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Protozooplankton combined with magnetic-EDTA@chitosan nanocomposite as a novel system for fish farms wastewater remediation

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ABSTRACT

In Egypt, most fish farms use water of agricultural drainage originated from agricultural activities that may contaminate farmed fish. This work was conducted to assess a novel wastewater treatment using combined protozooplankton with magnetic-EDTA@chitosan nanocomposite (ME@CS NC), to dwell with high concentrations of heavy metals and ammonia. A highly significant reduction (p<0.01) was observed in the concentrations of Pb, Mn, Al and Cd on applying the treatment. The estimated decrease percentages were 99.9, 99.6, 98.8 and 93.3, respectively, whereas Ni showed a reduction of 90.3%. Simultaneously, ammonia in wastewater was significantly reduced (p<0.01). Water environmental parameters of EC, pH, TDS, and NO_2 were reduced by 2.4, 3.7, 7.4, and 43.3%, respectively, while PO_4, and NH_3 were reduced by 76.7 and 94.4%, respectively. Moreover, COD, nitrite, nitrate and phosphate physicochemical parameters were significantly reduced (p<0.05), by 41.4, 54.9, 63.3, 64.4%, respectively. Hence, the combined treatment of ME@CS nano-composite and protozooplankton is recommended to be implemented in semi-intensive and modern intensive aquaculture systems to manage less water consumption and attain healthy fish for human consumption.

INTRODUCTION

Aquaculture has been known in Egypt for a long time, but lately, modern methods have been implemented to maximize its production **(Shaalan *et al.*, 2018)**. The Egyptian aquaculture sector has expanded over the last two decades because of a model shift from traditional extensive to semi-intensive and modern intensive aquaculture systems **(FAO, 2020)**.

Therefore, aquaculture production in Egypt has witnessed a vast growth; production elevated from 139,389 tons in 1998 to 1,561,457 tons in 2018, which is 71% of the total aquaculture manufacture in Africa **(FAO, 2020)**. The fisheries and aquaculture sector are essential to improve food security and human nutrition having an increasingly important role in fighting hunger, as articulated in the 2030 agenda. People have never consumed as much fish as they do today, with global fish consumption being doubled since 1960s **(FAO, 2018)**. Egypt's aquaculture industry has grown rapidly since 1998 because of the Egyptian government's consistent and cumulative interventions over the years, as well as growing a private sector-driven investment **(Soliman & Yacout, 2016)**.

The increasing of fish farming production is needed to develop culture systems and meanwhile manage waste efficiently to limit environmental degradation caused by aquaculture wastes and ensure its sustainability **(Dauda *et al.*, 2019)**. Most fish farms in Egypt use water from agriculture drainage. The used water is an outcome of agricultural activities identified as sources of pesticide residues and runoff derived fertilizers and metals that may contaminate farmed fish **(Khallaf & Authman, 2018)**.

Most heavy metals are bound to particles in the sediment, while some quantities are dissolved in water, and hence, can spread widely in the food chains **(Khadr, 2005)** especially those on top of the food chain **(El-Nemeki *et al.*, 2008)**. Metal ions can be incorporated into food chains and concentrated in aquatic organisms to a level that affect their physiological state **(Zhao *et. al.*, 2011)**.

Heavy metals are considered serious pollutants to aquatic ecosystems due to their long-term persistence, toxicity, bioaccumulation, and bio magnification along the water, sediments, and aquatic food chain causing sublethal effects or loss in fish populations besides being considered as carcinogenic elements **(Yi *et al.*, 2011**). Cadmium, lead, and chromium exhibit great toxicity even at lower levels **(Jaishankar *et al.*, 2016)**.

Poor water quality in fish farms is usually triggered by fish farmers who have inadequate knowledge and skills on proper pond management **(Ngugi *et al.*, 2007)**. The excessive addition of pond inputs (fertilizers either organic (manure) or inorganic and fish feeds) influence both the chemical and physical balances in water **(Hossain *et al.*, 2011)**.

The presence of various inorganic and organic pollutants in aquatic streams has already increased because of industrialization and urbanization (Sarkar and Adhikari, 2018). Protozooplankton are aquatic microscopic organisms that have developed excellent survival strategies, which help them tolerate environmental stresses. Moreover, the biodegradation ability of such protozoan organisms serves as suitable candidate to be used in environmental biotechnology and clearing effluents. Therefore, they can act as natural bio-control in complex ecological systems **(Syed *et al.*, 2019)**. Protozooplankton is an important component of the aquatic microbial food webs and its composition, density, and distribution that reflect the chemical, physical, and biological aspects of the environment **(Bagatini, *et al.*, 2013)**.

Nanoparticles have a great potential to be used in wastewater treatment. Its unique characteristic of having high surface area can be used efficiently for removing toxic metal ions, disease causing microbes, organic and inorganic solutes from water. Various classes of nano-materials are also proved to be efficient for water treatment, such as metal-containing nanoparticles, carbonaceous nano-materials, zeolites and dendrimers **(Pranjali *et al.*, 2013)**.

Currently, a special attention has been drawn to the environmental contamination with heavy metals due to their high toxicity and non-biodegradability. Notably, inefficient ways of managing heavy metal ions in wastewater may cause long-term risk to both the ecosystem and humans. Effluents containing heavy metal ions are widely discharged from industrial process, such as electroplating and mining; thus, the ions need to be removed before being discharged into water to protect human health and environment **(Morsi *et al.*, 2018)**.

The fishery industry faces a huge economical loss caused by hazardous and heavy metals in such waters leading to growth retardation and fish death. In aquaculture, nanotechnology has core applications for water treatment to provide favorable and safe habitat for fish breeding **(Shah & Mraz, 2020)**.

Chitosan is generally recognized as safe (GRAS) by the **US FDA (2001)**. Chitosan has been widely used in water treatment to eliminate heavy metal and radioactive pollutants. Chitosan is a coagulant that is able to capture substances such as colloids and suspended solids in water, and then it would sink or float. Chitosan nano-composite is one of the most effective adsorbents in removing pollutants from the aquatic environments **(Divya & Jisha, 2018)**.

Nano-spherical necklaces (NSN) of inorganic α-Fe core-organic shell and ethylenediaminetetraacetic acid (EDTA) were fabricated for capturing/trappingAs(V) and Cr(VI) species from water sources. The α-Fe core and the dressing shell of EDTA provided numerous active sites for adsorption, which led to 100% adsorption uptake of these toxic ions. Treated water showed a removal of >95% and 94% of the As (V) and Cr(VI) species from this water sources using NSN adsorbent. This obtained result could be used as a basis to provide effective and low-cost products for the purification of wastewater resources from toxic metals **(Azzam *et al.*, 2017)**.

The aim of the present work was to fabricate magnetic-EDTA@chitosan (ME@CS) nano-composite as a novel adsorbent and measure its efficiency for treating draining water of fish farms and improve the treatment process by protozooplankton for safety water reuse to provide unconventional water sources.

MATERIALS AND METHODS

Water samples, collection and analysis

In the present study, wastewater and fish samples were collected from fish farms in Kafr El-Sheikh Governorate, Egypt. Chemical oxygen demand (COD), NH_3, NO_3, and PO_4 were detected in collected water at Faculty of Sciences, Ain-Shams University. Chemical composition (Al, Mn, Fe, Ni, Cu, Cd, and Pb) of water samples was recorded using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Prodigy High Dispersion ICP, Leeman, USA) in Central Laboratory for Isotope Applications, Egyptian Atomic Energy Authority.

Water protozooplankton examination

Water protozoan organisms were collected, examined and identified. The wheat media was prepared, providing a nutritive medium for protozooplankton to accelerate the growth rate. Samples were fixed with mercuric chloride and stained with bromophenol blue for examination and enumeration **(Bagatini, *et al.*, 2013)**. The total count of each protozooplankton species was detected and recorded according to the identification keys of **Patterson and Hedley (1996)**.

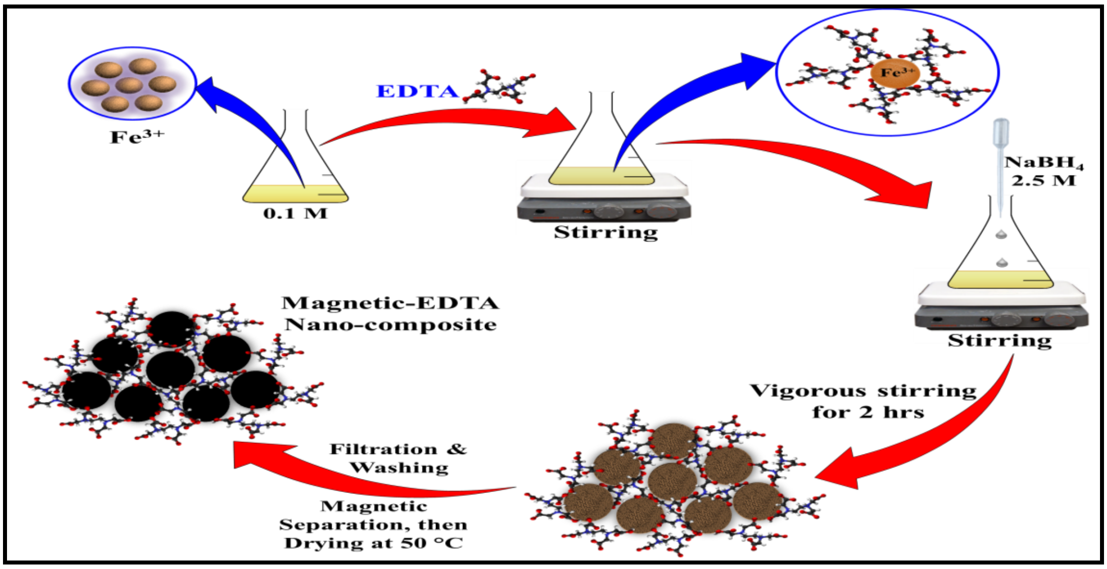
Synthesis of chitosan nanoparticles

The chitosan polymer was extracted from *Penaeus Japonicus* exoskeleton using the procedure of **Abd El-Fattah *et al.* (2016)**. The resulting chitosan was washed in running tap water till neutrality, rinsed with distilled water, filtered, and then dried at 60°C for 24 hrs. The chitosan powder after deacetylation was dissolved in 1M of HCl, and the solution was then stirred for more than 24 hrs until a pure viscous solution of chitosan was gained. Chitosan nanoparticles were prepared by adjusting the pH of a chitosan solution to 8.0 by adding NaOH, and thereafter the precipitate was washed and dried at 50°C for 24 hrs **(He *et al.*, 2016)**.

Synthesis of magnetic-EDTA nanocomposite (ME NC)

The magnetic nanoparticles were synthesized by modified wet chemical reduction technique according to **Chaki *et al.* (2015)** and **Azzam *et al.* (2017)**. In the synthesis, regarding solution (A), 500 ml of 0.1 M ferric chloride hexa-hydrate ($FeCl_3{\cdot}6H_2O$)

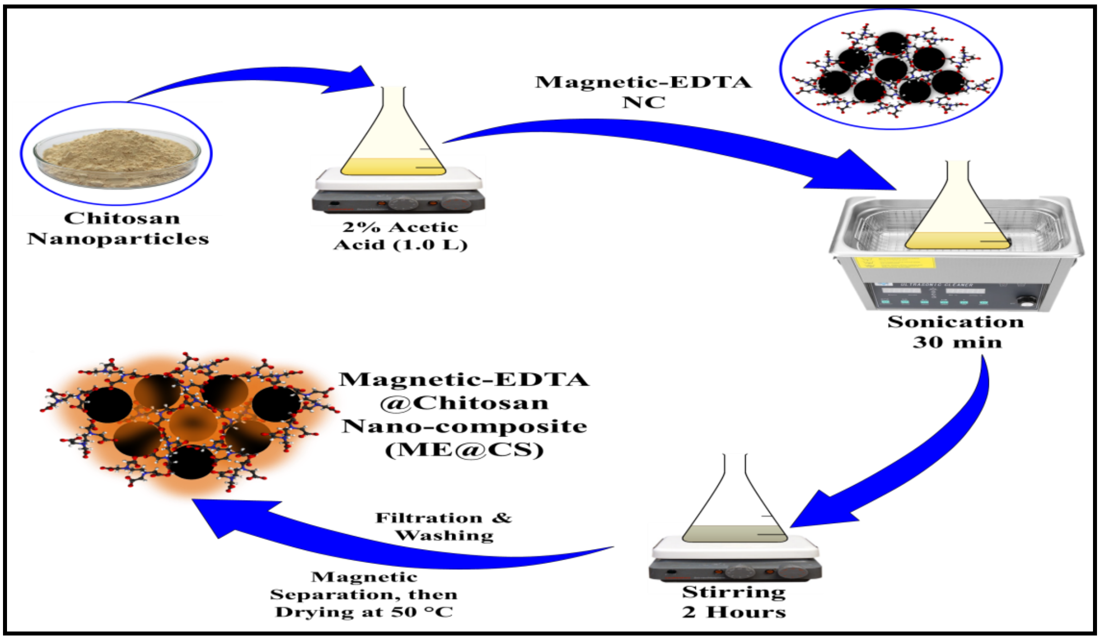
and 0.05 M sodium ethylenediaminetetraacetic acid (Na-EDTA) were mixed well. Then, solution (B) was prepared using 500 ml of 2.5 M sodium borohydride ($NaBH_4$) that was added drop by drop slowly to solution (A) while keeping up vigorous stirring for 2 hrs. At the end of this step, a dark colored precipitate formed, the magnetite. The magnetic-EDTA nano-composite was filtered and given multiple wash using distilled water and absolute methanol. The particles were dried in an oven overnight at 50°C (Scheme 1).



Scheme 1: Synthesis of magnetic-EDTA nano-composite (ME NC).

Preparation of magnetic-EDTA@chitosan nano-composite (ME@CS NC)

Magnetic-EDTA@chitosan nano-composite was successfully fabricated by a cross-link reaction between chitosan NPs and ME NC following the procedures of **Chen *et al* (2019)**. Chitosan NPs were firstly dissolved in acetic acid solution (2.0 wt %), then ME NC was fully suspended into chitosan solution by ultrasonic irradiation for 30 min. The mixture was continuously mechanically stirred for 2 hrs, followed by washing several times with DW to remove unreacted chitosan and separated with magnet. The purified gels were dried in a vacuum oven at 50°C for 24 hrs (Scheme 2).



Scheme 2: Preparation of magnetic-EDTA@Chitosan nanocomposite (ME@CS).

Characterization of synthesized nanomaterials

The structural characterization of the prepared nanomaterials was characterized with the help of multiple techniques. The surface morphology of nanomaterials was identified using a scanning electron microscopy (SEM) (JEOL JSM-5600). The particle size of the resulting nanomaterials was analyzed using transmission electron microscopy (TEM) (EM 208S Philips, Netherlands) connected to a high-resolution imaging system. Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to Chitosan NPs; Magnetic-EDTA NC and ME@CS NC were used to identify the functional groups by using Thermo Scientific Nicolet iS10 FTIR spectrometer via the KBr pressed disc method, with a range starting from 400 to 4000 cm^{-1}wave numbers.

Combination of protozooplankton and ME@CS nanocomposite to treat polluted drainage water in fish farm

After determining the optimum dose and time of protozooplankton wheat culture and ME@CS nano-composite for maximum removal of some pollutants, the combined treatment for wastewater was carried out. The concentrations of toxic heavy metal ions (Al, Mn, Fe, Ni, Cu, Cd and Pb) and physico-chemical parameter items (pH, EC, TDS, COD, NH_3, NO_2, NO_3 and PO_4) of fish farm water were determined before and after the application of treatment.

RESULTS AND DISCUSSION

Characteristic features of magnetic@chitosan core-shell nano-composite (ME@CS**)**

In the present study, scanning electron microscopy (SEM) images for characteristic features of synthesized nano-composite showed that, chitosan NPs were nonporous, spherical in shape witha smooth surface. While, the obtained magnetite EDTA nano-composite (ME NC) was spherical in shape and nanocrystal in form and aggregates up to 200 nm, assembling large aggregates. However, magnetic EDTA-chitosan nanocomposite (ME@CS NC) has a spherical shape but doesn’t have an identical surface. It possesses some differences and a rough and irregular surface, which indicates particles surface and pores filled with chitosan polymer (Fig.1). Moreover, transmission electron microscopy (TEM) images for characteristic features of the synthesized nano-composite revealed that chitosan NPs were spherically shaped and had a particle size ranging from 55 to 110 nm with an average size of 80 nm. Most ME NC exhibited spherical shape and had a particle size ranging from 15 to 40 nm with an average size of 26 nm, while ME@CS NC showed a spheroidal morphology in the range of 40-80 nm with an average size of 60 nm. Meanwhile, **Chen *et al.* (2019)** reported that TEM images showed uniformly dispersed Fe_3O_4 magnetic particles in good spherical shape with an average diameter of 200–500 nm, which is consistent with the SEM results. After the introduction of CS, the resulting Fe_3O_4-CS composite showed an irregular shape and some Fe_3O_4 particles were embedded into the light grey cross-linked CS matrix, which makes the spherical structure of Fe_3O_4 somewhat vague. The TEM pattern of Fe_3O_4-CS appeared similar to that of Fe_3O_4-CS/EDTA (Fig.2). However, **Asgari *et al.* (2020)** synthesized non-uniform surface of ME@CS NC in the range of 40.2–55.5 nm with good porosity and relatively uniform distribution throughout the surface.

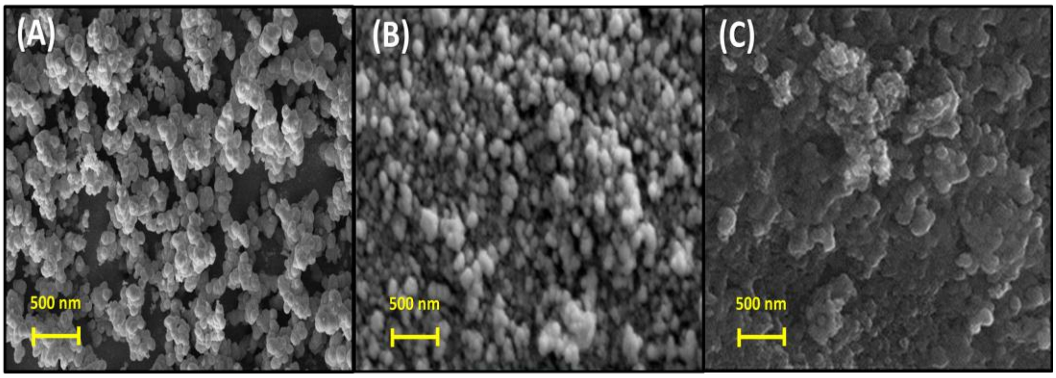


Fig.1: SEM images of A) Chitosan NPs; B) Magnetic-EDTA nano-composite and C) Magnetic-EDTA@Chitosan nano-composite

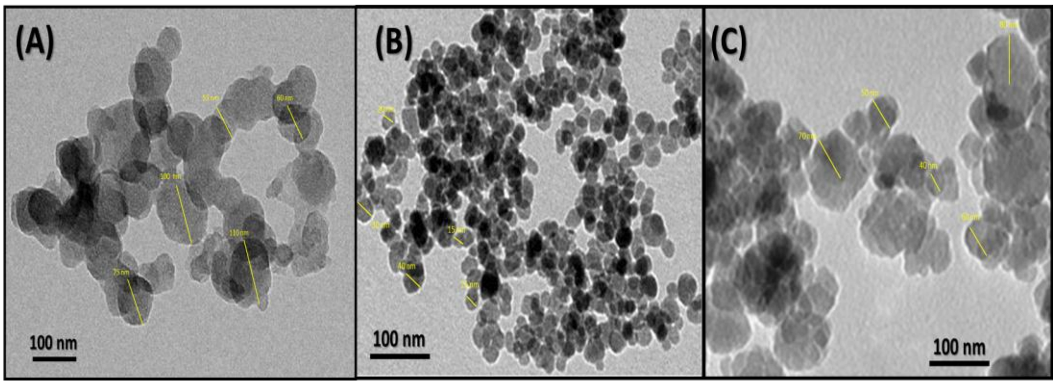


Fig.2: TEM images of A) Chitosan NPs; B) Magnetic-EDTA nano-composite and C) Magnetic-EDTA@Chitosan nano-composite

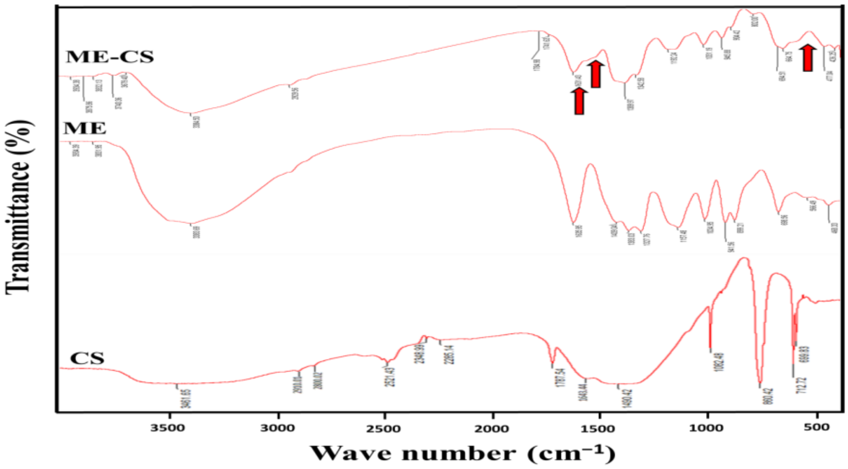


Fig. 3: FTIR spectrum of chitosan nanoparticles (CS); Magnetic-EDTA nano-composite (ME), and Magnetic-EDTA@Chitosan nanocomposite (ME@CS)

In the current study, the Fourier transforms infrared (FTIR) spectroscopy for chitosan NPs, ME NC and ME@CS NC showed that the successful combination between chitosan NPs and ME NC was obviously enhanced, where the peaks were determined at 1631 cm^{-1} (stretching vibration of carbonyl group in $-COO^{-}$ and amide bond) and 1565 cm^{-1} (N-H bending vibration of amide). This indicates the successful formation of amide bond between the amino group of CS and carboxyl group of magnetic EDTA NC through an amidation reaction. Moreover, the characteristic peak at around 1389 cm^{-1}, from stretching vibration of C-O in $-COO^{-}$, further confirmed the introduction of carboxyl group of EDTA to CS **(Chen *et al.*, 2019)**. However, new broad absorption band in the range of 694–426 cm^{-1} appeared in the FTIR spectra of Fe-O-CS NC due to formation of O-Fe-O bond. In addition, the peaks of C=O, $-NH_2$, 3′-OH, and 5′-OH groups were found due to hydrogen bonds between chitosan and Fe_3O_4 **(Guo *et al.*, 2010; Asgari *et al.*, 2020)** (Fig.3).

Treatment of fish farms wastewater using protozooplankton

Table (1) represents the water parameters of fish farm wastewater treated with 1% of protozooplankton wheat culture and incubated for 48 hrs. Water environmental parameters (NO_3, pH, NO_2, PO_4, and NH_3) were reduced by 1.1, 1.2, 6.7, 27.8, and 47.2%. However, heavy metals (Cd, Al, Mn, and Pb) were removed with 6.8, 20.0, 28.4, and 29.1%, while Ni and Fe were reduced by 68.3 and 43.0%. Whereas **Weisse *et al.* (2021)** demonstrated that improved estimates of peritrich ingestion and clearance rates further emphasize the significance of protozooplankton for the water purification process.

Effect of ME@CS nano-composite concentration

In this study, the effect of concentration 0.5 g/L of ME@CS NC on the removal of heavy metals from fish farm wastewater after an exposure of 60 min and pH 7.0 recorded high removal percentages with values of 99.9, 99.5, 99.3 and 99.1 % for Al, Pb, Cd and Mn ions, respectively. However, Ni, Fe and Cu ions were removed recording removal percentages of 70.8, 65.0 and 64.2%, respectively. For Fe, Ni and Cu ions, they were reduced significantly ($p<0.05$) at concertation 0.5 g/L (Fig. 4). Similarly, **Yuan *et al.* (2021)** recorded the adsorption capacity of Co(II) onto the Fe_3O_4@CS-EDTA and still retained 84.5% of the capacity of the fresh adsorbent, indicating that Fe_3O_4@CS-EDTA can be considered a promising recyclable adsorbent to remove heavy-metal ions from wastewater.

Table 1: Water parameters of fish farm wastewater treated with 1% wheat culture protozooplankton

Parameter	Before	After	Accepted value
pH	8.2	8.1	5.0 – 9.0#
EC	4.1	4.1	ms/cm
TDS	2.7	2.7	< 1.0 g/L#
COD	29	34	10 - 50 mg/L#
NH_3	0.36	0.19	< 0.1 mg/L#
Nitrite	3.0	2.8	< 0.1 mg/L#
Nitrate	0.91	0.90	< 50 mg/L#
Phosphate	0.90	0.65	< 0.1 mg/L#
Al	78.89	63.12	< 50 ppb##
Mn	1.41	1.01	< 10 ppb###
Fe	75.8	43.2	< 500 ppb###
Ni	9.94	3.15*	< 1000 ppb#
Cu	11.86	7.60	< 6.0 ppb###
Cd	0.118	0.110	< 0.2-8 ppb#
Pb	1.609	1.140	< 1.0-7.0 ppb#

N.B The incubation period: 48 hrs; ME-CS nanocomposite: 0.5 g/l contact time: 60 min; agitation rate: 200 rpm. *$p < 0.05$; **$p < 0.01$ ### Wedemeyer and Yasutake , 1977, ## FAO, 1993, # Water quality standards/criteria relevant to freshwater aquaculture in Australia (ANZECC, 2000) **(Wedemeyer & Yasutake, 1977; FAO, 1993; ANZECC, 2000)**

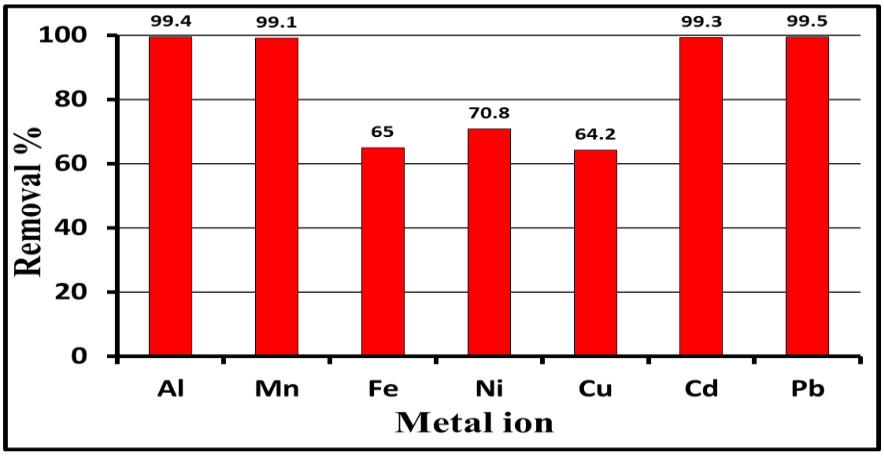


Fig. 4: Removal percentage of heavy metals from fish farm wastewater using ME@CS nano-composite at concentration 0.5 g/l, pH 7.7, and 60 min exposure time

Fig. (5) shows the effect of concentration 0.5 g/L of ME@CS NC on physicochemical parameters of fish farm wastewater after an exposure time of 60 min and pH 7.0. The ammonia and nitrate showed significant reduction (*$p<0.05$*) at concentrationof 0.5 g/L of ME@CS NC. However, the COD and phosphate showed non-significant reduction at the same concentration. The reduction percentages of ME@CS NC at concentration of 0.5 g/L were 63.3, 57, 28, 21.4 and 11.8 for NO_2, COD, NH_3, PO_4 and NO_3, respectively.

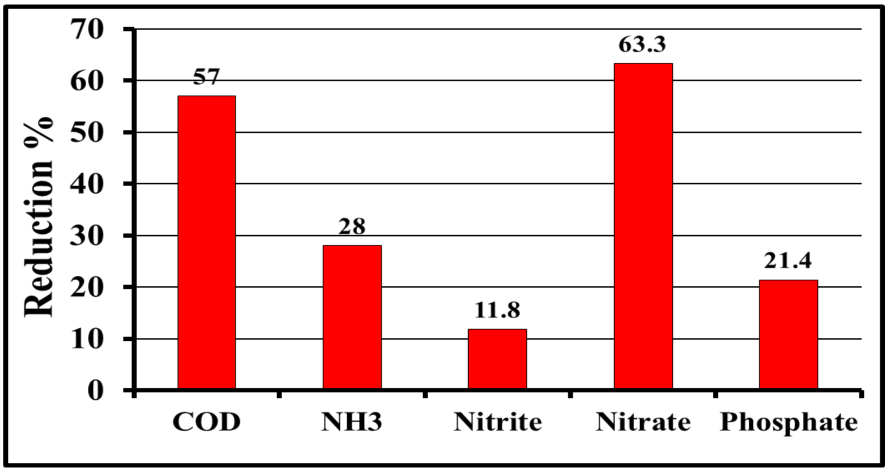


Fig.5: Reduction percentage of physicochemical parameters of fish farm wastewater using ME@CS nano-composite at concentration of 0.5 g/l, pH 7.7, and 60 min exposure time

Effect of protozooplankton combination with ME@CS nano-composite on the treatment of polluted drainage water of fish farms

Fish farm wastewater samples were treated by a combination of protozooplankton (1%) wheat culture for 48 hrs and ME@CS nanocomposite (0.5 g/L) for 60 min. Table (2) shows the water parameters of fish farm wastewater treated with 1% of protozooplankton wheat culture and incubated for 48 hrs, then treated with 0.5 g/L of ME@CS nano-composite for another one hour.

The ammonia showed highly significant reduction (p<0.01) after the application of the combined treatment. Meanwhile, other physicochemical parameters, such as COD, nitrite, nitrate and phosphate recorded significant reduction (p<0.05). However, water environmental parameters, such as EC, pH, TDS, and NO_2 were reduced by 2.4, 3.7, 7.4, and 43.3%, while COD, NO_3, PO_4, and NH_3 were reduced by 51.7, 53.8, 76.7, and 94.4% (Fig.6).

Meanwhile, the heavy metal (Al, Mn, Cd and Pb) concentrations recorded highly significant reduction (p<0.01) after implementation of the combined treatment while Fe and Ni showed significant reduction (p<0.05). On the other hand, the reduction percentage of the Cu was moderate. Moreover, the removal percentages recorded for Cu and Fe were 30.0 and 60.9%, while Ni, Cd, Al, Mn and Pb were reduced by 90.3, 93.3, 98.8, 99.7, and 99.9%, respectively. This finding may be attributed to the high level of water pH leading to low reduction of Cu ions, where the optimum removal value of Cu was at the acidic pH of4.0 **(Saad *et al.*, 2020)**.

Treatment of polluted drainage water of fish farm using ME@CS nano-composite followed by protozooplankton

Table (3) exhibits the water parameters of El-Shakhlopa (Sidi Salem) fish farm wastewater treated with 0.5 g/L of ME@CS nano-composite for one hour, then treated with 1% of protozooplankton wheat culture and incubated for 48 hrs.

Ammonia recorded highly significant reduction (p<0.01) after the previously mentioned combined treatment, while nitrite, nitrate and phosphate showed a significant reduction (p<0.05). However, water environmental parameters such as pH, EC, TDS, and COD were reduced by 2.4, 2.4, 3.7, and 41.4%, while NO_3, NO_2, PO_4, and NH_3 were declined by 54.9, 63.3, 64.4, and 86.1% (Fig. 7). Ammonia was successfully removed from wastewater by eco-friendly biosorbents bentonite–chitosan composite **(Yadi *et al.*, 2016)**.

Table 2: Treatment of polluted drainage water of fish farm using Protozooplankton followed by ME@CS nano-composite

Parameter	Before	After	Accepted value
pH	8.2	7.9	5.0 – 9.0#
EC	4.1	4.0	ms/cm
TDS	2.7	2.5	< 1.0 g/L#
COD	29	14*	10 - 50 mg/L#
NH_3	0.36	0.02**	< 0.1 mg/L#
Nitrite	3.0	1.7*	< 0.1 mg/L#
Nitrate	0.91	0.42*	< 50 mg/L#
Phosphate	0.90	0.21*	< 0.1 mg/L#
Al	0.081	0.001**	< 50 ppb##
Mn	60.31	0.16**	< 10 ppb###
Fe	1387	542*	< 500 ppb###
Ni	10.4	1.01*	< 1000 ppb#
Cu	4.57	3.20	< 6.0 ppb###
Cd	0.015	0.001**	< 0.2-8 ppb#
Pb	1.609	0.001**	< 1.0-7.0 ppb#

N.B: The incubation period: 48 hrs; ME-CS nano-composite: 0.5 g/l contact time: 60 min; agitation rate: 200 rpm. *$p < 0.05$; **$p < 0.01$ ### Wedemeyer and Yasutake , 1977, ## FAO, 1993, # Water quality standards/criteria relevant to freshwater aquaculture, in Australia **(Wedemeyer & Yasutake, 1977; FAO, 1993; ANZECC, 2000).**

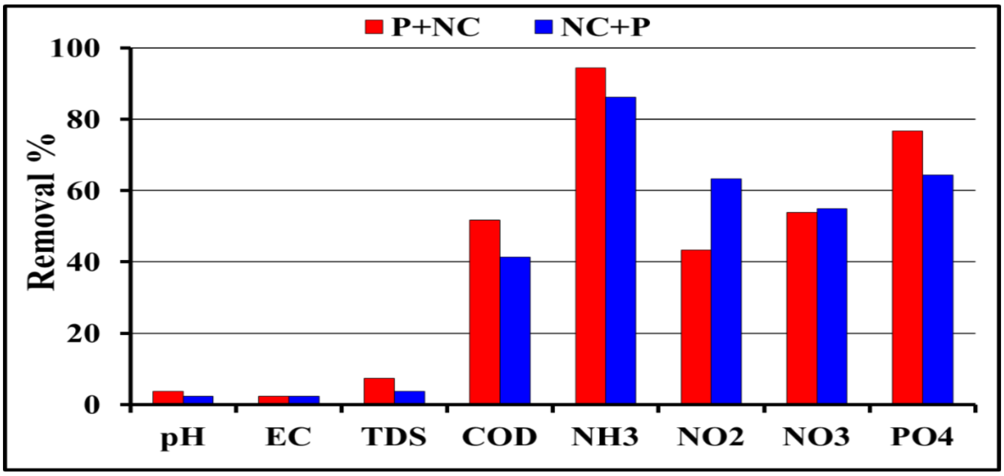


Fig. 6: Removal percentages of environmental parameters of fish farm wastewater using protozooplankton followed by ME@CS nanocomposite (P+NC) and ME@CS nano-composite followed by protozooplankton (NC+P)**.**

For heavy metals removal, the above-mentioned treatment removed metals as Pb, Mn, Cd, and Ni with a highly significant reduction (p<0.01), Al and Fe presented a significant one (p<0.05), while Cu showed no significant decline. Moreover, heavy metals such as Cu and Fe were removed with 16.8 and 43.5%, while Al, Ni, Cd, Mn and Pb were reduced by 84.0, 87.5, 93.3, 99.6, and 99.9% (Fig. 7). The superior adsorption performance of magnetic chitosan could be attributed to the electrostatic interaction and ion exchange between target pollutants and the grafted cationic polymer **(Zheng *et al.*, 2021)**.

Owing to its high adsorption capacity and rapid separation, ME@CS nano-composite has great potential for practical application in fish farms wastewater treatment. The present results could potentially satisfy the increasing need for the purification of wastewater resources contaminated with toxic metals.

Table 3: Treatment of polluted drainage water of fish farm using ME@CS nano-composite followed by protozooplankton

Parameter	Before	After	Removal %	Accepted value
pH	8.2	8.0	2.4	5.0 – 9.0#
EC	4.1	4.0	2.4	ms/cm
TDS	2.7	2.6	3.7	< 1.0 g/L#
COD	29	17	41.4	10 - 50 mg/L#
NH_3	0.36	0.05**	86.1	< 0.1 mg/L#
Nitrite	3.0	1.1*	63.3	< 0.1 mg/L#
Nitrate	0.91	0.41*	54.9	< 50 mg/L#
Phosphate	0.90	0.32*	64.4	< 0.1 mg/L#
Al	0.081	0.013*	84.0	< 50 ppb##
Mn	60.31	0.24**	99.6	< 10 ppb###
Fe	1387	784*	43.5	< 500 ppb###
Ni	10.4	1.3**	87.5	< 1000 ppb#
Cu	4.57	3.80	16.8	< 6.0 ppb###
Cd	0.015	0.001**	93.3	< 0.2-8 ppb#
Pb	1.609	0.001**	99.9	< 1.0-7.0 ppb#

(ME@CS nano-composite: 0.5 g/L with contact time: 60 min; Protozooplankton: 1% with incubation: 48 hrs; agitation time: 200 rpm).
*$p< 0.05$; **$p< 0.01$ ### Wedemeyer and Yasutake , 1977, ## FAO, 1993, # Water quality standards/criteria relevant to freshwater aquaculture, in Australia **(Wedemeyer & Yasutake, 1977; FAO, 1993; ANZECC, 2000).**

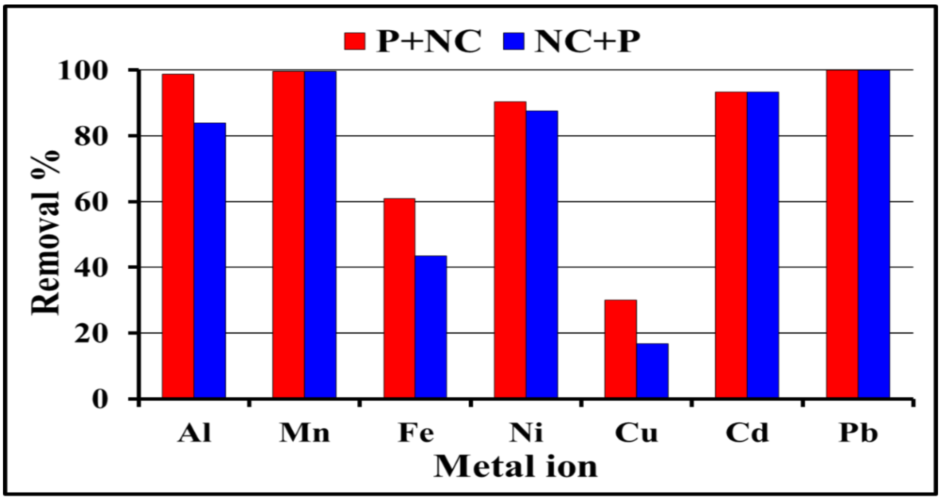


Fig. 7: Removal percentages of heavy metal ions from fish farm wastewater using protozooplankton followed by ME@CS nano-composite (P+NC) and ME@CS nano-compoite followed by protozooplankton (NC+P)

CONCLUSION

The present work proved that the combined treatment of protozooplankton and the nano-composite (ME@CS) is able to remove pollutants from fish farm wastewater. The study proved that wheat provided a suitable natural nutritive media to increase the number of protozoan organisms that have the ability to remove some pollutants from the wastewater. It is recommended to use the environmentally safe combined treatment using ME@CS nano-composite and protozooplankton, which showed promising results for the treatment of polluted drainage water used in open fish farms.The usage of ME@CS nano-composite and protozooplankton in semi-intensive and modern intensive aquaculture systems would lead to the optimization of water consumption, production of healthy fish and would consequently maintain safe human consumption of fish production.

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ARABIC SUMMARY

دمج الهائمات الأولية مع المركب النانومترى الإيديتا المغناطيسية-كيتوزان كنظام جديد لمعالجة مياه صرف المزارع السمكية

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الدراسات السابقة أثبتت أن معظم مزارع الأسماك في مصر تستخدم مياه الصرف التي تحتوي على نواتج الأنشطة الزراعية و الصناعية التي قد تلوث الأسماك المستزرعة. أثبتت الدراسة الحاليه أن المعالجة الثنائية بالهائمات ثم المركب النانومترى (ME@CS) كانت أكثر قدرة على إزالة الملوثات من مياه صرف المزارع السمكية وخاصة العناصر الثقيلة و الأمونيا. حيث سجلت تركيزات المعادن الثقيلة الالومنيوم (AL) والمنجنيز (MN) و الكادميوم (Cd) و الرصاص (Pd) إنخفاضا معنوياً كبيراً ($p<0.01$) بعد المعالجة المجمعة. وقد إنخفضت نسبة المنجنيز والرصاص 99.6 و 99.9 في المائة في حين إنخفضت عناصر النيكل (Ni) و الكادميوم (Cd) والالومنيوم (AL) بنسبة 90.3 و 93.3 و 98.8 في المائة على الترتيب. الأمونيا سجلت إنخفاضاً معنوياً كبيراً ($p<0.01$) بعد المعالجة المشتركة. انخفضت مؤشرات جودة المياه EC و pH و TDS و NO_2 بنسب 2.4 و 3.7 و 7.4 و 43.3 ٪. كما انخفض PO_4 و NH_3 بنسبة 76.7 و 94.4 ٪. كما تم تسجيل انخفاض معايير فيزيائية-كيميائية أخرى مثل COD والنترات والنترات والفوسفات معنوياً ($p<0.05$) إلى 41.4 و54.9 و 63.3 و 64.4٪ على الترتيب. تدعم نتائج هذا البحث إستخدام المعالجة المشتركة بين المركب النانومترى CS-ME والعوالق الأولية في نظم الاستزراع المائي المكثفة وشبه المكثفة والتى ستؤدي إلى تقليل إستهلاك المياه و إنتاج أسماك صالحة للاستهلاك البشري.