

Metal adsorption capabilities of clinoptilolite and selected strains of bacteria from mine water

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ABSTRACT

Small-scale mining has socio-economic advantages such as the reduction of unemployment and the general improvement of the economy. However, these operations if not properly managed or controlled have a potential to cause environmental damage, particularly with respect to the contamination of groundwater and water supplies that are not distant from where these mining activities take place. This paper focuses on metal removal from water contaminated by heavy metals emanating from small-scale mining operations using clinoptilolite and bacteria. Removal of As, Ni, Mn, Au, Co, Cu and Fe was carried out on mine water samples using original and HCl-activated (in 0.02 M and 0.04 M) natural clinoptilolite and bacterial strains (a mixed consortia of *Bacillus* strains (*Bacillus subtilis*, *Bacillus cereus*, *Bacillus firmus*, *Bacillus fusiformis*, *Bacillus macroides* and *Bacillus licheniformis*), *Pseudomonas* spp., *Shewanella* spp. and a mixed consortia of *Acidithiobacillus caldus*, *Leptospirillum* spp., *Ferroplasma* spp. and *Sulphobacillus* spp.). The purpose of the study was to compare the removal efficiencies of the bacterial strains versus natural clinoptilolite adsorbents for metal cations. The *Bacillus* consortia removed most of the metals up to 98% metal removal efficiency with the exception of nickel where clinoptilolite showed good removal efficiency. The 0.02 M HCl-activated clinoptilolite also demonstrated excellent removal capabilities with Cu, Co and Fe removal efficiency of up to 98%. Both clinoptilolite and bacteria demonstrated capabilities of removing Cu²⁺, Co²⁺, Fe²⁺, Mn²⁺, As³⁺ and Au from solution which augurs well for metal recovery from mining and mineral processing solutions, as well as in water decontamination.

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1. Introduction

The discharge of heavy metals into aquatic ecosystems has become a matter of concern globally over the past few decades. These metal pollutants are introduced into the aquatic systems mainly through industrial and mining operations causing water systems to be contaminated by species such as heavy metals. This paper focuses on contamination of aquatic systems by heavy metals emanating from small-scale mining operations. While small-scale mining has socio-economic advantages like the reduction of unemployment, such operations if not properly managed or controlled have a potential to cause environmental harm, particularly with respect to the contamination of groundwater and water supplies that are not distant from where these mining activities take place (Mulaba and Mamba, submitted for publication). The contamination of aquatic ecosystems poses health risks to human beings who may be exposed to it as well as to aquatic life. Commonly used methods for removing metals from aqueous streams include chem-

ical precipitation, lime coagulation, ion-exchange, reverse osmosis and solvent extraction (Rich and Cherry, 1987). The removal of these metals from water is costly due to the limitations of available technologies (Cohen, 2004). Physical parameters such as pH, conductivity and turbidity also affect the extent to which these metals can be removed from aqueous solutions.

The use of zeolites, especially natural zeolites, in the removal of metal ions from aqueous solution has not been largely explored. Zeolites are crystalline, naturally occurring hydrated aluminosilicate minerals of alkali and alkaline earth cations. Their structure is characterised by variable Si/Al ratios and the exchangeable ions which may be Na⁺, K⁺ and Mg²⁺ for high silica clinoptilolite or Ca²⁺ for low silica ones (Culfaz and Yagiz, 2004). Structurally, zeolites consist of a framework of aluminosilicates which is based on an infinite three-dimensional structure of SiO₄ and AlO₄ tetrahedral molecules linked together by shared oxygen. Most common zeolites are formed by alteration of glass-rich volcanic rocks with fresh water in playa lakes or by seawater (Almaraz et al., 2003). Natural zeolites, discovered in the 18th century, are three-dimensional, microporous, crystalline solids with well-defined structures that contain aluminium, silicon and oxygen atoms in a regular

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Table 1

Physical parameters of drinking water according to South African National Standards (SANS) (DWAF, 2005).

Physical parameter	Recommended concentration	Consumption period
Conductivity at 25 °C	<150 mS/m	7 years
pH at 25 °C	5.0–9.5 units	No limit
Turbidity	<1 NTU	No limit

framework (Korkuna, 2006). Natural clinoptilolite has been previously used as an ion-exchanger in hydrometallurgical processes. Zeolites can exchange the structural and the interstitial sodium for external cations in solution such as heavy metals in mine water (Thermstocleous, 1990). Also, zeolites have been employed in water treatment processes for their capability to adsorb metals (Kuronen, 2006) and are used for the concentration of Cu^{2+} , Fe^{2+} , Mn^{2+} , Ni^{2+} ions in solution (Jansen, 2006).

The use of bacteria on the other hand in metal removal/recovery is also not entirely a novel technology. Some bacterial strains have been successfully employed in ore leaching for centuries, though the mechanism remained unknown. Lots of reports have been documented on bacteria and its role in bioleaching including the oxidation of sulphur compounds to sulphuric acid resulting in acid mine drainage (Evangelou and Zhang, 1995). Further, remediation of acid mine drainage (AMD) has previously been attempted by treating acid mine water with micro-organisms (Acharya, 1990). Thus, biosorption of heavy metals is emerging as one of the most promising technologies being advanced in the removal of toxic substances from waste waters and has been receiving a great deal of attention in recent years, not only because of its scientific novelty

Table 2

Maximum allowable limits of metal species in drinking water (DWAF, 2005).

Metal	Max. allowable concentration for limited duration ($\mu\text{g L}^{-1}$)	Max. allowable concentration in ppm (mg L^{-1})	Max. water consumption period ^a
As	50	0.05	1 year
Mn	1000	1	7 years
Ni	1500	1.5	1 year
Au	Not available	Not available	Not available
Cu	2000	2	1 year
Co	2000	2	1 year
Fe	2000	2	7 years

^a Limits for the maximum consumption are based on the consumption of 2 L of water per day by a person of mass 70 kg over a period of 70 years.

but also for its potential application to diverse industries (Drogui et al., 2005). The biosorption process also exhibits several advantages such as low operating costs, high probability of metal recovery and potential biosorbent regeneration (Wase and Forster, 1997). Literature reports on the bacterial species *Pseudomonas* spp., suggest that these species remove significant amounts of heavy metals in solution through biosorption (Kumar et al., 2006).

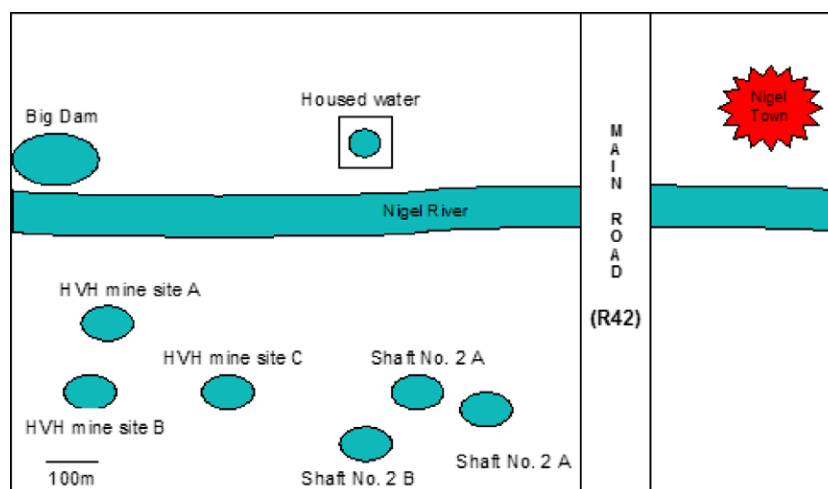
In this study, natural clinoptilolite and selected strains of bacteria were used to remove metals from mine water samples collected from selected mining sites in Johannesburg. The results obtained after removals were compared with the drinking water standards stipulated by the South African National Standards (SANS) part of which are shown in Tables 1 and 2. The values in these Tables were used as guidelines in the determination of the extent of contamination of the collected mine water samples. We used drinking water standards as a point of reference because it came to the researchers' attention that the contaminated water was being used for drinking purposes, hence it was justifiable to ascertain the extent of non-compliance.

2. Experimental

2.1. General

Water samples were collected from eight gold mining sites owned by HVH gold mining company located around Nigel Town as schematically drawn in Fig. 1. Nigel is a small mining town situated South-East of the City of Johannesburg. The sites labelled HVH are where small scale miners operate in order to recover remnant gold from previous large scale mining operations. Conductivity, pH and turbidity were measured on site. The water samples were stored in a refrigerator at a temperature of about 4 °C for 24 h to stabilize any chemical and biological activity that could be taking place. Sampling was done three times (before rains, after rains and in a dry season). They were then assayed for the initial metal content of As, Ni, Mn, Au, Co, Cu and Fe using Flame Atomic Absorption Spectroscopy (AAS).

There were notable activities observed at some sites which may have influenced the initial results obtained. The HVH sites A, B and C were the sites with lots of mining activities with site A being the most active and relatively the largest of the three sites. HVH site B was observed to have dried up when the second sampling was done. There were no lavatories of any kind around the area and the sites were exposed to faecal contamination, which could be

**Fig. 1.** Site map showing the sampling points around Nigel town.

transported as run-off during the wet seasons. The Shaft No. 2 sites are situated next to informal settlements and the contaminated water adjacent to the sites are treated as drinking water sources by the inhabitants. Activities observed in the vicinity of the Big Dam ranged from mining activities to dumping sites, as a result, the Nigel River flowing across the town becomes polluted.

2.2. Metal removal experiments

Removal of metals in water samples was carried out using as-received and HCl-activated clinoptilolite at 0.02 M and 0.04 M HCl activation concentrations and bacteria: a mixed consortia of *Bacilli* strains (*Bacillus subtilis*, *Bacillus cereus*, *Bacillus fusiformis*, *Bacillus macroides*, *Bacillus firmus* and *Bacillus licheniformis*), *Pseudomonas* spp., *Shewanella* spp. and a mixed consortia of *Acidithiobacillus caldus*, *Leptospirillum* spp., *Ferroplasma* spp. and *Sulphobacillus* spp.

2.2.1. Clinoptilolite as an ion-exchanger

In optimisation studies carried out in our laboratories when accessing the most suitable HCl concentration for the activation of the natural zeolite towards the adsorption of cobalt and copper from their aqueous solutions, it was found that 0.02 M and 0.04 M HCl-activated clinoptilolite exhibited a high removal efficiency of these metals from solution. Therefore in this study these concentrations were used as “ideal” concentrations for the activations of the zeolite for the removal of metal species from mine water. The ion-exchange processes using the clinoptilolite were conducted at room temperature. Glass columns of 2 cm diameter and 30 cm length were pre-loaded with 25 g of either as-received natural clinoptilolite or activated clinoptilolite of particle size range between 2.8 and 5.6 mm. Mine water samples were passed through each form of zeolite. These samples were afforded equal solution–zeolite contact time. The resultant solutions after having eluted through the zeolite-packed column were assayed using Atomic Absorption Spectroscopy (AAS) in order to ascertain the zeolite’s metal removal efficiency.

2.2.2. Culturing and growth of bacterial strains

Pseudomonas spp., *Bacillus* consortia and Mintek consortia.

A mixed strain culture of bacteria containing four types of bacterial strains namely *A. caldus*, *Leptospirillum* spp., *Ferroplasma* spp. and *Sulphobacillus* spp. was obtained from Mintek’s Bioleach division, (Randburg, South Africa). *Pseudomonas* spp. and *Bacillus* strains were isolated from soil and water sampled from mine dumps near Nigel Town. The *Pseudomonas* spp. were grown in *Pseudomonas* agar base while the *Bacillus* strains and Mintek consortia (*A. caldus*, *Leptospirillum* spp., *Ferroplasma* spp. and *Sulphobacillus* spp.) were grown at 37 °C (carefully controlled) in agitated liquid media containing (NH₄)₂SO₄ (1.0 g L⁻¹), KCl (0.1 g L⁻¹), K₂HPO₄ (0.5 g L⁻¹), MgSO₄ (0.5 g L⁻¹), elemental sulphur (8.5 g L⁻¹) and FeSO₄·7H₂O (7.5 g L⁻¹). Both media were sterilized by autoclaving using the HUXLEY HL 341 speedy autoclave at 121 °C for 15 min. The pH was adjusted to about 6.5 for the *Bacillus* and pH 2 for the Mintek consortia using diluted KOH solutions for the liquid media. Growth was allowed to proceed for 1, 2 and 3 days for *Pseudomonas* spp., Mintek constortia and *Bacillus* strains respectively. After one day the *Pseudomonas* spp. colonies were transferred into the nutrient broth, further incubated for 24 h, then concentrated by centrifugation and the resultant solid material was washed with deionised water. Glycerol stock cultures were prepared by adding a few drops of glycerol to the solid material followed by storage at –80 °C for further experiments. These stock cultures were defrosted by placing them at room temperature and were inoculated into nutrient broth (50 ml) with gentle shaking (100 rpm) for approximately 70 h before being utilized in further laboratory scale experiments.

2.2.3. *Shewanella* spp.

Shewanella spp. was isolated from soil samples obtained from a tailings dump at Palabora Mining Company (PMC) which is basically a copper mining company which also excavates vermiculite from surface and underground workings in Palabora. PMC is situated in the Limpopo, the Northern Province of South Africa, North East of Johannesburg. *Shewanella* spp. was then grown in nutrient broth, incubated for 24 h and then concentrated by centrifugation and the solid material obtained was washed with deionised water. Glycerol stock cultures were prepared by adding a few drops of glycerol to the solid material which was stored at –80 °C for further experiments. These stock cultures were defrosted at room temperature and inoculated into nutrient broth (50 ml) with gentle shaking (100 rpm) for approximately 70 h for further laboratory scale experiments.

2.2.4. Metal removal using bacterial strains

Metal removal experiments were performed by innoculating the mine water effluent with each of the grown bacterial species at a ratio of 1:3, 3:1 and 3:1 and metal removal rate was determined after 48 h. Flame Atomic Absorption Spectroscopy was used to analyse the water samples for the metal species. The percentage metal adsorbed by biomass was calculated as follows:

$$\% \text{ Metal adsorbed by biomass} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where C_i is the initial metal concentration and C_f is the final metal concentration.

3. Results and discussion

3.1. Physical parameters of sampled water

The physical parameters recorded during sampling, pH, conductivity and turbidity, are shown in Table 3.

The values obtained for all the physical parameters presented in Table 3 are average values obtained from three measurements at different points of the same site. Samples obtained from HVH mine site A, Nigel River, HVH mine site C and Shaft No. 2C were acidic. This could be emanating from acid mine drainage caused by the exposure of sulphide minerals, particularly pyritic and pyrrhotitic minerals to atmospheric oxygen and water. Conductivity was very high compared to the recommended limits, especially in drinking water. This suggests the presence in high amounts of anions, cations as well as organic pollutants. The sites that showed high turbidity could be as a result of dust particles or algal bloom around mine dumps. Nigel River receives seepages from most of these sites and thus the turbidity and conductivity of its water are raised and the pH is at 5.05. The fact that the river’s physical parameters are not significantly high compared to the feeding sites could be attributed to the filtration process that occurs as the water percolates the earth towards the river and the fact that river water is continuously flowing.

Table 3
Physical properties of sampled mine water.

Site	pH	Conductivity (S)	Turbidity (NTU)
Nigel River	5.05	1114	52.5
Big Dam	7.72	6.67	7.26
HVH mine site A	3.84	11.67	56.3
HVH mine site B	7.12	>3999	11.7
HVH mine site C	4.04	670	4.68
Shaft No. 2A	7.93	4.03	2.84
Shaft No. 2B	8.09	533	3.01
Shaft No. 2C	4.20	1265	3.32

3.2. Initial concentrations

The initial concentrations of the metal ions in the mine water were measured immediately after sampling and recorded. Table 4 shows the initial assay of the water samples.

Table 4

Initial concentrations of the metal ions (mg L^{-1}) in effluent mine water at selected sites.

Site	As	Mn	Ni	Au	Cu	Co	Fe
Nigel River	6	27	0	0.2	1	1	1
Big Dam	6	25	0	0.2	0	2	2
HVH mine site A	6	28	2	0.3	8	5	3
HVH mine site B	7	27	0	0.2	2	3	2
HVH mine site C	7	26	0	0.1	0	1	2
Shaft No. 2A	13	29	0	0.1	0	0	1
Shaft No. 2B	12	29	0	0.1	0	0	2
Shaft No. 2C	13	33	3	0.2	0	0	1

Considering Tables 4 and 3, the water samples from most of the sites were contaminated beyond the set SANS standards. Generally, HVH gold mine sites recorded high metal content than any other site. The most abundant compounds in the excavated gold mineral ore are pyrite, uraninite (UO_2), brannerite ($\text{UO}_3\text{Ti}_2\text{O}_4$), arsenopyrite (FeAsS), cobaltite (CoAsS), galena (PbS), pyrrhotite (FeS), gersdofite (NiAsS) and chromite (FeCr_2O_4) (Watson and Beharrell, 2006). These are most probably the sources of the metals present in the water samples collected from the gold mine sites.

3.3. Metal removal: clinoptilolite versus bacteria

The as-received and acid treated clinoptilolite and various strains of bacteria were used to remove metal ions from the eight sampling sites represented in Table 4. The next sections compare the individual metal removal capabilities of the bacterial strains and clinoptilolite from mine water containing As, Mn, Ni, Au, Cu, Co and Fe.

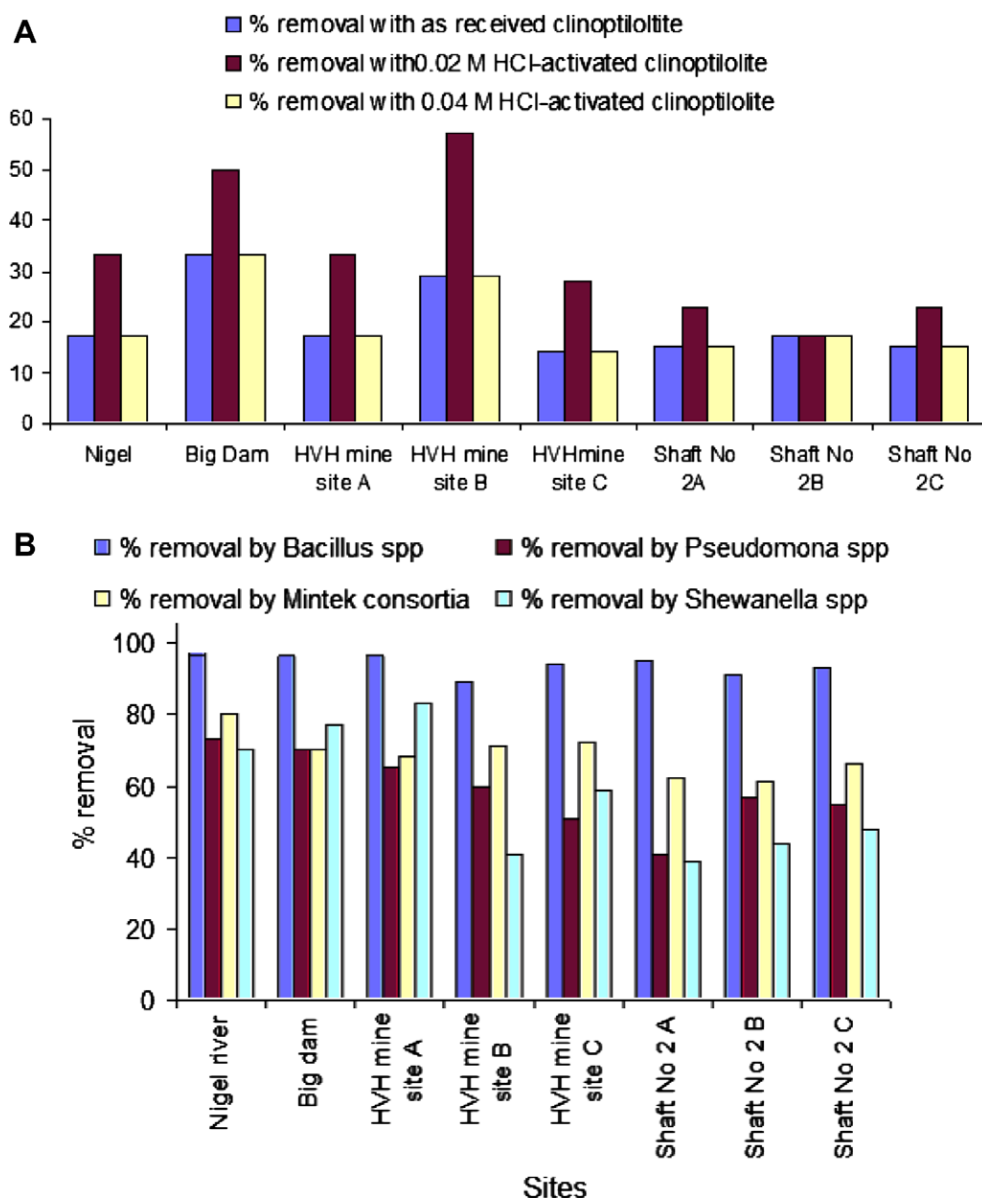


Fig. 2. (A) Arsenic removal using clinoptilolite. (B) Arsenic removal using bacterial strains.

3.3.1. Arsenic removal

Clinoptilolite and bacteria were used to remove arsenic from water which is one very toxic contaminant found in water. The removal efficiencies of the bacteria and clinoptilolite are shown in Figs. 2A and B.

The bar charts in Fig. 2A and B show results of both adsorbents in the removal of arsenic from water. Arsenic was found present in all water samples from all the sampled sites. Up to 100% arsenic was removed by the *Bacillus* strains followed by the *Shewanella* spp. and the Mintek consortia. *Pseudomonas* spp. removed this metal ion by up to 50%. Arsenic removal by *Bacilli* strains was highest (up to 100%) in samples collected from the HVH mine site A, Big Dam and Nigel River. This could be attributed to the fact that this consortium comprised six species of bacteria, each with referenced metal removal properties making the impact of the combined strains on metal removal high. *Shewanella* spp. has good toxicity tolerance properties which are demonstrated by its ability to

shorten itself when the conditions become unfavourable (Konishi et al., 2006) and the Mintek consortia on the other hand was also a mixed strain containing four strains which worked together in removing the metal ions from the water samples. The 0.02 M HCl-activated clinoptilolite showed better removal efficiency than the 0.04 M HCl-activated and the as-received clinoptilolite. This could be attributed to the fact that As^{3+} possesses stereochemically active lone pairs and prefers trigonal pyramidal soft ligand-binding sites (Touw et al., 2007).

3.3.2. Manganese removal

The results in Fig. 3A and B show that both adsorbents are capable of removing manganese in water. Manganese was present at very high concentrations in all the sampled sites. The *Bacillus* strains removed up to 96% manganese and *Pseudomonas* spp. removed 72% of Mn. The Mintek consortia and *Shewanella* spp. also showed good removal efficiency in that they were able to remove

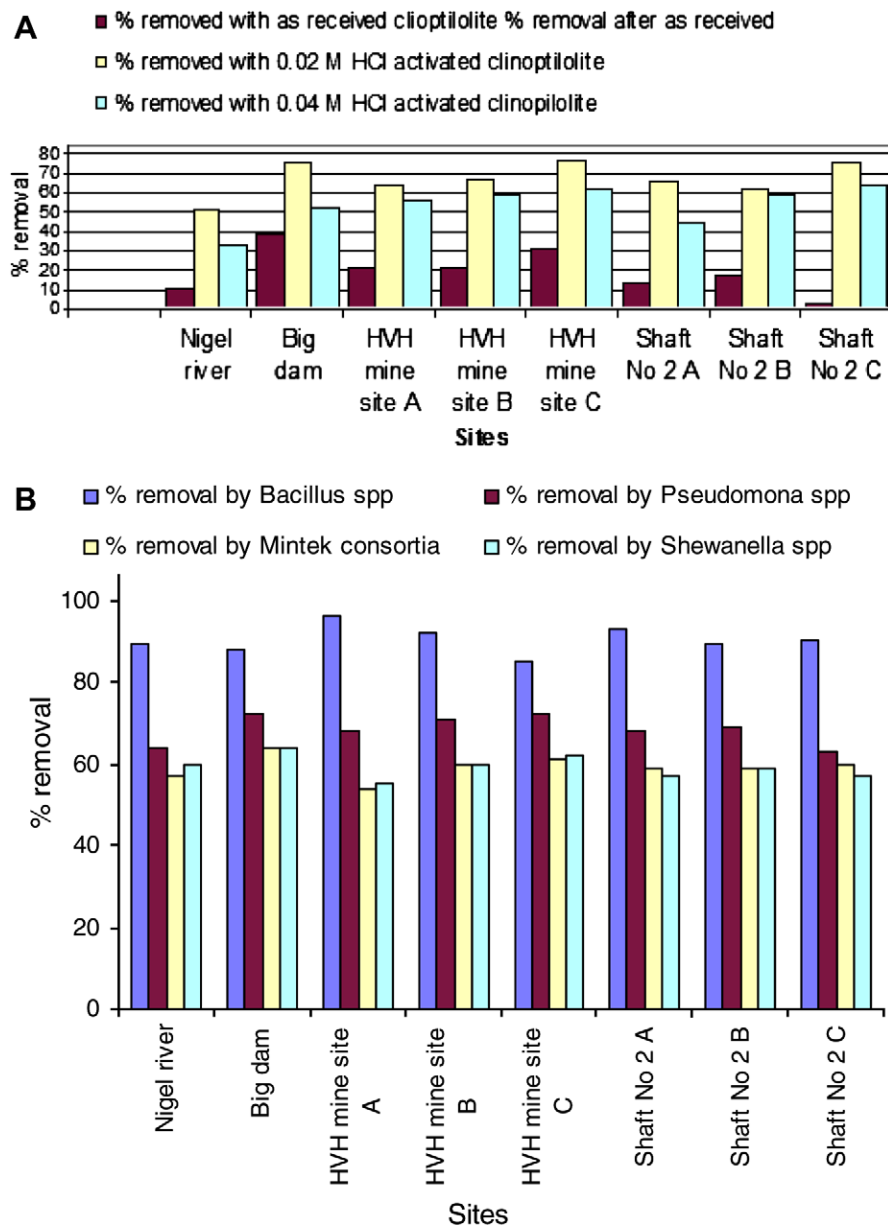


Fig. 3. (A) Manganese removal using clinoptilolite. (B) Manganese removal using bacterial strains.

about 60% manganese from the mine water samples. The metal removal by the *Bacillus* species can be attributed to the fact that the consortia consists of a mixture of strains and in the case of *Pseudomonas* spp., the absorption rate could be due to the high toxicity tolerance behaviour of this bacterial strain. *Pseudomonas* spp. isolated from tannery effluent has been used in the removal of cadmium from aqueous solutions (Srivastava et al., 2008). Cadmium is a very toxic metal and this demonstrates a high toxicity tolerance by the *Pseudomonas* spp. The concentration levels of manganese fell within the SANS maximum allowable limits after treatment with *Bacillus* strains and *Pseudomonas* spp.

The 0.02 M HCl-activated clinoptilolite showed better manganese removal efficiency (79%) than the 0.04 M HCl-activated (64%) and the as-received clinoptilolite (40%). Generally, a high oxidation state reduces the hydrated radius of the metal ion, thus making it highly favoured towards ion-exchange processes. If this is true Mn would be expected to be more easily removed than any other metal since it can have an oxidation state of up to +7. However, the fact that this oxidation state is not stable in solution cannot be ruled out. The highest most stable oxidation state that Mn has is +4, which is still higher than any of the oxidation states of any of the metals in this study. The oxidation state of the Mn in this study is most likely to be 2⁺ since the Mn removal was comparable to the other divalent ions (Cu²⁺ and Co²⁺).

3.3.3. Nickel removal

Most of the water sampled did not contain nickel with the exception of only two sites that contained this metal. Fig. 4 shows the removal efficiencies of the clinoptilolite and bacteria.

As observed in Fig. 4 the Mintek consortia together with *Bacillus* strains reduced nickel concentrations by up to 40% which fell within the acceptable levels. However none of the other strains were able to significantly reduce the nickel in the samples. This could be attributed to competition of the wider ionic radii of nickel compared to the other metal ions in solution which resulted in competition for biosorption sites causing a low uptake of nickel from the mine water.

The 0.02 M and 0.04 M acid activated clinoptilolite on the other hand removed up to 97% nickel from water in all the sites. Removal using bacteria as well as as-received clinoptilolite however, reduced the nickel concentrations to acceptable levels with reference to SANS guidelines.

3.3.4. Gold removal

All the sites sampled contained traces of gold and its removal was difficult with most of the adsorbents. Fig. 5A and B shows the removal efficiencies of the adsorbents. As shown in Fig. 5A, clinoptilolite recorded 0% removal for most of the mine water samples.

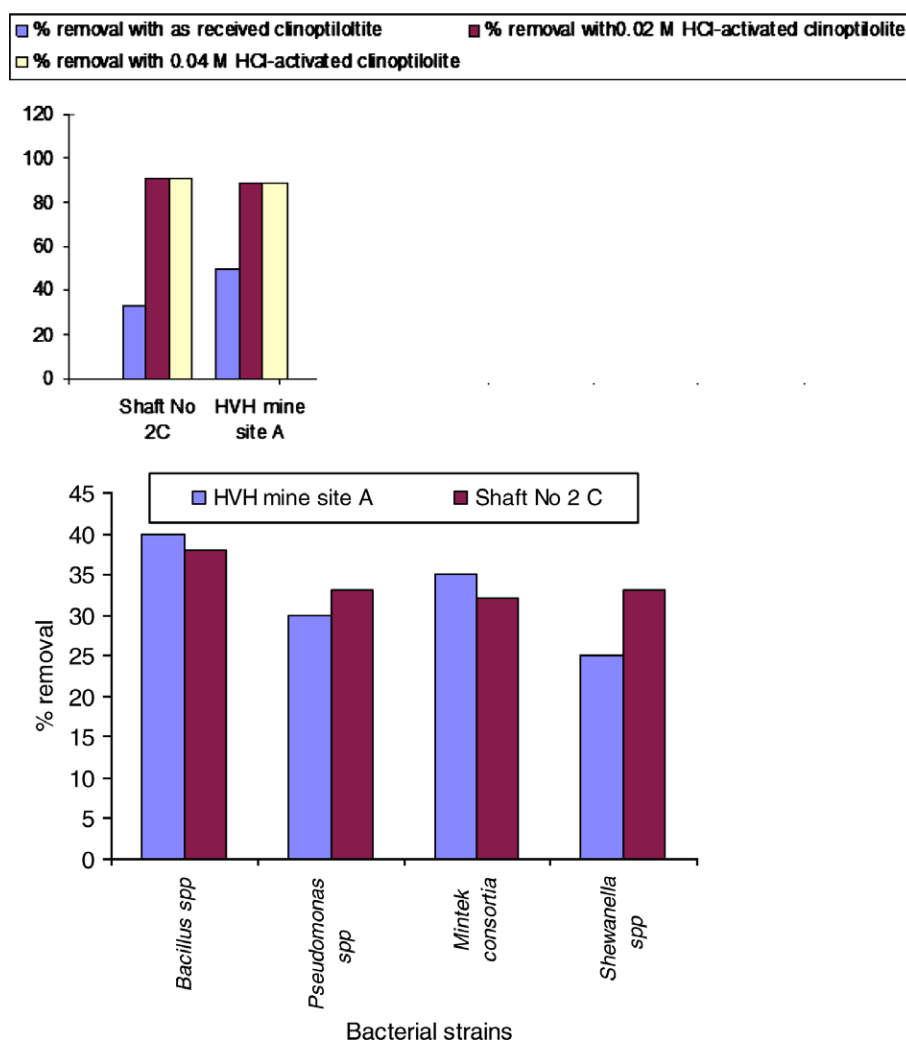


Fig. 4. (A) Nickel removal using clinoptilolite. (B) Nickel removal using bacterial strains.

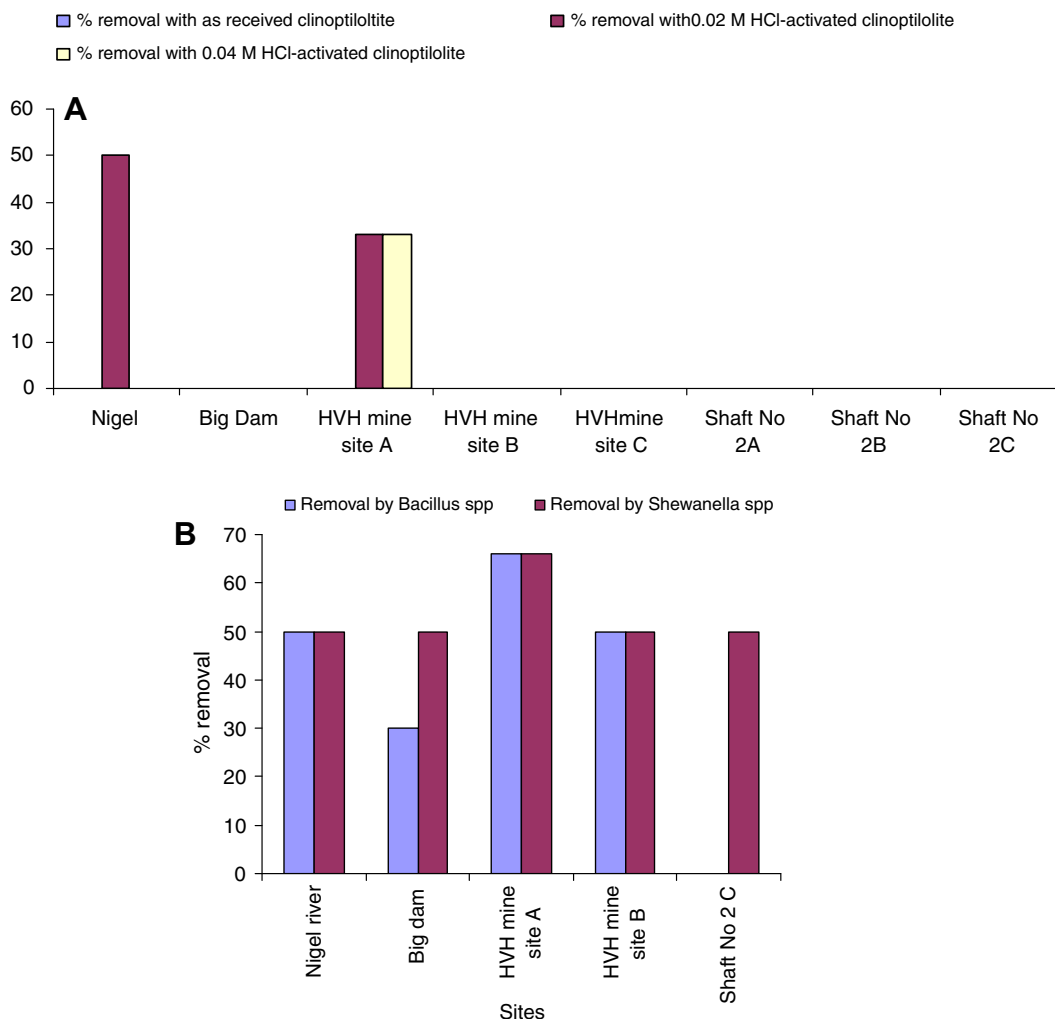


Fig. 5. (A) Gold removal using clinoptilolite. (B) Gold removal using bacterial strains.

Bacillus strains and *Shewanella* spp. were able to remove gold from the mine water. *Shewanella* spp. removed up to 67% of gold. This agrees with results obtained by Konishi and co-workers who recorded about 60% gold removal using *Shewanella* spp. from gold bearing solutions containing 0.1 M Au in solution. Gold is a noble metal and belongs to the group 1B elements; it hardly forms ions in aqueous solutions and therefore its removal by processes is not supported by a lot of mechanisms such as ion-exchange. *Shewanella* spp. was able to remove gold ions from the aqueous solution and as suggested by Konishi et al., reductive precipitation could be the mechanism by which this biosorption process occurs.

The 0.02 M and 0.04 M HCl-activated and as-received zeolites could not remove significant amounts of gold. This was expected because gold does not form ions in solution and therefore can not partake in an ion-exchange process.

3.3.5. Copper removal

Most of the sites did not contain copper and of the three sites that contained copper almost all the sites except for the highly concentrated HVH mine site A had copper reduced to acceptable levels after the use of different adsorbents (Figs. 6A and B).

Clinoptilolite removed up to 100% copper from most of the sites as shown in Fig. 6A. The strains from the *Bacillus* genus removed up

to 100% copper from the mine water while *Shewanella* spp. removed up to 90%; the Mintek consortia and *Pseudomonas* spp. also showed good removal efficiencies with up to 70% copper removed from the mine water. The *Pseudomonas* spp. showed poor copper removal capabilities in acidic conditions, as observed in the case of the HVH and River Nigel sites with low pH values. The effect of low pH on Cu^{2+} removal has also been reported in ion-exchange mechanisms using kyanite, where adsorption efficiency doubled as pH increased from 1 to 6 (Ajmal et al., 2001).

3.3.6. Cobalt removal

The results in Fig. 7A and B show that both adsorbents removed cobalt ions in water with clinoptilolite removing up to 98% of copper on average. The 0.02 M HCl-activated clinoptilolite removed 98% cobalt present in mine water samples.

Bacillus strains had a removal efficiency of up to 98% and *Pseudomonas* spp. removed up to 80% of the cobalt. The *Bacillus* strains and *Pseudomonas* spp. removed more cobalt from acidic water samples obtained from Nigel River (pH 5.05), HVH mine site A (pH 3.84) and HVH mine site C (pH 4.04). *Shewanella* spp. however thrived performed well with a wider pH range with more than 60% cobalt removed in all the sites. This could be due to the fact that *Shewanella* rods have a tendency of shortening themselves when conditions around them become unfavourable (Shewan,

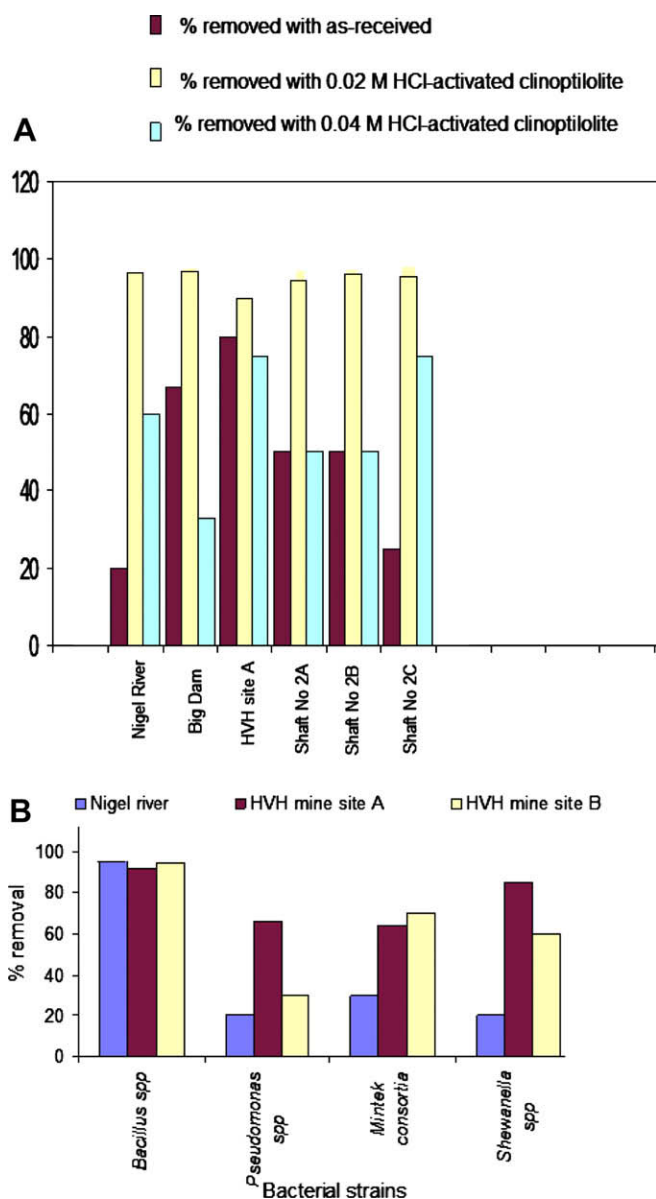


Fig. 6. (A) Copper removal using clinoptilolite. (B) Copper removal using bacterial strains.

1977). This trait enables *Shewanella* spp. to continue removing metals even at unfavourable conditions.

3.3.7. Iron removal

Iron was the most abundant metal in the mine water sampled area indicating the abundance of pyrite in the sampled area and all the sites contained this metal. Fig. 8 depicts that both adsorbents removed iron from the water.

Bacillus spp. and *Pseudomonas* spp. removed up to 90% iron whereas *Shewanella* spp. also removed up to 30% Fe^{3+} . The other bacterial species could not remove the metal ions in solution. This is probably due to the fact that Fe^{3+} has a higher coordination number which is expected to hinder the biosorption process. In the case of *Bacilli* rods, the biosorption which is the mechanism by which bacteria adsorb metals involves the contribution of other factors such as diffusion, adsorption, chelation, complexation, coordination and microprecipitation mechanisms. Thus, even though Fe^{3+} has a higher coordination number and its adsorption is expected to be hindered, *Bacillus* strains were able to remove iron effectively.

3.4. Adsorbent selectivity for the metal ions and possible mechanisms for adsorption

The occupation of active sites on an adsorbent during ion-exchange is a competitive process whereby one cation is more favoured over the other. This could be attributed to cation–cation interactions as well as with the water molecules that surround the cations in the solution. Inglezakis et al. (2005) documented that selectivity for one ion over the other in a matrix is a result of physico-chemical and stereochemical factors which are hydrated radii, hydration enthalpy of the cation and the space requirements in the micropores of the adsorbent in connection with the incoming ions. Tansel et al. (2006) (Fig. 9) also reported that every metal cation in water possesses its own specific hydrated layer which has a characteristic thickness and degree of stability which determine its susceptibility to being up-taken by an ion-exchanger.

This is such that the larger anhydrite ions (more dispersed charge), the less strongly the water of hydration is held to the cation. This leads to the conclusion that the larger the ion, the less hydrated it is and the smaller the relative size of the hydrated ion and thus it would be easily picked. Therefore, ions with large radii often show large rejections while smaller ions are often favoured by adsorbents. The Eisenman–Sherry theory also attempts to explain a zeolite's selectivity for any two competing cations with characteristics such as size, charge density and free energy of hydration (Burgess, 1978; Caputo and Pepe, 2007). According to this theory, for divalent–divalent competitors, a zeolite exhibits a greater preference for larger cations, irrespective of their framework composition. This is probably due to the coordination numbers of the cations. Heavy metals are known to form hydrated ions with high coordination numbers because of the availability of d-orbitals. The coordination numbers determine: (i) the complex matrix of the cation i.e. whether it will form a complex with its competing ions or not (ii) the type of hydrated ions that the competing cations form with water molecules and (iii) the enthalpy of hydration of the competing ions present in the matrix. All but two (Au and As) of the cations identified in this study have high coordination numbers and are known to form stable complexes with coordination numbers of 4 and 6. Complexes formed in this manner are even more stable compared to complexes formed by a 6-coordinated cation $[M(H_2O)_x \cdot n(H_2O)_x]^{n+}$, M is the cation present in solution. The strength of the bond between the water molecules and the cation depends on the coordination number of the metal in the solution. Usually hexa-coordinated cations form labile complexes compared to tetra-coordinated ones. This phenomenon is also true for the Fe cation present in these solutions. If the Fe cation present in the matrix solution has a +3 oxidation state, the Fe cation would be expected to be the most favoured than its divalent counterparts according to Tansel et al. (2006). However, the Fe cation in this matrix is from a form of pyrite since pyrite is often associated with gold mines. From pyrites, Fe^{2+} forms $[Fe(H_2O)_4]^{2+}$ in solution. By interference, this could suggest that Cu^{2+} , Co^{2+} , Mn^{2+} , Fe^{2+} and Ni^{2+} form $[M(H_2O)_4]^{2+}$ in solution which is a stable complex which is not expected to hinder the removal of the individual metals from the mine water matrix.

As mentioned earlier, Touw et al. (2007) documented that As^{3+} possesses stereochemically active lone pairs and prefers trigonal pyramidal soft ligand-binding sites which is a bulky structure and thus expected to hinder ion-exchange. This makes As^{3+} to be less favoured for absorption by the clinoptilolite in ion-exchange processes compared to the other metal ions discussed in this study. Gold hardly forms ions in aqueous solutions and therefore its removal by ion-exchange processes is not supported. The selectivity series alluded to removal by clinoptilolite can therefore, be summarised as follows: $Cu^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+} > As^{3+} > Au$.

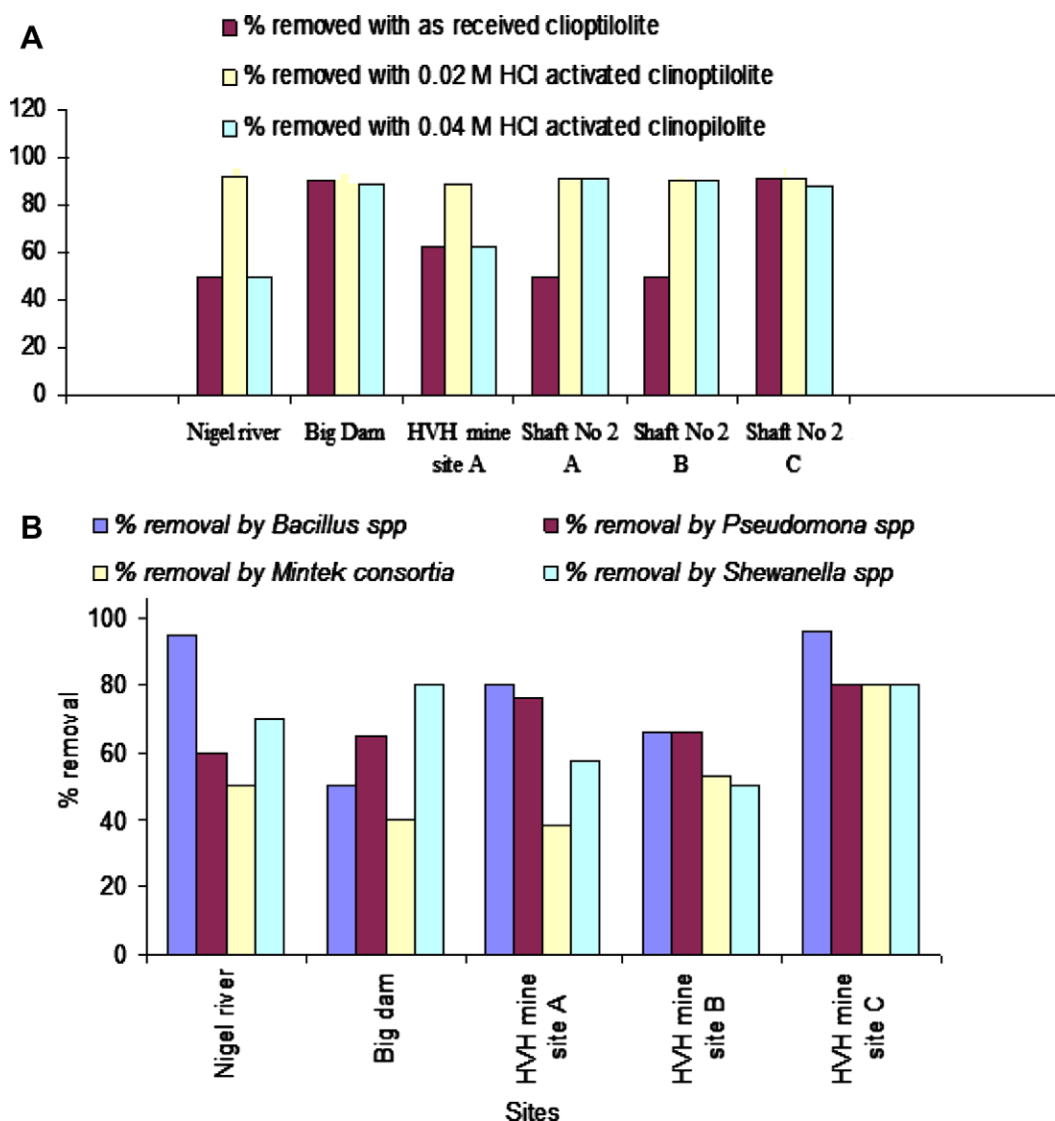
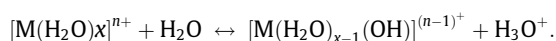


Fig. 7. (A) Cobalt removal using clinoptilolite. (B) Cobalt removal using bacterial strains.

From the results it can be deduced that the pH within the range of 3–8 favours heavy metal removal by clinoptilolite. This was evident with the more acid mine water of the HVH gold mine site (3.05). Therefore, H^+ cations could be responsible for the high efficiency of ion-exchange processes. Similar observations have been published on ion-exchange of metals by clinoptilolite (Ouki and Kavanagh, 1999; Feng et al., 2000; Panayotova, 2001; Inglezakis et al., 2003). According to these studies, there is no significant change in metal uptake in the range of acidity between pH 3 and 12, while a considerably lower uptake, up to 50% was observed in the case of Cu^{2+} at pH 3 in comparison to higher pH values. The effect of low pH on Cu^{2+} removal has also been reported in ion-exchange processes using kyanite, where adsorption efficiency doubled as pH is increased from 1 to 6 (Ajmal et al., 2001). Acidity tends to increase the efficiency of an ion-exchange process where hydration and hydrolysis occur according to the following reaction (Inglezakis et al., 2003):



At low pH the above equilibrium is shifted to the left and more highly charged metal complexes which are favoured by ion-exchange are formed.

4. Summary

The bacteria in general demonstrated higher metal removal capabilities when compared to clinoptilolite as summed up in Table 5.

Biosorption is a complex mechanism involving diffusion, adsorption, chelation, complexation, coordination and microprecipitation mechanisms. On the other hand clinoptilolite adsorbs via the ion-exchange mechanism; acid activation improves the zeolite by leaching out non-zeolitic minerals and expose ion-exchangeable sites thus increasing the zeolite's metal removal capabilities. Given these adsorption mechanisms by bacteria and zeolites it is not difficult to conclude why bacterial strains were better adsorbents.

5. Conclusions

When taking into account water scarcity and demand in view of the changes in climate due to global warming, it becomes imperative to manage the available water resources against an ever increasing demand due to population increase and industrialization, which is further made more complicated by the extent of

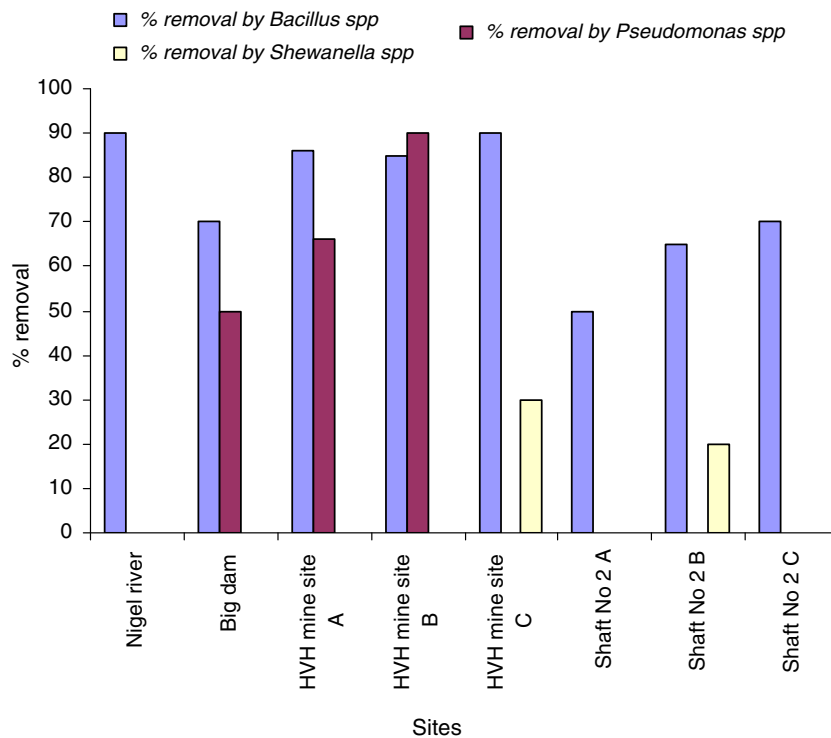
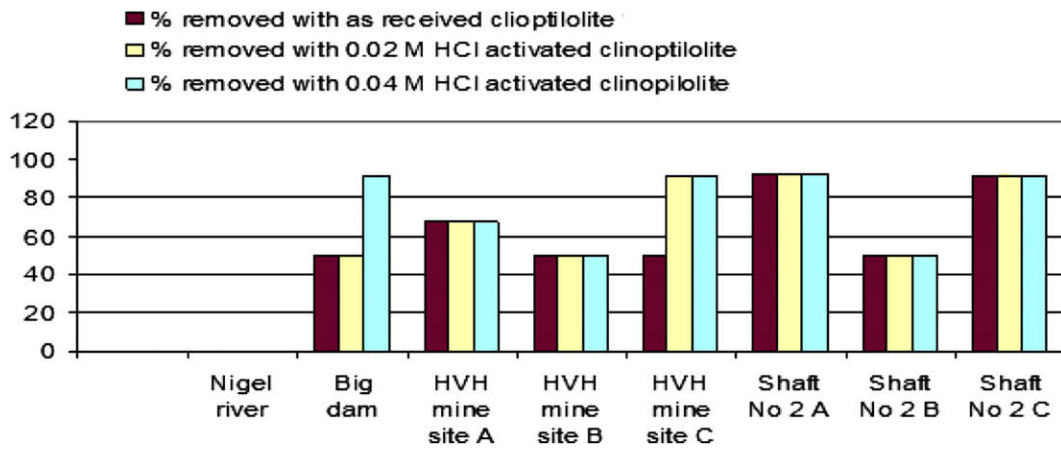


Fig. 8. (A) Iron removal using clinoptilolite. (B) Iron removal using bacterial strains.

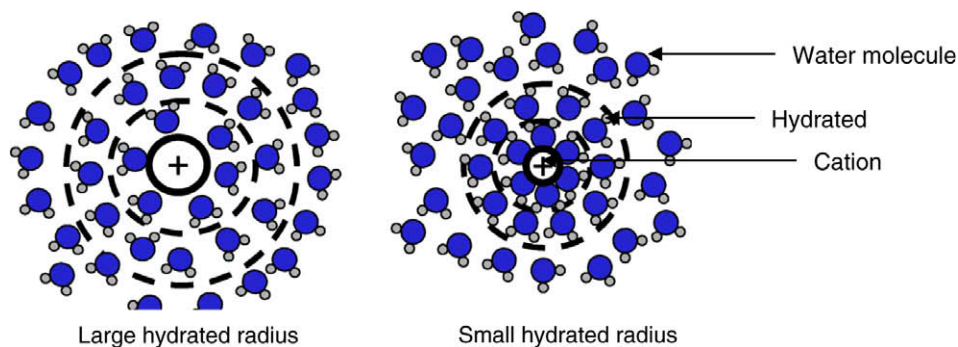


Fig. 9. The effect of hydrated radii on metal adsorption Tansel et al. (2006).

pollution of the water. The polluted water tested in this study as a result of small scale mining activities could have been used for irrigation, agricultural farming drinking by livestock and humans. However, such water should meet the minimum acceptable stan-

dards, in terms of quality, before being used for diverse purposes. It is envisaged that the use of micro-organisms and adsorbents such as zeolites, based on the results obtained from work thus far, could be viable alternatives for cheaper and affordable water

Table 5
Best adsorbents for the different metal ions.

Metal	Best adsorbent(s)
Arsenic	<i>Bacillus</i> strains (98%), <i>Shewanella</i> spp. (83%), Mintek consortia (80%)
Manganese	<i>Bacillus</i> strains (96%), <i>Pseudomonas</i> spp. (72%) and 0.02 M acid activated clinoptilolite (79%)
Nickel	0.02 M and 0.04 M acid activated clinoptilolite (98%)
Gold	<i>Shewanella</i> spp. (66%) and <i>Bacillus</i> strains (50%)
Copper	<i>Bacillus</i> strains (98%), <i>Shewanella</i> spp. (85%) and 0.02 M acid activated clinoptilolite (98%)
Cobalt	<i>Bacillus</i> strains (98%), <i>Pseudomonas</i> spp. (80%)
Iron	<i>Bacillus</i> strains (90%), <i>Pseudomonas</i> spp. (90%)

purification strategies. To satisfy the latter, studies on large scale water purification using zeolites as ion-exchange resins are currently underway.

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