Organochlorine Pesticides Occurrence In Fish Muscle And Their Public Health Risks In Northwest Ethiopia

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Abstract

Pesticides are the parent compounds, their metabolites, and associated impurities of agricultural and health chemical inputs. If they are found concentration level higher than the standard limits, they have potential negative impacts on ecosystem in general and in fish and humans in particular. This study investigates organochlorine pesticides (OCPs) residue occurrences in fish muscle and assesses their public health potential risks, in North West Ethiopia. The concentration of OCPs residue under GC-ECD detected in 37.84% fish muscle samples. The mean amount detected were Endosalfan I, 341.50 ± 32.19 μg/kg; Endosalfan II, 36.01 ± 2.3μg/kg; Endosalfan sulfate, 5.43 ± 4.06 μg/kg; 4,4, DDE, 64.01 ± 9.08μg /kg; 4, 4, DDD, 5.65 ± 3.12μg /kg; and 4,4, DDT, 1.58 ± 0.30μg/kg. The mean concentration of Endosalfan I tested fish muscle sample was higher than that of permissible limit of different international standards. However, due to low per capital consumption rate of fish origin food in Ethiopia, health risk index (HRI) ranges from 0.002-0.1275 which shows there is no public health risk. This study highlights the possibilities of chemical residues occurrence in fish food products and hence pesticide use regulations and monitoring concentration level should be implemented regularly to avoid human and environmental health risks.

Introduction

Pesticides are chemicals that can contaminant the aquatic and terrestrial environment. It is known that pesticides applied in an area reaches into aquatic environment through; drift, leaching and drainage (Srivastava et al., 2010). Pesticides mainly organochlorine pesticides (OCPs) are poorly hydrolyzed and slowly biodegrades in the environment. This chemical nature of OCPs contributes for their wide distributions in the environment thus are considered to be significant chemical food contaminants. These OCPs ultimately biomagnifies in various trophic levels and eventually high burden in fish and consequently threaten the health of human (Donaldson et al., 2010, Srivastava et al., 2010, Khodadadi et al., 2012).

The risks associated with consumption of animal origin food should be taken in to account in the agricultural sector by reasonable and achievable good practices in the agricultural industry. The maximum levels of OCPs residues in foodstuffs of animal origin should be set at the strictest possible level due to the lipophilic nature of these chemicals (USEPA, 2009, Fair et al., 2018). According to the WHO/FAO (2009), humans, animals and fishes are considered as at risk when they consume the OCPs residue level higher than maximum residue limits and acceptable daily intake.

The exposure to OCPs compounds is especially dangerous during prenatal development and infancy; as it causes irreversible changes in the central nervous system. Studies revealed that OCPs have strong potential to cross placental barriers even in minute concentration and cause serious neonatal damage (Jayara et al., 2016, Thompson et al., 2017). The Long term adverse health effects such as increased likelihood of reproductive disorder, respiratory failures, kidney and liver dysfunctions, nervous and birth defects, endocrine disruption, immune system dysfunction and are carcinogenic (Yazgan and Tanik, 2010, Okoffo *et al.*,2016). Besides, it causes cardiovascular diseases, disrupts heme biosynthesis and vitamin D metabolism and disrupts mineral metabolism (Jayara et al., 2016).

The problem of OCPs stems out not only from their toxic properties rather at low levels of exposure to these chemicals. Their effects can be observed only at the physiological or biochemical level. Acute effect of OCPs is intoxication of fish, human and other animals by direct contact or through the food chain (Jayara et al., 2016, Thompson et al., 2017). Numerous studies on both human and animals provide strong evidence of the toxic potential of OCPs (Ravindran et al., 2016). According to the study conducted by Yazgan and Tanik (2010), the acute toxicity effect of OCPs causes environmental risks that may cause for ecological damages including the flora and fauna of the entire eco-system.

Different studies indicated that level of OCPs in developing countries show an increasing level as it is still in use for agriculture and public health purposes but declining in developed countries environment (Sadasivaiah et al., 2007, WHO, 2010, Thompson et al., 2017). Ethiopia has a relatively well-developed pesticides legislation on registration and control of pesticides intended to address its environmental and health effects (Negatu *et. al.*, 2016). In addition, Ethiopia is signatory to the Stockholm Convention and agreed to support research on persistent organic pollutants. But there are gaps between policy and practical enforcement of prohibiting import and application of banned OCPs including DDT and Endosulfan (Negatu *et al.*, 2016). However, limited data is available about the occurrence and level of pesticides in aquatic environment specifically in fish tissue. In Ethiopia, studies in rift valley water bodies have revealed the contamination of the environment (sediment and fish) by pesticides (Deribe et al., 2014, Yohannes et al., 2014). Ethiopian environmental health issues especially OCPs requests further efforts of scholars for incessant investigation to draw a clear map about the situation (Thompson et al., 2017).

The first version of this study indicates that there is still an increasing trend of OCPs application for improving agricultural production in the catchments of Lake Tana (Birhan and Marshet, 2020). Accordingly, high concentrations of OCPs can be found in the environment and fish muscle of our study area. In Ethiopia the studies conducted to assess the contamination of OCPs in the environment is scarce; none has been conducted in Lake Tana. Dried fish produced is being exported to neighboring countries especially to Republic of Sudan and to Eritrea recently. Hence the research result will help to amend fish product export policy and associated environmental safety considerations regionally and locally. Therefore, the aim of this study was to assess OCPs accumulation in the muscle of fish and estimate its potential human health risks in fishes of Lake Tana, Northwest Ethiopia.

Methodology

Study Area

The study was conducted in Lake Tana, the headwater of the Blue Nile River. It is located in the province of North West Ethiopia. It is located approximately 580 km north west of the city of Addis Ababa. The Lake is one of the largest Lake (3600 km^2) and fishing sites in Ethiopia (Stave et al., 2017).

Geographically Lake Tana is found at latitude 12°1'35.75"N longitude 37°18'12.54"E. Mean annual temperatures range from 13°C to 22°C. The annual average rainfall of Lake Tana is 1248 mm per year (Stave et al., 2017).

The lake has three main commercially important fish groups such as: large *Labeobarbus* spp., African catfish (*Clariasgariepinus*) and Nile tilapia (*Oreochromisniloticus*). They are consumed by larger part of the community and traded widely in the region and even in to neighboring country Sudan in dry form. There are 55 fishery enterprises and a total of 21,084 beneficiaries directly dependent on the fishing activities (Shewit et al., 2018, Mengistu et al., 2017). The samples were taken from representative five landing sites of Lake Tana (Gorgora, Enfranze, Kidsthana, Agede-kergna, and Bahir Dar).

Study Design and Sampling Method

The cross-sectional study design was carried out from January 2018 to December 2020. Three commercially important fish species of the Lake including large Labeobarbus spp., African catfish *(Clarias gariepinus)* and Nile tilapia *(Oreochromis niloticus)* were sampled for this study. A total of 37 fish were sampled with an average size of 446g for *O. niloticus*, 675g of *C. garipinus* and 378g of Labeobarbus spp. five fishes were sampled from four of the sampling sites and seven fish were taken from the Bahir Dar gulf side of the Lake. Muscle tissue was taken from the dorso lateral sides of each sampled fish. The samples were wrapped in aluminum foil, packed in clean polyethylene bags, labeled and sealed, and then transported in a thermo-insulated container with ice packs. The Gas chromatography was used for the pesticide residue analysis. The GC-ECD processing and analysis was conducted in laboratories of JIJE analytical and testing service laboratory, Nifas silk, Lafto Sub-city, Addis Ababa Ethiopia. All glassware and containers were washed with detergent, rinsed with purified water and acetone and kept in an oven at 180°C for 2h for processing (Anastassiades et al., 2003).

Laboratory Procedures

Sample Extraction and Clean Ups

The extractions were carried out according to the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method described by Anastassiades et al. (2003), with some necessary modifications. Lyophilized dry fish muscle sample was chopped on a chopping board with a sharp knife and ground using mini-chopper, then 10 g of chopped sample was transferred to a 50 mL Teflon centrifuge tube. Then 10 mL of acetonitrile was added to the centrifuge tube and agitated well for proper mixing, followed by addition of 7.5 g of anhydrous $MgSO_4$ and 1 g of NaCl, and the centrifuge tube was vigorously shaken for 1 min. Later it was centrifuged at 5000 rpm for 5 min. After centrifugation, 2 mL of the supernatant was transferred to an Eppendorf tube containing 100 mg of primary secondary amine (PSA), 150 mg of $MgSO_4$, and 100 mg of charcoal for cleanup, followed by vigorous shaking for 2 min. Again, the prepared sample extract was centrifuged at 10,000 rpm for 5 min. afterward; the sample extract was filtered through a 0.45 μ m filter using a syringe and transferred to auto-sampler vials for further GC analysis.

GC Analysis

The OCPs residues were analyzed by a Shimadzu GC-2010 with an electron capture detector (ECD), an auto-injector (AOC 20i; Shimadzu, Kyoto, Kyoto Prefecture, Japan), and GC solution software. The capillary column used in ECD was Rtx-CL, 30.0 m length /0.25mm in diameter 0.32 μm film thickness. The GC was run under the following conditions: injector temperature: 250 ^{0}C; detector temperature 330 ^{0}C; oven temperature: 260 ^{0}C starting from 0 to 180 ^{0}C for 0.3 min and continued at 5 ^{0}C /min to 220 ^{0} C, held for 12 min, and continued at 5 ^{0}C /min to 260 ^{0}C; injected sample volume: 1–2 μ L; mode of injection: split; carrier gas: N_2 with a 77.8 kPa flow rate; runtime: 28 min. Standard peaks were detected by inserting a high concentration of the standard (1 ppm), and the retention time for OCPs was estimated.

The GC system was calibrated using an external standard technique. Individual standard stock solution (100 mg/ L) was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a Teflon-lined screw cap and dissolving the weighed standard in HPLC-grade methanol. To prepare primary dilution standards, stock standard solution was used. An appropriate volume of each individual stock solution was transferred to a volumetric flask and the solutions were mixed to acquire stock standard solution.

Public Health Risk Calculation

Comparison of observed concentrations of pollutants in the environment with established maximum permissible levels (MPL) is the basic approach used to assess the potential risk posed to ecosystems and human health by toxic effects of pollutants. Irrespective of the eating habits and consumption rate, Food and Agricultural Organization (FAO) and World Health Organization (WHO) recommend the acceptable daily intake (ADI) to assess human exposure to target contaminants. An individual's exposure to OCPs residues from the fish used for the study was achieved by calculating the **estimated daily intake (EDI)** in **μg/kg** body weight/day of OCPs by the equation:

$$\mathbf{EDI} = \frac{C \times D}{B}$$

where C, represent the concentration of OCPs residues in fish (μg/kg) on d weight basis, D average daily intake of fish estimated at 0.41g/person/day for adults and B average body weight considered to be 55kg for adults. To ascertain the potential public health risk of this estimated exposures, exposure values were compared to two benchmark concentrations for cancer and non-cancer health effects. These benchmarks were founded on standard toxicological references. A benchmark concentration represents the daily concentration of a contaminant below which there is a high probability of no adverse health effect (Liu et al., 2010 and Dougherty *et al.*, 2000). Health risk assessment of consumers from the intake of OCPs contaminated fish was characterized by using **health risk index (HRI)**. According to WHO/FAO, (2009), the estimated HRIs were obtained by dividing the EDI by their corresponding values of acceptable daily intakes (ADI) as shown by the equation;

$$\mathbf{HRI} = \frac{EDI}{ADI}$$

The food concerned is acceptable when the HRI is less than 1and is risk to the consumer if it is greater than one indicating that there is high probability of adverse health effect (Akoto et al., 2016).

Data Analysis

The data were entered in to an excel sheet and cleaned by EPI-InfoV.3.5.3; and exported by state transfer to be cleaned, edited and analyzed by using SPSS version 20. Data cleaning was performed to check for accuracy and consistencies and missed values.

Results And Discusion

Occurrence of pesticide Residues in fish Meat

Overall, Gas chromatography laboratory analysis of the 37 fish muscle samples 37.84% was positive for OCPs. This analysis study indicated that six pesticide residues including Endosalfan I; Endosalfan II, Endosalfan sulfate, 4, 4, DDE, 4, 4, DDD, and 4, 4, DDT residues were detected (Table 1). This study finding was different from Gustav Gbeddy *et al.*, (2012) who founds in 100% tested samples which is higher than the present study. Another higher frequency of occurrences of OCPs detected by Bedigama and Gabadage, (2018) in Srilanka was 80%.

The detection levels were from not detected for Dimethotes (detection limit = 0.001 µg/ kg), to 4041.73 µg/kg dry weight for Endosalfan I recorded in the present study (Table 1).

Table 1
Organochlorine pesticides (OCPs) detected in Lake Tana Fishes (n = 37)

No	OCPs	Range (µg/kg)	Over all Mean ± SD (µg /kg)
1	**Endosalfan I**	0.001–4041.73	341.50 ± 32.19
2	**Endosalfan II**	ND – 628.27	36.01 ± 2.3
3	**Endosalfan sulfate**	ND-146.69	5.43 ± 4.06
4	**4, 4, DDE**	ND -227.11	64.01 ± 9.08
5	**4, 4, DDD**	ND-55.59	5.65 ± 3.12
6	**4, 4, DDT**	ND to 42.76	1.58 ± 0.3

Endosalfan and its metabolites concentrations in fish muscle were recorded up to mean of 341.50 ± 32.19 µg/kg. A study conducted in Uganda by Bagumire et al., (2008) showed lower level (< 2µg/kg) of endosalfans in fish muscle; Polder et al., (2014) documented in fish muscle of lakes of Tanzania, that endosulfan level was from ND – to 405µg/kg. Study done by Deribe, et al., (2014) in fish muscle of lake Hawassa, Ethiopia noted Endosalfan, ND – 42.5µg/kg which is lower than the present result. In Gana, a study conducted by Kuranchie-Mensah et al., (2013) endosulfan reported a concentration of 0.01–7.52 µg/kg. Another study in Bénin by Yehouenou et al., (2014) noted endosulfan of 9–215 µg/kg concentration.

In our finding the mean concentration of 4, 4, DDT and its metabolites was recorded 1.58 to 64.01μg/kg (Table 1). Similar with our finding the study done by Deribe et al., (2014) in fish muscle of lake Hawassa, Ethiopia detects mean concentration range of 19–56 μg/kg of 4, 4, DDT. Another comparable study was done by Yohannes et al., (2014) in edible fish species from a Rift Valley lake-Lake Ziway in Ethiopia, the concentration of 4, 4; DDT was 0.77–61.9 μg/kg. Different studies done by different scholars globally including Gustav Gbeddy *et al.*, (2012) in Ghana was found the mean DDTs residue concentration in fish muscles ranges from 10 -1700.35 μg/kg; another study done by Adu-Kumi et al (2010) in lakes of Ghana reported up to 440.90 μg / kg and Polder et al., (2014) in Tanzania was recorded 7.2–319 μg/kg indicates the concentration levels of 4, 4, DDT was higher than our finding. A study done in Srilanka with an average of 735 μg/kg; 4, 4, DDT was detected which were higher than the present finding (Bedigama and Gabadage, 2018). Lower than our findings were detected in study done by Kasozi et al (2006), that DDTs in fish samples from Lake Victoria, Uganda was 0.80–0.86μg/kg. Another lower concentration of dry weight DDTs in fish muscle was reported in studies conducted in Africa; in Egypt by Yahia and Elsharkawy, (2014) noted 0.70–0.90 μg/kg; in Bénin by Yehouenou et al., (2014) stated 3.88–11.3 μg/kg and in Kenya study by Omwenga et al., (2016) quantified 2.098 μg/kg.

This high occurrence in our study area might be due to the long-term use of organochlorine pesticides to improve agricultural productivity in the catchments of the Lake. The high lipophilicity, bioaccumulation nature, long half-life and potential of long range leaching nature of the chemicals results in accumulation of persistence toxic substances in soil, water and air (USEPA, 2009, Agrawal et al., 2010, Ravindran et al., 2016). The tropical conducive pests' multiplication environment of the water shed of Lake Tana might lead to the use of OCPs frequently and indiscriminately by the farmers (Kannan *et al.*, 1995, Birhan and Marshet, 2020). In addition, lack of proper implementation of legislation, improper market regulations, poor knowledge and skill of safety use of OCPs and ignorance shown by the farmers, agricultural workers in the Lake Tana catchment may prone to experience high levels of agricultural chemicals, including OCPs (Mengistie et al., 2016, Beyene et al., 2016, Birhan and Marshet, 2020).

In our finding the detected level of 4, 4, DDT was relatively lower than its metabolites (4, 4, DDE and 4, 4, DDD) and also similarly the Endosalfan sulfate concentration in fish muscle was lower than its metabolites (Endosalfan I and Endosalfan II) (Table 1). This the lower concentration of the parent compounds might indicate that currently limited use of those persistence OCPs around Lake Tana catchments. The detected concentration may be due to previous historic event with long half-life (2-15Years) (Augustijn-Beckers et al., 1994). However non negligible concentration of parent compound of this persistence pollutants were detected that indicates still in use of these OCPs in the water shed of Lake Tana.

This study highlights that more pesticide was detected from African catfish (*Clarias gariepinus*) species than other main commercially important fish groups (Labeobarbus spp. and *O. niloticus*) Fig. 1.

Similar to our finding study conducted by Nicklisch et al., (2017) high variation in pesticide levels have been observed when examining different fish species. Spatial wise, occurrence was frequent at Gorgora, Enferanze and Bahir Dar sampling sites of the study Lake. This variability distribution of OCPs in our study area might be due to the environment as well as the biological factors like amounts of lipid in their body and difference in ecological characteristics of the fish species (Borga et al., 2004). Different studies indicated that factors that influence OCPs pollutant levels in fish muscle such as fish length, fish species, its trophic level, habitat type, feeding habitat, and fish movement patterns and residence time in contaminated areas (Borga et al., 2004, USEPA, 2009, Cardona, 2015, Bonito et al., 2016).

Public Health Risk

Humans represent one of the top receptors for aquatic contaminants and consumption of fish with high levels of contaminants may pose public health concerns. The consumption of contaminated fish may lead to serious public health treat if the concentration level is higher than maximum residue level (MRL) and acceptable daily intake (ADI) standards of FAO/WHO, (2009); U.S. FDA, (2009) and EU, (2013). The amount of OCPs residue concentration found in some fish meat samples of our study was higher than the recommended MRL of different standards. However, except Endosalfan I the overall mean value of concentration detected in our study was lower than MRL standards of FAO/WHO, (2009), U.S, FDA, (2009), EU, (2013)(Fig. 2).

These higher concentrations of endosalfan I in fish muscle originated from Lake Tan North West Ethiopia, indicating that it is alarming to the people who consume fish food.

Another method that health risk assessment of consumers from the intake of OCPs contaminated fish was done by calculating health risk index (HRI). In our finding the mean concentration detected was higher than the acceptable daily intake of WHO/FAO recommended. The acceptable daily intake of Endosalfan I; Endosalfan II, 4, 4, DDE, 4, 4, DDD recommended by WHO/FAO, (2009) is 20, 20, 5, 5 µg/kg respectively. Due to low per capital consumption rate of fish in Ethiopia, the present finding of a HRI ranges from 0.002 to 0.1275 indicating the present level has no public health risk (Table 2).

Table 2

ADIs, EDIs of OCPs residues and their HRI associated with the consumption of fish from the Lake Tana

Pesticides	Range (µg/kg)	Mean ± SD	ADI	EDI	HRI	Health Risk
Endosalfan I	0.001–4041.73	341.50 ± 32.19	20	2.55	0.1275	No
Endosalfan II	ND – 628.27	36.01 ± 2.3	20	0.27	0.0135	No
Endosalfan sulfate	ND-146.69	5.43 ± 4.06	20	0.04	0.1275	No
4, 4, DDE	ND -227.11	64.01 ± 9.08	5.0	0.48	0.096	No
4, 4, DDD	ND-55.59	5.65 ± 3.12	5.0	0.04	0.008	No
4, 4, DDT	ND to 42.76	1.58 ± 0.301	5.0	0.01	0.002	No

Where ADI: acceptable daily intake, EDI: estimated daily intake; HRI health risk index; SD: Standard deviation; ND: not detected

But these values may pose potential public health risk if the HRI is calculated considering local per capita consumption. This minimum exposure with no public health risk (HRI<1) was recorded indifferent studies; for instance in Ghana by Gbeddy *et al*., (2012) and Akoto *et al*., (2016). Differently to our finding; study done by Yohannes *et al*. (2014) from a Rift Valley Lake-Lake in Ziway, Ethiopia, indicated a potential cancer risk from consumption of the fish from the calculated hazard ratio. Another study in Srilanka by Bedigama and Gabadage, (2018) found HRI values more than 1.

As Limitation of this study; it is necessary to conduct further studies that assess the detection of OCPs residues in human serum to examine burden of these OCPs and integrating biomarkers of exposure and health risk effects, and modeling exposure routes via fish consumption.

Conclusion And Recommendations

The present research finding showed that high concentration of Endosalfan I residues concentrations in fish muscle were detected in fish samples which is higher than the standard limit level. However, as a result of low consumption rate of fish meat in the meantime, will have little or no significant adverse public health effects on consumers. So, applying more conservative threshold limit (WHO/FAO standard) implementation is necessary action by considering health risks of local consumers, other sources of exposure and vulnerable populations (children and pregnant women). The study highlights the presence of non-negligible level of OCPs in muscle of fish that indicates there is still in use of these pesticides in the catchments of Lake Tana, Northwest Ethiopia. Hence, to protect adverse effects on flora and fauna of the entire eco-system and public health hazards following one health intervention is recommended. Collaborative work on awareness creation about possible occurrence of OPCs residues should also be made among environmental health, agricultural and public health offices. In addition, further study of OCPs residue in other agricultural food items and its effects on ecology of aquatic environment is recommended.

Declarations

Abbreviations

"Not applicable"

Ethics approval and consent to participate

The study was granted an exemption from requiring ethics approval from the College of Agriculture and Environmental Science, Research ethics and Review Board.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Birhan Agmas and Marshet Adugna developed the research idea. Both of them contributed to collecting the materials and drafted the manuscript. All coordinated and supervised the study. BA and MA write the manuscript. All authors agreed with the results and conclusions and approved the final manuscript.

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Figures

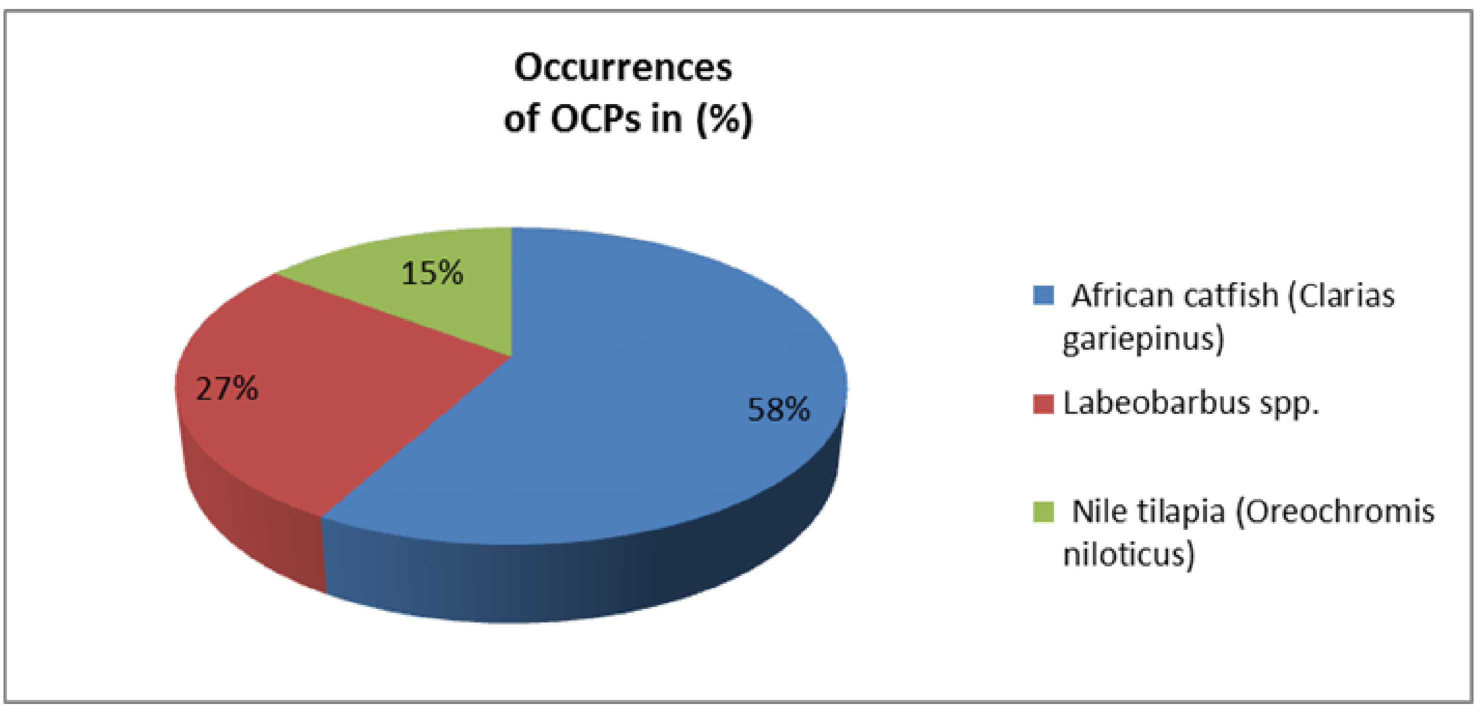

Figure 1

Comparing of OCPs detected with fish groups

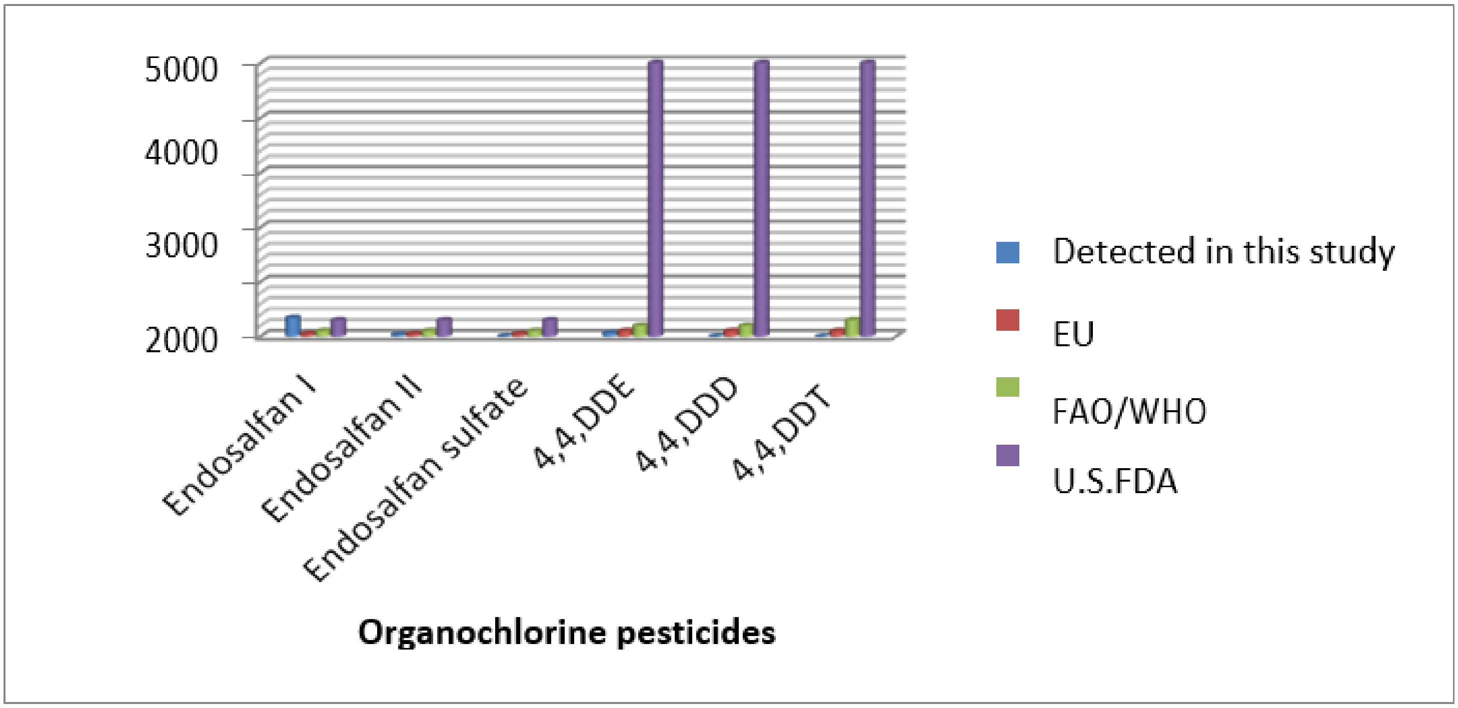

Figure 2

Comparing of OCPs detected with MRL of different standards