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Immune and Antioxidant-Related Genes in the Nile Tilapia (*Oreochromisniloticus*) Collected From Different Locations

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ABSTRACT

Immune and antioxidant-related genes reflect the immune response of fish against different ecosystem circumstances around it. The present study is concerned with the effect of industrial and sewage effluents in some water drainages on immune and antioxidant-related genes of the Nile tilapia (*Oreochromisniloticus*). Fish specimens were collected from three different places; the first group was collected from El-Rayah El-Towfeky stream, Tokh, which receives loads of sewage and agricultural effluents (site A). The second group was collected from El-Sharkawia stream,Shoubra El-Khama, which receives industrial wastes (site B). The third group (site C) was collected from a fish farm in Al-Fayoum City (supplied with clean water) and was considered the control group. The expression of some immune-related genes (*infl1α, csf1r, igmh*, and *il1b)* and antioxidant genes (*sod, cat,* and *gr*) was determined in the liver and head kidney of the fish. The results revealed that the highest expression of most immune-related genes in the liver and kidney of the Nile tilapia was recorded in fish collected from site B. Regarding antioxidant genes, the highest catalase (cat) expression was recorded in the liver and kidney of the Nile tilapia maintained in sites A and B, respectively. Again fish from site B showed the highest superoxide dismutase (sod) and glutathione reductase (gr) expression in the kidney compared to the fish from other sites (although the differences were not statistically significant). The only highest expression found in fish in site C was reported in superoxide dismutase (sod) and glutathione reductase (gr) expression in the liver. Interestingly, the results revealed the positive influence of industrial pollution in some drainages on the Nile tilapia health.

INTRODUCTION

Aquatic pollutants are considered dangerous to ecosystems either directly through their effect on living organisms or indirectly through adverse water quality which is used in drinking and other human needs. Pollutants may occur in the aquatic environment as a result of natural occurrences like blooming and flooding or as a result of wrong human

activities, viz. industrial waste disposal, heavy insecticides, pesticides, sewage waste disposal, and fertilizers in agriculture which pour in water resources **(Cuesta *et al.*, 2011)**. Moreover, the effect of pollutants on aquatic organisms is variable according to toxic substances, concentration, duration, self-life, and animal behavior in addition to biology **(Cuesta *et al.*, 2011)**. Long-term exposure to certain pollutants has been proven to adversely affect the health of some fish species, especially in the North Sea and Great **(Austin, 1998)**. However, the presence of a small amount of metals could improve the immune status of fish **(Gatta *et al.*, 2001; Guardiola *et al.*, 2015; Wang *et al.*, 2020)**.

The River Nile is the longest freshwater river in the world. It starts from middle Africa and flows northward through Egypt to drain into the Mediterranean Sea. Certainly, The River Nile is the main water resource for living organisms and human life in Egypt. According to studies carried by the National Water Research Center (**(NWRC) 2000)**, some streams of the River Nile in Egypt (from Aswan to El-Kanater) receive untreated wastewater discharge from 124 point sources, 67 of which are agricultural sources and the remainders are industrial sources. Furthermore, the excessive use of pesticides and fertilizer in agriculture poured in those streams presented another serious problem **(Wahaab & Badawy, 2004**).

Regarding the immunological point of view, several studies reported variable responses to fish toward pollutants; some of them were positive while the others were negative. In negative reaction, exposure to chromium (Cr) caused a reduction in the number and functions of phagocytes as well as induced cytotoxicity and cell death in common carp leucocytes **(Steinhagen *et al.*, 2004)**, while decreasing antibody production, lysozyme activity and reactive oxygen species (ROS) production in tilapia **(Prabakaran *et al.*, 2007)**. Contrarily, feeding rainbow trout diets containing 1540 to 4110 ppb Cr revealed a positive response. Where an increase in respiratory burst, phagocytic, and lysozyme activities were observed **(Gatta *et al.*, 2001)**.

Thus, the present study aimed to evaluate whether the industrial and sewage drainages have a positive or negative effect on some immune-related genes and antioxidant genes of head kidney and liver of the Nile tilapia compared with the same fish species reared in fish farms, which is supposed to get clean water resource.

MATERIALS AND METHODS

2.1. Fish, experimental design and sampling

Individuals of the Nile tilapia (*Oreochromisniloticus*) of average weight 90 ± 5g were collected from three different locations. The first group of fish (site A) was collected from El-Rayah El-Towfeky stream, Tokh, which receives loads of sewage and agricultural effluents. The second group (site B) was collected from El-Sharkawia stream, Shoubra El-Khama, which receives industrial wastes. The third group (site C) was collected from a fish farm in Al-Fayoum City and was considered the control group. Fish were anesthetized with MS-222 and sacrificed. Head kidney and liver from each fish were collected and placed in eppendorf tubes and immersed immediately in RNA later (Sigma) until use.

2.2. RNA extraction and reverse transcription

Total RNA was extracted from the head kidney and liver using TRIzol reagent **(Chomczynski, 1993)**. Briefly, RNA later was disposed, and samples were transferred to fresh eppendorf before homogenizing in 1ml of TRIzol reagent before centrifugation (12000g x 4ºC for 10min). The clear supernatant was transferred to a fresh eppendorf and allowed to rest at room temperature for 5min. Aliquots of 0.2ml chloroform were added to the supernatants and vortex for 15s before allowing to rest for 2- 15min at room temperature. Then, the mixture was centrifuged for 15min. (12000 x g at 4ºC). The aqueous phases were transferred to a fresh tube and mixed with 0.5ml of isopropanol and then left to rest for 5– 10min at room temperature, and the mixtures were centrifuged again as described above. The supernatants were removed, and RNA pellets were washed with 1ml of 75% ethanol. Samples were vortex and centrifuged (7,500 x g for 5min at 4°C). Afterwards the ethanol was replaced with fresh distilled water, and the quantity of RNA was measured using nanodrop before being stored at -80ºC until use.

RNA was treated with DNase I (Promega) to remove genomic DNA contamination. Reverse transcription was done with SuperScript III reverse transcriptase (Invitrogen, Spain) with an oligo-dT18 primer. Briefly, For this, an amount of 1µg of total RNA was added to 1µl of oligo (dT)18 primer and 1µl of dNTP Mix (10 mM), and the volume was made up to 12µl with DEPC-treated water. The mixture was heated to 65°C for 5min followed with a quick chill on ice. Then, the mixture of 4µl of 5X First-Strand Buffer, 2µl of 0.1 M DTT, 1µL of SuperScript™ II RT and 1µl of DEPC-treated water were added, spun and incubated for 1hr at 50°C. Finally, the mixture was incubated for 15min at 70°C.

2.3. Real -time PCR

The expression of Interferon 1 alpha-like(*infl1α*), Colony-stimulating factor 1 receptor (*csf1r*), Immunoglobulin heavy chain (*igmh*), Interleukin 1 beta(*il1b*), Catalase (*sod*), Superoxide dismutase (*cat*) and Glutathione reductase (*gr*) genes in head kidney and liver (Table 1) were analyzed by real-time PCR (ABI PRISM 7500 instrument, Applied Biosystems) using SYBR Green PCR Core Reagents (Applied Biosystems). A mixture was prepared from 10µl of SYBR Green supermix, 5 µl of primers (0.6 mM each) and 5 µl of cDNA template and incubated for 10min at 95°C, followed by 40 cycles of 15s at 95°C, 1min at 60°C, and finally, 15s at 95°C, 1 min at 60°C and 15s at 95°C. Gene expression was corrected by the reference gene, elongation factor 1 α (*ef1α*) in each sample. The relative quantification of gene expression among the different groups was calculated using the $2^{-\Delta\Delta Ct}$ method **(Livak & Schmittgen, 2001)**.

2.4. Statistical analyses

The significant difference between the gene expressions among the groups was statistically analyzed with one-way analysis (ANOVA) of variance and Tukey's test using the Minitab statistical software (Minitab, Coventry, UK). Differences were considered statistically significant when $P< 0.05$.

Table 1. Oligonucleotides used for real-time PCR

Gene name	Abbreviation	GenBank ID	Sequence (5′→3′)
Elongation factor 1-alpha	*ef1α*	AB075952	F: AGAACGTCTCCGTCAAGGAA R: TGATGACCTGAGCGTTGAAG
Immunoglobulin heavy chain	*igmh*	KJ676389	F: GCAAAGGGATGATGCTGTCT R: GAGGTCAATGCGGTTTTTGT
Interleukin 1 beta	*il1b*	KJ574402	F: ATAAGCGCTGAAAGCGACAT R: CGCTGTGCTGATGTACCAGT
Colony-stimulating factor 1 receptor	*csf1r*	GR682279	F: CAAGCTCATTTCAACGGTCA R: CGAAGGAAGTTCAGCAGGTC
Interferon 1 alpha-like	*infl1α*	GR673680	F: ATGGGAGGAGAACACAGTGG R: TGTCGTATTGCTGTGGCTTC
Catalase	*cat*	JF801726	F: ATGAGGAGGAGCGACAGAGA R: AATTCTCGACCATGCGTTTC
Superoxidedismutase	*sod*	JF801727	F: GGAGGTGAACCACAAGGAGA R: TACAGCCACCGTAACAGCAG
Glutathionereductase	*gr*	GR589931	F: TCTGCACGATCATGGTGATT R: TGCGATTTAGGTGACTGACG

RESULTS

The influence of pollutants on immune-related (*inf1a*, *ighm*, *csf1r*, and *il1b*) and antioxidative stress genes (*sod, cat,*and *gr*) on head kidney and liver of the Nile tilapia in the three different locations (A, B, and C) is expressed in Figs. (1- 4).

In the liver, the highest expression in all tested immune- related genes (*inf1a*, *ighm*, *csf1r* and *il1b*) (Fig.1) was recorded at site B, which receives industrial drainages. Additionally, site B showed the highest expression for *inf1a* and *csf1r* genes in the kidney (Fig.2). However, the expression of *ighm* and *il1b* genes in kidney was slightly higher at site A (received sewage drainage) and site B (received industrial drainage), respectively compared to site C (collected from fish farm), with no significant difference.

Regarding antioxidant genes, *sod* and *gr* were highly expressed in the liver (Fig. 3) of the Nile tilapia collected from fish farm (site C) compared to the other sites, but without significant differences. In the kidney (Fig.4). Moreover, Site B showed the highest *sod* and *gr* expression compared to the other sites (without significant differences). The highest *cat* expression in the liver and kidney of the Nile tilapia was recorded at sites A and B, respectively. Although a high expression was recorded at some sites, no significant difference was detected among them.

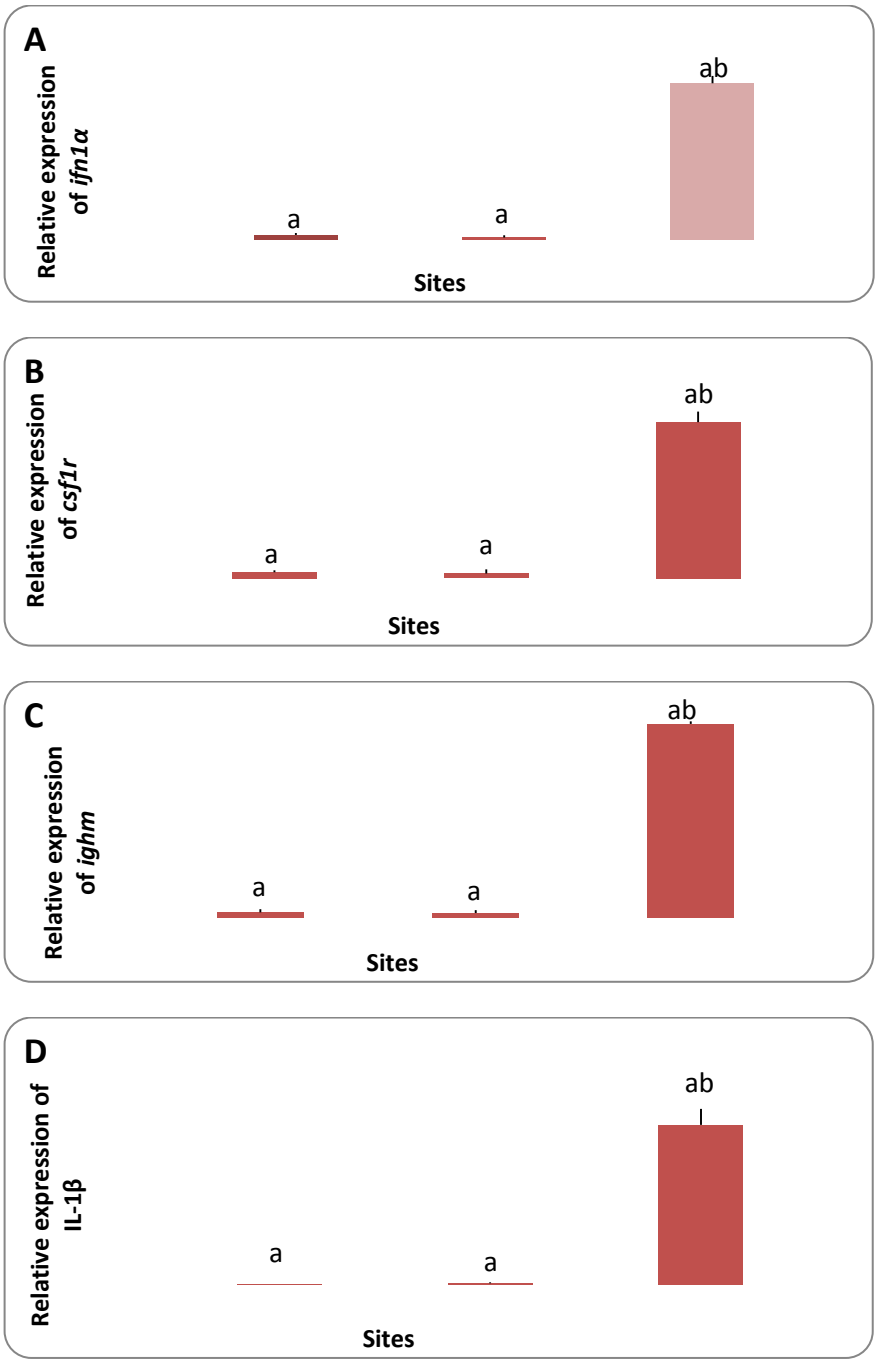

Fig. 1. Relative expression of *ifna* (A), *csf1r* (B), *ighm* (C), and *il1b* (D) in the liver of the Nile tilapia collected from the River Nile streams (site A and B) and from fish farm (site C). Significant differences between sites are represented by letters ($P< 0.05$). Bars= mean ± S.E. Bars= mean ± S.E.

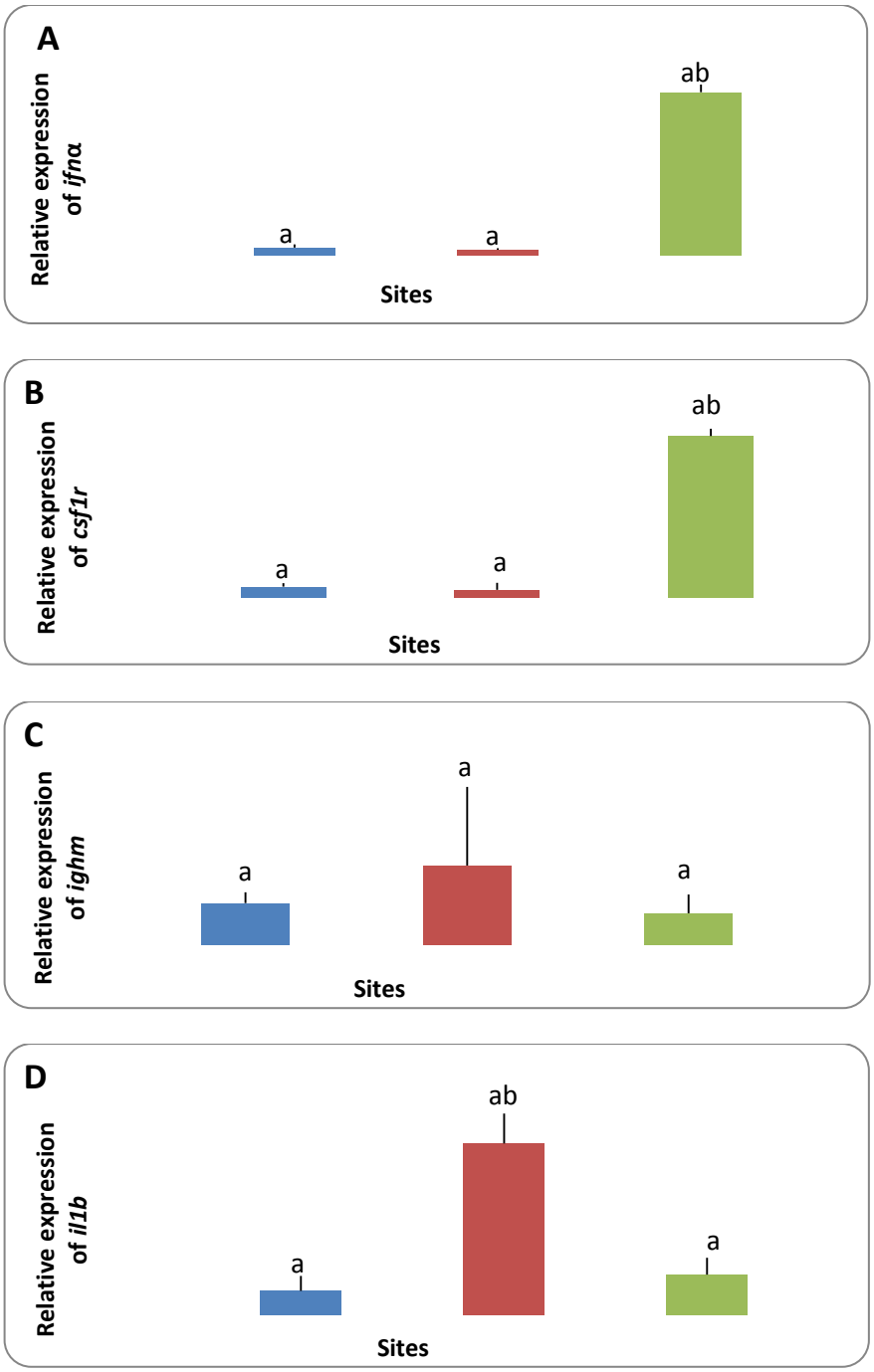

Fig. 2. Relative expression of *ifna* (A), *csf1r* (B), *ighm* (C), and *il1b* (D) in head kidney of the Nile tilapia collected from the River Nile streams (site A and B) and from fish farm (site C). Significant differences between sites are represented by letters ($P< 0.05$). Bars= mean ± S.E. Bars= mean ± S.E.

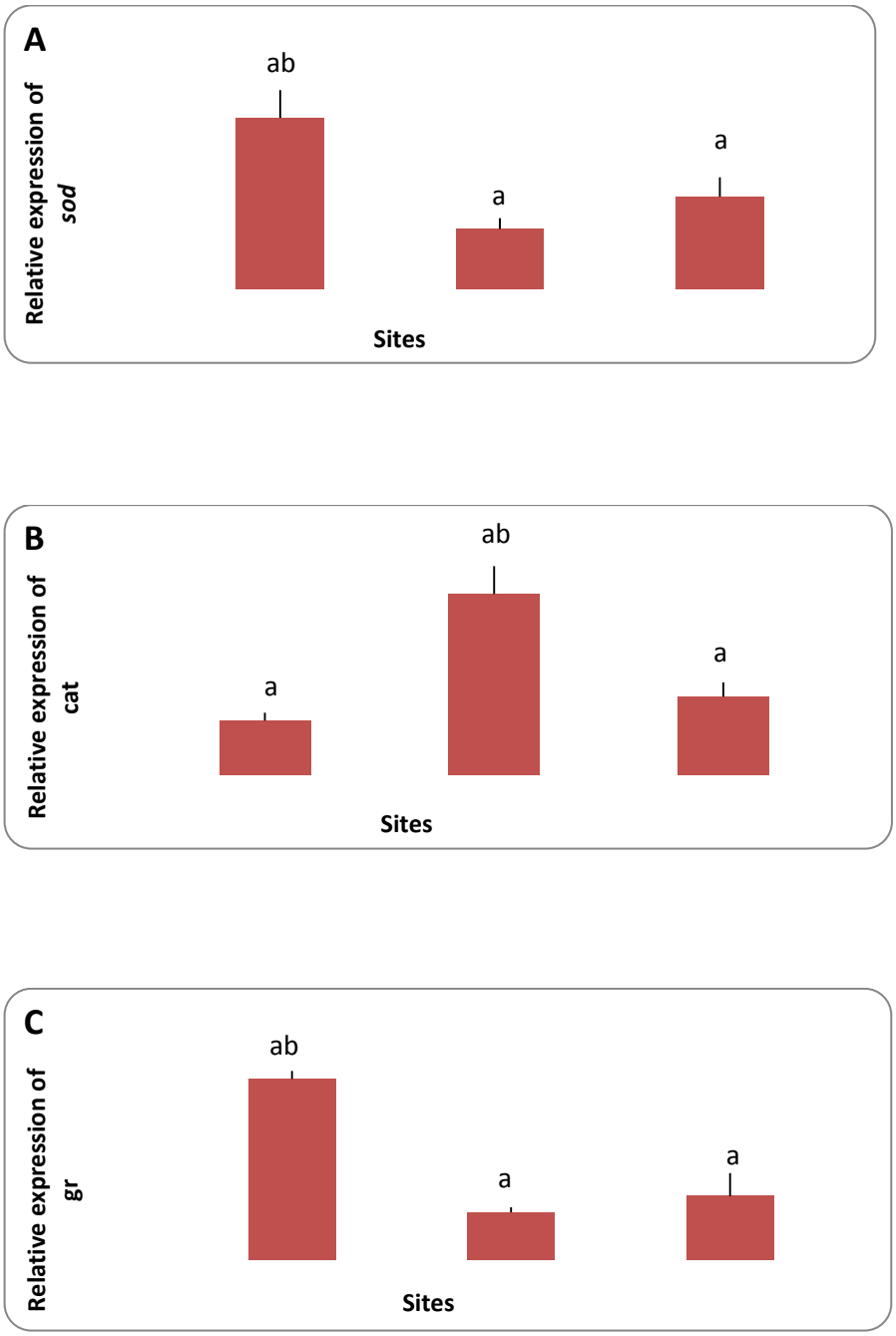

Fig. 3. Relative expression of *sod* (A), *cat* (B) and *gr* (C) in liver of the Nile tilapia collected from the River Nile streams (site A and B) and from fish farm (site C). Significant differences between sites are displayed in letters ($P< 0.05$). Bars= mean ± S.E. Bars= mean ± S.E.

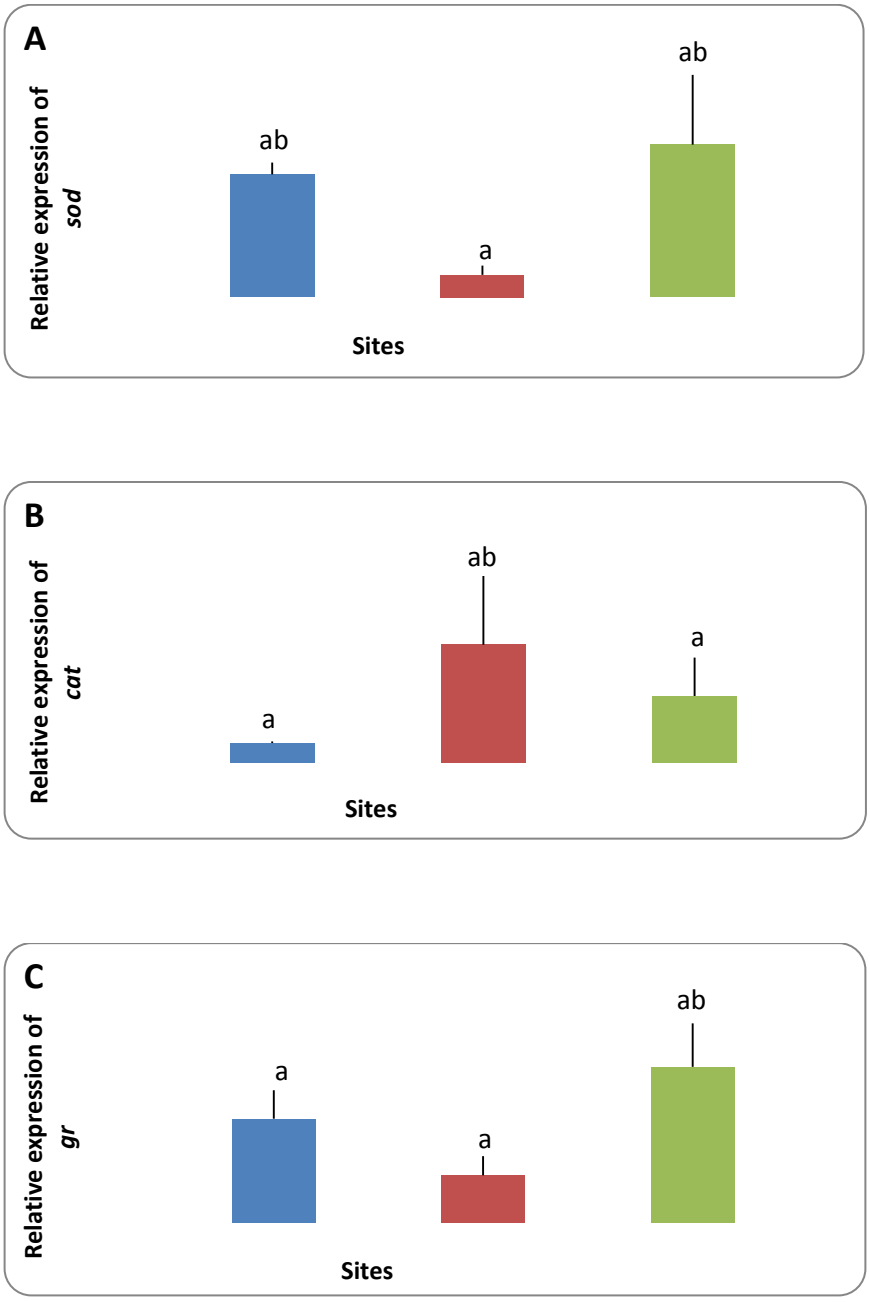

Fig. 4. Relative expression of *sod* (A), *cat* (B) and *gr* (C) in head kidney of the Nile tilapia collected from the River Nile streams(site A and B) and from fish farm (site C). Significant differences between sites are shown in letters ($P< 0.05$). Bars= mean ± S.E. Bars= mean ± S.E.

DISCUSSION

The influence of environmental toxicants on immune function and health status in fish varies with the type of materials and their concentrations in water. The concept that the fish immune system participates in a complex communication network with the neuroendocrine system has been strongly supported. This network is called the hypothalamus-pituitary-interrenal (HPI) axis **(Burnett, 2005**). For example, the fish endocrine system under stress conditions releases a great amount of cortisol into the bloodstream, depressing numbers of B-lymphocytes and antibody production; however, it rescues neutrophilic granulocytes, thereby favoring the innate immune response **(Engelsma *et al.*, 2002;Yada & Nakanishi, 2002)**. Many studies have documented immune modulation in response to metal, pesticide and organic contaminants in fish **(Dunier & Siwicki, 1993; Anderson & Zeeman, 1995; Bols *et al.*, 2001)**.

The immune system defense and antioxidant defense are considered among the substantial defenses in fish against the harmful effects caused by environmental contaminants. Thus, any disturbance in these defenses could act negatively on the health status of fish, reducing its ability to protect against infection **(Xu *et al.*, 2013)**. Therefore, in the present study, the expression of selected immune and antioxidant genes was evaluated in the liver and kidney of the Nile tilapia as a response to pollutants from different sources.

Both IFN and IL-1 are the earliest expressed pro-inflammatory cytokine that enables living organisms to respond instantly to infection **(Dinarello, 1997; Huising *et al.*, 2004; Robertsen, 2006)**. Notably, INF-1α plays a vital role in antigen presentation and inhibition of viral replication **(Gadan *et al.*, 2012)**. While, IL-1β plays different roles in anti-bacterial response **(Wang *et al.*, 2006)**. INF-1α and IL-1β are predominantly produced by monocytes and activated macrophages, granulocytes, T lymphocytes, and many other cell types **(Huising *et al.*, 2004; Robertsen, 2006)**. The *il1b* expression was high at sites B and A for the kidney and liver, respectively, compared to the control group (site C). Similarly, previous results carried on different sites of Lake Burullus in Egypt showed a significant up-regulation in *il1b* of the Nile tilapia liver **(Ghazy *et al.*, 2017).** The higher expression of *il1b* could be ascribed to the presence of some minerals and chemicals under the permissible limit, which enhances the immune response. Interestingly, insecticide (esfenvalerate) up-regulated the *il1b* gene in the kidney of juvenile Chinook salmon after exposing to for 20 days **(Eder *et al.*, 2008).** Contrarily, some minerals provoked suppression in the immune-related genes. For example, the Nile tilapia feeding Zn-enriched diet recorded a down regulation in *il1b* expression **(Wang *et al.*, 2020)**.

In the present study, the highest *ifn1a* expression was recorded in both kidney and liver from fish collected at site B, which receives industrial drainage. Some studies revealed an enhancement in fish *ifn1a* expression after exposure to some chemical compounds. For example, zebrafish exposed to organic compounds like di-n-butyl phthalate and diethyl phthalate revealed an over expression to the immune-related gene (*il1b*, *ifng*, *tnfa*, *lyz*,

and *c3b*) at the developmental stages **(Xu *et al.*, 2013).** Similarly, the *ifng* expressions of the Nile tilapia increased significantly after feeding with a diet containing Cu and Zn for 42 days **(Wang *et al.*, 2020)**. In addition, common carp exposure to various concentrations of Atrazine and chlorpyrifos and their mixture resulted in the upregulation of the *ifn1a* expression **(Wang *et al.*, 2011)**. On the contrary, the Nile tilapia exposed to LC_{50} of lambda cyhalothrin (insecticide) showed suppression in the *ifn1a* expression **(Khalil *et al.*, 2020)**.

Colony stimulating factor 1 receptor (*csf1r*), known also as macrophage colony-stimulating factor receptor (*m-csfr*), is required for the proliferation, survival and migration of macrophages **(Munugalavadla *et al.*, 2005)**. Oral administrations of diets contain different doses of microalgae (*Tetraselmischuii,* and *Phaeodactylumtricornutum*) **(Cerezuela *et al.*, 2012a)** and inulin with *Bacillus subtilis* **(Cerezuela *et al.*, 2012b)**, led to an upregulation of *csf1r* gene in gilthead seabream. Besides, our study recorded an increase in the *csf1r* expression in fish from site B, which receives industrial drainage. Eminently, the stress condition caused immunosuppressant to *csf1r* expression in fish **(de Mattos *et al.*, 2019)**. Although it seems that chemical material received from industrial drainage didn't induce stress conditions, on the contrary, it persuaded the immune response.

The IgM is an important gene in teleosts that can be used as an indicator for the ontogenesis of the immune system. It is the first formed antibody of the primary response in higher vertebrates **(Magnadóttir, 1998)**. An overexpression of *ighm* gene has been recorded in fish after the administration of immunostimulants **(Low *et al.*, 2003; Reyes-Becerril *et al.*, 2011; Cerezuela *et al.*, 2012b; Awad *et al.*, 2015)**. Our results demonstrated a higher expression of *ighm* in fish from site B in the liver. It was noticed that, this site is highly loaded with industrial effluents originated from numerous factories. The previous study carried out in this location recorded the presence of heavy metals (Zn, Ni and Cd) in water within the permissible limits **(Elgendy *et al.*, 2017)**, which could be responsible for the enhancement of the immune response in fish. Supporting this hypothesis, the gilthead seabream exposure to a small amount of heavy metals, such as As, Cd, and Hg, showed an increase in IgM levels, considering fish not exposed to those metals **(Guardiola *et al.*, 2015)**.

It is worthnoting that, the immune system plays an essential role in the recognition of pathogen, their phagocytosis, and the elimination of invaders microbes **(Monari *et al.*, 2007)**. During the process of phagocytosis, the oxygen molecule is converted into ROS to destroy the microbes **(Lambeth, 2004)**. Nevertheless, the excessive amount of ROS would cause cellular damage and malfunction in the immune system **(Malhotra & Kaufman, 2007)**. To maintain the balance of ROS and protect the host, the enzymatic antioxidants including SOD, CAT and GR are required to get rid of excessive ROS production **(Mates, 2000)**. Remarkably, CAT is an important member of the enzymatic antioxidants which catalyze the decomposition of hydrogen peroxide and keep balance in cell host, hence it is essential for innate immunity. In bivalves, CAT was proved to benefit the immune system defending against pathogen infection **(Wang *et al.*, 2013)**. Interestingly, using herbal immunostimulants in fish enhanced the immune parameters as well as the expression of *cat*, *gr* and *sod* genes **(Sönmez *et al.*, 2015; Hoseini & Yousefi, 2019; Zeilab Sendijani *et al.*, 2020)**. On the other hand, exposure of the aquatic

environment to pollutants affects the vital activities of fish, for example, the pollution with petroleum hydrocarbons interrupts the expression of antioxidant genes in the skin, gills, livers, and muscles of *Siganus canaliculatus* and *Epinephelus morio* caught from six areas along Jeddah and Yanbu coasts in Saudi Arabia **(Afifi *et al.*, 2017)**.

Our results demonstrated the highest expression in *sod* and *gr* genes in the liver of fish from site A and in the kidney of fish from site B. Additionally, fish from site A recorded the highest *cat* expression in both kidney and liver. The differences in these gene expression could be attributed to the difference in response, which varies between tissue & fish species and among complex mixtures of contaminants type, concentration and composition **(Avci *et al.*, 2005; Martínez-Álvarez *et al.*, 2005)**. Similarly, some differences in the oxidative stress enzymes and the innate immune response were recorded in gill and head kidney of wild-caught yellow perch collected from different sites of St. Lawrence River, Canada **(Dautremepuits *et al.*,2009)**.

It is worth mentioning that, the laboratory trials conducted on the influence of heavy metals on fish denoted an improvement in oxidative enzymes. For example, copper increased the expression of *sod*, *cat* and *gr* genes in jian carp brain **(Jiang *et al.*, 2014)**. In addition, previous studies elucidated that pacu fish fed diets containing different doses of organic selenium improved some immune parameters (lysozyme and respiratory burst) and antioxidant enzymes (CAT and Glutathione S-transferases, GST) **(Biller-Takahashi *et al.*, 2015)**. Similarly, sea bass exposed to chemically dispersed oil exhibited an improvement in innate immunity and antioxidant systems **(Dussauze *et al.*, 2015)**.

During the current study, an increase was detected in antioxidant enzymes genes associated with an increase in immune-related genes. Similar observations have been recorded in fish upon using immunostimulants and subsequently marking an increase in antioxidant enzymes genes. In this issue, rainbow trout fed doses of Dill showed stimulation in the immune response (lysozyme and complement activities) as well as an antioxidant enzyme (CAT and SOD) **(Zeilab Sendijani *et al.*, 2020)**. Correspondingly, feeding with diets containing fenugreek enhanced the immune response and antioxidant enzyme genes (*gr*, *cat*, and *sod*) in the liver of the gilthead seabream **(Awad *et al.*, 2015)**.

CONCLUSION

In short, fish raised under industrial drainage (site B) showed an up-regulatation in immune-related gene expression, with a marked increase in the liver, whereas fish raised in sewage water displayed a very similar gene expression profile compared to farmed fish (C). Remarkably, this study revealed that water in industrial drainage could have a positive impact on fish health, especially if the minerals are within the unpermissible limit.

REFERENCES

Afifi, M.; Alkaladi, A.; Zinada, O.a.A. and Couderchet, M. (2017). Alteration in antioxidant genes expression in some fish caught from jeddah and yanbu coast as a bio-indicator of oil hydrocarbons pollution. Saudi j. biol.Sci.,24: 1580-1587.

Anderson, D. and Zeeman, M. (1995). Immunotoxicology in fish. Fundam.Aquat.Toxicol.,2: 371-404.

Austin, B. (1998). The effects of pollution on fish health. J. Appl. Microbiol.,85: 234S-242S.

Avci, A.; Kaçmaz, M. and **Durak, İ.** (2005). Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicol.Environ.Saf.,60: 101-105.

Awad, E.; Cerezuela, R. and **Esteban, M.Á.** (2015). Effects of fenugreek (*trigonella foenum graecum*) on gilthead seabream (*sparus aurata* l.) immune status and growth performance. Fish Shellfish Immunol.45: 454-464.

Biller-Takahashi, J.D.; Takahashi, L.S.; Mingatto, F.E. and **Urbinati, E.C.** (2015). The immune system is limited by oxidative stress: Dietary selenium promotes optimal antioxidative status and greatest immune defense in pacu *piaractus mesopotamicus*. Fish Shellfish Immunol.,47: 360-367.

Bols, N.C.; Brubacher, J.L.; Ganassin, R.C. and **Lee, L.E**. (2001). Ecotoxicology and innate immunity in fish. Dev. Comp. Immunol.,25: 853-873.

Burnett, K.G. (2005). Impacts of environmental toxicants and natural variables on the immune system of fishes. Biochem.Molec.Biol.Fishes,6: 231-253.

Cerezuela, R.; Guardiola, F.A.; González, P.; Meseguer, J. and **Esteban, M.Á.** (2012a). Effects of dietary *bacillus subtilis, tetraselmis chuii*, and *phaeodactylum tricornutum*, singularly or in combination, on the immune response and disease resistance of sea bream (*sparus aurata* l.). Fish Shellfish Immunol.,33: 342-349.

Cerezuela, R.; Guardiola, F.A.; Meseguer, J. and **Esteban, M.Á.** (2012b). Increases in immune parameters by inulin and *bacillus subtilis* dietary administration to gilthead seabream (*sparus aurata* l.) did not correlate with disease resistance to *photobacterium damselae*. Fish Shellfish Immunol.,32: 1032-1040.

Chomczynski, P. (1993). A reagent for the single-step simultaneous isolation of rna, DNA and proteins from cell and tissue samples. Biotechniques,15: 532-4-536-7.

Cuesta, A.; Meseguer, J. and **Esteban, M.Á.** (2011). Immunotoxicological effects of environmental contaminants in teleost fish reared for aquaculture INTECH Open Access Publisher.

Dautremepuits, C.; Marcogliese, D.J.; Gendron, A.D. and **Fournier, M.** (2009). Gill and head kidney antioxidant processes and innate immune system responses of yellow perch (*perca flavescens*) exposed to different contaminants in the st. Lawrence river, canada. Sci. Total Environ.,407: 1055-1064.

De Mattos, B.O.; López-Olmeda, J.F.; Guerra-Santos, B., Ruiz, C.E.; García-Beltrán, J.M.; Ángeles-Esteban, M.; Sánchez-Vázquez, F.J. and **Fortes-Silva, R.** (2019). Coping with exposure to hypoxia: Modifications in stress parameters in gilthead seabream (*sparus aurata*) fed spirulina (*arthrospira platensis*) and brewer's yeast (*saccharomyces cerevisiae*). Fish Physiol. Biochem.,45: 1801-1812.

Dinarello, C.A. (1997). Interleukin-1. Cytokine & growth factor reviews,8: 253-265.

Dunier, M. and **Siwicki, A.K.** (1993). Effects of pesticides and other organic pollutants in the aquatic environment on immunity of fish: A review. Fish Shellfish immunol.,3: 423-438.

Dussauze, M.; Danion, M.; Le Floch, S.; Lemaire, P.; Pichavant-Rafini, K. and **Theron, M.** (2015). Innate immunity and antioxidant systems in different tissues of sea bass (*dicentrarchus labrax*) exposed to crude oil dispersed mechanically or chemically with corexit 9500. Ecotoxicol. Environ. Saf.,120: 270-278.

Eder, K.J.; Clifford, M.A.; Hedrick, R.P.; Köhler, H.-R. and **Werner, I.** (2008). Expression of immune-regulatory genes in juvenile chinook salmon following exposure to pesticides and infectious hematopoietic necrosis virus (ihnv). Fish Shellfish Immunol.,25: 508-516.

Elgendy, M.Y.; Soliman, W.S.; Abbas, W.T.; Ibrahim, T.B.; Younes, A.M. and **Omara, S.T.** (2017). Investigation of some virulence determents in *aeromonas hydrophila* strains obtained from different polluted aquatic environments. Jordan J. Biol. Sci.,10: 265-272.

Engelsma, M.Y.; Huising, M.O.; Van Muiswinkel, W.B.; Flik, G.; Kwang, J.; Savelkoul, H.F. and **Verburg-Van Kemenade, B.L**. (2002). Neuroendocrine–immune interactions in fish: A role for interleukin-1. Vet. Immun.Immunopath.87: 467-479.

Gadan, K.; Marjara, I.S.; Sundh, H.; Sundell, K. and **Evensen,** Ø. (2012). Slow release cortisol implants result in impaired innate immune responses and higher infection prevalence following experimental challenge with infectious pancreatic necrosis virus in atlantic salmon (*salmo salar*) parr. Fish Shellfish Immunol.,32: 637-644.

Gatta, P.; Piva, A.; Paolini, M.; Testi, S.; Bonaldo, A.; Antelli, A. and **Mordenti, A.** (2001). Effects of dietary organic chromium on gilthead seabream (*sparus aurata* l.) performances and liver microsomal metabolism. Aquaculture Res.32: 60-69.

Ghazy, H.A.; Abdel-Razek, M.A.; El Nahas, A.F. and **Mahmoud, S.** (2017). Assessment of complex water pollution with heavy metals and pyrethroid pesticides on transcript levels of metallothionein and immune-related genes. Fish Shellfish Immunol.,68: 318-326.

Guardiola, F.A.; Dioguardi, M.; Parisi, M.G.; Trapani, M.R.; Meseguer, J.; Cuesta, A.; Cammarata, M. and **Esteban, M.A.** (2015). Evaluation of waterborne exposure to heavy metals in innate immune defences present on skin mucus of gilthead seabream (*sparus aurata*). Fish Shellfish Immunol.,45: 112-123.

Hoseini, S.M. and **Yousefi, M.** (2019). Beneficial effects of thyme (*thymus vulgaris*) extract on oxytetracycline induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*oncorhynchus mykiss*). Aquaculture Nut.,25: 298-309.

Huising, M.O.; Stet, R.J.; Savelkoul, H.F. and **Verburg-Van Kemenade, B.L.** (2004). The molecular evolution of the interleukin-1 family of cytokines; il-18 in teleost fish. Develop. Comp. Immunol.,28: 395-413.

Jiang, W.-D.; Liu, Y.; Hu, K.; Jiang, J.; Li, S.-H.; Feng, L. and **Zhou, X.-Q.** (2014). Copper exposure induces oxidative injury, disturbs the antioxidant system and changes the nrf2/are (cuznsod) signaling in the fish brain: Protective effects of myo-inositol. Aquatic Toxicol., 155: 301-313.

Khalil, S.R.; Abd Elhakim, Y.; Abd El-Fattah, A.H.; Farag, M.R.; Abd El-Hameed, N.E. and **Abd Elhakeem, E.-M.** (2020). Dual immunological and oxidative responses in *oreochromis niloticus* fish exposed to lambda cyhalothrin and

concurrently fed with thyme powder (*thymus vulgaris* l.): Stress and immune encoding gene expression. Fish Shellfish Immunol., 100: 208-218.

Lambeth, J.D. (2004). Nox enzymes and the biology of reactive oxygen. Nat. Rev. Immunol.,4: 181-189.

Livak, K.J. and **Schmittgen, T.D.** (2001). Analysis of relative gene expression data using real-time quantitative pcr and the 2− ΔΔCT method. Methods,25: 402-408.

Low, C.; Wadsworth, S.; Burrells, C. and **Secombes, C.** (2003). Expression of immune genes in turbot (*scophthalmus maximus*) fed a nucleotide-supplemented diet. Aquaculture,221: 23-40.

Magnadóttir, B. (1998). Comparison of immunoglobulin (igm) from four fish species. Icel. Agric. Sci.,12: 47-59.

Malhotra, J.D. and **Kaufman, R.J.** (2007). Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? Antioxid. redox signal.,9: 2277-2294.

Martínez-Álvarez, R.M.; Morales, A.E. and **Sanz, A.** (2005). Antioxidant defenses in fish: Biotic and abiotic factors. Rev. Fish Biol.Fisher.,15: 75-88.

Mates, J. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology,153: 83-104.

Monari, M.; Serrazanetti, G.P.; Foschi, J.; Matozzo, V.;Marin, M.G. and **Cattani, O. (**2007). Effects of salinity on the clam *chamelea gallina* haemocytes. Part ii: Superoxide dismutase response. Marine Biol.,151: 1059-1068.

Munugalavadla, V.; Borneo, J.; Ingram, D.A. and **Kapur, R.** (2005). P85α subunit of class ia pi-3 kinase is crucial for macrophage growth and migration. Blood,106: 103-109.

Nwrc, N.W.R.C. (2000). Wl/delft hydraulics. "National Water Resources Plan for Egypt, Water Quality and Pollution Control". Technical Report No. 5.

Prabakaran, M.; Binuramesh, C.; Steinhagen, D. and **Michael, R.D. (**2007). Immune response in the tilapia, *oreochromis mossambicus* on exposure to tannery effluent. Ecotoxicol. Environ. Saf.,68: 372-378.

Reyes-Becerril, M.; Ascencio-Valle, F.; Tovar-Ramírez, D.; Meseguer, J. and **Esteban, M.Á.** (2011). Effects of polyamines on cellular innate immune response and the expression of immune-relevant genes in gilthead seabream leucocytes. Fish Shellfish Immunol.,30: 248-254.

Robertsen, B. (2006). The interferon system of teleost fish. Fish Shellfish Immunol.,20: 172-191.

Sönmez, A.Y.; Bilen, S.; Alak, G.; Hisar, O.; Yanık, T. and **Biswas, G. (**2015). Growth performance and antioxidant enzyme activities in rainbow trout (*oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. Fish Physiol. Biochem.,41: 165-175.

Steinhagen, D.; Helmus, T.; Maurer, S.; Michael, R.D.; Leibold, W.; Scharsack, J.P.; Skouras, A. and Schuberth, H.-J. (2004). Effect of hexavalent carcinogenic chromium on carp *cyprinus carpio* immune cells. Dis. Aquat. Org.,62: 155-161.

Wahaab, R.A. and **Badawy, M.I.** (2004). Water quality assessment of the river nile system: An overview. Biomed. Environ. Sci.,17: 87-100.

Wang, C.; Yue, X.; Lu, X. and **Liu, B.** (2013). The role of catalase in the immune response to oxidative stress and pathogen challenge in the clam *meretrix meretrix*. Fish Shellfish Immunol.,34: 91-99.

Wang, J.; Xiao, J.; Zhang, J.; Chen, H.; Li, D.; Li, L.; Cao, J.; Xie, L. and **Luo, Y.** (2020). Effects of dietary cu and zn on the accumulation, oxidative stress and the expressions of immune-related genes in the livers of nile tilapia (*oreochromis niloticus*). Fish Shellfish Immunol., 100: 198-207.

Wang, X.; Xing, H.; Li, X.; Xu, S. and **Wang, X.** (2011). Effects of atrazine and chlorpyrifos on the mrna levels of il-1 and ifn-γ2b in immune organs of common carp. Fish Shellfish Immunol.,31: 126-133.

Wang, Y.; Wang, Q.; Baoprasertkul, P.; Peatman, E. and **Liu, Z.** (2006). Genomic organization, gene duplication, and expression analysis of interleukin-1β in channel catfish (*ictalurus punctatus*). Molec.Immunol.,43: 1653-1664.

X**u, H.; Shao, X.; Zhang, Z.; Zou, Y.; Wu, X.** and **Yang, L.** (2013). Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. Ecotoxicol. Environ.Saf.,93: 39-44.

Yada, T. and **Nakanishi, T.** (2002). Interaction between endocrine and immune systems in fish. Int. Rev. Cytol.,220: 35-92.

Zeilab Sendijani, R.; Abedian Kenari, A.; Smiley, A.H. and **Esmaeili, M.** (2020). The effect of extract from dill *anethum graveolens* on the growth performance, body composition, immune system, and antioxidant system of rainbow trout. N. Am. J. Aquac.,82: 119-131.