Oxidative stress and antioxidant enzymes in *Oreochromis niloticus* as biomarkers of exposure to crude oil pollution

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ABSTRACT

In recent years, oil pollution has become a global environmental issue in that oceanic as well as inland aquatic breeding ecosystems are threatened greatly. Several aquatic bodies in Egypt are being affected by crude oil and hydrocarbons which are of anthropogenic contribution and a wide spectrum of petroleum contaminants are detected in water, sediment and animals. In the present study the fish *Oreochromis niloticus* was exposed to three sublethal concentrations (350, 1750 and 3500 ppm) of crude oil representing 5, 25 and 50 % of 96 hr.LC_{50} respectively. The activity of antioxidant enzymes and oxidative stress [superoxide dismutase (SOD), catalase (CAT), glutathione S. transeferase (GST)] and malondialdehyde (MDA) in the liver were investigated , as well as gills Na^+ / K^+ adenosine triphosphatase ($Na^+ / K+ATPase$) and serum electrolytes (Na^+ and K^+) were also measured after 5, 15 and 30 days of exposur. The results revealed that induction of SOD, CAT and GST enzymes activities in the liver of *Oreochromis niloticus* were increased with higher concentrations of the crude oil and time of exposure. Also lipid peroxidation was evolved through the elevation of MDA in the liver after 30 days of exposure to crude oil. Inhibition of Na^+/K^+ ATPase activities in the gills was recorded during the exposure period at different concentrations. Serum Na^+ and K^+ were decreased significantly (except K^+ after 30 days of exposure).From the results it can be concluded that the activities and expression levels of antioxidant enzymes and oxidative stress can be used as biomarkers to evaluate the influence of crude oil on the biochemical pathway and enzymatic function in *Oreochromis niloticus* that can be used for biological monitoring unacceptable levels of environmental contamination.

1. INTRODUCTION

Petroleum oil provides ingredients for thousands of products that we use everyday, but this involves various potential problems .In recent years, oil pollution has become a global environmental issue in that Oceanic ecosystem and inland aquatic breeding ecosystems are threatened greatly. Pollution by crude oil is wide spread and a common problem, and particularly endemic in countries whose economies are dependent on the oil industry. Such pollution arises either accidentally or operationally wherever oil is produced, transported, stored, processed or used. The constituents of crude oil are complex. It contains aliphatic, alicyclic, polyaromatic hydrocarbons, oxygen, nitrogen and sulphur containing substances.

The other components include toxic phenols and anilines (Traven, 1992). The content of components differs depending on the area of oil drilling. All crude oils and many refined products in sufficient concentrations are poisonous to aquatic organisms. Direct killing of aquatic organisms can occur through coating with oil and by asphyxiation (Hoong *et al*., 2001). In addition, the incorporation of finely dispersed particles of oil and oil products into organisms can negatively affect the body organs and systems, either directly or as a consequence of bioaccumulation processes (Lockhart *et al*., 1996).

On the other hand, there is a growing concern that several aquatic bodies in Egypt are being affected by crude oil and hydrocarbons which are suggested to be of anthropogenic contribution. Also a wide spectrum of petroleum contaminants in Egyptian aquatic environment are detected in water, sediment and aquatic animals (Hegazy, 2007). Fish responses have been used as biomarkers of aquatic pollution (Ek *et al.,* 2005) .It is important to examine the toxic effect of crude oil on fish since they constitute an important link in the food chain. The Nile tilapia *Oreochromis niloticus* are widely distributed freshwater fish that can persist in a highly polluted habitat and is possible to use as a potential bio-indicator for aquatic environmental contaminants including crude oil. The activities and expression levels of antioxidant enzymes and metabolic were used as biomarkers to evaluate the influence of pollution on the biochemical pathway and enzymatic function in fish (Sun *et al*., 2006 and Correia *et al.,* 2007) and also for monitoring unacceptable levels of environmental contamination. However, bioassay studies on the change in activities and expression levels of antioxidant enzymes in fish exposed to crude oil are limited, since most of such studies are concerned with endocrinology, hematology, respiration, osmoregulation and histopathology (Alkindi *et al*., 1996; Barron *et al*., 2003; Couillard, *et al*., 2005; Simonato *et al.,* 2008 and

Mohamed, 2009). Therefore, this study was designed to evaluate the time and concentration dependent changes in antioxidant enzymes (SOD, CAT and GST) activities as well as the concentration of (MDA) as a bi -product of lipid peroxidation in the liver of *Oreochromis niloticus* following crude oil exposure at sublethal concentrations for 30 days. Due to membrane function being a major indicator of radical injury, investigation of Na^+/K^+ ATPase in gills and serum electrolytes Na^+, K^+ were also assayed .

2. MATERIALS AND METHODS

2.1. Site of work: this study was carried out at the Barrage Fish Research Station, Al kanater Al-khairya Cairo, Egypt.

2.2. Pollutant: the crude oil used in this study was taken from Balayim field, Raas Ghareb .Red Sea

2.3. Experimental fish:

Healthy specimens of *Oreochromis niloticus* having a body weight of 82.4 $\pm$6.2 g. and length 17.0$\pm$1.2 cm were obtained from a fish farm at Al-kanater Alkairya. Fish were acclimated to the laboratory conditions for a period of 2 weeks in large fiberglass tanks containing well aerated dechlorinated tap water (temperature 25 $\pm$0.5C^{0,} pH, 7.4 $\pm$0.3 and oxygen concentration 7.7$\pm$0.2 mg/L). During acclimation, the fish were fed on commercial pellets (30 % protein) once per day. Water was renewed every 48 hr. with routine cleaning of aquaria leaving no faecal matter or unconsumed food .Two days prior to application of crude oil, fish were transferred from the stock tanks to the glass aquaria (100 Liters) provided with well aerated tap water.

2.4. Toxicity bioassay

Preliminary experiments were conducted to determine the median lethal concentration after 96 hr (96hr.LC_{50}) for crude oil. Various concentrations of crude oil (1000, 2000, 4000, 8000, 16000, and 32000 ppm) were prepared in 100 l. glass aquaria. Groups of 10 fish were exposed to each test concentrations of crude oil for 96 hr.

Another fish group served as a control. The median lethal concentration at 96 hr.(96 hr.LC_{50}) was calculated according to the method of American Public Health Association(APHA,1995) and was found to be 7000 ppm.

2.6. Crude oil exposure

In the present experiment, fish were divided into four groups in three replicates each group included 24 fish. The crude oil was added to the experimental glass aquaria one hour before the transfer of fish.

Group 1: Exposed to 350 ppm (5% 96 hr. LC_{50})

Group2: Exposed to 1750 ppm (25% 96 hr. LC_{50})

Group 3: Exposed to 3500 ppm (50% 96 hr.LC_{50})

Group 4: Served as a control.

Feeding was allowed in the experimental as well as control groups once per day.

2.7. Sampling and procedure

At the end of exposure period (5, I5 and 30 days) 8 fish from each group were taken and blood samples were withdrawn by cutting the caudal peduncle and collected in non heparinized tubes. Blood was allowed to coagulate at room temp for 2hr, then centrifuged at 3000 r.p.m for 15 min. Serum was then removed and used for electrolyte (Na^+ and K^+) analysis After dissection liver and gills tissues were carefully removed and washed with ice cold saline (0.7 Nacl). The gill filaments were separated from the gill arches, weighed to the nearest mg. Tissues (liver or gill filaments) were homogenized in 0.25 M sucrose buffer at pH 7.4 using a glass homogenizer and then centrifuged at 8, 000 r.p.m for 20 min. The supernatant was used for enzymes assays.

2.8. Enzymes assays

The activity of superoxide dismutase (SOD) in the liver tissues was determined spectrophotometrically at wave length 480 nm by epinephrine method according to Misra (1972) and expressed in units of enzymes activities per gram of tissues wet wt. The activity of catalase (CAT) in the liver was determined spectrophotometric at wave length 570 nm according to the method of Sinha (1972) and was expressed in ml mol of decomposed hydrogen peroxide per sec

per gram of tissues wet wt. The activity of glutathione S transferase (GST) was determined spectrophotometric at wave length 340 nm according to the method of Habig *et al.,* (1974) using 1-chloro- 2-4 dinitrobenzene (CDNB) as substrate. It was expressed in µ mol /min/mg protein wet wt. Malondialdehyde (MDA) was determined according to the method of Nair and Turner (1984). MDA derived from lipid peroxidation was determined with thiobarbituric acid (TBA). 0.5 ml homogenate without filtration was taken and 4.5 ml of TBA reagent was added. The mixture was heated using boiling water bath for 20 min, centrifuged at 2500 r.p.m for 10 min. The absorbance of supernatant was recorded at wave length 525nm MDA results were expressed as µ mol of MDA per g. wet wt.in the tissues. Na^+/K^+ ATPase activity in the gills was measured at $37C^0$ and calculated as the difference between rate of inorganic phosphate liberated in the presence and absence of Ouabain (Johnson *et al*., 1977). The released inorganic phosphate was measured spectrophotometric at wave length 740 nm according to the method of Beechey *et al*., (1975) and expressed as µ mol /mg protein/hr.

Serum sodium (Na^+) and potassium (K^+) were measured spectrophotometric at wave length 578 nm using the method of Tietz (1976) (SPECTRUM kit, Egypt), and the results expressed as ml mol /L. Each assay was carried out in replication.

2.9. Statistical analyses:

All values were expressed as mean $\pm$standard error. The significance of difference between control and experimental data was statistically analyzed using student $^{((}t^{))}$ test (Sendecor and Cochran, 1980).

3. RESULTS

The changes in SOD, CAT, GST enzymes and lipid peroxidation) (MDA) in the liver of *O. niloticus* exposed to three sublethal concentrations (350,1750 and 3500 ppm) of crude oil were presented in Tables (1&2). A slight non significant increase was

observed in SOD and CAT enzymes after 5 days of exposure to different concentrations of crude oil. However after 15 and 30 days of exposure the activity of SOD was increased significantly to (31.1-39.3 %) and (27.7-49.1), P$\leq$0.05. Similarly, CAT activity was increased significantly by (38.9–147%) and (61.6-178%) at low and high concentrations of crude oil. For GST the activity was significantly increased with the exposure concentration and duration time to (50-75%),(90-174%) and (104-205%) for 5,15 and 30 days respectively at 350 – 3500 ppm crude oil (P$\leq$0.05 and 0.01).

Table 1: Changes in superoxide dismutase (SOD) and Catalase (CAT) enzymes in the liver of _Oreochromis niloticus_ exposed to crude oil .

Conc.of crude oil	SOD(unit/g wet wt.)			CAT (ml mol/g wet wt.)		
	Exposure period(days)			Exposure period (days)		
	5	15	30	5	15	30
Control	343$\pm$2.6	351$\pm$0.5	346$\pm$1.7	72$\pm$1.4	72$\pm$2.6	73$\pm$3.7
350ppm	362$\pm$1.2	445$\pm$3.9	442$\pm$1.9	76$\pm$1.8	100$\pm$5.2	118$\pm$6
	(+5.54)	(+26.8)	(+27.7)	(+5.6)	(+38.9) *	(+ 61.6)*
1750 ppm	374$\pm$3.5	460$\pm$4.1	500$\pm$2.9	72$\pm$4.2	123$\pm$6.0	187$\pm$7
	(+9.03)	(+31.1)	(+44.51)*		(+70.8)**	(+156) **
3500ppm	374$\pm$2.3	489$\pm$7.2	516$\pm$4.1	80$\pm$1.6	178$\pm$5.1	203$\pm$6.0
	(+9.03)	(+39.3)*	(+49.1)*	(+11.1)	(+147)**	(+178.)**

Data are represented as mean $\pm$standard error (M$\pm$S.E) of 8 fish . Figures between brackets are % of change from control values. * Significant difference at P$\leq$0.05.   ** Highly significant at P$\leq$ 0.01

Table 2: Changes in glutathione S transferase (GST) and lipid peroxidation (LPO) in the liver of _Oreochromis niloticus_ exposed to crude oil.

Conc.of crude oil	GST (μ mol/min/mg protein wet wt .)			LPO (MDA) μ mol/g wet wt.		
	Exposure period (days)			Exposure period (days)		
	5	15	30	5	15	30
Control	1.0$\pm$0.07	0.95$\pm$0.02	0.98$\pm$0.03	52.$\pm$2.4	50$\pm$3.0	53$\pm$2.6
350ppm	1.5$\pm$0.17	1.8$\pm$0.03	2.0$\pm$0.12	55.6$\pm$3	54.6$\pm$3.2	64.6$\pm$3
	(+50)*	(+90)**	(+104)**	(+6.3)	(+9.2)	(+29.2)
1750 ppm	1.3$\pm$0.16	2.2$\pm$0.05	2.5$\pm$0.6	58.6$\pm$8.6	56$\pm$2.4	84$\pm$3.4
	(+30)	(+132)**	(+155)**	(+12)	(+12.8)	(56.7)**
3500ppm	1.75$\pm$0.12	2.6$\pm$0.15	2.9+0.14	54$\pm$3.2	52$\pm$6.1	90$\pm$5.0
	(+75)*	(+174)**	(+205)**	(+3.3)	(+4)	(+68)**

Data are represented as mean $\pm$standard error (M$\pm$S.E) of 8 fish. Figures between brackets are % of change from control values * Significant difference at P$\leq$0.05.   ** Highly significant at P$\leq$ 0.01

MAD level in the liver of _O. niloticus_ was not changed significantly at low dose of crude oil and short exposure time. However, there was a statistically significant increase in MAD to (56.7 – 68%) (P$\leq$ 0.01) at 1750 - 3500 ppm for 30 days of exposure.

The effect of crude oil exposure on Na$^+$/K$^+$ ATPase activity in the gills of _O. niloticus_ was presented in Table(3).

Table 3: Change in Na$^+$/K$^+$ ATPase enzyme in the gills of _Oreochromis niloticus_ exposed to crude oil.

Conc.of crude oil	Na$^+$/ K$^+$ ATPase (μ mol/mg protein/hr.)		
	Exposure period(days)		
	5	15	30
Control	9.0$\pm$0.1	9.4$\pm$0.5	9.2$\pm$0.l7
350ppm	6.1$\pm$0.17	6.8$\pm$0.12	5.8$\pm$0.16
	(-32)*	(-27.7)	(-37)
1750 ppm	5.4$\pm$0.15	4.6$\pm$0.22	4.4$\pm$0.15
	(-40)*	(-51.1)*	(-52.2)*
3500ppm	4.6$\pm$0.15	3.8$\pm$0.15	3.5$\pm$ 0.14
	(-49)*	(-59.5)*	(-62)*

Data are represented as mean $\pm$standard error (M$\pm$S.E) of 8 fish. Figures between brackets are % of change from control values * Significant difference at P$\leq$0.05.   ** Highly significant at P$\leq$ 0.01.

The results of the present study showed that the activity of Na$^+$/K$^+$ ATPase in the gills of *O. niloticus* was decreased significantly with the increase of crude oil concentrations and time of exposure to (32 - 49%),(27.7 -59.5) and (37-62%) , P$\leq$0.05.

The changes in serum electrolyte in *O. niloticus* exposed to crude oil was presented in Table (4). The results showed that there was a decrease in serum Na$^+$ but statistically not significant after 5, 15 and 30 days of exposure to low concentrations (350 and 1750 ppm) of crude oil. However, at very high concentration (3500ppm) there was a significant decrease (-32%) P$\leq$0.05 after 30 days of exposure. Also serum K$^+$ was decreased insignificantly after 5 and 15 days of exposure but after 30 days of exposure serum K$^+$ was increased significantly to (27.8 – 68.1%) P$\leq$0.01.

Table 4: Changes in serum sodium (Na$^+$) and potassium (K$^+$) of *Oreochromis niloticus* exposed to crude oil .

Conc.of crude oil	Na$^+$ ml mol/L			K$^+$ ml mol/L		
	Exposure period (days)			Exposure period (days)		
	5	15	30	5	15	30
Control	142$\pm$1.5	143$\pm$2	141$\pm$1.6	7.5$\pm$0.6	7.4$\pm$0.6	7.2$\pm$0.4
350ppm	135$\pm$2.4	139$\pm$1.6	137+1.3	6.4$\pm$0.1	5.8$\pm$0.3	9.2$\pm$0.1
	(-4.9)	(-2.8)	(-3.5)	(-14.7)	(-21.6)	(+27.8)
1750 ppm	131+1.8	123+1.3	118$\pm$2.4	6.3$\pm$0.2	5.7$\pm$0.2	11$\pm$0.3
	(-7.75)	(-14)	(-16.7)	(-16)	(-23)	(+53)**
3500ppm	132$\pm$3.1	108$\pm$0.8	98$\pm$2.1	6.3$\pm$0.4	5.8$\pm$0.1	12.1$\pm$0.2
	(-2.2)	(-24.5)	(-32)*	(-16)	(-21.6)	(+68)**

Data are represented as mean $\pm$standard error (M$\pm$S.E) of 8 fish. Figures between brackets are % of change from control values * Significant difference at P$\leq$0.05.   ** Highly significant at P$\leq$ 0.01

4. DISCUSSION

Under normal physiological status, the antioxidant defense systems including SOD, CAT and GST can be induced by a slight oxidative stress as a compensatory response, and thus the reactive oxygen species (ROS) can be removed to protect the organisms from oxidative damage (living stone, 2001). The activity of antioxidant may be increased or inhibited under chemical stress depending on the intensity and duration of stress applied as well as susceptibility of exposure species. The liver in fish is an organ that performs various functions associated with the metabolism of xenobiotics (Jiminez and Stegeman, 1990). Hepatocytes like other cells are dependent on antioxidant enzymes for the protection against reactive oxygen species produced during the bio transformation of xenobiotics (Londis and Yu, 1995). Among the enzymes that comprise this defense system are SOD and CAT. SOD is responsible for the removal of hydrogen peroxide which is metabolized to oxygen and water (Vander Oost *et al*., 2003). Also SOD is the enzyme metabolizing superoxide radical and its levels are directly related to CAT activity.

The control values of superoxide dismutase (SOD) and catalase (CAT) enzymes activities in the liver of *O. niloticus* ranged between (343$\pm$2.6 - 351$\pm$1.5 unit/g wet wt.) and (72$\pm$1.4 -73$\pm$3.7 m mol/g wet wt). respectively and was found to be within the same range for other fresh water fishes (Oruce and Usta, 2007; Talas *et al*., 2008; Metwaly 2009; Wenju *et al*., 2009 and Gad and Yacoub 2009). The present study revealed that SOD and CAT activities in the liver of *O. niloticus* exposed to crude oil were increased significantly (P$\leq$0.05 and 0.01) after 15 and 30 days of exposure. The induction of SOD and CAT in the present study suggests that oxidative stress response still works well under the current conditions, and the increase of antioxidative enzymes may be a physiological adaptation for the elimination of ROS generation. Similar results have been observed in gilthead sea bream (*Sparus aurata*) and *Carassius auratus* exposed to polyaromatic hydrocarbons as phenanthrene (Sun *et al.,*

2006 and Correia *et al.,* 2007). Halliwall (1994) reported that an increase in SOD is followed by a parallel increase in CAT, since both enzymes are linked functionally and occur in tandem. The induction of SOD and CAT enzymes in the present study agrees with the results obtained in gold fish exposed to diesel oil for 40 days (Zang *et al.*, 2003): in *Prochilodus Lineatus* exposed to diesel oil for 28 days (Simonato *et al.,* 2008); in Atlantic cod (*Codus morhua*) exposed to sea oil and alkyl phenol for 15 days (Sturve *et al.,* 2006); in hybrid tilapia exposed to phenanthrene for 14 days (Wenju *et al.,* 2009).

Biotransformation reaction of toxic chemical in the organisms occur in three phases (transformation, conjugation and excretion). Phase 2 enzyme system like glutathion S transferase enzyme (GST) facilitates the conjugation of electrophilic substances or groups to tripeptide glutathione in order to make the xenobiotic chemicals more hydrophilic for transportation or excretion (Egaas *et al.*, 1993).

The control values of GST in the liver of *O. niloticus* ranged between 0.95±0.02-1.0±0.7 µ/mg wet. wt. of tissues and was found to be within the same range of other freshwater fishes (Oruce and Usta 2007; Talas *et al.,* 2008;Wenju *et al.,* 2009 and Gad 2009).

In the present study, GST showed a time dependent increase in the liver of *O. niloticus* exposed to crude oil with a significant increase after 5 days of exposure and doubled after 30 days of exposure. This increase was also demonstrated after exposure of *Carassium auratus* fish to water soluble fraction of crude oil (Zang *et al.*, 2004), Other authors also found that the activity of detoxification enzymes such as GST are increased in the presence of polycyclic aromatic hydrocarbon (Vander Oost *et al.,* 2003). The increase in the activity of GST reported in the present study indicated the biotransformation pathway valid for crude oil used, a protective response in fish toward exposure to an oxidative stress inducing xenobiotics and showed that GST activity can be a good biomarker for contamination by crude oil

.The increase in GST reported in the present study agrees with the results obtained in rainbow trout exposed to nonylphenol (Uguz *et al.*, 2003); in Atlantic cod exposed to sea oil and alkaylphenoles for 15 days (Sturve *et al.*, 2006); in *Prochilodus Lineatus* exposed to diesel oil for 15 days (Simonato *et al.,*2008); in polar cod exposed to crude oil for 4 weeks (Nahragan *et al.,* 2010).

Lipid peroxidation is one of the main processes induced by oxidative stress. Lipid peroxides are formed from the oxidative deterioration of poly unsaturated lipids in the membranes of cells and organelles. Lipid peroxidation bi-products, such as a malondialdehyde (MAD), are used as indicators of increased concentration of cellular reactive oxygen species and a sign of cellular injuries (Christi and Costa, 1984). Diverse contamination can initiate lipid peroxidation, including organic compounds and heavy metals.

The control values of MDA in the liver of *O. niloticus* ranged between (50±3.2-53±2.6) µ mol/g wet wt. and was found to be within the same range of other fresh water fishes (Durmaz *et al.*, 2006, Sturve *et al.*, 2006 and Oruc and Usta 2007). In the present study, lipid peroxidation was not changed significantly after 5 and 15 days of exposure to crude oil. However lipid peroxidation was evolved as a bi product of MAD after 30 days of exposure. The elevation of lipid peroxidation in the liver of *O. niloticus* as indicated by increased MAD production in the present study, suggested participation of free radical induced oxidative cell injury in mediating the toxicity of crude oil. Also the observed inhibition in Na$^+$/K $^+$ATPase enzyme in the gills of *O. niloticus* in the present study initiates lipid peroxidation process. The observed increase in lipid peroxidation in the present study agrees with the results obtained in Atlantic cod exposed to sea oil and alkayl phenol for 15 days (Sturve *et al.,* 2006); in *O. niloticus* exposed to diazinon for 30 days (Durmaz *et al.*, 2006); and in *Cyprinus carpio* exposed to diazenon for 30 days (Oruc and Usta, 2007).

The gills are extremely important for respiration, osmoregulation, acid base balance and excretion of nitrogenous wastes in fish (heath, 1995), and represent the greatest surface area of the fish in contact with external environment.

Adenosine triphosphatase (ATPase) plays an important role in intracellular functions and critical for cellular viability because they control many essential cellular functions, and are considered to be a sensitive indicator of toxicity. Na^+/K^+ ATPase pumps Na^+ and K^+ ions against their concentration gradients for the ions, and produces a convenient driving force for the secondary transport of metabolic substrates such as amino acids and glucose (Koksoy, 2002). The control value of Na^+/K^+ ATPase activities in the gills of *O. niloticus* ranged between 9.0 ± 0.1 -9.4 ± 0.5 μ mol /mg protein and within the same range of other fresh water fish (Danasoury 1997: Assem *et al*., 1999; Salah El Deen *et al*., 2001; Oruc and Usta 2007). In the present study Na^+/K^+ ATPase activity in the gills of *O. niloticus* was decreased significantly during all the period of exposure at different concentrations of crude oil ($P \leq 0.05$ and 0.01). The observed inhibition in the activity of Na^+/K^+ ATPase reported in the present study could be due to tissues hypoxic condition (Das and Mukherjee, 2003).

Na^+/K^+ ATPase is the membrane bound enzyme and its activity dependes on the phospholipids of the membrane. Therefore, any change in the lipid component of the membrane will directly affect the activity of this enzyme (Sahoo *et al*., 1999). Two different mechanisms are thought to lead to Na^+/K^+ ATPase inhibition in the present study as a direct effect of reactive oxygen species and MAD level, and an indirect effect of changes in membrane fluidity. On the other hand, inhibition in ATPase activity initiates free radical production leading to lipid peroxidation (Rauchova *et al*., 1999) as reported in the liver of *O. niloticus* in the present study. The reported inhibition in Na^+/K^+ ATPase in the present work agrees with the results obtained in different freshwater fish exposed to different pollutants (Danasoury 1997; Assem *et al*.,1999; Salah El-Deen *et al*., 2001 and Oruc and Usta 2007).

The body fluids of freshwater fish are hyperosmotic compared to the aquatic medium. Therefore, these animals need to avoid water gain and loss of salts and contaminants present in the water can interfere with this osmotic ionic balance (heath, 1995). Disturbances in the concentrations of serum ions and /or in osmolarity have already been shown in various species of fish exposed to different pollutants (Marie *et al*., 1997; Assem *et al*., 1999; Oruc and Usta 2007; Gad, 2007; Firat and Kargin, 2010).

The present study indicated that serum Na^+ and K^+ was decreased significantly ($P \leq 0.05$) at different concentrations of crude oil after 5,15 and 30 days of exposure (except after 30 days for K^+) compared to the control group. The observed decrease in serum Na^+ and K^+ may be due to inhibition of ion uptake and increased ion flux (Marie 1997). It is important to mention that the branchial ion regulation (especially Na^+ and K^+) in fish is accompanied by the activity of Na^+/K^+ ATpase.

On the other hand, the increase in serum K^+ reported in the present study after 30 days of exposure to crude oil may be due to the increased intravascular damage to erythrocyte membrane following stress and subsequent leakage of K^+ into the plasma (Abo-Hegab *et al*., 1990). Also hyperkalemia can occur typically in acute and chronic renal failure. In normally functioning, fish kidney K^+ are reabsorbed from the glomerular filtrate, since the crude oil damages the renal tubules and induced renal failure, the increase in K^+ might be due to this factor (renal dis function). The present result agrees with those obtained in different freshwater fish exposed to different pollutants (Danasoury, 1997; Marie, 1997; Assem, 1999; Gad, 2007).

In conclusion this study demonstrated that crude oil at 350 -3500ppm concentration levels after 15-30 days can cause adverse

effects on *Oreochromis niloticus* including the induction of SOD, CAT, GST and lipid peroxidation in the liver, as well as inhibition of Na^+/K^+ ATPase enzyme activities in the gills, decreased serum sodium and potassium (except after 30 days for K+). The present results suggest that the activities and expression levels of antioxidant enzymes and oxidative stress can be used as biomarker to evaluate the influence of crude oil on the biochemical pathways and enzymatic function in the fish *Oreochromis niloticus* so it can be used as a biological indicator to monitor unacceptable levels of environmental contamination.

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