Occurrence of Banned and Currently Used Herbicides, in Groundwater of Northern Greece; A Human Health Risk Assessment Approach

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Occurrence of banned and currently used herbicides, in groundwater of Northern Greece; A human health risk assessment approach

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ABSTRACT

The presence of pesticide residues in groundwater, many years after their phase out in European Union verifies that the persistence in aquifer is much higher than in other environmental compartments. Factors such as limited degradation, and adsorption in phreatic horizon have resulted in frequent detection of pesticide residues and their metabolites in the saturated zone. Currently used and banned pesticides were monitored in Northern Greece aquifers and risk to human health was assessed. The target compounds were the herbicides metolachlor, terbuthylazine, atrazine and its metabolites Deisopropylatrazine (DIA), Deethylatrazine (DEA) and Hydroxyatrazine (HA). The area's aquifer has been extensively studied over the past 20 years. Eleven sampling sites were selected in order to have representatives of different type of wells. Namely, five drinking water, two irrigation wells and four experimental boreholes located close to Greek/Turkish/Bulgarian borders were monitored and fifty-four samples were analyzed. Pesticides were extracted by solid-phase extraction and analyzed by liquid chromatography. Metolachlor was detected in 100% of water samples followed by ATR (96.4%), DEA and HA (88.6%), DIA (78.2%) and TER (67.5%). Atrazine, DIA, DEA, HA, MET and TER mean concentrations detected were 0.18, 0.29, 0.14, 0.09, 0.16 and 0.15 μg/L, respectively. Obtained results were compared with historical data from our previous monitoring studies (1999-2003 and 2010-2012) and temporal trends were assessed. Preferential flow was the major factor facilitating pesticide leaching within the month of herbicide application. Moreover, apparent age of groundwater and the reduced pesticide dissipation rates on aquifers resulted of long-term detection of legacy pesticides. Although atrazine had been banned more than 15 years ago, it was detected frequently during our monitoring campaign and their concentrations in some cases were over the maximum permissible limit. Furthermore, human health risk assessment of pesticides was calculated for two different age groups though drinking water consumption. The presence of atrazine residues causes concerns related with chronic toxicity.

Keywords: herbicides; metabolites; banned pesticides; groundwater; preferential flow; leaching.

Introduction

Safe drinking water from surface- and ground-water is essential for human health, quality of life and socio-economic development of humanity and is a prerequisite factor for the human population (Affum et al., 2018). Groundwater is the largest body of freshwater in the European Union. In Greece, 13.9% of total renewable resources is originated from groundwater. In Greece, the annual water consumption/requirements are mainly covered by groundwater use representing 36% in farming, 5% in public use and 1% in industrial production. Hence, the usual geophysical peculiarities of Greece render the groundwater pumping as the only source of drinking water (EUWI/MED, 2007; Vryzas et al. 2012c).

Herbicides are generally considered the most economical and effective way to control weeds in agricultural and non-crop environments. However, the increasing use of herbicides has caused water contamination and other environmental threats (Kalkhoff et al. 1998). Several studies have highlighted the potential risks that these compounds pose to public health; biodiversity; and non-target organisms, such as fish, algae and aquatic invertebrates (Papadakis et al. 2015; Singh et al. 2017). There are several factors that can affect pesticide and their metabolites behavior in the environment. Physicochemical properties of pesticides such as ionization, water solubility, volatility, octanol-water partition coefficient, thermo-, photo- and hydrolysis stability combined with the soil properties including organic carbon content, texture, pH, clay mineral type, dissolved organic matter and cation exchange capacity play important role on run-off, adsorption, or leaching potential. In addition, rainfall and irrigation intensity, biological processes (biodegradation) and the agricultural practices have influence on pesticide fate (Vryzas et al. 2007; Carazo-Rojas et al. 2018). Point or nonpoint source pesticide pollution can cause groundwater contamination through various leaching mechanisms. Pesticides residues can reach groundwater in a short time following various paths, they are able to move

through soil matrix, rapidly by macropores with reduced possibility to be absorbed by soil or to be biodegraded. Macropores are caused by worm activity, roots, cracks, shrinkage of clay minerals and voids in soil (Vryzas et al. 2012b; Vryzas et al. 2012c). Otherwise, pesticides move through soil micropores slowly (matrix flow) and are available to interact with soil particles and microorganisms (Hasegawa and Sakayori, 2000).

Herbicides such as terbuthylazine (TER) (N-tert-butyl-6-chloro-N) -ethyl-1,3,5-triazine-2,4-diamine), metolachlor (MET) [2-chloro-N- (2-ethyl-6methylphenyl) - N- (2-methoxy-1-methylethyl) acetamide) and atrazine (ATR) (2-chloro-4-ethylamino-6isopropiamino-s-triazine), have been widely used for weed control in many crops in Greece, EU and around the world. However, Commission decision 2004/248/EC banned the use of active substance atrazine in EU (Charizoupoulos and Papadopoulou-Mourkidou 1999; Kolpin et al. 1998; Cerejeira et al. 2003; Kostantinou et al. 2006; Vryzas et al. 2009;) and terbuthylazine became the main herbicide used instead of atrazine after its withdrawal.

During the last three decades, various directives regulated the presence of pesticides in groundwater such as Ground Water Directive (EC 2006), Drinking Water Directive (EC, 1998), Water Framework Directive (EC, 2000) and Directives about priority substances and environmental quality standards in the field of water policy (EC 2008). The quality standards of drinking water, related to pesticides in EU, were set with maximum concentration of 0.1 μg / L and 0.5 μg / L of the presence of individual and total pesticides and metabolites, respectively (EC 1998). In addition, EU has set environmental quality standards (EQS) for surface water bodies in the field of water policy for priority substances and certain other pollutants, including pesticides. According to this directive the annual average EQS for atrazine has set to 0.6 μg/L and the maximum allowable EQS to 2 μg/L (EC 2008). U S Enviromental Protection Agency (USEPA) has set Maximum Contaminant Levels (MCLs) and Maximum Contaminant Level Goals (MCLGs) of atrazine to 3 μg/L (USEPA, 2019).

The greatest part of the available information about atrazine toxicity impacts are coming from animal studies. Although, there are a few toxic (at cellular level) and epidemiological (case) studies considering the direct atrazine exposure implications on human health. Recent research

showed that atrazine exposure to population may threaten public health. Mainly, atrazine is considered as an endocrine disruptor causing disfunction in extreme exposing conditions at normal human reproduction and development for both genders. Furthermore, atrazine exposure was correlated to potential neurological and liver problems (Singh et al. 2018; Yang et al. 2019). For atrazine transformation products there are only animal tests, which indicates similar effects with parent compound (Stoker et al. 2013).

Farmers are usually exposed to terbuthylazine through inhalation and dermal contact, whereas the main exposure pathway for people no related to agriculture is considered to be the oral route by contaminated drinking water consumption and less often by dermal route (USEPA, 1995). Published data about terbuthylazine impacts on human are limited. The terbuthylazine and its metabolite desethyl-terbuthylazine detection in hair samples of exposed farm workers was not related to significant health problems but only slight to moderate irritation to the eyes and skin were observed (Mercadante et al. 2017).

Metolachlor belongs in Toxicity Category III for acute dermal, oral, and inhalation effects and is in Toxicity Category IV for dermal and eye irritation (USEPA 2009). Thorpe and Shirmohammadi (2005) showed that children who were exposed to a mixture of herbicides that contained metolachlor had a 7.6-fold increased chance of developing bone or brain cancer, leukemia, and lymphoma compared to unexposed children, while herbicide applicators in Iowa and North Carolina had increased risk of lung and prostate cancer when exposed to metolachlor (Rusiecki et al. 2006).

Northern Evros is one of the most important regions of agricultural economy in Greece. In addition to the extensive agricultural activity, the vicinity with transboundary rivers and different agricultural practices followed in Bulgaria and Turkey increase the complexity of studying the origin of pesticide pollution.

Previous monitoring studies of Northern Evros showed medium frequency detection of atrazine, metolachlor, terbuthylazine and atrazine metabolites, deisopropylatrazine (DIA) (amino-2-chloro-6-ethylamino-s-triazine), deethylatrazine (DEA) (2-amino-4-isopropylamino-6-chloro-s-triazine) and hydroxyatrazine (HA) 4-(Ethylamino)-2-hydroxy-6-(isopropylamino)-

129 1,3,5- triazine (Papastergiou and Papadopoulou-Mourkidou 2001; Vryzas et al. 2012c). Also,
130 these compounds have been frequently detected in high concentrations including water quality
131 standards exceedances in various European countries such as Spain (Menchen et al. 2017),
132 Slovenia (Korosa et al. 2016), Hungary (Szekacs et al. 2015) and Portugal (Sanchez-Gonzalez
133 et al. 2013). Consequently, these compounds are considered to be of the main pollutants
134 detected in groundwater bodies all over the world. Therefore, the aim of this study was to
135 investigate the water quality, the presence and the persistence of these compounds, to
136 characterize their temporal and spatial variability in the aquifer and to characterize atrazine and
137 its metabolites behavior 15 years after atrazine's ban. Last but not least, a chronic risk
138 assessment of side effects on human health by consumption of contaminated drinking water
139 was conducted. According to our knowledge, this study is the first one which is related with
140 human health risk assessment in combination with the determination of pesticides residues in
141 groundwater in Greece.
142
143 **Materials & Methods**
144 *Studying area*
145 The choice of sampling area was based on results of previous studies which indicate the
146 presence of target pesticides in groundwater, the intensive agricultural activity in Northern
147 Evros and neighboring countries Bulgaria and Turkey (Fig 1). Samples were taken from 3
148 Groups of 11 sampling points, at Ardas Valley. A sampling network of shallow groundwater
149 was established by our research team 20 years ago (Vryzas et al. 2012c) consisted of 4
150 experimental boreholes (Group A). Furthermore, 5 drinking water wells were included, which
151 supply Orestiada town and local villages with potable water (Group B). In addition, two active
152 irrigation wells were chosen (Group C) to include all available well types. The Groups form
153 was: (Group A): A1, A2, A3, A4, (Group B): B1, B2, B3, B4, B5 and (Group C): C1, C2 (Table
154 1). Sampling points are located close to villages Rizia, Keramos, Plati, Fylakio, Elia, Arzos and
155 Kastanies (Fig. 1). The study included 5 sampling campaigns and 50 samples were collected
156 and analyzed.

157 Each sample was collected in triplicate (3 sub-samples of 1 L volume each one), transported in
158 ice-boxes and stored under refrigeration until analyzed. Experimental borehole samples were
159 manually pumped using an experimental tube. The drinking water and irrigation wells were
160 equipped with a pump system and samples were collected automatically before chlorination
161 stage.
162
163 *Reagents and chemicals*
164 The pesticide standards had the highest available purity (>97%) and were purchased by Dr.
165 Ehrestrofer GmbH (Augsburg, Germany). The HPLC grades, acetonitrile, ethyl acetate, water
166 and methanol for liquid chromatography were purchased by Riedel de Haen (Seelze, Germany).
167 LiChrolut® EN Polymer-based solid-phase extraction cartridges with 200 mg absorbent and 3
168 ml volume were purchased by Merck (Darmastdt, Germany). Individual pesticide standard
169 solution in 1, 10, 50, 100 μg/ml, in methanol, for HPLC analysis. Mixed pesticide standard
170 solutions in different concentrations were prepared, too. All standard solutions were stored at -
171 20 ^{o}C. Physicochemical properties of studied compounds are shown in Table 2.
172
173 *Sample preparation*
174 Groundwater samples were prepared for HPLC analysis using Solid Phase Extraction (SPE) for
175 the multi-residue analysis. Water samples of 1 L were extracted by cartridges which were
176 preconditioned with adding of 4 ml methanol followed by 4 ml deionized water. Samples were
177 passed through cartridges at a flow rate of 5 ml/min. Target compounds were eluted with 7 ml
178 methanol followed by 3 ml ethyl acetate. Next, samples were concentrated under nitrogen
179 stream at 50 ^{o}C. Finally, samples were dissolved with 1.25 ml of the initial HPLC mobile phase
180 and stored at -20 ^{o}C until instrumental analysis (Papadakis et al. 2006).
181
182 *Instrumental analysis*
183 Samples were analyzed by a HPLC/DAD equipped with autosampler (Finnigan Surveyor,
184 Thermo Scientific). The analytical column C18 Speedcore 100 x 4.6 mm was purchased by

185 Fortis Technologies Ltd. (Cheshire, UK). Chromatographic data were processed by the
186 ChromQuest 5.0 software (Finnigan Surveyor, Thermo Scientific). The mobile phase was
187 consisted of acetonitrile (A) and water (W). The flow was set at 1.0 ml/min and the gradient
188 included the following steps: the elution began at 20-80/A-W, 20-80/A-W (0-20 min.), 95-5/W-
189 A (20-25 min.), 95-5/A-W (25-26 min.) and 20-80/A-W (26-33 min). Total run time was 40
190 min. The injection volume was 25μl. The column oven temperature was adjusted at 30 ^{o}C.
191 Metolachlor, terbuthylazine, DEA and DIA were detected at 220 nm, while atrazine and HA at
192 240 nm. For further confirmation of the target peaks, the UV absorption spectra taken at the
193 apex of each sample were compared with those obtained from the standard solutions and control
194 spiked samples. The quantification was done using external working standard calibration curves
195 (1, 10, 50, 100 μg/ml). The accuracy (recovery) and precision (repeatability) of the analytical
196 method were evaluated with the analysis of fortified (at 0.1 μg/g and 0.5 μg/g) tab water samples
197 in sextuplicate. The limits of detection (LOD, μg/L) were determined as the lowest
198 concentrations giving a response of three times the baseline noise of the analysis of three control
199 samples. The limits of quantification (LOQ, μg/L) were determined as the lowest
200 concentrations of a given compound in fortified samples that could be quantified with relative
201 standard deviation lower than 20%. Positive detections of atrazine, DIA, DEA, MET and TER
202 were also confirmed with Gas chromatographic analysis using a Trace 2000 gas chromatograph
203 connected with the GCQ plus ion-trap mass spectrometer (Thermoquest, Austin, Texas, USA).
204 Gas chromatographic analysis was carried out on a 30 m × 0.25 mm I.D., 0.25 μm film thickness
205 CP-SIL 8 CB (5% phenyl, 95% dimethylpolysiloxane) low bleed/MS column (Varian
206 Analytical Instruments, The Netherlands) and the GC and MS operational conditions were
207 those mentioned by Vryzas et al. (2009).
208
209 *Human Health Risk Assessment*
210 Human health risk assessment was conducted for atrazine, metolachlor and terbuthylazine.
211 According to Li and Qian (2011) human health risk assessment of pesticides can provide
212 information about the probability and the kind of effects to human population. In our case, oral

exposure through drinking water consumption was considered as pathway to people. Risk assessment was divided to carcinogenic and non-carcinogenic one and two age groups, adults and children. Drinking water is provided to local population by wells located close to villages Elia, Arzos, Fylakio, Rizia and Kastanies (Fig. 1)

Chronic daily intake (CDI)

CDI shows the estimated intake amount of pesticide per kilogram body weight Eq. 1.

$$CDIi = \frac{D_{IP}\ x\ EF_i\ x\ ED_i}{BW_i\ x\ AT} \quad (1)$$

The determination of the average daily intake (D_{IP}) was estimated using the Eq. 2. This equation is suggested by Muhammad et al. (2011), Papadakis et al. (2015) and Ali et al. (2017)

$$D_{IP} = C_i \text{ x } IR_i \quad (2)$$

where C_i (μg/L) represents extreme and mean concentration of pesticide residues and IR_i shows the intake rate of water (0.87 L/day for children and 1.41 L/day for adults liters per day). EF_i is the exposure frequency (365 days per year for both age groups), ED_i is the exposure duration (6 and 70 years for adults and children, respectively), BW_i is equal to 70 Kg for adults and 20 Kg for children and AT is the average lifespan (2190 and 25550 days for children and adults, respectively).

Hazard Quotient (Non-carcinogenic risk assessment)

To calculate the Hazard Quotient (HQ), CDI was divided with the respective reference dose of each compound (Eq. 3)

$$HQ = CDI_i/RfD \quad (3)$$

where RfD is the acute toxicity reference dose (USEPA 1999).

The RfD values for atrazine, metolachlor, terbuthylazine were 0.035, 0.015 and 0.008 (mg/Kg-day), respectively (IRIS 1994; FOOTPRINT 2014). When HQ values are equal or greater than 1, the exposed part of population is under health risk.

Multiple pesticides residues risk (HQs) can be calculated by the sum of HQ for individual pesticide using the Eq. 4.

$$HQs = \sum_{i=1}^{n} HQi \quad (4)$$

Carcinogenic risk assessment

Carcinogenic risk (R) was calculated by the Eq. 4 (Kim et al. 2013; Papadakis et al 2015).

$$R = CDI \times SF \times ADAF \quad (5)$$

where SF is the cancer slope factor (mg/Kg-day), which reflects the possibility of the individual pesticide to cause cancer and ADAF is an age factor considering the early life pesticide exposure (3 for children and 1 for adults). Among the studied pesticides the only available SF is for atrazine with value 0.22, provided by IRIS.

Results & Discussion

Concentrations and detection frequency

For all compound the LODs were ranged from 0.001 to 0.005 μg/L and LOQs from 0.01 to 0.05 μg/L. The recoveries were higher than 86% for all compounds with RSD lower than 15% at the two fortification levels tested. The sampling sites were in the Ardas valley, an aquifer vulnerable to pesticide contamination according to previous studies (Papastergiou et al. 2001; Vryzas et al. 2012c). All the target pesticides were detected in all group of groundwater samples (Table 3). Metolachlor was detected in 100% of water samples followed by ATR (96.4%), DEA and HA (88.6%), DIA (78.2%) and TER (67.5%). Atrazine, DIA, DEA, HA, MET and TER mean concentrations detected were 0.18, 0.29, 0.14, 0.09, 0.16 and 0.15μg/L, respectively (Table 4). Atrazine mean concentration exceeded the maximum permissible limit of 0.1 μg/L in experimental boreholes A2 (0.23 μg/L) and A4 (0.28 μg/L), DIA concentration was found over the limit in A4 (0.14 μg/L), DEA in A1 (0.13 μg)/L) and A4 (0.18 μg/L) and HA in A2 (0.30 μg/L). As far as drinking water wells, atrazine was detected in concentration higher than 0.1 μg/L in B1 (0.23 μg/L), B2 (0.45 μg/L) and B5 (0.30 μg/L), DIA in B1 (0.14 μg/L), B3 (0.26 μg/L) and B5 (0.22 μg/L) and HA mean concentration was lower than limit in all of

267 drinking water wells. The results of irrigation wells indicated exceedances for atrazine in C1
268 (0.20 μg/L) and C2 (0.14 μg/L), DIA in C1 (1.99 μg/L), DEA in C1 (0.23 μg/L) and C2 (0.20
269 μg/L) and HA in C1 (0.13 μg/L). In experimental boreholes A2, A4, in drinking water wells
270 B1, B2 and B3 and in irrigation well C1 were observed exceedances for terbuthylazine. Also,
271 metolachlor was detected in concentrations higher than 0.1 μg/L in all wells apart from B2, B3,
272 B4 (Table 4). The most frequent exceedances of the maximum permissible limit of 0.1 μg/L for
273 drinking water of all compounds were observed in drinking water well B5 followed by C1, C2,
274 B1 and A4. The fact that target compounds reached concentrations above the quality standard
275 values for drinking water indicates that prediction made during pesticides registration process
276 are not always complied with the results from monitoring studies. It is estimated that less than
277 1% of the pesticides applied reach the target pest and the remaining distributed to various
278 environmental compartments including groundwater bodies (Pimentel and Levitan 1986). The
279 most frequent exceedances of the maximum permissible limit of 0.1 μg/L for drinking water
280 were observed in irrigation well of Fylakio C1 (for all 6 compounds), followed by the
281 experimental borehole Fylakio A4 (for all compounds apart the HA). In a previous study was
282 found that waters of that site consisted of a mixture of waters with different residence time and
283 various leaching mechanisms are involved to the pollution of groundwater (Vryzas et al.
284 2012a). The only well with concentrations of the studied compound lower than 0.1 μg/L, was
285 the drinking water in Arzos B4.
286 The active substance terbuthylazine is approved and applied by farmers in agricultural area of
287 Ardas valley as a pre-emergence herbicide for maize, corn and beet cultivation in April,
288 replacing the banned atrazine (Fig. 2). This explains why its highest concentrations occur one
289 month after its application. In the last decade, s-metolachlor (metolachlor isomer) has been used
290 instead of metolachlor.
291 Irrigation (repeated each week, during summer) is usually carried out by a self-propelled
292 sprinkler irrigation system. These sprinkler irrigation systems provide high volumes of high-
293 pressure water, which in combination with rainfall can exacerbate the phenomenon of leaching
294 (Vryzas et al. 2012b). Furthermore, there are a few paddies which are irrigated by basin

295 irrigation systems. Nouma et al. 2016 mentioned that basin irrigation systems are the most
296 factor that determine pesticides leaching.
297 The highest concentration of atrazine (1.81μg/L) was found in drinking water well of Fylakio
298 (B2) on third sampling, while atrazine did not detect in drinking water of Fylakio (B2) and
299 Rizia (B1) on first sampling. DIA presented the highest concentration (2.58μg/L) in irrigation
300 well of Fylakio (C1) and detected in all sampling points with the lowest concentration in
301 experimental boreholes. DEA had the highest concentration (0.65μg/L) in experimental
302 borehole of Fylakio (A4) at second sampling and wasn't detected in experimental boreholes of
303 Fylakio (A4), Plati (A3) and Keramos (A2) at second and third sampling campaigns. The
304 highest concentration of HA (0.30μg/L) detected in irrigation well of Fylakio C1 and wasn't
305 detected in drinking water and irrigation well of Fylakio (A4 andC1) and in drinking water well
306 and experimental borehole of Rizia (A1 and B1) at first and second sampling. The highest
307 concentration of metolachlor (0.93 μg/L) was detected in experimental borehole of Fylakio
308 (A4). Terbuthylazine presented the highest concentration (1.00μg/L) in the experimental
309 borehole of Keramos (A2). It is worth noting that atrazine's metabolites were often found in
310 higher concentrations than their parent compound.
311 The annual average concentrations for atrazine, DIA, DEA, HA, metolachlor and
312 terbuthylazine were 0.17 μg/L, 0.28 μg/L, 0.13 μg/L, 0.06 μg/L, 0.09 μg/L and 0.14 μg/L,
313 respectively. The annual average concentration of atrazine and its maximum concentration
314 detected were below the annual average EQS for atrazine (0.6 μg/L) and the maximum
315 allowable EQS (2 μg/L), respectively (EC 2008).
316 The distribution of pesticide concentrations in all wells was examined by applying the Box and
317 Whisker Plot (Fig. S1). Figure S1 shows the distribution of median, quartile, non-outlier,
318 outlier and extreme concentrations of compounds found in the studied wells.
319 The maximum concentrations detected in this study are within the range of concentrations
320 detected in groundwater samples at the European level. Menchen et al. (2017), has recorded the
321 maximum concentrations for atrazine (0.38 μg/L), metolachlor (0.23 μg/L), DEA (0.12 μg/L),
322 DIA (0.21 μg/L) and terbuthylazine (0.90 μg/L). According to Meffe et al. (2014), the

maximum concentrations for terbuthylazine in Italian groundwater was 29.05 μg/L. Considerable higher maximum concentrations were found by Jurado et al. (2012), atrazine (3.45 μg/L), metolachlor (5.37 μg/L), DEA (1.98 μg/L) and terbuthylazine (1.27 μg/L). Hernandez et al. (2008) found that DIA was the most frequent detected compound (72%), followed by terbuthylazine (50%), with maximum concentrations of 1.42 μg/L for DEA 0.4 μg/L for DIA and 0.46 μg/L for terbuthylazine. Also, a third Spanish study in agricultural areas showed maximum concentration 0.327 μg/L for atrazine 0.369 μg/L for DEA, 0.335 μg/L for terbuthylazine and 0.548 μg/L for metolachlor with detection frequency ranged from 4% (DEA) to 68% (metolachlor). The same study presented results from Portuguese groundwater in agricultural areas. Terbuthylazine had the highest concentration (1.885 μg/L) with detection frequency reached 56%, followed by atrazine (0.191 μg/L) and detection frequency 25%. DEA and metolachlor concentration were lower than 0.1 μg/L (Samchez-Gonzalez et al. 2013). According to Korosa et al. (2016), in groundwater samples from Slovenia atrazine and DEA were detected at concentrations up to 0.228μg/L and 0.103 μg/L and their frequency of detection was 94.6% and 98.2% respectively. On another study which was conducted in United Kingdom and France, the highest concentrations from British groundwaters for atrazine, DIA, and DEA were 0.2, 0.1 and 0.16 μg/L, respectively. On the other hand, the highest concentrations were found lower than 0.1 μg/L, in France (Lapworth et al., 2015).

Historical vulnerability of the transboundary aquifer to contamination by pesticide residues

Target compounds had been monitored previously (between 1999-2003), at the same locations, before atrazine ban in EU. Also, a similar study was conducted between 2010-2012 (data not shown), confirming the occurrence of atrazine, DEA, DIA, and metolachlor (Vryzas et al. 2012c). In order to have a better perspective on pollution temporal trends, our data were compared with those of 1999-2003. Fifteen to nineteen years ago metolachlor had been detected at least once in 63 % of the wells followed by atrazine (61%), DEA (50%), alachlor (47%) and DIA (34%). According to Vryzas et al. (2012c), maximum concentrations for atrazine (1.48 μg/L), DEA (0.76 μg/L), DIA (0.071 μg/L) and metolachlor (1.54 μg/L) had been detected at

351 the same drinking water wells sampled in this study and considerable higher pesticide
352 concentrations were detected in shallow groundwater from experimental boreholes (Table 3).
353 Vulnerability of the aquifer to pollution depend on the land uses, soil properties, geological
354 characteristics of the unsaturated zone, the hydraulic properties, the depth of the vadose zone
355 and the leaching potential or physicochemical properties of the contaminant.
356 Due to the metolachlor and atrazine effectiveness against corn weeds and the limited available
357 herbicides, both of them were extensively used during the period 1980-2005. The atrazine
358 withdrawal in 2004, bring out the terbuthylazine as the most used herbicide, until nowadays.
359 The cropping system and major crops has been gradually changed from 2005 till now. However,
360 field crops are still the major crops in the area and the irrigation practices are the same used 20-
361 40 years ago (frequent sprinkler irrigation.
362 According to previous studies focused in this area, atrazine degraded faster than metolachlor in
363 all soils of the vadoze zone and the biotransformation rates of both compounds decreased as
364 the soil depth increased. Hence, the chronic presence of atrazine in field is indicated by the
365 higher biotransformation rate of atrazine in soil taken from the middle of a studied field in
366 comparison with soil sampled from the field margins (Vryzas et al. 2012a). The major
367 metabolites of atrazine and metolachlor were found at higher concentrations in the 10–20 cm
368 layers of all soil cores studied (0-110 cm bgs). However, the enhanced biodegradation rates of
369 atrazine in these soils is not enough to prevent the contamination of groundwater bodies.
370 Similar results have been observed by other studies. According to McMahon et al. (1992);
371 Kolpin et al. (1997) and Steele et al. (2008), degradation rates of triazine parent compounds are
372 slower than their transport rates in groundwater.
373 Adsorption studies of atrazine, DEA, DIA, HA and metolachlor were also conducted in soils
374 from five (0-10, 10-20, 20-40, 40-80, 90-110 cm bgs) different depths (Vryzas et al. 2007).
375 This study revealed that when pseudo-equilibrium stage reached, the amount of compounds
376 adsorbed accounted only for 10, 14, 27, 43 and 94% of the initial amount of DEA, DIA,
377 atrazine, metolachlor and HA, respectively, spiked to the soils. According to this study, it was
378 expected that more than 57 and 73% of the applied dose of metolachlor and atrazine,

379 respectively, to be desorbed into the soil water and be available for leaching to deeper soil
380 layers (Vryzas et al. 2007). In addition, to low adsorption capacity of atrazine and metolachlor
381 within soil profile of the studied area, it was proved that the preferential flow is a major
382 pesticide leaching mechanism in this area since pollutants can reach the saturated zone of the
383 aquifer through preferential flow paths (shrinkage of the clay minerals, plant roots, earthworms
384 forming burrows) without going through chromatographic flow within unsaturated zone and
385 thereby circumventing the degradation processes (Vryzas et al.2012b). Studies on the apparent
386 age of the studied aquifers shown that the residence time of groundwater bodies ranged from
387 1.2 to 50 years (Vryzas et al. 2012c). The leaching mechanisms prevailed in this area has been
388 also studied in an extensive four-year field experiment focused on soil water samples taken
389 from 0-25, 35, 60, 100 and 160 cm bgs (Vryzas et al. 2012b). According to this study,
390 metolachlor, atrazine, DEA and DIA were detected in more than 67% of the total soil water
391 samples. The main conclusion of this study was that the corn-applied herbicides have been
392 leached below the surface soil via macropore-dominated pathways in less than one month after
393 their application. Agricultural practices (application of pesticides and sprinkler irrigation) used
394 in this area, soil structure and hydrogeological conditions increase the leaching potential of
395 pesticides in the studied area. It is worth notice that alachlor another banned herbicides, with
396 very limited half-life period ($DT_{50field}$ = 14 days) had been detected in soil water of the studied
397 area at concentrations greater than 0.1 mg/L up to 40 months after its application.
398 Also, as recommend by Vryzas, et al. (2012b), the late pesticide application, use of drip instead
399 of sprinkler irrigation and delayed first irrigation seem to be the major management actions
400 according to good agricultural practice that prevent pesticide leaching to groundwater in a
401 semiarid Mediterranean region. The limited spatial and temporal variation of concentration
402 levels observed in studied wells indicates a continuous load of the aquifer with the target
403 compounds. The continuous use of high amounts of atrazine for more than 30 years was enough
404 to contaminate the soil and aquifer and to be detected with its metabolites in groundwater 15
405 years after its last use (2004). However, illegal applications cannot be excluded since the
406 studied area is 20 km from Greek/Turkish/Bulgarian borders and illegal trade of banned

407 pesticides had been observed. Metolachlor has been used in the area for more than 40 years and
408 terbuthylazine is mainly used the last 15 years.
409 Contrary to the results obtained 15 to 19 years ago extreme concentrations were not observed
410 in this study, indicate the absence of point source pollution sites nearby the studied wells.
411 Moreover, the studied compounds (metolachlor and terbuthylazine) were used in reduced
412 quantities due to the changes of crop profile of the area or not used at all (ban of atrazine)
413 compared the situation prevailed when previous studies were conducted (Vryzas et al 2012a).
414
415 *DEA to atrazine ratio (DAR)*
416 DEA to atrazine ratio (DAR) has been used to categorize point- and non-point source pollution
417 of groundwater and in order to characterize the degradation and transport of atrazine in response
418 to its metabolite DEA. This ratio can give us an indication of the major leaching mechanisms
419 contribute to the pollution of groundwater and the capacity of the unsaturated zone to
420 biodegrade atrazine to DEA. During the transport of atrazine through chromatographic flow
421 within the biological more active unsaturated zone it could be metabolized in significant
422 amounts by microorganisms to DEA (Adams and Thurman 1991; Goolsby et al. 1997; Vryzas
423 et al. 2012b). In such cases the DAR would have values higher than 0.4 or even close 1.
424 Contrary, when atrazine bypasses the vadose and enters the saturated zone through preferential
425 flow the contact time between atrazine and soil microbial community could be shorter and,
426 therefore, the DAR ratio would be less than 0.4. The DAR ratio can provide information about
427 atrazine leaching behavior based on the fact that atrazine represents a closer adsorption capacity
428 to DEA than to HA in spite of HA was found as the main metabolite of atrazine at same area.
429 In addition, this soil can adsorb higher amount of atrazine than DEA. Therefore, DEA can be
430 leached faster than atrazine through chromatographic or preferential flow (Vryzas et al. 2007).
431 The calculated DAR in this study was found to be higher than 1 in some cases and lower the 1
432 in most samples (Table 5) indicating that contamination in some cases comes from diffuse
433 sources but most probably the bound atrazine was gradually desorbed from the soil matrix to
434 the soil water and moved to groundwater through preferential flow (Hildebrandt et al. 2008;

435 Vryzas, et al 2012b; Koch-Shulmeyer et al. 2014; Vonberg et al. 2014). Our results are in
436 agreement with those of Vryzas et al. (2012a) conducted in the same area 15-19 years ago who
437 found similar DAR values few months after the application of atrazine. Overall, atrazine's
438 degradation products showed similar and, in few cases, higher concentrations than did the
439 parent compound. DIA exhibits a large range of concentrations varying between 0.01 μg/L and
440 2.91 μg/L. According to biotransformation studies conducted in the soil profile of the studied
441 area HA was the most frequently detected metabolite and with the highest concentrations. The
442 second most frequently detected degradation product in soil was DEA, followed by rare DIA
443 detections (Vryzas et al. 2012). The overwhelming majority of soil water samples with DEA
444 presence, showed DEA had greater concentrations than DIA and the ratio values CDEA/CDIA
445 reached 33 (Vryzas et al. 2012b). Similarly, DEA (50% of groundwater samples) was more
446 frequently detected than DIA (34% of groundwater samples) in an extensive groundwater
447 monitoring program conducted in the same area 15-19 years ago (Vryzas et al. 2012c). Contrary
448 to previous reported data, in our study, atrazine and its metabolites were detected with similar
449 frequency of detection.
450
451 *Risk assessment*
452 An extended discussion was preceded related to the presence, occurrence and distribution
453 reasons of target pesticides at studied area. Results on human health risk assessment are
454 presented in Table 6. Although, the HQ values for individual pesticide did not exceed the value
455 1, the estimated non-carcinogenic risk for children was higher, when compared to adults. The
456 HQ values for mean pesticides concertation were ranged between 0.0171 to 0.1913 for adults
457 and between 0.0393 to 0.5752 for children. The highest mean values are reported to metolachlor
458 and the lowest to atrazine. The highest HQ values were determined in drinking water well close
459 to Rizia namely, 0.2507 and 0,7817 for adults and children, respectively. Similar HQ values for
460 atrazine and metolachlor in drinking water were reported by Papadakis et al. (2015). The risk
461 level for terbuthylazine is low with HQ values lower than 0.6.

462 The sum of HQ values did not reach the unity in all studied wells. The greatest cumulative
463 potential risk was determined in the Rizia well with values 0.2836 and 0.8299 for adults and
464 children, respectively. The lowest potential risk has the Elia well, with values lower than 0.4.
465 Consequently, according to the acute risk assessment, the studied drinking water wells were
466 characterized safe.
467 Oppositely, the carcinogenic risk assessment showed high values. In all cases, atrazine R values
468 were higher than the parametric one of 1 x 10^{-6} recommended by USEPA, for both age groups,
469 showing that the local population is under carcinogenic risk (table 6). The water consumption
470 through Fylakio well presents the highest risk, while Arzos well the lowest. The R values are
471 ranged between 0.002-0.0018 for adults and 0.0012- 0.0181 for children. Papadakis et al.
472 (2015), in a similar study, indicate high carcinogenic risk only for children.
473
474 **Conclusions**

475 - Although agricultural use of atrazine has been banned in Greece for more than 15 years
476 ago, atrazine and its metabolites residues are still detected in groundwater of the region,
477 indicating their high persistence in saturated zone.
478 - Among the compounds included in this study metolachlor was detected in 100% of
479 samples followed by atrazine (96.4%), DEA and HA (88.6%), DIA (78.2%) and
480 terbuthylazine 67.5%.
481 - Atrazine, DIA, DEA, HA, MET and TER mean concentrations detected were 0.18,
482 0.29, 0.14, 0.09, 0.16 and 0.15 μg/L, respectively
483 - DIA, terbuthylazine, atrazine, metolachlor, DEA and HA exceeded the critical
484 pesticide limit for drinking water of 0.1 μg/L in 58%, 50.5%, 38%, 35.9%, 30% and
485 15.5% of the total number of samples for each compound, respectively.
486 - All pesticides were detected in both shallow and deep ground-water bodies
487 (experimental boreholes, drinking or irrigation water wells).

- Although the repeated application of studied pesticides could lead to enhanced biodegradation, as previously reported in the studied area, the remaining amounts of bound residues was gradually desorbed from the soil matrix to the soil water and moved to groundwater through preferential or chromatographic flow.
- Due to the presence of occasional point-sources pollution were detected extreme concentrations.
- The drinking water consumption for local people is safe considering the acute risk assessment.
- The atrazine R values suggested high carcinogenic risk.

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Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Authors Contributions

Parlakidis Paraskevas, Alexoudis Christos, Fernández-Cirelli Alicia and Vryzas Zisis conceived and planned the experiments, Parlakidis Paraskevas and Alexoudis Christos made the sampling, Parlakidis Paraskevas, Rodriguez M. Soledad, Perez-Rojas Greivin and Perez-Villanueva Marta made the extractions and instrumental analysis. Parlakidis Paraskevas, Zisis Vryzas, Rodriguez M. Soledad, Perez Carrera Alejandro and Fernández-Cirelli Alicia wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

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Competing Interests

Authors have no other competing interests

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

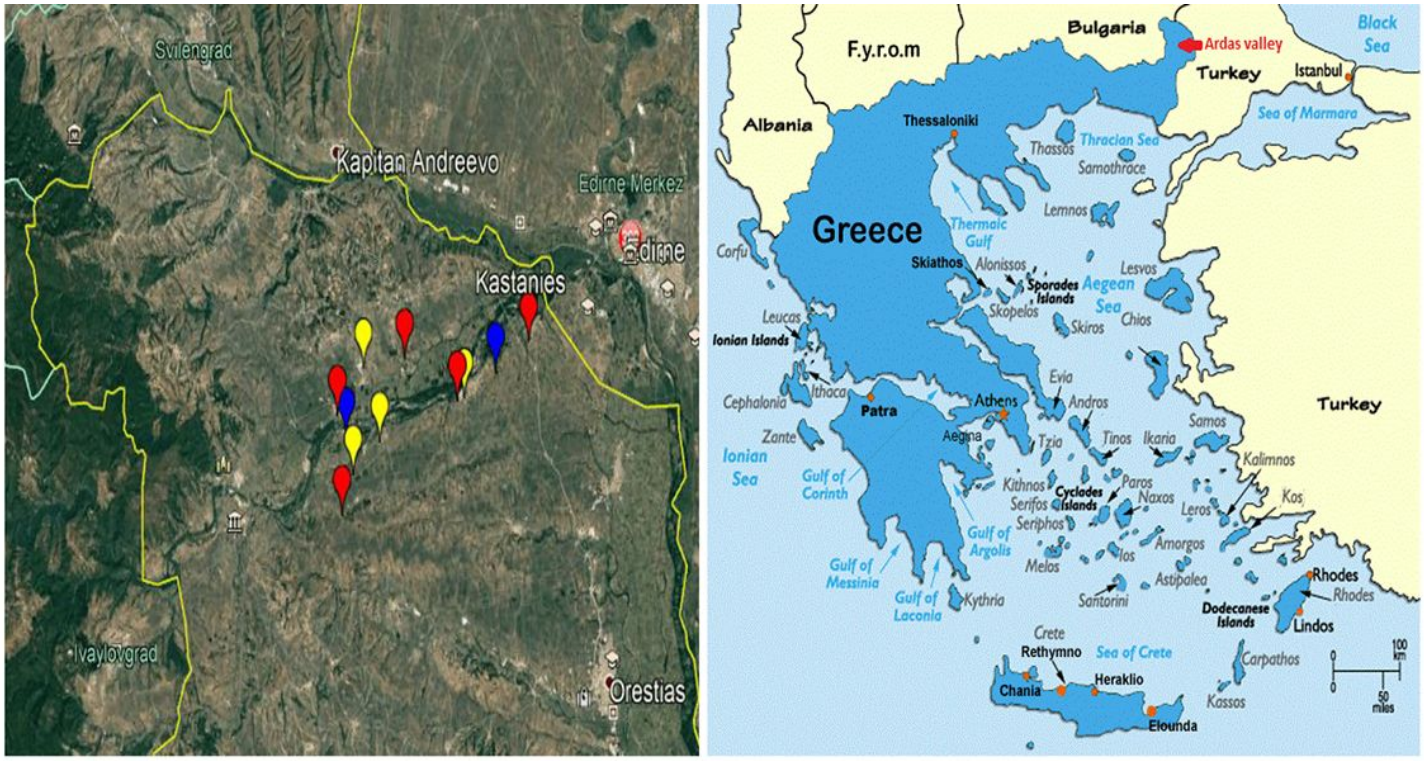

Figure 1

Sampling area of a transboundary aquifer, among Greece, Turkey and Bulgaria (picture on right). Yellow points: Experimental boreholes. Red point: drinking water wells. Blue points: irrigation wells (picture on left). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

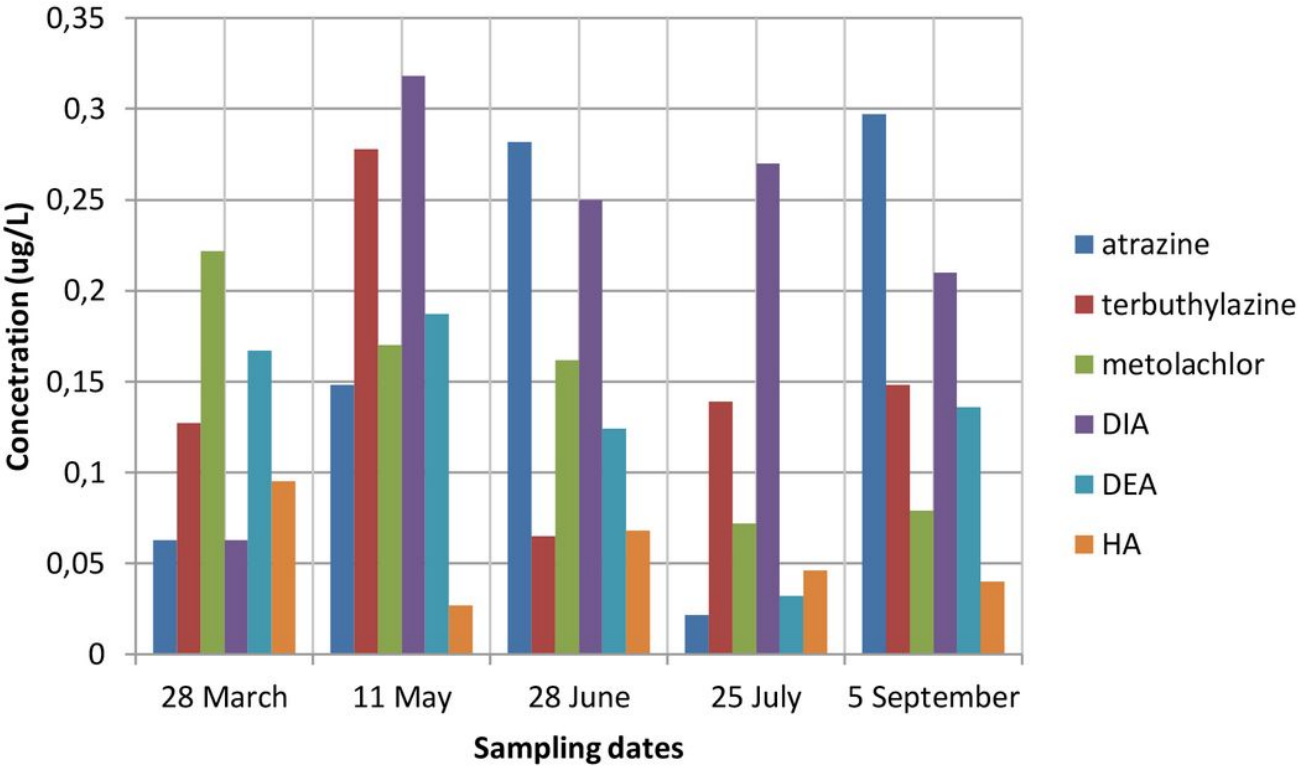

Figure 2

Mean compound concentrations in each sampling date.

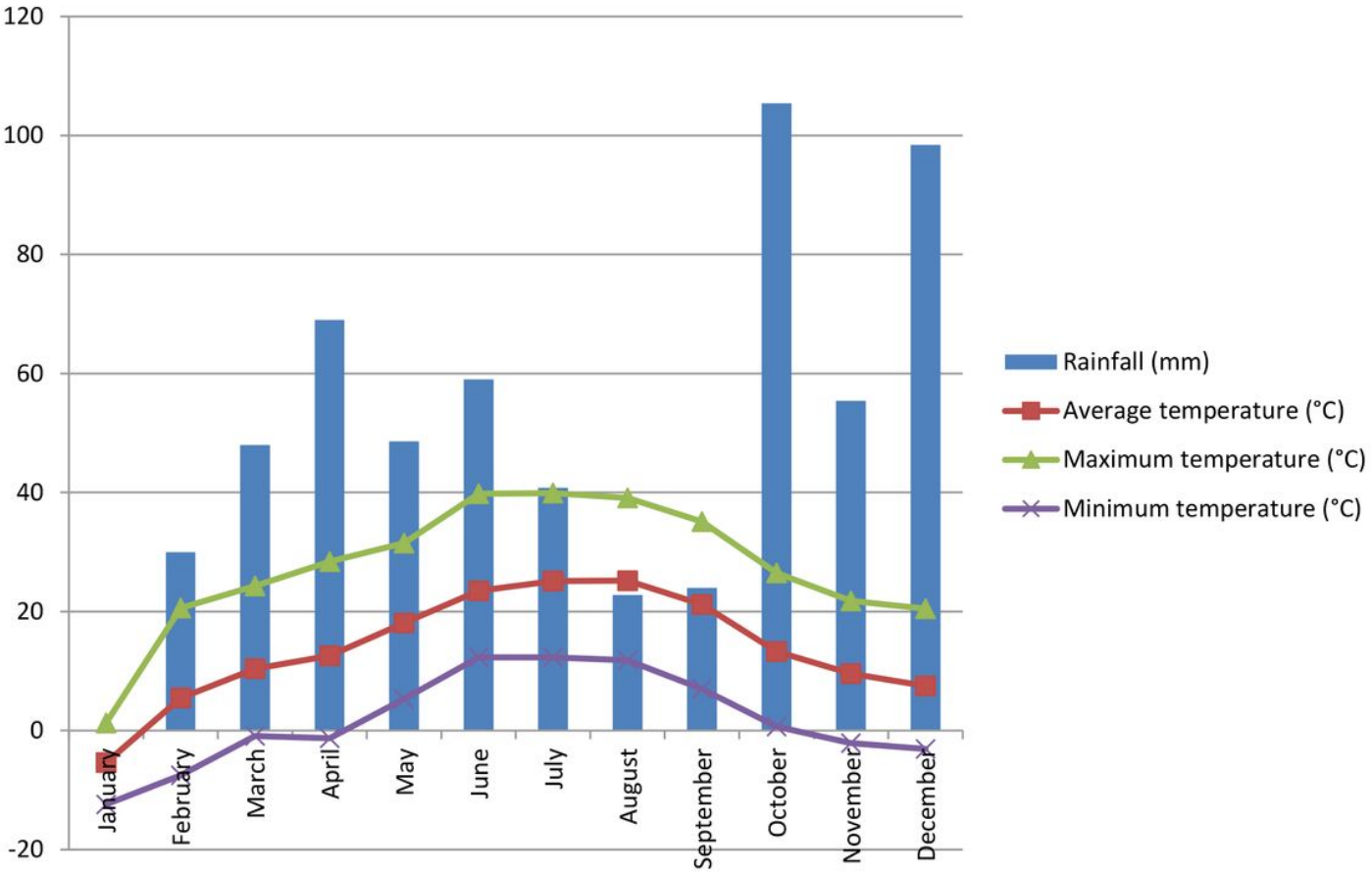

Figure 3

Meteorological data during the studying growing season.

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