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Advanced nanoparticle-based treatment of aquafarm and hatchery effluents: The role of chitosan and chitosan TPP in water purification

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Abstract

A healthy economy and the multifaceted growth of society depend on access to clean, safe water. Rapid population growth, growing industrialization, urbanization, and widespread agricultural activities have led to the production of wastewater that has made the water not only contaminated or lethal, but also dirty or polluted. Every year, millions of people pass away from diseases spread by drinking water tainted with harmful microorganisms. Although many different approaches to wastewater treatment have been researched over the past few decades, their application is constrained by a number of factors, such as the need for chemicals, the production of disinfection by-products (DBPs), the length of the process, and the cost. In order to create new structures, devices, and systems with superior electronic, optical, magnetic, conductive, and mechanical properties, nanotechnology, which involves manipulating matter at the molecular or atomic level, is becoming more and more popular. This promising technology has accomplished amazing feats in a number of industries, including wastewater treatment. Nanomaterials are well suited for use in wastewater treatment because of their high surface to volume ratio, high sensitivity and reactivity, high adsorption capacity, and simplicity of functionalization. The methods being explored for wastewater treatment utilizing nanotechnology have been discussed in this article and are based on adsorption and biosorption, nano-filtration, photocatalysis, disinfection, and sensor technologies. The fate of the nanoparticles in wastewater treatment and the dangers of their use are also highlighted in this review. The present study carried by Evaluation of various physico-chemical parameters of shrimp farm and hatchery effluents such as alkalinity, electrical conductivity, total hardness, total suspended solids, total ammonia, BOD, COD, was done before and after treatments in laboratory scale. From the results of the present investigations chitosan and chitosan TPP nanoparticles showed good coagulating properties, and has many advantages compared to chemical coagulants and does not affect the pH, alkalinity or conductivity of the water. Further multifunctional environmentally friendly chitosan should play a larger role in the recycling of aquaculture wastewater.

Keywords: Aquafarm wastewater, Hatchery wastewater, Chitosan, Chitosan TPP nanoparticles, Physico chemical parameters

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Introduction

Because there appears to be a detrimental impact on the environment from the release of waste water, the aquaculture business has been a target for criticism from environmental groups (Doupe *et al*., 1999; Gandhi *et al*., 2013). To be in compliance with the Water Resource Act (1995) for resource protection and sustainable development, fish farming enterprises in developing nations like Canada are required to obtain a certificate of approval. For the treatment and disposal of waste water, an aquaculture industrial management plan was created recently (Fernandes *et al*., 2001; Priyamvada *et al*., 2013; Gandhi *et al*., 2024a). In order to safeguard the aquatic ecosystem and allow for the reuse of water sources, aquaculture not only needs clean water to be supplied to it, but it also needs to release that water into the environment. Environmental control organizations around the world put a lot of pressure on aquaculture to purify wastewater before releasing it into the environment (Bunting *et al*., 2001; Gandhi *et al*., 2024b; Priyamvada *et al*., 2012). Volumes of material have been written about the potential environmental implications of aquaculture industrial operations on freshwater and marine systems as a result of the industry's fast global expansion. The main waste water problems are hyper-nutrification and eutrophication, which lead to algae blooms, oxygen depletion, and deprivation of benthic habitat in the vicinity of open cage operations with no waste collection system and inadequate flushing (Boyd, 2001; Gandhi *et al*., 2016). As a result, the aquaculture sector has realised that in addition to a constant supply of clean water, they also need to create technologies for wastewater treatment. The improvement of wastewater treatment technology in the aquaculture sector will reduce environmental and social issues and increase the industry's long-term economic security (Doupe *et al*., 1999; Gandhi *et al*., 2018a).

Aquaculture wastewater

Aquaculture's operation from hatcheries and farmed systems results in wastewater creation. There are typically three operating systems for aquaculture, as depicted in Figure 1. Depending on the nature and location of the aquaculture system, different amounts and types of effluent are produced during aquaculture operations (Dochoda *et al*.,1999; Gandhi *et al*., 2024a). In terms of waste quality and quantity, the waste water from a hatchery differs from that of a production farm (Oberdorff and Porcher, 1994; Vinusha *et al*., 2017). Better technology is also required for the regulation of waste water in pond or tank systems, such as those typically used to raise catfish and tilapia. Because the cage and pen systems used to produce salmon and other species are relatively open to natural water, they have the potential to leak untreated effluent into the environment (Rebecca and Triplett, 1997; Gandhi *et al*., 2024b).

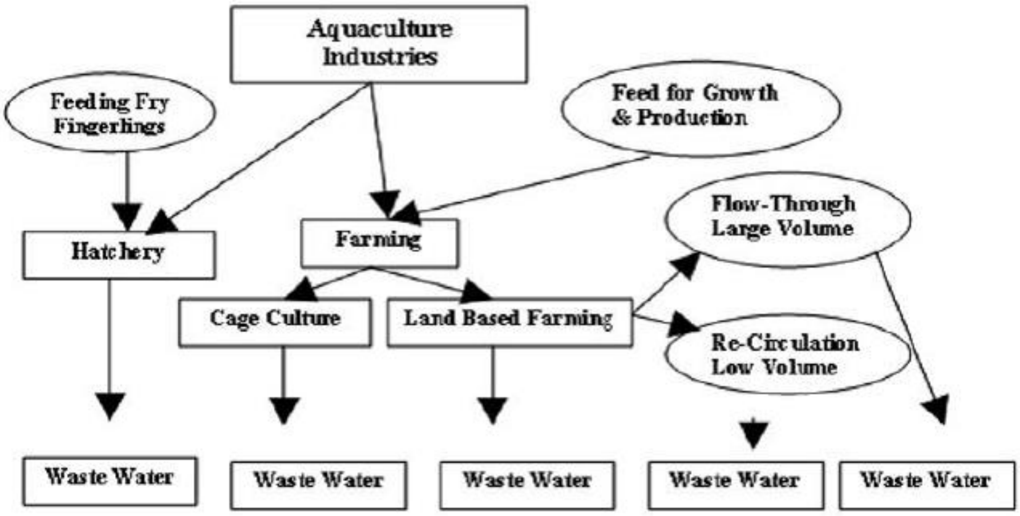

Figure 1: Various sources of generation of wastewater at aquaculture industries.

Aquaculture wastewater composition

The type of feed provided to the species being raised, the amount fed, and the type of system in use are all directly related to the composition of the wastewater produced by aquaculture. The primary sources of waste from aquaculture are untreated water including excrement, faeces, and uneaten fish feed. However, the overall organic output from a salmon farm may be close to 2.5 tonnes wet weight for every tonne live weight fish, according to estimates (Ackefors and Enell, 1994). Aquaculture effluent primarily contains the elements nitrogen and phosphorus (Axler *et al*., 1996). The majority of the suspended solids are made up of food and faeces. Aquaculture waste is therefore influenced by the feed's composition and feeding methods. Aquaculture wastes are kept within the system if the concentration of suspended solids (SS), chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen, and total phosphorus in the exit from the aquaculture plant is lower than in the intake. As a result, aquaculture wastewater treatment technology depends not only on the culture system but also on the makeup of the wastewater generated by the specific fish production facility.

Every day, wastewater effluents from aquaculture ponds are released into the environment, containing a small amount of persistent organic pollutants. They harm the entire ecology, without a doubt. Ion exchange and reverse osmosis haven't been able to effectively remove ammonia from wastewater effluents because of their intrinsic limitations, claim Seruga *et al*. (2019).If the issue is not urgently solved, the occurrence of ammonia linked to persistent organic pollutants (POPs) in waste water is likely to continue in the future. Wastewater poses a severe threat to both human health and the environment, and if it is not properly treated, it has the potential to drastically change the planet's whole ecology (Gandhi *et al*., 2024c).

Wastewater treatment technology for land based aquaculture industry

Municipal wastewater treatment has been heavily adapted for use in wastewater treatment technologies for land-based aquaculture. One of the simplest ways to lessen waste generated by the aquaculture sector is sedimentation. The fundamental idea behind this technique is to let solid waste, primarily uneaten food and human waste, settle out before releasing effluent water into the environment. In this system, floatable particles can gather on the water's surface while settleable items can sink (Czysz *et al*., 1989; Gandhi *et al*., 2022a). The segregated wastes are taken from the aquaculture chambers' top and bottom and may get additional treatment before being disposed of. As the aquaculture business has grown, numerous technologies have been used to remediate waste water (Daniel and Trudell, 1990; Gandhi *et al*., 2022b).

Since sedimentation requires no energy input and no particular operating knowledge, it is frequently used in commercial fish farming (Daniel and Trudell, 1990; Vinisha *et al*., 2023). The drawback of sedimentation systems is that they frequently need substantial amounts of land (Pilay *et al*., 1992; Gandhi *et al*., 2019). System design, building method, and system operation all affect how much waste is removed by sedimentation in aquaculture. The sinking velocity depends on the diameter and density of the suspended particles (Czysz *et al*., 1989; Gandhi *et al*., 2018b). Small particles or dissolved garbage in water cannot be effectively removed by sedimentation.

The coagulation/flocculation process is a useful technology currently in use. Utilizing organic polymers, such as alum, may speed up settling, save expenses, and enhance sludge quality. Unwanted compounds may unfortunately result from their reactions with additional chemicals introduced during wastewater treatment. When it comes to specific kinds of colloids, they show some selectivity. They add to the organic load and, under certain circumstances, can cause cancer (Mallevialle, 1984; Kawamura, 1991). A significant portion of all the wastewater treatment difficulties are being resolved thanks to the development of new technologies known as clean technologies. Chitin and chitosan are examples of natural polymers that can be employed as flocculent and coagulant aids. Here, chitosan is suggested as a solution to all the issues with synthetic polyelectrolytes and metallic trivalent salts. These substances aid in the bacteria' ability to digest sludge because they are biodegradable. Natural organic polymers lower the amount of sludge generated in comparison to alum. In addition to being an abundant and renewable resource, non-petroleum based, non-toxic, proved to encourage plant growth, re-generable by desorption, and effective in cold water, chitosan has attracted increasing interest in the field of water treatment.

Chitosan and chitosan nanoparticles are utilized in the treatment of wastewater and drinking water as an efficient coagulant/flocculant substitute for typical inorganic coagulants like alum and ferric chloride (Pan *et al*.,1999;

Gandhi *et al*., 2021). Because it is a natural substance that is completely biodegradable and non-toxic, chitosan is ideal for various uses. Chitosan is exceptional in that it can attach to oils, heavy metals, and negatively charged particles. It has been demonstrated to be safe for fish at the usual dosage and is frequently used as a filtration aid in commercial aquariums to produce high clarity water (Rinaudo *et al*., 1989).

The pH of the solution is a key factor in how well conventional coagulants work, hence the pH of the raw water must frequently be carefully adjusted. A high residual concentration of Al^{+3} or Fe^{+3} ions are also produced by the use of alum or ferric chloride in the treated water. Due to its potential link to Alzheimer's disease, the high residual aluminium level in drinking water treatment raises a public health concern. The treated water's Al^{+3} and Fe^{+3} ions may precipitate in the distribution system, resulting in a smaller pipe diameter and a reduction in hydraulic power. Because these metal ions are difficult for microbes to absorb, their buildup in natural water bodies is of concern. The disposal of the sludge generated during the coagulation process is another issue associated with the use of typical iron or aluminium coagulants because both aluminium and iron salts are not biodegradable (Guibal *et al*., 2007). In addition to making the treated water safer, using chitosan as a coagulant/flocculant also helps to prevent a number of issues with sludge disposal from water or wastewater treatment facilities. Because chitin is naturally abundant, using chitosan instead of alum or ferric chloride is more advantageous economically (Iyamua *et al*., 2019; Gandhi *et al*., 2018c).

For the adsorption of dyes and heavy metals in waste water treatment, chitosan composites have been explored (Ravi Kumar, 2000; Vinusha *et al*., 2023). It appears that no significant research has been done on the use of chitosan and chitosan TPP nano particles in the coagulation and flocculation process to clear aqua effluents. As a result, this study was conducted to evaluate the role of chitosan and chitosan TPP nanoparticles in the flocculation process used to clarify aqua effluents under various experimental settings.

Objectives of the study includes

1) Collection of aqua pond and hatchery effluents from Nellore District
2) Treatment of effluent samples with chitosan and chitosan TPP nanoparticles
3) Analysis of various physiochemical parameters before and after treatment

Materials and methods

Collection of aquaculture pond and hatchery effluent samples

Samples of aquaculture pond effluent were taken in duplicate at random times and places around the Nellore area. Kotha Koduru, Muthukuru, Mypadu, Kota, and Gangapatnam are the locations. In the Nellore District, samples of hatchery effluent were taken at the C.P., B.M.R., Mahitha, Alpha, and Blue parks. Samples of effluent were taken from January to May 2017. Sample bottles were cleaned beforehand by soaking them in detergent for 24 hours, then rinsing them in tap water, 5% nitric acid, and then distilled water (APHA, 1992). After gathering the samples, the bottles were filled with the sample, with just a tiny air gap remaining at the top. Paraffin wax was used to stopper and seal the sample vials. Every sample was labeled properly (Table1). Samples were kept in a portable cooler containing ice, to maintain an inert temperature condition for the effluent and were transported to the laboratory for analysis.

Table1: Sampling sites with sampling code in detail (Shrimp Pond and Hatchery effluent sample).

S.No	Effluent Source	Sample ID	Sampling site
01	Pond effluents	A1	Kothakoduru
02		A2	Muthukuru
03		A3	Mypadu
04		A4	Kota
05		A5	Gangapatnam
06	Hatchery effluents	H1	Cp hatchery (Thupilipalem)
07		H2	BMR hatchery (Ramatherdam)
08		H3	Mahitha hatchery (Mypadu)
09		H4	Alpha hatchery (Indukurpet)
10		H5	Blue Park (Vidavaluru)

Treatment of effluent samples with chitosan and chitosan TPP nano particles

In the trials, chitosan and chitosan TPP nanoparticles were utilized to coagulate a sample of aqua effluent waste water utilizing a typical jar test device. It was conducted as a batch test using eight beakers and eight spindle steel paddles in succession. Chitosan and chitosan TPP nanoparticles were prepared and characterized by Vinusha *et al*., (2015), Vinusha and Vijaya (2019), and Vinusha *et al*. (2020). The varying concentrations of nanoparticles (5, 10, 15, 20 mg/mL) were applied to effluent samples (100 mL). After shaking the beakers, 300 rpm flash mixing started right away and continued for 10 minutes. After then, the mixing speed was lowered to 30 rpm and maintained there for 20 minutes. Finally, a 30-minute quiet settling period was permitted. At the end of the settling period, a sample of the supernatant was analyzed for the different various physico-chemical parameters, (Maram *et al*., 2022; Gandhi *et al*., 2016). All tests were performed at an ambient temperature in the range of 26-30°C.

Determination of physico-chemical parameters of the waste water

Different physicochemical characteristics, including ammonia, electrical conductivity, total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), alkalinity, and total hardness, were measured in the samples. The collection, preservation, and analytical methodologies and techniques were carried out in accordance with APHA (2002). To remove large-sized suspended particles, the collected samples were first filtered through a 0.1 mm mesh sieve. According to the appropriate standard procedures, the physio-chemical parameters were assessed before and after treatment with chitosan and chitosan TPP nanoparticles.

Statistic evaluation

GraphPad Prism V5's statistical software programme was utilized to measure the treatment impact by analysis of variance (ANOVA). When the p-values were 0.05 or lower, differences were deemed to be significant.

Results and discussion

Changes in concentration of ammonia (ppm) in aqua pond and hatchery effluents with chitosan and chitosan TPP nano particle application

The amount of ammonia present in the effluents of selected aqua ponds and hatcheris before and after treatment with chitosan and chitosan TPP nanoparticles was estimated and presented in the form of % removal and represented in Figure 2. Before treatment the ammonia concentration ranged from 1.8 to 3.0 ppm in aqua pond effluents and 2.0 to 2.8 ppm in hatchery effluents. The ammonia values were too high in all the samples analyzed. After treatment with chitosan and chitosan TPP nanoparticles ammonia concentration is reduced up to 0.2 ppm in aqua pond effluents and 0.5 ppm in hatchery effluents. The results proved that both chitosan and chitosan TPP nanoparticles showed higher removal of ammonia from aqua pond and hatchery effluents (Figs. 2 and 3). Chitosan TPP nanoparticles showed high efficiency than chitosan.

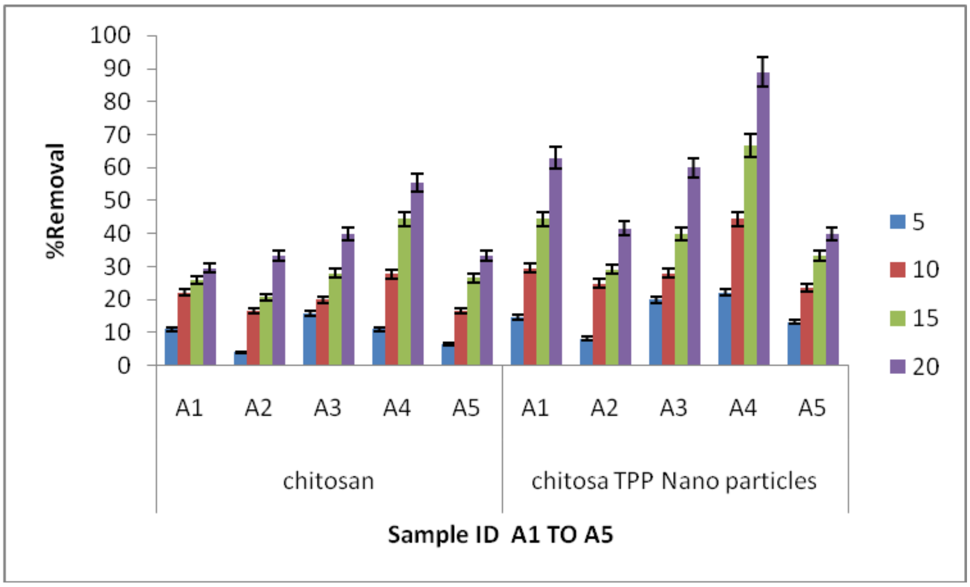

Figure 2: Removal effeciency of ammonia at different dosage of chitosan and chitosan TPP nanoparticles from various aquapond wastewater.

Organic matter in the wastewater often consumes large quantities of oxygen, further inhibits nitrification, and conceals pathogens (Chen, 1995). Hence, their removal is essential regardless of whether they are degradable or non-degradable organic compounds. N-containing compounds are other worrisome substances if the effluent of aquaculture waste water is to be recycled to cultivate aquatic organisms. Among the compounds, NH_3 is usually regarded as the most toxic to aquatic organisms (Handy, 1993; Parvez and Vijaya, 2020). An NH_3 concentration of less than 2.0 mg/L is recommended during the cultivation process (Zweig ,1999).

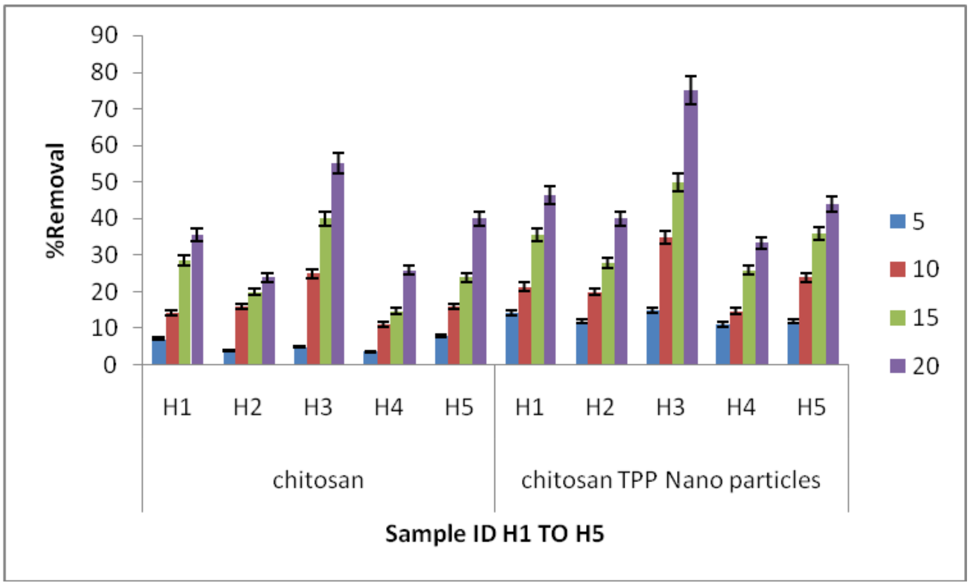

Figure 3: Removal effeciency of ammonia at different dosage of chitosan and chitosan TPP nanoparticles from various hatchery industry effluents.

Two-way ANOVA was employed to find the significant differences in removal of ammonia from aqua pond and hatchery effluents over a concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles (treated). Bonferroni Post-T treplicates by row were performed to compare the significance of means. The considered significant level was at $p<0.05$ by using the statistical software package GraphPad Prism V5 (Figs. 4 and 5).

Changes in electrical conductivity (μs/m) of aqua pond and hatchery effluents with chitosan and chitosan TPP nano particle application

Electrical conductivity (EC) is the ability of an aqueous solution to conduct the electric current. Electrical conductivity ranged from 348-370 μs/cm in aqua effluents and 345-380μs/cm in hatchery effluents. Influence of varied chitosan and Chitosan TPP nanoparticles concentrations on the electrical conductivity of effluents, as well as the electrical conductivity reduction is presented in Figure 6 and Figure 7. Results revealed that the electrical conductivity of effluents has been decreased with increasing chitosan and chitosan TPP nanoparticles concentrations because of the chelation between chitosan and salts as a result to sedimentation of salts then separate via filtration, this process led to decrease the electrical conductivity. After treatment with chitosan and chitosan TPP nanoparticles EC reduced to 78 µs/cm in a pond aqua effluents and 75 µs/cm in hatchery effluents.

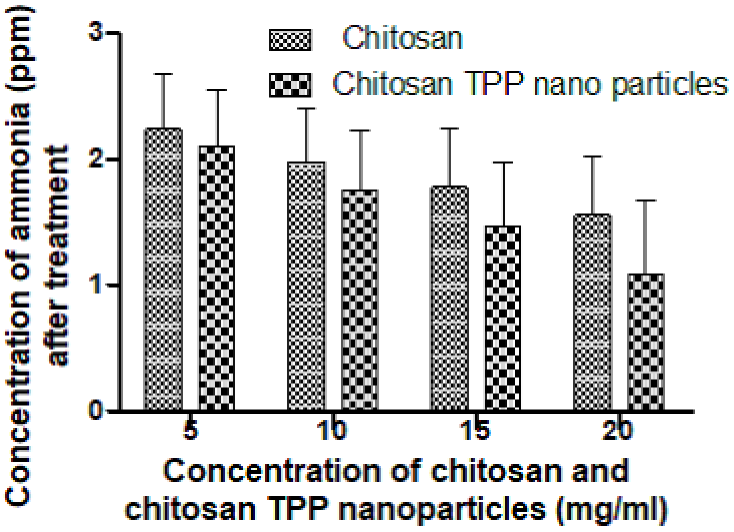

Figure 4: Effect of chitosan and chitosan TPP nanoparticles dosage on Ammonia (PPM) in aqua pond effluents.

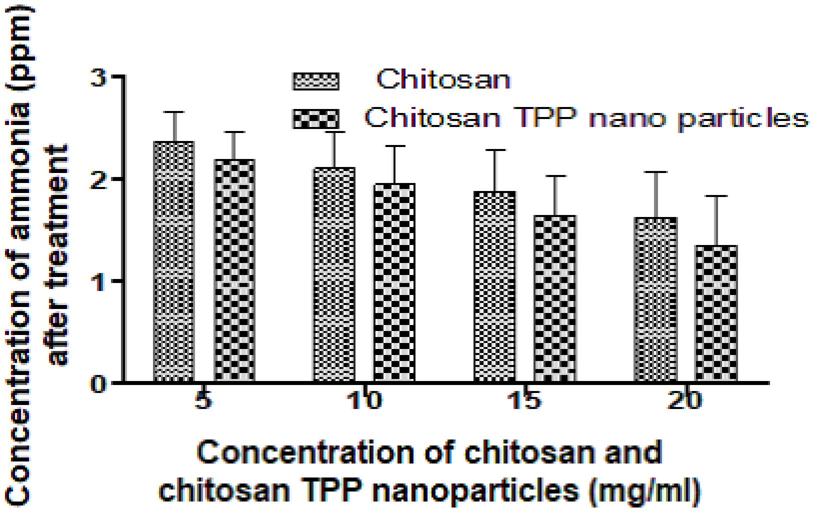

Figure 5: Effect of chitosan and chitosan TPP nanoparticles dosage on Ammonia (PPM) in hatchery effluents.

Chitosan TPP nanoparticles showed more efficiency compared to chitosan in the reduction of EC of effluent samples. According to WHO the desirable limit for EC is 200µs/cm. Compared to the desirable limit, the values of the samples were found to lie within the limit after treatment with chitosan and chitosan TPP nanoparticles and was satisfactory.

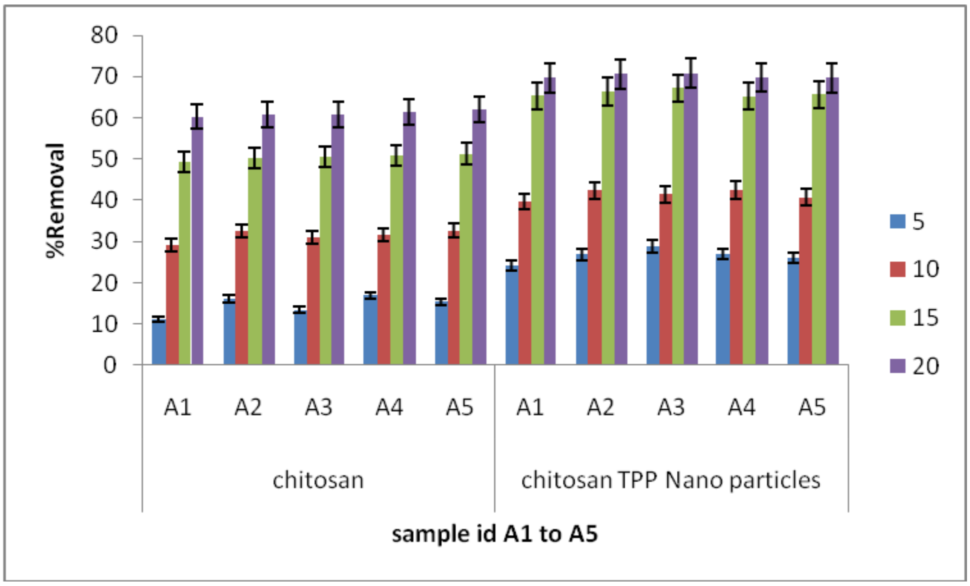

Figure 6: Change in Electrical conductivity (%) with treatment of different dosage of chitosan and chitosan TPP nanoparticles of samples colleted from various aquapond wastewater.

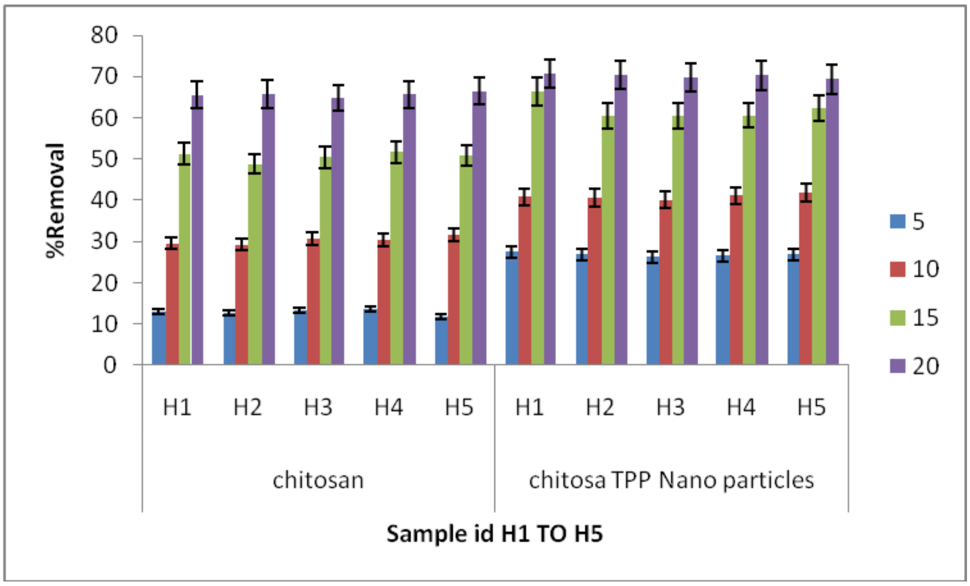

Figure 7: Change in Electrical conductivity (%) with treatment of different dosage of chitosan and chitosan TPP nanoparticles of samples colleted from various hatchery industries.

Two-way ANOVA was employed to find the significant differences in the Electrical conductivity from aqua pond and hatchery effluents over a concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles, Bonferroni Post-T replicates by row were performed to compare the significance of means. The considered significant level is at $p<0.05$ by using the statistical software package Graph Pad Prism version 5.0. (Figs. 8 and 9).

Changes in COD (mg/L) of aqua pond and hatchery effluents by chitosan and chitosan TPP nano particle application

Chemical Oxygen Demand is an alternative method for measuring the organic content in a waste water. It is an important pollutant parameter.

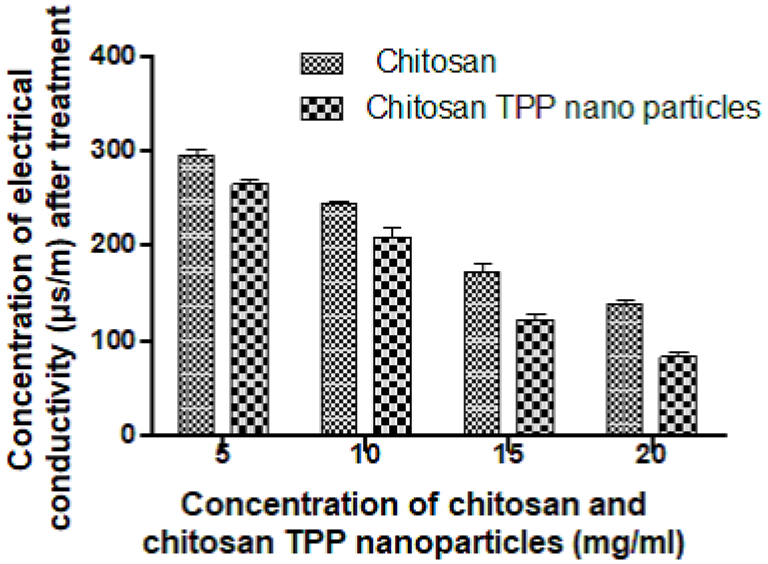

Figure 8: Effect of chitosan and chitosan TPP nanoparticles dosage on electrical conductivity (µs/m) in aqua pond effluents.

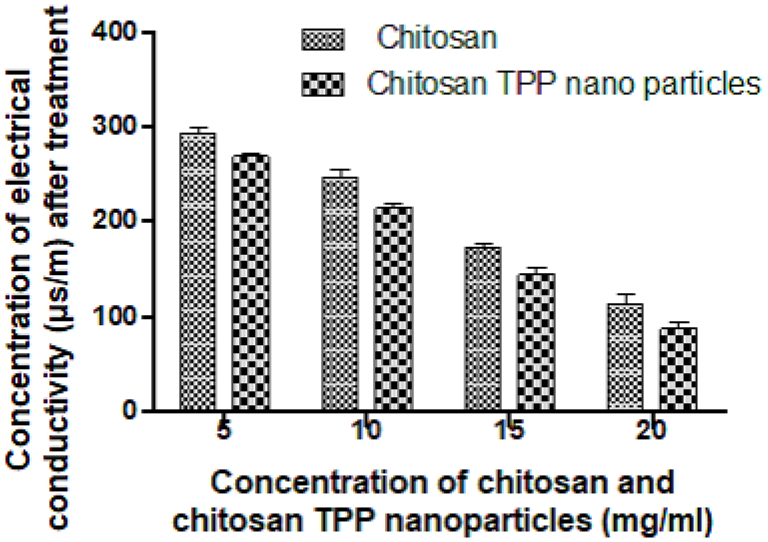

Figure 9: Effect of chitosan and chitosan TPP nanoparticles dosage on Electrical conductivity (µs/m) in hatchery effluents.

The COD of an effluent is usually higher than BOD as the number of compounds that can be chemically oxidized is greater than those that can be degraded biologically. COD ranges from 340-354 mg/L in aqua pond effluents and 342-350 mg/L in hatchery effluents. After treatment with chitosan and chitosan TPP nanoparticles, the COD reduced to 100 mg/L in aqua effluents and 100 mg/L in hatchery effluents. Chitosan TPP Nanoparticles showed more efficiency than Chitosan (Figs. 10 and 11).

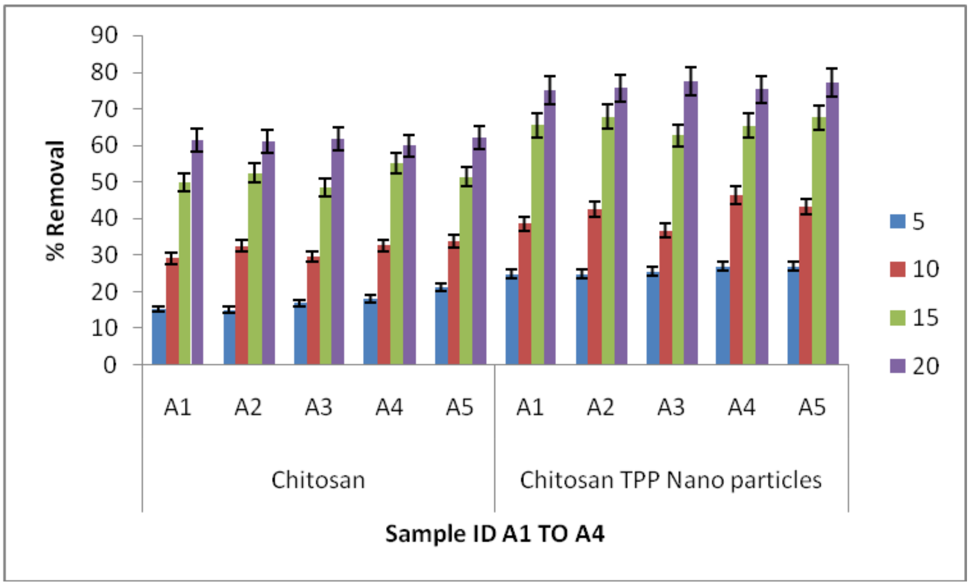

Figure 10: Removal effeciency of COD at different dosage of chitosan and chitosan TPP nanoparticles from various aquapond wastewater.

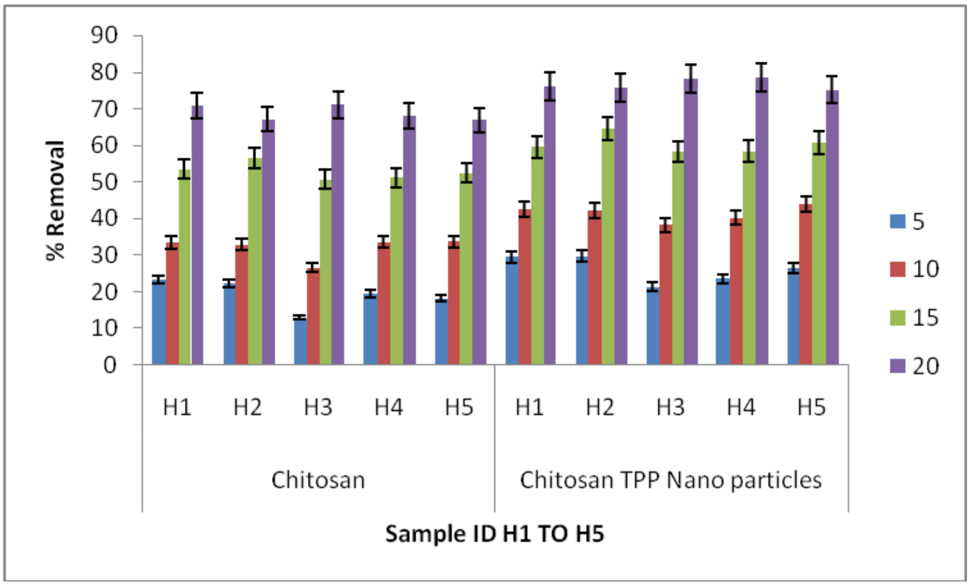

Figure 11: Removal effeciency of COD at different dosage of chitosan and chitosan TPP nanoparticles from various hatchery industry effluents.

According to APHA (2002) the desirable limit for COD is 250 mg/L. Compared to the desirable limit, the values of the samples were found to lie within the limit after treatment with chitosan and chitosan TPP nanoparticles and was satisfactory. Two-way ANOVA was employed to find the significant differences in the Electrical conductivity from aqua pond and hatchery effluents over a concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles. Bonferroni Post-T replicates by row were performed to compare the significance of means. The considered significant level is at $p<0.05$ by using the statistical software package Graph Pad Prism V5. (Fig. 12 for aqua pond effluent and Fig. 13 for hatchery effluent).

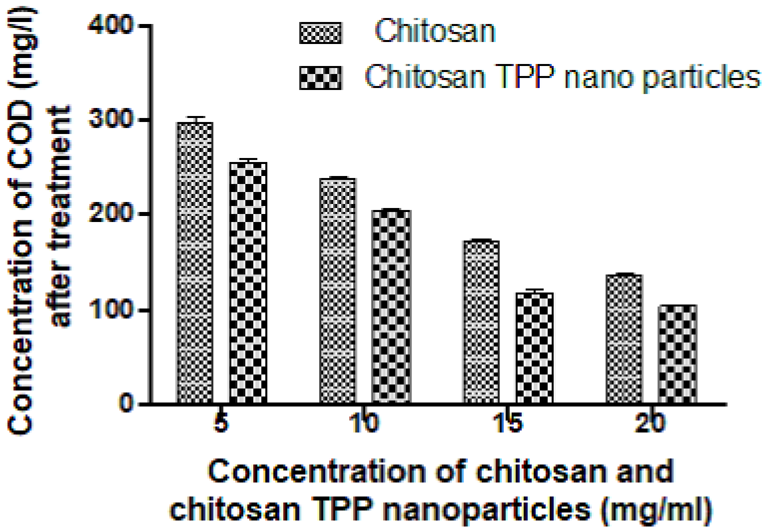

Figure 12: Effect of chitosan and chitosan TPP nanoparticles dosage on COD (mg/L) in aqua pond effluents.

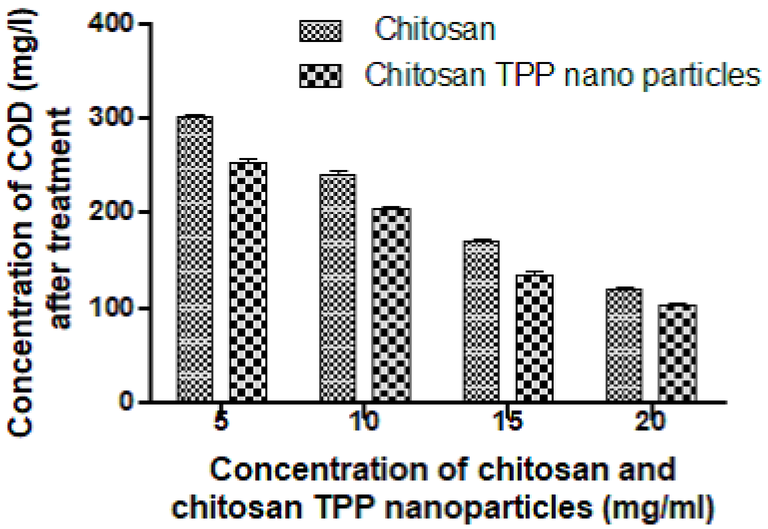

Figure 13: Effect of chitosan and chitosan TPP nanoparticles dosage on COD (mg/L) in hatchery effluents.

dChanges in BOD (mg/L) of aqua pond and hatchery effluents with chitosan and chitosan TPP nano particle application

Biochemical oxygen demand (BOD) estimates the degree of contamination by measuring the oxygen required for oxidation of organic matter by aerobic metabolism of the microbial flora. It is also taken as a measure of the concentration of organic matter present in any water. BOD is the most reliable parameter for judging the extent of pollution in the water (Mishra and Saksena,1991). The greater the decomposable matter present, the greater the oxygen demand and the greater the BOD values (Ademoroti, 1996). In aquaculture processing effluent biochemical oxygen demand originates from the carbonaceous compounds which are used by microorganisms as their substrate and from the nitrogenous compounds such as proteins and volatile amines. Waste waters from aqua culture operations can be very high in BOD (Lawrence *et al*., 2005). In present study BOD values of aqua pond effluents is fairly low compared with the hatchery effluents. In aqua pond effluents it varied from 160 mg/L to 220 mg/L (Fig. 14), whereas in hatchery effluents it ranges from 195 mg/L to 215mg/L. After treatment with chitosan and chitosan TPP nanoparticles, BOD reduced to 21 mg/mL in aqua effluents and 28 mg/L in hatchery effluents (Fig. 15).

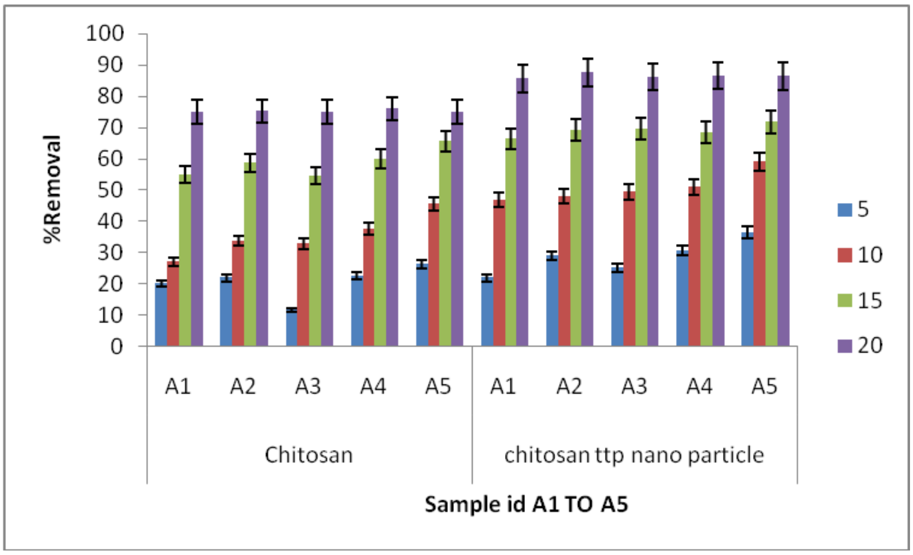

Figure 14: Removal effeciency of BOD at different dosage of chitosan and chitosan TPP nanoparticles from various aquapond wastewater.

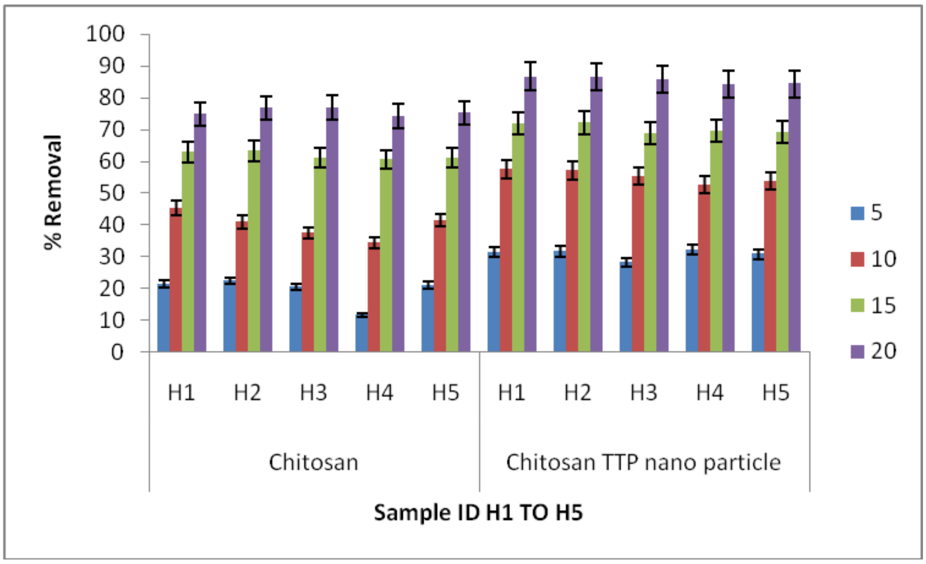

Figure 15: Removal effeciency of BOD at different dosage of chitosan and chitosan TPP nanoparticles from various hatchery industry wastewater.

Chitosan TPP nanoparticles showed more efficiency compared to Chitosan. According to APHA (2002) the desirable limit for BOD is 30 mg/L. Compared to the desirable limit, the values of the samples were found to lie within the limit after treatment with chitosan and chitosan TPP nanoparticles and was satisfactory.

Two-way ANOVA was employed to find the significant differences in the BOD from aqua pond and hatchery effluents over a concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles. Bonferroni Post-T replicates by row were performed to compare the significance of means. The considered significant level is at $p<0.05$ by using the statistical software package GraphPad Prism V5 (Figs. 16 and 17).

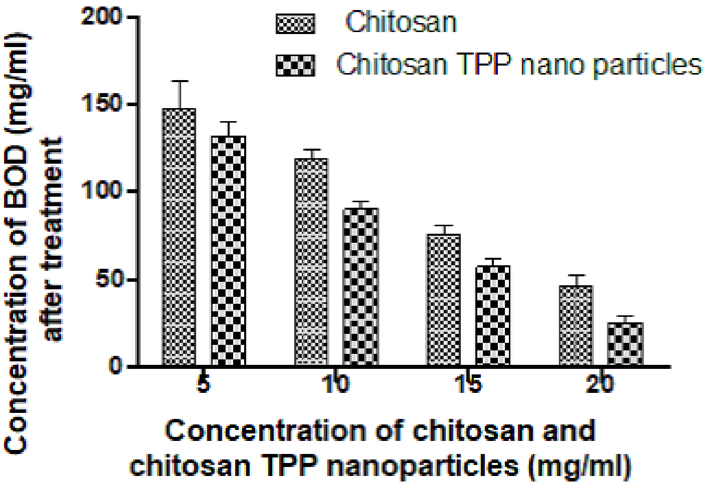

Figure 16: Effect of chitosan and chitosan TPP nanoparticles dosage on BOD (mg/L) in aqua pond effluents.

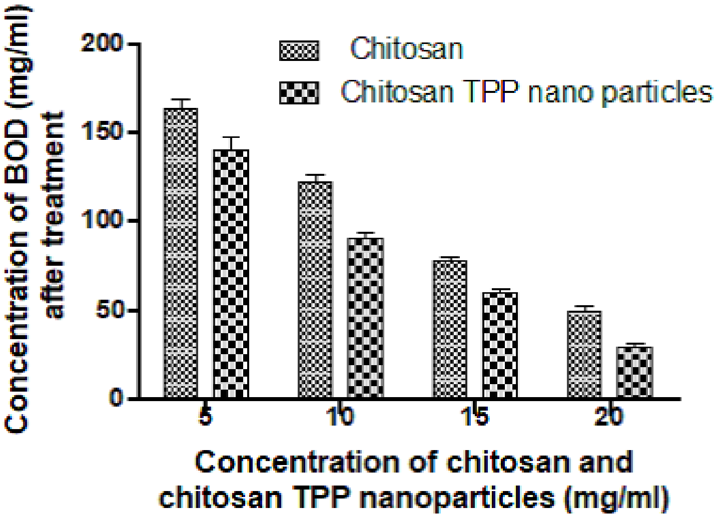

Figure 17: Effect of chitosan and chitosan TPP nanoparticles dosage on BOD (mg/L) in hatchery effluents.

Changes in Total Suspended solids (mg/L) of aqua pond and hatchery effluents with chitosan and chitosan TPP nano particle application

Waste water contains variety of solid materials. Total solids are determined as residue left after evaporation of unfiltered samples. These wastes contribute significantly to the suspended solid concentration of the waste stream. There is a significant variation between different samples studied. TSS ranges from 175 -190 mg/L in aqua pond effluents (Fig. 18) and in hatchery effluents it ranges from 200-179 mg/L (Fig. 19).

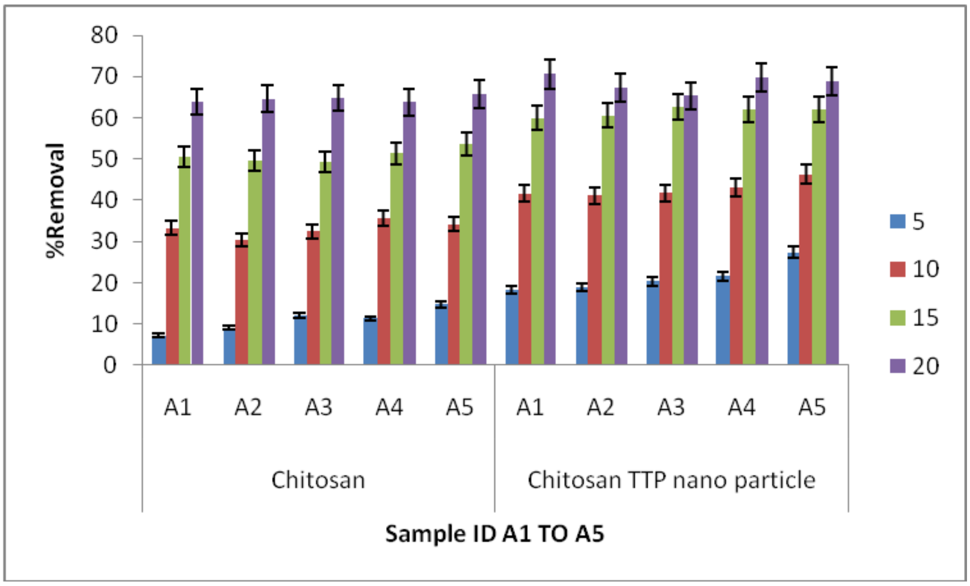

Figure 18: Removal effeciency of TSS at different dosage of chitosan and chitosan TPP nanoparticles from various aquapond wastewater.

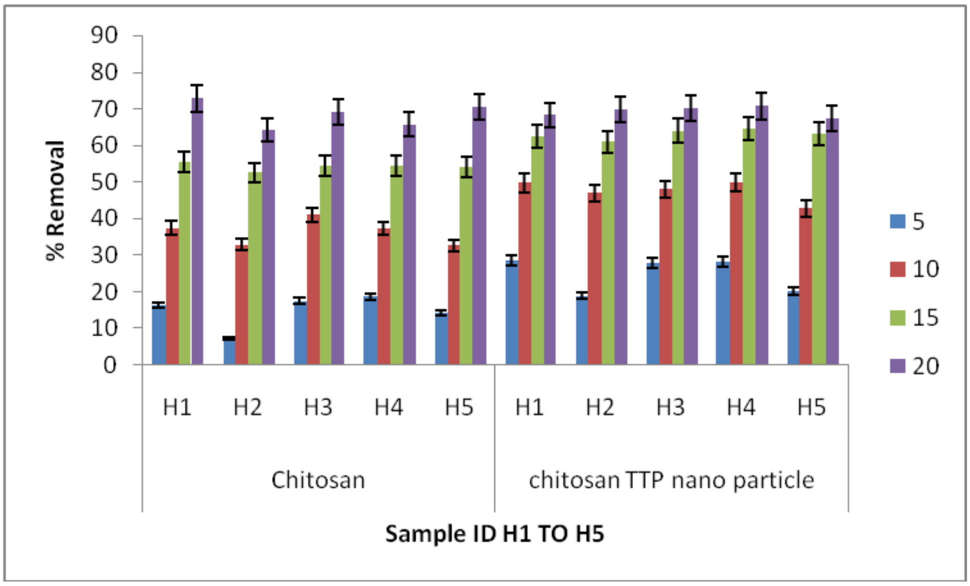

Figure 19: Removal effeciency of TSS at different dosage of chitosan and chitosan TPP nanoparticles from various Hatchery industry effluent.

After treatment with chitosan and chitosan TPP nanoparticles, Concentration of TSS reduced to 53 mg/L in aqua pond effluents and 54 mg/L in hatchery effluents. After treatment with chitosan and chitosan TPP nanoparticles, all the analysed samples possess a lower value of TSS than the standard value of 100 mg/L (APHA, 1998). Water that contains less than 100 mg/L of TSS was considered as fresh water generally satisfactory for the domestic use and other industrial purposes (Ackefors, 1994). Water that contains more than 100 mg/L of suspended solids usually contains minerals that give it a distinctive taste or make it unsuitable for human consumption. A similar observation was reported by Singh *et al*. (2010) for waste water of Raniganj industrial area in India. Two-way ANOVA was employed to find the significant differences in the TSS from aqua pond and hatchery effluents over a concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles. Bonferroni Post-T replicates by row were performed to compare the significance of means. The considered significant level is at $p<0.05$ by using the statistical software package GraphPad Prism V5 (Figs. 20 and 21).

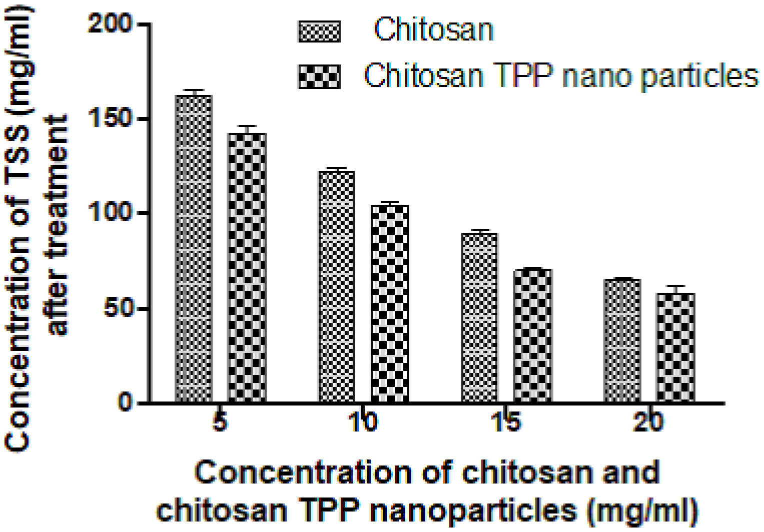

Figure 20: Effect of chitosan and chitosan TPP nanoparticles dosage on TSS (mg/L) in aqua pond effluents.

Changes in Alkalinity (mg/L) of aqua pond and hatchery effluents with chitosan and chitosan TPP nano particle application

The amount of alkalinity concentration of the effluent samples collected in the study area ranged from 1550 to 1600 mg/L. after treatment with chitosan and chitosan TPP nanoparticles, the alkalinity reduced to 239 mg/L in aqua effluents (Fig. 22) and 235mg/L in hatchery effluents (Fig. 23). According to APHA (2002) the desirable limit for total Alkalinity is 350 mg/L as $CaCO_3$.

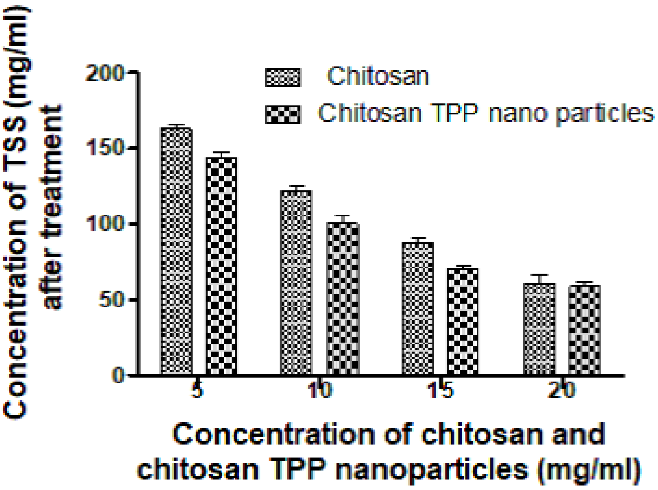

Figure 21: Effect of chitosan and chitosan TPP nanoparticles dosage on TSS (mg/L) in hatchery effluents.

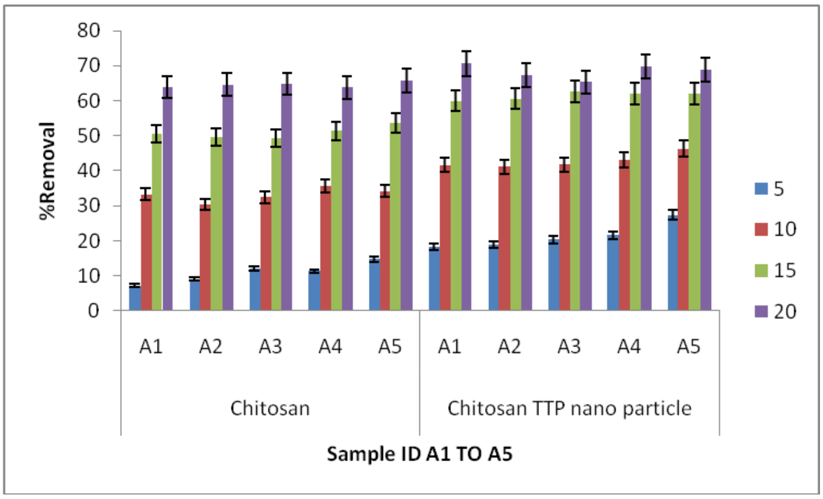

Figure 22: Removal effeciency of alkalinity at different dosage of chitosan and chitosan TPP nanoparticles from various aquapond wastewater.

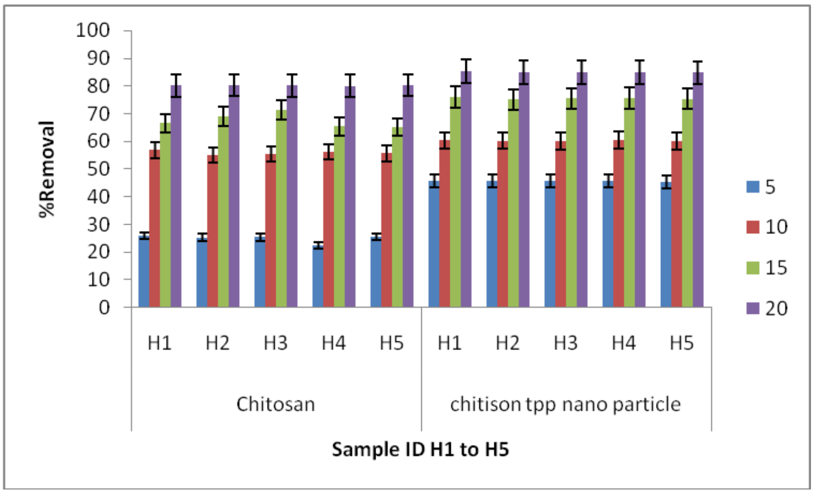

Figure 23: Removal effeciency of alkalinity at different dosage of chitosan and chitosan TPP nanoparticles from various hatchry industry effluent.

Compared to the desirable limit, the values of the samples were found to lie within the limit after treatment with chitosan and chitosan TPP nanoparticles and was satisfactory.

Two-way ANOVA was employed to find the significant differences in the alkalinity from aqua pond and hatchery effluents over a concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles. Bonferroni Post-T replicates by row were performed to compare the significance of means. The considered significant level is at *p*<0.05 by using the statistical software package GraphPad Prism V5 (Figs. 24 and 25).

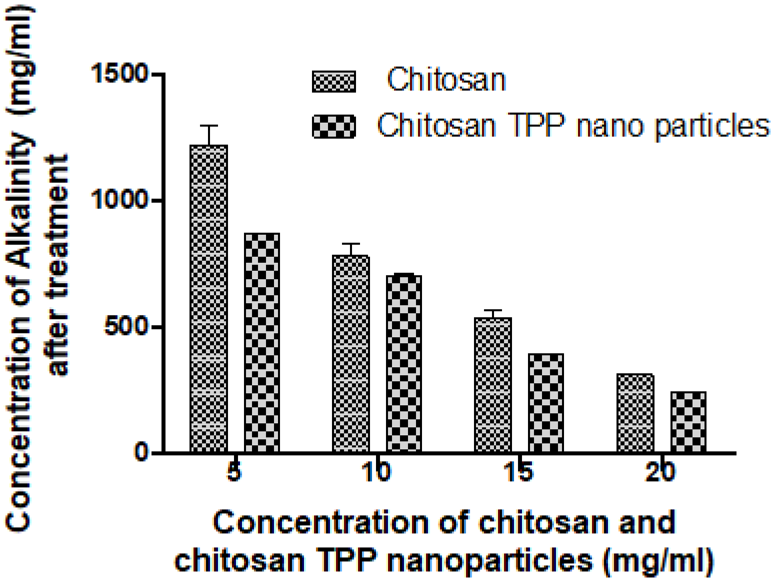

Figure 24: Effect of chitosan and chitosan TPP nanoparticles dosage on alkalinity (mg/L) in aqua pond effluents.

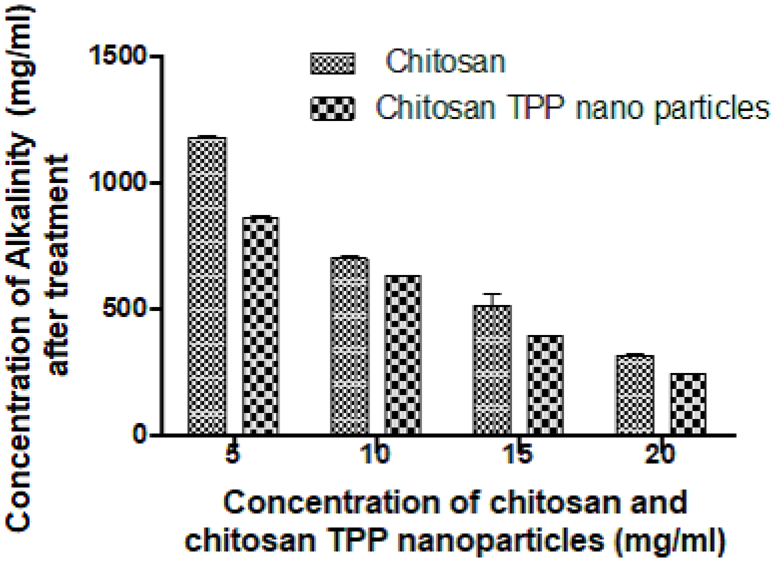

Figure 25: Effect of chitosan and chitosan TPP nanoparticles dosage on alkalinity (mg/L) in hatchery effluents.

Changes in Total Hardness (mg/L) of aqua pond and hatchery effluents with chitosan and chitosan TPP nano particle application

Hardness of water is a measure of its capacity to form precipitates with soap and scales with certain anions present in the water. Hardness concentration values ranged from 6670 to 4560 mg/L in the study area. After treatment with chitosan and chitosan TPP nanoparticles it reduced to 1120 mg/L in aqua pond effluents (Fig. 26) and 2241 mg/L in hatchery effluents (Fig. 27). Chitosan TPP nanoparticles showed more efficiency than chitosan in the removal of Hardness.

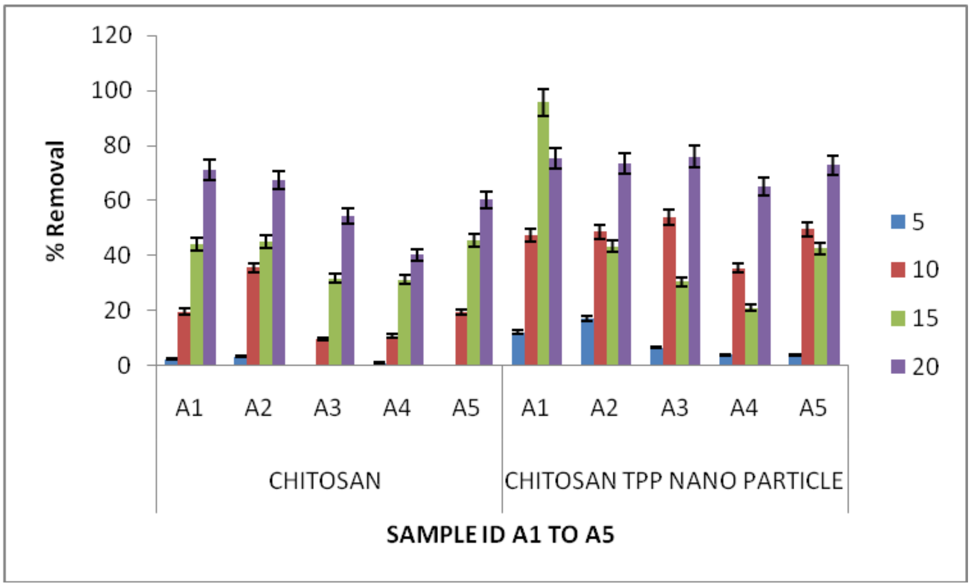

Figure 26: Removal effeciency of hardness at different dosage of chitosan and chitosan TPP nanoparticles from various aquapond wastewater.

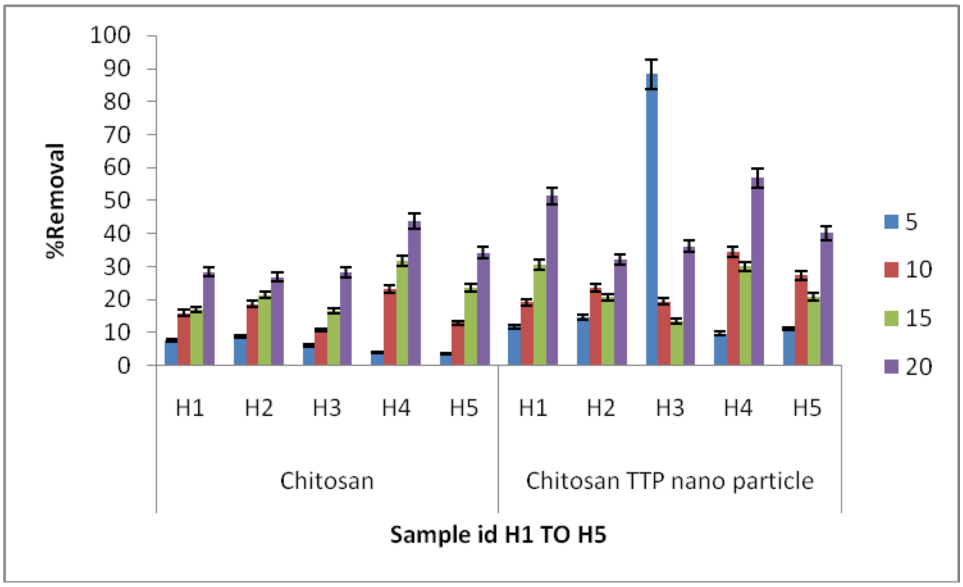

Figure 27: Removal effeciency of hardness at different dosage of chitosan and chitosan TPP nanoparticles from various hatchery industrial effluents.

Two-way ANOVA was employed to find the significant differences in the total hardness from aqua pond and hatchery effluents over a Concentration of 5, 10, 15 and 20 between chitosan and chitosan TPP nano particles. Bonferroni Post-T replicates by row were performed to compare the significance of means. The considered significant level is at $p<0.05$ by using the statistical software package GraphPad Prism V5 (Figs. 28 and 29).

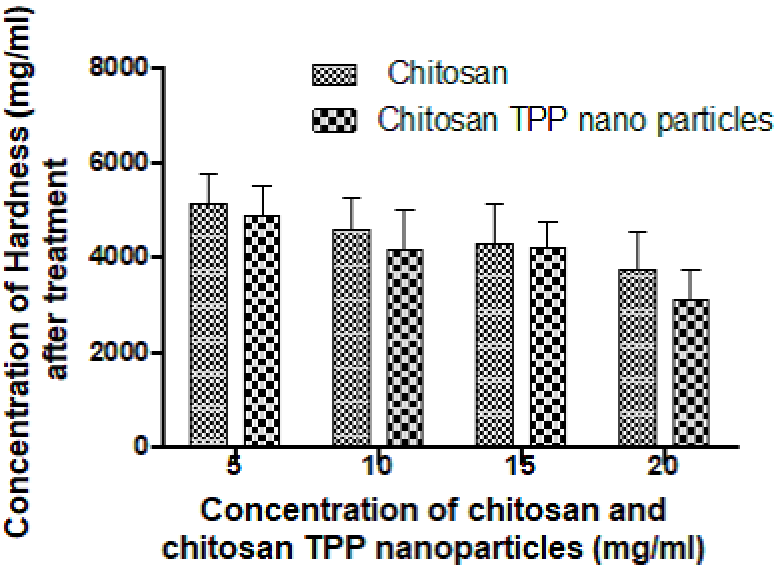

Figure 28: Effect of chitosan and chitosan TPP nanoparticles dosage on total hardness (mg/L) in aqua pond effluents.

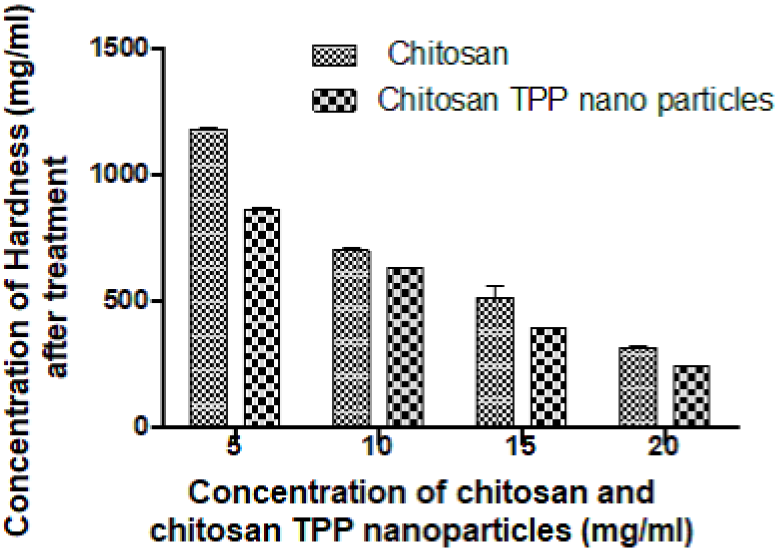

Figure 29: Effect of chitosan and chitosan TPP nanoparticles dosage on total hardness (mg/L) in hatchery pond effluents.

Conclusions

- Evaluation of various physico-chemical parameters of shrimp farm and hatchery effluents such as alkalinity, electrical conductivity, total hardness, total suspended solids, total ammonia, BOD, COD, was done before and after treatments in laboratory scale.
- Chitosan TPP nanoparticles showed high efficiency than chitosan. Data obtained from physical and chemical measurements were statistically analyzed. Two- way ANOVA were used to determine the statistical significance of the independent variables and their interactions. ANOVA showed that all effects were statistically significant (p<0.05) at 95% confidence levels.
- Chitosan and chitosan TPP nanoparticles exhibited efficient removal of various pollutants when it was applied in aquaculture waste waters originating from the effluent of shrimp culture pond. It played various roles in the coagulation, adsorption, bacteriostasis, and even combination processes to achieve the recycling of aquaculture wastewater. The physio-chemical characterization of the treated effluent is below the standard limit prescribed by Andhra Pradesh Pollution Control Board.
- From the results of the present investigations chitosan and chitosan TPP nanoparticles showed good coagulating properties, and has many advantages compared to chemical coagulants and does not affect the pH, alkalinity or conductivity of the water. Further multifunctional environmentally friendly chitosan should play a larger role in the recycling of aquaculture wastewater.

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