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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The effect of mercury, copper, and zinc on paraoxonase (PON) enzyme activity in Bonito (*Sarda sarda*) fish

Palamut *(Sarda sarda)* balığında paraoksonaz (PON) enzim aktivitesi üzerine cıva, bakır ve çinkonun etkisi

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Abstract: Since heavy metal dirtiness, which we frequently encounter in environmental pollution causes harmful effects on the organism through biochemical enzyme reactions, in this study, the effects of mercury (Hg^{+2}), copper (Cu^{+2}), and zinc (Zn^{+2}) heavy metal ions, which are common in environmental pollution, on PON (paraoxonase) enzyme activity in muscle tissue of bonito (*Sarda sarda*) were investigated. In the study, 25 bonito (*S. sarda*) fish muscle tissues freshly obtained from the Samsun region sea were used. The changes in PON enzyme activity were determined by adding different volumes of heavy metal solutions. PON enzyme activities of Hg^{+2} heavy metal ion used in different volumes were calculated as 30.9383 U/mLdak, 29.0598 U/mLdak, 26.3799 U/mLdak, 23.9443 U/mLdak, 20.6725 U/mLdak, PON enzyme activities of Cu^{+2} heavy metal ion used in different volumes were calculated as 19.7949 U/mLdak, 19.4807 U/mLdak 19.1864 U/mLdak, 19.1200 U/mLdak, 18.9037 U/mLdak and PON enzyme activities of Zn^{+2} heavy metal ion used in different volumes were calculated as 23.8305 U/mLdak, 23.0781 U/mLdak, 22.9073 U/mLdak, 22.4324 U/mLdak, 21.8159 U/mLdak. As a result of these obtained data, activity (%) values were calculated and activity (%) graphs were drawn. As a result of the study, it was determined that increasing concentrations of Cu^{+2} and Zn^{+2} heavy metal ions caused a decrease in PON enzyme activity, but there was no statistically significant difference between the different concentrations used. It was determined that increasing concentrations of Hg^{+2} heavy metal ion inhibited the PON enzyme activity, caused a statistically significant decrease between the activities depending on the different concentrations used ($p < 0.05$).

Keywords: Bonito (*Sarda sarda*), heavy metals, copper (Cu), mercury (Hg), zinc (Zn), paraoxonase (PON)

Öz: Çevre kirliliğinde sık rastlanan ağır metal kirlilikleri, biyokimyasal enzim reaksiyonları üzerinden organizmada zararlı etkiler meydana getirdiği için bu çalışmada çevre kirliliğinde sık rastlanan cıva (Hg^{+2}), bakır (Cu^{+2}) ve çinko (Zn^{+2}) ağır metal iyonlarının palamut (*Sarda sarda*) balığı kas dokusunda bulunan PON (paraoksonaz) aktivitesi üzerine etkileri araştırılmıştır. Çalışmada Samsun bölgesi denizinden taze olarak temin edilen 25 adet palamut balığının kas dokuları kullanıldı. Ağır metal çözeltilerinden farklı hacimlerde eklenerek PON enzim aktivitesindeki değişiklikler tayin edildi. Farklı hacimlerde kullanılan Hg^{+2} ağır metal iyonunun PON enzim aktiviteleri 30,9383 U/mLdak, 29,0598 U/mLdak, 26,3799 U/mLdak, 23,9443 U/mLdak, 20,6725 U/mLdak olarak hesaplandı, farklı hacimlerde kullanılan Cu^{+2} ağır metal iyonunun PON enzim aktiviteleri 19,7949 U/mLdak, 19,4807 U/mLdak 19,1864 U/mLdak, 19,1200 U/mLdak, 18,9037 U/mLdak olarak hesaplandı ve farklı hacimlerde kullanılan Zn^{+2} ağır metal iyonunun PON enzim aktiviteleri 23,8305 U/mLdak, 23,0781 U/mLdak, 22,9073 U/mLdak, 22,4324 U/mLdak, 21,8159 U/mLdak olarak hesaplandı. Elde edilen bu veriler sonucunda % aktivite değerleri hesaplanarak % aktivite grafikleri çizildi. Çalışma sonucunda Cu^{+2} ve Zn^{+2} ağır metal iyonlarının artan derişimlerinin enzim aktivitesinde azalmaya neden olduğu fakat kullanılan farklı derişimler arasında istatistiksel olarak anlamlı bir fark olmadığı belirlendi. Hg^{+2} ağır metal iyonun artan derişimlerinin PON enzim aktivitesini inhibe ettiği, kullanılan farklı derişimlere bağlı olarak aktiviteler arasında istatistiksel olarak anlamlı bir azalmaya neden olduğu belirlendi ($p < 0.05$).

Anahtar kelimeler: Palamut (*Sarda sarda*), ağır metal, bakır (Cu), cıva (Hg), çinko (Zn), paraoksonaz (PON)

INTRODUCTION

Paraoxonase (PON) is a serum esterase with arylesterase and paraoxonase activities (aryldialkylphosphatase; E.C.3.1.8.1), which is expressed from the liver and able to hydrolyze paraoxone, which the active metabolite of parathion (Primo-Parma *et al*., 1996; Memişoğulları and Orhan, 2010). The paraoxonase enzyme hydrolyzes toxic oxon metabolites of insecticides such as chlorpyrifos, parathion, and diazinon (Mackness *et al*., 1996), OP (organophosphate) nerve agents such as sarin, tabun, and soman (Broomfield and Ford, 1991; Baillie *et al*., 1993), and ester substrates such as phenylacetate (Eckerson *et al*., 1983; Sorenson *et al*., 1995). The gene which is responsible for PON activity has three members and is called PON1, PON2, PON3 (Primo-Parma *et al*., 1996). After PON1 and PON3 are synthesized in the liver, they are delivered to the blood, the plasma is transported together with HDL (high density lipoprotein). PON2, on the other hand, is not found in serum and synthesized in many different tissues (*Teiber et al*., 2007). It has been hypothesized that the receptor that provides the relationship between PON and HDL is scavenger

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receptor B1 (SR-B1). It has been reported that this receptor HDL binding to the membrane of the cell and providing material exchange between the cell and lipoproteins and that PON is synthesized abundantly from the liver (Deakin and James, 2004).

There are many defense mechanisms to prevent the formation of reactive oxygen species (ROT) in living and the damage which is caused (Prior and Cao, 1999). One of them is the PON enzyme (Costa *et al*., 1999; La Du *et al*., 1999). The paraoxonase enzyme exists depending on HDL and has a protective effect against the formation of lipid peroxides by oxidation of LDL (low density lipoprotein) (Mackness *et al*., 2001; Carey *et al*., 2005). It shows an antioxidant property by limiting lipid oxidation in LDL thanks to HDL, which is dependent on the structure of this enzyme (Elana *et al*., 2006).

Heavy metals are defined as metals which have toxic or poisoner effects even at low concentration and they have a density of more than 5 g/cm^3 in terms of physical properties (Kahvecioğlu *et al*., 2003; Seven *et al*., 2018). These metals, even if found in small quantities, accumulate in the body of aquatic organisms at increasing rates and reach levels that will do a toxic effect (Ikuta, 1985). Although some metals, like copper and iron, are necessary for life at certain levels, metals in the state of mercury and lead can be toxic even at trace levels (Hu, 2002). Heavy metals are taken from the external environment by fish and are transported to tissues and organs through the blood tract by binding with carrier proteins, reaching high concentrations holding by metal-retaining proteins in tissues (Kaptan, 2014). Metals of physiological importance are stored from metals that can be excreted out of the body by participating in different metabolic processes in the living structure, but if these are toxic metals, these can disrupt enzyme structures (Yazkan *et al*., 2004). Mercury (Hg) levels in the tissues of fish found in these regions have also increased due to the increase of industrial enterprises on the sea coast (Vural, 1993). Hg is highly toxic to fish, even in very low amounts. Organic Hg compounds usually enter the living body through food, causing chronic toxic disorders after accumulating (Dökmeci, 2001; Kaya and Akar, 2002). Copper (Cu) is taken from the environment by fish usually through the gill and food, its excretion outside the body is through feces and urine (Sağmanlıgil, 1994; Cicik, 2003). Excess copper in the water accumulates in the gill tissue within a short period, as well as reaching higher concentrations in the liver tissue depending on the duration of action (Kalay and Erdem,1995). Besides, the Cu metal increases the outflow of sodium ions by fracking the energy-bound sodium/potassium pump in the gill tissue (Cicik, 2003; Coppock and Nation, 2007). In addition to the fact that the mechanism of action of zinc (Zn) metal in fish is not fully known, it has been found to cause damage to gill tissue (Cicik, 2003; Kaya and Akar, 2002; Ağcasulu, 2007).

Heavy metals, which reach from the first step to the last step of the food chain and showing accumulation, in bonito (*S. sarda* Bloch, 1793) fish, which is of great importance in protein-rich human nutrition, the accumulation in increasing concentrations shows a toxic effect and negatively affects human health. To find a solution to such a problem, further research of some metals is needed. How the activity of the PON enzyme with physiological function in metabolism, is affected by some heavy metals is extremely important for animal and environmental health. This study has investigated the effects of mercury (Hg^{+2}), copper (Cu^{+2}), and zinc (Zn^{+2}) heavy metal ions on PON enzyme activity in muscle tissue of bonito (*S. sarda*) fish.

MATERIAL AND METHODS

A total of 25 bonito (*S. sarda*) fish, which an average weight of 600-800 g and a length of 40 to 45 cm consisted of the material of the study. In the study, muscle tissue of bonito (*S. sarda*) fish freshly taken from the sea of Samsun region in October, which is the seasonal season of this fish species, and brought to the laboratory environment by the cold chain in a short time was used. Tissue samples taken from bonito (*S. sarda*) were weighed 0.3 g and taken into dry centrifuge tubes, then 1.5 mL Tris-HCl buffer was added to them and homogenized. The homogenized tissues were centrifuged in a cooled centrifuge at +4 °C and 3000 rmp speed for 30 minutes and supernatants were separated. Then, the separated supernatants were used on the same day.

PON enzyme activity determination method recommended by Gülcü and Gürsu (2003), was used for PON enzyme activity determination. In activity determination, 50 µL Tris-HCl buffer, 50 µL substrate (calcium chloride+paraoxone) solution were added to cuvettes and the value at 405 nm absorbance was read in ELISA. 50 µL supernatant solution was added to the measured cuvettes and the change occurring in the absorbance at 405 nm, at 37 °C in ELISA it was read in 30 seconds. In this way, the enzymatic conversion speed of paraoxone to p-nitrophenol was defined as PON activity (1 U/L).

The activity measurement in the paraoxonase enzyme of the mercury ıı chloride ($HgCl_2$) solution: In paraoxonase enzyme activity determination, 50 µL tris-HCL buffer, 50 µL substrate (calcium chloride+paraoxone) solution, and 50 µL supernatant solution were added to the cuvettes, and the value at 37 °C and 405 nm absorbance in ELISA it was read in 30 seconds. Then, the change in enzyme activity was determined, after adding different volumes 10 µL, 20 µL, 30 µL, 40 µL, 50 µL 0.001 M $HgCl_2$ solution to the measuring cuvette. The enzyme activity determined in the environment without inhibitor was used as 100% activity.

The activity measurement in the paraoxonase enzyme of the copper ıı chloride ($CuCl_2$) solution: In paraoxonase enzyme activity determination, 50 µL tris-HCL buffer, 50 µL substrate (calcium chloride+paraoxone) solution, and 50 µL supernatant solution was added to the cuvettes and the value at 37 °C and 405 nm absorbance in ELISA it was read in 30 seconds. Then, the change in enzyme activity was

determined, after adding different volumes 10 µL, 20 µL, 30 µL, 40 µL, 50 µL 0.001 M $CuCl_2$ solution to the measuring cuvette. The enzyme activity determined in the environment without inhibitor was used as 100% activity.

The activity measurement in the paraoxonase enzyme of the zinc ıı chloride ($ZnCl_2$) solution: In paraoxonase enzyme activity determination, 50 µL tris-HCL buffer, 50 µL substrate (calcium chloride+paraoxone) solution, and 50 µL supernatant solution were added to the cuvettes, and the value at 37 °C and 405 nm absorbance in ELISA it was read in 30 seconds. Then, the change in enzyme activity was determined, after adding different volumes 10 µL, 20 µL, 30 µL, 40 µL, 50 µL 0.001 M $ZnCl_2$ solution to the measuring cuvette. The enzyme activity determined in the environment without inhibitor was used as 100% activity.

Measurement of the total oxidant capacity (TOC): The TOC was measured by a colorimetric method based on the cumulatively oxidize of ferrous ion to the ferric ion of oxidant molecules present in the supernatant (Erel, 2004, 2005). Calculations were made according to the formula in the procedure.

Measurement of the total antioxidant capacity (TAC): The TAC was measured by the method of decolorization of the colored radical in proportion to the total concentration of the antioxidant molecules by reducing dark blue-green colored ABTS cationic radical of all antioxidant molecules present in the supernatant (Erel, 2004, 2005). Calculations were made according to the formula in the procedure.

Calculation of the oxidative stress ındex (OSI): The OSI was obtained by calculating from the Total oxidant capacity (µmol H_2O_2Equiv/L)/Total antioxidant capacity (mmol TroloxEquiv/L) × 10 formulation.

SPSS statistical (version 22) package program was used for statistical analysis. The analysis of the samples was done with a One-Way Analysis of Variance (One-Way Anova). While the difference between the samples was not significant for $p>0.05$ value, the difference was regarded to be significant for $p<0.05$ value. For the samples with a value of $p<0.05$, the homogeneity of the samples was examined using the homogeneity of variance test, and the samples with a significant difference between them were compared with the Tukey test.

RESULTS

The enzyme activity table and graph were drawn by repeating the measurements made by taking different volumes from $CuCl_2$, $HgCl_2$, and $ZnCl_2$ solutions and studying in duplicate and calculating the average of the results.

Paraoxonase enzyme activity (%) values obtained by using $HgCl_2$ solutions of different concentrations (0.6×10^{-4} M, 1.1×10^{-4} M, 1.6×10^{-4} M, 2.1×10^{-4} M, 2.5×10^{-4} M) were given in Table 1.

Table 1. Activity (%) values of paraoxonase enzyme determined in $HgCl_2$ environment

Heavy metal	Tris-HCl Buffer (µL)	Supernatant Solution Volume (µL)	Substrate Solution Volume (µL)	Metal Solution Volume (µL)	Metal Solution concentration ($1x10^{-4}$ M)	ΔOD (405nm)	Activity (U/mL dak)	Activity (%)
Hg	50	50	50	---	---	1.1822	19.3910	100.00
				10	0.6	1.8862	30.9383	159.55
				20	1.1	1.7717	29.0598	149.86
				30	1.6	1.6082	26.3799	136.04
				40	2.1	1.4598	23.9443	123.48
				50	2.5	1.2603	20.6725	106.61

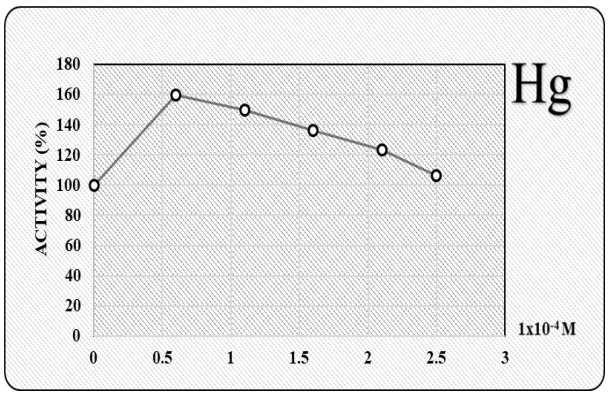

Figure 1. Activity determination for paraoxonase enzyme in $HgCl_2$ environment

The solution concentrations and activity (%) graph specified in Table 1 for paraoxonase enzyme in $HgCl_2$ environment were given in Figure 1.

After determining enzyme activity, PON enzyme activity was calculated as 159.55% when added a 10 µL-0.6×10^{-4} M $HgCl_2$ solution to cuvettes, while enzyme activity decreased as a result of increased concentrations and was calculated as 106.61% when added a 50 µL-2.5×10^{-4} M $HgCl_2$ solution. When the activity (%) graph was examined as a result of the measurements made, it was determined that Hg^{+2} heavy metal ion caused a decrease in paraoxonase enzyme activity ($p<0.05$). Paraoxonase enzyme activity (%) values obtained by using $CuCl_2$ solutions of different concentrations (0.6×10^{-4} M, 1.1×10^{-4} M, 1.6×10^{-4} M, 2.1×10^{-4} M, 2.5×10^{-4} M) were given in Table 2.

Table 2. Activity (%) values of paraoxonase enzyme determined in $CuCl_2$ environment

Heavy Metal	Tris- HCl Buffer (µL)	Supernatant Solution Volume (µL)	Substrate Solution Volume (µL)	Metal Solution Volume (µL)	Metal Solution Concentration ($1x10^{-4}$ M)	ΔOD (405nm)	Activity (U/mL dak)	Activity (%)
Cu	50	50	50	---	---	1.0730	17.5997	100.00
				10	0.6	1.2068	19.7949	112.49
				20	1.1	1.1877	19.4807	110.69
				30	1.6	1.1697	19.1864	109.02
				40	2.1	1.1657	19.1200	108.64
				50	2.5	1.1525	18.9037	107.41

The solution concentrations and activity (%) graph specified in Table 2 for paraoxonase enzyme in $CuCl_2$ environment were given in Figure 2.

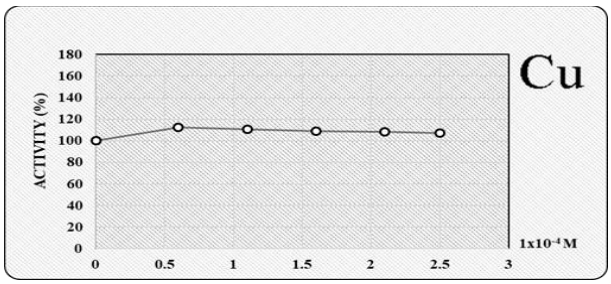

Figure 2. Activity determination for paraoxonase enzyme in $CuCl_2$ environment

After determining enzyme activity, PON enzyme activity was calculated as 112.49% when added a 10 µL-0.6 × 10^{-4} M $CuCl_2$ solution to cuvettes, while enzyme activity decreased as a result of increased concentrations and was calculated as 107.41% when added a 50 µL-2.5 × 10^{-4} M $CuCl_2$ solution. When the activity (%) graph was examined as a result of the measurements made, it was determined that Cu^{+2} heavy metal ion caused a decrease in paraoxonase enzyme activity but there was no statistically significant difference ($p>0.05$).

Paraoxonase enzyme activity (%) values obtained by using $ZnCl_2$ solutions of different concentrations (0.6 × 10^{-4} M, 1.1 × 10^{-4} M, 1.6 × 10^{-4} M, 2.1 × 10^{-4} M, 2.5 × 10^{-4} M) were given in Table 3.

Table 3. Activity (%) values of paraoxonase enzyme determined in $ZnCl_2$ environment

Heavy Metal	Tris- HCl Buffer (µL)	Supernatant Solution Volume (µL)	Substrate Solution Volume (µL)	Metal Solution Volume (µL)	Metal Solution Concentration ($1x10^{-4}$ M)	ΔOD (405nm)	Activity (U/mL dak)	Activity (%)
Zn	50	50	50	---	---	1.3021	21.3574	100.00
				10	0.6	1.4529	23.8305	111.58
				20	1.1	1.4070	23.0781	108.06
				30	1.6	1.3958	22.9073	107.26
				40	2.1	1.3676	22.4324	105.03
				50	2.5	1.3300	21.8159	102.15

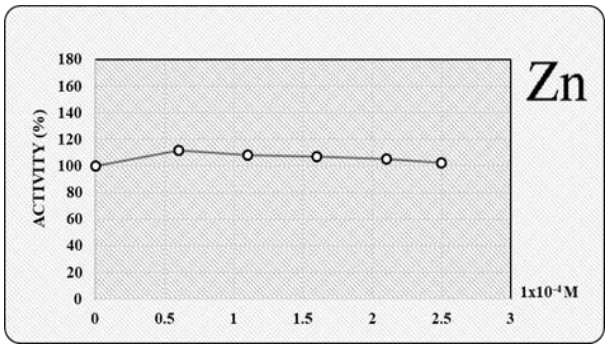

Figure 3. Activity determination for paraoxonase enzyme in $ZnCl_2$ environment

The solution concentrations and activity (%) graph specified in Table 3 for paraoxonase enzyme in $ZnCl_2$ environment were given in Figure 3.

After determining enzyme activity, PON enzyme activity was calculated as 111.58% when added a 10 µL-0.6 × 10^{-4} M $ZnCl_2$ solution to cuvettes, while enzyme activity decreased as a result of increased concentrations and was calculated as 102.15% when added a 50 µL-2.5 × 10^{-4} M $ZnCl_2$ solution. When the activity (%) graph was examined as a result of the measurements made, it was determined that Zn^{+2} heavy metal ion caused a decrease in paraoxonase enzyme activity but there was no statistically significant difference ($p>0.05$). The TOC, TAC, and OSI index values obtained as a result of the analyses were given in Table 4.

Table 4. Total antioxidant capacity (TAC), total oxidant capacity (TOC), and oxidative stress index (OSI) values (mean values ± standard error)

		Mean ± SE
TAC	(mmol TroloxEquiv/L)	0.55 ± 0.29
TOC	(µmol H_2O_2Equiv/L)	10.72 ± 3.52
OSI	(AU)	2.45 ± 1.41

DISCUSSION AND CONCLUSION

Recently, the pollution of surface water resources by heavy metals, which is one of the common environmental pollutants, has started to increase all over the world (Uncumusaoglu *et al*., 2016; Mutlu and Kurnaz, 2017; Huang et al., 2020) and the level of metals in the environment has become very dangerous for the health of water and land life (Pamukoglu and Kargi, 2007). Especially, It has been shown in many studies that fish, which are among aquatic organisms, are the living group most affected from heavy metal pollution (Yilmaz et al., 2016; Aytekin and Kargın, 2019; Çoğun and Kargın, 2020). Among marine creatures, fish accumulate pollutants from the aquatic environment and therefore widely used in pollution monitoring systems of the aquatic environment (Henry *et al*., 2004). Due to pollution from chemicals and waters, fish are constantly exposed to heavy metals (Ashraf, 2005). An increasing heavy metal accumulation in the bodies of fish can disrupt the structure of enzymes by showing toxic effect (Mackness *et al*., 2000; Mackness *et al*., 2001). Heavy metals exhibit toxicity by complexing with organic compounds and if they bind to these groups, they can switch to the inactive enzyme form (*Ekinci et al.,* 2007). Since PON activity is important in environmental pollution, in this study the effects of Hg^{+2}, Cu^{+2}, and Zn^{+2} heavy metal ions on the activity of paraoxonase enzyme, which has detoxification, antioxidant and antibacterial activity in muscle tissue of bonito (*S. sarda*) fish was examined and the oxidative stress index of the muscle tissue at normal levels was determined.

When the effect of Cu^{+2} heavy metal ion on PON enzyme activity, it was determined that increasing concentrations caused a decrease in PON enzyme activity, but there was no statistically significant difference between increasing concentrations of Cu^{+2} heavy metal ion. Sayın *et al.* (2012), in a study, examined in vitro the inhibition effect of Cu^{2+}, Ni^{2+}, Cd^{2+}, and Hg^{2+} metal ions on PON enzyme activity in *Scyliorhinus canicula* fish. Cu^{2+}, Ni^{2+}, Cd^{2+}, and Hg^{2+} metal ions showed an inhibitory effect on this enzyme activity and were determined that the strongest effect was by Cu^{2+} (Sayın *et al*., 2012). But when the activity (%) graphs were examined in this study, it was found that Cu^{+2} heavy metal ion did not cause a significant difference between enzyme activities, but Hg^{+2} heavy metal ion significantly caused a decrease in enzyme activity, causing a greater effect on activity. Hg is a metal that acts toxic by binding to thiol groups and inactivating proteins and enzymes when it enters the cell (Misra, 1992). Erdös *et al.* (1960), in a study, stated that Hg, Cu, and Ni salts inhibit PON1 activity at low concentration due to interacting with a thiol group at the catalytic center (Erdös *et al*., 1960). Therefore, Hg heavy metal ion is thought to inactivate the enzyme by binding to the free sulfhydryl (thiol) group in the cysteine at the 284 th position of the PON enzyme. During the oxidation of Cu^{+1}/Cu^{+2} ions, it has also been reported that it may be responsible for the partial inactivation of PON, replacing the Ca ion required for PON's paraoxonase activity (Aviram, 1999). In this study, when the activity (%) graph was examined, It was determined that the Cu^{+2} heavy metal ion acted in this way, causing a partial decrease in paraoxonase enzyme activity but there was no significant difference between statistically measured activities.

When the effect of Zn^{+2} heavy metal ion on PON enzyme activity was examined, it was determined that increasing concentrations caused a decrease in PON enzyme activity, but there was no statistically significant difference between increasing concentrations of Zn^{+2} heavy metal ion. In a study investigating the effect of zinc on PON enzyme activity in fish, plasma PON activity of *Capoeta capoeta* fish kept in tanks containing 5 and 10 mg/L $ZnSO_4$ for 10 days decreased compared to the control group. They reported that this decrease in PON enzyme activity may be due to the absorption relationship between Ca^{+2} and Zn^{+2} cations (Deveci *et al*., 2015). In this study, when the activity graph of the Zn^{+2} heavy metal ion was examined, it was observed that it caused a decrease in PON enzyme activity, and this decrease in activity was thought to be due to the absorption relationship between Ca^{+2}/Zn^{+2} ions. Because the Ca^{+2} ions required for the stability and activity of the PON enzyme due to HDL in plasma are directly related to the absorption of Zn^{+2} ions (Babacan *et al*., 2011; Cicik, 2003). Also, Zn is taken by an apical Ca^{+2} channel found in mitochondrial-rich ion carrier cells in the gills of fish (Bury *et al*., 2003; Zhang and Wang, 2006).

It was observed that Hg^{+2} heavy metal ion showed a statistically significant decrease in PON enzyme activity compared to Cu^{+2} and Zn^{+2} ions due to increasing concentrations and inhibited the enzyme. Especially when enzyme activities were compared, it was determined that there were significant differences between the 0.6×10^{-4} M and $2.1\text{-}2.5 \times 10^{-4}$ M samples used and between 1.1×10^{-4} M and 2.5×10^{-4} M samples. According to the results obtained, it was seen that the Hg^{+2} heavy metal ion inhibited by showing more effect compared to Cu^{+2} and Zn^{+2} ions on enzyme activity. In a study examined the effect of different metals on paraoxonase enzyme activity in carp (*Cyprinus carpio)* fish, it was determined that Co, Hg, Cu, and Cd heavy metals inhibited different levels of PON activity (Beyaztaş *et al*., 2007).

In another study in scaly carp (*C. carpio*) fish, the effect of malathion, which is known to be toxic such as heavy metals on paraoxonase and arylesterase enzyme activities were researched and it was observed that PON activity decreased due to increasing concentrations of malathion (Kılıç and Yonar, 2017). In a study using the chromium oxide (CrO_3)

form of chromium, a heavy metal such as copper, mercury and zinc, it was determined that chromium applied at concentrations of 15, 30 and 60 ppb for 28 days reduced the activity of carp (*C. carpio*) serum PON (Yonar *et al.*, 2012).

In a study conducted in bonito (*S. sarda*) fish, it was examined the effect of some heavy metals on glutathione transferase enzyme, which has detoxification and antioxidant properties such as the PON enzyme. Güller *et al.* (2014), in this study, the effects of Pb^{2+}, Cr^{2+}, Fe^{3+} Ag^{+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} metal ions on the enzyme activity were examined and it was reported that Cu ion showed the strongest inhibitor effect and, Zn ion showed weakest inhibitor effect (Güler *et al.*, 2014). When the activity (%) graphs were examined also in our study, it was found that Zn heavy metal ion caused a more significant decrease in PON enzyme activity compared to Cu heavy metal ion.

Besides studies carried out among fish, in the studies also carried out in rats, humans, bull, and sheep, the effect of heavy metals on PON enzyme activity was investigated. Pla *et al.* (2007), in a study, examined the effects of some metal ions on PON1 enzyme activity purified from rat liver. In the present study they have done, inhibition effects of Mn^{+}, Cu^{+2}, Hg^{+2} ve Co^{+2} heavy metal ions were determined and it was determined that Hg^{+2} heavy metal ion was the strongest inhibitor and Cu^{+2} ion was the weakest inhibitor for PON1 (Pla *et al.*, 2007). In this study, when the activity (%) graphs were examined, it was determined that the inhibitor effect of Hg^{+2} ion on the enzyme activity more than the effect of Cu^{+2} ion. When looked the effect of Hg^{+2} and Cu^{+2} heavy metal ions on PON enzyme activity studied in bonito (*S. sarda*) fish, it is seen that it causes a decrease in enzyme activity similar to the PON1 enzyme activity in rats. Samra *et al.* (2010), examined the in vitro inhibitor effects of some metal ions, at 1.0 mM concentration on human PON1 enzyme activity. It was determined that Mg^{+2} and Mn^{+2} ions did not show any effect on human PON1 enzyme activity, Pb^{+2}, Co^{+2}, and Zn^{+2} ions decreased the activity, while Ni^{+2}, $Cd^{+2,}$ and Cu^{+2} ions inhibited the PON1 enzyme activity (Samra *et al.*, 2010). Dedeoğlu *et al.* (2014), in a study, determined changes occurring in PON1 enzyme activity purified from bull semen in the presence of Cu^{+2}, Mn^{+2}, Cd^{+2}, Zn^{+2}, Ni^{+2}, and Pb^{+2} heavy metal ions in different cuvette concentrations. While Cd^{+2} ions increased PON1 activity, other heavy metal ions were found to inhibit PON1 at micromolar levels (Dedeoğlu *et al.*, 2014). It was determined that Cu^{+2} and Zn^{+2} heavy metal ions used in this study also caused at low levels decrease of PON enzyme activity in bonito (*S. sarda*) fish but there was no statistically significant difference. Therefore, it is seen that Cu and Zn heavy metal ions affect PON enzyme activity studied in bonito (*S. sarda*) fish, similar to that of human PON1 enzyme and PON1 enzyme activity purified from bull semen. Erol *et al.* (2013), in another study, examined the effect of some metal ions on PON1 enzyme activity purified from blood samples taken from Merino and Kivircik sheep breeds. It was determined that Mn^{+2}, Hg^{+2}, Co^{+2}, Cd^{+2}, $Ni^{+2,}$ and Cu^{+2} metal ions showed different levels of inhibition effect on PON enzyme activity and Cu^{+2} heavy metal ion caused strongest inhibitor effect for PON (Erol *et al.*, 2013). When examined the effects of heavy metal ions used in this study on PON enzyme activity in bonito (*S. sarda*) fish, it is seen that Cu^{+2} metal ion caused a partial decrease in the enzyme activity compared to other heavy metal ions (Hg^{+2}, Zn^{+2}) used in the study. Therefore, it is seen that the Cu^{+2} heavy metal ion caused a decrease at different levels of PON enzyme activity in Merino and Kivircik sheep breeds by the PON enzyme of bonito (*S. sarda*) fish.

In the study, the effect of Hg^{+2}, $Cu^{+2,}$ and Zn^{+2} heavy metal ions was researched on paraoxonase enzyme activity, which an antioxidant enzyme in muscle tissue of bonito (*S. sarda*) fish, and it was found Cu^{+2}, Zn^{+2} ions caused a statistically insignificant decrease in enzyme activity. It is thought that the inhibitory effect of Cu and Zn ions, which are present in excess amount in the environment, on the PON enzyme may be due to the substitution of Ca ions, which are the cofactor of the enzyme. Therefore, it is thought of these ions cause a decrease in activity by causing inhibition of enzymatic activity. It is thought that Zn^{+2} and Cu^{+2} ions to be used in higher concentrations in studies where the effects of heavy metals on PON enzyme activity will be investigated, may cause a significant decrease in enzyme activity. Hg^{+2} heavy metal ion is thought to inhibit the enzyme activity and show its inhibitory effect on the enzyme by binding to the free sulfhydryl (thiol) group in the cysteine at the 284 th position of the PON enzyme. It is thought that controlled experimental studies may be conducted to investigate the effects of heavy metals taken in higher concentrations on TOC, TAC and PON enzyme activity and our study may also create an example.

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