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Physiological Response of Mugil Capito Fingerlings (*Liza ramada;* Risso, 1826) to Physico-Chemical Characteristics of Wastewaters of Two Hot Spots, West of Alexandria, Egypt

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Abstract: The western Mediterranean coast of Alexandria received huge quantities of waste effluents from two sources, El-Umoum drain and West-Noubaria canal. The Physico-chemical characteristics of wastewaters of these two water bodies reflect high eutrophication conditions, toxicity due to concentrations of NO_2, TDN and disturbance in phosphorous / nitrogen ratio (P / N). The West-Noubaria canal water exhibits higher toxicity than El-Umoum water because of the high toxicity of unionized ammonia UIA (NH_3). The acute toxicity test (96-hours period) showed that fish fingerlings tolerated the different concentrations of both wastewaters and survived with 100 % percentage. While, the survival rates of fish in the chronic experiments (28-days) showed that they tolerated El-Umoum drain wastewater more thane that of West-Noubaria canal. Fish exposed to different concentrations of wastewater of both water bodies exhibited a significant decrease, with increasing concentrations, in growth performance indices: body weight increase percent (BWI %), average daily gain (ADG) and significant growth rate (SGR %) and in protein utilization index: protein efficiency ratio (PER). Food utilization index: food conversion ratio (FCR) exhibits a significant increase, only, with increasing concentrations of West-Noubaria canal wastewater. Meanwhile, condition factor (K) exhibits a significant increase with increasing concentrations of wastewaters of both water bodies. Muscle total protein and gross energy were only changed significantly after exposure to El-Umoum drain wastewater in comparison with control. In conclusion, food intake, metabolism and growth of fish fingerlings had been ad versed under the stress effect of both wastewaters.

Key words: Physico-chemical characteristics · El-Umoum drain · West-Noubaria canal · *Liza ramada* · growth performance indices

INTRODUCTION

Growth of human settlement along the coast, the concentration of industrial development in coastal areas and the wealth of exploited living marine resources in coastal waters, all justify the concern felt for the quality of the coastal marine environment and its resources [1] The coastal area of West-Alexandria is suffering from discharge of huge quantities of agricultural, industrial and sewage wastes. The main sources of these wastes are El-Umoum drain, which discharge its effluents into El-Mex Bay (76.4 m^3 s^{-1}) and West-Noubaria canal, which discharge its effluents into the Mediterranean coast at the 21 Km region (1 m^3 s^{-1}), in addition to the maritime activities in western harbor [2]. Accordingly, eutrophication has become a persistent problem in this coastal area of West-Alexandria because of continuous enrichment of nutrients. There is a wealth of data on the physical, chemical and biological characteristics of this aquatic system. Numerous studies have been conducted on the physico-chemical conditions in this area [2-11]. Zakaria *et al.* [12] identified four types of water in El-Mex Bay based on the salinity values. Youssef [13] recorded the maximum average ratios of specific alkalinity at regions affected by the drainage water. El-Rayis [14] and Abuldahab *et al*. [15] studied the levels of heavy metals in El-Mex Bay ecosystem including marine organisms of different trophic levels. Tayel and Shiradah, [16] and Halim *et al*. [17] studied concentrations of heavy metals in fish tissues. However, yet no information has been conducted to relate the physico-chemical characteristics of El-Umoum drain and West-Noubaria canal wastewaters and their impact on fish.

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The aim of this work was to assess the physico-chemical characteristics of El-Umoum drain and West-Noubaria canal wastewaters and determine the median lethal concentrations (96-hours LC_{50}) of these wastewaters for Mugil capito fingerlings. In addition, investigate the influence of raw wastewaters and different dilutions, on growth performance, food and protein utilization, the condition of fish fingerlings and fat and protein contents of their muscles was another target.

MATERIALS AND METHODS

This work was carried out in Fish Physiology Laboratory through the project: "The impact of industrial activities, marine transport and tourism on the physico-chemical and biological characteristics of seawater and fish groups in western zone of Alexandria" financed by NIOF during the period from June 2005-May 2006.

Physico-chemical Analyses: The physico-chemical characteristics of control seawater and experimental wastewaters were determined according to Standard Methods for the Examination of Water and Wastewater [18]. Samples of wastewater from El-Umoum drain and West-Noubaria canal and seawater from Eastern Harbor coast in front of NIOF were collected using Niskin's bottles samplers of 3 liters capacity. The pH, dissolved oxygen (DO) and salinity were measured using CTD (Model YSI XL600). Nitrate (NO_3), nitrite (NO_2), ammonia (NH_4), reactive silicate (SiO_4) and phosphate (PO_4) were determined spectrophotometrically according to Grassohoff [19]. The percent of unionized ammonia UIA (NH_3), total dissolved nitrogen (TDN) and phosphorous / nitrogen ratio (P / N) were calculated. Total alkalinity was measured by titration against standard HCl according to Strickland *et. al*. [20]. Oxidizable organic carbon (OOC) was determined by the method of Carlberg [21]. Heavy metals were determined according to Riley and Taylor [22] using Chalex 100. Water samples were filtered using 0.45 µm membrane filter paper and the filtrate, which represents the dissolved phase, was used for determining the metals. Ni, Mn, Fe, Cu, Zn and Cd metals were determined by atomic absorption spectrophotometer (AAS) Perkin El-mer.

Fish: Fingerlings of Mugil capito (*Liza Ramada,* family: *Mugilidae*) with length ranged between 3.4-4.5 cm and weight ranged between 1.27-1.66 g were collected from the seacoast of Eastern Harbor, in front of NIOF and were used in this work. Fish fingerlings were acclimatized for two weeks before carrying out the experiments. They were daily fed with fishmeal composed of: dry matter 88.96%, crude protein 22.92%, crude fat 9.67%, crude fiber 8.49%, ash 9.34%, nitrogen free extract 49.58 and calculated gross energy 429.12 Kcal/ 100g dry matter.

Aquaria: 12 glass aquaria (40 x 30 x25 cm) were used, having 20 liters of seawater for control aquaria and 20 liters of a mixture of seawater and wastewater of El-Umoum drain or West-Noubaria canal with concentrations 20, 40, 60, 80 and 100 % for experimental aquaria. The aquaria were provided with sufficient aeration by using electric aerators. Large samples of wastewater from El-Umoum drain, West-Noubaria canal and seawater from seacoast of Eastern Harbor were weekly collected in plastic containers for experimental purpose.

Experiments: 1-Acute experiments (96-hours period) for determining the median lethal concentration (LC_{50}) of wastewater of both El-Umoum drain and West-Noubaria canal for the experimental fingerlings according to Reish and Oshida [23]. The fish were stopped to feed 24 hours before starting and during the four days period of the acute experiment. The acute experiment was carried out in duplicate.

Chronic experiments (28-days period) to determine the impact of both wastewaters on growth performance indices: body weight increase percentage (BWI %), average daily gain (ADG) and specific growth rate (SGR %); food utilization index: food conversion ratio (FCR); protein utilization index: protein efficiency ratio (PER) and fish condition factor (K) of Mugil capito fingerlings. These indices were determined according to Jauncy and Ross [24].

The chronic experiment was carried out in duplicate. Fish were fed once daily, 6 days a week at a rate of 5% of fish group body weight in each concentration and weighed at the end of every week. The experimental and control water were changed once a week. Water temperature during the experimental period varied between 20-23°C.

Protein and Fat Analyses: Five alive fish were taken from, only, control and raw wastewaters (100 % concentration) of both water bodies at the end of chronic experiment and frozen at-20°C for protein and fat content analyses. Proximate analyses of protein and fat in body fish carcass were performed according to standard methods AOAC [25]. Gross energy was calculated according to NRC [26] using the calorific values 5.65 and 9.45 k. cal. /g whole body of protein and fat respectively.

Statistical analyses of data were performed only for fish groups that survived the completely chronic experimental period and calculated by the analysis of variance (ANOVA-two ways) according to Snedecor and Cochran [27], Duncan's multiple ranges test [28] and t-test.

RESULTS

The physico-chemical characteristics of control seawater and wastewaters of El-Umoum drain and West-Noubaria canal are shown in Table (1). El-Umoum drain water recorded relatively low values of pH (7.74) and salinity (5.1), low oxygenation (1.59 mg/l) and high alkalinity (451.0 mg/l) as compared with those of Noubaria water (8.08, 7.1, 5.79 mg/l and 210.0 mg/l, respectively) and control water (8.19, 37.9,6.91 mg/l and 295.0mg/l, respectively). Both El-Umoum and West-Noubaria wastewaters revealed richness with the nutritive salts. They recorded concentrations of NH_4 (86.32), NO_2 (13.31), NO_3 (16.21), PO_4 (13.73) and SiO_4 (139.96 µM) for El-Umoum water and 9.24, 3.47, 29.76, 7.13 and 62.07 µM for West-Noubaria water, respectively. While for the control seawater, the respective values were 3.06, 0.22, 3.69, 1.47 and 0.63 µM. The calculated TDN concentrations in both wastewaters and control water take the order: El-Umoum (115.84) > Noubaria (42.47) > control (6.97 µM), while the P/N ratios take the opposite order: El-Umoum (0.119) < Noubaria (0.167) < control

Table 1: The physico-chemical characteristics of control seawater and El-Umoum Drain and West-Noubaria canal wastewaters.

Parameter	Control	El-Umoum drain	West-Noubaria canal
pH	8.19	7.74	8.08
Salinity	37.90	5.10	7.10
DO	6.91	1.59	5.79
Alkalinity(mg /l)	295.00	451.00	210.00
NH4 (µM)	3.06	86.32	9.24
NO2 (µM)	0.22	13.31	3.47
NO3 (µM)	3.69	16.21	29.76
PO4 (µM)	1.47	13.73	7.13
SiO4 (µM)	0.63	139.96	62.07
TDN(µM)	6.97	115.84	42.47
P/N	0.21	0.119	0.167
OOC (mg O2/l)	2.86	7.24	2.66
Nickel (µg/l)	3.55	9.75	12.29
Manganese (µg/l)	1.62	4.46	4.73
Iron (µg/l)	9.80	25.33	25.46
Copper (µg/l)	6.25	12.62	11.23
Zinc (µg/l)	11.63	23.62	42.32
Cadmium (µg/l)	ND	0.91	0.03

ND = Not detected

Table 2: Survival rate percentages of fish exposed to different concentrations of El-Umoum drain and West- Noubaria canal wastewaters during the period of chronic experiment

			7 - days			14 - days			21- days			28 - days		
wastewater	concentration	Initial no. of fish	dead	alive	Survival %	dead	alive	Survival %	dead	alive	Survival %	dead	alive	Survival%
El-Umoum drain	Control	18	0	18	100.0	0	18	100	0	18	100	0	18	100
	20%	18	0	18	100	0	18	100	0	18	100	0	18	100
	40%	18	0	18	100	1	17	94.4	4	14	77.8	11	7	38.9
	60%	18	2	16	88.9	8	10	55.6	9	9	50	12	6	33.4
	80%	18	0	18	100	2	16	88.9	18	0	0			
	100%	18	0	18	100	0	18	100	0	18	100	1	17	94.4
West-Noubaria canal	Control	18	0	18	100	0	18	100	0	18	100	0	18	100
	20%	18	9	9	50	18	0	0						
	40%	18	1	17	94.4	9	9	50	12	6	33.4	12	6	33.4
	60%	18	6	12	66.7	18	0	0						
	80%	18	5	13	72.2	15	3	16.7	18	0	0			
	100%	18	1	17	94.4	10	8	44.5	10	8	44.5	12	6	33.4

Tab. 3: Average total length and weight, food weight as dry matter and weight of protein intake per fish in the control and different wastes concentrations during the period of experiment

		Initial time		7-Days				14-Days			
Wastewater	Conc. %	Av.Fish Tot.L. (cm)	Av.Fish Tot.Wt. (g)	Av.Fish Tot.L. (cm/7-d)	Av.Fish Tot.Wt. (g/ 7-d)	Food as D M (g/f/ 7-d)	Protein Intake (g/f/ 7-d)	Av.Fish Tot.L. (cm/14-d)	Av.Fish. Tot Wt. (g/14-d)	Food as D M (g/f/14-d)	Protein Intake (g/f/14-d)
El-Umoum Drain	Control (seawater)	3.8	1.41	4.2	1.56	0.375	0.086	4.6	1.73	0.792	0.181
	20	3.5	1.31	3.9	1.44	0.350	0.080	4.4	1.64	0.734	0.168
	40	3.4	1.27	3.7	1.36	0.338	0.077	3.9	1.44	0.701	0.161
	60	3.6	1.34	3.7	1.36	0.357	0.082	4.2	1.57	0.721	0.165
	80	3.9	1.46	4.0	1.48	0.388	0.089	4.1	1.53	0.783	0.179
	100	3.6	1.33	3.7	1.36	0.354	0.081	4.1	1.52	0.718	0.164
West-Noubaria Canal	Control (seawater)	4.2	1.58	5.0	1.85	0.421	0.097	5.8	2.18	0.915	0.210
	20	3.8	1.42	4.1	1.54	0.380	0.087				
	40	3.8	1.43	4.2	1.55	0.383	0.088	4.1	1.52	0.797	0.183
	60	4.1	1.53	4.3	1.59	0.409	0.094				
	80	4.5	1.66	4.8	1.79	0.443	0.102	4.6	1.73	0.922	0.211
	100	4.1	1.51	4.6	1.71	0.403	0.092	5.1	1.90	0.859	0.197

		Initial time		21-Days				28-Days			
Wastewater	Conc. %	Av.Fish Tot.L. (cm)	Av.Fish Tot.Wt. (g)	Av.Fish Tot.L. (cm/21-d)	Av.Fish Tot.Wt. (g/ 21-d)	Food as D M (g/f/ 21-d)	Protein Intake (g/f/ 21-d)	Av.Fish Tot.L. (cm/ 28-d)	Av.Fish. Tot Wt. (g/ 28-d)	Food as D M (g/f/ 28-d)	Protein Intake (g/f/ 28-d)
El-Umoum Drain	Control (seawater)	3.8	1.41	5.6	2.10	1.254	0.287	6.0	2.24	1.815	0.416
	20	3.5	1.31	5.3	1.96	1.171	0.268	5.6	2.09	1.695	0.388
	40	3.4	1.27	4.1	1.54	1.086	0.249	4.4	1.64	1.498	0.343
	60	3.6	1.34	3.9	1.47	1.140	0.261	3.7	1.38	1.531	0.351
	80	3.9	1.46								
	100	3.6	1.33	4.8	1.80	1.124	0.258	5.6	2.08	1.604	0.368
West-Noubaria Canal	Control (seawater)	4.2	1.58	6.9	2.59	1.496	0.343	7.4	2.77	2.187	0.501
	20	3.8	1.42								
	40	3.8	1.43	4.2	1.57	1.203	0.276	4.6	1.70	1.621	0.372
	60	4.1	1.53								
	80	4.5	1.66								
	100	4.1	1.51	5.9	2.20	1.366	0.313	6.8	2.53	1.953	0.448

(0.21). El-Umoum wastewater is loaded with OOC (7.24) compared with Noubaria wastewater (2.66) and control (2.86 mg O_2/l). The abundance of measured heavy metals in control, El-Umoum drain and Noubaria canal waters is found to be Ni (3.55, 9.75 and 12.29, respectively); Mn (1.62, 4.46 and 4.73 respectively); Fe (9.80, 25.33 and 25.46, respectively); Cu (6.25, 12.62and 11.23, respectively); Zn (11.63, 23.62and 42.32, respectively) and Cd (ND, 0.91 and 0.03 mg/l, respectively).

The 96-hours acute toxicity test revealed no mortalities among Mugil capito fingerlings exposed to different concentrations of wastewaters of both water bodies. It has been confirmed that, there are no median lethal concentrations (96-hours LC_{50}) for fish fingerlings under the effect of all concentrations of both wastewaters.

The mean survival rates of Mugil capito fingerlings in the chronic experiment are shown in Table (2). At the end of the first week, all fish groups in different concentrations of El-Umoum wastewater survived with percentage 100 % except that in concentration 60 %, which showed survival rate 88.9 %. Meanwhile, they suffered mortalities in Noubaria wastewater and revealed survival rates 50.0, 94.4, 66.7, 72.2 and 94.4 % in concentrations 20, 40, 60, 80 and 100 % respectively.

At the end of second, third and fourth weeks, fish groups in different concentrations of both wastewaters suffered mortalities. Compared with control, at the end of chronic experiment, fish showed survival rates 100.0, 38.9, 33.4 and 94.4 % under concentrations 20, 40, 60 and 100 % of El-Umoum wastewater and 33.4 and 33.4 % under

Table 4: Changes in body weight increase percentage (BWI %) after exposure to different concentrations of El-Umoum drain and West-Noubaria canal wastewaters

Area	Concentration	7-days	14-days	21-days	28-days
El-Umoum drain	Control	10.72[b]	22.93	48.94[ab]	58.79[ab]
	20%	9.84[b]	25.11	49.70[b]	59.46[b]
	40%	7.17	13.48	21.48	29.36
	60%	1.68	17.16	9.45[a]	3.23[a]
	100%	2.34[b]	14.45	35.34[b]	56.13[b]
West-Noubariacanal	Control	17.09[b]	37.83	63.85[ab]	75.46[ab]
	40%	8.60	6.45	9.56[a]	18.88[a]
	100%	12.97	25.83	45.70	67.77

Body weight increase (BWI %) = (live body gain wt. / initial wt.) x 100.

El-umoum Drain Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (sig.).

F (cal.) = rows (4.48), col. (9.34); F (crit.) = rows (3.26), col. (3.49); P = rows (0.0191), col. (0.0018)

Duncan's Shortest Significant Range:

For rows (conc.)-for 2, 3, 4and5 means at $p<0.05$, df 15 = 27.87, 29.26, 30.10, 30.65

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 16 = 20.82, 21.79, 22.86

West-noubaria Canal Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (sig.)

F (cal.) = rows (9.85), col. (6.40); F (crit.) = rows (5.14), col. (4.76); P = rows (0.0127), col. (0.0268)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2 and3 means at $p<0.05$, df 9 = 33.18, 34.64

For columns (weeks)-For 2, 3 and 4 means at $p<0.05$, df 8 = 41.83, 43.62, 44.65

a-Significant with the control row.

b-Significant with the 7-days column.

Table 5: Average daily gain (ADG) of fish after exposure to different concentrations of El-Umoum drain and West-Noubaria canal wastewater

Area	Concentration	7-days	14-days	21-days	28-days
El-Umoum drain	Control	21.59[a]	23.10[a]	32.86[a]	29.60
	20%	18.41	23.49	31.01	27.82
	40%	13.02	12.23[a]	12.99	13.32[a]
	60%	3.21	16.43	6.03[a]	1.55[a]
	100%	4.44[ab]	13.73	22.38[ab]	26.66[b]
West-Noubaria canal	Control	38.57[a]	42.70[a]	48.04[a]	42.58[a]
	40%	17.56[a]	6.59[a]	6.51[a]	9.64[a]
	100%	27.98[a]	27.86[a]	32.86[a]	36.55[a]

Average daily gain (ADG mg / day / fish) = (live body gain wt. (g) / exp. period (d) x 1000.

El-umoum Drain Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.).

F (cal.) = rows (9.20), col. (2.54); F (crit.) = rows (3.26), col. (3.49); P = rows (0.0012), col. (0.1056)

Duncan's Shortest Significant Range:

For rows (conc.)-for 2, 3, 4and5 means at $p<0.05$, df 15 = 9. 51, 9. 99, 10. 27, 10. 46

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 16 = 12. 96, 13. 56, 14. 00

West-noubaria Canal Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.).

F (cal.) = rows (43.84), col.(0.35); F(crit.) = rows (5.14), col. (4.76); P = rows (0.0003), col. (0.7884)

Duncan's Shortest Significant Range:

For rows (conc.)-for 2 and3 means at $p<0.05$, df 9 = 7. 14, 7. 45

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 8 = 32. 44, 33. 83, 34. 63

a-Significant with the control row

b-Significant with the 7-days column.

Table 6: Specific growth rate (SGR %) of fish after exposure to different concentrations of El-Umoum drain and West-Noubaria canal wastewaters.

Area	Concentration	7-days	14-days	21-days	28-days
El-Umoum drain	Control	1.45[a]	1.47	1.90[a]	1.65
	20%	1.34	1.60	1.92	1.67
	40%	0.99	0.90	0.93[a]	0.92[a]
	60%	0.24[ab]	1.13[b]	0.43[a]	0.11[a]
	100%	0.33[ab]	0.96	1.44[b]	1.59[b]
West-Noubaria canal	Control	2.25[a]	2.29[a]	2.35[a]	2.01[a]
	40%	1.18[a]	0.45[a]	0.43[a]	0.62[a]
	100%	1.74[a]	1.64[a]	1.79[a]	1.85

Specific growth rate (SGR %) = {Ln final wt.-Ln initial wt. / time (d)} x 100.

El-umoum Drain Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.).

F (cal.) = rows (8.69), col. (1.72); F (crit.) = rows (3.26), col. (3.49); P = rows (0.0016), col. (0.2151)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2, 3, 4and5 means at $p<0.05$, df 15 = 0. 53, 0. 56, 0. 58, 0. 59

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 16 = 0.76, 0.79, 0. 82

West-noubaria Canal Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.)

F(cal.) = rows (46.41), col. (0.78); F(crit.) = rows (5.14), col. (4.76); P = rows (0.0002), col. (0.5481)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2 and3 means at $p<0.05$, df 9 = 0. 36, 0. 38

For columns (weeks)-For 2, 3 and 4 means at $p<0.05$, df 8 = 1. 55, 1. 62, 1. 66

a-Significant with the control row.

b-Significant with the 7-days column.

Table 7: Food conversion ratio (FCR / d) after exposure to different concentrations of El-Umoum drain and West-Noubaria canal wastewater.

Area	Concentration	7-days	14-days	21-days	28-days
El-Umoum drain	Control	2.48[a]	2.45	1.82	2.19[a]
	20%	2.71	2.23	1.80	2.18
	40%	3.71	4.10	3.98	4.02
	60%	15.88[ab]	3.13[b]	9.00[b]	35.34[ab]
	100%	11.39	3.73	2.39	2.15
West-Noubaria canal	Control	1.56	1.53[a]	1.48[a]	1.83[a]
	40%	3.11	8.64[a]	8.80[a]	6.00[a]
	100%	2.06	2.20	1.98	1.91

Food conversion ratio (FCR / d) = food as DM (g) / live body gain wt. (g).

El-umoum Drain Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (insig.), col. (insig.).

F (cal.) = rows (2.99), col. (0.94); F (crit.) = rows (3.26), col. (3.49); P = rows (0.631), col. (0.4522)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2, 3, 4and5 means at $p<0.05$, df 15 = 9. 87, 10. 36, 10. 66, 10. 86

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 16 = 10. 83, 11. 34, 11. 70,

West-noubaria Canal Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.)

F(cal.) = rows (12.82), col. (0.97); F(crit.) = rows (5.14), col. (4.76); P = rows (0.0068), col. (0.4665)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2 and3 means at $p<0.05$, df 9 = 2. 48, 2. 59,

For columns (weeks)-For 2, 3 and 4 means at $p<0.05$, df 8 = 5. 84, 6. 09, 6. 23

a-Significant with the control row.

b-Significant with the 7-days column.

Table 8: Protein efficiency ratio (PER) of fish after exposure to different concentrations of El-Umoum drain and West-Noubaria canal wastewater

Area	Concentration	7-days	14-days	21-days	28-days
El-Umoum drain	Control	1.76	1.78	2.40[a]	1.99
	20%	1.61	1.96	2.43	2.01
	40%	1.18	1.06	1.10[a]	1.09[a]
	60%	0.27[b]	1.39[b]	0.48[a]	0.12[a]
	100%	0.38[b]	1.17	1.82[b]	2.03[b]
West-Noubaria canal	Control	2.80[a]	2.85[a]	2.94[a]	2.38[a]
	40%	1.40[a]	0.50[a]	0.50[a]	0.73[a]
	100%	2.12[a]	1.98[a]	2.20[a]	2.29

Protein efficiency ratio (PER) = live body gain wt. (g) / protein intake (g).

El-umoum Drain Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.).

F (cal.) = rows (7.90), col. (1.76); F (crit.) = rows (3.26), col. (3.49); P = rows (0.0023), col. (0.2075)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2, 3, 4and5 means at $p<0.05$, df 15 = 0. 70, 0. 73, 0. 75, 0. 77

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 16 = 0. 96, 1. 00, 1. 03

West-noubaria Canal Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (insig.)

F(cal.) = rows (41.70), col. (0.70); F(crit.) = rows (5.14), col. (4.4.76); P = rows (0.0003), col.(0.5858)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2 and3 means at $p<0.05$, df 9 = 0. 47, 0. 49

For columns (weeks)-For 2, 3 and 4 means at $p<0.05$, df 8 = 1. 96, 2. 04, 2. 09

a-Significant with the control row.

b-Significant with the 7-days column.

Table 9: Fish condition factor (K) after exposure to different concentrations of El-Umoum drain and West-Noubaria canal wastewater

Area	Concentration	7-days	14-days	21-days	28-days
El-Umoum drain	Control	2.11[b]	1.78	1.20[ab]	1.04[ab]
	20%	2.43[b]	1.92	1.32[b]	1.19[b]
	40%	2.69[b]	2.43	2.24[a]	1.93[ab]
	60%	2.69[b]	2.12	2.47[a]	2.73[ab]
	100%	2.69	2.21	1.63[b]	1.18[b]
West-Noubaria canal	Control	1.48[a]	1.12[a]	0.79[a]	0.68[a]
	40%	2.10[a]	2.21[a]	2.11[a]	1.75[a]
	100%	1.75	1.43	1.07	0.81

Fish condition factor (K) = {weight (g) / length (cm)^3} x 100.

El-umoum Drain Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (sig.).

F (cal.) = rows (6.78), col. (8.17); F (crit.) = rows (3.62), col. (3.49); P = rows (0.0043), col. (0.0031)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2, 3, 4and5 means at $p<0.05$, df 15 = 0. 73, 0. 77, 0. 79, 0. 81

For columns (weeks)-for 2, 3 and 4 means at $p<0.05$, df 16 = 0. 66, 0. 69, 0.71

West-noubaria Canal Wastewater:

Two factors ANOVA F at $p<0.05$ for rows (sig.), col. (sig.)

F (cal.) = rows (35.92), col. (8.75); F (crit.) = rows (5.14), col. (4.46); P = rows (0.0005), col. (0.0131)

Duncan's Shortest Significant Range:

For rows (conc.)-For 2 and3 means at $p<0.05$, df 9 = 0. 54, 0. 56

For columns (weeks)-For 2, 3 and 4 means at $p<0.05$, df 8 = 1. 05, 1. 09, 1. 12

a-Significant with the control row.

b-Significant with the 7-days column.

Table 10: Chemical composition of fish muscles after exposure to wastewaters of El-Umoum drain and West-Noubaria canal.

Parameter	Control	El-Umoum drain	West-Noubaria canal
Total protein	18.41±2.89	15.49±2.07*	16.75±3.02
Total lipid	1.43±0.49	1.60±0.48	1.75±0.48
Gross energy	117.36±19.45	102.42±9.68**	111.00±18.59

Average of 5 fish, * $p<0.05$, ** $p<0.01$

concentrations 40 and 100 % of West-Noubaria wastewater, respectively. Fish group under concentration 80 % of El-Umoum wastewater suffered 100 % mortalities at the end of third week, meanwhile, fish groups under concentrations 20 and 60 % of West-Noubaria wastewater suffered 100 % mortalities at the end of second week and fish group under concentration 80 % of the same wastewater suffered 100 % mortalities at the end of third week. Table (3) shows the results of growth rates in length and weight in addition to the results of food intake as dry matter and protein intake for all fish groups in different concentrations of both wastewaters at the end of every week of the experimental period.

Results of the physiological response of *Liza ramada* fingerlings exposed to different concentrations of both wastewaters are shown in Tables (4-9). Changes of growth performance indices: BWI percentage (Table 4), ADG (Table 5) and SGR percentage (Table 6) showed significant decrease with increasing concentrations as compared with control group every week. Meanwhile, with increasing time, only the BWI percentage index exhibited a significant increase comparing rates of growth at 14, 21 and 28 days with 7 days. Results of food utilization index FCR / d (Table 7) showed increased values with increasing concentrations as compared with control group every week. Meanwhile, they showed (in general) decreased values with increasing time except fish groups under concentrations of 40 and 60 % of El-Umoum wastewater and 40 % of West-Noubaria wastewater, which showed increasing changes with increasing time. Statistically, the increased changes of this index with increasing concentrations for fish under the effect of wastewater of West-Noubaria were the only significant changes.

Results of protein utilization index PER (Table 8) showed changed values taking the opposite trend compared with FCR index for both wastewaters. They showed decreased values with increasing concentrations as compared with control group every week and increased values (in general) with time except fish groups under concentrations of 40 and 60 % of El-Umoum wastewater and 40 % of West-Noubaria wastewater, which showed increased values with increasing time. Statistically, the decreased changes of this index with concentrations were significant, while those increased with time were insignificant for both wastewaters.

Values of condition factor K (Table 9) for fish groups exposed to different concentrations of both wastewaters statistically, exhibited significance for both the increased changes with increasing concentrations and the decreased changes with increasing time.

Table 10 shows changes of chemical composition of fish muscles after exposure to El-Umoum and West-Noubaria raw wastewaters compared with control fish. Results showed that protein synthesis rate had decreased and lipid synthesis rate had increased in fish exposed to both wastewaters than in control fish. Statistical analysis of results using t-test shows that, muscle total protein and gross energy only changed significantly after exposure to El-Umoum drain wastewater in comparison with control values.

DISCUSSION

The physico-chemical features showed pH values 8.19, 7.74 and 8.08 and alkalinity values 295.00, 451.00 and 210.00 mg/l for control seawater, El-Umoum and West-Noubaria wastewaters, respectively. According to Boyd [29], fish grow between 6.5 and 9 pH values and prefer slightly alkaline, close to neutral, water. Total alkalinity over 40 mg/l of $CaCO_3$ is considered more productive for biological purposes [29]. Therefore, El-Umoum wastewater may be considered more appropriate for fish than Noubaria wastewater. Fish tolerated wide range of salinity, during the acute toxicity test, varied from 5.1 and 7.1 for both raw wastewaters and higher concentrations of their different dilutions up to 37.9 for the control seawater. Variations of salinity for fish groups in different concentrations of both wastewaters may affect the osmoregulation in fish and may be considered as one of fish mortalities reasons in the chronic experiment. The DO content recorded lowest values (1.59, 5.79 mg/l) in El-Umoum and West-Noubaria wastewaters reflecting their eutrophication condition, while recorded 6.91 mg/l in control water. The depleted DO concentrations in both experimental wastewaters had no serious effects on fish because they were compensated by aeration.

The distribution of NH_4, NO_2, NO_3, PO_4 and SiO_4 levels was found as 86.32, 13.31, 16.21, 3.73 and 139.96 µM in El-Umoum water and 9.24, 3.47, 29.76, 7.13 and 62.07 µM in Noubaria water, compared to control levels 3.06, 0.22, 3.69, 1.47 and 0.63 µM, respectively. In natural waters where decomposition of organic matter takes place, NH_4 level is increased and is often concomitant with decrease in DO. The unionized ammonia UIA (NH_3) mainly causes the toxicity of ammonia. Meade [30] observed that NH_3 is 300-400 times more toxic than NH_4. Tiews [31] stated that UIA is highly toxic for fish at levels less than 1.4 µM and its toxicity effect increases at higher pH. The amount of UIA at different levels of pH can be calculated from the dissociation constant (pKa) values for ammonia according to the formula used by EIFAC [32]:

$$\%UIA = 100/1 + (\text{antilog } pKa - pH),$$

where:
pKa = 9.40 at 20°C

According to this formula, the percent of UIA (NH_3) was found (5.8 %) in control, (2.14 %) in El-Umoum drain and (4.57 %) in Noubaria canal waters at 20°C. This means that, the Noubaria canal water (higher pH and UIA %) exhibits higher toxicity than El-Umoum drain water (lower pH and UIA %).

Nitrite is present in natural waters only in small quantities. It has been found to be toxic to fish, as NO_2 combines with haemoglobin and forms methhaemoglobin, causing the brown coloration of blood [33]. In contrast to ammonia, nitrite toxicity increases at lower pH levels [33]. The presence of chloride [34] and calcium ions [35] inhibits nitrite toxicity. Thus, lower pH and salinity levels in El-Umoum wastewater lead to higher toxicity for fish than in West-Noubaria wastewater. Nitrate is the major form of nitrogen used by phytoplankton. No toxic effects to fish have been reported at NO_3 level below 100 mg (1.4 µM) [36].

The most common form of phosphorus is present in natural waters as ortho-phosphate (reactive inorganic phosphate). Biological productivity is mostly limited by the amount of phosphorus in water. Levels of phosphorus over 0.4 mg/l in fishpond water were observed to be not useful in increasing the productivity [37]. Both phosphorus and nitrogen levels are important to fish and a healthy P / N ratio of 1 / 4, (0.25) in water is proposed [37]. In the present work, the P / N ratio in El-Umoum drain (0.119) and in Noubaria canal (0.167) wastewaters showed disturbed unhealthy values for fish compared with control water (0.21).

Reactive silicates in natural fresh waters (mostly as ortho-silicates) are next to carbonates in abundance. The major source of silicate is through dissolution of rocks in drainage basins. Silicate is important because of its influence on diatom growth.

The oxidizable organic carbons (OOC) showed highest content in El-Umoum drain water (7.24 mg O_2/l) compared with their content in West-Noubaria (2.66 mg O_2/l) and control waters (2.86 mg O_2/l). Water temperature is the most effective factor on the OOC content [38], as their decomposition rate increased at high temperature. In the present work, aeration of the experimental wastewaters helps in oxidizing OOC and limiting their toxic effects on fish.

The abundance of measured heavy metals in control, El-Umoum drain and El-Noubaria canal wastewaters, revealed that Ni, Mn, Fe, Cu, Zn and Cd concentrations, in spite of still below the maximum permissible limits, could exert stress on fish and affect their growth performance.

In general, the results of the physico-chemical analyses reflect high eutrophication condition, toxicity due to concentrations of NO_2, TDN, high percentage of UIA (NH_3) and disturbance in P / N ratio in both wastewaters of El-Umoum drain and West-Noubaria canal.

The 96-hours acute toxicity test revealed no mortalities among fish fingerlings exposed to different concentrations of El-Umoum drain and West-Noubaria canal wastewaters. It has been confirmed that, there are no median lethal concentrations (96-hours LC_{50}) for *Liza ramada* fingerlings and they can tolerate and survive with percentage of 100 % for 4 days under the effect of all concentrations of wastewaters of both water bodies in spite of their inappropriate physico-chemical characteristics. Therefore, the toxicity tests (Bioassay) are necessary in water pollution evaluation because chemical and physical measurements alone are not sufficient to assess potential effects on aquatic biota [39].

Comparing the survival rates of fish in different concentrations of wastewaters of both water bodies during the four weeks period of the experiment, revealed that fish tolerated El-Umoum wastewater more than that of West-Noubaria canal. At the end of the first week, all fish groups in different concentrations of El-Umoum wastewater survived with percentage 100 % except that in concentration 60 %, which showed survival rate 88.9 %. Meanwhile, in spite of fish tolerated and survived in the different concentrations of West-Noubaria wastewater for 96 hours in the acute toxicity test, they suffered mortalities at the end of the first week. They revealed survival rates 50.0, 94.4, 66.7, 72.2 and 94.4 % in concentrations 20, 40, 60, 80 and 100 %, respectively.

At the end of second, third and fourth weeks, fish groups in different concentrations of both wastewaters suffered mortalities, but still the tolerance ability of fish groups for El-Umoum wastewater higher than that of fish groups for wastewater of West-Noubaria canal. Compared with control, fish groups in different concentrations of both wastewaters, at the end of chronic experiment, showed survival rates 100.0, 38.9, 33.4, 94.4 % under concentrations 20, 40, 60, 100 % of El-Umoum wastewater and 33.4 and 33.4 % under concentrations 40 and 100 % of West-Noubaria wastewater, respectively. Fish group under concentration 80 % of El-Umoum wastewater suffered 100 % mortalities at the end of third week, meanwhile, fish groups under concentrations 20 and 60 % of West-Noubaria wastewater suffered 100 % mortalities at the end of second week and fish group under concentration 80 % of the same wastewater suffered 100 % mortalities at the end of third week

The high mortality rates among fish groups in different concentrations of El-Noubaria wastewater could be attributed to the higher percentage of UIA (4.57 %) which is 300-400 more toxic than NH_4 [30] and pH (8.08) which increases the toxicity of UIA [31] and also, may be due to other not detected harmful substances.

Changes of growth performance indices for experimental fish, statistically, exhibited a significant decrease of BWI percentage, ADG and SGR percentage with increasing concentrations as compared with control groups every week. Meanwhile, with increasing time, only the BWI percentage index exhibited a significant increase comparing rates of growth at 14, 21 and 28 days with 7 days.

The observed decline in growth performance indices BWI %, ADG and SGR % for fish groups in different concentrations of wastewaters of both water bodies as compared with control fish groups, in spite of the gradual weekly grow in each fish group, manifest the stress effect of wastewaters on the growth rate of fish. This decline may reflect a change in feeding patterns towards decreasing feed intake [40] or an increase in the metabolic rate and, at the same time, depletion of energy reserves in response to stress [41].

Results of food utilization index FCR / d showed increased values with increasing concentrations as compared with control groups every week. Meanwhile, they showed (in general) decreased values with increasing time except fish groups under concentrations of 40 and 60 % of El-Umoum wastewater and 40 % of West-Noubaria wastewater, which showed increasing values with increasing time. Statistically, compared with control groups every week, the increased changes of this index with increasing concentrations for fish under the effect of wastewater of West-Noubaria canal were the only significant changes.

Results of protein utilization index PER showed changed values taking the opposite direction compared with FCR index values for wastewaters of both water bodies. They showed decreased values with increasing concentrations (rows), as compared with control groups every week and increased values (in general) with time except fish groups under concentrations of 40 and 60 % of El-Umoum wastewater and 40 % of West-Noubaria wastewater which showed increased values with increasing time. Statistically, the decreased changes of this index with concentrations were significant, while those increased with time were insignificant for both wastewaters.

Values of condition factor K for fish groups exposed to different concentrations of both wastewaters statistically, exhibited significance for both the increased changes with increasing concentrations and the decreased changes with increasing time. Comparing with the previous indices results, it can be noticed that K values seem to take the opposite trend to the values of growth performance indices (BWI %, ADG and SGR %) and protein utilization index (PER) and the same trend as (FCR) values. This means that the increased values of K with increasing concentrations does not reflect an increase in fish growth but reflects the eutrophication conditions of both wastewaters that may cause unhealthy increase in water and lipid contents in fish tissues.

The changes of chemical composition of fish muscles after exposure to raw wastewaters of El-Umoum drain and West-Noubaria canal compared with control fish showed decrease of protein synthesis rate and increase of lipid synthesis rate in fish exposed to wastewaters of both water bodies than in control fish. Statistical analysis of results shows that, muscle total protein and gross energy only changed significantly after exposure to El-Umoum drain wastewater in comparison with control values. This means that, more protein was metabolized rather than being used for growth.

In conclusion, the results of environment impact assessment on the status of El-Umoum drain and West-Noubaria canal wastewaters reflect the high eutrophication conditions, the adverse effects on feeding, metabolism and growth performance patterns of Mugil capito fingerlings and the urgent need to establish a follow up surveys and treatment for wastewaters of both water bodies.

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