



Reduction of Heavy Metal Residues in Nile Tilapia from Egyptian Aquaculture Using Chelating Acid Treatments



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Abstract

Tilapia stands among the most widely consumed fish, yet the threat of heavy metal (HM) contamination from human activities poses serious risks to both aquatic life and public health. This study investigates the levels of key heavy metals—lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and chromium (Cr)—in water and Nile tilapia harvested from two major aquaculture regions in Egypt. Additionally, we explore the effectiveness of a simple 10-minute soaking treatment using a chelating solution of ascorbic acid, citric acid, and acetic acid in reducing HM residues in fish muscle. Our findings reveal that, while most HM concentrations in water samples were within WHO limits (with the exception of Fe and Cd at one site), Pb levels in all fish samples exceeded FAO/WHO safety thresholds (ranging from 0.3885 to 0.6835 mg/kg). Remarkably, the chelating treatment significantly reduced HM residues in fish, with reductions of up to 97% for Zn, 89% for Fe, 82% for Ni, and substantial decreases for other metals. These results highlight the potential of chelating acid solutions as a practical and effective method to enhance the safety of tilapia for consumers, supporting sustainable aquaculture practices in Egypt.

Keywords: Nile tilapia, Heavy metals, Chelating agents, Residue reduction, Egyptian aquaculture

Introduction

Fish are high-nutrient foods that help people maintain a healthy, balanced diet. They are also regarded as a source of high-biological-value protein, which has a good balance of essential amino acids and fats with high levels of healthy polyunsaturated fatty acids. These help people stay healthy by lowering their risk of cardiovascular diseases [1-3]. Fish are known for their tenderness, ease of digestion, and high levels of minerals such as zinc, sodium, cobalt, phosphorus, iron, copper, potassium, magnesium, iodine, and fluorine. They are also a good source of B-complex vitamins. Fish can be used as an alternative to red meat as an animal protein source because of its tasty flavor and affordable price, which gives consumers a chance to satisfy their daily nutritional needs [4]. According to Mohamed et al. [5], fish make up approximately 45% of animal protein consumed by Egyptians. In 2018, Egypt ranked sixth globally and top in Africa in aquaculture [6]. In 2019, Egypt produced more than two million tons of fish [7]. In 2017, Egyptians consumed 23.69 kg of fish per person; by 2021, that amount had increased to 25.3 kg. Egypt ranks 49th out of 165 countries in terms of fish consumption per capita, indicating that consumer interest in this type of food is steadily increasing annually [8]. Tilapia are warm-water fish that have a mild flavor, a high yield, and the ability to withstand low water quality [9,10]. Egypt is one of about 140 countries that cultivate tilapia [11]. The FAO estimates that about 80% of Africa's total production comes from Egypt alone [12]. The qualitative structure of the most important farmed fish species in Egypt in 2022 is depicted in Figure 1.

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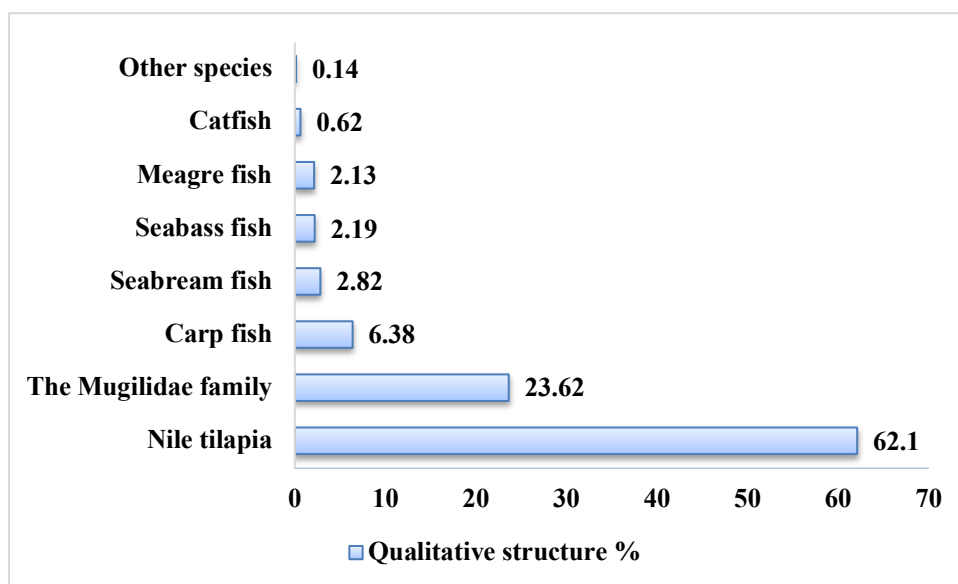


Figure 1. Qualitative structure of the most significant farmed fish species in Egypt [8].

Industrial and agricultural processes, agrochemicals, vehicle emissions, deforestation, and landfills are from the ways that HMs find their way into water bodies [13,14]. Many studies have demonstrated that fish muscles and tissues can accumulate HMs like Pb, Cr, Zn, Fe, Cd, Cu, and Mn. The contents of HMs in fish meat depend on their habitat and species. It has been discovered that the HMs levels, especially cadmium, in certain fish species are higher than the advised limits. Inappropriate treatment or lack of recognition of heavy metals can result in serious illnesses. The liver, lungs, kidneys, endocrine glands, bones, cardiovascular, gastrointestinal systems, and central nervous system can all be harmed by acute heavy metal intoxication. Physical, muscular, Alzheimer's, Parkinson's, muscular dystrophy, multiple sclerosis, and cancer can all result from prolonged exposure [4,15]. Thus, eating fish is a significant way for people to be exposed to heavy metals and the risks that come with it [16-18]. HMs can be effectively removed with chelating agents. Chelating agents are pliable compounds that can create covalent bonds with metal ions. They can have functional groups, like carboxylic or amine. By attaching to metal ions and blocking their ability to bind to cellular proteins and enzymes, chelating agents play a crucial role in eliminating metal toxicity [19,20]. The use of chelating agents has been used in numerous investigations.

One of the chelating agents, acetic acid, can lower the pH of fish tissue and combine with metal ions to form complexes that decrease the solubility and bioaccessibility of those ions [21]. Citric acid is one of the chelating agents that effectively eliminates HMs over a wide pH range [22]. Also, antioxidant compounds, such as ascorbic acid and E, have been demonstrated in studies to reduce the toxicity of HMs in different fish tissues by their chelating abilities [23-25].

The study aims to determine the contamination levels of Pb, Ni, Fe, Zn, Mn, Cd, Cu, and Cr in the water and muscles of tilapia fish from Egypt's two most productive districts, El-Faiyum Kafr and El-Sheikh. Another goal is to develop a method to safely extract these heavy metals from contaminated fish. Examines how chelating agents (acetic acid, ascorbic acid, and citric acid) can lower metal level and then compares the residues to the safe permissible limits set by some international organizations.

2. Materials and methods

2.1. Site description

Two aquaculture sites in Egypt's Kafr El-Sheikh (Sidi Salem and Al-Hamol) and two sites in El-Faiyum governorates (Qarun Lake and Wadi El Rayan) were chosen for the current study. The chosen sites were well-known for their high production rates, aside from their potential for contamination with high concentrations of harmful elements because they used agricultural drainage water for production.

2.2. Chemicals

Every chemical that was used was analytical grade. Ascorbic acid, citric acid, acetic acid, hydrogen peroxide, and concentrated HNO_3 were purchased from El-Gomhoriya Company for Chemicals and Drugs, Cairo, Egypt.

2.3. Water and fish samples

From the chosen sites, one-litre samples of water were gathered in sterile glass bottles. Following their labelling, the bottles were moved to the research lab. To get rid of sand and debris, water was filtered and kept at 4°C until analyzed. Professionals with expertise in the Department of Fish Production collected fish with an average body weight of 200–250 g, and then transferred them to the Department of Food Science and Technology Laboratory, in an icebox. Tilapia fish (*Oreochromis niloticus*) were cleaned and kept at -18°C until analysis.

2.4. Methods

2.4.1. Preparation of treatment solution

The treatment solution was designed by dissolving acetic acid at a concentration of 0.5%, ascorbic acid at 1%, and citric acid at 1% in 2000 ml of water.

2.4.2. Treatment of fish samples

All that was required was a thorough 10-minute immersion of the fish in the treatment solution, followed by repeated washings with tap water to remove the liquid portion that contained the majority of the metals.

2.5. Digestion and analysis

Using the method of APHA [26], fish and water samples were digested. After passing through the Whatman filter paper (No. 1), roughly 10 ml of collected water was digested at 100 °C with 5 ml of concentrated HNO₃. Hydrogen peroxide drops were added until there were no more brown fumes. After that, the digested solution was filtered and then analyzed by an atomic absorption spectrophotometer (AAS). The fish samples were oven-dried for 24 hours at 105°C, then ground into uniform powder, and placed in clear plastic bags. In 50 mL digestion vessels, approximately 0.5 g of dried samples were added to ultrapure HNO₃ (70%, 5 mL) and H₂O₂ (30%, 1 mL). The samples were heated until digested and then cooled at room temperature. The digested solutions were blended with 1% HNO₃ to a volume of 25 ml. Following the AAS operator manual, blank and standard solutions were made using the same chemicals and in the same way as applied for digestion. The concentrations of Fe, Zn, Cu, Mn, Ni, Cd, Pb, and Cr were measured after the digest blanks and standard solutions using an AAS (VARIAN, Australia, model AA240 FS). Metal levels were expressed in milligrams per kilogram.

2.6. Statistical analysis

All experimental data were statistically analyzed using SPSS software version 22. The results are presented as means ± standard deviation (SD). To determine significant differences among groups, a one-way analysis of variance (ANOVA) was conducted, followed by Duncan's multiple range test for post-hoc comparisons. Statistical significance was set at $p < 0.05$. This approach ensured robust evaluation of the effects of chelating treatments on heavy metal residues in both water and fish samples, providing clear insights into the efficacy of the interventions.

3. Results and discussion

3.1. Heavy metal levels in water

Both natural (geological activity) and man-made (domestic discharge, agricultural practices, and industrial operations) sources are responsible for the presence of HMs in aquatic environments [27,28]. The statistical evaluation of heavy metals is presented and compared with the WHO [29], USEPA [30,31], CCME [32], and EWQS [33] guidelines in Table 1, followed by a graphical presentation in Figure 2.

Table 1. Concentration of HMs in water with the international permissible limits (mg/l).

Concentration of HMs in water with the international permissible limits (mg/l).									
Heavy metal	Farms sites				El	Permissible levels			
	Sidi Salem	Al-Hamol	Qarun Lake	Wadi Rayan		WHO (2022)	USEPA (2018, 2022*)	CCME (2021)	EWQS (2007)
Fe	0.6513	0.1747	0.2610	0.0568		NGL	0.3	0.3	0.3
Zn	0.0159	0.0164	0.0111	0.0105		4	5	0.007	3
Cu	0.0035	0.0048	0.0043	0.0049		2	1.3	0.004	2
Mn	0.0573	0.0448	0.0616	0.0072		0.08	0.05	0.43	0.1
Ni	0.0232	0.0197	0.0206	0.0238		0.07	0.1	0.15	0.02
Cd	0.0031	0.0029	0.0022	0.0018		0.003	0.001	0.003	0.003
Pb	0.0026	0.0018	0.0023	0.0029		0.01	0.06	0.007	0.01
Cr	0.0176	0.0102	0.0178	0.0149		0.05	0.01	0.05	0.05

NGL: The WHO has not set any health-based standards, but concentrations more than 0.3 mg/l may have an impact on water acceptability.

The water samples exhibit a wide range for Fe, Zn, Cu, Mn, Ni, Cd, Pb, and Cr concentrations, as given in Table. The HMs levels in samples were discovered to be between 0.0568–0.6513, 0.0105–0.0164, 0.0035–0.0049, 0.0072–0.0616, 0.0197–0.0238, 0.0018–0.0031, 0.0018–0.0029, and 0.0102–0.0178 mg/l for Fe, Zn, Cu, Mn, Ni, Cd, Pb, and Cr, respectively. The four sites' average levels were determined to lie in the following hierarchical order: Fe > Mn > Ni > Cr > Zn > Pb > Cu > Cd. El-Batrawy et al. [34] corroborated these findings, stating that in Burullus Lake water, the sequence of the HMs showed higher levels of Fe and lower for Cd. Abbas et al. [35] discovered a variety of HMs in water, with Cd having the lowest amounts and Fe having the highest. Comparable findings were documented by Pinkey et al. [36], who discovered that the HMs levels in the water show a decreasing order: Fe > Zn > Pb > Cr > Mn > Cu > Cd. Notably, in comparison to the results of other domestic and foreign investigations, these levels are comparatively acceptable. For example, Abdel-Hamed et al. [37], Ramadan et al. [38] for Bahr Mouse water, Pan et al. [27] for the Yellow River in China, and Pobi et al. [39] for the natural stream of Durgapur, India.

The findings indicated that the concentrations of HMs varied in the water samples taken from the various locations. In terms of mean HMs detection frequency, site 1 had the greatest overall, followed by sites 3 and 2, with site 4 showing the lowest frequency. Despite the lack of WHO [29] health-based recommendations for iron, 25% of water (site 1) samples were deemed unfit for domestic use, due to the iron concentration being greater than 0.3 mg/l (record 0.6513 mg/l). Additionally, water samples at this location exceeded the upper allowable levels (0.3 mg/l) by the USEPA, CCME, and EWQS recommendations (Figure 2). Anthropogenic activities like industrial emissions and runoff from agriculture, as well as natural sources such as geological formations, may be responsible for elevated iron levels. According to Bhuiyan et al. [40], basic elements found in the Earth's crust, Mn, Fe, and others, are frequently abundant in aquatic settings.

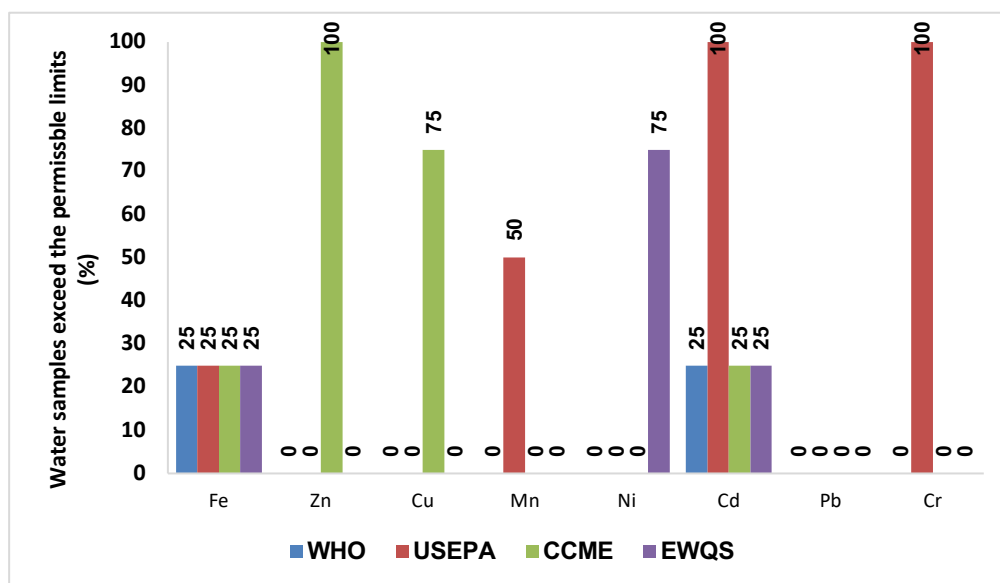


Figure 2. Water samples (%) that are higher than the international permissible limits for HMs.

In terms of zinc level, all samples were within the permitted thresholds established by the USEPA, WHO, and EWQS; nevertheless, all samples (100%) were above the CCME (0.007) limits (Figure 2). All samples had Cu contents below USEPA, WHO, and EWQS standards, but 75% of samples had more than the CCME [32] permissible level of 0.004 mg/l (Figure 2). The results of Mn show that the content for water samples was below the allowable levels (0.1, 0.08, and 0.43 mg/l) set by the EWQS [33], WHO [29], CCME [32], respectively, but 50% of samples were above the USEPA (0.05 mg/l) guidelines in sites 1 and 3 (Figure 2). For Ni levels, 75% of water samples (sites 1, 3, 4) were above the allowable limit set by the EWQS (Figure 2). While 100% of samples were lower than the acceptable limit of the WHO (0.07 mg/l), USEPA (0.1 mg/l), and CCME (0.15 mg/l) guidelines. For Cd content, 100% of samples were above the acceptable limit (0.001 mg/l) set by the USEPA and 25% above the limit (0.003 mg/l) set by WHO [29], CCME [32], and EWQS [33] (Figure 2). Given Cd's strong affinity for organic materials, the increased Cd content in water is caused by Cd from trash and fertiliser-containing phosphates. The Pb levels in all samples were lower than the acceptable levels of the WHO (0.01 mg/l), USEPA (0.06 mg/l), CCME (0.007), and EWQS (0.01 mg/l) guidelines (Table 1). For Cr content, 100% of samples exceeded the USEPA permissible level (0.01 mg/l), while all samples were below the standard permissible limits of the WHO, CCME, and EWQS (0.05 mg/l) (Table 1).

3.2. Heavy metal concentration in fresh fish samples

Table 2 shows the variations in HM content in the fish samples. All samples contained Fe, Zn, Zn, Cu, Mn, Ni, Cd, Pb, and Cr, and the individual metals were ordered in the following: Fe > Zn > Mn > Pb > Cr > Ni > Cu > Cd based on their mean

concentration. HMs' values were contrasted with standards for food safety for fish established by the European Commission [41], FAO/WHO [42,43], FAO [44], and EOS [45]. Iron had the largest concentrations, and this may be related to rising overall dissolved Fe contents in the water, which may enhance the quantity of free Fe and improve its absorption by fish organs. The results concur with Radwan et al. [46] and Abbas et al. [35]. Additionally, zinc and manganese showed high contents in the majority of cases because fish feed contains these elements since they are necessary for numerous biological functions [35,47].

Table 2. Tilapia heavy metal levels (mg/kg) with the usual allowable limit.

Heavy metal	Farms sites				Tolerable and permissible levels of HMs			
	Sidi Salem	Al-Hamol	Qarun Lake	Wadi El Rayan	EC 2023	FAO/WHO 2019; 2021	FAO 1983	EOS 1993
Fe	45.883	49.309	13.294	6.854		100		-
Zn	2.312	31.211	47.056	13.833		40		40
Cu	0.1015	0.1310	0.1475	0.3235		30		20
Mn	0.856	0.946	0.6315	0.665		1		-
Ni	0.1005	0.1260	0.1255	0.946		30		10
Cd	0.0310	0.0355	0.0365	0.0380	0.05	0.5		0.1
Pb	0.6835	0.6685	0.3885	0.5460	0.3	0.3		0.1
Cr	0.260	0.3205	0.2480	0.2730		-	0.15	0.15

The European legislation (EU) 2023/915.

The Egyptian Organisation for Standardisation and Quality Control.

Iron levels in fish samples ranged between 6.854 and 49.309 mg/kg. Fe exhibited the highest level of 49.309 mg/kg in samples of site 2, followed by 45.883 mg/kg in site 1 and 13.294 mg/kg in site 3, meanwhile, the lowest (6.854 mg/kg) was noted in site 4. Al-Sisi et al. [48] found similar concentrations of Fe ranging from 1.5 to 87.6 mg/kg in tilapia samples from the Rosetta Nile. According to the permissible limit set by FAO/WHO, Fe levels in all fish were lower than the allowable limit of 100 mg/kg (Table 2).

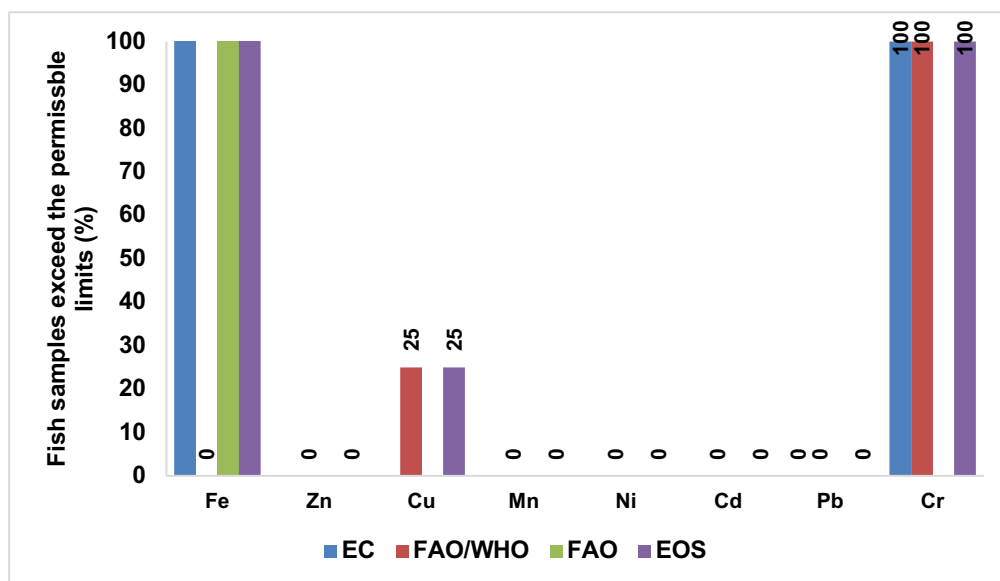


Figure 3. Fish samples (%) that are above the international allowable levels for heavy metals.

Zinc concentration ranged from 2.312 to 47.056 mg/kg. According to Rizk et al. [49], tilapia collected from Lake Nasser, Egypt, had similar concentrations of Zn, where the concentrations range from 8.5 to 15.7 mg/kg. According to the allowable limit published by FAO/WHO [42,43] and EOS [45], Zn concentrations in fish samples were below the allowable limit of 40 ppm, except 25% of fish samples in site 3 exceeded the permissible limit and recorded 47.056 mg/kg (Figure 3). The highest level of Cu (0.3235) was detected in site 4, while the lowest was found in site 1 (0.1015). Other studies found similar concentrations of Cu; the study of Rizk et al. [49] found the concentration of Cu in the muscle tissue of tilapia collected from different locations on Lake Nasser in Egypt was higher (1.2 to 9.17 mg/kg) than our findings. According to FAO/WHO [42,43] and EOS [45], the Cu levels were below the permissible limit of 30 and 20 mg/kg. The Mn concentration ranged

between 0.665 to 0.946 mg/kg. According to the permissible limit set by FAO/WHO [42,43], Mn levels were below the allowable limit of 1 ppm (Table 2).

The Ni levels in the fish ranged from 0.1005 to 0.946 mg/kg, which was below the threshold considered to be safe according to the FAO/WHO and EOS guidelines; therefore, it was 100% of the fish samples were considered safe. In an investigation by Mohiuddin et al. [50] and Akter et al. [51], the Ni levels varied between 0.233 and 0.688 mg/kg, close to the value of the present study. The Cd levels ranged between 0.0310 and 0.0380 mg/kg. Alam et al. [52] discovered that variation in Cd levels varied between 0.017 and 0.049 mg/kg, which was consistent with the current results. The findings show that all fish samples (100%) fell below the FAO/WHO, EOS, and EC guidelines (Figure 3). Cd may enter fish through diet, discarded sediments, and other sources.

The Pb levels in the muscle of tilapia ranged from 0.3885 to 0.6835 mg/kg. According to Hossain et al. [53], the range of Pb levels in fish muscles was 0.202 to 0.68 mg/kg. Hasanein et al. [54] found higher levels of Pb (1.74 mg/kg) in tilapia from Lake Mariut. The results show that all fish samples (100%) were higher than the allowable levels (0.3 mg/kg) set by the FAO/WHO, (0.3 mg/kg) set by the EC, and (0.1 mg/kg) set by the EOS guidelines (Figure 3). Pb accumulation in fish is caused by both the feed and the culture system sediment. The levels of Cr ranged from 0.2480 to 0.3205 mg/kg. Our findings are lower than those of Alam et al. [52], who discovered that the average Cr level in *C. Cirrhosus* (0.654 mg/kg). Furthermore, 100% of the samples exceeded the permissible levels (0.15 mg/kg) established by FAO [44] and EOS [45] guidelines (Table 2). The high levels of Cr may have come from contaminated feed, water and other sources. Furthermore, metal levels in fish could be affected by their habitat.

3.3. Effect of treatments on metal contents of fish

Figure 4 shows that after a 10-minute contact period, treatment (acetic acid, ascorbic acid, and citric acid) was effective in lowering HMs residues in fish samples. All HMs contents in fish samples are positively impacted when samples are submerged in a treatment solution. The soaking treatment used in this study resulted in a significant decrease in HMs concentration, as shown in the table. Because various metals have varying capacities when coupled with other chemicals, the proportion that occurs in the elimination of heavy metals varies. Heidari et al. [55] reported that there is no public hierarchy of organic acids' effectiveness in lowering HMs concentrations. In our study, in terms of heavy metal removal efficiency, $Zn > Fe > Ni > Mn > Cu > Cd > Pb > Cr$ was the order.

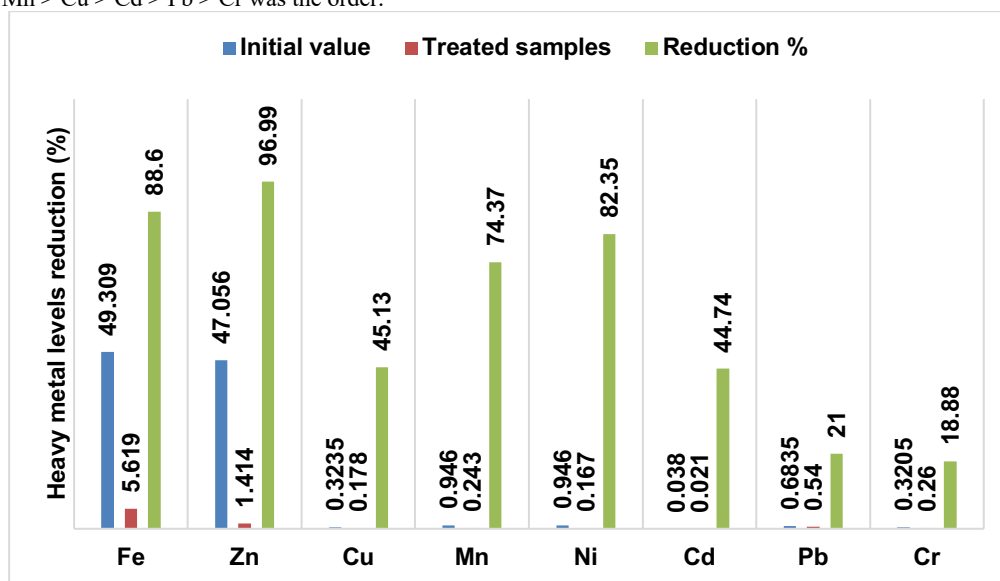


Figure 4. Heavy metal levels in treated samples (after soaking)

When fish was immersed in treatment solution, the Fe level reduced from 49.309 mg/kg to 5.619 mg/kg (88.6%), the Zn from 47.056 to 1.414 mg/kg (96.99%), the Cu from 0.3235 to 0.178 mg/kg (45.13%), the Mn from 0.946 to 0.243 mg/kg (74.37%), and the Ni from 0.946 to 0.167 mg/kg (82.35%), the Cd from 0.038 to 0.021 mg/kg (44.74%), the Pb from 0.6835 to 0.540 mg/kg (21%), and the Cr from 0.3205 to 0.260 mg/kg (18.88%). These findings support previous studies on the use of organic acids for fish decontamination. After being rinsed in an acetic acid solution (5%), Elnimr [56] observed a 58.3% decrease in cadmium levels in purposefully contaminated tilapia fillets. Abdullah et al. [57] investigated the use of sodium acetate and trisodium citrate to remove HMs from *T. thynnoides*, and they found sodium acetate eliminated 73.04, 50.46, and 80.94% of Cd, Cu, and Ni, respectively, while trisodium citrate eliminated 66.58, 49.63, and 19.26% of these metals. After soaking, Zn was removed from fish samples by 96.99%, which is less than the WHO/FAO levels of 40 mg/kg. Lead in fish samples was removed by 21% after soaking, but the content remains above the allowable levels (0.3 mg/kg). This removal result is below that stated by Anjana et al. [58], who discovered that Pb was reduced by 96.5% after 48 hours of UV depuration. Swastawati et al. [59] discovered that the use of liquid smoke at 4, 5, and 6% reduced Pb contents in blood cockles by 27.64-78.04%.

Furthermore, after soaking, the Cr content in fish samples was reduced by 18.88%, but it remains above the FAO and EOS permissible limit of 0.15 mg/kg. An excess of Cr in the body leads to cancer because it prevents the benzopyrene hydroxylase enzyme from functioning [60].

The amount of Cd in fish should be decreased even when the levels have not gone beyond the allowable limit. The human body may collect cadmium if the fish are polluted. The Cd concentration before the soaking treatment was 0.038 mg/kg. After soaking, its amount was reduced by 44% to 0.021 mg/kg. This indicates that the amount of Cd in the sample was still within the 0.5 and 0.05 mg/kg allowable limits established by the FAO/WHO and the EU Commission Regulation. Cadmium is a carcinogen, as stated by the International Agency for Research on Cancer [61]. As reported by Elhefnawy et al. [62], after 30 minutes of treatment with 5% acetic acid, the Cd levels decreased to 11.3 mg/kg (a reduction of 43.5%). Elnimr [56] reported acetic acid pretreatment of the fleshy sections of Bolti fish decreased the Cd level from 0.12 to 0.07 mg/kg (58.3%), Mn from 0.08 to 0.02 (25%), Pb from 0.83 to 0.62 (24%), and Zn from 0.62 to 0.41 (66%). Our results outperform those of Suprapti et al. [63], who found that soaking greencockle with 25% acetic acid reduced heavy metal levels of Cd by 37.88%.

Cadmium levels are decreased because of the nature of the ions, which influences the adsorbent's ability to bind. According to Mopoung and Kengkhetkit [64], adsorbents absorb Pb^{2+} more effectively than Cd^{2+} in aqueous solution. The ionic radius discrepancies between Pb^{2+} and Cd^{2+} cations are most likely the cause of this. A greater ionic radius reduces the electrostatic characteristics of metal ions while fostering covalent bonds with the functional groups of the surface of the adsorbent. The acid's chelating properties will confine the heavy metal cations' content until equilibrium is reached. As a result, chelating effectiveness is crucial for lowering metal content. By binding the ion with the deprotonated group, the ions of metal are liberated and attached to the acid groups, which is the mechanism of Pb transfer. Because of the free electrons in the acid group, which take the form of deprotonated, Pb ions will change and bond with acidic substances [56]. The carboxyl group becomes a negatively charged group that will adhere to Pb metal when the acid group is deprotonated by the presence of OH ions [59]. A small rise in Pb removal was linked to a slight drop in pH [55]. A greater amount of acetic acid and longer application times may improve Pb removal [63]. Acetic acid's chelating qualities, which allow heavy metals to be removed during processing by creating soluble complexes with them, account for these reductions [65]. The rate at which the acid and metal ions react increases with an increase in the amount of acid present and the length of immersion time [62, 66].

4. Conclusions

This study underscores the persistent challenge of heavy metal contamination in Egyptian aquaculture, particularly concerning lead levels in Nile tilapia. However, our results offer a promising solution: a brief, straightforward soaking of fish in a chelating acid mixture can dramatically reduce the accumulation of hazardous metals in fish muscle. By achieving significant reductions in iron, zinc, nickel, and other metals, this method provides a practical step forward for both producers and consumers seeking safer, healthier fish. Implementing such treatments at the post-harvest stage could play a crucial role in safeguarding public health and promoting the sustainability of Egypt's vital aquaculture sector.

Competing interests:

The authors declare no competing interests.

Availability of data and materials:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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