



# PHYSICO-CHEMICAL CHARACTERISTICS AND PHYSIOLOGICAL CHANGES IN *OREOCHROMIS NILOTICUS* FROM ROSETTA BRANCH OF THE RIVER NILE

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The River Nile is the essence of life in Egypt, but during the last decades its water quality has been changed by several factors, as a result of anthropogenic activities. The western branch of the River Nile is Rosetta Branch receives different types of pollution. The main origins of most pollutants are El-Rahawy drain and industrial activities in Kafr El-Zayat city. Water samples were analysed for physico-chemical parameters and blood samples for biochemical parameters of the Nile Tilapia; *Oreochromis niloticus*, to investigate the responses of fish towards these different types of pollution. Samples were collected from three sites from Rosetta branch of the River Nile during summer 2014 and winter 2015. S2 and S3 recorded an increase in water electrical conductivity (EC), total dissolved solids (TDS), biological oxygen demand (BOD) and chemical oxygen demand (COD), while dissolved oxygen (DO) and pH have been depleted. Nitrite, nitrates and ammonia levels also showed an elevation, especially in winter. Moreover, fundamental biochemical parameters such as; glucose, total protein, total lipid, albumin, cholesterol, triglycerides, kidney functions and liver functions in blood serum of *O. niloticus* recorded remarkable alterations, indicating stressful conditions, caused by the profound pollutants and poor water quality of water at these sites.

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## INTRODUCTION

Water quality in Egypt is a major issue since water resources are limited to the River Nile, groundwater in the Delta, western desert and Sinai and the rainfall. The severity of water quality problems in Egypt varies among the different water bodies depending on water flow, population density, the extent of industrialization, social and economic conditions. The human population continuously loads the aquatic environment with foreign chemicals (pollutants) released by urban communities and industries. Pollution load in the Nile ecosystem (main River Nile, canals, and drains) has increased in the past few decades despite all the programs for pollution control. Consequently, quality of the Nile water worsened dramatically in the past few years.<sup>1,2</sup> It is anticipated that the dilution capacity of the River Nile system will diminish as the program to expand irrigated agriculture moves forward and the growth in industrial capacity increases the quantity of pollutants discharged into the River Nile.<sup>3</sup> After passing Cairo, the River Nile pursues a north westerly direction, and divides at El-Qanater Barrage into two branches, each of which runs separately to Mediterranean Sea, forming the Delta region between both branches. The western branch is Rosetta Branch and the Eastern branch is Damietta branch. Rosetta Branch of River Nile begins from El-Qanater Barrage at south and ends at Rosetta estuary in the Mediterranean Sea. It exhibits the worst water quality along the River Nile.<sup>4,5</sup> Rosetta Branch receives about 12 billion m<sup>3</sup> year<sup>-1</sup> of seeping water from agriculture fields, 75 % of it is in the Nile delta region.<sup>6</sup> This drainage water contains dissolved salts washed from

agricultural lands as well as residues of pesticides and fertilizers, pathogens, toxic organic and inorganic pollutants.<sup>7</sup> Industrial wastewater is the second main source of pollution because of the toxic chemicals and organic loading. Moreover, The Branch suffers from discharges of untreated sanitary and industrial wastewaters from municipalities, heavy navigation of cruises and commercial transportations.<sup>8-10</sup> In Egypt, fish is considered as a cheap protein food if compared with others of animal sources. Fish are very intimate contact with their environment; only a thin epithelial membrane separates the blood of the fish from the water. Fish are very susceptible to physical and chemical changes which reflected in their blood components.<sup>12-14</sup> Changes in the biochemical characteristics of blood and tissues are important diagnostic tool, indicate the changes in metabolism and the physiological processes of fish caused by environmental pollutants, detection and diagnosis of metabolic disturbance, disease and health during stress conditions.<sup>15-19</sup> Analysis of biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals and it may also provide an early warning signal in stressed organisms.<sup>20-23</sup>

Our objective in this study was to assess the impact of some physico-chemical characteristics on *O. niloticus* physiology.

## EXPERIMENTAL

Rosetta Branch of River Nile is about 220 Km long and about 180 m wide with an average depth of 2.0 to 2.3 m. It starts from EL-Qanater EL-Khayria and ends at Rosetta Estuary. It passes through six governorates viz., EL-Qalubia, EL-Menofiya, EL-Giza, EL-Gharbiya, Kafr El-Shiekh and EL-Boheira. There are three main sources of pollution discharged into Rosetta Branch which, potentially affects and deteriorates the water quality of the branch (1) various small agricultural drains and sewage from several cities and

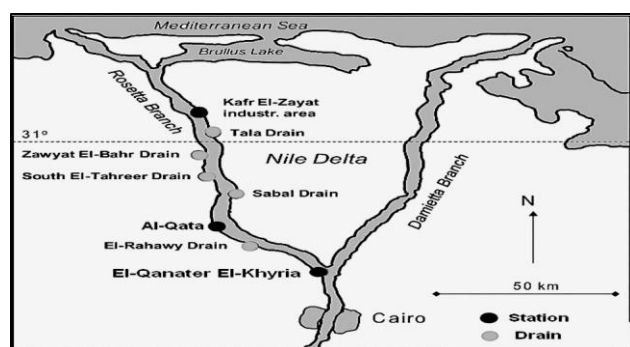
its neighboring villages, which discharge their wastes directly into the branch without treatment, (2) El-Rahawy drain and (3) Kafr El-Zayat industrial area.

### Area of investigation

Three stations from Rosetta branch were selected according to pollution type. S1 - El-Qanater El-Khyria before bifurcation (relatively unpolluted site), S2 - in front of Al-Qata (affected by El-Rahawy drain) and S3 - in front of the Kafr El-Zayat industrial area (loaded with industrial effluents from factories) Table 1 and Figure 1.

**Table 1.** The latitude and longitude of sampling stations at Rosetta Branch (GPS).

Sites	Latitude	Longitude
S1	30° 10' 22.4''	31° 8' 34.0''
S2	30° 13' 18.7''	30° 58' 30.3''
S3	30° 49' 33.7''	30° 48' 23.6''



**Figure 1.** Map of northern Egypt showing the area of study and sampling stations in the Rosetta Branch.

### Collection of samples

Water and fish samples were collected from S1, S2 and S3 in summer 2014 and winter 2015. Some physico-chemical parameters of water were done in field such as: water temperature, hydrogen ion concentration (pH), Electrical conductivity (EC), total dissolved solids (TDS), using Orion Research Ion Analyzer 399A. Water samples were stored at 4°C and transported to the laboratory for analysis of other parameters. Water samples were analyzed according to standard method for examination of water and wastewater.<sup>24</sup> Determination of dissolved oxygen (DO) was carried out by using Winkler Azide titration method<sup>25</sup> (the modified method). Chemical oxygen demand (COD) and biological oxygen demand (BOD) and nutrient salts (nitrite, nitrate, and ammonia) were measured using Standard Methods.<sup>24</sup>

Blood samples (proximally 3 mL) were collected immediately from alive *O. niloticus*; blood was withdrawn from the caudal vein using a syringe. Blood was left to clot then centrifuged at 3000 r.p.m for 10 min. Supernatant serum was obtained using micropipette and stored at 4 °C till determination of biochemical parameters [glucose, liver functions (alanine amino transferase (ALT), aspartate amino transferase (AST), total protein and albumin, kidney functions (urea, creatinine and uric acid) and lipid profile (cholesterol and triglycerides)] in blood serum were assayed by spectrophotometer (model Jenway 6800UV/Vis double

beam), and using commercially available kits in Egypt (spectrum). The experimental data were subjected to statistical analysis by one-way analysis of variance (ANOVA), the significant of difference was analyzed by the Dunnett test (compare data of all vs. control) was done by using a software program (GraphPad InStat Software, Inc.). The difference checked by one-way (ANOVA) was significance when ( $P \leq 0.05$ ), each reading represents (Mean  $\pm$  S.E) of 8 fish. Correlation Matrix was assigned using "corrplot" (Version 0.84).<sup>26</sup>

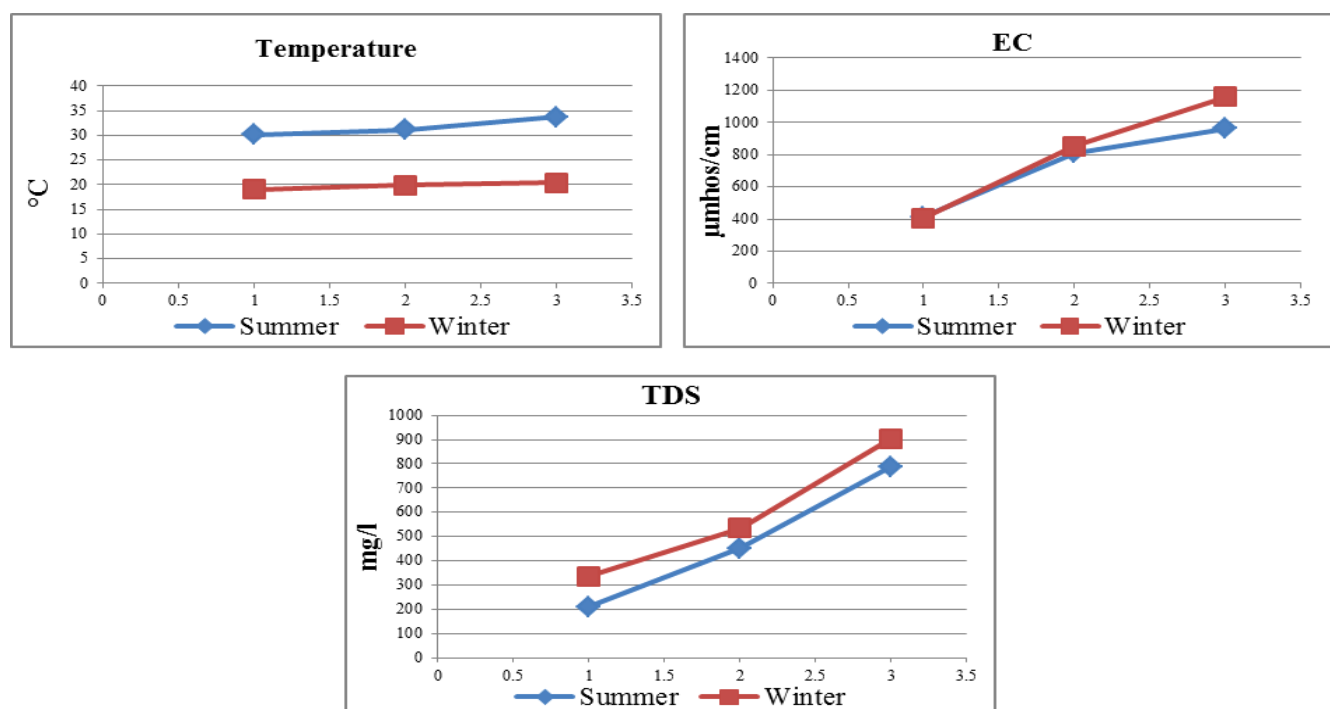
## RESULTS AND DISCUSSION

### Physical characteristics

Water temperature is known as a key factor, controlling the physiology, distribution, and behavior of fishes. In temperate and subtropical regions, which are characterized by seasonal fluctuations in water temperature, the effect of temperature is manifested at every level of biological organization. Changes in temperature affect the fluidity of lipid membranes, the conformational mobilities and activities of proteins, and the stability of DNA duplexes.<sup>27</sup> Water temperature in the present study followed the Egyptian climate with low values during winter season, and increased in summer (Figure 2). Temperature ranged from a lowest value of 19.1 °C during winter at S1 to highest value 33.7 °C during summer at S3 (Figure 2). Changes in water temperature could be due to some variables such as season, day time, depth, wind, current and water inflow. Temperature was positively correlated with pH ( $r = 0.86$ ) and negatively with DO ( $r = 0.75$ ) (Figure 8). Temperature was a water quality indicator that exhibited little variance between the sites and declared seasonal variations.

Electrical conductivity (EC) measures the ability of aqueous solution to carry the electrical current, solution of most inorganic compounds and more abundant ions have higher conductivity. In the present study, water samples collected from Rosetta Branch showed a highly significant increase in EC ( $P \leq 0.01$ ) compared to S1. The reported increase in EC in the present study at S2 varied between 677.3  $\mu\text{mhos cm}^{-1}$  in summer and 853.2  $\mu\text{mhos cm}^{-1}$  in winter. At S3, EC recorded a highly significant increase 1520.5  $\mu\text{mhos cm}^{-1}$  in summer and 1657.6  $\mu\text{mhos cm}^{-1}$  in winter (Figure 2). The increase in EC at S2 and S3 mainly attributed to the sewage, domestic, agricultural and industrial effluents discharged from El-Rahawy and Kafr El-Zayat industries drains, respectively in this area, increasing the ability to convey electrical current. These results coincident with that finding by many authors.<sup>28-30</sup>

Total dissolved Solids (TDS) refer to suspended and dissolved matter in water. In the present work TDS showed a highly significant increase ( $P \leq 0.01$ ) in water samples collected from S2 and S3. TDS was 3533.9  $\text{mg L}^{-1}$  and 904.3  $\text{mg L}^{-1}$  in winter at S1 and S2, respectively (Figure 2). This revealed high level of organic and inorganic matters produced by living organisms in this area, as well as the increase in cations and anions concentration of water as a result of winter closure.<sup>31</sup> TDS showed a strong positive correlations with EC ( $r = 0.94$ ), that means both EC and TDS depend on each other (Figure 8). TDS in S2 and S3 exceeded the permissible limits (500  $\text{mg L}^{-1}$ ) according to Egyptian Governmental Law.<sup>32</sup>



**Figure 2.** Seasonal variations of physical parameters: temperature, EC and TDS in Rosetta branch water during summer 2014 and winter 2015.

### Chemical characteristics

Hydrogen ion concentration (pH) of natural water affects biological and chemical reaction, control the solubility of metal ions, affects fish and natural aquatic life.<sup>33</sup> The regional and seasonal pH values showed a tendency towards alkaline side, varied between (7.1 to 7.88) during the investigation period (Figure 3). There was a remarkable decrease in pH value (7.3 and 7.4), recorded at S2 and S3 respectively, during winter. The decrease in pH values is due to the formation of organic acids through methane and hydrogen sulphide release, by bacteria and fungi in El-Rahawy drain discharges, as well as the industrial effluents from Kafr El-Zayat Companies.<sup>28</sup> The pH values were within the permissible range (6.5 to 8.5) according to Egyptian Governmental Law.<sup>32</sup>

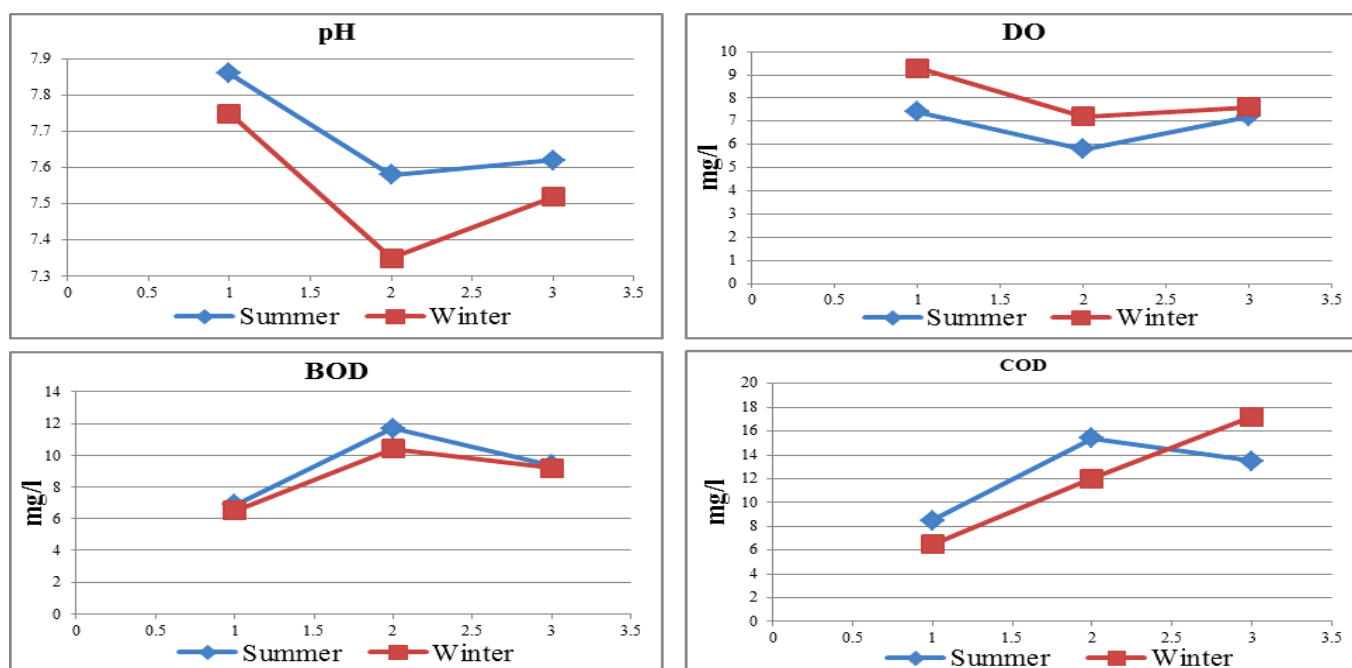
Dissolved oxygen (DO) is essential for a healthy aquatic ecosystem and for fish and most of aquatic animals to survive. Oxygen tends to be less soluble as temperature increases. Low levels of (DO) causes hypoxia, mortality and a massive reshaping of fish communities.<sup>34</sup> In the present study DO showed a negative correlation with temperature and pH ( $r=-0.75$  and  $-0.74$ ) (Figure 8).

DO in S2 and S3 showed a significant decrease ( $P \leq 0.01$ ), comparing to S1. DO range was between  $5.8 \text{ mg L}^{-1}$  at S2 in summer and  $9.3 \text{ mg L}^{-1}$  at S1 in winter (Figure 3). Low DO in S2 is due to the extended impact of El-Rahawy drain, which directly discharges organic and inorganic pollutants into the branch water. These pollutants consume the dissolved oxygen during oxidation of nitrogenous compounds and lead to increase in ammonia processes especially during hot seasons and decrease of phytoplankton and hydrophytes. DO in the present study were within the acceptable limit ( $\geq 5 \text{ mg L}^{-1}$ ) recommended by Egyptian Governmental Law.<sup>32</sup>

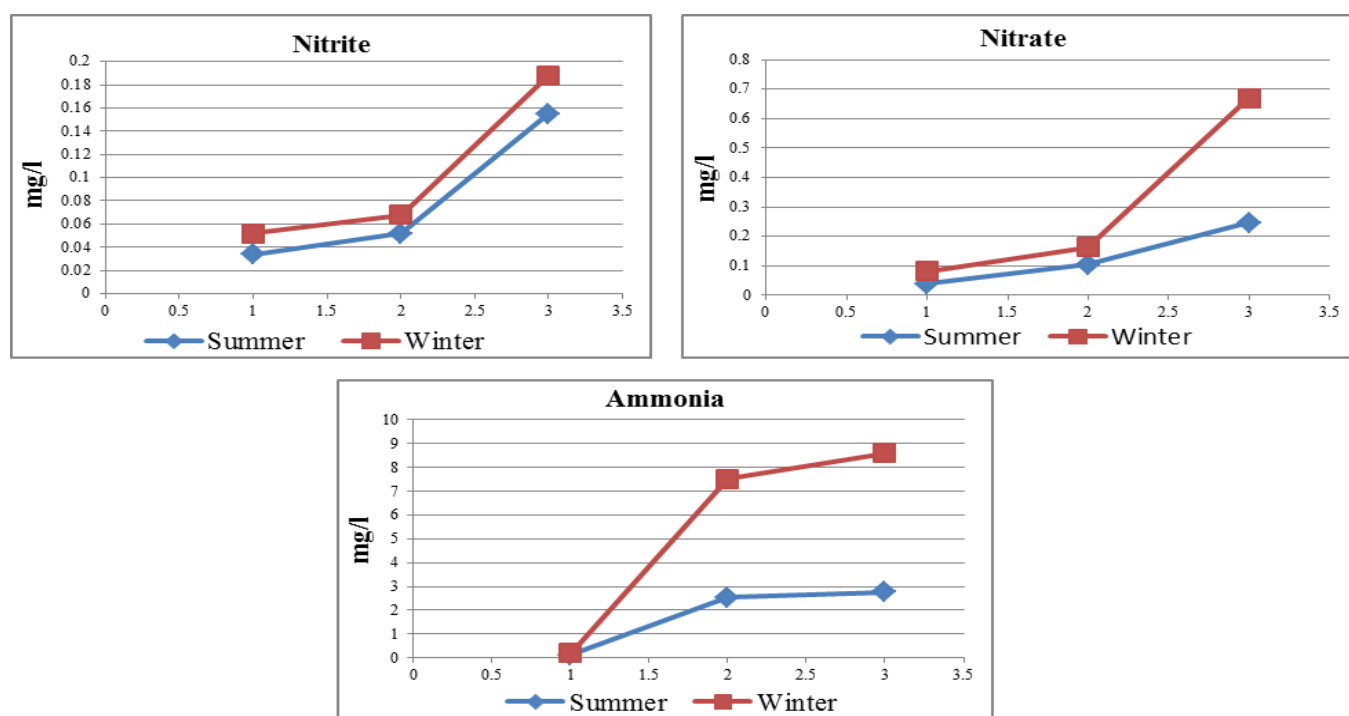
Biological oxygen demand (BOD) is an important parameter for indicating organic pollution level, it determines the dissolved oxygen consumed by microorganisms to stabilize any biodegradation of organic materials.<sup>35</sup> A higher value of BOD indicates a decline in DO and increase the amount of organic matter, and a large number of microorganisms, which in turn shows a high level of pollution.<sup>36,37</sup>

Chemical oxygen demand (COD) is a measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide and water. It's an important parameter for stream and industrial wastes study, also serves as an index for organic matter production.<sup>38</sup> In the present study BOD is correlated positively with COD ( $r=0.79$ ) (Figure 8). The BOD varied between  $6.5$  and  $11.7 \text{ mg L}^{-1}$  and COD ranged between  $6.5$  and  $17.2 \text{ mg L}^{-1}$  (Figure 3). Both BOD and COD showed a significant increase ( $P \leq 0.01$ ) at S2 and S3 as compared to that at S1 reflecting the effect of the high load of organic matter, sewage, domestic, agricultural and industrial wastes discharged into Rosetta branch via El-Rahawy and Kafr El-Zayat industrial drains. BOD and COD in polluted sites exceeded the permissible limits recommended by Egyptian Governmental Law.<sup>32</sup>

Nitrite ( $\text{NO}_2^-$ ) is an intermediate oxidation state between ammonia and nitrate. Nitrite is an invisible killer of fish because it oxidizes haemoglobin to methemoglobin in the blood, turning the blood and gills into brown and hindering respiration, also damage the nervous system, liver, spleen and kidneys of the fish.<sup>39</sup> Nitrate ( $\text{NO}_3^-$ ) is the final oxidation product of nitrogen compounds in the aquatic environment, at the same time nitrate is considered as the only thermodynamically stable form of nitrogen in the absence of oxygen and also, in the major nitrogenous compound in the aquatic environment.<sup>40</sup> In the present study nitrite and nitrate peaks were  $0.188$  and  $0.669 \text{ mg L}^{-1}$ ,



**Figure 3.** Seasonal variations of pH, DO, BOD and COD in Rosetta branch water during summer 2014 and winter 2015.

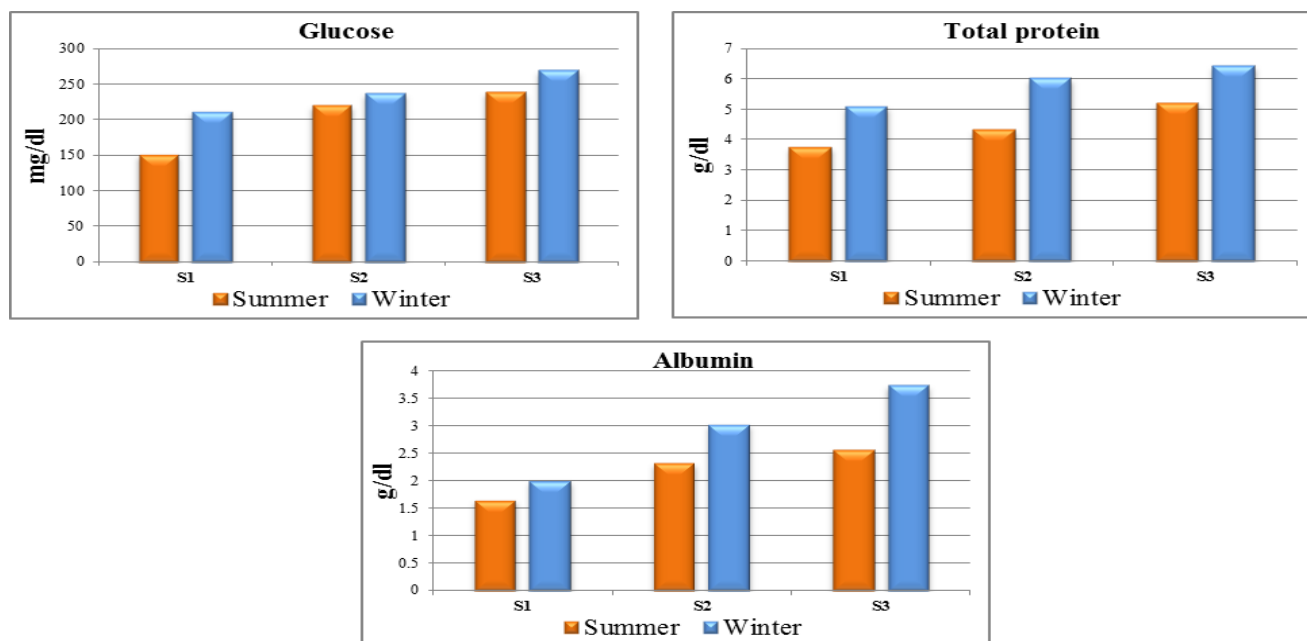


**Figure 4.** Seasonal variations of chemical parameters: nitrite, nitrate and ammonia in Rosetta branch water during summer 2014 and winter 2015.

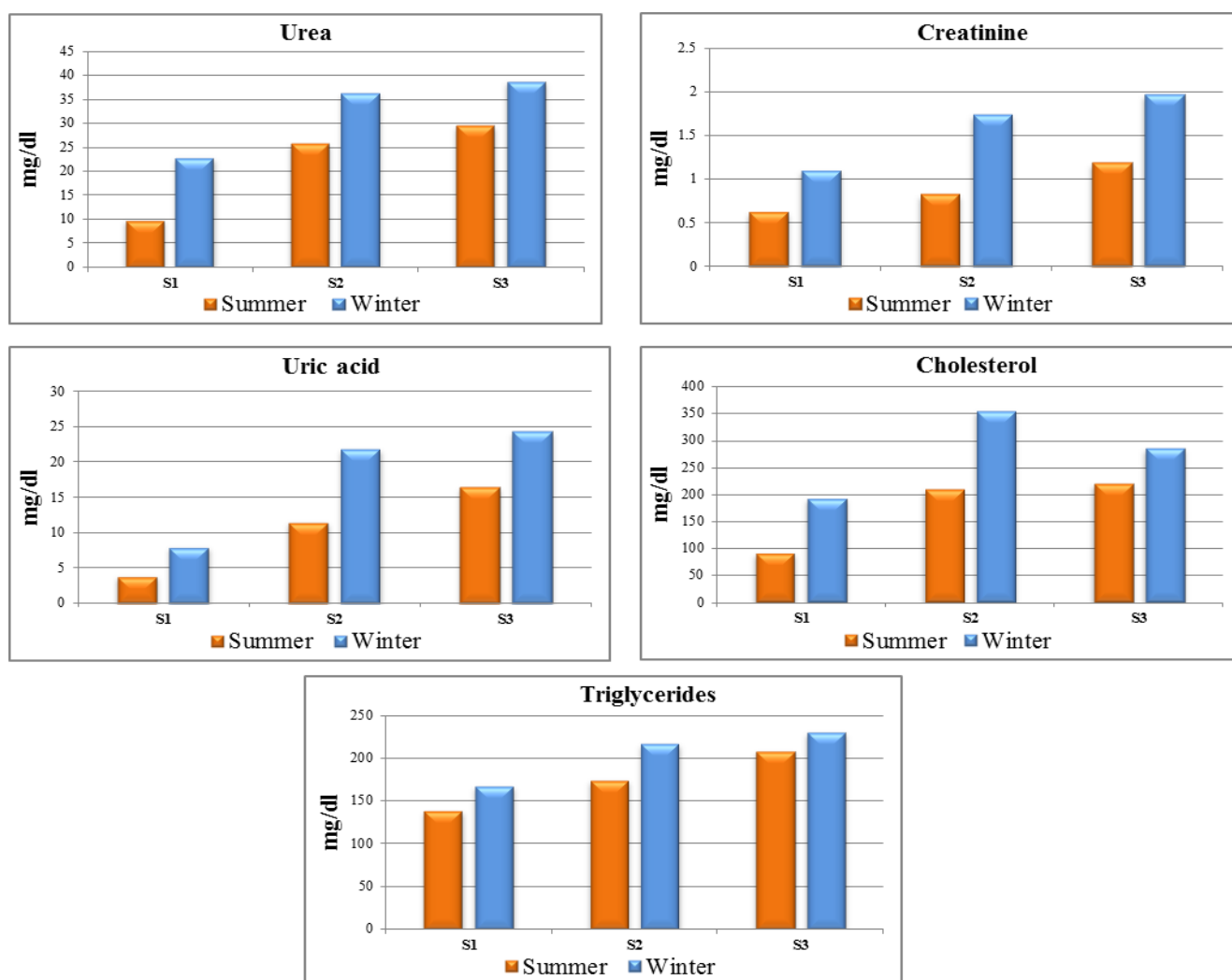
recorded at S3 during winter (Figure 4). Nitrate concentrations were found to be higher than nitrite at all stations due to the fact that  $\text{NO}_3^-$  is the final stable form of nitrogen. Nitrite and nitrate were correlated positively ( $r = 0.91$ ) (Figure 8).  $\text{NO}_2^-$  and  $\text{NO}_3^-$  inhibited a significant increase ( $P \leq 0.01$ ) at S2 and S3, comparing to S1, due to the oxidation of ammonia by nitrifying bacteria and biological nitrification at S2 from El-Rahawy drain discharges.

Moreover, the nitrogenous effluents at S3 from industrial area Kafr El-Zayat city producing metals, dyes and celluloid.<sup>41</sup>

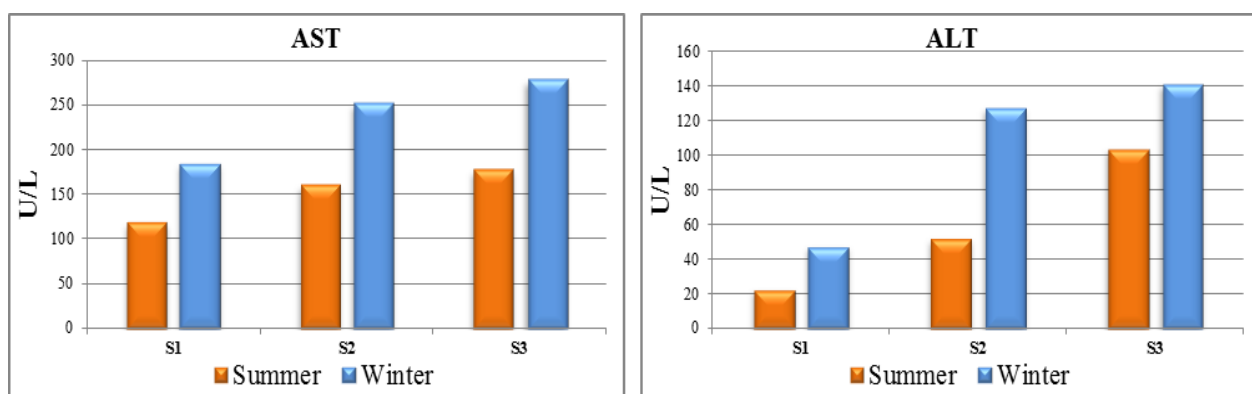
Ammonia is the third form of nitrogen compounds which occurs naturally in water bodies, arising from the breakdown of nitrogenous organic and inorganic matter in sediment and water, excretion by biota. The most important source of



**Figure 5.** Seasonal variations of biochemical parameters: glucose, total protein and albumin (Mean  $\pm$  S.E) in blood serum of *O. niloticus* from Rosetta branch water during summer 2014 and winter 2015.



**Figure 6.** Seasonal variations in biochemical parameters: uric acid, cholesterol and triglycerides (Mean  $\pm$  S.E) in blood serum of *O. niloticus* from Rosetta branch water during summer 2014 and winter 2015.



**Figure 7.** Seasonal variations in biochemical parameters: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) (Mean  $\pm$  S.E) in blood serum of *O. niloticus* from Rosetta branch water during summer 2014 and winter 2015

ammonia is the ammonification of organic matter, so, occurrence of ammonia in the waters used as an indicator for organic pollution.<sup>40</sup> In the present study ammonia range at S2 and S3 were 7.5 and 8.6 mg L<sup>-1</sup> during winter (Figure 4), exhibited a highly significant increase ( $P \leq 0.01$ ), at both sites, compared to that in S1. This may be related to the large impact of the domestic discharge from El-Rahawy

drain, which is highly loaded with organic matter and bacteria, leading to decomposition of the organic matter, exhausting dissolved oxygen and produce high level of ammonia,<sup>37</sup> and the industrial effluents of Kafr El-Zayat factories discharged into the branch water. The concentration of ammonia in water samples exceeded the permissible limits (0.5 mg L<sup>-1</sup>) of Egyptian Governmental Law.<sup>32</sup> EC, TDS, nitrite, nitrate and ammonia in the present study recorded the highest values in winter, this may be due to winter closure which lowers water levels in the River Nile, leading to an increase in pollutants load which reduces the dilution effects.<sup>30</sup> This is in agreement with Ashry *et al.*<sup>42</sup>

Fish are very susceptible to physical and chemical changes which reflected in their blood components,<sup>13</sup> that are considered pathophysiological indicators of the whole body. The present study recorded a pronounced increase in the studied biochemical parameter in blood serum of *O. niloticus*, for example: serum glucose level at polluted sites S2 and S3 were (221.1 and 240.1 mg dL<sup>-1</sup> in summer) and (238.6 and 270.4 mg dL<sup>-1</sup> in winter), respectively. Both sites showed a significant increase ( $P \leq 0.01$ ), as compared to S1 (Figure 5). Blood glucose is a sensitive and reliable indicator of environmental stress in fish.<sup>43</sup>

The hyperglycemic response illustrated in the present study indicated that, fish generated more glucose to produce the energy used in combating the stress induced by the environmental pollution, that possibly due to the induction of hepatic gluconeogenic enzymes and increasing substrate supply by cortisol hormones,<sup>44</sup> elevate the breakdown of liver glycogen or the synthesis of glucose from adipose tissue and others extrahepatic tissues, provide liver with lactate and amino acids, which serve as gluconeogenic substrates for hepatocytes to synthesize more glucose.<sup>41</sup> Moreover the pancreatic cell injury caused by pollutants in

water enhanced glycogen breakdown in liver, and reduced insulin activity.<sup>45</sup>

Total serum protein is a useful indicator in diagnosis of fish disease. The majority of plasma proteins which are synthesized in the liver are used as indicators of liver impairment.<sup>43</sup> Also, serum albumin in fish is involved in metabolism and plays an important role in transport functions of exogenous chemicals and endogenous metabolites. Thus, serum albumin is considered as a diagnostic tool which reflects the liver function, metabolic

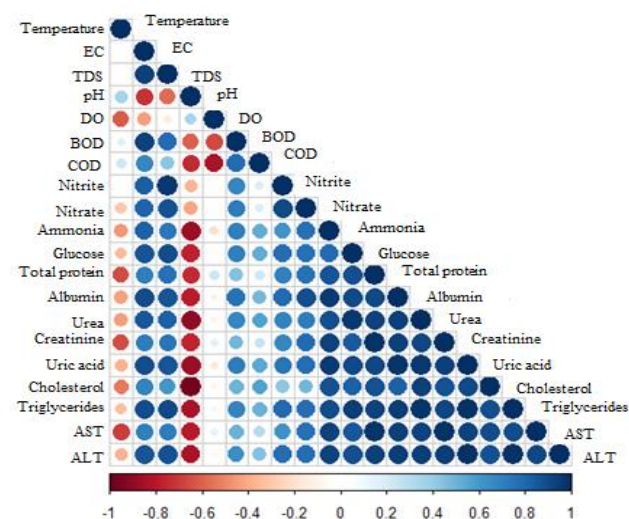
status and stress conditions in fishes.<sup>44,45</sup> In the present study, highest level of serum total protein and serum albumin of *O. niloticus* fish collected from Rosetta Branch of River Nile were 6.04 g dL<sup>-1</sup> and 3.57 g dL<sup>-1</sup> at S3 in winter, showed a highly significant increase ( $P \leq 0.01$ ) as compared to S1 (Figure 5). The observed hyperproteinaemia and hyperalbuminemia in the present study may be due to activation of metabolic systems as a response to pollutants exposure, or the induction of protein synthesis and degradation of the cellular material in the liver caused by liver damage, due to the stressful condition of water pollution.<sup>46,47</sup>

Urea, creatinine and uric acid are non-protein nitrogenous compounds and products of metabolism. Creatinine is a waste product largely from the muscles breakdown. Urea is the primary metabolite derived from dietary protein and tissue protein turnover.<sup>48</sup> Uric acid is formed by fish from exogenous and endogenous purines. It is converted in the liver to urea for excretion by the gills.<sup>49</sup> Urea, creatinine and uric acid are useful in diagnosis of renal function impairment, renal insufficiency, renal tubular necrosis, muscle tissue damage as well as impaired nitrogen metabolism.<sup>50</sup>

Concerning, the effect of water pollutants on kidney functions of *O. niloticus* collected from Rosetta Branch at S3, the obtained results revealed that, serum urea, creatinine and uric acid were 29.54, 1.19 and 16.5 mg dL<sup>-1</sup> in summer and 38.78, 1.98 and 24.4 mg dL<sup>-1</sup> in winter, respectively. S3 inhibited a significantly increased ( $P \leq 0.01$ ) comparing to S1 during both the seasons (Figure 6). This response may be attribute to an impairment of renal functions and acute renal

failure, due to necrosis of renal tubules which was associated with decrease in urea, creatinine and uric acid excretion, leading to their increase in plasma.<sup>51</sup> Moreover, kidney damage caused by pollutants may reduce the renal blood flow and glomerular filtration rates, resulting in azotemia, which is characterized by increase in blood urea nitrogen uric acid and creatinine.<sup>52</sup> The reported renal failure in *O. niloticus* collected from Rosetta branch of the River Nile are in accordance with previous findings in serum of *O. niloticus* collected from different lakes (Maryut, Manzala, El-Burullus, Edku and Qarun) and the River Nile,<sup>53</sup> and in *O. niloticus* exposed to aquatic pollution.<sup>54</sup>

The main lipids classes in plasma or serum are cholesterol and triglycerides. Cholesterol is an essential structural component of cell membranes and is precursor of all steroid hormones. The outer layer of plasma is lipoproteins.<sup>55</sup> The level of triglycerides and cholesterol in plasma and tissues of fish are sensitive to environmental pollutants, depending on many factors such as the types of contaminants, the concentration, mode of its action, duration of exposure and fish species.<sup>56</sup> Both serum cholesterol and triglycerides levels in blood serum of *O. niloticus* in the present study, showed a highly significant increase ( $P \leq 0.01$ ) at S2 and S3, during both sampling seasons, comparing to S1 (Figure 6). This increase may be due to the liver failure and renal dysfunction. The damaged liver cells release cholesterol and other lipids constituents into the circulation, also renal dysfunction may increase the total cholesterol and triglycerides in serum.<sup>57</sup> In addition, the increased triglyceride and cholesterol concentration in blood serum of *O. niloticus* may suggest that, a general increase in lipid mobilization must have taken place simply to fulfill the increasing demand for energy to cope with the stress of wastewater toxicity, this is in accordance with the hyperglycemic response recorded in blood serum of glucose is converted to pyruvate in the glycolytic pathway, that metabolized to acetyl-CoA in aerobic tissues and used as a precursor in the synthesis of cholesterol and fatty acids in the citric acid cycle.<sup>58</sup>



**Figure 8.** Correlation matrix between the physio-chemical parameters of water and the biochemical parameters in blood serum of *O. niloticus* from Rosetta branch water, during summer 2014 and winter 2015. Positive correlations are displayed in blue and negative correlations in red colour. Colour intensity and the size of the circle are proportional to the correlation coefficients.

All chemical reactions in the cell are catalyzed by enzymes and introduction of foreign chemicals in the cell generally disturbed enzyme functions. The transaminases (AST and ALT) are key enzymes known to play a key role in mobilizing of L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological conditions.<sup>59</sup> Serum enzymes such as AST and ALT could be used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscles and gills.<sup>60, 61</sup> AST and ALT used as biomarkers in ecotoxicology, because they provided an early warning of potentially hazardous alterations in contaminated aquatic organisms.<sup>62</sup> Serum AST increased by 1.3 and 1.4 folds at S2, and 1.4 and 1.6 folds at S3 in summer and winter, respectively. On the other hand, serum ALT increased 2.2 and 2.6 folds at S2, and 4.4 and 3 folds at S3 in summer and winter, respectively. AST and ALT activities in blood serum of *O. niloticus* in the present study showed a significant increase ( $P \leq 0.01$ ) during the study at S2 and S3 comparing to S1 (Figure 7).

The activities of serum AST and ALT in *O. niloticus* collected from downstream Rosetta Branch of the River Nile as compared to S1 are another diagnostic indicator of the liver damage in fish collected from the polluted sites (S2 and S3). Moreover, muscle damage, intestinal and hepatopancreatic injury and myocardial impairment, leads to extensive liberation of these enzymes into the blood circulation.<sup>63, 64</sup> Our findings are in agreement with these tilapia sp. collected from wadi El-Rayan and Lake Qarun.<sup>65, 66</sup> The studied biochemical parameters in blood serum of *O. niloticus* collected from Rosetta branch showed a positive correlation with EC, DO, BOD, COD, TDS, nitrite, nitrate and ammonia in water, that means these characteristics affect and relate to each other (Figure 8).

## CONCLUSION

Water downstream in Rosetta branch is continuously loaded with pollutants. Particularly, at Al-Qata (S2), which receives water rich in domestic wastewaters discharged from El-Rahawy drain and agricultural wastewater coming from many small agriculture drains, in addition to industrial effluents from the industrial area of Kafr El-Zayat (S3). Increased nutrients are expected to affect biological community, fish health and water quality of Rosetta branch. This hypothesis is strengthened by finding that *O. niloticus* collected from polluted sites S2 and S3 suffers from organ dysfunction (liver and kidney), that threatens the fish health in Rosetta branch of River Nile.

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