Volume 248

Pim de Voogt *Editor*

Reviews of Environmental Contamination and Toxicology

Springer

Reviews of Environmental Contamination and Toxicology

VOLUME 248

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Reviews of Environmental Contamination and Toxicology

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Volume 248

Springer

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ISSN 0179-5953 ISSN 2197-6554 (electronic)
Reviews of Environmental Contamination and Toxicology
ISBN 978-3-030-14705-1 ISBN 978-3-030-14706-8 (eBook)
https://doi.org/10.1007/978-3-030-14706-8

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Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife, and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the Earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated (eco)toxicological consequences. Such articles facilitate the often complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the 50+ years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews)* was first published, the number, scope, and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing people worldwide. This fact, and the routine discovery and reporting of emerging contaminants and new environmental contamination cases, creates an increasingly important function for *Reviews.* The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. *Reviews* addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities, nongovernmental organizations, or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans, and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific environmental research is to enhance understanding of the environment in ways that allow the public to be better informed or, in other words, to enable the public to have access to sufficient information. Because the public gets most of its information on science and technology from internet, TV news, and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is an important global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the twenty-first century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, because the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists. New legislation that will deal in an appropriate manner with this challenge is currently in the making or has been implemented recently, such as the REACH legislation in Europe. These regulations demand scientifically sound and documented dossiers on new chemicals.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can be either general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, (eco)toxicology, and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the editor and the editorial board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of anthropogenic chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their scope.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the Editor-in-Chief is recommended before volunteered review manuscripts are submitted. *Reviews* is registered in WebofScience™.

Inclusion in the Science Citation Index serves to encourage scientists in academia to contribute to the series. The impact factor in recent years has increased from 2.5 in 2009 to 7.0 in 2017. The Editor-in-Chief and the Editorial Board strive for a further increase of the journal impact factor by actively inviting authors to submit manuscripts.

Amsterdam, The Netherlands Pim de Voogt
August 2018

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The Toxicity of Nanoparticles to Organisms in Freshwater

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P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 248*, Reviews of Environmental Contamination and Toxicology Volume 248,
https://doi.org/10.1007/398_2018_18

1 Introduction

Nanoparticles (NPs) have existed for millions of years, formed from natural phenomena such as weathering, volcanic activities and formation of colloids in rivers (Sharma et al. 2015). The use of manufactured NPs started recently (Warheit 2018) and represents a human-made material which is being used increasingly. Currently, nanotechnology is a trillion dollar industry which is increasing exponentially. It can be assumed that naturally produced NPs have been in a form of equilibrium in nature, but engineered NPs are a growing concern among institutions and the public due to their possible negative consequences on living organisms (Moore 2006; Tiede et al. 2009). The knowledge that the scientific world has acquired to date is inadequate to draw conclusions on the actual release and fate of NPs in the natural environment, actual environmental exposure to NPs and the magnitude of harm they incur to living beings (Gottschalk and Nowack 2011; Bäuerlein et al. 2017). Establishing the safety of nanomaterials is important to protect the environment and health of organisms. The effects of NPs depend on many factors including their intrinsic properties, fate and bioavailability in the respective environment and response of the receptor organisms (Lapresta-Fernández et al. 2012). The toxicity of NPs to organisms has been the subject of study for the past decade (Klaine et al. 2008; Moore 2006; Levard et al. 2012); however, coherent, consistent and well-founded data are still lacking (Selck et al. 2016; Giese et al. 2018). Currently available data on exposure to NPs and effects on organisms are currently insufficient to conclude on the risks involved (Ma et al. 2013; Holden et al. 2014; Skjolding et al. 2016; Hjorth et al. 2017a, c), and the detection and quantification of NPs in the environment are challenging (Bundschuh et al. 2016).

The authors appreciate attempts made by several authors in reviewing published literature on freshwater nanoecotoxicology (Handy et al. 2008a, b; Baun et al. 2008; Fabrega et al. 2011; Ma et al. 2013; Vale et al. 2016; Rocha et al. 2017; Lei et al. 2018; Goswami et al. 2017). This review was written in view of giving the reader an exhaustive, holistic and comparable understanding on the effects of NPs on three major groups of freshwater organisms of various classes, in contrast to the majority of reviews which are devoted to specific classes of NPs. By doing this, authors address the issue of information and data heterogeneity. Also, care has been taken in the review to appreciate and stress the importance of the physicochemical characteristics and transformation of NPs in the environment with respect to their toxicity. Furthermore, the number of publications on nanoecotoxicity is increasing every year, and therefore, a concerted effort is needed to cohesively analyse them to better understand potential risks of NPs in a background of ongoing, increasing research and development of nanotechnology applications. This review discusses our current understanding of environmental exposure to NPs, physicochemical characteristics of NPs and the aquatic environment that influence toxicity, their bioavailability, trophic transfer, toxicity, mechanisms of toxicity and behavioural toxicity in view of some key environmentally relevant freshwater organisms. The paper also identifies gaps in research and provides recommendations for future research needs to effectively develop our understanding on the risks of NPs to freshwater aquatic organisms and develop effective strategies to mitigate those risks. This review examined more than 350 articles including review papers with majority of those published in the last decade.

NPs are released into all ecosystems including freshwater, marine water, soil and air. However, the behaviour of NPs in the freshwater environment is likely to differ in each due to their unique environmental characteristics. For example, high salinity in the marine environment causes increased agglomeration, aggregation and precipitation of NPs which affects the bioavailability of NPs (Keller et al. 2010; Gambardella et al. 2015; Buffet et al. 2013). High surface area increases the potentiality of ion release from NPs (Mudunkotuwa and Grassian 2011), while higher aggregation reduces surface area for dissolution and any metal cations released from NPs are likely to be complexed by free chloride (Cl^-) ions present in saltwaters (Baker et al. 2014). Moreno-Garrido et al. (2015) report that the EC_{50} values of NPs for marine algae species are twofold higher than for freshwater species as per the published literature. Also, they claim that OECD documents on safety and toxicity tests for NPs do not have any specific references to marine water. The major source of NPs to soil is through the disposal of wastewater treatment plant (WWTP) sewage sludge, and NPs are unlikely to enter the soil in their original form due to organically rich reactive environments in the WWTP. The attachment of NPs to soil colloids is rapid, and therefore, the mobility of NPs away from the point of source could be limited in soils (El Hadri et al. 2018). Also, the assessment of the form of NPs in soil matrices is hampered by the relative lack of procedures for their characterization compared to aqueous media (Tourinho et al. 2012; Kraas et al. 2017). Due to the variations in the fate and behaviour of NPs and mode of organism exposure in different spheres, the authors have restricted this review to the freshwater environment only. However, this does not undermine the importance for more research related to the nanotoxicity to organisms in other environments which attract comparatively less nanoecotoxicology studies (Minetto et al. 2016).

Metal oxide NPs, metal NPs and carbon nanotubes (CNT) are the most relevant materials in terms of worldwide production volumes and exposure (Bundschuh et al. 2018; Tiede et al. 2016), while the OECD has highlighted silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO2) and cerium dioxide (CeO_2) NPs as high interest due to their widespread use, commercial importance and inherent properties (Baker et al. 2014). Rocha et al. (2015) reported that 85% of toxicological studies on marine bivalves are based on inorganic NPs and only 15% are on organic NPs. Also, more than 70% of inorganic NPs examined in saltwater are metal oxides and metals that mainly consist of TiO_2, Ag, Au, ZnO and CuO NPs (Minetto et al. 2016; Rocha et al. 2015). A large number of studies (~80%) on the effects of inorganic NPs on the organisms considered in this review also reflect the production, release and exposure risk concerns of inorganic NPs in the freshwater environment (Fig. 2 and Table 2). However, a large proportion of those inorganic NPs are coated with organic capping agents. As an example, citrate and carboxylic acids are the most used reductant and capping agents in the synthesis of AgNPs (Sharma et al. 2014).

Low-throughput tests such as microcosms, mesocosms or field-scale studies are more representative of actual environmental conditions in comparison with high-throughput tests such as in vitro tests which lack environmental complexity. Therefore, using widely accepted key environmental organisms in ecotoxicology with corresponding in vivo tests is still highly regarded in environmental risk assessment of NPs. The European Chemical Agency mentions that in vitro data are relevant information for aquatic toxicity assessments, but also note that there are no EU/OECD guidelines for in vitro tests at the moment (Hjorth et al. 2017b). Therefore the authors have restricted this review to in vivo studies. In vitro assays have a role in hazard identification, but their usefulness in environmental risk assessment is limited (SCENIHR 2009; Mattsson and Simkó 2017). There is a limited correlation between in vitro and in vivo toxicity results (Sharifi et al. 2012). These reasons may have influenced the large number of in vivo studies published compared to in vitro studies as reflected in this review. However, this does not undermine the importance of alternative testing strategies in nanoecotoxicology risk assessment since there is an ongoing discussion and proposals to use those effectively (Hjorth et al. 2017b). Also, certain aspects of NP toxicity studies such as shape-dependent toxicity are based on in vitro experiments (Sharifi et al. 2012; Forest et al. 2017).

2 Nanoparticles (NPs) in the Environment

Several types of NPs are present in the environment and exposure to those particles is a reality. Therefore, it is important to understand the flow of NPs to the environment and exposure to assess the risks (Scown et al. 2010). Release of NPs to the environment may occur from the manufacturer through to the end user who consumes NP-enabled products (Sun et al. 2014). The majority of the products containing NPs belong to cosmetics and personal care products with sunscreen representing the dominant application (Boxall et al. 2007). A significant fraction of the NPs released to soil and air would end up in waterbodies as well while cosmetics, coatings, paints and pigments alone contribute 89–97% of total NP emissions to water (Keller et al. 2013). The data related to NP production and released volumes in the literature have large variations, and Giese et al. (2018) provide a comprehensive summary based on data from literature, their own surveys and modelling. About 250,000 metric tonnes are released to landfills, soil and air every year (Keller and Lazareva 2013), and it is predicted that about 69,000 metric tonnes of NPs are released globally to surface waters directly. This amount is increasing since the predicted NP production in 2019 is close to 600,000 metric tonnes with an annual growth rate of 21.1% of NP production (Vale et al. 2016). It is estimated that around 10–30% of NPs released into the environment would end up in waterbodies in Asia, while it is 3–17% in Europe and 4–19% in North America (Keller and Lazareva 2013).

Usage of NPs are increasingly popular in several consumer and industrial sectors including health and fitness, home and garden, automotive, electronics, contaminant remediation and food and beverage (Vance et al. 2015; Cecchin et al. 2016). The number of inventories with catalogue products which contain NPs is rising globally (Hansen et al. 2016; Vance et al. 2015; Mcgillicuddy et al. 2017). Hansen et al. (2016) summarized the number of products listed yearly until 2015 in the Consumer Product Inventory (CPI 2018) and Nanodatabase (2018) which list products containing NPs or are based on nanotechnology available to the European market. The number of products listed in the Nanodatabase has increased from 2,231 to 3,038 from 2015 to date. There are several classes of engineered nanoparticles (NPs) based on chemical composition and morphology (Kümmerer et al. 2011). Though metal oxide NPs, SiO_2 NPs and CNTs are the most produced worldwide (Fig. 1), Silver (Ag) NPs are the most used in consumer products representing 25% of the products containing NPs (Bondarenko et al. 2013; Vance et al. 2015; Bundschuh et al. 2018). Due to their excellent antimicrobial action, Ag NPs are increasingly popular in medicines, cosmetics, personal care and certain clothing products (Boxall et al. 2007; Yameen et al. 2014). Manufacturers are not required to report the use of NPs in products except for a few NPs in some countries (e.g. carbon nanotubes in the USA). Also, manufacturers are not legally bound to label products that contain NPs (Kessler 2011) or may be ignorant with respect to specific information (Giese et al. 2018). A survey conducted by Piccinno et al. (2012) found that the manufacturers are reluctant to provide production amounts with respect to NPs. As per the listed NP-containing products in the Nanodatabase for 2018, the constituent NPs in 64% of the consumer products have not been disclosed (The

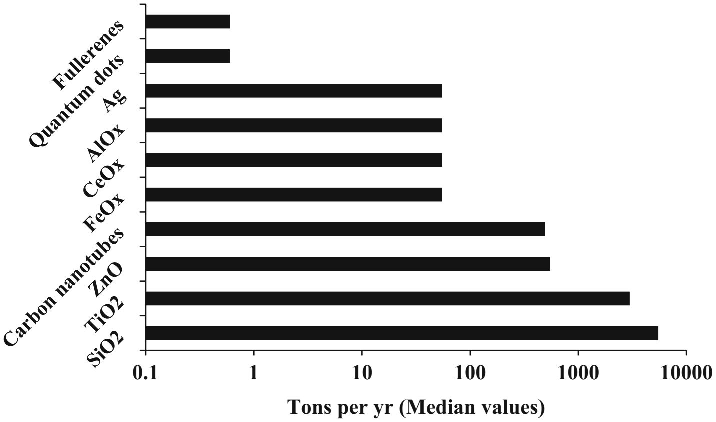

Fig. 1 Annual production volumes of nanoparticles (adapted from Piccinno et al. 2012)

Nanodatabase 2018), while metallic NPs (19.5%), metal oxide NPs (6.5%) and other types including organic NPs (9.5%) constitute the rest of the products. The majority of organic NPs are constituted of carbon (present in 2% of products), carbon nanotubes (2.1%), bamboo charcoal (1.4%), graphite (0.6%), carbon black (0.5%) and fullerene (0.3%).

Emission and environmental concentration levels are mainly estimated by using material flow models following the NP life cycle (Mueller and Nowack 2008; Boxall et al. 2007; Gottschalk et al. 2013; Sun et al. 2014, 2017; Piccinno et al. 2012; Markus et al. 2016, 2017; Jiménez et al. 2016) and analytical methods (Gottschalk et al. 2013; Chang et al. 2017; Aznar et al. 2017; Laborda et al. 2016a, b; Majedi and Lee 2016; Venkatesan et al. 2018; Vidmar et al. 2017; Yang et al. 2016; Gondikas et al. 2014, 2018; Folens et al. 2018; Markus et al. 2018; Hartmann et al. 2013; Chen and Ding 2012; Astefanei et al. 2014). However, there are number of known incorrect assumptions in all the models (Giese et al. 2018). Measured field data are essential to validate predicted environmental concentrations of NPs (Bäuerlein et al. 2017). Most NP analytical studies have so far concentrated on method development, but a rise of efforts to apply these methods to measure actual concentrations in the environment is observed (Aznar et al. 2017; Bäuerlein et al. 2017; Venkatesan et al. 2018; Peters et al. 2018). However, limitations in analytical methods in discriminating engineered NPs from naturally occurring NPs have caused results of models difficult to validate (Giese et al. 2018; Gondikas et al. 2014; Wagner et al. 2014). Also, factors such as transformation of NPs in the environment, aggregation and the copresence of dissolved ions may cause measurement of NP properties and concentrations less accurate (Majedi and Lee 2016). The physicochemical characteristics of the surrounding environment and NP properties such as coating agent and size have a huge impact on their fate and behaviour in the environment which demands careful attention in both modelling and analytical efforts (Ellis et al. 2016; Pu et al. 2016; Luo et al. 2018).Once released in to the environment, NPs undergo transformation and change their characteristics such as size compared to their pristine form (Nowack et al. 2012). For example, NPs may agglomerate in the environment, and the modelling considers agglomerates larger than 100 nm as well and thus targets the complete NP pool. In contrast, analytical methods may consider only the nanofraction, and therefore, the measured concentrations may indeed be smaller than the actual concentrations. However, this was true for certain types of NPs that were measured in a recent study conducted by Bäuerlein et al. (2017) in the Dutch environment, but the measured concentrations of AgNPs were higher than the predicted concentrations in sewage treatment plant effluent.

From various sources, Gottschalk et al. (2013) summarized predicted environmental concentrations of TiO_2 (10^{-3}–10^{1} μg L^{-1}), Ag (10^{-5}–10^{0} μg L^{-1}), ZnO (10^{-4}–10^{-3} μg L^{-1}), CNT (10^{-6}–10^{-3} μg L^{-1}), fullerenes (10^{-5}–10^{-4} μg L^{-1}) and CeO_2 NPs (10^{-3}–10^{-1} μg L^{-1}) in freshwater. In the year 2017, the global predicted environmental concentrations of SiO_2, CeO_2 and Ag NPs in the freshwater were 5,300, 7.0 and 0.3 ng L^{-1}, respectively, and predicted to increase up to 25,300, 46.7 and 2.1 ng L^{-1} in 2050, respectively. These increased concentrations correlate with the predicted increased release of NPs into the environment (Giese et al. 2018).

Based on the per capita contributions from the households, Markus et al. (2018) estimated the concentrations of ZnO, TiO_2 and AgNPs in the river Dommel in Netherlands to be 1.4 μg L^{-1}, 1.0 μg L^{-1} and 13.0 ng L^{-1}, respectively. Relatively few reports are available on actual application of analytical methods to determine the presence, properties and concentrations of NPs in freshwater samples collected from the environment. The concentration of nC_{60} was found up to 98 ng L^{-1} by using LC-MS in surface water samples collected from a creek in Hsinchu Science Park, Taiwan (Chen and Ding 2012). Comparatively, C_{60} and C_{70} concentrations were reported in using an UPLC-MS method with concentrations between 25 and 330 pg L^{-1} in freshwater samples collected from several ponds located around Barcelona's airport (Astefanei et al. 2014). Folens et al. (2018) reported Pt NP concentrations in the range of 0.05–0.9 ng L^{-1} by measuring with spICP-MS in the road dust leachate of Ghent, Belgium and Gothenburg, Sweden, which might be released into aquatic environment. Peters et al. (2018) reported actual environmental concentrations in the range of 0.3–2.5 ng L^{-1} for Ag NPs, 1.3–5.2 ng L^{-1} for CeO_2 NPs and 0.2–8.1 μg L^{-1} for TiO_2 NPs measured using spICP-MS in the samples collected from the rivers IJssel and Meuse in the Netherlands. Venkatesan et al. (2018) found TiO_2 particle concentration in the range of 260–659 ng L^{-1} in the Salt River, Arizona, USA, by measuring with spICP-MS. The morphological features of those particles were similar to the NPs present in sunscreens. In the last decade, predicting environmental concentrations of NPs by modelling has received considerable attention, but determining actual concentrations is critical for validating those estimates and reliable risk assessment and for regulating NP industry. However, the development of real-world parameters of NPs and concentrations remains scarce due to several limitations such as lack of appropriate analytical methods and complexity of the real sample matrices (Gondikas et al. 2018).

3 Why Be Concerned About NPs?

Engineered nanoparticles (ENPs) have been around for quite a while, but concerns about the risks associated with them rose a few years ago. Despite huge concerns, due to a lack of sample-related certified standards, analytical procedures and reliable units of measure (Mottier et al. 2017), the presence of NPs in the sources and receiving bodies like waste effluents, surface or groundwaters and sediments was not well documented (Mirzajani et al. 2013; Mitrano et al. 2012). However, the presence of NPs in the environment is a proven phenomenon as per recent studies, as discussed earlier. Bulk materials are usually defined in terms of properties like density, resistivity, magnetism and dielectric constant which are averaged for the whole unit. Compared to their bulk form, NPs possess unusual and different properties which cannot be explained with Newtonian mechanics, but only with quantum mechanics (Throbäck et al. 2007; Bhushan 2010; Poole and Owens 2003). Compared to naturally available NPs, engineered NPs may have different physical and chemical characteristics (Handy et al. 2008b). Unpredictable consequences due to their colloidal nature and the dynamics of NPs in receiving environments represent a huge challenge in assessing their toxicity (Service 2004; Nowack and Bucheli 2007; Blaser et al. 2008; Diegoli et al. 2008; Hassellöv et al. 2008; Tiede et al. 2008). Chemical and physical properties like zeta potential and metal binding capacity are determined by the size of the particles (Madden et al. 2006) which varies significantly in NPs. Due to their small size, the behaviour of NPs in the environment and effects on organisms are different to those of conventional xenobiotics (Scown et al. 2010; Klaine et al. 2012). High surface to volume ratio and abundant reactive sites on the surface are some unique characteristics of NPs, and these along with their mobility could result in unexpected health hazards (Maynard et al. 2006; Wiesner et al. 2006). Also, physicochemical characteristics of both NPs and their surrounding environment and modalities of the suspension decide the attributes of the dispersed nanophase.

It was reported that creation of free radicals and oxidative damage is the main cause of adverse effects in cells (Auffan et al. 2009). Since NPs have a very large surface area in relation to volume, they may cause direct generation of oxyradicals which can attack DNA, proteins and membranes (Brown et al. 2001). Once in the cell, NPs may embed within the cell functional machinery resulting in different toxicological responses compared to conventional toxicants (Moore 2006). In addition to their own toxicity effects, NPs also influence the toxicity of other contaminants which are harmful to aquatic organisms (Tan and Wang 2014; Fan et al. 2016). NPs have the potential to bind toxicants and may carry them to sites in cells where these chemicals would not normally travel (Cheng et al. 2004; Pelkmans and Helenius 2002).

The risk assessment methodologies of NP exposure are still at the research and development level. A number of authors have proposed approaches to NP risk assessment (Dekkers et al. 2016; Domercq et al. 2018; Garner et al. 2017; Hristozov et al. 2016), although a comprehensive risk assessment of NPs, data requirements, models and advancement related to NP production, release, exposure, fate and behaviour, risk characterization, etc. (Scott-Fordsmand et al. 2017) has not yet been developed. The models that are proposed each have their own limitations, and the tools used in characterization of NPs in exposure assessment and toxicology tests are not sufficient for risk assessment (Garner et al. 2017; Mattsson and Simkó 2017). Several parameters influence NP toxicity tests, but there exists a lack of scientific understanding of the importance of each parameter or the interactions between them for the toxicity endpoints in current test guideline (Hjorth et al. 2017c). The available test guidelines are not sufficient to analyse the behaviour of NPs in test media. For example, Wasmuth et al. (2016) concluded that the available OECD guidance document No. 29 which was designed to determine the rate and extent of ion release from metals does not cover analytical methods for NPs. Also, the development of toxicity test guidelines is still at early stages with only a few guidelines available, published recently (OECD 2017a, b, c). Accordingly, the lack of NP toxicity test data which are suited for regulatory decision-making is still a pressing issue (Hjorth et al. 2017c). Also, the currently available remediation or purification technologies may not reduce NPs to environmental permissible levels.

Furthermore, increasing NP release may cause further issues in wastewater and sewage sludge treatment plants which may pose a risk to microbes in the digestion systems (Wang and Chen 2016).

4 Physicochemical Characteristics of NPs on Ecotoxicity

NPs exhibit unique physicochemical properties compared to their bulk counterparts. Several researchers have tried to investigate the influence of physicochemical characteristics of NPs such as size, shape, surface properties and charge that could change their toxicity to organisms. Though the results seem to be inconsistent and conflicting, they suggest that the physicochemical properties of NPs could affect the toxicity to organisms. Therefore, these parameters need to be considered in environmental risk assessments and demand further research.

4.1 Size

The uptake and toxicity of NPs depend on the inherent properties of NPs and also the chemistry of the surrounding environment (Park et al. 2015). The behaviour of NPs depends on factors like size, shape, surface chemistry, surface area, functional groups, coatings, charge, aggregation, solubility, photochemistry, crystallinity and the presence of other compounds (Scown et al. 2010; Albanese et al. 2012; Shang et al. 2014; Fröhlich 2012; Clément et al. 2013; Hund-Rinke and Simon 2006; Barbero and Yslas 2016; Garner and Keller 2014). The size of NPs determines the physicochemical properties, adsorption, distribution, metabolism and excretion in the biological systems (Qu et al. 2017). The toxicity of AgNPs is size dependent with smaller particles which are more active (Lok et al. 2007). Choi and Hu (2008) observed that the inhibition of nitrifying organisms correlated with a fraction of AgNPs less than 5 nm in size. This was achieved through examining the correlation between nanoparticle size distribution, photocatalytic reactive oxygen species (ROS) generation, intracellular ROS accumulation and nitrification inhibition. They concluded that NPs of this size could be more toxic to bacteria than any other fractions of NPs.

Choi et al. (2008) saw no indication of internalization of AgNPs (14 nm in size) into the bacteria *Escherichia coli* since internalization of NPs into bacteria cells depends on the size of the NPs and only smaller NPs (<10 nm in size) could enter (Morones et al. 2005). Jiang et al. (2008) found that binding and activation of membrane receptors and subsequent protein expression in mammalian cells depend on nanoparticle size. Hoecke et al. (2009) reported that the toxicity of CeO_2 NPs to *Raphidocelis subcapitata* increased with decreasing particle size. Lei et al. (2016) observed increased toxicity of zero-valent iron (nZVI) NPs to *Chlorella pyrenoidosa* with decreasing particle size. Hartmann et al. (2010) studied the ecotoxicity of three

TiO_2 NPs with different sizes, to the alga *Pseudokirchneriella subcapitata*. They found that the smallest particle type (<10 nm) resulted in higher inhibition than the other two types (3–300 nm). Kim et al. (2010) investigated the effects of particle size of TiO_2 NPs on *Daphnia magna*. They showed that the particle fraction in between 400 and 800 nm increased antioxidant enzyme activities in comparison with the NPs which were less than 400 nm in size. Cui et al. (2017) reported that the longer Ag nanowires (NWs) (20 μm) were more toxic to *Daphnia magna* and *Daphnia galeata* than those that were shorter (10 μm). Similarly, Chae and An (2016) showed that larger Ag nanowires (AgNW) (20 μm) were more toxic to aquatic organisms than smaller ones (10 μm) by exposing *Chlamydomonas reinhardtii* and *Daphnia magna* and to Ag NWs.

However, Matzke et al. (2014) observed no clear-cut relationship between NP toxicity and size of NPs when the bacterium *Pseudomonas putida* was exposed to AgNPs. Li et al. (2010a) studied the effects of three different sized (36, 52 and 66 nm) Ag NPs but concluded that the toxicity was not a function of size possibly due to the large degree of aggregation of NPs in synthetic freshwater. Also, Lopes et al. (2014) studied the effects of ZnO NPs with two different particle sizes (30 and 80–100 nm) and ionic Zn. They found that the acute toxicity of ZnO NPs did not depend on particle size. Iswarya et al. (2017) exposed the alga to PVP-coated Au NPs in different sizes but observed no size-dependent toxicity. However, the toxicity of citrate-coated Au NPs depended on the size with the smaller particles being less toxic. The smaller-sized NPs reacted rapidly with the substances in the solution causing aggregation which may have caused less toxicity. Wiench et al. (2009) reported that the acute toxicity to *Daphnia magna* was independent of particle size, type of coating, aggregation of particles or the type of medium for TiO_2 and ZnO NPs.

4.2 Shape

Peng et al. (2011) observed that rod-shaped zinc oxide NPs (ZnO NPs) were more toxic to the alga *Phaeodactylum tricornutum* than sphere-shaped NPs. Bacchetta et al. (2018) observed higher internalization of spherical- and tube-shaped CNTs into *Daphnia magna* compared to the cubic NPs. They also reported that NP shape influenced the severity of pathogenesis with cubic NPs being more effective in terms of physical damage and cellular degeneration. Liu et al. (2018) observed that star-shaped Au NPs were more toxic to the fungus *Aspergillus niger*, *Mucor hiemalis* and *Penicillium chrysogenum* compared to the toxicity of spherical-shaped ones. Also, the toxicity of star-shaped NPs increased with smaller sizes. Nasser et al. (2016) suggested that shape and charge played an important role in the toxicity and uptake of Au NPs to *Daphnia magna*. Abramenko et al. (2018) observed higher toxicity of spherical-shaped Ag NPs to *Danio rerio* embryos compared with Ag nano-plates. In contrast Dai et al. (2015) saw no effect of NP form or shape on the toxicity of CuO NPs to *Capitella teleta*. Also, Silva et al. (2014) claimed that particle shape did not contribute to the toxicity of organo-coated Ag NPs to *Escherichia coli* and *Daphnia magna*. Chauhan et al. (2011) claimed that the rod-shaped CdSe/CdS NPs penetrated tumour cells more rapidly than spherical NPs. Truong et al. (2015) suggested that nonspherically shaped, such as filamentous, discs or wormlike NPs were better as drug delivery carriers. However, Chithrani et al. (2006) observed higher uptake of spherical-shaped Au NPs into mammalian cells than the rod-shaped Au NPs.

4.3 Surface Properties

Though NP size still remains central in determining toxicity, studies suggest that other inherent factors like coating agents should be considered in toxicity studies (Silva et al. 2014). The role of the surface properties of NPs is poorly understood and cannot be generalized to determine the risks (Baumann et al. 2014; Saei et al. 2017). Surface properties of NPs are key factors in determining behaviour of NPs; multiple types of surface ligands pose new challenges in understanding the toxicity of NPs (Yu et al. 2013). NPs are highly reactive because of their large surface area. The surface chemistry and reactivity of NPs determine their interactions with the surface lining layers of biological tissues (Hoet et al. 2004) and transfer of NPs to higher levels through the food web (Geitner et al. 2016). Many NPs which are in development are complex and carry different coatings which can alter their surface properties (Nune et al. 2009; Daima et al. 2014). Currently many different types of compounds are being used as capping agents in commercial NP production (Table 1). The physicochemical characteristics of these different coatings lead NPs to behave differently in the environment. Different ligands impart different chemical properties and affect charge, particle size, surface area and aggregation of NPs (Elsaesser and Howard 2012; Lapresta-Fernández et al. 2012; Rana and Kalaichelvan 2013; Cupi et al. 2016b). NPs are stabilized against aggregation

Table 1 Different types of capping agents of NPs (Park et al. 2011; Sapsford et al. 2013; Singla and Kumar 2009; Shukla et al. 2008; Song et al. 2011; Levard et al. 2012; Sharma et al. 2014)

Category	Compound
Carboxylic acids	Citrate, oleic acid, mercaptosuccinic acid
Polymers	Polyvinylpyrrolidone, polyacrylate, polyvinylalcohol, polyacrylamide, polylactic acid, polyvinyl chloride, polystyrene, dodecanothiol
Polysaccharides	Gum Arabic, sophorolipids, chitosan, heparin, hyaluronic acid, cellulose, starch, alginic acid, dextran, maltose
Biological molecules	Bovine serum albumin, fatty acids, tyrosine
Inorganic coatings	Silver carbonate
Surfactants	Sodium dodecyl sulphate, cetyltrimethylammonium bromide, polyoxyethylene sorbitane monooleate
Organic coatings	Plant extracts, whole plant extracts, food sources from plant origin, triethanolamine, thioglycerol, hexamine, sodium dodecylbenzenesulfonates

and other chemical reactions like oxidation and sulfidation through adsorption or covalent attachment of organic compounds which provide electrostatic, steric or electrosteric repulsive forces between particles (Phenrat et al. 2008; Hotze et al. 2010). However, the impacts of different coatings on toxicity have been scarcely explored (Dominguez et al. 2015). It was shown that fullerene can cause oxidative damage in mammalian cells, and their toxicity is related to lipophilicity; reduction of lipophilicity with modification of the surface of fullerene by introducing aliphatic and hydroxyl groups resulted in reduced toxicity (Colvin 2003; Sayes et al. 2004). It has been reported that uncoated colloidal fullerenes may damage the brain of largemouth bass (Oberdörster 2004). Iron oxide NPs coated with ascorbate and dextran have been shown to be more toxic to the freshwater cladoceran *Daphnia magna* in comparison with the same NPs coated with citrate and polyvinylpyrrolidone (Baumann et al. 2014). Bozich et al. (2014) found that both the type of ligand and the charge of the NP surface affected the toxicity of Au NPs to *Daphnia magna* at acute and chronic level. Bone et al. (2012) found that the silver speciation from silver NPs (Ag NPs) varied significantly by coating type (gum Arabic and polyvinylpyrollidone) and the presence of plants (*Potamogeton diversifolius* and *Egeria densa*) in the medium, which reduced the toxicity of NPs to *Daphnia magna*. Interestingly, the fate and behaviour of NPs are changed by organisms as well. Adeleye and Keller (2016) observed charge reversal and change of surface properties of TiO_2 NPs by the extracellular polymeric substances (EPS) produced by *Chlamydomonas reinhardtii.* The presence of EPS may affect the bioavailability of NPs, their interactions with organisms and overall effects. Therefore, the authors suggested that the fate and effects of NPs cannot be simply predicted by the physicochemical characteristics of NPs.

4.4 Charge

The surface charge of NPs, measured as zeta potential, contributes to the adhesion of NPs on cell surfaces and hence is important in the toxicity of NPs. The NPs with the highest positive charge are the most toxic to the algae cells. Algal cells, having a negative charge on their surface, attract positive NPs to neutralize the charge, and this causes surface alterations resulting in cell death (Karunakaran et al. 2015). El Badawy et al. (2010) observed a surface charge-dependent toxicity of Ag NPs to bacteria (*Bacillus* sp.) when they were exposed to four different Ag NPs with different surface charges. Dominguez et al. (2015) showed that different types of coatings and the charge of NPs had an impact on ROS formation and gene expression in *Daphnia magna*. Nasser et al. (2016) suggested charge played an important role in toxicity and uptake of Au NPs to *Daphnia magna*.

5 Effects of the Surrounding Environment on NP Toxicity

OECD (2014) highlights the importance in identifying transformation, degradation and dissolution in the characterization of NPs in toxicity tests (Cupi et al. 2016a). Transformation of Ag NPs affects their surface properties, transportation, reactivity and toxicity in the environment (Xiu et al. 2011; Liu et al. 2010; Levard et al. 2011a, b). It is important to further assess the effects of the transformed NPs as well as fresh NPs to clearly understand how the transformation of NPs in the aquatic environment affects organisms (Levard et al. 2012). Biological systems have not evolved in the presence of ENPs which are produced today, and hence, the lack of knowledge about transport and fluxes of such particles present problems (Hoet et al. 2004; Dowling 2004). Generally, abiotic factors like pH, salinity, hardness of water and chemical oxygen demand (COD) influence the aquatic toxicity of chemicals (Li et al. 2013; Fabrega et al. 2011). The fate and toxicity of NPs in the aquatic environment are governed by physicochemical pathways which include aggregation and subsequent sedimentation, dissolution, adsorption to particulate and other solid surfaces, stabilization via surfactants and binding to natural organic matter (NOM) (Wang et al. 2016c; Boncel et al. 2015; Apul and Karanfil 2015; Köser et al. 2017; Ellis et al. 2018). Biological degradation, abiotic degradation, oxidation and reduction could also be of concern in some aquatic environments (Batley and Mclaughlin 2007). It was reported that the surface coatings change or are replaced with new coatings during their transit in water (Jarvie and King 2010). Less is known about the comparative toxicity of metallic NPs and their ionic forms (Xiu et al. 2012; Wang et al. 2016a). It has been found that NPs release ions into water over time and the rate and the degree of dissolution depend on their surface functionalization. Therefore, the biological toxicity of aged and freshly prepared NPs differ (Kittler et al. 2010). Strigul et al. (2009) studied the toxicity of boron NPs (B NPs) to *Daphnia magna*. Depending on the age of the test solution, the calculated 48 h LC_{50} values for B NPs ranged from 56 to 66 mg/L, and the difference in toxicity was attributed to dissolution of NPs releasing free ions. Once released into the environment, the toxicity also depends on the oxidation state of the NPs (Conway et al. 2015). Lei et al. (2016) found that the toxicity of nZVI to the alga *Chlorella pyrenoidosa* decreased after NPs was aged for 3 months in the medium, in comparison with the toxicity of fresh NPs. They attributed this to the surface oxidation of the NPs.

5.1 Media and Exposure System

Abiotic factors like pH, salinity, water hardness, temperature, different organic ligands and the components in the media affect the ecotoxicity of NPs (Handy et al. 2008b; Jin et al. 2010; Djurišić et al. 2015). The fate and transport of NPs in aquatic systems largely depend on the chemical characteristics of water (Garner and Keller 2014). Physicochemical factors in freshwater are different from brackish or seawater. Therefore the behaviour and effects of NPs identified in one medium cannot readily be applied to other media.

Li et al. (2011) assessed the toxicity of ZnO NPs to *Escherichia coli* in five different media (ultrapure water, 0.85% NaCl, phosphate-buffered saline (PBS), minimal Davis (MD) and Luria-Bertani (LB)). They observed different toxicity levels in a range of media and recommended that attention be paid to the physicochemical characteristics of NPs and media in bacterial toxicity tests. Li et al. (2013) found that the toxicity of ZnO NPs to *Escherichia coli* depended on the dissolution of NPs. Interestingly, toxicity was reduced by the presence of Ca^{2+} and Mg^{2+} in the medium which could compete with toxic Zn^{+} ions for binding sites on the organisms. Lopes et al. (2012) observed higher bacterial toxicity in Milli-Q water than in ASTM hard water which may be due to the interference of ions in ASTM hard water causing higher aggregation. von Moos et al. (2015) exposed *Chlamydomonas reinhardtii* to CuO NPs (10 mg/L) in five different exposure media. They observed that the media was decisive in determining toxicity regardless of the effects from NPs or ions. Similarly, Aravantinou et al. (2015) observed that the different sensitivity of the algae *Chlorococcum* sp. and *Scenedesmus rubescens* to ZnO NPs strongly depended on the algae medium. Zhang et al. (2016a) observed that media chemistry had profound effects on aggregation, dissolution and toxicity of TiO_2, ZnO and Ag NPs and CNTs to *Chlorella pyrenoidosa*. Seo et al. (2014) observed different toxicity levels in different media (ISO and moderately hard water (MHW)) when *Daphnia magna* was exposed to Ag, Cu and Zn NPs. Though the dissolution rate of NPs was higher in ISO medium, the toxicity was highest in MHW. Muna et al. (2017) found increased total Cu body burden from Cu NPs after exposing *Daphnia magna* in natural freshwater compared with OECD202 artificial medium. The Cu body burden in daphnids in natural freshwater bodies may be higher than laboratory predictions carried out using artificial media. Also, the total Cu body burden was higher in daphnids exposed to Cu NPs than Cu salt. Hu et al. (2017) reported higher toxicity of AgNPs and $AgNO_3$ to *Daphnia magna* in M4 medium in comparison with the surface water. For both forms of Ag, daphnids took up less and depurated more in the surface water. The authors suggested a reduced toxicity for the observation. However, Salieri et al. (2015) did not observe any significant influence of test media on toxicity of TiO_2 NPs to *Daphnia magna*. They believe this may be due to fast and strong agglomeration of NPs in all media, creating secondary particle size in the micrometre range. They did however report that the exposure volume of the medium had a significant influence on toxicity.

Nicolas et al. (2016) conducted standard algal growth inhibition tests (OECD 2011) with *Raphidocelis subcapitata* to test how the exposure system (24-well microplate, cylindrical vials and Erlenmeyer flasks) influenced the toxicity of TiO_2 NPs. They found that the exposure system significantly affected the results and recommended attention be paid during the algal growth inhibition test. Sørensen and Baun (2015) exposed the alga *Raphidocelis subcapitata* to AgNPs and $AgNO_3$ for 2 and 48 h in standard algal toxicity tests. Similar toxicity levels were observed for Ag^{+} in the two tests, whereas the toxicity of AgNPs was less toxic in 2 h test compared to 48 h test. Interestingly, ageing AgNPs in the medium for 48 h before performing the 2 h test increased the toxicity, while ageing beyond 48 h prior to testing reduced the toxicity. Xiao et al. (2018) observed higher toxicity in a dynamic exposure system with a vibration speed of 140 rpm in comparison with static exposure when *Daphnia magna* was exposed to CuO NPs. The aggregation of NPs in the dynamic system was less, and therefore, they hypothesized that the reduced toxicity may be due to the lower hydrodynamic diameter (HDD) of NPs. Sørensen et al. (2016b) claimed that the acute toxicity of Ag NPs and CuO NPs to *Daphnia magna* after pulse exposure (1–2 h) was comparable to the effects levels of 24 h continuous exposure. They attributed this to rapid toxicokinetic and toxicodynamic features of NPs causing the same level of toxicity following a few hours of exposure, concluding that the dissolved fractions of NPs are responsible for the toxicity. With this, they suggested that the use of pulse exposure was more environmentally relevant for NP toxicity assessments than standard continuous exposure tests.

Media critically influence the toxicity of NPs; this is due to several reasons. The physicochemical characteristics of the medium affect the fate and behaviour of NPs. The constituents of the medium may react with dissolved ions from NPs causing complexation and aggregation or compete with them for binding sites on the organisms. In addition, an organism's sensitivity and response to NP exposure also depend on the medium. Due to these reasons, the toxicity of NPs in one medium cannot be readily applied to other media. Other than the media, the outcome of toxicity testing of NPs is highly influenced by the test duration, the time from the moment NPs are added to the test medium, dynamic vs static exposure and pulse vs continuous exposure.

5.2 *Natural Organic Matter*

Studies suggest that the presence of some organic and inorganic substances in the medium could change the properties of NPs which contribute to determining the fate and toxicity of NPs (Metreveli et al. 2016; Gunsolus et al. 2015; Wang et al. 2018; Luoma et al. 2016). NPs may be more stable in natural waters than in synthetic waters where no NOM is present (Batley and Mclaughlin 2007). NOM present in media could form a layer on NPs and increase the stability of NPs (Baalousha and Lead 2013; Omar et al. 2014). Once released to the aquatic environment, NOM coated on NPs changes the reactivity and bioavailability of NPs to organisms (Aiken et al. 2011). Also, DOM may promote the mobility of the NPs in the aquatic environment (Ren et al. 2017). Liberation of ions from NPs is influenced by the presence of NOM in water (Wang et al. 2015). Jiang et al. (2015) observed that NOM affected the dissolution kinetics of ZnO NPs and found that the dissolution rate constants and dissolved Zn concentrations increased with increased NOM concentration. In addition, they found that the aromatic carbon content of NOM played a key role in promoting dissolution. Li et al. (2016b) studied the effects of DOM in the medium on the generation of ROS and the acute toxicity of metal oxide NPs to *Escherichia coli*. They observed that different photo-reactivity of humic and fulvic acids resulted in different effects on ROS generation and acute toxicity of NPs. Seitz et al. (2015) found that the pH and dissolved organic matter (DOM) in water considerably influenced the acute and chronic toxicity of Ag NPs to *Daphnia magna*. Xiao et al. (2018) observed that the toxicity of CuO NPs to *Daphnia magna* was mitigated in the presence of DOM. There are different views about the effects of NOM on the toxicity of NPs. However, NOM have been shown to influence the stability, dissolution, reactivity, bioavailability and mobility of NPs which directly or indirectly affected the toxicity.

5.3 Sulfidation

In natural waters, Ag NPs will preferentially transform to Ag_2S or AgCl as per the thermodynamic constraints. Also, the transformation will depend on pH and redox potential (Eh) and the composition of natural waters; by knowing those values, it is possible to predict the speciation of silver in simple systems. Under aerobic conditions, formation of silver chloride species is predicted, but under anaerobic conditions, sulfidation is predicted (Levard et al. 2012). Bioavailability and toxicity of ions change with sulfidation, and it was found that the toxicity of Ag^+ to *Daphnia magna* decreased by about fivefold in the presence of environmentally relevant levels of sulphide (Bianchini and Wood 2008; Bianchini et al. 2002). Guo et al. (2017) observed that the toxicity depended on sulfidation rate when the bacteria *Escherichia coli* was exposed to Ag NPs. Reinsch et al. (2012) observed decreasing toxicity of Ag NPs to *Escherichia coli* with increasing sulfidation (Ag_2S:Ag^0 ratio).

5.4 Other Factors

The interactions between NPs and bacteria can be affected by several other factors such as the pH and ionic strength of the medium and the photocatalytic activity of NPs under different irradiation conditions (Djurišić et al. 2015). Pagnout et al. (2012) observed toxicity changed with different electrolytes (NaCl, $CaCl_2$, Na_2SO_4) in the medium when *Escherichia coli* was exposed to TiO_2 NPs. Also, they observed that the toxicity changed with pH which may cause changes in the surface charge of NPs resulting in different interactions between bacteria and NPs. Bhuvaneshwari et al. (2015) observed increased toxicity of ZnO NPs to *Scenedesmus obliquus* under UV-C irradiation compared with that under visible light. They ascribed this to increased ROS production in UV-C irradiated algal cells compared with cells under other irradiation conditions. Sendra et al. (2017) reported a significant increase in the toxicity of TiO_2 NPs and bulk TiO_2 under UVA irradiation in comparison with that observed invisible light. Ratti et al. (2016) observed light-enhanced antimicrobial activity of NPs when *Escherichia coli* and

Bacillus subtilis were exposed to AgNPs. Lee and An (2013) exposed *Raphidocelis subcapitata* to ZnO and TiO_2 NPs under visible, UVA and UVB irradiation conditions. Though the growth of algae was inhibited under all conditions, there was no significant toxicity difference among the light conditions.

Physicochemical characteristics of NPs alter upon environmental release with time under the influence of the surrounding environment, thereby affecting their impact on organisms. Several environmental factors such as media composition, exposure scenario, sulfidation, irradiation, pH and ionic strength of media influence the toxicity of NPs. Most ecotoxicological studies (Table 2) to date have focused on the effects of as-prepared NPs on organisms; few studies have evaluated the effects of the transformation of NPs on toxicity. More studies focusing on this aspect which are biologically and environmentally relevant are warranted.

5.5 NP Stability and Aggregation

Aggregation, sulfidation and oxidation are examples of changes that could happen to varying degrees (Fortner et al. 2005; Brant et al. 2005; Teeguarden et al. 2007; Garner and Keller 2014; Conway et al. 2015). Size and aggregation are the crucial factors in determining the ecotoxicity of carbon NPs, while solubility and speciation determine the toxicity of metal oxide NPs (Blinova et al. 2010). The degree, kinetics and size range of aggregates depend on the characteristics of the particles, the characteristics of the environment and the concentrations of the particles (Phenrat et al. 2007; Hyung et al. 2007). Negatively charged NPs are electrostatically stabilized when the negative charge is strong enough to repel NPs from each other to overcome attractive forces. However, the presence of counterions in the solution will reduce the repulsive forces resulting in decreased stability. Several researchers provided supportive evidence for this phenomenon claiming that the different ionic strengths of the environment affect the aggregation and stability of NPs (El Badawy et al. 2010; Li et al. 2010c; Liu et al. 2011; Delay et al. 2011). Even a slight increase in salinity decreases colloids by particle aggregation and precipitation (Stolpe and Hassellöv 2010). The ionic strength of freshwater systems ranges from 1 to 10 mM and that of seawater is about 700 mM (Levard et al. 2012). However, there is a tendency for less aggregation when NPs are stabilized sterically other than solely by surface charge. Attachment of certain polymers causes steric stabilization, and several researchers demonstrated that adsorption of compounds in natural waters induced steric forces that resist aggregation (Fabrega et al. 2009; Delay et al. 2011; Chinnapongse et al. 2011; Cumberland and Lead 2009). Polyelectrolytes exhibit additional electrosteric forces in addition to steric stabilization which makes them excellent in stabilizing NPs (Badawy et al. 2010).

Aggregation is a crucial factor in determining NP toxicity. In general, the majority of studies support the idea that the aggregation of NPs reduces the toxicity to organisms though some researchers have claimed otherwise. Several researchers reported a correlation between aggregation of NPs and their toxicity to isolated

Table 2 Summary list of NP effect studies involving freshwater species of bacteria, algae, *Daphnia* and bivalves

Reference	Materials tested	Organisms	Effects studied	Endpoints
Bacteria				
Hwang et al. (2008)	Ag and Au NPs, $AgNO_3$	*Escherichia coli*	Toxic mode of action	Oxidative stress, membrane/protein damage, DNA damage
Strigul et al. (2009)	B, TiO_2 and Al NPs	*Vibrio fischeri*	Toxicity of NPs	60 days EC_{50} (mg/L) – B NPs, 56–66; TiO_2 NPs, ND; Al NPs, ND
Jiang et al. (2009)	Al_2O_3, SiO_2 and ZnO NPs	*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*	Toxicity of NPs and mechanisms of toxicity	Mortality% *B. subtilis*: Al_2O_3, 57%; SiO_2, 40%; ZnO, 100% *E. coli*: Al_2O_3, 36%; SiO_2, 58%; ZnO, 100% *P. fluorescence*: Al_2O_3, 70%; SiO_2, 70%; ZnO, 100% Membrane damage (cell pitting) by NP attachment
Baek and An (2011)	CuO, ZnO, NiO and Sb_2O_3 NPs	*Escherichia coli*, *Bacillus subtilis*, *Streptococcus aureus*	Toxicity of NPs	24 h EC_{50} (mg/L) – *E. coli*: CuO, 28.6; ZnO, 15.7; NiO, 160.2; Sb_2O_3, 265.5 *B. subtilis*: CuO, 61.1; ZnO, 85.8; NiO, 121.9; Sb_2O_3, 144.7 *S. aureus*: CuO, 65.9; ZnO, >125; NiO, 121.1; Sb_2O_3, 324.0
Choi et al. (2008)	Ag NPs, $AgNO_3$, AgCl colloides	Nitrifying bacteria, *Escherichia coli*	Size, bioavailability and ROS-dependent toxicity of NPs	Nitrifying bacteria (% inhibition): AgNPs, 86; Ag^+, 42; AgCl, 46 *E. coli* (% inhibition): AgNPs, 55; Ag^+, 100; AgCl, 66 Membrane damage (cell pitting) by NP attachment
Lopes et al. (2012)	Sodium dodecyl sulphate/didodecyl dimethylammonium	*Vibrio fischeri* and *Salmonella typhimurium*	Toxicity of NPs and genotoxicity of NPs	30 min EC_{50} (mg/L) (*V. fischeri*) ASTM medium – SDS/DDAB, 24.5; Mo/NaO, 36.5; TiO_2, NT; $TiSiO_4$, NT;

	bromide (SDS/DDAB), monoolein and sodium oelate (Mo/NaO), TiO_2, $TiSiO_4$, CdSe/ZnS, Au nanorods			CdSe/ZnS, 0.15; Au nanorods, 0.22 Milli-Q – SDS/DDAB, 13.6; Mo/NaO, 68; TiO_2, NT; $TiSiO_4$, 51; CdSe/ZnS, 0.08; Au nanorods, 0.22
Heinlaan et al. (2008)	ZnO, CuO and TiO_2 NPs, ZnO, $ZnSO_4$, $CuSO_4$, TiO_2, CuO	*Vibrio fischeri*	Toxicity of NPs	72 h EC_{50} (mg/L) – ZnO, 1.8; ZnO NPs, 1.9; $ZnSO_4$, 1.1; CuO, 3,811.0; CuO NPs, 79; $CuSO_4$, 1.6; TiO_2, ND
Fabrega et al. (2009)	Ag NPs	*Pseudomonas fluorescens*	Toxicity of NPs	Growth inhibition
Jin et al. (2010)	Ag NPs	*Bacillus subtilis*, *Pseudomonas putida*	Toxicity of NPs, effect of water chemistry on toxicity	Membrane damage, survival
Li et al. (2013)	ZnO NPs	*Escherichia coli*	Effect of water chemistry on dissolution and toxicity of NPs	Percentage mortality
Choi et al. (2010)	Ag NPs, $AgNO_3$	*Escherichia coli*	Toxicity of NPs in planktonic and biofilm cultures	Minimum bactericidal concentration (MBC) (mg/L) AgNPs, planktonic, 38 biofilms, 10 Ag^+, planktonic, 2.4 biofilms, 1.2
Lok et al. (2006)	Ag NPs, $AgNO_3$	*Escherichia coli*	Mode of antibacterial action	Membrane damage
Binaeian et al. (2012)	Ag NPs, $AgNO_3$	*Vibrio fischeri*	Toxicity of NPs	30 min EC_{50} (mg/L) – AgNPs (biological method), 34.5; AgNPs (chemical reduction method), 29.3; Ag^+, 1.8
Greulich et al. (2012)	Ag NPs, $AgNO_3$	*Escherichia coli*, *Staphylococcus aureus*	Toxicity of NPs	MBC (mg/L) (for both types of bacteria) AgNPs, 12.5–50; Ag^+, 0.5–5.0
Li et al. (2010b)	Ag NPs	*Escherichia coli*	Growth, permeability and morphology	Leakage of reducing sugars and proteins, change of respiratory chain dehydrogenases, membrane damage (cell pitting)
Sondi and Salopek-Sondi (2004)	Ag NPs	*Escherichia coli*	Mechanism of antimicrobial toxicity of NPs	Growth inhibition, membrane damage (cell pitting), NP accumulation in the membrane

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Su et al. (2009)	Ag/silicate nanohybrids	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyrogens*	Mode of antibacterial action Toxicity of NPs	Growth inhibition, cell death, membrane damage due to ROS production by NPs
Brayner et al. (2006)	ZnO NPs	*Escherichia coli*	Toxicity of NPs	Membrane damage, NP accumulation in the membrane, cellular internalization of NPs
Feng et al. (2015)	Au NPs	*Bacillus subtilis*, *Shewanella oneidensis*	Charge and ligand type on surface binding and toxicity	Viability, membrane damage by associated NPs on bacterial cells, NP localization in cells
Hossain and Mukherjee (2012)	CdO NPs	*Escherichia coli*	Toxicity of NPs on growth, morphology and cell division	Growth inhibition, morphological changes, membrane damage by ROS generation, effects on cell division
Thill et al. (2006)	CeO_2 NPs	*Escherichia coli*	Toxicity of NPs	Growth inhibition, membrane damage by adsorption of NPs on bacteria cells
Kumar et al. (2011)	TiO_2 and ZnO NPs	*Escherichia coli*	Toxicity of NPs by oxidative stress and DNA damage	Cell viability, membrane damage, cellular intake, enzyme activity, DNA damage
Li et al. (2011)	ZnO NPs	*Escherichia coli*	Toxicity of NPs in different media	Mortality, growth inhibition
Reinsch et al. (2012)	Ag NPs	*Escherichia coli*	Effect of sulfidation on acute toxicity	Growth inhibition
Pagnout et al. (2012)	TiO_2 NPs	*Escherichia coli*	Role of electrostatic interactions on toxicity	Viability, growth inhibition
El Badawy et al. (2010)	Ag NPs	*Bacillus* sp.	Surface charge-dependent acute toxicity	Viability, oxygen consumption
Guo et al. (2017)	Ag NPs	*Escherichia coli*	Acute toxicity in the presence of anions	Viability

Choi and Hu (2008)	Ag NPs	Nitrifying bacteria	Size- and ROS-dependent toxicity	30 min EC_{50} (mg/L) – AgNPs, 0.14; AgCl, 0.25; Ag^+, 0.27; membrane damage (cell pitting)
Li et al. (2016b)	Metal oxide NPs	*Escherichia coli*	Influence of DOM on photogenerated ROS formation and acute toxicity of NPs	Survival rate, ROS generation
Xiu et al. (2012)	Ag NPs, $AgNO_3$	*Escherichia coli*	Particle-specific toxicity, effect of size and coating	Survival rate, effects of NP morphology
Kvitek et al. (2008)	Ag NPs	*Escherichia coli*	Effect of surfactants and polymers on stability and antibacterial activity of NPs	Growth inhibition
Lok et al. (2007)	Ag NPs, $AgNO_3$	*Escherichia coli*	Oxidation and antibacterial activity of NPs	Growth inhibition
Werlin et al. (2011)	CdSe Qds	*Pseudomonas aeruginosa*	Trophic transfer of NPs from bacteria to protozoa and biomagnification	Bioaccumulation of QDs in *P. aeruginosa* and trophic transfer to *Tetrahymena thermophila*
Jaiswal and Mishra (2018)	Ag NPs, $AgNO_3$	*Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*	Toxicity of NPs with improved stability	MIC and minimum biocidal concentration (MBC), Growth inhibition, cytotoxicity MIC (mg/L) – Cur-AgNPs: *P. aeruginosa*, 10; *E. coli*, 5; *B. subtilis*, 2.5; *S. aureus*, 5 MIC (mg/L) – AgNPs: *P. aeruginosa*, 10; *E. coli*, 15; *B. subtilis*, 10; *S. aureus*, 10 MIC (mg/L) – Ag^+: *P. aeruginosa*, 1.25; *E. coli*, 0.6; *B. subtilis*, 2.5; *S. aureus*, 1.25
Matzke et al. (2014)	Ag NPs, $AgNO_3$	*Pseudomonas putida*	Toxicity of differently sized and coated NPs	72 h EC_{50} (μg/L) – AgNPs, 0.25–13.4; Ag^+, 0.16
Akhil and Sudheer Khan (2017)	ZnO NPs	*Pseudomonas aeruginosa*, *Staphylococcus aureus*	Effects of humic acid on toxicity of NPs	Percentage toxicity, ROS, lipid peroxidation

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Miao et al. (2018)	TiO_2 NPs, CeO_2 NPs	*Cytophagaceae*, *Methylotenera* genera	Effect on metabolic activities of microbial communities	Enzyme activities, quantitative PCR, and high-throughput sequencing
Miao et al. (2018)	Ag NPs	Benthic microbial communities	Influence of NPs on benthic oxygen consumption	Oxygen concentration distributions
Algae				
Taylor et al. (2016b)	CeO_2 NPs	*Chlamydomonas reinhardtii*	Molecular toxicity of NPs with exposure concentration	Transcriptomics and metabolic profiling, NP internalization
Hoecke et al. (2009)	CeO_2 NPs	*Raphidocelis subcapitata*	Acute toxicity of NPs	72 h IC_{50} (mg/L) – 10.2–19.1
Rogers et al. (2010)	CeO_2 NPs	*Raphidocelis subcapitata*	Physicochemical behaviour and toxicity of NPs	72 h IC_{50} (mg/L) – 10.3 Membrane permeability, ROS production
Oukarroum et al. (2012)	Ag NPs	*Chlorella vulgaris*	Toxicity of NPs	Cell viability, ROS production, enzyme activity, chlorophyll content
Navarro et al. (2008)	Ag NPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Acute toxicity	1–5 h EC_{50} (nM) – AgNPs, 829.0–3,300.0 1–2 h EC_{50} (nM) – Ag^+ ($AgNO_3$), 184–188 1–5 h EC_{50} (nM) – Ag^+ (AgNPs), 8–33
Miao et al. (2010)	Ag NPs	*Ochromonas danica*	Toxicity and intracellular uptake of NPs	7 days EC_{50} (pM) – dissolved Ag^+ (from AgNPs), 1.27 7 dEC_{50} (nM) – Ag^+, 49.1
Angel et al. (2015)	CeO_2 NPs	*Raphidocelis subcapitata*	Acute toxicity, mechanism of toxicity	72 h IC_{50} (mg/L) – 7.6–28 ROS production, hyperspectral imaging
Adeleye and Keller (2016)	TiO_2 NPs	*Chlamydomonas reinhardtii*	Influence of algal EPS on TiO_2 Nanoparticles	Interaction, surface charge and aggregation of NPs
Zhou et al. (2016)	Ag NPs, $AgNO_3$	*Chlorella pyrenoidosa*	Role of EPS on bioaccumulation and toxicity of Ag nanoparticles	96 h IC_{50} (mg/L) – AgNPs, 39.2–140.0; Ag^+, 12.0 Interactions, accumulation of Ag
Ribeiro et al. (2014)	Ag NPs, $AgNO_3$	*Raphidocelis subcapitata*	Toxicity of NPs	96 h EC_{50} (μg/L) – AgNPs, 32.40; Ag^+, 33.79

Rodea-Palomares et al. (2011)	CeO_2 NPs	*Raphidocelis subcapitata*	Toxicity (growth rate measured by different methods) of NPs	24 h EC_{50} (mg/L) – OD, 0.88–16.5; cell counting, 4.25–56.7; ATP, 2.4–20.3
Hartmann et al. (2010)	TiO_2 NPs	*Raphidocelis subcapitata*	Algal toxicity of different types of NPs and influence of NPs on Cd toxicity	72 h EC_{50} (mg/L) – Degussa P25, 71.1; LW-S, 145.0; UV 100, 241.0 Cd toxicity
Lei et al. (2016)	Nano zero-valent iron, Fe_2O_3 and Fe_3O_4 NPs, $FeCl_3$	*Chlorella pyrenoidosa*	Effects of particle size, crystal phase, oxidation state and ageing of NPs on toxicity	96 h IC_{50} (mg/L) – ZVI NPs, 3.2–91.3; Fe_2O_3 NPs, 71.0–132.0; Fe_3O_4 NPs, ~32.0; Fe^{2+}, 1.0
Nicolas et al. (2016)	TiO_2 NPs	*Raphidocelis subcapitata*	Influence of exposure system on NP toxicity	72 h EC_{50} (mg/L) – 24 well plates, 8.5–>50; cylindrical vials, 2.7–39; Erlenmeyer flasks, >50
von Moos et al. (2015)	CuO NPs	*Chlamydomonas reinhardtii*	Sublethal effects in different media	Cellular growth, chlorophyll autofluorescence, intracellular oxidative stress and membrane integrity
Lee et al. (2015)	Au NPs	*Euglena gracilis* and *Chlamydomonas reinhardtii*	Toxicity and trophic transfer of NPs from algae to *Daphnia*	48 h EC_{50} (mg/L) *E. gracilis* – 9.18; *C. reinhardtii*, ND Trophic transfer from algae to *D. magna*
Bouldin et al. (2008)	Carboxyl NPs	*Raphidocelis subcapitata*	Toxicity and trophic transfer of NPs from algae to *Daphnia magna*	96 h EC_{50} (μg/L) – 37.1 Uptake of NPs
Zhao et al. (2016)	CuO NPs	*Chlorella pyrenoidosa*	Adhesion, uptake and toxicity of NPs	72 h EC_{50} (mg/L) – 45.7 NP internalization, membrane damage, ROS production, mitochondrial dysfunction
Aruoja et al. (2009)	CuO, ZnO and TiO_2 NPs, CuO, TiO_2, ZnO, $ZnSO_4$	*Raphidocelis subcapitata*	Toxicity of NPs	72 h EC_{50} (mg/L) – ZnO NPs, 0.042; TiO_2 NPs, 5.83; CuO NPs, 0.71; Zn^{2+}, 0.037–0.042; Ti^{2+}, 35.9; Cu^{2+}, 0.02–11.5
Ji et al. (2011)	Al_2O_3, SiO_2, ZnO, TiO_2	*Chlorella* sp.	Toxicity of NPs	6 days EC_{30} (mg/L) – ZnO NPs, 20.0; TiO_2 NPs, 30.0; Al_2O_3 and SiO_2 NPs, ND

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Sørensen et al. (2016a)	Pt NPs	*Raphidocelis subcapitata*, *Chlamydomonas reinhardtii*	Toxicity of NPs	48 h EC_{50} (mg/L) – *P. subcapitata*, 15; *C. reinhardtii*, 201
Dorobantu et al. (2015)	AgNPs, $AgNO_3$	*Chlorella protothecoides*, *Euglena gracilis*	Toxicity of NPs and mechanisms of toxicity	Growth inhibition, membrane damage
Aravantinou et al. (2015)	ZnONPs	*Chlorococcum* sp., *Scenedesmus rubescens*	Effect of media on toxicity of NPs	Growth inhibition 48 h IC_{50} (mg/L) – *Chlorococcum* sp., 0.77–7.99; *S. rubescens*, 42.68–300.59; 72 h IC_{50} (mg/L) – *Chlorococcum* sp., 1.78–2.03; *S. rubescens*, 22.56–>810.0; 96 h IC_{50} (mg/L) – *Chlorococcum* sp., 0.75–893.55; *S. rubescens*, 14.27–>810.0
Aruoja et al. (2015)	Al_2O_3, Co_3O_4, CuO, Fe_3O_4, MgO, Mn_3O_4, Sb_2O_3, SiO_2, ZnO, TiO_2, WO_3 and Pd NPs	*Raphidocelis subcapitata*	Toxicity of different NPs	Growth inhibition 72 h EC_{50} (mg/L) – CuO, ZnO, Pd NPs; 0.1–1.0; Co_3O_4, Fe_3O_4, Mn_3O_4, TiO_2 NPs; 1.0–10.0, Al_2O_3, SiO_2, WO_3 NPs; 10.0–100.0, MgO, Sb_2O_3 NPs; >100.0
Becaro et al. (2015)	AgNPs	*Raphidocelis subcapitata*	Toxicity of NPs	Growth inhibition 7 days EC_{50} (mg/L) – 1.09
Iswarya et al. (2017)	Au NPs	*Scenedesmus obliquus*	Modulatory effects of Zn^{2+} on the toxicity of NPs	Acute toxicity
Karunakaran et al. (2015)	Al_2O_3, SiO_2	*Porphyridium aerugineum*, *Geitler*	Toxicity of NPs based on size, contact angle and zeta potential	Growth inhibition, chlorophyll content and protein content of algae
Lee and An (2013)	ZnO, TiO_2	*Raphidocelis subcapitata*	Effect of NPs under different irradiation conditions	72 h EC_{50} (mg/L) – ZnO NPs – visible, UVA, UVB: <0.05; 72 h EC_{50} (mg/L) – ZnO NPs – visible, 2.53; UVA, 3.0; UVB, 2.95
Li et al. (2015b)	AgNPs, $AgNO_3$	*Euglena gracilis*	Toxicity and association of NPs	1 h EC_{50} (nM) – Ag NPs, 1,858.0; Ag^+, 85 2 h EC_{50} (nM) – Ag NPs, 1,487.0; Ag^+, 89 Cell morphology, uptake

Müller et al. (2016)	Cu NPs, Cu^{2+}	*Chlamydomonas reinhardtii*	Toxicity of NPs	Photosynthetic yield 1 h EC_{50} (μM) – Cu NPs, 101.0–219.0; Cu^{2+} (dissolved), 1.23–1.29; Cu^{2+}, 1.12; 2 h EC_{50} (μM) – Cu NPs, 29.0–30.0; Cu^{2+} (dissolved), 0.23–0.4; Cu^{2+}, 0.39
Nogueira et al. (2015)	Graphene oxide NPs	*Raphidocelis subcapitata*	Toxicity of NPs	Growth inhibition, ROS production, membrane damage
Oukarroum et al. (2017)	NiO NPs	*Chlorella vulgaris*	Toxicity of NPs	Cell viability, chlorophyll content, ROS production
Qian et al. (2016)	AgNPs, $AgNO_3$	*Microcystis aeruginosa*, *Chlorella vulgaris*	Toxicity of NPs and detoxification strategies	Growth inhibition, ROS production, gene transcription and protein expression analysis, chlorophyll content 48 h EC_{50} (nM) – *M. aeruginosa*: AgNPs, 731.7; Ag^+, 146.0; 96 h EC_{50} (nM) – *M. aeruginosa*: AgNPs, 351.4; Ag^+, 44.6; EC_{50} – *Chlorella vulgaris*: ND
Sendra et al. (2017)	TiO_2 NPs, Bulk TiO_2	*Chlamydomonas reinhardtii*	Toxicity of NPs under different irradiation conditions	Growth inhibition, yield reduction, ROS production, membrane damage, production of exopolymeric substances (EPS) 72 h EC_{50} (mg/L) – UVA + visible light – TiO_2 NPs, 2.3; bulk TiO_2, 1.35; 72 h EC_{50} (mg/L) – UVA – TiO_2 NPs, 551.7; bulk TiO_2, 423.7
Sørensen and Baun (2015)	AgNPs, $AgNO_3$	*Raphidocelis subcapitata*	Effect of timing in algal toxicity testing of NPs	Growth inhibition 2 h EC_{50} (μg/L) – AgNPs, 50–710; Ag^+, 6.0; 48 h EC_{50} (μg/L) – AgNPs, 75–310; Ag^+, 4.9; 2 h EC_{50} (μg/L) – AgNPs + cysteine, 1,000.0–1,800.0; Ag^+ + cysteine, >240.0; 48 h EC_{50} (μg/L) – AgNPs + cysteine, 670.0–3,700.0; Ag^+ + cysteine, 270.0
Stevenson et al. (2013)	AgNPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Mitigation of AgNP toxicity by EPS	Chlorophyll concentration

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Taylor et al. (2016a)	AgNPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Toxicity of NPs and effects on EPS	Growth inhibition, cell viability, ROS production
Wang et al. (2016b)	AgNPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Cellular internalization and biotransformation of NPs	Uptake, distribution and speciation analysis of NPs
Yue et al. (2017)	AgNPs, $AgNO_3$	*Euglena gracilis*	Interaction of NPs with algal cells	Uptake, enzyme activity, interaction with proteins
Zhang et al. (2017)	TiO_2 NPs, organochlorine	*Chlorella pyrenoidosa*	Toxic interactions between NPs and OC contaminants	Oxidative stress, bioaccumulation
Zhang et al. (2016a)	TiO_2, ZnO, Ag NPs, CNTs	*Chlorella pyrenoidosa*	Physicochemical transformation and toxicity of NPS	Cell viability
Dauda et al. (2017)	TiO_2 NPs	*Chlorella vulgaris*	Toxicity of NPs	Oxidative stress
Zhang et al. (2016b)	Fe_xO_y NPs	*Raphidocelis subcapitata*	Toxicity of NPs	72 h EC_{50} (mg/L) – 0.05–0.09
Metzler et al. (2018)	TiO_2 NPs	*Raphidocelis subcapitata*	Influence of algae age and population on toxicity of NPs	Growth inhibition, chlorophyll content, oxidative stress
Yu et al. (2018)	TiO_2, SiO_2 and Ag NPs, CdTe/CdS QDs	*Chlamydomonas reinhardtii*	Effects of NPs on Cd toxicity	Growth inhibition, photosynthesis, bioaccumulation, oxidative stress
Ozkaleli and Erdem (2018)	TiO_2 NPs	*Raphidocelis subcapitata*	Mechanisms of NP toxicity	Growth inhibition, lipid peroxidation 24 h EC_{50} (mg/L) – 18.12–98.3, 72 h EC_{50} (mg/L) – 3.58–12.14
Bhuvaneshwari et al. (2017)	nZVI	*Scenedesmus* sp.	Trophic transfer of NPs to *Ceriodaphnia dubia*	Acute toxicity, uptake, enzyme activity 72 h EC_{50} (mM) – 7.12–24.29
Bhuvaneshwari et al. (2018b)	TiO_2 NPs	*Dunaliella salina*	Trophic transfer of NPs to *Artemia salina*	Acute toxicity, uptake, enzyme activity 72 h EC_{50} (mg/L) – 11.35
Akhil and Sudheer Khan (2017)	ZnO NPs	*Chlorella pyrenoidosa*	Effects of humic acid on toxicity of NPs	Chlorophyll content, ROS, lipid peroxidation

Cladocerans				
Allen et al. (2010)	Ag NPs, $AgNO_3$	*Daphnia magna*	Effects of filtration, coating and presence of food on toxicity of NPs	48 h LC_{50} (μg/L) – Ag NPs, 0.7–16.7; Ag^+, 1.1–1.4
Zhu et al. (2010b)	TiO_2	*Daphnia magna*	Trophic transfer of TiO_2 NPs from *D. magna* to *D. rerio*	Accumulation of NPs in *D. magna* and *D. rerio*
McTeer et al. (2014)	Ag NPs, $AgNO_3$	*Daphnia magna*	Trophic transfer of Ag from algae to *Daphnia*, feeding behaviour	Feeding behaviour, accumulation of Ag NPs in *D. magna*
Skjolding et al. (2014a)	Au NPs	*Daphnia magna*	Uptake and depuration of NPs	Uptake, depuration
Skjolding et al. (2014b)	ZnO NPs, $ZnCl_2$	*Daphnia magna*	Influence of surface functionalization on acute toxicity, uptake and trophic transfer of NPs	Acute toxicity – 48 h LC_{50} (mg/L) – Zn^{2+}, 0.41; ZnO NPs, 1.9–15.5 Uptake, trophic transfer from *D. magna* to *D. rerio*
Rosenkranz et al. (2009)	Carboxylated polystyrene NPs	*Daphnia magna*	Influence of size of NPs on uptake, accumulation and depuration of NPs	Uptake, accumulation, depuration
Heinlaan et al. (2008)	ZnO, CuO and TiO_2 NPs, $ZnSO_4$, $CuSO_4$	*Daphnia magna*	Acute toxicity and bioavailability of NPs	48 h EC_{50} (mg/L) – ZnO, 8.8; ZnO NPs, 3.2; $ZnSO_4$, 6.1; CuO, 165; CuO NPs, 3.2; $CuSO_4$, 0.17; TiO_2, ND
Dabrunz et al. (2011)	TiO_2 NPs	*Daphnia magna*	Acute toxicity, biological surface coating and moulting inhibition	72 h EC_{50} (mg/L) – 3.8, 96 h EC_{50} (mg/L) – 0.73 Moulting disruption
Lu et al. (2017)	Cu and Cr NPs	*Daphnia magna*	Acute and chronic toxicity	48 h EC_{50} (mg/L) – Cu NPs, 0.63; Cr NPs, 1.57 Feeding behaviour, enzyme activity (AChE, SOD,CAT and GST)
Zhao and Wang (2011)	Ag NPs, $AgNO_3$	*Daphnia magna*	Acute and chronic toxicity of NPs and Ag^+, effects of diet-borne and waterborne NPs and Ag^+	48 h EC_{50} (μg/L) – Ag^+, 2.51; Ag NPs, ND Acute toxicity, growth, reproduction, uptake

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Rainville et al. (2014)	AgNPs, $AgNO_3$	*Daphnia magna*	Acute toxicity (lethal and sublethal)	48 h LC_{50} (μg/L) – Ag^+, 4.5; Ag NPs, 47.2–429.9 Enzyme activity (CAT and GAPDH), protein identification
Li et al. (2010a)	Ag, Au and Ag-Au NPs	*Daphnia magna*	Comparative toxicity of NPs, effects of size of NPs	48 h LC_{50} (μg/L) – Au NPs, 65–75; Ag NPs, 3–4; Ag-Au NPs, 12–59
Shen et al. (2015)	Ag NPs and $AgNO_3$	*Daphnia magna*	Acute toxicity of NPs and Ag^+	8 h LC_{50} (μg/L) – Ag^+, 0.4; Ag NPs, 5.0–144.2
Adam et al. (2015a)	ZnO and CuO NPs, $ZnCl_2$, $CuCl_2{\cdot}2H_2O$	*Daphnia magna*	Uptake and elimination of NPs under chronic exposure	Uptake and elimination of NPs
Wiench et al. (2009)	TiO_2 and ZnO NPs	*Daphnia magna*	Acute and chronic effects on mobility and reproduction	Mortality, reproduction
Kim et al. (2010)	TiO_2 NPs	*Daphnia magna*	Oxidative stress based on size of NPs	Enzyme activity (CAT, GST, GSH and GPx)
Dominguez et al. (2015)	Au NPs	*Daphnia magna*	Effects of charge and surface ligand properties of NPs on oxidative stress and gene expression	ROS production, gene expression
Strigul et al. (2009)	TiO_2, B and Al NPs	*Daphnia magna*	Acute toxicity based on ageing of NPs	48 h LC_{50} (mg/L) – B NPs, 6.7; TiO_2 NPs, ND; Al NPs (ALEX), 7.4; Al NPs (L-ALEX), 107.5
Adam et al. (2014)	ZnO NPs, $ZnCl_2$	*Daphnia magna*	Chronic toxicity, NP aggregation and dissolution	21 days EC_{50} (reproduction) (mg/L) – ZnO NPs, 0.112; Zn^{2+}, 0.082 Growth, reproduction, accumulation
Adam et al. (2015b)	CuO NPs and $CuCl_2{\cdot}2H_2O$	*Daphnia magna*	Chronic toxicity of NPs and Cu^{2+}	21 days EC_{50} (reproduction) (mg/L) – CuO NPs, 1.041; Cu^{2+}, 0.022 Reproduction, length

Conine and Frost (2017)	Ag NPs	*Daphnia magna*	Effects of food and nutrition in responding to NP exposure	48 h LC_{50} (μg/L) – AgNPs (without food), 5.2; AgNPs (with food), 17.0
Tan et al. (2016b)	TiO_2 NPs, Cd	*Daphnia magna*	Toxicity of Cd in the presence and absence of well-dispersed NPs	Toxicity, uptake and accumulation of Cd
Lu et al. (2017)	Cu and Cr NPs	*Daphnia magna*	Toxicity of NPs	48 h LC_{50} (μg/L) – Cu NPs, 0.63; Cr NPs, 1.57 Chronic toxicity of NPs (AChE, CAT, SOD, GST), feeding rate
Nasser et al. (2016)	Au NPs	*Daphnia magna*	Effect of shape and charge of NPs on acute toxicity, oxidative stress and moulting	24 h LC_{50} – Au NPs (positively charged), 6.1–50.0 μg/L; Au NPs (negatively charged), 10–50 mg/L ROS production; moulting inhibition
Fan et al. (2016)	TiO_2 NPs, Cu	*Daphnia magna*	Influence of NPs on Cu toxicity	Uptake and bioaccumulation of Cu, enzyme activity
Sørensen et al. (2016c)	Ag and CuO NPs, $AgNO_3$, $CuCl_2$	*Daphnia magna*	Acute and chronic effects of NPs and ions after standard and pulse exposure	48 h LC_{50} (μg/L) – CuONP, 5.2; AgNP, ND; Ag^+, 4.0; Cu^{2+}, 0.05, 24 h LC_{50} (μg/L) – CuONP, 6.5; AgNP, 130; Ag^+, 4.5; Cu^{2+}, 0.1 EC_{50} values after 1, 2 and 3 h pulse exposure, moulting inhibition, body length, offspring production
Georgantzopoulou et al. (2016)	Ag NPs, $AgNO_3$	*Daphnia magna*	Influence of NPs on multixenobiotic resistance	Inhibition of multixenobiotic resistance transporters
Iswarya et al. (2016)	TiO_2 NPs (anatase and rutile)	*Ceriodaphnia dubia*	Individual and binary mixture toxicity of anatase and rutile NPs	48 h LC_{50} (visible irradiation) (mg/L) – anatase NPs, 37.0; rutile NPs, 48.0 48 h LC_{50} (UV irradiation) (mg/L) – anatase NPs, 22.5; rutile NPs, 23.7 LC_{50} values for binary mixtures

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Bhuvaneshwari et al. (2016)	ZnO NPs, Zn^{2+} ions	*Ceriodaphnia dubia*	Relative contribution of dissolved ions and NPs to acute toxicity and accumulation	48 h LC_{50} (mg/L) – ZnO NPs, 0.431–0.701; Zn^{2+}, 1.048–2.046 Bioaccumulation
Sakka et al. (2016)	AgNPs	*Daphnia magna*	Behaviour and chronic effects of differently stabilized NPs	Reproduction, mass balance, uptake, depuration
Tan et al. (2016a)	TiO_2 NPs	*Daphnia magna*	Influence of Ca in the medium on NP uptake and uptake routes	Accumulation, depuration
Chen et al. (2016)	Fullerene (nC_{60}) NPs	*Daphnia magna*	Effect of subcellular distribution on NP uptake and transfer efficiency from algae to *Daphnia*	Bioaccumulation in *Scenedesmus obliquus* and *D. magna*
Botha et al. (2016)	Au NPs	*Daphnia magna*	Adsorption, uptake and distribution of NPs	Reproduction, imaging
Zhao and Wang (2012a)	Ag NPs, Ag^{+}	*Daphnia magna*	Influence of surface coating on acute toxicity	48 h LC_{50} (μg/L) – AgNPs, 1.1–28.7; Ag^{+}, 0.57–1.1 Bioaccumulation
Zhu et al. (2010a)	TiO_2 NPs	*Daphnia magna*	Toxicity and bioaccumulation	72 h EC_{50} (mg/L) – 1.62, 72 h LC_{50} (mg/L) – 2.02 Bioaccumulation, offspring production, reproductive defects, growth retardation
Blinova et al. (2010)	CuO and ZnO NPs, $CuSO_4$ and $ZnSO_4.7H_2O$	*Daphnia magna*	Toxicity of NPs in natural waters	48 h EC_{50} (mg/L) – CuO NPs, 2.6–24; ZnO NPs, 2.6–9.0; Cu^{2+}, 0.07–0.92; Zn^{2+}, 1.4–2.0
Baumann et al. (2014)	Iron Oxide NPs	*Daphnia magna*	Effects of coating on acute toxicity	96 h EC_{50} (mg/L) – 27.9–>100

Bozich et al. (2014)	Au NPs	*Daphnia magna*	Effects of surface chemistry, charge and ligand type on acute and chronic toxicity	Mortality, reproduction, body size
Bone et al. (2012)	Ag NPs	*Daphnia magna*	Effects of biotic and abiotic interactions on fate and toxicity of NPs	Mortality (percentage)
Seo et al. (2014)	Ag, CuO and ZnO NPs	*Daphnia magna*	Effects of physiochemical properties of media on toxicity	48 h EC_{50} (µg /L) – AgNPs, 4.94; CuO NPs, 980.0; ZnO NPs, 1,950.0
Salieri et al. (2015)	TiO_2 NPs	*Daphnia magna*	Influence of exposure mode on toxicity	96 h EC_{50} (mg/L) – 32–82
Seitz et al. (2015)	Ag NPs, $AgNO_3$	*Daphnia magna*	Effects of NP properties, media pH and dissolved organic matter on toxicity	48 h EC_{50} (µg /L) – AgNPs, 3.9–33.4, Ag^+: 1.7–3.0
Rosenfeldt et al. (2015)	TiO_2 NPs	*Daphnia magna*	Role of crystalline phase of NPs and OM on Cu toxicity	Acute toxicity of Cu
Kim et al. (2016)	Ag NPs	*Daphnia magna*	Influence of NP on As, Cu and Cd toxicity and bioaccumulation	Acute toxicity of Cu, Ad and Cd
Liu et al. (2015)	TiO_2 NPs and nanosheets	*Daphnia magna*	Influence of different facets of NPs on NP and cu toxicity	Enzyme activity, bioaccumulation of NPs and Cu
Simon et al. (2015)	MWCNT	*Daphnia magna*	Population level effects of MWCNTs in *D. magna* exposed to pulses of triclocarban	96 h EC_{50} (µg /L) – 12.65–33.16 Size, reproduction
Wang et al. (2014)	MWCNT, Ni	*Daphnia magna*	Effects of MWCNTs on the toxicity of Ni under different pH levels	Acute toxicity and bioaccumulation of Ni
Li et al. (2015a)	Ag NPs, $AgNO_3$	*Daphnia magna*	Toxicity at sublethal levels by NMR-based metabolomics	Metabolic profile

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Sakamoto et al. (2015)	Ag NPs, Ag^+	*Daphnia magna*, *Daphnia galeata*, *Bosmina longirostris*	Role of free silver in acute and chronic toxicity of NPs	48 h EC_{50} (µg/L) – AgNPs: *D. magna*, 2.43; *D. galeata*, 2.16; *B. longirostris*, 2.90 Ag^+: *D. magna*, 0.25; *D. galeata*, 0.16; *B. longirostris*, 0.22 Swimming behaviour, offspring production
Strigul et al. (2009)	B NPs, TiO_2 NPs, Al NPs	*Daphnia magna*	Toxicity of NPs	48 h LD_{50} (mg/L) – B NPs, 6.7; Al NPs, 7.48–107.58; TiO_2 NPs, ND
Hoecke et al. (2009)	CeO_2 NPs	*Daphnia magna*	Acute and chronic toxicity of NPs	21 days EC_{50} (mg/L) – survival, 36.9–71.1 Reproduction, 20.5–42.7
Ribeiro et al. (2014)	Ag NPs, $AgNO_3$	*Daphnia magna*	Acute toxicity	48 h LC_{50} (µg/L) – without food – AgNPs, 11.02; Ag^+, 1.05; with food AgNPs, 72.0; Ag^+, 3.38 24 h EC_{50} (feeding inhibition) (µg/L) – AgNPs, 13.64; Ag^+, 2.03 21 days EC_{50} (reproduction) (µg/L) – AgNPs, 1.0; Ag^+, 0.385
Lee et al. (2015)	Au NPs	*Daphnia magna*	Toxicity and trophic transfer of NPs from algae from *E. gracilis* and *C. reinhardtii*	48 h LC_{50} (mg/L) *Daphnia magna* – 0.02 Trophic transfer from *E. gracilis* and *C. reinhardtii* to *D. magna*
Bouldin et al. (2008)	Carboxyl NPs	*Ceriodaphnia dubia*	Toxicity and trophic transfer of NPs from algae *Raphidocelis subcapitata* to *Daphnia*	48 h LC_{50} (µg/L) – >110.0 Localization of NPs in *Daphnia*
Lovern et al. (2008)	Au NPs	*Daphnia magna*	Intake of NPs	Localization of NPs, intake and retention of NPs
Wray and Klaine (2015)	Au NPs	*Daphnia magna*	Influence of physicochemical properties on NP uptake and elimination	Uptake, elimination, bioaccumulation

Scanlan et al. (2013)	Ag NPs, $AgNO_3$	*Daphnia magna*	Acute toxicity and internalization of NPs	Gene expression, NP localization
Ribeiro et al. (2017)	Ag NPs, $AgNO_3$	*Daphnia magna*	Bioaccumulation from waterborne and dietary NPs	Uptake, elimination
Stanley et al. (2016)	MWCNTs	*Daphnia magna*	Acute toxicity and sublethal effects	48 h LC_{50} (mg/L) – 29.3, 48 h EC_{50} (swimming velocity) (mg/L), 6.7; 14 days LC_{50} (mg/L), 4.3; 14 days EC_{50} (reproduction) (mg/L), 5.0 Feeding rate
Lovern and Klaper (2006)	C_{60} and TiO_2 NPs	*Daphnia magna*	Acute toxicity, swimming behaviour	48 h LC_{50} (mg/L) – filtered TiO_2, 5.5; unfiltered TiO_2, ND 48 h LC_{50} (μg/L) – filtered C_{60}, 460; 48 h LC_{50} (mg/L) – unfiltered C_{60}, 7.9 Swimming behaviour
Pokhrel and Dubey (2012)	Ag NPs	*Daphnia magna*	Swimming behaviour in the presence of *Anax junius*	Survival, reproduction, swimming behaviour
Artells et al. (2013)	CeO_2 NPs	*Daphnia similis*, *Daphnia pulex*	Effects of NPs on acute toxicity and swimming behaviour	48 h LC_{50} (mg/L) – *D. similis*, 0.26; *D. pulex*, 91.79 Swimming velocity, localization of NPs
Lovern et al. (2007b)	TiO_2 and C_{60} NPs, $C_{60}H_xC_{70}H_x$	*Daphnia magna*	Behavioural and physiological changes	Hopping frequency, feeding appendage and postabdominal curling movement, heart rate
Noss et al. (2013)	TiO_2 NPs	*Daphnia magna*	Effects of NPs on swimming behaviour	Swimming behaviour and velocity
Qin et al. (2015)	AgNPs	*Daphnia carinata*	Impact of predator cues on responses to NPs	48 h LC_{50} (μg/L) – predator absent, 1.75; predator present, 4.61 Enzyme activity (SOD, CAT), offspring production
Bacchetta et al. (2018)	CNPs and CNCs	*Daphnia magna*	Influence of shape on acute toxicity, uptake and morphological alterations	48 h LC_{35} (mg/L) – CNP, 50; 48 h LC_{20} (mg/L) – CNC, 50 Uptake, microscopic and histological analysis

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Cui et al. (2017)	Ag NPs, Ag NWs, Ag PLs, $AgNO_3$	*Daphnia magna*, *Daphnia galeata*	Dimension-dependent acute toxicity	48 h LC_{50} (μg/L) – AgPLs: *D. magna*, 39.42; *D. galeata*, 34.90; AgNPs, *D. galeata*, 36.51; AgNWs (10 nm), *D. galeata*, 214.19; AgNWs (20 nm), *D. galeata*, 149.26; Ag^+, *D. magna*, 3.09; *D. galeata*, 1.94
Lv et al. (2017)	C_{60}	*Daphnia magna*	Mechanisms underlying the acute toxicity of NPs	72 h EC_{50} (mg/L) – 14.9, 72 h LC_{50} (mg/L) – 16.3 Feeding rate, digestive enzyme activities, morphological changes, oxidative stress
Das et al. (2013)	AgNPs, capped and uncapped TiO_2 NPs	*Daphnia magna*	Acute and chronic toxicity	48 h LC_{50} (μg/L) – AgNPs, 2.75 48 h LC_{50} (mg/L) – capped TiO_2 NPs, ND, uncapped TiO_2 NPs, 7.75
Becaro et al. (2015)	AgNPs	*Daphnia similis*	Toxicity of NPs	Acute toxicity 48 h EC_{50} (μg/L) – 0.26
Bacchetta et al. (2016)	ZnO NPs, $ZnSO_4$	*Daphnia magna*	Role of Zn^{2+} in Zn NP cytotoxicity	Acute toxicity, cytotoxicity 48 h EC_{50} (mg/L) – Zn^{2+}, 0.99; ZnO NPs, ND 48 h LC_{50} (mg/L) – Zn^{2+}, 1.37; ZnO NPs, ND
Conine and Frost (2017)	AgNPs	*Daphnia magna*	Effects of algal particles and animal nutrition on toxicity	Acute toxicity, growth 48 h LC_{50} (μg/L) – algae fed (continuous), 17.0; nonfed, 5.2 48 h LC_{50} (μg/L) – algae fed (2 h intervals), 3.9; nonfed, 4.5
Cupi et al. (2016a)	Zn, TiO_2, AgNPs	*Daphnia magna*	Influence of pH and media composition on toxicity of NPs	48 h EC_{50} (μg/L) – AgNPs: US-EPA medium, 51.0; M7 medium, 66.0 48 h EC_{50} (mg/L) – TiO_2NPs: US-EPA medium, 14.0; M7 medium, >100.0 48 h EC_{50} (mg/L) – ZnONPs: US-EPA medium, 0.047; M7 medium, 4.9

Lu et al. (2017)	Cu NPs, Cr NPs	*Daphnia magna*	Acute and chronic toxicity of NPs	Acute toxicity, enzyme activity, food intake
Lv et al. (2017)	C_{60} NPs, Si NPs	*Daphnia magna*	Mechanisms of NP toxicity	Acute toxicity, accumulation, feeding rate, enzyme activity, oxidative stress 48 h EC_{50} (mg/L) – 28.5, 72 h EC_{50} (mg/L) – 16.3
Vijayakumar et al. (2016)	ZnO NPs	*Ceriodaphnia cornuta* *Moina micrura*	Protective effects of chitosan against ZnO NP toxicity	Bioaccumulation, behavioural effects, mechanical damage
Wu et al. (2017a)	CuO NPs	*Daphnia magna*	Effects of waterborne and dietary exposure of NP	Chronic toxicity, bioaccumulation
Xiao et al. (2018)	Cu NPs	*Daphnia magna*	Impact of water chemistry on particle-specific toxicity of NPs	Acute toxicity 48 h LC_{50} (mg/L) – static exposure and no DOC, 0.024–0.076 48 h LC_{50} (mg/L) – static exposure and DOC, 0.5–3.5 48 h LC_{50} (mg/L) – dynamic exposure and no DOC, 0.02–0.15 48 h LC_{50} (mg/L) – dynamic exposure and no DOC, 0.3–2.1
Zhang et al. (2016b)	Fe_xO_y NPs	*Daphnia magna*	Toxicity of NPs	72 h EC_{50} (mg/L): 20.8–65.9
Sá-Pereira et al. (2018)	TiO_2 NPs	*Daphnia magna*	Protein profiling as a biomarker for NP toxicity	Acute toxicity, uptake, distribution, protein profiling 48 h EC_{50} (mg/L) – 30.0
Brun et al. (2017)	Polystyrene NPs	*Daphnia magna*	Brood pouch-mediated NP uptake	Uptake
Gökçe et al. (2018)	TiO_2, ZnO NPs	*Daphnia magna*	Effects of NPs on population dynamics	Acute toxicity, chronic toxicity 96 h LC_{50} (mg/L) – TiO_2NPs, 1.8; ZnONPs, 0.7 21 days LC_{50} (mg/L) – TiO_2NPs, 1.0; ZnONPs, ND

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Gonçalves et al. (2018)	ZnO NPs	*Daphnia magna*	Comparative assessment of toxicity	Acute toxicity, chronic toxicity 48 h EC_{50} (μM) – 27.1–34.5
Heinlaan et al. (2017)	Co_3O_4 NPs Mn_2O_3 NPs	*Daphnia magna*	Body burden of NPs	Acute toxicity, ROS generation, feeding behaviour, bioaccumulation 48 h LC_{50} (mg/L) – Co_3O_4 NPs, >100.0; Mn_2O_3 NPs, >100.0
Hu et al. (2017)	Ag NPs, $AgNO_3$	*Daphnia magna*	Toxicity of NPs and Ag^+	Acute toxicity, chronic toxicity, bioaccumulation, biomagnification
Jensen et al. (2017)	Au NPs	*Daphnia magna*	Uptake of NPs and related artefacts	Microscopic analysis
Kim et al. (2017)	CuO and ZnO NPs $CuSO_4$, $ZnSO_4$	*Daphnia magna*	Time-dependent toxicity of NPs	48 h EC_{50} (μg/L) – CuO NPs, 1,090.0; Cu^{2+}, 56.0 ZnO NPs, 1,400.0; Zn^{2+}, 800.0
Liu et al. (2017)	Graphene oxide NPs	*Daphnia magna*	Transgenerational effects of NPs	Mortality, body size, time to first brood
Pacheco et al. (2018)	Au NPs	*Daphnia magna*	Toxicological interactions by chronic exposure to NPs and microplastics	Mortality, growth inhibition, number of broods, total offspring
Tan and Wang (2017)	TiO_2	*Daphnia magna*	Influence of NPs on dietary metal uptake	Metal assimilation
Bhuvaneshwari et al. (2018a)	ZnO NPs, ZnO	*Ceriodaphnia dubia*	Trophic transfer of NPs from alga *Scenedesmus obliquus*	Toxicity, uptake, algae cell viability
Bhuvaneshwari et al. (2017)	nZVI	*Ceriodaphnia dubia*	Trophic transfer of NPs from alga *Scenedesmus* sp.	Acute toxicity, uptake, enzyme activity 48 h EC_{50} (mM) – 14.22–15.64
Gaiser et al. (2011)	AgNPs, CeO_2 NPs	*Daphnia magna*	Acute and chronic toxicity	Acute toxicity (percentage), moulting and growth inhibition, mortality, feeding rate, swimming velocity

Blinova et al. (2017)	Fe_3O_4 NPs, Fe_3O_4	*Daphnia magna*	Toxicity of NPs	Acute toxicity, reproduction 48 h EC_{50} (μg/L) – Fe_3O_4 NPs, > 100, Fe_3O_4, > 100
Blinova et al. (2013)	Ag NPs, $AgNO_3$	*Daphnia magna*	Toxicity of NPs in different media	Acute toxicity, reproduction 48 h EC_{50} (μg/L) – AgNPs, 40.2–236.3; Ag^+, 2.2–12.9
Khan et al. (2014)	Au NPs	*Daphnia magna*	Bioaccumulation and epithelial uptake	Uptake, elimination, internalization
Muna et al. (2017)	CuO NPs, $CuSO_4$	*Daphnia magna*	Acute toxicity and effect of medium on Cu body burden	Percentage mortality, body burden, body length, dry weight
Xiao et al. (2015)	Cu NPs and ZnO NPs	*Daphnia magna*	Toxicity and accumulation of NPs and dissolved ions	Acute toxicity, accumulation, 48 h LC_{50} (mg/L) – CuNPs, 0.093; Cu^{2+}, 0.028 48 h LC_{50} (mg/L) – ZnONPs, 0.99; Zn^{2+}, 1.01
Akhil and Sudheer Khan (2017)	ZnO NPs	*Daphnia magna*	Effects of humic acid on toxicity of NPs	Percentage mortality, accumulation, morphology changes

ACP acid phosphatase, *CAT* catalase, *CNC* cubic-shaped carbon nanoparticle, *CNP* carbon nanopowder, *CSP* caspase, *DOM* dissolved organic matter, *EC* effective concentration, *EPS* extra polymeric substances, *GPx* glutathione peroxidase, *GST* glutathione S-transferase, *HSP* heat shock protein, *IC* inhibitory concentration, *LD* lethal dose, *LDH* lactate dehydrogenase, *LPO* lipid peroxidation, *MBC* minimum bactericidal concentration, *MIC* minimum inhibitory concentration, *MT* metallothionein, *MWCNT* multi-walled carbon nanotube, *ND* not defined, *NT* not tested, *nZVI* nano zero-valent iron, *OM* organic matter, *QD* quantum dots, *ROS* reactive oxygen species, *SOD* superoxide dismutase strains of bacteria (Kvitek et al. 2008; Lok et al. 2007; Bradford et al. 2009). They demonstrated that aggregation mitigates the potential toxicity of NPs. It is generally accepted that aggregation reduces toxicity to aquatic organisms. Low environmental concentrations lead to less aggregation, and hence, unlike traditional toxicants, it is possible that low concentrations are more toxic than higher concentrations with time (Tiede et al. 2009). Lok et al. (2007) observed higher aggregation in high salt media resulting in loss of antibacterial activities of AgNPs to *Escherichia coli*. Fernandes et al. (2006) suggested that NPs would disaggregate in the presence of household or industrial detergents. Limbach et al. (2008) found that protein breakdown products and surfactants in wastewater change the zeta potential of NPs causing stabilization. Oleszczuk et al. (2015) found that certain surfactants decrease the toxicity of TiO_2 NPs to *Daphnia magna*, and they hypothesized that the surfactants increase the aggregation of NP, reducing the bioavailability to daphnids. In contrast, there are instances where higher toxicity was observed with the aggregation of NPs. In one such study, Kashiwada (2006) found increased salinity caused higher toxicity by NPs to medaka eggs though NPs showed higher aggregation in saline media.

5.6 Influence of NPs on Other Contaminant Effects

There is also evidence that NPs influence the toxicity of other contaminants (Deng et al. 2017) and that influence is mitigated by the characteristics of the aquatic environment, such as the presence of organic matter. Fan et al. (2016) observed a reduction in Cu accumulation in *Daphnia magna* in the presence of TiO_2 NPs, but humic acids decreased that reducing effect. In a similar study, Rosenfeldt et al. (2015) observed a twofold decrease in Cu toxicity to *Daphnia magna* in the presence of TiO_2 NPs in the medium. They attributed this to the adsorption of Cu to NPs leading to a reduction in the bioavailability of Cu as the cause of toxicity reduction. In another study, Liu et al. (2015) found that TiO_2 NPs increased Cu accumulation in *Daphnia magna*, while TiO_2 nanosheets decreased Cu accumulation. Interestingly, the presence of Cu^{2+} in the medium caused agglomeration and sedimentation of TiO_2 NPs causing decreased NP bioaccumulation. Hartmann et al. (2010) investigated the toxicity of cadmium (Cd^{2+}) ions in the presence of TiO_2 NPs. Toxicity from Cd was reduced in the presence of TiO_2 NPs compared to Cd alone due to the decreased bioavailability of Cd resulting from the sorption of Cd to NPs. Kim et al. (2016) observed decreased bioaccumulation of Cu, while both acute toxicity and bioaccumulation of Cd increased in the presence of citrate-coated Ag NPs after 24 h exposure of *Daphnia magna*. Simon et al. (2015) observed a reduction in the toxicity of triclocarban to *Daphnia magna* in the presence of multiwalled carbon nanotubes (MWCNT). In contrast, Wang et al. (2014) observed increased toxicity of Nickel (Ni) to *Daphnia magna* in the presence of hydroxylated MWCNTs. They found that this was due to the uptake of Ni-adsorbed NPs. NPs could also influence the multixenobiotic resistance (MXR) of aquatic organisms. Georgantzopoulou et al. (2016) reported similar findings when they exposed *Daphnia magna* to Ag NPs.

Zhang et al. (2017) evaluated the joint toxicities of TiO_2 NPs with four different organochlorine contaminants (OC) towards the alga *Chlorella pyrenoidosa*. The results indicated that there were synergistic, antagonistic and additive effects between TiO_2 NPs and OCs on the alga. Similarly, Yu et al. (2018) reported synergistic and antagonistic effects of TiO_2, SiO_2 and Ag NPs and CdTe/CdS QDs on Cd^{2+} toxicity towards alga *Chlamydomonas reinhardtii*. Li et al. (2017a) also observed increased toxicity of Cd^{2+} ions to *Daphnia magna* in the presence of TiO_2 NPs. These studies show that the NPs influence the effects of existing environmental contaminants on organisms and therefore highlight the importance of systematic studies of toxicological effects of NPs due to their own effects plus their influence on other contaminant effects in environmental risk assessment.

6 The Toxicity of NPs to Freshwater Organisms

Human and industrial wastes enter waterways, and hence, it is inevitable that NPs also end up in waterbodies due to the mass use of products containing NPs (Daughton 2004; Moore 2002). Ingestion and inhalation are considered as the major routes of NP uptake by terrestrial organisms (Dowling 2004; Warheit 2004; Moore and Allen 2002). In addition, there might be other routes of exposure in aquatic organisms, such as uptake through gills and surface epithelia (Moore 2006; Oberdörster 2004). Once internalized into invertebrates, the gut epithelium, the cellular immune system and the hepatopancreas are likely targets for reactive mechanisms, while the liver is a probable target in fish (Moore 1990; Moore et al. 2004). Eukaryotes have developed advanced mechanisms, endocytosis (100 nm or less) and phagocytosis (100–10,000 nm) for cellular internalization of particles (Na et al. 2003; Pelkmans and Helenius 2002; Moore 2006). Contamination of waterways is not only harmful for aquatic biota but also to terrestrial organisms including humans by direct or indirect exposure to NPs via direct ingestion, inhalation of water aerosols, skin contact or food (Daughton 2004). The ability of water treatment plants to treat NPs is still doubtful, and in particular, uncharged or anionic NPs could pass through into the sewage effluent. Also, some studies showed that there is a potential for NPs to harm important bacteria in sewage treatment plants (Choi et al. 2008; Kwak et al. 2001; Ghafari et al. 2008; Nyberg et al. 2008) which may put freshwater aqueous ecosystems under threat from other contaminants.

Metallic NPs have the potential to dissolve and release ions into the aquatic media. Some researchers claim that these liberated ions are the only cause of toxicity to aquatic organisms, while other studies indicate that NPs are the major cause of toxicity (Li et al. 2017b; Abramenko et al. 2018). This debate is still prevalent though the effects of metallic NPs have been intensively studied in the past decades (Wang et al. 2016a). In general, the toxicity of NPs is compared to the toxicity of the counterpart bulk material, usually metal salts, to test this hypothesis (Djurišić et al. 2015). Evaluation of acute and chronic toxicity and mechanism of toxicity is crucial in environmental risk assessment of NPs in protecting the organisms and setting up guidelines. A considerable number of studies have been undertaken to date in assessing the toxicity of different NPs and the sensitivity of organisms to NPs and on the mechanisms of toxicity.

6.1 The Toxicity of NPs to Bacteria

Toxicity to bacteria is an area of concern due to the possibility that important biogeochemical processes and other organisms in the aquatic environment may be affected detrimentally by the release of NPs to aquatic systems (Neal 2008). Several studies have evaluated the effects on non-pathogenic or environmentally relevant bacteria (Bondarenko et al. 2013; Hwang et al. 2008; Strigul et al. 2009; Jiang et al. 2009; Bradford et al. 2009; Baek and An 2011; Choi et al. 2008; Lopes et al. 2012; Heinlaan et al. 2008; von Moos and Slaveykova 2014). Also, due to the antimicrobial effects of certain NPs, the effects on pathogenic bacteria have been heavily studied due to the potential applications of NPs in medical and healthcare products. Studies on silver dominate these as a result of the excellent antimicrobial properties of Ag NPs (Marambio-Jones and Hoek 2010; Rai et al. 2009; Atiyeh et al. 2007; Durán et al. 2010; Fabrega et al. 2009).

6.1.1 Acute and Chronic Toxicity

More than 60% of the studies have looked into the acute toxicity of NPs to bacteria, while approximately 40% of studies have assessed other cellular effects such as membrane damage, morphological changes, oxidative stress, uptake, internalizations, enzyme activity, protein damage and DNA damage (Table 2). Currently, there are no standard bacterial species recommended for toxicity assessments. However, *Escherichia coli* has been the most preferred organism since 45% of studies have used it to assess the effects of NPs on bacteria (Fig. 2a). More than 40% of studies have assessed the effects of Ag NPs on bacteria (Fig. 2d) possibly due to the increased antimicrobial properties of Ag compared to other metals which is a huge concern in environmental toxicity and also due to the importance of Ag NPs in the medical field. Additionally, ZnO and TiO_2 NPs which have antimicrobial properties have been used in 15% and 11% of studies, respectively. Most studies have focused on single species, while there are a few studies at the community level (Miao et al. 2018; Colman et al. 2012; Kumar et al. 2012; Pradhan et al. 2011; Ge et al. 2013; Frenk et al. 2013; Li et al. 2016a; Asadishad et al. 2018; Ma et al. 2015) which report on changes in microbial biomass, community activity, community composition, microbial diversity, community richness, genome and structural diversity. Risk assessment of the effects of NPs on bacterial communities is particularly important due to the detrimental effects on treatment processes.

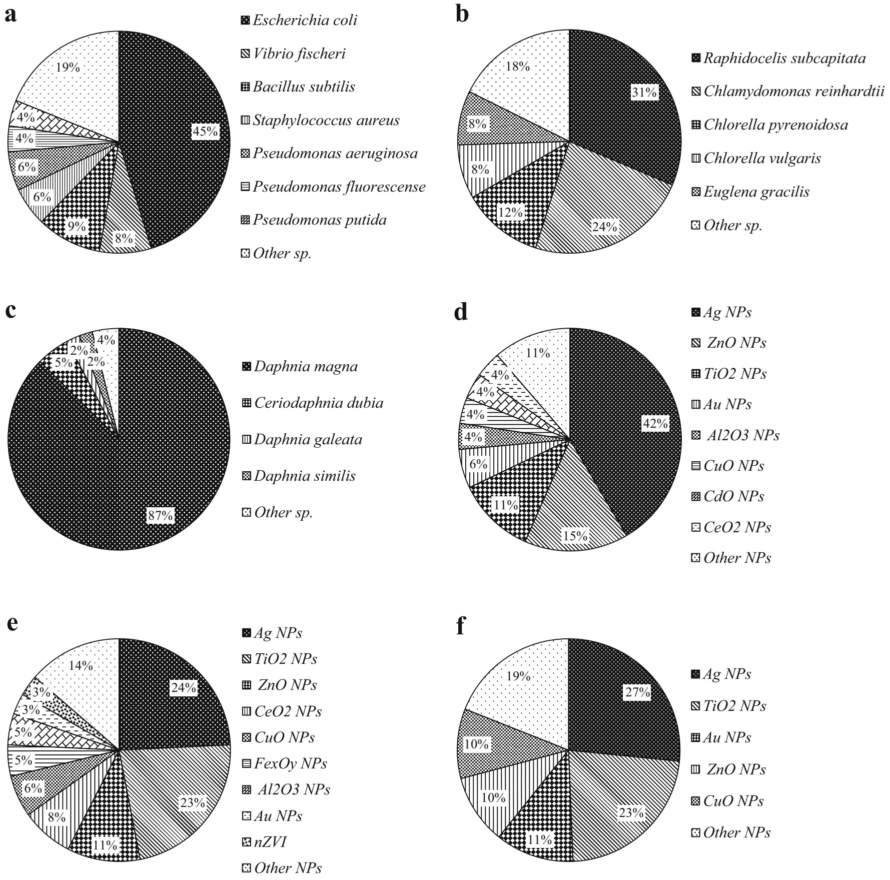

Fig. 2 (**a**) Percentage use of different (**a**) bacteria (**b**) algae and (**c**) *Daphnia* species in NP studies; percentage use of different types of NPs to study effects on (**d**) bacteria, (**e**) algae and (**f**) daphnia in NP studies (the data are based on the previous publications referred in this review)

6.1.2 Toxicity from NPs or Ions

The toxicity of NPs is widely attributed to the dissolved ions rather than NPs themselves (Jiang et al. 2009). Fabrega et al. (2009) investigated the impact of Ag NPs on *Pseudomonas fluorescens* in the presence of river humic acids (RHA) at different pH values. They found that the bacterial growth was entirely inhibited at 2 mg/L under all conditions and adversely affected at 0.2 mg/L concentration under some conditions. A similar toxicity was observed in the absence of RHA at pH 9 only. With these results, the authors concluded that there was a specific nanoparticle effect which could not be explained by just dissolved Ag^{+}. Jin et al. (2010) studied the influence of inorganic aquatic chemistry on Ag NP stability and bacterial viability. They found that the antibacterial activity of Ag NPs was much lower than Ag^+, when compared on the basis of mass added. Choi et al. (2010) found Ag^+ was more toxic to *Escherichia coli* biofilms than Ag NPs with minimum bactericidal concentrations of 1.2 mg/L and 10 mg/L, respectively. Ag NPs were highly aggregated in the presence of biofilms causing increased size of NPs by a factor of 40 causing reduced toxicity. The aggregation may be due to a change of ionic strength in the medium caused by biofilms and interactions with various complexing agents produced by biofilms. Xiu et al. (2012) suggested that the antimicrobial effects of AgNPs are primarily from Ag^+ released from NPs rather than the morphological properties of the particles after exposing *Escherichia coli* to AgNPs with different coatings and sizes. Dorobantu et al. (2015) observed no damage to membranes of the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* when exposed to Ag NPs. However, though AgNPs were toxic to bacteria, only $AgNO_3$ caused membrane damage, and therefore, they decided that only Ag^+ ions were responsible for biological action against microorganisms. However, Lok et al. (2006) claimed that Ag NPs were more toxic to *Escherichia coli* than Ag^+ though they found the mode of action of NPs was similar to Ag^+. The effective concentrations for toxicity of AgNPs and Ag^+ were at nanomolar and micromolar level, respectively. Similarly, Baek and An (2011) attributed the toxicities to particle-specific toxicity rather than ionic effects when *Escherichia coli*, *Bacillus subtilis* and *Streptococcus aureus* were exposed to metal oxide NPs. Most studies on metallic NP toxicity support the hypothesis that the toxicity of NPs to bacteria is primarily caused by the ions released from NPs and the toxicity of NPs is less than their bulk form. However, as above, there are instances where authors conclude that the NPs are more toxic than the ions.

6.1.3 Mechanisms of Toxicity

Sondi and Salopek-Sondi (2004) found that Ag NP-treated *Escherichia coli* cells were damaged, while Ag NPs were found accumulated in the bacterial membrane. They concluded that such changes in morphology would significantly increase the permeability of the membrane resulting in death of the cell. Jiang et al. (2009) suggested that the toxicity to *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* was affected by the attachment of NPs to the surface of bacteria upon exposure to Al_2O_3, SiO_2 and ZnO NPs. Thill et al. (2006) found that CeO_2 NPs come into contact with *Escherichia coli* cells to cause toxicity. They observed a strong absorption of NPs to the surface of bacterial cells and reduction of NPs which was correlated with the cytotoxicity. Su et al. (2009) found that the nanohybrids made up of Ag and silica were adhered to the surface of the bacteria cells. Subsequent toxicity studies revealed that the toxicity was related to the cell death caused by loss of membrane integrity due to the formation of ROS. Lok et al. (2006) observed that AgNPs destabilized the outer membrane, collapsed the plasma membrane potential and depleted the levels of intracellular ATP when *Escherichia coli* was exposed to AgNPs and Ag^+. Li et al. (2010b) observed that NPs destroyed the permeability of the membrane of *Escherichia coli* and some membrane enzymes were depressed upon exposure to AgNPs. Brayner et al. (2006) observed the membrane disorganization of *Escherichia coli* cells as a result of exposure to ZnO NPs which led to NP accumulation in the bacterial membrane and internalization as verified by TEM images. Feng et al. (2015) studied the toxicity of cationic and anionic Au NPs to the Gram-negative bacteria *Shewanella oneidensis* and the Gram-positive bacteria *Bacillus subtilis*. Au NPs coated with cationic polyelectrolyte PAH were associated most with the bacterial surfaces and caused greatest membrane damage causing highest toxicity. Hossain and Mukherjee (2012) observed morphological changes of *Escherichia coli* cells to the filamentous form followed by filamentation-associated clumping with increased CdO NP concentrations. Also, the cell surface was severely damaged, and cell division proteins were affected upon exposure to NPs. The researchers attributed intracellular oxidative stress as a cause of these changes. Kumar et al. (2011) assessed the toxicity of TiO_2 and ZnO NPs to *Escherichia coli* and observed that the exposure caused oxidative stress leading to genotoxicity and cytotoxicity. Both NPs caused induction of reactive oxygen species (ROS) and DNA damage. Genotoxic effects were also reported by Lopes et al. (2012) when bacterium *Salmonella typhimurium* was exposed to sodium dodecyl sulphate and didodecyl dimethylammonium bromide NPs. The mechanisms of toxicity of NPs to bacteria are complex although membrane damage by the production of ROS and physical damage by NPs have attracted most attention (Hwang et al. 2008). Membrane damage causes severe effects including the inability to properly regulate transportation through the plasma membrane. Attachment of NPs onto the surface of the bacteria is emphasized, while accumulation of NPs in the membrane has also been observed. In addition, some researchers have reported additional adverse effects such as DNA and protein damage and enzyme inactivation (Kumar et al. 2011).

6.2 *The Toxicity of NPs to Freshwater Algae*

Freshwater microalgae are primary producers in the environment and hence carry out a pivotal role in the food chain. Therefore, any abnormal structural or population changes of the organism will affect higher organisms which directly or indirectly feed on them (Nyholm and Peterson 1997). This highlights the importance of assessing any causes for such changes and the effects due to such causes. Their main habitats, freshwater bodies, are always under threat of chemicals released by households and industries. Also, toxicity tests with algae are recommended internationally by organizations as a source of basic information to understand environmental hazards (OECD 2011; ASTM 2012).

6.2.1 Acute and Chronic Toxicity

Most acute toxicity tests have been performed over 72 h (>40%), a time recommended for algae by the OECD; 18 and 15% of studies report 96 and 48 h toxicity tests, respectively. Several methodologies have been used to measure the acute toxicity of NPs to algae, although growth inhibition has been predominantly used. Growth inhibition can be evaluated using several techniques and methodologies including cell counting, ATP measurement, optical density measurement and chlorophyll content. Other endpoints assessed include membrane damage, oxidative stress, uptake, accumulation, cell morphology, mitochondrial dysfunction, cellular growth and metabolic profiling (Table 2). *Raphidocelis subcapitata* (31%), *Chlamydomonas reinhardtii* (24%), *Chlorella pyrenoidosa* (12%), *Chlorella vulgaris* (8%) and *Euglena gracilis* (8%) have been the most preferred species in NP studies (Fig. 2b). Most studies on algae have assessed the effects of Ag NPs (24%) followed by TiO_2 (23%), ZnO (11%) and CeO_2 (8%) (Fig. 2e).

6.2.2 Toxicity from NPs or Ions

Lee and An (2013) exposed the alga *Raphidocelis subcapitata* to ZnO NPs and concluded that the observed toxicity was almost entirely caused by the dissolved free Zn^{2+} ions. Li et al. (2015b) exposed the alga *Euglena gracilis* to AgNPs in the presence and absence of cysteine which is a strong silver ligand. The effects of NPs on photosynthesis decreased in the presence of cysteine suggesting that the effects of AgNPs were mediated by the dissolved Ag^+. Müller et al. (2016) exposed the alga *Chlamydomonas reinhardtii* to Cu NPs and corresponding dissolved fraction of Cu^{2+} ions and observed that the toxicity was similar. Also, when the same experiments were performed in the presence of EDTA which is a strong metal ion chelator, the toxicity of both NPs and Cu^{2+} decreased. These results indicated that the toxicity of Cu NPs arises mostly from the dissolved fraction of Cu^{2+} ions. Despite Zn^{2+} being toxic, Iswarya et al. (2017) saw a reduction in toxicity of Au NPs to the alga *Scenedesmus obliquus* with the addition of Zn^{2+} ions to the medium. Navarro et al. (2008) examined the short-term toxicity of Ag^+ and Ag NPs to photosynthesis in *Chlamydomonas reinhardtii*. They found that the toxicity of Ag^+ in terms of EC_{50} was about 18 times higher compared to Ag NPs. However, the observed toxicity by Ag NPs could not be fully explained relative to the Ag^+ measured in the Ag NP suspension, and the toxicity of Ag NPs appeared to be much higher when compared as a function of Ag^+ concentration. When the alga *Raphidocelis subcapitata* was exposed to Ag NPs, the toxicity from 2 to 48 h did not increase at the corresponding ionic release rate. Also, the addition of cysteine in equimolar concentrations to silver did not eliminate toxicity. Therefore, Sørensen and Baun (2015) suggested that the dissolution cannot be the only process which contributes to the algal toxicity.

6.2.3 Mechanisms of Toxicity

Angel et al. (2015) found that the presence of dissolved organic carbon (DOC) reduced the toxicity of NPs to *Raphidocelis subcapitata*. The presence of DOC substantially reduced the sorption of NPs to the algal cells, and therefore, they concluded that sorption was the cause of the toxic mechanism. However, though they stopped ROS generation by using UV filters, the toxicity observed was still similar to the levels when ROS was present. They concluded, in contrast to many other findings, that the toxicity was not caused by localized exposure to ROS. Rogers et al. (2010) assessed the effects of CeO_2 NPs and CeO_2 macro-particles (<5 μm) to *Raphidocelis subcapitata*. They concluded that the effects were due to membrane damage of cells by lipid peroxidation caused by the production of hydroxyl radicals. Sørensen et al. (2016a) observed growth inhibition in *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii* following exposure to Pt NPs and attributed toxicity to oxidative stress caused by ROS production. Higher body burden of NPs was found in *Raphidocelis subcapitata*, possibly due to favoured binding of NPs to the polysaccharide-rich cell wall. Interestingly, the accumulation of intracellular ROS levels was comparatively less in *Raphidocelis subcapitata* though it was the most sensitive species. Membrane damage was not observed in both algae species. Bhuvaneshwari et al. (2015) noted significant toxicity correlated with intracellular ROS generation in the alga *Scenedesmus obliquus* when exposed to ZnO NPs. Substantial membrane damage and a significantly enhanced lactate dehydrogenase (LDH) enzyme release into the medium were also observed. Nogueira et al. (2015) exposed the alga *Raphidocelis subcapitata* to grapheme oxide NPs and observed increased ROS production and membrane damage in algal cells which was suggested as the cause of observed growth inhibition. Oukarroum et al. (2017) suggested that several cellular alterations, such as the inhibition in cellular division processes, the deterioration of photosynthetic apparatus and the generation of ROS, caused the cell viability in alga *Chlorella vulgaris* to decrease when exposed to NiO NPs. Qian et al. (2016) saw increased ROS production and lipid peroxidation in the cyanobacterium *Microcystis aeruginosa* when exposed to AgNPs. They also showed that ROS inhibited SOD and POD transcription and expression. In contrast, ROS production was mediated by the induction of SOD and POD activity and the expression of the antioxidant enzyme glutamine synthetase in *Chlorella vulgaris* at same exposure scenario. Dauda et al. (2017) reported a significant increase in GST and peroxidase (POD) enzymes in *Chlorella vulgaris* upon exposure to TiO_2 NPs.

Miao et al. (2010) studied the behaviour and toxicity of Ag NPs to the freshwater alga *Ochromonas danica* to determine whether there were any other mechanisms in algal toxicity other than due to the Ag^+ liberated outside the cells. They demonstrated that the Ag NPs were taken inside the cells where they exerted their toxic effects. However, they did not discuss how the NPs exerted toxic effects inside the cells. Dorobantu et al. (2015) observed that Ag NPs caused membrane damage in the alga *Euglena gracilis*, but not in *Chlorella protothecoides*. In addition Ag NPs caused morphological changes in *Euglena gracilis* altering the shapes from spindle to round with the cells showing increased diameter. Ag^+ ions from $AgNO_3$ caused membrane damage in both algae causing intracellular material leaking out of the cells resulting in a depressed volume of cells. Hartmann et al. (2010) evaluated the toxicity of TiO_2 NPs to the alga *Pseudokirchneriella subcapitata* and suggested that the observed decreased growth rate could be caused by the adhesion of NPs onto the algal cell surfaces. Ozkaleli and Erdem (2018) observed lipid peroxidation of the alga *Raphidocelis subcapitata* cell membrane upon exposure to TiO_2 NPs, resulting in the deformation of the membrane structure. Li et al. (2015b) reported a doubling of cell volume when the alga *Euglena gracilis* was exposed to AgNPs. They suggested that the enlargement was a result of unspecific interactions of Ag^+ ions released from AgNPs with the thiol groups of glycoproteins in the pellicle. However, they did not observe any internalization of NPs into the algal cells. Ji et al. (2011) excluded the effects of ions or shading for the observed toxicity of ZnO NPs to the alga *Chlorella* sp. but concluded that the toxicity was caused by entrapping and wrapping by the NPs.

Zhou et al. (2016) observed increased toxicity and cell internalization of Ag NPs in the absence of EPS compared to the presence of EPS. EPS could bind both NPs and Ag^+ reducing the internalization and toxicity. Stevenson et al. (2013) investigated the toxicity of Ag NPs to the populations of the alga *Chlamydomonas reinhardtii* at different phases of batch culture and found that the toxicity was highest for the cultures at early phases in growth. Dynamic process modelling, incorporating algal growth rate, dissolution, bioaccumulation and extracellular DOC production, revealed that the DOC was a strong factor mitigating the toxicity of NPs. Kadukova (2016) exposed the alga *Parachlorella kessleri* to $AgNO_3$ and noted that the Ag^+ was removed from the medium by biosorption by algae. Interestingly, the majority of Ag was released back into the medium in the next 14 days, while the algal cells had formed Ag NPs inside, within that period. Those NPs were comparatively less toxic against algal cells than Ag^+ ions at the same Ag concentrations. The surface charge of the algae cells and the NPs is a major determinant in causing toxicity. There is a high tendency for negatively charged NPs to bind on the positively charged algal surfaces. Also, once bound to the cells, the charge density of the NPs decreases which favours the adsorption of more NPs resulting in large clusters. It is widely accepted that the sorption of NPs on the algal surfaces facilitates the localized exposure to ROS resulting in oxidative damage to the cell membranes. However, sorption of NPs might cause toxicity even without the production of ROS. Certain other effects were also reported including adverse effects to morphology, cell division, gene expression and even physical effects. Also, algae have their own mechanisms to mitigate the NP toxicity into certain extent.

6.3 The Toxicity of NPs to Daphnia

Invertebrates are the most widely distributed living macroorganisms on earth. Their presence in almost all ecological niches, fast and high rate of reproduction, short life span and relatively high sensitivity to pollutants make them excellent candidates for ecotoxicological studies. Among invertebrates, *Daphnia* sp. is the first choice for standard toxicity tests among control agencies (Jonczyk and Gilron 2005). Except in extreme environments, this organism is present in all aquatic habitats and possesses all the above-mentioned positive characteristics for standard tests (Cattaneo et al. 2009). Daphnids exert strong grazing effects and support the aquatic food web. They feed on several sources like bacteria, algae, other invertebrates and plants and enter the trophic chain at intermediary level by being a preferential prey for larger organisms like fish, birds and humans. This also makes them a possible important linkage for passing contaminants through the food chain which should be studied for any such contaminants which are suspected of being capable of bioconcentration and bioaccumulation (Zhu et al. 2010b).

6.3.1 Acute and Chronic Toxicity

The *Daphnia* spp. 48 h acute test is one of the most widely used aquatic standardized tests, and this is reflected in NP toxicity studies. However, there are some suggestions to improve the sensitivity by prolonged exposure up to 72 h or the 48 h test duration followed by a 24 h recovery period (Novak et al. 2018). LC_{50} and EC_{50} are the most common endpoints used, while other effects such as uptake, accumulation, feeding rate, reproduction, enzyme activity, oxidative stress and morphological changes are reported (Table 2). More than 87% of studies have used *Daphnia magna* as the test species (Fig. 2c) possibly as a result of its inclusion in regulatory chemical testing, guidelines and international standards (Baun et al. 2008). The majority of studies have tested against Ag NPs (27%) followed by TiO_2 (23%), Au NPs (11%) ZnO NPs (10%) and CuO NPs (10%) (Fig. 2f).

6.3.2 Toxicity from NPs or Ions

There are different views on whether NPs or liberated ions from NPs cause the toxicity to *Daphnia* sp. Some evidence suggests that the ions are the cause and the NPs merely represent a source of ions, while several other studies suggest cumulative effects or more adverse effects from NPs. Li et al. (2015a) observed significant changes in the metabolomic profile of *Daphnia magna* after exposure to Ag NPs and Ag^+ for 48 h. The changes in metabolites of daphnids exposed to Ag NPs were identical to those exposed to Ag^+, and therefore, they concluded that Ag^+ is the dominant cause of toxicity. Sakamoto et al. (2015) observed higher toxicity for *Daphnia magna*, *Daphnia galeata* and *Bosmina longirostris* after exposure to Ag NPs compared to Ag^+. However, the 48 h EC_{50} values of Ag NPs based on Ag^+ concentrations were comparable with those of Ag^+, and therefore, they concluded that the effects of NPs were due to liberated Ag^+ from AgNPs. Zhao and Wang (2011) observed no toxicity from AgNPs to *Daphnia magna* when the liberated Ag^+ ions were complexed by cysteine, suggesting that the toxicity was primarily caused by Ag^+. Shen et al. (2015) exposed *Daphnia magna* to seven types of Ag NPs with different sizes and coatings in $NaNO_3$ medium for 8 h to identify Ag species responsible for acute toxicity. The LC_{50} values of the seven Ag NPs as free Ag^+ agreed well with that of $AgNO_3$, and therefore, they concluded that the Ag^+ is exclusively responsible for acute toxicity. Bacchetta et al. (2016) noted the toxicity of ZnO NPs to *Daphnia magna* was similar to the toxicity from Zn^{2+} and therefore concluded that the toxicity was caused by released ions from NPs. Adam et al. (2014) found chronic effects of ZnO NPs (EC_{50}, 0.112 mg/L) and ionic Zn (EC_{50}: 0.082 mg/L) in *Daphnia magna* following exposure for 21 days. They studied the influence of free, dissolved and aggregated Zn fractions in the medium and concluded that the dissolved fraction was largely responsible for the chronic toxicity. Adam et al. (2015b) concluded that the ions from the dissolution of Cu NPs caused toxicity to *Daphnia magna* by exposing them to CuO and ZnO NPs and Cu and Zn salts for 21 days.

In contrast, there are reports by some researchers regarding the toxicity of NPs which cannot be explained by ionic effects (Navarro et al. 2008; Fabrega et al. 2009; Yin et al. 2011). Allen et al. (2010) observed that coffee-coated AgNPs were more toxic to *Daphnia magna* than Ag^+. Pakrashi et al. (2017) observed that AgNPs significantly affected the reproduction process of the first two broods in comparison with $AgNO_3$ which affected only the first brood. Based on this, they suggested that AgNPs may have longer adverse effects than Ag^+ ions. Bhuvaneshwari et al. (2016) claimed that the relative contribution of dissolved ions from NPs towards acute toxicity to *Ceriodaphnia dubia* was less than that of ZnO NPs. When *Daphnia magna* was exposed to CuO NPs and $CuSO_4$, Kim et al. (2017) observed that the dissolved Cu^{2+} ion concentration from CuO NPs after 72 h was much less than the 72 h median effective concentration of $CuSO_4$. These authors therefore suggested that the observed median toxicity of CuO NPs at 72 h was caused by the particles rather than by the dissolved ions. Xiao et al. (2015) reported that the relative percentage contributions of dissolved ions from CuO and ZnO NPs were 26% and 31%, respectively, when *Daphnia magna* was exposed to NPs. Therefore, they concluded that the particles rather than the dissolved ions were the main source of toxicity.

6.3.3 Ingestion into *Daphnia*

Ingestion via active and passive diffusion is the most common way of NP uptake by daphnids. Many NPs are lipophilic, and the ingested NPs are highly likely to be found in storage cells which contain lipids such as triacylglycerol and glycogen (Goulden and Hornig 1980; Moore 2006). The size of the particles daphnids can uptake depends on their body size. *Daphnia magna* can ingest particles up to about 70 μm, and the minimum size depends on the distances between setulae on thoracic limbs, which do not depend on the age or size since the gap is constant throughout

(Burns 1968; Geller and Müller 1981). Zhao and Wang (2011) observed a linear and positive correlation between Ag concentration in the daphnids and the concentration in the medium after exposing *Daphnia magna* to Ag NPs. Also, at same Ag exposure concentration levels, the Ag body burden from Ag NPs was two to three orders of magnitude higher than that from $AgNO_3$, showing the potential of daphnids to accumulate Ag NPs due to ingestion of NPs into their gut environment. Zhao and Wang (2012b) demonstrated that the Ag NP influx rate of *Daphnia magna* decreased with increased NP size. Also, they found 60% of Ag distributed in the gut of daphnids and concluded that ingestion was the dominant uptake pathway. Similarly, Skjolding et al. (2014a) observed a higher uptake of smaller mercaptoundecanoic acid-coated Au NPs than bigger particles. However, no such correlation was observed for citrate-coated Au NPs. In contrast, Rosenkranz et al. (2009) reported a lower uptake of smaller carboxylated polystyrene NPs (20 nm) in terms of mass compared to larger particles (1,000 nm). Tan et al. (2016b) observed that the uptake of polyacrylate-coated TiO_2 NPs by *Daphnia magna* depended on the calcium concentration in the medium. At low Ca concentrations, NPs were ingested via endocytosis and passive drinking and distributed throughout the body, with the highest NP concentration at the abdominal zone and gut. In contrast, NPs were actively ingested and concentrated only in the gut at high Ca concentration levels in the medium. Conine and Frost (2017) found that the presence of food reduced the toxicity of AgNPs in terms of the growth and survival of *Daphnia magna*. They also found that toxicity was greater for animals fed with P-rich algae compared to P-poor algae. The algal-bound AgNPs were not toxic at any tested concentrations, and they suggested that the reduced toxicity in daphnids fed with P-rich algae was due to higher removal efficiency of Ag NPs by P-rich algae from the medium leaving less for uptake by daphnids. They also suggested that the algae may convert NPs to non-toxic form to daphnids, while the nutrition and overall health of daphnids also play a role in responding to NPs. Skjolding et al. (2014b) studied the influence of surface functionalization of ZnO NPs and observed fast uptake of ZnO NPs and ZnO-octyl NPs compared to ZnO-OH NPs. Daphnids ingest NPs via active and passive diffusion, while the body size of the daphnids and the concentration of NPs in the medium positively correlate with ingestion. The body burden of NPs may be higher than their bulk counterparts due to the higher NP accumulation in the guts. The size of the NPs influences the ingestion though there are conflicting views on the correlation of size and ingestion rate. Also, several other factors such as media composition, the presence of food and the surface functionalization of NPs influence the ingestion.

6.3.4 Mechanisms of Toxicity

The widely accepted key toxic mechanism for acute toxicity from metals and metal NPs to invertebrates such as daphnids is the inhibition of Na^+/K^+-ATPase activity and the prevention of the absorption of Na^+ ions which could induce ionoregulatory failure and finally cause the death of the organism (Bianchini and Wood 2003; Kennedy et al. 2012; Rüdel et al. 2015). In addition to this, several other effects are reported at acute and chronic level. Bacchetta et al. (2016) exposed *Daphnia magna* to ZnO NPs and noted morphological changes in the digestive epithelium. They attributed these effects to the dissolved Zn^{2+} from NPs. Zn^{2+} ions enter into the gut enterocyte cytoplasm and resulted in altered mitochondria membrane permeability causing ROS production, which stimulates the extensive autophagy process eventually causing cell and animal death. Chae and An (2016) reported structural damage to the digestive organs of *Daphnia magna* along with the production of lipid droplets and concluded that AgNPs adversely affected nutrient uptake leading to immobility and death. Das et al. (2013) suggested that the observed decreased reproduction, growth inhibition and erratic behaviour of *Daphnia magna* from chronic exposure to TiO_2 and Ag NPs could be due to the uptake of NPs in their gut plus decreased enzyme activity. Zhu et al. (2010a) observed growth retardation and reproductive defects in *Daphnia magna* upon exposure to TiO_2 NPs. A significant amount of NPs accumulated in the body interfered with food intake which could conceivably be the cause. Blinova et al. (2017) saw long-term effects on reproductive potential with decreased number of neonates hatched from ephippia when *Daphnia magna* was exposed to Fe_3O_4 NPs. Lv et al. (2017) observed reduced digestive enzyme activities in *Daphnia magna* upon exposure to C_{60} and Si NPs. They also reported a concentration-dependent increase in SOD and LPO levels. However, the SOD activity decreased at a higher dose of C_{60} exposure after 72 h along with increased levels of MDA. They suggested this may be due to the breakdown of the antioxidant system at high concentrations over lengthy exposures. Ulm et al. (2015) found increased GSH, CAT and AChE activity levels in *Daphnia magna* upon exposure to TiO_2 NPs. When Dabrunz et al. (2011) exposed *Daphnia magna* to TiO_2 NPs for 96 h, they observed that the second moulting was disrupted due to the biological surface coating of NPs on the daphnids. Disruption to moulting directly results in reduced reproduction rates. Vijayakumar et al. (2016) noted ingestion of ZnO NPs in *Ceriodaphnia cornuta* and *Moina micrura* which caused blackening of the intestine, rupture of intestinal wall, shrinkage of the abdomen and loss of carapace and antennae leading to structural deformities. Rainville et al. (2014) reported increased protein carbonylation indicating ROS, changed vitellogenin levels and higher haemoglobin levels indicating cellular respiration from Ag NP exposure in *Daphnia magna*. NPs would have adverse effects on ionoregulatory processes, digestive system, growth, reproduction, behaviour, oxidative stress and moulting. *Daphnia* acute and chronic tests are widely used by regulatory regimes. However, the toxicity of NPs might not be reflected within the scope of the tests, and careful consideration of the mechanisms of toxicity is important to analyse effects. It is also reported that daphnids release certain proteins creating eco-corona around NPs (Nasser et al. 2016) resulting in heightened uptake and toxicity which warrants careful investigation of NP risks under environmentally relevant scenarios.

6.4 Bioaccumulation and Trophic Transfer of NPs

NPs in current use are expected to persist in the aquatic environment in different forms. Bioaccumulation of NPs is significant and calls for more research even though the emission of NPs to the aquatic environment is low, because of their limited degradability combined with the probability that they will be fed on by many invertebrates (Baun et al. 2008). To understand the trophic transfer of NPs through the food web, it is important to understand the mode of action at cellular and higher levels within individual organisms (Aschberger et al. 2011). Cells use different routes to internalize NPs, and a particular preferred route is chosen based on NP properties like size, shape and surface characteristics (Yameen et al. 2014). Any foreign materials which the cell finds harmful are transported to the lysosomes where they are digested. Therefore, in medical nanotechnology, many NPs are designed to enter the target cell through the caveolae to avoid degradation (Na et al. 2003; Panyam and Labhasetwar 2003). Once they are released into the environment after use, their non-degradative nature might negatively affect aquatic organisms. Bioaccumulation and biomagnification of NPs through the food webs are yet to be properly understood, with more research required on the influential physicochemical characteristics of NPs and trophic transfers (Zhu et al. 2009). The potential of accumulation and biomagnification of NPs may be higher in comparison with conventional contaminants, but the current testing paradigms do not emphasize the importance of evaluating the ecological impacts in this context (Wu et al. 2017a).

6.4.1 Bioaccumulation of NPs

Cellular uptake and accumulation of NPs may determine the toxicity (Taylor et al. 2016a). However, studies show contradictory results regarding the internalization mechanisms and where NPs accumulate inside the algae cells. Some reports claim that NPs enter into the cells, while others claim that they are just absorbed onto the cellular surface of algal cells or restricted to the outer region including the cell wall or periplasmic space. Taylor et al. (2016a) noted NPs in the periplasmic space of *Chlamydomonas reinhardtii* algal cells when they were exposed to Ag NPs. However, there were no Ag NPs accumulated inside the vesicle or the endosome around the cell, excluding the possibility of endocytosis or passive diffusion which is proposed to be the most feasible route (von Moos et al. 2014; Behra et al. 2013) for cellular internalization. In contrast, they observed Ag_2S particles in the cytoplasm which they suggested were present as a result of sulfidation of Ag^+ ions from Ag NPs. Sulfidation is widely accepted as a mechanism for the complexation and sequestration of heavy metals in plants to mitigate the toxicity (Chen et al. 2013). Lee et al. (2015) found that the bioaccumulation of Au NPs in *Euglena gracilis* was higher than in *Chlamydomonas reinhardtii* and noted that the reason might be the difference in the physical structure of organisms and the surface area available for interaction with NPs. They also observed the transfer of NPs to *Daphnia magna* after feeding them with Au NP-treated algae. Zhao et al. (2016) observed internalization of CuO NPs into *Chlorella pyrenoidosa* cells by endocytosis followed by storage in the vacuole. Yue et al. (2017) observed cell-associated Ag in the alga *Euglena gracilis* when exposed to Ag NPs. However, Ag NPs did not enter into the algal cells, only absorbed onto the algal surface.

Several studies have looked into the bioaccumulation of NPs in daphnids. Waterborne exposure and diet are the major routes for uptake of NPs in daphnids. NPs may enter the body or be retained by attaching to the body surface including the carapace. The concentration of NPs, media composition and physicochemical characteristics of NPs such as size and charge influence the uptake and retention of NPs in daphnids. Ribeiro et al. (2017) concluded that waterborne exposure to Ag NPs causes more accumulation of Ag than dietary exposure in *Daphnia magna*. However, more Ag from $AgNO_3$ was accumulated through the diet. Similarly, Wu et al. (2017a) observed a higher uptake, retention of NPs and attachment to the carapace surface of *Daphnia magna* upon waterborne dermal exposure to CuO NPs when compared to feeding exposure. Oral exposure was predominant in feeding exposure through NP-treated algae, and the ingested Cu was regulated within the body and transferred to other biological compartments such as neonates and carapaces which may have caused less toxicity. Botha et al. (2016) observed that the uptake of Au NPs into *Daphnia magna* was related to NP concentration in the medium and the charge of NPs. NPs were seen adsorbed to the surface of daphnids and in the gut, but there were no evidence of NP internalization into the body cavity. No effects on reproduction or moulting patterns were observed. Wray and Klaine (2015) observed that the uptake and elimination of Au NPs by *Daphnia magna* were influenced by the size and surface charge of NPs, whereas shape of NPs was non-influential. However, they also found no evidence for NP internalization into the body with NPs restricted to the gut lumen and the carapace. Adam et al. (2014) found increased concentrations of Zn in *Daphnia magna* with increased ZnO NPs and Zn^{2+} concentrations in the media after exposing them for 21 days. In a similar study, Adam et al. (2015a) observed localization of CuO NPs in the gut of *Daphnia magna* when they were exposed to CuO NPs for 10 days. However, CuO were not internalized in the cells and were easily eliminated. Khan et al. (2014) observed accumulation of Au NPs in the gut lumen of *Daphnia magna*, but there was no internalization into the gut epithelial cells. Zhu et al. (2010a) found that significant amount of TiO_2 NPs accumulated in the body and *Daphnia magna* and had difficulty in eliminating these NPs. Tan et al. (2016a) reported that Ca concentration in the medium influenced NP uptake into *Daphnia magna*. They observed TiO_2 NPs distributed throughout the daphnid while NPs were concentrated in the gut at high Ca concentrations. Vijayakumar et al. (2016) observed the bioaccumulation of ZnO NPs in the gut region of *Ceriodaphnia cornuta* and *Moina micrura*. Pakrashi et al. (2017) exposed *Daphnia magna* to AgNPs and saw the NPs accumulated in the gut and non-gut tissues. Interestingly, a higher degree of positive correlation between the concentration of Ag in the non-gut tissue was found. Xiao et al. (2015) reported that the bioaccumulation of NPs or dissolved ions from NPs were concentration dependent. At low concentrations, *Daphnia magna* accumulated more dissolved ions from

Cu and ZnO NPs (0.05 and 0.5 mg/L, respectively), while the particles were accumulated more at high concentrations (0.1 and 1 mg/L). Scanlan et al. (2013) observed similar or higher concentrations of Ag levels in the haemolymph of *Daphnia magna* in comparison with the initial concentration of Ag NWs in the medium indicating effective bioaccumulation during filter feeding. Lovern et al. (2008) used electron microscopy to observe accumulation and to investigate the presence and distribution of Au NPs in gut tissues of *Daphnia magna* exposed for 24 h. They observed movement of NPs to the posterior region of the gut, and there were no large blockages, and minimal deaths were observed. Therefore, they concluded that the particles are cleared with time in waste pellets.

Correlation between accumulation of NPs in daphnids and their eggs is also reported. Sá-Pereira et al. (2018) found NPs in the digestive tract, mainly in the gut and in the eggs of the brood pouch of *Daphnia magna* when exposed to TiO_2 NPs. Also, the penetration of Ti into epithelial region was higher at higher concentration levels. When *Daphnia magna* was exposed to polystyrene NPs, Brun et al. (2017) noted accumulation of NPs in or on the lipophilic cells in the early stages of embryonic development, while the embryo is still surrounded by a chorion. However, they did not observe any NPs accumulated neither in the gut epithelium nor in lipid droplets in the adults. Sakka et al. (2016) observed higher mortality and reproductive effects in *Daphnia magna* correlated with the uptake of Ag NPs.

6.4.2 Trophic Transfer of NPs

Studies show that NPs are taken up and accumulated inside organisms which are transferred to higher trophic levels. Transfer of nanoparticles up through the food chain is a primary concern since it affects the balance of the ecosystem putting ecosystem health at risk (Bhuvaneshwari et al. 2018a; Wu et al. 2017b). Since organisms may feed on NP-contaminated food, it is important to understand the role of the trophic route (Bour et al. 2015). Dietary intake of NPs may cause significant effects on growth, survival and reproduction (Bhuvaneshwari et al. 2018a). Certain metals and NPs are accumulated more through dietary intake than waterborne exposure (Ribeiro et al. 2017), while certain other NPs are accumulated more through the waterborne exposure (Bhuvaneshwari et al. 2018b). The effects from NPs ingested via dietary intake may have different mechanism of toxicity compared to direct exposure (Bour et al. 2015). Werlin et al. (2011) showed that the CdSe quantum dots can be transferred from the bacteria *Pseudomonas aeruginosa* to the protozoa *Tetrahymena thermophile* with the Cd concentration in the protozoa five times higher than that found in the bacteria. Chae and An (2016) observed that the Ag NWs were transferred from the alga *Chlamydomonas reinhardtii* to *Danio rerio* through *Daphnia magna*. Renault et al. (2008) showed that Au NPs were transferred from the freshwater alga *Scenedesmus subspicatus* to *Corbicula fluminea*. Bouldin et al. (2008) observed the transfer of carboxyl quantum dots from *Raphidocelis subcapitata* to *Ceriodaphnia dubia* through dietary intake. Chen et al. (2016) found that the trophic transfer of fullerene NPs from *Scenedesmus obliquus* to

Daphnia magna was dependent on subcellular distribution of NPs in alga cell. They observed that the highest NP transfer occurs via the cell wall followed by cell organelle and cell membrane. McTeer et al. (2014) reported the transfer of Ag to *Daphnia magna* from AgNP- and $AgNO_3$-treated alga, *Chlamydomonas reinhardtii*. Bhuvaneshwari et al. (2018a) observed the transfer of ZnO NPs from the alga *Scenedesmus obliquus* to *Ceriodaphnia dubia* with the BMF found to be nearly one causing ultrastructural damage and degradation of internal organs in *Daphnia*. Larguinho et al. (2014) reported that Au NPs transferred from the alga *Dunaliella salina* to the bivalve *Mytilus galloprovincialis*. However, they did not observe any significant morphological alterations in mussel digestive glands or activation of any antioxidant enzymes tested. Zhu et al. (2010b) observed trophic transfer of TiO_2 NPs from *Daphnia magna* to *Danio rerio* by dietary exposure. Although they observed lower biomagnification from dietary intake than from aqueous exposure, the higher body burden in the dietary exposure group led them to conclude that trophic transfer is a major route of potential NP exposure. Skjolding et al. (2014b) observed trophic transfer of ZnO NPs and ZnO-octyle NPs from *Daphnia magna* to *Danio rerio*. However, daphnids did not uptake ZnO-OH NPs, and therefore, these NPs were not available for trophic transfer. This demonstrates that surface functionalization influences the trophic transfer of NPs. Cano et al. (2018) observed the trophic transfer of MWCNTs from *Daphnia magna* to *Pimephales promelas* which was found to be dependent on the size of the particles. However, Bhuvaneshwari et al. (2017) did not observe any transfer of nZVIs from the treated alga *Scenedesmus* sp. to *Ceriodaphnia dubia* though the algae had taken up NPs. Similarly, Bhuvaneshwari et al. (2018b) did not observe any trophic transfer of TiO_2 NPs from the treated alga *Dunaliella salina* to *Artemia salina*. However, Hu et al. (2017) observed the transfer of Ag from AgNP-treated *Chlorella pyrenoidosa* to *Daphnia magna*. In this case the biomagnification factor (BMF) was 0.5, and therefore, they concluded that there was no biological magnification of NPs from algae to daphnids.

6.5 *Effects of NPs on Behaviour of Aquatic Organisms*

In addition to the direct effects of the contaminants to organisms, behavioural effects are also critically important. Behaviour is a sensitive measure of an organism's response to stress, and noticeable changes can be observed at concentrations of contaminants which are orders of magnitude less than that which cause mortality (Weis and Candelmo 2012). Behavioural ecotoxicity tests are becoming increasingly popular because of their high sensitivity at low concentrations and early response (Yeardley et al. 1996). Though the importance of behavioural tests is appreciated in ecotoxicology tests, far less attention has been received (Melvin and Wilson 2013). Most currently available standard tests mention the obligation to document abnormal behaviour, but this is not quantitatively sufficient for any risk assessments (Postma and Keijzers 2014). Most of the behavioural activities which are used in experiments are related to feeding (feeding rate, filtration rate, predator response) or movement (swimming, avoidance, burrowing).

There are a few studies on the effects of the NPs on the behaviour of aquatic species with most relating to daphnids. The adhesion of aggregates of NPs to the exoskeleton of *Daphnia* sp. may lead to different probability of survival, loss of mobility and physical damage (Baun et al. 2008). Stanley et al. (2016) exposed *Daphnia magna* to multiwalled carbon nanotubes (MWCNTs) for 48 h and found LC_{50} as 29.3 mg/L and EC_{50} (swimming velocity) as 6.7 mg/L. They concluded that behavioural tests are more sensitive than traditional acute toxicity tests for materials which are toxic physically rather than chemically. Also, they suggested that use of only survival endpoints to set environmental guidelines could underestimate potential hazards and risks of NPs to the environment. Lovern and Klaper (2006) observed *Daphnia magna* showing abnormal behaviour such as sporadic swimming in small circles, persistent ramming to vessel walls and inability to swim down from the surface when exposed to fullerene NPs. Artells et al. (2013) studied the effects of CeO_2 NPs on the survival and swimming behaviour of *Daphnia similis* and *Daphnia pulex*. Swimming velocities decreased in the range of 30–40% in both species when treated with 1 mg/L NPs. At higher concentrations (10 and 100 mg/L), the swimming velocity of *Daphnia similis* was more impacted than *Daphnia pulex*. Noss et al. (2013) studied the swimming behaviour of *Daphnia magna* after treating with TiO_2 NPs. They observed a treatment-dependent swarming in the centre of the test vessels during the initial period. The swimming velocities increased with increased body length but significantly reduced after 96 h of exposure. Vijayakumar et al. (2016) observed abnormal behaviour in *Ceriodaphnia cornuta* and *Moina micrura* upon exposure to ZnO NPs. The restricted and reduced movements were attributed to the adhesion and agglomeration of NPs on the carapace and the filter apparatus. When Strigul et al. (2009) exposed *Daphnia magna* to 2.5 mg L^{-1} B NPs, they were actively swimming compared with the control group. However, when the concentration increased to 8 mg L^{-1}, they were less active, while they were very slow at 25 mg L^{-1}.

O'Keefe et al. (1998) suggested that the predation risk of daphnids depends on their swimming behaviour. Pokhrel and Dubey (2012) investigated the potential impacts of citrate-coated Ag NPs on the behaviour of *Daphnia magna* in the presence of the predatory dragonfly *Anax junius*. In the absence of Ag NPs, daphnids avoided predators with both horizontal and vertical movements which are different to the control. However, they did not show any difference in vertical movement when treated with Ag NPs suggesting that Ag NPs may have potential implications on daphnid populations with increased vulnerability to predation. Lovern et al. (2007a) quantified the behavioural responses of *Daphnia magna* at sublethal concentrations of TiO_2 and fullerene NPs. Both treatments caused significant increase in hopping frequency and appendage movement suggesting increased risk of predation and reproductive decline. Lu et al. (2017) observed a decrease in the ingestion and filtration rate of algae by *Daphnia magna* upon exposure to increased concentrations of TiO_2 NPs, and the researchers attributed this to the observed chronic toxicity. Similarly, Lv et al. (2017) saw a reduction in ingestion and filtration rate of *Daphnia* *magna* upon exposure to C_{60} and Si NPs. Heinlaan et al. (2017) suggested that the reduced algal feeding rate of Co_3O_4 and Mn_2O_3 NPs-exposed *Daphnia magna* was not particle specific since similar results were obtained for daphnids exposed to relevant metals. Gaiser et al. (2011) observed reduced feeding in *Daphnia magna* when they were exposed to CeO_2 NPs which was ascribed to potential replacement or coating of algae by NPs and filling the intestine with particles. Zhu et al. (2010a) observed drastic reductions in food intake when *Daphnia magna* was exposed to TiO_2 NPs. The chronic toxicity of NPs was ascribed to poor food intake and malnutrition. McTeer et al. (2014) observed a significant reduction of feeding when *Daphnia magna* were fed with Ag NP- and $AgNO_3$-contaminated algae compared to the control. They concluded that this reduction was due to the presence of Ag in algae.

In general, behavioural tests are fast and more sensitive than conventional acute and chronic ecotoxicity tests. These characteristics are particularly useful in assessing NP toxicity. NPs tend to transform and aggregate in the medium exerting huge challenges in assessing toxicity by conventional tests. Also, due to numerous types of NPs entering into the market, it is a huge challenge to assess the toxicity due to time consuming nature and the cost of conventional tests. These issues can be overcome by choosing comparatively faster and cheaper behavioural tests. There is a growing interest to develop lab-on-a-chip behavioural tests (Wang et al. 2017; Cartlidge et al. 2017) which are fast and sensitive with added advantages.

7 Conclusions and Recommendations

1. Nanotechnology is a booming industry and more applications are continuously being found. Therefore, release of NPs to the aquatic environment during their manufacture or use is unavoidable. The exact concentrations of NPs in waterbodies are yet to be assessed, and only limited predicted data are available with huge assumptions since there is also a lack of published data on NP-containing products. There has been increasing interest in research on the fate and effects of NPs in the environment, but the scientific community has not been able to come to a general consensus to accurately design regulatory requirements or guidelines.
2. Efforts have been taken to assess the flow of NPs into the environment and the exposure levels. Recent developments in material flow modelling are noteworthy. Also, recent efforts to accurately measure the environmental concentrations of NPs by analytical methods are a positive sign which also support in verifying the values predicted by models. However, factors such as the complexity of real sample matrices, transformation and aggregation of NPs once released into the environment and limitations in the analytical methods are causing a huge challenge in accurately measuring the environmental concentrations, while there is considerable uncertainty in models resulting in lack of reliable data. Therefore, estimates of more refined levels are needed, and further

research is needed for determination of actual environmental concentrations of NPs for reliable risk assessment and for regulating NP industry.

3. NPs possess special physicochemical characteristics due to their smaller size which may have different effects on organisms compared to their bulk counterparts. However, the presence of NPs in the environment is still not well documented due to a lack of sample-related certified standards, analytical procedures and reliable units of measurement. Also, toxicity tests and risk assessment methodologies specific to NPs are still at the research and development stage. Further, the available technologies are not sufficient to remediate them to environmental permissible levels.
4. The effect of the particle properties of NPs on toxic responses has been heavily studied in the last decade, and it has been found that certain physicochemical properties such as size, shape and surface functionality of NPs influence toxicity. However, conflicting and inconsistent results demand further research to make sound conclusions to protect the organisms from adverse effects of NPs. It is recommended to focus on the NP properties that are already known to influence toxicity. In the meantime, it is required to put more attention into systematic approaches to design NP structures with minimal adverse effects to the environment.
5. The surrounding environment largely influences the transformation of NPs once they are released into the aquatic environment. The presence of NOM, media constituents and kinetics of transformations make it significantly difficult to predict transformations in complex natural environments. Further research is required to develop methodologies and generate data on the fate and transport of NPs in the environment and how these affect organisms. Site-specific studies are recommended since mechanisms of transformation depend on the characteristics of any particular environment. Also, knowledge gaps exist, and further studies are required regarding effects of ageing of NPs since kinetics of particle degradation and kinetics of biological impact are extremely important to tease out mechanisms of interaction and mode of action. NPs interact with other chemicals in the environment. These interactions influence their very own toxicity and also the effects of those chemicals to aquatic organisms. Therefore, it is required to take multiple chemical interactions into account in environmental risk assessment of NPs.
6. Opinions still differ in what causes the effects of metallic NPs on living organisms. Contradictory views on whether NPs, ions released from NPs or a combination of both cause toxicity is still an issue to understand the nature of the metallic NPs' toxicity as well as their toxicity mechanisms. In general, the effects seem to depend on several factors such as the type of NPs, the surrounding environment and the organism. Therefore, more focused research is required to address this to better understand the toxic potential of the metallic NPs and make accurate risk assessments on them.
7. The toxicity data generated even with standard toxicity tests are not consistent. Compared with the traditional contaminants, there are several variables in assessing the effects of NPs to organisms. The influence of particle properties

on the toxicity of NPs is a major challenge to compare and make conclusive decisions based on obtained acute and chronic toxicity values. Therefore, it is necessary to develop robust procedures to generate data with a high degree of credibility. With such variable results, it is difficult to extrapolate the sensitivity of different species, and therefore, it is recommended that toxicity tests be conducted with a broad range of taxa to protect different organisms in aquatic environments.

8. Mechanisms of NP toxicity are also still not well understood. The underlying mechanisms for the toxic interactions of NPs are complex, possibly involving various processes mediated through reactive oxygen species (ROS) generation and oxidative stress. These mechanisms are currently regarded as the best-developed paradigm for NP toxicity. Other toxicity mechanisms include membrane damage, protein denaturation, DNA damage, behavioural effects, physical damage, etc. Improved understanding on the mechanisms of NP toxicity is crucial in risk assessment of NPs since conventional toxicity tests may not reflect the risks associated with NPs.
9. NPs can be ingested and accumulated inside the organism or absorbed onto the surface which may lead to trophic transfer of NPs through the food web. Studies on the bioaccumulation and trophic transfer of NPs are very limited, and therefore, more research is recommended to understand the effects on organisms at higher levels.
10. Due to the low NP concentrations in field conditions, the toxicity or any other physiological effects in organisms are unlikely to be prominent enough for detection. Behavioural effects may be more sensitive and would be efficient in certain situations to evaluate effects. Also, behavioural toxicity tests are fast and cheaper which could be helpful in assessing the toxicity of ever-increasing varieties of NPs. Further, behavioural tests may be more relevant in addressing challenges posed by NPs such as transformation and aggregation. However, attention to such tests is still lacking, and further research is recommended.

8 Summary

Both the use and the number of applications of nanoparticles have expanded rapidly in recent years. This has led to increasing concern regarding the impact of nanoparticles on ecosystem health. Toxicological research in this area is therefore of utmost importance in order to determine the risks of nanoparticles to organisms in the environment. The goal of this review is to analyse recent literature in this interdisciplinary research field, with special focus on the freshwater environment. The paper begins with summarizing knowledge of current production and use of nanoparticle production and exposure concerns in the environment. The major physicochemical characteristics of NPs are examined and their subsequent fate, behaviour and toxicity to aquatic organisms. We review literature regarding the toxicity of nanoparticles to freshwater organisms at different trophic levels involving studies on bacteria, algae and *Daphnia*. Finally this review examines the less understood behavioural effects of nanoparticles on freshwater organisms. This aspect necessarily focuses on inorganic nanoparticles due to their industrial use and production although the effects of organic nanoparticle should not be overlooked. It is a huge challenge to accurately predict the environmental concentrations of nanoparticles, their fate and behaviour in the environment and to assess the risks posed to aquatic organisms. However, the work carried out by the nanotoxicology community over recent years is commendable. Through analysis, this review contributes to improved understanding on the effects of NPs while also identifying current research gaps and suggesting future research areas in nanotoxicology.

Acknowledgements This material is based upon work which was supported by the Australian Government to Sam Lekamge through an “Australian Government Research Training Program Scholarship”. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the Australian Government.

Conflicts of Interest The authors declare that they have no conflict of interest.

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Environmental Water Pollution, Endocrine Interference and Ecotoxicity of 4-*tert*-Octylphenol: A Review

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P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 248*, Reviews of Environmental Contamination and Toxicology Volume 248,
https://doi.org/10.1007/398_2018_20

1 Introduction

High production volume (>1,000 tonnes/year) pollutants from agrochemicals, industrial wastes and personal care products as well as from poorly treated effluents from wastewater treatment plants are continuously being released into waterbodies, and these have the potential to impact negatively on humans as well as aquatic wildlife and ultimately upset the entire ecosystem (reviewed by Olaniyan et al. 2016). 4-tert-Octylphenol or 4-(1,1,3,3-tetramethylbutyl) phenol (4-tOP) (Fig. 1) exemplifies high production volume chemical sourced from breakdown product of alkylphenol polyethoxylates (APEOs) the non-ionic surfactants (Chokwe et al. 2017). Early work (de Voogt et al. 1997) has shown that ethoxylates and alkylphenols are degradation products of APEOs. Kinetic studies (Jonkers et al. 2001) from aerobic biodegradation of nonylphenol ethoxylates further showed that the degradation is initiated by ω-carboxylation of the individual ethoxylate chains generating the long- and short-chain carboxylated ethoxylates as well as alkyl chain of varying lengths. 4-tOP is also a raw material for a number of industrial, agrochemical and domestic applications (Tamagawa et al. 2007; Petrovic et al. 2002). It is a base compound for phenol formaldehyde (Novolac) resins used for a wide range of products including automobile tyres, carbonless copy paper, printing inks, detergents and personal care products. 4-tOP belongs to the class of chemicals of very high health concern (EU 2006) following its demonstrated oestrogen receptor-mediated endocrine disruption effects both in vitro and in vivo (Bian et al. 2006; Zhou 2006; Routledge and Sumpter 1997) with evidence of thyroidal activity in amphibians (Evans et al. 2011; ECETOC 2009). It has a high detection frequency probably worldwidely in aquatic system including surface water and river sediments (Liu et al. 2017; Zhong et al. 2017; Salgueiro-Gonzalez et al. 2015b; Oketola and Fagbemigun 2013), wastewater effluents and sewage sludge (Bina et al. 2018), marine water (Mayer et al. 2007), drinking water (Zhong et al. 2017; Kovarova et al. 2013; Sharma et al. 2009) and healthcare facility wastewaters (Nagarnaik et al. 2010) albeit at ultra-trace (ng/L) levels. The compound and its parent APEOs have been placed under restrictions by some countries such as those of European Union (Directive 2003/53/EC 2003; Directive 2000/60/EC 2000), but it is still being detected in various environmental matrices such as water and soil in some developing nations including South Africa (Zhong et al. 2017; Aneck-Hahn et al. 2009). In sewage treatment plant (STP) effluents, APEOs are degraded to shorter-chain and more resistant alkylphenols such as 4-tOP and 4-nonylphenol. Nonylphenol is the major (80%) transformation

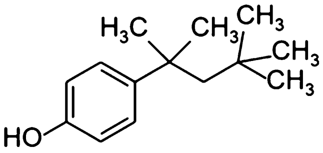

Fig. 1 Structure of 4-tert-octylphenol (CAS 140-66-9)

product of APEO, while 4-tOP is the minor product (Chokwe et al. 2017; White et al. 1994). Although good removal mechanisms for 4-tOP from wastewaters have been proposed and demonstrated, they still require development and adaptability for large-scale applications. Structurally, the 4-tOP phenolic head and its tertiary-branched alkyl tail are important for oestrogen receptor (ER) activation (Routledge and Sumpter 1997); the branched tail (Fig. 1) also confers hydrophobicity or lipophilicity ($K_{\mathrm{ow}} > 4$) (Table 1).

The hydrophobicity plays an important role in its partitioning between water and sediments and is therefore expected to significantly sorb onto sediments than solubilize in water. The hydrophobicity may account for its reported ability to interact with biological membranes (Diao et al. 2017). Also its potential to bioaccumulate in tissues of aquatic animals such as fish has been widely reported (Madsen et al. 2006; MITI-List 2002; Ferreira-Leach and Hill 2001; Tsuda et al. 2001; Tsuda et al. 2000) fueling the conception that it may be toxic to both human and wildlife as a secondary poison. The presence of electron-donating methyl groups contributes to its lower acidity (high p*K*a) (Table 1) when compared with phenol (p*K*a 10) or with p-chlorophenol (p*K*a 8.6) which otherwise contains electron-withdrawing chlorine species. This amphipathic nature probably accounts for its ubiquity in environmental matrices (air, soil, water).

Reports from epidemiological studies appear to give fillip to the view that environmental exposure to endocrine disruptors (EDs) is a risk factor to human non-communicable diseases such as cancers, reproductive disorders (Hatch et al. 2010; Meeker 2010; Soto and Sonnenschein 2010; Swan 2008; Skakkebaek et al. 2001) and diabetes (Lin et al. 2013). Toxicity biomarkers of endocrine disruption in wildlife following exposure to 4-tOP have been reported (Liu et al. 2017; Salgueiro-Gonzalez et al. 2015c; Zhong et al. 2014). A population-based study carried out by

Table 1 Some chemical properties of 4-tert-octylphenol

Property	Value	Reference
Molecular formula	$C_{14}H_{22}O$	
Molecular weight (g/mol)	206.3	Brooke et al. (2005)
Water solubility	19 mg/L at 22°C; 62 mg/L at 25°C	Brooke et al. (2005), Scifinder Scholar (2011)[a]
Partition coefficient octanol/water (K_{ow})	4.12 (20°C); 5.5^{b}; 5.18	Brooke et al. (2005), Ferguson et al. (2000)
Boiling point	280–283°C	Brooke et al. (2005)
Melting point	79–82°C	Brooke et al. (2005)
Density	0.92 g/mL	Brooke et al. (2005)
p*K*a	10.33; 10.39 (25°C)	Brooke et al. (2005), Bledzka et al. (2009)
Vapour density	3.6×10^{-4} mmHg	Salapasidou et al. (2011)
Henry's law constant	195 ± 68; (145 M atm^{-1} at 25°C)	Xie et al. (2004), Nguyen et al. (2014)

[a] https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf

[b] Syracuse Research Corporation (Syrres), http://www.syrres.com/esc/physprop.htm

Qin et al. (2013) reported a direct relationship between urinary 4-tOP levels and infertility in males. Cell-based studies have shown that the endocrine disruption activity of 4-tOP is mediated by ER signalling pathway though its activation is weaker than 17β-oestradiol (E2), the natural ER agonist. Evidence of other mechanisms of endocrine disruption by 4-tOP has been presented (Jambor et al. 2016), but other signalling pathways such as (anti-)/androgen receptors, aryl hydrocarbon receptors, pregnane X receptors and aromatase pathways (Kjeldsen et al. 2013) have not really received as much attention as ER pathway. Thus, current knowledge about consequences of 4-tOP exposure in human and wildlife health is limited. Analyses regarding mitigation strategies of its impacts on the ecosystem appear not properly coordinated. We carried out a literature review of the activities of 4-tOP in environmental waters, available removal mechanisms, biological activities and relevance to toxicities.

2 Modes of Exposure to 4-*tert*-Octylphenol

Some personal care products such as lipsticks branded with detergents may contain as high a concentration of 4-tOP as 10 μg/g (Liao and Kannan 2014), which is absorbable through dermal route. Figure 2 shows mode of exposure to 4-tOP by human and aquatic wildlife. Considering its relatively high vapour pressure, 4-tOP can volatilize and be airborne either as aerosol or as particulate matter which could

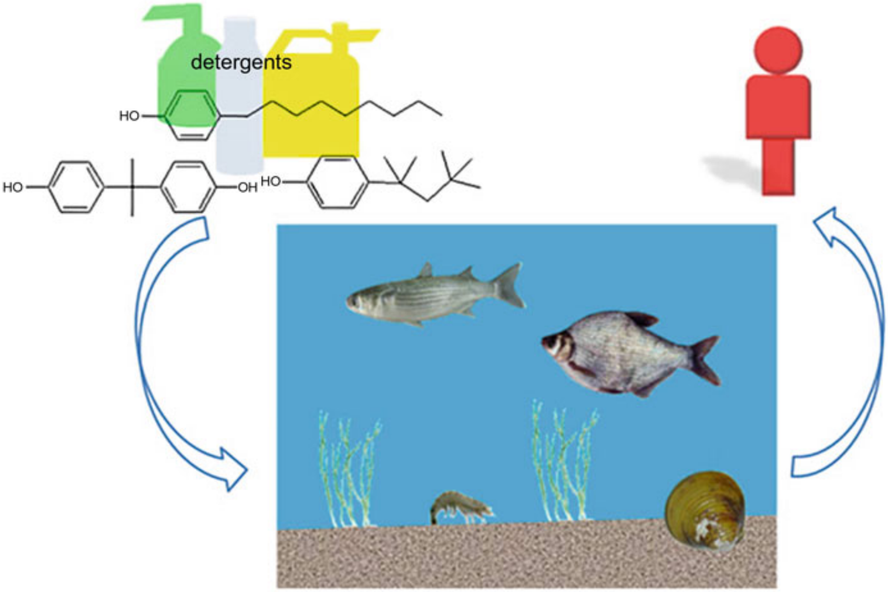

Fig. 2 4-tert-Octylphenol exposure mode to human and aquatic lives (Diao et al. 2017)

reach humans by inhalation (Mandin et al. 2016; Salgueiro-Gonzalez et al. 2015a). Indoor air concentrations of 45.7 ng/m^3 (Saito et al. 2004) and 3.2 ng/m^3 (Mandin et al. 2016) have been reported. Table 1 contains a few of the chemical properties of 4-tOP relevant to its exposure routes. Since 4-tOP is a synthetic chemical compound, its presence in aquatic ecosystems marks an anthropogenic input. Following ubiquity of 4-tOP, environmental waters are open to contamination by this compound. Table 2 shows the concentrations of 4-tOP in aquatic systems around the globe. Leaching from 4-tOP-containing industrial as well as household materials may be an important source of contamination (Phan et al. 2016). Table 3 summarizes the concentrations of 4-tOP found in seafood worldwide. Human consuming seafood sourced from polluted water (Wu et al. 2016; Tsuda et al. 2000) may be exposed to the deleterious effects of 4-tOP (Fig. 2). Complete removal of this micro-pollutant by wastewater treatment plants has not been achieved (Céspedes et al. 2008); thus, discharges from wastewater treatment plants continue to impact on the river water level.

The presence of 4-tOP in drinking water (Table 2) may have resulted from incomplete removal by wastewater treatment plants. The environmental quality standard set by European Commission for 4-tOP is 0.01 μg/L (EC 2013), but higher levels have been reported in some water resources (Table 2).

The compound potency for persistence as well as generation of toxic by-products following interaction with residual chlorine in drinking water has been demonstrated (Richardson and Postigo 2011; Gibs et al. 2007). The presence of the chemical in sediments and sludge has implications on human health following consumption of plants cultivated on the biosolid-amended soil.

The detection of 4-tOP in human biological samples as summarized in Table 4 may suggest significant exposure to the pollutant. Since the compound traverses membrane barriers such as the placental (Mannelli et al. 2015) and blood brain (Bianco et al. 2011) barriers, its presence in amniotic fluid and cord blood means exposure to the developing foetus whose drug-metabolizing pathways are underdeveloped and consequently affecting critical developments (Schug et al. 2011). Its presence in breast milk (Table 4) may also be a significant source of exposure to the infants. An average daily intake of 0.006 μg 4-tOP/kg body weight has been reported in infants (≈5 kg body weight) consuming 350 mL milk per day (Ademollo et al. 2008). The concentration of 4-tOP in the urine of the exposed school-aged children ranged from 0.2 to 20.6 ng/mL (Calafat et al. 2008). But Choi et al. (2014) reported that 4-tOP levels in the urine and the serum were unrelated to diabetes among children. Tolerable daily intake of 0.42 mg 4-tOP/kg and no observed adverse effect level of 125 mg/kg/day have been reported (Sahambi et al. 2010; Bian et al. 2006), while 10 μg/kg was proposed by Danish Institute of Safety and Toxicology following toxicological studies (Ferrara et al. 2008). Extent of exposure to pollutants in aquatic animals is generally estimated using bioconcentration factor (BCF) which measures the ratio of the concentration of the pollutant in the animal tissues to the average concentration in water (Salgueiro-Gonzalez et al. 2015c). BCF of a chemical exceeding 5,000 is considered as an indicator of bioaccumulation (Weisbrod et al. 2010). However, we believe BCF does not really provide information on exposure

Table 2 Concentrations of 4-tert-octylphenol detected in some aquatic environments

Aquatic resource	Number of sampling sites	Concentration range (ng/L)	Mean concentration (ng/L)	Median value (ng/L)	Frequency of detection (%)	References
WWTP influents						
Urban	4	86.1–183.3				Bina et al. (2018)[a]
Rural	2	35–51.5				Bina et al. (2018)
Livestock	1	686.4				Bina et al. (2018)
Hospital	1	139.4	4			Bina et al. (2018)
Commercial	1	718.7				Bina et al. (2018)
Tannery	2		73			Pothitou and Voutsa (2008)
Textile	2					Pothitou and Voutsa (2008)
WWTP effluents						
Urban	4	12.8–54.8				Bina et al. (2018)
Rural	2	11.2–14.6				Bina et al. (2018)
Livestock	1	5.4				Bina et al. (2018)
Hospital	1	22.2	4			Bina et al. (2018)
Commercial	1	40.2				Bina et al. (2018)
Tannery	2					Pothitou and Voutsa (2008)
Textile	2		51			Pothitou and Voutsa (2008)
Surface waters						
Haihe River (China)	13	18.0–20.2	19.2	19.3		Jin et al. (2004)
Shijing River (China)	4	33.1–3,150	433	159	100	Zhao et al. (2011)
Zhujiang River	3	3.08–27.1	10.6	7.74	100	Zhao et al. (2011)
Ialomita River (Romania)	3[b]	140[c]				Cruceru et al. (2012)
River Ogun (Nigeria)	10	57.1–68.3				Oketola and Fagbemigun (2013)

Gulf-Gdanski (Poland)	5	<LOQ-65.9	24.6			Staniszewska et al. (2014)
Estuaries (China)	6	1,265–15,700			100	Zhong et al. (2017)
Underground waters						
23 European countries	164[d]	41[c]	1	0	23	Loos et al. (2010)
Drinking waters						
	2[d]	<LOD-1530				Furtado and von Mühlen (2015)
Tap water (Spain)	6	2.6–9.7	4.9	4.3	100	Esteban et al. (2014)
Ground/surface water	10	0.2–5.0	2	1.8		Kuch and Ballschmiter (2001)
Sediments[e]						
Shijing River (China)	4	103–979	385	309	100	Zhao et al. (2011)
River Ibeche (Nigeria)	10	9.2–24.5				Oketola and Fagbemigun (2013)
Pearl River Delta (China)	7	0.1–4.5	0.3–3.0			Chen et al. (2014)
Po River (Italy)	5	Nil-15.8			20	Luigi et al. (2015)
Estuaries (China)	6	2.7–42			100	Zhong et al. (2017)

[a] 24-h composite sampling; LOQ-Limit of quantitation = (5 ng/L)

[b] 12 samples/site

[c] Maximum concentration

[d] Number of samples; LOD-Limit of detection = 140 ng/L

[e] ng/g dry weight

Table 3 Measured 4-*tert*-octylphenol levels in biota consumed by humans as foods

Name of biota	Number of samples	Concentration range (ng/g)[a]	Mean (ng/g)[a]	References
Fish				
Mugil cephalus	16	<0.11–0.17	0.13	Diao et al. (2017)
Parabramis pekinensis	12	<0.11–0.3	0.16	Diao et al. (2017)
Lateolabrax japonicus; grass carp (muscle)	10[b]	nd–4.4	0.66	Liu et al. (2017)
Herring (whole body)	10	2.5–50.2	19	Staniszewska et al. (2014)
Flounder (muscle)	11	1.0–19.2	10.2	Staniszewska et al. (2014)
Flounder (liver)	11	<LOQ–45.2	16.1	Staniszewska et al. (2014)
Shrimps				
Penaeus chinensis	12	<0.11–0.61	0.33	Diao et al. (2017)
Molluscs				
Corbicula fluminea	24	<0.11–0.48	0.21	Diao et al. (2017)
Corbicula fluminea	11	19.9–112		Salgueiro-Gonzalez et al. (2015c)

[a] Dry weight; LOQ – Limit of quantitation = (0.8 ng/g)
[b] Grams; nd = not detected

level; in fact it may have underestimated the level. The tissue level of any pollutant for that matter represents a balance between intake and metabolism, and BCF does not take this into consideration. Also the average concentration of the pollutant in the animal tissues needs be defined to standardize inter-laboratory comparison.

3 Pathways Responsible for 4-*tert*-Octylphenol Attenuation in the Environmental Waters

Wastewater treatment plants are normally designed for nutrient (carbon, nitrogen and phosphorus) removal, but partial ED removal is often achieved (Phan et al. 2016) and efficiency of removal dependent on treatment technology (Matamoros and Salvadó 2013; Phan et al. 2016). Removal during secondary biological treatment is through the waste sludge, biological or chemical degradation, transformation and volatilization during aeration (Mastrup et al. 2001; Johnson et al. 2000a, b).

Tables 5 and 6 illustrate the 4-tOP removal mechanisms and their efficiencies. Tertiary treatment systems employing coagulation/flocculation and filtration-UV-chlorination processes poorly remove 4-tOP (Matamoros and Salvadó 2013). Ozonation also appears to remove 4-tOP poorly in water (Table 6). Loss of 4-tOP through hydrolysis appears insignificant given its lipophilic nature ($K_{OW} > 4$), but reduction through sorption onto the sediments and finally to sludge during treatment may be a

Table 4 4-*tert*-Octylphenol levels in human samples

Samples	Number of samples	Concentration range (ng/L)	Mean (ng/L)	References
Adipose tissue	20	nd-8.68[a]	4.58[a]	Lopez-Espinosa et al. (2009)
Amniotic fluid	40		2,960[b]	Shekhar et al. (2017)
Breast milk	10	nd-200[c]	120	Ademollo et al. (2008)
	59		1.29[a]	Chen et al. (2010)
	26	nd-1.1[d]		Lin et al. (2009)
Cord blood	180[e]	<50 to 1,150		Tan and Mohd (2003)
	201	$(40–1{,}250) \times 10^3$	840×10^3[f]	Li et al. (2013)
Maternal blood	40		3,090[b]	Shekhar et al. (2017)
	201	$(100–2{,}970) \times 10^3$	$1{,}400 \times 10^3$[f]	Li et al. (2013)
Serum				
(Normal)	58	18,360–67,000	40,330	Choi et al. (2014)
(Obese)	69	74,390–24,510	441,502[g]	Choi et al. (2014)
Urine				
	2,517[h]	1,600–3,200	2,200[f]	Calafat et al. (2008)
Obese	985	560–640	600[b]	Qin et al. (2013)
Maternal (creatinine-adjusted)	58	nd-48,890	9,040	Choi et al. (2014)
	69	nd-36,810	7,480[i]	Choi et al. (2014)
	1,100	150–42,490	1,510[j,b]	Lv et al. (2016)

[a] ng/g lipid
[b] Geometric mean
[c] nd: < limit of detection (LOD) = (0.01 ng/mL)
[d] LOD = 0.2 ng/g
[e] Detection frequency (d.f.) 17%
[f] 95th percentile
[g] $p = 0.06$ compared with the normal controls
[h] d.f. 57%
[i] $p = 0.69$ compared with the normal controls
[j] ng/g creatinine; nd = not detected

quantitatively removal mechanism (Czech et al. 2016; Korner et al. 2000). In natural waters, 4-tOP can be transformed by photolysis such as exposure to sunlight (Ahel et al. 1994; SIDS 1994). Laboratory evidence had shown that photolysis by both visible and ultraviolet (UV) radiations was efficient in removing 4-tOP under alkaline medium (Huang et al. 2013; Environment Agency UK 2005; Mazellier and Leverd 2003) but generated more toxic phenol, hydroquinone, 4-tert-octylcatechol and certain hydroxylated compounds as the photolytic products (Gurban et al. 2015; Huang et al. 2013; Lu and Gan 2013; Bledzka et al. 2009; Mazellier and Leverd 2003). The mechanism of 4-tOP photolysis is thought to be by attack on the susceptible aromatic ring by hydroxyl radicals being generated from water. The proposed structures of the products of 4-tOP photo-degradation are as shown in Fig. 3.

Table 5 Mechanisms of 4-*tert*-octylphenol removal from wastewaters

Method	Efficiency (%)	References
Coagulation-flocculation	49	Matamoros and Salvadó (2013)
Conventional activated sludge process	65	Luo et al. (2014)
Biofiltration	78	Balest et al. (2008)
Fenton process (Fe^{3+}+H_2O_2)	70	Wu et al. (2013a)
Membrane bioreactor	>99	Trinh et al. (2012)
PAC+ MBR	>95	Nguyen et al. (2014)
Photocatalysis using TiO_2/$NaBiO_3$	>90	Chang et al. (2010), Wu et al. (2012)
Ultraviolet irradiation	>80	Lu and Gan (2013), Gurban et al. (2015)

PAC powdered activated charcoal, *MBR* membrane bioreactor

Table 6 Analysis of water samples from a waterworks for 4-tert-octylphenol (ng/L) (Wenzel et al. 2003)

Raw water	After ozonation	After GAC	After chlorination	Tap water	LOQ
4.1	1.2	nd	nd	nd	0.5

GAC granular activated carbon filtration, *LOQ* limit of quantitation, *nd* below LOQ

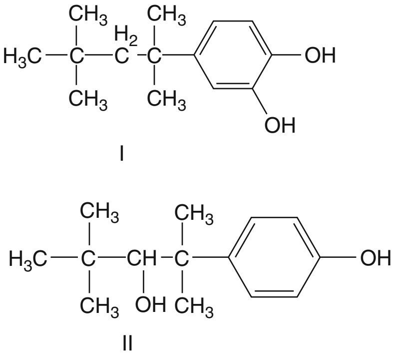

Fig. 3 Proposed structures of photolytic degradation of 4-tert-octylphenol (Wu et al. 2012)

Advanced oxidation processes (AOP) are technologies using the mechanism involving ultraviolet radiation, ozone, hydrogen peroxide and oxygen in treating water to remove pollutants such as 4-tOP. Fenton process, an aspect of AOP under UV radiation, has been shown to generate hydroxyl radicals which are suspected to be responsible for significantly (>70%) degrading 4-tOP (Wu et al. 2013b). Certain organic compounds such as nitrates, nitrites and sulphates when irradiated have been found to generate these radicals (Huang et al. 2013; Maddigapu et al. 2010; Calza et al. 2008; Vione et al. 2006, 2007). However, the kinetics of the photolytic reactions is presently not well understood, in particular the hydroxyl radical generation from organic compounds. Also the chemistry of 4-tert-octylcatechol, the presumed product of 4-tOP photolysis and the mechanism of toxicity is presently obscure. Degradation using AOP technologies increases appreciably when MnO_2, TiO_2 or $NaBiO_3$ is used as photocatalyst (Wu et al. 2012; Chang et al. 2010). The use of transitional metal oxide sensors is being developed for the attenuation of 4-tOP in water. In all photolytic treatments, the generation of more toxic by-products is a concern for the health of the aquatic taxa. 4-tOP is biodegradable in aquatic medium under aerobic conditions (Ying and Kookana 2003; Gledhill 1999) by bacterial (Toyama et al. 2011) and fungal activities (Rajendran et al. 2017) through their enzymatic activities. Preliminary studies by Qhanya et al. (2017) on four bacterial isolates recovered from South African soil and belonging to *Pseudomonas*, *Enterobacteria* and *Stenotrophomonas* species may be useful in degrading alkylphenols, the group to which 4-tOP belongs. The ligninolytic enzyme laccase and manganese peroxidase synthesized extracellularly by white rot fungi have been shown to effectively degrade 4-tOP (Chang et al. 2016; Tamagawa et al. 2007). Also non-ligninolytic fungi such as *Umbelopsis isabellina* offer higher advantages such as faster metabolic processes and rapid colonization of environmental matrices over ligninolytic fungi (Krupinski et al. 2014). It is not precisely known whether recent claim of degradation by ligninolytic fungi in aquatic medium provides a contrary opinion (Marco-Urrea et al. 2015) given that the white rot fungi thrive well on lignin-rich medium such as wood. Laboratory demonstrations defining optimum conditions such as optimum 4-tOP concentration and conditions for optimum cell growth are required. 4-tert-octylcatechol, 4-hydroxybenzaldehyde, hydroquinone and 1,2,4-trihydroxybenzene have been reported as the products of biodegradation (Rajendran et al. 2017; Cajtham 2015) with projected reduction of oestrogenicity (Janicki et al. 2016; Moon and Song 2012). However, toxicities of these degradation products have not been fully investigated. There are reports of successful application of crude plant enzymes (peroxidases) in 4-tOP reduction process in water (Reis et al. 2014; Reis and Sakakibara 2012) which could also be relevant for wastewaters. Table 6 provided removal efficiencies by aquatic plant enzymes. Asides the projected environment-friendly by-products (Chai et al. 2003), the wide pH range spanning acidity to alkalinity under which the catalysis takes place shows that the effectiveness of the remediation process is unlikely to be grossly inhibited by the dissociation constants (pKs) of the pollutants. The technique is still in its infancy for large-scale application.

4 Fates of 4-tert-Octylphenol

The fate of this compound or any one for that matter depends on the environmental medium in which it is found, liability to physicochemical reactions and tendency to bioaccumulate. In view of the high distribution constant between sediments and water (Salgueiro-Gonzalez et al. 2015c) as well as its hydrophobicity, the pollutant is expected to sorb more on to the river sediments than dissolved in water (Czech et al. 2016). Thus the compound finally ends up in biosolids. During secondary treatment, significant amount of the pollutant is sorbed to the sludge which is often disposed of in the soil or reused for agricultural purposes (Kelessidis and Stasinakis 2012). Besides biodegradation, adsorption to sludge is the most effective process of attenuation of the pollutant in water.

5 Endocrine Interference Activities of 4-*tert*-Octylphenol

4-tOP is an oestrogen receptor agonist (half inhibition concentration IC_{50}, of 3.47 μM) (Li et al. 2012), and the compound has been reported to have equal affinities for the two isomers of oestrogen receptors (ERα and ERβ) in reporter cell lines (Paris et al. 2002). The interaction with these ERs is thought to account for some of its adverse effects including effect on aromatase activity (the conversion of androgens to oestrogens) as well as on endogenous level of oestrogen (Dong et al. 2014) which affect hormone production, induction of reproduction toxicities involving spermatogenesis in mammals (Bian et al. 2006; Bendsen et al. 2001), reduced egg production in the female fish (Zoller 2006), induction of VTG (vitellogenin, a phosphoprotein produced by the liver in the oviparous vertebrates) in male fish (Madsen et al. 2006) as well as in amphibians (Porter et al. 2011) and other endocrine-related effects (Dang 2016). Prior exposure of murine splenocytes to E2 reduces 4-tOP toxicity (Blake et al. 1997) showing that the latter has weaker oestrogenic activity in these cells. The reported proliferation of MCF-7 cells through the mechanism of cyclin D1 expression on exposure to 4-tOP appears to support the suspicion of its carcinogenicity in some cells. The reversibility of the cyclin D1 expression by ER antagonist, ICI 182,780 (Lee et al. 2014) shows that the pathway is ER related. But in bovine oocytes, low concentrations (0.1–1 ng/mL) of 4-tOP have been reported to downregulate ERα genes leaving ERβ unaffected (Pocar et al. 2003) possibly showing ability to downplay E2-induced proliferation in these cells (Helguero et al. 2005). Oestradiol equivalent (EEQ) is an index of relative oestrogenic activity and is reported as oestrogen equivalent factor (EEF) (Tables 7 and 8).

EEF is a ratio of EC_{50} (median effective concentration) of E2 to EC_{50} of the compound and can differ markedly among laboratories as the result of using different testing protocols or different in vitro assays (Compare Tables 7 and 8). E2 is known to exert adverse effects in vivo at concentration above 1 ng/L (Environmental Agency 2004); consequently in vitro assay reporting EEQ of a compound above 1 ng/L is taken as injurious in vivo. The in vitro report from reporter cell lines by Paris et al. (2002) and reduced plasma testosterone in fish (Du et al. 2008) seem to suggest antiandrogenic activity of 4-tOP purporting that the compound displaces androgens from their receptors. The observation is supported by Ahbab et al. (2017) who reported reduced androgenic activities in prepubertal male rats exposed to high (125 mg/kg/day for 90 days) 4-tOP dosage. But increased androgenic hormone (testosterone) production in the rat Leydig cells reported by Jambor et al. (2016) could be associated with ER-independent mediated androgenicity or weak oestrogenicity in the testicular cells. The molecular docking

Table 7 4-*tert*-Octylphenol removal efficiency by enzymes sourced from aquatic plants (Reis et al. 2014)

Crude enzyme	Plant source	Removal efficiency (%)
Peroxidase (soluble)	Hornwort (*Ceratophyllum demersum*)	98.1
	Crystalwort (*Riccia fluitans*)	99.3
	Amazon frogbit (*Limnobium laevigatum)*	76.6
	Giant Duckweed (*Spirodela polyrhiza*)	87.8
Peroxidase (ionically cell wall-bound)	Hornwort (*Ceratophyllum demersum*)	95.6
	Crystalwort (*Riccia fluitans*)	94.9
	Amazon frogbit (*Limnobium laevigatum)*	30.1
	Giant Duckweed (*Spirodela polyrhiza*)	59.4
Peroxidase (covalently cell wall-bound)	Hornwort (*Ceratophyllum demersum*)	78.9
	Crystalwort (*Riccia fluitans*)	96.6
	Amazon frogbit (*Limnobium laevigatum*)	24.8
	Giant Duckweed *(Spirodela polyrhiza)*	44.8
Polyphenol oxidase	Hornwort (*Ceratophyllum demersum*)	31.1
	Crystalwort (*Riccia fluitans*)	37.4
	Amazon frogbit (*Limnobium laevigatum*)	79.8
	Giant Duckweed *(Spirodela polyrhiza)*	70.2
Glutathione-S-transferase	Hornwort (*Ceratophyllum demersum*)	18.6
	Crystalwort (*Riccia fluitans*)	2.80
	Amazon frogbit (*Limnobium laevigatum*)	7.40
	Giant Duckweed *(Spirodela polyrhiza)*	3.84
Plant laccase	Hornwort (*Ceratophyllum demersum*)	10.6
	Crystalwort (*Riccia fluitans*)	20.6
	Amazon frogbit (*Limnobium laevigatum*)	10.2
	Giant Duckweed (*Spirodela polyrhiza*)	11.9

of 4-tOP with androgen receptors conducted by Rehan et al. (2015) showed that the compound has the potential to interfere with testosterone (the natural agonist) occupation of androgen receptor-binding sites indicating anti-androgenicity and thus ability to interfere with male reproductive process. Data from models such as in silico as well as in vitro studies which to our knowledge are lacking should assist in shedding more lights on the potency of 4-tOP as AR ligand. There are reports linking 4-tOP to delayed metamorphosis in amphibians which is normally regulated by the thyroid hormones (Croteau et al. 2008; Crump et al. 2002). The decreased plasma thyroxine levels reported in the amphibian embryos exposed to the pollutant (Haselman et al. 2016) could be associated with the effect of the pollutant on the

Table 8 Relative oestrogenic potency (relative potency) of 4-tert-octylphenol and nonylphenol

In vitro tests										
					Zonal radiata					
					Proteins					
	YES		E-Screen		Production		MELN		RTG-2	
Compounds	Relative potency	EC_{50} (nM)[a]	Relative potency	EC_{50} (nM)	Relative potency	EC_{50} (nM)	Relative potency	EC_{50} (nM)	Relative potency	EC_{50} (nM)
4-tOP	2×10^{-3}	27,000	6×10^{-4}		3×10^{-5}	32,000	5×10^{-6}		1×10^{-3}	155
	8×10^{-6}[a]									
NP	6×10^{-4}	8,400			8×10^{-5}	14,000	10×10^{-6}		1×10^{-3}	182
	3×10^{-5}[a]									
E2	1.0	0.21	1.0		1.0	1.05	1.0		1.0	0.2
	1.0[a]									

Values are in oestradiol equivalent factor EEF (Leusch et al. 2010; Jarošová et al. 2014)

Relative potency = oestrogenic potency relative to E2

YES yeast oestrogen screen, *E-Screen* oestrogen screen, *RTG-2* rainbow trout gonad cell line, *MELN* transfected human breast cancer cell line

[a] Data after Rutishauser et al. (2004)

activities of iodothyronine deiodinases (Croteau et al. 2009). Activities of deiodinases (Types I and II) can lead to activation of pro-hormone thyroxine producing triiodothyronine or may lead to the generation of inactive metabolites through loss of iodine residue. Thus, 4-tOP perturbs thyroid homeostasis.

6 4-*tert*-Octylphenol Metabolism and Toxicity

4-tOP is rapidly metabolized in humans and rats regardless of the route of administration into polar glucuronide (Hamelin et al. 2008; Kawaguchi et al. 2007; Upmeier et al. 1999). In humans in addition to sulphate conjugation (Shangari et al. 2005), glucuronidation in the liver microsomes is the predominant detoxification pathway, whereas it occurs mainly in the intestinal microsomes in monkey and mouse suggesting involvement of species in the detoxification process (Hanioka et al. 2017). The conjugated 4-tOP is voided via urine (Calafat et al. 2008). Reports of pharmacokinetic studies in rats (Upmeier et al. 1999; Certa et al. 1996) show that strain and sex may play a role in the metabolic process. The activity of cytochrome P450 (CYP) is reduced in male SD rats exposed to 4-tOP (Hanioka et al. 2000). Expectedly, hydroxylation of 4-tOP necessary for its conjugation is reduced which may account for the reported toxicity in the animal exposed to the compound (Saggu et al. 2014). 4-tOP is an agonist of the pregnane X receptor and the constitutive androstane receptor, both of which are capable of regulating several detoxification enzymes such as those of the CYP2B and CYP3A families responsible for the metabolism of steroids (Kretschmer and Baldwin 2005), indicating ability of the pollutant to interfere with detoxification of the steroids as well as other compounds sharing metabolic pathways with the steroids. Exposure to 4-tOP is a matter of concern also because of its potential to interfere with normal metabolism in the taxa (Wang et al. 2012; Bonefeld-Jørgensen et al. 2007) producing undesirable physiological effects. Inhibitory effect of 4-tOP on algae has been demonstrated using *Skeletonema pseudocostatum* ($EC_{50} = 5.6$ μM) (Petersen et al. 2014) a marine alga found in non-polar regions of the world (Kooistra et al. 2008). The activity of the pollutant in algae is known to affect photosynthesis and energy flow through photosystem II (Perron and Juneau 2011), decreases the ratio of variable and maximal fluorescence, causes thickening and wrinkling of the cell wall matrix and increases the number of starch granules with a reduced size (Zhou et al. 2013). The adverse effect of the pollutant on the algae is of a considerable threat to the overall well-being of the taxa and the ecosystem (El Gamal 2010). In crabs (*Uca pugilator*) exposed to 4-tOP, shedding of eggshells is delayed (Zou and Fingerman 1999a, b), while growth is delayed in *nauplius* copepods (*Acartia tonsa*) (Andersen et al. 2001). 4-tOP interference with thyroid hormones in tadpoles leading to impaired metamorphosis is well known (Croteau et al. 2009). Adult *Xenopus tropicalis* frog developed male oviduct and VTG in froglets when exposed to 4-tOP (Porter et al. 2011) which could worsen the declining amphibian population (Houlahan et al. 2000) and in synergy with similar activities if unaddressed may drive the taxonomic group into the verge of extinction. The embryos of zebra fish exposed to doses higher than 5 µM 4t-OP for 3 days exhibited 100% mortality (Saputra et al. 2016) showing early-life toxicity. Toxic endpoints such as liver damage (Kaptaner 2016) and anaemia (Vázquez and Nostro 2014) have been reported in fish exposed to water containing 4t-OP. Increased VTG induction, inhibition of the secondary sexual characteristics and increased sex ratio were reported in fish at very low 4t-OP exposure concentrations (OECD 2010; Länge et al. 2001). Intersex was reported in fish containing high levels of 4-tOP in their bile (Bizarro et al. 2014; Puy-Azurmendi et al. 2013). Hepatotoxicity via oxidative stress was recorded in rats exposed orally to >400 µM 4-tOP/kg body weight for 8 days (Saggu et al. 2014), but minimum effects on oestrous cycle were recorded at >600 µM/kg for 35 days (Sahambi et al. 2010) showing failure to induce toxic effect on reproductive organs via oestrogen-mediated pathway in the female rats. Exposure of rat mast cells to 10–20 µM 4-tOP for 1 h promoted degranulation without being cytotoxic (Kennedy et al. 2012). Degranulation is a route through which oestrogenic chemicals are thought to precipitate allergic diseases (Narita et al. 2007) suggesting modulating effect on immune system. 4-tOP induced apoptosis in rat Sertoli cell (Qian et al. 2006) as well as in human embryo stem cells (Kim et al. 2006). In testis, the mechanism of cell death induction may involve inhibition of endoplasmic reticulum Ca^{2+} pump (Hughes et al. 2000). Kinetics study by Xie et al. (2013) showed 4-tOP potentials to bind human serum albumin. This may suggest a toxicity process in human beings because protein secondary structure (conformation) and ultimately its metabolic functions such as transportation of endogenous molecules become impaired as the result (Qin et al. 2010). Recent epidemiological studies have associated urinary 4-tOP with balance disorder in adult humans (Shiue 2013), but no conclusion can be drawn at present in view of paucity of data as well as poor understanding of its biological mechanisms. In order to evaluate the potential risk to ecosystems, chemical-specific monitoring trigger quotients (Sengupta et al. 2014) or hazard quotient (HQ) otherwise called risk quotient (RQ), defined as the ratio of the maximum environmental concentration (MEC) to the predicted no effect concentration (PNEC), is recommended by European Union (EU 1994). The PNEC value is based on toxicity assays on different organisms (Xu et al. 2011). At HQ value less than 0.1, the risk to the environment is minimal and is medium if the value is between 0.1 and 1. The risk is high when this value is greater than 1 (Chen et al. 2014; Blair et al. 2013). The differing protocols in measuring toxicity are the greatest undoing of this index of potential risk.

7 4-tert-Octylphenol Specific Toxicities

Many of the reported 4-tOP toxicities are mediated by ER signalling pathway. The compound adverse effects on sexual development and reproduction reported in rats such as testicular dysfunction in males (Bian et al. 2006; Blake et al. 2004), anovulation following ovarian disturbance (Katsuda et al. 2000) as well as reduction of uterine contractibility (Kim et al. 2014; An et al. 2013) in the females are thought to be mediated by ER pathway. Cell-based studies so far have shown that 4-tOP plays significant roles in carcinogenesis via a number of mechanisms which include ER-mediated signalling pathways. The mechanism of the increased proliferation of BG-1 ovarian cancer cell by 4-tOP (1 μM) is thought to be downregulation of transforming growth factor β receptor 2 (TGF-β R2) and upregulation of the oncogene *c-myc* (Park et al. 2011), while metastasis is produced in the cell by increasing the expression of N-cadherin (Shin et al. 2016), a protein regarded as an inducer of epithelial-mesenchymal transition (Hotz et al. 2007) which is a critical event in cancer progression. The expression of cathepsins B and D in human breast cancer cells is via ER-mediated signalling pathway (Lee and Choi 2013). These works appear to support the reports that 4-tOP plays a definitive role in cancer progression (Table 8) by upregulating oncogene expression through abnormal activation of ER-mediated signalling pathway (Jung et al. 2012; Pisapia et al. 2012; Hwang et al. 2011). In wildlife such as fish, the expression of gene encoding O^6-methylguanine-DNA methyltransferase (O^6-MGMT), a DNA repair enzyme, is repressed by 4t-OP. The activity of this enzyme decreases in some tumour tissues and cell lines, and its inactivation is thought to be related to cancer progression (Chen et al. 1992). 4-tOP at the micro level has been reported (Lee et al. 2014) as capable of altering breast cancer cell (MCF-7) cycle by upregulating the expression of cyclin D1 and downregulating expression of p21 on exposure for 1 week. Cyclin D1 is a factor responsible for cell-cycle progression from G1 to S phase, while p21 is a potent cyclin-dependent kinase inhibitor that arrests the cell cycle in G1 phase in response to stimuli (Hwang et al. 2012). Perturbation of cell cycle is the hallmark of cancer development and progression (Junk et al. 2013) (Table 9).

Genotoxicity involves genetic alterations in somatic and germ cells, and these have been associated with serious health effects including cancer, degenerative diseases, reduced fertility and inherited diseases. Somewhat evidence of genotoxicity by 4-tOP appears to have been provided in rat orally exposed to 250 mg/kg for 4 weeks (Ulutas et al. 2011). The toxicity has been recorded in tissues of fish from water containing significant amount of 4-tOP; however, no direct evidence was presented linking the chemical with the genotoxicity (Sunjog et al. 2016). A recent report showed lack of unequivocal data supporting genotoxicity of 4-tOP (Mertens et al. 2016).

8 Conclusion and Future Direction

4-tOP is a multimedia compound having been detected in all environmental media such as air, soil and water. Data are accumulating in support of the view that the compound interferes with endocrine homeostasis and subsequently exerts toxicity via multiple pathways. The compound oestrogenicity is weak; its activation of ER being less than that of natural oestrogen E2 and could be more of (anti)androgen. Current knowledge about modes of endocrine interference and by extension toxicity is

Table 9 Reported 4-*tert*-octylphenol toxicities

Biological sample	OP concentration (mg/L)	Exposure	Toxicity	Reference
Breast cancer cell (MCF-7)	>0.21	6 days	Cell proliferation; increased expression of cathepsins	Lee et al. (2014), Lee and Choi (2013)
Human placental choriocarcinoma JEG-3 cell line	>4.13	24 h	Decreased aromatase activity	Pérez-Albaladejo et al. (2017)
Hepatocyte (*Alburnus tarichi*)	>2.06	24 h	Cytotoxic; oxidative stress	Kaptaner (2016)
Zebra fish (*Danio rerio*) embryos	>0.21	3 days	Mortality	Saputra et al. (2016)
Rat liver	0.10 kg/day	8 weeks	Oxidative stress; DNA fragmentation	Saggu et al. (2014)

therefore still hazy. Although the compound is biodegradable, it is capable of bioaccumulation in living tissues leading to toxicity. So much attention has been focused on ER signalling pathway to the near neglect of other possible pathways such as thyroid receptor signalling pathway. This has limited our current knowledge on its toxicity which may be a dangerous dimension as it may frustrate efforts at ridding the environment of the pollutant. There is need for concerted effort on removal strategies. Several of the demonstrated removal techniques need to be developed for large-scale use. Strategies for removing this compound from aquatic resource using enzymes such as plant peroxidases hold promise following their efficient removal and generation of less toxic by-products and should be fully explored. WWTPs are designed primarily not to remove EDs, their effluents are sources of surface water micropollutants and their degradation products. Their toxicological potential deserves consideration especially in the design option of WWTPs.

9 Summary

4-tert-Octylphenol (4-tOP), a degradation product of the non-ionic surfactants alkylphenol polyethoxylates, is a multimedia compound detectable in all environmental compartments such as water, air and soil at ultra-low (ng/L) levels. Following its exposure to human, 4-tOP is detectable in physiological samples such as blood, breast milk and urine. Their toxicity includes endocrine disruption and is mediated by oestrogen receptor signalling which has been demonstrated in vitro and in vivo. Data are increasing supporting its role in carcinogenesis. The compound is biodegradable, and a number of removal mechanisms have been demonstrated of which the use of plants and fungal enzymes appears most promising. However, toxicity of most of the degradation products including those from biological process has not been ascertained.

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The Impact of Metal-Rich Sediments Derived from Mining on Freshwater Stream Life

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P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 248*, Reviews of Environmental Contamination and Toxicology Volume 248,
https://doi.org/10.1007/398_2018_21

Abbreviations

AMD	Acid Mine Drainage
AMDI	Acid Mine Drainage Index
AMR	Average Metabolism Response
ANZECC	Australian and New Zealand Environment and Conservation Council
ARISA	Automated Ribosomal Intergenic Spacer Analysis
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
ASPT	Average Score Per Taxon
AVS	Acid volatile sulphides
AVS-SEM	Simultaneously extracted metals released from a sediment sample during AVS extraction
AWIC	Acid Water Indicator Community
BLM	Biotic Ligand Models
BMWP	Biological Monitoring Working Party
BSI	Biotic Sediment Index
CCAR	Chronic Criterion Accumulation Ratio

CCME	Canadian Council of Ministers of the Environment
CCU	Cumulative Criterion Unit
CMD	Community Metabolism Diversity
DARLEQ	Diatoms for Assessing River and Lake Ecological Quality
DNA	Deoxyribonucleic acid
DOM	Dissolved organic matter
EPA	US Environmental Protection Agency
EPT	Number of Ephemeroptera, Plecoptera and Trichoptera taxa
EQG	Environmental quality guidelines
EQS	Environmental quality standards
FIAM	Free Ion Activity Model
HA	Humic acid
IOBS	Oligochaete Index of Sediment Bioindication
LC_{50}	Lethal concentration (that will kill 50% of the population)
LEAFPACS	Assessment method for macrophytes (UK)
LOEC	Lowest observable effect concentration
NOEC	No observed effect concentration
NTAXA	Number of scoring families
O/E	Observed/expected
PCBs	Polychlorinated biphenyls
PEC	Predicted environmental concentration
PICT	Pollution-induced community tolerance
PNEC	Predicted environmental no effect concentration
QMCI	Quantitative Macroinvertebrate Community Index
RIVPACS	River Invertebrate Prediction and Classification System
RNA	Deoxyribonucleic acid
SEM	Simultaneously extracted metals
SFG	Scope for growth
SIGNAL-MET	Stream Invertebrate Grade Number Average Level–metals
SQC	Sediment Quality Criteria
SQG	Sediment Quality Guidelines
SSD	Species Sensitivity Distributions
TITAN	Threshold Indicator Taxa Analysis
TOSC	Total Oxyradical Scavenging Capacity
T-RFLP	Terminal Restriction Fragment Length Polymorphism
WFD	Water Framework Directive (of the European Union)
WFD-UKTAG	Water Framework Directive – UK Technical Advisory Group
WHAM	Windermere Humic Aqueous Model
WoE	Weight of evidence
WQC	Water quality criteria
WQG	Water quality guidelines

1 Introduction

Metal-rich sediments have the potential to impact upon life in freshwater streams and rivers and, thereby, to inhibit recovery of ecological conditions after any remediation of mine water discharges. The extent to which such metal-rich sediments are causing impacts is as yet unknown. Metal-rich sediments by definition consist of sediments with high concentrations of trace metals, all of which are toxic to biota above a certain threshold level. In mining-impacted rivers, metals tend to be strongly associated with sediment, and these high affinities mean that these metal-rich sediments do not give up their associated metals easily, even when relatively metal-free water is flowing over them, for example, after remediation. Thus, the sediments remain metal-rich over long time periods (Macklin et al. 2006) and have long-term potential ecotoxicological interactions with local biota, unless the sediments themselves are physically removed by active remediation or move downstream by strong water flow to be replaced by new less metal-rich sediment.

The composition of riverbed sediments will influence biota even in the absence of metal contamination (Jones et al. 2012a, b, 2014; Kemp et al. 2011), and it is necessary in any investigation of the effect of metal-rich sediments on biota to tease out the separate effects of any modification of bed sediments per se from the effects of any sediment-associated metals. Furthermore, it is necessary to separate the effects of any sediment-associated metals from any other effects associated with mining activities (low pH water, channel modification, industrial pollutants) and the development of mining communities (organic pollution).

Laboratory-derived environmental quality standards are difficult to apply to the field situation as many complicating factors exist in the real world, such as physiological acclimation, water chemistry, metal interaction effects and particularly the ingestion of sediment by deposit-feeding biota, which are at particular ecotoxicological risk in the presence of metal-rich sediments (Environment Agency 2009). Thus, as alternatives to laboratory-derived standards, there is a strong case to consider other field-relevant measures of toxic effects in freshwater streams and to seek better biological tools to detect, diagnose and ideally predict community-level ecotoxicological impairment. Ecological principles need to be integrated into ecotoxicological research if there is to be a full understanding of the effects of contaminants in real environmental situations (Clements and Rohr 2009). Luoma and Rainbow (2005, 2008), recognising these challenges, advocated the use of risk assessment approaches with a stronger integration of field observations into decision-making processes. Such a ‘lateral risk’ assessment and risk management process, encompassing hitherto separate approaches and using several lines of evidence, recognises the strong potential contribution of observational data from nature in such a decision-making process.

Against this context, the aim of this review was to concentrate on field measures of toxic effects of trace metal-rich sediment in freshwater streams, with less emphasis on laboratory-based toxicity testing approaches. To this end, this review provides an overview of how metal-rich sediments impact on freshwater stream life, incorporating recent developments in the field, focusing on biological impacts linked to trace metal contamination of sediment.

1.1 General Concepts of Metal Ecotoxicity

Trace metals (and metalloids) – those that are typically present in organisms in trace amounts (typically, but not always, less than 0.01% by dry weight in an organism) – are most commonly the focus of mining activity. However, definitions of 'trace metals' and other terms, e.g. 'heavy metals' (see Luoma and Rainbow 2008), are inconsistent. For the purposes of this review, we exclude major metals such as sodium, magnesium, potassium and calcium and also the rare earth elements (lanthanides and actinides). Thus, the metals of concern potentially include cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), tin (Sn), vanadium (V) and zinc (Zn) and the metalloids arsenic (As) and antimony (Sb). These metals may be either the focus of mining activity or present in other material and for convenience are all referred to here as trace metals (Luoma and Rainbow 2008).

The toxicity of trace metals is an obvious major driver of the detrimental effects of metal-rich sediments derived from mining on the biota of local freshwater streams. All trace metals, whether essential or non-essential, are toxic to life above a certain bioavailable threshold concentration (Fig. 1). This bioavailable threshold concentration may be extremely low or even absent in the case of non-essential metals (Fig. 2), but it is likely that environmentally low bioavailable concentrations of a

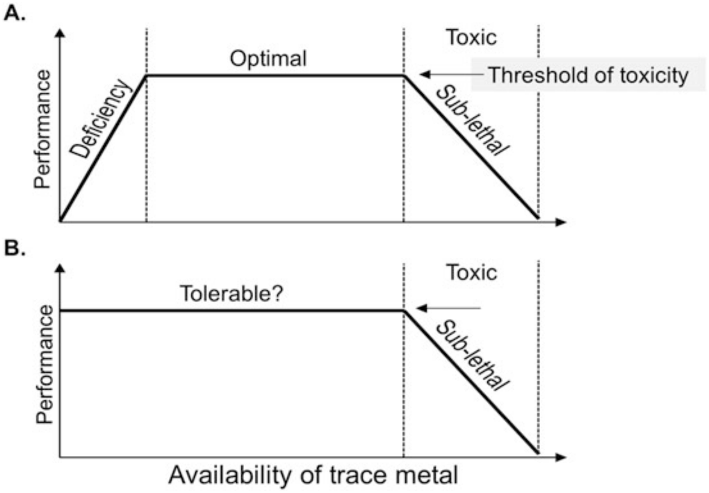


Fig. 1 Effects of increasing availability of (**a**) essential and (**b**) non-essential trace metals on the performance (e.g. growth, production, fecundity, survival, etc.) of an organism (from Luoma and Rainbow 2008)

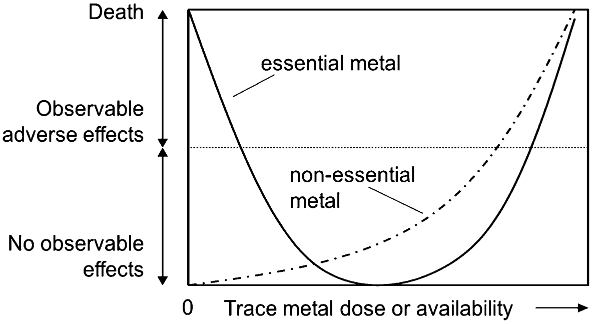


Fig. 2 Biological response of an organism to increasing dose or availability of an essential or non-essential trace metal (from Luoma and Rainbow 2008)

non-essential metal are tolerable without significant sublethal physiological cost to an organism (Fig. 1). In the case of essential trace metals, a very low metal bioavailability will cause negative performance effects as a result of deficiency, whilst toxic effects will occur at bioavailable concentrations above the toxic threshold (Figs. 1 and 2). Once toxic effects are apparent (initially sublethal and ultimately lethal), these effects will increase in a dose-related manner for both essential and non-essential metals (Fig. 2). It does not follow that non-essential metals are generally more toxic than essential metals, Cu being both essential in small doses and yet one of the most toxic metals at higher doses. Nevertheless, it is true that the non-essential metals Hg and Ag do top most relative toxicity tables of trace metals (Luoma and Rainbow 2008).

Trace metals are toxic as a result of their chemical properties, especially their preference for binding to the elements sulphur (S) and nitrogen (N). S and N are present in the make-up of many biological macromolecules, especially in proteins, vital to metabolism not least in their guise as enzymes. This affinity for S and N actually underlies many of the essential roles of trace metals in metabolism, their controlled presence in a protein structure enabling them to act as a catalytic centre in an enzyme or to bind oxygen, as in the respiratory proteins haemoglobin (Fe) and haemocyanin (Cu). The downside of the affinity of trace metals for S and N is that trace metals present in excess, unless prevented from doing so, will inevitably bind to S- and N-containing groups in biological molecules in the wrong place at the wrong time. They might replace another trace metal playing a key essential role in a protein, thereby inhibiting the catalytic activity of an enzyme, or they might bind elsewhere on the protein, distorting its structure and preventing its biochemical function. Either way, a toxic effect has been caused.

Life has evolved in the presence of trace metals, and many have become incorporated into biochemistry as essential metals playing key metabolic roles. Correspondingly organisms have evolved physiological mechanisms to prevent a build-up of all potentially toxic metals in cells in a metabolically available form that gives them uncontrolled access to bind to the wrong molecule. In practice, trace metals, essential and non-essential, are typically bound in cells to selected molecules that hold them out of harm’s way – either irreversibly or perhaps reversibly in the case of essential metals. These metals have been detoxified by binding to sacrificial sites with a high affinity for that metal, perhaps a special protein (e.g. metallothionein) or to an inorganic granule such as an insoluble metal pyrophosphate. Metals newly entering an organism, for example, an aquatic invertebrate, via permeable external surfaces or within the gut are initially in metabolically available form with the potential to bind anywhere. They are usually excreted quickly or detoxified, to be removed from causing damage (Fig. 3).

Toxicity occurs when the total rate of metal uptake combined across all routes into an organism exceeds the combined rates of excretion and physiological detoxification (Luoma and Rainbow 2008). Uptake rates are typically not under the control of the organism and increase passively with the site-specific bioavailabilities of the metals in the local environment. Under most circumstances the combined rate of metal uptake across all metal sources is easily matched by some combination of the rates of excretion and detoxification of the metal. Under extremely high local metal bioavailabilities, toxicity ensues. At and above the toxic threshold (Figs. 1 and 2), the rate of metal uptake is greater than the combined rates of excretion and detoxification (Casado-Martinez et al. 2010b; Croteau and Luoma 2009). Under these circumstances accumulated metals build up intracellularly in a metabolically available form, and these metals bind where they are not wanted, with toxic effects (Fig. 4). Since the rate of uptake of metal increases with increased local metal bioavailability and since, above the toxic threshold, toxicity is related to uptake

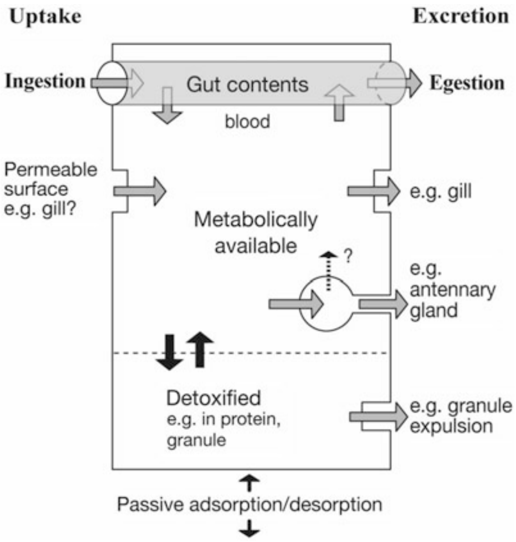


Fig. 3 A schematic representation of the body metal content of an aquatic invertebrate such as a decapod crustacean. When metal first enters the body, it will initially be metabolically available, before potentially being stored in detoxified form, probably elsewhere in the body after internal transport via body fluids. Detoxified storage may be permanent or temporary. Trace metals taken up into the body may or may not be excreted, either from the metabolically available component or from the detoxified store (from Luoma and Rainbow 2008)

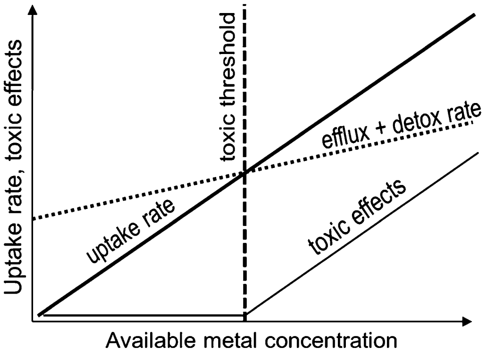


Fig. 4 Schematic representation of how the uptake rate (combined across all routes of uptake) of a trace metal and hence (after a threshold) the manifestation of toxic effects will increase with the availability of the trace metal to an aquatic organism. Toxic effects occur when the uptake rate exceeds the combined rates of efflux and detoxification (after Luoma and Rainbow 2008)

rate, it follows that within the toxic range of exposures, toxicity increases with metal bioavailability, although not necessarily linearly (Fig. 4).

1.2 Uptake and Accumulation of Metals in Organisms

Whilst the different possible routes of entry of metals across a cell membrane into a cell and, therefore, into an organism are not of specific relevance here (but see Luoma and Rainbow 2008), it is relevant to consider the different sources of metals available to organisms inhabiting mine-affected freshwater systems. Any organism bathed in water will take up trace metals from solution across the external membrane of cells in contact with the medium. In the case of multicellular animals, a typical route is via the surfaces of respiratory organs, which are particularly permeable, but uptake through other exposed soft tissues does occur. Furthermore, animals will take up metals in the alimentary tract, from ingested material (with availability potentially enhanced after digestion) and water (Croisetière et al. 2006; Poteat and Buchwalter 2014; Wang 2013; Wang et al. 1995): the gut wall is specifically adapted for the uptake of materials, making this route especially vulnerable. In the case of sediment-dwelling animals, potential routes of metal uptake are direct from solution (typically the water column even if transported into burrows by irrigation currents (de Jonge et al. 2009) and, to a lesser or negligible extent, from pore water in the sediment) and from the diet, especially from ingested sediment in the case of deposit feeders. In addition to uptake from solution, sediment-associated herbivores will take up dietary metals from primary producers such as benthic diatoms, macrophytic algae, moss or higher plants associated with sediment. Predators accumulate metals from prey (Croisetière et al. 2006), whether herbivores, deposit feeders or animals at other trophic positions in the food web. Predatory invertebrates appear to be less susceptible to the toxic effects of metals than primary consumers (Liess et al. 2017), possibly as a consequence of less metals in their diet than that of invertebrates which feed on basal resources.

Once metals have been taken up by organisms, they may be excreted but are more typically accumulated, a process that necessarily involves the storage of metals in detoxified form (Fig. 3). If excretion balances uptake, organisms are said to regulate the body concentration of the metal. Regulation of body metal levels is typically restricted to essential trace metals and to date is a feature only found in vertebrates and a few invertebrates, such as decapod crustaceans (Luoma and Rainbow 2008). Net accumulation patterns vary from weak to strong accumulation, according to the relative rates of metal uptake and excretion, the difference necessarily being made up for by the rate of detoxification. Any accumulator detoxifies the extra metal. If this is stored permanently, then body metal content increases continuously. In some cases, animals can excrete metal in detoxified form, for example, as metal-rich granules from the gut or Malpighian tubules. In this case, accumulated concentrations may reach steady-state levels that vary with local metal uptake rates and thus local conditions.

With the exception of those few examples where invertebrates are capable of regulation, aquatic invertebrates will have accumulated metal concentrations that vary with local metal bioavailabilities. Toxicity will occur once local bioavailability rises above the toxic threshold. When the concentration of metabolically available metal rises before the extra incoming metal can be excreted or detoxified, the metabolically available fraction can cause sublethal and ultimately lethal toxic effects. The total accumulated metal concentration, however, will depend on the previous history of metal exposure of the invertebrate, and death can occur at any total accumulated metal concentration, depending only on the potentially toxic, much smaller metabolically available concentration. Thus, in contrast with the case of organic contaminants, in any organism that uses detoxification, there is no critical body concentration of an accumulated metal at which death occurs (despite there being a critical concentration of metabolically active metal) – in practice this is true of nearly all invertebrates (Adams et al. 2010; Luoma and Rainbow 2008).

2 Sources, Behaviour and Consequences of Sediment-Associated Metals in Mine-Impacted Streams

2.1 Sources of Metals in Mine-Impacted Streams

The earliest evidence of extractive metallurgy originates from Eastern Europe around 7,000 years ago (Radivojević et al. 2010), and since early inception, the industry has been associated with metalliferous mineral mining. The extent to which mining of metal ores occurs depends on both geological and economic factors. In

Britain, the birthplace of the industrial revolution, metal ores have been mined for more than 4,000 years, with nearly 5,000 mine sites identified (Environment Agency 2008a, b, 2012), although currently very few metal mines are active. A long history of industrialisation has resulted in historical extraction of hundreds of thousands of tonnes of Cu, Pb and Zn ore from the major mining regions, together with other metals including Ag, As, Au, Cd, Fe, Sn and Ni (Environment Agency 2008b). Despite closure of almost all metal mines in the UK the early twentieth century, abandoned metal mines still contribute significantly to the metal pollution entering British rivers (Environment Agency 2008a; Mayes et al. 2010: Fig. 5).

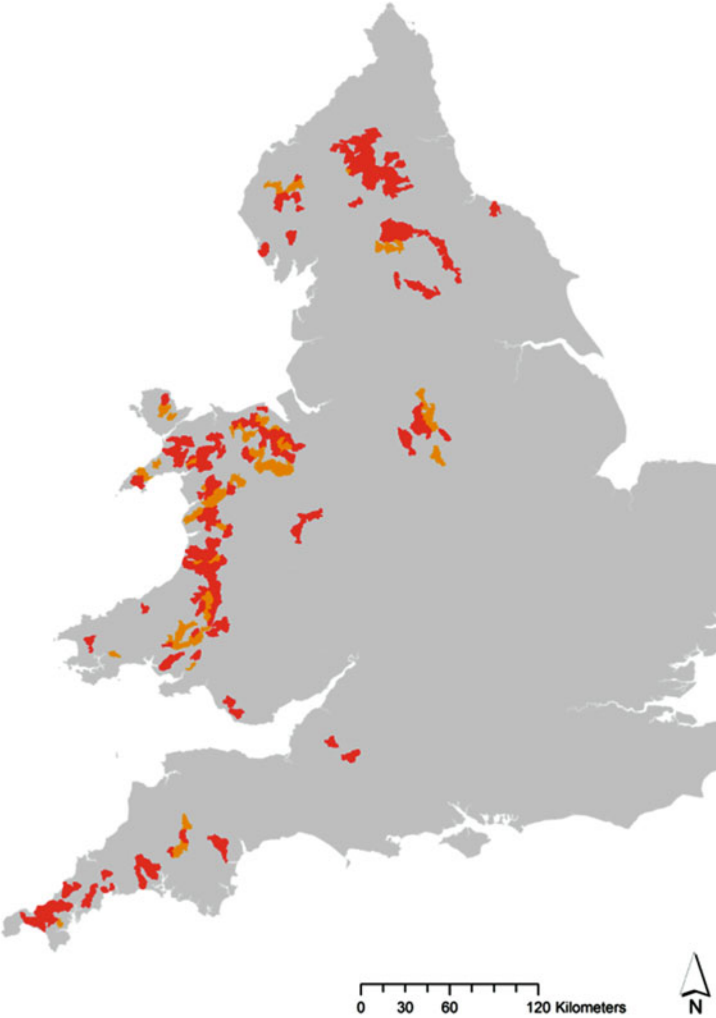


Fig. 5 River catchments in England and Wales at risk (red) or probably at risk (orange) of damage from abandoned metal mines (data from Environment Agency)

Although metals are naturally present at elevated environmental concentrations in certain geologies (Schmidt et al. 2012b), metals are liberated into the environment both in dissolved and particulate form by mineral extraction and a range of ore-processing activities, including smelting, calcination and milling (Environment Agency 2012; Mayes et al. 2009; Mighanetara et al. 2009). When metal-bearing sulphide minerals, e.g. pyrite (FeS), galena (PbS) and sphalerite (ZnS), are exposed, they are oxidised in the presence of oxygen and water both abiotically and biotically (promoted by Fe-oxidising *Thiobacillus* bacteria) to release SO_4^{2-}, associated metal cations and colloidal Fe (Maia et al. 2012; Singh et al. 1999; Younger et al. 2002). This often results in a discharge of water which typically has a low pH (pH 2–4), a high ionic strength and a high dissolved metal load, termed 'acid mine drainage' (AMD) (Butler et al. 2009; Jones et al. 2013). However, mine water discharges are circum-neutral where pH is buffered by the dissolution of carbonate-rich bedrock (e.g. Hiller et al. 2012; Jones et al. 2013). The concentrations of dissolved free metal ions are dependent upon pH; the concentration of other ions, particularly cations; and concentrations of dissolved organic ligands (Tipping et al. 2016). As mine water enters the oxic upland stream environment typical of mining regions, a number of processes occur including a decrease in stream pH, precipitation of Fe oxyhydroxides and the co-precipitation and/or adsorption of dissolved metals to those oxyhydroxides. These precipitates settle out onto the streambed, coat other mineral and sand grains or can be dispersed considerable distances downstream in suspension (Butler et al. 2009; Hudson-Edwards et al. 1999; Palumbo-Roe et al. 2010). In addition to these fine-grained, ochreous sediments, mining waste including slag and cinder heaps, tailings ponds, gangue and overburden can also provide a source of both fine- and coarse-grained metal-rich sediments to the aquatic environment (Macklin et al. 2006; Mighanetara et al. 2009; Villarroel et al. 2006). Consequently, numerous point and diffuse sources of metals may be active, and fluxes of dissolved and particulate metals are highly variable (Gozzard et al. 2011; Mayes et al. 2009). Seasonal increases in precipitation and groundwater flow and extreme storm/flood events increase the resuspension of precipitates and the erosion of mining waste materials, as well as the potential collapse of mines and failure of tailings dams (Dennis et al. 2003; Hudson-Edwards et al. 1999; Mighanetara et al. 2009) releasing large quantities of metal-rich sediment to the catchment. Furthermore, increased flows can result in the remobilization of previously deposited metal-rich particulates from the riverbed and floodplain. The geochemistry and physical composition of mine-impacted fluvial sediments can be highly heterogeneous, both spatially and temporally.

Streambed sediments comprise detrital mineral grains eroded from mine waste, e.g. cerussite ($PbCO_3$) and arsenopyrite (FeAsS) (Palumbo-Roe et al. 2010; Rieuwerts et al. 2014), and Fe-rich sediments precipitated from AMD which, although often described simply as Fe-hydroxides or ferrihydrite, include a wide range of Fe oxyhydroxide, carbonate and hydroxysulphate minerals, including schwertmannite, goethite, jarosite, lepidocrocite and feroxyhyte (Singh et al. 1999). In addition, sediments may include efflorescent Fe sulphate precipitates and secondary minerals formed from the oxidation and dissolution/precipitation of detrital mineral grains and Fe oxyhydroxides, e.g. scorodite ($FeAsO_4{\cdot}2H_2O$: Hudson-Edwards et al. 1999). Consequently, metals can be partitioned to all sediment fractions including carbonates, Fe/Mn oxyhydroxides, sulphides and the residual sediment fractions (e.g. Bird et al. 2010; Byrne et al. 2010; Maia et al. 2012; Wang et al. 2012) with implications for the mobility and bioavailability of metals. However, the association of metals with the sediment is highly dynamic and is influenced by overlying water chemistry, hydrological conditions and underlying geology. For example, changes in stream pH, ionic strength and dissolved organic carbon content can all result in release of metals to the overlying water column (Butler et al. 2009; Byrne et al. 2010; Palumbo-Roe et al. 2010). Changes in hydrological conditions, for example, seasonally or during storm/flood events, can disturb settled sediment, changing redox conditions and resulting in the oxidative release of metals (Gozzard et al. 2011; Ranville et al. 2004) and dissolution of efflorescent salts (Mighanetara et al. 2009), as well as the migration of metals to less strongly bound mineral phases. Low flow conditions have also been shown to increase the attenuation of metals, as the overlying waters can become supersaturated resulting in the precipitation of Fe oxyhydroxides and co-precipitation/adsorption of other metals; conversely, high flow conditions can result in metal release (Byrne et al. 2013b). As a result, metal-rich sediments stored in the catchment in streambed deposits, floodplains and overbank deposits can present a long-term (100s to 1,000s of years) diffuse source of metal contamination to the catchment (Bird et al. 2010; Lecce and Pavlowsky 2014). These sediment stores can be eroded, transported, dispersed and redeposited throughout the catchment. Their deposition is highly variable, dependent upon fluvial processes, resulting in 'hotspots' of contamination (Dennis et al. 2009; Macklin et al. 2006). Hence, representative field sampling can be challenging.

2.2 Bioavailability of Toxic Metals in Mining-Affected Streams

Bioavailability describes a relative measure of that fraction of the total ambient metal that an organism actually takes up when encountering or processing environmental media, summated across all possible sources of metal, including water and food as appropriate (Luoma and Rainbow 2008). Metals taken up by organisms are handled by the physiological processes of the body, typically to be accumulated by most aquatic invertebrates. Metals from different routes of uptake in aquatic invertebrates (from solution via permeable external surfaces or from the diet via the alimentary tract) may, or may not, share the exact same physiological routes in the body leading to excretion or detoxified accumulation. Nevertheless, the accumulated metal concentrations are additive, irrespective of the route into the body.

Metals associated with metal-rich sediments can be bioavailable to the local biota in a variety of ways. Classically, it has been considered that metals in solution offer the most significant source of metals to aquatic invertebrates, including those living in or on sediments. Thus, the pore water of sediments has been highlighted (not least by environmental regulators) as a very important source of metals to infaunal animals (Besser et al. 2015).

The basic assumption has been that the total concentration of metal in sediment does not matter. It was assumed that the variable driving toxicity was the distribution of metal between sediment particles and water, and exposure was limited to pore waters: this is termed the equilibrium partitioning theory of bioavailability (e.g. di Toro et al. 1991). In practice, the last decade has brought about a paradigm change in our understanding of the significance of the diet in providing an ecotoxicologically significant (often the major) source of metal to aquatic invertebrates (Luoma and Rainbow 2008; Luoma et al. 2005; Wang 2002, 2013; Wang and Ke 2002) and fish (Kraal et al. 1995; Langevoord et al. 1995; Oyoo-Okoth et al. 2012). Thus, it has been shown that diet is by far the most important exposure route for Cd and Cu uptake in five species of grazing mayfly larvae (Cain et al. 2011). Metal concentrations in the benthic invertebrates of the mining-affected Animas River, Colorado, USA, were influenced by feeding habit, with concentrations of Zn, Cu and Cd highest in grazing mayflies and concentrations of Pb highest in a detritivorous stonefly (Besser et al. 2001). Furthermore, Cu and Cd were efficiently transferred to fish via dietary exposure (Besser et al. 2001). Dietary uptake of Cd by fish (common carp, *Cyprinus carpio*) feeding on invertebrate prey can be as important as direct uptake from the water (Kraal et al. 1995) and is affected by prey type (Langevoord et al. 1995) and gut parasites (Oyoo-Okoth et al. 2012). Even in planktonic organisms, feeding strategy influences metal uptake, with metal concentrations in the omnivorous copepods *Centropages typicus* intermediate between herbivores and carnivores, highlighting the importance of diet (Battuello et al. 2017). For the predatory alderfly *Sialis velata*, food (prey) is the almost exclusive source of As, Cd, Co, Cu and Zn and the source of 94% of its Pb (Croisetière et al. 2006).

Furthermore, it has become increasingly appreciated (de Jonge et al. 2011; Luoma and Rainbow 2008) that burrowing aquatic invertebrates (including soft-bodied worms with potentially permeable body walls) are not actually in contact with undisturbed pore water containing dissolved metal in equilibrium with adjacent metal-rich particles. Burrowing animals live in burrows which are irrigated by a flow of oxygenated water from the overlying water column, so that they can breathe. The contribution of pore water to such bathing water in the burrow is usually negligible, and pore water can, in essence, be considered insignificant as a source of metals to the burrowing animals. Thus, even burrowers are only exposed to dissolved metals from the water column lying above the sediment. Similarly, processes at the root-sediment interface, rather than pore water concentrations, appear to dictate the uptake of metals by aquatic plants rooted into contaminated sediments (Teuchies et al. 2012).

Calculations of how much metal is released from sediment particles into the pore water, and the effects of changes in redox conditions in the pore water, or the sediment content of chemical species such as acid volatile sulphides (AVS) that might bind these metals, are to all extents and purposes irrelevant when considering sources of metals to burrowing invertebrates (Luoma and Rainbow 2008). It is true that in anoxic sediments, one of the most important factors controlling the transfer of metals from sediment to pore water is the presence of AVS in the sediment, operationally defined as the amount of sulphides volatilized by the addition of 1 N HCl and mainly consisting of Fe, Mn and Al sulphides (Ankley et al. 1991, 1994; di Toro et al. 1992; Hansen et al. 1996). Sulphides bind strongly with metals and inhibit metal exchange from sediment to the pore water, resulting in greatly reduced metal concentrations in the pore water in the zone of anoxia. When such sediments are homogenised and used in toxicity tests, the reduced pore water concentrations correspond to declines in toxicity (Ankley et al. 1996). In 10-day whole sediment toxicity tests, acute toxicity is typically not observed when the molar concentration of AVS is greater than the molar concentration of the simultaneously extracted metals (SEM) released from a sediment sample during AVS extraction (di Toro et al. 1991). The body of literature relating sediment toxicity to SEM-AVS in whole sediment bioassays is large, but the conceptual basis of the SEM-AVS approach is flawed because burrowing animals live in oxidised subsections (burrows) of otherwise anoxic sediments and are not bathed in anoxic pore water. Toxicity tests with homogenised sediments break down this substructure and have no relevance to field conditions. Despite these constraints, the whole sediment bioassay paradigm has been repeatedly used (Ankley et al. 1996; Besser et al. 2013), and equilibrium partitioning was assumed to be the basis of the toxicity of sediment-associated metals to burrowing animals (di Toro et al. 1992; Hansen et al. 1996).

de Jonge et al. (2009) evaluated the influence of AVS on accumulation of sediment-bound metals in sediment burrowing invertebrates (midge larvae *Chironomus* gr. *thummi* and oligochaete worms *Tubifex tubifex*) under field conditions (17 historically polluted Flemish rivers). They showed that AVS was not a significant variable in describing variation in metal accumulation and clearly demonstrated that burrowing invertebrates can accumulate metals from field sediments even when there is an excess of AVS present in the sediments. Supporting evidence comes from much related literature (de Jonge et al. 2011; Hare et al. 1994; Ingersoll et al. 1994; Lee et al. 2000a, b, c, 2001; Yoo et al. 2004). Similarly, de Jonge et al. (2010) evaluated the relationship between AVS and metal accumulation in the two above species and a further four freshwater species; they concluded that when uptake through food becomes the dominant metal exposure route, metal accumulation in an organism is not controlled by AVS concentrations in the sediment.

The diet is a, if not the, major source of trace metals to aquatic invertebrates (de Forest and Meyer 2015; Lee et al. 2000a; Poteat and Buchwalter 2014). Supporting evidence is provided from the results of biodynamic or biokinetic modelling (Luoma et al. 2005; Wang et al. 1996). This predominance of diet as a metal source is even more exaggerated for deposit feeders, which ingest large amounts of fine-grained sediment with a high surface area to volume ratio and typically high organic content, both attributes promoting the adsorption of high concentrations of trace metals. Biodynamic modelling takes into account such high ingestion rates as well as the assimilation efficiency of the ingesting animal, together with the high metal concentrations in sediments, to show that the sediment ingestion route often provides in excess of 90% of total metal uptake in aquatic deposit feeders (Casado-Martinez et al. 2009, 2010a; Rainbow et al. 2009).

Nevertheless, we still suffer from the legacy of acute dissolved toxicity tests in setting environmental quality standards, on the long-held assumption that dissolved metal represents the most significant source of metals to aquatic invertebrates (e.g. Besser et al. 2015). Whilst this is true in the artificial experimental set-up used for acute laboratory toxicity testing, it is a false premise when attempting to predict the onset of toxicity in field situations, where it is clear that accumulation of metals from sediments can occur even where they are undetectable in the water (Roig et al. 2016). The paradigm needs to continue to change as more ecology is introduced into ecotoxicology (Clements and Rohr 2009; Luoma and Rainbow 2010). There is an urgent need for environmental quality standards based on the concentration of metals in sediment.

2.3 Biomonitors

Most aquatic invertebrates are accumulators of trace metals, storing most of the metal taken up in detoxified form (Luoma and Rainbow 2008; Rainbow and Luoma 2011a). Strong accumulators excrete very little of the metal accumulated, whereas weaker accumulators lose some metal from the body, perhaps still in detoxified form. In the latter case, there will be a turnover of metal, but the body concentration will still reflect local bioavailabilities, the standing stock of metal in the body during turnover being higher when bioavailability is higher. Any organism that is a metal accumulator has the potential to act as a biomonitor.

A *biomonitor* is defined here as an organism that accumulates trace metals in its tissues and the accumulated metal concentration provides a relative measure of the total amount of metal taken up via all routes by that organism, integrated over a preceding time period (Luoma and Rainbow 2008). Thus, the bioaccumulated concentrations are integrated measures of the uptake and accumulation of metals from all sources to that organism. The use of biomonitors can, therefore, identify areas of high or low metal bioavailability to that chosen biomonitor and identify changes of metal bioavailability in space and/or time. Such information is prerequisite to an efficient subsequent search for (sublethal) ecotoxicological effects.

2.4 Ecotoxicological Effects: Biomarkers

Whilst biomonitors will provide information on when, where and how much of a metal in bioavailable form(s) is present in a habitat, biomonitoring does not directly address the consequences in terms of adverse effects. Biomarkers are used to determine whether an observed high bioavailability of a metal is actually having an ecotoxicological effect on a member of the local biota and the severity (ecotoxicological significance) of that effect.

A *biomarker* is a biological response (e.g. a biochemical, cellular, physiological or behavioural variation) that can be measured at the lower levels of biological organisation, in tissue or body fluids or at the level of the whole organism (Amiard-Triquet et al. 2015). In short, a biomarker is a measurable biological response to the local presence of a bioavailable quantity of a contaminant (a toxic metal in this context).

Ecotoxicological effects occur at different levels of biological organisation – through the molecular, biochemical, cellular, physiological, individual organism, population and community levels (Fig. 6). Typically the molecular response is the most sensitive, followed in turn by effects progressing up the hierarchy of biological organisation. However, the relationships between effects across different scales of biological organisation are not necessarily straightforward. Whilst biomarkers at the lowest levels of biological organisation are often the most sensitive (Fig. 7), it is the toxicological effects reflected by biomarkers at the highest level (the community) that are most ecotoxicologically significant: although biomarkers at lower levels (e.g. molecular) can be useful as early warning indicators of exposure to a stressor, they do not enable prediction of the occurrence or relevance of effects at higher levels of organisation. If metal pollution (high metal bioavailability) is producing ecotoxicological effects observable at the community level, then biota are being greatly affected. The task facing environmental regulators is to detect where the ecotoxicological effects of metal contamination are causing profound damage to biota.

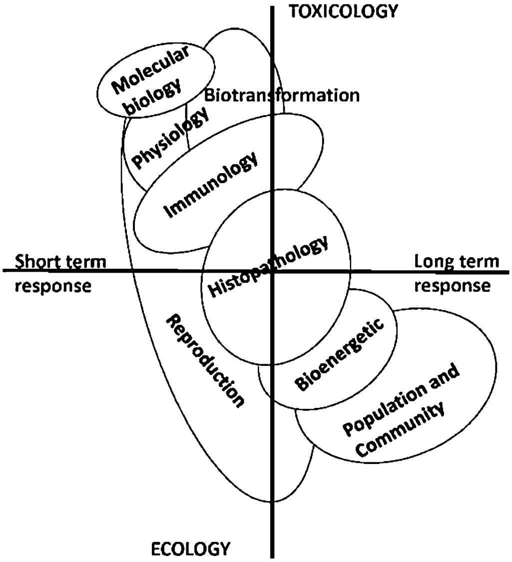
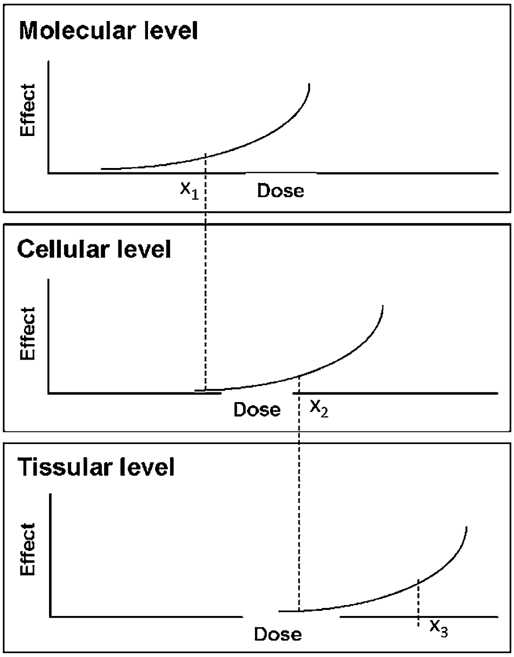


Fig. 6 Biomarkers: latency between exposure of fish to pollutants and the occurrence of biological effects at different levels of biological organisation (from Amiard-Triquet and Amiard 2013b; after Adams et al. 1989)

Fig. 7 Biomarkers: progression of the dose-effect relationship from low to higher levels of biological organisation (from Amiard-Triquet and Amiard 2013b)



3 Environmental Assessment of Mining-Affected Streams

3.1 Introduction

Metal contamination of streams and rivers produces ecological damage (Environment Agency 2008a, 2009). Whilst individual case studies may not include all ecological symptoms and the relationship between the onset of negative ecological effects and metal concentrations in water or sediment (the dose response) will vary from site to site, a combination of several signs is diagnostic of the ecotoxicological effects of mining on streams and rivers (Luoma and Rainbow 2008). These effects include reduced numbers and diversity of aquatic flora and invertebrates (Batty et al. 2010; Clements 2004; Clements et al. 2000; Clements and Ranville 2002; Environment Agency 2008a, 2009; Jarvis and Younger 1997; Say and Whitton 1981a, b; Loayza-Muro et al. 2014a, b), especially reduced species richness and abundance of mayfly larvae (order Ephemoptera), associated with decline in numbers, or often complete absence, of specific sensitive species like ephemerellid and heptageniid mayflies (Cain et al. 2011; Clements 1991, 2000; Clements et al. 2000; Gower et al. 1994; Loayza-Muro et al. 2010). Similar effects are apparent where high concentrations of metals are naturally present due to geological processes acting on metal-rich rock types (Loayza-Muro et al. 2010, 2014a; Schmidt et al. 2012b). Fish are typically of low abundance with fish mortalities, particularly of more sensitive salmonid species (e.g. rainbow trout *Oncorhynchus mykiss* in the

Clark Fork River, USA; Marr et al. 1995), reducing the number of fish species, whilst substrate clogging by metal-rich sediment may cause loss of spawning gravels and nursery streams for fish reproduction (Environment Agency 2008a; Luoma and Rainbow 2008; Malouf 1974).

Historically, amongst the earliest observations of the ecotoxicological effects of mining activities on British water catchments was the work of Carpenter in the 1920s in the then active mining regions of West Wales (Carpenter 1924; Environment Agency 2009; Kelly 1988). Carpenter (1924) noted that the flora in Pb- and Zn-contaminated streams downstream of mines was very reduced, typically restricted to the red algal genera *Batrachospermum* and *Lemanea*, together with some mosses and liverworts. The number of species increased with distance downstream and, after closure of mines, over time in the upper reaches (Kelly 1988).

Whilst such characteristic changes in the flora and fauna of metal-contaminated sites have long been recognised (e.g. Carpenter 1924; Environment Agency 2009; Kelly 1988), attributing any observed differences to the toxic effects of metals rather than other factors (de Haas and Kraak 2008; Klok and Kraak 2008), particularly those associated with mining or where mines are, remains a challenge. Evidence of ecotoxicological effects of metals can be obtained at various levels of biological organisation (Fig. 6), with inevitably some degree of overlap between levels. Here we consider the use of biomarkers at various levels for the assessment of the impacts of metal pollution on streams and rivers.

3.2 Sub-organism-Level Effects

Biomarkers are increasingly being suggested for use in freshwater biological monitoring (Chaumot et al. 2015; Christophe et al. 2015). Here, we discuss biomarkers because of their potential use as evidence linking the effects of metal contamination to community-level impacts. Many biomarkers of toxic metal pollution in aquatic habitats have been proposed, assessed and are now being widely employed (Amiard-Triquet and Amiard 2013b). An ideal biomarker in ecotoxicology would be toxicant-specific (e.g. d-aminolevulinic acid dehydratase for lead exposure: Amiard-Triquet and Amiard 2013b), but most biomarkers lack specificity, being responsive to more than one stressor and indicative of the general health status of an organism. Nevertheless, these biomarkers are of considerable value as a measure of exposure in ecotoxicological assessment. A biomarker is of particular use if it is contaminant-sensitive (and therefore detectable at low levels of biological organisation; Fig. 7) and links (almost certainly correlational by necessity, e.g. Fig. 8) can be established between its detection in exposed organisms in the field and consequent ecotoxicological effects at higher levels of biological organisation and although the evidence of such biomarkers is scarce, ultimately up to the population, community and eventually ecosystem (Amiard-Triquet and Amiard 2013b; Durou et al. 2007; Moore et al. 2013).

At the molecular level, metals may bind to DNA and interrupt normal metabolic functioning, with the ultimate ecotoxicological effect being carcinogenesis (the

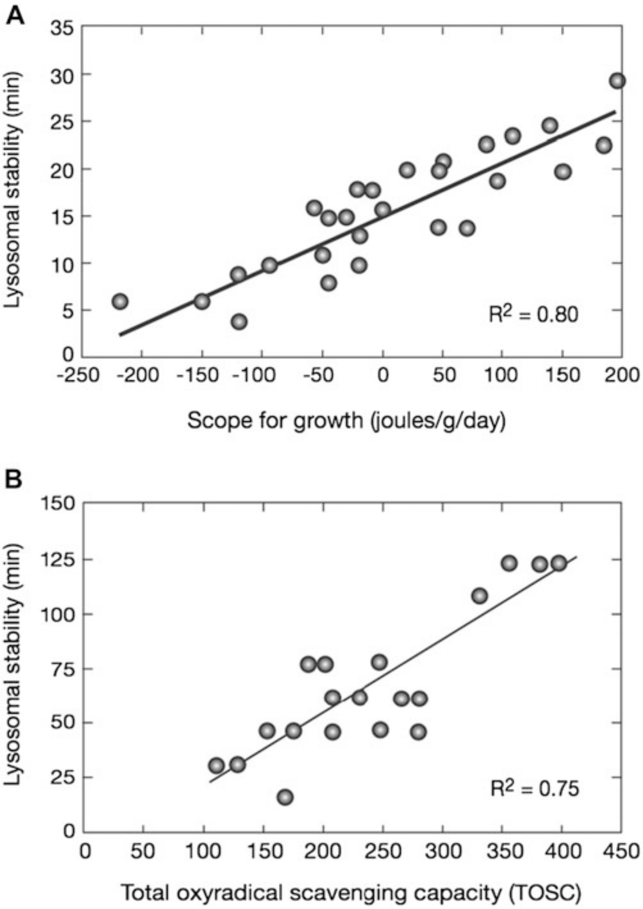


Fig. 8 Significant correlations of biomarkers from low to higher levels of biological organisation. (**a**) Between scope for growth and lysosomal stability in the mussel *Mytilus edulis* (Allen and Moore 2004). (**b**) Between lysosomal stability and Total Oxyradical Scavenging Capacity (TOSC) in mussels (after Moore et al. 2006a; Regoli 2000)

development of tumours), heritable mutations and teratogenesis (the malformation of embryos), expressed at higher levels of organisation. The comet assay is a commonly used biomarker of the early stages of damage to DNA, for it uses electrophoresis to identify the presence of broken DNA fragments in individual cells of exposed organisms (Vasseur et al. 2013) and has been used to identify potential impacts of metals in contaminated river sediments (Barjhoux et al. 2017; Garcia et al. 2017; Sunjog et al. 2016). The micronucleus assay, which is sensitive to chromosome damage or mitotic spindle dysfunction, has been shown to respond to metals in *Daphnia* (Barka et al. 2016).

Metallothioneins are non-enzymatic proteins that are induced by, and bind to, particular trace metals such as Ag, Cd, Cu, Hg and Zn (Roméo and Giambérini

2013). They are of low molecular weight (12–15 kDa), have a high content of the sulphur-containing amino acid cysteine (providing binding sites for the trace metals), no aromatic amino acids and heat stability (Amiard et al. 2006). Metallothioneins play a role in the homeostasis of essential metals like Cu and Zn and in the cellular detoxification of both essential and non-essential metals. The induction and metal-binding properties of metallothioneins, which are widespread in invertebrates and vertebrates, initially generated a great deal of excitement in their potential for use as specific biomarkers for toxic metal exposure in the field (Amiard et al. 2006). Whilst there are field examples of the induction of metallothioneins in organisms in contaminated field conditions, metallothionein induction can be variable, for example, with species, metal, exposure concentration and the different metallothionein isoforms (different forms of the same protein resulting from co-occurring slightly different genes) that might be present (Amiard et al. 2006). Moreover, it is now appreciated that metallothioneins can also be induced by other stress factors that are not related to metal physiology, such as anoxia, handling, starvation, freezing and the presence of antibiotics, herbicides or vitamins (Amiard et al. 2006; Luoma and Rainbow 2008). Furthermore, like other proteins, metallothioneins are turned over in the cell, a process usually involving breakdown in lysosomes (autophagic vesicles in cells), and an increased rate of metallothionein synthesis on metal exposure may be associated with an increased rate of turnover but not an increased concentration, which is the measure typically used as a biomarker (Amiard et al. 2006; Luoma and Rainbow 2008). Thus, metallothioneins are no longer considered to be the specific biomarkers of toxic metal exposure once hoped, but any observed induction of metallothioneins in organisms at sites under investigation for metal ecotoxicity may contribute to an increasing package of relevant field observations to this end.

More general biochemical stress responses that are used as biomarkers include stress proteins and biochemical defences against oxyradicals, which can be generated by trace metal exposure as well as by other stressors, particularly organic contaminants (Amiard-Triquet et al. 2013). Stress proteins make up a set of protein families originally called heat-shock proteins but now known to be induced by exposure to many stressors including, for example, organic compounds, ultraviolet radiation and salinity change, as well as toxic metals. Antioxidant defences against oxyradicals (which may, for example, damage DNA) include primary antioxidant enzymes, like superoxide dismutase and catalase, which have been measured as biomarkers for some time (Roméo et al. 2009). Other biomarkers include malondialdehyde, a breakdown product of lipid peroxidation caused by oxyradicals, and glutathione, an oxyradical scavenger (Roméo et al. 2009). A widely used biomarker is the Total Oxyradical Scavenging Capacity (TOSC), an integrated measure of antioxidant defence, to which toxic metal exposure will contribute (Regoli 2000; Regoli et al. 2002, 2011). Again, these more general biochemical stress biomarkers may not be specific to toxic metal exposure, but they can contribute to a general battery of biomarkers assessing the general health status of an organism being investigated for potential ecotoxicological stress.

Transcriptomic (the set of all RNA molecules produced, reflecting the genes actively being expressed) and metabolomic (the complete set of small molecule metabolites found) studies are typically still at the laboratory stage of investigation. Nevertheless, some approaches, such as the use of rainbow trout gill cell culture to assess the expression of genes for metallothioneins, Zn transporters and glutathione-S-transferase (Bury et al. 2008; Schnell et al. 2016; Walker et al. 2007, 2008), have been used to assess natural waters polluted with metals (Minghetti et al. 2014; Schnell et al. 2015). Transcriptomics and metabolomics do have particular potential for use in biomarker studies in the field, as we try to understand which genes are induced or show reduced transcription and which metabolites are present under conditions of raised trace metal exposure (Gonzalez and Pierron 2015). This potential is now being explored, with the expression of genes involved in cysteine metabolism (a precursor of metallothionein) and metabolomics used as response endpoints of laboratory-bred chironomids (*Chironomus tepperi*) exposed to sediment experimentally contaminated with Cu in field microcosms and subsequently compared with effects at the individual (survival and dry weight), population (reproduction) and community levels (Jeppe et al. 2017b).

Biomarkers have also been developed at the cytological level. Genotoxic damage referred to above can lead to chromosomal changes visible in cells under the microscope. One common cytological manifestation of such damage is the presence of micronuclei, displaced from the main nucleus of the cell (Vasseur et al. 2013). Michailova et al. (2009) identified aberrations in the structure of salivary gland chromosomes of larvae of the chironomid *Chironomus acidophilus* in Afon Goch, a small river draining the former Cu mining area of Parys Mountain in Anglesey, UK, with high water concentrations of Fe, Mn and Zn, as well as Cu. Similarly, chromosomal and somatic aberrations have been associated with sediments variously contaminated with Ca, Cu, Cr, Mn, Pb and Zn in mining-impacted rivers in Poland and Bulgaria (Ilkova et al. 2017; Michailova et al. 2015, 2016; Szarek-Gwiazda et al. 2013).

Other widespread cytological biomarkers of toxic metal pollution in aquatic habitats centre on *lysosomes*, membrane-delimited organelles containing hydrolytic enzymes. Lysosomes occur in nearly all cells of eukaryotic organisms and serve to break down redundant or damaged cell organelles and proteins (including metallothioneins) for recycling (Moore et al. 2006a, b, 2013). There are several identified responses of lysosomes to stress that have potential as biomarkers, including changes in lysosomal size and number in particular cells, production of lipofuscin and destabilisation of the lysosomal membrane (Moore et al. 2006a, b, 2013). Lipofuscin is a pigment that is the end product of lipid peroxidation of cell components brought about by reactive oxygen species, and its increased production in lysosomes indicates increased lysosomal turnover activity, as might be caused by exposure to both toxic metals and organic contaminants. Lipofuscin granules may indeed contribute to the detoxification of excess cellular trace metals, for example, as part of the turnover of metallothioneins. The functional stability of the lysosome membrane (and hence its permeability) changes with degree of exposure to contaminants including toxic metals, and the assessment of lysosomal stability based on the dye neutral red method (Neutral Red Retention NNR) has become widely used in ecotoxicology because it is both simple and quantitative (Moore et al. 2006b, 2013; Svendsen et al. 2004). Measurement of lysosomal stability is a sensitive, low organisational-level biomarker, and excitingly, its quantification has been correlated with other biomarkers at other levels of biological organisation, including TOSC and Scope for Growth (SFG) (Fig. 8: Moore et al. 2006b, 2013).

3.3 Organism-Level Effects

Biomarkers at the biological organisation level of the organism can be morphological or physiological. There are limited records of morphological abnormalities of organisms resulting from field exposure to high availabilities of toxic metals, and these usually concern diatoms or chironomid larvae. Falasco et al. (2009) have produced a wide-ranging review of abnormally developed (teratological) forms of diatoms in the field, and exposure to toxic metals figures strongly as a cause of these morphological abnormalities (e.g. valve distortion and abnormal valve striation). However, other stressors, such as excess light, nutrient depletion and low pH, can also induce deformities, confounding interpretation (Falasco et al. 2009). Whilst some field studies indicate a correlation between diatom abnormalities and metal contamination (Cantonati et al. 2014; Lavoie et al. 2018; Pandey et al. 2018), others do not, despite being conducted in severely polluted river basins (Fernandez et al. 2018; Lavoie et al. 2012). Better resolution may be achieved by classifying deformities by type/severity or the species they occur in (Lavoie et al. 2017).

In the case of chironomid larvae, deformities of the mouthparts have long been known to occur in midge larvae living in contaminated sediments (Warwick 1988). Cause/effect relationships between the presence of such deformities and equivalent sediment concentrations of metals (particularly Cu) have been demonstrated in the laboratory (Martinez et al. 2003; di Veroli et al. 2012) and field (Beghelli et al. 2018; di Veroli et al. 2014). Mouthpart deformities of *Chironomus tentans* larvae include fused teeth, split teeth, missing teeth, extra teeth and abnormally shaped teeth on the mandible, with apparently different effects being associated with different metal exposures (Martinez et al. 2003, 2004, 2006). de Pauw and Heylen (2001) used a measure of Percentage of Mentum Deformities (Warwick 1988) in unspecified *Chironomus* larvae as a contributing index in a biological assessment of the environmental quality of freshwater sediments in Flanders. Nevertheless, some workers have shown poor relationships with metal concentrations (Langer-Jaesrich et al. 2010). It appears that chironomid mouthpart deformities may offer relevant contributing evidence in any ecotoxicological study of metal-rich stream sediments (Ilkova et al. 2017), but further work is needed.

Physiological biomarkers include physiological condition indices (particularly for fish (Christophe et al. 2015), *Daphnia* and floating plants (Gerhardt et al. 2008), growth rates, feeding rates and the popular measure ‘Scope for Growth’ (SFG). The latter two at least, however, involve collection of animals for subsequent laboratory experimentation, reducing their ease of applicability in any field study. As regards growth rates, Faria et al. (2007, 2008) measured the growth rates in situ of *Chironomus riparius* larvae in mining-affected streams in Portugal, showing inhibition of growth rate (body length increase) in metal-contaminated streams. Incidentally, the results of Faria et al. (2007) confirmed that the midge larvae were more affected by metals entering the body through ingested sediment than by metals dissolved in the water column. Brown trout (*S. trutta*) in the metal-mining-affected Clark Fork River in Montana, USA, were smaller than fish of the same age in carefully chosen reference sites (Tohtz 1992).

The most common in situ bioassay involves exposure of the zooplankter *Daphnia,* although caution is recommended by Pereira et al. (2000) as mine-impacted sediment, besides being an important contaminant source, can become significantly altered with sampling, transport, storage and manipulation during the set-up of experimental enclosures and confound the results. Similar caution is recommended by Peeters et al. (2001) who found that bioassays (using *Daphnia magna*, the bacteria *Vibrio fischeri* and *Chironomus riparius*) explained little of the variation in macroinvertebrate community composition in the enclosed Rhine-Meuse delta.

Feeding rates feature strongly in the literature of the effects of trace metal contaminants in freshwater, for example, in the case of freshwater gammarid crustaceans, whether standing alone (e.g. Taylor et al. 1993) or as part of the calculation of SFG (Chaumot et al. 2015; Maltby et al. 1990a, b). Maltby et al. (2002) found inhibition of the in situ feeding rates of *Gammarus pulex* downstream of point source effluent discharges in UK rivers, and Dedourge-Geffard et al. (2009) showed reduced feeding rate of *Gammarus fossarum* at metal-contaminated sites in the Amous River, France, receiving drainage from a former Pb-Zn mine.

SFG integrates different physiological measures in a calculation of the energy balance of an organism (Luoma and Rainbow 2008). SFG is an estimate of the surplus energy available to an animal for growth and reproduction, calculated from the difference between energy assimilated from food and the energy used in respiration. SFG is interpreted to decrease when energy is required to cope with the extra physiological cost of handling (detoxifying or excreting) high amounts of toxicants taken up in contaminated environments. Thus, Maltby et al. (1990a, b) showed that the freshwater amphipod *Gammarus pulex* had reduced SFG when exposed to 3 mg L^{-1} Zn. SFG is a very useful integrated measure of how well an animal is coping with a high bioavailability of a contaminant such as a toxic metal, but it is not easy to measure, requiring rapid access to laboratory facilities to measure such physiological parameters as feeding rate, assimilation efficiency and respiration rate. Nevertheless, a correlational link has been established between lysosomal stability and SFG in mussels *Mytilus edulis* in the marine environment (Fig. 8; Moore et al. 2006a, 2013), again linking biomarkers at different levels of biological organisation.

Given appropriate observational facilities, the behaviour of an animal is another integrative response at the organism level that can be used as a biomarker (Amiard-Triquet 2009; Amiard-Triquet and Amiard 2013a). Behavioural changes at an individual organism level have the potential to induce knock-on effects at population and community levels (Amiard-Triquet and Amiard 2013a) as in the case of a reduced swimming capacity of a fish to capture prey and avoid predators (Weis et al. 2001, 2003). Furthermore, behavioural disturbances originate in biochemical and physiological impairments such as neurotoxicity disrupting the function of sensory systems and endocrine disruption, thereby linking responses at different levels of biological organisation (Amiard-Triquet and Amiard 2013a). There have been many examples of dissolved trace metals upsetting behaviour of fish (Amiard-Triquet and Amiard 2013a; Hansen et al. 1999) and invertebrates (Gerhardt 1995, 1996, 2001, 2007), particularly in the laboratory, and at concentrations of the order of magnitude of those encountered at contaminated sites in the field (e.g. de Bisthoven et al. 2004). High trace metal concentrations in sediments and diet can also affect the behaviour of invertebrates and vertebrates (Amiard-Triquet and Amiard 2013a). Nevertheless, the links between behavioural modification and other biological parameters on the sub-organismal as well as the population level are still largely lacking (Gerhardt 2007). Observation of behavioural changes associated with exposure of animals to metal-rich sediments can therefore contribute valuable information to a portfolio of evidence on the ecotoxicological effects of such sediments, but again, it would be necessary to use observational (laboratory) facilities after field collection of affected individuals.

3.4 Population-Level Effects

Ecotoxicological effects observed at the population level have clear ecological significance for the biota in a contaminated habitat, with obvious implications for local community structure, via loss of species and/or abundances of individuals of the species present. Such ecotoxicological effects might be on the numbers of individuals present, population age structure, reproductive rates or recruitment rates. The selection of metal-tolerant strains is another population-level effect dealt with in more detail below. Laboratory-based studies have shown that metal exposure can affect population parameters such as rate of population increase (e.g. the freshwater amphipod *Gammarus pulex*; Maund et al. 1992) or life-table parameters like percentage hatch, juvenile survival, fecundity, time to maturity, etc., in the case of the freshwater gastropod mollusc *Biomphalaria glabrata* (Salice and Miller 2003). Field-based evidence is less forthcoming. Nevertheless, Schmidt et al. (2013) provided evidence on the emergence of adult insects of many families from aquatic larvae along a gradient of metal contamination in streams in Colorado, USA. Larval densities decreased in non-linear fashion with water metal concentrations with a precipitous fall above a threshold of combined modelled metal bioavailabilities. Adult emergence also showed a non-linear threshold response with a steeper decline below this same threshold and a more modest decline thereafter (Schmidt et al. 2013). Schmidt et al. (2013) concluded that adult emergence (a population-level parameter) was a more sensitive indicator of the effect of low metal concentrations on aquatic insect communities than larval density, presumably because successful emergence is limited by a combination of larval survival and other factors. Thus, the ecotoxicological effects of metal exposure on the populations of the insect larvae were not all manifested until later in life, during metamorphosis and emergence.

The application of biomarkers in the ecotoxicological assessment of contaminants in freshwater lags behind that in coastal environments, but there are specific projects actively pursuing this topic (Collier et al. 2013). Biomarkers in several freshwater fish species have been developed to assess the effects of contaminants with the potential to be used in environmental regulation (Sanchez et al. 2013; Sanchez and Porcher 2009). These biomarkers have included those of oxidative stress, neurotoxicity and reproductive and immunological disruption. Allan et al. (2006) concluded that biomarkers have shown potential as sensitive methods for the detection of pollution and suggested that they could become important regulatory tools as part of the evidence linking pollutants to community-level impacts.

3.5 Community-Level Biological Monitoring

Community-level biological monitoring is based on the assumption that the composition and organisation of communities reflect local environmental conditions and respond to any significant anthropogenic alterations to these conditions (Clements and Newman 2002). A second assumption is that species differ in their sensitivity to anthropogenic stressors, such as metals. Hence, an ecotoxicologically significant dose of a metal will change the numbers of species present and the abundances of those remaining. Measures of the numbers of species present and their abundance have been the first steps in any quantitative analysis of community structure (e.g. Clapcott et al. 2016; Malmqvist and Hoffsten 1999). *Species richness*, the number of species present, is dependent on sampling effort and increases asymptotically with sample size and number of individuals collected. Hence, a standardised sampling protocol (e.g. Furse et al. 1981) is necessary when comparing across sites. However, measures of species richness do not take into account differences in abundance amongst the species present. Measures of *species diversity* have been used to compare communities from locations differentially contaminated with metals (e.g. Amisah and Cowx 2000; Gray and Delaney 2008; Hirst et al. 2002), although they have been criticised using theoretical, statistical and conceptual arguments (Clements and Newman 2002).

Multivariate techniques provide community-level analyses to determine relationships between sites, samples, species and variables such as metal contamination (e.g. Beltman et al. 1999; Gower et al. 1994; Hirst et al. 2002). Gower et al. (1994) used canonical correspondence analysis to show that variation in macroinvertebrate communities in metal-contaminated streams in South West England was best explained by four stream chemistry variables (Cu, Al, alkalinity, pH). Similarly, de Jonge et al. (2008) demonstrated the influence of Zn and water chemistry on macroinvertebrate and diatom community compositions in a study of metal pollution in a lowland river in Flanders, and Loayza-Muro et al. (2010, 2014a, b) demonstrated community differences attributable to metals and altitude in Andean streams.

Theoretical and empirical studies indicate that some communities show abrupt, non-linear changes in structure or function in response to perturbations (Clements and Rohr 2009; Luoma and Rainbow 2010; Rainbow et al. 2012; Schmidt et al. 2013). These ecological discontinuities or thresholds are defined as significant changes in an ecological state variable as a result of a continuous change in an independent environmental variable (Clements and Rohr 2009). The threshold is the point at which any rapid change initially occurs. Typically, in any ecological study assessing the effects of contaminant stressors on resident communities, not all the factors that might affect the communities are measured, and statistical distributions of ecological data have unequal variation as a result of complex interactions between these factors (Cade and Noon 2003; Schmidt et al. 2012a). Such unequal variation can be examined by quantile regression analysis which estimates multiple rates of change (slopes) from the minimum to maximum response and provides a more complete picture of the relationships between variables missed by other regression methods (Cade and Noon 2003). Schmidt et al. (2012a) used quantile regression to measure risks to aquatic life exposed to metals in a study of the population densities of two mayflies and a caddisfly, metals and other environmental variables in 125 streams in Colorado, USA. In accordance with the concept of ecological thresholds, the most obvious effects on mayfly populations were at upper quantiles and not mean density (Schmidt et al. 2012a).

3.5.1 Macroinvertebrate Biotic Indices

The community-based indices above give all species equal weight irrespective of their sensitivity to anthropogenic contamination and may not show responses if, for example, sensitive species are simply replaced by contaminant-insensitive species. Therefore, different *biotic indices* have been developed to assess the state of the community based on the relative abundance of sensitive and insensitive species, particularly in the case of *benthic macroinvertebrates* in streams and rivers.

The simplest expression of such an approach lies in the concept of indicator species. An *indicator species* is a species, the presence or absence of which is indicative of a particular habitat, community or set of environmental conditions. In an ideal world, there would exist a species whose presence or high abundance would indicate a high local bioavailability of toxic metal(s) in a particular habitat, in our case a freshwater stream. However, there is the need to distinguish the relative significance of an anthropogenic stressor from the effects of the many other biotic and abiotic factors that influence the presence or absence of a species (Clements and Newman 2002; Schmidt et al. 2012b): the alga *Stigeoclonium tenue* present at high abundance in the Zn-rich streams of the River Nent system, UK, is relatively insensitive to other forms of pollution as well as high Zn levels (Armitage 1980). The so-called pollution-sensitive species are frequently sensitive to other disturbances, natural or anthropogenic, and the absence of such a species provides only weak support for the hypothesis that its absence is a result of the local presence of a particular toxic contaminant (Clements and Newman 2002).

It is well known that mayflies (Ephemoptera), stoneflies (Plecoptera) and caddis flies (Trichoptera) are relatively sensitive to the low oxygen concentrations caused by organic enrichment, whilst chironomids (Diptera) are generally insensitive to organic pollution. The *EPT score* (Lenat 1988) makes direct use of these relative sensitivities to create a biotic index, which has been used by several workers to assess the impacts of metals (e.g. Gray and Delaney 2008; Malmqvist and Hoffsten 1999), despite *EPT score* being a generic measure of degradation. It must be remembered that relative sensitivities of species do depend on the contaminants of concern. As many of the biotic indices developed for use in freshwater systems so far are for the assessment of the effects of organic pollution (Jones et al. 2010), it should not be surprising if they are not suitable for assessment of toxic metal pollution, and vice versa. Nevertheless, relationships between the extent of metal pollution and indices related to organic pollution (Blanco and Becares 2010; de Jonge et al. 2008, 2013; Gerhardt et al. 2004) and measures of invertebrate diversity and richness (Blanco and Becares 2010; de Jonge et al. 2008, 2013; Hirst et al. 2002) have been recorded.

The *Biological Monitoring Working Party* (BMWP) *score* (Biological Monitoring Working Party 1978) was developed as an index to assess the ecological effects of organic pollution and, therefore, cannot be expected from first principles to be suitable as a biotic index for mining effluent pollution. Nevertheless, the use of BMWP has been evaluated with respect to (often metal-rich) coal mine water discharges (Environment Agency 2009; Davies et al. 1997; Jarvis and Younger 1997, 2000) and AMD (Gray and Delaney 2008). Jarvis and Younger (1997) showed a strong statistical inverse correlation between BMWP score and Fe concentration in coal mine-affected streams. However, the physical blanketing by ochre may have been the main cause of decreases in faunal abundance and diversity (Environment Agency 2009), as opposed to any toxic metal effect that might be more typical of mining-affected streams with metal-rich sediments. In the case of discharges from metal mines, any relationships between dissolved concentrations of metals such as Zn, Pb and Cd and BMWP scores were far more ambiguous than for coal mine drainage, with no clear strong inverse relationships present (Environment Agency 2009; Jarvis and Younger 2000). A specific study of streams in mining-affected regions of Wales drew similar conclusions; there was a correlation between BMWP score (but not ASPT) and total Zn concentration, albeit with considerable scatter in the data, but no clear relationship between any other trace metal (As, Cd, Cu, Fe, Mn, Ni, Pb, as total or dissolved fraction) and BMWP score, ASPT or number of taxa present (Environment Agency 2009). In this study there was a potentially strong confounding effect present, in the form of a strong inverse relationship between pH and BMWP score. The low pH of AMD has been highlighted as the main driver of invertebrate (Clapcott et al. 2016) and algal (Schowe and Harding 2014) diversity and community composition. Furthermore, the potential inappropriateness of the BMWP score for use as a biotic index in mining-affected streams is probably also a factor producing the reported lack of relationships investigated.

Gray and Delaney (2008) did find BMWP to be responsive to AMD in the River Avoca in Ireland, draining a region of abandoned Cu sulphide mines. However, the effects of AMD are driven by a number of factors, particularly water acidity, in addition to salinisation, metal toxicity and metal precipitation, confounding the direct ecotoxicological effects of metal-rich sediments (Clapcott et al. 2016; Schowe and Harding 2014). On the other hand, ASPT was not correlated with any measured parameter such as pH, sulphate, Zn or Fe concentrations nor, unlike the BMWP score, with any of the diversity indices measured (Gray and Delaney 2008), suggesting that differences in taxonomic richness were driving change in BMWP, a known weakness in this and many other total score indices (Armitage et al. 1983). Faria et al. (2006) found that the Iberian equivalents, IBMWP and IASPT, showed some ability to discriminate metal-contaminated sites from uncontaminated sites but that the indices were confounded by organic pollution (specifically ammonia concentrations). Similarly, Hickey and Clements (1998) found that the New Zealand quantitative macroinvertebrate community index (QMCI), designed to assess organic pollution, was unresponsive to metals. Gray and Delaney (2008) concluded that specific biotic indices do need to be developed that measure the expected community structure of benthic macroinvertebrates arising from AMD impact more precisely than the existing metrics.

The biotic indices discussed above are not particularly useful as stand-alone measures as there is natural variation in the occurrence of species and, hence, index scores vary naturally dependent upon the natural characteristics of the river being investigated. To assess the extent of pollution at a site, it is necessary to determine if the community structure of benthic macroinvertebrates (or other biota) deviates from the community that would be expected in the absence of pollution. The *River Invertebrate Prediction and Classification System* (RIVPACS: Wright et al. 1984) represented a major step forward because it adopted a 'reference condition' approach. The fauna at a site under investigation is compared with fauna at similar 'reference sites' that are not subject to any apparent environmental stress, site quality being measured as a ratio (the observed/expected score) where the expected score has been predicted by RIVPACS based on the fauna at similar sites with matching physical, chemical and geographical characteristics (Armitage et al. 1987; Jones et al. 2010; Moss et al. 1987). Similar tools have been developed in Australia, Canada and the USA (Nichols et al. 2017). Using the RIVPACS approach, de Jonge et al. (2013) demonstrated a negative relationship between dissolved metal concentrations and the observed/expected of the indices NTAXA and BMWP and invertebrate metal body burdens in rivers in Northern England.

Although RIVPACS and other predictive models were developed for the assessment of organic pollution, and not specifically for the assessment of the ecotoxicological effects of trace metals derived from mining, RIVPACS models produce predictions of the invertebrate community expected at a site in terms of the species present and their abundance (Jones et al. 2010), which facilitates the inclusion of new indices. Of relevance to waters receiving mine drainage is the Acid Water Indicator Community (AWIC) index, which addresses the effects of acidification (including AMD) on river benthic invertebrate communities (Davy-Bowker et al.

2005; Murphy et al. 2013). AWIC has been used to great effect in determining the extent of recovery of acidified sites (Murphy et al. 2014) and could be used to assess the pH effects of AMD.

In Australia, a diagnostic index for acid mine drainage, SIGNAL-MET, was developed where taxa were scored using correlation with distance downstream of mine discharges (Chessman and McEvoy 1998). This index (which is indicative of the various stressors associated with mining activity) has been tested using an Australian predictive model, AUSRIVAS, which is based on RIVPACS (Sloane and Norris 2003): 70% of the variation in observed/expected was explained by variables associated with mine pollution (Cu, Cd, Pb and Zn in the sediment, Cd and Zn in the water and pH). Sloane and Norris concluded that the predictive modelling (RIVPACS) approach is appropriate for assessing the degree of impairment from previous mining activity. In New Zealand, the Acid Mine Drainage Index (AMDI) developed using field data from AMD-affected sites (Gray and Harding 2012) does not use a reference condition approach and may be more influenced by low pH than the toxic effects of metals (Clapcott et al. 2016).

Whereas the above has largely focussed on the effects of waterborne or dissolved pollutants, here we are concerned about riverbed sediments contaminated with metals as a consequence of mining activities. Various indices have been developed to relate benthic macroinvertebrate communities to fine sedimentation stress (e.g. Extence et al. 2013; Hubler et al. 2016; Murphy et al. 2015). Although these indices purport to be able to assess damage from fine sedimentation stress, none addresses the issue of contaminated sediment, which is most relevant to sites affected by metal mining.

Whilst the Australian SIGNAL-MET index is associated with the various impacts of mining, not just metal-rich sediments, in both France and Belgium, there have been initiatives to develop biotic indices of sediment quality in rivers, the *Oligochaete Index of Sediment Bioindication* (IOBS) in France (Rosso et al. 1994) and the *Biotic Sediment Index* (BSI) in Belgium (de Pauw et al. 2001; de Pauw and Heylen 2001). The IOBS aims to assess the general quality of sediments by calculating the percentage of species in the oligochaete worm family Tubificidae relative to the total number of oligochaete species found. A larger percentage of tubificids returns a lower index score – interpreted as poor quality sediment, particularly in terms of organic enrichment. The proportion of tubificids that have setae (hairs) is also assessed: the lack of setae is taken to be an indication of pollution of the sediments by toxic metals and organic contaminants (PCBs) (Prygiel et al. 1999; Rosso et al. 1994). Thus, Prygiel et al. (1999) used the IOBS to assess the ecotoxicological status of sediments of rivers and canals in the Artois-Picardie water basin. They concluded that there was an inverse relationship between the IOBS score and sediment trace metal concentrations and a correlation between these concentrations and the percentage of tubificids without setae (Prygiel et al. 1999). The IOBS thus shows promise as a biotic index of the ecotoxicological effects of metals in sediment, although this study (Prygiel et al. 1999) was confounded by the additional contamination of the sediments by polyaromatic hydrocarbons (PAH) and PCBs. Furthermore, it should be noted that this index requires a far higher degree of taxonomic resolution than is typically used; in most countries Oligochaeta are rarely taken above class.

Using field data from Northern Spain, Costasa et al. (2018) used a variety of multivariate and univariate procedures, including Threshold Indicator Taxa Analysis (TITAN), to establish a set of invertebrate families sensitive to sediment metal (Au, Cu, Hg) concentrations, most of which corresponded to EPT families. Whilst this approach did not investigate the bioavailability of the metals, it represents a significant step towards developing a reliable invertebrate indicator of metal pollution.

Archaimbault et al. (2010) investigated the ecological quality of stream sediments from sites on 150 mountain streams in France, again using benthic macroinvertebrate communities. Sites were preassigned to one of four toxic quality classes ranging from high to poor on the basis of concentrations of toxic substances (trace metals, PAH and PCB) in the sediment. A non-parametric multiple comparison statistical procedure was used to compare relative abundances of different biological traits of the macroinvertebrates (e.g. habitat preference, contaminant resistance, biogeographic distribution, etc.) between groups of sites assigned to the different four toxic quality classes above. This was done in order to identify the combinations of traits that best separated sites between adjacent toxic quality classes. Archaimbault et al. (2010) were ultimately able to allocate sites to toxic quality classes from the biological attributes of their invertebrate communities with confidence, and their tool has considerable potential, after development, as an in situ functional tool of stream sediment contamination assessment at community level.

3.5.2 Other Biological Groups

Benthic macroinvertebrates have been the target community in the biotic indices discussed above, but several different biological quality elements could be assessed in addition to invertebrates. Hirst et al. (2002) investigated the responses of diatom communities to dissolved metal concentrations in Welsh and Cornish streams in metal-mining areas but showed that changes in pH and conductivity best explained variations in diatom assemblage compositions. Species diversity, species richness and evenness did not vary with metal concentrations (Hirst et al. 2002). Conversely, the single strongest predictor of the structure of diatom assemblages was the Cumulative Criterion Unit (CCU) score, a measure of total stream metal concentration and toxicity (see later). de Jonge et al. (2008) included diatom communities in their biological assessment of a gradient of metal pollution in the River Dommel in Belgium. Significant variables explaining diatom community structure were conductivity (16.5%), chloride (11.4%), ammonium (10.6%) and Zn (5.9%), and the diatom community structure better reflected the metal gradient than the macroinvertebrate community structure (de Jonge et al. 2008). Nevertheless, other factors can influence the response of diatom communities to metals, such as nutrients (Ivorra et al. 2002b) and exposure history (Ivorra et al. 2000). One solution may be to use translocated test communities (Ivorra et al. 1999).

Diatoms do appear to be sensitive to the degradation caused by mining impacts and recovery once mitigated (DeNicola et al. 2012; Gray and Vis 2013), and diatom-based indices have been developed to assess the impact of AMD in the USA (Zalack et al. 2010), New Zealand (Schowe and Harding 2014), China (Dong et al. 2015) and South Africa (Riato et al. 2018). However, only the Índice de Contaminación por Metales (Metal Pollution Index; Fernandez et al. 2018) developed in the severely contaminated Tinto and Odiel river basins of Spain has been tested against metal toxicity, such that the impact of low pH per se, which is the main driver of change in both diatom community and such indices (Clapcott et al. 2016), cannot be discounted. Separating the effects of chemical (dissolved metal, low pH) and physical (deposition of metal oxides) stress emanating from mine drainage on benthic algae, mosses and liverworts can be difficult (Niyogi et al. 2002). Nevertheless, Blanco and Becares (2010) found strong correlations between existing diatom-based indices (mostly developed for assessing nutrient pollution) and dissolved metal concentrations but again identified little impact on diversity. Similarly, Ivorra et al. (2002b) found an impact of metals on diatom indices used to assess nutrient and organic pollution. It does therefore appear that a biotic index based on diatom community composition may well be a valid assessment of the effects of high metal bioavailabilities in streams affected by mining, albeit that the evidence so far concerns dissolved metal concentrations.

Although mosses and liverworts may be sensitive to metal pollution, the UK Environment Agency concluded that their biological assessment tool based on macrophytes (LEAFPACS) showed less potential than that based on diatoms (DARLEQ) for development as a biotic index for metal pollution (Environment Agency 2009). This was because of the relative lack of macrophytes present in upland streams typically affected by mining and the difficulty of accurate identification of filamentous green algae to species.

Fish are a further biological group that are sensitive to mining impacts and could be used to assess damage. Predatory fish are typically of low abundance in mine-impacted streams, and fish that are present are often small or have shortened life spans. In the metal-contaminated Clark Fork River system and other mine-impacted North American streams, it is not unusual to find only the most metal-insensitive species of trout, the brown trout (*Salmo trutta*), although the habitat might otherwise be expected to contain rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarki lewisi*), bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) (Goldstein et al. 2001; Woodward et al. 1997). Furthermore, adult Chinook salmon (*Oncorhynchus tshawytscha*) returning to spawn avoid metal-rich environments (Goldstein et al. 1999). Even brown trout are absent from extremely metal-contaminated streams. It appears that the ingestion of metal-contaminated prey is the significant source of stress to the fish: brown trout fed on metal-rich invertebrates (collected from metal-contaminated regions of the Clark Fork River) showed elevated biochemical dysfunctions, histological abnormalities, reduced growth and poor survival rates (Farag et al. 1994, 1998, 2000; Woodward et al. 1995).

A study of pollutants from the coal industry affecting a small river in the South Wales coalfield, in the UK, showed that brown trout (*S. trutta*) occurred at very low densities downstream of acid and ferruginous drainage, apparently as a result of impoverished food supply (Scullion and Edwards 1980a, b). Nevertheless, a biotic index based on freshwater fish communities has yet to be developed that might be used to assess the ecotoxicological effects of metal-rich sediments in streams.

Whilst *meiofauna* are not often used for biological assessment, Burton et al. (2001) did investigate the relationship between the composition of freshwater meiofaunal communities and trace metal contamination, again in the streams of Cornwall, using univariate and multivariate statistical techniques including non-metric MDS. Dissolved Cu concentration (singly or in combination with Al, Zn or dissolved organic carbon) was the most important correlate with meiofaunal community composition. Metal contamination did alter the composition of the stream meiofaunal assemblages significantly, with cyclopoid copepods abundant at high metal concentrations, but not diversity (Burton et al. 2001).

The development of molecular techniques to analyse the taxonomic composition of *microbial communities* (Liu et al. 2007; Nocker et al. 2007) has greatly opened up the potential to use differences in these communities that might occur in response to anthropogenic contamination. Thus Automated Ribosomal Intergenic Spacer Analysis (ARISA; Fisher and Triplett 1999) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) were used to analyse the biofilm bacterial and ciliate protozoan communities at 23 urbanised stream sites in the Auckland region of New Zealand variously impacted by Cu, Pb and Zn (Ancion et al. 2013). Concentrations of the three metals in biofilms explained 7% of the variation in the bacterial biofilm communities and 9% of that in ciliate protozoan communities (Ancion et al. 2013). In laboratory experiments, significant differences in bacterial community structure occurred within 3 days of exposure to metals and remained detectable at least 14 days after transfer to uncontaminated water (Ancion et al. 2010).

In summary, appropriate indices for the assessment of metal pollution are almost entirely lacking and thus urgently needed, especially for invertebrates.

3.6 Tolerance as an Indicator of Significant Ecotoxicological Selective Pressure

The presence of a metal-tolerant population of an organism in a particular habitat is evidence that local bioavailabilities of that toxic metal are of ecotoxicological significance, clearly to that species but potentially also to other members of the local biota (Luoma 1977). Thus, the local raised metal bioavailability has acted as a selection pressure, selecting for biochemical and physiological traits that are the most metal-tolerant in the gene pool of a local population of organisms, leading then to the establishment of a local metal-tolerant population (Klerks and Weis 1987; Luoma 1977).

It needs to be restated here that, in this review, the term metal-tolerant is used to refer to a particular metal-exposed population, whether or not that tolerance is inheritable after selection over several generations or has been derived by physiological acclimation and is restricted to the one exposed generation (Amiard-Triquet et al. 2011). The term metal-insensitive was used earlier to refer to species which survive relatively well in conditions of metal contamination, without specific physiological acclimation or genetically based adaptation to the high metal bioavailability in a particular local habitat.

The presence of metal-tolerant populations has long been recorded from UK streams affected by mining activities (Kelly 1988). Harding and Whitton (1976) showed that populations of the alga *Stigeoclonium tenue,* abundant in Zn-contaminated streams in the Northern Pennines, showed lower sensitivity to exposure to dissolved Zn over populations in streams with lower Zn levels. Say et al. (1977) added the algae *Hormidium rivulare* and *H. flaccidum* to the list of species with Zn-tolerant populations in North East England. Ivorra et al. (2002a) showed that a Zn-tolerant strain of the diatom *Gomphonema parvulum* (induced by chronic exposure to Zn in the field) was sensitive to previously unexperienced high concentrations of Cu. Foster (1977) reported on a Cu-tolerant population of another alga, the green alga *Chlorella vulgaris*, from the River Hayle draining disused Cu mines in Cornwall, the relevant physiological mechanism of tolerance being Cu exclusion. In a follow-up study, Foster (1982a) identified 19 metal-tolerant strains of green algae (Chlorophyta) from the River Hayle and the nearby Pb-rich River Gannel, with several of the Cu-tolerant strains from the Hayle also showing a co-tolerance to Pb, and used these experimental results to explain the distribution of algal taxa in the field (Foster 1982b).

In a parallel study of the freshwater isopod crustacean *Asellus meridianus* in these rivers, Brown (1977) also demonstrated the presence of tolerance and co-tolerance (tolerance to more than one metal in the same population). The Hayle isopod population was tolerant to both Cu and Pb, although the River Hayle contains low Pb and high Cu concentrations; the Gannel population was tolerant only to Pb, the Pb-rich River Gannel lacking high Cu concentrations (Brown 1977). Thus, it appears that the mechanism for Cu tolerance in the River Hayle population has simultaneously achieved Pb tolerance in the absence of raised Pb bioavailability but that the mechanism for Pb tolerance in the River Gannel population is not associated with simultaneous Cu tolerance in the absence of raised Cu bioavailability. Tolerance to Zn and Cd has been recorded in chironomids in the River Dommel, Belgium, exposed to effluent from a factory smelting metal ores, although interbreeding with drifting individuals from non-adapted populations upstream leads to dilution and variation in expression (Groenendijk et al. 2002). Loayza-Muro et al. (2014a) found that only a single tolerant strain of chironomids could survive in most metal-rich sites in the Andes, and it should be noted that here (and elsewhere), tolerance may have evolved in response to naturally occurring metal-rich environments and, thus, may be much older than the human-induced changes due to mining.

Tolerance to metals has been identified in populations of brown trout (*S. trutta*) in the USA (Farag et al. 1995), Norway (Hansen et al. 2006) and the UK (Webster et al.

2013). A wider study of brown trout populations in the rivers of Cornwall, UK, indicated that populations in metal-contaminated rivers are genetically distinct from those in uncontaminated rivers and that divergence between these populations coincided with the industrial revolution, when metal mining increased in the region (Paris et al. 2015). Transcriptomic work on the trout from the River Hayle has highlighted the molecular mechanism underlying this tolerance (Webster et al. 2013). Similarly, tolerant populations of three-spined sticklebacks (*Gasterosteus aculeatus*) from metal-contaminated sites have been identified (Webster et al. 2017).

The argument that the evolution of metal tolerance is a manifestation that local metal exposure is of ecotoxicological significance can be extended to the structure of the local biotic community. Blanck et al. (1988) proposed that the selection pressure associated with an ecotoxicologically significant toxicant bioavailability will lead to an increased average tolerance to that toxicant amongst all species within that local community. Such *Pollution-Induced Community Tolerance* (PICT) is therefore a potential ecotoxicological tool to assess the effects of a toxicant on communities (Blanck and Wangberg 1988; Blanck et al. 1988; Clements and Newman 2002; Luoma and Rainbow 2008; Schmitt-Jansen et al. 2008). PICT is tested by comparing communities collected from contaminated and reference sites to contaminant exposure under controlled conditions, and increased community tolerance that results from the elimination of more sensitive species is considered to be strong evidence that community restructuring has been caused by the contaminant (Clements and Newman 2002; Clements and Rohr 2009). The need to carry out experiments to measure the difference in tolerance between communities does constrain the application of PICT as an assessment tool, although PICT has been tested in several different communities beyond the marine periphyton community on which the PICT hypothesis was first developed (Blanck et al. 1988; Clements and Newman 2002). Such communities include lentic periphyton, lentic phytoplankton, marine phytoplankton, lotic microalgae, estuarine nematodes and freshwater and coastal macroinvertebrates, and the contaminants concerned include the trace metals As, Cd, Cu and Zn (Clements and Newman 2002; Clements and Rohr 2009; Luoma and Rainbow 2008). Amongst the assumptions behind PICT is that the communities most likely to be suitable for PICT assessment are those that show a large amount of variation in sensitivity amongst species (Clements and Newman 2002), and PICT may prove particularly suitable for comparison of microbial populations collected from different field sites (Tlili and Montuelle 2011; Virsek et al. 2013).

An alternative approach to testing PICT on individual community samples is to establish species sensitivities a priori and use these to estimate the community-level tolerance. This approach works best when field-based responses to the stressor are used to establish sensitivity, as this avoids the complications of extrapolating from the laboratory to the field, but cannot account for local adaptation of tolerance.

3.7 Metal Ecotoxicity and Ecosystem Functioning

This review has focussed on how trace metal contamination of sediments affects community structure in freshwater streams, highlighting the observed community shift from sensitive to insensitive species. Another approach is to consider the ecotoxicological effects of stressors on ecosystem processes (Clements and Rohr 2009). Not all species are equally important for the functioning of an ecosystem, although communities are likely to express some functional redundancy where species share functional characteristics (Reiss et al. 2009). An abrupt decrease in ecosystem function is likely at a critical threshold of species loss, once any functional redundancy has been exceeded (Clements and Rohr 2009).

Several studies have taken an ecosystem function approach when investigating the ecotoxicological effect of metal pollution in streams and rivers (Ferreira et al. 2016). For example, Schultheis et al. (1997) showed that Cu pollution in East Prong Creek, Virginia, USA, derived from an abandoned pyrite mine, reduced taxonomic richness and abundance of benthic macroinvertebrates. Leaf decomposition rates were also decreased at the affected sites, apparently by interruption of the action of shredders processing leaf material in the streams (Schultheis et al. 1997). Maltby et al. (2002) similarly reported that the decrease in rates of feeding (leaf shredding) by *Gammarus pulex* downstream of point source discharges in UK rivers was correlated with a decrease in local leaf decomposition rates. Hunting et al. (2013) noted that sublethal effects of metals decouple invertebrate community structure and ecosystem functioning. Furthermore, Carlisle and Clements (2005) showed that the secondary production of macroinvertebrate shredders was negatively correlated with dissolved Zn concentrations in Colorado streams affected by mining, with associated declines in leaf litter breakdown rates and microbial respiration. Carlisle and Clements (2005) concluded that some shredder species contribute disproportionately to leaf litter breakdown and that the functionally dominant taxon (the stonefly *Taenionema pallidum*) was also the most sensitive to metal contamination. There was no functional redundancy in leaf litter breakdown in the study streams, and leaf litter breakdown was highly sensitive to metal contaminant-induced alterations in community structure (Carlisle and Clements 2005). Although Carlisle and Clements (2005) argue for the necessity to measure ecosystem function as well as community structure in assessments of the ecotoxicological effects of anthropogenic contaminants in stressed ecosystems, a meta-analysis of metal impacts on leaf litter breakdown suggested that most of the effect of metals is through changes in invertebrate biomass (Ferreira et al. 2016). Whilst conceptually appealing, the use of measures of ecosystem function to detect pollution problems is difficult, as attribution of an observed change to a specific cause is not straightforward; frequently there are multiple different potential mechanisms that could result in the same observed change (e.g. Niyogi et al. 2001).

In a review of freshwater macroinvertebrate trait-based community descriptors, Menezes et al. (2010) highlighted that the ecosystem functional trait approach is one of the most promising tools emerging for the biological monitoring of freshwater ecosystems. However, further research is still required to develop a broad unified monitoring tool, not least for the detection of specific stressor impacts.

As for community structure analysis, there is tremendous scope for the further expansion of genomic and metagenomic approaches to investigate the ecosystem functional processes at the microbial level, involving such indices as the Average Metabolism Response (AMR) and Community Metabolism Diversity (CMD).

3.8 Separating Effects of Metal-Rich Sediments from Other Effects of Mining

In the real world, any assessment of the ecotoxicological effects of mining-associated metal-rich sediments in streams will be confounded by the presence of other mining-associated stressors with potential detrimental effects on biotic communities. By definition, one such stressor will be the very presence of the sediment itself.

3.8.1 Sediment

Certain mining practices can result in excess sediment entering rivers and dispersing downstream through the river network (Palmer et al. 2010). Furthermore, the impact of metals on terrestrial vegetation can lead to exposed soils and erosion of fine sediments. The accumulation of excess fine sediment, even in the absence of any significant contaminant loading, will itself cause changes in the expected biotic communities of streams (Murphy et al. 2015), by direct effects such as burial, clogging and associated reduction in oxygen availability or by indirect effects via changes in habitat or food availability (Jones et al. 2012b). Hence, sedimentation has the potential to threaten ecological status in riverine systems (Collins and Anthony 2008; Collins et al. 2009). Beyond the benthic macroinvertebrate community, fine sediment in rivers will also impact on fish (Kemp et al. 2011), macrophytes (Jones et al. 2012a) and diatoms (Jones et al. 2014).

In a synthesis of the literature on the effects of sediment deposition on benthic macroinvertebrates and fish in rivers, Collins et al. (2011) briefly reviewed the key factors controlling the impacts of sediment on freshwater biota and international approaches in setting sediment targets for rivers to protect or enhance their ecological status (in light of the needs of water management policy). A feature of existing international guidelines relating to critical sediment concentration thresholds is that they are founded on a direct (linear) relationship between sediment concentration and ecological impact (Collins et al. 2011). As exemplified several times in this review, such a simple linear model is undoubtedly an oversimplification, and there is a pressing need for revised sediment targets in rivers to inform river catchment water policy with toolkits founded on the coupling of sediment pressures and biological response (Collins et al. 2011).

3.9 Other Covarying Stressors

There will be other covarying stressors potentially present when carrying out an ecotoxicological assessment of the effect of metal-rich sediments in streams affected by mining. These will need to be recognised and evaluated in any attempt to identify those ecotoxicological effects specifically down to the presence of toxic metal-contaminated sediments.

Physical stressors will include the composition of the streambed and alteration of the channel (e.g. channelization, dams) and consequent changes to flow regimes. Even these stressors will covary, for example, the composition of the streambed and sediment loading.

Chemical stressors will include levels of dissolved trace metals and their dissolved bioavailabilities, linked in turn to pH (as in AMD) which has a pronounced effect on bioavailability (see Sect. 5.3). Nevertheless, low pH will have a direct effect on biota even in the absence of high metal bioavailabilities (Gerhardt 1993; Murphy et al. 2013). Although metals do cause impacts in mining systems with neutral drainage (Byrne et al. 2013a; Jones et al. 2013), the effects of increased acidity due to mine drainage may have as much impact on biota as the toxic effects of metals (Hogsden and Harding 2012; Clapcott et al. 2016). Dissolved organic matter (DOM), perhaps derived from the sewage of mining communities, can affect biota directly as well as indirectly by chelating dissolved metals and reducing their dissolved bioavailabilities (see Sect. 5.3). Levels of organic matter in the sediment will also affect the community structure of macroinvertebrates, as seen in the use of many of the biotic indices discussed above. Urban development around mines (to house workers) can lead to increased sewage inputs in the vicinity of the mine. Other anthropogenic contaminants like PAHs or organochlorine compounds are likely to be introduced into river systems, although these are generally at relatively low levels in upland streams draining orefields with abandoned mines.

4 Legislation

Due to the well-known risks to both the environment and human health, metals feature highly in environmental legislation worldwide (e.g. the US Clean Water Act 33 U.S.C. §1,251 et seq. (1972)). In particular, quality criteria describing acceptable levels of contamination in the environment are frequently defined for those metals considered to present a high risk. In most countries, although protecting biota is an aim of legislation, achievement of the relevant environmental policy targets is assessed through chemical monitoring of metal concentrations relative to these quality criteria (Cooter 2004). However, in Europe, the Water Framework Directive (WFD) commits member states to achieving *Good* status of all water bodies using a formal classification scheme that characterises the ecological status of each water body based on biological quality elements and the hydromorphological, chemical and physico-chemical elements that support the biological elements (European Parliament 2000). Here, the status is compared with a reference condition, which represents the condition of a water body considered to be in a relatively un-impacted state. Both chemical and biological quality elements need to be at least good status to achieve a *Good* classification. The WFD uses ambient environmental standards to classify water bodies on the basis of chemical contamination, and these standards are set to prevent ecological damage from contaminants like trace metals. A particular focus is given to establishing maximum acceptable concentrations of 'priority hazardous substances', which present a particularly strong environmental risk (European Council 2007; European Parliament 2001, 2013); the measurement of these substances in surface waters constitutes a major tool for regulatory agencies in their efforts to achieve the requirements of the WFD.

Environmental quality standards (EQS) or guidelines (EQG) are very significant in assessing the extent of contamination of a water body. For reasons explained above, not least the significant role of the diet, in addition to dissolved metal in providing ecotoxicologically significant doses of toxic metal to local stream biota, it is not easy to translate the toxicity performance of a defined dissolved metal concentration from toxicological action in the laboratory to ecotoxicological action in the field. Nevertheless, EQS for dissolved trace metals in the aquatic environment have been defined for the UK and other member states (Table 1). Similar environmental standards have been published for North America and Australasia (Table 1). However, such standards are less frequently defined for the metal content of sediments (Table 2) and are typically based on less evidence than those for water (e.g. ANZECC and ARMCANZ 2000). With the exception of proposed SQG for the Flanders region of Belgium (Table 2), Europe currently lacks Sediment Quality Criteria/Guidelines (SQC/SQG). Yet, dissolved metal EQS alone may have little ecotoxicological relevance given that the sediment is often the environmental compartment of concern.

Environmental standards are only one of several benchmarks. Success is typically judged by the achievement of ecological goals, rather than by meeting purely chemical standards. Thus, the WFD places a legal obligation on EU nations to use biota to assess the ecological quality of a water body (European Parliament 2000), and this is achieved largely through community composition analysis. The WFD defines the biological quality elements to be assessed as benthic macroinvertebrates, phytoplankton, macrophytes and phytobenthos (including algae, bryophytes and angiosperms) and fish, depending on the type of water body. Phytoplanktons are not relevant to rivers. Nevertheless, a water body will still fail to be classified as 'Good' if the average concentration of any metal listed as a priority substance exceeds the relevant environmental quality standard for that specific metal. As the WFD requires efforts to restore 'Good' ecological status, biological techniques are now urgently required that can predict the consequences and cost-effectiveness of water body management measures (Jones et al. 2010). Thus, we need more biological monitoring tools to predict and support the return of 'Good' ecological status to contaminated water bodies.

Table 1 Freshwater environmental standards for metals and metalloids (in µg L^{-1})

	Hardness (mg $CaCO_3$ L^{-1})	UK EQS[a,b,c,d]		Australia and NZ 95% trigger[e]	USA[f]		Canada[g]	
		Long term (mean)	Short term (95%ile)	Annual average	Chronic CCC	Acute CMC	Long term	Short term
Aluminium				55[h]			5[h]	
Arsenic		50			150	340	5	
Arsenic III				24				
Arsenic V				13				
Boron				370			29,000	1,500
Cadmium	<40	≤0.08	≤0.45	0.2				
	40–50	0.08	0.45				0.09	1
	50–100	0.09	0.6					
	100–200	0.15	0.9		0.25	2		
	>200	0.25	1.5					
Chromium III		4.7	32		74	570	1.0	
Chromium IV		3.4		1	11	16	8.9	
Cobalt		3[i]	100[i]					
Copper	0–50	1 bioavailable		1.4			2	
							2–4[j]	
							2–4[j]	
							4	
Iron		1,000			1,000		300	
Lead		1.2 bioavailable		3.4	2.5	65	1[j]	
Manganese		123 bioavailable		1,900				
Mercury		0.05	0.07	0.6	0.77	1.4	0.026	
Molybdenum								73
Nickel		4 bioavailable		11	52	470	25[j]	

(continued)

Table 1 (continued)

	Hardness ($mg\ CaCO_3\ L^{-1}$)	UK EQS[a,b,c,d]		Australia and NZ 95% trigger[e]	USA[f]		Canada[g]	
		Long term (mean)	Short term (95%ile)	Annual average	Chronic CCC	Acute CMC	Long term	Short term
Selenium				11			1	
Silver		0.05[i]	0.1[i]	0.05		3.2	0.1	
Tin		25[i]						
Vanadium	0–200	20[k]						
	>200	60[k]						
Zinc		10.9 bioavailable + ambient background concentration						
	0–50			8			30	
	100–250				120	120		

[a] WFD-UKTAG (2008) Proposals for Environmental Quality Standards for Annex VIII Substances Final (Revised)

[b] DEFRA and the Welsh Government (2014) Water Framework Directive implementation in England and Wales: new and updated standards to protect the water environment. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/307788/river-basin-planning-standards.pdf

[c] European Parliament (2013) Directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. COM (2011) 876 final

[d] WFD-UKTAG (2014) River & Lake Assessment Method Specific Pollutants (Metals). Metal Bioavailability Assessment Tool (M-BAT). http://wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat

[e] ANZECC and ARMCANZ [Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand] (2000) *Australian and New Zealand guidelines for fresh and marine water quality. Volume 1, The guidelines*. National water quality management strategy; no.4

[f] US EPA National Recommended Water Quality Criteria http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm

[g] Canadian Council of Ministers of the Environment (1999) Canadian water quality guidelines for the protection of aquatic life. Canadian Council of Ministers of the Environment, Winnipeg. http://ceqg-rcqe.ccme.ca/

[h] $pH < 6.5$ Australia and New Zealand, $pH < 5$ Canada ($100\ \mu g\ L^{-1}$ for $pH > 5$)

[i] Non-statutory. Dangerous Substances Directive (76/464/EEC)

[j] Variable dependent on measured hardness

[k] Statutory. Dangerous Substances Directive (76/464/EEC)

Table 2 Freshwater sediment quality guidelines for metals and metalloids (in mg/kg dry wt)

	Australia and New Zealand[a]		Belgium (Flanders)[b]	Canada[c]		USA[d]
	Low trigger value (10% probability of effect)	High trigger value (50% probability of effect)	SQG	Interim sediment quality guidelines	Probable effect level	Probable effect level
Cadmium	1.5	10	1	0.6	3.5	0.99
Chromium	80	370	62	37.3	90	43.4
Copper	65	270	20	35.7	197	31.6
Lead	50	220	40	35	91.3	35.8
Mercury	0.15	1	0.55	0.17	0.486	0.18
Nickel	21	52	16			22.7
Silver	1	3.7				1
Zinc	200	410	147	123	315	121
Antimony	2	25				2
Arsenic	20	70	19	5.9	17	9.8

[a] ANZECC and ARMCANZ [Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand] (2000) *Australian and New Zealand guidelines for fresh and marine water quality. Volume 1, The guidelines*. National water quality management strategy; no. 4

[b] Flemish sediment quality guidelines as published on July 9th 2010 (de Deckere et al. 2011)

[c] Canadian Council of Ministers of the Environment (1999) Canadian sediment quality guidelines for the protection of aquatic life: Winnipeg

[d] Consensus-based sediment quality guidelines for freshwater ecosystems (Dalu et al. 2017; MacDonald et al. 2000): there are no promulgated federal criteria for sediments in the USA

5 Explanation and Prediction of Metal Ecotoxicity

To explain and predict how metal-rich sediments in mining-affected streams exert ecotoxicological effects on the local biota, there is a need to understand how metal bioavailability and toxicity causes effects at the community level (Fig. 9). Thus, we need to demonstrate what ecological effects are caused by metals, at what concentration and under what conditions – i.e. how much metal is a problem, with an evidence base behind the choice of measure. This review will consider community-level biological methods to assess the ecological status of streams with metal-rich sediments, but it will also make use of the established correlations between biomarkers at low and high levels of biological organisation (Amiard-Triquet et al. 2013) to assess the predictive applicability of new biological tools.

Can we use a relatively simple biological assay of local metal bioavailability to interpret analysis of community composition? Subsequently, we can then address the question ‘Can we determine where (and how much) restoration effort is needed by understanding the link between bioavailability, toxicity and community ecological effects?’

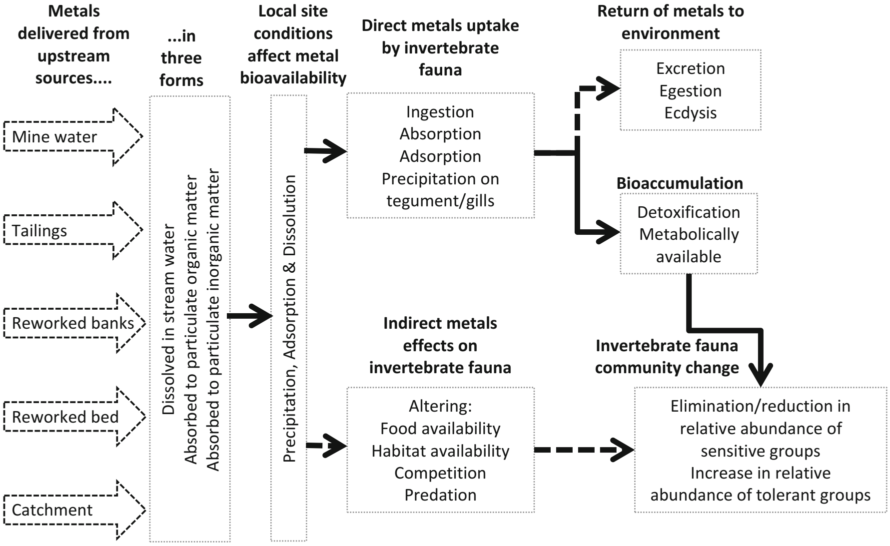


Fig. 9 Schematic diagram of how metals derived from mine workings and the catchment impact the invertebrate community. The bioavailability of metals from upstream sources is influenced by local site conditions, which influence the direct uptake of metals by invertebrate fauna (from particulates, primarily through ingestion). Some of the metals taken up are returned to the environment, the remainder are bioaccumulated (either detoxified in the tissues or metabolically active and, hence, toxic). The toxicity of available metals results in changes in the invertebrate fauna. Although toxicity of metals taken up by direct consumption is the main route of impact, indirect effects of metals can also impact the invertebrate fauna. For simplicity feedback loops are not illustrated (e.g. bioaccumulation of metals alters food availability and competition [by elimination/reduction of sensitive groups] and the metal content of available prey, which combine to alter the rate of metal uptake through ingestion)

In this section we introduce existing methods used to address these questions and attempt to draw summarised conclusions on a resource-efficient way forward.

5.1 Laboratory Testing: Bioassays

Historically, environmental regulations have drawn on a database of laboratory-based acute toxicity testing using high dissolved concentrations of toxic metals. Such acute toxicity tests have served to rank metals in order of (dissolved) toxicity and to rank species in order of sensitivity to such dissolved toxic metals. The classic toxicity test is the acute (dissolved) toxicity test over 4–96 h with death as the endpoint, aiming to provide an estimate of the (lethal) concentration that will kill 50% of the population – the LC_{50}. Traditional single species toxicity testing has been extended to include sediments and to cover multiple species and more sensitive endpoints than lethality with longer duration tests at lower exposure concentrations to detect sublethal effects (Besser et al. 2015; Luoma and Rainbow 2008). Such tests have the advantage of providing environmental regulators with single numbers to define toxicity, and it is typical to use chronic toxicity data to derive regulatory thresholds, rather than acute data.

We now, however, appreciate the limitations in extrapolating the results of such toxicity testing to real field situations (Luoma and Rainbow 2008). Toxicological responses derived under controlled laboratory conditions and continuous exposure, whilst defining absolute limits, are likely to be overprescriptive for variable natural conditions, where spatial patchiness and behaviour of organisms can influence exposure. This disconnect between toxicity tests and field responses is not restricted to metals; similar difficulties in interpretation of toxicity tests have been noted for organic pollution (Jones et al. 2008). Furthermore, animals obtain a significant (often majority) source of metals from their diet, and it is no longer accepted that only metals taken up from solution are ecotoxicologically significant (Cain et al. 2011; Cardwell et al. 2013; Croisetière et al. 2006; Erickson et al. 2011; Farag et al. 1994; Kiser et al. 2010; Poteat and Buchwalter 2014). This is particularly true in the case of sediment-ingesting deposit-feeding animals. Thus, a single dissolved metal concentration derived in the laboratory has little direct relevance to the field, particularly given the variation in physico-chemical conditions amongst contaminated sites, which influence the bioavailabilities of metals. Furthermore, geochemical differences will occur between sediments in the field and sediments tested in the laboratory (including effects of collection and transport of natural sediments; Pereira et al. 2000), affecting the local exchange of sediment-associated metals with the water column and more importantly the trophic availability of metals to sediment-ingesting infauna like oligochaete worms and other test organisms (Méndez-Fernández et al. 2017). Extra metals are often spiked to increase the range of metal exposure concentrations to be tested (Schlekat et al. 2016; Simpson et al. 2011; Vangheluwe et al. 2013). Such spiking does have negative implications for the partitioning of added metals: a better approach might be to mix sediments with higher and lower metal concentrations collected from the same water body (such as a particular river or estuary) but otherwise very similar physico-chemical characteristics for use in toxicity tests (e.g. Casado-Martinez et al. 2010b). Furthermore, biology intervenes – the relative importance of different metal uptake routes will vary for different organisms, causing interspecific differences in ecotoxicological responses to the suite of bioavailable metal sources active in the field. This feature is particularly important if inappropriate species have been used in the original laboratory toxicity testing, as in the (at one time common) exposure of the planktonic swimmer *Daphnia* to metal-rich sediment (although an alternative, benthic, cladoceran has been suggested; Dekker et al. 2006). Given the enormous diversity of responses to metal exposure amongst fauna and flora, it is not practical for all species to be tested. Time scales of laboratory and field exposures are inevitably different, with most toxicity tests using exposure times of less than the generation time of the test organism as is the case in nature.

These limitations are well recognised now for metals and other pollutants (e.g. Jones et al. 2008), and systematic adjustments (application factors) are typically made to laboratory-derived toxicity measures to take into account potential underestimations of toxicity, and chemical test conditions are set for worst possible cases (Cairns and Mount 1990). Decades of toxicity testing have allowed databases for many metals to grow and include many species. *Species Sensitivity Distributions* (SSD) can provide evidence on the dissolved metal concentration that would have no ecotoxicological effect (NOEC) on, for example, 95% of species, the percentage defined by the US Environmental Protection Agency (EPA) to be used in protecting aquatic communities (Posthuma et al. 2002). SSD plot the NOEC of a metal for different species against the cumulative rank of the value amongst species (Fig. 10:

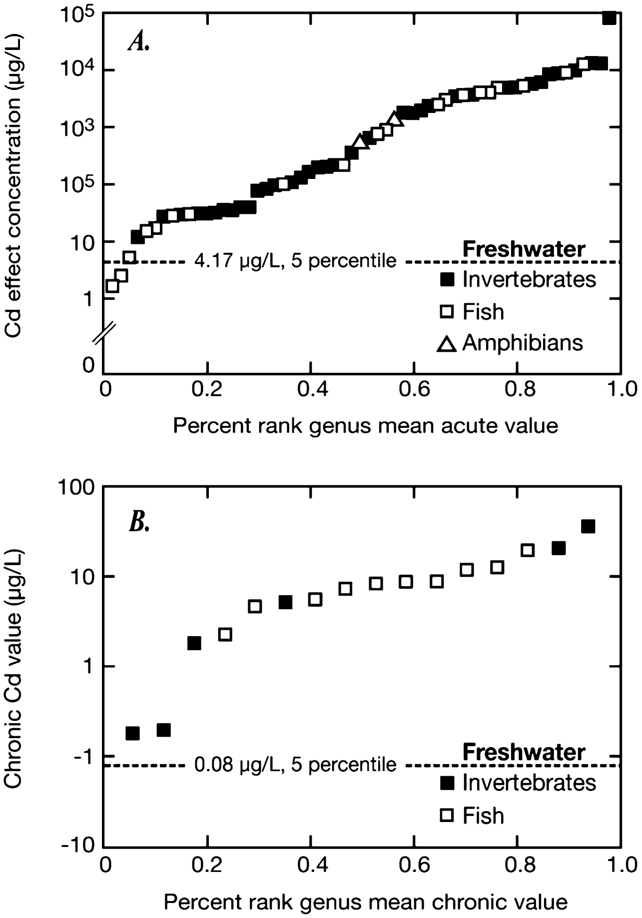


Fig. 10 Species Sensitivity Distributions (SSD) of (**a**) concentrations of Cd causing acute toxicity ranked for different genera of invertebrates, fish and amphibians. This SSD can be used to derive a freshwater final acute value for Cd – the Cd concentration at which toxicity was observed for the most sensitive 5% of taxa (4.17 µg/L at 50 mg/L hardness). (**b**) SSD based on chronic toxicity tests. Sensitivity at the fifth percentile occurs at 0.08 µg/L at 50 mg/L hardness (after USEPA 2001)

Brix et al. 2001), and risk is estimated as the percentage of species expected to be affected at any dissolved concentration. The SSD method is the preferred approach to setting environmental quality standards; however, it is often not used for sediments (e.g. ANZECC and ARMCANZ 2000).

5.2 *Extrapolation from Laboratory to Field*

Given that EQS for dissolved metals in freshwaters were based mainly on laboratory toxicity data with associated uncertainties as to their extrapolation to the field, Crane et al. (2007) analysed a dataset from England and Wales of dissolved metal (Cd, Cr, Cu, Fe, Ni, Pb, Zn) concentrations and associated benthic invertebrate community metrics. Crane et al. (2007) used piecewise (‘broken stick’) regression, quantile regression and available data on metal concentrations consistent with good quality status (WFD-UKTAG 2008) and showed that proposed dissolved metal EQS available were similar to dissolved metal concentrations in rivers with unimpaired benthic macroinvertebrate assemblages. Field tests of laboratory derived EQS for sediments have yet to be undertaken.

5.2.1 In Situ and Mesocosm Toxicity Tests

Improvements on laboratory toxicity testing have been the development of in situ sediment testing, using microcosms to better reflect field situations. In situ toxicity testing involves the exposure of test organisms within or on the sediments of the water body of interest, typically by caging organisms in direct contact with intact sediments, better simulating natural exposures (Besser et al. 2015). In situ bioassays with fish have a long history, but methods for testing macroinvertebrates in sediments have been developed more recently (Burton et al. 2003, 2012). In situ protocols typically require survival of control organisms for 10–28-day exposure periods, necessitating the use of test organisms that can survive handling stress and fluctuating field conditions. A challenge then is to distinguish between the effects of the metal contamination of the sediment and the effects of handling or responding to test conditions. For example, Olsen et al. (2001) transplanted fourth instar *Chironomus riparius* larvae for 48 h at 13 uncontaminated river sites across South East England. Activities of two enzymatic biomarkers varied almost twofold across the sites, with statistically significant differences detectable between sites. Olsen et al. (2001) concluded that biomarker results must be treated with caution because natural variability in responses can occur even in the absence of toxicant exposure.

Mesocosms can be used to test ecotoxicological questions that single species bioassays cannot, such as, for example, secondary ecological effects on communities (Clark and Clements 2006). Although mesocosm studies can be costly, labour-intensive, difficult to control and difficult to replicate (and therefore rarely used in routine toxicity testing), mesocosms are well suited to testing process questions (Cadmus et al. 2016; Clements et al. 1990, 2013; Kiffney and Clements 1996). Clements et al. (1988), Kiffney and Clements (1994) and Clements (2004) used multispecies experimental mesocosms to investigate field toxicities of metals in Rocky Mountain streams in the USA, and Cadmus et al. (2016) assessed the relative influence of metals and other mining-associated stressors. Multispecies assemblages were collected by placing boxes of stones in uncontaminated streams for 40 days to allow colonisation by local fauna. The boxes were then transferred intact to the laboratory to be submerged in experimental mesocosm streams for 10 days. Results from such experiments established a hierarchy of vulnerability to metal exposure amongst the local taxa inhabiting streams, in line with relative metal sensitivities discussed above. Thus, mayflies and stoneflies showed reduced abundance and species richness at the lowest metal concentrations in the mesocosms, and heptageniid mayflies such as *Epeorus longimanus* were particularly sensitive to the metals, in line with field surveys of metal-contaminated streams (Clements 2004). Although the size of individuals influenced sensitivity (Kiffney and Clements 1996), numbers of overall taxa, numbers of mayfly taxa and EPT showed statistically significant metal concentration – response relationships. The mesocosm experiments could be used to generate EC_{10} values (effect concentrations affecting 10% of the population), which correspondingly showed higher values for combined taxa than for single sensitive species like *E. longimanus* (Clements 2004).

Mesocosms have also been used to investigate the effects of mixtures of metals (e.g. Zn, Cu and Cd) in comparison to those of single metal exposures (Clements 2004). The Cumulative Criterion Unit (CCU) is the additive measure of toxicity used in such cases. The dissolved concentration of each metal is divided by the US EPA chronic criterion value for that metal derived from single species toxicity tests but adjusted for water hardness by formula, and the ratios for each metal are summated to give the CCU (USEPA 1986). Macroinvertebrate responses to a mixture of the three metals Zn, Cu and Cd were generally greater than responses to either Zn alone or to Zn + Cd (Clements 2004).

The same mesocosm approach to investigate the effects of metal contamination in New Zealand streams (Hickey and Clements 1998; Hickey and Golding 2002) has led to similar conclusions, particularly that abundance and species richness of mayflies are sensitive measures of metal effects in streams. The wide generality of such conclusions offers confidence that, although community-level responses are to some extent context dependent (Clements et al. 2016), biological responses to metal contamination in streams are predictable.

Community-level mesocosm experiments by Clements et al. (2013) in Colorado have shown that EC_{50} values, defined as the metal concentrations that reduced abundance of stream insects by 50%, were several orders of magnitude lower than values derived for single species from previous laboratory testing. Clements et al. (2013) hypothesised that the short duration of laboratory toxicity tests and the lack of evaluation of effects on early life history stages are the primary factors behind the mismatch between laboratory and field measures. The mesocosm results of Clements et al. (2013) suggest that US water quality criteria for Zn would protect most aquatic species but that Cu was highly toxic to some species at concentrations near to the published Cu water quality criteria. Similar conclusions were arrived at by Iwasaki et al. (2018) when comparing the results of the mesocosm experiments by Clements with field community data: the ranked sensitivity of some taxa differed between the two approaches, potentially due to short-term experiments not accounting for dietary exposure, rapid recolonisation and sensitive life stages.

Although the use of mesocosms as described here still involves only the dissolved concentrations of toxic metal as measures of contaminant input, mesocosms are more representative of field conditions, and the results from mesocosm experiments, together with field data, can provide more precise estimates of 'safe' metal concentrations than laboratory toxicity tests alone (Cadmus et al. 2016; Clements 2004). Nevertheless, the extrapolation of the results of any laboratory controlled experiment with, for example, continuous exposure/concentration to the field, where concentrations, environmental conditions and behavioural responses are variable, is not straightforward.

5.3 Modelling of Dissolved Bioavailability

As defined in the introduction, bioavailability describes a relative measure of that fraction of the total ambient metal concentration that an organism actually takes up when encountering or processing environmental media, summated across all possible sources of metal, including water and food as appropriate. Specifically, in the case of dissolved bioavailability, it is now recognised that only a fraction of the total dissolved metal is available for uptake across a cell membrane into an organism, i.e. is bioavailable. A good model for the dissolved bioavailable fraction of many trace metals is the free metal ion according to the Free Ion Activity Model (FIAM; Campbell 1995).

The release of the free metal ion in the various chemical equilibria affecting a dissolved metal is very dependent on the physico-chemistry of the medium. Dissolved organic matter, especially humic acid (HA), tends to complex metal ions, thereby reducing the percentage of free metal ion in the total dissolved metal concentration. Acid conditions often promote the availability of the free metal ion, and the presence of other dissolved metals may also affect the release of the free ion from different forms of organic and inorganic complexation. Thus, the same total dissolved metal concentration may have very different concentrations of free metal ions, according to local physico-chemical conditions.

In the absence of analytical techniques to measure the concentrations of all the different forms (chemical species) of metals dissolved in natural waters, speciation modelling has proved a very successful alternative (where sufficient data are available). Thus, the activities of free metal ions and other chosen low molecular weight metal complexes can be calculated from first chemical principles, by considering the total concentration of dissolved metal, the concentrations of potential ligands (ions or molecules that form a complex with a metal ion) and the stability constants defining the affinity of each ligand to the metal. One of the most successful and commonly used metal speciation models in freshwaters is WHAM, the Windermere Humic Aqueous Model (Tipping 1994; Tipping et al. 1998) This model includes very thorough analyses of metal-organic (especially metal-HA) interactions and is updated regularly (e.g. Lofts and Tipping 2011; Stockdale et al. 2010; Tipping et al. 2011, 2016). A tenet of the WHAM model is that the dissolved metal cation binding sites of aquatic invertebrates can be modelled by the functional groups of humic acid, and WHAM can model how much metal will bind to HA under different physico-chemical circumstances. The predictor 'bioavailable' dissolved metal concentrations calculated by WHAM in its different updated guises are usually better predictors of the toxicological effects of dissolved metals than are total dissolved metal concentrations. In Colorado, Iwasaki et al. (2013) found that predictor concentrations of Zn, Cd and Cu derived from the WHAM 7 model of Tipping et al. (2011) provided better estimates of metal effects observed in mesocosm experiments than three other measures – total metal concentrations, free metal ion concentrations and the CCU. Stockdale et al. (2010) developed WHAM into the WHAM-F_{TOX} model to describe the bioavailability and toxicity of proton and metal mixtures to aquatic organisms, by incorporating a toxic potency coefficient for each metal. Stockdale et al. (2014), however, had variable levels of success in applying this model to sites in UK and Norwegian streams and suggested that the presence of other unmodelled factors was further repressing species richness at some sites.

Biotic Ligand Models (BLMs) are a theoretical offshoot of the FIAM (Paquin et al. 2002), which predict dissolved metal toxicity on the basis of calculated (modelled) free metal ion activities as affected by the competitive effects of major ions and pH (critically important in freshwaters). BLMs were developed to explain how water chemistry affects the toxicity of dissolved metals, toxic exposure being expressed by the occupancy by the metal of a key (biotic) ligand. In the regulatory arena, BLMs can be used to incorporate site-specific physico-chemical conditions, using geochemical modelling to calculate metal speciation (WFD-UKTAG 2014). The BLM approach is used to set EQS for most metals across the European Union (see Table 1). An advantage of the BLM is that it shifts emphasis from the exposure solution to a postulated biological receptor. Although based on chronic exposures to dissolved metals, dietary metal uptake is typically not included, and uptake properties have only been characterised for a few species. Schmidt et al. (2010) developed a toxic unit model of additive trace metal toxicity (the Chronic Criterion Accumulation Ratio, CCAR) derived from BLM outputs to estimate the toxicity of trace metal mixtures to benthic communities (e.g. Schmidt et al. 2012b).

There is no doubt that the predictor concentrations coming from dissolved metal speciation models such as WHAM, WHAM-F_{TOX} and the BLM are superior estimators of dissolved metal bioavailabilities than total metal concentrations. However, it still needs to be remembered that such models concern dissolved metal concentrations and ignore the ecotoxicologically significant role of metal uptake from sediments, particularly via the diet, in the real contaminated world. Similar constraints apply to the use of passive samplers employing diffusive gradients in thin film technology to model dissolved metal bioavailabilities (Costello et al. 2012).

5.4 Bioaccumulation, Biomonitors and Identification of Ecotoxicological Effects

Aquatic invertebrates take up trace metals in relation to the total metal bioavailabilities (e.g. dissolved and dietary) to which they are exposed. They are typically net accumulators of trace metals, the strength of the accumulation after uptake being controlled by the subsequent physiological balance between uptake rates, excretion rates and storage detoxification rates (Luoma and Rainbow 2008). The metal accumulation patterns of aquatic invertebrates vary between metals and invertebrate species and will lie on a gradient from weak to strong accumulation. Different invertebrate species have different rates of turnover of metals in their bodies and will achieve different accumulated body metal concentrations under the same metal exposure conditions (Méndez-Fernández et al. 2017).

These principles allow the use of aquatic invertebrates as biomonitors of trace metal bioavailabilities. Biomonitors are of great utility in biological monitoring programmes, for their use can identify areas of high metal bioavailability (strictly to that chosen biomonitor but interpreted more generally) and identify changes in metal bioavailability over space and time (see Introduction). It must be remembered, however, that a highly accumulated body concentration of a metal does not per se indicate that the invertebrate (or other organism such as a plant) is suffering an ecotoxicological effect. Such a demonstration requires the application of biomarkers from any level from the molecular to the community.

It is not surprising that the changes in the summated bioaccumulated metal concentration of all invertebrate species in a community will not automatically be a good predictor of altered macroinvertebrate community structure (Beltman et al. 1999). The metal concentration of a pooled invertebrate sample will depend not only on local metal bioavailabilities but also on other factors such as the presence and relative contribution of the different invertebrate species present in the sample (with different associated metal accumulation patterns), life history stage, size, age, etc. (Beltman et al. 1999; Hare 1992; Kwan et al. 2003; Shoults-Wilson et al. 2015). The community composition will be changed on metal exposure in ways that will inevitably affect the total metal bioaccumulation ability of the community (Beltman et al. 1999).

The key to the use of biomonitors and metal bioaccumulation in ecotoxicological studies is to interpret accumulated metal concentrations at the species level. Thus, it is well established that the use of individual species as biomonitors provides key information on differences in metal bioavailabilities over space and/or time (Wilson et al. 2018). Biomonitoring of a water system may make use of the in situ fauna, as in the case of the caddis flies *Plectrocnemia conspersa* (Gower and Darlington 1990) and *Hydropsyche* spp. (Awrahman et al. 2016) in the mining-impacted rivers of Cornwall, UK, or baetid mayflies and the amphipod *Gammarus fossarum* (Fiałkowski et al. 2003a, b; Fiałkowski and Rainbow 2006) and again *Hydropsyche* spp. (Awrahman et al. 2016) in metal-rich streams in Poland. Mussels (Bivalvia) are the most frequently used biomonitors in both marine (e.g. Mandich 2018) and freshwater systems (e.g. Kraak et al. 1991; Shoults-Wilson et al. 2015; Wagner and Boman 2004; Wong et al. 2017; Yologlu et al. 2018), although they are often lacking from the coarse substrate rivers typical of hard rock metal-mining areas. Alternatively, members of a control population of invertebrates may be deployed at different sites for a time period before analysis (e.g. the amphipod crustacean *Hyalella azteca* by Couillard et al. (2008) in mining-affected river systems in Quebec).

As discussed earlier, it is not possible to correlate a specific total accumulated body concentration of an aquatic invertebrate in the field with the onset of toxic effects in that individual, there being no critical (lethal) total body concentration of a metal in an invertebrate using (temporary or permanent) storage detoxification (Adams et al. 2010; Casado-Martinez et al. 2010b; Rainbow and Luoma 2011b). So, there is no specific ecotoxicological information in a bioaccumulated metal concentration relevant to the biomonitor itself. Studies are now showing, however, that it is possible to calibrate accumulated metal concentrations in specific biomonitors against ecotoxicological changes occurring in the local biological community, typically the benthic macroinvertebrate community (Awrahman et al. 2016; Luoma et al. 2010; Rainbow et al. 2012). Luoma et al. (2010) found a correlation between the bioaccumulation of Cu in larvae of metal-hardy *Hydropsyche* caddis flies and the number of species and abundance of more metal-sensitive ephemerellid and heptageniid mayflies at sites in the Clark Fork River system, Montana, USA. Rainbow et al. (2012) found a similar response between the bioaccumulated concentrations of toxic metals in the caddisfly *Hydropsyche siltalai* (as indicators of metal bioavailabilities) and ecotoxicological responses of mayfly larvae in metal-contaminated streams of Cornwall, England. Mayfly larvae were always sparse where metal bioavailabilities, hence bioaccumulated metal concentrations in the caddis flies, were high and were abundant where metal bioavailabilities were low, a pattern particularly evident when the combined abundance of ephemerellid and heptageniid mayflies was the response variable (Rainbow et al. 2012). Furthermore, Rainbow et al. (2012) were able to identify threshold bioaccumulated concentrations in *H. siltalai* (especially of Cu and As, the apparent major ecotoxicological metal drivers in many Cornish streams – see also Gower et al. 1994), corresponding to the elimination of mayflies of these two families through the ecotoxicological effects of the reflected high metal bioavailabilities (Fig. 11). Rainbow et al. (2012) used these threshold bioaccumulated concentrations (170 μg Cu g^{-1}, 85 μg As g^{-1}, 300 μg Zn g^{-1}, 300 μg Pb g^{-1}) to define toxic units (Adams and Rowland 2003) for each metal by dividing the mean accumulated concentration in *H. siltalai* at each site by this threshold concentration for the relevant metal. The toxic units for different metals can be summed at each site to assess the additive ecotoxicological effects of the metals (Fig. 11). Awrahman et al. (2016) successfully used a similar approach to combine data from England and Poland and, thus, identify threshold community response.

Schmidt et al. (2011) have addressed the same principle and showed that whole body Zn concentrations in three aquatic insect taxa (heptageniid mayflies *Rhithrogena* spp., ephemerellid mayflies *Drunella* spp. and the caddisfly *Arctopsyche grandis*) could be used to predict ecotoxicological effects on stream

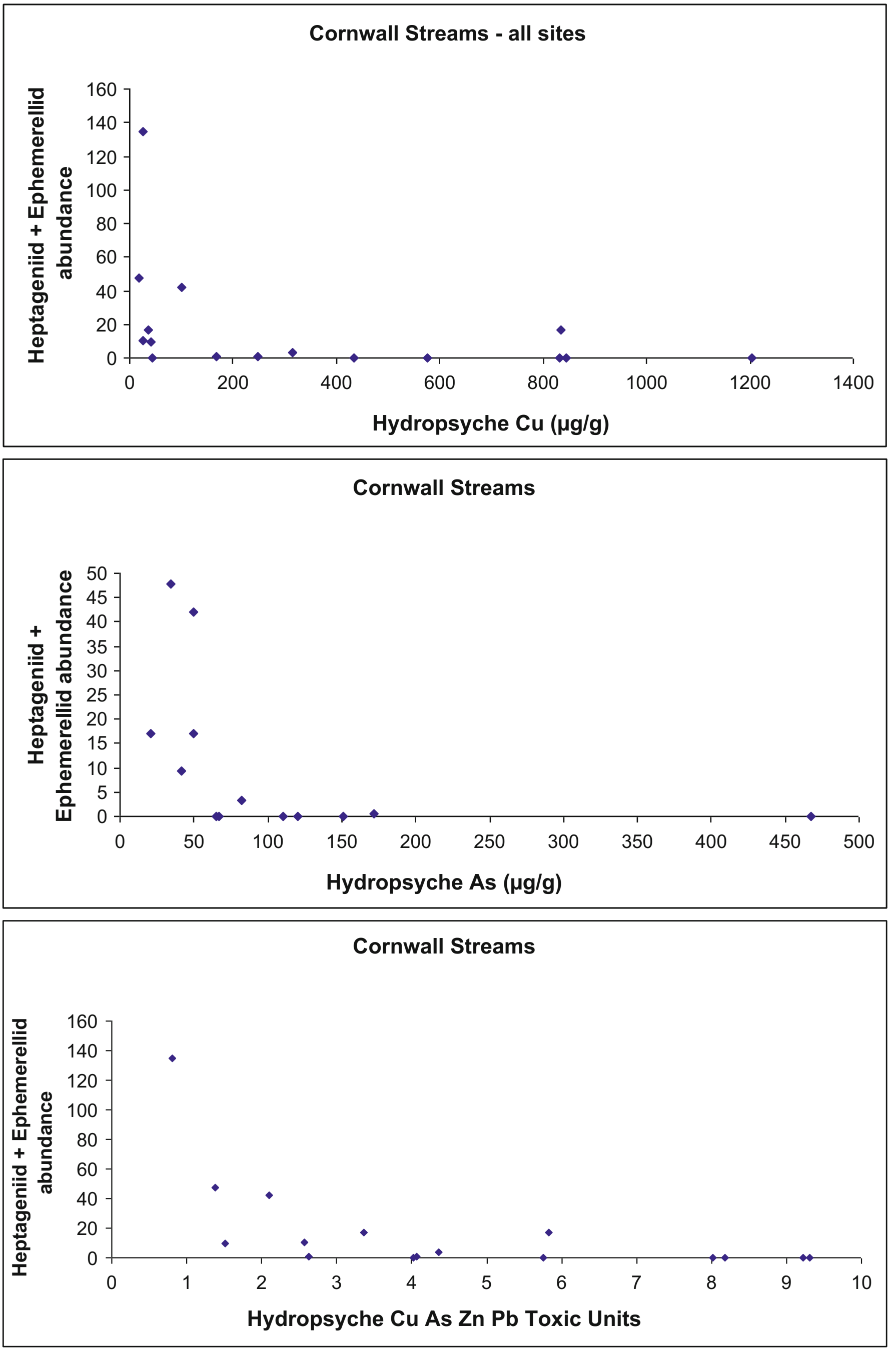


Fig. 11 Plots of mean accumulated Cu and As concentrations and combined mean accumulated metal concentrations expressed as toxic units, in larvae of the caddisfly *Hydropsyche siltalai* from sites in metal-contaminated Cornish rivers against combined abundance of heptageniid and ephemerellid mayfly larvae (mean number of mayfly larvae in 1 min kick sample) (from Rainbow et al. 2012)

communities in the Rocky Mountains, USA. Schmidt et al. (2011) were also able to define threshold bioaccumulated concentrations (critical tissue residues) of Zn in the biomonitors that were associated with specific percentage declines in mayfly densities and taxonomic completeness (the ratio of expected and observed numbers of taxa).

de Jonge et al. (2013) also showed that the metal body burdens of specific biomonitors can be used to predict metal-induced effects on macroinvertebrate communities in upland streams in North West England, using quantile regression analysis for comparison against community structure parameters including taxonomic completeness (RIVPACS) and BMWP scores. The study did not identify biomonitor species but, nevertheless, showed significant relationships between Cu, Zn and Pb body burdens in the stonefly *Leuctra* sp. (Zn, Pb), the heptageniid mayfly *Rhithrogena* sp. (Cu, Zn, Cu + Zn) and mixed simuliid blackflies (Zn, Pb) and both taxonomic completeness and BMWP scores (de Jonge et al. 2013).

Using a similar approach, Bervoets et al. (2005) could relate parameters of fish community structure to summated toxic units of toxic metal accumulation in fish livers in metal-contaminated river systems in Flanders, Belgium. However, this specific approach does lack the benefit of obtaining a measure of high metal bioavailabilities in the absence of the species directly affected by those high bioavailabilities.

It appears, therefore, that bioaccumulated concentrations of metals in metal-insensitive biomonitors can be calibrated to diagnose ecotoxicological impacts on stream benthos from metal stressors. There is considerable potential for further extension of the number of biomonitors to be calibrated against observed ecotoxicological responses of benthic macroinvertebrates. In addition to the species and genera mentioned above, potential biomonitors include the zebra mussel *Dreissena polymorpha* and species of chironomid midge larvae (de Jonge et al. 2012), larvae of the caddis flies *Plectrocnemia conspersa* (Rainbow et al. 2012) and *Hydropsyche* spp. (Awrahman et al. 2016) and mayfly larvae of the genus *Baetis*, as well as gammarid amphipods.

5.5 Weight of Evidence (WoE) Approach

The determination of the ecotoxicological potential of metal-contaminated sediment ultimately requires the integrated use of a battery of techniques – a weight of evidence (WoE) assessment combining evidence from different lines of evidence (Benedetti et al. 2012; Chapman 2007a; Chapman and Anderson 2005; Piva et al. 2011). WoE determinations include both chemical and biological measurements and typically include both laboratory and field components which may be observational or involve experimental manipulation (Chapman 2007a). The original Sediment Quality Triad of Long and Chapman (1985) was a pioneer of this approach and comprises evidence of the extent of contamination, evidence of toxicity (at field concentrations, usually derived through laboratory experimentation) and evidence of change in benthic communities (from field data, including the contaminated sites). These three lines of evidence provide eight different outcomes on which to base management decisions and, when they coincide as positive, the clearest evidence of the impact of contaminated sediment (Long and Chapman 1985). Fewer lines of evidence cannot support the conclusion that any potential impacts actually occur in the receiving environment (Chapman 2007b). On the other hand, more lines of evidence provide more potential outcomes and stronger evidence of impact, enabling cause and effect to be evidenced: the original triad has now been expanded, to include, for example, biomarkers (Chapman 2007a; Chapman and Hollert 2006).

There are examples of the integration of laboratory and field approaches to assess the impact of metal pollution in mining-impacted streams. Thus, Clements and Kiffney (1994) measured water metal concentrations, bioaccumulation of metals by periphyton and selected benthic macroinvertebrates and parameters (e.g. abundance, species richness and percentage Ephemeroptera) of the community structure of benthic invertebrates at sites in the Arkansas River, USA, impacted by historic mining activities. In the laboratory, 7-day toxicity tests were carried out with the planktonic cladoceran *Ceriodaphnia dubia* exposed to river water (Clements and Kiffney 1994). In this early integrated study, Clements and Kiffney (1994) were able to conclude that an integrated approach be recommended for assessing effects of metals in streams. In subsequent years, Clements and colleagues took this recommendation forward with great success in the Colorado area, incorporating the more ecologically relevant results from mesocosm experiments to provide necessary toxicity test data (e.g. Clements et al. 2013; Schmidt et al. 2011).

Leslie et al. (1999) used the sediment triad approach to identify the impact of Cr on the Chusovaya River, Russia, using evidence from community analysis together with biomonitoring using the caddis fly *Hydropsyche pellucidula*, which in turn exhibited physical abnormalities (gill damage and discoloration of anal papillae). Wolfram et al. (2012) combined a great deal of laboratory-based and field evidence in an impressive WoE approach to assess the ecotoxicological impact of sediment contamination on benthic invertebrate communities in three river basins (Elbe, Scheldt and Llobregat). Chemical analyses of sediments were integrated with a battery of sediment toxicity tests, encompassing bacteria (*Vibrio fischeri*), benthic invertebrates of varying field relevance (the universal laboratory nematode model *Caenorhabditis elegans*, the gastropod mollusc *Potamopyrgus antipodarum*, the oligochaete *Lumbriculus variegatus* and the midge larva *Chironomus riparius*) and fish embryos (*Danio rerio*), together with univariate and non-parametric statistical analyses of biological data on the benthic macroinvertebrates (Wolfram et al. 2012). A selective approach based on this study by Wolfram et al. (2012) but with the addition of biomarkers (Allan et al. 2006) has considerable potential for a WoE assessment of metal-contaminated sediments in mining-affected streams.

6 Conclusions

It is possible finally to draw conclusions from this review of available tools for the ecotoxicological assessment of metal-contaminated sediments in mining-affected water systems and propose a WoE toolbox that would meet the requirements of environmental policy.

6.1 Sediment Metal Concentrations

Typically, legislation requires chemical data, and it is appropriate to measure the trace metal concentrations of sediments collected from sites under investigation. These can be compared against SQG (or suchlike), but it must be remembered that total metal concentrations are not measures of bioavailable metal concentrations. Extractions to model bioavailable concentrations in sediments have some value given an understanding of the routes of metal uptake used by different organisms under investigations. Indices such as the AVS index, however, are based on a flawed understanding of the biological processes affecting the uptake of metals and are to be avoided (de Jonge et al. 2009).

6.2 Bioaccumulated Metal Concentrations in Biomonitors

A real measure of bioavailable metals in a habitat is provided by the bioaccumulated metal concentrations in selected biomonitors of known biology and metal accumulation physiology and kinetics (Luoma and Rainbow 2008). Biomonitors provide an integrated measure of the uptake and subsequent accumulation of toxic metals from different sources of metal (e.g. solution, diet) according to the biomonitor chosen, a carefully chosen suite of biomonitors covering all potential routes of uptake (i.e. bioavailable metal sources).

Biomonitors provide vital information on the geographical and temporal variations in metal bioavailabilities, in this case in a freshwater system, without direct information as to whether that biomonitor is itself suffering ecotoxicological effects of the metal exposure and subsequent metal uptake and accumulation. However, it has now been shown that absolute bioaccumulated metal concentrations in specific hardy biomonitors can be correlated with ecotoxicological changes in the habitat. This approach, after calibration, offers enormous scope for relating the measurement of bioaccumulated concentrations in such selected biomonitors to the community structure of local benthic macroinvertebrates.

Species of the caddis fly genus *Hydropsyche* are prime candidates for this role (Awrahman et al. 2015, 2016), although other biomonitors (e.g. mussels) also offer potential, perhaps in habitats where these caddis flies might be absent, due to factors such as high local sedimentation. *Hydropsyche* species spin nets to collect food, and it can be expected that fine (re)suspended sediment particles will be ingested by these caddis flies. Other potential biomonitors are species of the stonefly genus *Leuctra* in upland streams (de Jonge et al. 2013), species of the mayfly *Baetis* (Fiałkowski et al. 2003b) which are less metal-sensitive than many other mayflies (Gower et al. 1994; Rainbow et al. 2012) or amphipods (Couillard et al. 2008; Fiałkowski et al. 2003a).

6.3 Toxicity Tests

The original Sediment Quality Triad (Long and Chapman 1985) invoked the use of laboratory-based toxicity data. Given their extent and availability, there is still an attraction to use such data to express comparative toxicities of metals to different organisms, not least freshwater benthic macroinvertebrates in the establishment of Species Sensitivity Distributions (SSD) or to contribute to the development of biotic indices. For assessment of sediment toxicities, laboratory whole sediment toxicity tests are by definition more relevant than dissolved toxicity tests. Nevertheless, more ecologically realistic and relevant data can now be obtained from in situ toxicity testing or mesocosm toxicity tests. These are the way forward if comparative ecotoxicity data are required in a particular WoE assessment.

6.4 Biomarkers

Biomarkers do provide evidence of the presence of an ecotoxicological effect on metal-exposed organisms and have great potential for successful inclusion in biological monitoring programmes (Allan et al. 2006; Amiard-Triquet et al. 2015; Chapman and Hollert 2006; Collier et al. 2013).

Biomarkers at the lower levels of biological organisation (molecular, biochemical and cytological) are more sensitive than the more obviously ecologically relevant biomarkers at higher levels (e.g. population). Although the relationships between effects across different scales of biological organisation are not necessarily straightforward, some lower-level biomarkers have been correlated with higher organisational-level effects (Moore et al. 2013) and are not difficult to measure in these days of ‘omics’ (e.g. Jeppe et al. 2017a). Without resource to ‘omics’, attractive biochemical-level biomarkers include primary antioxidant enzymes like catalase, the oxyradical scavenger glutathione and the Total Oxyradical Scavenging Capacity (TOSC). The metal-binding protein biomarker should only be employed with care given the variability in its induction properties. Of the cytological biomarkers available, the measurement of lysosomal stability using the neutral red retention (NRR) assay has much to recommend it (Moore et al. 2013), after extension to suitable candidates from relevant stream biota. Invertebrates with blood cells are the best candidates and so might include *Gammarus* species, chironomid midge larvae or tubificid oligochaetes. In the latter case, it would be possible to draw on the extensive experience of soil ecotoxicologists using the NRR assay in earthworms, the oligochaete relatives of freshwater tubificids (Bundy et al. 2004; Spurgeon et al. 2003; Svendsen et al. 2004). Biomarkers of genotoxicity that are well tested include the comet assay.

Moving up the hierarchy of biological organisation, potential metal-affected biomarkers at the level of the organism are the distorted morphology of the mouthparts of chironomid midge larvae (di Veroli et al. 2014; Martinez et al. 2003).

6.5 Biotic Indices

Biotic indices are widely used to meet legislated requirements for assessment of biota in freshwaters and historically have long been used with benthic macroinvertebrate communities to assess the extent of pollution. Such biotic indices, however, have typically been developed to address organic pollution, although general degradation may be interpreted (e.g. through NTAXA). A biotic index for metal pollution is still to be developed (Clarke et al. 2011), a non-trivial task. However, the Australian experience with a diagnostic index for acid mine drainage, SIGNAL-MET (Chessman and McEvoy 1998), in combination with a RIVPACS style predictive model of community structure, where 70% of the variation in O/E was attributable to mining impacts, indicates that this approach is highly appropriate for assessing the degree of impairment from previous mining activity (Sloane and Norris 2003).

In the absence of a specific biotic index for metal-contaminated sediments, several authors have used a comparative multimetric statistical approach for the comparison of macroinvertebrate communities at stream sites under different conditions of metal exposure (e.g. de Jonge et al. 2013; Gower et al. 1994; Hirst et al. 2002). Mondy et al. (2012) used a multimetric approach to assess the ecotoxicity of contaminated sediments to benthic macroinvertebrates in French mountain streams, but this study considered a mixture of toxicants, involving organic contaminants such as PAHs and PCBs as well as toxic metals. A biotic index developed by linking community-level responses to measures of bioavailability and ecotoxicological responses would provide the best route for a rapid, easy to use method to assess the impact of metal-contaminated sediments on biota.

6.6 Remediation

Together with the need to identify where sites are being impacted by metal-contaminated sediments (Clements et al. 2010), there is a clear ongoing need for a low-cost system that is capable of assessing the effectiveness of any remediation undertaken to mitigate the impact of metal-contaminated sediments (and mine waters) on river ecosystems. This system should be capable of rapid and easily repeatable assessments that can be undertaken through time to determine the rate of improvement of mining-affected stream sites after mitigation and, in particular, after any event that may influence the delivery of metal-contaminated sediment from the catchment.

7 Summary

Mining can result in metal-rich sediments entering freshwater streams and rivers via a variety of routes. Such metal-rich sediments present a potential issue for environmental managers as they might impact upon life in freshwaters and inhibit recovery of ecological condition after any remediation of mine water discharges. Many case studies indicate that metal-rich sediments can cause ecological impacts: the extent to which such impacts are causing management issues in the field is, as yet, largely unknown. As diet represents a, if not the, major source route of uptake of metals from sediments, acute dissolved toxicity tests are not suitable for setting environmental quality standards for sediments. Furthermore, translating laboratory-derived environmental quality standards to the field situation is difficult, as many complicating factors exist in the real world. There is a strong case to consider other, field-relevant measures of toxic effects as alternatives to laboratory-derived standards. Biomonitors, species that accumulate metals in their body tissues, offer a potential route to assess bioavailability of metals under field conditions, which, in turn, can be compared with measures of ecotoxicological effects (biomarkers) to determine thresholds. There is also a need for better biological tools to detect those sites suffering from ecotoxicological impairment due to metal-contaminated sediments, a challenging task as the toxic effect of metals is only one of the impacts on freshwaters associated with mining. Biotic indices may provide regulatory authorities with a low-cost approach for initial detection of problems. Nevertheless, it is clear that the way forward in the ecotoxicological assessment of mining-affected stream sediments is via an integrated approach involving a multiplicity of tools from different disciplines such as biology, ecology, geochemistry and toxicology, as reflected in a WoE approach (Chapman 2007a, b).

Acknowledgements We acknowledge the UK Department for Food and Rural Affairs (Defra) for funding and supporting the project WT0970 Characterisation and targeting of measures for (non-coal) polluted mine waters – impacts of contaminated sediment on ecological recovery, which supported this work – together with the Coal Authority, the Environment Agency and Natural Resources Wales for their support. The views expressed in this paper are those of the authors, and not necessarily Defra, the Coal Authority, the Environment Agency or Natural Resources Wales. ALC is supported by the UK Biotechnology and Biological Sciences Research Council (BBSRC) through the Soil to Nutrition strategic programme (BBS/E/C/000I0330) at Rothamsted Research.

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P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology Volume 248*, Reviews of Environmental Contamination and Toxicology 248,
https://doi.org/10.1007/978-3-030-14706-8