RISK ASSESSMENT OF DICHLORODIPHENYLTRICHLOROETHANE RESIDUES IN SOIL AND RICE SAMPLES HARVESTED IN SELECTED RICE FARM OF BENUE STATE, NIGERIA

ABSTRACT

This research aim at investigating the level of Dichlorodiphenyltrichloroethane (DDT) residues in soil and rice samples collected from otukpo farmland area of Benue State. Ten soil samples and five different species of rice samples were collected on the farmlands to determine the concentration of DDT residues. Standard analytical methods were employed for the determination of some physicochemical parameters (pH, total organic carbon, moisture content and cation exchange capacity) of the samples. Collected samples were analyzed for residues of total dichlorodiphenyltrichloroethane using gas chromatography with mass spectrometric detector (GC-MS) after careful extraction and cleanup. Risk assessment was carried out by determining the hazard indices. The results of the physicochemical analysis showed that the mean pH value of soil samples is 6.2 indicating slight acidity as compared to rice 7.34 which is approximately neutral and within WHO acceptable limits. The mean total organic carbon (TOC) value is 14.57% while the mean cation exchange capacity is 7.85 meq/100g in soil. DDT was detected with average concentration of 10.5 mg/kg in soil and 3.41 mg/kg in rice which is above the EU/WHO and MRL recommended rate of 0.1 mg/kg and 0.05 mg/kg. Harzard indices employed in this research shows that the some rice species are fit for consumption with the exception of those with Hazard Index level greater than one in children. The contamination levels of DDT in the analyzed soil based on national standards are considered toxic for crop production but analysis shows that the uptake level of DDT from soil to crop is very low which makes the rice cultivaled fit for consumption.

Keywords: *Organochlorine, DDT, ILCR, Hazard Index*

1.0 Introduction

Due to shortage of farm yield caused by pest and increase in population, the demand of food commodity has been on the increase than its supply which requires urgent attention in most countries. In order to meet this demand, the use of synthetic chemical known as pesticides were employed in agricultural practices to increase the yield of most crops. Pesticides are chemicals which are used to eliminate or control the growth and reproduction of pests, certain plants and animals [1], while the term Pesticide residues refer to the break-down products of pesticides that may remain on soil and food after application to food crops [2]. Contamination caused by pesticides in surface and ground waters affects aquatic life, plants and human health due to public consumption of water [3]. With increased agriculture productivity, pesticides has helped to reduced insect-borne diseases as well as restoration of plantations, forests, harvested wood products and fiber [4]. The usage of pesticides containing DDT has been associated with various human health hazards, such as headaches, constipation and chronic chronic impacts such as cancer, and endocrine disruption when present in food items. Survey also shows that major pesticides used in Nigeria are organochlorine (13.5%), Organophosphorous (21.1%) and others [1]; [5]. Agricultural chemicals like pesticides and fertilizers are major source of chemical residues which has pose serious environmental threat such as soil contamination even when their proposed function is to minimize the actions of pests [6]. Dichlorodiphenyltrichloroethane (DDT), an example of Organochlorine are persistent organic pollutants which generate significant hazards when they are released into the environment. They are applied as dusts or sprays and widely used as contact insecticides against beetles, flies and bugs [1]. Due to high rate of persistency, toxicity and difficulty in recovery, usage of pesticides containing DDT was banned in many countries, but was substituted by other chemicals as toxic organochlorine [7,8]. According to WHO 2010, DDT residues are soluble in fats and lipids of animals and plants; therefore, when water is contaminated, fish and other aquatic organisms have the capacity to absorb them from water and concentrate them in their fatty tissues. According to [6],

DDT interferes with the reproductive system of birds, fishes, and mammals. The effects were noted when DDT interfere with calcinations of birds egg. This study is focused on determining the concentration of DDT in the samples, the level of uptake from soil to crop and to estimate the risk assessment of DDT residues in various species rice collected using USEPA method.

2.0 Material and methods

2.1 Equipments and chemical reagents used

The equipments used in the study include: GC-MS machine, 150 mL beaker, Blender, 0-1000 micropipette, Reagent bottles, Separating funnel , 2 in 1 magnetic stirrer and heater, 500 mL and 25 mL measuring cylinder, Sample bottles, Test tubes, Stirrer, Micro pipette, Syringe, Sonicator, Filter paper and centrifuge. While the chemical reagents used in this research are: Acetone, Ethyl acetate, Sodium sulphate, Silica gel, Sodium acetate, Acetonitrile, Magnesium sulphate, Distill water, anhydrous magnesium and n-Hexane.

2.2 Study area

The location of sample collection is Otukpo in Benue state. Its geographical coordinates are 7^{o} $27^{!}$ North, 7^{o} $58^{!"}$ East. Otukpo is a local government in Benue State located in the Middle Belt Region of Nigeria as shown in Figure 1. Otukpo is the headquarters of the Otukpo Local Government Area and remains an important town in Idoma land. It is an area dominated by the Idoma speaking people, though with diverse dialects of Idoma land. Otukpo Idoma language is the major dialect.

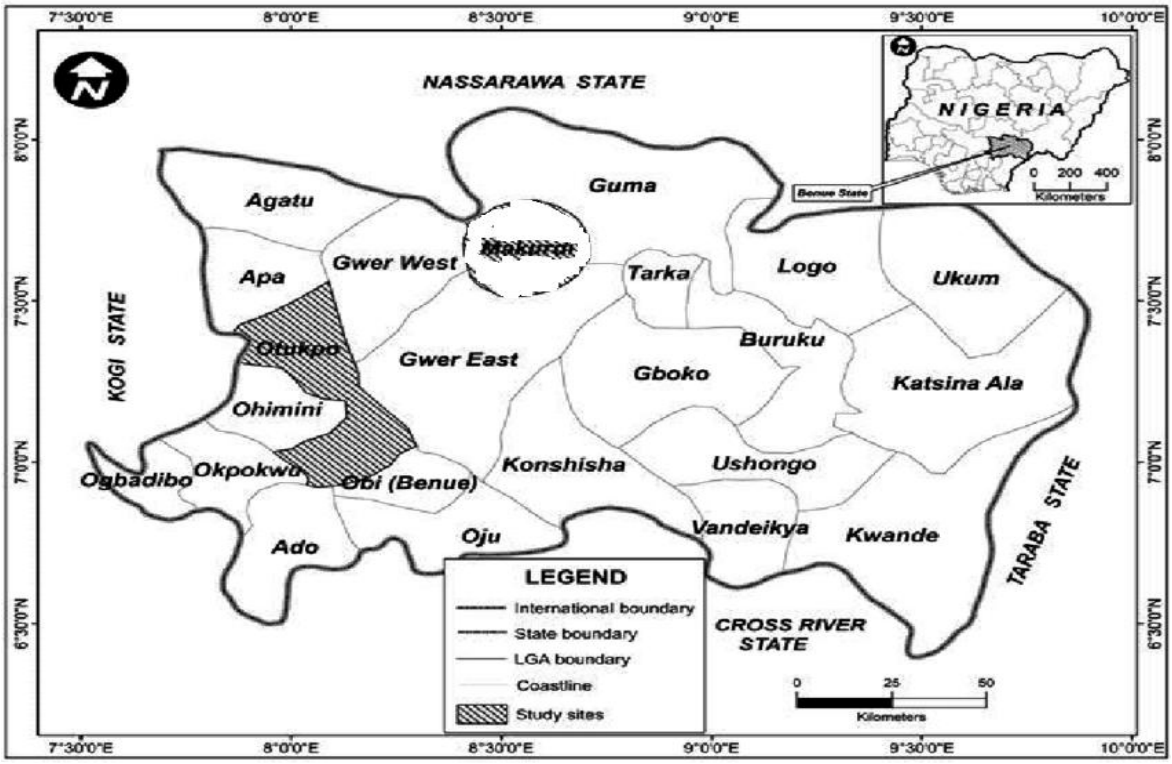

Fig 1: Map of study area showing Otukpo Local Government

2.3 Sample collection and treatment

Soil profile of depth of 20cm was dug and soils were collected. Eight soil samples (labeled S_A - $S_{H)}$ and two control samples (labeled as CR_1 AND CR_2) were collected from different soil profiles, during dry season, then package in a sterile amber sample collection bottle and transported to Maeve Research

Laboratory for analysis. The soil samples was air-dried in the laboratory for one week, picked for obvious non-soil and extraneous materials, ground in agate mortar and sieved through a 2mm mesh. These were stored in black polythene bags prior to analysis. Different species of local rice (R_1, R_2, R_3, R_4 and R_5) were collected randomly from the selected farmland of Adoka community. Fifty grams of harvested samples were collected from the farmland and transported to the laboratory. These samples were chopped, sub-sampled and preserved in a freezer till further processing.

2.4 Physicochemical analysis of sample

"The pH and cation exchange capacity of the soil samples was determined by standard method as described by A.O.A.C (2016) [9,10], while the wet oxidation method of Walkley and Black (2004) was used to determine the total organic carbon contents from which organic matter content was calculated. Furthermore, the moisture content was determined by thermo-gravimetric method" [11].

2.5 Laboratory Procedure

2.5.1 Extraction of organochlorine pesticides residue from soil samples

"All the reagents used were of analytical grade and glass wares used for the study were cleansed as prescribed by Method 1699 of USEPA (2007). Extraction of the soil samples was carried out by the National Environmental Method Index (NEMI) method described by Parveen" [12] . "Ten grams of each sample and 20 g of anhydrous sodium sulphate was grounded into dry powder. The grinded sample was extracted with 150 mL of a mixture of Acetone and n-Hexane (2:1). After extraction, the extract was transferred into a round bottomed-flask connected to a pre-weighed receiver through a Liebig condenser and concentrated to about 20mL on a water bath maintained between 50^oC and 55^oC. The solvent left in the concentrated extract were evaporated using a rotary evaporator. The almost - dry extracts were cleaned up in a micro-columns. Two grams of activated silica gel was packed into a chromatographic micro-column of 10 mm internal diameter and approximately 10 cm long. The silica gel was conditioned with 10mL n-Hexane, while the sample extracts were dissolved in 5mL n-Hexane before they were loaded onto the separate micro-column. Elution of each of the sample was done with 50 mL of ethyl-acetate: hexane mixture (9:1). The eluents were placed on a rotary evaporator at about 45^0C and under a stream of nitrogen gas. The almost-dry concentrates were then dissolved in 2ml acetone and were transferred into vials for subsequent injection into the Gas Chromatograph using a Shimadzu GCMS-QP2010SE model, equipped with auto samples and using the MS detector for analyzing the OCPs".

2.5.2 Extraction of organochlorine pesticides residue from rice samples

Fifty grams each of rice samples collected from the farmland was transported to the laboratory. These samples were chopped, sub-sampled and preserved in a freezer till further processing. The methods of Tahir were followed for extraction and clean up of samples [13]. Fifty grams of the sample was chopped and mixed thoroughly. A subsample of 25 grams were measured and mixed with 50 mL of acetone, 50 grams of anhydrous sodium sulphate and 50 mL of a mixture of Cyclohexane and Ethylacetate (1:1). The mixture was still-stand for 5mins until a clear supernatant was formed and thirty (30) mL supernatant solution was extracted into a volumetric flask. Drops of 10% propandiol in ethylacetate and 4-6 glass beads were added. The solvent was evaporated at 40°C under vacuum in rotary evaporator. The solvent was reconstituted in 6 mL of cyclohexane and ethylacetate and passed through high-flow super cells. Two mL of this sample was introduced on GPC column for further cleanup. After passing through GPC column, the samples were dried under vacuum and restored in 1mL Ethylacetate for analysis on Gas Chromatograph (GC) with mass spectrometric detector.

2.6 Statistical Analysis

The data obtained were computed as mean ± standard deviation (SD) and One Way ANOVA was used to compare obtained means and to test for the significant differences for the physicochemical properties. Values with $P \leq 0.05$ were considered statistically significant.

2.7 Human health risk assessment model

"Human health risk assessment was carried out to deduce the nature and extent of adverse health effects in humans as a result of exposure to OCPs through soil and rice around the vicinity of the study areas. Assessment was carried out for both adults and children for carcinogenic health risk. The Incremental lifetime cancer risk (ILCR) represents the incremental chance that an individual will be affected by cancer during his lifetime as a result of exposure to a potential chemical carcinogen" [14]. Therefore, the ILCR of the three pathways within the scope of the study was calculated using the following equations adopted from the USEPA standard models using the equation below:

$$\text{ILCR–ingestion} = \left(\frac{\text{CS} \times \text{CSF} \times \sqrt[3]{\frac{BW}{70}} \times \text{IRsoil} \times \text{EF} \times \text{ED}}{\text{CF} \times \text{BW} \times \text{AT}}\right) \quad (1)$$

$$\text{ILCR–dermal} = \left(\frac{\text{CS} \times \text{CSF} \times \sqrt[3]{\frac{BW^1}{70}} \times \text{SA} \times \text{FE} \times \text{AF} \times \text{ABS} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT} \times \text{CF}}\right) \quad (2)$$

$$\text{ILCR} - \text{inhalation} = \left(\frac{\text{CS} \times \text{CSF} \times \sqrt[3]{\frac{BW}{70}} \times \text{IRair} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT} \times \text{PET}}\right) \quad (3)$$

where CS is the concentration of the contamination in soil and rice (mg/kg); CSF is the carcinogenic slope factor (1/(mg/kg/d)); BW is the average body weight (kg); IR_{soil} is the ingestion rate of soil (mg/d); EF is the exposure frequency (d/yr); ED is the exposure duration (yr); AT is the average life span (d), SA is the surface area of the skin that contacts the soil (cm^2/d); FE is the fraction of dermal exposure ratio to soil; CF is the conversion factor (106 mg/kg); AF is the skin adherence factor for soil (mg/cm^2); ABS is the dermal absorption factor (chemical specific); IRair is the inhalation rate (m^3/d); and PET is the particle emission factor (m^3/kg). The total risks in different age groups were estimated as the sum of individual risk for the three exposure pathways and the reference used for estimating the ILCR is listed in Table 1 and 2.

Table 1: Reference values of the exposure parameters for the estimation of the incremental lifetime cancer risk [15].

Exposure parameters	Childhood	Adolescence	Adult
BW	10	47	60
(IR_{soil})	200	100	100
EF*	350	350	350
ED	6	14	30
AT	LT×365	LT×365	LT×365
LT	72	72	72
SA	2800	2800	5700
FE	0.61	0.61	0.61
AF	0.2	0.2	0.07
ABS	0.13	0.13	0.13

IRair	10.9	17.7	17.5
PET	1.36×10^{9}	1.36×10^{9}	1.36×10^{9}

*same value for soil and rice

Table 2: The carcinogenic slope factor (1/ (mg/kg/d)) of DDT through ingestion, dermal contact and inhalation.

OCPs	$CSF_{ingestion}$	CSF_{dermal}	$CSF_{inhalation}$
Total DDT	3.40E−01	4.86E−01	3.40E−01

2.7.1 Health risk assessment for rice

Body weights (BW) of 10 kg for children and 60 kg for adult were used, C is the concentration of DDT in rice (mg/kg), ADI is the acceptable daily intake (MAL 0.02 mg/kg), IR is rice ingestion or rice consumption rate which was estimated to be 70 mkg/day, ED is exposure duration (70yrs), Consequently, for each type of DDT exposure, the estimated daily intake (mg/kg) was obtained using the following equations below:

$$\text{Estimated Daily Intake} = \left(\frac{C \times IR}{BW}\right) \quad (4)$$

$$\text{Hazard Indices} = \left(\frac{EDI}{ADI}\right) \quad (5)$$

2.8 Sampling techniques

The sampling technique employed in this research work is random sampling due to the difference in texture of soil samples and sizes of rice samples. Therefore every sample has equal probability of being selected.

2.9Analytical quality assurance and quality control

Proper quality assurance procedures and precautions were taken to ensure the reliability of the results. The samples were carefully handled to avoid any external influences that could interfere with the integrity of the sample and hence contaminate it. Glass wares were properly cleaned, and reagents were of analytical grades. The use of Deionized water was employed throughout the study.

3.0 Results and Discussion

3.1 Physicochemical Parameters of Soil and Rice sample

Physicochemical parameters analyzed in soil samples include; pH, Cation exchange capacity (CEC) and total organic carbon while pH and moisture content were analyzed in the rice samples. A summary of the mean and standard deviation of the physical parameters of Soil and Rice samples are presented in Table 3 and Table 4 respectively. The results obtained showed that pH values analyzed were slightly acidic and slightly basic for different samples being analyzed ranging from 5.0± 2.0 to 7.0± 1.0 in Table 3 and 6.7± 0.11 to 8.0± 0.13(rice samples) in Table 4. The mean values of organic carbon were observed to vary significantly from one area site to another, and values randomly differ within the site, hence there is no consistent sequence. OCPs i.e DDT have the tendency to be absorbed by soil organic matter (SOM) due to their hydrophobicity, and high soil organic matter content provides adequate carbon for soil microbes to facilitate the degradation of OCPs. This was determined for each soil by summing up the values of exchangeable base and exchangeable acidity for each soil in each sample plot as described by [10]. Results of CEC shows that soils from the farmland has moderate values of CEC ranging from 5.60±0.13 to 12.0 ± 0.33 meq/100g as shown in Table 3. The high value of CEC in the soils indicates that farmland has higher

nutrient storage capacity; therefore, collected soil samples are more fertile compared to control sites. "The CEC of soil greater than 10 meq/100g is regarded as being suitable for crop production" [16]. For soil with lower CEC, it is advisable to increase the clay content of the soil.

Table 3: Physicochemical parameters of soil samples

Soil samples	PH	TOC (%)	CEC (cmol/kg)
Sample A	5.0±2.0	23.80±0.18	8.30±0.62
Sample B	7.0±1.0	19.40±0.19	9.80±0.43
Sample C	6.0±1.0	22.80±0.10	6.10±0.55
Sample D	8.0±1.0	21.30±0.06	7.40±0.23
Sample E	6.0±1.0	10.60±0.55	9.50±0.41
Sample F	6.0±1.0	14.10±0.11	12.0±0.33
Sample G	6.0±1.0	11.00±0.22	8.90±0.62
Sample H	6.0±1.0	8.90±0.31	7.20±0.22
Control site 1	7.0±0.0	6.90±0.31	5.90±0.11
Control site 2	7.0±1.0	6.90±0.305	5.60±0.13
MEAN	6.2	14.57	7.85
WHO (1993)	6.5 – 8.5	-	-

Results presented as mean ± standard deviation of three replicates. No significant difference at $p<0.05$.

Table 4: Physicochemical parameters of Rice samples

Rice samples	pH	MOISTURE (%)

R_1	8.0±0.13	7.90±0.02
R_2	7.6±0.05	4.80±0.03
R_3	6.8±0.12	8.07±0.02
R_4	6.7±0.11	4.80±0.015
R_5	7.5±0.11	2.60±0.02
MEAN	7.34	5.63

Results presented as mean ± standard deviation of three replicates. No significant difference at $p<0.05$.

3.2 Concentrations of DDT in soil and rice samples

Table 5 reveals the mean concentrations of DDT analyzed in soil and rice samples. The concentration of OCPs was quantified using the peak area on the chromatogram. The concentration of DDT was found to be high in some samples, low in some samples and was not detected at all in some samples (both soil and rice). In the rice sample, the lowest concentration was detected in R_1 (1.9 mg/kg), the highest concentration was detected in R_5 (6.74 mg/kg) and there was no OCP detected in Mars. These concentrations when compared were found to be above the EC MRLs (0.05 mg/kg) and FAO/WHO MRLs (0.1 mg/kg) which is capable of posing serious carcinogenic risk when consumed or exposed to target populations.

Table 5: Concentration of DDT residues in soil in mg/kg

SOIL SAMPLES	Total DDT (mg/kg)	**RICE SAMPLES**	Total DDT (mg/kg)
S_A	1.9	R_1	1.9
S_B	ND	R_2	2.77
S_C	32.4	R_3	ND
S_D	41.3	R_4	5.64
S_E	29.35	R_5	6.74
S_F	ND	AVERAGE	3.41
S_G	ND	FAO/WHO (2005)	0.1
S_H	ND		
CR1	ND		
CR2	ND		
AVERAGE	10.5		

FAO	0.1
WHO (2004)	0.1
EC MRLs	0.05

ND= Not Detected or below detection limit

The ILCR in this study was calculated to interprete the lifetime risks of exposure to soil-borne OCPs through the pathways of ingesting, dermal contact and inhalation. The total risk is the sum of all the risk associated with the three exposure pathway which reveals that from the calculated results in this research, adolescence are more prone to carcinogenic health effect caused by DDT associated with the farmland under study while children are least prone to lifetime risks of exposure i.e children adult < adolescence as shown in Table 6 below:

Table 6: Exposure risk of DDT

OCPs	Exposure pathway	Dermal	Inhalation	Ingestion	**Total risk**
DDT	Child	6.300×10^{-3}	1.197×10^{-11}	2.814×10^{-3}	$\mathbf{9.114 \times 10^{-3}}$
	Adolescence	5.250×10^{-3}	1.617×10^{-10}	1.170×10^{-2}	$\mathbf{1.700 \times 10^{-2}}$
	Adults	6.300×10^{-3}	2.900×10^{-10}	2.130×10^{-2}	$\mathbf{2.760 \times 10^{-2}}$

3.3 Exposure status of DDT

Results indicate that health risk mainly occurs from dermal uptake and ingestion. Direct Inhalation contributed least to the total risk, but is still a concern. This result contrasts with some previous studies that consider ingestion and inhalation as the predominant exposure pathways ([17]; [13]; [18]; [19]), likely because most of these assessments considered food chains as the exposure pathway. In this study, food chains was not prioritize as an exposure pathway when predefining the exposure conditions; because the study area is within the farmland, where most of the residents rice are transported from within the geographical area, thus little health risk came from Inhalation intake. According to US Environmental protection agency, it considers a cancer risk above 1×10^{-4} as harmful while cancer risk less than 1×10^{-6} as negligible [20] which when compared to the results obtained in this research shows that the exposure route through inhalation is harmless but dermal and ingestion contact are said to be harmful and could cause serious carcinogenic effect. It can also be deduced that the species of rice that can cause carcinogenic effect is R_4 and R_5 due to the fact that their HI value is greater than one in children. Therefore life consumption of the rice containing a measured level of DDT in children has the tendency to pose serious health risk such as endocrine disruption in children. So it is advised that the consumption of such species of rice be minimized in children. The various values of the Hazard indices are as shown graphically in fig 2.

Table 7: Concentrations, EDI, and health risk estimation for OCP residues detected in rice samples

Samples	Pesticides	Concentration	EDI	ADI		HI
R1	DDT	1.9	2.22×10^{-3}	0.02	Adults	1.11×10^{-1}
			1.33×10^{-2}		Children	6.65×10^{-1}
R2	DDT	2.77	3.23×10^{-3}	0.02	Adults	1.62×10^{-1}
			1.94×10^{-2}		Children	9.70×10^{-1}

R3	DDT	-	-	0.02	Adults	-
			-		Children	-
R4	DDT	5.64	6.58×10^{-3}	0.02	Adults	3.29×10^{-1}
			3.95×10^{-2}		Children	$*\mathbf{1.97 \times 10^{0}}$
R5	DDT	6.74	7.86×10^{-3}	0.02	Adults	3.93×10^{-1}
			4.72×10^{-2}		Children	$*\mathbf{2.36 \times 10^{0}}$

** HI value greater than one*

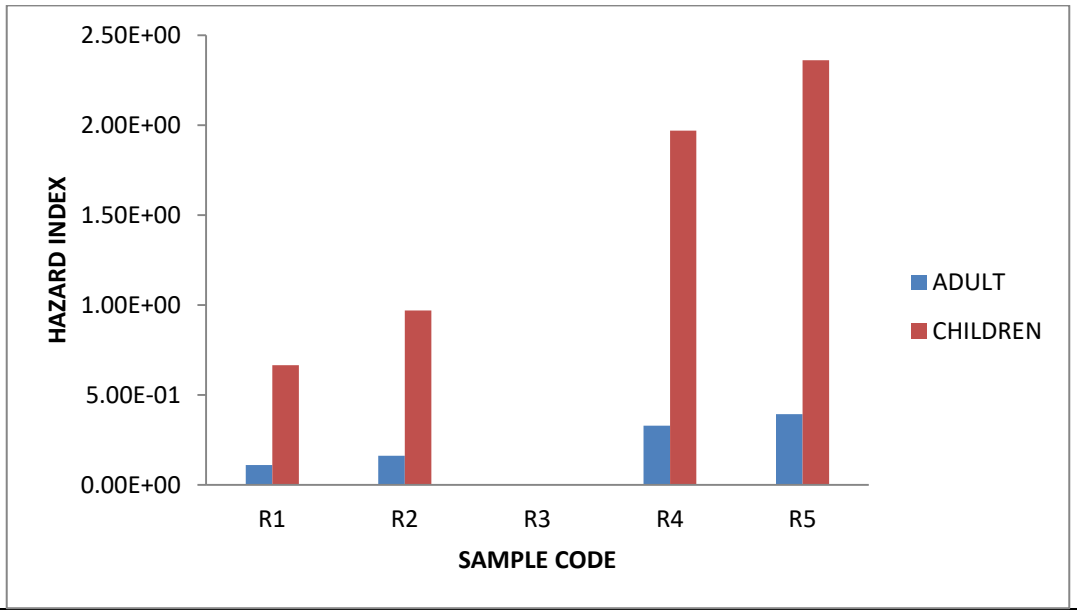

Fig 2: Hazard index of DDT in adult and children

4.0 Conclusion

This research shows that the rice cultivated in the farmland under study is concentrated with residues of pesticides containing DDT which is capable of posing serious health effect on consumption over a long period of time. The application of pesticides contributes in the accumulation of pesticide residues in food associated with variety of human health hazards, including damage to central and peripheral nervous systems, and disruption of the immune system. Hazard index shows that children are more prone to health challenges than adult when exposed to dichlorodiphenyltrichloroethane through consumption of rice. The impact of pesticide residues generally can be minimized by introduction of preventive measures such as use of natural pesticides and bio-pesticides and strict implementation of pesticide-related laws. Routine analysis should also be carried out to check the extent of degradation of OCPs in soil and crop cultivated on the land.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the

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Data Availability Statement
All data and materials are available