Chapter 2
Advances in Bioremediation of Toxic Heavy Metals and Radionuclides in Contaminated Soil and Aquatic Systems

Evans M. Nkhalambayausi-Chirwa, Pulane Elsie Molokwane, Tshilidzi Bridget Lutsinge, Tony Ebuka Igboamalu, and Zainab S. Birungi

Abstract Metals are used in several products essential to humans. However, processes for extraction of the metals generate effluents containing chemical by-products many of which are toxic to living organisms and are disruptive to ecosystems. Processes used in the creation of useful products from the metals leave a legacy of pollution that may take generations to clear. Metals such as mercury, cadmium, lead, chromium, and uranium, and a range of metalloids such as arsenic and selenium, are widely known for their acute toxicity at high doses and carcinogenicity at low doses. Several technologies for treatment of land and water that have been contaminated with toxic heavy metals have been proposed. Other metallic elements, although possessing no significant chemical toxicity to organisms, occur as radioactive isotopes that impart oxidative stress on organisms leading to increased incidence of mutations and carcinomas in animal tissue. The main difficulty in the treatment of metals is that the metals cannot be degraded or mineralized as is the case with organic pollutants. Metallic elements can only be oxidized or reduced to forms that are less mobile and easier to extract from the environment. This chapter is compiled from information from projects in which metals were either oxidized or reduced to less mobile and less toxic states using pure or consortium cultures of bacteria followed by immobilization or extraction using physical or biological media. The uptake of metals for reuse was attempted using bioengineered molecular adsorbents on cell surfaces. The latter process was developed to facilitate selective uptake of different metallic species as a low energy biorefinery.

E. M. Nkhalambayausi-Chirwa (✉) · T. B. Lutsinge · T. E. Igboamalu · Z. S. Birungi
Water Utilization and Environmental Engineering Division, Department of Chemical Engineering, University of Pretoria, Pretoria, Gauteng, South Africa
e-mail: evans.chirwa@up.ac.za

P. E. Molokwane
Water Utilization and Environmental Engineering Division, Department of Chemical Engineering, University of Pretoria, Pretoria, Gauteng, South Africa

Oloenviron Consultancy, Johannesburg, South Africa

R. N. Bharagava, G. Saxena (eds.), *Bioremediation of Industrial Waste for Environmental Safety*, https://doi.org/10.1007/978-981-13-3426-9_2

Keywords Toxicity pathways · Bioremediation · Bioseparation · Toxic metals · Bioengineering · Enzyme kinetics

1 Background

Metals are used in industrial processes either as structural components such as steel or as additives for modification of strength properties, corrosion resistance, and control of chemical reactions and for ornamental purposes. Metals such as chromium (Cr) and radium (Rd) have been used in the making of paints and pigments, wood preservation, and luminescence in displays (Beszedits 1988). Addition of metals and carbon compounds is critical in the formation of stronger alloys that are less susceptible to corrosion. For example, metals such as chromium, silver, and tin have been widely used to give steel a polished silvery mirror coating or to galvanize it against corrosion. Ornamental use of Cr and Pb includes the production of emerald green (glass) and synthetic rubies in the case of Cr (Morrison and Murphy 2010) and giving glass a reddish tinge in the case of Pb (Francis and Croft 1849). The discovery and utilization of metals by humans is so important such that at least three of the human eras of civilization have been named after a metal, i.e., iron age, bronze age, and nuclear age. One evolutionary epoch, the Anthropocene age, is associated with the humans' ability to harness the power of the atom through fission of the uranium atom which is also loosely associated with our ability to impact the global climate (Wilke and Johnstone 2017).

The production of metals and utilization of dissolved metals typically produces contaminated wastewater (Goutam et al. 2018; Bharagava et al. 2017a; Gautam et al. 2017; Saxena and Bharagava 2015; Saxena et al. 2016). Metals can serve as oxidants or reductants in environmental systems resulting in damage to cells or inhibition of cellular processes (Jaishankar et al. 2014). At high concentrations, metals such as Cr, U, Pb, Hg, Se, and As can cause acute toxicity resulting in death of organisms (Chirwa and Molokwane 2011; Mtimunye and Chirwa 2013; Brink et al. 2017; Bansal 2010; Wessels 2017). At low concentrations, metals can cause mutagenicity, carcinogenicity in organisms, and teratogenicity in mammals (Flessel 1979). For this reason, all national and international environmental regulatory organizations place stringent regulations on the disposal of metals to the environment (Federal Register 2014; Hiz and Aki 2015; Gao et al. 2014; ACGIH 2004).

2 Mining of Ores and Environmental Impacts

During the mining operation for any particular metal, the target metal is extracted together with several metal pollutants from geological seams. For example, chromium is mined from seams containing trace amounts of platinum group metals and other base metals. In South Africa, the country with the largest reserve of chrome ore, the main seams include:

- Chromite: $(Mg,Fe^{2+})(Cr,Al,Fe^{3+})_2O_4$, the main component of Merensky Reef of the Bushveld Igneous Complex in South Africa (von Gruenewaldt and Hatton 1987; Wagner 1923)
- Barbertonite: $Mg_6Cr_2(CO_3)(OH)_{16}.4H_2O$, the major component of the Eastern Bushveld Igneous Complex (Meli 2009; Calas et al. 1984)
- Nichromite: $(Ni,CoFe^{2+})(Cr,Fe^{3+},Al,)_2O_4$, which was discovered first in the Bon Accord nickel deposit in Barberton District, South Africa (Tredoux et al. 1989)

In Brazil, the richest chrome ore seam is in the Campo Formoso layered intrusion which also contains chromite (Cramer et al. 2004; Boukili et al. 1984). Consequently, tailing dumps and process waste stockpiles at chrome mining and ferrochrome processing sites contain significant levels of other platinum group and rare earth metals (Chirasha and Shoko 2010; von Gruenewaldt and Hatton 1987). Chromium in the tailing dumps can exist either as Cr(III) or Cr(VI) depending on the environmental conditions within the dump. Interaction with other metals at different oxidation states can influence the stability of the oxidation state of the chromium species inside the waste dump and its ability to leach into the surrounding water bodies (Tiwary et al. 2005; Ma and Garbers-Craig 2006). In areas where the leachate water enters agricultural supply water, there is a high risk of contamination of food products and bioaccumulation into higher order organisms (Chirasha and Shoko 2010).

3 Metal Polluting Activities

Due to the wide range of uses of metals, pollution comes in many forms. The most important feature is whether a metal poses a particular threat and how such a threat could be best dealt with. In the following subsections, we classify pollutants based on whether they are toxic to organisms due to chemical poisoning or radiation poisoning. Chemical toxicity is best dealt with by neutralization using reducing or oxidizing agents, whereas radiation toxicity, on the other hand, is remediated by isolation of the radiation source and confinement for a period equivalent to the time required for the radiation to dissipate.

3.1 Chemically Toxic Elements

There are various metallic elements that confer chemical toxicity. Technically, any element at excessive loading can cause toxicity. For some metals, the levels at which they produce observable negative impacts are very low. For metals such as Cr(VI), Hg(II), Cd(II), and Pb(II), the concentrations at which they produce observable negative effects are quite low. Their high toxicity thus serves as an example and warrants special consideration for treatment in this chapter.

3.1.1 Chromium

Chromium has been used extensively in industrial processes such as leather tanning, electroplating, negative and film making, paint and pigment processing, and wood preservation (Beszedits 1988). Additionally, chromium has been used as a metallurgical additive in alloys (such as stainless steel) and metal ceramics. Chromium plating has been widely used to give steel a polished silvery mirror coating. The radiant metal is now used in metallurgy to impart corrosion resistance. Its ornamental uses include the production of emerald green (glass) and synthetic rubies. Due to its heat-resistant properties, chromium is included in brick molds and nuclear reactor vessels (Dakiky et al. 2002).

Through the above and many other industrial uses, a large amount of chromium (approximately 4500 kg/d) is discharged into the environment making it the most voluminous metallic pollutant on earth. Almost all chromium inputs to the natural systems originate from human activities. Only 0.001% is attributed to natural geologic processes (Merian 1984).

Chromium from the anthropogenic sources is discharged into the environment mainly as hexavalent chromium [Cr(VI)]. Cr(VI) – unlike Cr(III) – is a severe contaminant with high solubility and mobility in aquatic systems. Cr(VI) is a known carcinogen classified by the US EPA as a Group A human carcinogen based on its chronic and subchronic effects (Federal Register 2004). It is for this reason that most remediation efforts target the removal of Cr(VI) primarily.

3.1.2 Uranium

Uranium exists in the environment mainly as oxides, organic or inorganic complexes, and rarely as a free metal ion (Mtimunye and Chirwa 2013). Free elemental uranium primarily exists in higher oxidation states typically bound to oxygen. The oxygen bound uranium exists mainly as uraninite (UO_2), triuranium octaoxide also known as pitchblende (U_3O_8), and uranium trioxide (UO_3) (Stefaniak et al. 2009). U_3O_8 is relatively insoluble in water and relatively stable over a wide range of environmental conditions. UO_2 on the other hand is not as stable as U_3O_8 in the environment as it may undergo alteration under various environmental conditions (Senanayake et al. 2005). Upon exposure to air, UO_2 is subjected to oxidation and as a result produces a secondary mineral (UO_2^{2+}) which complexes easily with phosphates, carbonates, silicates, and sulfates (Senanayake et al. 2005; Stefaniak et al. 2009).

3.1.3 Selenium

There are many natural pathways in which selenium is released into the environment. The processes range from volcanic activity; wildfires; volatilization from soils, plants, and water bodies; and weathering of rocks (Sandy and DiSante 2010).

Selenium occurs naturally in soil, but at low concentrations, ranging between 0.1 and 2 μg/g soil, whereas in seleniferous soil the concentration can go up to 320–324 μg/g soil (Girling 1984). Of the selenium released into the environment, 37.5–40.6% can be ascribed to anthropogenic activities. It is released from the earth's crust by the mining of coal, by oil production, through the use of agricultural products, as well as during the melting of nonferrous metals (Lenz and Lens 2009). Landfill ash disposal generating toxic leachate poses a risk of groundwater contamination (Lemly 2004). According to different studies, acid mine drainage waters contain selenium at concentrations ranging between 2.0×10^{-4} and 6.2×10^{-3} mM (reported as total selenium) (Lenz et al. 2008). Wastewater from oil refineries in the San Francisco Bay (USA) contains relatively low concentrations of selenium of about 50–300 μg/L (Lawson and Macy 1995). The wastewater from a selenium refinery plant in Japan contained an average of 30 mg/L selenium, with the most of it present as selenite (Satoshi et al. 2012).

3.1.4 Mercury

Mercury is released to the environment from activities such as alkali and metal processing, incineration of coal, and medical waste and other wastes. In the Southern hemisphere, a lot of mercury is released into the environment as effluents from the mineral purification processes. Mercury is used extensively in the separation of gold from other metallic impurities. The process involves extraction of gold in liquid mercury and boiling away the mercury to leave behind the pure gold. Fumes produced result in atmospheric mercury pollution. Once in the atmosphere, mercury is widely disseminated and can circulate for years. Other sources of atmospheric mercury include natural sources such as volcanoes, geologic deposits, and volatilization from the ocean. Although all rocks, sediments, water, and soils naturally contain small but varying amounts of mercury, scientists have found some local mineral occurrences and thermal springs that are naturally high in mercury. In many relatively pristine areas, however, mercury concentrations have actually increased because atmospheric deposition has increased. For instance, concentrations of mercury in feathers of fish-eating seabirds from the northeastern Atlantic Ocean have steadily increased for more than a century. In North American sediment cores, sediments deposited since industrialization have mercury concentrations about 3–5 times those found in older sediments. Some sites may have become methylmercury hot spots inadvertently through human activities (Gilmour and Henry 1991). Lake acidification, addition of substances like sulfur that stimulate methylation, and mobilization of mercury in soils in newly flooded reservoirs or constructed wetlands have been shown to increase the likelihood that mercury will become a problem in fish. Although scientists from USGS and elsewhere are beginning to unravel the complex interactions between mercury and the environment, a lack of information on the sources, behavior, and effects of mercury in the environment has impeded identification of effective management responses to the nation's growing mercury problem (USGS 1995).

3.1.5 Lead

Natural lead occurrence is not common on earth surface. Anthropogenic lead is introduced through the use of products containing lead additives such as paints, gasoline, and metal works (Gioia et al. 2006; Boyle et al. 2014). Metallic lead and lead alloys are extensively used for storage battery plates, sheathing for electrical cables, small-caliber ammunition, shielding for X-ray apparatus and atomic reactors, type metal, bearing metal, and solder. Lead is also released as a waste product from extraction of metals such as silver, platinum, and iron from their respective ores. In car engines, the burning of fuel in the presence of other impurities (chlorines, bromines, oxides) produces lead salts (Wang et al. 2010). The lead salts enter the environment through the exhausts of the cars in particulate form. Lead is also released from combustion of coal during electric power generation in coal-powered plants. Typical concentrations of lead released from coal-powered plants have been reported at 7–110 mg/kg with release rates up to 11,200 tons lead/year (UNEP 2010).

The increased non-localized outputs of lead to the environment have generated a lot of concern mainly due to its toxicity to living organisms. Lead contamination of water and land is known to produce sub-chronic effects at low concentrations (Supanopas et al. 2005), acute effects at moderately high concentrations (Papp et al. 2006), and catastrophic changes to ecosystems at very high concentrations (Eisler 1988). Lead concentration above 1000 ppm can wipe out almost the entire ecosystem, whereas, concentrations lower than 10 mg/L can result in endocrine disrupting activity in sensitive species. In many areas around the United States, accumulation of lead in aquatic systems has risen to levels detrimental to human inhabitants (Renner 2010).

3.2 Radioactive Elements

In high temperature gas-cooled reactors (HTGR), also known as fast reactors, graphite is utilized as the moderator of the nuclear reaction. The graphite is either used as part of the structural materials for the reactor core vessel or as fuel containment elements in the form of pebbles. The graphite used from natural sources contains non-carbon impurities within the carbon matrix. Among these impurities are oxygen and nitrogen from entrapped air, cobalt, chromium, calcium, iron, and sulfur (Khripunov et al. 2006).

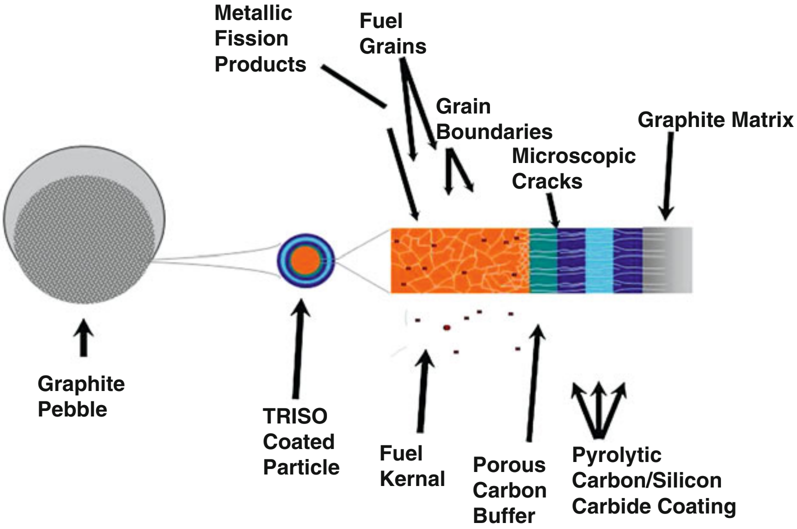

Fig. 2.1 Propagation of fission products and impurities in a graphite-regulated high temperature (gas)-cooled reactor fuel element

The radioactive fission products are created within the fuel grains and migrate through grain boundaries and then through microscopic cracks in the graphic matrix (Fig. 2.1). Most of the fission products are entrained in the matrix – a small proportion escapes through the outer layers into the gas phase.

Reprocessing of irradiated nuclear graphite entails the separation of the metallic radionuclides from the graphite matrix and reducing the amount of C-14. Impurities in the fuel itself include (1) metallic fission products (Mo, Tc, Ru, Rh, and Pd) which occur in the grain boundaries as immiscible micron- to nanometer-sized metallic precipitates (ε-particles); (2) fission products that occur as oxide precipitates of Rb, Cs, Ba, and Zr; and (3) fission products that form solid solutions with the UO_2 fuel matrix, such as Sr, Zr, Nb, and the rare earth elements (Buck et al. 2004; Bruno and Ewing 2006).

3.2.1 Uranium-238 and Transuranic Elements

Uranium is the heaviest naturally occurring element in the solar system. All other elements with higher molecular weight than uranium are referred to as transuranic (TU) elements and are synthetically produced. Uranium and all transuranic elements are highly fissionable, that means, they can be split into smaller elements releasing neutrons and large amounts of energy in the process. Among the milliard of fission products, there are a few common species that are persistent in nature due to their relatively long half-lives such as strontium-90 (Sr-90), cesium-120 (Cs-120), and radiocarbon-14 (C-14).

Uranium used in electricity generation in nuclear reactors is generally sourced from ores with uranium oxide concentrations up to 10%. However, lower grade ores with uranium oxide concentration of 0.2% or less are the most common and the most mined (Ross 2015; Thomas 1981). The uranium concentrate used in nuclear reactors is typically at 75–95% uranium oxide (U_3O_8). Mining of such readily available lower-grade uranium ore to achieve about 75–95% U_3O_8 concentrate used in nuclear power plants results in the release of large amounts of waste rock tailings with significant environmental consequences due to the presence of residual uranium in the rocks (Thomas 1981).

Uranium is sourced from rich ores with concentrations up to 10%. However, ores with uranium oxide concentration as low as 0.2% are also mined and are the most common (Sovacool 2008; Tudiver 2009). Uranium producers have been able to utilize ores with uranium oxide concentration as low as 0.0004%. Uranium is recovered from ore by comminution of the rocks followed by leaching using alternative solutions of acid and/or alkaline chemicals. The end product from ore milling and leaching results into a bright yellow powder called yellow cake (U_3O_8) which is about 75–90% uranium oxide (Sovacool 2008). Before this uranium oxide concentrate can be used in a reactor for generating electricity, it must first be converted into uranium hexafluoride (UF_6), which is used in a gaseous diffusion enrichment process. During the uranium enrichment process, U-235 concentration is increased to least 3.5% for atypical commercial light-water reactor and up to 4–5% for other modern reactors, while at the same time, the U-238 isotope is decreased notably. Suffice to say, U-235 is the only natural occurring isotope that can sustain a fission chain reaction by capturing neutrons and splitting into two parts yielding large amount of energy (Soudek et al. 2006; WNA 2008). On average, the specific radioactivity of natural uranium is 25 kBq/g, double that of U-238. During its decay process, uranium may generate 0.1 watts/tonne which is enough to warm the earth's mantle (WNA 2008).

After the enrichment process, about 85% of oxide comes out as waste in the form of depleted UF_6, and the remaining 15% emerges as enriched uranium and is converted into ceramic pellets of UO_2. Fresh UO_2 which contains up to 5% of U-235 is then packaged in zirconium alloy tubes and bundled together to form fuel rod assembles for reactors. Thereafter, the used reactor fuel which contains up to 95% U-238, 3% fission products and transuranic isotopes, 1% plutonium, and 1% U-235 is removed and stored to be reprocessed prior to disposal (Soudek et al. 2006; WNA 2008). During the reprocessing stage, uranium (U-235) and plutonium (Pu-239) are separated from the spent fuel using the PUREX method and then reused as mixed-oxide (MOX) fuel in the reactor. This process is referred to as the closed fuel cycle (Fig. 2.2). The majority of radioactive organic waste is produced in the enrichment and reprocessing operations. All values in the tables are reported in cubic meters per Gigawatt electricity-year (m^3/GWe-year).

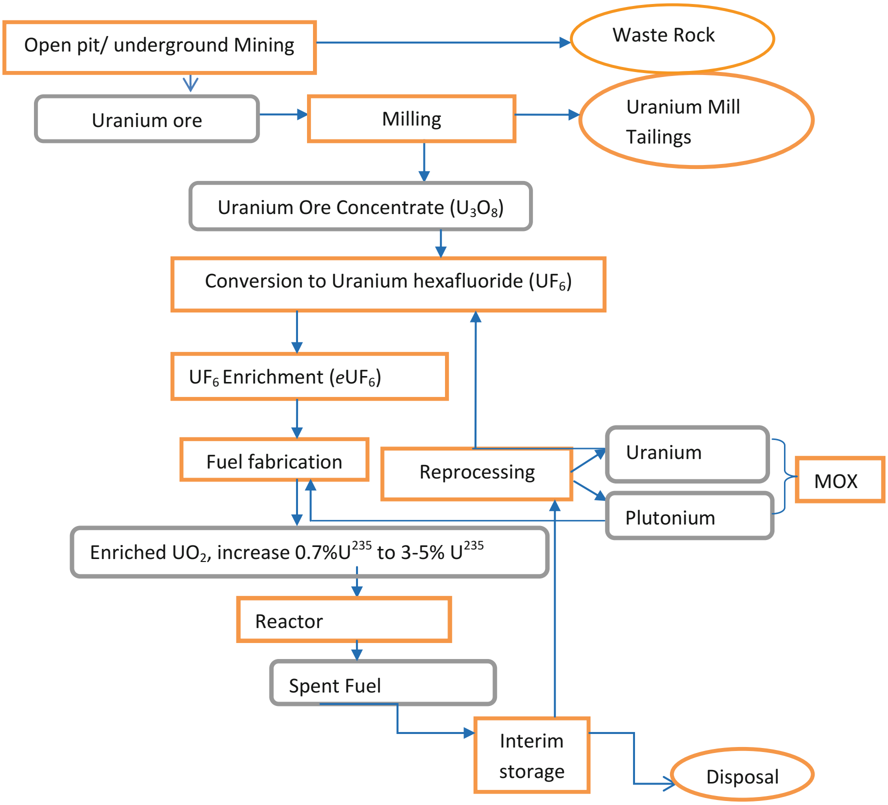

Fig. 2.2 Nuclear fuel closed cycle

3.2.2 Sr-90and Cs-120

The radioactive isotope of strontium (Sr-90) is of great concern, and its toxic effects on human beings are well documented (Chen 1997; Kossman and Weiss 2000; Noshchenko et al. 2001; Greve et al. 2007). Strontium can be highly mobile in both soil and groundwater systems, and it has a half-life of 28 years. Due to its chemical similarity with calcium, it is easily incorporated into bone material in mammals. When incorporated in the organisms in this manner, it continues to irradiate localized tissues with the eventual development of bone sarcoma and leukemia (Chen 1997). The main disadvantages of using conventional adsorbents, such as zeolites and synthetic organic ion exchangers, for strontium removal from radioactive waste is their unsuitability at high pH, high sodium concentrations, and in irradiated environments (Chaalal and Islam 2001). However, the main disadvantages with the above methods is their unsuitability at high pH, high sodium concentrations, and in irradiated environments.

Table 2.1 Carbon-14 production mechanisms and thermal cross sections

Target isotope	Mechanism	Thermal cross section (barns)	Isotopic abundance (%)*
^{14}N	$^{14}N(n, p)^{14}C$	1.81	99.6349
^{12}C	$^{12}C(n, \gamma)^{14}C$	n/k	n/k
^{13}C	$^{13}C(n, \gamma)^{14}C$	0.0009	1.103
^{17}O	$^{17}O(n, \alpha)^{14}C$	0.235	0.0383

Adapted from International Union of Pure and Applied Chemistry
n/k Not known

3.2.3 Radiocarbon-14 and Irradiated Organic Pollutants

In nuclear fuel processing, the problem is the generation of large volumes of the partially water-soluble wastes that often contain toxic organic compounds. The use of decontamination reagents such as carbon tetrachloride (CCl_4) together with phenolic tar results in wastewater with high content of chlorophenols (Makgato and Chirwa 2015). Chlorophenols are compounds of serious environmental concern due to their toxic and carcinogenic impact on living organisms (Olaniran and Igbinosa 2011). Operation of reactors at high neutron influx can result in the transmutation of C-12 to C-14, a common problem in the graphite-moderated generation-IV nuclear reactors. Additionally, upon exposure to high neutron flux, most of the impregnated nonmetallic impurities are expected to transmute to unstable radioactive forms. For example, experimental exposure of graphite in nuclear reactors has shown that the stable forms of oxygen, nitrogen, and C-12 are converted to radiocarbon-14 (C-14) as shown in Table 2.1. This table shows the available natural process that lead to production of C-14. Although not a heavy metal, the removal of C-14 can be managed together with the remediation of other radioactive metallic impurities (Molokwane and Chirwa 2007).

4 Conventional Treatment of Toxic Metals

Dissolved metallic species are commonly treated by chemical conversion to precipitable chemical species and followed by extraction at pH ranges where the metal exists as a solid precipitate. For example, a metal such as Cr(VI) could be reduced to Cr(III) at a relatively low pH through the following reduction-oxidation (redox) reaction:

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O + 1.33v\left(E^0\right) \tag{2.1}$$

(Garrel and Christ 1965), followed by precipitation as chromium hydroxide ($Cr(OH)_3(s)$) at a higher pH. Because of the difference in electric potential between the two states, substantial amounts of energy are needed to overcome the activation energy for the reduction process to occur. It is therefore assumed that spontaneous reduction of Cr(VI) to Cr(III) never occurs in natural aquatic systems at ambient pH and temperature. Common reducing agents such as iron sulfide (FeS) and pyrite (FeS_2) have been used to precipitate Cr economically since both are naturally occurring reductants. The resultant reactions using FeS and FeS_2 are shown below:

$$\mathrm{Cr(VI)(aq)} + \mathrm{3Fe(II)(aq)} \rightarrow \mathrm{Cr(III)(aq)} + \mathrm{3Fe(III)(aq)} \tag{2.2}$$

$$\mathrm{xCr(III)} + (1-\mathrm{x})\mathrm{Fe(III)} + 3\mathrm{H_2O} \rightarrow \left(\mathrm{Cr_xFe^{1-x}}\right)\mathrm{(OH)_3(s)} + 3\mathrm{H^+} \tag{2.3}$$

The shortcoming of chemical processes for the treatment of metals is that the process is environmentally intrusive due to the production of large volumes of toxic sludge which is difficult to dispose. Additionally, the treatment becomes increasingly expensive as the remaining concentration of the pollutant becomes smaller.

5 New Treatment Approaches

In order to overcome the challenges posed by conventional methods of treatment of toxic metals, biological processes have been proposed (Shen and Wang 1993; Chirwa and Wang 1997, 2001; Igboamalu and Chirwa 2017). Biological treatment is based on the principle of emulating the natural occurring processes to treat waste. During 3 billion years of existence, microorganisms have evolved mechanisms to survive in hostile environments and to adapt to changes in the environment (Bush 2003). Environmental engineers around the world have undertaken to find ways to tap into the mysteries of nature by diligently studying the action of microorganisms as they adapt to extreme conditions.

One of the most conserved mechanisms in the living cell is the biochemical pathway for electron transport through the cytoplasmic membrane to conserve energy through the oxidation of an electron donor and reduction of an electron acceptor such as oxygen. This process has been conserved over billions of years, such that, to this day, all life on earth depends on variants of this pathway (Bush 2003; Thomas et al. 1985; Nealson 1999; Kalckar 1974). Most biochemical processes for degradation and/or detoxification of compounds in are linked to the above process.

Lately, microorganisms have been isolated that are capable of reducing the toxic forms of heavy metal and transitional metal elements to less mobile precipitable forms (Foulkes et al. 2016). Other researchers have found microbial cultures with the capability to resist high radiation doses (Battista 1997; White et al. 1999).

6 Bioremediation Processes for Removal of Toxic Metals

Bioremediation is the use of microbes and plants to degrade/detoxify the organic and inorganic pollutants in contaminated matrix (Bharagava et al. 2017b, c; Saxena and Bharagava 2017; Chandra et al. 2015). As indicated above, the utilization of biological systems to remove toxic metals from water, soil, and environmental systems has been investigated extensively since the late 1980s. Metals can be removed through reduction to precipitable species and oxidation to precipitable species or by biosorption taking advantage of the ion-exchanging properties of cell surfaces of bacteria, fungi, and/or algae. The following are examples of processes that have been studied extensively by our research group at the University of Pretoria and our collaborators overseas.

6.1 Biological Reduction, Separation, and Recovery

Any highly oxidized metallic element such as Cr(VI), U(VI), Tc(VII), and Se(VI) can be reduced to a lower oxidation state utilizing the microbial cell's NADH-dehydrogenase (NADH+-dh) (Fig. 2.3). $NADH^+$ is readily oxidized to NAD, thereby donating two electrons to the membrane electron-transporting proteins such as $NADH^+$-dh, ubiquinone, and cytochrome$c$-c_3, which in turn channels electron to the target metallic species directly or through an enzyme typically known as a M^{n+} reductase where M^{n+} is the target metal of the valency state n (Cervantes et al. 2001; Barak et al. 2006). For hexa- and heptavalent metals, it was demonstrated that living cells of metal-reducing organisms can reduce the metals, either as a necessity to detoxify the cell's immediate environment (Cervantes 1991) or as a source of energy for cell growth and maintenance (Horitsu et al. 1987). Energy for M^{n+} reduction is

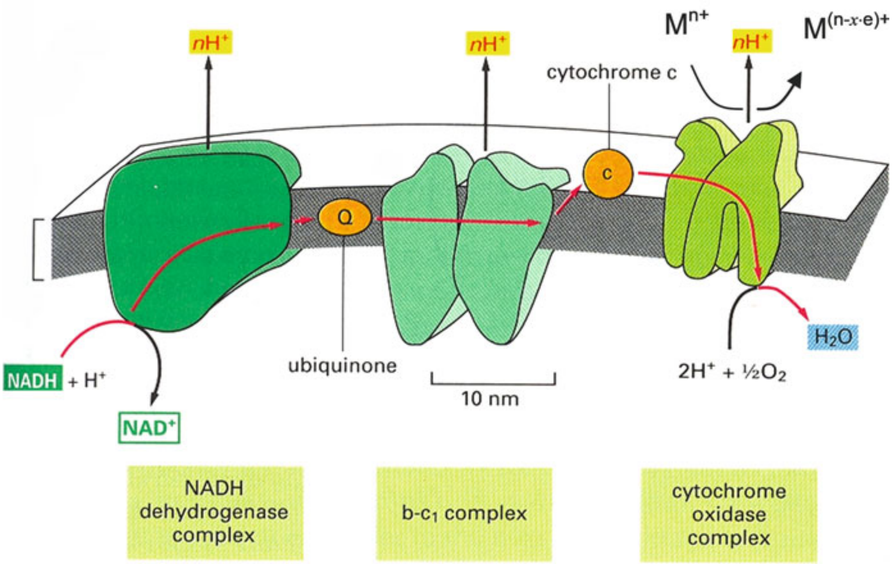

Fig. 2.3 Electron flow pathway resulting in reduction of a metal species. The n-valent metal is reduced to an (n-x·e) valent species by receiving x·e electrons from electron donors in the system

derived from the oxidation of $NADH^+$ to release electrons to the cytochrome c,c_3 system which functions as a conduit for electron flow to M^{n+} reductase (Lovley and Philips 1994; Chirwa and Wang 1997; Mtimunye and Chirwa 2014).

The results obtained from the U(VI) reduction (Mtimunye and Chirwa 2014), Cr (VI) reduction (Chirwa and Molokwane 2011), and reduction of other metallic species (Cervantes and Silver 1992; Lloyd et al. 1999; Yong et al. 2002) showed that the reduction pathway for most metals is non-specific and is closely related to the sulfate reduction transmembrane electron shuttle.

6.2 Biological Oxidation

The detoxification of arsenic by oxidation of As(III) to As(V) is used here as an example of the bioremediation through an oxidation reaction process. In this example, As(III) served as an inorganic electron donor for beneficial oxidation to less toxic and immobile As(V). Thermodynamically, the conversion of As(III) to As (V) is an exothermic reaction and could generate considerable amount of energy ranging from – (254 to 468) KJ/mol for cell growth and metabolism (Dastidar and Wang 2010; Wang et al. 2013).

The first heterotrophic As(III) oxidation was observed in a cow dip in South Africa in 1918 (Green 1918), whereas an autotrophic As(III) oxidation was observed in 1981 (Ilialetdinov and Abdrashitova 1981). Heterotrophic As (III) oxidation may represent a detoxification reaction on the cell's cytoplasmic (inner) membrane, whereas autotrophic As(III) oxidation releases energy that is used for CO_2 fixation and cell growth under both aerobic and anaerobic conditions (Santini et al. 2000). In 2010, Dastidar and Wang reported that about 256 KJ/mol energy can be generated during oxidation of As(III) to As(V) by a purified culture of *Thiomonas arsenivorans* strain b6 (Dastidar and Wang 2010). Further studies showed that about 467.95 KJ of energy could be generated in the process (Wang et al. 2013). The redox conversion of As(III) in aqueous environment was represented by the following equation:

$$As^{3+} \rightarrow As^{5+} + 2e^{-} + \text{energy} - (254\text{ to }468)\text{KJ/mol}(3)$$

The oxidation of As(III) could operate in competition with the oxidation of the other organic and inorganic electron donors as energy sources. For example, nitrate ($NO_3{}^-$) and chlorate ($ClO_3{}^-$) in solution can act as competitive electron donors to As(III) (Sun et al. 2010). In the recent study, the use of As(III) as an electron donor for reduction of Cr(VI) was demonstrated using a consortium of As(III) oxidizing species isolated from a cow dip in Tzaneen, Limpopo Province (South Africa) (Fig. 2.4) (Igboamalu and Chirwa 2017). The predominance of As(III) as an electron donor for metabolic processes over other electron donors in the system has not been investigated.

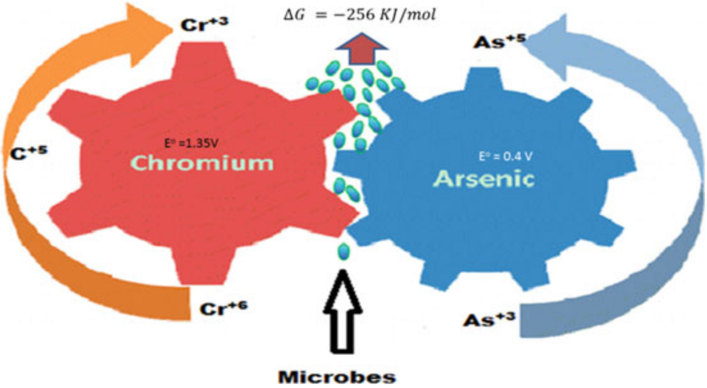

Fig. 2.4 Conceptual representation of biologically catalyzed Cr(VI) reduction with A(III) as an electron donor

6.3 Methylation

Partitioning of metallic species from the aquatic phase can be achieved through biologically mediated methylation followed by volatilization. Detoxification by methylation has been observed for mercury and selenium. For selenium, it is firstly necessary to reduce all forms of selenium to elemental selenium (Se(0)) in order to achieve methylation and volatilization. Methylation is identified as a critical step in the transport of selenium out of terrestrial and aquatic environments contaminated with selenium. Methylation occurs in water, sediment, and soil and is mostly a biotic process. A wide range of organisms from bacterial, algal, and fungal groups are capable of catalyzing the methylation process of selenium. Examples of the compounds that are formed from the methylation of selenium include dimethylselenide (DMSe), dimethyldiselenide (DMDSe), dimethylselenone, trimethylselenonium, selenous acid, selenium dioxide, and selenic acid, to name a few.

6.4 Biosorption

Traditional treatment technologies such as membrane filtration, GAC adsorption, precipitation-sedimentation, flotation, ion-exchange, and electrochemical deposition systems have been used for decades for the removal of toxic heavy metals from water (Veglio et al. 2003; Wang et al. 2004; Aziz et al. 2008). Unfortunately, these technologies are associated with high cost for processing of metal concentrations, lack of specificity, production of large volumes of sludge, and low performance at low metal concentrations (Kotrba 2011; Gaur et al. 2014). Adsorption processes are proving to be the most efficient for advanced wastewater treatment (Anastopoulos et al. 2015). Adsorption is a more advanced reaction-mediated process at the surface of a biosorbent such as granular-activated carbon (GAC), natural clay materials such as kaolinite and zeolite, biomass, and other natural and artificial materials (Borda and Sparks 2008; Gadd 2009; Gaur et al. 2014).

6.4.1 Conventional Adsorbents

Recent adsorption technologies which act as possible alternatives and/or complementary to conventional technologies include granular-activated carbon (GAC), nanotechnology, and biological adsorbent systems. GAC is the most commonly used adsorbent due to its large surface area and high adsorption capacity attaining high metal removal efficiency. However, the cost of production and regeneration of spent carbon is still high and has limited its use in wastewater treatment (Babel and Kurniawan 2004; Fu and Wang 2011). Nanotechnology offers great promise in treatment of wastewater containing inorganic pollutants but faces some challenges of cost-effectiveness and potential environmental and human risk (Qu et al. 2013). The current cost of most nanomaterials is significantly high and owing to the small size of these particles may facilitate transport of toxic materials in the environment causing harm to cellular materials. Biological adsorbent systems use materials of biological origin either dead or live for the treatment of wastewater containing heavy metals. The technology can be categorized under biosorption and bioaccumulation.

6.4.2 Novel Biosorption of Metallic Species

The first major challenge in the biosorption field is to screen and select the most promising biomass with high binding capacity for metals and possibility of reuse (Kratochvil and Volesky 1998). A broad range of biomass types have been tested including fungi, bacteria, yeast, and agricultural wastes such as cane molasses (Abdel-Rahman et al. 2016), maize stalks (Haryanto et al. 2017), wood chips, grass, and maize tassels (Guyo et al. 2015). The sorption of metals onto these biomaterials is attributed to the constituents of the cell wall which are mainly composed of carbohydrates, proteins, and phenolic compounds (Choi and Yun 2006).

The biosorption isotherms are used for basic evaluation of sorption systems under optimal environmental conditions. Any comparison done at two different sorption systems can only be done at the same initial concentration for screening of sorbents with highest sorption capacity. These experiments are usually carried in batch reactors as an initial step before application into the dynamic continuous systems (Gadd 2009). Equilibrium isotherm models are classified into empirical and mechanistic equations. In the mechanistic models, mechanisms for biosorption are explained and can predict the experimental behavior (Pagnanelli et al. 2001; Volesky 2007).

6.4.3 Algae as Biosorbents

In our latest studies, we evaluated the performance of several species of algae in the bioflocculation and biosorption of toxic metals and rare earth metals (REMs). Empirical models were used to evaluate the biosorption kinetics and biosorption capacity of wet and dry cells. The Langmuir and Freundlich are the most widely used and accepted simplistic mathematical models in literature as they usually fit the experimental done relatively well (Volesky and Holan 1995; Arıca et al. 2001; Mehta and Gaur 2005). These isotherm models reflect metal binding as a function of equilibrium concentration of a known metal in solution. A Langmuir model assumes monolayer adsorption solutes onto surface containing a finite number of identical sites with homogenous distribution of adsorption energy and no transmigration of adsorbed ions. Freundlich model assumes the energy distribution is heterogeneous with binding sites having a higher affinity for the metal first occupied (Selatnia et al. 2004; Goksungur et al. 2005; Stumm and Morgan 1995).

Other common isotherm models used include Temkin model whose consideration is given to the interaction between adsorbate and adsorbent; Dubinin-Radushkevich (D-R) model which expresses the adsorption mechanism with a Gaussian energy distribution on a heterogeneous surface, Table 2.2 (Gadd 2009; Pagnanelli 2011).

Adsorption capacity and affinity of the different algal species were evaluated for removal/recovery of lanthanum (La), thallium (Tl), and cadmium (Cd). The Langmuir model performed better than the Freundlich model with a correlation coefficient of ≥ 0.90 (Table 2.2). The results indicated that *Chloroidium saccharophilum* had the highest potential for metal removal of La, Cd, and Tl with a sorption capacity ($q_{\max}$) of 129.81, 1000, and 128.21 mg/g, respectively (Table 2.3). Tl adsorption capacity was significantly higher than other metals with a $q_{\max}$ in the range of 833 and 1000 mg/g. *Desmodesmus multivariabilis* showed a relatively higher affinity for La and Cd adsorption at 4.55 and 1.49 L/g, respectively. The algal species with both a higher $q_{\max}$ and higher affinity were considered as they indicate possible removal and recovery of metals (Birungi and Chirwa 2014, 2015).

6.5 *Electrokinetic Mobilization*

The first electrokinetic phenomenon was observed at the beginning of the nineteenth century when Reuss in 1808 applied a direct current to a clay water. However,

Table 2.2 Most common equilibrium models for single metallic studies

Isotherm type	Linearized equation
Langmuir	${}^{C_e}/_{q_e} = {}^{C_e}/_{q_{\max}} + {}^{1}/_{\mathrm{bq}_{\max}}$
Freundlich	$\log q_e = \log k + {}^{1}/_{n} \log C_e$
Dubinin-Radushkevich (D-R)	$\mathrm{In} q_e = \mathrm{In}\, X_m + \beta\varepsilon^2$
Temkin	$q = B\,\mathrm{In}\, A_T + B\,\mathrm{In}\, C_e$

Table 2.3 Langmuir parameters for La, Tl, and Cd adsorption using microalgae

	La adsorption		Thallium adsorption		Cd adsorption	
Adsorbent	$q_{\max}$ (mg/g)	b (L/g)	$q_{\max}$ (mg/g)	b (L/g)	$q_{\max}$ (mg/g)	b (L/g)
Desmodesmus multivariabilis	100	4.55	909.1	0.524	35.57	1.49
Chloroidium saccharophilum	129.87	0.142	1000	1.667	128.21	0.016
Scenedesmus acuminutus	111.1	0.12	833.3	0.290	–	
Stichococcus bacillaris	51.02	4.56	833.3	0.293	–	0.049

experimenting with the electroosmotic velocity of fluid and the zeta potential under an imposed electric gradient was first proposed by the two scientists, namely, Helmholtz and Smoluchowski [cica. 1879], as quoted in Walls (2010). The technology was demonstrated to be successful in removing large quantities of heavy metals from the soil by electric fields in laboratory studies. The technology involves the application of low-voltage direct current through electrodes that are placed across a section of contaminated ground, and the charge moves the contaminant. The principle of the technology is an electric current is used to mobilize ions. In order for electrokinetic remediation to be carried out, the pore fluid should be present as it has the following functions: conducting the electrical field, transporting species that are injected, and controlling and modifying the electrode reactions.

Ions are transported by electromigration, electroosmosis, electrophoresis, and other modes of transport like diffusion. Electromigration of ionic species is defined as the movement of ions in the pore fluid of the soil under the influence of an electric current; cations move toward the cathode and anions move toward the anode. Cationic and anionic contaminants are both removed by electromigration. Compared to electroosmosis in terms of cation contaminant migration, electromigration has been reported to have greater charge of ionic species present, field strength, and ionic concentration which influences electromigration during electrokinetics (Page and Page 2002). The movement is described by the following equation:

$$u_m = vE \tag{2.4}$$

where u_m is the velocity of an ion and v is ionic mobility (Page and Page 2002). Factors such as concentration, ionic charge, and temperature affect the electrical conductivity of the solution, and in turn this is related to the ionic mobility.

The second transport mechanism is electroosmosis which is the movement of pore water under an electrical potential difference from the anode to the cathode. This process is affected by the soil porosity and the zeta potential of the soil medium. It occurs due to the drag interaction between the bulk of the liquid in the pore and a thin layer of charged fluid next to the pore wall. The ions move under the action of the electric field in a direction parallel (Probstein and Hicks 1993; Reddy and Parupudi 1997). The electroosmotic flow rate depends on the balance between the electrical force on the liquid and the surface of the soil particles (Page and Page 2002). Electroosmosis has been found to be effective in removing cation at low concentrations. The rate of electroosmotic flow in the soil is described by Darcy's law for hydraulic flow (Page and Page 2002):

$$q_A = -k_e E A \tag{2.5}$$

where k_e is the coefficient of electroosmotic permeability (or conductivity), E is the electric field strength or negative potential gradient, and A is the total cross-sectional area normal to the flow direction.

Electrophoresis refers to the transport of charged particles under the influence of an electric current, these charged particles colloid in soil-liquid mixture, and it is an important mechanism in remediation of sludge. The drawback of this mechanism is that in compact systems like clay soil, movement of contaminants is restrained. However electrophoresis is important in remediating colloids that have contaminants adsorbed to them (Pamukcu and Wittle 1992; Reddy and Parupudi 1997).

Other mechanisms that are involved in electrokinetics are diffusion which plays a significant role in cationic and anionic contaminant transport, advection which moves soil moisture or groundwater due to hydraulic forces, and finally convection which is responsible for the movement of soil moisture or groundwater due to buoyancy forces. Trivalent chromium migrates toward the cathode due to electromigration, and CrO_4^{2-} and Cl^- migrate toward the anode due to electromigration, and the negative-charged colloids move due to electrophoresis.

The electrolysis reaction at the electrodes generates hydrogen ions (H^+) and oxygen gas at the anode and hydroxyl ions (OH^-) and hydrogen gas at the cathode. The oxygen gas produced at the anode and the hydrogen gas produced at the cathode escape out of the soil. The hydrogen ions in the anode attempt to migrate through the soil toward the cathode, whereas the hydroxyl ions in the cathode attempt to migrate through the soil toward the anode. The degree at which the H^+ and OH^- ions migrate depends on the buffering capacity of the soil. An acid front is produced at the anode, and at the cathode a base front is produced, and these two fronts move toward opposite directions. Page and Page (2002) have found that the acid front moves faster than the base front due to the fact that the mobility of H^+ exceeds that of OH^-, and electroosmotic flow is generally toward the cathode.

During electrokinetics the pH of the soil becomes acidic with the reading at the anode dropping to around 2 and the pH at the cathode increases to above 10. The rate of acid and base production depends on the current density (Castillo et al. 2012). Precipitated hydroxides occur at the point where the pH change occurs as the solubility of metal ions is at a minimum. In order to enhance the electrodes, reduce the pH at the cathode, and increase the pH at the anode, an alkaline solution needs to be added at the anodic compartment, and an acid solution needs to be added to the cathode compartment.

According to Acar et al. (1995), the application of electric current has the following effects:

1. It produces an acid in the anode compartment that is transported across the soil and desorbs contaminants from the surface of soil particles.
2. It initiates electromigration of species available in the pore fluid and those introduced at the electrodes.
3. It establishes an electric potential difference which may lead to electroosmosis-generated flushing of different species.

In order to remove the contaminants from the soil by electrokinetics, the contaminants should exist in pore water in dissolved ionic form so that they are transported to either the cathode or anode. In a study by Reddy and Chinthamreddy (2003), they found that in order to improve the performance of electrokinetics, the following can be done: changing the operating conditions such as switching the electrodes, prolonging the processing time, and increasing electric gradient or by controlling the reservoir fluid pH.

Trivalent chromium behaves differently under electrokinetics in different soil types. In a study conducted by Reddy and Chinthamreddy (1999), they found that during the electrokinetics of both Cr(III) and Cr(VI), the pH near the anode decreased to a value between 2 and 3, and at the cathode the pH increased between 11 and 12; due to the high pH at the cathode, a precipitate of chromium hydroxide was formed that clogged the pore space of the cathodic base front. The limitation of this process is the near-anode focusing effect which results in the formation of a precipitate layer block around the anode resulting in the reduction of efficiency with time (Shen et al. 2007; Li et al. 2011).

The two most common occurring valence states of chromium trivalent exist in the form of cationic hydroxides such as $Cr(OH)_3$ which will migrate toward the cathode during electrokinetic remediation (Fig. 2.5). However, chromium(VI) exists as CrO_4^{2-} at high pH and as $HCrO_4^-$ at low pH and other forms of oxyanions such as CrO_4^{2-} which migrate toward the anode; however it is adsorbed by the soil in the low pH regions, and it stops the complete removal of Cr (VI) from the soil. Electrokinetics is highly dependent on the acidic condition which favors the re-solubilization of heavy metal precipitated contaminants into the solution phase which makes it easier to transport; this can be done by acidification.

The problem encountered during electrokinetic mobilization and recovery of metallic species in soil is mainly due to accumulation of precipitates and increase

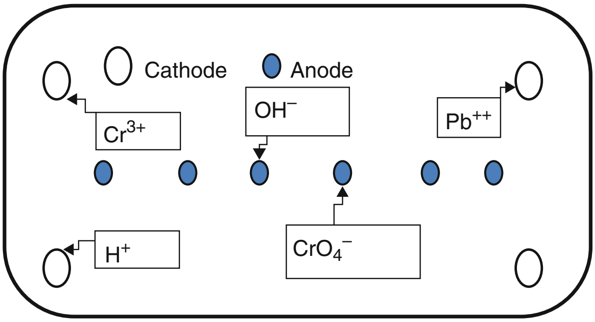

Fig. 2.5 Movement of ionic species under an electrokinetic gradient

in pH at the negatively charged electrode zone (anode zone) of the field. The accumulation of divalent metal precipitates reduces the permeability of the soil around the anode, thereby increasing the electric potential required to continue the mobilization and recovery process to the point where further increase of potential is not economically feasible. The inhibition of transport of dissolved charged species due to precipitate accumulation at the anode is called the "near-anode focusing" (Li et al. 2015). Strategies such as pulse charge (Reddy and Karri 2006), charge reversal (Hunter and James 1992), and acid enhancing (Maturi and Reddy 2008) have been applied in certain scenarios in the past to increase the recovery times by decreasing or all together eliminating the near-anode focusing effect.

6.6 Biomineralization and Biocrystallization

The process of biomineralization and biocrystallization attempts to reduce the metallic species to the zero-valent state and recover the metal in its pure form. The metal can be recovered by physical means such as scrapping or used as an in situ conductor collecting electrons (Yong et al. 2002; Foulkes et al. 2016; Lloyd et al. 2008). This biotechnological innovation has shown potential in the recovery of precious metals (Mabbett et al. 2006) or in the assembly of biosynthetic electrodes in microbial fuel cells (Yong et al. 2002). In the study by Yong et al. (2002), the researchers managed for the first to demonstrate the biological reduction of palladium from palladium (II) to palladium (0) which resulted in the production of the metallic form – Pd(0) – which was detected on cell surfaces using XRD and EDX methods. The crystalline nature of the precipitate was confirmed by SEM-XRD scans where the predominant element on the cell wall deposits was shown to be palladium (0).

The process of biocrystallization will definitely be of economic importance in the field of bio-purification and biorecovery of several beneficial products from the energy conservation processes.

7 Environmental Application Strategies

As indicated above, the utilization of biological systems to remove toxic metals from water, soil, and environmental systems has been investigated extensively since the late 1980s. Metals can be removed through reduction to precipitable species and oxidation to precipitable species or by biosorption taking advantage of the ion-exchanging properties of cell surfaces of bacteria, fungi, and/or algae. The following are examples of processes that have been studied extensively by the research group at the University of Pretoria and our collaborators overseas:

7.1 In Situ Bioremediation

In situ biological permeable reactive barriers (BPRBs) have been used mainly for removal of toxic organic compounds by introducing organisms or by enhancing the activity of the portion of the indigenous community possessing inherent capability to degrade recalcitrant organic compounds (Ramsburg et al. 2004; Steffan et al. 1999). Specific application of BPRB systems for the removal of Cr(VI) in groundwater has only been attempted lately at pilot level by Jeyasingh et al. (2011). The slow progress toward full implementation of biological barriers for remediation of Cr(VI) and toxic metals has been both due to the unavailability of microorganisms capable of growing under nutrient-stressed conditions and lack of information on the speciation and mobility of the reduced metal ion species in the soil.

During preliminary investigations in the laboratory at the University of Pretoria, microorganisms capable of reducing and immobilizing Cr(VI) were isolated from dried sludge and introduced in columns packed with aquifer media to remove Cr (VI) from water flowing through the system (Fig. 2.6). The isolated bacteria survived under minimal nutrient conditions and were able to reduce Cr(VI) as water flowed through the inoculated microbial barrier (Molokwane et al. 2008; Molokwane and Chirwa 2009). The findings implied that autotrophic organisms utilizing hypocarbonate as carbon sources can be used in in situ remediation of groundwater without the need of supplementing the groundwater with additional nutrients. However, in spite of these promising findings, the fate of reduced chromium species and the activity of the organisms inside the columns were not investigated.

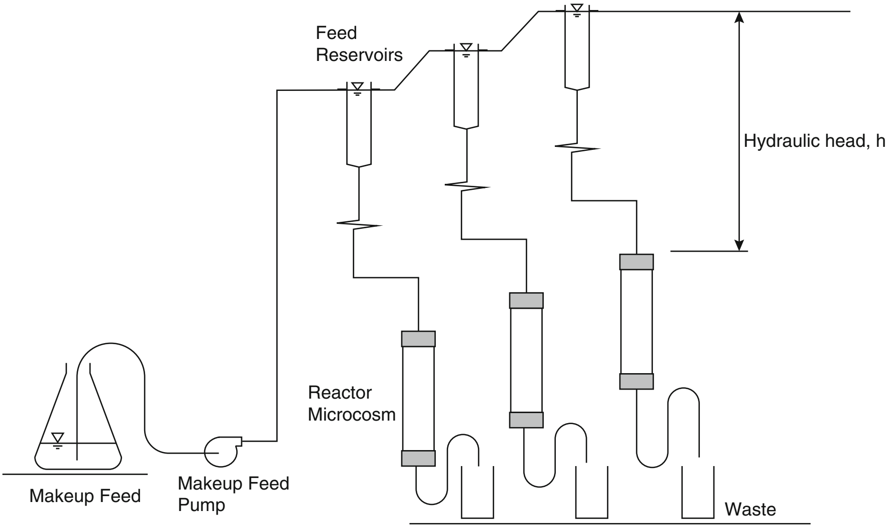

Fig. 2.6 Experimental setup for testing microbial inoculated aquifer medium using gravity fed reactor columns packed with aquifer material. (Adapted from Molokwane and Chirwa 2009)

7.2 In Situ Bioaugmentation

This entails identifying indigenous species of bacteria within the vicinity of the contaminated site and determining the critical carbon sources and nutrients that could be supplied to encourage the growth of the target species. When the selected nutrients are introduced into the environment, either by injection into boreholes or by spreading on the ground, the target species will out-compete other species and will be able to degrade the contaminants. The potential problem with this form of bioaugmentation is that the nutrients may be viewed as pollutants in their own right especially at the beginning of the bioaugmentation process when microbial loading is very low. The nutrients such as NO_3^- and SO_4^{2-} have an undesirable pollution effects on receiving water bodies such as eutrophication of streams receiving the base flow from the remediated areas. However, if applied successfully, in situ bioaugmentation is the only environmentally nonintrusive process that avoids the introduction of alien species into the environment. Most countries prohibit the introduction of organisms foreign to a particular region or country (Federal Register 1999).

7.3 Ex Situ Bioremediation

Ex situ methods of remediation are conventionally used in the treatment of contaminated groundwater through a pump-and-treat process which involves the extraction of contaminated water from the aquifer, treatment above ground, and injection of the treated water back into the aquifer (Milkey 2010; Wittbrodt and Palmer 1992). The treatment above ground can be conducted via both chemical and biological means. Chemical treatment processes employed in the remediation of toxic metals produce unwanted chemical by-products which result in the production of toxic sludge (Gonzalez et al. 2003; Shakoori et al. 2000). Therefore, the chemical processes are viewed as costly and environmentally intrusive. Alternative biological treatment methods have been suggested using biotransformation followed by bioseparation using biomass (Chirwa and Wang 1997; Chrysochoou et al. 2013; Jin et al. 2008). These methods may be applied ex situ (Colica et al. 2010; Zakaria et al. 2007). The ex situ remediation processes are used rarely for treatment of soil which requires excavation of large volumes of soil, treatment in off-site facilities, and backfilling. The whole process for treatment of contaminated soil is too expensive to be undertaken on a large scale. For the treatment of the aquatic phase, experience at sites where pump-and-treat remediation groundwater contaminated with metals and radionuclides showed that, although it is feasible to remove large volumes of the contaminant from the subsurface, it becomes more and more difficult to remove the remaining pollutants as concentration decreases (EPA-Odessa 2005). The biological removal by reactors that can be applied ex situ has been demonstrated at the laboratory scale for chromium, uranium, selenium, and lead (Hawley et al. 2004; Mtimunye and Chirwa 2014; Wessels 2017; Chirwa and Wang 2001; Brink et al. 2017).

8 New Developments in Metal Bioremediation

With the increasing global population, fast-tracked industrialization, and discovery of new technologies, the volume and complexity of metal pollution is bound to increase in the future. In addition to the increasing trajectory in the generation of conventional metallic pollutants, future energy demands may result in a transitional period where nuclear energy will become predominant. This will require environmental engineers and scientist to delve deeper into research on environmentally friendly processes to counter the effects of both conventional and nonconventional pollutants such as heavy metals and recalcitrant organic compounds in the environment. Advanced microbial cultures will be sought to treat a wider variety of recalcitrant pollutants. Discussions on the possibility of genetically engineering specialized cultures for the purpose are not new in the environmental engineering fraternity. However, the application of ideas bears a large ethical burden as it is forbidden in almost all countries in the world to introduce genetically engineered organisms into the environment. Less aggressive methods for dealing with the problem without violating ethics include in situ bioaugmentation and molecular bioaugmentation to a certain extent.

8.1 Molecular Bioaugmentation

The molecular bioaugmentation process utilized genetic carriers such as transposons and plasmids to shuttle genetic information for toxic metal remediation into native species to the environment or species already adapted to the target environment. Several species of bacteria are capable of picking up and retaining circular fragments of DNA called broad-host-range plasmids which may be engineered to carry specific genes for the degradation of xenobiotic compounds and transformation of toxic metals (Weightman et al. 1984; Vincze and Bowra 2006). The same process can be applied using genetically engineered linear DNA called transposons. Although studies have been conducted using these techniques in laboratory microcosms, the application in actual environments has not been attempted (Hill et al. 1994). In the future, it is foreseeable that these methods will find wide application for the new pollutant varieties that may be untreatable by conventional methods.

8.2 Biofractionation and Bioseparation of Radioactive Elements

A very little understood application of bioseparation involves using microorganisms to discriminate radioisotopes by size. So far, this application has remained conceptual due to limited understanding on the structure and function of organisms that are suspected to achieve biofractionation (Molokwane and Chirwa 2007). In the latter study, Molokwane and Chirwa observed with a modest degree of certainty that microbial cells previously isolated from a high radiation-exposed facility accumulated C-14 while growing on a C-14/C-12 carbon matrix from powdered nuclear graphite. The experiment was conducted in a closed loop chemostat system equipped with biofilters for collection of suspended matter for analysis. The observed metabolic activity in the cells indicated that the process was possible under very low dissolved oxygen suggesting that microorganisms preferred inorganic forms of carbon as the primary carbon source. Bacteria that utilize inorganic carbon sources such as CO_2 and HCO_3^- as primary carbon sources – known as autotrophic organisms – favor anaerobic conditions for growth. However, in this preliminary study, the amount of C-14 remaining in solution was not measured which could be required to draw a mass balance on C-14 in the system. These preliminary results on C-12/C-14 bioseparation hold promise for development of the decontamination and reuse process for graphite in model HTGR nuclear reactors that produce large amounts of radioactive nuclear graphite from expired fuel containment (pebbles). Success in the above process is also important for the decontamination and recovery of nuclear graphite decommissioned plants for reuse in new reactors.

9 Conclusion

Since the first Cr(VI)-reducing bacteria were isolated in the 1970s and U(VI)-reducing bacteria were isolated in the late 1980s, a lot of progress has been made in isolating and developing higher performing cultures adapted to various environments. New research using genetic tools has yielded new cultures and new understanding of the metal removal processes both at the molecular level [through genetic studies] and at culture community level [through genomics and proteomics]. Pure and mixed cultures of bacteria have been applied successfully in treating industrial effluents containing Cr(VI), U(VI), As(III), and many other metals. However, application of biological systems in the remediation of contaminated environments still faces a challenge. Although culture performance under natural conditions has been evaluated using laboratory microcosms, more research is still required to elucidate the fate and possibility of recovery of artificial microbial barriers. The question of the fate of reduced species and what to do about the foreseeable blockage by metal hydroxides, metal sulfides, and other complexing agents remains unanswered. In order for the in situ bioremediation technology to work for Cr(VI), U(VI), Se(VI), Tc(VII), and other toxic heavy metals, a solution must be found for feasible recovery of the barrier zones involving remobilization of reduced Cr species. So far, in the current studies, this was achieved by applying an electric potential across a section of contaminated soil. More work is required in the above process to prevent localized precipitation of metals around the electrodes which eventually stopped the migration of metals from the aquatic phase.

Acknowledgment The research was funded by the National Research Foundation (NRF) of South Africa through the Incentive Funding for Rated Researchers Grant No. IFR2010042900080 and Competitive Programme for Rated Researchers Grant No. CPR2011060300001 awarded to Prof. Evans M.N. Chirwa of the University of Pretoria. Postdoctoral fellow working on this project – Dr Zainab Birungi – was funded by the National Research Foundation and the University of Pretoria Postdoctoral Fellowship Programme.

References

Abdel-Rahman GN, Nassar NRA, Heikal YA, Abou-Donia MAM, Naguib MM, Fadel M (2016) Effect of different treatments on heavy metal concentration in sugar cane molasses. Int J AgriBiosys Eng 10(1):43–48

Acar YB, Gale RJ, Alshawabkeh AN, Marks RE, Puppala S, Bricka M, Parker R (1995) Electrokinetic remediation: basics and technology status. J Hazard Mater 40(2):117–137

ACGIH (American Conference of Governmental Industrial Hygienists) (2004) Threshold limit values for chemical substances and physical agents and biological exposure indices. ACGIH, Cincinnati, OH, USA

Anastopoulos AG, Papoutsis AD, Papaderakis AA (2015) Differential capacitance and electrochemical impedance study of surfactant adsorption on polycrystalline Ni electrode. J Solid State Electrochem 19(8):2369–2377

Arıca MY, Kaçar Y, Genç Ö (2001) Entrapment of white-rot fungus Trametes versicolor in Ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. Bioresour Technol 80(2):121–129

Aziz HA, Adlan MN, Ariffin KS (2008) Heavy metals (Cd, Pb, Zn, Ni, Cu and Cr(III)) removal from water in Malaysia: post treatment by high quality limestone. Bioresour Technol 99 (6):1578–1583

Babel S, Kurniawan TA (2004) Cr (VI) removal from synthetic wastewater using coconut shell charcoal and commercial activated carbon modified with oxidizing agents and/or chitosan. Chemosphere 54(7):951–967

Bansal N (2010) Determination of trace amounts of mercury (II) by catalytic kinetic method in environmental samples. World Rev Sci Technol Sustain Dev 7(4):316–327

Barak Y, Ackerley DF, Dodge CJ, Banwari L, Alex C, Francis AJ, Matin A (2006) Analysis of novel soluble chromate and uranyl reductases and generation of an improved enzyme by directed evolution. Appl Environ Microbiol 72(11):7074–7082

Battista JR (1997) Against all odds: the survival strategies of Deinococcus radiodurans. Annu Rev Microbiol 51:203–224

Beszedits S (1988) Chromium removal from industrial wastewaters, In: Nriagu O, Nieboer E (eds) Chromium in the natural and human environments. Wiley, New York. , pp 232–263. isbn:978-0471856436

Bharagava RN, Saxena G, Mulla SI, Patel DK (2017a) Characterization and identification of recalcitrant organic pollutants (ROPs) in tannery wastewater and its phytotoxicity evaluation for environmental safety. Arch Environ Contam Toxicol. https://doi.org/10.1007/s00244-017-0490-x

Bharagava RN, Saxena G, Chowdhary P (2017b) Constructed wetlands: an emerging phytotechnology for degradation and detoxification of industrial wastewaters. In: Bharagava RN (ed) Environmental pollutants and their bioremediation approaches, 1st edn. CRC Press/Taylor & Francis, Boca Raton, pp 397–426. https://doi.org/10.1201/9781315173351-15

Bharagava RN, Chowdhary P, Saxena G (2017c) Bioremediation: an ecosustainable green technology: its applications and limitations. In: Bharagava RN (ed) Environmental pollutants and their bioremediation approaches, 1st edn. CRC Press/Taylor & Francis, Boca Raton, pp 1–22. https://doi.org/10.1201/9781315173351-2

Birungi Z, Chirwa E (2014) The kinetics of uptake and recovery of lanthanum using freshwater algae as biosorbents: comparative analysis. Bioresour Technol 160:43–51

Birungi ZS, Chirwa EMN (2015) The adsorption potential and recovery of thallium using green micro-algae from eutrophic water sources. J Hazard Mater 299:67–77

Borda M, Sparks D (2008) Kinetics and mechanisms of sorption–desorption in soils: a multiscale assessment. Biophys Chem Process Heavy Metals Metalloids Soil Environ 19(8):2369–2377

Boukili H, Novikoff A, França J (1984) Mineralogie et géochimie des chlorites et hydroxycarbonateschromitifères de Campo Formoso, Bahia, Brésil. Cahiers ORSTOM Série Géologie 14(2):141–152

Boyle EA, Lee J-M, Echegoyen Y, Noble A, Moos S, Carrasco G, Zhao N, Kayser R, Zhang J, Gamo T, Obata H, Norisuye K (2014) Anthropogenic lead emissions in the ocean: the evolving global experiment. Oceanography 27(1):69–75

Brink HG, Lategan M, Naudé K, Chirwa EMN (2017) Lead removal using industrially sourced consortia: influence of lead and glucose concentrations. ICheaP-13, Milan, 28-31 May 2017. Chem Eng Trans 57:409–414

Bruno J, Ewing RC (2006) Spent nuclear fuel. Elements 2:343–349

Buck EC, Hanson BD, McNamara BK (2004) The geochemical behaviour of Tc, Np and Pu in spent nuclear fuel in an oxidizing environment. In: Gieré R, Stille P (eds) Energy, waste, and the environment: a geochemical perspective, vol 236. The Geological Society of London Special Publication, London, pp 65–88

Bush MB (2003) Ecology of a changing planet, 3rd edn. Prentice Hall, New Jersey

Calas G, Manceau A, Novikoff A, Boukili H (1984) Chromium behavior in alteration minerals of the Campo Formoso chromite deposit, Bahia, Brazil. Bull Minéral 107(6):755–766

Castillo AN, García-Delgado RA, Rivero VC (2012) Electrokinetic treatment of soils contaminated by tannery waste. Electrochim Acta 86:110–114

Cervantes C (1991) Bacterial interaction with chromate. Anton Leeuw 59(4):229–233

Cervantes C, Silver S (1992) Plasmid chromate resistance and chromate reduction. Plasmid 27 (1):65–71

Cervantes C, Campos-García J, Devars S, Gutiérrez-Corona F, Loza-Tavera H, Torres-Guzmán JC, Moreno-Sánchez R (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 25(2):335–347

Chaalal O, Islam MR (2001) Integrated management of radioactive strontium contamination in aqueous stream systems. J Environ Manag 61(1):51–59

Chandra R, Saxena G, Kumar V (2015) Phytoremediation of environmental pollutants: an eco-sustainable green technology to environmental management. In: Chandra R (ed) Advances in biodegradation and bioremediation of industrial waste, 1st edn. CRC Press/Taylor & Francis, Boca Raton, pp 1–30. https://doi.org/10.1201/b18218-2

Chen JP (1997) Batch and continuous adsorption of strontium by plant root tissues. Bioresour Technol 60(3):185–189

Chirasha J, Shoko NR (2010) Zimbabwe alloys ferro chromium production: from cradle to grave sustainably. In: Proc 12th International Ferroalloys Congress sustainable future. June 6–9, 2010, Helsinki, Finland, pp 391–400

Chirwa EMN, Molokwane PE (2011) Chapter 5: Biological Cr(VI) reduction: microbial diversity, kinetics and biotechnological solutions to pollution. In: Sofo A (ed) Biodiversity. InTech Online Publishers, Cambridge, pp 75–100

Chirwa EMN, Wang YT (1997) Hexavalent chromium reduction by Bacillus sp. in a packed-bed bioreactor. Environ Sci Technol 31(5):1446–1451

Chirwa EN, Wang YT (2001) Simultaneous chromium(VI) reduction and phenol degradation in a fixed-film coculture bioreactor: reactor performance. Water Res 35(8):1921–1932

Choi SB, Yun Y (2006) Biosorption of cadmium by various types of dried sludge: an equilibrium study and investigation of mechanisms. J Hazard Mater 138(2):378–383

Chrysochoou M, Zhang X, Amador J (2013) Comparison of Cr(VI) reduction by aerobic bacteria in culture and soil conditions. Soil Sediment Contam 22(3):273–287

Colica G, Mecarozzi PC, De Philippis R (2010) Treatment of Cr(VI)-containing wastewaters with exopolysaccharide-producing cyanobacteria in pilot flow through and batch systems. Appl Microbiol Biotechnol 87(5):1953–1961

Cramer LA, Basson J, Nelson LR (2004) The impact of platinum production from UG2 ore on ferrochrome production in South Africa. J South Afr Inst Min Metall 104(9):517–527

Dakiky M, Khamis M, Manassra A, Mer'eb M (2002) Selective adsorption of Cr(VI) in industrial waste water using low-cost abundantly available adsorbents. Adv Environ Res 6(4):533–540

Dastidar A, Wang YT (2010) Kinetics of arsenite oxidation by chemoautotrophic Thiomonasarsenivorans strain b6 in a continuous stirred tank reactor. J Environ Eng 136 (10):1119–1127

Eisler R (1988) Lead hazards to fish, wildlife, and invertebrates: a synoptic review. Organization series: contaminant hazard reviews-contaminant hazard reviews-report 14. Biological Report 85 (1.14). U.S. Department of the Interior, Fish and Wildlife Service

EPA-Odessa (2005) Pump and treat and in situ chemical treatment of contaminated groundwater at the Odessa Chromium II South Plume Superfund Site Odessa, Ector County, Texas. U.S. Environmental Protection Agency. Available online at http://costperformance.org/pdf/odessa_ii_06-30-2005.pdf

Federal Register (1999) Executive order 13751- safeguarding the nation from the impacts of invasive species (Dec 5, 2016). https://www.invasivespeciesinfo.gov/laws/execorder.shtml. Last accessed 14/02/18, 3:00 PM

Federal Register (2004) Occupational Safety and Health Administration. Occupational exposure to hexavalent chromium. 69 Federal Register 59404. October 4, 2004

Flessel CP (1979) Trace metals in health and disease. Raven Press, New York, pp 109–122

Foulkes JM, Deplanche K, Sargent F, Macaskie L, Lloyd JR (2016) A novel aerobic mechanism for reductive palladium biomineralization and recovery by Escherichia coli. Geomicrobiol J 33 (3–4):230–236

Francis W, Croft H (1849) The chemical gazette, or, Journal of Practical Chemistry, in all its applications to pharmacy, arts and manufactures, vol. 7. London, R. and J. E. Taylor [etc., 1842-59]

Fu F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. J Environ Manag 92(3):407–418

Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84(1):13–28

Gao Y, Truong YB, Cacioli P, Butler P, Kyratzis IL (2014) Bioremediation of pesticide contaminated water using an organophosphate degrading enzyme immobilized on nonwoven polyester textiles. Enzym Microb Technol 10(54):38–44

Garrels RM, Christ CL (1965) In solutions, minerals and equilibria. Harper and Row, New York, pp 403–435

Gaur N, Flora G, Yadav M, Tiwari A (2014) A review with recent advancements on bioremediation-based abolition of heavy metals. Environ Sci Process Impacts 16(2):180–193

Gautam S, Kaithwas G, Bharagava RN, Saxena G (2017) Pollutants in tannery wastewater, pharmacological effects and bioremediation approaches for human health protection and environmental safety. In: Bharagava RN (ed) Environmental pollutants and their bioremediation approaches, 1st edn. CRC Press/Taylor & Francis, Boca Raton, pp 369–396. https://doi.org/10.1201/9781315173351-14

Gilmour CC, Henry EA (1991) Mercury methylation in aquatic systems affected by acid deposition. Environ Pollut 71(2–4):131–169

Gioia SMCL, Pimentel MM, Tessler M, Dantas EL, Campos JEG, Guimarães EM, Maruoka MTS, Nascimento ELC (2006) Sources of anthropogenic lead in sediments from an artificial lake in Brasília–central Brazil. Sci Total Environ 356(1-3):125–142

Girling CA (1984) Selenium in agriculture and the environment. Agric Ecosyst Environ 11 (1):37–65

Gonzalez CF, Ackerley DF, Park CH, Matin A (2003) A soluble flavoprotein contributes to chromate reduction and tolerance by Pseudomonas putida. Acta Biotechnol 23(2–3):233–239

Goutam SP, Saxena G, Singh V, Yadav AK, Bharagava RN (2018) Green synthesis of TiO_2 nanoparticles using leaf extract of *Jatropha curcas* L. for photocatalytic degradation of tannery wastewater. Chem Eng J 336:386–396. https://doi.org/10.1016/j.cej.2017.12.029

Green HH (1918) Description of bacterium which oxidizes arsenite to arsenate, and one which reduces arsenate to arsenite, isolated from a cattle dipping-tank. S Afr J Med Sci 14:465–467

Greve K, Nielsen E, Ladefoged O (2007) Evaluation of health hazards by exposure to strontium in drinking water. Toxicol Lett 172(Suppl 1):S210–S210

Guyo U, Makawa T, Moyo M, Nharingo T, Nyamunda BC, Mugadza T (2015) Application of response surface methodology for Cd(II) adsorption on maize tassel-magnetite nanohybrid adsorbent. J Environ Chem Eng 3(4):2472–2483

Haryanto R, Tambun R, Haloho H, Panjaitan F, Sitorus S (2017) Investigation on the ability of a natural adsorbent corn stalk in removing heavy metal ions from aqueous solution. ARPN J Eng Appl Sci 12(18):S263–S270

Hawley EL, Deeb RA, Kavanaugh MC, Jacobs JRG (2004) Treatment technologies for chromium (VI). In: Cr(VI) Handbook/L1608_C08, vol 8. CRC Press LLC, New York, pp 273–308

Hill KE, Fry JC, Weightman AJ (1994) Gene transfer in the aquatic environment: persistence and mobilization of the catabolic recombinant plasmid pDlO in the epilithon. Microbiol 140:1555–1563

Hiz MM, Aki C (2015) The nightmare: genetically modified organisms as alien species. Transylvan Rev Sys Ecolog Res 16(1). https://doi.org/10.1515/trser-2015-0008

Horitsu H, Futo S, Miyazawa Y, Ogai S, Kawai K (1987) Enzymatic reduction of hexavalent chromium by hexavalent chromium tolerant Pseudomonas ambigua G-1. Agric Biol Chem 51 (9):2417–2420

Hunter RJ, James M (1992) Charge reversal of kaolinite by hydrolyzable metal ions: an electro-acoustic study. Clay Clay Miner 40(6):644–649

Igboamalu TE, Chirwa EMN (2017) As (III) oxidation and Cr(VI) reduction insight in an indigenous mixed culture of anaerobic bacteria from a local environment. PRES'17, Tiajing, China, 23-26 August 2017. Chem Eng Trans 61:259–264

Ilialetdinov AN, Abdrashitova SA (1981) Autotrophic oxidation of arsenic by a culture of Pseudomonas arsenitoxidans. Mikrobiologiya 50:197–204

Jaishankar M, Tseten T, Anbalagan N, Blessy B, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7(2):60–72

Jeyasingh J, Somasundaram V, Philip L, Bhallamudi MS (2011) Pilot scale studies on the remediation of chromium contaminated aquifer using bio-barrier and reactive zone technologies. Chem Eng J 167(1):206–214

Jin S, Fahlgren PH, Morris JM, Gossard RB (2008) Biological source treatment of acid mine drainage using microbial and substrate amendments: microcosm studies. Mine Water Environ 27:20–30

Kalckar HM (1974) Origins of the concept oxidative phosphorylation. Mol Cell Biochem 5 (1–2):55–63

Khripunov VI, Kurbatov DK, Subbotin ML (2006) C-14 production in CTR materials and blankets. In: Proc 21st Fusion Energy Conference (FEC2006), 16–21 October 2006, Chengdu, China. SE/p2-3. Available online at http://www-pub.iaea.org/MTCD/Meetings/FEC2006/se_p2-3.pdf

Kossman SE, Weiss MA (2000) Acute myelogenous leukemia after exposure to strontium-89 for the treatment of adenocarcinoma of the prostate. Cancer 88(3):620–624

Kotrba P (2011) Microbial biosorption of metals-general introduction. In: Microbial biosorption of metals. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-0443-5_1

Kratochvil D, Volesky B (1998) Advances in the biosorption of heavy metals. Trends Biotechnol 16(7):291–300

Lawson S, Macy JM (1995) Bioremediation of selenite in oil refinery wastewater. Appl Microbiol Biotechnol 43(4):762–765

Lemly AD (2004) Aquatic selenium pollution is a global environmental safety issue. Ecotoxicol Environ Saf 59(1):44–56

Lenz M, Lens PNL (2009) The essential toxin: the changing perception of selenium in environmental sciences. Sci Total Environ 407(12):3620–3633

Lenz M, Van Hullebusch ED, Hommes G, Corvini PFX, Lens PNL (2008) Selenate removal in methanogenic and sulfate-reducing upflow anaerobic sludge bed reactors. Water Res 42 (8-9):2184–2194

Li S, Li T, Li F., Liang L, Li G, Guo S (2011) Application of sequential extraction analysis to electrokinetic remediation of chromium contaminated soil. In: Bioinformatics and Biomedical Engineering, (iCBBE) 2011, 5th International conference, pp 1–4

Li D, Huang T, Liu K (2015) Near-anode focusing phenomenon caused by the coupling effect of early precipitation and backward electromigration in electrokinetic remediation of MSWI fly ashes. Environ Technol 37(2):216–227

Lloyd JR, Ridley J, Khizniak T, Lyalikova NN, Macaskie LE (1999) Reduction of technetium by Desulfovibrio desulfuricans: biocatalyst characterization and use in a flow through bioreactor. Appl Environ Microbiol 65(6):2691–2696

Lloyd JR, Pearce CI, Coker VS, Pattrick RADP, van der Laan G, Cutting R, Vaughan DV, Paterson-Beedle M, Mikheenko I, Yong P, Macaskie LE (2008) Biomineralization; linking the fossil record to the production of high value functional materials. Geobiology 6(3):285–297

Lovley DR, Phillips EJP (1994) Reduction of chromate by Disulfovibrio vulgaris and its c3 cytochrome. Appl Environ Microbiol 60(2):726–728

Ma G, Garbers-Craig AM (2006) Cr(VI) containing electric furnace dusts and filter cake from a stainless steel waste treatment plant Part 1 – Characteristics and microstructure. Ironmak Steelmak 33(3):229–237

Mabbett AN, Sanyahumbi D, Yong P, Macaskie LE (2006) Biorecovered precious metals from industrial wastes: single-step conversion of a mixed metal liquid waste to a bioinorganic catalyst with environmental application. Environ Sci Technol 40(3):1015–1021

Makgato SS, Chirwa EMN (2015) Photoassisted biodegradation of irradiated organics in simulated nuclear wastewater. Water Environ Res 87(5):392–403

Maturi K, Reddy KR (2008) Cosolvent-enhanced desorption and transport of heavy metals and organic contaminants in soils during electrokinetic remediation. Water Air Soil Pollut 189 (1–4):199–211

Mehta S, Gaur J (2005) Use of algae for removing heavy metal ions from wastewater: Progress and prospects. Crit Rev Biotechnol 25(3):113–152

Meli KC(2009) Microbial Cr(VI) reduction in indigenous cultures of bacteria: characterisation and modelling. M.Sc. Dissertation, University of Pretoria, Pretoria, South Africa

Merian E (1984) Introduction on environmental chemistry and global cycles of arsenic, beryllium, cadmium, chromium, cobalt, nickel, selenium, and their derivatives. Toxicol Environ Chem 8 (1):9–38

Milkey NE (2010) Remediation of a hexavalent chromium release to groundwater using ion-specific resins. In: Proc. 25th Annual International Conference on Soils, Sediments, Water and Energy, October 19–22, 2009, Amherst, Massachusetts, vol. 15, article 7. Available at: http://scholarworks.umass.edu/soilsproceedings/vol15/iss1/7

Molokwane PE, Chirwa EM (2007) Development of a Carbon-14 bioseparation technique for clean-up of nuclear graphite. In: Proc 11th International conference on Environmental Remediation and Radioactive Waste Management (ICEM2007), Bruges, Belgium, September 2–6, 2007, pp 113–118

Molokwane PE, Nkhalambayausi-Chirwa EM (2009) Microbial culture dynamics and chromium (VI) removal in packed-column microcosm reactors. Water Sci Technol 60(2):381–388

Molokwane PE, Nkhalambayausi-Chirwa EM, Meli KC (2008) Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa). Water Res 42(17):4538–4548

Morrison D, Murphy BL (2010) Environmental forensics: contaminant specific guide. Academic Press, New York

Mtimunye PJ, Chirwa EMN (2013) Chapter 8: Bioremediation of radiotoxic elements under natural environmental conditions. In: Patil YB, Rao P (eds) Applied bioremediation – active and passive approaches. InTech Open Publishers, Rijeka, pp 177–206

Mtimunye PJ, Chirwa EMN (2014) Biochemical optimisation and control of uranium (VI) reduction in facultative uranium VI reducing cultures. Chemosphere 113:22–29

Nealson KH (1999) Post-Viking microbiology: new approaches, new data, new insights. Origins of life and evolution of the biosphere. Orig Life EvolBiosph 29(1):73–93

Noshchenko AG, Moysich KB, Bondar A, Zamostyan PV, Drosdova VD, Michalek AM (2001) Patterns of acute leukaemia occurrence among children in the Chernobyl region. Int J Epidemiol JID-7802871

Olaniran AO, Igbinosa EO (2011) Chlorophenols and other related derivatives of environmental concern: properties, distribution and microbial degradation processes. Chemosphere 83 (10):1297–1306

Page MM, Page CL (2002) Electroremediation of contaminated soils. J Environ Eng 128 (3):208–219

Pagnanelli F (2011) Equilibrium, kinetic and dynamic modelling of biosorption processes. In: Anonymous microbial biosorption of metals. Springer, Dordrecht, pp 59–120

Pagnanelli F, Trifoni M, Beolchini F, Esposito A, Toro L, Veglio F (2001) Equilibrium biosorption studies in single and multi-metal systems. Process Biochem 37(2):115–124

Pamukcu S, Wittle JK (1992) Electrokinetic removal of selected heavy metals from soil. Environ Prog 11(3):241–250

Papp A, Pecze L, Szabó A, Vezér T (2006) Effects on the central and peripheral nervous activity in rats elicited by acute administration of lead, mercury and manganese, and their combinations. J Appl Toxicol 26(4):374–380

Probstein RF, Hicks RE (1993) Removal of contaminants from soils by electric fields. Science 260 (5107):498–503

Qu X, Alvarez PJ, Li Q (2013) Applications of nanotechnology in water and wastewater treatment. Water Res 47(12):3931–3946

Ramsburg CA, Abriola LM, Pennell KD, Löffler FE, Gamache M, Amos BK, Petrovskis EA (2004) Stimulated microbial reductive dechlorination following surfactant treatment at the Bachman Road site. Environ Sci Technol 38(22):5902–5914

Reddy KR, Chinthamreddy S (1999) Electrokinetic remediation of heavy metal-contaminated soils under reducing environments. Waste Manag 19(4):269–282

Reddy KR, Chinthamreddy S (2003) Sequentially enhanced electrokinetic remediation oh heavy metals in low buffering clayey soils. J Geotech Geoenviron Eng 129(3):263–277

Reddy KR, Karri MR (2006) Effect of voltage gradient on integrated electrochemical remediation of contaminant mixtures. Land Contam Reclam 14(3):685–698

Reddy KR, Parupudi US (1997) Removal of chromium, nickel and cadmium from clays by in-situ electrokinetic remediation. Soil Sediment Contam 6(4):391–407

Renner R (2010) Exposure on tap: drinking water as an overlooked source of lead. Environ Health Perspect 118(2):A68–A74

Ross DA (2015) Technical report on the Patterson Lake South property, Northern Saskatchewan, Canada, NI 43-101 Report. Available online at http://www.fissionuranium.com/_resources/reports/RPA_Fission_U_Patterson_Lake_South_Technical_Report_FINAL_Feb_2015.pdf

Santini JM, Sly LI, Schnagl RD, Macy JM (2000) A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: phylogenetic, physiological and preliminary biochemical studies. Appl Environ Microbiol 66:92–97

Satoshi MS, Hisamitsu T, Tsubasa K, Masaki M, Emi N, Ike SKIN (2012) Biotreatment of selenium refinery wastewater using pilot-scale granular sludge and swim-bed bioreactors augmented with a selenium-reducing bacterium *Pseudomonas Stutzeri* NT-1. Jpn J Water Treatment Biol 48 (2):63–71

Saxena G, Bharagava RN (2015) Persistent organic pollutants and bacterial communities present during the treatment of tannery wastewater. In: Chandra R (ed) Environmental waste management, 1st edn. CRC Press/Taylor & Francis, Boca Raton, pp 217–247. https://doi.org/10.1201/b19243-10

Saxena G, Bharagava RN (2017) Organic and inorganic pollutants in industrial wastes, their ecotoxicological effects, health hazards and bioremediation approaches. In: Bharagava RN (ed) Environmental pollutants and their bioremediation approaches, 1st edn. CRC Press/Taylor & Francis, Boca Raton, pp 23–56. https://doi.org/10.1201/9781315173351-3

Saxena G, Chandra R, Bharagava RN (2016) Environmental pollution, toxicity profile and treatment approaches for tannery wastewater and its chemical pollutants. Rev Environ Contam Toxicol 240:31–69. https://doi.org/10.1007/398_2015_5009

Selatnia A, Bakhti M, Madani A, Kertous L, Mansouri Y (2004) Biosorption of Cd^{2+} from aqueous solution by a NaOH-treated bacterial dead Streptomyces rimosus biomass. Hydrometallurgy 75 (1–4):11–24

Senanayake R, Rousseau D, Colegrave H, Idriss H (2005) The reaction of water on polycrystalline UO_2: pathways to surface and bulk oxidation. J Nucl Mater 342:179–187

Shakoori AR, Makhdoom M, Haq RU (2000) Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries. Appl Microbiol Biotechnol 53(3):348–351

Shen H, Wang YT (1993) Characteristic enzymatic reduction of hexavalent chromium by Escherichia coli ATCC 33456. Appl Environ Microbiol 59(11):3771–3777

Shen Z, Chen X, Jia J, Qu L, Wang W (2007) Comparison of electrokinetic soil remediation methods using one fixed anode and approaching anodes. Environ Pollut 150(2):193–199

Soudek P, Valenova S, Vavrikova Z, Vanek T (2006) Study of radio-phyto-remediation on heavily polluted area in South Bohemia. J Environ Radio 88(3):236–250

Sovacool BK(2008) Valuing the greenhouse gas emissions from nuclear power: acritical survey. Energy Policy 36, Elsevier

Stefaniak EA, Alsecz A, Frost R, Mathe Z, Sajo IE, Torok S, Worobiec A, Grieken R.V (2009) Combined SEM/EDX and micro-Raman spectroscopy analysis of uranium minerals from a former uranium mine. Hazard Mater, 168:416-423.

Steffan RJ, Sperry KL, Walsh MT, Vainberg S, Condee CW (1999) Field-scale evaluation of in situ bioaugmentation for remediation of chlorinated solvents in groundwater. Environ Sci Technol 33(16):2771–2781

Stumm W, Morgan JJ (1995) Aquatic Chemistry: chemical equilibria and rates in natural waters, 3rd edn. Wiley, New York/London

Sun W, Sierra-Alvarez R, Hsu I, Rowlette P, Field JA (2010) Anoxic oxidation of arsenite linked to chemolithotrophic denitrification in continuous bioreactors. Biotechnol Bioeng 105(5):909–917

Supanopas P, Sretarugsa P, Pokethitiyook P, Upatham ES (2005) Acute and subchronic toxicity of lead to the spotted Babylon, Babylonia areolata Neogastropoda, Buccinidae. J Shellfish Res 24 (1):91–98

Thomas KT (1981) Management of wastes from uranium mines and mills. IAEA Bull 23(2):33–35

Thomas PG, Quinn PJ, Williams WP (1985) The origin of photosystem-I mediated electron transport stimulation in heat-stressed chloroplasts. Planta 167(1):133–139

Tiwary RK, Dhakate R, Rao A, Singh VS (2005) Assessment and prediction of contaminant migration in ground water from chromite waste dump. Environ Geol 48(4–5):420–429

Tredoux M, De Wit MJ, Hart RJ, Armstrong RA, Lindsay NM, Sellschop JPF (1989) Platinum group elements in a 3.5 Ga nickel-iron occurrence possible evidence of a deep mantle origin. J Geophys Res 94(B1):795–813

Tudiver S (2009) Greenhouse gas emissions from nuclear power in 2030: examining emissions estimates and projected growth. Yale J Int Aff 4(2):100–111

UNEP (2010) Final review of scientific information on lead. United Nations Environment Programme, Chemicals Branch, DTIE. Available at http://www.environmentalhealthnews.org/ehs/news/2013/pdf-links/UN%202010%20report.pdf. Last accessed November 9, 2014

USGS (1995) Mercury contamination of aquatic ecosystems, USGS fact sheet 216-95. Complied by Krabbenhoft DP and Rickert DA. US Geological Survey report

Veglio F, Quaresima R, Fornari P, Ubaldini S (2003) Recovery of valuable metals from electronic and galvanic industrial wastes by leaching and electrowinning. Waste Manag 23(3):245–252

Vincze E, Bowra S (2006) Transformation of Rhizobia with broad-host-range plasmids by using a Freeze-Thaw method. Appl Environ Microbiol 72(3):2290–2293

Volesky B (2007) Biosorption and me. Water Res 41(18):4017–4029

Volesky B, Holan Z (1995) Biosorption of heavy metals. Biotechnol Prog 11(3):235–250

Von Gruenewaldt G, Hatton CJ (1987) Platinum-group metals-a resource in the tailings of chromium mines in South Africa. J South Afr Inst Mining Metallurgy 87(9):265–268

Wagner PA (1923) The chromite of the Bushveld complex. S Afr J Sci 20:223–235

Wall S (2010) The history of electrokinetic phenomena. Curr Opin Colloid Interface Sci 15 (3):119–124

Wang D, Sun W, Xu Y, Tang H, Gregory J (2004) Speciation stability of inorganic polymerflocculant-PACl. Colloids Surf Physicochem Eng Aspects 243:1–10

Wang Y, Xie Y, Li W, Wang Z, Giammar DE (2010) Formation of lead(IV) oxides from lead (II) compounds. Environ Sci Technol 44(23):8950–8956

Wang Z, Bush RT, Sullivan LA, Liu J (2013) Simultaneous redox conversion of chromium(VI) and arsenic(III) under acidic conditions. Environ Sci Technol 47(12):6486–6492

Weightman AJ, Don RH, Lehrbach PR, Timmis KN (1984) The identification and cloning of genes encoding haloaromatic catabolic enzymes and the construction of hybrid pathways for substrate mineralization. In: Omenn GS, Hollaender A (eds) Genetic control of environmental pollutants. Plenum Press, New York/London, pp 47–80

Wessels CE(2017) Bioremediation of seleniferous water is gaining more momentum, especially when it comes to bacterial reduction of the selenium oxyanion, Masters dissertation, University of Pretoria, South Africa

White O, Eisen JA, Heidelberg JF, Hickey EK, Peterson JD, Dodson RJ, Haft DH, Gwinn ML, Nelson WC, Richardson DL, Moffat KS, Qin H, Jiang L, Pamphile W, Crosby M, Shen M, Vamathevan JJ, Lam P, McDonald L, Utterback T, Zalewski C, Makarova KS, Aravind L, Daly MJ, Minton KW, Fleischmann RD, Ketchum KA, Nelson KE, Salzberg S, Smith HO, Venter JC, Fraser CM (1999) Genome sequence of the radio resistant bacterium Deinococcus radiodurans R1. Science 286(5444):1571–1577

Wilke S, Johnstone J (2017) Readings in the anthropocene: the environmental humanities, German studies. Bloomsbury, New York

Wittbrodt PR, Palmer CD (1992) Limitations to pump-and-treat remediation of a chromium contaminated site. Aquifer restoration: pump-and-treat and the alternatives. In: Proc National Groundwater Association Convention, 30 Sept.–2 Oct. 1992, Las Vegas, NV

WNA (2008) World Nuclear Association information report: http://www.world-nuclear.org/info/inf88.html

Yong P, Rowson NA, Peter J, Farr G, Harris IR, Macaskie LE (2002) Bioreduction and biocrystallization of palladium by Desulfovibrio desulfuricans NCIMB 8307. Biotechnol Bioeng 80(40):369–379

Zakaria ZA, Zakaria Z, Surif S, Ahmad WA (2007) Biological detoxification of Cr(VI) using wood-husk immobilized Acinetobacter haemolyticus. J Hazard Mater 148(1–2):164–171