# Deliverable Report

| | |
|---|---|
| **Deliverable D 2.20** | Overview of (abiotic) transformation reactions in the aquatic environment which are relevant for nanomaterial risk assessment |
| **Due date of deliverable** | *2020/09/30* |
| **Approved by MC** | *2020/12/17 (via written procedure)* |
| **Actual submission date** | *2020/12/23 (submission to portal)* |
| **File name** | D2.20_G4N_Overview of (abiotic) transformation reactions in the aquatic environment which are relevant for nanomaterial risk assessment |

| | |
|---|---|
| **Author(s) and beneficiary acronym:** | Stetten, L., Tepe, N. and von der Kammer, F., UNVIE |
| **Work package/task:** | WP2 / Task 2.7 |
| **Document status:** | final |
| **Confidentiality:** | confidential / restricted / <u>public</u> |
| **Key words:** | Geochemical transformation, aquatic environment, nanomaterial behaviour |

DOCUMENT HISTORY

| *Version* | *Date* | *Reason of change* |
|---|---|---|
| 1 | 2020/09/17 | Draft submitted to Elisabeth Heunisch, Thomas Kuhlbusch and Eric Bleeker |
| 2 | 2020/10/08 | Comments received from Eric Bleeker |
| 3 | 2020/10/27 | Draft resubmitted to Melanie Müller, Eric Bleeker and Thomas Kuhlbusch for further comments |
| 4 | 2020/11/11 | Comments received from Thomas Kuhlbusch |
| 5 | 2020/11/30 | Draft resubmitted to PO |

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement 814401

**Lead beneficiary for this deliverable: University of Vienna, 19-UNIVIE**

| Owner(s) of this document | |
|---|---|
| Owner of the content | University of Vienna, UNIVIE |
| Co-Owner 1 | - |
| Co-Owner 2 | - |
| Co-Owner 3 | - |
| ….. | |

| Dissemination Level: | | | |
|---|---|---|---|
| PU | Defined in the DoA | Public | PU<br>PP<br>RE<br>CO |
| PP | | Restricted to other programme participants (including the Commission Services) | |
| RE | | Restricted to a group specified by the consortium (including the Commission Services) | |
| CO | | Confidential, only for partners of the consortium (including the Commission Services) | |
| | | | |

| | | |
|---|---|---|
| 1. | Gov4Nano Advisory Board | Yes |
| 2. | NMBP-13 projects collaboration core groups | Yes |
| 3. | | |
| 4. | | |
| 5. | | |
| 6. | | |
| 7. | | |

# Table of Contents

**TABLE OF CONTENTS ......... 3**

**1 SUMMARY ......... 4**

**2 DESCRIPTION OF TASK ......... 4**

**3 DESCRIPTION OF WORK & MAIN ACHIEVEMENTS ......... 5**

3.1 BACKGROUND OF THE TASK ......... 5

3.2 DESCRIPTION OF THE WORK CARRIED OUT ......... 6

3.2.1 Theoretical background on transformations of nanomaterials in aquatic environments ......... 6

3.2.1.1 Dissolution ......... 6

3.2.1.2 Phosphatation ......... 7

3.2.1.3 Sulphidation ......... 8

3.2.1.4 Other (re-)precipitation processes ......... 8

3.2.1.5 Adsorption and complexation ......... 8

3.2.1.6 Bio-geochemical redox processes ......... 9

3.2.2 Overview of the composition of natural waters ......... 9

3.2.2.1 European surface waters and specific rivers ......... 9

3.2.2.2 Geochemical conditions in redox dynamic aquatic systems ......... 15

3.3 RESULTS ......... 16

3.3.1 Evaluation of suitable media for experiments and targeted nanoparticles ......... 16

3.3.1.1 Artificial surface waters ......... 17

3.3.1.2 Anoxic pore waters ......... 18

3.3.1.3 Targeted nanoparticles ......... 19

3.4 EVALUATION AND CONCLUSIONS ......... 19

3.4.1 Upcoming experimental directions ......... 19

3.4.1.1 Dynamic dissolution experiments ......... 20

3.4.1.2 Static exposure experiments ......... 20

3.4.2 Conclusion ......... 21

**4 DEVIATION FROM THE WORK PLAN ......... 21**

**5 PERFORMANCE OF THE PARTNERS ......... 21**

**6 REFERENCES ......... 21**

**7 LIST OF ABBREVIATIONS ......... 26**

# 1 Summary

This report presents an overview of (abiotic) transformations that nanomaterials can undergo in the aquatic environment. The major transformation mechanisms likely to occur in specific aquatic compartments (i.e. surface waters and pore waters) and having an impact on nanomaterials fate will be identified. Based on this knowledge, the identification of important geochemical and physicochemical parameters that could influence the transformations of nanomaterials in aquatic media will be highlighted. Besides existing databases, geochemical composition of European Surface waters, as well as the specific rivers Danube, Rhine, and Elbe, are presented. The main goal of this work is to combine our knowledge on the geochemical reactivity of nanomaterials with knowledge on the typical geochemistry of both surface waters and pore waters to underscore the main drivers of nanomaterials transformations in natural aquatic media. On this basis, we propose suitable exposure media compositions to study nanomaterials transformations under environmentally-relevant conditions. Finally, preliminary experimental concepts adapted to study nanomaterials transformations under environmentally relevant scenarios, that will be developed in the next tasks of Work Package 2.7 are presented.

# 2 Description of task

The overall goal of task 2.7 is guidance on how to address nanomaterials transformations in environmental media within standard risk assessment testing protocols. Specific focusses are on the major transformation pathways and the development of standard transformation protocols (i.e. experimental layout, conditions, and analysis). This relates to the report of OECD ENV/CHEM/NANO(2015)31 in which the requirement to develop methods adapted to test environmental transformation of nanomaterials has been outlined.

Objective of Deliverable 2.20 is:

- To deliver a set of risk assessment relevant transformation reactions and pathways they relate to and possible reaction products.
- To define suitable media for risk assessment testing strategies

Literature research combined with an international expert workshop on NMs transformations will serve as the basis for identifying transformation reactions of NMs, which are relevant for risk assessment and lead to a significant modification of NM behaviour and toxicity. The workshop will serve to identify the concentrations of aquatic species required for transformation reactions and to compare those with reported environmental concentrations (literature, databases).

Currently not well investigated NM transformations as e.g. formation of secondary NMs, transformation into metal-phosphates, -carbonates, -oxides and other phases with low solubility will be investigated in batch and flow-through systems (input from Gracious, NanoFASE) using a set of different media compositions (natural and artificial). The newly formed phases will be identified using different solid-phase analytical and speciation techniques like XRD, EM, CHNSO analysis, TGA, EM, and XAS (based on availability) and suitable wet-chemistry methods.

Approach:

- Transformed NMs are expected to be low soluble compounds, their nature depending on the medium in which it is formed (e.g. Ag → AgS or $Cd_{QD}$ → $Cd_3(PO_3)_2$).
- Literature study of nano and bulk material transformations under environmental aquatic conditions. Collecting data on solubilities and use geochemical modelling to find stable products which might form in the respective environment

- Detailed analysis of trace compounds in surface waters, which lead to the formation of new mineral phases (dissolution-precipitation pathway), transform a particle on the molecular scale or adsorb to surfaces.
- Results in a report that details the concentration ranges of constituents representing the factors driving the transformation of NMs in environmental aquatic media: media definition, expected time frames of reactions, expected transformation products.

# 3 Description of work & main achievements

## 3.1 Background of the task

Predicting the fate of nanomaterials (NMs) in natural environments is crucial to assess the risks associated with it, especially since models reported significant releases of engineered nanomaterials (ENMs) in sub-surface environments. In aquatic environments, dissolution mechanisms are important processes, being the main drivers for the release of dissolved species often referred to as the cause of the toxicity. However, solid-phase transformation mechanisms play also an important role, controlling the fate of NMs and mitigating their toxicity by limiting the release of aqueous species due to the formation of stable secondary solid forms. Despite a large number of studies focused on NMs behaviour in synthetic exposure media, environmentally-relevant laboratory experiments assessing the behaviour of NMs in natural waters are less documented. Strongly dependent on environmental parameters (i.e. geochemical composition of the environment, physicochemical properties, presence of organic and inorganic colloids), information on the transformations of NMs should then be addressed using experimental approaches, mimicking environmental conditions.

The overall goal of Work Package 2.7 (WP 2.7 “Abiotic Transformation of Nanomaterials in Environmental Aquatic Media”) is the generation of information on the diversity of geochemical transformations of NMs likely to occur in aquatic environments. This includes both surface waters and sub-surface groundwaters. Such work aims to support the development of an OECD guidance document on environmental (abiotic) transformations of NMs. In that regard, the objectives of this deliverable are (1) to give an overview of geochemical transformations of NMs likely to occur in aquatic environments, (2) to identify the major driving forces affecting the behaviour of NMs in natural waters, and (3) to define environmentally-relevant chemical composition of artificial media suitable to assess the transformation of NMs in specific aquatic environments. Finally, conclusions of this work, which combined literature and database researches, will be considered for the development of suitable experimental approaches and conditions for the testing of NMs transformations in risk assessment studies.

This work benefits from the knowledge gained in previous EU projects. The NanoFASE project, focused on ENPs transformations in freshwater, estuaries, and marine waters and the Gracious project mainly focused on the dissolution of different NM classes under relevant exposure media, mimicking exposure media relevant for humans and the environment. Within these projects, the dynamic flow-through system has been optimized and media compositions relevant for dissolution, including background electrolytes and pH variations, have been defined. While NanoFASE and Gracious mainly focused on dissolution mechanisms and suitable experimental set-ups for studying dissolution of NMs, WP 2.7 and its respective deliverables will focus on (abiotic) transformation processes (i.e. phase transformation, dissolution and (re-)precipitation) in the aquatic environment. We will use the knowledge gained from experimental work performed in NanoFASE and Gracious to improve our experimental set-ups to study NM transformation. However, we will also monitor the dissolved-phase composition to better understand solid-phase NM transformation processes.

## 3.2 Description of the work carried out

### 3.2.1 Theoretical background on transformations of nanomaterials in aquatic environments

#### 3.2.1.1 Dissolution

Among the diversity of geochemical processes that nanoparticles can undergo, dissolution is a major mechanism, occurring in all aquatic compartments, from surface waters to unsaturated and saturated soils and sediments. The lifetime of an ENM in aquatic environments is directly controlled by its solubility and dissolution rate. Solubility is an intrinsic property and is measured in equilibrium saturated suspensions, while the dissolution rate is considered as an extrinsic property and is measured in non-equilibrium conditions, under the saturation limit (Koltermann-Juelly et al., 2018). Intrinsic particle properties, such as size, crystallinity, state of aggregation/agglomeration, and coating of the particle, are known to influence the dissolution of nanoparticles (Borm et al., 2006; Bian et al., 2011; Misra et al., 2012). Extrinsic parameters such as the chemical composition and physical properties of the surrounding environment (e.g. ionic strength, ligands, pH, organic matter, and temperature) play also an important role (Skjolding et al., 2016, Hartmann et al., 2014; Misra et al., 2012; Bian et al., 2011;).

Some ENMs are classified as insoluble and recalcitrant to dissolution in environmental media, such as $TiO_2$ NPs, which have been found in the surface waters of the Old Danube Lake, Austria (Gondikas et al., 2014) and in urban runoff samples (Kägi et al., 2008). To the opposite, it has been widely agreed in the scientific literature that Ag, ZnO and Cu NPs will undergo dissolution in natural aquatic media. Indeed, a fast dissolution of ZnO NPs is reported in seawater (Miller et al., 2010) and carbonate-rich waters (Sivry et al., 2014). The dissolution of Cu NPs is considered to be a very slow process in natural waters (Keller et al., 2017). However, in a freshwater stream field exposure, rapid dissolution of $Cu(OH)_2$ NPs is reported and to a lesser extend of Cu/CuO NPs (Kent & Vikesland, 2016). While dissolution mechanisms do not require any redox reactions for ZnO, $Cu(OH)_2$ and CuO NPs, Ag NPs dissolution is mainly driven by an oxidative dissolution pathway in surface waters, *via* the formation of an $Ag_2O$ layer, and the subsequent $Ag^+$ dissolution, enhanced at low pH (Levard et al., 2012; Dobias & Bernier-Latmani, 2013; Zou et al., 2017). Field exposure experiment combined with laboratory work also suggested that $Ag^+$ desorption processes could outcompete the low oxidative dissolution of Ag NPs in alkaline natural waters ($7.9 < pH < 8.4$) (Dobias & Bernier-Latmani, 2013), chemisorbed $Ag^+$ at the surface of Ag NPs being documented (Henglein et al., 1998). While it has been shown that Ag NPs can reach a complete dissolution in deionized water or environmentally-relevant exposure media (Liu et al., 2010), a study by Li & Lenhart, (2010) showed that Ag NPs dissolution and release of $Ag^+$ is significantly limited in natural river waters. It has been argued that aggregation/agglomeration of Ag NPs may hamper dissolution due to a substantial decrease of specific surface area (Skjolding et al., 2016), and this phenomenon has been shown through dissolution testing of Ag NPs in different ionic strength media (Gondikas et al., 2012). However, the study by Kent and Vikesland (2012) found that dissolution of Ag NPs was dependent on the particle size rather than subsequent agglomeration, suggesting that there might not be a clear link between aggregation/agglomeration of the ENMs and their dissolution rates.

The presence of natural organic matter (NOM) may significantly impact the behaviour of NMs by inhibiting or promoting dissolution. Indeed, NOM could adsorb on NMs surfaces inhibiting its dissolution. On the other hand, the complexation of NOM with metal ions could promote dissolution of metallic NMs (Yu et al., 2018). Slower dissolution of Ag NPs was observed in littoral lake mesocosms, likely due to a high content of dissolved organic carbon (DOC) and natural colloids leading to hetero-agglomeration and stabilization of Ag NPs (Furtado et al., 2014). Conway et al. (2015) also observed a lower dissolution of CuO NPs in organic-rich natural waters, explained by the chelation and the coating of the particle surface. Using natural river waters, Blinova et al. (2010) reached the same conclusion for CuO nanoparticles. To the opposite, ZnO NPs dissolution is promoted by the presence of NOM in aqueous solutions (Jiang et al., 2015, Yu et al., 2018). However, in natural waters harbouring different DOC contents, no significant differences in ZnO NPs dissolution were reported by Odzak et al. (2015). Finally, pH is also an important factor controlling dissolution of ENMs (Bian et al., 2011; Dahle et al., 2015; Peng et al., 2017; Fernando et al., 2019). Higher dissolutions of Ag NPs, ZnO NPs and CuO NPs were reported in natural water harbouring low pH values (Odzak et al., 2015;2017). In soils, lower pH has also been shown to promote rapid dissolution of CuO NPs (Sekine et al., 2017).

Under anoxic conditions, usually found in groundwater, wetlands, sediment pore waters and water bottom layer of meromictic lakes, the solubility of redox-sensitive NMs is modified. For example, thanks to the limited oxidation of $Ag^0$ into soluble $Ag^+$ by $O_2$, $Ag^0$ NPs are much less soluble (Zou et al., 2017). The release of aqueous species can be also limited *via* the formation of secondary species, harbouring a lower solubility. The diversity of NM transformations related to specific mechanisms likely to occur in natural aquatic media is reported in Figure 1 and will be presented in detail in the following sections.

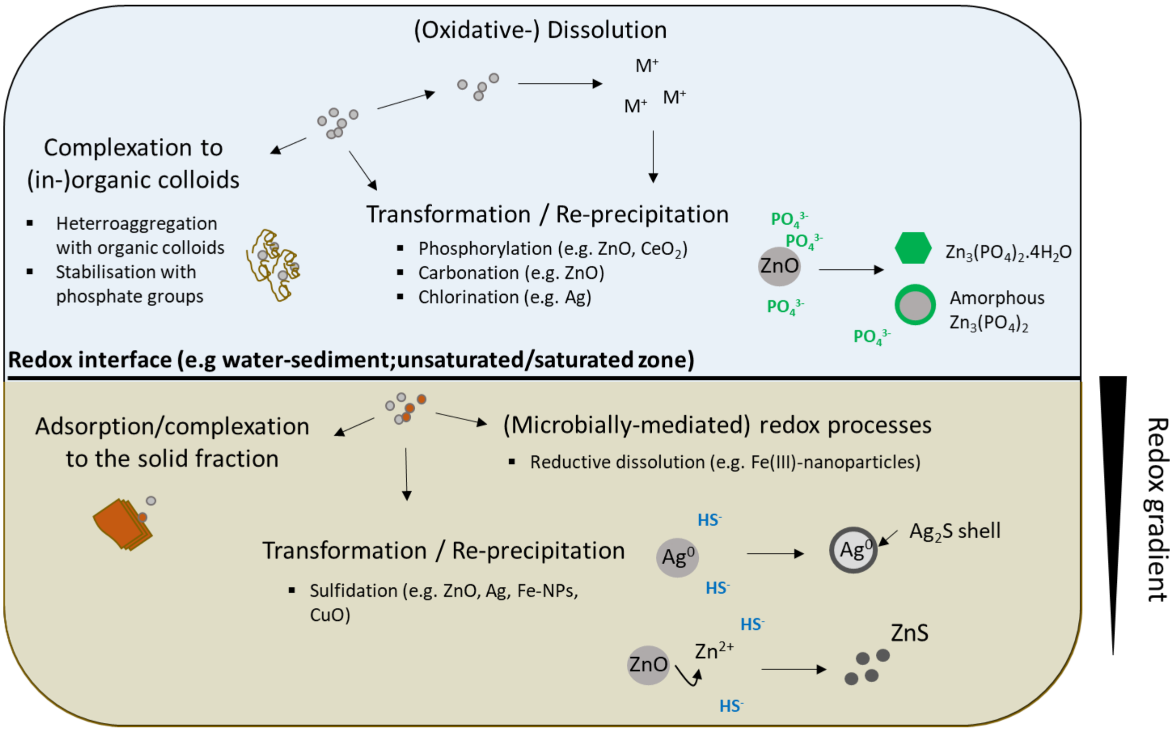


Figure 1: Conceptual illustration of transformations that NMs can undergo in the aquatic environment (i.e. surface waters, soils and sediments).

### *3.2.1.2 Phosphatation*

In phosphate-rich media, experimental studies have shown the phosphatation of ZnO NPs, leading to a decrease of $Zn^{2+}$ release (Lv et al., 2012; Li et al., 2012). Both, highly crystalline Zn-phosphate species (i.e. hopeite-like mineral $Zn_3(PO_4)_2 \cdot 4H_2O$) and amorphous Zn-phosphate species forming a halo around a preserved ZnO core could precipitate, depending on the pH and the phosphate concentration of the media (Rathnayake et al., 2014). Interestingly, in water-saturated sand column experiments, it has been documented that phosphates enhance ZnO NPs mobility (Li et al., 2014). Such mobilization could be explained by the high affinity of phosphate groups to sorb onto the surface of ZnO NPs (Lv et al., 2012), limiting the deposition of ZnO NPs onto the column matrix. The dissolution of ZnO NPs is also limited by the formation of an amorphous Zn phosphate layer at the surface of the NP. In the case of $CeO_2$ NPs, despite the possible adsorption of phosphate groups at the surface of $CeO_2$ NPs (Dahle et al., 2015), the reduction of Ce(IV) to Ce(III) is needed to enhance the formation of $CePO_4$ species. Reduction of Ce(IV) has been already reported by Geitner et al. (2018) in wetland mesocosms. Unfortunately, the molecular environment of Ce was not investigated, preventing the authors to conclude on the nature of the Ce(III) species. Even though the transformation of $CeO_2$ NPs into $CePO_4$ was shown under laboratory conditions (Römer et al., 2019), the phosphatation of $CeO_2$ NPs in phosphate-rich natural environments is, to date, not clearly stated.

#### 3.2.1.3 Sulfidation

Sulfidation mechanisms of NMs composed of chalcophile elements (e.g. Ag, Cu, Zn) have been largely studied under laboratory-controlled experiments, especially for Ag NPs (Liu et al. 2011; Levard et al. 2011 & 2012), ZnO NPs (Ma et al. 2013) and CuO NPs (Ma et al. 2014; Gogos et al. 2018). In natural settings, some studies reported the sulfidation of NMs. For instance, Lowry et al., 2012 reported the transformation of Ag NPs into $Ag_2S$ in freshwater wetland mesocosms. Besides, Khaksar et al. (2015) investigated the transformations of Ag NPs along the water-sediment continuum in marine and freshwater sediments. Using XANES spectroscopy, the authors showed an increase of the extent of sulfidation with depth, interpreted to be controlled by the progressive release of sulphide during sulphate-reduction processes. Sulfidation mechanisms can also occur in unusual sulphide-rich environments such as wastewater treatment plants (WWTPs). Indeed, ZnS was found as a significant Zn species after the amendment of ZnO NPs in sludges (Lombi et al., 2012b; Ma et al., 2013). After incineration of sewage sludge initially spiked with either CuO NPs or ZnO NPs, sulfidic species (i.e. CuS and ZnS) are also reported (Wielinski et al., 2019). In addition to the transformation of ZnO into ZnS species, these studies also reported the formation of $Zn_3(PO_4)_2$ and Zn associated Fe oxy/hydroxide species, with proportions depending on redox conditions and moisture content (Lombi et al., 2012b; Ma et al., 2013; Wielinski et al., 2019). Such results confirm that in complex natural systems such as anoxic sediment and soils, sulfidation processes may compete with other dissolution and (re-)precipitation processes, mainly depending on the presence of ligands such as organic compounds, phosphates and carbonates.

#### 3.2.1.4 Other (re-)precipitation processes

In carbonate-rich environments, such as rivers and groundwaters, carbonate species can also form. For example, the formation of Zn carbonate species such as hydrozincite $Zn_5(OH)_6(CO_3)_2ZnO$ from initially ZnO NPs has been shown by Sivry et al. (2014) *via* experimental investigation of ZnO behaviour in natural river samples from the Seine river.

In chloride-rich media, the behaviour of Ag NPs is modified due to the formation of soluble $AgCl_x^{(x-1)-}$ species as well as solid AgCl species, depending on Cl/Ag ratio (Levard et al., 2013). Indeed, AgCl formation and persistence were observed under acidic conditions (pH = 5.7), whereas sulphur-bound forms of Ag dominated in neutral and alkaline soils (pH = 6.9 and 7.8) (Sekine et al., 2015). Dissolution of Ag NPs in chloride-rich natural waters has not been reported yet. Hence, the dissolution rate of Ag NPs in aerobic natural aquatic media containing chloride is difficult to predict. However, it has been shown that Ag NPs dissolution is strongly reduced due to the precipitation of insoluble AgCl at the surface of the Ag NPs at low Cl:Ag ratios, limiting particle (oxidative-)dissolution, whereas Ag NPs dissolution is enhanced at high Cl:Ag ratios, due to the formation of soluble $AgClx^{(x-1)-}$species. (Li et al., 2010; Ho et al., 2010; Levard et al., 2013).

#### 3.2.1.5 Adsorption and complexation

Adsorption of NMs onto organic matter and inorganic surfaces is also an important mechanism to be considered, especially in soils and sediments. Laboratory studies using natural waters have demonstrated the important role of natural organic compounds by reducing the aggregation and stabilizing $TiO_2$, ZnO and $CeO_2$ nanoparticles (Keller et al., 2010). Whereas in natural organic-rich settings, interactions of ENM with organic compounds have been shown to stabilize particles and reduce the release of aqueous metals (Keller et al., 2010; Aiken et al. 2011), ENM-organic colloids interactions can also enhance nanoparticle mobility (Johnson et al., 2009; Cornelis et al., 2011; Chen et al. 2012). In soil batch experiments, Cornelis et al. (2011) suggested colloid-mediated mobility of $CeO_2$ and Ag NPs, NPs being associated with mobile Al-Si-

Fe-Ca-K-C-rich colloids (i.e. hetero-aggregation). Chen et al. (2012) investigated the effect of humic acid (HA) on the transport and the retention of $TiO_2$ nanoparticles in saturated sand columns. The authors showed an increase of $TiO_2$ mobility in the presence of HA due to the adsorption of HA onto $TiO_2$ NPs. They also showed that electrostatic repulsion of $TiO_2$ NP to sand is increased at alkaline conditions, naturally enhancing their mobility. Conversely, soils and sediments may also reduce ENMs mobility. Zhao et al., 2012 have shown limited mobility of ZnO NPs in natural soils due to the association of ZnO NPs with soil components. Recently, it has also been shown that PVP-coated Ag NPs retention is enhanced in carbonate dominated porous media, mimicking natural carbonate-rich aquifers and that Ag NPs mobility is reduced in the presence of soil organic matter (Adrian et al., 2019). There is clear evidence that the behaviour of ENMs in soil and sediments involves a range of interacting processes depending on the properties of both ENM and solid components. In this context, soil column experiments or soil and sediments incubation experiments should be used to identify general parameters influencing the transport of ENMs.

Finally, adsorption and complexation processes could also play a subsequent role limiting the migration of aqueous species issue from the dissolution of ENM. Recently, Sekine et al. (2017) investigated the fate of Cu-based NPs in oxic soils, showing that CuO NPs are preserved longer in high pH soils. However, after 4 months, CuO NPs were completely transformed into Cu-bound to FeO(OH) or organic species. Indeed, in complex systems, the dissolution of ENMs is going to result in the formation of both mineral and sorbed species into either phyllosilicates, Fe-oxyhydroxides mineral and organic matter (Wielinski et al., 2019; Lowry et al., 2012a; Lombi et al., 2012b; Ma et al., 2014), that could limit the migration of dissolved species.

#### 3.2.1.6 Biogeochemical redox processes

In natural sub-surface environments, direct or indirect microbially-mediated redox processes play a major role in NMs transformations. While oxidation processes are effective in natural waters and aerated soils and sediments where they could enhance NP dissolution, in reducing environments such as water-saturated soils and sediments (i.e. groundwaters), reduction mechanisms could occur. Redox processes lead to a release of various inorganic ligands (i.e. $HCO_3^-$, $HS^-$) that can indirectly enhance the transformation of NMs (i.e. carbonation and sulphidation), as demonstrated by the progressive sulphidation of Ag NPs due to the production of sulphides along with a sediment profile (Khaksar et al. (2015). Also, redox-sensitive elements such as Ce, Fe and Ag are likely to be directly subjected to redox processes. As an example, redox reactions of the Ce(III)/Ce(IV) redox couple can easily occur in the environment as reported by Geitner et al. (2018). Moreover, the reduction of $Cu^{2+}$ to $Cu^{+}$ combined with the oxidation of sulphide to sulphate has been already reported during the sulphidation of CuO NPs (Ma et al., 2014). Under reducing conditions, Fe(III)-bearing nanoparticles such as ferrihydrite, goethite and hematite can also undergo a reductive dissolution process, due to the reduction of Fe(III) into soluble Fe(II) (Kumar et al., 2018), that have crucial environmental importance due to the expanding use of Fe(III) nanomaterials for remediation and the potential release of adsorbed contaminants.

### 3.2.2 Overview of the composition of natural waters

#### 3.2.2.1 European surface waters and specific rivers

To study and anticipate major NM transformations occurring in natural aquatic environments, information on the physicochemical properties and the chemical composition of natural waters is essential. In this section, values for *in-situ* parameters, chemical elements and compounds known or suspected to play a role in NMs transformations in surface waters are presented, for (1) European surface waters in general and (2) the selected rivers, Danube, Rhine, and the Elbe. As additional information, individual elemental concentrations and *in-situ* parameters obtained for rivers worldwide (Berner and Berner, 1996; Gaillardet et al., 2014) are presented.

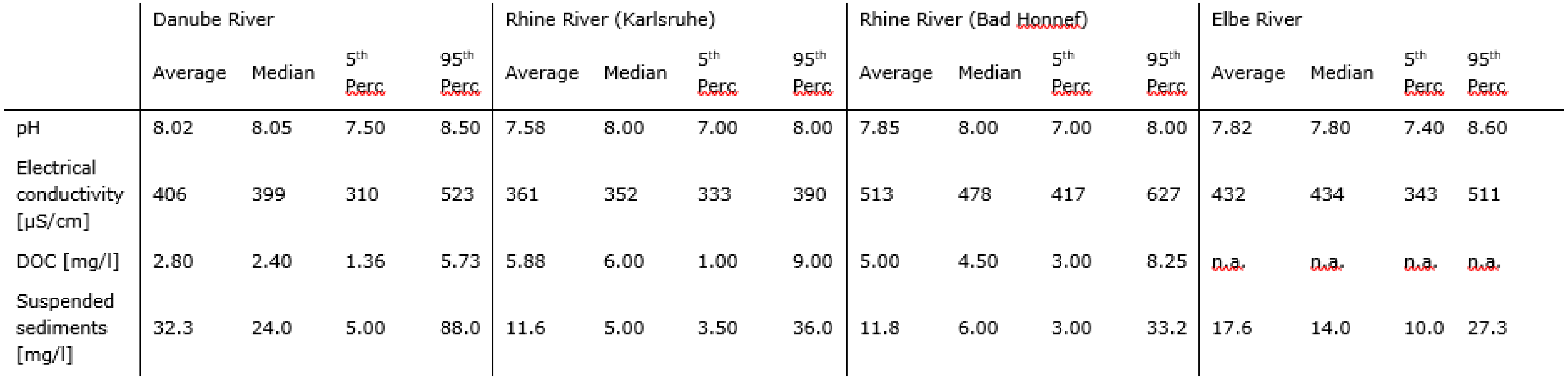
Data representative of European surface waters were collected from the FOREGS database, issue from the Geochemical Atlas of Europe (Salminen et al., 1998 & 2005) and the EEA's database (European Environment Agency) “Waterbase”(https://www.eea.europa.eu/data-and-maps/data/waterbase-rivers-10). Data from the FOREGS database correspond to elemental concentrations and *in-situ* parameters of stream water samples collected in 25 European countries from 1997 to 2001. Data from the EEA database correspond to the average of annual mean concentrations for groundwater bodies/river stations/lake stations collected in 26 European countries from 2000 to 2012. Data for the Danube river were collected from the TransNational Monitoring Network (TNMN) dataset of the International Commission for the Protection of the Danube River (ICPDR) database (https://www.icpdr.org/wq-db/). This dataset represents the elemental concentrations and *in-situ* parameters collected from 1996 to 2017 in 79 monitoring locations with up to three sampling points across the Danube and its main tributaries. For the Rhine river, data were extracted from the FGG Rhein database (http://fgg-rhein.bafg.de/dkrr/) which provides data at individual measuring stations along the Rhine and its tributaries. Here, elemental concentrations and *in-situ* parameters were extracted from 2016 at the sampling locations Karlsruhe and Bad Honnef. The Rhine and its tributaries are significantly influenced by human activities. Potential (anthropogenic) influence in the hydrochemistry of the Rhine could then potentially be assessed by comparing two sampling locations, that differ because of their localization more or less upstream of the source, but also because of the mixing of the main river and its tributaries. In the present case, the change in the geochemical composition of the Rhine due to the mixing with the main tributary the Main can be assessed. Data for the river Elbe were obtained from the specialized information system (FIS) of the FGG Elbe (https://www.elbe-datenportal.de/) which have been collected at measuring stations in the area of the Elbe catchment within the national measuring programs. In this report, data from 2016 are presented.

For all the databases evaluated, the calculated median, average, minimum (5th percentile) and maximum (95th percentile) values for major and minor elements, $Na^+$, $K^+$, $HCO_3^-$, $PO_4^{3-}$, $NO_3^-$, $Mg^{2+}$, $Ca^{2+}$, $SO_4^{2-}$, $Cl^-$, Fe as well as trace elements Ti, Cu, Zn, Ag are presented (Tables 1 and 3). Tables 2 and 4 also reported the calculated median, minimum (5th percentile), and maximum (95th percentile) values for the electrical conductivity, pH, NPOC/DOC and suspended sediment.

Table 1**.** Median, average, 5th percentile and 95th percentile values for major, minor and trace element concentrations obtained for the specific rivers Danube, Rhine and Elbe. “n.a.” stands for “not available”.

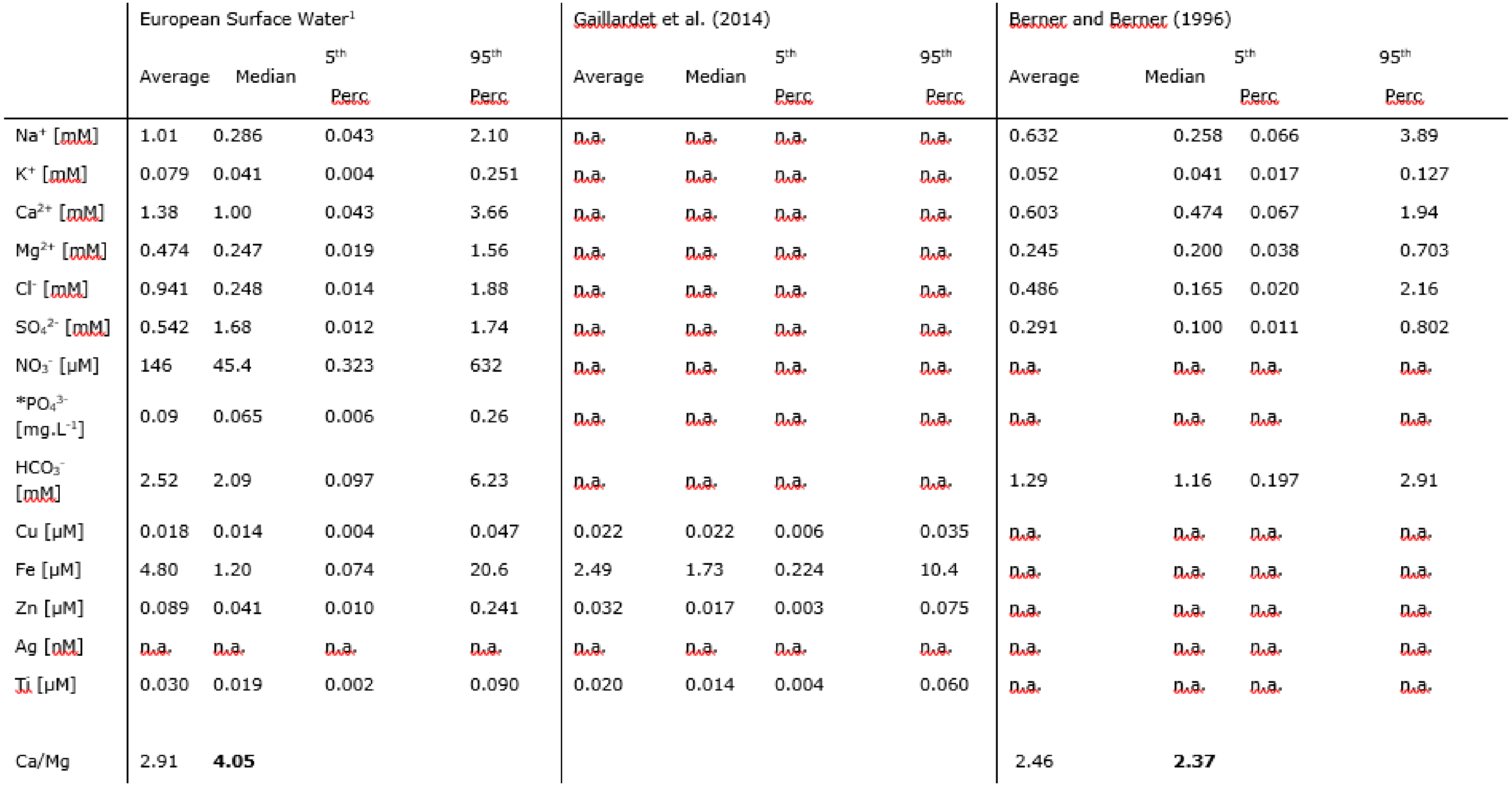
| | Danube River | | | | Rhine River (Karlsruhe) | | | | Rhine River (Bad Honnef) | | | | Elbe River | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average | Median | 5th Perc | 95th Perc | Average | Median | 5th Perc | 95th Perc | Average | Median | 5th Perc | 95th Perc | Average | Median | 5th Perc | 95th Perc |
| $Na^+$ [mM] | 0.802 | 0.652 | 0.478 | 1.81 | 0.458 | 0.478 | 0.287 | 0.583 | 0.970 | 0.957 | 0.574 | 1.34 | 1.17 | 1.17 | 0.913 | 1.44 |
| $K^+$ [mM] | 0.096 | 0.087 | 0.075 | 0.170 | 0.037 | 0.026 | 0.026 | 0.051 | 0.153 | 0.179 | 0.026 | 0.230 | 0.144 | 0.148 | 0.110 | 0.174 |
| $Ca^{2+}$ [mM] | 1.35 | 1.20 | 0.923 | 2.04 | 1.28 | 1.27 | 1.19 | 1.38 | 1.69 | 1.72 | 1.54 | 1.82 | 1.15 | 1.15 | 0.948 | 1.33 |
| $Mg^{2+}$ [mM] | 0.601 | 0.576 | 0.488 | 0.788 | 0.282 | 0.288 | 0.247 | 0.288 | 0.427 | 0.411 | 0.354 | 0.510 | 0.398 | 0.411 | 0.333 | 0.453 |
| $Cl^-$ [mM] | 0.699 | 0.639 | 0.372 | 1.13 | 0.425 | 0.423 | 0.265 | 0.564 | 1.03 | 1.02 | 0.598 | 1.47 | 1.04 | 1.02 | 0.790 | 1.30 |
| $SO_4^{2-}$ [mM] | 0.374 | 0.349 | 0.198 | 0.616 | 0.247 | 0.250 | 0.208 | 0.289 | 0.493 | 0.479 | 0.354 | 0.629 | 0.695 | 0.697 | 0.560 | 0.802 |
| $NO_3^-$ [µM] | 27.3 | 24.6 | 11.0 | 51.0 | 71.5 | 64.5 | 17.7 | 142 | 76.6 | 72.6 | 16.1 | 136 | 235 | 210 | 177 | 306 |
| $PO_4^{3-}$ | 0.603 | 0.495 | 0.105 | 1.37 | 171 | 179 | 73.7 | 250 | 491 | 390 | 278 | 828 | 2.46 | 2.63 | 0.758 | 3.90 |
| $HCO_3^-$ [mM] | 2.99 | 3.00 | 2.29 | 3.75 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 1.71 | 1.80 | 1.41 | 1.97 |
| Cu [µM] | 0.235 | 0.087 | 0.022 | 0.787 | 1.05 | 1.32 | 0.120 | 1.49 | 0.055 | 0.047 | 0.035 | 0.079 | 0.018 | 0.014 | 0.004 | 0.047 |
| Fe [µM] | 9.00 | 5.46 | 0.895 | 27.0 | 2.25 | 1.11 | 0.541 | 8.03 | 5.38 | 3.85 | 2.24 | 14.4 | 3.86 | 2.51 | 0.537 | 10.9 |
| Zn [µM] | 0.753 | 0.306 | 0.046 | 2.91 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 0.270 | 0.168 | 0.072 | 0.709 |
| Ag [nM] | 12.2 | 9.27 | 0.056 | 32.3 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Ti [µM] | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 0.140 | 0.054 | 0.021 | 0.439 |
| **Ca/Mg** | 2.25 | **2.08** | | | 4.54 | **4.41** | | | 3.96 | **4.18** | | | 2.89 | **2.80** | | |

Table 2: Median, average, 5th percentile and 95th percentiles values for pH, electrical conductivity, DOC and suspended sediments obtained for the specific rivers Danube, Rhine and Elbe. "n.a." stands for "not available".



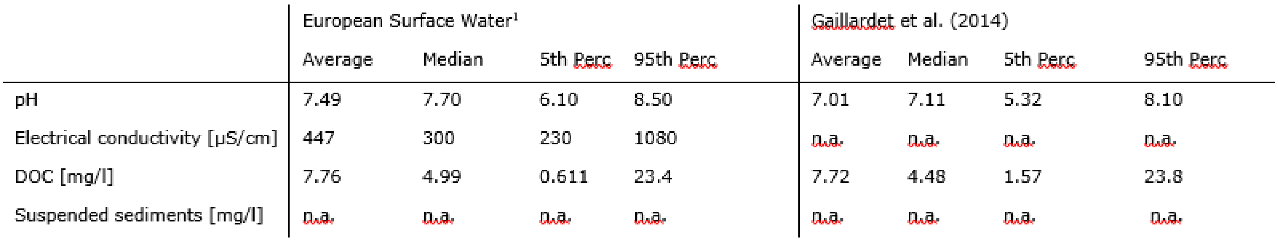
| | Danube River | | | | Rhine River (Karlsruhe) | | | | Rhine River (Bad Honnef) | | | | Elbe River | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average | Median | 5th Perc. | 95th Perc. | Average | Median | 5th Perc. | 95th Perc. | Average | Median | 5th Perc. | 95th Perc. | Average | Median | 5th Perc. | 95th Perc. |
| pH | 8.02 | 8.05 | 7.50 | 8.50 | 7.58 | 8.00 | 7.00 | 8.00 | 7.85 | 8.00 | 7.00 | 8.00 | 7.82 | 7.80 | 7.40 | 8.60 |
| Electrical conductivity [µS/cm] | 406 | 399 | 310 | 523 | 361 | 352 | 333 | 390 | 513 | 478 | 417 | 627 | 432 | 434 | 343 | 511 |
| DOC [mg/l] | 2.80 | 2.40 | 1.36 | 5.73 | 5.88 | 6.00 | 1.00 | 9.00 | 5.00 | 4.50 | 3.00 | 8.25 | n.a. | n.a. | n.a. | n.a. |
| Suspended sediments [mg/l] | 32.3 | 24.0 | 5.00 | 88.0 | 11.6 | 5.00 | 3.50 | 36.0 | 11.8 | 6.00 | 3.00 | 33.2 | 17.6 | 14.0 | 10.0 | 27.3 |

Table 3. Median, average, 5th percentile and 95th percentiles values for major, minor and trace element concentration obtained for European surface waters and a data compilation for rivers worldwide from Berner and Berner, 1996 and Gaillardet et al., 2014 . "n.a." stands for "not available". The FOREGS database used to represent European surface waters does not provide any information about PO4$^{3-}$. * Data from the EEA database.



| | European Surface Water[1] | | | | Gaillardet et al. (2014) | | | | Berner and Berner (1996) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average | Median | 5th Perc | 95th Perc | Average | Median | 5th Perc | 95th Perc | Average | Median | 5th Perc | 95th Perc |
| $Na^+$ [mM] | 1.01 | 0.286 | 0.043 | 2.10 | n.a. | n.a. | n.a. | n.a. | 0.632 | 0.258 | 0.066 | 3.89 |
| $K^+$ [mM] | 0.079 | 0.041 | 0.004 | 0.251 | n.a. | n.a. | n.a. | n.a. | 0.052 | 0.041 | 0.017 | 0.127 |
| $Ca^{2+}$ [mM] | 1.38 | 1.00 | 0.043 | 3.66 | n.a. | n.a. | n.a. | n.a. | 0.603 | 0.474 | 0.067 | 1.94 |
| $Mg^{2+}$ [mM] | 0.474 | 0.247 | 0.019 | 1.56 | n.a. | n.a. | n.a. | n.a. | 0.245 | 0.200 | 0.038 | 0.703 |
| $Cl^-$ [mM] | 0.941 | 0.248 | 0.014 | 1.88 | n.a. | n.a. | n.a. | n.a. | 0.486 | 0.165 | 0.020 | 2.16 |
| $SO_4^{2-}$ [mM] | 0.542 | 1.68 | 0.012 | 1.74 | n.a. | n.a. | n.a. | n.a. | 0.291 | 0.100 | 0.011 | 0.802 |
| $NO_3^-$ [µM] | 146 | 45.4 | 0.323 | 632 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| *$PO_4^{3-}$ [mg.L$^{-1}$] | 0.09 | 0.065 | 0.006 | 0.26 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| $HCO_3^-$ [mM] | 2.52 | 2.09 | 0.097 | 6.23 | n.a. | n.a. | n.a. | n.a. | 1.29 | 1.16 | 0.197 | 2.91 |
| Cu [µM] | 0.018 | 0.014 | 0.004 | 0.047 | 0.022 | 0.022 | 0.006 | 0.035 | n.a. | n.a. | n.a. | n.a. |
| Fe [µM] | 4.80 | 1.20 | 0.074 | 20.6 | 2.49 | 1.73 | 0.224 | 10.4 | n.a. | n.a. | n.a. | n.a. |
| Zn [µM] | 0.089 | 0.041 | 0.010 | 0.241 | 0.032 | 0.017 | 0.003 | 0.075 | n.a. | n.a. | n.a. | n.a. |
| Ag [nM] | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Ti [µM] | 0.030 | 0.019 | 0.002 | 0.090 | 0.020 | 0.014 | 0.004 | 0.060 | n.a. | n.a. | n.a. | n.a. |
| Ca/Mg | 2.91 | **4.05** | | | | | | | 2.46 | **2.37** | | |

Table 4. Median, average, 5th percentile and 95th percentiles values for pH, electrical conductivity, DOC and suspended sediments obtained for European surface waters and data compilation for rivers worldwide from Gaillardet et al., 2014. n.a." stands for "not available".



| | European Surface Water$^1$ | | | | Gaillardet et al. (2014) | | | |
|---|---|---|---|---|---|---|---|---|
| | Average | Median | 5th Perc | 95th Perc | Average | Median | 5th Perc | 95th Perc |
| pH | 7.49 | 7.70 | 6.10 | 8.50 | 7.01 | 7.11 | 5.32 | 8.10 |
| Electrical conductivity [µS/cm] | 447 | 300 | 230 | 1080 | n.a. | n.a. | n.a. | n.a. |
| DOC [mg/l] | 7.76 | 4.99 | 0.611 | 23.4 | 7.72 | 4.48 | 1.57 | 23.8 |
| Suspended sediments [mg/l] | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |

In contrast to river-specific databases, the FOREGS database presents a wide heterogeneity of values for pH, electrical conductivity, DOC and elemental concentrations (Tables 3 and 4), that can be explained by the large representativity of the FOREGS database in terms of geochemical background (i.e. geological environments of low or high buffering capacity) and types of environments (i.e. organic carbon concentrations). For example, based on the FOREGS data, the majority of European stream waters pH values range between 6 and 9 (Table 4, Figure 2). There is also a low but significant proportion of values ranging between 5 and 6. A very small minority of pH values, with only three sampling points, range between 4 and 5, that could be attributed to exceptional environments such as acid mine drainage rather than natural surface waters. A lower pH variation is observed for specific rivers (Table 2, Figure 2), indicating small heterogeneities probably attributed to the sampling point location and the sampling period or particular events.

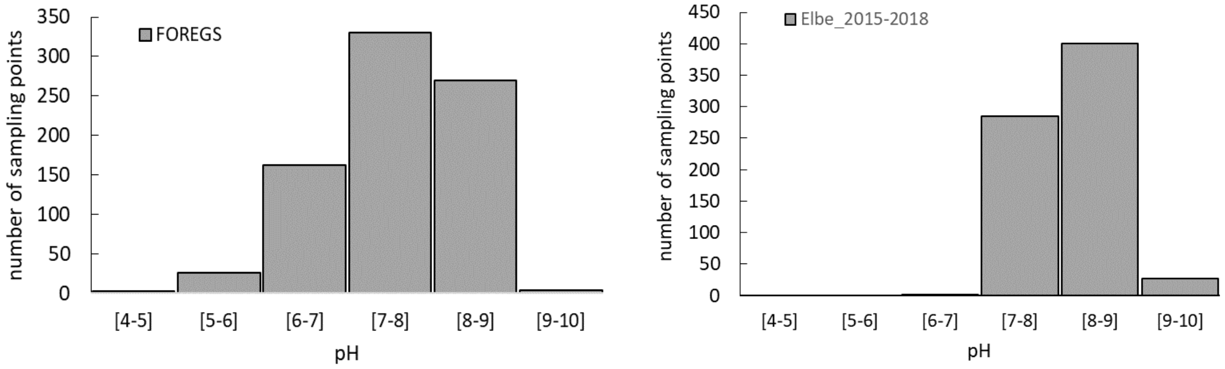


Figure 2: Histograms of the number of pH values in the different pH range for the FOREGS database (left) and measured pH in the Elbe river (right).

The FOREGS database is, to date, the most relevant database to define artificial media composition mimicking a large pool of natural surface waters, from alkaline rivers such as Danube and Rhine (Table 1 and 2) to more acidic surface waters mostly present in base-poor buffering capacity regions and/or acid buffering regions due to high contents of dissolved organic species. For studies aiming to understand the behaviour of NMs in a specific environmental compartment, the use of defined values according to a selected river, using specific databases instead of the FOREGS large scale average, is recommended. However, in the context of an OECD guidance document adapted for the testing of NMs and more generally for studies aiming to understand NMs behaviour in a large range of aquatic environments, the FOREGS database should be used to define a background composition of the exposure media with pertinent parameters to be varied to represent all the diversity of stream waters. Indeed, large heterogeneities in pH, NOM and specific ions concentrations are important to consider when it comes to studying NMs behaviour.

In section 5, artificial media composition will be defined based on the FOREGS database to investigate NM transformations in surface waters.

#### *3.2.2.2 Geochemical conditions in redox dynamic aquatic systems*

Among the diversity of aquatic environments, sub-surface terrestrial environments are important aquatic compartments often acting as water reservoirs. Freshwater sediments are believed to be a sink for ENMs after being transported by surface waters (Gottschalk et al., 2009). The accumulation of NMs in these systems is therefore a major concern due to the potential risk of toxic compounds released in the pore waters that could be threatening for the aquatic ecosystem. Aquatic sub-surface environments (i.e. wetland, aquifers, bottom lake sediment) are known to encounter specific geochemical and redox conditions, differing from surface waters. It is therefore important to evaluate the fate of ENMs under such particular geochemical conditions to build adapted management strategies.

In redox-dynamic aquatic media, a sequence of redox biogeochemical processes occurs, controlled by microbial activities (Figure 3). These redox gradients are typically found in all water-unsaturated/saturated boundaries (i.e. water-table) as well as at the interface water-sediment and in some particular cases in the bottom water layer of meromictic lakes such as Lake Pavin.

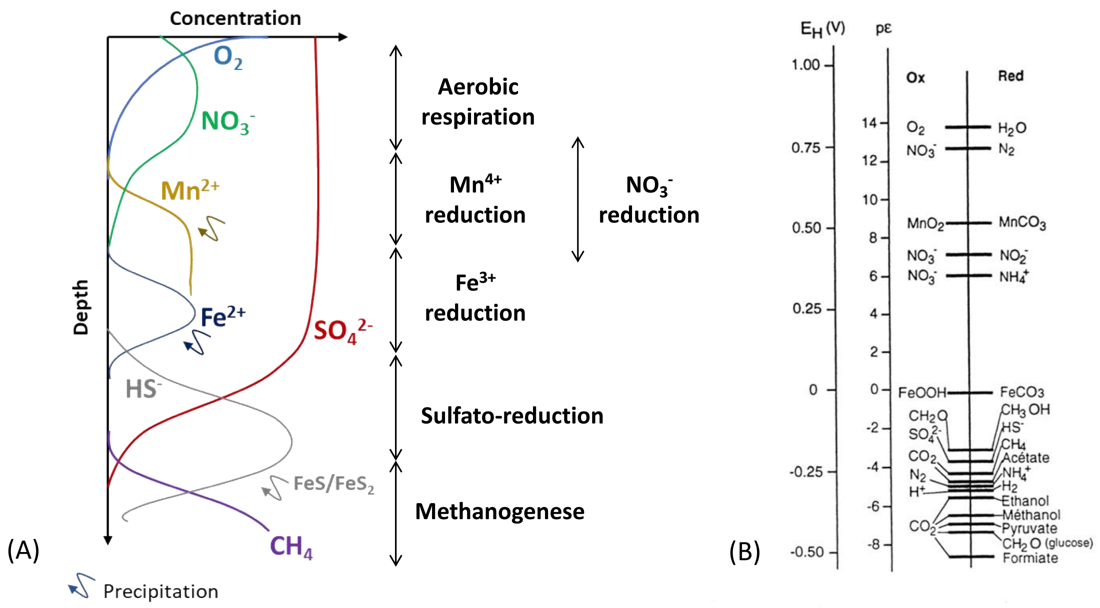


Figure 3: (A) Schematic representation of concentration profiles encountered in the pore waters of sediment during early diagenesis; (B) Redox sequence involved in oxidation-reduction reactions in lake sediments. Modified from Froelich, 1975 and Ogier, 1999.

All biogeochemical mechanisms associated to such microbial activities, which involve a multitude of redox-reactive species such as $O_2/H_2O$, $Fe^{3+}$ /$Fe^{2+}$ and $SO_4^{2-}/HS^-$ and are combined with the mineralization (oxidation) of organic matter, are known as early diagenesis. Under undisturbed and water-saturated conditions, the total consumption of $O_2$ by aerobic microorganisms and the low diffusion of $O_2$ leads to the development of anoxic conditions (Figure 3), where the spatial extent of this gradient largely depends on the local conditions. Under such anoxic conditions, anaerobic respiration processes could then occur. Major oxidation-reduction processes found in anoxic aquatic environments are Mn(IV) and Fe(III) reduction processes, catalysed by a wide range of metal-reducing bacteria and $SO_4^{2-}$ reduction processes catalysed by sulphate-reducing bacteria. Important changes could also occur regarding the mineralogical composition of the surrounding environment, due to the dissolution of iron Fe(III) and Manganese Mn(IV) oxides and the precipitation of sulphides species such as $FeS_2$.

NPs can undergo a series of environmental transformation processes in sub-surface aquatic environments, such as aggregation/attachment to the solid phase, dissolution and loss of coating. Stabilization of NPs by NOM adsorption (*i.e.* NOM overcoating) and/or incrustation with iron-oxides precipitates could also occur. These transformations can consequently influence their fate, transport, bioavailability and toxicity. When freely-dispersed in the sediment/soil pore water, advective and diffusive transport can expose changing redox conditions to the NPs with effects on the NPs, which are barely documented or understood. In addition, important factors that could affect NMs behaviour in such environments are the anaerobic reducing conditions and the presence of specific inorganic ligands such as $HCO_3^-$, $Fe^{2+}$ and $HS^-$, released from microbial redox processes. For example, an important mechanism that could occur in redox-dynamic environments is the reduction of sulphates ($SO_4^{2-}$), leading to the release of sulphide ($HS^-$), highly reactive with chalcophile elements. Indeed, sulfidation of chalcophile NMs such as Ag NPs or ZnO NPs is expected in sulphate-reducing aquatic environments, as already reported by several studies (Lowry et al., 2012; Khaksar et al., 2015). Reducing conditions could also directly impact the fate of redox-sensitive NMs such as Ag, $Fe^{3+}$, $Cu^{2+}$ and $Ce^{4+}$ -bearing NMs.

When it comes to defining representative pore water's chemical composition, one limiting aspect is the lack of databases that collect data on such environments, probably due to the high complexity when it comes to sampling and the large heterogeneities of chemical composition due to the geochemical background and the mineralogical composition of the matrix (soil/sediment). Indeed, pore waters may vary in composition as a result of the respective mineralogical and chemical composition of the solid phase. The time the pore water has been in contact with the soil or aquifer minerals is also an important factor. The major dissolved components of groundwaters include the anions $HCO_3^-$, $Cl^-$, and $SO_4^{2-}$, and the cations $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $K^+$. These constituents are typically present at concentrations in the range of a few $mg.L^{-1}$ to several hundred $mg.L^{-1}$ and the normal range for pH is 6 to 8.5 (Chapman, 1996). In addition, it appears as the prime of importance to consider reduce ligands released from early diagenetic processes when it comes to mimic reducing environments. An important reduced species is $HS^-$, that could enhance sulphidation of Ag, Fe, Cu, and Zn- bearing NPs. It is thus crucial to evaluate the role of $HS^-$ under environmentally relevant concentrations and conditions. In sulphate-reducing environments, sulphide concentrations ranging from 1 to 100 $\mu g.L^{-1}$ are reported (WHO, 2003). Higher sulphide concentrations, from a few $\mu g.L^{-1}$ up to tens of $mg.L^{-1}$ are also reported in sewage systems and WWTPs (Liu et al., 2011).

## 3.3 Results

### 3.3.1 Evaluation of suitable media for experiments and targeted nanoparticles

Recently, Geitner et al. (2020) presented some recommendations for relevant and consistently characterized standard media for the testing of NMs, previously discussed in the "Environmental Nano Testing Media Harmonization" workshop in 2016, and addressing the need for media harmonization. To simulate freshwaters, the authors recommend preparing very soft EPA water and moderately hard EPA water. These media are commonly used as test water in risk assessments and are intended to simulate freshwater with low hardness (i.e. alpine streams) and higher hardness waters. However, this later is recognized to be not fully representative of surface waters, mostly due to a Ca:Mg ratio different to 4:1, and should not be used in OECD and ISO testing. To date, OECD Test Guideline 318 (OECD, 2017) proposed specific hydro-chemical conditions for the testing of nanomaterials dispersion stability. Varying parameters such as pH, ionic strength and presence of dissolved organic matter (DOM), influencing the surface charge and the colloidal stability of many nanomaterials (Keller et al., 2010; French et al., 2009) are proposed, based on the FOREGS database (Salminen et al., 2005). Briefly, according to OECD TG 318, experiments should be conducted for 3 different pH values, namely 4, 7 and 9 with 0 to 10mM of $Ca(NO_3)_2$ as an electrolyte solution. The OECD Test Guideline 318 proposes also additional alternative testing media, more relevant to the geochemical conditions found in natural waters, with the additional presence of $Mg^{2+}$ and $SO_4^{2-}$ ions ($Ca^{2+}/Mg^{2+}$ 4:1, $NO_3^-/SO_4^{2-}$ 10:1).

Such latest background electrolyte is relevant for surface waters, keeping concentrations of Ca and Mg in the range of the FOREGS average values. However, we may point here that the pH values recommended by the OECD guideline to perform experimental testing are also not fully relevant to natural freshwater systems. Indeed, as mentioned in the section above, the majority of European stream waters pH values range between 5 and 9. Thus, it appears unnecessary to performed experiment testing under extreme pH values below 5, in case of environmentally relevant scenarios.

Taking all information gathered regarding surface and sub-surface waters geochemistry and NPs transformation behaviour (Figure 1), we propose alternative exposure media for the testing of NMs, more relevant to natural waters, e.g. changing the pH range from 4-7-9 (which is common for OECD testing guidelines and guideline documents) to the pH range from 5-7-8.5. Figure 4 gives an overview of the main characteristic of surface and pore waters that need to be considered when studying NMs behaviour in natural aquatic systems. Such parameters will be considered in the next deliverables, for the testing of NM transformations. More details on the composition of the artificial media (Figure 5) are also presented in the sections below.

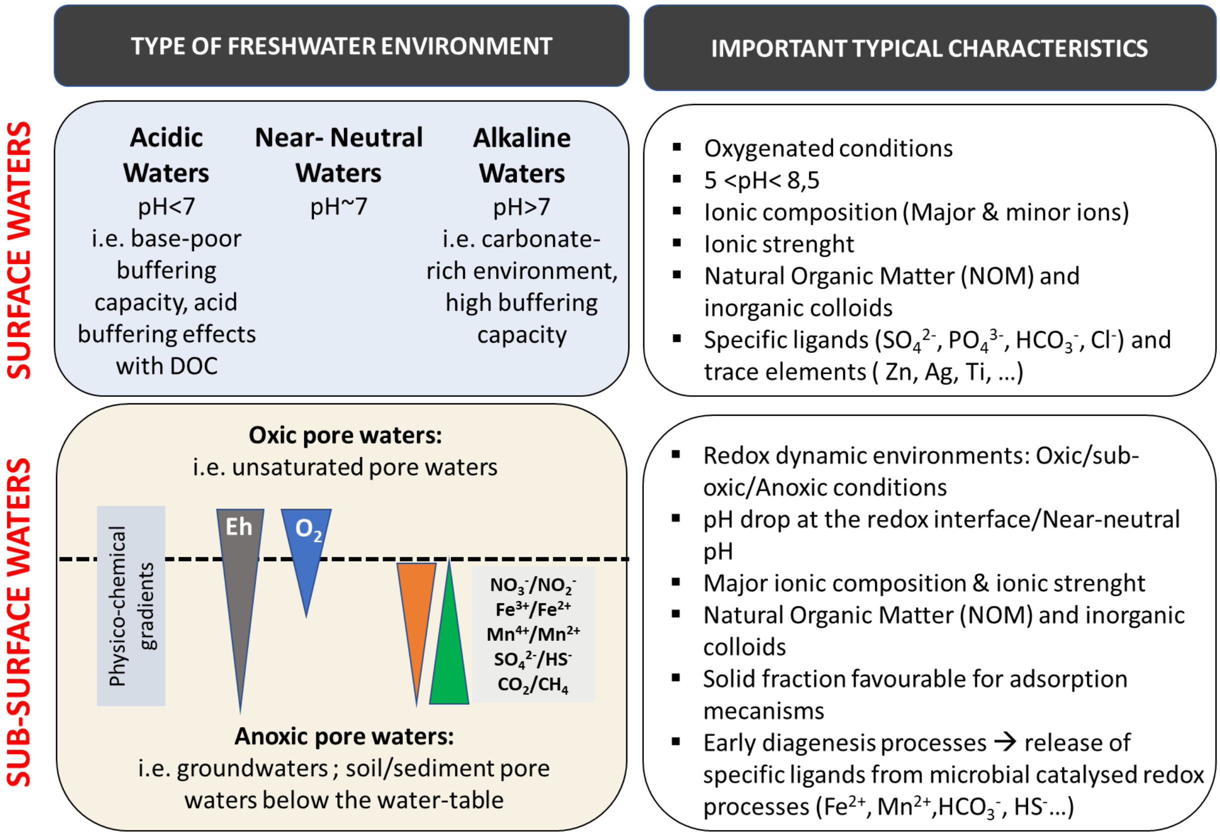


Figure 4: Overview of important characteristics and parameters defined for specific aquatic compartments, excluding extreme conditions not fully representative of the typical aquatic environments.

#### 3.3.1.1 Artificial surface waters

First, based on knowledge obtained on surface water geochemistry, to simulate the ionic background composition of surface waters we propose to use an electrolyte solution containing 2 mM of $Ca(NO_3)_2$ and 0.5 mM of $MgSO_4$. Such values are based on $Ca^{2+}$ and $Mg^{2+}$ concentrations reported by the FOREGS database, with an average concentration of 1.4 mM and 0.5 mM for $Ca^{2+}$ and $Mg^{2+}$, respectively (Salminen et al., 2005). They are slightly higher than the median values obtained from the FOREGS database (1mM and 0,25 mM for $Ca^{2+}$ and $Mg^{2+}$, respectively). However, such a chemical composition allows reaching a Ca:Mg ratio of 4:1, which is similar to the Ca:Mg ratio of the median values 4.05 in the FOREGS database and is also in the range of the Ca:Mg ratios reported for river-specific databases (Ca:Mg from 2.08 to 4.41). Such a background electrolyte is not supposed to play a direct role in the solid-phase transformation of NMs. However, considering that (homo/hetero)agglomeration of NMs can significantly affect dissolution kinetics, it is thus as prime of importance to include this parameter to study transformations pathways occurring in natural waters. Close to the average major ions' composition of natural waters, such a background solution should remain constant to investigate specific transformations *via* the addition of specific ligands in the exposure media, suspected to play a role in abiotic transformations of NMs.

As for the previous OECD guidelines, three 'categories' of artificial waters based on pH values are optimal to represent all type of surface waters. First, to mimic acidic natural waters (i.e. peats, acid mine drainage and low buffering capacity stream waters), a media at pH 5 should be used. Second, to simulate near-neutral rivers, the same artificial media should be prepared, adjusted at pH 7-7.5 by using a low concentration of $HCO_3^-$ as a buffer (< 2 mM $HCO_3^-$). In contrast, to simulate alkaline surface waters (pH 8.5), 2.5 to 5 mM $HCO_3^-$ should be added to the exposure media, which is consistent with the high buffer capacity of some surface waters (Tables 1 and 3) frequently due to the presence of carbonates species (Salminen et al., 2005). To define exposure media composition adapted to study NM transformations, it is primordial to select major NM transformations that are likely to occur in aquatic environments. Based on the collected knowledge presented in section 3, dissolution mechanisms are likely to occur in all surface waters and are strongly dependent on pH and the presence of organic compounds and phosphate. Natural organic matter, significantly present in surface waters (Tables 2 and 4) plays an important role since it is known to stabilize ENMs by complexation, steric effects, or transforming the surface coating of the particle. In that regard, for a more realistic scenario, a test medium should be prepared with 5 to 10 mg.L$^{-1}$ of DOC in addition to the background electrolyte solution of 2 mM of $Ca(NO_3)_2$ and 0.5 mM of $MgSO_4$. Besides, phosphatation may also occur (Khaksar et al., 2015; Sivry et al., 2014; Reed et al., 2012; Lv et al., 2012; Li et al., 2014) in phosphate-enriched waters. The number of phosphate-rich environments is increasing, mainly due to the widespread use of phosphorus fertilizer. In agricultural soil solutions that receive sewage sludge and/or chemical fertilizers, mM levels of phosphate have been reported (Bierman et al., 1995). Significant concentrations of orthophosphate are also reported in European surface waters (Table 3, EEA, 2019). Such levels can also significantly impact the dissolution of nanoparticles (Dahle et al., 2015; Lv et al., 2012; Li et al., 2014) even at low concentrations (Conway et al., 2015). Indeed, phosphate concentrations are significant, with values ranging between 0.006 (5th perc) to 0.26 (95th perc) mg.L$^{-1}$ (Table 3). Regarding the expected concentration of ENMs into aquatic environments, for example, a maximum of 150 ng.L-1 for ZnO NPs (Dumont et al., 2015), phosphate can thus play an important role. The addition of 0.01 to 0.1 mg.L$^{-1}$ inorganic phosphate is then also pertinent to investigate and predict the transformations of NM in aquatic environments. Figure 5 provides an overview of the composition of artificial media to be used in future experiments, with specific NM transformations that are expected.

#### 3.3.1.2 Anoxic pore waters

As part of Gov4Nano, transformations of NMs in anoxic pore waters will be first assessed in a natural system, through the incubation of sediment columns, that will help us to define artificial pore waters (APW) composition and refine experimental setups. Thanks to this work, we will (1) define an adapted background electrolyte, (2) choose suitable concentration range of relevant ligands to study NMs transformations. In addition, this experimental approach will allow us to expose NMs into a redox gradient occurring below the water-sediment interface (i.e. rapid $O_2$ consumption in the first millimetres of sediment, a progressive increase of $Fe^{2+}$, $HCO_3^-$, $HS^-$), still challenging to simulate experimentally.

In parallel to this work, preliminary artificial anoxic pore water will be tested. The sulfidation process is a major transformation that could occur in such systems. Consequently, sulphide will be first considered in the composition of APW. To represent anoxic pore waters, we propose to prepare an artificial solution composed of a background electrolyte of 2 mM of $Ca(NO_3)_2$ and 0.5 mM of $MgSO_4$, using $O_2$-free deionized water (Figure 5). The preparation of the solution should be performed in an anaerobic glove box to avoid any oxygen contamination. All experiments using such artificial media should also be performed in an anaerobic glove box. Finally, DOC, P and $HCO_3^-$ should also be added to the system for a more realistic scenario.

#### *3.3.1.3 Targeted nanoparticles*

As part of Gov4Nano, we will select NMs which are, to our knowledge, the most relevant to assess the feasibility of experimental approaches proposed and to study the major mechanisms occurring in natural waters. The NMs of interest to be used to tackle T2.7 are ZnO (for studying phosphatation and sulfidation), Ag (for studying sulfidation), Au and Ti (for representing inert and reference NMs), $Fe_2O_3$ (for representing redox-sensitive NMs and sulfidation). All of these NMs have been used in the Testing Programme of the OECD Working Party on Manufactured Nanomaterials (WPMN).

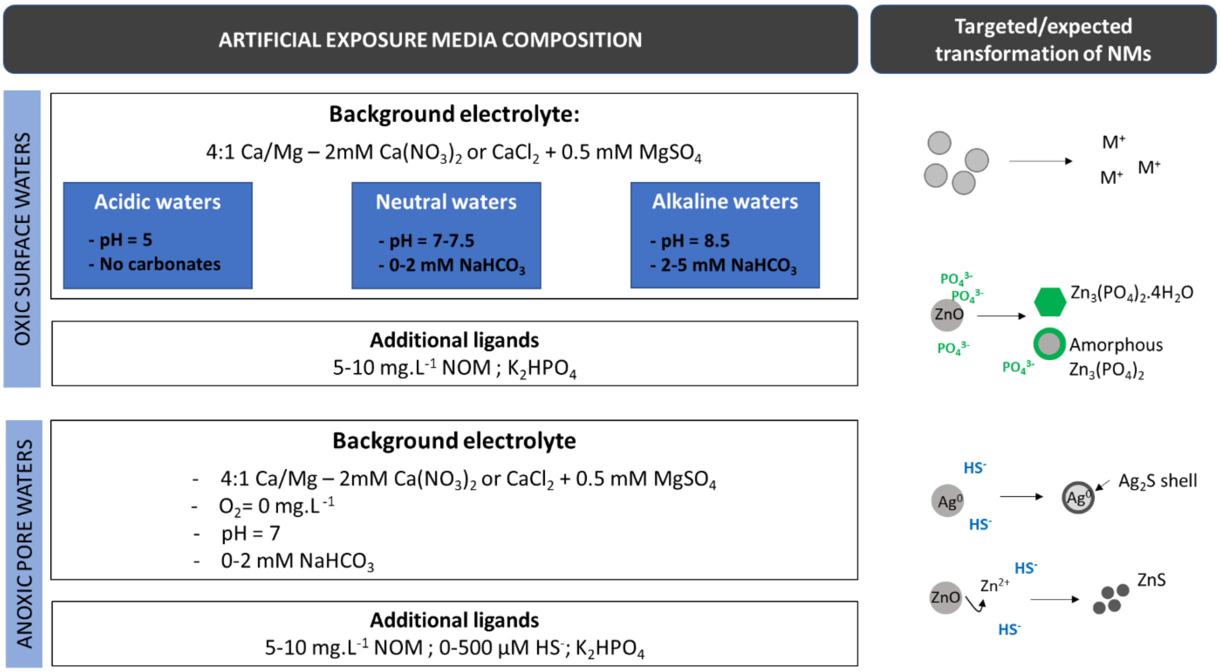


Figure 5: Selection of artificial media composition adequate to simulate surface waters and anoxic pore waters.

## 3.4 Evaluation and conclusions

### *3.4.1 Upcoming experimental directions*

A high degree of uncertainty remains when it comes to understanding transformation pathways in aquatic environments, mostly due to the lack of experimental studies that use (1) natural or artificial waters representative of the heterogeneity of the world's aquatic environments and (2) adapted experimental setups allowing to work under environmentally-relevant conditions. Indeed, one of the main challenges when studying transformations of NMs under environmentally-relevant conditions is to find a good compromise between keeping the relevant geochemical composition of the exposure media and working with adapted NMs concentrations allowing to observe the anticipated transformation. To date, studies are working with relatively high NM concentrations, especially when it comes to investigating the end product of a transformation, keeping relevant NM:reactant ratios to enhance NP transformation. In this context, our aim for the upcoming deliverables is to create experimental concepts allowing to work with very low NMs concentrations and thus environmentally relevant artificial media to track environmental transformations.

#### 3.4.1.1 Dynamic dissolution experiments

The dynamic dissolution test systems are considered to be more appropriate for dissolution rate measurements under an environmentally-realistic system. One of the main advantages of the dynamic flow-through system is that it allows working with low concentrations of ENMs, and when using appropriate analytical instrumentation, it allows detection of low levels of dissolved species. Thus, low elemental concentrations in the exposure medium can be used, allowing to mimic the elemental composition of natural waters in an environmentally relevant scenario due to a constant and unlimited intake of the eluent. These results can finally serve as inputs into environmental fate modelling.

The flow-through setup has been described in ISO TR 19057 (ISO, 2017) and has been applied in several recent studies. For instance, Koltermann-Juelly et al. (2018) studied dissolution rates for 24 (nano)forms of 6 different substances in phagolysosomal simulant fluid, and Bove et al. (2017) investigated dissolution of Ag ENMs in an *in vitro* assay simulating conditions which likely occur in human digestion. However, these studies applied relatively high concentrations of ENMs which would not be translatable to environmentally-relevant conditions. Previous work from the NanoFASE and the Gracious projects showed that the flow-through dynamic dissolution setup could be adapted to determine dissolution rates of ENMs under environmentally-relevant conditions, using artificial media mimicking natural waters. The main advantages of the presented setup are (1) an unlimited supply of exposure media, (2) the possibility to expose a very small amount of ENMs and (3) the instantaneous separation between the dissolved fraction and the solid phase. The very low concentration of ENMs used with such a setup is, indeed, relevant to mimic natural environments harbouring low concentrations of ENMs. The continuous flow setup employed herein maintained unchanged undersaturated conditions during the time of the experiment. However, several experimental aspects need to be improved. The improvement of such a technique is the principal scope of the UBA Dissolution project, focussing on the dissolution of NMs. As part of Gov4Nano (i.e. following Task 2.7.2), a close collaboration with the UBA Dissolution project will be ensured to adjust such an experimental setup to be able to follow solid-phase transformations of NMs. For using the continuous flow setup for studying solid-phase transformation processes, we will have to increase the amount of NMs injected to be able to monitor solid-phase NM transformation with the available suite of instruments and we will be working in more complex exposure media than for the dissolution of NMs.

#### 3.4.1.2 Static exposure experiments

Static batch experiments can be a relatively simple and straight forward method to assess NMs transformations. Indeed, they lead to a direct measure of the solubility of the NM in a specific aqueous medium and allow to determine the final speciation of the exposed NM using different analytical methods such as SEM, TEM and XAS. However, the progressive changes due to ENM dissolution and/or the re-precipitation of secondary species may lead to a change of the medium composition, which could then influence the behaviour of the tested NM in the course of the experiment. Besides, such tests are performed with relatively high concentrations of NM, suitable to study a specific transformation mechanism but not relevant when it comes to mimic natural media. An important part of this T2.7 will be dedicated to the development of an experimental method aiming to investigate NPs transformations under static conditions, keeping a relevant environmental scenario of aquatic natural systems. For this purpose, the development of an adapted NPs support that can be used as a probe was considered as a first step. As candidates for NP support, TEM-grids were chosen. NPs initially attached to a TEM-grid will be exposed to different exposure media. Preliminary results obtained in the European Horizon2020 project

NanoFASE showed that TEM grids are a suitable support that allows following NPs transformations in different exposure media. These results are promising and will especially be used to investigate NPs transformations in sediment pore waters, *via* laboratory and *in situ* experiments. In this regard, sediment column experiments are planned as a continuation of these investigations.

### 3.4.2 Conclusion

Based on the information gathered in this deliverable, the assessment of NMs transformations in aquatic systems can be first anticipated using the existing knowledge. However, knowledge on transformations of NMs in the environment still needs to grow and methods to assess such environmental questions still need to be improved, from their applicability to NMs to the attention of keeping representative scenarios of natural environments. Dissolution behaviour needs to be carefully assessed for NMs, always considered as the main factor of ENM toxicity. However, determination of the nature of the end product is also crucial, since solid-phase transformations are likely to occur in all aquatic compartments and since most NMs dissolution would also act as an intermediate process leading in the formation of secondary species that could mitigate or increase the risk associated to an NM. It appears also necessary to have such speciation information to feed predictive models.

In a first step, to create relevant scenarios of NM exposures in natural waters, the range values of dominant hydro-chemical parameters affecting NM's behaviour in stream waters and anoxic pore waters have been identified. Based on this work, environmentally-relevant exposure media are proposed. Method development will be assessed in the next tasks to propose novel experimental approaches suitable to assess NM transformations under environmentally relevant conditions. Preliminary developments are showing great promise for applicability and are very encouraging for our further work. Eventually, the results of this work will be fed into the development of an OECD guidance document "Environmental transformation of Nanomaterials".

# 4 Deviations from the work plan

No deviations occurred from the work plan.

# 5 Performance of the partners

The deliverable 2.20 is a scientific report written by members of the Gov4Nano consortium that are employed at the University of Vienna. Due to the issue of finding a qualified postdoc candidate for this project (hired in M10 instead of M1) and the current COVID-19 pandemic, the deliverable has been delayed.

# 6 References

Adrian, Y.F.; Schneidewind, U.; Bradford, S.A.; Šimůnek, J.; Klumpp, E.; Azzam, R. Transport and retention of engineered silver nanoparticles in carbonate-rich sediments in the presence and absence of soil organic matter. *Environ. Pollut.* **2019***, 255*, 113124.

Aiken, G.R.; Hsu-Kim, H.; Ryan, J.N. Influence of Dissolved Organic Matter on the Environmental Fate of Metals, Nanoparticles, and Colloids. *Environ Sci Technol.* **2011,** *45*(8), 3196-3201.

Berner, E. K.; Berner R. A. *Global Environment: Water, Air, and Geochemical Cycles.* Prentice Hall, New York, 1996.

Bian, S.-W.; Mudunkotuwa, I. A.; Rupasinghe, T.; Grassian V. H. Aggregation and Dissolution of 4 nm ZnO Nanoparticles in Aqueous Environments: Influence of pH, Ionic Strength, Size, and Adsorption of Humic Acid. *Langmuir* **2011**, 27, 6059-6068.

Bierman, P.; Rosen, C.; Bloom, P.; Nater, E. Soil Solution Chemistry of Sewage-Sludge Incinerator Ash and Phosphate Fertilizer Amended Soil. *J. Environ. Qual.* **1995**, *24*, 279-285.

Blinova, I.; Ivask, A.; Heinlaan, M.; Mortimer, M.; Kahru, A. Ecotoxicity of Nanoparticles of CuO and ZnO in Natural Water. *Environ. Pollut.* **2010,** *158*, 41-47.

Borm, P.; Klaessig, F.C.; Landry, T.D.; Moudgil, B.; Pauluhn, J.; Thomas, K.; Trottier, R.; Wood, S. Research strategies for safety evaluation of nanomaterials, part V: role of dissolution in biological fate and effects of nanoscale particles. *Toxicol Sci.* **2006,** *90*(1), 23-32.

Bove, P.; Malvindi, M.A.; Kote, S.S.; Bertorelli, R.; Summa, M.; Sabella, S. Dissolution test for risk assessment of nanoparticles: a pilot study. *Nanoscale*, **2017**,9, 6315-6326.

Chapman, Deborah V, World Health Organization, Unesco & United Nations Environment Programme. (1996). Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring / edited by Deborah Chapman, 2nd ed. London : E & FN Spon. https://apps.who.int/iris/handle/10665/41850.

Chen, G.; Liu, X.; Su, C. Distinct effects of Humic acid on transport and retention of TiO2 rutile nanoparticles in saturated sand columns. *Environ Sci Technol.* **2012**, *46*, 7142-7150.

Conway, J.R.; Adeleye, A.S.; Gardea-Torresdey, J.; Keller A. A. Aggregation, Dissolution, and Transformation of Copper Nanoparticles in Natural Waters. *Environ. Sci. Technol.* **2015,** *49* (5), 2749-2756. Dobias, J.; Bernier-Latmani, R. Silver Release from Silver Nanoparticles in Natural Waters. *Environ. Sci. Technol.* **2013**, *47*(9), 4140-4146.

Dahle, J.T.; Livi, K.; Arai, Y. Effects of pH and phosphate on CeO2 nanoparticle dissolution, *Chemosphere* **2015,** *119,* 1365-1371.

European Environment Agency (EEA), Nutrients in freshwater in Europe (CSI 020/WAT 003), 2019. Data from Waterbase-Water Quality. Available online:https://www.eea.europa.eu/data-and-maps/indicators/nutrients-in-freshwater/nutrients-in-freshwater-assessment-published-9 (accessed on 20 February 2020).

Fernando, I.; Zhou, Y. Impact of pH on the stability, dissolution and aggregation kinetics of silver nanoparticles. *Chemosphere* **2019**, *216*, 297-305.

French, R. A.; Jacobson, A. R.; Kim, B.; Isley, S. L.; Lee Penn, R.; Baveye, P. C. Influence of Ionic Strength, pH, and Cation Valence on Aggregation Kinetics of Titanium Dioxide Nanoparticles *Environ Sci Technol.* **2009** *43* (5), 1354-1359 DOI: 10.1021/es802628n.

Froelich P. N., Klinkhammer G. P., Bender M. L., Luedtke N. A., Heath G. R., Cullen D., Dauphin P., Hammond D., Hartam B., Maynard V. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim. Cosmochim. Acta* **1975,** 43, 1075-1090.

Furtado, L.M.; Hoque, E.; Mitrano, D.F.; Ranville, J.F.; Norman, B.; Paul, E.; Frost, P.; Marguerite, C.; Xenopoulos, M.; Holger, C.; Metcalfe, C. The persistence and transformation of silver nanoparticles in littoral lake mesocosms monitored using various analytical techniques. *Environ. Chem.* **2014**, *11*(4), 419-430.

Gaillardet, J.; Viers, J.; and Dupré, B. Trace elements in river waters. In: *Treatise on geochemistry* (2e). Edited by H. Holland and K. Turekian. Elsevier Ltd., the Netherlands. pp. 195–235, 2014.

Geitner, N.K.; Cooper, J.L.; Avellan, A.; Castellon, B.T.; Perrotta, B.G.; Bossa, N.; Simonin, M.; Anderson, S.M.; Inoue, S.; Hochella, Jr., M.F.; Richardson, C.J.; Bernhardt, E.S.; Lowry, G.V.; Ferguson, L.P.; Matson, C.W.; King, R.S.; Unrine, J.M.; Wiesner, M.R.; Hsu-Kim, H. Size-Based

Differential Transport, Uptake, and Mass Distribution of Ceria (CeO2) Nanoparticles in Wetland Mesocosms *Environ Sci Technol.* **2018**, *52*(17), 9768-9776.

Geitner, N.K., Ogilvie Hendren, C., Cornelis, G., Kaegi, R., Lead, J.R., Lowry, G.V., Lynch, I., Nowack, B., Petersen, E., Bernhardt, E., Brown, S., Chen, W., de Garidel-Thoron, C., Hanson, J., Harper, S., Jones, K., von der Kammer, F., Kennedy, A., Kidd, J., Matson, C., Metcalfe, C.D., Pedersen, J., Peijnenburg, W.J.G.M., Quik, J.T.K., Rodrigues, S.M., Rose, J., Sayre, P., Simonin, M., Svendsen, C., Tanguay, R., Tefenkji, N., van Teunenbroek, T., Thies, G., Tian, Y., Rice, J., Turner, A., Liu, J., Unrine, J., Vance, M., White, J.C., Wiesner, M.R. Harmonizing across environmental nanomaterial testing media for increased comparability of nanomaterial datasets. *Environ. Sci.: Nano* **2020,** 7, 13–36.

Gogos, A.; Voegelin, A.; Kaegi, R. Influence of organic compounds on the sulfidation of copper oxide nanoparticles. *Environ. Sci.: Nano* **2018,** *5,* 2560-2569.

Gondikas, A.P.; Morris, A.; Reinsch, B.C.; Marinakos, S.M.; Lowry, G.V.; Hsu-Kim, H. Cysteine-induced modifications of zero-valent silver nanomaterials: implications for particle surface chemistry, aggregation, dissolution, and silver speciation. *Environ. Sci. Technol.* **2012**, *46*, 7037-7045.

Gondikas, A. P.; von der Kammer, F.; Reed, R. B.; Wagner, S.; Ranville, J. F.; Hofmann T. Release of TiO2 Nanoparticles from Sunscreens into Surface Waters: A One-Year Survey at the Old Danube Recreational Lake. *Environ Sci Technol.* **2014**, *48*(10), 5415-5422.

Gottschalk, F.; Sonderer, T.; Scholz, R.W.; Nowack, B. Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, fullerenes) for different regions. *Environ. Sci. Technol.* **2009**, *43*, 9216-9222.

Hartmann, N.I.B.; Skjolding, L.; Hansen, S.; Baun, A.; Kjølholt, J.; Gottschalk, F. *Environmental fate and behaviour of nanomaterials: New knowledge on important transformation processes*. Environmental Project No. 1594, 2014 (The Danish Environmental Protection Agency, 2014).

Henglein, A. Colloidal silver nanoparticles: Photochemical preparation and interaction with O2, CCl4, and some metal ions. *Chem. Mater.* **1998,** *10*(1), 444−450.

Ho, C. M.; Yau, S.K.W.; Lok, C.N.; So, M.H.; Che, C.M. Oxidative dissolution of silver nanoparticles by biologically relevant oxidants: A kinetic and mechanistic study. *Chem.−Asian J.* **2010,** *5*(2),285−293.

Jiang, C.; Aiken, G.R.; Hsu-Kim, H. Effects of Natural Organic Matter Properties on the Dissolution Kinetics of Zinc Oxide Nanoparticles *Environ. Sci. Technol.* **2015,** *49* (19), 11476-11484.

Johnson, R.L.; O'Brien Johnson, G.; Nurmi, J.T.; Tratnyek P.G. Natural Organic Matter Enhanced Mobility of Nano Zerovalent Iron. *Environ. Sci. Technol.* **2009**, *43*(14), 5455-5460.

Kaegi, R.; Ulrich, A.; Sinnet, B.; Vonbank, R.; Wichser, A.; Zuleeg, S.; Simmler, H.; Brunner, S. ; Vonmont, H.; Burkhardt, M.; Boller, M. Synthetic TiO2nanoparticle emission from exterior facades into the aquatic environment. *Environ. Pollut.* **2008**, *156*(2), 233−239.

Keller, A.A.; Wang, H.; Zhou, D.; Lenihan, H.S.; Cherr, G.; Cardinale, B.J.; Miller, R.; Ji Z. Stability and Aggregation of Metal Oxide Nanoparticles in Natural Aqueous Matrices. *Environ. Sci. Technol.* **2010,** *44* (6), 1962-1967.

Keller, A. A.; Adeleye, A. S.; Conway, J. R.; Garner, K. L.; Zhao, L.; Cherr, G. N.; Hong, J.; Gardea-Torresdey, J. L.; Godwin, H.A.; Hanna, S.; Ji, Z.; Kaweeteerawat, C.; Lin, S.; Lenihan, H. S.; Miller, R. J.; Nel, A. E.; Peralta-Videa, J. R.; Walker, S. L.; Taylor, A. A.; Torres-Duarte, C.; Zink, J. I.; Zuverza-Mena, N. Comparative environmental fate and toxicity of copper nanomaterials. *NanoImpact,* **2017,** *7*, 28-40.

Kent, R.D.; Vikesland, P.J. Dissolution and Persistence of Copper-Based Nanomaterials in Undersaturated Solutions with Respect to Cupric Solid Phases. *Environ. Sci. Technol.* **2016,** *50* (13), 6772-6781

Khaksar M.; Jolley D.F.; Sekine R.; Vasilev K.; Johannessen B.; Donner E.; Lombi E. In Situ Chemical Transformations of Silver Nanoparticles along the Water–Sediment Continuum. *Environ. Sci. Technol.* **2015,** *49*(1), 318-325.

Koltermann-Jülly, J.; Keller, J.G.; Vennemann, A.; Werle, K.; Müller, P.; Ma-Hock, L.; Landsiedel, R.; Wiemann, M.; Wohlleben, W. Abiotic dissolution rates of 24 (nano) forms of 6 substances compared to macrophage-assisted dissolution and in vivo pulmonary clearance: Grouping by biodissolution and transformation. *NanoImpact* **2018,** *12,* 29-41.

Kumar, N.; Pacheco, J.L.; Noël, V.; Dublet G.; Brown, Jr. G.E. Sulfidation mechanisms of Fe(III)-(oxyhydr)oxide nanoparticles: a spectroscopic study. *Environ. Sci.: Nano* **2018**, *5*, 1012-1026.

Levard, C.; Reinsch, B. C.; Michel, F. M.; Oumahi, C.; Lowry, G. V.; Brown, G. E., Jr. Sulfidation processes of PVP-coated silver nanoparticles in aqueous solution: Impact on dissolution rate. *Environ. Sci. Technol.* **2011**, *45*(12), 5260−5266.

Levard, C.; Hotze, E.M.; Lowry, G.V.; Brown, G.E., Jr. Environmental Transformations of Silver Nanoparticles: Impact on Stability and Toxicity*. Environ. Sci. Technol.* **2012**, *46,* 6900−6914.

Levard, C.; Mitra, S.; Yang, T.; Jew, A.D.; Badireddy, A.R.; Lowry, G.V.; Brown, Jr. G.E. Effect of Chloride on the Dissolution Rate of Silver Nanoparticles and Toxicity to E. coli. *Environ Sci Technol.* **2013,** *47* (11), 5738-5745.

Li, L.; Schuster, M. Influence of phosphate and solution pH on the mobility of ZnO nanoparticles in saturated sand. *Sci. Total Environ.* **2014**, *472*, 971-978.

Li, M.; Zhu, L.; Lin, D. Toxicity of ZnO nanoparticles to Escherichia coli: mechanism and the influence of medium components. *Environ Sci Technol.* **2012**, *45*, 1977-1983.

Li, X.; Lenhart, J.J.; Walker, H.W. Dissolution-accompanied aggregation kinetics of silver nanoparticles. *Langmuir* **2010**, *26*(22), 16690-16698.

Liu, J.Y.; Hurt, R.H. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ. Sci. Technol.* **2010**, *44*(6), 2169-2175.

Lombi, E.; Donner, E.; Tavakkoli, E.; Turney, T. W.; Naidu, R.; Miller, B.W.; Scheckel, K.G. Fate of Zinc Oxide Nanoparticles during Anaerobic Digestion of Wastewater and Post-Treatment Processing of Sewage Sludge. *Environ. Sci. Technol.* **2012,** *46*(16), 9089−9096.

Lowry, G.V.; Espinasse, B.P.; Raju Badireddy, A.; Richardson, C.J.; Reinsch, B.C.; Bryant, L.D.; Bone, A.J.; Deonarine, A.; Chae, S.; Therezien, M.; Colman, B.P.; Hsu-Kim, H.; Bernhardt, E.S.; Matson, C.W.; Wiesner, M.R. Long-Term Transformation and Fate of Manufactured Ag Nanoparticles in a Simulated Large Scale Freshwater Emergent Wetland. *Environ. Sci. Technol.* **2012,** *46*(13), 7027-7036.

Lv, J.; Zhang, S.; Luo, L.; Han, W.; Zhang, J.; Yang, K.; Christie, P. Dissolution and microstructural transformation of ZnO nanoparticles under the influence of phosphate. *Environ Sci Technol.* **2012,** *46*, 7215-7221.

Liu, J.; Pennell, K.G.; Hurt, R.H. Kinetics and mechanisms of nanosilver oxysulfidation. *Environ. Sci. Technol.* **2011**, *45*(17), 7345-7353.

Ma, R.; Levard, C.M.; Michel, F.M.; Brown, G.E., Jr.; Lowry, G.V. Sulfidation mechanism for zinc oxide nanoparticles and the effect of sulfidation on their solubility. *Environ. Sci. Technol.* **2013**, *47*(6), 2527−2534.

Ma, R.; Stegemeier, J.; Levard, C.; Dale, J.; Noack, C.; Yang, T.; Brown, G.; Lowry, G. Sulfidation of copper oxide nanoparticles and properties of resulting copper sulfide. *Environ. Sci.: Nano* **2014**, *1*, 347-357.

Miller, R.J.; Lenihan, H.S.; Muller, E.B.; Tseng, N.; Hanna, S.K.; Keller A.A. Impacts of Metal Oxide Nanoparticles on Marine Phytoplankton. *Environ. Sci. Technol.* **2010,** *44*(19), 7329-7334.

Misra, S.K.; Dybowska, A.; Berhanu, D.; Luoma, S.N.; Valsami-Jones, E. The complexity of nanoparticle dissolution and its importance in nanotoxicological studies. *Sci. Total Environ.* **2012**, *438*, 225–232.

Odzak N.; Kistler D.; Behra R.; Sigg L. Dissolution of metal and metal oxide nanoparticles under natural freshwater conditions. *Environmental Chemistry* **2014, *12,*** 138-148.

Odzak N.; Kistler D.; Sigg L. Influence of daylight on the fate of silver and zinc oxide nanoparticles in natural aquatic environments. Environmental Pollution, **2017**, *226*, 1-11.

Ogier S. (1999) Diagenèse précoce en domaine lacustre : étude des composés minéraux et organiques des sédiments récents du lac d'Aydat (Puy de Dôme, France). Géologie appliquée. Université d'Orléans, <tel-00002273>.

Peng, C.; Shen, C.; Zheng, S.; Yang, W.; Hu, H.; Liu, J.; Shi, J. Transformation of CuO Nanoparticles in the Aquatic Environment: Influence of pH, Electrolytes and Natural Organic Matter. *Nanomaterials* **2017**, *7*, 326.

Rathnayake, S.; Unrine, J.M.; Judy, J.; Miller, A.F., Rao, W., Bertsch, P.M. Multitechnique investigation of the pH dependence of phosphate induced transformations of ZnO nanoparticles. *Environ. Sci. Technol.* **2014**, *48*, 4757-4764.

Römer, I.; Briffa, S.M.; Arroyo Rojas Dasilva, Y.; Hapiuk, D, Trouillet, V.; Palmer, R.E.; Valsami-Jones, E. Impact of particle size, oxidation state and capping agent of different cerium dioxide nanoparticles on the phosphate-induced transformations at different pH and concentration. *PLOS ONE* **2019**, *14*(6): e0217483.

Salminen, R.; Tarvainen, T.; Demetriades, A.; Duris, M.; Fordyce, F.M.; Gregorauskiene, V.; Kahelin, H.; Kivisilla, J.; Klaver, G.; Klein, H.; Larson, J.O.; Lis, J.; Locutura, J.; Marsina, K.; Mjartanova, H.; Mouvet, C.; O'Connor, P.; Odor, L.; Ottonello, G.; Paukola, T.; Plant, J.A.; Reimann, C.; Schermann, O.; Siewers, U.; Steenfelt, A.; Van der Sluys, J.; Williams, L. FOREGS Geochemical Mapping Field Manual. Geological Survey of Finland, Espoo, **1998**, Guide 47, pp. 36.

Salminen, R.; Batista, M.J.; Bidovec, M.; Demetriades, A.; De Vivo, B.; De Vos, W.; Duris, M.; Gilucis, A.; Gregorauskiene, V.; Halamic, J.; Heitzmann, P.; Lima, A.; Jordan, G.; Klaver, G.; Klein, P.; Lis, J.; Locutura, J.; Marsina, K.; Mazreku, A.; O'Connor, P.J.; Olsson, S.Å.; Ottesen, R.-T.; Petersell, V.; Plant, J.A.; Reeder, S.; Salpeteur, I.; Sandström, H.; Siewers, U.; Steenfelt, A.; Tarvainen, T. Geochemical Atlas of Europe. Part 1 – Background Information, Methodology and Maps. Geological Survey of Finland, Espoo, Finland**, 2005**, pp. 526.

Sekine, R.; Brunetti, G.; Donner, E.; Khaksar, M.; Vasilev, K.; Jämting, Å.K.; Scheckel, K. G.; Kappen, P.; Zhang, H.; Lombi E. Speciation and Lability of Ag-, AgCl-, and Ag2S-Nanoparticles in Soil Determined by X-ray Absorption Spectroscopy and Diffusive Gradients in Thin Films. *Environ. Sci. Technol.* **2015,** *49,* 897-905.

Sekine, R.; Marzouk, E.R.; Khaksar, M.; Scheckel, K.G.; Stegemeier, J.P.; Lowry, G.V.; Donner, E.; Lombi, E. Aging of dissolved copper and copper-based nanoparticles in five different soils: short-term kinetics vs. long-term fate. *J. of Environ. Qual.* **2017**, *46*(6). 1198-1205.

Sivry, Y.; Gelabert, A.; Cordier, L.; Ferrari, R.; Lazar, H.; Juillot, F.; Menguy, N.; Benedetti, M. F. Behavior and fate of industrial zinc oxide nanoparticles in a carbonate-rich river water. *Chemosphere,* **2014,** *95*, 519-526.

Skjolding, L.M.; Sørensen, S.N.; Hartmann, N.B.; Hjorth, R.; Hansen, S.F.; Baun, A. Aquatic ecotoxicity testing of nanoparticles—the quest to disclose nanoparticle effects. *Angew Chem Int Ed Engl.* **2016**, *55*(49), 15224-15239.

Wielinski, J.; Gogos, A.; Voegelin, A.; Müller, C.; Morgenroth, E.; Kaegi, R. Transformation of Nanoscale and Ionic Cu and Zn during the Incineration of Digested Sewage Sludge (Biosolids). *Environ Sci Technol.* **2019**, *53*(20), 11704-11713.

World Health Organization & International Programme on Chemical Safety. Chou, C. H.; Selene J. *Hydrogen Sulfide: Human Health Aspects*. World Health Organization (WHO), Geneva, 2003. Available online: https://apps.who.int/iris/handle/10665/42638.

Yu, S.; Liu, J.; Yin, Y.; Shen, M. Interactions between engineered nanoparticles and dissolved organic matter: A review on mechanisms and environmental effects. *Journal of Environmental Sciences* **2018**, *63*, 198-217.

Zou, X.; Li, P.; Lou, J.; Fu, X.; Zhang, H. Stability of single dispersed silver nanoparticles in natural and synthetic freshwaters: Effects of dissolved oxygen. *Environ. Pollut.* **2017**, *230,* 674-682.

Zhao, L.J.; Peralta-Videa, J.R.; Hernandez-Viezcas, J.A.; Hong, J.; Gardea-Torresdey, J.L. Transport and Retention Behavior of ZnO Nanoparticles in Two Natural Soils: Effect of Surface Coating and Soil Composition. *J. Nano R.* **2012**, *17*, 229-242.

# 7 List of abbreviations

| | |
|---|---|
| APW | Artificial Pore Water |
| DOC | Dissolved Organic Carbon |
| EDXS | Energy-dispersive X-ray spectroscopy |
| EEA | European Environmental Agency |
| ENM | Engineered Nanomaterial |
| EPA | (United States) Environmental Protection Agency |
| FOREGS | Forum of European Geological Surveys |
| HA | Humic Acid |
| ICP-MS | Inductively Coupled Plasma-Mass Spectrometry |
| ISO | International Organization for Standardization |
| JRC | Joint Research Centre |
| NM | Nanomaterial |
| NOM | Natural Organic Matter |
| NP | Nanoparticle |
| NPOC | Non-purgeable organic carbon |
| OECD | Organization for Economic Co-operation and Development |
| SEM | Scanning Electron Microscopy |
| STEM | Scanning Transmission Electron Microscopy |
| TEM | Transmission Electron Microscopy |
| UBA | Umweltbundesamt, i.e. the German Environment Agency |
| WHO | World Health Organization |
| WP | Work Package |
| WPMN | Working Party on Manufactured Nanomaterials |
| WWTP | Waste Water Treatment Plant |
| XAS | X-Ray Absorption Spectroscopy |