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Nitrogen isotopes of *Eichhornia crassipes* (water hyacinth) confirm sewage as leading source of pollution in Hartbeespoort Reservoir, South Africa

Nitrogen (N) isotopes of aquatic organisms offer a way of differentiating sources of dissolved nitrate species in water. Water quality in the Hartbeespoort Reservoir has been a problem for many decades, causing excessive growth of algae and water hyacinth, both of which further cause human health issues, degradation of environmental water quality, and recreational hazards. Six boreholes and four surface water locations were sampled and analysed for certain water quality parameters and stable water isotopes (H and O). Electrical conductivity and pH were acceptable, but faecal coliforms and *Escherichia coli* were high in the Crocodile River. δD and $\delta^{18}O$ showed that there is little groundwater input to the reservoir and the surface water experiences significant evaporation. Six samples of water hyacinth were analysed for C and N stable isotopes. The $\delta^{15}N$ values ranged from 20‰ to 33‰, indicating sewage or manure as the primary source of dissolved N in Hartbeespoort Reservoir. As high dissolved N concentrations cause water hyacinth growth to outstrip any manual, chemical or biological control measures, it is suggested that efforts to control the water hyacinth infestation on Hartbeespoort Reservoir focus on informal settlement sanitation and upgrades to sewage treatment works in the Crocodile River catchment.

Significance:

This work is possibly the first report on nitrogen isotopes in plant material to trace water pollution in South Africa. It presents a new line of evidence regarding eutrophication in the Hartbeespoort Reservoir. It indicates the optimal management method for controlling water hyacinth on this and other waterbodies. The study has relevance for agriculture, urban wastewater management, informal settlement sanitation, invasive alien plant control, recreation and tourism.

Introduction

Declining water quality due to human activities is a global trend of increasing concern.¹ This phenomenon has been known for decades^{2,3} and awareness to address the issue has extended into the less industrialised parts of the world, including Africa⁴⁻⁶. One of the key pollutants in surface, groundwater and coastal water across the world is nitrogen (N), in the form of various dissolved species, such as nitrate, nitrite and ammonium.⁷⁻⁹ Nitrate is the dominant form in environmentally active waters, as it is the most oxidised species. Sources of nitrate in water include sewage (human faeces), manure (animal faeces), compost (plant wastes), inorganic fertiliser (N-P-K type fertilisers), N-based explosives and natural nitrogen-fixing bacteria in plant roots.

Nitrogen has two stable isotopes: ¹⁴N (99.64%) and ¹⁵N (0.36%). Nitrogen is unusual in that the most abundant isotope has an uneven number of neutrons (7). As nitrogen moves through the biosphere and hydrosphere, fractionation of these two isotopes can take place during chemical and physical reactions, resulting in different substances with different abundances of each isotope. Nitrogen isotopes can therefore be used as tracers to determine the path taken by nitrogen compounds through inorganic, organic and biological processes. As there is already a large difference in the concentrations of the two isotopes, the relative change in concentrations, compared to a standard, provides a much better measure of the isotope ratios in different substances than absolute amounts of each isotope. $\delta^{15}N$ is a measure of the deviation in the ¹⁵N/¹⁴N ratio in samples, compared to a standard. For this purpose, the standard used is the atmosphere (AIR), and the isotopic abundances are reported in delta (δ) units in parts per thousand (‰) deviation from the standard:

$$\delta^{15}N_{\text{sample}} = 1000 \times \left(\frac{{}^{15}N/{}^{14}N_{\text{sample}}}{{}^{15}N/{}^{14}N_{\text{standard(AIR)}}} - 1 \right)$$

The $\delta^{15}N$ values of the various sources of nitrate vary such that some of the sources may be identified, but others may have a substantial overlap in values.⁸ This variation in isotope composition has been used to recognise the type of activity responsible for nitrate in water resources¹⁰, be it natural or anthropogenic^{7,8}, or even to distinguish the type of anthropogenic source, such as that done by Costanzo et al.¹¹ to identify sewage affecting the marine environment. A complicating factor is the fractionation between the dissolved nitrogen species being used by the plants (e.g. nitrate and ammonia) and the nitrogen compounds in the plants themselves. However, Deutsch and Voss¹² showed that minimal isotope fractionation occurs during uptake of nitrogen by aquatic plants. Similarly, Lee et al.¹³ found fractionation between the dissolved species in water and various trophic levels of organism (mussels and fish) to be minor, meaning the $\delta^{15}N$ values in organisms approximately represent the $\delta^{15}N$ values of the source dissolved species.

Water quality has been a problem in the Hartbeespoort Reservoir for a long time, due to the catchment being largely affected by human activity, including agriculture, mining, industry and urbanisation.¹⁴ Research on pollution of the reservoir has been done over the years, including on phosphorus¹⁵, organic contaminants (PAH – polycyclic aromatic hydrocarbons and PCB – polychlorinated biphenyls)¹⁶ and source attribution from acid mine waters¹⁷. Recent work shows that water quality problems, including algal blooms, are still dire and many water quality parameters exceed irrigation guidelines.¹⁸

Water hyacinth (*Eichhornia crassipes*) is a floating freshwater plant originally from South America; it is an aggressive invader in warm regions, and is the world's worst invasive aquatic plant.^{19,20} The plant was introduced to South Africa about 100 years ago as an ornamental garden plant and is now a well-established weed in many waterways. Water hyacinth has been a serious problem in Hartbeespoort Reservoir for many decades, causing reduction in recreational usage of the waterbody, reduction in light and increase in consumption of oxygen, that together limit growth of other organisms.^{21,22} It is well known that water hyacinth prefers warm, nutrient-rich water to grow in and experiments have shown that nutrient concentrations are the primary determinant of growth rates.²³

Previous work on the water quality of the Hartbeespoort Reservoir used flow rates and analysis of nitrogen (N) and phosphorus (P) content of tributaries, including effluent from sewage works, to determine that sewage works are the primary source of N and P.²⁴ However, as shown in the same study, N and P concentrations decreased substantially over the length of river flow from the Kempton Park sewage works in Johannesburg to the Rietvlei Reservoir south of Pretoria, which lies on the Hennops River, a tributary of the Crocodile and therefore also the Hartbeespoort Reservoir. Significant contributions of N and P from agricultural and urban drainage may therefore be offsetting declines in original sewage contributions. The extent to which sewage works contribute to the total N and P load flowing into Hartbeespoort, and other reservoirs, can benefit from further work.

In this study, we aimed to use nitrogen isotopes of water hyacinth in the Hartbeespoort Reservoir as a new line of evidence to support the generally held view that sewage works are the primary cause of the hypertrophic state of the water body.

Study area

The Hartbeespoort Reservoir is situated in the North-West Province of South Africa, about 30 km west of Pretoria. The dam is built into the quartzite that forms the mountainous ridge of the Magaliesberg, with the water of the northwards flowing Crocodile River backing up to the south of the ridge (see Figure 1).

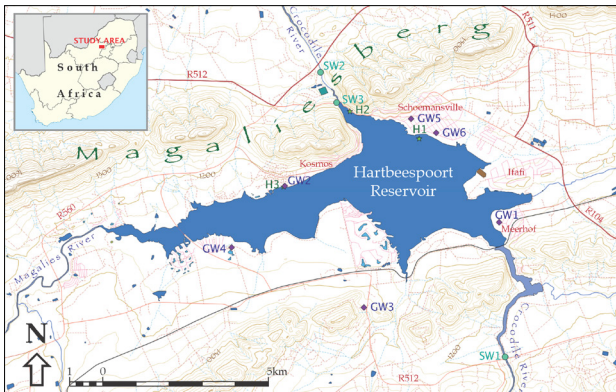


Figure 1: Map of the study area, showing sample locations. See Table 1 for an explanation of the sample codes.

Geology

The geology of the region is dominated by the Pretoria Group of the Transvaal Supergroup, a well-preserved, relatively undeformed Archaean to Proterozoic sequence of metamorphosed volcano-sedimentary rocks.²⁵ Large volumes of concordant, mafic sills occur within the stratigraphy. In the vicinity of the Hartbeespoort Reservoir, the geology strikes east-west and dips gently northwards, a tilting caused by gravitational warping of the crust when the Bushveld Igneous Complex intruded to the north, cooled and subsided. The Hartbeespoort Reservoir is sited on shale of the Silverton Formation and mafic sills. To the south and north occur quartzite ridges of the Daspoort and Magaliesberg Formations, respectively, with the latter being the rock used to site and anchor the dam wall. To the south, lower in the stratigraphy of the Pretoria Group, andesite, shale, sandstone and other rock types are found, and further south dolomite of the Malmani Subgroup of the Chuniespoort Group is also found.²⁶ Even further south in the Johannesburg area, the catchment is underlain by granite-gneiss terrain, minor greenstones and quartzites of the Witwatersrand Supergroup. To the north of the Magaliesberg Formation lie the coarse-grained mafic and ultramafic rocks of the Bushveld Igneous Complex.

The area is faulted, with the dominant faults being two NNW-SSE striking normal faults that create a graben structure, displacing the Magaliesberg ridge visibly southwards (see Figure 1) and causing the gap through which the Crocodile River flows and where the Hartbeespoort Dam was built.²⁷

Climate

The region experiences a seasonal, dry subtropical climate with convective summer rain. Daily minimum to maximum temperatures average 5 °C to 24 °C in winter (May to July) and 16 °C to 30 °C in summer (November to January)²⁸ (Figure 2). Frost does occur on winter mornings, but is uncommon. The rainy season typically commences in October and extends until March or April, and the mean annual rainfall is about 670 mm, with most of this associated with thunderstorms. Winds are very light, except for downdraughts during thunderstorms.²⁹

Hydrology

The Hartbeespoort catchment is 4144 km² in size and extends southwards from the dam, incorporating the Crocodile River (including the Jukskei and Hennops Rivers) and Magalies River, as well as the minor, non-perennial Leeuspruit and Swartspruit streams.²⁴ The Hartbeespoort Dam was completed in 1923 and, after raising the wall in 1971, now stores 195 GL when full, with an average depth of 9.6 m.³⁰ The flows of the tributaries have been substantially altered by urban, agricultural and industrial activity. In particular, winter (dry season) flows are larger than natural flows due to continuous urban stormwater and sewage inputs.

Hydrogeology

The area has several different types of aquifers. Near the surface, primary porosity is developed in surficial deposits (alluvium and colluvium) and weathered material.³¹ Adjacent to the reservoir, the quartzites of the Pretoria Group are fractured and provide a secondary porosity aquifer of reasonable yield. Further south, the high-yielding Malmani Subgroup dolomite aquifer occurs.

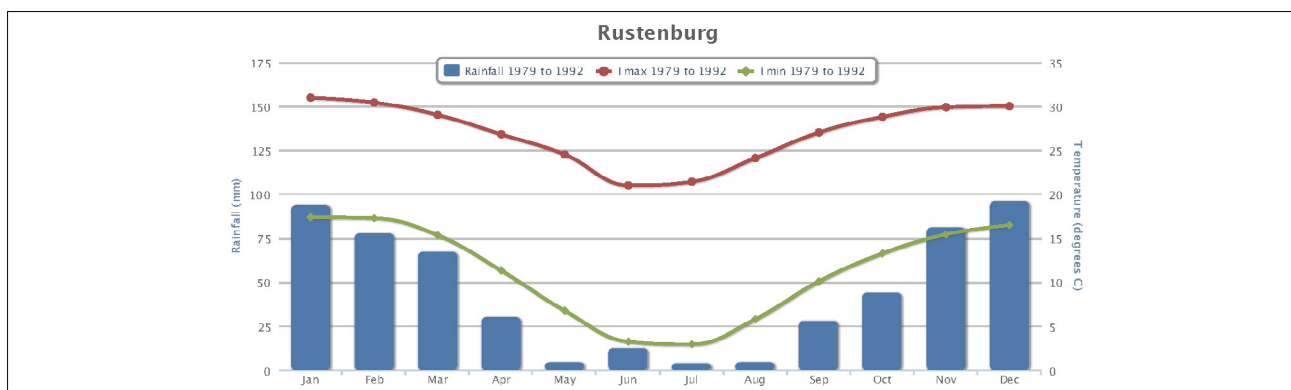


Figure 2: Temperature and rainfall averages for Rustenburg, 40 km northwest of the study area.²⁸

Groundwater quality is generally good, but with localised pollution or risk of pollution, due to the highly populated character of the area.³¹

Methods

For details of the sampling locations, refer to Figure 1 and Table 1. Surface water was sampled at four locations: a short distance upstream of the reservoir in the Crocodile River, a short distance downstream of the dam in the Crocodile River, and in the reservoir at the wall at surface and at 15 m depth. Groundwater was sampled from six boreholes located all around the reservoir, from very nearby, to almost 2 km away. Water hyacinth was sampled at three locations, and duplicates were taken at each location.

Water samples were analysed in the field using ExTech field probes for temperature, electrical conductivity (EC), pH, redox potential (Eh) and dissolved oxygen. Samples were taken and a lab analysis was conducted for microbial parameters and stable isotopes. Water samples were analysed for faecal coliforms and *Escherichia coli* at the CSIR Pretoria laboratory. An appropriate volume of water sample (250–500 mL) was filtered through a membrane filter upon which bacteria were entrapped. The filter was then placed on a selective growth medium and incubated at 44.5 °C for 18–24 h, after which colonies characteristic of faecal coliforms were counted. The number of faecal coliforms is reported per 100 mL of the original sample. Colonies from the membranes in the test for faecal coliforms were then picked and inoculated into tubes containing tryptone water. The tubes/bottles were then incubated at 44.5 °C ± 1 °C for 24 h. After incubation, Kovac's reagent was added. Tubes producing a red layer were positive for *E. coli*.

Table 1: Sample locations and descriptions

ID	Sample type	Location	Latitude	Longitude
H1	Hyacinth	Schoemansville Oewer Club	25° 44' 08" S	27° 52' 14" E
H2	Hyacinth	Scott Street (R104)	25° 43' 41" S	27° 51' 05" E
H3	Hyacinth	Metsi A Me offices	25° 44' 56" S	27° 49' 59" E
SW1	Surface Water	Crocodile River upstream of reservoir	25° 47' 45" S	27° 53' 40" E
SW2	Surface Water	Crocodile River down stream of reservoir	25° 43' 02" S	27° 50' 36" E
SW3.1	Surface Water	Above dam at surface	25° 43' 32" S	27° 50' 52" E
SW3.2	Surface Water	Above dam at 15 m depth	25° 43' 32" S	27° 50' 52" E
GW1	Groundwater	Meerhof School	25° 45' 31" S	27° 53' 34" E
GW2	Groundwater	Metsi A Me offices	25° 44' 55" S	27° 50' 00" E
GW3	Groundwater	African Swiss Restaurant	25° 46' 56" S	27° 51' 19" E
GW4	Groundwater	Lakeland Estate	25° 45' 56" S	27° 49' 07" E
GW5	Groundwater	Schoemansville	25° 43' 48" S	27° 52' 06" E
GW6	Groundwater	Schoemansville	25° 44' 02" S	27° 52' 31" E

Stable hydrogen and oxygen isotopes were analysed at the University of Pretoria. Each water sample was extracted into a 5-mL container and labelled prior to the isotope analyses. The water samples were run using a Los Gatos Research laser cavity ringdown instrument. Five working standards were used to calibrate the results: LGR Working Std 1 ($\delta^2\text{H}=-154.1\text{‰}$, $\delta^{18}\text{O}=-19.57\text{‰}$), LGR Working Std 2 ($\delta^2\text{H}=-117\text{‰}$, $\delta^{18}\text{O}=-15.55\text{‰}$), LGR Working Std 3 ($\delta^2\text{H}=-79\text{‰}$, $\delta^{18}\text{O}=-11.54\text{‰}$), LGR Working Std 4 ($\delta^2\text{H}=-43.6\text{‰}$, $\delta^{18}\text{O}=-7.14\text{‰}$), and LGR Working Std 5 ($\delta^2\text{H}=-9.8\text{‰}$, $\delta^{18}\text{O}=-2.96\text{‰}$).

The water hyacinth samples were separated into roots, stems and leaves, left in an oven to dry at 70 °C for 48 h and crushed into a powder. About 1.1–1.2 mg of the powder was loaded into tin capsules pre-cleaned in toluene, combusted at 1020 °C in a Flash EA1112 elemental analyser and fed, via a ConFlo IV system, directly into a Delta V Plus stable light isotope mass spectrometer. Laboratory standards Merck Gel ($\delta^{13}\text{C}=-20.26\text{‰}$, $\delta^{15}\text{N}=7.89\text{‰}$, C=41.28%, N=15.29%) and DL-Valine ($\delta^{13}\text{C}=-10.57\text{‰}$, $\delta^{15}\text{N}=-6.15\text{‰}$, C=55.50%, N=11.86%) were used and a blank sample was run after every 11 unknown samples.

Results and discussion

Water quality

The water quality and stable isotope results are shown in Table 2 and the water hyacinth analyses in Table 3. The pH of the water is neutral to slightly alkaline, with surface water having the more alkaline values, but all samples are well within drinking water guidelines of 6.0–9.0³² (Figure 3). The EC (measured as mS/m) also shows the samples are fresh water, generally acceptable for drinking (Figure 3).³² The total dissolved solids was calculated from the EC by the ExTech field probe, and so is an approximation. The freshest water (279 mg/L) was found in GW3, the borehole to the south of the reservoir, and probably reflects freshly recharged groundwater from the Witwatersberg hills to the south, which comprise Daspoort Formation quartzite. Fast flow through fractures and the lack of chemical input from weathering due to the quartzitic rock probably account for the freshness of the groundwater in this borehole. The highest dissolved content occurs in GW5, northeast of the reservoir in Schoemansville and is probably due to this borehole being drilled into the Silverton Formation, a shale dominated layer, which encourages evaporation prior to recharge and causes addition of dissolved matter from weathering and concentration of this dissolved matter by slow groundwater flow.

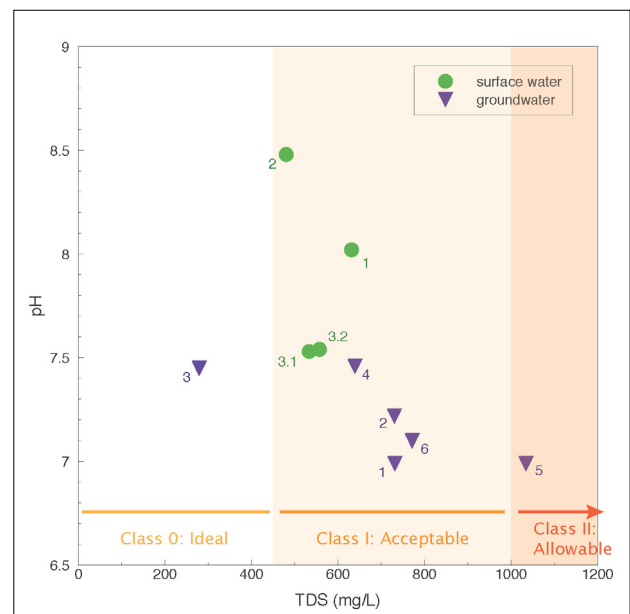


Figure 3: TDS (total dissolved solids) and pH values for groundwater and surface water with the SANS 241 water quality guideline values shown.²⁷ The pH is acceptable throughout.

Based on the microbiological analyses, the groundwater appears safe to drink; however, the surface water is not. The inflowing water from the Crocodile River is the most polluted by microbes, with outflowing reservoir water less so, and, interestingly, the water in the reservoir appears to have no coliforms. This is perhaps due to competition with algae or hyacinth, or consumption by other microbes, or destruction by sunlight (UV radiation).

Water stable isotopes

The stable isotopes of water show a very clear differentiation between the groundwater and surface water samples. The surface water samples are relatively enriched in the heavier isotopes, and plot to the right of

Table 2: Water quality and stable isotope results for groundwater and surface water samples

Sample	T °C	pH	EC mS/m	TDS mg/L	DO mg/L	Eh mV	δD ‰	δ ¹⁸ O ‰	FC /100 mL	<i>E. coli</i> /100 mL
GW1	22.2	6.9	104	731	1.84	160	-11.9	-3.15	<1	<1
GW2	20.0	7.2	99	730	2.88	163	-21.5	-4.58	<1	<1
GW3	23.8	7.4	40	279	4.74	168	-28.9	-5.90	<1	<1
GW4	20.8	7.5	88	639	4.70	170	-14.9	-3.38	<1	<1
GW5	21.0	7.0	143	1034	4.88	163	-7.4	-2.92	<1	<1
GW6	21.9	7.1	110	771	2.60	160	-17.4	-3.72	<1	<1
SW1	17.9	8.0	79	631	3.56	176	-4.5	-0.98	1400	420
SW2	17.5	8.5	66	480	5.60	152	+1.1	-0.85	41	41
SW3.1	18.3	7.5	79	533	2.92	151	+2.8	-0.90	<1	<1
SW3.2	17.9	7.5	78	557	2.40	160	+0.3	-1.03	<1	<1

T, temperature; EC, electrical conductivity; TDS, total dissolved solids; DO, dissolved oxygen; Eh, redox potential; FC, faecal coliforms
TDS was calculated from EC

the local meteoric water line (LMWL), which here is the Johannesburg LMWL³³ (Figure 4). This is a sign of evaporation having taken place since precipitation occurred, which is to be expected for water in rivers and reservoirs.³⁴ The groundwater samples all plot close to the JLMWL, which is a sign that minimal evaporation takes place prior to recharge. Interestingly, GW3 has the most negative δ values. This is usually a sign of either recharge at higher altitude (an isotopic altitude effect), or heavy rainfall events (an isotopic amount effect).³⁵ Recharge on top of the Witwatersberg could account for a part of this, as the slightly higher, cooler and wetter location on top of this ridge would drive the isotope composition of precipitation (and therefore recharge) towards more negative δ values. This confirms the conclusions drawn from the chemistry data, that this borehole contains groundwater that was recharged faster, through fractures in the quartzite of the Daspoort Formation.

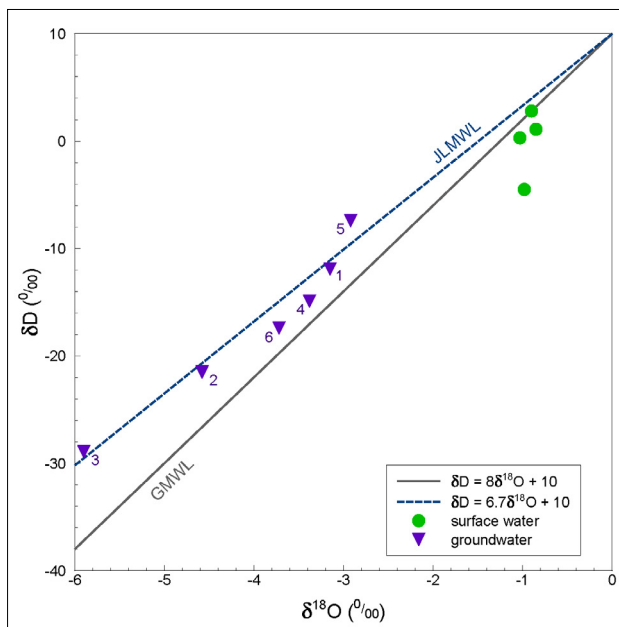


Figure 4: Stable isotope composition of surface and groundwater samples, with the Johannesburg Local Meteoric Water Line (JLMWL)³³ and Global Meteoric Water Line (GMWL)³⁷ shown for reference.

Water hyacinth stable isotopes

Table 3 and Figure 5 show the C and N stable isotope results for the water hyacinth samples. It can be seen in Table 3 that there is little

variation in the δ¹³C values, the range being 25–30‰, and they fall into the typical range for C3 metabolism plants. The δ¹⁵N values range from 20‰ to 33‰, with an average of 26.4‰. The values are displayed in Figure 5, where it is apparent that there is no systematic variation, either by sample location, or by plant part.

Table 3: Water hyacinth stable isotope analyses

Sample	Weight g	δ ¹⁵ N ‰	N %	δ ¹³ C ‰	C %	C/N
1.1R	1.12	23.13	3.3	-25.79	38.5	13.5
1.2R	1.18	32.53	3.0	-28.34	35.9	13.9
2.1R	1.17	30.87	3.1	-26.00	38.8	14.6
2.2R	1.13	28.71	2.4	-27.26	40.6	19.7
3.1R	1.16	26.21	2.6	-27.50	41.2	18.5
3.2R	1.15	20.90	2.7	-25.35	38.3	16.7
Mean R	1.15	27.06	2.8	-26.71	38.9	16.2
1.1S	1.16	22.55	3.3	-27.08	34.1	12.1
1.2S	1.14	29.04	2.5	-27.98	34.4	15.8
2.1S	1.18	29.10	1.9	-26.70	35.1	21.4
2.2S	1.12	27.68	1.9	-29.60	36.6	22.8
3.1S	1.17	24.81	1.9	-28.10	35.8	22.2
3.2S	1.13	21.78	2.6	-27.22	38.4	17.3
Mean S	1.15	25.83	2.3	-27.78	35.7	18.6
1.1L	1.18	20.89	4.9	-27.15	43.2	10.2
1.2L	1.11	33.39	3.9	-28.60	42.0	12.6
2.1L	1.13	31.64	4.1	-27.52	42.6	12.2
2.2L	1.16	28.83	3.4	-29.89	41.5	14.1
3.1L	1.16	22.24	3.6	-28.28	41.9	13.4
3.2L	1.13	20.35	4.2	-26.69	42.9	11.9
Mean L	1.15	26.22	4.0	-28.02	42.3	12.4

Plants take up nitrogen as dissolved species, such as nitrate or ammonia, in soil water, or, in the case of aquatic plants, from surface water. The $\delta^{15}\text{N}$ in a plant will therefore reflect the $\delta^{15}\text{N}$ of the dissolved species, but not be exactly the same, due to fractionation. Fractionation is dependent upon factors such as concentration of the dissolved species, water movement, temperature and organism specific factors. However, Deutsch and Voss¹² and Lee et al.¹³ showed that, generally, fractionation is minor and the resultant $\delta^{15}\text{N}$ values in organisms reflect approximately that of the original dissolved species in the water.

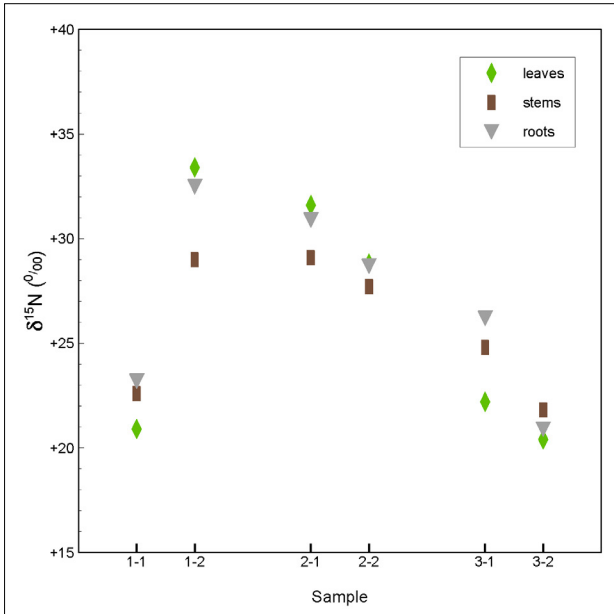


Figure 5: Nitrogen isotope composition of water hyacinth from Hartbeespoort Reservoir.

Figure 6 shows the variation in $\delta^{15}\text{N}$ values across a range of different natural and synthetic materials, as well as the water hyacinth analyses from this study. The results from this study plot outside most of the known ranges, but are closest to that for sewage or manure. This confirms the assertions of previous researchers that the main factor causing poor water quality in Hartbeespoort Reservoir is effluent from sewage works, mainly those servicing Johannesburg.³⁶

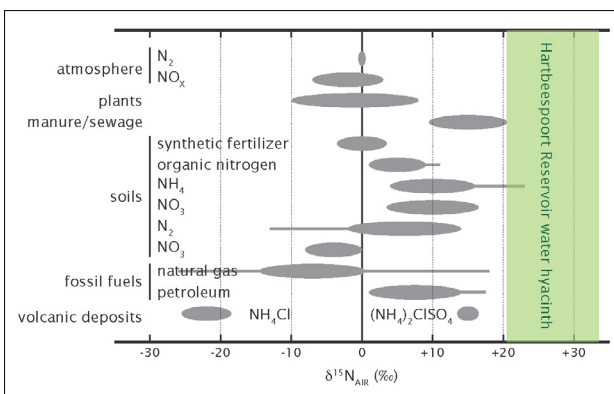


Figure 6: Diagram showing the nitrogen isotope composition of various materials.³⁸

Conclusions

Simple water quality parameters such as EC (40–140 mS/m) and pH (6.9–8.5) are within acceptable ranges in surface water and groundwater of the Hartbeespoort Dam area; however, faecal coliforms and *E. coli* measurements show the surface water, particularly the inflowing Crocodile River, to be high risk to human health. The relatively fresh

nature of the water indicates minimal contribution from industrial or mine effluents, including acid mine drainage, which usually have elevated EC values and, in the case of acid mine drainage, low pH.

Stable isotopes of water (H and O) reveal evaporated surface waters and a variation in groundwater due to the varied geology and landscapes of the area. The clear divide between the δD and $\delta^{18}\text{O}$ values for groundwater and surface water show that the Hartbeespoort Reservoir is primarily surface water fed, with negligible groundwater input.

Nitrogen isotopes of water hyacinth reflect the isotope composition of the dissolved nitrogen species (nitrate etc.) in the reservoir. The $\delta^{15}\text{N}$ averages 26‰, which matches most closely to that for manure or sewage. This confirms assertions of previous researchers that sewage works, mostly those servicing Johannesburg, are the primary cause of poor water quality in the Hartbeespoort Reservoir. As high nutrient levels are the main determinant of water hyacinth growth rates²³ and manual, chemical and biological control struggle to control the infestations, it is clear that any water hyacinth control efforts should target sanitation in informal settlements and the various sewage treatment works flowing into the Crocodile River catchment.

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Competing interests

We have no competing interests to declare.

Authors' contributions

R.G.: Conceptualisation, field work, initial analysis and writing. R.D.: Conceptualisation, some field work, funding, final analysis and article writing (including graphics).

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