THE OCCURRENCE AND BIOACCUMULATION OF SELECTED METALS AND RADIONUCLIDES IN AQUATIC AND TERRESTRIAL ECOSYSTEMS ON THE WITWATERSRAND

By

LOUIS PETRUS DANIËL DE WET

THESIS

presented in partial fulfilment of the degree

PHILOSOPHIAE DOCTOR

in

ZOOLOGY

in the

FACULTY OF NATURAL SCIENCES

at the

RAND AFRIKAANS UNIVERSITY

SUPERVISOR: PROF. HJ SCHOONBEE
CO-SUPERVISOR: DR. CAR BAIN

May 1996

PART 1

INVESTIGATIONS INTO THE ACCUMULATION AND CONCENTRATION RATIOS OF SELECTED RADIONUCLIDES IN AQUATIC ECOSYSTEMS AFFECTED BY MINE DRAINAGE EFFLUENTS WITH REFERENCE TO THE STUDY OF POTENTIAL PATHWAYS TO MAN

CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would hereby like to express my sincere appreciation to:

- Our Lord and Saviour without which given health and strength, nothing would be possible.
- Prof. H.J. Schoonbee for his years of unselfish devotion, encouragement, criticism and tuition. Mrs. Schoonbee is also sincerely thanked for her hospitality.
- Dr. C.A.R. Bain of the Atomic Energy Corporation of South Africa for his critical remarks and contributions during the execution and compilation of this study and especially his contributions on dose assessment modelling.
- The steering committee of the Water Research Commission, and in particular Dr. H. Saayman for financial support and valued advice respectively.
- My dearest wife, Louise, for her years of unconditional encouragement, love and devotion, and my dearest little children, Stefan and Anina, for their unconditional love.
- My dearest parents, for a lifetime of unselfish devotion, love and understanding. In particular, my sincere appreciation for their financial assistance during my years of study.
- My sisters, Theresa and Geraldine for their valued encouragement during this project.
- My parents in law, Wynand and Ina Bezuidenhout, for their support.
- The support and friendship of Elsabe' and Piet, Anita and Nelis and Sonia and Fanus.
- Mr. Kobus Hancke of the Atomic Energy Corporation of South Africa, for valued advice and his participation in the collection and analyses of radionuclide-containing material. Also, sincere thanks for his friendship.
- Mr. Fred Delport for his friendship and being an example of personal perseverance and motivation.
- Mr. D.J. Breedt formerly of Hoër Seunskool Hugenote, for his much appreciated guidance during my youth and for introducing me to the wonderful world of science.
- Mr. Johan du Preez (Genmin) and Mr. Andy Smithen (Steffen Roberston & Kirsten Consulting Engineers) for their valued advice and enthusiasm during this project.
- My dear colleagues of Waterlab Research, Mr. Nic Thirion, Mr. Rien van der Meer and Molly, Karen, Maretha, Albert, Arend and George for their particular friendship and support, especially during difficult times.

- Maretha Bonthuys of Waterlab Research for her assistance in the typing of this manuscript.

- The Department of Zoology, Rand Afrikaans University for their laboratory and computer facilities.

- The Division of Earth and Environmental Technology, Atomic Energy Corporation, for the supply of analytical and sample collection facilities.

- Waterlab Research and Geustyn Forsyth & Joubert consulting engineers for supplying a computer and a printer to complete this work. Also, for assistance to complete some of the chemical analyses of the present investigation.

- The Foundation for Research Development, the Rand Afrikaans University and the City Council of Germiston for financial support, respectively.

- The following farmers, for supplying sample material of soils and vegetable crops:

  - Mr. Jose' Geral, Marievale Farm
  - Mr. Frankie Inago, Vlakfontein Farm
  - Mr. Nic Coetzee, Luipaarsdvlei
  - Mr. Lourens du Plessis, Luipaardsvlei
  - Mr. Willie du Preez, Luipaardsvlei
  - Mr. Manny Bowwrageiro and Frankie da Costa, Crocodile Farm

ooOoo

A special tribute to Mr. J.G. de Klerk who sadly passed away on 22/01/96.
His friendship will always be remembered.

ooOoo

GENERAL INTRODUCTION

GENERAL INTRODUCTION TO RADIONUCLIDE AND METAL POLLUTION OF AQUATIC ECOSYSTEMS BY MINES AND INDUSTRIES ON THE WITWATERSRAND, GAUTENG PROVINCE

Introduction

Water, as a life-giving substance can be considered as the most essential natural resource in South Africa. Not only does water comprise the backbone of our social welfare, but also forms an integral and vital component of the South African economy. However, with an increasing growth in population and a subsequent diversification in the possible requirements and uses of water, increasing pressure is being placed on this natural resource.

The progressive salination and pollution (organic, inorganic and bacteriological) of our water resources by potentially toxic metals and other pollutants are currently common phenomena in South Africa as well as in the rest of the world. This is commonly associated with the intensive use and re-use of our limited water resources by industry, mining, agriculture and sewage purification works (Department of Water Affairs, 1986).

In his wisdom, but perhaps also rather unknowingly, Georgius Agricola stated in "De Re Metallica" (1556) the following: *"The miner should try to obtain a mine, to which access is not difficult, in a mountainous region, gently sloping, wooded, healthy, safe and not far distant from a river or stream by means of which he may convey his mining products to be washed and smelted. This indeed, is the best position. As for the others, the nearer they approximate to this position the better they are; the further removed, the worse".* In a way, some of these principles still apply today. Since 1556 however, the situation has changed dramatically. The additive effects of population and technological explosions have induced a greater need for water and mineral resources, which in turn caused an increase in the organic (sewage effluents and pesticides) and inorganic (salts, metals, etc.) loads reaching previously undisturbed surface and underground resources. These pollutant and toxicant loads entering water systems, mainly originate from industrial, mining, and sewage purification activities. An additional contribution is made by effluents and seepage water originating from domestic and industrial dump-sites, as well as by agricultural activities which, through the addition of plant fertilizers, add to the nutrient enrichment or eutrophication of our water resources.

South Africa currently reserves the first position in world gold production (Förstner & Wittmann, 1981 ; Elevatorski, 1984) and owns the largest platinum reserves in the world (Cousins, 1973). Kurian (1979) and van Biljon (1981) placed South Africa first on the international rank of gold production, while this country also occupied this position according to the 92nd yearly report of the Chamber of Mines of South Africa (1981). South Africa still reserves this position since 1981, followed by the USA, Australia, the CIS (Commonwealth of Independent States: Part of former USSR), Canada, China and Brazil (Chamber of Mines, 1993). In this respect, the mining industry can still be regarded as the economic backbone of South Africa (Murray, 1987).

The Witwatersrand Basin, the world's biggest treasure room of precious metals is situated on the Transvaal highveld at an average height of 1340 to 1820 metres above sea-level (Jeppe, 1946 ; Coetzee, 1976). This gold-reef has an average length of 350km in a southwest to north-easterly direction and a width of 200km south-east, stretching from the Witwatersrand, through the boundaries of the Gauteng Province into the Free-State (Haughton, 1964 ; Wittmann & Förstner, 1977 a,b ; Winterbach, 1984). The Witwatersrand Supergroup can be divided into seven main goldfields: Evander, East Rand, Central Rand, West-Rand, Far West-Rand, Klerksdorp and Orange Free-State (Jeppe, 1946 Haughton, 1964 ; Coetzee, 1976 ; Wittmann & Förstner, 1977 a,b ; Lurie, 1979 ; Winterbach, 1984). Any of these fields are more productive than goldfields in the remainder of the world (Wittmann & Förstner, 1976 ; 1977 a,b ; Winterbach, 1984). More than 150 mines, of which a quarter are still actively producing, are present on these goldfields (Winterbach, 1984).

After the chemical extraction of gold, excess sludge is pumped into slimes dams where it is stored. A serious problem arises when any cyanide- or pyrite containing effluents or seepage water escaping from the slimes dams, may directly or indirectly reach the aquatic environment (Murray, 1987 ; Jones *et al.*, 1989) Underground water pumped from disused mines to the surface, may also cause a significant pollution problem. This water usually is rich in dissolved salts, metals and other cations which may be potentially detrimental to the environment.

Due to the high pollution risk of old slimes dams and sand piles (Henzen & Pieterse, 1978 ; Kempe, 1983), the Chamber of Mines with the aid of the Department of Water Affairs already attempted to combat this problem (Chamber of Mines, 1979 ; Blight & Cladwell, 1984). Steps taken consisted of the covering of slimes dams by vegetation (Cook, 1973) or the containment thereof (Cartwright, 1983). With the treatment of precipitated sludge with a lime solution, a process known as the "High Density Sludge" (HDS) process (Van Staden, 1979), a number of mines on the Rand was able to restrict environmental pollution (Tyser, 1976 ; Bosman, 1983).

According to Dolan (1961) and Kempe (1983), the presence of pyrite (FeS_2) in gold ore and discarded sludge can be regarded as the prime source of gold mine pollution. Pyrites is the descriptive term for three minerals (Murray, 1987) with a sulphur content of greater than 25%. It consists of:

- Pyrite (FeS_2), when pure contains 46,6% iron and 53,4% sulphur. It has a copperish colour and is associated with small amounts of arsenic, copper, nickel, cobalt, zinc, tin and gold.

- Marcasite (FeS_2), which has the same chemical composition as pyrite, but possesses a light yellow to white colour, and

- Pyrrothyte ($Fe_{11}S_{12}$), which contains about 60% iron and 39% sulphur. It is of a bronze colour and is often associated with copper, nickel and cobalt.

Of the pyrites mentioned, the mineral pyrite constitutes the main component of the minerals found in association with Witwatersrand gold-ores. Acid mine-water is the consequence of the oxidation of iron-pyrites with the subsequent formation of sulphuric acid (Unz & Dietz, 1986). Consequently, effluents and seepage waters with high levels of metals, salts and sulphates are able to reach the aquatic environment. According to various authors (Matic & Mrost, 1964 ; Berner, 1970 ; Wittmann & Förstner, 1976 ; Ivarson *et al.*, 1978 ; Johnson *et al.*, 1979 ; Thompson, 1980 ; Kempe, 1983 ; Noike *et al.*, 1983 ; Murray, 1987), the oxidation of pyrites to sulphuric acid can be summarized as follows:

$2FeS_2 + 7O_2 + 2H_2O \text{ ---> } 2FeSO_4 + 2H_2SO_4$--[1]

$4FeSO_4 + 2H_2SO_4 + O_2 \text{ ---> } 2Fe_2(SO_4)_3 + 2H_2O$--[2]

$3Fe(SO_4)_3 + 12H_2O \text{ ---> } 2HFe_3(SO_4)_2(OH)_6 + 5H_2SO_4$---------------------------------------[3]

Reactions [1] and [2] are oxidation reactions which take place under aerobic conditions, while reaction [3] is a hydrolytic reaction taking place under anaerobic conditions (Ivarson *et al.*, 1978 ; Thompson, 1980). Reactions [1] and [3] can also be accelerated in the presence of bacteria, but reaction [2] requires the acidophyllic obligatory chemo-autotrophic bacteria *Thiobacillus ferrooxidans* responsible for the oxidation of iron-compounds in an acid medium where auto-oxidation is not possible (Mrost & Lloyd, 1971 ; Matic & Mrost, 1964 ; Wittmann & Förstner, 1976 ; Johnson *et al.*, 1979 ; Thompson, 1980 ; Kempe, 1983 ; Murray, 1987 ; Wagner & Van Niekerk, 1987).

During the release of acidic mine-water, the blending thereof with other water including the role of $CaCO_3$ present in rivers and streams, result in the pH thereof often rising above 4. Consequently, a yellow-brown gelatinous precipitate known as "Yellow-boy" is often found in the vicinity of mines. This usually consists of the hydroxides of iron and aluminium formed during the following reactions:

$Fe(SO_4)_3 + 3Ca(OH)_2 === \underset{\text{Insoluble}}{2Fe(OH)_3} + \underset{\text{Slightly soluble}}{3CaSO_4}$--[4]

$A\ell(SO_4)_3 + 3Ca(OH)_2 === \underset{\text{Soluble}}{2A\ell(OH)_3} + \underset{\text{Slightly soluble}}{3CaSO_4}$--[5]

These precipitates, rich in $A\ell$ and Fe and being slightly soluble to soluble, may also add to the potential mineralization of aquatic ecosystems in the vicinity of gold mines.

Another potential source of pollution originating from mining effluents and slimes dams seepage water is uranium and the radioactive daughter products thereof. Almost all uranium produced locally is a by-product of the Witwatersrand gold-mining industry (Feather & Koen, 1975 ; Wittmann & Förstner, 1977 ; Malan, 1981 ; de Jesus *et al.*, 1987)

According to de Jesus *et al.* (1987), gold and uranium mining has been in progress over the past 80 years on the Witwatersrand during which a total of 2500 million tonnes of processed sludge have been dumped on slimes dams. These slimes dams, which cover an approximate total area of 6500 hectares, stretching over an 80 km-long strip in the proximity of approximately 3,8 million people residing there.

The pollution risk is caused by the presence of ^{226}Ra which is a known alpha-emitter and possesses the ability to accumulate in bone-tissue (Malan, 1981). Discovered in 1898 by Marie Curie (Curie *et al.*, 1898), this element, as well as a number of other radionuclides, are the daughter products of the ^{238}U decay series. However, during the recovery of gold and uranium from ore, no attempt is made to remove ^{226}Ra from the discarded sludge, largely due to its low economic value. Consequently, considerable amounts of this radionuclide is present in mine discard (Malan, 1981). The presence of ^{226}Ra as well as its decay product, ^{222}Rn (radon), which is a radioactive gas, do not only pose a pollution potential to the aquatic environment, but also create a health risk to humans residing in the immediate vicinity of mine discard dumps and slimes dams (Spiers, 1968).

According to Malan (1981), Swanson (1985) and de Jesus *et al.* (1987) various pathways exist by which these radionuclides can reach humans either directly or via the aquatic environment. ^{222}Rn-gas can be inhaled by humans where this gas, as well as its toxic decay-products, can pose serious health risks. On the other hand, ^{238}U and ^{226}Ra as well as their decay products can be present in significant amounts in the abiotic (water and sediments) and biotic (insects, plants, fish and birds) environment from where it can be transferred to other components of either the aquatic or terrestrial environment (Polikarpov, 1967 ; Swanson, 1985 ; Lauria & Godoy, 1988 ; Platford & Joshi, 1988).

Malan (1981), Tracey *et al.* (1983) and de Jesus *et al.* (1987) also found that ^{226}Ra might be present in vegetables and grain products where mine-polluted water is pumped from locally affected rivers utilized for the irrigation of agricultural land. It was also found that the transfer of these radionuclides may directly take place from these products to humans or, indirectly via sheep and cattle grazing on radionuclide contaminated fodder (Swanson, 1985 ; de Jesus *et al.*, 1987).

The establishment of gold mines in South Africa during the past century led to the creation of several industrial zones surrounding some of the major cities, which directly or indirectly supported the mining industry. Many of these were metal-processing factories. Processed and unprocessed effluents and seepage waters from both the mines and industries discharged into streams and were found to contain variable amounts of heavy metals (Wittman and Förstner, 1976 a-d, 1977 a,b ; Kempster *et al.*, 1982 ; Van der Merwe *et al.*, 1990). The pollution from the mining areas in particular led to severe acidification of a number of tributaries of the Vaal River (Harrison, 1958, 1961 ; Schoonbee and Van der Merwe, 1989). Water pumped from some mines varied in pH, affecting the solubility and the consequent deposition in stream and lake sediments as well as the uptake of certain metals by the flora and fauna of the affected water bodies. Estimates of the volume of water pumped from mines reaching the Elsburgspruit in Gauteng alone amounted to as much as 10 938 m^3/d (Wells, 1989), directly and/or indirectly through seepage and effluents from existing mine dumps.

The effect of mine effluent water containing metals on stream ecosystems has received considerable attention elsewhere in the world (Eyres and Pugh-Thomas, 1978 ; Salomons and Mook, 1980 ; Norris, 1986). From these investigations it is clear that certain metals may have a pronounced effect on the composition and presence of certain aquatic biota in lakes and rivers and that changes in the water pH and associated physical and chemical variables which affect this parameter may have a direct bearing on the toxicity of some metallic ions. Research by Abo-Rady (1980) and Mortimer (1985), to name but two researchers, showed that rooted aquatic macrophytes can be used as bio-indicators to evaluate the extent of metal pollution in aquatic ecosystems. It is also known that floating aquatic weeds can be employed in the removal of potentially toxic metals from polluted waters (Muramoto and Oki, 1983 ; Abbasi and Nipanei, 1985 ; Jain *et al.*, 1989). Some aquatic invertebrates were also found to be extremely sensitive to metal pollution (Bryan and Ward, 1965 ; Burrows and Whitton, 1983 ; Yasuno *et al.*, 1985). Aquatic vertebrates such as amphibia and fish have also been studied to determine the various routes of metal uptake via their food (Baudin, 1989), gills (Matthiesen and Brafield, 1977), through the drinking of water (Eddy, 1981) and simply through absorption via the skin. The release of toxic metals into the aquatic environment was also shown to be detrimental to aquatic birds (Koeman *et al.*, 1972 ; Bull *et al.*, 1983).

In view of the increasing threat of metal-containing effluents to the water quality conditions of wetland areas on the Witwatersrand in particular and because of the planned and unplanned residential encroachment on these potential recreational areas and wildlife sanctuaries, the need was expressed by researchers at the Rand Afrikaans University to evaluate stream conditions and, where possible, to establish further research programmes on the problem of radionuclide and metal pollution of the aquatic environment in this region.

Objectives and Scope

The investigations reported here conducted during 1990-1994 had the following objectives:

- **Selection of representative localities in impoundments and rivers in wetlands affected by radionuclide- and metal-containing effluents and seepage water from mines and metal-processing industries.**

- **To determine the occurrence and concentration of radionuclides and selected metals in mine- and industry-polluted waters and sediments in catchments of the Blesbokspruit, the Klip River and the Crocodile River systems.**

- **To establish radionuclide and metal concentrations in some semi-aquatic and aquatic weeds in these mine- and industry-polluted waters with observations on the ability of some plants to accumulate certain metals in their roots and/or shoots.**

- **To evaluate some benthic macro-invertebrate organisms as possible indicators of radionuclide and metal pollution in the affected streams.**

- **To determine the radionuclide and metal concentrations in selected vegetable crops irrigated with mine- and industry-polluted water.**

The localities where the investigation took place were mainly in wetland regions on the East and West Rand. Water of polluted streams traverse expansive wetlands containing floating, emergent and submerged aquatic vegetation. These plants play an important role in the recovery from pollution of the affected waters. Pollutants are also available to and accumulated by other aquatic organisms such as crabs, fish and birds. In addition, the same water is often used for the irrigation of vegetable crops, thereby creating potential pathways through which these pollutants may be consumed by humans.

All the above objectives have largely been met in as such that potential sources of pollution were identified and pollutants detected in the abiotic (water and sediments) environment. These pollutants were also found in wetland biota as well as agricultural crops irrigated with contaminated water. Potential pathways for the transfer of these pollutants were identified and dose assessment modelling was conducted.

The present report is presented in two sections, namely:

Part 1: **Investigations into the accumulation and concentration ratios of selected radionuclides in aquatic ecosystems affected by mine drainage effluents with reference to the study of potential pathways to man.**

and

Part 2: **Investigations into the accumulation and concentration ratios of selected metals in aquatic ecosystems affected by mine drainage effluents with particular reference to the uptake of metals by vegetable crops.**

REFERENCES

ABASSI, S.A. & NIPANEY, P.C. 1985. Waste-water treatment using aquatic plants: Survivability and growth of *Salvinia molesta* (Mitchell) over waters treated with Zinc (II) and the subsequent utilization of the harvested weeds for energy (biogas) production. **Resources Conserv.**, 12: 47 - 55.

ABO-RADY, M.D.K. 1980. Aquatic macrophytes as indicators for heavy metal pollution in the River Leine, West Germany. **Arch. Hydrobiol.**, 89: 387 - 404.

BAUDIN, J.P. 1987. Investigation into the retention of ^{65}Zn absorbed by the trophic pathway in *Cyprinus carpio* L. Influence of the ingestion frequency and the radiozinc content of food. **Water Res.**, 21(3): 285 - 294.

BERNER, R.A. 1970. Sedimentary pyrite formation. **Am. J. Sci.**, 268(1): 1 - 23.

BLIGHT, C.E. & CLADWELL, J.A. 1984. The abatement of pollution from abandoned gold-residue dams. **J. S. Afr. Inst. Min. Metall.**, 84(1): 1 - 9.

BOSMAN, D.J. 1983. Lime treatment of acid mine water and associated solids/liquid separation. I.A.W.P.R. Conf., **Wat. Sci. Tech.**, 15(2): 71 - 84. In: "Mine Water Pollution", P.E. ODENDAAL (Ed.)

BRYAN, G.W. & WARD, E. 1965. The absorption and loss of radioactive and non-radioactive manganese by the lobster *Homarus vulgaris*. **J. Mar. Biol. Ass.**, 45: 65 - 95.

BULL, K.R., EVERY, W.J., FREESTONE, P., HALL, J.R. & OSBORN, D. 1983. Alkyl lead pollution and bird mortalities on the Mersey Estuary, UK, 1979-1981. **Environ. Pollut. Ser. A**, 31: 239 - 259.

BURROWS, I.G. & WHITTON, B.A. 1983. Heavy metals in water, sediments and invertebrates from a metal-contaminated river free of organic pollution. **Hydrobiologia**, 106: 263 - 273.

CARTWRIGHT, F.D. 1983. Some seventy years in the shrouding of mining residue deposits for environmental protection. I.A.W.P.R. Conf., **Wat. Sci. Tech.**, 15(2): 85 - 102. In: "Mine Water Pollution", P.E. ODENDAAL (Ed.)

CENTRAL STATISTICAL SERVICES, REPUBLIC OF SOUTH AFRICA. 1982. **South African Statistics.** Government Printer. Pretoria.

CHAMBER OF MINES OF SOUTH AFRICA. 1979. **Handbook of guidelines for environmental protection. Vol 1. The design, operation and closure of residue**

deposits. Chamber of Mines. Johannesburg.

CHAMBER OF MINES OF SOUTH AFRICA. 1981. **92nd. Annual Report.** Chamber of Mines. Johannesburg.

COETZEE, C.B. (Ed.). 1976. **Delfstowwe van die Republiek van Suid-Afrika.** Department of Mines. Geological Survey. Pretoria.

COUSINS, C.A. 1973. Platinoids in the Witwatersrand system. **J. S. Afr. Inst. Min. Metall.**, 73(6): 184 - 199.

CURIE, M., CURIE, P. & BEMONT, G. 1898. Another new radio-active element. **C.R.**, 127: 1215 - 1217.

DE JESUS, A.S.M., MALAN, J.J., ELLERBECK, V.T., VAN DER BANK, D.J., MOOLMAN, E.W. 1987. **An assessment of the radium-226 concentration levels in tailings dams and environmental waters in the gold/uranium mining areas of the Witwatersrand.** PER-159. Pelindaba.

DEPARTMENT OF WATER AFFAIRS. 1986. **Management of the Water Resources of the Republic of South Africa.** CTP Book Printers. Cape Town.

DOLAN, J. 1961. Water problems of the Transvaal and Orange Free State mines. Trans. **Seventh Commonwealth Mining Metallurgical Congr.**, 3: 1357 - 1388.

EDDY, F.B. 1981. Effects of stress on osmotic and ionic regulation in fish. In: **Stress and fish.** Pickering, A.D. (ed.). Academic Press. New York.

ELEVATORSKI, E.A. 1984. **Economic gold deposits.** MINOBRAS.

EYRES, J.P. & PUGH-THOMAS, M. 1978. Heavy metal pollution of the River Irwell (Lancashire, UK) demonstrated by analysis of substrate materials and macroinvertebrate tissue. **Env. Poll.**, 16: 129 - 136.

FEATHER, C.E. & KOEN, G.M. 1975. The mineralogy of the Witwatersrand reefs. **Minerals Sci. Engng.**, 7(3): 189 - 224.

FöRSTNER, U. & WITTMANN, G.T.W. 1981. **Metal pollution in the aquatic environment.** Springer-Verlag. Berlin.

HARRISON, A.D. 1958. The effects of sulphuric acid pollution on the biology of streams in the Transvaal, South Africa. **Verh. internat. Verein. Limnol.**, 13: 603 - 610.

HAUGHTON, S.H. (Ed.). 1964. **The geology of some ore deposits in Southern Africa.** Hortors. Leader Building. Johannesburg.

HENZEN, M.R. & PIETERSE, J.J. 1978. Acidic mine drainage in the Republic of South Africa. **Prog. Wat. Tech.**, 9: 981 - 1000.

IVARSON, K.C., ROSS, G.J. & MILES, N.M. 1978. Alterations of micas and feldspars during microbiological formation of basic ferric sulphates in the laboratory. **J. Soil Sci. Soc. of Amer.**, 42(3): 518 - 524.

JAIN, S.K., VASUDEVAN, P. & JHA, N.K. 1989. Removal of some heavy metals from polluted water by aquatic plants: studies on duckweed and water velvet. **Biol. Wastes**, 28(2): 115 - 126.

JEPPE, C.B. 1946. **Gold Mining on the Witwatersrand.** Vol. 1. Transvaal Chamber of Mines.

JOHNSON, D.B., KELSO, W.I. & JENKINS, D.A. 1979. Bacterial streamer growth in a disused pyrite mine. **Environ. Pollut.**, 18: 107 - 118.

JONES, G.A., BRIERLY, S.E., GELDENHUIS, S.J.J. & HOWARD, J.R. 1989. **Research on the contribution of mine dumps to the mineral pollution load in the Vaal Barrage.** Report to the WRC by Steffen Robertson & Kirsten Inc. WRC Report No. 136/1/89. Pretoria.

KEMPE, J.O. 1983. Review of water pollution problems and control strategies in the South African mining industry. **Wat. Sci. Tech.**, 15: 27-58.

KEMPSTER, P.L., HATTINGH, W.A.J. & VAN VLIET, H.R. 1982. **Summarised water quality criteria. Department of Water Affairs.** South Africa. Technical Report No. TR108. 45pp.

KOEMAN, J.H., BOTHOF, T.H., DE VRIES, R., VAN VELZEN-BLAD, H. & VOS, J.G. 1972. The impact of persistent pollutants on piscivorous and molluscivorous birds. **TNO-Nieuws**, 11: 561 - 569.

KURIAN, G.T. 1979. **The book of rankings.** MacMillan Reference Books. The MacMillan Press Ltd.

LAURIA, D.C. & GODOY, J.M. 1988. Determinacado de U-238, U-234, Th-228, Ra-228 e Ra-226 em aguas minerals do Planalto de Pocos de Caldas. **Ciência e Cultura.**, 40(9): 906 - 908.

LURIE, J. 1979. **South African geology for mining, metallurgical, hydrological and civil engineering.** McGraw-Hill Book Company. Johannesburg.

MALAN, J.J. 1981. **Die bepaling van radium-226 in die uitskot van goudmyne en hulle omgewings.** M.Sc. Tesis. Universiteit van Pretoria.

MATIC, M. & MROST, M. 1964. In situ leaching of uranium from gold mine residue dams. **S. Afr. Indust. Chemist.**, 18: 127 - 133.

MATTHIESSEN, P. & BRAFIELD, A.E. 1977. Uptake and loss of dissolved zinc by the stickleback *Gasterosteus aculeatus* L. **J. Fish Biol.**, 10: 399 - 410.

MORTIMER, D.C. 1985. Freshwater aquatic macrophytes as heavy metal monitors - the Ottawa River experience. **Environmental Monitoring and Assessment**, 5: 311 - 323.

MROST, M. & LLOYD, P.J. 1971. **Bacterial oxidation of Witwatersrand slimes.** IAEA-SM-135/2. Vienna.

MURAMOTO, S., & OKI, Y. 1983. Removal of some heavy metals from polluted waters by water hyacinth *(Eichhornia crassipes)*. **Bull. Environ. Contam. Toxicol.**, 30: 170 - 177.

MURRAY, K.A. 1987. **Wastewater treatment and pollution control.** Heer Printing Co. (Pty) Ltd. Pretoria.

NOIKE, T., NAKAMURU, K. & MATSUMOTO, J. 1983. Oxidation of ferrous iron by acidophilic iron-oxidizing bacteria from a stream receiving acid mine drainage. **Water Res.**, 17: 21 - 27.

NORRIS, R.H. 1986. Mine waste pollution of the Molonglo River, New South Wales and the Australian Capitol Territory: Effectiveness of remedial works at Captains Flat mining area. **Aust. J. Mar. Freshw. Res.**, 37: 147 - 157.

PLATFORD, R.F. & JOSHI, S.R. 1988. Dose rates to aquatic life near a uranium waste site. **Health Phys.**, 54(1): 63 - 68.

POLIKARPOV, G.C. 1967. Regularities of uptake and accumulation of radionuclides in aquatic organisms. **Proc. Int. Symp. on Radioecological Concentration Processes.** Stockholm, Permagon, Oxford. p. 819.

SALOMONS, W. & MOOK, W.G. 1980. Biogeochemical processes affecting metal concentrations in lake sediments (Iisselmeer, The Netherlands). **The Science of the Total Environment**, 16: 217 - 229.

SCHOONBEE, H.J. & VAN DER MERWE, C.G. 1989. Investigations into the effects of sewage, industrial and gold mine effluents on the water quality and faunal conditions of a stream in the Transvaal, South Africa. **Proceedings of the 4th International Conference on Environmental Quality and Ecosystem Stability**. Jerusalem, Israel, IV/A:401-418.

SPIERS, F.W. 1968. **Radioisotopes in the human body.** Academic Press. New York.

SWANSON, S.M. 1985. Food-chain transfer of U-series radionuclides in a northern Saskatchewan aquatic system. **Health Phys.,** 49(5): 747 - 770.

THOMPSON, J.G. 1980. Acid mine waters in South Africa and their ameriolation. **Water SA,** 6(3): 130 - 134.

TRACY, B.L., PRANTL, F.A. & QUINN, J.M. 1983. Transfer of ^{226}Ra, ^{210}Pb and uranium from soil to garden produce: assessment of risk. **Health Phys.,** 44(5): 469 - 477.

TYSER, J.A. 1976. **Colloquium on "Pumping, settling, treatment and clarification of underground water".** The South African Institute of Mining and Metallurgy. Kelvin House. Johannesburg. 17 November, 1976.

UNZ, R.F. & DIETZ, J.M. 1986. Biological applications in the treatment of acidic mine drainages. **Biotechnology and Bioengineering Symp.**, No. 16: 163 - 170.

VAN BILJON, W.J. 1981. **Our future in science and technology.** Presidential address to the Scientific and Technical Societies of South Africa. Annual Proceedings 1981/82.

VAN DER MERWE, C.G., SCHOONBEE, H.J. & PRETORIUS, J. 1990. Observations on concentrations of the heavy metals, zinc, manganese, nickel and iron in the water, in the sediments and in two aquatic macrophytes, *Typha capensis* (Rohrb.), N.E. Br. and *Arundo donax* L.M. of a stream affected by goldmine and industrial effluents. **Water SA,** 16: 119 - 124.

VAN STADEN, C.M. 1979. How ERPM uses lime to solve unique underground water problem. **Coal Gold and Base Minerals of Southern Africa,** 27(5): 100 - 109.

WAGNER, J.C. & VAN NIEKERK, A.M. 1987. Quality and treatment of effluents originating from mine and municipal solid waste sites. **Proc. Int. Conf. on Mining and Industrial Waste Management.** Johannesburg. 1: 283 - 288.

WELLS, J.D. 1989. Personal communication.

WINTERBACH, D.J. 1984. Geological setting of mineral deposits. **Archimedes,** 26(1): 4 - 37.

WITTMANN, G.T.W. & FöRSTNER, U. 1976a. Metal accumulations in acidic waters from gold mines in South Africa. **Geoforum,** 7: 41 - 49.

WITTMANN, G.T.W. & FöRSTNER, U. 1976b. Metal enrichment of sediments in Inland waters - The Jukskei and Hennops river drainage systems. **Water SA**, 2: 67 - 72.

WITTMANN, G.T.W. & FöRSTNER, U. 1976c. Heavy metal enrichment in mine drainage. I. The Rustenburg Platinum mining area. **S. Afr. J. Sci.**, 72: 242 - 246.

WITTMANN, G.T.W. & FöRSTNER, U. 1976d. Heavy metal enrichment in mine drainage. II. The Witwatersrand Goldfields. **S. Afr. J. Sci.**, 72: 365 - 370.

WITTMANN, G.T.W. & FöRSTNER, U. 1977a. Heavy metal enrichment in mine drainage. III. The Klerksdorp, West Wits and Evander goldfields. **S. Afr. J. Sci.**, 73: 53 - 57.

WITTMANN, G.T.W. & FöRSTNER, U. 1977b. Heavy metal enrichment in mine drainage. IV. The Orange Free State goldfield. **S. Afr. J. Sci.**, 73: 374 - 378.

YASUNO, M., HATAKEYAMA, S. & SUGAYA, Y. 1985. Characteristic distribution of chironomids in rivers polluted with heavy metals. **Verh. Internat. Verein. Limnol.**, 22: 2371 - 2377.

SUMMARY

SUMMARY

Investigations were made into the occurrence and concentration values of ^{226}Ra and uranium in some gold and uranium mine polluted aquatic environments in the Transvaal. An overview is given on aspects of the possible environmental effects of ^{226}Ra and uranium as well as background data on factors affecting the biological uptake and transport of both radionuclides in some terrestrial and aquatic organisms. A brief discussion is given on the prevailing physical and chemical conditions of the irrigation water and agricultural soils at three localities where analyses were made for the presence and concentrations of both radionuclides in the water, in stream sediments, in selected aquatic plants, in a decapod macro-invertebrate, in fish and water birds. In order to evaluate the potential effects of these radionuclides on man, studies were also conducted on the concentration ratios of ^{226}Ra and uranium in these organisms and the abiotic environment in which they occur, including selected vegetable crops irrigated with mine polluted river water. The experimental uptake of radium by beetroot and cabbage under controlled environmental conditions was also investigated. Concentration Ratios (CR) for each of the biota were calculated. Concentration data was subsequently used in a dose assessment model looking at various potential pathways of both radionuclides to man including via soil, drinking water, vegetables, a cereal and fish.

Field results obtained showed that in virtually all cases the presence and concentrations recorded for both radionuclides were (and possibly are also at other affected areas) at least an order of magnitude lower than concentrations found in countries like Japan, Germany and the USA, and that in all cases concentration values found for both radionuclides in the present study fall below the maximum recommended guideline values laid down by the Council for Nuclear Safety (CNS) of South Africa. The dose assessments for the scenarios chosen indicate that the annual effective dose is a fraction of the limit for members of the public. However it is possible that certain site specific concentration values may require controls to limit exposure.

OPSOMMING

Ondersoeke is gedoen na die voorkoms en konsentrasiewaardes van ^{226}Ra en uraan in sommige goud- en uraanmyn besoedelde akwatiese omgewings in die Transvaal. 'n Oorsig word gegee oor aspekte van moontlike omgewingseffekte van ^{226}Ra en uraan sowel as agtergrondsdata oor faktore wat die biologiese opname en transport van beide radionukliede in sommige terrestriële en akwatiese organismes raak. 'n Beknopte bespreking word gegee oor oorheersende fisiese en chemiese toestande van die besproeiingswater en landbougrond by drie verskillende lokaliteite waar analises uitgevoer is vir die aanwesigheid en konsentrasies van beide radionukliede in die water, stroomsedimente sowel as in geselekteerde akwatiese plante, in 'n dekapood makroïnvertebraat, in vis en watervoëls. Met die oog op die evaluering van die potensiële effekte van hierdie radionukliede op die mens, is ondersoeke gedoen na die konsentrasieverhoudings van ^{226}Ra en uraan in hierdie organismes in verhouding tot die in die abiotiese omgewing waarin hulle voorkom insluitend geselekteerde groentegewasse wat met ^{226}Ra en uraan mynbesoedelde water besproei is. Die eksperimentele opname van radium deur beet en kool onder beheerde omgewingstoestande is ook ondersoek. Konsentrasieverhoudings vir alle biotiese komponente is ook bereken. Al hierdie data is daarna gebruik in 'n dosisevalueringsmodel wat die onderskeie potensiële weë waarlangs beide radionukliede die mens bereik insluitende via die grond, drinkwater, groente, 'n graangewas en vis.

Resultate verkry dui daarop dat in bykans al die gevalle die aanwesigheid en konsentrasies gevind vir beide radionukliede in die huidige ondersoek (en waarskynlik ook in ander geaffekteerde gebiede in Suid Afrika), waardes van minstens een ordegrootte kleiner is as vergelykbare konsentrasies in lande soos Japan, Duitsland en die VSA. Resultate toon ook dat in alle gevalle in die huidige ondersoek dat konsentrasiewaardes gevind vir beide radionukliede binne die maksimum neergelegde grense val van die Raad vir Kernveiligheid van Suid-Afrika.

INTRODUCTION

1

1 INTRODUCTION

Gold mining on the Witwatersrand has been in progress since 1884 and by 1987 a total of 3 x 10^9t of tailings were produced, covering a surface area of about 6500 ha (de Jesus *et al.*, 1987). These tailings contain about 120 000t of unrecovered uranium whilst the radium residues of a further 30 000t of recovered uranium have been dumped with milled residue on slimes dams since 1952.

In the processing of South African gold-ores, uranium occurs as a by-product (Feather & Koen, 1975 ; Wittmann & Förstner, 1977 ; Malan, 1981) while several other radioactive isotopes from the uranium and thorium decay chains are also present. The average uranium concentration in the ores ($< 0{,}01\%$) is at least an order of magnitude lower than that mined in the rest of the world (Funke, 1990). The large volumes of ore processed for gold production, however, result in an appreciable production of uranium. As a result, South Africa was the third largest producer of uranium in the world in 1987 with a quantity of approximately 5000t of U_3O_8. Although the uranium production of South Africa decreased to approximately 2000t in 1992, it is at this level still appreciable in volume (Ainslie, 1992).

If an equilibrium exists in the natural decay chain of ^{238}U, every kilogram of ^{238}U is associated with 3,3 x 10^{-4}g of ^{226}Ra. As there is no present economic use for radium, the ^{226}Ra occurring with the uranium is usually returned to the tailings dams. ^{226}Ra has a half-life of 1620 years and decays to ^{222}Rn (radon), which is a gas and which can then emanate from slimes dams. ^{222}Rn can have detrimental effects on man when its solid radioactive decay products (^{218}Po, ^{214}Pb, ^{214}Bi, ^{214}Po, ^{210}Po) attach themselves to airborne particles which can be inhaled. It is estimated that these radioactive particles are by far the largest contributor of total radiation exposure to man (90% and over) (Funke, 1990). However, because radon gas has a short half-life of 3,8 days, most of it decays into solid decay products before it reaches the surfaces of slimes dams (Funke, 1990). The total amount of ^{226}Ra associated with the recovered or unrecovered uranium is approximately 50 000g, or an equivalent of 1,85 x 10^{15}Bq.

Because the volume of solid wastes in tailings is so immensely large, it is impossible to isolate these tailings from the environment over a prolonged period of time (de Jesus *et al.*, 1987). In addition, these mining activities and tailings on the Witwatersrand are in the close proximity of densely populated areas as well as agricultural land. These mines and tailings are spread over an 80 km-long strip with approximately 3,8 million people living within 20 km of this area. It is therefore unavoidable that radium and uranium contaminated leachate may reach dams, lakes and streams which are accessible to either the public or agricultural land. Contamination of surface waters such as lakes and rivers may also occur by direct discharges of large volumes of radioactive process water or underground mine water into such water bodies. Affected streams usually bypass tailings dams which have a normal adsorption capacity for ^{226}Ra (Funke, 1990).

It is therefore inevitable that radioactive material can enter aquatic ecosystems such as lakes, rivers and wetlands in the Transvaal. These radionuclides can furthermore be present in irrigation water pumped from these water bodies when it is utilized by farmers and, in the process, are transferred to the terrestrial environment. Once in the terrestrial environment, these contaminants can then be taken up by livestock or accumulated by crops and vegetables via the soil.

^{226}Ra, being an alpha emitter and a bone seeker with a biological half-life of 48 years, is known to possess a high degree of radiotoxicity and exhibits a health hazard when it enters the human body either by ingestion or inhalation. Uranium is also known to have both radiotoxic and chemotoxic properties.

Taking into account the potential radiotoxicity of ^{226}Ra and uranium, some local studies have in the past been conducted to determine the occurrence of ^{226}Ra in the environment as well as in agricultural produce (Malan, 1981; de Jesus *et al.*, 1987; Funke, 1990). However, none of these were aimed at determining the Concentration Ratios (CR) for transfer of these radionuclides to crops and livestock and the subsequent potential dose to humans.

This project was initiated on the basis of this background, and with the following research aims in mind:

- **Selection of suitable localities where ^{226}Ra and uranium might be present in significant concentrations.** Such localities are those which are in the close proximity of gold and uranium mines as well as agricultural areas located in the immediate vicinity of such mines.
- **Monitoring of these radionuclides from the point of release and, where possible, via aquatic to terrestrial ecosystems.**
- **Determination of the levels of these radionuclides in water, soil and agricultural products such as vegetables, milk and meat from farms potentially affected by contaminated effluents from mines.**
- **Investigation of an experimental study to determine the amount of ^{226}Ra uptake through the leaves and roots of two vegetables.** For the purpose of this experiment, two commercial strains of beetroot and cabbage were chosen.
- **Calculation of the potential dose to man using mathematical models.**

LITERATURE REVIEW

2

2 LITERATURE REVIEW

2.1 Uranium resources in South Africa

The most extensive uranium deposits are mostly found in South Africa, Niger, France, Czechoslovakia, Australia, Brazil, Canada, Namibia and USA (Fisher, 1988). South Africa has the third largest uranium resource (294 000 t) in the Western World (Figure 2.1).

An estimated 68% of the South African low cost reserves occurs in the Witwatersrand conglomerates and tailing dumps, 29% in Karoo sandstone and coal deposits and 3% in the Phalaborwa complex (Ainslie, 1993). Estimates on the average content of uranium in the Witwatersrand ores (usually uraninite) vary between 0,05 and 0,2 kg $U_3O_8.t^{-1}$ ore (Smit, 1989).

2.2 Mode of occurrence of uranium

Minute amounts of uranium are present in every type of rock and soil in the earth's crust. It is also found in natural fresh water as well as sea water (von Backström, 1976). Soil concentrations typically range between 1 to 4 ppm ($\mu g.g^{-1}$) (Kathren, 1984).

Uranium is more abundant than the elements Hg, Sb, Ag or Cd in nature, but similar in abundance to Mo and As (Weigel, 1983). It is found in greater concentrations in acidic rocks with elevated silicate levels, such as granite (3-5 ppm) (von Backström, 1976) as well as in shales and phosphate formations (Kathren, 1984). It also occurs to a lesser extent in basic rocks, such as basalt and sandstone.

Two types of uranium ore-minerals are usually found, namely primary (originally formed) and secondary or oxidized minerals (derived form primary minerals) (von Backström, 1976). It is however the secondary uranium minerals which increasingly become important sources of this element. The most important of these are carnotite (potassium-uranium vanadate), uranophane and beta-uranophane (calcium-uranium phosphate), torbernite (copper-uranium phosphate) and gummite (calcium-lead-uranium silicate) (Liebenberg, 1972 ; de Waal, 1973). In contrast to the dull brown and black primary minerals, the secondary uranium minerals display vivid colours in visible and ultraviolet light. For instance, uranophane ($Ca(UO_2)_2Si_2O_7.6H_2O$), beta-uranophane and tyuyamunite ($Ca(UO_2)_2(VO_4)_2.5\text{-}8H_2O$) are bright yellow in colour, while autunite ($Ca(UO_2)_2(PO_4)_2.10\text{-}12H_2O,CO_3$) and carnotite ($K_2(UO_2)_2(VO_4)_2.3H_2O$) typically display yellowish-green to greenish-yellow colours. Many of these secondary minerals fluoresce in yellowish to greenish hues under short wave ultraviolet light

2.3 Deposits of uranium in South Africa

The auriferous conglomerates in the four contiguous Precambrian systems, namely the Dominion Reef, Witwatersrand, Ventersdorp and Transvaal contain the major concentrations of uranium minerals in South Africa. Of these, the Dominion Reef and Witwatersrand

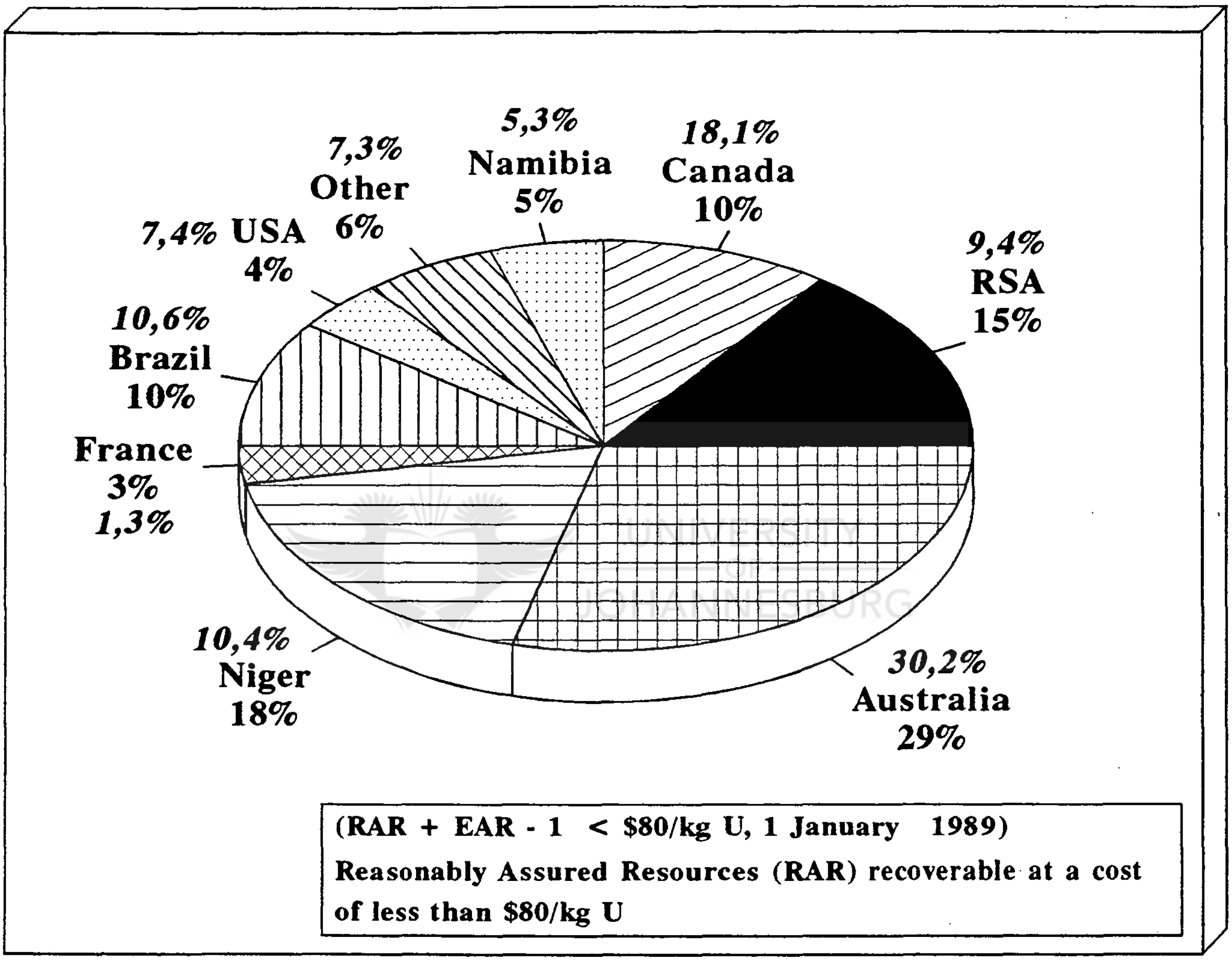


Figure 2.1 World uranium resources of World Outside Centrally Planned Areas (WOCA) (After NEA/IAEA, 1990)
Figures in italics indicate resouces during 1992 (After NEA/IAEA, 1993)

complex are the most important uranium resources. Although not geologically conformable, these four systems together cover tens of thousands of square kilometres in the Transvaal and the Orange Free State (von Backström, 1976). The most important deposits of uranium in South Africa, the minerals found at each deposit as well as the geographical position of each are illustrated in Table 2.1 and Figure 2.2 (After de Waal, 1973 ; Funke, 1990).

Table 2.1 Uranium deposits and minerals in Southern Africa (After de Waal, 1973).

Genetic type	Deposit	Minerals
Igneous	Phalaborwa carbonatite	Uranothorite
Metamorphic	Rössing alaskite Pegmatites of the North-western Cape, northern Transvaal and Namibia	Uraninite, betafite, monazite & zircon Euxenite, samarskite, pyrochlore, davidite
Detrital	Witwatersrandquartz-pebble conglomerate	Uraninite, brannerite[(1)], zircon, coffinite, monazite, xenotime, uranothorite, columbite, euxenite, uraniferous leucoxene[(2)]
Hydrogenic, Syngenetic (?) or Epigenetic	Karoo carbonaceous sediments	Coffinite
Hydrogenic Supergene	Surface limestones and gypcretes in the vicinity of Rössing, Namibia and in the northwestern Cape	Carnotite

(1)Secondary weathering products are: beta-uranophane, metahaiweeite, uranophane, carnotite, thorogummite and gummite

(2)Most probably formed by sorption of U_4^+ on titanium dioxide minerals

2.3.1 The Dominion Reef System

The Dominion Reef comprises a basal group of conglomeratic, arkosic and quartzitic rocks which form a layer about 100 meters thick. It is covered by about 900m of lava which is of andesitic or acidic composition (von Backström, 1976). From an economic point of view, the base of this reef which is formed by sedimentary rocks, constitutes the most important part of the Dominion Reef system. This layer mostly consists of disintegrated material from the basement granite. The sediments consist of medium-grained to coarse-grained feldspathic

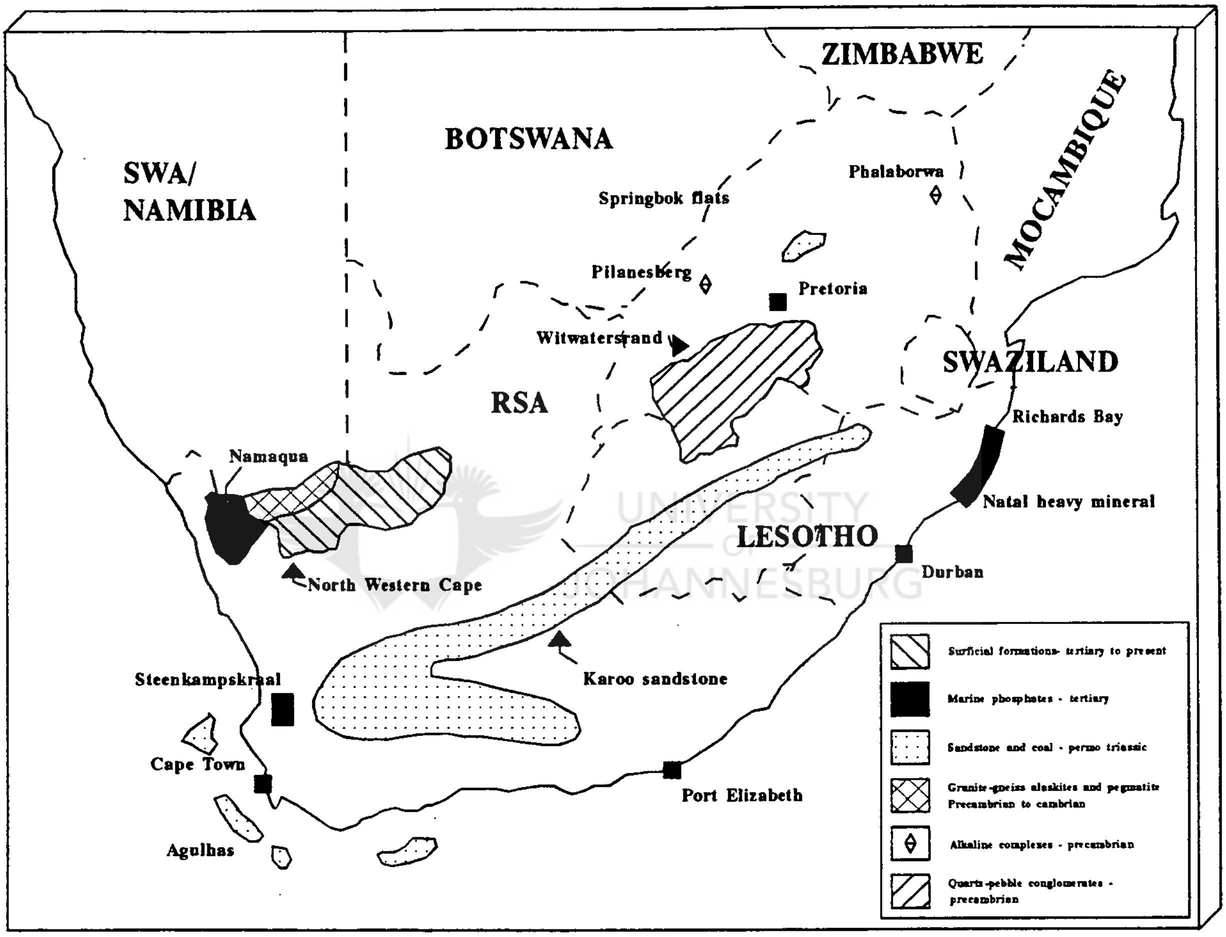


Figure 2.2 Important uranium deposits in South Africa (After Funke, 1990)

and somewhat micaceous sandstone or quartzite in which lenticular bodies of conglomerate and grit occur.

Two conglomerate bands, known as the Upper and Lower reefs, which are along or close to the base of the arenaceous sediments, are the most important economically and have been prospected and mined for gold and uranium. The Lower Reef, situated at the base of the conglomerate zone, occupies depressions in the erosion surface of the basement granite. This layer is lenticular and is known and utilized as the main gold bearing ore body in the area. The Upper Reef is situated about 18m higher up in the succession of conglomeratic, arkosic and quartzitic rocks. This reef, consisting of small-pebble conglomerates, varies in thickness from about 15 to 120 cm. Where this layer is thin, the concentration of uraninite may be as high as 1% uranium. This reef is the main carrier of uranium, but apart from gold and uranium, also contains relatively high concentrations of detrital monazite and cassiterite as well as minor amounts of detrital chromite, garnet, zircon, ilmenite and columbite-tantalite.

2.3.2 The Witwatersrand System

In this geological system, economically important uranium and gold-bearing conglomerate bands known as reefs or bankets occur on flat planes of intraformational diastems, disconformities and unconformities. Each conglomerate band represents a deposit in the process of sedimentation.

The ore-bearing conglomerates consist mainly of pebbles of vein-quartz, chert, quartzite, red jasper and quartz porphyry. Quartz is the most abundant constituent of the matrix, bit it is also associated with phyllosilicates such as sericite and pyrophyllite as well as lesser quantities of chlorite, chloritoid and tourmaline. Hydrocarbons containing minute particles of uraninite (thucholite) are also commonly found as granules and less often as thin layers or bands.

These conglomerates form only a minor part of the total Witwatersrand System. The amount of conglomerates varies in abundance in the different regions of the system, but are found more frequently in the Central Witwatersrand. In this region, the conglomerates comprise about 600 meters of the total of 7500 meters of sediments. The conglomerates are found most commonly in the Upper Division of the Witwatersrand System. Most of these conglomerates contain both gold and uranium, but not all of them are economically viable. By far the greatest amount of gold and uranium produced so far originates from the Afrikaner and Inner Basin Reefs in the Government Series west of Klerksdorp.

2.4 Production of uranium in South Africa since 1952

Uranium production in South Africa showed a significant increase (250%) between 1975 and 1980 because of the demand for it during this period. Since then, production of U_3O_8 declined to 3472 metric tonnes in 1989 (Figure 2.3).

Uranium in South Africa is closely associated with the gold-mining industry (Figure 2.4) which produces 97% of this resource compared to the remaining 3% which is recovered from

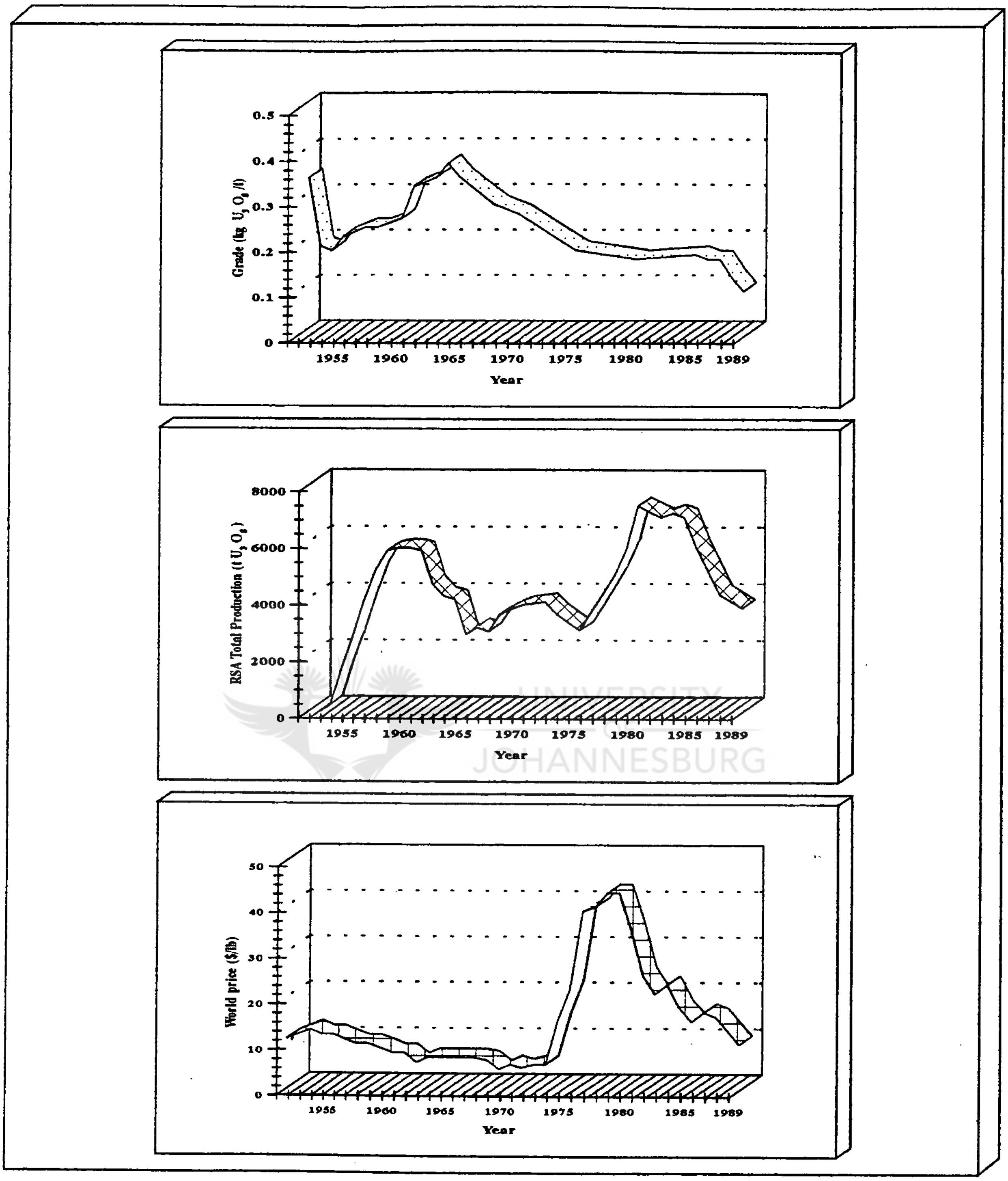


Figure 2.3 RSA uranium (grade, production and world prices: 1952-1989) (After Smit, 1989)

Notes: World price:
1952-1968: US AEC Contract price
1969-1989: NUEXCO Exchange price
COM: SA Chamber of Mines

the base metal ores in the Phalaborwa complex. According to Smit (1989), during 1989 seven producers extracted uranium at nine plants which included Freegold, Vaal Reefs, Hartebeesfontein, Buffelsfontein, Western Areas, Ergo and Palabora. Of these plants, only Freegold and Ergo recover uranium mainly or only from slimes dams (Table 2.2 : Figure 2.5).

The largest uranium-producing group in South Africa during 1989 was the Anglo-American Corporation of SA Ltd which contributed 69% of South Africa's production. It was followed by the General Mining Union Corporation Ltd (11%), Anglovaal Ltd (10%), Johannesburg Consolidated Investment Company Ltd (7%) and Palabora Mining Company Ltd (3%) (Smit, 1989).

During the latter period (1989), the Klerksdorp areas of the Western Transvaal produced about 72% of South Africa's uranium. It was followed by the Orange Free State (13%), West and Far West Rand (7%), the East Rand (5%) and the North Eastern Transvaal (3%) (Smit, 1989).

Table 2.2 Major uranium producers in South Africa (1989). (After Smit, 1989).

Producer	Production (t U_3O_8)	Ore treated (Kt)	Recovery grade ($g.t^{-1}$)
GOLD ORE			
Buffelsfontein	388	2325	167
Hartebeesfontein	344	3180	108
Vaal Reefs	1785	9195	194
Western Areas	225	626	359
Subtotal	2742	15326	179
GOLD TAILINGS			
ERGO(+)	173(x)	21416	8
Freegold(*)	444	16826	26
Palabora(**)	113(**)	11848	9
Subtotal	730	50090	15
RSA TOTAL	**3472**	**65416**	**53**

SOURCES:
Chamber of Mines (1989)
(x) East Rand Gold and Uranium Co Ltd (1989)
(**) Palabora Mining Company (1990)

NOTES:
(+) Recovery of uranium from slimes dams only
(*) Metallurgical scheme
(xx) Primarily a copper producer

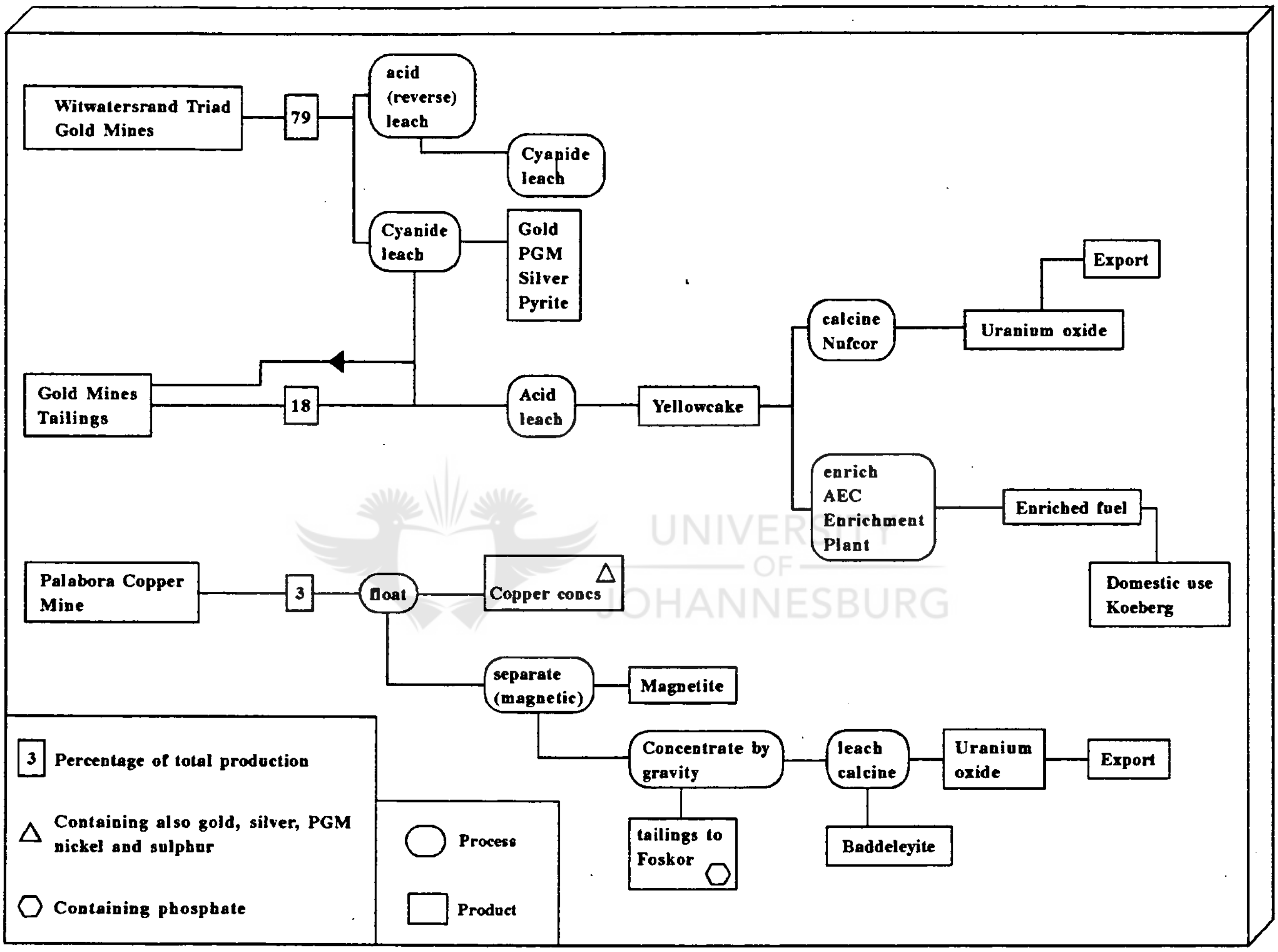


Figure 2.4 The close association between the South African gold and uranium industries (After Smit, 1989)

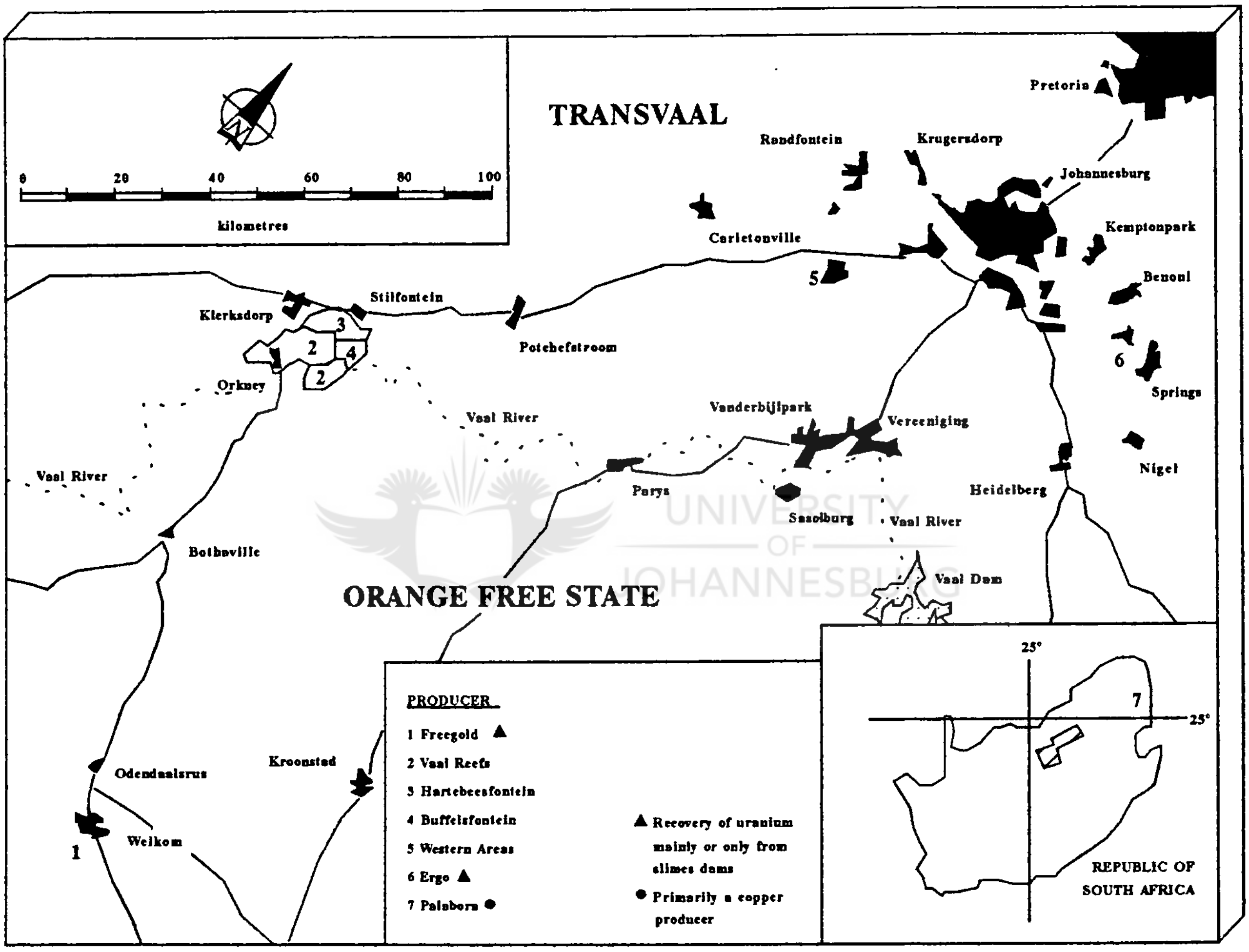


Figure 2.5 Uranium producing mines and recovery plants in South Africa (After Smit, 1989)

As a result of negotiations between USA and Russia, major nuclear defence conversions were made and the so-called "swords to ploughshare" attitude was adopted. This resulted in the conversion of 500t Russian highly enriched uranium (HEU) to low enriched uranium (LEU) by some american facilities. In addition, this move created a negative international market sentiment causing a depression of uranium spot prices (Harding, 1994).

In South Africa, these developments resulted in a U_3O_8 production decrease of 1464t (42%) since 1989. Currently, uranium is mainly produced by the Hartbeesfontein (329,4t U_3O_8), Vaal Reefs (1313,4t U_3O_8) and Western Areas (270,1t U_3O_8) mines. Smaller mines account for the remaining 94,8t U_3O_8 being processed (Chamber of Mines, 1993 ; Harding, 1994).

2.5 The extraction of uranium

The mineral uraninite is the chief uraniferous constituent of Witwatersrand ores. It usually occurs in the compact matrix of the conglomerates unless it is associated with carbon (Wittmann & Förstner, 1977).

Gold is frequently included in the uraninite and gold and uraninite appear to vary sympathetically, i.e. although they occur as individual grains they tend to occur together and distributed together (Jeppe, 1946 ; Liebenberg, 1972).

The uraniferous hydrocarbon which is described as thucolite (derived from the constituent elements Th-U-H-C-O) commonly includes minute crystals of uraninite. Sulphides are usually associated thucolite, but are often displaced by gold (Wittmann & Förstner, 1977). It was also recently found that the titanate uranium-bearing mineral brannerite, is most commonly encountered in the Western Witwatersrand (Feather & Koen, 1975).

The mineral uraninite has a variable composition, $xUO_2.yUO_3$. Whereas uranous oxide, UO_2, is only partially soluble in dilute mineral acids, uranyl oxide, UO_3, dissolves readily in dilute acids. Therefore, the complete dissolution of uraninite requires oxidizing conditions during extraction. Several oxidizing agents such as the ferric ion, sodium chlorate and manganese oxide (pyrolusite : MnO_2) are effective for the oxidation of uranium in the +IV oxidation state (e.g. UO_2) to the uranyl ion (UO_2^{2+}), which is the soluble species of uranium in the hexavalent state.

Pyrolusite, which is used in the extraction of uranium, occurs in association with the dolomites of Transvaal and is therefore a relatively cheap and freely available source of oxidant. The acid medium provided in the form of sulphuric acid is obtained by the roasting of pyrite, FeS_2 which is freely available in local gold ores (Funke, 1990).

The acid leaching of uranium from the milled ore entails the oxidation of uranium (IV) to uranium (VI) during the following reaction:

$$UO_2 + MnO_2 + 2H_2SO_4 \dashrightarrow UO_2SO_4 + MnSO_4 + 2H_2O$$

The recovery of uranium from the pregnant solution is done at a pH of 1,3. Uranyl sulphate is obtained by leaching low-grade uranium ores with sulphuric acid and is absorbed as the uranyl (UO_2^{2+}) ion on undissociated sulphide (UO_2SO_4) (Ayres & Westwood, 1957 ; Pryor, 1965). The acid oxidative leaching with steam injection to raise temperature and concentration of the dissolved uranyl sulphate is done in a series of flat-bottomed pachucas (Funke, 1990).

The separation of the pregnant solution from the barren slime is effected by the use of drum filters, horizontal belt filters, disc filters or counter-current decantation (CCD). This process is usually followed by sand-bed filtration.

For the acid leaching of uranium oxide, the uranyl ion is first leached from the slime by sulphuric acid/manganese dioxide and the pulp is then treated with lime and cyanide for gold extraction. This process is known as the reverse leaching procedure. In the forward leaching procedure, the gold is leached first, and the uranium afterwards (Wittmann & Förstner, 1977).

The final stage in the process is the recovery of the uranium from the OK liquor by the addition of NH_3 and subsequent precipitation of ammonium diuranate (ADU) or yellow cake (Funke, 1990). The ADU slurry containing about 30% U_3O_8 is sent to the Nuclear Fuels Corporation of South Africa (NUFCOR) where it is dried and calcined to produce a U_3O_8 concentrate of about 95%.

2.6 Chemical, physical and nuclear properties of uranium

The element Uranium, named after the planet Uranus which was observed only a few years earlier, was discovered in 1789 in pitchblende samples by the German metallurgist and chemist Klaproth.

Uranium, with an atomic number of 92 and an atomic weight of 238,04, is the fourth member of the actinide series. Uranium, a heavy (sg = 18,95), silvery-white metal is pyrophoric (ignites spontaneously and forms an oxide in the presence of oxygen) in finely divided form (Fisher, 1988 ; Burkart, 1991). It has a melting point of 1132 °C and a boiling point of 3818 °C. It is very electropositive (oxidation states range between +2 to +6), malleable, ductile and a poor conductor of electricity (Long, 1987 ; Fisher, 1988 ; Burkart, 1991). Uranium is insoluble in hot and cold water, alkalines and alcohols, but dissolves in acids (Long, 1987 ; Fisher, 1988).

Natural uranium occurs as a mixture of three isotopes, ^{234}U, ($t_{1/2} = 2,5 \times 10^5$ a : 0,0057%), ^{235}U, ($t_{1/2} = 7,1 \times 10^8$ a : 0,72%) and ^{238}U, ($t_{1/2} = 4,5 \times 10^9$ a : 99,27%). Isotopes of uranium with masses ranging between 227 and 240 are known (Long, 1987). Of all the isotopes of uranium, ^{235}U is the most important as it undergoes fission by slow neutrons and is used for power generation.

Uranium-238 has a long half life of 4,5 x 10^9 years. Because of its low specific (alpha only) activity, uranium itself is regarded as having a relatively low radiotoxicity. Its radionuclides, however, decay into a complex series of isotopes of the elements thorium, protactinium, radium, radon, polonium, bismuth, thallium and lead, some of which are significant sources of natural radiation in the environment, or in the human body, following its dietary intake (Long, 1987).

It is possible to identify individual members of the ^{238}U decay series (Figure 2.6). However, the series is generally not in isotopic equilibrium and therefore it is difficult to accurately predict the relative concentrations of the individual members. Uranium and radium present in soils leach at a much faster rate than thorium resulting in a disequilibrium. The formation of gaseous radon is also a major source of disequilibrium as its half-life is sufficiently long to allow a significant diffusion of it into the atmosphere. A disequilibrium therefore develops between the radon precursors and radon daughters (Figure 2.6).

2.7 Uranium technology

2.7.1 Commercial uses

Most natural uranium is mined as the fissionable properties of ^{235}U make it suitable as a principal fuel for nuclear power reactors Plöger & Vietzke, 1983; Saager, 1984; Fisher, 1988). Highly enriched ^{235}U has various military uses. It is either used as a bomb fuel for fission bombs or to ignite hydrogen bombs. Depleted uranium (^{238}U by-product of the uranium enrichment process) is used in other minor applications such as armour-piercing shells, ship ballast and counterweight for aircraft. It is also used as a negative contrast in electron microscopy, as tile glazes and as glass colourants.

2.7.2 Uranium waste products

Even though uranium has some technological applications, there is little commercial use for the radioactive decay product of this element. Radium-226, which is a highly radiotoxic decay product of uranium, was previously used in luminous paint on dials of watches. It was also used in radiotherapy where ^{226}Ra was applied in the treatment of tumours (Burkart, 1991). The daughter product of ^{226}Ra, ^{222}Rn, with a half-life of 3,8 days is still used today for local irradiation by applying minute tubes (seeds or needles filled with radon gas (Burkart, 1991).

Because of the low economic potential of ^{226}Ra and ^{222}Rn, the mining and milling of uranium produce large quantities of low-level radioactive waste which consists mostly of these radionuclides. Contamination of the environment may therefore be caused by the leaching of ^{226}Ra by rain water into drinking water or the emanation of ^{222}Rn from uranium mill tailings (Burkart, 1991). The use of mine tailings as building subsoil has also been shown to cause elevated and dangerous levels of ^{222}Rn and its decay products in indoor air (NCRP, 1984).

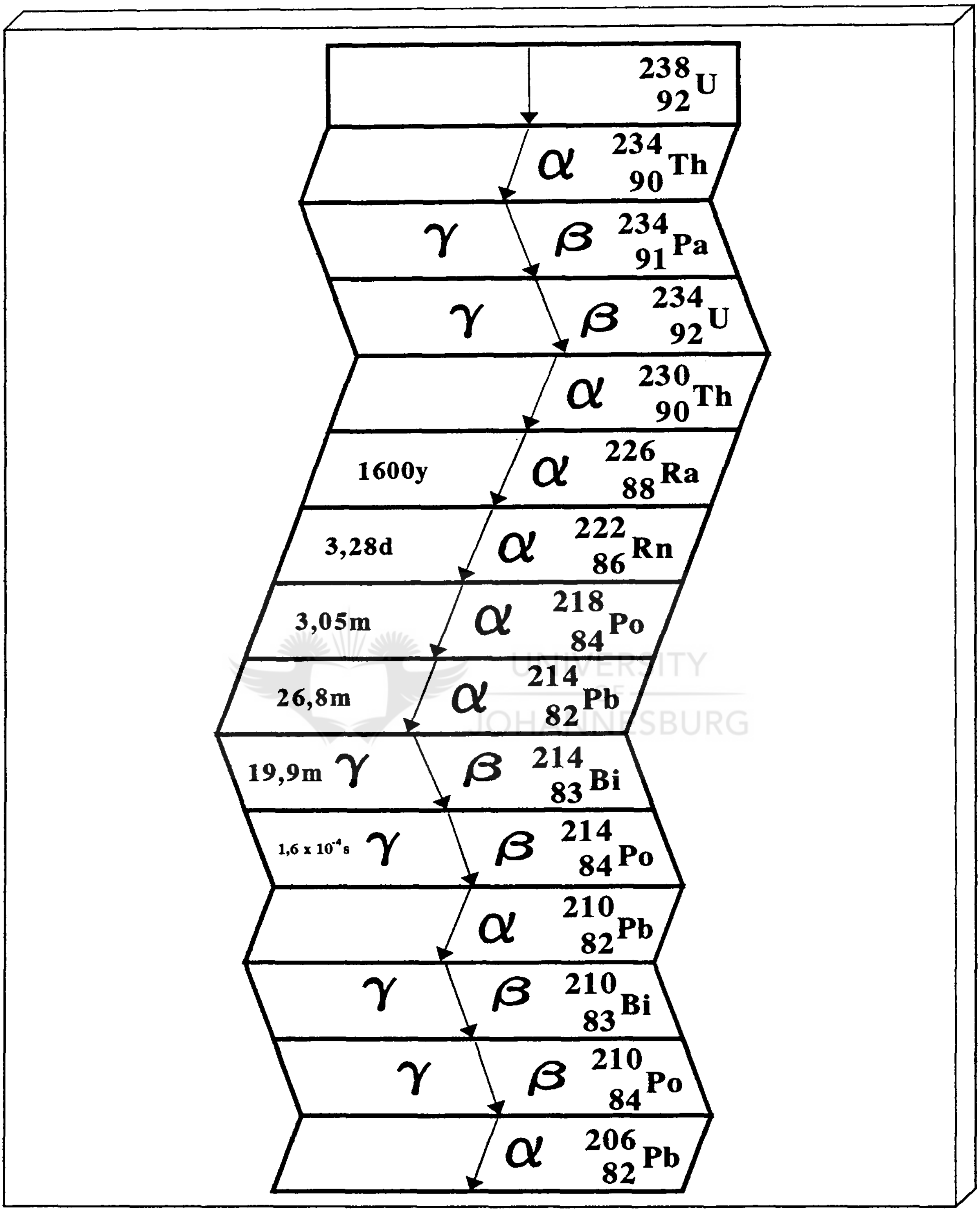


Figure 2.6 The U-238 decay series with an indication of emitted particles and waves as well as the duration of the half-lifes of several important radionuclides (After Malan, 1981)

2.7.3 Occupational exposures to uranium

Workers in the uranium-processing industry are potentially most likely to be exposed to uranium compounds. The commercial uranium fuel cycle usually involves the following steps: the mining of ore, refinement or milling of uranium oxide of uranium oxide (yellowcake: U_3O_8 or $(NH_4)_2U_2O_7$), fluorination of yellowcake to UF_6, isotopic enrichment to increase the relative content of ^{235}U and uranium oxide fuel production (Fisher, 1988). The inhalation of airborne uranium dusts is the primary cause of occupational exposure. Situations which are known to have the greatest potential for intake of uranium compounds are, amongst others, the packaging of yellowcake in uranium mills, the unloading of barrels containing and filling containers with UF_6 at conversion plants, all areas of enrichment plants as well as the power and pellet production areas of fuel fabrication facilities (Stoetzel *et al.*, 1981). Grinding, machining or incineration of uranium metal oxides may also potentially present a threat to workers, as they are exposed to uranium aerosols Fisher, 1988).

2.7.4 Potential health hazards

The kidney, which is the major route of excretion in the human body, is the critical organ in terms of uranium toxicity (Long, 1987; Fisher, 1988). The intake of uranium by man may therefore cause damage to renal proximal tubules resulting in nephritis. Tissue damage and a loss of renal function is also marked by morphological changes, enzymuria, glucosuria and an increased excretion of amino acids and small proteins (Thun *et al.*, 1985). Severe exposure to uranium may result in albuminuria and acute renal failure (Blantz, 1975).

2.8 Uptake, metabolism and physiology of uranium in plants, animals and humans

2.8.1 Effects on micro-organisms and plants

According to Burkart (1991), no information exists on the chemotoxic or radiotoxic effects from natural and industrial sources of U, Th and their decay products on micro-organisms or plants.

However, Wildung and Garland (1974) suggest that in the case of transuranic elements, toxicity towards plants does occur in the case of plutonium, americium and neptunium. These authors found that the concentration ratio for uptake of plutonium by barley decreased by an order of magnitude when the soil concentration of $^{239+240}Pu$ increased from 0,05 to 10 $\mu Ci.g^{-1}$. This phenomenon may therefore indicate toxic effects of the plutonium causing selective inhibition of uptake, or it may suggest a concentration dependent change in the availability of Pu in the soil.

Garland *et al.* (1974) also found that the seed production of soyabeans may be inhibited about 70 days after the application of $^{239+240}Pu$. Studies done by Cline (1968) further suggest that genetic damage occurred in pea seedlings during a toxicity study of ^{241}Am relative to ^{239}Pu. These radionuclides were applied at a concentration of 0,1 mCi of nuclide per litre of solution. The results of this experiment was that the seedling radicles grew about 1 cm following ^{241}Am treatment, but with no secondary root formation. No mitotic activity was also observed in the root tissue. Pea-seedlings treated with ^{239}Pu showed diminished cell division for about one day, but nearly normal secondary root growth occurred (Cline, 1968). The latter author ascribed the phenomenon to the solubility differences based on the specific activities of the radionuclides, but Cline (1968) suggests that a greater uptake increased toxicity of ^{241}Am could also have been caused by the differences in chemical behaviour of these two isotopes.

2.9 Physiology, biokinetics, metabolism, nephrotoxicity and acute effects of uranium in humans

The chemical form and solubility in body fluids of uranium determines the biokinetics and metabolism of this element in man (Scott, 1973). Soluble compounds such as UF_6, $UO_2(NO_3)_2$, UO_2CL_2, UO_2F_2, and uranyl acetates, sulphates and carbonates are highly transportable from the lung to other parts of the body. The compounds UF_4, UO_2, $UO_4(NH_4)_2U_7$, UO_3 and uranyl nitrates are moderately transportable in the human body, while U_3O_8 and uranium hydrides and carbides are mostly insoluble and only slightly transportable (Morrow *et al.*, 1966). According to Kalkwarf (1979), uranium oxides usually contain mixed fractions of soluble and insoluble constituents. Research by the ICRP (1966) also reveals that particle size of uranium aerosols plays a definite role in lung deposition. The clearance or removal of uranium from the lungs to the body may occur in the following manner: mucociliary particle transport into the gastrointestinal (GI) tract, absorption into blood draining the lungs, macrophage phagocytosis or by removal by the lymphatic system. Inhaled insoluble uranium compounds may remain in the lungs and the tracheobronchial lymph nodes for years after inhalation (ICRP, 1979a-c).

Biologically soluble uranium is absorbed into the blood from where it is filtered out by the kidneys and excreted in urine. A certain amount of uranium present in the bloodstream is also taken up by the skeleton where it first deposits on the bone surface, but later redistributes in the bone volume (Bernard & Struxness, 1957).

Uranium also tends to deposit in soft tissues in the body, especially in the kidneys. The metabolism and excretion of hexavelant uranium by the kidney has been described by Morrow *et al.* (1982). Complexed U(VI) is filtered from the blood by the glomeruli of the kidney whereafter the uranium concentrates in the proximal tubule, as water is resorbed. The increased acidity caused by the presence of U(VI) breaks the complex down, and U(VI) recombines with proteins or epithelial tissue. The U(VI) is then either retained in the kidney or is excreted in the urine. Figure 2.7 illustrates the main pathways of soluble U-compounds in the human body. Also shown are the biological half-lifes in critical areas (Burkart, 1991). Hodge (1973) has thoroughly described the renal toxicity of uranium in man and animals. In as little as 24 hours after acute exposure to uranium, kidney damage may be evident (Yuile, 1973). Due to kidney damage, water consumption increases and urinary output may multiply by as much as a factor of 5. According to Morrow *et al.* (1982), tissue damage caused by the presence of uranium may be indicated by failure of the kidneys to resorb urinary protein, glucose, catalase, phosphate, citrate and creatinine.

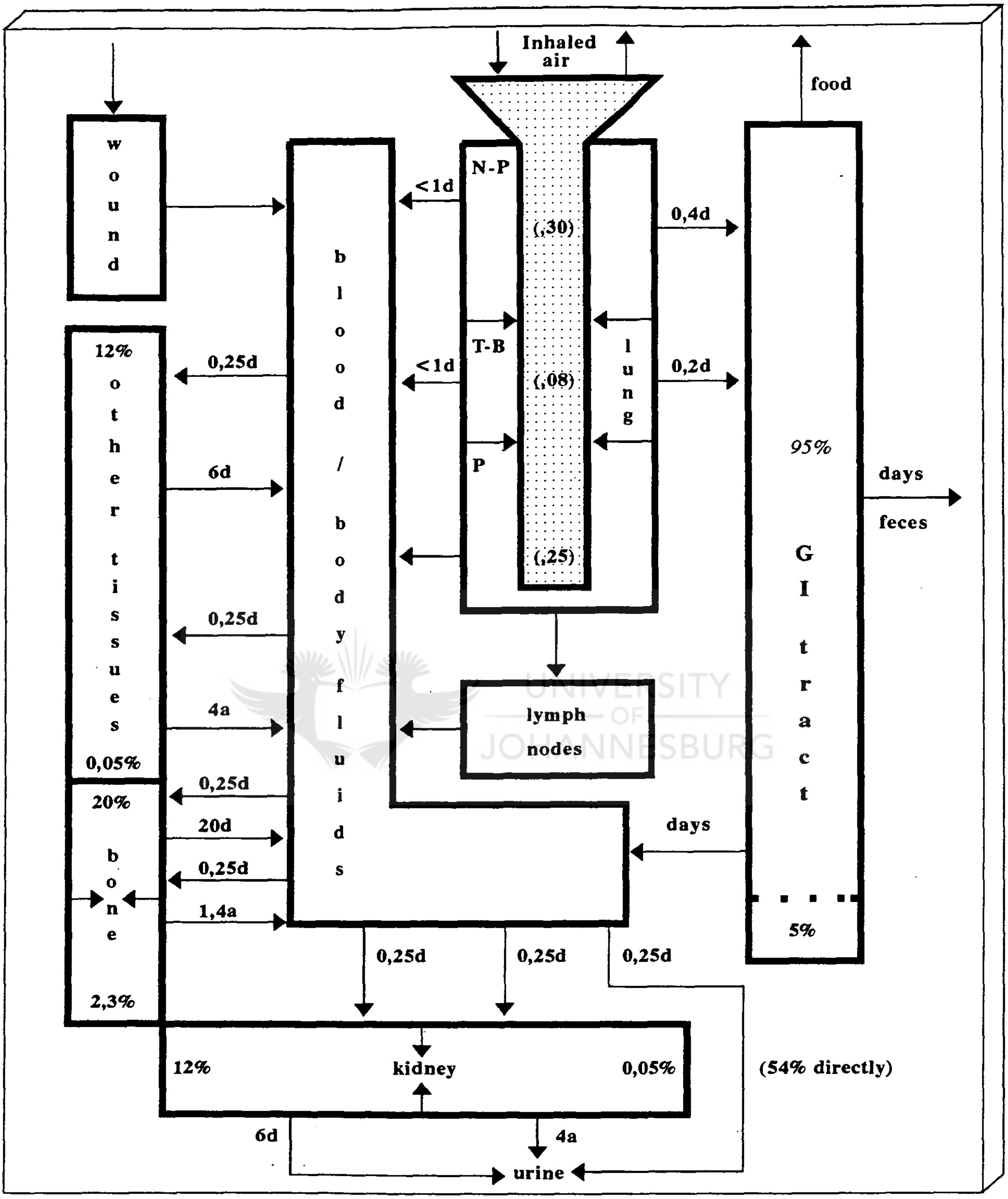


Figure 2.7 **Body compartments, pathways and half-lifes of transfer for soluble uranium (After Burkart, 1988)**

Numbers in lung air space represent fractions of inhaled activity retained in the different areas for 1μm-diameterparticles

Explanation: N-P: naseopharyngeal, T-B: tracheobronchial, P: pulmonary

For percent values in tissues, the fraction reaching the blood is set at 100%

Acute or lower chronic dosages of soluble uranium compounds may also cause the death of proximal renal tubule cells. Histological examinations of the kidney by Yuile (1973) also show that multiple sites of uranium-induced disfunction of the kidney involve both the glomeruli and renal tubule cells.

According to Hodge (1973), high doses of uranium exposure to man also has an effect on the blood vasculature throughout the body. Because of the effects of uranium on blood chemistry, capillary permeability, blood pressure and edema may increase while the clotting ability of blood may decrease. Minor damage in liver and muscle tissue may also be induced by uranium. The effects of uranium on the nervous system may be similar to those from poisoning by other metals (Yuile, 1973).

During the experimental application of UF_6 to rats and dogs it was found that total urinary output and glucose and protein excretion declined towards the 14th day following the exposure to uranium (Morrow *et al.*, 1982). This suggested a possible repair process and regeneration of the tubules. However, these authors suggest that the injury to the kidney tissues is not necessarily transient or reversible.

The exposed animal will not survive if a critical mass of kidney cells die as a result of exposure. In the case of a non-critical exposure, damage kidney cells are replace by atypical new cells which lack the functional ability of the original cells (Wrenn *et al.*, 1985). Morrow *et al.* (1982) found that after acute exposure, some kidney function appear normal, but tolerance by recovery was not uniformly observed in animal experiments which indicated that the kidneys of the exposed animals may have remained impaired.

2.9.1 Mutagenic, carcinogenic and teratogenic effects

All substances known to emit ionizing radiation are considered as potentially mutagenic and carcinogenic. Teratogenic affects are induced at high doses of ionizing radiation (Coggle, 1983). These may also result in developmental defects leading to impaired brain functions such as severe mental retardation. A case which clearly illustrates this phenomenon was the occurrence of severe mental retardation among the prenatally exposed survivors of the atomic explosions as Hiroshima and Nagasaki during the second world war (Otake *et al.*, 1987).

Although uranium is known to be chemotoxic and radiotoxic, the unstable decay products of this element are more important both in the occupational and non-occupational environments as they are major contributors to the total radiation dose (Burkart, 1991). These decay products (^{226}Ra, ^{222}Rn, ^{210}Pb, ^{210}Po) only produce radiological effects. Due to its alkaline earth behaviour, ^{226}Ra in drinking water leads to its exposure to the human skeleton (Burkart, 1991). In the workplace, a number of cases of bone and head sarcomas occurred in persons in the radium-dial painting industry (Rowland *et al.*, 1978). The decay product of ^{226}Ra, ^{222}Rn as well as its short-lived decay products are mostly found in mines and the indoor environment where ^{222}Rn occurs as a gas. The presence of ^{222}Rn decay products in mines and the occurrence of lung cancer amongst mine workers are strongly correlated. This form of lung cancer is currently the best quantified occupational disease (Thomas *et al.*, 1985 ; McMichael, 1989).

2.9.2 Other toxic effects

According to Hodge (1973), high doses of uranium exposure to man also has an effect on the blood vasculature throughout the body. Because of the effects of uranium on blood chemistry, capillary permeability, blood pressure and edema may increase while the clotting ability of blood may decrease. Minor damage in liver and muscle tissue may also be induced by uranium. The effects of uranium in the nervous system may be similar to those from poisoning by other metals (Yuile, 1973).

2.10 Tolerance levels and limiting concentrations of uranium

2.10.1 Normal daily intake and assessment of uranium levels in food groups

The average dietary intake of uranium from foods and water is about 1,75 μg.day^{-1}, while the population-weighted average for drinking water in the United States is 1,2 μg U.L^{-1} (Wrenn *et al.*, 1985).

Hamilton (1972) determined the uranium content of a variety of foodstuffs representative of the United Kingdom diet including cereals, vegetables, meat, eggs, fish and milk (Table 2.3). The average intake (g/person/day) is also represented while annual intake (μg) and the percentage annual intake was also calculated. Results show that starchy roots (138 μg : 35%) represent the biggest portion of annual intake of uranium, while items such as eggs (3 μg: 0,9%), fish (3 μg : 0,08%) and milk (2 μg : 0,5%) provided the lowest portions of annual intake.

In an extensive environmental impact study of a uranium mine in Okayama, Japan, Morishima *et al.* (1977) determined uranium concentrations in leafy and root vegetables (Table 2.4). These results showed that the uranium content of leafy and root vegetables was significantly higher than those of berries and grains.

A comprehensive investigation on the uranium content of human dietary items was conducted by Welford & Baird (1967). This study was primary aimed at establishing typical levels of uranium intake by man and was conducted for typical diets in New York City, Chicago and San Francisco (Table 2.5). These results showed that although there where geographical variations in uranium content of individual foods, the total annual intakes for the three cities were nearly similar. Milk made a large contribution towards the uranium intake of San Francisco, while there were considerable variations in the uranium content of flour and shellfish in all three cities. More than seventy percent of the annual intake of foodstuffs came from potatoes, meat, fresh fish and bakery products. Based on these figures, the Calculated daily intake of uranium was 1,3 μg.day^{-1} for New York, 1,4 μg.day^{-1} for Chicago and 1,3 μg,day^{-1} for San Francisco. It should also be noted that U is regarded as 2 million times less radiotoxic than ^{226}Ra (Williams, 1984).

Table 2.3 Concentration of uranium in individual diet items (After Hamilton, 1972).

Item	Uranium (ng.g^{-1})
Tea	5
Coffee	6
Parsley	60
Thyme	90
Pepper (red)	5
Pepper (white)	0,2
Mixed spice	30
Paprika	20
Ginger	40
Cloves	8
Taragon	30
Beef extract	20
Mustard	0,2
Salt (table)	40
Salt (cooking)	40
Salt (iodized)	40

Table 2.4 **Concentration of uranium in leafy and root vegetables (After Morishima *et al.*, 1977).**

Vegetable	Mean concentration ($\mu g.g^{-1}$)
Spinach	0.81 / 0,24 / 0,10
Scallion	0,29 / 0,088 / 0,11
Cabbage	0,055 / 0,066 / 0,081
Carrot	0,54 / 0,033
Radish	0,061 / 0,04
Turnip	0,086 / 0,017
Potato	0,19 / 0,14 / 0,052
Sweet potato	0,029
Tomato	0,062 / 0,13
Egg plant	0,088 / 0,11
Pumpkin	0,041 / 0,063 / 0,017
Cucumber	0,087 / 0,057
Watermelon	0,2
Fruits	0,043
Rice	0,0053 / 0,02
Wheat	0,049

Uranium concentration expressed as $\mu g.g^{-1}$ of ash.

Table 2.5 **Annual intake of uranium through various agricultural crops from New York, Chicago and San Francisco. (After Welford & Baird, 1967).**

Group	Uranium intake $\mu g.year^{-1}$		
	New York	Chicago	San Francisco
Bakery products	66	58	58
Grain products	16	16	16
Eggs	3,4	3,4	3,4
Fresh vegetables	25	25	44
Root vegetables	12	9,4	12
Milk	16	31	62
Poultry	2,7	8,4	5,6
Fresh fish	3,4	6,8	4,5
Flour	23	14	8,4
Macaroni	1,2	1,4	1,9
Rice	5,8	18	4,3
Meat	79	104	46
Shellfish	9,5	29	31
Dried beans	4,5	11	11
Fresh fruit	76	76	42
Potatoes	111	101	101
Canned fruit	4,2	6,4	4,0
Fruit juices	1,1	1,1	3,4
Canned vegetables	4,0	2,0	2,0
Total	**463,8**	**522,9**	**462,5**

Figures based on average food consumption (USDA).

2.10.2 Toxicity threshold to uranium

Although little data is available on humans, extensive research on animals has been conducted on the threshold tolerance limits for exposure to uranium. The renal injury threshold is estimated at 10 μg U (as UO_2F_2).kg^{-1} (body weight) for dogs and about 100 μg U.kg^{-1} for rats (Morrow *et al.*, 1982). The threshold level for man is therefore estimated to be between that for rats and dogs, that is about 70 μg.kg^{-1} (body weight). This estimated value corresponds to a renal injury threshold concentration value of 16,3 μg U.g^{-1} kidney tissue in man. However Morrow *et al.* (1982) found that a steady-state concentration of 3 μg UO_2F_2.g^{-1} (tissue) was sufficient to produce the typical steady-state symptoms of uranium poisoning in animal experiments. These results therefore raised questions on the suitability of currently recommended exposure limits.

The toxicity of uranium varies according to its compound form. Insoluble uranium compounds are the least toxic, increasing with chemical solubility. According to Morrow *et al.* (1982), the most toxic uranium compound in UO_2F_2, possibly due to additive effects of exposure to HF which originates from the original uranium compound in solution.

2.11 Historical introduction and overview of radium

2.11.1 Physical, chemical and nuclear properties of radium

Radium (L. radius : ray), element 88 is a radioactive alkaline earth metal belonging to group 11A of the periodic table (Williams & Kirchmann, 1990; Molinari & Snodgrass, 1990). The atomic weight of radium is 226,05 and the electronic configuration of the Ra-core is $7s^2$ (Drew, 1972). The ionic radius of radium is 1,40 Å with a melting point of 700°C and boiling point of 1150°C. At 20°C, the specific gravity of radium is 6,0. Pure radium metal is brilliant white but blackens in air. It is prepared by the electrolysis of a fused radium salt. Both radium metal and radium salts exhibit strong luminescent properties (Moore, 1972 a,b,c).

Altogether twenty-five isotopes of radium have been identified. The most abundant of these naturally occurring isotopes are ^{226}Ra(^{238}U daughter : α-emitter : half-life of 1622 years) and ^{228}Ra (^{232}Th daughter (mesothorium) : β-emitter : half-life of 5,8 years) (Williams & Kirchmann, 1990).

2.11.2 Medical and industrial uses of radium

In 1898 Pierre and Marie Curie discovered the new element polonium and "a new element with very curious properties" (Curie *et al.*, 1898) which was found to be nine hundred times (later found to be over a million times) more radioactive than uranium. It was also found to be chemically similar to element 56 in group IIa, barium. This element was named "radium" after the term "radioactivity" which was used to describe the spontaneous disintegration of matter.

Radium became a very useful element. It was utilized as a source of radioactivity for industrial and medical radiography. It also provided very valuable information about radioactivity and the structure of matter. In the field of medicine, radium was especially used in the treatment of cancer. Medical doctors also recommended radium treatment for most incurable diseases like arthritis, neuritis, poliomyelitis, hypertension, menopausal complaints, Hodgkins disease, debutantes fatigue and dementia praecox (Evans, 1933). A medical doctor claimed that radium cured up to 160 known diseases while another cured "all forms of cancer" (Williams & Kirchman, 1990). Radium-containing products such as bath- and drinking water, tonics, tissues, facial creams, salves, hair tonics, mouthwashes, tooth pastes, candy bars and fertilizers were sold to the public (Williams, 1990).

2.11.3 Realizing the dangers, health hazards and environmental effects of radium

Although the applications of radium provided a great benefit to medicine and industry, the hidden dangers of this element soon became apparent. Following the subsequent illness of Marie Curie after handling quantities of this element, a New York dentist in 1924 described a disease in a luminescent dial painter as "radium jaw". This was followed by a description of anaemia resulting from occupational poisoning (Martland *et al.*, (1925) and five deaths from "jaw necrosis" in a factory (Flinn, 1926). Following these deaths and many other radium poisoning related cases, the International Committee on X-Ray and Radium Protection, the forerunner of the present ICRP (International Commission on Radiological Protection) was established. At this time (1940), only 1,4 kg of radium has been extracted from the earth, but it was responsible for the deaths of more than one hundred people (Williams & Kirchmann, 1990).

A few years later after the discovery of radium in uranium ores, it was commonly found in the environment. Tsivoglou *et al.* (1958) were the first to identify radium as an environmental pollutant during investigations in the Colorado Plateau area in the USA. Since similar reports by Havlik (1970) in Czechoslovakia and Kirchmann *et al.* (1973) in Belgium, radium contamination of the aquatic and terrestrial environment originating from uranium, gold and phosphate mining and milling has been identified in many areas over the world including South Africa (Malan, 1981 ; de Jesus *et al.*, 1987).

2.12 Biological uptake and transport of radium by aquatic plants and animals

2.12.1 The mechanisms of radium intake

Due to the similar chemical properties of radium and calcium as well as the other alkaline earth elements such as Mg, Sr and Ba, it can be anticipated that radium may be taken up by cells via the same mechanism as the latter elements (Williams, 1984). Accordingly there could be a correlation between radium uptake across cell membranes and/or its distribution within tissues with that of calcium (Williams, 1984).

According to the ICRP (1975), 99% of the body calcium in man is distributed in the skeleton. The relative proportions of the other elements are: Sr:99% ; Pb:92% ; Ba:91%

; P:90% : Ra:87% ; Mg:58%. All the other elements known to be in the human body occur in lower portions in the skeleton. Fish also have a relatively high proportion of body radium in the skeleton (Anderson *et al.*, 1963), whilst freshwater mussels accumulate Ra with Ca, Mg, Ba, Mn, Fe and P in granular form which are then distributed throughout the body. According to Jeffree (1990), there is a positive correlation between the Ra concentration and Ca and Ba concentrations in mussels. Ra, however, is not correlated with Mg-concentrations.

This should however not be confused with the inverse correlation which exists between the Ca concentration of the organism's medium and the radium uptake. Various studies (De Bortoli & Gaglione, 1972 ; Jeffree, 1990) indicated that the uptake of radium is suppressed by a high calcium concentration in the organism's medium.

Radium in plants presents a somewhat different situation. Investigations conducted by Williams (1982) showed that among 40 samples of aquatic and semi-aquatic macrophytes which represented 19 species, there was no significant correlation between the distribution of Ra and the elements Ca, Mg, Na, Cu, Zn, Fe and S. However a positive correlation existed between Ra and Mn concentrations.

There are three major physico-chemical mechanisms of mineral uptake (including radium) by living organisms, namely:

- adsorption (a surface process)
- absorption (a metabolically controlled process)
- association (a structural factor) (Williams, 1984).

Investigations by Havlik (1971) showed that in 5 out of 7 species of unicellular algae more radium was accumulated by adsorption than by absorption. This may be ascribed to a high surface area-to-volume ratio of unicellular algae and a strong adsorption capacity of radium (Benes, 1984). In higher organisms the surface area-to-volume ratio is smaller and the distribution between organ more important. Absorption of minerals by the higher organisms is therefore the dominant process (Williams, 1984). Uptake of radium by association occurs where contaminated material is associated with a food item without being taken up by it (Williams, 1984). For example, sediment accumulates on the structural surfaces (roots, rhizomes, stems) of submerged aquatic macrophytes and semi-aquatic macrophytes and therefore radium present in this sediment can become part of a herbivorous diet simply by association. A similar situation exists where animals with digestive systems may carry undigested material containing radium in their guts. Even though the radium present in the gut may be discriminated against in the digestive process, it still remains incorporated in the diet of any predator which may feed on this animal (Williams, 1984).

2.13 Concentration ratios

Available published data on the biological uptake of radium consists mostly of field survey results where the radium concentrations of the diet items [Q] is compared to that of the supporting water medium or sediment [C]. From this, the Concentration Ratio (CR) or Bio-concentration Factor (BF) for radium can be calculated:

$CR = [Q] / [C]$ -----------(1)

According to the ICRP (1979a-c) and USNRC (1980), a possible linear relationship exists between [Q] and [C] (CR is independent of [C]). In the expression of results on the Ra uptake by algae, aquatic vascular plants, insects, fish and mussels the data are usually assembled in log[Q] vs. log[C] plots and the linearity of the graph tested by comparing the slope of the line against unity using the standard error of regression.

Organisms like fish which are exposed to radium via water and food, and aquatic macrophytes which are exposed via water and sediments, are examples of organisms which take up this element from either or both of the two sources. If the concentration ratios are therefore independent of the concentrations on both exposure sources, then:

$[Q] = a_1[C_1] + a_2[C_2]$ ----------(2)

A multiple regression of [Q] on $[C_1]$ and $[C_2]$, constrained through the origin of the graph, can facilitate the determination of the two concentration ratios, a_1 and a_2. This model has an important application to irrigation. Using this model, Myttenaere *et al.* (1969) was able to demonstrate that the transfer of Cs and Co to rice from irrigation water was 10-100 times greater than from soil. However, Williams (1981) states that there is insufficient data on radium available to describe its transfer during irrigation.

In the following subsections (algae, etc.), the goodness of fit of the regressions was estimated by the coefficient of determination r^2 which was expressed as a percentage. This provided an indication of the percentage of the variance that was accounted for by the regression. The statistical significance of the regression was taken from the square root of the coefficient of determination, which was the correlation coefficient, r. The significance was shown as a p value, being the probability that the r value occurred by chance (Williams, 1984)

2.13.1 Algae

Data from the literature presented in Figure 2.8 covers a concentration range of 7 orders of magnitude. A very good fit to the following regression was obtained:

$[Q] = 441[C]^{1,02}$ ----------(3)
With: $r^2 = 94\%$ $(p << 0,001)$

The value of the regression coefficient (b = 1,02) is not significantly different from 1. it can therefore be concluded that uptake of Ra from water by algae is a linear process (Williams, 1984). The equivalent linear regression therefore is:

$[Q]a = 432[C]$ ----------(4)

and the concentration ratio is:

$CR = [Q] / [C] = 432$ ----------(5)

2.13.2 Aquatic vascular plants

Aquatic vascular plants (macrophytes) obtain radium from the surrounding water (w) and from the underlying sediment(s) via the roots. The relationship between foliage and sediment is illustrated in Figure 2.9 (Williams, 1984). The regression of this relationship is:

$[Q] = 0{,}39[C_s]^{0{,}49}$ ----------(6)
With: $r^2 = 22\%$ and $p < 0{,}001$

Figure 2.10 illustrates the relationship between foliage and water and the relationship is:

$[Q] = 81[C_w]^{1{,}21}$ ----------(7)
With: $r^2 = 50\%$ and $p < 0{,}001$

The regression coefficient, $b = 1{,}21$ is not significantly different from 1 and the corresponding linear equation is therefore:

$[Q] = 55[C_w]$ ----------(8)

The two-source model (2) can be fitted to this data and regression is therefore:

$[Q] = 8{,}56[C_w] + 0{,}014[C_s]$ ---------(9)
With: $r^2 = 51\%$ and $p = 0{,}01$ for a1 : 0,02 for a^2 $(N = 37)$

This suggested model is linear, but does not take into account the non-linearity as seen in Figure 2.9 for the plant-sediment relationship. According to Williams (1984), this model suggest that at an average water concentration of 0,21 Bq.1^{-1}, and an average sediment concentration of 94 Bq.kg^{-1}, 58% of the radium will be contributed by the sediment (Williams, 1984), is a well known fact that total sediment concentration of an element, $[C_s]$, is not a good index of the amount actually available to plant roots. Therefore, the non-linearity if Figure 2.9 may be due to the physical and chemical differences between the natural and effluent streams from which material was collected (van Dorp *et al.*, 1979).

2.13.3 Insects

Data collected for aquatic insects from field surveys (Figure 2.11) give a good fit to the regression:

$[Q] = 186[C]^{0{,}09}$ ----------(10)
With: $r^2 = 81{,}2\%$ $p << 0{,}001$

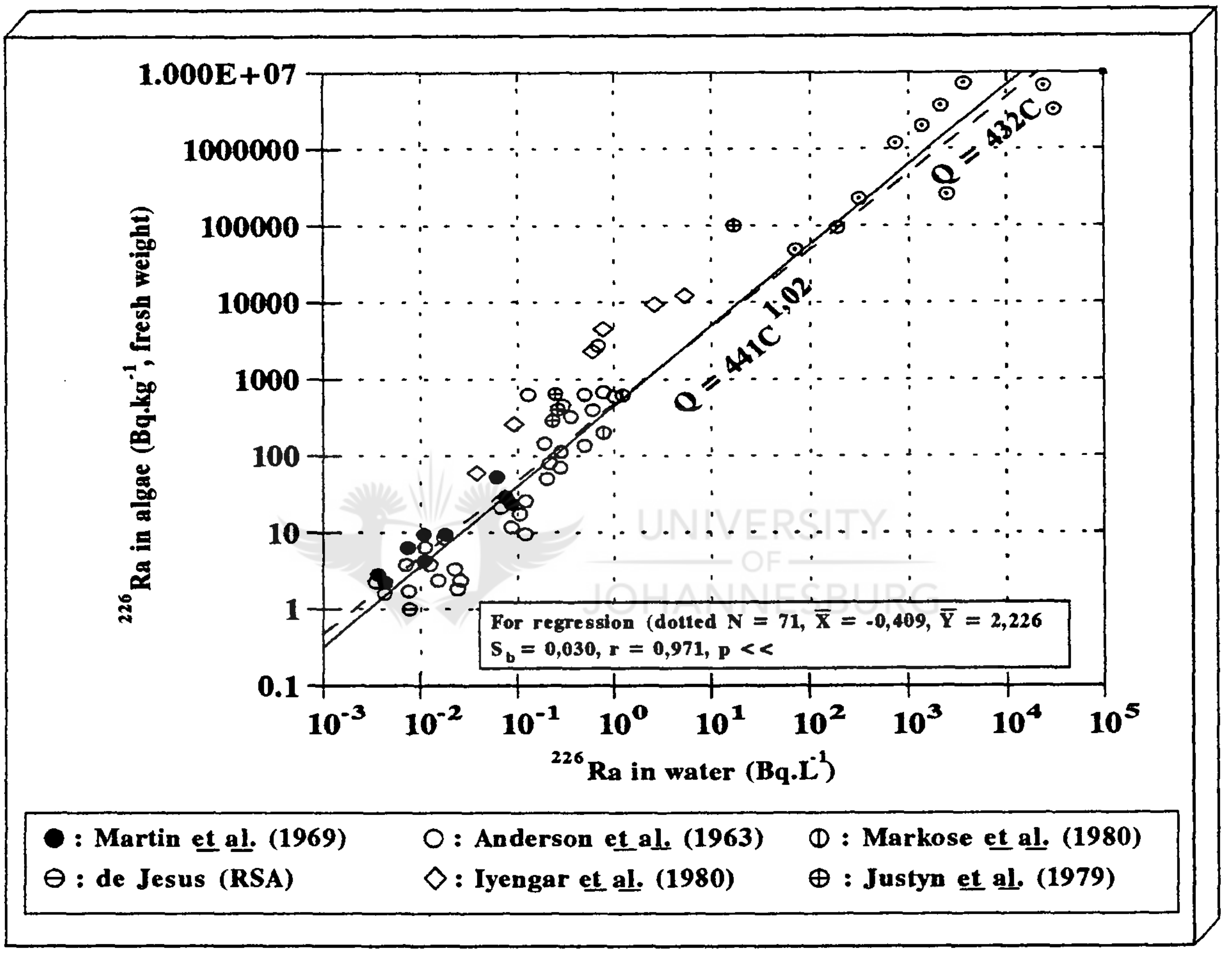
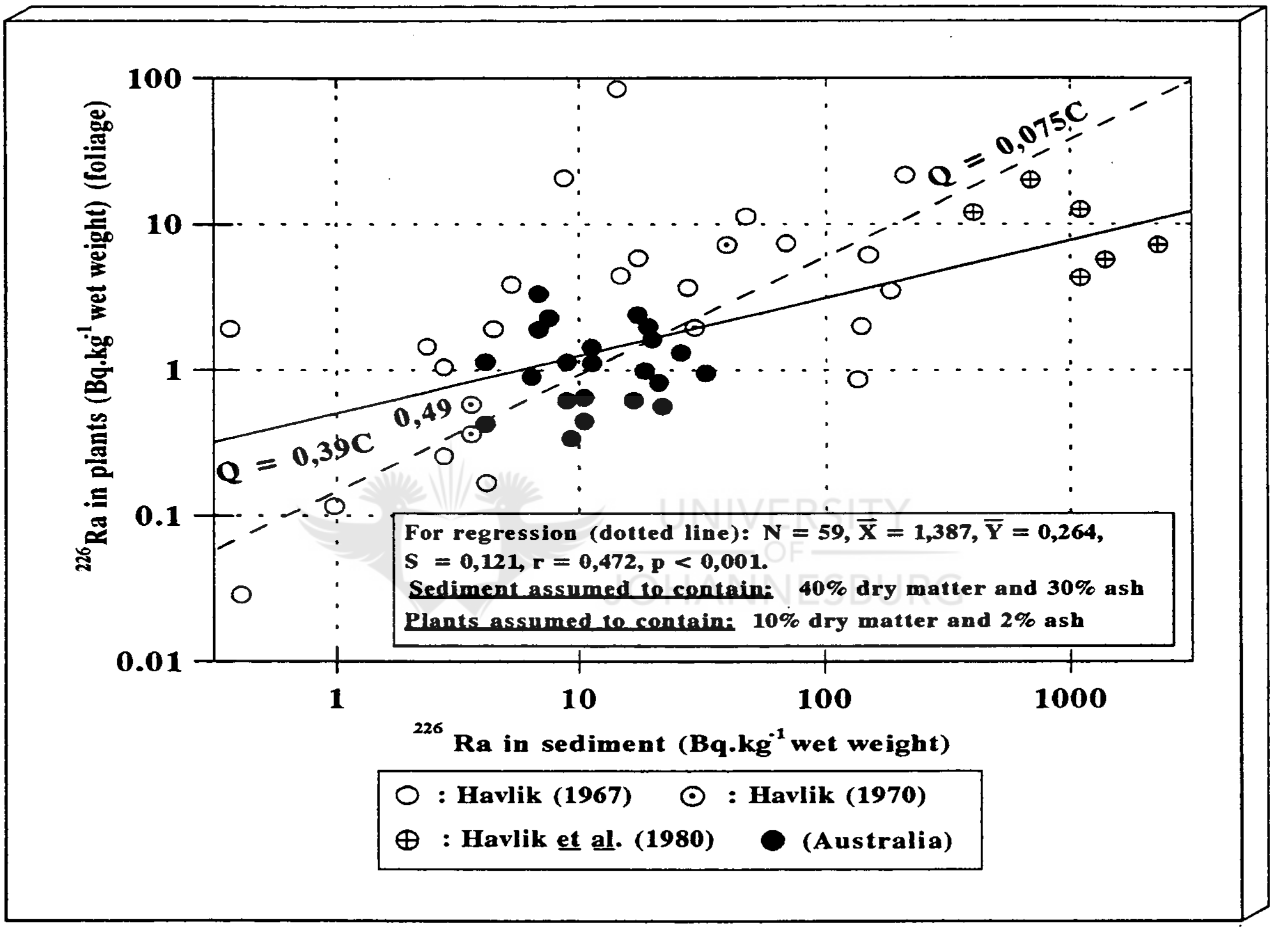


Figure 2.8 **Relationship between radium concentration in algae and in surrounding water (After Williams, 1984)**



For regression (dotted line): N = 59, $\overline{X}$ = 1,387, $\overline{Y}$ = 0,264, S = 0,121, r = 0,472, p < 0,001.

<u>Sediment assumed to contain:</u> 40% dry matter and 30% ash

<u>Plants assumed to contain:</u> 10% dry matter and 2% ash

Figure 2.9 Relationship between radium concentrations in aquatic macrophytes and in supporting sediments (After Williams, 1984)

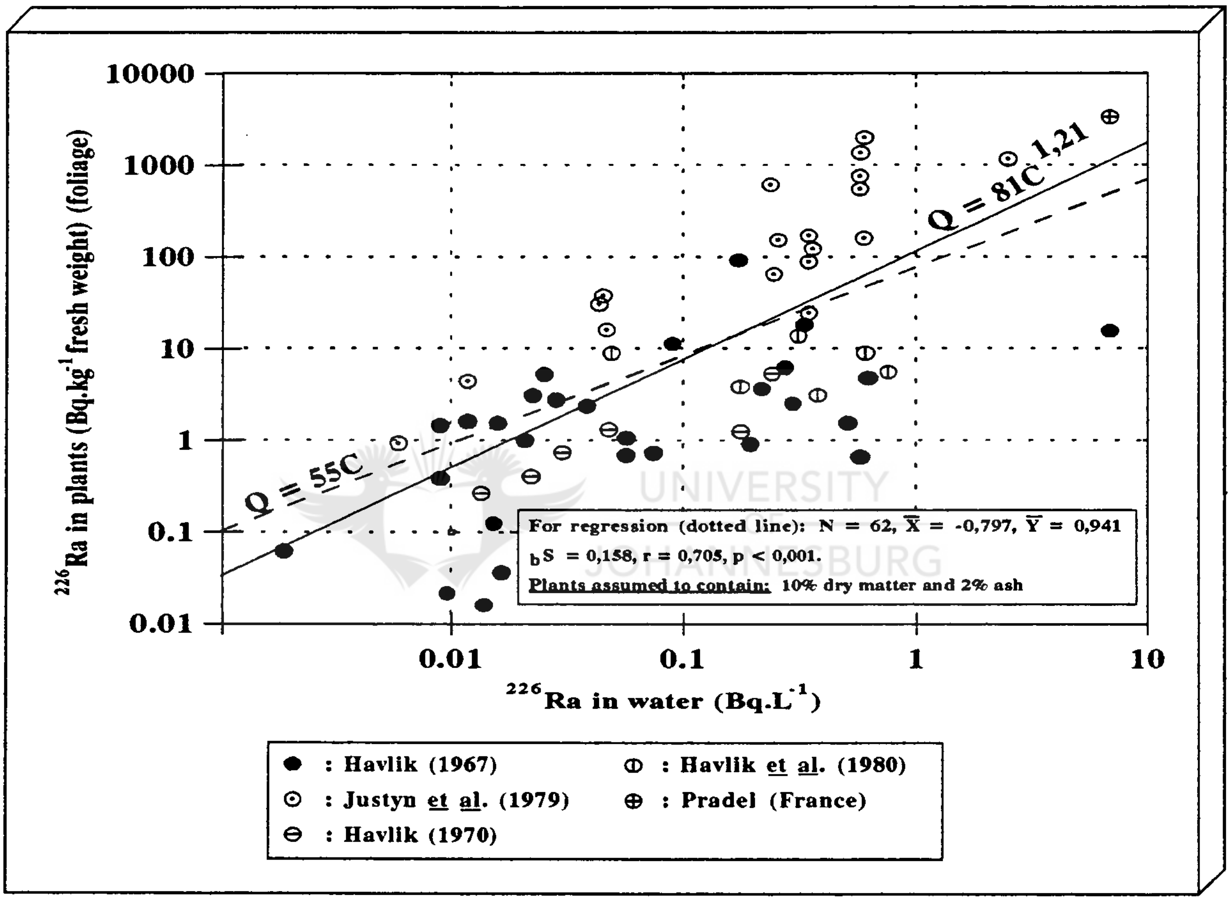


Figure 2.10 Relationship between radium concentrations in aquatic macrophytes and in supporting water (After Williams, 1984)

Because the regression coefficient ($b = 0{,}91$) is not significantly different from 1, the corresponding linear regression model is:

$[Q] = 247[C]$ ----------(11)

The concentration ratio is therefore 247 and it can be assumed that the uptake process for insects is linear if the radium concentration ranges between 10^{-2} to 10^{2} Bq.1^{-1}.

2.13.4 Fish

Data for fish collected from field surveys and/or field experiments give a good fit to the regression:

$[Q] = 13[C]^{0{,}068}$ ----------(12)
With: $r^2 = 54{,}5\%$ $p << 0{,}001$

However, the regression coefficient ($b = 0{,}68$) is significantly different from 1 ($p < 0{,}001$). The concentration ratio is therefore:

$BF = [Q] / [C] = 13[C]^{-0{,}32}$ ----------(13)

According to Williams (1984), the function varies from 119 at 0,001 Bq.1^{-1} to 13 at 1 Bq.$\ell 1^{-1}$ and is therefore highly non-linear (Figure 2.12). The different intercepts and slopes of the pooled data sets are due to the variation in the diets and behaviour of the different fish species. Pooling is however justified on the grounds that a significant general trend is produced and that the variation in some data sets spans the whole range (Davy & Conway, 1974 ; Davy & O'Brein, 1975).

2.13.5 Mussels

The freshwater mussel ***Velesunio angasi*** is a significant dietary item for the Aboriginal people in the Arnhem land region of Northern Australia. According to Fox *et al.* (1977) this area is developed for the large scale mining of uranium. Figure 2.13 illustrates available field data for this species. The best-fit regression is:

$[Q] = 203[C]^{0{,}78}$ ----------(14)
With: $r^2 = 39{,}2\%$ $p < 0{,}001$

The regression coefficient ($b = 0{,}78$) is not significantly different from 1 and therefore the equivalent linear model is:

$[Q] = 347[C]$ ----------(15)

According to Williams (1984), this mussel species is apparently second only to algae in its ability to accumulate radium.

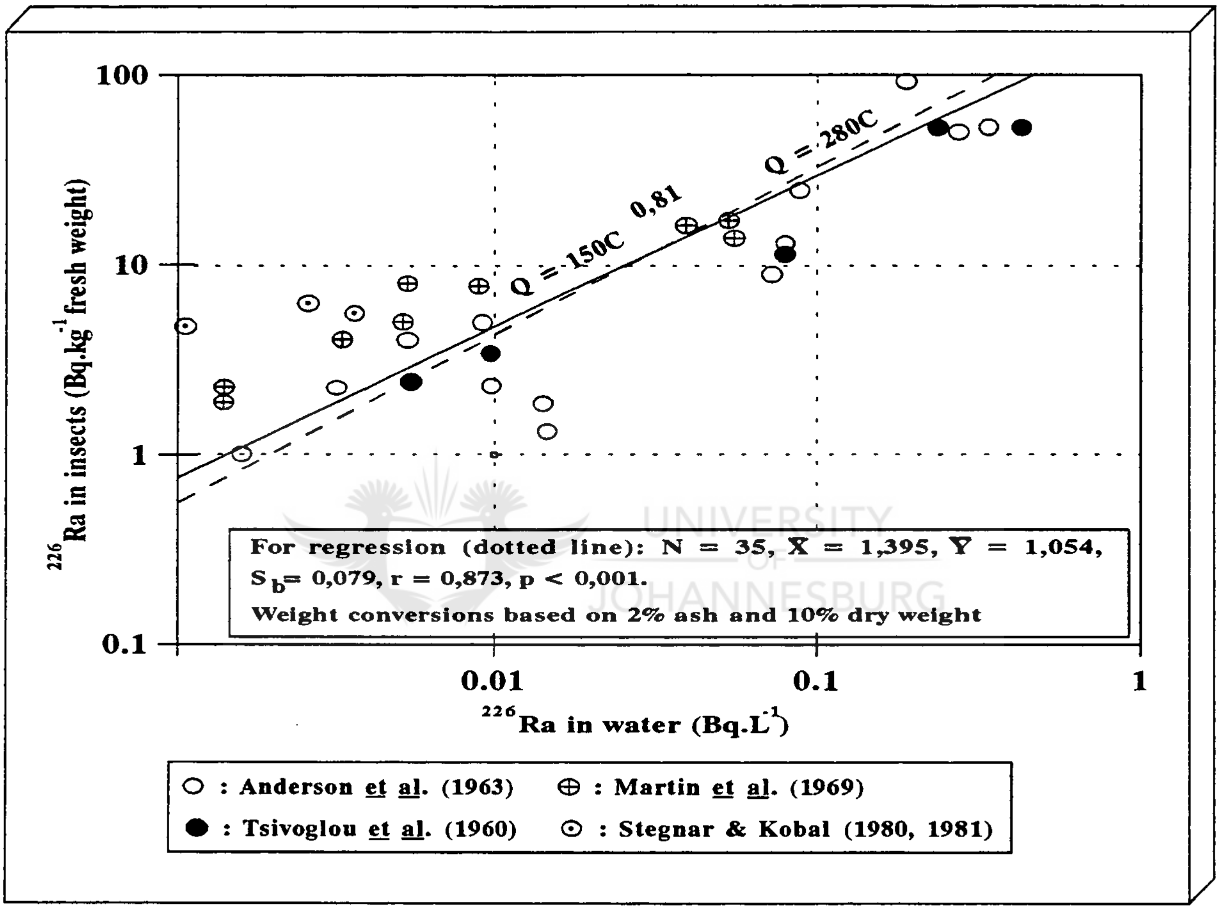


Figure 2.11 Relationship between radium concentrations in aquatic insects and in the surrounding water (After Williams, 1984)

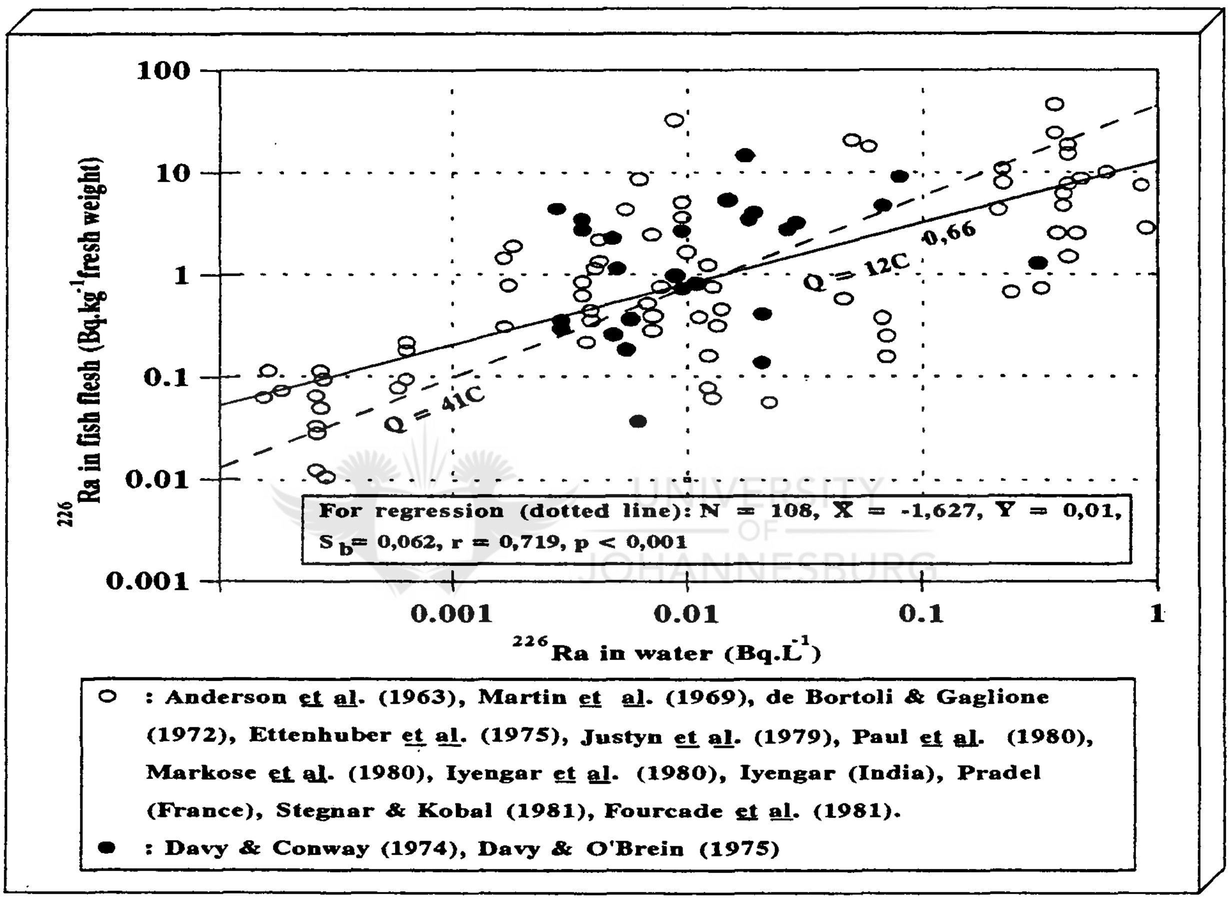


Figure 2.12 Relationship between radium concentrations in fish flesh and in the surrounding water (After Williams, 1984)

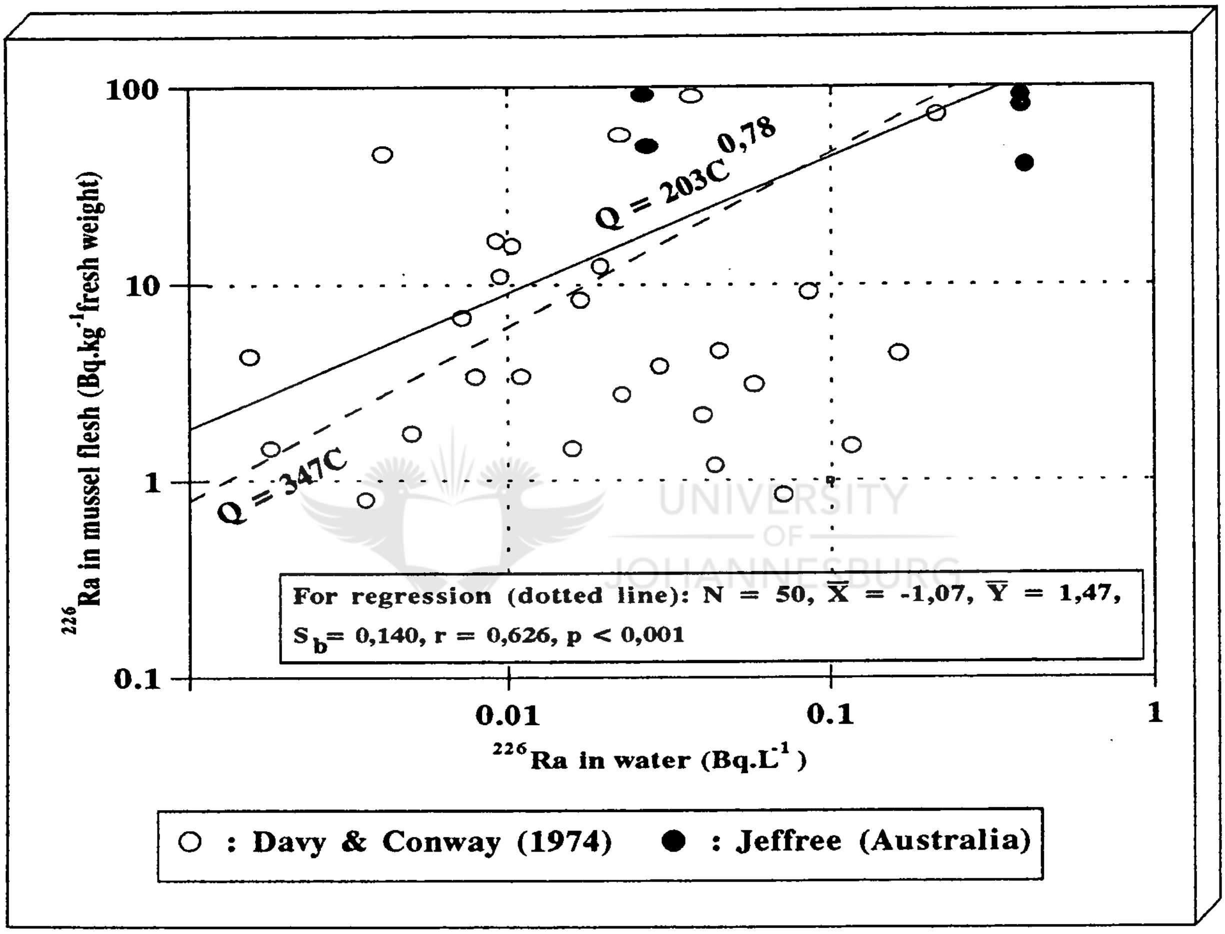


Figure 2.13 Relationship between radium concentrations in mussel flesh and in surrounding water (After Williams, 1984)

2.14 Biological uptake of radium by aquatic plants

A certain number of qualitative and quantitative factors exist which either promote and constrain radium accumulation by aquatic plants, namely:

- at cellular level
- the rate and extent of radium accumulation by the whole plant
- accumulation from sediment and translocation within plants, and
- the role of plants in radium cycling within the aquatic environment (Williams, 1990).

It has, however, been stated by Coughtrey & Thorne (1983) that no attempt has yet been made to construct generalized dynamic models of accumulation and retention of radionuclides by aquatic plants. This is especially true for radium and where there is no information available, general principles are illustrated from relevant information on other trace metals such as radioactive Sr (Pally & Foulquier, 1983) and Ca.

2.14.1 The plant-water interface

During the transfer of radium from the water column to aquatic plants, it first encounters the cell wall of the parent plant or its epiphytic community. A cell wall composed of a porous matrix of gelatinous poly-saccharide fibres usually surrounds the plasmalemma or cell membrane (Alberts, 1983). According to Epstein (1972), the plant cell wall is considered to be freely accessible to water and solute transport throughout the terrestrial plant. Investigations done by Stary *et al.* (1983) on the kinetics of accumulation of a range of cationic, anionic and neutral metal ions and complexes by the cell walls of freshwater algae showed that separate sites are involved for the three different ionic groups. The metal cations are accumulated best. Of the alkaline and alkaline earth metals, radium is the most strongly accumulated (Stary *et al.* 1984). Investigations done by Havlik & Robertson (1971) revealed that in algae, the cell wall plays a major role in radium uptake.

2.14.2 Intracellular absorption of radium

It is known that cell metabolism controls processes within the cells of plants. However, it is not clear which metabolite affects the movement of non-nutrient trace metals like radium (Williams, 1990). Whicker & Schultz (1982) demonstrated that Sr metabolism follows that of calcium fairly closely. The possibility thus exists that radium may also be influenced by calcium metabolism. According to Clarkson & Hanson (1980), calcium is a component of cell walls and cell membranes and is accumulated in large concentrations as inorganic crystalline deposits (Arnott & Pautard, 1970). It can therefore be assumed that dividing cells utilize more calcium than non-dividing cells. Although it has not yet been demonstrated for radium, Rice (1956) found that Sr uptake in actively dividing cell cultures was over one hundred times greater than in non-dividing cultures. According to Penot *et al.* (1976) the translocation of calcium in vascular plants occurs via the phloem. This process is also dependent upon protein metabolism. As calcium in one of the least mobile plant nutrients, it usually remains where it was originally deposited (Penot *et al.*, 1976 ; Clarkson & Hanson, 1980).

Even though adsorption of radium has been demonstrated in plants, the role of cell metabolism in radium has not been investigated directly. Investigations done by Emerson & Hesslein (1973), Havlik (1971) and Havlik & Robertsons (1971) have shown that some algal species accumulate all of their radium by surface adsorption. However other species significantly accumulated radium by absorbtion. In this case, radium uptake was influenced by metabolic control.

2.14.3 Uptake of radium from sediments

No experimental data on the relative contribution of radium by sediment and water to plants is available (Williams, 1990). As a result, little information is available on the role of sediment in the functioning of rooted aquatic plants (Cooper *et al.*, 1981; Williams, 1982). The oxidation state of sediments (aquatic) and soil (terrestrial) may be the most obvious factor causing differences in the availability of radium to plants. Sediments are usually anoxic. A lack of oxygen in the sediment causes a reduced state of the major chemical processes (Ponnamperuma, 1972). Under reducing conditions, radium remains a divalent cation, but other sediment constituents may also be reduced and could therefore indirectly affect the availability of radium to plants. Other sediments constituents which are easily reduced include Mn and Fe, while sulphates can be reduced to sulphates (Williams, 1990). A quantity of 10-13% of radium was found to be bound to iron/manganese oxides (Cooper *et al.*, 1981) while Hesslein & Slavicek (1984) discovered variable radium concentrations in deep lake water which could have been affected by changes in the oxidation states or iron and manganese. Radium which is already co-precipitated with calcium and barium sulphates could possibly be released under reducing conditions because the carbonates of these elements are much more soluble than their sulphates (Linke, 1985).

According to Sheppard (1980), an inverse relationship exists between the mobility of radium and other alkaline earths (Ca & Mg) in terrestrial soils. An inversely proportional relationship between combined Ca and Mg concentrations and the radium concentrations in the water extracts from abandoned uranium tailings supporting ***Typha spp.*** was also observed by Kalin & Sharma (1982), suggesting that exchange processes may dominate over changes in solubility.

In recent years it has also become evident that some aquatic plants are able to provide an oxygen supply to the surrounding sediments (Ponnamperuma, 1972) by a variety of structural and metabolic methods (Dacey, 1981; Howes *et al.*, 1981; Raskin & Kende, 1985). The ability of these plant species to aerate the sediment may therefore influence the availability of radium and other metals to the plant.

2.14.4 Translocation of radium in the plant

According to Williams (1990), no published data are available on the extent of translocation of radium within aquatic plants. however, much of the word on trace metal uptake by aquatic plants has been carried out on algae where translocation of elements in plants are largely restricted to terrestrial plants. Investigations done by Verkhovskaya et al., (1967) showed a decline of radium content in the leaves of the stems of sunflowers with an increase in length. This phenomenon may by explained by the fact that radium was not remobilized once is had been laid down and that accumulation of Ra might be a function of leaf age. Alternatively, the rate of translocation of trace elements may decline with the age of the plant (Gunn & Mistry, 1970).

2.15 Biological uptake of radium by terrestrial plants

2.15.1 The extent of radium accumulation

The extent of contamination of terrestrial plant by radium sources can according to Simon & Ibrahim (1990), be discussed under four major categories:

- In natural environments under normal conditions
- In regions with naturally high radioactivity
- In laboratory, greenhouse and field studies
- At uranium mill tailing sites.

2.15.2 Radium in plants in natural environments

According to the NCRPM (National Council for Radiation Protection and Measurement) (1975), the world average concentration of ^{226}Ra in soil is 0,00003 Bq.g^{-1} while typical levels in most components of the human diet range between 0,00004 and 0,0006 Bq.g^{-1} (United Nations, 1977). Table 2.6 provides some data on the concentration of radium in edible crop plants growing in normal soils at a number of locations in the world.

The Brazil nut tree is the plant considered to be the most effective in accumulating radioactivity. Turner *et al.* (1958) first observed the high ^{226}Ra content of Brazil nuts while Mercer *et al.* (1962) indicated that the pericarp of the Brazil nut contained a total alpha activity of 0,0003-0,016 Bq.g^{-1}. The endosperm contained 0,0009-0,11 Bq.g^{-1} while an alpha activity of 0,0003-0,0012 Bq.g^{-1} was measured in the leaves. However, the average soil concentration was about 0,0009 Bq.g^{-1} of total alpha activity and could therefore only contribute a small fraction towards this value. An investigation done on Brazil nuts from the Amazon Valley by Penna Franca *et al.* (1968), revealed that although the soil did not contain significantly high levels of^{226}Ra and ^{228}Ra, concentrations in the nuts ranged from 0,003 to 0,05 Bq.g^{-1}. It was also discovered during the same investigation on the Brazil nut tree that it has a high capacity for absorbing barium and that a positive correlation exists between radium and barium in the nut as well as in different parts of the tree. Turner *et al.* (1958) and Penna Franca *et al.* (1968) subsequently suggested that the high radium content of the Brazil nut was due to barium which functioned as a carrier for radium. Smith (1971) reported high concentrations of radium in the leaves of Brazil nuts (0,24 Bq $^{226}Ra.g^{-1}$) suggesting the formation of organic complexes within the plant which may therefore increase the mobility of the heavier alkaline earth ions. This may also explain the abnormally high concentrations of Barium present in these plants.

Results for other plants grown in natural environmental conditions (Table 2.6) generally show that where CR values for Ra were calculated, it was usually low or normal, ranging between 0,06 and 1,3.

2.15.3 Radium contents of plants from regions with naturally high radioactivity

Data obtained from literature on edible plants grown in regions which contain higher than normal levels of uranium and thorium is summarized in Table 2.7. In certain areas of Iran where a high natural background of radium is found, Khademi *et al.*, (1980) measured ^{226}Ra concentrations in the soil ranging from 2,2 to 37 $Bq.g^{-1}$. The ^{226}Ra concentrations in the plants ranged between 0,007 and 0,24 $Bq.g^{-1}$ and therefore, although the sediment concentrations were high, CR-values for these plants were relatively low, ranging between $<$ 0,001 and 0,043.

Work done by Eisenbud *et al.* (1964) on the ^{228}Ra and ^{226}Ra concentrations of vegetables collected in natural thorium deposits in Brazil showed that ^{228}Ra was higher than ^{226}Ra in all plants and that the content in leafy vegetables (cabbage and lettuce) was higher than in other vegetable crops. Research done by Linsalata *et al.* (1984) in the same Brazilian region also showed that the CR values for ^{228}Ra were about a factor of 2 greater than those for ^{226}Ra in vegetables. Vasconellos *et al.* (1987), investigating the uptake of ^{226}Ra by crops cultivated on the Pocos de Caldas plateau, an area in Brazil with a high natural radioactivity, showed that the maximum ^{226}Ra content of the vegetables analyzed were an order of magnitude higher than normal regions. The CR values for these vegetables were also low, ranging between 0,001 and 0,006.

2.15.4 Radium uptake in laboratory, greenhouse and field plot studies.

Table 2.8 provides a summary of radium uptake by plants from laboratory, greenhouse and experimental plots. Investigations done by D'Souza & Mistry (1970) showed that ^{226}Ra uptake in beans grown in a spiked radium solution was considerably higher than in plants grown in soil. The CR-values (85 : Shoots; 1787 : roots) for this plant reflect the enhanced uptake of ^{226}Ra in a solution. The lack of exchange sites and complexing ligands in the solution known to be present in soils, could explain the high ^{226}Ra uptake in solution.

Experimental exposure of a variety of crops to Ra-contaminated soil by Popova *et al.* (1964), yielded very low CR-values (0,00012 - 0,011) even though the ^{226}Ra content of the soil was very high (39,2 $Bq.g^{-1}$). As the chemical form of radium was unknown in this experiment, it could possibly explain the low CR-values for these plants. This particular chemical species was for some reason not accessible to the exposed plants. Investigations done by Kirchman *et al.* (1980), revealed that the root of the sugar beet has a lower ^{226}Ra content than the above-ground foliage (dry weight basis). Kirchman *et al.* (1966) also observed that the tubers of potatoes contained ten times less radium than the leaves. However, contradictory observations showed that roots contain higher amounts of ^{226}Ra than the shoots (Popova *et al.*, 1964; Verkhovskaya *et al.*, 1967; Gunn & Mistry, 1970).

2.15.5 Uptake of radium from uranium mill tailings

Uranium milling and extraction causes residues (tailings) which usually contain elevated ^{226}Ra levels which are several orders of magnitude above that in normal soil. Therefore, uranium mill tailings could provide a significant source of ^{226}Ra contamination for terrestrial and some aquatic plants. Simon & Ibrahim (1990) report that ^{226}Ra content in plants growing at uranium mill sites is highly variable. Substrate concentrations, sampling locations, the degree of leaf-surface contamination, the method used to clean vegetation prior to ^{226}Ra analysis and the milling process (acidic or alkaline) are some of the factors which may contribute to the variability in Ra-concentrations amongst the different studies found in the literature. Surveys conducted by Holtzman *et al.* (1979), showed Ra-concentrations (Table 2.9) in crops grown near uranium mill tailings which range between 0,000041 and 0,00038 Bq.g^{-1}. These were similar to those collected in regions with high natural radioactivity such as Brazil (Eisenbud *et al.*, 1964). Bio-concentration factors obtained for crops collected at a mill site in Germany (Schuttelkopf & Kiefer, 1982) (Table 2.9) also showed a relatively close correlation to the CR-values for crops collected in areas of high natural radioactivity as well as those exposed under experimental conditions.

2.15.6 Factors affecting the availability of radium from soil to plants

A large array of physical, chemical , biological and environmental factors paly a role in the availability of trace elements from the soil for plant uptake to occur, namely:

- Release of ions from the solid of the soil solution
- Movement (involving diffusion and mass flow) of these ions to locations where plant roots exists
- Exchange of ions onto the root surfaces and transport across the root membranes
- Translocation into plant tissues

Terms such as "availability" and "mobility" are often helpful in the description of the processes and mechanisms that determine the potential of ions for plant uptake (Simon & Ibrahim, 1990). The "available" nutrients in soils are defined as those belonging to an ionic pool which contribute to cation uptake by plants grown in that soil (Roberts & Menzel, 1965). The "labile ionic pool", which provides the available fraction of elements to plants, is composed of ions in the soil solution and those readily exchangeable ions on mineral or organic surfaces.

Table 2.6 **Concentrations and concentration ratios (CR) of ^{226}Ra in plants and edible crops under normal environmental conditions. (After Simon & Ibrahim, 1990)**

Country	Food item	n	Weight basis	Plant conc. (Bq.g^{-1}) AM(68% CI) Probability	Plant conc. (Bq.g^{-1}) GM(68% CI) Probability	Soil (Bq.g^{-1})	CR
Fed. Rep. Germany	Cabbage	2	F	0,000063 (0,00003 - 0,00011)			
Fed. Rep. Germany	Carrot	3	F	0,00011 (0,00002 - 0,00022)	0,000096 (0,000037-0,00018)		
USSR	Corn						0,07
USA	Corn	1	F	0,000056			
Italy	Fruit (mix)		F	0,000014		0,0265±0,0042	
USA	Lettuce	2	F	(0,00017 - 0,0002)			
USA	Root vegetables	2	D	0,000089 (0,00007 - 0,00011)	0,000089 (0,000070-0,00011)		
Italy	Vegetables (mix)		F	0,000036 (0,000027 - 0,000044)		0,0265 ± 0,0042	
USA	Vegetables (mix)	6	F	0,000048 (0,000019 - 0,000078)	0,000041(0,000023-0,000078)		
India	Wheat	5	D	0,00045 (0,00033 - 0,00056)	0,00043 (0,00033-0,00059)		
USSR	Wheat grain						1,0

Abbreviations: **A:** ash; **D:** dry; **F:** fresh; **a:** plants without surface contamination; **b:** plants with surface contamination; **AM:** Arithmetic Mean; **GM:** Geometric Mean; **CI:** Confidence Interval; **n:** number of samples

Table 2.7 Concentrations and concentration ratios (CR) of ^{226}Ra in plants and edible crops from regions with high natural radioactivity. (After Simon & Ibrahim, 1990)

Location and Condition	Plant or crop	Weight basis	n	Conc. $(Bq.g^{-1})$ Plant (*)	Conc. $(Bq.g^{-1})$ Soil (*)	CR (*)	Reference
Iran (Area high in natural radium)	Bean	A		0,0074 - 0,11	0,78 - 14,7	0,007 - 0,026	Khademi *et al.* (1980)
	Corn	A		0,03	0,022	0,036	
	Cucumber	A		0,007 - 0,02	0,78 - 14,7	0,001 - 0,009	
	Eggplant	A		0,028	0,81	0,035	
	Garlic	A		0,16	2,2 - 14,7	0,01 - 0,07	
	Herb	A		0,021 - 0,21	0,89 - 21,0	0,00 - 0,023	
	Onion	A		0,007	0,85	0,008	
	Orange	A		0,013 - 0,18	0,85 - 21,0	0,008 - 0,015	
	Peach	A		0,05	1,9	0,027	
	Pomegranate	A		0,037 - 0,24	0,85 - 37,0	0,005 - 0,043	
	Potato	A		0,03	0,85	0,029	
	Salad	A		0,03 - 0,078	0,81 - 14,7	0,005 - 0,036	
	Tomato	A		0,01 - 0,19	0,85 - 14,7	0,012 - 0,017	
Brazil (Area elevated in natural thorium	Banana	F	2	0,0002			Eisenbud (1964)
	Cabbage	F	2	0,00052			
	Corn	F	1	0,0002			
	Lettuce	F	1	0,0039			
	Potato	F	1	0,0003			
	Tomato	F	1	0,0001			
Brazil (Area elevated in natural uranium	Bean	F	6	0,00044	0,03 - 0,503	0,006	Vasconcellos *et al.* (1987)
	Carrot	F	4	0,00041	0,03 - 0,503	0,004	
	Potato	F	16	0,0001	0,03 - 0,503	0,001	
	Corn	F	9	0,0001	0,03 - 0,503	0,001	

Abbreviations: **D:** dry; **A:** ash; **n:** number of samples; *: single value or range

Table 2.8 Concentrations and concentration ratios (CR) of ^{226}Ra in plants and edible crops found under experimental conditions. (After Simon & Ibrahim, 1990)

Experimental conditions	Plant Group	Weight basis	n	Conc. $(Bq.g^{-1})$ Plant	Conc. $(Bq.g^{-1})$ Substrate	CR
Nutrient solutions containing carrier free ^{226}Ra	Bean (kidney)	D D				85 (shoots) 1787(roots)
Contaminated pots containing bean (*Vicia faba*)	Leaves (young)	A		0,459	37,0	0,012
	Leaves (old)	A		2,73	37,0	0,074
	Stem	A		1,65	37,0	0,045
	Fruit	A		0,992	37,0	0,027
	Roots	A		13,2	37,0	0,358
Contaminated soil, unknown chemical form of radium	Bean	D		0,056	39,2	0,0015
	Cabbage	D		0,056	39,2	0,0015
	Barley	D		0,044	39,2	0,0012
	Squash	D		0,407	39,2	0,011
	Lettuce	D		0,210	39,2	0,0057
	Spinach	D		0,067	39,2	0,0018
	Sunflower	D		0,063	39,2	0,0017
Field plots, phosphate fertilizer added	Wheat	A	5	0,018	0,03	0,61
	(straw)	D		0,0015	0,03	0,05
	Wheat	A	6	0,0089	0,03	0,30
	(grain)	D		0,0001	0,03	0,005
	Sugar beet	A	5	0,0093	0,034	0,27
	(foliage)	D		0,0015	0,034	0,04
	Sugar beet	A	5	0,023	0,034	0,68
	(root)	D		0,00063	0,034	0,02

Abbreviations: D: dry; A: ash; n: number of samples

Table 2.9 Concentrations and concentration ratios (CR) of ^{226}Ra in plants and crops grown on uranium mill tailings systems and under control conditions. (After Simon & Ibrahim, 1990)

Location and site	Plants	Weight basis	Conc. (Bq.g^{-1}) Y(a) Plant	CR (Y/X)
USA (New Mexico) Residential areas near mill site	Tomato	W	0,000041	
	Pea	W	0,000052	
	Pea pods	W	0,00013	
	Green bean	W	0,00022	
	Carrot	W	0,00022	
	Beet	W	0,00038	
	Grass	D	0,018	
	Grass	D	0,007	
	Grass	D	0,840	
Fed. Rep. Germany (Black Forest, near U, Co and Au mines)	Vegetable (mix)	F	0,00004 - 0,0063	0,028
	Potato	F	0,00004 - 0,0015	0,011
	Fruit (mix)	F	0,0001 - 0,007	0,071
	Grass (hay)	F	0,0003 - 0,02	0,15

Abbreviations: (a): Y arithmetic mean (AM) of plant concentrations; (b): X arithmetic mean (AM) of soil concentrations; D: dry; F: fresh; W: wet; n: number of samples; Y/X: mean of ratios

However, the term "nutrient" may refer to a variety of elements absorbed by plant roots, either as required nutrients or as analogues (Simon & Ibrahim, 1990). The availability of nutrients to plants from soil is governed by the solubility of the element associated with the solid phase (Cataldo & Wildung, 1978) and by the thermodynamic activity of the uncomplexed ion (Jenne & Luoma, 1977). These factors which may influence root uptake are dependent on the existence of a soluble species adjacent to the root membrane. However, the degree of availability of nutrients in the soil solution is dependent on the soil-plant relationship (Bray, 1954). According to Menzel (1965), the root system of the plant may alter the pH of the soil, influencing nutrient uptake.

Mobility of nutrients is defined as the overall process whereby ions reach sorbing root surfaces and therefore making the sorption of these ions into the plants possible (Bray, 1954). Therefore, mobility is the process involving the movement of ions by diffusion into the film of water between the root surface and the soil. Radionuclides such as radium which are soluble in the soil solution or which are isotopes of elements that have metabolic function in plants are most readily absorbed by roots of plants (Menzel, 1965).

The retention and availability of radium for plant uptake is therefore dependent on sorption and desorption processes in the soil. Under natural conditions a number of factors may have an influence on radium availability and uptake. These include the effect of calcium and other cations, organic matter in the soil, the degree of leachability from soil and the chemical species (Simon & Ibrahim, 1990).

2.15.7 Calcium and other cations

As mentioned earlier, the sorption and desorption (exchange) of radium in soil are influenced by other cations in the soil-solution system. Cations such as calcium are held as exchangeable ions on the surfaces of negatively charged, colloidal, clay and organic matter in the soil. These calcium cations can, according to Bray (1954), be replaced by, and are usually in equilibrium with, the cations in the soil solution. When radium is present as the dominant cation in dilute solutions, such a high affinity for the adsorption surfaces of soil particles is exhibited by this element, that it is almost completely absorbed (Nathawani & Phillips, 1978). As more adsorption sites are filled by radium, there is progressively less change for the solute to find and occupy available sites. Although is was observed that radium was absorbed on soil in the presence of Ca^{2+}, the degree of radium adsorption decreased considerably as the calcium concentration increased in the soil (Nathawani & Phillips, 1978). Investigations done by Titaeva (1967) showed that radium originating from naturally contaminated water was readily sorbed by peat. The radium-peat bond was shown to be of the exchange type (readily displaced by H^+, K^+ or NH^{4+}). Radium, however, becomes non-exchangeable and is associated with insoluble residues if substantial amounts of calcium are present. It is speculated that at normal soil pH, radium may co-precipitate with calcium in the presence of natural soil sulphates to form an insoluble species. Due to differences in ionic size, the ability of Ca^{2+} to replace Ra^{2+} in soil my be very low (Simon & Ibrahim, 1990). According to Rusanova (1962) and Titaeva *et al.* (1978), a significant fraction of radium in the soil was sorbed by topsoil layers which were determined to contain the highest amount of calcium. In both these cases, the radium contamination was a result of the emergence of radioactive ground water to the soil surface. It was also found by Rusanova (1964) that there was an inverse proportional relationship between the quantity of radium extracted from the soil and calcium content of the soil.

2.15.8 Organic matter

Research conducted showed that organic matter and clays tend to be the major constituents contributing to the adsorption of ^{226}Ra onto soil (Simon & Ibrahim, 1990). Stevenson and Ardakani (1972) also state that in most mineral soils, practically all of the humic (organic) materials occur in association with clay minerals. Organic matter adsorbs about ten times more radium than clay, which is known to be more adsorptive than other soil minerals. This phenomenon is due to the high "cation exchange capacity" (CEC) or organic matter (Nathwani & Phillips, 1978). Therefore, in soil with low organic matter and low clay content, a few sites will be available for radium absorption.

At a neutral or normal soil pH, both the clay surfaces and humic (organic) substances are negatively charged. Electrostatic interaction is thus not in favour of the observed associations and therefore their stabilities may be based on ligand exchange involving polydentate groups of the humic material and/or of the formation of ternary surface complexes (Schindler, 1990). A proposed schematic structure of the clay-organic interface is illustrated in Figure 2.14 (Stevenson & Ardakani, 1972). Metal ions M are coordinated to both the clay surface and the polyelectrolyte.

Elevated soil pH-values assist the separation of the clay and humic fractions by (a) ligand exchange to replace the anchoring polydentate groups and (b) by enhancing the negative charge of the humic material and thus weakening the ternary complexes (Schindler, 1990). As seen in Figure 2.14, the functional -OH and -COOH groups which are exposes to the soil solution can coordinate further metal ions and metal-ligand complexes. This can lead to apparently unlimited multilayer structures.

Investigations done by Kirchmann *et al.* (1966), revealed that an inverse relationship existed between the log of the ^{226}Ra content in plants and the amount of sorptive material in the soil. In a three-year greenhouse study to compare Ra-uptake from soil with different organic matter contents, Grzybowska (1974) indicated that high radium uptake occurred with low organic matter content as organic matter tends to bind radium. However, Whicker & Schultz (1982) state that decaying plant material may increase the mobility of certain radionuclides by producing complexing agents of cations which tend to displace those adsorbed in the soil.

2.15.9 Radium leachability from soil

Qualitative and quantitative information about radium desorption and mobility from soil and the potential for plant uptake can be obtained from leaching tests (Simon & Ibrahim, 1990). The presence of radium detected in lysimetric water by Rusanova (1962) indicated that radium was leached into soil solution and was therefore available to plants. The same author also determined that various soil types differed in the degree of radium leachability.

2.15.10 Chemical form and other factors

The chemical forms of radium, the presence of iron, the soil pH and the soil texture may influence Ra-mobility in soil (Simon & Ibrahim, 1990). Variations in the quality of water extractable radium may, according to Kalin & Sharma (1982), have an influence on the chemical form of ^{226}Ra. These authors indicated that an inverse relationship existed between the tailings concentration and the water extractable ^{226}Ra and that its uptake was influenced by the water solubility of the element. The quantity of ^{226}Ra that can exist in solution, may also be influenced by the chemical species of this element. As a result of the milling process of uranium mines, some mill tailings may contain large quantities of sulphate ions. The presence of trace quantities of barium in soil may therefore induce the co-precipitation of radium resulting in the reduced leachability of radium (Nathwani & Phillips, 1979; Ibrahim *et al.*, 1984).

During the comparison of the leachability of radium from alkaline and acidic mill tailings, Dreesen *et al.* (1981) found that the leaching of the fractions from the tailings were 6% and 0,3% respectively. The chemical form of radium as well as the effect of pH may have played a major role in differences observed in the leachability of these two types of tailings. Research by Taskaev *et al.* (1976) has shown that the soil type influences the extraction of ^{226}Ra. The formation of soluble organo-iron compounds also influences the mobility of ^{226}Ra in an $FeCl_3$ medium. Nathwani & Phillips (1978) found that soil texture influenced the leachability of radium from soil. Coarser soils showed greater desorption properties to radium than finer soils.

2.16 Distribution of radium in plant tissue

Tissue barriers, specific co-ordinating substances, metabolic incorporation and other causes not fully understood (Biddulph, 1960) cause heterogenous concentrations and distribution patterns of elements in the different parts or organs of plants. An acropetal gradient, i.e. a decreasing concentration gradient from the oldest to youngest growth and towards the apex of the plant, can occur. In the case of radium a concentration gradient was also found from roots to stems and from stems to shoots in many different plant types under different growing conditions.

Leafy vegetables and beans grown in pots containing radium-contaminated soil showed an acropetal gradient (Popova *et al.*, 1964). Although the roots generally contained the highest radium of all the plant organs, these authors indicated that the radium content in the older leaves sometimes exceeded that of the roots. Exposure of barley, peas and maize (Gunn & Mistry, 1970) and kidney beans (D'Souza & Mistry, 1970) to radium containing nutrient solutions showed that radium was preferentially accumulated by the roots and prevented from entering the shoots.

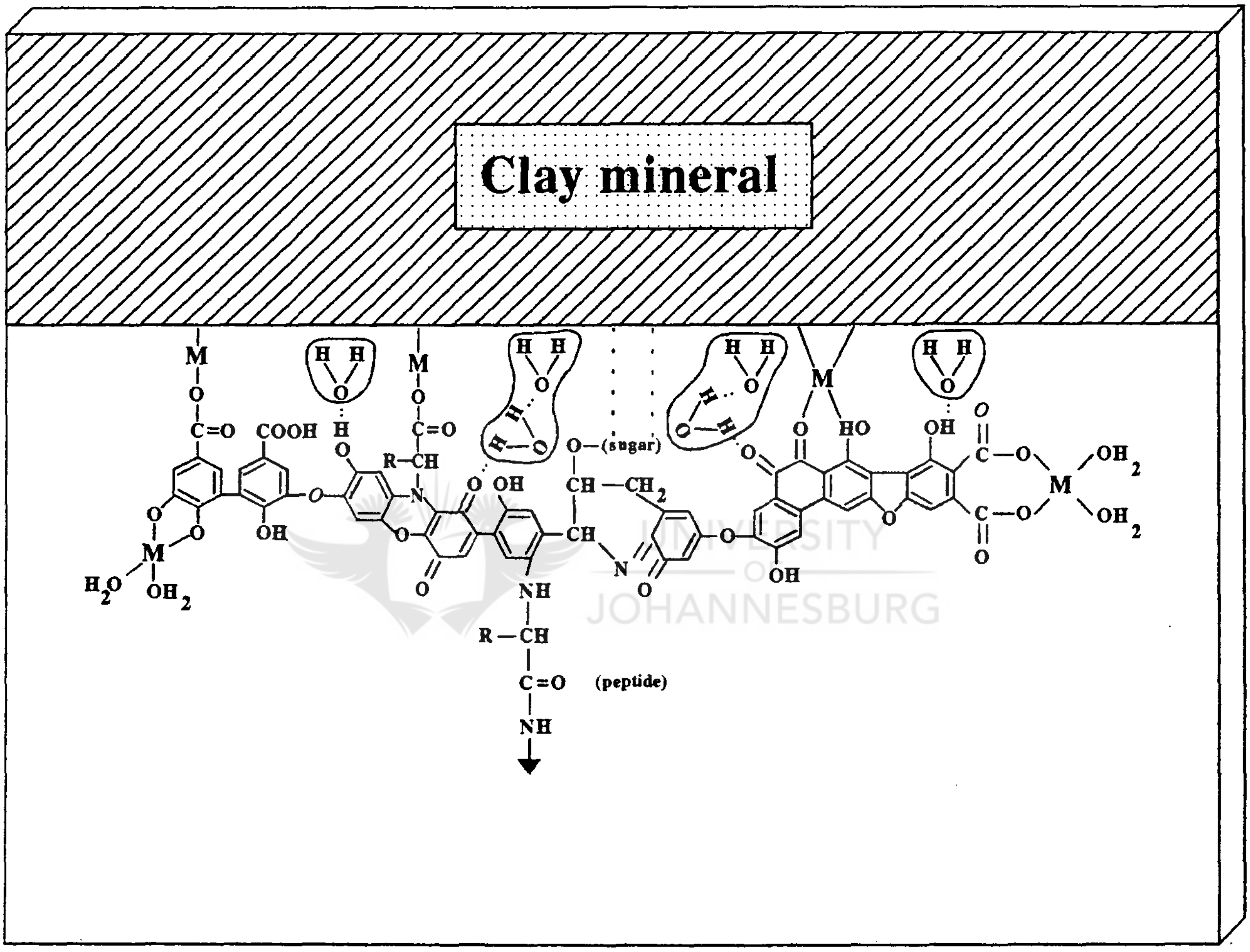


Figure 2.14 Schematic structure of the clay-humic acid complex (After Stevenson & Ardakani, 1972)

The existence of an acropetal concentration gradient suggests a low metabolic activity of radium resulting in no secondary distribution to other parts of tissues of the plant. Radium has been observed to flow unidirectionally (from roots towards shoots) and was also regulated by growth rate of the plant and leaf age. Once radium enters the transpiration stream, it remains in the leaves without further distribution. Furthermore, it does not enter the metabolic cycle which is known to redistribute some elements to areas of younger growth (Vavilov *et al.*, 1964).

Elements absorbed directly through the leaves of plants may have a different distribution pattern to those which are absorbed through the roots and stem base. While alkaline earths such as Ca, Sr and Ba are freely translocated through the xylem of the plant, their transportation encounters discrimination in the phloem. Immobilization and accumulation of Sr and Ca takes place when it is absorbed through the leaves of the plant (Biddulph, 1960).

No data from the literature is available on radium being absorbed through the leaves of plants. However, it is expected that radium would be immobile and demonstrate a similar behaviour to those of other alkaline earth elements (Simon & Ibrahim, 1990).

2.17 Speciation of radium in plants

The mobility of radium within the plant and the degree to which it is recycled from plants to soil is dependent on the chemical form or species of radium. Although no identification or characterization of the exact chemical species of radium in plants has been conducted, the results of some chemical speciation studies were however available. According to Verkhovskaya *et al.* (1969), a difference may exist between the chemical forms or species of radium deposited in different organs or plants. Comparisons between water-soluble, exchangeable and non-exchangeable fractions of radium have indicated that the relatively more mobile species localize in the plant tops rather than in the roots. Popova & Kyrchanova (1974), have indicated that mobile species of ^{226}Ra were mostly present in plants while Taskaev *et al.* (1977) determined that from 2 to 34% of ^{226}Ra in the green parts of plants occurs as a free ionic form, incorporated in soluble mineral salts and as organic complexes. Radium, however, also associates with carbonates, oxalates and other less soluble compounds (Simon & Ibrahim, 1990).

2.18 The behaviour, effects and radiation dosimetry of radium in man

During the assessment of radiation effects resulting from the intake of radionuclides, the following information is often required:

- The distribution and retention of the radionuclides in human tissues
- The position of sensitive cells in the human body in order to develop both stochastic (probabilistic) and non-stochastic (threshold) effects
- The relationship between radiation dose and risk (Stather, 1990)

There is little or no information available on the effects of most radionuclides on man. Therefore, animal studies provide insight into the assessment of possible consequences of human exposure to radionuclides and provide a basis for establishing limits on radionuclide intake (Stather, 1990). Substantial human data on both the adverse effects of radium, as well as its distribution and retention in the human body, is however readily or potentially available (Stather, 1990).

2.18.1 Behaviour and effects on the human body

After radium has entered the human body either through ingestion or inhalation, a fraction of it is translocated to the blood. The physico-chemical form of radium taken into the body and the affinity of the transportable fraction of radium for the biological transport system in the lung and gut determine the mount of radium absorbed by the blood after entry into the body. The skeleton acts as the main site of deposition after radium has entered the blood. Although a percentage of the radium present in the body is also absorbed by some soft tissues, a substantial fraction is however rapidly excreted through the gastrointestinal and urinary tracts (Stather, 1990).

2.18.2 Ingestion

During an investigation conducted by the ICRP (International Commission on Radiological Protection) Task Group on Alkaline Earth Metabolism in Adult Man (ICRP, 1973), fractional absorption values of radium from the adult GI (gastrointestinal) tract (f_l) were obtained which ranged between 0,15-0,21. From prisoners who ingested elevated levels of ^{226}Ra in drinking water (Stenhey & Lucas, 1956), the ICRP Task Group calculated an f_1 value of 0,21. The ICRP also derived an f_1 value of 0,15 for three adult control individuals which were presumed to be under equilibrium conditions.

No data on absorption of radium in children or young persons is however available (Stather, 1990). Because calcium is required for skeletal growth in young, and is chemically similar to that of radium, it can be expected that a higher level of uptake of radium would occur throughout childhood and into adolescence (Stather, 1990).

2.18.3 Inhalation

Little information is available on the clearance of inhaled radium for the respiratory system (Stather, 1990). Following the accidental exposure of six individuals, the transport of radium sulphate from the lung was measured by Marinelli *et al.* (1953). Data obtained by these authors suggests a moderate solubility of radium in lungs and that it has a half-time of clearance in the range of 32 - 140 days ($X = 120d$) within six months after exposure. Recommendations based on this case made by the ICRP suggest that about 12% of radium inhaled as an aerosol with an activity median aerodynamic diameter (AMAD) of 1 μm, would translocate to the blood within a year after intake.

2.18.4 Clearance from blood

After radium has entered the blood, there is a quantitative similarity but a qualitative difference between the behaviour of Ra and Ca. After intake, a rapid clearance of Ra and Ca as well as Sr and Ba takes place mainly from the blood to the skeleton. However, a substantial difference exists between the clearance rates of man and animals (Stather, 1990).

Comparing the clearance rates of ^{47}Ca, ^{85}Sr, ^{133}Ba and ^{223}Ra from the blood of a healthy 60-year-old man after intravenous administration, Harrison *et al.* (1967), facilitated the analysis by administering four injections in succession over ten weeks. A period of two to three weeks was allowed between each injection. Both ^{133}Ba and ^{223}Ra were more rapidly cleared from the plasma than either ^{47}Ca or ^{85}Sr. Any activity cleared from the plasma was either deposited in the tissues or excreted.

Considerable differences in the routes of excretion exist. Calcium is mainly excreted with the urine while radium tends to be present more in the faeces (Stather, 1990). Due to the high intestinal clearance of radium, much more radium was excreted than calcium (Stather, 1990). Measuring the excretion of ^{226}Ra in man (11 female : 1 male) after 103 and 104 days after radium intake, Rundo & Holtzman (1976) determined that faecal elimination was the main route of elimination, accounting for about 98% of the total radium activity lost from the body. At 104 days the overall rate of elimination was estimated as 1,5% of the body content per year, suggesting a biological half-time of about 46 years (Rundo & Holtzman, 1976).

2.18.5 Structure of and deposition in the human skeleton

Because the behaviour of radium is chemically similar to that of calcium in the human body, radium is often referred to as a bone volume seeking radionuclide (ICRP, 1979a-c). It is however necessary to know the role played by calcium in the development of the skeleton to fully understand the behaviour of radium in the human body.

Being a very specialized form of connective tissue, bone matrix contains deposits of a hard, complex mineral substance of a calcium, phosphate and carbonate combination known as hydroxyapatite. The mineral hydroxyapatite is deposited in a soft organic matrix consisting of 90% collagen fibres (Torrey & Feduccia, 1979 ; Stather, 1990). Two types of bone are found in the human skeleton:

- Hard compact bone (cortical bone) which encloses large marrow cavities and occurs in the shafts of the long bones.
- Cancellous bone (spongy or trabecular bone) which consists of a branching network of fine trabeculae of bone lamellae. These bone tissues surround bone cavities which contain either red or fatty (yellow) marrow. Cancellous bone is found in the vertebrae, most of the flat bones and in the ends of long bones. Both cancellous and

cortical bone tissue are found in the skeletal bones, but occur in varying proportions (Torrey & Feduccia, 1979 ; Freeman & Bracegridle, 1980 ; Jee *et al.*, 1985 ; Stahter, 1990).

Figure 2.15 illustrates a diagrammatic representation of a cross-section of adult cortical bone. The most anatomical and histological features as well as the different cell types which play a role in bone metabolism are also clearly indicated. According to Vaughan (1970) and Torrey & Feduccia (1979), three types of bone cells are present:

- Osteoblasts, which are responsible for laying down bone tissue on growing surfaces.
- Osteoclasts, which are responsible for resorbing bone tissue to allow for remodelling.
- Osteocytes, which are known to be responsible for maintaining calcium homeostasis in the skeleton.

Bone formation and resorption processes both occur on both the outer (periosteal) surface and the much more extensive inner (endosteal) surface of bone adjacent to the marrow cavity. New bone cells are laid down on the sites of bone formation, thus burying previously formed bone tissue. Within the bone volume in the Haversian canals, bone turnover (remodelling) usually takes place (Figure 2.15). The Haversian system is composed of a system of channels running through cortical bone. These canals are lined with bone cells which in active bone resorption or deposition occurs. These bone cells are nourished by blood vessels which run in the canals of the Haversian system. A number of osteocyte surround each Haversian system. The osteocyte are able to communicate with each other and receive their nutrients through canaliculi. A "cement line" surrounds and bounds the area around each Haversian system and area within this cement line is known as an "osteon" (Vaughan, 1970). Within each osteon, the degree of mineralization can be quite variable in that at any time the bone tissue can be in the process of being laid down or resorbed (Stather, 1990).

Frost (1967) has shown with the aid of ^{45}Ca experiments that the initiation of mineralization in any osteon is quite rapid at first. Within a few days, about 70% of mineralization could be complete, while the remaining mineralization could take weeks or months to complete. According to Parfitt (1983), the calculated overall rate of turnover of cortical bone in adults is about 3% per year.

Remodelling in cancellous bone usually takes place as a result of the action of osteoblast and osteoclasts. It includes either surface deposition and resorption or by replacement of trabeculae. Changes in the trabecular structure are necessary to accommodate varying stresses in the skeleton resulting from growth and ageing. With advancing age, osteoporosis usually develops, which is the result of the loss of cancellous bone. According to Parfitt (1983), the overall turnover rate of cancellous bone tends to be much faster than that of cortical bone. A calculated average rate of 24%.year^{-1} was reported in adults.

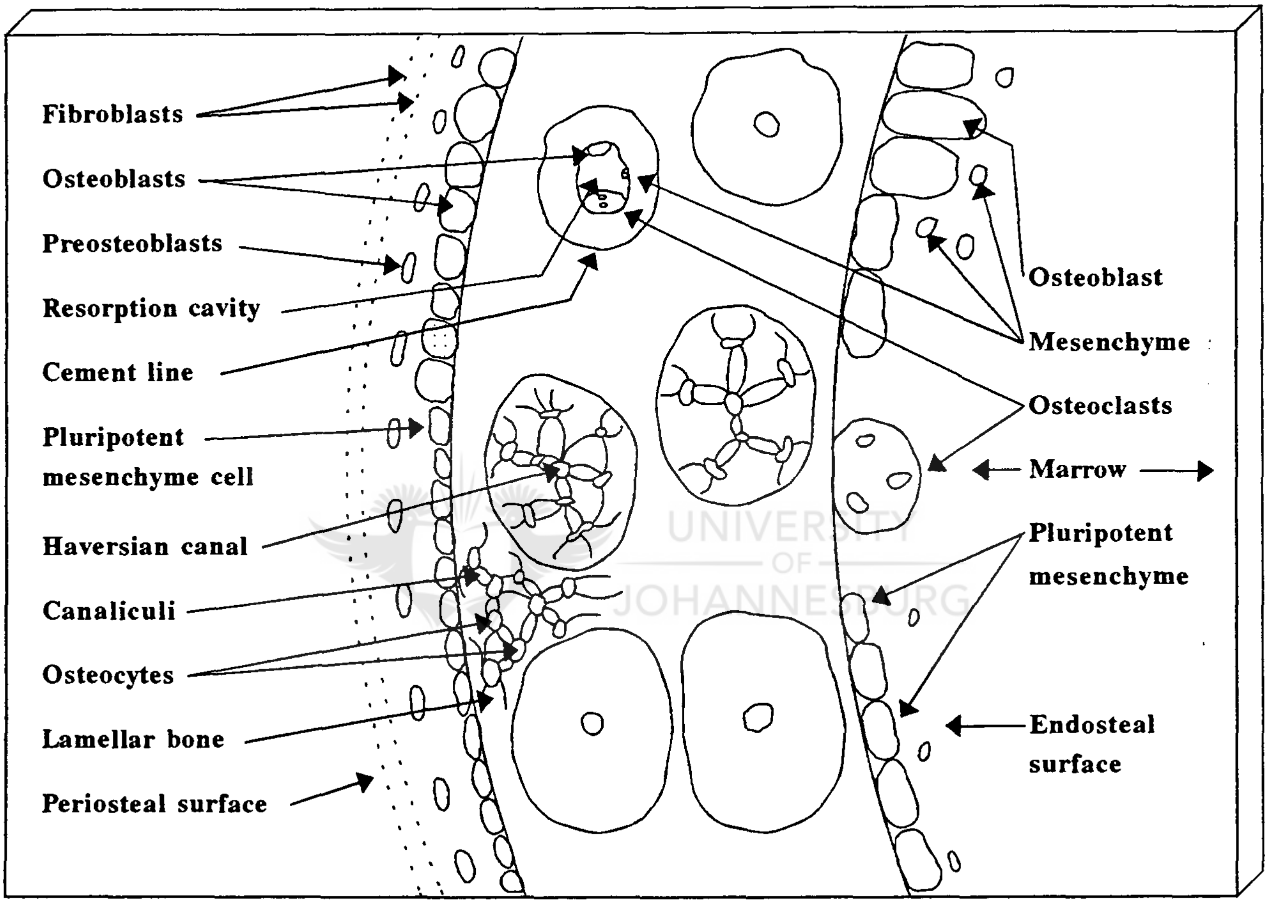


Figure 2.15 **Cross-section of adult cortical bone with an indication of the arrangement of Haversian canals, the resorption cavities and the different cell types (After Torrey & Feduccia, 1979 ; Stather, 1990)**

The main difference between bone and other living tissue is that hard bone mineral is laid down in an organic matrix largely consisting of collagen. The bone salt mostly consists of microcrystals of calcium hydroxyapatite of type $Ca_{10}(PO_4)_6(OH)_2$. Its chemical composition is largely determined by surface exchange, and to some extent, internal defects and substitutions. Amorphous calcium phosphate may also present together with the crystalline apatite (Termine & Posner, 1967).

Although the mechanism responsible for new bone formation is unknown, an increase in calcium in the area of formation takes place and apatite crystals form in the newly formed matrix. If radium or any other alkaline earths are present in the circulating blood and plasma, substitutions may be possible in the crystalline structure and these elements may therefore be incorporated into the bone crystals replacing calcium. Consequently, any bone being laid down during exposure and where high levels of radium has recently entered the blood, will contain much higher levels of activity than bone laid down when the levels of Ra in blood were low.

Any enhanced deposition occurs on all growing bone surfaces, but especially in the Haversian systems. In these Haversian systems, where active bone accretion takes place, these areas become labelled during radium intake. However, those which are in resting or resorbing phase retain only very small amounts as part of a more generalized "diffuse" deposit.

A constant exchange process is taking place between the Ca ions in the extracellular fluid and the Ca present in bone mineral. This exchange process is caused by diffusion of ions from the bloodstream to the extracellular fluids, therefore bathing the microcrystals of bone mineral. The small size of the crystals results in a large surface ion exchange area.

2.18.5.1 Summary

A short review has been presented on the information available on the presence and behaviour of radionuclides in the environment and the radiation effects and behaviour of radium in the human body. Data from the literature have shown numerous cases illustrating the effects of ^{226}Ra in the aquatic and terrestrial environments. Literature has also indicated that the substantial amounts of data available on the consequences provide a valuable source of information on the effects of incorporated radionuclides in man. It is known that radium is 2 milliom times more radiotoxic than uranium and thus has a more important potential environmental impact (Williams, 1984). The literature survey shows that there is less data available on uranium effects on the environment than radium. To fully understand the mechanisms involved in the transport and uptake of ^{226}Ra and uranium as well as the application of dose assessment modelling, as much information and data as possible needs to be gathered. This also applies to the South African situation. Based on previous investigations (Malan, 1981; de Jesus *et al.*, 1987) the present investigation was aimed at contributing to this pool of information, especially in the South African context.

The absence of literature on the uptake of ^{226}Ra from spray irrigation via plant leaves, together with a single reference to the effect of a higher CR for plants grown in spiked ^{226}Ra solutions (section 2.7.1.3) make it imperative for the present study to further investigate this gap.

2.18.6 Dose assessment and modelling

2.18.6.1 The concept of modelling

The different public exposure pathways of dust inhalation, radon and radon daughter inhalation and aquatic and food pathways need to be quantified for regulatory authorities and to assure adequate protection of the public and environment. Because of the complexity of environmental interactions and the varied modes of exposure, dose assessment is undertaken using mathematical computer models (Till, 1983).

A typical model developed for the assessment of radionuclide transfer in a terrestrial environment is composed of an ensemble of submodels and mathematical equations which represent a large number of different processes and mechanisms. Pathways for the transport of radionuclides from the source to humans is illustrated in Figure 2.16.

According to such models, the following pathways can be identified and mathematical models designed for each:

- Direct exposure
- Inhalation
- Atmosphere-to-vegetation transfer
- Atmosphere-to-soil transfer
- Soil-to-vegetation transfer
- Aquatic transfer
- Irrigation-to-vegetation transfer
- Animal products

Essentially, models consider release mode from the source (gaseous or liquid), transport, dilution and dispersion, reconcentration, bioaccumulation and transfer up the food chain and subsequent exposure of humans either directly or by inhalation and consumption of water and foodstuffs. The habits of the population and data on food types and masses or volumes consumed are important for realistic dose assessment.

The models usually have the option to evaluate the dose from a number of different nuclides to:

a) the maximum exposed individual,
b) the critical group, and
c) the collective dose to the wider population

Doses to adults, teenagers or children can be evaluated, both for the whole body or individual body organs. The internal dosimetry submodels are based on established transfer factors between conceptualized body compartments.

2.18.6.2 Model application

Various models have been developed world wide such as by the IAEA (1984) and the United States Nuclear Regulatory Commission (1982). Models that were available locally were LADTAP II (Simpson & McGill, 1980) from the Oak Ridge National Laboratory (U.S. Department of Energy), a mainframe version and GENII, the Hanford Environmental Radiation Dosimetry Software System (Napier, 1988) of Pacific Northwest Laboratory. The GENII code operates on a PC and was chosen for its ease of use for this project. It incorporates the internal dosimetry models recommended by the International Commission on Radiological Protection (ICRP).

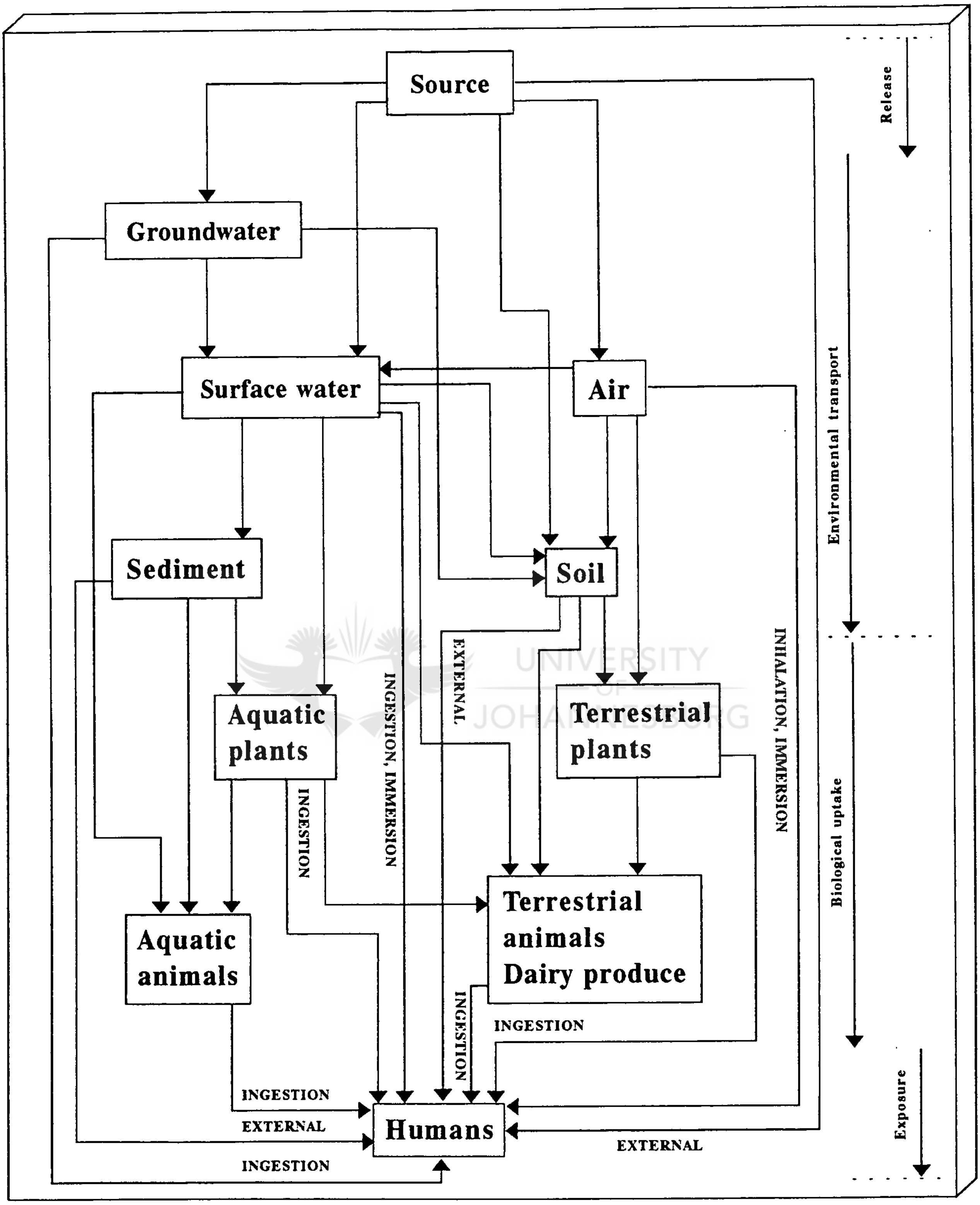


Figure 2.16 Pathways for the transport of radionuclides from the source to humans (After National Technical Planning Group, 1981)

MATERIALS AND METHODS

3

3 MATERIALS AND METHODS

3.1 Factors affecting the selection of sampling localities

The criteria for suitable sampling localities required that significant concentrations of radium and uranium should occur in surface water or sediments associated with mining activities on the Witwatersrand and that agricultural production occur using such contaminated water. In general, the localities should exclude mining land.

During the selection of suitable sampling locations, the following factors which may have had an influence on ^{238}U and ^{226}Ra activity and agricultural use at particular sites were taken into account:

3.1.1 Geology of the study area

Although the association between gold and uranium has been known since 1923 (Travener, 1957), the extraction of uranium from gold ore only started in the late forties. The main uraniferous component of Witwatersrand ores is the primary uranium mineral uraninite (usually black or dark brown, massive and non-crystalline) which usually occurs in a complex matrix of conglomerates (Liebenberg, 1955 ; 1972). The reefs of the West Rand mostly contain brannerite ($(U,Ca,Ce)(Ti,Fe)_2O_6$), which is a uranium-bearing titanate (Feather & Koen, 1975). The average uranium (as U_3O_8) concentrations in the Witwatersrand and the Far West Rand vary between 50 g.t^{-1} to about 200 g.t^{-1} (2 Bq ^{238}U.g^{-1}) ore (Funke, 1990). They can even be as high as 640 g.t^{-1} in the Klerksdorp area and 600 g.t^{-1} in the OFS goldfields. However, these figures are in contrast with those of Canada, USA, and Australia where the uranium ore content are two orders of a magnitude higher (Funke, 1990).

In contrast with uranium, the concentrations of ^{226}Ra are much lower and average about 50 μg.t^{-1} (2 Bq ^{226}Ra.g^{-1}) discarded sludge for a 200 g.t^{-1} ore. Analyses of slimes dams have shown that ^{226}Ra levels vary between 0,6 and 3,1 Bq.g^{-1} for the Klerksdorp and OFS mining areas where richer uranium ores are associated with the presence of gold. This is in contrast with the East Rand where activity levels between 0,2 and 0,6 Bq.g^{-1} were measured on slimes dams (Chamber of Mines of South Africa, 1979). These results are confirmed by de Jesus *et al.* (1987) who obtained the same tendency by measuring the ^{226}Ra activity on various slimes dams in these mining areas. On the East Rand (0,49 ± 0,16 Bq.g^{-1}) and Central Rand (0,41 ± 0,15 Bq.g^{-1}) a relatively low ^{226}Ra activity was measured while the Klerksdorp mining area produced the highest average ^{226}Ra activity of 1,84 ± 1,21 Bq.g^{-1} (Table 3.1). On the West Rand (1,18 ± 0,80 Bq.g^{-1}) and OFS-goldfields (1,23 ± 0,99 Bq.g^{-1}) the ^{226}Ra-activity was more or less the same (Table 3.1), but lower than that of Klerksdorp (de Jesus *et al.*, 1987). The same tendency has also been observed during an airborne gamma-survey conducted by Grundling & Schoeman (1989) from Nigel in the east to Stilfontein in the far west. These results also showed a relatively low gamma-activity in the Eastern and Central Rand, while the West Rand and Klerksdorp-Stilfontein areas showed relative gamma-activity varying from > 1000 to about 3600 counts per second.

Table 3.1 ^{226}Ra activity on slimes dams on the East, West and Central Rand as well as the Klerksdorp and OFS goldfields (After de Jesus *et al.*, 1987).

Mine	^{226}Ra Concentration (Bq.g^{-1})	Mine	^{226}Ra Concentration (Bq.g^{-1})
EAST-RAND		**WEST RAND**	
Brakpan	0,64 ± 0,05	West Rand Consolidated	1,22 ± 0,08
Geduld-East	0,58 ± 0,05	Randfontein Estates	2,69 ± 0,10
Grootvlei	0,58 ± 0,06	East Champ d'Or	0,53 ± 0,04
New State Areas	0,56 ± 0,06	Blyvooruitzicht (1)	0,64 ± 0,05
Daggafontein	0,38 ± 0,06	Blyvooruitzicht (2)	1,32 ± 0,07
Sallies	0,21 ± 0,06	West Driefontein	0,71 ± 0,05
Mean	**0,49 ± 0,16**	**Mean**	**1,18 ± 0,8**
CENTRAL RAND		**ORKNEY/KLERKSDORP**	
Vill. Main Reef (2)	0,47 ± 0,03	Buffelsfontein	3,40 ± 0,26
Vill. Main Reef (1)	0,43 ± 0,03	Vaal Reefs	2,69 ± 0,14
Vill. Main Reef (3)	0,34 ± 0,03	Western Reefs	1,69 ± 0,08
City Deep (3)	0,16 ± 0,01	Hartebeespoort	0,54 ± 0,05
City Deep (1)	0,22 ± 0,02	Stilfontein	0,86 ± 0,05
City Deep (2)	0,69 ± 0,04	**Mean**	**1,84 ± 1,21**
ERPM (3)	0,37 ± 0,02	**ORANGE FREE STATE**	
ERPM (4)	0,47 ± 0,03	Merriespruit	2,67 ± 0,15
ERPM (1)	0,48 ± 0,03	Harmony	1,09 ± 0,12
ERPM (5)	0,31 ± 0,03	Welkom	0,48 ± 0,04
ERPM (2)	0,59 ± 0,03	Western Holdings	0,69 ± 0,05
Mean	**0,41 ± 0,15**	**Mean**	**1,23 ± 0,99**

3.1.2 Agricultural conditions

Although the East, Central and West Rand as well as the Klerksdorp and OFS goldfields fall within a summer rainfall area with an average precipitation of 400-720 mm per year, there are, however, subtle differences between the rainfall and soil composition of these areas which may have an influence on the suitability of sampling locations. The East Rand (Nigel, Springs, Brakpan and Boksburg), Central Rand (Germiston and Johannesburg) and West Rand (Roodepoort, Krugersdorp and Randfontein) have a higher average precipitation than the Klerksdorp mining area and the OFS goldfields (Weather Bureau, 1992). The soil conditions in the East, Central and West Rand are also more favourable for agricultural and irrigation purposes as the soil contains a higher average organic content and has a longer retention time for moisture. In these areas, a number of dams and lakes are found whilst extensive wetland areas also supply water to farmers for irrigation. Therefore, the Witwatersrand is generally more suitable for vegetable production as well as fodder for dairy cattle. However, the far West Rand, Klerksdorp and OFS are known for soils which consist of weathered calcareous dolomite which is not effective in storing water. The Klerksdorp and OFS mining areas are therefore not quite suitable for the production of irrigation-intensive crops and are generally known as maize-producing areas which are not irrigation-intensive. Due to the bigger impact that mining might have on agriculture, the Witwatersrand area was selected for this study.

3.1.3 Aquatic pathways for radionuclide contamination of agricultural land.

Two possible pathways exist whereby suspended or dissolved ^{226}Ra and uranium can reach surface waters near gold and uranium mines:

3.1.3.1 Seepage from slimes dams

An extensive survey conducted by de Jesus *et al.* (1987) to determine ^{226}Ra-levels on slimes dams on the Witwatersrand has shown that levels vary from 0,2 Bq.g^{-1} on the East Rand to 3,1 Bq.g^{-1} on the West Rand. The average activity for all these slimes dams was about 0,5 Bq.g^{-1} (Table 3.1) compared to 0,04 - 0,08 Bq ^{226}Ra.g^{-1} for normal soil. Many of the slimes dams which have been sampled have fallen into disuse in the past few decades. The state of conservation of these slimes dams therefore varies considerably. Some of these slimes dams have been subjected to plant rehabilitation programmes while others have been covered by gravel or burnt-out coal to prevent any further wind or water erosion (Cook, 1973 ; Cartwright, 1983 ; Blight & Cladwell, 1984 ; Jones *et al.*, 1989). However, there are some slimes dams which show signs of erosion and silting. It is especially the finer fractions which are clearly visible around the slimes dams. However, experimental data obtained by de Jesus *et al.* (1987) has shown that the mobility of ^{226}Ra is about 1300-4500 times slower than that of water and that the ^{226}Ra-activity averaged $<$0,07 Bq.ℓ^{-1} in seepage water sampled from the bases of some slimes dams. This seepage water could reach agricultural land but large volumes are probably unlikely.

3.1.3.2 Contamination of surface waters

Contamination of surface waters (rivers, lakes and wetlands) usually occurs where mining process water is released in waterways (de Jesus *et al.*, 1987). Surveys done in the vicinity of various mines as well as several sampling locations downstream from the points of release of effluents have shown that, except for a few isolated cases, the ^{226}Ra-levels have decreased to such an extent in the water and sediments, that an activity of 0,04 Bq.ℓ^{-1} was measured within a few kilometres from the point of release. Samples taken from lakes in the vicinity of gold/uranium mines and which are accessible to the public, have shown that the ^{226}Ra-levels are nearly similar to that of natural waters (de Jesus *et al.*, 1987) (Table 3.2) but sediment values are elevated in some localities. The greatest potential impact on irrigated lands is thus within a few kilometres downstream of mine discharges, and sampling localities had to be selected where agricultural activities were closest downstream from mine discharge points.

These results show general trends and identify certain areas. While not pinpointing problem areas, they have been very valuable in aiding selection of suitable sampling locations.

3.2 Sampling localities

Bearing in mind the criteria for sample location and the factors elaborated on above, various in-field inspections were carried out as highlighted below.

3.2.1 East Rand

This investigation originated on the East Rand and the Marievale Farm in particular was selected. The farm is located near the Marievale gold mine in the vicinity of Springs and Nigel (Figure 3.1). This farm is situated about 2 km downstream from where Marievale mine releases its effluents. On the farm, vegetables such as lettuce, cabbage, cauliflower, beetroot and carrots are produced. Vegetables produced here are supplied to a local market as well as to a well-known supermarket group (Geral, 1990).

This investigation was preceded by a preliminary study on the ^{226}Ra and uranium levels in a number of selected aquatic plants, algae, macro-invertebrate fauna as well as fish and water birds occurring at localities in the Blesbokspruit, Elsburgspruit and Natalspruit catchment areas on the East Rand. These results are also considered in the present report.

Permission was obtained to sample oats plants grown on a slimes dam near Marievale. These plants were experimentally cultivated by a mine to determine the potential survival of different plant species on slimes dams. The slimes dam was divided into approximately 30 field plots (4 X 6m) onto which various different plants, shrubs and crops were planted. The experiment was conducted by the mine and it lasted for approximately two years. No irrigation was applied to these plants. Rain provided the only moisture to these plants.

Table 3.2 ^{226}Ra levels of dams and lakes in the study area accessible to the public (After de Jesus *et al.*, 1987).

Sampling point	^{226}Ra Conc. Water (Bq.ℓ^{-1})	^{226}Ra Conc. Sediment (Bq.g-1)
EAST RAND		
Middle Lake	0,01	N.S.
Apex Pan	0,05 ± 0,02	0,17 ± 0,01
Jan Smuts Dam	0,01	0,06 ± 0,01
Geduld Dam	0,01	N.S.
Cowles Dam	0,03 ± 0,01	N.S.
Vogelstruisbult Dam	0,12 ± 0,04	N.S.
Nigel Dam	0,11 ± 0,04	0,24 ± 0,02
Withokspruit	0,01	N.S.
Van Wyk Dam	0,01	N.S
Rooikraal	0,05 ± 0,02	N.S.
CENTRAL RAND		
Wemmer Pan	0,07 ± 0,02	0,24 ± 0,02
Victoria Lake	0,01	N.S.
Elsburg Spruit	0,05 ± 0,02	N.S.
Angelo Pan	0,13 ± 0,04	N.S.
Boksburg Lake	0,01	0,05 ± 0,01
Elsburg Dam	0,02 ± 0,01	0,22 ± 0,02
WEST RAND		
Florida Lake	0,10 ± 0,01	N.S.
Rand Leases Dam	0,01	N.S.
New Canada Dam	0,01	N.S.
KLERKSDORP		
Vaal River (Downstream)	0,02 ± 0,01	0,04
Vaal River (Upstream)	0,01	0,04
Jagspruit	0,24 ± 0,07	0,06 ± 0,01
ORANGE FREE STATE		
Sand River (Upstream)	0,01	0,04
Sand River (Adjacent)	0,01	N.S.
Sand River (Downstream)	0,01	0,04
Doring Pan	0,02 ± 0,01	0,32 ± 0,04

Abbreviations: N.S. : Not Sampled

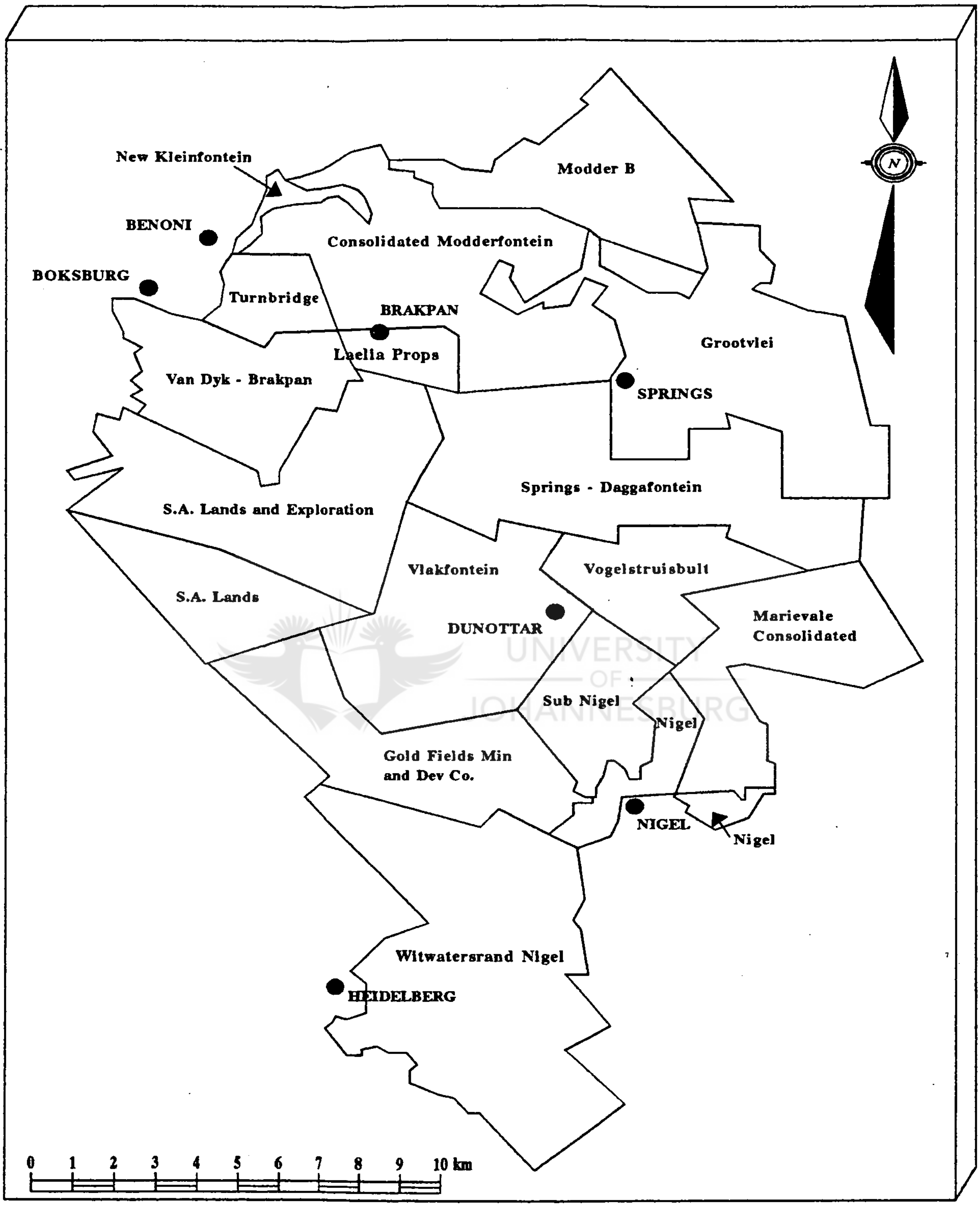


Figure 3.1 East Rand Goldfield locality map (After Antrobus, 1986 and Engelbrecht, 1989)

3.2.2 Central Rand

This area is also known to be active in gold and uranium production (Figure 3.2), but results obtained by de Jesus *et al.* (1987) and Grundling & Schoeman (1989) have shown that ^{226}Ra-activity to be the lowest of all the areas considered for the present investigation. Although extensive agricultural activities take place south of Johannesburg as well as Germiston, Alberton and Boksburg, nearly all the farmers utilize underground water for irrigation. As underground water is not representative of mining effluent, it was decided that these agricultural areas do not fall within the scope of this project. While, a few farmers were found to utilize water from the nearby wetlands, it was determined that ^{226}Ra-levels were negligible due to the filtering effects of these waters. During reconnaissance investigations several incidents of political violence were encountered which often made sampling dangerous in these areas.

3.2.3 West Rand

Two agricultural areas which seemed to be of specific interest were identified:

3.2.3.1 Luipaardsvlei

This agricultural area is situated south of Randfontein where it is surrounded by intensive gold and uranium mining activities (Figure 3.3). This area is characterized by vast underground aquifers which occur in dolomitic formations (Engelbrecht, 1989 ; & Funke, 1990). Significant amounts of water are pumped from underground compartments for mine dewatering as well as agricultural and domestic use.

A number of gold mines are active in this area, and U_3O_8, pyrite, silver and osmiridium are also produced as by-products (Engelbrecht, 1989). Some of these mines and a gold refinery are situated upstream from a river which drains through a series of dairy farms.

Most of the agricultural land is owned by farmers who specialize in milk production. Milk produced on these farms is delivered to a cheese factory (du Preez, 1991). The fodder produced is irrigated with water pumped from the nearby stream as well as with water received from a mine (du Plessis, 1991). Underground water is, however, unsuitable for human consumption (Coetzee, 1991).

3.2.3.2 Vlakfontein

This agricultural area is situated approximately 3-4 km southeast of Luipaardsvlei (Figure 3.3) and also receives effluent and seepage waters from a nearby goldmine for irrigation purposes. This vegetable farm produces lettuce, cabbage, cauliflower, beetroot, carrots, and spinach. A vegetable shop, open to the public, is situated on the farm next to a main route and vegetables are also supplied to a local market. The farmer makes exclusive use of flood irrigation as spray irrigation appears to damage or burn the leaves of the irrigated crops (Inago, 1992).

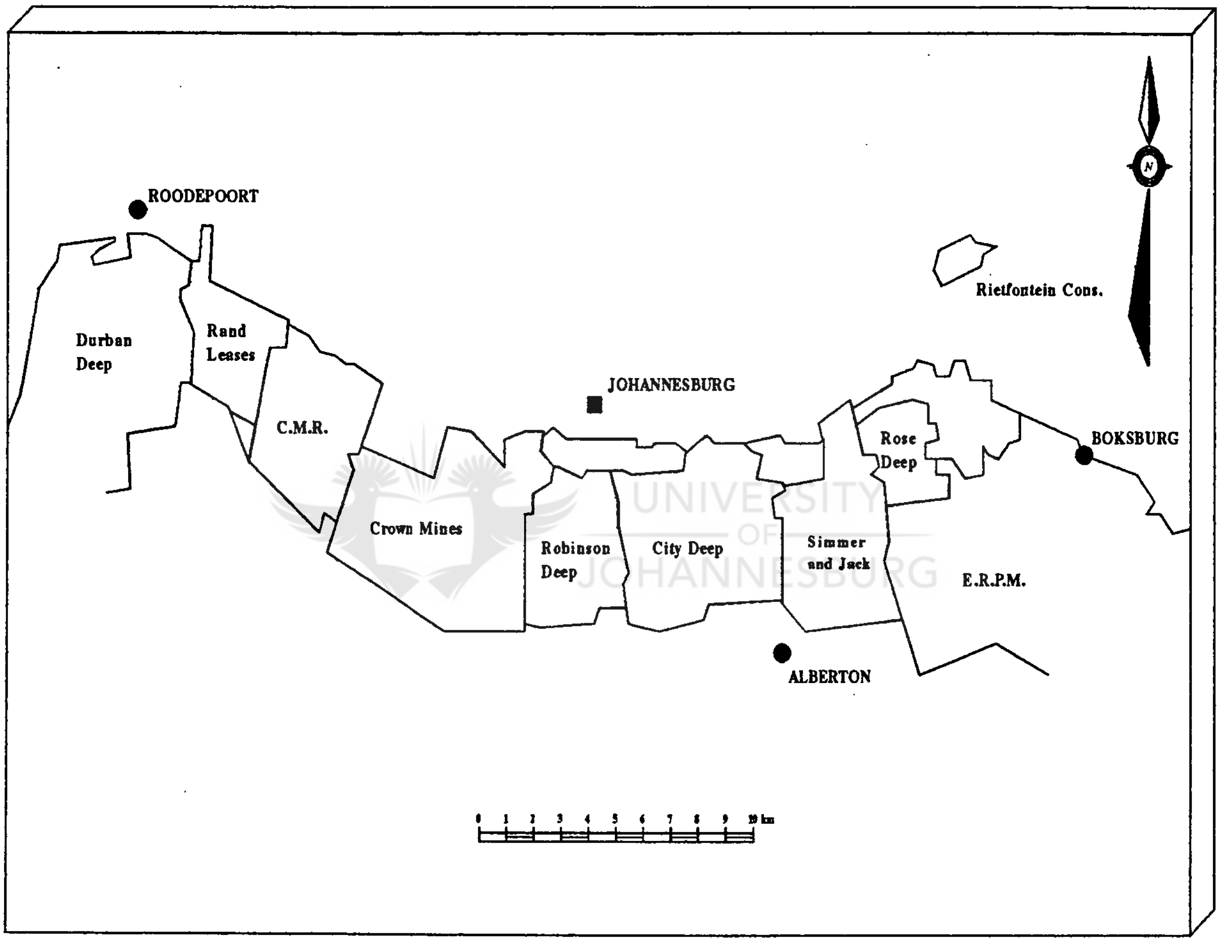


Figure 3.2 **Central Rand Goldfields locality map (After Antrobus, 1986 and Engelbrecht, 1989)**

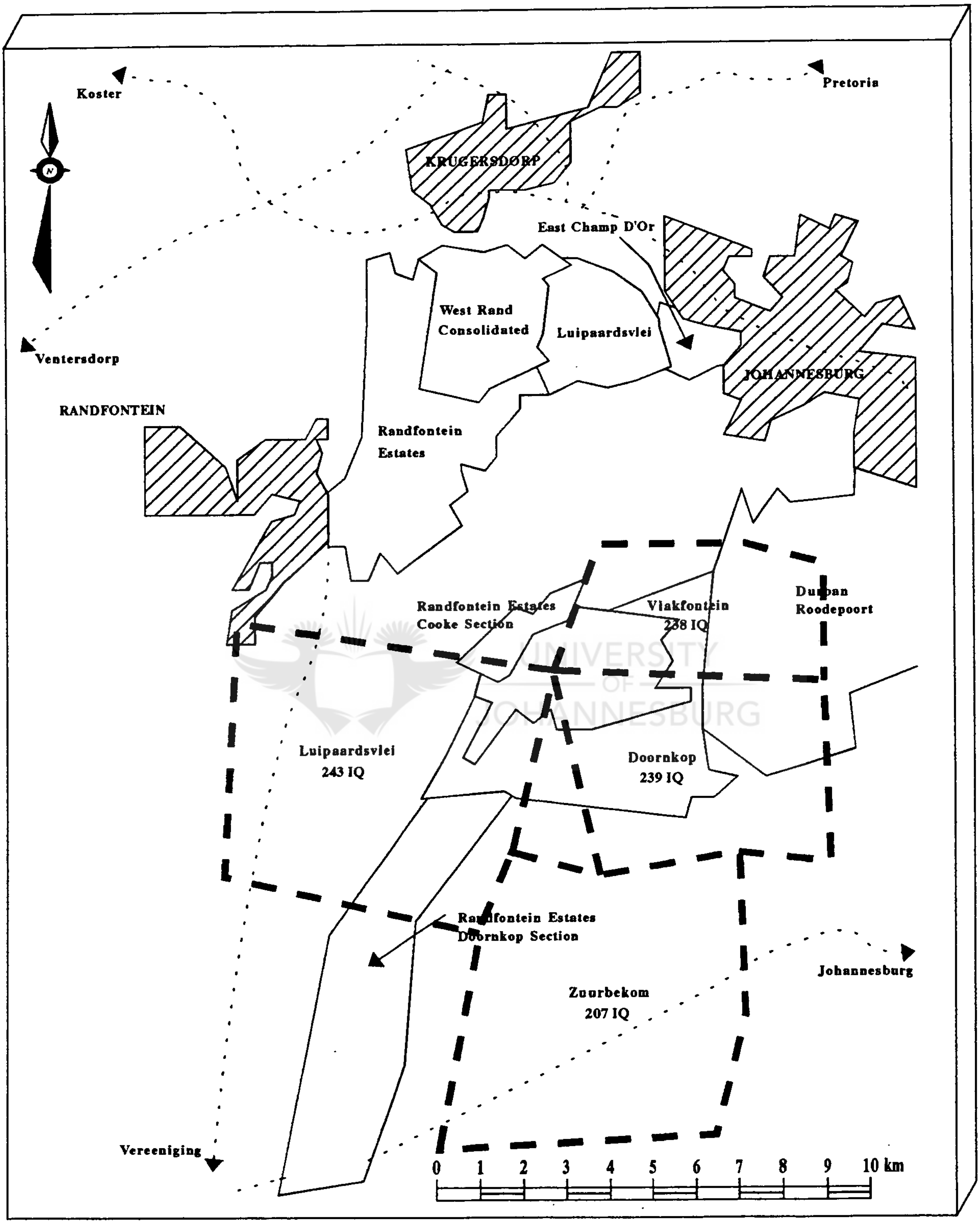


Figure 3.3 West Rand Goldfields locality map
(After Antrobus, 1986 and Engelbrecht, 1989)

3.2.4 Orkney/Klerksdorp and OFS goldfields

Although the highest ^{226}Ra-activity levels were reported in these areas (de Jesus e*t al.*, 1987), a number of factors render these areas unsuitable for agricultural activities. Although no dramatic differences exist between the amount of precipitation on the Witwatersrand and the Orkney/Klerksdorp (Figure 3.4) and OFS (Figure 3.5) goldfields areas, the latter tend to be drier and unsuitable for the production of vegetable crops. The drought during the time of sampling has even made it difficult to sustain dry land crops such as maize. It was therefore decided to discontinue any investigations in these specific areas.

3.3 Selection of radionuclides

Table 3.3 contains the radio isotopes which are formed in the important decay routes of ^{238}U. Based on annual limit on ingestion intake , the isotopes of concern are ^{226}Ra, ^{210}Pb and ^{210}Po.

Table 3.3 Radio isotopes formed by the decay of ^{238}U

Half-live(seconds)	Isotope	gram/ Becquerel	Annual limits on intake, ingestion in Becquerel (ICRP,1991)
1.41e+17*	^{238}U	8.05e-05	3e+06
2.08e+06	^{234}Th	1.17e-15	4e+06
2.43e+04	^{234}Pa	1.36e-17	4e+07
7.70e+12	^{234}U	4.32e-09	3e+06
2.43e+12	^{230}Th	1.34e-09	3e+05
5.05e+10	^{226}Ra	2.73e-11	9e+04
3.30e+05	^{222}Rn	1.74e-16	-
1.83e+02	^{218}Po	9.56e-20	-
1.61e+03	^{214}Pb	8.24e-19	1e+08
1.19e+03	^{214}Bi	6.09e-19	2e+08
1.64e-04	^{214}Po	8.41e-26	-
7.04e+08	^{210}Pb	3.50e-13	2e+04
4.33e+05	^{210}Bi	2.18e-16	1e+07
1.20e+07	^{210}Po	1.00e-14	9e+04

*1.41e+17 = 1.41 X 10^{17}

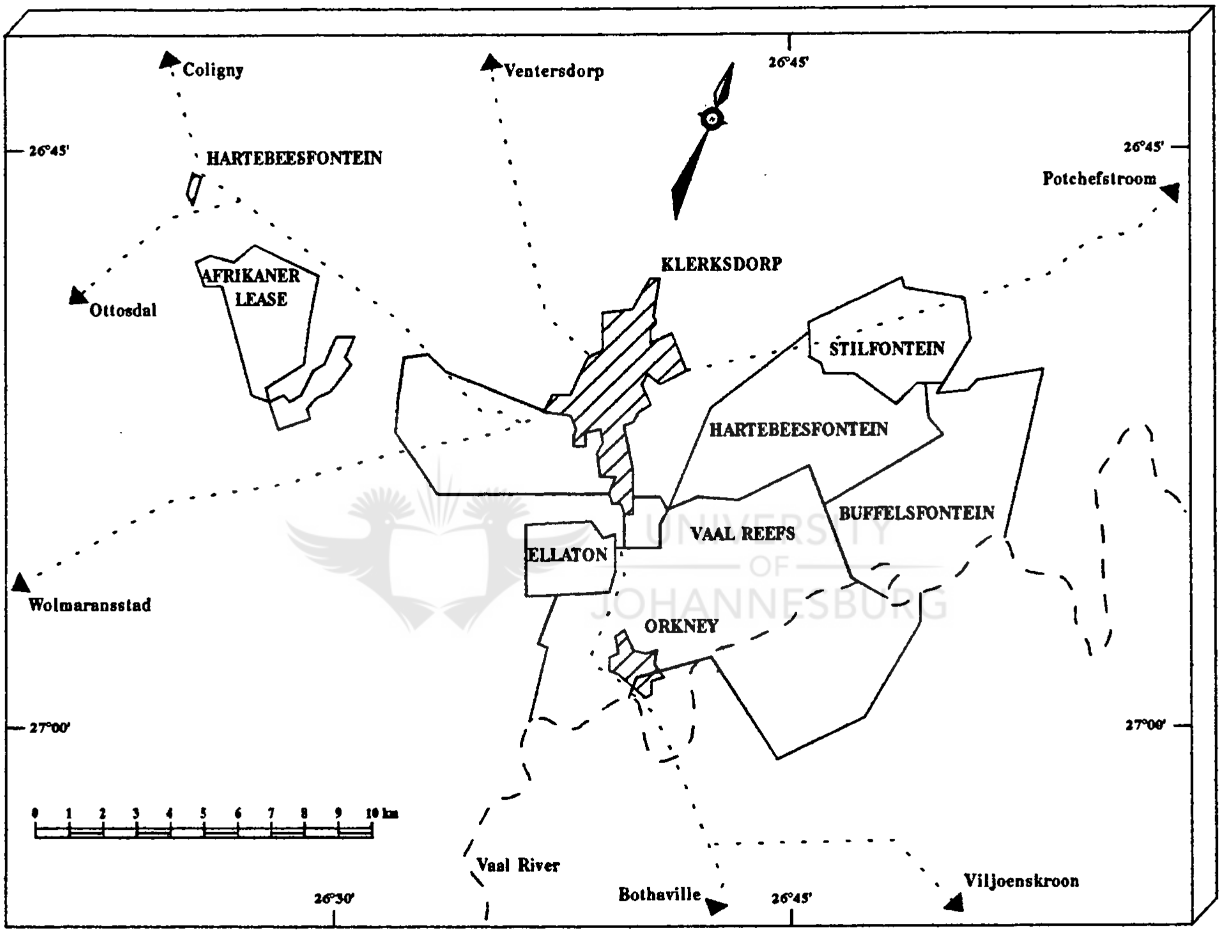


Figure 3.4 Klerksdorp Goldfield locality map
(After Antrobus, 1986 and Engelbrecht, 1989)

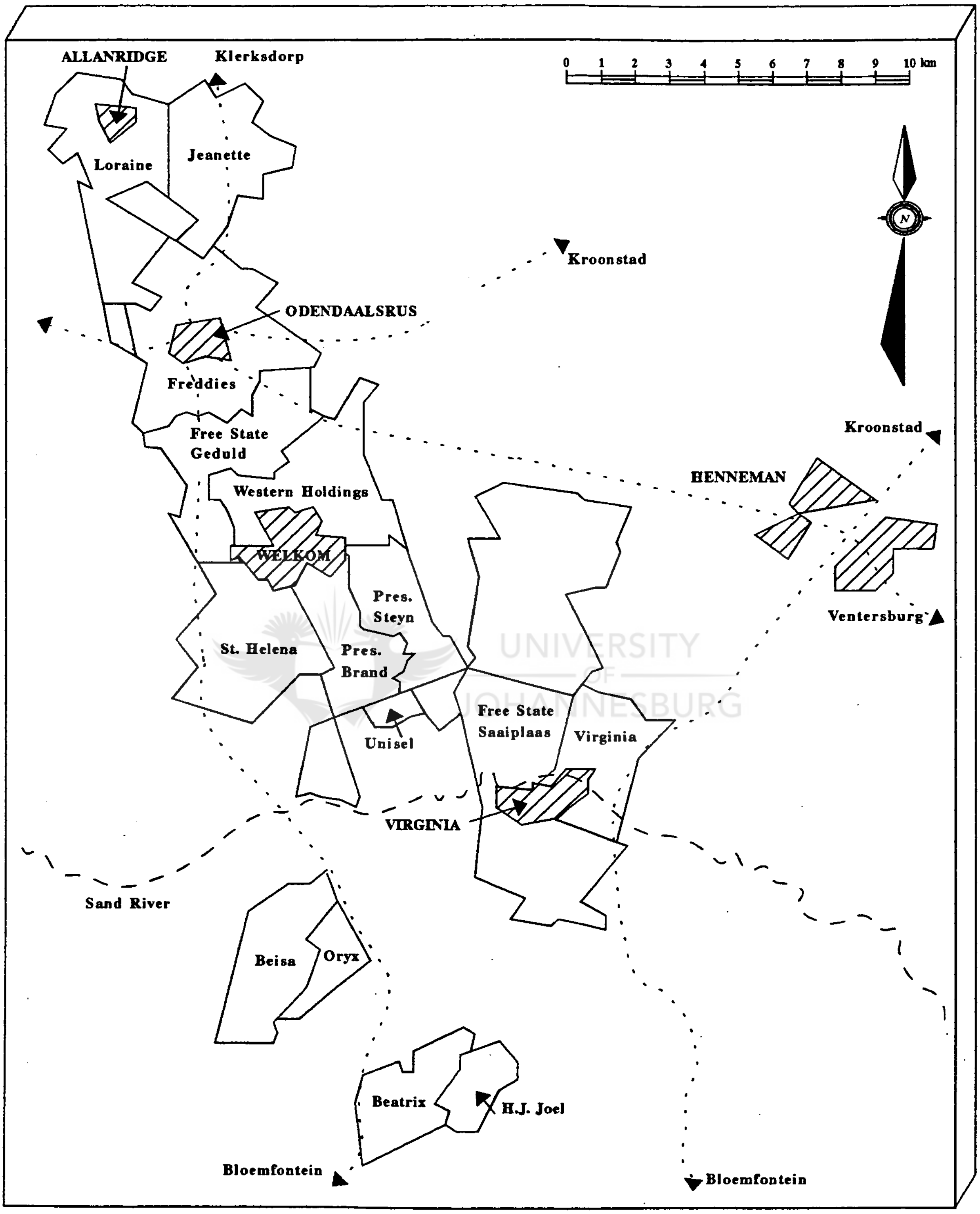


Figure 3.5 **Orange Free State Goldfields locality map (After Antrobus, 1986 and Engelbrecht, 1989)**

In the absence of ^{226}Ra, 1,0 Bq of ^{222}Rn will decay to form only about 0,00047 Bq of ^{210}Pb in about a month's time. ^{210}Pb and ^{210}Po can therefore only build up to appreciable quantities if ^{226}Ra is present. Furthermore, due to the fact that radon is a noble gas with a short biological half-life relative to its radiological half-life, almost all of the radon will diffuse out of living tissue before it decays to form its daughter isotopes (Napier *et al.*, 1988). The possibility of intake of appreciable amounts of ^{210}Pb and ^{210}Po through farm produce irrigated with water contaminated with mine effluents is hence small. ^{226}Ra could therefore represent the second part (last 9 daughter products) of the decay chain of ^{238}U.

The literature on uranium is less abundant than that of ^{226}Ra. The differences in the annual limit on intake of the first 5 isotopes in the ^{238}U decay chain is not as big as between the last 9 isotopes. ^{238}U itself can therefore represent the first part of the decay chain. The other usually important natural decay chain is that of ^{232}Th. However, the local gold-bearing reefs are associated with low concentrations of thorium, typically a factor 10 less than uranium concentrations and potential dose from the thorium decay chain can be neglected. This study therefore concentrated on uranium and ^{226}Ra as these would probably be adequate indicators of the extent of the possible effect of radionuclides on people living in the proximity of mining activities on the Witwatersrand.

3.4 Experimental uptake of ^{226}Ra by two selected vegetable crops

3.4.1 Aim of the experiment

As spray irrigation is often used on vegetables at the selected localities, the aim of this experiment was therefore to determine the difference in uptake of a known concentration of ^{226}Ra through the leaves and roots of two selected vegetable crops (one root and one leaf crop) under greenhouse conditions. Results obtained from the experiments were used to calculate concentration ratios (CR) for these crops and to assess effects of flood versus spray irrigation in agricultural production. The experimental uptake by uranium was not conducted due to little available literature.

3.4.2 Preparation of ^{226}Ra standard solutions for irrigation on experimental plants

A ^{226}Ra standard solution (dissolved Ra : acidic) of 4500 Bq (1 ℓ) was obtained from the AEC for the experimental study. Another stock solution for irrigation purposes was made by pipetting 5 mℓ from the original stock and making it up to 1 ℓ giving an irrigation stock of 22,5 Bq.ℓ^{-1} which, for comparison, is about 100 times higher than the drinking water limit. Therefore, a dosage of 0,0225 Bq was obtained per millilitre applied to the plants. A suitable irrigation scheme, giving a cumulative dosage of 5,67 Bq per plant at the end of the irrigation period was therefore calculated and used (Table 3.4).

3.4.3 Irrigation system and dose application

Four irrigation schemes were proposed for the conduction of the experiment:

1) Irrigation water enriched with ^{226}Ra sprayed on the leaves only of the plants.

2) Irrigation water enriched with ^{226}Ra applied to the roots only of the plants (via the soil).

3) Irrigation water enriched with ^{226}Ra sprayed on the leaves and applied to the roots of the plants.

4) Irrigation water not enriched with ^{226}Ra sprayed on the leaves and applied to the roots of the plants. These plants served as the control of the experiment.

Cabbage and beetroot seedlings were obtained from a farm remote from mining activities whereafter a certain number of these plants were kept for background analysis while the others were used for the experiment. For each irrigation scheme, ten plants of each type were used. Before the experiment commenced, all pots were cleaned with 10% formaldehyde to kill any pathogens. Plants used in the experiment were planted in these pots in fresh potting soil. These potted plants were kept in a greenhouse. After an acclimatization period of two weeks, the proposed irrigation schemes were commenced and application of the ^{226}Ra-enriched water continued daily for 6 weeks. At the end of the exposure period the plants were carefully removed from the pots with roots intact and prepared for analysis as per section 3.5.3.

Table 3.4 **^{226}Ra irrigation scheme showing the daily dosage and calculated weekly values for 6 successive weeks.**

Week	mℓ.day^{-1}	Bq.day^{-1}	Bq.week^{-1}
1	5	0,1125	0,7875
2	5	0,1125	0,7875
3	6	0,135	0,945
4	6	0,135	0,945
5	7	0,1575	1,1025
6	7	0,1575	1,1025
		Total	5,67

3.5 Collection, identification and preparation of water, sediment and biological samples for ^{226}Ra and uranium analysis

3.5.1 Water samples

At least 5ℓ composite water samples were collected at the various sampling localities (Figures

3.1 - 3.6). These were individually filtered in the field with a Sartorius pressure filter through 8 and 0,45 micron membrane filters to remove any suspended solids. 10 mℓ concentrated nitric acid was added to each sample to retain any ^{226}Ra present in solution. ^{226}Ra was determined in each water sample with the emanation method. In each case, a 400 mℓ sample was sealed in a bubble flask. After about three weeks, allowing for the build-up of ^{222}Rn to equilibrium level with the ^{226}Ra, the ^{222}Rn-gas was emanated into a scintillating flask. The concentration of the ^{222}Rn in the flask was then analyzed on a photomultiplier tube, after a further period of three hours storage. The scintillating flasks were calibrated using IAEA (International Atomic Energy Agency) certified ^{226}Ra solutions.

For uranium preparation and analysis, each water sample was evaporated on a thin plastic sheet. The sheet and solids on it were then ashed, and the resulting solid analyzed with Neutron Activation Analysis (NAA). Alternatively a 250 mℓ water subsample was run through an ion exchange column which was subsequently analyzed for uranium with NAA.

3.5.2 Sediment samples

After collection, all sediment samples were individually dried for 72h at 90 - 100 °C whereafter they were homogenized and sieved through a 1 mm sieve. Moisture, total organic content and pH of the sediment samples were also determined.

Each dried sample was sealed in a 30 mℓ glass bottle using epoxy glue. After about three weeks, allowing for the build-up of ^{214}Bi to equilibrium level with ^{226}Ra, the sample was analyzed on a gamma spectrometer with a Ge-Li detector. The 609 keV gamma of ^{214}Bi was used to determine the concentration. ^{226}Ra and ^{60}Co sources were used to do the energy calibration of the detector. An IAEA-certified ^{226}Ra standard, sealed in the same geometry as the samples, was used as a reference standard.

Uranium was determined using a delayed neutron version of NAA. A small amount of homogenized sample (200 mg) was sealed in an ampoule and irradiated with neutrons. The delayed neutrons emanating from ^{235}U were then quantitatively measured. The isotope distribution of natural uranium was then assumed to calculate the uranium in the sample.

3.5.3 Biological samples

Emergent, floating and submerged aquatic weeds were collected for ^{226}Ra and uranium analysis (Table 3.5). Identification of the aquatic weeds was done using Cook *et al.* (1974) and Van Wyk & Malan (1988). Freshwater fish species, which included several edible fish (Table 3.5), were collected from localities in the Blesbokspruit, Elsburgspruit and Natalspruit. While gill nets were used to collect the bigger specimens, smaller fish were mainly caught using a modified Moore type shocker (Moore, 1968) as well as a beach seine net of 5 mm stretched mesh. Fish species were identified using the methods described by Le Roux & Steyn (1968) and Jubb (1967) and placed into taxonomical order as described by Jackson (1975). The freshwater crab, *Potamonautes warreni* (Calman), which commonly occurs in the waters of the East Rand, was selected as a representative of the macro-

Table 3.5 Organisms collected for ^{226}Ra and uranium analysis.

Species	Common name	Family
PLANTS *Azolla filiculoides* Lam.	Water fern	Azollaceae
Marsilea macrocarpa (DC.) C. Presl	Water-clover	Marsileaceae
Lemna major L.	Duckweed	Lemnaceae
Lagarosiphon muscoides Harv.	Fine oxygen weed	Hydrocharitaceae
Potamogeton pectinatus L.	Fennel-leaved pondweed	Potamogetonaceae
Typha capensis (Rohrb.) N.E. Br.	Bulrush	Typhaceae
Arundo donax L.	Spanish reed	Poaceae
MACRO-INVERTEBRATES *Potamonautes warreni* (Calman)	Freshwater crab	Potamonautidae
FISH *Barbus aeneus* (Burchell, 1822)	Smallmouth yellowfish	Cyprinidae
Cyprinus carpio Linnaeus, 1758	Common carp	Cyprinidae
Clarias gariepinus (Burchell, 1822)	Sharptooth catfish	Clariidae
Tilapia sparrmanii Smith, 1840	Banded tilapia	Cichlidae
AQUATIC BIRDS *Fulica cristata* Gmelin, 1789	Redknobbed coot	Rallidae
Phalacrocorax carbo (Linnaeus), 1758	Whitebreasted cormorant	Phalacrocoracidae

invertebrate fauna (Table 3.5). Aquatic birds including the weedeating redknobbed coot, *Fulica cristata* and the piscivorous white breasted cormorant, *Phalacrocorax carbo* (Table 3.5), were collected for analysis after being shot with a 0,22 rifle.

Biological material was placed in plastic bags during collection. All samples were carefully rinsed and cleaned at the laboratory. Adequate quantities of fresh material of all the biological samples were individually weighed, then dried for 5-6 days at 90 °C (Ellerbeck *et al.*, 1990) and the moisture content determined. The dried material was ashed in a muffle for 1 h at 200 °C whereafter the temperature was increased to 500°C. The duration of the ashing was about 2 hours or until no black fragments of carbon remained in the samples (Moolman *et al.*, 1990).

Following ashing, the samples were individually weighed into cleaned glass beakers and a mixture of 1:1 HNO_3 (180 mℓ) : $HC\ell O_4$ (180 mℓ) was added to each of the samples and digested on a hotplate. After cooling, the samples were transferred to bubble flasks (100 mℓ or 400 mℓ, depending on the mass of the sample), sealed and stored for three weeks for ^{226}Ra and ^{222}Rn to reach equilibrium. The analysis of ^{226}Ra in the digested samples were the same as for water.

During the preliminary study samples were dried and sealed in the same containers as mentioned in 3.5.2. The ^{226}Ra concentrations proved to be below the detection limit of this method. During the main investigation, samples were digested as described above and the resulting solution/slurry was sealed in a bubble flask, and analyzed as for ^{226}Ra in water (3.5.1). For uranium analysis, subsamples of the ashed biological material were analyzed using the NAA technique as described in 3.5.2.

3.6 Concentration Ratios

Concentration ratios are discussed in section 2.6.3. In this study two types of concentration ratios were calculated, namely CRs and CRw which were the ratios between the plant or animal and soil and water concentrations respectively.

The CR values were calculated as following:

$$\mathbf{CRs = [Co]/[Cs]}$$

Where: [Co] = Concentration of radionuclide in organism
(U: $\mu g.g^{-1}$ (fresh) ; ^{226}Ra: $Bq.g^{-1}$ (fresh))
[Cs] = Concentration of radionuclide in soil
(U: $\mu g.g^{-1}$ (dry) ; ^{226}Ra: $Bq.g^{-1}$ (dry))

$$\mathbf{CRw = [Co]/[Cw]}$$

Where: [Co] = Concentration of radionuclide in organism .
(U: $\mu g.g^{-1}$ (fresh) ; ^{226}Ra: $Bq.g^{-1}$ (fresh))
[Cw] = Concentration of radionuclide in water
(U: $\mu g.m\ell^{-1}$; ^{226}Ra: $Bq.m\ell^{-1}$)
or: (U: $\mu g.g^{-1}$; ^{226}Ra: $Bq.g^{-1}$) as 1ml H_2O = 1g

Note in Tables 4.5 - 4.18 that the concentration data are given for ashed mass and per litre of water.

3.7 Scenarios for dose assessment modelling

The GENII code (Napier, 1988) allows several scenarios to be modelled. For this project the following options were chosen:

- Far field scenario
- Dose to a maximum individual adult from chronic exposure
- 1 year intake period
- 50 year committed dose period
- Exposure paths: a) drinking water
  b) consumption of irrigated foodstuffs
- Radionuclides, ^{238}U and ^{226}Ra

Exposure pathways such as inhalation and swimming in contaminated water were not considered. The option for transport in environmental media was not used because the radionuclide concentrations in the water and foodstuffs consumed were assumed known as determined by field and pot experiment measurements.

The quantitative details of the input parameters used for radionuclide concentrations in water and food, volume or mass of food consumed annually all as fresh mass are given in Table 3.6. Concentration values chosen are meant to reflect realistic values found in the field. The consumption values chosen are conservative and reflect a possible extreme case for potential exposure. Usually the water found in streams near mining areas on the Witwatersrand are not used for drinking. It is assumed that vegetables consumed are all from contaminated irrigation water. Consumption of oats is separately evaluated assuming a daily breakfast intake and a high concentration value based on the Marievale sample.

Table 3.6 **Input parameters for GENII dose assessment code**

Ingestion Parameter	Radionuclide	
	^{238}U	^{226}Ra
Drinking water		
Concentration (Bq/ℓ$^{-1}$)	0,5	0,04
Consumption (ℓ.y^{-1})	730	730
Vegetables (leafy)		
Concentration* (μg.g^{-1}) ; (Bq.g^{-1})	0,3 (0,024)	0,25
Consumption (kg.y^{-1})	30	30
Vegetables (root)		
Concentration* (μg.g^{-1}) ; (Bq.g^{-1})	0,3 (0,024)	0,25
Consumption (kg.y^{-1})	90	90
Cereal (oats)		
Concentration* (μg.g^{-1}) ; (Bq.g^{-1})	6,1 (0,494)	8,6
Consumption (kg.y^{-1})	80	80
Fish		
Concentration* (μg.g^{-1}) ; (Bq.g^{-1})	0,12 (0,010)	2,5
Consumption (kg.y^{-1})	25	25
Soil		
Concentration** (μg.g^{-1}) ; (Bq.g^{-1})	150 (12,2)	25
Consumption (mg.y^{-1})	410	410

*Concentration units per fresh mass **Concentration units per dry mass

RESULTS

4

4 RESULTS

Irrigation water and soil samples were collected for physical and chemical analysis at the same time as vegetable samples for radionuclide analysis. At the Marievale, Vlakfontein and Luipaardsvlei farms the same irrigation water samples collected according to procedures described in Chapter 3, were used for both the physical-chemical and radionuclide analysis.

4.1 Physical and chemical characteristics of irrigation water and soil samples collected at the Marievale, Vlakfontein and Luipaardsvlei farms

No samples of the water and sediments were collected for uranium and ^{226}Ra analysis during the preliminary survey of these radionuclides in selected aquatic organisms during 1989-90 (Table 4.5). However, during the main survey of 1990-92, irrigation water as well as soil samples were collected at the Marievale, Vlakfontein and Luipaardsvlei study areas for this purpose (Table 4.4). These are discussed in later sections.

4.1.1 Marievale farm

Results on the physical and chemical characteristics of the irrigation water and soil samples are summarized in Table 4.1. pH values for the six composite water samples were relatively constant, fluctuating between 7,5 and 7,9 (Table 4.1a). Electrical Conductivity of the samples were high, reflecting the high amount of Total Dissolved Salts (TDS) in the irrigation water. Values for this parameter ranged between 1400 and 1700 μS.cm^{-1} (Table 4.1a). Alkalinity values ranged between 72 and 102 mg.ℓ^{-1}. Total Hardness concentrations varied between 193 and 397 mg.ℓ^{-1} reflecting to some extent the effect of mining activities on the irrigation water. Ammonia, Nitrite and Nitrate values as well as concentrations of phosphates suggest some mild organic enrichment of the water (Table 4.1a).

Table 4.1a Water quality of the Marievale farm irrigation water

Water Quality Parameter	1	2	3	4	5	6
pH	7,5	7,6	7,6	7,6	7,7	7,9
E.C (μS.cm^{-1})	1700	1700	1600	1600	1500	1400
Alkalinity (mg.ℓ^{-1})	79	72	79	100	75	102
Total Hardness (mg.ℓ^{-1})	215	397	234	311	193	387
Ammonia (mg.ℓ^{-1})	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Nitrite (mg.ℓ^{-1})	<0,01	0,01	0,02	0,01	<0,01	0,01
Nitrate (mg.ℓ^{-1})	0,9	2,6	1,8	2,2	0,9	2,6
Phosphate (mg.ℓ^{-1})	0,3	0,2	0,6	0,3	0,6	0,3
Sulphate (mg.ℓ^{-1})	640	720	760	640	760	600

Soil pH values corresponded well with those of the irrigation water (Table 4.1b). A moderate percentage of organic material was present in the soils fluctuating between 0,43 and 0,70% (Table 4.1b).

Table 4.1b Soil pH and organic content of several field plots on the Marievale farm

Field plot	pH	Organic content (%)
Carrots	7,67	0,48
Beetroot	7,71	0,43
Lettuce	7,80	0,61
Cabbage	7,66	0,70
Cauliflower	7,73	0,68

4.1.2 Vlakfontein

At the Vlakfontein farm, the pH of the irrigation water was slightly alkaline with values for Electrical Conductivity (1400 μS.cm^{-1}), Hardness (1419 mg.ℓ^{-1}) as well as Sulphates (950 mg.ℓ^{-1}) clearly reflecting the effects of mining activity on the water quality of irrigation water used on this farm. The Alkalinity of the water was low (16 mg.ℓ^{-1}) with values for Ammonia (0,22 mg.ℓ^{-1}), Nitrite (0,01 mg.ℓ^{-1}), Nitrate (2,4 mg.ℓ^{-1}) and Ortophosphate (<0,01 mg.ℓ^{-1}) generally being of an acceptable quality (Table 4.2a).

The soil pH was alkaline at all the randomly selected sites on the farm where samples collected varied between 7,26 and 7,68 (Table 4.2b). The organic content of the soils were on the average slightly higher than that of the Marievale farm.

Table 4.2a Water quality of the Vlakfontein irrigation water

Water Quality Parameter	Irrigation water pump sample
pH	7,1
Electrical Conductivity (μS.cm^{-1})	1400
Alkalinity (mg.ℓ^{-1})	16
Total Hardness (mg.ℓ^{-1})	1419
Ammonia (mg.ℓ^{-1})	0,22
Nitrite (mg.ℓ^{-1})	0,01
Nitrate (mg.ℓ^{-1})	2,4
Phosphate (mg.ℓ^{-1})	<0,01
Sulphate (mg.ℓ^{-1})	950

4.1.3 Luipaardsvlei

The water quality parameters at the three adjacent farms as well as the soil conditions (Tables 4.3a &b) showed prevailing conditions to be mildly acidic to alkaline. Water pH values fluctuated between 6,4 and 8,1 (Table 4.3a). In all three cases, the effect of mine acid waters on the Electrical Conductivity, Hardness and, to a lesser extent, Sulphates are reflected in the results. The alkalinity of the irrigation water at all three farms remained below 50 mg.ℓ^{-1} (Table 4.3a).

Table 4.2b Soil pH and organic content of randomly collected soil samples at Vlakfontein farm

Sample No.	pH	Organic Content (%)
1	7,68	0,55
2	7,47	1,32
3	7,31	0,76
4	7,26	0,98
Mean		0,90

There was a considerable difference in the pH of the soils at the three farms fluctuating between 4,34 and 8,23 (Table 4.3b). These differences in soil pH may well have a marked effect on the solubility, speciation and bio-availability of uranium, ^{226}Ra and other trace elements at these sites.

Table 4.3a Water quality of the Luipaardsvlei irrigation water

Water Quality Parameter	LP1	LP2	LP3
pH	8,1	6,4	6,9
Electrical Conductivity (μS.cm^{-1})	800	1100	900
Alkalinity (mg.ℓ^{-1})	47	22	35
Total Hardness (mg.ℓ^{-1})	219	148	185
Ammonia (mg.ℓ^{-1})	0,039	0,116	0,142
Nitrite (mg.ℓ^{-1})	0,01	0,01	0,01
Nitrate (mg.ℓ^{-1})	0,6	1,1	0,9
Phosphate (mg.ℓ^{-1})	0,05	0,16	0,10
Sulphate (mg.ℓ^{-1})	140	165	125

Table 4.3b Soil pH and organic content of the three Luipaardvlei farms

Farm	pH	Organic Content (%)
LP1	8,23	0,67
LP2	4,34	0,65
LP3	5,30	0,88

4.2 Uranium and ^{226}Ra in the water and soils of the Marievale, Vlakfontein and Luipaardsvlei study areas

According to the results obtained, concentrations for both uranium and ^{226}Ra were significantly lower than their respective standards laid down for drinking water at both the Marievale and Vlakfontein farms. At Luipaardsvlei, however, the composite irrigation water samples obtained from three adjacent localities yielded variable results with two values for uranium of more than 50 $\mu g.\ell^{-1}$. The highest of these values exceeded 56 $\mu g.\ell^{-1}$ (Table 4.4a). In both cases the drinking water guideline value of 44 $\mu g.\ell^{-1}$ for uranium (Funke, 1990) was exceeded. Somewhat different conditions existed for ^{226}Ra, where the mean and individual concentrations obtained for the various composite water samples at both Marievale and Vlakfontein were less than one tenth of that laid down for drinking water standards (Table 4.4a). Even at the Luipaardsvlei localities, where comparatively high uranium concentrations were recorded (Table 4.4a), the concentrations for ^{226}Ra were on the average still at least one tenth of that of the drinking water guideline (Funke, 1990).

No specific standards are available for uranium and ^{226}Ra in soils. However, concentration values were obtained for soils in the vicinity of uranium mines in other countries (Morishima *et al.*, 1977 ; Tracy *et al.*, 1983) as shown in Table 4.4b. In the case of ^{226}Ra, concentrations for radium in natural soils are provided by the IAEA (1990). In uranium soil core samples an extremely high concentration for this radionuclide (3,8 - 760 $\mu g.g^{-1}$: Table 4.4b) was recorded in the USA (Tracy *et al.*, 1983). Concentration values for uranium was only obtained from the field plots at the Vlakfontein and Luipaardsvlei areas. In the case of Vlakfontein, individual and mean concentrations (6,08 $\mu g.g^{-1}$) all exceeded those found for soils in Japan (2,8 - 5,6 $\mu g.g^{-1}$: Morishima *et al.*, 1977) and in some cases the USA (3,8 - 760 $\mu g.g^{-1}$: Tracy *et al.*, 1983) (Table 4.4b). At the Luipaardsvlei farms, a considerable variation in uranium concentration was found in the soils, which varied from 3,1 to 40,2 $\mu g.g^{-1}$. Uranium concentrations for these soils exceeded in all three cases those recorded in Japan and USA (Table 4.4b)

Concentrations of ^{226}Ra per dry mass soil.

Mean and individual concentrations for ^{226}Ra in the soils at Marievale, Vlakfontein and Luipaardsvlei were all in the range of concentrations quoted for natural soil by the IAEA (1990) (Table 4.4b).

Table 4.4a Concentrations of uranium and ^{226}Ra in water samples collected at the Marievale, Vlakfontein and Luipaardsvlei farms.

Sampling locality	Radionuclide	
	Uranium (μg.ℓ^{-1})	Radium (Bq.ℓ^{-1})
Marievale Farm		
Irrigation cycle (h): 1	16,8 ± 0,6	< 0,007
Irrigation cycle (h): 2	17,3 ± 0,7	0,02 ± 0,01
Irrigation cycle (h): 3	16,5 ± 0,6	0,010 ± 0,007
Irrigation cycle (h): 4	15,6 ± 0,6	0,02 ± 0,01
Irrigation cycle (h): 5	16,2 ± 0,6	0,016 ± 0,007
Irrigation cycle (h): 6	17,2 ± 0,7	0,012 ± 0,003
Average	16,6	0,014
Vlakfontein Farm		
Farm average	28,2 ± 0,8	0,075 ± 0,014
Luipaardsvlei		
LP1	52,6 ± 1,4	0,018 ± 0,006
LP2	10,6 ± 0,3	0,022 ± 0,008
LP3	56,1 ± 1,5	0,018 ± 0,007
Average	39,8	0,019
Drinking water guideline values[1]	44	0,19

[1] Uranium and radium in drinking water (South Africa) (Funke, 1990)

4.3 Uranium and ^{226}Ra concentrations in some aquatic organisms

The concentrations of uranium and ^{226}Ra in selected aquatic plants, macro-invertebrates, fish and aquatic birds collected during the preliminary survey are listed in Table 4.5. Variable but relatively high concentrations were obtained for the emergent, floating and submerged aquatic weeds collected at four different localities from the mining areas of the East Rand. Concentrations for uranium fluctuated between a highest of 111 μg.g^{-1} (*Typha capensis* : Blesbokspruit) and a lowest of 12,5 μg.g^{-1} (*T.capensis* :Germiston lake) (Table 4.5a). In the case of ^{226}Ra, values for *T. capensis* were again the highest in the Blesbokspruit (0,153 Bq.g^{-1}) followed by *Arundo donax* with a ^{226}Ra concentration of 0,195 Bq.g^{-1} (Germiston lake) (Table 4.5a). Comparatively high concentrations of ^{226}Ra were recorded for submerged and rooted floating weeds. The ^{226}Ra concentration for *Marsilea macrocarpa* was 0,082 Bq.g^{-1} and for *Lagarosiphon muscoides* and *Potamogeton pectinatus* 0,305 Bq.g^{-1} and 0,178 Bq.g^{-1}, respectively (Table 4.5a).

Table 4.4b Concentrations of uranium and ^{226}Ra in soil samples collected at the Marievale, Vlakfontein and Luipaardsvlei farms.

Sampling location	Radionuclide	
	Uranium (μg.g^{-1})	Radium (Bq.g^{-1})
Marievale Farm		
Lettuce	---	0,025 ± 0,004
Cabbage	---	0,019 ± 0,012
Cauliflower	---	0,019 ± 0,012
Beetroot	---	0,029 ± 0,006
Carrots	---	0,034 ± 0,003
Average	---	0,025
Vlakfontein		
Field Plot No 1	5,63 ± 0,16	(0,01)
Field Plot No 2	7,75 ± 0,19	0,032 ± 0,016
Field Plot No 3	6,40 ± 0,17	(0,005)
Field Plot No 4	4,86 ± 0,17	0,023 ± 0,009
Irrigation Pump	5,77 ± 0,16	0,030 ± 0,01
Average	6,08	0,028
Luipaardsvlei		
LP1	3,1 ± 0,1	(0,03)
LP2	13,6 ± 0,3	(0,02)
LP3	40,2 ± 0,8	0,066 ± 0,012
Average	19,0	(0,039)
Sediment guideline values		
Natural soil[1]	---	0,004 - 0,126
Uranium mine[2]	2,8 - 5,6	---
Uranium control location[3]	1,5	---
Uranium core[4]	3,8 - 760	---

[1] Radium in natural soils (IAEA, 1990)

[3] Uranium in control soil (Tracy *et al.*, 1983)

[2] Uranium nearby U-mine in agricultural soil (Morishima *et al.*, 1977

[4] Uranium in soil core 15-30 cm underground (Tracy *et al.*, 1983)

During the main survey, uranium and ^{226}Ra analysis were also made on *Spirogyra* sp. and *P. pectinatus* from a stream at Luipaardsvlei. Concentrations for uranium was comparatively high for both *Spirogyra* sp. (52,6 μg.g^{-1}) and *P. pectinatus* (85,9 μg.g^{-1}) (Table 4.5b). In both cases (*Spirogyra* sp.: 1,27 Bq.g^{-1} ; *P. pectinatus:* 0,76 Bq.g^{-1}) values obtained for ^{226}Ra were, however, relatively low (Table 4.5b). Compared to the aquatic plants, the concentration of uranium in the freshwater crab, *Potamonautes warreni*, was considerably lower than in the plants (1,37 μg.g^{-1} : Natalspruit). Its concentration of ^{226}Ra was 0,033 Bq.g^{-1} (Table 4.5a).

Comparing both the uranium and ^{226}Ra concentrations obtained for the different fish species analyzed, both radionuclides appeared in both instances to be also distinctly lower than those found for the associated aquatic plants. This particularly applies to uranium, where values fluctuated between 4,99 μg.g^{-1} (*Clarias gariepinus*: Blesbokspruit) and 0,52 μg.g^{-1} (*Barbus aeneus* : Natalspruit) (Table 4.5a). The concentration data given here are based on complete fish samples which gives a higher value than for the edible portion on its own. This is because radium accumulates in the bone and invariably the gut contains sediment and higher concentrations than the flesh.

In the two aquatic birds analyzed for uranium, levels were distinctly lower for *Fulica cristata* (0,56 μg.g^{-1}) and *Phalacrocorax carbo* ($<$0,17 μg.g^{-1}) than for the fish collected. However, concentrations for ^{226}Ra in both these birds remained about the same as for the crab and the fish (Table 4.5a).

4.4 Uranium and ^{226}Ra in selected vegetable crops and cattle fodder from the three different farms at Marievale, Vlakfontein and Luipaardsvlei

During the growing season of 1989-1992 collections were made of vegetable crops and cattle fodder at the three farms where water and soil samples were also analyzed for uranium and ^{226}Ra. The first investigation into the occurrence of these radionuclides in the vegetable crops took place at the Marievale farm. At this farm, carrots used in the analysis were pooled from different areas of the land from which it was cultivated. The other crops were randomly collected from the various field plots and individually analyzed to determine possible variation in concentrations (Table 4.6 - 4.9).

All material collected of the different crops were first dried and ashed and then sealed in bottles for later analysis according to procedures described in Chapter 3. This procedure lead to values obtained for both uranium and ^{226}Ra which in most cases were below the detection limit for this particular analytical procedure. During the subsequent sampling and analysis of vegetable crops at Vlakfontein and Luipaardsvlei, all biological samples were ashed but the materials were then acid digested and analyzed as liquid samples according to procedures described in Chapter 3. This approach enabled the detection of lower concentrations of both radionuclides and a considerable increase in the accuracy of the results.

Note: Concentration results are given for ash mass for plants, dry mass for soil and liquid mass for water while concentration ratios use the fresh mass concentrations for plants.

4.4.1 Marievale farm

Uranium values for the different vegetable crops cultivated at the Marievale farm are listed in Tables 4.6 - 4.9. The root vegetables yielded the highest values for uranium namely 0,23 μg.g^{-1} for carrots, and 0,21 μg.g^{-1} to 0,36 μg.g^{-1} for beetroot. This was followed by cauliflower with uranium concentrations varying between 0,1 μg.g^{-1} and 0,17 μg.g^{-1}. Lowest concentrations occurred in cabbage where concentrations fluctuated between 0,02 μg.g^{-1} and 0,027 μg.g^{-1} (Table 4.8). Where values were obtained for ^{226}Ra, the concentration levels for

Table 4.5a Aquatic plants, macro-invertebrates, fish and aquatic birds collected for uranium and ^{226}Ra analysis during 1989-1990.

Organism	Blesbokspruit		Elsburgspruit		Germiston lake		Natalspruit	
	Uranium ($\mu g.g^{-1}$, ash)	^{226}Ra ($Bq.g^{-1}$, ash)	Uranium ($\mu g.g^{-1}$, ash)	^{226}Ra ($Bq.g^{-1}$, ash)	Uranium ($\mu g.g^{-1}$, ash)	^{226}Ra ($Bq.g^{-1}$, ash)	Uranium ($\mu g.g^{-1}$, ash)	^{226}Ra ($Bq.g^{-1}$, ash)
AQUATIC PLANTS *Azolla filiculoides*	47,9	<0,099	---	---	---	---	---	---
Marsilea macrocarpa	37,9	<0,092	58,4	0,082 ± 0,011	---	---	---	---
Lemna major	---	---	---	---	---	---	31,7	<0,109
Lagarosiphon muscoides	---	---	---	---	---	---	26,4	0,305 ± 0,017
Potamogeton pectinatus	---	---	7,7	<0,039	---	---	18,0	0,178 ± 0,009
Typha capensis	111	0,153 ± 0,031	18,5	<0,037	12,5	0,097 ± 0,013	15,3	<0,076
Arundo donax	37,7	<0,162	15,8	<0,071	18,1	0,195 ± 0,015	14,2	<0,087
MACRO-INVERTEBRATES *Potamonautes warreni*	---	---	---	---	---	---	1,37	<0,033
FISH *Barbus aeneus*	---	---	---	---	---	---	0,52	<0,064
Cyprinus carpio	0,89	<0,045	---	---	---	---	---	---
Clarias gariepinus	4,99	<0,036	---	---	2,42	<0,065	0,60	<0,063
Tilapia sparrmanii	1,33	<0,081	---	---	---	---	---	---
AQUATIC BIRDS *Fulica cristata*	---	---	---	---	---	---	0,56	<0,034
Phalacrocorax carbo	---	---	---	---	---	---	<0,17	<0,074

Table 4.5b Aquatic plants collected at Luipaardsvlei during 1991-1992 and analyzed for uranium and ^{226}Ra.

Aquatic plants	Uranium ($Bq.g^{-1}$)	Radium ($Bq.g^{-1}$)
Spirogyra sp.	52,6 ± 0,9	1,27 ± 0,01
Potamogeton pectinatus	85,9 ± 0,8	0,76 ± 0,01

Table 4.6 **Concentrations of uranium and ^{226}Ra in root vegetables (carrots and beetroot) collected at Marievale farm with an indication of CRs (soil) and CRw (water) values.**

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium ($\mu g.g^{-1}$ ash, soil) ($\mu g.\ell^{-1}$ water)	^{226}Ra ($Bq.g^{-1}$ ash, soil) ($Bq.\ell^{-1}$ water)
Soil: $\overline{X} \pm$ S.D.	—	—	—	—	0,034 ± 0,0003
Water: $\overline{X} \pm$ S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Carrots : $\overline{X} \pm$ S.D.	224,56	23,32	2,54	0,23 ± 0,04	(0,01)
CRs	—	—	—	—	(0,003)
CRw	—	—	—	0,16 ± 0,03	(7,10)
Soil: $\overline{X} \pm$ S.D.	—	—	—	—	0,029 ± 0,006
Water: $\overline{X} \pm$ S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Beetrt. 1 : $\overline{X} \pm$ S.D.	95,34	16,29	1,88	0,36 ± 0,05	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,43 ± 0,07	N.D
Soil: $\overline{X} \pm$ S.D.	—	—	—	—	0,029 ± 0,006
Water: $\overline{X} \pm$ S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Beetrt. 2 : $\overline{X} \pm$ S.D.	81,09	14,08	1,18	N.D	(0,01)
CRs	—	—	—	—	(0,005)
CRw	—	—	—	N.D	(10,5)
Soil: $\overline{X} \pm$ S.D.	—	—	—	—	0,029 ± 0,006
Water: $\overline{X} \pm$ S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Beetrt. 3 : $\overline{X} \pm$ S.D.	110,07	19,15	3,96	0,21 ± 0,04	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,46 ± 0,1	N.D
Soil: $\overline{X} \pm$ S.D.	—	—	—	—	0,029 ± 0,006
Water: $\overline{X} \pm$ S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Beetrt. 4 : $\overline{X} \pm$ S.D.	82,6	14,68	2,28	0,2 ± 0,04	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,33 ± 0,07	N.D
Soil: $\overline{X} \pm$ S.D.	—	—	—	—	0,029 ± 0,006
Water: $\overline{X} \pm$ S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Beetrt. 5 : $\overline{X} \pm$ S.D.	104,9	18,55	2,68	N.D	(0,01)
CRs	—	—	—	—	(0,006)
CRw	—	—	—	N.D	(12,0)

* Concentrations below the detection limit are indicated in brackets.
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as $\mu g.g^{-1}$ and $Bq.g^{-1}$ respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as $\mu g.\ell^{-1}$ and $Bq.\ell^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as $\mu g.g^{-1}$ and $Bq.g^{-1}$ respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

Table 4.7 **Concentrations of uranium and ^{226}Ra in lettuce collected at Marievale farm with an indication of CRs (soil) and CRw (water) values.**

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium (μg.g^{-1} ash, soil) (μg.ℓ^{-1} water)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ^{-1} water)
Soil: $\bar{X}$ ± S.D.	---	---	---	---	0,025 ± 0,004
Water: $\bar{X}$ ± S.D.	---	---	---	16,6 ± 0,6	0,014 ± 0,007
Lettuce 1 : $\bar{X}$ ± S.D.	238,33	9,64	1,36	0,09 ± 0,03	N.D
CRs	---	---	---	---	N.D
CRw	---	---	---	0,03 ± 0,01	N.D
Soil: $\bar{X}$ ± S.D.	---	---	---	---	0,025 ± 0,004
Water: $\bar{X}$ ± S.D.	---	---	---	16,6 ± 0,6	0,014 ± 0,007
Lettuce 2 : $\bar{X}$ ± S.D.	321,70	12,98	1,81	0,17 ± 0,04	N.D
CRs	---	---	---	---	N.D
CRw	---	---	---	0,06 ± 0,02	N.D
Soil: $\bar{X}$ ± S.D.	---	---	---	---	0,025 ± 0,004
Water: $\bar{X}$ ± S.D.	---	---	---	16,6 ± 0,6	0,014 ± 0,007
Lettuce 3 : $\bar{X}$ ± S.D.	326,01	12,84	2,09	N.D	(0,01)
CRs	---	---	---	---	(0,0025)
CRw	---	---	---	N.D	(4,5)
Soil: $\bar{X}$ ± S.D.	---	---	---	---	0,025 ± 0,004
Water: $\bar{X}$ ± S.D.	---	---	---	16,6 ± 0,6	0,014 ± 0,007
Lettuce 4 : $\bar{X}$ ± S.D.	309,51	12,42	1,61	N.D	(0,01)
CRs	---	---	---	---	(0,002)
CRw	---	---	---	N.D	(3,7)
Soil: $\bar{X}$ ± S.D.	---	---	---	---	0,025 ± 0,004
Water: $\bar{X}$ ± S.D.	---	---	---	16,6 ± 0,6	0,014 ± 0,007
Lettuce 5 : $\bar{X}$ ± S.D.	162,09	6,63	0,97	0,08 ± 0,03	N.D
CRs	---	---	---	---	N.D
CRw	---	---	---	0,03 ± 0,01	N.D

* **Concentrations below the detection limit are indicated in brackets.**
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as μg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as μg.ℓ^{-1} and Bq.ℓ^{-1} respectively.
Uranium and ^{226}Ra in vegetables expressed as μg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

Table 4.8 Concentrations of uranium and ^{226}Ra in cabbage collected at Marievale farm with an indication of CRs (soil) and CRw (water) values.

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium (μg.g^{-1} ash, soil) (μg.ℓ$^{-1}$ water)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ$^{-1}$ water)
Soil: $\overline{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\overline{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Cabbage 1 : $\overline{X}$ ± S.D.	219,33	18,28	1,87	(0,02)	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	—	N.D
Soil: $\overline{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\overline{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Cabbage 2 : $\overline{X}$ ± S.D.	229,07	18,47	1,65	0,022 ± 0,02	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,0095 ± 0,009	N.D
Soil: $\overline{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\overline{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Cabbage 3 : $\overline{X}$ ± S.D.	224,1	18,63	1,63	0,027 ± 0,02	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,012 ± 0,009	N.D
Soil: $\overline{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\overline{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,007
Cabbage 4 : $\overline{X}$ ± S.D.	232,38	18,58	1,82	N.D	0,01 ± 0,005
CRs	—	—	—	N.D	0,004 ± 0,003
CRw	—	—	—	N.D	5,6 ± 3,2

* Concentrations below the detection limit are indicated in brackets.
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as μg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as μg.ℓ$^{-1}$ and Bq.ℓ$^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as μg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

Table 4.9a Concentrations of uranium and ^{226}Ra in cauliflower collected at Marievale farm with an indication of CRs (soil) and CRw (water) values.

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium ($\mu g.g^{-1}$ ash, soil) ($\mu g.\ell^{-1}$ water)	^{226}Ra ($Bq.g^{-1}$ ash, soil) ($Bq.\ell^{-1}$ water)
Soil: $\bar{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\bar{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,0074
Caulif. 1 : $\bar{X}$ ± S.D.	190,56	18,24	2,18	0,1 ± 0,03	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,07 ± 0,02	N.D
Soil: $\bar{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\bar{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,0074
Caulif. 2 : $\bar{X}$ ± S.D.	146,32	13,96	1,65	0,17 ± 0,04	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,12 ± 0,03	N.D
Soil: $\bar{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\bar{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,0074
Caulif. 3 : $\bar{X}$ ± S.D.	188,77	18,08	2,11	N.D	(0,01)
CRs	—	—	—	—	(0,006)
CRw	—	—	—	N.D	(0,008)
Soil: $\bar{X}$ ± S.D.	—	—	—	—	0,019 ± 0,012
Water: $\bar{X}$ ± S.D.	—	—	—	16,6 ± 0,6	0,014 ± 0,0074
Caulif. 4 : $\bar{X}$ ± S.D.	191,02	18,28	2,22	0,13 ± 0,03	N.D
CRs	—	—	—	—	N.D
CRw	—	—	—	0,09 ± 0,02	N.D

Table 4.9b Concentrations of uranium and ^{226}Ra in oats collected at Marievale mine slimes dam with an indication of CRs (soil) values.

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium ($\mu g.g^{-1}$ ash, soil) ($\mu g.\ell^{-1}$ water)	^{226}Ra ($Bq.g^{-1}$ ash, soil) ($Bq.\ell^{-1}$ water)
Soil: $\bar{X}$ ± S.D.	—	—	—	165 ± 3,0	0,79 ± 0,06
Water: $\bar{X}$ ± S.D.	—	—	—	—	—
Oats. 1 : $\bar{X}$ ± S.D.	540	54,5	5,45	50,1 ± 0,9	0,85 ± 0,02
CRs	—	—	—	0,0031 ± 0,0001	0,011 ± 0,001
CRw	—	—	—	—	—

* Concentrations below the detection limit are indicated in brackets.
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as $\mu g.g^{-1}$ and $Bq.g^{-1}$ respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as $\mu g.\ell^{-1}$ and $Bq.\ell^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as $\mu g.g^{-1}$ and $Bq.g^{-1}$ respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

the different crops were similar, fluctuating around 0,01 Bq.g^{-1} for both the root and leafy vegetable crops (Table 4.8). As shown in Tables 4.6 - 4.9, only the water/vegetable ratio for uranium was calculated whereas both water and soil concentrations of ^{226}Ra were used to determine the concentration ratios (CR) for the different crops. Highest CRw values for uranium were recorded for beetroot fluctuating between 0,33 and 0,46 (Table 4.6). This was followed by carrots (0,16) with variable ratios obtained for cauliflower (0,07 - 0,12), lettuce (0,03 - 0,06) and cabbage (0,0095 - 0,12) (Tables 4.6 - 4.9). Although the results were limited, indications are that the highest CR-values for both radionuclides occurred in the root vegetables.

With the exception of beetroot, CR-values for ^{226}Ra for both the water and soil were not only variable in both cases, but did not display any distinct tendencies. In the case of beetroot the CRw values were approximately double that of the soil (Table 4.6). In all the other cases, CR-values for both water and soil were extremely low, varying between 0,002 (Lettuce : CRs) and 5,6 (Cabbage : CRw) (Tables 4.6 - 4.9).

From the CR-values obtained for both uranium and ^{226}Ra, it can be deduced that these crops were not very efficient in accumulating these radionuclides from either the irrigation water or the soils in which they were grown.

4.4.2 Vlakfontein farm

The concentrations of uranium and ^{226}Ra in selected vegetable crops cultivated on this farm are summarized in Table 4.10. In addition to carrots, beetroot and lettuce, spinach, leeks and turnips were additional vegetables investigated for their ability to accumulate uranium and ^{226}Ra from the soil.

Concentration values for uranium and ^{226}Ra in the different vegetable crops grown at the Vlakfontein farm revealed interesting results. Where the root vegetables at Marievale farm generally had higher concentrations of both uranium and ^{226}Ra compared to the leafy vegetables, this situation did not apply to the spinach grown at Vlakfontein. Spinach leaves had the highest concentrations of all the crops with 2,5 μg.g^{-1} (ash) for uranium and 0,023 Bq.g^{-1} (ash) for ^{226}Ra (Table 4.10). Values obtained for leeks and lettuce were correspondingly lower than those for beetroot and carrots, but turnips yielded intermediate concentrations of 0,13 μg.g^{-1} for uranium and 0,0014 Bq.g^{-1} for ^{226}Ra, which in both cases were between those for leafy and root vegetables (Table 4.10).

The CR-values for the soil and water were roughly reflected by the actual concentrations of both radionuclides in the soil and water. Highest CRw values of 1,75 for uranium was recorded for spinach with a corresponding 0,008 for the CRs. The second highest CRw value, namely 0,86 was obtained for carrots. The CRs of uranium in carrots was, however, very low (0,004) and of the same order as for beetroot (0,0047) (Table 4.10). In the case of leeks, turnips and lettuce, the CR-values remained low fluctuating between 0,023 and 0,056 for water and 0,00011 to 0,00026 for soil (Table 4.10).

Table 4.10 **Concentrations of uranium and ^{226}Ra in selected vegetable crops collected at Vlakfontein farm with an indication of CRs (soil) and CRw (water) values.**

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium (μg.g^{-1} ash, soil) (μg.ℓ$^{-1}$ water)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ$^{-1}$ water)
Soil: $\bar{X}$ ± S.D.	—	—	—	6,1 ± 0,2	0,027 ± 0,001
Water: $\bar{X}$ ± S.D.	—	—	—	28,2 ± 0,80	0,075 ± 0,014
Spinach: $\bar{X}$ ± S.D.	2593	227	51,1	2,5 ± 0,1	0,023 ± 0,001
CRs	—	—	—	0,008 ± 0,0006	0,02 ± 0,008
CRw	—	—	—	1,75 ± 0,01	6,0 ± 0,01
Soil: $\bar{X}$ ± S.D.	—	—	—	6,1 ± 0,2	0,027 ± 0,001
Water: $\bar{X}$ ± S.D.	—	—	—	28,2 ± 0,80	0,075 ± 0,014
Leek: $\bar{X}$ ± S.D.	880	131	8,3	0,07 ± 0,02	(0,0011)
CRs	—	—	—	0,00011 ± 0,00003	(0,00037)
CRw	—	—	—	0,023 ± 0,008	(0,14)
Soil: $\bar{X}$ ± S.D.	—	—	—	6,1 ± 0,2	0,027 ± 0,001
Water: $\bar{X}$ ± S.D.	—	—	—	28,2 ± 0,80	0,075 ± 0,014
Turnip: $\bar{X}$ ± S.D.	1613	130	19,5	0,13 ± 0,02	0,0014 ± 0,0002
CRs	—	—	—	0,00026 ± 0,00005	0,00063 ± 0,00042
CRw	—	—	—	0,056 ± 0,008	0,23 ± 0,006
Soil: $\bar{X}$ ± S.D.	—	—	—	6,1 ± 0,2	0,027 ± 0,001
Water: $\bar{X}$ ± S.D.	—	—	—	28,2 ± 0,80	0,075 ± 0,014
Lettuce: $\bar{X}$ ± S.D.	2369	96,9	18,5	0,17 ± 0,03	0,0017 ± 0,0004
CRs	—	—	—	0,00022 ± 0,00005	0,00048 ± 0,00036
CRw	—	—	—	0,047 ± 0,001	0,18 ± 0,010
Soil: $\bar{X}$ ± S.D.	—	—	—	6,1 ± 0,2	0,027 ± 0,001
Water: $\bar{X}$ ± S.D.	—	—	—	28,2 ± 0,80	0,075 ± 0,014
Beetroot: $\bar{X}$ ± S.D.	1487	256	24,8	1,7 ± 0,1	0,003 ± 0,0005
CRs	—	—	—	0,0047 ± 0,0004	0,0019 ± 0,0012
CRw	—	—	—	1,0 ± 0,005	0,67 ± 0,014
Soil: $\bar{X}$ ± S.D.	—	—	—	6,1 ± 0,2	0,027 ± 0,001
Water: $\bar{X}$ ± S.D.	—	—	—	28,2 ± 0,80	0,075 ± 0,014
Carrot: $\bar{X}$ ± S.D.	1456	171	20,7	1,7 ± 0,20	0,0041 ± 0,0005
CRs	—	—	—	0,004 ± 0,0006	0,0022 ± 0,0013
CRw	—	—	—	0,86 ± 0,009	0,78 ± 0,017

***** **Concentrations below the detection limit are indicated in brackets.**
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as μg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as μg.ℓ$^{-1}$ and Bq.ℓ$^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as μg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

CRs-values for uranium were low, with a highest CR of 0,008 recorded for spinach, followed by beetroot (0,0047) and carrots (0,004). CRs-values for leeks, turnips and lettuce were of a similar order of magnitude, fluctuating between 0,00011 and 0,00026, respectively (Table 4.10).

The CRw-values for ^{226}Ra for leeks, turnips, lettuce, beetroot and carrots were of the same order of magnitude, fluctuating between 0,14 and 0,78. The only exception was spinach, which yielded a value of 6,0 which was one order of magnitude higher than the latter. Nearly a similar picture emerged from the CRs-values for ^{226}Ra. A highest CRs of 0,02 was recorded for spinach, while the other CRs-values ranged between 0,00037 (leeks) and 0,0022 (carrots).

There was a difference in the types of irrigation used at these farms. Where flood irrigation was practised at Vlakfontein, spray irrigation was used at Marievale. Factors such as soil-pH and species of these radionuclides involved may well have affected their concentrations in the different crops.

4.4.3 Luipaardsvlei

At Luipaardsvlei, spray irrigated fodder produced on the three adjacent farms as well as the milk from cows which were fed on this fodder, were analyzed for uranium and ^{226}Ra. Cow dung was also analyzed to determine any possible declines in radionuclide concentrations during the digestion process. All results are summarized in Tables 4.11 - 4.13. Considerable variation existed in the concentrations of uranium in the fodder from the three adjacent Luipaardsvlei farms varying from 5,0 μg.g^{-1} to 107 μg.g^{-1} (Table 4.11). Concentration values obtained for ^{226}Ra varied much less with values fluctuating between 0,055 to 0,094 Bq.g^{-1} (Table 4.11).

Calculating the CR-values for fodder from the soil and water for both uranium and ^{226}Ra, comparatively high values were found in all cases. The CRw values at all three farms fluctuated between 0,46 and 92,2 for uranium and 22,9 and 41,0 for ^{226}Ra. CRs-values on the other hand were much lower for both uranium (0,0025 - 0,072) and ^{226}Ra (0,00011 - 2,5), being a reflection in the first instance of the actual concentration of these radionuclides in the soil (Table 4.11).

Milk produced by the cows on two of these farms was analyzed for the presence of uranium and ^{226}Ra (Table 4.12). In both cases the uranium and ^{226}Ra concentrations were considerably lower in the milk than the concentrations recorded in the fodder and the dung. Concentrations of both uranium and ^{226}Ra in milk were extremely low varying between 0,21 to 0,22 μg.g^{-1} (U) and 0,0051 to 0,005 Bq.g^{-1} (^{226}Ra) (Table 4.12). The milk/fodder CR-ratios were in both cases low, in particular uranium, where the CR-values fluctuated between 0,029 and 0,044. In the case of ^{226}Ra, the CR values were higher, fluctuating between 0,185 and 0,37 (Table 4.12).

Table 4.11 Luipaardsvlei (Localities 1-3 (LP1 - LP3): Water and sediment transfer to cattle fodder)

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium ($\mu g.g^{-1}$ ash, soil) ($\mu g.\ell^{-1}$ water)	^{226}Ra ($Bq.g^{-1}$ ash, soil) ($Bq.\ell^{-1}$ water)
Soil: $\overline{X} \pm$ S.D.	—	—	—	3,1 ± 0,1	(0,003)
Water: $\overline{X} \pm$ S.D.	—	—	—	52,6 ± 1,4	0,018 ± 0,006
Fodder(LP1): $\overline{X} \pm$ S.D.	2917	285	14,2	5,0 ± 0,2	0,094 ± 0,029
Crs	—	—	—	0,0079 ± 0,0006	(1,5)
CRw	—	—	—	0,46 ± 0,03	25,4 ± 0,02
Soil: $\overline{X} \pm$ S.D.	—	—	—	13,6 ± 0,3	(0,02)
Water: $\overline{X} \pm$ S.D.	—	—	—	10,6 ± 0,3	0,022 ± 0,008
Fodder(LP2): $\overline{X} \pm$ S.D.	1744	334	16	107 ± 1,5	0,055 ± 0,003
Crs	—	—	—	0,072 ± 0,003	(2,5)
CRw	—	—	—	92,2 ± 0,004	22,9 ± 0,01
Soil: $\overline{X} \pm$ S.D.	—	—	—	40,2 ± 0,8	0,066 ± 0,012
Water: $\overline{X} \pm$ S.D.	—	—	—	56,1 ± 1,50	0,018 ± 0,007
Fodder(LP3): $\overline{X} \pm$ S.D.	2032	283	24,6	8,15 ± 0,25	0,061 ± 0,018
Crs	—	—	—	0,0025 ± 0,01	0,00011 ± 0,00005
CRw	—	—	—	1,8 ± 0,0001	41,0 ± 0,03

Table 4.12 Luipaardsvlei (Localities 1-2 (LP1 - LP2): Cattle fodder to milk transfer)

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium ($\mu g.g^{-1}$ ash, fodder, milk)	^{226}Ra ($Bq.g^{-1}$ ash, fodder, milk)
Fodder(LP1): $\overline{X} \pm$ S.D.	2917	285	14,2	5,0 ± 0,2	0,094 ± 0,029
Milk (LP1): $\overline{X} \pm$ S.D.	5726	559	19,4	(0,21)	0,05 ± 0,02
CRmilk/fodder	—	—	—	(0,029)	0,37 ± 0,0013
Fodder(LP2): $\overline{X} \pm$ S.D.	1744	334	16	106,5 ± 1,5	0,055 ± 0,029
Milk (LP2): $\overline{X} \pm$ S.D.	1364	147	25	0,22 ± 0,2	0,0051 ± 0,020
CRmilk/fodder	—	—	—	0,044 ± 0,00003	0,185 ± 0,008

Table 4.13 Luipaardsvlei (Localities 1-3 (LP1 - LP3): Cattle fodder to faeces transfer)

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	Uranium ($\mu g.g^{-1}$ ash, dung, fodder)	^{226}Ra ($Bq.g^{-1}$ ash, dung, fodder)
Fodder(LP1): $\overline{X} \pm$ S.D.	2917	285	14,2	5,0 ± 0,2	0,094 ± 0,029
Dung (LP1): $\overline{X} \pm$ S.D.	4031	820	20,3	22,5 ± 0,35	0,19 ± 0,02
CRdung/fodder	—	—	—	4,66 ± 0,001	2,09 ± 0,004
Fodder(LP2): $\overline{X} \pm$ S.D.	1744	334	16	106,5 ± 1,50	0,055 ± 0,029
Dung (LP2): $\overline{X} \pm$ S.D.	2242	847	25,4	6,4 ± 0,2	0,04 ± 0,003
CRdung/fodder	—	—	—	0,07 ± 0,00002	0,898 ± 0,005
Fodder(LP3): $\overline{X} \pm$ S.D.	2032	283	24,6	8,15 ± 0,25	0,061 ± 0,018
Dung (LP3): $\overline{X} \pm$ S.D.	2163	2051	34,8	2,3 ± 0,1	0,032 ± 0,011
CRdung/fodder	—	—	—	0,375 ± 0,00034	0,697 ± 0,005

Uranium and radium concentrations obtained from dung samples from the three adjacent Luipaardsvlei farms as well as the dung/fodder ratios for these radionuclides are listed in Table 4.13. Variation was found to exist for uranium between the concentrations of the cow dung samples from the three adjacent farms. This also applied to the ^{226}Ra concentrations. Uranium concentration in the cow dung samples fluctuated between 2,3 and 22,5 μg.g^{-1} whereas the concentrations for ^{226}Ra varied between 0,032 and 0,19 Bq.g^{-1} (Table 4.13).

Comparing the CR dung/fodder values for uranium and ^{226}Ra, considerable variation was obtained for the different farms. There was no relation in values for these two radionuclides, suggesting a number of factors which may have affected the bio-availability of both these radionuclides (Table 4.13).

4.5 Experimental uptake of ^{226}Ra by cabbage and beetroot

The experimental setup and application procedures for ^{226}Ra during the pot plant experiment are described in Chapter 3. Background data on the ^{226}Ra concentrations of the seedlings are listed in Table 4.14 whilst those on the ^{226}Ra application on beetroot and cabbage are listed in Tables 4.15 - 4.18. Natural ^{226}Ra concentrations in the beetroot and cabbage from unpolluted soils differed slightly being the highest in the beetroot again reflecting the better ability of the roots of these vegetables to accumulate this radionuclide from the soils.
In the case of beetroot (Table 4.15 - 4.16) there were some slight differences in the concentrations of ^{226}Ra depending on the site of application of the radionuclide. In the case where the roots were irrigated, the concentrations of ^{226}Ra in the root tissue (0,063 Bq.g^{-1}) was slightly higher than in the leaves (0,056 Bq.g^{-1}). Transfer of this radionuclide from the irrigated leaves to the roots were very effective, leading again to the higher concentration of ^{226}Ra in the roots (0,111 Bq.g^{-1}). Where both roots and leaves were irrigated with the radionuclide containing water, the roots again were better able to bioconcentrate ^{226}Ra.

Table 4.14 Background values for vegetables used in experimental uptake of ^{226}Ra

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	^{226}Ra (Bq.g^{-1} ash)
Beetroot background	873	79,6	27,6	0,0091 ± 0,0006
Cabbage background	610	56,2	6,8	0,007 ± 0,001

In the control plants, the levels of the natural ^{226}Ra were also higher in the roots than in the leaves (Tables 4.15 - 4.16).

Where the roots were irrigated, the CRs-values showed no significant difference between the roots and the leaves. In the cases where the leaves and roots & leaves were irrigated, the

Table 4.15 Experimental uptake of ^{226}Ra by the roots of beetroot

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ$^{-1}$, water)
Soil: $\bar{X}$ ± S.D.	—	—	—	0,04 ± 0,015
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Beetroot (Roots irr.) : $\bar{X}$ ± S.D.	253	38,3	2,65	0,063 ± 0,006
CRs	—	—	—	0,016 ± 0,008
CRw	—	—	—	0,029 ± 0,003
Soil: $\bar{X}$ ± S.D.	—	—	—	0,084 ± 0,015
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Beetroot (Leaves irr.) : $\bar{X}$ ± S.D.	215	34,1	2,9	0,111 ± 0,007
CRs	—	—	—	0,018 ± 0,004
CRw	—	—	—	0,066 ± 0,004
Soil: $\bar{X}$ ± S.D.	—	—	—	0,082 ± 0,018
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Beetroot (Rt + Lvs irr.) : $\bar{X}$ ± S.D.	144	22,9	2,6	0,15 ± 0,009
CRs	—	—	—	0,033 ± 0,009
CRw	—	—	—	0,12 ± 0,007
Soil: $\bar{X}$ ± S.D.	—	—	—	(0,022)
Water: $\bar{X}$ ± S.D.	—	—	—	—
Beetroot (Control) : $\bar{X}$ ± S.D.	375	51,9	4,9	0,02 ± 0,003
CRs	—	—	—	(0,012)
CRw	—	—	—	—

* Concentrations below the detection limit are indicated in brackets.
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as μg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as μg.ℓ$^{-1}$ and Bq.ℓ$^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as μg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

Table 4.16 Experimental uptake of ^{226}Ra by the leaves of beetroot

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ$^{-1}$, water)
Soil: $\bar{X}$ ± S.D.	—	—	—	0,04 ± 0,015
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Beetroot (Roots irr.) : $\bar{X}$ ± S.D.	777	77,1	9,9	0,056 ± 0,004
CRs	—	—	—	0,018 ± 0,008
CRw	—	—	—	0,032 ± 0,002
Soil: $\bar{X}$ ± S.D.	—	—	—	0,084 ± 0,015
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Beetroot (Leaves irr.) : $\bar{X}$ ± S.D.	991	92,1	11,4	0,057 ± 0,003
CRs	—	—	—	0,0078 ± 0,002
CRw	—	—	—	0,029 ± 0,002
Soil: $\bar{X}$ ± S.D.	—	—	—	0,082 ± 0,018
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Beetroot (Rt + Lvs irr.) : $\bar{X}$ ± S.D.	1063	95,4	12,2	0,09 ± 0,004
CRs	—	—	—	0,013 ± 0,003
CRw	—	—	—	0,046 ± 0,002
Soil: $\bar{X}$ ± S.D.	—	—	—	(0,022)
Water: $\bar{X}$ ± S.D.	—	—	—	—
Beetroot (Control) : $\bar{X}$ ± S.D.	1033	88,4	8,9	0,012 ± 0,001
CRs	—	—	—	(0,0047)
CRw	—	—	—	—

- **Concentrations below the detection limit are indicated in brackets.**
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as μg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as μg.ℓ$^{-1}$ and Bq.ℓ$^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as μg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

Table 4.17 Experimental uptake of ^{226}Ra by the roots of cabbage

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ^{-1}, water)
Soil: $\overline{X}$ ± S.D.	—	—	—	0,034 ± 0,015
Water: $\overline{X}$ ± S.D.	—	—	—	22,5
Cabbage (Roots irr.) : $\overline{X}$ ± S.D.	96,8	15,1	1,5	0,113 ± 0,014
CRs	—	—	—	0,052 ± 0,03
CRw	—	—	—	0,078 ± 0,01
Soil: $\overline{X}$ ± S.D.	—	—	—	0,063 ± 0,014
Water: $\overline{X}$ ± S.D.	—	—	—	22,5
Cabbage (Leaves irr.) : $\overline{X}$ ± S.D.	134	21,5	3,5	0,106 ± 0,007
CRs	—	—	—	0,044 ± 0,01
CRw	—	—	—	0,123 ± 0,08
Soil: $\overline{X}$ ± S.D.	—	—	—	0,076 ± 0,014
Water: $\overline{X}$ ± S.D.	—	—	—	22,5
Cabbage (Rt + Lvs irr.) : $\overline{X}$ ± S.D.	156	24,8	5,3	0,262 ± 0,01
CRs	—	—	—	0,117 ± 0,03
CRw	—	—	—	0,396 ± 0,02
Soil: $\overline{X}$ ± S.D.	—	—	—	(0,014)
Water: $\overline{X}$ ± S.D.	—	—	—	—
Cabbage (Control) : $\overline{X}$ ± S.D.	94	13,3	1,1	(0,02)
CRs	—	—	—	(0,017)
CRw	—	—	—	—

* Concentrations below the detection limit are indicated in brackets.
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as µg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as µg.ℓ^{-1} and Bq.ℓ^{-1} respectively.
Uranium and ^{226}Ra in vegetables expressed as µg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 µg

Table 4.18 Experimental uptake of ^{226}Ra by the leaves of cabbage

Sample	Fresh mass (g)	Dry mass (g)	Ash mass (g)	^{226}Ra (Bq.g^{-1} ash, soil) (Bq.ℓ$^{-1}$, water)
Soil: $\bar{X}$ ± S.D.	—	—	—	0,034 ± 0,015
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Cabbage (Roots irr.) : $\bar{X}$ ± S.D.	909	90,3	9,0	0,057 ± 0,003
CRs	—	—	—	0,017 ± 0,008
CRw	—	—	—	0,025 ± 0,001
Soil: $\bar{X}$ ± S.D.	—	—	—	0,063 ± 0,014
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Cabbage (Leaves irr.) : $\bar{X}$ ± S.D.	976	96,5	11,3	0,028 ± 0,003
CRs	—	—	—	0,0051 ± 0,002
CRw	—	—	—	0,014 ± 0,002
Soil: $\bar{X}$ ± S.D.	—	—	—	0,076 ± 0,014
Water: $\bar{X}$ ± S.D.	—	—	—	22,5
Cabbage (Rt + Lvs irr.) : $\bar{X}$ ± S.D.	828	82,1	9,9	0,097 ± 0,012
CRs	—	—	—	0,015 ± 0,005
CRw	—	—	—	0,052 ± 0,006
Soil: $\bar{X}$ ± S.D.	—	—	—	(0,014)
Water: $\bar{X}$ ± S.D.	—	—	—	—
Cabbage (Control) : $\bar{X}$ ± S.D.	593	53,7	6,8	0,008 ± 0,001
CRs	—	—	—	(0,0066)
CRw	—	—	—	—

- **Concentrations below the detection limit are indicated in brackets.**
Standard deviations were calculated on counting statistics and not on replicate analysis.

—: = Not Determined N.D.: = Below detection limit but no estimated value could be given.

Uranium and ^{226}Ra in soil expressed as μg.g^{-1} and Bq.g^{-1} respectively for a dried sample.
Uranium and ^{226}Ra in water expressed as μg.ℓ$^{-1}$ and Bq.ℓ$^{-1}$ respectively.
Uranium and ^{226}Ra in vegetables expressed as μg.g^{-1} and Bq.g^{-1} respectively for an ashed sample.

CRs: Concentration Ratio of plant (fresh mass) (per gram) concentration over soil concentration (per gram)
CRw: Concentration Ratio of plant (fresh mass) (per gram) concentration over water concentration (per 1mℓ)

For ^{238}U: 1Bq = 80,9 μg

CRs (roots analyzed) were distinctly higher than the CRs (leaves irrigated) ratios. There was also a marked difference between the CRs (roots analyzed) and CRs (leaves analyzed) for the controls, which cannot be explained (Tables 4.15 - 4.16), except to say that the soil concentration was below the detection limit.

In the case of the CRw-values for beetroot a similar picture emerged, showing a slightly higher CRw (leaves analyzed) ratio (0,032) than the CRw (roots analyzed) (0,029). In the case of the irrigated leaves and roots & leaves, the ratio again was in favour of the roots.

A comparison of the ability of the roots and leaves of the cabbage plants to accumulate ^{226}Ra clearly showed the roots more capable to bioconcentrate this radionuclide. In all three types of irrigation systems the concentration ratio from soil to the roots were clearly the highest, varying between 0,044 (leaves irrigated) and 0,052 (roots irrigated) to 0,117 for roots and leaves irrigated (Tables 4.17 - 4.18). Control values also showed some difference, with the concentration of ^{226}Ra being low in both cases.

In the case of beetroot and cabbage the CRs and CRw values again confirmed the superior ability of the roots over the leaves to accumulate ^{226}Ra, being true for all three irrigation types. The difference in the concentration ratios from the soil to roots and leaves varies from a fraction to about 3 standard deviations. The differences are more apparent in the CRw to roots and leaves, due to the smaller standard deviations on the CRw's. The CRw values obtained were in all cases higher than those for CRs.

Another interesting observation is that the soil surrounding the roots of the plants in the leaves-only irrigation scheme afterwards also had an enhanced ^{226}Ra concentration. This concentration was in the case of both beetroot and cabbage higher than the soil from the roots-only irrigation scheme, but comparable to the roots and leaves irrigation scheme.

4.6 Dose assessment results

The dose results are interpreted in terms of the International Commission on Radiological Protection (ICRP, 1991) recommendations on dose limits. The effective dose limit for members of the public is 1 mSv in a year but in special circumstances a higher value of effective dose could be allowed in a single year, provided that the average dose over 5 years does not exceed 1 mSv per year. The limit applies to the sum of the doses from external exposure and 50 year committed dose from ingestion and inhalation.

For a single practice resulting in a radiation dose to the public the annual dose is further restricted to 250 μSv. This is to allow for additional doses from other sources such that the combined dose does not exceed the 1000 μSv per year limit. The exposure resulting from mining activities should be subject to this lower limit. Table 4.19 gives the annual effective dose D for ^{238}U and ^{226}Ra for the first year of exposure and the annual committed effective dose, D_{50}, over a committed period of 50 years, for ingestion of drinking water, vegetables, cereals and fish, from the concentrations and volumes mentioned in Table 3.6.

4.6.1 Drinking water and vegetable pathway

Field observations show that the two most likely significant ingestion pathways for this study are drinking water and vegetables. In these cases the first year exposure values are all very low being individually less than 1% of the 250 μSv limit. The annual committed effective dose provides higher values, but still well within the limit. The committed dose due to ingestion of radium is much higher than for uranium because radium is a bone seeker and has a much longer biological half life than uranium.

The doses from drinking water pathway are greater than for vegetable consumption.The dose from the drinking water is however exaggerated by deliberately assuming consumption values of 730 l per year, the value for standard man. In practice, surface water is not routinely used for drinking and cooking. The dose from the vegetable pathway assumes that all vegetable intake annually (30kg from leafy and 90kg from root crops) is from a contaminated source. In practice this is very unlikely. The model dose results will therefore decrease with lower expected consumption habits.

The conclusion is that the potential effective dose received from the most likely pathway of drinking water and consuming vegetables irrigated with contaminated water is well within limits set internationally.

4.6.2 Cereal pathway

This pathway was included because of an isolated occurrence of oats being experimentally cultivated directly on a mine dump. The concentrations of uranium and radium in the oats was the highest of any produce measured in this study and in fact ranks with the maximums encountered elsewhere in the world. For modelling purposes an annual consumption of 80 kg was assumed. The resulting effective dose for uranium is low being about 1% of the 250 μSv limit. The effective dose from radium is however much higher with the committed effective dose being 180 μSv.

At this particular site the area under cultivation was small, about 24 m^2 and took place for only 2 years and has been now discontinued. The occurrence however serves as a warning that any crop production on mine dump land should be controlled.

4.6.3 Fish pathway

In this study a limited number of fish were sampled in the field and the concentration data are for complete fish samples. However, from a previous confidential report other data for edible part of fish samples exists and for completeness sake a fish pathway was included in the modelling study. The assumed consumption of 25 kg fish annually is based on that of a sports fisherman. Probable species include mixed portions of the common carp, *Cyprinus carpio*, the african catfish, *Clarias gariepinus* and the smallmouthed yellowfish, *Barbus aeneus*. Once more the dose due to uranium can be neglected while the committed effective dose from radium is 16 μSv which is considerably below the limit. The amount of fish caught and consumed is however small compared to the amount of vegetables produced, and therefore the significance of this pathway should be negligible.

4.6.4 Miscellaneous pathways

By definition this project has excluded some pathways of public exposure such as the inhalation of radon gas and radioactive dust. Other components of aquatic pathways that are usually modelled are direct exposure due to swimming, boating and sunbathing on contaminated sediments. In this study the latter pathways were excluded since in any case they are usually a very small fraction of the different pathways. Other possible pathways are direct exposure from the contaminated irrigated lands and internal exposure from inadvertent soil ingestion. For the latter the model assumes a default value of 410 mg per day and for a soil radium concentration of 25 Bq.kg^{-1} the committed effective dose is 10 μSv. For direct exposure from such soil for 2000 hours per year the annual effective dose is 2,3E-2 μSv (Table 4.19).

4.6.5 Summary

In summary, the summation of doses for an assumed maximum exposed individual due to ingestion of water, vegetables as well as soil (accidental), plus direct exposure from soil will give an overall committed effective dose significantly less than 250 μSv and even more reduced for a more average individual. With the addition of the very unlikely pathway of either cereal (oats) grown on mine dump land or fish consumption from contaminated water the limit of 250 μSv/a may, however, be approached.

The collective dose for the population in general has not been estimated but for the aquatic pathway is considered to be low. Further downstream from mines the radionuclide concentrations decrease even more and the public at large is even less likely to consume surface water in large amounts. The relatively small mass of potentially contaminated food products will be greatly diluted in the large open market place and any possible dose will be greatly fractionated.

Note: The GENII code uses the organ weighing factors of the ICRP 26 and 30 publications. The more recent ICRP 60 of 1990 (ICRP, 1991) recommends changes in these factors. For radium being a bone seeker the important change is the reduction in the bone surface weighing factor from 0,03 to 0,01. This has the effect that the results reported here over estimate the committed effective dose. For uranium the kidney and stomach weighing factor changes of 0,06 to 0,025 and 0,06 to 0,12 respectively are relevant. These compensate for each other and significant changes in effective dose are not expected.

Table 4.19 Annual Effective dose (D) and Annual Committed Effective Dose (D50) from various ingestion pathways - Units: μSv.

Ingestion Parameter	Radionuclide	
	^{238}U	^{226}Ra
Drinking water		
D	1,7	0,88
D_{50}	2,3	7,8
Vegetables (leafy)		
D	0,042	0,22
D_{50}	0,057	1,95
Vegetables (root)		
D	0,12	0,65
D_{50}	0,17	6,0
Oats		
D	2,3	20
D_{50}	3,1	180
Fish		
D	-	1,8
D_{50}	0,02	16,0
Soil ingested		
D	-	1,3
D_{50}	-	10
Soil external		
D	1,3E-2	2,3E-2
D_{50}	-	-

DISCUSSION AND RECOMMENDATIONS

5

5 DISCUSSION AND RECOMMENDATIONS

5.1 Introduction

Due to the relatively uniform distribution of uraninite- and gold-containing conglomerates, virtually all uranium is produced as a by-product in South African gold mines. During the first part of local uranium mining and milling processes, environmental contamination of aquatic and terrestrial ecosystems may occur. Uranium-contaminated water usually contain a number of primordial radionuclides of which ^{226}Ra is considered to be the most radiotoxic element. This radionuclide which posesses no economic value, and is therefore not recovered during the actual extraction process of uranium, is usually discarded with processed sludge onto slimes dams. In addition, the risk of environmental contamination with ^{226}Ra is enhanced through the extraction of contaminated underground water as well as the release of mine process water onto arable land and public water sources (de Jesus *et al.*, 1987).

Concern about the potential radiotoxic effects of ^{226}Ra (and daughter nuclides) in the environment, as well as a lack of information on the behaviour of this element on tailings dams and in surface and underground waters, have prompted investigations by local researchers such as Malan (1981) and de Jesus *et al.* (1987). While investigations by Malan (1981) mainly concentrated on the occurrence of ^{226}Ra on and around gold/uranium mine tailings, de Jesus *et al.* (1987) extended the scope of their research towards the occurrence and concentration of this radionuclide in the abiotic and biotic components of affected aquatic and terrestrial ecosystems.

However, the need still existed to identify possible pathways of exposure and to model radionuclide concentrations in the environment in terms of exposure through consumption of contaminated water and agricultural crops. In addition to the latter, the aim of this investigation also included the expression of uranium and ^{226}Ra concentrations in biota (plants, animals and agricultural crops) in terms of concentrations measured in water and aquatic sediments or terrestrial soils. The resulting Concentration Ratio (CR) facilitated a comparison of results on an equal level with those obtained from research conducted in other countries.

It should be noted, however, that the average uranium concentration in South African ores (0,01%) is at least an order of magnitude lower than that mined in the rest of the world (Funke, 1990). It was therefore accepted that roughly the same estimate might be expected when comparing South African data on radionuclide concentrations of biota subjected to these radionuclides in nature compared to those recorded in some overseas countries.

The following sections of this chapter will be devoted to the discussion of results obtained during this study, the comparison between the latter and results from overseas studies, and where possible, applicable explanations and speculations to justify a number of phenomena obtained.

5.2 Submerged and rooted emergent aquatic macrophytes

Considering results obtained for uranium and ^{226}Ra concentrations in submerged algae collected in mine polluted rivers on the East Rand on the Witwatersrand goldfields, overall lower concentrations for both radionuclides were recorded than concentrations measured for related species in other countries. For example, *Spirogyra* sp. collected at Luipaardsvlei contained 1,27 Bq.g^{-1} ^{226}Ra and 52,6 μg.g^{-1} uranium whilst a mixture of the green filamentous algae, *Fontinalis antipyretica* L. (70%), *Platyhypnidium riparioides* (Hedw.) Podp. (23%), *Cladophora glomerata* (L.) Kutz. (3%), *Oedogonium capillare* Kutz (2%) and *Rhizoclonium hieroglyphicum* (Ag) Kutz (2%) from the uranium mine polluted Nedvedieka and Mze rivers in Moravia and Bohemia (Czechoslovakia) yielded values of 3,73 Bq.g^{-1} for ^{226}Ra and 600 μg.g^{-1}, uranium respectively (Justyn & Stanek, 1974). In both these cases, values were more than double those measured in *Spirogyra* sp. from the mine polluted rivers in South Africa. However, the above authors state that Czechoslovakian uranium mining and milling wastes are usually discarded into smaller rivers without being treated. Subsequently, these wastes are relatively little diluted by river water and ^{226}Ra and uranium concentrations of 265 Bq.g^{-1} and 100 μg.g^{-1}, respectively were recorded in the sediments of these affected rivers. The latter authors do not state water concentrations, but it can be accepted that significantly high concentrations of these radionuclides were present in this water. The above sediment concentrations are significantly higher than those obtained locally and can probably be ascribed to either of the following: (i) lower local ore-concentrations, or (ii) differences in effluent disposal practices between South Africa and Czechoslovakia. It is evident that effluent disposal and environmental management is not as controlled there as practiced locally, thereby increasing the risk of environmental contamination. The ability of uptake of the different algal species mentioned can be accepted as more or less similar.

Tsivoglou *et al.* (1960), monitoring the Animas river in the vicinity of uranium mills at Colorado, recorded a concentration of 0,148 Bq.g^{-1} for ^{226}Ra in algae (species not specified) from unpolluted sections of the river. However, mine polluted sections of the same river yielded algae with a ^{226}Ra concentration of 129,5 Bq.g^{-1}. *Spirogyra* sp. from uranium mine polluted waters in Jaduguda, India (Markose *et al.*, 1978), also showed relatively high ^{226}Ra concentrations, ranging between 2,51 and 89,3 Bq.g^{-1}, which in all cases (6 sampling localities), were generally higher than those recorded during this study for this genus in South Africa. In both the above cases, elevated sediment concentrations caused by inadequate mining practices led to enhanced accumulation of ^{226}Ra and uranium in the tissues of these algae.

Although concentrations of ^{226}Ra in *Spirogyra* sp. collected locally tended to be lower than most other values measured in overseas countries, an elevated accumulation of ^{226}Ra from the water and sediments by this algal species is evident (Figures 5.1 and 5.2). However, it is thought that the elevated ^{226}Ra levels in *Spirogyra* sp. cannot be ascribed to active uptake, but rather to one or both of the following factors:

- It was found (Havlik, 1971 ; Havlik & Robertson, 1971) in a number of cases that an unknown concentration of radionuclides and other elements may be bound to the outer surface of the cell wall of the plant. They are therefore not absorbed and accumulated by this algae, thus making it difficult to distinguish between the relative amounts adsorbed on the cell wall and those accumulated.

The cell wall, consisting of a porous matrix of gelatinous polysaccharide fibres surrounding the cell membrane is the first barrier between an aquatic plant and the radium entering it from the surrounding water column (Alberts *et al.*, 1983). The kinetics of accumulation of several cationic, anionic and neutral metal ions forming complexes with freshwater algal cell walls have been demonstrated by Stary *et al.* (1983). Their results showed that separate sites on the cell walls were selective to the three ionic groups. Of the three groups, the metal cations were the most strongly accumulated while radium was the best accumulated amongst the alkaline and alkaline earth elements (Stary *et al.*, 1984). By chemically removing loosely bound ^{226}Ra from the cell walls of a range of algae, Havlik (1971) and Havlik & Robertson (1971) could demonstrate that algal cell walls play a major role in ^{226}Ra capture which could therefore not be considered as bioaccumulation.

Differences in preparation techniques of plant samples have also produced large variations in results and therefore caution us in the interpretation of bio-accumulation investigations. By using different washing and drying techniques for the preparation of aquatic bryophytes, Wehr *et al.* (1983) produced results varying up to tenfold. During this investigation, the possible differences in preparation methods were not investigated.

- On the cell walls of algae and on the roots of aquatic macrophytes, epiphytes or aufwuchs (consisting of smaller algae and bacteria) develop rapidly and extensively. Besides being capable of accumulating radionuclides, it is also thought that the aufwuchs may in fact modify the actual capacity of these plants to accumulate elements from the water considerably. Although not proven yet, Emerson and Hesslein (1973) have reported significant accumulation of ^{226}Ra by epiphytes growing on rocks. In fact, no special studies on radium uptake by epiphytes growing on plants have been reported yet, and could well provide an interesting subject for future investigations.

When the abovementioned factors are taken into account and brought into relation with results obtained during this study, both could well have had an influence on the data as no special washing and drying procedures to remove aufwuchs were undertaken.

In the case of the aquatic macrophytes, both the concentrations of uranium and ^{226}Ra in *Potamogeton pectinatus* from Luipaardsvlei (0,76 Bq.g^{-1}), Elsburgspruit (<0,039 Bq.g^{-1}) and Natalspruit (0,178 Bq.g^{-1}) were lower than those recorded for the same species at Langley Bay, Canada, which is affected by uranium mine mill tailings (Waite *et al.*, 1988). While the concentrations of uranium and ^{226}Ra in the same aquatic weed at the Langley Bay site was 5130 μg.g^{-1} and 56,5 Bq.g^{-1} respectively, values were in both cases higher than those recorded for a nearby control locality (U: 70 μg.g^{-1} ; ^{226}Ra: 0,37 Bq.g^{-1}). It is however interesting to note that the uranium and ^{226}Ra concentrations obtained for *P. pectinatus* from the mine polluted Elsburgspruit and Natalspruit rivers were even lower than those obtained for the Langley Bay control values. Again, these lower concentrations could be ascribed to lower ore and environmental concentrations of uranium and ^{226}Ra. However, when comparing ^{226}Ra concentrations in the tissues of *P. pectinatus* and surrounding sediments (Figure 5.2), this macrophyte also exhibits the ability to accumulate ^{226}Ra at elevated levels. This plant also shows similar abilities to accumulate metals under local conditions as revealed in Part 2 of this study.

No data exist in the literature on the radionuclides uranium and ^{226}Ra accumulated by aquatic macrophytes such as *Lemna* sp., *Azolla filiculoides*, *Marsilea macrocarpa*, *Lagarosiphon muscoides* and *Arundo donax*. In the present study, all values found for these plants seemed to fall within the same order of magnitude for uranium and ^{226}Ra, respectively. In the case of the floating aquatic weeds, uranium (47,9 μg.g^{-1}) and ^{226}Ra (0,099 Bq.g^{-1}) recorded in *Azolla filiculoides* (Blesbokspruit) showed slight differences with those of *Lemna major* from the Natalspruit (U: 31,7 μg.g^{-1} ; ^{226}Ra: 0,109 Bq.g^{-1}). The rooted submerged aquatic fern, *Marsilea macrocarpa*, showed values ranging between 37,9 (Blesbokspruit) and 58,4 (Elsburgspruit) μg.g^{-1} for uranium while ^{226}Ra fluctuated between 0,082 (Elsburgspruit) and 0,092 (Blesbokspruit) Bq.g^{-1}. The other rooted submerged aquatic weed analyzed, *Lagarosiphon muscoides*, showed a somewhat different tendency. While the uranium (26,4 μg.g^{-1}) concentration seemed to be within range of the other aquatic weeds, this plant possessed a marked ability to accumulate ^{226}Ra (0,305 Bq.g^{-1}). Again, this phenomenon could, as was the case with *Spirogyra* sp., be attributed to either the presence of aufwuchs which enhances the "ability" of these plants to accumulate radionuclides, or the presence of the latter and other elements adsorbed on the outer cell walls.

Uranium and ^{226}Ra concentrations from the stems (U: 5 μg.g^{-1} ; ^{226}Ra: 1,3 Bq.g^{-1}) and roots (U: 85 μg.g^{-1} ; ^{226}Ra: 18,7 Bq.g^{-1}) of *Typha* sp. collected at Langley Bay, Canada, were generally higher than concentrations from whole composite (roots + stems) samples for the same species found in the Elsburgspruit (<0,037 Bq.g^{-1}), Germiston lake (0,097 Bq.g^{-1}) and the Natalspruit (<0,076 Bq.g^{-1}). The only exceptionally local high uranium value for *T. capensis* was recorded in the Blesbokspruit where a concentration of 111 μg.g^{-1} was recorded. It should, however, be noted that this locality in the Blesbokspruit is situated adjacent to a disused slimes dam. Often, during the rainy season, it was found that significant amounts of seepage water from this slimes dam reached the particular stream. During the period of sample collection, slimes dams recovery operations on this particular dam was also taking place. Using water cannons to collect the slime, significant uncontrolled runoff from the slimes dam was created, contaminating the stream and thereby providing these plants with a source of radionuclides. This also applied to *T. capensis,* which was also collected at the same locality at the same time.

A highest ^{226}Ra concentration of 0,195 Bq.g^{-1} was recorded for *A. donax* at Germiston lake while the same plant produced a lowest concentration of 0,071 Bq.g^{-1} at the Elsburgspruit. The higher ^{226}Ra concentration recorded for this plant in Germiston lake can most likely be ascribed to seepage from the slimes dams of the defunct Simmer and Jack mine, reaching this lake via a stream. As mining operations have ceased a number of years ago, no slimes dams management was practiced during the survey, which could possibly explain the elevated ^{226}Ra levels measured in *A. donax* from Germiston lake.

A comparison of the relationship between ^{226}Ra concentrations in the tissues of aquatic macrophytes and in the supporting sediments reveal that, except in the case of *Spirogyra* sp. and *P. pectinatus*, all plant ^{226}Ra concentrations measured during the present study generally fall within the vicinity of the regression line $Q = 0{,}39C^{0{,}49}$ presented in Figure 5.2. This supports the idea that for these plants, a positive relationship exists between sediment and plant tissue concentrations.

Some significant differences regarding sources of elemental uptake exist between the different plants found in a wetland ecosystem. Algae (*Spirogyra* sp.) and floating aquatic macrophytes (*A. filiculoides* and *Lemna major*) usually have no contact with underlying sediments, and therefore obtain most of their nutrition from the water column. On the other hand, submerged rooted aquatic macrophytes such as *P. pectinatus* and *M. macrocarpa* obtain nutrients and other elements from both the water column as well as sediments surrounding these plants. In the case of rooted emergent aquatic macrophytes such as *T. capensis* and *A. donax*, most nutrients and other elements are mostly obtained from the surrounding sediments. A small portion is obtained from the water column through sections of the stems which might be submerged, depending on the water level. In the case of these plants, most elements are stored in the rhizomes and translocated to aerial parts (leaves, stems, flowers) according to demand.

Aquatic sediments are generally anaerobic as they are saturated with water. Usually the levels of the sediments are below that of water mixing which would allow for some oxygenation to occur. In anaerobic sediments a number of electrochemical changes take place. Sediments usually tend to be in a reduced state (a well drained and oxygenated soil or sediment usually tends to be in an oxidized state). The low redox potentials (Eh = 0,2V to -0,4V) of submerged anaerobic sediments reflects the reduced state, while the high redox potentials (Eh = 0,8V to 3,0V) of aerobic media reflect an oxidized condition (Ponnamperuma, 1972). When the Eh drops below 0,2V (in anaerobic sediments), aquatic sediments (which include mud, clay, grains and organic material) undergo reduction, lose their absorptive capacity and release metals, toxicants, phosphates and silica into the water column and interstitial water. Consequently, an increase of available element concentrations as well as an increase in the bio-availability of elements and nutrients to aquatic fauna and flora can be expected. In most cases, concentrations of metals and other elements in water in contact with anaerobic sediments could be up to 10 times higher than in supernatant water (Reinhard & Förstner, 1976). Under such conditions, available radium remains a divalent cation, but its availability could indirectly be influenced by other sediment constituents such as iron, manganese and sulphates which also go into a reduced state (Cooper *et al.*, 1981 ; Hesslein & Slavicek, 1984).

It has been shown on numerous occasions that the growth of terrestrial and aquatic fauna and flora is affected to a significant extent by the oxidation-reduction status of soil and aquatic

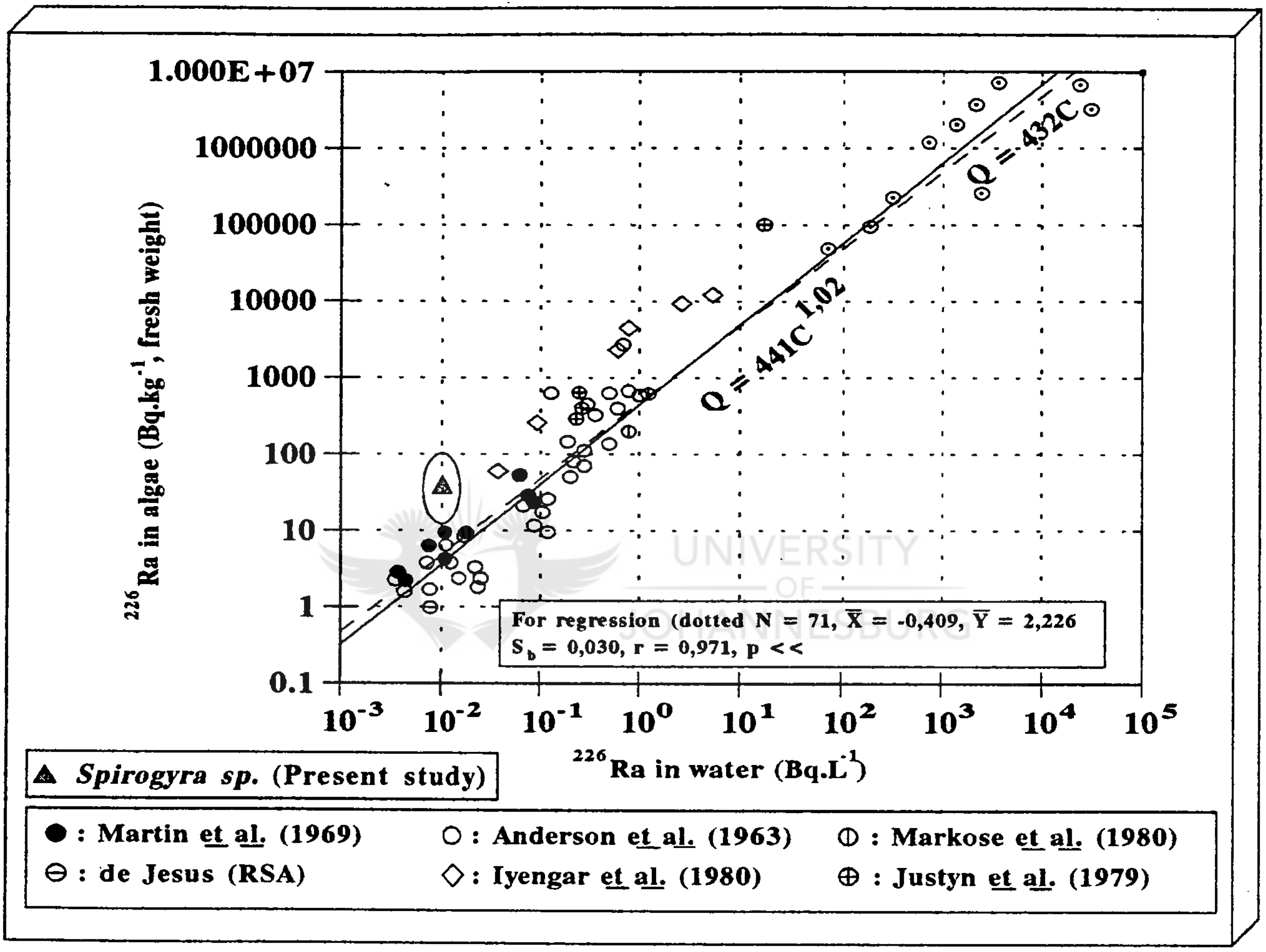


Figure 5.1 **The relationship between radium conecntrations in algae and in surrounding water: a comparison between results obtained during this investigation and those from the literature**

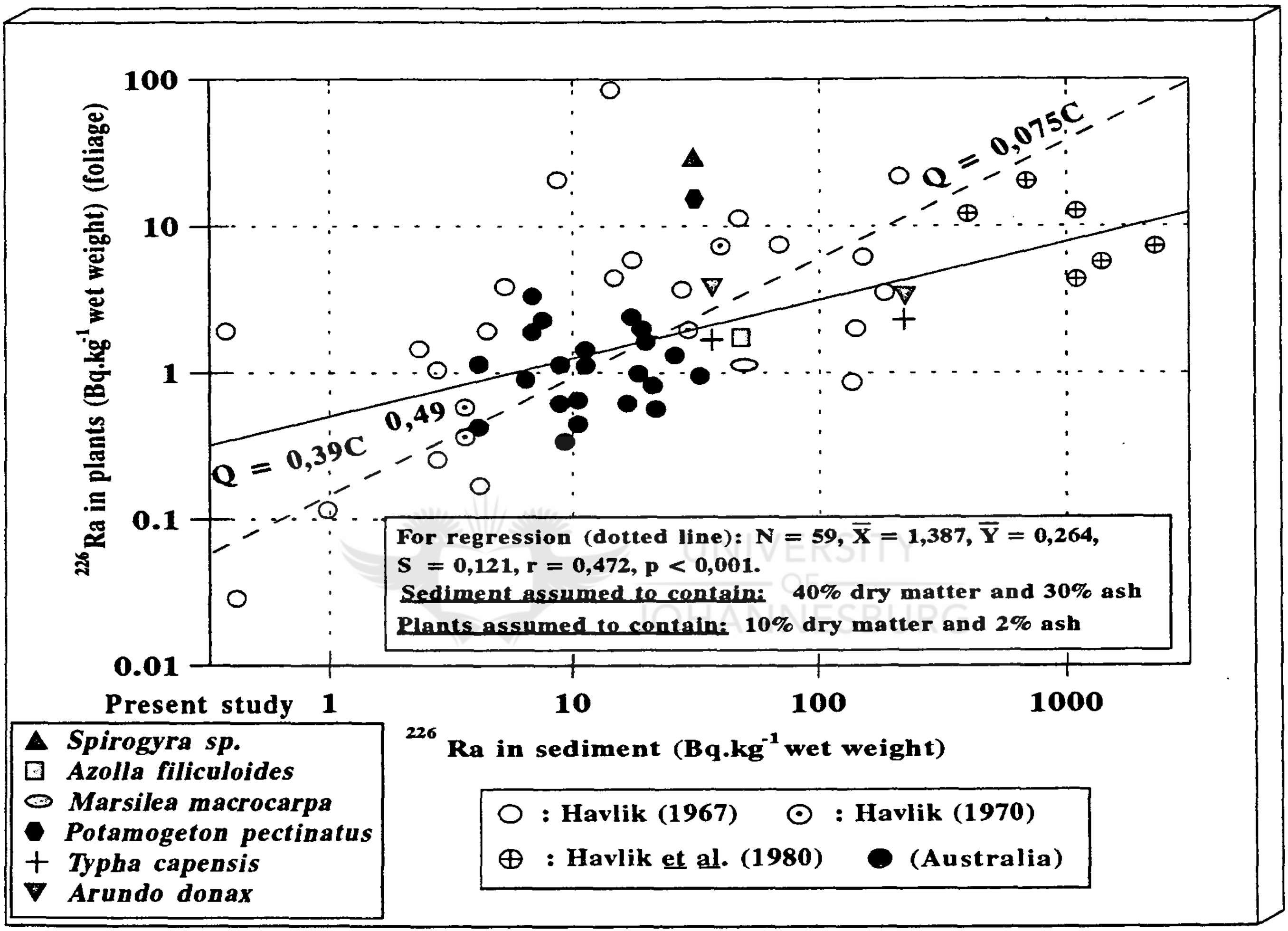


Figure 5.2 The relationship between radium concentrations in aquatic macrophytes and in supporting sediment: a comparison between results obtained from this investigation and those from the literature

sediments. Indeed, it plays a major role in the functioning and survival of plants in wetlands, lakes, rivers and agricultural soil. During this study, the potential of this parameter has not been recognized in full and would have made a valuable contribution to the interpretation of results. Therefore, due to its tremendous potential as an environmental parameter, it is vital to include Eh as a chemical variable during future environmental monitoring programmes.

Regarding observations made from the relationship between ^{226}Ra concentrations in plant tissues and surrounding sediment (Figure 5.2), the following comments need also be made:

Overall, as seen from results obtained from this study, rooted emergent aquatic macrophytes seemed to contain lower ^{226}Ra concentrations in their tissues than the submerged rooted macrophyte, *P. pectinatus*. It should be taken into account that in all these cases, whole plant analyses were conducted. In the case of the rooted emergent macrophytes, most elemental uptake are stored in the rhizomes, while it seems that these elements are rather evenly distributed in the various organs of submerged aquatic macrophytes. In the case of the rooted emergent macrophytes, translocation of elements to aerial foliar parts (stems, leaves and flowers) is executed according to need. During whole plant analyses, a "dilution" effect is thereby created due to:

- higher concentrations of these elements in the rhizomes,
- lower concentrations of these elements in the aerial organs, and
- the relatively large volume of these plants compared to submerged aquatic macrophytes.

Analyses of the different organs of these plants (roots, rhizomes, white stems, green stems, leaves and flowers) would have produced quite different results (This is clearly demonstrated during metal analyses of these plants as discussed in Part 2 of this study). Due to practical reasons which include:

- high costs of ^{226}Ra analyses,
- sample volume requirements for analyses, and
- requirements of the scope of this study;

it was not possible to explore these possibilities. However, some interesting opportunities arise for future investigations.

5.3 Vegetable crops

Little mention is made in local literature on water quality criteria regarding radionuclide concentrations during the evaluation of water for the irrigation of crops. Kempster *et al.* (1982) state that a maximum value of 0,2 Bq.ℓ^{-1} for radioactivity (alpha + beta) for irrigation purposes but do not highlight any specific radionuclides. No definite standards regarding radionuclides are either presented in the South African Water Quality Guidelines for Agricultural Use (Volume 4) (DWAF, 1993). Regarding maximum tolerable levels of elements in crops (Logan & Chaney, 1983), maximum permissible content of metals (and other elements) in soil (mg.kg^{-1}) and maximum rates of application (kg.ha^{-1}.yr^{-1}) (CEC, 1986 ; Vivier *et al.*, 1988) for metals and other elements for the RSA and the CEC (Commission of European Communities), no standards could be obtained for ^{226}Ra and uranium. Results obtained for vegetable crops during this study were therefore compared to similar data obtained from the literature, mainly originating from Germany, Japan, India and the USA.

5.3.1 Marievale Farm

All crops collected at this locality, which include root- (carrots and beetroot) and leafy vegetables (lettuce, cabbage and cauliflower), produced the same ^{226}Ra concentration of 0,01 Bq.g^{-1}. This phenomenon can, however, be ascribed to the analytical technique employed for the determination of ^{226}Ra concentrations in these crops. Due to the higher detection limit of the Gamma Spectrometrical analytical technique, concentrations obtained for these crops did not reflect their actual concentrations. In fact, it can be expected that the actual ^{226}Ra concentrations of these vegetable crops could be one order of magnitude lower, which is more or less the same as concentrations obtained by Tracy *et al.* (1983) (carrots and beetroot), Muth *et al.* (1960) (carrots and cabbage), and Straub *et al.* (1961) (Table 5.1). As explained in Chapter 3, a more sensitive technique was subsequently employed for the analyses of samples collected at Vlakfontein and Luipaardsvlei farms. In addition, it should be remembered that the Marievale farm is situated next to an extensive stretch of wetland which could explain low radionuclide concentrations in the soil and biota collected here.

When ^{226}Ra concentrations obtained for this farm are compared with concentrations obtained for crops collected in control values (Table 2.6: Literature Survey), it is evident that Marievale farm could not be classified as a control locality in the true sense. However, it should also be noted that values presented in Table 2.6 are mostly based on fresh and dry weight concentrations and that corrections should be made to ascertain the exact values. Due to a lack of information concerning the moisture contents of plants listed in Table 2.6, these corrections were not made. Comparing the ^{226}Ra concentrations obtained for crops collected at Marievale with those from regions with high natural radioactivity (Table 2.7), local concentrations are the same order of magnitude as minimum values in crops collected in Iran (Khademi *et al.*, 1980) (Bean: 0,0074 - 0,11 Bq.g^{-1} ; Cucumber: 0,007 - 0,02 Bq.g^{-1} ; Onion: 0,007 Bq.g^{-1}). Please note that values obtained for Iran are based on ash mass. Comparing Marievale crop concentrations with values obtained during experimental studies (based on ash mass), local values are well below those obtained for beans (0,459 - 13,2 Bq.g^{-1}) cultivated in artificially contaminated pots (Table 2.8).

To relate back to the above comparisons made between local plant concentrations and those obtained from the literature, Till and Meyer (1983) made the following comments which should be taken into account: The moisture content of vegetable crops may vary between 90 - 95% of the total weight, especially for crops such as lettuce and tomatoes. For crops such as cauliflower, cabbage and rooted vegetables, the moisture content is lower. When Concentration Ratios are expressed in either a wet, dry or ash weight basis, significant differences, which may be an order of magnitude, can be encountered. Therefore, caution should be exercised when a comparison of elemental content or Concentration Ratios are made. These authors provided representative fresh-to-dry-weight ratios to aid in the conversion between the different bases of measurement. However, these authors also warn that these values may vary by more than 30% depending upon the crop, time between harvest and weighing as well as climatic factors such as relative humidity. The above comparisons made for Marievale (as well as those for Vlakfontein and Luipaardsvlei farms) should therefore serve as a rough indication of radionuclide concentration capability.

In the case of uranium concentrations measured in crops on this farm, the root vegetables contained higher concentrations than those obtained from other regions. Carrots from Marievale farm (0,23 $\mu g.g^{-1}$) contained higher concentrations than those obtained by Morishima *et al.* (1977) in Japan (0,033 $\mu g.g^{-1}$) (Table 5.1), while beetroot (0,2 - 1,7 $\mu g.g^{-1}$) also contained higher concentrations than root vegetables (0,026 - 0,033 $\mu g.g^{-1}$) analyzed by Welford & Baird (1967). It is interesting to note that uranium concentrations in the leafy vegetables (cabbage: 0,02 $\mu g.g^{-1}$; lettuce: 0,08 $\mu g.g^{-1}$) collected from Marievale farm were significantly lower (one order of magnitude) than those obtained for the above root vegetables (Table 5.1). This can most probably be ascribed to the storage and elemental cycling functions of these plant organs. A number of authors have recorded similar trends (Drobkov, 1937 ; Kirchmann & Berino, 1965 ; D'Souza & Mistry, 1970 ; Markose *et al.*, 1993) where plant-root activity far exceed those of the foliar parts.

5.3.2 Vlakfontein Farm

Vegetables collected from Vlakfontein contained lower ^{226}Ra, but higher uranium concentrations than crops collected at Marievale farm. In fact, all concentrations recorded for uranium were one order of magnitude higher than those recorded for Marievale farm (Table 5.1). However, it should again be noted that differences in ^{226}Ra concentrations at these two locations can be ascribed to differences in analytical techniques employed. Although, differences due to analytical techniques are evident, it can be expected that ^{226}Ra concentrations would be the same order of magnitude at both these localities.

These elevated uranium concentrations can be ascribed to both intensive mining activities in the vicinity of Vlakfontein farm as well as the mobility of this element. In addition, sections of wetland present in the vicinity of Vlakfontein are not as extensive as those found at Marievale farm. Subsequently, lesser amounts of radionuclides and other contaminants are retained by the wetlands that are present.

Although regarded as potentially polluted, all ^{226}Ra concentrations were of the same order of magnitude as those found in the literature (Table 5.1). For instance, a ^{226}Ra concentration of 0,0041 $Bq.g^{-1}$ obtained for carrots at this farm resorted between a lower concentration of 0,0035 $Bq.g^{-1}$ (Tracy *et al.*, 1983) and a highest concentration of 0,0055 $Bq.g^{-1}$ obtained by Muth *et al.* (1960). In the case of beetroot, results obtained during this study for this locality (0,003 $Bq.g^{-1}$) was approximately half of that obtained by Tracy *et al.* (1983) (0,0067 $Bq.g^{-1}$) for the same crop (Table 5.1). These results clearly illustrate the regulation of contaminants from soil to plants under environmentally stressed conditions.

Turnip, another root vegetable, contained about a third (0,0014 Bq.g^{-1}) of the ^{226}Ra contained by the other root vegetables. However, no specific data in the literature could be obtained for this crop, but when compared to data for root vegetables in general (Table 5.1), it tends to be lower than the values obtained by Morse & Welford (1971) (0,0026 Bq.g^{-1}) and Hallden & Fisenne (1961) (0,0018 Bq.g^{-1}). The only explanation which seems to correlate with the results obtained is that differences in rate of accumulation do exist between the different rooted vegetable species.

Considering leafy vegetables, lettuce contained 0,0017 Bq.g^{-1} of ^{226}Ra which was about 6 times lower (but still the same order of magnitude) than that obtained by Straub *et al.* (1961) (0,0095 Bq.g^{-1}) for the same crop. On the other hand, spinach contained a ^{226}Ra concentration which was an order of magnitude higher (0,023 Bq.g^{-1}) than any other crop analyzed during this study on data presented in Table 5.1. However, recent investigations conducted by Markose *et al.* (1993) proved this plant, grown on agricultural field, to contain double the amount (0,044 Bq.g^{-1}) (Soil: 0,0013 Bq.g^{-1} ; CR: 0,029) of ^{226}Ra contained by Vlakfontein spinach. As reflected by these results, as well as results obtained during Part 2 of this study, it is clear that spinach can be regarded as an active accumulator of bio-available elements in the soil solution. However, although not measured, the contribution by atmospheric deposition should also be taken into account as a possible source of contamination. In this particular case, any contribution by spray irrigation and subsequent accumulation through the leaves can be excluded as flood irrigation was practiced at this farm.

The uranium content of beetroot (1,7 μg.g^{-1}) and carrots (1,7 μg.g^{-1}) were in both cases higher than those obtained for these crops by Morishima *et al.* (1977) (0,033 μg.g^{-1}) and Welford & Baird (1967) (0,026 - 0,033 μg.g^{-1}) respectively (Table 5.1). These high uranium concentrations can again be attributed to high environmental concentrations as well as active accumulation.

5.3.3 Luipaardsvlei

Some information regarding ^{226}Ra and uranium concentrations in cattle fodder, milk and excreta are available in the literature. Being a grain crop, concentrations of both radionuclides in cattle fodder was compared with other grain products such as corn, wheat, barley and grass (hay). At sampling locations LP1 to LP3 ^{226}Ra concentrations in fodder ranged from 0,055 to 0,094 Bq.g^{-1} which were one order of magnitude higher than grass grown on tailings ponds (0,0082 Bq.g^{-1}) (Markose *et al.*, 1993).

^{226}Ra concentrations obtained from milk samples collected from Luipaardsvlei (0,0051 - 0,05 Bq.g^{-1}) were however, considerably higher than a concentration of 0,00046 Bq.g^{-1} obtained by Morse & Welford (1967). The same applied to the uranium content of milk from Luipaardsvlei (0,21 - 0,22 μg.g^{-1}) compared to that obtained by Lal *et al.* (1982) (0,026 - 0,153 μg.g^{-1}) in India (Table 5.1). Although these values appear high in comparison with those in the literature, it should be mentioned that large error bars were encountered from

Table 5.1 **Concentrations of uranium and ^{226}Ra in vegetable crops from different countries of the world**

Crop	Uranium (μg.g^{-1})	Reference (U)	^{226}Ra (Bq.g^{-1})	Reference (Ra)
Carrots	0,033	Morishima *et al.* (1977) Japan	0,0035	Tracy *et al.* (1983) USA
			0,0055	Muth *et al.* (1960) Germany
		This study	**0,0041 - 0,01**	**This study**
Beetroots	---	---	0,0067	Tracy *et al.* (1983) USA
	0,2 - 1,7	**This study**	**0,003 - 0,01**	**This study**
Root vegetables	0,026-0,033	Welford & Baird (1967) USA	0,0026	Morse & Welford (1971) USA
			0,0018	Hallden & Fisenne (1961) USA
Cabbage	0,067	Morishima *et al.* (1977) Japan	0,0032	Muth *et al.* (1960) Germany
	0,02	**This study**	**0,01**	**This study**
Lettuce	---	---	0,0095	Straub *et al.* (1961) USA
Vegetable mix	0,078-0,22	Welford & Baird (1967) USA	0,0024	Klement (1982) USA
Milk	0,026-0,153	Lal *et al.* (1982) India	0,00046	Morse & Welford (1971) New York average
	0,21 - 0,22	**This study**	**0,081 - 0,05**	**This study**

the counting statistics when the milk samples were analyzed. Therefore, these values may appear confusing when they are compared to other values. However, it should be remembered that relatively high ^{226}Ra concentrations were also obtained for the fodder. It is therefore difficult to interpret these results when the above variables are taken into account.

Considerable interest as well as concern (Shukla *et al.*, 1994) exists regarding elemental and radionuclide metabolism by cattle as they constitute principal food and milk sources to man. Usually, these animals are quite easily exposed to a uranium (or other contaminants) contaminated diet (Chapman & Hammons, 1963 ; Shukla *et al.*, 1994). Generally, the existence of carnivores is dependent upon the maintenance of herb-foraging species.

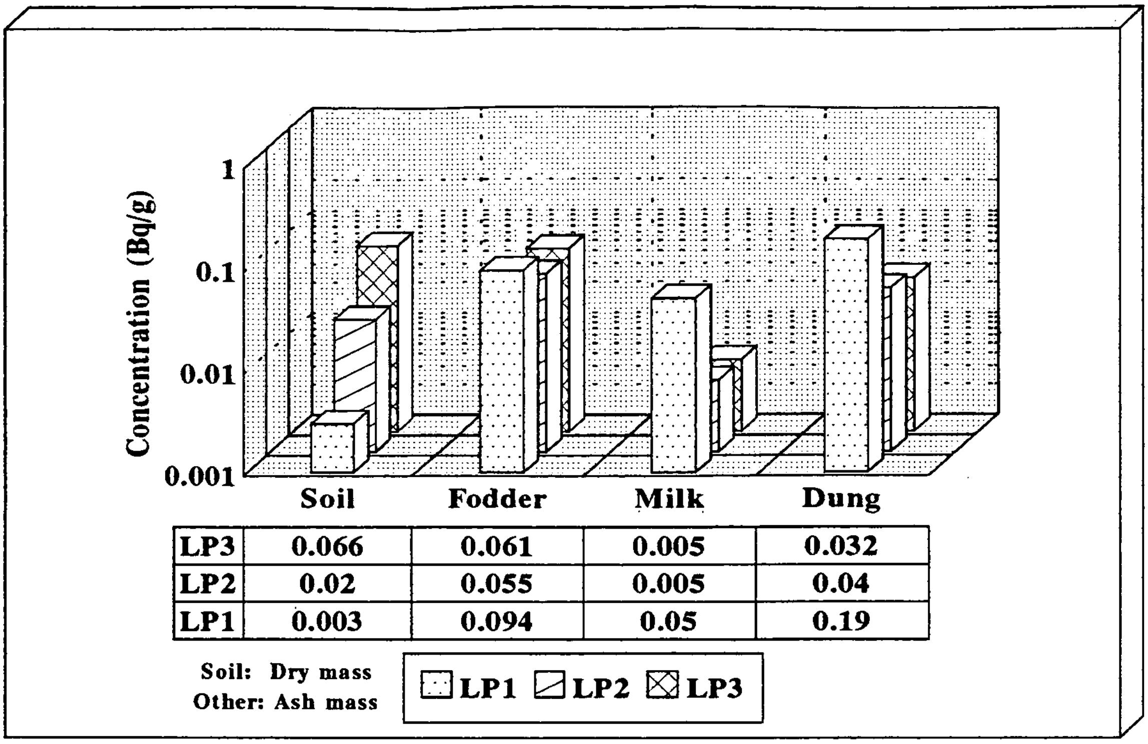
It is therefore often feared that due to differences between the digestion processes of humans and cattle, that the latter are able to concentrate higher amounts of bio-available elements or contaminants. In the rumen of cattle, micro-organisms solubilize five carbon atom carbohydrates which are indigestible to other mammals. After softening, fibrous vegetable matter is regurgitated, masticated and re-swallowed. This process as well as the passage through the reticulum and omasum into the abomasum for gastric digestion exposes the food to completely different solubilizing conditions and for longer periods of time than is the case of non-ruminants (Chapman & Hammons, 1963).

Considering elevated fodder concentrations as well as enhanced digestion processes, concern regarding possible contamination of edible meat as well as in milk can be justified. It is often the case that radium as well as other rare elements could also be present in the tissues of other farm animals such as steer, pigs and chickens (Linsalata *et al.*, 1989). As observed from results obtained during this study, it appears that the majority of ^{226}Ra and uranium was excreted through the faeces and a minor amount through the milk (Figures 5.3 & 5.4). However, the above cannot be accepted as actually taking place, as a full mass balance investigation of all the major organs and excretion pathways need to be conducted to prove this point.

Although relatively low, values obtained for ^{226}Ra in milk ranged from one to two orders of magnitude higher (0,005 - 0,05 $Bq.g^{-1}$) than those obtained by Morse & Welford (1971) (0,00046 $Bq.g^{-1}$), while uranium was approximately 1,4 times higher (0,22 $\mu g.g^{-1}$) than the maximum of 0,153 $\mu g.g^{-1}$ obtained by Lal *et al.* (1982) in India. Again, this can be attributed to elevated radionuclide concentrations in cattle fodder which were ingested by these cattle.

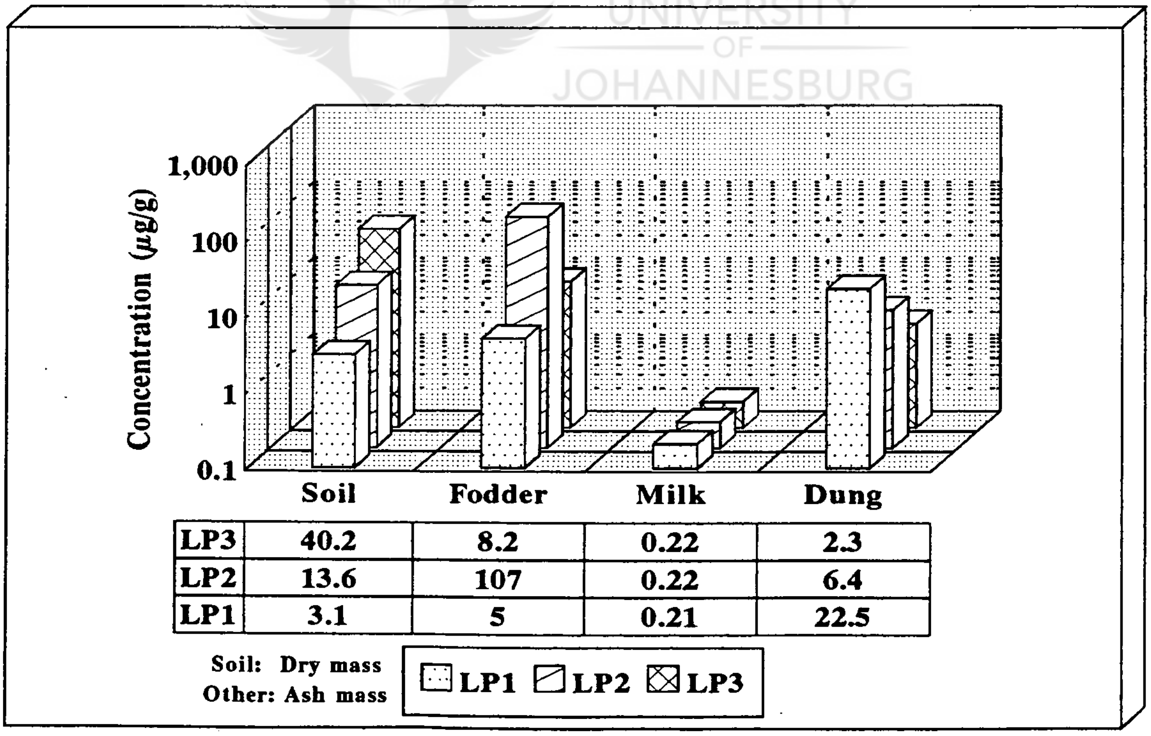
5.4 Concentration Ratios

Results obtained from ^{226}Ra concentrations in vegetable crops which include those from Marievale farm, Vlakfontein farm and the experimental study (edible portions of beetroot and cabbage) were submitted to linear fitting of the plot of Concentration Ratios as a function of soil concentrations. Subsequently, a positive trend with a y-intercept of 0,0017 and a slope



	Soil	Fodder	Milk	Dung
LP3	0.066	0.061	0.005	0.032
LP2	0.02	0.055	0.005	0.04
LP1	0.003	0.094	0.05	0.19

Figure 5.3 Excretion of radium through feces and milk obtained from contaminated fodder collected from Luipaardsvlei



	Soil	Fodder	Milk	Dung
LP3	40.2	8.2	0.22	2.3
LP2	13.6	107	0.22	6.4
LP1	3.1	5	0.21	22.5

Figure 5.4 Excretion of uranium through feces and milk obtained from contaminated from fodder collected at Luipaardsvlei

of 0,229 was obtained (Figure 5.5). However, a correlation coefficient of 0,44 was obtained which is low. This can, however, be ascribed to insufficient data points which do have a negative influence on trend statistics.

CRw values for the vegetable crops and fodder from the Marievale, Vlakfontein and Luipaardsvlei farms and the irrigation water were constantly higher than those of the corresponding CRs values. This is however not an indication that more ^{226}Ra or uranium is taken up from the water, but just that the concentrations in the water, expressed as activity/mℓ for the calculation of the concentration ratio's, is lower than the activity/g of soil. It is unlikely that the concentrations in the plants were in any of the cases in equilibrium, with the concentrations in the irrigation water which is a prerequisite to calculate the concentration ratio. The same apparent observation was made in the experimental part of this study. The CRw-values appeared to be higher than the CRs-values. This was true for cabbage and beetroot from all the different irrigation schemes investigated. Again this might just be that the soil is the dominating medium for transfer of ^{226}Ra to the vegetables. The ^{226}Ra concentrations [Q] in the vegetables are probably described by the relationship:

$$[Q] = CRs[Cs] + CRw[Cw]$$

If [Cs] and [Cw] are respectively the ^{226}Ra concentrations in the soil and in the irrigation water, and [Cw] is less than [Cs], then this relationship indicates that CRw has to be bigger than CRs for the irrigation water to contribute to the ^{226}Ra concentration in the plant.

In the experimental part the ^{226}Ra concentration in the irrigation water [Cw] was kept constant at 0,0225 Bq.mℓ^{-1}. If one assumes that CRw stays constant, then the equation becomes:

$[Q] = CRs[Cs] + k$: **Where:** $k = CRw[Cw]$ and is constant.

If one then plots [Q] against [Cs], then the slope is the concentration ratio from soil to the vegetable plant, and the intercept, k, divided by the concentration ratio of the ^{226}Ra in the irrigation water is the concentration ratio from water to the vegetable plant. If the ^{226}Ra in the irrigation water is not in equilibrium with the plant, it means that the ratio is valid only under similar circumstances (eg. irrigation method). This was done, and the best correlation that could be found was that between soil and beetroot ^{226}Ra concentration, and even in this "best" case the correlation coefficient was only 0,57. The reasons for this low coefficient is probably the lack of sufficient data points (6 in this case) and that the ^{226}Ra concentration in the independent variable (soil ^{226}Ra concentration) did not cover a wide range of values. The soil ^{226}Ra concentrations varied only between 0,04 and 0,08 Bq.g^{-1} which is in the range of normal soil concentrations of 0,004 to 0,125 Bq.g^{-1} given by Iyengar (1990).

If one nevertheless accept that these calculations do give a rough indication of the concentration ratios, the slope is $1 \pm 0,7$ and the intercept is $0,02 \pm 0,05$. The

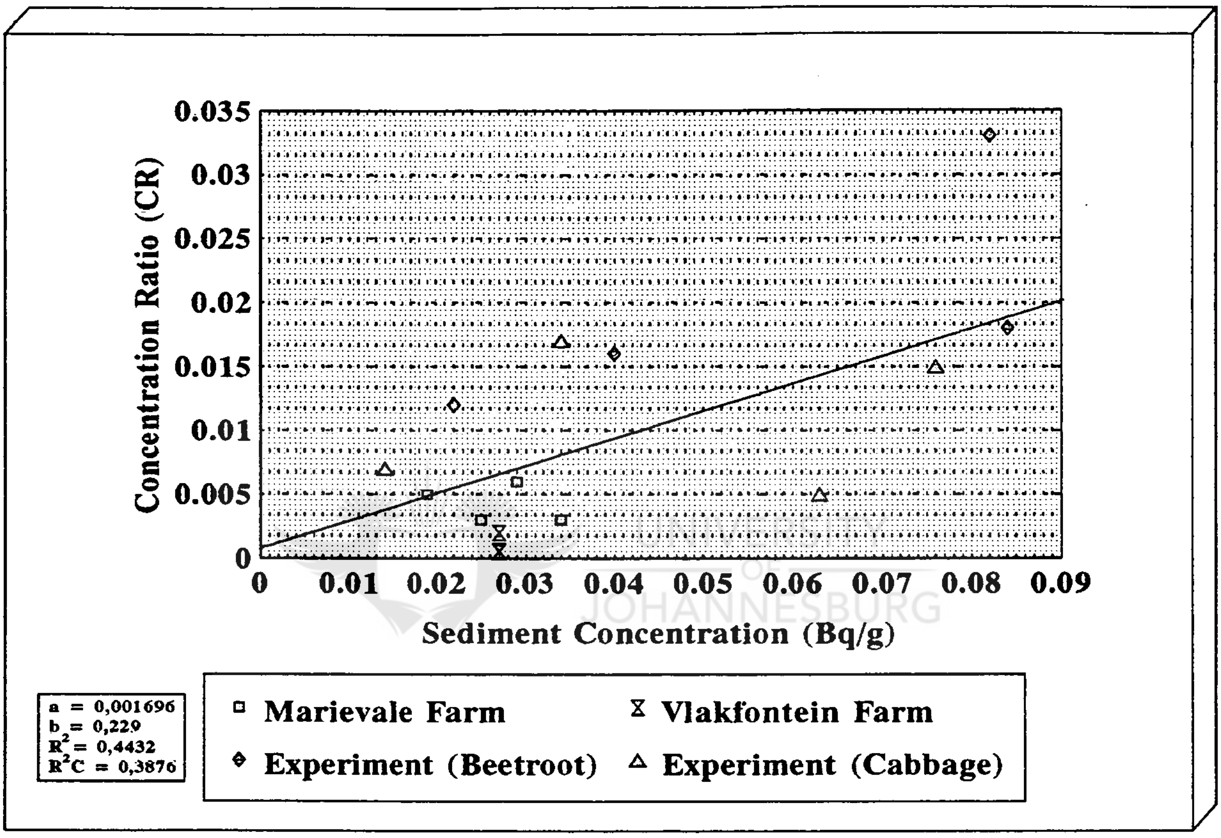


Figure 5.5 Relationship between crop concentration ratios and radium concentrations in soil obtained from results collected during this study

concentration ratio from soil to beetroot is therefore of the order of 1 and for the concentration ratio of water to beetroot the order of magnitude is also about 1 (0,02 / 0,0225). The concentration of ^{226}Ra in water has therefore to be of the same order as the concentration in the soil to contribute equally to the transfer of ^{226}Ra to the plant. This again is only valid according to the irrigation method used. If one has for example a soil ^{226}Ra concentration of 0,03 Bq.g^{-1}, then an irrigation water concentration of 0,03 Bq.mℓ^{-1} will contribute equally to the ^{226}Ra in the plant. Concentrations of 0,03 Bq.mℓ^{-1} or 30 Bq.ℓ^{-1} was never found in this study. The highest concentration used for irrigation by a farmer was of the order of 0,2 Bq.ℓ^{-1}, which is a hundred times lower than concentrations normally found for ^{226}Ra in soil for this study. Even if there is a factor 10 error in the calculation of the CRs and CRw, and CRs is really 10 times lower and CRw is really 10 times higher than calculated, the uptake of ^{226}Ra from the irrigation water will still only be of the same order as the transfer from soil.

One of the most important factors which may have had an influence on the CR values in the present study, is whether the plant and soil are in equilibrium. If in equilibrium, the CR will remain predictable (Whicker & Schultz, 1982). Another important factor is the choice of location and the soil sampling method. This especially applies to areas where soil contamination is non-uniformly distributed. In the case of spray and flood irrigated agricultural lands, the distribution of contaminated water should be relatively homogenous which most likely ensures a relatively uniform distribution of elements. During the collection of soil samples, particular attention was given to the randomness of the sampling programme. All subsamples were pooled, thoroughly mixed and homogenized to ensure an even better distribution of elements.

Even though the concentration ratio of ^{226}Ra from irrigation water to vegetables might be bigger than the same ratio from soil to vegetables, the biggest contribution to the ^{226}Ra concentration in vegetables will probably be from the soil. This is due to the fact that the ^{226}Ra concentration in the soil is usually a few orders higher than in the water used by farmers in the vicinity of the mines. For the same reason the buildup of ^{226}Ra in soils irrigated with mine water will be insignificant.

The concept of CR, however, has a serious weakness, namely the lack of physical meaning which makes the direction and degree of change if circumstances vary, difficult to predict. These circumstances may include soil type, physical and chemical characteristics of soil (pH, organic content etc.), physical and chemical characteristics of applied irrigation water, plant species and climate (Van Dorp *et al.*, 1979). Therefore, if the CR value is specified with respect to one or more of the variables which may have an influence on it, a huge amount of data will be stored which could make a database for radiological assessment very extensive, yet cumbersome.

It is suggested that although the concept of CR is a useful tool in predicting food chain transport, it needs further evaluation, modification and refinement. It is recommended that a CR is calculated for each main crop type or, if sufficient data exists, each main crop type and each associated main soil type (Ng *et al.*, 1990). An alternative approach for predicting concentrations of radionuclides in plants via uptake was suggested by Van Dorp *et al.* (1979). This proposed approach utilizes a selectivity coefficient, a transpiration coefficient as well as the annual production of dry matter. It seems however, that the actual equation for the calculation of a CR which takes all environmental factors in consideration, will be open for discussion for the long foreseeable future. However, Simon & Ibrahim (1990) stresses that at a minimum, the CR should be treated as a function of substrate concentration rather than a constant value. This is also, shown to a certain extent, by results obtained during this study (Figure 5.5) and is also illustrated through results obtained on metal concentrations measured in the same crops. More data points were obtained (Part 2: Chapter 4) and more clearly defined trends were obtained.

5.5 Aquatic macro-invertebrates

Some information is available on the presence and concentration of uranium and ^{226}Ra in aquatic macro-invertebrates. In the case of the freshwater crab, *Potamonautes warreni*, no local information was available on the accumulation of radionuclides. However, results showed that uranium concentrations were more than double those obtained for fish whilst ^{226}Ra concentrations were slightly lower but of a similar order than those found for fish and aquatic birds. Regarding accumulation compared to fish, the results can be justifed as these organisms are on a lower trophic level with some differences in their dietary habits. However, the comparative accumulation to that of birds cannot be explained.

5.6 Fish

Fish are considered to be the principal link in the food chain by which water-borne contamination can reach man directly. In this way, it is also believed to affect the extent of radiological exposure of a population (Justyn & Havlik, 1990). In uncontaminated water, the ^{226}Ra content of fish seem to be very low (thousandths to hundredths of Bq.g^{-1} dry mass) while in surface waters contaminated by uranium milling effluent, the ^{226}Ra content can be from 3 to 6 orders of magnitude higher in fish muscle (Paul *et al.*, 1980 ; Anderson *et al.*, 1963 ; Martin *et al.*, 1969 ; de Bortoli & Gaglione, 1972 ; Martinec, 1973 ; Davy & Conway, 1974 ; Davy & O'Brein, 1975 ; Ettenhuber *et al.*, 1975).

Since there are not many similarities between the fish fauna of the different countries, data obtained for South African fish species was compared with each other. It seems that physiological differences between species do not have such a great influence on the bioaccumulation of radionuclides as regional or geographical differences might have. In all cases, concentrations of ^{226}Ra for all fish species collected during the present survey were lower than 0,1 Bq.g^{-1} with values ranging between 0,036 Bq.g^{-1} (*C. gariepinus*) and 0,081 Bq.g^{-1} (*T. sparrmanii*). A comparison of these results with those obtained for other freshwater fish collected in Lake Athabasca, Canada (Swanson, 1985), showed that all values for the local fish species analyzed were between one and two orders of magnitude lower than those for Canada. Concentration values of ^{226}Ra in fish, collected in Lake Athabasca ranged between 0,15 Bq.g^{-1} (Lake Chub: *Covesius plumbeus*) and 4,0 Bq.g^{-1} (Spottail shiner: *Notropius hudsonius*). A similar tendency was observed for uranium uptake by fish in South

Africa with values ranging between 0,60 $\mu g.g^{-1}$ (*C. gariepinus* : Natalspruit) and 4,99 $\mu g.g^{-1}$ (*C. gariepinus* : Blesbokspruit). The uranium content of the Lake Athabasca fish were again one order of magnitude higher, fluctuating between 25,5 $\mu g.g^{-1}$ (*Covesius plumbeus*) and 78,3 $\mu g.g^{-1}$ (Small white sucker: *Catostomus commersoni*) (Swanson, 1985). Czechoslovak researchers who investigated the ^{226}Ra-concentrations in fish from reservoirs in the vicinity of uranium milling operations in Northern Bohemia also obtained values for radium which were at least one order of magnitude higher than those for fish from different mine polluted streams in South Africa. Justyn & Lusk (1976) recorded ^{226}Ra in fish varying between 0,185 $Bq.g^{-1}$ (*Esox lucius*) and 1,41 $Bq.g^{-1}$ (*Perca fluviatilis*). Other fish analyzed by these authors yielded more intermediate results with ^{226}Ra values for *Anguilla* (0,28 - 0,5 $Bq.g^{-1}$), *Gobio gobio* (0,3 - 0,425 $Bq.g^{-1}$) and *Leuciscus cephalus* (0,225 - 0,55 $Bq.g^{-1}$) falling within this range. Justyn *et al.* (1979) obtained nearly similar results for *Perca fluviatilis* (0,18 - 1,41 $Bq.g^{-1}$) and *Salmo trutta* (0,016 - 0,045 $Bq.g^{-1}$) while values of 1,75 $Bq.g^{-1}$ and 1,25 $Bq.g^{-1}$ was obtained for *Cyprinus carpio* and *Perca fluviatilis* respectively, by Justyn *et al.* (1985).

Comparing the above results obtained from local fish species with trends (showing ^{226}Ra in water ($Bq.\ell^{-1}$) against ^{226}Ra in fish flesh ($Bq.kg^{-1}$) fresh weight) obtained from the literature (Figure 5.6), it can clearly be seen that these data points closely fall within the limits of the trend. Both data points for *C. gariepinus* marginally fall below the $Q = 12C^{0,66}$ trend while data obtained for *T. sparrmanii* and *C. carpio* reflect a slightly higher intake and accumulation of ^{226}Ra. This can probably be ascribed to differences in feeding habits. *C. carpio* is a known bottom-feeder and most probably is exposed to higher levels of metals and other elements such as ^{226}Ra and uranium. Both *C. gariepinus* and *T. sparrmanii* have variable dietary intakes which include zooplankton, algae, detritus, other fish and aquatic insects which generally contain lower amounts of toxicants than bottom detritus.

It is important to evaluate the ^{226}Ra content in fish in relation to the species of radium in the water as well as the presence and species of other chemical substances in the water, namely calcium and barium. The pH, alkalinity and acidity of the water is also important as it influences the mobility, toxicity and bioavailability of radium to fish. Two pathways normally exist whereby fish and other aquatic animals can assimilate and bioconcentrate radium or non-nutritional elements, namely: direct uptake from the water through the skin or gills during water filtration ; uptake from food (Justyn & Havlik, 1990). However, uptake and bioconcentration of radium and other elements by fish are mainly dependent on a number of conditions: it has been found that differences exist between fish species and the extent of the concentration and uptake of radium from contaminated water. However, these differences can largely be related to the food and feeding habits. A low radium content and therefore a low concentration ratio was found in *Coregonus lavaretus maraena* Bloch, which is known to live in the water column and to feed mainly on zooplankton organisms. Other fish such as *Gobio gobio* L., *Leuciscus cephalus* L., *Thymallus thymallus* L. and *Anguilla* L. also showed relatively low radium contents. In the benthophagous and phytophagous *Tinca tinca* L., *Cyprinus carpio* L., *Rutilus rutilus* L. and *Carassius carassius*, a relatively high uptake of radium was observed. In predatory fish, the CR was higher in *Salmo trutta* m. *fario* L. than in *Esox lucius* L. (Martinec, 1973 ; Justyn & Lusk, 1976 ; Justyn *et al.*, 1979 ; 1985). These differences can, according to Justyn & Havlik (1990) primarily be

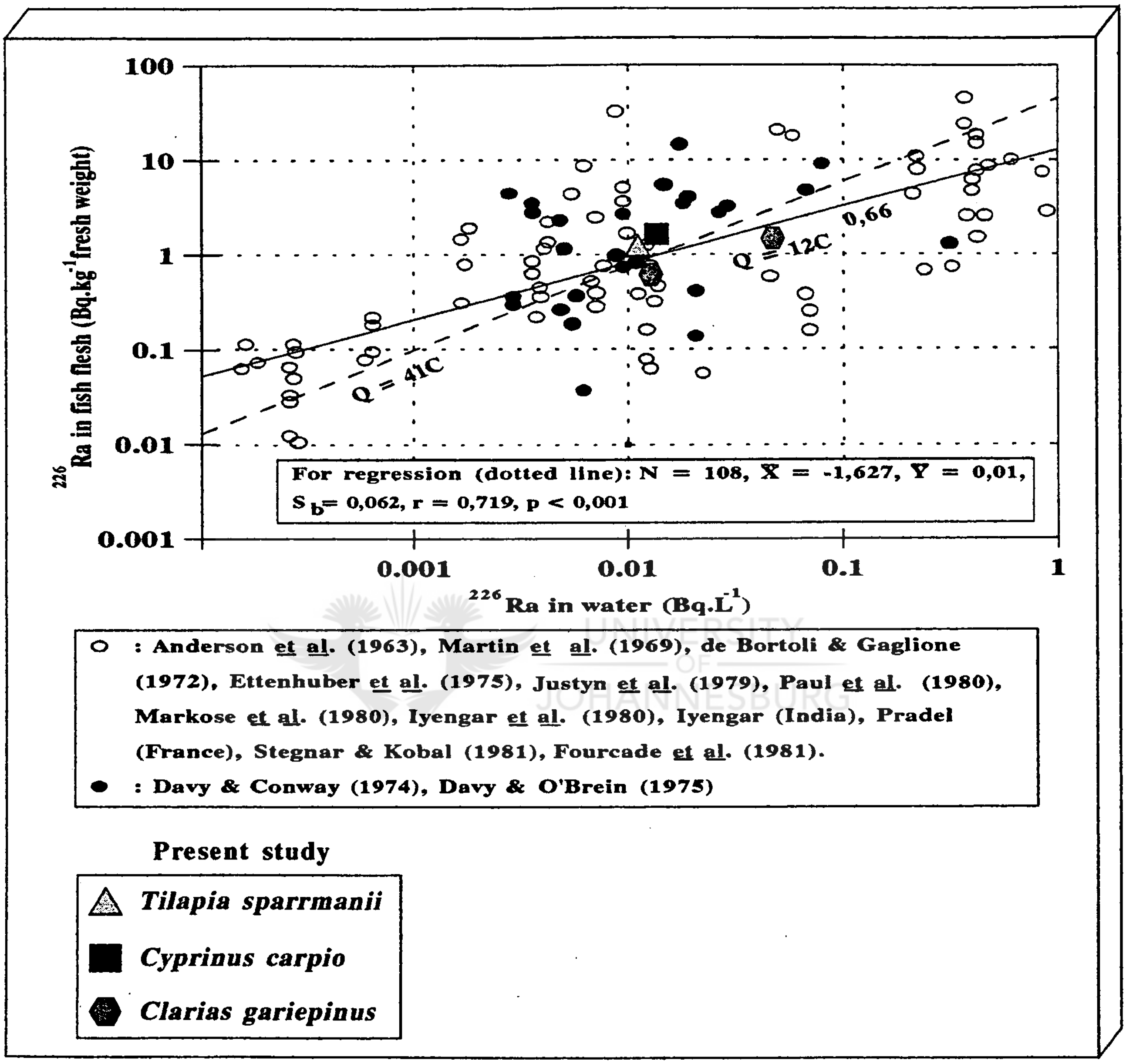


Figure 5.6 The relationship between radium concentrations in fish flesh and in surrounding water: a comparison between results obtained during this investigation and those from the literature

ascribed to lifestyle, food preference (zoophagous, phytophagous, benthophagous or predatory), rate of metabolism, growth and sexual maturity. In field tests with *Cyprinus carpio* stocks, Justyn *et al.* (1979) found that radium uptake in younger fish was almost double that in older fish. With a rise in water temperature a more rapid increase in ^{226}Ra volume activity was observed. Justyn *et al.* (1985) also noted that higher ^{226}Ra concentrations were observed in fish in the summer months than in winter. According to these authors, ^{226}Ra concentrations in fish can be related to the intensity of food intake during the active feeding summer period.

5.7 Birds

Although the previously mentioned organism groups are extensively covered in the literature, no data could however be found for uranium and ^{226}Ra concentration in aquatic birds. However, data collected from the Natalspruit on the coot *Fulica cristata* and the cormorant *Phalacrocorax carbo* seems to be a first definitive contribution to the investigation of the concentrations levels of these radionuclides in these species of birds. From the results for uranium it is interesting to note that the phytophagous *F. cristata* contains more uranium (0,56 μg.g^{-1}) than the fish eating *P. carbo* (0,17 μg.g^{-1}). This tendency can most likely be ascribed to the feeding habits of these two bird species as well as the uranium content of the food. However, this tendency is in the case of ^{226}Ra not so definite with *F. cristata* containing 0,034 Bq.g^{-1} and *P. carbo* 0,074 Bq.g^{-1}. It seems from these results that ^{226}Ra does not possess the same bioavailability as uranium does.

5.8 Dose Assessment Modelling

The dose assessment results for reasonably realistic yet conservative assumptions for water and vegetable consumption show that doses to a maximum exposed group will be of the order of 1% of the limit of 250 μSv. Radium, as expected, has a higher contribution than uranium. If, in the exposure scenario considered, the drinking water radium concentration were a factor 5 higher, i.e. equal to current EPA (USA) limit of $\approx$ 0,2 Bq.ℓ^{-1} then the annual committed effective dose would be 39 μSv, still well below the dose limit. Only infrequently and then very close to mine properties have such concentrations been observed locally. Similarly a factor 5 or 20 increase in vegetable concentrations will still leave a good margin below the limit. Obviously production of crops on mine dumps should be prohibited or strictly controlled as evidenced by the individual results from the oats production.

There is no obvious data to confirm the model's default value of 410 mg per day for soil ingestion. The associated committed effective dose value for radium of 10 μSv is the same order as the dose from drinking water and vegetable consumption. The control value for radium contamination of soil (or alpha contamination of soil) set by the Council for Nuclear Safety (CNS) is 100 Bq.g^{-1} or a factor four greater than the concentration used in the model. Such a limit would result in an ingestion dose of 40 μSv.

The data collected in this study in the Witwatersrand area shows that no member of the public is expected to be exposed via aquatic pathways to any significant dose. If mine effluents are controlled to less than 0,2 $Bq.\ell^{-1}$, radium and less than 0,5 $Bq.\ell^{-1}$ uranium the doses received from various aquatic pathways should be well within dose limits set. The concentration values quoted here are not intended to imply these should be concentration limits. Control limits are set by CNS based on site specific assessments.

5.9 Objectives of this study and the extent to which they have been achieved

5.9.1 Background

A number of local studies have in the past been conducted to assess the occurrence of ^{226}Ra and uranium in the environment as well as in agricultural produce potentially exposed to these radionuclides (Malan, 1981 ; de Jesus *et al.*, 1987 ; Funke, 1990). However, none of these investigations were aimed at determining the Concentration Ratios (CR) for transfer of these radionuclides to crops and livestock and the subsequent potential dose to humans.

This investigation therefore addressed these shortcomings for both uranium and radium. The need for investigation of such environmental impacts in South Africa has in recent years received fresh impetus by the requirements of the Council for Nuclear Safety, (for licensing affected mines under the Nuclear Energy Act) and the Government Mining Engineer, who requires an Environmental Management Review plan under the Minerals Act.

The objectives of this study have largely been achieved and are briefly summarized below:

5.10 Conclusions

The aim of this investigation was to obtain suitable sites for this particular investigation. To some extent, this was achieved. The combination of contaminated mining effluent being utilized for irrigation purposes as well as suitable vegetable crops or animal fodder were obtained. Although the intention of this study was to obtain site-specific information, valuable data was also obtained which could make a contribution to basic and applied science. These include:

- Radium concentrations obtained in rooted emergent aquatic macrophytes indicate that these plants scavenge radium and other elements from the water as well as anoxic sediments where reduced conditions prevail. This mechanism of element and toxicant scavenging by the roots and the storage of the above by the rhizomes of aquatic macrophytes play a very important role in the filtering effect and efficiency of wetlands. In a way, these plants indirectly protect other biota (aquatic and terrestrial) from ingesting or accumulating toxic levels of elements or contaminants. By returning elemental matter back to the soil, the wetland as such, is also protected.

- Uranium and radium concentrations in local aquatic macrophytes, invertebrates, fish and birds were generally lower than those obtained from other uranium mining countries. These results correlate to the much lower local ore concentrations. Significantly lower concentrations of these radionuclides were detected in fish and semi-aquatic birds, indicating the possible accumulation and bio-accumulation of radionuclides in aquatic sediments and biota respectively.

- The concentrations of uranium in farm grown vegetables had values generally greater than those from background areas in other countries. The radium values in the vegetables were similar to overseas values and in the case of lettuce, much lower. A statistically valid background site for data was not established in this study, so the high trend in uranium concentrations cannot be placed into perspective. However, these trends also indicate towards differences between the mobility of these two radionuclides in the environment with radium being the lesser mobile.

- The vegetable concentration ratio values (water) were generally greater than those relative to soil. This is, however, not an indication that more radium or uranium is taken up from the water, but just that the concentrations in the water are less than in the soil. In this study the highest concentration used for irrigation by a farmer was of the order of one hundred times lower than the radium concentrations in the soil. The crop uptake is more strongly dependent on the soil concentration than that in the water. The expected buildup of radium in soil due to irrigation is anticipated to be negligible. The concept of concentration ratios is useful but it has a limitation, particularly in the lack of easily interpreted physical meaning. It should preferably be defined together with other field variables.

- The pot experiments yielded useful data on radium uptake in roots and leaves of selected vegetables for flood and spray irrigation. Various aspects can be deduced from the pot experiments:

    - relative to high concentrations of radium in the water the results showed that the leaves of both beetroot and cabbage produced about the same CR, while the roots of cabbage produced a greater CR than those of beetroot.

    - the difference between the effects of root only (or flood only) irrigation and root plus leaf (or flood plus spray) irrigation was the greatest for the beetroot where there was about X2 increase in soil CR and X4 increase in water CR.

    - a transfer of radium from the leaves of beetroot to the roots of the same plant took place where spray irrigation was applied.

    - the actual concentration of radium in the beetroot vegetable was greater than that in the cabbage.

- The conclusion is that beetroot (and possibly other root crops) are more vulnerable to radium contamination than cabbage (or leaf crops) and that flood irrigation only is preferred to limit possible uptake. The latter is relevant to high water concentrations of radium but may not be the case for much lower concentrations found in practice.

- The dose assessment results for reasonably realistic, yet conservative assumptions for water and vegetable consumption show that doses to a maximum individual will be of the order of 1% of the annual limit of 250 μSv. Radium, as expected, has a higher contribution than uranium. If the ^{226}Ra exposure scenario is considered, the drinking water radium concentration were a factor 5 higher, i.e. equal to the current EPA (USA) limit of approximately 0,2 Bq.ℓ^{-1}, then the annual committed effective dose would be 39 μSv, still well below the dose limit. Only infrequently, and then very close to mine properties, have such concentrations been observed locally. Similarly a factor 5 or 20 increase in vegetable concentrations will still leave a good margin below the limit. Obviously, production of crops on mine dumps should be prohibited or strictly controlled as evidenced by the individual results from oats production.

- The data collected in this study in the Witwatersrand area shows that no member of the public is expected to be exposed via aquatic pathways to any significant dose. If mine effluents are controlled to less than 0,2 Bq.ℓ^{-1} radium and less than 0,5 Bq.ℓ^{-1} uranium, the doses received from various aquatic pathways should be well within dose limits set. The concentration values quoted here are not intended to imply these should be concentration limits. Control limits are set by CNS on site specific assessments. The collective dose to the high density population of the Witwatersrand on a whole was not considered in this study but for the aquatic pathway it is expected to be low.

5.11 Recommendations for future investigations

A few improvements to the present and possibly future investigations are hereby presented:

- Due to analytical uncertainty, low level measurements suffer from large errors. This fact can be demonstrated by some samples obtained from the Marievale farm which could not, due to the analytical technique produce any values above the detection limit, although it was expected that ^{226}Ra be present in all samples. Therefore it is recommended that a more sensitive analytical technique be used.

- It is often found with older literature that difficulties arise when different units of measurement and different terminologies are encountered. A vast amount of time is usually spent in converting such data to a more recent and acceptable format. Weight units (g,kg), weight basis (fresh, dry and ash) and the conversion factors are all very important components in report format. In this report, it was decided to use basic SI units of measurement. Therefore, in the case of ^{226}Ra content in solid matter (ash), concentrations were expressed in Bq.g^{-1} while concentrations in liquid samples (water) were expressed in Bq.ℓ^{-1} (CR-values were calculated using the fresh mass content of vegetables). This approach in measurement could therefore explain the differences often encountered in the CRs and CRw values. The difference in CRs and CRw values would therefore have been smaller if solid concentrations were expressed in Bq.kg^{-1}, or when Bq.g^{-1} and Bq.mℓ^{-1} was used. For the sake of uniformity, a few sacrifices had to be made and the use of basic SI units of measurement proved to be the best.

- Data are always expressed as $Bq.g^{-1}$ ash weight. Additionally wet, dry and the ash mass of each sample were also supplied. In the literature, conversion factors are often supplied, but in this report these figures were provided to facilitate calculations and make it more accurate.

- To further improve reporting, environmental conditions such as water quality (physical and chemical) as well as soil characteristics should be included. Often, this data could prove to be very helpful in explaining certain phenomena. The sources of radium contamination should also be determined to obtain more information about its chemical species.

- The term CR (Concentration Ratio) was also accepted as suggested by Simon & Ibrahim (1990). It should be accepted as a universal term. The term CR gives clarification on its meaning, i.e. implies a ratio, while terms as bioconcentration factor (BF) etc. could sometimes be cumbersome and misleading and does not specifically refer to a ratio.

- Additional consideration of the concept that the CR may be treated as a function rather than as a constant should also be followed.

Certain areas which justify additional research have also been identified. These include studies on:

- The differences in ^{226}Ra and uranium uptake between different plants species.

- The kinetics of the movement of ^{226}Ra across root membranes and within plants.

- The degree of foliar absorption of ^{226}Ra by plants. The influence of wax and hair layers on leaf surfaces on ^{226}Ra absorption.

- The influence of calcium and other alkaline earth elements on radium uptake by plants.

- Determination of the species of radium in soil and plants.

- The effect of organic matter, soil pH etc. on the mobility, activity and bioavailability of ^{226}Ra.

- Preparation and cleaning methods of algae and aquatic macrophytes prior to analysis.

- Accumulation and concentration of radionuclides by bone tissues of local aquatic vertebrates. Additional information on radionuclide metabolism may be gained through analyses of indivdual organs.

- More detailed investigations into the regulation and metabolism of radionuclides by cattle may be of valuable interest. A full mass-balance study may provide information on the consumability of meat- and milk products.
- The effectivity of wetlands in the removal of radionuclides from contaminated water need also be investigated.
- A range of other radionuclides (Th, Po, Pb, etc.) are also present in the underground mining situation where workers are possibly exposed to radiation. The presence and possible impact of these nuclides on workers as well as the environment need also be investigated.

REFERENCES

6

6 REFERENCES

AINSLIE, L.C. 1993. Atomic Energy Corporation of South Africa. Personal communication

ALBERTS, B., *et al.*, 1983. **Molecular Biology of the Cell.** Garland Publishing. New York.

ANDERSON, J.B., TSIVOGLOU, E.C., SHEARER, S.D. 1963. Effects of uranium mill wastes on biological fauna of the Animas River (Colorado-New Mexico). **Radioecology.** (Proc. 1st Natl. Symp. Fort Collins, CO, 1961) (SCHULTZ, V., KLEMENT, A.W. Eds.). Rheinhold an American Institute of Biological Sciences, Washington, DC 373 - 383.

ANTROBUS, E.S.A. (Ed.). 1986. **Witwatersrand Gold - 100 Years.** Geological Society of South Africa. Kelvin House. Johannesburg.

ARNOTT, H.J. & PAUTARD, F.G.E. 1970. **Biological Calcification: Cellular and Molecular Aspects.** pp. 375 - 446.

AYRES, D.E.R. & WESTWOOD, R.J. 1957. The use of ion exchange process in the extraction of uranium from Rand ores with particular reference to practice at the Randfontein uranium plant. In: **Uranium in South Africa 1946 - 1956.** Vol. 2. pp. 85 - 176. The Associated Scientific & Technical Societies of South Africa. Johannesburg.

BARANOV, V.I. 1939. Assimilation of radioactive elements by plants. **Doklady Akad. Nauk SSSR**, 24: 951 - 954.

BENES, P. 1984. Migration of Radium in the terrestrial hydrosphere. In: **Behaviour of Radium in Waterways and Aquifers.** IAEA-TECDOC-301. IAEA. Vienna. pp. 117 - 174.

BERNARD, S.R. & STRUXNESS, E.G. 1957. **A Study of the Distribution and Excretion of Uranium in Man.** ORNL-2304. Oak Ridge National Laboratory. Oak Ridge. Tennessee.

BIDDULPH, O. 1960. Radioisotopes in plants: Foliar entry and distribution. In: **Radioisotopes in the Biosphere.** (CALDECOTT, R.S. & SNYDER, L.A. Eds.). pp. 73 - 85. University of Minnesota Press. Minneapolis. MN.

BLANTZ, R. 1975. **J. Clin. Invest.**, 55: 621.

BLIGHT, C.E. & CLADWELL, J.A. 1984. The abatement of pollution from abandoned gold-residue dams. **J. S. Afr. Inst. Min. Metall.**, 84(1): 1-9.

BRAY, R.H. 1954. A nutrient mobility concept of soil-plant relationships. **Soil Sci.**, 78: 9 - 22.

BURKART, W. 1988. Radiotoxicity. In: **Handbook on Toxicity of Inorganic Compounds.** (SEILER, H.G. & SIGEL, H. Eds.). Marcel Dekker, Inc. New York.

BURKART, W. 1991. Uranium, Thorium and Decay Products. In: **Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance.** (MERIAN, E. Ed.). pp.1275 -1287. VCH Verlagsgesellschaft. Weinheim.

CARTWRIGHT, F.D. 1983. Some seventy years in the shrouding of mining residue deposits for environmental protection. I.A.W.P.R. Conf., **Wat. Sci. Tech.**, 15(2): 85-102. "Mine Water Pollution", (P.E. ODENDAAL Ed.).

CATALDO, D.A. & WILDUNG, R.E. 1978. Soil and plant factors influencing the accumulation of heavy metals by plants. **Environ. Health Perspect.**, 27: 149 - 159.

CHAPMAN, T.S. & HAMMONS, S. Jr. 1963. Some observations concerning uranium content of ingesta and excreta of cattle. **Health Phys.**, 9: 79 - 81.

Commission of the European Communities (CEC). 1986. Council Directive of June 12, 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in Agriculture. **Off. of the Eur. Communities.** No. L181/6-12.

CHAMBER OF MINES OF SOUTH AFRICA. 1979. The design, operation and closure of residue deposits. **Handbook of Guidelines for Environmental Protection.** 1.

CHAMBER OF MINES OF SOUTH AFRICA. 1989. **Analysis of Working Results: October to December 1989.** Chamber of Mines. Johannesburg.

CHAMBER OF MINES OF SOUTH AFRICA. 1993. **Analysis of Working Results: October - December 1993.** Chamber of Mines. Johannesburg.

CLARKSON, D.T. & HANSON, J.B. 1980. The mineral nutrition of higher plants. **Annu. Rev. Plant Physiol.**, 31: 239 - 298.

CLINE, J.F. 1968. **Uptake of Am-241 and Pu-239 by plants.** In: USAEC Rept. BNWL-714. pp. 8.24 - 8.25. Battelle Pacific Northwest Laboratory. Raichland. Washington.

COETZEE, N.J.C. 1991. Personal communication

COGGLE, J.E. 1983. **Biological Effects of Radiation.** Taylor & Francis Inc. New York.

CONWAY, N.F., DAVY, D.R., GILES, M.S., NEWTON, P.J.F. & POLLARD, D.A. 1974. **The Alligator rivers area fact finding study.** Four AAEC Reports. Australian Atomic Energy Commission. Research Establishment Lucas Heights.

COOK, W. 1973. Green grow the mine dumps. **Mining Survey**, 73: 21-23.

COOK, C.D.K., GUT, B.J., RIX, E.M., SCHNELLER, J. & SEITZ, M. 1974. **Water plants of the world: A manual for the identification of the genera of freshwater macrophytes.** Dr. W. Junk b.v, Publishers. The Hague.

COOPER, M.B., STANNEY, K.A. & WILLIAMS, G.A. 1981. **An Investigation of the Speciation of Radionuclides in Sediments and Soils.** Rep. ARL/TR-039. Australian Radiation Laboratory. Melbourne.

COUGHTREY, P.J. & THORNE, M.C. 1983. **Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems: A Critical Review of Data.** A.A. Balkema. Rotterdam.

CURIE, M., CURIE, P. & BEMONT, G. 1898. Another new radio-active element. **C.R.**, 127: 1215 - 1217.

DACEY, J.W.H. 1981. Pressurized ventilation in the yellow waterlily. **Ecology**, 62: 1137 - 1147.

DAVY, D.R., CONWAY, N.F. 1974. Environmental studies, Northern Territory Uranium Province 1971-73. **The Alligator Rivers Area Fact Finding Study**. Four Reports (CONWAY, N.F., DAVY, D.R., GILES, P., NEWTON, P.J.F, POLLARD, D.A. Eds). Rep. AAEC/E-305, Australian Atomic Energy Commission, Lucas Heights Research Laboratories, III>

DAVY, D.R., O'BRIEN, B.G. 1975. Radiological Aspects. In: **Rum Jungle Environmental Studies** (DAVY, D.R., Ed.). Rep. AAEC/E-365, Australian Atomic Energy Commission, Lucas Heights Research Laboratories, Chapter 9.

DE BORTOLI, M., GAGLIONE, P. 1972. Radium-226 in environmental materials and foods, **Health Phys.**, 22: 43 - 48.

DE JESUS, A.S.M., MALAN, J.J., ELLERBECK, V.T., VAN DER BANK, D.J., MOOLMAN, E.W. 1987. **An assessment of the radium-226 concentration levels in tailings dams and environmental waters in the gold/uranium mining areas of the Witwatersrand.** PER-159. Pelindaba.

DEPARTMENT OF WATER AFFAIRS. 1986. **Management of the Water Resources of the Republic of South Africa.** CTP Book Printers. Cape Town.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWA&F). 1993. **South African Water Quality Guidelines.** Vol. 4. Agricultural Use. Department of Water Affairs and Forestry. Pretoria.

DE WAAL, S.A. 1973. More light on the U clan. **Nuclear Active.** pp. 21-23.

DOLAN, J. 1961. Water problems of the Transvaal and Orange Free State mines. Trans. **Seventh Commonwealth Mining Metallurgical Congr.**, 3: 1357 - 1388.

DREESEN, D.R., WILLIAMS, J.M., MARPLE, M.L., GLADNEY, E.S. & PERRIN, D.R. 1981. **Mobility and Bioavailability of Uranium Mill Tailings Contaminants.** Rep. LA-UR-3733. Los Alamos National Laboratory. Los Alamos. NM.

DREW, I.M. 1972. Atomic number and the periodic table. In: **The Encyclopedia of Geochemistry and Environmental Sciences.** (FAIRBRIDGE, R.W. Ed.). pp. 43 - 48. Encyclopedia of Earth Sciences Series. Vol. IVA. Van Nostrand Reinhold. Princeton, NJ and New York.

DROBKOV, A.A. 1937. The influence of the radioactive elements uranium, radium, thorium and actinium on the yield of plants. **Doklady Akad. Nauk SSSR**, 17: 229 - 232.

D'SOUZA, T.J. & MISTRY, K.B. 1970. Comparative uptake of thorium-230, radium-226, lead-210 and polonium-210 by plants. **Radiat. Bot.**, 10: 293 - 295.

DU PLESSIS, L.J. 1991. Personal communication

DU PREEZ, W.J.S. 1991. Personal communication

EAST RAND GOLD AND URANIUM CO. LTD. (ERGO). 1989. **Annual Report.** 1989.

EISENBUD, M. *et al.* 1964. Naturally occurring radionuclides in foods and waters from the Brazilian areas of high radioactivity. In: **Natural Radiation Environment.** (Proc. Int. Symp. Houston. 1963.) (ADAMS, J.A.S. & LOWDER, W.M. Eds.) pp. 837 - 854. University of Chicago Press. Chicago. IL.

ELLERBECK, V.T., SMIT, M.C.B & GERMISHUYS, G. 1990. **Voorbereiding van plantmateriaal (Omgewingstudies).** Atoom Energie Korporasie van Suid-Afrika. Inligting en Handleidings: Verwysing 2/5/04.

EMERSON, S. & HESSLEIN, R. 1973. Distribution and uptake of artificially introduced radium-226 in a small lake. **J. Fish. Res. Board Can.** 30: 1485 - 1490.

ENGELBRECHT, C.J. 1989. **Operating Gold Mines and Gold Recovery Plants in the RSA.** Department of Mineral and Energy Affairs. Minerals Bureau. Braamfontein.

EPSTEIN, E. 1972. **Mineral Nutrition of Plants: Principles and Perspectives.** Wiley. New York.

ETTENHUBER, E., CLAJUS, P. & ROHNSH, W. 1975. Bestimmung der **Akkumulationfatoren Fisch/Wasser einiger fur die Strahlenbelastung des Menschen Wichtiger Radionuklide**. Rep. SAAS-175, Staatliches Amt fur Atomsicherheit und Strahlenschutz, Berlin.

EVANS, R.D. 1933. Radium poisoning: A review of present knowledge. **Am. J. Public Health,** XXII: 1017 - 1023.

FEATHER, C.E. & KOEN, G.M. 1975. The Mineralogy of the Witwatersrand reefs. **Miner. Sci. Engng.**, 7, 189-224.

FISHER, D.R. 1988. Uranium. In: **Handbook on Toxicity of Inorganic Compounds.** (SEILER, H.G. & SIGEL, H. Eds.). Marcel Dekker, Inc. New York.

FLINN, F.B. 1926. Radioactive material an industrial hazard? **J. Am. Med. Assoc.**, 87: 2078 - 2081.

FOX, R.W., KELLEHER, G.G. & KERR, C.B. 1977. **Ranger Uranium Environmental Inquiry.** Second Report. Australian Govt. Publishing Service. Canberra.

FREEMAN, W.H. & BRACEGIRDLE, B. 1980. **An Advanced Atlas of Histology.** Heinemann Educational Books. London.

FROST, H.M. 1967. The dynamics of osteoid tissue. In: **L'osteomalacie.** (HIOCO, D.J. Ed.). pp. 3 - 18. Masson. Paris.

FUNKE, J.W. 1990. **The water requirements and pollution potential of South-African gold and uranium mines.** WRC Report No. KV 9/90. Water Research Commission. Pretoria.

GARLAND, T.R., WILDUNG, R.E., NEEL, J.W. & CATALDO, D.A. 1974. **Factors affecting uptake and distribution of plutonium in barley and soybean plants.** In: Rept. BNWL-1950. Part 2. pp. 30 - 36. Batelle Pacific Northwest Laboratory. Richland. Washington.

GERAL, J. 1990. Personal communication

GRUNDLING, A. & SCHOEMAN, R.P. 1989. **Ondersoeke na geskikte woongebiede vanaf Nigel tot Klerksdorp vir binneshuise radonmetings.** Tegniese Nota No. KTAO-TN 89/5. Atoomenergie Korporasie van SA BPK. Julie 1989.

GRZYBOWSKA, D. 1974. Uptake of radium-226 by plants form contaminated soils. **Nukleonica**, 19(1): 62 - 68.

GUNN, K.B. & MISTRY, K.B. 1970. The effect of chelating agents on the absorption of radium by plants. **Plant Soil**, 33: 7 - 16.

HALLDEN, N.A., FISENNE, I.M. 1961. **Fallout Program Quarterly Summary.** Rep. HASL-112, United States Atomic Energy Commission, Washington, DC.

HAMILTON, E.I. 1972. **Health Phys.**, 22: 149 - 153.

HARDING, A.J. 1994. **Uranium. In: South Africa's Mineral Industry 1993/1994.** Department of Mineral and Energy Affairs. Mineral Bureau. Braamfontein.

HANFORD ENVIRONMENTAL RADIATION DOSIMETRY SOFTWARE SYSTEM. 1988. (Computer Programme for the Modelling of Radionuclides in Humans).

HARRISON, G.E., CARR, T.E.F. & SUTTON, A. 1967. Distribution of radioactive calcium, strontium, barium and radium following intravenous injection into a healthy man. **Int. J. Radiat. Biol.**, 13: 235 -247.

HAVLIK, B. 1967. Cumulation of radioactive substances by organisms of natural biocenosis of surface water and its significance for determination of radioactive lead. **J. Hyg. Epid. Microb. Immun.**, 2: 229 - 245.

HAVLIK, B. 1970. Radioactive pollution of rivers in Czechoslovakia. **Health Phys.**, 19: 617 - 624.

HAVLIK, B. 1971. Radium in aquatic food chains: Radium uptake by freshwater algae. **Radiat. Res.** 46: 490 - 505.

HAVLIK, B., ROBERTSON, E. 1971. Radium uptake by freshwater algae. In: **Radionuclides in Ecosystems** (Proc. 3rd Natl Symp. Oak Ridge, TN, 1971) (NELSON, D.J. Ed.). CONF-710501-P1. United States Atomic Energy Commission. Washington, DC. 372 - 380.

HAVLIK, B., HANUSOVA, J. & RALKOVA, J. 1980. Hygienic importance of increased barium content in some fresh waters. **J. Hyg. Epidemiol. Microbiol. Imunol.**, 24(4): 396 - 404.

HESSLEIN, R.H. & SLAVICEK, E. 1984. Geochemical pathways and biological uptake of radium in small Canadian Shield lakes. **Can. J. Fish. Aquat. Sci.** 41: 459 - 468.

HODGE, H.C. 1973. A History of Uranium Poisoning (1824 - 1942). In: **Uranium, Plutonium, Transplutonic Elements.** (HODGE, H.C., STANNARD, J.N. & HURSH, J.B. Eds.). pp. 5 - 68. Springer-Verlag. New York.

HOLTZMAN, R.B., URNEZIS, P.W., PADOVA, A. & BOBULA, C.M. 1979. **Contamination of the Human Food Chain by Uranium Mill Tailings Piles.** Rep. NUREG/CR-0758. ANL/ES-69. Argonne National Laboratory. Argonne. IL.

HOWES, B.L., HOWARTH, R.W., TEAL, J.M. & VALIELA, I. 1981. Oxidation-reduction potentials in a salt marsh: Spatial patterns and interaction with primary production. **Limnol. Oceanogr.**, 26: 350 - 360.

INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA). 1984. **The Behaviour of Radium in Waterways and Aquifers.** IAEA-TECDOC-301. IAEA. Vienna.

INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA). 1991. **The Environmental Behaviour of Radium.** Vols. 1 & 2. Technical Reports Series No. 310. International Atomic Energy Agency. Vienna.

IBRAHIM, S.A., CHURCH, S.L. & WHICKER, F.W. 1984. The effects of barium chloride treatment of uranium ore on Rn-222 emanation and Ra-226 leachability from mill tailings. In: **Management of Uranium Mill Tailings, Low-Level Waste and Hazardous Waste.** (Proc. 7th Symp. Fort Collins. CO. 1984). (NELSON, J.D. Ed.). pp. 327 - 334. Colorado State University. Fort Collins.

ICRP (International Commission on Radiological Protection). 1966. **Health Phys.**, 12: 173.

ICRP (International Commission on Radiological Protection). 1973. **Alkaline Earth Metabolism in Adult Man.** ICRP Publication No. 20. Permagon Press. Oxford.

ICRP (International Commission on Radiological Protection). 1975. **Report of the Task Group on Reference Man.** Publication No. 23. ICRP. Permagon. Oxford.

ICRP (International Commission on Radiological Protection). 1979a. **Radionuclide Release in the Environment: Assessment of Doses to Man.** ICRP Publication No. 29. Annals of the ICRP 2, 2C.

ICRP (International Commission on Radiological Protection). 1979b. **Limits on Intakes of Radionuclides by Workers.** ICRP Publication No. 30. Part 1. Vol 2. No. 3/4/ Permagon Press. Oxford.

ICRP (International Commission on Radiological Protection). 1979c. **Limits for Intakes of Radionuclides by Workers.** ICRP Publication No. 39. Part 1 - 3. Permagon Press. Oxford.

ICRP (International Commission on Radiological Protection). 1991. **1990 Recommendations of the International Commission on Radiological Protection.** ICRP Publication 60. Annals of the ICRP, 21 No. 1-3. Permagon Press, 1991.

INAGO, F. 1992. Personal communication

IYENGAR, M.A.R., RAJAN, M.P., GANAPATHY, S., KAMATH, P.R. 1980. **Sources of radiation exposure in a low monazite environment.** Natural Radiation Environment III (Proc. Int. Conf. Houston, 1978) (T.F. GESELL & W.M. LOWDER, Eds.) Vol. 2. CONF-780422. Technical Information Centre. US Department of Energy. Oak Ridge. TN. pp. 1090 - 1106.

IYENGAR, M.A.R., 1990. The natural distribution of radium. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. IAEA (International Atomic Energy Agency). Vienna.

JACKSON, P.B.N. 1975. **Common and scientific names of the fishes of Southern Africa. Part II. Freshwater Fishes.** Special Publication No 14. J.L.B. Smith Institute of Ichthyology. Rhodes University. Grahamstown.

JEE, W.S.S., DELL, R.B., PARKS, N.J., MILLER, S.C. & WRENN, M.E. 1985. Toxicity of plutonium and americium: Relationship of bone composition to location of ^{226}Ra, ^{239}Pu and ^{241}Am - induced bone sarcomas. In: **Metals in Bone.** (PRIEST, N.D. Ed.). pp. 155 - 174. MTP Press. Lancaster.

JEFFREE, R.A. 1990. Radium Uptake by Freshwater Invertebrates. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. IAEA (International Atomic Energy Agency). Vienna.

JENNE, E.A. & LUOMA, S.N. 1977. Forms of trace elements in soils, sediments and associated waters: An overview of their determination and biological availability. In: **Biological Implications of Metals in the Environment.** (Proc. 15th Annu. Hanford Life Sciences Symp. Richland. WA. 1975). pp. 110 - 143. CONF-750929. Technical Information Centre. United States Energy Research and Development Administration. Oak Ridge. TN.

JEPPE, C.B. 1946. Shafts. Sinking equipment and procedure. In: **Gold Mining on the Witwatersrand.** Vol 1. pp. 493 - 501. The Transvaal Chamber of Mines, Johannesburg.

JONES, G.A., BRIERLY, S.E., GELDENHUIS, S.J.J. & HOWARD, J.R. 1989. **Research on the contribution of mine dumps to the mineral pollution load in the Vaal Barrage.** Report to the WRC by Steffen Robertson and Kirsten (PTA) Inc. WRC Report No. 136/1/89. Water Research Commission. Pretoria.

JUBB, R.A. 1967. **Freshwater fishes of Southern Africa.** A.A. Balkema. Cape Town.

JUSTYN, J. & HAVLIK, B. 1990. Radium uptake by freshwater fish. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. Vol. 1. pp. 529 - 543. IAEA (International Atomic Energy Agency). Vienna.

JUSTYN, J., & LUSK, S. 1976. Evaluation of natural radionuclide contamination of fishes in streams affected by uranium ore mining an milling, **Folia Zool.**, 25(3): 265 -274.

JUSTYN, J. & STANEK, Z. 1974. Accumulation of natural radionuclides in the bottom sediments and by aquatic organisms of streams. **Int. Revue ges. Hydrobiol.**, 59(5): 593 - 609.

JUSTYN, J., MARVAN, P., ROSOL, J. 1979. Radioactive wastes and aquatic organisms, Vydal Vyzkumny ustav vodohospodarsky ve Statnim zemedolskem nakladatelstvi. Prague. **Prace a Studie, S.**, 151: 43 - 214.

JUSTYN, J., OLIVA, O., PIVNICKA, K. & SVATORA, M. 1985. Accumulation of natural radionuclides and growth of selected fish species in contaminated waters. **Vestn. Cesk. Spol. Zool.**, 49(4): 253 - 266.

KALIN, M. & SHARMA, H.D. 1982. Radium-226 and lead-210 uptake in Typha latifolia from inactive uranium mill tailings in Canada. In: **Environmental Migration of Long-Lived Radionuclides.** (Proc. Int. Symp. Knoxville. TN. 1981.). pp. 247 - 262. IAEA. Vienna.

KALKWARF, D.R. 1979. **Solubility Classification of Airborne Products from Uranium Ores and Tailings Piles.** NUREG/CR-0530. Pacific Northwest Laboratory. Richland. Washington.

KATHREN, R.L. 1984. **Radioactivity in the Environment.** Harwood Academic. London.

KEMPE, J.O. 1983. Review of water pollution problems and control strategies in the South African mining industry. **Wat. Sci. Tech.**, 15: 27-58.

KEMPSTER, P.L., HATTINGH, W.H.J. & VAN VLIET, H.R. 1982. **Summarized Water Quality Criteria.** Technical Report No. TR 108. Department of Environment Affairs. Pretoria.

KHADEMI, B., ALEMI, A.A. & NASSERI, A. 1980. Transfer of radium from soil to plants in an area of high natural radioactivity in Ramsar, Iran. In: **Natural Radiation Environment III.** (Proc. Int. Conf. Houston. 1978). (GESELL, T.F. & LOWDER, W.M. Eds.). Vol. 1. pp. 600 - 610. CONF-780422. Technical Information Centre. United States Department of Energy. Oak Ridge. TN.

KIRCHMANN, R. & BERINO, G. 1965. Comportement du ^{222}Rn chez *Pisum sativum* L. **Phyton**, 22: 137 - 139.

KIRCHMANN, R., BOULENGER, R. & LAFONTAINE, A. 1966. Uptake of Ra-226 by crop plants. **Health Phys.**, 12: 1805 - 1806. (abstract only).

KIRCHMANN, R., LAFONTAINE, A., CANTILLON, G. & BOULENGER, R. 1973. **Etude du cycle biologique parcouru par la radioactvite.** Rep. BLG-477. Belgian Nuclear Centre (CEN/SCK). Mol, Belgium.

KLEMENT, A.W., Jr. 1982. **Natural sources of environmental radiation**. CRC Handbook of Environmental Radiation (KLEMENT, A.W., Jr., Ed.). CRC Press. Boca Raton.

LAL, N., SHARMA, Y.P., SHARMA, P.K., TALWAR, I.M., NAGPAUL, K.K. & CHAKARVARTI, S.K. 1982. Uranium assay in milk. **Health Phys.**, 43(3): 425 - 428.

LE ROUX, P.J. & STEYN, L. 1968. **Fishes of the Transvaal.** Cape & Transvaal Printers Ltd. Cape Town.

LIEBENBERG, W.R. 1955. The occurrence and origin of gold and radioactive minerals in the Witwatersrand system, the Dominion reef, the Ventersdorp Contact and the Black reef. **Trans. Geol. Soc. S. Africa**, 58, 101-227.

LIEBENBERG, W.R. 1972. Mineralogical features of gold ores in South Africa. In: **Gold Metallurgy in South Africa.** Adamson, R.J. (Ed.). chap 11, pp. 352-426. Chamber of Mines of South Africa, Johannesburg.

LINKE, W.F. 1958. **Solubilities, Inorganic and Metal-Organic Compounds.** Van Nostrand. Princeton.

LINSALATA, P. *et al.* 1984. Radium, thorium and the light rare earth element in soils and vegetables grown in an area of high natural radioactivity. In: **Environmental Research for Actinide Elements.** (Proc. Symp. Hilton Head Island. SC. 1984). (PINDER, J.E. III *et al.* Eds.). CONF-841142. National Technical Information Service. Springfield. VA.

LINSALATA, P., MORSE, R., FORD, H., EISENBUD, M., PENNA FRANCA, P., DE CASTRO, M.B., LOBAO, N., SACHETT, I. & CARLOS, M. 1989. Transport pathways of Th, U, Ra, and La from soil to cattle tissues. **J. Environ. Radioactivity**, 10: 115 - 140.

LINSALATA, P., MORSE, R., FORD, H., EISENBUD, M., PENNA FRANCA, E., DE CASTRO, LOBAO, N., SACHETT, I. & CARLOS, M. 1991. Th, U, Ra and rare earth element distributions in farm animal tissues from an elevated natural radiation background environment. **J. Environ. Radioactivity,** 14: 233 - 257.

LOGAN, T.L. & CHANEY, R.L. 1983. Utilization of municipal wastewater and sludge on land - metals. In: **Utilization of Municipal Wastewater and Sludge on land.** University of California. Riverside. 235 - 323.

LONG, S.E. 1987. **Uranium and human diet: a literature review.** Chemical Analysis Group. Environmental and Medical Sciences Division. United Kingdom Atomic Energy Authority. Harwell Laboratory. Oxfordshire.

MALAN, J.J. 1981. **Die bepaling van radium-226 in die uitskot van goudmyne en hulle omgewings.** M.Sc. Tesis. Universiteit van Pretoria.

MARINELLI, L.D., NORRIS, W.P., GUSTAFSON, P.F. & SPECKMAN, T.W. 1953. Transport of radium sulphate from the lung and its elimination from the human body following single accidental exposures. **Radiology**, 16: 903 - 915.

MARKOSE, P.M., EAPPEN, K.P., VENKATARAMAN, S. & KAMATH, P.R. 1978. Distribution of radium and chemical toxins in the environment of a uranium complex. In: **Natural Radiation Environment III.** (Proc. Symp. Houston, 1978). Technical Information Centre. Springfield. CONF-780422 (1980). 1655 - 1667.

MARKOSE, P.M., BHAT, I.S. & PILLAI, K.C. 1993. Some characteristics of ^{226}Ra transfer from soil and uranium mill tailings to plants. **J. Environ. Radioactivity**, 21: 131 - 142.

MARTIN, S.S., HELM, W.T., SIGLER, W.F. 1969. Accumulation of 226-Ra in two aquatic ecosystems. **Radioecology.** (Proc. 2nd Natl. Sump., Ann Arbor, MI, 1967) (NELSON, D.J., EVANS, F.C., Eds.) CONF-670503, United States Atomic Energy Commission, Washington, DC, 307-318.

MARTINEC, M. 1973. Vliv tezby uranu na obsah 226-Ra v povrchove vode, rakosu a sladkovodnich rybach, **Cesk. Hyg.**, 18 (10): 475 - 478 (in Czech).

MARTLAND, H.S., CONLON, P. & KNEF, J.P. 1925. Some unrecognized dangers in the use of and the handling of radioactive substances, with especial reference to the storage of insoluble products of radium and mesothorium in the reticulo-endothelial system. **J. Am. Med. Assoc.**, 85: 1769 - 1776.

McMICHAEL, A.J. 1989. **The Contribution of Epidemiology to Understanding the Mechanisms of Action of Carcinogens.** Proceedings Vth International Congress of Toxicology. Brighton. pp. 25 - 35. Taylor & Francis. London-New York-Philadelphia.

MENZEL, R.G. 1965. Soil-plant relationships of radioactive elements. **Health Phys.**, 11: 1325 - 1332.

MERCER, E.R., MITCHELL, W.A. & SMITH, K.A. 1962. **Absorption by plants of naturally occurring radioactive materials.** Annual Report 1961 - 1962. Rep. ARCRL-8. pp. 82 - 92. Radiobiological Laboratory. Agricultural Research Council. Wantage. UK.

MOLINARI, J. & SNODGRASS, W.J. 1990. The Chemistry and Radiochemistry of Radium an the other Elements of the Uranium and Thorium Natural Decay Series. In: **The Environmental Behaviour of Radium.** Vol 1. pp. 11 - 56. IAEA (International Atomic Energy Agency). Vienna.

MOOLMAN, W., SMIT, M.C.B. & LABUSCHAGNE, M. 1990. **Verassing van dierlike weefsel.** Atoom Energie Korporasie van Suid-Afrika. Inligting en Handleidings: Verwysing 2/5/03.

MOORE, W.H. 1968. A light-weight pulsed d.c. fish shocker. **J. appl. Ecol.**, 5, 205 - 208.

MOORE, W.S. 1972a. Radioactive isotopes. In: **The Encyclopedia of Geochemistry and Environmental Sciences.** (FAIRBRIDGE, R.W. Ed.). pp. 987 - 990. Encyclopedia of Earth Sciences Series. Vol. IVA. Van Nostrand Reinhold. Princeton, NJ and New York.

MOORE, W.S. 1972b. Radium: element and geochemistry. In: **The Encyclopedia of Geochemistry and Environmental Sciences.** (FAIRBRIDGE, R.W. Ed.). pp. 1006 - 1007. Encyclopedia of Earth Sciences Series. Vol. IVA. Van Nostrand Reinhold. Princeton, NJ and New York.

MOORE, W.S. 1972c. Radon: element and geochemistry. In: **The Encyclopedia of Geochemistry and Environmental Sciences.** (FAIRBRIDGE, R.W. Ed.). pp. 1014 - 1015. Encyclopedia of Earth Sciences Series. Vol. IVA. Van Nostrand Reinhold. Princeton, NJ and New York.

MORISHIMA, H., KOGA, T., KAWAI, H., HONDA, Y. & KATSURAYAMA, K. 1977. **J. Radiat. Res.**, 18: 139 - 150.

MORROW, P.E., GIBB, F.R. & LEACH, L.J. 1966. **Health Phys.**, 23: 273.

MORROW, P.E., LEACH, L.J., SMITH, F.A., GELEIN, R.M., SCOTT, J.B., BEITER, H.D., AMATO, F.J., PICANO, J.J., YUILE, C.L. & CONSLER, T.G. 1982. **Metabolic Fate and Evaluation of Injury in Rats and Dogs Following Exposure to the Hydrolysis Products of Uranium Hexafluoride.** NUREG/CR-2268. New York.

MORSE, R.S. & WELFORD, G.A. 1971. Dietary intake of ^{210}Pb. **Health Phys.,** 21: 53 - 55.

MUTH, H., RAJEWSKY, B., HANTKE, H.-J., AURAND, K. 1960. The normal radium content and the Ra-226/Ca ratio of various foods, drinking water and different organs and tissues of the human body, **Health Phys.**, 2: 239 - 245.

MYTTENAERE, C., BOURDEAU, P & BITTEL, R. 1969. Importance relative de l'eau et du sol dans la contamination indirecte en radiocesium et radiocobalt de rivieres irriguees. In: **Environmental Contamination by Radioactive Materials.** (Proc. Seminar. Vienna). pp. 175 - 182. International Atomic Energy Agency. Vienna.

NAPIER, B.A., PELOQUIN, R.A., STRENGE, D.L., RAMSDELL, J.V. 1988. **GENII - The Hanford Environmental Radiation Dosimetry Software System.** Pacific Northwest Laboratory - BATTELLE. PNL-6584 UC-600. Vol 1. December 1988.

NATHAWANI, J.S. & PHILLIPS, C.R. 1978. Adsorption of Ra-226 by soils in the presence of Ca(+2) ions. Specific adsorption (II). **Chemosphere**, 5: 293 - 299.

NATIONAL TECHNICAL PLANNING GROUP. 1981. Report of the National Technical Planning Group on Uranium Tailings Research. Canada Centre for Mineral and Energy Technology (CANMET). Department of Energy, Mines and Resources. Ottawa.

NCRPM (National Council on Radiation Protection and Measurements). 1975. **Natural Background Radiation in the United States.** Rep. 45. NCRPM. Washington DC.

NEA/IAEA. 1990. **Uranium - Resources, Production and Demand.** OECD. Paris.

NEA/IAEA. 1993. **Uranium - Resources, Production and Demand.** OECD. Paris.

NG, Y.C., COLSHER, C.S. & THOMPSON, S.E. 1990. Transfer factors for assessing the dose from radionuclides. In: **Biological Implications of Radionuclides Released from Nuclear Industries.** Vol. 2. IAEA. Vienna. pp. 295 - 318.

OTAKE, M., YOSHIMARU, H. & SCHULL, W.J. 1987. **Severe Mental Retardation among the Prenatally Exposed Survivors of the Atomic Bombing of Hiroshima and Nagasaki: A Comparison of the Old and New Dosimetry Systems.** Radiation Effects Research Foundation RERF Technical Report. pp. 16 - 87. Hiroshima.

PALLY, M. & FOULQUIER, L. 1983. **Synthese bibliographique sur la capacite et les modalites de la fixation du radiostrontium par les vegetaux aquatiques.** Rep. CEA-BIB-238. CEA. Centre d'etudes nucleaires de Saclay. Gif-sur-Yvette.

PARFITT, A.M. 1983. The physiologic and clinical sigificance of bone histomorphometric data. In: **Bone Histomorphometry: Techniques and Interpretation** (RECKER, R.R. Ed.). pp. 143 - 223. CRC Press. Boca Raton. FL.

PAUL, A.C., LONDHE, V.S., PILLAI, K.C. 1980. Radium-228 and radium-226 levels in a river environment and its modification by human activities. **Natural Radiation Environment III** (Proc. Int. Conf. Houston, 1978) (GESELL, T.F., LOWDER, W.M., Eds.), Vol. 2, CONF-780422, Technical Information Centre, United States Department of Energy, Oak Ridge, TN 1633 - 1654.

PENNA FRANCA, E. *et al.*, 1968. Radioactivity of Brazil nuts. **Health Phys.**, 14: 95 - 99.

PENOT, M., FLOC'H, J.-Y. & PENOT, M. 1976. Etude comparee de l'absorption et de la redistribution du Ca-45 chez divers groupes de vegetaux. **Planta**, 129: 7 - 14.

PALABORA MINING COMPANY. 1990. **Annual Report**. 1990.

PLÖGER, F. & VIETZKE, H. 1983. Uranium and Uranium-Compounds (in German). In: **Ullmanns Encyklopädie der technischem Chemie.** 4th Ed., Vol. 23. pp. 457 - 490. Verlag Chemie. Weinheim-Deerfield Beach/Florida-Basel.

PONNAMPERUMA, F.N. 1972. The chemistry of submerged soils. **Adv. Agron.** 24: 29 - 96.

PONTIUS,F.W. **Journal AWWA**, Vol 83 no 4, April 1991.

POPOVA, O.N., KODANEVA, R.P. & VAVILOV, P.P. 1964. Distribution in plants of radium absorbed from the soil. **Sov. J. Plant Physiol.**, 11: 371 - 375.

POPOVA, O.N. & KYRCHANOVA, A.N. 1974. Forms of Ra-226 deposition in plants. **Agrokhimiya,** 2: 110 - 115. (in Russian). English translation. Chem Abs. 81. Abstr. No. 74116m.

PRYOR, E.J. 1965. Selected ore treatments. In: **Mineral Processing.** 3rd ed. chap 23. pp. 780 - 791. Elsevier. Amsterdam.

RASKIN, I. & KENDE, H. 1985. Mechanism of aeration in rice. **Science**, 228: 327 - 329.

REINHARD, D. & FöRSTNER, U. 1976. Metallanreicherungen in Sedimentkernen aus Stauhaltungen des mittleren Neckars. **Neues Jahrb. Geol. Palaontol. Monatsh.**, 5: 301 - 320.

RICE, T.R. 1956. The accumulation and exchange of strontium by marine planktonic algae. **Limnol Oceanogr.**, 12: 123 - 138.

ROBERTS, H.R. Jr. & MENZEL, R.G. 1965. Availability of exchangeable and non-exchangeable strontium-90 to plants. In: **Radioactive Fallout, Soils, Plants, Foods, Man.** (FOWLER, E.B. Ed.). Elsevier. New York.

ROWLAND, R.E., STEHNEY, A.F. & LUCAS, H.F. 1978. Dose-response relationships for female radium dial workers. **Radiat. Res.**, 76: 368 - 383.

RUNDO, J. & HOLTZMAN, R.B. 1976. Comparison of the late excretion of ^{226}Ra and ^{239}Pu by man. In: **Health Effects of Plutonium and Radium.** (JEE, W.S.S. Ed.). pp. 497 - 504. J.W. Press. salt Lake City. UT.

RUSANOVA, G.V. 1962. Study of leaching and migration of radium in soils. **Sov. Soil Sci.**, 9: 962 - 964.

SAAGER, R. 1984. Uranium. In: **Metallic Raw Materials Dictionary** (in German). pp. 165 - 169. Bank von Tobel. ZÜrich.

SCHINDLER, P.W. 1990. Co-adsorption of metal ions and organic ligands: Formation of ternary surface complexes. In: **Mineral-Water Interface Geochemistry.** (HOCHELLA, M.F. & WHITE, A.F. Eds.). Vol. 23. Chp. 7. pp. 281 - 307. Reviews in Mineralogy. Mineralogical Society of America. Washington DC.

SHUKLA, V.K., MENON, M.R., RAMACHANDRAN, T.V., SATHE, A.P. & HINGORANI, S.B. 1994. Natural and fallout radioactivity in milk and diet samples in Bombay and population dose rate estimates. **J. Environ. Radioactivity**, 25: 229 - 237.

SCHÜTTELKOPF, H. & KIEFER, H. 1982. Radium-226 contamination of the Black Forest and the radioecological behaviour of radium. In: **Environmental Migration of Long-Lived Radionuclides.** (Proc. Int. Symp. Knoxville. TN. 1981). pp. 345 - 352. IAEA. Vienna.

SCOTT, L.M. 1973. Environmental Monitoring and Personnel Protection in Uranium Processing. In: **Uranium, Plutonium, Transplutonic Elements.** (HODGE, H.C., STANNARD, J.N. & HURSCH, J.B. Eds.). pp. 271 - 294. Springer-Verlag. N.Y.

SHEPPARD, M.I. 1980. **The Environmental Behaviour of Radium.** Rep. AECL-6796. Whiteshell Nuclear Research Establishment. Atomic Energy of Canada Limited. Pinawa. Manitoba.

SHUKLA, V.K., MENON, M.R., RAMACHANDRAN, T.V., SATHE, A.P. & HINGORANI, S.B. 1994. Natural and fallout radioactivity in milk and diet samples in Bombay and population dose rate estimates. **J. Environ. Radioactivity**, 25: 229 - 237.

SIMON, S.L. & IBRAHIM, S.A. 1990. Biological Uptake of Radium by Terrestrial Plants. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. IAEA (International Atomic Energy Agency). Vienna.

SIMPSON, D.B. & McGILL, B.L. 1980. **Users Manual for LADTAP II. A computer program for calculating Radiation Exposure to man from routine release of nuclear reactor liquid effluents.** NUREG/CR-1276. ORNL 1980.

SMIT, D.S. 1989. Uranium. In: **South Africa's Mineral Industry.** (VAN ZYL, L., SCHREUDER, C.P. & VAN ZYL, W.J. Eds.). pp 61 - 66. Minerals Bureau. Department of Mineral and Energy Affairs. Braamfontein.

SMITH, K.A. 1971. The comparative uptake and translocation by plants of calcium, strontium, barium and radium. II. **Triticium vulgare** (wheat). Plant **Soil**, 34: 643 - 651.

STARY J., ZEMAN, A., HAVLIK, B. 1983. Radionuclides in the investigation of cumulation of toxic elements on algae and fish. **Isotopenpraxis**, 19(7): 243 - 244.

STARY, J., KRATZER, K. & PRASILOVA. 1984. The accumulation of radium, barium and lead in algae. **J. Radioanal. Nucl. Chem.**, 84(1): 17 - 21.

STATHER, J.W. 1990. The Behaviour, Effects and Radiation Dosimetry of Radium in Man. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. Vol. 2. pp. 297 - 343. IAEA (International Atomic Energy Agency). Vienna.

STEHNEY, A.F. & LUCAS, H.F. 1956. Studies on the radium content of humans arising from the natural radium of their environment. In: **Peaceful Uses of Atomic Energy.** (Proc. Int. Conf. Geneva. 1955.) Vol. II. pp. 49. United Nations. New York.

STEVENSON, F.J. & ARDAKANI, M.S. 1972. Organic matter reactions involving micronutrients in soils. In: **Micronutrients in Agriculture.** (MORTVEDT, J.J., GIORDANO, P.M. & LINDSAY, W.L. Eds.). Soil Science Society. Madison. Wisconsin.

STOETZEL, G.A., FISHER, D.R., McCORMACK, W.D., HOENES, G.R., MARKS, R.H., MOORE, R.H., QUILLICI, D.G. & BREITENSTEIN, B.D. 1981. **Occupational Exposures to Uranium: Processes, Hazards and Regulations.** USUR-01. U.S. Uranium Registry. Richland. Washington.

STRAUB, C.P., MURTHY, G., CAMPBELL, J.E. 1961. Radionuclides in foods, **J. Am. Diet. Assoc.**, 38: 15 - 21.

SWANSON, S.M. 1985. Food-chain transfer of U-series radionuclides in a northern Saskatchewan aquatic system. **Health Phys.,** 49(5): 747 - 770.

TASKAEV, A.I., OVECHENKOV, V.Ysa., ALEKSAKHIN, R.M. & SHUKTOMOVA, I.I. 1976. Effect of pH and cation composition of the liquid phase on the extraction of radium-226 from soil. **Pochvovedenie**, 12: 46 - 50.

TASKAEV, A.I., OVCHENKOV, V.Y., ALEKSAKHIN, R.M. & SHUKTOMOVA, I.L. 1977. Uptake of Ra-226 by plants and change in its state in the soil-plant tops-litter fall system. **Sov. Soil Sci.**, 2: 79 - 85.

TERMINE, J.D. & POSNER, A.S. 1967. Amorphous/crystalline interrelationships in bone mineral. **Calcif. Tissue Res.**, 1: 8 - 23.

THOMAS, D.C., McNEILL, K.G. & DOUGHERTY, C. 1985. Estimates of lifetime lung cancer risks resulting from radon progeny exposure. **Health Phys.**, 49: 825 - 846.

THUN, M.J., BAKER, D.B., STEENLAND, K., SMITH, A.B., HALPERIN, W. & BERL, T. 1985. **Scand. J. Work Environ. Health**, 11: 83.

TILL, J.E. & MEYER, H.R. 1983. **Radiological Assessment: A textbook on Environmental Dose Analysis.** U.S. Nuclear Regulatory Commission. Washington DC. NUREG/CR-332 ORNL-5968.

TITAEVA, N.A. 1967. Association of radium and uranium with peat. **Geochem Int.**, 4(4): 1168 - 1174.

TITAEVA, N.A., TASKAEV, A.I., OVCHENKOV, V.Ya, ALEKSAKHIN, R.M. & SHUKTOMOVA, I.I. 1978. Content and characteristics of U, Th, Ra and Rn uptake in plants growing under different radioecological conditions. **Sov. J. Ecol.**, 9: 328 - 334.

TORREY, T.W. & FEDUCCIA, A. 1979. **Morphogenesis of the Vertebrates.** John Wiley & Sons. New York.

TRACY, B.L., PRANTL, F.A. & QUINN, J.M. 1983. Transfer of ^{226}Ra, ^{210}Pb and uranium from soil to garden produce: assessment of risk. **Health Phys.,** 44(5): 469 - 477.

TRAVENER, L. 1957. An historical review of the events and developments culminating in the construction of plants for the recovery of uranium from gold ore residues. In: **Uranium in South Africa 1946-1956.** Vol 1, p. 1. The Associated Scientific and Technical Societies of South Africa, Johannesburg.

TSIVOGLOU, E.C., BARTSCH, A.F., RUSHING, D.E. & HOLADAY, D.A. 1958. Effects of uranium ore refinery wastes on receiving waters. **Sewage Industrial Wastes,** 30: 1012 - 1027.

TSIVOGLOU, E.C., STEIN, M. & TOWNE, W.W. 1960. Control of radioactive pollution of the Animas River. **J. Water Poll. Cont. Fed.,** 32: 262 - 287.

TURNER, R.C., BRADLEY, J.M. & MAYNEFORD, W.V. 1958. The naturally occurring alpha-ray activity of foods. **Health Phys.,** 1: 268 - 275.

UNITED NATIONS. 1977. **Sources and Effects of Ionizing Radiation** (Report to the General Assembly). Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). UN. New York.

USNRC (U.S. Nuclear Regulatory Commission). 1980. **Final Generic Environmental Impact Statement on Uranium Milling.** Vol. 3. NUREG-0706. Washington.

USNRC (U.S. Nuclear Regulatory Commission). 1982. Calculation Models for Estimating Radiation Doses to Man from Airborne Radioactive Materials Resulting from Uranium Milling Operations. Regulatory Guide 3.51. March. USNRC. Washington. DC.

VAN DORP, F., ELEVELD, R., FRISSEL, M.J. 1979. **A new approach for soil-plant transfer calculations, Biological Implications of Radionuclides Released from Nuclear Industries** (*Proc. Int. Symp. Vienna,* 1979), Vol. 2, IAEA, Vienna: 399-406.

VAN WYK, B. & MALAN, S. 1988. **Veldgids tot die veldblomme van die Witwatersrand- en Pretoria gebied.** Struik Uitgewers. Kaapstad.

VASCONCELLOS, L.M.H., AMARA, E.C.S., VIANNA, M.E. & PENNA FRANCA, E. 1987. Uptake of Ra-226 and Pb-210 by foodstuff cultivated in a high natural radioactivity region in Brazil. **J. Environ. Radioact.,** 5(4): 287 - 302.

VAUGHAN, J.N. 1970. **The Physiology of Bone.** Clarendon Press. Oxford.

VAVILOV, P.P., POPOVA, O.N. & KODANEVA, R.P. 1964. On the behaviour of radium in plants. **Sov. J. Plant Physiol.**, 157(4): 992 - 994.

VERKHOVSKAYA, I.N., VAVILOV, P.P. & MASLOV, V.I. 1967. The migration of natural radioactive elements under natural conditions and their distribution according to biotic and abiotic environmental components. In: **Radioecological Concentration Processes** (Proc. Int. Symp. Stockholm. 1966). (ABERG, B & HUNGATE, F.P. Eds.)..pp. 313 - 328. Permagon Press. Oxford.

VERKHOVSKAYA, I.N., *et al.* 1969. The content and translocation of natural radioactive elements in the system "soil-plants-animals" under natural and experimental conditions. In: **Radioecology.** (Proc. Int. Symp.). pp. 781 - 832. Department de protection sanitaire. CEA. Centre d'etudes nucleaires de Fontenay-aux-Roses.

VIVIER, F.S., PIETERSE, S.A. & AUCAMP, P.J. 1988. **Guidelines for the use of sewage sludge.** Paper presented at the Symposium on Sewage Sludge Handling. Nov. 15, 1988. Division of Water Technology. CSIR. Pretoria.

VON BACKSTRÖM, J.W. 1976. **Uraan.** Geological Survey of South Africa. Handbook 7. pp. 221 - 228.

WAITE, D.T., JOSHI, S.R. & SOMMERSTAD, H. 1988. The effect of uranium mine tailings on radionuclide concentrations in Langley Bay, Saskatchewan, Canada. **Arch. Environ. Contam. Toxicol.**, 17: 373 - 380.

WEATHER BUREAU. 1992. Pretoria. Personal Communication.

WEHR, J.D., EMPAIN, A., MOUVET, C., SAY, P.J. & WHITTON, B.A. 1983. Methods for processing aquatic mosses used as monitors of heavy metals. **Water Res.**, 17: 985 - 992.

WEIGEL, F. 1983. Uranium and Uranium Compounds. In: **Encyclopedia of Chemical Technology.** Vol. 23, 3rd ed., pp. 502 - 547. John Wiley & Sons, New York.

WELFORD, G.A. & BAIRD, R. 1967. Uranium levels in human diet and biological materials. **Health Phys.**, 13: 1321 - 1324.

WHICKER, F.W. & SCHULTZ, V. 1982. **Radioecology: Nuclear Energy and the Environment II.** CRC Press. Boca Raton.

WILDUNG, R.E. & GARLAND, T.R. 1974. Influence of soil plutonium concentration on plutonium uptake and distribution in shoots and roots of barley. **J. Agr. Food Chem.**, 22: 836 - 838.

WILLIAMS, A.R. 1981. **Biological uptake and transfer of ^{226}Ra: a review. Migration in the Terrestrial Environment of Long-lived Radionuclides from the Nuclear Fuel Cycle.** Proc. Int. Symp. Knoxville. International Atomic Energy Agency. Vienna. IAEA-SM-257.

WILLIAMS, A.R. 1982. Biological uptake and transfer of radium-226: A review. In: **Environmental Migration of Long-Lived Radionuclides.** (Proc. Int. Symp. Knoxville. TN, 1981). pp. 175 - 202. IAEA. Vienna.

WILLIAMS, A.R. 1984. Biological Uptake and Transport. In: **The Behaviour of Radium in Waterways and Aquifers.** pp. 177 - 202. IAEA-TECDOC-301. IAEA (International Atomic Energy Agency). Vienna.

WILLIAMS, A.R. 1990. Radium uptake by freshwater plants. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. Vol. 1. pp. 487 - 507. IAEA (International Atomic Energy Agency). Vienna.

WILLIAMS, A.R. & KIRCHMANN, R.J. 1990. Radium: A historical introduction. In: **The Environmental Behaviour of Radium.** Technical Reports Series No. 310. Vol. 1. pp. 3 - 10. IAEA (International Atomic Energy Agency). Vienna.

WITTMANN, G.T.W. & FöRSTNER, U. 1977. Heavy metal enrichment in mine drainage. III. The Klerksdorp, West Wits and Evander Goldfields. **S. Sfr. J. Sci.**, 73: 53 - 57.

WRENN, M.E., DURBIN, P.W., HOWARD, B., LIPSZTEIN, J., RUNDO, J., STILL, E.T. & WILLIS, D.L. 1985. **Health Phys.**, 48: 601.

YUILE, C.L. 1973. Animal Experiments. In: **Uranium, Plutonium, Transplutonic Elements.** pp. 165 - 196. (HODGE, H.C., STANNARD, J.N. & HURSH, J.B. Eds.). Springer-Verlag. New York.

PART 2

INVESTIGATIONS INTO THE ACCUMULATION AND CONCENTRATION RATIOS OF SELECTED METALS IN AQUATIC ECOSYSTEMS AFFECTED BY MINE DRAINAGE EFFLUENTS WITH PARTICULAR REFERENCE TO THE UPTAKE OF METALS BY VEGETABLE CROPS

CONTENTS

Page

PART 2: INVESTIGATIONS INTO THE ACCUMULATION AND CONCENTRATION RATIOS OF SELECTED METALS IN AQUATIC ECOSYSTEMS AFFECTED BY MINE DRAINAGE EFFLUENTS WITH PARTICULAR REFERENCE TO THE UPTAKE OF METALS BY VEGETABLE CROPS

LIST OF TABLES

LIST OF FIGURES

Page

SUMMARY

SUMMARY

Investigations were made into the occurrence and Concentration Ratios of the metals Fe, Cu, Ni, Pb, Zn, Mn and Cr in mine- and industry-polluted aquatic and terrestrial environments on the Witwatersrand, Gauteng Province. Four localities were identified for this study, namely the Crocodile farm (unpolluted), Marievale farm (moderately polluted), Vlakfontein farm (highly polluted) and Luipaardsvlei farm (moderately polluted). In addition to the physical and chemical conditions, which include analyses of the seven metals under consideration, investigations were also made on the occurrence and concentrations of these metals in selected aquatic fauna and flora as well as in vegetables irrigated with these metal polluted waters. In order to evaluate and classify the extent of pollution, use was made of an Aquatic Toxicity Index (ATI) system. The Concentration Ratios were also calculated for the various aquatic plants and animals as well as vegetable crops at each of the localities under consideration.

However, a combination of certain factors such as variations in pH, Dissolved Oxygen contents, Alkalinity and Total Hardness of the water as well as the role of benthic macro-invertebrate organisms on the bioturbation of metals in the soft-bottomed substrate of the aquatic habitats appear to have a direct and/or indirect effect on this parameter. As a result, the actual extent of metal bio-availability which has a direct bearing on Concentration Ratios in aquatic organisms, could not be accurately assessed. Until this is clarified, Concentration Ratios cannot be used with certainty to express the actual transfer of bio-available metals within food webs of aquatic ecosystems.

In view of the findings and shortcomings, recommendations are made on proposed projects which will solve particular problems encountered during the course of this study. This mainly deals with the refinement of the ATI, the role of aquatic fauna in the bioturbation of metals in the substrate of rivers, dams and wetlands and, the identification of indicator organisms for metal pollution in aquatic and terrestrial ecosystems. Proposals are made on the further refinement and use of Concentration Ratios in the evaluation of potential indicator organisms of metal polluted aquatic and terrestrial ecosystems.

OPSOMMING

Ondersoeke is gedoen na die voorkoms en konsentrasieverhoudings van die metale Fe, Cu, Ni, Pb, Zn, Mn en Cr in myn- en industrieëlbesoedelde varswater- en terrestriële omgewings aan die Witwatersrand, Gauteng Provinsie. Vier lokaliteite is uitgesonder vir die studie, naamlik die Krokodil- (onbesoedelde), Marievale- (ligbesoedelde), Vlakfontein- (hoogsbesoedelde) en Luipaardsvlei (ligbesoedelde) plase. Naas die fisies-chemiese toestande, wat onder meer analises ingesluit het van die sewe metale onder bespreking, is ondersoeke ook gedoen na die voorkoms en konsentrasieverhoudings van hierdie metale in geselekteerde akwatiese fauna en flora sowel as in groentegewasse wat met hierdie metaalbesoedelde water besproei is. Om die omvang van besoedeling te evalueer en te klassifiseer, is daar gebruik gemaak van 'n Akwatiese Toksisiteits Indekssisteem (ATI). Hiernaas is die konsentrasieverhoudings ook bereken vir die verskillende akwatiese plante en diere sowel as vir die groentegewasse wat by elk van die lokaliteite aangetref is.

Aanduidings is egter dat 'n kombinasie van sekere faktore soos variasies in pH, Opgeloste Suurstof, Alkaliniteit en Totale Hardheid van die water, sowel as die rol van bentiese makroinvertebraatorganismes in die bioturbering van metale in die sagtebodemsubstraat van die akwatiese habitatte, almal direk of indirek die waardes van hierdie parameter mag beïnvloed. Gevolglik was dit nie moontlik om 'n betroubare beeld te verkry van die werklike omvang van metaalbiobeskikbaarheid nie. Dit het 'n direkte invloed op die betroubare bepaling van konsentrasieverhoudings in akwatiese organismes. Totdat hierdie probleem opgelos is, kan die konsentrasieverhouding nie met sekerheid gebruik word om die werklike oordrag van biobeskikbare metale binne voedselkettings van akwatiese ekosisteme te bepaal nie.

In die lig van die bevindings en die tekortkominge wat in die ondersoeke voorkom, word aanbevelings gemaak ten opsigte van projekte wat hierdie betrokke probleme wat aangetref is tydens die studie, mag oplos. Dit geld veral vir verbeteringe aan die ATI, die rol van akwatiese fauna in die bioturbering van metale in die substrate van riviere, damme en vleilande asook die identifisering van indikatororganismes vir metaalbesoedeling in akwatiese en terrestriële ekosisteme. Voorstelle word ook gemaak oor die verdere verfyning en gebruik van konsentrasieverhoudinge in die evaluering van potensiële indikatororganismes van metaalbesoedelde akwatiese en terrestriële ekosisteme.

INTRODUCTION

1

1 INTRODUCTION

The economies of most modern nations rely heavily on the availability of metals and their compounds which find diverse uses in science, technology and medicine. Metals therefore have been key factors in the liberation of modern civilization from hunger, disease and discomfort. However, each industrial or mining process generates wastes which must be discharged into the environment, along with the growing list of new metallic compounds (Nriagu, 1988). A recent estimate shows that the toxicity of all the metals presently being released into the environment annually, already far exceeds the total combined toxicity of all existing organic and radioactive wastes. Potentially, toxic metals are non-degradable and their continuous buildup and accumulation in mankind's life-support systems, has assumed some considerable health implications (Nriagu, 1988).

Only a very small fraction of the total volume of the earth and its atmosphere is accessible to life. Our planet mostly consists of inorganic substances which supply the raw materials for the biotic environment. For normal life functions such as growth, maintenance and reproduction, living organisms need certain essential elements (Irgolic *et al.*, 1985). In the presence of non-essential elements, these functions may get partially impaired or, to such an extent, where all living functions cease to exist. An overview of these essential, toxic and radioactive elements are presented in Figure 1.1.

A continuous cycling of elements takes place in nature (Irgolic *et al.*, 1985 ; Figure 1.2). The majority of elements cycled are contained in the form of insoluble inorganic compounds in rocks, soil and sediments. Before biota can utilize these elements, the non-available inorganic substances must be mobilized and solubilized. This mobilization phenomenon is effected by weathering processes which involve both abiotic chemical reactions as well as biologically mediated reactions. The degradation of organic material and redox changes induce the release of metals from aquatic sediments and terrestrial soils (Bricker, 1985 ; Morgan *et al.*, 1985).

Numerous substances are released into the aquatic environment by human activities. After the mobilization process where insoluble substances are transformed into soluble substances, the primary products of the process are chemically changed to inorganic co-ordination, organometallic and organo-metalliodal compounds (Irgolic & *et al.*, 1985). These compounds then become chemically available in such a form where they then can interact with and be accumulated by, the biota. Such compound-biota interactions, being inimical or beneficial, either move to the outer surfaces of cells or are transported through membranes into cells (Wood & Wang, 1985). With the aid of cellular biochemical processes, these inorganic compounds may be converted to new chemical species. These newly created species may often be released into the environment through decay or excretion and consequently are directly available to other living organisms. Alternatively, the affected organism may die through toxic reactions where these compounds can be removed from circulation, for instance, by their deposition into sediments. In the sediments these compounds may join the pool of non-available substances awaiting remobilization (Irgolic *et al.*, 1985).

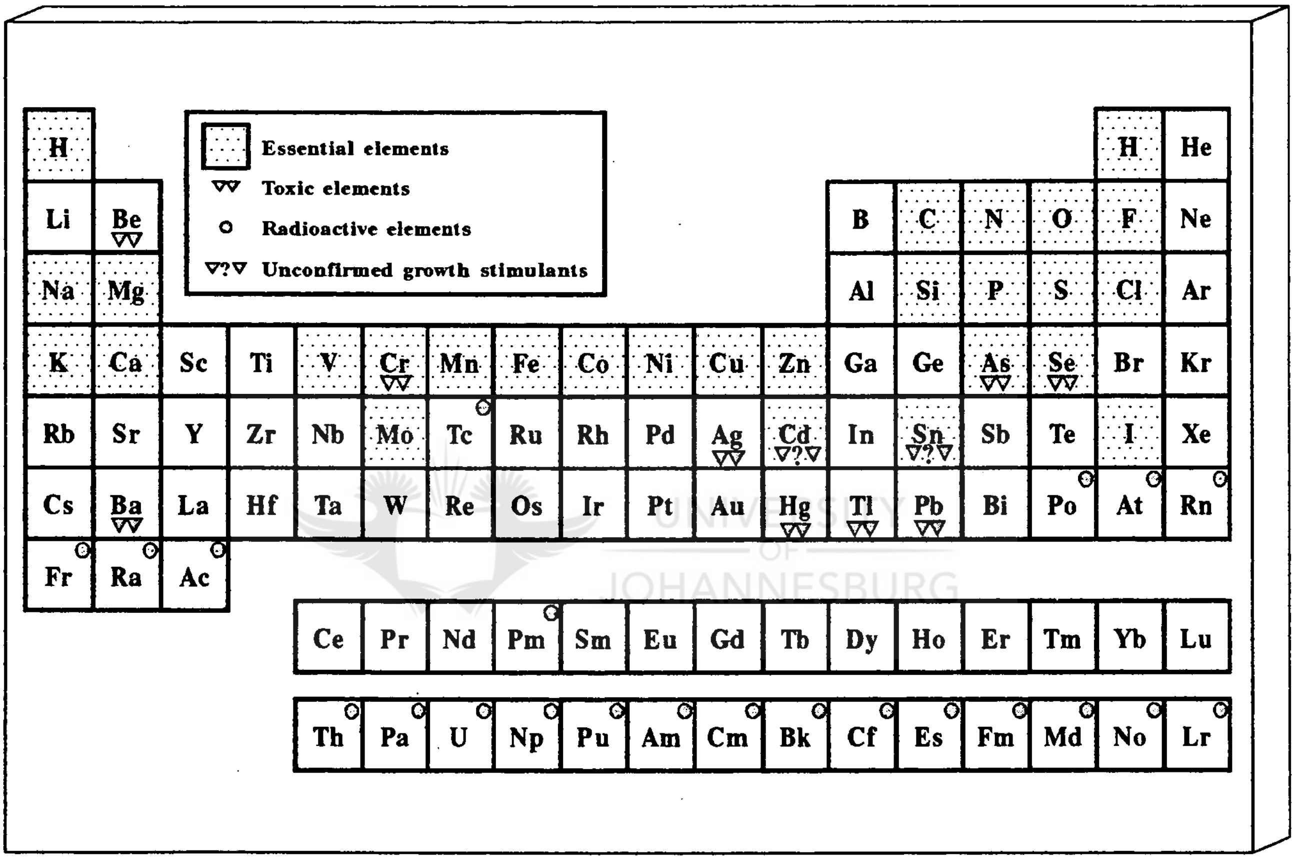


Figure 1.1 **Essential, toxic and radioactive elements in the environment (After Irgolic *et al.*, 1985)**

A more detailed description of the complexities of the many cycling processes which take place in the environment cannot be discussed in a general introduction of metal pollution of aquatic and terrestrial environments. However, the complexities of these systems, which form the domain of environmental inorganic chemistry, can briefly be summarized in an inspection of the major components of such a system:

- The building blocks of a large variety of compounds consist of eighty six naturally occurring elements. Among them 20 essential trace elements and 10 toxic elements occur. The majority of these elements are classified as metals. Upon mobilization, these elements form simple ionic compounds and more complex coordination compounds (Martell *et al.*, 1985). Some of the metallic elements (Fe, Cu, Ni, Pb, Zn, Mn, Cr, Cd, Co, Pt, Pd, Au, Hg, Ge, Sn, Sb, Bi & Po) and all the metalloids form element-carbon bonds. These compounds are sufficiently stable to survive hydrolysis and therefore are able to exist in aqueous media (Brinkman, 1985 ; Bellama, *et al.*, 1985 ; Sadler *et al.*, 1985).

- Man-made radioactive elements and technologically enhanced natural radionuclides not only add potentially toxic compounds to the environment, but may also emit biologically damaging radiation (Scoppa & Mytteneare, 1985 ; Choppin, 1985 ; Schulte, 1985 ; Raymond, 1985).

- A medium for chemical and biochemical reactions are provided by aqueous solutions of various compositions. These aqueous-based reactions usually proceed under the influence and control of other dissolved substances (Ahrland, 1985 ; Martell *et al.*, 1985 ; Hancock, 1985 ; Morgan *et al.*, 1985 ; Williams, 1985 ; Brinckman, 1985 ; Bellama *et al.*, 1985 ; Sadler *et al.*, 1985).

- Suspended solid matter or solid matter deposited in sediments offer adsorption sites for dissolved substances and may provide catalytically active surfaces which have an influence on reaction rates (*Morgan et al.*, 1985 ; Ching-I & Hingxiao, 1985).

- Biota (plants, animals, bacteria, etc.) which posses a tremendous variability in size, function, form and biochemistry, interact in a multitude of ways with inorganic compounds.

As stated earlier, complex interactions of biological and abiotic reactions are involved in environmental processes. Environmental inorganic chemistry is far from a detailed understanding of these interdependent and perhaps synergistic interactions (Irgolic & *et al.*, 1985). Experimental systems and environmental monitoring therefore assist us in providing our understanding of environmental processes. Under well-controlled laboratory conditions, experimental systems serve as models for environmental compartments. The results of such experimental studies of metals on aquatic life as well as field environmental monitoring programmes both contribute to the overall database of knowledge of metals in the aquatic environment. Results of experimental studies are further useful to interpret field observations made during environmental research programmes.

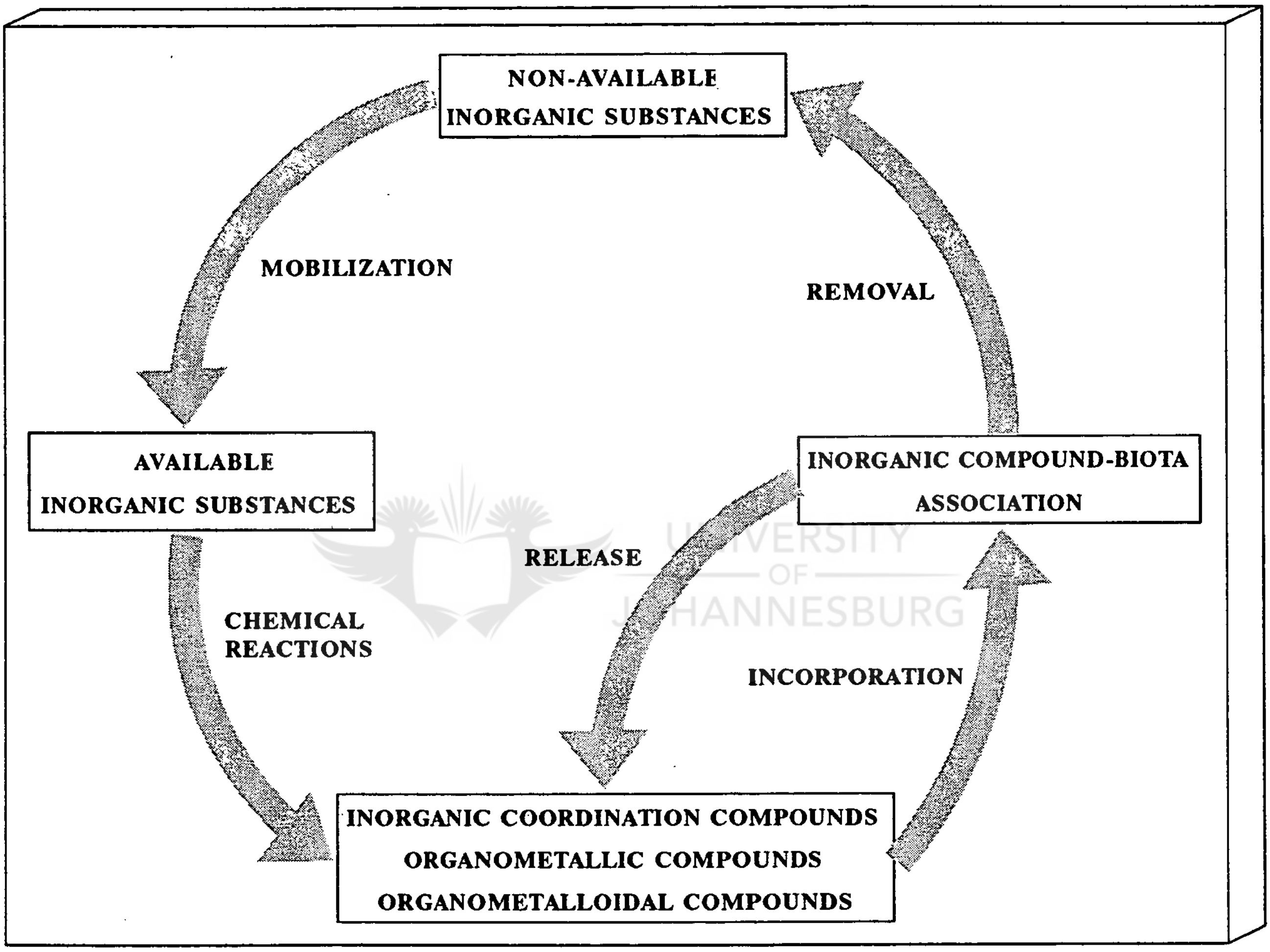


Figure 1.2 Cycling of inorganic, organometallic and organometalloid compounds in the environment (After Irgolic *et al.,* 1985)

The Research unit for Aquatic and Terrestrial Ecosystems at the Rand Afrikaans University has for the past seven years been involved in the investigation of metal transfer and accumulation in the aquatic environment and also the possible effects of metals on aquatic biota. Investigations showed that the metals Fe, Cu, Ni, Pb, Zn, Mn and Cr were largely associated with mine- and industrial pollution of rivers and lakes in the Gauteng Province. Several projects have contributed towards a better understanding of these complex interactions and mechanisms. These projects include work conducted on the Germiston Lake (de Wet, 1990a), Elsburgspruit (van der Merwe, 1990), Natalspruit (van Eeden, 1990), Florida Lake (Venter, 1991) and the Blesbokspruit (de Wet, 1990b). The present investigation, including results presented in Part 1 on the presence of radionuclides in the aquatic environment, was aimed at gaining a wider perspective of possible transfer mechanisms and pathways of metals from aquatic to terrestrial ecosystems as well as the potential effects on man. With this in mind, the research aims of the present investigation have been identified. The main objectives and recommendations of the present report on metals in mine-, sewage- and industry-polluted aquatic environments are dealt with under the following sections:

- **Establishment of the occurrence and concentration of selected metals in mine- and industry-polluted waters and sediments in catchments of the Blesbokspruit, Klip River and Crocodile River systems.** Selections were made of suitable sampling localities where these metals were present in significant concentrations. Such locations were those which were in close proximity of gold and uranium mines as well as agricultural areas located in the vicinity of such mines.

- **Determination of metal concentrations in selected semi-aquatic and aquatic weeds in these mine- and industry-polluted waters with observations on their ability to accumulate metals in their roots and/or shoots.**

- **Determination of the levels of these metals in irrigation water, soil and agricultural products potentially affected by contaminated effluents from mines and industries.**

- **Evaluation of some benthic macro-invertebrate organism groups as possible indicators of metal pollution in streams affected by mines and industries.** Specific attention was given to representatives of the Annelida, Crustacea and certain insect groups such as the Ephemeroptera, Hemiptera, Coleoptera, Trichoptera and Diptera. Mollusca (*Lymnaea* sp.) was also included in the study.

- **Determination of metal concentrations in the larvae of the guttural toad, *Bufo gutturalis* which occurred at the Luipaardsvlei sampling locality.**

- **Evaluation of Concentration Ratios (CR) of metals in the various organism groups in relation to these metals found in the bottom sediments of dams and rivers at those metal polluted localities where the aquatic fauna and flora were collected for this study. The CR (vegetable-soil) values were also calculated for**

carrots, beetroot, cabbage, lettuce, cauliflower, turnips, spinach and leeks.

- **Discussion of the major findings of the survey with recommendations on further investigations which need to be done in the areas surveyed.**

LITERATURE SURVEY

2

2 LITERATURE SURVEY

2.1 Introduction

For many years it has been known that trace amounts of some elements exert a positive or negative influence on plant, animal and human life (Förstner & Wittmann, 1981). Recently an increasing interest has also been taken into the specific role of these elements on human health.

Regarding the functions of metals, five behavioral categories for substances in living systems have been identified:

- Elements for which a deficiency results in impairment of function and which is relieved only by administration are known as **essential nutrients**. However, it is important to note that **essentiality** is organism-dependent and that care has to be taken to distinguish it from **stimulation**. There are for instance many essential and non-essential compounds which behave as stimulants (Sigel & Seiler, 1988).
- **Innocuous substances** on the other hand, exert no effect or are merely inert at the same concentrations.
- **Inert elements** such as gold, silver, platinum and tantalum pose a wide application in medical science and have often been used in surgical implants (Sigel & Seiler, 1988).
- **Therapeutic agents**, such as the use of arsenic and mercury compounds against parasites and lithium against manic depression have often been used in the past (Sigel, 1982).
- At **high concentrations**, however, most **substances become toxic** and harmfully affect the activity of organisms often in such a way that a loss of function, deformity or death is encountered.

2.2 Elements essential to plant and animal life

Contrary to the classical concept that inorganic chemistry is restricted to non-living chemical systems, metals play a significant role in the organic and biochemical reactions of living organisms. Therefore, a close interrelationship exists between applied inorganic chemistry and nature. Experimental evidence have shown that the role of metal ions in living systems follows the pattern of their natural availability and abundance in nature (Vahrenkamp, 1973; Williams, 1967 ; Wood, 1974 ; 1975)

Humans require for normal living processes relatively large amounts of nutrients such as carbon, nitrogen and phosphorous (Pier, 1975). Other nutrients, such as metals, are required to a much lesser extent, where typically two general types are commonly found, namely:

- Metals which are required in substantial amounts by the human body and for which it has a rather wide tolerance. Elements such as Fe, Na and K are typical of this category.

- Metals which are required in comparatively smaller amounts and for which the human body has a narrow range of tolerance. Metals such as Cu, Mn and Co are included in this category. These elements are normally essential for biochemical functions, but produce toxic effects when present at higher concentrations. Elements which are required in small or trace quantities are commonly referred to as "micro nutrients" (Pier, 1975). A brief summary of the functions of metals as essential nutrients are presented in Table 2.1

Table 2.1 Functions of metals as essential nutrients in man (Jessop, 1970).

Element	Function
Calcium	Building of skeletal structure; essential for blood clotting and nerve and muscle function; regulates cation balance between cells and milieu; enzyme cofactor
Magnesium	Important in ionic balance; enzyme cofactor
Potassium	Major intracellular cation; involved in ionic balance and in maintenance of electrochemical potential across cell membranes; promotes protein synthesis
Sodium	Major extracellular cation; important to water retention processes; promotes nuclear uptake of amino acids and osmotic balance; involved in cellular electrochemical intranuclear protein synthesis
Iron	Components of cytochromes, catalase, haemoglobin, myoglobin; essential for B vitamin synthesis
Manganese	Enzyme cofactor; promotes synthesis of vitamins; affects calcium metabolism
Copper	Required for synthesis of haemoglobin and iron-containing enzymes; enzyme component
Zinc	Enzyme cofactor; mitotic accelerator; required for synthesis of tryptophan and carboxylase
Cobalt	Vitamin B_{12} constituent; promotes synthesis of iron-containing pyrroles; enzyme activator
Molybdenum	Enzyme cofactor; essential in nitrogen cycle
Nickel	Required for insulin synthesis; enzyme activator; antianemic factor

In terms of typical human contact with metals, another category has to be introduced:

- Metals for which no essential functions in life processes have been determined and which are toxic at low levels. Elements such as Pb, Hg, Cd (and others) are included in this category.

It is, however, according to Pier (1975) important to recognize that failure to define an essential role may primarily be the result of our as yet inadequate understanding of life processes. Therefore, elements which are generally regarded as non-essential, wether toxic or not, may in fact fulfil essential roles in living organisms at very low concentration levels.

2.3 Metal essentiality and toxicity

When the nutritional supply becomes excessive, essential trace elements may become toxic. In trace amounts, a metal (usually less than 0.01 % of the mass of the organism) is essential when an organism fails to grow or to complete its life cycle in the absence of that metal (Förstner & Wittman, 1981). According to Venugopal and Luckey (1975), the same trace metal is toxic when concentration levels exceed those required for correct nutritional response by factors varying between 40- and 200-fold.

Figure 2.1a demonstrates the essentiality of trace metals as a dose-response curve ranging from deficiency to oversupply. An increase in the width of the plateau corresponds to a low inherent toxicity of the metal, while a narrow plateau reflects a small range between required and harmful doses (Förstner & Wittmann, 1981 ; Martin, 1986). It is also important to note that all life-essential metals become toxic when supplied or administered in concentrations which are in excess of those needed for optimum metabolic processes.

Figure 2.1b reflects the effect of metals which may not be as yet identifiable as serving biological functions and are regarded as bio-essential. The concept of essentiality is however under constant review (Förstner & Wittmann, 1981) together with the progress of research. An unequivocal classification of metals is therefore impossible.

2.4 Possible detoxification mechanisms

Most living vertebrates posses detoxification mechanisms which act as a response to foreign matter and serve to limit or even eliminate a toxic substance. By making use of their own properties (such as formation of insoluble compounds in the gut), many substances render themselves less damaging. From the gut, metals are transported by the blood to other tissues where they might be immobilized (as Pb^{2+} is immobilized in bone tissue), or be converted by the liver and kidney to some less toxic or disposable form (Martin, 1988). However, excess Zn^{2+}, toxic Cd^{2+}, Hg^{2+}, Pb^{2+} and other metal ions cause the human liver and kidney to respond by increased synthesis of metallothionein. Metallothioneines (MT) are small proteins in which approximately one-third of the 61 amino acid residues are cysteine (Martin, 1988). Metallothioneines are also characterized by a high occurrence and frequent juxtaposition of sulfhydryl groups which provide bindings sites for toxic metal ions.

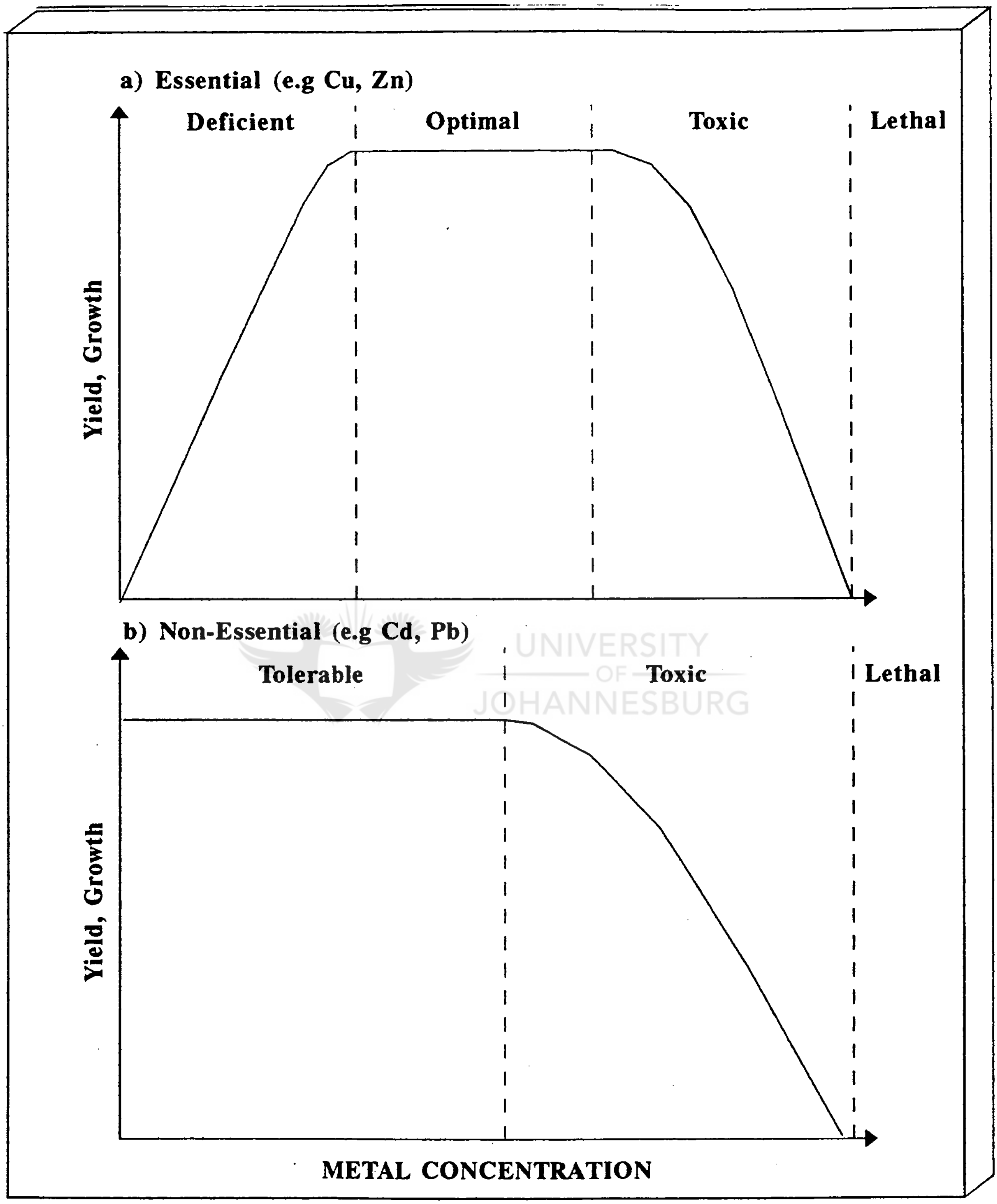


Figure 2.1 Deficiency and oversupply of essential and non-essential trace elements (After Baccini & Roberts, 1976)

Consequently, a reduction in the negative effects of metals is induced (Brown & Parsons, 1978). According to Martin (1988), metallothioneines are mostly synthesized in the liver and kidneys of mammals. Currently, only metallothioneines for Cd (Webb,1972 ; Olafson & Thompson, 1974 ; Rugstad & Norseth, 1975 ; Marafante, 1976 ; Overnell & Coombs, 1979 ; Thomas *et al.*, 1983), Cu (McCarter & Roch, 1983 ; Roch & McCarter, 1984), Hg (Brown & Parsons, 1978) and Zn (Kagi & Nordberg, 1979 ; Olafson *et al.*, 1979 a - b) had been isolated. However, Hennig (1986) has indicated that the possibility of such a protein for other metals also exists. A metal that does not induce MT-formation, is lead (Foulkes, 1981). According to Hildebrand *et al.* (1982), the mechanism involved with the detoxification or accommodation of metals by this protein on cellular level is extremely complex and still uncertain.

2.5 Beneficial and hazardous effects of metals on plants, animals and man

2.5.1 Copper

Copper has been detected in most living organisms (Jessop, 1970) and for several decades the essentiality of this metal in animal and human nutrition has been known. With a molecular weight of 63.54g, copper is widely distributed in most foods and has been found abundantly in grain, nuts, legumes, liver and shellfish. It is found in lesser abundance in muscle meat, milk, other dairy products and baked foods (Sandstead, 1975). Normal adults require 30 μg/kg per day (WHO, 1973), while the daily requirements for infants and children range between 50 - 100 μg/kg per day (Daniels & Wright, 1934). Copper is included as an essential element in important human proteins such as cerebrocuprein I, erythorcuprein, hemocuprein, hepatocuprein and mitochondrocuprein. To a number of enzymes, which include ceruloplasmin, tyrosinase, amino oxidase, cytochrome C oxidase, uricase and dopamine B-hydroxylase, copper is also an essential element (Davies, 1972). For proper functioning, copper and other metals are required. Copper is also the key metal component in the respiratory pigments of many invertebrates (Jessop, 1970).

In view of the fact that copper is closely involved in numerous biochemical systems in the body, inadequate dietary intake of this metal results in serious deficiency diseases. Copper deficiency symptoms include depigmentation of hair (the first symptom of deficiency), anemia, vascular abnormalities in bone formation, myocardial fibriasis, demyelination and gastro-intestinal disturbances (Pier, 1975)

Copper toxicity usually occurs when oral intake exceeds 400 - 1000 mg/day. At these concentrations, copper interferes with the absorption of iron and zinc (Linder, 1985). A decrease in the hepatic formation of ceruloplasmin results in an increase of unbound copper in the body and consequently, excessive deposition of this metal in the brain, skin, liver, kidney, pancreas and myocardium. This condition is commonly known as Wilson's disease (Pier, 1975 ; Sarkar, 1988). Acute toxicity of copper produces symptoms ranging from nausea, vomiting, severe abdominal pain, hemolysis, diarrhoea and coma (Johnson & Nordlie, 1977). Chronic toxication, however, produces milder symptoms resembling laryngitis, bronchitis and chromic intestinal colic (Chatterji & Ganguly, 1950).

2.5.2 Cobalt

Although cobalt is an essential element, it has not been investigated in the present study. All living organisms contain cobalt and is recognized as an intrinsic constituent of cobalamin - vitamin B12, which is life-essential (Melki *et al.*, 1987 ; Jessop, 1970). Vegetables and whole grains contain cobalt, while lesser amounts of this element is present in milk and milk products (Linder, 1985). The average diet contains 5 - 10 μg/day cobalt and most of it is absorbed in the inorganic form (Melki *et al.*, 1987). Cobalt binds to albumin in the blood and is distributed to several tissues: the muscles contain 43 % and bones contain 14 % cobalt. Smaller amounts are present in other tissues, especially in the kidneys. Cobalt mainly exists in the inorganic form in these tissues, while only 8 - 10 % exists in the vitamin form (Linder, 1985). Cobalt is mainly excreted via urine (Underwood, 1977). The major enzymes methylmelonyl-Co A mutase, methyl tetra hydrofolate oxido reductase, homocysteine methyltransferase and ribonucleotide reductase all need vitamin B12 for optimum functioning (Davies, 1972).

Cobalt deficiency in humans is rarely encountered as the requirement for this element is low (Jessop, 1970). Up to date only one case of cobalt deficiency has been known to have taken place in a Scottish infant living in a cobalt-deficient area. The infant had marked geophagia, but responded well to cobalt supplementation (Shuttleworth *et al.*, 1961)

Excessive intake of cobalt results to a toxicity which enhances the proliferation of erythropoietic cells in the bone marrow as well as the cells of the thyroid. Damage to the pancreatic inlets and heart muscle had also been encountered (Linder, 1985).

A very interesting case of cobalt poisoning was encountered in a series of instances of severe cardiac failure in persons consuming large amounts of beer. It was soon realized that the excessive amounts of beer had not been the direct cause of cardiac failure, but rather the treatment of the beer with soluble cobalt salts to stabilize the froth or "head" (Anon, 1968). The actual root of the problem was the use of detergents for the washing of the glasses. After rinsing, residual amounts caused the collapse of the froth and, as a result, cobalt salts had been used to counter this problem.

2.5.3 Manganese

Manganese is closely involved with several metalloenzymes which have key biological functions in the human body (Melki *et al.*, 1987). Some of these enzymes are pyruvate carboxylase which catalyses the transformation of pyruvate to oxaloacetate, superoxide dismutase which plays an essential role in free radical metabolism (Hurley, 1982) and glycosyl-transferase, which is important for the synthesis of glycoproteins and glycosoaminoglycans (Leach & Lilburn, 1978). Manganese is also known to play a role in the proper functioning of flavoproteins (Jessop, 1970). Manganese also exerts a catalytic effect on haemoglobin synthesis (Best & Taylor, 1966) and is involved in the synthesis of cholesterol (Davies, 1972).

The average human diet requires about 3-5 mg of manganese per day (Schroeder, 1972). Manganese gastrointestinal absorption is usually poor and is adversely affected by the presence of iron, phytates, phosphates and calcium in the diet. Bound to transferrin, manganese is transported to the pineal and pituitary glands, lactating breasts, liver, bone and pancreas where it accumulates in high concentrations. Secretion of manganese usually takes place via bile and the intestines (Linder, 1985).

Manganese deficiency has been described and experimentally observed in many animal species, but has not been well documented in humans. The first case of manganese deficiency in man has been described by Doisy (1972). Symptoms encountered included a hair colour change from black to red, retarded hair and nail growth, transient dermatitis as well as decreased serum levels of triglycerides and cholesterol.

Manganese-containing fumes occurring in mines are the primary cause of manganese toxicity (Underwood, 1977). Typical symptoms include fever, pneumonitis, rheumatoid arthritis and a Parkinson-like syndrome (Davies, 1972 ; Hopps, 1971). The wide industrial field of applications derived from manganese and its compounds induces serious concern to environmental contamination and subsequent effects on human health. Manganese finds numerous applications in the chemical, glass, dye, ceramic, metals and varnish industries. However, these applications have caused many industrial poisonings which resulted in symptoms similar to Parkinsonism. Some severe cases, including deaths have been recorded (Glaister & Rentoul, 1966 ; Smith, 1972)

2.5.4 Zinc

Zinc is a known essential nutrient for plant, animal and human life (Todd *et al.*, 1934). This metal is contained in various metalloenzymes which participate in carbohydrate, lipid, protein, haeme, and nucleic acid metabolism, as well as CO_2 transport (Prasad, 1976). Zinc also occurs in important enzymes such as alcohol dehydrogenase, carboxypeptidase, reverse transcriptase, alkaline phosphatase, DNA polymerase, δ-aminoleuvulinic acid dehydratase, superoxide dismutase and carbonic anhydrase (Melki *et al.*, 1987). This metal also participates in the metabolism of collagen (Solomons, 1981), cellular immunity (Golden *et al.*, 1978), maintenance of the functioning of cell membranes (Solomons, 1981), spermatogenesis as well as the development of male reproductive functions and hormone secretion and action (Agget & Harries, 1979)

The average human diet contains approximately 7 - 15 mg of elemental zinc per day. Only 30 % of the total amount of zinc ingested is absorbed from the gastrointestinal tract with the aid of an energy-dependent transporter. The average bodily content for zinc is about 2 - 2.5g. The highest amounts of zinc (± 75 %) occur in the muscle, skin, ligaments and nails (Agget & Harries, 1979). Excessive zinc is excreted from the body via the pancreatic digestive fluids and lost through the faeces. Minor losses occur via the sweat, urine and hair (Linder, 1985)

The prevalence of zinc deficiency cases is quite common all over the world. It is usually the result of poor intake associated with excessive losses. Zinc deficiency affects all ages, but is more often encountered during pregnancy and lactation. This is probably due to excessive losses in the urine and/or in the milk (Jameson, 1980). Other symptoms of zinc deficiency include impaired sexual development, chronic diarrhoea, growth retardation and numerous variations of skin changes. These skin changes are characterized by progressive bulbous-pustular dermatitis of the extremities of the oral, anal and genital areas, combined with patchy and generalized alopecia and paronychia (Melki *et al.*, 1987). Severe zinc deficiency, according to Prasad (1982), include corneal opacities, conjunctivitis, photophobia and blepharitis.

Little mention is made in the literature of zinc toxicity. Melki *et al.* (1987) however, make the following interesting statement namely that for most practical considerations, the homeostatic mechanisms regulating zinc absorption and retention operate with such efficiency that zinc overload is extremely unlikely and zinc can therefore be considered essentially non-toxic to humans.

2.5.5 Nickel

No clear metabolic function for nickel in man has been defined yet. However, Shnegg & Kirchessen (1978) observed that its deficiency resulted in a decrease in the activity of several hepatic dehydrogenase enzymes such as malate, glutamate, isocitrate, lactate and glucose-6-phosphate. Nickel is primarily obtained from plant foods with a resulting dietary content of 0.3-0.6 mg per day (Linder, 1985).

The average human body contains about 10 mg nickel and is mostly formed in the muscle, liver, bone and skin which contains the greatest amount (± 20 %) individually (Linder, 1985). Nickel is mostly disposed of from the body through urine.

Exposure to nickel and its compounds mostly takes place in the occupational environment and occurs in mining, processing and transport of nickel-containing ores, refining and smelting of nickel, electroplating of nickel, production and use of nickel catalysts, manufacture of nickel structures and parts by grinding, polishing, cutting, flame spraying and welding of nickel-containing alloys, spraying of paints which contain nickel (yellow nickel titanate pigment), recycling and/or disposal of nickel-containing products, manufacture of Ni-Cd batteries, and the construction of nickel molds in glass-bottle factories (Sunderman, 1977). Refinery workers are most often exposed to nickel substances and are more prone to cancers of the lung and nose (Doll *et al.*, 1977). A case of nickel toxicity was reported by Webster *et al.* (1980) when the leaching of a nickel-plated water heater tank contaminated the dialysate which were administered to 23 patients on chronic dialysis. Symptoms recorded by the main author included palpitations, headache, nausea, weakness and vomiting. Nickel also induces corrosive effects on the gastrointestinal and respiratory mucosae (Sunderman, 1977) and results in eczematous manifestations on the skin (Fregert *et al.*, 1969).

2.5.6 Chromium

When found to be part of the glucose tolerance factor (GTF), the importance of chromium to human and animal nutrition had been established (Anderson & Mertz, 1977). Chromium was also found to potentiate the effect of insulin on peripheral tissues, to enhance hepatic glycogenesis (Mertz, 1979), and to inhibit the action of hydroxymethyl-glutaryl-CoA reductase (Linder, 1985). It is also interesting to note that the presence of chromium has been secondary to a decrease in atherosclerosis and has been associated with a decreased incidence of coronary artery disease (Melki *et al.*, 1987).

Following an average dietary intake of 60-90 μg/day, only 1 % of chromium is absorbed by the gastrointestinal tract. Bound to transferrin, it is transported to the liver and incorporated into the glucose tolerance factor (GTF). A portion of the Cr-containing GTF present in the liver is subsequently secreted into the plasma compartment, where it enhances the action of insulin in the peripheral tissues (Linder, 1985).

Chromium deficiency is commonly related to an enhanced intake of fibre and phytate in the diet. Usually it also results during total parenteral nutrition when an enhanced supplement of glucose is administered together with inadequate supplementation of other nutrients (Jeejeebhoy *et al.*, 1977). Chromium deficiency is commonly characterized by hypercholestrolemia, glucose intolerance and impaired metabolism of amino acids (Linder, 1985). Chromium deficiency is infrequent in humans. It is associated with decreased glucose tolerance, diabetes and cardiovascular diseases (Gauglhofer & Bianchi, 1991).

The most common areas of exposure to chromium in the workplace include chromate production, chrome plating, chrome pigment production, tanning and leather industries, ferrochrome production and stainless steel welding (Stern, 1982). For over 2000 years, the toxic effects of exposure to high levels of this metal have been observed and recognized (Hamilton & Wetterhahn, 1988). Chromium and its compounds are recognized to be corrosive to skin and mucous membranes of the respiratory and gastrointestinal tracts (National Institute of Occupational Safety and Health (NIOSH), 1975). Allergic and eczematous dermatitis are the common symptoms of exposure to chromium (Fregert *et al.*, 1969). Without treatment, topical exposure of exposed skin can cause lesions which can develop into deep ulcers or "chrome holes" (Pederson, 1982 ; Burrows, 1983) Toxicity to the liver and kidneys are typical of systemic effects (NIOSH, 1975 ; Tandon, 1982). Contact dermatitis is caused in the chromium industries by products such as paint pigments, glues, wood preservatives, brewery yeast, cement and leather (Pederson, 1982 ; Polak, 1983).

2.5.7 Lead

Lead is considered as one of the "big four" (Pb, Hg, Cd and As) of metals which are of environmental concern. Numerous physiological disturbances which have occurred with its toxicity, has prompted an interest in lead. Although the essentiality of this metal has not been proved yet, lead does posses a number of beneficial properties which find application in industry.

The average dietary intake of lead is approximately 300 μg per day (Linder, 1985), which fortunately is well below the daily allowable limit of 490 μg/day recommended by both the World Health Organisation and the FAO (WHO, 1978).

An imbalance of excessive intake and an overexposure to environmental factors are known to cause lead toxicity and result in a serious health hazard. Due to the higher percentage of lead absorption, children are more sensitive to the mentioned environmental factors than adults (Melki *et al.*, 1987). A wide range of metabolic disorders and neuropsychological deficits are associated with environmental exposure to low levels of lead (National Academy of Sciences (NAS), 1980 ; Environmental Protection Agency (EPA), 1986). According to Rosen (1985), and Landsdown & Yuile (1986), well known toxic (metabolic and cellular) effects of lead in asymptomatic children include:

- **impairment of red blood cell nucleotides, haeme and vitamin D metabolism,**
- **perturbations of calcium homeostasis in the brain and bone cells and in the hepatocytes, and**
- **neurological damage.**

In addition, Linder (1985) reports that the most common consequences of lead toxicity are:

- **anemia**
- **hemolysis of red blood cells, and**
- **inhibition of δ-aminolevulinate dehydratase and ferrochelatase enzymes, which are involved in placing Fe^{2+} ions in porphyrin rings.**

Excess lead is also known to inhibit ATP-ase enzymes (Vallee & Ullmer, 1972). Furthermore, the presence of lead also impairs glucose and amino acid reabsorption in kidneys and decrease the ability of kidneys to convert Vit D_2 to Vit D_3 (Mahaffey *et al.*, 1982). Most important, lead involvement affect the Central Nervous System and can result in hyperactivity and stunted intellectual development in children (Harland *et al.*, 1982).

2.6 Biogeochemical cycling of metals in aquatic ecosystems

Numerous natural sources are responsible for the introduction of metals into the aquatic environment. The most significant of those natural sources include atmospheric deposition, volcanic activity, biodegradation and water-rock interaction (Hochella & White, 1990). However, anthropogenic addition of metals into aquatic ecosystems are becoming increasingly significant. Anthropogenic addition possesses the potential to outweigh natural introduction on a local, regional and even a global scale with dramatic proportions.

Anthropogenic introduction of metals usually results from industrial waste and effluents from mining and industrial activities, extraction processes, chemical weathering of landfills and mine tailings as well as from burning of fossil fuels and smelting ore which results in atmospheric deposition (Hochella & White, 1990). It is estimated (Moore & Luoma, 1990) that from milling and smelting operations alone, 7 to 70 x 10^3 metric tons of industrialized metals are daily released in the world into the aquatic environment.

Considerable evidence in the scientific literature exists which indicate that contaminants such as trace metals, phosphorous, pesticides, PCBs and polycyclic aromatic hydrocarbons are taken up and concentrated by particulate matter in the water column and sediments in aquatic systems (Bloom & Ayling, 1977 ; Jenne, 1977 ; Förstner & Wittmann, 1981). Within aquatic ecosystems, metal and contaminant transport and subsequent distribution are critically influenced by mineral-metal interaction. These minerals include hydroxides of iron and manganese, clay minerals and soil particles (Astruc, 1986 ; Förstner, 1986 ; Bewers *et al.*, 1987). The association of metals and other contaminants with particulate matter and the transport thereof represents a major pathway in the biogeochemical cycling of trace contaminants (Allan, 1979). Figure 2.2 illustrates a generalized global diagram of potential metal reservoirs and possible aquatic and terrestrial ecosystem interactions.

In a lake, or wetland ecosystem, settling bioinorganic particles attract metals and transfer them to deeper areas of the lake where they are either transformed into the sediments or partially mineralized (Morgan & Stumm, 1991). Photosynthetic production of plankton also play a major role in particle cycling in the sense that biologically they seem to be efficient scavengers for metals. It is believed that mineral surfaces (Morel & Hudson, 1985 ; Sigg, 1987 ; Goncalves *et al.*, 1987) also actively participate in the scavenging of metals. Occasionally, some autochthonous particles such as humic acids, clays and aluminium silicates may also participate in metal cycling.

The main reservoirs (or carriers) of trace metals in the aquatic environment have therefore been identified as:

- **the "dissolved phase" containing free metal ions (M^{2+}) and complexed and colloidally bound metal species (ML) which are in constant equilibrium (M^{2+}==ML)**
- **the particulate phase which can be separated into biotic (bacteria and abiotic particulates (organic and inorganic particles greater than 0.45 μm in size)**
- **bottom sediments (Hart, 1982).**

In the confines of the upper lake waters, physico-chemical sorption and active uptake of metals takes place by algal (phytoplankton) cells and other particulate materials (Figure 2.3). Subsequently bacterial decomposition or mineralization of phytoplankton takes place and metals are released into the water column as dissolved and particulate forms. A portion of the particulate matter may be lost to the sediments by sedimentation or it may stay in suspension to either:

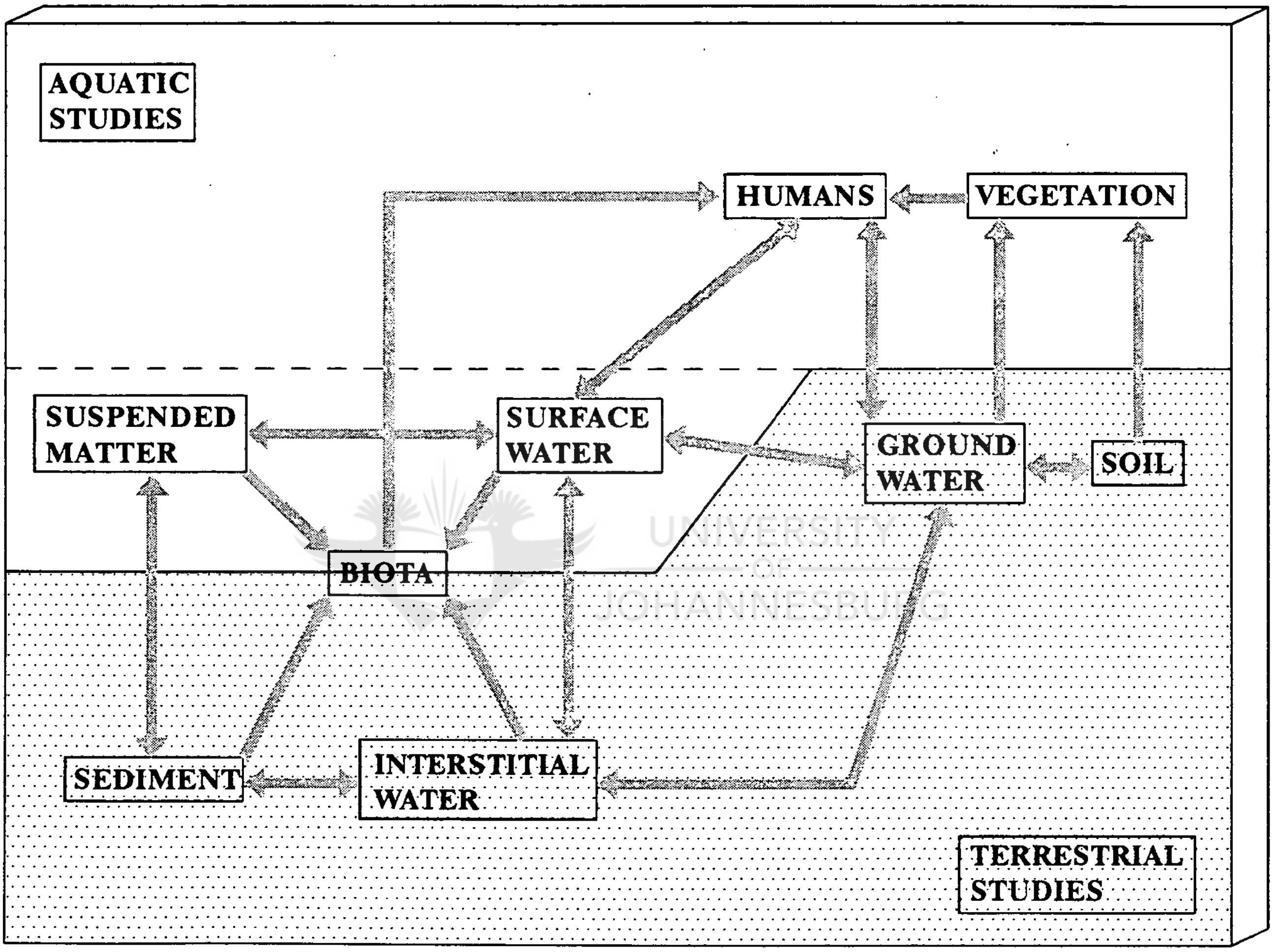


Figure 2.2 **Potential global metal reservoirs and possible interactions between aquatic and terrestrial ecosystems (After salomons & Förstner, 1984)**

- **accumulate more metals**
- **be incorporated into the food chain (fish and macro-invertebrates),**
- **be exported from the lake to another aquatic ecosystem, or**
- **be transported to terrestrial ecosystems (irrigation, drinking water, etc.)**

Simple metal ion species may also reach the sediment and adsorption may also occur. This, however, depends on factors such as lake depth and thermal stratification. Free metal ions and particulate complexes can also, under certain circumstances (bacterial action, physical-chemical conditions, etc.) be released from sediments and be incorporated in the aquatic food-chain, namely:

2.6.1 Algae, aquatic and semi-aquatic weeds

The ability of aquatic weeds to absorb and accumulate various metals from the aquatic environment has been demonstrated by a number of research workers (Dean *et al.*, 1972 ; Dietz, 1973 ; Erikson and Mortimer, 1975). It has also been shown that aquatic macrophytes can be reliable indicators of metal pollution in freshwater ecosystems (Ray and White, 1976 ; Abo-Rady, 1980 ; Franzin and McFairlane, 1980 ; Kovacs *et al.*, 1984 ; Mortimer, 1985). The latter author points out that the degree of metal uptake by some plants is largely dependent on the type of metal and the plant species involved. Guilizzoni (1975) showed that considerable variation in metal contents may occur between rooted submerged and rooted emergent aquatic weeds from the same environment. Whilst floating aquatic weeds may absorb metals mainly from the water column, rooted aquatic vegetation is capable of metal uptake through its roots, shoots and leaves (Gambrell *et al.*, 1976 ; Patrick *et al.*, 1977 ; Reddy and Patrick, 1977 ; Förstner and Wittmann, 1981). Investigations by Erikson and Mortimer (1975) showed that some submerged aquatic plants can absorb metals directly from the water column through their chlorophyll-containing leaves and stems and that the transfer of these metals can then take place to the roots where they are stored.

2.6.2 Benthic macro-invertebrates

The effect of metal pollution, and in particular pollution from mining areas on the benthic macro-invertebrate fauna of rivers received considerable attention by researchers in the past including De March (1976), Friberg *et al.* (1977), Slobodchikoff & Parrot (1977), Winner *et al.* (1980) and Steenkamp *et al.* (1993). Yasuno *et al.* (1985) found that the larvae of some species of Chironomidae were able to survive in seriously metal-polluted waters and that some of these species were able to bio-accumulate most of these metals in their tissues. Yamamura *et al.* (1983) indicated that *Chironomus yoshimatsui*, which occurred in heavily metal-polluted waters, was not able to bio-accumulate these metals at any high concentrations. Occhiogrosso *et al.* (1979) found that the dominant aquatic macro-invertebrate groups in a metal polluted part of Hudson River, New York, mainly consists of representatives of the Oligochaeta and Chironomidae. A similar tendency was reported by

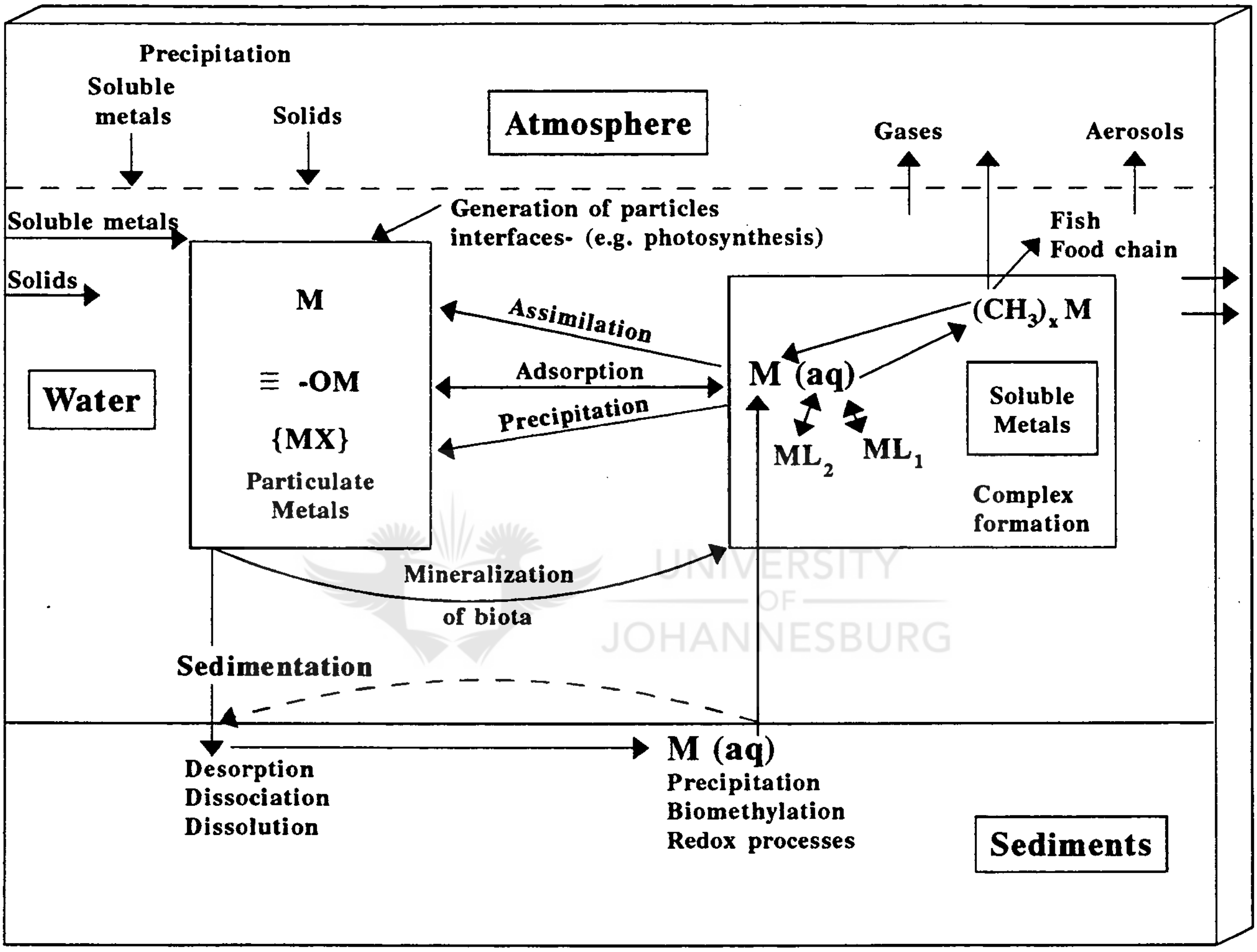


Figure 2.3 Biogeochemical cycling of metals in lakes and wetlands with an indication of abiotic and biotic components participating in metal cycling (After Morgan & Stumm, 1991)

Dixit & Witcomb (1983) for the metal-polluted Irwell River in England. The tolerance of the Oligochaeta and in particular the Tubificidae against metal pollution in aquatic ecosystems is well-known in the literature. Whitley (1967) showed that Tubificidae, consisting of *Tubifex sp.* and *Limnodrilus sp.*, were highly tolerant to abnormal concentrations of lead and zinc in rivers. The ability of the benthic macro-invertebrates to accumulate certain metals from the aquatic environment has been demonstrated by a number of authors including Eyres & Pugh-Thomas (1978) and Lynch *et al.* (1988). Brown (1977) showed that free-living larvae of Trichoptera were able to accumulate relatively high concentrations of copper, zinc and iron in their tissues. Eyres & Pugh-Thomas (1978) found mean concentrations of 43,5 $\mu g.g^{-1}$ lead, 17,5 $\mu g.g^{-1}$ copper and 127 $\mu g.g^{-1}$ zinc in the tissues of certain macro-invertebrate organisms from metal-polluted aquatic environments. Yasuno *et al.* (1985), Nehring *et al.* (1979) and Burrows & Whitton (1983) recorded similar findings for chironomid larvae from metal-polluted aquatic habitats.

2.6.3 Higher trophic biota including fish, amphibia and aquatic birds

Effluents from gold mines and metal-processing industries contributed greatly to the acidification and metal contamination of streams and lakes in the Gauteng Province, South Africa (Harrison, 1961 ; Schoonbee and Van der Merwe, 1989 ; Van der Merwe *et al.*, 1990 ; Steenkamp *et al.*, 1994a,b). Once released into freshwater ecosystems, Ph changes in the water may result in the deposition of metals in stream and lake sediments (Förstner and Prosi, 1979 ; Campbell and Tessier, 1985), from where they can affect the ecology of certain aquatic biota (Harrison, 1958 ; Nehring, 1976 ; Whitton *et al.*, 1981). Such metals may thus be transferred from aquatic plants and macro-invertebrate fauna to freshwater fish via several pathways in the food chains of such affected ecosystems (Heath, 1987). It has been demonstrated that fish which are present in such waters may obtain these metals by means of diffusion through gill and skin surfaces (Matthiessen and Brafield, 1977 ; Heath, 1987) or, from their natural food (Mathis and Cummings, 1973 ; Moore and Ramamoorthy, 1984 ; Villegas-Navarro and Villareal-Trevino, 1989).

Conflicting reports exist concerning the mechanism involved, and/or the ability of fish to bioconcentrate the various metals in their organs and tissues. Some researchers have found that a positive correlation exists between fish body mass and metal concentration (Phillips *et al.*, 1980; Mohamed *et al.*, 1990), while others (Goodyear and Boyd, 1972 ; Johnson, 1987) recorded no correlation between these two parameters. In some cases, however (Chernoff and Dooley, 1979 ; Anderson and Spear, 1980 ; Memmert, 1987 ; de Wet *et al.*, 1994), a definite inverse relationship was found to exist between body mass and metal concentration in fish.

Investigations on the uptake of selected metals by the frog and tadpole stages of the platanna *Xenopus laevis* (Fleischer, 1993 ; van Eeden, 1994) showed that certain organs and tissues had much higher concentrations of some of the metals than others. This can be linked to sites of uptake, storage and excretion as well as the possible processes of metal bioregulation and does not necessarily reflect the extent of metal pollution in the aquatic environment. Whole-body metal analysis of the frog and of the larval stages of both the platanna and *Rana* *angolensis* (van Eeden 1994) yielded data which more closely approximated metal concentration ratios in the water and sediments of the areas of study. Preliminary conclusions by this author are that both the adults as well as larvae and juveniles of these and other amphibia would be extremely useful in the evaluation of metal contamination of aquatic ecosystems, especially as they would provide more information on the ability of amphibia to bioregulate metals in their bodies.

Various studies on the concentrations of a number of metals in a variety of bird species have suggested that the diets of birds were most probably the major source of these metals (Hulse *et al.*, 1980 ; Cheney *et al.*, 1981 ; Nicholson, 1981 ; Reid and Hacker, 1982 ; Lock *et al.*, 1992). These authors also suggested that the specific dietary preferences among different bird species as well as among individuals of the same species could have a pronounced effect upon the concentrations of these metals in the bird's organs and tissues. It would also appear that the effects of diet are greater than taxonomic differences in evaluating organ and tissue metal burdens. For instance, the Reed Cormorant (fish predator) might exhibit higher metal levels as a consequence of their specific diet rather than as a result of any intrinsic function of being a Reed Cormorant. It is therefore important that the dietary preferences as well as the occurrence and concentrations of certain metals in various food items of each of aquatic bird species studied to be known by the researcher (Van Eeden, 1994).

2.7 Metals in terrestrial ecosystems

2.7.1 Behaviour of metals and metal compounds in terrestrial soils

Anthropogenic introduction of metals and other contaminants into aquatic ecosystems can also ultimately lead to contamination of arable soil if it is irrigated with such polluted water. Once introduced into soil, a number of processes regarding the fate of these metals can take place, namely:

- **metals can be precipitated along with other compounds to soil particles**
- **sorbed onto exchange sites: metal oxides and hydroxides, as well as clay minerals and organic matter can serve as exchange sites**
- **dissolved into the soil solution, either in the aqua-form or complexed with inorganic or organic ligands**
- **transferred to, and accumulated by micro-organisms, plants or animals (Schmitt & Sticher, 1991).**

Metals introduced into terrestrial ecosystems do not passively accumulate into the soil medium. In fact, most of the time they remain mobile and chemically active. Under these circumstances, these metals tend to be bioavailable to living organisms. However, the degree of mobility, activity and bioavailability of these metals can, according to Trangmar *et al.* (1985) and Wopereis *et al.* (1988) be influenced by a number of factors which include:

- **pH of soil**
- **temperature of soil**
- **redox potential of soil**
- **cation exchange capacity of the solid phase (soil particles)**
- **competition between different metal ions**
- **ligation of cations by anions (humic and fulvic acids, etc.)**
- **composition and quantity of the soil solution**

Within the soil system, a number of interactions take place between the solid and liquid phases which have important consequences on metal bio-availability. Due to the close contact between the solid and liquid phases, the latter, which is a polar solvent, interacts with the surface structures of the solid phase and is capable of enveloping negatively charged sites. Usually the solid phase is composed by all, or either clays, metal oxides and organic matter which in most cases carry an excess negative charge. The nett negative charge of the solid phase leads to a surface potential which determines the distribution of cations and anions in the liquid phase. The latter therefore acts as a transport medium for ions in exchange and adsorption processes (Schmitt & Sticher, 1991). As already mentioned in Part 1, availability and mobility of metals are influenced to a great extent by the redox potential (Eh) of the soil. In oxidized soils, the redox potential may reach 0,7V (700mV), while it could drop to -0,4V (-400mV) in strongly reduced soils. Eh is very pH-sensitive and varies with -59mV per pH-unit (Gambrell & Patrick, 1978). Using the negative common log of the aqueous free electron activity, redox potential can be expressed as a pE value where a positive value indicates oxidized conditions and a smaller value reduced conditions. According to Sposito (1981), the following soil conditions can be encountered:

- **oxidized soils: $+7 < pE < +13,5$**
- **moderately reduced soils: $+2 < pE < +7$**
- **reduced soils: $-2 < pE < +2$**
- **highly reduced soils: $-6,8 < pE < -2$**

The status of several metal ions is affected by redox conditions. In particular, Fe and Mn are affected, but also Cr, Cu, As, Hg and Pb to some extent. For example, Fe^{3+} is usually stable under strongly acidic and oxidizing conditions, but is otherwise reduced to soluble Fe^{2+}:

$$FeOOH + e^- + 3H^+ == Fe^{2+} + 2H_2O$$

Under strong reducing conditions, the formation of $Fe(OH)_2$, $FeCO_3$, FeS and FeS_2 may occur. In the case of Mn, the solubility thereof increases with increasing acidity and reducing conditions. Under these circumstances, the Mn^{2+} ion is the only significantly soluble ion as Mn^{4+} is very insoluble (Schmitt & Sticher, 1991). According to these authors, organic matter, Fe(II) minerals and other reducing agents in soil are known to reduce $Cr(VI)(CrO^{2-}{}_4)$ to more soluble Cr(III) under acidic conditions.

2.7.2 Uptake and accumulation of metals by terrestrial plants

A number of factors play a role in the uptake of metals from soil by plants through their roots to their above-ground parts or underground storage organs. They include:

- **the total amount of metals present in the soil,**
- **the proportion of the total amount which is accessible to plant roots, and**
- **the ability of plants to transfer metals across the soil-root interface (Berrow & Burridge, 1991).**

A close interaction exists between the above factors and can therefore not be accepted as independent. The chemical form of an element as well as its location within the soil determines its accessibility to plants. The elements in the soil solution, i.e. those found in the ionic state and those found as soluble organic matter complexes are those which are the most readily available to plants. The least available are those elements bound within the structure of solids, for example within the crystal lattice of primary rock minerals (Berrow & Burridge, 1991). Situated between these extremes, the largest and most important pool of available metals is those which are associated with charged sites on the surfaces of very small particles such as silt and clay, as well as organic material. Together, these components comprise the "exchange-complex" (Berrow & Burridge, 1991). These sites, as their name implies, are able to release one ion in exchange for another. Conditions such as acidity of the soil, organic matter content and drainage status are, according to Mitchell (1964), among other factors, responsible for the availability of metals to plants.

According to Crooke (1964), the soil-root interface cannot be considered as a passive, inert sieve. The plant root surface is rather seen as an active boundary with variable characteristics depending on the plant species as well as the particular element. The same author also states that cation exchange capacity is a property of plant roots which can be reproducibly measured. It is also interesting to note that cation exchange capacity is generally greater for dicot than monocot plants. Additionally, plant root exudates are also able to influence the soil environment immediately adjacent to the roots (Linehan *et al.*, 1985 ; Merckx *et al.*, 1986 a,b) in such a way that apart from biochemical processes of transfer across cell walls within the roots, chemical processes such as dissolution, chelation and precipitation outside the root also occur. The above authors also state that some elements such as $A\ell$, Cu and Fe are able to accumulate on plant roots without being transferred to above-ground tissues even when poor growth has occurred.

A number of elements such as Aℓ, As(III), B, Be, Cd, Co, Cr(VI), Cu, I, Mo, Ni, Se(IV) and Tℓ can be harmful to crops, even at low concentrations (Bowen, 1979). Although toxic in some cases, the same elements are also essential for sustainable growth. Toxicity mechanisms in plants may include to following:

- **alteration of the permeability of cell membranes through the formation of antimetabolites,**
- **reaction with essential metabolites, or by**
- **substituting in part for other essential ions.**

At abnormally high concentrations in soil, most metals can cause visible injuries to plants, either by damage to roots which inhibit plant growth, or by crop failure. However, in some cases healthy plants could contain metal concentrations tolerable to plants, but potentially toxic to grazing animals or humans using these plants as crops (Berrow & Burridge, 1991).

2.7.3 Exposure routes of metals and bioavailability to humans

2.7.3.1 Introduction

Numerous potential pathways exist whereby humans (and other biota) can be exposed to metallic compounds as well as other contaminants. These contaminants are, however, subject to the amount in which these metals are used by human society or to which they speciate in the environment (Jaworski *et al.*, 1984). According to the above authors, an environmental pathway can be defined as the route that a substance follows between a source of emission and either a geological depot or a biological receptor. Simple pathways may involve only one environmental medium (e.g. a compound emitted into the atmosphere may be transported to the lungs where it is inhaled and subsequently absorbed by the receptor), while more complex pathways may involve two or more environmental media and transfer occurs between different compartments of the media (e.g. a compound present in a river may be irrigated onto soil where it is absorbed by crops and subsequently eaten).

2.7.3.2 Identification of significant pathways

For any compound, being a metal- or organic contaminant, the relative importance of a given pathway is determined by the following:

- **flux (the amount per unit time of a compound which is transferred to a deposition or absorption site)**
- **rate of absorption, and**
- **the destination in the body (Jaworski *et al.*, 1984)**

However, the following information must be available to identify a critical pathway for a given metal species:

- **the possible critical effect of a metal species or compound as well as its location within the body**
- **the relative absorption rates of metal species from organs such as gut, skin and lungs, as well as the translocation rate from the absorption site to the site of action, and**
- **the relative fluxes of metal species via different pathways to the sites of absorption (Venugopal & Luckey, 1978)**

However, the above factors must be comprehended in the term "bio-availability" which can be considered as a quantitative parameter expressing the combined total effect of environmental mobility, absorption/excretion, transport within the body as well as binding to the site of action or target site. Therefore, two components of bio-availability can be identified which include:

- **Outside the organism: factors which increase or decrease exposure and absorption, and:**
- **Inside the organism: factors which increase or decrease distribution and binding to the sites of action (Bowen, 1979 ; Jaworski *et al.*, 1984)**

In addition the following characteristics regarding the physical and chemical nature of a substance have to be considered to augment its bio-availability:

- **Particle size: smaller particle size ensures long-range transport and deposition into the respiratory tract as well as deposition onto food**
- **Volatility: vapours of a compound are as small particles subject to long-range transport. Usually these compounds also have high rates of respiratory absorption**
- **Aqueous/lipid solubility: depending on the nature of the target site, compound bio-availability may be enhanced by its solubility in either body-fluids or fatty tissue**
- **Complexation: elemental complexes with specific net charges or molecular sizes can diffuse by passive transport across biological membranes more easily than others. On the other hand, complexation may also strongly reduce transfer across biological membranes, and**
- **Mimicry: some elements or elemental complexes may mimic essential nutrients.**

Consequently, they may encounter enhanced absorption rates as transport processes may prefer these compounds.

2.7.3.3 Factors affecting pathway flux

In an environmental pathway, the flux of a metal species or other compound is influenced by a number of factors which either diminish or augment the transfer from one environmental compartment to another. Table 2.2 presents a number of chemical, physical and biological which can act as partial or full barriers to the movement of metals along their pathway to humans.

The physical processes influencing metal flux include adsorption, filtration and sedimentation, while the chemical processes involve redox reactions, complexation and precipitation. The biological reactions tend to be more complex and include rejection at membranes, biotransformation, restricted distribution among receptor tissues and death or growth inhibition of food source organisms.

The intention on this section of pathway assessment was not to provide a detailed picture on this aspect, but rather a short overview. The reason for this being that this aspect has not been addressed to the extent to which it has been applied in Part 1.

Table 2.2 **Barriers to increased flux of metals through environmental pathways (After Jaworski et al., 1984)**

Environmental medium	Transfer affected	Type of effect*
Air	long-range transport	P: settling of larger particles, filtration of air
Water	sediment/water	P: adsorption, filtration C: precipitation
	water/biota	C: formation of poorly absorbed complexes, inter-element interaction B: rejection at gill membrane
	biota/man	C: as above P: avoiding ingestion of contaminated organs or tissue
Soil	soil/soil solution	P: adsorption onto clay C: binding by humic substances, formation of precipitates
	soil solution/root	C: intermetallic interactions B: root membrane rejection
	plant/man or plant/animal	C: formation of precipitate or adsorbate in roots (no translocation) B: death or lack of productivity before toxic levels reached in edible tissue P: food processing (rejecting contaminated tissues)
	animal/man	B: as above P: as above

***P = physical ; C = chemical ; B = biological**

MATERIALS AND METHODS

3

3 MATERIALS AND METHODS

3.1 Sampling localities

All sampling locations discussed in Part 1 apply to this section, with the exception of Crocodile farm located on the Crocodile River (Broederstroom) near Hartebeespoortdam.

3.2 Collection of water samples

Spray or flood irrigation water samples were collected during the time of crop sampling and irrigation. The type of water sample depended on the method of irrigation practised on the particular farm. During each collection, the pH and electrical conductivity was determined on site.

For laboratory chemical analyses, water samples were collected in cleaned 1ℓ sample bottles. At the laboratory each sample was filtered through a Whatmann No. 1 filter paper to remove any suspended matter. Routine chemical analyses of water samples were conducted on each of the samples according to methods as prescribed by APHA (1989). All chemical analyses performed as well as their units of measurement are summarized in Table 3.1.

For metal analyses, unfiltered water samples (total metal content) were placed into 100 mℓ volumetric flasks and 10 mℓ concentrated HNO_3 added to keep any metals in solution. Afterwards, samples were placed into clean metal-free glass bottles for later metal-analyses.

During Atomic Absorption analyses of water samples, corrections were made for the increased volumes of the samples.

3.3 Collection of aquatic sediments and agricultural soil

Grab samples of aquatic sediments were collected from the streams where water were pumped for agricultural use. Soil samples were randomly collected from the fields where crops were grown. After collection all samples were placed in marked, cleaned, metal-free glass bottles and transported to the laboratory. At the laboratory the sediment and soil samples were individually dried in an oven at 90°C for 48 hours or until the samples have reached stable dry mass. After drying, each sample was homogenized and sifted through a 1 mm sieve. Approximately 1 gram of each sample was weighed off to the nearest 4th decimal on a Sartorius Model No R200D electronic balance in a clean metal-free digestion vessel and stored for later acid digestion.

3.4 Collection of biological material

3.4.1 Aquatic macro-invertebrates

Macro-invertebrate organisms from the soft bottomed substrate were collected with the aid of a Birge-Eckman grab with a bite of 225 cm^3. Live organisms were separated from the

Table 3.1 **Chemical analyses performed on each water sample as well as units of measurement.**

Chemical parameter	Unit of measurement
pH	-
Electrical Conductivity	µS.cm-1
Total Dissolved Solids	mg/ℓ
Total Alkalinity	mg/ℓ
Total Hardness	mg/ℓ
Calcium Hardness	mg/ℓ
Magnesium Hardness	mg/ℓ
Ammonia-N	mg/ℓ
Nitrate-N	mg/ℓ
Nitrite-N	mg/ℓ
Orthophosphate as PO_4	mg/ℓ
Sulphate as SO_4^{2-}	mg/ℓ
Turbidity	mg/ℓ
Sodium as Na	mg/ℓ
Calcium as Ca	mg/ℓ
Magnesium as Mg	mg/ℓ
Iron as Fe	µg/ℓ
Copper as Cu	µg/ℓ
Nickel as Ni	µg/ℓ
Lead as Pb	µg/ℓ
Zinc as Zn	µg/ℓ
Manganese as Mn	µg/ℓ
Chromium as Cr	µg/ℓ

sediment and other material by mechanical stirring and flotation. Following separation, all living organisms were transferred to 500 mℓ jars and Rose Bengal added to colour any chitin-containing organisms. Afterwards, all samples were preserved with 10% buffered formalin and stored for later analyses.

Sorting of macro-invertebrates into taxonomic groups were done with the aid of a moveable stereo-microscope mounted on a light platform. Where possible, all organisms were identified to family or genus level (Crass, 1947 ; Usinger, 1956 ; Harding, 1961 ; Pennak, 1978).

For metal analyses however, organisms were largely grouped into pooled family levels to accumulate the maximum combined weight possible. After grouping, the wet weight of the samples were recorded and the samples subsequently dried in a drying oven at 90 °C for 48 hours. After drying, the dry weight of each sample was determined and the moisture content calculated. Each sample was weighed off to the nearest 4th decimal on a Sartorius Model

No R200D electronic balance in a clean metal-free digestion vessel and stored for later acid digestion.

3.4.2 Aquatic macrophytes and edible crops

In the case of vegetable crops, the edible portions were collected for metal analyses. With the collection of aquatic macrophytes however, the plants were divided into roots, rhizomes, white stems, green stems and leaves. After thorough washing, the wet mass of each sample was determined. After drying in an oven at 90 °C for 48 hours, the dry mass and moisture content of each sample was determined.

Approximately 1 gram of each sample was weighed off to the nearest 4th decimal on a Sartorius Model No R200D electronic balance in a clean metal-free digestion vessel and stored for later acid digestion.

3.5 Metal analyses of samples

3.5.1 Acid digestion of organic and inorganic samples

After weighing and storing, digestion of each sample was conducted in clean (Giesy & Wiener, 1977) glass beakers (250 $m\ell$) whereby a mixture of 1:2 perchloric ($HC\ell O_4$ - 60%) and nitric acid (HNO_3 - 55%) was added (Van Loon, 1980 ; Houba *et al.*, 1983).

All glass beakers were covered with watchglasses and digestion was conducted on a hotplate at a temperature of 250 °C. The digestion process lasted about 1 - 6 hours or until the samples appeared clear and transparent.

After acid digestion the solutions were left to cool down whereafter all the samples were filtered through 0,45 μm filter papers. The filter papers were carefully washed during filtration to remove any traces of metal from the filter paper. The filtrates were transferred into A-grade 100 $m\ell$ volumetric flasks and filled up to mark. The samples were then transferred to clean metal-free glass containers for Atomic Absorption Analysis.

3.5.2 Atomic Absorption Analyses of acid digested samples

The total metal concentrations of all samples were determined with the aid of a Varian Spectra 10 Atomic Absorption Spectrophotometer. To calibrate the AAS, suitable concentration ranges of analytical standards for each metal were used. Analytical standards were created using Holpro chemically pure analytical stock solutions (1000 ppm).

All concentrations were determined on the AAS in $mg.\ell^{-1}$. As all the organic and inorganic material were in solid form, all measurements had to be converted from $mg.\ell^{-1}$ to $\mu g.g^{-1}$. For the water samples, all measurements had to be converted to $\mu g.\ell^{-1}$.

The conversions from mg.ℓ^{-1} (AAS-measurement) to μg.ℓ^{-1} (in the case of water samples) were achieved as follows:

mg.ℓ^{-1} / 10 = mg100mℓ^{-1} / digested volume (mℓ) = mg.ℓ^{-1}(1000) = μg.ℓ^{-1}.

The conversions from mg.ℓ^{-1} (AAS-measurement) to μg.g^{-1} (in the case of soil, sediment and biological samples) were achieved as follows:

mg.ℓ^{-1} / 10 = mg100mℓ^{-1} / digested mass (g) = mg.g^{-1}(1000) = μg.g^{-1}

3.6 Processing and interpretation of water quality data and metal analyses

3.6.1 Application of the Concentration Ratio (CR) concept

The purpose of the Concentration Ratio (CR) concept is to determine the ratio of element per unit weight of organism compared to that of equal weight of surrounding medium, being water, sediment or soil. A CR is only valid if a condition of equilibrium exists between the concentrations in the organism and in the water, sediment or soil. Refer to section 2.6.3 of Part 1 for further information on the philosophy of the CR concept.

The CR-values, where applicable, were calculated as following:

$$CR = [C_o] / [C_s]$$

Where:
CR = Concentration Ratio
$[C_o]$ = Concentration of metal in organism (μg.g^{-1} dry weight)
$[C_s]$ = Concentration of metal in sediment or soil (μg.g^{-1} dry weight)

If: $CR < 1$: Indicates little uptake or accumulation of metals by the organism. If CR values are very low (eg. CR = 0,015) concentrations are of the same magnitude as trace elements.

If: $CR \geq 1$: Indicates active uptake or accumulation of metals. In the case of primitive plants and macro-invertebrates, CR-values could be two orders of magnitude greater than 1.

3.7 Development and application of an Aquatic Toxicity Index (ATI)

3.7.1 Introduction

Very often the need arises to describe general water quality conditions with a single figure which provides an overall picture of the current water quality situation in a particular water body at a particular time. The earliest attempts to quantify water quality conditions into a single index-value included the saprobic system developed by Kolkwitz and Marsson (1908

; 1909) and Kolkwitz (1950) with reference to the presence or absence and numbers of macro-invertebrates in the benthic regime. These indices served as indicators for different forms of organic pollution in running and standing waters. Liebmann (1951), Patrick (1950 ; 1951), Wurtz (1955) and Hynes (1964) also made efforts to refine the saprobic system to quantify toxic pollution in fresh water systems.

Although these systems are very handy and applicable to environmental monitoring, it only uses one particular parameter to measure the consequences of pollution. These systems do not take into account that the type of pollution cannot be organic in origin and that it could possibly be of inorganic origin. Therefore, in addition to a biotic indexing system, it is necessary to introduce a numbering system to take into account the complexities of inorganic toxicants and other physical-chemical parameters which may have an influence on aquatic life.

During water quality management of a particular water resource (being stream, river, wetland or lake), scientists, engineers and managers are often confronted with an overwhelming array of numerical (chemical) data. Therefore, the need often arises to integrate this data into a simplified, yet understandable figure which provides an instant quantitative picture of water quality at a specific site and time (Smith, 1990). This number is commonly known as a Water Quality Index (WQI). According to House (1989), the use of a WQI bridges the gap between the extremes of water quality monitoring and reporting.

For many years, efforts have been made to quantify water quality data into a single descriptive index. Probably the first significant effort have been made by Brown *et al.* (1970 ; 1972) and has since been followed by many others (Walski & Parker, 1974 ; Bhargava, 1983 ; 1985 ; Landwehr & Deininger, 1976). It was, however, the efforts of Smith (1990), House (1989), House & Ellis (1980) and Tyson & House (1989) which set the standard for water quality modelling which is commonly accepted. The publication by House (1989) was considered as the basis for a proposed Aquatic Toxicity Index (ATI) which has been applied during this investigation.

It is therefore evident that this system of indexing, and particularly the use of an Aquatic Toxicity Index (ATI), could be used as an additonal tool during the evaluation of the ecological, biological and chemical conditions of a water resource.

3.7.2 Theoretical basis for the development of an ATI

The term water quality is seldom defined and in many instances the Water Quality Index produced is an attempt to pool several aspects of water uses, including pollution (House & Ellis, 1980), the reason for this being that different water users have different water quality requirements. During the development of an Aquatic Toxicity Index (ATI), the toxic effects of various water quality parameters on aquatic organisms, specifically fish, were taken into account. Due to the extensive toxicity database available on fish, the index was developed for the latter (Wepener *et al.*, 1992 ; Van Vuuren *et al.*, 1994). In general, this index could also be applied to aquatic invertebrates, but it is envisaged that the effects of water quality variables on these organisms would be incorporated into the ATI in future (Van Vuuren *et al.*, 1994).

To be of maximum value to the user of an ATI, the applicable index should comply to the following criteria:

- It must be based on legally adopted water quality standards and criteria. Else, in the case of aquatic life, it should be based on field monitoring data or data obtained from toxicity testing (House, 1989).
- It should contain information on the relevant use of the water associated with a particular water quality state; and
- Contain information on toxic determinants directly within its structure (House, 1989).

During the development of a Water Quality Index, or in this case, an ATI, four stages are identified:

(i) determinant selection
(ii) determinant transformation
(iii) determinant weighting
(iv) determinant aggregation

(i) Determinant selection

The determinants selected which are applicable to South African conditions comprized most of the commonly measured attributes of natural water as measured by the Department of Water Affairs and Forestry (DWA&F) (Van Vuuren *et al.*, 1994). The selected determinants included physical attributes of water, general chemical variables, and potentially hazardous (toxic) trace and heavy metals. According to Van Vuuren *et al.* (1994), each determinant had to conform to three basic requirements:

- the determinants should be readily available at frequent intervals,
- the determinants should be considered as important indicators of water quality change, and
- maximum permissible water quality criteria should be available for each of the determinants.

The following physical and chemical water quality parameters were selected as the most applicable in water quality modelling:

Physical: Temperature (°C), pH, Dissolved Oxygen (mg/ℓ)

Chemical: TDS (mg/ℓ), Fluoride (mg/ℓ), Potassium (mg/ℓ), Orthophosphates (mg/ℓ)

Metals: Cu, Ni, Pb, Zn, Mn, Cr (all μg/ℓ)

The above set of determinants as well as their rating functions are listed in Table 3.4.

(ii) Determinant transformation

The selection or obtaining of suitable rating curves for each of the determinants could be considered as the most efficient method of transforming information on individual determinant concentrations (Walski & Parker, 1974 ; House, 1989 ; Smith, 1990). However, before a rating curve could be developed, it was necessary to define the scale on which the ATI was to operate:

(i) Defining an ATI scale

The purpose of determinant transformation is to transform information of individual determinant concentrations to graphs which all posess the same Y-scale. Consequently, each rating curve posesses its own individual X-axis (e.g. pH: 0-14 and TDS: 0-1200), but share similar Y-axes ranging from 0 - 100. A scale from 0 - 100 was selected for the ATI with a score of 0 reflecting water similar to crude sewage and a score value of 100 that of water with a pristine quality (House, 1989). The scale proposed by House (1989) has been defined in terms of a range of possible water uses which include potable water supply, recreation and the protection of aquatic life (Table 3.2).

To be more applicable to South African conditions (which take mining and industrial effluents into account), a scale was proposed by Wepener *et al.* (1992) which to an extent is similar to that developed by Smith (1990) for a Water Quality Index. This scale, also ranging between 0 and 100, was based on salmonid spawning conditions. In this case, a score of 10 reflects water which is totally unsuitable for fish life, while a score of 100 reflects pristine conditions suitable for the growth and reproduction of any fish life. The interpretation and classification of the ATI scale (Wepener *et al.*, 1992) for local conditions is presented in Table 3.3.

(ii) Selection of ATI rating curves

According to Wepener *et al.* (1992) and Van Vuuren *et al.* (1994), two different methods were employed during the selection of ATI rating curves:

(a) The use of existing WQI rating curves

Existing curves obtained from the literature that were incorporated into this system were those for pH (Walski & Parker, 1974), Dissolved oxygen (Smith, 1990), Temperature (Walski & Parker, 1974), TDS (Moore, 1990), Orthophosphates (Walski & Parker, 1974) and Potassium (Workshop: Sensitive Fish Species, 1991).

Table 3.2 **Interpretation and classification of the WQI and ATI scales (House, 1989).**

WQI Class	WQI Range	Interpretation
I	71 - 100	Indicates water of high quality suitable for all high value uses including Potable Water Supply, (PWS), game fisheries, contact recreation and high quality industrial abstractions at low cost.
II	51 - 70	Indicates water of reasonable quality suitable for high value uses including PWS after conventional treatment, good coarse fisheries, indirect contact sports and most industrial abstractions at moderate cost.
III	31 - 50	Indicates polluted water with generally moderate value including PWS after advanced treatment, indirect contact sports, reasonable to sporadic coarse fish populations and some industrial abstractions at high treatment costs.
IV	10 - 30	Indicates badly polluted water of low economic value requiring substantial investment in treatment facilities if it is to be upgraded. Use generally restricted to non-contact recreational uses, sewage transport and navigation.

Table 3.3 **Interpretation and classification of the ATI scale for local conditions (Wepener *et al.*, 1992).**

ATI Scale	Interpretation
60 - 100	Indicates water of suitable quality for all fish life.
51 - 59	Indicates quality of water suitable only for hardy fish species e.g. adult *Oreochromis mossambicus* and adult *Clarias gariepinus*.
0 - 50	Indicates water quality which is totally unsuitable for normal fish life

Table 3.4 Rating functions for chemical variables used in the Aquatic Toxicity Index (ATI).

Chemical parameter	Input range	Rating function	a	b	c
Temperature (°C)	0-40	$f(T)=((400-(T-20)^2)/400)*100$	-	-	-
pH	2-12	$f(pH)=((25-(pH-7)^2)/25)*100$	-	-	-
Dissolved Oxygen (mg/ℓ)	0-8	$f(DO)=\exp(a(DO-8))*100$	0.3	-	-
TDS (mg/ℓ)	0-6500	$f(TDS)=\exp(a+bTDS)$	4.597	$-5.5x10^{-4}$	-
Phosphates (mg/ℓ)	0-5	$f(P)=\exp(aP)*100$	-2.5	-	-
Potassium (mg/ℓ)	0-250	$f(K)=a+b*K+c/K$	38.72	-0.1819	1565
Fluoride (mg/ℓ)	0-6	$f(F)=a+b*F+c*F^2$	95.91	-33.16	3.003
Copper (μg/ℓ)	0-1200	$f(Cu)=a+b/Cu+c/Cu^2$	-3.325	6891	-0.6891
Nickel (μg/ℓ)	0-1200	$f(Ni)=a*b^{Ni}*Ni^c$	157.4	0.9976	-0.07576
Lead (μg/ℓ)	0-1200	$f(Pb)=a+b*Pb+c*Pb^2$	95.06	-0.1707	0.0008362
Zinc (μg/ℓ)	0-1200	$f(Zn)=101*\exp(b*Zn)$	101.3	-0.002541	-
Manganese (μg/ℓ)	0-1200	$f(Mn)=a+b*Mn+c*Mn^2$	109.7	-0.1235	0.00004112
Chromium (μg/ℓ)	0-1200	$f(Cr)=a+b*Cr+c*Cr^2$	95.3	-0.1486	0.000061

a - c are numerical constants used in equations (Table 3.4 ; Column 3)

(b) Development of ATI rating curves

Rating curves were obtained as follows (Wepener *et al.*, 1992): Blank graph formats were taken on which the Y-axis represented the suitability-for-use (index rating score) and ranged from 0 - 100. The X-axis represented the range of determinant concentrations or values likely to have an effect on fish. In all the cases the curves were to be plotted through a fixed point, i.e. the index rating score of 60 and the X-axis value representing the water quality standard for that determinant; this corresponds with the lowest value in the "suitability for all fish life" category (Table 3.3). The remainder of the concentrations of the specific determinants and their corresponding rating scores were plotted on the graph by employing current toxicity data in the form of LC50 and No Observed Effect Concentration (NOEC) values.

Using the above-mentioned method, rating curves were obtained for Fluoride, Cu, Ni, Pb, Zn, Mn and Cr. Using existing toxicological data from the literature (Smith *et al.*, 1985 ; Hellawell, 1986 ; Mance, 1987 ; Van de Meent *et al.*, 1990), LC50 and NOEC values were obtained for the construction of rating curves. The toxicological data employed, were obtained using the following fish species: *Lepomis macrochirus* (bluegill), *Pimephales promelas* (fathead minnow), *Oncorynchus mykiss* (rainbow trout), *Ictalurus punctatus* (channel catfish) and the local *Oreochromis mossambicus* (Mozambique tilapia) (Wepener *et al.*, 1992 ; Van Vuren *et al.*, 1994).

The concentration on the X-axis for the index rating score of 60 (Y-axis value) for each determinant was derived by combining and aggregating the water quality standards of the UK (Gardiner & Zabel, 1989), Netherlands (Van de Meent *et al.*, 1990), Australia (Hart, 1974), Canada (Environment Canada, 1987), European Inland Fisheries Advisory Commission (EIFAC, 1980), South Africa (Kempster *et al.*, 1982) and water quality guidelines for the Olifants River (Moore *et al.*, 1991). The rating score of 60 is regarded as the minimum ATI value where the water quality is suitable for all fish species.

A graphical summary of all rating curves employed for this purpose, as well as rating curve functions are illustrated in Figures 3.1 a-f.

(iii) Incorporation of an Expert System in the construction of rating curves and the use of the system in the prediction of the effects of water quality variables on fish

The rules-based expert system developed by Wepener *et al.*, (1992), allows for the prediction of the effects of water quality variables on fish, should the "suitability-for-use" value be exceeded. These principles were also taken into account during the design of the ATI. The rule base is based on "if" and "then" statements. The "if" part of the rule is referred to as the condition, while the "then" part is the action. The initial rules set were obtained from toxicological and physiological data in the literature and are summarized in Van Vuuren *et al.* (1994) for D.O., pH, Mn, Ni, F, Cr, Pb, NH_4-N, Cu, Zn, PO_4-P, K, Turbidity and TDS.

(iii) Determinant weighting

Many attempts have been made to develop a weighting scale reflecting the relative importance placed on individual determinants (House, 1989). This was undertaken by supplying operational management personnel with questionnaires and requesting them to rank a set of determinants in order of their importance as indicators of water quality change. A final set of weightings had been proposed (House, 1989) and is in use for conditions in the UK. However, local authors (Wepener *et al.*, 1992) admit that insufficient knowledge and expertise is available locally to propose a set of weightings for water quality determinants.

Although not ideal, this investigation was also an attempt to present a set of determinant weightings, and is presented in Table 3.5.

(iv) Determinant aggregation

House (1989) has, during an extensive comparison of aggregation equations, found that the modified arithmetic equation, first used by the Scottish Development Department (SDD), produced the best results. The aggregation equation proposed has been adopted by House and was also tested on local data:

$$ATI = \frac{1}{100} \left(\sum_{i=1}^{n} q_i w_i\right)^2 \text{---} [1]$$

Where: q_i represents the rating of the ith determinant

w_i represents the weighting of the ith determinant

n represents the number of determinants

However, the Additive function originally proposed by Brown *et al.* (1970 ; 1972) still seemed to pose some merit for investigation and was therefore also included:

$$ATI = \frac{1}{100} \sum_{i=1}^{n} q_i w_i \quad [2]$$

Where: qi represents the rating of the ith determinant

wi represents the weighting of the ith determinant

n represents the number of determinants

Table 3.5 Proposed weights (w_i) attached to each of the chemical parameters used in the ATI.

Chemical parameter	Weight (w_i)
Temperature (°C)	0.05
pH	0.1
Dissolved oxygen (mg/ℓ)	0.06
TDS (mg/ℓ)	0.1
Phosphates (mg/ℓ)	0.01
Potassium (mg/ℓ)	0.1
Fluoride (mg/ℓ)	0.1
Copper (μg/ℓ)	0.1
Nickel (μg/ℓ)	0.06
Lead (μg/ℓ)	0.1
Zinc (μg/ℓ)	0.06
Manganese (μg/ℓ)	0.06
Chromium (μg/ℓ)	0.1

3.8 The concept of the Sodium Adsorption Ratio (SAR) (Sodicity) and its application to the suitability of irrigation water

Although the Aquatic Toxicity Index gives a clear indication of the suitability of water for use by aquatic organisms, it has little application towards the suitability-evaluation of water for use on crop irrigation. Therefore, a similar modelling system to evaluate the suitability of water for irrigation was utilized and evaluated.

Irrigation of agricultural soil with sodic (sodium-rich) water induces soil sodicity (McKee & Wolf, 1963 ; van Tonder, 1980 ; Dept. of Water Affairs and Forestry, 1993). Soils affected by excessive amounts of sodium exhibit impaired soil physical conditions. The most

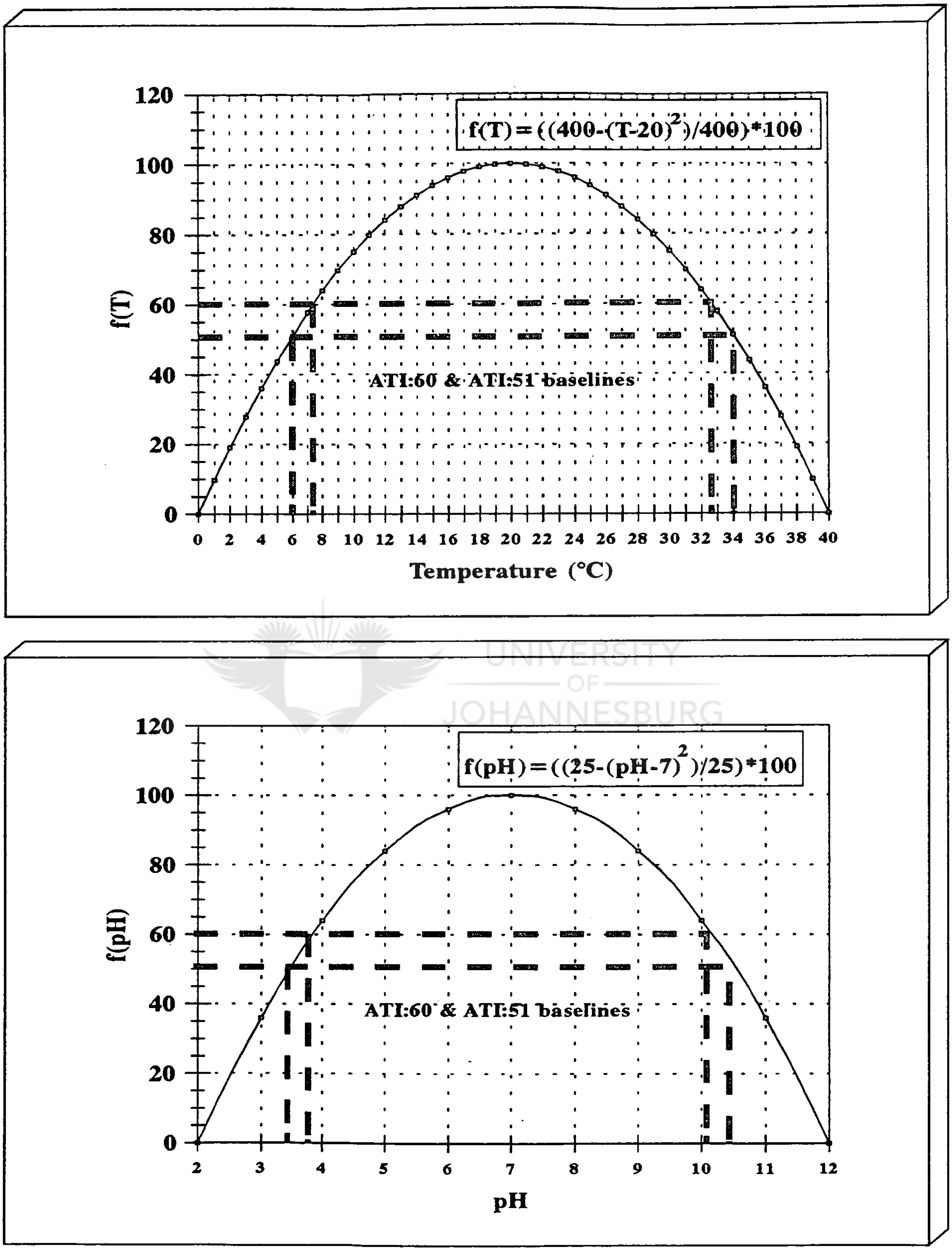


Figure 3.1a ATI rating curves for temperature and pH

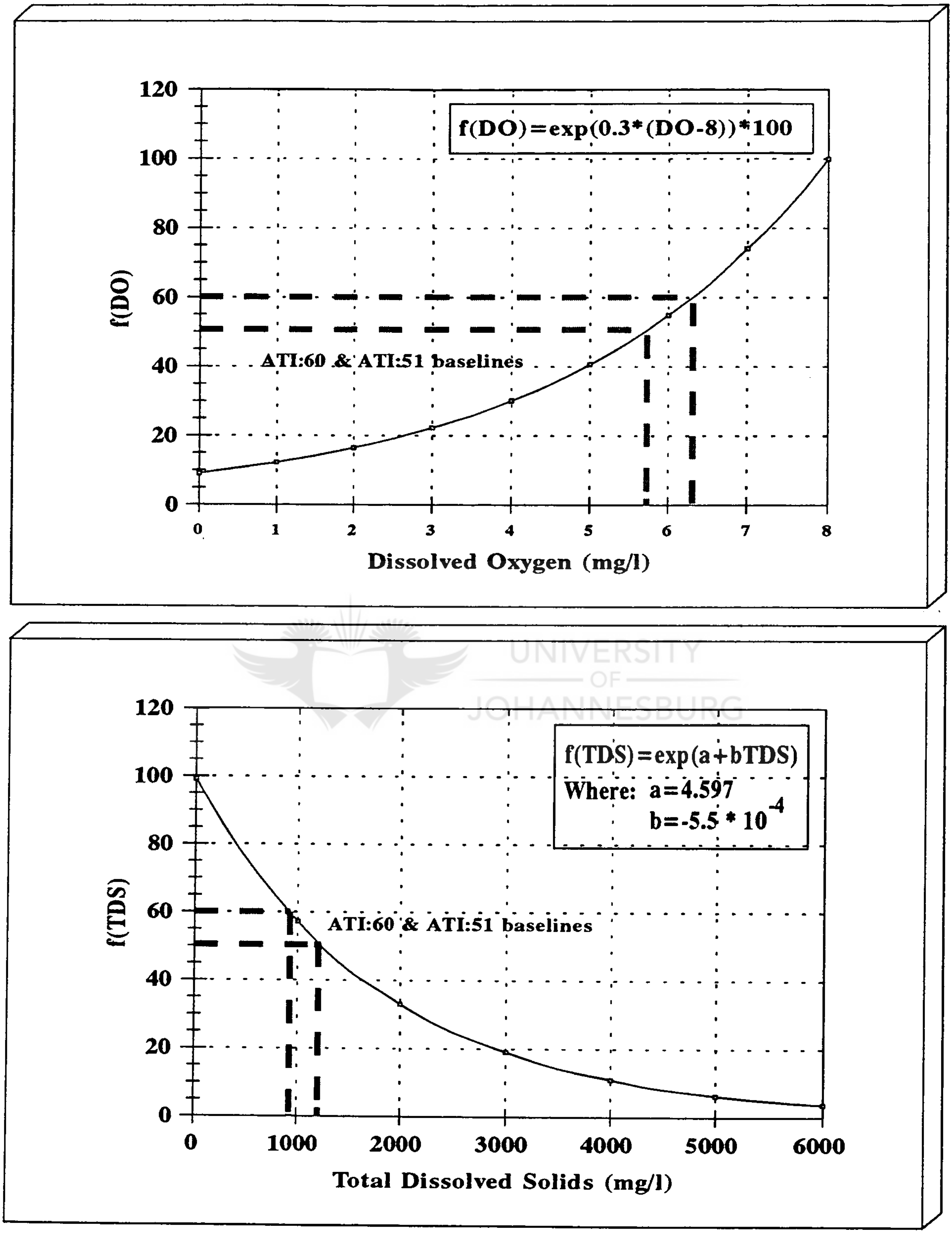


Figure 3.1b ATI rating curves for dissolved oxygen and TDS

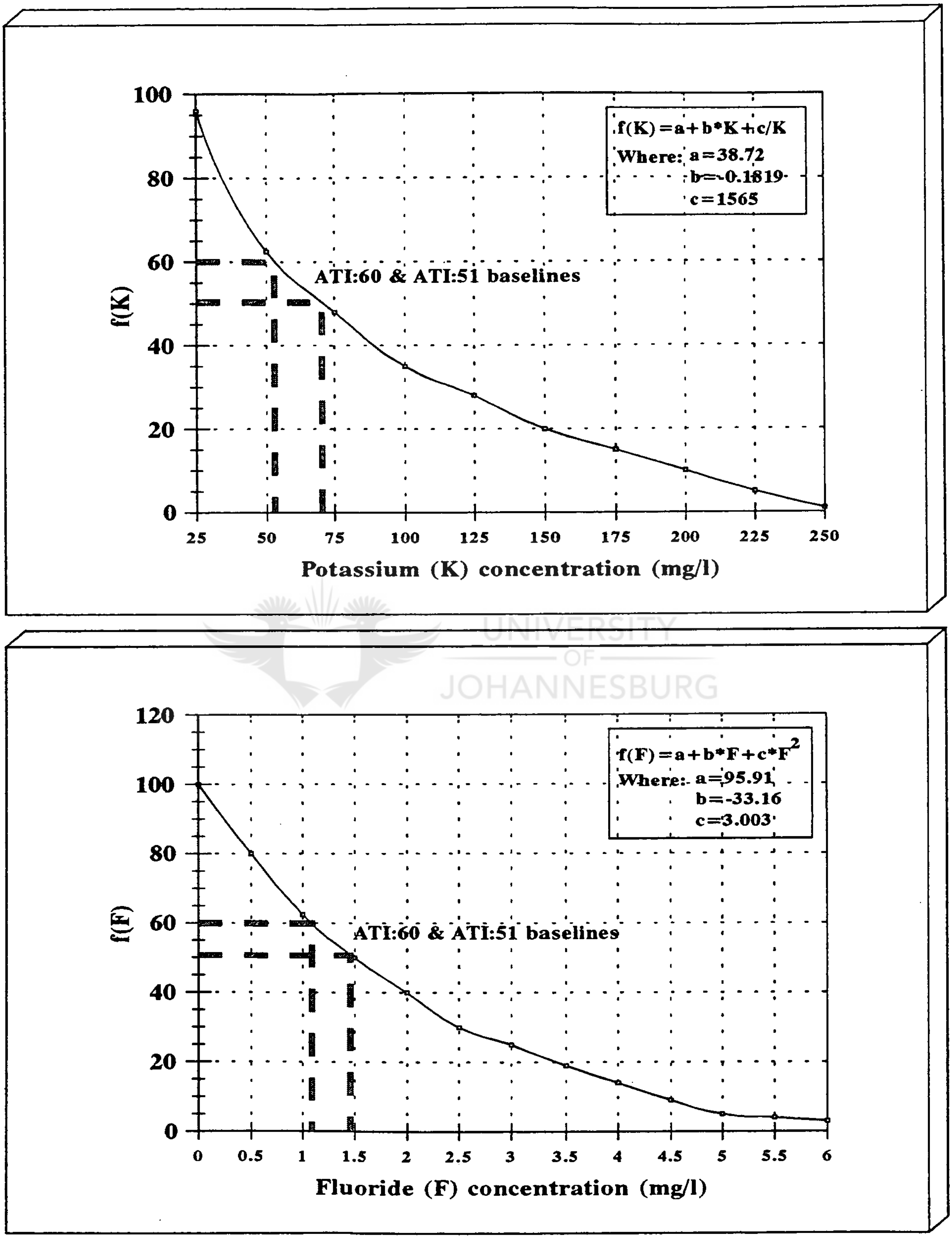


Figure 3.1c ATI rating curves for Potassium and Flouride

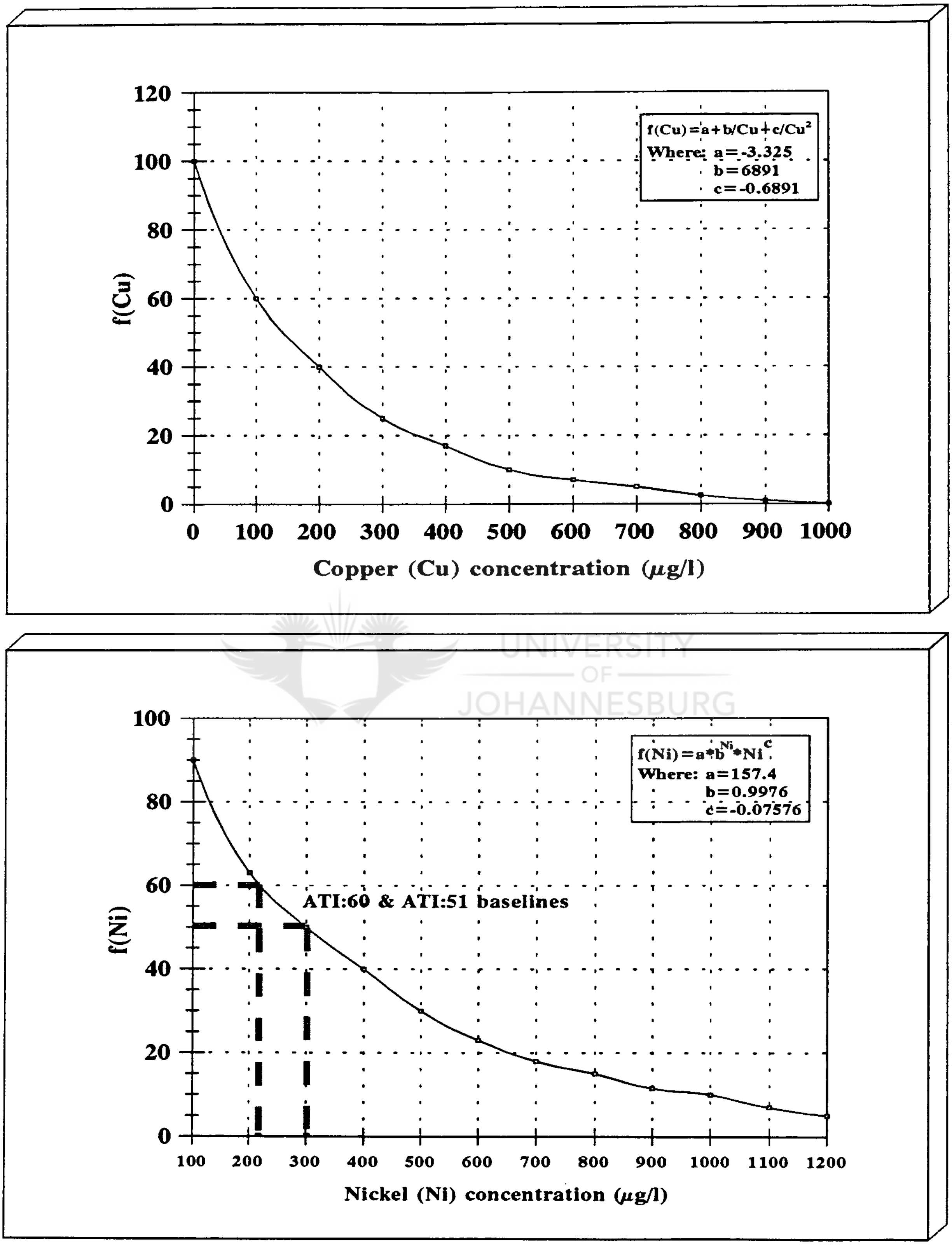


Figure 3.1d ATI rating curves for Copper and Nickel

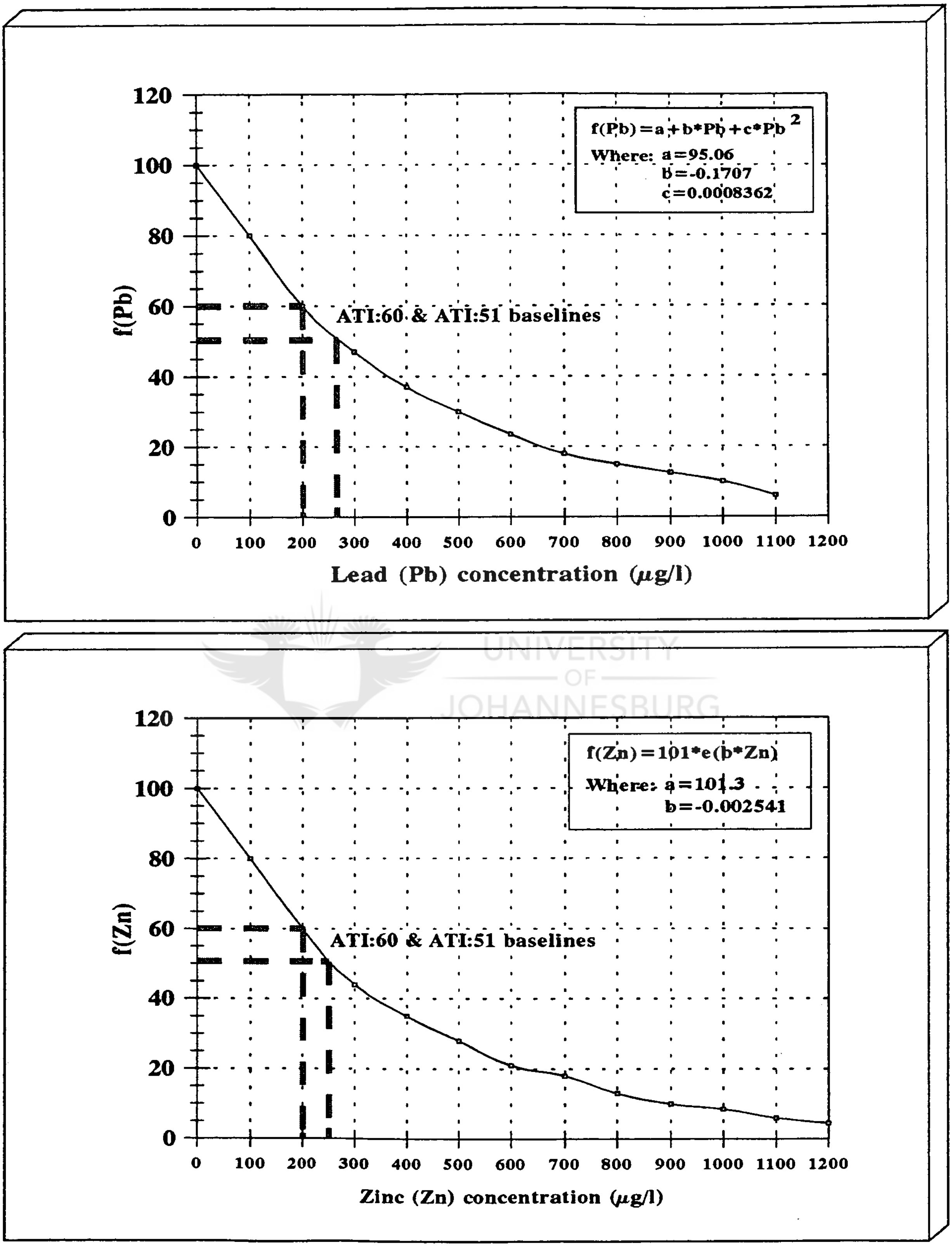


Figure 3.1e ATI rating curves for Lead and Zinc

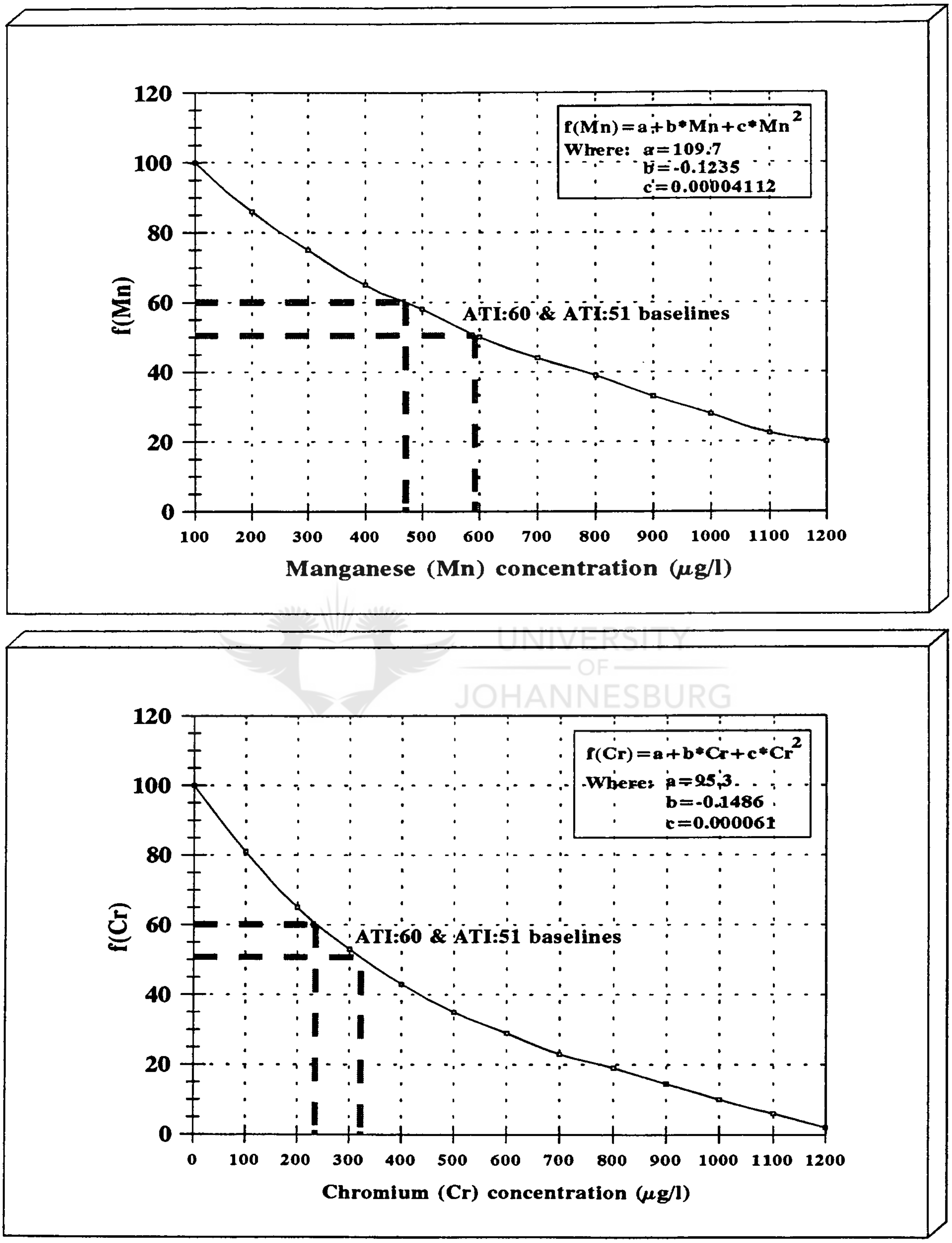


Figure 3.1f ATI rating curves for Manganese and Chromium

prominent symptom of physically impaired soil is the reduction of soil permeability which could, in turn, result in soil unable to absorb sufficient water to supply in crop water requirements.

The SAR therefore is an index of the potential of a given irrigation water to induce sodic soil conditions (DWA&F, 1993). The SAR is calculated using the milli-equivalent (mmol/ℓ) concentrations of sodium, calcium and magnesium in water. The concentration of sodium

is usually determined using an AAS. The concentration for Ca and Mg can be determined using the same technique, but is usually determined by measuring the total- and calcium hardness of the water. The following relationship exists:

$$^{*}\text{Total hardness} = \text{Calcium hardness} + \text{Magnesium hardness} \tag*{[1]}$$

As total, Ca and Mg hardness are expressed as mg/ℓ $CaCO_3$, conversions for Ca- and Mg hardness have to be made to determine the milli-equivalent Ca and Mg concentrations:

$$[\text{Ca}] = \text{Ca-hardness} / 2{,}5 \tag*{[2]}$$

$$[\text{Mg}] = \text{Mg-hardness} / 4{,}12 \tag*{[3]}$$

**Note that total hardness is not entirely comprised of Ca and Mg ions, but other cations as well. However, the concentrations of these ions are often so low that they can be considered as insignificant.*

The SAR (mmol/ℓ) was subsequently calculated using the following equation:

$$\text{SAR} = \text{Na} / (\text{Ca} + \text{Mg})^{0,5} \tag*{[4]}$$

Where: Na, Ca and Mg are concentrations (mmol/ℓ) of Na, Ca and Mg in solution.

To convert normal mg/ℓ concentrations to milli-equivalent concentrations (mmol/ℓ), the following equation was used:

$$\text{SAR} = \frac{[\text{Na}] \times 0.0435}{\sqrt{\dfrac{([\text{Ca}] \times 0.0499) + ([\text{Mg}] \times 0.08224)}{2}}} \tag*{[5]}$$

Also important to note is the relationship between sodium concentration and electrical conductivity of a water sample. It is found that an increase in Na concentration coincides with an increase in electrical conductivity and a subsequent increase in the SAR index value. Therefore, another scheme has been developed (McKee & Wolf, 1963 ; van Tonder, 1980) which takes the SAR and electrical conductivity into account. This scheme is illustrated in Figure 3.2 which classifies water into conductivity (C) and salinity (S) classes.

Conductivity

Low-salinity water (C1)	can be used for irrigation on most crops on most soils with little likelihood that soil salinity will develop. Some leaching is required, but this occurs under normal irrigation practices except in soils of extremely low permeability. Suitable for the irrigation of lemons, grapes, peaches, apricots, pears, plums, eggfruit, potatoes and green beans.
Medium-salinity water (C2)	can be used if a moderate amount of leaching occurs. Plants with moderate salt tolerance (lucerne, tomatoes, asparagus, grain sorghum, maize, corn, sunflowers, carrots, spinach, pumpkin, onions and russians) can be grown without special practices for salinity control.
High-salinity water (C3)	cannot be used on soils with restricted drainage. Even with adequate drainage, special management for salinity control may be required and plants with good salt tolerance (sugarbeet, cauliflower and cotton) should be selected.
Very high-salinity water (C4)	is not suitable for irrigation under ordinary conditions, but may be used occasionally under very special circumstances. The soils must be permeable, drainage must be adequate, irrigation water must be applied in excess to provide considerable leaching, and very salt-tolerant crops should be selected.

Sodium

The classification of irrigation waters with respect to SAR is primarily based on the effect of exchangeable sodium on the physical condition of the soil. Sodium-affected soil may therefore cause injury to sodium-sensitive plants which is the result of sodium accumulation in plant tissues.

Low-sodium water (S1)	can be used for irrigation on almost all soils with little danger of the development of toxic levels of exchangeable sodium.
Medium-sodium water (S2)	present an appreciable sodium hazard in fine-textured soils with a high cation-exchange-capacity, especially under low-leaching conditions. This water may be used on coarse-textured or organic soils with good permeability.

High-sodium water (S3)	may produce harmful levels of exchangeable sodium in most soils and will require special soil management such as good drainage, high leaching and organic matter additions.
Very high-sodium water (S4)	is generally not suitable for irrigation purposes except at low and perhaps medium salinity.

3.9 Corrosivity of irrigation water

The potential corrosivity of irrigation water was also determined in order to determine the possible effects on irrigation pipes as well as physical damage to plant tissue. In order to determine corrosivity, pH, TDS, alkalinity and calcium hardness were used as input data in the STASOFT computer programme (Loewenthal *et al.*, 1988). Using this programme, precipitation potential and pHs (saturation pH) were determined. Using pH and pHs, Langelier and Ryznar indices were calculated to assess potential corrosivity.

3.9.1 Langelier Saturation Index (LSI)

The Langelier Saturation Index gives an indication of the over- or undersaturation of water with respect to calcium carbonate content. This index (Langelier, 1936) is defined as follows:

$$\text{Langelier Saturation Index (LSI)} = \text{pH}_{(\text{actual})} - \text{pHs}$$

The index value is negative for undersaturated water (corrosive water which dissolve calcium carbonate) while a positive value indicates towards supersaturated (scale-forming) waters (Langelier, 1936 ; McKee & Wolf, 1963 ; Loewenthal *et al.*, 1986)

In practice, however, the index may merely be considered as an indication of a tendency, not of a capacity, to resist change. Water containing high concentrations of carbonate and calcium ions has a better chance of resisting change in its composition than water containing low concentrations of these ions. This property of water is commonly referred to as its buffer capacity (Loewenthal *et al.*, 1986).

3.9.2 Ryznar Stability Index

Ryznar on the other hand, proposed an empirical stability index in order to exclude the possibility of interpreting a positive saturation index as indicating non-corrosive tendencies in cases where the water actually has corrosive properties:

$$\text{Ryznar Stability Index} = 2\text{pHs} - \text{pH}$$

The Ryznar index value is positive in all cases. Index values falling between 6,5 and 7,5 reflects water fairly stable with regard to $CaCO_3$, while values below 6,5 indicate scale-forming properties. Index values above 7,5 reflect water with corrosive properties (McKee & Wolf, 1963 ; Loewenthal *et al.*, 1986)(Table 3.6).

3.10 Graphic representation of data and text editing

Graphic illustration of figures and data were done using Harvard Graphics 3.0 software package. All typing of text were done using Wordperfect 4.0.

Table 3.6 Chemical stability or corrosivity of water according to the Ryznar Index (After McKee & Wolf, 1963 ; Loewenthal *et al.*, 1986)

Ryznar Index	Tendency for corrosivity or scale formation
>8,5	Highly corrosive
7,5 - 8,5	Slightly corrosive
6,5 - 7,5	Chemically stable
5,5 - 6,5	slightly depositing or scale-forming
<5,5	highly depositing or scale-forming

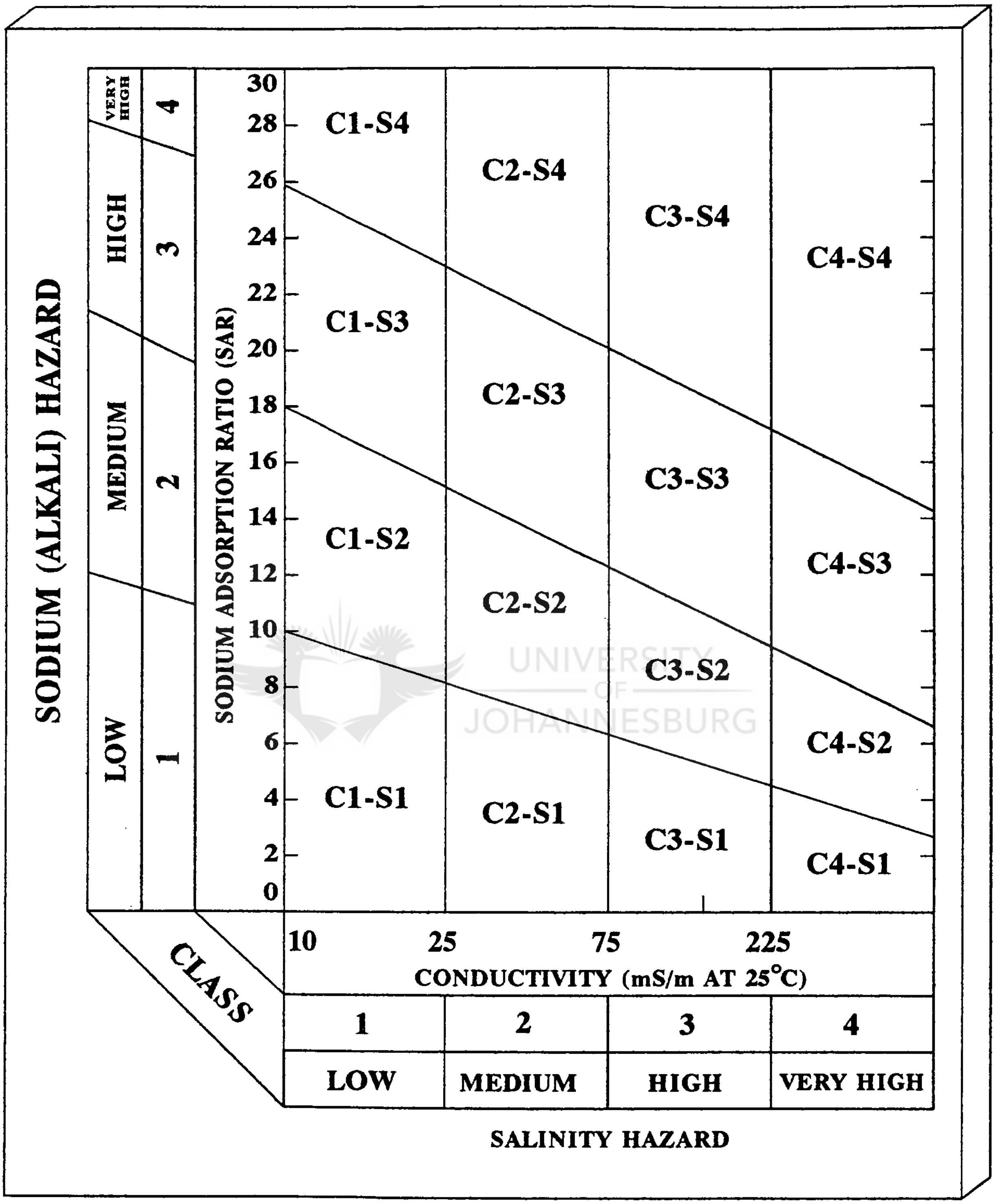


Figure 3.2 The classification of irrigation waters according to sodium content and conductivity (After McKee & Wolf, 1963 ; van Tonder, 1980)

RESULTS

4

4 RESULTS

4.1 Physical-chemical characteristics of irrigation water collected from the control locality as well as localities affected by mine drainage effluents

4.1.1 Crocodile Farm

During the time of the survey, the overall water quality at this site proved to be of a quality suitable for the irrigation of vegetable crops (Table 4.1). The pH (7,9) and electrical conductivity (EC) (650 μS/cm) further indicated water which complied with the general guidelines for suitability of water for irrigation purposes (Department of Water Affairs and Forestry, 1993 ; Kempster *et al.*, 1982) (Table 4.5). Although the alkalinity (79 mg/ℓ), total hardness (142 mg/ℓ), nitrogenous compounds (Ammonia-N: 0,13 mg/ℓ ; Nitrite-N: 0,06 mg/ℓ ; Nitrate-N: 4,0 mg/ℓ) and Orthophosphate (0,05 mg/ℓ) levels usually don't have a direct impact on the suitability of irrigation water (or are considered to be potentially toxic), the concentrations recorded of these nutrients generally reflected water of a good quality which can also be considered as suitable for the sustainment of aquatic life. Fluoride (0,2 mg/ℓ) and chloride (41 mg/ℓ) concentrations also complied as suitable for this purpose according to the general guidelines, while the relatively low sulphate concentration (85 mg/ℓ) suggests the low level or even absence of specific mining activities.

The metal concentrations recorded (Table 4.1) were all of such comparatively low values that they generally reflected water belonging to Class I irrigation water, being highly suitable for all types of vegetable crops (Table 4.5). This is also confirmed by a calculated Sodium Absorption Ratio (SAR) value of 0,48 which reflects a water of medium salinity, but low sodium content. A precipitation potential of -0,84 mg/ℓ (as $CaCO_3$), Langelier index of -0,1 and a Ryznar Index of 8,1 further suggest that this water was only mildly corrosive to irrigation pipes as well as plant leaves and that it would pose little if any effect on the production of vegetable crops (Table 4.1).

An inspection of the Aquatic Toxicity Index (ATI) values obtained for the water of the Crocodile farm revealed some interesting trends. All but one index value (Additive Unweighted: 91,52 ; Additive Weighted: 70,82 ; Solway Unweighted: 83,76) (Table 4.1) fell within the general guideline values for the Class I (ATI > 70) category for suitability-of-use of water (Table 3.2). Only the Solway Weighted index produced a value lower than 70 (50,16), placing it in Class II water. According to these index values, water from this river is of a relatively high quality suitable for drinking water supply, game fisheries, contact recreation and high quality industrial abstractions at low cost (Table 3.2). This water is therefore generally suitable for the sustainment of aquatic life (Table 3.5).

4.1.2 Marievale Farm

Compared to the water of the Crocodile River control sampling locality, the water used for irrigation at Marievale showed a significant increase in the amounts of total dissolved solids

Table 4.1 **Chemical quality of irrigation water collected from Crocodile Farm during the summer of 1992**

Water Quality Parameter	Concentration	ATI Rating (0-100)	
		Weighted	Unweighted
Temperature (°C)	21,0	49,88	99,75
pH	7,9	96,76	96,76
Dissolved Oxygen (mg/ℓ)	6,7	40,62	67,71
Electrical Conductivity (µS/cm)	650	-	-
Total Dissolved Salts (mg/ℓ)	416	78,90	78,90
Alkalinity (mg/ℓ)	79	-	-
Total hardness (mg/ℓ)	142	-	-
Ca-hardness (mg/ℓ)	78	-	-
Mg-hardness (mg/ℓ)	64	-	-
Ammonia-N (mg/ℓ)	0,13	-	-
Nitrate-N (mg/ℓ)	4,0	-	-
Nitrite-N (mg/ℓ)	0,06	-	-
Orthophosphate-P (mg/ℓ)	0,05	8,82	88,25
Sulphate-SO_4 (mg/ℓ)	85	-	-
Fluoride-F (mg/ℓ)	0,2	89,40	89,40
Chloride-Cℓ (mg/ℓ)	41	-	-
Iron (µg/ℓ)	18	-	-
Copper (µg/ℓ)	7	100,00	100,00
Nickel (µg/ℓ)	6	60,00	100,00
Lead (µg/ℓ)	3	94,55	94,55
Zinc (µg/ℓ)	84	49,10	81,83
Manganese (µg/ℓ)	14	60,00	100,00
Chromium (µg/ℓ)	18	92,64	92,64
Sodium (mg/ℓ)	13,2	-	-
Potassium (mg/ℓ)	0,5	100,00	100,00
S.A.R.	**0,48**	**Medium Salinity**	
Irrigation Class	**C2-S1**	**Low Sodium**	
pHs	**8,00**		
Precip. Pot. as $CaCO_3$ (mg/ℓ)	**-0,84**		
Langelier Index	**-0,1**	**Slightly**	
Ryznar Index	**8,1**	**Corrosive**	
ATI: Additive Unweighted	**91,52 (Class I)**	**MINOP: 67,71**	
ATI: Additive Weighted	**70,82 (Class I)**	**MINOP: 8,82**	
ATI: Solway Unweighted	**83,76 (Class I)**	**MINOP: 67,71**	
ATI: Solway Weighted	**50,16 (Class II)**	**MINOP: 40,62**	

present (E.C: 1540 μS/cm ; TDS: 986 mg/ℓ). The ammonia concentration was moderately high (<0,1 mg/ℓ) and with a higher nitrate concentration (3,3 mg/ℓ) suggest eutrophic conditions which prevailed in the water of the Blesbokspruit wetland at this sampling station. A relatively moderate alkalinity of 82 mg/ℓ indicated a slightly reduced buffer capacity of the water at this site. This in turn is also reflected by its slight corrosivity (Table 4.2). Fluoride (0,6 mg/ℓ) and chloride (55 mg/ℓ) concentrations both complied to the values of the general guidelines for irrigation (Table 4.4), while a higher sulphate concentration of 624 mg/ℓ strongly suggests the presence of mining activity in the vicinity of this farm. Although the metal concentrations of the water here were higher than those of the control sampling locality (Fe: 386 μg/ℓ ; Ni: 58 μg/ℓ ; Zn: 70 μg/ℓ ; Mn: 112 μg/ℓ) (Table 4.2), all values still fell within limits of the general guidelines proposed for irrigation purposes (Table 4.5).

A relatively high sodium concentration (342 mg/ℓ) as well as a high E.C (1540 μS/cm) produced a SAR-value of 8,80 which according to general standards for irrigation water, points towards advancing sodicity of the soil taking place. The Langelier (0,05) and Ryznar (7,7) indices suggest a slight corrosivity of the water towards irrigation pipes and also to vegetable leaves. ATI-values recorded for this sampling locality produced a somewhat different picture with the Additive Unweighted Index (84,11) of the water falling into Class I, both Additive Weighted (66,91) and Solway Unweighted (70,74) in Class II and Solway Weighted (44,77) in Class III (Table 4.2). Minimum Operators for the indices showed that for this locality, low dissolved oxygen concentrations and elevated Orthophosphate concentrations affected the water quality negatively. According to the guidelines (Tables 3.2 and 3.5) (House, 1989 ; Wepener *et al.*, 1992) this water at Marievale is still of a reasonable quality suitable for drinking water supply following conventional treatment, for good coarse fisheries (under local conditions suitable for hardy fish species such as the common carp, *Cyprinus carpio* as well as the sharptooth catfish, *Clarias gariepinus*), indirect contact sports as well as for most industrial abstractions at moderate cost.

4.1.3 Vlakfontein Farm

The relatively high electrical conductivity (1400 μS/cm) obtained here, combined with low alkalinity (16 mg/ℓ) and a relatively low total hardness (142 mg/ℓ) showed this water to be not only highly corrosive to irrigation pipes but also potentially damaging to vegetable leaves (Langelier: -1,52 ; Ryznar: 10,1 ; Precipitation Potential: -6,35 mg/ℓ as $CaCO_3$) (Table 4.3). It also produced a SAR-value of 4,72 (High salinity, Low sodium) which placed it in irrigation Class III water, rendering it relatively unsuitable for the spray irrigation of crops (Table 4.5). During collection of samples, it was also noted that only flood irrigation is practised, as spray irrigation tended to damage or burn the leaves of the crops (Inago, 1993 : pers. comm.). This farmer mentioned that he was anxious to dispose of his farm as severe crop losses were experienced as a result of the problems encountered.

Comparing water metal concentrations with the general guidelines for irrigation water (Table 4.5), Cu (340 μg/ℓ), Ni (4690 μg/ℓ), Pb (328 μg/ℓ), Zn (1820 μg/ℓ), Mn (20810 μg/ℓ) and

Table 4.2 Chemical quality of irrigation water collected from Marievale Farm during the summer of 1992

Water Quality Parameter	Concentration	ATI Rating (0-100)	
		Weighted	Unweighted
Temperature (°C)	21,5	49,72	99,44
pH	7,8	97,44	97,44
Dissolved Oxygen (mg/ℓ)	5,5	28,34	47,24
Electrical Conductivity (µS/cm)	1540	-	-
Total Dissolved Salts (mg/ℓ)	986	57,67	57,67
Alkalinity (mg/ℓ)	82	-	-
Total hardness (mg/ℓ)	285	-	-
Ca-hardness (mg/ℓ)	172	-	-
Mg-hardness (mg/ℓ)	113	-	-
Ammonia-N (mg/ℓ)	<0,1	-	-
Nitrate-N (mg/ℓ)	3,3	-	-
Nitrite-N (mg/ℓ)	0,01	-	-
Orthophosphate-P (mg/ℓ)	0,3	4,72	47,24
Sulphate-SO_4 (mg/ℓ)	624	-	-
Fluoride-F (mg/ℓ)	0,6	77,10	77,10
Chloride-Cℓ (mg/ℓ)	55	-	-
Iron (µg/ℓ)	386	-	-
Copper (µg/ℓ)	7	100,00	100,00
Nickel (µg/ℓ)	58	60,00	100,00
Lead (µg/ℓ)	6	94,04	94,04
Zinc (µg/ℓ)	70	50,88	84,79
Manganese (µg/ℓ)	112	57,83	96,38
Chromium (µg/ℓ)	22	92,06	92,06
Sodium (mg/ℓ)	342	-	-
Potassium (mg/ℓ)	2.4	100,00	100,00
S.A.R.	8,80	High Salinity	
Irrigation Class	C3-S1	Low Sodium	
pHs	7,75		
Precip. Pot. as $CaCO_3$ (mg/ℓ)	0,81		
Langelier Index	0,05	Slightly	
Ryznar Index	7,7	Corrosive	
ATI: Additive Unweighted	84,11 (Class I)	MINOP: 47,24	
ATI: Additive Weighted	66,91 (Class II)	MINOP: 4,72	
ATI: Solway Unweighted	70,74 (Class I)	MINOP: 47,24	
ATI: Solway Weighted	44,77 (Class III)	MINOP: 28,34	

[*] Chemical value in bold exceeds guideline value for the protection of aquatic life (Kempster *et al.*, 1982)

Cr (180 μg/ℓ) all exceeded the proposed guideline values laid down by Kempster *et al.* (1982), with a relatively large margin. The effect of these elevated metal concentrations in the irrigation water can also clearly be seen in the results obtained for metal accumulation in the vegetable crops concerned (Table 4.18).

ATI-values for this locality show in some sense an entirely different picture than those obtained for the Crocodile Farm. Only the Additive Unweighted Index value (54,37) falls within Class II water while all the other (Additive Weighted: 42,13 ; Solway Unweighted: 29,56 ; Solway Weighted: 17,75) resorted to Class III. Excessive Nickel concentrations produced Minimum Operator values of zero for several ATI's. However, the low index values recorded for dissolved oxygen, copper, lead and manganese need also be mentioned (Table 4.3). Overall, these index values suggest polluted water with a general limited value, being only suitable for potable water supply following advanced treatment, indirect contact sports and some industrial abstractions at high treatment costs (Table 3.2). According to guidelines for local conditions (Table 3.2), this water is unsuitable for the sustainment of normal fish life as well as for aquatic invertebrate life in general.

4.1.4 Luipaardsvlei Farm

The Total Dissolved Solids concentration (704 mg/ℓ) of water from this sampling locality (Table 4.4) fell between that of the control sampling locality (Table 4.1) and that of the Marievale Farm (Table 4.2). Relatively moderate Ammonia (0,12 mg/ℓ), Nitrate (1,1 mg/ℓ) and Orthophosphate ((0,16 mg/ℓ) concentrations suggest some organic enrichment and therefore mild eutrophication in the water of this stream ecosystem. Relatively low alkalinity (22 mg/ℓ) and total hardness (148 mg/ℓ) values indicate a slightly corrosive tendency of this water (Langelier: -1,66 ; Ryznar: 9,72) (Table 4.4).

Sodium content (13,2 mg/ℓ) and electrical conductivity (1100 μS/cm) produced a SAR of 0,08 and a C3-S1 (High salinity ; Low sodium) irrigation class (Table 4.4). Overall, total metal concentrations complied to guideline values for aquatic life (Table 3.4) as well as for irrigation (Table 4.5).

Looking at the metal concentrations in the irrigation water analyzed at Luipaardsvlei, most values recorded were relatively low with highest values for Zn (84 μg/ℓ) and to a lesser extent, Mn (55 μg/ℓ). A lowest value of 9 μg/ℓ was recorded for Pb.

ATI-values showed both unweighted indices (Additive Unweighted: 88,98 ; Solway Unweighted: 70,27) to fall within Class I (Table 4.4) while index values for ATI: Additive Weighted (69,75) as well as ATI: Solway Weighted (47,93) all fell within Classes II and III, respectively. On average, this water is still of reasonable quality, suitable for potable water supply after conventional treatment, coarse fisheries, indirect contact sports and most industrial abstractions at moderate cost (Table 3.2). This water also tends to be suitable for the sustainment of life of most local fish species (Table 3.5).

Table 4.3 Chemical quality of irrigation water collected from Vlakfontein Farm during the summer of 1993

Water Quality Parameter	Concentration	ATI Rating (0-100)	
		Weighted	Unweighted
Temperature (°C)	18,0	49,50	99,00
pH	7,1	99,96	99,96
Dissolved Oxygen (mg/ℓ)	4,6	21,64	36,06
Electrical Conductivity (μS/cm)	1400	-	-
Total Dissolved Salts (mg/ℓ)	896	60,59	60,59
Alkalinity (mg/ℓ)	16	-	-
Total hardness (mg/ℓ)	142	-	-
Ca-hardness (mg/ℓ)	88	-	-
Mg-hardness (mg/ℓ)	54	-	-
Ammonia-N (mg/ℓ)	0,22	-	-
Nitrate-N (mg/ℓ)	2,4	-	-
Nitrite-N (mg/ℓ)	0,01		
Orthophosphate-P (mg/ℓ)	<0,01	9,75	97,53
Sulphate-SO_4 (mg/ℓ)	950	-	-
Fluoride-F (mg/ℓ)	1,2	60,44	60,44
Chloride-Cℓ (mg/ℓ)	70	-	-
Iron (μg/ℓ)	117	-	-
Copper (μg/ℓ)	**340**	16,25	16,25
Nickel (μg/ℓ)	**4690**	0,0	0,0
Lead (μg/ℓ)	**328**	48,07	48,07
Zinc (μg/ℓ)	**1820**	0,6	0,99
Manganese (μg/ℓ)	**20810**	10,42	17,37
Chromium (μg/ℓ)	**180**	70,53	70,53
Sodium (mg/ℓ)	129	-	-
Potassium (mg/ℓ)	8.6	100,00	100,00
S.A.R.	**4,72**	**High Salinity**	
Irrigation Class	**C3-S1**	**Low Sodium**	
pHs	**8,62**		
Precip. Pot. as $CaCO_3$ (mg/ℓ)	**-6,35**		
Langelier Index	**-1,52**	**Highly**	
Ryznar Index	**10,1**	**Corrosive**	
ATI: Additive Unweighted	**54,37 (Class II)**	**MINOP: 0,0**	
ATI: Additive Weighted	**42,13 (Class III)**	**MINOP: 0,0**	
ATI: Solway Unweighted	**29,56 (Class III)**	**MINOP: 0,0**	
ATI: Solway Weighted	**17,75 (Class III)**	**MINOP: 0,0**	

[*]Chemical values in bold exceed guideline values for the protection of aquatic life (Kempster *et al.*, 1982)

Table 4.4 **Chemical quality of irrigation water collected from Luipaardsvlei during the summer of 1993**

Water Quality Parameter	Concentration	ATI Rating (0-100)	
		Weighted	Unweighted
Temperature (°C)	21	49,88	99,75
pH	6,4	98,56	98,56
Dissolved Oxygen (mg/ℓ)	6,7	40,62	67,71
Electrical Conductivity (μS/cm)	1100	-	-
Total Dissolved Salts (mg/ℓ)	704	67,34	67,34
Alkalinity (mg/ℓ)	22	-	-
Total hardness (mg/ℓ)	148	-	-
Ca-hardness (mg/ℓ)	112	-	-
Mg-hardness (mg/ℓ)	36	-	-
Ammonia-N (mg/ℓ)	0,116	-	-
Nitrate-N (mg/ℓ)	1,1	-	-
Nitrite-N (mg/ℓ)	0,01	-	-
Orthophosphate-P (mg/ℓ)	0,16	6,70	67,03
Sulphate-SO_4 (mg/ℓ)	165	-	-
Fluoride-F (mg/ℓ)	0,2	89,40	89,40
Chloride-Cℓ (mg/ℓ)	14	-	-
Iron (μg/ℓ)	96	-	-
Copper (μg/ℓ)	12	100,00	100,00
Nickel (μg/ℓ)	25	60,00	100,00
Lead (μg/ℓ)	9	93,53	93,53
Zinc (μg/ℓ)	84	49,10	81,83
Manganese (μg/ℓ)	55	60,00	100,00
Chromium (μg/ℓ)	25	91,62	91,62
Sodium (mg/ℓ)	13,2	-	-
Potassium (mg/ℓ)	1,0	100,00	100,00
S.A.R.	**0,08**	**High Salinity**	
Irrigation Class	**C3-S1**	**Low Sodium**	
pHs	**8,06**		
Precip. Pot. as $CaCO_3$ (mg/ℓ)	**-32,9**		
Langelier Index	**-1,66**	**Slightly**	
Ryznar Index	**9,72**	**Corrosive**	
ATI: Additive Unweighted	**88,98 (Class I)**	**MINOP: 67,03**	
ATI: Additive Weighted	**69,75 (Class II)**	**MINOP: 6,70**	
ATI: Solway Unweighted	**70,27 (Class I)**	**MINOP: 67,34**	
ATI: Solway Weighted	**47,93 (Class III)**	**MINOP: 40,62**	

Table 4.5 Water quality guidelines for irrigation (After Department of Water Affairs and Forestry, 1993).

Water quality parameter	Water quality guideline for irrigation water			
	Class I	Class II	Class III	Class IV
	Salinity and sodicity			
Salinity (EC) (μS/cm)	0 - 400	400 - 900	900 - 2700	2700 - 5400
Sodicity (SAR)(mmol/$\ell^{0,5}$)	0 - 1,5	1,5 - 3,0	3,0 - 5,0	5,0 - 10,0
	Potentially toxic ions			
Boron (B, mg/ℓ)	0 - 0,2	0,2 - 0,9	0,9 - 1,5	1,5 - 3,0
Chloride (Cℓ, mg/ℓ)	0 - 105	105 - 140	140 - 350	>350
Sodium (Na, mg/ℓ)	0 - 69	69 - 115	115 - 161	161 - 207
	Trace elements (mg/ℓ)			
Aluminium	0 - 5,0	0 - 5,0	5,0 - 10,0	10,0 - 20,0
Arsenic	0 - 0,1	0 - 0,1	0,1 - 1,0	1,0 - 2,0
Beryllium	0 - 0,1	0 - 0,1	0,1 - 0,25	0,25 - 0,5
Cadmium	0 - 0,01	0 - 0,01	0,01 - 0,025	0,025 - 0,05
Chromium	0 - 0,1	0 - 0,1	0,1 - 0,5	0,5 - 1,0
Cobalt	0 - 0,05	0 - 0,05	0,05 - 2,5	2,5 - 5,0
Copper	0 - 0,2	0 - 0,2	0,2 - 2,5	2,5 - 5,0
Fluoride	0 - 2,0	0 - 2,0	2,0 - 7,5	7,5 - 15,0
Iron	0 - 5,0	0 - 5,0	5,0 - 10,0	10,0 - 20,0
Lead	0 - 0,2	0 - 0,2	0,2 - 1,0	1,0 - 2,0
Manganese	0 - 0,2	0 - 0,2	0,2 - 5,0	5,0 - 10,0
Mercury	*	*		
Molybdenum	0 - 0,01	0 - 0,01	0,01 - 0,025	0,025 - 0,05
Nickel	0 - 0,2	0 - 0,2	0,2 - 1,0	1,0 - 2,0
Selenium	0 - 0,02	0 - 0,02	0,02 - 0,025	0,025 - 0,05
Uranium	0 - 0,01	0 - 0,01	0,01 - 0,05	0,05 - 0,1
Vanadium	0 - 0,1	0 - 0,1	0,1 - 0,5	0,5 - 1,0
Zinc	0 - 1,0	0 - 1,0	1,0 - 2,5	2,5 - 5,0
	Miscellaneous parameters			
Nitrogen	0 - 5	5 - 30	>30	
pH	6,5 - 8,4			

*No level at present available

4.2 Concentrations and Concentration Ratios of selected metals in aquatic and semi-aquatic macrophytes collected from the control locality as well as from the other localities affected by mine drainage effluents

4.2.1 Submerged and floating aquatic weeds

Due to stream and habitat conditions, submerged and floating aquatic weeds were only available at the localities of the Marievale and Luipaardsvlei sampling sites. Comparative results on the metal contents in the sediments and in the plant tissues from these localities are summarized in Table 4.6.

In the Blesbokspruit near Marievale farm, Fe was detected at a concentration of 27303 $\mu g.g^{-1}$ in the sediments. This was followed by Mn (2261 $\mu g.g^{-1}$), Zn (403 $\mu g.g^{-1}$), Ni (369 $\mu g.g^{-1}$), Cr (195 $\mu g.g^{-1}$), Cu (49 $\mu g.g^{-1}$) and Pb (20 $\mu g.g^{-1}$). It is interesting to note that of the aquatic plants analyzed, the duckweed *Lemna gibba* collected at Marievale contained the highest concentrations of Pb (27 $\mu g.g^{-1}$), Zn (871 $\mu g.g^{-1}$), Mn (24605 $\mu g.g^{-1}$) and Cr (81 $\mu g.g^{-1}$), while the fennel-leaved pondweed, *Potamogeton pectinatus* contained the largest amount of Fe (7955 $\mu g.g^{-1}$) per equivalent dry mass. The alga, *Spirogyra sp.* shared a highest Cu content (57 $\mu g.g^{-1}$) with *P. pectinatus*, but contained the highest concentration of Ni (443 $\mu g.g^{-1}$) (Table 4.6). Although *L. gibba* collected at Marievale contained the highest concentrations of a number of metals as indicated in Table 4.6, it nevertheless contained the lowest concentrations of Fe (1088 $\mu g.g^{-1}$) and Cu (27 $\mu g.g^{-1}$). The water clover, *Marsilea macrocarpa* contained lowest concentrations of Ni (377 $\mu g.g^{-1}$), Pb (14 $\mu g.g^{-1}$) and Mn (4541 $\mu g.g^{-1}$), while Zn (217 $\mu g.g^{-1}$) and Cr (21 $\mu g.g^{-1}$) were measured in the smallest concentrations in the red water fern, *Azolla filiculoides* (Table 4.6).

At Luipaardsvlei, only *Spirogyra sp.* and *P. pectinatus* were collected. In the case of four metals, namely Cu (415 $\mu g.g^{-1}$), Ni (217 $\mu g.g^{-1}$), Zn (217 $\mu g.g^{-1}$) and Mn (6702 $\mu g.g^{-1}$), *Spirogyra sp.* contained the highest metal concentrations, while in the other three, *P. pectinatus* contained the highest concentrations, namely Fe (11577 $\mu g.g^{-1}$), Pb (113 $\mu g.g^{-1}$) and Cr (19 $\mu g.g^{-1}$), respectively (Table 4.6).

Looking at Concentration Ratios for all the submerged and floating aquatic weeds investigated at both these localities, it is evident from the results that manganese produced the highest ratios (Table 4.6). This was generally followed by an intermediate zone with no definite dominance by a specific metal, consisting of copper, nickel, zinc and lead. At the lowest two positions, i.e. metals producing the lowest Concentration Ratios, chromium and iron dominated, but again with no definite pattern. These tendencies in Concentration Ratio patterns exhibited by the various metals are analyzed in more detail and compared with those in aquatic weeds from other metal polluted aquatic habitats in the discussion which will follow later.

4.2.2 Rooted emergent aquatic macrophytes

The following rooted aquatic weeds were collected for analyses where they occurred at the

Table 4.6 **Concentration levels and Concentration Ratios of selected metals in the tissues of various submerged and floating aquatic weeds collected at Marievale and Luipaardsvlei sampling localities during the summers of 1992 - 1993**

Parameter	Sampling locality	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	Marievale	27303	49	369	20	403	2261	195
Spirogyra sp.		5881	57	443	18	416	18176	43
CR		0,215	1,163	1,201	0,900	1,032	8,039	0,221
Sediment	Marievale	27303	49	369	20	403	2261	195
L. gibba		1088	27	407	27	871	24605	81
CR		0,040	0,551	1,103	1,350	2,161	10,882	0,415
Sediment	Marievale	27303	49	369	20	403	2261	195
A. filiculoides		5745	52	441	17	217	4705	21
CR		0,210	1,061	1,195	0,850	0,538	2,081	0,108
Sediment	Marievale	27303	49	369	20	403	2261	195
M. macrocarpa		6563	46	377	14	312	4541	27
CR		0,240	0,939	1,022	0,700	0,774	2,008	0,138
Sediment	Marievale	27303	49	369	20	403	2261	195
P. pectinatus		7955	57	403	18	397	6733	64
CR		0,291	1,163	1,092	0,900	0,985	2,978	0,328
Sediment	Luipaardsvlei	33405	289	209	96	254	1044	97
Spirogyra sp.		8533	415	217	102	217	6702	12
CR		0,255	1,436	1,038	1,063	0,854	6,420	0,124
Sediment	Luipaardsvlei	33405	289	209	96	254	1044	97
P. pectinatus		11577	385	187	113	194	2516	19
CR		0,347	1,332	0,895	1,177	0,764	2,410	0,196

different localities : the spanish reed, *Arundo donax*, the cock's comb, *Polygonum lapathifolium*, bulrush, *Typha capensis*, the common reed, *Phragmites australis* and the water plantain, *Alisma plantago-aquatica*.

4.2.2.1 Crocodile Farm

Metal concentrations in aquatic sediments

At the control locality, namely the Crocodile farm, the bottom sediments contained 30667 $\mu g.g^{-1}$ of Fe. The second highest concentration of 918 $\mu g.g^{-1}$ was recorded for Mn, followed by Ni (302 $\mu g.g^{-1}$), Zn (256 $\mu g.g^{-1}$), Cu (155 $\mu g.g^{-1}$), Cr (122 $\mu g.g^{-1}$) and Pb (68 $\mu g.g^{-1}$), respectively (Tables 4.7 and 4.8).

Metal concentrations in and Concentrations Ratios of metals in aquatic macrophytes

Arundo donax collected at the Crocodile farm produced highest concentrations of all metals in the roots, namely Fe (5428 $\mu g.g^{-1}$), Cu (284 $\mu g.g^{-1}$), Ni (96 $\mu g.g^{-1}$), Pb (27 $\mu g.g^{-1}$), Zn (132 $\mu g.g^{-1}$), Mn (1065 $\mu g.g^{-1}$) and Cr (20 $\mu g.g^{-1}$). Generally, this was followed by lower metal concentrations in the white submerged stems (min - max values indicated) (1 $\mu g.g^{-1}$ (Cr) - 583 $\mu g.g^{-1}$ (Fe)), still lower in the green emergent stem (0,9 $\mu g.g^{-1}$ (Cr) - 337 $\mu g.g^{-1}$ (Fe)) and lowest in the emergent leaves (0,4 $\mu g.g^{-1}$ (Cr) - 245 $\mu g.g^{-1}$ (Fe)) (Table 4.7).

Concentration Ratios obtained for the same organs of this plant produced a similar sequence of results. CR_{RT} values ranging between 0,164 (Cr) and 1,832 (Cu) were followed by those for CR_{WS} (0,008 (Cr) - 0,393 (Mn)), CR_{GS} (0,007 (Cr) - 0,141 (Zn)) and lastly, a lowest CR_{LF} of 0,003 (Cr) - 0,174 (Cu) (Table 4.7).

In contrast to the above results, *Polygonum lapathifolium* collected at the Crocodile farm produced a somewhat different trend. Highest metal concentrations, ranging between 81 $\mu g.g^{-1}$ for Pb and 24043 $\mu g.g^{-1}$ for Fe were recorded in the root tissue for these metals. This was followed by a decline in the mean metal content of the white stem tissues which ranged between 7 $\mu g.g^{-1}$ (Cr) and 4876 $\mu g.g^{-1}$ (Fe). This trend partly continued, with lower concentrations for Ni (98 $\mu g.g^{-1}$), Pb (23 $\mu g.g^{-1}$), Zn (136 $\mu g.g^{-1}$), Mn (3606 $\mu g.g^{-1}$) and Cr (6 $\mu g.g^{-1}$) recorded in the green stem tissues (Table 4.8). The exceptions were Fe (9323 $\mu g.g^{-1}$) and Cu (1521 $\mu g.g^{-1}$) which both showed an increase in concentration in the green stem compared to values recorded in the white stem (Table 4.8). Looking at the metal contents of the leaves of this plant, an upward metal transfer is encountered from the green stem showing an increase in the concentrations of Cu (2938 $\mu g.g^{-1}$), Ni (137 $\mu g.g^{-1}$), Pb (27 $\mu g.g^{-1}$), Zn (182 $\mu g.g^{-1}$) and Mn (5335 $\mu g.g^{-1}$) in the leaves of this plant. Only in the case of Fe (8280 $\mu g.g^{-1}$) and Cr (1,8 $\mu g.g^{-1}$) a relative decrease in metal concentrations were encountered between the green stems and leaves (Table 4.8). In all cases, lower metal concentrations, relative to those measured in the leaves, were recorded in the flowers, ranging between 1,7 $\mu g.g^{-1}$ (Cr) and 8096 $\mu g.g^{-1}$ (Fe) respectively (Table 4.8).

The CR-values for the metals analyzed in the different tissues of *P. lapathifolium* followed an almost similar pattern with highest CR-values recorded for the root tissues (min-max values) (CR_{RT}: 0,983 (Ni) - 28,68 (Cu)). This was followed by a downward trend for CR_{WS} (0,057 (Cr) - 6,084 (Cu)) and CR_{GS} (0,048 (Cr) - 9,813 (Cu)). Similar to metal concentrations, this was followed by a slight increase in CR_{LF} (0,015 (Cr) - 18,955 (Cu)) and a decrease in CR_{FL} (0,014 (Cr) - 16,755 (Cu)) (Table 4.8).

4.2.2.2 Marievale Farm

Metal concentrations in aquatic sediments

At the Marievale farm, an almost similar pattern (to that obtained for Crocodile farm) for metal contents of the sediments was obtained. A highest Fe concentration for this locality (27303 $\mu g.g^{-1}$) was followed by those for Mn (2261 $\mu g.g^{-1}$), Zn (403 $\mu g.g^{-1}$), Ni (369 $\mu g.g^{-1}$), Cr (195 μg.g-1), Cu (49 $\mu g.g^{-1}$) and Pb (20 $\mu g.g^{-1}$) (Tables 4.9 - 4.12). It must be noted that Fe, Cu and Pb concentrations at this site were generally lower in the sediments than those recorded at the control locality.

Metal concentrations in and Concentrations Ratios of metals in aquatic macrophytes

Typha capensis collected at the Marievale farm showed a rather similar pattern to plants collected at the Crocodile farm when their concentrations in the different organs are compared. In the case of Cu (RT: 822 $\mu g.g^{-1}$; WS: 235 $\mu g.g^{-1}$; GS: 57 $\mu g.g^{-1}$; LF: 54 $\mu g.g^{-1}$), Ni (RT: 418 $\mu g.g^{-1}$; WS: 84 $\mu g.g^{-1}$; GS: 75 $\mu g.g^{-1}$; LF: 69 $\mu g.g^{-1}$), Zn (RT: 694 $\mu g.g^{-1}$; WS: 372 $\mu g.g^{-1}$; GS: 276 $\mu g.g^{-1}$; LF: 129 $\mu g.g^{-1}$) and Cr (RT: 11 $\mu g.g^{-1}$; WS: 1 $\mu g.g^{-1}$; GS: 1 $\mu g.g^{-1}$; LF: 1 $\mu g.g^{-1}$) invariably constant downward trends in metal concentrations for these organs listed here, were encountered (Table 4.9). The remaining metals analyzed did not exactly follow this particular trend, producing elevated concentrations in the foliar organs. For instance, Fe showed a decrease in concentration from the roots (6607 $\mu g.g^{-1}$) to the green stem (218 $\mu g.g^{-1}$) followed by a subsequent slight increase in the leaf tissue (300 $\mu g.g^{-1}$). In the case of Pb and Mn respectively, elevated concentrations were recorded in the green stem (7 $\mu g.g^{-1}$) and leaf tissues (13837 $\mu g.g^{-1}$), respectively (Table 4.9).

Corresponding to trends obtained for metal concentrations, CR-values showed downward trends for Cu (max-min values) (CR_{RT} (16,770) - CR_{LF} (1,110)), Ni (CR_{RT} (1,134) - CR_{LF} (0,187)) and Zn (CR_{RT} (1,722) - CR_{LF} (0,320)). Concentration Ratios for Fe, Pb, Mn and Cr on the other hand, showed upward trends for some of the plant organs. In the case of Fe, the CR_{LF} (0,011) showed a slight increase relative to that of CR_{GS} (0,008), while the CR_{GS} for Pb (0,330) was significantly higher than the corresponding CR_{WS} (0,280) for the same metal (Table 4.9). The CR-values for Mn displayed a somewhat different picture with a constantly increasing trend from CR_{WS} (4,730), through CR_{GS} (5,040) to CR_{LF} (6,120) (Table 4.9).

Almost similar trends for metal concentrations were obtained for the tissues of *Phragmites australis* collected in the Blesbokspruit wetland flowing next to the Marievale farm. Again, highest metal concentrations for certain metals in the root tissues (min-max values) (13 μg.g^{-1} (Cr) - 15533 μg.g^{-1} (Mn)) were followed by those in the white stems (2 μg.g^{-1} (Cr) - 2853 μg.g^{-1} (Mn)). Again, the same trends did exist with the leaf tissue of this plant containing higher concentrations of Fe (1584 μg.g^{-1}), Cu (370 μg.g^{-1}) and Mn (6455 μg.g^{-1}) relative to those of the green stems (Table 4.10).

Similar to metal concentrations, highest CR-values were recorded for the root tissue, ranging between a minimum of 0,067 (Cr) and a maximum of 19,780 (Cu). A lowest CR-value for the white stem tissue (Cr: 0,008) and a highest of 4,811 (Cu) was followed by a lowest of 0,003 (Cr) and a highest of 3,960 (Cu) for the green stem tissues. In the case of the CR_{LF} values, increases relative to those in the green stems were encountered for Fe (0,058), Cu (7,559), Mn (2,855) and Cr (0,004) (Table 4.10).

Alisma plantago-aquatica collected at the same locality also contained highest concentrations of Fe (792 μg.g^{-1}), Cu (648 μg.g^{-1}), Ni (7077 μg.g^{-1}), Pb (83 μg.g^{-1}), Zn (3490 μg.g^{-1}), Mn (18698 μg.g^{-1}) and Cr (107 μg.g^{-1}) in the root tissues. Again, a decrease in metal concentrations were recorded in the white stems but with peak values (lower than those in the root tissues, but higher than in the white stems) emerging for Fe (300 μg.g^{-1}), Cu (298 μg.g^{-1}), Ni (366 μg.g^{-1}), Pb (8 μg.g^{-1}), Zn (931 μg.g^{-1}) and Mn (1967 μg.g^{-1}) in the leaves of this plant (Table 4.11). The concentrations of all metals in the flowers (except Cr and Mn) showed a decline, relative to those of the leaves. It is however interesting to note that following the virtual absence of Cr in the white and green stems, significant concentration of this metal occurred in the leaves (2 μg.g^{-1}) and flowers (4 μg.g^{-1}), respectively (Table 4.11).

Trends similar to those obtained for the actual metal concentrations were exemplified by the CR-values of the root tissues. The highest CR-values ranged between 0,029 (Fe) and 19,180 (Ni). A downward trend in the CR-values for metals in the white stems and green stems were followed by peak CR-values for the leaves, ranging between 0,011 (Cr) and 6,090 (Cu), respectively (Table 4.11).

During sampling, no flowers were present on *Polygonum lapathifolium*. Subsequently, only roots, white stems, green stems and leaves were collected for analyses. As was the case with all the other plants, highest metal concentrations were again recorded in the root tissues (Cr: 12 μg.g^{-1} ; Pb: 24 μg.g^{-1} ; Zn: 540 μg.g^{-1} ; Ni: 611 μg.g^{-1} ; Cu: 1421 μg.g^{-1} ; Mn: 11463 μg.g^{-1} ; Fe: 21133 μg.g^{-1}) (Table 4.12). Relative to the other plant organs (roots excluded), the leaves contained highest concentrations of Fe (11194 μg.g^{-1}), Cu (887 μg.g^{-1}) and Mn (11102 μg.g^{-1}). In contrast, white stem tissue contained the highest concentrations of Ni (255 μg.g^{-1}), Pb (14 μg.g^{-1}), Zn (278 μg.g^{-1}) and Cr (8 μg.g^{-1}) in this case (Table 4.12).

P. lapathifolium, as in all the other plants, produced highest CR_{RT} values, ranging between 0,061 (Cr) and 29,010 (Cu). A lowest CR_{WS} for Cr (0,039) and a highest CR_{WS} for Cu (6,986) were followed by a lower CR_{GS} for Cr (0,017) but a higher CR_{GS} for Cu (11,361). A lowest CR_{LF} for Cr (0,014) was also the lowest of all the CR-values recorded, while a CR_{LF} for Cu (18,096) was the second highest of all CR-values (Table 4.12).

Table 4.7 **Concentration levels and Concentration Ratios of selected metals in organs of *Arundo donax* collected in the vicinity of Crocodile Farm during the summer of 1992**

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	30667	155	302	68	256	918	122
Root (RT)	5428	284	96	27	132	1065	20
White stem (WS)	583	35	11	13	65	361	1
Green stem (GS)	337	16	5	6	36	126	0,9
Leaves (LF)	245	27	6	3	23	111	0,4
Flowers (FL)	-	-	-	-	-	-	-
CR_{RT}	0,177	1,832	0,318	0,397	0,516	1,160	0,164
CR_{WS}	0,019	0,226	0,036	0,191	0,254	0,393	0,008
CR_{GS}	0,011	0,103	0,016	0,088	0,141	0,137	0,007
CR_{LF}	0,008	0,174	0,020	0,044	0,090	0,121	0,003
CR_{FL}	-	-	-	-	-	-	-

Table 4.8 Concentration levels and Concentration Ratios of selected metals in organs of *Polygonum lapathifolium* collected in the vicinity of Crocodile Farm during the summer of 1992

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	30667	155	302	68	256	918	122
Root (RT)	24043	4445	297	81	362	5214	253
White stem (WS)	4876	943	194	46	190	4767	7
Green stem (GS)	9323	1521	98	23	136	3606	6
Leaves (LF)	8280	2938	137	27	182	5335	1,8
Flowers (FL)	8096	2597	100	26	154	4230	1,7
CR_{RT}	0,784	28,68	0,983	1,191	1,414	5,680	2,074
CR_{WS}	0,159	6,084	0,642	0,676	0,742	5,193	0,057
CR_{GS}	0,304	9,813	0,325	0,338	0,531	3,928	0,048
CR_{LF}	0,270	18,955	0,454	0,397	0,711	5,812	0,015
CR_{FL}	0,264	16,755	0,331	0,382	0,602	4,608	0,014

Table 4.9 **Concentration levels and Concentration Ratios of selected metals in organs of *Typha capensis* collected in the Blesbokspruit in the vicinity of Marievale Farm during the summer of 1992**

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	27303	49	369	20	403	2261	195
Root (RT)	6607	822	418	16	694	48840	11
White stem (WS)	655	235	84	6	372	10695	1
Green stem (GS)	218	57	75	7	276	11395	1
Leaves (LF)	300	54	69	3	129	13837	1
Flowers (FL)	-	-	-	-	-	-	-
CR_{RT}	0,242	16,770	1,134	0,779	1,722	21,601	0,055
CR_{WS}	0,024	4,790	0,227	0,280	0,923	4,730	0,006
CR_{GS}	0,008	1,160	0,203	0,330	0,686	5,040	0,003
CR_{LF}	0,011	1,110	0,187	0,162	0,320	6,120	0,004
CR_{FL}	-	-	-	-	-	-	-

Table 4.10 **Concentration levels and Concentration Ratios of selected metals in organs of *Phragmites australis* collected in the Blesbokspruit in the vicinity of Marievale Farm during the summer of 1992**

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	27303	49	369	20	403	2261	195
Root (RT)	7836	969	375	35	637	15533	13
White stem (WS)	1201	236	82	10	310	2853	2
Green stem (GS)	846	194	68	10	255	2634	1
Leaves (LF)	1584	370	65	5	117	6455	1
Flowers (FL)	-	-	-	-	-	-	0
CR_{RT}	0,287	19,780	1,016	1,770	1,581	6,870	0,067
CR_{WS}	0,044	4,811	0,221	0,497	0,770	1,262	0,008
CR_{GS}	0,031	3,960	0,185	0,480	0,633	1,165	0,003
CR_{LF}	0,058	7,559	0,176	0,270	0,290	2,855	0,004
CR_{FL}	-	-	-	-	-	-	-

Table 4.11 Concentration levels and Concentration Ratios of selected metals in organs of *Alisma plantago-aquatica* collected in the Blesbokspruit in the vicinity of Marievale Farm during the summer of 1992

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	27303	49	369	20	403	2261	195
Root (RT)	792	648	7077	83	3490	18698	107
White stem (WS)	355	435	245	10	1197	506	0
Green stem (GS)	137	105	59	2	459	717	0
Leaves (LF)	300	298	366	8	931	1967	2
Flowers (FL)	218	288	310	6	794	4009	4
CR_{RT}	0,029	13,220	19,180	4,140	8,660	9,270	0,551
CR_{WS}	0,013	8,870	0,663	0,505	2,970	0,224	0
CR_{GS}	0,005	2,144	0,161	0,117	1,140	0,317	0
CR_{LF}	0,011	6,090	0,993	0,394	2,310	0,870	0,011
CR_{FL}	0,008	5,870	0,840	0,295	1,970	1,773	0,019

Table 4.12 **Concentration levels and Concentration Ratios of selected metals in organs of *Polygonum lapathifolium* collected in the Blesbokspruit in the vicinity of Marievale Farm during the summer of 1992**

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	27303	49	369	20	403	2261	195
Root (RT)	21133	1421	611	24	540	11463	12
White stem (WS)	4532	342	255	14	278	11034	8
Green stem (GS)	8546	557	160	7	206	9082	3
Leaves (LF)	11194	887	182	8	267	11102	3
Flowers (FL)	-	-	-	-	-	-	-
CR_{RT}	0,774	29,010	1,656	1,194	1,340	5,070	0,061
CR_{WS}	0,166	6,986	0,691	0,692	0,690	4,880	0,039
CR_{GS}	0,313	11,361	0,433	0,340	0,512	4,017	0,017
CR_{LF}	0,410	18,096	0,492	0,407	0,663	4,910	0,014
CR_{FL}	-	-	-	-	-	-	-

4.2.2.3 Vlakfontein Farm

Metal concentrations in aquatic sediments

Vlakfontein farm, which can be considered as being impacted by mining activities at the time of the survey, contained the highest amounts of Fe (49788 $\mu g.g^{-1}$) of all the metals in the sediments analyzed. A relatively low Mn concentration (240 $\mu g.g^{-1}$) was recorded in the bottom sediments which is actually lower than that of the control locality. Other metal concentrations included Pb (130 $\mu g.g^{-1}$), Cr (227 $\mu g.g^{-1}$), Zn (248 $\mu g.g^{-1}$), Cu (344 $\mu g.g^{-1}$) and Ni (361 $\mu g.g^{-1}$) (Table 4.13).

Metal concentrations in and Concentrations Ratios of metals in aquatic macrophytes

At the Vlakfontein farm, where water is pumped from a nearby stream, *Arundo donax* was the dominating aquatic macrophyte species present. As was the case with this plant species collected at the Crocodile farm, the root tissue contained the highest concentrations for all the metals analyzed (Fe: 22056 $\mu g.g^{-1}$; Cu: 8077 $\mu g.g^{-1}$; Ni: 509 $\mu g.g^{-1}$; Pb: 266 $\mu g.g^{-1}$; Zn: 477 $\mu g.g^{-1}$; Mn: 2122 $\mu g.g^{-1}$ and Cr: 21 $\mu g.g^{-1}$). However, significantly lower metal concentrations were recorded in the white stems, ranging between 6 $\mu g.g^{-1}$ (Cr) and 7418 $\mu g.g^{-1}$ (Fe) respectively. A further decrease was recorded in the green stems for Fe (3883 $\mu g.g^{-1}$), Cu (1386 $\mu g.g^{-1}$), Ni (103 $\mu g.g^{-1}$), Pb (73 $\mu g.g^{-1}$), Zn (191 $\mu g.g^{-1}$), Mn (358 $\mu g.g^{-1}$) and Cr (4 $\mu g.g^{-1}$). In some cases (Ni: 82 $\mu g.g^{-1}$; Pb: 50 $\mu g.g^{-1}$; Zn: 87 $\mu g.g^{-1}$), a decline in some values of these metals was recorded in the leaves relative to the corresponding concentrations in the green stems, while the other remaining metals (Fe: 4580 $\mu g.g^{-1}$; Cu: 2897 $\mu g.g^{-1}$; Mn: 727 $\mu g.g^{-1}$; Cr: 5 $\mu g.g^{-1}$) showed an increase in concentrations relative to those in the green stems (Table 4.13).

Concentration Ratios for the different organs of this plant showed trends similar to those obtained for the actual metal concentrations. A highest CR-value of 0,443 for Fe was recorded for the root tissue, while Cu (23,481), Ni (1,410), Pb (2,043), Zn (1,922), Mn (8,840) and Cr (0,095) all produced highest CR-values for the same organ. This was followed by a decrease in all the CR-values encountered between the white stems and green stems, respectively, with the leaves producing higher CR-values relative to those of the CR_{GS} (Fe: 0,092 ; Cu: 8,422 ; Mn: 3,030 ; Cr: 0,020) (Table 4.13).

4.2.2.4 Luipaardsvlei Farm

Metal concentrations in aquatic sediments

In the case of the Luipaardsvlei sampling site, the sediment metal concentrations were again characterized by a relatively high Fe concentration of 33405 $\mu g.g^{-1}$. The second-highest was recorded for Mn with a concentration of 1044 $\mu g.g^{-1}$. This was followed by Cu (289 $\mu g.g^{-1}$), Zn (254 $\mu g.g^{-1}$), Ni (209 $\mu g.g^{-1}$), Cr (97 $\mu g.g^{-1}$) and Pb (96 $\mu g.g^{-1}$) in descending order of concentration (Tables 4.14 & 4.15).

Metal concentrations in and Concentrations Ratios of metals of aquatic macrophytes

At the stream flowing through the Luipaardsvlei farming area, *Typha capensis* was the dominant aquatic macrophyte. A few specimens of *A. plantago-aquatica* were also collected for sampling. Roots, being the primary organ for mineral uptake form the sediments, contained the highest amounts of Fe (9253 $\mu g.g^{-1}$), Cu (6774 $\mu g.g^{-1}$), Ni (217 $\mu g.g^{-1}$), Pb (96 $\mu g.g^{-1}$), Zn (359 $\mu g.g^{-1}$), Mn (18886 $\mu g.g^{-1}$) and Cr (3 $\mu g.g^{-1}$) for *T. capensis* (Table 4.14). Only in the case of Fe and Zn, a constant downward trend was observed when the relative concentrations of these metals were compared in the different plant organs (Table 4.14). In the case of the leaves, significantly higher concentrations were recorded for Cu (609 μg.g-1), Ni (38 $\mu g.g^{-1}$), Mn (5196 $\mu g.g^{-1}$) and Cr (0,4 $\mu g.g^{-1}$) relative to the same concentrations of these metals in the green stem of this plant (Table 4.14).

Similar trends were obtained for the metal concentrations in the different organs of *T. capensis* collected at this locality. Highest CR-values for all metals were again recorded in the white stems for Fe (CR_{WS}: 0,051 ; CR_{GS}: 0,016), Cu (CR_{WS}: 6,691 ; CR_{GS}: 2,040), Ni (CR_{WS}: 0,193 ; $CR_{GS:}$ 0,150), Zn (CR_{WS}: 0,771 ; CR_{GS}: 0,470) and Cr (CR_{WS}: 0,004 ; CR_{GS}: 0,002) and the green stems for Pb (CR_{GS}: 0,502 ; CR_{WS}: 0,336) and Mn (CR_{GS}: 4,282 ; CR_{WS}: 3,166), respectively (Table 4.14). Only in the case of Cu (2,107), Ni (0,182), Mn (4,977) and Cr (0,004), higher CR_{LF}-values were obtained relative to the corresponding CR_{GS}-values (Table 4.14).

Being the primary uptake and storage organ, the roots of *A. plantago-aquatica* collected at the Luipaardsvlei locality contained the highest metal amounts for all this plants organs ranging between 19 $\mu g.g^{-1}$ for Cr and 5573 $\mu g.g^{-1}$ for Cu (Table 4.15). This was followed by trace amounts of Cr (< 1 $\mu g.g^{-1}$) in the white and green stems of this plant, respectively. A similar decrease in all other metal concentrations occurred when the concentrations for the same organs are compared, namely roots > white stems > green stems.

In the case of Fe (735 $\mu g.g^{-1}$), Cu (2809 $\mu g.g^{-1}$), Ni (176 $\mu g.g^{-1}$), Pb (85 $\mu g.g^{-1}$) and Zn (307 $\mu g.g^{-1}$) peak concentrations in the leaves, relative to the same metals' concentrations in the green stems, were recorded (Table 4.15). Following these peak values, significantly lower values were recorded for the same metals in the flowers of this plant. Only in the case of Mn (922 $\mu g.g^{-1}$) and Cr (1,1 $\mu g.g^{-1}$), did peak values occur in the flowers of this plant (Table 4.15).

Trends similar to those obtained for the metals were obtained for the Concentration Ratios of each of the plant organs. Again, highest CR-values for the roots (min-max values) (0,056 (Fe) - 19,283 (Cu)) were followed by those for the white stems (0 (Cr) - 11,020 (Cu)) and green stems (0 (Cr) - Cu (4,551)) (Table 4.15). Peak CR_{LF}-values (relative to CR_{GS}) were also recorded for Cu (9,720), Ni (0,840), Pb (0,882) and Zn (1,209) while CR_{FL} produced peak CR values (relative to CR_{LF}) for Mn (0,883) and Cr (0,011) (Table 4.15).

4.3 Concentrations and Concentration Ratios (CR) of selected metals in crops collected from the control locality and from the localities affected by mine drainage effluents

4.3.1 Crocodile Farm

Comparing the metal concentrations obtained from soil samples of this locality, Fe was consistently the highest in all the field plots sampled ranging between 3072 - 4563 μg.g^{-1}, with a mean concentration of 3831 μg.g^{-1} (Table 4.16). This was followed by Mn (Range: 563 - 1539 μg.g^{-1} ; X: 880 μg.g^{-1}), Zn (Range: 80 - 341 μg.g^{-1} ; X: 208 μg.g^{-1}), Ni (Range: 86 - 156 μg.g^{-1} ; X: 107 μg.g^{-1}), Cr (Range: 64 - 158 μg.g^{-1} ; X: 96 μg.g^{-1}), Pb (Range: 60 - 111 μg.g^{-1} ; X: 86 μg.g^{-1}) and Cu (Range: 50 - 102 μg.g^{-1} ; X: 79 μg.g^{-1}).

The metal concentrations in the different vegetable crops showed a relatively consistent pattern with the highest concentrations mostly occurring in the leafy vegetables. Highest concentrations (X ± S.D.) for Fe (296 ± 17 μg.g^{-1}), Ni (36 ± 4 μg.g^{-1}) and Pb (36 ± 4 μg.g^{-1}) were obtained in the tissues of cauliflower, while lettuce contained the highest concentrations of Zn (152 ± 16 μg.g^{-1}), Mn (84 ± 5 μg.g^{-1}) and Cr (19 ± 2 μg.g^{-1}). Lettuce shared the highest Ni concentration of 36 ± 7 μg.g^{-1} with cauliflower. The only exception was in the case of beetroot which contained the highest overall concentration of Cu (33 ± 6 μg.g^{-1}) (Table 4.16).

Carrots and cabbage generally contained the lowest metal concentrations of the vegetables analyzed. Iron (167 ± 14 μg.g^{-1}), Zn (42 ± 4 μg.g^{-1}) and Mn (19 ± 3 μg.g^{-1}) occurred in the lowest concentrations of all seven metals analyzed in this vegetable. This tendency was also shared by Ni (21 ± 2 μg.g^{-1}), Pb (21 ± 3 μg.g^{-1}) and Cr (15 ± 2 μg.g^{-1}) in the case of cabbage. Both vegetables had, of all the metals analyzed, the lowest copper concentration namely 12 μg.g^{-1} (Table 4.16).

Following a similar trend as for metal concentrations, the Concentration Ratios were the highest in the leafy vegetables and the lowest in the root vegetables. Lettuce produced the highest CR-values for Fe (0,083), Cu (0,486), Ni (0,421), Zn (0,824), Mn (0,116) and Cr (0,297). In the case of Pb, the highest CR-value of 0,450 was obtained for cauliflower (Table 4.16). In most cases, carrots was the vegetable which produced the lowest CR-values for Fe (0,040), Cu (0,120), Pb (0,246), Zn (0,122) and Mn (0,020). Nickel (0,154) and Cr (0,123) however, produced the lowest CR-values in beetroot (Table 4.16).

4.3.2 Marievale Farm

Iron concentrations were consistently the highest in the soil, ranging between 4640 - 4950 μg.g^{-1}, with an average of 4805 μg.g^{-1}. This level is approximately 970 μg.g^{-1} higher than that of the control location. Iron was followed by Mn (Range: 156 - 332 μg.g^{-1} ; X: 267 μg.g^{-1}), Cr (Range: 66 - 76 μg.g^{-1} ; X: 72 μg.g^{-1}), Zn (Range: 28 - 92 μg.g^{-1} ; X: 55 μg.g^{-1}), Ni (Range: 42 - 51 μg.g^{-1} ; X: 48 μg.g^{-1}), Pb (Range: 27-35 μg.g^{-1} ; X: 31 μg.g^{-1}) and Cu (Range: 20 - 26 μg.g^{-1} ; X: 24 μg.g^{-1}) (Table 4.17). It is interesting to note that except for

Table 4.13 **Concentration levels and Concentration Ratios of selected metals in organs of *Arundo donax* collected in the vicinity of Vlakfontein Farm during the summer of 1993**

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	49788	344	361	130	248	240	227
Root (RT)	22056	8077	509	266	477	2122	21
White stem (WS)	7418	2635	122	80	217	426	6
Green stem (GS)	3883	1386	103	73	191	358	4
Leaves (LF)	4580	2897	82	50	87	727	5
Flowers (FL)	-	-	-	-	-	-	-
CR_{RT}	0,443	23,481	1,410	2,043	1,922	8,840	0,095
CR_{WS}	0,149	7,660	0,337	0,615	0,874	1,773	0,026
CR_{GS}	0,078	4,030	0,284	0,559	0,770	1,490	0,017
CR_{LF}	0,092	8,422	0,226	0,382	0,351	3,030	0,020
CR_{FL}	-	-	-	-	-	-	-

Table 4.14 **Concentration levels and Concentration Ratios of selected metals in organs of *Typha capensis* collected in the vicinity of Luipaardsvlei during the summer of 1993**

Plant organs and CR	**Metal concentrations in plant organs (μg.g^{-1})**						
	Fe	**Cu**	**Ni**	**Pb**	**Zn**	**Mn**	**Cr**
Sediment	33405	289	209	96	254	1044	97
Root (RT)	9253	6774	217	96	359	18886	3
White stem (WS)	1704	1934	40	32	196	3305	0,4
Green stem (GS)	534	590	31	48	119	4470	0,2
Leaves (LF)	301	609	38	20	72	5196	0,4
Flowers (FL)	-	-	-	-	-	-	-
CR_{RT}	0,277	23,440	1,038	1,004	1,412	18,090	0,034
CR_{WS}	0,051	6,691	0,193	0,336	0,771	3,166	0,004
CR_{GS}	0,016	2,040	0,150	0,502	0,470	4,282	0,002
CR_{LF}	0,009	2,107	0,182	0,207	0,282	4,977	0,004
CR_{FL}	-	-	-	-	-	-	-

Table 4.15 **Concentration levels and Concentration Ratios of selected metals in organs of *Alisma plantago-aquatica* collected in the vicinity of Luipaardsvlei during the summer of 1993**

Plant organs and CR	Metal concentrations in plant organs ($\mu g.g^{-1}$)						
	Fe	Cu	Ni	Pb	Zn	Mn	Cr
Sediment	33405	289	209	96	254	1044	97
Root (RT)	1871	5573	2389	924	1009	4184	19
White stem (WS)	568	3185	153	100	357	120	0
Green stem (GS)	367	1315	24	39	169	101	0
Leaves (LF)	735	2809	176	85	307	535	0,6
Flowers (FL)	301	2352	149	76	196	922	1,1
CR_{RT}	0,056	19,283	11,433	9,623	3,972	4,008	0,192
CR_{WS}	0,017	11,020	0,731	1,037	1,407	0,115	0
CR_{GS}	0,011	4,551	0,117	0,411	0,667	0,097	0
CR_{LF}	0,022	9,720	0,840	0,882	1,209	0,512	0,006
CR_{FL}	0,009	8,140	0,712	0,795	0,773	0,883	0,011

Fe, all other metal concentrations in the soil samples were lower than those obtained at the control sample locality. This tendency is considered in more detail in the discussion (Chapter 5).

The various patterns observed for metal concentrations in the soils from both farms followed a similar trend for the metal concentrations measured in the crops from these two localities. All concentrations of metals in crops from the Marievale farm were, however, lower than those obtained from the control locality (Tables 4.16 & 4.17). (It was observed that the specific farmer at the Marievale farm applied considerable quantities of organic compost to the agricultural land. The soils of the control locality was also of a more sandy nature than the rich loamy soils of the Marievale farm). The highest and lowest metal concentrations obtained for crops at the Marievale farm revealed a somewhat contradictory pattern when compared with values found at the Crocodile farm. Lettuce at Marievale showed the highest concentrations for Fe (314 ± 73 $\mu g.g^{-1}$) and Zn (90 ± 13 $\mu g.g^{-1}$), while beetroot, cauliflower, cabbage and carrots showed the highest concentrations for Cu (10 ± 4 $\mu g.g^{-1}$), Ni (18 ± 4 $\mu g.g^{-1}$), Pb (24 ± 3 $\mu g.g^{-1}$) and Mn (18 ± 4 $\mu g.g^{-1}$), respectively. Carrots, cabbage and cauliflower shared a highest concentration of 13 $\mu g.g^{-1}$ for Cr (Table 4.17).

The lowest metal concentrations in the vegetables from Marievale showed a more consistent pattern, with cabbage producing the lowest concentrations for Fe (154 ± 25 $\mu g.g^{-1}$), Cu (3 ± 0,4 $\mu g.g_{-1}$), Zn (39 ± 10 $\mu g.g^{-1}$) and Mn (16 ± 4 $\mu g.g^{-1}$). Cabbage and beetroot shared a lowest concentration of 12 ± 2 $\mu g.g^{-1}$ for Ni while lettuce and beetroot shared a lowest concentration of 11 ± 2 $\mu g.g^{-1}$ for Cr. A lowest Pb concentration of 17 ± 2 $\mu g.g^{-1}$ was measured in beetroot (Table 4.17).

Carrots consistently produced the highest CR-values for Ni (0,371), Pb (0,821), Zn (1,525) and Mn (0,401), while lettuce, beetroot and cauliflower produced the highest CR-values for Fe (0,066), Cu (0,422) and Cr (0,180), respectively. In the case of most metals (Fe: 0,031 ; Cu: 0,135 ; Ni: 0,235 ; Mn: 0,048), cabbage produced the lowest CR-values. Beetroot had the lowest CR-values for Pb (0,503) and Zn (0,590), while a lowest value of 0,155 was also obtained for Cr (Table 4.17).

4.3.3 Vlakfontein Farm

In most cases, the metal concentrations measured in soil samples from this farm were generally the highest for all the localities investigated. The only exceptions were Pb (Range: 76 - 123 $\mu g.g^{-1}$; X: 83 $\mu g.g^{-1}$) and Zn (Range: 76 - 123 $\mu g.g^{-1}$; X: 93 $\mu g.g^{-1}$), which were lower than those found at the Crocodile farm but which were clearly higher than those recorded from the Marievale farm. In this case, a highest Fe concentration (Range: 5907 - 6796 $\mu g.g^{-1}$; X: 6331 $\mu g.g^{-1}$) was followed by Mn (Range: 440 - 1783 $\mu g.g^{-1}$; X: 974 $\mu g.g^{-1}$), Ni (Range: 132 - 243 $\mu g.g^{-1}$; X: 183 $\mu g.g^{-1}$), Cr (Range: 94 - 291 $\mu g.g^{-1}$; X: 178 $\mu g.g^{-1}$), Zn (Range: 76 - 123 $\mu g.g^{-1}$; X: 93 $\mu g.g^{-1}$), Pb (Range: 72 - 96 $\mu g.g^{-1}$; X: 83 $\mu g.g^{-1}$) and Cu (Range: 46 - 121 $\mu g.g^{-1}$; X: 81 $\mu g.g^{-1}$) (Table 4.18).

Considering the highest and lowest concentrations as well as Concentration Ratios of the various metals in the vegetable crops, interesting and rather consistent patterns were observed. Spinach produced six of the seven highest metal concentrations and consequently also dominated as the crop with the highest corresponding CR-values. Another interesting pattern emerged when comparing the lowest metal concentrations and CR-values for the vegetable crops at the Vlakfontein farm: all crops which contained the lowest metal concentrations, also produced the lowest CR-values.

Except in the case of beetroot in which the highest Cu concentration (38 ± 2 $\mu g.g^{-1}$) was measured, spinach produced the highest concentration for all the other metals analyzed. Lowest concentrations for Fe (236 ± 15 $\mu g.g^{-1}$) and Cu (8 ± 1 $\mu g.g^{-1}$) were measured in cabbage, while leeks produced the lowest concentrations for Ni (26 ± 2 $\mu g.g^{-1}$), Zn and Mn (29 ± 8 $\mu g.g^{-1}$). Lowest concentrations for Pb (16 ± 2 $\mu g.g^{-1}$) and Cr (6 ± 1 $\mu g.g^{-1}$) were measured in beetroot and turnips, respectively (Table 4.18).

As already mentioned, lowest CR-values corresponded with lowest metal concentrations. Spinach produced the highest CR-values throughout, ranging between 0,573 (Ni) and 6,013 (Zn), respectively. Cabbage produced the lowest CR-values for Fe (0,039) and Cu (0,093), while Ni (0,105), Zn (0,542) and Mn (0,019) were the lowest CR-values for leeks. Beetroot produced a lowest CR-value of 0,203 (Pb), while a low CR-value of 0,021 (Cr) was characteristic for turnips (Table 4.18).

4.3.4 Luipaardsvlei Farm

As discussed earlier, the Luipaardsvlei group of farms (LP1, LP2 & LP3) mainly concentrated on the production of fodder for use by dairy cattle. Milk produced is sold to a feta-cheese factory. Other small agricultural practices include some sheep and chicken farming. However, these animals are fed using commercial food and tap water. As a result, this aspect was therefore not included in the present study as it was not regarded as a significant pathway for the possible transfer of metals to humans. However, results obtained on the metal content of fodder, milk and dung at LP3 provided a useful picture of metal accumulation in fodder, milk and dung and are therefore presented here. Results obtained for metal concentrations in the soil, fodder, milk and dung collected during this survey showed that, as was the case for the other localities, that the iron concentration (4106 $\mu g.g^{-1}$) was the highest in the soil. This was followed by Mn (533 $\mu g.g^{-1}$), Ni (93 $\mu g.g^{-1}$), Cr (71 $\mu g.g^{-1}$), Zn (69 $\mu g.g^{-1}$), Pb (52 $\mu g.g^{-1}$) and Cu (41 $\mu g.g^{-1}$) (Table 4.19). Metal concentrations in the fodder ranged between 15 $\mu g.g^{-1}$ (Cu & Cr) and 234 $\mu g.g^{-1}$ (Fe), 0,8 $\mu g.g^{-1}$ (Cu) and 19 $\mu g.g^{-1}$ (Mn) in the milk and 5 $\mu g.g^{-1}$ (Cu) and 81 $\mu g.g^{-1}$ (Fe) in the cattle dung (Table 4.19).

Concentration Ratios for these different materials analyzed also revealed some interesting tendencies. Results show lower $CR_{fodder/soil}$ values than $CR_{dung/fodder}$ ratios for potentially toxic or immobile metals such as Ni ($CR_{fodder/soil}$: 0,280 ; $CR_{dung/fodder}$: 0,538), Pb ($CR_{fodder/soil}$: 0,346 ; $CR_{dung/fodder}$: 0,556) and Cr ($CR_{fodder/soil}$: 0,211 ; $CR_{dung/fodder}$: 0,467) (Table 4.19). In all cases, $CR_{milk/fodder}$ were the lowest, ranging between 0,053 (Cu) and 0,115 (Ni).

Table 4.16 Concentration levels and Concentration Ratios of selected metals in crops collected from Crocodile Farm during the summer of 1992

Samples	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Soil	3161	50	86	92	185	730	64
Lettuce: $\bar{X}$ ± S.D.	263 ± 16	24 ± 2	36 ± 7	28 ± 2	152 ± 16	84 ± 5	19 ± 2
: Range	242 - 286	20 - 27	28 - 47	25 - 32	128 - 179	76 - 91	16 - 21
: C.V.	6,1	7,4	18,7	7,0	10,4	6,0	8,4
: CR	0,083	0,486	0,421	0,303	0,824	0,116	0,297
Soil	3072	56	100	60	80	563	80
Cabbage: $\bar{X}$ ± S.D.	188 ± 7	12 ± 3	21 ± 2	21 ± 3	65 ± 11	38 ± 4	15 ± 2
: Range	175 - 199	8 - 15	19 - 24	17 - 26	51 - 89	29 - 39	12 - 18
: C.V.	4,0	20,2	7,5	15,4	16,4	11,4	12,9
: CR	0,061	0,221	0,210	0,357	0,817	0,060	0,193
Soil	4192	85	97	79	126	647	93
Cauliflower: $\bar{X}$ ± S.D.	296 ± 17	15 ± 1	36 ± 4	36 ± 4	62 ± 3	42 ± 5	17 ± 1
: Range	259 - 320	12 - 16	28 - 42	27 - 42	56 - 67	36 - 54	15 - 19
: C.V.	5,7	8,6	11,2	10,1	5,1	11,9	6,8
: CR	0,071	0,173	0,374	0,450	0,490	0,065	0,183
Soil	4563	102	156	88	309	1539	158
Beetroot: $\bar{X}$ ± S.D.	189 ± 8	33 ± 6	24 ± 2	22 ± 3	137 ± 15	61 ± 6	19 ± 2
: Range	177 - 200	24 - 44	21 - 27	17 - 27	119 - 173	51 - 69	18 - 22
: C.V.	4,2	18,0	7,8	11,6	10,8	9,2	7,5
: CR	0,041	0,328	0,154	0,247	0,443	0,040	0,123
Soil	4168	102	98	111	341	923	84
Carrots: $\bar{X}$ ± S.D.	167 ± 14	12 ± 2	24 ± 3	27 ± 5	42 ± 4	19 ± 3	16 ± 3
: Range	140 - 185	9 - 15	21 - 31	20 - 39	36 - 48	15 - 24	12 - 22
: C.V.	8,5	14,8	11,5	17,5	8,8	15,3	17,4
: CR	0,040	0,120	0,246	0,246	0,122	0,020	0,192

Table 4.17 Concentration levels and concentration ratios of selected metals in crops collected from Marievale Farm during the summer of 1992

Samples	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Soil	4751	26	51	29	67	267	73
Lettuce: $\overline{X}$ ± S.D.	314 ± 73	9 ± 0,7	14 ± 1	23 ± 3	90 ± 13	24 ± 8	11 ± 2
: Range	215 - 494	8 - 10	12 - 17	20 - 30	75 - 120	20 - 48	9 - 16
: C.V.	23,1	7,9	9,8	14,7	14,9	33,8	18,2
: CR	0,066	0,350	0,279	0,794	1,346	0,090	0,155
Soil	4950	24	50	33	44	332	72
Cabbage: $\overline{X}$ ± S.D.	154 ± 25	3 ± 0,4	12 ± 2	24 ± 2	39 ± 10	16 ± 4	13 ± 2
: Range	135 - 208	3 - 4	8 - 15	18 - 27	30 - 65	11 - 25	11 - 17
: C.V.	15,9	12,1	16,3	10,2	25,7	24,0	14,3
: CR	0,031	0,135	0,235	0,714	0,890	0,048	0,179
Soil	4950	24	50	33	44	332	72
Cauliflower: $\overline{X}$ ± S.D.	270 ± 56	4 ± 0,5	18 ± 4	19 ± 3	63 ± 7	25 ± 6	13 ± 4
: Range	205 - 374	3 - 5	13 - 29	16 - 23	52 - 73	18 - 38	10 - 23
: C.V.	21,4	13,8	23,2	13,0	11,3	25,3	27,7
: CR	0,055	0,160	0,367	0,575	1,441	0,076	0,180
Soil	4640	24	48	35	92	246	66
Beetroot: $\overline{X}$ ± S.D.	185 ± 31	10 ± 4	12 ± 2	17 ± 2	54 ± 12	17 ± 6	11 ± 2
: Range	137 - 247	6 - 20	10 - 14	15 - 21	32 - 75	11 - 27	7 - 13
: C.V.	16,9	37,8	12,4	10,4	21,2	33,7	16,9
: CR	0,040	0,422	0,248	0,503	0,590	0,067	0,170
Soil	4735	20	42	27	28	156	76
Carrots: $\overline{X}$ ± S.D.	162 ± 19	7 ± 2	16 ± 2	23 ± 2	43 ± 8	63 ± 35	13 ± 1
: Range	139 - 199	5 - 12	12 - 17	19 - 25	33 - 62	32 - 158	11 - 15
: C.V.	11,7	29,9	10,5	7,4	18,0	55,4	9,2
: CR	0,034	0,328	0,371	0,821	1,525	0,401	0,169

Table 4.18 Concentration levels and concentration ratios of selected metals in crops collected from Vlakfontein Farm during the summer of 1993

Samples	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Soil	5907	46	158	72	82	561	94
Lettuce: $\bar{X}$ ± S.D.	402 ± 12	18 ± 2	38 ± 3	35 ± 5	121 ± 14	242 ± 49	20 ± 1
: Range	384 - 420	16 - 21	31 - 42	27 - 44	100 - 150	153 - 305	18 - 21
: C.V.	3,0	9,1	7,8	14,5	11,4	20,2	3,7
: CR	0,068	0,385	0,241	0,482	1,475	0,432	0,211
Soil	6023	87	218	91	76	1783	211
Cabbage: $\bar{X}$ ± S.D.	236 ± 15	8 ± 1	63 ± 22	25 ± 3	95 ± 13	63 ± 14	16 ± 2
: Range	213 - 254	7 - 10	35 - 117	19 - 30	69 - 116	43 - 79	12 - 20
: C.V.	6,5	10,7	35,7	13,4	13,9	21,4	14,9
: CR	0,039	0,093	0,287	0,277	1,261	0,036	0,075
Soil	5907	46	158	72	82	561	94
Spinach: $\bar{X}$ ± S.D.	3539 ± 154	33 ± 4	90 ± 4	44 ± 4	492 ± 16	575 - 19	26 ± 2
: Range	3361 - 3791	26 - 38	83 - 95	36 - 50	449 - 511	532 - 603	24 - 30
: C.V.	4,3	11,3	4,5	9,7	3,3	3,3	6,3
: CR	0,599	0,710	0,573	0,606	6,013	1,026	0,279
Soil	6796	121	243	96	123	1516	291
Leeks: $\bar{X}$ ± S.D.	402 ± 41	18 ± 2	26 ± 2	24 ± 3	67 ± 11	29 ± 8	16 ± 3
: Range	367 - 521	15 - 23	22 - 31	19 - 28	54 - 89	16 - 46	10 - 22
: C.V.	10,2	12,4	9,4	11,1	16,5	26,1	18,6
: CR	0,059	0,147	0,105	0,252	0,542	0,019	0,054
Soil	6796	121	243	96	123	1516	291
Turnips: $\bar{X}$ ± S.D.	284 ± 16	20 ± 2	60 ± 13	29 ± 4	143 ± 39	75 ± 20	6 ± 1
: Range	255 - 305	17 - 24	41 - 87	20 - 33	78 - 195	39 - 116	5 - 7
: C.V.	5,7	11,0	21,0	13,0	27,0	26,8	12,2
: CR	0,042	0,164	0,246	0,297	1,163	0,050	0,021

Table 4.18 (Continued) **Concentration levels and concentration ratios of selected metals in crops collected from Vlakfontein Farm during the summer of 1993**

Samples	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Soil	6443	74	132	77	83	440	134
Beetroot: X ± S.D.	350 ± 8	38 ± 2	42 ± 2	16 ± 2	149 ± 6	359 ± 21	13 ± 2
: Range	341 - 366	35 - 43	39 - 46	12 - 20	138 - 159	340 - 395	10 - 17
: C.V.	2,3	6,1	5,5	14,9	4,3	5,9	17,1
: CR	0,054	0,522	0,322	0,203	1,793	0,817	0,100
Soil	6443	74	132	77	83	440	134
Carrots: X ± S.D.	295 ± 8	28 ± 3	55 ± 10	20 ± 2	126 ± 9	190 ± 59	14 ± 1
: Range	282 - 310	24 - 37	40 - 77	16 - 22	110 - 141	98 - 293	12 - 15
: C.V.	2,8	12,2	17,9	8,2	6,9	31,0	8,7
: CR	0,046	0,381	0,421	0,252	1,509	0,432	0,104

Table 4.19 Concentration levels and Concentration Ratios (CR) of selected metals in fodder, milk and cattle dung collected at Luipaardsvlei during the summer of 1993

Samples	Fe (μg.g^{-1})	Cu (μg.g^{-1})	Ni (μg.g^{-1})	Pb (μg.g^{-1})	Zn (μg.g^{-1})	Mn (μg.g^{-1})	Cr (μg.g^{-1})
Soil	4106	41	93	52	69	533	71
Fodder	234	15	26	18	71	212	15
Milk	17	0,8	3	1	8	19	1
Dung	81	5	14	10	25	79	7
$CR_{fodder/soil}$	0,057	0,366	0,280	0,346	1,029	0,398	0,211
$CR_{dung/fodder}$	0,346	0,333	0,538	0,556	0,352	0,373	0,467
$CR_{milk/fodder}$	0,073	0,053	0,115	0,056	0,113	0,090	0,067

4.3.5 Comparison of the calculated CR-values of the different metals for the various crops at the four sampling localities

According to Brooks (1972), nearly all chemical elements and their concentrations have been determined in large numbers of species of terrestrial and aquatic vegetation. It is alsotrue to state that for every element there may be at least one or more of plant species capable of concentrating it in a spectacular manner. It is therefore possible that certain definite patterns may emerge concerning the uptake ability of metals by plants from surrounding terrestrial environment. This phenomenon of preferential enrichment of certain elements by plants is also known in the literature as the Goldschmidt enrichment principle (Goldschmidt, 1937). This principle is based on the ratio between metal concentrations in plants compared to those of the surrounding soil. The ratio of these two concentrations can then also be taken as an indication or measure of the biogenic characteristics of each element. Being biogenic, implies that an element plays a physiological role in the plant or, alternatively that it is concentrated to an appreciable degree whether or not it participates in the plant's metabolic processes. Ballast elements, on the other hand, are accumulated without having any requirement by plants (Frey-Wyssling, 1935). Table 4.20 lists a number of so-called biogenic, intermediate and non-biogenic elements which may be found in plants. This includes the metals Fe, Cu, Ni, Pb, Zn, Mn and Cr analyzed in aquatic plants and vegetable crops during this study.

Within the arrangement presented in Table 4.20, it is somewhat arbitrarily assumed that elements with concentration ratios of 0,10 or greater can be classified as biogenic, while those with concentration ratios of less than 0,01, as non-biogenic. Between these two groups occurs a group of metals known as intermediate elements.

Table 4.20 **Biogenic elements, intermediate elements and non-biogenic elements with an indication of typical CR-values of some terrestrial plants under natural environmental conditions (After Hutchinson, 1943 ; Cannon, 1960).**

Biogenic elements		Intermediate elements		Nonbiogenic elements	
Element	**CR**	**Element**	**CR**	**Element**	**CR**
B*	1,70	Mo*	0,040	Na	0,007
S*	0,96	Mg*	0,034	Rb	0,007
Zn*	0,90	Ni	0,030	Cr	0,003
P*	0,88	Co	0,020	Li	0,0015
Mn*	0,40	U	0,020	Si	0,0006
Ag	0,25	Fe*	0,012	V	0,0006
Ca*	0,14			Ti	0,0003
Sr	0,13			Aℓ	0,0003
Cu*	0,13				
K*	0,12				
Ba	0,12				
Se	0,10				

*Essential elements

The various metals analyzed in the vegetable crops as well as their respective CR-sequences recorded at the four sampling localities are listed in Tables 4.21 to 4.24. Looking at these results, and bearing in mind the classification by Hutchinson (1943) and Cannon (1960) of these metals according to their status as either biogenic, intermediate or non-biogenic elements (Table 4.20), the following tendencies can be observed for the crops at the various localities:

4.3.5.1 Crocodile Farm

With one exception (Carrots: Pb), Zn clearly produced the highest CR-value for all the other vegetable crops. Taking the lower end of the CR-values, Fe and Mn were the metals which were least concentrated by the five vegetable crops under consideration. No particular clear pattern of dominance in terms of CR were recorded for the metals Cu, Ni, Pb and Cr (Table 4.21). These were considered as intermediate in terms of CR-values obtained in the order of highest to lowest values for this parameter.

However, using these CR-values and comparing them with those proposed for the biogenic elements (Table 4.20), the metals Zn, Cu, Ni, Pb and Cr all potentially qualify as biogenic elements with none of the Cr-values being lower than 0,1. The metals Fe and Mn both fall within the category of intermediate elements (Table 4.20)

Table 4.21 Relative positions of concentration ratios of selected metals for each of the vegetable crops collected at the Crocodile Farm (control locality)

Vegetable crop	Metals: Descending order of CR-values						
	>	>	>	>	>	>	>
Lettuce	Zn 0,824	Cu 0,486	Ni 0,421	Pb 0,303	Cr 0,297	Mn 0,116	Fe 0,083
Cabbage	Zn 0,817	Pb 0,357	Cu 0,221	Ni 0,210	Cr 0,193	Fe 0,061	Mn 0,060
Cauliflower	Zn 0,490	Pb 0,450	Ni 0,374	Cr 0,183	Cu 0,173	Fe 0,071	Mn 0,065
Beetroot	Zn 0,443	Cu 0,328	Pb 0,247	Ni 0,154	Cr 0,123	Fe 0,041	Mn 0,040
Carrots	Pb 0,246	Ni 0,246	Cr 0,192	Zn 0,122	Cu 0,120	Fe 0,040	Mn 0,020

4.3.5.2 Marievale Farm

In the moderately polluted Marievale farm, an almost similar pattern of distribution of metals were found to exist according to the CR-values (Table 4.22). Zinc again clearly occupies the highest position for all five vegetable crops, followed by Pb. The lowest CR positions were mostly occupied by Mn and Fe with again a variable and intermediate position of subdominance by the metals, Pb, Cu, Ni and Cr. Comparing the metal CR-values of these crops with the classification of Hutchinson (1943) and Cannon (1960), all metals, with the exception of Fe and Mn, again qualify as biogenic elements with Fe and Mn falling into the category of intermediate elements (Table 4.20).

4.3.5.3 Vlakfontein Farm

Metal concentrations (CR-values) in the vegetable crops from the highly polluted Vlakfontein farm clearly showed Zn to be the best accumulated by all seven crops investigated here. Although Pb still featured prominently in the case of lettuce, leeks and turnips, CR-values for the metals Mn, and to a lesser extent Fe, clearly produced different scattered patterns in terms of concentration ratios, producing high values in spinach (Mn and Fe), lettuce (Mn), beetroot (Mn) and carrots (Mn) (Table 4.23).

Using the CR-values, Cr, Fe and Mn now mainly fell within the category classified as intermediate elements. Metal pollution as such at this locality, therefore seriously interfered with the CR-patterns observed at the unpolluted Crocodile farm and at the mildly polluted Marievale and Luipaardsvlei farms.

Table 4.22 **Relative positions of concentration ratios of selected metals for each of the vegetable crops collected at the Marievale Farm**

Vegetable crop	Metals: Descending order of CR-values						
	>	>	>	>	>	>	>
Lettuce	Zn 1,346	Pb 0,794	Cu 0,350	Ni 0,279	Cr 0,155	Fe 0,066	Mn 0,040
Cabbage	Zn 0,890	Pb 0,714	Ni 0,235	Cr 0,179	Cu 0,135	Mn 0,048	Fe 0,031
Cauliflower	Zn 1,441	Pb 0,575	Ni 0,367	Cr 0,180	Cu 0,160	Mn 0,076	Fe 0,055
Beetroot	Zn 0,590	Pb 0,503	Cu 0,422	Ni 0,248	Cr 0,170	Mn 0,067	Fe 0,040
Carrots	Zn 1,525	Pb 0,821	Mn 0,401	Ni 0,371	Cu 0,328	Cr 0,169	Fe 0,034

Table 4.23 **Relative positions of concentration ratios of selected metals for each of the vegetable crops collected at the Vlakfontein Farm**

Vegetable crop	Metals: Descending order of CR-values						
	>	>	>	>	>	>	>
Lettuce	Zn 1,475	Pb 0,482	Mn 0,432	Cu 0,385	Ni 0,241	Cr 0,211	Fe 0,068
Cabbage	Zn 1,261	Ni 0,287	Pb 0,277	Cu 0,093	Cr 0,075	Fe 0,039	Mn 0,036
Spinach	Zn 6,013	Mn 1,026	Cu 0,710	Pb 0,606	Fe 0,599	Ni 0,573	Cr 0,279
Leeks	Zn 0,542	Pb 0,252	Cu 0,147	Ni 0,105	Fe 0,059	Cr 0,054	Mn 0,019
Turnips	Zn 1,163	Pb 0,297	Ni 0,246	Cu 0,164	Mn 0,050	Fe 0,042	Cr 0,021
Beetroot	Zn 1,793	Mn 0,817	Cu 0,522	Ni 0,322	Pb 0,203	Cr 0,100	Fe 0,054
Carrots	Zn 1,509	Mn 0,432	Ni 0,421	Cu 0,381	Pb 0,252	Cr 0,104	Fe 0,046

Metal analyses of fodder at Luipaardsvlei farm (Table 4.24) again confirmed the dominance of Zn as a metal which is the highest accumulated, followed by Mn. Cr-values for Cu, Pb, Ni and Cr in this case all qualified as biogenic elements if the classification of Hutchinson (1943) and Cannon (1960) is considered, with Fe being an intermediate element (Table 4.20).

Table 4.24 Relative positions of concentration ratios of selected metals in fodder collected at the Luipaardsvlei Farm

Crop	Metals: Descending order of CR-values						
	>	>	>	>	>	>	>
Cattle fodder	Zn 1,029	Mn 0,398	Cu 0,366	Pb 0,346	Ni 0,280	Cr 0,211	Fe 0,057

Briefly, the data obtained on the CR-values for the various metals and a comparison of these with the classification of Hutchinson (1943) and Cannon (1960) suggest that the CR-ranges proposed by the latter authors not necessarily fit in with the results obtained on the vegetable crops during this study. There are however, some broad agreements in the tendencies observed. Zinc, Cu, Cr and Ni usually qualify on the basis of their values as biogenic elements, whilst Mn and Fe both qualify as intermediate elements. Theoretically, Pb qualified on the basis of CR-values as a biogenic element.

4.4 Concentrations and Concentration Ratios (CR) of metals in some macro-invertebrate organisms collected from the control sampling locality and from the localities affected by mine drainage effluents

4.4.1 Crocodile Farm

Bottom sediments collected at this sampling locality yielded 30667 μg.g^{-1} Fe, 918 μg.g^{-1} Mn, 302 μg.g^{-1} Ni, 256 μg.g^{-1} Zn, 155 μg.g^{-1} Cu, 122 μg.g^{-1} Cr and 68 μg.g^{-1} Pb (Table 4.25).

Metal concentrations recorded from the macro-invertebrate fauna at this site ranged between 283 μg.g^{-1} (Pb & Cr) and a highest of 25735 μg.g^{-1} (Fe) in the Chironomidae. Lowest and highest concentrations of 241 μg.g^{-1} (Pb) and 16358 μg.g^{-1} (Fe) were recorded in the Lymnaeidae (Mollusca). In the Chironomidae, lead (4,154) produced the highest CR-value. This was followed by Cu (3,827), Cr (2,315), Ni (1,777), Zn (1,545), Mn (0,892) and Fe (0,839) in declining order (Table 4.20). Concentration Ratios for the Lymnaeidae followed nearly a similar pattern with Pb (3,549) again producing the highest value. This was followed by the CR-value for Ni (3,095), Cr (2,748), Cu (2,336), Zn (1,744), Mn (0,745) and Fe (0,533) in descending order (Table 4.25).

4.4.2 Marievale farm and other nearby localities on the Blesbokspruit

Data obtained on the metal concentrations in the macro-invertebrate fauna in the metal polluted Blesbokspruit was collected over a period of 2 years. A number of sampling localities were included in this particular study, stretching from the Van Ryn and Homestead lakes in Benoni, Cowles Dam, Springs Bird Sanctuary and from a portion of the Blesbokspruit wetland in the vicinity of Springs, the Nigel Dam and the Marievale Bird Sanctuary in Nigel.

Metal concentrations in the sediments of Van Ryn lake ranged between 39 $\mu g.g^{-1}$ (Cr) and 28168 $\mu g.g^{-1}$ for Fe. Considering metal concentrations in the tissues of macro-invertebrate fauna collected at this locality, highest concentrations of Ni (310 $\mu g.g^{-1}$), Pb (186 $\mu g.g^{-1}$), Zn (902 $\mu g.g^{-1}$) and Mn (380 $\mu g.g^{-1}$) were measured in the Hirudinea. Highest concentrations of Cr (84 $\mu g.g^{-1}$) and Fe (14228 $\mu g.g^{-1}$) were measured in the Tubificidae, while Cu (214 $\mu g.g^{-1}$) was the only metal which occurred in the highest concentration in a non-annelid, namely in the freshwater crab, *Potamonautes warreni*. In most cases (Ni: 54 $\mu g.g^{-1}$; Pb: 66 $\mu g.g^{-1}$; Zn: 172 $\mu g.g^{-1}$; Cr: 34 $\mu g.g^{-1}$) this crab contained the lowest metal concentrations of all the macro-invertebrates analyzed (Table 4.26).

A similar pattern was obtained for the Concentration Ratios and the actual metal concentrations in the macro-invertebrate fauna of Van Ryn lake (Table 4.26). The Hirudinea produced the highest CR-values for Ni (1,572), Pb (2,421), Zn (2,592) and Mn (0,349), while the other Annelida (Tubificidae) produced the highest CR-values for Fe (0,505) and Cr (2,142) (Table 4.26). Again, lowest CR-values for *P. warreni* were recorded for Ni (0,275), Pb (0,851), Zn (0,493) and Cr (0,859). The lowest Fe concentration (3319 $\mu g.g^{-1}$) and its corresponding CR-value (0,118) was obtained for the Hirudinea (Table 4.26).

As was the case with metal concentrations in Van Ryn lake, sediments collected in the Homestead lake a similar tendency, with Fe being the highest (37880 $\mu g.g^{-1}$) followed by Mn (8961 $\mu g.g^{-1}$), Ni (487 $\mu g.g^{-1}$), Zn (388 $\mu g.g^{-1}$), Cu (338 $\mu g.g^{-1}$), Pb (318 $\mu g.g^{-1}$) and Cr (178 $\mu g.g^{-1}$) was observed (Table 4.26). Of the metal concentrations in the macro-invertebrate fauna of this lake, highest concentrations of Fe (20278 $\mu g.g^{-1}$), Ni (1059 $\mu g.g^{-1}$), Pb (955 $\mu g.g^{-1}$) and Cr (399 $\mu g.g^{-1}$) were measured in *Micronecta sp.* (Hemiptera) while *Baetis sp.* (Ephemeroptera) produced highest concentrations for Cu (453 $\mu g.g^{-1}$) and Mn (10368 $\mu g.g^{-1}$). The highest Zn concentration of 570 $\mu g.g^{-1}$ was measured in the Veliidae (Hemiptera) (Table 4.26). In all the other macro-invertebrate fauna, the Hemiptera also contained the lowest metal concentrations. In the case of Fe (3843 $\mu g.g^{-1}$), Cu (202 $\mu g.g^{-1}$), Zn (424 $\mu g.g^{-1}$) and Cr (141 $\mu g.g^{-1}$), the lowest concentrations for these metals were measured in *Plea sp.* (Hemiptera), while the Veliidae (Hemiptera) produced lowest concentrations for Ni (242 $\mu g.g^{-1}$), Pb (272 $\mu g.g^{-1}$) and Mn (246 $\mu g.g^{-1}$) (Table 4.26).

As expected, patterns for CR-values roughly corresponded with the actual metal concentrations recorded. Highest CR-values for *Micronecta sp.* (Hemiptera) were found for Fe (0,535), Ni (2,175), Pb (3,003) and Cr (2,243), while *Baetis sp.* (Ephemeroptera) produced highest CR-values for Cu (1,340) and Mn (1,157). In contrast, lowest CR-values were produced by *Plea sp.* for Fe (0,101), Cu (0,598), Zn (1,093) and Cr (0,794) as well as in the Veliidae (Hemiptera) for Ni (0,497), Pb (0,855) and Mn (0,027), respectively (Table 4.26).

Table 4.25 Concentration levels and Concentration Ratios of selected metals in the tissues of macro-invertebrate fauna collected in the vicinity of Crocodile Farm during 1992

Parameter	Sampling location	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Sediment	Crocodile River:	30667	155	302	68	256	918	122
Diptera: Chironomidae	Crocodile Farm	25735	593	537	283	396	819	283
CR	(Pretoria)	0,839	3,827	1,777	4,154	1,545	0,892	2,315
Sediment	Crocodile River:	30667	155	302	68	256	918	122
Mollusca: Lymnaeidae	Crocodile Farm	16358	362	935	241	447	684	335
CR	(Pretoria)	0,533	2,336	3,095	3,549	1,744	0,745	2,748

Table 4.26 Concentration levels and Concentration Ratios of selected metals in the tissues of macro-invertebrate fauna collected at various sampling localities on the Blesbokspruit as well as in the vicinity of Marievale Farm during 1991 - 1992

Parameter	Sampling location	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Sediment	Van Ryn Lake (Benoni)	28168	79	197	77	348	1088	39
Annelida: Tubificidae		14228	96	205	90	561	283	84
CR		0,505	1,211	1,040	1,172	1,611	0,260	2,142
Sediment	Van Ryn Lake (Benoni)	28168	79	197	77	348	1088	39
Annelida: Hirudinea		3319	147	310	186	902	380	77
CR		0,118	1,854	1,572	2,421	2,592	0,349	1,963
Sediment	Van Ryn Lake (Benoni)	28168	79	197	77	348	1088	39
Hemiptera: *Sphaerodema* sp.		3796	195	125	102	435	207	68
CR		0,135	2,466	0,636	1,327	1,249	0,190	1,747
Sediment	Van Ryn Lake (Benoni)	28168	79	197	77	348	1088	39
Crustacea: *Potamon* sp.		4080	214	54	66	172	262	34
CR		0,145	2,714	0,275	0,851	0,493	0,241	0,859
Sediment	Homestead Lake (Benoni)	37880	338	487	318	388	8961	178
Hemiptera: *Micronecta* sp.		20278	417	1059	955	434	8247	399
CR		0,535	1,233	2,175	3,003	1,119	0,9220	2,243
Sediment	Homestead Lake (Benoni)	37880	338	487	318	388	8961	178
Hemiptera: *Notonecta* sp.		17767	331	893	893	447	8170	331
CR		0,469	0,981	1,834	2,809	1,151	0,912	1,862
Sediment	Homestead Lake (Benoni)	37880	338	487	318	388	8961	178
Hemiptera: *Plea* sp.		3843	202	268	283	424	4535	141
CR		0,101	0,598	0,550	0,889	1,093	0,506	0,794
Sediment	Homestead Lake (Benoni)	37880	338	487	318	388	8961	178
Hemiptera: Veliidae		5626	261	242	272	570	246	160
CR		0,149	0,771	0,497	0,855	1,468	0,027	0,899

Table 4.26 (Continued) **Concentration levels and Concentration Ratios of selected metals in the tissues of macro-invertebrate fauna collected at various sampling localities on the Blesbokspruit as well as in the vicinity of Marievale Farm during 1991 - 1992**

Parameter	Sampling location	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Sediment	Homestead Lake	37880	338	487	318	388	8961	178
Ephemeroptera: *Baetis* sp.	(Benoni)	13255	453	623	604	472	10368	226
CR		0,350	1,340	1,279	1,899	1,216	1,157	1,272
Sediment	Cowlesdam	33203	874	1390	106	596	1148	177
Annelida: Tubificidae	(Springs)	6773	1562	2193	87	1244	722	159
CR		0,204	1,787	1,578	0,821	2,087	0,629	0,898
Sediment	Cowlesdam	33203	874	1390	106	596	1148	177
Annelida: Hirudinea	(Springs)	4899	971	1530	59	671	483	97
CR		0,148	1,11	1,101	0,557	1,126	0,421	0,492
Sediment	Cowlesdam	33203	874	1390	106	596	1148	177
Diptera: Chironomidae	(Springs)	5953	1229	1977	103	981	651	143
CR		0,179	1,406	1,422	0,972	1,646	0,567	0,808
Sediment	Springs Bird Sanctuary	4885	136	434	54	238	1204	147
Crustacea: Cladocera	(Springs)	1636	195	359	210	496	2090	169
CR		0,335	1,436	0,826	3,887	2,082	1,736	1,150
Sediment	Springs Bird Sanctuary	4885	136	434	54	238	1204	147
Hemiptera: *Sigara* sp.	(Springs)	1985	210	334	245	385	587	93
CR		0,406	1,543	0,770	4,533	1,616	0,487	0,634
Sediment	Blesbokspruit: R29 Road	27872	50	362	25	309	146	181
Annelida: Tubificidae	crossing: Grootvlei	4553	21	229	16	347	78	88
CR	(Springs)	0,163	0,429	0,634	0,640	1,125	0,535	0,485
Sediment	Blesbokspruit: R29 Road	27872	50	362	25	309	146	181
Diptera: Chironomidae	crossing: Grootvlei	22917	156	1568	130	1510	315	534
CR	(Springs)	0,822	3,139	4,333	5,232	4,895	2,161	2,947

Table 4.26 (Continued) Concentration levels and Concentration Ratios of selected metals in the tissues of macro-invertebrate fauna collected at various sampling localities on the Blesbokspruit as well as in the vicinity of Marievale Farm during 1991 - 1992

Parameter	Sampling location	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Sediment	Nigel Dam	24220	98	158	13	509	282	219
Annelida: Tubificidae	(Nigel)	6921	188	268	16	1306	197	207
CR		0,286	1,918	1,696	1,231	2,566	0,699	0,945
Sediment	Nigel Dam	24220	98	158	13	509	282	219
Diptera: Chironomidae	(Nigel)	5918	169	193	7	1162	214	129
CR		0,244	1,724	1,222	0,538	2,283	0,759	0,589
Sediment	Marievale Bird Sanctuary	27303	49	369	20	403	2261	195
Trichoptera: Hydroptilidae	(Nigel)	35068	149	1109	113	2081	1005	348
CR		1,284	3,064	3,008	5,687	5,167	0,444	1,783
Sediment	Marievale Bird Sanctuary	27303	49	369	20	403	2261	195
Mollusca: Lymnaeidae	(Nigel)	17125	98	1046	63	738	1540	508
CR		0,627	2,001	2,839	3,142	1,831	0,681	2,597

At Cowlesdam, situated approximately 15km downstream from the above lakes and in the vicinity of a paper factory, three main organism groups, consisting of the Tubificidae (Annelida), Hirudinea (Annelida) and Chironomidae (Diptera) were collected.

Metal concentrations in the sediments of this dam mostly followed a different pattern from those recorded in the previously discussed lakes. A highest concentration for Fe (33203 $\mu g.g^{-1}$) was followed by Ni (1390 $\mu g.g^{-1}$), Mn (1148 $\mu g.g^{-1}$), Cu (874 $\mu g.g^{-1}$), Zn (596 $\mu g.g^{-1}$), Cr (177 $\mu g.g^{-1}$) and Pb (106 $\mu g.g^{-1}$) in descending order (Table 4.26).

The metal concentrations and respective CR-values obtained for the macro-invertebrate fauna followed a pattern nearly similar than for those observed for the Van Ryn lake fauna. In all cases, except for Pb, the Tubificidae contained the highest metal concentrations in this dam (Table 4.26). This was followed by the Chironomidae, which contained the second highest quantities of Fe (5953 $\mu g.g^{-1}$), Cu (1229 $\mu g.g^{-1}$), Ni (1977 $\mu g.g^{-1}$), Zn (981 $\mu g.g^{-1}$), Mn (651 $\mu g.g^{-1}$) and Cr (143 $\mu g.g^{-1}$). In all cases, the Hirudinea contained the lowest metal concentrations (Fe: 4899 $\mu g.g^{-1}$; Cu: 971 $\mu g.g^{-1}$; Ni: 1530 $\mu g.g^{-1}$; Pb: 59 $\mu g.g^{-1}$; Zn: 671 $\mu g.g^{-1}$; Mn: 483 $\mu g.g^{-1}$ and Cr: 97 $\mu g.g^{-1}$) (Table 4.26).

The Tubificidae usually produced the highest CR values, ranging from 0,629 for Mn to 2,087 for Zn. Again, this was followed by the Chironomidae and then the Hirudinea with CR values which ranged from 0,148 for Fe to 1,126 for Zn (Table 4.26).

The ability to remove metals from the water column by the Blesbokspruit wetland ecosystem can clearly be observed when sediment metal concentrations from the Cowlesdam are compared with those collected from the Blesbokspruit at the R29 road crossing on the Blesbokspruit approximately 8km downstream. The sediment iron concentration decreased to 27872 $\mu g.g^{-1}$, while Cu (50 $\mu g.g^{-1}$), Ni (362 $\mu g.g^{-1}$), Pb (25 $\mu g.g^{-1}$) and Mn (146 $\mu g.g^{-1}$) also showed significant decreases at the lower station.

Contrary to metal concentrations obtained for the Tubificidae and Chironomidae at the Cowlesdam, these organisms collected at the R29 road crossing produced contrasting results. In all cases, the highest metal concentrations, ranging between 130 $\mu g.g^{-1}$ (Pb) and 22917 $\mu g.g^{-1}$ (Fe) were measured in this case of the Chironomidae while the Tubificidae produced the lowest metal concentrations ((Fe: 4553 $\mu g.g^{-1}$; Cu: 21 $\mu g.g^{-1}$; Ni: 229 $\mu g.g^{-1}$; Pb: 16 $\mu g.g^{-1}$; Zn: 347 $\mu g.g^{-1}$; Mn: 78 $\mu g.g^{-1}$ and Cr: 88 $\mu g.g^{-1}$) (Table 4.26).

As expected, CR-values yielded similar results as those obtained for the corresponding metal concentrations with those to the Chironomidae being the highest, ranging from 0,822 (Fe) to 5,232 (Pb). The Tubificidae produced the overall lowest CR values, ranging from 0,429 (Cu) to 1,125 (Zn) (Table 4.26).

The occurrence of industrial and mining activities in the immediate vicinity of the Nigel Dam was reflected by a significant increase in the concentrations of Cu (98 $\mu g.g^{-1}$), Zn (509 $\mu g.g^{-1}$), Mn (282 $\mu g.g^{-1}$) and Cr (219 $\mu g.g^{-1}$) in the bottom sediments. Various metal processing plants and heavy metal industries reside at the Vorsterskroon industrial area near the shore of Nigel Dam which probably accounted for the increased levels of Cu and Zn in the sediments. The presence of a tannery which employed chromium salts during the processing of hides, may account for the elevated concentrations of this metal in the sediments of this dam. Elevated Zn and Mn concentrations in the sediments can mainly be ascribed to gold and uranium mining activities in the immediate vicinity (Table 4.26). Yet another contradictory pattern to that obtained at the R29 road crossing but similar to that obtained for the Cowlesdam, was obtained for the Tubificidae and Chironomidae of Nigel Dam. With the exception of Mn (197 $\mu g.g^{-1}$), the Tubificidae contained the highest metal concentrations ranging from 16 $\mu g.g^{-1}$ (Pb) to 6921 $\mu g.g^{-1}$ (Fe). Lowest concentrations for Fe (5918 $\mu g.g^{-1}$), Cu (169 $\mu g.g^{-1}$), Ni (193 $\mu g.g^{-1}$), Pb (7 $\mu g.g^{-1}$), Zn (1162 $\mu g.g^{-1}$), and Cr (129 $\mu g.g^{-1}$) were measured in the tissues of the Chironomidae (Table 4.26).

The CR values obtained for the macro-invertebrate organisms collected at the Nigel Dam produced a similar pattern as that obtained for the metal concentrations. In the case of the Tubificidae, highest CR values were calculated for Fe (0,286), Cu (1,918), Ni (1,696), Pb (1,231), Zn (2,566) and Cr (0,945), while the Chironomidae only produced a highest CR value of 0,759 for Mn.

At the Marievale Bird Sanctuary, which is in the close proximity of the Marievale farm, elevated levels of Zn (403 $\mu g.g^{-1}$) and Mn (2261 $\mu g.g^{-1}$) in the bottom sediments indicated the presence of mining activities. At this sampling locality, significant numbers of the Hydroptilidae (Trichoptera) and Lymnaeidae (Mollusca) occurred which were collected for metal analyses. Except in the cases of Mn (1005 $\mu g.g^{-1}$) and Cr (348 $\mu g.g^{-1}$), highest concentrations of Fe (35068 $\mu g.g^{-1}$), Cu (149 $\mu g.g^{-1}$), Ni (1109 $\mu g.g^{-1}$), Pb (113 $\mu g.g^{-1}$) and Zn (2081 $\mu g.g^{-1}$) were measured in the Hydroptilidae. All the other metal concentrations, except Mn (1540 $\mu g.g^{-1}$) and Cr (508 $\mu g.g^{-1}$) were the lowest in the Lymnaeidae (Table 4.26).

For the various metals, the CR values were the highest in the Hydroptilidae, ranging between 1,284 (Fe) and 5,687 (Pb). Lowest CR-values for the above organisms were obtained for Mn (0,444) and Cr (1,783). Contrary to the latter organism group, highest CR values for Mn (0,681) and Cr (2,597) were obtained for the Lymnaeidae. In the case of all the other metals (Fe, Cu, Ni, Pb and Zn), CR-values obtained for this taxon were the lowest, ranging between 0,627 (Fe) and 3,142 (Pb) (Table 4.26).

4.4.3 Vlakfontein Farm

Compared to values obtained for the control locality (Crocodile Farm), sediment metal concentrations at the Vlakfontein Farm were, except in the cases of Zn and Mn, higher than those recorded at the Crocodile Farm. A highest Fe concentration of 49788 $\mu g.g^{-1}$ was followed by those for Ni (361 $\mu g.g^{-1}$), Cu (344 $\mu g.g^{-1}$), Zn (248 $\mu g.g^{-1}$), Mn (240 $\mu g.g^{-1}$), Cr (227 $\mu g.g^{-1}$) and Pb (130 $\mu g.g^{-1}$), respectively (Table 4.27).

Metal concentrations in the macro-invertebrate fauna collected at this locality ranged between 84 $\mu g.g^{-1}$ (Pb) and 29408 $\mu g.g^{-1}$ (Fe) for the Tubificidae, while the Chironomidae contained between 154 $\mu g.g^{-1}$ for copper and 39914 $\mu g.g^{-1}$ for iron (Table 4.27). In the case of the Tubificidae, Mn (0,750) produced the highest CR value. This was followed by Ni (0,660), Pb (0,646), Fe (0,591), Cr (0,482), Zn (0,441) and Cu (0,262), respectively. The CR for the Chironomidae on the other hand, showed a somewhat different pattern with Pb (3,039) being the highest followed by those for Ni (1,747), Zn (1,587), Cr (1,327), Mn (0,922), Fe (0,802) and Cu (0,447) in descending order (Table 4.27).

4.4.4 Luipaardsvlei Farm

Metal concentrations of the bottom sediments at this locality were somewhat lower than those recorded from the Vlakfontein Farm and generally ranged between values recorded for the Crocodile and the Marievale farms, respectively. A highest concentration for Fe (33405 $\mu g.g^{-1}$) was recorded here followed by the concentrations for Mn (1044 $\mu g.g^{-1}$), Cu (289 $\mu g.g^{-1}$), Zn (254 $\mu g.g^{-1}$), Ni (209 $\mu g.g^{-1}$), Cr (97 $\mu g.g^{-1}$) and Pb (96 $\mu g.g^{-1}$), in that order (Table 4.28).

In the tissues of the aquatic Coleoptera at this site, metal concentrations ranged between 24 $\mu g.g^{-1}$ (Pb) and 7544 $\mu g.g^{-1}$ (Fe) for the Dytiscidae and 310 $\mu g.g^{-1}$ (Cu) and 5534 $\mu g.g^{-1}$ (Fe) for the Haliplidae. The Hydroptilidae contained metal concentrations ranging between 229 $\mu g.g^{-1}$ for Cr and 13947 $\mu g.g^{-1}$ for Mn (Table 4.28).

The only lower vertebrate collected at this sampling locality was larvae of the common toad, *Bufo gutturalis*. The larvae of this frog contained a (lowest) Pb concentration of 111 $\mu g.g^{-1}$ and a (highest) Fe concentration of 5492 $\mu g.g^{-1}$ (Table 4.28).

Concentration Ratio patterns showed that Mn was bio-accumulated the most by all macro-invertebrates collected at this locality. Fe was accumulated in relatively smaller quantities in at least two cases, namely the Haliplidae and the larvae of the common toad. In the case of *B. gutturalis*, Cr (3,102) was concentrated the most, followed by Mn (2,457), Zn (2,322), Pb (1,159), Cu (1,144), Ni (0,993) and Fe (0,164) (Table 4.28).

Table 4.27 Concentration levels and Concentration Ratios of selected metals in the tissues of macro-invertebrate fauna collected in the vicinity of Vlakfontein Farm during 1993

Parameter	Sampling location	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Sediment	Vlakfontein	49788	344	361	130	248	240	227
Annelida: Tubificidae	(Roodepoort)	29408	90	238	84	109	180	109
CR		0,591	0,262	0,660	0,646	0,441	0,750	0,482
Sediment	Vlakfontein	49788	344	361	130	248	240	227
Diptera: Chironomidae	(Roodepoort)	39914	154	640	394	394	221	301
CR		0,802	0,447	1,774	3,039	1,587	0,922	1,327

Table 4.28 Concentration levels and Concentration Ratios of selected metals in the tissues of macro-invertebrate fauna collected in the vicinity of Luipaardsvlei during 1993

Parameter	Sampling location	Fe ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Ni ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Mn ($\mu g.g^{-1}$)	Cr ($\mu g.g^{-1}$)
Sediment	Luipaardsvlei (Randfontein)	33405	289	209	96	254	1044	97
Coleoptera: *Dytiscus* sp.		7544	106	41	24	123	1406	30
CR		0,226	0,366	0,194	0,248	0,482	1,347	0,306
Sediment	Luipaardsvlei (Randfontein)	33405	289	209	96	254	1044	97
Coleoptera: Haliplidae		5534	310	499	361	878	5017	344
CR		0,166	1,072	2,388	3,765	3,456	4,806	3,549
Sediment	Luipaardsvlei (Randfontein)	33405	289	209	96	254	1044	97
Trichoptera: Hydroptilidae		4445	881	664	383	423	13947	229
CR		0,133	3,048	3,175	3,993	1,667	13,360	2,359
Sediment	Luipaardsvlei (Randfontein)	33405	289	209	96	254	1044	97
Amphibia: *Bufo gutturalis*		5492	331	208	111	590	2565	301
CR		0,164	1,144	0,993	1,159	2,322	2,457	3,102

DISCUSSION AND RECOMMENDATIONS

DISCUSSION AND RECOMMENDATIONS

5 & 6

5 DISCUSSION

It is often stated that wetlands can be considered as nature's own water purifiers (Reed, 1988 ; de Wet *et al.*, 1991). In practice, wetlands are also often utilized for the purification of sewage (Gersber *et al.*, 1983 ; Coghlan, 1991 ; Gersber *et al.*, 1986 ; Wood, 1991 ; 1993 ; 1994 a,b), industrial (Fourie, 1991 ; Wood & Pybus, 1992) and mining (Gusek, 1991 ; US Bureau of Mines, 1991) effluents. However, the following pertinent questions need to be adressed in the evaluation of some of the physical, chemical and biological mechanisms involved in the rehabilitation of wetland ecosystems from the various forms of pollution:

- ***"Why are rivers and lakes as such not considered as efficient biological filters compared to typical wetlands"?, and***
- ***"What features of wetlands characterise and distinguish them as efficient biofiltration systems for the removal of nutrients as well as potentially toxic inorganic substances such as metals (and even radionuclides) from a polluted aquatic environment"?***

A number of answers may be obtained when various aspects regarding the physical, chemical and biological interactions of a wetland are considered. Results obtained during the present study as well as in those obtained from the literature will be incorporated into this part of the discussion to find possible answers to some of the above questions encountered during this study.

In contrast to rivers and streams, typical wetlands are generally shallow, spread over a wide surface area and therefore posess a high surface area/depth ratio (Wood & Pybus, 1992). The above physical characteristics of a wetland generally result in a low speed of water traversing the wetland system. This in turn, facilitates a high rate of deposition of suspended particles (flocs to which contaminants are adsorbed) as well as a maximum contact time between the water/sediment and water/biota (floral and faunal communities) of a wetland. Not only does it ensure a high rate of deposition of suspended particles, but also induce the physical trapping of larger particles between rooted emergent, rooted submerged and floating aquatic macrophytes (Chambers & Eadie, 1981 ; Charlton, 1983). In addition, a maximum contact time between water and biota ensures optimum interaction during which a variety of contaminants are abstracted from the water column and in the process taken up by either aquatic macrophytes or benthic macro-invertebrate organisms through different food-chain systems (Luoma & Bryan, 1979 ; O'Connor & Conolly, 1980 ; Hart, 1982 ; Sly, 1982 ; Salomons & Förstner, 1984).

During this investigation, the following characteristics were observed at some of the various sampling localities which supported the above statements:

- Generally, the wetlands investigated, ranged in width between approximately 20 metres to as much as 2000+ metres. The average depth varied between 0,1 and 0,4 metres, while an average flow-rate of $<0{,}1$ m.s^{-1} prevailed during non-flood conditions.

- In addition, the wetland flora was dominated by dense growths of the aquatic macrophytes *Phragmites australis* and *Typha capensis*. These plants not only occur in vast numbers, but exist in highly physically inpenetrable densities in these wetlands through which water constantly filters. In addition, these plants are particularly adapted to wetland conditions which include: (i) prolific growths of roots and rhizomes which form a dense layer of plant tissue covering the wetlandfloor, and (ii) long subsurface as well as hollow and herbaceous chlorophyll containing stems with apical leaves to trap sunlight. These aquatic weeds have been observed in particular in the Blesbokspruit wetland to occur in uninterrupted stretches of over 8km long. At the southern border of the Marievale section of this wetland, water flowing from this wetland ecosystem was at times found to posess a turbidity of less than 10 NTU and in a single case, even less than 1 NTU. This in particular, illustrates the remarkable ability of wetland macrophytes to remove suspended particles from an affected water column.

Due to the relatively low solubilities of most toxic organic and inorganic chemicals, many of these compounds associate with solid phases in the aquatic environment. Generally, suspended particles in the water column and bottom sediments can be regarded as the solid phase. The constant or periodic supply and sedimentation of toxicant-solids complexes may eventually lead to the buildup of high (relatively to the concentrations in the overlying water column) concentrations of metals and other toxicants in the bottom sediments of an aquatic ecosystem (Förstner & Wittmann, 1981 ; Hart, 1982 ; de Wet *et al.*, 1993 a,b ; Allan, 1986).

Unfortunately, especially in the case of polluted aquatic environments, the major route by which particle-associated contaminants are introduced into the biotic components of the aquatic environment, is usually via the bio-accumulation thereof by aquatic fungi, bacteria and benthic macro-invertebrate organisms which together form one of the lowest levels in aquatic food webs (Förstner & Wittmann, 1981 ; Allan, 1986). Usually, in an unpolluted aquatic environment, essential (and non-essential) elements (metals) occur in very low concentrations and are sufficient to be utilized in trace amounts by the aquatic organisms. Under these conditions, the enrichment of the biota with these metals does exceed the level which allows the enzyme systems of these organisms to function without interference (Förstner & Wittmann, 1981).

If a larger-than-necessary abundance of essential (and non-essential) metals exists in the aquatic environment, homeostatic control mechanisms may operate to regulate the metal content of the organism (Bryan & Hummerstone, 1973). However, these homeostatic control mechanisms cease to function when the metal concentration at the source of supply (e.g. water, nutrients) becomes too high. Under these conditions, these metals may act in an either acutely or chronically toxic manner (Förstner & Wittmann, 1981).

Most important, little indication and information about the toxicity and bio-availability of metals to aquatic organisms can be provided by depending only on a basic analysis of metals in the water column itself. The extent of toxicity and bio-availability of a metal is generally determined by the particular species in which a metal occurs and this in turn is usually governed by a combination of prevailing physico- chemical as well as biological conditions of the aquatic environment (Förstner & Wittmann, 1981 ; Allan, 1986 ; Allan & Ball, 1990). The latter authors also agree that although Atomic Absorption Spectrometry may provide a useful tool to determine the overall metal concentrations of water samples, it fails however to produce sufficient evidence of chemical speciation and the actual bio-availability of a particular fraction of the total metal concentration measured.

In the discussion that will follow, the logical sequence of the various aspects covered by this investigation is considered under the following headings as previously indicated in the introduction (Chapter 1) of this study:

- Evaluation of water quality conditions, as reflected by this study, with specific reference to the application of an Aquatic Toxicity Index used as a measure of the prevailing conditions which existed during the time of the study.

- Metal concentrations in the aquatic environment with specific reference to their concentration, bio-accumulation and bio-availability in the organs and tissues of floating, rooted submerged and rooted emergent aquatic macrophytes at the various sampling localities.

- Metal concentrations in the terrestrial environment with specific reference to their concentration, bio-accumulation and bio-availability in the tissues of vegetable crops produced at the various sampling localities.

- Metal concentrations in the aquatic environment with specific reference to their concentration, bio-accumulation and bio-availability in the tissues of selected benthic macro-invertebrate organisms present at the various sampling localities.

- A brief reference to the bio-accumulation of metals by the lower aquatic vertebrate organism, namely the guttural toad, *Bufo gutturalis* collected at Luipaardsvlei.

- Discussion of the phenomenon and potential value of the Concentration Ratio as well as problems encountered with the interpretation of this particular parameter.

5.1 Evaluation of water quality conditions and metal concentrations, as reflected by this study, with specific reference to the use of Aquatic Toxicity Indices and compliance evaluation

5.1.1 The use of an Aquatic Toxicity Index in the modelling of water quality data

Very often, scientists, engineers and managers involved in the field of water quality control and management are confronted with large arrays of data which at times can be very confusing. To the average lay-person, a set of figures are usually overwhelming, and often no attempt is made to gain an understanding of their actual relevance. The past few decades, numerous attempts have been made in integrating a set of selected water quality parameters into a single, descriptive value which reflects the suitability-of-use of a particular water. Depending on the potential use of water, which may include recreation, irrigation, potable water supply, industrial abstractions as well as the protection of aquatic life, different Water Quality Indices (WQI) using separate water quality variables, have been developed. The primary aim of the development of WQI's was therefore an attempt to depart from the so-called "data rich but information poor" syndrome (Ward *et al.*, 1986), often encountered in water quality management. Ultimately, information will be available in a simple, yet understandable and useful format.

It is however important to ensure that a suitable water quality index type is applied to a particular target group or audience. For instance, the technical Aquatic Toxicity Index (Solway Unweighted) which incorporates a number of mathematical equations, cannot be published on a weekly basis in a local newspaper to describe water conditions to the public. Being technical, large amounts of space would be required to explain the mechanism of such an index. Consequently, costs would be high, making the publication thereof impractical. On the other hand, only a small group of people would be able to interpret the meaning of such an index.

The Department of Water Affairs and Forestry (DWA&F), in conjunction with the Springs town council and the Springs Advertiser, which is a local newspaper, have started with a weekly report of water quality conditions in the Blesbokspruit presented in a short, simple, yet understandable format. Basically, only two parameters are measured, namely sodium concentrations and the presence of faecal coliform organisms. While the latter parameter gives an indication of the suitablity-of-use of water for recreation, sodium concentrations are measured to give an indication of the suitablity of water for irrigation. Four categories for the description of water quality conditions have been developed, and include the following:

- **Ideal:** water quality which will not result in any negative consequence
- **Acceptable:** use of water may only result in insignificant negative consequences
- **Tolerable:** water in this category may result in limited to serious negative consequences depending on the vulnerability of the user
- **Maximum allowable:** use of water over a short period of time may result in limited to serious negative consequences
- **Unacceptable:** use of water may result in immediate serious negative consequences for the user *(Springs Advertiser: 5 April 1996)*

According to representatives of the DWA&F (Viljoen, 1996: personal communication), this system of water quality reporting has been positively accepted by the local community, and serves as a useful indicator for suitablity-of-use of water for recreation and agriculture.

In contrast to the above system, the Aquatic Toxicity Index (ATI), has also been applied on the Blesbokspruit data during this study as well as in separate investigations on this wetland and other local aquatic ecosystems (de Wet, 1996 a-f).

The combination of four index systems as used in the present study have been employed with a marked degree of success to evaluate the general water quality conditions at the four sampling localities, namely Crocodile farm, Marievale farm, Vlakfontein farm and Luipaardsvlei farm (Refer to Chapter 4 : Tables 4.1 - 4.4). A subsequent inspection and evaluation of the performance of the different indices has revealed that the Solway Unweighted ATI proved to be the most acceptable index of choice, mainly due to the following reasons:

- The Solway Unweighted ATI can be seen to share Class categories with at least two to three of the other ATI systems. For instance, at Crocodile farm a Class I water was recorded for the Additive Unweighted, Additive Weighted and Solway Unweighted indices (Table 5.1). At Marievale farm, the Solway Unweighted shared a Class I classification with Additive Unweighted. Vlakfontein farm which had polluted water, shared a Class III with all indices except the Additive Unweighted ATI. Luipaardsvlei produced the same results as the Marievale farm (Table 5.1).
- Due to the sensitivity of the Solway Unweighted ATI, a clear discrimination between unpolluted (Class I : Crocodile farm), moderately polluted (Class II : Marievale and Luipaardsvlei farms) and polluted water (Class III : Vlakfontein farm) was obtained.
- The Solway Unweighted index system was applied to evaluate conditions in the Blesbokspruit where toxic mining effluents were released into the wetland. Results obtained from toxicity tests conducted on the same water, closely correlated with ATI values obtained for the same samples (de Wet, 1996b). Practically applied during various field investigations on other water systems (de Wet, 1996 a-f), this index system proved to be suitable and reliable to evaluate different intensities of water pollution.

It must be accepted that no index system is perfect, neither fool-proof. The Solway Unweighted system is constantly being re-evaluated and efforts are presently being made to further improve it. These improvements, which will be incorporated into the system in future, will adress the following aspects:

- **The present lack of sufficient knowledge or experience by local researchers to assign correct weights to each of the parameters.** Weighting relies on local conditions and may vary geographically. The ultimate aim is therefore to arrive at a point where a reliable set of weightings can be attached to each of the parameters. Eventually, with more information available, the Solway Weighted Aquatic Toxicity Index may possibly be utilized for future water quality evaluations in place of the

Solway Unweighted index system.

- **The role of pH, total hardness and alkalinity of water in the toxicity and bio-availability of metals.** These have not yet been quanitified for local conditions. This aspect should also be taken into consideration and incorporated into the ATI system.

- **Investigation of a number of additional water quality parameters which include Cd and Hg for possible inclusion into the ATI.** This process requires extensive literature surveys, etc. and will in time produce suitable rating curves for each of the parameters employed.

Table 5.1 Comparison between Aquatic Toxicity Index values and water quality classes for each of the sampling localities investigated

Aquatic Toxicity Index	Crocodile farm		Marievale farm		Vlakfontein farm		Luipaardsvlei farm	
	ATI	Class	ATI	Class	ATI	Class	ATI	Class
Additive Unweighted	91.25	I	84.11	I	54.37	II	88.98	I
Additive Weighted	70.82	I	66.91	II	42.13	III	69.75	II
Solway Unweighted	83.76	I	70.74	I	29.56	III	70.27	I
Solway Weighted	50.16	II	44.77	III	17.75	III	47.93	III

5.1.2 Compliance analysis of metal concentrations in water collected at each of the sampling localities

By using a compliance analysis based on the metal water quality criteria for the protection of aquatic life of river and dam water by Kempster *et al.* (1982) (Table 5.2), an almost similar picture emerged concerning the metals Fe, Cu, Ni, Pb, Zn, Mn and Cr at the four localities investigated during this study. Interestingly enough, the water at the Crocodile farm and that of Luipaardsvlei farm both produced a quality of water which now resembles a Class I type with a 100% compliance with the standards laid down. The Marievale farm (86% compliance) resembles on the basis of metal concentrations a Class II water. On the same grounds, water from Vlakfontein farm (14% compliance) resembles a Class III water (Table 5.2). Therefore, on the basis of metal concentration compliance, a reasonably reliable agreement now exists between the ATI system and compliance analysis.

5.2 Metal concentration in the aquatic environment with specific reference to the concentration, bio-accumulation and bio-availability in the organs and tissues of floating, rooted submerged and rooted emergent aquatic macrophytes

In a submerged soil, such as in the case of wetlands where a submerged sediment exists, gas exchange between sediments and the atmosphere is drastically curtailed. Only through molecular diffusion in the interstitial water can oxygen and other atmospheric gases enter the subsurface sediments. According to Lemon & Kristensen (1960) and Greenwood (1961), this

Table 5.2 **Compliance analysis of metal concentrations of water collected at the four sampling localities during this investigation**

Metal	Crocodile Farm	Marievale Farm	Vlakfontein Farm	Luipaardsvlei Farm
Fe	+*	+	+	+
Cu	+	+	-	+
Ni	+	-	-	+
Pb	+	+	-	+
Zn	+	+	-	+
Mn	+	+	-	+
Cr	+	+	-	+
Compliance (%)	**100**	**86**	**14**	**100**

*+ Parameter complies ; - Parameter does not comply (According to values by Kempster *et al.*, 1982)

process of molecular diffusion is approximately 10000 times slower than the same process in terrestrial agricultural soil. In addition, micro-organisms in wetland sediments utilize oxygen present in the water or oxygen trapped in the sediments which may render the sediments devoid of molecular oxygen. Under these conditions, the sediments can be regarded as anoxic. During the present study a scan of oxygen levels of sediments at randomly selected sites in the various localities investigated, revealed concentrations of less than 0,1 mg.ℓ^{-1} throughout. The literature also indicate similar results. For instance, Yamane (1958) reported the absence of oxygen in flooded rice fields, while Yunkervich *et al.* (1966) and Armstrong & Boatman (1967) could not detect any dissolved oxygen in bogs filled with stagnant water. Mortimer (1941 ; 1942) reported the absence of oxygen 1cm below the surface of submerged lake muds, while Scholander *et al.* (1955) recorded no oxygen levels in a mangrove swamp. In addition, low oxidation-reduction potentials reported by Hutchinson (1957) in lake muds and by Ponnamperuma (1965) in rice paddies suggest the absence of molecular oxygen in waterlogged soils and sediments. However, these observations don't imply anything if a link between physical- chemical conditions in wetland sediments and metal uptake by aquatic fauna is not established. In what follows, an attempt will be made to adress this specific aspect by integrating the data of this study and illustrating the concept of metal cycling in the process.

In addition to an absence of dissolved oxygen, the most important chemical difference between submerged sediments and drained, oxygenated soil is that submerged sediments are in a reduced state. Except for posessing a low oxidation-reduction potential, it also contains the reduced counterparts of NO^{3-}, SO_4^{2-}, Mn^{4+}, Fe^{3+}, NH_4^+, H_2S, Mn^{2+}, Fe^{2+} and CH_4 (Ponnamperuma, 1972). Reduction of submerged sediments, in the absence of molecular oxygen, is according to the above author, largely the consequence of anaerobic respiration by bacteria. Together with a decrease in the redox-potential of sediments, an increased mobility and bio-availability of bottom-sediment associated toxic metals is observed (Ponnamperuma, 1972 ; Lin & Benjamin, 1992 ; Rav-Acha & Rebhun, 1992 ; Yoshiyuki *et al.*, 1992). The redox potential, according to the above authors, is also important in the mobilization of molecular iron which, in turn, plays a major role in the bio-availability of several potentially toxic metals.

Numerous investigations in the past have shown that aquatic macrophytes posess the ability to concentrate metals from water and sediments and to produce internal concentrations greater than their surroundings (Abo-Rady, 1980 ; Mortimer, 1985 ; Sridhar, 1986 ; Lyngby & Brix, 1989 ; Van der Merwe *et al.*, 1990 ; de Wet *et al.*, 1990 ; de Wet, 1990 a,b ; Venter, 1991 ; de Wet *et al.*, 1993a ; de Wet, 1994 ; de Wet, 1996b). According to Kovacks *et al.* (1984), aquatic macrophytes are employed with success to evaluate the level of pollution in an affected environment as these plants are often able to accumulate metals in higher concentrations than those present in the water column. However, Mortimer (1985) suggests that the degree of metal uptake is dependent on both the particular metal as well as the plant species accumulating the metal. This view is supported by Guilizzoni (1975) and Ray & White (1976) who recorded variations in metal concentrations between rooted submerged and rooted emergent aquatic macrophytes in the same aquatic environment. This point of view is also shared by local investigations conducted by Van der Merwe (1990), Van der Merwe *et al.* (1990), de Wet (1990 a,b), de Wet *et al.* (1990) and Venter (1991) as well as in the present study which showed marked differences in the metal accumulation abilities of algal, rooted submerged, rooted emergent and floating aquatic weeds. For instance, the above authors unanimously concluded that the freshwater algae, *Spirogyra* sp. posessed an excellent ability to accumulate metals and that submerged aquatic macrophytes such as *P. pectinatus*, *L. muscoides* and *M. macrocarpa* also were efficient accumulators of metals. The present study generally confirmed the results of these authors, showing *Spirogyra* sp. to be amongst the most efficient of all aquatic plants investigated in its concentration of Ni, Cu, Zn and Mn (Table 4.6). The submerged fennel-leaved pondweed, *P. pectinatus* was further shown to be equally successful in the bio-accumulation of Fe, Pb and Cr. However, when metal accumulation by rooted submerged plants is compared with that of rooted emergent macrophytes (*T. capensis*, *P. australis*, etc.), no significant differences could be observed, despite the differences in their ecological status. This, however, does not remove the fact that the rooted component of rooted emergent aquatic weeds are extremely capable of bio-accumulating metals compared to the other organs. This is clearly demonstrated by the present study when the concentrations of all metals are compared for *A. donax* (Tables 4.7 and 4.13), *P. lapathifolium* (Tables 4.8 and 4.12), *T. capensis* (Tables 4.9 and 4.14), *P. australis* (Table 4.10) and *A. plantago-aquatica* (Tables 4.11 and 4.15). However, contradictory views exist about the above observations. For instance, Denny (1980) states that metal enrichment tends to be plant species-specific which is the result of differences in physiological processes and morphological structures such as the thickness of the cuticula, the structure of the vasculature system as well as differences in specific metal absorption structures such as roots and young shoots. On the other hand, it is the opinion of Aulio & Salin (1982) that no significant differences in metal accumulation of plants exists, irrespective the differences of ecological status of rooted submerged, rooted emergent and floating aquatic macrophytes. In the case of all the above observations and statements made, it is evident that submerged emergent and floating aquatic macrophytes also accumulate metals and other nutrients through either submerged stems or leaves and that a relatively smaller fraction could be accumulated through the roots itself. A possible explanation for this could probably be that a larger surface of stem and leaf area is exposed to the water column, especially in the case of totally submerged weeds such as *P. pectinatus*.

It is, however, controversial when the above observations are compared with results obtained during the present investigation (as well as by de Wet, 1990 a,b ; Venter, 1991 ; Van der Merwe, 1990 ; Van der Merwe *et al.*, 1991) for rooted emergent plants where a relatively small portion of the stem tissue is exposed to the water column. It appears in this case that metals are rather primarily accumulated via the submerged roots and rhizomes, and to a lesser extent through the submerged portions of shoots and leaves. In all cases during the present study (Tables 4.7 - 4.15), it was recorded that highest metal concentrations occurred in the roots compared to other organs of these plants. Partially supporting these observations, the results of Erikson & Mortimer (1975) and Ray & White (1976) are mentioned in which it is stated that the roots of emergent aquatic macrophytes may accumulate metals in higher amounts in the roots relative to other plant organs. However, Mortimer (1985) indicates that metals present in above-water (foliar) parts of a plant may not mainly originate from the root tissues, but in fact from the submerged stems and leaves. Through laboratory investigations, the latter author has shown that metals present in the submerged above-sediment plant organs tended to be translocated downwards in the direction of the roots. In effect, Mortimer (1985) actually states that metals present in water is either actively or passively absorbed through submerged stems and leaves and transported to the roots where they are returned to the sediments when the plants die down. This implies that the roots alone may not necessarily be such important organs in metal uptake in these plants, considering the actual component of the roots as part of the total plant. This however, may differ between plant species, depending on the densities and biomass of the root systems. The results obtained during the present investigation supports this statement namely that relatively high metal concentrations are found in the roots and relatively lower concentrations in the emergent foliar parts of emergent aquatic weeds.

To relate to physical and chemical (redox) conditions encountered in wetland sediments discussed previously, it can be accepted that the majority of available metals are, depending on their species, absorbed through the roots of submerged emergent aquatic macrophytes. In the roots, metals are probably transformed to less toxic species (if potentially toxic species are absorbed), where it is speculated that a number of processes may take place:

- metals may be retained in storage organs such as rhizomes. These organs may eventually die off when such metals can be returned to the sediments. It is thought that these metals may either be transformed into another non-toxic species or be bound to organic ligands such as humic or fulvic acids which posess the ability to detoxify metals.

- Being a dynamic process, metal exchange between sediments and roots may constantly take place, during which potentially toxic metal species are accumulated, detoxified within the roots, and returned to the sediments as non-toxic species.

- Active translocation of metals through the stems and leaves of these plants may also take place in which these plant organs may act as temporary reservoirs or storage sites for metals. This mechanism probably takes place during times when over-concentration of metals occurs in the sediments. By transporting metals to other parts of a plant, a dilutive effect is obtained whereby root and rhizome tissues are protected against possible toxification. It can also be speculated that these metals temporarily stored in other organs may eventually be returned to either the sediments or rhizomes.

5.3 Metal concentrations in the terrestrial environment with specific reference to their concentration, bio-accumulation and bio-availability in the tissues of vegetable crops produced at the various sampling localities

5.3.1 Comparison of results on metal concentrations in agricultural soils and vegetable crops obtained during this study with maximum allowable levels

Potentially toxic metals, especially Cu, Mo, Pb, Ni, Co, Cd, Zn and Cr are often encountered in either sewage sludge applied to agricultural land or in irrigation water contaminated with mining and/or industrial effluents. Consequently, metals may be transmitted to various components of the aquatic and terrestrial food chain. Due to their potential toxicity at certain concentration levels, these metals might pose a threat to crop production as well as to human and animal health. Due to the high mobility of cadmium in the soil, this metal is considered as an element which should be limited during sludge disposal on soil (Page & Chang, 1981 ; Korentajer, 1991) as well as in contaminated effluents irrigated on crops. Other metals, such as Zn, Ni and Cu, (due to their potential phytotoxicity), and Mo (to its toxicity to livestock), are also considered as limiting when released in abnormal quantities into the terrestrial environment (CAST, 1976).

Comparing soil metal concentrations measured at the different farms investigated, it is evident that in a number of cases, the South African limits for the maximum permissible content of metals in soil (Table 5.3), were exceeded. Even in the case of the control location (Crocodile farm) (Table 4.16), concentrations of Ni and Pb exceeded the maximum permissible levels for metal in agricultural soil (Table 5.3). In the case of the soil plots where beetroot and carrots were sampled, Cr and Cu also exceeded these limits (Table 4.16). The same applied to the Cr concentrations of the soil where cauliflower was cultivated. These high metal concentrations found could probably be attributed to a number of chemical industries a considerable distance upstream. In the case of metals in the soils of the Marievale Farm (Table 4.17), only Ni exceeded the South African maximum permissible limits for agricultural soil (Table 5.3). Although the high levels of these metals can be connected to the presence of gold and uranium mining activities, it can also be attributed by the presence of a base-metal refinery in the vicinity of this farm.

The situation at the Vlakfontein Farm seemed somewhat worse compared to the other sampling localities. Nickel, Pb and Cr did not in any case (Table 4.18) comply with the maximum permissible soil metal concentrations for South Africa (Table 5.3). Again, in this case, high Fe, Mn, Ni, Pb and Cr levels could have originated from mining and industrial effluents (Förstner & Wittmann, 1976 ; Wittmann & Förstner, 1976). At Luipaardsvlei (Table 4.19) farm, only Ni exceeded the maximum allowable limits for metal content in agricultural soils (Table 5.3).

Considering the high metal concentrations which were encountered in the soils of some of the sampling localities, one would expect that significantly higher metal concentrations could be found in the tissues of exposed vegetable crops. The most effective way to evaluate this was to compare metal concentrations in exposed crops to maximum tolerable levels of metals in plant tissues, either utilized as vegetable crops or as livestock forage (Table 5.4) (After Logan & Chaney, 1983 ; Korentajer, 1991). These guideline values provide an indication of the so-called "phytotoxic level" which is nothing more than a concentration level at which physiological processes in plants are inhibited.

Table 5.3 South African and EEC limits for the maximum permissible content of metals in agricultural soil (After [1]Vivier *et al.*, 1988 ; [2]CEC, 1986)

Metal	[1]RSA ($\mu g.g^{-1}$)	[2]EEC ($\mu g.g^{-1}$)
Cadmium	2	1 - 3
Cobalt	20	-
Chromium	80	-
Copper	100	50 - 140
Mercury	0,5	1 - 1,5
Molybdenum	2,3	-
Lead	56	50 - 300
Nickel	15	30 - 75
Zinc	185	150 - 300

When results obtained during the present study for metal concentrations in the vegetable crops were compared with those in Table 5.4, it was surprising to note, that despite relatively high metal concentrations in the soils, none of the metal concentrations in vegetable tissues from all the farms investigated (Tables 4.16 - 4.19) exceeded any of these guideline values. Contrary to the aquatic macrophytes investigated, it is further evident from the results that vegetable crops do not appear to posess the same ability to concentrate metals from their surrounding environment.

Several factors are known to regulate the availability of metals to terrestrial plants. These include soil pH, organic matter content of the soil as well as the soil cation exchange capacity

(Korentajer, 1991). It is also known that phytological and environmental factors may prevent the excessive accumulation of some metals in plant tissues which might be potentially toxic to animals and man. This phenomenon, known as the "soil-plant barrier" (Sommers & Barbarick, 1986) (Table 5.4) is commonly known to be effective in the regulation of Fe, Ni, Pb, Zn, Cr, B, V and As in some plant tissues. However, this barrier is also known to be ineffective in the regulation of Cd, Mo and Co in plants. The latter metals are therefore capable of accumulating to toxic levels in plants (Korentajer, 1991).

Table 5.4 Maximum tolerable levels of metals (or ranges) in plant tissues either utilized as vegetable crops or as livestock forage (After Logan & Chaney, 1983 ; Korentajer, 1991) with an indication of the failure or success of the "Soil-Plant Barrier".

Metal	Success or failure of the "Soil-Plant Barrier"	Tolerable level ($\mu g.g^{-1}$)
Cadmium	Fails	5 - 700
Cobalt	Fails?	25 - 100
Molybdenum	Fails	100
Selenium	Fails	100
Copper	Succeeds	25 - 40
Manganese	?	400 - 2000
Nickel	Succeeds	50 - 100
Lead	Succeeds	-
Zinc	Succeeds	500 - 1500

Another important mechanism utilized by terrestrial plants as a barrier for the transfer of metals is their ability to restrict their translocation from plant roots to the shoots and fruits. Generally, a distinction can be made between accumulator, indicator and excluder plants. The former accumulate metals in their tissues compared to concentrations in the soil in which they grow while the metal content of indicator plants roughly correspond to that of the soil in which they grow. Contrary to the above, excluder plants are able to discriminate to some extent against some metals by accumulating it in their roots from where they transport metals to edible parts such as leaves and fruit. However, an exhaustion of this storage capacity may eventually lead to a breakthrough of metals to other plant tissues such as leaves and fruits (Pettersson, 1976 ; Baker, 1981 ; Verloo *et al.*, 1983).

Maximum tolerable levels for metals in vegetable crops and plants utilized as livestock forage are summarized in Table 5.4. The existence of the "Soil-Plant Barrier" is also demonstrated in this table. Considering results obtained during the present study, it was evident that all vegetables collected from the different farms, typically behaved as excluder plants. The results also indicate that, although high levels of metal contamination was evident (for the metals investigated) in the irrigation water and soils of most of these farms, the "Soil-Plant Barrier" functioned effectively in regulating potentially toxic metals in these tissues of the vegetable crops analyzed.

5.4 Metal concentrations in the aquatic environment with specific reference to their concentration, bio-accumulation and bio-availability in the tissues of benthic macro-invertebrate fauna collected at the various sampling localities

Compared to the aquatic flora, there is a greater variety of pathways in which metals can be accumulated by the various aquatic macro-invertebrate organisms. In all of these, three basic mechanisms can be identified during the metal accumulation process. This may be mainly via gills and skin surfaces involved in the respiratory process, by adsorption onto the body surface of the organism, and thirdly from solid and/or dissolved nutrients (Förstner & Wittmann, 1981 ; Allan, 1986 ; Allan & Ball, 1990). Respiration in all aquatic fauna permits the uptake of metals from the surrounding water containing the dissolved bio-available metal species. It is, however, the metal uptake from nutrients which is considered to be of greatest importance because of the variations in metal concentrations in the different types of dietary items of the aquatic fauna.

It has been shown that in polluted freshwater ecosystems investigated, metal concentrations in the aquatic fauna may occasionally be higher than those in the sediments, particulate matter and detritus (Förstner & Wittmann, 1981 ; Allan, 1986 ; Allan & Ball, 1990). It is therefore very important to take into account the particular feeding habits and dietary preferences of the specific aquatic fauna which are investigated. Some organisms are for instance phytophagous, as in the case of the gastropod, *Lymnaea* sp. (Förstner & Wittmann, 1981), while the oligochaetes such as the Tubificidae, also investigated during the present study, are known as typically sediment detrital feeders (Appelby & Brinkhurst, 1970 ; Fisher *et al.*, 1980 ; Förstner & Wittmann, 1981 ; Allan, 1986 ; Allan & Ball, 1990). Algal, bacterial and detritus feeders include chironomid larvae and ephemeropteran (*Baetis* sp.) nymphs analysed during this study, whereas the Hemiptera (*Micronecta* sp., *Notonecta* sp., *Plea* sp. and the Veliidae) as well as the Hirudinea are considered to be typically carnivorous organisms. The crustacean, *Potamonautes warreni*, a scavenger, also utilizes benthic macro-invertebrate organisms as food (van Eeden, 1994).

Looking at the numerically dominant taxa of the fauna investigated during the present study, namely the aquatic oligochaeta (Tubificidae) and Chironomidae, certain tendencies concerning the ability and/or the extent of metal accumulation by them were observed. Highest concentrations for most metals were recorded in the Tubificidae from the dam ecosystems (Nigel and Cowles Dam) (Table 4.26). In contrast, in the typical wetland areas such as the Blesbokspruit, the Chironomidae were the organisms which generally contained the highest concentrations of the metals analyzed (R29 Road Crossing: Table 4.26 & Vlakfontein: 4.27). The carnivores such as the Hirudinea occupied variable positions (Van Ryn Lake & Cowles Dam: Table 4.26) in terms of the extent of metal accumulation. The Hemiptera did not show any particular tendency in terms of metal accumulation ability for specific metals. Only *Micronecta* sp. showed a more successful ability to accumulate Fe, Ni, Pb and Cr (Homestead Lake: Table 4.21).

The Tubificidae being active burrowers with their anterior ends down in the sediments where they feed, are also known as "conveyer-belt species" (Fisher *et al.*, 1980 ; Robbins, 1982

; Ankley *et al.*, 1994 ; Reible *et al.*, 1996) digesting and transferring sediment-bound metals from the subsurface layers and depositing faecal pellets on the surface of the substrate of rivers, lakes and wetlands. In the case of the present study, these organisms occurred in extremely high densities in the sediments of wetland areas. Since their dietary habits play a very important role in the bioturbation (Ankley *et al.*, 1994 ; Reible *et al.*, 1996) of organic as well as inorganic (including metals) contaminants from the sediments, they are also potentially capable of translocating bio-available metal species formed in the sediments, to the surface layers of these water bodies from where organisms such as the Chironomidae and even the Ephemeroptera may absorb these metals in a direct (food) or indirect (detritus) way. This may explain the exceptionally high concentrations of all metals recorded for the Chironomidae in the Blesbokspruit (R29 road crossing) (Table 4.21) and from the wetland area near the Vlakfontein farm (Table 4.22).

The crustacean, *Potamonautes warreni*, collected in Van Ryn Lake appears to bio-regulate most metals successfully, except for Cu. Concerning the bio-accumulation of various metals by this organism, the present results showed that the metals Ni, Pb, Zn and Cr in particular are the best bio-regulated by this organism, substantiating the findings of van Eeden & Schoonbee (1991) on this organism from the metal polluted Natalspruit wetlands. It is known that decapod Crustaceans have evolved mechanisms to regulate concentrations of essential, but in some cases potentially toxic metals (Bryan, 1964, 1967). Regulatory mechanisms may involve biochemical detoxification processes (Simkiis, 1981) as well as the production of physiologically inert, insoluble or granular deposits (Rainbow, 1985). Metal-binding proteins may also be involved in the process (Guary & Negrel, 1980).

5.5 The phenomenon and potential value of the Concentration Ratio as well as problems encountered with the interpretation of this particular parameter.

Although the CR-values for the sediments/soils were used to evaluate the ability (or potential) of the aquatic flora and vegetables as well as the fauna to bio-accumulate metals, there were a number of factors which affected the application of CR as a measure to evaluate plants and animals as indicator species for metal pollution in the aquatic and terrestrial environments. Bain *et al.* (1994) discussed the merits and weaknesses of the CR and its use to demonstrate the possible transfer of radionuclides in the food-chain system with special reference to the transfer of uranium and ^{226}Ra from contaminated water to vegetable crops. A serious weakness in the present study was the unqualified use of CR as such, without taking into consideration the speciation of metals and the potential bio-availability of only a fraction of the total metal concentration measured in the sediments at a particular site. This clearly leads to the distortion of the actual picture of the ability of aquatic and terrestrial vegetation as well as the aquatic fauna to accumulate metals in their organs and tissues. It also clearly influences the entire interpretation of the actual bio-magnification of metals in organisms and the transfer of potentially available toxic metals within the food web of aquatic and terrestrial ecosystems.

Judged by the possible effects of the combination of physical, chemical and biological factors which all may play a role in concert in the mechanisms of speciation of metals in the terrestrial and aquatic environments, it is inevitable not to over-estimate the actual values obtained for CR in this investigation as well as in many of these research projects where CR is purely calculated for a metal in the abiotic and biotic environments. As a result, no accurate reliable construction of pathways of metals within the aquatic and terrestrial environments can yet be made.

6 RECOMMENDATIONS

From the results obtained, a number of weaknesses and/or shortcomings in the research approach have been identified and also highlighted during the discussion. They are:

- **Refinement of the Aquatic Toxicity Index (ATI) system.** This is presently based on the total metal contents of the water column of affected waters. Attention must be given to the interaction of parameters such as Dissolved Oxygen, pH, Total Hardness, Alkalinity as well as the direct and indirect involvement of the aquatic fauna and flora in the manipulation of factors which affect the chemical speciation and consequently the bio-availability of particular metals.

- **Bioturbation of the bottom sediments by aquatic fauna.** It is known that the soft-bottomed substrate of aquatic habitats can serve as a sink for metals. It is also known that a combination of physical, chemical and biological mechanisms such as bioturbation may be responsible to return these metals to the water column from where thay can be re-utilized and circulated in the water-biota interface. Much more need to be done to evaluate the extent of this recirculation process of metals deposited in the bottom sediments of metal polluted rivers, dams and wetlands.

- **Metals which cross the soil-plant-barrier.** These metals which include Cd and Hg need also be investigated to determine concentration levels at which this this barrier is broken.

- **Identification of indicator organisms for metal pollution in aquatic and terrestrial ecosystems.** The present study has already shown that certain aquatic plants, vegetables as well as aquatic fauna may be extremely useful as potential indicator organisms for metal pollution in mine- and industry polluted areas. The time has come to combine field data with experimental evidence on these and other organisms to evaluate their ability to accumulate the bio-available components of metals which occur in industrial and mine effluent. Proposed indicator aquatic plants identified during the present study to evaluate metal accumulation ability are listed in Table 6.1. Regarding vegetable crops, spinach, carrot and beetroot posess some potential to be regarded as vegetable indicator species.

- **Refinement of the concept of the Concentration Ratio.** The value and potential usefulness of the Concentration Ratio must, on results obtained during the present study, as well as work done by other research workers, be emphasized. However, it still requires a considerable refinement before its unqualified acceptance as a measure of the ability of organisms to accumulate metals in their organs and tissues. In order to achieve this objective, a better understanding of the bio-availability as well as absorption of metals by the affected plants and animals is required.

Table 6.1 Proposed aquatic macrophytes for use as indicator species for the detection of metal pollution in the aquatic environment

Plant species	Common name	Ecological status	Season for best results	Accumulative organs	Metals accumulated*	References
Spirogyra sp.	Spirogyra	SA	All year	WP	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 2, 6, 10
Nitella sp.	Coarse alga	SA	All year	WP	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 10
Azolla filiculoides	Water fern	FA	Summer	WP	Fe, Zn, Mn, Cu, Pb (Cr, Ni)	2, 3, 10
Lemna gibba	Duckweed	FA	All year	WP	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	2, 10
Potamogeton pectinatus	Fennel-leaved pondweed	SA	All year	ST, LF	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 6, 8, 9, 10
Typha capensis	Bulrush	EA	Summer	RT, RZ, GS, WS, LF, FL	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 2, 4, 5, 6, 7, 8, 9, 10
Phragmites australis	Common reed	EA	Summer	RT, RZ, GS, WS, LF, FL	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 2, 4, 5, 6, 7, 8, 9, 10
Arundo donax	Spanish reed	EA	Summer	RT, RZ, GS, WS, LF, FL	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 4, 5, 10
Polygonum lapathifolium	Cock's comb	MP	Summer	RT, RZ, GS, WS, LF, FL	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 2, 7, 8, 10
Alisma plantago-aquatica	Water plantain	EA/MP	Summer	RT, RZ, GS, WS, LF, FL	Fe, Zn, Mn (Cu, Ni, Pb, Cr)	1, 2, 7, 8, 9, 10

*Metals accumulated in lower concentrations indicated in brackets

Abbreviations: SA = Submerged Aquatic ; FA = Floating Aquatic ; EA = Emergent Aquatic ; MP = Mud Plant (Venter 1991b)

Plant organs: WP = Whole Plant ; ST = Stem ; LF = Leaves ; RT = Roots ; RZ = Rhizome ; GS = Green Stem ; WS = White Stem ; FL = Florescence

References: [1]de Wet (1990a) ; [2]de Wet (1990b) ; [3]de Wet *et al.* (1990) ; [4]Van der Merwe (1990) ; [5]Van der Merwe *et al.* (1990) ; [6]Venter (1991a) ; [7]de Wet *et al.* (1993a) ; [8]de Wet (1994) ; [9]de Wet (1996b) ; [10]Schoonbee *et al.* (1996)

REFERENCES

7

7 REFERENCES

ABO-RADY, M.D.K. 1980. Aquatic macrophytes as indicators for heavy metal pollution in the River Leine, West Germany, **Arch. Hydrobiol.**, 89: 387 - 404.

ADRIANO, D.C. 1986. **Trace Elements in the Terrestrial Environment.** Springer-Verlag. New York.

AGGET, P.J. & HARRIES, J.T. 1979. Current status of zinc in health and disease states. **Arch. Dis. Child.**, S4: 909-917.

AHRLAND, S. 1985. **Inorganic Chemistry of the Ocean.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

ALLAN, R.J. 1979. **Sediment-related fluvial transmission of contaminants: some advances in 1979.** Scientific Series No. 107. Environment Canada. Ottawa.

ALLAN, R.J. 1986. **The Role of Particulate Matter in the Fate of Contaminants in Aquatic Ecosystems.** Scientific Series No. 142. Inland Waters Directorate. National Water Research Institute. Canada Centre for Inland Waters. Burlington. Ontario.

ALLAN, R.J. & BALL, A.J. 1990. An overview of toxic contaminants in water and sediments of the Great Lakes. **Water Poll. Res. J. Canada**, 25(4): 387 - 505.

ANDERSON, R.A. & MERTZ, W. 1977. Glucose tolerance factor: an essential dietary agent. **Trends Biochem. Sci.**, 2: 277-279.

ANDERSON, P.D. & SPEAR, P.A. 1980. Copper pharmacokinetics in fish gills - I. Kinetics in pumpkinseed sunfish, *Lepomis gibbosus*, of different body sizes. **Water Res.**, 14: 1101 - 1105.

ANKLEY, G.T., LEONARD, E.N. & MATTSON, V.R. 1994. Prediction of bioaccumulation of metals from contaminated sediments by the oligochaete, Lumbriculus variegatus. **Water Res.**, 28(5): 1071 - 1076.

ANON. 1968. Epidemic cardiac failure in beer drinkers. **Nutr. Rev.**, 26: 173.

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA) 1989) **Standard methods for the examination of water and wastewater.** 17th Edition. American Public Health Association, Washington.

APPLEBY, A.G. & BRINKHURST, R.O. 1970. Defecation rate of three tubificid oligochaetes found in the sediment of Toronto Harbour, Ontario. **J. Fish Res. Board Can.**, 27(11): 1971 - 1982.

ARMSTRONG, W. & BOATMAN, D.J. 1967. **J. Ecol.**, 55: 101 - 110.

ASTRUC, M. 1986. Evaluation of methods for the speciation of cadmium. In: **Cadmium in the Environment** (H. MISLIN & O. RAVERA (Eds.). Birkhauser Verlag. Basel. pp. 12 - 17.

AULIO, K. & SALIN, M. 1982. Enrichement of copper, zinc, manganese and iron in five species of pondweeds (*Potamogeton* spp.). **Bull. Environ. Contam. Toxicol.**, 29: 320 - 325.

BACCINI, P. & ROBERTS, P.V. 1976. Die belastung der gewasser durch metalle. **Beil. Forsch. Tech. Neue Zurcher Z.**, 18: 57-58.

BAIN, C.A.R., SCHOONBEE, H.J., DE WET, L.P.D. & HANCKE, J.J. 1994. **Investigations into the Concentration Ratios of selected Radionuclides in Aquatic Ecosystems affected by Mine Drainage Effluents with reference to the study of Potential Pathways to Man.** WRC Report No. 313/1/94. Water Research Commission. Pretoria. 95pp.

BAKER, A.J.M. 1981. Accumulators and excluders - strategies in the response of plants to heavy metals. **J. Plant Nut.**, 3: 643 - 654.

BELLAMA, J.M., JEWETT, K.L. & NIES, J.D. 1985. **Rates of Methylation of Mercury (II) Species in Water by Organotin and Organosilicon Compounds.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

BERROW, M.L. & BURRIDGE, J.C. 1981. **Persistence of Metals in Available Form in Sewage Sludge Treated Soils under Field Conditions.** In: Proceedings of International Conference on Heavy Metals in the Environment. Amsterdam. pp. 202 - 205. CEP Consultants Ltd. Edinburg.

BEST, C.D. & TAYLOR, N.B. 1966. **The physiological basis of medical practice.** Williams & Wilkins Co. Baltimore.

BEWERS, J.M., BARRY, P.J. & MacGREGOR, D.J. 1987. Distribution and cycling of cadmium in the environment. In: **Cadmium in the Aquatic Environment.** (J. NRIAGU & J. SPRAGUE. Eds.). Wiley Interscience. New York. pp. 1 - 18.

BHARGAVA, D.S. 1983. Use of a water quality index for river classification and zoning of Ganga River. **Environ. Pollut.**, 6: 51-67.

BHARGAVA, D.S. 1985. Expression of drinking water supply standards. **J. Env. Eng.**, 111(3): 304-316.

BLOOM, H. & AYLING, G.M. 1977. Heavy metals in the Derwent Estuary. **Environ. Geol.**, 2: 3-22.

BOWEN, H.J.M. 1979. In: **Environmental Chemistry of the Elements.** p. 333. Academic Press. London.

BRICKER, O.P. 1985. **Environmental Factors in the Inorganic Chemistry of Natural Systems: The Estuarine Benthic Sediment Environment.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

BRINKMAN, F.E. 1985. **Environmental Inorganic Chemistry of Main Group Elements with Special Emphasis on Their Occurrence as Methyl Derivatives.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

BROWN, B.E. 1977. Effects of mine drainage on the River Hayle, Cornwall. Factors affecting concentrations of copper, zinc and iron in water, sediments and dominant invertebrate fauna. **Hydrobiologia**, 52(2-3): 221 - 233.

BROWN, R.M., McCLELLAND, N.I., DEININGER, R.A. & TOZER, R.G. 1970. A water quality index - do we dare? **Water Sewage Wks.**, 117: 339-343.

BROWN, R.M., McCLELLAND, N.I., DEININGER, R.A. & O'CONNOR, M.F. 1972. A water quality index - crashing the psychological barrier. **Proc. 6th Annual Conference "Advances in Water Pollution Research".** pp. 787-794.

BROWN, D.A. & PARSONS, T.R. 1978. Relationship between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncorynchus keta*) exposed to mercury in a controlled ecosystem. **J. Fish. Res. Bd. Can.**, 35: 880.

BRYAN, G.W. 1966. The metabolism of Zn and 65Zn in crabs, lobsters and freshwater crayfish. In: **Radio-ecological concentration processes.** (B. ABERG & F. HUNGATE. Eds.). Proceedings of the International Symposium. Stockholm. Permagon Press. Oxford: 1005 - 1016.

BRYAN, G.W. 1976. **Some aspects of heavy metal tolerance in aquatic organisms. In: Effects of pollutants on aquatic organisms.** (A.P.M. LOCKWOOD. Ed.). Cambridge University Press. Cambridge. pp. 7 - 34.

BRYAN, G.W. & HUMMERSTONE, L.G. 1973. Adaptation of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of zinc and cadmium. **J. Mar. Biol. Assoc.**, 53: 839 - 857.

BURROWS, D. 1983. In: **Chromium: Metabolism and Toxicity.** CRC, Boca Raton, Fla.

BURROWS, I.G. & WHITTON, B.A. 1983. Heavy metals in water, sediments and invertebrates from a metal-contaminated river free of organic pollution. **Hydrobiologia**, 106: 263 - 273.

Council for Agricultural and Scientific Technology (CAST). 1976. **Application of sewage sludge to cropland: Appraisal of potential hazards of heavy metals to plants and animals.** Counc. for Agric. Sci. Techn. No. 64 Ames, Iowa. USA.

CAMPBELL, P.G.C., & TESSIER, A. 1985. Metal speciation in natural waters: Influence of environmental acidification. In: **Sources and Fates of Aquatic Pollutants** Chapter 7 (Eds: HITES, RA & EISENREICH, SJ) Amer. Chem. Soc., Washington DC:185-207.

Commission of the European Communities (CEC). 1986. Council Directive of June 12, 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. **Off. of the Eur. Communities**, No. L181/6-12.

CHAMBERS, R.W. & EADIE, B.J. 1981. Nepheloid and suspended particulate matter in south-eastern Lake Michigan. **Sedimentology**, 28: 439 - 447.

CHARLTON, M.N. 1983. Downflux of sediment, organic matter and phosphorous in the Niagra River area of Lake Ontario. **J. Great Lakes Res.**, 9(2): 201 - 211.

CHATTERJI, S.K. & GANGULY, H.D. 1950. Copper in human urine and faeces. **Indian J. Med.**, 38: 303-314.

CHENEY, M.A., HACKER, C.S. & SCHRODER, G.D. 1981. Bioaccumulation of lead and cadmium in the Louisiana Heron (Hydranassa tricolor) and the Cattle Egret (Bubulcus ibis). **Ecotoxicol. Environ. Safety**, 5: 211 - 224.

CHERNOFF, B. & DOOLEY, J.K. 1979. Heavy metals in relation to the biology of the mummichog, *Fundulus heteroclitus*. **J. Fish. Biol.**, 14: 309 - 328.

CHUTTER, F.M. 1972. An empirical biotic index of the quality of water in South African streams and rivers. **Wat. Res.**, 6: 19-30.

CHING-I, L. & HONGXIAO, T. 1985. **Chemical Studies of Aquatic Pollution by Heavy Metals in China.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

CHOPPIN, G.R. 1985. **Speciation of Plutonium in Seawater and Freshwater.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

COGHLAN, A. 1991. Europe plans for cleaner water. **New Scientist**, 16 March 1991. 38 - 42.

CRASS, R.S. 1947. The May-Flies (Ephemeroptera) of Natal and the Eastern Cape. **Ann. Natal Mus.**, Part 1. 11: 37 - 110.

CROOKE, W.M. 1964. The Measurement of Cation-exchange Capacity of Plant Roots. **Plant Soil**, 21: 43 - 49.

CUMMINGS, J.M. No Date. **Trace elements in the brain in health and in neurological disease. The scientific basis of medicine.** University of London. London.

DANIELS, A.L. & WRIGHT, O.E. 1934. Iron and copper retention in young children. **J. Nutr.**, 18: 125-138.

DAVIES, L.J.T. 1972. **The Clinical Significance of the Essential Biological Metals.** Wm. Heinemann Medical Books. London.

DEAN, J.C., BOSQUI, F.L. & LANOUETTE, K.H. 1972. Removing heavy metals from wastewater. **Environ. Sci. & Technol.**, 6: 518 - 522.

DE MARCH, B.G.E. 1976. Spatial and temporal patterns in macrobenthic stream diversity. **J. Fish. Res. B. Can.**, 33: 1261 - 1270.

DENNY, P. 1980. **Biol. Rev.**, 55: 65.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWA&F). 1993. **South African Water Quality Guidelines. Volume 4. Agricultural Use.** Department of Water Affairs and Forestry. Pretoria.

DE WET, L.M. 1990a. **Akkumulering van swaarmetale in 'n myn- en nywerheidsbesoedelde meerekosisteem.** M.Sc. Thesis. Randse Afrikaanse Universiteit. Johannesburg.

DE WET, L.M., SCHOONBEE, H.J., DE WET, L.P.D. & WIID, A. 1994. Bioaccumulation of metals by the southern mouthbrooder, Pseudocrenilabrus philander (Weber, 1897) from a mine-polluted impoundment. **Water SA**, 20(2): 119 - 126.

DE WET, L.P.D. 1990b. **'n Ekologiese Studie van die Blesbokspruitvlei-ekosisteem aan die Oos-Rand met Spesiale Verwysing na die Besoedelingstoestande in die Cowles- en Nigeldamme.** M.Sc. Thesis. Randse Afrikaanse Universiteit. Johannesburg.

DE WET, L.P.D., SCHOONBEE, H.J., PRETORIUS, J. & BEZUIDENHOUT, L.M. 1990. Bioaccumulation of selected heavy metals by the water fern, Azolla filiculoides Lam. in a wetland ecosystem affected by sewage, mine and industrial pollution. **Water SA.**, 16(4): 281 - 286.

DE WET, L.P.D., SCHOONBEE, H.J., DE WET, L.M. & VAN DER MERWE, C.G. 1991. Wetlands as water purification systems with special reference to the potential of some of its components to capture heavy metals. **Proceedings of the East Rand Wetlands Symposium.** 17 Oct. 1991. pp. 14 - 33. Suikerbosrand Nature Reserve. Heidelberg.

DE WET, L.P.D., SCHOONBEE, H.J., PRETORIUS, J. & DE WET, L.M. 1993a. Distribution and accumulation of selected metals in some organs and tissues of semi-aquatic macrophytes from a metal polluted wetland ecosystem. **Southern African Society of Aquatic Scientists. Annual Conference on Aquatic Ecosystems.** July 6-9, 1993. Johannesburg.

DE WET, L.P.D., SCHOONBEE, H.J. & DE WET, L.M. 1993b. Observations on the accumulation of selected metals in sediment layers of a mine and industrial polluted wetland ecosystem. **Southern African Society of Aquatic Scientists. Annual Conference on Aquatic Ecosystems.** July 6-9, 1993. Johannesburg.

DE WET, L.P.D., SCHOONBEE, H.J., PRETORIUS, J. & DE WET, L.M. 1993c. Bioconcentration of selected metals in vegetable crops affected by goldmine and industrial effluents. **Southern African Society of Aquatic Scientists. Annual Conference on Aquatic Ecosystems.** July 6-9, 1993. Johannesburg.

DE WET, L.P.D. 1994. **Impact of the Blesbokspruit on the natural environment: application of integrated water quality monitoring in the assessment of ecosystem health.** Waterlab Research (Pty) Ltd Environmental Report BB/1/94.

DE WET, L.P.D. 1995. **The possible effects of elevated temperature and TDS concentrations on the life history stages (egg-adult) of fish and benthic fauna in the Blesbokspruit.** Waterlab Research (Pty) Ltd Environmental Report BB/1/95.

DE WET, L.P.D. 1996a. **Ecosystem health assessment and baseline conditions of the stream flowing through Blyvooruitzicht Gold Mine: Carletonville.** Waterlab Research (Pty) Ltd Environmental Report BV/1/96.

DE WET, L.P.D. 1996b. **Environmental Impact Assessment of underground mine water on the aquatic health and integrity of the Blesbokspruit wetland.** Waterlab Research (Pty) Ltd Environmental Report BB/1/96.

DE WET, L.P.D. 1996c. **Eco-Health Assessment of the Waterval River and tributaries in the vicinity of the Evander Goldfield.** Waterlab Research (Pty) Ltd Environmental Report WH/1/96.

DE WET, L.P.D. 1996d. **Ecosystem Health Assessment of the Mpisi and Ngulane rivers in the vicinity of Mpisi Collieries.** Waterlab Research (Pty) Ltd Environmental Report MP/1/96.

DE WET, L.P.D. 1996e. **Ecosystem Health Assessment and Environmental Impact Assessment of a proposed cement mining operation in the vicinity of the Ngwenyana River, Kidds Beach (East London).** Waterlab Research (Pty) Ltd Environmental Report BC/1/96.

DE WET, L.P.D. 1996f. **Environmental Impact Assessment of the Herbert Bickley sewage works effluent on the Blesbokspruit.** Waterlab Research (Pty) Ltd Environmental Report HB/1/96.

DIETZ, F 1973. The enrichment of heavy metals in submerged plants. In: S.M. JENKINS (Ed.) **Advances in water pollution research.** Pergamon Press. 6: 53 - 62.

DIXIT, S.S. & WITCOMB, D. 1983. Heavy metal burden in water, substrate, and macroinvertebrate body tissue of a polluted River Irwell (England). **Environ. Pollut.**, B6: 161 - 172.

DOISY, E.A. 1972. **Micronutrient controls on biosynthesis of clotting proteins and cholestrol.** In: Trace substances in environmental health-VI. (D.D. HEMPHILL, Ed.). University of Missouri Press. Columbia.

DOLL, R., MATHEWS, J.D. & MORGAN, L.G. 1977. Cancers of the lung and nasal sinuses in nickel workers: a reassessment of the period of risk. **Br. J. Ind. Med.**, 34: 102-105.

Environment Canada. 1987. **Canadian Water Quality Guidelines.** Report prepared by the Task Force on water quality guidelines of the Canadian Council of Resource and Environment Ministers. 407 pp.

EPA, 1986. **Air quality criteria document for lead.** Environmental Criteria and Assessment Office. US Environmental Protection Agency. Research Triangle Park. North Carolina.

ERIKSON, C. & MORTIMER, D.C. 1975. Mercury uptake in rooted higher aquatic plants; laboratory studies. **Verh. Internat. Verein. Limnol.**, 19: 2087 - 2093.

European Inland Fisheries Advisory Commission (EIFAC). 1980. **Water Quality Criteria for European Freshwater Fish. Report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water.** EIFAC Technical Paper No. 37. Rome: European Inland Fisheries Advisory Commission, Food and Agriculture Organisation of the United States.

EYRES, J.P., & PUGH-THOMAS, M. 1978. Heavy metal pollution of the River Irwell (Lancashire, UK) demonstrated by analysis of substrate materials and macroinvertebrate tissue. **Environ. Pollut.**, 16: 129 - 136.

FISHER, J.B., LICK, W.J., MCcCALL, P.L. 1980. Vertical mixing of lake sediments by tubificid oligochaetes. **J. Geophys. Res.**, 85(7): 3997 - 4000.

FLEISCHER, C.L. 1993. **Bio-akkumulering van metale in organe en weefsels van die Platanna Xenopus laevis in myn- en nywerheidsbesoedelde varswaterekosisteme.** Unpublished M.Sc. thesis, Rand Afrikaans University, Johannesburg, 260 pp.

FöRSTNER, U. 1976. Lake sediments as indicators of heavy-metal pollution. **Naturwissenschaften**, 63: 465-470.

FöRSTNER, U. 1986. Cadmium in sediments. In: **Cadmium in the Environment** (H. MISLIN & O. RAVERA. Eds.). Birkhauser Verlag. Basel. pp. 40 - 46.

FöRSTNER, U. & PROSI, F. 1979. **Heavy metal pollution on freshwater ecosystems.** In: Ravera, O (ed.) Biological Aspects of Freshwater Pollution. Pergamon Press, Oxford.

FöRSTNER, U. & WITTMANN, G.T.W. 1981. **Metal Pollution in the Aquatic Environment.** Springer-Verlag. Berlin.

FOULKES, E.C. (Ed.). 1981. **Biological roles of metallothionein.** Elsevier. New York.

FOURIE, H. 1991. Using plants to clear water. **Resource**, May-June 1991. 12 - 13.

FRANZIN, W.G. & McFARLANE, G.A. 1980. An analysis of the aquatic macrophyte, *Myriophyllum exalbescens*, as an indicator of aquatic ecosystems near a base metal smelter. **Bull. Environ. Cont. Toxicol.**, 24: 597 - 605.

FREGERT, S., HJORT, N. & MAGNUSSON, B. 1969. Epidemiology of contact dermatitis. **Trans. St. Johns Hosp. Derm. Soc.**, 55: 71-86.

FRIBERG, F., NILSSON, L.M., OTTO, C., SJöSTRöM, P., SVENSSON, B.W., SVENSSON, B.J. & ULFSTRAND, L. 1977. Diversity and environments of benthic invertebrate communities in south Swedish streams. **Archiv. fuer Hydrobiol.**, 81: 129 - 154.

GAMBRELL, R.P., KHALID, R.A., COLLARD, V.R., REDDY, C.N. & PATRICK, W.G. Jr. 1976. The effect of pH and redox potential on heavy chemistry in sediment-water systems affecting toxic metal bioavailability. **Dredging: Environmental Effects and Technology, Proceedings ofthe World Dredging Conference**, July 10-12, 1976. San Francisco, CA Wodcon Assn. 1976: 579 - 604.

GAMBRELL, R.P. & PATRICK, W.H. 1978. In: (D.D. HOOK & R.M.M. Eds.). **Plant Life in Anaerobic Environments.** pp. 375 - 423. Ann Arbor Publishers. Ann Arbor. Michigan.

GARDINER, J. & ZABEL, T. 1989. **United Kingdom Water Quality Standards arising from European Community directives - an update.** Water Research Centre Report No. PRS 2287-M.

GAUGLHOFER, J. & BIANCHI, V. 1991. Chromium. In: **Metals and Their Compounds in the Environment.** (E. MERIAN. Ed.). VCH. Weinheim. 1438 pp.

GERSBERG, R.M., ELKINS, B.V. & GOLDMAN, C.R. 1983. Nitrogen removal in artificial wetlands. **Water Res.**, 17(9): 1009 - 1014.

GERSBERG, R.M., ELKINS, B.V., LYON, S.R. & GOLDMAN, C.R. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. **Water Res.**, 20(3): 363 - 368.

GLAISTER, J. & RENTOUL, E. 1966. **Medical jurisprudence and toxicology.** E. & S. Livingstone, Ltd. London.

GOLDEN, M.N.H., GOLDEN, B.E. & HARLAND, P.S.E.G. 1978. Zinc and immuno competence in protein-energy malnutrition. **Lancet**, 1: 1226-1227.

GONCALVES, M.L.S., SIGG, L. & STUMM, W. 1987. Metal Ion Binding by Biological Surfaces; Voltammetric Assessment in Presence of Bacteria. **W. Nurnberg Spec. Issue, Sci. Total Environ.**, 60: 105-119.

GOODYEAR, C.P. & BOYD, C.E. 1972. Elemental composition of largemouth bass (*Micropterus salmoides*). **Trans. Amer. Fish. Soc.,** 3: 545 - 547.

GUARY, J.C. & NEGREL, R. 1980. Plutonium and iron association with metal-binding proteins in the crab *Cancer pagurus* (L.). **J. Exp. Mar. Biol. Ecol.**, 42: 87 - 98.

GUILIZZONI, P. 1975. Manganese, copper and chromium content in macrophytes of Lake Endine (Northern Italy). **Mem. 1st. Ital. Idrobiol.**, 32: 313 - 332.

GUSEK, J. Constructed wetlands - passive treatment of mine drainage. **Supplement to Mining Journal**, 316(8110): 6.

HAMILTON, J.W. & WETTERHAHN, K.E. 1988. **Chromium.** In: Handbook on Toxicity of Inorganic Compounds. (H.G. SEILER, & H. SIGEL, Eds.). Marcel Dekker, Inc. New York.

HANCOCK, R.D. 1985. **Factors Influencing the Coordination Chemistry of Metal Ions in Aqueous Solutions.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

HARDING, J.P. 1961. Some South African Cladocera collected by Dr. A.D. Harrison. Part III. **Ann. S.A. Mus.**, XLVI: 35 - 46.

HARLAND, B.F., HARWOOD, J.P. & THATCHER, R.W. 1982. **Hair cadmium and lead in learning-disabled children.** In: Trace metabolism in man and animals (TEMA-4). (J.McG. HOWELL, J.M. GAWTHORNE & C.L. WHITE, Eds.). pp. 407-408. Australian Academy of Science, Canberra.

HARRISON, A.D. 1958. The effects of sulfuric acid pollution on the biology of streams in the Transvaal, South Africa. **Verh. internat. Verein. Limnol.**, 13: 603 - 610.

HARRISON, A.D. 1961. **Some environmental effects of coal and gold mining on the aquatic biota.** In: Biological Problems in Water Pollution: The Relaxation of Land Use to Aquatic Environment. Third seminar, PAS Publ. 999-WP-25:270-274.

HART, B.T. 1974. **A Compilation of Australian Water Quality Criteria.** Australian Water Resources Council Technical Paper No. 7.

HART, B.T. 1982. Uptake of trace metals by sediments and suspended particulates: a review. **Hydrobiologia**, 91: 299-313.

HEATH, A.G. 1987. **Water Pollution and Fish Physiology.** CRC Press, Inc. Boca Raton, Florida.

HELLAWELL, J.M. 1986. **Biological Indicators of Freshwater Pollution and Environmental Management.** Elsevier Applied Science Publishers Ltd. London. 546pp.

HENNIG, H.F.-K.O. 1986. Metal-binding proteins as metal pollution indicators. **Environ. Health Perspect.**, 65: 175-187.

HILDEBRAND, C.E., GRIFFITH, J.K., TOBEY, R.A., WALTERS, R.A. & ENGER, M.D. 1982. 1982. **Molecular mechanisms of cadmium detoxification in cadium-resistant cells: Role of metallothionein and other inducible factors.** In: (H.G. SEILER & H. SIGEL, Eds.). Biological roles of metallothionein. Marcel Dekker Inc. New York.

HOCHELLA, M.F. & WHITE, A.F. (Eds.). 1990. **Mineral-Water Interface Geochemistry. Reviews in Mineralogy.** Vol. 23. 603 pp.

HOPPS, H.C. 1971. **Environmental geochemistry in health and science.** (H.L. CANNON & H.C. HOPPS, Eds.). Geological Soc. Am. Memoir 123. Boulder.

HOUBA, C., REMACLE, J., DUBOIS, D. & THOREZ, J. 1983. Factors affecting the concentrations of cadmium, zinc, copper and lead in the sediments of the Vedre River. **Wat Res.**, 17(10): 1281-1286.

HOUSE, M.A. & ELLIS, J.B. 1980. Water quality indices: an additional management tool? **Prog. Wat. Tech.**, 13: 413-423.

HOUSE, M.A. 1989. A water quality index for river management. **J. IWEM.**, 3: 336-344.

HULSE, M., MAHONEY, J.S., SCHRODER, G.D., HACKER, C.S. & PIER, S.M. 1980. Environmentally acquired lead, cadmium and managanese in the Cattle Egret, *Bubulcus ibis*, and the Laughing Gull, *Larus atricilla*. **Arch. Environ. Contam. Toxicol.**, 9: 65 - 78.

HURLEY, L.C. 1982. **Clinical and experimental aspects of manganese in nutrition.** In: Clinical, biochemical and nutritional aspects of trace elements. (A.S. PRASAD, Ed.). pp. 369-378. Alan R. Liss, Inc. New York.

HUTCHINSON, G.E. 1957. **A Treatise on Limnology.** Vol 1. Wiley. New York.

HYNES, H.B.N. 1964. The use of biology in the study of water pollution. **Chem. Ind.**, 435-436.

IRGOLIC, K.J., MARTELL, A.E. and Workshop Participants. 1985. **Environmental Inorganic Chemistry: Workshop Summary.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

JAWORSKI, J.F. 1984. **Routes of Exposure to Humans and Bioavailability.** In: Changing Metal Cycles and Human Health. (J.O. NRIAGU, Ed.). pp. 375 - 388. Dahlem Konferenzen 1984. Berlin. Springer-Verlag.

JAMESON, S. 1980. **Zinc and pregnancy**. In: Zinc and the environment, part II: health effects. (J.O. NRIAGU, Ed.). pp. 183-197. John Wiley. New York.

JEEJEEBHOY, K.N., CHU, R.C. & MARLISS, E.G. 1977. Chromium deficiency, glucose intolerance and neuropathy reversed by chromium supplementation in a patient receiving long-term total parenteral nutrition. **Am. J. Clin. Nutr.**, 30: 531-538.

JENNE, E.A. 1977. **Trace element sorption by sediments in soils: sites and processes.** In: Symposium on Molybdenum in the Environment. Vol. 2. (W. CHAPELL & K. PETERSON, Eds.) Marcell Dekker. New York.

JESSOP, N.M. 1970. **Biosphere - a study of life.** Prentice-Hall, Inc. Englewood Cliffs.

JOHNSON, W.T. & NORDLIE, R.C. 1977. Differential effects of Cu^{2+} on carbamoyl phosphate: glucose phospho-transferase and glucose-6-phosphate phosphohydrolase activities of multi-functional glucose-6-phosphatase. **Biochemistry**, 16: 2458-2466.

JOHNSON, M.G. 1987. Trace element loadings to sediments of fourteen Ontario lakes and correlations with concentrations in fish. **Can. J. Fish. Aquat. Sci.**, 44: 3 - 13.

KAGI, J.H.R. & NORDBERG, M. (Eds.). 1979. **Metallothionein: Proceedings of the First International Meeting on metallothionein and other low molecular weight metal-binding proteins.** Zurich, July 17-22. 1978. Birkhauser Verlag. Boston.

KEMPSTER, P.L., HATTINGH, W.A.J. & VAN VLIET, H.R. 1982. **Summarised Water Quality Criteria.** Department of Water Affairs. South Africa. Technical Report No. TR108. 45pp.

KOLKWITZ, R. & MARSSON, M. 1908. Okologie der pflanzlichen Saprobien. **Ber. dt. bot. Ges.**, 26: 505-519.

KOLKWITZ, R. & MARSSON, M. 1909. Okologie der tierische Saprobien. Beitrage zur Lehre von der biologische Gewasserbeurteilung. **Int. Rev. Hydrobiol.**, 2: 126-152.

KOLKWITZ, R. 1950. Oekologie der Saprobien. Uber die Beziehungen der Wasserorganismen zur Umwelt. **Schr. Reiche Ver. Wasserhyg.**, 4: 64.

KORENTAJER, L. 1991. A review of the agricultural use of sewage sludge: benefits and potential hazards. **Water SA**, 17(3): 189 - 196.

KOVACS, M., NAYARY, I. & TOTH, L. 1984. The microelement content of some submerged and floating aquatic plants. **Acta Bot., 30**: 173-185.

LANDSDOWN, R. & YULE, W. 1986. **Lead toxicity.** John Hopkins University Press. Baltimore.

LANDWEHR, J.M. & DEININGER, R.A. 1976. A comparison of several water quality indexes. **J. Wat. Pollut. Control Fed.**, 48: 954-958.

LANGELIER, W.F. 1936. The analytical control of anti-corrosion water treatment. **AWWA**, 28: 1500.

LEACH, R.M. & LILBURN, M.S. 1978. Manganese metabolism and its function. **World Rev. Nutr. Diet.**, 32: 123-134.

LEMON, E. & KIRSTENSEN, J. 1960. **Trans. Int. Congr. Soil. Sci.**, 7th, 1960. Vol. 1. pp. 232 - 240.

LIEBMANN, H. 1951. **Handbuch der Frischwasser und Abwasserbiologie.** Oldenbourg. Munchen.

LIN, C. & BENJAMIN, M.M. 1992. The effects of strongly complexing ligands on the adsorptive partitioning of metal ions. **Water Res.**, 26(4): 397 - 407.

LINDER, M.C. 1985. **Nutrition and metabolism of the trace elements.** In: Nutritional biochemistry and metabolism: with clinical applications. (LINDER, M.C. Ed.) pp. 151-197. Elsevier. New York.

LINEHAN, D.J., SINCLAIR, A.H. & MITCHELL, M.C. 1985. Mobilization of Cu, Mn and Zn in the Soil Solutions of Barley Rhizospheres. **Plant Soil**, 86: 147 - 149.

LOCK, J.W., THOMPSON, D.R., FURNESS, R.W. & BARTLE, J.A. 1992. Metal concentrations in seabirds of the New Zealand region. **Environ. Pollut.**, 75: 289 - 300.

LOEWENTHAL, R.E., WIECHERS, H.N.S. & MARAIS, G.v.R. 1986. **Softening and Stabilization of Municipal Waters.** Water Research Commission Report. Pretoria.

LOEWENTHAL, R.E., EKAMA, G.A. & MARAIS, G.v.R. 1988. **STASOFT: An Interactive Computer Program for Softening and Stabilization of Municipal Waters.** Water Research Commission Report. Pretoria.

LOGAN, T.L. & CHANEY, R.L. 1983. Utilization of municipal wastewater and sludge on land - metals. In: **Utilization of Municipal Wastewater and Sludge on Land.** University of California. Riverside. 235 - 323.

LUOMA, S.N. & BRYAN, G.W. 1979. Trace metal availability: modelling chemical and biological interactions of sediment-bound zinc. In: Chemical Modelling in Aqueous Systems. (E.A. JENNE. Ed.). **ACS Symp. Ser.**, 93: 577 - 604.

LYNCH, T.R., POPP, C.J. & JACOBI, G.Z. 1988. Aquatic insects as environmental monitors of trace metal contamination: Red River, New Mexico. **Water, Air, and Soil Pollution**, 42: 19 - 31.

LYNGBY, J.E. & BRIX, H. 1989. Heavy metals in eelgrass (Zostera marina L.) during growth and decomposition. **Hydrobiologia**, 176/177: 189 - 196.

MAHAFFEY, K.R., ROSEN, J.F. & RUSSEL, M.D. 1982. Association between age, blood lead concentration, and serum 1,25-dihydrocholecalciferol levels in children. **Am. J. Clin. Nutr.**, 35: 1327-1331.

MANCE, G. 1987. **Pollution Threat of Heavy Metals in Aquatic Environments.** London: Elsevier Applied Science.

MARAFANTE, E. 1976. Binding of mercury and zinc to cadmium-binding protein in liver and kidney of goldfish (Carassius auratus). **Experienta**, 32: 149.

MARTELL, A.E., MOTEKAITIS, R.J. & SMITH, R.M. 1985. **Speciation of Metal Complexes and Methods of Predicting Thermodynamics of Metal-Ligand Reactions.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

MARTIN, R.B. 1986. In: **Vol 20: Metal Ions in Biological Systems.** Chap. 2.

MARTIN, R.B. 1988. **Bioinorganic Chemistry of Toxicity.** In: Handbook on Toxicity of Inorganic Compounds. (H.G. SEILER, & H. SIGEL, Eds.). Marcel Dekker, Inc. New York.

MATHIS, B.J. & CUMMINGS, T.F. 1973. Selected metals in river sediment, water and animals. **J. Water Pollut. Contr. Fed.**, 45: 1573 - 1583.

MATTHIESSEN, P. & BRAFIELD, A.E. 1977. Uptake and loss of dissolved zinc by the stickleback *Gasterosteus aculeatus* L. **J. Fish Biol.**, 10: 399 - 410.

McCARTER, J.A. & ROCH, H. 1983. Hepatic metallothionein and resistance to copper in juvenile coho salmon. **Comp. Biochem. Physiol.**, 74C: 133.

McKEE, J.E. & WOLF, H.W. 1963. **Water Quality Criteria.** Publication No. 3-A. Resources Agency of California. State Water Quality Control Board.

MELKI, I.A., BULUS, N.M. & ABUMRAD, N.N. 1987. **Trace Elements in Nutrition.** In: (N.N. ABUMRAD, Ed.). Nutrition in Clinical Practice. Invited Review: American Society for Parenteral and Enteral Nutrition.

MEMMERT, U. 1987. Bioaccumulation of zinc in two freshwater organisms (*Daphnia magna*, Crustacea and *Branchydanio rerio*, Pisces). **Water Res.**, 21(1): 99 - 106.

MERCKX, R., VAN GINKEL, J.H., SINNAEVE, J. & CREMERS, A. 1986a. Plant-induced Changes in the Rhizosphere of Maize and Wheat I. Production and Turnover of Root-derived Material in the Rhizosphere of Maize and Wheat. **Plant Soil**, 96: 85 - 93.

MERCKX, R., VAN GINKEL, J.H., SINNAEVE, J. & CREMERS, A. 1986a. Plant-induced Changes in the Rhizosphere of Maize and Wheat II. Complexation of Cobalt, Zinc and Manganese in the Rhizosphere of Maize and Wheat. **Plant Soil**, 96: 95 - 107.

MERTZ, W. 1972. Human requirements: basic and optimal. **Ann. N.Y. Acad. Sci.**, 199: 191-199.

MERTZ, T. 1979. **Chromium, an overview.** In: Chromium in nutrition and metabolism. (D. SHAPCOTT & J. HUBERT, Eds.). pp. 1-14. Elsevier Biomedical. New York.

MITCHELL, R.L. 1964. **Trace Elements in Soils.** In: Chemistry of the Soil. (F.E. BEAR. Ed.). 2nd Ed. ACS Monograph 160. pp. 320 - 368. Reinhold. New York.

MOHAMED, A.E., AWADALLAH, R.M. & GABR, S.A. 1990. Chemical and ecological studies on *Tilapia nilotica*. **Water SA**, 16(2): 131 - 134.

MOORE, C.A. 1990. **Water Quality Requirements of the Biota of the Kruger National Park River.** Report presented at the Workshop on the Preliminary Water Quality Guidelines for the Kruger National Park Rivers. 23 - 24 October 1990. Pretoria. 27pp.

MOORE, J.N. & LUOMA, S.N. 1990. Hazardous wastes from large-scale metal extraction: A case study. **Environ. Sci. Technol.**, 24(2): 116 - 121.

MOORE, J.W. & RAMAMOORTHY, S. 1984. **Heavy metals in natural waters.** Springer-Verlag, New York. 267 pp.

MOORE, C.A., VAN VEELEN, M., ASHTON, P.J. & WALMSLEY, R.D. 1991. **Preliminary Water Quality Guidelines for the Kruger National Park Rivers.** Kruger National Park Rivers Research Programme Report No. 1. 91pp.

MOREL, F.M.M. & HUDSON, R.J.M. 1985. **The Geobiological Cycle of Trace Elements in Aquatic Systems: Redfield Revisited.** In: Chemical Processes in Lakes. Wiley-Interscience. (W. STUMM, Ed.). New York.

MORGAN, J.J., SUNG, W. & STONE, A. 1985. **Chemistry of Metal Oxides in Natural Water: Catalysis of the Oxidation of Manganese (II) by τ-FeOOH and Reductive Dissolution of Manganese (III) and (IV) Oxides.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

MORGAN, J.J. & STUMM, W. 1991. **Chemical Processes in the Environment, Relevance of Chemical Speciation.** In: Metals and Their Compounds in the Environment. (E. MERIAN, Ed.). VCH Verlagsgesellschaft. Weinheim.

MORTIMER, C.H. 1941. **J. Ecol.**, 29: 280 - 329.

MORTIMER, C.H. 1942. **J. Ecol.**, 30: 147 - 199.

MORTIMER, D.C. 1985. Freshwater aquatic macrophytes as heavy metal monitors - the Ottawa River experience. **Environmental Monitoring and Assessment** 5: 311 - 323.

NAS. 1980. **Lead in the Human Environment.** US National Academy of Sciences. Washington DC.

National Institute of Occupational Safety and Health (NIOSH). 1975. **Occupational exposure to chromium (VI).** Washington, DC.

NEHRING, R.B. 1976. Aquatic insects as biological monitors of heavy metals pollution. **Bull. Environ. Contam. Toxicol.**, 15(2): 147 - 154.

NEHRING, R.B., NISSON, R. & MINASIAN, G. 1979. Reliability of aquatic insects versus water samples as measures of aquatic lead pollution. **Bull. Environm. Contam. Toxicol.**, 22: 103 - 108.

NICHOLSON, J.K. 1981. The comparative distribution of zinc, cadmium and mercury in selected tissues of the Herring Gull (*Larus argentatus*). **Comp. Biochem. Physiol.**, 68C: 91 - 94.

NRIAGU, J.O. 1988. A silent epidemic of environmental metal poisoning? **Environ. Pollut.**, 50: 139-161.

O'CONNOR, D.J. & CONOLLY, J.P. 1980. The effect of concentration of absorbing solids on the partition coefficient. **Water Res.**, 149: 1517 - 1523.

OCCHIOGROSSO, T.J., WALLER, W.T. & LAUER, G.J. 1979. Effects of heavy metals on benthic macroinvertebrate densities in foundry cove on the Hudson River. **Bull. Environm. Contam. Toxicol.**, 22: 230 - 237.

OLAFSON, R.W.K. & THOMPSON, J.A.J. 1974. Isolation of heavy metal binding proteins from marine vertebrates. **Mar. Biol.**, 28: 83-86.

OLAFSON, R.W.K., ABEL, K. & SIM, R.G. 1979a. Prokaryotic metallothionein: Preliminary characterization of a blue-green alga heavy metal-binding protein. **Biochem. Biophys. Res. Commun.**, 89: 36-43.

OLAFSON, R.W.K., KEARNS, A. & SIM, R.G. 1979b. Heavy metal induction of metallothionein synthesis in hepatopancreas of the crab *Scylla serrata*. **Comp. Biochem. Physiol.**, 62B: 417-424.

OVERNELL, J. & COOMBS, T.L. 1979. Purification and properties of plaice metallothionein, a cadmium-binding protein from the liver of the plaice (Pleuronectes platessa). **Biochem. J.**, 183: 277.

PAGE, A.L. & CHANG, A.C. 1981. Trace metals in soils and plants receiving municipal wastewater irrigation. In: (F.M. D'ITRI. Ed.). **Municipal Wastewater in Agriculture.** Academic Press. New York. 351 - 372.

PATRICK, R. 1950. Biological measure of stream conditions. **Sewage ind. Wastes**, 25: 210-214.

PATRICK, R. 1951. A proposed biological measure of stream conditions. **Verh. int. Verein. theor. angew. Limnol.**, 11: 299-307.

PEDERSON, N.B. 1982. **Top. Environ. Health**, 5: 249.

PENNAK, R.W. 1978. **Freshwater invertebrates of the United States.** Ronald Press Company. New York.

PETTERSSON, O. 1976. Heavy-metal ion uptake by plants from nutrient solutions with metal ion, plant species and growth period variations. **Plant Soil**, 45: 445 - 459.

PHILLIPS, G.R., LENHART, T.E., GREGORY, R.W. 1980. Relation between trophic position and mercury accumulation among fishes from the Tongue River reservoir, Montana. **Environ. Res.**, 22: 73 - 80.

PIER, S.M. 1975. The role of heavy metals in human health. **Texas Rep. Biol. Med.**, 33(1): 85-105.

POLAK, L. 1983. In: **Chromium: Metabolism and Toxicity.** CRC, Boca Raton, Fla.

PONNAMPERUMA, F.N. 1965. In: **The Mineral Nutrition of the Rice Plant.** pp. 295 - 328. Johns Hopkins Press. Baltimore. Maryland.

PONNAMPERUMA, F.N. 1972. The chemistry of submerged soils. **Adv. Agron.**, 24: 29 - 96.

PRASAD, A.S. 1976. **Deficiency of zinc in man and its toxicity.** In: Trace elements in human health and disease. (A.S. PRASAD, Ed.). Academic Press. New York.

PRASAD, A.S. 1982. **Clinical and biochemical spectrum of zinc deficiency in human subjects.** In: Clinical, biochemical and nutritional aspects of trace elements. (A.S. PRASAD, Ed.). Alan R. Liss, Inc. New York.

RAINBOW, P.S. 1985. Accumulation of Zn, Cu and Cd by crabs and barnacles. **Estuar. Coast. Shelf Sci.**, 21: 669 - 686.

RAV-ACHA, C. & REBHUN, M. 1992. Binding of organic solutes to dissolved humic substances and its effects on adsorption and transport in the aquatic environment. **Water Res.**, 26(12): 1645 - 1654.

RAYMOND, K.N. 1985. **Specific Sequestering Agents for Iron and Actinides.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

RAY, S.N. & WHITE, W.J. 1976. Selected aquatic plants as indicators for heavy metal pollution. **J. Environ. Sci. Health**, AII. 11: 717 - 725.

REED, S.C. 1988. **Natural Systems for Waste Management and Treatment.** McGraw Hill Book Co. New York.

REIBLE, D.D., POPOV, V., VALSARAJ, K.T., THIBODEAUX, L.J., LIN, F., DIKSHIT, M., TODARO, M.A. & FLEEGER, J.W. 1996. Contaminant fluxes from sediment due to tubificid oligochaete bioturbation. **Water Res.**, 30(3): 704 - 714.

REID, M. & HACKER, C.S. 1982. Spatial and temporal variation in lead and cadmium in the Laughing Gull, Larus atricilla. **Mar. Pollut. Bull.**, 13: 387 - 389.

ROBBINS, J.A. 1982. Stratigraphic and dynamic effects of sediment reworking by Great Lakes zoobenthos. **Hydrobiologia**, 92: 611 - 622.

ROCH, M. & McCARTER, J.A. 1984. Hepatic metallothionein production and resistance to heavy metals by rainbow trout (Salmo gairdneri). II. Held in a series of contaminated lakes. **Comp. Biochem. Physiol.**, 77C: 77.

ROSEN, J.F. 1985. **Metabolic and cellular effects of lead: a guide to low level lead toxicity in children.** In: Dietary and environmental lead: Human health effects. (K.R. MAHAFFEY, Ed.). Elsevier. Amsterdam.

RUGSTAD, H.E. & NORSETH, T. 1975. Cadmium resistance and content of cadmium-binding protein in cultured human cells. **Nature**, 257: 136-137.

SADLER, P.J., HIGHAM, D.P. & NICHOLSON, J.K. 1985. **The Environmental Chemistry of Metals with Examples from Studies of the Speciation of Cadmium.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

SALOMONS, W. & FöRSTNER, U. 1984. **Metals in the Hydrological Cycle.** Springer-Verlag. Berlin. 350 pp.

SANDSTEAD, H.H. 1975. Some trace elements which are essential for human nutrition. **Prog. Food Nutr. Sci.**, 1: 371-391.

SARKAR, B. 1988. **Copper.** In: (SEILER, H.G. & SIGEL, H. Eds.). Handbook on Toxicity of Inorganic Compounds. Marcel Dekker, Inc. New York.

SCHOONBEE, H.J. & VAN DER MERWE, C.G. 1989. Investigations into the effects of sewage, industrial and gold mine effluents on the water quality and faunal conditions of a stream in the Transvaal, South Africa. **Proceedings of the 4th International Conference on Environmental Quality and Ecosystem Stability**. Jerusalem, Israel, IV/A:401-418.

SCHROEDER, H.A. 1972. **The Poisons Around Us - Toxic Metals in Food, Air and Water.** Indiana University Press. Bloomington-London.

SCHULTE, E.H. 1985. **Bioturbation and Fate of Radionuclides in Benthic Marine Ecosystems.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

SCOPPA, P. & MYTTENAERE, C. 1985. **Behaviour of Radionuclides in the Marine Environment: Present State of Knowledge and Future Needs.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

SHROEDER, H.A. 1973. **The trace elements and man: some positive and negative aspects.** (H.A. Shroeder, Ed.). Devin-Adair Co. Old Greenwich, CT.

SHUTTLEWORTH, V.S., CAMERON, R.S. & ALDERMAN, G. 1961. A case of cobalt deficiency in a child presenting as "earth eating". **Practitioner**, 186: 760-764.

SHNEGG, A. & KIRCHESSNER, M. 1978. **Nickel metabolism.** In: Trace element metabolism in man and animals. (M. KIRCHESSNER, Ed.). p. 236. US Department of Health, Education and Welfare. Washington, DC.

SIGEL, H. (Ed.). 1982. **Inorganic Drugs in Deficiency and Disease.** Vol 14 of Metal Ions in Biological Systems. Marcel Dekker. New York.

SIGEL, H. & SEILER, H.G. 1988. **Scope and use of the Handbook.** In: Handbook on Toxicity of Inorganic Compounds. (H.G. SEILER, & H. SIGEL, Eds.). Marcel Dekker, Inc. New York.

SIGG, L. 1987. **Surface Chemical Aspects of the Distribution and Fate of Metal Ions in Lakes.** In: Aquatic Surface Chemistry. (W. STUMM, Ed.). Wiley-Interscience. New York.

SIMKISS, K. 1977. Bioaccumulation and detoxification. **Calcif. Tiss. Res.**, 24: 199 - 200.

SLOBODCHIKOFF, C.N. & PARROT, J.E. 1977. Seasonal diversity in aquatic insect communities in an all-year stream system. **Hydrobiologia**, 52: 143 - 151.

SLY, P. (Ed.). 1982. Sediment/Freshwater Interaction. Proc. 2nd Int. Symp., Kingston, Ontario. **Hydrobiologia**, 91/92. 700pp.

SMITH, R.G. 1972. In: **Metallic contaminants and human health.** (D.H.K. LEE, Ed.). Academic Press, New York.

SMITH, L.R., HOLSEN, T.M., IBAY, N.C., BLOCK, R.M., BLOCK, A.B. & DE LEON, A.B. 1985. Studies on the acute toxicity of Fluoride ion to Stickleback, Fathead minnow and Rainbow trout. **Chemosphere**, 14(9): 1383 - 1389.

SMITH, D.G. 1990. A better water quality indexing system for rivers and streams. **Wat. Res.**, 24(10): 1237-1244.

SOLOMONS, N.W. 1981. **Zinc and copper in human nutrition**. In: Nutrition in the 1980s: constraints on our knowledge. (SELVEY, N. & WHITE, P.L. Eds.). pp. 97-127. Alan R. Liss. New York.

SOMMERS, L.E. & BARBARICK, K.A. 1986. Constraints to land application of sewage sludge. In: Utilization, Treatment and Disposal of Waste on Land. Proc. Workshop (Chicago, III). Madison. WI. USA. **Soil. Sci. Soc. Am.**, p. 193 - 216.

SPOSITO, G. 1981. **The Thermodynamics of Soil Solution.** Oxford University Press. Oxford-New York.

SRIDAR, M.K.C. 1986. Trace element composition of Pistia stratiotes L. in a polluted lake in Nigeria. **Hydrobiologia**, 131: 273 - 276.

STEENKAMP, V.E., DU PREEZ, H.H. & SCHOONBEE, H.J. 1994a. Bioaccumulation of copper in the tissue of *Potamonautes warreni* (Calman) (Crustacea, Decapoda), from industrial, mine and sewage polluted freshwater ecosystems. **S. Afr. J. Zool.**, 29(2): 152 - 161.

STEENKAMP, V.E., DU PREEZ, H.H., SCHOONBEE, H.J. & VAN EEDEN, P.H. 1994b. Bioaccumulation of manganese in selected tissues of the freshwater crab *Potamonautes warreni* (Calman) from industrial and mine-polluted freshwater ecosystems. **Hydrobiologia**, 288: 137 - 150.

STERN, R.M. 1982. **Top. Environ. Health**, 5: 5.

SUNDERMAN, F.W. 1977. A review of the metabolism and toxicology of nickel. **Ann. Clin. Lab. Sci.**, 7: 377-398.

TANDON, S.K. 1982. **Top. Environ. Health**, 5: 209.

TANIZAKI, Y., SHIMOKAWA, T. & YAMAZAKI, M. 1992. Physico-chemical speciation of trace elements in urban streams by size fractionation. **Water Res.**, 26(1): 55 - 63.

THOMAS, D.G., SOLBE, J.F., KAY, J. & CRYER, A. 1983. Environmental cadmium is not sequestered by metallothionein in rainbow trout. **Biochem. Res. Comm.**, 110: 584.

TODD, W.R., ELVEHJEM, C.A. & HART, E.B. 1934. Zinc in the nutrition of the rat. **Am. J. Physiol.**, 107: 146-156.

TRANGMAR, B.B., YOST, R.S. & UEHARA, G. 1985. Application of Geostatistics to Spatial Studies of Soil Properties. **Adv. Agron.**, 38: 45 - 94.

TYSON, J.M. & HOUSE, M.A. 1989. The application of a water quality index to river management. **Wat. Sci. Technol.**, 21: 1149 - 1159.

UNDERWOOD, E.J. (Ed.). 1977. Trace elements in human and animal nutrition. pp. 170-190. Academic Press. New York.

U.S. BUREAU OF MINES. 1991. **Constructed Wetlands Reduce Cost of Treating Coal Mine Drainage.** Technology Transfer Announcement. U.S. Bureau of Mines.

USINGER, R.L. 1956. **Aquatic insects of California.** University of California Press. Los Angeles.

VAHRENKAMP, H. 1973. Metalle in lebensprozessen. **Chemie Unserer Zeit**, 7: 97-105.

VALLEE, B.L. & ULMER, D.D. 1972. Biochemical effects of mercury, cadmium and lead. **Annu. Rev. Biochem.**, 41: 91-128.

VAN DE MEENT, D., ALDENBERG, T., CANTON, J.H., VAN GESTEL, C.A.M. & SLOOF, W. 1990. **Desire for Levels: Background study for the policy document "Setting Environmental Quality Standards for Water and Soil".** Report No. 670101002. Bilthoven: National Institute of Public Health and Environmental Protection.

VAN DER MERWE, C.G. 1990. **Die effek van myn-, nywerheids- en rioolafloopwaters op die waterkwaliteit van die Elsburgspruit, Germiston met spesiale verwysing na die voorkoms en akkumulering van geselekteerde swaarmetale in die ekosisteem.** Ph.D. Thesis. Randse Afrikaanse Universiteit. Johannesburg.

VAN DER MERWE, C.G., SCHOONBEE, H.J. & PRETORIUS, J. 1990. Observations on concentrations of the heavy metals, zinc, manganese, nickel and iron in the water, in the sediments and in two aquatic macrophytes, *Typha capensis* (Rohrb.), N.E. Br. and *Arundo donax* L.M. of a stream affected by goldmine and industrial effluents. **Water SA**, 16: 119 - 124.

VAN EEDEN, P.H. & SCHOONBEE, H.J. 1991. Bio-accumulation of heavy metals by the freshwater crab *Potamonautes warreni* from a polluted wetland. **S. Afr. J. Wildl. Res.**, 21: 103 - 108.

VAN EEDEN, P.H. 1994. **Bio-accumulation of selected metals in the organs and tissues of the Redknobbed Coot, *Fulica cristata*, Reed Cormorant, *Phalacrocorax africanus* and Sacred Ibis, *Threskiornis aethiopicus*, in mine and industrial polluted freshwater ecosystems.** Unpublished Ph.D. Thesis, Rand Afrikaans University. 165 pp.

VAN LOON, J.C. 1980. **Analytical Atomic Absorption Spectroscopy. Selected methods.** Academic Press. New York.

VAN TONDER, G.J. 1980. Toets vir geskiktheid van besproeiingswater. **Landbouweekblad**, 25 Januarie 1980.

VAN VUUREN, J.H.J., DU PREEZ, H.H. & DEACON, A.R. 1994. **Effects of Pollutants on the Physiology of Fish in the Olifants River (Eastern Transvaal).** WRC Report No 350/1/94. Water Research Commission. Pretoria.

VENTER, A.J.A. 1991a. **'n Ekologiese studie van die Floridameer, Roodepoort met spesiale verwysing na die biologiese beheer van probleemwaterplante.** M.Sc Thesis. Rand Afrikaans University. Johannesburg.

VENTER, S. 1991b. Distribution and possible role of aquatic macrophytes in the Middle Letaba Dam. In: SAAYMAN *et al.* (1991). **A post impoundment ecological study of the Middle Letaba Dam, Gazankulu, with special reference to its fish population potential.** University of the North Report to the Department of Development Aid, Pretoria.

VENUGOPAL, B. & LUCKEY, T.D. 1975. **Toxicology of non-radioactive heavy metals and their salts.** In: Heavy Metal Toxicity, Safety and Hormology. (T.D. LUCKEY, B. VENUGOPAL & D. HUTCHESON, Eds.). Thieme. Stuttgart.

VERLOO, M., COTTENIE, A. & LANDSCHOOT, G. 1983. Analytical and biological criteria with regard to soil pollution. **Landwirt.**, F. 39: 394 - 403.

VENUGOPAL, B. & LUCKEY, T.D. 1978. **Metal Toxicity in Mammals.** Vols. 1 and 2. New York. Plenum Press.

VILLEGAS-NAVARRO, A. & VILLARREAL-TREVINO, C.M. 1989. Differential uptake of zinc, copper and lead in Texas cichlid *Cichlasoma cyanoguttatum.* **Bull. Environ. Contam. Toxicol.**, 42: 761 - 768.

VIVIER, F.S., PIETERSE, S.A. & AUCAMP, P.J. 1988. **Guidelines for the use of sewage sludge.** Paper presented at the Symposium on Sewage Sludge Handling. Nov. 15, 1988. Division for Water Technology. CSIR. Pretoria.

WALSKI, T.M. & PARKER, F.L. 1974. Consumers water quality index. **J. Env. Eng. Div.**, 100(EE2): 593-611.

WARD, R.C., LOFTIS, J.C. & McBRIDE, G.B. 1986. The "Data-rich but Information-poor" syndrome in water quality monitoring. **Environ. Manage.**, 10(3): 291 - 297.

WEBB, M. 1972. Binding of cadmium ions by rat liver and kidney. **Biochem. Pharmacol.**, 21: 2751-2756.

WEBSTER, J.D., PARKER, T.F. & ALFREY, A.C. 1980. Acute nickel intoxication by dialysis. **Ann. Int. Med.**, 92: 631-633.

WEPENER, V., EULER, N., VAN VUUREN, J.H.J., DU PREEZ, H.H. & KöHLER, A. 1992. The development of an aquatic toxicity index as a tool in the operational management of the water quality in the Olifants River (Kruger National Park). **Koedoe**, 35: 1 - 9.

WHITLEY, L.S. 1967. The resistance of Tubificid worms to three common pollutants. **Hydrobiologia**, 32: 193 - 205.

WHITTON, B.A., SAY, P.J. & WEHR, J.D. 1981. **Use of plants to monitor heavy metals in rivers.** In: SAY, P.J. & WHITTON, B.A. (Eds.) Heavy Metals in Northern England: Environmental and Biological Aspects. University of Durham, Department of Botany, Durham.

WINNER, R.W., BOESEL, M.W. & FARREL, M.P. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. **Can. J. Fish. Aquat. Sci.**, 37: 647 - 655.

WITTMANN, G.T.W. & FöRSTNER, U. 1976. Metal accumulation in acidic waters from goldmines in South Africa. **Geoforum**, 7: 41 - 49.

World Health Organization (WHO). 1973. **Trace elements in human nutrition**. World Health Organization Technical Report. Series 532.

WHO. 1978. **Evaluation of certain food additives and contaminants.** Twenty-second report of the joint expert committe on food additives. Food and agriculture organization. Geneva.

WILLIAMS, R.J.P. 1967. Heavy metals in biological systems. **Endeavour**, 24: 96-100.

WILLIAMS, P.A. 1985. **Secondary Minerals: Natural Ion Buffers.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

WOOD, A. 1993. Wastewater treatment performance of artificial wetlands investigated. **SA Waterbulletin**, January/February 1993. 21 - 23.

WOOD, A. 1994a. Localising wastewater treatment opportunities. **Munisipale Ingenieur**, Maart 1994. 38 - 41.

WOOD, A. 1994b. Natural wastewater treatment. **Water Sewage and Effluent**, 14(1): 16 - 21.

WOOD, J.M. 1974. Biological cycles for toxic elements in the environment. **Science**, 183: 1049-1052.

WOOD, J.M. 1975. Biological cycles for elements in the environment. **Naturwissenschaften**, 62: 357-364.

WOOD, A. & PYBUS, 1992. **Artificial Wetland use for Wastewater Treatment: Theory, Practice and Economic Review.** WRC Report No: 232/1/93. Water Research Commission. Pretoria.

WOOD, J.M. & WANG, H.K. 1985. **Microbial Resistance to Heavy Metals.** In: Environmental Inorganic Chemistry. (K.J. IRGOLIC & A.E. MARTELL. Eds.). VCH Publishers. Florida.

WOPEREIS, M.C., GASCUEL-ODOUX, C., BOURRIE, G. & SOIGNET, G. 1988. Spatial Variability of Heavy Metals in Soil on a One-hectare Scale. **Soil Sci.**, 146: 113 - 118.

Workshop: Sensitive Fish Species. 1991. **Skukuza: Kruger National Park Rivers Research Programme.** October 1991.

WURTZ, C.B. 1955. Stream biota and stream pollution. **Sewage ind. Wastes**, 27: 1270-1278.

YAMAMURA, M., SUZUKI, K.T., HATAKEYAMA, S. & KUBOTA, K. 1983. Tolerance to cadmium and cadmium-binding proteins induced in the midge larva, *Chironomus yoshimatsui* (Diptera: Chironomidae). **Comp. Biochem. Physiol.**, 75C: 21 - 24.

YAMANE, I. 1958. **Soil Plant Food**, (Tokyo). 4: 25 - 31.

YASUNO, M., HATAKEYAMA, S. & SUGAY, Y. 1985. Characteristic distribution of chironomids in a river polluted with heavy metals. **Verh. Internat. Verein. Limnol.**, 22: 2371 - 2377.

YUNKERVICH, I.D., SMOLYAK, D. & GARIN, B.E. 1966. **Sov. Soil Sci.**, 2: 159 - 167.