## EXECUTIVE SUMMARY


Heavy metal contamination of wastewaters is a major source of environmental pollution and represents a loss of a vital resource since water availability and quality will be a significant factor in the future socio-economic growth of South Africa. The unsuitability of metal-contaminated water for reuse in industrial processing is also an important economic consideration for many industries because of the escalating cost of fresh water supplies. Numerous studies, included those in our laboratory, have shown that biotechnology-based processes may provide effective and inexpensive means of removing heavy metals from water when compared to more traditional methods such as ion-exchange and chemical precipitation.

The objectives of this study were to:

1. Provide a means of cost-effective treatment of heavy metal containing effluents, both for the removal of metals to reduce environmental pollution and to facilitate effective disposal of such effluents, and for the possible recovery and the recycling of the metals in certain instances.
2. Provide a technology for the potential reuse in industrial operations of water that is currently disposed of.
3. Evaluate the potential toxicity of heavy metals to micro-organisms such as bacteria and algae used in bioremediation processes.
4. Train researchers through the participation in the project in the technology of bioremediation of water systems contaminated with heavy metals and thereby develop a local expertise in this field of biotechnology.
5. Develop expertise and capacity in this field of technology amongst black and female students and assist in developing a research culture in this field at institutions such as technikons which were not previously involved in such initiatives.
6. Transfer technology to collaborating industries in the first instance.

In initial studies on the binding and accumulation of heave metals by biomass of the alga, *Spirulina* sp., metal binding was rapid with saturation reached in 30 minutes, and followed an affinity series of Pb>Cu>Zn>>Fe. The binding capacity of the *Spirulina* for each of the metals was relativity low when compared to a range of other biosorbents. The treatment of an effluent from a base metal mine by direct contact with *Spirulina* cultures was effective for

a period of time, although most of the metal removal could be attributed to alkaline precipitation as a result of the high carbonate content of the growth medium.

The toxicity data show that copper and zinc are both acutely toxic to *Spirulina* with threshold toxicity levels of below 10 μmoles of metal per gram of biomass. The mechanism of toxicity appears different, with the results suggesting copper affects the photosynthetic pigments and the integrity of the cell membrane, resulting in chlorophyll breakdown and the leakage of cell contents. Lead was less toxic, but did induce considerable changes in cell morphology with the cells becoming highly distorted. Lead has been reported to affect membrane stability and the SEM images suggest that leakage of water and cellular components induced a loss of turgor pressure in the lead-treated cells. The algae were therefore not suitable for application in a treatment system in which they came into direct contact with the toxic metals.

A significant finding was the ability of the algae to increase the pH of the surrounding medium. This occurred as a result of the accumulation of inorganic carbon, for bicarbonate, as a response to low concentrations of carbon dioxide in the medium, The release of a hydroxide ion into solution led to the increase in pH. The increase in pH was shown to be due to a reduction in acidity, rather than an increase in alkalinity. The enzyme carbonic anhydrase was shown to be pivotal in this system. Attempts to determine the enzyme activity directly were unsuccessful, due to the inherent inaccuracy of the assay system. An indirect method of determining enzyme activity, by measuring changes in the carbonate species equilibrium, was developed. Under optimal conditions *Spirulina* was able to reduce the acidity by an amount equivalent to the addition of 3670 μmoles NaOH $g^{-1}h^{-1}$. Predictive modelling showed that this enhanced the potential of the medium to effect metal precipitation.

In order for such as system to be sustainable, a readily available source of bicarbonate is needed, Clearly the continuous addition of bicarbonate salts would not be a cost-effective solution. The biological generation of bicarbonate by sulphate reducing bacteria (SRB) was investigated in another study. While sulphate reducing bacteria have been the focus of a considerable amount of research, the majority of this has focused on desalination and the production of sulphide for metal precipitation. For most carbon sources, the amount of bicarbonate generated per unit of sulphate reduced is significantly greater than the sulphide produced. The SRB reactor designed for bicarbonate production generated a ratio of bicarbonate to sulphide of 6:1, three times higher than expected.

Pilot plant studies were subsequently undertaken and demonstrated the potential of an integrated biological system for the treatment of acid mine drainage. The *Spirulina* based system was effective, but suffered from problems associated with maintaining the algae in suspension and preventing biomass loss. These problems were overcome by using *Oscillatoria* in the simulated stream reactors and the system was operated without a loss of efficiency for 90 days. The anaerobic digested component of the integrated system was able to consistently generate bicarbonate at a rate of 4200 μmoles/l/day. The combination of the nitrogen sparging step and the action of the algae was able to reduce the bicarbonate concentration by over 50% and increase the carbonate concentration from 0.07 mM to 12.65 mM. In terms of metal removal, the selective precipitation of over 99% of copper and lead, using hydrogen sulphide gas, was consistently achieved. The blending of the hydrogen sulphide treated acid mine drainage and the algal overflow at an acidity to alkalinity ratio of 1:2 resulted in the precipitation of all the heavy metal, except manganese, to concentrations below the acceptable environmental risk level. It was shown that a portion of the overflow stream from the precipitator could be enriched with a carbon source, ammonia and phosphate, and used as a feed for the anaerobic digester. The remainder of the stream could be discharged to a final polishing step, possibly an anaerobic wetland.

The integrated biological treatment system performed well, effectively treating an effluent modelled closely on the quality of the water being discharged from the East Rand Basin. The cost of such a system would be considerably less than a "high tech" physico-chemical system. This, coupled with the potential long term sustainability of a biological system, would make it a potentially attractive option for the treatment of future acid mine drainage discharges.

In terms of the objectives of this study, the potential toxicity of heavy metals in effluents to organisms used in bioremediation systems was demonstrated and a system of non-contact between the effluent and the organisms was designed. A cost-effective, "low-tech" treatment system for heavy metal containing effluents was also demonstrated and also provided for a potential technology for reuse in industrial operations of water which currently disposed. In respect of training of researchers and capacity development, this study facilitated the development of local expertise in this field of biotechnology. A number of black and female students participated and were trained on this project as were staff and students at the Vaal Triangle Technikon.

In summary, there are no major obstacles to the scaling up of an integrated biological system such as the one proposed here. The individual reactor components are relatively "low-tech", as are the required control mechanisms. The system has the additional advantage that once the biological components are established, the system is essentially self-sustaining, provided a suitable carbon source is provided. It has been previously noted that considerable research is being conducted into the utilisation of low cost and waste carbon sources as feeds for sulphate reducing systems. The results presented in this study clearly indicate that this type of system could be a viable treatment option for the uncontrolled, long term discharge of acid mine drainage from abandoned mines.

This technology is in the process of being transferred to two major mining concerns. Further studies on the implementation of the process will need to examine the exact composition of the effluents and to model the associated water chemistry in order to optimise the reactor design and operation.

This report will deal with experimental studies designed to achieve these. An overview of the previous studies and literature related to this field is presented followed by a series of chapters daeling with the toxicity of heavy metals to algae, the possible direct treatment of acid mine drainage by algal biomass, and algal modification of the aqueous environment to facilitate heavy metal precipitation. The final chapter deals with the use of pilot scale integrated biological systems for the treatment of acid mine drainage. Each chapter has a relevant introduction section followed by a detailed description of materials and methods, results and conclusions.