Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(2): 859 – 875 (2022)
www.ejabf.journals.ekb.eg

Impact of chitosan and chitosan nanoparticles on reducing heavy metals in the Nile tilapia, *Oreochromis niloticus*

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ARTICLE INFO

Article History:
Received: March 9, 2022
Accepted: April 14, 2022
Online: April 29, 2022

Keywords:
Chitosan nanoparticles,
Heavy metals,
Alleviation,
Nile tilapia,
Amelioration,
Fish quality

ABSTRACT

The current study was conducted to assess the ability of chitosan and chitosan nanoparticles to alleviate the effect of heavy metals in the Nile tilapia (*Oreochromis niloticus)*. The experimental design was completely randomized with a 2 x 3 factorial design; two protein sources (fish and gluten meals) and three forms of chitosan "CS" (zero-chitosan, CS, and CS nanoparticles "CSNP"). A total number of 270 Nile tilapia fingerlings were randomly distributed among 18 tanks (water capacity = 55 L). The overall duration of the experiment was 82 days.

Results revealed that fish fed on a fish meal-based diet recorded the highest retained heavy metals followed by those fed gluten meals. Moreover, supplementation of diets with CS and CSNP lowered the concentration of the retained heavy metals in the fish's whole body, especially CSNP.

Consequently, it is recommended to use CS and CSNP, especially CSNP as feed additives for fish cultured in agricultural and sewage wastewaters, loaded with high concentrations of heavy metals to ameliorate the fish quality.

INTRODUCTION

The past couple of decades witnessed a remarkable spread of aquaculture in Egypt **(Kaleem and Sabi, 2021)** that surpassed the capture of fisheries. It is expected that aquaculture will be the main source of aquatic animal nutrition in the coming years **(Ahmed *et al.*, 2020)**. Since the Egyptian irrigation law prohibits the usage of Nile water in fish farming, agricultural drainage water has become Egypt's primary aquaculture source **(Soliman & Yacout, 2016)**. Interestingly, this source is contaminated with domestic industrial effluents, sewage, and agrochemicals (**Khallaf *et al.*, 1998)** as well as elevated concentrations of pesticides, runoff-derived fertilizers, and heavy metals **(Authman *et al.*, 2013)**. These contaminants have been

shown to have negative impacts on the quality of farmed fish and, consequently, on human health as an end user.

Heavy metals are categorized into essential and non-essential. Essential heavy metals (as, Cu, Zn, Fe, Cr, and Mn) are required in low concentrations for the body welfare **(Santos *et al*., 2014).** On the other hand, non-essential heavy metals (as, Cd and Pb) do not have biological functions in living organisms **(Gati *et al*., 2016).** Heavy metals enter the aquatic food chain via direct consumption of water or food (**Emam and Soliman, 2021**). Being enduring and non-biodegradable, heavy metals can cause various diseases to living organisms once accumulated in the aquatic ecosystem (**Bayomy *et al*., 2015**). Accumulation of heavy metals in tissues and organs of fish entering the food chain has been reported to reach the highest rate in relation to the end users **(Akan *et al*., 2009)**.

Recently, interest in removing heavy metal ions from aqueous solutions has increased, both for the control of pollution and for the recovery of raw materials. Adsorption-based technologies have emerged as one of the most effective alternatives for treating industrial wastewater contaminated with a variety of pollutants, both inorganic (**Fu & Wang, 2011**) and organic (**Vocciante *et al*., 2019**), due to the availability of various types of low-cost and environmentally friendly adsorbents, their ease of operation, as well as their low processing and instrumentation costs. Metal oxides, polymers, activated carbon, carbon nanotubes, wastes of agriculture, and engineered as well as natural clays **(Chen *et al*., 2011)** have been effectively used to adsorb heavy metals from aqueous solutions. This becomes even more noteworthy when using low-cost sorbents derived from industrial waste (**Pietrelli *et al*., 2019**), as it would provide a double benefit to the environment while adhering to operational principles, such as the "Circular Economy" and "near-zero discharge" of harmful waste **(Pietrelli *et al*., 2018)** mandated by the most recent European legislation. One of the highly promising and economical substances in this area is chitosan (CS) **(El-Naggar *et al*., 2019).**

Chitin is the second most abundant natural linear homopolysaccharide after cellulose **(Fadlaoui *et al*., 2019)**. Chitosan, (β-(1→4)-2-amino-2-deoxy-β-D-glucose), is the partially N-deacetylated analog of chitin and is nontoxic biodegradable hydrophilic heteropolysaccharide (**Robertson, 2014**). CS has a high potential for metal ion adsorption because of its amino and hydroxyl groups which can function as chelation sites for metal ions. It combines with several heavy metals to generate complexes, and its amine groups serve as heavy metal coordination sites **(Wan Ngah & Fatinathan, 2010**; **Chauhan *et al*., 2012)**. Amongst the most intriguing benefits of chitosan is its adaptability, since the material can be readily and physically changed to produce various polymer shapes (as beads **(Chiou & Li, 2003)**, membranes **(Pietrelli & Xingrong, 2004)**, and sponges **(Ko *et al*., 2010)**) for different applications. Over and above, chitosan can be easily modified chemically to increase its applications **(Guibal, 2004)**. Numerous critical evaluations on the diverse applications of chitosan as an ecologically benign biomaterial have recently been published, spanning from the medical industry to environmental

protection and food technology **(Gamage & Shahidi, 2007**; **Zhao *et al*., 2018**; **Darweesh *et al*., 2020; Salaah *et al*., 2021**; **El-Naggar *et al*., 2020, 2022)**.

The field of nanotechnology has experienced a remarkable growth **(El-Naggar *et al*., 2019)**. Nanoparticles have the ability to treat both human and animal diseases as well as many other health-related problems **(El-Naggar *et al*., 2020)**. Their small size increases the available surface area to interact with biological systems, facilitates efficient cellular uptake and deep penetration into target sites, and increases the bioavailability of essential compounds **(Alishahi *et al*., 2011)**. The efficacy of chitosan nanoparticles (CSNP) has been demonstrated, serving as immune enhancers in *Oreochromis niloticus* (**El-Naggar *et al*., 2021**), as a feed additive that improves the growth and meat quality of *O. niloticus* (**El-Naggar *et al*., 2022**), and as a chelating agent for heavy metals in water treatment (**Abd-Elhakeem *et al*., 2016**).

Nile tilapia (*Oreochromis niloticus*) is the main fish farmed in East Africa and the third most important fish group cultured globally after salmon and carp **(El-Sayed, 2006)**. Due to its low cost, it is regarded as the most popular source of protein in Egypt **(Kaleem and Sabi, 2021)**. The success of *O. niloticus* in aquaculture is due to its ability to eat different types of foods, survive in low-oxygen environments **(El-Sayed, 2019)**, and its strong immune system that increases the ability to withstand stress conditions **(Abdel-Tawwab *et al*., 2008; Salaah, 2021)**.

Unfortunately, there isn't any prior research to use as a guide for the efficiency of chitosan (CS) and chitosan nanoparticles (CSNP) in eliminating heavy metals from *O. niloticus*. Accordingly, the current study aimed for the first time to evaluate the effect of CS and CSNP in the removal of heavy metals from *O. niloticus*.

MATERIALS AND METHODS

1. Experimental fish and culture technique

The present study was carried out at the Fish Nutrition Laboratory, Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt. Mixed sex *O. niloticus* fingerlings, with an average (±SE) initial body weight of 15.3 (±0.08) g, were collected from a local hatchery located in Kafr El-Sheikh Governorate, Egypt. Under controlled thermal conditions, a total of 270 *O. niloticus* fingerlings were randomly distributed into 6 different treatments with a triplicate of 15 fish each. The water capacity of each tank was 55 L. Fish were grown out under laboratory recirculating aquaculture system (RAS) with a flow rate of 0.4 L/min. Fish were acclimatized to the experimental conditions for a week prior to the feeding trial. Fish were fed a floating diet of 30% crude protein at 2% of the fish body weight twice daily (10 a.m. and 4 p.m.). During the experiment, the fish were fed till apparent satiation. Fish weight was measured every 15 days, and the number of dead fish was recorded daily. The overall experiment lasted 82 days.

2. Experimental design and diets

The experimental design was completely randomized with a 2 x 3 factorial design, with two protein sources, fish meal (FM) and gluten meal (GM), and three forms of chitosan (zero-chitosan, CS, and CSNP). Fish meal basal diet was formulated and 100% of fish meal was replaced with GM in gluten meal-basal diet. Each of the basal diets were supplemented with CS or CSNP (0.5%) to design six isonitrogenous (30% crude protein) and isocaloric (4500 kcal/kg) for *O. niloticus* **(NRC, 2011)**. The dose was managed according to **Wang and Li (2011)**.

The proximate composition of the experimental diets (**Table 1**) was analyzed in the Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture. The experimental diets were prepared by blending the ingredients into a homogeneous mixture and then passing it through a local minced meat machine, dried overnight at room temperature and stored in plastic bags at 4°C till furtherly used. The extraction of CS from *Procambarus clarkii* wastes and the preparation of CSNP as well as their characterization were profoundly discussed in the study of **El-Naggar *et al*. (2019).**

Table 1. Formulation and proximate composition of fish meal and gluten meal-based diets supplemented with CS or CSNP

Ingredient (g/100g)	FM-based diet			GM-based diet		
	FM	**FMCS**	**FMCSNP**	**GM**	**GMCS**	**GMCSNP**
Fish meal	14	14	14	-	-	-
Gluten	-	-	-	14	14	14
Soybean	34	34	34	34	34	34
Corn	8.43	7.93	7.93	8.43	7.93	7.93
Oil	6	6	6	6	6	6
Bran	35	35	35	35	35	35
Vitamin and mineral premix*	2	2	2	2	2	2
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05
Carboxy methyl cellulose	0.50	0.50	0.50	0.50	0.50	0.50
Butylated hydroxytoluene	0.02	0.02	0.02	0.02	0.02	0.02
Chitosan	-	0.50	-	-	0.50	-
Chitosan nanoparticles	-	-	0.50	-	-	0.50
Total	**100**	**100**	**100**	**100**	**100**	**100**
Proximate composition (% dry matter)						
▪ **Moisture**	7.8	7.9	7.7	7.8	8	8.6
▪ **Crude protein**	30.7	30.5	30.8	29.1	29.5	29.5
▪ **Crude lipid**	8.62	8.46	8.57	8.22	8.23	8.17
▪ **Ash**	6.8	6.9	6.8	5	5.3	5.3
▪ **Crude fiber**	6.73	6.99	6.94	7.02	6.91	6.89
▪ **Nitrogen free extract (NFE)**	39.35	39.25	39.19	42.86	42.06	41.54
▪ **Gross energy kcal/kg**	4469	4449	4472	4501	4486	4458

***** Provides per kg of diet: retinyl acetate, 3,000 IU; cholecalciferol, 2,400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49 % ascorbic acid), 120 mg; cyanocobalamin, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1,200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3,077 mg; ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 20 % Fe), 65 mg; manganese sulfate ($MnSO_4$, 36 % Mn), 89 mg; zinc sulfate ($ZnSO_4 \cdot 7H_2O$, 40 % Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1,000 mg Agri-Vet Co., Cairo, Egypt.

3. Water quality parameters

Water temperature (°C) was registered using Senso Direct Oxi 200. The pH value was determined using Milwaukee-pH600 digital pH meter twice a week. The alkalinity (mg/L) was estimated once a week by titration with sulfuric acid till the pH reached 4.5 **(Boyd & Tucker, 1992)**. The total ammonia nitrogen (NH_3-N; mg/L) and nitrite (NO_2^-; mg/L) values were evaluated according to **Boyd and Tucker (1992)**, using water analysis photometer (MultiDirect Lovibond) once a week. Following the instruction manual of the MultiDirect Lovibond, the total ammonia nitrogen concentration, including the ionized (NH_4^+) and unionized (NH_3) forms, was calculated by multiplying the resultant value of NH_3-N by a conversion factor, 1.29 and 1.22, for NH_4^+ and NH_3, respectively.

4. Heavy metals analysis

Along with the 270 fingerling samples, a sample of thirty fish was gathered to serve as an initial one at the beginning of the experiment. At the end of the experiment, fish were anesthetized using clove oil (40 mg/L), and then six fish from each tank were randomly selected to serve as a final sample. The fish were then killed, oven-dried at 60−70°C, ground into homogenous powder and stored at -20ºC until further analysis.

The determination of the heavy metals concentrations, in their total form, in fish carcass involves two main steps; namely, digestion and analysis. The digestion of the fish carcass, as well as heavy metals analysis were measured according to the Standard Method of the American Public Health Association **(APHA-3111B, 2017)** at the Central Laboratory of Faculty of Science, Ain Shams University. The digestion was performed using CEM Microwave Sample Preparation System (MDS-2000, USA).

Procedure of digestion: One gram of the ground samples was placed in vessels and concentrated nitric acid (HNO_3) was added. The vessels were left overnight to allow sufficient reaction. Afterwards, the vessels were placed into a turntable connected to the system and a heating program and left to run till digestion completion. Eventually, the samples were left to cool for 5min, and the turntable was removed from the system. Heavy metals concentrations of the fish carcass were measured using Flame Atomic Absorption Spectrophotometer (Savant AA, GBC Scientific Equipment). The equipment is provided with acetylene as a source of fuel and air as an oxidant.

Procedure of analysis: The digested samples were added to 25ml distilled water and placed in the spectrophotometer. The concentration of each heavy metal was measured at a certain wavelength and slit width. Six heavy metals were analyzed (Cu, Zn, Fe, Cd, Pb, Cr, and Ni), and the resultant concentrations were expressed in mg/kg.

The retained concentrations of heavy metals (X; mg/kg) were calculated according to **Hernández and Roman (2016),** with slight modifications using the following formula

$$\mathbf{X = (BW * HM)_{Final} - (BW * HM)_{Initial}}$$

where, **BW:** fish body weight (g); **HM:** heavy metal concentration (mg/kg)

5. Statistical analysis

The experiment was conducted on triplicates. Jeffreys's Amazing Statistics Program (JASP 0.14) was used to carry out two-way analysis of variance (ANOVA) to detect the impact of the dietary protein source, chitosan forms, and their interactions on the heavy metals' retention in the whole fish. Tukey's test **(Abdi & Williams, 2010)** was used to determine the differences among the experimental treatments.

RESULTS AND DISCUSSION

1. Water quality parameters

Water quality has a great impact on aquaculture; it affects the health status, behavior, and growth of fish **(Osman *et al.*, 2021).** Generally, water temperature has a remarkable impact on the initiation and the course of a number of fish diseases, with a considerable effect on the growth rate. The optimum temperature required, for rearing *O. niloticus*, varies from 25 to 27°C according to **DeWalle *et al.* (1995).** In the present experiment, the readings of water temperature varied from 21.53 to 26.90 °C, with an average of 24.51°C (**Table 2**).

Throughout the experiment, the pH values were alkaline (**Table 2**) fluctuating between 7.97 to 8.45 with an average of 8.28 . The optimal pH range for *O. niloticus* is from 6 to 9 **(DeWalle *et al.*, 1995)**. Destruction and mortality of fish species are associated with the alkaline pH values above 9.2 and acidity below 4.8 **(FAO, 1993)**.The gills are the most susceptible organs to a great damage due to extremely high or low pH values, where hemorrhages may occur in the gills and on the lower part of the body. Moreover, excessive mucus secretion, often containing blood, can be observed in post mortem examination of the gills and skin **(FAO, 1993)**.

During the current study, the total alkalinity fluctuated from 299.2 to 396 mg/L with a mean of 334.53 mg/L, and this confirms that the aquaria water is on the alkaline side (**Table 2**). The total alkalinity of water is the concentration of titratable bases, mainly carbonates (CO_3^{-2}) and bicarbonates (HCO_3^-), which is expressed as $CaCO_3$ equivalents. In order to achieve an optimum fish growth performance, water used for aquaculture must have a total alkalinity ≥ 20 mg/L $CaCO_3$ (in fresh water) (**Andrade *et al.*, 2007**). Notably, water with low total alkalinity is more susceptible to acidification than water with high total alkalinity.

Total Ammonia nitrogen (NH_3-N) includes both the ionized form (ammonium, NH_4^+) and the unionized form (ammonia, NH_3). The latter form is highly toxic for fish (**Chervinski, 1982**). The toxicity of the total ammonia nitrogen is highly apparent if the level is higher than 1.0 mg/L **(DeLong *et al.*, 2009)**. It is worth mentioning that the toxicity of ammonia is inversely proportional with the dissolved oxygen and CO_2, whereas directly proportional with the pH value (**Chervinski, 1982**). In the present study, NH_3-N concentrations varied from 0 to 0.31 mg/L with a mean of 0.14 mg/L (**Table 2**). NH_4^+ concentrations varied from 0 to 0.38 mg/L with a mean of 0.18 mg/L, while the concentrations of NH_3 ranged from 0 to 0.40 mg/L, with an average of 0.17 mg/L.

During the nitrification process, ammonia is oxidized into nitrite (NO_2) then converted into nitrate (NO_3) through nitrifying bacteria grown on suspended organic matter. Nitrite has a toxic effect on fish, including tilapia, since it causes growth retardation and disturbs the physiological functions of the fish as well **(Sudharsan *et al.*, 2000)**. On the other hand, nitrate is relatively non-toxic to tilapia; however, chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile *O. niloticus* in recirculating aquaculture systems (**Monsees *et al.*, 2017).** Throughout the experiment, the level of nitrite (NO_2) ranged from 0.04 to 1.35 mg/L, with an average of 0.42 mg/L. The optimum tolerable range of nitrite (NO_2) for the culture of *O. niloticus* is from 0.08 to 1.0 mg/L **(Otoo *et al.*, 2019)** (**Table 2**). Nitrite concentration above 5.0 mg/L is extremely toxic for tilapia **(DeLong *et al.*, 2009)**.

The present study showed that the values of water quality parameters used for rearing *O. niloticus* under RAS system were quite adequate (**Fig. 1**). This result is coincided with that of **Cruz and Ridha (2001)**.

Table 2. Values of water quality used in the recirculating aquaculture system during the experimental study

Parameters	Measured Values Min – Max (Mean)	Optimum values (Reference)	
Water Temperature (°C)	21.53 – 26.90 (24.51)	25 – 27	**(DeWalle *et al.*, 1995)**
pH	7.97 – 8.45 (8.28)	6 – 9	**(DeWalle *et al.*, 1995)**
Total alkalinity (mg/L)	299.2 – 396 (334.53)	≥ 20	**(Andrade *et al.*, 2007)**
Total ammonia nitrogen (mg/L)	0 – 0.31 (0.14)	< 1	**(DeLong *et al.*, 2009)**
Nitrite (mg/L)	0.04 – 1.35 (0.42)	0.08 – 1	**(Otoo *et al.*, 2019)**

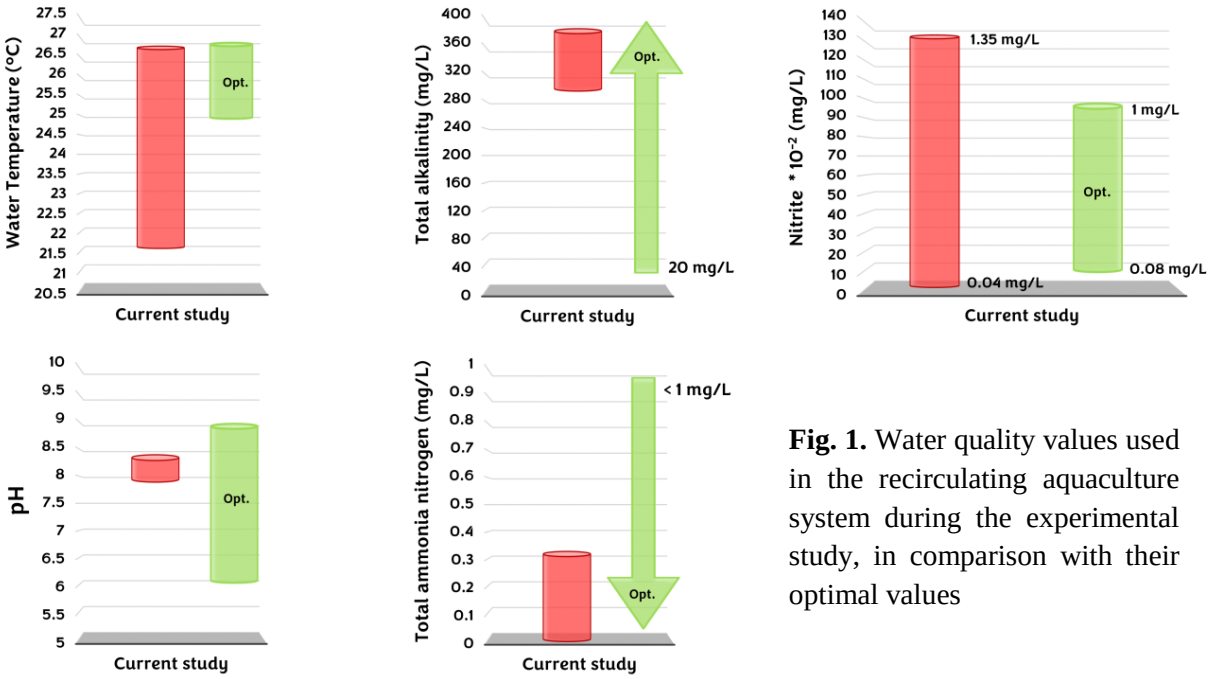

Fig. 1. Water quality values used in the recirculating aquaculture system during the experimental study, in comparison with their optimal values

2. Heavy metals analysis

In the aquatic ecosystem, heavy metals are regarded as the paramount pollutants, since they are present throughout the ecosystem and are detectable in trace amounts **(Authman *et al.*, 2015)**. They are detrimental for fish health, either above the threshold level, as in case of non-essential heavy metals, or below and above the permissible concentration, for essential heavy metals **(Sfakianakis *et al.*, 2015)**. Most of these metals are accumulated in tissues, causing fish poisoning, inducing pathological changes, affecting the reproduction and suppressing the immune system. Consequently, fish are used as bio-indictors for monitoring heavy metal pollution **(Authman *et al.*, 2015)**.

The effect of CS and CSNP on heavy metals' retention in *O. niloticus* (whole fish) fed fish meal and gluten meal-based diets is summarized in **Table (3)**. Results showed that both chitosan forms significantly affected Cd and Ni retention (**Fig. 2**). The highest significant retained heavy metal concentration was recorded in fish fed fish meal-based diet, followed by those fed gluten meal-based diet. Moreover, the dietary fortification with CS and CSNP lowered the retention of the Cd and Ni, especially CSNP supplementation.

On the other hand, no significant difference was recorded upon using CS and CSNP on the Cu, Zn, Fe, Pb, and Cr retention in *O. niloticus* (**Fig. 2**). However, they differed numerically; fish fed fish meal-based diet recorded the highest retained heavy metals, followed by those fed gluten meal-based diet. Furthermore, the retention of the afore-mentioned heavy metals was numerically declined upon fortifying diets with CS and CSNP. Moreover, the lowest retained heavy metal concentration was recorded in fish fed diets supplemented with CSNP, especially

gluten meal supplemented diets. Our study anticipates that both dietary chitosan and nano chitosan have the capacity to reduce metals' accumulation in the body and protect the vital organs function against metal intoxication.

Table 3. Impact of CS and CSNP on heavy metal retention (mg/kg) in *O niloticus* (whole fish) fed fish meal and gluten meal-based diets

HM	FM-based diets			GM-based diets			MSE	*P*-value		
	FM	FMCS	FMCSNP	GM	GMCS	GMCSNP		Protein source effect	CS forms effect	Protein source X CS forms effect
Cd	0.010^{a}	0.005^{b}	0.004^{b}	0.006^{ab}	0.006^{ab}	0.004^{b}	0.001	0.400	0.007	0.017
Ni	0.022^{ab}	0.021^{b}	0.017^{cb}	0.019^{cb}	0.015^{cb}	0.012^{c}	0.002	0.009	0.051	0.582
Cu	0.144	0.082	0.066	0.096	0.078	0.066	0.017	0.582	0.101	0.855
Zn	0.224	0.120	0.107	0.171	0.122	0.042	0.071	0.520	0.261	0.885
Fe	0.395	-0.183	-0.413	-0.252	-0.396	-0.484	0.091	0.536	0.061	0.910
Pb	0.067	0.058	0.036	0.054	0.043	0.040	0.007	0.172	0.020	0.310
Cr	0.012	0.007	0.011	0.012	0.008	-0.448	0.185	0.331	0.391	0.387

Each value denotes means (n=3).
Different superscripts in each row designate significant difference ($P < 0.05$) by Tukey test.
MSE: Mean standard error.

CS and CSNP are regarded as impressive metal ligands, giving rise to stable complexes with multiple metal ions **(Gamage & Shahidi, 2007)**. CS possesses an elevated adsorption capacity for various metal ions, such as Ni^{2+}, Zn^{2+}, Fe^{2+}, Mg^{2+}, and Cu^{2+} in acidic condition. Accordingly, it has been used for the recovery of metal ions in several industries **(Kurita, 1998)**. The detoxification process in aquatic animals have been well documented by two main mechanisms, comprising intracellular ligands: cytosolic metal binding compounds, such as metallothionein proteins and biomineralization; the relative relevance of both detoxification mechanisms varied according to species **(Marigómez *et al*., 2002)**. CS has the tendency to form coordinate bond with the heavy metal forming a complex through the donation of its lone pair to the vacant orbital of the heavy metal **(Hussein *et al*., 2012)**. The mechanism of chelation of CS and CSNP in vivo can be interpreted as follows: in vivo, the total metal breaks down through the digestion process, then absorbed through the gastrointestinal tract; mainly the small intestine, entering the tissues in its active ionic (cationic) state **(Goff, 2018)** forming coordinate bonds with CS and CSNP.

Additionally, dietary CS and nano chitosan were found to enhance the antioxidant defense system in fish **(El-Naggar *et al*., 2021; Salaah *et al*., 2021)**. One of the main antioxidant protective molecules in cells are thiols. Thiol groups are essential for metal detoxification in the liver, which may involve in the removal of metals from the body through the urine and gut (**Eliaz *et al*., 2006**).

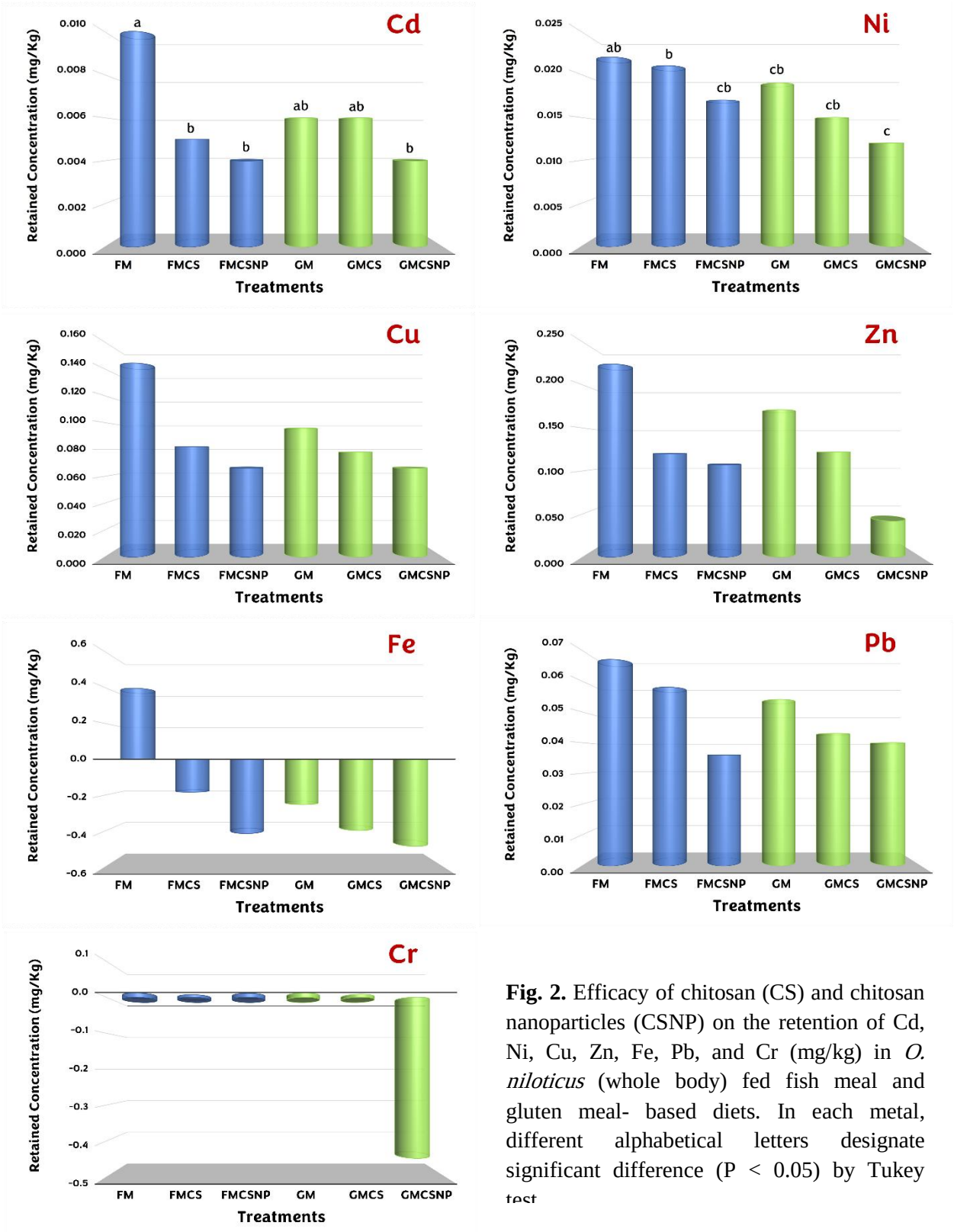

Fig. 2. Efficacy of chitosan (CS) and chitosan nanoparticles (CSNP) on the retention of Cd, Ni, Cu, Zn, Fe, Pb, and Cr (mg/kg) in *O. niloticus* (whole body) fed fish meal and gluten meal- based diets. In each metal, different alphabetical letters designate significant difference ($P < 0.05$) by Tukey test

Thilagar and Samuthirapandian (2020) documented low Pb accumulation in the fish receiving dietary chitosan supplements. **Setiyorini *et al.* (2022)** studied the use of chitosan nanoparticles as a therapeutic agent for metal exposure in rats. Low molecular weight nano chitosan performed well in absorbing harmful metal in the blood circulation and gradually lowering its concentrations in the body. The reduction in metal content in the body was followed by a progressively improved body weight over the six-week nano chitosan supplementation.

Ismaiel *et al.* (2015) compared plant protein-based diets to fishmeal-based diets and suggested that the latter might accumulate harmful substances, causing organs histopathological alterations. Moreover, it was noted that, the ash content was higher in FM-based diet than GM-based diet (**Table 1**). Thus, it is more efficient to supplement fishmeal-based diet with CSNP that has higher effective chelation capacity for heavy metal compared to CS. Small size, large surface and high stability may be the reasons for CSNP high ability of chelation **(Seyedmohammadi *et al.*, 2016; Zareie *et al.*, 2019)**. The higher density of adsorption sites of CSNP increases the probability of coordinate bond formation between the CSNP functional group and heavy metals compared to CS (**Yu *et al.*, 2013)**.

CONCLUSION

In conclusion, it was found that the retained heavy metals in fish fed GM-based diets were lower than those fed FM-based diets. Moreover, the supplementation of CS and CSNP to fish meal and gluten meal-based diets lowered the concentrations of the retained heavy metals in the fish whole body, particularly CSNP. So, it is preferable to use the gluten meal in the fish diet, as it has lower levels of heavy metals, and it is cheaper than the fish meal. Moreover, it is recommended to use CS and CSNP as feed additives for fish cultured in agricultural and sewage wastewaters, which are always loaded with high levels of heavy metals. Furthermore, CS and CSNP can be used in fish finisher diets to maintain the accumulated heavy metals at permissible levels for the sake of the consumer's safe and health.

ACKNOWLEDGMENTS

This work was funded by Ain Shams University's Scientific Research Sector. Special thanks to Dr. Rania Ahmed (Associate Professor of Fish Nutrition, Fish Nutrition Lab, Department of Animal production, Faculty of Agriculture, Cairo University) for her valuable insight and expertise that greatly assisted the research.

REFERENCES

Abd-Elhakeem, M.A.; Ramadan, M.M. and **Basaad, F.S. (2016).** Removing of heavy metals from water by chitosan nanoparticles. Journal of advances in Chemistry, 11**(7)**: 3765−3771. https://doi.org/10.24297/jac.v11i7.2200

Abdel-Tawwab, M.; Abdel-Rahman A.M. and **Ismael, N.E.M. (2008).** Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture, 280 **(1−4)**: 185−189. https://doi.org/10.1016/j.aquaculture.2008.03.055

Abdi, H. and **Williams, L.J. (2010).** Newman-Keuls Test and Tukey Test. In: Salkind, N. (Ed.), Encyclopedia of Research Design, Thousand Oaks, CA: Sage. https://personal.utdallas.edu/~herve/abdi-NewmanKeuls2010-pretty.pdf

Ahmed, I.; Reshi, Q.M. and **Fazio, F. (2020)**. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquaculture International, 28: 869−899. https://doi.org/10.1007/s10499-019-00501-3

Akan, J.C.; Abdulrahman, F.I.; Sodipo, O.A. and **Akandu, P.I. (2009).** Bioaccumulation of some heavy metals of six fresh water fishes caught from Lake Chad in Doron Buhari, Maiduguri, Borno State, Nigeria. Journal of Applied Sciences in Environmental Sanitation, 4**(2)**: 103−114.

Alishahi, A.; Mirvaghefi, A.; Tehrani, M.R.; Farahmand, H.; Shojaosadati, S.A.; Dorkoosh, F.A. and **Elsabee, M.Z. (2011).** Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles, Food Chemistry, 126 **(3)**: 935−940. https://doi.org/10.1016/j.foodchem.2010.11.086

American Public Health Association (APHA-3111B) (2017). Standard methods for the examination of water and wastewater. 23rd edition. Rice, E.W.; Baird, R.B. and Eaton, A.D (eds.). Washington, D.C., 40 pp.

Andrade, L.S.; Andrade, R.L.B.; Becker, A.G.; Rossato, L.V.; Rocha, J.F. and **Baldisserotto, B. (2007).** Interaction of water alkalinity and stocking density on survival and growth of Silver catfish, *Rhamdia quelen*, juveniles. Journal of the World Aquaculture Society, 38**(3)**: 454−458. https://doi.org/10.1111/j.1749-7345.2007.00118.x

Authman, M.M.; Abbas, H.H. and **Abbas, W.T. (2013).** Assessment of metal status in drainage canal water and their bioaccumulation in *Oreochromis niloticus* fish in relation to human health. Environmental Monitoring and Assessment, 185: 891−907. https://doi.org/10.1007/s10661-012-2599-8

Authman, M.M.N.; Zaki, M.S.; Khallaf, E.A. and **Abbas, H.H. (2015).** Use of fish as bio-indicator of the effects of heavy metals pollution. Journal of Aquaculture Research and Development, 6(**4**): 1000328. http://dx.doi.org/10.4172/2155-9546.1000328

Bayomy, M.F.F.; Alne-na-ei, A.A.; Gaber, H.S.; Sayed, H.A. and **Khairy, D.M. (2015).** Environmental and physiological impacts of heavy metals on Nile tilapia (*Oreochromis niloticus*). Journal of Bioscience and Applied Research, 1**(2)**: 52−58. http://dx.doi.org/10.4172/2155-9546.1000328

Boyd, C.E. and **Tucker, C.S. (1992)**. Water Quality and Pond Soil Analysis for Aquaculture. Boyd, C.E. (ed.). Alabama Agricultural Experiment Station, Auburn University, Alabama. 183 pp.

Chauhan, D.; Jaiswal, M. and **Sankararamakrishnan, N. (2012).** Removal of cadmium and hexavalent chromium from electroplating waste water using thiocarbamoyl chitosan. Carbohydrate Polymers, 88**(2)**: 670−675. https://doi.org/10.1016/j.carbpol.2012.01.014

Chen, L-F.; Liang, H-W.; Lu, Y.; Cui, C-H. and **Yu, S-H.** (**2011).** Synthesis of an attapulgite clay@carbon nanocomposite adsorbent by a hydrothermal carbonization process and their application in the removal of toxic metal ions from water. Langmuir, 27**(14)**: 8998–9004. https://doi.org/10.1021/la2017165

Chervinski, J. (1982). Environmental physiology of Tilapias. In: "The Biology and Culture of Tilapias". Pullin, R.V.S. & Lowe-McConnell, R.H. (eds). ICLARM Conference Proceedings No. 7, ICLARM, Manila, Philippines, pp. 119-128.

Chiou, M.S. and **Li, H.Y. (2003).** Adsorption behavior of reactive dye in aqueous solution on chemical cross-linked chitosan beads. Chemosphere, 50**(8)**: 1095–1105. https://doi.org/10.1016/S0045-6535(02)00636-7

Cruz, E.M. and **Ridha, M.T. (2001).** Growth and survival rates of Nile tilapia *Oreochromis niloticus* L. juveniles reared in a recirculating system fed with floating and sinking pellets. Asian fisheries science, 14: 9−16. https://doi.org/10.33997/j.afs.2001.14.1.002

Darweesh, W.; Mohamed, S.; Emam, W.; Abd El-Moniem, O. and **El-Khateeb, M. (2020).** Evaluation of Antibacterial Activity of Chitosan against Different Bacterial Strains. Egyptian Journal of Chemistry, 63**(7)**: 2265−2275. https://dx.doi.org/10.21608/ejchem.2019.20467.2227

DeLong, D.P.; Losordo, T.M. and **Rakocy, J.E. (2009)**. Tank culture of Tilapia. Southern Regional Aquaculture Center, 282. https://wkrec.ca.uky.edu/files/tilapiatankculture.pdf

DeWalle, D.R.; Swistock, B.R. and **Sharpe, W.E. (1995).** Episodic flow – duration analysis: a method of assessing toxic exposure of brook trout (*Salvenius fontinalis*) to episodic increases in aluminium. Canadian Journal of Fisheries and Aquatic Sciences, 52(**4**): 816−827. https://doi.org/10.1139/f95-081

Eliaz, I.; Weil, E.; and **Wilk, B. (2006).** Integrative medicine and the role of modified citrus pectin/alginates in heavy metal chelation and detoxification-five case reports. Forschende Komplementarmedizin, 14**(6)**: 358−364. https://doi.org/10.1159/000109829

El-Naggar, M.M.; Abou-Elmagd, W.S.I.; Suloma, A.M.; El-Shabaka, H.A.; Khalil, M.T. and **Abd El-Rahman, F.A.A. (2019).** Optimization and physicochemical characterization of chitosan and chitosan nanoparticles extracted from the crayfish *Procambarus clarkii* wastes. Journal of Shellfish Research, 38**(2)**: 385−395. https://doi.org/10.2983/035.038.0220

El-Naggar, M.M.; Haneen, D.S.A.; Mehany, A.B.M. and **Khalil, M.T. (2020).** New synthetic chitosan hybrids bearing some heterocyclic moieties with potential activity as anticancer and apoptosis inducers. International Journal of Biological Macromolecules, 150: 1323−1330. https://doi.org/10.1016/j.ijbiomac.2019.10.142

El-Naggar, M.M.; Medhat, F. and **Taha, A. (2022).** Applications of chitosan and chitosan nanoparticles in fish aquaculture. Egyptian Journal of Aquatic Biology and Fisheries, 26**(1)**: 23−43. https://dx.doi.org/10.21608/ejabf.2022.213365

El-Naggar, M.M.; Salah, S.; El-Shabaka, H.A.; Abd El-Rahman, F.A.A.; Khalil, M.T. and **Suloma, A. (2021).** Efficacy of dietary chitosan and chitosan nanoparticles supplementation on health status of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture Reports, 19: 100628. https://doi.org/10.1016/j.aqrep.2021.100628

El-Sayed, A-F.M. (2006). Tilapia Culture. CABI Pub., Wallingford, GB, 360 pp.

El-Sayed, A-F.M. (2019). Tilapia culture. Academic Press. https://doi.org/10.1016/C2017-0-04085-5

Emam, W.W.; El-Kafrawy, S.B. and **Soliman, K.M. (2021).** Integrated geospatial analysis linking metal contamination among three different compartments of Lake Edku ecosystem in Egypt to human health effects. Environmental Science and Pollution Research, 28: 20140–20156. https://doi.org/10.1007/s11356-020-11661-8

Fadlaoui, S.; El Asri, O.; Mohammed, L.; Sihame, A.; Omari, A. and **Melhaoui, M. (2019).** Isolation and characterization of chitin from shells of the freshwater crab *Potamon algeriense*. Progress on Chemistry and Application of Chitin and its Derivatives, XXIV: 23–35. http://dx.doi.org/10.15259/PCACD.24.002

Food and Agricultural Organization (FAO) (1993). Water quality and fish health. Technical paper No. 54, Rome, Italy, 71pp.

Fu, F. and **Wang, Q. (2011).** Removal of heavy metal ions from wastewaters: A review. Journal of Environmental Management, 92(**3**): 407–418. https://doi.org/10.1016/j.jenvman.2010.11.011

Gamage, A. and **Shahidi, F. (2007).** Use of chitosan for the removal of metal ion contaminants and proteins from water. Food Chemistry, 104 **(3)**: 989−996. https://doi.org/10.1016/j.foodchem.2007.01.004

Gati, G.; Pop, C.; Brudaşcă, F.; Gurzău, A.E. and **Spînu, M. (2016).** The ecological risk of heavy metals in sediment from the Danube Delta. Ecotoxicology, 25: 688–696. https://doi.org/10.1007/s10646-016-1627-9

Goff, J.P. (2018). Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. Journal of dairy science, 101**(4)**: 2763−2813. https://doi.org/10.3168/jds.2017-13112

Guibal, E. (2004). Interactions of metal ions with chitosan-based sorbents: a review. Separation and purification technology, 38**(1)**:43−74. https://doi.org/10.1016/j.seppur.2003.10.004

Hernández, A.J. and **Roman, D. (2016)**. Phosphorus and nitrogen utilization efficiency in rainbow trout (*Oncorhynchus mykiss*) fed diets with lupin (*Lupinus albus*) or soybean (*Glycine max*) meals as partial replacements to fish meal. Czech Journal of Animal Science, 61**(2)**: 67−74. https://doi.org/10.17221/8729-CJAS

Hussein, M.H.M.; El-Hady, M.F.; Sayed, W.M. and **Hefni, H. (2012)**. Preparation of some chitosan heavy metal complexes and study its properties. Polymer Science Series A, 54**(2)**: 113−124. https://doi.org/10.1134/S0965545X12020046

Ismaiel, Y.M.; Khedr, N.A and **Ahmed, T.E. (2015).** Effect of fish meal and plant protein alternatives on the histological picture of different organs on Nile tilapia in Egypt. Benha Veterinary Medical Journal, 28(**2**): 273−282. https://dx.doi.org/10.21608/bvmj.2015.32513

Kaleem, O. and **Sabi, A-F.B.S. (2021).** Overview of aquaculture systems in Egypt and Nigeria, prospects, potentials, and constraints. Aquaculture and Fisheries, 6(**6**): 535−547. https://doi.org/10.1016/j.aaf.2020.07.017

Khallaf, E.A.; Galal, M. and **Authman, M. (1998).** Assessment of heavy metals pollution and their effects on *Oreochromis niloticus* in aquatic drainage water. Journal of the Egyptian-German Society of Zoology, 26 (**B**): 39−47.

Kurita, K. (1998). Chemistry and application of chitin and chitosan. Polymer Degradation and Stability, 59 **(1−3)**: 117–120. https://doi.org/10.1016/S0141-3910(97)00160-2

Marigómez, I., Soto, M., Cajaraville, M.P., Angulo, E. and **Giamberini, L. (2002).** Cellular and subcellular distribution of metals in molluscs. Microscopy research and Technique, 56**(5)**: 358−392. https://doi.org/10.1002/jemt.10040

Monsees, H.; Klatt, L.; Kloas, W. and **Wuertz, S. (2017).** Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (Oreochromis niloticus L.) in recirculating aquaculture systems. Aquaculture Research, 48**(7)**: 3482−3492. https://doi.org/10.1111/are.13174

NRC (National Research Council) (2011). Nutrient Requirements of Fish and Shrimp. Washington, DC: The National Academies Press. https://doi.org/10.17226/13039

Osman, A.G.M.; Farrag, M.M.S.; Badrey, A.E.A.; Khedr, Z.M.A. and **Kloas, W. (2021).** Water quality and health status of the monosex Nile Tilapia, *Oreochromis niloticus* cultured in aquaponics system (ASTAF-PRO). Egyptian Journal of Aquatic Biology & Fisheries, 25**(2)**: 785−802. https://dx.doi.org/10.21608/ejabf.2021.169899

Otoo, N.E.G.; Sanful, P.O.; Iddrisu, W.A.; Amfoh, S. and **Boateng, O.K. (2019).** Understanding water quality dynamics in aquaculture ponds in Sunyani, Ghana: Insights

from Partial Least Squares (PLS) - Path Modeling. Journal of Fisheries and Aquaculture Research, 4**(1)**: 28−42.

Pietrelli, L. and **Xingrong, L. (2004).** Chitosan membrane: Tool for chromium (III) recovery from aqueous solutions. Annali di chimica, 94**(5−6)**:389–398. https://doi.org/10.1002/adic.200490048

Pietrelli, L.; Ferro, S. and **Vocciante, M. (2018).** Raw materials recovery from spent hydrochloric acid-based galvanizing wastewater. Chemical Engineering Journal, 341: 539–546. https://doi.org/10.1016/j.cej.2018.02.041

Pietrelli, L.; Ippolito, N.M.; Ferro, S.; Dovì, V.G. and **Vocciante, M. (2019).** Removal of Mn and As from drinking water by red mud and pyrolusite. Journal of environmental management, 237: 526–533. https://doi.org/10.1016/j.jenvman.2019.02.093

Robertson, G.L. (2014). Food packaging. In: Van Alfen, N.K. (Ed.), Encyclopedia of Agriculture and Food Systems, Academic Press, Oxford, pp. 232–249. https://doi.org/10.1016/B978-0-444-52512-3.00063-2

Salaah, S. (2021). The influence of global warming on oxidative stress and antioxidant defense system in blue tilapia (*O. aureus*). Aswan University Journal of Environmental Studies, 2**(4)**: 240−248. https://dx.doi.org/10.21608/aujes.2021.91839.1037

Salaah, S.M.; El-Gaar, D.M. and **Gaber, H.S. (2021).** Potential effects of dietary chitosan against lead-induced innate immunotoxicity and oxidative stress in Nile tilapia (*Oreochromis niloticus*). The Egyptian Journal of Aquatic Research, 48**(2)**: 123−129. https://doi.org/10.1016/j.ejar.2021.10.004

Santos, I.; Diniz, M.S.; Carvalho, M.L. and **Santos, J.P. (2014).** Assessment of essential elements and heavy metals content on *Mytilus galloprovincialis* from River Tagus estuary. Biological trace element research, 159**(1−3)**: 233–240. https://doi.org/10.1007/s12011-014-9974-y

Setiyorini, Y.; Anggraeni, A. and **Pintowantoro, S. (2022).** In-Vivo study of nano chitosan as therapeutic agent for toxic metal implant. Results in Engineering, 13: 100352. https://doi.org/10.1016/j.rineng.2022.100352

Seyedmohammadi, J.; Motavassel, M.; Maddahi, M.H. and **Nikmanesh, S. (2016)**. Application of nanochitosan and chitosan particles for adsorption of Zn(II) ions pollutant from aqueous solution to protect environment. Modelling Earth Systems and Environment, 2: 165. https://doi.org/10.1007/s40808-016-0219-2

Sfakianakis, D.G.; Renieri, E.; Kentouri, M. and **Tsatsakis, A.M. (2015).** Effect of heavy metals on fish larvae deformities: A review. Environmental Research, 137: 246-255. https://doi.org/10.1016/j.envres.2014.12.014

Soliman, N.F. and **Yacout, D.M.M. (2016).** Aquaculture in Egypt: status, constraints and potentials. Aquaculture International, 24:1201−1227. https://doi.org/10.1007/s10499-016-9989-9

Sudharsan, R.; Rani, A.S.; Reddy, T.N.; Reddy, P.U.M. and **Raju, T.N. (2000)**. Effect of nitrite toxicity on the dehydrogenases in the fish *Tilapia mossambica*. Journal of Environment and Pollution, **7(2)**: 127−130.

Thilagar, G. and **Samuthirapandian, R. (2020).** Chitosan from crustacean shell waste and its protective role against lead toxicity in *Oreochromis mossambicus*. Toxicology reports, 7: 296−303. https://doi.org/10.1016/j.toxrep.2020.02.006

Vocciante, M.; Finocchi, A.; D'Auris, A.F.; Conte, A.; Tonziello, J.; Pola, A. and **Reverberi, A.P. (2019).** Enhanced Oil Spill Remediation by Adsorption with Interlinked Multilayered Graphene. Materials, 12**(14):** 2231. https://doi.org/10.3390/ma12142231

Wan Ngah, W.S.W. and **Fatinathan, S. (2010).** Pb (II) biosorption using chitosan and chitosan derivatives beads: Equilibrium, ion exchange and mechanism studies. Journal of Environmental Sciences, 22**(3)**: 338−346. https://doi.org/10.1016/S1001-0742(09)60113-3

Wang, Y. and **Li, J. (2011)**. Effects of chitosan nanoparticles on survival, growth, and meat quality of tilapia, *Oreochromis nilotica*. Nanotoxicology, 5**(3)**: 425−431. https://doi.org/10.3109/17435390.2010.530354

Yu, K.; Ho, J.; Mccandlish, E.; Buckley, B.; Patel, R.; Li, Z. and **Shapley, N.C. (2013)**. Copper ion adsorption by chitosan nanoparticles and alginate microparticles for water purification applications. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 425: 31−41. https://doi.org/10.1016/j.colsurfa.2012.12.043

Zareie, C.; Eshkalak, S.K.; Darzi, G.N.; Baei, M.S.; Younesi, H. and **Ramakrishna, S. (2019)**. Uptake of Pb (II) ions from simulated aqueous solution via nanochitosan. Coatings, 9**(12)**: 862. https://doi.org/10.3390/coatings9120862

Zhao, D.; Yu, S.; Sun, B.; Gao, S.; Guo, S. and **Zhao, K. (2018).** Biomedical Applications of Chitosan and Its Derivative Nanoparticles. Polymers, 10**(4)**:462−479. https://doi.org/10.3390/polym10040462