Biogeochemical factors affecting groundwater quality in central Tanzania

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Abstract: Analysis of groundwaters from the Makutuapora aquifer in the Dodoma region of central Tanzania has revealed a relationship between mineral–water interactions, water chemistry, bedrock geology, and microbiology. Groundwaters were slightly alkaline (pH 6–7.8) and essentially Na-Ca-HCO_3-Cl, with minor K, Mg, F, and SO_4^{2-}. Variations in water chemistry, particularly Ca/Na and Mg/Ca, ratios are related to the progressive alteration of feldspars and ferromagnesium minerals. The constant Na/Ca and Mg/Ca ratios noticed over mature aquifers and wells indicates that a steady-state is attained between aluminosilicates and groundwater. While erratic Fe/Mg and Na/K ratios denote a more open system or rather a greater diversity in minerals hosting these elements participating in mineral–water reactions. In places total concentrations of Fe, Mn, and Al can each exceed 1 mg l^{-1} with most of the metal held in particulate form (> 0.45 μm). The increase in metals suggests an imbalance in the steady-state reactions between magmatic minerals and leachate, possibly related to microbial activity. Fifty percent of the groundwaters were contaminated by significant numbers of thermotolerant coliforms indicating considerable risk of contamination by faecal pathogens. Numbers of faecal coliforms were positively correlated with K, Na, NO_3^-, PO_4^{3-} and BOD. Groundwater chemistry also affected the activity of the indigenous microbial community. Microbial biomass appeared to be unaffected by differences in groundwater chemistry. The numbers of selected physiological bacterial types (e.g. organisms contributing to the nitrogen and sulphur cycles) and the range of protist morphotypes, isolated from the tropical groundwater systems, were broadly similar to those found in temperate groundwater. Total concentration of metals such as Al, Fe, Co and Mn certainly exceed levels at which these metals could be considered toxic although if these metals are present in non-labile forms (as suggested by other studies) then the potential toxicity would be negligible. At present the major concerns for health are high seasonal salinities in the groundwaters and high faecal contamination.

Groundwater makes up over 95% of the world's available freshwater resources and is the main source of drinking water for a large percentage of the world's population. Historically groundwater has been considered a safe source of water protected by the soil layer which removes pollutants as the water percolates downwards. In many developing countries groundwater is not treated or even monitored prior to consumption. If these groundwaters contain high concentrations of potentially toxic elements (PTEs) or faecal contaminants there may be serious health implications.

Current understanding of the biogeochemical processes occurring in subsurface environments is based largely on studies carried out in temperate regions. Little is known about these processes in tropical regions where environmental conditions are thought to play an important role in determining the quality of groundwater; rocks weather more quickly and leaching is more intense than in temperate zones (Trescases 1992). Recent research has highlighted the high concentrations of potentially toxic elements in African groundwaters (Ogbukagu 1984; Lahermo *et al.* 1991; McFarlane & Bowden 1992; Bowell *et al.* 1994).

A wide range of microbial types contributes to

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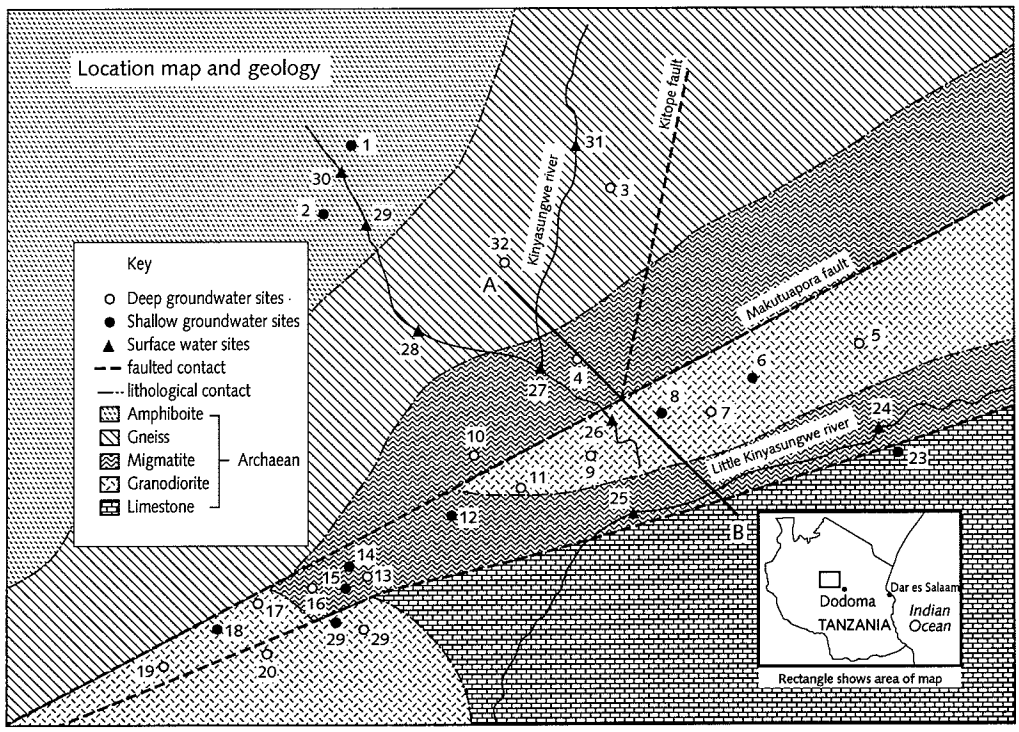


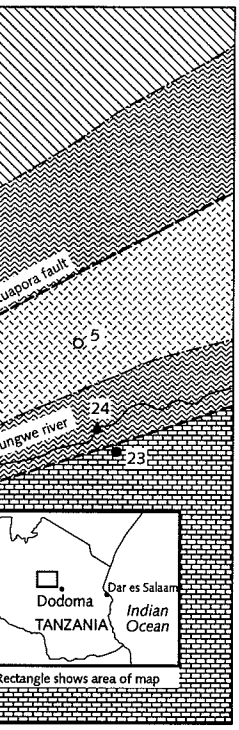
Fig. 1. Bedrock geology map of the crystalline basement in the Makutuapora area, Dodoma, Tanzania.

the biogeochemical cycles through oxidation/reduction reactions, alkylation and dealkylation processes, fossilization and solubilization, or mineralization reactions. Some microorganisms contribute to all the elemental cycles, e.g. heterotrophic bacteria are involved in the degradation and mineralization of organic matter, while others contribute significantly to one cycle, e.g. ammonia oxidizing bacteria to the nitrogen cycle. The presence of diverse and complex microbial communities in soils (Smith 1982), and shallow and deep groundwater systems is well established (Sinclair & Ghiorse 1989; Johnson & Wood 1992). It is likely that many different microbial populations within these communities contribute to element transformations and cycling in groundwater systems. This may have implications for the fate of PTEs in groundwater. Contamination of groundwater by faecal pathogens has considerable implications for human populations using the groundwater resource. The longevity of enteric pathogens in groundwater systems is determined by environmental factors including geochemistry and the indigenous microbial community.

Recent studies have shown that eukaryotic microorganisms (protists) are also widely distributed in subsurface sediments (Sinclair & Ghiorse 1989; Novarino *et al.* 1994). Protists are important bacterial predators in aquatic and terrestrial environments and may play a significant role in the population dynamics of groundwater bacteria. They have also been used in bioassays for a range of organic and inorganic elements and compounds. Consequently it may be possible to use protists as indicators of bacterial activity and to monitor any variations in groundwater chemistry which will influence biogeochemical processes. The aim of this research was to study the relationships between water chemistry and microbiological activity in deep (collected below 30 m, hosted by unweathered bedrock) and shallow (above 30 m, hosted in the weathered rocks and soils) groundwaters of the main aquifer in the Dodoma region of Tanzania at Makutuapora (Fig. 1). An assessment is made of possible advantages in using groundwater for drinking water rather than the traditional supplies of potable water available at the surface.

Methodology

Groundwater, sediment, soil and rock samples were collected in two field seasons in 1992:



a, Dodoma, Tanzania.

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soil and rock samples field seasons in 1992:

April–May (wet season) and October–November (dry season). Thirty-two sites were sampled comprising 8 surface water sites, 11 shallow groundwater sites (34 samples from various depths) and 13 deep groundwater sites (22 samples from various depths). Sites designated as deep groundwater holes are those which draw water from below 30 m. Above this the boreholes were generally cased with clay or PVC lining. Exceptions to this were three holes which had yet to be cased and from which samples could be taken from the water-table to a depth of between 75 and 100 m. The shallow groundwater sites were designated as those which drained the weathering profile and were generally drilled by a WATERAID Landrover-mounted soil auger. None of these holes was lined.

Groundwater samples were collected by one of three methods: (1) From pre-drilled concrete-lined boreholes using hand-, wind- or diesel-operated pumps; (2) from traditional hand-dug wells; (3) from freshly-drilled aquifer material collected either by hand augering or percussion drilling.

In some cases a stainless steel sampler was employed for collecting water at known depths down the water column. In the monitoring study of two newly drilled boreholes (sites 2 and 17) sampling was conducted at the surface during the first five days of pumping. For microbiological sampling all equipment was sterilized beforehand whenever possible.

For each sample of water three sub-samples were taken; unfiltered and unmodified (natural water); unfiltered and acidified with 10% (v/v) HNO_3; filtered through a 0.45 μm Durapore membrane filter and acidified with 10% (v/v) HNO_3. Field measurements of Eh, pH, electrical conductivity (EC), temperature and biological oxygen demand (BOD) were made at each site using OAKTON field instruments.

Mineralogy and lithogeochemistry

Mineral identification was carried out by optical and scanning electron microscopy and confirmed by X-ray diffraction (XRD) and Fourier transform infra-red spectroscopy. Chemical analysis of the rocks and soils was carried out by inductively coupled plasma atomic emission spectrometry (ICPAES, Fisons ARL3410 Minitorch). Elements were extracted from 5 g of sediment by digestion with 15 ml of HNO_3 (70% v/v) 15 ml of HF (40% v/v) and 15 ml of perchloric acid (70% v/v). Precision of the technique was checked against known standards (Williams *et al.* 1993).

Hydrogeochemistry

All waters were analysed by ion-chromatography (Dionex-300) using an AS4A-AMMS column with Na_2CO_3 (1.8 mM) eluent at a flow rate of 2.5 ml min^{-1} for anion analysis, a CS 12 column with methane sulphonic acid eluent (20 mM) at a flow rate of 2 ml min^{-1} for group I/II cation analyses. A pulsed electrochemical detector in conductivity mode was used for detection. Transition metal analysis was accomplished with a Dionex CS 5 column with pyridine-di-carboxylic acid eluent and 4(2-pyridylazo) resorcinol post-column derivitization and measurement by a variable wavelength detector in the range 520–530 nm.

Microbiology

Bacterial enumeration was performed on representative samples (4) of selected soils and borehole sediments (deep 30–100 m; shallow 0–30 m). One gram (wet weight) of each sample was suspended in phosphate buffered saline (pH 7.3) and prepared as a dilution series (to 10^{-6}). Bacterial numbers were then determined as follows.

(i) Total viable count (TVC) for aerobic heterotrophs. One ml volumes of sample dilutions were grown on peptone yeast extract agar at 26°C for 7 days before counting. The results were expressed as colony forming units (CFU) g^{-1} (wet weight) soil.

(ii) Denitrifying bacteria. Replicate (5) MPN (Most Probable Number) tubes containing nutrient broth supplemented with 10 mM KNO_3 were inoculated with 1 ml of sample dilutions and incubated at 20°C to 22°C for 7 days. Gas production indicated the presence of denitrifying bacteria.

(iii) Ammonia-oxidizing bacteria. One ml volumes of diluted sediment/soil samples were inoculated into Alexander and Clarke medium containing 0.05% (w/v) $(NH_4)_2SO_4$. Five 200 μl volumes of each sample were transferred to MPN microtitre plates which were incubated in a humid environment at 20–22°C for 14 days. Griess-Ilosvay reagent was added to determine the presence of nitrite.

(iv) Nitrite-oxidizing bacteria. Microtitre plates were prepared and incubated as described above using Alexander and Clarke medium containing 0.006 g KNO_2 per 1 l medium. The presence of nitrite was examined (above).

(v) Sulphate reducing bacteria (SRB). Replicate (5)' sediment/soil sample dilutions were prepared in 5 ml volumes of anoxic Baars medium supplemented with yeast extract and

reducing agents. The tubes were incubated anaerobically for 14 days at 20°C to 22°C before determining the presence of SRBs by the formation of black coloration.

The numbers of bacteria, determined by MPN methods, were expressed as numbers per gram wet weight of sediment/soil.

Faecal coliforms were enumerated in the field using a DelAgua water testing kit (DelAgua Ltd, University of Surrey, Guildford, UK). Immediately upon collection, water samples (100 ml, 50 ml or 10 ml) were passed through a 0.45 μm sterile filter membrane. Each membrane was placed onto a pad containing Membrane Lauryl Sulphate Broth, and incubated for 14 to 18 hours at 44°C. Colony forming units of thermotolerant faecal coliform bacteria were then counted and expressed as number per ml of sample.

Bacterial biomass and activity were determined by the analysis of biochemical markers. Biomarkers used for biomass determination were DNA and lipid phosphate (a cell membrane component). Adenylates (ATP, ADP and AMP) and RNA were used as indicators of activity. Samples were freeze dried and the biomarkers were extracted by sonication in a suspension of chloroform, methanol and 10 mM phosphate buffer (pH 7.4) in a ratio of 1:2:0.8 for 2 hours at room temperature. The phases were then separated and reduced to dryness by rotary evaporation. The upper aqueous phase (containing DNA, RNA and adenylates) was resuspended in 18 MΩ water and the chloroform phase (containing lipid phosphate) was filtered and resuspended in chloroform.

The aqueous phases were mixed with 10 mg ml^{-1} polyvinyl polypyrollidone (PVPP) and centrifuged to remove humic contaminants from the extracts. Nucleic acids were extracted from the supernatant by adding buffered chloroform (pH 8). The phases were separated and the nucleic acid precipitated from the aqueous phase, and resuspended in 10 mM phosphate buffer (pH 7.4). The DNA content of each sample was quantified after binding with Hoechst 33258 by fluorescence detection at 350 nm excitation and 450 nm emission. Total nucleic acids were measured by absorbance at 260 nm and RNA concentration was then derived by difference. DNA and RNA content in the samples were expressed as ng g^{-1} dry weight sediment/soil.

AMP, ADP and ATP extracted from the aqueous phase were derivatized using chloracetaldehyde to produce the fluorescent N6 etheno derivatives. The derivatized samples were separated by ion pairing on HPLC and quantified by fluorescence at an excitation of 280 nm and an emission of 400 nm. After peak area integration, AMP, ADP and ATP sample content was expressed as p mole g^{-1} dry weight sediment/soil. The energy charge was also calculated as (ATP + 0.5 ADP)/(ATP + ADP + AMP).

Lipid phosphate present in the chloroform fraction after soil extraction was quantified using persulphate digestion and the phosphomolybdate reaction followed by reaction with malachite green. Absorbance was measured at 615 nm. Lipid phosphate soil content was expressed as n moles g^{-1} dry weight soil/sediment.

Samples were screened for the presence of protists. One gram of sediment or 1 ml of groundwater was transferred into Erdschreiber's Soil Extract medium in a sterile Petri dish and incubated at 20°C for 4–7 days. Dishes were examined daily by light microscopy for the presence of protists. Uniprotistan cultures were obtained either by micromanipulation of individual cells with a micropipette or by the 'dilution to extinction' method.

Geology of the Dodoma region

The lithologies of the Dodoma area are part of the Archaean–Lower Proterozoic Tanzanian shield (Cahen & Snelling 1984) which extends north beneath Lake Victoria and into southern Uganda and Kenya. In the Dodoma area the lithologies consist of migmatitic gneisses, amphibolites, feldspathic quartzites, siliceous marbles, garnet–kyanite schists, and quartz–feldspathic gneisses, metamorphosed to amphibolite and granulite facies. They represent an ancient granitoid–supracrustal terrane dated at 2850 and 2600 Ma (MADINI 1992).

Due to the extreme weathering which has occurred in central East Africa, a thick weathering mantle has been developed above the bedrock. This varies in thickness from usually 30 m but up to 40 m on the plains to less than 1 m on steep slopes. The bulk change to bedrock occurs as the coherent, dense, rock composed largely of anhydrous or weakly hydrated minerals are altered to friable units with a lower density consisting mainly of microcrystalline strongly hydrated phyllosilicates, oxides and hydroxides. The major mineralogical variations in the weathering profile are shown in Fig. 2. The main geochemical changes in the conversion of bedrock to saprolite and soils is the loss of elements such as Ca, Mg, Rb and retention of more immobile elements such as Ti, Fe and Al (Table 1). On plateaus a laterite ferricrete is present and consists largely of Fe and Al oxides

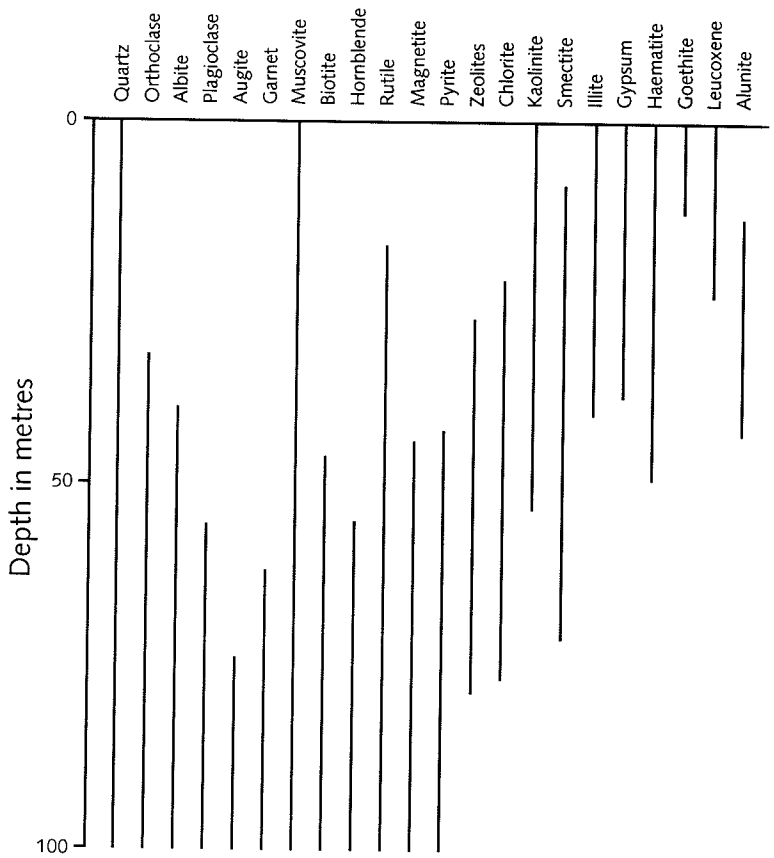

Fig. 2. Schematic representation of mineralogical variations with depth of the Makutuapora core samples.

and hydroxides. The soils in the area are largely ferrasols and vertisols but andosols and nitosols are present on peneplains (Bowell *et al.* 1995).

From mass balance reactions (Brimhall & Dietrich 1987; Brimhall *et al.* 1988) of the Dodoma weathering profiles, element behaviour can be quantified (Table 2). Elements such as Ca, Na, and K show a strong depletion as intensity of weathering increases. This is a reflection of the instability of primary silicate minerals (Velbel 1989, 1992). Aluminium is enriched in the clay-rich saprolite and surface soils. Similarly Si is retained in the saprolite and clay-rich soils but is lost from the surface soils. Iron is enriched during weathering with a larger enrichment in the ferricrete horizon, or cuirasse (over 600% v. average hypogene concentration) at the top of the mottled clay zone, possibly as a result of iron hydrolysis at the water table, or 'ferrolysis' (Mann 1983). However, the mass of added iron required to account for the enrichment in the overlying soils is much too great to be an *in situ* product. Given the sluggish mobility of iron in the oxide zone (Mann, 1983), some of the added iron must be due to mechanical concentration, possibly related to profile denudation. Trace elements such as Mn are largely depleted in the profile, although a Mn enrichment is observed in the zone of ferrolysis (ferricrete, Table 2) and is probably related to the same mechanism as Fe enrichment (Mann 1983). Like Zr, Nb is largely immobile but the strain indicators based on Nb v. Zr reveal a collapse zone in the soils.

The Dodoma region is similar to other areas of crystalline basement rocks in Africa, with groundwater occurring in two forms (Farquharson & Bullock 1992; Wright 1992). Superficial deposits above the granitic basement are composed of thin sand and gravel which retain infiltration water in its downward movement. These formations are shallow and are similar to palaeodambos identified elsewhere (Wright 1992). The recharge of these aquifers is depen-

Table 1. *Geochemistry of protolith, saprolite and soils from the Makutuapora area, Dodoma, Tanzania*

Soil	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	Na_2O	K_2O	CaO	MgO	P_2O_5	Zr	Nb	V	Cr	Zn	Cu	Rb
vertisol surface	44.90	28.80	14.50	0.98	0.08	1.25	1.56	0.08	0.05	0.29	349	115	41.3	39	12	10	3.2
vertisol 0.5 m	46.45	28.30	13.90	0.81	0.09	1.32	1.65	0.19	0.09	0.21	345	114	38.7	36	12	10	3.2
vertisol 1.0 m	47.21	27.60	14.00	0.79	0.08	1.31	1.67	0.23	0.09	0.20	346	104	39.5	36	14	12	4.6
ferralsol surface	26.06	27.55	36.90	1.24	0.05	0.08	0.09	0.04	0.03	0.16	511	45	115	123	8	7	1.2
ferralsol 0.5 m	24.45	28.65	38.65	1.29	0.05	0.11	0.09	0.04	0.04	0.13	510	37	109	86	14	9	1.8
ferralsol 1.0 m	23.64	29.55	38.95	1.31	0.08	0.12	0.14	0.04	0.04	0.13	510	28	107	100	23	10	1.9
ferricrete	16.89	30.87	39.20	1.76	0.08	0.14	0.23	0.06	0.07	0.20	508	30	121	139	27	13	3.8
clay zone 10 m	62.03	18.23	12.95	0.68	0.16	1.48	1.51	0.23	0.08	0.15	345	98	30.8	40	16	12	11
clay zone 15 m	63.59	17.96	11.23	0.65	0.18	1.91	1.56	0.26	0.09	0.19	340	87	35.9	34	18	15	12
saprolite 10 m	65.61	15.91	10.98	0.51	0.21	2.11	2.11	0.27	0.10	0.16	340	96	34.2	33	20	21	15
saprolite 20 m	65.11	15.65	9.85	0.48	0.38	3.58	2.69	0.28	0.11	0.18	343	99	32.3	29	21	22	16
saprolite 25 m	66.04	15.22	8.55	0.36	0.42	4.23	2.96	0.31	0.12	0.20	340	105	29.3	27	21	24	19
saprolite 30 m	67.57	14.95	5.32	0.37	0.46	5.46	3.71	0.38	0.14	0.21	342	110	30.6	33	25	26	25
granodiorite	66.01	12.55	2.61	0.37	0.45	6.10	3.95	0.49	0.16	0.23	340	112	28.9	28	28	29	65
gneiss	59.55	25.93	2.71	0.38	0.50	5.23	2.55	0.33	0.16	0.21	506	108	112	39	27	14	71
pyx-gneiss	50.96	20.22	10.70	0.93	0.69	1.29	2.11	5.12	5.39	0.19	80	10.9	244	191	63	335	36
quartzite	89.00	2.69	2.18	0.18	0.11	0.48	1.26	1.30	1.21	0.39	255	24	65	62	10	12	16
marble	16.23	2.31	2.29	0.05	0.12	0.58	0.19	24.22	20.83	0.18	150	18	19	12	59	34	19
schist	56.23	24.23	4.21	0.25	0.63	3.88	4.68	1.96	1.81	0.23	187	39	36	78	98	89	46

Oxide concentration in wt% and element concentration in mg/kg. All analyses by ICPAES.

Table 2. *Element mass balance* in Dodoma weathering profiles (all rock types)*

Element	Saprolite	Clay zone	Ferricrete	Soil
SiO_2	98	112	65	82
Fe_2O_3	139	180	438	374
Al_2O_3	109	148	108	180
TiO_2	103	105	114	151
MnO	85	70	108	43
CaO	88	33	6	1
MgO	65	19	2	1
Na_2O	62	10	3	4
K_2O	81	52	15	3
P_2O_5	77	39	28	22
Zr	100	100	100	100
Nb	105	100	97	180
Cr	109	167	355	305
V	110	119	276	198
Cu	93	86	17	7
Rb	65	12	2	1
Sr	61	15	3	1
Ba	78	54	22	11

*Mass balances calculated using equations of Brimhall & Dietrich (1987) and Brimhall *et al.* (1988). T% of protolith concentration, assuming no variation in Zr concentration.

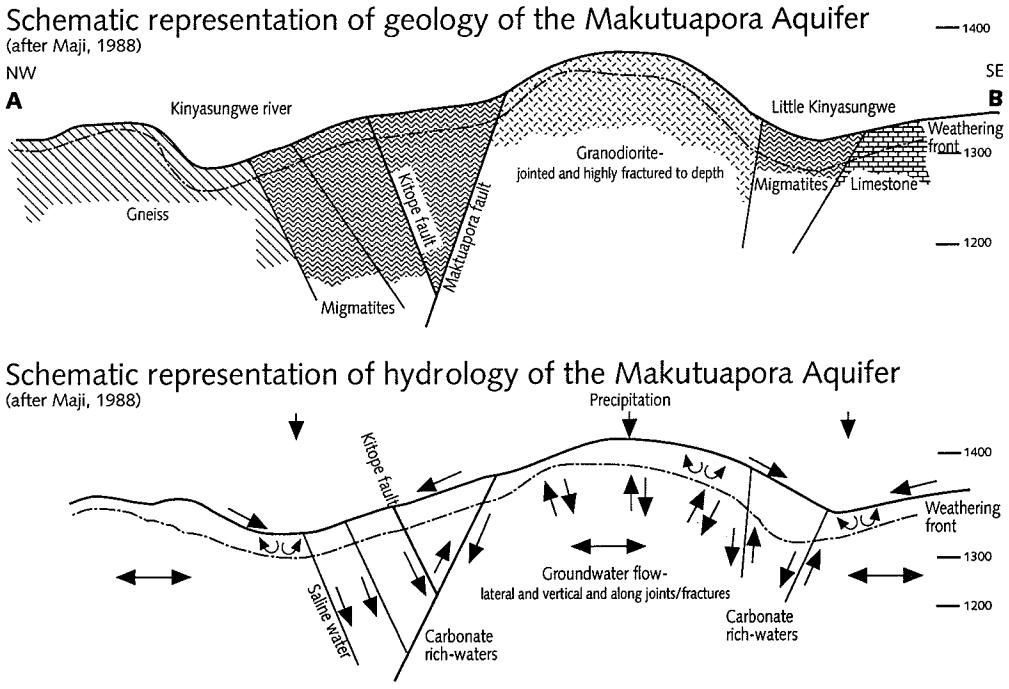

Fig. 3. Schematic cross-section and hydrological section of the Makutuapora aquifer (after MAJI 1988). **(a)** Geology: A–B represents position of cross-section as shown in Fig. 1; **(b)** hydrology: arrows show direction of flow.

Oxide concentration in wt% and element concentration in mg/kg. All analyses by ICPAES.

Table 3. *Geochemistry of deep groundwaters (analysed by ICPAES and ion chromatography, n = 22)*

	Wet season		Dry season	
	Min.	Max.	Min.	Max.
pH	5.5	8.2	5.0	8.5
EC	200	2750	262	43200
Eh	198	622	211	708
Temp. °C	19	42	20	42
TDS	50	1450	43	1670
DOC	< 0.1	0.2	< 0.1	0.15
BOD	0	2.2	0	1.2
FC	0	88	0	50
HCO_3^-	23	320	36	350
SO_4^{2-}	1.9	22	5.9	39
NO_3^-	< 0.01	0.18	0.09	0.31
PO_4^{3-}	0.1	0.42	0.25	0.53
Li	< 0.01	1.6	0.23	2.21
Na	93	231	148	265
K	2	16	12	24
Mg	16	40	22	44
Ca	23	91	43	116
Sr	0.27	2.18	0.18	2.97
Ba	0.09	0.42	0.05	0.23
B	0.88	1.13	0.57	0.98
Al	0.12	33.95	1.51	21.40
Si	16.95	42.85	12.34	19.58
Mn	0.013	0.221	0.012	0.162
Fe	0.11	1.21	0.19	0.65
Cu	< 0.01	0.19	< 0.01	0.06
Zn	< 0.01	0.39	< 0.01	0.30

All anion and element concentrations in $mg\,l^{-1}$.
EC, electrical conductivity in $\mu S\ cm^{-1}$; Eh, redox potential in mV; TDS, total dissolved solids in $mg\,l^{-1}$; DOC, dissolved organic content in $mg\ l^{-1}$ (technique of Ertel *et al.* 1986); BOD, biological oxygen demand, a measure of biological activity, in $mg\,l^{-1}$; FC, faecal coliform counts expressed as number of coliforms in 100 ml of water. Elements analyzed below detection: Br, I (< $0.1\,mg\,l^{-1}$), Be, Sc, Zr, V, Cr, Mo, Co, As (< $0.01\,mg\,l^{-1}$).

dent on infiltration rates and is subject to large seasonal fluctuations. Due to the variable morphology of the region these aquifers have a wide distribution. They give a low yield ($4.5\,l\,min^{-1}$ per well on average) and are exploited by means of manual hand pumps. The major aquifers in the region occur within fractured crystalline rocks, granites and their metamorphosed equivalents, such as at Makutuapora. From a study of drill core from Makutuapora, a schematic cross-section was constructed (Fig. 3a). Most drill sites which hit water were closely associated with the major Kitoe fault system or within subsidiary fractures (Fig. 1).

Hydrogeochemistry

The physicochemical characteristics of groundwater and some surface waters from the Makutuapora aquifer are shown in Tables 3–5 for wet and dry season sampling. In general surface waters were weakly alkaline, at ambient temperature and had a high total dissolved solid concentration. The particulate solid load was slightly greater in the wet season than in the dry season, but the dissolved load was higher in the dry season. Most of the deep groundwaters were above pH 7 and were essentially Na–Ca–HCO_3^-–Cl, with minor K, Mg, F and SO_4^{2-}. In the dry season the higher salinity reduces water quality and would be a major factor in determining the use of water resources from individual wells as well as the whole aquifer. Most groundwaters had a lower range of total dissolved solid (TDS) content and metal content than surface water and shallow groundwaters.

Seasonal variations existed in the wells for trace element concentration depending upon whether or not the element was associated

Table 4. *Geochemistry of shallow groundwaters (analysed by ICPAES and ion chromatography, n = 34)*

	Wet season		Dry season	
	Min.	Max.	Min.	Max.
pH	4.6	6.9	4.7	6.5
EC	157	680	187	822
Eh	221	565	228	608
Temp. °C	15	29	19	33
TDS	60	720	73	895
DOC	0.15	0.39	0.23	0.45
BOD	0.4	6.9	0	6.2
FC	0	128	0	50
HCO_3^-	45	346	50	225
F^-	< 0.1	1.2	< 0.1	2.3
Cl^-	10.6	178	12.9	212
SO_4^{2-}	0.9	16.2	0.8	15.8
NO_3^-	< 0.01	0.39	0.05	0.49
PO_4^2	0.1	0.56	0.36	0.95
Li	< 0.01	0.98	0.15	0.89
Na	36	295	42	312
K	10.5	21	13	26.50
Mg	12	72	16	81
Ca	20	63	23	68
Sr	0.15	0.96	0.15	1.01
Ba	0.05	0.31	0.06	0.23
B	0.65	0.89	0.58	0.72
Al	3.97	34.56	3.21	25.97
Si	9.5	28.7	6.58	30.10
Mn	0.016	0.079	0.011	0.060
Fe	0.39	1.98	0.16	0.79
Cu	< 0.01	0.25	< 0.01	0.16
Zn	< 0.01	0.46	< 0.01	0.39

All anion and element concentrations in mg l^{-1}.
EC, electrical conductivity in $\mu S\ cm^{-1}$; Eh, redox potential in mV; TDS, total dissolved solids in mg l^{-1}; DOC, dissolved organic content in mg l^{-1} (technique of Ertel *et al.* 1986); BOD, biological oxygen demand, a measure of biological activity, in mg l^{-1}; FC, faecal coliform counts expressed as number of coliforms in 100 ml of water. Elements analyzed but below detection: Br, I (< 0.1 mg l^{-1}), Be, Sc, Zr, V, Cr, Mo, Co, As (< 0.01 mg l^{-1}).

largely with the dissolved or particulate fraction. For Fe, Al, Mn and SiO_2, the dominant fraction was in the particulate load and concentrations were higher in the wet season than in the dry season (Table 4). Other trace metals, such as V, Cr, Co, Pb, and Mo were all below the analytical detection limit of 0.01 mg l^{-1} in both bulk sample and filtrate. The subsurface or shallow groundwaters were more acidic with pH as low as 4.6, probably due to high levels of dissolved organic acids. An attempt was made to analyse dissolved organic acids although the rapid degradation of the acids and their labile nature made the analyses unreliable. Carboxylic and phenolic acids were identified and if these were similar to the organic acids in the original waters then they could represent a potential mechanism for Fe, Mn, Zn and Al mobility (Shotyk 1984).

From correlation coefficients a number of relationships can be observed in the Makutuapora groundwaters (Table 6). Bicarbonate content is positively correlated to pH, Na, Ca and Mg and inversely correlated to Al, Fe and sulphate. A positive correlation occurs between the anions nitrate, phosphate, chloride and sulphate. This correlation is probably biased by the shallow groundwaters which will reflect rainfall recharge, the main source of chloride and sulphate, to a greater extent than deeper groundwaters. Shallow groundwaters will also be more contaminated by soil leachates, reflected in the nitrate and phosphate content probably arising from decaying organic matter and leaching of fertilizers. The possible contamination of groundwater by faecal matter and biological waste is reflected by the positive correlation between biological oxygen demand (BOD), thermotolerant faecal coliforms (FC) with

Table 5. *Geochemistry of surface waters (analysed by ICPAES and ion chromatography, n = 8)*

	Wet season		Dry season	
	Min.	Max.	Min.	Max.
pH	7.5	8.0	7.0	8.0
EC	705	1900	700	2000
Eh	125	225	195	286
Temp. °C	11	22	18	38
TDS	270	960	295	1080
DOC	0.53	0.75	0.51	0.66
FC	112	> 250	100	> 250
HCO_3^-	250	652	165	655
F^-	< 0.1	0.2	< 0.1	0.3
Cl^-	11.9	16.5	12.6	59.5
SO_4^{2-}	1.8	30	2.2	33.5
NO_3^-	0.09	0.69	0.18	0.86
PO_4^{3-}	0.81	1.29	0.98	1.98
Li	< 0.01	0.51	< 0.01	0.63
Na	115	178	169	226
K	18.90	23.20	19.50	24.50
Mg	10	68.50	28	72
Ca	26	119	30	136
Sr	0.78	0.83	0.77	0.82
Ba	0.12	0.22	0.15	0.24
B	0.61	0.64	0.52	0.55
Al	< 0.01	1.30	< 0.01	1.0
Si	8.70	11.20	5.80	7.90
Mn	0.017	0.31	0.029	0.196
Fe	0.11	0.56	< 0.01	0.39
Cu	< 0.01		< 0.01	
Zn	< 0.01		< 0.01	

All anion and element concentrations in $mg\,l^{-1}$.
EC, electrical conductivity in $\mu S\ cm^{-1}$; Eh, redox potential in mV; TDS, total dissolved solids in $mg\,l^{-1}$; DOC, dissolved organic content in $mg\,l^{-1}$ (technique of Ertel *et al.* 1986); FC, faecal coliform counts expressed as number of coliforms in 100 ml of water.
Elements analyzed but below detection: Br, I (< 0.1 $mg\,l^{-1}$), Be, Sc, Zr, V, Cr, Mo, Co, As (< 0.01 $mg\,l^{-1}$).

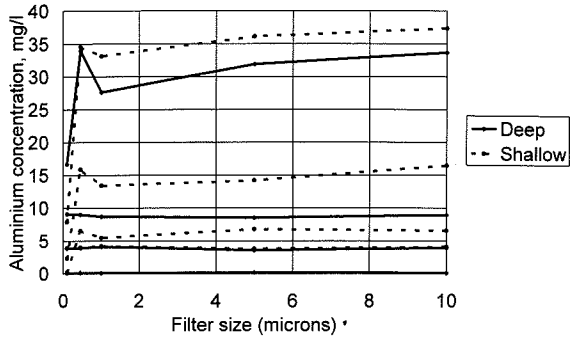

Fig. 4. Distribution of Al concentration with filter pore size after filtration of Makutuapora groundwaters.

Table 6. *Correlation matrices for Makutuapora groundwaters (all groundwaters)*

	pH	alk	NO_3^-	PO_4^{3-}	SO_4^{2-}	Cl^-	BOD	FC	Na	K	Ca	Mg	Al	Mn	Fe
pH	1.000														
alk	0.765	1.000													
NO_3^-	0.034	0.180	1.000												
PO_4^{3-}	0.129	0.186	0.321	1.000											
SO_4^{2-}	−0.411	−0.379	0.251	0.236	1.000										
Cl^-	−0.143	0.192	0.311	0.192	0.419	1.000									
BOD	−0.066	0.018	0.562	0.505	−0.119	−0.078	1.000								
FC	−0.032	0.012	0.505	0.479	−0.205	−0.082	0.765	1.000							
Na	0.019	0.615	0.211	0.211	0.046	0.298	0.231	0.297	1.000						
K	0.024	0.124	0.369	0.176	0.036	0.196	0.363	0.289	0.311	1.000					
Ca	−0.238	0.795	0.211	−0.231	0.110	0.158	0.150	0.212	0.389	0.175	1.000				
Mg	−0.241	0.699	0.059	−0.142	0.150	0.127	0.137	0.188	0.212	0.163	0.721	1.000			
Al	−0.569	−0.279	0.021	−0.561	0.213	0.159	0.119	0.154	−0.129	0.113	0.119	0.121	1.000		
Mn	−0.512	−0.196	−0.119	−0.126	0.105	0.131	0.158	0.139	−0.071	0.069	0.182	0.198	0.318	1.000	
Fe	−0.611	−0.312	−0.126	−0.685	0.200	0.172	0.167	0.127	−0.095	0.093	0.169	0.198	0.495	0.621	1.000

Pearson correlation coefficients calculated using TECHBASE software package. Number of samples = 56; r95% = 0.238.
BOD, biological oxygen demand; FC, faecal coliform.

Table 7. *Proportion of total metal concentration in < 0.45 μm filtrate*

Sample	Depth (m)	pH	Fe (%)	Al (%)	Mn (%)
4 deep groundwater	100	7.8	1.3	0.5	2.0
3 deep groundwater	93	7.5	1.9	1.3	13.6
4 deep groundwater	89	8.0	nd	nd	0.1
3 deep groundwater	75	7.6	1.6	1.2	6.5
7 deep groundwater	70	7.9	nd	nd	0.4
19 deep groundwater	65	7.0	6.7	4.9	30.0
9 deep groundwater	62	7.7	0.9	1.8	6.4
11 deep groundwater	50	7.5	1.5	1.4	16.5
17 deep groundwater	45	7.6	1.6	1.2	9.6
5 deep groundwater	40	7.3	2.3	1.9	20.7
5 deep groundwater	36	7.4	2.2	2.2	22.8
21 deep groundwater	30	6.8	11.9	6.2	33.8
2 shallow groundwater	25	5.8	19.2	8.9	46.8
6 shallow groundwater	20	4.9	37.8	12.7	57.0
8 shallow groundwater	15	6.0	14.6	7.4	45.4
14 shallow groundwater	8	5.7	22.4	9.3	47.7
8 shallow groundwater	5	5.6	19.5	10.8	48.7
24 surface water	0	7.0	5.6	3.2	26.6
30 surface water	0	7.7	0.8	1.1	4.3
28 surface water	0	8.0	nd	0.1	0.1

nd, not detected. All values expressed as a % of total metal concentration in groundwater.

nitrate and phosphate. Faecal coliform numbers are also positively correlated to Na and K, and BOD is positively correlated to K (Table 6) (see below).

Aluminium, Fe and Mn are all positively correlated to each other suggesting they are influenced by similar mechanisms in the groundwaters. Additionally all metals are inversely correlated to pH as would be anticipated since mobility is enhanced at low pH (Jones *et al.* 1974; Jeffries & Hendershot 1981; Lindsay & Walthall 1981). However, none of these elements is significantly positively correlated with any anion (Table 6) so mobilization may be as hydroxide, organically-bound species (Shotyk 1984; Smith *et al.* 1996) or by microbial activity (see below). Both Al and Fe are strongly positively correlated with phosphate, as high dissolved Al and Fe in groundwaters would lead to Fe/Al-oxide precipitation and phosphate retention through surface adsorption processes (Chesworth *et al.* 1989). In acidic shallow groundwaters total concentrations of Al, Fe and Mn can exceed $1\,mg\,l^{-1}$, the majority of which is retained by the 0.45 μm filter and is considered to be colloidal (Table 7). From the filtration of Al into several fractions it is clear that much of the groundwater Al is held in particulate forms (Fig. 4). The transport and mobilization of Al, Fe and Mn can be inferred from the chemistry and mineralogy of sediments collected from Dodoma water pipes and Makutuapora water storage tanks (Table 8). Sediments are composed of evaporite salts, amorphous material, clay minerals (from the wall of the pipes) and Al–Fe hydroxides and sulphates. Additionally, other trace metals are also mobilized based on the chemistry of the sediments (Table 8). It is not possible to infer what proportion of these elements has been leached away from the clay pipes and what has migrated in the waters from the aquifer.

From three boreholes (9, 11 and 22, all in granodiorite lithology) sampling was possible from the water-table to 98 m as the holes were unlined (waters represent both deep, > 30 m, and shallow, < 30 m, groundwaters). The hydrochemistry of the three holes is shown in Fig. 5. Levels of Al, Fe, and Mn decreased with depth in the aquifer, with the highest concentration at the weathering front, in the ferricrete at *c.* 20 m depth (Figs 5e, 5h, 5f respectively). At this point pH was lowest (Fig. 5a) promoting dissolution and mobilization of the metals. Silica concentration was relatively static throughout the water column with a maximum at 85 m depth (possibly a density effect within the brine). Like Na, Ca and sulphate alkalinity, Mg and B also showed no systematic variation with depth. As would be expected biological oxygen demand and dissolved oxygen decreased with depth, although the apparently high values below 75 m depth agreed with the surprising observation of active protists deep in the aquifer.

%)	Mn (%)
	2.0
	13.6
	0.1
	6.5
	0.4
	30.0
	6.4
	16.5
	9.6
	20.7
	22.8
	33.8
	46.8
	57.0
	45.4
	47.7
	48.7
	26.6
	4.3
	0.1

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deep in the aquifer.

Table 8. *Mineralogy and geochemistry of pipe precipitates from Makutuapora aquifer, Dodoma, Tanzania*

Site	Mineralogy	SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO	Na_2O	K_2O	SO_4	Mn	Co	Cu	Zn
storage tank	goethite, quartz, kaolinite, jarosite-alunite	36.9	21.5	20.5	0.9	0.49	1.58	2.32	3.4	23450	79	112	76
clay pipe 1.5 km	goethite, copiapite, aluminite, manganite, halite, gypsum, quartz, sylvite, natron	21.2	23.9	23.9	1.7	0.58	1.96	3.20	8.8	32250	96	36	97
clay pipe 5 km	goethite, halite, gypsum, sylvite, illite, natron-trona, gibbsite	8.9	12.9	18.6	16.44	2.92	8.22	2.12	12.8	120	19	15	25

Mineralogy by XRD and geochemistry by ICPAES (oxides shown in wt% and elements in mg/kg).

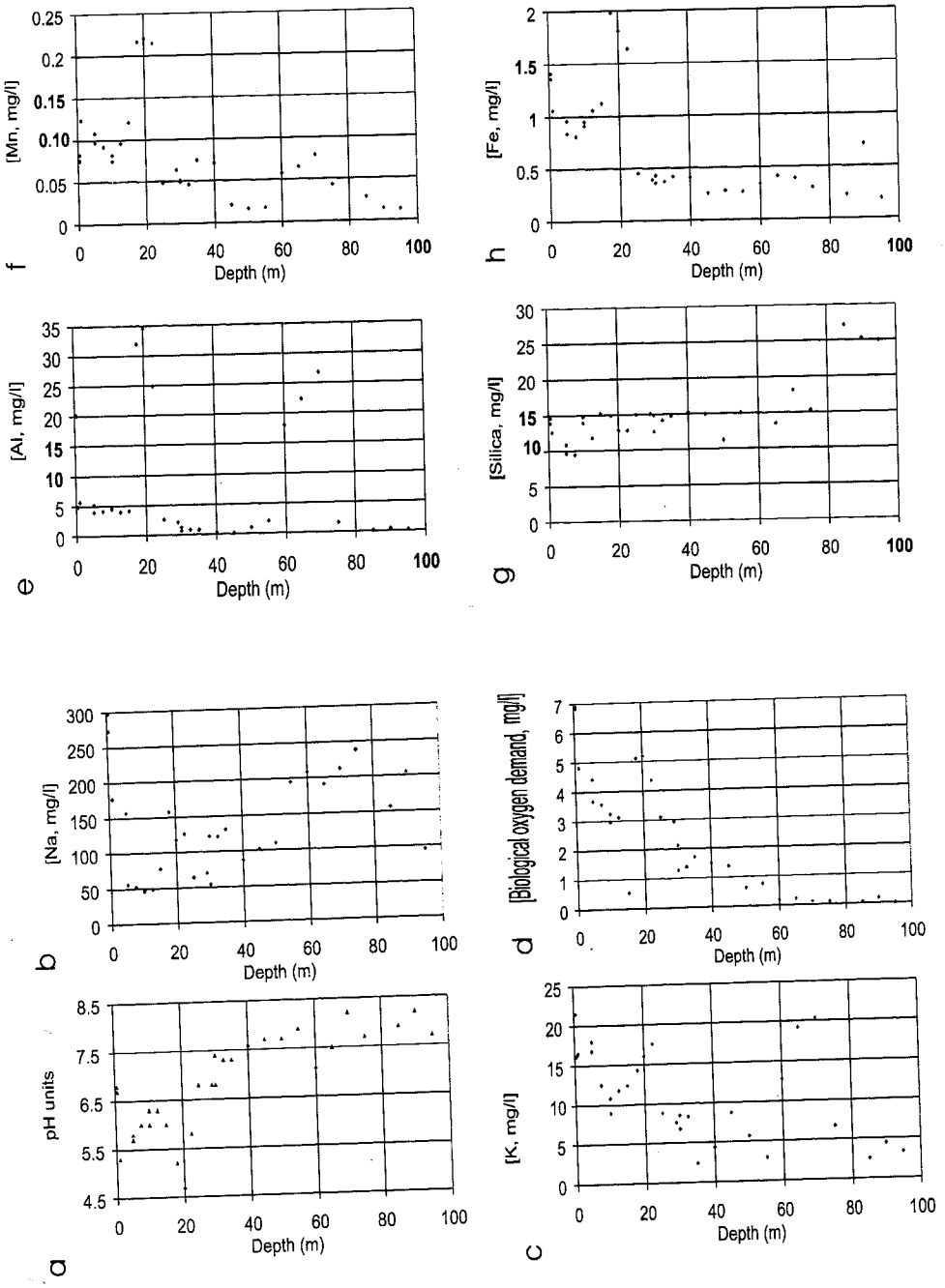
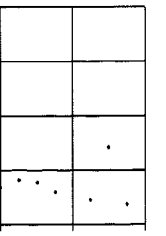
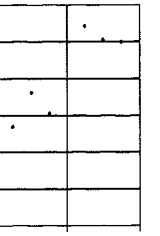
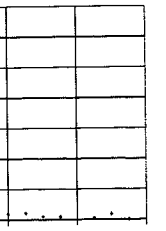
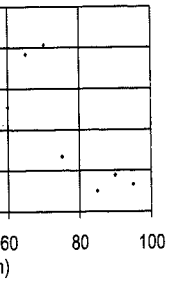


Fig. 5. Hydrochemical and biological oxygen demand variations with depth from sites 9, 11 and 22.









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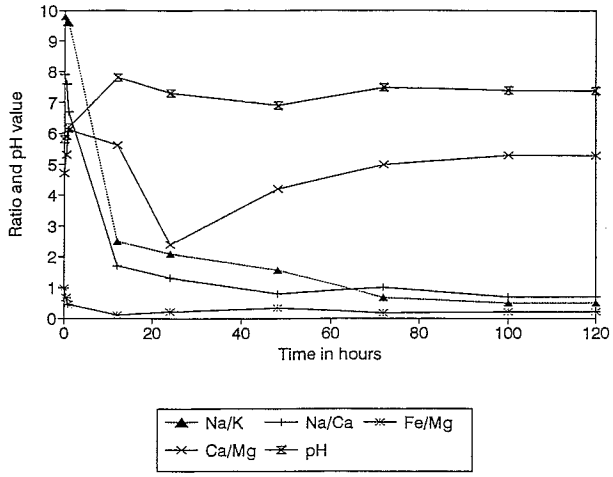

Fig. 6. Change in element ratios and pH with time from site 17 hosted by granodiorite.

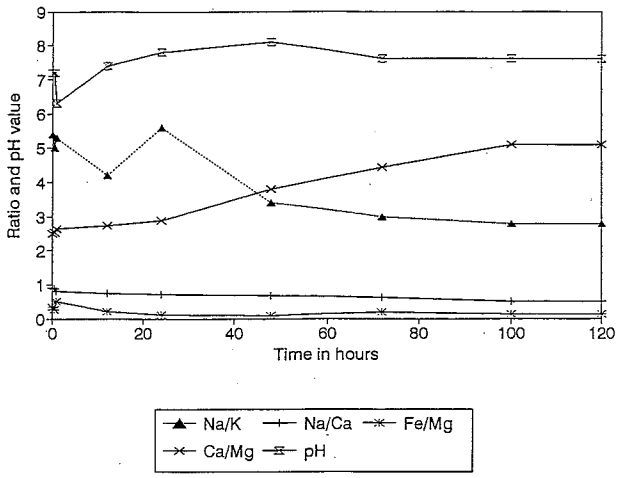

Fig. 7. Change in element ratios and pH with time from site 2 hosted by amphibolite.

Intriguingly, this apparent increase correlates to a relative increase in sulphate, chloride and K (Fig. 5) at a depth of 70–80 m in the groundwaters.

During the course of fieldwork two new boreholes were sampled immediately following initial contact with the aquifer over a 5 day period. The first hole was in the granodiorite (site 17) and the second in the amphibolite (site 2). At the initial stage of water release at site 17, fluid chemistry was dominated by Na and bicarbonate (not shown), but after 12 hours K and Ca increased in concentration and this can be observed in the variation in Na/K and Na/Ca ratios (Fig. 6). The initial Na/K ratio was 9.8 while the initial Na/Ca ratio was greater than 5 but this decreased after 12 hours to 2.5 for Na/K and less than 2 for Na/Ca. After 100 hours of continuous pumping the ratios stabilized at Na/K = 0.5 and Na/Ca = 1 (Fig. 6). No stability was observed in the Ca/Mg or Fe/Mg ratio. Over the same period the level of total Fe dropped from 18.2 mg l^{-1} to less than 0.2 mg l^{-1} and pH increased from 5.8 to 7.4 (Fig. 6). These ratios were also recorded when the well was resampled five months later. This high initial Fe level may explain the poor taste often experienced with newly drilled wells. Another newly

drilled well was monitored from an initial point (0.5 hours after water was hit) but with amphibolite as the host rock. Here the fluid chemistry was dominated by Ca and bicarbonate over the whole 100 hour period of continuous pumping and also in samples collected 5 months later. The Na/Ca ratio over a 100 hour period decreased from 0.9 to 0.5 while the Ca/Mg ratio increased from 2.5 to 3.8 in the first 48 hours (Fig. 7). The Fe/Mg ratio was even more erratic than in the granodiorite well and pH was largely constant varying from an initial 7.2 to 7.6 60 hours later (Fig. 7). The stabilization of the ratios occurred after about 100 hours in these wells.

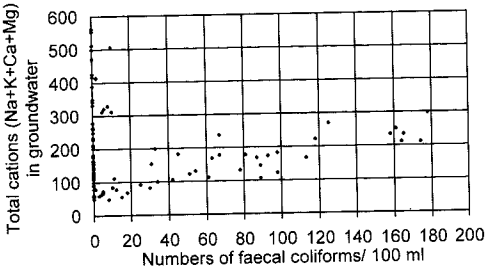

Fig. 8. Total alkalis v. faecal coliform counts for Makutuapora groundwaters.

Microbiology of Dodoma aquifers

Significant numbers of thermotolerant faecal coliforms (> 10 per 100 ml) were present at 50% of the sites studied. This indicates excessive faecal contamination of groundwater sites, particularly in shallow aquifers. The numbers of faecal coliforms showed positive correlations with NO_3^- (r = 0.505), PO_4^{3-} (r = 0.479), Na (r = 0.297), K (r = 0.289) and BOD (r = 0.765) in the Makutuapora groundwaters (Table 6). There was a relatively complex relationship between the total content of the cations Na, Ca, K and Mg and numbers of faecal coliforms in the Makutuapora groundwaters. Faecal coliform numbers of between 1 and 80 per 100 ml groundwater were present over the whole range of cation concentrations found. The highest numbers of faecal coliforms (100–180 per 100 ml groundwater), however, were only found to be present in samples with the higher cation content (< 300 mg/l, Fig. 8). The numbers of bacteria capable of aerobic heterotrophic growth (i.e. utilizing organic carbon as a carbon and energy source) in the surface and subsurface borehole soils were in the range 9.0×10^5 to 2.3×10^7 CFU g^{-1} wet weight soil and was not clearly related to any geochemical characteristics of the groundwater systems.

Bacteria involved in the cycling of nitrogen were isolated from all the sampling sites. Dentrifying bacteria were most abundant with 2.0×10^2 to $1.8 \times 10^5 g^{-1}$ wet weight groundwater material. Nitrifying bacteria were also present at all sample sites, including both ammonia oxidizing bacteria (in general $< 2.0 \times 10^0 g^{-1}$ wet weight soil), and nitrite oxidizing bacteria (in general $< 2.0 \times 10^0 g^{-1}$ wet weight soil).

Sulphate reducing bacteria, which contribute to the cycling of sulphur, were present in low numbers between 2.0×10^0 to $1.4 \times 10^3 g^{-1}$ wet weight soils at all sample sites studied.

The biomass measured by lipid phosphate determination was similar for all the sample sites, 171 and 172 nMole g^{-1} dry weight (Table 9). This suggests a far more constant microbial community size than demonstrated through culture techniques, which varied by several orders of magnitude between sites (above). The amount of DNA, another measure of biomass, also varied with sample. There was a range of DNA content from 158–418 ng g^{-1} dry weight sample (Table 9). The amount of biomass measured by lipid phosphate or DNA did not appear to be related to the geochemical characteristics of the groundwater system and was independent of depth.

RNA, an indicator of microbial activity, showed an exceptionally wide variation between sites (< 1–2827 ng g^{-1} dry weight sample). The amounts of individual adenylates (ATP, ADP, AMP) fluctuated with sample as did the total adenylates (Table 9). In a number of samples ATP was below the levels of detection. The calculated EC ratios for the Tanzanian aquifer (only calculated for those sites with measurable ATP) were all below 0.5. Again there was no apparent relationship between the depth the sample was taken from and activity. Some relationships did exist between geochemical characteristics in the groundwater and microbial activity by RNA and adenylate measurement. Microbial activity measured by total adenylates (Fig. 9a) and RNA (Fig. 9b) tended to be highest at the lowest groundwater concentration of the halides F^- and Cl^-. There was a positive relationship between metals, particularly Al, and total adenylates reaching a plateau at adenylate concentrations above 10 p mole per gram dry weight sample (Fig. 10a). A positive relationship between RNA and all three metals

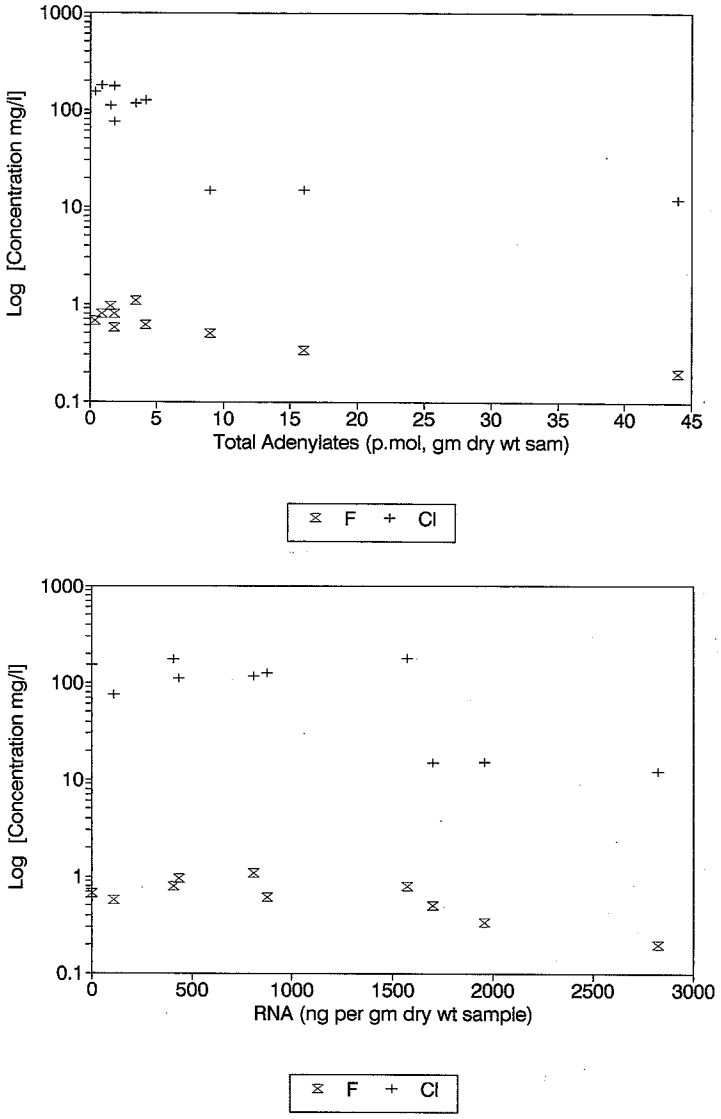

Fig. 9. Halides F^- and Cl^- v. microbial activity measured by biomarkers for Makutuapora groundwaters; (**a**). total adenylates v. F and Cl; (**b**) RNA v. F and Cl.

was also found (Fig. 10b). It seems clear that there was a relationship between microbial activity in groundwater and the concentration of Al, Mn and Fe.

Protists were isolated in enrichment cultures from 22 out of 32 sites suggesting that they are widespread in the subsurface throughout the region. Representatives of three main morphological groups were present: flagellates, ciliates and amoebae. Of these, flagellates were by far the most abundant. All protists which could be recognized are known to be bacterivorous.

Discussion

Microbiological health of Dodoma groundwaters

The microbiological health of groundwater is controlled by a variety of biological and abiotic parameters. These factors include the frequency

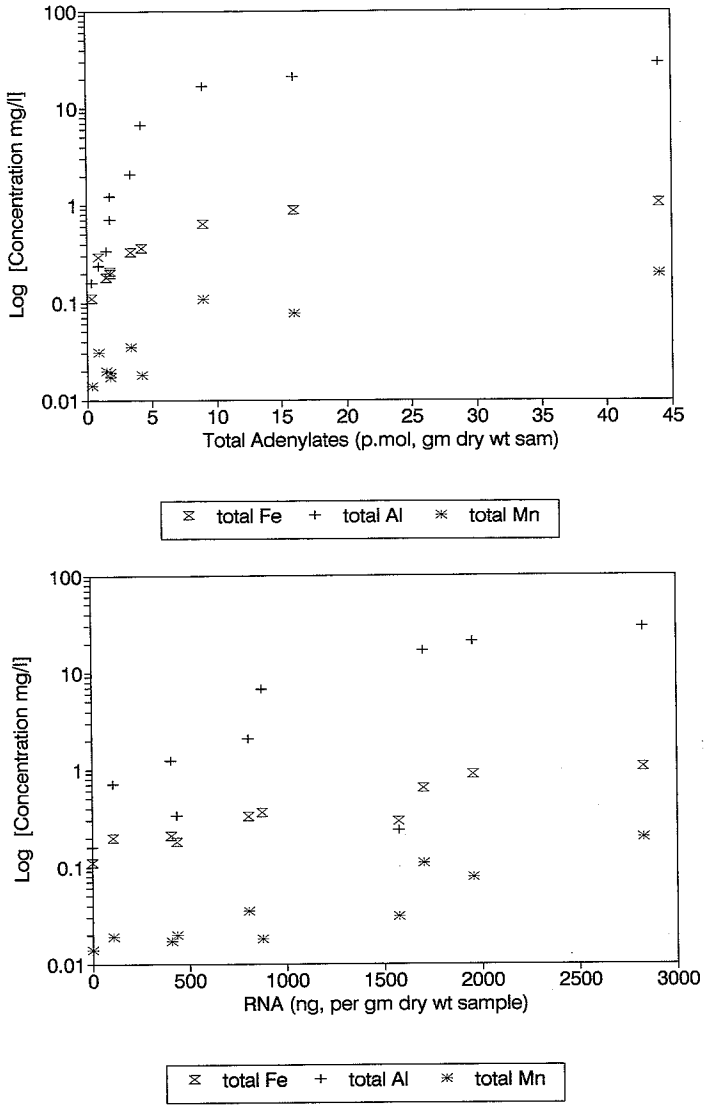

Fig. 10. Total metal concentration v. microbial activity measured by biomarkers for Makutuapora groundwaters; (**a**) total adenylates v. Al, Fe and Mn; (**b**) RNA versus Al, Fe and Mn.

of faecal contamination of groundwater from human or animal sources and the longevity of enteric pathogens in groundwater. The survival of microbial pathogens is influenced by the abiotic conditions in the groundwater and the biomass and activity of the indigenous microbial community.

The faecal pollution indicated by the presence of thermotolerant coliforms in the Tanzanian aquifers is likely to have originated from both animal and human sources. Animal sources may be particularly important as the land in the region is heavily grazed. The presence of these indicators of faecal pollution suggests a considerable risk of contamination of these aquifers by enteric pathogens.

Among the abiotic factors that may influence the survival of thermotolerant coliforms and faecal pathogens, are the geochemical characteristics of the aquifer. There was a positive correlation between the numbers of thermotolerant faecal coliforms and the concentrations of NO_3^- and PO_4^{3-}. It is possible that higher soil concentrations of these anions have a protective role aiding survival of thermotolerant coliforms in soils. The longevity of enteric pathogens

Table 9. *Microbial biomass and activity measured by biomarkers at selected sites in the Makutuapora area*

Sample	Lipid phosphate (in moles)*	DNA (ng)*	RNA (ng)*	AMP (p mole)*	ADP (p mole)*	ATP (p mole)*	Total adenylates (p mole)*	Energy charge†
2 shallow	172	220	2827	30.4	8.5	5.1	44.0	0.21
7 deep	172	235	1701	4.3	4.0	0.7	9.0	0.30
8 shallow	171	276	806	2.7	0.7	nd‡	3.4	nc§
10 deep	172	165	407	1.4	0.4	nd	1.8	nc
10 deep	171	418	878	3.4	0.8	nd	4.2	nc
14 shallow	172	320	1959	5.6	6.2	4.2	16.0	0.46
14 shallow	172	193	436	0.6	0.4	0.5	1.5	0.47
15 shallow	171	305	110	0.9	0.9	nd	1.8	nc
15 shallow	172	158	< 1	0.4	nd	nd	0.4	nc
23 shallow	172	181	1573	0.5	0.4	nd	0.9	nc

*Measurements per g/dry weight sediment; †energy charge calculated as (ATP + 0.5 ADP)/(ATP + AMP + ADP); ‡nd, not detected; §nc, not calculated.
Samples taken at the same site, e.g. 10, 14 and 15, were from different depths in the profile.

may, also, be increased in this way. A positive correlation was also observed between numbers of thermotolerant faecal coliforms and BOD (Table 6). The higher concentrations of BOD are likely to be due to elevated concentrations of organic matter, arising from faecal contamination, acting as a source of nutrients for the growth of indigenous microorganisms (Harvey *et al.* 1984; Madsen *et al.* 1991).

The biomass and activity of the soil microbial community are also likely to affect the longevity of thermotolerant coliforms and enteric pathogens in the groundwater systems, since the indigenous community tends to eliminate foreign microorganisms. The microbial biomass measured by lipid phosphate was remarkably constant between samples and with depth (Table 9). Lipid phosphate is considered a relatively reliable measure of biomass due to its rapid turnover and loss on cell death (White *et al.* 1979). Measures of DNA can also be taken to provide an estimate of biomass (Paul & Meyers 1982) and have been correlated with direct bacterial counts (McCoy & Olson 1985). The use of DNA as a measure of biomass is slightly complicated, however, by the increase in DNA in a cell just prior to division during cell growth (Neidhardt *et al.* 1990). This may result in a rise in DNA measured but no actual increase in cell numbers, although cell mass will increase, until after cell division has occurred. In addition, DNA may be released from microorganisms and other soil or surface organisms on their death. This DNA, which is not cell associated, may persist for a period in the environment. Any free DNA in the aquifer would influence estimates of biomass by DNA measures. Given that the lipid phosphate remains relatively constant between aquifer samples in the Tanzanian study, the higher values of DNA in some samples i.e. $> 300\,ng\,g^{-1}$ dry weight sediment (Table 9) compared to others, may indicate that the bacteria at these sites were actively dividing or that there was variation in the amount of free DNA with sample.

The amount of RNA (Karl *et al.* 1981) and adenylates (Karl & Holm-Hansen 1978) present in samples gives information on the physiological status of the bacterial community. High RNA levels are found in active cells and drop rapidly at the onset of starvation or other stress (Harder *et al.* 1984). There is a clear divergence in RNA content with sample indicating considerable fluctuations in microbial activity (Table 9). ATP is a precursor to cell nucleic acids (DNA and RNA), as well as providing energy for general cell biosynthesis (Harder *et al.* 1984). Levels of adenylates fluctuate considerably with nutritional and physiological conditions and provide information on the physiological status and activity of the microbial community. There were variations in the levels of individual and total adenylates with sample (Table 9). The highest values of ATP and total adenylates were in general found in those samples with the largest RNA content, confirming these samples as potentially the most microbially active. EC (see methods) is a measure of the physiological state of the organisms within a sample and is independent of population size (Karl & Holm-Hansen 1978). EC could only be calculated for four of the Tanzanian samples studied since ATP in all the remaining samples was below levels of detection. The calculated EC values were < 0.5 suggesting relatively low activity in these samples. There is some discrepancy be-

tween this and the RNA results. It is possible that EC determinations are in fact influenced by the role of ATP as a precursor for the nucleic acids. In natural environments ATP and other adenylates may have a rapid turnover rate in cells resulting in the detection of only low concentrations.

The environmental characteristics of the groundwater system are likely to be an important determinant of the activity of the microbial community. It is often difficult to find correlations between geochemical factors and microbial growth and activity since many different factors interact to determine levels of bacterial activity. Even so, a slight inverse relationship was observed between microbial activity (biomarker data) and concentrations of the halides F^- and Cl^- in the groundwater (Fig. 9). Data on interactions between halides and microorganisms are relatively limited. A number of reactions, however, have been described. These include microbial halide binding (Bors *et al.* 1984), oxidation/reduction reactions (Tsungai & Sase 1969; Gozlan & Margalith 1973), and alkylation reactions (Harper & Hamilton 1988; Manley & Dastoor 1988). In addition, the halides are highly efficient disinfectants which may partly explain the inverse relationship between activity and F^- and Cl^-. Positive relationships between microbial activity, measured by either RNA or adenylate biomarkers, and the concentration of Al, Mn and Fe were found (Fig. 10a, b). Manganese and Fe can be oxidized by certain heterotrophic and autotrophic bacteria as part of their normal physiological processes, although the latter organisms are unlikely to be present in these tropical groundwaters. In addition, microorganisms can reduce oxidized forms of these metals (Beveridge & Doyle 1989; McEldowney *et al.* 1993). Iron and Mn are also required by microorganisms as trace elements i.e. micro-nutrients, and therefore their availablity may have an impact on microbial activity. Aluminium has no known microbiological function. Microbial activity may, however, affect the fate of Al in tropical groundwater (see below).

Eukaryotic microbes (protists) were found to be widespread throughout the Makutuapora aquifer. Until relatively recently protists were thought to be absent in subsurface environments. However, evidence is now accumulating that protists comprise a substantial part of many subsurface microbial populations (Sinclair & Ghiorse 1987, 1989; Kinner *et al.* 1992; Novarino *et al.* 1994). Previous studies of subsurface protists have been confined almost exclusively to temperate regions. This is thought to be the first record of protists in tropical groundwaters.

Recent research indicates that the vast majority of groundwater protists have a strong predilection for surfaces and are usually found attached to, or closely associated with, the aquifer sediment (Harvey *et al.* 1992; Novarino *et al.* 1994). Few protists are found free-swimming in groundwaters. Collection of subsurface protists therefore requires that cores of aquifer material are taken. Access to freshly-collected aquifer material was available at only a few sites in the Makutuapora aquifer. Sampling procedures mostly consisted of pumping groundwater to the surface. Therefore, it is assumed that the protists isolated during the present study represented only a small proportion of those present in the aquifer.

The vast majority of the protists isolated from the Makutuapora aquifer were heterotrophic. This is consistent with previous studies carried out in temperate regions (Sinclair & Ghiorse 1987; Novarino *et al.* 1994). Furthermore, all the protists identified during the present study were bacterivorous. Consquently, these may directly influence the levels of bacterial activity in the subsurface environment thereby indirectly affecting biogeochemical processes.

Mineral–water interactions

The major changes in the mineral assemblages are the loss of feldspar, pyroxene, biotite, and hornblende with the formation of zeolites (natrolite), hydrated phyllosilicates (kaolinite, sericite, vermiculite, montmorillonite and chlorite), oxides and hydroxides (goethite, gibbsite, hematite, and leucoxene). These changes are a function of mineral–water interactions in the bedrock aquifer and tropical weathering in the upper layers of the weathering profiles. Silicate minerals such as feldspar are being altered to hydrated forms, such as albite or microcline to kaolinite. This can also be inferred from the systematic changes in Na/K and Na/Ca ratios for newly drilled wells. Initially there is leaching of alkalis and alteration of oligoclase to albite and paragonite with time. In the amphibolites a similar variation in pumped water chemistry from a freshly drilled aquifer was noticed by variation in Na/Ca ratio, related to the alteration of feldspars and in the Fe/Mg and Ca/Mg ratio related to the alteration of ferromagnesium silicates augite and bronzite. The lack of constancy in the Fe/Mg ratio could be due to dissolution of ilmenite–magnetite as well as pyroxenes. The constant Na/Ca and Ca/Mg ratios noticed in established wells (those drilled prior to sampling seasons) indicate that a steady-

state is attained between aluminosilicates in the wallrock and leachate chemistry. These steady states are attained instantly on geological time scales, within the space of a few hundred hours.

Biogeochemistry of Makutuapora waters

The biomass and activity of the microbial community has an impact, not only on the survival of enteric pathogens, but also on mobility, form and fate of organic and inorganic compounds in groundwater. This has health implications when water is extracted for drinking. There is a paucity of information available on the biomass and activity of microbial communities in tropical groundwater systems (see above), and the microbial community structure of soils and sediments associated with tropical aquifers.

This study demonstrated that the heterotrophic bacterial population present in the Tanzanian surface and subsurface soils associated with boreholes is towards the upper end of the range reported for aerobic heterotrophs isolated from shallow (Balkwill & Ghiorse 1985) and deep (Albrechtsen & Winding 1992; Fredrickson *et al.* 1989) groundwater in non-tropical regions. Heterotrophic bacteria play an important role in the degradation of organic compounds.

Bacteria involved in the nitrogen biogeochemical cycle were present at all sites examined. Denitrifying bacteria represented a significant fraction of the heterotrophic bacterial population. Denitrifying bacteria are commonly found to be present and active in groundwater systems in temperate environments (Francis *et al.* 1989; Johnson & Wood 1992). Nitrifying bacteria, both ammonia oxidizing bacteria and nitrite oxidizing bacteria, were present at the Tanzanian sites. It has previously been found that the population density of nitrifying bacteria in temperate groundwater sediments is low, e.g. $10\,g^{-1}$, even when ammonia is not limiting. This has been attributed to competition for oxygen with aerobic heterotrophs (Fredrickson *et al.* 1989). Ammonia oxidizing bacteria are unable to dominate over heterotrophs particularly at available organic carbon levels below $1–2\,mg\,l^{-1}$ (McCarthy *et al.* 1981). The heterotrophic numbers isolated from the Tanzanian aquifer were high and the explanation for the low numbers of nitrifying bacteria may be the result of adverse competition for oxygen.

Sulphate reducing bacteria (SRB) have been isolated from shallow and deep aquifers, their numbers often increasing with depth (Jones *et al.* 1989; Johnson & Wood 1992). Their distribution and numbers have previously been related to the clay content of sediment, and have been difficult to correlate with groundwater sulphate concentration (Jones *et al.* 1989). Similarly, there was no significant correlation between groundwater sulphate concentration and the numbers of sulphate reducing bacteria at Makutuapora. In fact, the number of SRBs was low at most sites.

There is an apparent contradiction in the variation in isolated microbial numbers with sample and the relatively constant estimate of biomass shown in the lipid phosphate determinations. This discrepancy is probably simply related to the inevitably selective nature of culture media and chosen growth conditions failing to permit growth of many types of bacteria.

The presence of Fe and Al minerals in mineral precipitates in the feeder pipes between Dodoma and Makutuapora suggest that some mechanism exists for mobility of both metals. Similarly high levels of Mn and other metals occur in the pipe precipitates as well. A similar phenomenon observed in Wales has also been reported by Fuge *et al.* (1992) and likewise used to support the mobilization of metals in groundwater. Previous studies in temperate regions have shown that in carbon-limited habitats such as the subsurface, organic contamination of faecal origin often results in a significant increase in microbial activity (Harvey *et al.* 1984; Madsen *et al.* 1991). Increased microbial activity may in turn influence the mobility of certain elements in the aquifer through a variety of mechanisms discussed previously. Elevated levels of dissolved organic matter released by microbial activity in the groundwaters may also explain the observed relationships between elevated metal concentration and high BOD in some groundwaters.

Most of the aluminium was associated with particles in the size range $0.1–10.0\,\mu m$, which is colloidal either mineralogical or bacterial. The source of the aluminium may have been from the congruent dissolution of kaolinite in the upper soils. The dissolution of aluminium may also have been assisted by indigenous microorganisms, a phenomenon previously reported by McFarlane & Heydeman (1984) and by McFarlane & Bowden (1992). There is, however, little information on interactions between bacteria and aluminium in the environment. The importance of microorganisms in the mobilization of aluminium in deeply weathered profiles of the African surface has been highlighted by McFarlane (1987) and McFarlane & Bowden (1992). In these studies it was found that aluminium was leached from kaolinite by the action of indigenous populations of microorganisms, and further-

more that the aluminium remained mobile within the groundwaters, probably as a result of microbially-mediated organic complexing. Bacteria can accumulate metals at their surface and internally (Beveridge & Doyle 1989). Internal metal accumulation by bacteria is normally an energy dependent process and increases with time. Even potentially toxic metals with no physiological function can be taken up internally. Cell surface sorption of metals is a physicochemical process and tends to be relatively rapid, sometimes reaching equilibrium within minutes of metal exposure (McEldowney *et al.* 1993). Aluminium at the levels present in Makutuapora groundwater could be considered 'toxic' (WHO 1971; Connery 1990). Microorganisms within these groundwaters may assist in attaining these conditions and further, may be involved in the transportation and deposition of the metals. However, more recent work suggested that in tropical waters much of the Al is present in a non-labile form of relatively low potential toxicity (Smith *et al.* 1996).

Conclusions

In this study the geochemistry and microbiology of groundwaters and surface waters of the Makutuapora aquifer have been studied. Groundwater is essentially Na-Ca-Cl-HCO_3^- with minor K, Mg, F and SO_4^{2-}. Water chemistry is largely influenced by mineral–water interactions and less so by microbial activity, although the concentrations of Al, Mn and Fe in groundwater may be related in part to microbial activity.

The biochemical marker analysis suggests that the bacterial community is of comparable size between the different study sites, but that the physiological status of the community varies. This is undoubtedly related to variations in the physicochemical and nutrient conditions encountered by the bacteria at the sites. The numbers of nitrifying and denitrifying bacteria and SRBs isolated from the tropical groundwater systems were broadly comparable with numbers found to be present in temperate systems. By contrast, numbers of heterotrophs appeared relatively high in these tropical systems. In common with temperate groundwaters, there were no apparent correlations between the geochemical characteristics of the tropical groundwater and the numbers of different physiological types. There was considerable contamination of the groundwaters by thermotolerant coliforms suggesting a significant health risk from enteric pathogens in these groundwaters. The survival of the thermotolerant coliforms appeared to be linked with some of the geochemical characteristics of the groundwaters. Heterotrophic protists were widespread throughout the aquifer and are likely to influence bacterial activity by their predatory behaviour.

Metal content of the groundwaters can exceed $1\,mg\,l^{-1}$ of Al, Mn and Fe. These metals are present largely in a particulate, possibly colloidal form. The increase in metals suggests an imbalance in the steady-state reactions between groundwaters and magmatic minerals. This imbalance could in part be in response to microbial activity.

At present only weak correlations can be drawn from the field data which could be coincidental rather than casual and experimental work will be required to prove these possible relationships and to aid modelling of the biogeochemical cycles in the Makutuapora groundwaters.

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References

ALBRECHSTEN, H-J. & WINDING, A. 1992. Microbial biomass and activity in subsurface sediments from Vezen, Denmark. *Microbial Ecology*, **23**, 303–317.

BALKWILL, D. L. & GHIORSE, W. C. 1985. Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. *Applied and Environmental Microbiology*, **50**, 580–588.

BEVERIDGE, T. J. & DOYLE, R. J. 1989. *Metal Ions and Bacteria*. Wiley, New York.

BORS, J., MASTENS, R. & KUHN, W. 1984. Investigations on the influence of micro-organisms on the translocation of radio-iodide in soil. *In:* BONNYS-VAN GELDER, E. & KIRCHMAN, R. (eds) *Role of Microorganisms on the Behaviour of Radionuclides in Aquatic and Terrestrial Systems and their Transfer to Man*, Proc. Workshop Int. Union of Radioecologists, Brussels, 25–27 April 1984, 219–227.

BOWELL, R. J., MCELDOWNEY, S. & WARREN, A. 1995. *Biogeochemical Factors Affecting Water Quality in Tanzanian Waters*. Final report of NHM-Water-

Aid-University of Westminster project, 1992/93 (unpublished).

———, MORLEY, N. H. & DIN, V. K. 1994. Arsenic speciation in porewaters, Ashanti, Ghana. *Applied Geochemistry*, **9**, 15–23.

BRIMHALL, G. H. & DIETRICH, W. E. 1987. Constitutive mass balance relations between chemical composition, volume, density, porosity, and strain in metasomatic hydrochemical systems: results of weathering and pedogenesis. *Geochimica et Cosmochimica Acta*, **51**, 567–587.

———, LEWIS, C. J., AGUE, J. J., DIETRICH, W. E., HAMPEL, J., TEAGUE, T. & RIX, P. 1988. Metal enrichment in bauxites by deposition of chemically mature aeolian dust. *Nature*, **333**, 819–824.

CAHEN, L. & SNELLING, N. J. 1984. *The Geochronology and Evolution of Africa*. Clarendon, Oxford.

CHESWORTH, W., VAN STRAATEN, P. & SEMOKA, J. M. R. 1989. Agrogeology in East Africa. *African Journal of Earth Sciences*, **9**, 352–362.

CONNERY, J. 1990. Summary report of workshop on aluminium and health, Oslo, May 2–5 1988. *Environmental Geochemistry and Health*, **12**, 179–196.

ERTEL, J. R., HEDGES, J. I., DEVOL, A. H., RICHEY, J. E. & RIBEIRO, M. de N. G., 1986. Dissolved humic substances of the Amazon river system. *Limnology and Oceanography*, **31**, 739–754.

FARQUHARSON, F. A. & BULLOCK, A. 1992. The hydrology of basement complex regions of Africa with particular reference to southern Africa. *In:* WRIGHT, E. P. & BURGESS, W. P. (eds) *Hydrogeology of Crystalline Basement Aquifers in Africa*, Geological Society, London, Special Publication, **66**, 59–76.

FRANCIS, A. J., SLATER, J. M. & DODGE, C. J. 1989. Denitrification in deep subsurface sediments. *Geomicrobiology Journal*, **7**, 103–116.

FREDRICKSON, J. K., GARLAND, T. R., HICKS, R. J., THOMAS, J. M., LI, S. W. & MCFADDEN, K. M. 1989. Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. *Geomicrobiology Journal*, **7**, 53–66.

FUGE, R. F., PEARCE, N. J. G. & PERKINS, W. T. 1992. Unusual sources of aluminium and heavy metals in potable water. *Environmental Geochemistry and Health*, **14**, 15–18.

GOZLAN, R. S. & MARGALITH, P. 1973. Iodide oxidation by a marine bacterium. *Journal of Applied Bacteriology*, **36**, 407–417.

HARDER, W., DIJKHUIZEN, L. & VELDKAMP, H. 1984. Environmental regulation of microbial metabolism. *In:* KELLY, D. P. & CARR, N. G. (eds) *The Microbe 1984. II Prokaryotes and Eukaryotes*. Cambridge University Press, Cambridge.

HARPER, D. B. & HAMILTON, J. T. G. 1988. Biogenesis of halomethanes by fungi. *In:* CRAIG, P. J. & GLOCKING, F. (eds) *The Biological Alkylation of Heavy Elements*. Royal Society of Chemistry, Special Publication, **66**, 197–200.

HARVEY, R. W., SMITH, R. L. & GEORGE, L. 1984. Effects of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod, Mass., aquifer. *Applied and Environmental Microbiology*, **48**, 1197–1202.

———, KINNER, N. E., BUNN, A. L. & MACDONALD, D. 1992. Transport of protozoa through an organically contaminated sandy aquifer. *In: First International Conference on Groundwater Ecology, US EPA*. American Research Association, 111–118.

JEFFRIES, D. S. & HENDERSHOT, W. H. 1981. Aluminium geochemistry at the catchment scale in watersheds influenced by acidic precipitation. *In:* SPOISTO, G. S. (ed.) *The Environmental Chemistry of Aluminium*, CRC, Boca Raton, 279–302.

JOHNSON, A. C. & WOOD, M. 1992. Microbial potential of sandy aquifer material in the London Basin. *Geomicrobiology Journal*, **10**, 1–13.

JONES, B. F., KENNEDY, V. C. & ZELLUREGE, G. W. 1974. Comparison of observed and calculated concentrations of dissolved Al and Fe in stream water. *Water Resources Research*, **10**, 791–793.

JONES, R. E., BEEMAN, R. E. & SUFLITA, J. M. 1989. Anaerobic metabolic processes in deep terrestrial subsurface. *Geomicrobiology Journal*, **7**, 117–130.

KARL, D. M. & HOLM-HANSEN, O. 1978. Methodology and measurement of adenylate energy charge ratios in environmental samples. *Marine Biology*, **48**, 185–197.

———, WINN, C. D. & WONG, D. C. L. 1981. RNA synthesis as a measure of microbial growth in aquatic environments: evaluation, verification, and optimization of methods. *Marine Biology*, **64**, 13–21.

KINNER, N. E., BUNN, A. L., HARVEY, R. W., WARREN, A. & MEEKER, L. D. 1992. Preliminary evaluation of the relationships among protozoa, bacteria and chemical parameters in sewage contaminated groundwater at Otis Air Base, Massachusetts. *In:* MULLARD, G. E. & ARONSON, D. A. (eds) *USGS Toxic Substances Hydrology Program Proc. Tech. Mtg.* WRI Report 91-4034, 148–151.

LAHERMO, P., SANDSTROM, H. & MALISA, M. 1991. The occurrence and geochemistry of fluoride in natural waters in Finland and East Africa with reference to geomedial implications. *Journal of Geochemical Exploration*, **4**, 65–79.

LINDSAY, W. L. & WALTHALL, P. M. 1981. The solubility of aluminium in soils. *In:* SPOISTO, G. S. (ed.), *The Environmental Chemistry of Aluminium*, CRC, Boca Raton, 221–240.

MCCARTHY, P. L., REINHARD, M. & RITTMANN, B. E. 1981. Trace organics in groundwater. *Environmental Science and Technology*, **15**, 40–51.

MCCOY, W. F. & OLSON, B. 1985. Fluorometric determination of the DNA concentration in municipal drinking water. *Applied Environmental Microbiology*, **49**, 811–817.

MCELDOWNEY, S., HARDMAN, D. J. & WAITE, S. 1993. *Pollution: Ecology and Biotreatment*. Longman Scientific & Technical, Harlow, 261–289.

MCFARLANE, M. J. 1987. The key role of microorganisms in the process of bauxitisation. *Modern Geology*, **11**, 325–344.

——— '& BOWDEN, D. J. 1992. Mobilization of aluminium in the weathering profiles of the African surface in Malawi. *Earth Surface Pro-*

cesses and Landforms, **17**, 789–805.

——— & HEYDEMAN, M. T. 1984. Some aspects of kaolinite dissolution by a laterite-indigenous micro-organism. *Geo-Eco-Trop.*, **8**, 73–91.

MADSEN, E. L., SINCLAIR, J. L. & GHIORSE, W. C. 1991. In situ biodegradation: microbiological patterns in a contaminated aquifer. *Science*, **252**, 830–833.

MADINI. 1992. *Stratigraphy and Geochronology of Dodoma, Tanzania*, Unpublished report.

MAJI. 1988. *Hydrogeology of the Makutuapora aquifer: Results of deep drilling*. Government printers, Dares Salaam.

MANLEY, S. L. & DASTOOR, M. N. 1988. Methyl iodide (CH_3I) production by kelp and associated microbes. *Marine Biology*, **98**, 477–482.

MANN, A. W. 1983. Hydrogeochemistry and weathering on the Yilgarn block, Western Australia-ferrolysis and heavy metals in continental brines. *Geochimica et Cosmochimica Acta*, **47**, 181–190.

NEIDHARDT, F. C., INGRAHAM, J. L. & SCHAECHTER, M. 1990. *Physiology of the Bacterial Cell. A Molecular Approach*. Sinauer Associates Inc., Sunderland, Massachusetts.

NOVARINO, G., WARREN, A., KINNER, N. E. & HARVEY, R. W. 1994. Protists from a sewage-contaminated aquifer on Cape Cod, Massachusetts, U.S.A. *Geomicrobiology Journal*, **12**, 23–36.

OGBUKAGU, I. K. 1984. Hydrology of groundwater resources of the Aguta area, SE Nigeria. *Journal of African Earth Science*, **2**, 109–117.

PAUL, J. H. & MEYERS, J. 1982. Fluorimetric determination of DNA in aquatic microorganisms by use of Hoechst 33258. *Applied and Environmental Microbiology*, **43**, 1393–1399.

SHOTYK, W. 1984. Metal-organic species in natural waters. *In:* FLEET, M. E. (ed.), *MAC Short Course Handbook, Environmental Geochemistry*. 45–66

SINCLAIR, J. L. & GHIORSE, W. C. 1987. Distribution of protozoa in subsurface sediments of a pristine groundwater study site in Oklahoma. *Applied and Environmental Microbiology*, **53**, 1157–1163.

——— & ——— 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. *Geomicrobiology Journal*, **7**, 15–31.

SMITH, B. J., BREWARD, N., CRAWFORD, M. B., GALIMAKA, D., MUSHIRI, S. M. & REEDER, S. 1996. The environmental geochemistry of aluminium in tropical terrains and its implications to health. *This volume*.

SMITH, O. L. 1982. *Soil Microbiology: a Model of Decomposition and Nutrient Cycling*, CRC, Boca Raton.

TRESCASES, J-J. 1992. Chemical Weathering. *In:* BUTT, C. & ZEEGERS, H. (eds), *Regolith Exploration Geochemistry in Tropical and Subtropical terrains. Handbook of Geochemistry, vol. IV*, 25–40.

TSUNGAI, S. & SASE, T. 1969. Formation of iodide-iodine in the ocean. *Deep Sea Research*, **16**, 484–487.

VELBEL, M. A. 1989. Mechanisms of saprolization, isovolumetric weathering, and replacement during weathering – A review. Geochemistry of the earth's surface *Chemical Geology*, **84**, 17–18.

——— 1992. Constancy of silicate-mineral weathering-rate ratios between natural and experimental weathering implications for hydrologic control of differences in absolute rates. *Chemical Geology*, **105**, 89–99.

WHITE, D. C., BOBBIE, R. J., HERRON, J. S., KING, J. & MORRISON, S. 1979. Biochemical measurements of microbial biomass and activity from environmental samples. *In: Native Aquatic Bacteria: Enumeration, Activity and Ecology*. ASTM, Special Technical Publication, **695**, American Society for Testing and Materials, Philadelphia, 69-81.

WHO 1971 *International Standards for Drinking Water*. Geneva.

WILLIAMS, C. T., SYMES, R. F. & DIN, V. K. 1993. Mobility and fixation of a variety of elements, in particular B, during the metasomatic development of adinoles at Dinas Head, Cornwall. *Bulletin of the Natural History Museum (Geology Series)*, **49**, 81–98.

WRIGHT, E. P. 1992. The hydrogeology of basement complex regions of Africa with particular reference to southern Africa. *In:* WRIGHT, E. P. & BURGESS, W. P. (eds) *Hydrogeology of Crystalline Basement Aquifers in Africa*, Geological Society, London, Special Publication, **66**, 21–58.