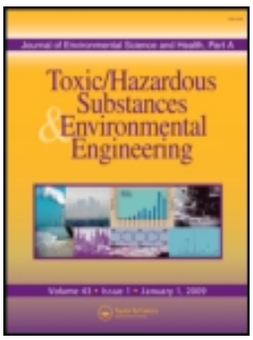

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STUDIES ON THE USE OF NEEM PRODUCTS FOR REMOVAL OF AMMONIA FROM BRACKISHWATER

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ABSTRACT

Laboratory experiments were carried out to investigate the ammonia removal from brackishwater (Salinity 16 ± 1 ppt) using natural plant products such as seed powder and seed oil from neem (*Azadirachta indica*) and commercially available neem products—neemazal and neemgold. The experimental results showed that ammonia removal was effective with 90 mg/l of neem oil, whereas, neem seed powder at 90 mg/l registered an increase in ammonia levels throughout the course of the experiment. Neem oil, neemazal, and neemgold at 90 mg/l were effective in decreasing the total ammonia nitrogen (TAN) level of 0.40–0.45 mg/l in 96 h. The effect of initial ammonia concentrations on the ammonia removal using neem oil revealed that percentage ammonia removal decreased with an increase in initial ammonia concentration.

Key Words: Neem products; Total ammonia nitrogen; Brackishwater

INTRODUCTION

Traditional aquaculture activities require a considerable amount of land and water resources. Consequently, they are becoming relatively unecon-

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omical due to increasing costs and decreasing availability of these resources. A recent trend is to practice semi-intensive and intensive aquaculture that requires a significantly less amount of land and water resources (1–2). High-density aquaculture in a limited aquatic space may lead to a deterioration of pond water quality. A major issue to be addressed first in connection with the aquaculture water quality is the high ammonia concentration (3–4). Ammonia in the aquaculture ponds is generated primarily by high excretion and decomposition of unconsumed food. Total ammonia consists of the relatively nontoxic ionized form in equilibrium with the highly toxic un-ionised form. The acceptable levels of this chemical compound in the aqueous solution for a normal shrimp and fish aquaculture activity is quite low (4–6). Ammonia removal thus becomes necessary if the water quality is to be sufficiently maintained for a normal aquacultural activity (1–2). This can prevent the deterioration of pond conditions, and thereby, reduce the stress on the animals (7).

Several methods have been used for removal of total ammonia nitrogen (TAN). The most common procedures are water exchange, aeration (8–9), and ion exchange using zeolite (10–12). Water exchange can be effective only if sufficient water of a lower TAN concentration is made available to rapidly exchange a large volume of the pond water (8). Aeration would increase the removal of ammonia, but it has little promise for reducing TAN concentrations in ponds (8). Natural and synthetic zeolites are ion-exchange media that cannot reduce ammonia concentrations effectively in ponds (8). The amount of zeolite required to significantly reduce TAN concentrations in ponds is prohibitively large and would be impractical. Bacterial augmentation products are sometimes applied to shrimp ponds in an effort to remove ammonia concentration (13). However, application of these products seems redundant, as bacteria in these products are already available naturally in ponds (14). Further, there are no conclusive reports of the benefits of bacterial augmentation of ponds for ammonia removal (8). Nitrification processes (15–17) and ozonation (18) are other methods sometimes used for ammonia removal. Although these methods have been found to be efficient for ammonia removal, they are relatively expensive. However, the use of herbal products and medicinal plants is safe, ecofriendly, and cheap (19). In the present study, an attempt has been made to use various natural plant products, such as seed powder and seed oil from neem (*Azadirachta indica*), and commercially available neem-based natural products, such as neemazal and neemgold, to treat brackishwater containing a high concentration of total ammonia nitrogen.

MATERIALS AND METHODS

Fresh dried seeds of neem (*A. indica*) were crushed and powdered in an electric mixer and passed through a 60 mesh sieve. This seed powder was used

in the experiment. In order to prepare neem oil, the powdered material was extracted with hexane (HPLC grade, E. Merck). The solvent was allowed to evaporate, and the remaining oil was transferred to a volumetric flask and stored in a refrigerator.

Commercially Available Neem Products

Neemazal

This is a neem oil-based antifeedant pesticide preparation that contains 1% azadirachtine, 46.3% neem oil, and 49.7% polyethylene monosorbital oleate.

Neemgold

This is also a neem oil-based antifeedant pesticide preparation that contains 0.03% azadirachtine and other relevant ingredients, 90.57% neem oil, 5% hydroxy EL, 0.5% epichlorohydrin, and 3.9% aromox.

The brackishwater for the experiment was prepared by mixing sea water with fresh water to attain a salinity of 16 ± 1 ppt. An appropriate amount of ammonium sulfate (E. Merck) was added in brackishwater to attain various initial TAN concentrations ranging from 0.16 to 0.806 mg/l. For each experimental run, 2 l of the prepared water was poured into the conical flask. Thereafter, varying amounts of the neem-based natural and commercial products were added to the flasks and covered with aluminum foil. Further, water samples were analyzed for measurement of TAN at daily intervals for a period of 6 days.

Analytical Methods

Ammonia was determined by the standard method (20) using a spectrophotometer (Hitachi U-2000). Other parameters such as pH, dissolved oxygen (DO), alkalinity, and salinity were analyzed by standard methods (20,21) at an interval of 2 days. Duncan's multiple range test was deployed for the statistical analysis of the results.

RESULTS AND DISCUSSION

Determination of Effective Amount of Neem Oil for Ammonia Removal

A preliminary experiment was conducted to ascertain the optimum dose of neem seed oil for decreasing ammonia levels. The effect of various

concentrations of neem oil ranging from 10 to 180 mg/l on the removal of 0.575 mg/l TAN in 120 h is shown in Figure 1. TAN level decreased from 0.575 to 0.476 (17%), 0.326 (43%), 0.296 (48%), 0.257 (55%), 0.27 (53%), 0.273 (53%), and 0.241 (58%) in 48 h with 10, 30, 60, 90, 120, 150, and 180 mg/l of neem oil, respectively. There was a further decline in TAN level, and after 96 h, ammonia concentrations were 0.473 (18%), 0.246 (57%), 0.187 (67%), 0.12 (79%), 0.15 (74%), 0.157 (73%), and 0.118 (79%) mg/l with 10, 30, 60, 90, 120, 150, and 180 mg/l of neem oil, respectively. After 96 h, there was no significant reduction in TAN concentration. From this, it is evident that oil at 90 mg/l is effective in decreasing a TAN level of 0.45 mg/l in 96 h.

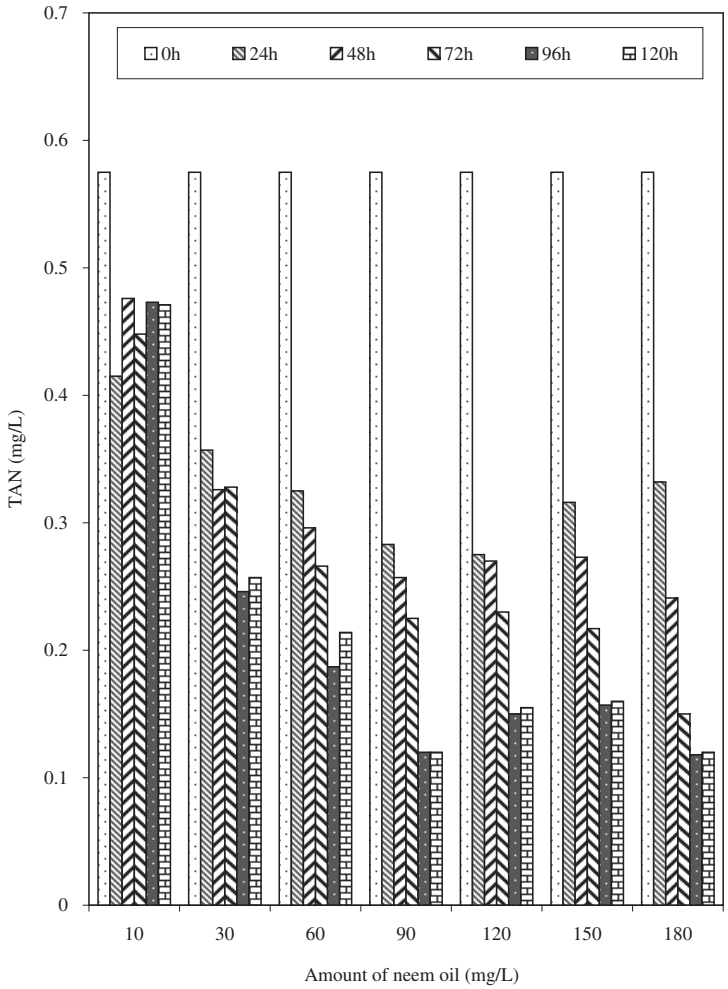

Figure 1. Determination of effective amount of neem oil for ammonia removal (Initial TAN 0.575 mg/l).

Effect of Other Neem Products on Ammonia Removal

Studies on the use of other neem products, such as neem seed powder and commercially available neem products such as neemazal and neemgold at 90 mg/l, were conducted with 0.575 mg/l concentration of ammonia. The results are shown figuratively in Figure 2. It is evident that both treatments registered a decrease in TAN level. Neemazal and neemgold decreased the ammonia (TAN) from 0.575 to 0.275 mg/l (52%) and 0.185 mg/l (68%) within 48 h, respectively. After 96 h, there was a removal to the extent of 69 and 79%. From Figure 2, it is evident that these products at 90 mg/l are effective in ammonia removal of 0.4–0.45 mg/l in 96 h. In the case of treatment with neem seed powder (at 90 mg/l), the ammonia level was found to increase substantially from 0.575 mg/l to as high as 1.18 mg/l (105%) in 48 h and 1.4 mg/l (143%) in 96 h.

Effect of Initial Ammonia Concentration on Ammonia Removal Using Neem Oil

The effect of initial ammonia concentrations at 0.16, 0.215, 0.375, 0.428, 0.48, 0.575, 0.806, and 1.74 mg/l on the ammonia removal with neem oil at 90 mg/l at $29^{\circ}C \pm 1$ is shown in Figure 3. These initial concentrations were selected on the basis of efficacy of neem products for ammonia removal. In

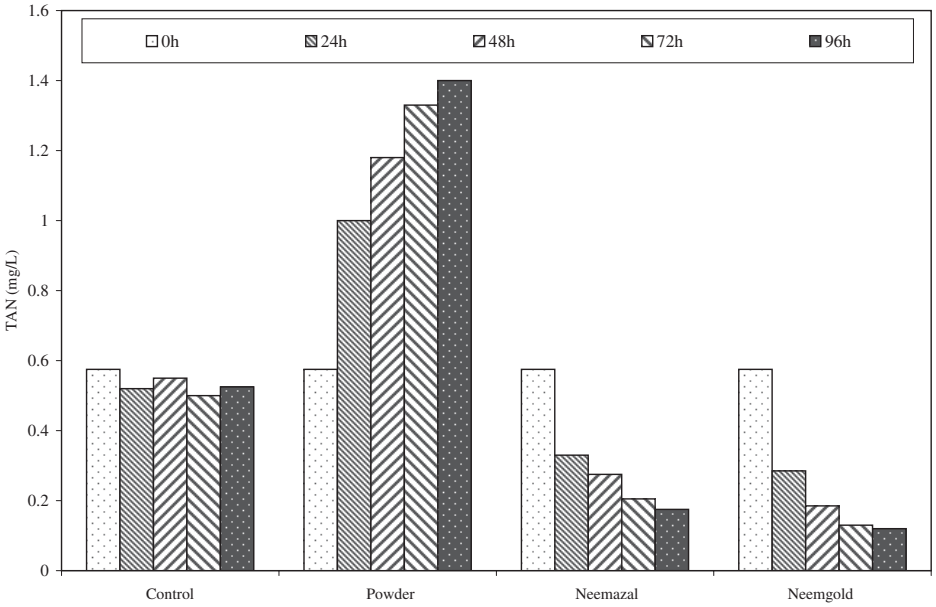

Figure 2. Effect of three neem oil-based products on ammonia removal (Initial TAN 0.575 mg/l).

this case, the percentage ammonia removal appears to be decreasing from 0.16 to nil (100%), 0.215 to 0.05 (77%), 0.375 to 0.057 (85%), 0.428 to 0.072 (83%), 0.48 to 0.139 (71%), 0.575 to 0.258 (55%), 0.806 to 0.573 (29%), and 1.74 to 1.411 (19%) in 48 h, respectively. There was further removal, and after 96 h, ammonia decreased up to nil (100%) for initial concentrations 0.16, 0.215, 0.375, and 0.428 mg/l. For 0.48, 0.575, 0.806, and 1.74 mg/l initial ammonia concentrations, ammonia decreased up to 0.035 (93%), 0.12 (79%), 0.493 (39%), and 1.289 (26%), respectively. This indicated that percent ammonia removal decreases with increasing initial ammonia concentrations from 0.16 to 1.74 mg/l up to 96 h of the treatment (Figure 4).

Effect of Initial Ammonia Concentration on Ammonia Removal Using Commercially Available Neem Products

Studies on the use of neemazal and neemgold at 90 mg/l were conducted with 0.375, 0.428, 0.48, 0.575, and 0.806 mg/l initial concentration of

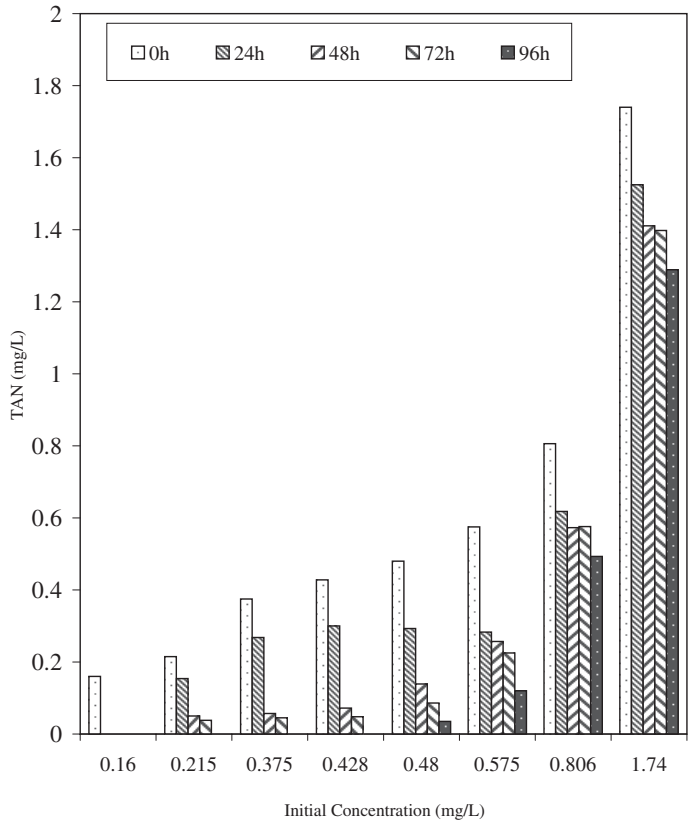

Figure 3. Effect of initial concentration on TAN removal using neem oil.

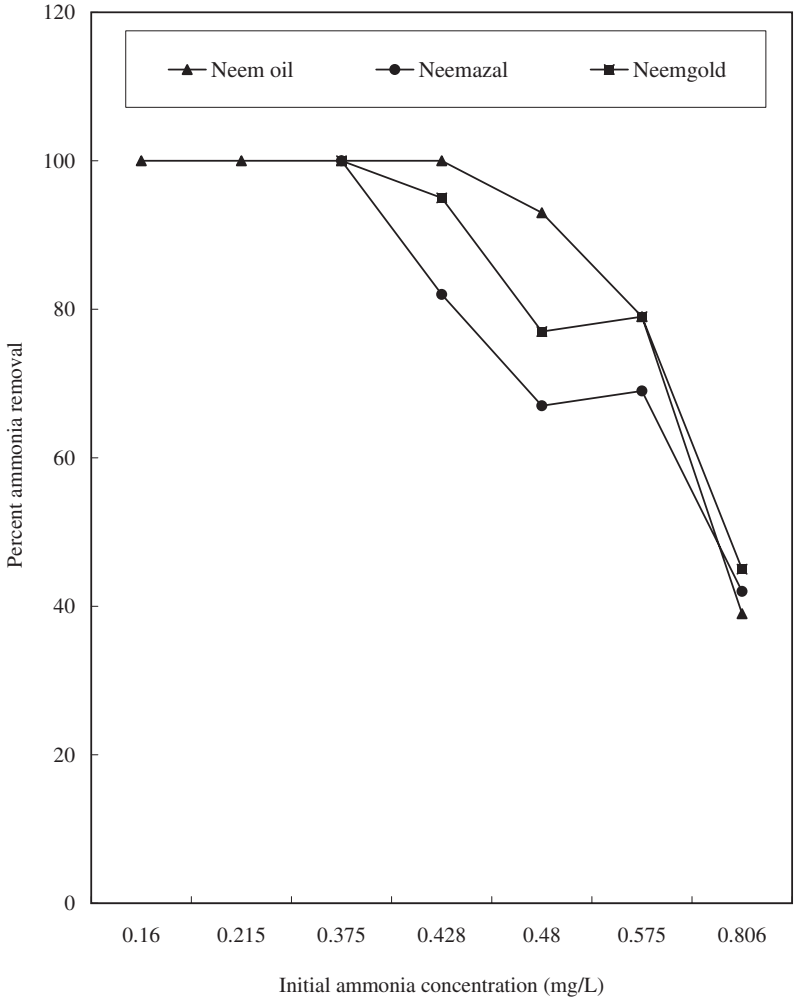

Figure 4. Effect of initial concentrations on percent TAN removal after 96 h using neem oil and commercially available neem products.

ammonia, and the results are shown in Figures 5(a) and 5(b). From this, it is evident that neemazal and neemgold decreased the ammonia (TAN) by 82 and 95% for 0.375 mg/l initial TAN, 65 and 84% for 0.428 mg/l TAN, 51 and 66% for 0.48 mg/l TAN, 52 and 68% for 0.575 TAN, and 26 and 35% for 0.806 mg/l initial ammonia concentration within 48 h, respectively. There was further removal, and after 96 h, there was 100% removal for 0.375 mg/l TAN for both the products, 82 and 95% removal for 0.428 mg/l TAN, 67 and 77% removal for 0.48 mg/l TAN, 69 and 79% removal for 0.575 mg/l TAN, and 42 and 45% removal for 0.806 mg/l TAN concentration. Figure 4 showed that percent ammonia removal decreases with an increase in initial concentration of ammonia from 0.375 to 0.806 mg/l.

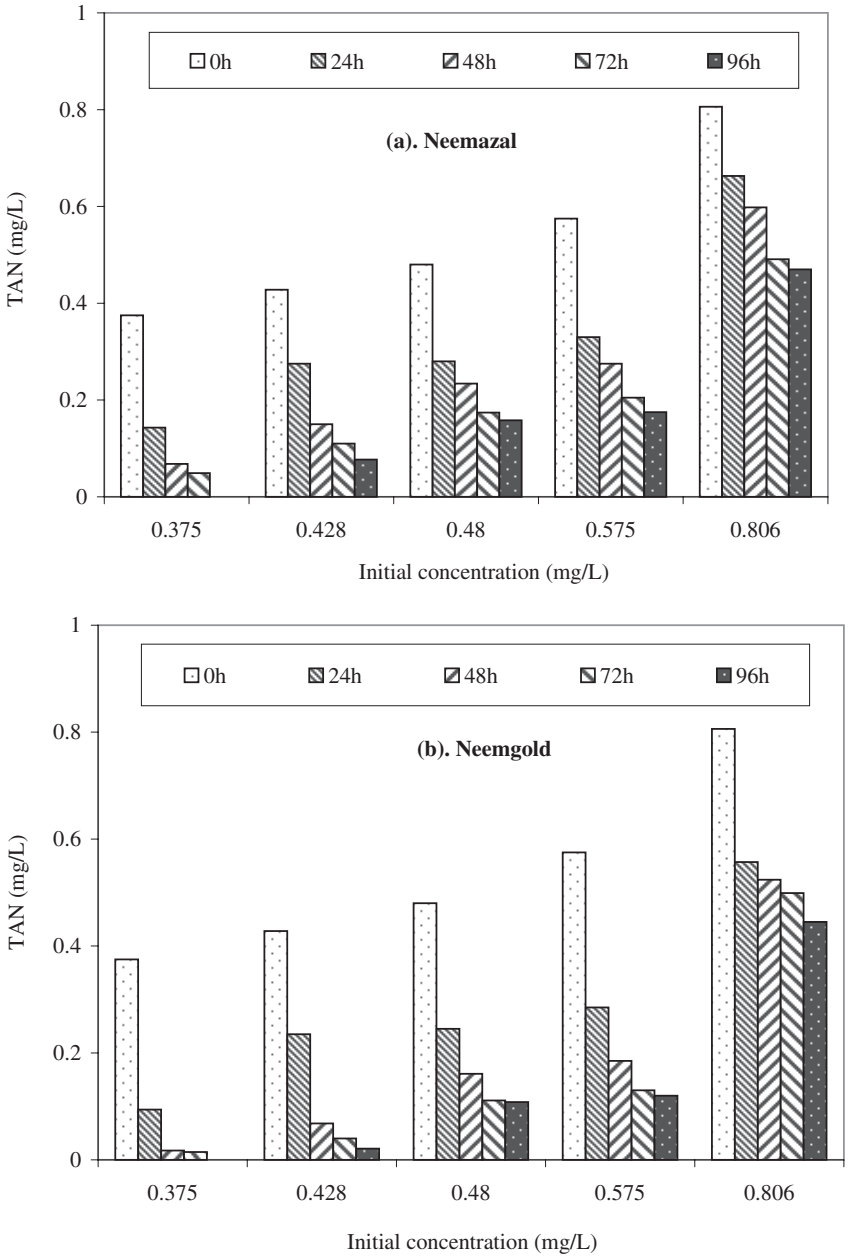

Figure 5. Effect of initial concentration on TAN removal using neemazal and neemgold.

Statistical data for the comparison of efficacies of three neem products with respect to initial ammonia concentrations are given in Table 1. From this, it is evident that there was significant difference in the efficacies of neemgold and neemazal for 0.428 and 0.575 mg/l initial TAN

Table 1. Duncan's Multiple Range Test for the Comparison of Efficacies of the Three Neem Oil-Based Products

Initial TAN (mg/l)	Neem Oil	Neemgold	Neemazal
0.375	0.1445^{A}	0.1002^{A}	0.1243^{A}
0.428	0.1696^{AB}	0.1586^{B}	0.2080^{A}
0.48	0.2066^{B}	0.2210^{AB}	0.2652^{A}
0.575	0.2920^{AB}	0.2590^{B}	0.3123^{A}
0.806	0.6132^{A}	0.5662^{A}	0.6056^{A}

Values in the horizontal row followed by different letters are significantly different ($p \leq 0.05$).

concentrations, whereas, efficacy of neem oil was not significantly different from these products. In the case of 0.48 mg/l initial TAN concentration, the difference in efficacies of neem oil and neemazal was significant, whereas, efficacy of neem gold was not significantly different from these products. As far as the initial TAN concentrations, viz. 0.375 and 0.805 mg/l, were concerned, there were no significant differences in the efficacies of these neem products, and all of them were equally effective in removing ammonia.

Effect of Neem Products on Other Water Quality Parameters

During the course of the experiment, water quality parameters such as temperature, pH, salinity, dissolved oxygen (DO), and alkalinity were also monitored at 2-day intervals. It has been found that except in control, dissolved oxygen was found to decrease in all the treatments. Neem oil has slightly decreased the DO from 7.0 to 5.0 mg/l in 96 h. However, plant seed powders registered a substantial decrease in DO up to 2.8 mg/l. Other parameters such as temperature, pH, salinity, and alkalinity did not show many changes among all the treatments throughout the course of the experiment, and they ranged from 28–30°C, 7.0–8.3, 16–18 ppt and 111–121 as milligrams $CaCO_3$ per litre, respectively.

CONCLUSIONS

Ammonia removal by neem products was investigated. Neem seed powder has been found to substantially increase the ammonia level of brackishwater with low dissolved oxygen, which can be attributed to the conversion of nitrogen containing compounds present in the seed powder into total ammonia-N. Neem oil and other neem-based commercial products were found to be capable of removing ammonia from brackishwater. It has

been found that 90 mg/l oil can remove 0.40–0.45 mg/l ammonia from brackishwater. Percentage ammonia removal was found to decrease with an increase in initial ammonia concentration. Although there was a slight decrease in DO with these treatments, it was well within optimum levels. Further studies are in progress to find out the actual compound/class of compounds of neem oil and, for ammonia removal from brackishwater and its mechanism.

Neem trees help to protect the environment not only by reducing soil erosion but also by helping to keep the atmosphere free of pollutants, moderating temperature and also mitigating air pollution. Neem seeds contain 35–40% oil. They have been widely used in the traditional Indian system of medicines like Ayurveda. Scientific research has confirmed that Neem seed oil and oil extracts are nontoxic to mammals and are effective organic antifungal (22,23), antibacterial (24,25), and antiviral (26) agents. In recent years, neem products are increasingly being used in aquaculture as antibacterial agents (27) and immunostimulants. The main constituents of this oil are glycerides. They contain saturated and unsaturated fatty acids. Neem oil and its formulated product contain the active ingredient azadirachtin, which has proven insecticidal activity. It breaks down rapidly in 100 h in water or light and would not cause long-term effects. The potential for mobility in soil is very low for the formulated product, and accumulation in the environment is not expected. Hence, the use of neem-based products may offer a selective, harmonious and eco-friendly approach to remove toxic ammonia in water quality management. Although neem oil has been made safe for consumption, internal use cannot be recommended without carrying out the bioassays and toxicological studies. Large-scale field testing for suitability is mandatory, to screen various neem formulations biologically.

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