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Research Article

Biosorption of some heavy metals (chromium and copper) by microalgae, amphipoda, and beluga in the Caspian Sea

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Huso huso

Abstract

The absorption or accumulation of metals by the organisms of an ecosystem will have a different effect on their food chain. This research compares the heavy metal absorption of copper and chromium in algae, amphipods, and sturgeon species. In Materials and Methods respectively, included the preparation stage including algae cultivation, catching and propagating gammarus and fish and the digestion stage including initial digestion by nitric acid and the final digestion by Header Digest. In this research, algae had a better efficiency in chromium absorption at the initial time. A significant difference was observed in the absorption of algae between copper and chromium (p<0.01). On the contrary, gammarus had a better efficiency in copper absorption, and the absorption process increased during the time. A significant difference was observed in the biosorption of gammarus between heavy metals copper and chromium (p<0.01). The accumulation of heavy metals in the liver was more than in muscle. There was a significant difference between fish tissues in the accumulation of heavy metals (p<0.01). In body tissue, copper accumulates more than chromium. A significant difference was observed, the comparison between the accumulation of copper and chromium metals in fish tissues (p<0.01). The highest absorption and accumulation of metal was observed by algae and gammarus respectively, in fish body tissues. Algae are strong metal absorbers from the environment and remove metal from the fish body. Gammarus absorbs the metals from the environment and transports them to the fish body. The absence of Spirulina algae in the coastal ecosystem of the Caspian Sea causes the direct transfer of heavy metals to small organisms such as gammarus, and the consumption of these organisms by fish will have harmful effects on their health and ultimately on humans.

Article info

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Introduction

The Caspian Sea is a closed basin and the majority of its water supply comes from river inflows. The water entering the southern part of the Caspian Sea contains a large amount of urban and agricultural wastewater due to human activities. With the rapid development of industries, especially in developing countries, heavy metal wastes are discharged directly or indirectly into the environment. Organic and mineral materials are discharged into the environment due to domestic, agricultural, and industrial activities and finally turn into organic and mineral pollutants (Abdel-Raouf *et al*., 2012). Unlike organic pollutants, heavy metals are not degradable and tend to accumulate in organisms. Therefore, heavy metals remain in wastewater and combine with inorganic nitrogen and phosphorus, damaging the food chain (Souza *et al*., 2012).

Copper is an essential element for all organisms because the lack of this mineral can cause enzyme dysfunction. However, the same metal is toxic to fish in high concentrations in water (Pickering and Henderson, 1966). The importance of testing copper metal because it is essential for organisms and extensive agricultural activity due to pesticides, as well as the entry of detergents from urban sewage into the southern part of the Caspian Sea.

Chromium metal exists in several oxidation states in the environment and usually appears as trivalent and hexavalent (Ghosh and Singh, 2005). Hexavalent chromium is toxic to all plants and animals, highly carcinogenic, and has high solubility and accessibility in water and soil (Goyer and Clarkson, 2001). Due to the presence of a factory near the Tajan River that discharges waste containing chromium into this watershed, the study of this metal has received attention.

Bioremediation is used by natural mechanisms, including microorganisms and plants, to remove dangerous pollutants (Sibi, 2019). Biosorption has particular importance due to its contribution to the environment and its excellent performance in removing heavy metals (Wang *et al.*, 2006). The essential advantages of this method can be attributed to its effectiveness in reducing the concentration of metal ions to shallow levels, beneficial live microorganisms, simple technology, nutrient recycling, adsorbent regeneration capability, no production of sludge, the possibility of metal recycling, and the use of cheap absorbent materials such as natural algae (Herrero *et al*., 2006). In biosorption efficiency, several factors are influential, such as the initial concentration of metal ions, contact time, pH, temperature, the concentration of the biological absorbent, the rate of turbulence, and the competition of ions (Salam, 2019).

Spirulina microalga species (*Arthrospira platensis*) are used in the food industry and bioabsorption of heavy metals in wastewater. In recent years, algae have been used to assess ecological risks and effects of heavy metals, herbicides, and other pollutants in aquatic systems for sensitivity to metal pollutants (Levy *et al*., 2007). In other words, algae can provide suitable places for connection metals due to having polysaccharides, protein, and fat on the surface of their cell walls, which have functional groups such as amine, hydroxyl, carboxyl, and sulfate (Rajfur *et al*., 2011).

Therefore, algae have always been of interest due to their high absorption capacity and availability in almost unlimited amounts (Klimmek *et al*., 2001). It should be mentioned that the effect type of heavy metals in different concentrations is diverse on the biological and biochemical properties of microscopic algae (Soeder *et al*., 1978).

The absorption of heavy metals in the body of Creatures is mostly through receive of metals in food and water in the form of suspended or dissolved. Excessive absorption of essential and unnecessary metals accumulates in different tissues (Canli and Atli, 2003). Micro-benthic species (*Pontogammarus maeoticus*) are crustaceans and one of the most abundant amphipods with wide distribution and high density at the edge of the Caspian Sea. There are more than 100 freshwater, brackish, and marine species in the Northern Hemisphere. They are ecologically significant in the Caspian Sea because they are fed by many economically valuable fish, including sturgeons, kutum, mullet, perch, anchovy kilka, and carp. Also, these animals transfer pollutants to the food chain through contact with water, seafloor sediments, and the consumption of phytoplanktons and zooplanktons containing heavy metals (Ghareyazie and Mottaghi, 2012). Due to their wide distribution, importance in the food web, and sensitivity to a wide range of pollutants, they are essential bioindicators for water quality assessment (Gerhardt *et al*., 2011).
In fish, the digestive system absorbs heavy metals in food, and the gills absorb heavy metals in water. At the end of the absorption process, heavy metals usually accumulate in the liver and muscle tissue (Rajeshkumar and Li, 2018). High concentrations of metals cause changes in the biological activities of fish (Canli and Atli, 2003). Humans can cause serious health problems by consuming metal-contaminated fish (Kamaruzzaman *et al*., 2011). The hazardous effects of metals on fish at the top of the food chain are greater than on terrestrial vertebrates (Kousar and Javed, 2014). Beluga fish (*Huso huso*) are among the predatory and carnivorous creatures of the Caspian Sea. They feed on the creatures around them at different life stages depending on the place and location. They usually feed on benthic invertebrates, insects, and crustaceans at a young age. They feed on small and large fish in their habitat, including big-scale sand smelt (*Atherina boyeri*), common roach (*Rutilus rutilus*), and so forth in old age. Beluga fish is on the Red List of the International Union for Conservation of Nature (IUCN) and is currently in the status of critically endangered (CR) species (Gessner *et al*., 2022).

Bioaccumulation of heavy metals by organisms may be passive or selective. When the excretory, metabolic, Storage, and detoxification mechanisms cannot resist absorption, the toxic effects of these substances become visible. These effects ultimately lead to physiological and histological changes (Jia *et al*., 2017). Therefore, researchers believe that the transfer of heavy metals from the lower to the upper levels of the food chain causes danger in the ecosystem because these substances tend to accumulate and transfer from one to another food chain (Martin and Griswold, 2009).

According to Norian *et al*. (2022), the observed increase in the Trophic Diatom Index (TDI) at the lower trophic levels of the Arvand River food web is indicative of heightened organic and nutrient pollution, resulting from anthropogenic pressures such as untreated wastewater discharge, aquaculture effluents, and other land-based sources of contamination.

The purpose of this research is to compare and influence the amount of gammarus (*P. maeoticus*) and spirulina microalgae (*A. platensis*) in the maximum concentration of biosorption and also the final lethality limit of heavy metals chromium and copper. At the end of this stage was the process and amount of heavy metals absorbed in the diet of beluga fish (*H. huso*) at the top of the food pyramid of the Caspian Sea.

Materials and methods

Preparation

In the spirulina algae (*Arthrospira platensis*) experiment, pre-cultivated algae in the number of treatments placed inside the Erlens Mayer containing heavy metals chromium and copper (1 ml/L) with proper aeration and light (Budi *et al.*, 2020). Algal biomass was removed using a 5 micron (µm) filter during periods of zero (less than one hour), 24 and 48 hours. Algae samples were removed to complete the preparation stage of the experiment dried and finally powdered in an oven at 50°C for 24 hours. For accurate and reliable calculation of the experiment, the water sample separated from the algae fixed with nitric acid (1.5 ml per liter) to calculate the amount of remaining or unabsorbed metal. Gammarus (*Pontogammarus maeoticus*) specimens were caught in the Tejan River estuary into the Caspian Sea with a 1 mm net and kept in an aquarium under laboratory conditions. To start the Gammarus experiment, the amount of Gammarus calculated according to the number of treatments based on the heavy metals chromium and copper (1 mL/L) was inside Erlen. Gammarus specimens were removed during periods of zero (less than one hour), 24, and 48 hours, and dried and powdered in an oven at 50°C for 48 hours to complete the experiment preparation stage. Similar to the previous experiment, the water sample isolated from Gammarus was fixed with nitric acid (1.5 ml/l) for the accuracy of the experiment. In the co-cultivation experiment, similar to the previous two methods were performed, with the difference that algae and Gammarus samples were placed in direct competition and with a typical tank, separated by the type of metal and periods. In the beluga fish (*Huso huso*) experiment, heavy metals chromium and copper with a ratio of one milliliter of metal in one liter of water (mg/L) were separately absorbed and finally dried by adsorbents. Powdered adsorbent samples were introduced, including algae (treatment 1), Gammarus (treatment 2), co-culture of algae with Gammarus (treatment 3), and control or food containing two metals, chromium and copper (treatment 4) to this stage. Each of the treatments was mixed with fish food at a ratio of 3 grams per kilogram. For treatment 4, One milligram of metal was added per kilogram of fish food. After two months of feeding based on fish body weight, 10 fish with an approximate weight of 100 grams from each pool were transferred to the Caspian Sea Ecology

Research Institute with dry ice and kept at -20°C until the experiment. At the end of this stage, the liver and muscle tissues of the fish separated, and their wet weight was measured with a digital scale (AND, Japan); then the samples were transferred to a freeze dryer (Christ, Germany) for drying. At the end of this stage, muscle tissue samples were crushed using an electric shredder (Moulinex, France), and liver tissue samples were powdered using a mortar (Moopam, 1999).

Digestion

At this stage of the experiment, the powdered samples of algae, gammarus, and the liver and muscle tissue of beluga fish were placed separately in the amount of 0.3 grams of each sample into the laboratory tubes. Then, 4 ml of nitric acid 65% was added to each of the samples, and the digestion process was performed for one hour. In the following, laboratory tubes for 3 hours were placed into the digestion device (Header Digest) at 90°C to final digestion. Finally, the cooled samples were put into a 50 ml volumetric flask with Whatman filter paper and made up to volume with distilled water. In the algae and gammarus of the experiment, 100 ml of water samples were passed through filter paper, and then initial digestion was done with 5 ml of 65% nitric acid. The samples were transferred to a water bath with a temperature of 100°C for final digestion. After 7 hours, the water samples were brought to the required volume of 15 ml. Each sample was filled with distilled water in a 100 ml volumetric flask (Moopam, 1999).

Numerical and statistical calculations

The device for evaluating the amount of absorption of heavy metals in the samples was carried out by an atomic absorption spectrometer (Solaar.M5, Thermo Electron Company, America) in both flame and furnace methods (Crump, 1993; Parsons, 2013). After the samples were prepared, they were transferred to the atomic absorption laboratory to read the amount of metal absorption. The amount of metal absorption observed for algae and gammarus by flame method (mg/kg) and for body tissue by graphite furnace method (µg/kg):

$C_r = C_i \times V/m$

C_i= number of the device, V= final volume of the sample (50 mL), m= dry weight of the sample (0.3 or 0.1 g), C_r= final number

One-way analysis of variance (ANOVA) was used to identify significant differences among treatments. To compare the means, Duncan's test was applied at a significance level of 0.05. The statistical analyses were carried out using SPSS 19 and Excel 2010 software. A significant difference was determined with a confidence level of 95% and a p number.

Results

Spirulina algae (type of metal and time)

Chromium metal absorption was more than copper by algae. A significant difference was observed in the absorption of algae between the heavy metals copper and chromium (p<0.01). In the comparison between time intervals, the highest absorption of copper metal by algae was seen in 24 hours; it showed a downward trend over time. The highest absorption of chromium metal was seen in 48 hours; it showed an upward trend over time. Nevertheless, No difference was observed in the absorption of two metals, copper and chromium, by algae (Table 1; $p>0.05$) (Hajilee *et al.,* 2024).

Gammarus (type of metal and time)

In the comparison between heavy metals, the biosorption of copper (Cu) metal was more than that of chromium (Cr) by gammarus. A significant difference was observed in the biosorption of gammarus between heavy metals copper and chromium ($p<0.01$). In the comparison between the periods, the highest absorption of copper and chromium metals by gammarus was seen in 48 hours; it showed an upward trend over time. A difference was observed in the comparison between periods for the absorption of copper and chromium by gammarus (Table 1; $p<0.05$) (Güven *et al*., 1999).

Table 1: Comparison of metal biosorption periods by Spirulina algae and gammarus culture (mg/L).

Time (h)	Algae		Gammarus	
	Cu	Cr	Cu	Cr
0	0.365 ± 0.06^{a}	0.601 ± 0.02^{a}	0.203 ± 0.003^{a}	0.117 ± 0.004^{a}
24	0.582 ± 0.13^{a}	0.791 ± 0.21^{a}	0.541 ± 0.09^{b}	0.398 ± 0.1^{ab}
48	0.501 ± 0.06^{a}	0.836 ± 0.12^{a}	0.613 ± 0.07^{b}	0.462 ± 0.09^{b}
Total	0.483±0.12	0.742±0.15	0.452±0.20	0.325±0.17

Lowercase letters on each column indicate significant difference between the data.

Co-cultivation (algae and gammarus)

Metal type

In general comparison, chromium absorption was observed more than copper metal. A significant difference was found in the absorption of heavy metals between heavy metals ($p<0.05$). In the comparison of algae absorption between heavy metals, the absorption of chromium (Cr) was seen more than copper metal (Cu) A significant difference was observed in the absorption of heavy metals by algae ($p<0.05$). In comparing the absorption of gammarus between heavy metals, the absorption of copper (Cu) was relatively higher than that of chromium metal (Cr). No difference was observed in the absorption of heavy metals by gammarus ($p>0.05$). The highest initial absorption of copper (Cu) and chromium (Cr) metal was in zero hours (under one hour). At this time, A significant difference was seen between the two metals ($p<0.01$). The maximum copper and chromium metals absorption was observed in 24 hours. At this time, there was no significant difference between the two metals ($p>0.05$). The downward course of absorption of copper and chromium metals, mainly algae, was in 48 hours. At this time, there was no significant difference between the two metals ($p>0.05$) (Table 2).

Absorbents

In the general comparison of the biosorption of heavy metals between the adsorbents, the absorption of algae was more than that of gammarus. A significant difference was observed between algae and gammarus ($p<0.01$). The maximum absorption of heavy metals between the adsorbents was to algae at zero (under one hour) and 24 hours. A significant difference was found between gammarus and algae in both periods ($p<0.01$). The relative superiority of metal absorption by algae was in 48 hours. No significant difference was found between Gammarus and algae (p>0.05). The separate comparison of copper (Cu) and chromium (Cr) metals absorption between adsorbents showed absorption algae more than Gammarus. There was a significant difference in metal absorption between algae and Gammarus (Table 2; p<0.01).

Periods

In general comparison, the highest absorption of heavy metals was at the initial time. It showed the maximum absorption of metal in 24 hours by algae and its continuation up to 48 hours by Gammarus. There was no significant difference in the absorption of heavy metals between periods (p>0.05). The maximum absorption of heavy metals by Gammarus was in 48 hours. It showed a significant difference in the absorption of heavy metals by Gammarus between periods (p<0.01). The maximum absorption of heavy metals by algae was in 24 hours. There was no significant difference in the absorption of heavy metals by algae between periods (p>0.05). In separate comparisons of copper and chromium between time intervals, the initial maximum absorption was achieved in less than one hour and the final limit was measured in 24 hours. No significant difference in heavy metals were observed between periods (Table 2; p>0.05).

Table 2: Comparison of periods based on heavy metals and adsorbents in co-cultivation (mg/L).

Time (h)	Cu		Cr	
	Algae	Gammarus	Algae	Gammarus
0	0.3± 0.012[a]	0.1± 0.018[a]	0.51±0.01	0.1±0.02[a]
24	0.51±0.002[b]	0.2± 0.03[b]	0.53±0.07	0.25±0.08[ab]
48	0.35±0.03[a]	0.3±0.017[c]	0.45±0.01	0.32±0.07[b]
Total	0.39±0.1	0.2± 0.09	0.5±0.07	0.23±0.11

Lowercase letters on each column indicate significant difference between the data.

Beluga fish

Absorbent (treatments)

In general comparison, Gammarus had the highest and algae had the lowest accumulation of heavy metals shown in fish tissue. There was no significant difference between absorbent samples in the levels of heavy metals and fish tissues (p>0.05). Algae had the highest absorption, and Gammarus had the highest accumulation of heavy metals among absorbent samples in liver and muscle tissue. There was no significant difference between absorbent samples in fish tissues (p<0.05). Algae had the highest absorption, and Gammarus had the highest concentration of heavy metals among the adsorbent samples in copper and chromium metals. No significant difference was observed between absorbent samples in both metals (Table 3; p<0.05).

Table 3: Comparison of treatments based on heavy metals and fish tissues (mg/L).

Treatments	Muscle		Liver	
	Cu	Cr	Cu	Cr
Algae	0.004±0.001[a]	0.0004±0.00007	0.054±0.01[a]	0.0039±0.0003
Gammarus	0.024±0.004[b]	0.002±0.001	0.305±0.007[c]	0.011±0.0007
Co-culture	0.011±0.002[ab]	0.0013±0.0001	0.161±0.03[b]	0.006±0.002
Control	0.013±0.004[ab]	0.0013±0.0001	0.163±0.004[b]	0.0073±0.003
Total	0.013±0.008	0.0014±0.001	0.171±0.09	0.0071±0.003

Lowercase letters on each column indicate significant difference between the data.

Fish tissues

In general comparison, the amount of accumulation of heavy metals was in the liver more than in muscle tissue. Statistically, there was a significant difference between fish tissues in the accumulation of heavy metals ($p<0.01$). The accumulation of heavy metals for copper and chromium was in the liver more than in muscle tissue. There was a significant difference between the two fish tissues in both metals (Table 3; $p<0.01$). The accumulation in adsorbent samples (algae, Gammarus, co-culture, and control) showed liver more than muscle tissue. Also, algae had the highest absorption, and Gammarus had the highest accumulation of heavy metals among absorbent samples in two fish tissues. There was a significant difference between fish tissues in each absorbent sample (Table 4; $p<0.01$).

Table 4: Comparison of heavy metals and fish body tissue based on different treatments (mg/L).

Tissues	Metals	Algae	Gammarus	Co-culture	Control
Muscle	Cu	0.004±0.001[a]	0.02±0.004[b]	0.01±0.002[a]	0.01±0.005[a]
	Cr	0.0004±0.007[a]	0.002±0.001[a]	0.001±0.0001[a]	0.001±0.001[a]
Liver	Cu	0.05±0.01[b]	0.3±0.007[c]	0.16±0.03[b]	0.16±0.005[b]
	Cr	0.003±0.0003[a]	0.01±0.0007[ab]	0.006±0.002[a]	0.007±0.003[a]

Lowercase letters on each column indicate significant difference between the data.

Type of metal

In general comparison, the amount of accumulation in fish tissues was copper (Cu) higher than chromium metal (Cr). There was a significant difference between copper and chromium metals in fish tissues ($p<0.01$). The accumulation in fish tissues showed copper more than chromium metal. A significant difference was seen between copper and chromium metals in both fish body tissues (Table 3; $p<0.01$). The accumulation in adsorbent samples (algae, Gammarus, co-culture, and control) showed copper more than chromium metal. Also, algae, the lowest, and Gammarus, the highest concentration of heavy metals, showed among the absorbent samples of fish tissue. There was a significant difference between heavy metals in each absorbent sample (Table 4; $p<0.01$).

Comparison of experiments

In the overall comparison, the highest absorption of heavy metals copper (Cu) and chromium (Cr) was observed in algae and the lowest in Gammarus, followed by liver and muscle tissue of fish. There was a significant difference between the samples absorbed in each metal (Fig.1; $p<0.01$).

The amount of absorption of heavy metals in algae culture was higher for chromium than copper metals. It showed its effect in inhibiting heavy metals in the liver and muscle tissue. A significant difference was between absorbed samples ($p<0.01$). The absorption of heavy metals in Gammarus culture was observed to be higher for copper than chromium metal. It showed its effect on the transfer of metals in the liver and then in the muscle tissue. A significant difference was between absorbed samples ($p<0.05$). In co-culture, chromium metal was observed to have the highest metal uptake by algae, and copper metal was observed to have the highest metal accumulation by body tissues. Algae are effective in inhibiting the transfer of metals to Gammarus in co-culture. A significant difference was between absorbed samples (Table 5; $p<0.01$).

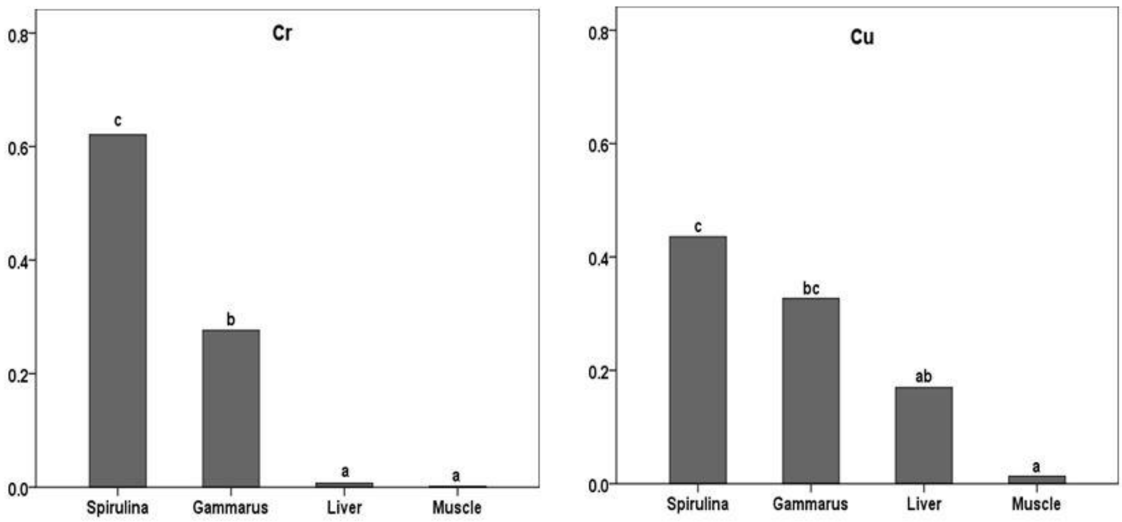

Figure 1: Comparison of copper (Cu) and chromium (Cr) absorption in different samples (mg/L).

Table 5: Comparison of heavy metal absorbing samples based on metal-containing cultures (mg/L).

Samples	Algae culture	Gammarus culture	Co-culture
Algae	0.61 ± 0.19^{b}	-	0.44 ± 0.06^{c}
Gammarus	-	0.38 ± 0.18^{b}	0.21 ± 0.09^{b}
Beluga liver	0.2 ± 0.01^{a}	0.15 ± 0.16^{ab}	0.07 ± 0.08^{ab}
Beluga muscle	0.002 ± 0.001^{a}	0.01 ± 0.01^{a}	0.006 ± 0.005^{a}

Lowercase letters on each column indicate significant difference between the data.

Discussion

In this research, an experiment was to compare the bioabsorption of heavy metals copper and chromium by adsorbents including algae, Gammarus, and co-culture based on time zero (less than one hour), 24 and 48 hours. In the present research, the absorption of copper and chromium heavy metals by adsorbents was observed according to the periods for Algae > Algae in co-culture> Gammarus> Gammarus in co-culture. The initial absorption rate in algae was higher than other adsorbents, but it showed a downward trend over time. Similar to this opinion, Sayadi (2018) reported in their articles that the absorption rate of algae in the first minutes is high, and the metal removal efficiency is almost constant over time (Sayadi and Shekari, 2018). According to Alowitz and Scherer (2002), the reason for the high amount of metal absorption in the first minutes was the presence of empty pores on the surface of the absorbent, which were occupied by chromium and copper metal ions over time, and it decreased the amount of metal absorption (Alowitz and Scherer, 2002).

In another experiment from this research, the initial rate of metal absorption by amphipod specimens was observed to be lower than that of algae, but it showed an upward trend over time. According to the present study, Attaran (2016) introduced time as a function of metal absorption by Gammarus. It concluded that as time increases, this increases the accumulation of absorbed metal (Attaran, 2016). According to Felten (2008) and Vellinger *et al*. (2012), any increase in the amount of metal, whether gratis in the environment or the form of food in the diet, as well as skin absorption, increases the rate of metal absorption in the body of these organisms (Felten *et al.*, 2008; Vellinger *et al*., 2012). According Rainbow (1989) several crustaceans, especially Amphipoda, they concluded that all the metal absorbed by the creature remained in their body without any excretion (Rainbow and White, 1989).

In the present study, maximum bioabsorption by adsorbents (algae and Gammarus) was observed in the first period (early hours). Hu *et al*. (2005) concluded that 90% of chromium were removed in the first minutes. In the beginning, the removal rate was very high, and then it decreased, and at the end of the time, it reached equilibrium (Hu *et al*., 2005). According to Utomo *et al.* (2016), the absorption process of a metal was speedy, which was done within a few minutes and remained constant during the absorbable time (Utomo *et al*., 2016). According to Nourisepehr *et al.* (2016) the speed of metal absorption increases with time, but this increase over time has been almost uniform and has not had a significant effect (Nourisepehr *et al*., 2016). These results mean that when heavy metals enter the water environment in the early times, it will affect the organisms of that area, including algae and gammarus.

In this research, a comparison was made of the biosorption of heavy metals copper and chromium by adsorbents, including algae, Gammarus, and co-culture. According to the present research, absorption of heavy metals in two metals, copper and chromium, by adsorbents was observed respectively for Algae> Algae in co-culture> Gammarus> Gammarus in co-culture. According to the present study, Kanamarlapudi *et al*. (2018) found algae to be better absorbents than other biological absorbents for absorbing metal ions (Kanamarlapudi *et al*., 2018). According to Almomani and Bhosale (2021), microalgae can remove several types of heavy metals from wastewater at the same time (Almomani and Bhosale, 2021). In the articles of Chen *et al.* (2021), Yang *et al.* (2021), and Ahmad *et al.* (2022a) algae could absorb and accumulate significant amounts of heavy metals (Chen *et al*., 2021; Yang *et al*., 2021; Ahmad *et al*., 2022a). According to Putri (2015), the high absorption of algae is considered due to the wide surface and empty spaces in the cell walls (Putri, 2015). According to Mane and

Bhosle (2012), algae protect themselves against the toxicity caused by heavy metals by several mechanisms, including removal, absorption to the cell surface, or intracellular accumulation (Mane and Bhosle, 2012). Also, the competition factor in co-cultivation will have a negative effect on metal absorption by algae.

In this research, chromium absorption was observed more than copper metal in algae, and the maximum absorption of both metals was in 24 hours. Putri (2015) and Sayadi and Shekari (2018) found algae to be an adsorbent with better absorption efficiency for chromium metal, and the reason for that was the large size of copper compared to chrome metal (Putri, 2015; Sayadi and Shekari, 2018). According to Duffus (1980), algae can absorb chromium metal about 4,000 times more than their water environment (Duffus, 1980). The highest chromium absorption has been reported at the lowest level of the food pyramid, namely algae (Avenant-Oldewage and Marx, 2000). According to Mane and Bhosle (2012) and Salmani *et al.* (2018), the microalgae spirulina (*A. platensis*) was found to be algae with high efficiency in removing chromium (Mane and Bhosle, 2012; Salmani *et al*., 2018). In the research of Mane and Bhosle (2012), the absorption of copper was much higher than that of chromium metal by algae (Mane and Bhosle, 2012). According to Mane and Bhosle (2012) and Putri (2015), algae act selectively in the absorption of some metals, and its cause is related to the detoxification mechanism of metals in the cell wall of algae with metal-binding peptides or proteins such as melatonin (Mane and Bhosle, 2012; Putri, 2015).

Contrary to the results of this study, in the other study, the uptake of copper (II) metal was more efficient than chromium (VI) and chromium (III) by the microalgae Spirulina (*A. platensis*) (Gelagutashvili *et al*., 2017). The maximum absorption of both metals by Gammarus was in 48 hours (maximum test time). Higher uptake of copper than chromium by Louisiana crab (*Procambarus clarkii*) has been previously reported (El Qoraychy *et al*., 2015). A study conducted for LC50 tests reported that organisms from the groups Gastropoda, Crustacea, and Oligochaeta were sensitive to copper (Flemming and Trevors, 1989). Research conducted on Gammarus and crustaceans has reported that these organisms cannot regulate the concentration of non-essential metals (such as chromium) in their bodies, and no mechanism has yet been identified to control these metals (Rainbow and White, 1989). According to Khaksar (2014), the concentration of copper metal in Gammarus was higher than that of sediments, which indicates the high capacity of Gammarus to accumulate copper metal (Khaksar, 2014). According to the standards expressed by the European Union, American, and Australian organizations for the accumulation of metals in the body of animals, copper metal has a high concentration in the body of Gammarus (Indonesia ASTM, 2013; Indonesia BSN, 2013; Australian Government, 2015). Hemocyanin is a copper-containing respiratory protein found in arthropods, and about 0.17% of their body weight is copper (Redfield, 1934). Therefore, the amount of copper injected into the culture medium is small compared to the natural levels found in their bodies and can be neglected.

In this research, an experiment was conducted to compare the biosorption of heavy metals copper and chromium by adsorbents including algae (treatment 1), Gammarus (treatment 2), co-culture (treatment 3), and control (treatment 4) in muscle and liver tissue of beluga fish. In the present research, the absorption of heavy metals in fish tissue by adsorbents is seen in the order of Algae > co-culture > control > Gammarus. According to the present study, Khalila (2018) and Abdel-Motleb (2022) found that the injection of spirulina algae (*A. platensis*) through feed to fish causes severe changes in the histopathological reduction caused by heavy metals in the tissues of tilapia fish (*Oreochromis niloticus*). Also, the consumption of algae in fish diets reduces the accumulation of heavy metals in their tissues (Khalila *et al*., 2018; Abdel-Motleb, 2022). Specific strategies to prevent the accumulation of heavy metals in the food chain using high concentrations of microalgae have been reported. Living algae remove heavy metals by two biochemical methods: biosorption (metal uptake at the cell surface) or bioaccumulation (metal uptake within the cell) (Ahmad *et al*., 2022b). It has been reported that microalgae can reduce the toxic effects of heavy metals on fish. They stated that this is due to their ability to sequester heavy metals from water (Kaoud *et al*., 2012).

The amount of chromium and copper absorbed by the adsorbent in fish body tissue was compared with experiments. It was observed that chromium metal was absorbed more by the adsorbents than copper metal in fish body tissue. According to the results of previous experiments, chromium absorption was reported more than copper. Algae may absorb the metal at their cellular level and then excrete them from the fish's body. Chromium uptake was calculated to be higher than copper. Algae may absorb the metal at their cellular level and then excrete copper from the fish. According to the present study, Tran *et al*. (2016) and Ashfaq *et al*. (2017), the algal diet reduced metal accumulation in fish tissues. Metal elimination is also mainly through excretion from the gastrointestinal tract (Tran *et al*., 2016; Ashfaq *et al*., 2017). Fish also excrete chromium in the urine, which has been reported to be due to increased bile secretion after consumption of metal-contaminated food or water (Mertz, 1969). Based on previous experiments, algae performed better in chromium absorption than other absorbers, and this caused the lower amount of chromium observed in the fish tissue.

The accumulation of heavy metals (copper and chromium) in muscle tissue and liver of beluga fish was compared. In the present study, the accumulation of heavy metals was observed to be greater in liver tissue than in muscle. According to the present research, Safahieh *et al*. (2011), Rajeshkumar and Li (2018), and Parvin *et al*. (2019) stated that the concentration of heavy metals in the liver is higher than in other parts of the fish's body, and this tissue is used to investigate the accumulation process. It has been reported that liver, kidney, gill, and muscle tissues have the highest concentrations of heavy metals, respectively (Safahieh *et al*., 2011; Rajeshkumar and Li, 2018; Parvin *et al*., 2019). According to Hashemi *et al*. (2016), there is a significant difference in the amount of metal accumulation between liver and muscle tissues, and the amount of metal in muscle tissue is meager (Hashemi *et al*., 2016). According to Tayel *et al*. (2018), the liver tissue plays a vital role in the detoxification and storage of heavy metals through the blood that comes from the intestine, and this shows that it is the primary tissue in receiving heavy metals through the consumption of metal-contaminated food (Tayel *et al*., 2018). In several other studies, heavy metal concentrations were higher in the liver than in muscle tissue (Canli and Atli, 2003; El-Shaer and Alabssawy, 2019). Yilmaz (2003) reported that muscle tissue has the lowest accumulation of heavy metals among fish tissues. Also, this tissue often does not reflect increased metal accumulation when exposed to high levels of heavy metals in the external environment (Yilmaz, 2003).

In the present research, algae had the lowest, and Gammarus had the highest concentration of heavy metals in fish tissues. According to the current research, in the Al-Weher (2008) article, crustaceans and mollusks store metals and other pollutants, and fish transfer heavy metals to their body while feeding on these organisms (Al-Weher, 2008). According to Yabanli *et al.* (2014) and Gharedaashi *et al.* (2013) on the food pyramid, Gammarus are considered primary consumers for juvenile fish such as white fish (kutum), mullet, and sturgeon, and they were considered this as a warning sign for the transfer and accumulation of heavy metals in the body of the fish (Gharedaashi *et al*., 2013; Yabanli *et al*., 2014). According o Mohanty *et al.* (2009) and Shuhaimi -Othman *et al*. (2010), the contamination of Gammarus with heavy metals is the result of the transfer of pollution through feeding to fish and then to humans (Mohanty *et al*., 2009; Shuhaimi-Othman *et al*., 2010). In the present study, the accumulation of copper was higher than chromium metal in fish tissues. The reason for this could be that algae absorb copper more than chromium. Gammarus accumulates in fish body tissue due to its high amount of copper metal in fish feeding. Another reason could be the necessity of copper absorption in fish body tissues. According to the present study, Rajeshkumar and Li (2018) and Zidan and El-Zaeem (2020) reported the liver as the tissue with the highest copper accumulation compared to other fish body tissues (Rajeshkumar and Li, 2018; Zidan and El-Zaeem, 2020). In researching the effect of heavy metals on the white muscle of the Caspian Sea fish, Mousavi Moghadam *et al.* (2018) stated that copper metal accumulates twice as much as chromium metal in the muscle tissue (Mousavi Moghadam *et al*., 2018). It has been reported that the higher accumulation rate of copper than chromium is due to its necessity for homeostatic regulation in all living organisms (Goyer and Clarkson, 1996). Also, according to Bennani *et al*. (1996) and Duffus (1980), copper is a trace element necessary for the natural growth and metabolism of plants, animals, and most microorganisms (Duffus, 1980; Bennani *et al*., 1996). Miller *et al.* (1993) found a direct relationship between copper metal in liver tissue and fish diet (Miller *et al*., 1993). It is considered the high affinity of metallothioneins from copper and other heavy metals in liver tissue (Carpene and Vašák, 1989; Klerks and Levinton, 1989).

According to Doudoroff and Katz (1953), hexavalent chromium metal is identified as toxicological different from most heavy metals. Hexavalent chromium metal can penetrate the gill membranes by passive diffusion and concentrate at higher levels in different organs and tissues (Doudoroff and Katz, 1953). Also, Avenant-Oldewage and Marx (2000) stated in their article that the absorption of chromium metal is not transferred through the food chain. In their research, they experimentally transferred chromium metal directly to the intestine, and the result of this action was the immediate removal of chromium without accumulation in other tissues. They identified gills as the primary source of chromium absorption (Avenant-Oldewage and Marx, 2000). Buhler *et al.* (1977) introduced the high concentration of chromium metal accumulation in the gills and liver due to the slow rate of removal of chromium metal from the body tissue of the fish (Buhler *et al*., 1977). According to Oldewage (2000), some fishes are considered capable of accumulating high levels of chromium, nearly 100 times the concentration in water (Oldewage and Marx, 2000). In a previous article by Duffus, he discussed the dangerous accumulation of chromium in many living organisms (Duffus, 1980).

Conclusions

There are two possibilities for the effect of algae on fish body tissue. In the first possibility, the algae absorb the metals from the environment, and during consumption in the fish diet, it is not transferred to the fish body. Finally, it is excreted from the fish body (the possibility of this research). In the second possibility, the algae absorb the metals from the fish's body and finally, the algae with metals are removed from the fish's body (need more time). Also, there are two possibilities for the effect of Gammarus on fish body tissue. In the first possibility, Gammarus absorbs the maximum metals from the environment and finally transfers all the absorbed metals as food to the fish (the possibility of this research). Another possibility is that fish digest and absorb Gammarus better than algae, thereby providing conditions for maximum metal uptake into fish tissue (this would require experiments on the digestion process and its effect on metal uptake). As a result, if algae are not present in an ecosystem, it causes the direct transfer of heavy metals to tiny organisms such as Gammarus. The consumption of these organisms by fish and, finally humans, it will have harmful effects on health in the long term. Therefore, algae cannot be directly food for fish in nature, but in the water ecosystem chain, they refine heavy metals for fish by directly absorbing metal from the environment and also by consuming algae by tiny organisms such as Gammarus as mediators.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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