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Decontamination of Nitrogenous Toxicants from Brackish Water Using Plant and Animal Extracts

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Ammonia is the main nitrogenous product excreted by crustaceans and additionally is produced from the ammonification of organic matter in a culture system (Chen and Lei 1993; Frais-Espericueta et al. 1999). Total ammonia consists of the relatively less toxic ionized form in equilibrium with the highly toxic unionized form. Ammonia can be oxidized in to toxic nitrite. Nitrogen in the form of ammonia and nitrite may be toxic to fish / shrimp and can accelerate eutrophication processes. The acceptable levels of these chemical compounds in the aqueous solution for a normal fish and shrimp aquaculture activity are quite low (Chen and Lei 1993; Frais-Espericueta et al. 1999). Ammonia and nitrite removal thus become necessary if the water quality is to be sufficiently maintained for a normal aquaculture activity (Krishnani et al. 2002). Most of the previous works highlight the use of commercially activated carbons and ion exchange resins, which need high capital investment and regeneration costs (Boyd 1995). Conversely, bioremediation is a constructive approach for the treatment of water contaminated with pesticides (Gupta et al. 2001), heavy metals (Rehman and Shakoori 2001) and toxic nitrogenous compounds (Krishnani et al. 2002). In the present study, seed oil from custard apple (*Annona squamosa*) and lipids from squid (*Lolligo* sp.) and clam (*Meritrix costa*) tissues were used to treat brackish water (20±1 ppt) containing high ammonia and nitrite concentrations.

MATERIALS AND METHODS

The fresh dried seeds of custard apple (*Annona squamosa*) were ground into a fine powder and then mixed with hexane (HPLC grade, E. Merck) and placed on the shaker for 1 hour. The resulting suspension was poured into a large Buchner funnel under vacuum. After solvent removal, the resulting seed cake was extracted with hexane and the process was repeated a total of four times. The combined fractions of solvent was allowed to evaporate and the oil remaining was transferred to a volumetric flask. Lipids from animal tissues were extracted using Bligh and Dyer method (1959). The brackish water for the experiment was prepared by adding appropriate amount of ammonium sulphate and potassium nitrite in brackish water to attain various initial ammonia concentrations ranging from 0.55 to 1.95 ppm and nitrite concentration of 0.92 ppm. For each experimental run, 2 litre of the prepared water was poured into the conical flask. Thereafter, varying amounts of seed oil from custard apple and lipids from clam and squid were added to the flasks and covered

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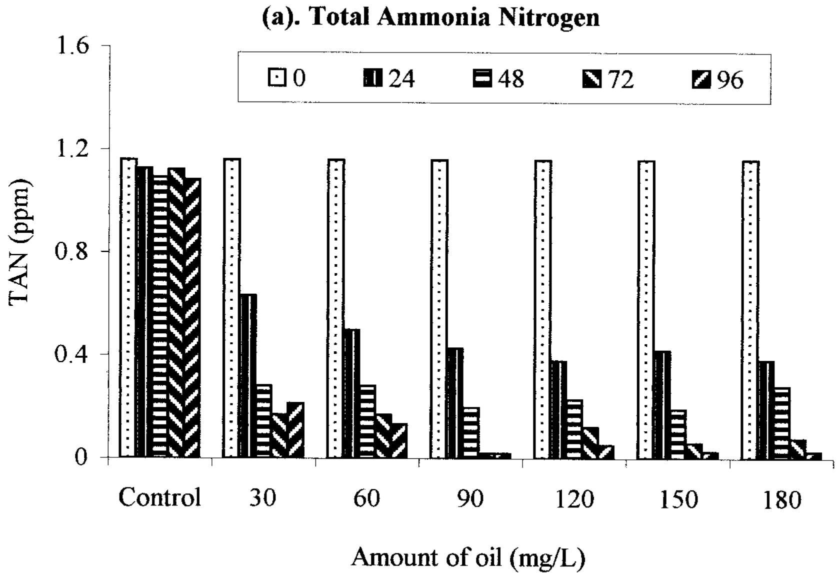

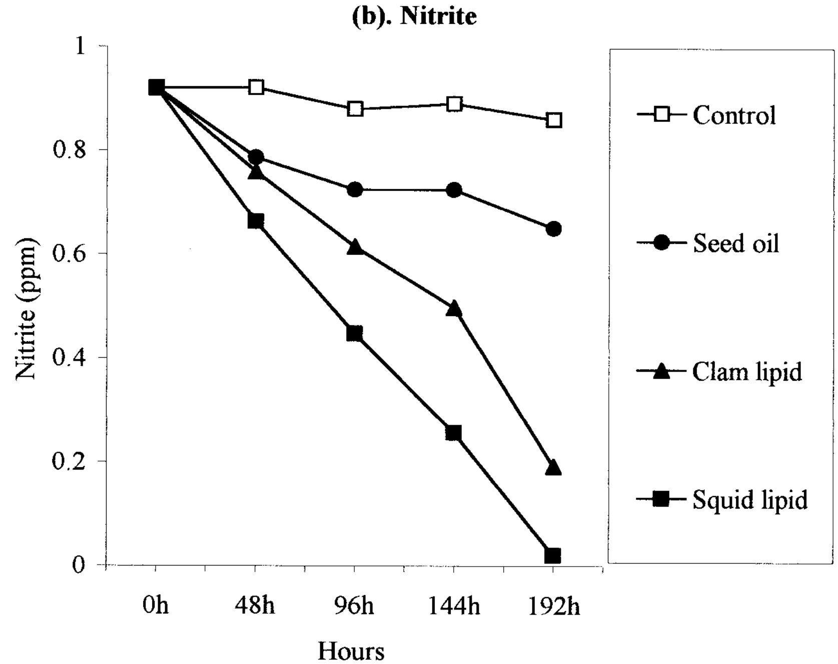

Figure 1. Effect of plant and animal extracts on ammonia and nitrite removal from brackishwater (a). Determination of effective amount of custard apple seed oil for ammonia removal (Initial TAN 1.16 ppm)
(b). Effect of seed oil & lipids on nitrite removal (Initial nitrite = 0.92 ppm).

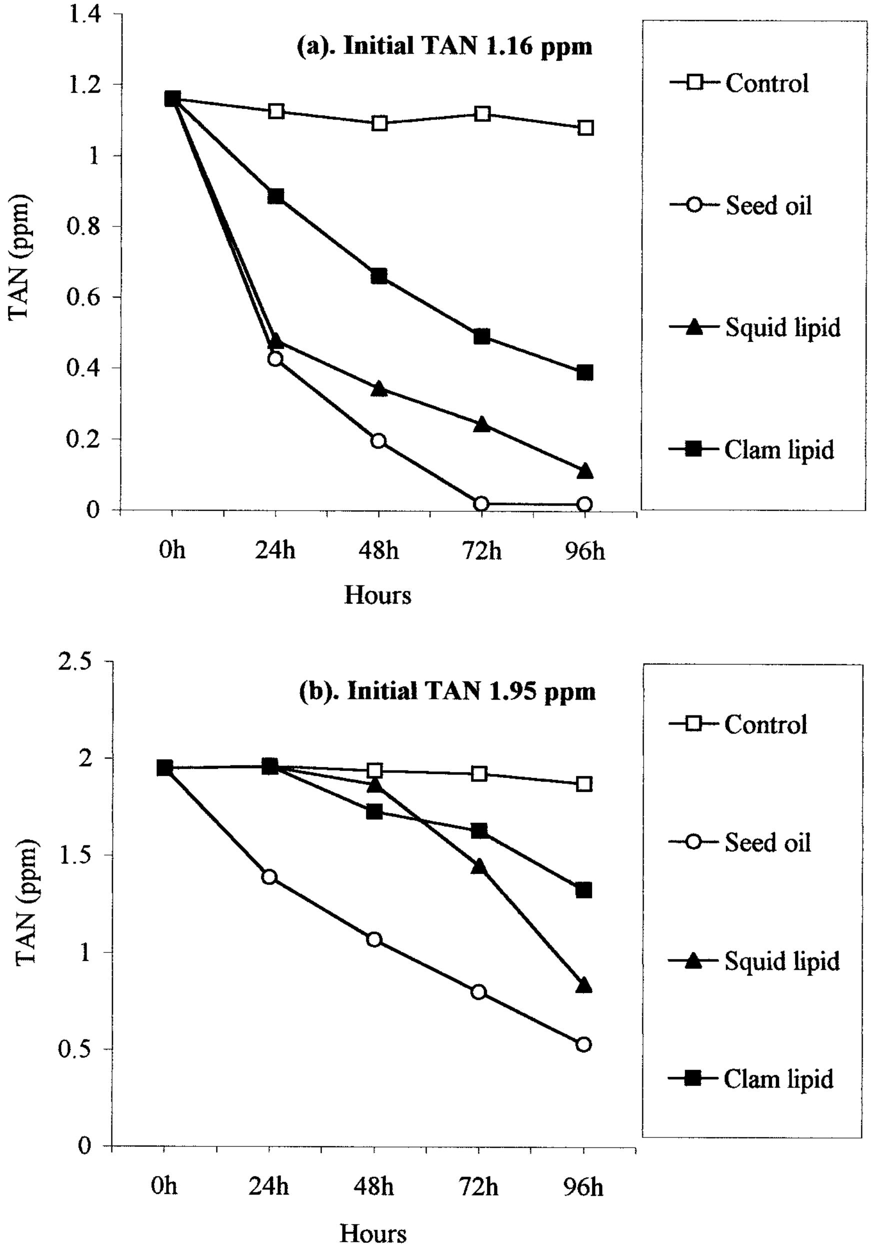

Figure 2. Effect of plant & animal extracts on ammonia removal.

with aluminum foil. Further, water samples were analyzed for measurement of ammonia and nitrite at daily intervals by the standard method (Strickland and Parsons 1972) using spectrophotometer (Hitachi U-2000). Other water quality parameters such as pH, salinity, alkalinity and dissolved oxygen (DO) were also analyzed by standard methods (Strickland and Parsons 1972) at an interval of two days.

The data was statistically analyzed using 5 x 3 Factorial Completely Randomized design with two replications. Duncan's multiple range test was applied to identify significant differences between main effects and interaction effects. M-STATC statistical software was employed to perform statistical analysis.

RESULTS AND DISCUSSION

A preliminary experiment was conducted to arrive at the optimum dose of custard apple seed oil in decreasing ammonia level. The effect of various concentrations of oil ranging from 30 to 180 mg/L on the removal of 1.16 ppm ammonia is presented in Fig.1(a). This shows that ammonia removal was maximum within 24 hours and this has decreased 1.16 ppm to 0.633 (44%), 0.5 (56%), 0.427 (63%), 0.379 (66%), 0.42 (63%) and 0.381 ppm (66%) with 30, 60, 90, 120, 150 and 180 mg/L of oil respectively. There was a further decline and after 48 hours, total ammonia nitrogen (TAN) levels were 0.282 (75%), 0.282 (75%), 0.196 (83%), 0.229 (80%), 0.19 (83%) and 0.28 ppm (75%) and after 96 hours, levels were 0.214 (81%), 0.134 (88%), 0.02 (98%), 0.05 (96%), 0.025 (98%) and 0.025 ppm (98%) respectively. Thereafter, there was no significant reduction in TAN concentration. From the figure, it is evident that oil @ 90 mg/L is effective in decreasing TAN level of 1.16 ppm to bare minimum in 96 hours.

The effects of seed oil from custard apple @ 90 mg/L on nitrite removal (initial concentration 0.92 ppm) is presented in Fig.1(b). This showed that seed oil decreased the nitrite from 0.92 ppm to 0.786 (15%) within 48 hours. There was further removal and after 96 hours, nitrite level was 0.725 (21%).

The effects of seed oil from custard apple and lipids from squid and clam tissues @ 90 mg/L on ammonia removal (initial concentration 1.16 ppm and 1.95 ppm) is presented in Fig.2. This shows that all the treatments registered decrease in ammonia levels. Squid lipid and clam lipid decreased the ammonia from 1.16 ppm to 0.345 (70%) and 0.662 ppm (43%) respectively within 48 hours. There was further removal and after 96 hours, ammonia levels were 0.115 (90%) and 0.392 ppm (66%) (Fig.2a). Thereafter, there was no significant reduction in ammonia level. In the case of 1.95 ppm ammonia concentration, 57 % and 32 % ammonia removal (Fig.2b) were achieved with squid and clam lipids respectively, in 96 hours.

Statistical analysis for the comparison of efficacies of plant and animal extracts with respect to 1.16 and 1.95 ppm initial ammonia concentrations are given in the Table-1. For both the initial ammonia concentrations, duration and materials have significant ($p<0.05$) effects on ammonia removal. Interaction between duration and materials was also found to be significantly different ($p<0.05$). It has been observed that the rate of

Table 1. Comparison of efficacies of the plant and animal extracts for the removal of ammonia and nitrite from brackish water.

Extract	Mean		
	Initial TAN 1.16 ppm	Initial TAN 1.95 ppm	Initial Nitrite 0.92 ppm
Custard apple seed oil	0.3646^{C}	1.148^{C}	0.7612^{A}
Squid Lipid	0.4688^{B}	1.613^{B}	0.4614^{C}
Clam Lipid	0.7184^{A}	1.719^{A}	0.5966^{B}

Extract	Hours	Mean		Hours	Mean
		Initial TAN 1.16^{A} ppm	Initial TAN 1.95^{A} ppm		Initial nitrite 0.92^{A} ppm
Custard apple seed oil	24h	0.4270^{DE}	1.39^{C}	48	0.786^{B}
	48h	0.1960^{G}	1.070^{D}	96	0.725^{C}
	72h	0.02^{I}	0.8^{E}	144	0.725^{C}
	96h	0.02^{I}	0.5310^{F}	192	0.65^{D}
Squid lipid	24h	0.4790^{D}	1.958^{A}	48	0.663^{D}
	48h	0.3450^{F}	1.868^{A}	96	0.447^{G}
	72h	0.2450^{G}	1.45^{C}	144	0.257^{H}
	96h	0.1150^{H}	0.838^{E}	192	0.02^{J}
Clam lipid	24h	0.8850^{B}	1.96^{A}	48	0.759^{B}
	48h	0.6620^{C}	1.728^{B}	96	0.615^{E}
	72h	0.4930^{D}	1.63^{B}	144	0.497^{F}
	96h	0.3920^{EF}	1.326^{C}	192	0.192^{I}

Means in the vertical row with different superscripts are significantly different ($p \leq 0.05$)

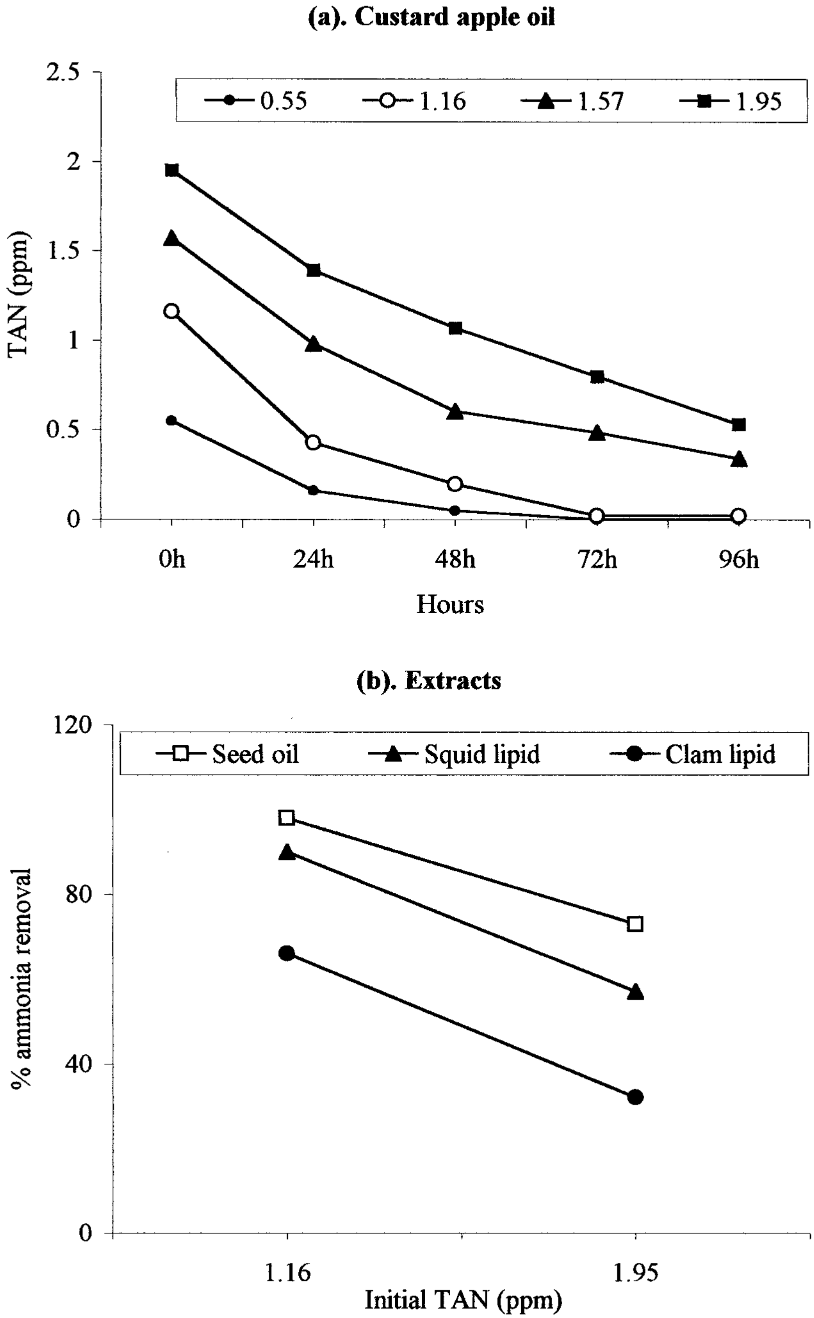

Figure 3. Effect of initial concentration on TAN removal using custard apple seed oil and lipids from squid and clam tissues.

ammonia removal for all the three materials has been decreased as time period increases. However, in the cases of initial ammonia concentrations of 1.16 ppm with seed oil treatment and 1.95 ppm with squid lipid treatment, there were no significant difference between 72 and 96 hours and 24 and 48 hours respectively. Similarly, in the case of clam lipid, ammonia removal after 48 and 72 hours were also not significantly different. For both the initial ammonia concentrations, ammonia removal by clam lipid after 96 hours can be achieved using the custard apple seed oil within 24 hours. Similarly, the amount of ammonia removal by squid lipid after 72 hours can be achieved using seed oil within 24 to 48 hours. From these analyses, it is evident that ammonia removal was highest for custard apple seed oil followed by squid lipid throughout the experiment.

The effects of lipids from clam and squid tissues @ 90 mg/L on nitrite removal (initial concentration 0.92 ppm) is presented in Fig.1(b). This showed that squid lipid and clam lipid decreased the nitrite from 0.92 ppm to 0.663 (28%) and 0.759 ppm (18%) respectively within 48 hours. There was further removal and after 96 hours, nitrite levels were 0.447 (51%) and 0.615 ppm (33%) in respectively. Squid and clam lipids have further decreased the nitrite levels and after 144 hours they were 0.257 (72%) and 0.497 ppm (46%) and after 192 hours, levels were 0.02 (98%) and 0.192 ppm (79%) respectively. Nitrite removal was highest for squid lipid followed by clam lipid. This also has been substantiated by statistical analysis (Table-1).

The effect of initial ammonia concentrations on the ammonia removal for custard apple seed oil, squid and clam lipids (@ 90 mg/L) is shown in Fig.3. From this it is evident that seed oil decreased the ammonia (TAN) by 71%, 63%, 37%, 29% within 24 hours and 91%, 83%, 61% and 45% within 48 hours for 0.55, 1.16, 1.57 and 1.95 ppm initial ammonia concentrations respectively. There was further removal in ammonia and after 96 hours, 100%, 98%, 78% and 73% were achieved (Fig.3a). Figure-3 showed that percent ammonia removal decreases with increase in initial concentration of ammonia with seed oil and lipids @ 90 mg/L.

During the course of experiment, other water quality parameters such as pH, salinity and alkalinity did not show much changes among all the treatments throughout the course of the experiment and they ranged from 7.37-7.89, 19-21 ppt and 96-102 ppm respectively. Oil from custard apple seeds and lipids from squid and clam tissues decreased dissolved oxygen (DO) from 4.8 to 4.0, 4.4 and 4.6 ppm respectively in 48 hours.

To summarize, we can infer from the present study that oil from custard apple seeds and lipids from animal tissues are capable of removing ammonia from brackish water. Percentage ammonia removal was found to decrease with an increase in initial ammonia concentration. Lipids from squid and clam tissues decreased nitrite effectively. Although there was a slight decrease in dissolved oxygen (DO) with these treatments, but it was within safe permissible limit. Essential oils from some plants possess antimicrobial activity (Cowan 1999). Custard apple products also are effective organic antibacterial (Vohra et al. 1975), antiparasitic (Tiangda et al. 2000) and antiviral agents (Wu et al. 1996). In addition, lipids are used in animal feed for the optimum growth of shrimp and fishes. Hence the use of natural products from plant and animals may offer a selective, harmonious and eco-friendly approach to remove toxic nitrogenous compounds from brackish water aquaculture systems. However, internal use cannot be recommended without carrying out the bioassays and toxicological studies.

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