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PATHOLOGICAL CHANGES IN DIFFERENT SPECIES AND EMBRYOLOGICAL STAGES OF FISH INDUCED BY UREA - FERTILIZER WASTEWATER.

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ABSTRACT

The raw waste of H A C or L A C is highly toxic. Raw effluent with H A C has L C_{50} values in the range of 1.7 - 1.5 % vol. / vol. while for L A C ranging from 7.6 - 9.2 % vol. / vol.

Ammonia at very low concentration down to 0.04 mg/L in presence of traces of Cr, Pb, Cu, and Zn caused hypertrophy of gill filament and hyperplasia of the epithelial surface of respiratory lamellae for fingerlings of **Sparus aurata** with respect to fry **Mugil capito** gill filament and lammeller organization were distorted by severe edema and separation of respiratory epithelium.

Liver was also affected at the same tested concentration of 4 % vol. / vol. of waste as indicated by abnormal accumulation of lipid in **Sparus aurata** ranging from moderate to severe. **Mugil capito** small intestine show a forthy vacuolated mucosal epithelium and loss brush border. Treatment of this effluent eliminated this effect.

INTRODUCTION

Agriculture is one of the basic pillars of the Egyptian economy. There is always continuous efforts to increase the productivity of agricultural land through the use of natural and man made fertilizers. Abu-Kir fertilizers plant is one of the leading plants for production of urea-fertilizers. This plant utilizes the natural gas produced at Abu-Kir Bay off-shore field in the production of urea-fertilizer. Liquid ammonia is an intermediate chemical to be produced for the final processing of urea through its conversion to ammonium carbonate and finally to urea and water in the urea reactor. This final reaction is highly exothermic and the urea formation is never a 100% conversion process. WAter is added to maintain the proper temperature in the reactor and to dissolve all unreacted CO_2 as carbonates.

Urea processing utilizes excessive amounts of water for cooling purposes. Cooling water withdrawn from Rakta canal is subjected to extensive treatment before use as process and cooling water. Cooling water is partially recycled and its overflow is discharged to Abu-Kir Bay with its luden of ammonia and urea due to some operational troubles and leakage from the processing reactors.

Fish toxicity assays were carried to determine the toxicity of final effluent on **Sparus aurata, Cyprinus carpio, Mugil capito,** and **Mugil cephalus.** Eary life stages of fish were used to provide good estimate of the chronic toxicity of raw waste. Serial dilutions were tested to show the effect of waste when diluted with the bay water on the living fish due to the importance of Abu-Kir Bay for Egypt as a marine fishing ground.

Gill hyperplasia is one of the acute effects on fish due to ammonia irretation as indicated by Nystrom and Post (1982).

Alternaion in liver glycogen and blood glucose concentration as shown by Mcleay (1973), Mcleay and Brown (1975) as well as decrease in ventilatory water volume and oxygen uptake (Davis, 1973), are all acute effects that can be grouped under the concept of general stress syndrome. Behavioural changes also were noticed during the course of this study.

Impaired liver function in the studied species of fish exposed to the final effluent of Abu-Kir fertilizer plant were observed in an effort to develop more specific bioassay for detecting and monitoring the sublethal impacts of this waste.

MATERIALS AND METHODS

Bioassays were conducted with hatched embryo, larvae, fry of **Cyprinus carpio, Mugil capito, Mugil cephalus** and fingerlings of **Sparus aurata.**

Cyprinus **carpio** fry were obtained by induced spawning. Adult females adapted to laboratory condition were injected intermuscularly with 3000 IU of human chorionic gonadotropin hormone to insure egg maturation. Fertilization was affected by hand stripping the female and mixing milt from infected males by using the dry method. After mixing the sexual product the brackish water was added and the fertilized eggs were placed in groups in petri dishes. Hatched embryos (2 days old) larvae (7 days old) and fry (1 month old) were tested to determine the toxicity of Abu-Kir fertilizer raw waste to the previous stage and fry **Mugil capito, Mugil cephalus** and fingerlings **Sparus aurata.**

Fry **Mugil capito** and **Mugil cephalus** were collected from El-Max fish farm, fingerlings **Sparus aurata** were collected from El-Ratama drain. These fry were acclimated to laboratory condition for one week before exposure. Developmental stages were selected for testing because they are often more sensitive than adult stages to toxic substance and their perish affect the fish productivity.

Acute 96-h static toxicity tests were conducted in accordance with the Standard Methods (APHA, 1978). Toxicity was determined by exposing 10 larvae or fry per aquarium of eight liter capacity. Different concentrations (0, 3, 125, 12.5, and 25%) vol/vol of raw waste were tested. Each experiment was repeated 10 times to determine the average chlorides, DO, pH and ammonia concentrations of each tested dilution. Results are summarized in table (1).

TABLE (1)

DO, Chloride, pH and Ammonia Content of Different Waste Dilutions

Parameter	25	12.5	6.25	3.125	1.65	D.T.W.
Average D.O	I 6.9	8.0	8.2	8.5	8.6	8.7
	F 6.0	7.7	7.5	8.0	8.3	8.4
Average chloride mg/l	S 350	255	115	82	70	50
pH	I 9.1-7.8	8.8-7.7	8.4-7.6	8.2-7.3	8.0-7.3	7.2
	F 9.0-7.6	8.6-7.5	8.3-7.3	8.0-7.3	8.0-7.3	7.2
Ammonia	H 215-250	118-140	52-69		23-30	12-14
mg/l	L 1-2.5	0.5-1.2 /	0.25-0.7	0.1-0.3	0.05-0.15	

N.B.

D.T.W. = Dechlorinated Tap Water.
I = Start of Experiment.
F = End of Experiment.
H = High Ammonia Content. (465-830 mg/l). (Hamza, 1983).
L = Low Ammonia Content. (3-8 mg/l).

In case of **Sparus aurata** sea water was used for preparing the serial dilutions. The different raw waste effluents were characterized as high and low ammonia waste based on their chemical analysis. The LC_{50} for each tested stage of the different species were determined.

To determine the cause of mortality and pathological changes inducing it, the LC_{50} and lower dilutions were prepared and fry **Mugil capito** and fingerlings of **Sparus aurata** were exposed to them. Concentrations tested were 2 and 4 % vol/vol of raw waste charcterized by low ammonia concentration.

After 96 hours, the tested organisms were fixed in Davidson's fixative for 24 hours, then transferred to 70% ethyl alchohol, and processed for sectioning for pathalogical examination. Sections were stained with Mallory's stain. Tissue and organs studied histologically were: gills, liver (for **Sparus aurata**) gill, liver and digestive system for fry of **Mugil capito**. This was repeated on the air stripped waste as suggested treatment (Hamza, 1983). Stripping was carried out for 4 hours in case of low ammonia content cooling tower effluent while the time was extended for 24 hours in case of high ammonia concentration effluent. The effect of treatment was evaluated on mortality and pathological changes.

RESULTS AND DISCUSSION

The raw combined effluent is characterized by fluctuation in a wide range (for ammonia, it varied from 68-613 mg/l). The total dissolved solid content also varied between 855-2878 mg/l with a relatively high content of suspended solids from 86 to 105 mg/l. The organic load of the waste is relatively low. As a result, the D.O. content is always present with an average of 6.4 mg/l. Regarding the discharge of this waste to the sea the only limit will be its harmful effect on biological species living in the receiving bay water.

The raw waste average content of heavy metals was Cr 62 µg/l, Pb 33 µg/l, Cu 456 µg/l, Ni 147 µg/l, Cd 8.3 µg/l, Zn 547 µg/l and Fe 803 µg/l (Hamza, 1983). Although the present concentrations of each metal is low and they will be further reduced by dilution in the bay water, yet their accumulative effect on the sea funa and flora cannot be neglected on the long run.

Abu-Kir fertilizer raw waste induced highly toxic effects on **Cyprinus capito** different developmental stages as well as hatched embryo (2 days old), larval stage (7 dayes old) and fry (2 months old).

The toxicity of the waste at 25% vol/vol of (LAC) low ammonia concentration effluent was markedly different in hatched embryo and larval stage of **Cyprinus capito**. The hatched embryos were highly affected by this concentration. Increasing dilution of the effluent, this effect tends to diminish as shown in Fig. (1) which demonstrates the decrease in the LT_{50} by increasing concentration of the waste.

Fry of **Cyprinus carpio** were more resistant than the hatched embryo and larval stage when exposed to serial dilution of LAC effluent since there were no observed mortality as shown in Table (2).

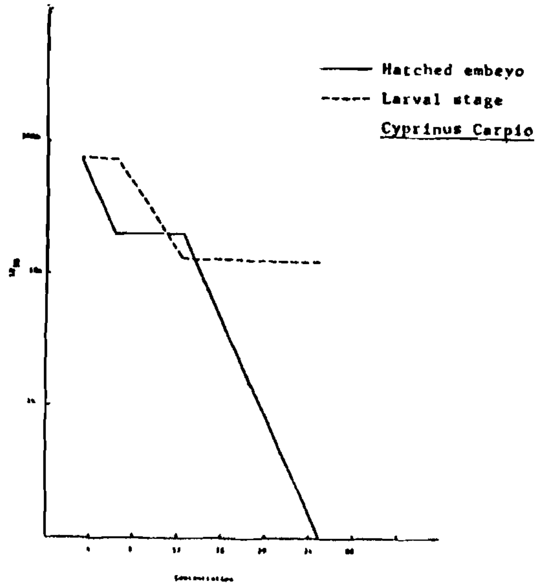

Fig. (1)
Effect of diluting low ammonia effluent on LT_{50} values in **Cyprinus carpio** developmental stages.

TABLE (2)

THE LC_{50} VALUES OF RAW AND TREATED EFFLUENT IN DIFFERENT DEVELOPMENTAL STAGES OF TESTED SPECIES.

Species	Stages	Age	Length	96h LC_{50} raw waste vol/vol		96h LC_{50} after treatment % vol/vol of waste	
				HAC* 830-465 mg/l	LAC* 10-4 mg/l	HAC 3-8 mg/l	LAC 0.1-0.3 mg/l
<u>Cyprinus carpio</u>	Hatched	2 days	0.5-0.5 cm		5.25		
	Larval stage	7 days		190	6.25	10 %	20 %
	Fry	1 month	3 -4.5 cm				
<u>Mugil capito</u>	Fry	About 1.5 month	2 -3 cm	1.1	8.05	10 %	23.0
<u>Mugil cephalus</u>	Fry	About 1 month	1.9-2.5 cm	1.5	9.2	14.2	20.0
<u>Sparus aurata</u>	Fingerlings	About 4 month	7.2-9 cm	1.5	7.8	12.5	22.5

Note : Fish toxicity will be rated : high when LC_{50} 9-20 % vol/vol waste.
moderate when LC_{50} varies from 20-75 % vol/vol waste.
low when LC_{50} varies from 75-100 % vol/vol waste.

*HAC : High Ammonia Concentration.

*LAC : Low Ammonia Concentration.

The 96 hours L C_{50} value to hatched embryo and larval stages of Cyprinus **carpio** were very low (6.25% vol/vol) indicating high toxicity effluent. With respect to fry **Cyprinus carpio** the LAC waste was moderately toxic.

The second experiment was conducted on the fry of **Mugil cephalus, Mugil capito** and fingerlings **Sparus aurata** using raw waste of high ammonia concentration (HAC) (465 - 830 mg/l). The L T_{50} for **Mugil cephalus** was 0.08 hour at 25% concentration and 0.4 hour at 3.125%. The same result was observed in case of **Sparus aurita** fingerlings and **Mugil capito** fry.

In case of LAC waste, **Sparus aurita** fingerlings were more tolerent than fry of **Mugil capito** at high concentration (25%) of LAC raw effluent, Fig. (2).

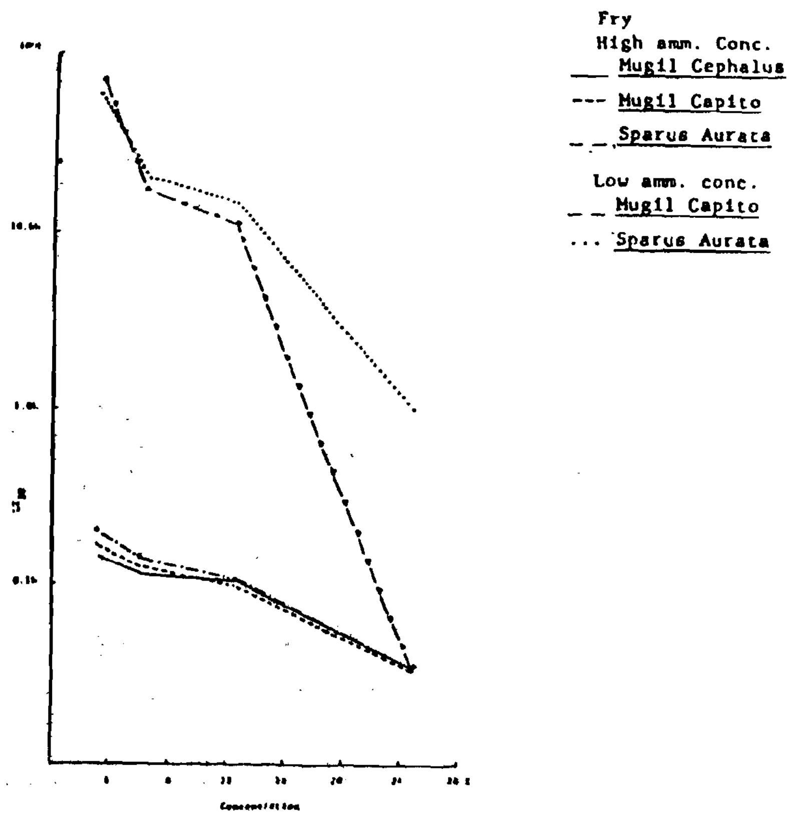

Fig. (2)
Effect of dilution of LAC and HAC on LT_{50} values
of **M. cephalus, M. capito and S. aurata.**

The L C_{50} value of Abu-Kir fertilizer raw effluent of LAC with respect to **Mugil capito** was 8.85% vol/vol. and 9.2% vol/vol. for **Mugil cephalus** and 7.6% vol/vol. for **Sparus aurata**. The L C_{50} values for raw effluent of HAC ranged from 1.1% to 1.5% vol/vol. for **M. capito** and **Sparus aurata**, respectively as shown in Table (2).

These low values of L c_{50} indicate high toxicity to the tested species according to IERI - RTP manual (1980).

The tested organisms when exposed to the different serial dilutions in the range of 3.12% to 25% vol/vol. of waste showed a rapid increase in ventilation rate. The increase was evident in both opercular and mouth movement and greater lifting of the hypomandibular and operculum and mouth. Fish usually swam disoriently at the surface of the water in the aquaria for a while before dying.

Thurston (1978) found that the LC_{50} value for the cutthroat were 0.5 to 0.8 mg/L unionized ammonia and for 36 days were 0.3 to 0.5 mg ammonia/L. Additionally, histological examination of fish exposed for 29 days to 0.34 mg ammonia/L showed degradative change in gills, kidney and liver tissue.

Vamos et al. (1974) reported that free ammonia was more toxic to fish in summer when oxygen concentration were low.

Spehar (1978) reported that the maximum accetaple toxicant concentration (MATC) for Cd between 4.1 and 8.1 µg/l and the MATC value for Zn were between 75 and 139 µg/l. Benoit (1975) investigated the chronic effect of Cu on survival, growth and reproduction of the blue gill in soft water. It was reported that concentration of 40 to 162 µg/l caused significant quantities of Cu to accumulate in gill and liver and adversely affected larval survival after 90 days. Although the waste dilutions contained lower concentrations of heavy metals than the MATC of each metal, yet their presence in combination with the relatively high ammonia content can not be neglected.

Histopathology:

Microscopic Examination:

A- Gills:

The gills are among the most delicate structures of the teleost body. Their vulnerability is thus considerable because their external location and necessarily intimate contact with the water means that they are liable to damage by any irritant whether dissolved or suspended in the water. External irritants are the most frequent causes of significant gill pathological changes.

The pseudobranchepithelium **Mugil capito** and **Sparus aurata** in normal case consists of plump, easinophilic cells that surround lamellar capillaries, (Figs. 3 and 4), respectively.

Mugil capito fry exposed to 4% LAC waste of 0.4 mg/l ammonia for 24 hours and sacrificed while still alive. Gill spot showed pseudobranch lamellar epithellial membrane distorted and separated from other gill tissue, (Fig. 5).

Exposing **Mugil capito** for raw waste dilution of 4% at ammonia concentration (0.4 mg/l) severe gill damage were observed after 4 days of exposure, (Fig. 6). Gill filament and lamellae organization were distorted by severe edema. Complete separation of squamous respiratory epithelium and capillary tissues in the gill lamellae occured.

Fig. (3)
Section of gill from normal control Sparus aurata, note the pseudobranch epithelium consist of plump, easinophilic cells surrounding lamellar capillaries.

Fig. (4)
Section of gill from normal control **Mugil capito**.

Fig. (5)
Pseudobranch alternation observed in fry spot **M. capito** exposed to 4 µg/l ammonia for 24 h. and sacrificed alive. Lamellar epithelial membranes are distorted and separated from other pseudobranch tissues.

Fig. (6)
Gill filament and lamellae from fru M. capito spot exposed to 4 µg/l ammonia for 96 h. and sacrificed alive. Filament and lamellae organization are distorted by severe edema and separation of respiratory epithelium.

Microscopic examination of **Sparus aurata** gills revealed hypertrophy of gill filaments and hyperplasia of the epithelial surface of respiratory lamellae and interlamellar filament epithelium. Necrotic and sloughed respiratory epithelium were also observed after 96 hours exposure at concentration of 4% vol/vol of defined low ammonic concentration effluent as shown in Fig. (7). This is in agreement with Nystrom and Post (1982) findings with respect to damage caused by ammonia.

Air stripped waste initially with defined HAC were highly toxic to fish even at 16% vol/vol of waste as their LC_{50} values were lower than 29% vol/vol of waste as shown in Table (2). Smart(1978)reported that gill damage resulting from acute exposure to 0.5 - 0.65 mg/l ammonia was relatively minor and was not thought to be the primary cause of death. He observed histopathological changes in gill structure when rainbow trout (**Salino gairdneri**) were exposed to 0.25 - 0.3 mg/l ammonia for longer than 14 days. This severe gill damage was observed in case of **Sparus** aurata and **Mugil capito** after 4 days of exposure to a lower concentration of ammonia 0.4 mg/l in either the LAC raw effluent or treated HAC effluent. This indicate a synergistic effect from suspended solids and trace metals in the waste.

Fig. (7)
Section of gill from **Sparus aurata** exposed to 4% LAC vol/vol which contains 4 μg/l ammonia for 96 h. and sacrificed alive. Hypertrophy of gill filaments and hyperplasia of the epithelial surface of respiratory lamellae and interlamellar filament epithelium.

B- Liver:

Microscopic examination of **Sparus aurata** liver sacrificed after exposure to LAC raw effluent concentration of 4% vol/vol for 96 hours revealed the extensive vacuolation of liver parenchyma probably due to abnormal accumulation of lipid and individual hepatocysts. This means alternation of liver from moderate to severe fatty accumulation, (Fig. 8).

No pathological effect was observed when examining liver of **Mugil capito** exposed to the 4% waste dilution.

C- Small Intstin:

Pathological examination of **Mugil capito** small intestin sections exposed to 4% vol/vol raw waste of LAC for 96 hours revealed forthy vacuolated mucosal epithelium and loss of bursh border. Raw waste caused degeneration of tips of villi of intestinal epithelium. These changes were not found in control fish nor in treated LAC low effluent, (Fig. 10).

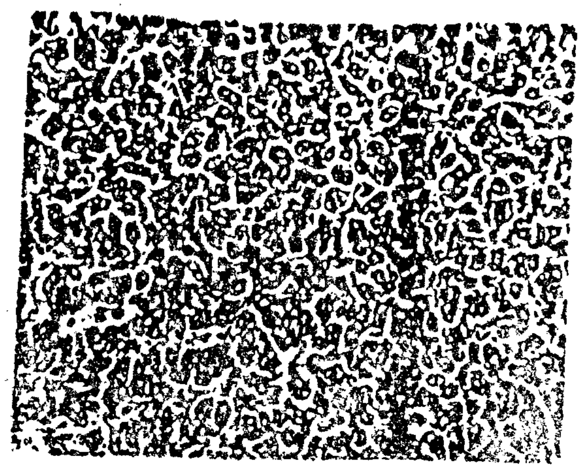

Fig. (8)
Section from normal liver of *Sparus aurata* from control aquaria.

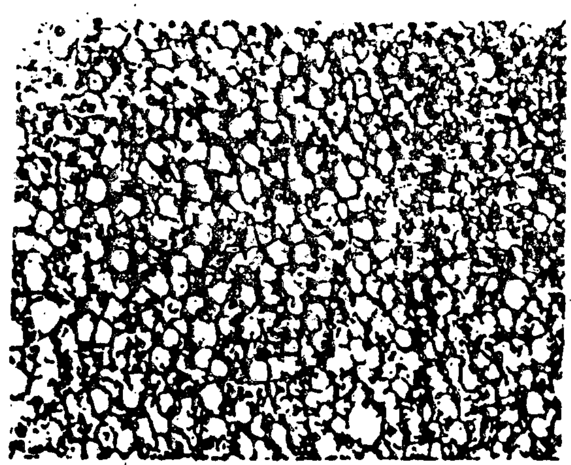

Fig. (9)
Section from liver of *Sparus aurata* exposed to LAC which contains 4 µg/l ammonia. Note extensive vacuolation of liver parenchyma probably due to abnormal accumulation of lipid.

Fig. (10)
Section of small intestin from *M. capito* exposed to 45 LAC contained 4 µg/l ammonia for 96 h. and sacrificed alive. Note degeneration of tips of villi of intestinal epithelium.

CONCLUSIONS

The raw waste either of HAC or LAC is highly toxic. The leathal time for 50% of the tested organisms was within 3 - 5 minute of exposure depending on the range of ammonia present.

Raw effluent with HAC has LC_{50} values in the range of 1.7 - 1.5 % vol/vol of waste. This means if this waste to be discharged to a body of water dilution for at least 100 times by volume should be provided to eliminate toxic effects on aquatic organisms.

Treatment of such waste by air stripping for 24 hours for HAC was able to reduce its ammonia content to a range of 3 - 8 mg/l. Dilution of this treated effluent is still needed to overcome the residual toxicity of the effluent. Dilution for at least 25 times the discharged volume will be mandatory to overcome any possible toxic and or pathological damage to the living types of tested fish.

Effluent with LAC had LC_{50} values ranging from 7.6 - 9.2 % vol/vol indicating high toxicity effluent.

Air stripping for 4 hours for LAC was able to reduce the final effluent content to 0.1 - 0.3 mg/l and raise its LC_{50} value to 20 - 23% vol/vol of waste and by that the treated LAC effluent can be rated as moderately toxic. Dilution of this effluent more than 10 times would be required to reduce its leathal and pathological effects to fish stock.

Ammonia at very low concentrations down to 0.04 mg/l in presence of traces of Cr, Ni, Pb, Cu and Zn caused hypertrophy of gill filaments and hyperplasia of the epithelial surface of respiratory lamellae for fingerlings of **Sparus aurata**.

With respect to fry **Mugil capito** gill filament and lammellar organization were distorted by severe edema, and separation of respiratory epithelium.

Liver was also affected at the same tested concentration of 4% vol/vol of waste as indicated by abnormal accumulation of lipid in **Sparus aurata** ranging from moderate to severe.

Mugil capito small intestin showed a forthy vacuolated mucosal epithelium and loss of bursh border when exposed to 4% vol/vol of LAC waste. Treatment of this effluent eliminated this effect.

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