

Full Length Research Paper

Multi residue analysis of pesticides in pre and postharvest wheat grains in Misha Woreda, Hadiya Zone, Ethiopia

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This study was conducted to assess the presence of pesticides residues in wheat from Misha woreda, Haiya Zone, Ethiopia. Samples of wheat were collected during the period December 2015 to August 2016. Gas chromatograph (GC-MS) detection was employed after Liquid-Liquid extraction (LLE). The concentration levels of eight pesticides have been investigated in both wheat samples in farm and purchased from storage facilities. Since the method was out of scope, it was validated before application to the analysis of these pesticides. The spiked recovery results for five analytes were within the acceptable international standard. The LOD value and LOQ value are below the LCC and MRL of the analyte. Method validation for this study maintained a RSD <20% and percentage recoveries were in the range of 80-110%. The %RSD of all detected analytes was less than the acceptable international standard. Results show that out of the residues of eight analytes determined, four analytes (2,4-D; aldrin; Endosulfan; p,p-DDT) were detected in both field (pre harvest) samples and stored (post harvested) sample, whereas the remaining analytes were not detected at all. In general, the concentrations of these analytes in the wheat samples are in the order: 2, 4-D >aldrin>p,p-DDT >Endosulfan. The study observed that from the detected analytes, none of them are above the international MRL values and suggested that stringent monitoring of use of pesticides in agriculture and food storage is required.

Key words: Pesticide residue, wheat, Gas chromatography–mass spectrometry (GC-MS), method validation, maximum residue limit.

INTRODUCTION

Modern agricultural practices often include the extensive use of a wide range of pesticides for increased crop production as well as for greater yield by controlling pests

(Nilufar, 2005; Ghosh and Philip, 2006). The use of pesticides, therefore, continues to exist as world population and the demand for food production continues to grow.

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In 1995, conventional pesticide use in USA amounted to about 1.22 billion pounds, which was one-fifth of the world's use of such chemical. In spite of the undeniable advantages that pesticides have brought to modern agricultural economy for controlling pests, they can generate a series of problems for untargeted organisms, if the necessary precautions are not taken during applications and storages (Abdulahi, 1997). In general, agricultural chemicals, while often benefiting farming productivity, can have determinable environmental effects when applied improperly. Therefore, pesticides and their potentially undesirable effects on the environment, aquatic organisms and human health has been one of the major concerns of recent research. At present, pesticides of 32 billion dollars have been marketed in the world (Lesan and Bhanderi, 2000).

Nowadays, more than 1100 pesticides are possibly used in various combinations and at different stages of cultivation and during postharvest storage to protect crops against a range of pests and fungi and/or to provide quality preservation. Pesticide residues in cereals samples, which might pose a potential risk for human health due to their sub acute and chronic toxicity, could possibly end up in the final products of crops. Contaminants of animal feed can cause harmful health effects in the animals and may be harmful to people through secondary exposure of consumers to products deriving from these animals. Contamination of feedstuffs may include both naturally occurring and synthetic toxic compounds (Walorczyk, 2008).

Since these pesticides provide unquestionable benefits for increasing agricultural production, cereal grains however, usually receive direct application of pesticides in the field or in post-harvest treatment and may retain a proportion as residues in or on the edible portion delivered to the consumer (Seyed and Somashekar, 2010). Therefore, public concern about the contamination of food by pesticides has been increasing over the past years due to the uncertainty about the adverse effect those residues may pose over a long-time exposure. The toxicity of most pesticides and consumption of raw cereal grains reinforce the concern for contamination of these food substances over other foods (Jos'e et al., 2004). As a result, levels of pesticides in different food item are regulated by international and national organizations in order to protect human health (Flemming, 2000). That is, for the protection of the public against the toxic effects of pesticides, regulatory agencies in many countries have established standards specifying the residue levels of each pesticide in various foodstuffs. At an international level, the WHO, in conjunction with the FAO, has been convening Joint FAO/WHO Meetings on Pesticide Residues annually since 1991. At these meetings, the toxicological and related data are evaluated for the establishment of an acceptable daily intake or provisional tolerable daily intake. Almost every country either imports or exports food. Especially, many African countries like

Ethiopia do not have adequate food security, resulting in a situation where at least 60% of the food supply is imported to supplement local production (Gayathri et al., 2007). Thus guaranteeing the safety of both imported and locally produced food begins on the farm or on the port and follows through the entire food chain until meals. In Ethiopia, cereals, among which are teff, barley, maize, sorghum, oats, and wheat, make up 85 and 90% of the total cultivated area and total production of field crops respectively and accounts for over 90% of modern input consumption (FAO/WHO, 2008; 2010).

Wheat is the most important cereal crop and constitutes the main component of millions of heads world over. It is frequently stored for long periods of time with consequent risk of heavy insect infestation. Storage in transit is also a critical stage and must be given full attention. It has therefore, become a normal agricultural practice to spray or fumigate stored grains, particularly prior to shipment with contact insecticides so that they reach the port of destination free from any pest infestation (Daglish et al., 2003). Soils often receive different fungicides and effect of these has been studied on wheat crop. There are two principal sources of pesticides in wheat grain; firstly, spraying pesticide to growing crops. Secondly, the admixture of pesticides is used on stored commodities. But the main route of this contamination is grain protectants. Cereal grains are treated with degradable pesticides, including organophosphate pesticides, carbamates, synthetic pyrethroids and insect growth regulators to prevent insect infestation during storage period (Fatiha et al., 2006). Cereal grain might be contaminated with several pesticides, which can enter in food chain of human consumption with its consequential hazard. In addition to grain handlers and traders, cereal processing companies and regulatory agencies are potential users of these methods. For processors, analysis of baked or cooked end products and intermediate products such as flours are also important. Wheat is the major staple food in Ethiopia which is the major wheat producer in sub-Saharan Africa. Hadiya zone, especially Misha woreda is the largest area of wheat producing zones of Ethiopia. Despite the documented frequent and occasionally heavy use of pesticides in Ethiopia, there is limited information on the level of pesticide residues in wheat samples from some localities in the region to explore the level of contamination and determine the risk potential.

Thus, it is deemed absolutely necessary to establish several reliable, rapid, inexpensive and effective analytical methods for simultaneous determination of the residues of many pesticides. The main objective of this research study was to assess the concentration of pesticide residues in Wheat (*Triticum aestivum* L.) and estimate the potential health risks associated with the pesticide residue with regard to consumers. The selected pesticides in this study are being used to a large extent in Hadiya Zone, Misha woreda for grain protection, and

technical data is not fully available on them. In order to achieve this goal for proper recommendations, knowledge of these aspects is very important.

MATERIALS AND METHODS

Description of the study area

Hossana, the capital city of Hadiya zone of North West Ethiopia, is 235 km South from capital city, Addis Ababa, located at 7° 33' 32" N Latitude and 37° 51' 58" E Longitude, with subtropical climate along with a mean annual temperature of 17.1°C and annual average rainfall ranging from 920.4 mm to 1436.5 mm. The highest temperature is experienced between January and March and the lowest between July and September. Also, Misha is one of the woredas in Hadiya Zone and has been divided into 11 local government areas (LGAs). Misha is bordered on the south by the Gomibora, on the southwest by Gibe, on the west by the Yem special Woreda, on the north by the Gurage Zone, on the east by the Silt'e Zone, and on the southeast by Limo. Based on 2007 census results with regional average growth rate of 2.9%, the population of Hossana town was estimated to be 83,046 in 2013.

Sampling methods

Samples of wheat were collected in both field and storage facilities (that is, merchants around Hadiya Zone and wheat factories in the region) in six local areas around Hadiya Zone, Ethiopia. The markets were assessed for the suitability of proper sampling and the merchants around Hadiya Zone are orally interviewed on the origin of their wheat. Sampling was conducted in two phases. Firstly, during the harvest periods (November - December 2015) from eighteen different farms (three each at the LGAs) and secondly, four months postharvest period at storage and preservation facilities by using stratified random sampling method. The postharvest samples consists of samples not limited to the last farm harvests. Wheat samples were purchased, first by selecting the shops using convenient sampling technique. For purchasing maximum of 6 kg of wheat, three shops were sampled from the 15 shops. Thereafter, the samples were packed with polyethylene plastic bag and were kept in a refrigerator for a week until sample preparation started for the analysis. Before sample preparation, the samples were further stratified by the size (weight) into three portions as large, medium and small to make homogeneous sample. Also, about 300 to 350 g was taken from each portion to make a sub-sample of 1 kg (QSAE, 2005).

Chemicals and materials

Pesticide standards

Pesticide standards with the corresponding purity were obtained from Adamitulu Pesticide Preparation Company, Zuway, Ethiopia.

Standard pesticide solutions preparation

Pesticide stock solution of 1000 mg/ L was prepared by dissolving 25 mg of each pesticide in 25 ml of acetone.

Organic solvents and reagents

Acetone, n-hexane, diethyl ether, and sodium chloride of analytical

grade was obtained from Sigma–Aldrich (Steinheim, Germany). Other necessary materials Na₂SO₄ (99.0 – 100.5%, Merck, extra pure), Florisil adsorbent (60–100 mesh, Merck, residue grade), Diatomaceous earth were obtained from BDH chemical Ltd Poole England. Florisil were activated by taking 1 kg of its reflex in 2.5 L of distilled water for one hour. (Jos'e et al., 2004).

Analytical methods/techniques

Sample extraction and enrichment techniques pesticide residues appear in environmental samples at trace levels, which are well beyond the detection capability of most available analytical techniques. Some of the techniques commonly used for extraction/preconcentration of pesticide residues from aqueous matrices include liquid–liquid extraction (LLE).

Sample preparation

In pesticide residues analysis, basically there are three steps represented by the following flow diagram (Figure 1). Under the sample preparation step, there are also sub-steps like homogenisation, extraction, concentration and clean up. Each of them will be sequentially done in the experimental work.

Sample extraction

An international standard method of sample extraction for wheat (cereal grains) was used in this study (Renato et al., 2004). This method is described briefly as follows: A total of 1 kg of wheat samples were chopped and then blended for 3–5 min using Waring blender. A 20 g portion of the homogenate was weighed and transferred to a 250-mL beaker, followed by addition of 20 mL water and kept for 2 h for equilibration. It was then extracted twice with 100 mL and 50 mL of acetone. During each time of extraction, it was homogenized for 3 min using magnetic stir bar and suction filtered using Whatman No. 42 filter paper containing 1 cm diatomaceous earth. The filtrate was transferred to evaporatory flask and the solution was evaporated at 40°C to 30 mL. The 30 mL extract was transferred to 500-mL separatory funnel containing 100 mL of 10% sodium chloride solution. Thereafter, it was partitioned using 100 mL of n-hexane after shaking for 5 min. The n-hexane layer was transferred to a conical flask; anhydrous sodium sulfate were added until freely flowing and kept for 15 min by intermittent shaking. The content was suction filtered in an evaporatory flask and evaporated to dryness. The residue were dissolved in 5 mL n-hexane and proceeded to clean up step.

Clean-up

Florisil column preparation: a homemade glass column (1.5 cm i.d.-40 cm length) containing a piece of glass wool were filled with 10 g of Florisil in n-hexane and about 5 g of anhydrous sodium sulfate was topped on the florisil. The following clean-up processes were used before chromatographic analysis: 5 mL of the extract were transferred to the Florisil column; the pesticide residues were eluted with 200 mL of n-hexane–diethyl ether (17:3, v: v) mixture and the eluate was collected in a conical evaporating flask. The eluate was concentrated in a vacuum rotary evaporator to approximately 1 mL at 40°C. Thereafter, the eluate was N₂ –gas dried and the residue were dissolved in 2 mL n-hexane for chromatographic analysis.

Gas chromatography-mass spectrometry (GC-MS)

The popularity of GC is based on a favourable combination of very

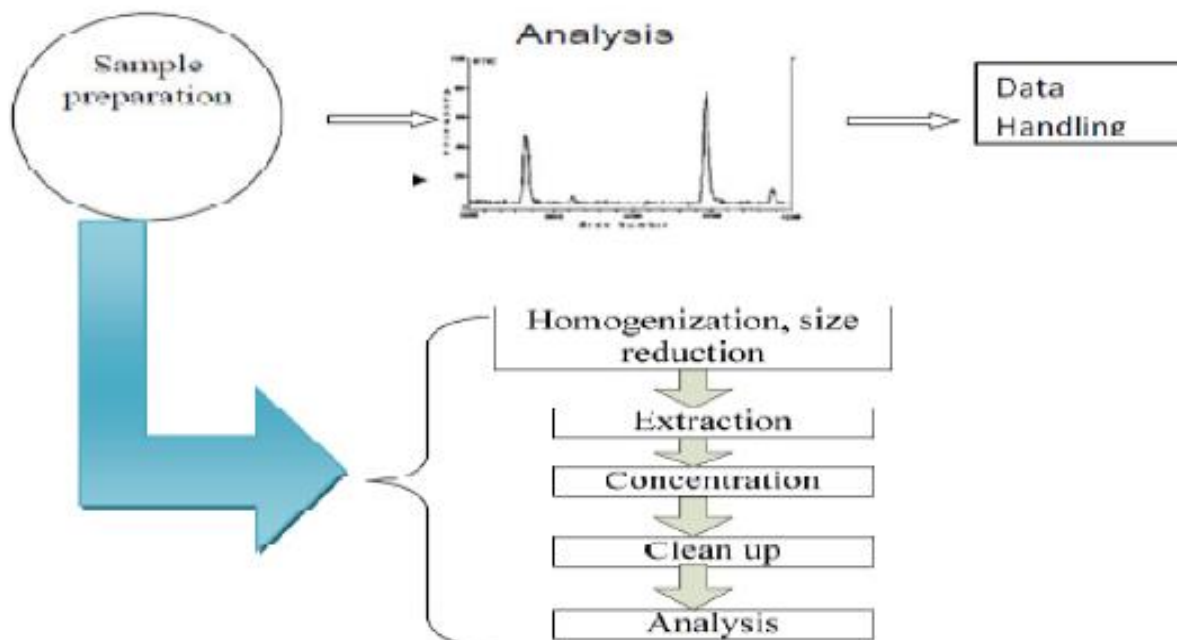


Figure 1. Steps in the analysis of pesticide residue in cereal crop.
Source: Quality Control Procedures for Pesticide Residues Analysis, SANCO (2003).

high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity. At present, more than 60% of registered pesticides and/or their metabolites are amenable to GC analysis. This has made GC the analytical method of choice for most analytical work involving pesticides. Fused-silica capillary columns have almost replaced the packed columns, allowing the separation of a large number of analytes with similar physico-chemical characteristics (Andrue and Pico, 2004).

Statistical data analysis

The data obtained were recorded, organized and summarized in simple descriptive statistics methods using SPSS-PC statistical package (SPSS version 20 for windows). Results were presented in tables and ANOVA for mean comparison among parameters. Moreover, interpretations of data were done by their magnitudes such as concentration of the level of pesticide residue per analyzed wheat sample.

RESULTS AND DISCUSSION

The objective of this research was determination of the state of contamination of wheat samples with pesticides. Organochlorines, organophosphates, carbamates and pyrethroids pesticides are the common and major classes of pesticides applied to food crops especially wheat in the field and at storage. The eight pesticides, mainly as insecticides, determined in this study covered these common and major classes of pesticides. Residues of eight targeted pesticides were detected in the analyzed samples, that is, α -HCH, 2,4-D, aldrin, Cyhalothrin, Endosulfan, Dieldrin, Malathion and p,p-DDT. The

discussion of the present study was focused on two areas, that is, quantitative evaluations of the pesticide residue results compared to the Maximum Residue Limits (MRLs) of the Codex Committee which is the standard that is supposed to be followed locally to protect the Ethiopian consumers. The pesticide residue results were also compared to the EU (2010) MRLs which apply on the Ethiopian exports to the European countries. However, before the analyses were done, the methods used for the analysis were validated. For the validation purpose, five common parameters were selected.

Method validation

Calibration curve for determination of the analytes

The calibration curves were obtained using analytical solutions of the mixture of the pesticides prepared in pure solvent and prepared in the extract of the matrix in the concentration range (Bliesner, 2006). A correlation coefficient of > 0.999 is generally considered acceptable. The y-intercept should be less than a few percent of the response obtained from the target level (Anagilda et al., 2004). Accordingly, the calibration curves were obtained from a running of seven point calibration solutions having a concentration range of 5 to 2000 ng/mL (in the case of 2,4-D and for Malathion, the range were 10 to 2000 ng/mL). The lowest concentration level in the calibration curve was established as a practical determination limit for the instrument. Linearity was evaluated by the

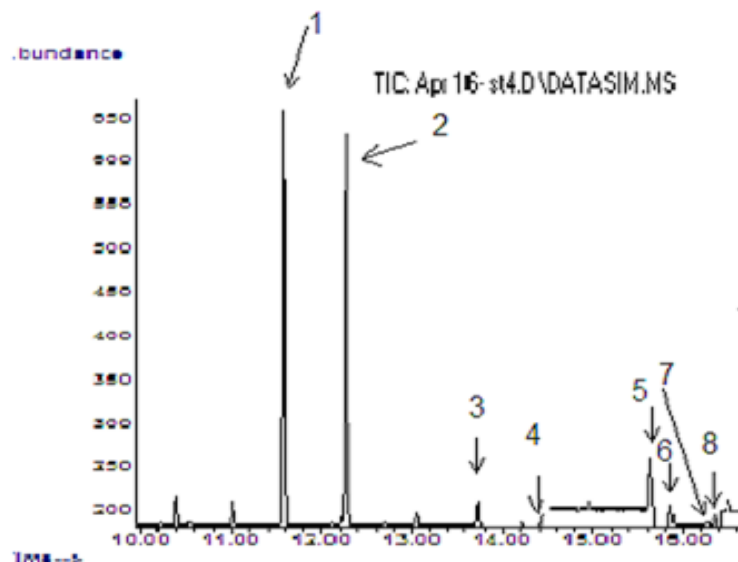


Figure 2. A representative chromatogram of 100 g/L standard analyzed for 1: α -HCH; 2: 2, 4-D; 3: aldrin; 4: Cyhalothrin; 5: Endosulfan ; 6: Dielderin; 7: Malathion; 8: p,p-DDT.

Table 1. Calibration parameters, LOD and LOQ.

Pesticide	r^2	Linear range (ng/mL)	Wheat sample from farm/ field (WSF)		Wheat sample from storage facilities (WSS)	
			LOD (mg/L)	LOQ (mg/L)	LOD (mg/L)	LOQ (mg/L)
α -HCH	0.999	5-2000	0.003	0.010	0.002	0.007
2,4-D	0.999	10-2000	0.004	0.014	0.004	0.013
Aldrin	0.998	5-2000	0.003	0.011	0.004	0.016
Cyhalothrin	0.996	5-2000	0.004	0.013	0.004	0.015
Endosulfan	0.995	5-2000	0.003	0.008	0.002	0.005
Dielderin	0.990	5-2000	ND	ND	ND	ND
Malathion	0.989	10-2000	ND	ND	ND	ND
p,p-DDT	0.999	5-2000	0.002	0.007	0.0003	0.001

calculation of a seven-point linear plots of the peak height (as observed in Figure 2 representative chromatogram from the seven concentration range) against concentration based on linear regression and squared correlation coefficient, r^2 , which should be > 0.990 . The linearity range and r^2 values are given in Table 1.

Limits of detection and quantification

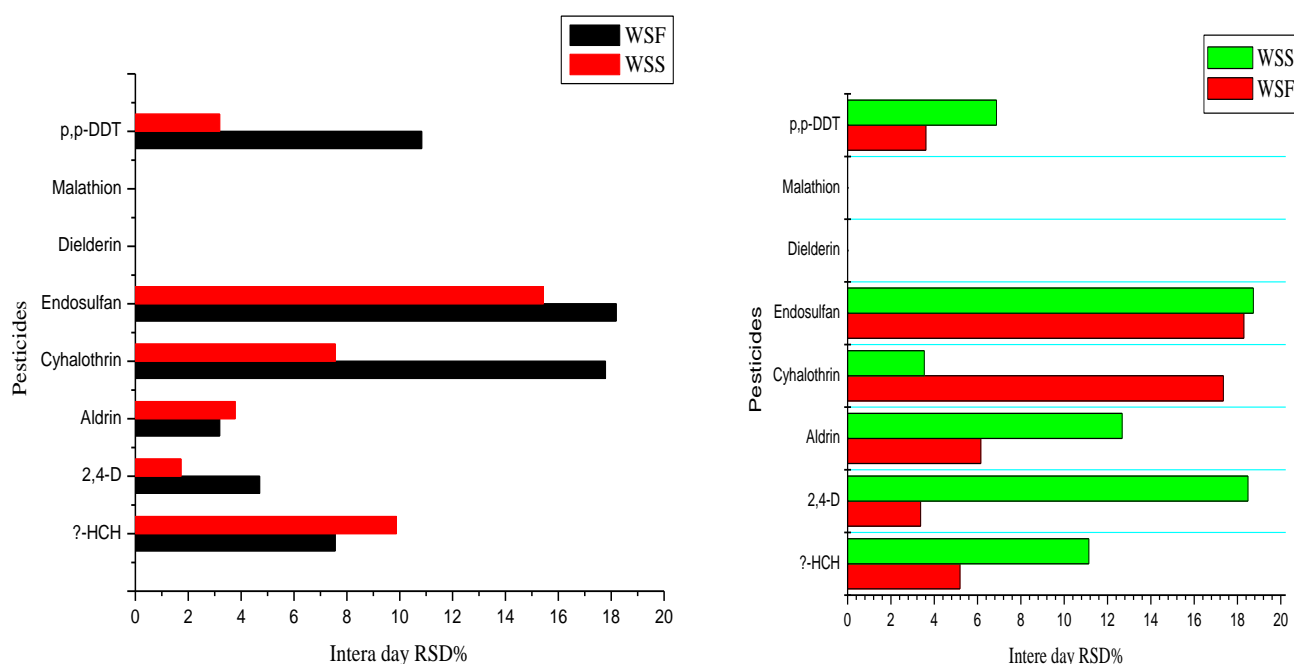
The limits of detection and limit of quantification values for the detected pesticides in the proposed method are depicted in Table 1. Limit of detection is expressed as the analyte concentration corresponding to the sample blank value plus three standard deviation or sample blanks fortified at lowest acceptable concentration (i. $LOD \equiv x_{ib} + 3s$ or ii. $0 + 3s$), and Limit of quantification is the analyte

concentration corresponding to the sample blank value plus ten standard deviations ($LOQ \equiv x_{ib} + 10s$), where x_{ib} is the mean concentration of the fortified sample blank and s is the standard deviation of the fortified sample blank (Bliesner, 2006).

Thus, determination of limits of detection and quantification were calculated according to EURACHEM (1998) recommendations. The LOD and LOQ were determined in full scan mode from samples of wheat fortified at a 10 ng/mL. From the table above, it is possible to observe that the values of limit of detection for both samples were below the lowest calibration level for all of the pesticides detected in the samples. In the same manner, when the limit of detection of the detected analytes was compared to the international MRL values set by codex, EU and Japanese MRL, it was lower value. This clearly shows that it is possible to determine the

Table 2. Repeatability and reproducibility of the studied pesticides in fortified wheat samples extracted by LLE, and analyzed using GC-MS.

Compound	Intraday RSD%		Interday RSD%	
	WSF	WSS	WSF	WSS
α -HCH	7.560	9.877	5.188	11.139
2,4-D	4.702	1.729	3.372	18.483
Aldrin	3.190	3.780	6.152	12.675
Cyhalothrin	17.786	7.564	17.346	3.537
Endosulfan	18.189	15.437	18.296	18.730
Dieldrin	-	-	-	-
Malathion	-	-	-	-
p,p-DDT	10.835	3.191	3.6134	6.876

**Figure 3.** Intraday and Interday RSD % of the studied pesticides in fortified wheat samples.

residual levels of the pesticides in these food items to much lower than the international MRLs. However, the limits of detection and quantification of Dieldrin and Malathion were not determined because the amount might be below the detection limit.

Precision

The precision of a method is the measure of agreement or closeness of analyte concentrations to each other when the analyses were performed using identical conditions, that is, the same method, same sample, same operator, and same laboratory conditions over a short period of time. This is known as repeatability (Anagilda et al., 2004). Reproducibility is data collection using the same

sample and the same method but a different operator, another set of laboratory conditions, and a different period of time (days or even weeks). The precision data is generally obtained from triplicate analyses of spiked samples.

Repeatability and reproducibility

The repeatability of the method (intra-day) was studied by running five extractions which was spiked with 0.1 mg/L of pesticides mixture within one day of extraction time. The %RSD value of all the pesticide was below 20% as shown in Table 2 and Figure 3. On the other hand, the reproducibility of the method (inter-day) was investigated by running five extractions of 0.1 mg/L spiked samples in different days of extraction time. The result of %RSD was

Table 3. Recoveries of the studied pesticides in fortified wheat samples extracted by LLE, and analyzed using GC-MS.

Pesticide	Spiked Level (µg/g)	Spiked Mean Concentration (µg/g)		Blank Mean Concentration (µg/g)		Mean Recovery%	
		WSF	WSS	WSF	WSS	WSF	WSS
<i>α-HCH</i>	0.01	0.013	0.007	ND	ND	130	78.8
<i>2,4-D</i>	0.01	0.030	0.080	0.018	0.069	112.3	105.3
<i>Aldrin</i>	0.01	0.035	0.043	0.029	0.037	70	60
<i>Cyhalothrin</i>	0.01	0.014	0.02	ND	ND	140	199
<i>Endosulfan</i>	0.01	0.004	0.003	0.001	0.0005	37	18
<i>Dielderin</i>	-	-	-	-	-	-	-
<i>Malathion</i>	-	-	-	-	-	-	-
<i>p,p-DDT</i>	0.01	0.007	0.003	0.001	0.001	60	20

also below 20%, which indicates that the method is reasonably repeatable and reproducible under the laboratory conditions available in the program (SANCO/10232., 2006).

Method bias study

Recovery

Method validation for this study consisted of sample runs for spiked analytes quality control, which was maintained at < 20% deviation. The percentage recoveries were in the range of 80 -110%. Based on a confidence level of 95%, the uncertainty of measurement is within the acceptance criteria. Wheat samples in both farm and stored was fortified at 0.01 µg/g of a mixed standard solution. The recovery rate was replicated and the data are presented in Table 3 and Figure 4. The table shows that the recovery rate for four pesticides (*α-HCH*, 2, 4-D, Aldrin and p-DDT) out of six were within acceptable range for wheat from the stored facilities (Serrahima et al., 2008). Recovery for Endosulfan was below the acceptable level, whereas that of Cyhalothrin is above acceptable value. The low recovery for Endosulfan may be due to the analyte retained in the clean-up column or during filtration or in any of the extraction step but the case of Cyhalothrin may be due to some experimental error; therefore, these has to be checked further. On the other hand, the recoveries of only three pesticides (*α-BHC*, 2, 4-D and aldrin) were within the acceptable range for the wheat samples from the farm / field (European Union SANCO, 2000). But Endosulfan and DDT were below the standard range whereas Cyhalothrin was above the recovery range.

This method is, therefore, applicable for the determination of *four pesticides* in both cultivated and stored wheat samples reasonably. Due to shortage of time and budget it was not possible for us to look into the case for others. A number of factors such as non-availability to farmers and or non-application of these insecticides during the period of study, and also from low

concentration levels below the limits of quantitation may be responsible of the non-detection of Endosulfan and DDT in both the pre storage and postharvest wheat samples analysed.

Analysis of wheat sample

After the validation of the method, wheat samples were collected from farm/ field and storage facilities were analyzed for the eight pesticide residues. In this study, samples of wheat will be collected in both field and storage facilities before sampling merchants and wheat flour factories were interviewed on the origin of their products. According to the responses of the most interviewees, wheat are coming to this market from around Hadiya zone, especially Misha woreda. Only very few of them were not sure about the origin of their products. From this preliminary assessment it is possible to conclude that the Hossaina Market is the best place to collect wheat samples for pollution study. Therefore, it was decided to purchase wheat samples from this market place.

Analysis were done three times and during all of the analysis, *α-BHC*, Aldrin, Endosulfan and DDT were detected in farm/field samples and 2,4-D was detected only in the third and fourth analysis for farm/field samples. On the other hand, 2, 4-D, Aldrin, Endosulfan and DDT were detected in the stored samples in all of the experimental analysis as the recovery of Endosulfan was not good in both samples. Whereas the recovery of DDT in farm sample are also below the standard, it was not possible to correctly determine what was available in the original sample. However, 2, 4-D, Aldrin, Endosulfan and p,p- DDT were detected only in the pre and post storage wheat samples. Pesticides residues in crops at harvest may result from uptake by the plant of soil-applied pesticides or otherwise, translocation of pesticides applied to the plant before the edible part of the plant is formed.

Residues at harvest from these circumstances are usually low and often below the limit of determination, but

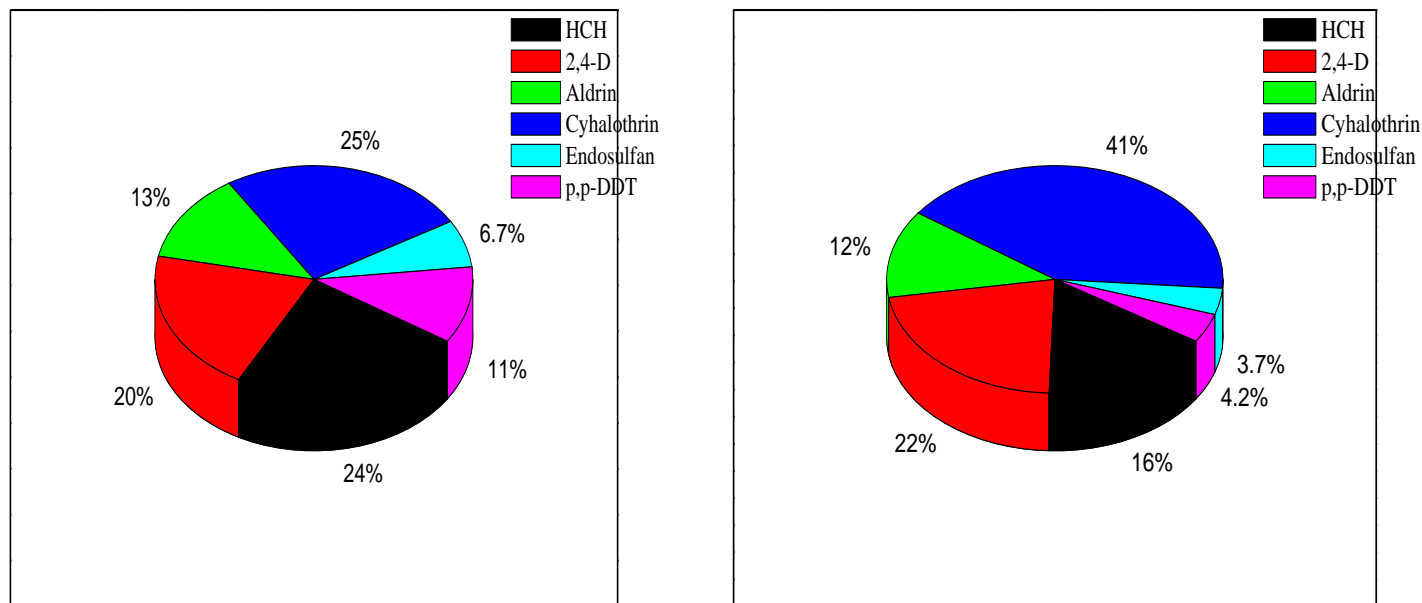


Figure 4. Percent recoveries of the studied pesticides in fortified wheat samples extracted by LLE, and analyzed using GC-MS.

Table 4. FAO/WHO, Japanese and EU MRLs of pesticides in wheat samples and concentration of analytes in the sample.

Pesticide	FAO/WHO MRLs (mg/kg)	Japanese MRLs (mg/kg)	EU MRLs (mg/kg)	WSF Mean concentration (mg/kg)	WSS-Mean concentration (mg/kg)
<i>α</i> -HCH	3.0	0.01	0.01	ND	ND
2,4-D	0.5	0.01	0.03	0.010	0.035
Aldrin	0.02	0.05	0.02	0.020	0.010
Cyhalothrin	0.2	0.02	0.02	ND	ND
Endosulfan	0.2	0.5	0.5	0.007	0.001
Dieldrin	0.02	0.05	0.02	ND	ND
Malathion	2.0	0.5	0.2	ND	ND
p,p-DDT	0.1	0.1	0.02	0.002	0.003

the majority of significant residues at harvest result from applications when the edible part of the plant is already present. DDT, 2, 4-D, and Endosulfan are obviously heavily utilized in this region due mainly to their easy availability and dependability. This is also reflected in the almost similar trend in their concentrations across the sampling locations of the present study.

However, the basic processes acting on pesticide residues in the field can continue to operate after crops are harvested. These include: volatilization, hydrolysis, penetration, metabolism, enzymatic transformation, oxidation and photodegradation, which generally ceases or is greatly reduced once a crop is removed from the field situation. These were generally observed in trends of DDT, Aldrin, Endosulfan and 2,4-D, but the higher accumulation of 2,4-D in wheat samples may be due not only to the volume of applications but also to the

solubilities of 2,4-D than rest. Residues of postharvest insecticide treatment on stored staples foodstuff generally decline only rather slowly as the rate of reductions were rather low. The regulations for all controls on pesticide residues in crops are generally based on Maximum Residue Limits (MRL's) which are set using field trial data for a particular pesticide to arrive at the highest residue levels expected under use according to Good Agricultural Practice (GAP).

Comparison of results obtained with MRLs values of different source

Table 4 and Figure 5 shows the mean value of the three experimental analyses against the MRL values from different sources.

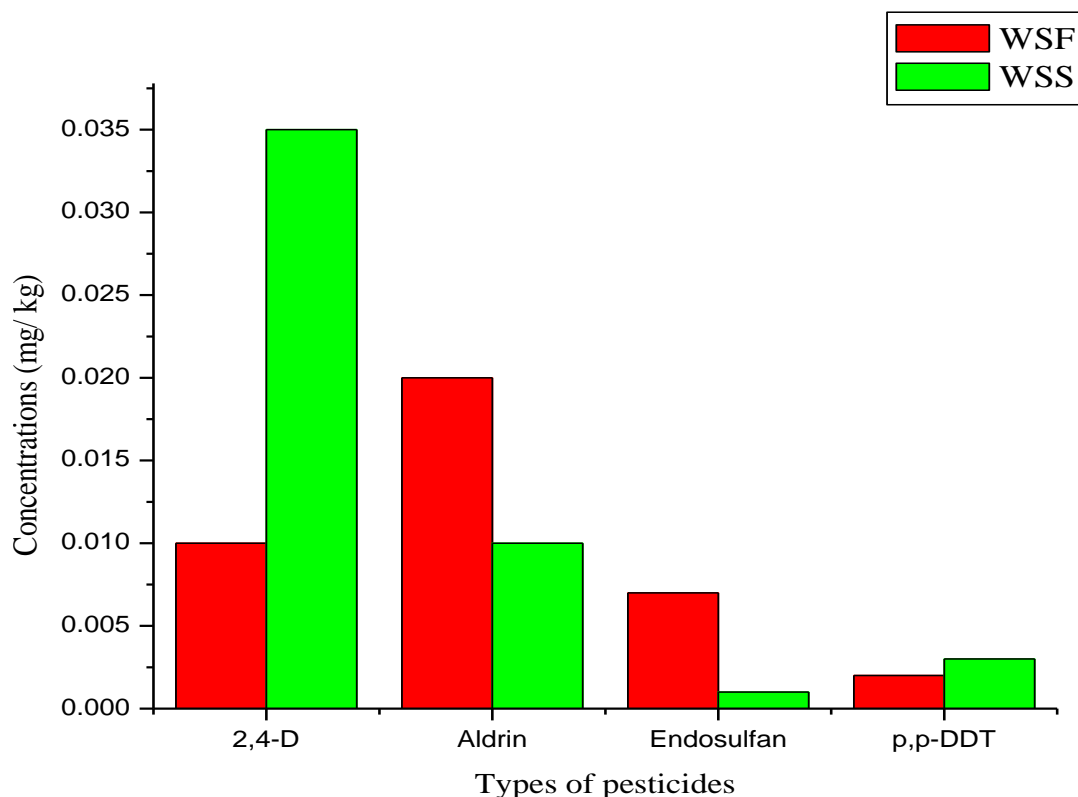


Figure 5. Mean concentrations of analytes samples from farm/ field and storage facilities.

None of them were above the MRL of different sources as can be seen from Table 4. These results are an indication that there might be a proper usage of pesticides in the production of these food items or it due to low use of pesticides. However, wheat samples available for consumption were contaminated with 2, 4-D, Aldrin, Endosulfan, and DDT pesticides. Thus, the cumulative and synergetic toxic effect of these pesticides on the consumer is not yet determined.

Figure 6 shows the overall percentage difference of pesticide residues concentrations between the PRE and POST samples of wheat. 2, 4-D increased by about 71% between PRE and POST samples analysed, while Aldrin, p,p- DDT and Endosulfan decreased by about 20, 6.6 and 2% respectively. In general, the order of concentration for these analytes in the wheat samples is 2, 4-D > Aldrin > p, p- DDT > Endosulfan.

CONCLUSIONS AND RECOMMENDATIONS

Pesticide residues in or on plants may be unavoidable even when pesticides are used in accordance with Good Agricultural Practice. That is why in this research the main purpose is determination of these pollutants particularly in wheat. However, before the determination of target contaminant the methods used for the analysis

was validated to test whether or not they were fit for the intended purpose. The result of these parameters indicated that the values of LOD for all analytes were lower than the LCC. Also, all the LOD value of the detected analytes were less than the MRL set by codex and JFCRF as shown in Table 4. Therefore, this method can be used to detect four pesticides found in wheat sample even below their MRL.

The mean recoveries were also within the standard range for four pesticides in farm/cultivated samples and three for stored samples. However, the recoveries of Chlordane, Endosulfan and DDT in farm sample whereas, chlordane and Endosulfan in stored sample are out of the standard range as the result were indicated in Table 3. The repeatability and reproducibility of the data in both analysis showed that the method is fit for the analysis, that is, the intraday and inter day %RSD value are < 20% as shown in Table 2. Therefore, these results depicted that the analysis method is appropriate for four pesticides out of eight targeted pesticides.

The sample analysis result shows that farm/field ready for consumption were contaminated with Aldrin, 2, 4-D, Endosulfan and p,p- DDT. Also, analyses for the same target pesticide in commercially available wheat sample collected from Hossaina Market revealed that 2, 4-D, Aldrin, Endosulfan and DDT were found as the contaminant as shown in Table 4. However, the

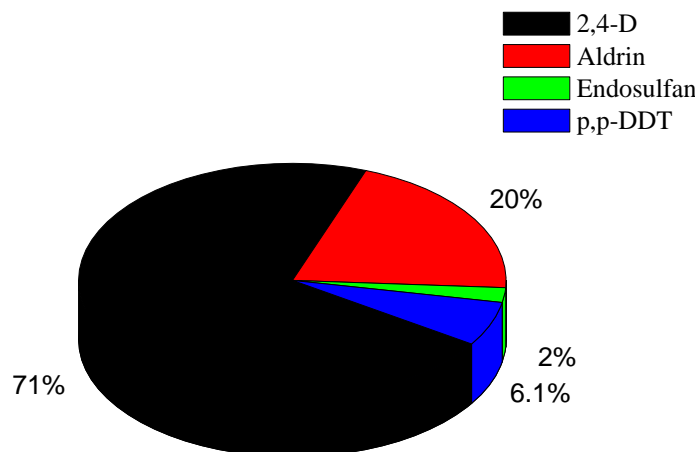


Figure 6. Percentage variations of pesticide residues concentrations between the PRE (farm) and POST (stored) wheat samples.

contamination status of these pesticides in both samples was lower than when compared to the WHO codex, EU codex and Japanese MRL value.

Thus, all concerned bodies of the country need to play their crucial role of ensuring that foods consumed by the general public are not of health concern, even though the concentrations of the detected analytes are lower than the reference MRL. Therefore, in order to have safe fresh produce, a variety of measures such as laws, regulations, standards, and a system of effective inspection and laboratory analysis are urgently needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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