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EFFECT OF INDUSTRIAL EFFLUENT (TRUST TEXTILE FACTORY) ON FINGERLINGS OF MARINE FISH, <u>MUGIL SEHELI</u>

BY

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Keyword: industrial effluent, textile, effect, nutrients, metals, toxicity, fish, histology, ovary, liver, gills, kidney.

ABSTRACT

The present study aimed to assessment the quality of effluent of Trust Textile Factory which discharges its effluent in the western side of the Suez Bay (northern part of the Gulf of Suez), and its effects on fingerlings of marine fish, <u>Mugil seheli</u>. Physicochemical conditions, nutrient salts and metals concentrations were determined in the effluent before and after mixing with seawater, high levels of most of the studied parameters were recorded. The LC_{50} of raw effluent to fingerlings of fish Mugil seheli were 13.3, 8.4 and 7.2 % for 24, 48 and 72 hours, respectively. Different concentrations of effluents had a damage effect on ovary, liver, gills and kidney. These effects were representing in loose of ovary wall, degenerations of oogonia and their divisions. Also some early and late perinucleolus oocytes become atretic. More hazardous effects were recorded for other organs of fish.

INTRODUCTION

In natural aquatic ecosystems, most of water constituents occur in low concentrations, normally at the nanogram to microgram per liter. Recently, a problem of increasing water constituents concentrations was developed. This situation originates as a result of the rapid growth of population, increased urbanization and expansion of industrial activities discharged effluents as well as the lack of implementing environmental regulations (Ride, 1997). Contamination of aquatic environment leads to deleterious effects from localized inputs, which may be acutely or chronically toxic to aquatic life within the affected area. Suez Bay is a small extension of the Gulf of Suez, where most of the industrial activities are presented in the western side of the bay. As well as a new industrial region is constructed at the same side of the bay. One of these industries is Trust Textile Factory, which discharges its effluent directly into the bay (Fig., 1), which consists mainly of

polyethylene fibers with an average industrial effluent amount of 240 m^3/hr . The untreated waste effluents of this type of industry results in several hazardous effects to the aquatic environment (Mahmoud, 2002). On the other hand, Suez Bay is characterized by high productive artisanal fishery and act as a nursery ground for common fishes especially the *Mugil seheli*.

The present work aims to study the physico-chemical characteristics including nutrient salts and metals concentrations in the effluent of Trust Textile factory. As well as its toxicity (LC_{50}) to fingerlings of the fish *Mugil seheli*, and its effects on histological characters of the ovary, liver, gill and kidney.





S1: raw effluent before mixing, S2: after mixing

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The present work aims to study the physico-chemical characteristics including nutrient salts and metals concentrations in the effluent of Trust Textile factory. As well as its toxicity (LC_{50}) to fingerlings of the fish *Mugil seheli*, and its effects on histological characters of the ovary, liver, gill and kidney.



Fig. 1: Map of Suez Bay showing the site of Trust textile factory and the area of its water effluent.

S1: raw effluent before mixing,

S2: after mixing

MATERIALS AND METHODS

1- Water effluent

Two water samples were collected from the waste effluents of "Trust Textile factory" before and after mixing with the Suez Bay water. pH and temperature were measured using portable digital Orion pH meter model 230A, salinity was measured using benchtop digital Orion Conductivity/Salinity/TDS meter model 150. Nutrient salts (phosphate, nitrite, nitrate and ammonia), oxidizable organic matter and total metal concentrations were measured according to APHA (1989).

2- Toxicity test

Fingerlings of *Mugil seheli* were collected during summer 2001 from the western side of coastal area of the Suez Bay, transported to the wet lab, and acclimatized to the laboratory conditions for one week. Toxicity test was carried out in glass tanks to determine the 24, 48 and 72 h LC_{50} "as a percentage of the raw effluent". Batches of ten fish were picked from the stock tank and placed in a series of tanks containing different concentrations (up to 70 %) of raw effluent including a control. Observations for mortality were made four times daily at regular intervals, and the dead fish were counted and removed. The test was terminated after 72h. The percentage mortality in each concentration was corrected for control mortality using Abbott's formula (Finney, 1971) as follows:

C = O - X/100 - X

where, C is the corrected mortality,

O is percentage of observed mortality, and

X is percentage of the control animals which have died at the relevant observation time.

 LC_{50s} of raw effluent were estimated by probit methods (Finney, 1971) using graphical analysis.

3- Histology

Fish samples were collected during the period of toxicity test. Ovary, liver, gills and kidney were fixed in Bouin's solution. The specimens were dehydrated, cleared and embedded in paraffin wax, then sectioned at 5 μ m. the sections were stained with Ehrlich haematoxylin and Eosin.

RESULTS AND DISCUSSION

1- Water effluent

Table (1) shows the average concentrations of the different parameters analyzed in water samples of Trust Textile factory comparing to previous study of the Suez Bay and Gulf of Suez. The temperature of raw water was 39.0 °C, it was higher than that reported for Misr-Iran textile factory, 29.0 °C (Mahmoud, 2002). The pH value of raw water (11.20) was close to that found by Mahmoud (2002) which was 11.60 for Misr-Iran textile factory. While at the mixing area (9.03), it was higher than that recorded by Amer, 1994 (8.14) and Belal, 1995 (7.34 - 8.66). Salinity in the raw effluent was relatively higher than the range of fresh water (2.3 %). This value leads to decrease the salinity at the mixing area to 37 %. which is lower than those found by Abd El-Rahman, 1993 (42.73 ‰) and Amer, 1994 (41 ‰). Although, the value of oxidizable organic matter (40.32 mg/L) in raw effluent was higher than recorded by Amer. 1994 (4.21 mg/L), it showed a very low concentration than those recorded for Misr-Iran textile factory, 339 mg/L (Mahmoud, 2002). This result may be attributed to that Trust textile industry based on polyethylene fibers, while Misr-Iran textile industry depends on cotton materials. The concentration of phosphate, nitrate and ammonia were 3.44, 5.49, 9.04 and 23.64 μ M in the raw effluent, which was reduced to 1.41, 1.76, 2.69 and 14.07 uM at the mixing area, respectively. The level of metals were 7.29, 31.77, 27.39, 136.52, 16.18, 14.91, 25.17, 27.17 and 618.92 ppb for Cd. Pb. Cu. Zn. Cr. Co. Ni, Mn and Fe, respectively. These values were reduced to about 10% of its original concentrations as a result of mixing with the water of Suez Bay. Except Cu, Ni and Mn; levels of other metals and nutrient salts at the mixed area were higher than that recorded by Amer (1994) and Hamed and El-Moselhy (2000) for the Suez Bay and Gulf of Suez water.

During the toxicity test, the chemical and physical parameters were analyzed in the different tanks concentration (Table 2). It was found that there was a gradual increase in the concentrations of phosphate, nitrite and nitrate with increasing the concentration of waste effluent in the experimental tanks. While this sequence was only reversed with any anamonia due to its change to unionized-anymonia. This reaction may occur due to the presence of high pH value and the alkaline sodium hydroxide. Also it was noticed that the concentrations between 20% and 30% were nearly to that found at the mixing area. Also the pH values in the different concentrations (ranging from 8.44 to 8.95) were higher than that of normal seawater.





3- Histological studies

A. ovary

Control ovary

Ovary of fingerlings of *Mugil seheli* consists of a very thin two thread structure under the elementary canal. In histological section, each one is enclosed in a fibrous connective tissue tunica albuginea. The lumina surface of the tunica albuginea folds into ovigerous lamellae, inside it there are three types of oocytes. The first one is oogonia which found in groups or solitary lining the ovigerous lamellae, and their divisions are numerous. The second and third are early perinucleolus and late perinucleolus oocytes, their cytoplasm are stained with pale purple colour (Fig. 3).

Treated ovary

Ovaries of fingerlings exposed to different concentrations of effluent showed different histological forms as shown in Fig. (4, 5 and 6). It can observe that, the tunica albuginea is loose, the oogonia are destroyed while disappeared in exposure to high concentrations. The divisions of oogonia are not found. The cytoplasm of early and late perinucleolus oocytes is stained deep purple. Also, the early perinucleolus oocytes appeared as a solid mass, while, the late perinucleolus oocytes become atretic. These lesions may be attributed to the direct and/or indirect effect of high value of pH and high concentrations of nutrients and heavy metals in the effluent. Kumar and Pant (1984) found that 2- to 4- month exposures of an Indian teleost to copper, zinc, or lead caused disappearance of oocytes in the ovaries. Murugesan and Haaniffa (1992) recorded a serious effect of paper-mill and textile-mill on gonadotropin of *Heteropneustes fossilis*, which affects the process of gonad recrudescence.

B-Liver

Control liver

Histological sections of control liver of *Mugil seheli* consist of plates of two or three hepatocytes, and blood sinusoids are found between these plates. The hepatocytes contain a central nucleus, which contains one nucleolus. A very few numbers of vacuoles are found in hepatocytes (Fig. 7).

Treated liver

The results of the present study showed that, the gradual increase in the concentrations of the effluent lead to increase in the effect on liver. Fig. (8) shows that, in 10 % and 20 % concentrations of the raw effluent, the spaces between plates of

Parameter	Raw industrial effluent	Mixed water	Suez bay and Gulf of Suez *		
<u>Physical and chemical</u> parameters:					
Temperature (°C)	39	30	28.8		
pH	11.20	9.03	8.14		
Salinity (%0)	2.3	37	41		
00M (mg/l) **	40.32	46.44	4.21		
Phosphate (µM)	3.44	1.41	0.72		
Nitrite (µM)	5.49	1.76	0.45		
Nitrate (µM)	9.04	2.69	1.04		
Ammonia (µM)	23.64	14.07	11.71		
Trace metals (ppb):					
Cd	7.29	0.82	0.662		
Рb	31.77	2.89	1.350		
Cu	27.39	2.98	5.600		
Zn	136.52	16.9	15.46		
Cr	16.18	1.55	0.628		
Co	14.91	1.2	0.568		
Ni	25.17	2	4.650		
Mn	27.17	3.61	5.810		
Fe	618.92	98.15	16.440		

Table 1: Average concentrations of different parameters in the effluent of Trust Textile factory

* cited from Amer (1994); Hamed and El-Moselhy (2000)

****** OOM: Oxidizable organic matter

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Concentration Parameter	Control tank	10%	20%	30%	40%	50%	60%	70%
pH	8.23	8.44	8.46	8.46	8.49	8.7	8.86	8.95
Phosphate (µM)	0.80	0.33	0.58	1.41	1.80	3.49	6.14	8.23
Nitrite (µM)	0.73	1.06	1.51	1.98	2.80	5.42	8.33	11.28
Nitrate (µM)	1.62	2.24	2.88	3.36	5.86	7.85	11.82	13.71
Ammonia (µM)	13.46	11.43	10.86	9.90	9.50	9,79	9.00	8.00
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Table 2: Levels of pH and nutrient salts in different tanks of the toxicity test.

2-Toxicity test

The toxicity tests are necessary in water pollution evaluation because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota (Tarzwell, 1971). Fig. (2) shows the trend of the effect of different concentrations (as % v/v, raw effluent/seawater) of raw effluent of Trust textile factory on the mortality rate of the fingerlings of Mugil seheli during 24, 48 and 72 hours of the experiment time. The obtained data for LC_{50} of effluent show decreasing pattern with increasing the exposure time, and its value were 13.3, 8.4 and 7.2 % for 24, 48 and 72 hours, respectively. Estimating the correlation coefficient between LC_{50} and exposure times gave a negative relationship (r = -0.944), this inverse relationship results from decreasing the tolerance of fingerlings of fish with the time of exposure. Mahmoud (2002) reported that the observed 96 h LC_{50} for Misr-Iran of textile raw effluent to mullet fingerlings was 118.0 ml/l (11.8 %), and attributed the differences in the mortality between high and low concentrations to the pH values. Experimental studies on the effect of alkaline solution at high pH have indicated that fish damage generally begins at pH 9.0 (Hartwell et al., 1986). Jordan and Lioyed (1964) reported fish mortality at levels of pH up to 10 and they attributed it to pH and salinity. Tabat (1962) concluded that free ammonia (NH_3) was significantly more toxic to fish than ammonium ion (NH4), and stated that the great increase in toxicity of free ammonia was due to increasing pH value. Smart (1975), Bower and Bidwell (1978) and FAO (1993) reported that pH and free ammonia had a complex toxic effect on fish.





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hypatocytes are increased with the presence of white area. The nucleus is eccentric and the thickness of cytoplasm in some hepatocytes decreased. In some areas there is destroyed hepatocytes with pyknotic nucleus. In 30 %, 40 % and 50 % the extent of damage increased, as there is a destruction of great area of hepatocytes, and the number of cells with pyknotic cells increased (Fig. 9). While in 60 % and 70 % the destruction increased more, such as increasing the area between plates of hepatocyte and decreasing the size of hepatocytes (Fig. 10). These observations are in agreement with those of Mahmoud (2002), who found that textile effluent had a damaging effect on liver tissue of *Mugil seheli*.

C- gills

Control gills

Fig. (11) shows the structure of control gills of *Mugil seheli*. It consists of primary lamellae project from the posterior edge of gill arch and ended by a marginal blood channel, and secondary lamellae which originate from primary lamellae. A thin epithelial covering of the secondary lamellae lies on a basement membrane supported by pillar cells, which separated by spaces called lacunae.

Treated gills

The gills of fish exposed to 10 %, 20 % and 30 % concentrations of effluent showed different abnormalities as shown in Fig. (12). The marginal blood channel is congested. The lamellar epithelium cells are hypertrophy and lifting the secondary lamellae. The destruction of secondary lamellae and a beginning of lamellar fusion is observed in fish exposed to 40 % (Fig. 13). Hyperplasia of lamellar epithelium and increasing in lamellar fusion are found at concentrations 60 % and 70 % (Fig. 14). The previous abnormalities are in agreement with that recorded by Mahmoud (1994 and 2002). According to Malatt (1985), lifting of epithelia could be serve as a mechanism of defense, because the separation epithelial of the secondary lamellae increases the distance across where water-borne irritants must diffuse to reach the blood stream.

D- kidney

Control kidney

Fig. (15) shows the structure of control kidney tubules, which is formed of lumen surrounded by columnar epithelial cells with apical nucleus.

Treated kidney

Many abnormalities occurred in the kidney of *Mugil seheli* fingerlings as affected with the different concentrations of effluents. Granulated cytoplasm of columnar cells, separation of these cells from the basement membrane are found in 10 % and 20 % exposure (Fig. 16). More degeneration of collecting tubules occurred in 30 %, 40 % and 50 % concentrations (Fig. 17). In 60 % and 70 % the degeneration increased and there were completely destructed collecting tubules (Fig. 18). These observations are in agreement with those of Mahmoud (1994) who revealed widespread necrotic alterations in the proximal segments and explained it due to the presence of nitrogenous compounds in the sewage water. While Sedrak (1992) reported a reduction in haemopoeitic tissue and lyses tubular epithelial cells.

CONCLUSION

From the obtained data in the present study, it can be stated that, the treatment of the Trust textile factory effluent is insufficient. Whereas, it has a high toxic effect on the marine fish, *Mugil seheli*, as well as damaging effects on its organs (ovary, liver, gills and kidney). Also, it was found that, the allowable limits of some discharged water parameters on the marine environment according to the environment law No. 4/1994 are insufficient to save the aquatic marine life.

It is recommended that, there is a dire need to increase the efforts of inspections for the industries. Also it is necessary to modify the allowable limits for discharging wastewater into the marine environment.



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LIST OF FIGURES

- Fig. 3. T. S. of control ovary. 1- ovigerous lamellae; 2- tunica albuginea; 3- oogonia; 4division of oogonia; 5- early perinucleolus oocyte; 6- late perinucleolus oocyte. (H & E, X100)
- Fig. 4. T. S. of treated ovary. 1- loose ovigerous lamellae; 2- loose tunica albuginea; 3atretic oocytes. (H & E, X100)
- Fig. 5. T. S. of treated ovary. 1- thin layer of ovigerous lamellae; 2- destroyed oogonia; 3- solid mass of oocytes. (H & E, X100)
- Fig. 6. T. S. of treated ovary. 1- loose ovigerous lamellae; 2- loose tunica albuginea; 3all oocytes as solid mass and disappeared of normal cells. (H & E, X100)
- Fig. 7. Section of control liver. 1- cords of hepatocytes; 2- sinusoids containing red blood cells. (H & E, X250)
- Fig. 8. Section of treated liver represents the effect of 10% & 20% concentrations of effluent. 1- white area between cords of hepatocytes; 2- necrotic hepatocytes. (H & E, X250)
- Fig. 9. Section of treated liver represents the effect of 30%, 40% & 50% concentrations of effluent . 1- hepatocytes with pyknotic nucleus and absence of cytoplasm. (H & E, X250)
- Fig. 10. Section of treated liver represents the effect of 60% & 70% concentrations of effluent . 1- white area between hepatocytes; 2- destroyed hepatocytes. (H & E, X250)
- Fig. 11. Sagital section of control Gill filament. 1- primary lamella; 2- secondary lamella; 3- epithelial cell; 4- pillar cell; 5- lacuna (capillary lumen); 6erythrocyte within capillary lumen; 7- chloride cell; 8- marginal blood channel. (H & E, X50)

- Fig. 12. Sagital section of treated gill filament represents the effect of 10%, 20% and 30% concentrations of effluent. 1- primary lamella; 2- secondary lamella; 3hypertrophy epithelial cells; 4- lamellar sinus constricts. 5- epithelial lifting; vascular congestion. (H & E, X100)
- Fig. 13. Sagital section of treated gill filament represents the effect of 40% and 50% concentrations of effluent. 1- primary lamella; 2- destruction of secondary lamella; 3- hypertrophy epithelial cells; 4- lamellar sinus constricts; 5- epithelial lifting; 6- beginning of lamellar fusion.; 7- vascular congestion. (H & E, X100).
- Fig. 14. Sagital section of treated gill filament represents the effect of 60% and 70% concentrations of effluent. 1- primary lamella; 2- destruction of secondary lamella; 3- hypertrophy epithelial cells; 4- lamellar sinus constricts; 5- epithelial lifting; 6- lamellar fusion.; 7- chloride cell proliferation. (H & E, X100).
- Fig. 15. T. S. of control kidney tubules. 1- columnar epithelial cells; 2- basement membrane. 3- red blood cells. (H & E, X250).
- Fig. 16. T. S. of treated kidney tubules represents the effect of 10% & 20% concentrations effluent. 1- separation of columnar epithelial cells from the basement membrane. 2- destroyed of some tubules. (H & E, X250).
- Fig. 17. T. S. of treated kidney tubules represents the effect of 30%, 40% & 50% concentrations effluent. 1- separation of columnar epithelial cells of the basement membrane. 2- vacuolization in the columnar epithelial cells of the tubules; 3- the destroyed of some tubules with presence of nucleus only. (H & E, X250).
- Fig. 18. T. S. of treated kidney tubules represents the effect of 60% & 70% concentrations effluent. 1- separation of columnar epithelial cells of the basement membrane. 2- vacuolization in the columnar epithelial cells of the tubules; 3- completely destroyed of some tubules. (H & E, X250).

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