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Chitosan and Moringa Oleifera Seeds Controlling Heavy Metal Residues in Water and Fish Meat

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ABSTRACT

The present work was organized to explore the efficacy of chitosan (CS) and *Moringa oleifera* seeds (MOS) on heavy metal levels ((Pb) lead, (Cd), cadmium, (Cu) copper, (Zn) zinc, and (Fe) iron on five fish farms in the El-Fayoum Government. Heavy metal residues were analyzed in the fish muscle of *Tilapia Nilotica*, and water was collected from a commercial fish farm. The results demonstrated that iron was the most abundant metal in fish muscle and water samples from all farms. The highest levels were detected in fish and water from farm 2, with values of 1267.4±57.2 ppm and 3000±189 µg/l, respectively. Pb and Cd were not found in the Nile tilapia fish muscle. Our results revealed that metals were arranged in the following manner: iron, zinc, copper, lead, and cadmium for all farms, with farm 2 having the most significant quantities of heavy metals. Several chitosan and *Moringa oliefera* seeds (0.5 g/l, one g/l, and two g/l as powder and 1%, 2%, and 3% as extract) were used for water treatment. The highest efficient dosages of chitosan and *Moringa oleifera* seeds in heavy metal removal were two g/l as powder and 3% as an extract. Chitosan showed the highest cadmium removal rate (100%), whereas *Moringa oliefera* seeds recorded the highest Cd removal rate (79%). In conclusion, chitosan was a more efficient adsorbent for heavy metal elimination than Moringa. The highest elimination of metal ions from wastewater by chitosan powder 2g\l was 42.8, 100, 80.2, respectively, for lead, cadmium, and copper, while the highest elimination for zinc and iron was 62.54, and 55.9% by chitosan extract 3%. Moringa seeds and chitosan had the following affinity for cations adsorbed selectively: Cd > Cu > Zn > Fe > Pb.

INTRODUCTION

Fish individual is one of the most nutritious foods of animal origin because of its high biological value of protein, lipid, vitamin and essential fatty acid content as well as the availability of Omega-3 fatty acids, which have several health benefits (**Dalia *et al.*, 2019**). In contrast, fish can store heavy metals in their tissues at greater levels that are hundreds of times more concentrated than the water medium by absorption along the gill surface and gastrointestinal tract wall **(Burger & Gochfeld, 2005)** which differed according to species, age, gender and organs **(Younis *et al.*, 2012)**. Contamination of the marine environment is a warning signal that might cause global concerns. The most significant number of manufacturing, cultivated and commercial compounds in the marine environment has various harmful effects on aquatic creatures. As a result, heavy metal residue in aquatic systems has piqued the interest of various academics **(Kadim & Risjani, 2022)**.

Heavy metals pollute the marine environment, including industrial waste, household fumes, products that are metal-based and electronic waste. Aquaculture is the farming of marine organisms. Toxicity with heavy metals leads to a decrease in aquaculture populations and causes organisms' physical deformation, and these toxic metals cause several diseases in marine organisms. Thus, food chains are severely affected by the intrusion of these metals into the aquatic environment. Heavy metals are of great concern to the environment since they persist for long periods and can bioaccumulate, leading to water degradation **(Sonone *et al.*, 2021)**. Notably, most heavy metals are associated with sediment particles; only a tiny amount dissolves in water and is distributed far up through food chains **(El-Nemeki *et al.*, 2008)**. In addition, rivers and ponds are excellent water sources for fish farms **(Gabbadon & De Souza, 2008)**.

Moringa oleifera seed (MOS) is legal, inexpensive, lighter, and more easily used than substances like carbon. MOS granules remove heavy metals from water effectively by adsorbing these metal ions to be safe for humans and also can be reused several times without needing expensive regeneration technology **(Khader *et al.*, 2022)**. Previous studies have shown that MOS were excellent adsorbents for removing metal ions from aquatic systems **(Sharma *et al.*, 2006)**. The chemistry of MOS reveals more potent antioxidants that prevent the toxicity of heavy metals as it has potent "oxygen and nitrogen" ligands that bind to heavy metals. **(Bhatti *et al.*, 2007)**.

Chitosan (CS) is a polymer characterized by many properties, like biodegradability and biocompatibility among others, and the ability to interact with other polymer fragments through hydrogen bonds because of the presence of "hydroxyl and amino groups" or a high charge density. In recent decades, the attention of the scientific community has been attracted to the physicochemical properties of CS, which depend on the "hydroxyl and amino groups". Their use in many research studies as the presence of these groups in glucosamine units allows the adsorption of minerals via numerous methods, such as electrostatic interactions, metal chelation, ion pairing and ion exchange **(Carmen *et al.*, 2020)**. Therefore, materials containing CS have been extensively studied because they are environmentally friendly and are used for water treatment as an effective adsorbent. CS is pH dependent, e.g., insoluble at alkaline and neutral pH but soluble at acidic PH. The change in chitosan's physical and chemical composition improves its properties and ability to adsorb metals. It is a promising material for eliminating heavy metals from water **(Gamage *et al.*, 2023)**. This study looks at the prospects of CS and MOS which are inexpensive adsorbents for the treatment of polluted water with heavy metals,viz. (Pb), Lead, (Cd) Cadmium, (Cu) Copper, (Zn) Zinc and (Fe) Iron gathered from a fish farm at El-Fayoum.

MATERIALS AND METHODS

Sampling and preparation of samples

Two hundred Nile tilapia (*Oreochromis niloticus*) samples (40 samples from each farm) with weights from 190– 200g and lengths from 19– 22cm and 50 water samples (10 samples from the sediment of different sites from each farm) were collected from five separate fish farms in El-Fayoum Government, Egypt (farm 1, farm 2, farm 3, farm 4 and farm 5). Sampled fishes were individually packed into impermeable polyethylene bags, and then labeled and transported without delay at 4°C in an ice box to the laboratory and kept at -20^0C until analysis. Water was taken using 0.5-liter bottles pre-cleaned with polyethylene and acidified with 5ml of concentrated HNo3 and stored in a refrigerator.

Heavy metal residue in fish samples

Fish samples were dried at 105^0C in an oven. Representative samples (10 g fresh muscle) were collected from the fish in dry form. Samples were digested as described in the study of **Goldberg (1963)**.

The guidelines of **APHA (2005)** were used to analyze water samples**.**, and the analysis was performed at the "Animal Health Research Institute, Dokki, Giza, Egypt," using a UNICAM 969 atomic absorption spectrophotometer (AAS).

Treatment of water

Water samples gathered from farm two were used for the treatment using the two adsorbents, *Moringa oliefera* seeds and chitosan.

Preparation of *Moringa Oleifera* Seeds (MOS)

Dried MOS were obtained from the NRC, the National Research Center in Dokki Giza, Cairo. The seed's coats were removed, then the white kernel was ground into fine particles using an electric crusher and sieved through a 0.8-mm sieve, and then the MOS powder was directly applied to the examined water in the amounts of 0.5, 1, and 2 g/l, and the mixture was shaken for five minutes. Moringa seed extract (MSE) was also prepared by mixing MOS powder with distilled water at 1, 2, and 3% dosages, using a magnetic stirrer for 60 minutes and settling for 20 minutes. *Moringa oleifera* aqueous extract was finally filtered through a 20µm paper filter **(Ravikumar & Sheeja, 2013)**.

Preparation of chitosan solution (CS)

CS was gained from the Egyptian Petroleum Research Institute and produced from shrimp shells with an MW molecular weight of 110 KDa and an 85% degree of de-acetylation **(Hussien *et al.*, 2012)**. The powder of chitosan was prepared using an electric crusher and sieved through a 0.8mm sieve. Then, the powder was immediately applied to the examined water with amounts of 0.5, 1 and 2g/ l and shaken for 5 minutes. In addition, 2% chitosan extract was prepared by dissolving the powder in acidic medium by using 0.1M HCl and adjusting the PH of chitosan solution **(Divakaran & Pillai, 2002)**. The suspension was vigorously shaken for five minutes before the filtration process and then used immediately in doses of 1%, 2%, and 3% as an extract.

Statistical analysis

SPSS version 16.0 statistical software was used to analyze data obtained from five dairy farms to evaluate if the results were statistically significant, one-way ANOVA and two-tailed Pearson correlation with a *P*-value of 0.05 were used. Furthermore, the data were displayed as a Mean±Standard Error **(SPSS, 2007)**.

RESULTS

Table (1) shows that the highest amounts of Cu and Fe in fish's muscles were detected in farm 2 as follows: 9.24±0.7 and 1267.4±57.2 mg/kg. At the same time, Zn concentration was recorded at the highest level in the fish muscle samples from farm 3 (56.6±1.1 mg/kg). The obtained results illustrated that the metal ion levels were arranged in the following manner (Fe> Zn > Cu> Pb and Cd) for all fish farms. In contrast, "lead and cadmium" were not detected in the Nile tilapia fish's muscle collected from all farms.

Table 1. Heavy metal levels mg/kg in fish muscle samples

Fish farm	Heavy metals				
	Pb	Cd	Cu	Zn	Fe
Farm1	ND	ND	4.105 ± 0.5^{bcde}	23.42 ± 1.00^{bce}	253 ± 12.4^{bd}
Farm2	ND	ND	9.24 ± 0.7^{acd}	45.7 ± 1.06^{acde}	1267.4 ± 57.2^{acde}
Farm3	ND	ND	7.13 ± 0.5^{ab}	56.6 ± 1.1^{abde}	241.95 ± 9.7^{bd}
Farm4	ND	ND	7.55 ± 0.43^{ab}	20.2 ± 1.10^{bce}	1132.95 ± 57.23^{abce}
Farm5	ND	ND	8.39 ± 0.60^{a}	40.25 ± 1.9^{abcd}	220 ± 8.6^{bd}
MPL*	[a]0.30 mg/kg	[a]0.050 mg/kg	[b]20 mg/kg	[b]40 mg/kg	[b]30 mg/kg

*Maximum permissible limit (MPL) was assessed according to ([a]EOS. 2010, [b]EOS. 1993). The mean values across the Table with different subscripts in the same column are significantly different at the 0.05 level.

Table (2) illustrates that farm 2 had the highest amounts of metal ions in water, which were 40±1.42, 8.65±0.74, 130±10.1 and 3000±189 µg/l, respectively, for Pb, Cd, Cu and Fe. At the same time, Zn content was most significant in water samples from farm three (323±36.4 µg/l). The heavy metals were arranged for all five farms as follows: Fe > Zn > Cu > Pb > Cd. Furthermore, copper and zinc contents were below the allowed limits of the Food and Agricultural Organization **(FAO, 2007)**. While, iron content was within the limits permitted by **FAO (2007)**. In contrast, lead and cadmium levels were above the allowed limits set by **FAO (2007)**.

Table 2. Heavy metal levels µg/l in the examined water samples

Fish farm	Heavy metals				
	Pb	Cd	Cu	Zn	Fe
Farm1	39.3±1.1^{de}	7.1±0.59^{e}	66.35±4.9^{bde}	179±19^{c}	1618.5±158.1^{be}
Farm2	40±1.42^{cde}	8.65±0.74^{de}	130±10.1^{acde}	214.5±5.1^{cd}	3000±189^{acde}
Farm3	36.3±1.134^{bcde}	7.66±0.7^{e}	80.75±7.5^{be}	323±36.4^{abce}	1407.6±87.2^{bd}
Farm4	21.1±0.95^{abc}	6.25±0.40^{be}	90.3±8.6^{ab}	136±9.3^{bce}	1810±96.1^{cbe}
Farm5	24.1±1.2^{abc}	4.105±0.5^{abcd}	103.8±8.13^{abc}	200±22.3^{c}	1160.25±35.9^{abd}
MPL*	10 µg/l	3 µg/l	1000 µg/l	3000 µg/l	500-50000 µg/l

Table 3. Reduction percentages of heavy metals residues (µg/l) in water samples after using different concentrations of MOS powder

Heavy metal	MOS powder (g/l)	Concentration		
	Treated dose	Before treatment	After treatment	Reduction %
Pb	0.5	40±1.42	35.8±1.3	10.5
	1		32.4±1.2	19
	2		28±0.99	30
Cd	0.5	8.65±0.74	8.65±0.74	0.00
	1		8.65±0.74	0.00
	2		4.8±0.41	45.0
Cu	0.5	130±10.1	84.3±6.5	35.2
	1		84.3±6.5	35.2
	2		67.08±0.6	48.4
Zn	0.5	214.5±15.1	179.751±12.7	16.2
	1		140.93±9.9	34.3
	2		113.3±7.98	47.2
Fe	0.5	3000±188.5	2700±169.7	10
	1		2133±134	28.9
	2		1599±100.5	46.7

Tables (3, 4) reveal that the heavy metal content in wastewater (Pb, Cd, and Cu) was dramatically reduced by increasing the addition of the researched materials, MOS and CS powder or extract. For lead and copper, MOS powder outperformed seed extract at a dosage of 2g/ l. However, in the instance of Cd, MOS extract outperformed seed powder at a dosage of 3%, with a removal percentage of 79%. Nevertheless, the content of metals in waste water (Zn and Fe) was dramatically reduced by increasing the addition of the test substance (MOS and CS), either as a powder or as an extract. MSP proved to be more efficient than MSE in eliminating Zn and Fe at a dosage of 2g/ l.

As shown in Tables (5, 6), CS operates as ordinary adsorbent to eliminate metal ions from water samples. Cs powder was more successful than extract for lead and copper removal, with the maximum percentages (42.8 and 80.2%) at doses of 2g/ l for Pb and Cu. While for Cd, chitosan extract outperformed powder at 1, 2 and 3% doses, with removal percentages of 50, 70 and 100%, respectively. In addition, CS extract was more successful than powder in removing Zn and Fe with the maximum percentages (62.54 and 55.9%) at a dose of 3%.

Table 4. Reduction percentages of heavy metals residues (µg/l) in water samples after using different concentrations of MOS extract

Heavy metal	MOS **extract** (g/l)	Concentration		
	Treated dose	Before treatment	After treatment	Reduction %
Pb	1	40±1.42	40±1.42	0.00
	2		35.9±1.27	10.25
	3		29.8±1.03	25.6
Cd	1	8.65±0.74	6.83±0.6	21.0
	2		5.02±0.43	42.0
	3		1.8±0.15	79.0
Cu	1	130±10.1	101.41±7.9	22.05
	2		84.3±6.5	35.2
	3		77.93±6.05	40.1
Zn	1	214.5±15.1	124.41±8.9	42
	2		180.61±12.7	15.8
	3		152.5±10.7	28.92
Fe	1	3000±188.5	2430.6±152.7	18.9
	2		2364±148.5	21.2
	3		1986±124.8	33.8

Table 5. Reduction percentages of heavy metals residues (µg/l) in water samples after using different concentrations of Cs powder

Heavy metal	Cs powder (g/l) Treated dose	Concentration Before treatment	After treatment	Reduction %
Pb	0.5	40±1.42	32.84±1.2	17.9
	1		27.72±0.98	30.7
	2		22.88±0.81	42.8
Cd	0.5	8.65±0.74	6.92±0.6	20
	1		6.49±0.6	25
	2		0.00	100
Cu	0.5	130±10.1	76.31±5.9	41.3
	1		55.64±4.3	57.2
	2		25.74±1.9	80.2
Zn	0.5	214.5±15.1	179.4±12.6	16.38
	1		150.5±10.6	29.85
	2		109.2±7.7	49.08
Fe	0.5	3000±188.5	2451±154	18.3
	1		1866±117.2	37.8
	2		1524±95.8	49.2

Table 6. Reduction percentages of heavy metals residues (µg/l) in water samples after using different concentrations of Cs extract

Heavy metal	Cs extract (g/l) Treated dose	Concentration Before treatment	After treatment	Reduction %
Pb	1	40±1.42	35.1±1.23	12.25
	2		30.16±0.11	24.61
	3		24.42±0.9	38.95
Cd	1	8.65±0.74	4.33±0.4	50
	2		2.595±0.2	70
	3		0.00	100
Cu	1	130±10.1	77.48±6.01	40.4
	2		44.46±3.5	65.8
	3		29.51±2.3	77.3
Zn	1	214.5±15.1	171.11±12.04	20.23
	2		124.1±8.7	42.15
	3		80.35±5.7	62.54
Fe	1	3000±188.5	2605.8±163.7	13.14
	2		2202±138.4	26.6
	3		1323±+83.1	55.9

DISCUSSION

Food safety has been integrated into the necessary manufacturing procedures to meet the significant negative reactions of consumers and consumer organizations to recent crises in the food-animal sector **(Mulder, 2011)**. Furthermore, Table (1) shows the the levels of metals (Fe, Zn, Cu, Pb and Cd) in all fish farms under study. Our study is consistent with the findings of **Belal *et al.* (2021)** who analyzed heavy metals in major drainage systems and farms in Fayoum Government and found that lead and cadmium were not detected in the fish muscle of the Nile tilapia. In addition, our results agree with those of **Mendoza *et al.* (2023)** who investigated that lead was not detected in fish meat. But these result disagrees with those of **Stancheva *et al*. (2013)**, **Helmy *et al.*, (2018)** and **Khalid *et al.*, (2019)** who detected lead and cadmium in fish muscles with various concentrations.

Furthermore, the current results were much higher than those of **El Degwy *et al.* (2023)**, confirming that the concentrations of the examined heavy metals in the Nile tilapia muscle collected from Mariout Lake were 15.60, 6.14, 1.23, 1.44, and 0.80µg/ g, respectively, for Fe, Zn, Cu, Pb and Cd. Furthermore, iron contents in fish muscle samples taken from all five farms exceeded the permissible limit recorded by **EOS (1993)**. Additionally, Zn levels in fish muscle collected from farms 2 and 3 were above the permissible limit recorded by **EOS (1993)**. In contrast, Cu levels were below the permissible limit recorded by **EOS (1993)**. According to **Wei *et al.* (2014)**, the ability of metals to undergo bioaccumulation in the food chain, as well as differences in fish habitats, environmental needs, metabolic capability and feeding habits may all contribute to the variation recorded in heavy metal contents in muscle tissue among the examined fish. Heavy metals in the marine environment are a significant issue because of their harmfulness and risk to animal life, which disrupts the average environmental balance. The residue is the net gathering of chemicals from water in marine organisms caused by increased absorption and sluggish removal of such compounds **(Bhattacharya *et al.*, 2008)**. Table (2) elucidates that the heavy metal state in all fish farms was in the sequence of Fe > Zn > Cu > Pb > Cd. Our results are consistent with those of **Ali and Abdel-Satar (2005)**, who analyzed water from different fish farms in El-Fayum (El-shura, Goda 1, 2, and Shalakan) for the presence of heavy metals like iron, manganese, zinc, copper, lead and cadmium). Furthermore, the current outcomes coincide with those of **Belal *et al.* (2021)**, who investigated heavy metals in the Faiyoum Government drainage system and farms.

Additionally**, Abdel-Satar *et al.* (2010)** and **Gohar *et al.* (2018)** addressed heavy metal pollution in water samples from El-Wadi and El-Bats drainage canals. Additionally, **Monier *et al.* (2023)** documented that the metal levels in water samples gathered from 4 sites nearby Damietta port during the winter and spring seasons were in the following arrangement:Fe > Zn > Cd > Pb > Cu) in the winter season. While, the order was as follows Pb > Fe > Zn > Cd > Cu during the spring season. Furthermore, our results concur with those of **El-Degwy *et al.* (2023)** who postulated that, the concentration ranges of the studied heavy metals in the water of Lake Mariut were 266.50–1145.40, 18.00–130.40, 5.00–23.23, 10.55–59, 51 and 3.30–9.90µg/ L, respectively, for Fe, Zn, Cu, Pb and Cd during the academic year. Furthermore, our findings indicated that lead and cadmium levels exceeded the acceptable limits set by **FAO (2007)** in water; samples were taken from all fish farms. While copper, zinc and iron levels were within permissible limits recorded by **FAO (2007)**. Because any contaminants in water have a detrimental influence on fish and hence public significance, water must be cleaned off such pollutants as heavy metals. Thus, the current study was concerned with treating fish farm water using simple and inexpensive approaches, such as treatment with MOS and CS powder and extract.

MOS powder was more successful than seed extract in eliminating lead and copper at doses of 2g/ l, with 30% and 48.4% removal percentages, respectively (Table 3). These results might be due to MOS adsorptive potential, which contains significant amounts of cellulosic fibers interconnected with lignin. Lignin is a heterogeneous complex biopolymer molecule with multiple functional groups, including phenolic, carboxyl and aliphatic groups **(Shin & Rowell, 2005)**. While, the moringa powder removed 45% of cadmium. Correspondingly, **Mataka *et al.* (2010)** showed that increasing the dose of MOS powder improved the percentage of Cd elimination from about 20% to about 58%. The results recorded in the study of **Khader *et al.* (2022)** assessed that the maximum elimination efficacy of heavy metal ions was 93, 96 and 93%, respectively, for Cu2+, pb2+ and F-1 by MOS powder, and that was much higher than our results. At the same time, the most significant percentages of Zn and Fe (47.2 and 46.7%, respectively) were found in MOS powder at 2g/ l. The current findings reveal that the adsorbent's metal absorption capability improves with dose because the quantity of functioning sites presented for metal grows with adsorbent quantity. These findings agree with those reported in the work of **Guibal (2004)**. In addition, our findings agree with those of **Seif and Howayda (2022)** who found that, MOS treatment removes several heavy metals from the water of the Ismailia Canal. MOS extract at 3% exhibits lead and copper removal percentages of 25.6% and 40.1%, respectively. While, the moringa extract removed 79% of cadmium (Table 4). This conclusion matches with the results of **Sajidu *et al.* (2005)** and **Subramanium *et al.* (2011)**, who stated that MOS could eliminate heavy metals from wastewater. Adsorption is how molecules and ions attach to the proteins of seeds through specific methods. A high level removes heavy metals and solid particles with a significant charge on moringa’s colloidal surface. The positive metal ion creates a channel between the anionic polyelectrolyte and the negatively charged, efficient groups of protein on the surface of colloidal particles, thereby initiating the adsorption of heavy metals **(Benes & Steinnes, 1995)**.

MOS generally prefer cations in the following sequential order: Cd, Cu, Zn, Fe and Pb. The present findings are consistent with those of **Abdeen (2016)** who reported that

MOS usage removed heavy metals from wastewater. Additionally, the current results revealed that the highest concentration of Cs powder (2 g/l) or extract (3%) removed 100% of the cadmium. Moreover, Cs was more efficient than MOS in removing lead, with a marginal percentage removal (42.8%) achieved with two g/L Cs powder (Tables 5, 6) and that is consistent with the study of **Reddy *et al.* (2010)** who examined the pb+2 bio sorption from watery solutions of M. olive grove. The present findings match with those of **Abdeen (2016)** who found that chitosan (CS) usage removed heavy metals from wastewater. In addition, **Varma *et al.*, (2004)** observed that CS could chelate or bind with various metals such as cadmium, copper and lead given that the amine groups in the polymer sequence have a high affinity for metal ions. Furthermore, the present results showed that the maximum proportion of Zn and Fe (62.54 and 55.9%) was achieved at 3% of CS extract matched to 2g/ l of CS powder, which recorded 49.08 and 49.2%, respectively. The percentage of elimination of such metal ions increased with increasing adsorbent substance doses. Based on our findings, Cd levels were below the permitted limits set by **FAO (2007)** in water samples gathered from farm two following treatment with MOS extract 3%, CS powder 2 g/l or extract 3%.

CONCLUSION

The current study explored the influence of CS and MOS on the proportions of lead, cadmium, copper, zinc and iron in samples gathered from five fish farms in the El-Fayoum Government. The principal treatment findings revealed that increasing the quantity of the additional materials reduced the content of heavy metals in wastewater substantially. MOS and CS were tested in various dosages as powder or extract. The findings revealed that the most efficient doses of CS and MOS seeds in heavy metal removal were 2g/ l as powder and 3% as an extract.

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