fe(II). In the HCO_3^- amended systems pH is lowered to pH 8.5-9.5, and Cr(VI) removal is largely completed within 14 days. However Cr(VI) removal is accompanied by a rapid increase in the proportion of acid extractable iron in the lower Fe(II) oxidation state. The sterile control for the HCO_3^- amended microcosms exhibited a large drop in Cr(VI) upon heat treatment, but over the test duration the proportion of acid extractable Fe in the lower Fe(II) oxidation state decreased steadily. Thus it is inferred the increase in extractable Fe(II) in these tests is most likely due to microbial Fe(III)-reduction [8].

In the pH-unamended system without acetate, Cr(VI) reduction occurs more slowly and is complete after 29 days. Evidence for Microbial Fe(III) reduction occurring is less clear than in the HCO_3^- amended systems as extractable Fe(II) increases only slightly. In the pH-unamended microcosm containing acetate Cr(VI) reduction is even slower and is only complete after 101 days, but it is inappropriate to ascribe too much importance to this difference in rate as only single replicates were conducted for the microcosms with unamended pH. Interestingly, however, a larger increase in extractable Fe(II) was observed in this longer duration test.

All freshwater growth media incubations containing Fe(III) citrate with sediment from the HCO_3^- amended microcosms and either acetate or lactate as the electron donor scored positive for growth (darkening of media indicating conversion of Fe(III) $\rightarrow$ Fe(II)) within 30 days. No growth was observed in media with potassium chromate as the only electron acceptor. These incubations indicate that the HCO_3^- amended microcosms, and thus the grey clay, contained a viable microbial community capable of iron reduction at pH 7.3 with either acetate or lactate as the electron donor.

Discussion:

The water emerging from the front face of the waste pile is likely to have at least two sources; water leaking from the canal, and rainwater. Both are likely to be contributing to the seepage, but the precise balance between these sources is unclear at this stage. In any engineering intervention to remediate this site it would be relatively easily to reduce rainfall infiltration by capping, and to intercept any contaminated groundwater still emerging from the sides of the tip and treat it. What will be less easy will be to prevent any water that enters the side of the waste from leaching downwards. Unrestricted, this would be a significant environmental issue as the leaching tests on the unweathered waste indicate that such water will have very high pH and TDS values and a high Cr(VI) concentration.

The soil immediately beneath the waste (assumed to be the topsoil layer prior to waste tipping) appears to be acting as a natural reactive zone beneath the waste as it is accumulating chromium. This finding is supported by the microcosm results, where Cr(VI) is removed from all the systems studied. This behaviour can be explained by an abiotic reaction with the Fe(II) present in the soil (it contains 30-70% acid extractable iron as reduced Fe(II)), with differences in the rate of Cr(VI) removal between the microcosms explained by variation in the solubility and/or accessibility of the Fe(II). However the source of this Fe(II) in the soil is, on first inspection, something of a mystery because, as a former near-surface layer, it would be expected that most of the amorphous iron would have originally been in the Fe (III) oxidation state prior to burial.

A mass balance calculation readily shows that the amount of chromium that has accumulated in the soil

could not have been reduced by amorphous iron present in the soil (it requires three equivalents of Fe(II) to reduce one equivalent of Cr(VI) to Cr(III). Thus the stock of Fe(II) in the soil must be being replenished. In the HCO_3^- amended microcosms there was a significant increase in the proportion of acid extractable Fe as Fe(II) as time progressed and in the pH-unamended microcosms there was a small increase in the %Fe(II) over time which, taken together, are clear evidence that Fe(III) was being reduced within these alkaline microcosms.

The only reasonable explanation for soil beneath the COPR having a high proportion of its acid extractable iron in the lower Fe(II) oxidation state is that iron reducing bacteria are respiring, albeit at a slow rate, under the prevailing high pH conditions and replenishing Fe(II) consumed by Cr(VI) reduction. Initially this finding may seem surprising, but this study has clearly shown that the grey clay contains a bacterial population that will readily reduce iron if the pH is reduced. Table 4, which reports the reduction potentials of microbially significant half-reactions, indicates that iron reduction coupled to the oxidation of organic compounds is still thermodynamically favourable at a pH of 10.5. Indeed iron reduction is more thermodynamically favourable than sulphate reduction, which is a widely reported bacterial process in alkaline soda lakes [e.g. 17]. Recently two alkaliphilic bacterial species in the order Clostridialles have been identified in the literature, which have been shown be capable of Fe(III) reduction at a pH value of 10.5 [18, 19]. However phylogenetic similarity of the 16S rDNA sequences isolated from grey clay to these alkaliphilic iron reducing bacteria should not be used to imply that they possess similar metabolic pathways, and elucidation of the species actually responsible for iron-reduction and the metabolic pathways involved, will require further work.

While the fortuitous formation of a natural barrier beneath this site will certainly reduce the impact of the waste on the wider environment. Its presence should not be taken for granted, as factors determining its long-term viability are unknown. For example any increase in the Cr(VI) flux may easily overwhelm its treatment capability, and changes in the influent water, particularly with regards to any electron donor, may threaten its long-term survival.

Conclusions:

The former top-soil layer beneath the COPR tip appears to be acting as a natural reactive zone preventing the downward movement of chromate leached from the waste. The mechanism appears to involve Cr(VI) reduction to Cr(III), which is then precipitated. Cr(VI) reduction is most likely to be an abiotic reaction with the Fe(II) present in the soil. It is thought that Fe(II) in the soil is being replenished by microbial iron reduction despite the pH of 10.5.

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