

REMEDICATION OF METAL AND METALLOID CONTAMINATED GROUNDWATER

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Abstract. This chapter describes the remediation of groundwater polluted by heavy metals. Special attention is paid to ‘pump and treat’ methods and to different *in situ* approaches. Emphasis is on microbial processes and their combination with physico-chemical systems.

Keywords: heavy metals; groundwater; water treatment; *in situ* bioremediation; *on site* treatment; soil washing and flushing; sulfate reduction; iron reduction; biosorption; bioprecipitation; immobilization; permeable reactive barriers; reactive zones; electron donors; sand filtration; bioreactors; upflow anaerobic sludge blanket reactor; natural attenuation

1. Introduction

In general more than 60% of contaminated sites in the world have problems with the presence of toxic metals such as cadmium, lead, copper, zinc, mercury and nickel. These metals are considered to be the most hazardous and are included on the US Environmental Protection Agency (EPA) list of priority pollutants (Cameron, 1992). Groundwater pollution is caused mostly by leaching of metals by infiltrating rainwater from the contaminated soil to the groundwater. Metal surface treatment activities have frequently caused pollution with toxic chromium (VI) and many large urban sites are contaminated by the same metal due to the leather industry (Saha and Ali, 2001). Other important industrial sectors that use heavy metals in their production processes are the non-ferrous industry and mining activities. In many cases, metals are solubilized by using acid process waters (e.g. sulfuric acid). This leads to groundwaters low in pH (between 2.5 and 6.5), rich in sulfate (between 100 and 5000 mg SO₄²⁻/l) and high in dissolved metals (mostly in the range of 100 to 2000 µg/l). Other

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co-pollutants may also be present (e.g. trichloroethylene used as degreaser in surface treatment). In the case of the mining industry, acid mine drainage (AMD) is responsible for widespread contamination. AMD is the result of a natural-occurring process when a metal sulfide mineral, particularly pyrite ores (e.g. chalcopyrite, sphalerite, etc.) are exposed to oxygen and water in the presence of naturally occurring sulfur-oxidizing bacteria such as *Acidithiobacillus ferrooxidans* and *A. thiooxidans* that act as biological catalysts (Johnson and Hallberg, 2005). The components of AMD have a deleterious influence on the biota of streams which receive it (Kontopoulos, 1988). In the case of uranium mines, uranium and radium are leached into the drainage water, poisoning the groundwater with radioactivity.

Toxic metals were distributed in the past via aerial-emissions (ancient pyrometallurgical processes) and have caused large-scale diffuse pollution. In this case a slow process of metal infiltration eventually will lead to leaching of metals into the surface water. As a first step the metals migrate from the unsaturated zone into the saturated zone, sorbing to aquifer material until all binding sites are saturated. Once the sorption capacity of the soil is exhausted, elevated metal concentrations can be transported through the groundwater and, in the long term, reach surface waters. Figure 1 illustrates metal distribution over different soil compartments as a function of time. Waste heaps and landfills can leak and lead to point sources leaching high concentrations of metals into the groundwater. Landfills containing jarosite, goethite, gypsum, slags or fines can, especially in the presence of organics, lead to solubilization of metals and metalloids, the contamination of surface water and contamination of groundwater due to anaerobic leaching. Metals from mines are more easily

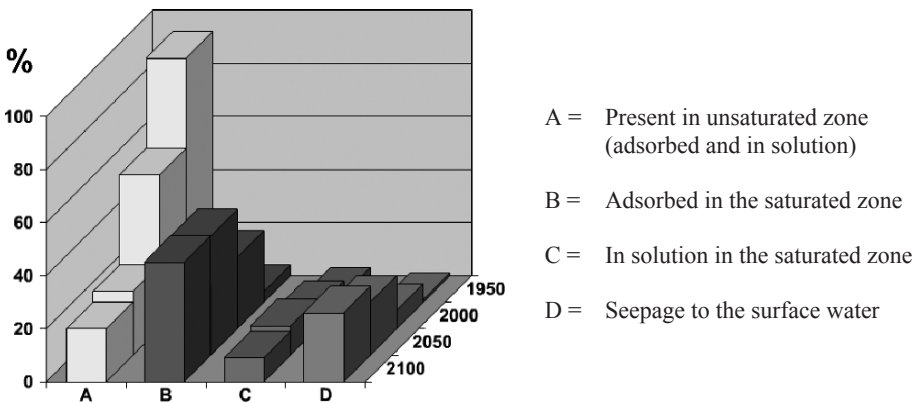


Figure 1. Expected evolution over time of the distribution of cadmium in the Kempen groundwater bodies if no remedial actions are undertaken (adopted from Schmidt, 2002)

transported by surface water and groundwater and rare events such as dam ruptures can lead to flooding of large areas contaminating the soil and the groundwater. An example of such a disaster was the mine tailings accident in Aznacollar (Grimault et al., 1999).

The current chapter reviews both full-scale and developing technologies that are available. The remediation of metal pollution can be based on extraction and physical separation, precipitation, immobilization and toxicity reduction. The selection of the most appropriate method depends on aquifer characteristics, pollutant concentration, types of pollutants to be removed, and the use of the contaminated medium. As an example, the evolution of the distribution of cadmium in groundwater bodies in The Kempen (Belgium) is presented in Figure 1. In order to avoid infiltration to the groundwater or seepage into the surface water, the metals can either be removed from the groundwater (decrease in C in Figure 1) or the metals can be adsorbed (immobilization) on the aquifer of the saturated zone (increase in B in Figure 1). In the approach, a distinction can be made between methods based on groundwater extraction, which remove metals from the water (decrease in C) or methods aimed at immobilizing the metals (increase in B) in the aquifer preventing further distribution of the contaminants. The first approach is an *on site* treatment method; the second approach an *i* method designed to reduce migration risks of the metals. Several physico-chemical and biological immobilization methods exist; here we will only focus on those methods that involve biological processes.

2. *On Site* Treatment Methods

The currently used pump and treat technology removes large volumes of groundwater from an aquifer and, if the water has to be returned to the aquifer, it must be treated in such a way that the metal concentrations fall below the standards for surface water or potable water. The above-ground treatment must lower the metal content to below the standards for surface water or drinking water. As these discharge standards are very stringent, a very expensive treatment will be necessary. If, after treatment, the water quality is inferior to the drinking water quality it has to be discharged into sewers or as surface water.

2.1. PUMP AND TREAT

Pump and treat methods are relatively easy to implement and control, but consume large pump energies, require high treatment costs and produce large amounts of waste products and water that must be discharged. A high volume

discharge of water to the surface water can result in problems with ecotaxes. Also high-rate aquifer pumping can result in decreasing water tables, causing undesirable drainage of land or land subsidence.

Still, in some cases, pump and treat technology can be of interest for treatment of contaminant sources. An example is the full-scale groundwater treatment based on sulfate-reducing bacteria (Webb et al., 1998; Weijma J. et al., 2002; Greben et al., 2000). The system is composed of an UASB (Upflow Anaerobic Sludge Blanket) reactor with a three phase separator on the top. The excess of hydrogen sulfide that is produced is oxidized in a biological sulfide oxidation reactor. In this reactor, the bacteria and the sulfur adhere to the packing material until shear forces caused by the stirring action of the forced air stream detach the solids. Oxygen for the reaction is supplied as air and the carbon source (carbon dioxide) and nutrients (N, P) are present in the water. At high redox potentials and high oxygen levels, sulfide can be further oxidized to sulfate. To avoid an increased concentration of sulfate in the water, this reaction is minimized by controlling the supply of oxygen. A tilted-plate settler removes the solids that consist of sulfur produced in the biological oxidation process and metal sulfides and biomass flushed along with the effluent. A DynaSand filter further removes the suspended solids from the water. The method efficiently removes metals from the water, e.g. from 230 mg Zn/l to < 0.3 mg Zn/l. This THIOPAQ[®] system has proven to be reliable in treatment of sulfate containing, metal-contaminated water up to a scale of 400 m³/h for more than 10 years.

Another biological treatment method is based on the use of microorganisms that induce biosorption and bioprecipitation on their surface. Biosorption is a biological treatment method which involves the adsorption of metals onto biomass such as algal, fungal or bacterial cells that can be dead or alive. If large-scale, inexpensive production techniques for the biomass are developed, this heavy metal treatment is promising.

Special biomass production can be avoided in the MERESAFIN (Metal Removal by Sand Filter Inoculation) process (Pümpel et al., 2001a; Diels et al., 2001). In this, bacteria able to biosorb or bioprecipitate heavy metals grow in a biofilm on a supporting material (e.g. sand). During contact with heavy metal-containing wastewater the biofilm adsorbs the metals. Subsequently the metal-loaded biomass is removed from the supporting material by the sand filter airlift and the resting biomass residual on the substratum can be re-used, after re-growth, for a subsequent treatment cycle. The supporting material can be sand or other materials retained within a moving bed sand filter which is based on a counter-flow principle (Figure 2). The water to be treated is admitted through the inlet distributor (1) in the lower section of the unit and is cleaned as it flows upward through the sand bed, prior to discharge through the filtrate outlet (2) at the top. The sand containing the heavy metals bound to the biofilm is conveyed

from the tapered bottom section of the unit (3) by means of an airlift pump (4) to the sand washer (5) at the top. Cleaning of the sand starts in the pump itself, in which metal-loaded biofilms are separated from the sand grains by the turbulent mixing action. The contaminated sand spills from the pump outlet into the washer labyrinth (6), in which it is washed by a small flow of clean water. The metal-loaded flocs are discharged through the washwater outlet (7), while the grains of sand with a partly removed biofilm are returned to the sand bed (8). As a result, the bed is in constant downward motion through the unit. In this concept water purification and sand washing both take place continuously, enabling the filter to remain in service without interruption. In such a complete water treatment system groundwater is pumped through the Astrasand filter and purified. The wash water, containing the metal-loaded biomass, is drained to a lamella separator or settling tank. The water, coming from the thickener, is reintroduced in the sand filter. The sludge coming from the thickener is treated further in a filter press of lime. The filter cake (30% dry weight) obtained in this way, containing the metals (in some cases up to 10%), is recycled in a pyrometallurgical treatment facility (shaft furnace) of a non-ferrous company (Woebking and Diels, 2000).

- 1. Inlet distributor
- 2. Outlet
- 3. Dirty sand
- 4. Air-lift pump
- 5. Sand washer
- 6. Washer labyrinth
- 7. Wash water outlet
- 8. Cleaned sand

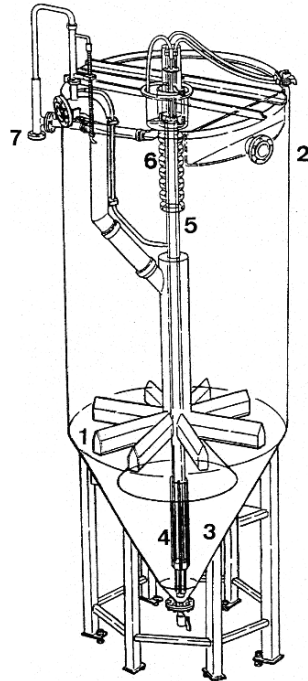


Figure 2. Moving bed sand filter concept (Diels et al., 2003)

Several other treatment technologies have been tested and prove OK at full scale or at pilot scale and described by Pümpel and Paknikar (2001). At the Homestake Mine at Lead, South Dakota, a rotating biological contactor (RBC) was immobilized with bacteria from the genus *Pseudomonas* that grow predominantly in biofilms. They are responsible for the degradation of free and metal complexed cyanide and thiocyanate and for the removal of heavy metals by biosorption in an aerobic process. Due to the slightly alkaline pH (7.5 to 8.5) and HCO_3^- produced within the biofilm there is a strong likelihood that precipitation of metal hydroxides and carbonates also contributes to metals removal following destruction of the metal-cyanide complexes.

The METEX[®] anaerobic sludge reactor is a cylindrical, Upflow Anaerobic Sludge Blanket (UASB) reactor filled with anaerobic sludge from standard sewage treatment plants. Slowly moving stirrers prevent the formation of short-circuit channels through the sludge bed, but keep the desired vertical gradients. From bottom to top subsequent zones with different metabolic activities and also different groups of microorganisms may develop in the sludge bed, depending on nutrients and on the electron acceptors available in the water (e.g. aerobic, denitrifying, sulfate reducing zone). The anoxic sulfate reducing zone is the most important one with respect to heavy metal removal in the METEX reactor, promoting the formation of highly insoluble metal sulfides. Further, bioprecipitation of metal carbonates, and biosorption/adsorption of dissolved metal species were shown to contribute to the overall metal removal process. The Bio-substrat[®] anaerobic micro-carrier reactor (Füerst and Burggräf, 2000) is similar to the METEX reactor but differs in two major aspects: the slowly stirred upflow reactor is filled with a granular microcarrier material with high sorption capacity (zeolite), and natural microorganisms, which have been adapted to the particular (ground)water matrix, are grown on the micro-carriers instead of using anaerobic sludge.

Wagner-Döbler et al. (2000) have demonstrated a bioreactor inoculated with a mixture of seven mercury-resistant, non-pathogenic *Pseudomonas* strains, isolated from mercury-rich environments. This reactor was especially designed for treatment of mercury-contaminated water.

2.2. SOIL WASHING AND FLUSHING

Metals, dissolved in the groundwater, are in equilibrium with the aquifer material which has a certain metal sorption capacity depending on the mineralogy and organic matter content. Since water solubility and desorption rates control metal removal from aquifers during pump and treat, additives are sometimes used to enhance water solubility and removal efficiencies. Metal desorption rates can be increased by a factor of more than 100-times by soil

washing and flushing techniques. Soil washing and *in situ* flushing involve the injection of water with or without additives including organic and inorganic acids, sodium hydroxide (which can dissolve organic soil matter), water soluble solvents, e.g. methanol, non-toxic cations, and complexing agents, e.g. such as ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetate (NTA). High clay and organic matter are particularly detrimental. Once the water is pumped from the soil, it must be extracted and then treated to remove the metals in wastewater treatment facilities or re-used in the flushing process (Mulligan et al., 2001). In general, soils with low contents of cyanide, fluoride and sulfide, a CEC of 50-100 meq/kg and particle sizes of 0.25-2 mm, with contaminant water solubility larger than 1000 mg/l, can be most effectively cleaned by soil washing (Mulligan et al., 2001).

Several groundwater treatment technologies exist such as sodium hydroxide or sodium sulfide precipitation, ion exchange, activated carbon adsorption, ultrafiltration, reverse osmosis, electrolysis/electrodialysis and biological systems (Patterson, 1985). As mentioned earlier, biosorption and bioprecipitation methods can be used. Merten et al. (2004) used *Escherichia coli* and fungal (*Schizophyllum commune*) biomass to adsorb uranium and rare earth elements from seepage water from a former uranium mining site in Eastern Thuringia in Germany.

A special approach is necessary when organic complexing agents (e.g. EDTA, NTA, sophorolipids) are used. The treatment method can be based on bacterial breakdown of the organic component followed by adsorption of the metal to the biomass in the water treatment plant. The problem is that very often these complexes are stabilized by the metal and biodegradation, normally feasible with the sodium or magnesium complex, will be prevented by the metal. However, in some cases bacteria can be isolated that are able to cleave heavy metal-organic complexes (Francis and Dodge, 1993).

3. *In Situ* Treatment Methods

Heavy metals can occur in several forms in the groundwater and on the aquifer. In many cases the aquifer-groundwater zone is stratified, having layers that are aerobic, nitrate-reducing, iron-reducing and/or sulfate-reducing depending *on site*-dependent circumstances. *In situ* treatment aims at immobilizing the metals in the aquifer. The methods used to cause precipitation determine the exact reaction mechanisms occurring, which in their turn determine the long-term stability of the metal precipitates. The technique is only acceptable as a viable remedial option if very stable precipitates are formed.

To determine the speciation of metals in aquifers, specific extractants are used since they are supposed to solubilize metal fractions present in different chemical environments (each with a specific availability/solubility). By

sequentially extracting the aquifer with solutions of increasing strength, a more precise evaluation of the different fractions can be obtained (Tessier et al., 1979). An aquifer is shaken over time with a weak extractant, centrifuged, and the supernatant is removed by decantation. The pellet is washed in water and the supernatant removed and combined with the previous supernatant. Next the procedure is repeated with a stronger extractant. Extraction reagents can be (from weak to strong): water, $MgCl_2$, sodium acetate, hydroxylamine and ammonium acetate, HCl. This procedure allows determination of the leachable/exchangeable fractions, carbonate fraction, Fe-Mn-oxide (reducible) fraction and organic fractions (oxidizable fraction).

3.1. NATURAL ATTENUATION

Natural attenuation is a process in which metals are immobilized by naturally-occurring chemical, biological and physical processes. Metals can be complexed by binding to carboxylic or phenolic groups of humic acids ($Fe > Cu >> Zn >> Mn$) and can precipitate as hydroxides, oxides, carbonates, phosphates and sulfides. Microbial sulfate reduction can lead to the precipitation of metal sulfides: some metalloids such as As can co-precipitate in Fe_2O_3 or MnO_2 . Other metals can adsorb to $Fe(OH)_3$. Metals can also be taken up by plants. Suspended or colloidal materials can be filtered by their passage through the soil matrix while alkalinity generation by, e.g. dolomite or calcareous materials, can lead to pH increase and subsequent metal precipitation. Microorganisms can also play an important role in adsorbing or bioprecipitating metals and also influence the toxicity and speciation. Microorganisms can oxidize some metals such as iron and manganese and make them insoluble. Arsenic can be oxidized from arsenic (III) to arsenic (V) making it less toxic. Arsenic (V) will also co-precipitate on/with iron. Chromium (VI) can be reduced to chromium (III) which is more insoluble and less toxic.

The consulting company Tauw developed (with the support of SKB in the Netherlands) an expert system called BOSS for evaluation of natural immobilization of heavy metals in aquifers. This system requires the following parameter inputs: $Fe^{2/3+}$, SO_4^{2-} , HCO_3^- , DOC, soil organic matter, clay, and iron and aluminum oxides (Steketee, 2004).

3.2. *IN SITU* BIOPRECIPITATION

In situ metal (bio)precipitation (ISMP) is the process in which sulfate-reducing bacteria are grown by the addition of electron donors as molasses, lactate, HRC[®] (Koenigsberg et al., 2002), MRC[®] (Koenigsberg, 2002), ethanol and/or other carbon sources. The bacteria oxidize the electron donor and use the

released electrons to reduce the sulfates present in the water. The formed sulfides cause a precipitation of the metals from solution (Hao, 2000; Janssen and Temminghoff, 2004).

A few conditions are required for the process. Sulfate reducing bacteria must be present in the aquifer. Sulfate must be present in sufficient concentration (mostly > 200 mg /l). A not too extreme pH (5 – 8) is necessary as is a minimum content of nutrients (N and P), no oxygen and a low redox potential. The general principle is presented in Figure 3. The following reactions can take place:

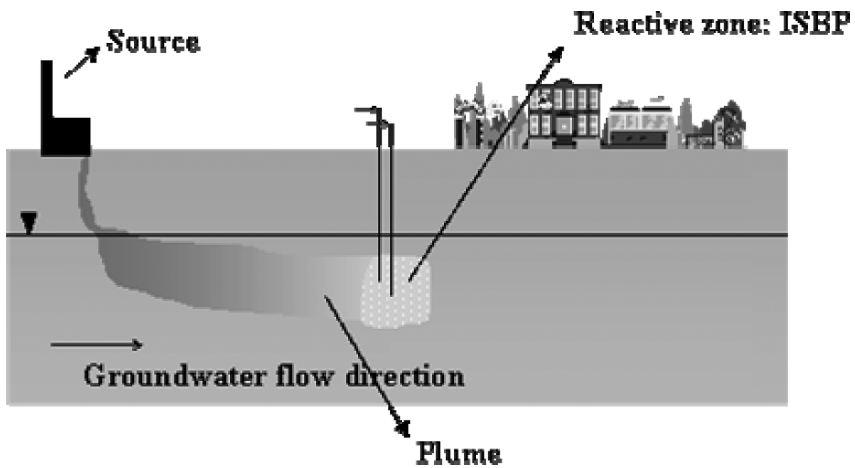
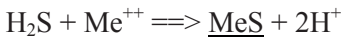
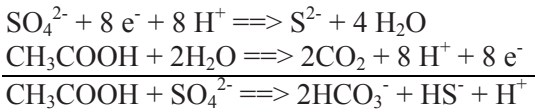


Figure 3. General principle of an *in situ* bioprecipitation (ISBP) treatment method

Several lab-scale tests (batch and column tests) are currently available to study the feasibility of the process. However, only a few field tests have been performed up to now. One field test was at a non-ferrous industrial site in Belgium, with groundwater contamination of Cd, Zn, Ni and Co. Another site in Belgium, contaminated with chromium (VI), was treated by molasses injection in order to reduce chromium (VI) to chromium (III). A third demonstration was obtained at a metal surface treatment site, contaminated by Zn, in Dieren in The Netherlands.

The following part will give a summary of results and important parameters (Diels et al., 2005a). The sulfate concentration is important in relation to the electron donor. If the sulfate concentration is low ($< 200 \text{ mg SO}_4^{2-}/\text{l}$) the sulfate-reducing process will not start unless a sulfate-reducing inoculum is added. Further, at low sulfate concentrations the sulfate reduction could only be started if hydrogen was added as electron donor. The electron donor concentration is important since an excess can lead to methanogenic conditions. In addition, high concentrations of acetate could lead to inhibition of certain SRB strains. Moreover, the use of acetate always leads to a very slow precipitation process because only few SRBs have the ability to use acetate as carbon source. High concentrations of molasses can cause a pH decrease due to fermentation processes. A lowering of pH can lead to metal release from the aquifer into the groundwater. High molasses concentrations also lead to the complexation of chromium (III) and hence to its solubilization. A wide range of electron donors has been proved useful in the process, varying from expensive pure substrates such as ethanol, lactate (Hammack and Edenborn, 1992), and hydrogen (Van Houten et al., 1994) to economically more favorable waste products, with or without enrichment with pure substrates or inoculation with monocultures or media (manure, sludge, soil) containing SRB (Maree and Strydom, 1987; Prasad et al., 1999; Annachhatre and Suktrakoolvait, 2001).

Sulfate-reducing bacteria can be enriched at a pH between 4 and 8. At neutral pH, SRB from different origins could be detected (Groudev et al., 2005a). At low pH only *Desulphotomaculum* and *Desulphorosinus sp.* could be detected (Geets et al., 2004). Molecular biology techniques can be used to identify the SRB and study their diversity. Either 16 S rRNA gene-based primers can be used or *dsr*-based (dissimilatory sulfite reductase) primers, one of the prime enzymes in sulfate reduction. It has been concluded that only by using *dsr*-based DGGE a real biodiversity could be observed. Temminghof and Janssen (2005) stated that at a location in The Netherlands (Dieren) with an initial pH of 3.9, the ISBP process could only be started after addition of an SRB inoculum. A redox potential $< -200 \text{ mV}$ is necessary to grow the SRBs. Temminghof and Janssen (2005) used Na_2S to reduce the E_h . Diels et al. (2005b) used other redox manipulating compounds to reduce the E_h , especially in low pH conditions.

Janssen and Temminghoff (2004) discuss the need for specific SRB medium to stimulate the growth of SRB. This indicates that, in specific cases, nutrients (N and P) need to be added and sometimes also other trace elements. From our experience, we can say that at appropriate redox potential and pH, the sulfate reducing process can be induced in nearly all aquifers.

The ISBP process was investigated for Cu, Zn, Cd, Ni, Co, Fe, Cr, and As. The first field tests showed that ISBP is feasible as a strategy for sustaining groundwater quality (Ghyoot et al., 2004). However, some questions remain to be answered, especially about the pH decrease due to molasses fermentation, the stability of the Ni and Co precipitates and the type, amount and injection frequency of electron donor.

In order to define the stability of the immobilized metals it is important to analyze the metal precipitates by sequential extraction (Tessier et al., 1979). A detailed study was produced by Diels et al. (2005b). A summary of these results is given below. An aquifer from a non-ferrous contaminated site was used in a column study. Groundwater from the site was pumped over the aquifer-loaded columns over two years. The columns were treated with different electron donors including molasses and lactate. Besides lactate, lactate containing additional nutrients N and P (a mixture of ammonium nitrate and orthophosphate) was also added in order to avoid nutrient limitation for the SRB population. A control column without electron donor was also operated which was representative of a natural attenuation process (NA). In the electron donor amended columns, the redox potential decreased to -250 mV, the initial pH of about 4 went up to 6 and most of the metals were removed. In the NA column metals were not removed. Different carbon sources like molasses, HRC[®] and lactate (with or without N/P nutrients) promoted sulfate reduction within 8 weeks, with average sulfate-removal efficiencies of 50%. At the same time, substantial removal of Cd, Zn (at least 75%) and Co (at least 45%) took place, whereas attenuation of Ni was still difficult and unstable. In the following weeks, molasses failed to maintain sulfate reduction, and metal concentrations quickly increased in the column effluent. After 15 weeks, the sulfate reduction process in the HRC[®] amended column also showed a decreasing efficiency of metal removal, whereas the ISBP process seemed to be stable in the lactate-amended columns (Vanbroekhoven et al., 2005a). Table 1 presents the results of the sequential extraction for Zn of the column operated with molasses as substrate and compares the results of sequential extraction of Zn of the aquifer at T0. At T2, after two years of operation, the columns were stopped and samples were taken at four places (C1, C2, C3 and C4) in the column. C1 is the inlet of the column and C4 is the outlet, and C2 – C3 intermediate. Different extractions were made in order to define the speciation of the metal: leachable fraction (extraction with water), exchangeable fraction (extraction with $MgCl_2$), carbonate fraction (extraction with sodium acetate), Fe-Mn oxide fraction or reducible fraction (extraction with $NH_4OH.HCl$), organic or oxidizable fraction (extraction with NH_4 -acetate) and the residual fraction. The sum of all the

fractions was compared with a second sample treated by *aqua regia* (HCL and HNO₃) in order to control the mass balance. It was found that the mass balance was good since the aquifer was not homogeneously contaminated. There was a large difference (increase of metal deposition) between T0 and T2 as during those two years metals were precipitated continuously on the aquifer. The metal concentrations were highest at the inlet (C1) of the column as there the oxygen was first consumed and redox potential decreased first. Leachable, exchangeable and carbonate fractions were decreased compared to the original situation. This indicated that all metals had moved into a more tightly-bound precipitate. Nearly all the metals were found in the reducible (Fe-Mn oxide) and mostly in the oxidizable fraction (organic). In fact, the metal sulfide precipitates were expected in the oxidizable fraction.

TABLE 1. Sequential extraction of Zn (mg/kg dm) from aquifer derived from the column operated with molasses as electron donor

Sample	Leachable	Exchangeable	Carbonate	Fe-Mn oxide	Organic	Residual	Total Seq. Ext.	Total Extraction
T0	227	809	93	144	69	75	1417	1630
C1	71	262	51	689	3 153	356	8583	8400
C2	80	308	38	625	2 783	287	4121	4520
C3	77	281	41	571	1 536	201	2708	3410
C4	42	155	20	422	2 647	260	3546	3950

T0 is the metal distribution over the different fractions at the start up of the column experiment at time zero. C1, C2, C3 and C4 present the sequential extractions of Zn in the columns under respectively abiotic conditions, natural attenuation, with molasses and fed with lactate (+N/P).

Table 2 presents a comparison of sequential extractions for four columns operated under abiotic conditions, natural attenuation conditions or with molasses or lactate (+N/P) as the electron donor. The leachable and exchangeable fractions were stable in the NA column and were reduced in the molasses and lactate columns. However the reduction was always higher in the lactate column compared to the molasses column. The same was true for the carbonate fractions, but the differences were smaller. It was also observed that the Zn was precipitated in the reducible fraction and the highest amount was recovered from the oxidizable fraction (ZnS). However, for Ni, this was recovered in the reducible and mostly in the oxidizable fraction in the lactate-amended column. Only very small amounts of Ni could be recovered from the aquifer of the molasses-amended column. Here a large difference was observed between molasses and lactate as electron donors. Lactate always tends to precipitate the

metals in a more stable form and this was especially true for Ni. Ni removal and stable precipitation is rather poor in the case of molasses-amended columns while optimal conditions could be obtained with lactate. A complete discussion of these results is presented in Diels et al. (2005b).

TABLE 2. Sequential extraction for Zn and Ni from aquifer-derived material from columns operated with molasses or lactate as electron donor compared to natural attenuation

	Zn (mg/kg dm)			Ni (mg/kg dm)		
	NA	Molasses	Lactate N/P	NA	Molasses	Lactate N/P
Leachable						
T0	227	227	227	81	81	81
C1	267	71	16	93	77	103
C2	306	80	1	111	75	35
C3	292	77	1	108	75	12
C4	293	42	2	107	76	10
Exchangeable						
T0	809	809	809	22	22	22
C1	763	262	45	23	23	81
C2	890	308	0	31	37	46
C3	828	281	0	62	46	19
C4	720	155	0	21	39	17
Carbonate						
T0	93	93	93	22	22	22
C1	119	51	83	23	23	81
C2	124	38	9	31	37	46
C3	191	41	6	62	46	19
C4	75	20	2	21	39	17
Fe-Mn oxide						
T0	144	144	144	5	5	5
C1	189	689	445	189	11	126
C2	199	625	430	7	11	95
C3	198	572	381	7	12	39
C4	265	423	228	26	16	32
Organic						
T0	69	69	69	3	3	3
C1	69	3153	2652	4	6	3425
C2	83	2783	1594	5	7	1706
C3	91	1536	684	5	10	794
C4	99	2647	1217	8	44	530

In situ precipitation of metals from contaminated groundwater by acceleration of biogeochemical processes that may occur naturally, is a promising sustainable technology to remediate sites polluted by metals. However, often these aquifers contain high concentrations of Fe, presumably present as Fe(III) minerals which may compete with the *in situ* bioprecipitation process by sulfate-reducing bacteria. Based on thermodynamics, microbes are supposed to use the electron acceptors resulting in the highest energy yield. Once oxygen is completely used or becomes limiting, microbes utilize nitrate, followed by iron and later on sulfate. Dissimilatory iron reduction has indeed often been assumed to be a competitive process in our studies since high concentrations of Fe-likely to be Fe(II) at neutral or slightly acidic pH have been measured in the groundwater used in ISBP process. Often it could be found that the iron reduction took place in the beginning of the stimulation of the process at redox potentials higher than -250 mV. In fact by using specific 16S rRNA gene fragments as primers, Geobacteriaceae could be detected (Vanbroekhoven et al., 2006). In the *in situ* processes the alkalization, as a result of the iron or nitrate reduction process, aided in the precipitation of the metals as hydroxides or carbonates and explains why not always the metals are found in the oxidizable fraction but sometimes in the reducible fraction.

3.3. PERMEABLE REACTIVE BARRIERS

Permeable Reactive Barriers (PRB) are installed perpendicular to the groundwater flow direction. The barrier is composed of a reactive material that allows the removal of the pollutant (e.g. heavy metals) from the groundwater. The PRB filler material can be an adsorbent (e.g. silicates, zeolites, hydroxyapatite) to which the metals can bind via ion exchange to functional groups or precipitate via ligand complexation processes (Benner et al., 1997; Waybrant et al., 2002). The material can also be limestone that leads to precipitation of metals due to pH-neutralization. The PRB filler material can also be zero-valent iron. This material can remove metals by the processes of reduction, sorption or cementation. In reduction, metals can be reduced by the electrons that escape due to oxidation of the iron. This is the case for the reduction of soluble Cr (VI) to the less soluble and less toxic Cr (III) (Powell et al., 1995). Uranyl ions [U(VI)] can be reduced to non-soluble uraninite [U(IV)]. Metals can also just sorb onto the iron surface. Metal ions like Cu^{2+} , Ni^{2+} and Cd^{2+} can be reduced to their zero-valent form by oxidation of the iron at the same time and allowing the electrons to travel from the iron to the metal. This process is known as 'cementation'. Diels et al. (2005a) compared different materials to induce cementation. An organic material such as peat was compared with an iron oxide (Ferrosorp), zero-valent iron (ZVI), hedulite (a titanium oxide waste product)

and a commercial arsenic-sorbent ('P.As'). The analysis was carried out after 6 hours incubation. Metals could be removed from the leachate by Ferrosorp, ZVI and 'P.As'. In the case of hedulite it was observed that some metal (Zn) was leached into the water. Other newer results were presented by Van Roy et al. (2005a). Munro et al. (2004) described a PRB filled with Bauxsol™ mixed with sand (in order to keep a high permeability). Bauxsol™, a product made from seawater-neutralized red mud (a by-product of alumina refining) buffers the pH at 8.8 and has been shown to remove > 99% of heavy metal loadings > 1000 meq/kg, which would make it an ideal medium for PRB. Bastiaens et al. (2005) proposed an alkalinity generating PRB, based on crushed limestone, which can help to increase the pH in order to start biological reactions.

On the other hand, PRB can be filled with organic materials or combinations of materials in order to allow bacterial processes to take place. These processes involve sulfate and iron reduction, oxidation and reduction processes and adsorption processes. Groudev et al. (2004, 2005a) studied the removal of uranium and other heavy metals from an acid mine drainage contaminated groundwater in a so-called "Multibarrier" system. Gilbert et al. (2002) have described the combination of several materials in a PRB for treatment of a metal contaminated groundwater.

3.4. COMBINED CHEMICAL AND MICROBIAL PROCESSES

Acid mine drainage (AMD) waters contain a mixture of contaminants. For example this is the case at the Curilo deposit near Sofia in Bulgaria. The AMD contains radionuclides (uranium, radium), toxic heavy metals (mainly iron, manganese, copper, zinc and cadmium) and sulfates. Groudev et al. (2005a) have reported the use of a so-called 'Multibarrier' PRB in which different passive water treatment processes are combined either successively (i.e. sequentially) either mixed. The first system described was a successive combination of four units. The first unit (I) was an aerobic barrier in which most of the ferrous iron was turned into the ferric state as a result of oxidations carried out by acidophilic chemolithotrophic bacteria (*Acidothiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*). The second unit (II) was a barrier in which most of the ferric ions were precipitated as $\text{Fe}(\text{OH})_3$ as a result of chemical neutralization in the presence of crushed limestone. The third unit (III) was an anaerobic barrier for microbial dissimilatory sulfate reduction. It contained a mixture of slowly biodegradable solid organic substrates (plant and spent mushroom compost, cow manure, sewage sludge, hay) and was inhabited by a consortium of sulfate-reducing bacteria and other metabolically interdependent-microorganisms. In this barrier, the non-ferrous metals were precipitated mainly as insoluble sulfides, and uranium was precipitated as uraninite (UO_2) as a

result of the reduction of the hexavalent uranium to the tetravalent form. Radium was removed mainly as a result of adsorption by the organic matter and clay minerals present in the barrier. The effluents from this barrier were enriched in dissolved organic compounds but still contained manganese in concentrations higher than the permissive levels. In the last unit (IV), the dissolved bivalent manganese was oxidized under aerobic conditions by different heterotrophic bacteria to Mn^{4+} , which was precipitated as MnO_2 . The dissolved organic compounds were also removed by the heterotrophs inhabiting this barrier. Table 3 presents the results of the water after treatment in the permeable Multibarrier. Groudev et al. (2005b) isolated the predominant organisms occurring in the different units: Fe^{2+} -oxidizing chemolithotrophs in the first unit: nearly no bacteria were found in unit II, cellulose-degrading, sulfate reducing

TABLE 3. Metal concentrations and physico-chemical parameters of drainage water during their treatment in a Multibarrier at the Curilo deposit

Parameters	Before treatment	Effluents from the barriers			
		I	II	III	IV
pH	2.71 - 2.90	2.73 - 3.41	4.55 - 5.10	7.25 - 7.58	7.32 - 7.65
Eh, mV	(+325) - (+484)	(+488) - (+594)	(+240) - (+293)	(-235) - (-260)	(+257) - (+286)
Dissolved O_2 , mg/l	2.4 - 2.8	4.6 - 5.3	2.1 - 2.5	0.2 - 0.4	2.4 - 3.0
TDS, mg/l	1184 - 1720	1144 - 1680	820 - 1076	532 - 684	541 - 701
Solids, mg/l	28 - 59	27 - 64	46 - 104	35 - 77	37 - 71
Dissolved organic C, mg/l	0.6 - 0.9	0.7 - 1.0	0.6 - 0.8	51 - 140	14 - 21
Sulfates, mg/l	671 - 932	664 - 923	532 - 695	293 - 406	302 - 410
U, mg/l	2.40 - 3.87	2.30 - 3.61	1.64 - 2.75	<0.1	<0.1
Ra, Bq/l	0.35 - 0.55	0.35 - 0.50	0.25 - 0.35	<0.05	<0.05
Cu, mg/l	1.40 - 2.84	1.37 - 280	1.04 - 2.13	<0.1	<0.1
Zn, mg/l	12.5 - 20.8	12.2 - 19.9	11.6 - 17.0	<1.0	<1.0
Cd, mg/l	0.06 - 0.10	0.06 - 0.10	0.04 - 0.08	<0.01	<0.01
Pb, mg/l	0.28 - 0.64	0.28 - 0.60	0.25 - 0.53	<0.05	<0.05
Ni, mg/l	0.91 - 1.78	0.90 - 1.7	0.82 - 1.52	<0.1	<0.1
Co, mg/l	0.71 - 1.50	0.70 - 1.43	0.64 - 1.22	<0.1	<0.1
Fe, mg/l	257 - 590	251 - 578	21 - 41	<1.0	<1.0
Mn, mg/l	8.2 - 20.3	8.1 - 19.8	7.9 - 17.6	0.5 - 3.5	0.2 - 0.8
As, mg/l	0.23 - 0.45	0.21 - 0.41	0.14 - 0.28	<0.05	<0.05

and methanogenic bacteria occurred in unit III, and aerobic heterotrophic bacteria (with some $S_2O_3^{2-}$ -oxidizing chemolithotrophic bacteria) were found in unit IV.

In another approach Groudev et al. (2005b) combined an alkaline limestone drain with an anaerobic section. The effluent from the barrier was allowed to flow through a wetland system used as a polishing step before final discharge of the mine drainage water to surface water. The wetland removed small traces of metals by adsorption and uptake by plant material. However, plant uptake was very important in removing radium (between 110 and 610 Bequerel/kg dry plant matter). Uranium can be reduced by SRBs and also some combinations with zero-valent iron in which the iron reduces the uranium to an insoluble form of uraninite, can be mentioned as interesting new emerging technologies (Mallants et al., 2002).

The Bitterfeld site in Germany is a large industrial site contaminated by chlorinated aliphatics and aromatics. At some locations heavy metal pollution is also present. It is well known that chlorinated aliphatics can be dehalogenated under sulfate-reducing or methanogenic conditions (El Fantroussi et al., 1998). However in the simultaneous presence of CAH and heavy metals it was shown that no degradation took place, presumably due to toxicity of the metals toward the dehalogenating bacteria. Vanbroekhoven et al. (2005b) found that no detectable amounts of PCE degrading bacteria were present. The addition of a dehalogenating inoculum led to dehalogenation only in the absence of metals. Only after addition of a suitable medium to induce sulfate-reducing bacteria could CAH dehalogenation and metal sulfide precipitation take place.

A groundwater, contaminated by a landfill in Poland (Tarnowie Gory), contained zinc, copper and sulfate, and also boron, barium and strontium. The quaternary aquifer was composed either of sand or clay. In both aquifer textures, the ISBP could be used to immobilize the metals in the presence of molasses, lactate or HRC[®]. However B, Ba and Sr were not removed and even lead to release in the presence of molasses (pH decrease) on the sandy aquifers (Diels et al., 2005a; Van Roy et al., 2005b). Therefore, some adsorbents were added to the test systems, yielding a combined system of adsorption and ISBP. The results are presented in Table 4. Here Sr, and Ba were removed completely due to adsorption on zeolite and B to about 35%. In case of boron, a combination of different adsorbents was necessary to remove the different chemical forms of boron (e.g. borate). Zeolite was successful to some extent and the combination of anionic Metasorb with Apeyron PAsXP lead to relatively good B adsorption. Dolomite as an adsorbent had no effect on the removal process. It was also observed that the ISBP was slightly inhibited by the addition of zeolite. In these tests also MRC[®] (Metal Removing Compound, Regenesis) was used and similar results were obtained as with HRC[®].

TABLE 4. ISBP of non-ferrous metals and B, Ba and Sr in mesocosms containing groundwater and aquifer in combination with adsorbents

Metal	No addition	HRC [®] , MRC [®] , lactate, molasses	C-source + dolomite, diatomaceous earth	C-source + zeolite
SO ₄	-	+++	+++	+
Zn	-	+++	+++	+
Sr	-	-	-	+++
B	-	-	-	0.35
Ba	-	-	-	+++

+++; very fast; ++; fast; ± removal; - no removal

C-source = molasses or HRC[®]

4. Concluding Remarks

This chapter attempts to give an overview of the different existing or developing methods for treatment of groundwater contaminated with heavy metals or radionuclides. The classical remediation method is based on the pumping of groundwater. In some cases the efficiency of metal removal by pumping can be improved by using additives to increase the water solubility of the pollutants. In this way, the number of aquifer volumes that need to be pumped can be reduced. Different methods exist for treatment of pumped-up metal-contaminated water. This paper focused on biological methods, and sulfate reduction followed by metal sulfide precipitation is especially presented as a very efficient technology. Another technology is based on metal adsorption and precipitation properties of several bacteria. This principle was used in a moving bed reactor to allow a process of biomass withdrawal at a rate depending on biomass growth. This active bacterial process allowed a continuous growth of biomass, followed by metal binding, metal precipitation and heavy metal loaded biomass removal. It was also shown that the metal-loaded biomass could be used in a pyro-metallurgical treatment plant. Extensive attention was paid to the development of *in situ* methods to immobilize metals in the aquifer by using the sulfate reducing capacity of the aquifer microbial biomass leading to metal sulfide bioprecipitation. We described the different parameters that are important to keep the processes running and sustainable (stable precipitates). Finally some typical combinations of processes were presented, i.e. the removal of radionuclides from AMD by sulfate reduction compared with oxidation and adsorption processes or the removal of heavy metals together with boron and barium by a combination of sulfate reduction and adsorption processes. This

paper furthermore focused on the combination of the ISBP-process with other techniques and dedicated special attention to the longevity and sustainability of the process. The parameters pH, redox potential, and sulfate concentration seem to play a very important role in the induction of the sulfate reduction process. The selection of the electron donor is important to maintain the process. Combinations of electron donors may turn out to give best results, as suggested by Agathos (2005) for anaerobic dehalogenation. They suggested induction of the process with lactate to stimulate a large variety of dehalogenating bacteria. Later on, the process could be kept going by adding much cheaper methanol. Some experiments also reveal the importance of certain trace elements that may be necessary in order to render the process sustainable. This was shown by some initial results that indicated that the addition of a nitrogen and phosphorus source to lactate kept the process much more stable. It is possible that other metals or elements can become limiting too. However, the mineral aquifer can probably be a source of slowly released micro-elements. Another point that needs evaluation is the comparison between the regular injection of electron donor and slow-release based processes. In fact, all column experiments in the present study were based on simultaneous addition of the electron donor with the groundwater, so the effects of slow release versus immediate injection could not be seen. The regular injection of an electron donor has some drawbacks as it is laborious and even when fully automatically operated needs maintenance. A regular injection of a carbon source in an injection or monitoring well leads to risks related to bacterial growth on the housing of the injection well which can lead to biofouling and blocking of the filters. On the other hand, slow release compounds are more expensive but need only to be injected once or twice per year by a direct-push system (e.g. a Geoprobe system) without risk of clogging or biofouling.

As many sites are contaminated by several metals (anions and cations or radionuclides) by activities such as non-ferrous metals processing, surface treatment and mining activity (including coal mining in some cases), pump and treat methods cannot always deliver an economically acceptable solution. Therefore the development of *in situ* technology is becoming increasingly important. The results and problems encountered up to now indicate that in many cases the combination of different passive systems (also in combination with wetlands) will be necessary (a detailed account of wetlands has not been included in this review). The use of *in situ* bioremediation processes combined or not with other passive systems such as wetlands, PRB, etc. will allow an economically acceptable management of risks related to metals and acid spreading into the environment from large contaminated sites. Pump and treat technology can be useful in source-removal whereas the passive treatment systems will be more applicable for the treatment of diffuse pollution of large affected sites.

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