

POTENTIAL SAFETYNESS OF SODIUM ALGINATE IN ALLEVIATION OF AMMONIA AND HEAVY METALS RESIDUES IN FISH PONDS OF *OREOCHROMIS NILOTICUS*

M. S. Gado¹; N. M. El-Bahy²; A. T. Ibrahim³ and M. T. Shehab El-Din⁴

1. Fac. Vet. Med., Tanta Univ., Dept. Poultry and Fish. 2. Fac. Vet. Med., EL-Menofia Univ., Dept. Parasitology. 3. Animal Health Research Institute; Giza.
4. Animal Health Research Institute, Kafr EL-Sheikh.

ABSTRACT

This study was carried out to explore the efficacy of sodium alginate - a byproduct of sea weeds - in combating some pollutants in fish cultures, typically ammonia and heavy metal residues in different stages of fish life as well as the effect of alginate on the overall fish health, monitored by certain serum enzymes and some hematological investigations.

Alginate alleviates ammonia at ratios ranging from 52.3 - 60.5% in fry aquaria, fed either on ration or sabla respectively and consequently an improvement in the DO concentration percentage with 16 - 15.7% when matched with the counterpart control of both food regimes, on a similar manner, in the fingerlings groups; an amelioration in ammonia ranged from 16.2 - 23%, pursuit with 15 - 11.5% improvement in the DO percent.

Studying alginate with frequent doses, with an interval of 10 days on adult fish; an improvement of ammonia concentration had been noted revealing 48.2, 46.2 and 41.9% for the single, double and triple dosing with also, a subsequent improve in DO concentrations as 11.7, 10.8 and 5.6% respectively. The binding capacity of alginates for heavy metals residues (Cadmium, Nickel, Lead, Manganese, Iron and Copper) in fish, showed a significant effect only in diminishing Lead, Manganese and Iron in fry fed ration and highly significant effect for all metals except Lead in fry fed sabla. The significant effect of

alginate was only restricted to Manganese and Iron in fingerlings fed ration, whilst had no significance for that fed sabla.

Exploring the general health condition of adult fish supplemented with frequent doses of alginates; exhibited a gradual increase in the erythrocytes and leucocytes counts in all doses as well as an increase in the hemoglobin content in the fish's blood. On the other hand, estimation of some liver enzymes (AsT and ALT) in the fish's sera, revealed declined values, compared with that of control, even though cessation of administration of alginate 10 days later, creatinine as one of the kidney functions, infrequently fluctuates, but returned back to normal as that of the control. The albumin concentration in the fish's sera was also decreased than the control value; from all of which, alginate can provide a safety support in fish culture for health and performance.

INTRODUCTION

Pollution is one of the hidden black faces of the moon of the industrial development all over the world. Environmental contamination from human activity and any resulting public health problems do not arise in a vacuum but rather are side stream effects or the economic and cultural activities of the whole society (**Mushak, 1992**).

Since the excessive use of the recycled drained fresh water previously used in irrigating reclaimed agricultural lands, polluted with wastes from factories and sewage systems, the hazard of some pollutants like heavy metals might be higher than the permissible limits reaching human consumers have been existing especially, with continuous addition of organic fertilizers like litters of poultry farms to fish culture ponds (**Forstner and Wittmann 1983**).

Recently the use of sodium alginate in fish production has attracted many people. Due to their binding effect, and ionic exchange capacity, sodium alginate has an anti-pollutant effect, since the pollution in the fish culture ponds was sharply reduced by removing undesired heavy

metal pollutants, carcins and toxins(*Protan,1987;Murray and Pizzoms, 1991 and Lau, 1992*) and was helpful in combating organic matter and ammonia in aquacultures (*Kareeda, 1987; Remane, 1997 and Dawoud, 2000*).Another field of interest is the use of sodium alginate in the enhancement of immunogenic activities via the proliferative response of spleen cells, associated with B-cell,not T-cell,populations,(*Nordmo et al.,1995; Fujiki and Yano, 1997; Okai et al., 1998; and Gado and Azza, 2004*).

In this study, the effect of sodium alginate on some water pollutants and fish health were investigated on the popular Egyptian fish species; *Oreochromis niloticus*. **Through;** studying the effect of sodium alginate supplement on the heavy metals residues in fish at different life stages, as well as on the undesirable effects of dissolved ammonia in the water. Exploring the safety-by work of sodium alginate on the fish's health via the measurement of some hematological and serological investigations of some liver and kidney enzymes.

MATERIALS AND METHODS

1- Fish and aquaria:

A total number of 80 adult fish, weighing $65 \pm 5g$ at a presumptive age of approximately 150 days old, a 200 fingerlings weighing $15 \pm 5g$ at a presumptive age of approximately 100 days old as well as a 905 fry weighing $0.04 \pm 0.015g$ with a presumptive age of approximately 25 days old;of apparently healthy Nile Tilapia,*Oreochromis niloticus*(*O.niloticus*). All fish stages were transferred to the laboratory in separate plastic bags provided with aeration by a battery aerator (Beauty, Italy). The fish were left 10 days for acclimation prior to experiments,according to *Plumb and Bowser (1982)*.

Twelve glass aquaria of 40x50x100cm dimensions were used for the experimental work. They were supplied with chlorine free water with continuous aeration; using an electric air pumping compressors (RENA -

France) according to *Innes (1966)*, and water temperature was thermostatically controlled at $22 \pm 1^\circ\text{C}$ (type CMI, Germany).

2- Sodium Alginate:

Biopolym FZT, (Schulze and Hermsen Co.Germany) liquid(0.5% sodium alginate concentration).

3- Diet:

The fry, fingerling and adult were fed on:commercial ration,not less than 40, 30 and 25% total protein content respectively, manufactured by kafr El- Sheikh Government Ration Factory for fry, fingerlings and adult fish. Feed (ration and sabla) samples were succumbed for heavy metals analysis at the beginning of experiments. Poultry droppings (sabla), containing not less than 19-20% nitrogenous wastes. The fry, fingerlings and adult fish were fed either on ration or sable at a rate of 10,5 and 3% respectively of the fish's biomass as twice daily for fingerlings and adult fish while as fry were fed Quarterly per day along the experimental period. (*Jauncey, 1982; NRC, 1983 and Teshima et al., 1986*).

4- Fish experiments:

4-1- Fry expriments:

A total number of 905 fry at a presumptive age of approximately 25 days old, apparently healthy of *O. niloticus* obtained from a fish hatchery were used.They were subdivided into 222,230,225 and 228 for each group respectively in a succession of G1,G2,G3 and G4,in separate glass aquaria. The first two groups (G1 and G2) were fed on a commercial ration, while the other two groups (G3 and G4) were fed on sabla. G2 and G4 were supplied with a single dose of sodium alginate supplement at a rate of 0.01 ml/25L of aquarium water,(calculated according to the recommended dose by the manufacturer; 2 L/Feddan). While G1 and G3 were non treated (control groups). Samples of aquarium water were obtained every 10days for analysis of ammonia concentration (ppm), DO (mg/1), salinity (ppt)

and pH. Heavy metals analyses were carried out for aquarium sediments at the end of experiments(70 days old).Fish samples were taken for heavy metals analysis every 10 days from the beginning of experiments.

4-2- Fingerlings experiments:

A total number of 200 fingerlings at a presumptive age of approximately 100 days old, of apparently healthy *O. niloticus* obtained from a fish farm were used. They were grouped into 4 groups. They were subdivided into 50 fish for each group respectively in a succession of G1,G2,G3 and G4,in a separate glass aquarium.The first two groups(G1 and G2)were fed on commercial ration, while the other two groups, (G3 and G4) were fed on sabla.G2 and G4 were supplied with a single dose of sodium alginate supplement at a rate of 0.05 ml/125L of aquarium water.While G1 and G3 were non treated (control groups). Samples of aquarium water were obtained every 10 days for analysis of ammonia concentrations(ppm),DO (mg/l), salinity (ppt) and pH. Heavy metals analyses were carried out for aquarium sediments at the end of experiments (140 days old). Fish samples were taken for heavy metals analysis every 10 days from the beginning of experiments.

4-3- Adult fish experiments:

A total number of 80 adult fish at a presumptive age of approximately 150 days old, apparently healthy *O. niloticus* obtained from a fish farm, were used. They were grouped into 4 groups. They were subdivided into 20 fish for each group in a succession of G1, G2,G3 and G4,in a separate glass aquaria. All groups were fed commercial ration. The fish groups; (G2), (G3) and (G4) were supplied respectively with a single, double and triple dose of sodium alginate supplement with an interval of one week at a rate of 0.04ml/100L of aquarium water. The first group (G1) was kept as a control non treated group. Blood samples were obtained at the age of 175 and 185 days for some hematological studies(RBCs, WBCs and Hb) and some serum enzymes analysis for the liver and kidney functions, (AsT, AlT, total albumin and ceatinine).

5-Blood sampling:

The blood samples were collected from the adult fish groups by disposable plastic syringes using heart puncture method, for the first time at the age of 175 days old and the fish were sacrificed at the age of 185 days old for obtaining the second blood sample by severing the caudal vessels, according to (*Leid et al.1975*).The collected pooled blood samples of each group were subdivided into two portions; the first portion was left to coagulate aside at the room temperature and then centrifuged for obtaining a clear sera for serum enzyme analysis (SGOT, SGPT, creatinine and total albumin) whilst, the second portion was mixed with EDTA as an anticoagulant for hematological investigations (RBCs and WBCs count and Hb estimation) according to *Smit and Hattingh (1980)*.

5-1- Erythrocytic and total Leukocytic counts:

The number of erythrocytes (RBCs) and total Leukocytes (WBCs) were counted and expressed in millions and thousands per microliter of blood respectively, according to *Natt and Herick (1952)*.

5-2- Estimation of Hemoglobin content:

The hemoglobin(Hb)was estimated by the Sahli's method expressed as gram per deciliter (g/100ml).

5-3- Determination of some serum components:

In the adult fish's sera, the total amounts of AsT (iu) and AIT (iu) were determined colorimetrically at a wave length of 340nm.The Albumin, (g/dl) at 545nm and creatinine (mg/ml) at 492nm.

6- Analysis of Heavy metals:

Representative specimens from fry and, fingerling groups, commercial ration,sabla and aquarium sediments were obtained. The heavy metals residues were determined in fry and fingerling specimens every 10 days from the beginning of the experiments.The residues in the ration and sabla were determined at beginning of the experiments; while as the aquarium sediments were detected at the end of experiments.

The collected specimens were dried in an oven at 70°C for overnight, after complete dryness the remnants were milled in a stainless steel mill. A 0.5g of the specimen in a conical flask was digested in a 10ml of H₂SO₄ for few minutes with shaking till complete digestion. To the digested fluid, 1ml of HCl₄ was added. In a plastic cuvette the obtained clearly digested specimen was completed to a 100ml solution by addition of distilled water, then measured in an Atomic Absorption Technique Unit, for the determination of Manganese (Mn), Copper (Cu), Iron (Fe), Lead (Pb), Cadmium (Cd) and Nickel (Ni): *Kendal and Scanlon (1982)*. The residual level was obtained from the following equation:

Residual level (ppm) = reading of atomic absorption (ppm) /100 X volume (v) of digested specimen /weight of dried specimen (w) X 1000.

7-Water analysis:

The aquarium water of all fish groups experiments were measured digitally (direct reading) For DO concentration (mg/l), water salinity (ppt) and pH.

A series of standardized concentration of working ammonia solutions (0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0 and 4ml) were diluted, up to 50ml with ammonia free water and consequently the concentration of ammonia became (0.0, 0.04, 0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 mg) respectively; a 1ml of Nessler's reagent was added to each of the final ammonia concentration then after 20 minutes the optical density was read colorimetrically at 425nm. From the previous readings a standard curve was designed. To each of the test water sample (50ml), 1ml of Nessler's reagent was added in a large Nessler's tube (100ml), thereafter 20 minutes the optical density was similarly recorded. The concentration of ammonia of each water sample was determined from the standard curve, according to the method recommended by *A.O.A.C. (1975)*.

8-Statistical analysis

Statistics were carried out with Chi square according to *Snedecor and Cochran (1976)*.

RESULTS

1- Water analysis:

1-1- Fry experiments:

The aquarium waters of all groups were submitted for the analysis of DO, pH, salinity and ammonia throughout the experimental period (35-70 days).

The determined DO concentrations in G2 were 5, 4.1 and 2.8mg/l whileas in G1 were 5.1, 3.2 and 1.7 mg/l respectively. On the other side G4 were 4.7, 3.1 and 2.4mg/l compared with 4, 2.8 and 1.8mg/l in G3 at the ages of 45, 55 and 70 days old respectively, table (1).

The hydrogen ion concentrations (pH) in G2 were 7.6, 7.8 and 8.6. However in G1 were 7.4,7.6 and 8.1 successively. Mean while in G4 were 7.8, 8 and 8.3 as well as G3 were 7.9, 8.2 and 8.6 respectively; at the ages of 45, 55 and 70 days old, table (1).

The salinities measured in ppt in G2 and G1 ranged from 2.2-2.4 while in G4 and G3 ranged from 2.3-2.6ppt respectively along the experimental period, table (1).

The concentrations of ammonia in G2 were 0.24, 0.38 and 0.4ppm whileas in G1 were 0.5, 0.54 and 1.1ppm respectively. On the other hand; in G4 were 0.32, 0.4 and 0.52ppm versus to 0.6, 0.74 and 1.8ppm in G3 at the ages of 45, 55 and 70 days old respectively, table (1).

In fry experiments there was non-significant effect of sodium alginates in the elimination of Cd and Cu fed on a commercial ration, although the alginates had a highly significant effect on the release of Pb and Mn and only a positive significant effect for iron, alginate had a negative significant effect for Ni.

The fry fed on poultry sabla; alginate revealed a highly positive significance in the elimination of Cd, Ni, Mn, Cu and iron while as, alginate has a highly negative significance for Pb, the values of each element were compared with the corresponding value in the aquarium sediment.

1-2 Fingerlings experiments:

The ammonia concentration in G2 were 0.50, 0.72, 1.02, 1.28 and 1.36ppm, while as in G1 were 0.58, 0.75, 1.06, 1.40 and 2.01ppm, respectively. On the other side in G4 were 0.62, 0.85, 1.05, 1.20 and 1.93ppm when compared with 0.60, 0.82, 1.16, 1.44 and 2.6ppm at the ages of 110, 130, 140, 150 and 160 days old respectively, table (2).

The dissolved oxygen (DO) concentrations in G2 were 5.1, 4.3, 3.1 and 2.6mg/l, where even in G1 were 5.8, 5.2, 4.5, 3.9 and 2.8mg/l respectively. In G4 the DO, were 5.3, 5.4, 4.7, 3.8 and 3.4mg/l when compared with that of G3 were 5.1, 5.3, 4.1, 3.4 and 2.1mg/l respectively, table (2).

The pH in G2 were 7.4, 7.6, 8.1, 8.4 and 8.6 where as in G1 were 7.5, 7.7, 8.0, 8.1 and 8.5 mg/l respectively. While in G4 were 7.4, 7.8, 8.2, 8.4 and 8.8 mg/l when compared with G3; 7.6, 7.9, 8.5, 8.6 and 8.9 mg/l respectively, table (2).

The measured salinities in G2 showed an increasing values from 2.5ppt at the begining of the experiment (110 days old) to 4.2ppt at the age of 160 days old, as well as in G1, from 2.6 to 4.3ppt . This increasing pattern of salinity were also exhibited in G4 (2.5-4.1ppt) and in G3 (2.7-4.5ppt), as shown in table (2).

In fingerling experiments fed on a commercial ration and poultry sabla, the alginate showed a nonsignificant effect for Cd, Ni, Pb and Cu. While as had a highly significant effect for the elimination of Mn and iron in fingerlings fed commercial ration and the reverse is true in fingerlings fed poultry sabla, the levels of each metal were matched with its corresponding in the aquarium sediments.

1-3- Adult fish experiments:

The dissolved oxygen concentration(DO)were 5.8, 5.9 and 5.4mg/l; 5.9, 5.8 and 5.1mg/l and also, 6.1, 5.8 and 4.1mg/l for G2, G3 and G4 respectively, when compared with 5.7, 5.3 and 4.1mg/l of the control group (G1) , table (3).

The pH concentrations ranged from 7.3-7.7, 7.5-7.8 and 7.4-8.3 for G2, G3 and G4 respectively, while as, the pH range of G1 was 7.5-8.7, table (3).

The water salinities for G2, G3 and G4 ranged from 3.3-3.9, 3.5-4.3 and 3.2-4.3ppt, respectively although the water salinity the control (G1) ranged from 3.4-4.2, table (3).

The concentrations of ammonia were 0.24, 0.80 and 1.02ppm; 0.40, 0.80 and 0.94ppm as well as 0.44, 0.81 and 1.06ppm in G2, G3 and G4 supplemented with a single, double and triple doses of sodium alginate, at the ages of 160, 170 and 185 days respectively when compared with that of the control (G1) as 1.0ppm, table (3).

2- Analysis of Heavy metals:

2-1- fry experiments:

On analysis of ration fed for fry the heavy metal constituents were 0.001, 4, 22, 18, 44 and 458 ppm for Ni, Pb, Cd, Cu, Mn and Fe respectively. Whereas the analysis of poultry sabla were 16, 16, 22, 22, 130 and 844 ppm for the afore mentioned elements. Analysis of the aquarium sediments evoked 12, 36, 26, 110, 423 and 454 ppm for G1, 30, 84, 24, 122, 418 and 162 ppm for G2, 22, 54, 18, 120, 390 and 1834 ppm for G3, as well as 44, 288, 6, 142, 458 and 2480 ppm for G4 for the previously mentioned elements.

The heavy metals residues in fry fed commercial ration, statistically showed a highly significant difference between the treated and nontreated groups, G2 and G1 for Ni, Pb and Mn and only a significant difference for Fe while as there was no significance for Cd and Cu, table (4).

In fry fed poultry sabla; there was a highly significant differences between the treated and non treated groups (G4 and G3) for all elements, table (5).

2-2- Fingerling experiments:

On analysis of the commercial ration fed for fingerlings the heavy metal constituents were. 0.9, 1.1, 0.1, 1.5, 20.5 and 26.6 ppm for Ni, Pb, Cd, Cu, Mn and Fe respectively, where as the analysis of poultry sabla were 1.0, 2.5, 0.2, 0.5, 3.2, and 27.9 ppm for the afore mentioned elements.

Analysis of the aquarium sediments evoked 3.3,3.8, 0.3, 1.5,9.9 and 14.6 ppm for G1, 1.9, 2.7, 0.4, 1.8, 12.3 and 6.4 ppm for G2, 2.2, 2.0,0.3, 3.7, 32.2 and 6.0 ppm for G3 as well as 2.2, 3.6,0.3, 3.6,31.6 and 6.6ppm for G4 for the previously mentioned elements.

The heavy metals residues in fingerlings fed commercial ration, statistically showed a highly significance between the treated and non treated groups (G2 and G1) for Mn and Fe. There was no significance for the reminders, table (6).

The fingerlings fed poultry sabla showed a highly significant differences between the treated and non treated groups; (G4 and G3) for Mn and Fe, and had no significance for the other elements, table (7).

3- Some hematological and serum investigations

The hematological studies represented by the RBCs, WBCs counts and Hb estimation revealed an ascending increase in the RBCs count, 4.5, 5.5 and 6.1 million/ml for G2, G3 and G4, post administration of sodium alginate at the age of 175 (frist blood sample), when compared with the control group (G1), 3.5 million/ml, mean while at the age of 185 days (second blood sample.) the RBCs count were declined to 5.6, 5.3 and 5.1 million/ml in G2, G3 and G4 respectively.

The WBCs count exhibited an increase in G2,8.2 thousand/ml.On the conterary it was declined in the other groups either in the first blood sample or the second one, even though still higher rather than the control group (G1).

The Hb content had an increased amount in G3 and G4,9.05 and 11.22 g/100ml in the first blood sample then declined in the second blood sample, but sustained higher than the control G1, as presented in table (8).

The investigated serum enzymes AsT and AIT showed a lowered values in all groups in both blood samples,when compared with the control G1.

The serum albumin in all treated groups vibrated around that recorded of control group (G1);simillarly, the serum creatinine exhibited no valuable differences among all treated groups when compared with the control group (G1), as shown in table (8).

DISCUSSION

Water pollution often stems from the same sources as habitat degradation. Although, pollution and water quality are generally linked, they should be treated jointly for the sustainable management of a considered water body.

In Egypt, the main origins of aquaculture pollution are the agriculture run-off of fertilizers, pesticides, organic matter heavy metals and the industrial wastes. The later and the organic matter, represented by poultry droppings (sabla); introduced to the fish farms are, however a hot spots, still higher and because of their toxicity, persistence and high risk of bioaccumulation through the food chain, with a potential risk for human consumption; a preventive control measures are strongly recommended. Since, human activities have a tendency to degrade rather than to conserve, and the effects of such pollution and degradation on fish stocks in particular and aquatic environments in general are multifold. Moreover, curative measures prove to be more costly than preventive measures; therefore an on going monitoring the environment to maintain the ecological balance of the ecosystem is needed.

In order to minimize the negative impacts of organic compounds, ammonia and heavy metal pollution, on the aquatic fauna; the toxicities of various pollutants and their elimination from the ecosystem had been researched by *Remane, (1997)*.

In the present study, sodium alginate (Bioplym FZT liquid); a salt of alginic acid derived from brown sea weeds were used to determine its efficacy in combating the suspended or soluble pollutants in aquacultures harmful to fish specifically, ammonia and heavy metals.

Ammonia is the primary nitrogenous metabolic waste of fish, but also formed by the decay of organic matter and may be present in the aquaculture inflowing water, especially where agricultural run-off is encountered, or high levels may simply indicate overstocking or over feeding of fish (*Meade, 1985; Brown, 1993 and Noga, 1996*). The unionized form of ammonia, the toxic form (NH_3); rather than its pathological deterioration effect on fish, caused a reduced ability of fish to take up oxygen, with reduced activity and growth rate. (*Samylin, 1969; Fromm, 1970; Smith and Piper, 1975; Robinette, 1976; and Thurston et al., 1978*). On the other hand, low levels can produce chronic stress to fish (*Rice and Bailey, 1980*).

The fry, fingerlings and adult fish experiments, revealed a decreased values of total ammonia in fish groups supplemented with sodium alginate when compared with that of controls. (tables, 1, 2 and 3); indeed a more pronounced reduction had been noticed with that fish provided with an alginate supplement but fed on sabla; this might explain the raised survival percent of fish between the control and supplemented group of each treatment. While as, the survival percent of adults were comparatively lowered in fish group (G3) supplied with a double dose of alginate rather than in G2 and G4 supplied with a single and triple doses of alginate. Possibly due to the cumulative effect of accumulated nitrogenous compounds in the aquaria as a result of slightly increased salinities hence, ammonia stress on fish ensue. This concise the fulfilled investigations by *Katz and Pierro (1967); Emerson et al. (1975) and Meade (1985)* who reported that the amount of unionized ammonia in the water as well as its toxicity to fish depends mainly upon pH, temperature and salinity. In addition, *Brown, (1993)* emphatically said that, the ammonia levels in a fish pond, will vary with pH and temperature changes, being minimized by values of both these parameters. Moreover, *Svobodova et al., (1993)*, after explaining the mechanism of fish's ammonia intoxication, mentioned that the ratio between the two forms of ammonia water; the

ionized form (NH_4^+) and the molecular form (NH_3), were dependant largely beside other stressors on pH and temperature of water. Concerning the pH changes in adult experiments (table, 3) there was a steady increase in the control groups either fed ration or sabla (G 1 and G 3) which were comparatively higher than that of sodium alginate supplemented groups (G 2 and G 4) where still ambient higher, that may share in explaining the role of alginates in conservation of the surrounding pH or in other words; keeping pH changes at the minimum fluctuations, via its ionic exchange capacity.

Regarding the temperature, in all experiments, the temperature was made constant at 22°C (nearly simulating the nature in most events of summer-rearing months of fish) to neglect the temperature fluctuations, would impact the results, first, because temperature fluctuations would in turn fluctuate the DO concentration, pH and salinity and the linkages increasingly or decreasingly of such parameters with ammonia second, as fish was pikilothermic animals, the temperature oscillation might alternate the hematological parameters as well as the serum enzymatic reactions in adult fish specifically-will be discussed later-that falsely judge the periodic, sodium alginate effect on fish health.

There were a remarkable improvement in DO concentrations in fish either fry, fingerling or adult groups supplemented with sodium alginate supplement or almost nearer to that of control non supplemented groups this may be attributed to the constancy of temperature as well as the minor fluctuations in pH and salinity.

The reduction effect of alginates, on ammonia might be attributed to its ionic exchange capacity, forming complex with ammonia ion (NH_4) hence, prevent the evolution of free ammonia (NH_3), *kareeda*, (1987); *Gurk and Brunsch*; (1994), *Dawoud*, (2000) and *Patnaiks et al.* (2001) there by, keeping ammonia levels as low as possible which is advisable.

Heavy metals, rank as major polluting chemicals in both developed and developing countries. In our country, the sources of heavy metals arise from the tailing ponds, where waste crushed ore is settled out, from fuel combustion and from water drained from spoil heaps which continue to discharge heavy metals into water courses long after. Another important source; is the industries that use these metals in various processes like electroplating and galvanizing iron, where the wastes are discharged without treatment. This problem was recognized in the last century and formed one of the first topics for research on the effects of their pollution on fish and consequently on human health.

The uptake of metals occurs across the body surfaces that are in contact with the environment. There are both passive and active mechanisms involved in the absorption and transport of metals and these mechanisms are dependant on water quality, metabolic activity and developmental stages of fish. In fish the possible areas for absorption of dissolved metals are the gills, intestine and skin. Hence, fish-in the field practice in our country-may be fed on a formulated ration and conjuncted with poultry sabla or exclusively on poultry sabla excessively, that might increase the heavy metal pollution to fish, besides the other concurrent sources in the water current. Therefore, the experiments were designed to evaluate the effect of sodium alginate on the heavy metal residues in fish (fry and fingerlings). To reconnoiter the role of sodium alginate in alleviation of heavy metal residues in fry fed either on ration or sabla; the results showed a highly significant effect of alginates in the elimination of Lead and Manganese and only a significant effect for iron in fry fed ration, table (4), but on the other hand, the fry fed sabla, there were a highly significant role for the elimination of Cadmium, Nickel, Manganese, Copper and Iron, table (5).

In the fingerlings fed on ration, sodium alginate had a limited highly significant effect in diminishing the metal residues only of Manganese and Iron, table (6). While as, sodium alginate had no significance in the removal of residues in fingerlings fed sabla, table (7).

In this study sodium alginate statistically presented to some extent; a limited value in the elimination of heavy metals inside the fish body in the different treatment methods either in fry or fingerlings fed on ration or sabla; this may be attributed to the physico-chemical conditions of the aquatic environment, thus variables such as pH changes, water hardness, temperature, ionic compositions, concentration of particulate matter especially the organic matter as well as other complexing agents in the water, might determine the speciation of the metals and thereby their availability to fish; such all factors made the explanation, for the toxicity of fish with heavy metals more complex. Apparently pH plays an important role in the interaction between heavy metals and organic compounds represented by ration debris, fish excreta and sabla. Therefore, the organic substances in the water and the changes in pH could strongly influence the adsorption and desorption of metals and hence, high pH values resulted in decreased free metal ions as well decreased its availability to fish. *Dodge and Theis (1979), Port and Wikmark (1984), Block (1991) and Mc Donald et al. (1989)*, and noted that pH plays an important role in the interaction between heavy metals and organic matter ; more added if the concentration of soluble organic compounds is low in water, the complications of heavy metals with organic substances generally lead to reduced uptake by fish.

Notably, fry either fed ration or sabla, showed a great affinity for the bio-accumulation of heavy metals and fortunately sodium alginate

exerts well its action within. This was also precepted by many authors; *Svobodova et al. (1993)* and *Brown (1993)* who mentioned that the toxic action of metals is particularly pronounced in the early stages of development of the fish life.

Another explanation would be accepted which may play a role in solubility and in turn the availability of heavy metals to fish; is the salt content in water. In the present experiments, there were a steady increase in the salt content in the aquarium waters, simultaneously with the increased amounts of organic matter which hinder the availability of heavy metals by fish as discussed before. This met with that recorded by *Part et al. (1985)* that mentioned, the high salt content increases pH and consequently lowers the heavy metals solubility.

Hence, sodium alginate exerts its action in the elimination of heavy metals by its bounding activity via complexing free ions of metals in the water by adsorption, rendering them unavailable to fish, many factors-which may be a matter of further studies-may impair to some extent its bounding action for metals. In our experiments, that explains why sodium alginate bestowed a diminished effect probably due to the presence of a large number of these polyvalent ions and this had been detected by many authors; *Costa and Leite (1991)* and *El-Sheikh (2004)*.

Exploring the impact of sodium alginate on fish health calls for monitoring its effect on some blood parameters and some serum enzymes. There were a remarkable decrease in serum liver enzymes (SGOT and SGPT) and total albumin whereas serum kidney enzyme (Creatinine) exhibited an amphoteric pattern nevertheless, still around that of the control (*Seal and Mathers 2001*).

An eye - catching sign was conducted to the declined values of the albumin in the fish's sera implicating an immunological dimension of alginate, which may accordingly lead to elevated levels of globulins that will be highlighted in further studies, *Nordmo et al. (1995)* and *Dawoud (2000)*.

The RBCs, WBCs and Hb counts showed a gradual increase in the first blood samples (at 175 days) for fish provided with a single, double and triple doses of sodium alginate though, remains higher than the control 10 days later (time of the second blood sample). These results would be received a longwise, due to the reduction of ammonia levels and accordingly increased DO concentrations that might be reflected worthily on the fish health.

A tangible interesting hint should be deposited on probation, was the obtaining of an even homogenous weights for fish supplemented with sodium alginate; either in fry or fingerlings.

In conclusion, sodium alginate is a herbal extract obtained from sea weeds, would be considered as a promising prebiotic in fish aquaculture practice. However, in the modern world, it is impossible naturally to escape contamination from heavy metals as far as fish; alginate binds the toxic polyvalent metals such as; Cadmium, Nickel, Lead, Manganese, Copper and Iron either in the ecosystem, rendering such metals unavailable to fish or in the fish's body, forming complexes with the metals that have been excreted later. Another fruitful profit of alginate, is the combating of ammonia in fish ponds, that all aqua-culturists agitating against; which in turn improving the aqua-cultural circumstances; like DO and pH which are important factors for fish's productivity, health and life and consequently on man.

Table (8): Efficacy of sodium alginate on some liver and kidney functions and some haematological parameters of adult fish (175-185)days.

Serum enzymes and hematological parameters	175 days				185 days		
	G 1	G 2	G 3	G 4	G 2	G 3	G 4
AsT (iu)	160.5	110.3	130.1	136	149.8	155.3	140.9
AIT (iu)	39.66	23.40	30.2	25.1	28.1	24.2	35.1
Albumin (g/dl)	0.96	0.83	0.77	0.52	0.80	0.70	0.90
Creatinin (mg/ml)	1.20	1.27	1.60	1.20	1.40	1.2	1.2
RBCs (million/ml)	3.50	4.50	5.50	6.10	5.60	5.50	5.10
WBCs (thousand/ml)	4.18	4.20	8.20	7.80	7.30	5.80	5.40
Hb (g/100ml)	5.10	8.10	9.05	11.22	8.90	8.50	8.20

AsT Aspartate Transaminase

AIT Alinine Transaminase

RBCs Red Blood cells

WBCs White Blood cells

Hb Haemoglobin

REFERENCES

- *Association of official analytical chemists (A.O.A.C.), (1975):* Official methods of analysis of the association of official analytical chemists 12th ed., A.O.A.C., Washington, D.C., U.S.A.
- *Block, M.(1991):* Uptake of cadmium in fish: effects of xanthates and dieth-ylodithiocarbamate. PhD thesis, university of Uppsala, Sweden. pp 37.
- *Brown, L. (1993):* Aquaculture for veterinarians: Fish Husbandry and Medicine Pergamon press Ltd, the Headington Hill Hall, Oxford OX3 OBW, England, pp: 447.
- *Costa, A.C.A. and S.G.F., Leite (1991):* Metals bioabsorption by sodium alginate immobilized *Chlorella homosphaera* cells. Biotechnol. Bioen., 34: 990-999.
- *Danecker, E. (1964):* The jauche poisoning of fish: an ammonia poisoning. Osterr. Fischerei., 3/4: 55-68.

- **Dawoud, A.S. (2000):** Comparative clinicopathological studies between some medicaments controlling ammonia in poultry farms. Egyptian Journal. Comp. Path. and Clinic. Path. Volume 13. No. 2 p. 156-162.
- **Dodge, E. E. and T. C., Theis (1979):** Effect of chemical speciation on the uptake of copper by *Chironomus Tentans*. Environ. Sci. and Techn., 13, 1287
- **El-Sheikh, M.H. (2004):** The use of alginate as indicator for environmental pollution. (An assay) published in: Alam Alkemya magazine. No. 28 (January-February-March) 2004. pp: 28-30.
- **Emerson, K ; R.C., Russo; R.E., Lund and R.V., Thurston, (1975):** Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J. fish Res. Board of Canada, 32: 2379 – 2383.
- **Forstner, N. and G.T.W., Wittmann (1983):** Metal pollution in the aquatic environment. Springer-Verlag, Berlin.
- **Fromm, P.O. (1970):** Effect of ammonia on trout and goldfish. In: Toxic action of water soluble pollutants on freshwater Fish, Washington DC, US Environmental Protection Agency, pp. 9-22 (EPA Water Pollution Control Research Series No. 18050DST 12/70).
- **Fujiki, K. and T. Yano (1997):** Effects of sodium alginates on the non-specific defence system of the common carp (*Cyprinus carpio L.*). Fish and Shellfish Immunology 7 (6): 417-427.
- **Gado, M.S. and Azza, A.K. (2004):** Augmentation of specific and non-specific protection in *Oreochromis niloticus* treated with some prebiotics and/or bacterin. Kafr El-sheikh Vet. Med. J., Vol. (2) No. 2
- **Gurk, S. and R., Brunsch (1994):** Stall atmosphere improvement institute for applied science of farm animals. Humboldt University of Berlin.

-
- **Innes, W.T. (1966):** Exotic Aquarium Fishes. 19th Ed. Aquarium Incorporated New Jersey P.1 (12): 24-25, 29-30 and 530-533.
 - **Jauncey, K. (1982):** The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile Tilapia (*Sarotherodon mossambicus*), *Aquaculture*, 27: 43 - 54.
 - **Kareeda, H. (1987):** Uses of *Yucca Schidigera* in animal feeds. *Poultry science*, 4: 165-168.
 - **Katz, M. and R.A., Pierro (1967):** Estimates of the acute toxicity of ammonia-urea plant wastes to Coho salmon (*Oncorhynchus kisutch*), Seattle, Washington, Fisheries Research Institute, College of Fisheries, University of Washington, 15 pp (Final report).
 - **Kendall, R.J. and P.F., Scanlon (1982):** A rapid method for analysis of tissues for heavy metals using atomic absorption spectrophotometer. *Northwest Sci.* 56, 265-267.
 - **Lau, B. (1992):** Edible plant extracts modulate macrophage activity and bacterial mutagenesis. *Int. J. Clin. Nut.*, 12: (3): 147-155.
 - **Lied, E.; Z., Gzerde and O.R., Braskhan (1975):** "Simple and rapid technique for repeated blood sampling in rainbow." *J. Fish. Res. board of Canada*, 32 (5): 669-701.
 - **Meade, J.W. (1985):** Allowable ammonia for fish culture prog. *Fish-culturist*, 47, 135-145.
 - **McDonald, D.G.; J. P., Reader and T. R. K., Dalziel (1989):** The combined effects of pH and trace metals on fish ionoregulation. In: Morris, R.; Taylor, E. W.; Brown, D.J.A. and Brown, J. A. (eds) *Acid toxicity and Aquatic Animal*. Cambridge university press, Cambridge, pp: 221 - 242.

-
- **Murray, M. and J., Pizzoms (1991):** Encyclopedia of natural medicine. Prima publishing, Rocklin, CA.
 - **Mushak, P. (1992):** Defining Lead as the premiere environmental health issue for children in America: Criteria and their quantitative application. Environmental Research 59: 281-309.
 - **National Research council (NRC), (1983):** Nutrient requirements of warm water fishes and shell fishes. National Academy Press., Washington, D. C. 102 P.
 - **Natt, M.P. and C.A., Herrick (1952):** A new blood diluents for counting the red and white blood cells of chickens . Poult. Sci., 31, 335.
 - **Noga, E.J. (1996):** Fish diseases : Diagnosis and treatment. By-year boox, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri, 63146, U. S. A . pp: 367.
 - **Nordmo, R.; J.M., Holth and B.O., Gabrielsen (1995):** Immunostimulating effect of alginate feed in Atlantic salmon (*Salmon salar L*) challenged with *Aeromonas salmonicida*. Molecular Marine Biology and Biotechnology. 11: 232-235.
 - **Okai, Y.; K., Higashi-Okai and S., Ishizaka (1998):** Possible immunomo-dulating activities in an extract of edible brown alga; *Hijikia fusiforme (Hijiki)* J. Sci. food Agric. 76:56-62.
 - **Part, P.; O., Svanperg and A., Keissling (1985):** The availability of cadmium to perfused rainbow trout gills in different water qualities. Water research, 19: 427-434.
 - **Patnaiks, sarkar R. and Mitra A. Aug (2001):** Alginate immobilization of *spirulina platensis* for wastewater treatment. Indian J. Exp.Biol.;39(8):824-6.

-
- **Plumb, J.A. and P.R., Bowser (1982):** A Laboratory Manual of Microbial Fish Diseases. Auburn Univ. Auburn, Alabama, p. 77.
 - **Port, P.and G.,Wikamrk(1984):** The influence of some complexing agents (EDTA) and citrate on the uptake of cadmium in perfuse rainbow trout gills. Aquatic toxicology, 5, 277-289.
 - **Protan(1987):** Protanal alginates for cell immobilization.Drammen, Norway, Protan A/S, 3P.
 - **Remane,k.(1997):**African inland fisheries,aquaculture and the environment. FAO and fishing new books,a division of Black well scientific Osney Mead, Oxford OX2 OEL, England, pp: 384.
 - **Rice, S.D. and J.E., Bailey (1980):** Survival, size, and emergence of pink salmon,*Oncorhynchus gorbuscha,alevins* after short-and long-term exposures to ammonia. Fish Bull., 78(3): 641-648.
 - **Robinette, H.R. (1976):** Effect of selected sublethal levels of ammonia on the growth of channel catfish (*Ictalurus punctatus*). Prog. Fish-Cult., 38(1): 26-29.
 - **Samylin, A.F. (1969):** Effect of ammonium carbonate on early stages of development of salmon. Uchen. Zap. Leningr. Gos. Pedagog. Inst. Im. A. I. Gertsena, 422: 47-62 (in Russian).
 - **Seal C.J.and J.C.Mathers(2001):**Comparative Gastrointestinal and plasma cholesterol responses of rats fed on cholesterol – free diets supplemented with guar gum and sodium alginate. British J. of Nutr., 85 (3): 317 - 324.
 - **Smit, G. L. and J.,Hattingh(1980):** Hematological assessment of generally used fresh water fish blood anticoagulants. J. Fish Biol., 17, 337-341.
 - **Smith,C.E.and R.G.,Piper(1975):** Lesions associated with chronic exposure to ammonia. In: Ribelin, W.E. and Migaki, G., ed. The

pathology of fishes, Madison, Wisconsin, University of Wisconsin Press, pp. 497-514.

- *Snedecor G.H. and W., Cochran (1976):* Statistical methods. 6th Ed., Iowa State Univ. Press. Ames., Iowa U.S.A.
- *Svobodova, Z; R, Lloyd; J, Machova and Vykusova, B. (1993):* Water quality and fish health. FAO fisheries Dept., Rome, Italy. pp: 67.
- *Swift, D.J. (1982):* The blood haemoglobin concentration of the Atlantic mackerel *Scomber scombrus* L.. Comp . Biochem. Physiol., 73 A., 229-232.
- *Teshima, S.; A. kanazawa and y. uchiyama (1986):* Effect of severral protein sources and other factors on the growth of *Tilapia nilotica*. Bull. Jpn. Soc. Sci. fish., 52: 525 - 530.
- *Thurston, R.V.; R.C., Russo and Smith C.E. (1978):* Acute toxicity of ammonia and nitrite to cutthroat trout fry. Trans. Am. Fish. Soc., 107(2): 361-368.

التأثير الآمن لألجينات الصوديوم في تخفيف معدلات الأمونيا و بقايا المعادن الثقيلة في أحواض اسماك البلطي النيلي

محمد سعيد جادو¹، نصر معوض الباهي²، أحمد توفيق إبراهيم³، محمد تاج الدين شهاب الدين⁴

1- كلية الطب البيطري-جامعة طنطا-قسم الدواجن و الأسماك، 2- كلية الطب البيطري-جامعة المنوفية-قسم الطفيليات، 3- معهد بحوث صحة الحيوان بالقاهرة ، 4- معهد بحوث صحة الحيوان بكفر الشيخ

أجريت هذه الدراسة على مادة الأليجينات (أليجينات الصوديوم) كمنتج من منتجات الطحالب البحرية لإستبيان مدى كفاءتها في إزالة أو تخفيف بعض الملوثات المائية، مثل الأمونيا و العناصر المعدنية الثقيلة على الأسماك والمياه في مزارع الأسماك في مراحل نمو الأسماك المختلفة (زريعة، إصبغيات وكاملة النمو) وكذا تأثير هذه المادة على الحالة الصحية العامة للأسماك.

وجد أن لهذه المادة تأثير جيد في إزالة الأمونيا بنسب تتراوح ما بين 52.3 - 60.5 ٪للزريعة التي غذيت على علف وسبلة على التوالي، كما وجد تحسنا ملحوظا في نسبة الأوكسجين الذائب بتلك المجموعات يتراوح ما بين 16-15.7٪ على التوالي أيضا في مجموعات الإصبغيات التي غذيت على

علف وسبلة كان التحسن في نسبة الأمونيا يتراوح ما بين 16.1 - 23% واستتبعه تحسنا في نسبة الأكسجين الذائب تتراوح ما بين 15 - 11.5%.

عند دراسة تأثير هذه المادة بجرعات متكررة ما بين كل منها عشرة أيام، على أسماك كاملة النمو وجد أن تحسن الأمونيا في المجموعة ذات الجرعة المقررة كان 48.2% وكان في الجرعة المزدوجة 46.2% وفي المجموعة ثلاثية الجرعة كان 41.9%، كما كانت نسب التحسن في الأكسجين الذائب هي 11.7، 10.8 و 5.6% لكل جرعة على التوالي.

عند دراسة أثر هذه المادة على بقايا العناصر المعدنية الثقيلة (كاديوم، نيكل، رصاص، نحاس والحديد) وجد إحصائيا أن لها تأثير إيجابي في تقليل تركيزات الرصاص، المنجنيز والحديد في الزريعة المغذاة على العلف، أما في الزريعة المغذاة على السبلة كان أكثر إيجابية على كل العناصر ماعدا الرصاص أما في الإصبعيات المغذاة على السبلة فكان تأثيرها الإيجابي إحصائيا منحصرا في المنجنيز والحديد ولم يبدو لها تأثير إيجابي في الإصبعيات المغذاة على السبلة.

لبيان أثر الليجينات الصوديوم على الصحة العامة لأسماك كاملة النمو والتي أعطيت الأليجينات بصورة متكررة وجد ارتفاعا ملحوظا في عدد خلايا الدم الحمراء والبيضاء في كل الجرعات و كذا ارتفاع نسبة الهيموجلوبين. وعند قياس بعض إنزيمات وظائف الكبد للأسماك وجد انخفاضا ملحوظا في مستوى تلك الإنزيمات ويقاس إحدى وظائف الكلى وهو الكرياتينين وجد تذبذبا (ارتفاعا وانخفاضا) بين مجموعات الجرعات عند مقارنته بالمجموعة الضابطة. أظهرت نسبة الزلال في مصل دماء تلك الأسماك انخفاضا ملحوظا عن مثيلاتها في المجموعة الضابطة مما يعطى ظاهريا أمانا لاستخدام تلك المادة في الإستزراع السمكي.