

Methodological Aspects of the Extended Transformation-Dissolution Protocol

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Summary

The Extended Transformation/Dissolution Protocol (T/DP-E) is proposed for use in the context of hazard classification of metals based on their removal rates from the water column (Burton et al., 2019b). This note clarifies two methodological aspects that have been identified as outstanding issues: the selection of a representative substrate and a consideration of the impact of different stirring conditions.

We applied the T/DP-E to a set of 12 substrates and confirmed that metal binding is related to the contents of Fe, Al, Mn, organic carbon, and fine particles in the substrate. Referring to existing databases (most notably FOREGS), the contents of Fe and organic carbon were identified as the most suitable basis for substrate selection criteria.

A suitable substrate was identified as having iron content around or below 13 g/kg, and organic carbon content around or below 0.84%. This ensures that the substrate is representative of reasonable worst case conditions for metal binding, corresponding approximately to the metal binding properties of the 10th percentile of European sediments.

The T/DP-E was developed without stirring, in order to mimic particle settling. Modelling showed that continuous stirring accelerates metal removal, because the kinetic convection-diffusion of dissolved metal ions towards the particles is faster. This is in line with previous findings that vigorous resuspension of settled substrates in the T/DP-E results in some release of Fe, but not of Cu. A static incubation during the T/DP-E seems therefore appropriate, since it represents the condition of lowest metal binding.

Questions addressed

This note clarifies issues related to the test conditions in the extended T/Dp, in particular:

- Standardization and relevance of the test substrate used in the extended T/Dp
- The relevance of the lack of stirring during the settling phase

Selecting a representative substrate for the T/DP-E

The T/DP-E protocol requires an initial seeding of a substrate, allowing for a metal binding to occur (Huntsman et al., 2019). It should be ascertained that this substrate is representative of reasonable worst case conditions for metal binding, which implies that it represents a situation with relatively limited metal binding. We developed criteria that define the selection of a suitable substrate, using properties of European sediments as basis.

Substrate properties that are likely to affect metal binding include the content of organic carbon (OC) and oxyhydroxides of Fe, Al, and Mn. European sediment types vary widely and

have been grouped into metalloregions, reflecting geographic differences in mineralogy (ICMM, 2007). Sediments in some parts of Europe have high levels of the proposed substrate selection criteria, while others have lower levels. Several databases with information on these properties of European sediments were identified (Annex 1). The most complete database with consistent sampling across Europe is the FOREGS database. It should be noted, in addition to the above properties, also pH and acid volatile sulphide (AVS) may affect metal binding. These factors already reflect reasonable worst case conditions as dictated by the experimental set-up of the T/DP-E: pH is standardized at the value minimizing metal binding (usually pH 6), and drying of the substrate causes oxidation and removal of any AVS that is present.

Further work confirmed the link between the abovementioned substrate properties and metal binding in the T/DP-E. Twelve substrates were identified representing a wide range of physicochemical properties, but mostly having relatively low contents of Fe, Al, Mn, organic matter, and fine particles. These substrates were evaluated using the T/DP-E to determine removal rates of metals using copper as example, and removal rates were compared to the substrate properties (Annex 2 and Burton et al., 2019a). Regression analysis shows that the contents of all considered properties were significantly ($p < 0.05$) related to Cu removal. This confirms that metal removal is slower in substrates with low concentrations of Fe, Al, Mn, organic carbon, and fine particles.

Iron and organic carbon were identified as the most suitable properties for developing criteria for substrate selection in the T/DP-E, as they can be used as surrogates for the contents of Mn, Al, and fine particles. Substrates with low Fe content also tend to have low Al and Mn contents, which is confirmed by strong correlations ($r = 0.72$ and 0.56) in the FOREGS database. Furthermore, substrates with low organic carbon also tend to have low contents of fine particles ($r = 0.65$ in the Burton et al., 2007 dataset). It is proposed that suitable substrates for the T/DP-E have total recoverable iron contents around or below 13 g/kg, and organic carbon content around or below 0.84%. These values reflect the 25th percentile of the FOREGS data. The combination of these two criteria ensures that less than 10% of the sediments in Europe are retained (71 out of 844 FOREGS samples), meaning that the metal binding properties of acceptable substrates are below the 10th percentile of those encountered in Europe. This reflects reasonable worst-case conditions. Seven out of the 12 studied substrates in Annex 2 fulfil the above criteria.

In addition to the Fe and organic carbon criteria, it is recalled that the T/DP-E method specifies suitable substrates should not induce unacceptable pH drift in the T/DP-E, and should come from an uncontaminated source with concentrations of the metal of interest close to natural background.

The stirring conditions in the T/DP-E

The T/DP-E includes incubation of metal ions and substrate without stirring in the 28-day period after initial homogenization. A question was raised about the impact of continual agitation/stirring on metal removal. The rationale behind not stirring was to allow for loss of particles and particle-associated metals by settling, a process that is well-established for lakes and rivers. Agitation after 28-days in the second T/DP-E phase was included to assess episodic resuspension events.

The T/DP-E experiment results indicate loss of dissolved trace metals over the 28-days static incubation via adsorption and settling from the water column, with negligible releases during subsequent agitation (Huntsman et al., 2019). Continual stirring would limit settling and keep most of the substrate suspended in the water column. Model simulations indicate that, under these conditions, dissolved metal loss still occurs through metal ion adsorption to suspended substrate particles (see Annex). In fact, without settling, higher water column substrate concentrations increase the rate of metal ion loss via adsorption. Particle-associated (and generally non-bioavailable) metal will remain suspended in the water column as long as agitation occurs. When agitation stops, particles and associated metal will settle very rapidly, as observed in Huntsman et al. (2019). In summary, continual stirring does not substantially change dissolved metal removal, and settling of particles is an established process which transfers metal to its ultimate sink (i.e., the sediment). Therefore, specifying static incubation conditions in the settling phase of the T/DP-E is justified.

Conclusions

The contents of iron and organic carbon were identified as suitable criteria for substrate selection in the T/DP-E. These are related to the measured metal removal in the T/DP-E, and can serve as surrogate for other substrate properties. Based on a sediment survey for Europe (FOREGS), a reasonable worst case (10th percentile) substrate has iron content around or below 13 g/kg, and organic carbon content around or below 0.84 g/kg. These criteria may be included in the T/DP-E protocol.

The experimental conditions of the T/DP-E settling phase, consisting of a 28-day static incubation phase, are justified. Model simulations confirm that continual stirring does not substantially change metal removal, and settling of particles is an established process which transfers metals to its ultimate sink, the sediment.

References

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Annex 1 – Properties of stream sediments in Europe

The available databases on stream sediments in Europe were investigated in order to evaluate the properties of a suitable substrate for use in the T/DP-E test. The following data sources were identified:

1. The most extensive database (circa 850 data points) on physico-chemical parameters in stream sediments is from the FOREGS Geochemical Baseline mapping program, which aimed to provide high quality, multi-purpose geochemical baseline data for Europe (Salminen et al, 2005).
2. Sediment samples from a monitoring survey conducted in 2007 (Vangheluwe et al, 2008) by the United Kingdom (Environment Agency), Finland (Geological Survey of Finland) and Spain (Ministerio de Medio Ambiente) established baseline concentration for several sediment parameters from ecoregions (Finland: 25 data points, Spain: 20 data points and United Kingdom: 16 data points).
3. Burton et al (2007) measured several sediment characteristics in wadeable headwater streams of a wide variety of ecoregions of Western Europe (84 sites, 10 countries, nine ecoregions). This database is referred to as “EU headwaters”. Since this database focused on headwater streams, gravel and sandy gravel sediments are likely overrepresented.
4. Data on organic carbon and AVS content are available for Flanders (Belgium: 200 data points) (Vangheluwe et al, 2005).

Tables 1A through 1D report the 10th, 25th, 50th (median), 75th, and 90th percentile of sediment properties according to these databases.

Table 1A: Fe concentrations (mg/ kg dry wt., measured in aqua regia extract)

Database	# data points	P10	P25	P50	P75	P90
FOREGS	845	7540	12500	19700	28600	39600
UK	16	12920	22525	26050	32375	40700
Finland	25	34620	38900	54200	64600	74700
Spain	20	12830	14375	18100	23950	27160
EU headwaters	84	3328	6903	12050	27350	45490

Table 1B: Al concentrations (mg Al/kg dry wt., measured by X-Ray Fluorescence)

Database	# data points	P10	P25	P50	P75	P90
FOREGS	850	10280	17730	27254	35453	42071

Table 1C: Mn concentrations (mg/ kg dry wt., measured in aqua regia extract)

Database	# data points	P10	P25	P50	P75	P90
FOREGS	845	163	264	452	795	1405
UK	16	242	342	520	834	1048
Finland	25	852	1020	1620	3540	5156
Spain	20	152	191	255	331	516
EU headwaters	84	89	113	245	644	1178

Table 1D: Organic Carbon (OC) concentrations (%)

Database	# data points	P10	P25	P50	P75	P90
FOREGS	847	0.41	0.84	1.7	2.9	4.4
UK	16	2.0	2.6	5.5	7.9	10.7
Finland	25	2.2	2.6	4.9	7.1	8.9
Spain	20	1.1	1.2	1.7	2.6	3.8
Flanders	200	0.3	0.5	1.2	2.4	3.8
EU headwaters ^A	84	0.13	0.35	1.1	3.6	7.2

^A Database focused on headwater streams, and therefore includes a large number of gravel and sandy gravel sediments

Annex 2: Metal binding in the T/DP-E is related to substrate properties

Table 2 shows the 12 substrates for which metal binding was evaluated according to the T/DP-E method. The concentrations of Fe, Al, and Mn were measured in aqua regia extracts, and removal rate of copper is expressed as half-life. The correlations between the copper removal and substrate properties are shown in Table 3 and Figure 1. All relationships are statistically significant at the level of $p < 0.05$, although the relationship with Mn content is only borderline significant. Data from Burton et al., 2019a.

Table 2: Properties of various substrates, and corresponding copper removal (as half-life) as measured in the T/DP-E.

Sample	Fe mg/kg	Mn mg/kg	Al mg/kg	OC %	Fine particles %	Cu half-life h
TSL	6347.5	83.7	14718.0	17.4	16.0	0.46
SAL	22857.3	651.1	6392.5	11.2	37.6	0.52
RBR	4334.3	170.3	1670.4	0.8	8.0	0.52
RBS	7563.6	506.0	1303.0	5.9	2.7	2.59
RSH	3931.0	156.5	1105.3	0.6	1.1	5.41
POR	3036.2	43.1	833.7	0.5	1.6	6.91
MLC	8181.7	242.8	1056.5	1.6	1.1	9.50
FLE	4594.6	135.3	1298.1	0.9	0.9	9.56
FIS	1086.9	14.6	1373.5	2.4	2.2	21.53
SPB	2644.2	71.4	801.9	0.9	0.4	73.58
SPR	2930.0	61.7	705.2	0.9		90.85
MAP	2367.6	41.6	1191.8	0.3		94.59

Table 3: Correlation analysis between substrate properties and copper removal, after log-transformation of the data.

	Correlation coefficient (r)	p-value
Fe	-0.66	0.018
Mn	-0.58	0.048
Al	-0.74	0.006
OC	-0.64	0.024
Fine particles	-0.90	0.0004

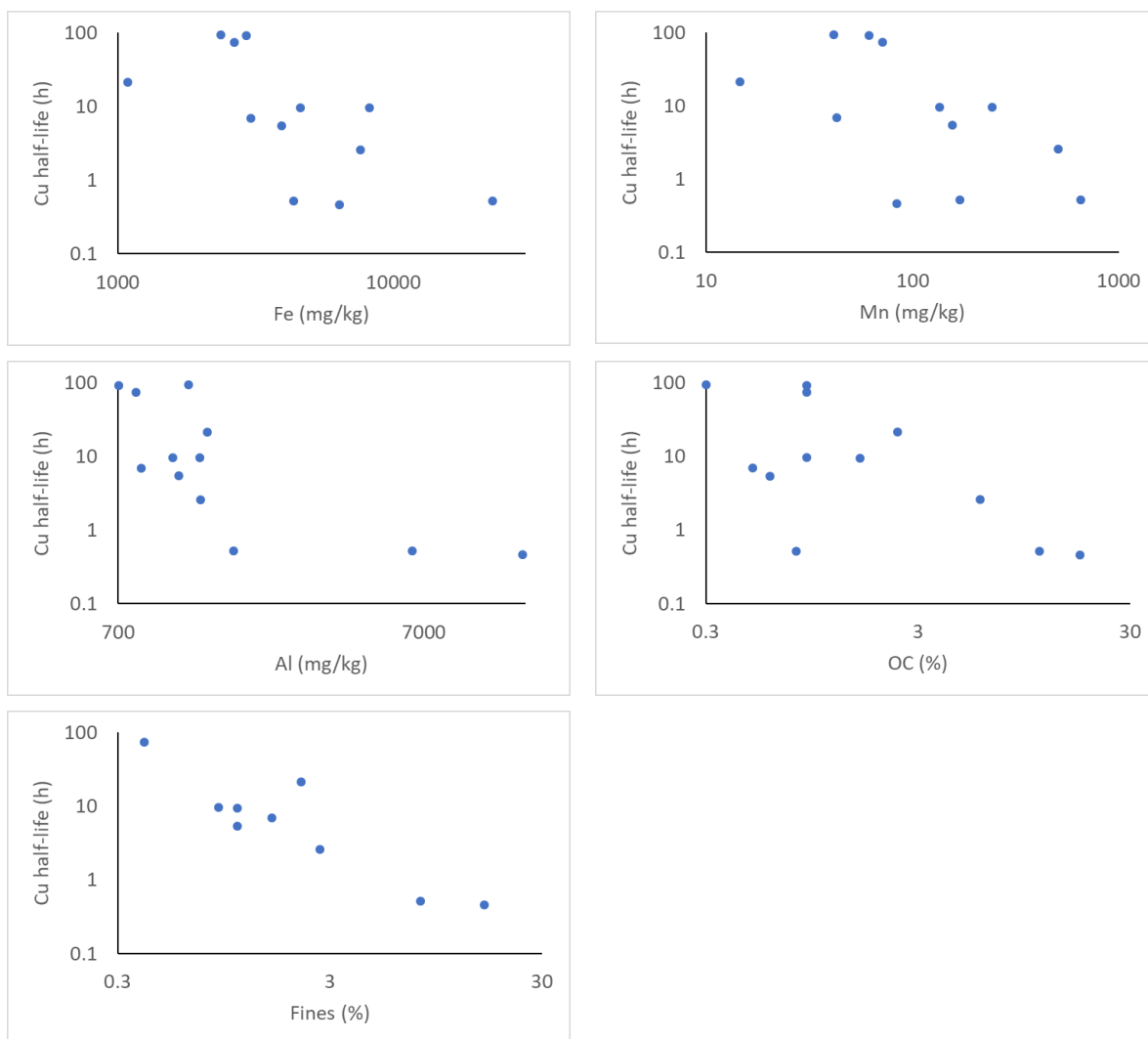


Figure 1: Plots of metal removal in the T/DP-E (using copper as example and expressed as half-life) versus various substrate properties.

Annex 3: The impact of stirring conditions in the T/DP-E

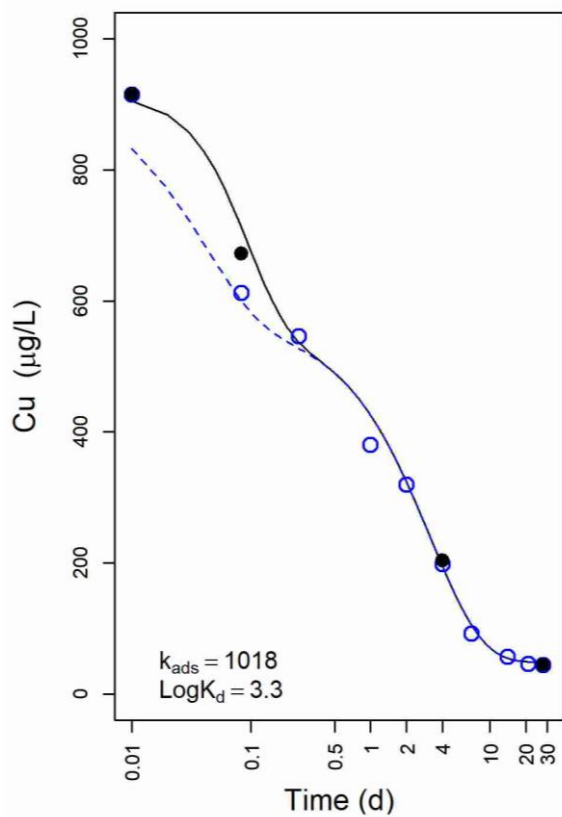
The loss of solids and metal from the water column of lakes and rivers is well-established and discussed in seminal water quality modeling texts (e.g., *Principles of Surface Water Quality Modeling* by Thomann and Mueller). As discussed in Burton et al. (2019b), metals form bonds with various functional groups present on suspended particulate matter (SPM). Particle-associated metal resulting from this interaction is then subject to the same physical transport process as the SPM including erosion and sedimentation (settling). Settling transports SPM from the water column to the sediment, thereby representing a key removal process for SPM and any associated metals.

To assess the key removal process of settling of particle-associated metal in the T/DP-E, stirring was omitted during the 28-day period following initial mixing of substrate particles throughout the water column. Mechanistic modeling of the T/DP-E experiments supported the conclusion that settling of particulate metal was indeed an important process but it was short-lived given the rapid settling of particles. The model indicated that metal transport to and direct adsorption by settled substrate was an additional important removal process causing water column dissolved metal concentrations to decrease (see Burton et al. (2019b) for more details).

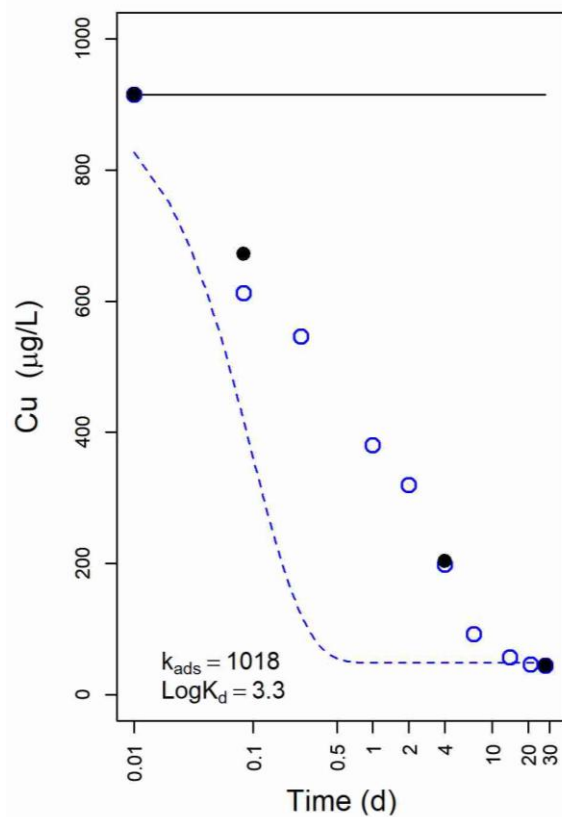
Hydrodynamic conditions and morphology of surface waters dictates transport of SPM. Shear forces and turbulence can result in limited settling of SPM and even resuspension/erosion of settled particles. Conditions favoring either particle sedimentation or erosion vary temporally and spatially. For example, high gradient streams are typically comprised of “riffles” and “pools.” Within the “riffles” where fast-moving water is flowing over a hard bottom, SPM are expected to remain suspended and be carried to “pooling” sections of the stream and deposit. Sediment deposited in the pools can vary from coarse sands to fine-grained cohesive material depending on the composition of the suspended solids load. High gradient streams typically feed lower gradient rivers/estuaries or impoundments where water velocity can slow and allow for particle settling. So, settling remains as key aspect of metal fate.

The model developed for the T/DP-E experiments was used to provide insight into the behavior of metals in a hypothetical system stirred continuously during the 28-day period following substrate addition. It was assumed that continual stirring would preclude settling of substrate particles and the associated metal and represent conditions of metal release in a high gradient stream. This was modeled by setting the particle settling velocity to zero in the model. Figure 2 below shows copper T/DP-E simulation results with settling (i.e. no stirring) and without settling (continual stirring). With settling (i.e. the default T/DP-E scenario) total and dissolved copper concentrations decrease as a function of time as described in Burton et al. (2019) (Figure 2A). Without settling, the total amount of copper in the water column remains constant, but the *dissolved copper decreases* as it binds to the suspended substrate particles (Figure 2B). In fact, the rate of dissolved copper removal in the continual stirring simulation (blue dashed line in Figure 2B) is *faster* than in the no stirring case (blue dashed line in Figure 2A) because the particle concentration in the water column stays at its initial value. Also noteworthy, is that the dissolved concentration at day 28 is approximately the same in both simulations. This is because mass transfer (diffusive exchange and adsorption) is rapid enough that copper in both systems achieves the same solid-solution equilibrium. Since dissolved metal is generally considered to be the bioavailable portion, the expected result in both the no stirring and continual scenarios is the same: detoxification.

A



B



- Measured Total Metal
- Measured Dissolved Metal
- Model Water Column Total Metal
- - - Model Water Column Dissolved Metal

Figure 2: Copper T/DP-E simulation results for a system with (A) no stirring and (B) continual stirring during the 28 days following substrate addition. The measured data (closed and open circles) reflect the default T/DP-E approach with no stirring.