Sulfamethoxazole contamination of a deep phreatic aquifer

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Abstract

Groundwater samples were obtained from the water table region of a phreatic aquifer (unsaturated zone depth up to 28 m) under land irrigated with wastewater effluents for about 5 decades and a relatively deep pumping well (109 m), used as a drinking water source till 2007, located downstream (1300 m) of wastewater effluent and sludge infiltration facilities. Sulfamethoxazole (SMX) concentrations in secondary effluents varied between 90 and 150 ng/L. SMX was extracted using SPE and was analyzed by HPLC-MS/MS. SMX (maximum concentration of 37 ng/L) was detected in the water table region, in two monitoring wells, after an unsaturated zone transport period of about 16 years. The maximum SMX concentration detected in the pumping well was of 20 ng/L. These results question wastewater effluent disposal strategies including the suitability of irrigation with effluents on the replenishment area of an aquifer supplying drinking water.

Introduction

Antibiotics are extensively used for human therapy and veterinary medicine and since they are only partially metabolized they accumulate in the environment. More than 90% of the metabolized and unmetabolized antibiotics are secreted through urine and feces and are detected in wastewater treatment plants where even the best tertiary treatment method is not designed to effectively disable their activity (McArthur and Tuckfield, 2000; French et al., 1987). Therefore, recharge of effluents into aquifers and irrigation with sewage effluents on the replenishment area of aquifers may lead to groundwater contamination by antibiotics.

The Sulfonamides antibacterial group is considered to be one of the first antimicrobial drugs used, and paved the way for the antibiotic revolution in medicine. It was intensively manufactured since the mid 40s. Presently, only few sulfonamides derivatives are still in clinical use because of developed bacterial resistance (Tilles, 2001). Sulfamethoxazole (SMX, C10H11N3O3S), a representative antibacterial compound of the sulfonamides group, is considered as an emerging micro-contaminant due to its potential adverse effects on ecosystems and human health (Hirsch et al., 1999; Lindsey et al., 2001).

The presence of antibiotic residues in groundwater was intensively investigated mainly below effluent recharge ponds (e.g., Hirsch et al., 1999; Sacher et al., 2001), feedlots (e.g., Zhu et al., 2001; Hamscher et al., 2002; Lindsey et al., 2001; Batt et al., 2006), landfills (e.g., Holm et al., 1995) and riverbanks (e.g., Vogel et al., 2005; Sacher et al., 2001). In many cases, where geohydrological information is available, it can be seen that antibiotic residues were mainly detected in shallow phreatic aquifers 3–6 m below ground surface (e.g., Batt et al., 2006; Krapac et al., 2002).

In this paper we report on SMX contamination of: (a) a 28 m deep water table region under land irrigated with sewage effluents since the late 1950s and, (b) a 109 m deep pumping well located downstream of the irrigated land and of wastewater infiltration lagoons and a sludge disposal site.

Study site

The study was conducted in farmland at Gil Yam, situated 15 km north of Tel Aviv and 2 km inland from the Mediterranean Sea (Figs. 1 and 2). The field overlies a portion of Israel’s phreatic Coastal Plain aquifer, which stretches along the Mediterranean coast and is one of the most important fresh water resources of the country. The aquifer is composed of clastic sediments of Pleistocene age overlying impervious clays interbedded with permeable beds of sand and lumachelle of Pliocene age (Issar, 1968). Towards the west, the aquifer is partially divided by intervening clay layers into sub-aquifers, of which the lower one is confined. At the study site, the aquifer thickness is about 130 m and the depth to the water table is about 30 m (Fig. 3; Ronen et al., 1987a,b,c). Since the early 1960s a groundwater depression developed in the Herzlia area as a result of over pumping. Consequently, the general direction of groundwater flow has been north–east to north (Fig. 2).

The area studied, is irrigated with wastewater effluents from the city of Herzlia. The wastewater treatment plant (WWTP, Fig. 1), a concrete
Fig. 1. Aerial photograph [2007] of the study area showing the location of the sampled wells (WT2, WT4 and GY2), the Waste Water Treatment Plant (WWTP), the Effluent Infiltration Lagoons (EIL), the Sludge Disposal Site (SDS) and the Spreading Ponds (SP).

Fig. 2. Groundwater level map (meters) showing: (a) the direction of groundwater flow towards the Herzlia groundwater depression, (b) the location of the cross section (Fig. 3), and (c) the location of the research (WT2 and WT3) and pumping wells (GY2), the wastewater treatment plant (WWTP), the effluent infiltration lagoons (EIL), the sludge disposal site (SDS) and the spreading pond (SP). WWTP, EIL, SDS and SP are not to scale.

Please cite this article as: Avisar D, et al, Sulfamethoxazole contamination of a deep phreatic aquifer, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.03.032
layered oxidation pond, has been in operation since the early 1960s (Ronan and Magaritz, 1985). At that time, part of the effluents were utilized for irrigation and the remainder was infiltrated into the aquifer through a sandstone quarry (SDS, Fig. 1) during the summer season, May to November. In winter, the effluents were discharged into the sea. In 1977, an extended aeration treatment plant and 7 infiltration lagoons (EIL, Fig. 1) replaced the old facilities and in 1987 the Herzlia WWTP was upgraded to activated sludge treatment. However, the general scheme of effluent utilization did not change. The infiltration lagoons were active till 1991 when they were replaced by a nearby spreading pond (SP, Fig. 1) which was active till 2000. In 1982 the effluent-irrigated area was 75 ha and it increased to 86 ha in 2008. The sludge, about 600 tons dry matter/year (in 1981), was discharged into the sandstone quarry (SDS) till the year 2000. During the period 1960 to 1988, about 20×10^6 m^3 of effluents were infiltrated into the aquifer while 8×10^6 m^3 were utilized for irrigation. No quantitative data is available for the period 1988 to 2000 concerning artificial infiltration through the SDS and EIL. For the period 1988 to 2008, it was estimated that the amount of sewage effluent utilized for irrigation was of 10×10^6 m^3.

3. The sampled wells

Two monitoring wells (WT2 and WT4) and one pumping well, Gil Yam 2 (GY2) were sampled in the course of this study. WT2 and WT4 were drilled in 1984 and 1987, respectively, with the purpose of studying the water table region under land irrigated with sewage effluents (Ronan, 1988). The screens, having an internal diameter of 8.0 cm penetrate 5 m (WT2) and 17 m (WT4) below the water table (Fig. 3). GY2 is a 109 m deep pumping well with screens between 65 m and 105 m (average depth of screens below the water table about 55 m).

Monitoring well WT2 is located at the edge of a 37 ha field of turf (peanuts, cereals and potatoes in the past). Monitoring well WT4 is located in the middle of 49 ha of orange and grapefruit orchards. Both fields are irrigated with secondary effluents by sprinklers (6000 m^3/ha and 8000 m^3/ha, respectively, in 2008).

Pumping well GY2 (drilled in 1956) is located 500 to 880 m downstream of the monitoring wells (Fig. 1), in an area devoted to the cultivation of vegetables and irrigated since 1960 with fresh water by sprinklers. This well was the main drinking water supply to the Glil Yam village from 1960s till 2007 (average amount of water pumped between October and April, 350,000 m^3/year) when it was shut down due to high nitrate levels (69 mg/l).

4. Materials and methods

4.1. Sampling and sample storage

Groundwater samples were collected with a 2 L bailer from a depth of 2 m below the water table in research wells WT2 (water table depth 26.8 m) and WT4 (water table depth 28.7 m). About 60 L of groundwater (equivalent to about 5 well volumes, till a depth of 3 m below the water table) were extracted from each well before sampling. Samples were also collected from GY2 after operating the well for 2 h at a rate of 159 m^3/h. Three samples where collected from WT2 and WT3 (on August 23, September 24 and October 12, 2008) and two samples were collected from well GY2 (on February 2 and September 15, 2008).

Samples of secondary wastewater effluents from four municipal WWTPs located in the Costal Plain aquifer of Israel (up to 15 km north and south of the study area) were collected in 2008. For technical reasons it was impossible to sample the nearby Herzlia WWTP (Fig. 1). It is important to stress that all sampled WWTPs treat municipal sewage by the same treatment technology (activated sludge) as the Herzlia WWTP.

All samples were inserted into clean 2.5 L amber glass bottles, kept in an ice box and immediately transferred to the laboratory. At the laboratory, the wastewater effluents were filtered firstly through a 0.7 μm glass fiber filter to remove suspended solids. Both, wastewater effluents and groundwater samples were filtered through 0.45 μm glass fiber filter (Whatman GFC, 0.45 μm) and stored overnight at 4 °C until extraction.

4.2. Sample extraction

Samples were pre-concentrated using solid phase extraction (SPE). SPE was performed using 500 mg/6 ml Chromabond HR-P (Macherey Nagel) cartridges connected to a 24-port SPE manifold and a vacuum pump. Before SPE, water samples were heated to room temperature and pH was adjusted to 4. The SPE cartridges were conditioned with 5 ml n-Hexane, 5 ml Ethyl acetate and 5 ml HPLC grade water at pH 4. Extraction of 0.5 L samples was carried out under vacuum at a flow-rate of approximately 5 ml/min. After loading the samples, the cartridges were washed with 10 ml HPLC grade water at pH 4 and air-dried for 5 min. The analytes were eluted with 20 ml of DCM: MeOH (80:20), collected in 20 ml glass tubes and dried to zero under a stream of 5/9 nitrogen gas. Finally, the extracts were reconstituted with 0.5 ml HPLC grade water and transferred to HPLC vials.

4.3. Sample analysis

The HPLC-MS/MS method was adapted from Lester et al. (2008) with some modifications. In brief, SMX was detected and quantified by HPLC (diode array detector) Agilent 1100 and MS Finnigan LCQ, using an ACE-RP C18 column 250×2.1 mm. The column temperature was 28 °C, the flow rate was 0.5 ml/min and the volume injected was 100 μl. The UV absorption of the SMX was recorded at 260 and 280 nm. The HPLC mobile phase consisting of water (A) and AcN (B) was adjusted to pH 3.3 by the addition of formic acid. The eluent gradient method is presented in Table 1.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Composition of the mobile phase</th>
<th>Eluent A%</th>
<th>Eluent B%</th>
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<tr>
<td>0</td>
<td>65</td>
<td>35</td>
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</tr>
<tr>
<td>2</td>
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<td>85</td>
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<td>5</td>
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<td>7</td>
<td>65</td>
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<tr>
<td>17</td>
<td>65</td>
<td>35</td>
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</table>
The LCQ mass spectrometer was used in positive electro-spray ionization (ESI) mode and the probe temperature was set to 220 °C. The flow from the HPLC was passed through a split connector with 60 μl/min of effluent introduced into the ESI interface. MS/MS tune file was created for the SMX standard in continuous flow mode, and the optimum capillary voltages, lens settings, collision energies, and the product ions of the highest intensities were determined. The sheath gas flow was 93 (arbitrary units), auxiliary flow was 26 (arbitrary units) and capillary temperature was set at 200 °C. The spray voltage was set to 4.5 kV. Instrument control, data acquisition and evaluation were performed with Xcalibur software. The achieved LOD and LOQ are 5 ng/L and 8 ng/L, respectively.

5. Result and discussion

SMX was detected in the municipal secondary wastewater effluents of all four different sampled WWTPs located in the Coastal Plain of Israel. SMX concentration varied between 90 and 150 ng/L. In the studied area, SMX was detected in all wells sampled (Fig. 4). The highest SMX concentrations (37 ng/L) were detected at the water table region (about 29 m below ground surface) in research well WT4, under land irrigated with sewage effluents. In the pumping well, specially operated for this study, SMX was up to 20 ng/L.

Variability in the concentration of chemical components at the water table region (Ronen et al., 1987a,b,c) and in pumping wells as observed for SMX in this study, was detected in the Coastal Plain aquifer for other chemical components and is related to the chemical heterogeneity of groundwater. In the past and for the same study area, it was demonstrated that: (1) dissolved organic carbon (DOC) from sewage effluents is not biodegraded in the unsaturated zone because of low moisture content (Amiel et al., 1990); (2) the total amount of DOC present in the sedimentary unsaturated zone column of WT2 (at the time of drilling, 1984) amounts to 59% of the total DOC input, and (3) anoxic conditions develop at the water table region because of the biodegradation of DOC when moisture content increases in the capillary fringe (Ronen et al., 1987a,b,c).

SMX is water soluble, has a half life of 19 days under sunlight and is highly resistant to further biodegradation in the subsurface (Lam et al., 2004). It has a low Kow (~0.1 to 1.7; Primor, 2008), and is considerably hydrophilic and polar. Such properties enable SMX to be transported over long distances without being adsorbed to sediments (Perez et al., 2005; Lindsey et al., 2001). Furthermore, under typical environmental pH conditions (pH ~7–8) SMX is negatively charged (95–100%; Primor, 2008) a property that can increase its transport velocity in porous media due to anion exclusion.

The sediment interstitial water obtained while drilling WT2 was used to reconstruct the chronological transport record through the unsaturated zone (Gvirtzman et al., 1986) using the difference between environmental tritium content of rain and irrigation water. Evidences of anion exclusion were detected along the 10 m thick clay loam layer where the vertical velocities of water and anions (Cl⁻ and SO₄²⁻) were calculated to be 0.7 m/year and 1.4 m/year, respectively. The vertical velocities of both water and anions through the rest of the profile (17.5 m of sand) were estimated to be 2 m/year. Therefore, we estimate that at the study areas and under land irrigated with sewage effluents SMX arrives at the water table region after a transport period of about 16 years. We also assume that because of its high resistance to biodegradation SMX is not affected by the anoxic conditions at the water table region.

It is important to note that evidence for the deep penetration of xenobiotic organic contaminants in this area, under land irrigated with sewage effluents, was already reported in the past (Muszkat et al., 1993). For example, toluene (up to 50 μg/L) and N-butyl benzenesulfonamide (up to 140 μg/L) were detected in 1993 in the water table region (sampling depth below the water table 0.5 m) of research well WT2. Moreover, 30 μg/kg of rometon, a triazine herbicide, was detected at a depth of 20 m below ground surface in sediments of the unsaturated zone of a borehole (UZ3; drilled with a hollow-stem auger driller equipped with a split-spoon sampler) just near WT4.

SMX was synthesized in 1957 (Kano and Ogata, 1957) and approved by the FDA in 1961. Recall that the WWTP in the study area was in operation since the early 1960s when also, the groundwater depression developed in Herzlia (Fig. 2). Since the horizontal component of groundwater flow velocity under natural gradient flow conditions in the study area can be of ~50 m/year (well WT4; transport distance of 2400 m in 48 years), it is reasonable to assume that the detected concentrations of SMX in the 109 m deep GY2 well are the result of both the extensive irrigation with wastewater effluents since the early 1960s and the upstream infiltration of wastewater effluents through the infiltration lagoons and sludge disposal site (Fig. 1). Compared to other studies (Table 2) where, in most cases, SMX was detected in relatively shallow aquifers, our results suggest that SMX may penetrate into relatively deep subsurface environments and be transported over relatively long distances (about 1.3 km) in saturated porous media.

![Fig. 4. SMX concentrations detected in the research wells (WT2 and WT3; sample 1 - August 23, sample 2 - September 24 and sample 3 - October 12, 2008) and pumping well (GY2, sample 1 - September 15 and sample 2 - February 2, 2008). Error bars denote standard deviation of 4 replicates for GY2 and 3 replicates for WT2 and WT3.](image)

### Table 2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Aquifer type</th>
<th>Aquifer thickness (m)</th>
<th>Depth of water table (m)</th>
<th>Monitoring well-sample depth (m)</th>
<th>Distance and type of contamination source (m)</th>
<th>Concentration of SMX (μg/L)</th>
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<td>Lindsey et al., 2001</td>
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<td>-</td>
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<td>-</td>
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<td>Vogel et al., 2005</td>
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<td>-</td>
<td>10–15 70</td>
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<td>-</td>
<td>Bank infiltration n.d.</td>
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<td>3–5</td>
<td>-</td>
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<td>Animal or human wastewaters 0.04–0.47</td>
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<td>Barnes et al., 2008</td>
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<td>-</td>
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<td>Animal or human wastewaters &lt;1.1</td>
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<td>Stoller et al., 2004</td>
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<td>-</td>
<td>-</td>
<td>n.d.</td>
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<tr>
<td>Ternes et al., 2007</td>
<td>Sand</td>
<td>-</td>
<td>1–2</td>
<td>5</td>
<td>Human wastewater effluent and sludge</td>
<td>0.02–0.1</td>
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Please cite this article as: Avisar D, et al, Sulfamethoxazole contamination of a deep phreatic aquifer, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.03.032
Many studies suggest that of all emerging contaminants, antibiotics are of greatest concern, since their emission in the environment can increase the occurrence of resistant bacteria (e.g., Hirsch et al., 1999; Lindsey et al., 2001; Petrović et al., 2003; Soto-Chinchilla et al., 2007; Burns et al., 2008 and Kemper, 2008) and enhance the transfer of genes that resist antibiotics between different bacteria taxonomic affiliations (Davidson, 1999). Chee-Sanford et al. (2001) demonstrated that antibiotic genes occur in groundwater as a direct result of effluent infiltration into aquifers and suggested that also groundwater may be a potential source of antibiotic resistance bacteria.

Therefore, our findings are of concern since: (a) well GY2 was a drinking water source during the period 1956 to 2007. Thus, it is reasonable to assume, that for a considerable period of time the local population was consuming routinely nanogram quantities of sulfa drugs. (b) large volumes of groundwater in the Herzlia depression (Fig. 2), where other pumping wells are located (not sampled in this study and never been monitored for antibiotics), may be presently contaminated by SMX and other antibiotic residues, and (c) wastewater effluents are used for agriculture irrigation above large areas of the phreatic Coastal Plain aquifer of Israel since the early 1960′s. Thus groundwater contamination by antibiotic residues may be a widespread phenomenon.

Acknowledgements

The authors would like to thank the Israeli water authority and the Gil Yam farmers (Dror & Shlomi) for allowing us free access to the groundwater wells for sampling and measurements.

References

Primor O. Sorption of antibiotic to clay. Master thesis, 2008; Tel Aviv University.
Ronen D. Chemical processes and transport phenomena in the water table region of phreatic aquifers. Ph.D. dissertation, 1988; Weizmann Institute of Science, Israel (pp. 138).