Heliyon 7 (2021) e06076

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Atrazine in fish feed and african catfish (*Clarias gariepinus*) from aquaculture farms in Southwestern Nigeria

Isaac Olufemi Olatoye [a], Reuben Chukwuka Okocha [b,e,*], Olayinka Ayotunde Oridupa [c], Charles Nnachetam Nwishienyi [a], Adebisi Musefiu Tiamiyu [d], Olufemi Bolarinwa Adedeji [a]

[a] Department of Veterinary Public Health and Preventive Medicine University of Ibadan, Nigeria
[b] Landmark University SDG 14 (Life Below Water), Omu-Aran, Kwara State, Nigeria
[c] Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Nigeria
[d] Department of Biological Sciences, University of Medical Sciences, Ondo City, Nigeria
[e] Department of Agriculture, Landmark University, Omu-Aran, Kwara State, Nigeria

ARTICLE INFO

Keywords:
Atrazine detection
Contaminant
Concentration
Commercial farms
ELISA

ABSTRACT

Extensive use of atrazine as herbicide in crop farming in Nigeria may lead to its accumulation in fish feed ingredients or aquatic ecosystem from aerosol or by runoff resulting in its residue in aquatic animals. Atrazine residues were determined in fish feed and catfish (*Clarias gariepinus*) fillets from commercial aquaculture farms in Southwestern Nigeria by matrix solvent particle dispersion and quantification using an ELISA kit. The mean atrazine concentrations in feed and fish were about 1.3–1.5 μg/kg and 1.4–1.8 μg/kg respectively. Atrazine was mostly detected in catfish from Ogun State (91.3%) and feed from Lagos State (80.0%) with mean concentrations of 1.4 ± 0.4 μg/kg and 1.5 ± 0.5 μg/kg, respectively. Mean atrazine concentration in catfish samples from Lagos State was significantly higher ($P < 0.05$) than the mean concentration in catfish samples from Ogun State. This study showed that the Estimated Average Daily Intake (EADI) of atrazine in fish samples from the selected states were below the Acceptable Daily Intake (ADI) value of 6 μg/kg for herbicide residues and thus within safe limit but their presence in fish is a cause for concern.

1. Introduction

Atrazine belongs to the group of triazine herbicides (others include simazine, promazine). It is useful in the prevention of pre- and early post-emergence broadleaf weeds in cultivated plants including maize (corn), sugarcane and on turf, including golf courses and residential lawns (EPA, 2006). It is the most commonly patronised herbicide in Nigeria (Adesina et al., 2014). It has been detected in surface and ground water due to its mobility in soil, where fresh water vertebrates are exposed to its toxic effects (Alvarez and Fuiman, 2005; Sherchan and Bachoon, 2011). Monitoring carried out in a number of countries indicates that concentrations of atrazine in groundwater and surface water rarely exceed 2 μg/l and are commonly well below 0.1 μg/l, although concentrations may be higher in agricultural areas where large amounts of atrazine are used (WHO, 2011). A study conducted from April 1993 to April 1994 in streams draining agricultural areas in Colorado detected a median atrazine concentration of 0.12 mg/L. Atrazine was detected in agricultural soils collected from southern areas of Poland at 0.69–19.59 μg/g. Soil samples taken across Canadian agricultural areas reported atrazine concentrations of 32.2 and 0.99 ng/g in samples collected from St. Anicet and Baie St. Francis in 2005 (National Center for Biotechnology Information, 2020).

Atrazine is considered a priority substance by the United States Environmental Protection Agency, Agriculture Canada and the European Commission (Lazarko-Connon and Achari, 2009) and has been classified as a class III toxic substance (on scale of I to IV, I being the highest toxicity class) (EPA, 2006). The potential health dangers presented by triazine chemicals to the complete environment have continued to attract attention from scientists throughout the world as many herbicides have been suspected of functioning as potential endocrine disruptors, that is substances interfering with the body's endocrine system and resulting in adverse developmental, reproductive, neurological and immunological effects in both humans and wildlife. Potential carcinogenic effects have been investigated in the laboratory as well as epidemiological studies (Klementova and Keltnerova, 2015).

* Corresponding author.
E-mail address: okocha.reuben@lmu.edu.ng (R.C. Okocha).

https://doi.org/10.1016/j.heliyon.2021.e06076
Received 23 May 2020; Received in revised form 11 July 2020; Accepted 20 January 2021

Water contamination by pesticides whether directly or indirectly can cause fish death, low fish productivity and increased levels of unwanted compounds in wholesome fish tissue which can adversely affect the health of humans via consumption (Adedeji and Okocha, 2012). Atrazine is highly persistent in the environment due to its resistance to abiotic hydrolysis (stable at pH 5, 7, and 9) and to direct aqueous photolysis (stable under sunlight at pH 7) (Liu, 2014). Atrazine and its metabolites can persist in water and soil for decades. Even more than 18 years after it was banned in Germany, atrazine remains the most abundant pesticide in groundwater samples (Jablonowski et al., 2011). Being a persistent and toxic pesticide, atrazine poses serious environmental and public health hazards as it is transmitted along the food chain from aquatic edible zooplanktons and vertebrates including fish to the consumers (Berntssen et al., 2005).

It has been reported to cause demasculinization and complete feminization of frogs at low doses (Hayes et al., 2011; Lazarko-Connon and Achari 2009; Hayes et al., 2011, 2011; Sene et al., 2010). Regarding the effective doses, the demasculinization effects of atrazine were produced at low ecologically relevant doses (e.g.2.5 μg/L or below) in amphibians (Hayes et al., 2011). According to the United States Department of Health and Human Services toxicology report notes, with respect to fetal and childhood exposures; maternal exposures to drinking water containing atrazine is characterized by reduced fetal weight and heart, urinary and limb disorders in humans (ATSDR, 2003). It also serves as a potent endocrine disruptor by shortening the length of estrous cycle and attenuation of the leutenising hormone surge, reducing pituitary hormone levels and causing ovarian histopathology (ATSDR, 2003). Further studies indicated that atrazine triggers aromatase activity in human ovarian cancer cells (Fan et al., 2007; Albanito et al., 2008). Other toxic effects of atrazine include poor semen quality and increased incidence of testicular cancers (Ohlson and Hardell, 2000).

Although the US Environmental Protection Agency Pesticide Programs concluded that atrazine is safe when used as indicated on the label, its common detection in drinking water and its action as a cancer-promoting aromatase stimulator made Cancer Advocacy Organizations to denounce the regulatory standards of continuous use in the country (Breast Cancer Action, 2008). However, it has been banned by the European Union for its persistent groundwater contamination (European Commission, 2004; Hamkim, 2015).

The indiscriminate use of atrazine for weed control by Nigerian farmers and the feeding of fish with ingredients exposed to atrazine create avenues for environmental and aquatic contamination and eventual health risks (Berntssen et al., 2005). The consequent, bioaccumulation and biomagnification along the food chain especially in fish portend toxicological exposure to consumers (Hamkim, 2015). In view of potential health effects to humans, animals and the environment, it is essential that food products destined for human consumption are routinely monitored for atrazine. This study therefore focused on ascertaining the levels of atrazine in catfish tissue and feed from aquaculture farms in Southwestern Nigeria.

2. Materials and methods

2.1. Study area

The study was carried out on catfish and feed from commercial farms located in Lagos, Ogun and Oyo States of Southwestern geopolitical zone of Nigeria. The zone is located between longitude 2°31′E and 6°00′E and Latitude 6°21′N and 8°37′N with a total land area of 77,818 square kilometers and an estimated population of 28, 767, 752 in 2002. The zone shares border with Edo and Delta States in the East, Kwara and Kogi States in the North, the Republic of Benin in the West and the Gulf of Guinea in the South (Faleyimu et al., 2013). The zone is endowed with many aquaculturable lands. It is an important region where intensive aquacultural activities form a central pivot. Also, small, medium and large scale catfish productions are practiced with growing numbers of fish farms and hatcheries as well as retail outlets of fresh and ready-to-eat catfish markets and restaurants (Figure 1).

2.2. Atrazine ELISA kit and standards

Enzyme-linked Immunosorbent Assay (ELISA) is a quantitative laboratory method for detection of atrazine residues in water and foods using polyclonal antibodies, which bind atrazine enzyme conjugates for a restricted number of antibody binding sites. Antibodies that bind atrazine compounds are immobilized to the inside of the wells. This assay is widely used as a test for the rapid screening as it is quantitative, easy to use, allows many samples to be run, and is less expensive for the determination of atrazine herbicide residues. ELISA Microplate Reader (ELX800, [BIOTEK], China) and Atrazine ELISA test kit (Abraxis LLC, United Kingdom) [containing 8 strips of 12 antibody-coated wells each in a strip holder; one vial of each of these: negative control (0.0 ppb atrazine), atrazine-enzyme HRP-conjugate, atrazine assay buffer, colour solution, and stop solution; and vial of each 0.05, 0.1, 0.25, 1.0, 2.5 and 5.0 ppb atrazine standards were used for detecting the residues in sample extracts.] The Plate Kit was stored at 6 °C until analysis.

2.3. Reageants

All reagents were of analytical reagent grade. All the plastics and glassware were acid–washed and rinsed with distilled water before being used.

2.4. Sampling, sample collection and storage

The list of the registered aquaculture farms in selected states were consulted from the Federal Department of Fisheries. A total of one hundred and thirty seven aquaculture farms were randomly selected for the study; Lagos State (n = 65), Ogun State (n = 46) and Oyo State (n = 26). Sampling was carried out between January and May, 2017 since atrazine fits a wide variety of cropping systems; atrazine can be applied by farmers prior to, during or after crop planting, or after crop emergence. Heavy rain following atrazine applications can result in runoff from farm fields into nearby streams and reservoirs which could be used as sources of water for fish farming.

A total of 10 adult catfish (*Clarias gariepinus*) were collected from ponds in each farm (1,370 catfish with an average body weight of 1007.08 ± 47.16 g) and placed in clean, inert containers offering adequate protection from contamination and against damage in transit. One composite feed sample from each farm was collected in food grade bags, sealed and affixed with code numbers. Samples were transported to the Food and Meat Hygiene Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for storage and analysis.

2.5. Sample homogenization

The fillets of individual fishes from the same farm were homogenized using a laboratory blender and 2g of composite sample of fish tissue per farm was obtained in duplicates using a mettler balance (Toledo B3002 Deltarange®)., wrapped in aluminum foil, labelled and stored at -20 °C pending extraction.

The composite feed sample from each farm were homogenized using a laboratory blender into fine powder and 2g feed sample per farm was obtained in duplicates using a mettler balance (Toledo B3002 Deltarange®), wrapped in an aluminium foil, placed in food grade plastic bags, appropriately labeled and stored away from light to avoid deterioration pending further analysis. Ethical approval of this study was obtained from the Animal Ethics Committee (Approval number: UI-ACUREC/18/0075) at the University of Ibadan.

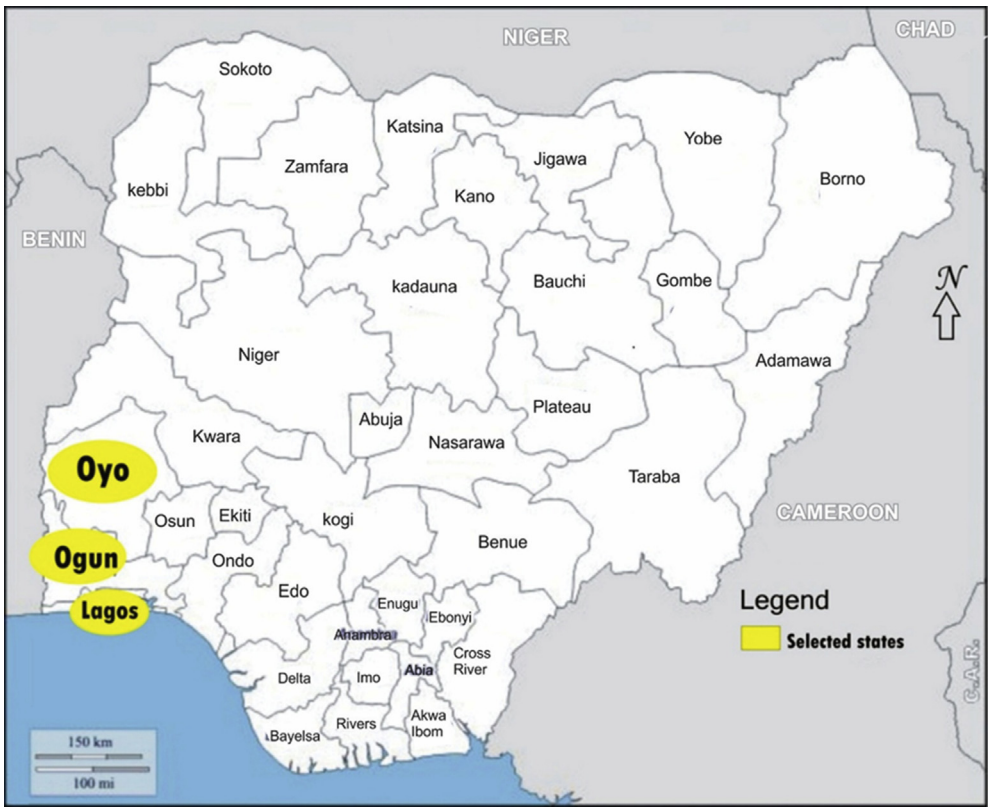

Figure 1. Map of study location.

2.6. Sample extraction

Matrix solvent particle dispersion (MSPD) as described by Barker (2006) was used for extraction of atrazine from the samples. About 2g of homogenized samples was placed in a laboratory mortar containing 2g of C18 silica, then grinded using a pestle, which applied mechanical and hydrophobic forces to disrupt membranes and release lipids.

2.7. Clean up of extracts

Cleanup was achieved using a 10 ml MSPD C18 extraction column as described by Barker (2006). Paper filters (Whatman No. 1.5 cm) were placed in the base of the columns; this was then topped with 2g activated florisil (an efficient adsorbent for triazines). The blended homogenates and C18 silica mixture were added and a Whatman filter was placed on top. Atrazine elution was achieved using 8ml of a polar mixture of 9:1 Acetonitrile and methanol. Eluents were evaporated to 1ml then collected for immunoassay.

2.8. Immunoassay procedure

Twenty-five μL of the assay buffer was added into each individual well with the aid of a multi-channel pipette. This was followed by the addition of 25 μL of the standard solutions, the controls or the samples into the wells of the test strips based on the working scheme. 50 μL of enzyme conjugate solution was placed in individual wells successively with the aid of a multichannel Pipette. The wells were covered up with parafilm and the contents mixed by moving the plate in a circular direction on the bench top for 30 s. It was then incubated for 30 min at room temperature. It was opened and the contents decanted into a sink. The strips were cleaned three times with the washing buffer solution. The left over buffer in the wells was poured out by patting the plate dry on a stock of paper, followed by the addition of 100 μL of substrate (color) solution to the wells succesively. The wells were sealed with parafilm and the contents mixed by moving the plate in a circular direction on the bench top for 30 s. The strips were incubated for 20 min at room temperature unexposed to sunlight. 50 μL of stop solution was placed in the wells in the same sequence as for the substrate solution. The absorbance was read at 450 nm with a microplate ELISA photometer within 10 min of adding the stopping solution.

2.9. ELISA assay quantification

The optical density (OD) of each well content was read from the ELISA plate reader. The OD of each set of calibrators and samples was averaged and used to determine the % Bound for the zero (Bo) using semi-log curve with data reduction capabilities fit for the standard curve.

$$\%\text{Bo} = \frac{\text{Average OD of calibrator or sample}}{\text{Average OD of negative control}} \times 100$$

The %Bo determination is used as a way of equalizing various runs of assay, also %Bo relationship of calibrators and samples to the negative control were kept moderately stable. The mean absorbance for each standard was calculated by dividing by the mean absorbance value for the Zero Standard (Standard 0).

Optical densities were obtained from ELISA readings of the six atrazine standards. A standard curve was constructed by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis against the corresponding atrazine level on horizontal logarithmic (x) axis. The $\%B/B_0$ for controls and samples were interpolated by using the standard curve to yield concentrations in ppb of Atrazine. The concentrations of the samples were determined with the aid of the standard curve. Samples showing a lower level of atrazine compared to the standard 1 (0.05 ng/mL) were

regarded negative. The concentration of the negative and positive controls was in the range provided in the test instruction (±20%).

2.10. Estimated Average Daily Intake (EADI) of atrazine in fish

The Estimated Average Daily Intake (EADI) for each study location was found by dividing the product of the mean atrazine concentration (μg/kg) and the fish consumption rate (Kg/day) by the body weight of an adult person (WHO, 1977; Fianko et al., 2011).

$$\text{EADI } (\mu g/kg/day) = \frac{\text{Mean atrazine concentration} \times \text{Food consumption}}{\text{Body weight}}$$

The food and agricultural organization (FAO, 2011) quotes the per capita consumption of fishes in Nigeria as 9 kg, while body weight was set at 70 kg for adult population group.

2.11. Statistical analysis

Quantitative data was analyzed using the SPSS Version 22 for Windows (IBM Inc., Chicago) and represented as proportion, mean ± standard deviation and range. Statistical difference between mean concentrations in catfish and feed from different states was analyzed using one way ANOVA on GraphPad Prism Version 4.00 (GraphPad Software Inc.) software at 95% confidence level. Levene test was used to assess the homogeneity of the variance and Tukey post hoc test was used to verify the differences between the means.

3. Results

3.1. Atrazine concentration in fish and feed from aquaculture farms in Southwestern Nigeria

The results of atrazine concentrations obtained in fish and feed in this study are presented in Table 1 with fish samples from Ogun State having the highest prevalence of atrazine (91.3%) at mean detectable concentration of 1.4 ± 0.4 μg/kg (range of 0.6–2.1 μg/kg). Also, atrazine was mostly detected in fish feed from Lagos State (80.0%) with mean concentration of 1.5 ± 0.5 μg/kg (range of 0.6–3.2 μg/kg).

Atrazine concentrations in fish samples from Oyo State ranged from 0.6 to 3.0 μg/kg, while the range in feed from Ogun and Oyo States were 0.6–3.0 μg/kg and 0.6–2.2 μg/kg respectively. There was no significant difference in the mean atrazine concentrations in catfish from Lagos and Oyo States, but the mean concentration in catfish samples from Lagos State was significantly higher than the mean concentration in catfish samples from Ogun State ($P < 0.05$). There was no significant difference in the mean atrazine concentration in fish feed samples across the states.

3.2. Human health risk associated with the consumption of catfish from aquaculture farms in Southwestern Nigeria

Human health risk estimates presented in Table 2 showed that EADI for atrazine in catfish from Lagos (0.23 μg/kg), Ogun (0.18 μg/kg) and Oyo (0.21 μg/kg) States were higher than the ADI of 6 μg/kg for 70 kg body weight of an adult person.

4. Discussion

Results of this study indicated that atrazine was detected in most of catfish and fish feed samples from selected states. Similarly, previous studies conducted by Osibanjo et al. (2002), Adeyemi et al. (2008) and Ezemonye et al. (2015) have also shown the detection of pesticides in sampled fishes from Nigeria.

The high prevalence of atrazine across the states shows evidence of exposure of fishes to exogenous sources, and this may be related to bioaccumulation (through feed) leading to bio-magnification of the pesticide in the environment (Ezemonye et al., 2015), since atrazine is the most widely used herbicide in Southwestern Nigeria, thus a common environmental contaminant. In the study conducted by Osibona et al. (2009), a higher lipid content in *C. gariepinus* (9.3%) than in *Tilapia zillii* (1.1) % was observed. The high lipid content of *Clarias gariepinus* (Ezemonye et al., 2015) favours their trapping of pesticides in their lipid stores. This is supported by Romanic et al. (2014), who observed a positive correlation between lipid content of fish muscle and bioaccumulation of pesticide. The bottom feeding modes of *Clarias gariepinus* can also be attributed to the higher prevalence of atrazine in the fishes due to the likelihood of exposure to contaminated sediments and sinking feeds as reported by Biego et al. (2010).

The mean atrazine concentration in catfish samples from Lagos State was significantly higher than mean atrazine concentrations in catfish samples from Ogun State ($P<0.05$). Mean atrazine concentrations in fish samples in this study were lower than 0.63 ± 0.28 μg/g obtained by Ezemonye et al. (2015) who determined atrazine level in catfish from Ogbese River in Edo State, and the range of 0.01–8.92 ppm by Adeyemi et al. (2008) who assessed organochlorine pesticides in fishes from Lagos Lagoon.

Atrazine was also detected in fish feed from aquaculture farms in this study. Lagos State had the highest prevalence (80%) in fish feed while Oyo State had the least prevalence (69%). The highest prevalence of atrazine in the fish feed samples suggested a likely contamination from ingredients that were used in the feed manufacturing process and environmental pollution. The mean atrazine concentration in fish feed samples obtained across the states were not significantly different, although Lagos State had the highest prevalence. Atrazine concentration in fish feed across the states could be due to feed produced locally (especially indigenous and locally formulated feed) in the country where atrazine is commonly used in agriculture, thereby contributing to the concentration of the analyte in the feed samples. Some farms make use of different feed from different vendors at different stages of production. Fishes are raised using a predetermined quantity of food supply (consisting of fish oil and fish meal-high in animal protein and fat as well as plant by-products) which have been documented as the main source of lipophilic pollutants in fish feeds (Berntssen et al., 2005). Thus, the feed could have been contaminated by chemicals from sprayed crops, raw materials as well as contaminated fish by-products used in formulating such feed.

Positive correlations between total pesticide levels in fish muscle and the level in the supplied feeds have been obtained by a number of investigations. Karl et al. (1998) discovered that the fish oil used in the process of production of the distributed fish feeds was responsible for chlordane concentrations in cultured Salmon. Hites et al. (2004) observed that concentrations of dieldrin in Salmon tissue were as a result of excess concentrations of dieldrin in feeds from Canada. Guo et al.

Table 1. Atrazine concentrations in catfish and fish feed samples from aquaculture farms in Southwestern Nigeria.

Location	N	Concentration in Fish			Concentration in Feed		
		No. positive	Mean ± SD (μg/kg)	Range (μg/kg)	No. positive	Mean ± SD (μg/kg)	Range (μg/kg)
Lagos State	65	57 (87.7%)	[a]1.8 ± 0.7	0.5–3.5	52 (80.0%)	1.5 ± 0.5	0.6–3.2
Ogun State	46	42 (91.3%)	[a]1.4 ± 0.4	0.6–2.1	36 (78.3%)	1.3 ± 0.5	0.6–3.0
Oyo State	26	23 (88.5%)	1.6 ± 0.6	0.6–3.0	18 (69.2%)	1.4 ± 0.5	0.6–2.2

[a]Indicates significant difference (P˂0.05).

Table 2. Human health risk associated with the consumption of catfish from aquaculture farms in Southwestern Nigeria.

Location	N	Mean atrazine concentration in fish (μg/kg)	FAO consumption rate (Kg/capita/year)	Body weight of an adult person (kg)	ADI (μg/kg)	EADI (μg/kg)
Lagos State	65	1.8	9	70	6	0.23
Ogun State	46	1.4	9	70	6	0.18
Oyo State	26	1.6	9	70	6	0.21

ADI: Acceptable daily intake; EADI: Estimated average daily intake.

(2009) concluded that the feeds intended for freshwater aquacultures had lower total DDT levels than those proposed for marine fish, by comparing contaminant levels in feeds for marine and freshwater fish and by-products of the fish industry used in the production of feeds in China.

The agricultural use of herbicides and pesticides in crop production in the country has increased steadily over the years. The consequent contamination and persistence in aquatic habitats and biota have heightened concerns due to their acute and chronic impacts on both humans and animals. The Acceptable Daily Intake value (ADI) of 6 μg/kg for herbicide residues is considered safe for consumption but their presence in fish is a cause for concern (Akan et al., 2019). In the present study, the EADI of atrazine in fish samples from the selected states were below the ADI value of 6 μg/kg for herbicide residues and thus within safe limit.

Atrazine is an herbicide of choice in Nigeria, thereby constituting a major pollutant in the environment (Ezemonye et al., 2015). Fishes are exposed to different sources of pesticides from direct ingestion of substances found in both the water and in the fish diet (Guo et al. 2009). The extensive use of atrazine and other herbicides in agriculture, despite being banned in international markets, can also be attributable to the aggressive marketing strategies of the representative of the manufacturers in Nigeria who promise farmers less toiling with much better results without considerably researching on possible toxicities and highlighting the possible dangers these chemicals pose to man and his environment. Uncontrolled use of atrazine in agriculture creates avenues for water and food contamination as well as eventual health risks as continuous exposure to pesticides via consumption of fish as obtained in this study may increase the risk of adverse effects of such chemicals on the consumers. The negative impacts to foreign trade of banned pesticides found in aquaculture products which are destined for exports to the international markets cannot be overemphasized.

5. Conclusion

This study revealed that atrazine is a contaminant of catfish and feeds resulting from agricultural practices in Southwestern Nigeria. The mean concentrations of atrazine in fish samples from the selected states were however below the ADI value of 6 μg/kg for herbicide residues and thus within safe limit but their presence in fish is a cause for concern. Control of pesticide use with the appropriate legislation, routine monitoring of aquaculture products for pesticides, as well as corrective measures to minimize residue occurrence with appropriate sanctions for non-compliance across the country will enhance food safety and public health. It is expected that this result will inspire a series of further studies on the production and supply chains of fish feeds and the assessment of atrazine levels in sediments and pond water.

Declarations

Author contribution statement

Isaac Olufemi OLATOYE: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Reuben Chukwuka OKOCHA, Charles Nnachetam NWISHIENYI:- Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Olayinka Ayotunde ORIDUPA: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Adebisi Musefiu TIAMIYU: Analyzed and interpreted the data.

Olufemi Bolarinwa ADEDEJI: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors acknowledge the following: (i) Federal Department of Fisheries, Nigeria for the logistics (coordination) and (ii) Catfish Farmers Association of Nigeria for the fish samples.

References

Adedeji, O., Okocha, R.C., 2012. Overview of pesticide toxicity in fish. Adv. Environ. Biol. 6, 2344–2351.

Adesina, J., Olabode, O.S., Sangodele, A.O., 2014. Performance evaluation of brands of atrazine in South West Nigeria. Int. J. Environ. Sci. Technol. 3, 85–92.

Adeyemi, D., Ukpo, G., Anyakora, C., Unyimadu, J.P., 2008. Organochlorine pesticide residues in fish samples from Lagos Lagoon, Nigeria. Am. J. Environ. Sci. 4, 649–653.

Agency for Toxic Substances and Disease Registry (ATSDR), 2003. Toxicological Profile for Atrazine. *U.S.* Department of Health and Human Services, Public Health Service, Atlanta, GA, p. 99.

Akan, J.C., Inuwa, L.B., Chellube, Z.M., Mahmud, M.M., Abdulrahman, F.I., 2019. Assessment of the levels of herbicide residues in fish samples from alau dam, maiduguri, borno, state, Nigeria. Int. J Environ. Chem. 3, 53–58.

Albanito, L., Lappano, R., Madeo, A., Chimento, A., E.R, Prossnitz, A.R., Capello, V., Dolce, S., Abonante, V., Pezzi, Maggiolini, M.G., 2008. Retracted: g-protein-coupled receptor 30 and estrogen receptor-α are involved in the proliferative effects induced by atrazine in ovarian cancer cells. Environ. Health Perspect. 116, 1648–1655.

del C, Alvarez M., Fuiman, L.A., 2005. Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae. Aquat. Toxicol. 74, 229–241.

Barker, S.A., 2006. Use of matrix solid phase dispersion for determining pesticides in fish and foods. In: Martinez Vidal, J.L., Frenich, A.G. (Eds.), Pesticide Protocols, Methods in Biotechnology, 19. Humana Press.

Berntssen, M.H.G., Lundebye, A.K., Torstensen, B.F., 2005. Dioxin and dioxin-like polychlorinated biphenyls (PCBs) in Scottish farmed salmon (Salmo salar): effects of replacement of dietary marine fish oil with vegetable oils. Aquaculture 243, 304–314.

Biego, G.H.M., Yao, K.D., Ezoua, P., Kouadio, L.P., 2010. Assessment of organochlorine pesticide residues in fish sold in Abidjan markets and fishing sites. Afr. J. Food Agric. Nutr. Dev. 10, 3.

Breast Cancer Action, 2008. A Hormonal Seesaw: the Atrazine and AI Connection. Newsletter No. 101. Available online at: http://www.bcaction.org/index.php?page=newsletter-101f.

a, 2006. "Decision Documents for atrazine." Office of Prevention, Pesticides, and Toxic Substances. U.S. Environmental Protection Agency. April 6, 2006. http://www.epa.gov/oppsrrd1/REDs/atrazine_combined_docs.pdf.

European Commission, 2004. Commission decision of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisation for plant protection products containing this active substance, 2004/248/EC. Off. J. Eur. Union 1, 53–55.

Ezemonye, L., Ogbeide, O., Tongo, I., 2015. Distribution and ecological risk assessment of pesticide residues in surface water, sediment and fish from Ogbesse River, Edo State, Nigeria. J. Environ. Chem. Ecotoxicol. 7, 20–30.

FAO, 2011. Fishery and Aquaculture Statistics. 2009. Statistics and Information Service of the Fisheries and Aquaculture Department/service. 2009. Rome/Roma, FAO. 2011, p. 78.

Faleyimu, O.I., Agbeja, B.O., Akinyemi, O., 2013. State of forest regeneration in Southwest Nigeria. Afr. J. Agric. Res. 8, 3381–3383.

Fan, W., Yanase, T., Morinaga, H., Gondo, S., Okabe, T., Nomura, M., Komatsu, T., Morohashi, K.I., Hayes, T., Takayanagi, R., Nawata, H., 2007. Atrazine-induced aromatase expression is SF-1 Dependent: implications for endocrine disruption in wildlife and reproductive cancers in humans. Environ. Health Perspect. 115, 720–727.

Fianko, R.J., Augustine, D., Samuel, T.L., Paul, O.Y., Eric, T.G., Theodosia, A., Augustine, F., 2011. Health risk associated with pesticide contamination of fish from the densu river basin in Ghana. J. Environ. Prot. Sci. 2, 115–123.

Guo, Y., Yu, H.Y., Zhang, B.Z., Zeng, E.Y., 2009. Persistent halogenated hydrocarbons in fish feeds manufactured in South China. J. Agric. Food Chem. 57, 3674–3680.

Hakim, D., 2015. A Pesticide Banned, or Not, Underscores Trans-Atlantic Trade Sensitivities. New York Times. February 23. https://www.nytimes.com/2015/02/24/business/international/a-pesticide-banned-or-not-underscores-trans-atlantic-trade-sensitivities.html.

Hayes, T.B., Anderson, L.L., Beasley, V.R., de Solla, S.R., Iguchi, T., Ingrahamf, H., Kestemontg, P., Kniewaldh, J., Kniewaldh, Z., Langloisi, V.S., Luque, E.H., McCoy, K.A., Munoz-de-Toro, M., Oka, T., Oliveira, C.A., Orton, F., Ruby, Sylvia Suzawa, M., Tavera-Mendoza, L.E., Trudeau, V.L., Victor-Costam, A.B., Willingham, Emily., 2011. Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J. Steroid Biochem. Mol. Biol. 127, 64–73.

Hites, R.A., Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., Schwager, S.J., 2004. Global assessment of organic contaminants in farmed salmon. Science 303, 226–229.

Jablonowski, N.D., Schäffer, A., Burauel, P., 2011. Still present after all these years: persistence plus potential toxicity raise questions about the use of atrazine. Environ. Sci. Pollut. Res. 18, 328–331.

Karl, H., Lehmann, I., Oetjen, K., 1998. Levels of chlordane compounds in fish muscle, meal, oil and feed. Chemosphere 36, 2819–2832.

Klementova, S., Keltnerova, L., 2015. Triazine herbicides in the environment. In: Price, A., Kelton, J., Sarunaite, L. (Eds.), Herbicides, Physiology of Action, and Safety. TechOpen, 978-953-51-2217-3.

Lazarko-Connon, S., Achari, G., 2009. Atrazine: its occurrence and treatment in water. Environ. Rev. 17, 199–214.

Liu, J., 2014. Atrazine. Encyclopedia of Toxicology, third ed., pp. 336–338

National Center for Biotechnology Information, 2020. PubChem Compound Summary for CID 2256. Atrazine. https://pubchem.ncbi.nlm.nih.gov/compound/Atrazine.

Ohlson, C.G., Hardell, L., 2000. Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. Chemosphere 40, 1277–1282.

Osibanjo, O., Bashin, N., Onyoyo, H., Bouwman, H., Yive, R., Okond"Ohoka, J., 2002. UNEP/GEF regionally based assessment of persistent toxic substances, Sub-Saharan Africa Regional Report.

Osibona, A.O., Kusemiju, K., Akande, G.R., 2009. Fatty acid composition and amino acid profile of two freshwater species, african catfish *(Clarias gariepinus*) and tilapia (*Tilapia zillii*). Afr. J. Food Agric. Nutr. Dev. 9, 608–621.

Romanic, S.H., Herceg, H.D., Lazar, B., Klincic, D., Mackleworth, P., Fortuna, C.M., 2014. Organochlorine contaminants in tissues of common bottle nose dolphins *Tursiops truncatus* from the northeastern part of the Adriatic sea. Environ. Toxicol. Pharmacol. 38, 469–479.

Sene, L., Converti, A., Secchi, G.A.R., Simao, R.C.G., 2010. New aspects of atrazine biodegradation. Braz. Arch. Biol. Technol. 53.

Sherchan, S.P., Bachoon, D.S., 2011. The presence of atrazine and atrazine-degrading bacteria in the residential, cattle farming, forested and golf course regions of Lake Oconee. J. Appl. Microbiol. 111, 293–299.

World Health Organization (WHO), 1997. Guidelines for Predicting Dietary Intake of Pesticide Residues (Revised) Global Environmental Programme (GEMS/Food) in Collaboration with Codex Committee on Pesticide Residues. Programme of Food Safety and Food Aid 1–44.

World Health Organization (WHO), 2011. Atrazine and its Metabolites in Drinking-Water Background Document for Development of WHO Guidelines for Drinking-Water Quality. https://www.who.int/water_sanitation_health/dwq/chemicals/antrazine.pdf.