



Evaluating the trophic transfer of zinc and lead in aquaculture using *Hydrilla verticillata* as a feed source for common carp

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Abstract

This study was conducted in the Fish Nutrition Laboratory of the Animal Production Department, College of Agriculture and Forestry, University of Mosul, to investigate the bioaccumulation of zinc and lead in the environment, food, and carp bodies. To implement this study, three experiments were conducted to grow, produce, and feed *Hydrilla* plants, and to determine the concentrations of zinc and lead and trace their movement through the food chain. In the first experiment, *Hydrilla* plants were grown using three sources of organic and compound fertilizers (cow manure, sheep manure, and NPK fertilizer), and the yield of *Hydrilla* was determined. In the second experiment, *Hydrilla* plants were grown at two planting levels (2 and 4 grams of *Hydrilla*/liter of water), and subsequently planted at the optimal fertilizer level and density. Sheep manure was applied at a rate of 2 g of *Hydrilla* per liter of water; the resulting yield and heavy metal (Zn and Pb) levels were then determined and used in the carp feeding experiment. The results showed an increase in zinc concentrations as the plant grew in the medium. However, lead concentrations were unaffected across all growth periods (0-15 days). Conversely, higher lead concentrations were observed in carp diets containing 15 and 20% *hydrilla* compared with the control treatment, whereas zinc concentrations did not differ at the 0.05 significance level ($p \leq 0.05$) between treatments. Zinc and lead concentrations in carp bodies differed from those in the diets. Zinc concentrations increased in fish fed diets containing 15% and 20% *hydrilla*. In contrast, lead levels in the fish remained unaffected despite variations in *hydrilla* content in the diets (within the permissible limits for fish feed, 20-150 mg/kg).

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Introduction

Submerged and floating aquatic plants are considered direct feed sources and are used as feed ingredients in fish feed, as they are rich in protein and other nutrients (1). Among these plants is *hydrilla*, a nutrient-dense species that can be used as a feed ingredient. Wild fish feed is also beneficial (2). The percentages of nutrients available in the *hydrilla* plant were 92.29% moisture and 7.71% dry matter (3). The percentages of nutrients in the dry matter were as follows: ash, 20.84%; crude protein, 18.15%; crude fibre,

15.49%; crude fat extract, 1.24%; and nitrogen-free extract, 59.55%. The *hydrilla* plant also contains numerous minerals, such as sodium, potassium, calcium, and phosphorus (4). Carp is a freshwater fish that requires an ideal temperature range of 20-28°C for growth (5). *Hydrilla* performs two tasks simultaneously, playing a positive role in conserving natural resources by recycling waste in water and converting it into biomass that can be used as animal feed. Aquatic plants can remove lead from water for vegetative reproduction and accumulate it in cell walls to protect the plant from lead's toxic effects (6). The concentration of lead in plants is 7

mg/kg (7). Lead is a heavy metal that hurts living organisms and the entire food chain (8). Lead is regarded as the most toxic metal to fish due to its adverse effects, including delayed embryonic development, reduced reproduction, impaired growth, increased mucus production, inhalation of enzymes, and impaired kidney function (9). Its danger lies in its accumulation in human tissues, especially in the bones, teeth, and liver (10). Its danger lies in its accumulation in human tissues, especially in the bones, teeth, and liver (10). There has been increasing public interest in fish farming in both artificial and natural environments, including inland lakes, floodplains, rivers, coastal lakes, permanent or temporary lakes, and dams. However, human and natural sources of toxic elements pollute surface and groundwater (11). (6) also emphasized the role of aquatic plants in removing zinc from water, with the removal rate reaching 85.71%, to meet the needs of new growing plants in building their membranes, chlorophyll, amino acids, and carbohydrates. Zinc also plays a role in activating numerous enzymes essential for plant vital processes. Generally, the concentration of zinc in plants ranges from 20 to 120 mg/kg of dry matter, as noted by (12). The permissible intake of zinc and lead by humans in their diet, according to the recommendations of the World Health Organization, is 150 mg/60 kg/day for zinc, and 1.5 mg/60 kg/day (From the trial period for lead, based on an average human weight of 60 kg (13).

This study was therefore designed to assess the transfer and bioaccumulation of Zn and Pb from water to *Hydrilla* and subsequently to common carp (*Cyprinus carpio*), to understand the pathways and potential risks associated with the use of aquatic plants in fish feed.

Materials and methods

Study site

The study was conducted in the Fish Laboratory of the Department of Animal Production, College of Agriculture and Forestry, University of Mosul.

Experimental fish

Common carp were obtained from a hatchery and private farm located in Erbil Governorate, Iraq. Upon arrival at the laboratory, they were placed in 80-litre plastic tanks containing a portion of the water from the transport vehicle to maintain the fish's environment unchanged for a period and to allow the fish to recover from transport stress. The fish were placed in tanks containing 3% salt to eliminate external parasites and then transferred to ceramic tanks, where they remained until the experiment was conducted.

Hydrilla cultivation and experimental experiment

This experiment was conducted in the field in plastic tanks to grow *Hydrilla* plants. During this experiment, all measurements related to growth and production processes

were taken, as were the concentrations of zinc and lead in the aquatic medium and in the plant. The resulting plant was then fed to fish at varying levels. During the feeding period, production and physiological measurements were taken, as well as the concentrations of zinc and lead in the fish's body.

Experimental design

In this study, experimental treatments were designed at two levels as follows: first, *Hydrilla* cultivation and production. In this part, *Hydrilla* was cultured using three types of fertilizers (cow manure, sheep manure, and NPK fertilizer). After conducting this experiment, the most effective fertilizer was selected, and *Hydrilla* was cultured at two levels (2 and 4 grams of *Hydrilla* per liter of water). Subsequently, the optimal culture level and fertilizer type were selected, and *Hydrilla* was produced. Second, in this part, *Hydrilla* protein was replaced with soybean protein in the feed at 10, 15, and 20%. These feeds were fed to carp for 56 days, during which the percentage of heavy elements in the fish's bodies was measured.

Measuring the concentration of heavy elements in the water

The concentrations of zinc and lead in the filtered water (tap water) were estimated by adding 1.5 mmol/L nitric acid concentrate to 50 ml of the sample (14). The water was then refrigerated and subsequently sent to the general laboratory at the University of Mosul for analysis by atomic absorption spectrophotometry.

Determination of heavy elements in feeds:

The heavy metals zinc and lead were determined on a dry-matter basis in the feeds and fish.

Ingredients of the experimental fish feeds manufactured for the fore treatments:

Ratios of feed ingredients and chemical composition of the treatments in the first experiment: Substitution of soybean meal protein with *hydrilla* protein instead of soybean meal protein (Table 1).

Determination of heavy elements in fish

Heavy elements in fish were determined within the fish muscles, which represent the edible portion of the fish. 0.5 g of each sample was taken to determine the concentration of zinc and lead. The sample was digested with concentrated sulfuric and perchloric acids on a hot plate until the solution became clear. The solution was filtered, and the volume was adjusted to 50 mL with distilled water. The results were measured in the central laboratory at the College of Agriculture and Forestry. Element concentrations were determined from the element's absorption spectrum using a Japanese-made atomic absorption spectrometer, following standard methods approved in (16).

Table 1: The proportions of feed components and chemical compositions of the experimental treatments

Feed Material	Control	10%	15%	20%
Protein concentrate, vitamin and mineral mixture	12	12	12	12
soybean meal	30	27	25.5	24
Hydraulic	0	5.8	8.7	11.8
bran	25	25	25	25
yellow corn	10.5	7.7	6.3	4.7
barley	20	20	20	20
Insurance	1	1	1	1
salt	0.5	0.5	0.5	0.5
limestone	0.5	0.5	0.5	0.5
binding material	0.5	0.5	0.5	0.5
Crude protein	25.35	24.87	24.64	24.42
moisture	9.58	9.67	9.72	9.77
raw fat	2.04	2	1.98	1.96
crude fiber	6.71	8.4	9.24	10.15
raw ash	8.2	8.94	9.31	9.72
soluble carbohydrates	48.12	46.12	45.11	43.98
* Energy represented (MJ/kg)	12.08976	11.71012	11.5208	11.3168

* Metabolic energy was calculated based on equation (15), $ME(MJ/Kg) = Protein \times 18.8 + Fat \times 33.5 + NFE \times 13.8$.

Determination of heavy elements in *Hydrilla*

The concentrations of the heavy metals zinc and lead in the plant were determined on a dry-matter basis. 0.5 g of *hydrilla* was taken, and 5 ml of concentrated sulfuric acid and 2 ml of concentrated perchloric acid were added. The solution was heated on a hot plate until it became clear and transparent. The solution was left to cool and filtered. The solution volume was then increased to 50 mL and sent to the Pollutant Treatment Centre/Scientific Instruments Laboratory of the Environment and Water Department/Ministry of Science and Technology/Baghdad. Elemental concentrations were determined from the element's absorption spectrum using a Japanese-made atomic absorption spectrometer, following the standard method approved in (16).

Statistical analysis

Data were analysed using a completely randomised design (CRD) in SPSS (version 25). Analysis of variance (ANOVA) was used to study the experimental parameters. Differences among application means were tested using Duncan's multiple-range test (Duncan, 1955) at the significance level $P \geq 0.05$, based on the linear model $Y_{ij} = \mu + T_i + e_{ij}$. Where: Y_{ij} : Observed value, μ : Overall mean, T_i : Effect of treatment, e_{ij} : Experimental error.

Results

Estimating the concentration of heavy elements, zinc and lead (mg/L), in the *Hydrilla* growth water

Table 2 shows the concentrations of zinc (Zn) and lead (Pb) in the *Hydrilla* growth medium over different time periods, from the day of planting (day zero) to the fifteenth

day. The results show apparent variation in the behaviour of each element in the culture medium, reflecting the mechanisms of plant uptake and the effects of these heavy elements over time. For zinc (Zn), a concentration of 0.0028 ppm was recorded on day 0, representing the initial zinc concentration in the medium before plant uptake or any biological reactions occurred. On the third day, a significant decrease in zinc concentration ($P \leq 0.05$) was observed, with a value of 0.00043 ppm. This is the lowest value recorded during the experiment, indicating rapid and significant zinc uptake by *Hydrilla* in the first days after planting. On day 6, the zinc concentration increased significantly to 0.0055 ppm, which may indicate that zinc was released back into the medium, either due to the decomposition of plant cells or to biological reactions in the medium, such as microbial activity. On day 9, the concentration remained unchanged relative to day 6 (0.005 ppm), indicating temporary stabilisation of zinc levels. On day 12, the slight significant increase continued to 0.0067 ppm, followed by the highest recorded concentration on day 15 (0.0077 ppm). Notably, the essential differences between periods indicate that the change in zinc concentration is statistically significant, confirming the plant's influence on the dynamics of the element in the aquatic medium. This gradual, continuous change in concentration after day 3 reflects a complex process of balancing absorption and excretion, or may be related to different mechanisms of balance between or release by *hydrilla*.

As for lead (Pb), the initial concentration was 0.0222 ppm on day 0. On the third day, the concentration increased slightly to 0.0239 ppm, and then rose further on the sixth day to 0.0286 ppm. Despite this initial gradual increase, it was not statistically significant, as evidenced by the statistical

analysis across these periods. On the ninth day, the lead concentration decreased to 0.0156 ppm, and the highest observed concentration was recorded on the twelfth day (0.123 ppm). However, this increase was also not significant. On the fifteenth day, the lead concentration decreased again to 0.0165 ppm. The changes in lead concentration were not statistically significant, despite the observed numerical changes, indicating relative stability or irregular fluctuations in lead behaviour in the medium. These results suggest that *Hydrilla* may be better able to absorb or interact with zinc than with lead. Zinc exhibited significant changes, reflecting the plant's active interaction with it, whereas lead concentrations showed no significant differences. This may indicate a relative resistance of the plant to its absorption, or that lead is bound in a non-biologically available form in the medium. The changes in both elements over time underscore the importance of studying the behaviour of heavy elements over time to understand the dynamics of interactions between aquatic plants and environmental pollutants, thereby paving the way for the use of these plants in bioremediation and the removal of heavy elements from polluted marine environments.

Table 2 shows the heavy elements present in the *Hydrilla* water growth medium during different periods.

	Zn ppm	Pb ppm
Day 0	0.0028 ± 0.0003 d	0.0222 ± 0.004 a
Day 3	0.0043 ± 0.00005 c	0.0239 ± 0.01 a
Day 6	0.0055 ± 0.0007 b	0.0286 ± 0.004 a
Day 9	0.0055 ± 0.0003 b	0.0152 ± 0.006 a
Day 12	0.0067 ± 0.0003 ab	0.123 ± 0.005 a
Day 15	0.0077 ± 0.0001 a	0.0165 ± 0.007 a

Means with different letters vertically within the same trait mean significant differences at the level ($P \leq 0.05$).

Table 3 shows that the final concentrations of these elements in the *Hydrilla* plant itself were very low, with the zinc concentration in plant tissues reaching 0.00337 mg/kg (equivalent to 0.00000337 ppm), and lead 0.000130 mg/kg (equivalent to 0.00000013 ppm), which are much lower than the concentrations recorded in the medium water. This significant disparity between the concentrations of the elements in the water and in the plant indicates that *Hydrilla* has a low capacity to absorb or accumulate zinc and lead from the aqueous medium, at least under the experimental conditions. On the other hand, the low zinc concentration in the water on the third day may indicate partial absorption by the plant. Still, its concentration in the plant tissues remained very low, supporting the hypothesis of weak accumulation. As for lead, despite its relatively high levels in the aquatic environment, its concentration in the plant remained very low. This confirms the plant's limited ability to absorb this toxic element, or may indicate the presence of resistance mechanisms that prevent its internal accumulation.

Table 3 shows the actual concentrations of zinc and lead in the *hydrilla* plant.

	Zn (mg/Kg)	Pb (mg/Kg)
Plants	0.00337	0.000130

Estimating the concentration of heavy elements, such as zinc and lead, in mg/L in fish experimental diets.

Table 4 shows the effect of adding *Hydrilla* at different rates (10%, 15%, and 20%) to fish diets compared to the control treatment (without addition) on the concentrations of zinc (Zn) and lead (Pb) in mg/kg. The results showed that zinc concentration was not significantly affected by *Hydrilla* addition, with values ranging from 0.002127 to 0.002620 mg/kg, indicating that rates up to 20% did not alter the diet's zinc content. This may be attributed to low zinc concentration in the plant or to limited bioavailability. In contrast, lead concentrations differed significantly among treatments, with the control treatment recording the lowest concentration (0.004573 mg/kg), whereas concentrations increased with increasing *hydrilla* percentage. The 10% *hydrilla* treatment recorded (0.006883 mg/kg), which did not differ significantly from the control treatment. In comparison, the highest concentrations were recorded in the 15% and 20% treatments (0.009973 and 0.009790 mg/kg, respectively), which were significantly higher than the control treatment, indicating a direct relationship between the percentage of *hydrilla* in the feed and the increase in lead content. As shown in Table 4, the higher the *hydrilla* content in the feed components, the higher the concentrations of zinc and lead. This indicates that *hydrilla* increases the concentrations of lead and zinc in feeds containing it. The lead concentrations in the experimental treatments shown in Table 4 are within the permissible limits for fish feed, ranging from 20 to 150 mg/kg. The zinc concentration in the treatments is also within the allowable limits for fish feed, ranging from 5 to 10 mg/kg (17). The results of the two experiments are consistent with the findings of (18), which indicate that the higher the concentration of *Azolla australica* in the feed, the higher the concentrations of zinc and lead.

Table 4: Zinc and lead concentrations in fish feeds (mean ± standard error)

	Zn (mg/Kg)	Pb (mg/Kg)
Control	0.002620 ± 0.0001 a	0.004573 ± 0.0002 b
10%	0.002293 ± 0.00007 a	0.006883 ± 0.002 ab
15%	0.002317 ± 0.00002 a	0.009973 ± 0.0008 a
20%	0.002127 ± 0.0005 a	0.009790 ± 0.0001 a

Means with different letters vertically within the same trait mean significant differences at the level ($P \leq 0.05$).

Estimating the concentration of heavy elements, represented by zinc and lead, mg/L, in fish bodies:

Data in Table 5 indicate an evaluation of the effect of different proportions of *Hydrilla* added to carp feed on zinc and lead concentrations in their bodies. The results showed that zinc concentrations were very low across all treatments, ranging from 0.00002 to 0.00003 parts per million, with no significant differences. Treatments three (15%) and four (20%) with *Hydrilla* differed significantly from the control and the second treatments, indicating an increase in zinc concentration attributable to the addition of *Hydrilla*. However, this increase was not biologically significant, given the similar values and overall low concentrations. Regarding lead, the results showed no significant differences between all treatments, including the control, indicating that the addition of *Hydrilla* at 10%, 15%, and 20% did not significantly affect lead accumulation in fish bodies. This stability in the concentration of these two elements indicates that the *hydrilla* plant used in the experiment was not a source of heavy metal contamination for the fish. Instead, it may have contributed to mitigating their accumulation or maintaining their natural levels within safe limits. This enhances the feasibility of its use as a natural alternative ingredient in fish feed without causing cumulative toxic or harmful effects on fish health or on the end consumer, mainly since the concentration remained at very low levels that do not pose an environmental or health risk. This stability in the concentration of these two elements indicates that the *hydrilla* plant used in the experiment was not a source of heavy metal contamination for the fish. Instead, it may have contributed to mitigating their accumulation or maintaining their natural levels within safe limits. This enhances the feasibility of its use as a natural alternative ingredient in fish feed without causing cumulative toxic or harmful effects on fish health or on the end consumer, mainly since the concentration remained at very low levels that do not pose an environmental or health risk.

Table 5: Shows the heavy elements present in the body of carp (mean \pm standard error)

	Zn (mg/Kg)	Pb (mg/Kg)
Control	0.000651 \pm 0.00003b	0.00244 \pm 0.000047a
10%	0.000667 \pm 0.00002 b	0.00331 \pm 0.000045a
15%	0.000794 \pm 0.00002 a	0.00333 \pm 0.000045a
20%	0.000832 \pm 0.00002a	0.000391 \pm 0.00056a

Means with different letters vertically within the same trait mean significant differences at the level ($P \leq 0.05$).

Discussion

The results show that *Hydrilla* exhibited a distinct zinc uptake pattern, with a sharp decrease in its concentration in the growing water during the first days of cultivation, reflecting the plant's high efficiency in taking up this element

during the initial growth phase (19,20). Aquatic plants rapidly take up trace elements during their early stages. However, this uptake did not translate into clear internal accumulation, as the concentration of zinc in plant tissues remained very low, indicating that *Hydrilla* may not possess a long-term accumulation mechanism for this element, but rather is involved in a vital balance between uptake and release. This balance may be attributed to plant cell decomposition processes or microbial activity (7) or to the solubility of zinc resulting from the slow decomposition of organic matter (21,22). It falls within the permissible limits for fish feed, ranging from 20 to 150 mg/kg (23). The study also indicated that zinc concentration remained high in carp tissues relative to other elements, consistent with its everyday use to maintain fish health and vitality. In contrast, lead exhibited a different behavior, with no significant changes in its concentration in the aquatic medium, despite numerical fluctuations during specific periods. This states that the plant did not interact with it by absorption. The *hydrilla* possesses defense mechanisms that reduce lead absorption or prevent its accumulation by inhibiting its uptake at the root level or by binding it to non-transportable organic compounds. This hypothesis was supported by laboratory measurements, which showed very low lead concentrations in plant tissues, indicating limited absorption or low bioavailability in the medium (19-24).

When analyzing the effect of introducing *hydrilla* into fish feed, it was observed that zinc was not significantly affected by increasing plant levels in the feed. This is attributed to the low zinc concentration in *hydrilla* itself; therefore, its bioavailability was insufficient to produce a significant effect on the feed. Lead showed a gradual response with increasing plant content, with its concentration in feeds significantly increasing when higher proportions of *hydrilla* were used. This indicates a correlation between plant lead content and its concentration in the feed, although both remained within the permissible limits for fish nutrition (17). This increase is attributed to small lead deposits in *hydrilla*, which may be released during processing or during mixing with other feed components (18).

Regarding the accumulation of elements in fish bodies, the results showed that both zinc and lead remained at very low levels, with no significant differences recorded. This indicates that the inclusion of *hydrilla* in the feed did not contribute to the transfer or accumulation of these elements in fish tissues. This refers to the safety of using *hydrilla* as a feed ingredient, which does not cause toxic accumulation in the food chain. Aquatic plants with low heavy metal content can be safely used without compromising environmental or animal health. These findings support the potential of using *hydrilla* for the bioremediation of aquatic pollutants, on the one hand, and for use in fish feed as a safe plant-based alternative, on the other, without significant environmental or health risks (25).

Conclusions

The study showed that hydrilla rapidly absorbs zinc from water without long-term internal accumulation. In contrast, it showed no significant ability to absorb lead due to resistance mechanisms or low bioavailability. When used in fish feed at 20% concentration, it did not affect the feed's zinc concentration, and lead increased slightly but remained within safe limits. Neither element accumulated in fish tissue, confirming its environmental and nutritional safety and demonstrating the feasibility of incorporating it into aquaculture systems without the risk of heavy metal accumulation in the food chain.

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Conflict of interest

No conflict of interest.

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وسماد NPK، وتم تقدير إنتاجية نباتات الهيدريلا. في التجربة الثانية، زُرعت نباتات الهيدريلا في مستويين للزراعة (٢ و ٤ غرام من نبات الهيدريلا/لتر ماء) و تم زرع نباتات الهيدريلا في أفضل مستوى تسميد وأفضل كثافة، وتم تقدير إنتاجيتها ومستويات العناصر الثقيلة فيها (الزنك والرصاص) واعتمد سماد الأغنام عند مستوى زراعة ٢ غرام من نبات الهيدريلا لكل لتر ماء تم أخذ نتائج هذه التجربة من نباتات الهيدريلا واستخدامها في التجربة لتغذية أسماك الكارب. أظهرت النتائج زيادة في تركيزات الزنك مع تقدم فترة نمو النبات في الوسط. ومع ذلك، لم تتأثر تركيزات الرصاص طوال جميع فترات النمو (من صفر إلى ١٥ يوماً). وعلى العكس من ذلك، وُجدت تركيزات أعلى من الرصاص في علائق أسماك الكارب التي تحتوي على ١٥ و ٢٠% من الهيدريلا مقارنة بمعاملة الضبط، بينما لم تختلف تركيزات الزنك عند مستوى معنوياً بين المعاملات. اختلفت تركيزات الزنك والرصاص في أسماك الكارب التي تغذى على الجسم عن تركيزاتها في العلائق. زادت تركيزات الزنك في علائق الأسماك التي تحتوي على ١٥ و ٢٠% من الهيدريلا، بينما ظلت مستويات الرصاص في الأسماك دون تأثير على الرغم من اختلاف محتوى الهيدريلا في العلائق (يقع ضمن الحدود المسموح بها في علف الأسماك، والتي تتراوح من ٢٠ إلى ١٥٠ ملغم/كغم).

تقييم النقل الغذائي للزنك والرصاص في تربية الأحياء المائية باستخدام نبات الهيدريلا فيرتيسيلاتا كمصدر تغذية لأسماك الشبوط الشائعة

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الخلاصة

أجريت هذه الدراسة في مختبر تغذية الأسماك التابع لقسم الإنتاج الحيواني، كلية الزراعة والغابات، جامعة الموصل، لدراسة التراكم الحيوي للزنك والرصاص في البيئة والغذاء وأجسام أسماك الكارب. ولتنفيذ هذه الدراسة، أجريت ثلاث تجارب لزراعة وإنتاج وتغذية نباتات الهيدريلا، وتحديد تراكيز الزنك والرصاص، وتتبعهما في السلسلة الغذائية. في التجربة الأولى، زُرعت نباتات الهيدريلا باستخدام ثلاثة مصادر من الأسمدة العضوية والمركبة سماد الأبقار، وسماد الأغنام،