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Assessment of Lindane pesticide residue in Cocoa beans in the Twifo Praso district of Ghana

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ABSTRACT

In this study samples of Cocoa Beans were analyzed for concentrations of lindane (organochlorine pesticide) residue. Samples were gathered from five communities within the Twifo Praso district in the Central Region. Quantitative determination of the lindane was performed by using the Gas Chromatography Thermal-Capture Detection (GC-TCD) at the Tema Oil Refiner (TOR). Samples from all five communities contained no detectable concentrations of lindane hence concentrations below the standard value (Acceptable Daily Intake of 0.001 mg/lg and the Maximum Residual Limit in cocoa beans which is 1.0 mg/kg) provided by Codex Alimentarius Commission (CAC).

Key Words: Lindane, organochlorine pesticide, Gas Chromatography, cocoa.

INTRODUCTION

Pesticides have been used in the public health sector for disease vector control and in agriculture to control and eradicate crop pests for the past several decades in Ghana [1]. The majority of pesticides used in agriculture are employed in the forest zones located in the Ashanti, Brong Ahafo, Western, and Eastern Regions of Ghana [2].

Endosulfan, marketed as thiodan, is widely used in cotton growing areas on vegetable farms, and on coffee plantations. Organochlorine pesticides such as DDT, lindane and endosulfan are also employed to control ectoparasites of farm animals and pets in Ghana [3]. In the public health arena, pesticides have been used by the Onchoccerciasis Programme in the Volta Basin for control of black flies (*Simulium* spp) which transmit Onchoccerciasis (African river blindness) to human beings and for control of domestic pests, e.g. cockroaches, flies, mosquitoes, ectoparasites including ticks, and other insects. Pesticides have also been used to control black flies along the banks of the Tano and Pra Rivers [3].

Cocoa farmers are a wide range of pesticides to limit losses from pests and diseases in cocoa agriculture. Prominent among these are: Copper sulphate (a fungicide popular in the treatment of black pod infection; Benzene Hexachloride (BHC) (an insecticide for control of cocoa mirids); Aldrin/Dieldrin or Aldrex 40 (to control mealy bugs); Carbamate Unden, (an insecticide which is effective in controlling cocoa mirids in West African countries) [4]. Others are Kokotine, Apeco, Perenox, Arkotine, Didimac 25, Basudin and Brestan. Pesticide use is associated with risk and can be hazardous if not handled properly. Cocoa farmers using pesticides containing Aldrin, Gamma BHC, Cuprous oxide, Copper sulphate, etc. face constant exposure to these pesticides [5]. Since 1957 when Lindane was recommended, spraying with synthetic insecticides has been the only effective method for controlling capsids on cocoa in West Africa. Presently, spray teeatment with Gammalin 20 (Lindane) at 280g a.i. /ha or 1.4 litres/ ha and Unden 20 (Propoxur) at 210g a.i. / ha or 1.1 litres/ ha applied at monthly intervals from August to December, is the only protection measure recommended in Ghana [3].

Although the organochlorines are banned from importation, sales and use in Ghana, there are evidence of their continued usage and presence in the ecosystem. Work already done in some farming communities in the Ashanti Region of Ghana and some other countries indicate the presence of organochlorine pesticide residues in fish [6], vegetables, water, sediments, mother's milk, blood smaples [3].

Lindane is listed among the Prior Informed Consent (PIC) pesticides, and all agricultural uses of lindane have been banned in 52 countries due to its hazardous nature. Pharmaceutical uses of lindane have also been banned in some countries [14].Many organochlorines which over the years have been linked to major health and environmental problems have been banned or are no longer used. Included in this catalogue are aldrin, dieldrin and endrin which have virtually disappeared, and DDT, heptachlor and toxaphene which have been banned in many countries but are still used quite extensively particularly in some developing countries. Lindane is a neurotoxin that interferes with GABA neurotransmitter function by interacting with the $GABA_A$ receptor-chloride channel complex at the picrotoxin binding site. It has an oral LD_{50} of 88 mg/kg in rats and a dermal LD_{50} of 1000 mg/kg. In humans, lindane primarily affects the nervous system, liver and kidneys, and may be a carcinogen and/or endocrine disruptor [3]. This paper therefore assesses the level of lindane pesticide residues in cocoa beans in five farming communities in the Twifo Praso district of Ghana.

EXPERIMENTAL SECTION

Sample collection

Cocoa beans samples were collected from five farming communities in Twifo Praso district. These communities are Aboabo, Amampoma, Womaso, Bepobeng and Ayaase. The sampling was performed randomly from twenty inorganic cocoa farms (four farms were selected from each community) in February, 2008. Table 1 presents the designation of the sampling sites across the studied communities. A total of 20 cocoa pods were collected within the sampling period and were dispatched to the laboratory for preparation and subsequent residue analysis.

Table. 1. Communities and their designated sampling site

Community	Aboabo	Amampoma	Womaso	Bepobeng	Ayaase
Sampling site	1	2	3	4	5

Sample Preparation

Cocoa beans were air dried for a period of 21 days. The dry samples were separately milled and this step was carried out two times for each sample to achieve better extracts. 20 g of each ground sample was transferred into 250 mL Erlenmeyer flask. 100 mL of chloroform was then added. The Erlenmeyer flasks were placed on a shaker at a go for 4 to 6 hours to allow full penetration of solvent that followed by 5 min motionless state.

Using Buckner funnel and filter paper the samples were filtrated. The solvent was evaporated using a rotary vacuum evaporator. In order to remove co-extractives from the analyte, the extracts were cleaned up using pre-conditioned C-18 Solid Phase Extraction bond columns and residues recovered by adding hexane because have aliphatic stretchers and appropriate solubility in organic solvents. The procedure was repeated for four other samples. The residual contents were poured into separately labelled sample tubes to desired volume for GC analysis. The samples were analysed for lindane residue at the Tema Oil Refinery (TOR) GC laboratory, Accra. Table 2 contains the apparatus and reagents that were involved the preparation of sample assays and standard solutions.

Table 2. Reagents and apparatus used at the TOR GC laboratory

Reagents	Apparatus
Chloroform (96%)	Adventurer Pro AV 412 analytical balance
Petroleum ether (96%)	Sabatar – 144 rotary vacuum evaporator
Hexane (96%)	IKA KS 260 basic shaker
	Erlenmeyer (250 mL), beakers (250 mL)
	sample tubes (10 mL),measuring cylinder (100 mL)
	C-18 solid phase extraction bond columns
	Buchner funnel
	Gas chromatography (Agilent 6890N series)

Preparation of Lindane Standard

0.1g of lindane (0.38%) in 10 mL chloroform to obtain 10 g/LA. 1 mL of A (10 mg) was dissolved in 100 mL chloroform (10 mg/mL) to obtain 0.1 g/LB. 1 mL of B (0.01 mg) was dissolved in 100 ML chloroform (0.1 mg/10 mL) to obtain 10 mg/LC. 1 mL of C (0.01 mg) was dissolved in 10 mL (1 mg/L) to obtain D.

Residue analysis by Gas Chromatography

Detection limit of the GC-TLC was determined for lindane by the successive dilution of the standard mixed pesticide solution, followed by injection into the GC-column, for which the retention time was recorded at 26.904 minute and peak area 4064.76172µVs. Then 1µL of each cleaned up test samples were injected into the GC-TCD. The residue was identified by comparing the retention time of sample peaks with that of the standard.

RESULTS AND DISCUSSION

The results from the gas chromatography analysis of cocoa beans samples for lindane residue are presented by Fig 2, Fig. 3, Fig. 4 and Fig. 5. Depicted on Fig. 1 is the the signal result for the standard lindane solution whereas the rest of the signal spectra are for the sampling sites. In all the five cocoa beans sample analyzed no peaks were found at retention time of 26.904 minutes which meant residual lindane was not detectable. This can imply no oral acute toxicity [7, 8].

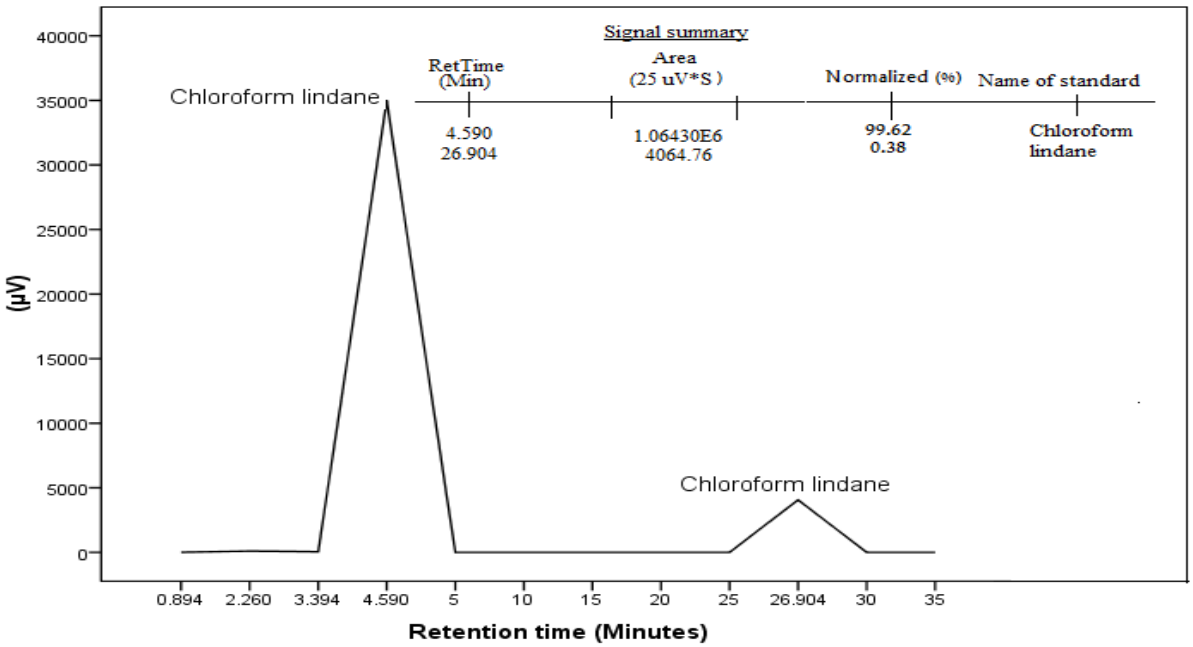

Fig.1. Chromatograph of lindane standard and summary of GC signal.

From the chromatogram of the standard, it peak area was detected to be 4064.76172µVs, and was measured at a retention time of 26.904 minutes but all the five samples were below the detection limit when compared with the standard generated on the chromatogram. Lindane has been banned in Ghana since 2003 which meant that its application on most cocoa farms had been stopped. It's apparent absent in the sample analyzed shows to indicate that once it is not being

used; at least no residue will be suspected in the product. Lindane was banned because of scientific and anecdotal evidence which links lindane with serious health problems including aplastic anaemia, and breast cancer, nausea, dizziness, tremors and muscular weakness.

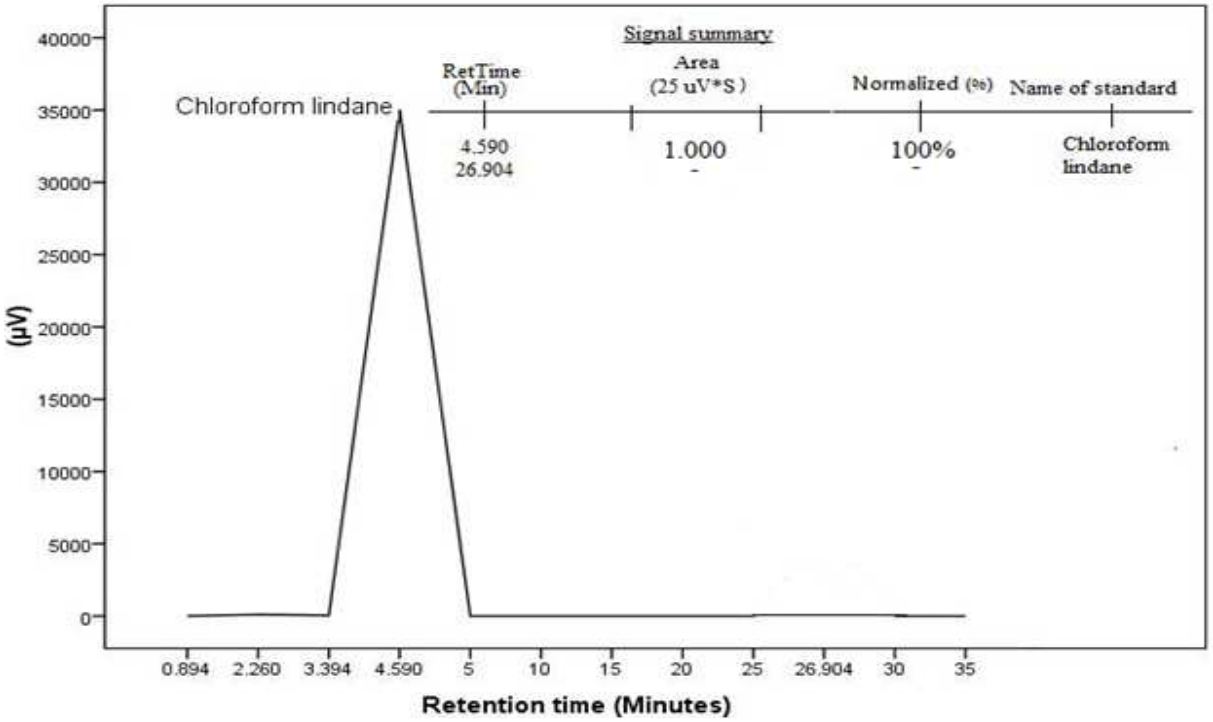

Fig.2. Chromatograph of cocoa beans sample from Aboabo and summary of GC signal.

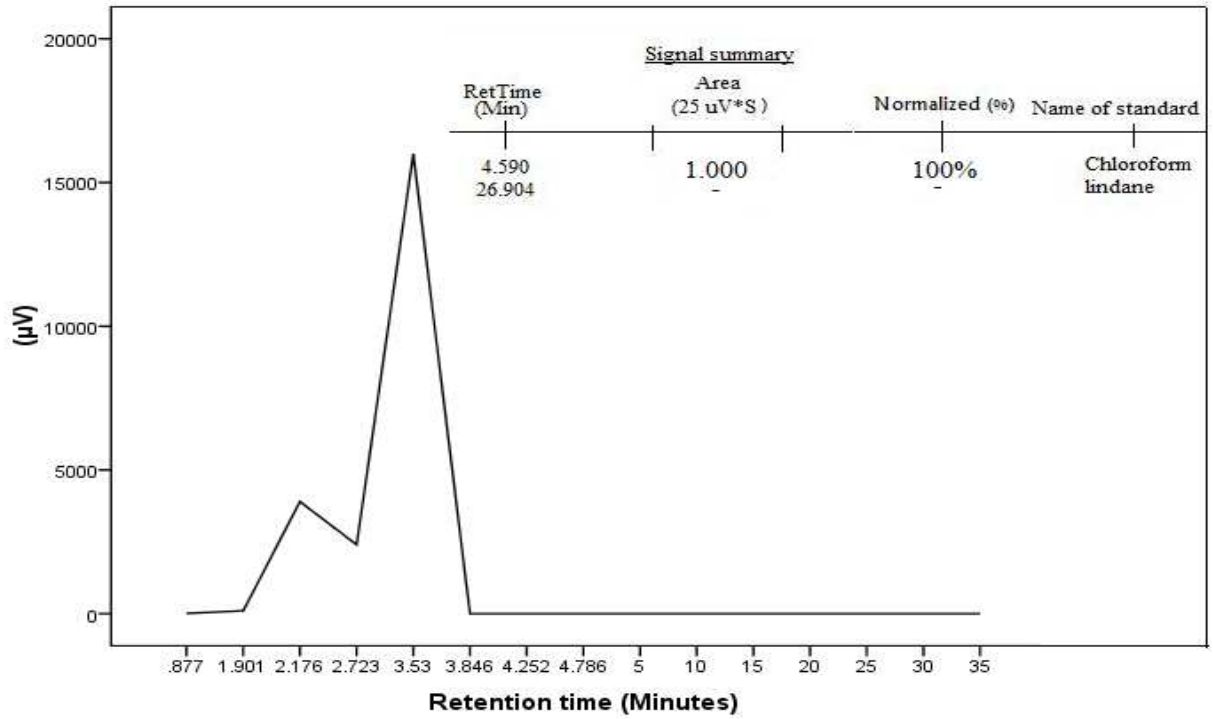

Fig.3. Chromatograph of cocoa beans sample from Amampoma and summary of GC signal.

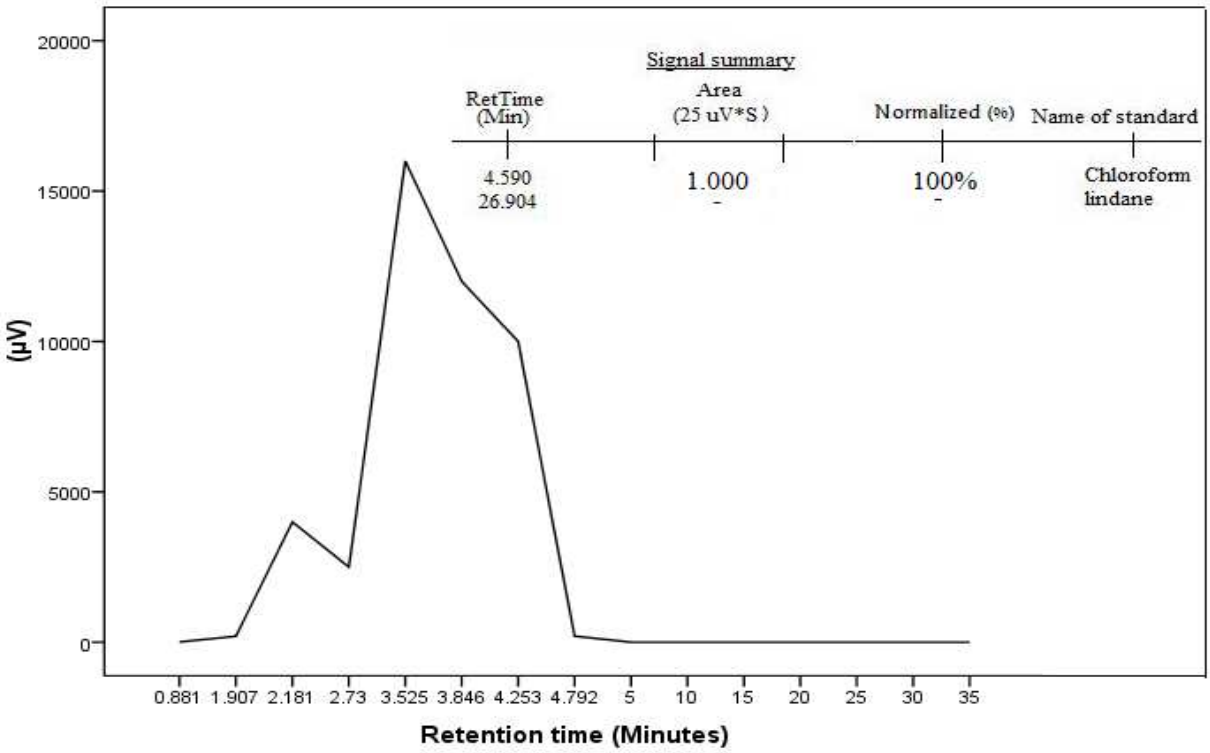

Fig.4. Chromatograph of cocoa beans sample from Womaso and summary of GC signal.

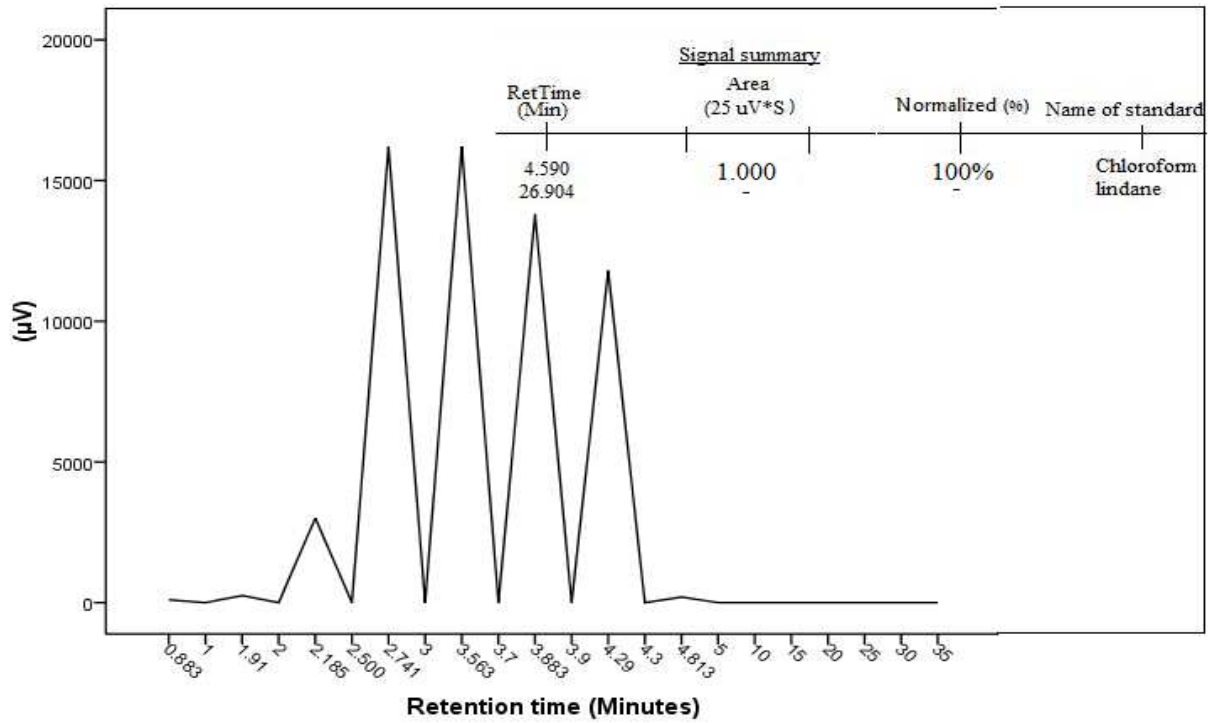

Fig.5. Chromatograph of cocoa beans sample from Bepobeng and summary of GC signal.

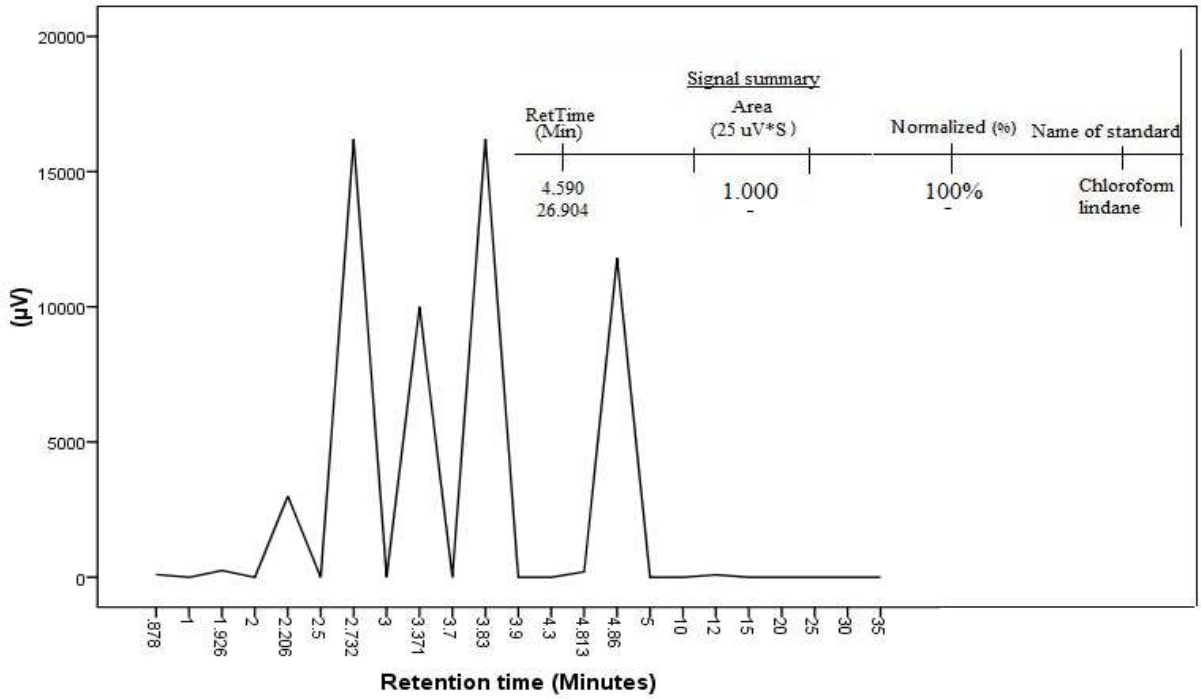

Fig.6. Chromatograph of cocoa beans sample from Ayaase and summary of GC signal.

Factors such as the following may have contributed to the low levels of lindane pesticide residue in the sampled cocoa beans. These factors may be:

1. In a series of dissipation studies with lindane, it was demonstrated that persistent pesticides such as lindane dissipate much faster in the tropics than in temperate climates, probably owing to a large extent to volatilization [9].
2. Soil characteristics such as pH, temperature, clay fraction, moisture content, and particularly organic matter content also influence the uptake of pesticides by plants [10].
3. In addition, the type of pesticide, the pesticide formulation, the method of application, and the mode of action affect plant uptake [11]. Once absorbed by the plant a pesticide can be metabolized and is lost.
4. Biological degradation as a result of microbial metabolism of pesticides, and is often the main source of pesticide degradation in soils [12]. It occurs when fungi, bacteria, and other microorganisms in the soil use pesticides as food or other energy source, or consume the pesticides along with other sources of food or energy. Soil organic matter content, moisture, temperature, aeration, and pH all affect microbial degradation [12].
5. Pesticides applied on leaves to crop residues are subject to interception by the plant material. These pesticides remain on the plant surface until they are removed by intercepted rainfall. The wash-off of these pesticides will amend the pesticide load in the soil and thus will alter the amount of pesticide available for movement by runoff and sediment. In the studies conducted by Haque and Freed [12], their studies indicated that pesticide wash-off was rapid during the first few increments of rainfall and diminished quickly thereafter. They concluded that the amount of rainfall rather than the rainfall intensity was the predominant factor in pesticide wash-off.

CONCLUSION

In all sampling sites, lindane concentrations did not exceed the Codex Alimentarius Commission (CAC) [13], acceptable daily intake of 0.001 mg/kg and the Maximum Residual Limit in cocoa beans which is 1.0 mg/kg. In this study, almost all the sites (Aboabo, Amampoma, Womaso, Bepobeng and Ayaase) had values which were lower than 1.0 mg/kg of the maximum residual limit in cocoa beans. Based on the results of the current concentrations of lindane residue in the Twifo Praso district, it is safe to state that lindane does not pose a serious threat to the cocoa industry and that lindane contamination should not be a limiting factor for it uses in chocolate, milo, cocoa powder, cocoa creams and others.

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