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Method Development for Determining the Removal of Metals from the Water Column under Transformation/Dissolution Conditions for Chronic Hazard Classification

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Abstract

An extension of the transformation/dissolution protocol (T/DP) was developed and evaluated as a tool to measure the removal of metals from the water column for chronic aquatic hazard classification. The T/DP extension (T/DP-E) consists of 2 parts: T/DP-E Part 1, to measure metal removal from the water column via binding of metals to a substrate and subsequent settling, and T/DP-E Part 2, to assess the potential for remobilization of metals following resuspension. The T/DP-E methodology (672 hr (28-day) removal period, a 1 hr resuspension event, and a 96 hr resettling period) was tested using Cu, Co and Sr solutions in the presence of a substrate. The metal removal rates varied from rapid removal for Cu to a much slower rate of removal for Sr. The resuspension event did not trigger any increase in dissolved Cu, Co or Sr. Additional 96 hr experiments were conducted using dissolved Ni, Pb, Zn and Ag and supported the conclusion that the T/DP-E is sufficiently robust to distinguish removal rates between metals with a wide range of reactivities The proposed method provides a means to quantify the rate of metal removal from the water column and evaluate remobilization potential in a standardized and reliable way.

Keywords: metals, water quality, hazard/risk assessment

INTRODUCTION

The Transformation/Dissolution Protocol (T/DP), developed within the framework of the Organization for Economic Cooperation and Development (OECD, 2001) measures the rate and extent to which metal-bearing substances can produce soluble ionic species in aqueous media under a set of standard laboratory conditions. The data generated from the T/DP examination are then compared with ecotoxicity reference values (ERVs) to determine the short and long-term potential aquatic hazard of the metal-bearing substance for hazard classification purposes. The T/DP has been included in the United Nations Globally Harmonized System (UN GHS) as Annex 10 (UN, 2017). The T/DP has been successfully applied to derive hazard classifications on a wide range of metal-bearing substances including metals, metal compounds, mattes, alloys and concentrates (Huntsman et al., 2018; Skeaff et al., 2008, 2011, 2014).

The UN GHS (UN, 2017) and the EU Classification, Labelling and Packaging (CLP) (EU, 2013) hazard classification schemes include the concept of degradation whereby rapid degradation from the water column for organics (greater than 70% removal to CO_2 in 28 days) results in one category difference in the classification scheme. For metals and inorganic metal compounds, the rapid and irreversible removal of the metal from the water column can serve as a surrogate for the rapid degradation concept for organics. Unlike organic substances, metal ions are not degraded in the strict sense, but metals are transformed through a wide range of processes such as sorption, sedimentation, and mineralization. This leads to a reduction in bioavailability and toxicity of the metal, and therefore constitutes a reduction in long-term hazard potential (Burton et al, this issue).

The aim of this work was to evaluate if an extension of the T/DP (T/DP-E) could be used to assess the removal of metals from the water column in a standardized way, analogous to the concept of "degradability" for organic substances mentioned in the UN GHS and EU CLP classification systems. To this end, the T/DP method was extended with 2 parts:

- T/DP-E Part 1, to measure metal removal from the water column through binding to a substrate with subsequent settling (removal part)

- T/DP-E Part 2, to assess the remobilization of metals following resuspension of substrate (irreversibility part)

The experimental set up of these extensions was developed to provide a standardized approach which includes the most relevant mechanisms for metal removal occurring in aquatic systems: (1) changes in metal speciation in the presence of a substrate and the associated removal due to these speciation changes, and (2) the potential for remobilization of metals upon resuspension. While several more complex processes may occur in real aquatic systems (see Burton et al., this issue), the experimental set-up strikes a balance between 1) key processes, and 2) achieving a reasonable level of standardization and reproducibility. A standard methodology allows for metals to be assessed in a uniform, repeatable, and comparative way. This forms a key part of the rationale for assessing removal from the water column for metals. The following sections describe the protocol development, the rationale for selecting test parameters and the method application and results for the T/DP-E. It is important to note that the original T/DP was not modified, therefore, all elements mentioned in the T/DP continue to be applicable here.

MATERIAL AND METHODS

Proposed test method

The T/DP calls for the addition of three loadings of a metal-bearing substance into an aquatic media, with agitation, followed by the evaluation of metal concentrations in solution over a 28-day (672 hr) period. Introduced in the T/DP-E, is the addition of substrate particles into a solution with a dissolved metal, with a period of rapid mixing and then settling.

One litre Schott-Duran flasks were used for the T/DP-E and all flasks were rinsed with about 200 mL of Aqua Regia to ensure that glassware used in the tests were clean. The flasks were shaken and then allowed to stand for about 16 hr in a fumehood. Flasks were then rinsed thoroughly with tap water and then with deionized water. Finally, the flasks were filled with deionized water, capped and stored until used.

The 10x dilute OECD 203 (OECD, 1992) medium at pH 6 was prepared in 20 litre Nalgene carboys by adding the weighed reagents (Table 1) to deionized water and filtering through a 0.2 µm 90 mm diameter membrane filter, which also served to sterilize the medium. The composition of the 10x dilute OECD media was obtained by applying the FactSage system (FactSage, 2009) to calculate a pH of 6.09 when equilibrated with an atmosphere containing 0.5% CO_2 with the balance as air. The medium has low water hardness, is relatively dilute and, as such, will have limited buffering capacity. As a guide for pH control, the T/DP proposes a variability of ±0.2 pH units over a period of one week. It also indicates that the pH should not be adjusted during the test using an acid or alkali. Consequently, no attempt was made to adjust pH during the T/DP-E tests.

A total of nine Schott-Duran glass flasks containing one litre of aqueous medium were conditioned to pH 6 by bubbling with the 0.5% CO_2 with the balance as air for 24 hrs. Six of these flasks contained 10x dilute OECD 203 medium with an additional 1 mg/L concentration of the metal of interest, prepared from a soluble metal salt with a counter-ion that is not expected to affect the results of the test (Supplemental Data, ST1). Of these six flasks, three had substrate added and three of these flasks were substrate blanks, with no substrate added to correct for the dilution caused by sampling and replenishment of test medium during the experiment. The remaining three flasks of the nine contained 10x dilute OECD 203 medium only for the procedural blanks. A procedural blank is processed identically to and at the same time as the other flasks. The blanks represent the background concentrations associated with factors such as glassware cleanliness, sampling, handling and solution analysis. The test scheme is shown in Table 2. For the duration of the experiment, all flasks were bubbled with gas containing 0.5% CO_2 with the balance air.

At 0 hrs, prior to substrate addition, pH, temperature and dissolved oxygen of the test solutions were measured with a HACH HQ4Od multimeter with a pH probe PHC10101 and a dissolved oxygen (D.O.) probe LD010101. At this point, triplicate 12 mL solution samples were drawn with a 15 mL syringe, passed through a 0.8/0.2 µm Acrodisc syringe filter into a 15 mL sample tube, and acidified with two drops of Fisher Trace Metal grade nitric acid (68-71%), which lowers the pH to approximately 1.5.

Ten grams of the air-dried substrate was then added to each of the three flasks containing 1 mg/L metal solution following the scheme shown in Table 2. The flasks were agitated on the orbital shaker for 1 minute at 100 rpm to ensure mixing of the sediment in the

solution. The pH, temperature and dissolved oxygen of the test solutions were measured at each sampling time of 2, 6, 24, 48, 96, 168, 336 and 672 hrs and the solutions analysed for the metal of interest. Selected samples were analyzed for total metal concentrations (including measurement of Fe as a fingerprint of the substrate). Flasks were replenished after each sampling point with the 10x dilute OECD 203 up to the one litre mark to maintain the same substrate to solution ratio throughout the experiment.

In the T/DP, the test solution is agitated throughout the test method in order "*to maintain the flow of aqueous medium over the test substance*". As the T/DP-E is initiated with a solution of dissolved metal salt, this is no longer relevant. Furthermore, one of the aims of the T/DP-E is to evaluate settling of metal-binding substrate under standardized conditions. Therefore, the T/DP-E test is performed under static conditions, with the exception of the initial mixing (T/DP-E part 1) and the agitation to mimic a resuspension event (T/DP-E part 2).

Part 2 of the T/DP-E mimics a resuspension event, which aims to address metal remobilization under such conditions. After completion of the T/DP-E, part 1, the flasks were agitated vigorously, e.g. in an orbital shaker at 150 rpm for 1 hr, to mimic a resuspension event. The solutions were then allowed to react for 4 days (96 hrs) in static conditions. The solutions were sampled periodically at 0.5, 1, 2, 6, 24, 48, and 96 hrs after completion of the resuspension event. Samples were analyzed for dissolved metal concentrations, pH, dissolved oxygen concentration, and temperature; selected samples were analyzed for total metal concentrations.

Processing and analysis of substrates

Geochemical data provided by the Geological Survey of Canada (GSC) of over 50 sediments collected from across Canada was evaluated for potential substrate use in the T/DP-E. The sediments, collected for exploration purposes, from both streams and lakes, had been air dried and stored in sample bags. Based on the geochemical composition, three sediments were then selected to represent a low binding substrate (LBS), high binding substrate (HBS) and a substrate of intermediate composition (IBS). To remove the larger pebbles, shells and twigs, the substrate was screened using a 14 mesh (1.41 mm) sieve and the material passing the 14 mesh was then used for the T/DP-E examination.

This substrate material was analyzed in triplicate by CanmetMINING's Analytical Services Group (ASG). The material was subjected to a microwave-accelerated four (4) acid digestion (HCl, HNO_3, HF and H_3BO_3) system (ASG 2013a) followed by analysis using an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) for total Al, Mn and Fe and using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Co, Ni, Ag, Cu, Zn and Pb. An additional Aqua Regia digestion followed by analysis for Fe was conducted in order to compare with the Fe values in the ARCHE (2013) report. Total Carbon (TC) and total organic carbon (TOC) were measured using an ELTRA CS-2000 (ASG 2013b). For the particle size analysis of the substrate, a Fritsch Analysette 22 Laser Particle Sizer Dry Dispersing was used.

Analysis of solutions

CanmetMINING's Analytical Services Group (ASG) used either a Thermo-Fisher X-series II ICP-MS or a Perkin Elmer Elan 6100 ICP-MS to determine metal concentrations in the µg/L range. During each analysis run, the ASG followed established quality control measures (Supplemental Information S1) to verify the initial instrument calibration and to monitor instrument stability.

Calculation of aqueous metal concentrations

For each individual test on each loading, a within-vessel average concentration was calculated as the arithmetic mean of the analytical determinations of the three sample solutions drawn at a particular sampling time. A between-vessel average was also calculated as the arithmetic mean of the average of the three within-vessel values. To calculate the net average concentration ($Me_{(aq)}$), for each loading at each sampling time, the between-vessel average for the procedural blanks was subtracted from the corresponding concentrations in loaded tests. The standard deviations and the coefficients of variation were calculated.

Calculation of dilution factor for volume correction

The purpose of the substrate blank (1 mg/L metal solution in 10x dilute OECD 203 medium without substrate addition) was to allow for the calculation of a dilution factor. Each time a sample was taken, the solution was replenished with the 10x dilute pH 6 OECD medium. The dilution factor was calculated by correcting for the volume taken at each sampling time (see Supplemental Data ST2). This factor was then applied to the flasks with substrate loadings by multiplying the metal concentration by the dilution

factor at any given time. This step is also useful to determine if metals are being removed from solution by variables other than the substrate (for example sorption onto the glass). If metal concentrations in the substrate blank remain stable after correction for dilution, then no other process is removing metals from solution.

METHOD DEVELOPMENT RATIONALE

Rationale for initial 1 mg/L dissolved metal concentration

In the original T/DP, the test is initiated by adding the solid metal-bearing test substance to the aquatic medium. However, in the T/DP-E, the test is initiated with a solution of 1 mg/L of soluble metal, prepared from a soluble salt with a counter-ion that is not expected to affect the results of the test. This modification was introduced to ensure that only metal removal was being measured, not a combination of both dissolution of the solid metal-bearing test substance and removal processes acting on the solubilized metal. The rationale for this approach is that in the T/DP, environmental toxicity and classification is determined at the level of substances. In contrast, metal removal from the water column can be determined at the level of dissolved metal ions. Therefore, it is appropriate for the development of the test method, to initiate the test with a dissolved metal. When integrated in a testing scheme, the outcome of the T/DP will be used directly for the T/DP-E phase. The highest metal substance loading in the chronic hazard classification test scheme is 1 mg/L and therefore, this was selected for this purpose as the target dissolved metal ion concentration.

Rationale for 10 g/L substrate addition

In aquatic systems, suspended solids are added from external sources (e.g., inflow and surface runoff) as well as internal sources (e.g. algal production, resuspension). Suspended solids leave the water column through settling and washout (in surface waters). In mechanistic lake models, solid balances considering the above sources/sinks are often assumed to be at steady-state. Consequently, the suspended solids concentration is constant and there is continual transport of solids out of the water column via settling.

From the perspective of metal added to the water column, adsorption to suspended solids and subsequent settling represents a continual removal process. Added metal is transported on a continuous "solids conveyor belt" out of the water column. This flux of solids can be quantified by multiplying the suspended solids concentration (m_{s1}) by the settling velocity (w_s): $Fs = m_{s1} \times w_s$. Typical values for m_{s1} and w_s for a generalized waterbody in the EUSES model are 15 mg/L for the suspended solids concentration, and 2.5 m/d for the settling velocity (EC 2004; European Chemicals Agency 2010). These values yield a solids flux (F_s) of 37.5 g/m^2/d.

For practical purposes, the T/DP-E entails a single addition of substrate rather than a continuous addition. After mixing, this substrate is allowed to settle. Therefore, the substrate concentration in the experimental system is not constant, but rather it decreases from the initial value. The above solids flux value was used to inform selection of an initial solids concentration that yields a similar integrated solid load leaving the water column. The T/DP-E (Part 1) has a duration of 28 days. The integrated areal load of solids based on the above solids flux is 1,050 g/m^2. The required initial solids

concentration to produce this integrated areal load in the T/D P-E (Part 1) was evaluated by multiplying the integrated areal load value (1,050 g/m^2) by the ratio of the experimental flask area to experimental flask volume (0.00709 m^2/L), (see Supporting Information S2 for details) The resulting solids concentration was 7.4 g/L. A rounded value of 10 g/L was selected as substrate loading rate to use in the T/DP-E experiments.

Rationale for the selection of a low binding substrate

In the T/DP-E, it is proposed to use a substrate that has relatively low or weak metal binding properties in order to be conservative with respect to metal removal. This is referred to as a low binding substrate (LBS). The substrate is characterized by comparison to typical European sediment characteristics (ARCHE, 2013) (Table 3). The main properties that drive metal binding were selected as Fe content and total organic carbon (TOC) content. It should be noted that pH and acid volatile sulphide (AVS) content also drive metal binding in sediments. These were not considered here, because 1) the pH is generally buffered at a given value in the T/DP-E, and 2) as a worst-case condition, the T/DP-E is conducted under oxic conditions where AVS, that may bind metals, may not be present. The 10th percentile of the Fe concentrations (measured in aqua regia extract) and TOC concentrations are 0.75% and 1.3%, respectively (ARCHE, 2013). Correlation analysis showed that these two parameters are good proxies for other constituents causing metal binding (e.g., Al, Mn). Considering these substrate parameters, a selection of three substrates were identified, collected and tested and the LBS selected based on the bulk chemistry (Table 4).

Substrates were evaluated under a variety of T/DP-E conditions, including different substrate loading levels (1 mg to 10 g), varying pH and metal concentrations (Huntsman, unpublished data). As expected, pH control is an important criterion for the T/DP-E, just as in the T/DP. For some substrates, it was difficult to maintain pH of the solution at the target value of 6 for the entire duration of the experiment. Therefore, an additional criterion for the selection of an appropriate substrate was that it should not cause a pH increase of more than 0.5 units. This resulted in substrate LBS being selected for use in the T/DP-E in the majority of the experiments, as pH did not increase above 6.

Rational for experimental pH

The aqueous test media used in the T/DP and in the T/DP-E are based on the aquatic toxicity testing medium OECD 203 (ISO 6341). The pH of an aqueous solution is established by i) its composition, ii) the composition of the atmosphere with which it is in equilibrium and iii) any solids that may react with the solution. In the T/DP, the pH is controlled by equilibrating CO_2 with the bicarbonate in the media. The T/DP tests are carried out at a pH that maximizes the concentration of the dissolved metal ions within a pH range of 6 to 8.5. Extensive T/DP examinations of metal-bearing substances have shown that, for most metals, tests conducted at pH 6 result in greater metal dissolution rates than at higher pHs (Skeaff et al., 2008, 2011, 2014). Furthermore, ample data in real aquatic environments have demonstrated that for most metals, the dissolution increases with decreasing pH (Burton et al., this issue and references therein). For this reason, the T/DP-E was developed at a relatively low pH of 6.

Rationale for static conditions during the metal removal step

In the T/DP, the test solution is agitated throughout the test in order "to maintain the flow of aqueous medium over the test substance". As the T/DP-E is initiated with a solution of dissolved metal salt, this is no longer relevant. Furthermore, one of the aims of the T/DP-E is to evaluate settling of metal-bearing substrate under standardized conditions. Therefore, the T/DP-E test is performed under static conditions, with the exception of the initial mixing (T/DP-E part 1) and the agitation to mimic a resuspension event (T/DP-E part 2). It should be noted, however, that the continuous bubbling of the T/DP-E solution does induce some minimal mixing.

Rationale for resuspension conditions to check for potential remobilisation

Surficial sediments may be resuspended and metals potentially remobilized into the overlying water. Sediment disturbances, such as resuspension due to propeller wash events and episodic hydrologic events, may expose sediments to surface waters with higher concentrations of dissolved oxygen and oxidation of reduced ligands could increase metal solubility and cause toxicity to aquatic organisms (Chapman et al., 1998).

In natural ecosystems, suspended particulate matter (SPM) concentrations rarely exceed 0.8–1 g/L during resuspension events (Duran et al., 2012; Ludwig and Probst, 1998) and easily exchangeable fractions of metals are quickly released and then complexed.

Acute criteria are appropriate for assessments as the exposures are for seconds to a few hours. Dredging operations rarely have resuspension of sediment in one location

exceeding one hour (Duran et al., 2012). Based on the above, the T/DP-E Part 2 resuspension time of 1 hour was selected as a representative duration.

 METHOD APPLICATION AND RESULTS

Experiments were conducted on Cu, Co and Sr using the T/DP-E parts 1 and 2 and consisted of 28 day (672 hr) full test removal period, a 1 hr resuspension event, followed for an additional 96 hr period to assess remobilization and irreversibility. Additional 96 hr removal experiments were also conducted on Cu, Co, Ni, Zn, Pb and Ag to determine the applicability of the method to a range of metals.

Substrate analysis

Results from the geochemical analyses of three substrates are found in Table 4. Based on TOC and % Fe values, the LBS substrate was found to be good match with the 10th percentile of typical European sediment characteristics (ARCHE, 2013) (Table 3). Additionally, the IBS and HBS substrates correspond well with the ARCHE 2013 average and 90th percentile values respectively. From the results of the particle size analysis, substrate LBS could be described as a medium sand, substrate IBS as a coarse sand and substrate HBS as predominantly silt and clay (Supplemental Data, ST3).

Results for Cu removal and remobilisation

A summary of dissolved and total metal concentrations corrected for dilution for the 1 mg/L Cu, Co and Sr solutions with LBS can be found in the Supplemental data (ST4).

Measurements taken during the Cu test show D.O. varied between 7.8 and 9.3 mg/L (average 8.2 mg/L). The average temperature during the same time period was $22.5^{o}C$

with a range of 20.0 – 23.2°C. The average pH in the three procedural blanks (10x dilute OECD pH 6 medium, no metal and no substrate) was 6.10 with a range of 5.90 – 6.28. The average pH for the flasks with 10 g of the LBS was 6.05 with a range of 5.82-6.21.

For the three procedural blanks, with the exception of elevated Cu readings at 0 and 2 hr which may be due to carryover on the ICP-MS, the average absolute concentration of Cu was ≤1.0 µg/L throughout the duration of the 672 hr rapid removal examination.

For the 1 mg/L Cu solutions with the 10 g/L LBS loading, the net average $Cu_{(aq)}$ concentration at 0 hr was 915 µg/L ($\sigma\% = 2$). This decreased to 198 µg/L ($\sigma\% = 33$) by 96 hr which would represent a 78% removal of Cu over 96 hrs. By 672 hrs, the $Cu_{(aq)}$ concentration was 44 µg/L ($\sigma\% = 8$) (corrected for dilution) which would represent 95% removal of Cu. Dissolved Fe concentrations were also measured, peaking at 6 hr at 24 µg/L (Figure 1). Fe measurements were considered optional in this study but do provide an additional weight of evidence, particularly as a marker for substrate resuspension.

At select times, samples were also collected for total Cu and total Fe (Table ST4). Initially, total Cu is greater than dissolved Cu indicating some fraction of the total Cu in the system is associated with particles. At 96 hrs, the two values are more comparable. At 672 hrs, essentially all of the water column Cu is dissolved. However, total Fe concentrations of 439 µg/L were measured at 2 hr, suggesting that fine grained Fe-bearing minerals were still in solution.

After the 672 hr reading was taken, the flasks were shaken for 1 hr. The shaking was stopped and samples were collected within 0.5 hr and subsequently at 1, 2, 6 24, 48 and 96 hr. The net corrected average $Cu_{(aq)}$ concentration at 672 hr (or 0 hr before

resuspension) was 44 µg/L ($\sigma\% = 8$). After shaking, this increased slightly to 53 µg/L

($\sigma\% = 59$) at 1 hr (after resuspension) but then continued to decline to 33 µg/L ($\sigma\% = 14$)

by 96 hrs after resuspension (Figure 1). Therefore, it is shown that the resuspension of the

substrate does not release many of the Cu ions into solution. Total Cu concentrations at

0.5 hr after resuspension increase to 105 µg/L compared to the dissolved concentration of

34 µg/L. However total Fe concentrations increase to 1980 µg/L at the same sampling

time compared to the dissolved concentrations of 41 µg/L.

Results for Co removal and remobilisation

Measurements during the entire test show D.O. varied between 8.0 and 9.0 mg/L

(average 8.4 mg/L). The average temperature during the same time period was 21.5°C

with a range of 19.1 –22.1°C. The average pH for the flasks with 10 g of the LBS was

6.12 with a range of 5.94-6.29.

For the three procedural blanks, with the exception of elevated Co readings at 0 and 2 hr

(as with Cu) which may be due to carryover on the ICP-MS, the average absolute

concentration of Co was ≤1.2 µg/L throughout the duration of the 28-day rapid removal

examination.

For the 1 mg/L Co solutions with the 10 g/L LBS loading, the net average $Co_{(aq)}$

concentration at 0 hr was 983 µg/L ($\sigma\% = 1$) (Figure 2). This decreased to 533 µg/L ($\sigma\%$

$= 2$) by 96 hr which would represent a 46% removal of Cu (corrected for dilution) over

96 hrs. By 672 hr, the $Co_{(aq)}$ concentration was 453 µg/L ($\sigma\% = 7$) (corrected for dilution)

which would represent 54% removal of Co at 672 hrs. Dissolved Fe concentrations were

also measured, peaking at 6 hr at 17 µg/L. At select time periods, samples were also

collected for total Co and total Fe. Total Co concentrations in the 96 hr samples are slightly greater than the dissolved Co suggesting some particulate Co still in solution. Total Fe concentrations are higher than the equivalent dissolved concentration (Table ST4).

After the 672 hr reading was taken, the flasks were shaken for 1 hr. Once the shaking stopped, samples were collected with 0.5 hr. The net corrected for dilution average $Co_{(aq)}$ concentration at 672 hr (0 hr before resuspension) was 453 µg/L ($\sigma\% = 7$). After the resuspension, this continued to decline to 416 µg/L ($\sigma\% = 3$) by 96 hr (after resuspension). Therefore, it is shown that the resuspension of the substrate does not significantly release Co ions into solution.

Total Co concentrations at 0.5 hr after resuspension increase to 517 µg/L compared to the dissolved concentration of 450 µg/L. However total Fe concentrations increase to 1560 µg/L at the same sampling time compared to the dissolved Fe concentrations of 36 µg/L (Table ST4).

Results for Sr removal and remobilisation

Measurements during the entire test show D.O. varied between 8.1 and 9.0 mg/L (average 8.5 mg/L). The average temperature during the same time period was 21.7°C with a range of 20.6 –22.5°C. The average pH for the flasks with 10 g of the LBS was 5.90 with a range of 5.61 - 6.19.

For the three procedural blanks, the average absolute concentration of Sr was ≤1.8 µg/L throughout the duration of the 672 hr rapid removal examination. For the 1 mg/L Sr

solutions with the 10 g/L LBS loading, the net average $Sr_{(aq)}$ concentration at 0 hr was 916 µg/L (σ% = 2). This decreased to 581 µg/L (σ% = 3) by 96 hrs (Figure 3), which would represent a 37% removal of Sr over 96 hrs. By 672 hrs, the $Sr_{(aq)}$ concentration was 541 µg/L (σ% = 5) which would represent 41% removal of Sr. The average absolute Sr concentration in substrate blank flasks (Sr solution with no substrate) decreased from 913 µg/L to 628 µg/L over the course of 672 hrs primarily because of sampling and the addition of the 10x dilute OECD pH 6 media. The corrected Sr concentration in the substrate blank at 672 hrs is 802 µg/L which would suggest that some Sr (approximately 5- 10%) is being removed by another mechanism other than dilution, possibly sorption on to the glass or precipitation. The corrected (for both dilution and additional losses) 672 hr removal of Sr is 34%. Dissolved Fe concentrations were also measured, peaking at 6 hr at 21 µg/L. At select time periods, samples were also collected for total Sr and total Fe. Total Sr values are generally slightly higher than dissolved values whereas total Fe concentrations are consistently higher than the dissolved Fe values (Table ST4).

Immediately after the resuspension, the net corrected average $Sr_{(aq)}$ concentration was 508 µg/L (σ% = 1) compared to 541 µg/L before suggesting that the increased agitation increased metal removal and that the resuspension of the substrate does not release Sr ions into solution. Total Sr concentrations at 0.5 hr after resuspension increase slightly to 517 µg/L compared to the dissolved concentration of 508 µg/L. However total Fe concentrations increase to 1090 µg/L at the same sampling time compared to the dissolved Fe concentrations of 41 µg/L.

Further application of the method

A series of 96 hr experiments were conducted on Cu, Co, Ni, Zn, Pb and Ag. The purpose of this was to determine, in a shorter timeframe than the full 672 hr test, if the method is sufficiently robust to distinguish between those metals that are rapidly removed from the water column and those that are not. In all other aspects, the setup remained identical to the 672 hr test described above.

For the Cu 96 hr experiment using the LBS, a 78% removal of Cu was observed compared to 87% removal at the 96 hr sampling point in the 672 hr experiment. The experiment was repeated at the University of Michigan and 83% Cu was removed within 96 hrs. For Co, using the LBS, a 46% removal of Co was observed both when the experiment was run and stopped at 96 hrs and also at 96 hrs when the experiment was run for the full 672 hrs. For Ni, using the LBS, 55% removal was observed when the experiment was run at CanmetMINING and 61% at the University of Michigan. This would suggest good reproducibility of the test method between labs.

Ni removal was examined over a 96 hr period using the three different substrates; LBS, IBS and HBS. Measurements show D.O. varied between 7.9 and 8.6 mg/L (average 8.4 mg/L). The average temperature was $21.6^{o}C$ with a range of $20.5 - 22.0^{o}C$. The average pH in the three procedural blanks (10x dilute OECD pH 6 medium) was 6.06 with a range of 5.97 – 6.17. The average pH in the three substrate blanks (1 mg/L Ni solution with no substrate) was 6.14 with a range of 6.04 – 6.20. The measured pH values averaged among each substrate loading are plotted as a function of time in Figure 4a. The graph clearly demonstrates that while addition of substrate IBS results in a pH increase to

6.6 and addition of substrate HBS results in a decrease in pH down to 5.6, the samples with substrate LBS remained stable in the range between 5.9 – 6.1.

For the three procedural blanks, the average absolute concentration of Ni was ≤1.5 µg/L throughout the duration of the T/DP-E examination. The kinetic data for $Ni_{(aq)}$ are presented in Figure 4b. For the 1 mg/L Ni solutions with the 10 g/L LBS loading, the $Ni_{(aq)}$ concentration at 0 hr was 959 µg/L ($\sigma\% = 2$). This decreased to 429 µg/L ($\sigma\% = 4$) by 96 hr which would represent a 55% removal of Ni. With the 10 g/L IBS loading, the $Ni_{(aq)}$ concentration at 0 hr was 960 µg/L ($\sigma\% = 1$). By 96 hrs, the $Ni_{(aq)}$ concentration was 214 µg/L ($\sigma\% = 8$) which would represent 78% removal of Ni. For the 1 mg/L Ni solutions with 10 g/L of sediment HBS, the $Ni_{(aq)}$ concentration at 0 hr was 965 µg/L ($\sigma\% = 2$). By 96 hr, the $Ni_{(aq)}$ concentration was 492 µg/L ($\sigma\% = 5$) which would represent a 49% removal of Ni over 96 hrs.

A regression analysis of the Ni kinetic data was performed followed by extrapolation to 672 hrs using TableCurve 2D v5.01.01. Comparing the 672 hr Ni concentrations derived from the regression analysis and extrapolation, the LBS is predicted to be the worst performer with respect to metal removal, although at 96 hr it is the HBS that has the lowest rate of metal removal.

Additional 96 hr removal experiments were conducted using solutions of Zn, Pb and Ag and using the LBS. For procedural blanks, the average absolute concentration of Zn was ≤6.2 µg/L, Pb ≤5.8 µg/L and Ag ≤0.02 µg/L throughout the duration of the T/DP-E examination.

For the 1 mg/L Zn solutions with the 10 g/L LBS loading, the $Zn_{(aq)}$ concentration at 0 hr was 1135 μg/L (σ% = 2). This decreased to 492 μg/L (σ% = 3) by 96 h which would represent a 57% removal of Zn over 96 hrs (Figure 5a).

The $Pb_{(aq)}$ concentration at 0 hr was 640 μg/L (σ% = 1). This decreased to 32 μg/L (σ% = 35) by 96 hr which would represent a 95% removal of Pb over 96 hr (Figure 5b), mostly within the first 2 hours of the experiment. It is important to note that although sufficient $Pb(NO_3)_2$ was added to the 10x dilute OECD aquatic medium to make a 1 mg/L Pb solution, it is believed that a quantity of Pb ions precipitated out of solution. Unlike the Ag solution however, this solution was not filtered prior to the T/D examination. However, the fact that the substrate blank (corrected for dilution and the blank) remained stable over the 96-hr period would suggest that no further precipitation of Pb occurred which could be mistaken for Pb removal by the sediment.

It is important to note that due to the precipitation of Ag from solution, it was not possible to prepare a solution of a concentration greater than 0.1 mg/L. Sufficient $AgNO_3$ was added to the 10x dilute OECD aquatic medium to prepare a 1 mg/L solution but this was then filtered to remove any precipitate. There is a very slight decline of Ag in the corrected sediment blank from 50 to 46 μg/L which might suggest some further, but very minor precipitation. For the 1 mg/L Ag solutions with the 10 g/L LBS loading, the $Ag_{(aq)}$ concentration at 0 hr was 50.2 μg/L (σ% = 1). This decreased to 5.5 μg/L (σ% = 29) by 96 hrs which would represent a 89% removal of Ag over 96 hr (Figure 5c).

DISCUSSION

This study was designed to assess whether an extension to the T/DP (T/DP-E) could be used to measure the rate and extent of metal removal from the water column and the potential for remobilization. It was recognized that the T/DP-E needed to be sufficiently robust as to be able to distinguish between metals with a wide range of reactivities in order to be able to yield consistent hazard classification outcomes for each metal. The primary difference between the T/DP and the T/DP-E is that the former measures the reaction kinetics of metal release from a metal-bearing substance into an aquatic media whilst the latter measures metal removal in the presence of a substrate.

Previous studies (Huntsman, 2018; Skeaff et al., 2008, 2011, 2014) have demonstrated that properly equipped and operated laboratories can apply the T/DP to metal-bearing substances to yield acute and chronic aquatic hazard classification outcomes that are reliable, consistent and in agreement with other laboratories. In the development of the T/DP-E, it was therefore important to maintain as many elements of the original T/DP as possible. The procedure for blanks and replicates, in addition to the schedule for sampling, remained the same in order to maintain the same level of robustness.

As expected, pH control is an important criterion for the T/DP-E. With some substrates, it was difficult to maintain pH of the solution at the target value of 6 for the entire duration of the experiment. Higher pH values, near pH 7, increase metal partitioning as complexation sites increase. This phenomenon supported selection of LBS for use in the T/DP-E, as pH did not increase above 6.

In many aquatic systems, anoxic or anaerobic conditions prevail in buried sediments and gradients in oxygen availability develop. The proposed test method needed to strike a balance between 1) recognizing the complexity of aquatic systems, and 2) standardization of the conditions, repeatability, reproducibility, and comparability between different metals. The test method does include the relevant processes for metal removal (binding to substrate, settling, resuspension), but anoxic reactions are excluded. This may lead to an underestimation of the removal and detoxicification of some metals (those that are strongly removed under anoxic conditions) in aquatic systems.

From the 96 hr Ni removal data using the three different substrates, it is clear that substrate selection is a key factor influencing the outcome of the experiments. For the T/DP-E to become an accepted "standard" methodology, a universally accepted, widely-available substrate must be identified. On-going research is focusing on the identification of the properties of an appropriate natural or artificial substrate for global use in hazard classification under the GHS or CLP. This research is also looking to identify optimal parameters describing a suitable LBS for use in the T/DP-E to allow for laboratories to identify a local sediment for use in the protocol. The parameters used to select should be simple from an analytical perspective; to allow for a wide range of laboratories to identify local, suitable sediments.

Results from the T/DP-E Part 1 with the initial 1 mg/L Cu solution demonstrate that Cu reaches 95% removal from the water column within the 672 hr testing period using the LBS. The Cu test did not demonstrate remobilization of Cu during the resuspension event, unlike dissolved and total Fe concentrations, which increased following

resuspension. Fe concentrations also increased following resuspension with the Co and Sr experiments.

Following the same procedure for Sr as for Cu, it was shown that only 34% of Sr was removed due to binding to the substrate. This clearly demonstrates that the newly developed T/DP-E is sufficiently robust to be able to distinguish between those metals that are rapidly removed from the water column and those that are not. This was demonstrated again with the 96 hr experiments with Pb and Ag showing a very rapid and then readily perceivable reduction in removal rates between days 1 to 4, with dissolved concentrations appearing to start levelling off as equilibrium is approached. The experimental data for Ni and Zn do not demonstrate this same effect.

Based on the data reported here, a numerical model was developed to provide mechanistic insight into important processes controlling dissolved metal removal from the water column during the experiments performed using the T/DP-E (Part 1) (see Burton et al., this issue). This model indicates that, depending on the rate of metal adsorption (relative to the rate at which substrate particles settle), some of the metal in the water column adsorbs to the particles and settles with them. Once most of the particles have settled from the water column, the dissolved and total metal concentrations become equal indicating essentially all water column metal is dissolved. Despite the lack of suspended particles in the water column, metal removal continues via transport to and direct adsorption by the settled substrate particles which form the sediment layer. So, the key phenomena responsible for removal of metals were (1) kinetically-controlled adsorption to rapidly-settling substrate particles and (2) transport to and direct adsorption by the settled substrate particles.

CONCLUSIONS

The dominant processes affecting metal fate and effects in aquatic environments are effectively demonstrated in the T/DP-E, including such processes as metal binding to suspended solids with associated complexation and transformation processes rapidly occurring, settling of metal associated suspended solids, and initial diagenesis reactions resulting in decreased remobilization potential.

This work was aimed at extending the T/DP to establish the rate and extent of partitioning of metals from the water column so that the resulting data can be used in metals chronic hazard classification. Through the addition of a substrate, data on water column residence times and processes can be generated. The data presented in this study supports the use of the proposed extension to the T/DP method (TD/P-E), thus providing a means to quantify the rate of metal removal from the water column and addressing this important process in the determination of the chronic classification of metals.

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Figures

Figure 1: Net corrected Cu(aq) and dissolved Fe data for the 1 mg/L Cu solutions at pH 6 using the LBS for T/DP-E Part 1 and 2. Blue shaded area indicates the re-suspension event.
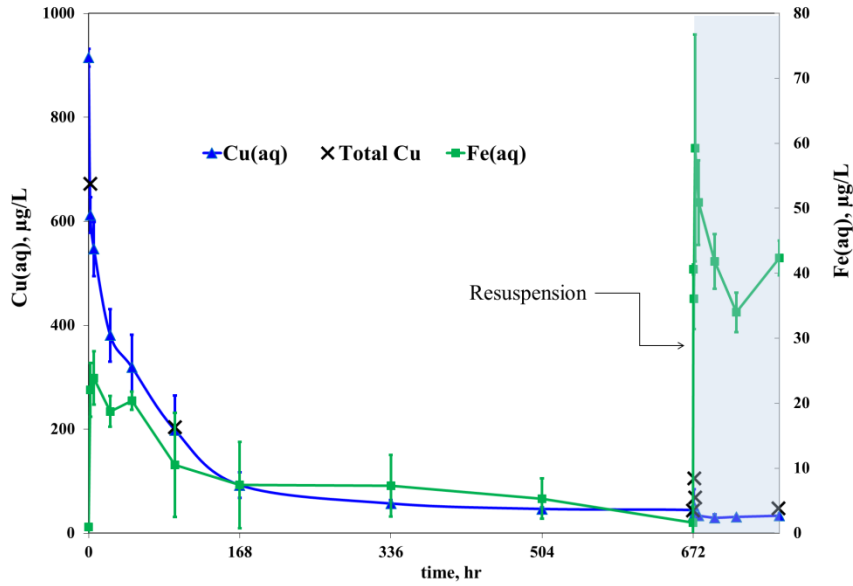

Figure 2: Net corrected Co(aq) and dissolved Fe data for the 1 mg/L Co solutions at pH 6 using the LBS for T/DP-E Part 1 and 2. Blue shaded area indicates the re-suspension event.
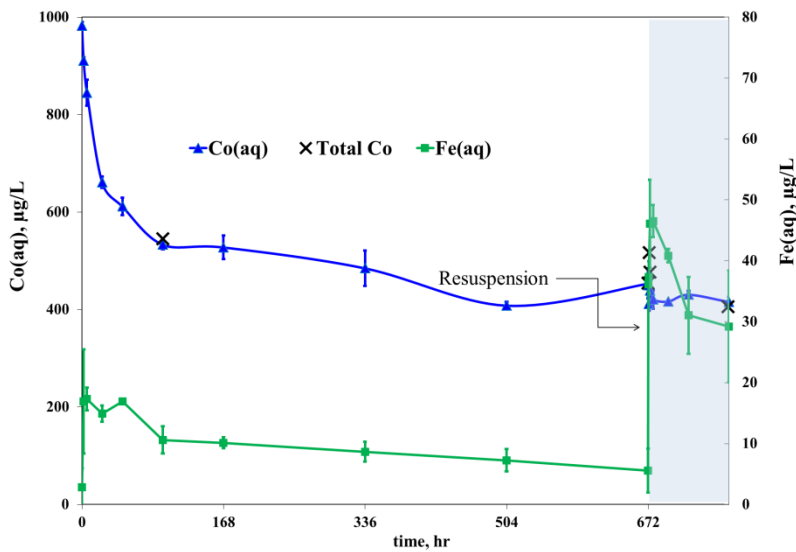
Figure 3: Net corrected Sr(aq) and dissolved Fe data for the 1 mg/L Sr solutions at pH 6 using the LBS for T/DP-E Part 1 and 2. Blue shaded area indicates the re-suspension event.
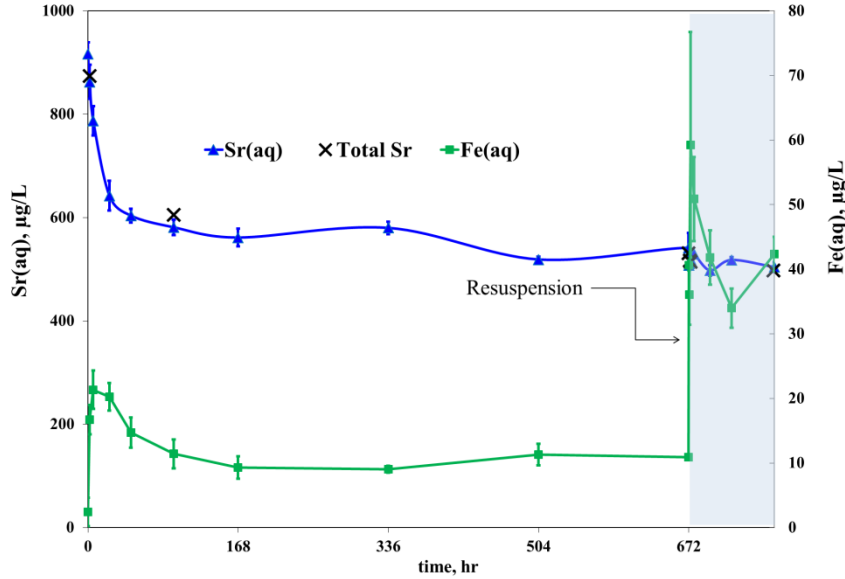

Figure 4. (a) variation in measured pH as a function of time for the 1 mg/L Ni solutions with the 3 different substrates and (b) net kinetic Ni(aq) data for the 1 mg/L T/D solutions at pH 6 in the presence of three different substrates. The dashed lines indicate results uncorrected for the dilution.
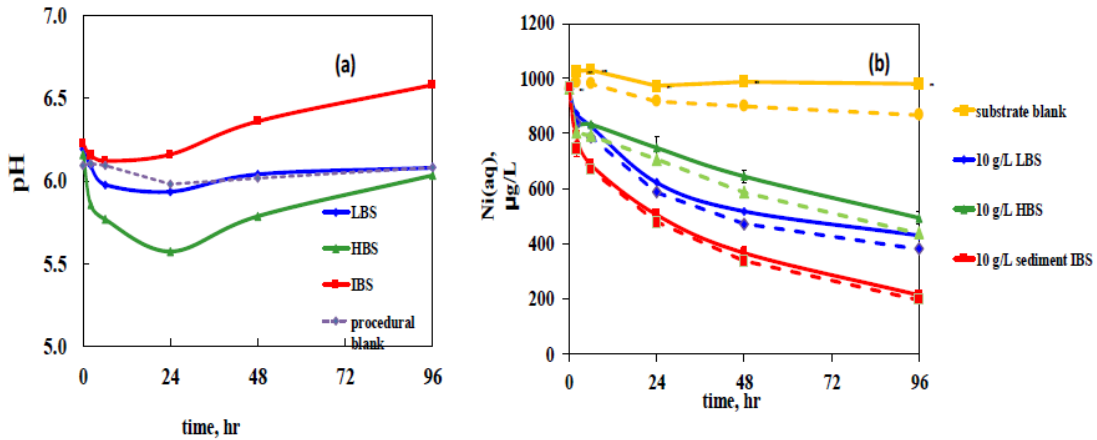
Figure 5. Net T/D kinetic Zn(aq) (a), Pb (b) and Ag (c) data for the 1 mg/L solutions at pH 6 in the presence of no substrate – orange line and LBS – blue line. The dashed lines indicate results uncorrected for the dilution.
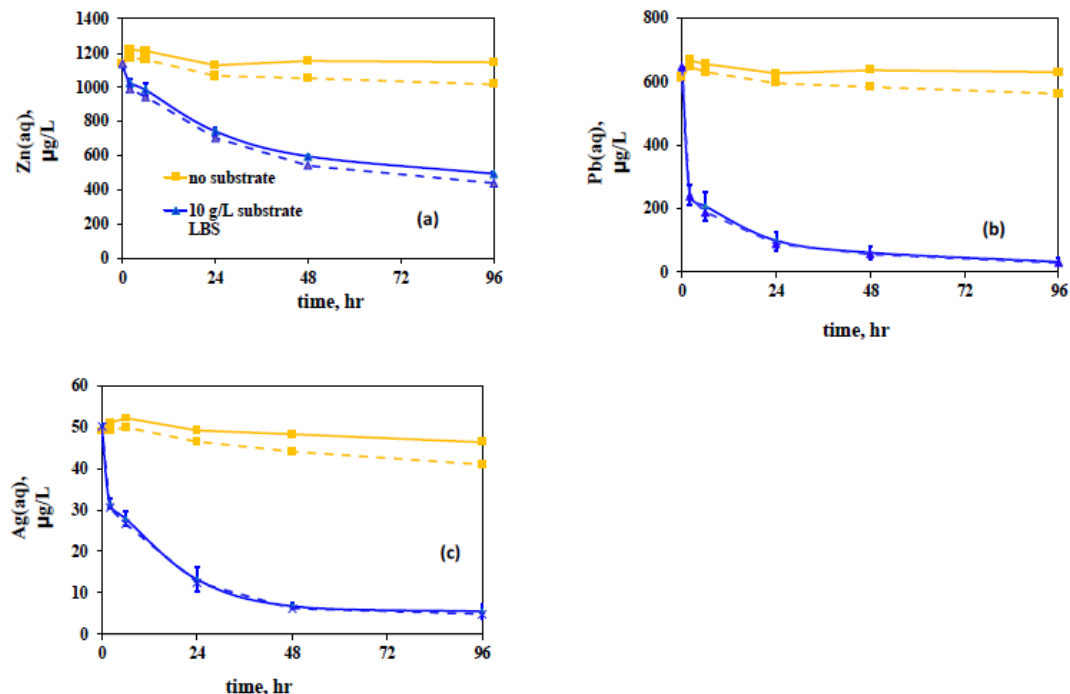

Table 1: Composition of 10X dilute OECD 203 (ISO 6341) aqueous medium (no micronutrients) for a target pH of 6

Component	M.W.	mg/L	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	SO_4^{2-}	Na^+	K^+	mmol/L
$CaCl_2 \cdot 2\,H_2O$	147.01	29.40		8	14.18					0.20
$MgSO_4 \cdot 7\,H_2O$	246.47	12.33	1.22				4.80			0.05
$NaHCO_3$	84.01	6.48				4.70		1.77		0.077
KCl	74.55	0.58			0.27				0.30	0.0077
totals, mg/L			1.22	8.01	14.45	4.70	4.80	1.77	0.30	
mmol/L			0.05	0.20	0.41	0.077	0.05	0.077	0.0077	
hardness as $CaCO_3$, mg/L		25.0								

calculated* pH of medium when in equilibrium with 0.5% CO_2-balance air at 21.5°C: 6.05

* FactSage 5.5. 2007 www.factsage.com.

Table 2: T/DP extension testing scheme at pH 6

Cu test no. TRS-	procedural blank: no metal, no substrate	metal loading 1 mg/L, no substrate	metal loading 1 mg/L, substrate loading 10 g/L	Co test no. TRS-	procedural blank: no metal, no substrate	metal loading 1 mg/L, no substrate	metal loading 1 mg/L, substrate loading 10 g/L	Sr test no. SRS-	procedural blank: no metal, no substrate	metal loading 1 mg/L, no substrate	metal loading 1 mg/L, substrate loading 10 g/L
1	x			10	x			19	x		
2		x		11		x		20		x	
3			x	12			x	21			x
4	x			13	x			22	x		
5		x		14		x		23		x	
6			x	15			x	24			x
7	x			16	x			25	x		
8		x		17		x		26		x	
9			x	18			x	27			x

Table 3: Aqua regia extractable average concentrations of Fe, and TOC average concentrations in European sediments (ARCHE, 2013)

Percentile	(Aqua R) in %	TOC (%)
P10	0.75	1.3
Average	2.25	3.7
P90	4	6.7

Table 4: Results of substrate geochemical analysis

Substrate	Type	Fe (Aqua R) wt%	TOC (%)	TC(%)	Co (ppm)	Mn (ppm)	Ni (ppm)	Ag(ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)
LBS	Stream	1.56	1.2	1.1	11.9	506	34	0.05	18	54.7	13.5
IBS	Stream	3.61	2.5	3.4	17.4	1413	62	0.26	17		21.4
HBS	Lake	5.98	3.3	3.1	36.3	1147	94.6	0.23	87	14.5	15.0