Removal of Cadmium, Copper and Nickel in *Thynnichthys thynnoides* using Chelation Technique

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Abstract. *Thynnichthys thynnoides* is a freshwater fish that widely consumed by Malaysian society. Due to increasing in industrial activities, the presence of heavy metals in *T. thynnoides* has become a risk that can affect human health and environment. This research was studied on efficiency of chelation technique to remove heavy metals cadmium (Cd), copper (Cu) and nickel (Ni) from *T. thynnoides*. The treatment for chelation technique was observed under optimized conditions of trisodium citrate concentration of 400 ppm for five hours treatment at temperature of 29.50±0.50°C. Initially the concentration of Cd, Cu and Ni in *T. thynnoides* were 0.74 \(\mu\)g/g, 3.18 \(\mu\)g/g and 2.95 \(\mu\)g/g and successfully removed to 35.80%, 65.22% and 61.17% respectively.

1. Introduction

The universal problem is the environmental pollution and most important pollutants are the heavy metals in aquatic network because of their toxicity, accumulation and bio-magnification by marine creatures. The pollution has mainly been caused by industrial processes, industrial waste, mining industrial and agriculture. The heavy metal contamination of aquatic ecosystems has drawn the attention of many researchers. Heavy metals may accumulate in aquatic species, enter the food chain, and cause serious harm to human health when the contamination content and exposure are significant [1]. Fish can accumulate heavy metals in their tissues by absorption along gill surface and kidney, liver, and gut tract wall to higher levels than environmental concentration [2].

Fish can transport major dietary sources of arsenic and mercury to humans because of their higher uptake mechanism in their tissues towards these elements. The scheme of accumulation regarding heavy metals in the liver, gills and flesh tissues was found maximum for Cd and Pb respectively [3]. Previous studied have reported that several methods to remove toxic elements from food such as cooking [4], bioremediation [5] and depuration method [6]. However, these method are less effective...
and consume longer time for treatment. Recent study done by Abdullah et al (2020) [7] showed that catalytic chelation treatment is considered as promising and effective ways to treat heavy metal in fish. By using chelating agents, they are capable to bind with metal ions to form a ring complex structure. The chelating agents can act as ligands and they are able to make one or more bonds to the metal and this may distinguish their denticity such as monodentate, bidentate and polydentate. T. thynnoides which is known as ikan loma was obtained from Sungai Rui, Perak which contains high level of heavy metals due to mining activity. The level of heavy metals in Sungai Rui is above the prescribed limit based on research conducted by Orji et al (2013) [8]. This cause T. thynnoides often be exposed to high heavy metals from surrounding environment and cause consumer to be hesitant about the safety aspect of T. thynnoides for consumption. Unfortunately, there is no comprehensively studied related to the treatment developing in order to reduce the amount of heavy metal accumulated from T. thynnoides. Therefore, the purposes of this study were to determine amount of selected heavy metals (Cd, Cu and Ni) contain in T. thynnoides and also to remove the heavy metals by using several types of chelating agents via chelation technique.

2. Experimental

2.1 Sampling of T. thynnoides

Thynnichthys thynnoides were purchased from market and placed in the freezer for further analysis. The location of the samples was from Sungai Rui, Perak, Malaysia.

2.2 Treatment of T. thynnoides

The sample of T. thynnoides was washed with distilled water before the treatment. Only the flesh of the sample was taken and weighed at 1.0 g for each sample. A sack was used to hold T. thynnoides in 250 mL of trisodium citrate dihydrate solution in a beaker with the concentration of 400 ppm for 1 hour at ambient temperature (32.50±0.50°C). The sample was put in the chelating agent solution and was stirred for an hour. The treated sample then was washed with distilled water before proceed to digestion process.

2.3 Digestion of T. thynnoides

The untreated and treated samples were digested by using 8.0 mL of 65% nitric acid, HNO₃. They were digested on hot plate until fully digested and obtained a clear solution. Then, the samples were cooled and filtered using Whatman No 42-filter paper. The digested samples were diluted to 25 mL with ultra-pure water. The heavy metals such as Ni, Cd and Cu in the prepared samples were determined by using Flame Atomic Absorption Spectroscopy (FAAS). The mass of undigested sample on the dried filter paper was weighed to get the actual weight of the samples.

2.4 Analysis of Heavy Metals in T. thynnoides

The concentration of heavy metals in samples were carried out using flame atomic absorption spectroscopy (FAAS) for Cu, Cd and Ni determination. The initial concentration of heavy metals in samples was also carried out using FAAS to know the removal percentage of heavy metal after treatment.

3. Result and discussion

3.1 Heavy Metals Concentration in T. thynnoides

The initial concentrations of heavy metals in T. thynnoides and the permissible limits of Malaysia Food Regulation (MFR) and World Health Organization (WHO) for each heavy metals are presented in Table 1 [9, 10, 11]. The results show that the concentration of Ni in T. thynnoides exceeded the acceptable limit stated by MFR and WHO. The average initial concentration of Ni was 2.954 ± 0.200 µg/g which clearly higher than the permissible limit of MFR and WHO. The increasing in the
concentration of Ni was due to the location of the sample where it near mining spot and this leads to disposal of heavy metals into the river. For Cd, the concentration was higher than the permissible limits state by WHO which was $0.738 \pm 0.003 \ \mu g/g$. The capacity of accumulating metals may be different among the aquatic organisms because it depends on their filtering activity and their position in water column as explained by Abdullah et al. (2020) [7].

**Table 1.** Initial concentration of heavy metals in *T. thynnoides* and the permissible limits of MFR and WHO [9, 10, 11]

|               | Cd (µg/g)          | Cu (µg/g)          | Ni (µg/g)          |
|---------------|--------------------|--------------------|--------------------|
| Initial       | $0.738 \pm 0.003$  | $3.183 \pm 0.400$  | $2.954 \pm 0.200$  |
| Permissible   | MFR                | WHO                |                    |
| limits:       | 1                  | 30                 | 1                  |
| WHO           | 0.2                | 10                 | 0.5-0.6            |

### 3.2 Heavy Metals Concentration in *T. thynnoides*

*T. thynnoides* was treated with four different chelating agents namely trisodium citrate (TSC), sodium acetate (SAT), potassium acetate (PA) and disodium oxalate (DSO) with concentration of 400 ppm each for 1 hour at ambient temperature, $32.50 \pm 0.50 \ ^\circ\mathrm{C}$ in order to see the potential chelating agents on the removal percentage of heavy metals in the samples. Figure 1 shows the removal percentage of heavy metals after treated with different chelating agents. From the results, the best two chelating agents that successfully remove high amount of heavy metals in *T. thynnoides* are sodium acetate (SAT) and trisodium citrate (TSC) for one hour treatment at ambient temperature. SAT had successfully removed heavy metals Cd, Cu and Ni by 73.04%, 50.46% and 80.94% respectively while TSC also successfully removed each metal by 66.58%, 49.63% and 19.26% respectively. The high removal of heavy metal noted in TSC was attributed to the high stability of the ring structured metal-citrate complex produced from chelation which can form tridentate ligands [7].

![Figure 1](image1.png)

**Figure 1.** The percentage removal of heavy metals from *T. thynnoides* after treated with different chelating agents with 400 ppm each for one hour at ambient temperature ($32.50 \pm 0.50 \ ^\circ\mathrm{C}$)

### 3.3 Effect of Different Concentration of Chelating Agents

In order to determine the best chelating agents with the best concentration to successfully remove the heavy metals in *T. thynnoides*, the fishes were treated with sodium acetate and trisodium citrate in five different concentrations (200 to 600 ppm) for one hour at ambient temperature. Figure 2 and 3
show the removal percentage of Cd, Cu and Ni in *T. thynnoides* after treatment with sodium acetate and trisodium citrate with different concentration (200 to 600 ppm). According to Figure 2, sodium acetate with concentration of 200 ppm to 600 ppm were successfully remove heavy metals Cd and Cu. However, Ni was not successfully removed by sodium acetate for those concentrations that sample had treated with.

![Figure 2](image1.png)

**Figure 2.** The percentage removal of heavy metals from *T.thynnoides* after treatment using sodium acetate (200 ppm - 600 ppm) with stirring for 1 hour at ambient temperature

Figure 3 shows the removal percentage of heavy metals when treated with trisodium citrate with concentration of 400 ppm gave the best results where Cd, Cu and Ni were successfully removed by 66.58%, 49.63% and 19.26% respectively. This means that the concentration of 400 ppm of trisodium citrate is the optimum concentration in removing the heavy metals from *T.thynnoides*. The declined of heavy metal removal is possibly due to Le Chatelier’s principle which favor reaction to the right and forming the citric acid when citrate ion production was increased [7]. The reversible reaction decrease the formation of citrate ion as the active site to excrete the heavy metal in *T.thynnoides*.

![Figure 3](image2.png)

**Figure 3.** The percentage removal of heavy metals from *T.thynnoides* after treatment using trisodium citrate (200 ppm - 600 ppm) with stirring for 1 hour at ambient temperature

### 3.4 Effect of Treatment Time

Further investigating was done in the treatment time, which is varied to one, three and five hours which were treated with 400 ppm of trisodium citrate. The period for chelating agent to excrete heavy
metals ion is important because it will affect the percentage removal of heavy metals. Results showed that the removal percentage of heavy metals increased as the time increased as shown in Figure 4. The highest percentage removal of heavy metals was shown by five hours treatment of time (Cd: 41.13%, Cu: 67.09%, Ni: 65.36%). This is most probably that trisodium citrate is allowed to excrete more heavy metals from *T. thynnoides* within a longer period of time. As reported on the previous study, longer treatment duration would increase the frequency of chelating agent to reach out the metal ions to form complexes thus increased the percentage removal of heavy metals [12].

![Figure 4](image)

**Figure 4.** The percentage removal of heavy metals in *T. thynnoides* after treatment at different reaction times using trisodium citrate (400 ppm) with stirring at ambient temperature

### 3.5 Effect of Treatment Temperature

Treatment of temperature is a crucial factor that can affect the determination of percentage removal of heavy metals from *T. thynnoides* since it related with the habitat and environment of the sample. The percentage removal of heavy metals in *T. thynnoides* was studied based on three different treatment temperatures which were 29.50 ± 0.50 °C, 32.50 ± 0.50 °C and 36.50 ± 0.50 °C as the results shown in Figure 5. The trend for removal of heavy metals in *T. thynnoides* decreased from 29.50 ± 0.50 °C until 36.50 ± 0.50 °C. Based on the finding, 29.50 ± 0.50 °C gave the highest percentage removal of heavy metals which were 41.20 % for Cd, 53.73 % for Cu and 66.58 % for Ni. This means that 29.50 ± 0.50 °C is probably the same temperature as the habitat and the environment of the tiny scale barb fishes can survive. On the other hand, the decreased in percentage removal of heavy metal when temperature increased from 29.50 ± 0.50 °C due to high mucus gland from *T. thynnoides* which cover the flesh of the fish surface and prevent chelating agent to remove the heavy metals [5, 12].
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Figure 5. The percentage removal of heavy metals in *T. thynnoides* after treatment at different temperatures (29.50±0.50°C, 32.50±0.50°C, 36.50±0.50°C) using trisodium citrate (400 ppm) with stirring for 1 hour

3.6 Summary on Chelating Treatment

The chelating treatment of *T. thynnoides* was carried out using edible chelating agents namely trisodium citrate. The different parameter such as treatment time and temperature were investigated in order to choose the best condition for the chelation treatment of *T. thynnoides*. The results revealed that the heavy metals in sample was successfully removed by using chelation method under certain parameters. From the data obtained, the best condition of trisodium citrate was at 400 ppm concentration of chelating agent solution with 5 hours treatment time at 29.50 ± 0.50 °C. The best condition among the parameters were obtained based on the higher percentage removal of heavy metals that had been extracted from the *T. thynnoides* and gave final concentration of heavy metals (Cd: 0.47 μg/g, Cu: 1.10 μg/g, Ni: 1.74 μg/g). The results show that the chelation technique able to reduce the heavy metal in *T. thynnoides*. However the concentrations of Ni were still above the permissible limits of Malaysia Food Regulation (MFR) and World Health Organization (WHO).

Figure 6. The concentration of heavy metals in *T. thynnoides* before chelation treatment and after chelation treatment under the optimum conditions (trisodium citrate (400 ppm), 5 hours, 29.50 ± 0.50 °C)

4. Conclusion

The chelation technique was found to be potential method to remove the heavy metals in *T. thynnoides*. The optimum condition of treatment was obtained by having 400 ppm trisodium citrate, five hours treatment time and 29.50 ± 0.50 °C treatment temperature. Present study illustrates that the efficiency of the studied chelating agents in the order of trisodium citrate > sodium acetate > potassium acetate >
disodium oxalate. The trisodium citrate (400 ppm) gave the highest percentage removal of heavy metals (Cd: 66.58%, Cu: 49.63% and Ni: 19.26%). To conclude, the chelation technique has the ability to remove heavy metals from T. thynnoides. Nevertheless, for metal Ni, its concentration is still not achieve the permissible limits stated by MFR and WHO.

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