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# Determination of Some Pesticides Harmful To Environment and Human Health in Bogazköy (Turkey) Dam Water by LC-MS/MS

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**Abstract:** Pesticides are the most dangerous organic pollutants for human and environmental health. These are substances that are harmful to health, the use of which has increased in recent years, to kill pests in agricultural production. The areas of use of pesticides, apart from agricultural activities, are in the fight against mosquitoes, which cause malaria, which creates a significant health problem for human health in the landscape, construction industry, timber protection, forestry, control of aquatic organisms, industrial insect control, food storage, transportation, and community hygiene there are multiple uses such as. Excessive intake of the body leads to many diseases, cancer formation, even death. In this study, it was aimed to analyze the pesticides Atrazine, Chlorfenvinfos, Chlorpyrifos, Diuron, Isoproturon, and Simazine simultaneously with LC-MS/MS, in Boğazköy Dam in Bursa(Turkey) and the branches that feed the dam in Bursa, İnegöl district. The samples were taken from 12 locations and were given to the device by direct injection. Environmental Quality Standards were taken as reference for the calibration of the method. Validation studies such as LOD, LOQ, linearity, and recovery have been performed for the accuracy of the method. As a result, it has been determined that the pesticide derivatives examined in the waters feeding the Boğazköy Dam and the dam itself do not exceed the Environmental Quality Standards limit values and do not pose a risk in irrigation of agricultural areas and surface waters.

**Keywords:** Pesticides, Water Environment Pollution, Human Health, Agricultural water, LC-MS/MS.

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## INTRODUCTION

Controlling the environment has been the common goal of people for centuries. But day by day, it has reached incredible dimensions in terms of population growth, quality of life, purchasing, unplanned settlement. Accordingly, its impact on the environment has increased over time, putting life in danger(1). Organic pollutants pose a great danger to the environment and health as they remain active in the environment for a long time. For this reason, the determination of these pollutants is necessary for human health and pollution control. Measuring the concentrations of the priority substances mentioned in the above-ground water resources legislation of the Ministry of Forestry and Water Affairs is important for the control and prevention of existing and potential new pollution. Biological or chemical products used to control these pests that carry infectious diseases to foodstuffs, humans, and animals, used to remove or destroy microorganisms that damage the products during consumption, production, and storage of foodstuffs, in addition to ensuring proper growth of plants. It is called "pesticide" (2). In addition, agricultural activities, the use of pesticides in the fight against mosquitoes that cause malaria to create an important health problem for human health (3,4). There are multiple uses such as landscape, construction industry, timber protection,

forestry, aquatic organism control, industrial insect control, food storage, storage, transportation, and community hygiene. The majority of these pesticides that have been used in these areas for many years are dangerous substances in the hazardous class and are widely spread around the environment (5). Pesticides are among the most commonly used chemicals in the world and are also one of the most dangerous for human health. In order to protect human health and the environment, there are limit values that should not be exceeded in the legislation for priority substances and specific pollutants in water, soil, and biota samples. These limit values that should not be exceeded are expressed as an Environmental Quality Standard (6).

Pesticides are classified according to their molecular structure, appearance, and formulations, as well as their classification according to the active ingredient, toxicity effects, and usage patterns. The common classification is the classification made according to the active ingredient and the harmful substance groups in which it is used (7).

**1. Classification by Effective Groups**
a. Insecticides (insecticides)
b. Molluscides (molluscicidal)
c. Herbicides (herbicide)
d. Algicides (algae killer)
e. Rodenticides (rodent killer)
f. Nematocytes (killing roundworms)
g. Acaricides (mite killer)
h. Fungicides (fungicide)
I. Avicides (kills birds)
j. Actresses (tractors)
k. Bactericide (which kills bacteria)

**2. Classification According To The Effective Substance Group In Its Composition**.
This classification is the most scientific one (8).

**A.** Inorganic pesticides
I. Arsenic-containing pesticides
ii. Copper-containing pesticides
iii. Mercury-containing pesticides
iv. Elementary sulfur-containing pesticides
vi. Fluoride-containing pesticides

**B.** Synthetic organic-containing pesticides
I. Organophosphate pesticides
ii. Organochlorines
iii. Organosulfurs
iv. Carbamates

**C.** Natural organic-containing pesticides
I. Allethrin
ii. Pyrethrum
iii. Rotenone
iv. Nicotine

The direct harmful effect of the pesticides on the human body occurs when it enters the metabolism as a result of drinking or eating a pesticide-contaminated food. Pesticides have many negative effects on both human health and the health of other living bodies (9). Different results occur depending on age, race, and gender, as well as diet, economic status, disease status, exposure time, and pesticide concentration. Improper use of drugs in the fight against insects and some carelessly applied pesticides have caused and brought down the number of bird species that can be fed with seeds, predatory bird species, and the number of insect-eating birds. Although songbirds, heron species, and a strong structure, eagles are one of the bird species affected by pesticides (5).

The first used pesticides were sulfur and arsenic. Nicotine was the first plant-derived pesticide used. Pyrethrin, which is naturally found in chrysanthemum flowers and considered as an organic insecticide, has been used since the 19th century. Until the 1860s, copper-containing arsenic compounds called Paris green were used for potato beetle, which was common in the state of Colorado, USA. By the time, lead and mercury compounds started to be used (1). The use of pesticides against insects has increased since the mid-1940s. The Swiss chemist Paul Mueller described the properties of the pesticide known as DDT dichlorodiphenyltrichloroetamine in 1939 and was introduced to the market. In 1979, DDT was banned due to its accumulation in the living organism and its passage into the food chain. German scientists studied nerve gases during World War II and discovered the insecticide parathion, an organophosphate compound. In 1943, parathion was put on the market as an insecticide. USA and other countries, during World War II, turned to synthetic organic chemicals due to the difficulty of supplying the country with botanical based pesticides (5). The first use of pesticides in Turkey began in 1965. Great progress has been made in harvesting crops due to their use. According to the statistics of the Ministry of Agriculture and Forestry, approximately 30,000 to 35,000 tons of pesticides are used every year in our country (10). The first law on pesticides was enacted in the USA in 1947 and the Environmental Protection Agency (EPA) was established in 1970 (2).

Çağdar (11) aimed to make pesticide analysis in the soil in Amik Plain using QuEcheRs method and GC/MS and LC/MS/MS devices. As a result of the analysis, he found 10 different pesticide derivatives in soil samples. These are imidacloprid, dimethomorph, metolachlor, epoxiconazole, tebuconazole, acetochlor, clothianidin, captan, triflualin, and 4,4-DDT. It drew attention to the significant presence of the 4,4-DDT pesticide, although it was banned in 1985. He emphasized that the results are between the limit values required in the Water Regulation for Human

Consumption and the Regulation on Natural Mineral Water (11). Baloglu et al. developed a method for direct injection, LC-MS/MS device for drinking, using, and determination of pesticides in natural waters. In this method, they determined the recoveries as 84.6% to 109.2% and their precision values as 2.2% to 10.5%. They compared their results with the residual limit values required in the Regulation on Water for Human Consumption and the Regulation on Natural Mineral Waters, and found that the results were among these limit values (12). The levels of some organochlorine pesticides was investigated in the tap water samples taken from Asartepe Dam Lake and its vicinity in Ayas district of Ankara. In the analysis, GC-ECD device was used and liquid-liquid extraction. As a result of the analysis, they determined, α-BHC, β-BHC, γ-BHC, δ-BHC, DDD, DDE, DDT, heptachlor, heptachlorperoxide, aldrin, dieldrin, endosulfan-I, endosulfan-II, endrin, and aldehyde pesticides were determined. It has been determined that the amount of organochlorine pesticides in dam and drinking water exceeds the limits specified in the European Union's directive 76/464 / EEC 2006/11 / EC "Pollution caused by the discharge of hazardous substances to the aquatic environment" and "80/778 / EEC drinking water" directive (5,13).

Kapsi Tsoutsi et al. aimed to measure the residual levels of 3 different pesticides (fungicides, herbicides and insecticides) derivatives in samples taken from 3 different points of the Lourus River in Greece. They performed extraction before they introduced the samples to the device. They used the solid-phase extraction (SPE) method as the extraction method. They made the analysis using GC / MS and LC / MS device. As a result of the analyses, 25 pesticide derivatives were identified. The most common pesticide derivatives are quizalofop-ethyl, pendimethalin and trifluralin pesticides. They stated that the tebufenpyrad pesticide is found everywhere (14). Another revealed methods for measuring and identifying agricultural chemicals in the Jucar River basin of Spain. They took samples from 15 different points of the river. Samples were extracted with SPE cartridge and made the analysis using LC-MS / MS device. 20 of the 50 pesticide derivatives analyzed exceeded the detection limit. The concentrations determined were terbuthylazine-2-hydroxide in chlorfenvinphos: 0.05 ng/L, terbuthylazine diethyl: 13.0 ng/L, diazinone: 0.2 ng/L, and 0.66 ng/L. They found chlorpyrifos in all samples, etion in 87%, chlorfenvinphos and tolklofos-methyl pesticides in 80% (15). It was aimed to analyze the distribution of atrazine and simazine using different organic solvents in the study on residue determinations on foods. Shaking, microwave irradiation, ultrasonic assisted extraction, and soxhlet extraction were employed as extraction methods. The solvents used are acetone, chloroform, n-hexane, methanol, and acetonitrile. Following the extraction process, they aimed to develop a method for the determination of atrazine and simazine derivatives using the HPLC device In this method they developed, LOD and LOQ values for atrazine and simazine, respectively, LOD 0.2 µg/mL – 0.3 µg/mL, LOQ: 0.73 µg/mL – 1.12 µg/mL. In the soil samples examined, they determined the concentration of atrazine and simazine in the range of 3.45–8.60 µg/g, 11.9–13.03 µg/g, respectively (16). Mualefe Torto et al. (17) investigated pesticide derivatives with GC/ECD and GC-TOF/MS methods in water samples taken from Okavango delta in Botswana. As a result of the analyses, they detected 4,4'-DDD and 4,42-DDE pesticide derivatives from hexachlorobenzene, and t-chlorine. They found the pesticide concentration to be 61.4 µg/L, 3.2 µg/L, 2.4 µg/L and 5.3 µg/L, respectively. According to the results of the analysis, they concluded that the pesticide derivatives were far above the European Union Drinking Water Directive Limit (0.1 µg/L). Poolpak et al. analyzed 20 organochlorine pesticide derivatives in the sediment samples using a GC device in Mae Klong Creek in the center of Thailand between 2003-2005. They used solid-liquid extraction as the extraction method. In the sediment samples, the total organochlorine pesticide concentration was 4.12-214.9 µg/g and 3.26-215 µg/g. In the summer season, they detected organochlorinated pesticide residue at high concentrations in both periods. The most common pesticide derivative was heptachlor epoxide. Dieldrin and aldrin, the concentrations of which were found to be 0.001-0.17 µg/g, 0.001-2.38 µg/g, respectively (18). Sun et al.(19) investigated 13 organochlorine pesticide derivatives by taking samples from the sediment and surface waters of the Qiantang Creek in China in all seasons. They used a GC/ECD device after solid phase extraction as the method of analysis. They found the total organochlorinated pesticide concentration to be 7.68-269.4 ng/L in water samples and 23.11-1616.5 ng/L in sediment samples. Among the organochlorine pesticides, lindane and heptachlor were common in water samples, while HCH, DDT, and heptachlor were common in widespread samples. They observed that pollution was common in the summer and autumn seasons. They found that DDT concentration in water samples was 8.11 ng/L and HCH 75.2 ng/L. As a result of their study, they showed that pesticide residues with organochlorine exist in water and sediment. Aybala investigated the responsibilities brought by the relevant legal regulations to the countries, the Environmental Quality Statement examined national and international regulations that may be relevant and made a general evaluation. With the Directive 2013/39 / EU, the annual average (AA-EQS) and maximum (MAX-EQS) values of 45 priority substances, freshwater and saltwater related to these substances have been determined. However, they also developed CCT values in biota for 11 items, which are among 45 priority items. In order

to protect and maintain the environment and human health, the Environmental Quality Standard (CCS), which is named as the concentrations that the pollutant and pollutant groups should not reach in the samples of water, soil, or biota, basically refers to the quality status that should be provided in the receiving environments. In the water regulation, the EQS determines the limit values that should be taken into account when evaluating the water quality monitoring data, it is used in determining the quality to control the pollution in the water resources, and reveals the necessity of the protection and improvement studies needed in order to achieve environmental targets (6). Beale David et al. have presented a simple and relatively inexpensive method for detecting Atrazine, Simazine and Hexazinone pesticides in natural waters. In the method used, samples were injected directly into High Performance Liquid Chromatography (HPLC) device. They obtained the best results for this method in the mobile phase consisting of acetonitrile and water in the ratio of (30:70, (v/v). They were found the results as Atrazine: 5.7 µg/L, Simazine: 4.7 µg/L and Hexazinone: 4.0 µg/L (20). Diaz Llorca-Pórcel et al. have implemented a new method for the determination of pesticides in tap and treated wastewater using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method used has been validated according to ISO / IEC 17025: 1999. The most important feature of this method is detecting pesticides by separating them in a short time with electrospray ionization (ESI) MS-MS. They found the detection limits below 15 and the correlation coefficients of the calibration curves drawn between 30-2000 ng/L concentrations higher than 0.99. They determined LOD values of Atrazine 2 µg /L, Diuron 8 µg/L, Isoproturon 3 µg/L, Simazine 4 µg/L, Alaklor 6 µg/L, Chlorfenvinfos 4 µg/L and Chlorpyrifos 7 µg/L. Accuracy was verified by external evaluation and precision was found always under 20% (21).

Limit values for Environmental Quality Standards have been determined for Atrazine, Chlorfenvinfos, Chlorpyrifos, Diuron, Isoproturon and Simazine, which are included in the Surface Water Quality Regulation published by the Ministry of Forestry and Urbanization in 2016, which are certain pollutants for surface water resources. Information containing priority substances and environmental quality standards limit values for surface water resources is given in Table 1 (22).

**Table 1.** Priority Substances and Environmental Quality Standards Limit Values for Surface Water Resources.

| Pesticide's Name | CAS No | AA-EQS Rivers/Lakes (µg/L) | MAX- EQS Rivers/Lakes (µg/L) | AA-EQS Coastal and Transitional Waters (µg/L) | MAX-EQS Coastal and Transitional Waters (µg/L/) |
|---|---|---|---|---|---|
| Atrazine | 1912-24-9 | 0.6 | 2.0 | 0.6 | 2.0 |
| Chlorfenvinfos | 470-90-6 | 0.1 | 0.3 | 0.1 | 0.0 |
| Chlorpirifos | 2921-88-2 | 0.03 | 0.1 | 0.03 | 0.1 |
| Diuron | 330-54-1 | 0.2 | 1.8 | 0.2 | 1.8 |
| Isoproturon | 34123-59-6 | 0.3 | 1.0 | 0.3 | 1.0 |
| Simazine | 122-34-9 | 1.0 | 4.0 | 1.0 | 4.0 |

AA-EQS: Annual Average-Environmental Quality Standards
MAX-EQS: Maximum-Environmental Quality Standards

With the increasing use of pesticides in the world in recent years, a lot of studies have been started on pesticide analysis. The use of pesticides in agriculture is also of great importance in our country. Boğazköy Dam is a basin of Sakarya Region where this study will be carried out. Boğazköy Dam was built to irrigate agricultural lands and started to hold water since 2010. This dam is located on Göksu Stream, one of the side branches of Sakarya River in Northwest Anatolia. Within the boundaries of the basin, there is İnegöl District and villages connected to Bursa Province. In our study, it was aimed to identify some pesticide derivatives from the priority substances in the Surface Water Quality Regulation by taking samples from different parts of the Boğazköy Dam and the branches that feed the dam in Bursa İnegöl region. In addition, these pesticides will be compared with the Environmental Quality Standard values determined in the legislation. In the method, simple and cheap methods will be tried to measure potentially harmful permanent pesticides in order to reduce time, chemical and consumable expenses in pesticide determinations.

## MATERIAL AND METHOD

### Material

Samples of water were used in the determination of pesticide amounts for the thesis work; Samples were taken from Boğazköy Dam Basin and the feeding branches of the Basin in İnegöl Region, which is given from satellite in Figure 1, and 12 different locations (3 samples from one point). Water samples were taken in 250 mL glass bottles and stored at +4 °C until analysis.

**Figure 1.** Boğazköy Dam Basin, satellite view.
Source: https://images.app.goo.gl/NjQ8ceDuzc9KP3GK8

**Method**

Liquid Chromatography-Tandem Mass Spectrometry LC-MS/MS (Shimadzu (LC/MS-8040)) device was used to determine pesticides in aqueous samples and to determine the quantities. MS/MS device working method is given in Figure 2. The Chromatography Column is Restek (Biphenyl 2.7 µm 100 x 2.1 mm). Depending on the sample to be analyzed, ESI (Electrospray Ionization) or APCI (Atmospheric Pressure Ionization Source) ionization techniques were used. In general, the analysis of polar compounds such as amines, pesticides and proteins uses the ESI technique, and the APCI technique is used for apolar compounds such as steroids.

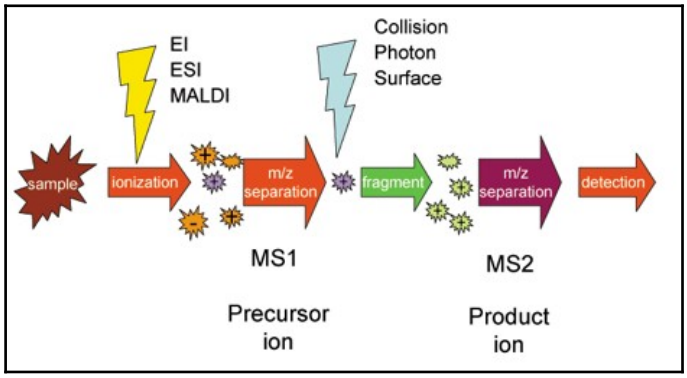


**Figure 2.** MS / MS Sequential Mass Analyzer.
https://images.app.goo.gl/2cQhe6ENzSmMu3W8A

**Chemical and Materials**

The chemicals and consumables used during the analysis are given respectively methanol (99 %w/w) and formic acid(>%98 ) were of high purity and of HPLC grade from Merck. Other pesticide standards; chlorpyrifos was 200 µg/mL n-hexane/acetone (80/20:w/w) from Restek, atrazine (100 µg/mL in methanol), klorfenvinfos (1000 µg/mL n-hexane/acetone (97.5/2.5:w/w), diuron (100 µg/mL in methanol), isoproturon (100 µg/mL in methanol), simazine (100 µg/mL in methanol) and they were Certified Reference Materials from AccuStandard (New Haven, CT 06513 USA). 0.22 µm 13 mm PTFE filter was provided from ChromXpert. The chromatographic conditions determined for the analysis in Table 2 and the other liquid chromatography flow chart is given in Table 3.

**Table 2.** Chromatographic Conditions for LC/MS-MS.

| Parameter | Chromatographic conditions |
|---|---|
| Flow Rate | 0.4 mL/min |
| Injecting Volume | 20.0 µL |
| Column Temperature | 35 °C |
| Analysis Time | 15.0 minutes |
| Mode | Gradient Flow |
| Ion Source | ESI |
| Dry Gas Flow Rate | 15.0 L/min |
| Initial Temperature | 250°C |
| Block Temperature | 400°C |
| Nitrogen Gas Flow Rate | 3 L/min |
| Mobile Phase A | Water 98%+Methanol 2%+Formic Acid 0.1% |
| Mobile Phase B | 100 % Methanol+Formic Acid 0.1% |

**Table 3.** Chromatographic Flow Table for LC-MS/MS.

| t (min) | Flow Rate (mL/min) | Mobile Phase A % | Mobile Phase B % |
|---|---|---|---|
| 1 | 0.4 | 90 | 10 |
| 3 | 0.4 | 45 | 55 |
| 10,5 | 0.4 | 0 | 100 |
| 12 | 0.4 | 0 | 100 |
| 12.01 | 0.4 | 97 | 3 |
| 15 | 0.4 | 100 | 0 |

**MRM optimization**

The optimum values were obtained as a result of the study with 98% water + 2% methanol containing 0.1% formic acid for mobile phase A, and 100% methanol containing 0.1% formic acid for mobile phase B. The Q1 and Q3 ions were determined by solving the pure standards to be analyzed in methanol and using MRM (multiple reaction monitoring) optimizations in a Shimadzu LC-MS 8040 Liquid Chromatography Triple Quadrupole Mass Spectrometer. For improving the SRM technique, mass spectra of the $[M+H]^+$ ions obtained by MS and MS/MS were registered in order to find convenient daughter ions for all the pesticides studied. The highest intensity Q1 and Q3 ions are given in Table 4.

**Table 4.** Optimization values and SRM parameters of the pesticide derivatives.

| Pesticides | Retention time (min) | Molecular weight (g/mol) | Precursor ion (m/z) | Daughter ion, (m/z) |
|---|---|---|---|---|
| Atrazine | 5.54 | 215.68 | 216.10$[M+H]^+$ | 174.10 |
| Chlorfenvinfos | 8.16 | 359.56 | 359.00$[M+H]^+$ | 169.50 |
| Chlorpyrifos | 9.37 | 350.59 | 350.00$[M+H]^+$ | 197.95 |
| Diuron | 5.50 | 231.10 | 233.00$[M+H]^+$ | 72.10 |
| Isoproturon | 5.65 | 206.30 | 207.20$[M+H]^+$ | 72.15 |
| Simazine | 4.96 | 201.65 | 202.10$[M+H]^+$ | 124.20 |

**Preparation of the calibration standard solutions**

The main stock solution concentration was 20.0 µg/ L. To generate the calibration curves of Atrazine, Chlorfenvinfos, Chlorpyrifos, Diuron, Isoproturon, and Simazine, standard pesticide solutions at concentrations of (0.1 µg/L; 0.3 µg/L; 0.5 µg/L; 0.8 µg/L; 1.0 µg/L; 2.0 µg/L; 5.0 µg/L; 10.0 µg/L) were prepared from the main stock solution (20.0 µg/L) diluted with 99.0% methanol, respectively.

**Preparation of Samples**

For the analysis, water samples kept at + 4 °C in a refrigerator were taken into vials by filtering through a 0.2 micron filter without any extraction. Then, these samples were prepared in 3 replicates and introduced to the LC/MS-MS by direct injection method for measurement.

## RESULTS AND DISCUSSIONS

In this study, pesticide analysis (Atrazine, Chlorfenvinfos, Chlorpyrifos, Diuron, Isoproturon and Simazine) was carried out in the Bogazkoy Dam Basin in the Inegol District of Bursa Province and its feeding branches. While determining the pesticide derivatives that need to be analyzed, priority substances were taken as reference for the Surface Water Resources mentioned in the Surface Water Quality Regulation (2016).

**Validation Parameters**

Six pesticides were selected for analysis under optimum conditions. In validation studies, the linearity of parameters, selectivity, detection limits (LOD), quantification limits (LOQ), accuracy (recovery), precision, and sensitivity parameters were carefully made and the results were given.

**Linearity**

The linear range is the range over which the relationship between the signal and analyte concentration in the sample medium of analysis results is proportional. Calibration curves are used to determine the linear range. The linear range was made to determine the concentration range at which the method quantitatively showed correct results. For this purpose, 6 pesticides prepared in the given concentration ranges (Atrazine 1.0-20.0 µg/L; Chlorfenvinfos and Chlorpyrifos 0.3-20.0 µg/L; Diuron 0.5-20.0 µg/L; Isoproturon 1.0-20.0 µg/L; Simazine 0.8-20.0 µg/L) were analyzed and calibration curves were drawn. Analysis was performed by making three measurements at each concentration level. While determining the calibration sub-points, environmental quality standards to be evaluated were taken as reference. Linearity was determined from the calibration curves drawn at the end of the analysis. The slope equation, Area and $R^2$ values of the calibration curves obtained as a result of the calibration studies are given in Table 5.

**Table 5.** Calibration Information of Pesticides.

| Pesticide's Name | $R^2$ | Equation of the curve |
|---|---|---|
| Atrazine | 0.9996 | y=75442x+12541 |
| Chlorfenvinfos | 0.9996 | y=29408x-5714 |
| Chlorpyrifos | 0.9999 | y=10937x+2524 |
| Diuron | 0.9998 | y=21915x-2384 |
| Isoproturon | 0.9996 | y=4352x+12528 |
| Simazine | 0.9997 | y=19445x+2598 |

**Selectivity(Specificity)**

Selectivity is the ability of the analytical method to accurately measure only the intended component or components in the presence of expected physical/chemical interferences. Therefore, blind water samples were analyzed by LC-MS/MS in order to observe possible noise from interference the studied water samples. According to the chromatograms examined, no interference originating from the matrix has been found for pesticide derivatives in the retention times determined. Blind sample chromatogram and standard sample chromatogram (10 µg/L) are given in Figures 3 and 4, respectively.

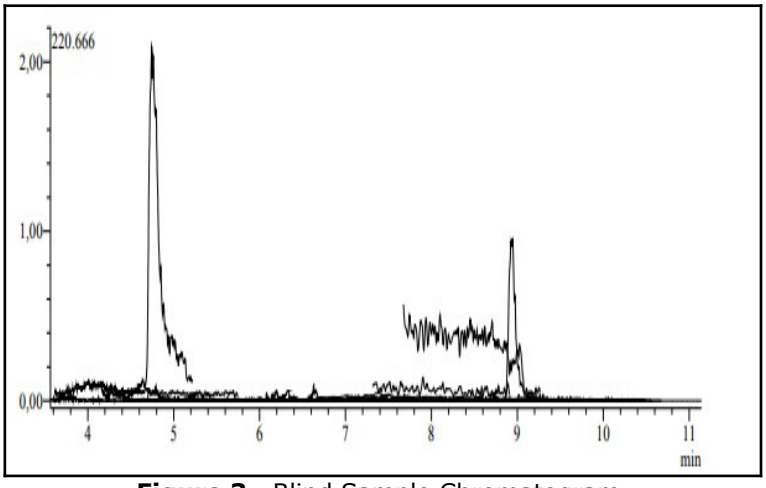


**Figure 3.** Blind Sample Chromatogram.

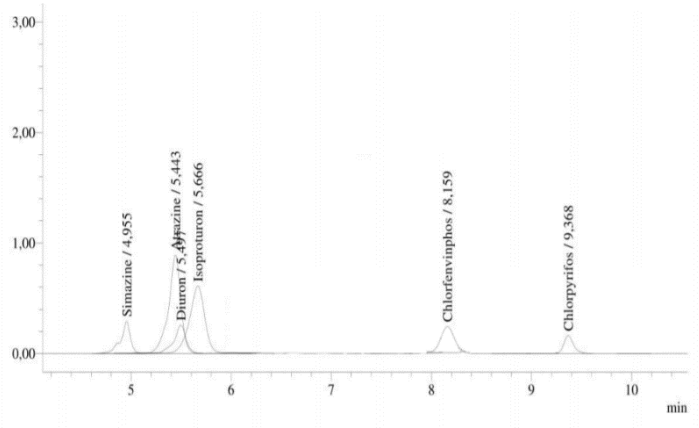


**Figure 4.** Standard Sample Chromatogram (10 µg/L).

**The limits of detection (LOD) and limits of quantification (LOQ)**

The lowest amount that the analyte signal that can be determined by the detector can be distinguished from the noise that can interfere with the background analysis is the diagnostic limit (LOD) of that substance. The lowest concentration value at which the reliable quantitative result for the substance analyzed can be obtained is the lower limit of the determination of that substance (LOQ).

Calculation of diagnosis and detection lower limit values;

LOD = Xbl + 3 Sbl

LOQ = Xbl + 10 Sbl

Xbl = Average of analyte-free measurements

Sbl = Standard deviation of analyte-free measurements

(LOD) the limits of detection and (LOQ) limits of quantification were calculated by considering the average and standard deviation measurements. LOD and LOQ studies the results of standard solutions (2 µg/L) for n=7 are given in Table 6.

**Table 6.** The limits of detection(LOD)and limits of quantification(LOQ) values

| **n=7** | Atrazine | Chlorfenvinfos | Chlorpyrifos | Diuron | Isoproturon | Simazine |
|---|---|---|---|---|---|---|
| LOD(µg/L) | 0.25 | 0.23 | 0.28 | 0.45 | 0.22 | 0.31 |
| LOQ(µg/L) | 0.86 | 0.79 | 0.96 | 1.51 | 0.75 | 0.89 |

**Accuracy (Recovery %)**

In determining the correctness of a method, a recovery study is required. It shows the closeness of the data to be obtained from the experiments to the correct value. The % recovery value rates should be between 70-120% according to the SANCO document (23). To determine the recovery values, 2 µg/L value of standard solution was added to the blind sample and chromatograms were taken, and the recovery was calculated. The accuracy (Recovery %) results studied are given in Table 7. Recovery rates were found in accordance with the 70-120% range specified for validation studies.

**Precision**

Precision shows the repeatability (closeness to each other) of experimental data. Precision is expressed in relative standard deviation. In the SANCO document published by the "General Directorate of Health and Consumer Protection of the European Commission" on November 19, 2013, the repeatability and reproducibility suitability is performed through SD and RSD% results (23). According to the same SANCO document, according to the acceptability criteria of the performance of a method, the relative standard deviation (%RSD) value should be ≤20. In experimental studies, depending on the conditions under which the experiment was performed, standard deviation (SD) and percent relative standard deviation (%RSD) values were calculated for the performance, that is, the repeatability of the method. It is given in Table 7.

**Table 7.** Pesticide Derivatives's Accuracy (Recovery%) and Precision results.

| **Pesticides** | **Recovery %** | **SD** | **RSD %** |
|---|---|---|---|
| Atrazine | 91.17 | $3.37x10^3$ | 4.3 |
| Chlorfenvinfos | 91.53 | $3.38x10^3$ | 21.2 |
| Chlorpyrifos | 84.95 | $5.28x10^3$ | 26.6 |
| Diuron | 101.2 | $2.36x10^3$ | 13.5 |
| Isoproturon | 84.85 | $2.37 x10^3$ | 11.7 |
| Simazine | 84.70 | $8.28 x10^3$ | 8.7 |

**Analysis of Dam Water Samples**

Following the validation studies, the samples were analyzed in 3 replicates on the same day, and the average of the data obtained from the studies was obtained. After the study was completed, it was observed that the pesticide amounts in the samples remained below the LOQ level. The analysis results of the samples are given in Table 8. Column charts including peak areas of pesticides with error bars represented ($n$=7) replicates in Figure 6.

**Table 8.** Quantities of pesticide samples determined in dam waters and their status according to the values given in the regulation.

| Water Samples | Atrazine (µg/L) | Chlorfenvinfos (µg/L) | Chlorpyrifos (µg/L) | Diuron (µg/L) | Isoproturon (µg/L) | Simazine (µg/L) |
|---|---|---|---|---|---|---|
| B1 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B2 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B3 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B4 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B5 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B6 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B7 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B8 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B9 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B10 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B11 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |
| B12 | <1 | <0.3 | <0.3 | <0.5 | <1 | <0.8 |

The analysis results of the chromatograms of the samples were given as follows in Figure 5.

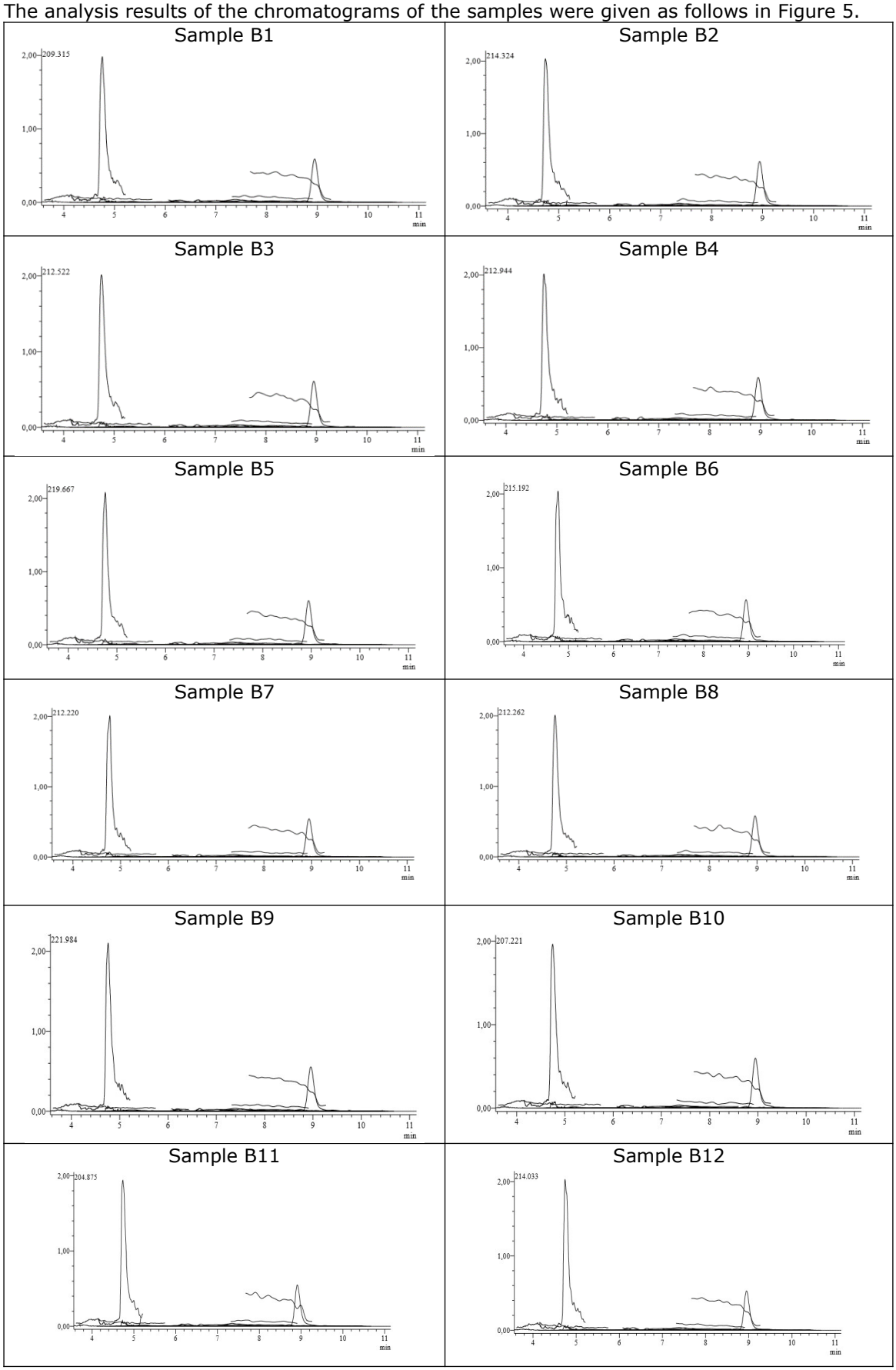


**Figure 5.** The chromatograms of the samples taken from dam water.

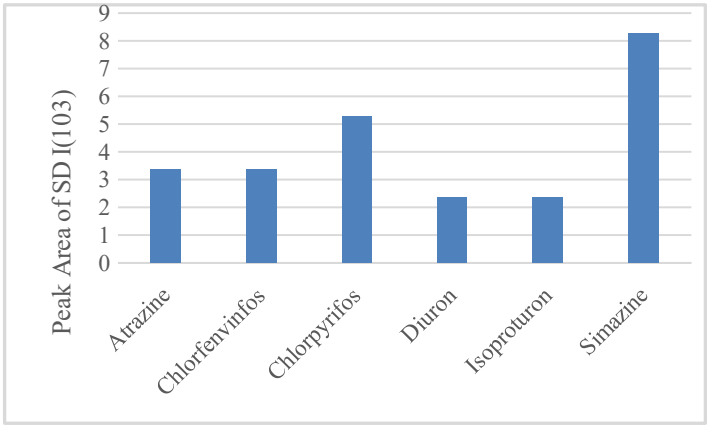


**Figure 6.** Column charts including peak areas of pesticides with error bars represented for $n$=7 replicates.

Calibration curves for pesticide derivatives were drawn first in the study. Limit values were taken as reference when drawing the calibration curves. For atrazine: 1 µg/L, for chlorfenvinfos: 0.3 µg/L, for chlorpyrifos: 0.3 µg/L, for diuron: 0.5 µg/L, for isoproturon: 1 µg/L, and for simazine: 0.8 µg/L values were determined. The correlation coefficients close to 1 proved that the linear relationship of the experimental data was strong. Calibration information and correlation coefficients are given in Table 5, respectively.

In the other part of the statistical calculations, the detection (LOD) and determination limits (LOQ) of the pesticides in the samples were made in the laboratory (see Table 6). The 20% RSD values, which should be according to the SANCO document, were below 20% in the calculations, as shown in Table 5. These results also prove the accuracy of the method. Each pesticide has been calculated and evaluated within its own area. Since the results (see Table 8) were below our LOD values, pesticides were not evaluated as quantitative analysis and were expressed as smaller (<) than the limit values (see Table 1).

When the results for the accuracy of the method were compared with SANCO documents, the recovery of pesticide derivatives was between 84% and 101%. It has been determined that it is compatible with the recovery values that should be between 70-120% according to SANCO Document (see Table 5).

Samples taken from 12 points were filtered through microfilters without extraction and were introduced to LC-MS/MS device. The analyzed pesticide derivatives were found below the Environmental Quality Limit Values. The analysis results of the samples are given in Table 8.

## CONCLUSION

As a result, it was observed that the pesticide derivatives (atrazine, klorfenvinfos, chlorpirifos, diuron, isoproturon, and simazine) analyzed in the waters taken from the Boğazköy Dam and the waters that feed the dam do not exceed the Environmental Quality Standards limit values in the Surface Water Regulation. It has been determined that Boğazköy Dam will not pose a risk in irrigation of agricultural lands and its use in surface waters in this region, considering agricultural products and human health, and therefore it can be easily used as irrigation and groundwater. Only in the region, the continuous production and continuation of agricultural production will bring pesticide use with it. Therefore, it should be remembered that periodic continuous monitoring of the region should not be ignored in terms of protecting agricultural products and human health.

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## CONFLICTS OF INTEREST

There is no potential or existing conflict of interest between our scientific work and our personal situation.

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