Microplastic Contamination in Karst Groundwater Systems

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Article Impact Statement: Plastics present a serious worldwide environmental problem. This article provides evidence that microplastics are present in karst groundwater.
Abstract

Groundwater in karst aquifers constitutes about 25% of drinking water sources globally. Karst aquifers are open systems, susceptible to contamination by surface-borne pollutants. In this study, springs and wells from two karst aquifers in Illinois, USA, were found to contain microplastics and other anthropogenic contaminants. All microplastics were fibers, with a maximum concentration of 15.2 particles/L. The presence of microplastic was consistent with other parameters, including phosphate, chloride and triclosan, suggesting septic effluent as a source. More studies are needed on microplastic sources, abundance, and impacts on karst ecosystems.

Introduction

Discarded plastic debris and microplastic contamination is widespread in surface-water environments and aquatic ecosystems worldwide (Gregory and Ryan 1997; Thompson et al. 2004; Barnes et al. 2009), including the open ocean, freshwater (streams and lakes), and soils (Law and Thompson 2014; Faure et al. 2015; McCormick et al. 2016; Rillig and Bonkowski 2018). Microplastics are contaminants of emerging concern and consist of plastic particles smaller than 5 mm (Moore 2008). Sources of microplastic to waterways include wastewater, fragmentation of large plastic litter to smaller pieces, and atmospheric deposition (Andrady 2017; Sheavly et al. 2007; O’Brien et al. 2010). Wastewater such as septic effluent, for example, can contain many thousands of microfibers made up of fine polymers (polyester and polyethylene) and fibers from the washing of synthetic fleece garments (Browne et al. 2011).
Ecological concerns related to microplastics include their ability to adsorb persistent organic pollutants (POPs) which can be transferred to animal tissues, affecting bioaccumulation of POPs (Rochman et al. 2013, Farrell et al. 2013), and irritation of digestive tissues following ingestion (Wright et al. 2013). Most research has determined that microplastic contamination is ubiquitous in ecosystems worldwide, however, no studies have examined the presence, abundance, or environmental drivers of microplastics in karst groundwater systems.

Karst aquifers are groundwater ecosystems made up of creviced carbonate rock commonly overlain by cover-collapse sinkholes. The open nature of karst aquifers makes them vulnerable to the rapid transport of surface-borne contaminants in dissolved and particulate forms (White 1988). Twenty percent of the world’s landmass is karst (White et al. 1988) and karst aquifers constitute about one quarter of the world’s drinking water sources (Ford and Williams 2007). In addition to being important drinking water resources, karst ecosystems are habitats for rare troglobitic faunal species that may be vulnerable to contamination (White et al. 1988).

The objectives of this study were to determine if microplastics are present in groundwater wells and springs in karst regions and if they co-occur with other anthropogenic contaminants. We hypothesized that microplastics were present in many karst groundwater systems due to 1) the open nature of karst systems to surface water and the atmosphere and 2) the prevalence of groundwater contamination by septic effluent. Our previous work demonstrated groundwater
from springs and wells in the study areas contained chemical and biological contaminants from agricultural activities, road salt runoff, and discharge of septic effluent (Hackley et al. 2007; Panno et al. 2017). Effluent from private septic systems and leaking sewage infrastructure suggests that groundwater may also contain microplastics. If so, microplastic presence could be related to contaminants associated with human sewage, including dissolved solids, chloride (Cl\(^-\)), nutrients, enteric bacteria, and pharmaceuticals and personal care products (PPCPs).

**Study Area**

Illinois has five distinct karst regions along its western and southern borders (Panno et al. 1997a). For this study we sampled groundwater in the Salem Plateau and the Driftless Area, which represent the two extremes of karst in the state (Figure 1). The Salem Plateau is in southwestern Illinois’ sinkhole plain where Mississippian carbonate bedrock of the St. Louis and Ste. Genevieve Limestone are overlain by about 10 m of fine-grained sediment, but may be exposed at and near the surface. Carbonate bedrock contains abundant fractures, crevices (approximately 30 - 60 cm wide), numerous long branchwork-type caves (up to 24 km) and large springs (Panno and Luman 2012). Unconsolidated materials overlying bedrock consist of glacial drift and loess (0-20 m) (Hansel and McKay 2010). About 86% of the Salem Plateau is farmland, most of which is dominated by row crop agriculture (Panno et al. 1997b). The area has over 18,000 individual cover-collapse sinkholes that are typically >100 m in diameter (Figure S1) (Panno and Luman 2018). Dye tracing of sinkholes by Aley and Moss (2001, 2009) revealed that
groundwater-basin recharge areas for springs within the Salem Plateau include numerous houses each with their own septic system.

The Driftless Area in northwestern Illinois was selected for this research because the karst features are more subtle. Bedrock is made up primarily of Ordovician and Silurian carbonate rock, while the fractured and creviced carbonate rocks of the Galena Dolomite constitute the aquifers used in the area (Figure S2). Bedrock is overlain by a relatively thin layer (0 – 8 m) of unconsolidated material (i.e., residuum and loess) (Piskin and Bergstrom 1975; Panno et al. 1997a, 2013, 2017). Crevices, caves, and associated sinkholes in the Driftless Area are smaller than those of the Salem Plateau. Most sinkholes are approximately 1 m wide, 1 m deep, and are often filled or partially filled with fine-grained sediment by anthropogenic activities and natural processes. Only about 30% of the Driftless Area in Jo Daviess County is used for row-crop agriculture, and 40% of the land is used for hayfields and pasture (Panno et al. 2015).

Previous studies have characterized the chemical and microbial composition of groundwater from springs and wells in the two study areas. Groundwater in karst areas of Illinois are Ca-HCO$_3$ and Ca-Mg-HCO$_3$ type waters with contaminants suggesting input from septic effluent and agricultural activities (Hackley et al. 2007; Panno et al. 2017; Panno et al. in review; Dodgen et al. 2017). Contaminants found include Cl$^-$, nitrate-nitrogen (NO$_3$-N), orthophosphate-phosphorous (o-PO$_4$-P), bacteria (including *Escherichia coli*), and PPCPs.
Research Method

Groundwater samples were collected in mid-November 2017 under low-flow conditions from eight springs and three shallow (< 65 m) wells in the Driftless Area, and from six springs in the Salem Plateau (Figure 1; Table S1). Groundwater from most of the sites had been analyzed for inorganic chemical composition, bacteria and PPCPs (Hackley et al. 2007; Dodgen et al. 2017; Panno et al. in review; Dodgen et al. (2017). Spring water samples were collected as close to spring mouths as possible. Well water samples were collected from taps where water did not go through water treatment equipment (e.g., water softeners). We measured temperature, pH, specific conductance (SpC), dissolved oxygen (DO), and oxidation-reduction potential (ORP) at the Driftless Area sites using a multi-sonde probe (Hydrolab MS5). Samples for microplastics were collected into 2-L HDPE bottles with the sampler wearing blue latex gloves. Bottles were capped immediately to prevent atmospheric contamination. Additional samples were collected at the Driftless Area sites and analyzed for 14 types of PPCPs. We collected samples in 1-L silanized amber glass bottles with Teflon-lined caps. All samples were transported to the analytical laboratories in ice-filled coolers and kept refrigerated at 4°C until analysis.

Microplastics

Microplastic samples were processed and identified at the Loyola University Chicago laboratory according to published methods (McNeish et al. 2018; Barrows et al. 2016). Each water sample
was filtered through a 0.45 µm filter (Whatman, Pittsburgh, Pennsylvania, USA), dried at 75°C for 24 h, and examined with a dissecting microscope at 25 – 50 × (Barrows et al. 2016) (Figure S3). We categorized particle shape as fiber, fragment, foam, bead, or film (Table S2), and we recorded color (Table S3). Colors are important because brown, for example, is often indicative of natural fibers. Other parameters for separating natural fibers from synthetic fibers include uniformity, thickness, and texture. Filters were checked by at least two laboratory technicians to ensure consistent quantification and identification of microplastic particles. We corrected microplastic concentrations for contamination with laboratory control samples (approximately 2 microplastic particles per filter) (McNeish et al. 2018; Hoellein et al. 2017).

To determine the chemical composition of the microplastic fibers, 20 microplastic samples from seven of the water samples were analyzed using pyrolysis gas chromatography mass spectrometry (py-GCMS; 5200 pyroprobe, CDS Analytical; 6890 gas chromatograph, Agilent; AutoSpec Ultima mass spectrometer; Waters Corporation). The microplastic fibers were removed from the filters and individually inserted into a quartz capillary tube with quartz wool plugs, then loaded into the pyroprobe and heated to 600°C for 90 s. The temperatures of the GC injection port and transfer line were constant at 300°C (split ratio of 5:1). Separation was performed on a capillary column ((30 m x 0.25-mm ID x 0.25 mm df); Rtx-5MS; Restek) with a helium carrier gas (flow rate of 1.0 mL/min). The oven was held at 40°C for 2 minutes and then heated at a rate of 10°C/min to 300°C, which was then held for 10 minutes.
Blanks were analyzed between each sample to check for carry-over and were continuously run until initial baselines were achieved. The mass spectrometer collected a mass to charge ion (m/z) range from 35 to 350. All sample data were background subtracted with data from a blank injection. Spectra were averaged across the entire chromatogram and searched in two pyrogram databases (CDS 2011 and in-house created) for the best match (McCormick et al. 2016: McNeish et al. 2018).

**Pharmaceuticals and Personal Care Products**

Analysis for 14 PPCPs (acetaminophen, caffeine, carbamazepine, diphenhydramine, erythromycin, fluoxetine, gemfibrozil, ibuprofen, naproxen, sulfamethazine, sulfamethoxazole, triclocarban, triclosan, and trimethoprim) was performed for samples from the Driftless Area only (samples 1-11). These compounds (plus ciprofloxacin) were analyzed for previously on samples from four of the sites in the Salem Plateau (sites 12-15) (Dodgen et al. 2017). Solid phase extraction of acidified 1-L water samples was used to concentrate the analytes and reduce matrix interference. Isotopic surrogates were used to correct for matrix effects and recovery (Li et al., 2013). Prepared samples were analyzed by liquid chromatography tandem-mass spectrometry (LCMS/MS). Full details of the extraction and analytical methods may be found in Dodgen et al. (2017).
Results

Sixteen of the 17 groundwater samples collected during this investigation contained microplastics (Table 1), with a median concentration of 6.4 particles/L and a maximum of 15.2 particles/L. Microplastics (65%) were blue and/or clear, whereas the other common colors were red (15%) and gray (13%). Four out of 20 microplastic samples were identified as polyethylene; these 4 samples were from two of the springs, both from the Salem Plateau (sites 15 and 16; Figure 1). The remainder of the microplastics were lost during analysis (i.e., combusted in the process of identification).

Table 1. Number of microplastics identified with microscopic techniques including the total number found in the water samples and the number/L. Because water sample volumes collected were over 2 L, concentrations are not integers. MP = microplastic.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Volume (L)</th>
<th>Fiber (No.)</th>
<th>Fragment (No.)</th>
<th>Bead (No.)</th>
<th>Foam (No.)</th>
<th>Film (No.)</th>
<th>MP (No.)</th>
<th>MP Conc. (No./L)</th>
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Results for PPCPs showed variability among study sites, with some patterns suggesting a positive relationship to microplastic concentrations. Five of the PPCPs were detected in at least one sample collected in the Driftless Area, including caffeine, carbamazepine, gemfibrozil, sulfamethoxazole, and triclosan (Figure 2).

We combined our microplastic data with previously collected water-quality data from springs in the Salem Plateau and the Driftless Area. Dodgen et al. (2017) measured concentrations of nutrients, PPCPs, and enteric bacteria from springs and caves in the Salem Plateau on five occasions during 2014 and 2015, including four of the six sampled in this study (sites 12-15). Combining our data with results from Dodgen et al. (2017) showed a positive pattern between microplastics concentration and $\text{o-PO}_4\text{-P}$ (although one outlier with Site 8, Figure 3A). In addition, we found a positive pattern between microplastic and $\text{Cl}^-$, although the relationship is different across $\text{Cl}^-$ concentrations. At lower $\text{Cl}^-$ concentrations (<40 mg/L) the pattern is positive and linear, the 2 sites with higher $\text{Cl}^-$ concentrations (>100 mg/L) do not follow the positive pattern, and Site 8 is an outlier (Figure 3B). Our previous work suggests the linear cluster where $\text{Cl}^- < 40$ mg/L is most likely due to input of septic effluent. Whereas, the sample from Site 8 is also < 40 mg/L, this site was found to be a special case because it was collected from a bedding
plane spring from bedrock just below the Lake Galena dam. It is possible that microplastics were filtered out as lake water migrated through fine bottom sediments adjacent to the dam before entering bedrock crevices and bedding planes (Panno et al., in review). Finally, we show what appears to be a positive pattern between microplastic concentrations and triclosan, which is a widely used antibacterial and antifungal agent found in consumer products (Figure 3C). Additional sampling may or may not confirm this relationship. Again, as with the other data, site 8 is an outlier.

**Discussion**

In this study, microplastics in karst aquifers have been observed and all of the detections were microplastic fibers. The presence of microplastics in groundwater suggests hydrogeologic connections from the surface to the underlying aquifers. Possible sources include anthropogenic litter (i.e., trash) and drainage of effluent from private septic systems. Our data do not show a definitive link to either source but offer patterns from which we can draw inferences about the hydrogeologic connections and show opportunities for future analyses of microplastic dynamics in subsurface waters.

The influence of septic effluent on groundwater chemistry in both study areas has been well documented (Panno et al. 2006, 2007, 2017, in review; Hackley et al. 2007; Dodgen et al. 2017), but the capacity of septic waste to be a source of microplastic particles is unknown. However,
previous research shows a strong link between machine washing synthetic fiber garments and microplastic in wastewater (Browne et al. 2011). Consequently, the gray-water component of septic effluent could yield large quantities of microplastics. It appears that sites with elevated levels of at least Cl\(^-\), \(\text{PO}_4\)\(^-\)-P and triclosan tended to have the highest concentrations of microplastics (Figure 3).

Variations in karst maturity within the two study areas offer some understanding of how surface-borne microplastics could migrate into groundwater. The relatively large crevices, conduits, branchwork caves, springs, and associated sinkholes of the Salem Plateau are likely points of entry for microplastics into the underlying limestone karst aquifers. This is in contrast with the much smaller crevices, sinkholes, springs, and discontinuous network caves of the dolomite karst aquifers of the Driftless Area. However, sinkholes in both areas serve as localized drainage features that lead directly to crevices that are part of the underlying karst aquifers. Anecdotally, we observed sinkholes in the Salem Plateau with anthropogenic litter that included cars, construction, household and farm-related refuse, and septic effluent discharge (Figure S4). Some of the anthropogenic litter items likely migrated, during recharge events, into caves via crevices associated with sinkholes, and microplastic from surface water recharge can follow the same path. In contrast, similar anthropogenic litter piles were not observed in the Driftless Area; this may be due to the smaller nature of sinkholes, crevices and caves in this area. Crevices and fractures near the bedrock surface in the Driftless Area are often filled or partially filled with
fine-grained sediment from the overlying soil zone (Panno et al. 2017) that could filter migrating microplastic fibers.

Because of the study design used here, we acknowledge variation in the timing of data collection in Figure 3 (A, B) (i.e., different collection times for microplastics and water chemistry metrics), which may weaken inferences drawn from these results. However, as stated previously, PPCP samples for the Driftless Area were collected at the time of microplastic sampling (Figure 3C) and the data indicate a relationship between PPCPs and micorplastics. We also carefully note that the overall scope and replication of this initial study on microplastic is modest. However, all samples were collected under low-flow conditions when ionic concentrations in groundwater in the study areas were relatively stable. With respect to these important caveats for data interpretation, we assert that the documented patterns are strongly indicative of ecological dynamics for microplastic in these karst ecosystems. We conclude that a likely source of the microplastic fibers in the groundwater in both karst regions is septic effluent, and suggest future studies are critical to examine the potential pathways in more detail.

Microplastic research conducted in surface waters of the upper Midwest offer context for the results of this study. Using the same collection method, McNeish et al. (2018) found microplastic concentrations in Lake Michigan tributaries were 2.9 - 89.6 particles/L. Microplastic particles isolated from rivers in the Chicago, IL region included common polymer types such as
polyethylene, polypropylene, and polystyrene (McCormick et al. 2016). We acknowledge the limited success in identifying the polymer types of particles reported as microplastic. It is possible that some particles we counted may actually be composed of naturally occurring fibers (e.g., cotton, viscose) or semi-synthetic mixtures of natural and plastic fibers (Lenz et al. 2015). However, we also note that natural or semi-synthetic fibers contain chemical additives such as dyes or flame-retardants (Barker 1975) and can be ingested by aquatic organisms (McNeish et al. 2018), and thus, are also ecologically meaningful to quantify. Overall, the polyethylene composition of microplastics and range of concentrations found in karst groundwater samples were consistent with previous research on surface water, and relationships of microplastics with PPCPs, Cl\textsuperscript{-} and \textit{O}-PO\textsubscript{4}\textsuperscript{-}-P suggest septic effluent as a source. However, this does not preclude the influence of surface water runoff.

The relative size of microplastic particles in this study offers some insight into their movement. The microplastic fibers in this study were smaller (< 1.5 mm) or similar to those collected from surface water bodies (Barrows et al. 2016; McNeish et al. 2017). We suggest that as water migrates from the surface to the subsurface via sinkholes (focused drainage features), it rapidly enters fractures and crevices of the underlying aquifers. Within the soil zone and along irregular surface of fractures and crevices, the larger size fraction of microplastics would be impeded and only the smaller size fractions of microplastics would reach and migrate through the karst aquifers. It is likely that additional retardation of microplastic fibers by rough surfaces of
fractures and crevices and by filtration by relatively small fractures would take place within the aquifers. Finally, fractured aquifers of any type may be vulnerable to contamination by microplastics as well. The ubiquitous nature of private septic systems and sinkholes in rural areas, leaky municipal sewage lines and surface runoff could provide a source. The similarities between karst of the Driftless Area and fractured aquifers, and the result of studies on human viruses found in confined, fractured aquifers (e.g., Borchardt et al. 2007) suggests that microplastics may not be limited to karst aquifers.

**Conclusions**

Our work shows that karst topography and associated aquifers allow for the movement of microplastic fibers into groundwater flow systems. Given the presence of PPCPs, nutrients, and enteric bacteria found in both karst systems, the source(s) of the microplastic fibers found in the karst aquifers strongly suggest they originated from septic effluent; however, surface runoff may also be a source. Future studies are needed to document microplastic dynamics of karst ecosystems in more depth and breadth, to determine microplastic sources to groundwater environments, and to measuring potential biological effects of microplastic on sensitive groundwater food webs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Sample numbers, field IDs, common names and locations of springs and wells sampled within the Driftless Area and springs sampled in the Salem Plateau.

**Table S2.** Pharmaceuticals and personal care products identified in springs and wells of the Driftless Area during this study.

**Table S3.** Field parameters and microplastic fiber concentrations for samples from the Driftless Area and the Salem Plateau.
Table S4. The number and colors of microplastic fibers present in each groundwater sample.

Figure S1. Aerial photograph of the Salem Plateau showing sinkholes (many filled with water) that are typically 100 m in diameter.

Figure S2. (A) Galena Dolomite showing crevices and fractures (both vertical and horizontal along bedding planes) within the Miner Road Quarry, Jo Daviess County, Illinois. (B) Croplines shown by alfalfa in thin soils overlying Galena Dolomite reveal the fractured and creviced nature of the aquifer.

Figure S3. (Left) Photographs of gridded filter and (Right) microscopic view of the filter showing silt particles and a microplastic fiber about 1.5 mm in length.

Figure S4. (A) Debris including tires, a filled black plastic garbage bag, a yellow plastic bottle and assorted household refuse within a sinkhole showing a portion of a bedrock crevice where runoff enters following rainfall events. (B) Discharge pipe from a private, aeration-type septic system draining into a sinkhole in the Salem Plateau.

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References


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**Figure Captions**

Figure 1. Karst regions of the Driftless Area (top) and the Salem Plateau (bottom) in Illinois, USA showing sinkhole regions with spring and well locations (large black dots) sampled in this study. The inset table includes sample numbers, common names and locations of springs and wells sampled.

Figure 2. Bar chart comparing microplastic concentrations with the most commonly detected PCPPs identified in springs and wells of the Driftless Area during this study.
Figure 3. Relationship between microplastic fibers and (A) ortho-phosphate ($\text{o-PO}_4^-$-P), (B) chloride (Cl$^-$) and (C) triclosan. Arrows on (B) show the site with the greatest Cl$^-$ concentrations that are related to road salt, whereas lower Cl$^-$ concentrations are related to septic effluent. Sites 4 and 7 (B) are wells, and Site 8 (A, B, C) is leakage along a bedding plane from an adjacent dam.
Bedrock Geology

Pennsylvanian

- Bond Fm (Pb)
- Shelburn-Patoka Fm (Psp)
- Carbondale Fm (Pc)
- Tradewater Fm (Pt)

Mississippian

- Lower Pope Group (Mpl)
- Genevieve Ls (Msg)
- St. Louis Limestone (Msl)
- Warsaw Fm. and Salem Ls. (Mws)
- Burlington-Keokuk Ls. (Mbk)

Silurian

- Silurian System (S)
- Ordovician
  - Maquoketa Shale Group (Om)
  - Galena Group (Og)
  - Kimmswick Limestone (Ok)
  - Ancell Group (Oa)
  - Platteville Group (Op)

Sample Locations

- Driftless Area:

  - Sample #
  - Common Name
  - Lat
  - Long

  1. Unnamed 42.371 -89.928
  2. Muna 42.487 -89.924
  3. H-Well 42.474 -89.991
  4. A-Well 42.459 -90.054
  5. Blacksmith Creek 42.364 -90.279
  6. Spring Street 42.411 -90.431
  7. B-Well 42.406 -90.436
  8. Spillway 42.402 -90.352
  9. Heaven's 42.388 -90.117
  10. Unnamed 42.356 -90.256
  11. Pleasant Valley 42.216 -90.034

- Sinkhole Plain:

  - Sample #
  - Common Name
  - Lat
  - Long

  12. Falling Springs 38.530 -90.183
  13. Sparrow Cr. 38.496 -90.164
  14. Stemler Cave 38.467 -90.161
  15. Camp Van 1 38.342 -90.204
  16. Camp Van 2 38.342 -90.205
  17. Belle Fontaine 38.327 -90.151

*All sample locations are springs, except for #3, 4, and 7 which are wells.

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