Fate of metallic engineered nanomaterials in constructed wetlands: prospection and future research perspectives

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Abstract

Metallic engineered nanomaterials (ENMs) undergo various transformations in the environment which affect their fate, toxicity and bioavailability. Although constructed wetlands (CWs) are applied as treatment systems for waste streams potentially containing metallic engineered nanomaterials (ENMs), little is known about the fate and effects of ENMs in CWs. Hence, literature data from related fields such as activated sludge wastewater treatment and natural wetlands is used to predict the fate and effects of ENMs in CWs and to analyze the risk of nanomaterials being released from CWs into surface waters. The ENMs are likely to reach the CW (partly) transformed and the transformations will continue in the CW. The main transformation processes depend on the type of ENM and the ambient environmental conditions in the CW. In general, ENMs are expected to undergo sorption onto (suspended) organic matter and plant roots. Although the risk of ENMs being released at high concentrations from CWs is estimated low, caution is warranted because of the estimated rise in the production of these materials. As discharge of (transformed) ENMs from CWs during normal operation is predicted to be low, future research should rather focus on the effects of system malfunctions (e.g. short-circuiting). Efficient retention in the CW and increasing production volumes in the future entail increasing concentrations within the CW substrate and further research needs to address possible adverse effects caused.

Key words

Nanoparticle, transformation, wastewater, effluent, discharge, TSS

1. Introduction

The growing production and widespread use of engineered nanomaterials (ENMs) in commercial products leads to increasing presence of these materials in the environment. The ENMs are defined by their small particle size (<100 nm) and large specific surface area which can increase their mobility, bioavailability and reactivity in comparison to their bulk counterparts (Christian et al. 2008; Navarro et al. 2008). Due to these characteristics ENMs pose a potential risk to the environment.

In principle, European environmental legislation for water, waste and air covers also ENMs and hence, this legislation would theoretically allow limiting the presence of ENMs in environmental media (Ganzleben et al. 2011). However in practice, this is difficult for several reasons. Firstly, suitable analysis methods are lacking to allow measuring low concentrations of ENMs in environmental samples and distinguishing between naturally occurring nano-scale particles from engineered nanomaterials. Secondly, due to the lacking analysis methodologies, there is no monitoring data of the presence of ENMs in surface waters nor is there enough evidence on ENM causing imminent risk in surface waters. Hence, ENMs cannot be listed as priority substances in the Water Framework Directive which would entail their monitoring in surface water and allow setting environmental quality standards. As a consequence, the measures to avoid potential emissions to the environment limit to risk management measures given in REACH. Through REACH companies are required to register the chemical substances, including ENMs, used in the products and provide risk management measures to the users (ECHA 2012).

ENMs can be discharged to the aquatic environment via the production, usage and disposal phases (Figure 1). For example, silver nanoparticles (Ag-NPs) and titanium dioxide nanoparticles (TiO_2-NPs) are used in common household products. As they are used in textiles, plastics and cosmetics, they can be released from the product during washing and end up in wastewater (e.g. Benn & Westerhoff 2008). Also, ENMs can be released from (weathered) materials, e.g. from painted facades during rainfall and then be transported with runoff (Kaegi et al. 2008). A large amount of ENM containing materials are disposed of in landfills (Keller and Lazareva 2013) and leaching could occur (Gottschalk and Nowack 2011).

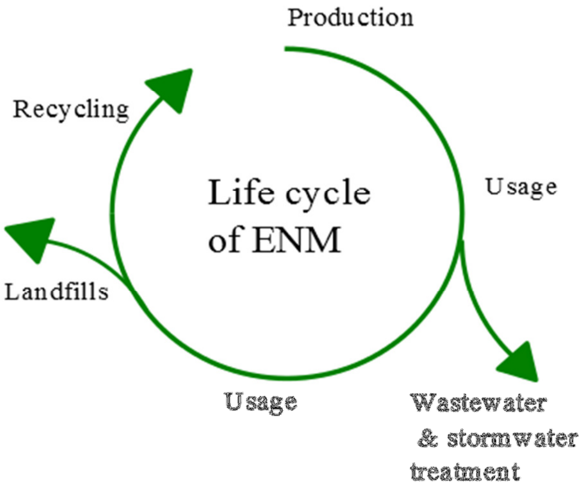


Figure 1: Possible release of ENM to the environment during their life cycle

A typical constructed wetland (CW) for wastewater treatment is a sub-surface flow CW, where water flows either vertically or horizontally through a gravel or sand bed. CWs are planted to increase the microbial activity in the bed, to increase nutrient removal and for aesthetic reasons. CWs are commonly used as decentralized wastewater treatment in rural areas because of, on one hand their robust treatment and low maintenance requirement and, on the other hand, because of their large land area requirement, which does restrict their use in urban areas. In addition to domestic wastewater, also industrial wastewater, stormwater and landfill leachate, i.e. potentially ENM bearing waste streams, are treated in CWs (Kadlec and Wallace 2009). When used for the treatment of domestic wastewater, CWs are most often used as a secondary treatment for settled wastewater but in some cases also as tertiary treatment after activated sludge treatment or rotating biological contactor. In France, constructed wetlands are often designed to treat domestic wastewater without primary settling (Molle et al. 2005). This creates a sludge layer on top of the wetland, which is disposed of about every 10 years. Although CWs constitute a possible source of ENMs in the environment, there is little data available in literature on the fate and removal efficiency of ENMs in CWs.

In this review, we provide an overview of transformation processes of ENMs and environmental factors affecting these processes based on literature data, and discuss the probable fate of ENMs in CWs. We also discuss the likelihood of ENMs being released from CWs and assess the potential toxicity of ENMs on the wastewater treatment processes occurring in CWs.

2. Predicted environmental concentrations of ENMs

At the moment, little is known about the environmental concentrations of ENMs because current analytical methodologies do not allow distinguishing between naturally occurring nanoscale materials and ENMs (von der Kammer et al. 2012). However, several calculations of predicted environmental concentrations (PEC) based on a probabilistic material flow analysis from a life-cycle perspective of ENM containing products have been performed. Blaser et al. (2008) estimated that the concentration of Ag-NPs in wastewater influent ranges between 2 to 18 µg/L which is somewhat higher than measured for nanosized Ag in real wastewater influent (< 1.5 µg/L; Li et al. 2013). Li et al. (2013) also showed that the concentration of the nanosized Ag decrease considerably during wastewater treatment, to < 12 ng/L. This is in agreement with modeling studies: predicted concentrations of 0.06-16 ng/L, 13-110 µg/L and 0.05-0.29 µg/L for Ag-NPs, TiO_2-NPs and zinc oxide NPs (ZnO-NPs) in wastewater effluent, respectively. The concentrations of ENMs decrease further upon discharge to surface water due to dilution. The concentrations of Ag-NPs, TiO_2-NPs and ZnO-NPs in European surface water were estimated to be very low, on average 0.66 ng/L, 0.53 µg/L and 0.09 µg/L, respectively (Sun et al. 2014). Furthermore, Markus et al. (2013) predicted that the contribution of Ag-NPs, TiO_2-NPs and ZnO-NPs to the current total load of these metals in the rivers Rhine and Meuse is insignificant. The ENMs which are removed during wastewater treatment in the CW are retained within the substrate possibly bringing the biofilm into contact with a growing concentration of ENMs over time (see further section 3.4).

The growing production and accumulation of ENMs in the CW substrate could thus cause toxic concentrations to appear in the long term. It must be noted however, that the concentration of the corresponding bulk metals is currently expected to be much higher than that of the nanoparticles. Lesage et al. (2007) who studied the accumulation of heavy metals in a horizontal sub-surface flow CW treating domestic wastewater reported average influent concentrations of 20 µg Cu/L, 120 µg Zn/L and 550 µg Fe/L, for example. Hence, the estimated concentration of ZnO-NPs in the influent is more than 100 times lower than that of its bulk counterpart. In case of Ag, Ag-NPs released from plastics and textiles can form a large fraction of the total Ag load in the influent if industrial wastewater is not treated in the CW.

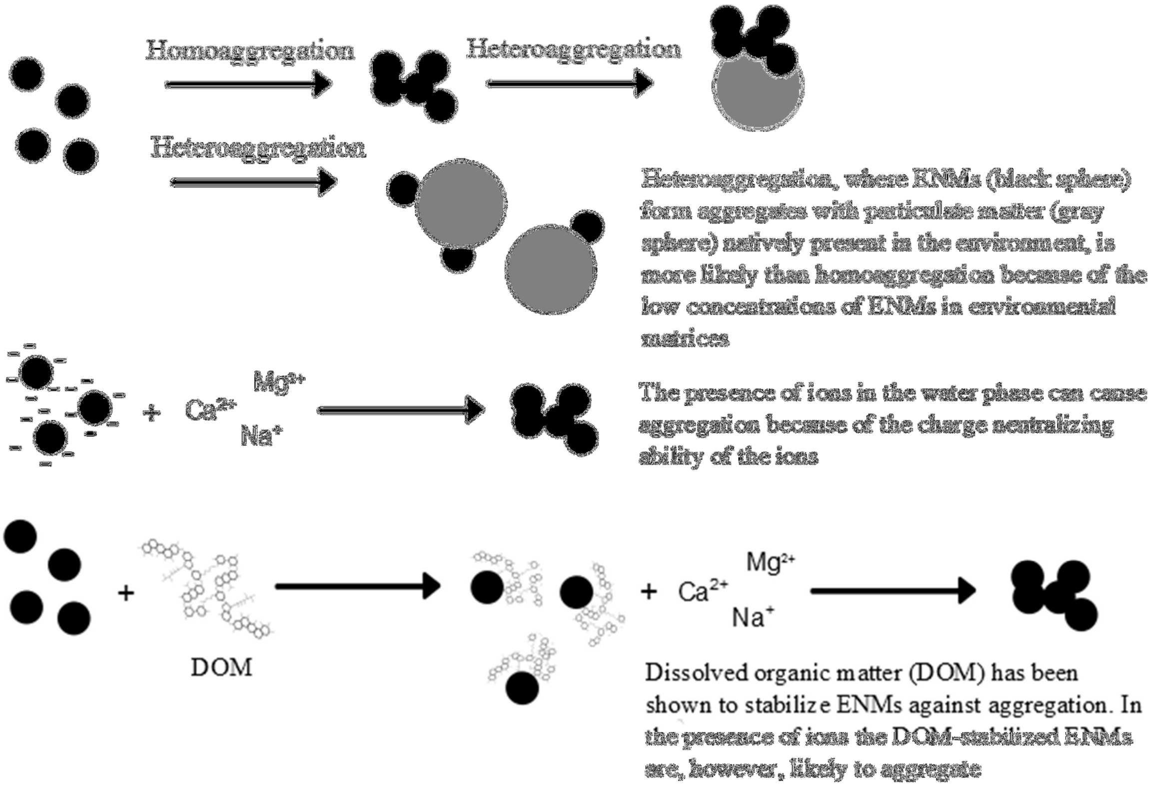
3. Possible transformation processes affecting the fate of ENMs in constructed wetlands

When ENMs are released to the aquatic medium they can undergo physical, chemical and biological transformations which affect their fate and behavior in the environment. These transformations include primarily processes such as dissolution, aggregation, sedimentation, sorption onto particulate matter and other solid surfaces and interaction with dissolved ions. The transformation processes are dependent on both the properties of the ENM (e.g. type, coating, size) and the prevailing environmental conditions (e.g. pH, presence of ions, natural organic matter and dissolved oxygen). In the following sections, the main transformation processes of different ENMs reported in earlier literature are reviewed and the environmental factors affecting these processes are discussed in relation to their possible fate in CWs.

3.1. Aggregation and sedimentation

Aggregation and subsequent sedimentation of ENMs decrease their mobility and bioavailability by restricting their distribution in the aquatic environment. Also, the surface area of the particles decreases due to aggregation which in turn decreases its reactivity, affecting the release of ions for instance (see further section 3.2).

Nanosized dispersed particles are not likely to settle. They move about in the dispersion by Brownian motion and along the streaming water. When this movement brings particle surfaces in contact, thermodynamic interactions allow aggregation to occur (Zhang 2014). ENMs can undergo two forms of aggregation in the environment, homo- and heteroaggregation (Figure 2). Whereas in homoaggregation particles of the same type collide and form aggregates, in heteroaggregation ENM aggregates with another kind of particulate or colloidal matter (Quik et al. 2014). When ENMs are scarcely present, as in most environmental media, the likelihood of homoaggregation is low (Hotze et al. 2010). In wastewater, for example, heterogenic particulate matter is ubiquitous and hence, aggregates formed with this matter and ENMs are much more likely to be formed than aggregates composed of only ENMs.



Heteroaggregation, where ENMs (black sphere) form aggregates with particulate matter (gray sphere) natively present in the environment, is more likely than homoaggregation because of the low concentrations of ENMs in environmental matrices

The presence of ions in the water phase can cause aggregation because of the charge neutralizing ability of the ions

Dissolved organic matter (DOM) has been shown to stabilize ENMs against aggregation. In the presence of ions the DOM-stabilized ENMs are, however, likely to aggregate

Figure 2 – Aggregation of ENMs in environmental matrices

Because the properties of ENMs are related to their small size and surface properties, the ENMs are mostly coated to enhance their dispersion stability, i.e. to prevent aggregation (Hotze et al. 2010). The mechanism of stabilization, electrostatic or steric, can have an impact on the behavior of the ENM in the environment (Christian et al. 2008). Electrostatically stabilized ENMs are kept in dispersion through the repulsion of similar surface charges and are thus more susceptible to changes in ionic concentrations in the media than sterically stabilized particles having an isolating polymer coating to keep them in dispersion. Several studies have investigated the effect of ionic strength on the aggregation behavior of different types of ENMs. Huynh & Chen (2011) and Li et al. (2010) studied the effects of NaCl, $MgCl_2$ and $CaCl_2$ on Ag-NPs with different coatings. The common conclusion that can be drawn from these studies is that the type of ion is more determinant of the aggregation than the mechanism of stabilization (Figure 2). For example, complete aggregation of citrate-coated (i.e. electrostatic stabilization) or polyvinylpyrrolidone (PVP)-coated (i.e. steric stabilization) Ag-NPs is achieved by >10 mM of monovalent ions, while only >1 mM of divalent ions is needed for complete aggregation. Divalent ions, such as e.g. Ca^{2+}, have a stronger charge neutralizing ability than monovalent ions, e.g. Na^+ (El Badawy et al. 2012). Also TiO_2-NPs, cerium dioxide NPs (CeO_2-NPs), copper oxide nanoparticles (CuO-NPs), copper nanoparticles (Cu-NPs) and ZnO-NPs have been observed to undergo aggregation and sedimentation at elevated ionic strength (Zhang et al. 2009; Zhou and Keller 2010; Gallego-Urrea et al. 2014). Although the natural concentrations of the ions are somewhat lower (in the range of 0.5 - 5 mM; Verbanck et al. 1989) than in the studies on synthetic waters, a cumulative effect can be expected in real freshwater and wastewater samples.

Similarly to coating, natural dissolved organic matter (DOM) which attaches on the surface of the ENMs has a stabilizing effect on the ENMs due to which the ENMs are more persistent to aggregation (Gallego-Urrea et al. 2014) (Figure 2). DOM has been shown to cause disaggregation and subsequent decrease in Ag-NP size distribution at concentrations 5 - 20 mg/L total organic carbon (TOC) as Suwannee river humic acid (SRHA) and up to 15 mg Ag/L (Fabrega et al. 2009; Kanel et al. 2015; Metreveli et al. 2015). Similarly, the presence of DOM has been shown to have a stabilizing effect on TiO_2-NPs (Zhang et al. 2009), CeO_2-NPs (Van Hoecke et al. 2011), CuO-NPs (Ben-Moshe et al. 2010), ZnO-NPs (Zhang et al. 2009), iron trioxide NPs (Fe_2O_3) (Zhang et al. 2009; Ben-Moshe et al. 2010), gold NPs (Au-NPs) (Stankus et al. 2011) and nickel oxide NPs (NiO-NPs) (Zhang et al. 2009). However, there are also contradictory results showing no significant impact of SRHA (2.5 - 10 mg TOC/L) on the aggregation state of carbonate-coated Ag-NPs or disaggregation of pre-agglomerated Ag-NPs (Piccapietra et al. 2012).

The dissolved fraction of the total natural organic carbon is some 20 – 30 % in wastewater influent but in effluent nearly 100 % (Dignac et al. 2000). Studies on dissolved organic carbon (DOC) removal in activated sludge treatment plants have reported concentrations of 10 – 20 mg DOC/L in wastewater effluent (Dignac et al. 2000; Katsoyiannis and Samara 2007) proving the relevance of the above mentioned studies in the context of wastewater treatment. In CWs similar removal of the particulate fraction is expected due to efficient removal of total suspended solids (TSS) (Vymazal 2009) but the composition and concentration of DOM may differ from that in an activated sludge treatment system. A study of Barber et al. (2001) on tertiary surface-flow CWs treating domestic wastewater, pulp mill and dairy effluent showed that CWs receiving treated wastewater can even produce an increase in DOC. The autochthonous DOM in CWs originates primarily from microbial activity (Quanrud et al. 2004), plant debris (Pinney et al. 2000) and plant root exudates (Glatzel et al. 2003). This indicates that the DOM discharged from CWs differs probably greatly from that of activated sludge treatment systems and may have a different impact on the stability of ENMs. However, in wastewater the ionic strength, especially the presence of Ca^{2+}, is probably more determinant of aggregation than DOM. Metreveli et al. (2015) showed that above a relatively low concentration of Ca^{2+} (~20 mg/L; moderately soft water) Ag-NPs aggregated until sedimentation independently of the presence or absence of SRHA.

3.2. Dissolution

When an ENM is dissolved, metal ions are released from its surface. The dissolution process of ENMs has environmental relevance from the risk analysis perspective because ions are often more toxic than ENMs (Sotiriou and Pratsinis 2010; Li et al. 2011; Levard et al. 2013a; Moore et al. 2016). However, dissolution can possibly reduce the mobility as Ag^{+} and Ce^{2+} appear to be more efficiently bound to soil/sediment than their nanoparticle counterparts (Cornelis et al. 2012; Van Koetsem et al. 2015).

Odzak et al. (2014) compared the dissolution tendencies of four different types of ENMs in artificial aqueous media chemically similar to environmental waters, and found clear differences in the dissolution rates of the ENMs. While Ag-NPs citrate, gelatin, PVP and chitosan coated Ag-NPs were very poorly soluble (only some %), ZnO-NPs were very rapidly largely dissolved. The dissolution of

CuO-NPs was found to be incomplete and faster than that of carbon-coated Cu-NPs but both dissolved to a smaller extent than ZnO-NPs. Quik et al. (2014) studied the environmental transformations of CeO_2-NPs in different surface waters and observed only minor dissolution (<0.4 %).

The dissolution rate is also related to particle size; small ENMs being dissolved faster than larger ones due to their greater surface-to-volume ratio and subsequent increased reactivity (Zhang et al. 2011). As a consequence, the dissolution rate of aggregated ENMs is reduced (Li and Lenhart 2012).

Odzak et al. (2014) observed that the dissolution tendency is to some extent also dependent on the coating of the ENM. The coating stabilizes the ENM against dissolution by isolating the metal core inside the coating (as was discussed in section 3.1). The presence of NOM in the water also inhibits the dissolution of ENMs and this is a dose dependent manner (Liu and Hurt 2010). The inhibition is likely caused by NOM attaching onto the surface of the ENM which results in steric stabilization analogue to a coating (Delay et al. 2011).

Ions dissolved in the water can affect the dissolution rate of ENMs. The reaction with sulfide, sulfidation, can either decrease or increase the dissolution process (Levard et al. 2011; Ma et al. 2013; Ma et al. 2014), as is also discussed in section 3.3. ZnO-NPs can react with phosphates to form precipitates which have lower solubility than the ZnO-NPs themselves (Rathnayake et al. 2014). The reaction between Ag-NPs and chloride ions can decrease the release of Ag^+ by formation of a solid AgCl precipitate on the outer layer of the ENM (Li et al. 2010) if the molar Cl/Ag ratio is ≤ 535) (Levard et al. 2013b). However, at environmentally relevant (low) concentrations of Ag-NPs (see section 2) in wastewater the Cl/Ag molar ratio is much higher (in the range of 10^5 if 1 µg Ag/L and 100 mg Cl/L is assumed) and this promotes the formation of readily soluble Ag-Cl complexes instead (Levard et al. 2013b).

Ag-NPs are more easily dissolved if there is oxygen present in the environment because oxygen causes the Ag-NPs to oxidize to silver oxides (Ag_2O) which easily dissolve upon contact with water (Levard et al. 2012). In CWs the dissolved oxygen concentration depends on the type (vertical sub-surface (VSSF) or horizontal sub-surface flow (HSSF)) and possible application of active aeration. Higher dissolved oxygen concentrations are usually measured in VSSF CWs than in HSSF CWs because the batch feeding mode applied in VF CWs allows the pores to be filled with air between feeding events.

3.3. Sulfidation

The reaction of ENMs with sulfides is interesting because sulfidation of Ag-NPs has been shown to decrease their toxicity (Reinsch et al. 2012; Levard et al. 2013a). When sulfide concentration is low, e.g. in surface water, sulfidation of Ag-NPs occurs via oxidative dissolution and subsequent precipitation (Liu et al. 2011) (Figure 3). First, Ag^+ is formed via oxidative dissolution in the presence of dissolved oxygen, after which these ions precipitate with sulfides to form silver sulfide (Ag_2S). For the reaction to occur, sulfide can be either free or in the form of metal sulfides (e.g. CuS, ZnS) (Thalmann et al. 2014). When sulfide concentration is high, e.g. within HSSF CWs, sulfidation of Ag-NPs occurs via a direct particle-fluid reaction where the Ag phase is converted to Ag_2S phase (Liu et al. 2011). The Ag_2S is very stable and does not dissolve even after prolonged aeration (Choi et al. 2009).

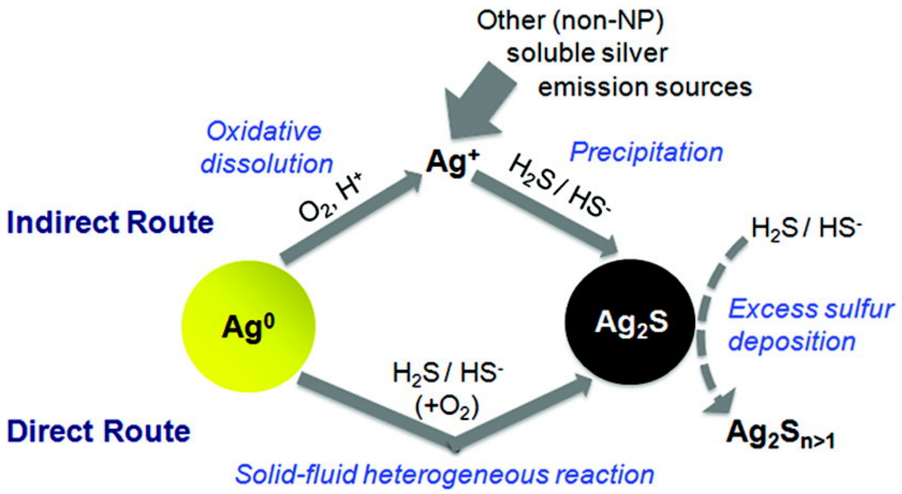


Figure 3 - Sulfidation pathways of Ag-NPs.
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Kinetics and mechanisms of nanosilver oxysulfidation. Environmental Science and Technology 45 (17), 7345-7353.
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Ma et al. (2013) studied the sulfidation mechanism of ZnO-NPs in the laboratory. While a solid Ag_2S phase is formed during the sulfidation of Ag-NPs, the ZnO core becomes covered by nanocrystalline zinc sulfide (ZnS). The ZnS shell does not quench the leaching of Zn^{2+} as efficiently as the Ag_2S phase (Levard et al. 2011). In contrast to Ag- and ZnO-NPs, the sulfides formed of CuO-NPs are more prone to dissolution than the original NP (Ma et al. 2014). The main reason is proposed to be the formation of poorly ordered easily soluble Cu_xS_y phases instead of poorly soluble CuS. The reaction occurs through both dissolution and reaction with sulfide as well as a direct solid-fluid sulfidation.

Sulfidation has been identified as one of the primary transformation mechanisms of Ag-NPs in the sewer systems (Kaegi et al. 2013) as well as in pilot- and full-scale activated sludge treatment systems (Kaegi et al. 2013; Kent et al. 2014) and in terrestrial and freshwater wetland mesocosms (Lowry et al. 2012a; Moore et al. 2016). Lombi et al. (2013) showed that Ag-NPs were sulfidized also in laboratory-scale anaerobic digestors fed with real sludge and wastewater and that the reaction occurred irrespective of surface functionalities or core composition (Ag or AgCl) of the NP. Lowry et al. (2012a) noticed however that sulfidation in the freshwater wetland mesocosms was slower than expected based on laboratory studies and that the sulfidation had occurred only partially after 18 months and leaching of Ag^+ was still possible. Once fully sulfidized, Ag_2S is very stable as it does not oxidize during prolonged aeration (Choi et al. 2009) and remains stable in wastewater sludge over more than 50 years (Donner et al. 2015).

ZnO-NPs are also prone to sulfidation but they may not be equally persistent as sulfidized Ag-NPs. In short-term, the sulfidation of ZnO-NPs seems to be efficient as Brunetti et al. (2015) showed that the spiked ZnO-NPs nearly completely sulfidized in a full-scale sewer experiment. Lombi et al. (2012) studied the fate of ZnO-NPs during anaerobic digestion of sewage sludge and in conditions simulating the post-processing of digested sludge in composting/stockpiling. First, the ZnO-NPs underwent sulfidation which represented 67 - 96 % of the total Zn. The Zn speciation was affected by the long (two months) incubation simulating composting/stockpiling and during this phase, ZnS was oxidized and Zn became associated with iron precipitates and complexed by phosphates.

The sulfide concentration of the wastewater (in sewer ~4 mg S/L; Kaegi et al. 2013) is expected to increase in CWs due to microbial sulfate reduction, where sulfate-reducing bacteria catalyze the production of sulfide from sulfate in the absence of dissolved oxygen (Wu et al. 2013). If we assume the sulfate removal efficiency to be ~30 % (based to laboratory-scale experiments of Wiessner et al. 2005) and an average sulfate concentration of ~30 mg S/L for domestic wastewater influent (Yoda et al. 1987), an increase of ~9 mg S/L can be expected in CWs. Bearing in mind the low predicted environmental concentrations of ENMs (see section 2), the S:Ag and S:ZnO molar ratio's would be in the range of 10^4. Since the molar ratios necessary for the sulfidation of Ag-NPs and ZnO-NPs is much lower (in the range of 0.5 - 1; Levard et al. 2011), (partial) sulfidation of these ENMs in CWs seems unavoidable.

3.4. Adsorption

In general, adsorption of molecules onto surfaces can result from three types of interactions, physical, chemical and electrostatic interactions (Voice and Weber 1983). In the environment, these interactions are usually involved in the sorption process simultaneously at varying degrees because of the heterogeneous chemical structure of natural (suspended) solids. In case of ENMs, their surface properties, such as surface charge and hydrophobicity/hydrophilicity, are likely to affect the adsorption efficiency (Lin et al. 2010). Hence, the coating of the ENM plays an important role in defining its sorption tendency. Song et al. (2011) investigated the effects of organic coatings of different hydrophobicity on Ag-NP adsorption onto chemically heterogeneous collector surfaces. The study indicated that ENM coatings with higher hydrophobicity (PVP and gum arabic (GA)) had greater attachment to hydrophobic surfaces than particles with a less hydrophobic coating (citrate). However, it is crucial to note that the surface properties of ENMs are affected by molecules present in the environmental matrix and hence, to predict the adsorption tendency of the ENMs based on their pristine structure is not possible. As discussed before, in the environment the ENMs are subjected to interactions with ions (e.g. sulfides, phosphates) and macromolecules (e.g. humic acids, proteins) which are likely to result in modifications in the (surface) structure and charge of the ENMs and hence, affect their fate in the environment (Lin et al. 2010; Lowry et al. 2012b).

ENMs are, in general, shown to attach efficiently to soil and sediment, especially to the organic fraction present (Cleveland et al. 2012; Coutris et al. 2012), although Ag-NPs and CeO_2-NPs have been shown to be less prone to adsorption than their ions, indicating that these ENMs have a higher mobility than their ions (Cornelis et al. 2012; Van Koetsem et al. 2015). In wastewater treatment, the organic matter functions as a sink for the ENMs (Table 1). The results in Table 1 showing that ENMs partition to great extent to organic matter, support the hypothesis that organic matter, when suspended, is likely to be a major transport medium for ENMs. Thus, enhancing the removal of TSS during wastewater treatment is likely to result in improved removal of ENMs (Kaegi et al. 2013).

The removal of TSS is mostly very efficient in CWs. The long-term monitoring of 10 Czech HSSF CWs treating domestic wastewater showed that the CWs reliably decreased the TSS concentration to mostly under 20 mg/L independent of the influent concentration (maximum reported concentration 1457 mg/L) (Vymazal 2011). During the life time of the CW, solids are building up within the substrate, especially at the inlet of the CW. Although the fraction of organic matter in these solids can vary greatly (5 – 82 %; Caselles-Osorio et al. 2007) since it is dependent on the type of wastewater and loading rate, these solids can form a sink for ENMs. The sludge layer on top of the French type VSSF CWs can function similarly reducing the amount of ENMs reaching the actual CW substrate. The surface of the substrate is inhabited by micro-organisms which form a biofilm. Extracellular polymeric substance (EPS) present in the biofilm efficiently adsorbs ENMs (Puay et al. 2015). In mature CWs plant roots form a large surface area which likely serves as a binding site for ENMs (Jacob et al. 2013), possibly (at least partly) due to biofilm formation on top of the root surface. In addition, metal retention in the roots can improve if iron plaque is formed on the roots (Jiang et al. 2009). This iron precipitate is caused by oxidation of ferrous iron in anoxic conditions (Jiang et al. 2009), e.g. upon exposure to wastewater from metallurgical industry or landfill leachate.

Table 1 – Retention of ENMs during wastewater treatment

Type of ENM	Process description	Coating	Retention (%)	Observations	Reference
Ag	Pilot-scale WWTP consisting of non-aerated tank, aerated tank and settler; average sludge age of 14 days	Polyoxyethylene fatty acid ester	85	Mass balance suggests that ~5 % of Ag left the WWTP with the effluent	Kaegi et al. 2011
	Batch-tests on activated sludge; contact time 24 h	Citrate, PVP	~99		Kaegi et al. 2013
	Batch-tests on activated sludge; contact time 3 h	Carboxyl	39		Kiser et al. 2010
		None	97		
	Simulated WWTP with primary settler, and SBR	Citrate	100	>90% of Ag-NPs remained in the water stream after primary clarification; complete removal after aeration and secondary clarification	Hou et al. 2012
TiO_2	Batch-tests on activated sludge; contact time 3 h	None	23		Kiser et al. 2010
CeO_2	Model WWTP with aeration tank and settler; sludge age not reported	None/surfactant	>94		Limbach et al. 2008
Cu	Batch-tests on activated sludge (filtrates); contact time 20 h	N/A	~95	Mainly removed by aggregation and settling rather than biosorption.	Ganesh et al. 2010
ZnO	Simulated WWTP with primary settler and SBR.	None	100	Simulated primary settler removed ~70% of ZnO-NPs. Further removal in SBR.	Hou et al. 2013
Au	Batch-tests on activated sludge; contact time 24 h		~99		Kaegi et al. 2013

3.5. Plant uptake

Some plant species are known to be able to take up metals and when the metals are principally accumulated in the aboveground biomass at high concentrations, one speaks of hyperaccumulators (for review see Krämer 2010). There is no one species which can accumulate different metals at equal amounts (Weiss et al. 2006). Generally, only free metal ions, soluble metal complexes and metal ions occupying exchangeable sites or attached on inorganic soil constituents are bioavailable for plants (Salt et al. 1995). Metals existing in the environment bound to organics, as precipitates or in the silicate mineral structure (indigenously present) are, in general, not bioavailable for plants. ENMs have been shown to be bioavailable to plants to some extent (Figure 4). Their bioavailability to plants is restricted by their size as particles up to only about 5 nm in diameter can traverse an undamaged cell wall efficiently (Dietz and Herth 2011). Also, the surface charge and hence, the coating, can affect the uptake efficiency and transport within the plant. Zhu et al. (2012), who studied the fate of Au-NPs with different surface functionalization, observed that the Au-NPs with a negatively charged coating were the least efficiently attached onto the root surface prior to uptake but more efficiently transported to leaves once inside the plant, compared to positively charged Au-NPs.

Van Koetsem et al. (2016) studied the uptake of citrate-coated Ag-NPs in a hydroponic culture of *Elodea canadensis* and observed a dose-dependent reduction of 20 – 57 % in the total Ag concentration in the growth medium within 72 h resulting in the accumulation of 25 – 110 mg Ag/kg in plant biomass. The uptake of Ag^+ was approximately 4 and 8 times higher than the uptake of Ag-NPs at the highest initial Ag doses tested (0.5 – 1 mg/L) (no significant difference at initial doses 0.05 – 0.25 mg/L). Lowry et al. (2012a) investigated the fate of PVP-coated Ag-NPs in a freshwater emergent wetland and found that only a few percent of the spiked Ag ended up in plant tissue of *Juncus effuses, Carex lurida, Panicum virgatum* and *Lobelia cardinalis*. Furthermore, the majority of the accumulated Ag was found in the roots. Low translocation of Ag-NPs to aboveground biomass has also been observed in other rooted plants, such as *Vigna unguiculata* and *Triticum aestivum* in terrestrial environments (Wang et al. 2015). In the same study of Wang et al. (2015), they observed that Ag_2S was taken up by the plant roots to a somewhat larger extent than Ag-NPs. Also CuO- and

ZnO-NPs are both mainly accumulated in the root zone of wetland plants *Phragmites australis* and *Schoenoplectus tabernaemontani* (Zhang et al. 2014, 2015).

Jacob et al. (2013) showed that high phosphorus concentration can limit the uptake of TiO_2-NPs in *Elodea Canadensis*. Van Koetsem et al. (2016) found no link between the uptake of Ag-NPs and phosphorus concentration but reported correlation between the uptake efficiency and conductivity and specific ion content. This likely evidences that the ENM uptake efficiency is dependent of on occurrence of environmental transformations, which are determined by the ambient conditions. In CWs, adsorption to organic matter will restrict plant uptake of ENMs as metal species bound to organics are unavailable to plants. Also, aggregation (occurring e.g. due to high ionic strength (conductivity)) for example can restrict plant uptake efficiency by increasing the particle size of ENMs.

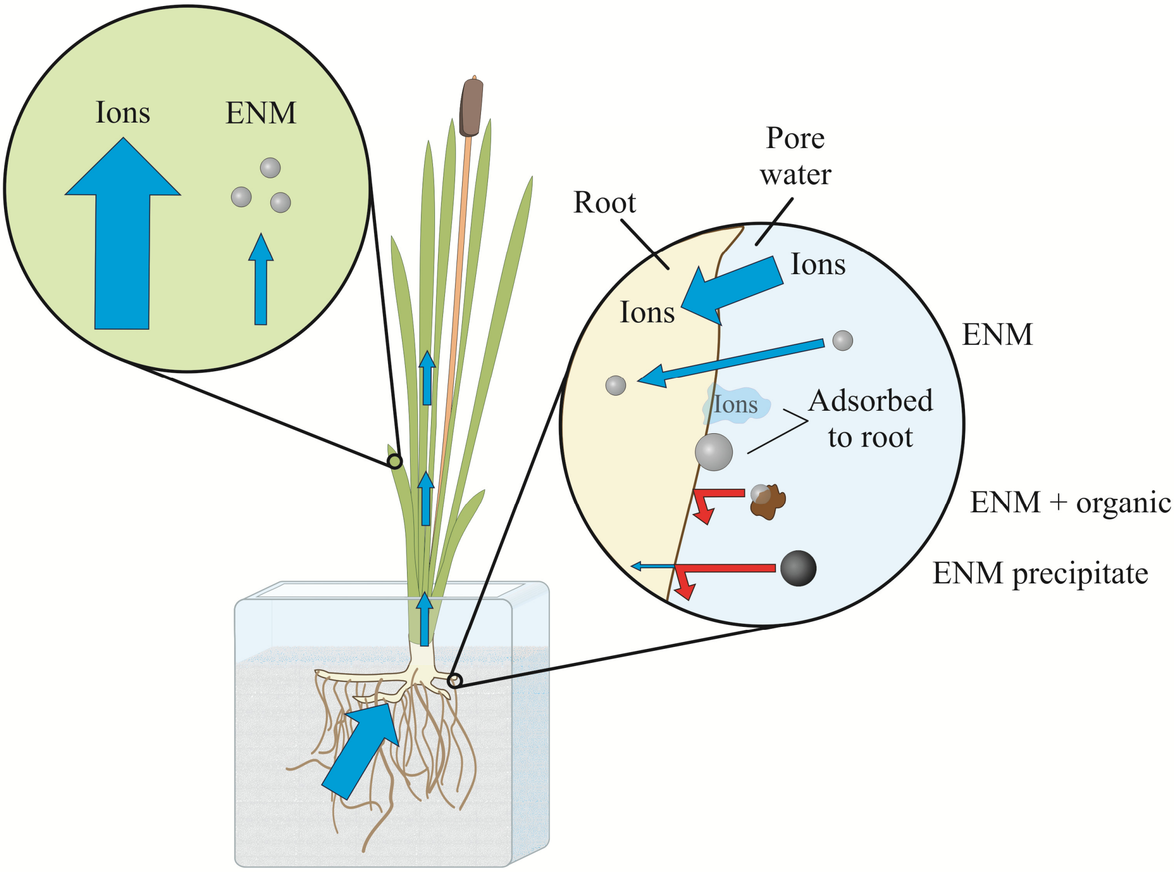

Figure 4 – Schematic illustration of the uptake of ENMs and metallic ions by plants. The translocation efficiency of the different species is indicated with the thickness of the arrows. The uptake and translocation of ENMs is restricted by their size and hence, it is likely that larger particles are less efficiently taken up by the plant and translocated within the plant than smaller ones.

4. Possible toxic effects of ENMs on the wastewater treatment process

The toxicity of ENMs is undoubtedly related to the toxicity of the metal it is composed of. There are thus ENMs which are composed of an element ubiquitous in nature (e.g. Ti), ENMs which contain elements essential for cell structures (e.g. Cu and Zn) and ENMs composed of metals toxic at low concentrations (e.g. Ag). It is thus logical that the toxic concentrations of the different ENMs vary greatly and that low concentrations of certain ENMs could even have positive effects on e.g. bacterial growth due to supplementation of essential elements. Several researchers have compared the toxicities of different types of ENMs. Luo et al. (2014) compared the toxicities of Au- and Ag-NPs on ammonia-oxidizing bacteria at concentrations 0.05 – 2 mg/L, and found no adverse effects of Au-NPs on the bacterial community. Instead, Ag-NPs decreased the bacterial abundance and diversity resulting in 19 and 46 % reduction in the ammoxidation activity at concentrations 0.05 and 2 mg Ag/L, respectively. Shah et al. (2014) observed adverse effects on the richness of a mixed soil microbial community when exposed to Ag- and TiO_2-NPs at a concentration of 0.0625 mg NPs/kg soil but ZnO- and Cu-NPs did not affect the bacterial community structure. Ma et al. (2015), who studied the effect of Ag (ion and NP), zero-valent Fe-NPs, TiO_2-NPs and CeO_2-NPs on the nitrification function and bacterial community structure, observed decreased nitrification efficiency and shifts in community structure only at prolonged exposure to high concentrations (20 mg/L) of Ag-species. In the study of Moore et al. (2016), the impacts on sediment microbial consortia exposed to pulse Ag- or Cu-NPs were rather short term and showed evidence of environmental resilience.

The actual mechanism causing the toxicity is not entirely clear. Although many studies show that the release of free ions from the particles is the main cause of toxicity (see section 3.2), there are also studies showing that the toxic effects cannot be explained only by the amount of ions leaching from the ENMs and hence, there are likely to be particle specific effects as well (Yuan et al. 2013).

Hence, the properties of an ENM can influence its toxicity as well. For example, El Badawy et al. (2011) showed that the toxicity of Ag-NP to *Bacillus sp.* is dependent on the surface charge of the ENM because the chances for cell-particle interactions increase with decreasing magnitude of the negative zeta-potential. As a consequence, the most negatively charged Ag-NPs (uncoated and citrate-coated) exhibited complete growth inhibition at 75 and 600 µg/L, the less negative PVP-coated Ag-NPs at 13 µg/L and the positively charged branched polyethyl-eneimine (BPEI)-coated Ag-NPs at 3 µg/L. Also the size of the ENM could be significant in defining its toxicity. Choi & Hu (2008) found that the fraction of Ag-NPs less than 5 nm in the suspension correlated with the inhibition of nitrifying bacteria. However, Levard et al. (2013a), who studied the transformations of Ag-NPs and their toxicity to higher organisms, found little correlation between the size of the aggregates and the observed toxicity and suggested that the reduction in Ag^+ due to AgCl formation had the dominant toxicity decreasing effect.

When ENMs undergo transformations in the environment, it is likely that their toxicity is altered. Sulfidation has been observed to be an important mechanism at decreasing the toxicity of Ag-NPs to bacteria (Reinsch et al. 2012). Moreover, Choi et al. (2009) showed that sulfide had the most pronounced effect on relieving the toxicity of Ag-NPs among the tested ligands (SO_4^{2-}, Cl^-, PO_4^{3-}, EDTA). In this study, the inhibition of nitrification was reduced by 80 % in the presence of 0.5 mg/L sulfide and 1 mg/L Ag (as Ag-NPs). In addition, other environmental factors such as dissolved oxygen concentration and ionic strength, can affect the dissolution/aggregation behavior of ENMs and thereby, affect their toxicity on organisms (see Section 3).

It should be noted that comparing the toxicities of ENMs and their transformation products is not straightforward since their toxicities can depend on the target organism among other factors. Choi et al. (2008) observed that AgCl colloids and Ag-NPs were less toxic to *Escherichia coli* than Ag^+ but nitrifying bacteria were especially susceptible to Ag-NPs, more than to Ag^+ and AgCl colloids: 1 mg Ag/L as Ag-NPs inhibited the microbial respiration by 86 % while in the case of Ag^+ and AgCl colloids the inhibitory effect was only approximately 45 %. In a similar experiment, Choi & Hu (2008) observed that the presence of 0.14 mg Ag/L of polyvinyl alcohol (PVA) coated Ag-NPs, 0.27 mg Ag/L of Ag^+ and 0.25 mg Ag/L of AgCl colloids inhibited the growth of nitrifiers by 50%.

There are some studies where the effects of ENMs on actual wastewater treatment processes have been investigated. Alito & Gunsch (2014) studied COD and ammonium removal efficiencies in laboratory-scale sequencing batch reactors (SBRs) which were spiked with citrate- and GA-coated Ag-NPs (0.2

mg/L). Although the treatment efficiency was recovered within 24 h, the COD and ammonium removal efficiencies were initially affected by 20 – 30 % and by 1 – 15 %, respectively. When the effect of CeO_2-NPs on the nitrogen removal process in an SBR was studied by Hou et al. (2015), they observed no significant effect on total nitrogen removal at 1 mg/L but at 10 and 50 mg/L the total nitrogen removal efficiency reduced by approximately 30 %. The resilience of the bacterial communities in sludge is probably related to the protection by the EPS matrix which accounts for the floc formation in activated sludge (Henriques and Love 2007). The adsorption of ENMs by EPS can decrease the diffusive transportation through the EPS (Choi et al. 2010) and thus, protect the bacteria from the ENMs.

In CWs, the cells habiting the biofilm are better protected against toxins than planktonic cells due to EPS (Choi et al. 2010), similarly to sludge flocs. A study on wastewater dwelling bacteria showing that the community profile of a biofilm culture did not change after exposure to 200 mg/L of Ag-NPs, while nearly all planktonic bacteria died at 1 mg/L illustrates the protective effects of EPS (Sheng and Liu 2011). However, the biofilm bacteria can still experience stress due to the presence of ENMs (Hou et al. 2015). Under stress, bacteria are unable to protect themselves from intracellular reactive oxygen species which can damage cellular structures when accumulating in the cell (Choi & Hu 2008). The concentrations of ENMs in the biofilm increase during the lifetime of a CW. Lesage et al. (2007) studied the accumulation of Zn and other bulk metals in a HSSF CW and measured concentrations reaching 1400 mg Zn/kg dry matter in the wetland substrate after 6 years of operation. During the sampling they measured an average Zn concentration of 120 µg/L in in the influent. As this is more than 100 times higher than the concentration predicted for ZnO-NPs in domestic wastewater (1 µg/L; calculated with a conservative 70 % removal efficiency (Markus et al. 2013) from a modeled value for wastewater effluent 0.29 µg/L (Sun et al. 2014)), the likelihood of NP induced toxicity seems irrelevant at current production volumes.

5. Possible release of ENMs from CW

Effluent, harvested plant material and removed sludge (French type CW) can be regarded as potential routes for ENMs to be released from CWs. Also, in cases where the CW is restored due to e.g.

clogging, by removing the gravel, washing it and returning it back to the CW, ENM containing wastewater can be produced.

Due to the affinity of ENMs for organic matter as discussed earlier (see section 3.4) and the efficient removal of suspended solids in CWs (e.g. Vymazal 2009) the concentrations of ENMs released from CWs are during normal operation likely to be low and comparable to activated sludge treatment systems. Over the years the CW bed becomes more filled with SS and plant roots and this can result in topographic and vegetative heterogeneities creating faster and slower flow paths through the wetland substrate (Dierberg et al. 2005). Fast flow of influent through the CW (i.e. short-circuiting) may decrease the removal efficiency of ENMs similarly to other contaminants. Also, active aeration may cause mixing of a fraction of influent with effluent especially in systems with a small length-to-width ratio. In addition, sudden changes in the influent flow rate or aeration may cause detachment of biofilm and subsequent increased discharge of ENMs embedded in the biofilm.

Conventional CWs sludge is not needed to be removed during operation. In French type VF CWs, where unsettled wastewater is treated, the sludge layer is occasionally removed, and in this case, if the sludge is applied to (agricultural) land, contamination of the soil by ENMs may occur (Deng et al. 2014). The mass of the sludge deposit corresponds to approximately 40 % of the SS introduced with the wastewater (Molle et al. 2005) and due to the mineralization of organic matter over the years, the metal concentration in the sludge deposit layer is bound to increase. Molle (2003) measured concentrations of e.g. Cu and Zn in a sludge deposit collected after 11 years of operation and stored since collection for 5 years. The concentrations found (196 mg Cu/kg dry matter and 221 mg Zn/kg dry matter) would not have restricted the application of the sludge on agricultural land (European Commission 1986). The European sewage sludge directive does not directly concern ENMs in sludge. Measuring the concentration of ENMs in sludge cannot be required because of the lack of reliable techniques to detect ENMs in environmental samples (Ganzleben et al. 2011).

Plants which are harvested at regular intervals from the CW may also contain ENMs which can be released upon composting. Vymazal et al. (2010) studied the accumulation of metals in the aboveground tissue of *Phalaris arundinacea* growing in a HSSF CW treating domestic wastewater.

The values for annual accumulated mass in the aboveground tissue per given area (so called standing stock) were for e.g. Zn and Cu 28.5 g Zn/m^2/a and 8.8 g Cu/m^2/a. If we assume similar uptake efficiency for ZnO-NPs as for Zn^{2+} and estimate the ZnO-NP:Zn ratio in domestic wastewater to be 0.008 (120 µg Zn/L (Lesage et al. 2007) and 1 µg ZnO-NPs/L; the latter calculated with a conservative 70 % removal efficiency (Markus et al. 2013) from a modeled value for wastewater effluent 0.29 µg/L (Sun et al. 2014)), the standing stock for ZnO-NP in *P. arundinacea* would be 0.2 g Zn/m^2/a. In comparison to the bulk metal counterpart, the mass of ENMs is thus predicted to be low and the risk posed by the ENM containing plant material from CWs seems insignificant.

6. Conclusions and perspectives

ENMs are present in our everyday household products. The increasing production volumes make it important to study the fate of ENMs in wastewater treatment systems to reliably estimate the environmental risk they pose.

During the usage of an ENM containing product, ENMs can be released to the washing water and end up in wastewater. In the sewer system, ENMs are likely to bind with organic matter and some types may sulfidized but little losses to the sewer biofilm are expected. The current estimates on the ENM concentration in wastewater reaching the treatment plant vary from some µg/L to hundreds of µg/L depending on the type of ENM. Although these values are low and unlikely to cause toxicity to microbial communities in CWs, caution is warranted due to the expected increase in the production of ENMs in the future.

The ENMs are expected to further transform in CWs. The type and extent of these transformations depend on the type of the ENM and the prevailing environmental conditions in the CW. Some differences in the fate of ENMs in HSSF CWs versus VSSF CWs can thus be expected due to e.g. different dissolved oxygen conditions. In general, important transformations that are likely to occur in CWs are sulfidation, sorption onto organic matter and other solids and heteroaggregation with suspended particles. Plant uptake is likely to have a minor role in the removal of ENM from the water phase, although adsorption of ENMs onto the root surfaces can be substantial. Hence, wetland plants can play an important role in ENM immobilization in the rhizosphere. The concentrations of ENMs released from a CW with effluent, harvested plant material and (in some special cases) sludge are expected to be low due to the efficient retention of ENMs within the substrate. CWs are thus not predicted to be potential point sources of ENMs in surface waters during normal operation.

Future research should focus on exploring (temporary) circumstances which possibly can cause the discharge of ENMs from CWs. Clogging and subsequent short-circuiting can decrease the overall treatment efficiency and hence, it can also affect the retention of ENMs. Biofilm which immobilizes ENMs can be detached from the CW substrate due to e.g. sudden changes in flow rate and aeration, and ENMs can also be discharged by being washed out from the CW. Although sulfidation in CWs seems unavoidable, it is impossible to say to what extent it occurs. This may be important for the bioavailability of the metal species as ions are better taken up by plants than ENMs or it may impact the toxicity caused on the biofilm. The biofilm is not predicted to be adversely affected by the ENMs in the water phase but in the long term the accumulating ENMs may become toxic to the micro-organisms. However, the fraction of ENMs in the total load of metals in CWs being very low, the primary toxic effects are likely to be caused by the bulk metals instead of ENMs at current production volumes.

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