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LEVELS OF TREATMENT ACHIEVED USING EFFECTIVE MICROORGANISMS IN SURFACE AND WASTE WATERS

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A Research Report submitted to the Faculty of Science in fulfilment of the requirements for the degree of Master of Science.

Johannesburg, 2010

DECLARATION

I declare that this research report is my own unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

_____ _____

Name Date

ABSTRACT

Effective Microorganisms (EM), a combination of decomposing microorganisms, are extensively used in many parts of the world but their success has not been thoroughly assessed within a Southern African context. Their purposes, mostly employed heuristically, include (a) the treatment of raw, polluted and municipal wastewater; (b) recycling of waste in livestock industries; (c) commercial composting of green wastes, garbage and other organic matter when used as compost inoculants; (d) helping to mitigate the effects of acid rain on crops, vegetation, water and soil; (e) reduction and/ or elimination of methane and harmful gas production in landfill sites; and (f) controlling odour and flies in landfill sites and other places where odour and flies are a problem (e.g., livestock industries). There are limited scientific publications on the subject and hence limited empirical evidence exists as to the efficacy of EM. In addition, limited empirical evidence exists as to under what conditions EM can be optimally employed. This study investigated EM by (a) undertaking an extensive literature research on the subject, (b) analysing the following physical and chemical parameters of raw water from different points of Zoo Lake in Johannesburg that had been regularly dosed with EM: pH, conductivity, turbidity, total suspended solids (TSS), ammonia, phosphates and heavy metals and (c) laboratory experiments under aerobic and anoxic conditions, analysing the same physical and chemical parameters (as done for the lake) of polluted municipal wastewater that had been dosed with EM. The results from the study showed a significant decrease in turbidity (for the aerobic and anoxic experiments), ammonia

and phosphates (for the aerobic experiment). The decrease in turbidity and phosphates was attributed to the EM but not that of ammonia as the control also decreased in the same manner. As such, the levels of treatment achieved by EM on surface and wastewaters were considered to be low as only two (turbidity and phosphates) out of seven parameters measured showed significant decreases. Based on this research, it is anticipated that better treatment efficiencies may be realised by combining EM with other complementary microbiological treatment agents and this is suggested for future research.

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God Almighty, for in Him I live and move and have my being…

TABLE OF CONTENTS

DECLARATION i
ABSTRACT ii
ACKNOWLEDGEMENTS iv
TABLE OF CONTENTS v
LIST OF FIGURES viii
LIST OF TABLES x

CHAPTER 1. INTRODUCTION 1
1.1. Background of the Research 1
1.2. Motivation 3
1.3. Aim and Objectives 4
1.4. Research Questions and Hypothesis 5
1.5 Structure of the Report 5

CHAPTER 2. LITERATURE REVIEW 6
2.1. The Concept of Effective Microorganisms 6
2.2. Biological Wastewater Treatment 7
2.3. Application of EM in Wastewater Treatment 9
2.4 Application of EM in Fields other than Wastewater Treatment 13
2.5. Summary of studies 23

CHAPTER 3. MATERIALS AND METHODS 24
3.1. Level of Investigation 24
3.2. Study Area 24
3.3. Field Study Applying EM to Surface Water 25
3.3.1. Preparation of Multi EM 25
3.3.2. Preparation of Bokashi 26
3.3.3. Collection of Water Samples for Testing 28
3.3.4. Laboratory Testing of Water Samples 29
3.4. Wastewater tests 35
3.5. Validating the EM prior to application 38
3.6 Data Analysis 39

CHAPTER 4. LEVELS OF TREATMENT ACHIEVED THROUGH THE APPLICATION OF EM TO SURFACE WATERS AT ZOO LAKE 40
4.1. Introduction 40
4.2 Results 40
4.2.1. pH 41
4.2.2. Conductivity 42
4.2.3. Total Suspended Solids (TSS) 43
4.2.4. Turbidity 44
4.2.5. Ammonia as N 45
4.2.6. Phosphate as P 46
4.2.7. Heavy Metals 47

CHAPTER 5. LEVELS OF TREATMENT ACHIEVED THROUGH THE APPLICATION OF EM TO WASTEWATER WITHIN AN ANOXIC ENVIRONMENT 49
5.1. Introduction 49
5.2. Results 50
5.2.1. pH 50
5.2.2 Conductivity 52
5.2.3 Total Suspended Solids (TSS) 53
5.2.4 Turbidity 55
5.2.5 Ammonia as N 56
5.2.6 Phosphate as P 57
5.2.7 Heavy Metals 58

CHAPTER 6. LEVELS OF TREATMENT ACHIEVED THROUGH THE APPLICATION OF EM TO WASTEWATER WITHIN AN AEROBIC ENVIRONMENT 63
6.1. Introduction 63
6.2. Results 63
6.2.1 pH 63
6.2.2 Conductivity 65
6.2.3 Total Suspended Solids (TSS) 66
6.2.4 Turbidity 67
6.2.5 Ammonia as N 68
6.2.6 Phosphate as P 70
6.2.7 Heavy Metals 71

CHAPTER 7 SUMMARY OF RESULTS AND DISCUSSION 76

CHAPTER 8. RECOMMENDATIONS AND CONCLUSION 89

REFERENCES 92

LIST OF FIGURES

Figure 2.1: Stages in conventional wastewater treatment 8

Figure 2.2: Analysis of water quality parameters 11

Figure 2.3: Manure treatments for Van Vliet *et al*.'s (2006) study 15

Figure 3.1: Tanks used to incubate Multi EM 27

Figure 3.2: Sampling points at Zoo Lake 28

Figure 3.3: Growth in Petri dishes showing that the EM is active 38

Figure 4.1: pH measurements for Zoo Lake 41

Figure 4.2: Conductivity measurements for Zoo Lake 42

Figure 4.3: TSS measurements for Zoo Lake 43

Figure 4.4: Turbidity measurements for Zoo Lake 44

Figure 4.5: Ammonia measurements for Zoo Lake 45

Figure 4.6: Phosphate measurements for Zoo Lake 46

Figure 5.1: pH results for anoxic experiment 50

Figure 5.2: Conductivity results for the anoxic experiment 52

Figure 5.3: TSS results taken over six weeks 53

Figure 5.4: Turbidity results taken over six weeks 55

Figure 5.5: Ammonia results over six weeks 56

Figure 5.6: Phosphate results taken over six weeks 57

Figure 5.7: Heavy metal results 59

Figure 6.1: pH results taken over six weeks 64

Figure 6.2: Conductivity results taken in six weeks 65

Figure 6.3: TSS results in six weeks 66

Figure 6.4: Turbidity results over six weeks 67

Figure 6.5: Ammonia results taken over six weeks 68

Figure 6.6: Phosphate results over six weeks 70

Figure 6.7: Heavy metal results 72

LIST OF TABLES

Table 2.1: Parameters of the fresh waste and composted waste 17

Table 2.2: Bacterial populations in six EM products 18

Table 2.3: Presence/absence of potentially pathogenic bacteria 19

Table 2.4: Yields of different crops (Mayer *et al*., 2008) 22

Table 2.5: Summaries, conclusions from literature review 23

Table 3.1: Summary of testing, Zoo Lake 30

Table 3.2: Summary of parameters tested, anoxic, aerobic expts. 37

Table 4.1: Heavy metal measurements for Zoo Lake 47

Table 4.2: Summary of levels of treatment achieved, Zoo Lake 48

Table 5.1: Tukey test for pH results 51

Table 5.2: Tukey test for conductivity results 53

Table 5.3: Tukey test for TSS results 54

Table 5.4: Tukey test for turbidity results 55

Table 5.5: Tukey test for ammonia results 56

Table 5.6: Tukey test for phosphate results 57

Table 5.7: Heavy metal results, anoxic experiment 58

Table 5.8: Tukey test for copper results 60

Table 5.9: Tukey test for iron results 61

Table 5.10: Tukey test for manganese results 61

Table 5.11: Tukey test for cadmium results 61

Table 5.12: Tukey test for lead results 61

Table 5.13: Summary of levels of treatment achieved, anoxic environment 62

Table 6.1: Tukey test for pH results 64

Table 6.2: Tukey test for conductivity results 65

Table 6.3: Tukey test for TSS results 66

Table 6.4: Tukey test for turbidity results 68

Table 6.5: Tukey test for ammonia results 69

Table 6.6: Tukey test for phosphate results 70

Table 6.7: Heavy metal results, aerobic experiment 71

Table 6.8: Tukey test for copper results 73

Table 6.9: Tukey test for iron results 73

Table 6.10: Tukey test for manganese results 74

Table 6.11: Tukey test for cadmium results 74

Table 6.12: Summary of levels of treatment achieved, aerobic environment 75

Table 7.1: Summary of levels of treatment achieved, surface and wastewaters 77

CHAPTER 1. INTRODUCTION

1.1. Background of the Research

Effective Microorganisms (EM) are a fermented mixed culture consisting of lactic acid bacteria, yeast, actinomycetes and fermenting fungi which are able to break down and decompose harmful microorganisms (Higa,1998). They have been in use in Japan for the past 28 years (Higa, 2007). EM owe their discovery to the work of Dr Teruo Higa, a microbiologist and organic farmer from the University of the Ryukyus in Okinawa, Japan (Higa, 1991; Higa and Wididana, 1991). Higa made an accidental discovery while researching the various beneficial aspects of isolated strains of microorganisms on soil composition and plant growth (Frietag and Meihoefer, 2000).

Based on research and development activities in biotechnology in some countries , EM is increasingly viewed as a means of providing solutions to many problems of food production, depletion of natural resources, environmental pollution, food safety and nutrition, human and animal health (Anthony, 2008). Currently, limited research exists on EM worldwide. This is acknowledged by Higa and Parr (1994:7). They conclude that,

"...it is difficult to demonstrate conclusively which microorganisms are responsible for the observed effects, how the introduced microorganisms interact with the indigenous species, and how these new associations affect the soil plant environment. Thus, the use of mixed cultures of beneficial microorganisms as soil inoculants to

enhance the growth, health, yield, and quality of crops has not gained widespread acceptance by the agricultural research establishment because conclusive scientific proof is often lacking."

The following are some of the environmental applications that EM have been used for:

- Recycling waste and controlling odours in livestock industries (an example is Vryburg Abattoir in Mafikeng where EM have been used to treat wastewater;
- To accelerate the commercial composting of green wastes, garbage and other organic matter when used as a compost inocculant;
- Wastewater purification, polluted rivers, lakes and streams;
- Mitigating the effects of acid rain on crops, vegetation, water and soil;
- The reduction and/ or elimination of methane and other harmful gases in landfill sites;
- To control odour and flies in landfill sites and other places where odour and flies are a problem.

Many towns and cities around the world use EM to reduce costs in the treatment of municipal wastewater (Joubert, 2008). With the use of EM, Joubert (Ibid) found that pollution of South African rivers and other waterways can be reduced. For this reason, Joubert (Ibid) encouraged municipalities and homeowners to look to EM to solve the ever-growing pollution problem.

There has been an increase in the use of microbes in biotechnology and microbiology in the last 50 years; animal health; food processing, safety and quality; genetic engineering; environmental protection; agricultural biotechnology and more effective treatment of agricultural and municipal wastes (Higa and Parr, 1994).According to Higa and Parr (1994), many of these technological advances would not have been practical and economically feasible solely using direct chemical and physical engineering methods. Higa and Parr (1994) also found that microorganisms are effective only when they are presented with suitable and optimum conditions for metabolizing their substrates including available water, oxygen (*i.e.*, depending on whether microorganisms are aerobic or anaerobic), pH and temperature of their environment.

1.2. Motivation

This study endeavours to investigate the validity and determine the effectiveness of EM as a viable agent for treating municipal wastewater or polluted surface waters within a South African context.

In many South African settlements, the issue of polluted surface water and/ or costly wastewater treatment is a serious problem (Joubert, 2008). Cholera outbreaks, which have erupted time and again (Mail and Guardian, 2009) are proof of the impact of polluted surface water, especially in rural and informal settlements. This is not only in rural and informal settlements but at the moment South Africa is experiencing a threat in terms of polluted water sources because of dams, sewage works and treatment plants that are poorly managed; this could lead to a major water crisis in

South Africa according to a Durban-based water researcher (Pure SA, 2009). EM is a cheap and affordable product which if effective, may be used to clean polluted water and reduce costs (time and money) in wastewater treatment especially in parts of the country that lag behind in service delivery.

1.3. Aim and Objectives

The aim of this research is to determine the levels of treatment achieved in surface and wastewaters injected with EM. Specific objectives of the research are:

- To undertake a literature review of the subject and document scientific findings on the use and effectiveness of EM.
- To undertake a laboratory experiment (within an aerobic and anoxic environment) involving the injection of EM in wastewater. The laboratory experiment will involve the measurement of the following parameters: total suspended solids (TSS), turbidity, pH, conductivity, phosphate, ammonia and heavy metals.
- To undertake a field study involving the injection of EM in a surface water body. This experiment will also involve the measurement of the parameters listed in the second bullet-point above. From the experiments above, to assess the levels of treatment achieved by EM for the above parameters

1.4. Research Questions and Hypothesis

Null Hypothesis, H_o: There is no statistically significant decrease in waste and/or surface water parameters before and after addition of EM.

Alternative hypothesis, H_a: There is statistically significant decrease in waste and/or surface water parameters before and after addition of EM.

Research questions:

- Are there differences in the following water quality parameters: TSS, turbidity, conductivity, pH, phosphates, ammonia and heavy metals after application of EM to surface and wastewater?
- Are the observed differences (positive and/ or negative) in water quality after EM treatment attributable to the effectiveness of EM?

1.5 Structure of the Report

Chapter 1. Background, motivation, aims and objectives of the research project.

Chapter 2. An overview of research conducted using EM

Chapter 3. Materials and methods employed to achieve the objectives of the research

Chapter 4. Surface water treatment using EM

Chapter 5. Wastewater treatment using EM (Anoxic conditions)

Chapter 6. Wastewater treatment using EM (Aerobic conditions)

Chapter 7. Discussion of results

Chapter 8. Summary of results, key issues, recommendations and conclusion

References

CHAPTER 2. LITERATURE REVIEW

2.1. The Concept of Effective Microorganisms

Dr Teruo Higa (Higa from hereon) developed the concept and technology of EM at the University of the Ryukyus in Okinawa, Japan in the late 1970's (Higa, 1991; Higa and Wididana, 1991). EM is a fermented mixed culture of beneficial microorganisms consisting of:

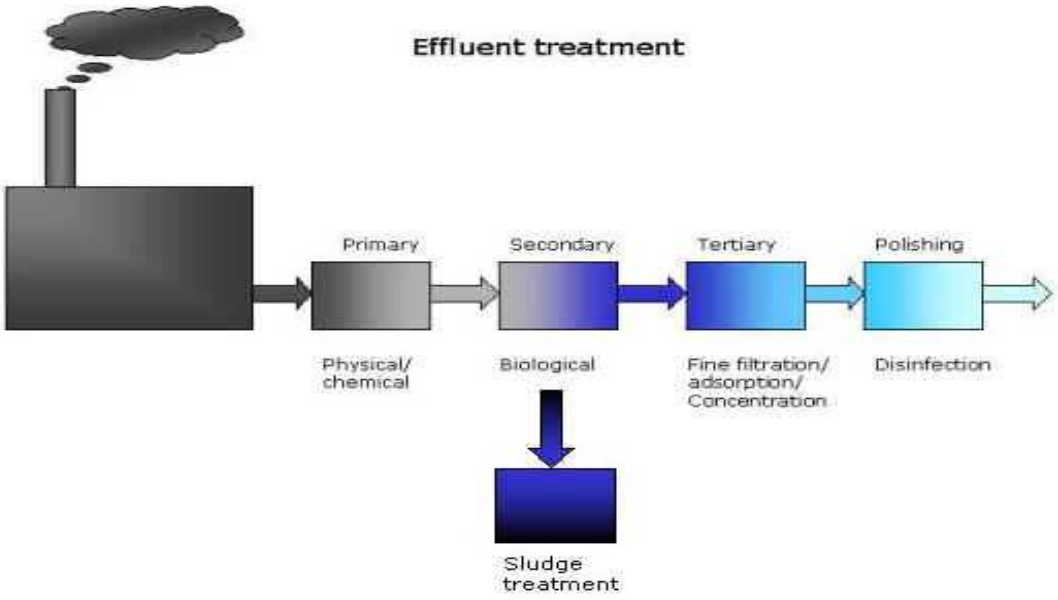
- Lactic acid bacteria- *Lactobacillus plantarum, L. casei, Streptoccus lactis,*
- Photosynthetic bacteria- *Rhodopsuedomonas palustrus, Rhodobacter spaeroides,*
- Yeasts- *Saccharomyces cerevisiae, Candida utilis,*
- Actinomycetes- *Streptomyces albus, S.griseus,*
- Fermenting fungi- *Aspergillus oryzae, Mucor hiemalis* (Diver, 2001).

The EM solution does not contain any genetically modified microorganisms (GMO) and these species are contained in an acidic medium (Higa, 2001). At the inception of the studies, Higa used 80 species to develop EM solutions from a candidate list of over 2000 species of microbes commonly utilized in the food and fermentation industries (Higa 2001). Further studies were carried out to simplify the process of developing EM and today, EM is developed using the three principle organisms: photosynthetic bacteria, lactic acid bacteria, and yeasts. These organisms are enriched naturally by other species such as Filamentous Fungi and *Actinomycetes* (Higa and Parr 1994; Higa, 2001).

The most outstanding characteristic of EM is that aerobic and anaerobic species coexist in a beneficial manner (Al-Taweil and Yusof, 2008). Al-Taweil and Yusof (2008) found that a positive feature of EM is its ability to secrete large amounts of nutrients such as organic acids, amino acids, chelated minerals, antioxidants, polysaccharides and vitamins when in contact with organic matter.

2.2. Biological Wastewater Treatment

The use of EM to treat wastewater in essence simulates the role of biological treatment in treating wastewater. Biological treatment is a process used in wastewater treatment plants as part of their water purification process and secondary treatment (Figure 2.1). It makes use of bacteria and other microorganisms to remove pollutants and impurities from water by digesting them; thereby getting rid of impurities in the water. Sand or carbon filters are used to provide a place on which the microorganisms grow (Schultz, 2005). The use of microorganisms to remove contaminants from wastewater has proven to be effective over time and is now used extensively. It has been in use in Europe since the early 1900s (Agriculture and Agri-Food Canada, 2007). It is not surprising therefore that biological treatment methods are leading in the secondary wastewater treatment sector as they are the most effective and eco-friendly option of the existing treatment processes (Wyszynska, 2006).



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Figure 2.1: Stages in conventional wastewater treatment, including biological treatment (Source: Lenntech, 2009a)

Bacteria have the capability of using different constituents found in wastewater to obtain energy for microbial metabolism and the raw materials for protein synthesis (Schultz, 2005). The biological-treatment process takes advantage of this metabolic activity. The bacteria consume and digest the organic material present in the wastewater and through their metabolism, the organic material is changed into cellular mass which is no longer in solution, but is settled at the bottom of a settling tank or container (Roisin, 2008).

Most organisms found in biological wastewater treatment processes are facultative, meaning that they can function aerobically in the presence of oxygen and anaerobically in the absence of oxygen (Eckenfelder, 1980). Nevertheless, an

adequate supply of oxygen is necessary because not only do they need organic material as food but also need oxygen to breathe (Roisin, 2008).

There are biological wastewater treatment processes that have been developed that are anaerobic in nature. They make use of a bacterial process that breaks down organic materials within waste in the absence of oxygen (Applications Water and Sewerage, 2002). Biological treatment improves water quality by reducing organic matter, dissolved organic carbon (DOC), colour and turbidity amongst others. When DOC levels are reduced for instance, this improves the taste and odour of the water (Agriculture and Agri-Food Canada, 2007).

2.3. Application of EM in Wastewater Treatment

The following are some of the studies that have been done on wastewater using EM:

Okuda and Higa's (1999) Study

Okuda and Higa (1999) intended to provide recycled water for agriculture using EM treatment at a sewage plant. The experiment was done at Gushikawa City Library in Japan and consisted of a primary, secondary and tertiary tank. 10L of EM1, 2L of EM2 and EM3 were initially added to the second tank in the sewage system. Subsequently (after every 3 months), 2L of EM1 and 0.5L of EM2 and EM3 were added to the same tank. 1L per sample of water was drawn from 3 tanks of the sewage system at monthly intervals from August 1995 to January 1997. 18 samples were procured from the primary tank, treated tank and final tank. Each sample was analysed 3 times for the following: Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids and total nitrogen and phosphate

concentrations. Fifty-four samples were analysed, each being considered a replicate. The pH after application of EM was reduced to 7.00 from 7.63. Application of EM to the second pond brought down the COD and BOD values by 93% and 20% respectively. The microbial breakdown of organic matter reduced the total solid content by 94%. It was also found that nitrogen and phosphorus contents in the second pond were reduced significantly after application of EM by about 55%. They concluded that the reductions could have been a result of microbial utilisation of the nitrogen and phosphorus, thus making the water fit for recycling and agricultural purposes.

Szyamanski and Patterson's (2003) Study

Szyamanski and Patterson (2003) conducted a study on EM and wastewater systems to find out whether EM was beneficial in reducing the volume of sewage sludge produced in on-site wastewater treatment systems (septic tanks). The other objective was to see whether the application of EM would bring about a change in pH, electrical conductivity and other physico-chemical indicators. In their study, 5 septic tanks were treated and sampled and an initial dose of 6 litres (L), of EM was applied to the septic tank at the inlet port. A week later, 3 L was added, with 350 mL doses

applied once each week for the next 3 weeks, 10 L over the 4 weeks was applied**.**

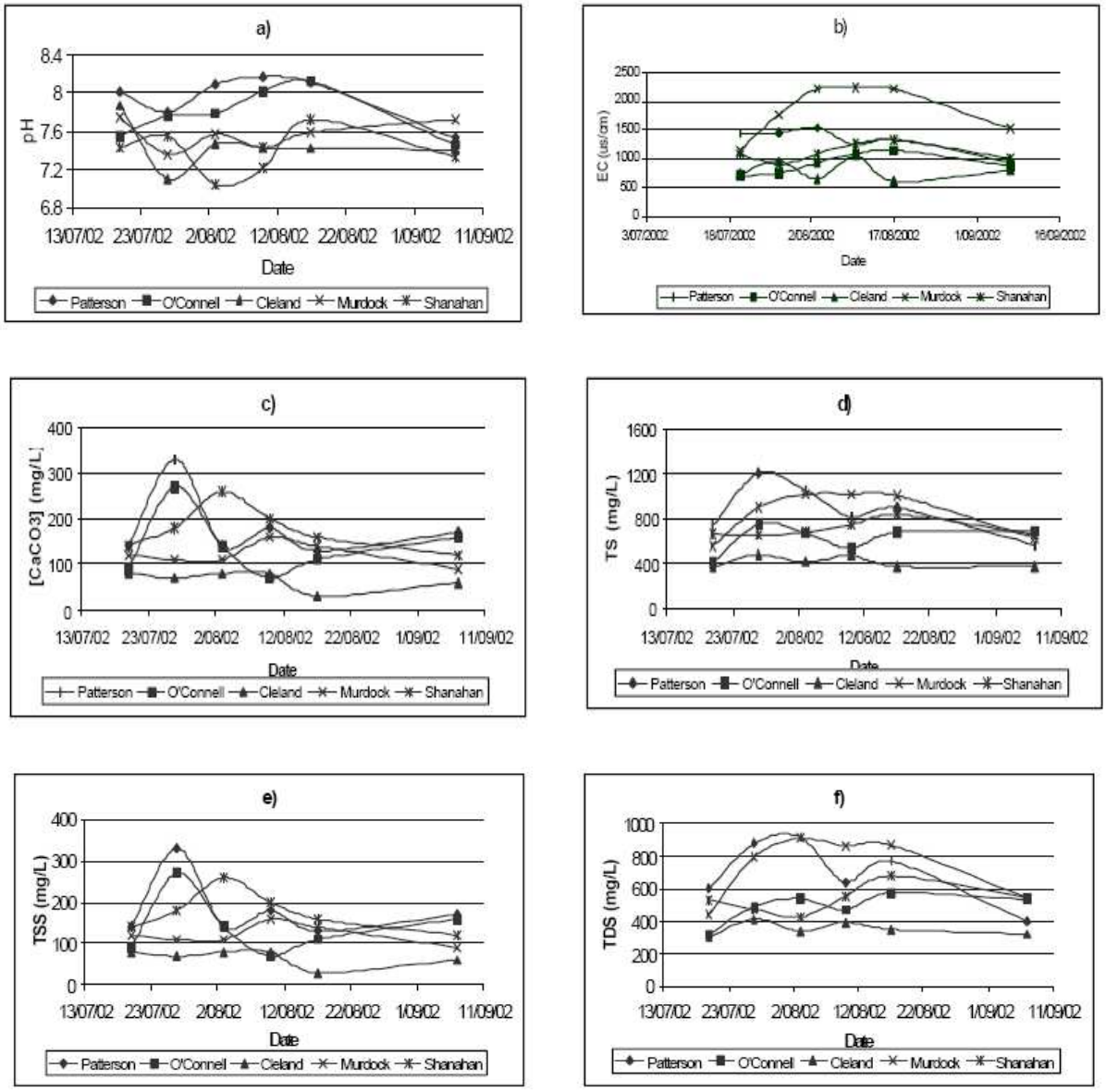


Figure 2.2: Szyamanski and Patterson (2003) - Analysis of water quality parameters (a) pH b) EC c) Alkalinity d) Total solids e)Total suspended solids f) Total dissolved solids) in septic tanks injected with EM

Addition of EM was undertaken on the same day each week. Samples were taken from the outlet pipe of each septic tank once per week before application and were analysed for pH, conductivity, total alkalinity, total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS). A final sample was taken three weeks after the last dose. The authors found that there was negligible reduction in suspended solids in the septic tanks and that influences on pH, alkalinity and electrical conductivity were minimal during the application of EM. The results from five septic tanks, Murdock, Shanahan, O'Connell, Patterson and Cleland were plotted against time (Figure 2.2).

Moyo et al.'s 2008 Study

EM has been used in Namibia in biological wastewater treatment and odour control (Moyo *et al*., 2008). Three biological treatments were used in that study: EM, EM mixed with fermented Moringa (an indigenous plant) and liquid bacteria (8 Alive). The aim was to assess the ability of these treatments to treat the wastewater in Ujams ponds and to see which one would be most effective. In addition, they wanted to reduce the strong odour emanating from the ponds as this was such an inconvenience to the surrounding communities. The 3 treatments (8 Alive, EM and EM with Moringa) were applied to wastewater. Each was replicated 5 times in buckets of 20 L capacity and there was a control alongside. The contents of the buckets were adjusted daily to simulate Ujams ponds – this was done by the removal of 25 % (5 L) of the volume followed by topping up the buckets with fresh wastewater (5 L) collected daily from Ujams. Before the buckets were refilled, the fresh wastewater was treated

with the 8 Alive, EM or the EM with Moringa. The process of taking out 5 L (outflow) and refilling (inflow) was done every day throughout the project period. The samples from the buckets were tested weekly from September until December 2007. The parameters that were monitored in this study were conductivity, pH, hydrogen sulphide, manganese, copper, zinc and chromium using the Hach system. It was found that effluent with 8 Alive was a better treatment than the other two and that the EM Plus with Moringa was better than the EM. It was felt that the effectiveness of the EM was affected by the presence of chromium and zinc that inhibit the growth of microorganisms and that in the absence of these metals the EM would have been more effective.

2.4 Application of EM in Fields other than Wastewater Treatment

Van Vliet et al.'s (2006) Study

Van Vliet *et al*. (2006) looked at microbial diversity after addition of EM to slurry manure. They investigated whether bacteria present in an activated EM suspension (EM-A) were able to maintain their populations and/ or multiply in slurry manure**.** Two hundred L of slurry manure were collected from a farm, the manure was homogenized using a cement mixer and sixteen 16 L buckets were each filled with 10 kg of slurry manure. Within half of the buckets, 1 mL of Agri-mest mixture (a natural mineral blend that stimulates the growth of microorganisms and increases the amount of energy available for anaerobic fermentation of manure by micro-organisms (Van der Stelt *et al*., 2006) was added while within the other half, 1 mL of deionised water was added. The Agri-mest mixture consisted of 4 g of mineral Agri-mest, 2 mL of

Agri-mest solution and 18 mL of lukewarm deionised water. The authors combined EM-A with Agri-mest. The addition of EM-A to the Agri-mest mixture energises the minerals thereby increasing the energy that is available to the manure environment thus leading to a better anaerobic fermentation by micro-organisms in the manure. The buckets were incubated for one week at 4 ^{o} C after which they were sampled for chemical analyses.

This set-up resulted in the following slurry manure treatments: Agri-mest mixture with EM-A (+A+EM), Agri-mest mixture without EM-A (+A-EM), EM-A without Agri-mest mixture (-A+EM), no Agri-mest mixture and no EM-A (-A-EM). Each treatment consisted of four replicates. All buckets were stored in a room at 20 ^{o} C for 6 weeks after which samples were taken for chemical and biological analyses. They found that even though several bacterial species were added to the slurry manure by the EM-A suspension, no differences in the bacterial diversity of the different slurry manure treatments were found. The EM-A microbes did not appear to multiply in significant numbers. In other words, the addition of an EM-A mixture had no effect on the bacterial diversity in the slurry manure (Figure 2.3) (van Vliet *et al*., 2006). Room temperature is generally considered to be 25^{o}C, so the experiment was conducted below room temperature (20^{o}C). Had they conducted it at a higher temperature than 20^{o}C, they could have obtained different results.

The effectiveness of EM is questionable as there was no effect of the EM-A mixture on the chemical composition of the manure (tests for pH; total carbon and nitrogen; mineral nitrogen; that is, NO_3^- and NH_4^+ concentrations were conducted) (van Vliet *et al*., 2006).

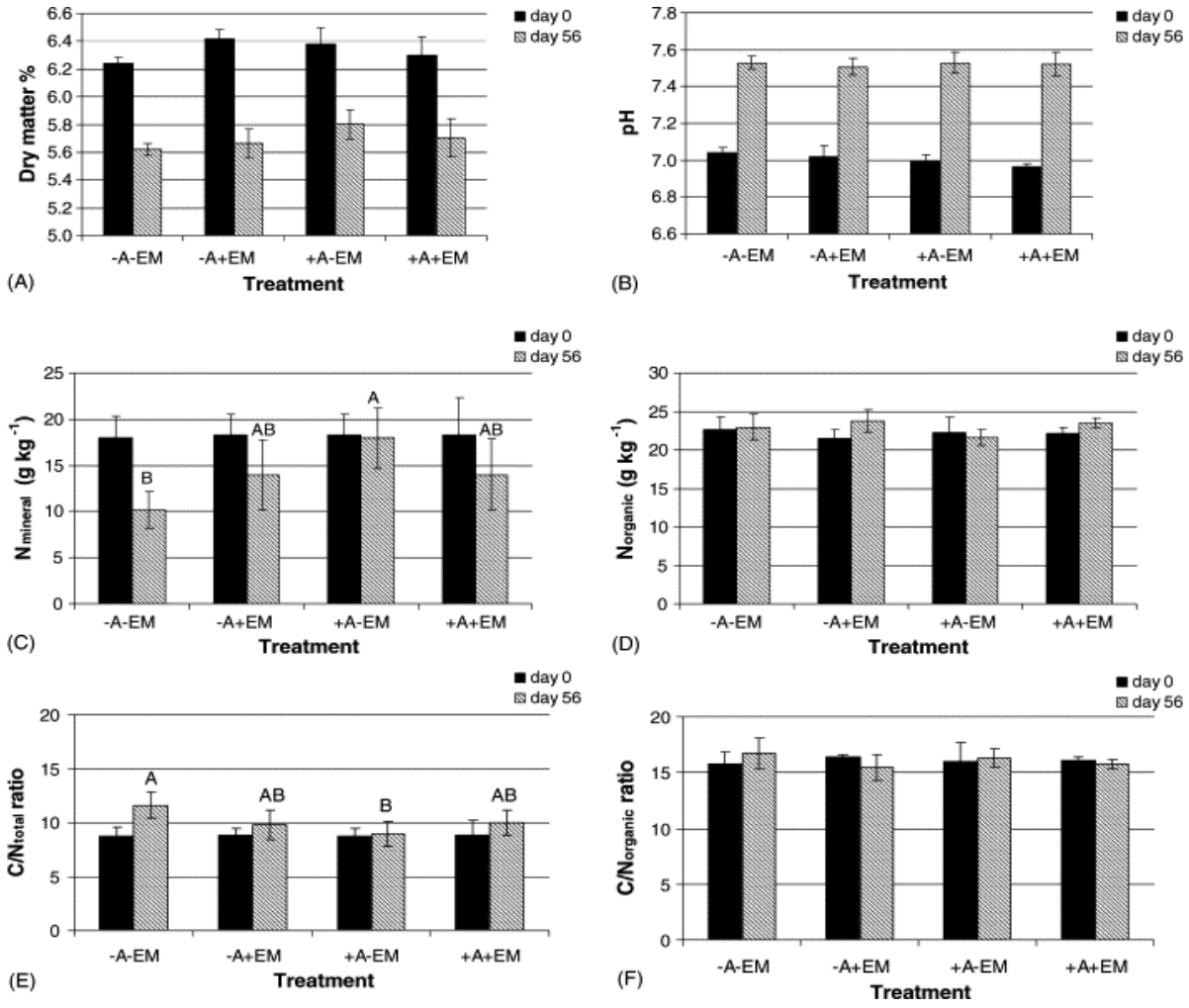


Figure 2.3: Four manure treatments at the start of Van Vliet *et al.*'s (2006) experiment and after 6 weeks of incubation.

NOTES

- *A = Agri-mest; EM= Activated Effective Microorganisms suspension; + = added; - = not added.*
- *Bars with different letters are significantly different ($p < 0.05$).*

Sekeran et al.'s (2005) Study

Sekeran *et al.* (2005) evaluated the composting of kitchen wastes using EM. Wastes from the canteen and hostels of a college campus were used A solution was prepared by mixing 30 L water and 1 L activated EM in a plastic bucket and about 15 L were sprayed on the clean 3 x 1 m composting site. A 15 cm thick base layer containing animal waste was spread over the site and the activated EM was sprayed over this layer. A second layer of solid waste, about 30 cm thick, was spread over the previous and also sprayed with the EM solution. This layering process was repeated up to a height of ~ 100 cm. The stack was completed with a final layer of 5 cm of animal dung. The entire unit was kept moist by spraying it with activated EM solution at regular intervals. After about 25 to 30 days, the volume of bed had dropped substantially and a sweet-smelling white mold appeared on the biomass. The finished compost was then collected and sieved. The compost was analyzed for pH, organic content, nitrogen (N), phosphorous (P), and potassium (K) (Table 2.1).

The carbon (C) content of the wastes decreased during composting indicating a greater mineralization rate for organic matter. However, the nitrogen (N) content only increased during composting. This demonstrated that increased microbial activity continued in the casts and resulted in a mineralization rate increase for organic nitrogen and consequently, further increase in the concentration of $NH4^+$. The C:N: ratio of the wastes was greater before composting than after. Low C:N ratios accelerated the decomposition rate. The control of the foul smells was attributed to the EM mediated decomposition process. A conclusion that can be drawn from this

study is that kitchen wastes provide a good environment for EM to grow and the production of high quality compost. Also, EM can lead to the detoxification of landfills and the promotion of highly sustainable, closed-cycle agricultural and organic waste treatment (Sekeran *et al*., 2005).

Table 2.1: Parameters of the fresh waste and composted waste (Sekeran et al., 2005)

No.	Parameters	% by Dry Mass for fresh waste	% by Dry Mass for composted waste
1	Carbon (C)	32.16	30.05
2	Nitrogen (N)	0.98	1.21
3	Phosphorous (P)	1.02	0.63
4	Potassium (K)	0.402	0.40
5	C: N	28: 1	26: 1
6	pH	7.4	8.4

Giovanelli et al.'s 2007 Study

Giovanelli *et al*. (2007) conducted a microbiological study with EM at the University of the Witwatersrand in Johannesburg, South Africa. Their study was aimed at determining bacterial populations and the presence/absence of potential food borne pathogens before and after fermentation of six EM-fermented products. The Six EM-fermented products were Multiplied EM, EM fermented Plant Extract, EM-Fermented Kitchen Garbage, EM-Fermented Fish, EM-Fermented Chicken Manure and EM-Bokashi. The presence/absence of *Salmonella*, *Listeria monocytogenes* and *E .coli* (disease causing pathogens) was detected for all EM products before and after fermentation by incubating on different plates of agar overnight.

Table 2.2: Bacterial populations before and after fermentation in six EM products (Giovanelli *et al.*, 2007)

EM Products	Bacterial Population	Before Fermentation (log cfu ml^{-1})	After Fermentation (log cfu ml^{-1})
Multiplied EM (M-EM)	Aerobic plate counts	4.64	6.48 increased
	Lactic acid bacteria	4.46	7.14 increased
	Nitrogen fixing bacteria	4.30	6.13 increased
	Actinomycetes	4.68	6.38 increased
	Gram negative bacteria	Below detection limit	Below detection limit
EM Fermented Plant Extract(EM-FPE)	Aerobic plate counts	5.21	7.03 increased
	Lactic acid bacteria	5.31	7.52 increased
	Nitrogen fixing bacteria	5.42	6.87 increased
	Actinomycetes	5.32	6.98 increased
	Gram negative bacteria	5.27	5.22 decreased
EM Fermented Kitchen Garbage (EM-FKG)	Aerobic plate counts	7.38	7.85 increased
	Lactic acid bacteria	7.50	5.07 decreased
	Nitrogen fixing bacteria	7.36	6.42 decreased
	Actinomycetes	7.54	7.82 increased
	Gram negative bacteria	6.48	7.42 increased
EM Fermented Fish (EM-FF)	Aerobic plate counts	7.55	5.65 decreased
	Lactic acid bacteria	5.78	5.42 decreased
	Nitrogen fixing bacteria	7.36	7.02 decreased
	Actinomycetes	7.86	4.85 decreased
	Gram negative bacteria	4.35	5.40 increased
EM Fermented Chicken Manure (EM-FCM)	Aerobic plate counts	7.32	5.13 decreased
	Lactic acid bacteria	4.28	3.00 decreased
	Nitrogen fixing bacteria	4.71	6.95 increased
	Actinomycetes	6.72	4.13 decreased
	Gram negative bacteria	2.94	2.68 decreased
EM Bokashi	Aerobic plate counts	4.23	6.71 increased
	Lactic acid bacteria	4.26	6.70 increased
	Nitrogen fixing bacteria	4.28	6.85 increased
	Actinomycetes	5.85	6.70 increased
	Gram negative bacteria	2.06	2.31 increased

The results obtained for potentially pathogenic fungi and bacteria were also summarised (Table 2.4). Population studies were conducted for general aerobic, lactic acid, nitrogen fixing, *Actinomycetes* and gram negative bacteria. Also, the presence of photosynthetic bacterial and fungal populations was noted. Common bacteria species

present in each EM product before and after fermentation was determined. (Table 2.3) (Giovanelli *et al*., 2007).

Table 2.3: Presence/absence of potentially pathogenic bacteria and fungi in six EM fermented products before and after fermentation (Giovanelli *et al*., 2007)

EM-Fermented Products	Potentially Pathogenic Bacteria						Potentially Pathogenic Fungi
	Salmonella		*Listeria monocytogens*		*Enterobacteriaceae*		
	Before	After	Before	After	Before	After	
Multiplied EM	-	-	-	-	-	-	-
EM Fermented Plant Extract	-	-	-	-	-	-	-
EM Fermented Kitchen Garbage	-	-	-	-	-	-	*Fusarium*
EM Fermented Fish	-	-	-	-	-	+	-
EM Fermented Chicken Manure*	+	+	-	-	+	+	-
EM Bokashi	-	-	-	-	-	-	*Fusarium*

**EM- Fermented Chicken Manure was inoculated with Salmonella S46 and E. coli E220 to create a hypothetical study on pathogens. Enterobacteriaceae were isolated from the general bacterial population study.*

- = absence

+ = presence

The EM fermentation products contain beneficial bacterial numbers that result in the decomposition of organic material and efficiency in their function of releasing nutrients for plants as was observed in the previous study. However, *Salmonella, E.coli* and other *Enterobacteriaceae* pathogens survived the fermentation steps. Thus, EM products may be unsafe for crop application as they can contain pathogens both fungal and bacterial that may be a threat to human health.

Hoyos et al.'s (2008) Study

The effectiveness of EM in environmental management and on the production and economic parameters of broiler chickens was assessed (Hoyos *et al*., 2008). An evaluation of two treatments in two batches of chickens with six replicates for each gender in each treatment was carried out. There were 24 experimental units observed for 35 days. They used EM containing lactic acid bacteria, yeasts, photosynthetic, fungi and *Actinomycetes* fermenters, at concentrations greater than 100,000 CFU/ml of solution (CFU = colony forming units). The production parameters that were evaluated were weight gain, feed conversion, cumulative mortality, economic performance and utility of EM in reducing the burden of total coliforms present in the bedding of chickens. After a five week study it was found that the EM significantly improved production parameters (weight gain, feed conversion rate, mortality) in male birds.

Female birds did not respond to treatment with respect to weight gain or feed conversion rate but their mortality rate was significantly lower. A reduction in burden of total coliforms present in the broiler chicken environment was achieved using EM. In sum, EM use lowered production costs and improved profits by 8.3% (Hoyos *et al*., 2008).

Mayer et al.'s 2008 Study

Mayer *et al*. (2008) assessed the effects of different preparations of EM on crop yields and on microbial parameters. This study was done over four years in Zurich,

Switzerland from 2003 to 2006. A field experiment was set up where preparations of EM Active (EM-A) (which is the same as EM Multi), as spraying agent, and Bokashi, as organic fertilizer, were applied. Treatments without EM and parallel treatments with autoclaved EM preparations (to separate the effect of the microorganisms from its substrate) were used as controls. Bokashi and the first EM-A spraying were applied at sowing. Further EM-A sprayings were spread during the vegetation period until flowering and after the cutting of Lucerne (a fodder plant). Potatoes were cropped in 2003 followed by winter barley in 2004, Lucerne in 2005 and winter wheat in 2006. Crop yields, microbial biomass, C by chloroform fumigation extraction (CFE) and soil basal respiration were determined. Soil samples (0-20 cm) were taken in March 2005, October 2005, immediately before and after sowing of winter wheat and in March 2006.

They observed from the study that potatoes showed no significant differences in yield in 2003. From 2004 to 2006, yields of the EM-A spraying treatments 2 and 3 showed no differences as compared with the untreated control (Table 2.2). It was shown that yields differed considerably in treatments with additional Bokashi application and this was attributed to the large amounts of nutrients like nitrogen, phosphorus, potassium etc., which were applied with Bokashi. The observed effects were attributed to the carrier substrate of Bokashi (Mayer *et al*., 2008).

Winter barley yields in 2004 increased compared to the control. The results were increases between 23% in treatment 6 (sp+bok+m) to 36% in treatment 4 (sp+bok). The comparatively high differences were however not significant (Mayer *et al*., 2008). Differences of winter wheat yield to the control in 2006 ranged between

13% in treatment 6 (sp+bok+m) and 23% in treatment 7 (sp+bok+m au) with significant differences recorded between the control, treatment 3 (sp au) and treatment 7 (sp+bok+m au) (Table 2.2). The results show that the additional application of manure to spraying combined with Bokashi application (sp+b+m), did not lead to crop yield increase. Lucerne yields in 2005 showed a similar pattern but differences between the treatments were small. There were no significant statistical differences between living EM with sterilized EM (treatment 2, 4, 6 vs. 3, 5 and 7) Mayer *et al*., 2008).

Table 2.4: Yields of different crops from 2003-2006 (Mayer *et al*., 2008).

Spraying treatment No.	**Spraying treatment**	**Potatoes 2003** (t FM ha^{-1})	**Winter barley 2004** (t FM ha^{-1})	**Lucerne 2005** (t DM ha^{-1})	**Winter wheat 2006** (t FM ha^{-1})
1	control	27.4 [a]	2.95 [a]	14.0 [a]	2.97 [a]
2	Sp	33.3 [a]	3.30 [a]	14.6 [a]	3.16 [ab]
3	Sp au	30.6 [a]	2.88 [a]	13.8 [a]	2.95 [a]
4	Sp+bok	27.0 [a]	4.00 [a]	14.5 [a]	3.53 [ab]
5	Sp+bok au	26.9 [a]	3.80 [a]	14.4 [a]	3.48 [ab]
6	Sp+bok+m	30.3 [a]	3.63 [a]	15.1 [a]	3.36 [ab]
7	Sp+bok+m au	29.0 [a]	3.75 [a]	14.7 [a]	3.64 [b]

[#]*Sp = spraying EM au= autoclave bok= bokashi m= manure*

Differing letters in a column show significant differences of means (Tukey, $p < 0.05$)

The effects of EM on soil microbial parameters are considered to be low. Mayer *et al*. (Ibid) concluded that in the temperate climate of Central Europe, under organic farming management regime, EM caused no significant effects on crop yields and soil microbial parameters. This was probably because the climate was too cold for EM

activity; the results would probably be different if the study was conducted in a warmer climate.

2.5. Summary of studies

Table 2.5: Summaries, conclusions and/or comments from literature review

	Summary/conclusions/comments from the study
Szyamanski and Paterson (2003)	There was negligible reduction in suspended solids in septic tanks and EM influence on pH, alkalinity and electrical conductivity were minimal.
Moyo *et al*. (2008)	Wastewater effluent with 8 Alive was a better treatment than EM Plus with Moringa and EM. EM Plus with Moringa performed better than EM. It was felt that the effectiveness of the EM was affected by the presence of chromium and zinc which inhibit the growth of microorganisms and that in the absence of these metals the EM would have been more effective
Okuda and Higa (1998)	After application of EM to the septic tanks; pH was reduced from 7.63 to 7, COD and BOD values were brought down by 93% and 20% respectively. Total solid content was reduced by 94%, nitrogen and phosphorus contents were reduced by about 55%.
Van Vliet *et al.* (2006)	The activated EM suspension had no effect on the chemical composition of the manure. The effectiveness of EM was questionable.
Hoyos *et al*. (2008)	EM significantly improved production parameters like weight gain, feed conversion rate and mortality in male birds. Female birds did not significantly respond to treatment with respect to weight gain or feed conversion rate but their mortality rate was significantly lower.
Sekeran (2005)	The carbon: nitrogen ratio of the wastes was lower after composting with EM. A low carbon: nitrogen ratio accelerated the rate of decomposition. EM was said to control the foul smell and the decomposition process was free of odours.
Mayer *et al*. (2008)	The effects of EM on soil microbial parameters were small. The conclusion was that EM caused no significant effects on crop yields and soil microbial parameters.
Giovanelli *et al.* (2007)	Since *Salmonella, E.coli* and other *enterobacteriaceae* survived the fermentation steps with EM, it was concluded that the EM products could be unsafe for crop application as they could contain both fungal and bacterial pathogens since the observed pathogens survived the fermentation steps.

CHAPTER 3. MATERIALS AND METHODS

3.1. Level of Investigation

The two levels of investigation used when undertaking this type of research include the census and sample levels (Williams, 2000). Sample level is a small representative subset of a population which involves selection of small areas where samples are collected, investigations are carried out in those samples, and the results from the samples are then applied to the entire area (Williams, 2000). The data collected from the samples normally gives a good indication of the measurement facts and other parameters of the entire population from which the samples were drawn (Watts and Halliwell, 1996). In this study, the sample level was used as it was more practicable and appropriate for this research.

3.2. Study Area

The field study was carried out at Zoo Lake (S 26.9' 35,00" E 28.1' 48,00") in Johannesburg, a recreational facility. City Parks, a subsidiary of the City of Johannesburg, commissioned a local contractor to treat the perceived polluted lake with EM. This is not the first time that EM has been added to the water at Zoo Lake. A similar project was initiated in 2002 in conjunction with the Zoo Lake users committee but the project was abandoned because of lack of funding (Davie, 2002). The 104 year old parkland where the Lake is situated stretches some 46 hectares and

is known as the Hermann Eckstein Park in memory of the man who donated the land to the city (Davie, 2002).

3.3. Field Study Applying EM to Surface Water

Two forms of EM were applied to the water: multiplied EM (Multi EM) and Bokashi. The liquid EM-multi was injected into the lake every week or every fortnight. The solid Bokashi was made into balls of ~ 100g each and the balls were all put into the lake on the first day of dosing, ~ 500 balls. They settled to the bottom of the lake and were slow releasing Both multi EM and Bokashi were made from stock EM, a liquid concentrate made from the cultivation of a mixture of aerobic and anaerobic microorganisms consisting of lactic acid bacteria, photosynthetic bacteria, yeast and fungi (Higa, 2001). The stock EM was obtained from Lindros Whole Earth Consultants.

3.3.1. Preparation of Multi EM

Two 5 000L tanks were set up within the greenhouse at the City Parks Training School at Zoo Lake. The Multi EM was prepared in these two tanks (Figure 3.1).

- **Materials**: Stock EM, pure liquid cane molasses, clean water.
- **Preparation**: The molasses was mixed with warm water to dissolve it. The stock EM was mixed into the molasses-water mixture and placed in the tank. The 5 000 L tank was filled with 4 500 L of warm water. There was air space left at the top of the tank and the solution was mixed well. The container was sealed (air tight).

A hole was drilled at the top of the tank to release the gas formed in the EM solution. A spaghetti tube was inserted through the hole and the other end was placed into a container filled with water in order for the gas to escape without any oxygen returning to the tank. A light bulb was placed inside the tank to provide light for the cultivation of the EM. The temperature was kept constant at 25^{o}C. The Multi EM was incubated for 14 days in a pH of not more than 3.7.

On average 500L of the Multi EM was released from the tank through the tap at the bottom every week or every fortnight and put into the lake at different points. Application of EM into the lake was carried out by the City Parks personnel as follows:

- 30 September 2008; 1000L EM
- 07 October 2008; 500L EM
- 28 October 2008; 500L EM
- O4 November 2008; 300L EM
- 15 November 2008; 400L EM

3.3.2. Preparation of Bokashi

Bokashi is a Japanese word meaning “fermented organic matter.” It is a system of odourless composting where carefully selected EM are allowed to decompose organic waste material. Bokashi was made using molasses, water, stock EM and wheat bran.



Figure 3.1: The 5000 L tanks used to incubate Multi EM

The molasses, water, stock EM and wheat bran were mixed thoroughly until the material was held together. The mixture was made into balls of ~ 100g each. The balls were covered and kept in closed polythene bags in a covered plastic container for 4-5 days to allow fermentation to take place, and after fermentation, the balls are called EM Bokashi (Majumdar, 2006). All the EM Bokashi balls (about 500 of them) were scattered into the lake covering as much of the lake's area as possible. They settled to the bottom of the lake and it was a slow release process over several weeks until they finally dissolved.

3.3.3. Collection of Water Samples for Testing

Four sampling points were chosen within the lake where water samples were taken at a depth of approximately three quarters of a meter and tested to observe the effect that the EM was having on the water (Figure 3.2).



Figure 3.2: Sampling points at Zoo Lake

One L samples were collected at each point and the following parameters were tested:

- pH
- Conductivity
- Turbidity
- Total Suspended Solids
- Ammonia

- Phosphate
- Heavy metals

Weekly measurements were carried out; heavy metals were tested at the beginning and at the end of the exercise. The initial state of the lake before the EM experiment commenced was measured based on the above parameters. The initial measurements were taken as the control. Thereafter, samples were collected for testing once a week.

Data collection was from the end of September to the end of November (two months) with an additional sample for heavy metals taken mid-December because it was not possible to test for them at the end of November.

3.3.4. Laboratory Testing of Water Samples

Analytical methods used in the analysis of water quality parameters are those stipulated in the Standard Methods for Water and Wastewater. The procedures for analysing the pH, conductivity, turbidity, total suspended solids, ammonia, phosphates, and heavy metals were adapted from the 20th edition of the Standard Methods for the Examination of Water and Wastewater (Eaton *et al.,* 1998), unless otherwise stated. A summary of testing for Zoo Lake is shown in Table 3.1.

1. **pH:** pH is a measure of the acidic or basic nature of a solution, the pH scale runs from 0 to 14 with 7 representing a neutral condition in most cases (Brady,1990). pH measurement is one of the most important and regularly used tests in water

analysis; pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes, because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures (Eaton, 1998).

Table 3.1: Summary of testing, Zoo Lake

	Sept.	**Oct.**				**Nov.**				**Dec**
Testing dates	**30th**	**7th**	**14th**	**21st**	**28th**	**4th**	**11th**	**19th**	**25th**	**12th**
	Before Ds	**Ds**	**Ds**	**Ds**	**Ds**	**Ds**	**Ds**	**Ds**	**Ds**	**Ds**
Zoo Lake sampling point 1	pH C NH3 PO4 T TSS H.M.	pH C NH3 PO4 T TSS	pH C NH3 PO4 TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	H.M.
Zoo Lake sampling point 2	"	"	"	"	"	"	"	"	"	"
Zoo Lake sampling point 3	"	"	"	"	"	"	"	"	"	"
Zoo Lake sampling point 4	"	"	"	"	"	"	"	"	"	"

Abbreviations:

TSS- Total Suspended Solids *H.M. - Heavy Metals* *C- Conductivity*
Wk- Week *NH_3- Ammonia* *T- Turbidity*
Ds- Dosing *PO_4- Phosphate*

- **Apparatus and Procedure:** A pH meter consisting of a potentiometer, a glass electrode, a reference electrode and a temperature–compensating device was used. The electrode system was calibrated against standard buffer systems

of known pH namely pH 7 and pH 4. Electrodes were kept wet by keeping them in a storage solution when pH meter was not in use. Before use the electrodes were removed from the storage solution, rinsed, blotted dry with a soft tissue' placed in initial buffer solution (pH 7) and then the isopotential point was set. Electrodes were removed from the first buffer, rinsed thoroughly with distilled water, blotted dry and immersed in second buffer (pH 4). The purpose of standardization is to adjust the response of the glass electrode to the instrument. Equilibrium is established between the electrodes and sample by stirring the sample to ensure homogeneity; it is stirred gently to minimize carbon dioxide being drawn into the sample. Readings were then taken after successful calibrations.

2. **Conductivity:** Conductivity, *k,* is a measure of the ability of an aqueous solution to carry an electric current (Eaton, 1998); this ability depends on the presence of ions, their total concentration, mobility and valence; and on the temperature of measurement. The units of *k* are1/ohm-cm. In the International System of Units (SI) the reciprocal of the ohm is the siemens (S) and conductivity is reported as millisiemens per meter (mS/m).

   - **Apparatus and Procedure:** The conductivity meter does not display the actual solution conductance, *G*, or resistance, *R*, it has a dial that permits you to adjust the internal cell constant to match the conductivity, k_s, of a standard. Once the cell constant has been set, the meter will display the conductivity of an unknown solution.

3. **Turbidity:** The clarity of a natural body of water is important in determining its condition and productivity. Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter and microscopic organisms (Davies and Day, 1998). Turbidity is an expression of the optical property in which light is scattered and absorbed rather than transmitted without a change in direction or flux level through a liquid.

   - **Apparatus:** An electronic laboratory nephelometer was used and the results were reported as nephelometric turbidity units (NTU). The apparatus consists of a light source for illuminating the sample. Sample cells, that is, tubes of clear colourless glass, were used to test samples. Matching pairs of cells were used for standardization and sample measurement.
   - **Procedure:** The manufacturer's operating instructions were followed in calibrating the nephelometer. For our standard, commercial gels of 10 NTU formazin were used. Turbidity was measured soon after samples got to the laboratory to prevent temperature changes and to prevent particle flocculation in the sample from changing the characteristics of the sample. To measure the turbidity, the sample was gently shaken then poured into a cell (glass tube) The turbidity was then read directly from the instrument display.

4. **Total Suspended Solids:** Total suspended solids (TSS) are particles that are suspended in water; they include tiny particles of silts, clays, living organisms and dead particulate organic matter (Davies and Day, 1998).

   - **Apparatus:** The following apparatus was used: Evaporating dishes made of heat resistant glass, filter paper, a dessicator, a drying oven for operation at 110^{o}C, an electric scale capable of weighing up to 0.1 mg, 250 ml measuring cylinders, a conical flask with filter disk connected to suction machine
   - **Procedure:** Each sample was shaken well and was placed in a 250ml measuring cylinder up to the 250ml mark. The same was done for a blank (a clear sample) made of distilled water, this blank was included to correct for the loss in weight of the filter paper. Evaporating dishes were taken and filter paper was placed on each then weighed together with the filter paper. Each of the samples and blank in the measuring cylinders were filtered through the conical flask (one after the other) with the weighed filter paper whilst the suction machine was on until all traces of water were removed. Samples were then placed in an oven at a temperature of 110^{o}C for 2 hours. They were then placed in a dessicator to cool and then weighed. The increase in weight of the filter paper and evaporating dish represented the total suspended solids.

5. **Phosphate as P:** Phosphorus occurs in natural water and wastewater as orthophosphates, condensed phosphates and organically bound phosphates. They are found in solution, in particles or in the bodies of aquatic organisms. Organic

phosphates are formed mainly by biological processes and are contributed to sewage by body wastes and food residues and may be formed from orthophosphates in biological treatment processes (Eaton, 1998).

- **Apparatus and Procedure:** A photometer was used at 690 nm. Acid washed glassware was used for determining low concentrations of phosphorus, as phosphate contamination is common because of its absorption on glass surfaces. The Merck spectroquant technique was used. Refer to 14848 P in the Merck Manual Photometer SQ118 for the procedure. To calculate the final reading in mg/l, the absorbance is multiplied by a built in factor to give the reading in mg/l.

6. **Ammonia as N:** The term ammonia refers to two chemical species which are in equilibrium in water (NH_3, un-ionized and NH_4^+, ionized (Eaton, 1998). Tests for ammonia usually measure total ammonia (NH_3 plus NH_4^+). The toxicity of ammonia is primarily attributable to the un-ionized form (NH_3), as opposed to the ionized form (NH_4). Ammonia is present naturally in surface and wastewaters. It is produced mostly by deamination of organic nitrogen-containing compounds and by hydrolysis of urea. Some water treatment plants even add ammonia to react with chlorine to form combined chlorine residual. Ammonia concentrations in water fluctuate from less than 10 mg ammonia nitrogen/L in some natural surface water to more than 30 mg/L in some wastewaters.

- **Apparatus and Procedure:** A spectrophotometer, for use at 690 nm with a light path of 1cm or more. The Merck spectroquant technique was used. Refer to 14752 NH_4^- in the Merck Manual Photometer SQ118 for the procedure. Just as in phosphate, the final readings in mg/l were calculated by multiplying the absorbance by a built in factor.

7. **Heavy Metals:** Heavy metals include metals with atomic weights greater than that of calcium. Metals such as iron, manganese, zinc, mercury and lead fall in this category. Trace elements include both metallic and non metallic elements. Some heavy metals at times find their way into water bodies through human activities. (Davies and Day, 1998).

    - **Apparatus and procedure:** An atomic absorption spectrophotometer was used for the testing of heavy metals. Each 100ml sample was mixed with 10 ml HNO_3. This was evaporated to the lowest volume possible (10 – 20 ml) before it started precipitating Digestion was shown to be complete by a light coloured clear solution. It was then made up to 100 ml.

3.4. Wastewater tests

A laboratory experiment was set up to inject wastewater with Multi EM (from the same source as Zoo Lake, the 5000L tanks) prior to any treatment at a sewage treatment works. The experiment was done in two stages, an anoxic and aerobic stage.

Wastewater was collected from Goudkoppies Wastewater Treatment Works in seven buckets and in a 20L container to top up the buckets. These were set up within the water quality laboratory located at the School of Civil and Environmental Engineering. The seventh bucket was a control also kept in the laboratory with no EM treatment in the duration of the study. The other buckets had the wastewater and EM added to them in different ratios. The same ratios of Multi EM were added to the buckets on a weekly basis over six weeks from 4th February 2009 to 10th March 2009 (Table 3.2). The buckets were stirred occasionally from Monday to Friday twice a day. This was the first stage, the anoxic part of the experiment,

In the second stage, that is the aerobic stage, water pumps were connected to each of the buckets via some tubes to provide aeration to the wastewater 24 hours a day. This was carried out over the same number of weeks as the first stage of the wastewater laboratory experiment from 19th March 2009 to 21st April 2009. Samples of wastewater from each of the buckets were analysed and compared with the control prior to addition of EM (Table 3.2). The same parameters as before were measured that is, pH, conductivity, turbidity, total suspended solids, ammonia, phosphates and heavy metals. The same standard methods for the examination of water and wastewater used for analysing the Zoo Lake samples were used to analyse the wastewater from the anoxic and aerobic experiments.

Table 3.2: Summary of parameters tested with different EM concentrations for the anoxic and aerobic experiments

Anoxic experiment						
	4 Feb. 09	**10 Feb.09**	**17 Feb.09**	**24 Feb. 09**	**3 Mar. 09**	**10 Mar. 09**
➢ Control: 1 bucket with no EM ➢ 2 buckets with EM in the ratio 1:1000 ➢ 2 buckets with EM in the ratio 1:500 ➢ 2 buckets with EM in the ratio 1:250	pH C NH3 PO4 T TSS H.M.	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS	pH C NH3 PO4 T TSS H.M.
Aerobic experiment						
	19 Mar. 09	**25 Mar. 09**	**1 Apr. 09**	**8 Apr. 09**	**15 Apr. 09**	**21 Apr. 09**
➢ Control: 1 bucket with no EM ➢ 2 buckets with EM in the ratio 1:1000 ➢ 2 buckets with EM in the ratio 1:500 ➢ 2 buckets with EM in the ratio 1:250	pH C NH_3 PO_4 T TSS H.M.	pH C NH_3 PO_4 T TSS	pH C NH_3 PO_4 T TSS	pH C NH_3 PO_4 T TSS	pH C NH_3 PO_4 T TSS	pH C NH_3 PO_4 T TSS H.M.

Abbreviations:
TSS-Total Suspended Solids H.M-Heavy Metals; Ds- Dosing; PO_4-phosphate; Wk-Week; NH_3-Ammonia; C-conductivity; T-turbidity

Materials:

- Four 1L plastic containers for collecting Zoo Lake samples
- a metal holder with a handle for immersing containers into the lake
- laboratory reagents and laboratory equipment for testing samples seven 10 L buckets for the laboratory experiment
- seven 1 L plastic containers for collecting samples from the buckets for analysis

- one 20 L container
- two 5000 L tanks for preparing the EM
- two drums for pouring the EM into the lake
- stock EM, molasses and wheat bran
- three aquatic pumps.

3.5. Validating the EM prior to application

To validate whether the EM that was going to be used in the studies was virulent, it was inoculated onto two Petri dishes with agar. One Petri dish was incubated at 37^{o}C and the other at 25^{o}C. After five days, there was growth on the agar indicating that the EM was alive and active (Figure 3.3).

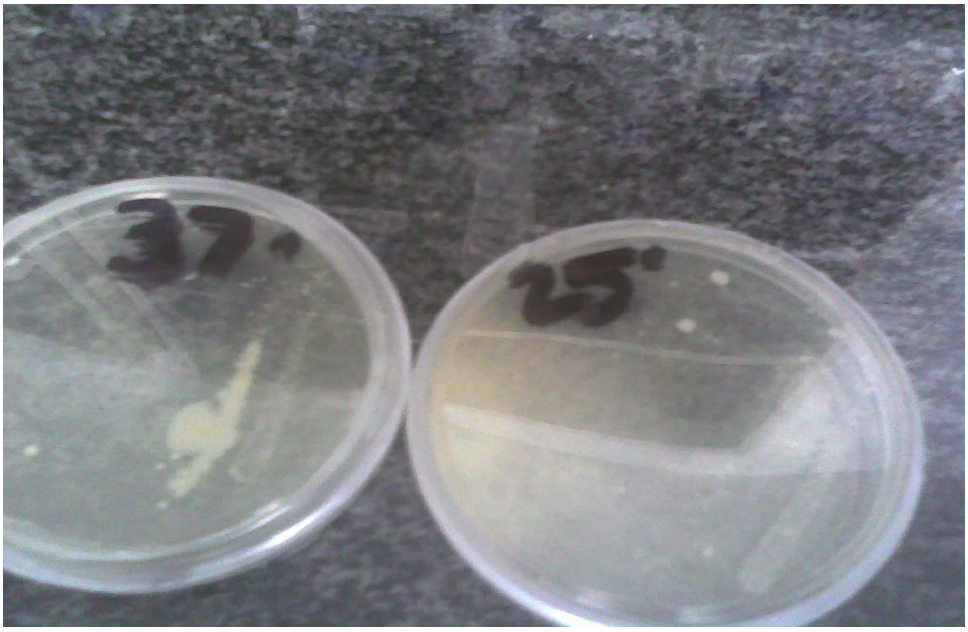


Figure 3.3: Growth in Petri dishes showing that the EM is active

3.6 Data Analysis

Statistica 6.0 was the statistics software programme that was used in the data analysis. Differences in mean measurements from the start and treatment were compared using one way ANOVA test at the standard 5% significance level. The resulting probability was used to make the decision to accept or reject the hypotheses put forward.

A one way analysis of variance (ANOVA) was also conducted with every analysis. Similar to the Zoo Lake study, this helped to validate, the results or *p* value obtained. In addition, a post hoc test (Tukey test) was conducted for the anoxic and aerobic experiments. The post hoc test was necessary as there were three treatment results emanating from the three EM ratios, therefore, the post hoc test helped to show the differences within the treatments as well.

The null hypothesis, H_o presented in the introductory chapter, stipulates that there is no statistically significant difference between the control and the treatment. A *p* value below 0.05 (5%) at 0.95 (95%) confidence interval shows differences are statistically significant and the null hypothesis, H_o is rejected. If it is above 0.05, there is no statistically significant difference in the results and the null hypothesis is accepted.

CHAPTER 4. LEVELS OF TREATMENT ACHIEVED THROUGH THE APPLICATION OF EM TO SURFACE WATERS AT ZOO LAKE

4.1. Introduction

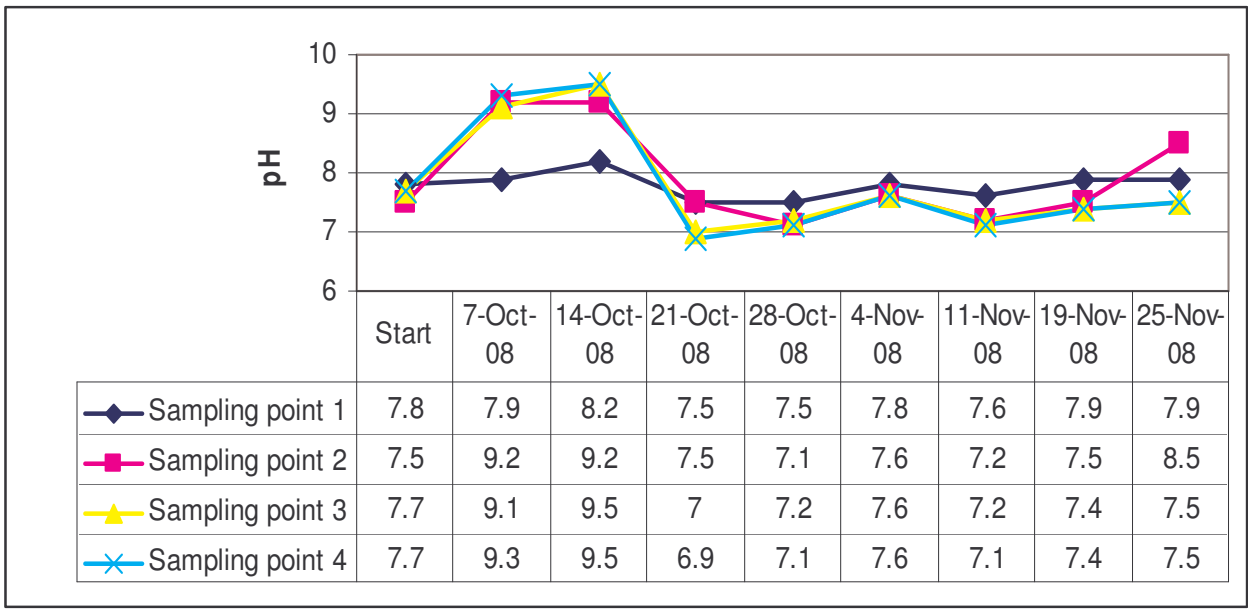
Four sampling points in the lake were chosen (c.f. Figure 3.2.). Baseline sampling was done before EM was added to the lake (starting point before treatment). Thereafter sampling was done on a weekly basis for two months. The combined tables and line graphs below show readings for the measured parameters from the 4 sampling points.

4.2 Results

Start refers to the state of the water before any EM was added. Treatment refers to the state of the water after dosing with EM.

4.2.1. pH

The pattern of pH change was the same in three sites, except site 1 (Figure 4.1). The one-way ANOVA showed that there were no significant differences in pH during the study in all the sites. F (1,6) 4.9091, p= 0.0686.

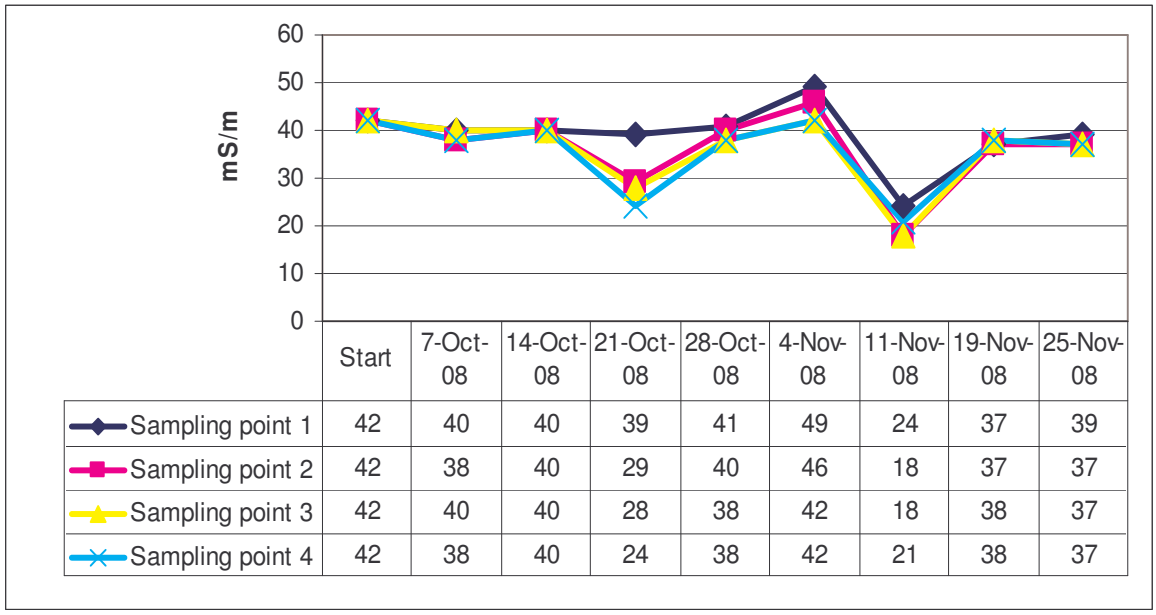


	Start	7-Oct-08	14-Oct-08	21-Oct-08	28-Oct-08	4-Nov-08	11-Nov-08	19-Nov-08	25-Nov-08
Sampling point 1	7.8	7.9	8.2	7.5	7.5	7.8	7.6	7.9	7.9
Sampling point 2	7.5	9.2	9.2	7.5	7.1	7.6	7.2	7.5	8.5
Sampling point 3	7.7	9.1	9.5	7	7.2	7.6	7.2	7.4	7.5
Sampling point 4	7.7	9.3	9.5	6.9	7.1	7.6	7.1	7.4	7.5

Figure 4.1: pH measurements for the four sampling points at Zoo Lake over nine weeks

4.2.2. Conductivity

Conductivity was more or less constant the first two weeks after addition of EM to the lake, but it subsequently fluctuated (Figure 4.2). The one-way ANOVA showed that there was a significant difference in conductivity between the start of the experiment and the treatments F (1,6) 45.696, p= 0.0005.

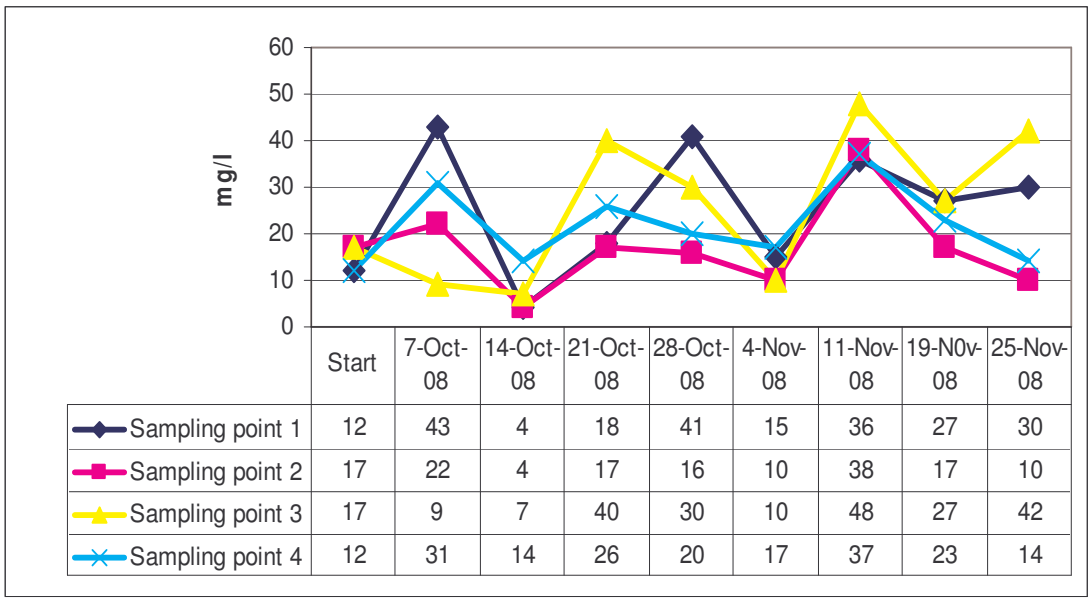


	Start	7-Oct-08	14-Oct-08	21-Oct-08	28-Oct-08	4-Nov-08	11-Nov-08	19-Nov-08	25-Nov-08
Sampling point 1	42	40	40	39	41	49	24	37	39
Sampling point 2	42	38	40	29	40	46	18	37	37
Sampling point 3	42	40	40	28	38	42	18	38	37
Sampling point 4	42	38	40	24	38	42	21	38	37

Figure 4.2: Conductivity measurements for the four sampling points at Zoo Lake over nine weeks

4.2.3. Total Suspended Solids (TSS)

TSS was erratic throughout the study. It was greater for most sampling points at the end of the study than it was at the beginning (Figure 4.3). There were no significant differences in mean TSS at the four sites during the study as the one-way ANOVA depicted F (1,6) 10.0091, p= 0.0195.

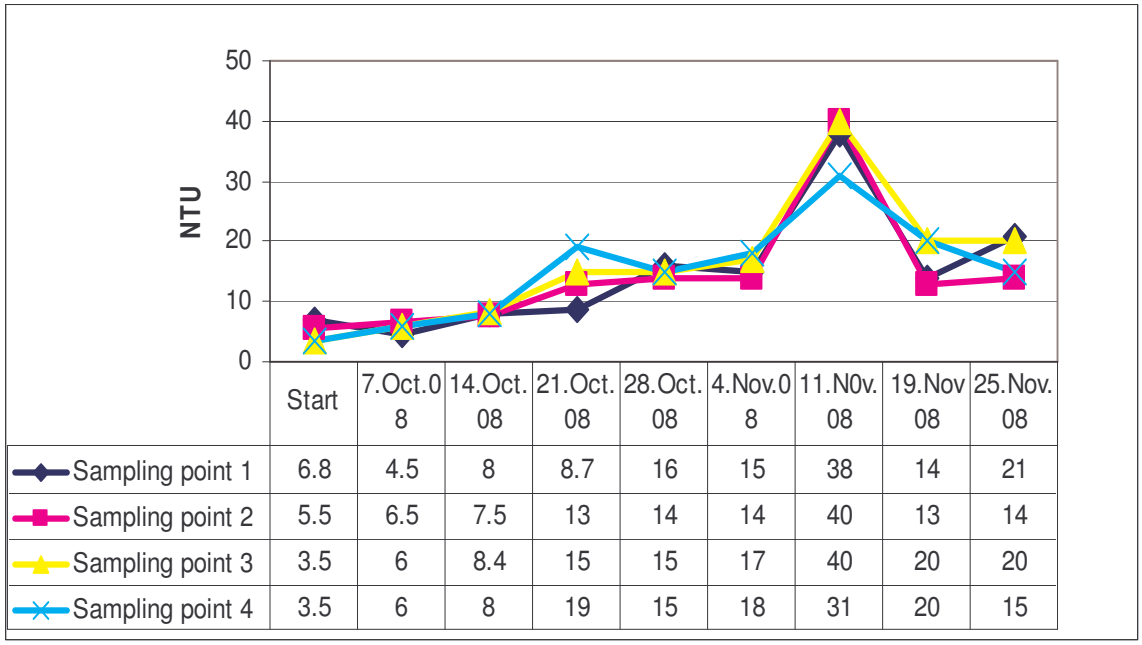


	Start	7-Oct-08	14-Oct-08	21-Oct-08	28-Oct-08	4-Nov-08	11-Nov-08	19-N0v-08	25-Nov-08
Sampling point 1	12	43	4	18	41	15	36	27	30
Sampling point 2	17	22	4	17	16	10	38	17	10
Sampling point 3	17	9	7	40	30	10	48	27	42
Sampling point 4	12	31	14	26	20	17	37	23	14

Figure 4.3: TSS measurements for the four sampling points at Zoo Lake over nine weeks

4.2.4. Turbidity

There was a gradual rise in turbidity, reaching its highest peak the week of 11th November (Figure 4.4). There was a statistically significant difference in turbidity with the start of the experiment before any addition of EM among the four sampling sites in the duration of the study as shown by the one-way ANOVA, F (1,6) 138.8358, p=0.00002.

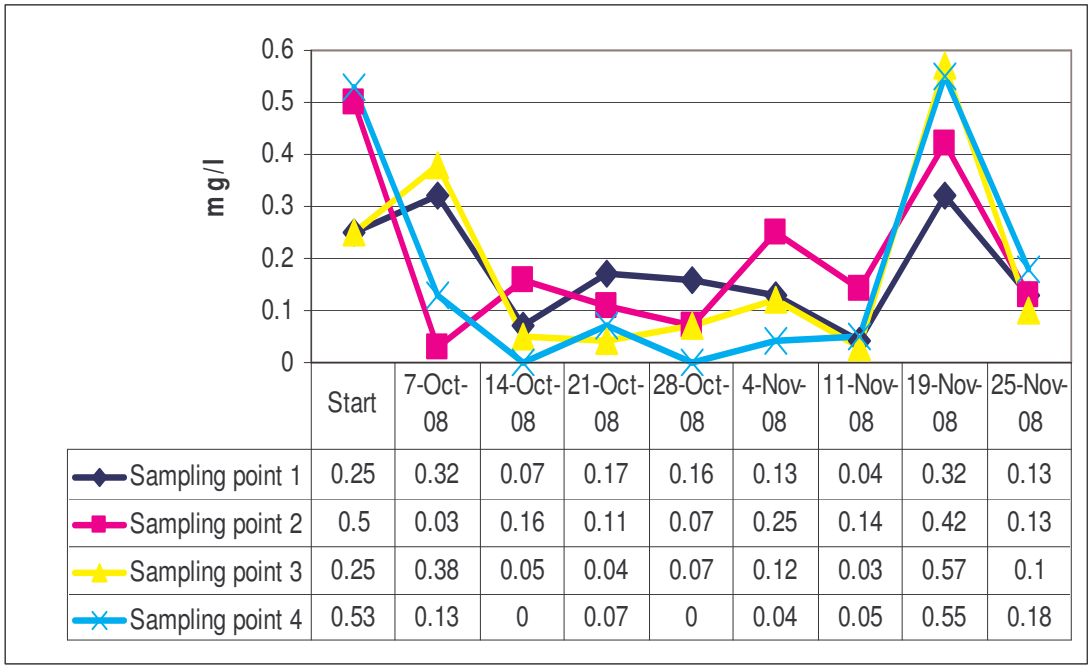


	Start	7.Oct.08	14.Oct.08	21.Oct.08	28.Oct.08	4.Nov.08	11.N0v.08	19.Nov 08	25.Nov.08
Sampling point 1	6.8	4.5	8	8.7	16	15	38	14	21
Sampling point 2	5.5	6.5	7.5	13	14	14	40	13	14
Sampling point 3	3.5	6	8.4	15	15	17	40	20	20
Sampling point 4	3.5	6	8	19	15	18	31	20	15

Figure 4.4: Turbidity measurements for the four sampling points at Zoo Lake over nine weeks

4.2.5. Ammonia as N

At each sampling point, the ammonia at the end of the study was less than at the beginning of the study (Figure 4.5). There was a significant difference in mean ammonia concentrations at the four sampling points between the start of the experiment and the treatments as the one-way ANOVA shows F (1,6) 7.8229, p= 0.0313.

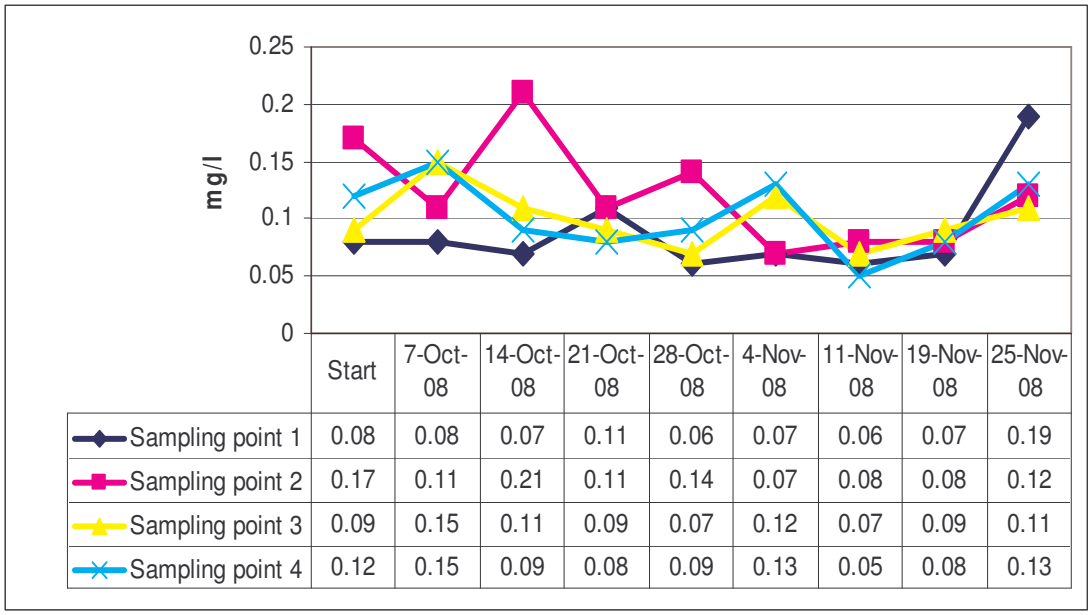


	Start	7-Oct-08	14-Oct-08	21-Oct-08	28-Oct-08	4-Nov-08	11-Nov-08	19-Nov-08	25-Nov-08
Sampling point 1	0.25	0.32	0.07	0.17	0.16	0.13	0.04	0.32	0.13
Sampling point 2	0.5	0.03	0.16	0.11	0.07	0.25	0.14	0.42	0.13
Sampling point 3	0.25	0.38	0.05	0.04	0.07	0.12	0.03	0.57	0.1
Sampling point 4	0.53	0.13	0	0.07	0	0.04	0.05	0.55	0.18

Figure 4.5: Ammonia measurements for the four sampling points at Zoo Lake taken over nine weeks

4.2.6. Phosphate as P

Phosphate went up slightly at the end of the study (except the second sampling point which was slightly reduced). The one-way ANOVA showed no statistically significant difference in phosphate between the start of the experiment and the treatments, F (1,6) 0.4324, p= 0.5352.



	Start	7-Oct-08	14-Oct-08	21-Oct-08	28-Oct-08	4-Nov-08	11-Nov-08	19-Nov-08	25-Nov-08
Sampling point 1	0.08	0.08	0.07	0.11	0.06	0.07	0.06	0.07	0.19
Sampling point 2	0.17	0.11	0.21	0.11	0.14	0.07	0.08	0.08	0.12
Sampling point 3	0.09	0.15	0.11	0.09	0.07	0.12	0.07	0.09	0.11
Sampling point 4	0.12	0.15	0.09	0.08	0.09	0.13	0.05	0.08	0.13

Figure 4.6: Phosphate measurements in for the four sampling points at Zoo Lake over nine wee

4.2.7. Heavy Metals

Table 4.1: Heavy metal measurements for the four sampling points at Zoo Lake. Readings were taken before the addition of EM (control) and at the end of the study

S/ point	Copper		Iron		Manganese		Cadmium		Lead	
	control	9 Dec.	control	9 Dec.	control	9 Dec.	control	9 Dec.	control	9 Dec.
1	0.00	0.00	0.95	0.56	0.55	0.1	0.00	0.002	0.07	0.00
2	0.00	0.00	0.94	0.71	0.14	0.14	0.00	0.003	0.08	0.00
3	0.00	0.00	1.2	0.9	0.12	0.18	0.00	0.007	0.09	0.00
4	0.00	0.00	1	1.02	0.16	0.24	0.00	0.01	0.11	0.00

Iron content decreased slightly at the end of the study. Manganese was more or less the same except the first sampling point which had a significant reduction. There were traces of cadmium at the end of the study but not at the beginning. Lead was there at the beginning of the study but not at the end. Iron and manganese showed no statistically significant differences in concentration between the start of the experiment and the treatments. Cadmium and lead showed statistically significant differences in cadmium and lead content between the control and treatment.

Results from the above analyses comparing the treatment and control at Zoo Lake are summarized (Table 4.2).

Table 4.2: A summary of the levels of treatment achieved from the application of EM to surface waters at Zoo Lake

Parameter	Period of sampling	Average of the four samples
pH	Start	7.67
	1 week later	8.87
	End	7.85
Conductivity (mS/m)	Start	42
	1 week later	39
	End	37.5
TSS (mg/l)	Start	14.5
	1 week later	26.3
	End	24
Turbidity (NTU)	Start	4.8
	1 week later	5.7
	End	17.5[#]
Ammonia (mg/l)	Start	0.38
	1 week later	0.21
	End	0.13
Phosphate (mg/l)	Start	0.11
	1 week later	0.12
	End	0.13
Copper (mg/l)	Start	0.00
	1week later	0.00
	End	0.00
Iron (mg/l)	Start	1.0
	1 week later	1.0
	End	0.8
Manganese (mg/l)	Start	0.24
	1 week later	0.24
	End	0.16
Cadmium (mg/l)	Start	0.00
	1 week later	0.00
	End	0.0055[#]
Lead (mg/l)	Start	0.087
	1 week later	0.087
	End	0.00[#]

[#]*Statistical significant difference*

##Start for the control and samples are the same value prior to the treatment of the four samples with EM

CHAPTER 5. LEVELS OF TREATMENT ACHIEVED THROUGH THE APPLICATION OF EM TO WASTEWATER WITHIN AN ANOXIC ENVIRONMENT

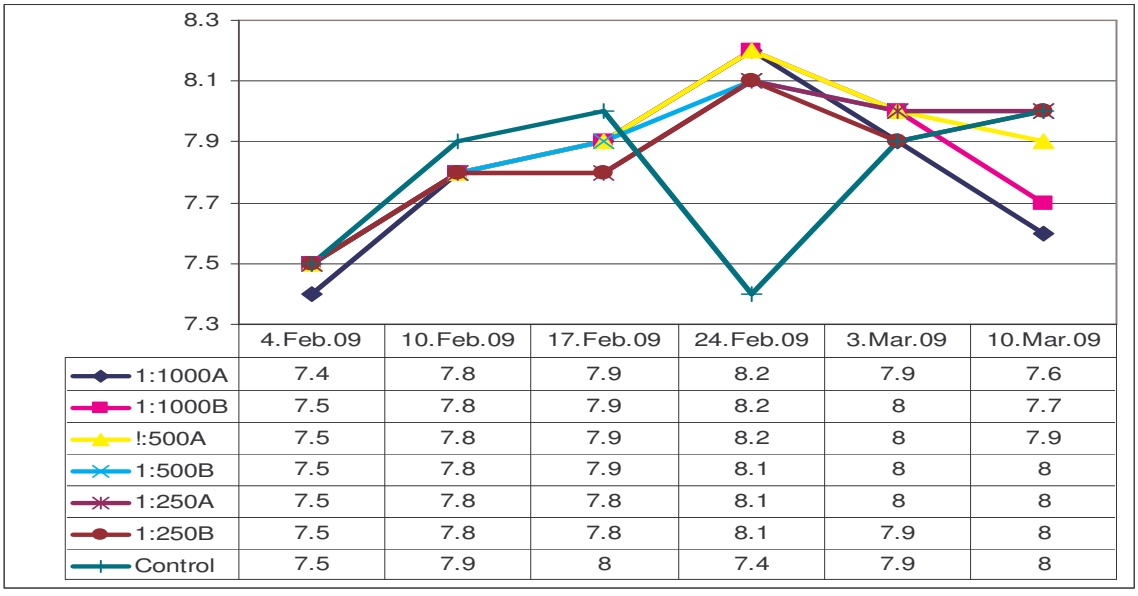
5.1. Introduction

A full description of how the anoxic experiment was carried out is outlined in section 3.4. Seven buckets of wastewater were set up in the water quality laboratory. Two buckets were dosed with EM in the ratio 1:1000, two in the ratio 1:500 and two in the ratio 1:250. The seventh bucket of wastewater was a control with no EM added to it throughout the testing period. The addition of EM and testing of samples was carried out on a weekly basis for six weeks. In this study, control refers to the wastewater during the study period that had no EM added to it. Treatment refers to the state of the water after addition of EM.

5.2. Results

5.2.1. pH

There was a continual rise in pH for three weeks after addition of EM to the buckets. This was observed in all samples including the control. There was a drastic drop in pH for the control from 8 to 7.4 in the fourth week. This went up to 7.9 the week after and to 8 in the last week. The EM with ratio 1:1000 saw a drop in pH from 8.2 in the fourth week to 7.6 and 7.7 in the sixth week. The EM with ratio 1:500 went up to 8.2 and 8.1 in the fourth week and dropped slightly to7.9 and 8 in the sixth week. Lastly, the EM with ratio 1:250 had pH rise from up to 8.1 in the fourth week and dropped slightly to 8 in the sixth week.



	4.Feb.09	10.Feb.09	17.Feb.09	24.Feb.09	3.Mar.09	10.Mar.09
1:1000A	7.4	7.8	7.9	8.2	7.9	7.6
1:1000B	7.5	7.8	7.9	8.2	8	7.7
!:500A	7.5	7.8	7.9	8.2	8	7.9
1:500B	7.5	7.8	7.9	8.1	8	8
1:250A	7.5	7.8	7.8	8.1	8	8
1:250B	7.5	7.8	7.8	8.1	7.9	8
Control	7.5	7.9	8	7.4	7.9	8

Figure 5.1: pH results for anoxic experiment taken over six weeks

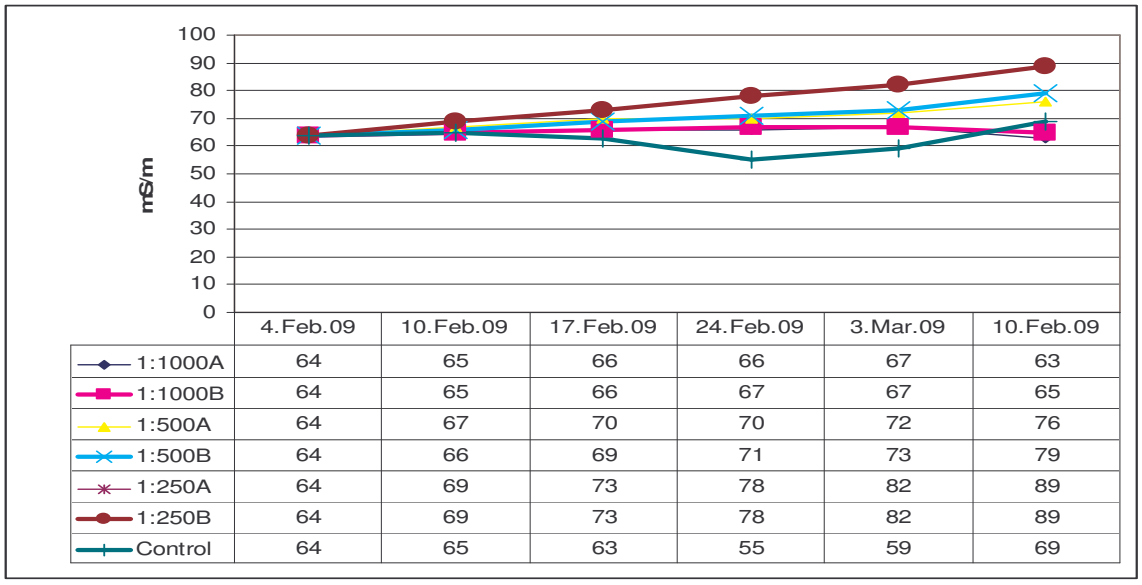
The one-way ANOVA showed that there was a statistically significant difference in pH, F (3,4) 71.5555, p = 0.0065. The control was not significantly different from ratio 1:1000 but was significantly different from ratios 1:500 and 1:250 as can be seen from Tukey's test (Table 5.1)

Table 5.1. Tukey test for pH results

Tukey HSD test; variable pH (Spreadsheet pH exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00025, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.062535	0.005755	0.016136
2	1:1000	0.062535		0.062535	0.355283
3	1:500	0.005755	0.062535		0.355283
4	1:250	0.016136	0.355283	0.355283	

5.2.2 Conductivity

The conductivity for the ratios 1:1000 rose slightly during the course of the study but by the end of the six weeks, it was similar to what it was at the beginning of the experiment. The conductivity for the ratios 1:500 and 1:250 rose steadily and was high by the end of the experiment. The control's conductivity was the only one that dropped during the study and this happened in the fourth week. It rose again and was the largest by the end of the experiment compared with the beginning (Figure 5.2).



	4.Feb.09	10.Feb.09	17.Feb.09	24.Feb.09	3.Mar.09	10.Feb.09
1:1000A	64	65	66	66	67	63
1:1000B	64	65	66	67	67	65
1:500A	64	67	70	70	72	76
1:500B	64	66	69	71	73	79
1:250A	64	69	73	78	82	89
1:250B	64	69	73	78	82	89
Control	64	65	63	55	59	69

Figure 5.2: Conductivity results for the anoxic experiment taken over six weeks

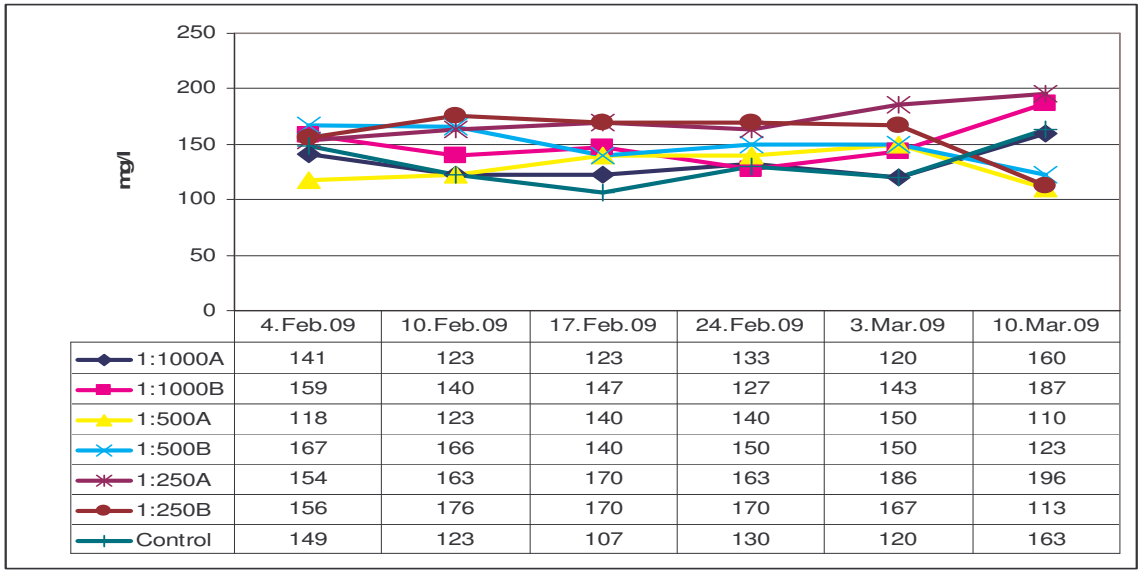
The one-way ANOVA and the Tukey test showed that there was a statistically significant difference between the control and treatments. The Tukey test further showed that this difference was between all the treatments and the control (Table 5.2).

Table 5.2. Tukey test for conductivity results

Tukey HSD test; variable mS/m (Spreadsheet Conductivity exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .09500, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.001868	0.000294	0.000291
2	1:1000	0.001868		0.000412	0.000291
3	1:500	0.000294	0.000412		0.000322
4	1:250	0.000291	0.000291	0.000322	

5.2.3 Total Suspended Solids (TSS)

The TSS content was erratic throughout the experiment for all the ratios including the control (Figure 5.3). The TSS content was greater at the end of the experiment than at the beginning for the ratios 1:1000, one of the 1:250 ratios and the control. The other 1:250 ratio and the ratios 1:500 had lower TSS at the end of the study than at the beginning.



	4.Feb.09	10.Feb.09	17.Feb.09	24.Feb.09	3.Mar.09	10.Mar.09
1:1000A	141	123	123	133	120	160
1:1000B	159	140	147	127	143	187
1:500A	118	123	140	140	150	110
1:500B	167	166	140	150	150	123
1:250A	154	163	170	163	186	196
1:250B	156	176	170	170	167	113
Control	149	123	107	130	120	163

Figure 5.3: TSS results taken over six weeks

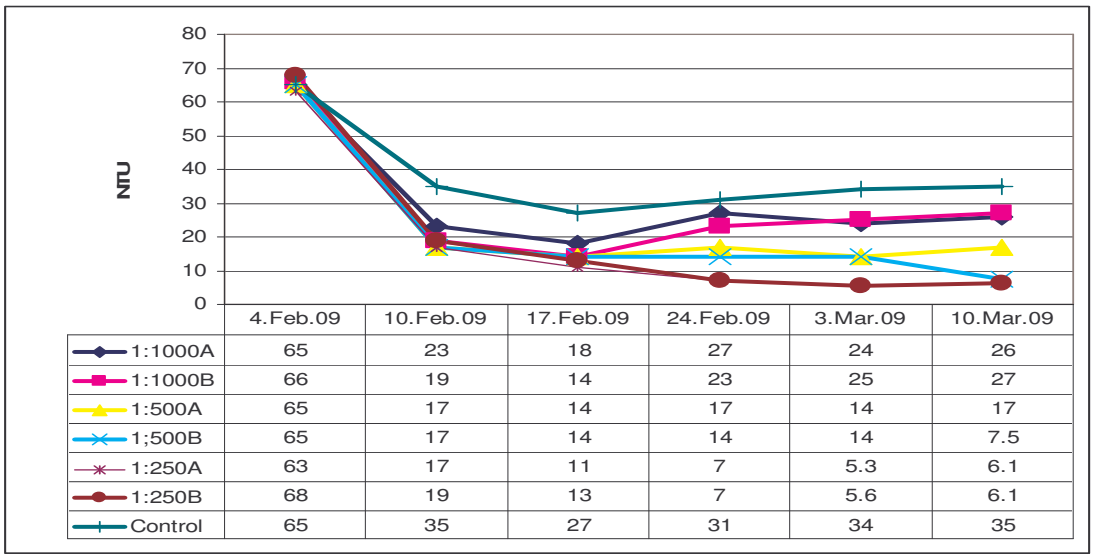
The one-way ANOVA showed no statistically significant difference (p = 0.0707) in TSS. Tukey's test further confirmed that there was no statistically significant difference between the control and treatments and within the treatments themselves (Table 5.3).

Table 5.3 Tukey test for TSS results

Tukey HSD test; variable mg/l (Spreadsheet TSS exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = 91.525, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.821847	0.871719	0.067607
2	1:1000	0.821847		0.999390	0.145267
3	1:500	0.871719	0.999390		0.130634
4	1:250	0.067607	0.145267	0.130634	

5.2.4 Turbidity

There was a significant drop in turbidity by the end of the study especially in the 1:500 and 1:250 ratios. The control had the highest turbidity by the end of the six weeks though its turbidity had also dropped but not as much as the treatments.



	4.Feb.09	10.Feb.09	17.Feb.09	24.Feb.09	3.Mar.09	10.Mar.09
1:1000A	65	23	18	27	24	26
1:1000B	66	19	14	23	25	27
1:500A	65	17	14	17	14	17
1;500B	65	17	14	14	14	7.5
1:250A	63	17	11	7	5.3	6.1
1:250B	68	19	13	7	5.6	6.1
Control	65	35	27	31	34	35

Figure 5.4: Turbidity results taken over six weeks

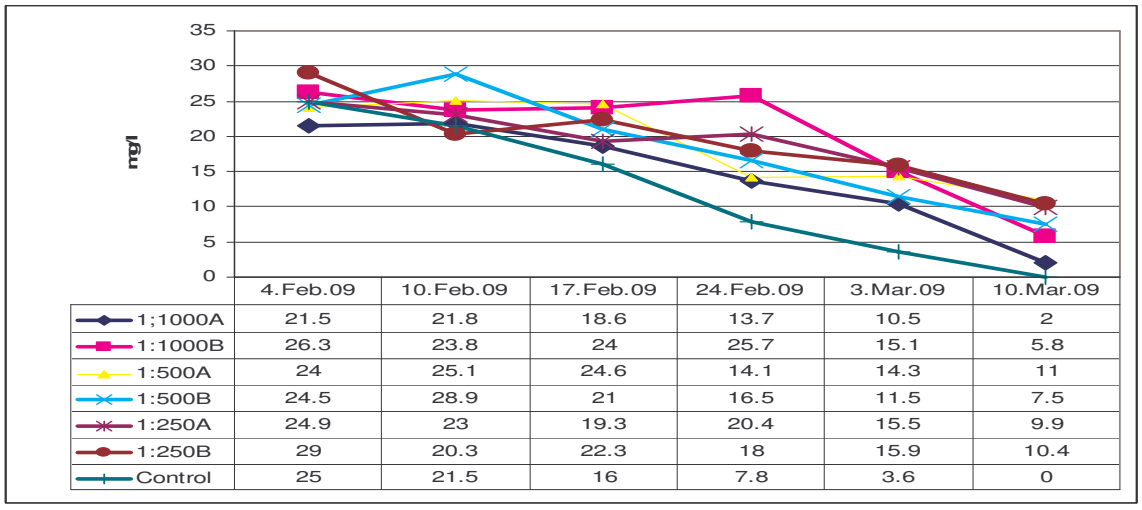
The one-way ANOVA showed that there was a statistically significant difference between the control and treatments p=0.00007. The Tukey test further confirmed that there was a statistically significant difference between the control and treatments and within the treatments themselves (Table 5.4).

Table 5.4 Tukey test for turbidity results

Tukey HSD test; variable NTU (Spreadsheet Ammonia exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = 1.3613, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.000908	0.000369	0.000307
2	1:1000	0.000908		0.008128	0.001507
3	1:500	0.000369	0.008128		0.046843
4	1:250	0.000307	0.001507	0.046843	

5.2.5 Ammonia as N

At the beginning of the study, ammonia was above 20mg/l for all the treatments and the control. By the end of the study, they were all below 10mg/l except one of the1:500 and 1:250 ratios. There were completely no traces of ammonia left in the control by the end of the study (Figure 5.5).



	4.Feb.09	10.Feb.09	17.Feb.09	24.Feb.09	3.Mar.09	10.Mar.09
1;1000A	21.5	21.8	18.6	13.7	10.5	2
1:1000B	26.3	23.8	24	25.7	15.1	5.8
1:500A	24	25.1	24.6	14.1	14.3	11
1:500B	24.5	28.9	21	16.5	11.5	7.5
1:250A	24.9	23	19.3	20.4	15.5	9.9
1:250B	29	20.3	22.3	18	15.9	10.4
Control	25	21.5	16	7.8	3.6	0

Figure 5.5: Ammonia results over six weeks

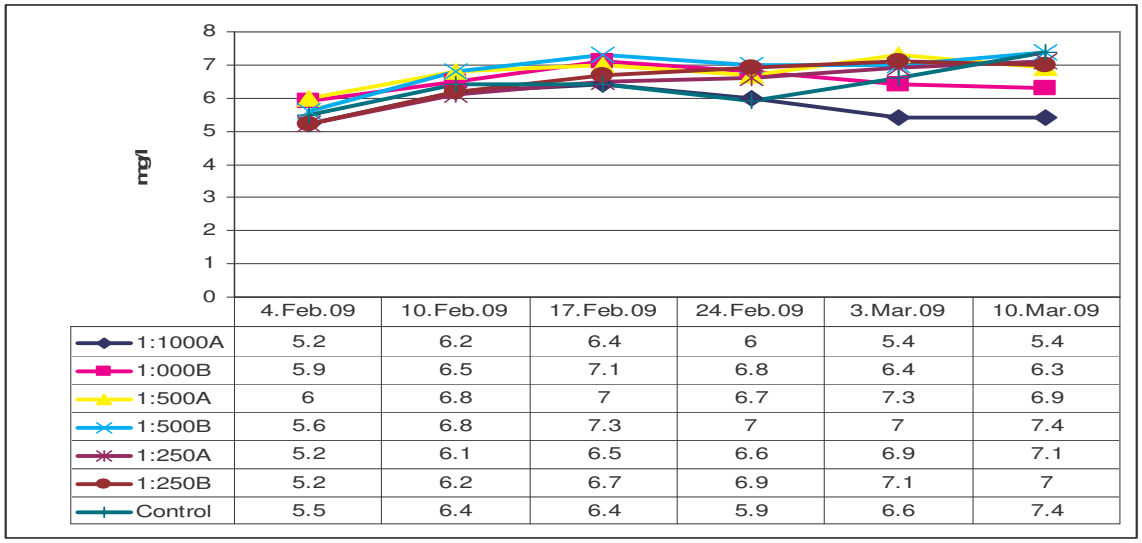
The one-way ANOVA showed no statistically significant differences in turbidity p= 0.1580. The Tukey test further confirmed that there was no statistically significant difference between the control and treatments and within the treatments themselves (Table 5.5).

Table 5.5 Tukey test for ammonia results

Tukey HSD test; variable mg/l (Spreadsheet Ammonia exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = 3.9862, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.353285	0.184351	0.180019
2	1:1000	0.353285		0.901187	0.891899
3	1:500	0.184351	0.901187		0.999994
4	1:250	0.180019	0.891899	0.999994	

5.2.6 Phosphate as P

The treatments and the control were between 5 and 6 mg/l at the beginning of the experiment. By the end of the study they had all gone up slightly and were now in the range of 6-7 mg/l except one of the ratios 1:1000 which had only gone up by 0.2 mg/l and was still below 6 mg/l (Figure 5.6).



	4.Feb.09	10.Feb.09	17.Feb.09	24.Feb.09	3.Mar.09	10.Mar.09
1:1000A	5.2	6.2	6.4	6	5.4	5.4
1:000B	5.9	6.5	7.1	6.8	6.4	6.3
1:500A	6	6.8	7	6.7	7.3	6.9
1:500B	5.6	6.8	7.3	7	7	7.4
1:250A	5.2	6.1	6.5	6.6	6.9	7.1
1:250B	5.2	6.2	6.7	6.9	7.1	7
Control	5.5	6.4	6.4	5.9	6.6	7.4

Figure 5.6: Phosphate results taken over six weeks

The one-way ANOVA showed no statistically significant differences ($p = 0.1413$) for the phosphates. The Tukey test further confirmed that there was no statistically significant difference between the control and treatments and within the treatments themselves (Table 5.6)

Table 5.6 Tukey test for phosphate results

Tukey HSD test; variable mg/l (Spreadsheet Phosphate exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .07410, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.968092	0.221486	0.633616
2	1:1000	0.968092		0.145811	0.432113
3	1:500	0.221486	0.145811		0.689100
4	1:250	0.633616	0.432113	0.689100	

5.2.7 Heavy Metals

The tests done on 4th of February were carried out before EM was added to the wastewater and those on the 10th of March after weekly addition of EM (Table 5.7).

Table 5.7: Heavy metal results taken at the beginning and at the end of the study

	Copper		Iron		Manganese		Cadmium		Lead	
Ratios	4 Feb	10 Mar	4 Feb	10 Mar	4 Feb	4 Mar	4 Feb	10 Mar	4 Feb	10 Mar
1:1000A	0.03	0.0615	3.1	3.6	0.4378	0.2435	0.0281	0.0176	0.1286	0.1511
1:1000B	0.0394	0.0666	3.5	4.1	0.472	0.3299	0.0114	0.0146	0.1302	0.1486
1:500A	0.0398	0.0198	3.5	3.7	0.4841	0.3265	0.0089	0.0151	0.1281	0.143
1:500B	0.0444	0.0267	3.6	3.9	0.4723	0.3649	0.0109	0.0165	0.1255	0.1389
1:250A	0.0365	0.0426	3.2	4.5	0.3977	0.3459	0.0116	0.0187	0.133	0.1334
1:250B	0.0335	0.0521	3.2	4	0.4134	0.4035	0.0128	0.0208	0.142	0.1312
Control	0.0372	0.0487	3.35	4	0.4462	0.3015	0.01395	0.0211	0.1312	0.2226

There was an increase in copper content for the ratios 1:1000, 1:250 and the control. There was a decrease in the 1:500 ratios. All samples including the control showed an increase in iron content. Manganese decreased in all samples. Cadmium increased in all samples except one of the 1:1000 ratios, which decreased. There was a slight increase in lead in all samples with the control having the highest increase

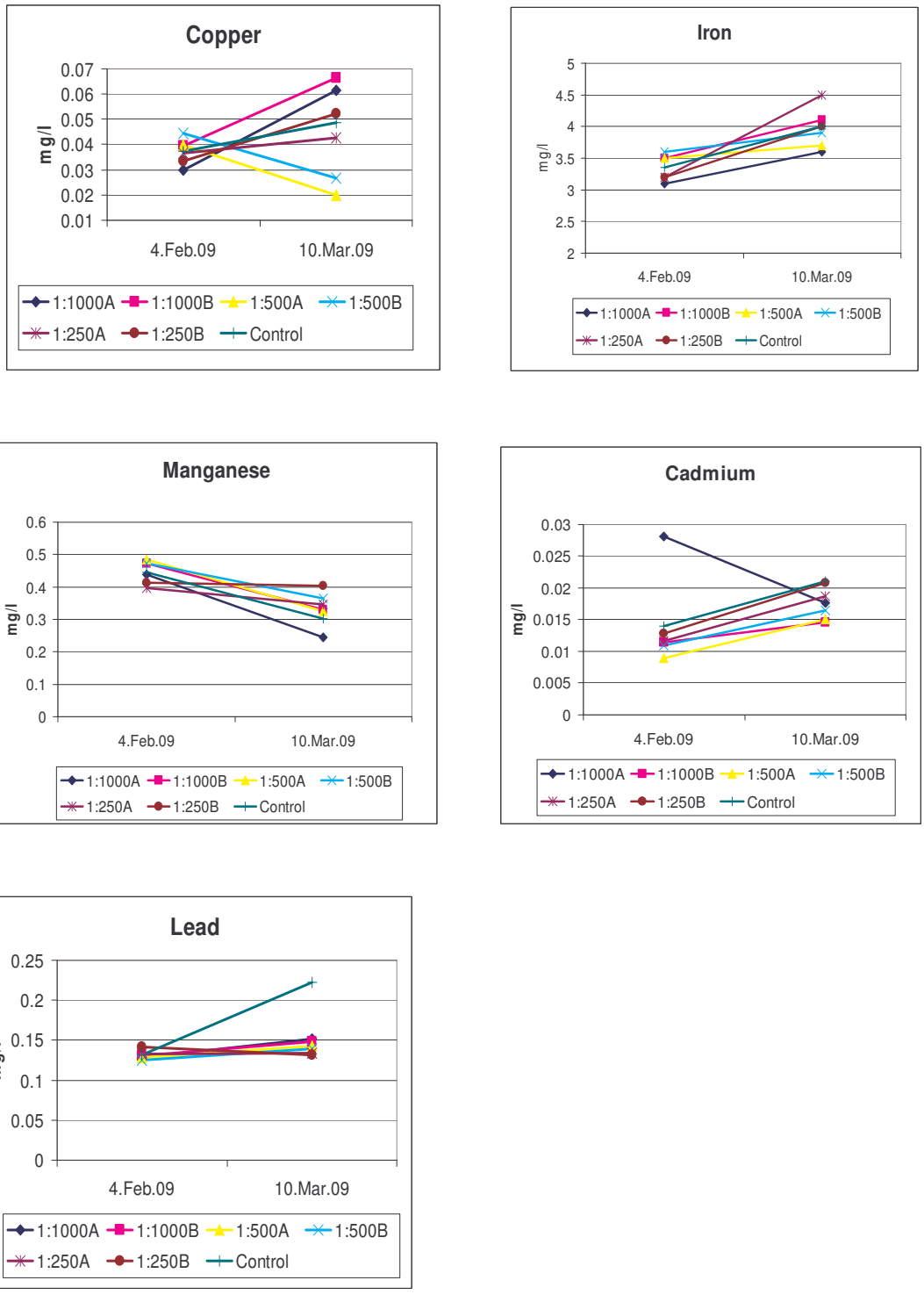


Figure 5.7: Heavy metal results taken at the beginning and at the end of the study

The one-way ANOVA results for copper showed a statistically significant difference (p = 0.0039) in copper content. The Tukey test (Table 5.7) however showed that it was the ratio 1:500 that was statistically different from the rest of the treatments and the control. The rest of the treatments (ratios 1:1000 and 1:250) and the control were not statistically significantly different from each other. The one-way ANOVA for iron showed there was no statistically significant difference between the control and treatments. This was confirmed using Tukey's test, which showed that there was no statistically significant differences between the control and treatments and among the treatments (Table5.8).It was the same for manganese as well with a p of 0.2389. For cadmium, the ANOVA showed a slightly significant difference (p = 0.0425). Tukey's test showed that there was no statistically significant difference between the control and treatments and among treatments (Table 5.9).. For lead, there was a statistically significant difference (p = 0.000003) in lead content. Tukey's test also confirmed that there was a statistically significant difference between the control and treatments and also among the treatments (Table 5.10).

Table 5.8 Tukey test foe copper results

Tukey HSD test; variable mg/l (Spreadsheet Copper exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00002, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.087859	0.016848	0.989486
2	1:1000	0.087859		0.003077	0.068193
3	1:500	0.016848	0.003077		0.020376
4	1:250	0.989486	0.068193	0.020376	

Table 5.9 Tukey test for iron results

Tukey HSD test; variable mg/l (Spreadsheet Iron exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .06750, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.933981	0.864650	0.777120
2	1:1000	0.933981		0.997112	0.495487
3	1:500	0.864650	0.997112		0.415469
4	1:250	0.777120	0.495487	0.415469	

Table 5.10 Tukey test for manganese results

Tukey HSD test; variable mg/l (Spreadsheet1 Manganese exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00153, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.979232	0.693956	0.364733
2	1:1000	0.979232		0.509918	0.253414
3	1:500	0.693956	0.509918		0.876448
4	1:250	0.364733	0.253414	0.876448	

Table 5.11 Tukey test for cadmium results

Tukey HSD test; variable mg/l (Spreadsheet Cadmium exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00000, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.073071	0.061072	0.771423
2	1:1000	0.073071		0.995898	0.175153
3	1:500	0.061072	0.995898		0.142986
4	1:250	0.771423	0.175153	0.142986	

Table 5.12 Tukey test for lead results

Tukey HSD test; variable mg/l (Spreadsheet Lead exp.1) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00000, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.000291	0.000291	0.000291
2	1:1000	0.000291		0.029807	0.002655
3	1:500	0.000291	0.029807		0.032805
4	1:250	0.000291	0.002655	0.032805	

Results obtained from the anoxic experiments were summarized (Table 5.13).

Table 5.13: A summary of the levels of treatment achieved from the application of EM to waste waters within an anoxic environment

		1:1000A	1:1000B	1:500A	1:500B	1:250A	1:250B	Control
pH	**Start**	7.4	7.5	7.5	7.5	7.5	7.5	7.5
	End	7.6	7.7	7.9	8.0	8.0	8.0	8.0
Conductivity	**Start**	64	64	64	64	64	64	64
	End	63	65	76#	79#	89#	89#	69
TSS	**Start**	141	159	118	167	154	156	149
	End	160	187	110	123	196	113	163
Turbidity	**Start**	65	66	65	65	63	68	65
	End	26#	27#	17#	7.5#	6.1#	6.1#	35
Ammonia	**Start**	21.5	26.3	24	24.5	24.9	29	25
	End	2.0#	5.8#	11	7.5#	9.9#	10.4	0#
Phosphate	**Start**	5.2	5.9	6	5.6	5.2	5.2	5.5
	End	5.4	6.3	6.9	7.4	7.4	7.1	7.4
Copper	**Start**	0.03	0.039	0.040	0.044	0.036	0.033	0.037
	End	0.06	0.067	0.02	0.027	0.043	0.052	0.049
Iron	**Start**	3.1	3.5	3.5	3.6	3.2	3.2	3.35
	End	3.6	4.1	3.7	3.9	4.5	4.0	4.0
Manganese	**Start**	0.438	0.472	0.484	0.472	0.398	0.413	0.446
	End	0.243	0.330	0.326	0.365	0.346	0.403	0.301
Cadmium	**Start**	0.028	0.011	0.009	0.011	0.012	0.013	0.014
	End	0.018	0.015	0.015	0.016	0.019	0.021	0.021
Lead	**Start**	0.129	0.130	0.128	0.125	0.133	0.142	0.131
	End	0.151	0.149	0.143	0.139	0.133	0.131	0.223

#Statistical significant difference

CHAPTER 6. LEVELS OF TREATMENT ACHIEVED THROUGH THE APPLICATION OF EM TO WASTEWATER WITHIN AN AEROBIC ENVIRONMENT

6.1. Introduction

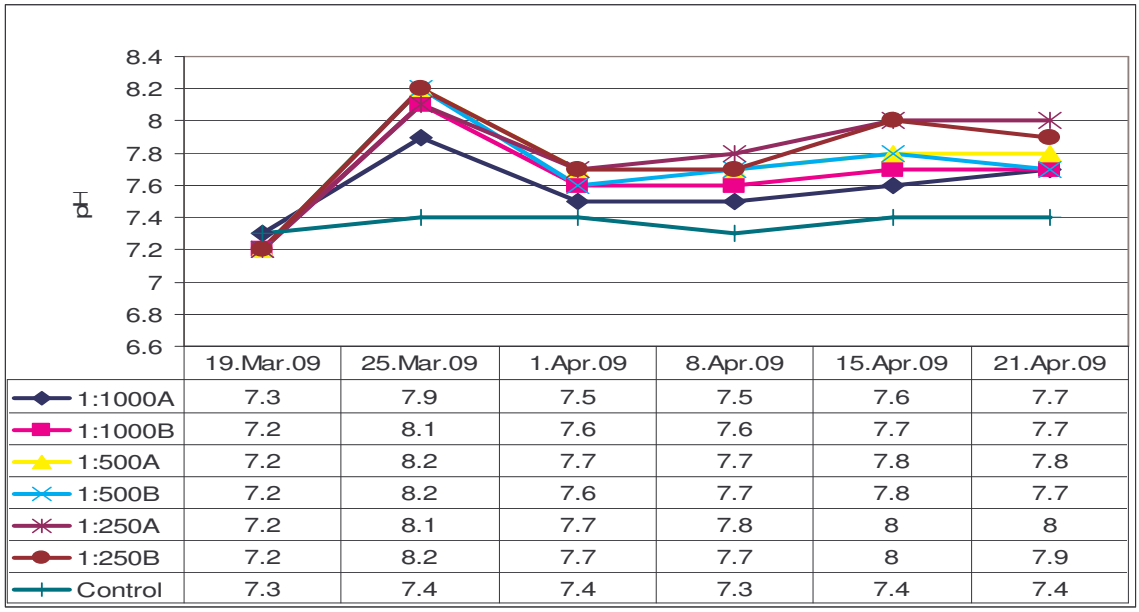
Just as in the anoxic experiment, seven buckets of wastewater were set up in the water quality laboratory where two were doused with EM in the ratio1:1000, two in the ratio 1:500 and two in the ratio 1:250. The addition of EM and testing of samples was carried out on a weekly basis for six weeks. The seventh bucket of wastewater was a control with no EM added to it throughout the testing period.

Air pumps were connected to each of the buckets giving 24-hour aeration to all seven buckets. This was the major difference between the anoxic and aerobic experiments. Just as in the previous study, control refers to the wastewater during the study period that had no EM added to it. Treatment refers to the state of the water after addition of EM.

6.2. Results

6.2.1 pH

The pH for all the treatments went up from 7.2 up to 8.2 a week after addition of EM and gradually came down in the following weeks. All treatments were above 7.6 at the end of the study. The control did not change much but fluctuated between 7.3 and 7.4 throughout the study period (Figure 6.1).



	19.Mar.09	25.Mar.09	1.Apr.09	8.Apr.09	15.Apr.09	21.Apr.09
1:1000A	7.3	7.9	7.5	7.5	7.6	7.7
1:1000B	7.2	8.1	7.6	7.6	7.7	7.7
1:500A	7.2	8.2	7.7	7.7	7.8	7.8
1:500B	7.2	8.2	7.6	7.7	7.8	7.7
1:250A	7.2	8.1	7.7	7.8	8	8
1:250B	7.2	8.2	7.7	7.7	8	7.9
Control	7.3	7.4	7.4	7.3	7.4	7.4

Figure 6.1: pH results taken over six weeks

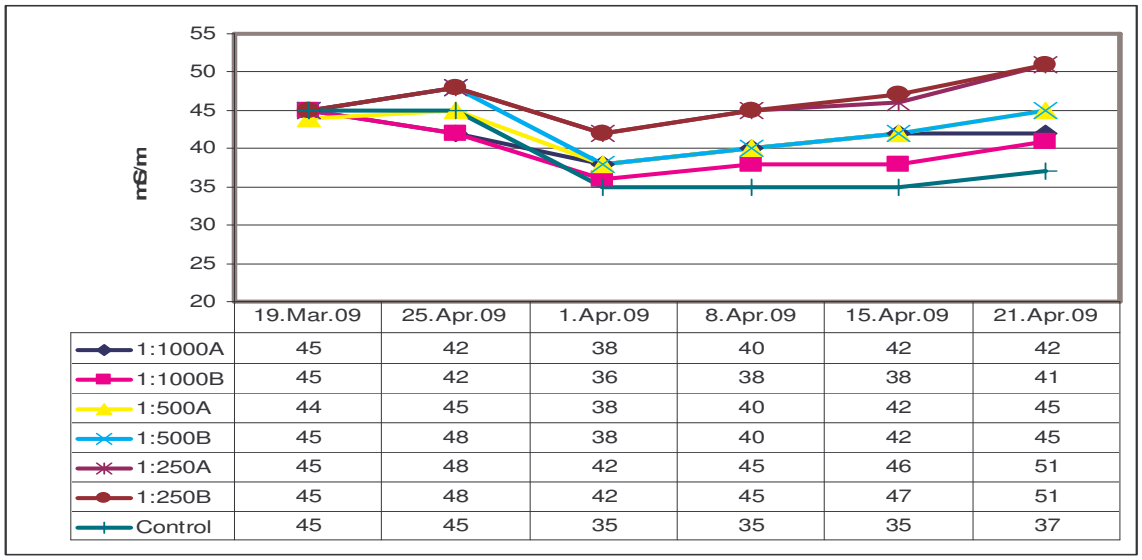
The one-way ANOVA indicated that there was a statistically significant difference (p = 0.0006) in pH. The control was significantly different from all other treatments. Ratios 1:500 and 1:250 were not significantly different from each other (Table 6.1)

Table 6.1 Tukey test for pH results

Tukey HSD test; variable pH (Spreadsheet pH exp.2) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00125, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.007443	0.001398	0.000719
2	1:1000	0.007443		0.043844	0.007443
3	1:500	0.001398	0.043844		0.145844
4	1:250	0.000719	0.007443	0.145844	

6.2.2 Conductivity

The control had the lowest conductivity of 37 mS/m by the end of the study. The ratios 1:1000 reduced slightly to 42 and 41 mS/m. The ratios 1:500 remained more or less the same at 45 mS/m and the ratios 1:250 increased to 51 mS/m (Figure 6.2).



	19.Mar.09	25.Apr.09	1.Apr.09	8.Apr.09	15.Apr.09	21.Apr.09
1:1000A	45	42	38	40	42	42
1:1000B	45	42	36	38	38	41
1:500A	44	45	38	40	42	45
1:500B	45	48	38	40	42	45
1:250A	45	48	42	45	46	51
1:250B	45	48	42	45	47	51
Control	45	45	35	35	35	37

Figure 6.2: Conductivity results taken in six weeks

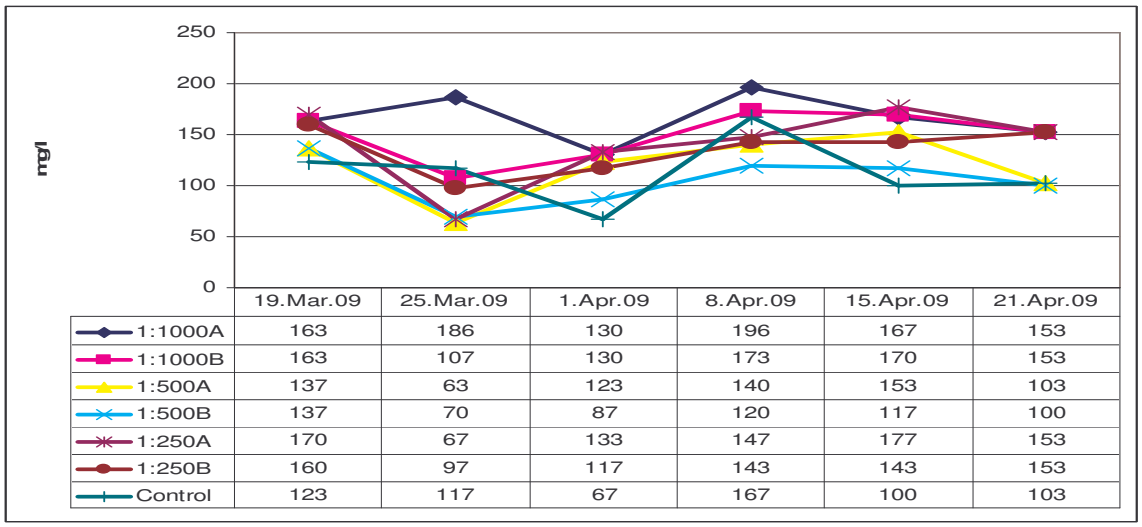
The one-way ANOVA showed that there was a statistically significant difference (p = 0.00088) in conductivity. The control was not significantly different from ratio 1:1000 but was significantly different from ratios 1:500 and 1:250 (Table 6.2).

Table 6.2 Tukey test for conductivity results

Tukey HSD test; variable mS/m (Spreadsheet Conductivity exp.2) Approximate Probabilities for Post Hoc Tests Error: Between MS = .45500, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.397581	0.020216	0.001298
2	1:1000	0.397581		0.076175	0.002306
3	1:500	0.020216	0.076175		0.011743
4	1:250	0.001298	0.002306	0.011743	

6.2.3 Total Suspended Solids (TSS)

The control and ratios 1:500 had the lowest TSS, ratios 1:1000 and 1: 250 had the highest TSS at the end of the study (Figure 6.3). The one-way ANOVA showed that there was a statistically significant difference (p = 0.0213) in TSS.



	19.Mar.09	25.Mar.09	1.Apr.09	8.Apr.09	15.Apr.09	21.Apr.09
1:1000A	163	186	130	196	167	153
1:1000B	163	107	130	173	170	153
1:500A	137	63	123	140	153	103
1:500B	137	70	87	120	117	100
1:250A	170	67	133	147	177	153
1:250B	160	97	117	143	143	153
Control	123	117	67	167	100	103

Figure 6.3: TSS results in six weeks

The Tukey test (Table 6.3) showed that the control was not significantly different from ratios 1:500 and 1:250 but was significantly different from ratio 1:1000.

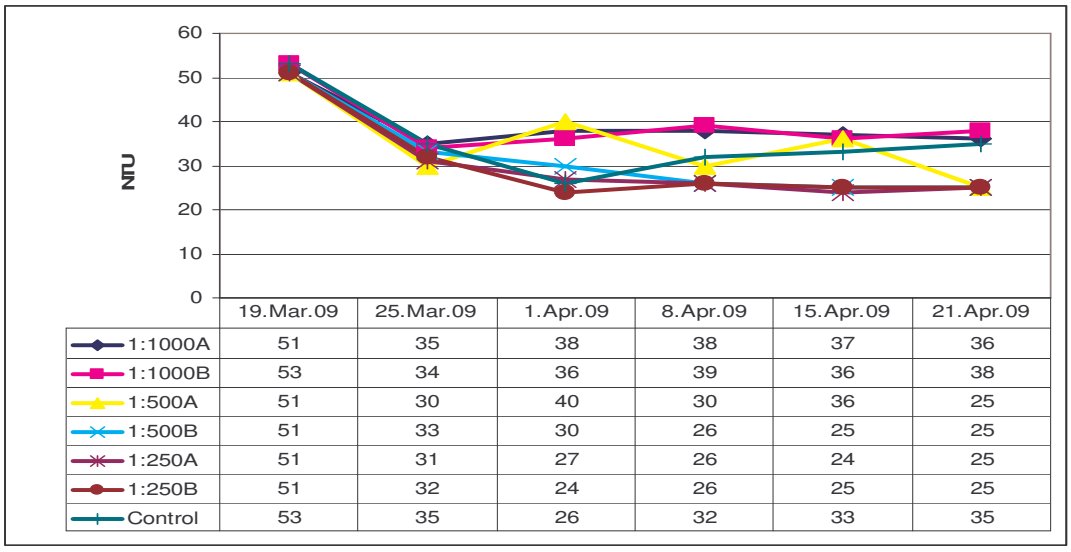
Table 6.3 Tukey test for TSS results

Tukey HSD test; variable mg/l (Spreadsheet TSS exp.2) Approximate Probabilities for Post Hoc Tests Error: Between MS = 90.370, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.033646	0.942716	0.282575
2	1:1000	0.033646		0.022970	0.206317
3	1:500	0.942716	0.022970		0.167211
4	1:250	0.282575	0.206317	0.167211	

6.2.4 Turbidity

Turbidity for the control and ratio 1:1000 dropped from the fifties to the thirties (NTU). That of ratios 1:500 and 1:250 dropped even further from the fifties to the twenties (NTU) (Figure 6.4). The one-way ANOVA showed that there was a statistically significant difference (p = 0.0078) in turbidity. Tukey's test further showed that this difference was between the control and ratio 1:250 and between ratio 1:1000 and ratios 1:500 and 1:250 (Table 6.4).

Turbidity for the control and ratio 1:1000 dropped from the fifties to the thirties (NTU). That of ratios 1:500 and 1:250 dropped even further from the fifties to the twenties (NTU) (Figure 6.4).



	19.Mar.09	25.Mar.09	1.Apr.09	8.Apr.09	15.Apr.09	21.Apr.09
1:1000A	51	35	38	38	37	36
1:1000B	53	34	36	39	36	38
1:500A	51	30	40	30	36	25
1:500B	51	33	30	26	25	25
1:250A	51	31	27	26	24	25
1:250B	51	32	24	26	25	25
Control	53	35	26	32	33	35

Figure 6.4: Turbidity results over six weeks

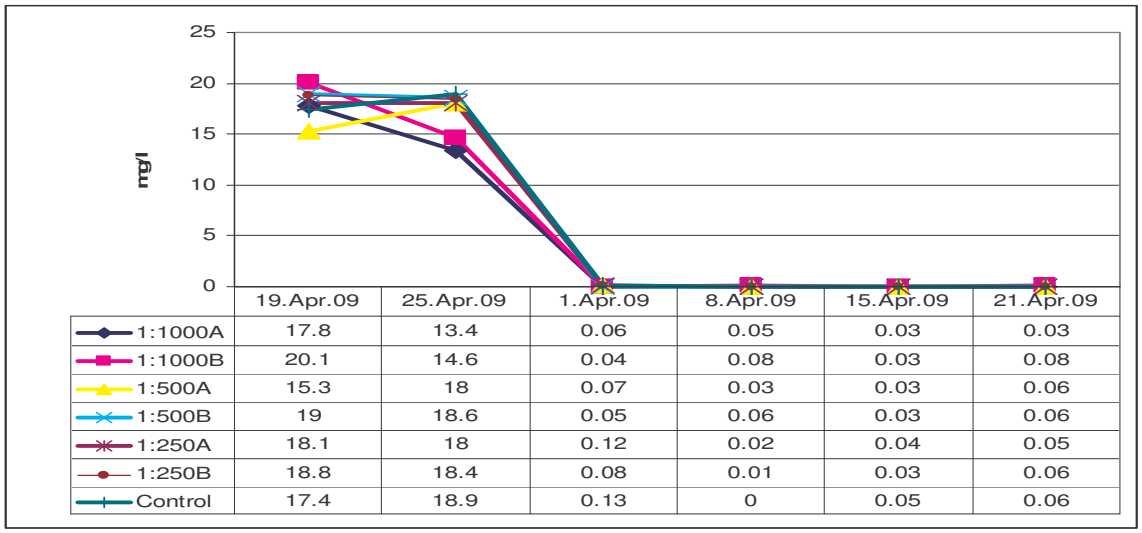
The one-way ANOVA showed that there was a statistically significant difference (p = 0.0078) in turbidity. Tukey's test further showed that this difference was between the control and ratio 1:250 and between ratio 1:1000 and ratios 1:500 and 1:250.

Table 6.4 Tukey test for turbidity

Tukey HSD test; variable NTU (Spreadsheet Turbidity exp.2) Approximate Probabilities for Post Hoc Tests Error: Between MS = 2.4300, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.913409	0.070113	0.014203
2	1:1000	0.913409		0.042029	0.009837
3	1:500	0.070113	0.042029		0.254131
4	1:250	0.014203	0.009837	0.254131	

6.2.5 Ammonia as N

The ammonia in all the treatments including the control reduced from the range of 15-20 mg/l to as low as 0.03 mg/l (Figure 6.5).



	19.Apr.09	25.Apr.09	1.Apr.09	8.Apr.09	15.Apr.09	21.Apr.09
1:1000A	17.8	13.4	0.06	0.05	0.03	0.03
1:1000B	20.1	14.6	0.04	0.08	0.03	0.08
1:500A	15.3	18	0.07	0.03	0.03	0.06
1:500B	19	18.6	0.05	0.06	0.03	0.06
1:250A	18.1	18	0.12	0.02	0.04	0.05
1:250B	18.8	18.4	0.08	0.01	0.03	0.06
Control	17.4	18.9	0.13	0	0.05	0.06

Figure 6.5: Ammonia results taken over six weeks

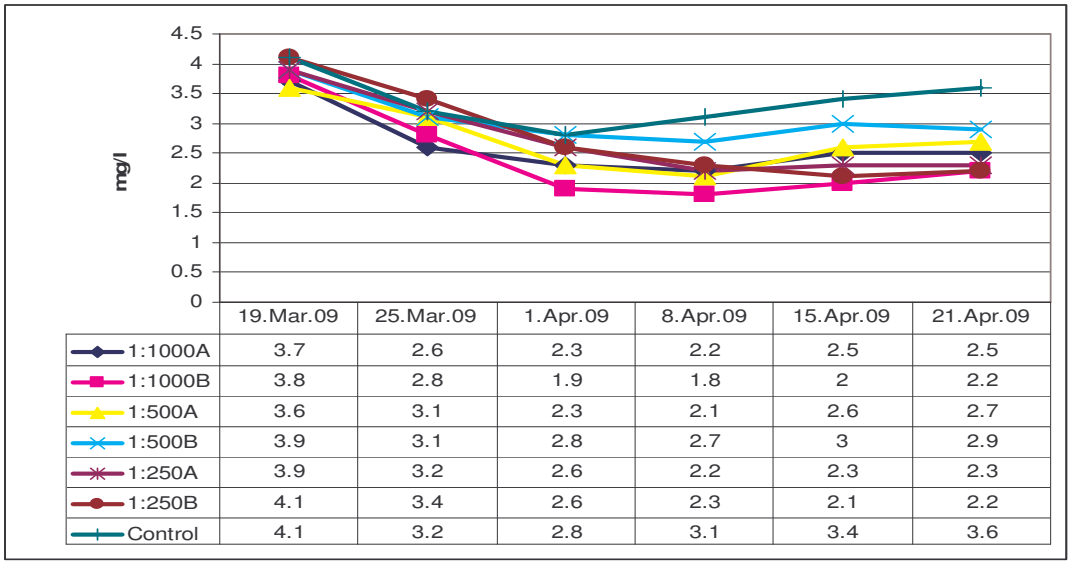
The one-way ANOVA showed that there was a statistically significant difference (p = 0.00003) in ammonia. Tukey's test showed that the difference was between the control and all the treatments (Table 6.5). There was no statistical difference between ratios 1:500 and ratios 1:250

Table 6.5 Tukey test for ammonia results

Tukey HSD test; variable mg/l (Spreadsheet Ammonia exp.2) Approximate Probabilities for Post Hoc Tests Error: Between MS = .01086, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.000292	0.000315	0.000314
2	1:1000	0.000292		0.004241	0.004519
3	1:500	0.000315	0.004241		0.998799
4	1:250	0.000314	0.004519	0.998799	

6.2.6 Phosphate as P

There was a decrease in phosphates by the end of the study in all the treatments. The control also had a decrease but not as much as the treatments (Figure 6.6).



	19.Mar.09	25.Mar.09	1.Apr.09	8.Apr.09	15.Apr.09	21.Apr.09
1:1000A	3.7	2.6	2.3	2.2	2.5	2.5
1:1000B	3.8	2.8	1.9	1.8	2	2.2
1:500A	3.6	3.1	2.3	2.1	2.6	2.7
1:500B	3.9	3.1	2.8	2.7	3	2.9
1:250A	3.9	3.2	2.6	2.2	2.3	2.3
1:250B	4.1	3.4	2.6	2.3	2.1	2.2
Control	4.1	3.2	2.8	3.1	3.4	3.6

Figure 6.6: Phosphate results over six weeks

The one-way ANOVA showed a statistically significant difference (p = 0.0087) in phosphates (Figure 6.6). The Tukey test revealed that the difference was between the control and treatments (Table 6.6).

Table 6.6 Tukey test for phosphate results

Tukey HSD test; variable mg/l (Spreadsheet Phosphate exp.2)) Approximate Probabilities for Post Hoc Tests Error: Between MS = .02425, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.007720	0.048593	0.018695
2	1:1000	0.007720		0.137792	0.494785
3	1:500	0.048593	0.137792		0.584529
4	1:250	0.018695	0.494785	0.584529	

6.2.7 Heavy Metals

Table 6.7: Heavy metal results taken at the beginning and at the end of the study

	Copper		Iron		Manganese		Cadmium		Lead	
Ratios	19 Mar.	29Apr.	19 Mar.	29 Apr.	19 Mar.	29 Apr.	19 Mar.	29 Apr.	19 Mar.	29 Apr.
1:1000 A	0.056	0.076	3.9	5	0.48	0.48	0.017	0.044	nil	nil
1:1000 B	0.049	0.074	3.9	5.2	0.41	0.47	0.022	0.045	nil	nil
1:500A	0.04	0.062	3.3	4.5	0.39	0.42	0.025	0.046	nil	nil
1:500B	0.043	0.057	3.4	3.8	0.4	0.41	0.029	0.052	nil	nil
1:250A	0.047	0.075	3.5	4.6	0.4	0.34	0.0316	0.053	nil	nil
1:250B	0.041	0.064	3.3	4.2	0.46	0.37	0.038	0.055	nil	nil
Control	0.045	0.085	3.5	4	0.43	0.39	0.042	0.07	nil	nil

Prior to addition of EM to the wastewater, tests were carried out on the 19th of March and at the end of the study on the 29th of April. There was an increase in copper content in all samples with the control having the highest increase. One of the ratios 1:1000 and 1:500 experienced the highest increase in iron content. The lowest increase was experienced by the other ratio1:500. For manganese, one of the ratios 1:1000 did not change, the other increased together with the ratios 1:500. The ratios 1:250 decreased together with the control. For cadmium, there was an increase in all the treatments including the control (Figure 6.7).

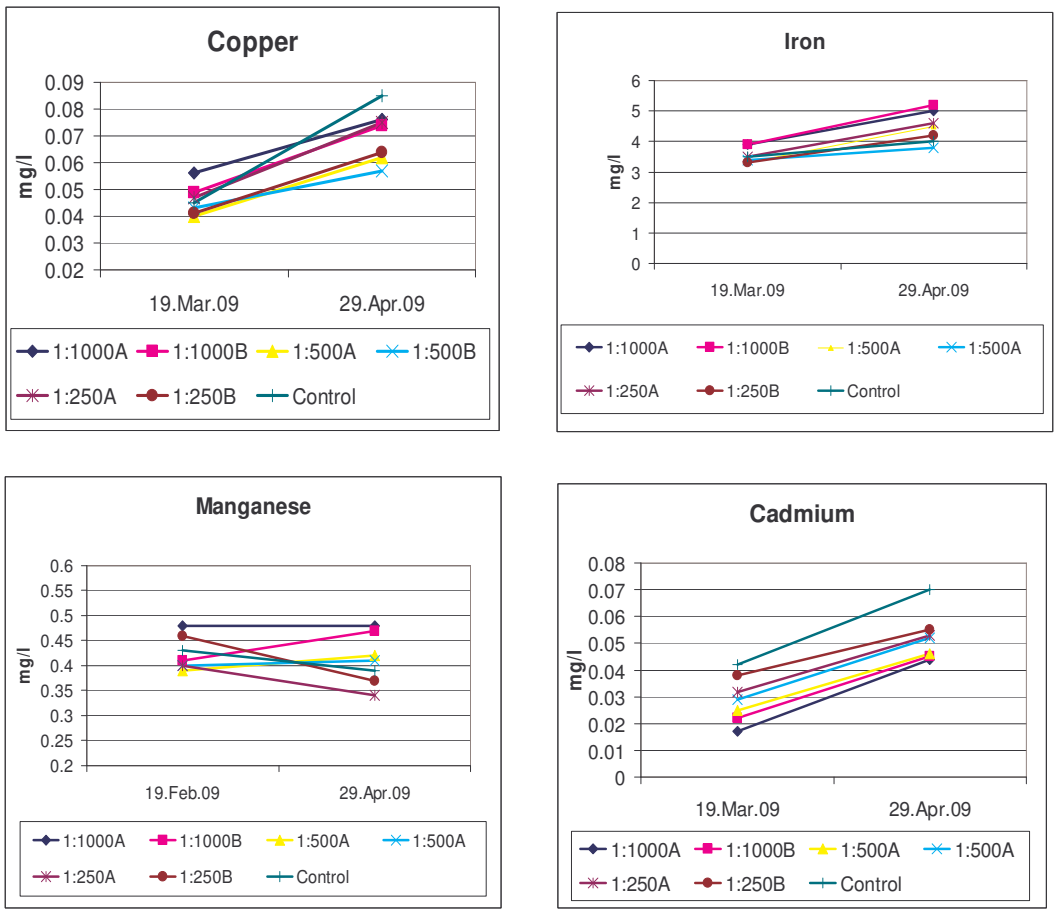


Figure 6.7: Heavy metal content- copper, iron, manganese, and cadmium

The one-way ANOVA for copper indicated that there was a statistically significant difference ($p = 0.0179$) in copper content. The Tukey test (Table 6.8) showed that the ratio 1:500 is the one that was statistically different from the rest of the treatments and from the control.

Table 6.8 Tukey test for copper results

Tukey HSD test; variable mg/l (Spreadsheet5) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00002, df = 4.0000				
	{1}	**{2}**	**{3}**	**{4}**
1		0.238874	0.014313	0.074797
2	0.238874		0.074797	0.623075
3	0.014313	0.074797		0.238874
4	0.074797	0.623075	0.238874	

The one-way ANOVA for iron showed that there was no statistically significant difference in iron content. The Tukey test confirmed that there was no significant difference in any of the treatments including the control.

Table 6.9 Tukey test for iron results

Tukey HSD test; variable mg/l (Spreadsheet Iron exp.2) Approximate Probabilities for Post Hoc Tests Error: Between MS = .08625, df = 4.0000				
	{1}	**{2}**	**{3}**	**{4}**
1		0.065122	0.952324	0.578005
2	0.065122		0.100810	0.222408
3	0.952324	0.100810		0.829268
4	0.578005	0.222408	0.829268	

The one-way ANOVA for manganese showed that there was a statistically significant difference ($p = 0.0022$) in manganese content. The Tukey test showed that the ratio1:1000 is the one that was significantly different from the rest of the treatments and from the control.(Table 6.9).

Table 6.10 Tukey test for manganese results

Tukey HSD test; variable mg/l (Spreadsheet Manganese exp.2)) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00014, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.006809	0.283509	0.126267
2	1:1000	0.006809		0.023392	0.001962
3	1:500	0.283509	0.023392		0.023392
4	1:250	0.126267	0.001962	0.023392	

Though the one-way ANOVA for cadmium showed that there was a statistically significant difference (p = 0.0013) in cadmium content, Tukey's test showed that there was no significant difference between the treatments and the control and among the treatments (Table 6.10).

Table 6.11 Tukey test for cadmium results

Tukey HSD test; variable mg/l (Spreadsheet7) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00001, df = 4.0000					
	treatments	**{1}**	**{2}**	**{3}**	**{4}**
1	control		0.001418	0.002780	0.007456
2	1:1000	0.001418		0.325808	0.045435
3	1:500	0.002780	0.325808		0.263280
4	1:250	0.007456	0.045435	0.263280	

A summary of the results from the aerobic experiment analyses is presented (Table 6.12).

Table 6.12. A summary of the levels of treatment achieved from the application of EM to waste waters within an aerobic environment

		1:1000A	1:1000B	1:500A	1:500B	1:250A	1:250B	Control
pH	**Start**	7.3	7.2	7.2	7.2	7.2	7.2	7.3
	End	7.7	7.7	7.8	7.9	8.0	7.9	7.4
Conductivity	**Start**	45	45	44	45	45	45	45
	End	42	41	45	45	51	51	37
TSS	**Start**	163	163	137	137	170	160	123
	End	153	153	103	100	153	153	103
Turbidity	**Start**	51	53	51	51	51	51	53
	End	36	38	25[#]	25[#]	25[#]	25[#]	35
Ammonia	**Start**	17.8	20.1	15.3	19	18.1	18.8	17.4
	End	0.03[#]	0.08[#]	0.06[#]	0.06[#]	0.05[#]	0.06[#]	0.06[#]
Phosphate	**Start**	3.7	3.8	3.6	3.9	3.9	4.1	4.1
	End	2.5[#]	2.2[#]	2.7[#]	2.9	2.3[#]	2.2[#]	3.6
Copper	**Start**	0.056	0.049	0.04	0.043	0.047	0.041	0.045
	End	0.076	0.074	0.062	0.057	0.075	0.064	0.085
Iron	**Start**	3.9	3.9	3.3	3.4	3.5	3.3	3.5
	End	5	5.2	4.5	3.8	4.6	4.2	4.0
Manganese	**Start**	0.48	0.41	0.39	0.4	0.4	0.46	0.43
	End	0.48	0.47	0.42	0.41	0.34	0.37	0.39
Cadmium	**Start**	0.017	0.022	0.025	0.029	0.032	0.038	0.042
	End	0.044	0.045	0.046	0.052	0.053	0.055	0.07
Lead	**Start**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	End	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[#]*Statistical significant difference*

CHAPTER 7 SUMMARY OF RESULTS AND DISCUSSION

A summary of the results obtained from the three experiments is presented in Table 7.1.

Table 7.1. Summary of the levels of treatment achieved from the application of EM to surface water (Zoo Lake) and wastewaters (within an aerobic and anoxic environment)

		1:1000A	1:1000B	1:500A	1:500B	1:250A	1:250B	Control
pH	**Start:**							
	• **Zoo Lake***	8.87						7.67
	• **Anoxic**	7.4	7.5	7.5	7.5	7.5	7.5	7.5
	• **Aerobic**	7.3	7.2	7.2	7.2	7.3	7.2	7.3
	End:							
	• **Zoo Lake***	7.85						7.67
	• **Anoxic**	7.6	7.7	7.9	8.0	8.0	8.0	8.0
	• **Aerobic**	7.7	7.7	7.8	7.9	8.0	7.9	7.4
Conductivity (mS/m)	**Start:**							
	• **Zoo Lake***	39						42
	• **Anoxic**	64	64	64	64	64	64	64
	• **Aerobic**	45	45	44	45	45	45	45
	End:							
	• **Zoo Lake***	37.5						42
	• **Anoxic**	64	64	64	64	64	64	64
	• **Aerobic**	42	41	45	45	51	51	37
TSS (mg/l)	**Start:**							
	• **Zoo Lake***	26.3						14.5
	• **Anoxic**	141	159	118	167	154	156	149
	• **Aerobic**	163	163	137	137	170	160	123
	End:							
	• **Zoo Lake***	24						14.5
	• **Anoxic**	160	187	110	123	196	113	163
	• **Aerobic**	153	153	103	100	153	153	103
Turbidity (NTU)	**Start:**							
	• **Zoo Lake***							
	• **Anoxic**	5.7						4.8
	• **Aerobic**	65	66	65	65	63	68	65
		51	53	51	51	51	51	53

		1:1000A	1:1000B	1:500A	1:500B	1:250A	1:250B	Control
	End:							
	• **Zoo Lake**[*]	17.5						4.8
	• **Anoxic**	26[#]	27[#]	17[#]	7.5[#]	6.1[#]	6.[#]	35
	• **Aerobic**	36	38	25[#]	25[#]	25[#]	25[#]	35
Ammonia (mg/l)	**Start:**							
	• **Zoo Lake**[*]	0.21						0.38
	• **Anoxic**	21.5	26.3	24.0	24.5	24.9	29.0	25.0
	• **Aerobic**	17.8	20.1	15.3	19.0	18.1	18.8	17.4
	End:							
	• **Zoo Lake**[*]	0.13						0.38
	• **Anoxic**	2.0[#]	5.8[#]	11.0	7.5[#]	9.9[#]	10.4	0.00[#]
	• **Aerobic**	0.03[#]	0.08[#]	0.06[#]	0.06[#]	0.05[#]	0.06[#]	0.06[#]
Phosphate (mg/l)	**Start:**							
	• **Zoo Lake**[*]	0.12						0.11
	• **Anoxic**	5,2	5.9	6.0	5.6	5.2	5.2	5.5
	• **Aerobic**	3.7	3.8	3.6	3.9	3.9	4.1	4.1
	End:							
	• **Zoo Lake**[*]	0.13						0.11
	• **Anoxic**	5.4	6.3	6.9	7.4	7.4	7.1	7.4
	• **Aerobic**	2.5[#]	2.2[#]	2.7[#]	2.9	2.3[#]	2.2[#]	3.6
Copper (mg/l)	**Start:**							
	• **Zoo Lake**[*]	0.00						0.00
	• **Anoxic**	0.03	0.039	0.04	0.044	0.036	0.033	0.037
	• **Aerobic**	0.056	0.049	0.04	0.043	0.047	0.041	0.045
	End:							
	• **Zoo Lake**[*]	0.00						0.00
	• **Anoxic**	0.06	0.067	0.02	0.027	0.043	0.052	0.049
	• **Aerobic**	0.076	0.074	0.062	0.057	0.075	0.064	0.085
Iron (mg/l)	**Start:**							
	• **Zoo Lake**[*]	1.0						1.0
	• **Anoxic**	3.1	3.5	3.5	3.6	3.2	3.2	3.35
	• **Aerobic**	3.9	3.9	3.3	3.4	3.5	3.3	3.5

		1:1000A	1:1000B	1:500A	1:500B	1:250A	1:250B	Control
	End:							
	• **Zoo Lake***	0.8						1.0
	• **Anoxic**	3.6	4.1	3.7	3.9	4.5	4.0	4.0
	• **Aerobic**	5	5.2	4.5	3.8	4.6	4.2	4.0
Manganese (mg/l)	**Start:**							
	• **Zoo Lake***	0.24						0.24
	• **Anoxic**	0.438	0.472	0.484	0.472	0.398	0.413	0.446
	• **Aerobic**	0.48	0.41	0.39	0.4	0.4	0.46	0.43
	End:							
	• **Zoo Lake***	0.16						0.24
	• **Anoxic**	0.243	0.330	0.326	0.365	0.346	0.403	0.301
	• **Aerobic**	0.48	0.47	0.42	0.41	0.34	0.37	0.39
Cadmium (mg/l)	**Start:**							
	• **Zoo Lake***	0.00						0.00
	• **Anoxic**	0.028	0.011	0.009	0.011	0.012	0.013	0.014
	• **Aerobic**	0.017	0.022	0.025	0.029	0.032	0.038	0.042
	End:							
	• **Zoo Lake***	0.0055						0.00
	• **Anoxic**	0.018	0.015	0.015	0.016	0.019	0.021	0.021
	• **Aerobic**	0.044	0.045	0.046	0.052	0.053	0.055	0.07
Lead (mg/l)	**Start:**							
	• **Zoo Lake***	0.087						0.00
	• **Anoxic**	0.129	0.130	0.128	0.125	0.133	0.142	0.131
	• **Aerobic**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	End:							
	• **Zoo Lake***	0.00						0.00
	• **Anoxic**	0.151	0.149	0.143	0.139	0.133	0.131	0.223
	• **Aerobic**	0.00	0.00	0.00	0.00	0.00	0.00	0.00

** **Unlike the anoxic and aerobic experiments which had different EM ratios added to the samples, the Zoo Lake experiment only had the treatment and control.***

*# **Statistical significant difference***

pH

The pH in the Zoo Lake waters rose sharply soon after dosing with EM. This may have been due to the bacteria in the EM mixture optimizing conditions in their new environment so that they could survive. Horikoshi (1999) found that alkaliphilic (alkali-loving) and neutrophilic (neutral pH range) bacteria could alter their environment by increasing the pH to conditions suitable for their growth. An observation made in support of this rise in pH was the rapid growth of green algae (not previously noticed) on the surface of the Zoo Lake waters a week after EM dosing commenced. Typically, green algae are abundant in alkaline environments of pH 7.5 to 13 and the increase in pH would have encouraged their growth. Another possible cause for, the rise in pH may be the algae in the waters using up carbon dioxide during photosynthesis. Carbon dioxide dissolved in water acts as carbonic acid (H_2CO_3), and when this carbon dioxide is consumed, the acidity of the water is reduced and pH levels increase (Michaud, 1991). After eight weeks of treating the water in Zoo Lake with EM, the average pH was 7.5, slightly higher than at the start of the study. Overall, there was no statistically significant difference in pH before and after dosing.

The pH for the anoxic experiment was generally higher after the five-week period. The implication of this was that the statistical difference between the control and the sample with EM in the ratio 1:1000 was not significant while the statistical differences between the control and the ratios 1:500 and 1:250 were significant. This may be explained by the fact that the samples with lower EM ratios (e.g. 1:1000) contain less EM and hence less ions, lower conductivity and lower pH than the

samples with higher EM ratios. Despite this, the pH means of all the ratios were below a pH of 8 and within the accepted water quality standard. The question might be asked, why a rise and not a drop in pH? We come back to the issue discussed earlier; that the bacteria in the EM mixture could be trying to find the optimum environment in which it could thrive. Alkali-loving bacteria can alter and increase the pH of their environment to a pH value appropriate for growth (Horikoshi, 1999). The concentration of EM was higher in the ratios 1:500 and 1:250 and it is here that the pH was higher.

The pH was greater at the end of the aerobic experiment just as in the Zoo Lake and anoxic studies. Unlike the anoxic study where the pH of the control also went as high as the treatments, the pH of the control in this study stayed more or less the same. There was a significant difference (p = 0.0006) in pH between the control and the treatments. We attribute this rise in pH to the EM trying to find the optimum pH in which to thrive.

Conductivity

The conductivity of most fresh water bodies range between 10 and 100 mS/m. The water in Zoo Lake before and after dosing with EM was within this range. The conductivity dropped from a mean of 42 mS/m (before dosing with EM) to a mean of 37.5 mS/m (at the end of dosing). This resulted in a statistically significant difference (p= 0.0005). Since the conductivity of water is highly dependent on its concentration of dissolved salts, the conductivity value is an indicator of how salt free, ion-free or impurity free the water sample is. Sterilized water has a lower conductivity because

there are very few ions in it compared with ionic solutions (Pashley, 2005). It can thus be assessed that the water in Zoo Lake was in a better state at the end of the study than it was at the beginning since the conductivity was lower. As indicated earlier, for the anoxic and aerobic experiments, conductivity was higher in the samples with higher EM ratios because of the presence of more EM (ions) in solution. Hence the samples at the start had a lower conductivity than at the end.

Conductivity is a measure of the ability of a sample of water to conduct an electric current and is thus a measure of the number of ions (charged particles) in solution. It can be said that since the control in the anoxic experiment had a lower mean concentration, the treatments had more ions due to the presence of the EM. It is interesting to note that conductivity was greater in the more concentrated solutions. Among the treatments, the ratio 1:1000 has the least conductivity and the ratio 1:250 has the highest conductivity.

We see an identical trend in conductivity between the anoxic experiment and the aerobic experiments. The conductivity ascended with the control having the lowest and the ratio1:250 having the highest conductivity. An indication that the more concentrated samples have more ions or total dissolved solids within them.

TSS and Turbidity

There was a rise in TSS in Zoo Lake; from a mean of 14.5 mg/l before application with EM to a mean of 24 mg/l after dosing with EM. The increase in TSS (and turbidity) may be attributed to the rainy season which began during the study. A lot of

debris was washed into the lake and there was regular agitation of sediments lying at the lake bottom.

Statistical analyses of anoxic samples indicated no significant difference in mean TSS between wastewater samples with and without EM. The control had a mean of about 149 mg/l, the ratios 1:1000 and 1:500 had means of about 140 mg/l and the ratio 1:250 had a mean of about 165 mg/l. The increase in TSS may have resulted from the change in the organic material within the wastewater that had been changed into cellular mass (Manahan, 2005; Roisin, 2008). The trend that is seen here of the higher concentrations having the higher TSS is explained by the fact that the samples with higher EM ratios have more EM acting on organic material within the wastewater.

There is no similar trend between the anoxic and aerobic experiments when it comes to TSS. In the aerobic experiment, the TSS reduced in all the samples by the end of the study unlike the anoxic experiment where the TSS increased. According to Manahan (2005), there are two pathways that are capable of taking place 1) oxidation of organic matter to provide energy for the metabolic processes of the microorganisms – no biomass is produced 2) synthesis, incorporation of the organic matter into cell mass – biomass is produced. In the first pathway, carbon is removed in the gaseous form as carbon dioxide, this is what took place in the aerobic experiment and that is why there was a reduction in TSS. In the second pathway, carbon is removed as a solid in biomass; this is what took place in the anoxic experiment.

Organic matter + O_2 → CO_2 + H_2O + energy in aerobic experiment

Organic matter + N + P trace elements → new cells in anoxic experiment

Turbidity is a good measure of the quality of water (Lenntech, 2009a; Minnesota Pollution Control Agency, 2008). There was a largely significant difference in turbidity (p = 0.00007) between the treatments and control at the end of the anoxic experiment. It is noticed that at high EM concentrations, turbidity is low. Comparing the end values with the control, it can be seen that samples with higher concentrations of EM had lower turbidity values and the turbidity value of the control at the end was significantly higher than all the samples with EM. An obvious implication of these observations is that the quality of water improved as a result of the EM and with increased EM, turbidity is lowered.

Turbidity in the aerobic experiment was lowest in the ratios 1:500 and 1:250. The control and ratio 1:1000 had similar results. This is almost similar with what was seen in the anoxic experiment. Turbidity as mentioned earlier is an important indicator of the quality of water and the fact that the control had more turbidity than the treatments 1:500 and 1:250 is an indication that there was an improvement in the water quality of the EM treated water.

Ammonia

There was a noticeable decrease in ammonia from a mean of about 0.38 mg/l before dosing with EM to a mean of about 0.15 mg/l at the end of the experiments. Oram (1999), shows that the lethal concentration of ammonia for a variety of fish species

ranges from 0.2 to 2.0 mg/l. It can therefore be said that the concentration of ammonia was reduced from a toxic level (before dosing the lake with EM) to a non-toxic level (by the end of the experiments). This result is of particular importance because of the population of fish that inhabits Zoo Lake.

In the anoxic experiment, there was a considerable reduction in ammonia in all treatments and the control, with the control being depleted completely. The means for the whole study period showed no significant difference (p = 0,148). The reason for the depletion of the ammonia could have been that the ammonia was converted to nitrate and nitrite in a process called denitrification (Hallin and Lindgren, 1999). This cannot be attributed to the EM since the control had an even greater depletion than the treatments. This conversion must have been carried out by denitrifying bacteria that were present in the wastewater. The ability to denitrify is a trait spread among many species within a wide variety of bacteria (Zumft, 1997) that are able to use various energy sources. The denitrification cycle provides a competitive advantage to these organisms in oxygen-limiting environments (Leta *et al*., 2004) such as the anoxic conditions in this experiment. The most concentrated (ratio 1:250), had the largest amount of ammonia (10 mg/l) at the end of the study and the least concentrated (ratio 1:1000) had the least amount (2 mg/l). This is a clear indication that the EM disturbed the action of the denitrifying bacteria in breaking down the ammonia.

For the aerobic experiment, there was no large difference in the final result of the control and the treatments (unlike the anoxic experiment) though the mean of the control was greater than the means of the treatments. They all started off with an

ammonia content of more than 15 mg/l which dropped to less than 0.09 mg/l for all the treatments and control. Under these aerobic conditions, nitrifying bacteria in the wastewater predominate and the toxic ammonia is converted to less toxic nitrite and then to the relatively harmless nitrates (Abel, 1996).

Phosphates

There was a negligible decrease in phosphates in Zoo Lake and this was not statistically significant (p = 0. 4943). When phosphates increase, the growth of plankton and aquatic plants is stimulated and this provides food for larger organisms, including: zooplankton, fish, humans, and other mammals. Initially, this increased productivity will cause an increase in the fish population and overall biological diversity of the system. However, as the phosphate loading continues and there is a build-up of phosphate in the lake or surface water ecosystem, the aging process of the ecosystem will be accelerated. Over production in the lake or water body can lead to an imbalance in the nutrient and material cycling process (Ricklefs, 1993). So the fact that there was a decrease in phosphates is a positive outcome as the chances of the above happening is minimised.

There was a slight increase in phosphates both in the control and the treatments for the anoxic experiment by the end of the study. There was no significant difference (p = 0.148) in the means. Wastewater discharges of phosphates to the environment are detrimental as it speeds up eutrophication as discussed earlier, so the increase in phosphates was not a desirable outcome.

The aerobic experiment had a decrease in phosphates as opposed to the anoxic experiment which had an increase, signifying that aerobic conditions are conducive for phosphate reduction.

Heavy Metals

Heavy metals are toxic minerals because they have the tendency to bioaccumulate (*i.e.*, they increase their concentration in an organism over time) (Lenntech, 2009b). Compounds accumulate in living organisms when they are taken up, and stored faster than they are broken down. Among the metals we tested, cadmium and lead are two of the three most polluting metals (mercury being the third). There were traces of lead at the beginning of the Zoo Lake study and none by the end of the study. The opposite was true for cadmium; there was no cadmium at the beginning. So, we see a positive outcome for lead and a negative outcome for cadmium which we would attribute as an effect of EM treatment. Cadmium could have been deposited by the water flowing into the lake or the result of the breakdown of the locked up metals in the water. Another positive result was the decrease in iron and manganese content. This decrease in iron and manganese and the disappearance of the lead could have been due to EM's ulilisation of these metals in their metabolism (Sheng *et al*., 2008).

For the anoxic experiment, there was a slight increase in iron and copper content except for the two 1:500 samples. The manganese content decreased, the sample with ratio 1:250 did not change. The lead content stayed more or less the same and the cadmium content increased slightly. The reason for the increase was discussed earlier.

There was essentially an increase in all the metals for the aerobic experiment. This could have been due to the release of the locked up metals in the wastewater.

CHAPTER 8. RECOMMENDATIONS AND CONCLUSION

Recommendations

- The Zoo Lake results were highly influenced by rainfall. Further research of a similar nature should be carried out during the dry season to rule out the influence of such external factors.
- It is interesting to note that among the biological processes, EMs have been identified to comprise the most effective application for heavy metal removal (Sheng, *et al*., 2008). This unfortunately was not entirely the case in this research as we had cases were the metals increased instead of decreasing. One crucial point to note is that if the concentration of heavy metals, such as copper, zinc, lead, tin, chromium, cadmium, and mercury in the wastewater reaches the heavy metal tolerance of EMs, its effectiveness for wastewater treatment decreases (Sheng *et al*., 2008). Sheng *et al.* suggest that heavy metals should be removed through a pre-treatment process if their presence has been detected in the wastewater. This step would improve the efficiency of EM wastewater treatment.
- Based on this and previous (Moyo *et al.*, 2008) research, it is anticipated that better treatment efficiencies may be realised by combining EM with other complementary microbiological treatment agents and this is suggested for future research.

- It was not possible to undertake BOD and COD analysis due to unavailability of equipment. The BOD and COD analysis should be included in future EM research as these are good indicators of effective organic waste break down.

Conclusion

Based on our Alternative Hypothesis, a statistically significant decrease in waste and/or surface water parameters after addition of EM indicates successful treatment. Turbidity (for the anoxic and aerobic experiments), ammonia and phosphates (for the aerobic experiment) are the parameters that were largely influenced (decreased) by the end of the anoxic and aerobic studies.

Both the anoxic and aerobic conditions seemed conducive for EM treatment in terms of turbidity, as turbidity decreased in both experiments. This is not surprising as EM contain both aerobic and anaerobic microorganisms (Al-Taweil and Yusof, 2008). Unfortunately, turbidity results at Zoo Lake were largely influenced by rain and hence, cannot be employed in this conclusion.

Decreases in ammonia cannot be attributed to EM as the control also decreased in the same manner.

The decrease in phosphates in the aerobic experiment may likely be indicative of EM treatment aided by the continual aeration of the effluent. The control, which was also aerated, also experienced a decrease in phosphates but was not as significant as the samples with EM treatment.

From this study, the levels of treatment achieved by EM on surface and waste waters are considered to be low. This is because treatment was only achieved on two

(turbidity and phosphates) out of the seven water quality parameters measured and within prescribed conditions (i.e. aeration). In conclusion therefore, the levels of treatment of EM in treating waste and surface waters within a South African context can thus be said to be low.

REFERENCES

Abel, P.D., 1996. *Water Pollution Biology*, 2nd Ed., London-Tylor and Francis, p 37

Agriculture and Agri-Food Canada, 2007. [online] Available at: www.agr.gc.ca . [Accessed 9 July 2009]

Al-Taweil, H.B. and Yusof, W.M., 2008. Effective Microorganisms Applications in a Sustainable Agriculture Crop Growth. *GRC Environment Research Bulletin* pp 8-12.

Anthony, D. 2008. Introduction to EM Technology [online] Available at: www.efficientmicrobes.co.za/news.aspx?tid=15 [Accessed 28 August 2008]

Applications: water and sewerage, 2002. Municipal wastewater treatment plant utilises anaerobic digestion, *World Pumps* 20(424), pp17-18.

Brady, J. 1990. *General chemistry: Principles and Structure*, 5th Ed. New York- John Wiley and Sons, p 516.

Braker, G., Fesefeldet, A. and Witzel, K.P., 1998 Development of primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples, *Applied and Environmental Microbiology* 64, pp 3769–3775.

Diver, S. 2001. *Nature farming and Effective Microorganisms* [Online] Available at http://ncatark.uark.edu/~steved/Nature-Farm-EM.html [Accessed 5 October 2008].

Davie, L., 2002. *A mud balls rescue for Zoo Lake*. [Online] Available at http://joburgnews.co.za/aug_2002/zoo_lake.stmht [Accessed 3 September 2008]

Davies, B. and Day, J. 1998. *Vanishing Waters,* Cape Town-UCT Press, p 45

Eaton, A. D., Clesceri, L.S. and Greenberg, A.E., 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th ed*.,* American Public Health Association, American Water Works Association, Water Environment Federation, Baltimore-United Book Press.

Eckenfelder, W.W., 1980: Principles of biological treatment in K.Curi and W.W. Eckenfelder, (eds) *Theory and practice of biological wastewater treatment*, Netherlands-Sijt

Frietag, D. and Meihoefer, H., 2002. *The Use of Microorganisms in Organic waste Management* [Online] Available at effectivemicroorganisms@emtrading.com. [Accessed 25 August 2008].

Giovanelli, J., Lindsay, D., Straker, C.J., 2007. *Determining bacterial populations and the presence/absence of potential food borne pathogens before and after fermentation with effective microorganisms.* BSc Hons. Research Project. University of the Witwatersrand, Johannesburg, South Africa.

Hallin, S. and Lindgren, P.-E., 1999. Detection of genes encoding nitrite reductase in denitrifying bacteria. *Applied and Environmental Microbiology* 65, pp 1652–1657

Higa, T., 1991. Effective microorganisms: A biotechnology for mankind. In: J.F. Parr, S.B. Hornick, and C.E Whitman (eds), *Proceedings of the First International Conference on Kyusei Nature Farming* , Washington D.C. pp 8-14.

Higa, T., 1998. Effective Microorganisms for a more sustainable agriculture, environment and society: Potential and prospects, in Parr, J.F. and S.B. Hornick,

(Eds.). *Proceedings of the Fourth International Conference on Kyusei Nature Farming*, Paris, pp 12-13.

Higa, T., 2001. *The Technology of Effective Microorganisms- Concept and Philosophy.* Okinawa-University of the Ryukyus.

Higa, T., 2007. Living a day. *Effective Microorganisms Research Organisation Newsletter*.

Higa, T. and Parr, J.F., 1994. *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment*. Japan-Atami, p 7.

Higa, T. and Wididana, G.N., 1991. The concept and theories of Effective Microorganisms. In: J.F. Parr, S.B. Hornick, and C.E. Whitman (eds.), *Proceedings of the First International Conference on Kyusei Nature Farming.* Washington D.C.

Horikoshi, K., 1999. Alkaliphiles: Some Applications of their Products for Biotechnology. Microbiology and Molecular Biology Reviews 64(4): 735-750

Hoyos D.H., Alvis N.G, Jabib L.R, Garcés, M.B., Pérez, D.F. and Salim Mattar, S.V., 2008. Utility of effective microorganisms in an avian farm of Cordoba: productive parameters and environmental control, *Revista Mvz Cordoba*, 13 (2) pp1369-1379.

Joubert, D., 2008. *Effective microorganisms reduce cost at sewage treatment plant* [Online] (Updated 10 July 2008) Available at http://www.kwevoel.co.za/?p=1929 [Accessed 25 August 2008].

Lenntech, 2009a. *Water treatment solutions* [Online] Available at http://www.lenntech.com/applications/waste/waste-water.htm [Accessed 11 September 2009].

Lenntech, 2009b. *Heavy metals*. [Online] Available at www.lenntech.com/processes/heavy/heavy-metals/heavy-metals.htm [Accessed 17 September 2009].

Leta, S., Gumaelius, L., Assefa, F. and Dalhammar, G., 2004. Identification of efficient denitrifying bacteria from tannery wastewaters in Ethiopia and a study of the effects of chromium III and sulphide on their denitrification rate, *World Journal of Microbiology and Biotechnology* 20, pp 405- 411.

Londt, J., 2008. *A useful moth-like fly* [Online] (Updated 20 May 2008) Available at http://www.witness.co.za/index.php?showcontent&global%5B_id%5D=7754. [Accessed 5 April 2009].

Mail and Guardian, 2009. Cholera death toll in South Africa rises. [Online] (Updated 14 January 2009) Available at http://www.mg.co.za/article/2009-01-14-cholera-death-toll-in-south-africa-rises

Manahan, S. E., 2005. *Environmental Chemistry*, 8th Ed. CRC Press, pp 214.

Mayer, J., Scheid, S. and Oberholzer, H.R., 2008. How effective are effective microorganisms? Results from an organic farming field experiment. In: *Proceedings of the 16th IFOAM Organic World Congress*, June 16-20, 2008, Modena, Italy.

Michaud, J.P., 1991. A citizen's guide to understanding and monitoring lakes and streams. Publ. #94-149. Washington State Dept. of Ecology, (360) pp 407-747:

Minnesota Pollution Control Agency, 2008. Turbidity: Description, impact on water quality, sources, measures- A general overview. *Water Quality/Impaired Waters*, 3 (21).

Moyo, C., Zimmermann, I., Pedro, M. and Kambuli, R., 2008. Biological wastewater treatment and odour control by use of effective microorganisms at Ujams ponds. In: *9th Waternet /WARFSA/GWP-SA Symposium*, 29- 31 Oct. 2008, Windhoek, Namibia.

Okuda, A and Higa, T., 1999. Purification of wastewater with effective microorganisms and its utilisation in agriculture. In Senanyake, Y.D.A., Sangakarra, U.R., (eds.). *Proceedings of the 5th Interntational Conference on Kyusei Nature Farming,* APNAN, Thailand., pp 246-253.

Oram, B. 1999. Wilkes University, centre for environmental quality, environmental engineering and earth sciences.

Pashley, R.M., 2005. De-gassed water is a better cleaning agent, *The Journal of Physical Chemistry,* 109, p 1231

Pure SA, 2009. *SA faces 'major water crisis'.* Available at http://www.puresa.co.za/SAWater.aspx. 20 March 2009. [Accessed 5 April 2009]

Ricklefs, R.E., 1993. *The economy of nature*, New York-W.H. Freeman and Co.

Roisin, B.C. 2008. *Biological wastewater treatment*, Thayer School of Engineering, Dartmouth College. http://engineering.dartmouth.edu/cushman/courses/eng37/BioTreatmentTypes.pdf

Sekeran, V., Balaji, C. and Bhagavathipushpa, G., 2005. T. Technical note: Evaluation of Effective Microorganisms (EM) in Solid Waste Management, *Electronic Green Journal* 1(21), pp 66-70.

Schultz, T.E. 2005. Biological wastewater treatment, *Chemical Engineering*, p 52.

Sheng,Z., Chaohai,W., Chaodeng, L. and Haizhen, W. 2008. Damage to DNA of effective microorganisms by heavy metals: Impact on wastewater treatment, *Journal of Environmental Sciences,*. 20, pp1514-1518.

Szyamanski, N. and Patterson R.A., 2003. Effective microorganisms (EM) and wastewater systems. Future Directions for On-site Systems: Best Management Practice. In: *Proceedings of On-Site Conference '03 conference.* , 30th Sept. – 2nd Oct., 2003. Armidale, Australia.

Tiedje, J.M., 1988. Ecology of Denitrification and dissimilatory nitrate reduction to ammonium. In A.J.B. Zehnder, (ed), *Biology of Anaerobic Microorganisms*,. New York-Wiley, pp 179–244.

Van der Stelt, B., Temminghoff, E.J.M., Van Vliet P.C.J., Van Riemsdijk, W.H., 2006. Volatalisation of ammonia from manure as affected by manure additives temperature and mixing. *Bioresource Technology*, 98 (18), pp 3449-3455.

Van Vliet, P.C.J., Bloem, J., de Goede, R.G.M., 2006. Microbial diversity, nitrogen loss and grass production after addition of effective microorganisms (EM) to slurry manure, *Applied Soil Ecology,* 32, pp 188-198.

Watts, S and Halliwell, L. (eds). 1996. *Essential Environmental Science: Methodology and Techniques.* London-Routledge.

Williams, M.K., 2000. *Research Design* [Online] Available at http://trochim.human.cornell.edu/kb/desintro.htm [Accessed 19 January 2009]

Wyszynska, M. 2006. Biological treatment continues to be the "bread and butter" technology for the European wastewater treatment equipment market, *European Environment Group*, p 1.

Zumft, W.G. 1992. The denitrifying prokaryotes, In Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. (eds), *The Prokaryotes*, 2nd ed, New York- Springer- pp. 554–582.

Zumft, W.G. 1997. Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Review*, 61, pp 533–616.