https://africanjournalofbiomedicalresearch.com/index.php/AJBR
*Afr. J. Biomed. Res. Vol. 28(2s) (February 2025); 1043-1047*
*Research Article*

# Pesticides Residue In Ghana Cocoa Beans

**Dadson[1], J. A., Fraikue[2], F. B., Dodoo[3], D. K. Essumang[3], D. K.**

[1]Department of Medical Laboratory Sciences, Takoradi Technical University, Takoradi
[2]Department of Hospitality Management, Takoradi Technical University, Takoradi
[3]Department of Chemistry, School of Physical Sciences, College of Agricultural and Natural Sciences, University of Cape Coast

**Corresponding Author:** Dadson James Atta
Faculty of Health and Allied Sciences, Takoradi Technical University, Takoradi

**Abstract**
Background and Objective: Pesticides have received considerable attention as environmental organic pollutant in many continents such as Africa, Europe and Asia. Many Pesticides compounds have been identified and quantified in virtually all segment of the environment due to their carcinogenicity, mutagenicity and cytotoxicity at very low concentrations. The objective of study is to analyse the cocoa beans for pesticides residue, across six different regions of Ghana and to assess the human health risk. Materials and Methods: Dry cocoa beans samples were collected from six cocoa-growing areas in six regions of Ghana. The extracts were analysed for pesticides using the Agilent 6890N GC-FID/MS. Results: The mean levels of pyrethroids in the Eastern, Western, Ashanti, Central, Volta BA ranged between BDL to 19.4 ng/kg, BDL -9.30 ng/kg, 0.20-18.10, BDL-3.90 ng/kg, 0.02-5.10 ng/kg, and BDL-34.00 ng/kg respectively. The total toxicity equivalencies for the seven U.S EPA priority carcinogens calculated was 1.91E-07 in the western region. The carcinogenic risk for an adult involved in a life time of 70 years ingestion of cocoa from the western region was also calculated to be 1.39E-06. The results reveal that the consumption of cocoa from these regions may not pose any significant carcinogenic risk to consumers since the risk value was within the U.S EPA unit risk of 1×10E-05.

*Received: 20/01/2025 Accepted: 03/02/2025*

*DOI: https://doi.org/10.53555/AJBR.v28i2S.6658*



## Introduction

In Ghana, cocoa is cultivated on about 1.5 million hectares of land, owned by some 800,000 farm families in 10 out of the 16 regions in the country (Cocoa board 2010). Over the years, cocoa has played very significant economic role for Ghana. These include being one of the highest contributors to Ghana's Gross Domestic Product (GDP), employing about 60% of the country's labour force (Denkyira et al 2016), source of income for roughly 800,000 smallholder farm families in Ghana, providing food, employment, tax revenue and foreign exchange earnings for the country (Appiah 2004, Anang 2011 Danso-Abbeam et al 2014). This is further supported by the report of Ghana COCOBOD which revealed that in Ghana, since independence, cocoa has been one of the biggest foreign exchange earners for country, and the share of cocoa in Ghana's GDP has been on the rise since the 2000s (Frimpong et al 2012). Production and post-harvest handling of cocoa affects the final quality of the produced beans. A number of contaminants such as Pesticides, weedicides among others are likely to be introduce into the cocoa beans during production, processing and post harvesting stage.

It has been reported(www.blog.worldcocoafounadtion) that approximately 30 to 40 percent of all potential cocoa production is lost to diseases and pests. Depending on tree crop variety and region, cocoa farmers can face a variety of fungal diseases and insect pests that attack the leaves, stems, trunks, or pods on their cocoa trees

(Adeniyi & Asogwa 2023). Farmers therefore resolved at using pesticides at controlling pests and disease attacks on their plantations to reduce loss. Pesticides work by attracting, seducing and then destroying or mitigating the pests (Mahmood et al., 2016). The pesticide used affect the produced beans, environment and the health of the person applying the pesticide. Most of the pesticides reach a destination other than their target, nonselective pesticides kill non-target plants and animals along with the targeted ones (Mahmood et al., 2016).

However, as a result of the extensive use and various application rates, there is a risk of crop and environmental contamination when pesticides are used in large quantities. According to previous researches, sprayed pesticides in cocoa fields may infiltrate and persist in soils, water bodies, and cocoa beans for several months or years following treatment (Frimpong, et al., 2012a, Dankyi et al 2014, Okoffo 2015, Fialor 2017, Boadu, V. G. & Boadu 2021).The biological environment and the natural environment are both challenged by the application of pesticides.

According to a study by (Gill & Garg 2014), when pesticide residues are ingested by plant roots, pods and leaves from soils, air, and other nutrient solutions, they have the potential to be hazardous to plants, their products, and pollute the food chain. A study by (Gill & Garg 2014), reported that exposure to pesticides through food and water can affect thyroid function, lower male sperm counts, birth defects, an increased risk of testicular cancer, immune system problems, endocrine disruptions, cancers, immunotoxicity, neurobehavioral and developmental disorders. Similar to this, some researchers asserted that the increasing concentration of these pesticide residues in food may cause major health risks if they are not digested by the body and build up in the soft tissues (Akan et al 2013, Agbeve et al 2014).

## Materials and Methods

### Sample Collection

Dry cocoa beans samples were collected from six cocoa-growing areas in six regions of Ghana. These farms were selected from all the six cocoa glowing regions of Ghana based on frequency pesticides usage. Six samples were randomly collected from each community in two crop seasons. The major season in October/November and the lean season in July/August.

### Analytical Reagent

Analytical-grade reagents supplied by BDH Chemical Limited in the UK were used. These reagents included dichloromethane, ethyl acetate, cyclohexane, anhydrous sodium sulfate, sodium chloride, methanol, and potassium hydroxide. Certified reference material(CRM 1941B).

### Extraction of Pesticides from cocoa beans

The ethylacetate method for extracting multi-residue pesticides in fatty food crops was employed (Luke, *et al.,* 1981). The extraction was carried out using ethylacetate as the extracting solvent. About 10g of sample was weighed into a mortar and 10g of anhydrous Sodiumtetraoxosulphate(VI) added and stirred thoroughly. The Mixture was allowed to dry in a dessicator for about 50 minutes. The dried mixture was quantitatively transferred into a 500 mL flat-bottomed flask and 200 mL ethylacetate was added. The resulting mixture was allowed to stand for 24 hours. The mixture was then fitted into a scientific flask shaker (model SFI) and shaken for 3 hours and filtered using Buchner funnel fitted with BUCHI 169 vacuum systems. The filtrate was quantitatively transferred into a 500 mL volumetric flask for concentration on the rotary evaporator. The crude extract was concentrated at 30 $^{o}$C to 1mL using a rotary evaporator (Rota vapor R-144, BUCHI Water Bath B480). Each concentrated crude extract was kept in a desiccator at room temperature prior to saponification.

### Saponification for Pesticides

About 50 mL of 50 % methanolic - Potassium Hydroxide solution was added to the crude concentrate followed by 40 mL 30 % Brine. The saponified mixture was transferred into 500 mL separating funnel containing 100 mL of distilled water. The flask was rinsed with 10 mL ethanol and twice with 10 mL ethylacetate and added to the content of the separating funnel. This was followed by the addition of 50 mL ethylacetate to make-up to the total 100 mL of ethylacetate in the separatory funnel. The separatory funnel was then shaken vigorously and allowed to stand for two hours.

The organic layer was drained into the ethylacetate contained in a 500 mL flat-bottomed flask.20 g of anhydrous sodium sulphate dried at 105 $^{o}$C for 24 hours, cooled and kept in a dessicator and was added to the organic layer and allowed to stand for 50 minutes to remove all traces of water. The dried extract was filtered again using a Buchner funnel fitted with a BUCHI B-169 vacuum System. The filtrate was transferred into another 500 mL round bottomed – flask and then fitted into a vacuum rotary evaporator (Model R-114) and concentrated to about 2 mL. The procedure described above was followed for all the samples and the 2 mL crude concentrates extract were kept in dessicator prior to a clean-up.

### Clean-up for pesticides samples

About 2 mL of each crude concentrate extracts were purified by taking them through column chromatographic clean-up process. A chromatographic column of 1.5 cm diameter and 50 cm high was packed with 30 g of florisil to a height of 30 cm. The florisil was activated in an oven for 2 hrs at 130 $^{o}$C before use. The activated florisil was then loaded into the column (dry method) that had its bottom blocked with enough glass-wool with constant tapping of the column to allow for maximum packing of the florisil. The top of the florisil was then sealed with enough glass - wool. The column was eluted with 30 mL dichloromethane cyclohexane mixture (1:1). The first 10 mL eluate was discarded. The extracts were dissolved in 5 mL of the dichloromethane and applied to the top of the column. The sample was then eluted with 30 mL of the dichloromethane and

cyclohexane mixture (1:1). The elution was repeated with 2 x 25 mL of the dichloromethane and cyclohexane mixture (1:1). The eluates were collected and quantitatively transferred into a flask and concentrated to 1mL at 30 ºC using the rotary evaporator. The final 1 mL extract was kept in a dessicator at room temperature for gas chromatographic analysis.

**GC Analysis**

The Gas Chromatographic-MS parameters were optimized prior to analysis of samples. A spitless glass liner with glass wool was chosen to avoid the contamination of the column since glass wool prevents the entrance of small particles to the column. The injection port temperature was set at 280 °C. Several temperature programs were experimented on to obtain the best resolution of Pesticides and the one found to be optimum was used for the determination of Pesticides in cocoa bean analysis.

**Risk Assessment**

Calculating carcinogenic risk using pyrethroids toxicity equivalent factors (TEF): Toxic equivalency factors (TEFs) have been developed for a number of individual Pesticides classified as potential carcinogens by a number of researchers and institutions, the factor for each of the pyrethroids expressing its potency relative to Pesticides a well-known potent carcinogen, which has a TEF of unity(Dankyi et al,. 2014). The concentration of each of the individual pyrethroids compounds is multiplied by its TEF (Table 1) and subsequently summed up to yield Pesticide equivalent concentrations, TEQ pyrethroids (Frimpong S. K. et al., 2012). By this means, the concentrations of a suite of Pesticide can be represented by a single concentration, which reflects the overall carcinogenic potential of the Pesticides within the sample for which TEFs have been assigned. This technique has in recent times been successfully applied in fresh seafood monitoring studies and other wider monitoring programmes.

TEQ- pyrethroids = Σ (TEFi ×Ci )----------(1)

MEQ- pyrethroids = Σ (MEFi ×Ci )----------(2)

where, Ci is the measured individual Bifenthrin concentrations for the 'ith' compound with the assigned TEFi . The TEF (for TEQ- pyrethroids) and MEF (for MEQ- pyrethroids) approach has been adopted in this study because pyrethroids contamination rarely consists of a single compound but rather of mixtures of compounds that can affect the environment and human health(https://inis.Iaea.org/search/search.aspx?orig-qcru:49076633).The assessment of individual Pesticide irrespective of their relative potency was believed to generate inaccurate or misjudged value for carcinogenic and mutagenic risk since it focuses on single compounds(Dankyi et al,. 2014). The calculated TEQ-pyrethroids and MEQ- pyrethroids for the seven U.S EPA classified carcinogens (mutagens) were used to estimate carcinogenic and mutagenic risk involved in ingestion of oysters used herein for life time of 70 years (Eudes et.al,.2023). The total risk due to exposure to mixtures of carcinogenic (or mutagenic) Pesticide is:

Risk (carcinogenic or mutagenic) = SF × pyrethroids equivalent pyrethroids dose of mixtures of Pesticides ----(3)

where, $SF_{pyrethroids}$ is the oral carcinogenic slope factor of pyrethroids (7.3 mg kgG1 /day). The pyrethroids equivalent daily dose of compound 'i' is given as:

Pyrethroids EQ Dosei = TEFi ×Dosei----------(4)

Hence the daily pyrethroids equivalent dose of mixtures of carcinogenic (mutagenic) Pesticide compounds was calculated for carcinogenicity and mutagenicity using Eq. 5

Pyrethroids Equivalent Dose of Carcinogenic (mutagenic) Pesticides = TEQ(or MEQ)xIRxEFxEDxCF/BWxAT--------5

These exposure assumptions were made to be consistent with EPA guidance on default assumption on "reasonable maximum exposure. Where IR is the ingestion or intake rate of carcinogenic (mutagenic) Pesticide in µg/day, EF is the exposure frequency to carcinogenic (mutagenic) Pesticides in days/year, ED is the exposure duration in years, CF is the conversion factor (i.e., 10G6 kg µgG1), BW is the average body weight of Ghanaian adult in kg and AT is the average life time of 70 year expectancy. Mean ingestion rate of 2 g/day calculated based on cocoa consumed by the average Ghanaian adult. Exposure frequency of 350 days/year, exposure duration of 30 years and average adult body weight of 70 kg were used for the risk assessment.

**Table 1: Levels of pyrethroids in cocoa from the six regions (ng / kg )**

| Pesticide | Eastern | Western | Ashanti | Central | Volta | BA | WHO/FAO |
|---|---|---|---|---|---|---|---|
| Alle | BDL | BDL | 1.30±0.0 | BDL | 0.30±0.0 | BDL | 10.00 |
| Bife | 12.50±0 | BDL | 2.60±0.0 | 2.30±0 | 0.02±0 | 1.20±0 | 10.00 |
| Lamb | 2.90±0 | 8.40±0 | 0.80±0 | 0.70±0 | 11.00±0 | BDL | 20.00 |
| Perm | 6.80±0 | 5.70±0 | 3.30±0 | 3.90±0 | 5.10±0 | 34.0±0 | 10.00 |
| Cyfl | 0.04±0 | 1.20±0 | 0.20±0 | 1.10±0 | 3.40±0 | 1.90±0 | 10.00 |
| Cype | 0.90±0 | 9..30±0 | 0.50±0 | 3.90±0 | 1.90±0 | 0.67±0 | 10.00 |
| Fenv | 0.60±0 | 0.70±0 | 0.20±0 | BDL | 0.42±0 | 0.10±0 | 2000 |
| Delt | 3.10±0 | 0.02±0 | 1.10±0 | 2.90±0 | 3.90±0 | 1.60±0 | 50.00 |
| Fenp | 19.40±0 | 0.20±0 | 18.10±0 | BDL | 0.12±0 | 8.70±0 | 10.00 |

KEY: Alle – Allethrin, Bife – Bifenthrin, Lamb - Lambda –Cyhalothrin, Perm- Permethrin,Cyfl- Cyfluthrin, Cype-Cypermethrin, Fenv-Fenvalerate, Delt-Deltamethrin, Fenp- Fenpropathrin

**Results and Discussion**

Cocoa beans from six regions: Eastern, Western, Central, Volta, Ashanti and Brong – Ahafo regions were analysed for the levels pesticide residues.

The analyses showed the presence of Allethrin, Bifenthrin, Lambda –Cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate, Deltamethrin, Fenpropathri in the cocoa beans sampled at very low concerntrations. The mean concentrations of all the pesticides were estimated in nanogram per kilogram (ng / kg) and were compared against the allowable residue threshold levels stipulated by the WHO/FAO to be in cocoa beans. Table 1 show Levels of pyrethroids in Cocoa from the six regions (ng/kg)

The mean levels of pyrethroids in the Eastern, Western, Ashanti, Central, Volta BA ranged between BDL to 19.4 ng/kg, BDL -9.30 ng/kg, 0.20-18.10, BDL-3.90 ng/kg, 0.02-5.10 ng/kg, and BDL-34.00 ng/kg respectively. All the concentration level of the pyrethroids were below the permissible level set by WHO/FAO ensuring high quality beans. Research carried out by Baah et al reported the presence of pirimiphos-methyl in cocoa beans from the Western North region of Ghana. They reported residue level of 0.01 mg/kg which was within the EU's MRL for cocoa beans. Villanueva et al also quantified pesticides residue of 9.8 µg/kg which was within EU's MRL for cocoa beans

**Conclusions**

The appreciable levels of pesticides were all below the permissible level set by WHO/FAO. This suggests a high quality of cocoa beans from these six regions of Ghana.

**References**


1. Adenyi & Asogwa, E. U. (2023). crop protection practices for cashew and cocoa production in Nigeria. Paper presented at the national workshop for farmers development unit (FADU) officials on coacoa and cashew production in nigeria,Ibada,May 2023.
2. Agbeve, S. K., Osei-Fosu, P. & Carboo, D., 2014. Levels of organochlorine pesticide residues in Mondia whitei, a medicinal plant used in traditional medicine for erectile dysfunction inGhana. *International Journal of Advance Agriculture Research,* Volume 1, pp. 9-16.
3. Akan, J. C., Jafiya, L., Mohammed, Z. & Abdulrahman, F. I., 2013. Organophosphorus Pesticide Residues in Vegetable and Soil Samples from Alau Dam and Gongulong Agricultural
4. Areas, Borno State, Nigeria. *International Journal of Environmental Monitoringand Analysis,* 1(2), p. 58.
5. Anang, B. T., 2011. Market structure and competition in the Ghanaian Cocoa Sector after partial liberalization. *Current Research Journal of Social Sciences,* 3(6), pp. 465 - 470
6. Appiah, M. R., 2004. *Impact of cocoa research innovations on poverty alleviation in Ghana.* Ghana: Academy of Arts and Science Publishers.
7. Baah, A. Yaayin, B. Hanson. R., 2023. Multi Residue, Analysis of Systemic Pesticide in Cocoa
8. Beans from Some Farms in the Western North Region, Ghana, *Food and Public Health*, Vol. 13 No. 1, 2023, pp. 6-14. doi: 10.5923/j.fph.20231301.02.
9. Boadu, V. G. & Boadu, M. O., 2021. Assessment of farmer's usage of pesticides on cocoa farms at Sefwi Wiawso district in the western region of Ghana. *Global Journal of Agricultural Research,* 9(2), pp.1-13.
10. COCOBOD, 2010. *Regional concentration of cocoa.* s.l.: s.n.
11. Dankyi, E., Gordon, C., Carboo, D. & Fomsgaard, I. S., 2014. Quantification of neonicotinoid insecticide residues in soils from cocoa plantations using a QuEChERS extraction procedure and LC-MS/MS. *Science of The Total Environment,* p. 276–283.
12. Danso-Abbeam, G., Setsoaia, E. D., Gershon, I. & Ansah, K., 2014. Modelling farmers investment in agrochemicals: the experience of small holder cocoa farmers inGhana. *Research in Applied Economics,* 6(4), pp. 1-15.
13. Denkyirah, E. K. et al., 2016. Modeling Ghanaian cocoa farmers' decision to use pesticide and frequency of application: the case of Brong Ahafo Region. *SpringerPlus,* pp. 1-17.
14. Eudes et.al,.2023 Screening for pesticide residues in cocoa(Theobroma Cocoa L) by portable infrared spectroscopy. Volume 257, 15 May, 2023, 124386.
15. Fialor, A. S., 2017. *Identification and concentration of selected pesticide residues in Ghanaian cocoa beans,* Legon-Accra: UGSpace.
16. Frimpong, S. K. et al., 2012a. Assessment of organochlorine pesticides residues in cocoa beans from Ghana. *Elixir Food Science,* Volume 50, pp. 10257-10261
17. Gill, H. K. & Garg, H., 2014. Pesticides: Environmental Impacts and Management Strategies. In: *Pesticides - Toxic Aspects.* s.l.: InTech, p.188– 230.
18. Grob, K., Artho, A., Biedermann, M. and Mikle, H. 1993. Contamination of hazelnuts and chocolate by mineral-oil from jute and sisal bags. Z LebensmittelUntersuchung und –
19. Forschung 197: 370-374.https://inis. Iaea.org/ search/search.aspx?orig-qcru:49076633 IARC Monographs on the evaluation of the carcinogenicity: an updating of IARC monographs ol 1-42, supplement 7. International Agency for Research on cancer, Lyon; 1987
20. Mahmood, I., Imadi, S.R., Shazadi, K., Gul, A. and Hakeem, K.R. (2016). Effects of Pesticides on Environment. In: Hakeem, K., Akhtar, M. and Abdullah, S., Eds., Plant, Soil and Microbes, Springer, Cham, 253-269.
21. Okoffo, E. D., 2015. *Pesticide use and pesticide residues in drinking water, soil and cocoa beans in the Dormaa west district of Ghana,* Accra: UGSpace

22. Villanueva, E. Glorio-Paulet, P. Giusti, M. M., Sigurdson G. T., Yao S., Rodríguez-Saona L. 2023 Screening for pesticide residues in cocoa (*Theobroma cacao* L.) by portable Infrared spectroscopy Volume 257, 15 May 2023, 124386