Comparative Biochemistry and Physiology, Part C 226 (2019) 108605

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Purification and characterization of the carbonic anhydrase enzyme from horse mackerel (*Trachurus trachurus*) muscle and the impact of some metal ions and pesticides on enzyme activity

Cuneyt Caglayan[a], Parham Taslimi[b,*], Cebrahil Türk[c], Fatih Mehmet Kandemir[d], Yeliz Demir[e], İlhami Gulcin[f]

[a] Department of Biochemistry, Faculty of Veterinary Medicine, Bingol University, 12000 Bingol, Turkey
[b] Department of Biotechnology, Faculty of Science, Bartin University, 74100 Bartin, Turkey
[c] Department of Fisheries, Genç Vocational School, Bingol University, 12500 Bingol, Turkey
[d] Department of Biochemistry, Faculty of Veterinary Medicine, Atatürk University, 25240 Erzurum, Turkey
[e] Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, 75700 Ardahan, Turkey
[f] Department of Chemistry, Faculty of Science, Atatürk University, 25240 Erzurum, Turkey

ARTICLE INFO

Keywords:
Trachurus trachurus
Horse mackerel
Enzyme purification
Enzyme characterization
Carbonic anhydrase
Heavy metal
Pesticides

ABSTRACT

In this paper, the total carbonic anhydrase (CA) enzyme was purified from horse mackerel (*Trachurus trachurus*) muscle with a specific activity of 23,063.93 EU/mg, purification fold of 551.08, total activity of 1522.22 EU/mL and a yield of 18.50% using sulfanilamide affinity column chromatography. For obtaining the subunit molecular mass and enzyme purity, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for this part was performed and a single band was clearly recorded. The molecular mass of this enzyme was found approximately 35 kDa. The optimum temperature and pH values were obtained from Arrhenius plot. In addition, the inhibitory effects of different heavy metal ions (Fe^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+} Hg^{2+} and As^{3+}) and some pesticides (thiram, clofentezine, propineb, deltamethrin, azoxystrobin and thiophanate) on horse mackerel (*Trachurus trachurus*) muscle tissue CA enzyme activities were investigated by utilizing esterase assay activity. The used metal ions and pesticides had IC_{50} values in the range of 0.21–13.84 mM and 3.78–70.58 mM, respectively.

1. Introduction

Heavy metals are pollutants for environment and can be defined in biological systems and environmental. Humans are exposed to them via contaminated food, water and air (Kirici et al., 2016a; Topal et al., 2014). Among them, lead (Pb) is exhibited toxic impacts on the kidneys, liver, bones and brain, which are thought the target organs for its effect. Lead exposure gives rise to an important reduce in hematocrit and hemoglobin values (Nikolić et al., 2015). Copper (Cu) is an important biometal for some plant species, people, and animals. Deficiency of copper can gives rise to anemia. Wilson's disease occurs excessive accumulation of copper in the body (Fuentealba et al., 2000). Iron (Fe) plays an important role for living organism such as gene regulation, DNA synthesis and transport, oxygen sensing and electron transport. However, the much concentration of its may be toxic (Mohanty and Samanta, 2018). Cobalt (Co) is the most toxic heavy metals. It is widely employed in the electroplate industry, in the food industries and chemistry as catalysts (Erturk et al., 2013). Mercury (Hg) is a common environmental contaminant that is stored and fixed in the tissues of organisms that directly affect the health of the individual. The sulfhydryl group of proteins interferes with mercury by removing other metals from their natural binding sites (Caglayan et al., 2019a; Chatterjee et al., 2014). Arsenic (As) is environmental toxic agent and available in the environment. It generates development of several risky impacts on human health via environmental or occupational processes (Ramanathan et al., 2003; Turk et al., 2019).

Pesticides are contaminants of the environment and present in samples from water, air, soil, animal and human tissues all over the world. They are extremely used in pest control, such as herbicides, fungicides, molluscicides, rodenticides, insecticides, and others (Kirici et al., 2017; Koksal et al., 2018; Özaslan et al., 2018). Deltamethrin is a synthetic pyrethroid insecticide. It is broadly employed in various home and agricultural pest control to exterminate the invasion of weevils mites, beetles and ants (Sharma et al., 2018b). Thiophanate is a methyl

* Corresponding author at: Faculty of Science, Department of Biotechnology, Bartin University, 74100 Bartin, Turkey.
E-mail addresses: ptaslimi@bartin.edu.tr, parham_taslimi_un@yahoo.com (P. Taslimi).

https://doi.org/10.1016/j.cbpc.2019.108605
Received 20 June 2019; Received in revised form 10 August 2019; Accepted 14 August 2019
Available online 15 August 2019

benzimidazole carbamate fungicide. It has been employed for over forty years to control of a number of vegetable and fruit pathogens (Sharma et al., 2018a). Propineb is commonly used as fungicide in the Mediterranean area, and because of its broad spectrum of effect on fungi an enlargement of the market is anticipated. Long-term exposure, by inhalation or oral is associated with teratogenicity, carcinogenicity, malformation of their vitals, and malfunction of the reproductive system (Kazos et al., 2007). Clofentezine is generally employed as a potent contact ovicide and is considered to behave by hindering cell differentiation and cell growth during the early larval development and final phases of embryonic (Demaeght et al., 2014). A sulfur fungicide, thiram, has been commonly employed as a protective agent on vegetables, fruits, and food crops. Thiram is slightly toxic by inhalation and ingestion (Sharma et al., 2003). Azoxystrobin is a fungicide. Due to large spectrum of soil-applied and systemic fungicide, it is used in over 85 different crops around the world (Cao et al., 2016).

Carbonic anhydrase (CA, E.C. 4.2.1.1) is found almost all tissues. The zinc enzyme catalyzes the reversible reactions of bicarbonate dehydration and carbon dioxide hydration, physiologically (Bayrak et al., 2019; Kucuk and Gulcin, 2016; Taslimi et al., 2017; Sağlık et al., 2019). Until now, 16 diverse CA isozymes have been detected in plants and animals. CA I, II, III, XIII, and VII are cytoplasmic, CA VI is secretory, CA V is mitochondrial, CA IV, IX, XII and XIV are membrane-associated, and CA VIII, XI, and X are noncatalytic isoenzymes (Caglayan and Gulcin, 2018; Taslimi et al., 2018b). CA is significant in the acid–base and osmotic regulation in the fish (Aslan et al., 2018; Kucukoglu et al., 2019; Türkeş et al., 2019). CAs participate in various significant biological mechanisms like bone resorption, ion transport and carbon dioxide, respiration, acid-base balance, gluconeogenesis, ureagenesis, body fluid generation and lipogenesis (Bayrak et al., 2017; Caglayan et al., 2019b; Gulçin et al., 2017; Taslimi et al., 2018a).

In this work, CA enzyme was purified from horse mackerel (*Trachurus trachurus*) muscle in a single step using affinity chromatography, and we investigated the in vitro impacts of some commonly used pesticides containing propineb, thiram, deltamethrin, clofentezine, azoxystrobin and thiophanate (Fig. 1), and heavy metal ions including Fe^{2+},Cu^{2+}, Co^{2+}, Pb^{2+} Hg^{2+} and As^{3+} on the purified CA enzyme.

2. Materials and methods

2.1. Chemicals

Thiram (Birgin Forte, 80 WP), clofentezine (Apofen), propineb (Antracol WP 70, Bayer), deltamethrin (Dentis 25 EC), azoxystrobin (Efdal Azbin SC) and thiophanate (Sumitop WP) were obtained from an agricultural pesticide shop. $Pb(CH_3COO)_2$, $NaAsO_2$, $CoCl_2$, $FeCl_2$, $CuSO_4{\cdot}5H_2O$, Sepharose-4B, protein assay reagents and chemicals for electrophoresis were purchased from SigmaAldrich (Taufk irchen, Germany). All another chemical materials utilized in this work were obtained from sigma and Merck (Germany, Darmstadt) and of analytical grade.

2.2. Horse mackerel (Trachurus trachurus)

Horse mackerel (*Trachurus trachurus*) were obtained from the Marmara Sea in the northwest Turkey.

2.3. Preparation of muscle homogenate

The muscle tissues used in the experiments was from fresh cropping. The muscles were kept in ice and transferred to the lab. They were cut into a piece of 20 g and kept at −80 °C for later use. 0.9% NaCl was washed three times. To prepare muscle homogenate, the specimen was cut into small pieces using a knife and then ground using liquid nitrogen and homogenized in a buffer solution of 25 mM Tris HCl/0.1 M Na_2SO_4 (pH 8.7). The suspension was centrifuged for 30 min at 13,500 ×*g* and this operation was performed three times. The supernatant was used for next analysis.

2.4. Purification of CA from horse mackerel (Trachurus trachurus) muscles samples

The pH of the homogenate obtained from horse mackerel (*Trachurus trachurus*) muscles was adjusted to 8.7 with solid Tris. Homogenate was applied to the column and washed with a solution of Tris-HCl and 0.4 L of 25 mM Na_2SO_4 (pH 8.7). Thus, enzyme purification was performed as in previous studies (Caglayan and Gulcin, 2018; Kucuk and Gulcin, 2016; Soyut and Beydemir, 2008).

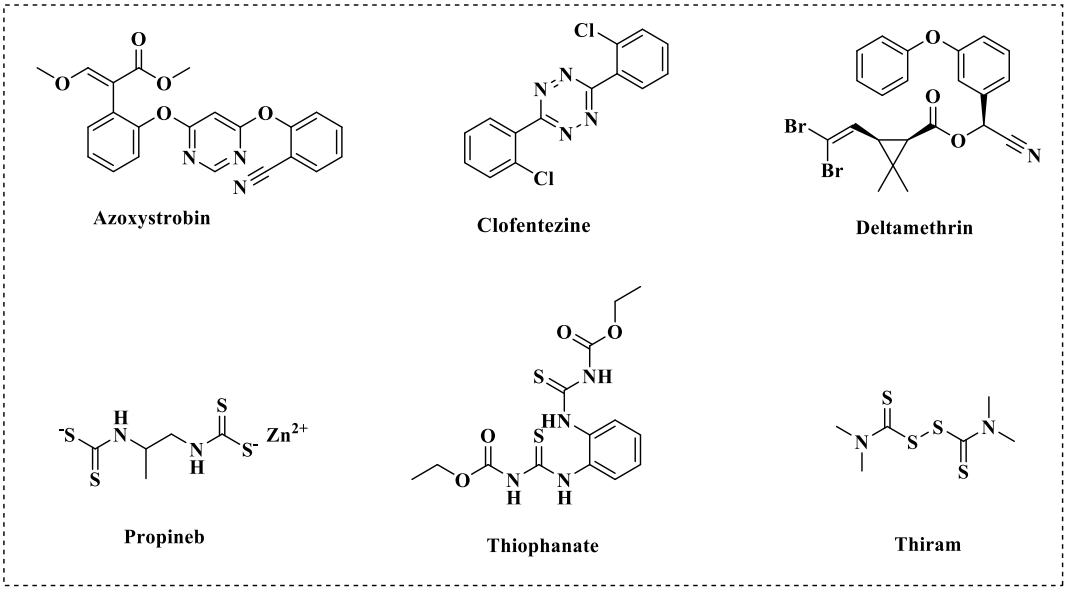

Fig. 1. Chemical structures of used pesticides including azoxystrobin, clofentezine, deltamethrin, propineb, thiophanate and thiram.

Table 1
Summary of purification of carbonic anhydrase (CA) enzyme from horse mackerel (*Trachurus trachurus*) muscle tissues.

Purification step	Activity (EU/mL)	Total volume (mL)	Protein (mg/mL)	Total protein (mg)	Total activity (EU/mL)	Specific activity (EU/mg)	Yield (%)	Purification fold
Homogenate	480.82	21	9.364	196.644	8229.90	41.85	100.00	1.00
Sepharose-4B-L- tyrosine sulfanilamide affinity chromatography	761.11	2	0.033	0.066	1522.22	23,063.93	18.50	551.08

2.5. Measurement of CA enzyme activity

CA enzyme activity can be measured in two ways: first one is esterase activity that can be carried out in vitro and followed spectrophotometrically, and the second is CO_2-hydratase activity, the physiological activity of the CA. Enzymes hydratase activities performed according to the assay explained by Wilbur and Anderson (1948). The CA enzyme activity was performed as in previous studies (Koçyiğit et al., 2018; Topal and Gülçin, 2014).

2.6. Protein determination

Quantitative amounts of protein were designated conforming to the Bradford (1976) procedure at 595 nm as described previously (Turkan et al., 2019).

2.7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) carried out to check the purity of the isozymes and calculate the subunit molecular weights. For this reason, 3 and 10% acrylamide respectively for stacking and running containing 0.1% SDS were prepared according to Laemmli (1970) procedure. Denaturized enzyme samples and protein markers were applied to the electrophoresis medium. The electrophoresis gel was held in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid in one night. The method was performed according to our previous studies (Beydemir and Demir, 2017; Demir and Beydemir, 2015; Demir, 2019). Used protein marker was the product with a catalog number of Thermo 26,616.

2.8. In vitro studies

Inhibitory effects of some commonly utilized pesticides and metal ions on CA enzyme activity purified from horse mackerel (*Trachurus trachurus*) muscles were tested in triplicate at each concentration used. CA activity was measured in the presence of different concentrations of inhibitors. Control activity was assumed to be 100% in the absence of inhibitor. For each compound, a percentage activity versus inhibitor concentration graph was drawn. For determination of K_i values, three different inhibitor concentrations were tested at five different substrate concentrations for each chemical. Lineweaver and Burk (1934) curves were used for the determination of K_i value and type of inhibition.

2.9. Kinetic studies

2.9.1. Optimum and stable pHs

For designation of optimum pH value, CA activities were measured in 1 M Na-phosphate buffers ranging from pH 5.0–8.0 and 1.0 M Tris-SO_4 ranging from pH 7.5–9.0, 1.0 M Glycine-NaOH ranging from pH 9.0–10.5 (GülçİN et al., 2005). On the other hand, for the designation of stable pH value, the CA enzymes activities were measured in these buffers. The activity measurements were performed at 24 h period during 3 days incubation using *p*-nitrophenolacetate (PNA) as the substrate under standard situations.

2.9.2. Optimum ionic strength and optimum temperature

For obtaining the optimum ionic strength value of the enzyme activities was used various concentrations of Glycine/NaOH buffer (pH 9.0) ranging from 0.1 M to 1.1 M. The horse mackerel (*Trachurus trachurus*) muscles CA enzyme activities were measured at various temperatures with an increase of 10 °C ranging from 0 to 80 °C for determining the optimum temperature.

2.9.3. Inhibition studies

The inhibitory effects of metal ions (Fe^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+} Hg^{2+} and As^{3+}) and pesticides (propineb, thiram, deltamethrin, clofentezine, azoxystrobin and thiophanate) were evaluated on CA enzyme activity purified from horse mackerel (*Trachurus trachurus*) muscle tissue. Metal ions and pesticides were dissolved in water and the effects of inhibition on the enzyme were investigated, IC_{50} and K_i studies were performed and the values were calculated and plotted (Kocyigit et al., 2018).

3. Result

3.1. Characterization results for CA enzyme

The CA enzyme was purified from horse mackerel (*Trachurus trachurus*) muscle with a specific activity of 23,063.93 EU/mg, purification fold of 551.08, total activity of 1522.22 EU/mL and a yield of 18.50% using sulfanilamide affinity column chromatography (Table 1). For determining the enzyme purity and molecular mass, SDS-PAGE was performed and single band was observed. The molecular mass of subunit was found approximately 35 kDa. Quantitative protein determination in enzyme solutions obtained by Bradford method was performed. A standard graph was prepared and quantitative protein determination of enzyme solutions obtained by affinity chromatography was found using this standard graph. SDS-PAGE method was used to check the purity of the eluates obtained by affinity of fish CA enzyme. Therefore, the electrophoresis system was installed and the enzyme samples were loaded into the wells in sequence and then carried out. The photograph showing the bands obtained is shown in Fig. 2. Optimal pH study was performed for CA enzyme purified from fish and their pH was determined spectrophotometrically using buffer solutions of 5–11(Fig. 3). The optimum pH was determined as 9.0 for CA enzyme purified from muscle tissue. In order to designate the optimal ionic strength for CA enzyme activity purified from fish muscle, solutions of different concentrations of Glycine/NaOH buffers, which were determined to be suitable in previous studies, were prepared. Activity measurements at different Glycine/NaOH concentrations were made and a graph of Glycine/NaOH concentration and activity values was plotted. As a result of the studies, the most suitable ionic strength for CA enzyme purified from fish muscle was determined as 1 M Glycine/NaOH (pH 9.0) buffer (Fig. 4). In this study, a stable pH study was performed to designate the stable pH of purified CA enzyme. The results are shown in Fig. 5. As a result of these studies, stable pH was determined as pH 8.0. Indeed, 1 M Glycine/NaOH (pH 9.0) buffer solution with optimum pH and appropriate ionic strength was used to determine the optimum temperature of the purified CA enzyme. Activity measurements were performed at 0 °C to 80 °C every 10 °C. The results are shown in Fig. 6. As a result of these studies, the optimum temperature was determined as 30 °C. The results of the CA enzyme characterization

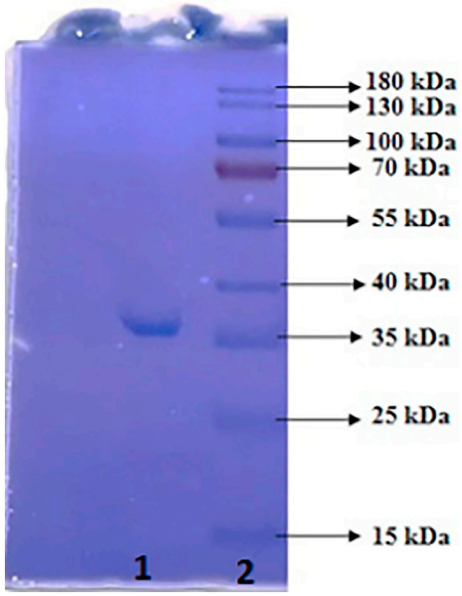

Fig. 2. SDS polyacrylamide gel electrophoresis of horse mackerel (*Trachurus trachurus*) muscle CA purified by Sepharose 4B-tyrosinesulfanilamide affinity gel. Line 1; horse mackerel (*Trachurus trachurus*) muscle CA. Line 2; The Protein standards (a: 180 kDa, b:130 kDa c: 100 kDa d: 70 kDa e: 55 kDa f: 40 kDa g: 35 kDa h: 25 kDa i: 15 kDa).

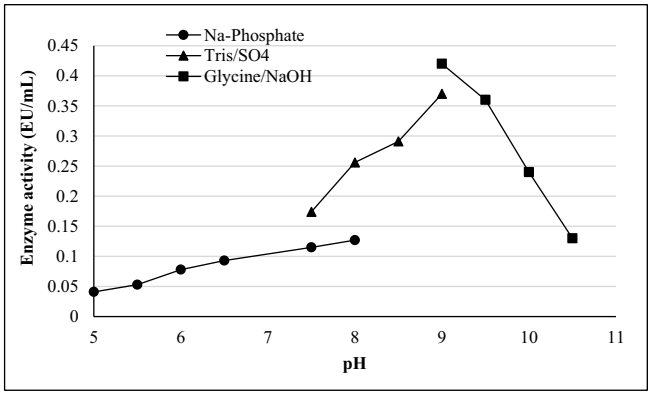

Fig. 3. Determination of optimum pH for from horse mackerel (*Trachurus trachurus*) muscle tissues in 1 M phosphate and 1 M Tris–SO_4 and glycine/NaOH buffers.

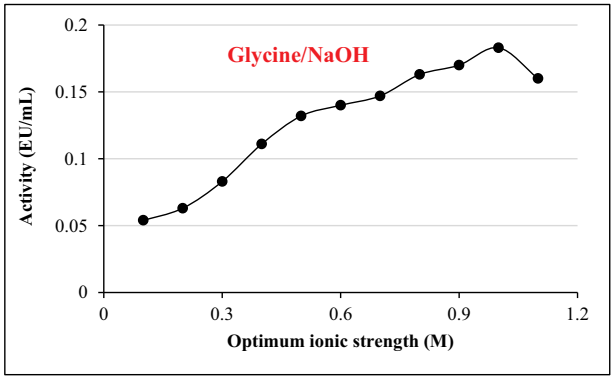

Fig. 4. Determination of optimum ionic strength (M, Tris–SO_4 Glycine/NaOH buffer) for from horse mackerel (*Trachurus trachurus*) muscle tissues.

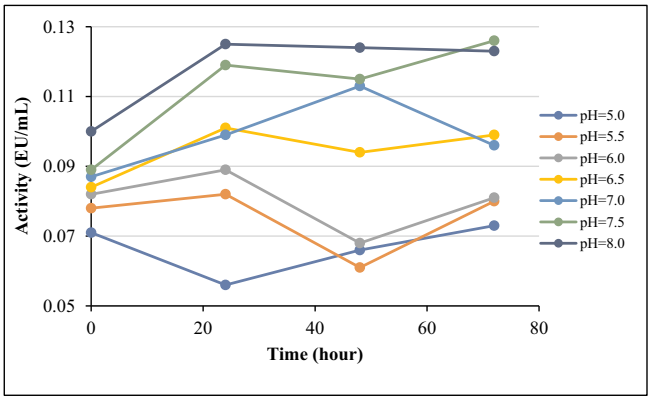

Fig. 5. Determination of stable pH graph of from horse mackerel (*Trachurus trachurus*) muscle tissues for three days.

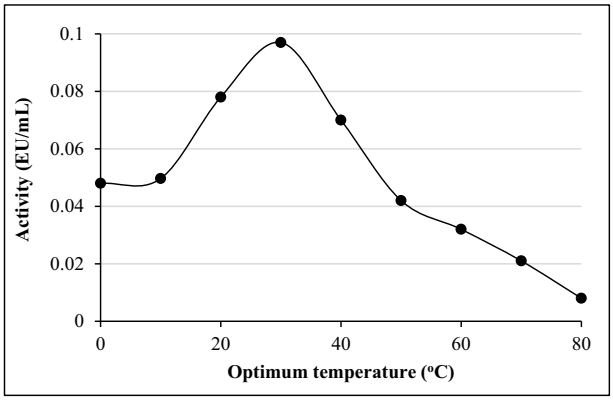

Fig. 6. The effect of temperature on CA enzyme activity from horse mackerel (*Trachurus trachurus*) muscle tissues.

from horse mackerel (*Trachurus trachurus*) muscle are shown in Figs. 1–6. The purity and molecule mass of the CA enzyme were absorbed as a single band. The molecular mass of the enzyme was calculated as approximately 35 kDa (Fig. 2). The recorded molecular mass was similar to CAs purified from many other tissues. For instance, sea bream gills CA 30.5 kDa (Kaya et al., 2013), zebrafish erythrocyte CA 29 kDa (Peterson et al., 1997) and Antarctica icefish gills CA 28 kDa (Rizzello et al., 2007) were determined respectively.

3.2. Inhibition results

In this work, we determined the CA inhibition effects of some pesticides (Table 2). The used some pesticides had IC_{50} values in the range of 3.78–70.58 mM. IC_{50} values of some pesticides exhibited the following order: Clofentezine (3.78 µM, r^2: 0.9775) < Thiram (5.77 nM, r^2: 0.9399) < Deltamethrin (8.55 nM, r^2: 0.9496) < Propineb (9.36 nM, r^2: 0.9507) < Azoxystrobin (30.01 nM, r^2: 0.9476) < Thiophanate (70.58 nM, r^2: 0.9547). On the other hand,

Table 2
IC_{50} and K_i values and inhibition types of some pesticides on carbonic anhydrase (CA) obtained from horse mackerel (*Trachurus trachurus*) muscle tissues.

Pesticides	IC_{50} (nM)	r^2	K_i (nM)	Inhibition type
Azoxystrobin	30.01	0.9476	30.74 ± 10.05	Competitive
Clofentezine	3.78	0.9775	2.23 ± 0.22	Competitive
Deltamethrin	8.55	0.9496	7.56 ± 1.14	Uncompetitive
Propineb	9.36	0.9507	9.98 ± 4.05	Uncompetitive
Thiophanate	70.58	0.9547	89.76 ± 32.23	Uncompetitive
Thiram	5.77	0.9399	5.71 ± 2.23	Uncompetitive

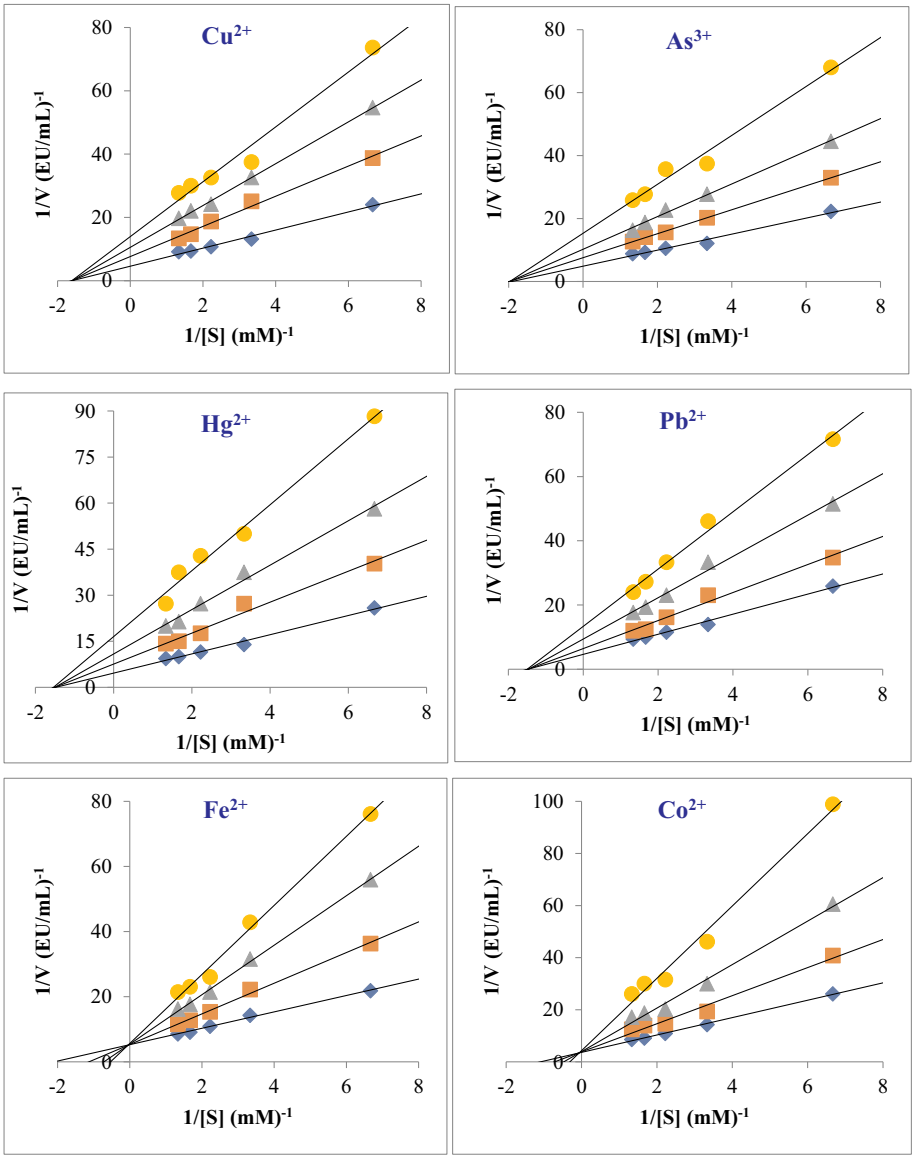

Fig. 7. K_i graphs of metal ions for horse mackerel (*Trachurus trachurus*) muscle tissues.

Table 3
IC_{50} and K_i values and inhibition types of some heavy metal carbonic anhydrase obtained from horse mackerel (*Trachurus trachurus*) muscle tissues.

Metal ions	IC_{50} (mM)	r^2	K_i (mM)	Inhibition type
Fe^{2+}	0.43	0.9603	0.36 ± 0.05	Competitive
Cu^{2+}	13.84	0.9815	17.83 ± 2.71	Uncompetitive
Co^{2+}	1.70	0.9904	1.14 ± 0.10	Competitive
Pb^{2+}	0.21	0.9483	0.32 ± 0.04	Uncompetitive
Hg^{2+}	8.63	0.9624	12.63 ± 0.97	Uncompetitive
As^{3+}	11.04	0.9670	12.80 ± 2.06	Uncompetitive

they demonstrated Ki values in the range of 2.23 ± 0.22–89.76 ± 32.23 nM (Table 2). Ki values of these some pesticides exhibited the following order: Clofentezine (2.23 ± 0.22 nM) < Thiram (5.71 ± 2.23 nM) < Deltamethrin (7.56 ± 1.14 nM) < Propineb (9.98 ± 4.05 nM) < Azoxystrobin (30.74 ± 10.05 nM) < Thiophanate (89.76 ± 32.23 nM) (Table 2 and Fig. 8).

Fe^{2+} and Co^{2+} tested metals inhibited the enzyme in a competitive manner. Our results indicate that these metal ions inhibit the fish CA enzyme in a similar manner to another α-CAs from mammals investigated earlier. Also, we define that these metal ions might be perilous at low millimolar concentrations for fish CA enzymes. The following metals, As^{3+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Fe^{2+}, and Hg^{2+} showed inhibitory effects on the enzyme. Pb^{2+}, Fe^{2+}, and Co^{2+} exhibited the strongest inhibitory action. Hg^{2+} was a moderate inhibitor, whereas other metals showed weaker actions. In this work, we determined the CA inhibition effects of metal ions (Table 2 and Fig. 7). The used metal ions had IC_{50} values in the range of 0.21–13.84 nM. IC_{50} values of some metal ions exhibited the following order: Pb^{2+} (0.21 nM, r^2: 0.9483) < Fe^{2+} (0.43 nM, r^2: 0.9603) < Co^{2+} (1.70 nM, r^2: 0.9904) < Hg^{2+} (8.63 nM, r^2: 0.9624) < As^{3+} (11.04 nM, r^2: 0.9670) < Cu^{2+} (13.84 nM, r^2: 0.9815). On the other hand, they demonstrated K_i values in the range of 0.32 ± 0.04–17.83 ± 2.71 nM (Table 3). Ki values of these metal ions exhibited the following order: Pb^{2+} (0.32 ± 0.04) < Fe^{2+} (0.36 ± 0.05) < Co^{2+} (1.14 ± 0.10) < Hg^{2+} (12.63 ± 0.97) < As^{3+} (12.80 ± 2.06) < Cu^{2+} (17.83 ± 2.71) (Table 3). Metal ions are considerable for their extensive environmental scattering from such activities as their trend to stack in selected tissue cells of the body and their overall potential to be toxic even at relatively minor levels of exposure. Additionally, several metals ions like iron and copper are necessary to live and play inevitable roles in several critical enzyme factors. Indeed, some metal ions have no beneficial roles in fishes and

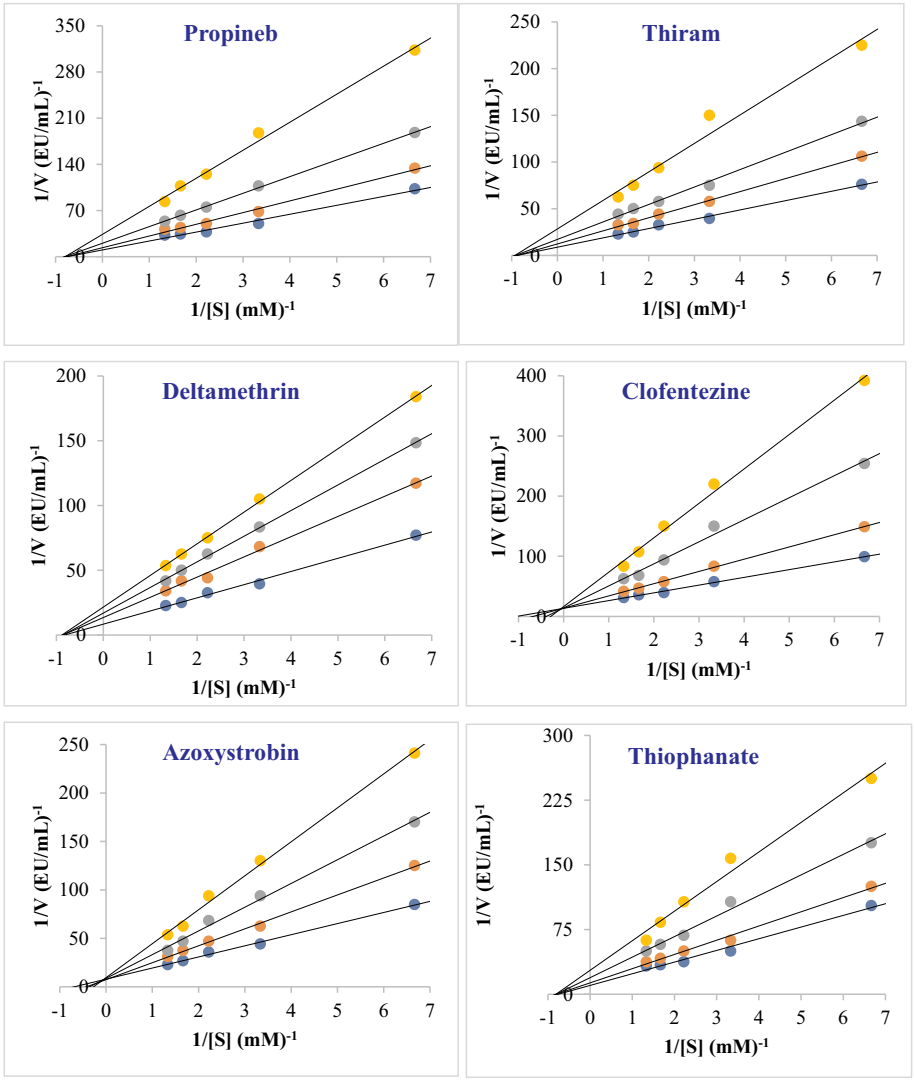

Fig. 8. nM should be written in 1 / S section of K_i graphs of pesticides for horse mackerel (*Trachurus trachurus*) muscle tissues.

human physiology and, even worse, as in the case of mercury and lead, may be toxic effect even at trace amounts of exposure (Coban et al., 2007; Ekİncİ et al., 2007).

4. Discussion

Recently, the great rate of addition in the human population and the rapid pace of industrialization have made the difficulty of access of wastewaters. Indeed, the domestic wastes and partially treated industrial effluents, supplemented with pollutants such as pesticides, heavy metal ions, and many organic molecules, have greatly contributed to great fish death of lentic ecosystems (Ekİncİ et al., 2007). These toxic metals and chemical compounds have changed the quality of water that affects the fish and other aquatic organisms. Heavy metals pass through water resources, industrial wastes or acid rains by dissolving the heavy metals present in the composition, and by dissolving heavy metals into rivers, lakes and groundwater (Kocyigit et al., 2018; Sivaperumal et al., 2007). Heavy metals transported to the waters are highly diluted and partially precipitate to the water base by forming solid compounds such as carbonate, sulfate, sulfur and enrich in this region. Due to the limited adsorption capacity of the sediment layer, the heavy metal concentration of the waters increases continuously. Metals in the sea accumulate as a result of many rivers carrying pollutants (Radwan and Salama, 2006). In addition, accumulation can be much higher due to human wastes as a result of these rivers passing through industrial or urban areas. Chemical pollutants are also highly contaminated by the atmosphere into the water environment. Because these elements in the atmosphere pass into the water with wind and rainfall over time. The mining industry is one of the leading sources of heavy metal pollution (Ekinci and Beydemir, 2010). Wastes generated during the recovery of metals from mineral ores are often activated by the processes to which they are subjected and become sources of pollution. These metals then dissolve with atmospheric effects and mix with the surface and groundwater. Heavy metal in fish samples from the Black Sea and Aegean Sea, in this study on the levels of heavy metal content of fish samples Cu: 0.73–1.83 μg/g, Cd: 0.45–0.90 μg/g, Pb: 0.33–0.93 μg/g, Zn: 35.4–106 μg/g, Fe: 1.28–7.40 μg/g, Cr: 0.95–1.98 μg/g, Ni: 1.92–5.68 μg/g, Mn: 68,6–163 μg/g reported as. As a result of the analysis, lead and cadmium levels in fish samples were found to be higher than acceptable limits for human consumption (Uluozlu et al., 2007). Akbulut and Tuncer (2011) in their study of the *C. tinca*, *C. capoeta* and L. *cephalus* fish from the Kızılırmak River in the muscle and gill tissues Co, Cr, Cu, Pb and Zn heavy metals were evaluated. According to the data obtained, the muscle was Zn > Cu > Pb > Cr > Co, and the gill was Zn > Pb > Cu > Cr > Co (Akbulut and Akbulut, 2010).

Pesticide compounds as insecticides and herbicides, can stack in aquatic ecosystems and exert toxic effects on aquatic organisms

(Ghasemzadeh et al., 2015). Also, these pesticide compounds can persist in the water bodies and environment such as lakes and rivers of agricultural lands. Pesticide compounds can contaminate the aquatic environment through run-off after application, mainly caused by intense irrigation and heavy rains. Indeed, washing of types of equipment and using containers can promulgate accidental spills, as well as aerosols that can also contaminate nearby water bodies (Koksal et al., 2018). Additionally, fish can be utilized as bioindicator factors in several aquatic ecosystems because they are fully susceptible to the presence of chemical compounds in water. Its situation is at the top of the aquatic trophic web when compared to other bioindicators. Consequently, the utilization of these animals as food source creates a risk to human health (Abdel-Moneim et al., 2012).

There are several studies on the inhibition of pesticides and metals on enzyme activity. For instance, Isik et al. (2005) evaluated the effects of diverse pesticides like fenarimol, nuarimol, 2,4-dichlorophenoxy acetic acid, and parathion-methyl on CA activity from seawater fish erythrocytes and some fresh water, and recorded that the pesticide compounds used inhibited the CA activity from various fish types to several degrees. It was obtained that IC_{50} values for fenarimol, nuarimol, parathion-methyl and 2,4-dichlorophenoxy acetic acid pesticides were 0.55, 0.38, 2.9 and 2.72 nM for *C. carpio* 0.59, 0.28, 2.45 and 1.73 nM for *Barbus barbus* 0.51, 0.23, 1.77 and 1.26 nM for *O. mykiss* 0.18, 0.20, 0.62 and 0.65 nM for *Scorpaena porcus*, 0.37, 0.38, 3.19 and 2.67 nM for *Diplodus vulgaris* CA, respectively. In another study, Kuzu et al. (2018) investigated in vitro inhibitory effect of arsenic (V) oxide, Cd^{2+}, Cu^{2+}, Ni^{2+} and Ag^{+} metal ions on CA enzyme activity purified from Van Lake fish (*Chalcalburnus Tarichi*) gills. They found Cd^{2+}, Cu^{2+}, Ni^{2+} and Ag^{+} showed inhibitory effects, but arsenic (V) oxide showed activation effect. Kirici et al. (2016b) studied in vitro effects of Cd^{2+}, Ni^{2+}, Fe^{3+} and Pb^{2+} on CA from *Capoeta umbla* gill. IC_{50} values were found in the range of 0.136–0.924 mM. Dinçer et al. (2015) determined the effects of Ni^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, and Ba^{2+} on gill of *Acipenser gueldenstaedtii*. IC_{50} values were found in the range of 0.2–1.7 mM.

5. Conclusions

In the current work, CA enzyme was purified from horse mackerel (*Trachurus trachurus*) muscle in a single step using affinity chromatography, and we investigated the in vitro toxicological effects of used some pesticides and some heavy metal ions on the purified CA enzyme. After plotting graphs, the results were calculated; inhibition results of the metal ions were obtained at the level of mM and pesticides at the level of nM. CA enzyme plays a significant role in mechanism base-acid adjustment in fish cells by providing base-acid equivalents for exchange with the environment. Indeed, unlike air-breathing vertebrate cells, which repeatedly utilize respiratory compensation to regulate base-acid condition, base-acid equilibrium in fish relies approximately entirely upon the direct exchange of base-acid equivalents with the environment, which is metabolic compensation.

Declaration of competing interest

The authors declare no conflict of interest.

References

Abdel-Moneim, A.M., Al-Kahtani, M.A., Elmenshawy, O.M., 2012. Histopathological biomarkers in gills and liver of Oreochromis niloticus from polluted wetland environments, Saudi Arabia. Chemosphere 88, 1028–1035.

Akbulut, A., Akbulut, N.E., 2010. The study of heavy metal pollution and accumulation in water, sediment, and fish tissue in Kızılırmak River Basin in Turkey. Environ. Monit. Assess. 167, 521–526.

Akbulut, N.E., Tuncer, A.M., 2011. Accumulation of heavy metals with water quality parameters in Kızılırmak River Basin (Delice River) in Turkey. Environ. Monit. Assess. 173, 387–395.

Aslan, H.E., Demir, Y., Özaslan, M.S., Türkan, F., Beydemir, Ş., Küfrevioğlu, Ö.I., 2018. The behavior of some chalcones on acetylcholinesterase and carbonic anhydrase activity. Drug Chem. Toxicol. 4, 1–7.

Bayrak, Ç., Taslimi, P., Gülçin, İ., Menzek, A., 2017. The first synthesis of 4-phenylbutenone derivative bromophenols including natural products and their inhibition profiles for carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase enzymes. Bioorg. Chem. 72, 359–366.

Bayrak, C., Taslimi, P., Karaman, H.S., Gulcin, I., Menzek, A., 2019. The first synthesis, carbonic anhydrase inhibition and anticholinergic activities of some bromophenol derivatives with S including natural products. Bioorg. Chem. 85, 128–139.

Beydemir, Ş., Demir, Y., 2017. Antiepileptic drugs: impacts on human serum paraoxonase-1. J. Biochem. Mol. Toxicol. 31, e21889.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Caglayan, C., Gulcin, İ., 2018. The toxicological effects of some avermectins on goat liver carbonic anhydrase enzyme. J. Biochem. Mol. Toxicol. 32, e22010.

Caglayan, C., Demir, Y., Kucukler, S., Taslimi, P., Kandemir, F.M., Gulçin, İ., 2019a. The effects of hesperidin on sodium arsenite-induced different organ toxicity in rats on metabolic enzymes as antidiabetic and anticholinergics potentials: a biochemical approach. J. Food Biochem. 43, e12720.

Caglayan, C., Kandemir, F.M., Yildirim, S., Kucukler, S., Eser, G., 2019b. Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats. J. Trace Elem. Med. Biol. 54, 69–78.

Cao, F., Zhu, L., Li, H., Yu, S., Wang, C., Qiu, L., 2016. Reproductive toxicity of azoxystrobin to adult zebrafish (Danio rerio). Environ. Pollut. 219, 1109–1121.

Chatterjee, S., Ray, A., Mukherjee, S., Agarwal, S., Kundu, R., Bhattacharya, S., 2014. Low concentration of mercury induces autophagic cell death in rat hepatocytes. Toxicol. Ind. Health 30, 611–620.

Coban, T.A., Senturk, M., Ciftci, M., Kufrevioglu, O.I., 2007. Effects of some metal ions on human erythrocyte glutathione reductase: an in vitro study. Protein Pept. Lett. 14, 1027–1030.

Demaeght, P., Osborne, E.J., Odman-Naresh, J., Grbić, M., Nauen, R., Merzendorfer, H., Clark, R.M., Van Leeuwen, T., 2014. High resolution genetic mapping uncovers chitin synthase-1 as the target-site of the structurally diverse mite growth inhibitors clofentezine, hexythiazox and etoxazole in Tetranychus urticae. Insect Biochem. Mol. Biol. 51, 52–61.

Demir, Y., 2019. The behaviour of some antihypertension drugs on human serum paraoxonase-1: an important protector enzyme against atherosclerosis. J. Pharm. Pharmacol. https://doi.org/10.1111/jphp.13144.

Demir, Y., Beydemir, Ş., 2015. Purification, refolding, and characterization of recombinant human paraoxonase-1. Turk. J. Chem. 39, 764–776.

Dinçer, B., Ekinci, A.P., Akyüz, G., Kurtoğlu, İ.Z., 2015. Characterization and inhibition studies of carbonic anhydrase from gill of Russian Sturgeon Fish (Acipenser gueldenstaedtii). J. Enzyme Inhib. Med. Chem. 31 (6), 1662–1665.

Ekinci, D., Beydemir, Ş., 2010. Purification of PON1 from human serum and assessment of enzyme kinetics against metal toxicity. Biol. Trace Elem. Res. 135, 112–120.

Ekİncİ, D., Beydemir, Ş., Küfrevİoğlu, Ö.İ., 2007. In vitro inhibitory effects of some heavy metals on human erythrocyte carbonic anhydrases. J. Enzyme Inhib. Med. Chem. 22, 745–750.

Erturk, F.A., Ay, H., Nardemir, G., Agar, G., 2013. Molecular determination of genotoxic effects of cobalt and nickel on maize (Zea mays L.) by RAPD and protein analyses. Toxicol. Ind. Health 29, 662–671.

Fuentealba, I.C., Mullins, J.E., Aburto, E.M., Lau, J.C., Cherian, G.M., 2000. Effect of age and sex on liver damage due to excess dietary copper in Fischer 344 rats. J. Toxicol.: Clin. Toxicol. 38, 709–717.

Ghasemzadeh, A., Jaafar, H.Z., Rahmat, A., 2015. Phytochemical constituents and biological activities of different extracts of Strobilanthes crispus (L.) Bremek leaves grown in different locations of Malaysia. BMC Complement. Altern. Med. 15, 422.

Gülçİn, İ., İrfan Küfrevİoğlu, Ö., Oktay, M., 2005. Purification and characterization of polyphenol oxidase from nettle (*Urtica dioica L.*) and inhibitory effects of some chemicals on enzyme activity. J. Enzyme Inhib. Med. Chem. 20, 297–302.

Gulçin, İ., Abbasova, M., Taslimi, P., Huyut, Z., Safarova, L., Sujayev, A., Farzaliyev, V., Beydemir, Ş., Alwasel, S.H., Supuran, C.T., 2017. Synthesis and biological evaluation of aminomethyl and alkoxymethyl derivatives as carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase inhibitors. J. Enzyme Inhib. Med. Chem. 32, 1174–1182.

Isik, M., Hudson, D., Coble, K.H., 2005. The value of site-specific information and the environment: technology adoption and pesticide use under uncertainty. J. Environ. Manag. 76, 245–254.

Kaya, E.D., Söyüt, H., Beydemir, Ş., 2013. Carbonic anhydrase activity from the gilthead sea bream (*Sparus aurata*) liver: the toxicological effects of heavy metals. Environ. Toxicol. Pharmacol. 36, 514–521.

Kazos, E.A., Stalikas, C.D., Nanos, C.G., Konidari, C.N., 2007. Determination of dithiocarbamate fungicide propineb and its main metabolite propylenethiourea in airborne samples. Chemosphere 68, 2104–2110.

Kirici, M., Kirici, M., Beydemir, S., Atamanalp, M. Purification of Carbonic Anhydrase from Capoeta umbla (Heckel, 1843) Gills and Toxicological Effects of some Metals on Enzyme Activity. Turk.J.FishAquat.Sci.16, 2016a, 169–175.

Kirici, M., Kirici, M., Demir, Y., Beydemir, S., Atamanalp, M., 2016b. The effect of Al 3 and Hg 2 on glucose 6-phosphate dehydrogenase from capoeta umbla kidney. Appl. Ecol. Environ. Res. 14, 253–264.

Kirici, M., Turk, C., Caglayan, C., Kirici, M., 2017. Toxic effects of copper sulphate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish Capoeta umbla (Heckel, 1843) tissues. Appl. Ecol. Environ. Res. 15, 1685–1696.

Kocyigit, U.M., Taslimi, P., Gulçin, İ., 2018. Characterization and inhibition effects of some metal ions on carbonic anhydrase enzyme from Kangal Akkaraman sheep. J.

Biochem. Mol. Toxicol. 32, e22172.
Koçyiğit, Ü.M., Durna Daştan, S., Taslimi, P., Daştan, T., Gülçin, İ., 2018. Inhibitory effects of some drugs on carbonic anhydrase enzyme purified from Kangal Akkaraman sheep in Sivas, Turkey. J. Biochem. Mol. Toxicol. 32, e22000.
Koksal, Z., Kalin, R., Gulcin, I., Ozdemir, H., 2018. Inhibitory effects of selected pesticides on peroxidases purified by affinity chromatography. Int. J. Food Propert. 21, 385–394.
Kucuk, M., Gulcin, İ., 2016. Purification and characterization of the carbonic anhydrase enzyme from Black Sea trout (*Salmo trutta Labrax Coruhensis*) kidney and inhibition effects of some metal ions on enzyme activity. Environ. Toxicol. Pharmacol. 44, 134–139.
Kucukoglu, K., Gul, H.I., Taslimi, P., Gulcin, I., Supuran, C.T., 2019. Investigation of inhibitory properties of some hydrazone compounds on hCA I, hCA II and AChE enzymes. Bioorg. Chem. 86, 316–321.
Kuzu M, Çomaklı V, Akkemik E, Çiftci M, Küfrevioğlu Öİ. 2018. Inhibitory properties of some heavy metals on carbonic anhydrase I and II isozymes activities purified from Van Lake fish (Chalcalburnus Tarichi) gill. Fish Physiol Biochem. 44(4), 1119–1125.
Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680.
Lineweaver, H., Burk, D., 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56, 658–666.
Mohanty, D., Samanta, L., 2018. Dietary supplementation of Spirulina ameliorates iron-induced oxidative stress in Indian knife fish Notopterus Notopterus. Environ. Toxicol. Pharmacol. 61, 71–78.
Nikolić, R., Krstić, N., Jovanović, J., Kocić, G., Cvetković, T.P., Radosavljević-Stevanović, N., 2015. Monitoring the toxic effects of Pb. Cd and Cu on hematological parameters of Wistar rats and potential protective role of lipoic acid and glutathione. Toxicol. Ind. Health 31, 239–246.
Özaslan, M.S., Demir, Y., Aksoy, M., Küfrevioğlu, Ö.I., Beydemir, Ş., 2018. Inhibition effects of pesticides on glutathione-S-transferase enzyme activity of Van Lake fish liver. J. Biochem. Mol. Toxicol. 32, e22196.
Peterson, R.E., Tu, C., Linser, P.J., 1997. Isolation and characterization of a carbonic anhydrase homologue from the zebrafish (*Danio rerio*). JMolE 44, 432–439.
Radwan, M.A., Salama, A.K., 2006. Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food Chem. Toxicol. 44, 1273–1278.
Ramanathan, K., Shila, S., Kumaran, S., Panneerselvam, C., 2003. Protective role of ascorbic acid and a-tocopherol on arsenic-induced microsomal dysfunctions. Hum. Exp. Toxicol. 22, 129–136.
Rizzello, A., Ciardiello, M.A., Acierno, R., Carratore, V., Verri, T., Di Prisco, G., Storelli, C., Maffia, M., 2007. Biochemical characterization of a S-glutathionylated carbonic anhydrase isolated from gills of the Antarctic icefish Chionodraco hamatus. Protein J. 26, 335–348.
Sağlık, B.N., Çevik, U.A., Osmaniye, D., Levent, S., Çavuşoğlu, B.K., Demir, Y., Ilgın, S., Özkay, Y., Koparal, A.S., Beydemir, Ş., Kaplancıklı, Z.A., 2019. Synthesis, molecular docking analysis and carbonic anhydrase I-II inhibitory evaluation of new sulfonamide derivatives. Bioorg. Chem. 91, 103153.
Sharma, V.K., Aulakh, J., Malik, A.K., 2003. Thiram: degradation, applications and analytical methods. J. Environ. Monit. 5, 717–723.
Sharma, G., Kumar, A., Devi, K., Sharma, S., Naushad, M., Ghfar, A.A., Ahamad, T., Stadler, F.J., 2018a. Guar gum-crosslinked-soya lecithin nanohydrogel sheets as effective adsorbent for the removal of thiophanate methyl fungicide. Int. J. Biol. Macromol. 114, 295–305.
Sharma, N., Banerjee, S., Mazumder, P.M., 2018b. Evaluation of the mechanism of anticancer activity of deltamethrin in Jurkat-J6 cell line. Pestic. Biochem. Physiol. 149, 98–103.
Sivaperumal, P., Sankar, T., Nair, P.V., 2007. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India Vis-a-Vis international standards. Food Chem. 102, 612–620.
Soyut, H., Beydemir, S., 2008. Purification and some kinetic properties of carbonic anhydrase from rainbow trout (*Oncorhynchus mykiss*) liver and metal inhibition. Protein Pept. Lett. 15, 528–535.
Taslimi, P., Caglayan, C., Gulcin, İ., 2017. The impact of some natural phenolic compounds on carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes: an antidiabetic, anticholinergic, and antiepileptic study. J. Biochem. Mol. Toxicol. 31, e21995.
Taslimi, P., Caglayan, C., Farzaliyev, V., Nabiyev, O., Sujayev, A., Turkan, F., Kaya, R., Gulçin, İ., 2018a. Synthesis and discovery of potent carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes inhibitors: the novel N, N′-bis-cyanomethylamine and alkoxymethylamine derivatives. J. Biochem. Mol. Toxicol. 32, e22042.
Taslimi, P., Osmanova, S., Caglayan, C., Turkan, F., Sardarova, S., Farzaliyev, V., Sujayev, A., Sadeghian, N., Gulçin, İ., 2018b. Novel amides of 1, 1-bis-(carboxymethylthio)-1-arylethanes: synthesis, characterization, acetylcholinesterase, butyrylcholinesterase, and carbonic anhydrase inhibitory properties. J. Biochem. Mol. Toxicol. 32, e22191.
Topal, M., Gülçin, İ., 2014. Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turk. J. Chem. 38, 894–902.
Topal, A., Atamanalp, M., Oruç, E., Demir, Y., Beydemir, Ş., Işık, A., 2014. In vivo changes in carbonic anhydrase activity and histopathology of gill and liver tissues after acute exposure to chlorpyrifos in rainbow trout. Arch. Ind. Hyg. Toxicol. 65, 377–385.
Turk, E., Kandemir, F.M., Yildirim, S., Caglayan, C., Kucukler, S., Kuzu, M., 2019. Protective effect of hesperidin on sodium arsenite-induced nephrotoxicity and hepatotoxicity in rats. Biol. Trace Elem. Res. 189, 95–108.
Turkan, F., Cetin, A., Taslimi, P., Karaman, M., Gulçin, İ., 2019. Synthesis, biological evaluation and molecular docking of novel pyrazole derivatives as potent carbonic anhydrase and acetylcholinesterase inhibitors. Bioorg. Chem. 86, 420–427.
Türkeş, C., Arslan, M., Demir, Y., Çoçaj, L., Rifati Nixha, A., Beydemir, Ş., 2019. Synthesis, biological evaluation and in silico studies of novel N-substituted phthalazine sulfonamide compounds as potent carbonic anhydrase and acetylcholinesterase inhibitors. Bioorg. Chem. 89, 103004.
Uluozlu, O.D., Tuzen, M., Mendil, D., Soylak, M., 2007. Trace metal content in nine species of fish from the black and Aegean seas, Turkey. Food Chem. 104, 835–840.
Wilbur, K.M., Anderson, N.G., 1948. Electrometric and colorimetric determination of carbonic anhydrase. J. Biol. Chem. 176, 147–154.