

Detection of Atrazine and Simazine in Ground Water of Delhi using High Performance Liquid Chromatography with Ultraviolet Detector

MOHD ASLAM^{1,2}, MASOOD ALAM² and SUMBUL RAIS^{2*}

¹Center of Excellence in Environmental Studies, King Abdulaziz University, Jeddah-21589, Saudi Arabia

²Department of Applied Sciences and Humanities, Faculty of Engineering and Technology, Jamia Millia Islamia, New Delhi - 110 025, India.

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ABSTRACT

Herbicide usage has increased dramatically during the last two decades coinciding with the change in farming practices and increasingly intensive agriculture. This study thus assesses herbicide occurrence in the ground water of Delhi i.e. Atrazine and Simazine herbicide. Liquid-liquid extraction with dichloromethane and methanol as extracting solvents were used. These extracted herbicides were separated and quantified by High Performance Liquid Chromatography (HPLC) with ultraviolet detector. The excellent results were achieved with spiked recoveries of 96.8% and 84.6 % for atrazine and simazine respectively. Analysis shows that the concentration of simazine was higher as compared to atrazine. The results obtained were compared with WHO limit of 0.002 mg/l and USEPA limit of 0.003 mg/l for Atrazine and 0.004 mg/l for Simazine. Highest concentration of atrazine was recorded in the north region of Delhi. Sample from the central Delhi did not reveal contamination from any of the herbicides being monitored. The results indicate that there is need for further work to identify sources and fate of herbicide contaminations. The findings of our investigation contribute to the knowledge of the extent of pollution in the groundwater of Delhi.

Key words: Herbicides, Simazine, Atrazine, Groundwater, HPLC

INTRODUCTION

Herbicides belong to the class of pesticides that are used to control undesirable or noxious plant growth, generally weeds, in the crop production. These are also used in non-crop areas, where it is necessary to limit the plant growth¹. They are, therefore, also called weed killers. Herbicide usage has increased dramatically during the last two decades coinciding with the change in farming practices and increasingly intensive agriculture². Herbicides show a wide range of beneficial effects such as improving the plant health, maintaining agro-ecosystems, food supply and other economical advantages³.

After application of herbicide on target weeds, the active ingredient is gradually lost as a result of breakdown, evaporation and leaching. The herbicide residue is the amount that remains on

the field after application and usage⁴. As some herbicides have long residual activity and they persist in the environment for long time. Others have low residual activity and disappear from the environment and produce low residual concentrations⁵. The identification and quantification of environmental pollutants are important in determining the extent of exposure to these compounds and in evaluating the hazard to humans and wildlife. Environmental pollution by pesticide residues is a major environmental concern due to their extensive use in agriculture and public health programs⁶. The extensive use of herbicides in agriculture and the high persistence of many of them have required rigorous control of environmental contamination, especially of ground water and drinking water sources⁷. Pesticide residues above the tolerance limits (MRL) are a cause of great concern globally as well as nationally. The intensive application of herbicides has resulted

in the contamination of the atmosphere, ground and waste waters, agricultural products such as wheat, corn, fruits, vegetables, etc. and biological systems⁸.

Pesticides monitoring of water is also possible with rapid and simple methods that use less sophisticated instruments which still provide reliable identification of analytes⁹. Thermo labile or non-volatile herbicides can be determined only by liquid chromatographic methods such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Reversed-phase HPLC is widely used in analyses of pesticides with high polarity, low volatility and thermal instability. Because of its higher sensitivity HPLC has been widely applied in the measurement of herbicide residues.

Where an MS detector is not available an ultraviolet (UV) or photodiode array detector (PDA) is frequently employed^{10, 11}. Liquid-liquid extraction is frequently used for the isolation of pesticides from water samples and dichloromethane is the most common solvent because it is capable of extracting compounds having a wide range of polarities while its volatility makes sample concentration easy¹².

The main chemical classes of herbicides include, triazine derivatives containing three heterocyclic nitrogen atoms in the ring structure (atrazine, simazine, etc. as shown in Fig. 1)

Atrazine (6-chloro-*N*²-ethyl-*N*⁴-isopropyl-1,3,5-triazine-2,4-diamine) is one of the most used pesticides worldwide^{13, 14} for pre and post emergence weed control amongst crops of corn, wheat, barley and sorghum. A large amount of applied atrazine and its degradation products remain in the soil even after 16 months, which suggest its potential to contaminate groundwater¹⁵. It can also be said that because of their relatively high water solubility symmetric triazine herbicides are agrochemical agents with a potentially high risk of leaching into surface and ground waters^{16, 17}. The Half- life of Atrazine was determined to be approximately 223 days¹⁸. According to USEPA the Potential Health Effects from long-term exposure above the MCL (unless specified as short-term) by atrazine is on Cardiovascular system and is also responsible for reproductive problems.

Simazine (6-chloro-*N,N*¹-diethyl-1-(1,3,5)triazine-2,4-diamine) is one of the most popular photosynthesis-inhibiting herbicides. It is used in many countries to kill broad-leaved weeds and also to control vegetation and algae in farm ponds, fish hatcheries, swimming pools, fountains, ornamental fish ponds & water-recirculating cooling towers. Although EU directives have banned the use of simazine on non-cropped land, its use is still permitted on cropped land and in ornamental water (ponds, aquariums)¹⁹. It affects the blood on Long-Term Exposure²⁰.

MATERIALS AND METHODS

Groundwater samples were collected from Delhi. The extraction procedure was undertaken within 72 h. The organic solvents, acetonitrile, methanol, methylenechloride and water, used were HPLC grade and were purchased from E. Merck. Pesticide standards were obtained from Accustandards with a purity of 95-99%. All solvents were filtered through Millipore membrane filters (polysulfone membrane with 0.45 µm pore size) before using as mobile phase. The samples were filtered using Millipore syringe filters (polysulfone membrane with 0.45 µm pore size) before injecting in the column.

Anhydrous sodium sulfate for the residue analysis, 60-100 mesh was maintained at 300°C overnight and stored in desiccators till used. Individual standard stock solutions of Atrazine and Simazine containing 1mg/ml in methanol were prepared and stored at 4°C. After the extraction and cleanup procedure, samples were stored in glass container at the recommended temperature.

Herbicides were extracted thrice from the groundwater sample (1000 ml) using liquid-liquid partitioning into dichloromethane (50 ml). After extraction, the supernatant was evaporated to dryness and redissolved in 5 ml of Methanol.

All samples were cleaned up by passing through anhydrous sodium sulfate (pretreated at 300°C) and eluted with 50 ml methanol. The organic phase thus obtained was evaporated to dryness in a rotary vacuum evaporator completely at a temperature of 40-45°C and then dissolved in methanol for HPLC analysis.

Perkin Elmer High Performance Liquid Chromatography (isocratic) having Ultraviolet detector, integrated with Totalchrom software. Stainless steel analytical column Spheri-10 Reverse Phase C18 of 250x4.6 mm i.d., particle size-10µm was used. The samples were injected manually through a Rheodyne injector. Working conditions for HPLC were as follows: The Eluent solvent was acetonitrile/water (60:40, v/v) at Flow rate 1ml min⁻¹, and the injection volume and detection wavelength were 20µl and 235 nm, respectively.

The identification of target pesticides were accomplished on the basis of the retention times of the analytes.

RESULTS AND DISCUSSION

Contamination of surface, ground and drinking water by the studied herbicides has been reported in many countries^{1, 20, 21, 22, 23}. The compounds are separated with good resolution and sharp peak by gradient HPLC using a simple mobile phase containing acetonitrile:water (60:40, v/v). The extraction procedure employed resulted recoveries of 96.8% and 84.6 % for atrazine and simazine respectively. Several mobile phase flow rates (0.5 – 2 ml/min) were evaluated. The best separation was achieved using a flow rate of 1.0 ml/min. Fig. 2a & 2b shows the chromatogram referring to HPLC analysis of atrazine and simazine herbicides respectively. Fig. 3 is the chromatogram obtained by analyzing analytical standard where as spiked water chromatogram is shown in Fig. 4. Fig. 5

represents the chromatograms for the water samples from the region of interest (Delhi). Analysis of the results on table 2 shows that the concentration of simazine was higher as compared to atrazine. Maximum Contaminant Level Goal (MCLG), the level of a contaminant in drinking water below which there is no known or expected risk to health, allow for a margin of safety and are non-enforceable public health goals. Maximum Contaminant Level (MCL) is however the highest level of a contaminant that is allowed in drinking water. The MCLG and MCL values for Atrazine and Simazine are 0.003 and 0.004 mg/l respectively²⁴. In the present study, highest concentration of atrazine was recorded in the north region of Delhi. Sample from the central Delhi did not reveal contamination from any of the herbicides being monitored. The atrazine was detected in 45% water samples analysed where as 35 % samples exceeded the WHO and USEPA limit. Simazine was detected in 85% analysed samples with 50% samples exceeding the limit according to USEPA and 55% exceeding the WHO limit (Table 2). The total mean concentrations of atrazine and simazine ranged from 0.00072 to 0.0173 mg/l and 0.00091 to 0.04097 mg/l respectively.

The findings of our investigation contribute to the knowledge of the extent of pollution in the groundwater of Delhi. The total mean concentration of atrazine ranged from 0.00072 to 0.0173 mg/l where as 0.00091 to 0.04097 mg/l are recorded as the mean concentration of simazine. It is important to monitor the pesticides and see whether they have caused contamination in the hydrological system

Table 1:Physicochemical properties of atrazine and simazine^a

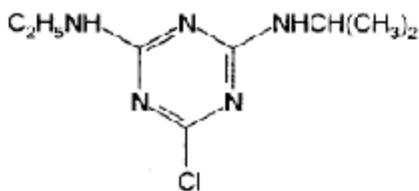
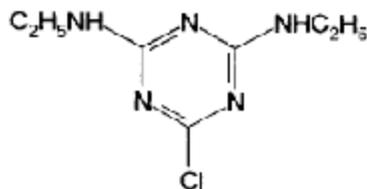
Property	Atrazine Value	Simazine Value
Mol. Wt.	215.7	201.72
Melting point	175–177 °C	25–227 °C
Density	1.187 g/cm ³ at 20 °C	1.302 g/cm ³ at 20 °C
Water solubility	33 mg/l at 20 °C	6.2 mg/l at 20 °C
pKa (Acid dissociation constant)	1.70 (base)	1.62 (base)
Log K_{ow} (Log octanol–water partition coefficient)	2.5	2.1
Vapour pressure	40 × 10 ⁶ Pa at 20 °C	8.1 × 10 ⁴ Pa at 20 °C

^a data obtained from Tran *et al.*, 2007

Table 2. Herbicide concentrations in the water samples of Delhi with statistical data

Sample No.	Atrazine (ppm)	Simazine (ppm)
Sample 1	ND	0.004326
Sample 2	ND	0.022492
Sample 3	0.0098	0.000912
Sample 4	0.017316	0.0098
Sample 5	0.00098	0.004008
Sample 6	0.00072	0.00154
Sample 7	0.00715	0.0014
Sample 8	ND	ND
Sample 9	0.010651	0.0106
Sample 10	ND	ND
Sample 11	0.009315	0.006255
Sample 12	ND	ND
Sample 13	0.00462	0.010714
Sample 14	ND	0.022415
Sample 15	ND	0.040979
Sample 16	ND	0.001722
Sample 17	0.00711	0.0017
Sample 18	ND	0.013587
Sample 19	ND	0.015409
Sample 20	ND	0.009714
Average	0.0033831	0.00887865
Maximum	0.017316	0.040979
Minimum	0	0
Std Deviation	0.00508707	0.010350463
Average Deviation	0.00422803	0.007580215
Variance population	2.45844E-05	0.000101775
Median	0	0.0052905
Mode	0	0
USEPA Limit	0.003 ppm	0.004 ppm
WHO Limit	0.002 ppm	0.002 ppm
% of samples exceeding USEPA limit	35	50
% of samples exceeding WHO limit	35	55

ND : Not Detected

**Atrazine****Simazine****Fig. 1: Structure of Atrazine and Simazine**

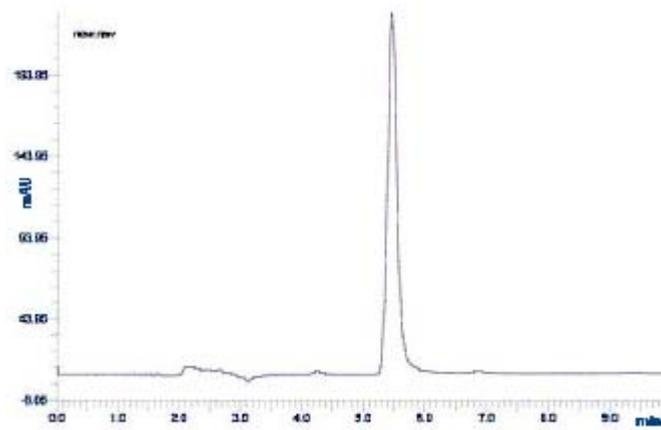


Fig. 2(a): HPLC Chromatogram obtained by analyzing Atrazine Standard.

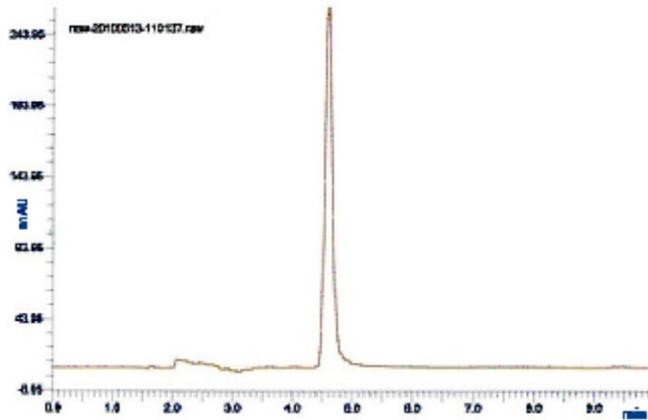


Fig. 2(b): HPLC Chromatogram obtained by analyzing Simazine Standard

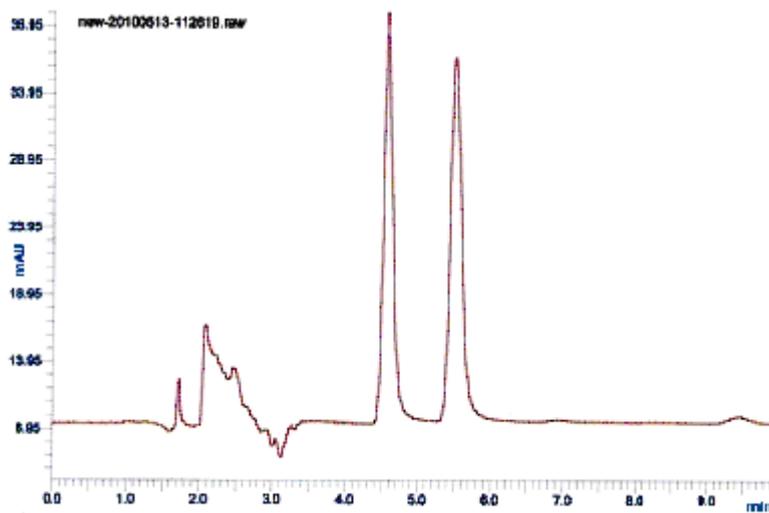


Fig. 3: HPLC Chromatogram obtained by analyzing Standard Simazine & Atrazine

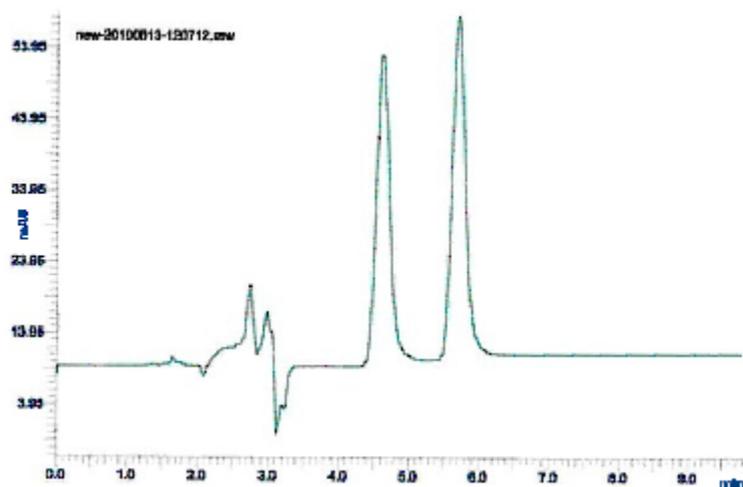


Fig. 4: HPLC Chromatogram obtained by analyzing Spiked water Sample

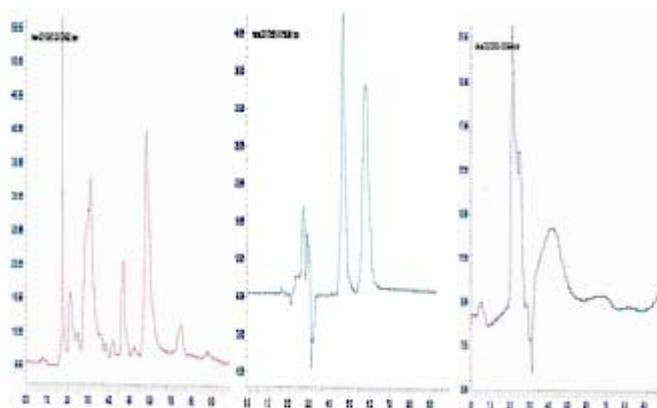


Fig. 5: HPLC Chromatogram a) Showing Atrazine concentration more than Simazine. b) Showing Simazine concentration more than Atrazine. c) Showing no peak in the interested area

this will also help to take necessary actions in minimising the chances of further deterioration and

prevent exposure of large population of Delhi from health hazards due to chemical contamination.

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