

Evaluation of endocrine disruptors in the environment of Eket metropolis in Nigeria: an insight into public health and risk assessment

Victor Eshu Okpashi^{1*}, Agnes Edet Asuquo Offiong², Etim Nkanu Efut², Ogre Gabriel Ogre²

1 Department of Biochemistry, Cross River University of Technology, Calabar, Nigeria

2 Department of Environmental Education, University of Calabar, Nigeria

* Corresponding author e-mail: vic2reshu@gmail.com

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Abstract

The adverse effect of some environmental contaminants on the endocrine system was investigated using the Risk Analysis Framework. The aim was to ascertain the level of EDCs in fish species and potable water. The research employed ecological examination approach. The samples from the environmental media with serious threat and links to humans and animals exposed to endocrine disruptors were water and fish. Twelve boreholes water samples were disproportionately and purposefully collected, while another twelve water samples were collected from Qua Iboe River one kilometer stretched forth. Also, six species of fresh fish including - Atlantic croaker (*Micropogonias undulates*), Catfish (*Clarias gariepinus*), African red snapper (*Lutjanus agennes*), Tilapia (*Oreochromis niloticus*), Yellowtail (*Seriola lalandi*), and Great barracuda (*Sphyrna barracuda*) were used. Both the water and fish samples were screened for endocrine disrupting substances with gas chromatography linking mass spectroscopy. The analysis (results) detected 8% phenol dichloro-4-nitro-, 1, 3, 5-Triazine, and 3% Triazine 2-chloro-4-, 6-bis-methylthio, octadecenamamide in borehole water. Butyl ethyl, hexyl phthalate, indolizine 6%, 4-methyl phenyl, cyclohexane, 3-dione 27%, 2-allylamine methylene 5, 5-hydroxyphenyl 8%, and menazon 17% were discovered in all the species of fish. Phthalate, butyl undecyl esters, 2-ethyl hexyl was 4%, and 7% of isohexyl propyl in Qua Iboe River. The results call for the attention of the regulatory body to put in place measures that will stem the impending disaster this may create in the reproductive capacity of the exposed population.

Keywords

endocrine disruptors; risk; monitoring, aquatic pollution, and hazard

Introduction

The public is worried about the effect of environmental contaminants on human's health. The public, health organization, and nonpublic sectors have shown tremendous zeal in populace healthcare. Taking the decision on the risk of exposure to endocrine disruptors is a beneficial framework for controlling and managing the potential effect on public health (Victor et al., 2018). For about thirty years, the emergence of developmental malformations substances and cancer-causing agents in the environment have become a source of worry in healthcare delivery. Investigations

on the animal model have indicated alterations at the gonadal reproductive insufficiency and hormones (Barron 2012). A number of abnormalities in the reproductive system of different species of animals correspond with abnormalities observed among humans (Mhananjayan and Muralidharan, 2012). These toxins in an environment including, food and edible products interfere with hormone biosynthesis and metabolism, leading to a deviation from normal homeostatic reproduction (Guarneri and Benvenga, 2007). Some reports from the fish analysis, human

clinical observation, and epidemiological investigations implicates ED chemicals as considerable toxins to public healthcare. Some researchers have also shown that estrogen receptors in the channel catfish immune system may change the responses of the immune system (Pluchino et al., 2002). These receptors are analogous to “on-off switches” that can be activated by lock and key mechanism. The report also considers the channel catfish due to their well-studied leukocyte cell lines. The defending of the body’s immune system cells against infectious disease and foreign invaders involves leukocytes (Obiakor et al., 2014). An EDC is produced by growth from superficial material or mixture stressors that modifies or mimics the role (s) of the endocrine gland and impact severe health consequence in an intact organism, its progeny or fill in-populations (IPCS, 2002). Also, EDC is a xenoestrogen that interposes with any part of the endocrine system (Zoeller et al., 2012). ED includes plasticizers, pesticides, cleaning agents, and pharmaceuticals. Fish belongs to the paraphyletic family which is gill-bearing aquatic craniate with dearth limbs and digits (Jock et al., 2015).

The changes that take place in the aquatic animal during gene expression and biosynthesis of hormone is a pointer to the availability of pollutants with EDC properties. The genes for aromatase and aldosterone is vulnerable to EDC effect after exposures, while estradiol and progesterone may produce significant changes (Tannia et al., 2008). The concept of monitoring environmental contaminants is key to articulating and regulating hazards caused to humans. The foresight concept is attuned to increasing the awareness of endocrine disrupting chemicals in the environment and their implication on public health. The utilization of this awareness will enable decisions on the risk of exposing one too, and/or from endocrine disrupting chemicals. EDs are different from one another and may not have structural resemblance apart from being tiny molecules or compounds (Sabine et al., 2015). It is difficult to predict if an EDC will exert endocrine-disrupting actions or not. However, EDCs such as pesticides, PBBs, dioxins, PCBs, may have halogen groups such as fluorine and bromine (Crews et al., 2003). They also have phenol moiety which interposed the normal steroid hormones, this enables the EDCs to bind with receptors as analog or antagonists (Dickerson and Gore, 2007; Watson et al., 2007).

The different spectrums of exposure to EDCs by organisms are different and vary based on location and activities. The condition is persistently developing because ten years ago, some EDCs were banned (Gore and Crews,

2009). Human and animal exposure to EDCs occurs via drinking polluted or untreated water supply, breathing polluted air, ingesting contaminated foods and making contact with polluted soil (Calafat and Needham, 2007). Individuals working in chemical industries are exposed to the high possibility of loss or injury and may possibly develop endocrine abnormalities (Enid, 2010). Some EDCs were formulated with long shelf-lives, this was useful for low application, but durable impart and persistent, this too, has become detrimental to wildlife and humans health (Porte et al., 2006). Also, some ED were traced to pristine environments at secluded locations they were not originally manufactured and used, they are now incorporated in the food chain of uncontaminated region (Stahlhut et al., 2009). Therefore this investigation was aimed at determining the EDCs in environmental media of Eket metropolis.

Materials and methods

Collection of samples from the study area

The water and fish samples were obtained from Eket area of Akwa Ibom State Nigeria. Eket is a growing town in Akwa Ibom State. It measures Northwards at Latitude $4^{\circ} 33''$ and $4^{\circ} 45''$ and Eastward at Longitude $7^{\circ} 52''$ and $5^{\circ} 02''$. On the north, Eket is bounded with Nsit Ubium, Esit Eket at the east, Onna at the west and Ibeno-Bright of Bonny at the south, see Figure 1. The investigation site consists of different points measured to cover possible polluted and unpolluted sites. Basically, twelve boreholes, twelve sampling points stretched forth the Qua Ibeo River - 1 Km aside and six different species of fresh fish were purposively collected (Okpashi et al., 2016).

Designation one. The upstream location was the first sampling point. It is found around the Ikot Dpe and Ikot Ikponding in Eket, at latitude $4^{\circ} 55.8''$ and longitude $7^{\circ} 40.8''$. The human activities in this area are fishing and boat making aside the river bank. The river is comparatively clean by eye-sight evaluation.

Designation two. This area takes in effluents from slaughterhouses along the Ikot Ukpong Village Road and Ikot Ebok. The household wastewater from residential bathrooms and cooking are discharged into the River in this location. The mining and conveyance of sand for business and industrial application is continuous. It measures one kilometer aside from destination one at latitude $4^{\circ} 22.9''$ and longitude $7^{\circ} 13.8''$.

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Designation three. This designation is around the Eket Nsiila area, near Ibeno/Bright of Bonny. Activities in this area involve washing of automobiles, washing of clothing, and bathing. The vegetation in this area appeared denser with bamboo trees shedding the aquifer and tributaries. It is measured one kilometer apart from destination two at latitude $4^{\circ} 23.2''$ and longitude $7^{\circ} 40.2''$.

Designation four. This is stretched to Upandong Village close to Ibeno /Bunny Bright, as illustrated in Figure 1. The human activities in this location are mining and fishing. The vegetation is dominated with mangrove. This location is turbid because of the discharge of waste or effluents; its long depth is due to the mining activities. Its measures one kilometer apart from designation three at latitude $4^{\circ} 43.5''$ and longitude $7^{\circ} 53.3''$.

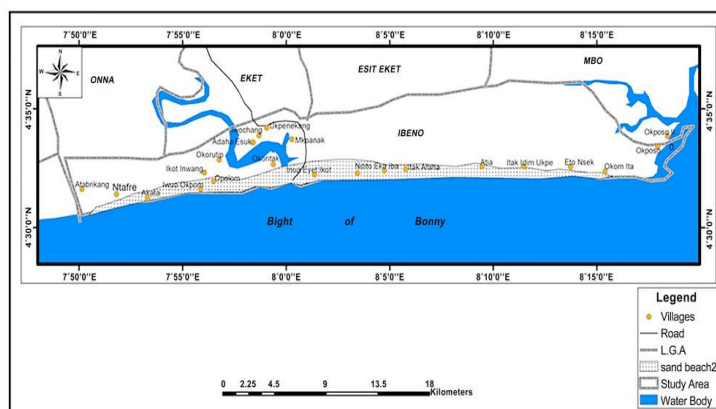


Figure 1. Map of Eket local government area of Akwa Ibom State. The figure above explained the locations where the groundwater – boreholes samples and river water samples were collected for study.

Collection of river water samples (surface water)

Twelve river water samples were obtained from the Qua Iboe River for EDC analysis. The sample bottles were sterilized using autoclave at 120°C in 15 minutes before taken for water samples collection. The bottles were conditioned with the water before collection in 1.0-litre amber glass bottles. Samples were preserved by adding drops of nitric acid at $\text{pH} < 2$ and stored below 4°C in a refrigerator prior to analysis.

Collection of fishes samples

Six species of fresh fish were randomly collected since they are mobile organisms. They include - African red snapper (*Lutjanus agennes*), yellowtail (*Seriola lalandi*), Great barracuda (*Sphyrnaena barracuda*), Atlantic croaker (*Micropogonias undulates*), Catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*) were hooked using a set of bent tool for catching. They were weighed and preserved for analysis in hexane-rinsed aluminum foil and kept inside a closed-glass vessel containing ice pack below -20°C . See Table 1 - dimensions of fish samples, their common names, scientific names, length (cm) and weight (g).

Dissection and extraction of the fish sample for chromatographic analysis

Prior to extraction, the scale and bones of the fish samples were removed using a knife. They were subsequently dissected to obtain the tissues. A quantity (15 g) of the tissue of each fish sample was placed in a clean mortar and grounded with a pestle, and 40 g of anhydrous sodium sulfate until the completely dry homogenized sample was obtained. The samples extraction was carried out using Dichloromethane (DCM). A 10 g of homogenized sample was placed in 50 ml extraction bottle and 1 ml of 60 ml of 1-chloro-octadecane surrogate standard was added in the extraction bottle. The content was agitated or vortexed for five hours and allowed to settle for one hour. The sample was carefully filtered through a funnel fitted with cotton wool, silica gel, and sodium sulfate Na_2SO_4 in a clean beaker or volumetric flask. The residue was washed and made up the volume using the extracting solvent. The samples were concentrated to a volume of 2 mL for EDC. A quantity ($1\mu\text{L}$) of the extracted sample was analyzed with Agilent US EPA 8270 gas chromatography-tandem mass spectroscopy, equipped with flame ionization detector (FID), has the following

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operational conditions: flow rate (H₂ 40mL/min, air 450ml/min), Nitrogen was used as a carrier gas at a flow rate of 40mL/s, injection temperature 300°C;

detector temperature (350°C). The chromatograms were quantified with internal standards.

| The common Name of fish | Scientific name | Length (cm) | Wet weight (g) |
|-------------------------|--------------------------------|-------------|----------------|
| Atlantic Crocker | <i>Micropogonias undulates</i> | 38 | 1184 |
| Catfish | <i>Clarias gariepinus</i> | 35 | 802 |
| Tilapia | <i>Oreochromis niloticus</i> | 24 | 456 |
| African red snapper | <i>Lutjanus agennes</i> | 28 | 543 |
| Yellowtail | <i>Seriola lalandi</i> | 37 | 902 |
| Great barracuda | <i>Sphyraena barracuda</i> | 56 | 1234 |

Table 1. Weight and length of fish samples.

Gas Chromatography linked with Flame Ionization Detector (GC-FID) technique

The GC-FID technique was utilized following the conditions stipulated below:

GC-FID-QP2010 Plus and ion source temperature - 200.00 °C, temperature of interface - 250.00 °C, solvent cut time - 2.50 min., mode detector gain – Mass spectroscopy, detector gain: 0.00 kV, threshold - 2000, initial temperature of column oven - 70.0 °C, injection: 250.00 °C, final temperature of injection mode - Split, flow control mode was linear velocity, pressure - 116.9 kPa, total flow - 40.8 ml/min, column flow - 1.80 ml/min, velocity of linearity - 49.2 cm/s, purge and trap flow - 3.0 ml/min, Split ratio - 20.0, injection at high pressure was switched OFF, the carrier gas was helium, splitter hold switched was put OFF, while the oven rating was: Oven Temperature Program Rate of

Temperature (°C), Hold Time (min) starting - 0.00 70.0 0.00, Finished - 10.0 280 5.00.

Results

The analyzed results are presented in Tables together with their sample number, percentage concentration (%) and species. Their concentration varies with location, age, and species of the fish.

The results revealed phthalate, butyl undecyl ester, phthalic acid, ethyl hexyl ester, and isohexyl propyl ester in Qua Iboe River. Butyl 2-ethyl, hexyl phthalate, indolizine, 2-(4-methyl phenyl), cyclohexane-1, 3-dione, 2-allylamine methylene 5, 5-hydroxyphenyl, and menazon were detected in all the fish species. Phenol, dichloro-4-nitro-, Triazine, chloro-4-, 6-bis (methylthio) - and octadecenamamide were screened in borehole water samples see Table 2.

| Sample 1, 3, 8, 10 Mean concentration (%) | Sample 2, 4, 5, 6, 8 Mean concentration (%) | Sample 3,1, 2, 11, 12 Mean concentration (%) | Sample 4, 6, 7, 9, 11 Mean concentration (%) | Sample 2, 3, 4, 7, 9, 12 Mean concentration (%) |
|---|---|--|--|--|
| Phenol -dichloro-4-nitro- (8%) | Phenol -dichloro-4-nitro- (10%) | Phenol -dichloro-4-nitro- (6%) | Phenol, 2, 6-dichloro-4-nitro- (2%) | 1, 3, 5-Triazine, 2-chloro-4-, 6-bis (methylthio) - (6%) |
| Triazine, 2-chloro-4-, 6-bis (methylthio)- octadecenamamide, (Z) - (3%) | Triazine, 2-chloro-4-,6-bis(methylthio)- octadecenamamide, (Z) - (6%) | Triazine, 2-chloro-4-,6-bis(methylthio)- Naphthalene (12%) | Triazine, 2-chloro-4-, 6-bis (methylthio) - (4%) | Octadecenamamide, (Z) - (2%) |
| Phenol -dichloro-6-nitro- (9%) | Phenol -dichloro-6-nitro- (12%) | Phenol -dichloro-6-nitro- (8%) | Phenol -dichloro-6-nitro- Acenaphthene (2%) | |

Table 2. Endocrine disrupting chemicals detected in groundwater (borehole) Samples and concentration (%).

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| Endocrine Disrupting Chemicals | Concentration (%) | Toxic Effect on target organs | Screened samples |
|--|-------------------|---|---|
| Butyl 2-ethyl, Hexyl phthalate | 15 | Liver, kidney, and lungs | Barracuda, catfish & tilapia |
| Dibutyl phthalate | 22 | Reproductive system | Catfish, yellowtail & red sniper Crocker, Tilapia, yellowtail & Catfish |
| Isohexyl propyl phthalate | 6 | Eyes (reduces or changes visual activity) | Barracuda, Tilapia, yellowtail & Catfish |
| Isolongifolan-8-ol Indolizine, 2-(4-methyl phenyl) | 13 | It is an insect repellent, which affects the skin | Tilapia, Catfish, and Barracuda |
| Cyclohexane-1,3-dione,2-allylamine omethylene 5,5- | 27 | It affects the urinary Secretion and the liver | Tilapia, Catfish, red sniper, barracuda, yellowtail and Crocker |
| dihydroxyphenyl (Benzoylmethyl)-6-methyl-2H-1,4 benzoxazin-3-one | 8 | It affects the liver, spleen, and pancreas | Crocker, Catfish and red sniper |
| Menazon | 17 | Used for treating Pakistanis disease Insecticide for induction of oxidative stress | Crocker, red sniper, tilapia, yellowtail, and Catfish |

Table 3. Endocrine-disrupting chemicals found in six species of fresh fish (%).

The analysis (results) revealed phthalate, butyl undecyl ester, phthalic acid, ethyl hexyl ester, and isohexyl propyl ester in Qua Iboe River. Butyl 2-ethyl, hexyl phthalate, indolizine, 2-(4-methyl phenyl), cyclohexane-1, 3-dione, 2-allylamine methylene 5, 5-hydroxyphenyl, and menazon were detected in all the fish species.

Phenol, dichloro-4-nitro-, Triazine, chloro-4-, 6-bis (methylthio) and octadecenamamide were screened in borehole water samples see Table 3.

Discussion

Twelve borehole water samples were used to screen for EDC and the result is presented in Table 2. The chemical constituents vary with boreholes. The presence and type of chemical were attributed to the dominant anthropogenic activities at that location. For instance, Phenol, dichloro-4-nitro-, Triazine, chloro-4-, 6-bis (methylthio), octadecenamamide and (Z) -Phenol, dichloro-6-nitro- were rummage in sample 1, 3, 8 and 10, respectively. Samples 2, 4, 5, 6 and 8 revealed Phenol, dichloro-4-nitro-1, 3, 5-Triazine, chloro-4-, 6-bis (methylthio), octadecenamamide and (Z)-Phenol, dichloro-6-nitro. While samples 1, 2, 3 and 11 showed

Acenaphthy. The degree of sustainability of the physical environment is an index of the survival and well-being of the entire components (Zoraida et al., 2013). Laboratory studies in rodents and other species have explained some of the mechanisms by which this occurs and strongly indicate how humans are also vulnerable to endocrine disruption. The application of hormones active compounds in medicine has revealed how developing fetus can be exposed to and be affected by endocrine disruptors, and that might take many years for adverse effects to manifest (Heather and Heather, 2009).

The EDCs detected in species of fish suggests that Qua Iboe River is highly polluted. This spells risk for the inhabitants of Eket who use the fishes for various purposes. Also based on the established tendency for the carcinogenicity of EDCs, continuous exposure to/and consumption of the fishes from these water bodies put the community at great risk of cancer and similar toxic effects. Considering the bioconcentration factors for these fishes, some synergistic toxicity of EDCs mixture may be inferred and this may have a possible future effect or a result of risk imposition and the sustenation of some fish species (Pluchino et al., 2014). The indirect releases

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of EDC to water bodies in Eket, Nigeria were mainly due to several crude oil spill incidents. The annual loss in economic terms by the residents of Eket and health risk caused by these spill events have not been evaluated and quantified. Therefore, this investigation was aimed at identifying EDC that have bioaccumulated in the biota in order to develop predictive bioaccumulation model as a screening tool for assessing contaminants' synergetic impact and effect on aquatic animals under future exposure conditions (Ezeonu et al., 2012).

The endocrine chemical that was screened in six different fish species such as croaker, tilapia, yellowtail, African red snipe, Great barracuda, and Catfish indicates a hazard to aquatic health. For example, Butyl 2-ethyl, Hexyl phthalate which has a potential effect on Liver, kidney, and lungs was found in barracuda, catfish, and tilapia. Dibutyl phthalate CAS000084-74-2, an ester of phthalic acid used especially as a plasticizer, a known potent chemical that affects the reproductive system in fishes and human was detected in catfish, yellowtail, and red snipe.

In tilapia, catfish, red snipe, barracuda, yellowtail, and Crocker result shows the presence of (benzoylmethyl)-6-methyl-2H-1, 4-benzoxazine-3-one, a potent Pakistanis disease therapy (Okpashi et al., 2016). According to Ezeonu et al, (2012) menazon insecticide as an analog

of heptachlor (pesticide), a cyclodiene chlorinated hydrocarbon pesticide $C_{10}H_5Cl_7$ that causes liver diseases in animals, a suspected carcinogen and for induction of oxidative stress. This was detected in Crocker, Catfish and red snipers differently, see Table 3. Even though the accessible reports are still inadequate to support a full-scale risk assessment profiling of EDC, substantiating incite to further study and address knowledge gaps and foresight actions against exposure to specific compounds in excess must be advanced (Selma et al., 2013). It is clearly beneficial to consider exposure of human to multiple compounds as a real-life condition or situation to give adequate attention to chemical or compounds that are widely present and persistent inedible stuff and to incorporate measurement of exposure to relevant indicators of biological effects. Such evidence should be considered as enough grounds for taking foresight approach to replace potential EDs.

ED means the ability of a chemical to interfere with normal hormonal systems (Elin et al., 2009). The screening of Qua Iboe river water for EDCs showed phthalates as a predominant pollutant and its derivatives see Table 4. It is responsible for a headache, dizziness, and hypertension and reduces or changes in visual activity in the organism.

| Detected Endocrine Disrupting Chemicals | Concentration (%) | Derivatives | Toxicity/effect of endocrine disruptor (ED) |
|---|-------------------|-------------|--|
| Phthalic, butyl undecyl ester(100308-91-2) | 4 | Phthalate | It is carcinogenic (i.e. can cause cancer of breast) the target organ is breast. |
| Phthalic acid, isobutyl octadecyl ester (1000309-06-1). | 7 | Phthalate | It is genotoxic (i.e. damages gene structure). The target organ is DNA |
| Phthalic acid, butyl tetradecyl ester(1000308-91-3) | 14 | Phthalate | Causes a headache, dizziness, and hypertension |
| Pentadecanoic acid (001002-84-2) | 6 | Not listed | effect not listed |
| n-Hexadecanoic acid (000057-10-3) | 9 | Not listed | effect not listed |
| Phthalic acid, isohexyl propyl ester(100308-98-9) | 8 | Phthalate | Reduces or changes visual activity in the organism. (The target organ is eyes). |
| Dibutyl phthalate (000084-74-2) | 12 | Phthalate | Not classifiable as for human carcinogen |
| Phthalic acid, 2-ethyl hexyl ester(1000309-02-5) | 7 | Phthalate | Displaces (3H) estradiol from its binding sites enhancing adenyl cyclase activity and inhibiting prostaglandin output, infertility, decreased sperm count. |

Table 4. Endocrine disrupting compounds detected in surface water (Qua Iboe River) samples (%).

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Phthalate displaces 3H estradiol from its binding sites enhancing adenylyl cyclase activity and inhibiting prostaglandin output, infertility and decreased sperm count (Curtis et al., 1999). Perturbation of this developmental progression can permanently alter the capacity for reproductive success (Sesukova et al., 2016). This plasticizer has been listed by the United State Environmental Protection Agency (USEPA) as one of the potent EDCs, because of its extreme capacity as carcinogenic and genotoxic. It is understood that upon exposure to EDC, changes may occur in the development, at the shape of the vertebrate brain, sex-specific physiology and behaviors. These usually occur in discrete developmental opening that span gestation to prenatal period and continues to puberty (Rutkowska et al., 2016). Frequent exposure to EDCs may show the consequential effect on the reproductive system and incite the features of the opposite sex, such as enlargement of breast in male and development of eggs in the testes of male fish. For example, xenoestrogenic EDCs are derived from different sources like natural estrogens to artificial medicament and agrochemicals which permeate the waterways. Research has reported that B-cells that generate antibodies, T-cells that control and coordinate immune responses and destroy cell infected by a virus, and macrophages that swallow up invaders, have varying arrays of estradiol receptors (Cynthia et al., 2004). It is possible to believe that these cells are directed by xenoestrogens differently and if affected by chemicals, adverse health effect will be the outcome. The presence of EDC may bring about different types of interactions such as additivity, antagonism, potentiation, and synergism. These interactions can cause damage to enzymes system, disruption of protein synthesis, production of reactive chemical species in cells and DNA damage (Ezeonu et al., 2012). Also, chronic exposure to EDC may result in physiological damage of cancer induction, mutation, and teratogenicity (Ranu et al., 2009). Studies in environmental endocrine disruption have contributed to the general knowledge as to how early life exposure to EDCs may alter the reproductive system through non-genomic, epigenetic mechanisms - such as DNA methylation and histone acetylation. These types of effects have the potential to impact future generations if the germ line is affected and extended to progeny (Sweeney et al., 2015). These findings did not only suggest that EDs may make fishes more vulnerable to disease, but it gives the basal information that improves our understanding to guide research with fish species and potable water samples, in order to investigate the

persistence of environmental endocrine disruptors, and to show their relationships between disease causation in the marine ecosystem, public healthcare, and EDs through food chain.

Conclusion

The investigation detected several EDCs in environmental media with concern to human health. The EDCs in fish and groundwater call for strict regulations and treatment of drinking water. These results call for the attention of the regulatory body to put in place measures that will stem the impending disaster this may create in the reproductive capacity of the exposed population.

Conflict of interest

The author (s) hereby declare no competing interest regarding the publication of this article.

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