

MONITORING RIVER WATER IN THE LOWER DANUBE FOR ATRAZINE CONTAMINATION

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SUMMARY

A GC–MS method is proposed for determination of atrazine in water. The limit of detection (*LOD*) is 0.0050 µg L⁻¹ and quantification is possible in the range 0.011 to 1.84 µg L⁻¹. The method was used for analysis of surface water sampled from Silistra and from Rousse, on the Lower Danube, over a two-year period. The concentrations of atrazine in the samples were from 0.389 to 0.673 µg L⁻¹, *RSD* 4.25%. The quantitative data obtained were processed statistically and showed seasonal and geographic changes in atrazine concentration. Important conclusions about the current status of the pollution were obtained from statistical and comparative analysis, as were other trends in the contamination of surface waters with atrazine.

INTRODUCTION

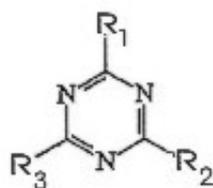
In comparison with the 1950s modern farmers invest 17 times more in pesticides, even allowing for inflation. Besides direct pollution of the environment, industrial agriculture has placed an extremely heavy burden on world water resources [1]. One of the main causes of pollution of the Earth's surface water with pesticides and herbicides is washing by rain which transfers the substances from treated fields to large water-supply basins and thus into surface water (lakes, rivers, streams, and drinking water) [2,3]. For this reason the Danube River seems to be an interesting matrix for investigation of pollution by organic compounds.

One modern class of herbicides is the triazines; they are widely used in agriculture and have been reported as contaminants in both surface and ground water [3]. The triazine compounds are ametryn, anilazine, atra-

zine, chlorinated isocyanurates, cyanazine, prometon, prometryn, propazine, simazine, and terbutryn. The structures of simazine, atrazine, and propazine are given in Table I [4–6]; the best known member of the group

Table I

The structures of some triazines



Compound	Substituent in position:		
	R ₁	R ₂	R ₃
Simazine	Cl	NH-C ₂ H ₅	NH-C ₂ H ₅
Atrazine	Cl	NH-C ₂ H ₅	NH-CH(CH ₃) ₂
Propazine	Cl	NH-CH(CH ₃) ₂	NH-CH(CH ₃) ₂

is atrazine (Fig. 1), which is registered and used as a tool for control of broad-leaved weeds and some herbaceous weeds. Atrazine was introduced in 1958 and is now one of the most widely used herbicides throughout the world. It is used in huge quantities in agriculture to kill weeds in corn and soybean fields; in USA approximately 75% of wheat areas are treated with atrazine [7,8].

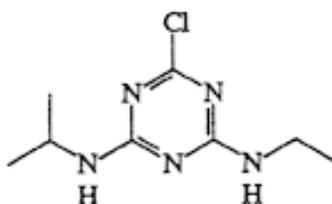


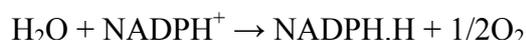
Fig. 1

The structure of atrazine

Although atrazine is poorly soluble in water, under the influence of rain it is transported down brooks and rivers to lakes and even enters drinking water. In Canada the maximum allowable concentration of atrazine in drinking water is 60 µg L⁻¹; in the European Community the level of atrazine and its metabolites has been set at 0.5 µg L⁻¹. When Ohio water

systems were tested in 1997, 94% contained traces of atrazine. The situation in Europe is similar. Its use is banned in Italy, the Netherlands, Germany, Sweden, Belgium, France, and Norway. Other European countries where its use is permitted allow only 1/30th the amount allowed in the United States. All representatives of triazines have been reported as contaminants of surface and ground water [4].

The triazines are strong inhibitors of photosynthesis that work by interfering with the Hill reaction, a water-splitting, light initiated reaction that results in the production of free oxygen by plants [9]:



The mechanism of action of atrazine seems to be herbicide-induced deficiency in growth regulator photosynthesis, as a result of this inhibition of photosynthesis.

It has been reported that atrazine is a reproductive carcinogen that causes dysgenesis and gene mutation even when contamination is below the maximum permitted. It is a weak toxin for birds and mammals, a moderately dangerous toxin for freshwater fish, and a strong toxin for water invertebrates. Atrazine is a very strong acute toxin for non-target terrestrial and water plants [7,8]. The long-term effects of atrazine are carcinogenic activity, presumable mutagenesis, production of immunotoxins, destruction of the adrenal glands, formation of bladder and urethral stones, teratogenic effects, destruction of the lungs and liver, suspected production of fetotoxins, sperm production disorders, and vitamin metabolism disorders. Immediate effects of atrazine are eye and skin irritation, nausea, vomiting, diarrhea, muscular debility, and salivation [10].

Because triazines are such widely used herbicides, in the last three decades a variety of methods has been developed for their analysis; these include UV-visible spectroscopy, gas-liquid chromatography (GLC), and thin-layer chromatography. During the last two decades capillary gas chromatography-mass spectrometry (CGC-MS) has found wide application [6]. The aim of this study was determination of atrazine pollution at two sampling points on the Lower Danube and estimation of seasonal periodic variations in atrazine concentration. For this purpose a GC-MS method for the analysis of atrazine in water extracts is proposed and the working conditions given. The internal standard (IS) method was used for quantitation of atrazine.

EXPERIMENTAL

Sample Preparation

The water sample (1 L) was transferred to a 2-L separation funnel and sodium sulfate (10 g) and internal standard working solution (α -hexachlorocyclohexane (α -HCH), 5 ppm, 20 μ L; final concentration 100 ng L⁻¹) were added. The sample was extracted with 100 mL dichloromethane and the combined extract was isolated and dried over anhydrous sodium sulfate. Hexane (100 μ L) was added as a keeper and the solvent was evaporated to 1 mL, of which 2 μ L was injected. A schematic diagram of the sample-preparation procedure is given in Fig. 2.

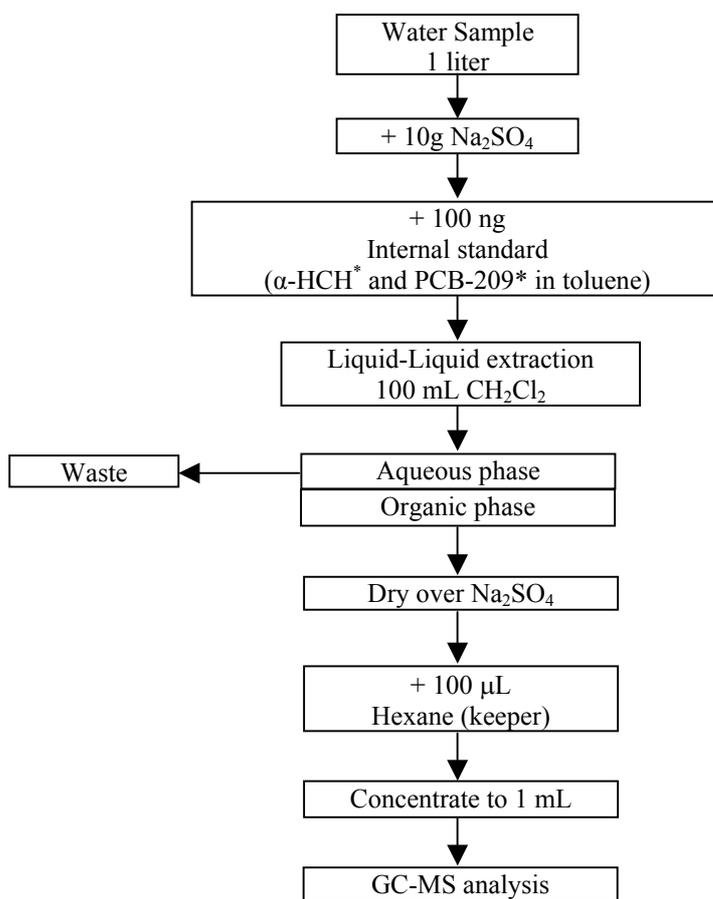


Fig. 2

Schematic diagram of the sample-preparation procedure

Capillary Gas Chromatography–Mass Spectrometry

Analysis was performed with a Hewlett–Packard (HP) model 5890 Series II gas chromatograph connected to a model 5972A quadrupole mass spectrometer equipped with a Hewlett–Packard data system (HPG1034C MS ChemStation, Version C.03.00). The chromatograph was fitted with a 30 m × 0.25 mm i.d. fused silica capillary column coated with a 1- μ m film of the polydimethylsiloxane HP5-MS (Hewlett–Packard). Preliminary data were also obtained by use of a 30 m × 0.25 mm i.d. column coated with a 0.25- μ m film of CP-SIL 8 CB-MS (Chrompack). Splitless injection (2 μ L) was performed at 280°C with an HP ALC autosampler; the splitless period was 0.75 min. Helium was used as carrier gas (44.8 cm s⁻¹, 0.7 mL min⁻¹ constant flow). The oven temperature was maintained at 120°C for 1 min after injection, then programmed at 30° min⁻¹ to 190°C, then at 4° min⁻¹ to 240°C, and finally at 45° min⁻¹ to 295°C.

For both qualitative and quantitative analysis the mass spectrometer was operated in electron-impact ionization mode. Quantitative analysis was performed by single-ion monitoring (SIM); the atrazine ions monitored were *m/z* 200 and 215 (settings SIM: Tgt, 200; Q1, 215; Q2, 202). The linearity of the method was evaluated by chromatographing 0.02, 0.04, 0.1, 0.2, 0.5, and 1 μ g mL⁻¹ calibration mixtures.

RESULTS AND DISCUSSION

Typical chromatograms obtained for a mixed pesticide standard by use of the HP5-MS and CP-SIL 8 CB-MS columns are shown in Figs 3 and 4. The retention times obtained for the components of the mixture are listed in Table II. From these data it is apparent that better resolution was obtained by use of the HP5-MS column. Under the conditions used for GC–MS the retention times of atrazine and α -HCH (internal standard; IS) were 11.96 and 11.54 min, respectively. Typical mass spectra are shown in Fig. 5. The detection limit obtained for atrazine was 0.005 μ g L⁻¹ (*SD* 0.0016, *RSD* 4.427; *n* = 10).

The Danube river (Fig. 6), total length 2857 km, is the second longest river in Europe after the Volga. The total water supply area of the Danube is 817 000 km². The right side of the river has a smaller water supply area but carries the greater part of the river's water-supply capacity (66% of the whole capacity of the influx water). The river water is contributed by 300 tributaries. The Danube basin is divided into three

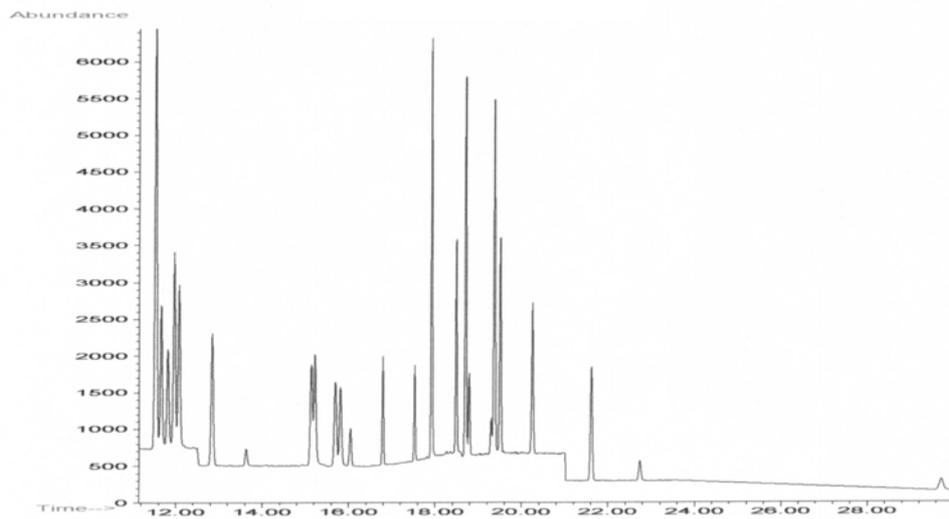


Fig. 3

SIM chromatogram obtained from 0.5 ppm mixed pesticide standard by use of the Hewlett-Packard HP5-MS capillary column

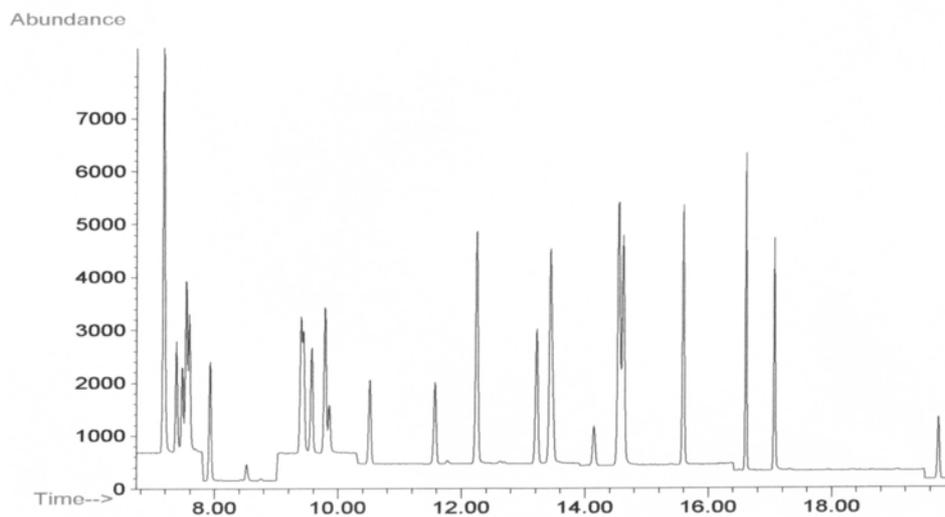


Fig. 4

SIM chromatogram obtained from 0.5 ppm mixed pesticide standard by use of the Chrompack CP-SIL 8-MS capillary column

Table II

Comparison of retention times on the two columns used

No.	Compound	Retention time	
		HP 5	Chrompack
1	α -HCH* (IS-1)	11.48	7.08
2	Prometon	11.61	7.29
3	Simazine	11.76	7.39
4	Atrazine	11.91	7.45
5	Propazine	12.04	4.51
6	Lindane	12.78	7.83
7	Ametryn	15.08	9.31
8	Prometryn	15.17	9.35
9	Heptachlor	15.64	9.48
10	Terbutryn	15.76	9.70
11	Fenitrothion	15.99	9.77
12	Endrin	16.75	10.42
13	Heptachlorepoxyde- <i>cis</i>	17.50	11.48
14	o,p-DDE	17.90	12.15
15	p,p-DDE	18.48	13.13
16	o, p-DDD	18.69	13.36
17	Dieldrin	18.77	13.41
18	Aldrin	19.28	14.05
19	p,p-DDD	19.35	14.47
20	p,p-DDT	19.49	14.54
21	o,p-DDT	20.23	15.53
22	Methoxychlor	21.59	16.36
23	Phosalone	22.71	17.03
24	PCB 209* (IS 2)	29.69	19.68

parts – the Upper Danube (977 km between the spring and the Morava river creek), the Middle Danube (the next 949 km to Turno Severin in Romania), and the Lower Danube (931 km) [11].

Our sampling points were situated near the towns Rouse and Silistra at 375.5 and 495 km, respectively, from the start of the Lower Danube. The region near Silistra is a horizontal, wide section of the river where we investigated three points (the Bulgarian coast, Talweg, and the Romanian coast). We also took one sample from a tributary of the Danube – the Ruse Lom River, near the town of Rouse. During the last few years there has been a tendency to reduce pesticide and herbicide use in the

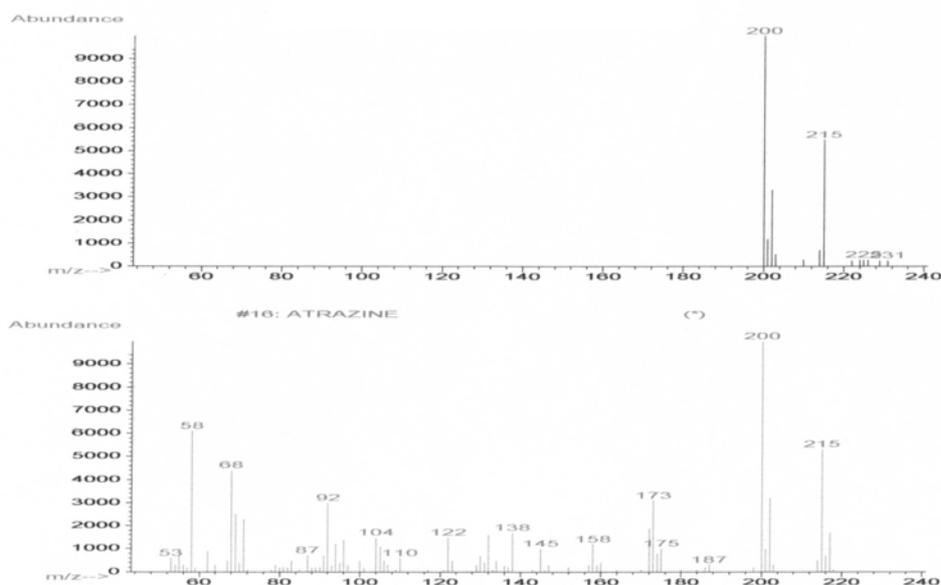


Fig. 5

Mass spectrum obtained from atrazine, and the spectrum from the mass spectral library

water supply area of the Lower Danube, mainly because of economic difficulties in the two neighboring countries (Bulgaria and Romania).

The results obtained from analysis of water samples from the Lower Danube are shown in Figs 7–10 and listed in Tables III and IV. The lowest values of the atrazine concentration were recorded in the first eight-month period of 2002, probably because of the higher level of the river during 2002, which reached record levels that year. The maximum value for 2001 was $1.840 \mu\text{g L}^{-1}$ whereas the maximum for 2002 was $1.024 \mu\text{g L}^{-1}$ (Table III). Seasonal variations were observed in atrazine contamination; maximum levels were observed in February and in June–July (Figs 7–9).

CONCLUSIONS

A GC–MS method has been developed for analysis of atrazine and used to monitor atrazine contamination of river water from the Lower Danube. The concentration of atrazine increases in the direction of the estuary of the river. The average annual concentration of atrazine at sampling points near the town of Silistra is 40% higher than that at the sampling point near Rousse. (The distance between the two sampling points is 120 km.)



Fig. 6

The Danube river basin

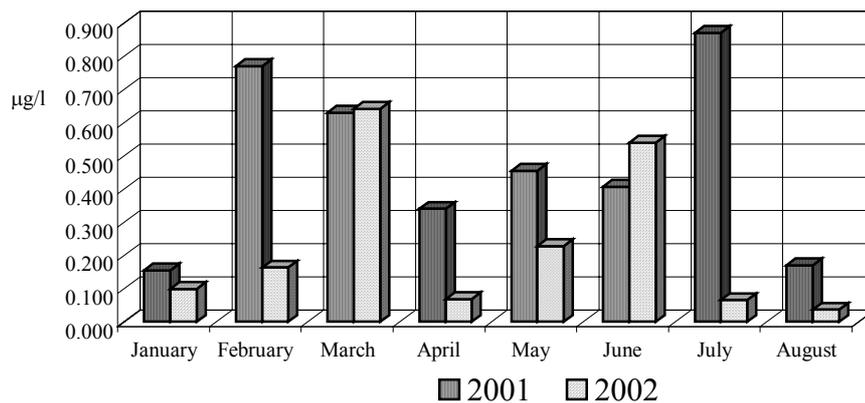


Fig. 7

Comparison of atrazine levels in the Danube river, at the Rousse sampling point (495 km), in 2001 and 2002

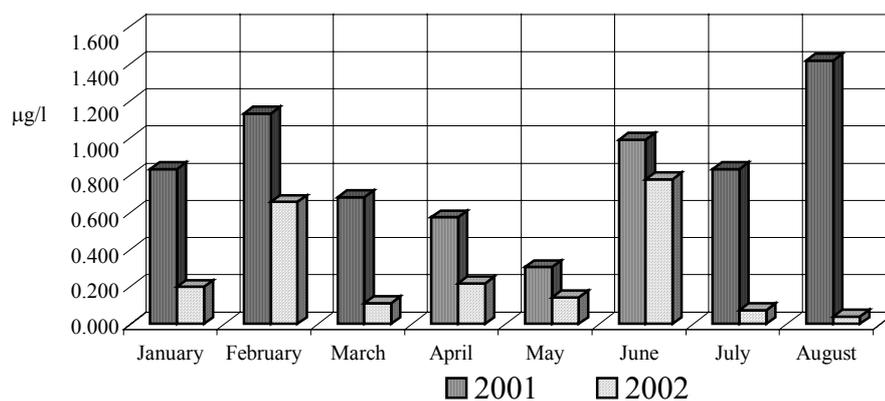


Fig. 8

Comparison of atrazine levels in the Danube river, at the Silistra sampling point (375.5 km), in 2001 and 2002

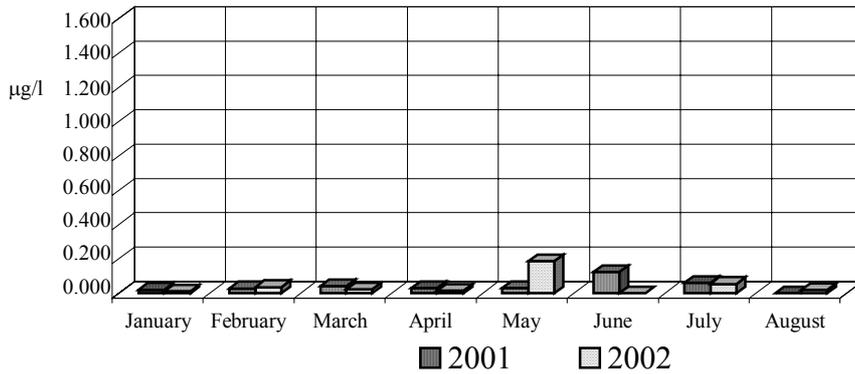


Fig. 9

Comparison of atrazine levels in the tributary of the Danube river, at the Rouse sampling point, in 2001 and 2002

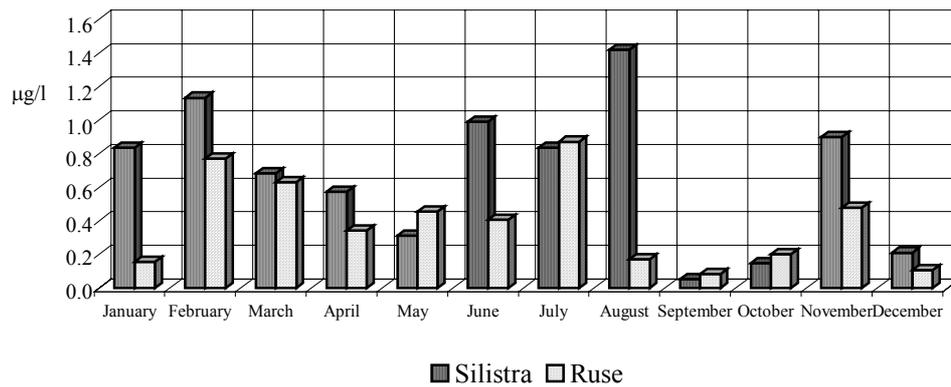


Fig. 10

Comparison of atrazine levels at the Silistra and Rouse sampling points in 2001

Table III

Comparison of atrazine levels ($\mu\text{g L}^{-1}$) in the Lower Danube in the first eight months of 2001 and 2002

	Bulgarian Coast		Talweg		Romanian coast		Rousse		Rusenski Lom	
	Silistra 375.5 km		Silistra 375.5 km		Silistra 375.5 km		495 km			
	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
January	0.900	0.192	0.740	0.194	0.860	0.210	0.155	0.099	0.021	0.011
February	1.182	0.47	0.929	1.024	1.285	0.474	0.770	0.163	0.027	0.035
March	0.804	0.112	0.550	0.113	0.690	0.107	0.630	0.641	0.040	0.022
April	0.640	0.213	0.510	0.217	0.570	0.223	0.340	0.068	0.030	0.015
May	0.314	0.146	0.310	0.142	0.293	0.141	0.455	0.228	0.030	0.185
June	1.240	0.719	1.380	0.823	0.350	0.835	0.407	0.539	0.123	0.025
July	0.860	0.075	0.880	0.067	0.760	0.071	0.870	0.065	0.060	0.053
August	1.840	0.043	1.240	0.036	1.170	0.035	0.170	0.036	<0.005	0.019
Min. value	0.314	0.043	0.310	0.036	0.293	0.035	0.155	0.036	<0.005	0.011
Max. value	1.840	0.719	1.380	1.024	1.285	0.835	0.870	0.641	0.123	0.185
Average	0.973	0.246	0.817	0.327	0.747	0.262	0.475	0.230	0.041	0.046

Table IV

Comparison of atrazine levels ($\mu\text{g L}^{-1}$) in the Lower Danube (495 km and 375.5 km) in 2001

	Bulgarian coast	Talweg	Romanian coast	Average value Silistra	Rousse
January	0.900	0.740	0.860	0.833	0.155
February	1.182	0.929	1.285	1.132	0.770
March	0.804	0.550	0.690	0.681	0.630
April	0.640	0.510	0.570	0.573	0.340
May	0.314	0.310	0.293	0.306	0.455
June	1.240	1.380	0.350	0.990	0.407
July	0.860	0.880	0.760	0.833	0.870
August	1.840	1.240	1.170	1.417	0.170
September	0.057	0.049	0.051	0.052	0.084
October	0.128	0.179	0.130	0.146	0.202
November	0.935	0.874	0.882	0.897	0.479
December	0.199	0.208	0.224	0.210	0.107

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