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Spectroscopic analyses and genotoxicity of dioxins in the aquatic environment of Alexandria

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ABSTRACT

Dioxins have global concerns because of the bioaccumulation tendency and persistency in the environment. Water, seabream *Pagrus auratus* and seabass *Dicentrarchus labrax* samples were collected from Abu Qir, Alexandria to evaluate the concentration of dioxin. Fourier Transform Infrared Spectrometer (FTIR) and molecular modeling was applied for elucidating the molecular structure of fish samples. Furthermore, HPLC with UV detection was used to determine the concentration of dioxins (2,8-dichloro dibenzo-p-dioxin). RT-PCR assay was conducted to verify the expression of some immune genes in the fish species as a result of water pollution. The average detected concentrations varied from 0.2 to 1.3 μg/l. Gene expression revealed that MHC class 1 and C3 were highly upregulated in liver and muscle of seabass and seabream while T2BP was highly regulated in seabass liver and seabream muscle and seabass muscle for transferrin, FTIR and molecular modeling indicate that dioxin finds its way to fish protein.

1. Introduction

Aquatic pollution is a fundamental problem due to its ubiquitous occurrence, recalcitrance properties, suspected carcinogenicity and mutagenicity to humans and biota. Moreover, water pollution with organic compounds is a hot topic of concern. *Polycyclic aromatic hydrocarbons* (PAHs) are included in the priority lists of poof the U.S. Environmental Protection Agency (EPA) and the European Union (Nassar et al., 2011; Li et al., 2012). Although some of such organic pollutants are naturally occurring, the majorities are anthropogenic and enter the environment through release of petroleum products (petrogenic sources) or by combustion of organic matter (pyrogenic sources) (Nassar et al., 2012). The Mediterranean Sea is a semi-closed sea, surrounded by highly populated and industrialized areas. For millenniums, the Mediterranean Sea has been the scenery of human development, which has extensively influenced the coastal areas. The Egyptian coast of the Mediterranean stretches for almost 1200 km from Rafah at the east to Sallum at the west (Emam et al., 2013). Alexandria is Egypt's largest city on the Mediterranean coast with 100 large factories and about 260 smaller ones (Abd-Alla, 1993), to cover about 40% of the Egypt's industry. It is also the main summer resort in Egypt were have two million visitors are visiting Alexandria in summer (Nasr, 1995). DNA barcodes analysis was used for molecular identification of some Mediterranean fish species in Alexandria as well as Gaeta coasts. Results recommended that, the genetic fish database improvement and basic data participated in policy interface for biodiversity conservation in addition to long-term human well-being (Giulia et al., 2017). The term 'Dioxin' covers a wide range of halogenated aromatic compounds, including polychlorinated dibenzo-p-dioxins (PCDDs). Dioxins are formed during industrial activities including incomplete combustion of hydrocarbons in the presence of chlorine in metal processing, and domestic waste incineration.

Dioxins are among the group of compounds known as Persistent Organic Pollutants (POPs). They are known to bio accumulate in the organisms as they prefer to accumulate in lipids, consequently their accumulation will results in health hazards which varies from organism to another depending on several factors including exposure time, life cycle of the organism, sexuality. Dioxins have high melting points and persistent to degradation which makes it last for quite long time in the environment. Dioxin impacts on gene expression in fish and other

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marine organisms were discussed in various literatures (Vannuccini et al., 2015; Velki et al., 2017; Liu et al., 2013). These impacts including alteration in gene expression reflected as up and down regulations in addition to hormonal and enzymatic disruptions.

The impact of toxic materials on the integrity and functioning of DNA has been investigated in many organisms (Ali et al., 2008; Abdel-Gawad et al., 2011; Bombail et al., 2001). Several biomarkers have been utilized as tools for detection of exposure to genotoxic pollutants such as gene expression which is increasingly became important tool in many research fields (Abdel-Gawad et al., 2016; Abdel-Gawad et al., 2014; Gontijo et al., 2003; Mocellin et al., 2003; Koyanagi et al., 2005). Reverse transcription polymerase chain reaction (RT-PCR) an established method commonly used to measure transcript abundance in biological samples. RT-PCR permits the simultaneous analysis of the expression levels of a small number of genes in many different samples (Ali et al., 2008; Ishii et al., 2007). Fourier-transform infrared spectroscopy (FTIR) and molecular modeling was coupled together to elucidate the molecular structure of many systems and structures in the aquatic environment (Ibrahim et al., 2008; Ibrahim et al., 2012; Ammar et al., 2014; Elhaes et al., 2014; Okasha et al., 2015; Ibrahim et al., 2015). Both could be coupled with analyses of water, sediment, fish (Abdel-Gawad et al., 2012) and correlate the fate and transport of pollutions in the aquatic environment (El-Bially et al., 2016).

Based upon the above consideration, the effect of dioxin upon marine fish in Alexandria is studied. Accordingly, FTIR is used to study marine fish, then molecular modeling at HF/3-21g** was used to model the interaction between hexahydrated dioxin and protein. To verify the model HPLC with UV was used to determine the concentration of dioxins while RT-PCR assay was conducted to verify the expression of some immune genes in the studied two fish species.

2. Materials and methods

2.1. Sampling site

Water and fish samples were collected from the Mediterranean coast of Alexandria at Abu Qir area as seen in Fig. 1. Water and two fish species (Seabream, *Pagrusaur Atus* and seabass, *Dicentrarchus labrax*) were collected monthly for one year. Pyrex amber glass containers previously rinsed with high-purity water (1 L) were used for water collection. Samples were transferred in an ice box to the hydrobiology laboratory, Centre of Excellence for Advanced Science, National Research Centre. Fish samples were (Seabass and Seabream) dissected then muscle and liver were isolated and used for RNA extraction and gene expression experiments.

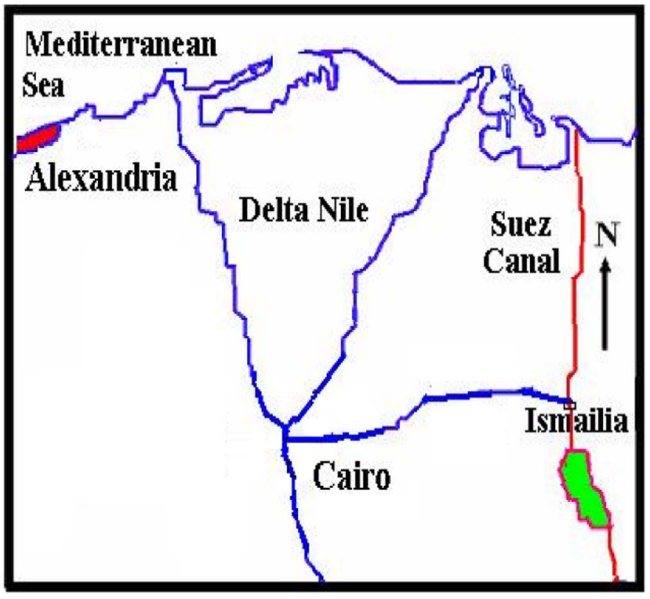

Fig. 1. Sampling site of water and fish samples along Alexandria coast at Abu Qir area, the area is marked with circle.

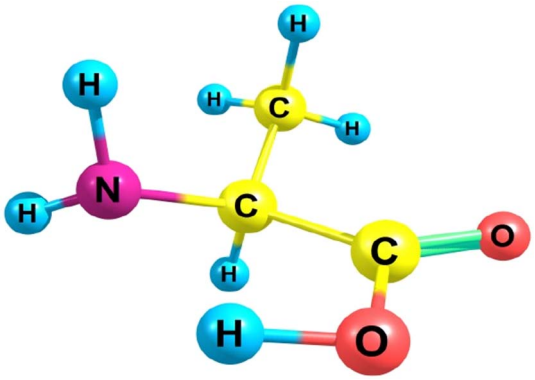

Fig. 2. HF/3-21g** optimized structure for the model molecule of alanine.

2.2. Molecular modeling calculations details

The geometries of alanine (simple amino acid used as a model molecule for protein), dioxin, alanine-dioxin and dioxin- alanine with six water molecules were optimized using the Gaussian09 program system at Spectroscopy Department, National Research Centre (Frisch et al., 2010). First each structure was optimized at the HF/3-21g** ab initio quantum mechanical method to locate the energy minimum then vibrational frequencies were calculated at the same level of theory. The optimized model molecules of alanine, dioxin, dioxin-alanine and dioxin-alanine with six water molecules are represented in Figs. 2, 3, 4 and 5 respectively. Total dipole moment, HOMO-LUMO band gap energy, bond lengths and bond angles for the studied structure were carried out at the same level of theory.

2.3. FTIR analysis of fish tissues samples

The muscle and liver samples were dried at 65 °C overnight, then grinded. Dried samples were then measured directly with Attenuated Total Reflection-Fourier Transformation Infrared spectroscopy ATR-FTIR (VERTEX 70 Bruker, Germany) at Spectroscopy Department, National Research Centre, Egypt.

2.4. Analysis of dioxins (2,8-dichloro dibenzo-p-dioxin) concentration

All water samples were filtered with high-pressure filtration equipment using pure cellulose membrane filters (pore size 0.45) and stored at 4 °C in the dark overnight till analyses. Aliquots of the filtered water samples were extracted by solid-phase extraction using C18 disks to a final volume of 1 mL in ethyl acetate prior to injection into the

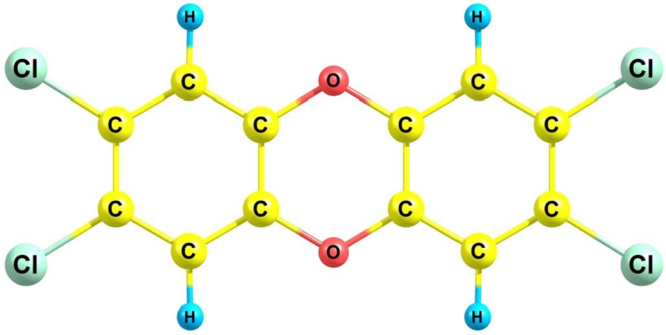

Fig. 3. HF/3-21g** optimized structure for the model molecule of dioxin.