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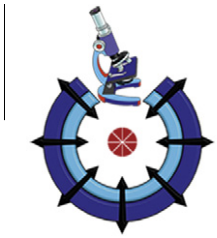

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ARTICLE

# Oxidative stress and DNA damage in relation to transition metals overload in Abu-Qir Bay, Egypt

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**Abstract** The aim of the present study is to evaluate the transition metals overload in Abu-Qir Bay in Egypt, as compared to a less polluted area (reference area) through some biomarkers of oxidative stress. Catalase enzyme activity, malondialdehyde (MDA) concentration and DNA damage (number of apurinic/apyrimidinic sites) were the tested biomarkers. The levels of iron and copper in *Mugil cephalus* liver tissues were significantly higher in samples from the polluted area as compared to the reference area: Fe: 407 ± 38 *vs.* 216 ± 21 μg/g wet wt; $p = 0.008$, Cu: 54 ± 6 *vs.* 17.7 ± 4 μg/g wet wt; $p = 0.0001$. This could account for the observed increase in MDA concentration (15.7 ± 5.7 *vs.* 2.5 ± 0.5 U/g; $p = 0.035$), and the elevated number of AP sites (13.9 ± 2.6 *vs.* 0.37 ± 0.2 AP site/$1 \times 10^5$ bp; $p = 0.0001$). Similarly, the activity of catalase enzyme responsible for the cellular defense was significantly high (58.3 ± 12.2 *vs.* 28.4 ± 4.0 U/mg; $p = 0.032$). The present data indicated a clear relationship between the pollution degree of the above marine environment and both biochemical and molecular responses of the piscine system.



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## 1. Introduction

The oceans are the ultimate sink for many chemicals of anthropogenic origin, such as metals [52]. Abu-Qir Bay is considered as one of the major hot spots along the Egyptian Mediterranean coast [53]. Along the coast of Abu-Qir Bay, there exist about 22 different factories representing four major categories of industrial wastes which are: food processing and canning, paper industry, fertilizers industry and textile manufacturing. The wastes of these industries are of either organic or inorganic nature and are pumped to the sea through El-Tabia pumping station; it pumps out an average amount of

1.5–2.0 million $m^3$ of polluted water per day [39]. The enrichment of trace metals in the bay has been shown by a number of studies [1,39]. These studies showed that the concentrations of trace metals (Fe, Cu, Zn, Pb, Mn, Cr, Co, Cd and Ni) have increased to a huge extent, deducing that industrial wastes deposited from several factories are the main contributors of the heavy metals characterizing the bay environment [2].

Unlike other classes of pollutants, which can be biodegraded and destroyed completely; deposited metals are not biodegradable and cannot be destroyed [55,56]. They may accumulate unnoticed in the aquatic environment to toxic levels. Some metals, such as Zn, Cu, Mn and Fe, are essential for aquatic organisms, but show toxic effects when organisms are exposed to higher abnormal concentrations [45]. Although their toxic and genotoxic effects on biological systems, transition metals; especially Cu and Fe, are of great interest and are considered as essential elements [5].

The toxicity of transition metals is often due to their great participation and action as catalysts in the production of the reactive oxygen species (ROS)[1] through the Fenton/Haber-wiess reactions, which are highly reactive chemicals containing oxygen; e.g. hydroxyl free radical $OH^{\bullet}$ that reacts easily with other molecules, resulting in potentially damaging modifications.

The accumulation of ROS in cells can lead to oxidative stress in which the effects of prooxidants (e.g. free radicals, reactive oxygen and reactive nitrogen species) exceed the ability of antioxidant systems to neutralize them [49]. In biological systems, oxidative stress has become of significant interest issue for environmental toxicology studies; especially oxidative damage induced by different classes of chemical pollutants [54]. Toxic consequences of oxidative stress at the subcellular level include lipid peroxidation, oxidative damage to DNA and proteins as well as alteration of the antioxidant enzymes responses [23].

Lipid peroxidation has been used successfully as a measure of xenobiotic-induced oxidative stress, especially by transition metals such as iron, mercury and copper [31]. In transition metals, catalyzed lipid per oxidation, $HO^{\bullet}$ is thought to be the primary initiating radical species [46]. Lipid per oxidation leads to destruction of membrane lipid, production of lipid peroxides and their by-products such as aldehydes. Malondialdehyde (MDA) is formed from the breakdown of polyunsaturated fatty acids (PUFA) and it serves as a convenient index for determining the extent of lipid peroxidation [22].

The production of $H_2O_2$ within the cell, may lead to the production of $HO^{\bullet}$ and subsequent cellular damage via the metal-catalyzed Haber-Weiss reaction. Thus, it is important to remove $H_2O_2$. Catalase; a heme-containing enzyme, functions to rapidly disintegrate $H_2O_2$ to water and oxygen ($k > 10^7\ M^{-1}\ s^{-1}$) [26]. It belongs to the cellular antioxidant system that counteracts the toxicity of ROS. Catalase is often induced as a result of oxidative stress, the decomposition of $H_2O_2$ is directly proportional to both the concentration of the enzyme and the concentration of substrate ($H_2O_2$) [57]. It is worth mentioning that, the measurement of catalase activity in aquatic organisms has been extensively used as biomarker of exposure in several biomonitoring studies on different marine organisms such as mussels, freshwater bivalve; *Uno tumidus*, sea fishes; *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area [14,38,12,30,17], and also on *Mugil cephalus* in Spain [44].

The most important and recently used biomarker of oxidation stress initiated by transition metals overload is the DNA damage. The increasing genotoxic risk in marine environment is motivating an intense and continuous ecosystem research in an effort to develop and apply new biomonitoring tools. Such biomonitoring research offers great potential for future applications [3]. As a molecular biomarker for genetic effects; DNA damage, integrity and repair processes, introduce a highly specific and very early signal of genotoxic xenobiotic effects in some organisms. Under the constant influence of xenobiotics on living organisms, measured DNA damage represents the dynamic state between constant DNA damage occurrence and its repair [3]. In such cases DNA damage could reflect the level of marine pollution by genotoxic xenobiotics. The genotoxic risk assessment and the estimation of endangered marine organisms are based on the monitoring of DNA damage frequencies caused by genotoxins. The modulation of DNA damage and repair mechanisms caused by genotoxic stress in the environment can predict future trends and are considered as early warning indicators [3,11].

One of the most prevalent lesions in DNA is the apurinic/apyrimidinic (AP) site, derived from the cleavage of the N-glycosyl bond by DNA glycosylase or by spontaneous depurination [20]. Under normal unstressed conditions, AP sites could be repaired by the base-excision repair mechanism; but under stress conditions, it was proven that the excision repair capacity of oxidative damage, a potentially protective cellular mechanism has been retained. Lack of such a repair capability can cause accumulation of this type of DNA base damage [11]. The oxidative damage of DNA is mainly caused by the hydroxyl ($OH^{\bullet}$) free radical [50]. Hydroxyl free radicals can attack the sugar-phosphate backbone of DNA, causing a different variety of lesions, including base free AP sites where the base has been removed by oxidant-mediated reactions [23]. In fact, AP-sites are one of the most frequently occurring lesions caused by ROS. ARP (Aldehyde Reactive Probe) reagent reacts specifically with an aldehyde group, which is the open ring form of the AP sites. The reaction makes it possible to detect DNA modifications that result in the formation of an aldehyde group, and that was the technique used to quantify AP-sites in the present study. After treating DNA containing AP sites with ARP reagents, AP sites are tagged with biotin residues, which can be quantified using avidin–biotin assay followed by a sensitive, rapid and easy colorimetric detection [37]. The danger of such damage in the DNA is that it may result in blockage of DNA replication and transcription; and therefore, cause lethal damage to the cell's DNA, when left unrepaired [18,6].

## 2. Materials and methods

### 2.1. Fish sampling and samples preparation

Ten fish individuals of *M. cephalus* species were collected from Abu-Qir Bay and Sidi-Barrani. Sampling areas are shown in

[1] *Abbreviations:* 8-OHdG, 8-hydroxyguanine; AAS, atomic absorption spectroscopy; AP site, apurinic/apyrimidinic site; ARP, *N*′-aminooxymethyl-carbonylhydrazino-D-biotin; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; MDA, malondialdehyde-bis-diethyl-acetal; PAHs, polycyclic aromatic hydrocarbons; POPs, persistent organic pollutants; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species.