Chapter 10
Nanotechnology in Contemporary Mine Water Issues

**Ken Oakes, Zhi Shan, Rajendran Kaliaperumal,
Shine Xu Zhang, and Martin Mkandawire**

Abstract Nanotechnology holds great promise in advancing affordable mine water treatment and remediation technologies by improving treatment efficiency and performance. Further, it promises to overcome major challenges faced by existing treatment technologies through provision of highly efficient, modular, and multifunctional processes facilitating new treatment capabilities. Such technologies could further allow economic utilization of mine water as a source of commercially viable products. This chapter provides an overview of recent and future developments in nanotechnology that would benefit contemporary mine water treatment regimes, while investigating novel abate approaches possible through the use of nanotechnology. The chapter discusses candidate nanomaterials, their advantages and limitations relative to existing processes, and the unique properties and surface-active mechanisms that enable their adoption in mine water treatment applications.

10.1 Introduction

Natural resources derived from the Earth's lithosphere, hydrosphere, biosphere, and atmosphere are the key building blocks of a sustainable human society. Historically, mining affects the surrounding environment during both active operation and

K. Oakes • Z. Shan • R. Kaliaperumal • M. Mkandawire (✉)
Verschuren Centre for Sustainability in Energy and the Environment, Cape Breton University, 1250 Grand Lake Road, Sydney, NS, Canada B1P 6 L2
e-mail: martin_mkandawire@cbu.ca

S.X. Zhang
Verschuren Centre for Sustainability in Energy and the Environment, Cape Breton University, 1250 Grand Lake Road, Sydney, NS, Canada B1P 6 L2

Waterloo Institute of Nanotechnology and Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, ON, Canada N2L 3G1

for extended periods following mine closure. Among the myriad of environmental impacts associated with mining, including disturbed landscapes, erosion, formation of sinkholes, depletion and contamination of water sources, and soil contamination, the long-term production of mine water poses one of the greatest concerns. Mine water refers to any surface or groundwater present at the mine site, which has been altered due to mining activities or influenced by mining-related processes such as milling, extraction, or percolation over mine waste. Mine water may be produced by contact with materials from long-closed mine sites, or it may refer to water produced during active mining by dewatering processes.

Contemporary mine water issues range from depletion and/or contamination of water resources during active mining, to acid mine drainage (AMD) and leachate from abandoned workings and waste mine rock heaps, to management of tailings post-mine closure. Notably, most closed mines are sources of multiple pollutants including inorganic metals and organic chemicals and acids used to extract the minerals. Despite the high risks they pose, many mine water discharges are insufficiently treated, if treated at all, due to the substantial number of sites on properties where mines closed without sufficient funds set aside during operation to address the high (and often on-going) cost of remediation. However, recent advances in nanotechnology offer opportunities to develop next-generation mine water remediation and management technologies, which promise affordable treatments offering improved performance. Nanotechnology may further overcome traditional challenges faced by existing treatment technologies through provision of highly efficient, modular, and multifunctional processes with the potential for economic utilization of mine water as source of commercially viable products.

This chapter provides an overview of recent and future developments in nanotechnology that would address contemporary mine water issues, while raising potential concerns that may accompany the non-precautious use of nanotechnology. The discussion covers candidate nanomaterials, properties, and mechanisms enabling the applications, advantages, and limitations relative to existing processes, and barriers and research needs for commercialization. By tracing these technological advances to the physicochemical properties of nanomaterials, the present chapter outlines the opportunities and limitations to further capitalize on these unique materials for sustainable mine water remediation and management.

The chapter is presented through several sentinel topics; beginning with a perspective on contemporary mine water issues through the lens of challenges faced both during active mining and post-closure. Types of mine water, as well as issues associated with mine water discharge, are next addressed, together with current technologies used to treat the contaminated waters. This is followed by an analysis of the current state of nanotechnology as applied to contemporary mine water issues, potential future applications, and an overview of potential issues associated with the application of nanotechnology to mine waters. The chapter will explore these topics from a broad-based, multidisciplinary perspective relevant for both engineers and scientists outside the field, and for students at both the undergraduate and graduate level.

10.2 Mining and the Mine Life Cycle

To understand contemporary mine water issues, an understanding of a mines' life cycle, including the mining techniques employed, are critical. While exact processes differ by mineral, depth, region, and available resources, the fundamentals are similar. They begin with the discovery of a commercially viable ore body, leading to development and extraction of minerals during the life of the mine, concluding with the closure of the mine, after which the site is either restored to its natural state, or reclaimed to a condition suitable for other land uses. While the discovery phase is a mixture of geological knowledge, exploratory drilling, and luck, the extraction of mineral resources is constrained to two common mining techniques, namely excavation and in situ leaching [1].

10.2.1 Excavation and Mining Techniques

Specific excavation techniques are used in surface or sub-surface mining applications, depending on the depth and quantity at which the mineral resource is found [2, 3]. Surface mining involves removing surface vegetation and overlying layers of bedrock to reach the ore deposits [4, 3]. Specific surface mining techniques include: (1) open pit mining, where relatively shallow ore is recovered from expansive open pits excavated into the ground; (2) quarrying of aggregate materials, from a usually shallow open pit mine; (3) strip mining which consists of stripping surface layers to reveal ore/seams underneath, and (4) mountaintop removal, commonly associated with coal mining, which involves removing the top of a mountain to access ore deposits at depth [3–6]. Conversely, sub-surface mining involves the digging of tunnels or shafts into the earth, often but not necessarily at significant depths, to access ore deposits. The targeted mineral-bearing ore and non-mineral-bearing waste rock are brought to the surface through the tunnels and shafts. Sub-surface mining can be further classified by the type of access shafts used, as well as the extraction method or technique used to reach the mineral deposit. For instance, drift mining utilizes horizontal access tunnels, while slope mining uses diagonally sloping access shafts, and shaft mining consists of vertical access shafts. Following excavation, the ore-bearing rocks are crushed and pulverized to facilitate mineral extraction. The target resource of interest is then recovered singularly, or with other minerals through combinations of several mechanical and/or chemical procedures [7–10]. Most metals are present in ores as oxides or sulphides, necessitating their reduction to their metallic forms via chemical means such as smelting or electrolytic reduction.

10.2.2 In Situ Leaching Technique

In situ leaching involves the drilling of holes into the ore deposit, with explosive or hydraulic fracturing potentially used to create openings in the deposit for a leaching solution to penetrate [1]. As the leaching solution is pumped into the deposit, it dissolves the target minerals and the solution carrying the dissolved ore content is pumped to the surface and processed [11]. In situ leaching allows for the extraction of metals and salts from an ore body without blasting, removing overburden and waste rock, or construction of underground mine infrastructure.

10.3 Mine Water and Contemporary Issues

For both excavative and in situ techniques, water is a major concern during both the active mining and post-closure periods. During active mining, both excavative and in situ techniques utilize dewatering processes, but excavative approaches typically produce more waste water as ore processing requires considerable quantities of water during washing and mineral extraction [5, 6]. Waste rock and tailing piles, if exposed to water, can leach contaminants. Mine flooding is common once the dewatering processes conclude with the cessation of active mining, leading to formation of underground mine water pools which remain a significant contemporary environmental issue [12]. With open pit mining, pit lakes may form by precipitation, surface runoff, or groundwater filling the open pit upon the completion of excavation activities [13, 14].

10.3.1 Classification of Mine Waters

Mine waters can be classified into numerous categories, varying in their quality, pH, and potential for environmental impacts. The definition of what actually constitutes mine water is still a major point of contention within scientific and engineering conversations and literature. Consequently, the term "mine water" has been used interchangeably with other terms like "mine drainage" and "mine pools" [15, 16]. In part, this confusion stems from the absence of a recognized body sanctioning terminology; so, the definition of mine water, as used in this chapter, will broadly constitute any water altered due to mining activities, or influenced by mining-related processes such as milling, extraction, or percolation over mine waste, as well as groundwater flooding active mine workings and subsequently de-watered [16, 17]. Consequently, "mine water" will generally refer to any surface or groundwater present at a mine site, while "mining water" will specifically refer to water that has come into contact with any mine workings. Within this framework,

mine water is the all-encompassing term that includes mining water [2, 17]. Mining water includes mill water, which refers to water used during the crushing and grinding of ore, and may also contain dissolved minerals or metals. Process water, another mining water used in the chemical extraction of metals, similarly frequently contains high burdens of dissolved metals and other chemicals. Leachate, water that has trickled through solid mine wastes or tailings, process chemicals, or metals. When the mining, mill, or process water is discharged to the surface, often after being treated, it is known as effluent. Ideally, all mine water should be collected and stored in tailings ponds before being treated and released to surface water, if necessary [15]. Mine drainage includes water contaminated by interactions with waste rock, within impoundments, or through interactions with geological formations in water-filled abandoned mine shafts. Mine drainage can be classified by its pH as either acid or neutral mine drainage, both containing metals/constituents at relatively high concentrations [15, 16].

10.3.2   Mine Water Issues

There are five outstanding mine water issues, mainly related to pollution monitoring and control that may be successfully addressed by the application of nanotechnology.

10.3.2.1   Acid Mine Drainage

AMD usually forms when sulphide minerals in rocks are exposed to oxidizing conditions during or after mining activities, leading to the production of sulphuric acid contaminated water known as acid rock drainage (ARD), which contributes to the acidity of interacting waters [18]. While ARD formation is a naturally occur process, it is markedly accelerated when sulphide-containing rocks are exposed to air and water through construction of shafts, tunnels, and collieries that maintain oxidizing conditions over greater geolithic surfaces leading to production of AMD [18, 19]. In most cases, AMD formation requires the presence of iron sulphides such as pyrite and marcasite (FeS_2), which are common in coal regions, but may also be formed by chalcopyrite ($CuFeS_2$), covellite (CuS), and arsenopyrite ($FeAsS$) (also see Table 10.1).

   The concentration and composition of metals in AMD is a function of both the type and amount of sulphide mineral, and the presence or absence of buffering alkaline materials, which regulate acidity. The oxidation of Fe disulphides and subsequent conversion to acid occur through several reactions. The first chemical process, iron sulphide oxidation, releases ferrous iron (Fe^{2+}), sulphate (SO_4^{2-}), and protons, as summarized in Eq. (10.1):

Table 10.1 Some important metal-sulphide minerals responsible for AMD at mining sites

Chemical formula	Mineral	Chemical formula	Mineral
FeS_2	Pyrite	MoS_2	Molybdenite
FeS_2	Marcasite	NiS	Millerite
Fe_xS_x	Pyrrhotite	PbS	Galena
Cu_2S	Chalcocite	ZnS	Sphalerite
CuS	Covellite	$FeAsS$	Arsenopyrite
$CuFeS_2$	Chalcopyrite	Cu_5FeS_4	Bornite

Source: Adapted from Ref. [20]

$$FeS_2 + \tfrac{7}{2}O_2 + H_2O = Fe^{2+} + 2SO_4^{2-} + 2H^+ \tag{10.1}$$
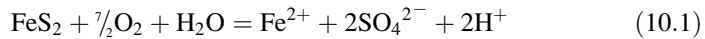
The resulting ferrous iron can further oxidize to ferric iron (Fe^{3+}), according to Eq. (10.2):

$$2Fe^{2+} + \tfrac{1}{4}O_2 + H^+ = Fe^{3+} + \tfrac{1}{2}H_2O \tag{10.2}$$
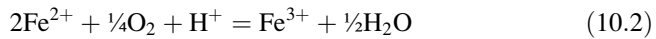
Ferric iron can then either be hydrolyzed to ferric hydroxide, $Fe(OH)_3$, increasing H^+ acidity (Eq. 10.3), or it can directly attack pyrite as a catalyst, generating much greater amounts of ferrous iron, sulphate, and acidity (Eq. 10.4).

$$Fe^{3+} + 3H_2O = Fe(OH)_3 + 3H^+ \tag{10.3}$$

$$FeS_2 + 14Fe^{3+} + 8H_2O = 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \tag{10.4}$$
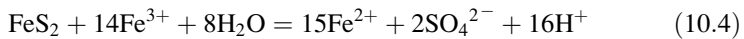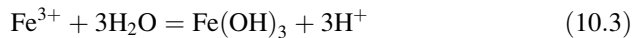
The generation of AMD would slow or cease when any of the constituent processes are inhibited as these steps are sequential and interdependent [21, 22]. Obviously, with removal of two of the three principal reactants, air and water from the system, pyrite would not be oxidized. The rate of pyrite oxidation depends on numerous variables such as the reactive surface area of pyrite, form of pyritic sulphur, oxygen concentrations, solution pH, catalytic agents, flushing frequencies, and the presence of Fe-oxidizing bacteria (FeOB). Under most conditions, the oxidation of Fe^{2+} to Fe^{3+} (Eq. 10.2) is the rate-limiting pyrite oxidation step as the conversion of ferrous iron to ferric iron is slow at pH values below 5 under abiotic conditions [21, 23]. However, FeOB, principally *Thiobacillus* sp., greatly accelerate the reaction, and are consequently critical regulators of AMD generation. Availability of oxygen may be the rate-limiting step in AMD formation within geological materials of low porosity and permeability, while in porous and permeable formations, especially those composed of coarse sandstone, air convection driven by the heat generated by pyrite oxidation provide high amounts of oxygen deep into the rocks, ensuring an oxidizing environment.

10.3.2.2   Alkaline Mine Drainage

Of perhaps less concern, but closely related to AMD is neutral or alkaline mine drainage (NAMD). NAMD has a pH of 6.0 or above, as a reflection of local alkalinity or proton buffering capacity attributable to the local presence of hydroxyl ion (OH^-), carbonate, silicate, borate, organic ligands, phosphate, and ammonia within the mine drainage [16, 24]. The principal source of alkalinity in mine water is dissolved carbonaceous compounds, which exist in solution as bicarbonate (HCO_3^-) or carbonate (CO_3^{2-}). The quality of mine water emanating from underground mines or backfills of surface mines is consequently dependent on both the acidic (i.e. sulphide) and alkaline (i.e. carbonate) materials contained in the disturbed geological material. It follows that NAMD may result from any combination of relatively low levels of sulphide minerals, a deoxygenated but sulphide-rich environment, the presence of monosulphides rather than pyrite or marcasite, a large pyrite grain-size limiting oxidation rate, the presence of highly reducing or alkaline influent water, neutralization of acid by carbonate or basic silicate minerals, and/or the neutralization of acid by naturally highly alkaline groundwater [25]. Other reactions that contribute to the formation of NAMD include the oxidation of other sulphides, dissolution of host rock minerals, precipitation and dissolution of iron hydroxide and hydroxysulphate, co-precipitation and adsorption of dissolved metals, precipitation and dissolution of iron sulphate, gypsum precipitation and dissolution, and CO_2 degassing [26]. Mine waters that contain alkalinity can also be contaminated with divalent metal ions including ferrous iron and manganese. None are contaminated with significant levels of dissolved ferric iron or aluminium because the solubility of these metal hydroxides is low in mine waters with pH values greater than 5.0.

10.3.2.3   Toxic Metal Contamination and Leaching

Both surface and underground as well as in situ leach mining approaches vary greatly but share risks for environmental contamination with toxic metals, metalloids, and radionuclides. Waste rock, common to the first two extraction techniques, accumulate in large quantities under fairly oxidizing conditions associated with surface storage in large piles, and often contain acid-generating sulphides, metals, and other contaminants. The waste rock and the exposed bedrock walls from which ore is excavated are the sources of most of the metal pollution. Toxic metal and metalloids such as arsenic, cobalt, copper, cadmium, lead, silver, iron, chromium, manganese, mercury, molybdenum, nickel, lead, selenium, vanadium and zinc as well as some radionuclides are liberated and move into mine water when excavated rock exposed in an underground mine come in contact with water [2, 27–31]. In open pit mines, new metals are in continuous contact with the surface air and water, and after mine closure, the metals continue to dissolve in the pit lake waters. With conventional extraction techniques, considerable quantities of tailings are generated, as the percentage of extractable material in ore rocks is usually very low.

For instance, high-grade uranium ore contains a maximum of 0.07 % extractable uranium [32]. As a result, uranium mining and processing inexorably leads to masses of geological materials, which are often surface stored long-term as mountains of waste. From these heaps, metals are leached out and carried downstream as water washes over the rock surface or seep through waste rock piles and tailings. Although metals can be mobilized at neutral pH, leaching is accelerated under low pH conditions, including those created by AMD.

Tailings also represent a major source of metal contamination of waterways. After the waste rock is removed and the ore is excavated, it is then processed to separate the target mineral from the valueless portion; the remaining ore becoming tailings. Mine tailings often contain the same toxic metals and acid-forming minerals that waste rock does. Tailings can also contain chemical agents used to process the ores, such as cyanide or sulphuric acid. With the in situ leaching technique, it is usually impossible to recover all the leaching solution from the ground. In most cases considerable volumes remain underground and continue dissolving rocks and releasing toxic metals and radionuclides into the ground that can have impacts for many generations after the active mining ceased.

10.3.2.4   Radioactive Contamination

A common misconception is that radioactive contamination is unique to uranium producing mines, when in fact, the potential to generate radioactive mine water is equally high at any abandoned mine as natural radioactivity is common in the geological materials of our planet. Radioactivity indicates that matter, often known as radionuclides, is decaying in order to reach, according to the law of physics, a more energetically stable state. As materials decay, they emit radiation—emission of gamma ray(s) or subatomic particles such as alpha or beta particles—which disintegrate entirely over short or long intervals (measured in seconds, years, or millennia). Although most mine water emits insignificant levels of gamma radiation [33, 34], the presence of short-lived alpha particles or radon emitted as a decay product of readily soluble isotopes of uranium and radium can pose a significant risk.

There are over 1,500 different radionuclides, with potassium (^{40}K) responsible for the highest radioactivity and being the most predominant radionuclide in soil and rock material [35]. However, more radioactive elements such as radium and radon in the ore arise from the radioactive decay of uranium over hundreds of millions of years. Most radioactive mine water, from both ground and open pit mining, results from the solubilization of radionuclide-containing minerals. Several studies have shown radionuclide contamination in coal mining is usually attributed to secondary mineralization of uranium with coal development [36]. Similarly, radioactivity has been observed in iron and phosphate due to mineralization processes started with localization of uranium and related nuclides co-precipitated with either iron and/or phosphorus [10, 37–40].

10.3.2.5 Processing Chemicals Contamination

Process chemical contamination occurs when chemical agents (such as cyanide or sulphuric acid) used by mining companies to separate the target mineral from the ore spill, leak, or leach from the mine and processing site.

10.4 State of Nanotechnology as Applied to Mine Water Issues

Advances in nanotechnology are enabling development of more efficient and cost-effective waste treatment and environmental remediation technologies. However, application of nanotechnology to mine water remediation, just as to mineral discovery, mining, extraction, and processing, has so far received little attention. To date, most applications of nanotechnology in mine water remediation have essentially followed the general application of nanomaterials in wastewater treatment and water purification. Most research in this area, especially that pertaining to mine water issues, has been performed in a small-scale laboratory context, with large-scale implementation of nanotechnology by the mining industry expected to take a few more decades.

10.4.1 Strategies in Water Treatment and Remediation

Some nanotechnology applications designed for wastewater treatment and water purification may be used in mine water treatment and remediation including nanofiltration, nanocatalysis, nanomagnetism, nanosensors, nanoextraction, and disinfection. Nanofiltration involves development of nanomaterial-enhanced membranes that mechanically exclude contaminating substances, thereby removing pollutants from water. Such filters and membranes are made from a variety of nanomaterials including carbon nanotubes, nanoporous ceramics (clays), dendrimers, zeolites, nanofibres, and nanosponges. Multi-tasking filtration systems can detect, separate out, and/or detoxify mixtures of contaminants. A key advancement is the development of nanowire membranes with tuneable wettability ranging from superhydrophobic to superhydrophilic, which have high potential as a clean-up media [41–43]. Further, dendritic nanomaterials, highly branched nanoscale polymers, have been successfully employed to "encapsulate" environmental pollutants. Dendritic nanomaterials are often recyclable, water-soluble, and have demonstrated great potential for removing inorganic pollutants, heavy metals, biological, and radiological compounds [44, 45]. During the last decade, significant advances have been made in the development of nanoscale supramolecular hosts that can serve as high capacity, selective, and recyclable ligands and sorbents for extracting valuable metal ions from artificially synthesized mine water based on

solutions and mixtures [44, 46–52]. For instance, dendrimer-based chelating agents and separation systems have been developed to recover valuable metal ions from mine water, such as Cu(II), Ni(II), Zn(II), Fe(III), Co(II), Pd(II), Pt(II), Ag(I), Au(I), Gd(III), and U(VI). Similarly, there have been advances in development of nanosorbents based on self-assembled monolayers on mesoporous supports for recovering a number metal ions, including most of those mentioned above.

Nanocatalysis, which utilizes nanoparticles with catalytic properties to chemically degrade pollutants, is a promising approach for treating waterborne contaminants present at very low levels. Magnetic nanoparticles have large surface areas to mass ratios, and easily bind with contaminants such as arsenic, AMD constituents, and oils, and following complexation, are easily removed from solution using a magnet, making them an appealing solution for mine water treatment [53–56]. Another example is nanoscale zero-valent iron (NZVI) particles, which are very efficient redox-active media for the degradation of organic contaminants, and can be applied to the treatment of AMD and other mine processing contaminants [53–57].

10.4.2 Detection and Monitoring

There have also been recent advances utilizing nanotechnology to develop small, portable sensors with enhanced capabilities for detecting trace biological and chemical contaminants in environmental matrices, including water. Nanotechnology-enabled sensors are enabling rapid and accurate detection of harmful compounds by exploiting a variety of sensing and detection modalities, such as chemical (e.g. molecular recognition), optical (e.g. fluorescence), and mechanical (e.g. resonance). Potential applications of engineered nanomaterials for mine water monitoring include detection of various compounds in gaseous, aqueous, or soil matrices; sampling and detecting mine water pollutants accumulated within biological systems (cells, organs, tissues, etc.), and monitoring of physical parameters (pressure, temperature, distance, etc.). To date, many excellent publications have been produced to address such topics as "Environmental sensors" or "Nanotechnology for environmental monitoring" [58–60], of which most emphasized the design, construction, and operational mechanisms of the nanostructure-based sensor, but very few focused on specific target analytes, especially those heavy metals species such as (Hg, As, Mn, Pb, Cd, Ni, Zn, Cu, Cr, U, Ra) and polycyclic aromatic hydrocarbons (PAHs) found in mine water.

10.5 Applicable Nanotechniques in Mine Water Issues

10.5.1 Detection and Monitoring Techniques

Monitoring mine water pollution with expensive and sophisticated instrumentation such as atomic absorption spectroscopy (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), mass spectroscopy (MS), and X-ray fluorescence
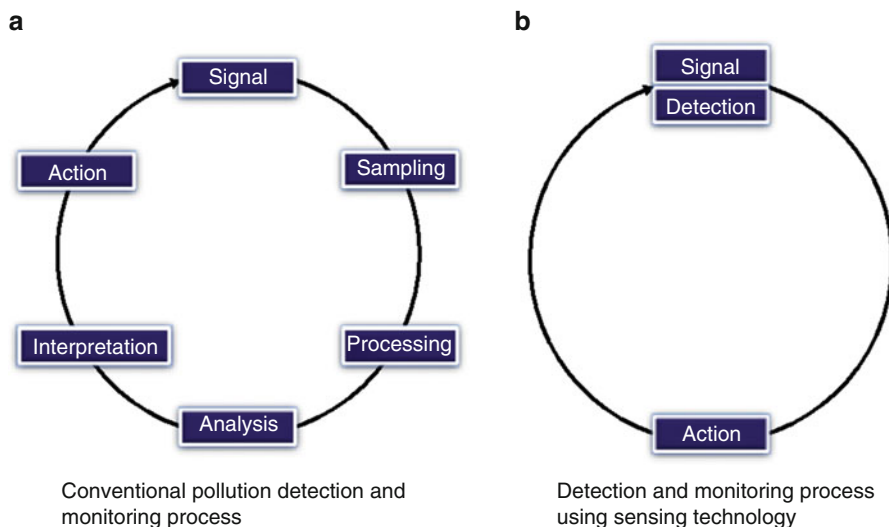
a

Signal

Action

Sampling

Interpretation

Processing

Analysis

Conventional pollution detection and
monitoring process

b

Signal

Detection

Action

Detection and monitoring process
using sensing technology

Fig. 10.1 Comparison of conventional (**a**) and sensing technology (**b**) processes in pollution detection and monitoring. In (**a**), the process has five steps, each having potential sources of error due to human and instrumentation factors; while in (**b**) the process is greatly simplified, reducing sources of errors while being relatively inexpensive

spectroscopy (R-FS) is still the standard approach. However, these instrumently intensive methods only measure total analyte concentrations in environmental samples, but provide no information on speciation. Further, these instruments often require intense sample preparation efforts. In short, the complexity of current instrumental approaches introduces many potential sources of error, and the expenses associated with monitoring mine water pollution preclude its application in many developing economies. Consequently, the availability of simple, portable, and affordable devices capable of on-site monitoring would be a critical advance in the monitoring of mine water pollution (Fig. 10.1). There are two approaches to develop on-site detection and monitoring apparatus; the first is simply to miniaturize current laboratory instrumentation (i.e. developing miniaturized gas- or liquid-chromatography/mass spectrometry instrumentation), the second is to develop devices such as micro- or nanosensors or sensor arrays. The idea underlying the former approach can be described as "top-down", while the latter is a "bottom-up" approach. Nanotechnology and nano-/micro-engineering methodologies are essential for both approaches, but in this chapter, we will focus on the development of nano-/micro-sensor technology for environmental monitoring.

The merit of the nanotechnology sensor-based approach and device development to monitor mine water pollutants can be demonstrated through recent progress made in this field. The following is not an exhaustive summary of all publications generated in this field; rather, we selected some published data since 2010 to exemplify the trend in the development of nanotech-based environmental sensing, updating the excellent review by Namour et al. [61].

The nanosensor technology normally involves nanostructured materials in either the recognition element or the signal-transducing element, so as to improve the performance of the sensor in terms of sensitivity, selectivity, reproducibility, portability (miniaturization), or throughput. When biomacromolecules or structures are incorporated into the recognition element for target recognition, the sensor is called a biosensor. Depending on the signal transduction mechanism [62], nanosensors can be categorized as optical sensors (i.e. fluorescent, colorimetric, SERS) or electrochemical sensors. Based on their configuration, there are simple nanoparticle-based sensors, nanomaterial-modified sensors/devices, and microfluidic sensors. We will briefly introduce the mechanisms of several representative nano-sensors and sensing devices in the following sections.

10.5.1.1 Optical Nanosensors

Optical nanosensors convert the recognition and binding of target analytes to an optical change in intensity, lifetime, fluorescence anisotropy, colour based on shift of light absorbance, or Raman Spectrum. Of these, colorimetric sensors are most desirable for on-site environmental monitoring due to their operational simplicity and convenience, as normally no power supply or other instruments are needed. While colorimetric sensors can detect analyte presence with the naked eye, instruments may be necessary to obtain more accurate quantification. In many cases, smartphones incorporating a digital camera could collect the data (colour or colour change) and send to the lab via wireless communication for accurate quantification. To construct colorimetric nanosensors, gold nanoparticles (AuNPs) serve as an excellent signal-transducing element owning to their unique optical properties. Due to the plasmonic effect, AuNPs ($d > 3.5$ nm) show specific absorbance dependent on inter-particle proximity. For example, 13 nm-sized monodispersed AuNPs in aqueous solution appear red in colour, but change to purple or blue due to shifts in surface plasmon resonance (SPR) peak in the case of AuNP aggregation. Herein, the aggregation of AuNPs could be induced by different targets when AuNPs are functionalized with the specific receptor (ligand) for a given analyte of interest, which forms the basis for the sensor design.

For heavy metal detection, the analyte recognition element immobilized on the AuNP surface is normally a chelating ligand that can specifically bind to the heavy metal ions. The red-to-blue colour change results from metal ion-induced AuNP aggregation by forming multidentate inter-particle complexes (Fig. 10.2) [63]. To date, small molecules [such as 11-mercaptoundecanoic acid (MUA) (for Pb^{2+}, Cd^{2+}, and Hg^{2+}), carboxylate, 15-crown-5 (for Pb^{2+}), cysteine (for Cu^{2+}), 5,5′-dithiobis (2-nitrobenzoic acid) (for Cr^{3+}), quaternary ammonium (for Hg^{2+}), tetramethylmalonamide (for Ln^{3+})], and biopolymer molecules [such as peptide (for Hg^{2+}), pentapeptide (CALNN, for Al^{3+}), single-stranded DNA] have been used as analyte recognizers on AuNP.

It is worth noting that single-stranded DNA (ssDNA) has been established as a powerful and versatile functional material serving as a recognition element for biosensor design [64]. The discovery of DNA aptamers [65], synthesized ssDNA
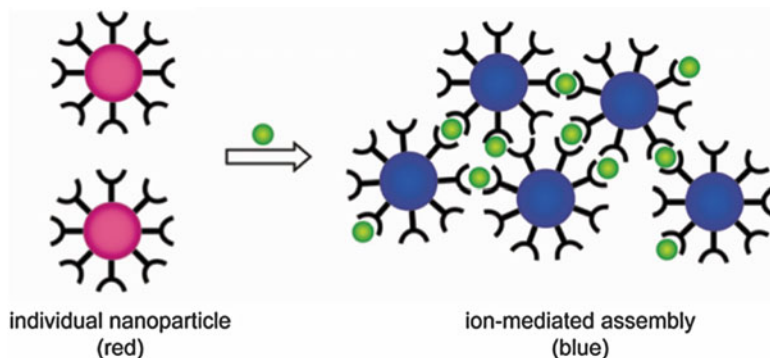
individual nanoparticle
(red)

ion-mediated assembly
(blue)

Fig. 10.2 Schematic of colorimetric sensing of metal ions (*red-to-blue*) using AuNPs functionalized with ligands. The ligand may be chelating molecules, peptides, or DNA strands. Adapted from Ref. [63]

that can specifically bind to a wide variety of metal ions or organic molecules or structures (similar to an antibody, but more chemically stable), paves the way for widespread use of ssDNA as a binding ligand for various targets including heavy metal ions. For example, the T-Hg^{2+}-T coordination chemistry has been widely employed to design DNA probes for sensor design [66]. In addition, DNAzyme, the catalytic DNA, has been incorporated into sensor design for signal-transducing [67–69]. For example, Pb^{2+} and UO_2^{2+} have been detected with the DNAzyme-based colorimetric sensors (blue-to-red transition), with excellent specificity against other metal ions such as Ca^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+}.

Nanomaterials such as AuNP, carbon nanotube, graphene, and graphene oxide are efficient fluorescence quenchers, while other nanoparticles such as inorganic quantum dots (QDs), carbon dots, nanoclusters, and up-conversion luminescent nanoparticles have been emerging as alternative fluorophores due to their improved optical properties, low background, multiple colours, and high photostability and brightness (quantum efficiency). As such, these nanomaterials are widely employed to construct fluorescent sensors, even for in vivo analysis. When designing fluorescent sensors with nanomaterials, the main relevant mechanisms are either "Förster resonance energy transfer (FRET)" or nanometal surface energy transfer (NSET) [70–72].

For example, Li et al. reported a DNA-directed QD–DNA–AuNP sensor for Hg^{2+} detection [72]. As shown in Fig. 10.3, QDs and AuNPs were functionalized with two complimentary oligonucleotide strands, each containing TTT as the recognition element for Hg^{2+} (based on T-Hg^{2+}-T coordination chemistry). The mixture of these two functionalized nanoparticles is fluorescent, as the proximity of AuNPs to QDs is not close enough to quench the fluorescence of the QDs. However, when the water sample containing Hg^{2+} ions were introduced into the fluorescent mixture, Hg^{2+} ions can link together the DNA strands on the AuNPs and QDs to form a stable duplex linking the AuNPs and QDs, which results in an energy transfer
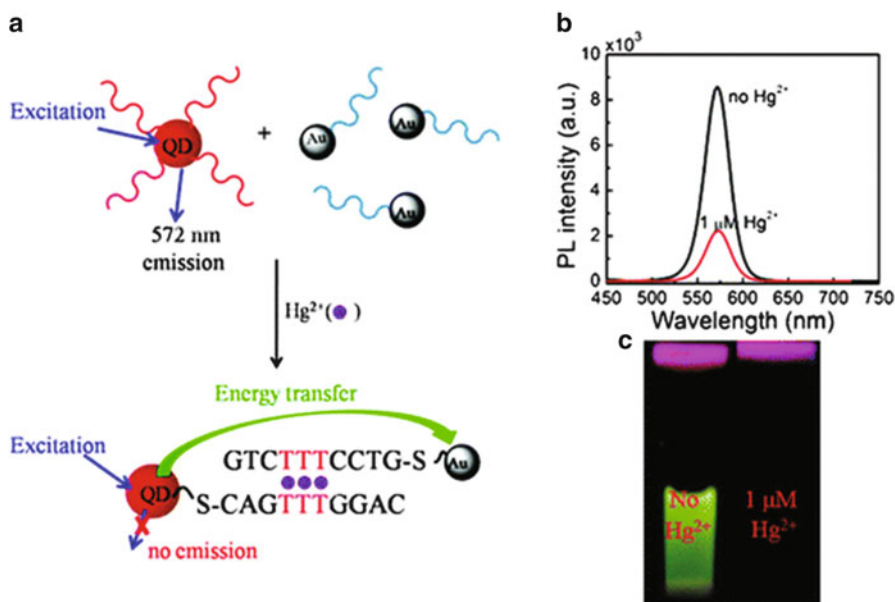
Fig. 10.3 The scheme of Hg^{2+} detection based on QD-DNA-AuNP sensor [72]

that quenches the QDs fluorescence. This sensor showed excellent sensitivity (LOD of 0.4 and 1.2 ppb Hg^{2+} in buffer solution and in river water, respectively) and specificity (Fig. 10.3).

In another example, graphene oxide (GO) was used as the fluorescence quencher [73]. In this case, the underlying sensor mechanism is the much lower affinity double-stranded DNA (duplex) demonstrates for GO than is displayed by ssDNAs. As shown in Fig. 10.4, a sensing system containing salt, fluorescein-labelled A15 (FAM-A15), T15, and GO, is fluorescent since FAM-A15 will bind to FAM-T15 to form a duplex, and is thus not likely to be adsorbed and quenched by GO. However, when a Hg^{2+}-containing sample was introduced, the Hg^{2+} induced T15 strands to form a T15/T15 duplex based on T-Hg^{2+}-T coordination. This leaves FAM-A15 single stranded, which was then adsorbed onto GO to quench the fluorescence. This sensor also showed good sensitivity (LOD in buffer: 0.5 nM) and selectivity.

Optical sensors, based on surface-enhanced Raman scattering (SERS), have been developed for heavy metal analysis, though by an indirect means. By exploiting the aggregation of silver nanoparticles (AgNPs), we can significantly enhance the SERS signal of the Raman reporter molecules (such as negatively charged 4-mercaptopyridine (4-MPY) on AgNPs) as Chen's group has done with its development of a SERS sensor for As^{3+} ions [74]. First, AgNPs were functionalized with both 4-MPY and glutathione (GSH), where 4-MPY not only serves as the Raman reporter but also provides negative charge to electrostatically stabilize
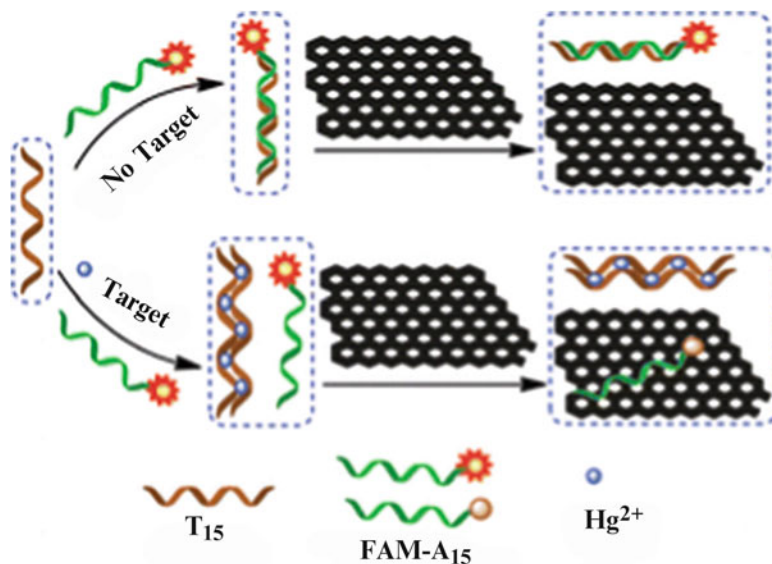
Fig. 10.4 The scheme of GO-DNA sensor for Hg^{2+} detection [73]

AgNPs, while GSH functions as a specific binding element for As^{3+} ions. When As^{3+} ions bind to GSH, their positive charges destabilize the AgNPs and induce aggregation, resulting in an enhanced SERS signal for 4-MPY molecules. The sensor showed good sensitivity (LOD: 0.76 ppb) and selectivity. Similarly, a SERS sensor for Cd^{2+} with Cd^{2+}-chelating polymer brush functionalized on AuNPs as the binding element was developed with good sensitivity and selectivity. More importantly, SERS provides structural information on the molecules, suggesting application potential for understanding heavy metal speciation, which is deserving of additional future research attention.

10.5.1.2 Electrochemical Nanosensors

For on-site detection of heavy metal ions and other charged pollutants in water samples, electrochemical sensing, such as potentiometry, voltammetry, coulometry, and amperometry, shows great potential. Owning to the rapid development of microelectronics, electrochemical sensors might be devised and fabricated commercially with high uniformity (no significant batch-to-batch variations). These sensors could be easily miniaturized and integrated into state-of-art wireless mobile technologies (such as smart phones) for fast detection and signal processing. As a result, long-term continuous on-line and remote monitoring may be practical, which could be very important for environmental or medical applications, for example,

monitoring glucose or hormone levels in blood with an implanted electrochemical sensor, or continuous monitoring of mine water discharges. The fundamental mechanism is the highly specific electrochemical detection of metal ions, suggesting a molecule recognition element might not be required to construct the electrochemical sensors, improving the sensor design simplicity by allowing for constant re-use, making these devices suitable for continuous on-line measurement over long lifespans. Nanomaterials have been widely exploited to upgrade traditional electrochemical sensing, with many review articles published over the last few years providing in-depth expertise on detailed aspects of this field. Herein, we provide only some simple examples to briefly overview the electrochemical sensing field.

One powerful technique is ion selective electrode (ISE), belonging to the potentiometric membrane sensors family, which detects specific target ions in water based on the selectivity of the ionophore membrane. Recently, significant improvements in the performance of carbon pastes were achieved by incorporating multi-walled carbon nanotubes (MWCNT), nanosilica, and functional polymer to form synergistic functional composites which serve as the potentiometric membrane for the detection of Yb^{3+}, Er^{3+}, Ho^{3+}, Pb^{2+}, and Cu^{2+}, so that the sensitivity and response time were enhanced [75–78].

For voltammetric sensors, which record current as a function of systematically varied potential, nanomaterials were widely adopted for electrode modification to achieve better performance. For example, by using carbon-based nanomaterials such as carbon nanotubes and graphene sheets with unique electric and chemical properties to modify the working electrode, the response time, sensitivity (signal to noise ratio), and stability could be significantly improved, presumably due to improved electron transfer rates, lower solution resistance, increased mass transfer kinetics, and enhanced chemical stability and mechanical strength. Some intriguing review articles may provide in-depth understanding in this regard [79–81].

When utilizing the nanomaterial-modified electrodes for stripping analysis, such as anodic stripping voltammetry, nanomaterials can serve as a sorbent, thus further improving the sensitivity of the assay. Compared to unmodified approaches, the carbon nanomaterial (graphene or CNTs)-modified electrode showed higher efficiency for stripping analysis (i.e. sensitivity was improved by thousands of times), which accumulates the heavy metal ions from the sample matrix prior to the reduction of those ions to the zero-valent metal. In this case, CNTs or graphene are often functionalized with various molecules to improve their affinity to target analyte ions. For example, when using cysteine-modified CNTs to modify the electrode surface, the detection limit for Pb^{2+} and Cu^{2+} reached 1 and 15 ppb, respectively [82]. Similarly, thiacalixarene-functionalized CNTs showed enhanced sensitivity towards Pb^{2+}, with the detection limit of 40 pM [83]. Likewise, a supersensitive and selective Hg^{2+} sensor (LOD: 3 ppt) was developed using layered titanate nanosheets, a typical cation-exchange material [84].

Field effect transistor (FET) has been adopted as a promising technological platform for real-time and label-free chemical- and bio-sensing for decades. Similar to CNT-modified electrodes, SWCNT-FET demonstrates promise for

heavy metal analysis. For example, SWCNT-FET was used to monitor Hg^{2+} ions in water up to 10 nM, based on the change in conductance of the CNTs on exposure to Hg^{2+} ions due to specific redox reactions between CNTs and Hg^{2+} [85].

10.5.1.3 Microfluidics-Based Sensor Devices

Microfluidics technology has demonstrated versatility in analytical chemistry, even for heavy metal detection. Based on the unique strengths of the miniaturized microfluidic platform, it is reasonable to expect on-site measurement and multiplexed detection of multiple analytes simultaneously. Currently, both optical (absorbance, SERS, and fluorescence) and electrochemical sensors have been incorporated into microfluidic platforms to achieve enhanced portability, energy- and time-efficiency. Date et al. [86] demonstrated a microfluidic immunoassay for heavy metal (Cd^{2+}, Cr^{3+}, and Pb^{2+}) absorbance measurement; herein, the bound and free AuNP-labelled antibody were separated by microparticles functionalized with the EDTA chelated metal ions, where the microparticles were entrapped in a dam-like structure in the microfluidic channel. When the heavy metal ion sample and AuNP-Abs mixture flow through the channel, the free AuNP-Abs (i.e. those in excess not binding to metal ions) are retained by the microparticles while the bound AuNP-Abs flow through the microparticles without retention. The retained AuNP-Abs are quantified by absorbance at 520 nm, demonstrating the simplicity and portability of the whole device. Other examples involve fabrication of on-chip quantitation devices such as micro-electrodes for electrochemical detection [86], fluorescence detection [87], and SERS [88]. To date, only simple chemical reactions have been realized on-chip and real environmental and biological samples were rarely tested against on-chip nanosensors.

10.5.2 Treatment and Remediation Techniques

10.5.2.1 Mine Water Remediation Strategies

To understand the potential of nanotechnology for mine water remediation and treatment, it is necessary to understand current techniques and strategies. Currently, there is a wide spectrum of mine water treatment and remediation technologies which have been developed, proven, and are being applied within different abandoned mine environments. The generic range of technologies is reflected in Fig. 10.5, and can be broadly classified into active, passive, and in situ treatment approaches [18, 19]. Nanotechnology has potential to enhance the capacity, as well as increase the efficiency, of all existing treatment technologies.
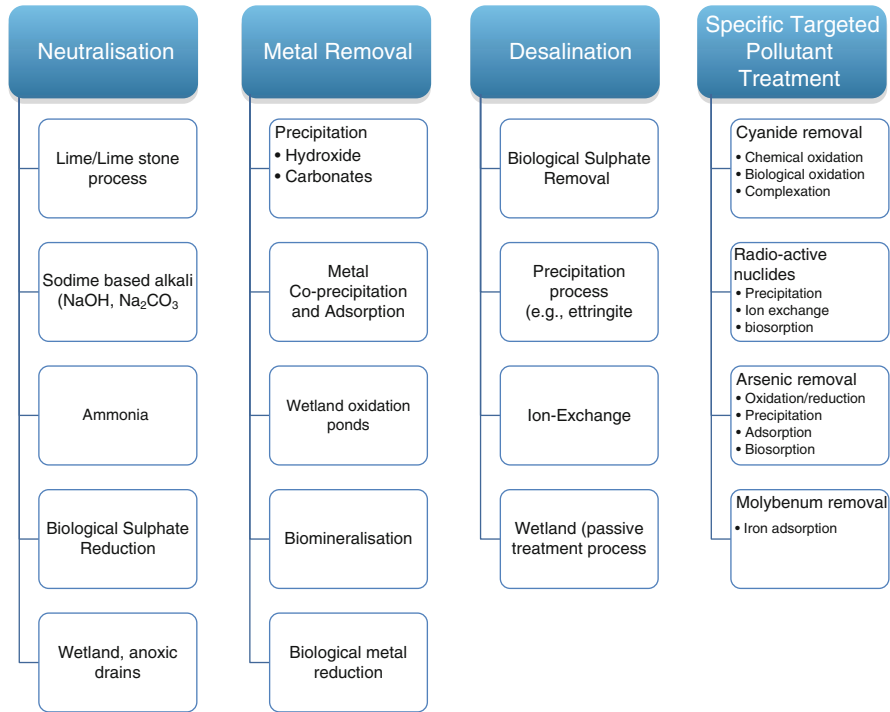
Neutralisation	Metal Removal	Desalination	Specific Targeted Pollutant Treatment
Lime/Lime stone process	Precipitation • Hydroxide • Carbonates	Biological Sulphate Removal	Cyanide removal • Chemical oxidation • Biological oxidation • Complexation
Sodime based alkali (NaOH, Na$_2$CO$_3$	Metal Co-precipitation and Adsorption	Precipitation process (e.g., ettringite	Radio-active nuclides • Precipitation • Ion exchange • biosorption
Ammonia	Wetland oxidation ponds	Ion-Exchange	Arsenic removal • Oxidation/reduction • Precipitation • Adsorption • Biosorption
Biological Sulphate Reduction	Biomineralisation	Wetland (passive treatment process	Molybenum removal • Iron adsorption
Wetland, anoxic drains	Biological metal reduction		

Fig. 10.5 Generic strategies in mine water treatment technologies. Modified from Ref [19]

Passive Treatment Systems: Passive treatment systems utilize chemical, biological, and physical removal approaches, many of which mimic or enhance naturally occurring processes in the environment, to modify influent characteristics. The major types of passive treatment applicable to mine water include:

1. Anoxic alkaline drains—used mainly in the treatment of AMD, this system utilizes trenches filled with alkaline material (often crushed limestone), sealed under plastic and geotechnical fabric and covered by soil, through which an un-aerated, contaminated influent stream flows by gravity. As AMD flows through the system, it gradually dissolves the limestone, releasing carbonate, which along with bicarbonate, raises effluent pH.
2. Constructed wetlands—ecological systems designed to optimize a variety of natural physical, chemical, microbial, and plant-mediated processes [89–93]. In a constructed wetland, influent mine water drains by gravity through the wetland, progressively undergoing metal removal in addition to pH neutralization. Metals are removed by precipitation, chelation, and exchange reactions, while neutralization is primarily achieved by the activity of sulphate-reducing bacteria (SRB), or by increasing alkalinity via chemical and microbial reactions including limestone dissolution.

3. Microbial reactor system—reactor comprised of a shell containing an electron-donating substrate (i.e. carbon donors), which supports the growth of microorganisms. Aerobic bacteria, such as sulphate-reducing bacteria, treat mine water (including AMD) by reducing sulphate to hydrogen sulphide [94–97]. The sulphide in turn reacts with metal ions leading to their precipitation as metal sulphides [91, 98]. Such bacterial activity consumes hydrogen ions by the reduction of substrates, thereby increasing the pH of the solution while producing carbon dioxide which contributes to the CO_2-bicarbonate-carbonate buffer system.

4. Biosorption systems—utilize living biomass including bacteria, algae, fungi, and yeasts to adsorb heavy metals and radionuclide pollutants from mine water. Such biosorption systems are limited to treating influent with metal concentrations below toxicity thresholds for each microorganism. Such systems are also seasonally limited, as biosorption by living biomass during the colder temperatures of winter are limited when growth is negligible. Consequently, biosorption systems cannot be effective stand-alone treatment systems for AMD, but are a viable alternative form of treatment, or for incorporation as a final polishing step.

Active Treatment Systems: Active treatment is the approach to improving mine water quality by the addition of chemical reagents (e.g. hydrogen peroxide, caustic soda) in the context of extensive infrastructure, energetic inputs, and engineered systems. Active treatment technologies include aeration, neutralization, metal precipitation, metals removal, chemical precipitation, membrane processes, ion exchange, and biological sulphate removal.

1. Aeration—this critical step increases dissolved oxygen, which is necessary as mine water from flooded shafts is often hypoxic or anoxic; this step promotes the oxidation of iron and manganese, and increases chemical treatment efficiency. Aeration also drives off dissolved CO_2, which is in dynamic equilibrium with carbonic acid in solution, thereby increasing the pH and significantly reducing reagent use. As the principal contaminant in AMD is often dissolved ferrous iron, and at average water temperatures, only about 10 mg/L of oxygen can dissolve to achieve saturation [19, 99]; aeration is required if there is more than ~50 mg/L of Fe^{2+} in the mine water. Aeration can be done before or during treatment, using gravity cascades or mechanical aeration mixing devices [19].

2. Neutralization and hydrolysis—key aspects of mine water treatment, which involves the addition of alkali materials such as limestone ($CaCO_3$), hydrated lime ($Ca(OH)_2$), un-hydrated (quick) lime (CaO), soda ash (Na_2CO_3), caustic soda ($NaOH$), magna lime (MgO), fly ash, kiln dust, or slag.

3. Metal removal—the classical approach to metals removal from mine drainage is based on chemical precipitation leading to the formation of solid particles containing metal precipitates. The process normally requires a pH adjustment to selectively remove metals of interest, as many metals are amphoteric.

Table 10.2 Theoretical minimum metal hydroxide solubility pH (pH$_{MMH}$) for some selected metals common in mine water

Metal	pH$_{MMH}$	Metal	pH$_{MMH}$
Ferric iron, Fe^{3+}	~3.5	Ferrous iron, Fe^{2+}	~8.0
Antimony, Sb^{2+}	~4.2	Zinc, Zn^{2+}	~8.5
Aluminium, Al^{3+}	~4.5	Nickel, Ni^{2+}	~9.3
Lead, Pb^{2+}	~6.5	Cadmium, Cd^{2+}	~10.0
Copper, Cu^{2+}	~7.0	Manganese, Mn^{2+}	~10.6

Amphoteric metals (M) decrease their solubility until a threshold pH is reached, above which the metal solubility increases again because of the formation of soluble complexes [18, 100]. Dissolved metals can form a number of insoluble compounds with anions, such as

$$\text{Hydroxides}: \quad M^{x+} + xOH^- \rightarrow M(OH)_x \tag{10.5}$$

$$\text{Carbonates}: \quad 2M^{x+} + xCO_3{}^{2-} \rightarrow M_2(CO_3)_x \tag{10.6}$$

$$\text{Sulphides}: \quad 2M^{x+} + xS^{2-} \rightarrow M_2(S)_x \tag{10.7}$$
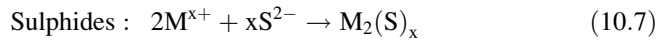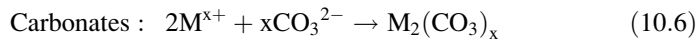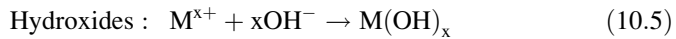
Table 10.2 presents pH values corresponding to the theoretical thermodynamic and minimum solubility of some selected metal hydroxides. Pre-oxidation of metals with multiple oxidation states prior to precipitation improves the process as the oxidized form of most metals have lower solubility with the exception of chromium, selenium, and uranium, which are more soluble in their oxidized forms [91, 101–103]. To enhance removal of targeted metals, chemical pre-treatment or co-precipitation strategies are often employed, such as aeration, iron addition to co-precipitate or adsorb certain metals onto ferric hydroxide precipitates, and chemical reduction or oxidation to alter the valence state of a target metal.

4. Chemical precipitation for sulphate removal—mine water may contain a wide range of anionic species, but sulphate is typical of many mine drainages and often represents the primary anion contaminant. Some sulphate is removed by gypsum precipitation during neutralization reactions if lime, limestone, or another calcium source is added during water treatment [26, 104]. In addition, a number of precipitation processes have been developed for specific application to high sulphate content mine waters, including: Barium sulphate process to re-precipitate sulphate [105, 106]; Ettringite ($Ca_6Al_2(SO_4)_3(OH)_{12}$•$26H_2O$) precipitation process, which is based on the addition of aluminium hydroxide in a high pH environment resulting in precipitation of ettringite (a hydrated calcium aluminosulphate mineral) [107]:

$$6Ca^{2+} + 3SO_4{}^{2-} + 2Al(OH)_3 + 38H_2O = Ca_6Al_2(SO_4)_3(OH)_{12} \bullet 26H_2O + 6H_3O^+ \tag{10.8}$$
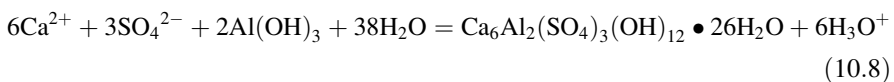
5. Membrane treatment—usually used to treat mine water that is brackish or saline. There are a wide range of existing membrane treatment technologies for mine drainage, but their application is challenging as mine water typically contains several compounds with fouling potential such as metals, sulphates, and carbonates [41, 108]. Thus, typical membrane treatments for mine water utilize spiral wound reverse osmosis or nanofiltration membranes, but other membrane processes such as electrodialysis reversal can also be applied to treat mine water, and are currently operational in large-scale desalination of mine drainage.

6. Ion exchange—involves a chemical reaction in which free mobile ions from a solid phase, the ion exchanger, are traded for different ions of similar charge from the solution. Like any ionic compound, the solid ion exchange material can dissociate and participate in ion exchange reactions, but without dissolving of the material. This property facilitates many specific phenomena and intriguing possibilities to design heterogeneous systems with ionic properties. The process has been traditionally used by mining companies in copper cementation or precipitation processes [53, 109–111]. Within these applications, metallic iron was placed in a copper-containing stream of leach solution from a waste or low-grade ore pile. Copper in solution would plate on the surface of the iron metal, and in the process, exchange electrons with the underlying iron, leading to irons oxidation and the reduction of copper to the metallic state. This process created a higher-value product from a waste product (e.g. precipitation of copper from waste) utilizing low-value scrap iron to remove dissolved copper from mine water. The reaction is

$$3Cu^{2+} + 2Fe^0 \rightarrow 3Cu^0 + 2Fe^{3+} \tag{10.9}$$
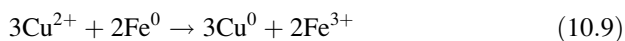
Recently, ion exchange processes using specialized resin materials have been developed for high sulphate mine water. There are both cation and anion exchange process; the cation resin exchanges Ca^{2+}, Mg^{2+}, and other cations (i.e. metal ions) by the following reaction:

$$2R - H + Ca^{2+} \rightarrow R_2 \bullet Ca + 2H^+ \tag{10.10}$$
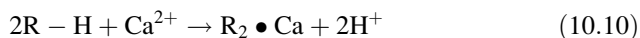
During the process, water acidifies requiring the degassing of CO_2. The anion resin exchanges SO_4^{2-}, Cl^-, and other anions by the following reaction:

$$2R - OH + SO_4^{2-} \rightarrow R_2SO_4 + 2OH^- \tag{10.11}$$
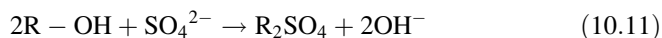
Many natural mineral compounds, such as clays (e.g. bentonite, kaolinite, and illite), vermiculite, and zeolites (e.g. analcite, chabazite, sodalite, and clinoptilolite) exhibit ion exchange properties.

7. Biological sulphate removal—a process based on sulphate reduction in an anaerobic reactor, which converts sulphate to sulphide. The process is mediated by SRB, which preferentially utilize substrates such as fatty acids, alcohols, and H_2 gas. The SRB includes a consortium of fermenting bacteria and methanogens, some of which help to hydrolyze and ferment complex carbon compounds to readily accessible substrates for the SRBs [97, 112]. In mine water applications, an electron-donating carbon source such as alcohol, sugar, H_2 gas, or even complex substrates such as sewage sludge, as well as nutrients including nitrogen, phosphate, potassium, and trace minerals should be added to the bioreactor to stimulate bacterial growth. Using this process, sulphide can be partially oxidized to sulphur in a carefully controlled micro-aerobic environment, with the potential for the sulphur to be separated as a potentially commercial by-product. Stripping of the sulphide and converting to sulphur involves a substitution of H_2S by CO_2, which results in an increase in carbonate alkalinity and potential precipitation of carbonates such as calcite [91, 98]. This can lead to biomineralization of a number of metallic contaminants present in the mine water.

8. Sulphide precipitation—this is a process used to remove metals such as lead, copper, chromium (VI), silver, cadmium, zinc, mercury, nickel, thallium, antimony, and vanadium from mine water, which works under the same basic principle as hydroxide precipitation [91, 98]. The precipitation process converts soluble metal compounds into relatively insoluble sulphide compounds through the addition of precipitating agents, such as sodium sulphide (Na_2S), sodium hydrosulphide (NaHS), ferrous sulphide (FeS), and calcium sulphide (CaS). Over a broad pH range, sulphides (S^{2-}, HS^-) are extremely reactive with metal ions but the precipitation occurs only near neutral conditions (pH 7.0–9.0) [91, 112]. The metal-sulphide precipitates are physically removed from the treated mine water through coagulation, flocculation, and clarification, or filtration thereby leaving a metal-sulphide sludge.

9. Biomineralization—a broad diversity of microorganisms influence and regulate geochemical processes such as the mineralization of inorganic materials [113, 114]. This process, known as biomineralization can be mimicked in mine water as a promising bionanotechnological method for contaminant removal. For instance, bacteria in natural systems can form calcium carbonate, influencing carbonate rock (i.e. limestone) and sediment formation. Such biominerals are hybrids of inorganic and organic components, and in living systems, are the basis for generating bones, teeth, or shells. The organic matrix contributing to these recalcitrant structures consists of proteins, lipids, or polysaccharides, and functions as a template or as nucleation sites for the mineralization of the inorganic phase. Minerals, synthesized by biomineralization processes include silica, iron oxides, hydroxyapatite, and calcium carbonate in various polymorphic orientations (e.g. calcite, aragonite, and vaterite) [115, 116]. Calcium carbonate mineralization by bacteria is regarded as a biologically induced and mediated process [113–115]. Microorganisms can influence most of the factors leading to precipitation, initially by bacterial cells acting as nucleation sites for metal accumulation. Positively charged ions
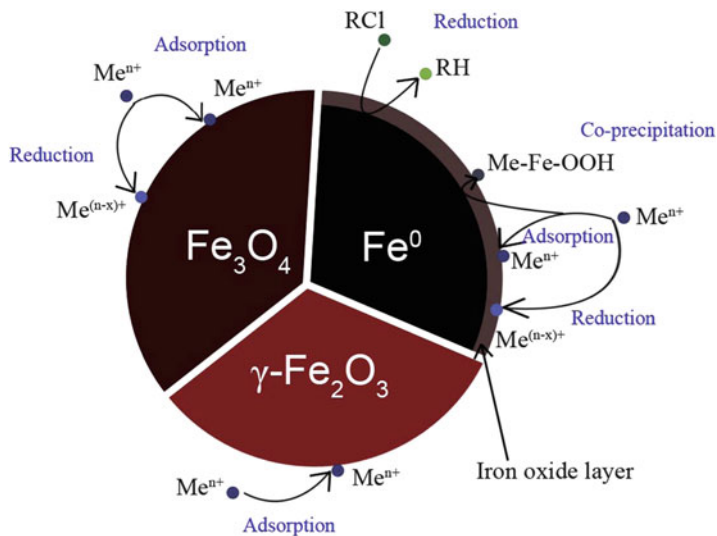
Fig. 10.6 Schematic of contaminant removal mechanisms from water by nanoscale iron and derivatives, illustrating nZVI in the core mainly provides the reducing power for reactions with contaminants while the oxide shell provides sorption sites. However, adsorption also occurs on the iron oxide (Fe_3O_4 and γ-Fe_2O_3) surfaces, while Fe_3O_4 also possesses reducing power. *Source*: Ref. [118]

like Ca^{2+} can be accumulated on negatively charged functional groups on the cell surface and subsequently react with anions to form insoluble inorganic solids like calcium carbonate [23, 113]. Metabolic pathways of heterotrophic bacteria, namely the nitrogen and the sulphur cycle, can be involved in biomineralization processes by the generation of (hydrogen-) carbonate ions as well as ammonia, influencing their surrounding medium [113, 114, 117].

10.5.2.2   Potential of Nanoscale Iron and Derivatives

Iron-based nanomaterials are gaining prominence in water nanoremediation, with the most popular being magnetic nanoparticles including nZVI, reactive nanoscale iron product (RNIP), magnetite (Fe_3O_4), and (γ-Fe_2O_3) nanoparticles [116, 118, 119]. These magnetic nanoparticles not only have a large removal capacity, fast kinetics, and high reactivity for contaminant removal due to their extremely small particle size and high surface-area-to-volume ratios but also one more important property, magnetism. Tang and Lo [118] have summarized the nanoremediation mechanism and processes involving nanoscale iron species (Fig. 10.6). The advantage of nanoscale iron is its ability to be reused by desorbing the contaminants and regaining removal capacity over successive treatment cycles [120].

(a) *Nanoscale Zero-Valent Iron*

NZVI particles range from 10 to 100 nm in diameter. They are usually synthesized through reductive precipitation of $FeCl_3$ with $NaBH_4$, or reduction of
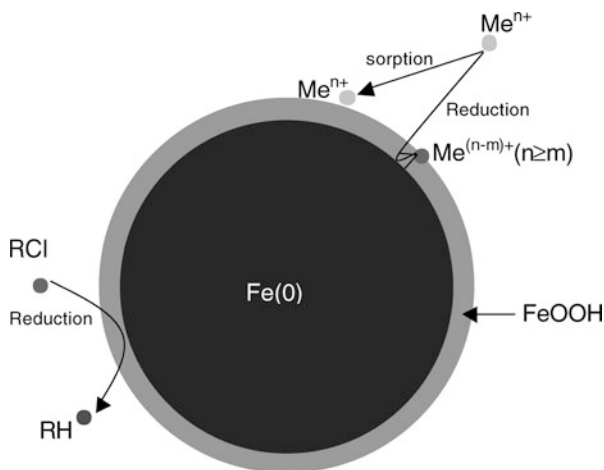
Fig. 10.7 Schematic representation of zero-valent iron nanoparticles illustrating the zero-valent iron core which provides the reducing power for reactions with environmental contaminants, and the iron oxide/hydroxide shell formed largely from the oxidation of zero-valent iron. Adapted from Ref. [55, 121]

goethite and hematite particles with H_2 at high temperatures (200–600 °C). They exhibit a typical core-shell structure, which consists primarily of zero-valent or metallic iron whereas the mixed valent (i.e. Fe (II) and Fe (III)) oxide shell is formed by oxidation of the metallic iron. The nZVI are efficient agents of nanoremediation due to their large surface area and large number of reactive sites, and their dual properties of adsorption and reduction [57, 121]. The use of nZVI in mine water remediation would benefit from iron being a strong reducing agent, reducing inorganic pollutants and immobilizing AMD-associated metals such as uranium, arsenic, and chromium, while degrading organic ore processing chemicals. For instance, in excess of 99 % of arsenic in water can be removed using 12 nm diameter iron oxide nanoparticles [57, 122–125], and they are highly effective for the transformation and detoxification of chlorinated organic solvents and polychlorinated biphenyls (PCBs). Further, surface sorption and co-precipitation of contaminants also occur due to the formation of an iron oxide or oxy-hydroxide shell when nZVI contacts air or water. Consequently, nZVI can be used in wide range of in situ decontamination applications including sub-surface injection into reactive treatment zones [18, 125]. Furthermore, they can be used as reactive barriers to control plumes migrating from contaminated sites and in filter systems (Fig. 10.7).

(b) *Reactive Nanoscale Iron Product*

In the presence of oxygen, nZVI is readily oxidized to Fe^{2+} and can be further oxidized to Fe^{3+} ions. The conversion from Fe^{2+} to Fe^{3+} is dominant under acidic and oxygenated conditions. Further, the oxidation of nZVI in the

Fig. 10.8 Schematic of an
eZVI droplet
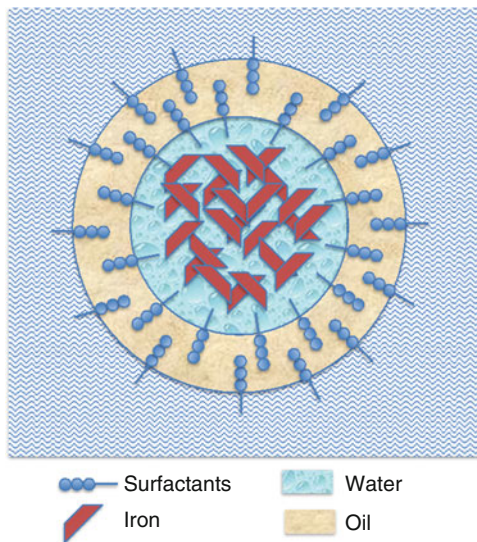
presence of water can also result in the production of hydrogen, causing the
nanoparticles to "burn out"; a major constraint when using nZVI for remedia-
tion of AMD. Providing surface coatings such as a thin layer of either Fe_3O_4,
silica, or polymers reduces oxygen contact with nZVI, retaining their reactivity
[120, 125, 126]. Particles formed when nZVI is coated with a thin layer of
magnetite (Fe_3O_4) are called Reactive Nanoscale Iron Products (RNIP)
[44, 116]. The RNIP consists of approximately a 50:50 mixture by weight of
elemental iron (α-Fe) as the core, surrounded by a Fe_3O_4 outer shell
[116]. Unfortunately, the reaction kinetics of RNIP are sometimes slower
than nZVI a lower accessibility of contaminants to Fe^0 due to the covering
Fe_3O_4 shell.

(c) *Emulsified Zero-Valent Iron*

Zero-valent iron can be modified to comprise catalysts like palladium, coatings
such as polyelectrolyte or triblock polymers, or they can be encased in emulsified
vegetable oil micelles. A potential benefit of eZVI (Fig. 10.8) over nZVI for mine
water applications is the hydrophobic membrane surrounding the nZVI, which
protects it from other waterborne constituents such as inorganic compounds that
might otherwise react with the nZVI, reducing its capacity or inactivating the iron
[116]. As with the addition of metal catalysts to nZVI particles, the formation of
emulsified zero valent iron (eZVI) also represents an enhancement to the existing
nZVI technology. In fact, such modifications are necessary to treat ore extracting
and processing chemicals in mine water, as well as treating petroleum by-products
as the surface of the nZVI nanoparticle requires modification to contain an
oil-liquid membrane. As the composition of this membrane is hydrophobic and
forms an emulsion with ZVI, the resulting eZVI can interact with non-aqueous
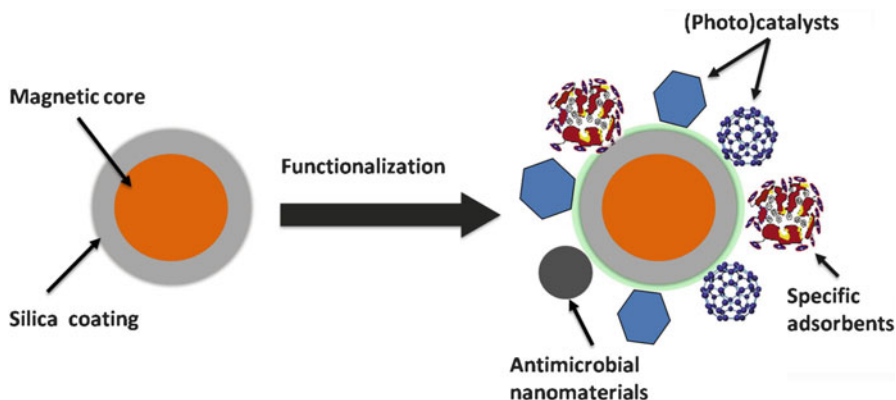phase liquid petroleum contaminants [127]. As the eZVI emulsion is miscible with

Fig. 10.9 Multifunctional magnetic nanoparticle showing the core material in shell nanoparticle structure where the shell provides the desired specialized function while the magnetic core facilitates magnetic separation. Silica coating helps with functionalization due to the rich silica chemistry. Adapted from Ref. [41]

hydrophobic contaminant, there is broad contact between the contaminant and the ZVI present within the oil emulsion droplet. While the ZVI in the emulsion remains reactive, contaminants are continuously degraded within the aqueous emulsion droplet, which produces a concentration gradient within the oil membrane. This gradient drives additional contaminant migration into the membrane, where continual degradation can occur.

(d) *Fe_3O_4 and γ-Fe_2O_3 Nanoparticles*

Fe_3O_4 and γ-Fe_2O_3 magnetic nanoparticles have many potential uses within mine water remediation applications due to their ability to remove metallic contaminants using both physical and chemical adsorption. Further, Fe_3O_4 and γ-Fe_2O_3 magnetic nanoparticles can also remove contaminants using ion exchange mechanisms, and can serve as carriers of other nanoparticles with complimentary functions in water treatment (Fig. 10.9). Fe_3O_4 nanoparticles use both physical and chemical adsorption to remove metals from water. For instance, Hu et al. [128] demonstrated that the $FeCr_2O_4$ crystalline structure formed on the surface of Fe_3O_4 nanoparticles was generated by the removal of Cr(VI) from water. The mechanism underlying the removal of Cr(VI) is its reduction to Cr(III), and the subsequent surface precipitation of Cr(III) onto the magnetic nanoparticles [118, 128].

Generally, the adsorption of inorganic contaminants onto magnetic nanoparticles is low at high pH values indicating that adsorption is largely through physicosorption mechanisms but posssibly with limited chemosorption. Since electrostatic interaction is one of the major removal mechanisms of magnetic nanoparticles, background electrolytes have little effect on removal performance when the affinity of pollutants for the nanoparticles exceeds their affinity for the background electrolytes. Natural organic matter (NOM) such as humic and fulvic acids affect pollutant extraction performance of nanoparticles

by slowing reaction kinetics through blocking sorption on the nanoparticle surface. pH dramatically affects the reduction of nZVI and adsorption performance of Fe_3O_4 and γ-Fe_2O_3 magnetic Nanoparticles the optimal pH for selective removal of Cr, Cu, and Ni determined to be 2.5, 6.5, and 8.5, respectively [116, 129, 130].

Similar to Fe_3O_4 nanoparticles, the dominant contaminant removal mechanism for γ-Fe_2O_3 nanoparticles is physical adsorption, with a limited amount of chemical adsorption contributing. The crystallite structure of γ-Fe_2O_3 nanoparticles does not change after the desorption, indicating that electrostatic interactions are the primary pollutant removal mechanism. Oxygen atoms on the surface of Fe_3O_4 and γ-Fe_2O_3 nanoparticles are polarized when ambient pH is below the zero point of charge (pHZPC), charging the iron oxides surface positive, which attracts negatively charged pollutants (e.g. Cr(VI) and As(V)) [41, 116, 131]. Consequently, the pollutant removal performance of Fe_3O_4 and γ-Fe_2O_3 nanoparticles is highly pH-dependent.

10.5.2.3 Potential of Bimetallic Nanoparticles and Other Metals

Another type of nanoparticle with strong potential for mine water remediation applications is the bimetallic nanoparticle (BNP). Bimetallic nanoparticles consist of elemental iron or other metals in conjunction with a metal catalyst, such as platinum (Pt), gold (Au), nickel (Ni), or palladium (Pd) [132]. BNPs can also be made using other noble metal catalysts, such as plutonium, gold, and nickel; however palladium-iron BNPs are the only particles commercially available, and are consequently the most common [118, 128, 133]. Metals such as zinc and tin possess similar reduction capabilities as iron, with all types of BNP nanoparticles increasing the oxidation–reduction (redox) reaction kinetics, thus catalyzing the reaction. Other inert metals, such gold and aluminium can also used to make BNP nanoparticles, but unlike iron, are non-reactive. Subsequently, gold and aluminium can only serve as a base metal core in conjunction with a catalyst coating. Nevertheless, their inclusion still increases the catalytic rates of the coating metal [46]. Various base metals can be fused with different noble metals to form a multitude of metal combinations [134, 133]. When metal catalysts are coupled with reactive metals such as iron, they serve to enhance the reactive properties of the iron. In contrast, when metal catalysts are coupled with non-reactive metals such as gold, they act as the catalysts themselves. Lately, palladium-coated gold nanoparticles have emerged as one of the most promising alternatives to nZVI and palladium-coated nZVI. The enhancing properties of gold, while not yet clearly understood, are illustrated by Pd-on-Au particles generating 100-fold greater reaction rates than palladium [135–137] alone, with a sub-monolayer of palladium on gold producing the highest catalytic rates [135–137].

There are several ways BNP can be used in mine water treatment, the most prominent application being in situ passive treatment utilizing slurry injections where particles dissipate into the ground. However, such an approach, while

Fig. 10.10 Mechanism for photo-induced formation of electron–hole pair in a semiconductor nanoparticle in the presence of a mine water pollutant, *P*
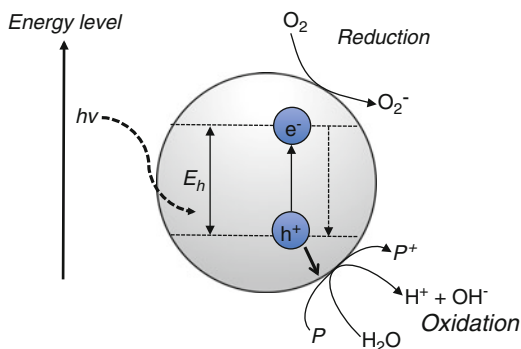
technically feasible, may not currently be cost-effective for Pd–Au nanoparticle applications. Interestingly, even expensive BNPs can be applied cost-effectively using two recent innovations. The first technology involves mounting the nanoparticles onto membranes, and then allowing contaminated mine water to be pushed through the membranes. The second technology binds the nanoparticles to a powder, which enables particle recovery from solution by filtration, a process unachievable with individual nanoparticles [138–140].

10.5.2.4 Nanoscale Semiconductor Photocatalysts

A number of materials, such as titanium dioxide (TiO_2), zinc oxide (ZnO), iron oxide (Fe_2O_3), cadmium sulphide (CdS), zinc sulphide (ZnS), and tungsten oxide (WO_3) can obtain energy by absorbing light, thereby acting as photocatalysts [141, 142]. These materials have semiconducting properties, and can act as sensitizers for light-induced redox processes due to their electronic structure, which is characterized by a filled valence band and an empty conduction band [46]. When a photon with energy of hv matches or exceeds the band gap energy, E, of the semiconductor, an electron, e^-, is promoted from the valence band, VB, into the conduction band, leaving a hole, h^+ behind (Fig. 10.10). Excited state conduction-band electrons and valence-band holes can recombine and dissipate the input energy as heat, get trapped in metastable surface states, or react with electron donors and electron acceptors adsorbed on the semiconductor surface or within the surrounding electrical double layer of the charged particles. In the absence of suitable electron and hole scavengers, the stored energy is dissipated within a few nanoseconds by recombination. If a suitable scavenger or surface-defect state is available to trap the electron or hole, recombination is prevented and subsequent redox reactions may occur. The valence-band holes are powerful oxidants (+1.0 to +3.5 V depending on the semiconductor and pH), while the conduction-band electrons are good reductants (+0.5 to −1.5 V). Most organic photodegradation reactions utilize the oxidizing power of the holes directly or indirectly; however, to prevent a build-up of charge one must also provide a

reducible species to react with the electrons. In contrast, on bulk semiconductor electrodes, only one species, either the hole or electron, is available for reaction due to band bending.

Semiconductor photocatalysts have the ability to oxidize pollutants into non-toxic or immobile forms, and for the reductive deposition of heavy metals from aqueous solution such as mine water to adjacent surfaces. Traditionally, TiO_2 has been used in advanced photochemical oxidation (APO) processes for environmental remediation because of its low toxicity, high photoconductivity, high photostability, availability, low cost, and ability in many cases to completely mineralize organic compounds [142]. TiO_2, for example, exhibits photoconductivity when illuminated by light with an energy level exceeding 3.2 eV, the band gap for TiO_2 [143, 144]. This energy level translates to light with a wavelength shorter than 387.5 nm, which falls into the category of UV light [142–144]. Doping particles or modifying the surfaces of the photocatalysts with metals has become an increasingly popular enhancement. Metals such as platinum, copper, silver, and gold have been tested for their ability to improve TiO_2 decontamination rates. Coupling TiO_2 with these metals can also induce a sensitivity and subsequent response to visible, rather than ultraviolet light. Such doping overcomes the traditional problem faced when utilizing semiconductors as photocatalysts, the requirement for UV light, which is a small component of incident solar radiation [143, 144]. However, by coupling TiO_2 with copper, we can utilize visible light for photocatalysis, including the remediation of metal contaminants like Cr(VI). Coupling TiO_2 with gold and silver produces similar reductive capabilities as that of TiO_2 and copper [52]. Further, if TiO_2 is used to coat silver and gold particles with a TiO_2 shell, reversing the traditional shell:core relationship may enhance photocatalytic activity and increase light absorbing capabilities. Other recent advancements include photocatalytic TiO_2-based p–n junction nanotubes containing platinum on the inside and TiO_2 on the outside [141, 142]. The nature of the p–n junction allows the outside of the tube to act as an oxidizing surface, while the inside of the tube acts as a reductive surface.

Recently, ZnO has been proposed as a dual function photocatalytic material. ZnO films possess both sensing and remediating potential for organic contaminants in mine water, including a high degree of sensitivity and ability to degrade aromatic compounds under UV light [145, 146]. These two features can facilitate monitoring of the degradation process, since as decontamination progressed, a direct change in emission intensity followed [52].

10.5.2.5   Nanocomposite Materials

(a) *Self-Assembled Monolayer on Mesoporous Supports*
    Self-assembled monolayers on mesoporous silica (SAMMS) have a structure resembling a hexagonal honeycomb with monolayers formed within the porous surfaces that adsorb or bind molecules [147, 148]. Synthesis of SAMMS involves multiple steps, mostly reliant on molecular self-assembly [53, 54].
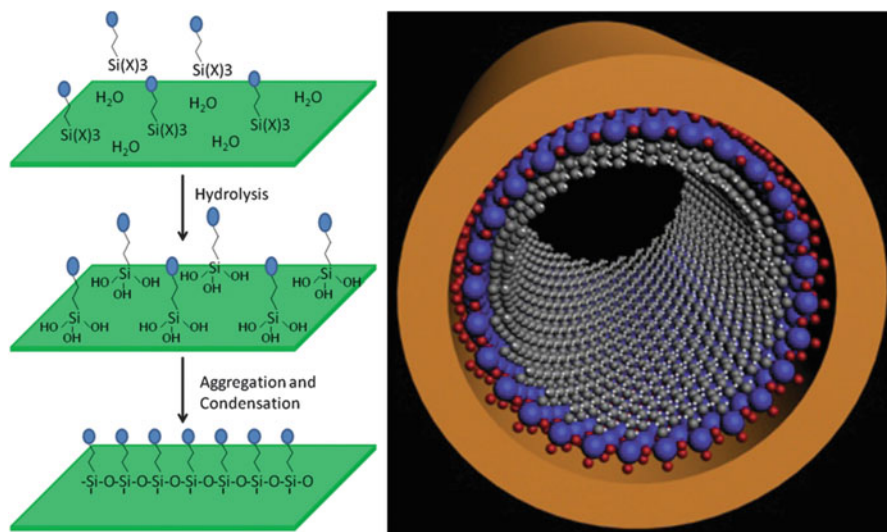
Fig. 10.11 Schematic of organosilane self-assembly on ceramic oxide surface, showing production stages (*upper illustration*, *top schematic*). Hydrolysis of organosilanes on the oxide surfaces to create the corresponding hydroxylsilanes, which then absorb on the surface via hydrogen bond (*upper illustration, middle schematic*). Aggregation and condensation of these hydrogen-bound species lead to a closely packed monolayers on the oxide surface (*upper illustration, lower schematic*). Self-assembled monolayer structure within a mesopore (*right illustration*), where the *red, blue, grew*, and *white* spheres indicate the oxygen, silicon, carbon, and hydrogen atom, respectively. *Source*: Ref. [147]

Figure 10.11 illustrates the process and steps involved; the first, self-assembly, involves the aggregation of the starting surfactant molecules into micelle templates of ordered liquid crystalline structures [148, 149]. Then, oxide materials are precipitated onto the surface of the micelles in the presence of solvents under mild hydrothermal conditions [147, 148]. This leads to formation of the preliminary mesoporous backbone, the second phase of self-assembly. The final step in creating the mesoporous ceramic requires the calcination of the organic-oxide material to remove the surfactants. Functionalized silane molecules are mixed in excess with the mesoporous ceramics and self-assemble in an ordered monolayer on the pore surfaces of the ceramic (Fig. 10.11). The biofunctional silanes used in the process can be engineered to have hydrophilic head groups that bind the target materials, and hydrophobic tail groups, which covalently bind to the ceramic substrate.

The potential of using SAMMS in mine water remediation is based on their ability to alter the exposed functional group of the monolayer in the porous surface, allowing potential binding of a broad range of contaminants [148, 150]. One of the most common functional groups is thiol (thiol-SAMMS), designed mainly for the sequestration of mercury. However, thiol-SAMMS can also bind other metallic cations, such as silver, cadmium, lead, molybdenum, and thallium.

Currently, there are Anion-SAMMS or metal-capped ethylenediamine (EDA) SAMMS which absorb anions, such as chromate and arsenate [148, 150]. Their derivative, chelate-SAMMS, contains only the EDA functional group, and can bind metals, such as copper, nickel, cobalt, and zinc (SAMMS Technical Summary). Cu-EDA-SAMMS (a type of anion-SAMMS) has been further functionalized to bind cesium by incorporating ferrocyanide-forming Cu-Ferrocyanide-SAMMS [116, 148, 150]. Furthermore, a variety of phosphonate and hydroxypyridone (HOPO) functionalized SAMMS have been created for the sequestration of actinides and lanthanides [150]. The advantage of SAMMS is twofold: they can be custom functionalized towards target contaminants for on-site remediation, while able to be recycled and reused in consecutive treatments [116]. However, mercury-laden SAMMS are tightly bound and cannot be reused, but must be disposed of [116]. SAMMS can be mixed with aqueous solutions where, following sequestration of targeted molecules, they can be filtered from solution and recycled. Acid-stripping causes SAMMS to release bound compounds, at which point they can be separated out of solution and reused.

(b) *Sol–Gel and Biocers*

In the sol–gel process, simple molecular precursors are converted into nanometer-sized particles to form a colloidal suspension, or sol. The colloidal nanoparticles are then linked with one another in a three-dimensional, liquid-filled solid network. This transformation to a gel can be initiated in several ways, but the most convenient approach is to change the pH of the reaction solution [151]. In general, the major processes involved are hydrolysis and condensation of alkoxide-based precursors such as Si (OEt) 4 (tetraethyl orthosilicate (TEOS)). The reactions involved in the sol–gel process are based on the hydrolysis and condensation of metal alkoxides $M(OR)_z$ and can be described as follows:

$$MOR + H_2O \rightarrow MOH + ROH \text{ (hydrolysis)} \tag{10.12}$$

$$MOH + ROM + ROH \text{ (condensation)} \tag{10.13}$$
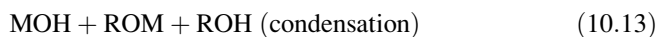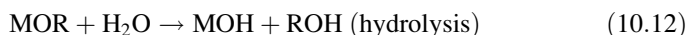
Sol–gel materials can be different forms, such as powders, films, fibres, and freestanding pieces of material called monoliths (Fig. 10.12). The sol–gel's properties are determined by the method used to remove liquid from a solid. For example, a gel can be dried in a sol–gel process to make aerogels, a special class of ultralow-density materials. Similarly, there are broad sets of sol–gel materials for applications ranging from optics, coatings, to energy storage, to ceramics, to nanoelectronics and to remediation. For example, specific forms of sol–gel material are designed to remove oil from water. Such sol–gel are designed porous and hydrophobic but absorb organics such as oil. A similar aerogel composite is used to remove contaminants such as uranium, chromium, copper, and arsenic from groundwater [152, 153]. Other new sol–gel materials include ultrathin films, which can be used to coat silicon wafers and protect
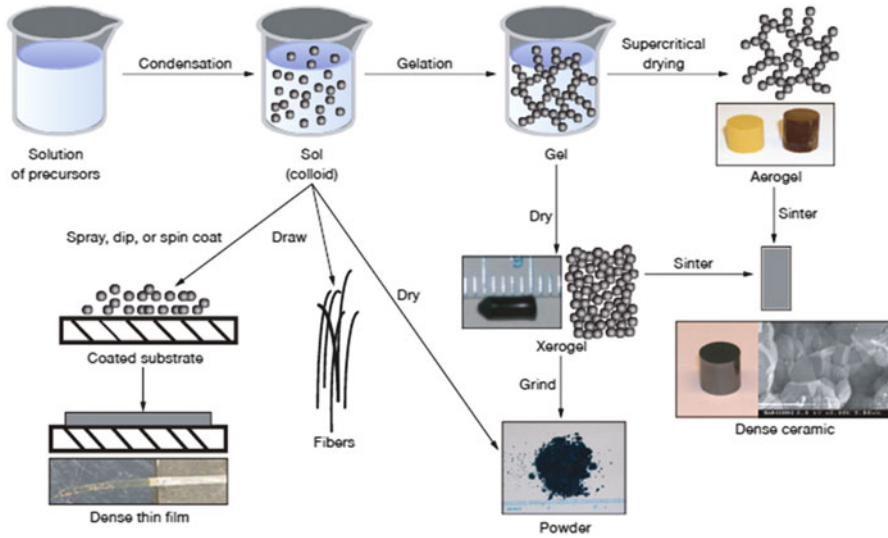
Fig. 10.12 Sol–gel chemistry involves molecular precursors, which are converted to nanometer-sized particles to form a colloidal suspension, or sol. Adding epoxide to the sol produces a gel network. The gel can be processed by various drying methods (shown by the *arrows*) to develop materials with distinct properties [153]
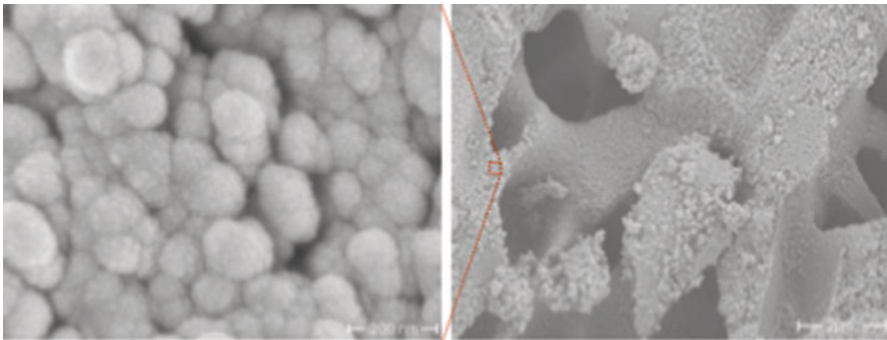

Fig. 10.13 Sol–gel material showing different porosity levels at nano- and meso-scale (courtesy of Martin Mkandawire—Habilitation research work)

optics, and sol–gel-derived powders, which can be used to produce ceramics with various properties (Fig. 10.13).

Sol-gels can be further modified to be biologically active by either incorporating bioactive molecules like proteins and enzymes, or by embedding them as an immobilizing matrix for living microorganisms. These types of sol–gel composite materials are popularly known as biological ceramic composites (biocers), and are made according to aqueous sol−gel protocols and can be used as selective metal-binding filters. Biocers are prepared by dispersing
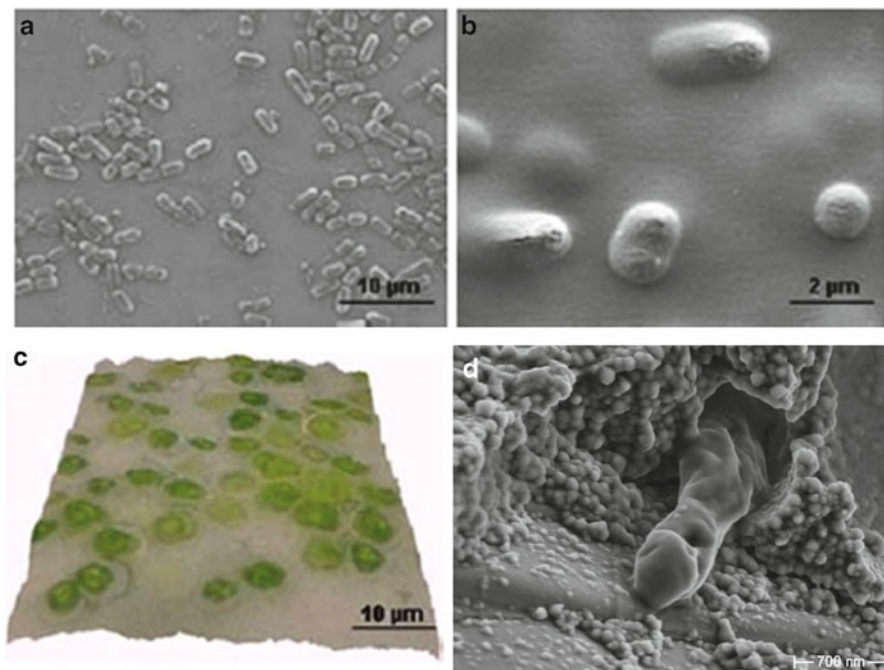
Fig. 10.14 Biological sol-gels (biocers) demonstrating different means of immobilizing living microorganisms: (**a**) surface immobilization of *Rhodoccocus ruber*, (**b**) bacteria under a thin film coating of biocer, (**c**) surface immobilization of algae, and (**d**) embedded bacteria growing in biocer. *Sources*: (**a–c**) Ref. [154] and (**d**) from Ref. [155]

vegetative cells, spores, and stabilized surface-layer proteins (S-layer) within aqueous silica nanosols prior to geling and drying. Both freeze-drying of prepared biocers as well as the addition of water-soluble compounds such as sorbitol lead to higher porosity and faster metal-binding properties. The biological component of the biocers can be customized for desirable adaptations in bacteria by isolating and incorporating those strains acclimated to contaminated sites or mine water discharges. Thereby, vegetative cells of algae, bacteria, and some fungus and their spores (where applicable) can be added to the biocer to selectively bind U, Cu, Al, Cd, and Pb in large amounts (Fig. 10.14).

10.5.2.6 Nanoscale Polymers and Nanosponges

(a) *Dendrimers*

Dendrimers are three-dimensional, highly branched, globular polymeric macromolecules usually comprised of three covalently bound components: a core, interior branch cells, and terminal branch cells (Fig. 10.15) [45, 156]. Flexibility during synthesis allows for customized molecular design, including size, shape, surface or interior chemistry, flexibility, and topology to be produced to
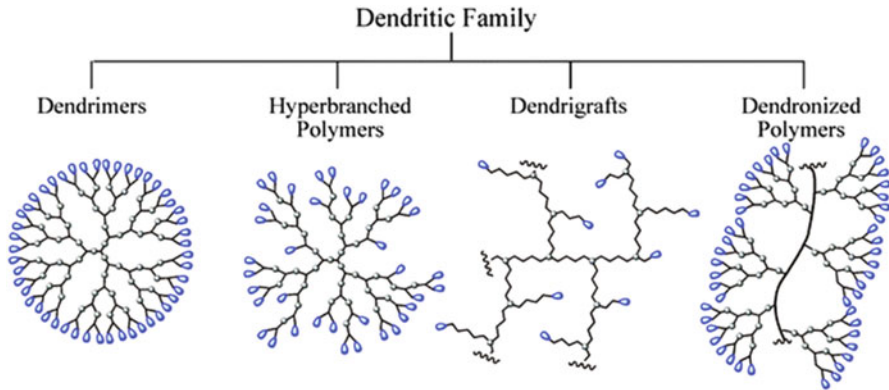
Fig. 10.15 Different dendritic polymers: dendrimer, core-shell tecto (dendrimer), dendrigraft polymer, hyperbranched polymer. *Source*: Carlmark et al. [45]

specifications [157, 158]. The size of dendrimers ranges between 2 and 20 nm, with common shapes including cones, spheres, and disc-like structures able to be generated. Dendrimers can be broadly applied to a variety of remediation applications, particularly in water treatment. For instance, poly(amidoamine) (a.k.a. PAMAM) are a type of dendrimer which have been used to treat wastewater contaminated with a variety of transition metal ions such as copper (Cu(II)) [159–161]. Diverse cores and functional groups that can be incorporated into dendrimers translate into equally diverse water remedial and biodetection applications [58, 159, 162]; for metal-remediating dendrimers, an EDA core is employed [157, 163]. The high concentration of nitrogen ligands within the interior branches makes PAMAM dendrimers particularly useful as chelating agents for metal ions [163–165] and the ability to choose a multitude of functional groups as terminal cells also contributes to their functionality as metal chelators. Surface terminal groups, including primary amine, succinamic acid, gycidol, hydroxyl, and acetamide have been tested successfully [165].

Currently, dendrimer-enhanced ultrafiltration (DEUF) is a recent remediation tool, which has emerged as a promising technology for removing metal ions from mine water streams. With DEUF, metal ions bind to the dendrimers allowing for removal of contaminants though membrane filtration. While the current status of dendrimer research for remediation only includes EDA core PAMAM dendrimers for copper and other metal ion recovery, PAMAM dendrimers can be functionalized with redox active metal clusters of FeS for reductive decontamination of organic pollutants [162].

(b) *Polymeric Nanoparticles*

Polymeric nanoparticles are molecules or molecular aggregates that possess amphiphilic properties originating from distinct segments present in the particle [166]. Individual nanoparticles contain both a hydrophobic and hydrophilic

region, which self-assemble in the presence of water to form polymer vesicles with diameters in the nanometer range, with hydrophobic segments oriented inwards, and hydrophilic segments forming the outer layer [166]. Polymeric nanoparticles offer a potential replacement for traditional surfactants commonly used to enhance the remediation of hydrophobic organic contaminants using pump-and-treat systems. They can be applied to treat mine process water as well as drilling oil contamination, which otherwise sorb strongly to soils or form non-aqueous phase liquids [166–168].

10.5.2.7 Bionanomaterials

(a) *Ferritin Nanoparticles*

Constituents of biological systems, including proteins such as ferritin, can regulate the formation of mineral structures. Iron, while an essential element for living organisms, is also highly toxic in unregulated, so living organisms store iron as ferritin to regulate iron homeostasis. The need to sequester iron, and hence the presence of ferritin, is found across phyla including the animal, plant, and microbial kingdoms. The structure of ferritin consists of a spherical protein shell (apoferritin) composed of 24 polypeptide subunits chains, surrounding an aqueous cavity with internal and external diameters of about 8 and 12 nm. The multi-subunit construction of the apoferritin shell facilitates the generation of eight hydrophilic channels, each of about 4–5 Å and leading to the protein cavity. Water, metallic cations, and hydrophilic molecules of appropriate size diffuse through these channels from the external matrix to the cavity, or vice versa. Consequently, iron molecules can diffuse into the ferritin cavity through channels in the protein shell, where mineralization converts the molecules into a nanoparticle of ferrihydrite, a ferric oxy-hydroxide.

Ferritin nanoparticles offer multiple potential applications for mine water remediation, the most promising being the photoreduction of contaminants in the presence of visible light or solar radiation [169–171]. Iron oxides in general have received a significant amount of attention for their potential remedial abilities. While able to carry out significant photochemical processes, Fe(III)-bearing iron oxides quickly undergo photoreduction to Fe(II), rendering the catalyst inactive [116]. However, ferritin naturally converts Fe(II) to Fe(III), preventing photoreduction of the iron oxide. While providing stability, the apoferritin cage does not inhibit photoreduction of environmental contaminants, conferring ferritin a significant advantage over traditional freestanding particles. In addition to iron hydroxide, the apoferritin protein cage supports the synthesis of the other metal hydroxides, such as $Mn(O)OH$, $Fe(O)OH$, and $Co(O)OH$ [116, 172]. Exploiting these capabilities could potentially expand this technology, thereby increasing remediation speed and effectiveness as well as expanding the list of contaminants that could be addressed. Further, ferritin can be employed in the production of both metallic and metal hydroxide nanoparticles like iron and cobalt metallic- and oxide-based nanoparticles.

(b) *Surface-Layer Proteins*

Surface layers (S-layers) form the outermost cell envelope of most archaea and many bacteria by serving as an interface between the bacterial cell and its environment. S-layers are macromolecular paracrystalline arrays of proteins or glycoproteins that self-assemble into two-dimensional semi-permeable mesh-works with regularly distributed pores of about 2–8 nm [151, 173]. The self-assembly consists of a spontaneous entropy-driven process with the size of each individual cell ranging from 2.5 to 35 nm, whereas the thickness of the layer varies between 5 and 70 nm [174]. The S-layers are about 50 % hydrophobic amino acids, 15 % glutamic and aspartic acids, and 10 % lysines [175]. Surface layer proteins can be extracted with chaotropic agents such as guani-dine hydrochloride from Gram-positive bacteria, or metal chelating agents (EDTA) from Gram-negative bacteria [174, 175]. Upon removal of the agent used for isolation, SLPs can be recrystallized to re-form meshworks identical to those on intact bacteria [175, 176]. The isolated S-layers can recrystallize into two-dimensional regular arrays in suspension or on various surfaces including silicon wafers, metal, glass, mica, or lipid, thus being an appropriate material for several bionanotechnological or potentially water treatment purposes.

Among the promising applications of S-layers is their use as biotemplates for capturing metal ions or for the synthesis of metal nanoclusters as they can be excellent bottom-up building blocks for metal immobilization structures and forming supramolecular scaffolding assemblies [176]. The high density of functional groups on the S-layer surface form matrices for controlled formation of inorganic nanocrystal superlattices (e.g. CdS, Au, Ni, Pt, or Pd), which are used for molecular electronics and non-linear optics [175, 177]. The S-layer's high capacity for binding metals is attributed to passive accumulation of metal on the surface of the bacteria cell, lending potential for applications removing toxic metals such as U, Cu, Pb, Al, Cd, Zn, and Ni [151, 174, 177, 178] from contaminated water, as well as for the recovery of precious metals (e.g. Au, Pt, Pd, Rh) from mine water [151, 174, 177, 178]. For instance, S-layers from different bacteria of *Lactobacillus kefir* bind Pb^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} while S-layers of *Lysinibacillus sphaericus* have high binding capacities towards UO_2^{2+} as well as Cu^{2+}, Pd^{2+}, Pt^{2+}, and Au^{3+} [151, 174, 177]. S-layer-metal interactions are mediated through coordination with side chain carboxyl groups of Asp and Glu residues, and NH groups from the peptide backbone while others with uranium are coordinated through carboxyl and phosphate groups (Fig. 10.16). Isolated S-layers can be used to nucleate biomineralization as they intrinsically tend to self-assemble in suspension into two-dimensional arrays and onto various surfaces, exhibiting pores of identical size and morphol-ogy and presenting functional groups aligned in a well-defined order and orientation [179]. Mineralization begins within the holes of the S-layer, where cations such as Ca^{2+} bind negative charges of the S-layer, followed by carbonate binding to initiate the formation of a mineral aggregate, dependant on the pH and ionic composition of the surrounding solution.
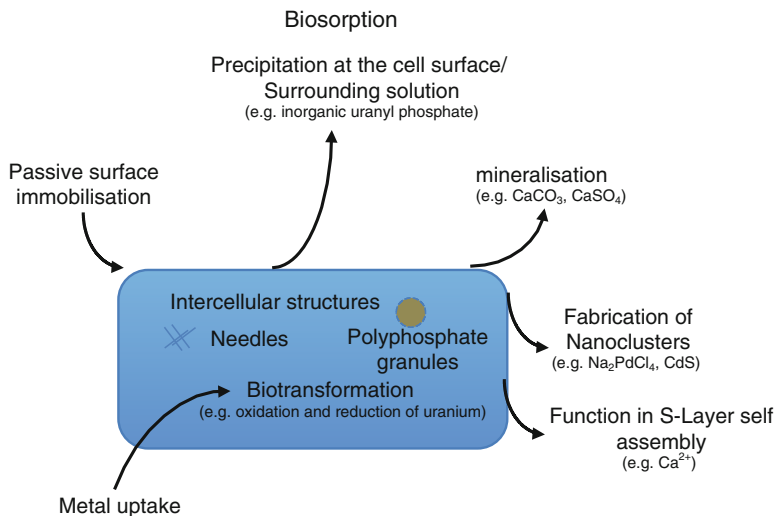
Biosorption

Precipitation at the cell surface/
Surrounding solution
(e.g. inorganic uranyl phosphate)

Passive surface
immobilisation

mineralisation
(e.g. CaCO$_3$, CaSO$_4$)

Intercellular structures
Needles   Polyphosphate
granules

Fabrication of
Nanoclusters
(e.g. Na$_2$PdCl$_4$, CdS)

Biotransformation
(e.g. oxidation and reduction of uranium)

Function in S-Layer self
assembly
(e.g. Ca^{2+})

Metal uptake

Fig. 10.16 Modes of interaction between metals and bacteria cells bearing S-layers, which can be exploited in mine water remediation. Modified from Ref. [181]

S-layers can be utilized in mine water remediation through construction of filter matrices with high metal-binding capacities possessing the ability to selectively and reversibly bind high amounts of toxic metals from mine water [180]. The S-layer can be engineered by inserting amino acid residues like cysteine and histidine carrying functional groups with a high affinity for heavy metals to metal sequestration. In other words, engineered recombinant S-layers enlarge the possibilities of designing nanoclusters with a higher affinity for metals.

(c) *Single-Enzyme Nanoparticles*

To increase longevity and enhance versatility, single enzymes are "caged" or encapsulated to create a class of catalysts called single enzyme nanoparticles (SENs) [182]. The "cage" is actually a nanostructured silicate shell, linked to the surface of the enzyme to protect the enzymes' catalytic function by allowing it to remain active for months relative to the normal proteinaceous enzymes life span of just a few hours. Consequently, these stable novel enzyme-containing nanoparticles are capable of significantly more catalytic activity, offering vast capabilities for chemical conversions, bio-sensing, and bioremediation. Their specificity and targeted effectiveness make them much more effective than synthetic catalysts. Synthesis of SENs initially involves covalent modification of the enzyme surface, creating vinyl group functionality and solubilization of the enzyme in a non-polar hydrophobic solvent such as hexane. Then, the modified enzymes are mixed with silane monomers with both vinyl and trimethoxysilane groups. Vinyl group polymerization creates linear polymers with free trimethoxysilane groups attached to the enzyme surface. Lastly, the cross-linked silicate shell resembling armour is created by hydrolysis of the trimethoxysilane groups and the subsequent condensation of the silanols [183]. The SENs offer a

variety of remediation advantages in being able to withstand more extreme conditions (pHs, high contaminant concentration, high salinity, and temperature) making them suitable for remediation of mine discharges and drainages with the contaminant of interest dictating the enzyme employed. Despite the benefits achievable through enzymatic remediation, concerns over enzyme stability and lifetime remain a cost-limiting factor for large-scale remedial applications.

(d) *Tunable Biopolymers*

Genetic and protein engineering have emerged as valuable tools for the construction of nanoscale materials that can be controlled precisely at the molecular level [184]. For instance, it is currently possible, using recombinant DNA techniques, to create "artificial" protein polymers with fundamentally new molecular organization. These nanoscale biopolymers can be specifically pre-programmed within a synthetic gene template and controlled precisely in terms of size, composition, and function at the molecular level [185]. In this manner, it is possible to design specific protein-based nano-biomaterials with both metal-binding and tuneable properties that can be used to selectively remove heavy metals from dilute solutions in a single process.

Elastin-like polypeptides (ELPs) are one such class of biopolymers that are of particular interest because of their "smart"-stimuli responsive-properties [185]. They are comprised of the repeating pentapeptide VPGVG and undergo a reversible phase transition from water-soluble forms or polymeric solutions into aggregates with increases in temperature [186]. The transition temperature of the ELP can be tuned/controlled by altering the chain length and sequence, but also with changes in pH, ionic strength, pressure, and covalent modifications [185]. The ELPs can be functionalized with other peptides or proteins, while still maintaining their temperature responsive characteristics. Tuneable biopolymers can be easily manipulated to selectively bind and remove heavy metals from water. For example, fusion of ELP with specific proteins creates biopolymers specifically targeting individual metals, such as cadmium, mercury, arsenic, and lead [184–186]. Currently, tuneable biopolymers that bind to cadmium and mercury are available and in use; those ELPs binding cadmium, for example, posses the repeating pentapeptide VPGVG, but can be tuned to incorporate a hexahistidine tail that serves as the cadmium-binding moiety [185, 186].

10.5.2.8   Nanocrystalline Zeolites

Zeolites are crystalline hydrated aluminosilicate materials composed of three-dimensionally connected framework structures constructed from corner-sharing tetrahedral coordinated Si and Al cations and oxygen [187]. These framework structures are composed of rings with 4–6 atoms of Si and Al, and large pore openings of 8-, 10-, and 12-rings. The specific surface area of zeolites is attributed to their porous crystalline structure. There are over 40 different kinds of natural

zeolites, and over 100 synthetics found in various part of the world. Conventional synthesis methods produce zeolites on the scale of 1–10 μm, however their pores are 0.4–1 nm, which are close to the molecular diameters of lighter hydrocarbons and consequently, zeolites are considered nanomaterials. The sizes of the intracrystalline pores and nanospaces depend on the type of zeolite. Zeolites have high cation-exchange capacities, high specific surface areas, and high hydrothermal stability [188]. The porosity of zeolite structure enables cations to migrate in and out freely, exchanging their cations for those of the surrounding fluid. The cation preference of a given zeolite can be due to ion sieving or competition between the zeolite phase and aqueous phase for the cations [189]. Strong acid sites exist on the nanopore surfaces, enabling the zeolites to be used as shape-selective catalysts. Zeolites are extremely active catalysts with selective catalysis-steric phenomena. They can be manipulated to catalyze reactions selectively by avoiding formation reactants or by preventing transition states from forming within the pores because of their size or shape.

Zeolite can act as a molecular sieve to produce sharp separations of molecules by size and shape while strong electrostatic fields within a zeolite cavity result in very strong interactions with polar molecules [189, 190]. However, they also effectively and strongly absorb non-polar molecules due to the polarizing power of the electric field. As a result, zeolites are excellent separators, even in the absence of steric hindrance. The unique properties of zeolites which make them effective sorbents and ion exchange media for metal ions, qualify them for use as a relatively inexpensive means of cleaning acidic mine water. Synthetic zeolites have been demonstrated to effectively remove Cr(III), Ni(II), Zn(II), Cu(II), and Cd(II) from electroplating waste water [190]. Regular-sized zeolites have the ability to remediate water containing cationic species, such as ammonium and heavy metals, as well as radioisotopes such as ^{137}Cs and ^{90}Sr [191, 192]. Zeolites can be used as permeable barriers to adsorb contaminants from groundwater and mine water as they pass to receiving environments. Overall, the separatory properties of zeolites based on their molecular sieve structure, electrostatic fields, and polarity enable their use for mine water treatment due to their contaminant separation and removal abilities.

10.6 Concerns with Nanomaterial Use in Mine Water

10.6.1 Perceived Nanotoxicological Risks

10.6.1.1 Toxicity Inherent to Nanoscale

The perceived risks of applying nanotechnology to contemporary mine water issues are inherent to characteristics and properties of materials at the nanoscale, and do not differ markedly from those associated with the use of nanomaterials in other

environmental applications. While nanoscale particles can be produced naturally by forest fires, geochemical reactions or as biological or abiotic particulates in aquatic systems, their distribution is usually limited in time and space [193]. Consequently, concerns surrounding the application of engineered nanoparticles to mine water issues are fuelled by the greater quantities and scope of use, as well as the uncertainties of short- and long-term behaviour and effects of the nanomaterials in the environment [194]. Production of anthropogenic nanoparticles and their resultant availability in the biosphere are indeed increasing at a remarkable rate. As of March 2011, 580 companies in 30 countries produced over 1,317 nanoparticle-based products from textiles to electronics to wound dressings [195]. The unique physicochemical properties of nanoparticles have resulted in a rapid expansion of their use, with nanoproduct development representing one of the fastest growing sectors of the high-tech economy [196, 197]. A recent estimate is that more than 40,000 tons of titanium nanoproducts were produced in the USA alone, a figure which is projected to reach 2.5 million tons annually by 2025 [198]. With increasing production and application of nanoparticles into diverse consumer products, their ultimate release to natural environments is inevitable [199]. Concern regarding releases of nanomaterials to aquatic systems, both at the consumer-level and through large-scale water treatment applications such as mine water, is growing among the scientific, academic, industrial, and regulatory community. As aquatic environments are the primary long-term sinks for nanomaterials, uncertainties regarding their fate and ecological effects are considerable [196, 197, 200].

Although the long-term impacts of the rapid and substantial loading of engineered nanomaterials to aquatic environments are largely unknown and difficult to quantify, they concentrate on the enhanced electrical, mechanical, and optical properties for which nanoparticles are designed. These same properties, desirable under the circumstances for which they were engineered, may elicit unintended consequences upon release to the environment, as will be expanded upon in detail in the following sections, but primarily revolve around three concerns. The first focus on the unique surface-reactive properties of nanomaterials, which can lead to unexpected consequences upon interaction with both abiotic and biological systems, including the generation of reactive oxygen species [196, 193, 200]. Further, not only are the materials themselves of concern, but the toxicity of ions dissolving off metallic nanomaterials, depending on the environmental conditions, are sometimes more toxic than the nanomaterials themselves [201]. Finally, nanomaterials in aquatic environments may easily serve as conduits to increase the availability, and hence potential for bioconcentration, bioaccumulation, and biomagnification of trace contaminants within the environment and their transfer to higher trophic levels [202, 203].

Although bulk materials themselves are well understood, when made progressively smaller, their surface chemical reactivity increases, as does the complexity of the processes regulating the nanoparticle surface chemistry upon contact with living systems, making it challenging, but crucial, to understand and predict possible toxicological effects [199, 204]. Complicating our understanding of these reactions

is the reality that nanoparticles are highly heterogeneous with none having identical size, shape, surface area or optical properties, although similar materials of similar size exhibit similar behaviours [202]. Toxicity of nanomaterials varies greatly with atomic composition, but to a large degree is contingent on whether the nanostructure is immobilized within or on the surface of a bulk material, or if they are free, unbound particles. For unbound particles with diameters in the nanometre range, nanomaterials and their degradation products are capable of great mobility within natural environments and organisms [204].

In surface waters, the consequences of unintentional exposure, particularly over the long term (as is the case with treated mine water discharges) is unknown. Most of the growing body of literature on nanocontaminants has focussed on short-term, acute toxicity assessments of nanoparticles to fish; however, it is likely that longer term exposure at lower concentrations is more ecologically relevant [205]. Unfortunately, few studies have investigated the consequences of long-term exposure to environmentally relevant concentrations of nanomaterials, so little is known of their chronic effects on aquatic organisms [196]. While much remains unknown, what seems clear is the distribution and potential for exposure to insoluble nanoscale particles of <50 nm is a new environmental phenomenon, which was non-existent in the pre-industrial biosphere of mere centuries ago [204]. Consequently, organisms have few, if any adaptive responses to accommodate exposure to nanomaterials [204]. If the widespread application and distribution of these relatively new products in consumer goods and potentially within water treatment systems is translated into widespread exposure and incorporation into organisms, potential nanotoxicity will be heightened by an inability to detoxify and/or remove these nanopollutants, the impacts of which are almost completely unknown [202].

As nanotoxicology is a relatively new field of study, a majority of toxicity investigations initially treated nanomaterials as if they behaved similarly to conventional chemicals or their bulk-sized counterparts rather than considering their distinctive physicochemical properties [202]. In not recognizing the unique surface chemistries of nanomaterials, and changes in their reactivities with changing environmental variables, many studies yielded inconclusive, controversial, and in many cases, conflicting results [202, 206, 220]. While we largely understand the properties of materials and/or chemicals at the molecular and bulk levels, there are novel and often distinct properties of materials in the zone between "molecule" and "bulk", the nanoscale. Specifically, the highly reactive surfaces of nanomaterials owing to their heightened surface area to volume ratios as well as wet biochemistry, both confer unique potential utility to nanoscale molecules, but also a means of interacting with biomaterials, which should be the focus of nanotoxicology [203, 206]. For example, the total surface area of nanoparticles may be a more sensitive metric for assessing the "dose" of nanomaterials than their mass, a paradigm-shifting perspective that causes no end of grief for classically trained toxicologists [204].

For example, Karlsson et al. [207] observed that CuO nanoparticles were much more toxic to mitochondria than micrometer-sized CuO particles; however, no size-related differences in toxicity were observed for iron oxides, with [208] also

reporting no discernible correlation in vivo between primary particle size and toxicity for TiO_2. In contrast, Karlsson et al. [207] found micrometer particles of TiO_2 caused more damage than TiO_2 nanoparticles, leading these authors to conclude that nanoscale nanoparticles are not always more toxic than micrometer particles. Such conclusions are common enough that Donaldson and Poland [209], while recognizing there are changes in quantum size effects for some particles at certain size thresholds, question the toxicological relevance of size changes. Specifically, these authors ask what changes in toxicity endpoints must be linked to nano-relative to macro-sized materials before we describe them as "nano-specific effects"? In most cases, Donaldson and Poland [209] believe the toxicity of nanomaterials is unlikely to differ from those established for larger particles. With caveats, other authors agree, with Bae et al. [200] stating that "the paradigm that smaller nanoparticles are more readily taken up, and thereby capable of inducing toxicity relative to bulk forms, is too simplistic, and may not always be true under specific experimental conditions (i.e. increased agglomeration reducing bioavailability, thereby limiting toxicity)".

Further complicating nanotoxicity assessments is the revelation toxicity from ions dissolving off metallic nanomaterials, depending on the environmental conditions, may be more toxic than the nanomaterials themselves. For example, in assessments with the green algal species *Chlorella*, Zn^{2+} was more toxic than nano-ZnO, which in turn was more toxic than bulk ZnO at concentration less than 50 mg/L. However, nanoscale ZnO was most toxic to *Chlorella* (then Zn^{2+}, then bulk ZnO) at concentrations >50 mg/L [210]. However, in other phyla TiO_2 nanoparticles were more acutely toxic than their bulk counterparts to zebrafish (*Danio rerio*), with the toxicity of ZnO nanoparticles and bulk ZnO being very similar [211]. In studies conducted by Heinlaan et al. [212], both bulk and nano TiO_2 were not toxic to any evaluated organism up to very high concentrations (<20 g/L), while ZnO in bulk and nanoscales was toxic to bacteria and crustaceans, but again only at very high (0.18–8.8 g/L LC_{50}) concentrations. Such clearly different experimental results with ZnO exposure across phyla also highlight differences in species uptake and sensitivity. With greater surface area per unit weight than their bulk counterparts, nanometal oxides such as ZnO, while having superior performance in many engineered respects, may in fact differ in biological availability, and hence toxicity, to different organisms depending on potential uptake mechanisms [199].

While nanoparticle size and surface area are significant factors modifying organismal uptake (and hence toxicity), rates of distribution and particle reactivity in solution allow many nanoparticles to easily enter living organisms and produce adverse reactions [193, 203]. Sun et al. [213] report that smaller nanoparticles including AuNPs, TiO_2NPs, SiO_2, quantum dots, and carbon nanoparticles can cross the placental barrier easier than larger ones, suggesting unique mammalian susceptibilities potentially impacting early developmental stages relative to other phyla. Female vertebrate populations are particularly vulnerable to nanotoxicity as female hypothalamic-pituitary-ovarian axis, and foetal development appear particularly sensitive to nanoparticles [213].

Hydroxyl radical generation by nanoparticles are at least partially responsible, if not the dominant mechanism of nanoparticle toxicity relative to bulk counterparts [214]. However, malondialdehyde (MDA) generation, a metric of oxidative damage in vivo, further illustrates the confusion surrounding size-dependent toxicity. For example, MDA concentrations in liver were 204 and 286 % elevated in zebrafish exposed to 5 mg/L ZnO NP and bulk ZnO respectively, suggesting bulk ZnO was more bioavailable and exerted greater oxidative damage-mediated toxicity than the nanoscale form [215].

10.6.1.2   Mechanism of Nanoscale Toxicity

(a) *ROS-Induced Toxicity*
   A wide range of NPs such as iron oxides, fullerenes, and TiO_2 [197, 216], aluminium oxide [217], and even talc and silica nanoparticles [217] and chitosan nanoparticles [219] induce the generation of reactive oxygen species (ROS). Many of these ROS, including singlet oxygen, superoxide anion, and hydroxyl radical, are extremely reactive due to unpaired electrons in their outer valence shells, conferring great reactivity, which is largely responsible for their toxicity. There are four possible mechanisms postulated by which nanomaterials may interact with biological tissue [220], the first three of which are directly ROS-mediated, including:

1. UV activation of electron hole pairs leading to ROS generation.
2. Composition of the nanomaterial itself, whereby discontinuous crystal planes and defects could generate active electronic configurations that could form ROS radicals.
3. Metals or metal coatings such as Fe could redox cycle forming ROS.
4. Dissolution of metal ions from particles to surrounding medium, which both releases toxic chemicals, but will also potentially changes surface charge.

   However, even the fourth mechanism, while not directly linked to ROS generation, may in fact also generate radicals indirectly in vivo. A prevalent suggestion is that free metal ions released from NPs exert their toxicity, at least in part, by crossing nuclear or mitochondrial membranes and reacting with endogenously generated hydrogen peroxide via Fenton-type processes, in turn inducing generation of highly reactive but short-lived ROS radicals such as hydroxyl radical and superoxide anion [220, 221]. However, some NPs as heterogeneous catalysts may have surface reactions that could generate ROS even in the absence of free ion dissolution, but more often than not, ion dissolution appears involved in nanomaterial toxicity, and it is the major postulated mechanism of metal oxide toxicity [221]. For instance, the antibacterial mechanism of AgNPs is related to the formation of free radicals and ROS-mediated membrane damage, likely as a function of Ag ion dissolution [200]. Silver nanoparticles and silver ions may in fact work synergistically to promote microbial membrane damage, as the ions move into the cells and

produce ROS, which in turn can increase cell permeability leading to uncontrolled AgNP and cellular constituent transport across the cell membrane leading to cell death [201]. A more than tenfold increase in ROS was observed with 15 nm silver nanoparticles relative to 55 nm, suggesting silver cytotoxicity is mediated by oxidative stress and AgNP size is critical [193]. Iron oxide nanoparticles, releasing metal ions in vivo, have been demonstrated to induce hydroxyl radical generation via Fenton-type processes under condoning biological microenvironments (physiological pH, reducing agent presence, etc.), which again damage plasma membranes leading to cell death. As alluded to previously, naturally occurring talc and silica nanoparticles both generate ROS to mediate their toxicity through oxidative stress as evidenced by lactate dehydrogenase leakage, lipid hydroperoxide increases, and depletion of reduced glutathione, although it is unclear if dissolved ions mediate this toxicity in any way [218].

(b) *Non-ROS-Mediated Toxicity*

While ROS-mediated toxicity is a prominent concern associated with in vivo nanoparticle exposure, nanomaterials can exert toxic effects by other significant pathway as well. Numerous studies have found NPs can induce cellular autophagy or cell death by free ion leaching, but these effects appear to be highly dependent on the nature of the cells [222]. It has been experimentally determined that negatively charged nanoparticles bound to fluid areas of a plasma membrane-induced localized gelation, whereas positively charged nanoparticles turned gelled areas into a fluid state for easier penetration. This phenomenon explains why cationic particles are more toxic than neutral or anionic analogues of the same size as positively charged NP surfaces induce holes in plasma membranes, while neutrals or negatives do not [223]. Biophysical structural impairment induced by nanomaterials may be a significant mechanism of toxicity; for example, a plasma membrane may suffer rapid strain-induced damage when nanomaterials bind, ion channels are blocked, or holes introduced to membranes [223].

Silver nanoparticles and silver ions also work synergistically to promote membrane damage in plants, although it is unclear if ROS mediates this toxicity. What is clear is the dynamic equilibrium between dissolved and nanoparticle-bound Ag allows the silver ions to be continuously replaced in solution as algal cells assimilate free Ag^+ ions, with nanoparticles serving as a reservoir of Ag^+ [224]. The efficient assimilation of Ag^+ by algae promotes further Ag dissolution from nanoparticles into solution, likely mediated by oxidation. Ironically, Ag^+-induced toxicity may be accelerated by the presence of algae due to their production of H_2O_2 as a metabolic product, which may be converted to hydroxyl radical in the presence of Ag^+ [224]. Published rates of H_2O_2 release are in excess of estimated Ag^+ releases, lending support to this theory [224]. Although only about 1 % of the silver in the algal media were present as Ag^+ ions, these dissolved ions were 18 times more toxic than the Ag nanoparticles, suggesting long-term toxicity is attributable to both the nanoparticles presence, and that of their dissolved free ions [224]. Gene expression profiles reveal AgNPs and

$AgNO_3$ (i.e. Ag^+ in solution) induce distinct expression profiles, suggesting differing modes of toxicity [225, 226]. Other studies confirm nanoparticle metal oxides such as ZnO and TiO_2 also exerted their algal toxicity through soluble metal ions continuously released from these nanoparticles [200]. Similarly, it is the free Cu^{2+} ions dissolved from CuO nanoparticles, rather than the NPs themselves, which inhibited cholinesterase activity in carp (*Cyprinus carpio*), suggesting the potential neurotoxicity is largely associated with exposure to the free metal species [205]. Aluminium oxide nanoparticles which leached aluminium ions after 48 h also crucially mediated toxicity in the freshwater crustacean *Ceriodaphnia dubia* [217]; however, other studies utilizing these and other metal oxide nanoparticles found negligible toxicity associated with ions dissolved from these nanoparticles to a suite of waterborne microorganisms [227].

Protein interactions with nanomaterials are another significant source of in vivo toxicity. As the dimensions of a nanoparticle are on par with that of a large plasma protein, several lines of evidence suggest the topographical features of NPs permit interactions with proteins in non-trivial ways [228]. For example, the formation of nanoparticle:protein complexes could enhance transport of nanoparticles, making them accessible to sites where larger particles could not reach. For many charged nanoparticles, especially those approximately the size of protein molecules, the interaction of proteins with their surface charges can alter the proteins' configuration, changing tertiary and quaternary structure, leading to loss of function or catalytic activity [204]. Conversely, smaller nanoparticles with their larger surface areas can enhance protein degradation, again leading to functional changes which do not occur with interactions on the relatively smaller surface areas of larger particles and bulk materials [193]. High and potentially deleterious local protein concentrations may result from proteins binding to nanoparticles by avidity effects, the sum total of the interactions between molecules at multiple binding sites [229]. Avidity effects are distinct from affinity, which is the strength of the interaction of one molecule with another at a single binding site, arising from close spatial repetition of the same protein. However, it is also possible proteins interacting with nanoparticles could produce co-operative effects such as promotion or inhibition of protein fibrillation or self-assembling of amino acid residues on a macromolecule serving as a template, such RNA. The large surface areas and high reactivity of nanoparticles also permit them to negatively interact with proteins and nucleic acids, thereby producing long-term adverse effects on living organisms [203]. Such interactions with DNA may be by direct intercalation or physical and/or electrochemical interactions, but recent evidence suggests nanoparticles may not necessarily need to permeate the cell nucleus to induce genotoxicity as this can be accomplished by ROS inducing oxidative stress within or adjacent to the nuclear membrane [204]. There are numerous and diverse means postulated whereby nanomaterials may exert toxicity together with, or independent of, ROS generation that do not fall cleanly into the preceding categories. Most nanoparticles stimulate the immune system, at

least to some extent, and elicit an inflammatory response (often including ROS generation) through the abnormal secretion of cytokines, while other nanopartoicles, such as cerium oxide, significantly suppress inflammation and ROS generation [193]. Due to surface charges, nanoparticle uptake by immune cells (monocytes, macrophages, leukocytes) may lead to inflammatory responses [193]. Such immune activation by nanomaterials may pose a carcinogenic risk, triggered by ROS released by macrophages attempting to destroy foreign material at the inflammation site, leading to DNA damage [223]. Nanometals are further suspected of inducing sublethal effects including respiratory toxicity, disturbances to trace element equilibriums in tissues, and inhibition of $Na^+K^+ATPase$ activities [196]. For example, exposure of zebrafish to TiO_2 NPs produced oedema and thickening of the gill lamellae, likely as a physical irritation response, but interestingly, increased Cu and Zn concentrations in the brain, clearly reflecting their uptake at the gill epithelium, but also their disruption of endogenous trace metal homeostasis [230]. $Na^+K^+ATPase$ activity was suppressed in the gills and intestine, while 2-thiobabituric acid reactive substances (TBARS), a metric of plasma membrane peroxidation, were elevated in the gill, intestine, and brain with exposure to TiO_2 NPs, suggesting cell membrane damage may have been linked to the increase in trace metal (Cu and Zn) concentrations [230].

10.6.1.3 Mine Water Conditions Mediating Nanotoxicity

The behaviour of NPs under real-world conditions has been under-investigated relative to laboratory simulations, but are known to be strongly influenced by the presence of NOM and salinity, which affect agglomeration, deposition, and adsorption [201]. The toxicity of nanomaterials may differ from their reactivity due to atomic composition, surface charge, particle agglomeration, and in natural systems, how these factors vary with environmental pH, presence of charged ions in solution, and through interactions with biomolecules in vivo [201]. For instance, ZnONPs and TiO_2NPs are more cytotoxic in the presence of sunlight, but cytotoxicity is ameliorated by the presence of humic acids [231]. Similarly, the potential toxicity of nanoparticles in mine waters is likely mediated by the interaction of the mine waters' and nanomaterials' physicochemical properties. Size, agglomeration rate (which decreases with increasing particle size), and adsorption properties towards dissolved and suspended organic material all determine the fate and ability of nanomaterials in mine water to interact with abiotic and biotic entities in receiving environments [201]. It is widely recognized that nanoparticles in a biological or environmental context never consist of "bare" particles as they rapidly contact atoms, atom clusters, single molecules, and macromolecules, which bind to the surface of the molecule and thereafter reflect the properties of the complex, rather than the pre-existing nanomaterial [204]. For example, in a biological environment where proteins are present, they will form a surface layer known as a corona that will ultimately define the surface properties of this new "particle + corona"

compound [204]. Consequently, to understand a particle's nanotoxicity, it is necessary to understand both the particle and its interactions with its environment. Natural organic material (NOM) likely rapidly coats NPs discharged into aquatic environments, which influences their availability and relative toxicity to aquatic organisms. This is well illustrated by both dissolved and suspended humic acids, when bound to surfaces of nanoparticles, greatly reduced their bonding to algal cells by increasing electrostatic repulsion [206]. As humic acids are the main NOM in environments impacted with mine water, their tendency to absorb or chelate metals, hydrous metal oxides, and multivalent cations to both alkyl and aromatic units of their humic acid skeleton suggests their presence will significantly affect both nanoparticles and metal pollutants within mine water. However, this relationship is dependent on pH, as many nanoparticles are negatively charged, or nearly uncharged at higher pH values [201]. Mechanisms underlying the myriad of potential interactions between nanoparticles and living systems are not yet fully understood, and may well never be. This complexity is largely due to the particles' ability to bind and interact with many forms of biological matter, and to change their surface characteristics, depending on the environment they are in [204]. Even particles of the same material can show completely different behaviour, and often toxicities, with slight differences in surface coating, charge, size, and especially environment [204].

10.6.2   *Risk of Nanoparticles as Pollution Vectors*

Untangling the mechanisms underlying the transport of contaminants in natural waters by colloidal particles is analogous to untying the mythical Gordian knot. Typical environmental colloids are nanoparticles consisting of silicates or oxides, organic colloids such as humic substances or polysaccharides, and biological particles (bacteria, viruses). Point source discharges such as municipal wastewater treatment plants, industry, and mine water are significant contributors of environmental colloids, which continually perfuse aquatic environments and expose biota with nanopollutants. However, the vast majority of nanomaterials in wastewater or in natural environments are retained among the biosolids and sediments [216]. Biosolids and sediments, in turn, can serve as a reservoir for non-point source, pulsatile nanomaterial release to surface waterways during rain events [195, 200, 216]. Considering how ubiquitous waterborne nanoparticles are in contemporary natural systems influenced by human activities, it is not surprising that they also influence the transport of other contaminants in the environment. However, the addition of significant quantities of engineered nanomaterials to aquatic systems may influence the migration of contaminants well beyond the role of natural nanoparticles or colloids, likely as highly efficient contaminant transport conduits. Traditionally, non-volatile contaminants within ground, surface, and mine waters are shuttled between the two-phase system of water and sediments by natural or anthropogenic nanoconduits linking the mobile aqueous phase and an immobile solid phase [232]. Sparingly

soluble or strongly adsorbing contaminants partition primarily to the later phase, and should, therefore, be sparingly detected in mine water. However, when contaminants interact with colloids in the aquatic phase, there is the formation of "pseudocolloids" or "eigencolloids" ("real colloids", or "intrinsic colloids") [233]. This leads to even sparingly soluble and strongly adsorbing contaminants becoming mobile, seemingly against the dictates of their individual physicochemical properties [232]. Conversely, dissolved contaminants may be removed from water and immobilized by adsorption onto colloids, which may aggregate leading to their sedimentation, thereby serving as a form of water treatment.

10.7   Concluding Remarks

Adopting nanotechniques for the monitoring and remediation of mine water pollution can radically reduce complexity, as well as material and energetic requirements relative to traditional approaches. Nanomaterials are uniquely surface-active due to their extensive surface area to volume ratios, which confer expansive active sites for sensing and/or binding proximal elements and compounds. Utilizing these unique attributes, specific nano- and biosensors can be developed to rapidly identify biological and chemical substances through variations produced upon interaction that alter physicochemical properties. Proximal sensing and signal change abilities, long-term stability, high adsorption efficiencies, extraordinary sensitivities, tuneable selectivity, and reusability are some of the highly desirable and useful attributes of engineered nanomaterials which facilitate their incorporation into a large suite of mine water treatments and sensors. As a result, nanosensors are expected to contribute to novel, revolutionary mine water applications, facilitating early and accurate detection of environmental pollutants. It follows that nanosensors, and nanoremediation have the potential not only to reduce the overall costs of cleaning up large-scale abandoned mining and processing sites but also to reduce clean-up time, eliminate the need for treatment and disposal of contaminated sludge, and reduce some contaminant concentrations in situ. Substantial initial investment would be needed to incorporate or switch to nanotechnology-based mine water treatments. However, once adopted, these techniques could considerably lower mine water treatment costs over the long term. Unfortunately, large-scale application of nanotechnology within mine water management has been extremely limited, as techniques are currently largely constrained to the bench-scale. However, it is anticipated that within the next two decades, such real-world applications will be more commonly adopted, as has been the paradigm in other fields. Prior to the wholesale adoption of large-scale nanotechnology-based mine water projects, further toxicological studies, including assessments of potential environmental impacts, must be considered. Relative to bulk material toxicity evaluations for which much is known and which have been well standardized against mass-based dose metrics, nanotoxicity assessments suffer from a paucity of data, and are made more complicated by the need to consider the influences of material, size, shape, surface charge, coating, dispersion, agglomeration, aggregation, concentration, and

matrix effects. Proper evaluation of materials used in nanoremediation, particularly full-scale ecosystem-wide studies, needs to be conducted to prevent potential adverse environmental impacts.

References

1. M. Gavrilescu, L.V. Pavel, I. Cretescu, J. Hazard. Mater. **163**, 475 (2009)
2. I.G. Carvalho, R. Cidu, L. Fanfani, H. Pitsch, C. Beaucaire, P. Zuddas, Environ. Sci. Technol. **39**, 8646 (2005)
3. R.R. Tatiya, *Surface and Underground Excavations*. Methods, Techniques and Equipment, 2nd edn. (CRC Press, London, 2013)
4. H.B.L. Pettersson, J. Koperski, Health Phys. **60**, 681 (1991)
5. E.G. Kelly, D.J. Spottiswood, *Introduction to Mineral Processing* (Wiley, Oxford, 1982)
6. B.A. Wills, T. Napier-Munn, *Wills' Mineral Processing Technology: An Introduction to the Practical Aspects of Ore Treatment and Mineral Recovery* (Butterworth-Heinemann, Oxford, 2006)
7. J. Ahn, Y. Park, J.-Y. Kim, K.-W. Kim, Environ. Geochem. Health **27**, 147 (2005)
8. J.A. Davis, D.E. Meece, M. Kohler, G.P. Curtis, Geochim. Cosmochim. Acta **68**, 3621 (2004)
9. R. Donahue, M.J. Hendry, Appl. Geochem. **18**, 1733 (2003)
10. M.C. Duff, J.U. Coughlin, D.B. Hunter, Geochim. Cosmochim. Acta **66**, 3533 (2002)
11. IAEA, *Manual of acid in situ leach uranium mining technology* (International Atomic Energy Agency, Vienna, Austria, 2001), p. 283
12. C. Wolkersdorfer, *Water Management at Abandoned Flooded Underground Mines: Fundamentals, Tracer Tests, Modelling, Water Treatment* (Springer, Heidelberg, 2008)
13. IAEA, *Guidebook on good practice in the management of uranium mining and mill operations and the preparation for their closure* (International Atomic Energy Agency, Vienna, Austria, 1998), p. 81
14. D.N. Castendyk, L.E. Eary (eds.), *Mine Pit Lakes: Characteristics, Predictive Modeling, and Sustainability* (Society for Mining, Metallurgy, and Exploration, Littleton, 2009)
15. P.L. Younger, N.S. Robins (eds.), *Mine Water Hydrogeology and Geochemistry* (The Geological Society, Brassmill Lane, 2002)
16. P.L. Younger, S.A. Banwart, R.S. Hedin (eds.), *Mine Water: Hydrology, Pollution, Remediation* (Springer, Heidelberg, 2002)
17. G.G. Bowen, C. Dussek, R.M. Hamilton, Geological Society, London, Special Publications **128** 93 (1998)
18. D.B. Johnson, K.B. Hallberg, Sci. Total Environ. **338**, 3 (2005)
19. INAP, Global Acid Rock Drainage Guide (GARD Guide) Global Acid Rock Drainage Guide (GARD Guide), ICARD 8. INAP: The International Network for Acid Prevention, Skelleftea, Sweden, 2009
20. A.S. Sheoran, V. Sheoran, Miner. Eng. **19**, 105 (2006)
21. R.E. Mielke, D.L. Pace, T. Porter, G. Southam, Geobiology **1**, 81 (2003)
22. A.R. Elsetinow, M.J. Borda, M.A.A. Schoonen, D.R. Strongin, Adv. Environ. Res. **7**, 969 (2003)
23. K. Benzerara, G. Morin, T.H. Yoon, J. Miot, T. Tyliszczak, C. Casiot, O. Bruneel, F. Farges, G.E. Brown Jr., Geochim. Cosmochim. Acta **72**, 3949 (2008)
24. R.S. Hedin, Mine Water Environ.ment **27**, 200 (2008)
25. N. Shikazono, M. Utada, Miner. Deposita **32**, 596 (1997)
26. C. Woulds, B.T. Ngwenya, Appl. Geochem. **19**, 1773 (2004)
27. H. Shaoping, C. Xincai, S. Jiyan, C. Yingxu, L. Qi, Chemosphere **71**, 2091 (2008)
28. K. Leopold, B. Michalik, J. Wiegand, J. Environ. Radioact. **94**, 137 (2007)

29. M.K. Banks, A.P. Schwab, G.R. Fleming, B.A. Hetrick, Chemosphere **29**, 1691 (1994)
30. E.R. Landa, E.J.P. Phillips, D.R. Lovley, Appl. Geochem. **6**, 647 (1991)
31. M. Mkandawire, *Environ Sci Pollut Res 1*, 2013
32. P. Diehl, Uranium Mining in Eastern Germany: The WISMUT Legacy. WISE Uranium Project, Arnsdorf, Germany, 2003, p. http://www.antenna.nl/wise/uranium/uwis.html.
33. H.M. Abdel-Aziz, J. Appl. Polym. Sci. **120**, 1547 (2011)
34. A. Abdelouas, Elements **2**, 335 (2006)
35. D.W. Engelkemeir, K.F. Flynn, L.E. Glendenin, Phys. Rev. **126**, 1818 (1962)
36. IAEA, World Distribution of Uranium Deposits (UDEPO) with Uranium Deposit Classification, International Atomic Energy Agency, Vienna, 2009.
37. S. Yusan, S. Erenturk, Desalination **269**, 58 (2011)
38. L.J. Qian, J.N. Zhao, P.Z. Hu, Y.X. Geng, W.S. Wu, J Radioanal Nucl Chem **283**, 653 (2010)
39. D.L. Guerra, A.A. Pinto, C. Airoldi, R.R. Viana, Inorganic Chemistry Communications **11**, 539 (2008)
40. T. Allard, P. Ildefonse, C. Beaucaire, G. Calas, Chem. Geol. **158**, 81 (1999)
41. X. Qu, P.J.J. Alvarez, Q. Li, Water Res. **47**, 3931 (2013)
42. X.G. Li, H. Feng, M.R. Huang, Chem. Eur. J **15**, 4573 (2009)
43. T.B. Henry, F.M. Menn, J.T. Fleming, J. Wilgus, R.N. Compton, G.S. Sayler, Environ. Health Perspect. **115**, 1059 (2007)
44. D.K. Tiwari, J. Behari, P. Sen, World Appl. Sci. J. **3**, 417 (2008)
45. A. Carlmark, C. Hawker, A. Hult, M. Malkoch, Chem. Soc. Rev. **38**, 352 (2009)
46. Y.C. Sharma, V. Srivastava, J. Chem. Eng. Data **56**, 819 (2011)
47. X.X. Wang, CLEAN—Soil Air Water **39**, 13 (2011)
48. Y.F. Lin, H.W. Chen, K.L. Lin, B. Chen, C. Chiou, J. Environ. Sci. **23**, 44 (2011)
49. X.G. Li, H. Feng, M.R. Huang, Chem. Eur. J **16**, 10113 (2010)
50. N.N. Nassar, Sep. Sci. Technol. **45**, 1092 (2010)
51. A. Eslami, S. Nasseri, B. Yadollahi, A. Mesdaghinia, F. Vaezi, R. Nabizadeh, S. Nazmara, J. Chem. Tech. Biotechnol. **83**, 1447 (2008)
52. N.S. Wigginton, K.L. Haus, M.F.H. Jr, J. Environ. Monit. **9**, 1306 (2007)
53. A.J. Du, D.D. Sun, J.O. Leckie, J. Hazard. Mater. **187**, 96 (2011)
54. H.J. Shipley, S. Yean, A.T. Kan, M.B. Tomson, Environ. Sci. Pollut. Res **17**, 1053 (2010)
55. X.-q. Li, D.W. Elliott, W.-x. Zhang, Critical Reviews in Solid State and Materials Sciences **31** 111 (2006).
56. C.-H. Liao, S.-F. Kang, Y.-W. Hsu, Water Res. **37**, 4109 (2003)
57. L.J. Dong, P.V. Zinin, J.P. Cowen, L.C. Ming, J. Hazard. Mater. **168**, 626 (2009)
58. O.A. Sadik, A.L. Zhou, S. Kikandi, N. Du, Q. Wang, K. Varner, J. Environ. Monit. **11**, 1782 (2009)
59. I. Linkov, J. Steevens, G. Adlakha-Hutcheon, E. Bennett, M. Chappell, V. Colvin, J.M. Davis, T. Davis, A. Elder, S. Hansen, P.B. Hakkinen, S.M. Hussain, D. Karkan, R. Korenstein, I. Lynch, C. Metcalfe, A.B. Ramadan, F.K. Satterstrom, J. Nanopart. Res **11**, 513 (2009)
60. D.G. Rickerby, M. Morrison, Sci. Tech. Adv. Mater. **8**, 19 (2007)
61. P. Namour, M. Lepot, N. Jaffrezic-Renault, *Sensors (Basel, Switzerland) 10 7947*, 2010
62. M. Li, H. Gou, I. Al-Ogaidi, N. Wu, ACS Sustain. Chem. Eng. **1**, 713 (2013)
63. K. Saha, S.S. Agasti, C. Kim, X. Li, V.M. Rotello, Chem. Rev. **112**, 2739 (2012)
64. J. Liu, Z. Cao, Y. Lu, Chem. Rev. **109**, 1948 (2009)
65. D.L. Robertson, G.F. Joyce, Nature **344**, 467 (1990)
66. X. Xue, F. Wang, X. Liu, J. Am. Chem. Soc. **130**, 3244 (2008)
67. J. Liu, Y. Lu, J. Am. Chem. Soc. **125**, 6642 (2003)
68. J. Liu, Y. Lu, J. Am. Chem. Soc. **126**, 12298 (2004)
69. J. Liu, A.K. Brown, X. Meng, D.M. Cropek, J.D. Istok, D.B. Watson, Y. Lu, Proc. Natl. Acad. Sci. **104**, 2056 (2007)

70. E. Oh, M.-Y. Hong, D. Lee, S.-H. Nam, H.C. Yoon, H.-S. Kim, J. Am. Chem. Soc. **127**, 3270 (2005)
71. C.S. Yun, A. Javier, T. Jennings, M. Fisher, S. Hira, S. Peterson, B. Hopkins, N.O. Reich, G.F. Strouse, J. Am. Chem. Soc. **127**, 3115 (2005)
72. M. Li, Q. Wang, X. Shi, L.A. Hornak, N. Wu, Anal. Chem. **83**, 7061 (2011)
73. J.R. Zhang, W.T. Huang, W.Y. Xie, T. Wen, H.Q. Luo, N.B. Li, Analyst **137**, 3300 (2012)
74. J. Li, L. Chen, T. Lou, Y. Wang, ACS Appl. Mater. Interfaces **3**, 3936 (2011)
75. F. Faridbod, M.R. Ganjali, M. Pirali-Hamedani, P. Norouzi, Int. J. Electrochem. Sci. **5**, 1103 (2010)
76. P. Norouzi, Z. Rafiei-Sarmazdeh, F. Faridbod, M. Adibi, M.R. Ganjali, Int. J. Electrochem. Sci. **5**, 367 (2010)
77. F. Faridbod, M.R. Ganjali, B. Larijani, M. Hosseini, P. Norouzi, Mater. Sci. Eng. C **30**, 555 (2010)
78. M.R. Ganjali, T. Poursaberi, M. Khoobi, A. Shafiee, M. Adibi, M. Pirali-Hamedani, P. Norouzi, Int. J. Electrochem. Sci. **6**, 717 (2011)
79. Y. Shao, J. Wang, H. Wu, J. Liu, I.A. Aksay, Y. Lin, Electroanalysis **22**, 1027 (2010)
80. A.K. Wanekaya, Analyst **136**, 4383 (2011)
81. D.W. Kimmel, G. LeBlanc, M.E. Meschievitz, D.E. Cliffel, Anal. Chem. **84**, 685 (2011)
82. J. Morton, N. Havens, A. Mugweru, A.K. Wanekaya, Electroanalysis **21**, 1597 (2009)
83. L. Wang, X. Wang, G. Shi, C. Peng, Y. Ding, Anal. Chem. **84**, 10560 (2012)
84. S. Yuan, D. Peng, D. Song, J. Gong, Sens. Actuators B **181**, 432 (2013)
85. T.H. Kim, J. Lee, S. Hong, J. Phys. Chem. C **113**, 19393 (2009)
86. Z. Zou, A. Jang, E.T. MacKnight, P.M. Wu, J. Do, J.S. Shim, P.L. Bishop, C.H. Ahn, IEEE Sensors J **9**, 586 (2009)
87. J.P. Lafleur, S. Senkbeil, T.G. Jensen, J.P. Kutter, Lab Chip **12**, 4651 (2012)
88. S.J. Lee, M. Moskovits, Nano Lett. **11**, 145 (2010)
89. L.C. Batty, P.L. Younger, Environ. Pollut. **132**, 85 (2004)
90. G.E. Dudel, C. Brackhage, H. Dienemann, M. Mkandawire, A. Weiske, in *Mine Water—Process, Policy and Progress*, ed. by A.P. Jarvis, B.A. Dudgeon, P.L. Younger (University of Newcastle Upon Tyne Press, Newcastle, 2004)
91. K.B. Hallberg, D.B. Johnson, Land Contam. Reclam. **11**, 213 (2003)
92. C.E. Rostad, B.S. Martin, L.B. Barber, J.A. Leenheer, Environ. Sci. Technol. **34**, 2703 (2000)
93. A. Zayed, S. Gowthaman, N. Terry, J. Environ. Qual. **27**, 715 (1998)
94. M. Morita, N.S. Malvankar, A.E. Franks, Z.M. Summers, L. Giloteaux, A.E. Rotaru, C. Rotaru, D.R. Lovley, *Mbio* **2**, 2011
95. H. Daims, M.W. Taylor, M. Wagner, Trends Biotechnol. **24**, 483 (2006)
96. U. Soltmann, H. Wand, A. Müller, P. Kuschk, U. Stottmeister, Acta Biotechnol. **22**, 161 (2002)
97. S. Dave, M. Damani, D. Tipre, J. Hazard. Mater. **173**, 231 (2010)
98. C. White, S.C. Wilkinson, G.M. Gadd, Int. Biodeter. Biodegrad. **35**, 17 (1995)
99. M. Kilic, M.E. Keskin, S. Mazlum, N. Mazlum, Chem. Eng. Process **47**, 31 (2008)
100. Y.B. Zang, W.G. Hou, J. Xu, Chin. J. Chem. **29**, 847 (2011)
101. S.K. Hamdona, O.A. Al Hadad, Desalination **228**, 277 (2008)
102. M. Saeedi, M. Hosseinzadeh, M. Rajabzadeh, Environ. Earth Sci **62**, 519 (2011)
103. G.C.L. Araujo, S.G. Lemos, A.G. Ferreira, H. Freitas, A.R.A. Nogueira, Chemosphere **68**, 537 (2007)
104. M. Islam, R. Patel, Desalination **256**, 120 (2010)
105. H. Beddow, S. Black, D. Read, J. Environ. Radioact. **86**, 289 (2006)
106. R.A.P. Thomas, L.E. Macaskie, Appl. Microbiol. Biotechnol. **49**, 202 (1998)
107. C. Bobirica, L. Bobirica, R. Stanescu, I. Constantinescu, Environ. Eng. Manag. J **8**, 759 (2009)
108. X.X. Guo, F.Z. Zhang, Q. Peng, S.L. Xu, X.D. Lei, D.G. Evans, X. Duan, Chem. Eng. J. **166**, 81 (2011)

109. N.C. Feng, X.Y. Guo, S. Liang, Y.S. Zhu, J.P. Liu, J. Hazard. Mater. **185**, 49 (2011)
110. V.E. Copcia, C.M. Hristodor, N. Bilba, E. Popovici, Rev. Chem **62**, 195 (2011)
111. M.L. Zhang, H.Y. Zhang, D. Xu, L. Han, D.X. Niu, B.H. Tian, J.A. Zhang, L.Y. Zhang, W.S. Wu, Desalination **271**, 111 (2011)
112. M.L. Coleman, D.B. Hedrick, D.R. Lovley, D.C. White, K. Pye, Nature **361**, 436 (1993)
113. K. Benzerara, J. Miot, G. Morin, G. Ona-Nguema, F. Skouri-Panet, C. Férard, Compt. Rendus Geosci. **343**, 160 (2011)
114. S. Mann, J. Webb, R.J.P. Williams, *Biomineralisation - Chemical and Biological pespectives* (Weinhein, VCH Verlagsgesellschaft, 1989)
115. M. Epple, E. Bäuerlein, W. Pompe (Ed.)^(Eds.), Handbook of Biomineralization. Wiley-VCH, Weinheim, 2009.
116. K. Watlington, Emerging Nanotechnologies for Site Remediation and Wastewater Treatment. National Network for Environmental Management Studies (NNEMS), 2005, p. 40.
117. G.M. Gadd, Microbiology **156**, 609 (2010)
118. S.C.N. Tang, I.M.C. Lo, Water Res. **47**, 2613 (2013)
119. N. Singh, G.J. Jenkins, R. Asadi, S.H. Doak, *Nano reviews 1*, 2010
120. C. Noubactep, Environ. Technol. **29**, 909 (2008)
121. C.S. Rajan, Int. J. Environ. Sci. Dev **2**, 3 (2011)
122. S. Shariati, M. Faraji, Y. Yamini, A.A. Rajabi, Desalination **270**, 160 (2011)
123. H. Parham, B. Zargar, Z. Heidari, A. Hatamie, J. Iran. Chem. Soc. **8**, S9 (2011)
124. J.R. Peralta-Videa, L.J. Zhao, M.L. Lopez-Moreno, G. de la Rosa, J. Hong, J.L. Gardea-Torresdey, J. Hazard. Mater. **186**, 1 (2011)
125. C. Noubactep, J. Radioanal. Nucl. Chem **267**, 13 (2005)
126. C. Noubactep, G. Meinrath, P. Dietrich, M. Sauter, B.J. Merkel, Environ. Chem **2**, 71 (2005)
127. S.O. Hara, T. Krug, J. Quinn, C. Clausen, C. Geiger, Remed. J. **16**, 35 (2006)
128. J. Hu, I.M.C. Lo, G. Chen, *Water Science and Technology 50, 50 139*, 2004
129. H.K. Boparai, M. Joseph, D.M. O'Carroll, J. Hazard. Mater. **186**, 458 (2011)
130. W. Wu, Q. He, C. Jiang, Nanoscale Res. Lett. **3**, 397 (2008)
131. R.A. Root, S. Fathordoobadi, F. Alday, W. Ela, J. Chorover, *Environmental Science & Technology*, 2013
132. A. Knauer, A. Thete, S. Li, H. Romanus, A. Csaki, W. Fritzsche, J.M. Kohler, Chem. Eng. J. **166**, 1164 (2011)
133. N. Savage, M. Diallo, J. Nanopart. Res **7**, 331 (2005)
134. M.S. Diallo, N. Savage, J. Nanopart. Res **7**, 25 (2005)
135. S.B. Shen, T.L. Pan, X.Q. Liu, L. Yuan, Y.J. Zhang, J.C. Wang, Z.C. Guo, J. Colloid Interface Sci. **345**, 12 (2010)
136. W.G. Shim, J.W. Lee, S.C. Kim, Appl. Catal. B Environ. **84**, 133 (2008)
137. Y. Wang, J.H. Qu, H.J. Liu, C.Z. Hu, Catal. Today **126**, 476 (2007)
138. C. Boschi, H. Maldonado, M. Ly, E. Guibal, J. Colloid Interface Sci. **357**, 487 (2011)
139. V.K. Gupta, B. Gupta, A. Rastogi, S. Agarwal, A. Nayak, J. Hazard. Mater. **186**, 891 (2011)
140. K. Jainae, K. Sanuwong, J. Nuangjamnong, N. Sukpirom, F. Unob, Chem. Eng. J. **160**, 586 (2010)
141. Q.J. Geng, Q.J. Guo, C.Q. Cao, H.Q. Wang, Chem. Eng. Tech **31**, 1023 (2008)
142. M.R. Hoffmann, S.T. Martin, W. Choi, D.W. Bahnemannt, Chem. Rev. **95**, 69 (1995)
143. V. Rodríguez-González, R. Zanella, G. del Angel, R. Gómez, J. Mol. Catal. Chem **281**, 93 (2008)
144. V. Stengl, S. Bakardjieva, T.M. Grygar, J. Bludska, M. Kormunda, Chem. Cent. J **7**, 41 (2013)
145. J.J. Chen, K. Wang, R. Huang, T. Saito, Y.H. Ikuhara, T. Hirayama, W.L. Zhou, IEEE Trans. Nanotech **9**, 634 (2010)
146. J.E. Rossini, A.S. Huss, J.N. Bohnsack, D.A. Blank, K.R. Mann, W.L. Gladfelter, J. Phys. Chem. C **115**, 11 (2011)

147. L. Wei, D. Shi, Z. Zhou, P. Ye, J. Wang, J. Zhao, L. Liu, C. Chen, Y. Zhang, Nanoscale Res. Lett. **7**, 334 (2012)
148. G. Fryxell, S. Mattigod, Y. Lin, H. Wu, S. Fiskom, K. Parker, F. Zheng, W. Yantasee, T. Zemanian, R. Addleman, J. Liu, K. Kemner, S. Kelly, X. Feng, J. Mater. Chem. **17**, 2863 (2007)
149. E. Blanco, A. Pineiro, R. Miller, J.M. Ruso, G. Prieto, F. Sarmiento, Langmuir **25**, 8075 (2009)
150. T.S. Zemanian, G.E. Fryxell, J. Liu, S. Mattigod, J.A. Franz, Z. Nie, Langmuir **17**, 8172 (2001)
151. U. Soltmann, J. Raff, S. Selenska-Pobell, S. Matys, W. Pompe, H. Böttcher, J. Sol-Gel Sci. Tech **26**, 1209 (2003)
152. A. Bögershausen, Ruprecht - Karls - Universität Heidelberg, Mannheim, 2004
153. K. Rath, *Science and Technology Review 24*, 2005
154. U. Soltmann, H. Böttcher, J. Sol-Gel Sci. Tech **48**, 66 (2008)
155. M. Mkandawire, J. Förster, D. Fiedler, H. Böttcher, W. Pompe, Int. J. Environ. Anal. Chem. **89**, 529 (2009)
156. T.J. Barnes, I. Ametov, C.A. Prestidge, Asia Pac. J. Chem. Eng **3**, 13 (2008)
157. C.C. Chu, N. Ueno, T. Imae, Chem. Mater. **20**, 2669 (2008)
158. M. Kubeil, H. Stephan, H.-J. Pietzsch, G. Geipel, D. Appelhans, B. Voit, J. Hoffmann, B. Brutschy, Y.V. Mironov, K.A. Brylev, V.E. Fedorov, Chem. Asian J. **5**, 2507 (2010)
159. W.-D. Jang, K.M. Kamruzzaman Selim, C.-H. Lee, I.-K. Kang, Prog. Polym. Sci. **34**, 1 (2009)
160. M. Gaab, S. Bellemin-Laponnaz, L.H. Gade, Chemistry **15**, 5450 (2009)
161. A.N. Petit, P. Eullaffroy, T. Debenest, F. Gagne, Aquat. Toxicol. **100**, 187 (2010)
162. N.M. Mahmoodi, B. Hayati, M. Arami, F. Mazaheri, J. Chem. Eng. Data **55**, 4660 (2010)
163. F. Ma, R.J. Qu, C.M. Sun, C.H. Wang, C.N. Ji, Y. Zhang, P. Yin, J. Hazard. Mater. **172**, 792 (2009)
164. A. Shakhbazau, D. Shcharbin, I. Seviaryn, N. Goncharova, S. Kosmacheva, M. Potapnev, B. Gabara, M. Ionov, M. Bryszewska, Mol. Biol. Rep. **37**, 2003 (2010)
165. T. Pietsch, D. Appelhans, N. Gindy, B. Voit, A. Fahmi, Colloids Surf. A Physicochem. Eng. Asp. **341**, 93 (2009)
166. M.J. DeMarco, A.K. SenGupta, J.E. Greenleaf, Water Res. **37**, 164 (2003)
167. A.A. Agrawal, B.J. Nehilla, K.V. Reisig, T.R. Gaborski, D.Z. Fang, C.C. Striemer, P.M. Fauchet, J.L. McGrath, Biomaterials **31**, 5408 (2010)
168. A.G. Cattaneo, R. Gornati, E. Sabbioni, M. Chiriva-Internati, E. Cobos, M.R. Jenkins, G. Bernardini, J. Appl. Toxicol. **30**, 730 (2010)
169. P. Arosio, S. Levi, Free Radic. Biol. Med. **33**, 457 (2002)
170. J.-F. Briat, M. Lebrun, Compt. Rendus Acad. Sci. III Sci. Vie **322**, 43 (1999)
171. E. Pilon-Smits, M. Pilon, Crit. Rev. Plant Sci. **21**, 439 (2002)
172. M. Uchida, S. Kang, C. Reichhardt, K. Harlen, T. Douglas, *Biochim Biophys Acta **1800***, 2010
173. Y. Gorby, J. McLean, A. Korenevsky, K. Rosso, M.Y. El-Naggar, T.J. Beveridge, Geobiology **6**, 232 (2008)
174. K. Fahmy, M. Merroun, K. Pollmann, J. Raff, O. Savchuk, C. Hennig, S. Selenska-Pobell, Biophys. J. **91**, 996 (2006)
175. N. Ilk, E.M. Egelseer, U.B. Sleytr, Curr. Opin. Biotechnol. **22**, 824 (2011)
176. V. Saravia, S. Küpcü, M. Nolte, C. Huber, D. Pum, A. Fery, U.B. Sleytr, J.L. Toca-Herrera, J. Biotechnol. **130**, 247 (2007)
177. M.L. Merroun, J. Raff, A. Rossberg, C. Hennig, T. Reich, S. Selenska-Pobell, Appl. Environ. Microbiol. **71**, 5532 (2005)
178. P.J. Panak, J. Raff, S. Selenska-Pobell, G. Geipel, G. Bernhard, H. Nitsche, Radiochimica Acta **88**, 71 (2000)
179. A. Martın-Molina, S. Moreno-Flores, E. Perez, D. Pum, U.B. Sleytr, J.L. Toca-Herrera, Biophys. J. **90**, 1821 (2006)

180. U. Jankowski, M.L. Merroun, S. Selenska-Pobell, K. Fahmy, Spectroscopy **24**, 177 (2010)
181. P. Mobili, M.D.L.A. Serradell, C. Mayer, V. Arluison, A. Gomez-Zavaglia, Int. J. Biochem. Res. Rev **3**, 39 (2012)
182. J. Kim, J.W. Grate, Nano Lett. **3**, 1219 (2003)
183. I. Perez-Baena, F. Barroso-Bujans, U. Gasser, A. Arbe, A.J. Moreno, J. Colmenero, J.A. Pomposo, ACS Macro Lett. **2**, 775 (2013)
184. S.R. MacEwan, A. Chilkoti, Biopolymers **94**, 60 (2010)
185. J. Kostal, A. Mulchandani, W. Chen, Macromolecules **34**, 2257 (2001)
186. J. Pu, K. Fukushi, Sci. World J **2013**, 6 (2013)
187. S. Akbar, R. Shahnaz, T.H. Shah, M. Sami, J. Chem. Soc. Pakistan **31**, 543 (2009)
188. A.I.P. Cordoves, M.G. Valdes, J.C.T. Fernandez, G.P. Luis, J.A. Garcia-Calzon, M.E.D. Garcia, Micropor. Mesopor. Mater. **109**, 38 (2008)
189. O. Gok, A. Ozcan, B. Erdem, A.S. Ozcan, Colloids Surf. A Physicochem. Eng. Asp. **317**, 174 (2008)
190. R. Apiratikul, P. Pavasant, Chem. Eng. J. **144**, 245 (2008)
191. V.K. Jha, M. Nagae, M. Matsuda, M. Miyake, J. Environ. Manage. **90**, 2507 (2009)
192. P. Misaelides, A. Godelitsas, A. Filippidis, D. Charistos, I. Anousis, Sci. Total Environ. **173–174**, 237 (1995)
193. I.D. Rastogl, J. Toxicol. Environ. Health Sci **4**, 1 (2012)
194. I. Ahmad, J. Biomed. Toxicol **7**, 32 (2011)
195. R. Zhang, Y. Bai, B. Zhang, L. Chen, B. Yan, J. Hazard. Mater. **212**, 404 (2012)
196. B.J. Shaw, R.D. Handy, Environ. Int. **37**, 1083 (2011)
197. B. Wang, J.-J. Yin, X. Zhou, I. Kurash, Z. Chai, Y. Zhao, W. Feng, J. Phys. Chem. **C117**, 383 (2012)
198. A. Pourmand, Abdollahi, M., Daru J. Pharm. Sci. **20** 95 (2012)
199. Y. Wang, W.G. Aker, H.-M. Hwang, C.G. Yedjou, H. Yu, P.B. Tchounwou, Sci. Total Environ. **409** 4753 (2011)
200. E. Bae, H.-J. Park, J. Yoon, Y. Kim, K. Choi, J. Yi, Korean J. Chem. Eng. **28**, 267 (2011)
201. H.C. Poynton, J.M. Lazorchak, C.A. Impellitteri, B.J. Blalock, K. Rogers, H.J. Allen, A. Loguinov, J.L. Heckman, S. Govindasmawy, Toxicogenomic responses of nanotoxicity in Daphnia magna exposed to silver nitrate and coated silver nanoparticles. Environ. Sci. Technol. **46**, 6288–6296 (2012)
202. K.J. Lee, L.M. Browning, P.D. Nallathamby, T. Desai, P.K. Cherukuri, X.-H.N. Xu, Chem. Res. Toxicol. **25**, 1029 (2012)
203. K.J. Lee, L.M. Browning, P.D. Nallathamby, X.-H.N. Xu, Chem. Res. Toxicol. **26**, 904 (2012)
204. A. Elsaesser, C.V. Howard, Adv. Drug Deliv. Rev. **64**, 129 (2012)
205. J. Zhao, Z. Wang, X. Liu, X. Xie, K. Zhang, B. Xing, J. Hazard. Mater. **197**, 304 (2011)
206. D. Lin, J. Ji, Z. Long, K. Yang, F. Wu, Water Res. **46**, 4477 (2012)
207. H.L. Karlsson, J. Gustafsson, P. Cronholm, L. Möller, Toxicol. Lett. **188**, 112 (2009)
208. A. Menard, D. Drobne, A. Jemec, Environ. Pollut. **159**, 677 (2011)
209. K. Donaldson, C.A. Poland, Curr. Opin. Biotechnol. **24**, 724 (2013)
210. J. Ji, Z. Long, D. Lin, Chem. Eng. J. **170**, 525 (2011)
211. D. Xiong, T. Fang, L. Yu, X. Sima, W. Zhu, Sci. Total Environ. **409**, 1444 (2011)
212. M. Heinlaan, A. Ivask, I. Blinova, H.-C. Dubourguuier, A. Kahru, Chemosphere **71**, 1308 (2008)
213. J. Sun, Q. Zhang, Z. Wang, B. Yan, Int. J. Mol. Sci. **14**, 9319 (2013)
214. M. Pumera, Chem. Asian J. **6**, 340 (2011)
215. D.W. Xiong, T. Fang, X.D. Chen, X.F. Sima, W.T. Zhu, Huan Jing Ke Xue **31**, 1320 (2010)
216. J.F. Reeves, S.J. Davies, N.J.F. Dodd, A.N. Jha, Mutat. Res. **640**, 13 (2008)
217. S. Pakrashi, S. Dalal, A. Humayun, S. Chakravarty, N. Chandrasekaran, A. Mukherjuu, PLoS ONE **8**(9), e74003 (2013)

218. I. Ahmad, I. Mohmood, C. Mieiro, J. Coelho, M. Pacheco, M. Santos, A. Duarte, E. Pereira, *Aquatic Toxicology*, 2011
219. Y.-L. Hu, W. Qi, F. Han, J.-Z. Shao, J.-Q. Gao, Int. J. Nanomed. **6**, 3351 (2011)
220. S. Arora, J.M. Rajwade, K.M. Paknikar, Toxicol. Appl. Pharmacol. **258**, 151 (2012)
221. T.P. Dasari, H.-M. Hwang, J. Environ. Sci. **25**, 1925 (2013)
222. S.J. Soenen, J. Demeester, S.C. De Smedt, K. Braeckmans, Nano Today **8**, 121 (2013)
223. Y.-L. Wu, N. Putcha, K.W. Ng, D.L. Leong, C.T. Lim, S.C.J. Loo, X. Chen, Acc. Chem. Res. **46**, 782 (2013)
224. E. Navarro, F. Piccapietra, B. Wagner, F. Marconi, R. Kaegi, N. Odzak, L. Sigg, R. Behra, Environ. Sci. Technol. **42**, 8959 (2008)
225. J. Choi, S. Kim, J. Ahn, P. Youn, J. Kang, K. Park, J. Yi, D.-Y. Ryu, *Aquatic Toxicology*, 2009
226. Y. Wu, Q. Zhou, H. Li, W. Liu, T. Wang, G. Jiang, *Aquatic Toxicology*, 2009
227. Y.-W. Baek, Y.-J. An, Sci. Total Environ. **409**, 1603 (2011)
228. T. Vorup-Jenson, D. Peer, Adv. Drug Deliv. Rev. **64**, 1661 (2012)
229. A.A. Shemetov, Nabiev, I., Sukhanova, A., ACSNano **6**, 4585 (2012).
230. G. Federici, B.J. Shaw, R.D. Handy, Aquat. Toxicol. **84**, 415 (2007)
231. V. Aruoja, H.-C. Dubourguier, K. Kasemets, A. Kahru, Sci. Total Environ. **407**, 1461 (2009)
232. K.-U. Ulrich, A. Rossberg, H. Foerstendorf, H. Zänker, A.C. Scheinost, Geochim. Cosmochim. Acta **70**, 5469 (2006)
233. K.J. Wilkinson, J.R. Lead (eds.), *Environmental Colloids and Particles Behaviour, Separation and Characterisation* (Wiley, West Sussex, 2007)