Testing atrazine biodegradation in water

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Large quantities of plant protection agents were used in the past in Slovenian agriculture. A survey of quantities used, based on the available data, has been carried out. Figure 1 shows the use of agents as given in the Statistical Yearbook of the Republic of Slovenia (1). It shows that the use of these agents is declining, which is the result of new legislation limiting the number and input of such substances in groundwater.

Figure 1 shows the tabulated values of the total use of plant protection agents in kg in Slovenia.

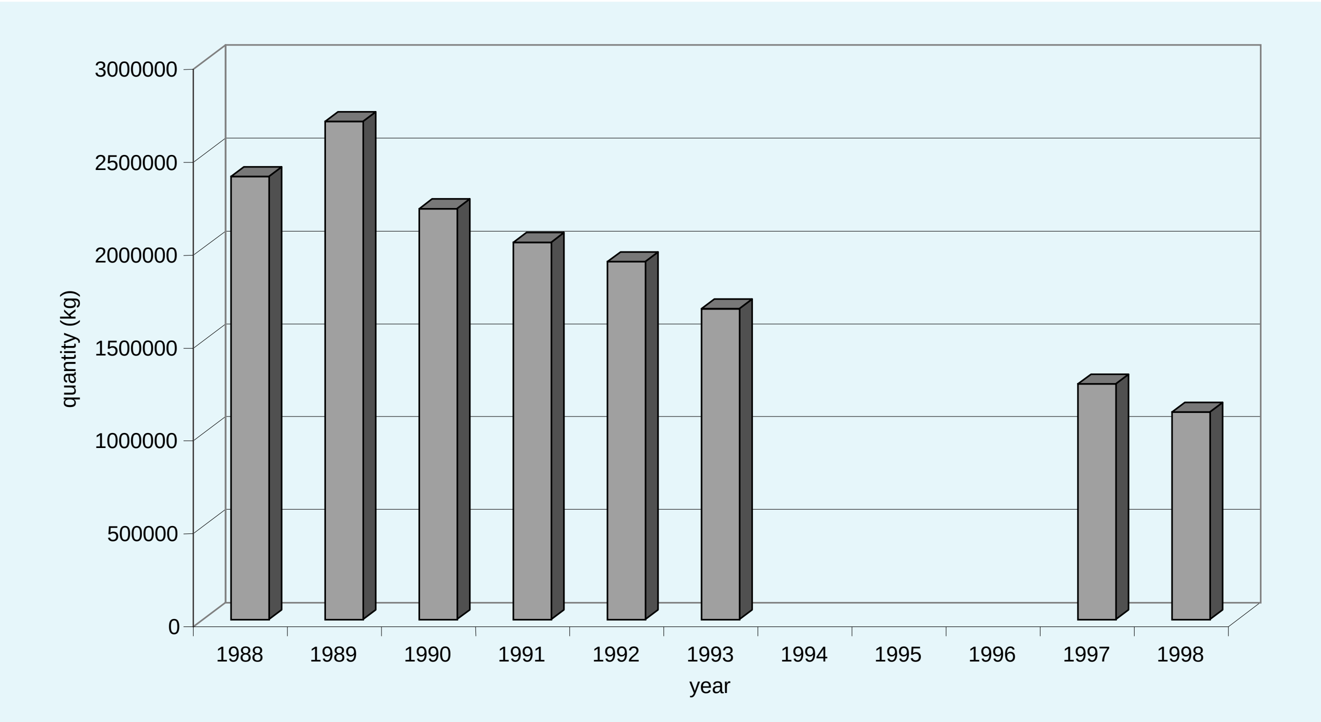

Figure 1. Tabulated values of the total use of plant protection agents in Slovenia

Figure 1 shows the decline in the amount of plant protection agents. Their actual use is probably higher: the data given was obtained by means of surveys while the real figures are slightly higher. Real data was assessed on the basis of imports of active substances, through factories importing these substances and preparing them for use, as well as from an assessment of individual and unregistered instances of import.

Monitoring of groundwater began many years ago in Slovenia through the analysis of certain chemical parameters. It was established that the groundwater was of relatively high quality, although there was an emerging trend of declining quality regarding some parameters.

Fearing that these substances had entered the groundwater, monitoring started in 1995, i.e. the first monitoring of As, Pb and $CHCl_3$ levels in drinking water.

The safety of drinking water is defined by a regulation (Official Gazette of the Republic of Slovenia, 46/97). This statutory act lays down the levels of certain parameters, permitting Pb levels of 10µg/l, As levels of 10 µg/l, and Cd levels of 3µg/l. It does not contain a specifically determined level for $CHCl_3$; it does, however, contain determined levels for CCl_4 (2 µg/l) and C_2HCl_3 (10µg/l).

The results of the monitoring of drinking water for levels of lead, arsenic, cadmium and chloroform are shown for all regions from 1995 to 2000 in Figures 2 to 6 and Tables 1 to 5.

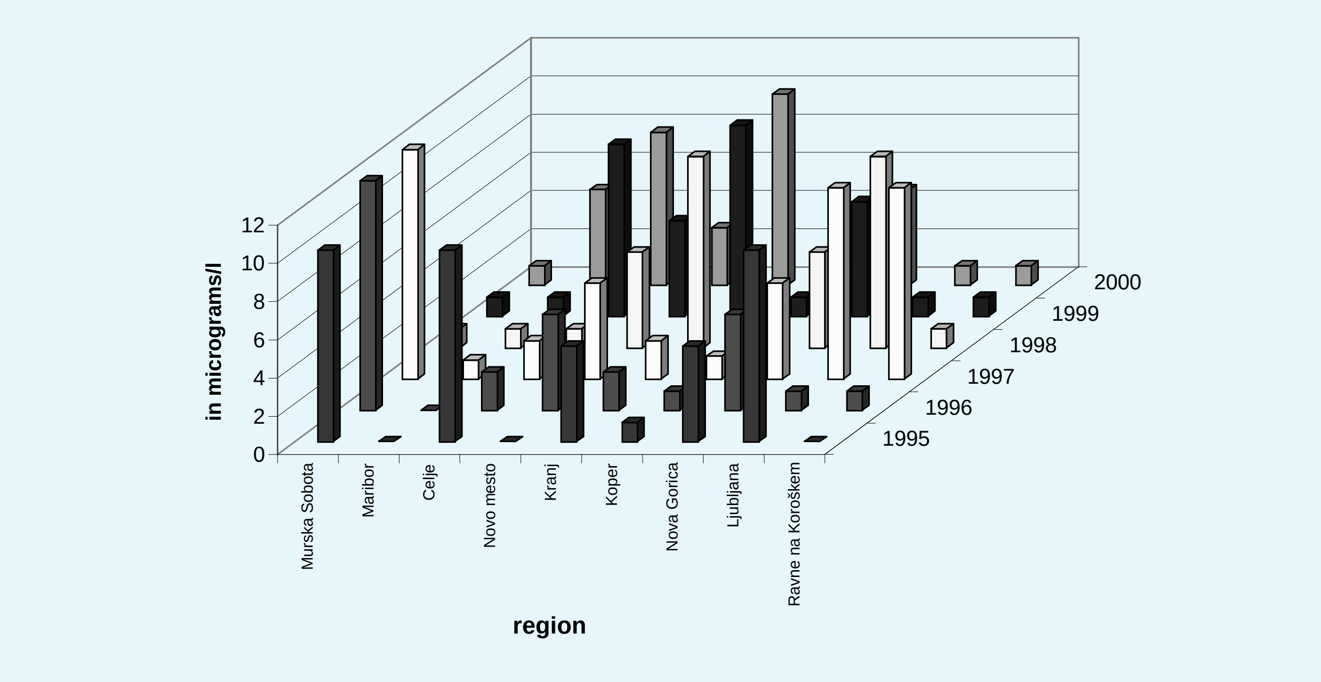

Figure 2. Quantity of Pb in drinking water in all regions from 1995 to 2000

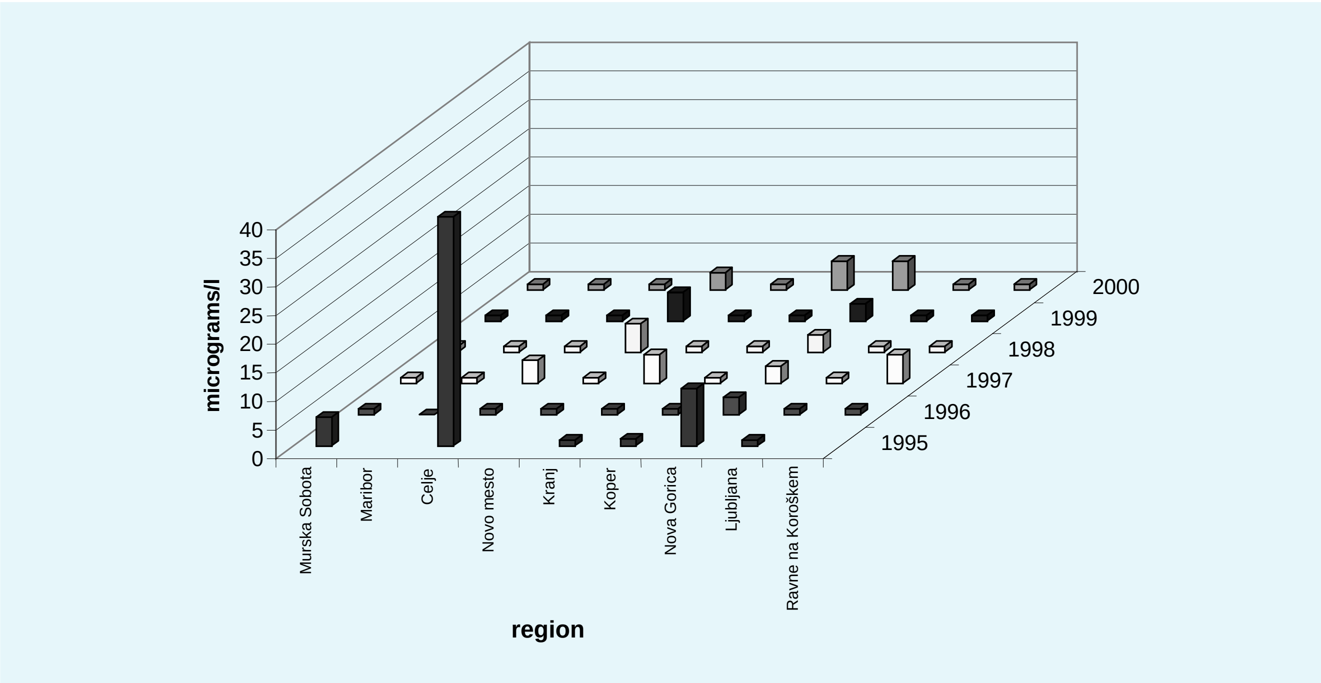

Figure 3. Quantity of As in drinking water in all regions from 1995 to 2000

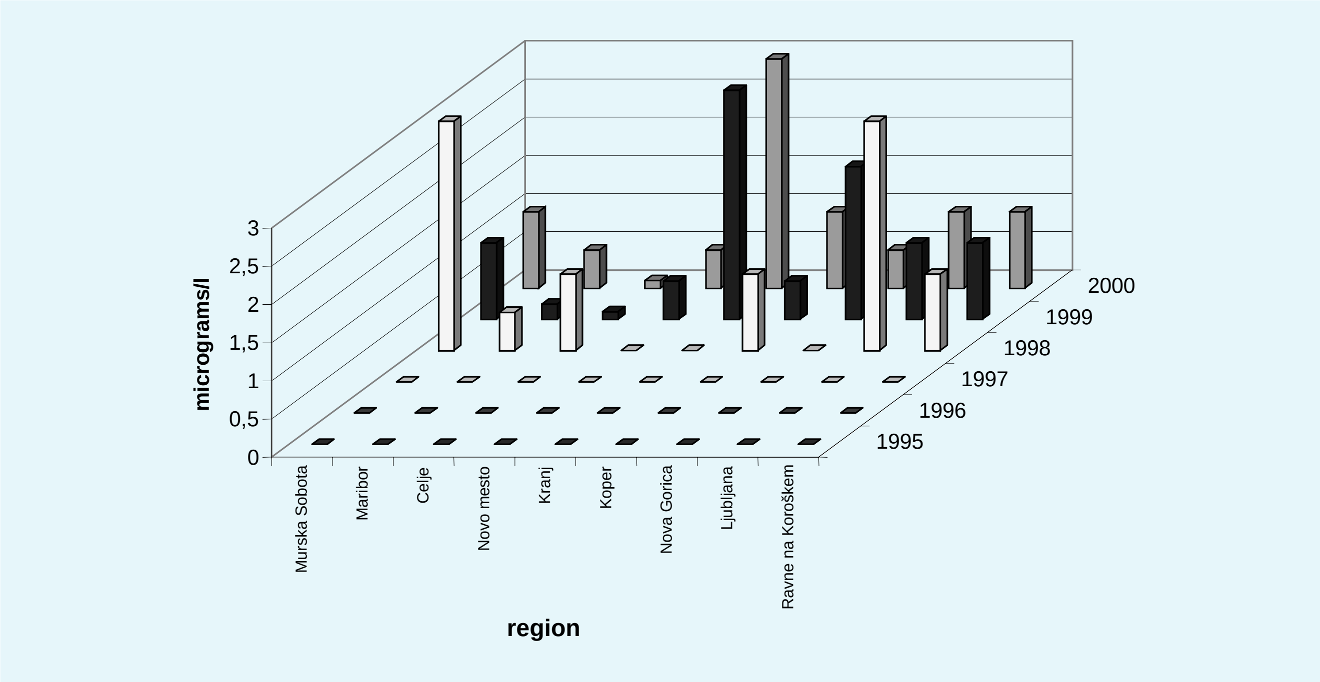

Figure 4. Quantity of Cd in drinking water in all regions from 1995 to 2000

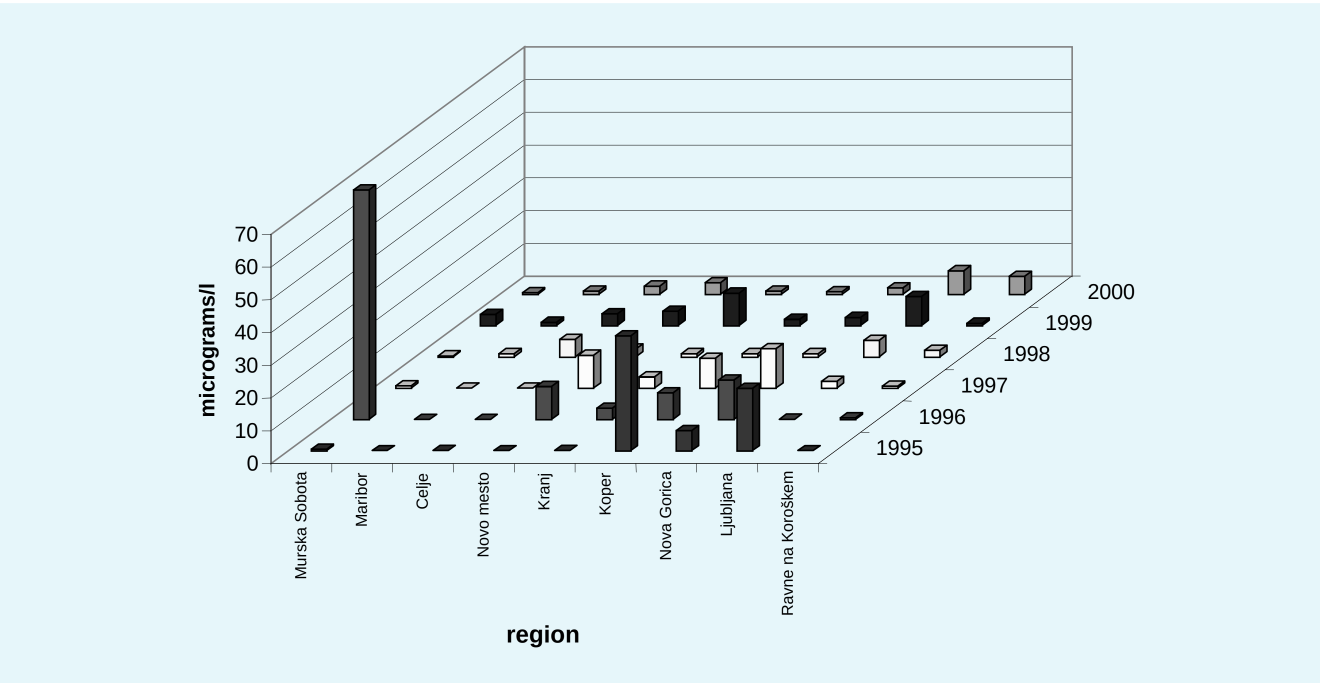

Figure 5. Quantity of $CHCl_3$ in drinking water in all regions from 1995 to 2000

Table 1. Pb (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	10	12	12	1	1	1
Maribor	0	0	1	1	1	5
Celje	10	2	2	1	9	8
Novo mesto	0	5	5	5	5	3
Kranj	5	2	2	10	10	10
Koper	1	1	1.2	2	1	1
Nova Gorica	5	5	5	5	6	5
Ljubljana	10	1	10	10	1	1
Ravne na Koroškem	0	1	10	1	1	1

Table 2. As (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	5	1	1	1	1	1
Maribor		0	1	1	1	1
Celje	40	1	4	1	1	1
Novo mesto		1	1	5	5	3
Kranj	1	1	5	1	1	1
Koper	1.2	1	1	1	1	5
Nova Gorica	10	3	3	3	3	5
Ljubljana	1	1	1	1	1	1
Ravne na Koroškem		1	5	1	1	1

Table 3. Cd (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	0	0	0	3	1	1
Maribor	0	0	0	0.5	0.2	0.5
Celje	0	0	0	1	0.1	0.1
Novo mesto	0	0	0	0	0.5	0.5
Kranj	0	0	0	0	3	3
Koper	0	0	0	1	0.5	1
Nova Gorica	0	0	0	0	2	0.5
Ljubljana	0	0	0	3	1	1
Ravne na Koroškem	0	0	0	1	1	1

Table 4. $CHCl_3$ (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	0.4	70	0.7	0.3	3.4	0.5
Maribor	0	0	0	1	1	1
Celje	0.01	0	0	5.3	3.6	2.5
Novo mesto	0	10	10	2.4	4.4	3.5
Kranj	0.01	3.4	3.4	1	9.9	1
Koper	35	8.1	9.1	1	1.9	0.8
Nova Gorica	6.1	12	12	1	2.5	2
Ljubljana	19	0.01	2.1	5	8.9	7.2
Ravne na Koroškem	0	0.5	0.6	2	0.7	5.5

As demonstrated by the Figures and Tables, Pb levels exceeded legal limits only in two cases (in the north-eastern region in 1996 and 1997), while the limit was reached nine times in the larger cities. This is the consequence of the chlorination of drinking water before its distribution through public water supply systems. Water supply systems in almost all cities consist of lead pipes.

Levels of As exceeded the limits only once: in the industrial town of Celje in 1995. All other values are below the legal limits.

Cd levels were generally not exceeded, and the limits were reached only in three cases. Industry in the town of Kranj, where the limit was reached twice, is well developed.

Chloroform levels increased in certain cases (Koper 1995, Ljubljana 1995, Murska Sobota 1996, Novo Mesto 1996 and 1997), which may be ascribed to the chlorination of drinking water before its distribution, since it is well known that when chlorinating agents are used for disinfection, trichalomethanes are formed.

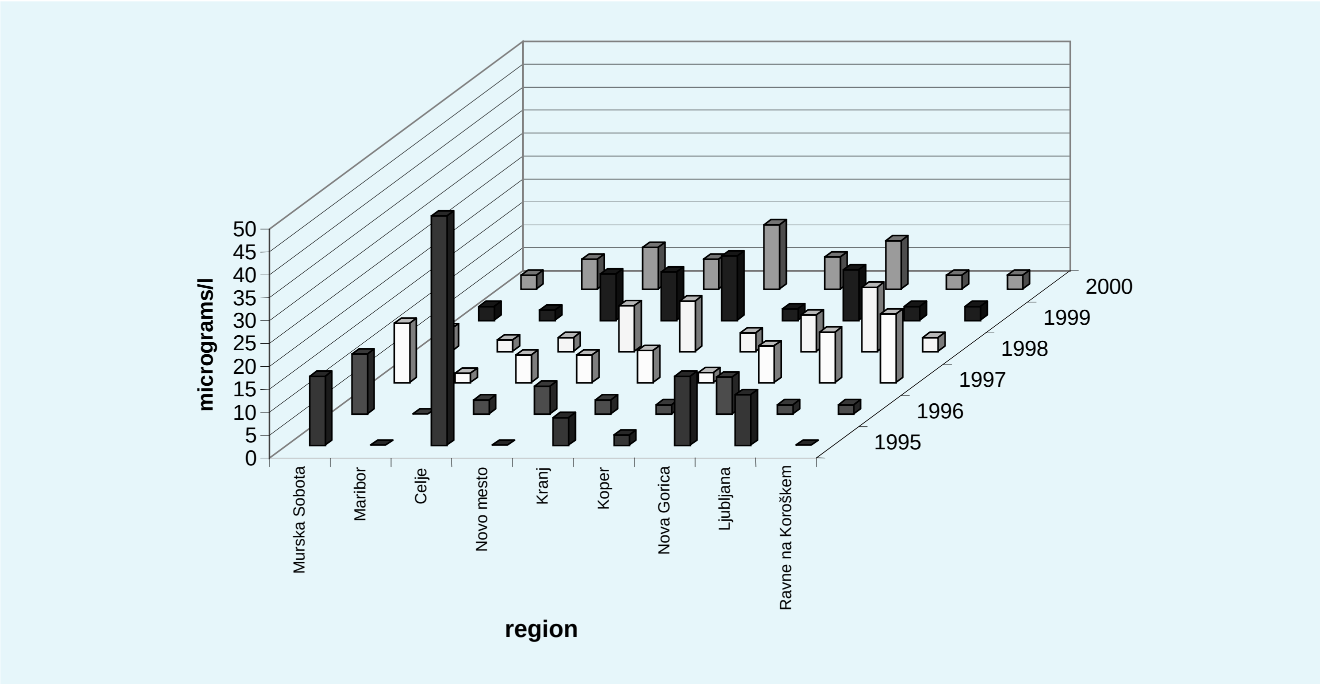

Figure 6. Amount of Pb+As+Cd in drinking water in all regions from 1995 to 2000

Table 5. amount of Pb+As+Cd (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	15	13	13	5	3	3
Maribor	0	0	2	2.5	2.2	6.5
Celje	50	3	6	3	10.1	9.1
Novo mesto	0	6	6	10	10.5	6.5
Kranj	6	3	7	11	14	14
Koper	2.2	2	2.2	4	2.5	7
Nova Gorica	15	8	8	8	11	10.5
Ljubljana	11	2	11	14	3	3
Ravne na Koroškem	0	2	15	3	3	3

Two years later (1997) the monitoring of drinking water was expanded by the analysis of two plant protection agents, atrazine and alachlor, and the two main metabolites of the former, desetil- and desisopropil-atrazine. Samples were taken from end-users of drinking water three times a year from the same end location in the nine regions into which Slovenia is divided for healthcare purposes. Each region has a health centre. The national centre is located in the capital, and the research work carried out locally by individual regional health centres is run from this institution.

In Slovenia the atrazine - 2-chloro-4-ethylamino-6-isopropilamino-1,3,5-triazine compound is used for spraying surfaces sown with corn. According to available data only 130 kg of atrazine and 8,355 kg of triazine herbicides were imported in 1997, and 71,920 kg of atrazine and 3,531 kg of herbicides (3) in 1998. We could not determine the amount of substances imported before 1997.

In Slovenia atrazine use is governed by statutory acts, i.e. the Decree on the Prohibition or Restriction of Trade and Use of Toxic Substances and Products Made Thereof Used as Plant Protection Agents (OGRS, 13/99), prohibiting the use of atrazine and atrazine agents in water-protection areas.

The quantity of atrazine in the soil is determined by the Decree on the Limit, Warning and Critical Values of Toxic Substances in Soil (OGRS, 68/96). The limit value for atrazine is 0.01 mg/kg, the warning value 3 mg/kg and the critical value 6 mg/kg.

The Rules on the Health Compliance of Drinking Water (OGRS, 46/97) determine the limit values for individual plant protection agents in drinking water, i.e. 0.1 μm/l and 0.5 μm/l for the sum of these agents.

The results of the monitoring of drinking water for the presence of atrazine, its metabolites and alachlor are shown for all regions from 1995 to 2000 in Figures 7 to 10 and Tables 6 to 9.

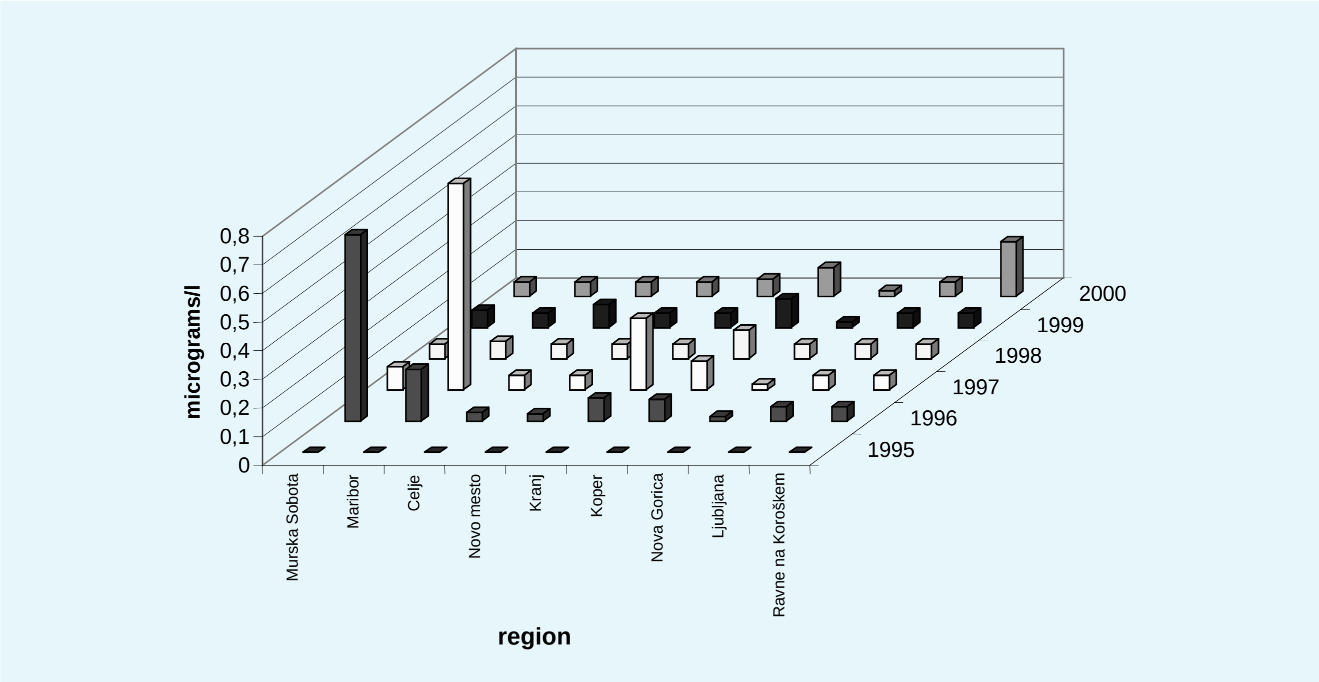

Figure 7. **Quantity of atrazine in drinking water in all regions from 1995 to 2000**

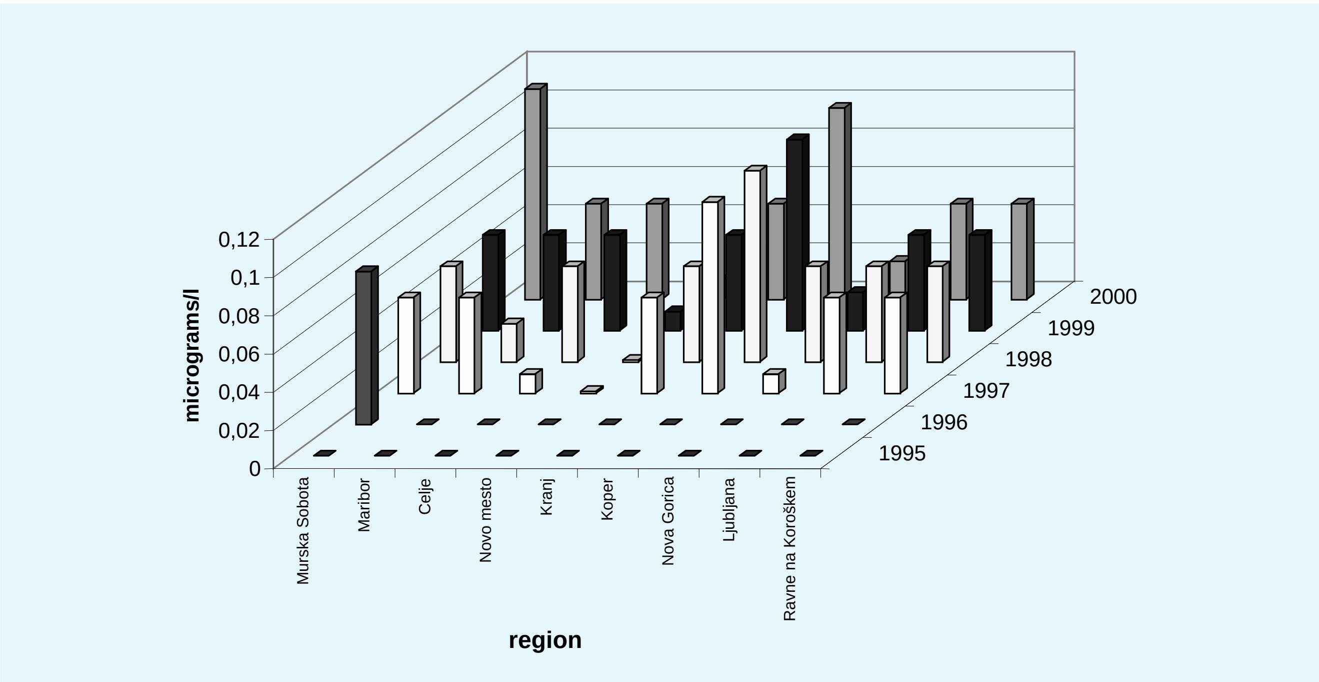

Figure 8. **Quantity of alachlor in drinking water in all regions from 1995 to 2000**

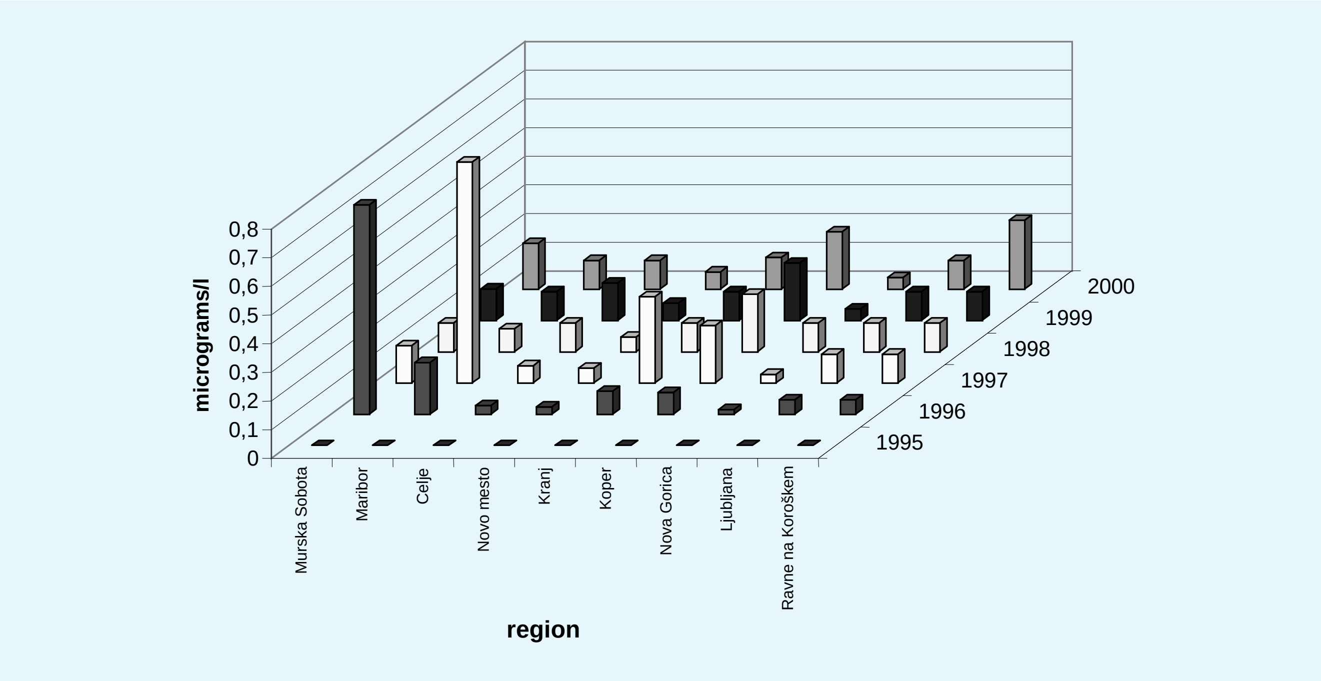

Figure 9. Amount of atrazine + alachlor in drinking water in all regions from 1995 to 2000

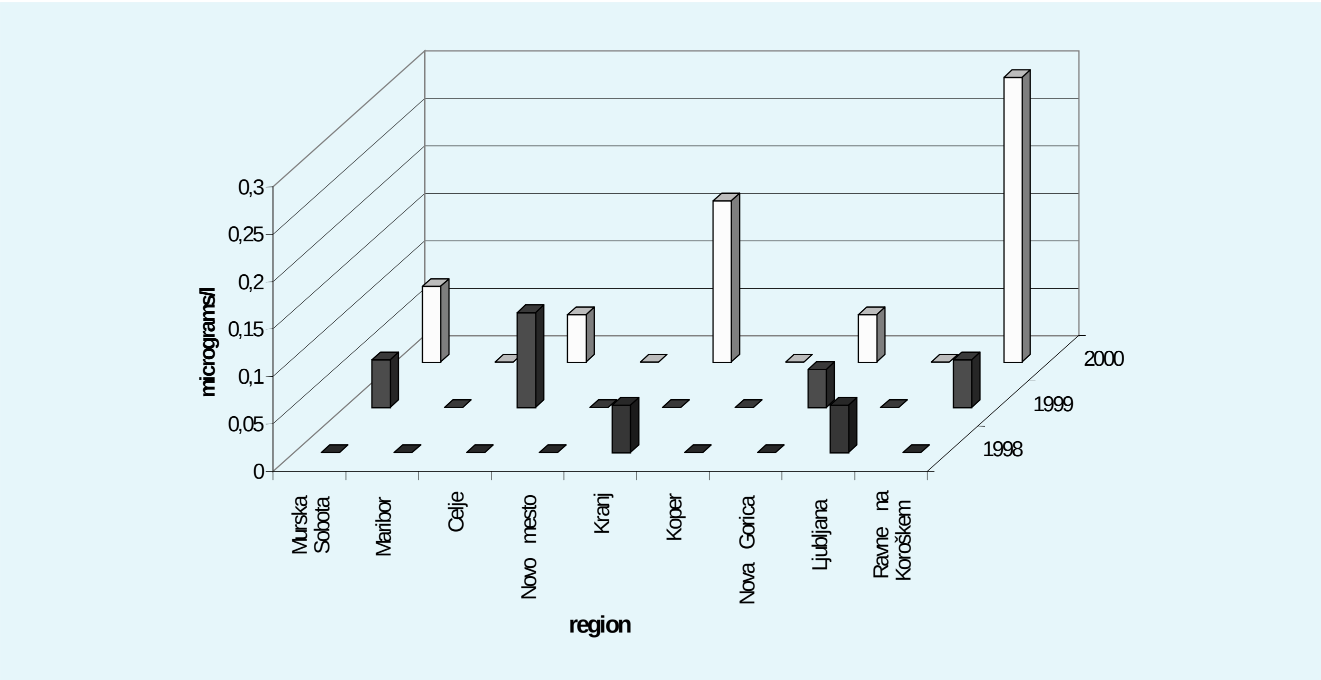

Figure 10. Quantity of desetilatrazine in drinking water in all regions from 1998 to 2000

Table 6. Atrazine (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	0	0.65	0.08	0.05	0.06	0.05
Maribor	0	0.18	072	0.06	0.05	0.05
Celje	0	0.03	0.05	0.05	0.08	0.05
Novo mesto	0	0.025	0.05	0.05	0.05	0.05
Kranj	0	0.08	0.25	0.05	0.05	0.06
Koper	0	0.075	0.1	0.1	0.1	0.1
Nova Gorica	0	0.016	0.02	0.05	0.02	0.02
Ljubljana	0	0.05	0.05	0.05	0.05	0.05
Ravne na Koroškem	0	0.05	0.05	0.05	0.05	0.19

Table 7. Alachlor (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	0	0.08	0.05	0.05	0.05	0.11
Maribor	0	0	0.05	0.02	0.05	0.05
Celje	0	0	0.01	0.05	0.05	0.05
Novo mesto	0	0	0.001	0.001	0.01	0.01
Kranj	0	0	0.05	0.05	0.05	0.05
Koper	0	0	0.1	0.1	0.1	0.1
Nova Gorica	0	0	0.01	0.05	0.02	0.02
Ljubljana	0	0	0.05	0.05	0.05	0.05
Ravne na Koroškem	0	0	0.05	0.05	0.05	0.05

Table 8. Atrazine + Alachlor (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	0	0.73	0.13	0.1	0.11	0.16
Maribor	0	0.18	0.77	0.08	0.1	0.1
Celje	0	0.03	0.06	0.1	0.13	0.1
Novo mesto	0	0.025	0.051	0.051	0.06	0.06
Kranj	0	0.08	0.3	0.1	0.1	0.11
Koper	0	0.075	0.2	0.2	0.2	0.2
Nova Gorica	0	0.016	0.03	0.1	0.04	0.04
Ljubljana	0	0.05	0.1	0.1	0.1	0.1
Ravne na Koroškem	0	0.05	0.1	0.1	0.1	0.24

Table 9. Desetilatrazine (mg/l)

Regions	1995	1996	1997	1998	1999	2000
Murska Sobota	0	0	0	0	0.5	0.08
Maribor	0	0	0	0	0	0
Celje	0	0	0	0	0.1	0.05
Novo mesto	0	0	0	0	0	0
Kranj	0	0	0	0.05	0	0.17
Koper	0	0	0	0	0	0
Nova Gorica	0	0	0	0	0.04	0.05
Ljubljana	0	0	0	0.05	0	0
Ravne na Koroškem	0	0	0	0	0.05	0.3

Of plant protection agents, atrazine and its metabolites desetil- and desisopropil-atrazine are the main polluters of groundwater and drinking water. The purpose of this article is to verify the possibilities for biological degradation of the substances in question and, consequently, the recovery of groundwater or drinking water. In so doing it is important that micro-organisms that are not harmful to people, or better, micro-organisms that have positive health effects on people, are used. For this purpose we selected a culture of lactic-acid bacteria, which has verified positive effects on the human body.

At the 5th International HCH and Pesticides Forum in Bilbao (2) we reported on the preliminary results of the biodegradation of atrazine using a lactic-acid culture developed by Kanne Brottrunk GmbH & Co., Selm-Bork, Germany. The culture was prepared for the lactic-acid fermentation of cereal, and contains lactic-acid bacteria, amino acids, minerals and vitamins.

The product is used in human food as a prebiotic.

An analysis of the product is given in Table 10. One hundred ml of the Kanne bread beverage contains on average:

Table 10.

PH value	2.90
Proteins	1.4 g
Fats	0.07 g
Carbohydrates	0.16 g
Lactic acid (D/L)	1.0 g
Minerals	200 g
Sodium	26.0 g
Potassium	24.5 g
Calcium	10.5 g
Magnesium	5.0 g
Iron	0.44 g
Manganese	0.09 g
Zinc	0.26 g
Copper	0.003 g
Chloride	70 g
Phosphorus	7.8 g

Vitamins:	
B1	10 g
Niacin	90 g
E	370 g
Pantothenic acid	10 g
B2	20 g
B6	40 g
B12	3 g

Amino acids	
Alanine	33 mg
Arginine	33 mg
Asparagine acid	40 mg
Glycine	42 mg
Glutamic acid	14 mg
Leucine	30 mg
Serine	39 mg
Threonine	30 mg
Tyrosine	20 mg
Valine	20 mg
Lysine	36 mg

The above-mentioned biodegradation experiments were carried out with relatively high atrazine concentrations, i.e. between 7.7 and 20 mg of atrazine per l of water solution, making the analytical monitoring of biodegradation easier.

We monitored changes to atrazine concentrations over time. In some samples we also determined the concentration of both metabolites.

Concentrations of atrazine and its two metabolites, desetil- and desisopropil-atrazine, in drinking water are in the microgram range; we therefore reduced the initial atrazine concentrations by a factor of 10^3 in later experiments. The initial concentrations were 5 and 10 µg atrazine/l of water. The same quantity of lactic-acid culture, i.e. 1 ml Kanne/1 l of water solution of atrazine, was always added to the solution of atrazine in water. The experiments were carried out in aerobic and non-sterile conditions. The concentrations of atrazine in samples were determined in intervals of between one and 14 days. In final samples (after 14 days) the concentration of both metabolites, desetil- and desisopropil-atrazine, was also determined. The results are assembled in Table 11.

Table 11.

Interval(days)	Atrazine concentration µg/l (initial concentration 10.5 µg of atrazine per l of water + 1 ml Kanne/l water solution of atrazine)	Atrazine concentration µg/l (initial concentration 5 µg of atrazine per l of water + 1 ml Kanne/l water solution of atrazine)
0	10.5	4.8
1	7.8	3.1
2	5.0	3.1
5	4.6	3.0
8	4.3	2.2
14	3.9	2.4

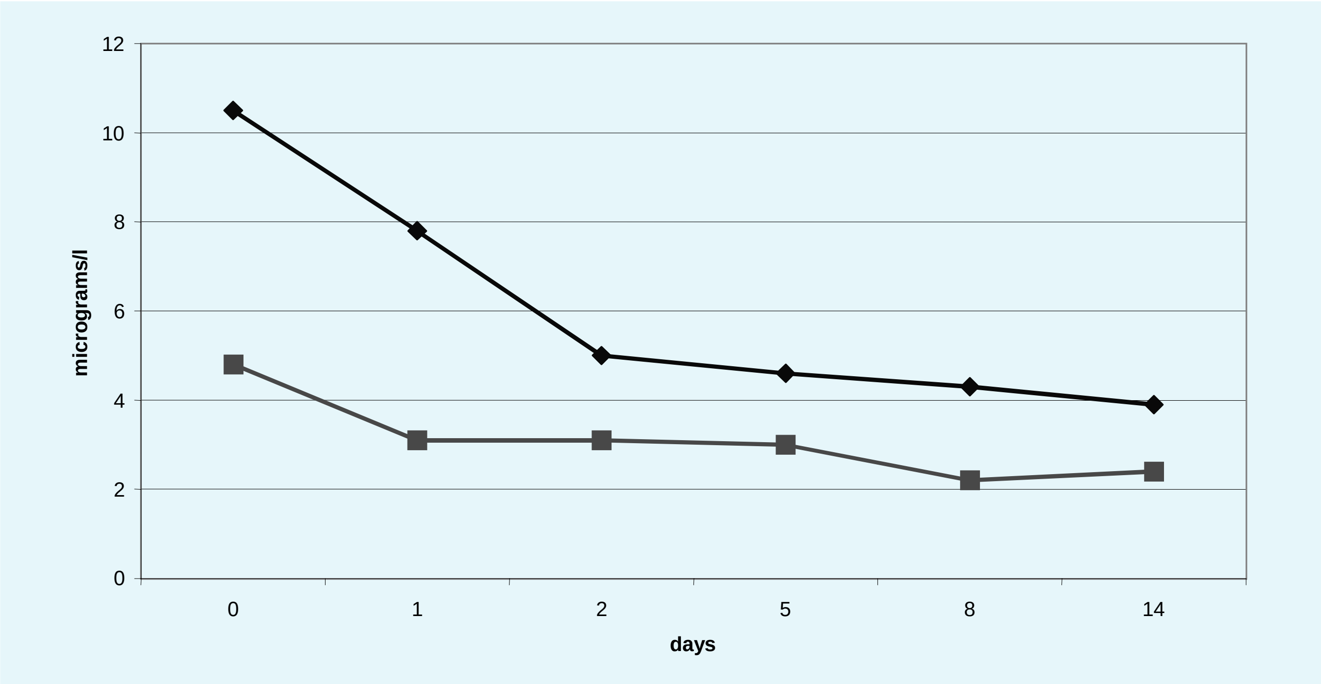

Figure 11.

Table 11 and Figure 11 demonstrate that in 14 days the concentration of atrazine in the abovementioned experimental conditions decreased considerably. The more intense decomposition of atrazine was observed with higher initial concentrations. In this case (initial concentration approx. 10.5 μg/l) biodegradation amounted to approximately 60 per cent; with a lower initial concentration (approx. 5 μg/l) it amounted to approximately 50 per cent. With higher concentrations (several mg) mentioned in the report from Bilbao (2), degradation was 79 per cent.

The decomposition time of atrazine (DT_{50}) is similar under aerobic and anaerobic conditions: 146 days.

In water, with natural light (pH 7), DT_{50} is 335 days; with mercury light (pH 7) it is 12.5 days (4).

Conclusion

The monitoring of drinking water for levels of the substances in question has confirmed the fact that water sources may be polluted for a variety of reasons. Although the quantities of individual substances generally do not exceed legally determined limits, it can be assumed that drinking water containing the measured quantities over many years could be hazardous to health because of the accumulation of heavy metals. Monitoring will be continued to at least the same parameter scope, or else the selection of parameters may even be expanded.

The biodegradation of atrazine in water is extremely accelerated in the presence of the KANNE lactic-acid culture. This fact is of great potential importance for the preventive protection of groundwater, which is the main source of drinking water. The substantial surface pollution of cultivated land with atrazine (and other pesticides) could be speedily reversed by the input (spraying) of the KANNE lactic-acid culture into the soil. The recovery of drinking water by adding this culture to drinking water tanks would be even simpler, but the regulations do not permit it. Consumption of the KANNE lactic-acid culture intended for human use is especially suitable if toxic substances enter the body via drinking water or food. Lactic-acid bacteria accelerate the decomposition of these substances.

Sources

1. Statistical Yearbook of the Republic of Slovenia, No. 37, Ljubljana 1998
2. B. Druzina, P. Bukovec, 5th International HCH and Pesticide Forum, Bilbao, Basque Country, 25-27 June 1998, IHOBE Forum Book, p. 265
3. Report on Atrazine Risk Assessment in the Republic of Slovenia, Ministry of Health, Chemicals Bureau, Ljubljana, February 2000
4. Fate and Distribution of Atrazine in the Environment, Part 1: Exposure Characteristics, Maribor Health Protection Institute and the Public Health Institute of the Republic of Slovenia, Maribor, December 1999