Online microdialysis-high performance liquid chromatography-inductively coupled plasma mass spectrometry (MD-HPLC-ICP-MS) as a novel tool for sampling hexavalent chromium in soil solution.

Dr. Elliott M. Hamilton[†‡], Dr. Scott D. Young[‡], Dr. Elizabeth H. Bailey[‡], Dr. Olivier S. Humphrey[†], Dr. Michael J. Watts[†]*

[†] Inorganic Geochemistry, Centre for Environmental Geochemistry, British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK.

[‡] School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK

Abstract

Conventional soil solution sampling of species-sensitive inorganic contaminants, such as hexavalent chromium (Cr^{VI}), may induce interconversions due to disruption of system equilibrium. The temporal resolution that these sampling methods afford may also be insufficient to capture dynamic interactions, or require time-consuming and expensive analysis. Microdialysis (MD) is emerging as a minimally invasive passive sampling method in environmental science, permitting the determination of solute fluxes and concentrations at previously unobtainable spatial scales and timeframes. This article presents the first use of MD coupled to HPLC-ICP-MS for the continuous sampling and simultaneous detection of Cr^{VI} in soil solution. The performance criteria of the system were assessed using stirred solutions; good repeatability of measurement (RSD < 2.5%) was obtained for Cr^{VI}, with a detection limit of 0.2 $\mu g\ L^{-1}$. The online MD-HPLC-ICP-MS setup was applied to the sampling of native Cr^{VI} in three soils with differing geochemical properties. The system sampled and analyzed fresh soil solution at 15-minute intervals, offering improved temporal resolution and a significant reduction in analysis time over offline MD. Simple modifications to the chromatographic conditions could resolve additional analytes, offering a powerful tool for the study of solute fluxes in soil systems to inform research into nutrient availability or soil-to-plant transfer of potentially harmful elements.

Introduction

The separation and quantification of trivalent (Cr^{III}) and hexavalent (Cr^{VI}) chromium (Cr) in soil is a burgeoning area of research motivated by the significant differences in toxicity and mobility between the two oxidation states.[1] Due to the negative charge of its compounds, typically chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$),[2] Cr^{VI} is more mobile and bioavailable in soil-water systems than Cr^{III}, [3] and is therefore more likely to be transferred from contaminated soil to drainage water and into plants.[4] The measurement of total Cr^{VI} in solid matrices presents a metrological challenge due to the potential for species interconversions during extraction and analysis,[5] which is further compounded when measuring changes in the bioavailable pool of Cr^{VI} in soil-pore water systems. This usually involves specialized extractions and/or separation steps which not only cause significant disruption to the equilibrium of the system,[6] but also produce large numbers of samples which are more susceptible to artefactual errors.

The kinetics of Cr species interconversions in soil-pore water systems are of particular importance when there is the potential for transportation of Cr^{VI} into groundwater and/or sediment systems.[7] Attenuation of Cr in these systems is dependent on the physiochemical properties of the water/soil and can be attributed to the formation of Cr^{III} following reduction of Cr^{VI}. Trivalent Cr is significantly limited in solubility due to its adsorption to mineral phases or co-precipitation with iron (Fe) (oxy-)hydroxides.[8] The adsorption of Cr^{VI} in soil-pore water systems is a slower process than for Cr^{III},

resulting in order-of-magnitude lower partition coefficients (K_d).[9] However, this process is accelerated through decreases in pH and increases in concentrations of soil organic carbon (SOC) and reducing inorganic components such as Fe(II) and sulfides.[10] Previously, the exchange kinetics between soil solution and mineral phases have been measured using diffusive gradients in thin-films (DGT).[11] This passive sampling technique involves the chelation of labile analytes on a resin implanted onto saturated soil, causing depletion around the DGT device and a shift in system equilibrium to resupply the soil solution from the solid phase.[12] This allows for the measurement of a range of kinetic parameters, including distribution coefficients (K_{dl}), remobilization fluxes and adsorption/desorption rate constants.[13] Despite its advantages, the technique suffers from spatial limitations (a large sampling area, of the order of cm^2, is required for successful device deployment), induces significant disruption to the equilibrium of the sampled soil and requires time-consuming offline sample preparation and analysis.[14] Depending on the temporal resolution required, a number of devices may need to be deployed which adds to the processing time, analytical requirements and overall cost.[15]

Microdialysis (MD) is another passive sampling technique that has been garnering increasing interest within the field of soil science[16-19] due to its high spatial and temporal resolution, and its preservation of the *in situ* dynamics of the system undergoing sampling.[20] Microdialysis uses a probe containing a semipermeable membrane with a specific molecular weight cut off (MWCO); the pumping of a perfusate solution into the probe creates a diffusion gradient within the sampled medium causing solutes to diffuse across the membrane.[21] The solution exiting the probe (dialysate), containing the sampled solutes, can then be analyzed using a suitable analytical technique.[22] The minimal disruption to the soil, coupled with the ability of the technique to sample soil solution at representative water contents (~50% water holding capacity (%WHC) and higher)[23] makes MD a very attractive tool to increase understanding of small-scale inorganic solute availability in soil.

The majority of MD sampling is undertaken offline through the collection of discrete samples over varying timescales (typically in the order of minutes to hours),[20] although recent articles have demonstrated the potential for hyphenating MD with analytical detectors such as electrothermal atomic absorption spectrometry (ETAAS)[24] and high performance liquid chromatography (HPLC).[25] Online MD sampling and simultaneous analysis, depending on the analytical technique being used, has the potential to overcome one of the biggest compromises in MD sampling- relative recovery (RR) versus perfusate flow rate.[26] The RR of a system can be defined as the ratio of the solute concentration in the dialysate to the solute concentration in the medium undergoing MD sampling, and is a function of the resistances that impede solute transport imposed by the external environment (R_{ext}), the MD probe membrane (R_m), the dialysate (R_d) and the perfusate flow rate (Q_p).[27] Therefore the lower the Q_p, the greater the likelihood of reaching a steady-state between the solute concentration in the external solution and the probe membrane, leading to RR values close to 100 % depending on the analyte being studied.[28] However, flow rates less than 5 µL min^{-1} are not practical for the majority of MD applications due to the increased time required to collect sufficient sample for analysis, and the subsequent impact on temporal resolution. Online systems, with careful optimisation of liquid handling steps, can allow for lower Q_p, increased RR and immediate analysis without adding additional time or cost constraints. The coupling of MD sampling to mass spectrometry was first conceived over 25 years ago,[29] but has seen limited application in soil since with no significant focus on inorganic solutes.[30]

The aim of this study was to couple MD to HPLC-ICP-MS (henceforth referred to as online MD-HPLC-ICP-MS) for continuous passive soil solution sampling and simultaneous analysis of Cr^{VI}. The objectives were:

(i) to undertake online MD calibration using stirred solutions of Cr^{VI};
(ii) to assess common performance characteristics (linearity, precision, limit of detection);
(iii) to apply the MD-HPLC-ICP-MS method to the sampling of Cr^{VI} in soils with differing geochemical characteristics.

Materials and Methods

Reagents.

All solutions were prepared in 18.2 MΩ cm ultrapure water (DDW, Merck Millipore, UK). Standards for Cr^{VI} were prepared through dilution of a commercially available solution (Greyhound Chromatography, UK). Ethylenediaminetetraacetic acid (di-ammonium salt, NH_4-EDTA), trisaminomethane (TRIS) and ammonium nitrate (NH_4NO_3) (Sigma Aldrich, UK) were used for the preparation of the chromatographic mobile phase.

Instrumental Apparatus and Analysis.

Separation and identification of Cr^{VI} in sampled soil solution was undertaken using a Dionex GP50 Gradient Pump (Dionex Corporation, USA) and a PRP-X100 anion exchange column (Hamilton Company, USA) coupled to an Agilent 8900 Triple Quad inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, Tokyo, Japan). A Rheodyne 7125 injector/switching valve (IDEX Corporation, USA) equipped with a 20 µL loop was used to interface the dialysate flow from the microdialysis probe with the column. The column was connected directly to the nebulizer of the ICP-MS instrument using a single piece of 0.18 mm internal diameter (ID) PEEK tubing.

The separation and identification of Cr^{VI} was achieved through isocratic elution using a mobile phase consisting of 40 mM NH_4NO_3, 50 mM TRIS buffer and 5 mM NH_4-EDTA, adjusted to pH 7.0 using concentrated nitric acid (HNO_3, Romil, UK). Mobile phase was introduced into the injector/switching valve at a flow rate of 1.2 mL min^{-1}, resolving Cr^{VI} within 5 min. The ICP-MS instrument was operated in collision cell mode, with the cell pressurized using helium (He) gas at a flow rate of 5.1 mL min^{-1}, to reduce the impact of polyatomic interferences on *m/z* 52 (e.g. $^{40}Ar^{12}C^{+}$).

Soil Sampling.

The physicochemical properties of the soil samples used to demonstrate the efficacy of the MD-HPLC-ICP-MS setup are summarized in Table 1. Samples were primarily chosen due to their total Cr^{VI} content, but also to provide a range of physicochemical properties to ensure a robust assessment of the MD-HPLC-ICP-MS setup.

Table 1. Soil physicochemical properties.

Soil ID	Country of Origin	Texture	pH	TOC (%)	LOI (%)	Total Cr (mg kg^{-1})	Cr^{VI} (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})
1	U.K. (Glasgow)	Silty Sand	7.37	3.1*	n/a	1750*	28.5	790*	41663*
2	U.S. (NJ)	N/A	9.33	0.1*	n/a	1055*	15.3	60.0*	5950*
3	Kenya	Sandy Clay Loam	5.77	n/a	6.4	329	2.0	2636	69449

* denotes a parameter that was not measured within author's laboratories.

Soil 1 was a sample of silty sandy soil from eastern Glasgow, held by the British Geological Survey (BGS) from a soil chemistry survey in 2018. Soil 2 (SRM2700) was purchased from NIST (National

Institute of Standards and Technology, U.S.), and was a soil matrix reference material intended for use in validating Cr^{VI} speciation data for soils and sediments. Soil 3 was a sample of sandy clay loam soil collected in Kakamega County, Kenya and retained by BGS. The methods used for the determination of total Cr, loss-on-ignition (LOI) and Cr^{VI} in these soils have been outlined previously.[31, 32] The presence of Cr^{VI} in soils 1 and 2 can be attributed to anthropogenic sources; the Cr^{VI} in soil 3 is of geogenic origin, possibly derived from ophiolitic parent material.[33, 34]

Maximum percentage water holding capacity (% WHC) was determined on 50 g subsamples of each soil, according to previously-outlined methods.[35] For each soil sampled by MD-HPLC-ICP-MS (n = 3 per soil), 10 g of soil was moistened to 70% WHC before being packed into polypropylene (PP) tubes (Sarstedt, UK), henceforth referred to as microcosms, for online microdialysis sampling.

Calibration of MD-HPLC-ICP-MS System in Stirred Solutions.

Prior to application of the online MD-HPLC-ICP-MS setup to soil solution sampling, the RR for a range of perfusate flow rates was calculated in stirred solutions to determine the optimum perfusate flow rate for the system. The Cr^{VI} solution (100 µg L^{-1}) was perfused with distilled deionized water (DDW, 18.2 MΩ cm at 25 °C, Millipore Merck, UK) at flow rates of 1, 3, 5, 7.5 and 10 µL min^{-1}, with each flow rate replicated 5 times. Solutions were stirred to remove the resistance contribution from the external environment (R_{ext}).[27]

The RR for each perfusate flow rate was calculated using Equation 1:

$$RR\ (\%) = 100 \times \frac{C_{dial}}{C_{std}} \quad (1)$$

where C_{dial} is the concentration (µg L^{-1}) of the analyte in the dialysate and C_{std} is the concentration (µg L^{-1}) of the analyte in the perfused solution.

Online Microdialysis Sampling.

The MD system consisted of a CMA 4004 syringe pump (CMA, Stockholm, Sweden) delivering perfusate (DDW) through a 10 mL syringe (BD Plastipak, US) into a CMA 20 microdialysis probe with a polyethersulfone membrane (10 mm length, 0.5 mm OD, 100 kDa MWCO). The dialysate flow was connected to the needle port of the injector/switching valve using a tubing adaptor (CMA, Stockholm, Sweden) and a #22 gauge square-cut end syringe needle. New probes were perfused at 10 µL min^{-1} with DDW. Prior to installation in soil microcosms, the online MD-HPLC-ICP-MS system was calibrated through perfusion and injection of stirred calibration standards containing $^{53}Cr^{VI}$ at 1, 10, 25, 50 and 100 µg L^{-1}. A more concentrated calibration standard (4 mg L^{-1}) was perfused after sampling each replicate for Soil 2, to extend the linear dynamic range whilst mitigating washout issues that could arise when considering the low perfusate flow rate and narrow diameter of the inlet and outlet probe tubing.

Fifteen minutes after probe installation- the total time taken to fill the volume of the outlet tubing and the 20 µL loop- the valve was switched to “inject” and the time resolved analysis (TRA) sequence was initiated on the ICP-MS software to begin data acquisition. The injector remained in this position until ten sample volumes of mobile phase had been pumped through the loop (equivalent to 10 seconds with a 20 µL loop), before being switched back to “load” to collect freshly-sampled dialysate for the next injection. These steps were repeated over a period of 2 hours, giving a total of 8 injections of passively-sampled soil solution. Each rewetted soil was sampled in triplicate using the online MD-HPLC-ICP-MS setup, with a new microcosm prepared for each replicate to minimize the potential for solute depletion associated with continuous sampling.[20]

Results and Discussion

Relative recovery of Cr^{VI} in solution.

The RR of Cr^{VI} (stirred solutions) displayed a non-linear decrease with increasing flow rate (Fig. 1); perfusate was delivered into the MD probe at flow rates of 1, 3, 5, 7.5 and 10 µL min^{-1} with collection and injection of the dialysate from each flow rate replicated 5 times.

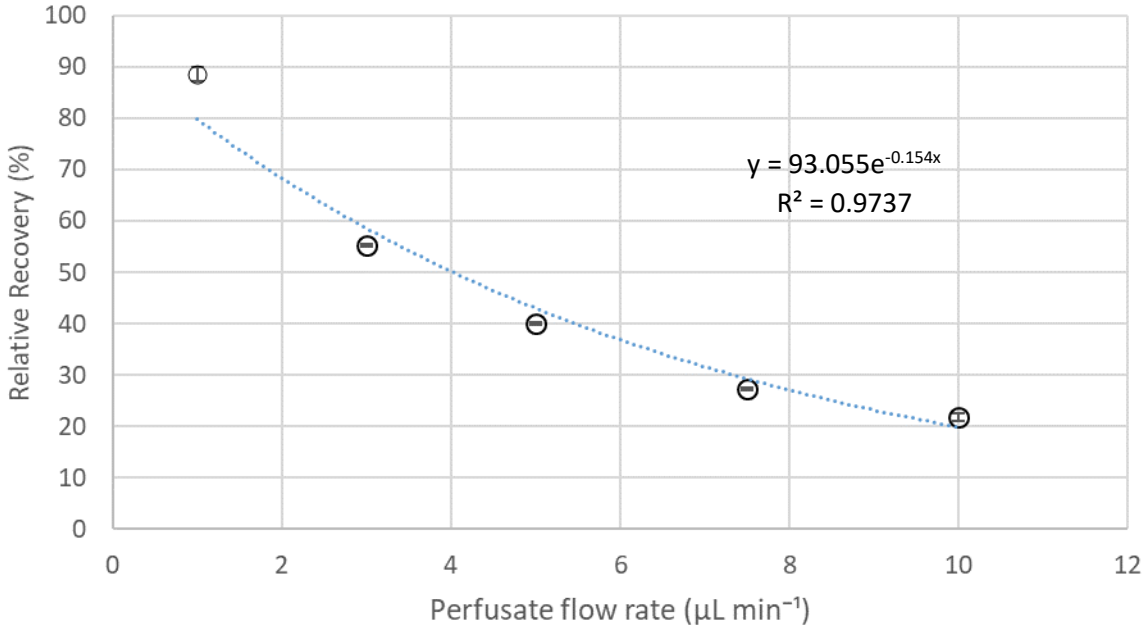

Figure 1. The effect of perfusate flow rate (µL min^{-1}) on relative recovery (%) of Cr^{VI} (100 µg L^{-1}) in stirred solutions (n = 5 for each flow rate). Exponential trendline and correlation coefficient (R^2 = 0.9737) are displayed within the chart. Error bars indicate ± standard error from 5 replicates.

Subsequent solution optimization and soil sampling were undertaken using a perfusate flow rate of 3 µL min^{-1}. This flow rate represented the best compromise between RR (approximately 55%) and the frequency with which freshly sampled soil solution could be injected into the HPLC column, otherwise known as the temporal resolution. The temporal resolution of the online MD-HPLC-ICP-MS system was 15 minutes, representing a significant improvement in sampling capability compared to conventional offline MD which can usually only sample in hour increments to ensure sufficient volume is collected for analysis.[36]

Previous studies have reported variability in RR due to inherent differences in probe structure arising from the manufacturing process.[17, 37] A significant advantage of the online MD-HPLC-ICP-MS method is that, once flow rate calibration has been carried out for the analyte of interest, a single injection of a perfused calibration standard prior to soil sampling can identify any variability or reduction in the performance of the probe before time-consuming and potentially expensive soil sampling and analysis is undertaken.

Analytical Figures of Merit.

The method detection limit (DL) was determined according to previously-outlined methods.[38] Briefly, 5 replicate injections of a perfused 0.5 µg L^{-1} Cr^{VI} standard were undertaken using the online MD-HPLC-ICP-MS setup. The DL was calculated using Equation 2:

192 $$DL = \frac{(t)(RSD)(C_{std})}{100\%} \quad (2)$$

193 where *t* is a confidence factor using Student t-distribution with α = 0.99 and n-1 degrees of freedom,
194 RSD is the relative standard deviation of the peak areas for the Cr^{VI} standard and C_{std} is the nominal
195 concentration of the injected Cr^{VI} standard; details of the precision of the injections are given in
196 Table 2. The DL was calculated as 0.2 µg L^{-1} Cr^{VI}. A similar exercise was previously undertaken to
197 establish the Cr^{VI} DL for the HPLC-ICP-MS setup without MD sampling; the DL for the HPLC-ICP-MS
198 setup was calculated as 0.05 µg L^{-1} Cr^{VI}.

199 **Table 2.** Precision of replicate injections of 0.5 µg L^{-1} Cr^{VI} standard (n = 5) used to calculate detection
200 limit for the online MD-HPLC-ICP-MS setup.

Spike Replicate	Peak Area Counts
1	3190
2	2779
3	2594
4	2785
5	2939
Standard Deviation	222
Average	2857
RSD (%)	8

201

202 The precision of the online MD-HPLC-ICP-MS setup was assessed at the same time as the RR. Across
203 each flow rate (1, 3, 5, 7.5, 10 µL min^{-1}), the 5 replicate injections displayed good precision,
204 demonstrating the repeatability of the technique for solution sampling (Table 3).

205 **Table 3.** Average RR and relative standard deviation (RSD, n = 5) for replicate injections at perfusate
206 flow rates of 1, 3, 5, 7.5 and 10 µL min^{-1}.

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Flow Rate (µL min^{-1})	Average RR (%)	RSD (%)
1	89	2.2
3	55	1.3
5	40	1.3
7.5	27	2.0
10	22	1.4

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212 The linearity of response was determined through injections of perfused Cr^{VI} standards at nominal
213 concentrations of 1, 10, 25, 50 and 100 µg L^{-1}. There was a strong positive linear correlation between
214 peak area counts and Cr^{VI} concentrations in stirred solutions (Fig. 2).

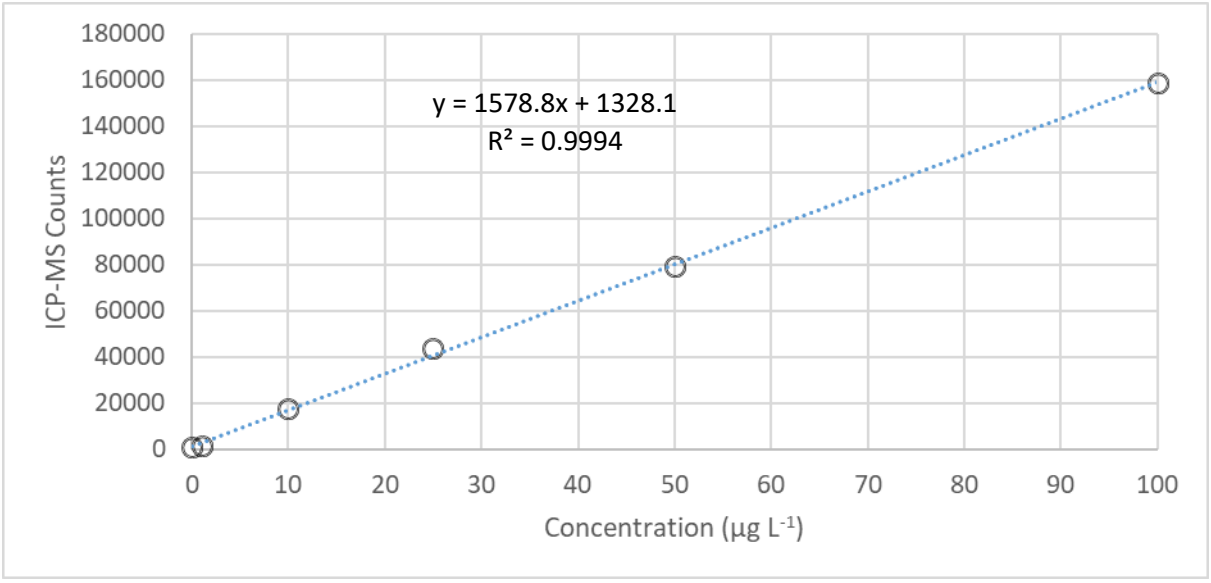

Figure 2. Calibration curve (R^2 = 0.9994) for online MD-HPLC-ICP-MS system at nominal concentrations of 1, 10, 25, 50 and 100 µg L^{-1}. MD probes were immersed in 50 mL plastic beakers containing Cr^{VI} solution, stirred and perfused at 3 µL min^{-1}.

Soil Solution Sampling.

The online MD-HPLC-ICP-MS setup was applied to the sampling and analysis of Cr^{VI} in soil solution from the previously detailed microcosms, using the perfusate flow rate (3 µL min^{-1}) and injector timings established during solution calibration (Fig. 3). The system was sensitive enough to sample Cr^{VI} in all microcosms at a temporal resolution of 15 minutes, a significant improvement compared to recent studies examining soil solution dynamics using offline microdialysis sampling and analysis.[39]

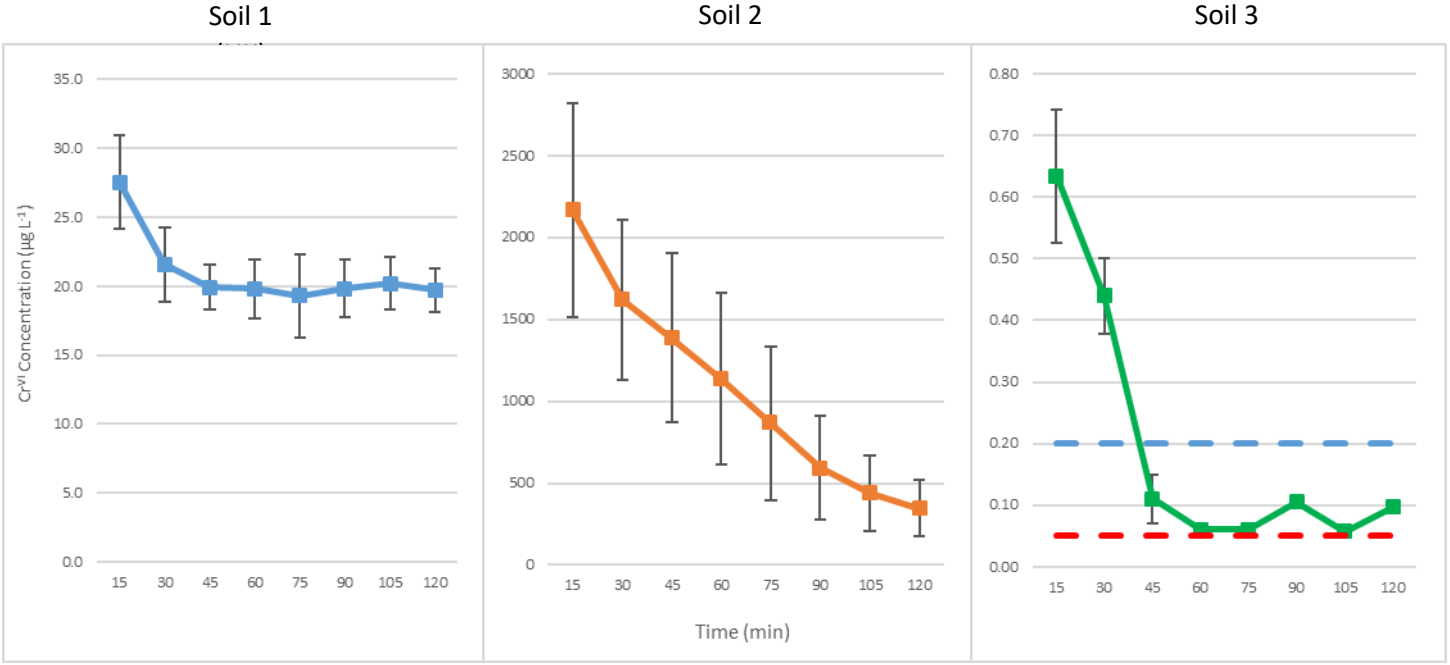

Figure 3. Soluble Cr^{VI} sampled using online MD-HPLC-ICP-MS technique. Markers represent mean values from triplicate measurements, and error bars indicate ± standard error (SE). The blue hatched

line in “Soil 3” graph is the detection limit for the online technique (0.2 μg L^{-1}), the red hatched line is the Cr^{VI} detection limit for the HPLC-ICP-MS system (0.05 μg L^{-1}).

The relevance and/or applicability of this temporal sampling resolution for monitoring the reduction of Cr^{VI} in the environment is dependent on the geochemical conditions of the system under investigation. From a solely-abiotic perspective, the presence of common electron donors (e.g. ferrous iron, soil organic matter (SOM)) will cause rapid (<5 minutes) reduction of Cr^{VI} up to pH 10, whereupon the ferrous iron will be oxidized by dissolved oxygen faster than by Cr^{VI}.[40] The rate of reduction of Cr^{VI} by SOM is also pH-dependent, decreasing with increasing pH but potentially occurring over timeframes of several weeks at neutral pH (depending on both the SOM and initial Cr^{VI} concentrations in the system).[41] In addition, microbial reduction of Cr^{VI} (both aerobic and anaerobic) can occur depending on both pH and the tolerance of the microorganism to Cr^{VI}.[42] These mechanisms are not as well-defined as abiotic pathways of reduction, but could occur over several hours at mg L^{-1} concentrations of Cr^{VI} depending on the bacterium and concentration of electron donors within the system.[43] Therefore, the temporal sampling resolution of the reported online MD-HPLC-ICP-MS setup should be sufficient to monitor these diverse processes, although specific studies may require modifications to be implemented if rapid turnover is expected.

The differing trends in sampled Cr^{VI} concentrations can be attributed to a combination of the physical particle size and the geochemical properties of each soil, as opposed to artefacts associated with the online MD-HPLC-ICP-MS setup. Soil 1 and Soil 3 had been sieved to ≤2 mm prior to sampling, whilst Soil 2 was used as received (milled material, packaged by NIST). The wider error bars for each sampled time point in Soil 2 are therefore due to increased R_{ext}, with the finer particle size of the material reducing the ability of Cr^{VI} to diffuse across the MD probe membrane.[44] This is also reflected in the trend of decreasing sampled Cr^{VI} over the 120-minute sampling period, due to the formation of a depletion zone around the MD probe arising from a combination of continuous sampling and impeded solute diffusion.[30] Similar depletion profiles have been reported for offline MD studies employing continuous sampling, and could be an informative artefact as nutrient uptake by plant roots is also governed by depletion and formation of diffusion gradients within the soil.[45] The majority of MD studies thus far have reconciled depletion zones in this way, due to their primary focus being the assessment of diffusive flux of high-turnover soil nutrients (e.g. plant-available nitrogen (N)).[46] However, further assessment of these depletion trends- through targeted studies into the significance of R_{ext}, alongside additional MD probe calibration strategies such as retrodialysis and/or no-net-flux techniques- are required to ensure wider adoption of MD by inorganic soil scientists. Due to the requirement of predictive solution metal speciation models (e.g. Windermere Humic Aqueous Model (WHAM), Visual MINTEQ) to be supplied with accurate estimates of labile pools of metal ion concentrations for site-specific bioavailability measurements,[47] the determination of free metal ion concentrations in dialysate samples will need to account for inherent changes in solute recovery due to the resistances imposed by R_{ext} and Q_p.

Sampling of Soil 1 was the most reproducible, with an average Cr^{VI} concentration of 19.8 ± 0.1 μg L^{-1} between 45 and 120 minutes, indicating that the sampled available pool of Cr^{VI} was resupplied consistently by diffusion within the microcosm towards the MD probe. The decrease from 27.5 ± 3.4 to 19.9 ± 1.6 μg L^{-1} between 15 and 45 minutes could indicate the sampling of a short-lived pool of immediately exchangeable Cr^{VI} following rewetting, although the online MD-HPLC-ICP-MS setup lacked the temporal resolution to confirm this. In comparison, Soil 3 had the lowest initial sampled Cr^{VI} concentration of 0.63 ± 0.11 μg L^{-1}, which decreased to below the online technique DL after 45 minutes of sampling; the sampled Cr^{VI} concentration remained below this for the duration of sampling for all 3 microcosm replicates. Due to the geochemical properties of this soil sample (high

Fe/Al/organic matter content, low pH), the observed rapid decrease is possibly due to Cr^{VI} adsorption to mineral solids[48] or reduction and subsequent precipitation as Cr^{III} compounds;[49] the precision of the microcosm replicates, combined with the inherently low sampled Cr^{VI} concentration, do not suggest that this quick temporal decrease is solely due to a depletion zone forming around the probe membrane.

Overall, the results of this study confirm that the online MD-HPLC-ICP-MS setup can be used to reproducibly sample and analyze soluble Cr^{VI} from a range of soils with different physicochemical properties. Differences in the efficacy of Cr^{VI} sampling between soil microcosms in this study were limited to particle size and/or geochemical factors influencing Cr^{VI} solubility, as opposed to artefacts associated with the MD-HPLC-ICP-MS system. Assessing the performance of the setup at different %WHC, alongside further method development to increase the temporal sampling ability and resolve more immediate pools of available Cr^{VI}, will contribute to the widespread adoption of the reported online MD-HPLC-ICP-MS technique for short-term nutrient availability studies.

Future Prospects for Optimization and Implementation of Online MD.

The use of MD for soil solution sampling is still an emerging technique (the first comprehensive review was published in early 2020) and so, to a certain extent, the future prospects and discussion points for online and offline MD are complementary.[20] One important consideration in the continuous sampling of soil solution is the increased likelihood of depletion zones forming around the probe due to removal of solute from solution.[46] The recharge of this zone is dependent on a number of factors, including the ability of the solute to diffuse from un-sampled areas within the medium, the concentration of solute within the medium and the diffusive resistance imposed by the soil. These factors may have significant ramifications for the interpretation of solute diffusive flux data when multiple MD probes are deployed. The proximity of one MD probe to its neighbor could create competing diffusion gradients and lead to a situation where probes with reduced permeability- due to manufacturing defects, continued use or implantation in heterogeneous portions of soil- would sample lower solute concentrations (Fig. 4). Depletion zones are a well-known component of MD, but a more empirical investigation is required to fully understand how they impact both diffusive flux measurements and the efficacy of probes that are in close proximity to each other.

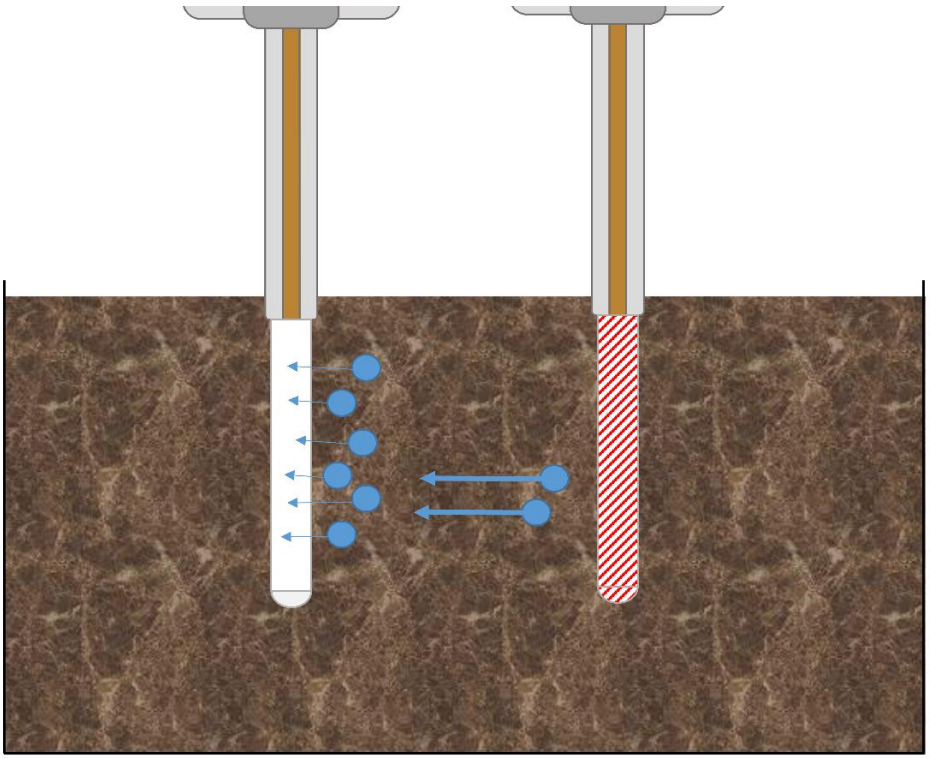

Figure 4. Diffusion of solutes (blue circles) towards probes implanted in soil matrix. Reduced permeability (represented by diagonal fill) in right MD probe shifts the diffusion gradient towards the left MD probe, therefore the right MD probe will sample lower concentrations of solute.

The temporal resolution of the online MD-HPLC-ICP-MS setup in this article represents a significant improvement over offline MD; further optimization of the instrumental setup could reduce this to sub-minute sampling frequencies. In recent years, the use of total consumption nebulizers has allowed sample volumes in the order of microliters to be introduced in to ICP-MS instruments.[50] The flow rates commonly used in MD are ideally suited to these sample introduction systems, with the potential for the outlet tubing from the MD probe to be interfaced directly with the nebulizer to monitor transient signals in real-time. Such a setup would also reduce the level of operator supervision required, as the only ‘hands-on’ task would be the installation of the probe into the microcosm prior to time-resolved analysis.

The spatial resolution of MD, combined with the greater sampling frequency afforded by online coupling to analytical systems, would allow for the investigation of solute turnover/removal at root- and microbe-relevant scales in near real-time. Microbial reduction of Cr^{VI} has been reported on numerous occasions, with incubation times varying from 45 min to 42 days[51] due to the significant variation in Cr^{VI} reduction efficiency between different strains.[52] Undertaking online MD-HPLC-ICP-MS on sterile and non-sterile soil could provide more information on the impact of microbial communities on Cr^{VI} reduction, with the ability to investigate parameters such as temperature, pH and soil type with greater replication than through batch experiments. Online monitoring would also allow for termination of the experiment once passively-sampled analyte concentrations reached DL, potentially saving days of time-consuming and costly experimentation and analysis.[53]

Understanding the mechanisms governing rapid soil fixation and speciation changes, for important redox-active micronutrients such as iodine (I) and selenium (Se), previously limited in terms of temporal resolution, may now be possible.[54 55] The use of stable and radio-isotope trials have confirmed that the removal of I and Se from soil solution, primarily through incorporation into the solid phase or immobilization by soil organic matter (SOM), is a rapid process which significantly reduces the bioavailability of these micronutrients.[56-58] Humphrey, et al.[54], in (at the time of the writing) the only application of offline MD to investigate I dynamics in soil solution, showed that adsorption was more rapid than previously reported. Increased frequency of sampling through online MD could further refine knowledge of the period over which soluble forms of I and Se are available for uptake by crops, leading to improvements in biofortification strategies intended to alleviate the prevalence of deficiency diseases.

The online MD-HPLC-ICP-MS system was only evaluated for Cr^{VI}, but through simple modification of the chromatographic conditions (column, mobile phase composition) the setup could be applied to the sampling and determination of other common inorganic species of interest, including compounds of arsenic, thallium and mercury, to better inform hazard assessment investigations. The soil solution dynamics of inorganic nutrients essential to human health (e.g. iodine, selenium) could be investigated at unprecedented temporal and spatial scales, allowing for more thorough assessments of the efficacy of staple crop biofortification strategies that are essential for the billions of people at risk of micronutrient deficiencies worldwide.

AUTHOR INFORMATION

Corresponding Author

Michael J. Watts- Inorganic Geochemistry, Centre for Environmental Geochemistry, British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK. Email: mwatts@bgs.ac.uk; telephone number: 0115 936 3042.

Authors

Elliott M. Hamilton- Inorganic Geochemistry, Centre for Environmental Geochemistry, British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK/School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK.

Scott D. Young- School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK.

Elizabeth H. Bailey- School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK.

Olivier S. Humphrey- Inorganic Geochemistry, Centre for Environmental Geochemistry, British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK.

Author Contributions

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript.

Conflict of Interest

The authors declare no competing financial interest.

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