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Research Article

Using algae and brine shrimp as food chain model for bioaccumulation and biomagnification of lead and cadmium

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Abstract
Bioaccumulation and biomagnification of heavy metals occur constantly in the aquatic environment. Therefore, this study aimed to simulate the environment in the laboratory conditions and study the amount of lead (Pb) and cadmium (Cd) accumulation in two types of algae microalgae *Dunaliella salina* and *Tetraselmis suecica*. These algae were exposed to metals for a short duration of 21 days. After this period, they used these algae as food for *Artemia salina* (brine shrimp). The results showed that Pb was bio-accumulated in *D. salina*, between 12.65 and 1262 ppm, respectively, when exposed to a concentration of 50 and 1500 ppm., while the *T. suecica* was bio-accumulated Pb at a concentration of 20 and 1363 ppm when exposed to the same concentrations respectively. Also, Cd bio-accumulated in *D. salina* between 9.99 and 1148 ppm, while bioaccumulated Cd in *T. suecica* at a concentration of 10.71 and 1110.8 ppm, respectively, while when *Artemia* feed on algae *(D. salina* and *T. suecica)*, that accumulated Pb and Cd after 21 days. The high biomagnification of *Artemia* for Pb (688.56 ppm) and the high bio-magnification of *Artemia* for Cd (700.99 ppm) when using *D. salina* as food. There are many environmental studies on the transfer of pollutants, especially heavy metals, in the food chain, but this study is one of the few studies that simulate the aquatic environment under controlled laboratory conditions for the accumulation of heavy metals in the food chain while minimizing the influence of interactions from other influences.

Keywords: Algae, *Artemia,* Bioaccumulation, Bio-magnification, Heavy metals

INTRODUCTION

Environmental pollutants of toxic heavy metals are present in high concentrations in the environment, such as Pb, Cd, Cr, Zn, Cu, Co, Fe, Ni, and As, contaminate the water, soil, fauna, and flora (Borgheipour *et al*., 2020). Plankton, situated at the base of food chains, can assimilate heavy metals and organic contaminants, which subsequently undergo biomagnification, resulting in substantial concentrations at higher trophic levels (Chevrollier *et al.,* 2022). Heavy metals have the potential to be introduced into an organism's body either by direct exposure to the abiotic environment through absorption and inhalation from water, sediments, and soil or by consumption of contaminated food or prey, which accumulate in their tissues as a result (Liu *et al*., 2020). The transmission of these types of pollutants through the food chain and their accumulation in the body of living organisms increasing from the lowest trophic level to the highest along the food chain is one of the most serious problems in the pollution of the aquatic environment (Huang *et al*., 2021). *Dunaliella salina* is one of the unicellular microalgae that can adapt to various salinity ranges. The cell is within the range of (5-25 µm and its width is from (3-15 µm.it has two flagella of length between 1 and 1.5 times that of the body (Rycroft, 2022). *Tetraselmis suecica* is one of the marine microalgae, one of the important species used as live feed in the shrimp culture industry (Bameri *et al.,* 2023). Brine shrimp (*Artemia*) is a crustacean type with major ecological importance in hypersaline ecosystems. It is used as a live food in aquaculture (Dong *et al.,* 2024). The present study aimed to determine the transfer of the heavy elements lead and cadmium through a laboratory food chain from water to algae and then to *Artemia.*

MATERIALS AND METHODS

The algae (*T. suecica* and *D. salina*) were selected as a primary product in the food chain, and the algae were obtained from UTEX at the University of Texas Austin (website https://utex.org/) The species were laboratory confirmed, cultured in Chu-10 medium, and left for at least two weeks before starting the experiment under constant laboratory conditions, including a temperature 21º C. The light was provided by fluorescent lamps with an intensity of 2840 lux, with a photoperiod of 12 h light:12 h dark. Dilutions were made during the first hour of the light period. The pH was maintained within the range of 7.6-8.0 (Bold and Wynne, 1978). Algae were treated with different concentrations of Pb and cd (50, 100, 250, 500, 750, 1000, 1500) ppm to study the accumulation performed by taking (3 g) of the powder of the algae sample to be digested was placed inside a (25 ml) (Griffin beaker) cup, then 3 ml of concentrated per Chloric acid solution was added to it and covered. The beaker was heated quietly on an electric hot plate using a watch glass, and we raised the temperature gradually to complete the digestion process. When the mixture reached the dry stage, the beaker was left to cool, and added 3 ml of a concentrated nitric acid solution again covered in the beaker and continued heating until the digestion process was over to get a mixture that was clear and colored in a light color. It was evaporated until it was close to the dry stage, and 5 ml of a solution of hydrochloric acid was added diluted with water in proportions (1:1), heated to dissolve the remaining sample after the digestion process, and then added distilled water. The volume of the solution was adjusted according to the expected concentration in the samples to a volume of 100 ml or 50 ml or less as the sample became ready for analysis. The absorption of these digested samples was measured using an atomic absorption device of the type (SHEMADZU AA 7000) at a wavelength of 228.8 nm for Cd, 283.3 nm for Pb, and the control group was exposed to element-free water (Anderson,1991) in addition to (Katz and Jennis, 1983) by using a Flame atomic absorption spectrophotometer,3111 metals by flame atomic absorption spectrometry (APHA,2017) after 21 days of treatment for Pb and Cd analysis. Brine Shrimp *(Artemia salina*) cysts were obtained from Brine Shrimp Direct company in Ogden, Utah, and were hatching under standardized conditions of hatching, temperature 30°C, salinity 35 g / L, light intensity 2840 lux, and pH 8 (Sorgeloos *et al.,* 1986). After that, the highest bioaccumulation concentration of Pb and Cd in algae was selected (Table 1) and used as Food type 1 (F1): *A. salina* which were fed on only algae treated with Pb or Cd three times a day in the amount 0.75 ml/l. Another way to feed *Artemia* was by mixing treated algae with other food (algae, soy protein, rice bran, and aquarium fish food) in a ratio of 2: 1:1: 1 (Food type 2 (F2) for 21 days. *Artemia* biomagnification of Pb and Cd inside their bodies was measured. An Atomic absorption spectrophotometer was used to measure Pb and Cd element concentrations (APHA, 2017). The bio-accumulation factor (BAF) was determined by dividing the concentration of the metal in the body of the organism (alga and *Artemia*) by the concentration of the metals in water (Bryan, 1979).

Statistical analysis

Using the Duncan test and correlation, the SPSS V 23 program was used to calculate the significant difference among metal concentrations.

RESULTS AND DISCUSSION

The results in Table 1 the treated algae *(D. salina* and *T. suecica*) with five concentrations (50, 100, 250, 500, 750, 1000, 1500) ppm and of Pb accumulation in *D. salina* ranged between 12.65 -1262.43 ppm for the concentration (50-1500) ppm and there were significant differences among control and all concentrations except 50ppm, while the BAF ranged between 0.253 - 0.84 ppm for the concentrations (50 – 1500) ppm, while *T. suecica* accumulation ranged between 20.39 -

Table 1. Showing different bio-accumulation concentrations for Pb and Cd in the algae (*D. salina* - *T. suecica*) after being treated for 21 days with the BAF

Metal	Alga		Metal concentrations (ppm)						
			Control	50	250	500	750	1000	1500
Pb	*D. salina*	Mean±SD	0 (ND) a	12.65±1.07a	60.96±2.93b	128.75±3.66c	313.72±2.58d	705.37±5.05e	1262.43±47.49f
		BAF		0.253	0.243	0.256	0.417	0.705	0.84
	T. suecica	Mean±SD	0 (ND) a	20.39±1.52b	57.55±2.435c	94.11±4.25d	367.66±0.217e	861.2±14.78f	1363.82±23.49g
		BAF		0.407	0.23	0.188	0.489	0.86	0.90
Cd	*D. salina*	Mean±SD	0 (ND) a	9.99±1.21a	45.46±3.64a	104.09±7.00ab	232.23±201.2b	669.93±10.80c	1148±24.30d
		BAF		0.199	0.181	0.208	0.309	0.66	0.77
	T. suecica	Mean±SD	0 (ND) a	10.71±0.75b	36.92±3.11c	125.09±4.07d	404.23±3.00e	747.87±6.84f	1110.8±11.89g
		BAF		0.214	0.147	0.25	0.538	0.74	74

* ND: No detection; Similar letters for each row mean they are not significantly different ($p < 0.05$)

1363.82 ppm for the concentrations (50 – 1500) ppm, there were significant differences among control and all concentrations. The BAF ranged between (0.407 to 0.90) ppm for the concentrations (50 – 1500) ppm. When treated both algae with Cd, the alga *D. salina* showed significant differences among control and concentrations (750,1000,1500) ppm, ranging between (9.99 - 1148) ppm for the concentration (50 – 1500) ppm with a BAF ranging between (0.199 - 0.77) ppm. Moreover, compared with the alga *T. suecica* there were significant differences with all concentrations and the ranged concentration of bioaccumulation between 10.71 – 1110.8 ppm for the concentrations (50 – 1500) ppm, with a BAF ranged between 0.214 – 74 ppm. Microalgae cells have a great potential to remove ion metals from aqueous solutions due to their large surface area and high binding affinity. Elleuchet *et al*. (2021) studied the capacity of *D. salina* to tolerate and remove Cd, and Pb at different concentrations, and the results showed that *D. salina* could survive at least for 14 days.

The results in Table 2 shows that when algae were treated with 1500 ppm of heavy metals, accumulated a quarter of the amount of Pb in *D. salina* with F1: 1262.43 ppm, F2: 474.4ppm and *T. suecica* with F1:1363.82 ppm, F2: 510.6 ppm, while when treated with Cd accumulate in *D. salina* with F1: 1148ppm, F2: 504.4ppm and *T. suecica* with F1: 1110.8 ppm, F2: 345.3ppm, this similar to the results of Kit and Chang, (2020) study that showed, the saturation of active functional groups existing on the *D. salina* surface at high concentrations of lead ions. The uptake efficiency was reduced when the lead ion concentration was increased. The behavior of reduced uptake efficiency when increasing the Pb ion concentration might be due to the saturation of the active functional groups on the *D. salina* surface with high concentrations of Pb ions. Also, Ziaei *et al*. (2023) mentioned similar results, stating that *D. salina* algae showed maximum Pb uptake of 96% efficiency. Zhu *et al*. (2020) mentioned that acute Cd toxicity impaired cell growth by increasing Cd bioaccumulation and lipid peroxidation, which reduced cellular pigment, total protein, and glutathione content.

Table 3 shows that *Artemia* had significant differences with both types of food when using F1 and with F2. A high concentration of biomagnification for Pb was seen in *D. salina* at F1: 688.56 ppm. *D. salina* at F1: 700.99 ppm showed a high concentration of biomagnification for Cd. Cd and Pb are toxic metals and can bioaccumulate in the body through exposure time. There are two types of transference in the trophic chain: positive transference (i.e., metal increased from seawater to phytoplankton and from crustaceans to fish), and negative transference (i.e., the shrimp had lower tissue concentrations of Pb than in his prey). And both of them are reflections of (biomagnification). Biomagnification is defined as the tendency of pollutants to increase body burden throughout the food chain. The microalga showed phenomenal removal efficiency (~80%) when exposed to 25 ppm of Cd. Additionally, the elevated level of ROS and antioxidant enzymes evidenced the activation of efficient antioxidant machinery for alleviating the Cd stress (Tripathi *et al*., 2021).

Table 2. Showing the accumulation concentrations in algae treated with 1500 ppm of lead and cadmium

Metal	Alga	Metal concentrations in D. salina and T. suecica (ppm)		
		Control	Food type 1	Food type 2
Pb	*D. salina*	0 (ND) a	1262.43±47.49 b	474.4 ± 2.48 c
	T. suecica	0 (ND) a	1363.82±23.49 b	510.6 ± 9.52 c
Cd	*D. salina*	0 (ND) a	1148±24.30 b	504.4 ± 7.25 c
	T. suecica	0 (ND) a	1110.8±11.89 b	345.3 ± 2.49 c

* ND: No detection; Same letters are not significantly different from each other ($p < 0.05$) for each row

Table 3. Showing the concentrations of biomagnification in *Artemia* fed on the algae (*D. salina* – *T. suecica*) treated with lead and cadmium.

Metal	Alga	Metal biomagnification in Artemia salina (ppm)		
		Control	Food type 1	Food type 2
Pb ppm	*D. salina*	0 (ND) a	688.56 ±2.77 b	315 ± 4.71 c
	T. suecica	0 (ND) a	639.94 ± 2.8 b	220.4 ± 5.61 c
Cd ppm	*D. salina*	0 (ND) a	700.99 ± 3.52 b	300.47 ±14.64 c
	T. suecica	0 (ND) a	516.19 ±13.94 b	125.1±3.77 c

* Same letters are not significantly different from each other ($p < 0.05$) for each row,

Conclusion

The present study showed heavy metal (Cd and Pb) concentrations' bioaccumulation and biomagnification in two algae species (*T. suecica* and *D. salina*) and *Artemia* showed appreciable accumulation of Pb and Cd through the levels of chain food in algae used as treated food with heavy metals and *Artemia* considered as the primary consumer for the algae in laboratory conditions for a short period of 21 days. Accumulation increased with an increase in the exposure period. It showed a high tendency for *Artemia* to accumulate heavy metals. The study also showed that a laboratory experiment simulated reality, showing the appreciable amount of accumulated metals that were transferred from water to the food chain consisting of two types of algae and *Artemia* under controlled laboratory conditions. As a result of the concentrated amount of heavy metals (Cd and Pb) present in food type 1, The results showed a higher level of bioaccumulation in the two types of algae (*D. salina* and *T. suecica*) and *Artemia* than in food type 2, which was less concentrated for the elements as a result of mixing with other nutrients. The results also showed that the bioaccumulation of lead in *T. suecica* was higher than that for the accumulation of Cd for the same algae when using both types of food. When compared with Pb, the *T. suecica* recorded the highest accumulation of algae, while for Cd, the opposite result appeared, as the *D. salina* recorded higher bioaccumulation than the *T. suecica*. The result of biomagnification showed that the highest level of biomagnification appeared when the *Artemia* fed by *D. salina* exposed to lead, compared to the *Artemia* fed by *D. salina,* the same algae exposed to cadmium.

Conflict of interests

The authors declare that they have no conflict of interest

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