Occurrence and Distribution of Hexachlorohexane (HCH) Concentration in Agricultural Soils in Albania Using Gas Chromatography

Armela Tafa, Iva Kertusha, and Ambra Xhafka

***Abstract*—Pesticides play an important role in agriculture to control or eliminate weeds, pests, insects and plant disease. Due to their wide usage in past years, pesticides are known as environmental pollutants. They can contaminate water, soil, biota and air. The objective of this study is to determine the occurrence and distribution of Hexachlorohexanes (HCH) isomers in soil samples. HCH are measured in soil samples collected from nine agricultural stations in Durres city in Albania. They are extracted using Soxhlet apparatus and Hexane as a solvent and analysed via GC–MS. The highest levels of HCH are detected in Station 8 due to the high agricultural activity in this area.**

***Index Terms*—GC–MS, Hexachlorohexane, soil samples, Soxhlet apparatus.**

I. Introduction

To protect plants and crops from weeds, different diseases or pests, farmers are using chemical substances named as *Pesticides* [1]. Pesticides are classified in several groups named: Insecticides, Herbicides, Fumigants, Rodenticides, and Fungicides. Herbicides are used to eliminate and control weeds in corn and vegetables. Insecticides are used to control parasites and insects in different plants and Rodenticides are used to control spread of mice and rats [2]. Insecticides, class of pesticides, include Organochlorines, Organophosphates, Carbamate Esters and Pyrethroids. They are used to Control and destroy parasites and insects in crops, plants, vegetables. Fungicides include Hexachlorobenzenes, Organomercurials, Dithiocarbamates, Pentachloro phenol and Phthalimides. They are used to Destroy Fungus in several plants and the grey mold that affect houses and plants. Herbicides include ChloroPhenoxy compounds and derivatives of Bipyridyl. They are used to control and destroy weeds and other vegetative parasites that can affect the growth of a certain plant. Rodenticides include Zinc phosphide and Fluroacetic acid. They are used to eliminate the spread of mice and rats in houses and crops. Fumigants include Phosphines, Ethylene dibromide and Dibromochloropropane. They produce gases / vapor to eliminate and destroy air / soil borne diseases and certain insects [2]–[5].

OPCs (organochlorine pesticides) are classified as very toxic substances with a high degree of toxicity related to target and non-target organisms [3]. When pesticides are released in the environment, they are distributed among five important sections: water, air, soil, flora and fauna. The distribution of the amount of pesticides in each section is variable based on physical and chemical properties of each compound. OPCs move with the water surface, rivers' water flow or they move through soil capillaries, reach the underground water and contaminate the wells sources or drinkable sources. They are known to have a high persistence in the environment and a long half-life. The persistence of pesticides is directly proportional to its half-life. Pesticides have a high degree of volatility. Some of them are known to be more volatile than others. The volatile pesticides can be converted into gas or vapors in conditions of low humidity and contaminate the air [4]. Pesticides are distributed into environment in different processes such as sedimentation, volatilization, absorption, wash off degradation, sorption / desorption, run off and plant uptake [5]. They are usually carried in rainwater runoff from farm fields, crops, and plants into the closest river, lake and creeks streams. This can cause pollution of environment and poison different living organisms in water or soil. Many ecosystems may be damaged and destroyed [6]. When pesticides are released in the environment with main intention to destroy or eliminate weeds, insects or rats, a very low concentration reaches the desired location. The major concentration is distributed in nature and causes pollution. Previous studies conclude that: When a pesticide is applied in plants, crops or vegetables, only 2% of this concentration reaches the plant leaves or stem. The remaining 98% of the released pesticide gets distributed in the environment and strongly pollutes air, water, soil and biota [7]. The objective of this study is to study the levels of Hexachlorohexanes (HCH) in soil samples. HCH are known as 666 or BHC, and are experimentally synthetized by chlorination of Benzene in presence of UV radiation. This reaction leads in a mixture of five stereoisomers. These stereoisomers are: α – HCH has a range 60–70 %, β – HCH has a range that varies between 5–12 %, γ – HCH varies between 10–12 %, δ – HCH varies in a range 6–10 % and ε – HCH varies in a range between 3–4 % [8]. HCH are known as persistent organic pollutants. δ – HCH and β – HCH are more stable in environment comparing with α – HCH and γ – HCH [9]. In agricultural soils the HCH isomers have the following half –life time: α – HCH half-life time is around 20-50 days, γ – HCH half-life time is around 100 days, β – HCH half-life time is around between 184 and 100 days, and δ – HCH half-life time is between 33.9 and 23.4 days [10]. HCH isomers are known to cause different diseases and illness when they are not used properly in agriculture. Hexachlorohexanes are classified as insecticides. There are

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five different Hexachlorohexanes isomers. Alpha hexachlorocyclohexane (α-HCH) is classified as an insecticide. It causes blood disorders, dizziness, headaches, endocrine system issues. If swallowed can cause death. α-HCH improper use may affect liver and kidneys [11]. Beta hexachlorocyclohexane (β-HCH) improper use may cause blood disorders, dizziness, headaches, endocrine system issues. If swallowed can cause death. It may affect liver and kidneys [11]. Beta hexachlorocyclohexane (β-HCH) is well known to be neurotoxic. It can damage the brain dopaminergic system and cause oxidative stress. It is closely related to Parkinson and Alzheimer diseases [12]. Epsilon hexachlorocyclohexane (ε-HCH) is not classified as carcinogenic. Its improper use can cause dizziness, headaches and vomiting [13]. Delta hexachlorocyclohexane (δ-HCH) causes blood disorders, dizziness, and headaches. It affects the endocrine system, damages liver and kidneys. δ -HCH is not classified as carcinogenic [13]. Lindane or gamma-hexachlorocyclohexane (γ-HCH) is considered as one of the most widely used insecticides in agriculture. It is toxic if swallowed. It can cause dermatitis when it comes in contact with the skin. Lindane affects the reproductive system. It is classified as carcinogenic and may cause liver and kidneys cancer [14].

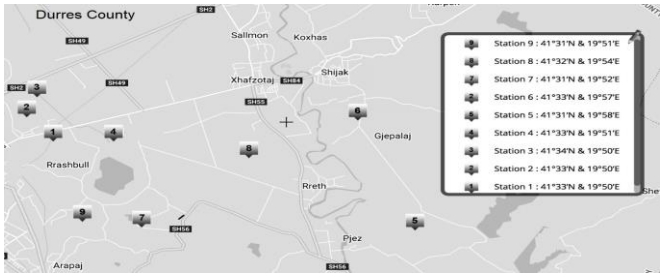

Fig. 1. Map showing the stations and their corresponding geographical coordinates (Latitude & Longitude).

Stockholm Convention is an agreement between 152 states and its major intention is to protect Human Health and to avoid environmental pollution from Persistent Organic Pollutants (POPs). This convention is focused on reducing releases of POPs in the environment and eliminating their usage [15]. Stockholm Convention held many meetings, but in our interest is their fourth meeting on May 2009. In this meeting, it was decided that three HCH isomers: α-HCH, β-HCH and γ-HCH, would be listed as persistent organic pollutants [16]. HCH have been classified as one of the most widely used pesticides in the past few decades. In 1950, different companies started producing γ-HCH and selling it as Lindane (its trade name) [17]. There are several countries known for legacy problems regarding pesticides, including USA, Ukraine, UK, The Netherlands, Switzerland, Turkey, Poland, Macedonia, Romania, Japan, Russia, Nigeria, Slovakia, South Africa, India, Spain, Brazil, China, Croatia, Argentina, Austria, Germany, Czech Republic, Italy, Azerbaijan, France and Albania. In Albania, Porto Romano region is considered as an environmental hot spot, due to several legacy issues regarding HCH and other pesticides.
Porto Romano is located north of Durres city, close to the city port. In 2006, around 313 716 kg of heavy pesticides and toxic chemicals, including large quantities of Lindane were repacked and stored in different warehouses away from inhabited areas [18]. The concentrations and the impact of pesticides in soil samples in Albania have been evaluated in many other studies. However, this is the first study that takes in consideration different agricultural areas in Durres city, and that investigates the concentrations of HCH. This study presents the contamination levels of HCH stereoisomers in soil samples in nine agricultural areas in Durres city. The aim of this study is: 1) to give information about concentration of α-HCH, β-HCH, δ-HCH and γ-HCH in soil samples and 2) to evaluate the soil samples pollution from HCH.

II. Study Area and Procedure of Collecting Soil Samples

Soil samples were collected from nine agricultural farms in Durres city in September 2019. Locations for each station are presented in Fig. 1.

These areas are known for high usage of pesticides in different agricultural processes. Durres is located in North-Western part of Albania. This city has a coastline in the Adriatic Sea. It has Mediterranean climate with dry summers and heavy rains during winter. Mediterranean climate, good soil conditions and watering systems are important factors in agricultural development [19]. In this city, many organic certified farms are established based on EU Regulation 2091/92 and IFOAM Standards. Agricultural farms, including the non-certified ones, are well known for production of grapes and wine, corn, vegetables, olives and olive oil [20]. Soil samples were collected 50 cm below the ground. Afterward, the samples were cleaned of manure, twigs and leaves. [21]. After taking 50g of soil using a small spatula, the samples were placed in polyethylene bags. Geographical coordinates, samples number and area were noted. The samples were stored at $+5^0$C for 5 days until drying. Sample collection took place in September, right after the collection of agricultural products in august and 15 days after the first rainfall.

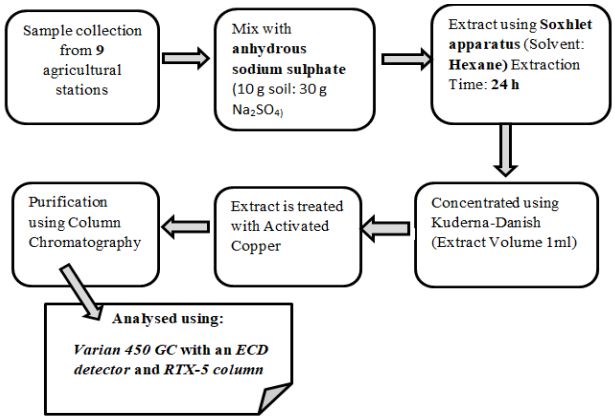

Fig. 2. Experimental procedure.

III. Experimental Procedure

The steps of this experiment are shown in Picture 2. Soil samples were initially mixed with anhydrous sodium sulphate (10 g soil: 30 g Na_2SO_4). The mixture was homogenized and the powder moved freely. The OC were extracted using Soxhlet apparatus and hexane as a solvent. 10g of soil was added into a thimble filter. The samples were extracted for 24 hours. The extracts were concentrated using

a Kuderna-Danish (K-D) concentrator until a volume 1ml of extract was obtained. To remove any dissolving sulphur, the extract was treated with activated cooper. For sample purification, column chromatography was prepared. Gravity column chromatography is a glass tube with length 33 cm and diameter 24 mm and in the bottom of the column, a small amount of cotton was placed. Column is filled halfway with silica gel and 0.5 cm of treated sodium sulphate. The column was washed with small amounts of hexane. Extract was transferred carefully in a chromatographic column and it was eluted with 50 ml of solvent (hexane) at a rate of 3mL/min [22]. OC were analysed using Varian 450 GC with an ECD detector and RTX-5 column (the columns dimensions are 30m x 0.25 mm x 0.25m). Initial oven temperature was 60^{o}C and was kept constant for one minute. Temperature increased with 7^{o}C/min until 220^{o}C and with 5^{o}C /min until 270^{o}C, and then it was increased with 10^{o}C until 300^{o}C and was maintained constant for 10 minutes. For the apparatus calibration, EPA 8081 standard solution was used [23].

IV. Results and Discussion

Soil samples were collected in nine different stations in Durres city, and the concentration of HCH for each sample was determined. In Table I, we are presenting the data for each HCH: their respective concentration range, mean and standard deviation.

TABLE I: HCH Concentrations in Soil Samples

HCH	*Range µg/Kg*	*Mean µg/Kg*	*Standard Deviation µg/Kg*
α – HCH	0 - 32.01	11.71	10.42
β – HCH	0 - 40.26	11.98	13.11
δ – HCH	0 - 2.3	0.45	0.75
γ – HCH	0 - 19.32	6.90	7.46

As shown in Fig. 3, HCH isomers are detected in all soil samples. β – HCH is not detected in station 4. δ – HCH is not detected in most of the stations: specifically in station 1, station 6, station 7 and station 9, and γ – HCH is not detected in stations 2 and 6. α – HCH is detected in all stations with a range that varies between 0.05 and 32.01 µg/Kg, with a mean 11.71 µg/Kg (as presented in Table I) and standard deviation is 10.42 µg/Kg. These values are higher than the ones represented in other previous studies in agricultural lands in Albania [24], but lower than the permissible values registered in Environmental Quality Standards *(EQS) [25]*. As presented in Fig. 3: β – HCH is detected in almost all stations, with a range that varies between 0.79 µg/Kg and 40.26 µg/Kg, with mean 11.98 µg/Kg and standard deviation 13.11 µg/Kg. β – HCH values registered in this area are higher than the ones registered in previous studies in other agricultural lands in Albania [24]. Concentration of β – HCH in soil samples is lower than the permissible standard values approved by EQS [25]. From Fig. 3 we conclude: δ – HCH is detected only in stations 2, 3, 4, 5, 8 and 9. Its range varies between 0.12 µg/Kg and 2.3 µg/Kg. Mean for δ – HCH concentrations in soil samples is 0.45 µg/Kg and standard deviation is 0.75 µg/Kg. We conclude that these values are slightly lower than the ones detected in previous studies in Albania [24]. γ – HCH (or Lindane) is detected in all stations except for stations 2 and 6. It varies in a range between 1.2 µg/Kg and 19.32 µg/Kg, with mean 6.90 µg/Kg and standard deviation 7.46 µg/Kg. Lindane is well known for being used as an insecticide in Albanian farms. The values presented in this study are similar with the ones from previous studies, in other areas in Albania [24]. Detected values are lower than the permissible ones approved by EQS. [25]. Fig. 4 presents the total concentration HCH for each station. From this graph, we conclude that station 8 is the most polluted station from HCH. The stations are arranged in descending order, based on their scale of pollution: Station 8 > Station 9 > Station 2 > Station 7 > Station 1 > Station 3 > Station 6 > Station 5 > Station 4. This order is related to the use of pesticides, and the crops production.

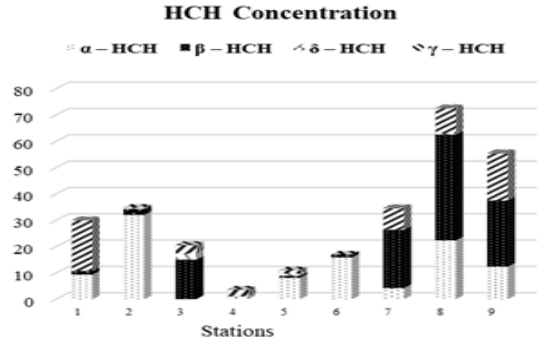

Fig. 3. HCH isomers concentration (µg/Kg) in soil samples.

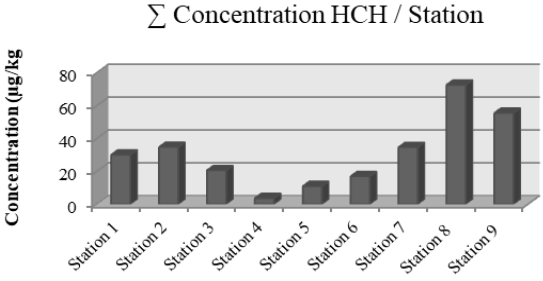

Fig. 4. Sum of HCH concentrations (µg/Kg) / station.

The farm in station 8 is well known for vegetable and fruits production, which is the reason why the soil in this farm results to be more polluted.

Station 4 is less polluted compared with other stations, since in this station the agricultural activity is lower. Station 4 includes in the major part of it high land surfaces cultivating Lucerne green grass. The farmers of this product in Albania prefer not to use pesticides or insecticides, since this kind of grass is usually used to feed cows and sheep. Station 8 has recorded the highest Total Concentration of HCH, which is the sum of β – HCH, α – HCH, γ – HCH, δ – HCH, with a concentration equal to 72.51 µg/Kg. This concentration is lower than the Soil Environmental Quality Standard value, which is 100 µg/Kg [26]. Fig. 5 presents the total concentration of HCH isomers in soil samples. We conclude that in higher concentration is detected β – HCH, with a total concentration 107.8 µg/Kg. The lowest concentration is registered for δ – HCH with a total concentration 4.05 µg/Kg in all soil samples. The descending scale is: β – HCH > α –

HCH > γ – HCH > δ – HCH. We can conclude that this order is related to their physical and chemical properties of HCH in Environment. The same results are observed in previous studies in other regions in Albania [24].

TABLE II HCH LEVELS IN REFERENCE STUDIES

	Kosovo [27] (City: Peje)		*Albania [28] (City : Lushnje)*	
	Min	**Max**	**Min**	**Max**
α–HCH	0.1	2.7	0	9.49
β–HCH	0.5	7.1	0	19.14
δ–HCH	0.1	1.1	9.08	16.34
γ–HCH	0.1	7.1	26.25	27.09

TABLE III: HCH TOTAL CONCENTRATIONS/ STUDIED AREA AND REFERENCE AREAS

	Kosovo (City :Peje)	*Albania (City : Lushnje)*	*Current study (Albania, City : Durres)*
	HCH Total Concentrations (μg/Kg)		
α – HCH	5	9.49	105.44
β – HCH	10.3	19.14	107.8
δ – HCH	1.7	53.34	4.05
γ – HCH	10	38.02	62.08
∑ HCH	**27**	**199.99**	**279.37**

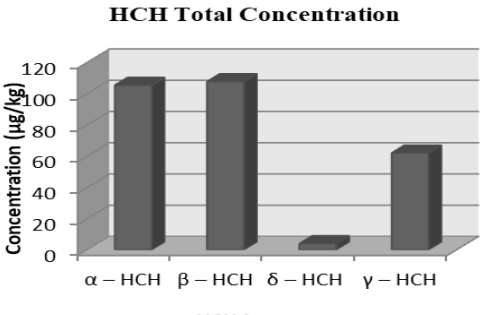

Fig. 5. HCH total concentration in soil samples.

In Table II, are presented concentrations of HCH isomers, taken from two different studies [27], [28]. As displayed in Table II and in Fig. 6, we have compared Minimum and Maximum values detected in Albania and in Kosovo. Higher concentration of α–HCH is detected in the current study in Durres city with a concentration of 32.01 μg/Kg, followed by Lushnje city with a concentration of 9.49 μg/Kg and then Peje city with a maximum concentration 2.7 μg/Kg. In Lushnje city, in all soil samples α–HCH is not detected. β–HCH highest concentration is detected in the current study- 40.26 μg/Kg, followed by Lushnje city - 19.14 μg/Kg followed by Peje city - 7.1 μg/Kg. In Lushnje, in all soil samples, β–HCH is not detected. Highest concentration of δ–HCH is detected in Lushnje city is 16.34 μg/Kg, followed by Durres city- 2.3 μg/Kg, and then Peje city - 1.1 μg/Kg. In Lushnje city is detected a high concentration of δ–HCH, with the minimum concentration detected in soil samples equal to 9.08 μg/Kg. γ–HCH is detected in all soil samples. We conclude that highest concentration is detected in Lushnje soil samples, with a minimum concentration 26.25 μg/Kg and maximum concentration 27.09 μg/Kg. A high concentration is observed in Durres soil samples as well. The highest concentration is observed in Durres soil samples due to the high intense of usage of insecticides in farms. In Lushnje city, samples are taken from Myzeqeja field, which is considered as one of the most important agricultural fields in Albania [28]. In general, the main source of HCH in these regions is primarily related to the use of different pesticides with main intention to protect crops from diseases and insects. The lowest concentrations are observed in Peje city, in Kosovo. As presented in Table III and in Fig. 7, we can conclude that the Total Highest Concentration of HCH is registered in Durres soil samples, followed by Myzeqeja soil samples and Peja soil samples. In Durres soil samples, the HCH isomers concentration in decreasing order is: β – HCH > α – HCH > γ – HCH > δ – HCH. In Myzeqeja soil samples, the HCH isomers concentration in decreasing order is: δ – HCH > γ – HCH > β – HCH > α – HCH. In Peje soil samples, the HCH isomers concentration in decreasing order is: β – HCH > γ – HCH > α – HCH > δ – HCH. As shown in Fig. 7, the most polluted area is Durres city, due to the high agricultural activity. The decreasing pollution order is: Durres soil samples > Lushnje soil samples > Peje Soil samples. By this comparison, we conclude that soil in the studied sites in Albania is more polluted than the soil in studied site in Kosovo.

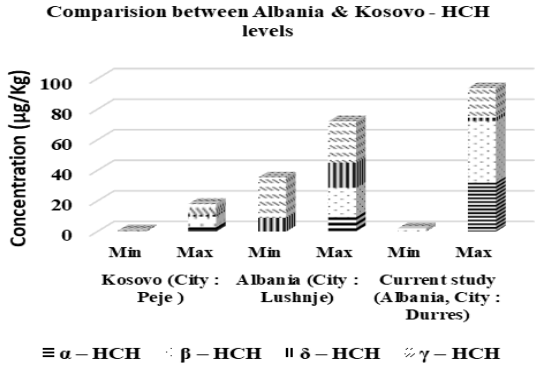

Fig. 6. Comparison between Albania and Kosovo - HCH Levels.

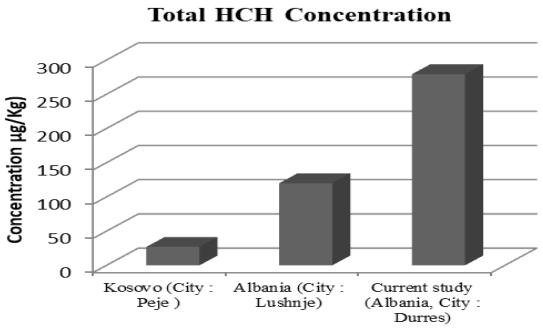

Fig. 7. Total HCH concentrations.

Nowadays, in Durres city many organic farms are producing vegetables, crops, olives and other products. Compared to other studied areas, Durres city has an active and intensive agriculture. If we compare between Albania and Kosovo, we will conclude that soil samples in Albania, for both cities, are more polluted. The main reason for this difference is the fact that Albania has a longer agricultural history and activity compared with Kosovo. This is primarily related to the Communism period in Albania. During communism, in Albania the main economical source was agriculture with big farms, where the most important was the product and not the environment. Farmers and the

population in general were not well informed about pesticides and their effect in the environment [29]. As a remaining trace from communism, farmers still use many pesticides, especially insecticides, without getting worried about their impact in health and environment.

V. Conclusions

In this study Hexachlorohexanes (HCH), also known as Benzenehexachloride (BHC), are analysed in soil samples in nine different agricultural stations in Durres city, Albania. This study has observed different concentrations of HCH isomers in most of the stations of the studied area. Among HCH isomers, we conclude that β – HCH is detected in higher concentrations compared with other isomers, followed by α – HCH, γ – HCH and δ – HCH. The main factor for this result is related to their physical and chemical properties, and their biodegradation and resistance in environment. Station 8, results more polluted than other stations and this is due to the higher agricultural activity in this farm.

This study also indicates that soil in studied sites in Albania is more polluted that soil in studied city in Kosovo. In Albania, agriculture is one of the main economical sources; for this reason the use of pesticides is higher than in Kosovo. This leads to higher concentrations of pesticides in environment and increases risk for disease especially among farmers. By comparing the detected concentrations with both EQS's standard values and other previous studies conducted in other farms and other cities, we conclude that our experimental values are slightly lower. These results indicate that the soil in studied agricultural areas is not polluted from HCH isomers. It is very important to monitor the levels of HCH in this area since they may increase after each season. This increment is caused mainly because the farmers grow different plants, depending on the season. This study concludes that pollution levels in agricultural lands in Durres are very low and right now this doesn't represent any risk related with environment or health. These conclusions may be setting stone for further studies in these areas.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Armela Tafa conceived the original idea and proposed the experimental procedure. The idea was initially discussed with Ambra Xhafka and then with Iva Kertusha. All authors discussed and agreed regarding the main idea of this research, area of study and experimental method.

Armela Tafa and Ambra Xhafka conducted the experimental research work. Iva Kertusha analyzed the data and performed the statistical analysis. Armela Tafa was focused on the literature survey and wrote the manuscript as well. Iva Kertusha conducted the proofreading. The final manuscript was read and approved by all authors.

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