You may have heard the saying “you are what you eat”. However, you are also what you drink.

What if we are filling our bodies with water flooded with plastic particles too small to see? I want to know what I am drinking and to find sources of water with the least amount of microplastics possible. Perhaps water from deep underground is less contaminated?

Thus, my research question was: Microplastics in Groundwater - How Do the Amounts and Type of Microplastics Compare Between Different Mineral Springs and Tap Water of the Hepburn-Daylesford Region?

The Hepburn-Daylesford region is a favourite Victorian tourist destination, and no visit is complete without a trip to the mineral springs. (My dog certainly enjoys our trips there). The mineral spring water, originally from deep groundwater, is purported to provide a range of health benefits, including improved muscle performance, digestion, bone and skin health and decreased blood pressure, as advertised by the Daylesford and Hepburn Mineral Springs Company. These surmised health effects stem from the presence of minerals.

It is possible, however, that microplastics have contaminated the waters. Microplastics are water-insoluble, solid plastic pieces conventionally defined as less than 5mm in length. They are categorised as either primary, intentionally manufactured to size, or secondary, microbially degraded from larger plastics. Unfortunately, mismanagement has spread them worldwide. According to studies by Barrett and Mason, respectively, the upper 9cm of ocean sediment holds an estimated 14.4 million tonnes of microplastics and an average of 325 were in every litre of 11 brands of bottled water across nine countries.

Microplastics can permeate soil and be transported into groundwater. They may be carried by water as it moves through the soil and fractures in rock or be ingested by creatures like earthworms and move with them. Numerous recent studies from around the world have found microplastics in groundwater, in widely varying amounts. A study in India found 19.9 particles/L, while a British study found only one fragment per thousand Litres of groundwater. In a 2022 study

from Bacchus Marsh, Samandra’s team found a range of 16 to 97 microplastics/L.

The health effects of microplastic consumption on humans are largely unknown, however, many studies have theorised their effects. Microplastics may induce an immune response and cause inflammation. Ones around 10µm may be able to access all organs, cross cell membranes and the blood–brain barrier. Viaroli and co- writers emphasised that microplastics can absorb hazardous chemicals, such as pesticides and heavy metals, potentially releasing them in the body. These are all scary possibilities. But even scarier – this year microplastics were found in human blood, with a mean concentration of 1.6 µg/mL in 22 healthy Dutch volunteers.

Therefore, it was important to test Hepburn-Daylesford’s mineral springs for microplastics. Knowing what is in our water empowers us to make choices about what we consume and advocate for improvements if necessary.

These mineral springs had never previously been tested for microplastics. So, I was excited to perform an experiment to quantify the amounts present. Six springs were tested: two from Hepburn (Sulphur, Wyuna), two in Daylesford (Central, Sutton) and two in the Regional Park (Tipperary, Jubilee). They have widely varying mineral content, suggesting water flow through different rocks and/or a differing aquifer source, which could result in varying microplastic amounts. Local tap water was also tested for comparison.

The water collection method was influenced by Uhl and co-writers’ Norwegian study on microplastics in drinking water. Immobile water was cleared from the outlet before sampling, and bottles were rinsed with sample water twice before collection.

12 samples from each spring and tap water were taken over four months to increase the findings’ reliability. I followed a rapid-screening method for microplastic identification developed by Maes’s team. A Nile Red/acetone solution was added to the samples and then they were filtered using a vacuum filtration apparatus.

Stained microplastics fluoresce when exposed to blue light under an orange filter. A 7x5 area of squares was examined under a microscope and the microplastics counted. There was a possibility of false-positive co-staining of chitin and cellulose. Chitin, found in shells, is unlikely to be present in spring water. Cellulose from plants or algae may be present, however, Wang and colleagues found cellulose was hardly stained by Nile Red. Microplastics were also categorised by size and shape, into fibres, fragments, microbeads and ‘other’.

While only 18% of each filter was examined, this is a representative sample, adopted by Maes for routine work. Using a microscopic magnification of X40 ensured one full square was in view, allowing me to orientate myself and reduce counting errors. However, only microplastics above approximately 50 micrometres could be seen.

To reduce potential bias, filters were labelled with random number codes after the filtration step, blinding me to the samples’ origin when counting.

All water samples in this study contained microplastics and the amounts are shocking. The highest median number, 1828, was found in tap water. Using this to calculate the concentration, tap water has over 100,000 microplastics/L. The mineral springs contain considerably less, as represented by the box plot.

These numbers are far higher than those found in the literature. Perhaps the Hepburn-Daylesford springs are more contaminated due to the popularity of the area. Alternatively, the method of staining using Nile Red, which was not used by these other studies, may have identified more microplastics or falsely stained some organic substances.

A Kruskal-Wallis test was performed to determine whether the mineral spring and/or tap water results were significantly different. Significantly different results in this study were defined as having a p- value < 0.05 and are highlighted in green. A highly significant difference was found from the Kruskal-Wallis test. A Dunn test was

then used to compare two groups at a time and found significant differences between tap water and every spring.

No significant difference was found between springs. This indicates that microplastic amounts are similar across the various springs, likely resulting from similar contamination methods, such as the flow of microplastics through soil into groundwater. In contrast, tap water was shown to be a different population. It originates in several open reservoirs, which are used for recreational fishing. Fishing line and plastic lures may be contributing to contamination.

The amount of microplastics present over time was also analysed. Three samples of each water type were taken monthly from February to May. A common trend was seen for tap water and all springs except Central - a rise from February to March, a fall to April, then a rise to May.

This appears to correlate with local rainfall. I have two hypotheses about this. Firstly, more rain may increase microplastic amounts if it rapidly washes them into groundwater. This theory is supported by a 2020 study, which found a positive linear relationship between millimetres of rainfall and microplastics/L in Lake Donghu in China.

Alternatively, rain may take weeks to reach groundwater and eventually decrease the amount of microplastics through dilution. Zhang and colleagues found that after simulated rainfall the maximum downwards migration of microplastics was only 4–7cm. I contacted a hydrologist and specialist of the Hepburn-Daylesford mineral springs, Dr Shugg, to ask about my hypotheses. His reply indicated that either theory is possible, depending on the exact state of the rocks in the region.

The most common type of microplastic found was “other” followed closely by fibres, contrasting with an American study which only found fibres in groundwater. There were very few beads and fragments. Similar types of microplastics were found in all the springs. Tap water contained larger amounts of fibres than the springs, highlighting that tap water is different and contains larger plastic pieces. These may be less likely to reach the mineral spring aquifers as they are halted by the pore-throats in soil and narrowed rock fissures.

Further research is required to determine where microplastics enter the tap water and groundwater systems.

If I am what I drink, I’m determined to be the least plastic I possibly can. This is why I have supplied key findings to the Mineral Springs’ Program Officer, who agreed that future investigations should be conducted into microplastics.

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