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Toxicity of the Secondary Treated Municipal Waste Water of Riyadh Region to Fish and Its Hematological and Biochemical Effects on Albino Mice

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Abstract. Toxicity of the secondary treated municipal wastewater (TMWW) of Riyadh region to fish (*Tilapia niloticus*) was estimated using the static bioassay test. The data showed that the lethal time required to kill 50% of fish (LT_{50}) was 22.3 to 24.1 days during two successive years. The experiments were performed also to determine the effect of TMWW on blood parameters of albino mice (*Mus musculus*), such as red blood cells (RBC's), white blood cell (WBC's), hemoglobin (HGB) concentration, hematocrite (HCT) value and mean cell volume (MCV). The toxicological effects of TMWW on delta aminolevulinic acid dehydratase (δ-ALAD), Adenosine triphosphatase (ATPase) and Acetyl cholinesterase (AChE) were also carried out. No significant differences between TMWW treated animals and the control groups, has been found except an activation of δ-ALAD activity after 30 days of treatment.

Introduction

Pollution of water and waste water usually resulted from industrial and agricultural processes which resulted in rising level of chemicals in the environment, particularly heavey metals and pesticides.

Some of these chemicals are highly persistant in the environment [1,2 p. 317] and have well-known toxical, clinical, enzymatological and hematological effects in the biological system [3,4] [5, p. 592] [6,7,8,9].

With increase in the number of synthetic toxicants introduced into the environment and the complexity of possible interactions, determintal effects of fish population, and the frequency of fish mortality are apparently increasing [10, pp. 1-7]. Thus, natural water as well as waste water could easily be contaminated with these compounds.

Therefore, this study was conducted to measure the toxicity of the treated municipal waste water (TMWW) of Riyadh region to fish. The effects of the TMWW on blood parameters of Albino mice and its interaction with some enzymatic systems are also investigated.

Materials and Methods

Toxicity of TMWW to *Tilapia niloticus*

Hatchery-reared *Tilapia niloticus* obtained from the Live Fish Center at Riyadh, was used as fresh water experimental fish. Glass aquaria (40 × 50 × 70 cm each) containing 90 liters of dechlorinated tap water were used for the acclimatization of the fish for three weeks before the beginning of the tests. The aquaria were aereated using air pumps connected with air stones during acclimatization and during the tests as well. The fish were daily reared by the addition of a suitable diet. In due course of the research, fish of one size (5.5 cm length, 2.5 gm weight each) was selected.

The test were carried out according to US-EPA method of Static bioassay [11]. Five replicates of spherical glass aquaria each containing 5 L of TMWW, which changed every other day were used. Dechlorinated tap water was served as control. Each aquarium was supplied with ten fingerlings of the fish and continuously aereated to maintain sufficient dissolved oxygen for fish. Values of LT_{50} (time required to kill 50% of the tested fish) were calculated after daily record of cumulative mortality.

Hematological and Biochemical Procedures

All the hematological and biochemical procedures used in this study were as described by Al-Rajhi [12].

1. Animal treatments

Females albino mice, *Mus musculus*, one month old, weighing 20-25 gm were used as an experimental animal throughout the present study. They were fed on commercial standard diet. Mice were divided into control and TMWW treated groups of 20 mice each and given distilled water (control) or TMWW as drinking water, at one day up to ninety days of gestation.

2. Sample preparations

After 30, 60 and 90 days of mice gestation, three mice from each group were sacrificed by decapitation. The blood was collected from each animal in citrated tube and used freshly for delta-amino levulinic acid dehydratase (δ-ALAD) assay and

hematological studies. Animals were dissected, brains and livers were kept at 20°C for biochemical determinations.

a) *Whole blood*

The blood samples were collected from each animals in 5 c.c. citrated tubes containing 250 ul of 3.8% sodium citrate. The blood was mixed carefully to avoid the formation of foams, then used freshly for δ-ALAD assay. White blood cells (WBC), and red blood cells (RBC) counts, haemoglobin (HGB) concentrations, were estimated using Hemacomp 5 instrument.

b) *Brain and liver tissues*

Brains and livers were obtained from dissected animals, homogenized at 1:5 (W/V) in ice-cold, 0.1M Tris-HCl buffer, pH 7.0 using Tekmar homogenizer and centrifuged at 6000 rpm for 10 min. using Beckman L5-75 Ultracentrifuge type 40 rotor. Pellets were discareded and supernatants were recentrifuged at 17,000 rpm for 30 min. Resultant pellets were suspended in the same buffer and kept at -4°C until use.

3. **Enzymes assay**

Enzymes were determined in brain and liver tissues, while δ-ALAD activity was assayed the in the whole blood. AChE was assayed using the method of Ellman *et al.* [13]. ATPase activity was determined according to the method of Koch *et al.* [14]. The erythrocyte δ-ALAD activity was assayed according to the method of Joseph *et al.* [15]. Protein concentrations were determined by the method of Lowry *et al.* [16].

Results and Discussion

Toxicity of TMWW to *Tilapia niloticus*

Determination of LT_{50} for *Tilapia niloticus* exposed to TMWW were carried out in order to investigate the possibility of using TMWW for culturing the fish. Table 1 summerizes the physico-chemical parameters ot TMWW used in the experiments.

Data in Fig. 1 indicate that, there was an increase in the percent mortality of fish with increase in exposure time to TMWW. LT_{50} values revealed 22.3 and 24.1 days during two successive years of 1409 and 1410 H. These results proved that, the secondary TMWW of Riyadh region could not be considered suitable for fish culturing.

Effect of TMWW on blood parameters

The experiments were conducted to study the effect of TMWW on the white blood cells (WBC's), red blood cells (RBC's), hemoglobin (HGB), hematocrit

Table 1. The physico-chemical characteristics of TMWW used in fish experiments during 1409-1410 H

Character		Constituent range	
		Minimum	Maximum
Conductivity	μmho/cm	1372.5	1647
Total hardness	mg/l. $CaCO_3$	370	440
Chloride	mg/L	110	130
PH		7.37	7.80
Temperature	°C	16	20
Nitrites	mg/L	5.5	11
TDS	mg/L	960.8	1153

Each figure is the mean of 14 replicates during two years.

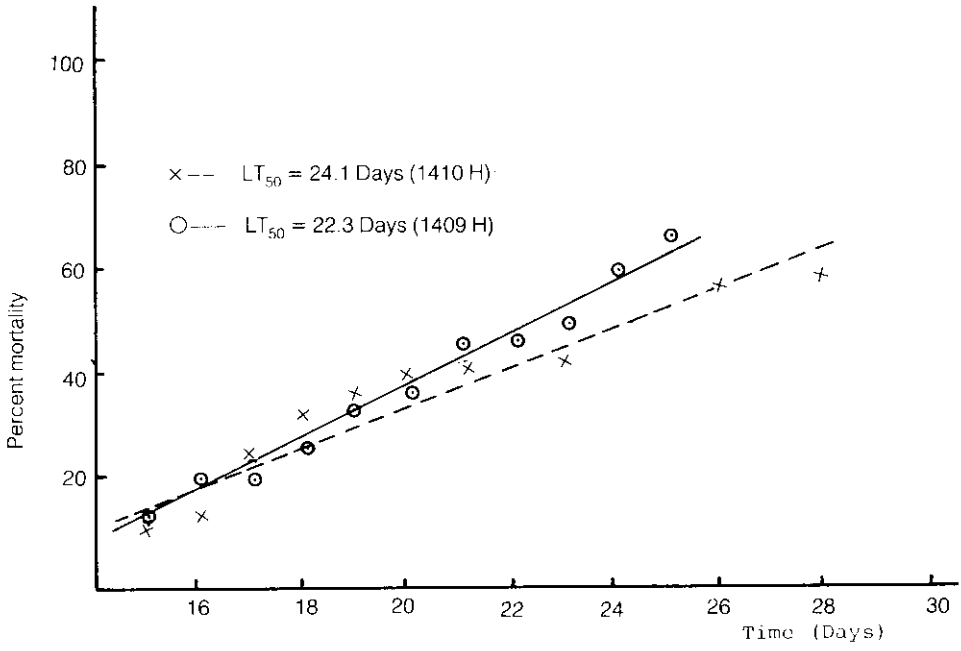

Fig. 1. Relationship between exposure time to TMWW and % mortality of fish.

(HCT), and mean cell volume (MCV) of albino mice blood. Data in (Table 2) show insignificant differences in the blood parameters between the control and the treated animals at any time of gestation up to 90 days. That is might be due to the minute amounts of contaminants present in the TMWW (unpublished data).

Table 2. *In Vivo* interaction of TMWW with albino mice blood parameters after 30, 60 and 90 days of gestastion

Parameter	Time elapsed after gestation (Days)					
	30		60		90	
	Control	Treated	Control	Treated	Control	Treated
WBD (10^3 Cell/C.C.)	10.37* ±2.32	9.40 ±2.22	6.88 ±1.44	5.07 ±1.10	5.83 ±1.37	5.23 ±3.23
RBC (10^6 Cell/C.C.)	6.79 ±0.68	7.18 ±0.03	7.38 ±0.23	7.46 ±0.44	7.74 ±1.05	7.51 ±0.35
HGB (Gm/dL)	11.57 ±1.10	12.27 ±0.21	15.15 ±1.18	15.48 ±0.87	11.23 ±1.64	11.20 ±0.80
HCT (%)	36.50 ±3.76	38.57 ±0.64	38.58 ±1.18	39.45 ±2.36	23.90 ±4.51	22.78 ±0.93
MCV (Micron^3/RBC)	53.67 ±1.15	53.67 ±1.15	52.59 ±1.29	53.00 ±1.41	31.67 ±1.54	30.25 ±0.95

Each figure is the mean of 4 replicates ± S.D.

Interactions of TMWW with δ-ALAD, ATPase, and AChE activities

Table 3 shows the activities of δ-ALAD, ATPase and AChE of mice brain and liver for the control and the treated groups after 30, 60 and 90 days of administration. Examination of the tabulated data indicated a significant activation of δ-ALAD in the treated groups. The activation value was 134.5% of the control after 30 days of TMWW administration. This activation was not observed after 60 and 90 days of treatment. The increase in δ-ALAD activity may be due to the presence of small amount of heavy metals in the TMWW that enhanced the synthesis of the enzyme. This finding was similar to that reported by Hiroyoshi *et al.* [17], who attributed the increase in δ-ALAD activity in the blood of rats to the stimulating effect of lead that animals treated with.

Table 3. Effect of TMWW on the activities of albino mice erythrocyte δ-ALAD, brain and liver ATP-ase after 30, 60 and 90 days of exposure

Time of exposure (days)	Treatment	δ-ALAD@	Specific activity of ATP-ase μ moles p_i /mg protein/hr.		AChE △ OD 412/mg protein/hr	
			Brain	Liver	Brain	Liver
30	control	20.69 ± 3.36	17.45 ± 1.60**	16.02 ± 2.97	13.63 ± 1.42	25.63 + 3.48
	treated	27.83 ± 1.68*	17.22 ± 3.69	15.50 ± 6.0	10.58 ± 2.00	18.68 + 6.74
60	control	54.99 ± 11.62	62.24 ± 4.60	6.53 ± 2.08	23.14 ± 1.86	44.34 + 7.33
	treated	48.99 ± 14.27	54.98 ± 7.53	11.43 ± 4.97	19.18 ± 2.21	38.45 + 8.81
90	control	84.33 ± 30.02	69.32 ± 1.59	11.54 ± 2.73	23.93 ± 2.80	44.11 + 2.39
	treated	75.84 ± 14.32	65.99 ± 9.10	10.46 ± 1.69	23.56 ± 2.04	38.07 + 4.09

**Each figure is the mean of 4 replicates ± S.D.

* Significantly different from the control by t-test at $P \leq 0.05$.

$$@ \text{ one enzyme unit} = 0D_{60} - 0D_0 \times \frac{100}{HCT} \times \frac{\text{Blood dilution}}{\text{Blood vol.} \times 60\,\text{min}} \times \frac{1}{0.036} \times 2$$

In general, the fish mortality data may enable us to say that the secondary TMWW of Riyadh region as it is could not be the suitable fish culturing water regardless to its positive effect on blood and enzyme systems of albino mice.

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سمية مياه الصرف الصحي المعالجة معالجة ثانوية على السمك، وتأثيراتها البيوكيماوية والهيماتولوجية على الجرذان البيضاء في منطقة الرياض

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ملخص البحث. قدرت سمية مياه الصرف الصحي المعالجة معالجة ثانوية لمدينة الرياض على سمك البلطي، وأظهرت النتائج أن الوقت اللازم لقتل ٥٠٪ من الأسماك المعرضة لهذه المياه تراوح بين ٢٢,٣ إلى ٢٤,١ يومًا. ودرس تأثير إعطاء هذه المياه إلى الجرذان كمياه للشرب لمدة ٩٠ يومًا على بعض الدلالات الدموية مثل عدد كرات الدم الحمراء والبيضاء، تركيز الهيموجلوبين، قيمة الهيماتوكريت ومتوسط حجم الخلايا. وتم دراسة تأثير هذه المياه على بعض الأنظمة للجرذان مثل إنزيم دلتا ـ أمينو حامض اللفيولينيك، الأدينوسين ثلاثي الفوسفات والأسيتايل كولين إستيريز. وقد أظهرت النتائج عدم وجود فروق معنوية بين الحيوانات المقارنة والمعاملة، ماعدا ازدياد نشاط إنزيم دلتا ـ أمينو حامض اللفيولينيك بعد ٣٠ يومًا من تعرض الحيوانات لهذه المياه.