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The Trace Elements in the Raft Cultivated Mussels (*Mytilus galloprovincialis* Lamarck, 1819) from Sinop Peninsula, in the Southern Black Sea

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Abstract: The concentration of cadmium (Cd), copper (Cu) and zinc (Zn) in the raft cultivated Mediterranean mussel *Mytilus galloprovincialis* from the Sinop Peninsula, in the Black Sea were investigated seasonally. The mean concentrations of Cd, Cu and Zn in the soft tissues were 2.53±0.19, 6.98±0.31 and 228.00±16.75 $\mu g\ g^{-1}$ dry weights (dw), respectively. There were no significant differences between the metal concentrations among the seasons ($p>0.05$). Cd and Zn levels were higher than legislative limits, while Cu level was below. The Cd and Zn concentrations in cultivated area were danger to human health or human consumption.

Key words: Raft cultivated mussel, heavy metals, *Mytilus galloprovincialis*, sinop peninsula, Black Sea

INTRODUCTION

The molluscs are among the most successfully cultured and commercially important types of shellfish and a large variety of different mollusc species are cultured throughout the world. The seafood notably fish, shrimp and molluscs are of value for both local consumption and export revenue.

Turkey is a developing country. Like other developing countries, its economy is also mainly based on agriculture. The population is increasing rapidly and it means simply more food production and more jobs. Although, vegetable production is enough for the present population and is likely to be for the future population, there is insufficient animal protein intake in spite of attempt made for 55 years. This gap has not been closed yet.

Aquaculture especially marine aquaculture in the Black Sea region is a relatively recent industry in Turkey, enjoying great potential for development and not yet widespread. Farming of the Mediterranean mussel (*Mytilus galloprovincialis*) is probably the most promising activity inthe region, although mussels are farmed commercially in Marmara and Aegean seas. Mussel aquaculture can be performed in various ways, including suspended culture (using rafts or longline) and bottom culture (by seeding intertidal area) (Hickman, 1992).

Farming of mussel (*Mytlius* sp.) is no doubt the most efficient way to convert the organic matter produced by marine organisms of the first link of the food chain (Phytoplankton and remains thereof) in to palatable and nutritious human food (Koringa,1976).

Mediterranean mussel is a filter feeding animal, which depends upon phytoplankton, organic detritus, bacteria and probably dissolved organic matter in the water as food source (Karayücel and Karayücel, 1997; Karayücel *et al*., 2003).

The marine mussels provide cheap source of protein for human consumption (Choo and Ng, 1990). In *M. galloprovincialis*, there was about 60.21±1.47 protein per 100 g (dry weight) of soft tissues (Karayücel *et al*., 2003). From the nutritional point of view, the mussel is an important food source for supplying essential trace metals and certain vitamins such as niacin, thiamine and ribofilavin (Cheong and Lee, 1984). More over, fish and shellfish may also contain the polyunsaturated n-3 fatty acids which are biologically important and have been associated with a decreased risk of cardiovasküler (Kromhout *et al*., 1985).

Mussels are commonly sessile forms, so they are directly influenced by environmental conditions. They are also used as bio monitors for environmental pollution by heavy metals (Phillips, 1977; Rainbow and Phillips, 1993).

Mediterranean mussel has been aquacultured in Turkey since, the 1995s. There is no commercial mussel aquaculture system in the Black Sea. The raft mussel production used in the present study is the first experimental system for this region. Although, there has been a wealth of literature dealing with wild mussels,

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there is no scientific data concerning heavy metal concentrations in cultivated mussels in Turkey. Even the processes that regulate the accumulation of metals are the same in raft cultivated and wild mussels, food acquisition, growth and in many cases, metabolism may be very different. Raft mussels feed mostly on phytoplankton, which is usually abundant and high in organic content and consequently, they grow faster than wild mussels (Labarta *et al*., 1997). As they are permanently submerged, they do not enter into anaerobic metabolism like the wild mussels do when they emerge, therefore, keeping their valves closed (Saavedra *et al*., 2004).

The present study aimed to determine the trace elements concentrations (Cd, Cu and Zn) in the soft tissues of Mediterranean mussel from raft aquaculture system inner Sinop Harbour (Black Sea, Turkey), which is residential area where untreated domestic wastes and ballast waters of fishing and transport vessels are dumped and to investigate whether these concentrations are within the permissible limits for human consumption.

MATERIALS AND METHODS

Raft system construction: The sac of raft system was shaped as 50×75×300 cm. About 4 mm bracket was assembled to the top corners of each rectangle sac prism. After two rectangle floats were combined with pipe by galvanization, three number iron sticks which's length and diameter were 400 cm and 8 mm, respectively were located upper part of combined floats with interval 100 cm. After that, pine woods (10×10×400 cm) were located on the iron sticks with screws (Fig. 1).

Sampling protocol: The study was carried out inner Sinop Harbour, in the Black Sea region (Fig. 2), Turkey. Sinop coasts are still unpolluted areas in terms of industry.

The samplings were conducted monthly, between May 2005 and 2006 at the depth of 13 m. Collected mussels were immediately put into an ice compartment and transported to the laboratory for further analysis. Shell Length (SL), Shell Height (SH) and Shell Width (SWi) of mussel were measured using a vernier caliper, while Total Weight (TW) and Tissue Weight (TiW) were determined using a chemical balance. Min-max length and weight of about 271 mussels were 25.70-83.10 mm; 1.45-36.35 g, respectively (Table 1). Samples were kept at -10°C for metal analysis. Before dissection, the mussel samples were thawed at room temperature (27°C) with the posterior margin facing downwards in order to allow excess water to drain away. About 15-25 mussels were selected and analysed for heavy metals. The soft tissues of mussels were dissected by removing the byssus and the shell. The total soft tissues were dried in an oven at 105°C to constant dry weight (Yap *et al*., 2004).

Fig. 1: The raft system for mussel culture in the study area

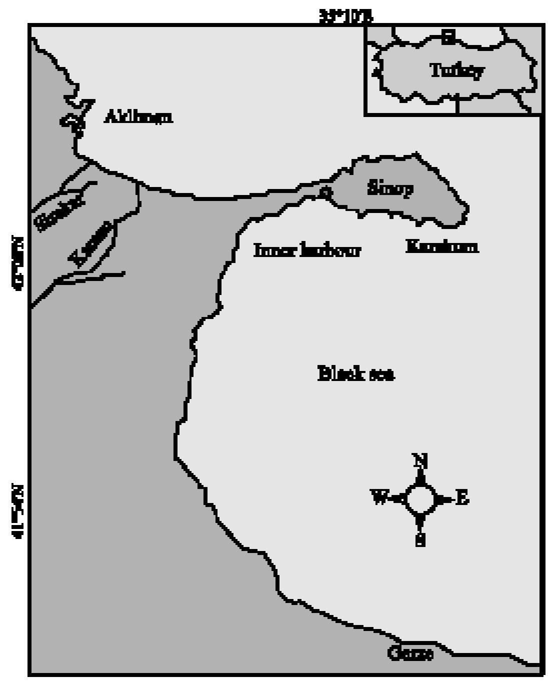

Fig. 2: Map showing the location of sampling, inner harbour, Sinop

Analysis of heavy metals: All samples were digested in concentrated HNO_3 (Merck Extra pure, 69%). They were placed in a hot block digester first at low temperature for 1 h and then they were fully digested at high temperature (140°C) for at least 3 h (Yap *et al*., 2004; Bernhard, 1976). The digested samples were then diluted to a certain volume with double-distilled water. After filtration, the prepared samples were determined for Cd, Cu and Zn by using an air-acetylene flame atomic absorption spectrophotometer (FAAS) UNICAM Model 929. The data were presented in $\mu g\ g^{-1}$ of sample dry weight (dw).

One way ANOVA in MINITAB software was used to analysis metal accumulation differences between seasons.

Table 1: Length (cm) and weight (g) measurements of mediterranean mussel (*M. galloprovincialis*)

	Spring	Summer	Autumn	Winter
SL	64.02±0.99	58.10±0.64	49.62±0.58	59.22±0.81
SH	23.07±0.46	21.00±0.27	17.85±0.25	21.21±0.35
SWi	30.61±0.46	29.09±0.46	24.70±0.26	29.01±0.42
TW	11.35±0.50	09.40±0.44	06.06±0.20	10.31±0.35
TiW	02.70±0.11	02.15±0.09	01.32±0.06	03.08±0.14

SL: Shell Length; SH, Shell Height; SWi: Shell Width; TW: Total Weigth; TiW: Tissue Weight

Table 2: Seasonal changes in heavy metal concentration of *M. galloprovincialis*

	Cd ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
Spring	2.35±0.30[a]	6.30±0.53[a]	221.09±17.74[a]
Summer	2.08±0.28[a]	6.90±0.57[a]	200.23±18.05[a]
Autumn	2.95±0.55[a]	7.92±0.70[a]	296.97±46.58[a]
Winter	2.51±0.31[a]	7.10±0.62[a]	182.21±31.60

[a]Values are mean±standard error; Common superscripts in the same column signify means which are not significantly different (p>0.05)

RESULTS AND DISCUSSION

The mean Cu, Cd and Zn were given in Table 2. There were no significant differences between the metal concentrations among the seasons (p>0.05).

Heavy metals are present in a significant concentration in the marine sediment; soil etc., from natural sources. Under changed environmental conditions, which are caused by secondary polluting compounds, such as photo-oxidation products of the petroleum, metals can be mobilized, dissolved and become bio available in the ecosystem (Bu-Olayan and Subrahmanyam, 1997).

Metal accumulations in bivalve and gastropods have been studied by several investigators in the Black Sea Region and the different areas of the Sinop Peninsula (Table 3). The present study showed that there was an increase in Cu, Cd and Zn for *M. galloprovincialis* comparing with the studies of Ünsal *et al.* (1995, 1998) (for Cu), Bat *et al.* (1999), Topçuoglu *et al.* (2002) (only for Cd) and Çulha *et al.* (2007). Same trend was also valid for *R. venosa*. The reason could be an increase in the discharge of untreated domestic wastes, harbour activities, dumping of ship wastes, other coastal activities and local population especially in the summer.

Human consumption: The potential hazard of metals has long been recognized. People can be exposed to toxic chemicals that accumulate in fish and shellfish taken from contaminated waters that are consumed (Svensson *et al.*, 1995). However, most current health risks associated with seafood safety originate in the environment. For example, Han *et al.* (1994) reported the copper intake and health threat by consuming seafood from copper-contaminated coastal environments (Erhjin Chi estuary) in Taiwan. Because of the incident of green oysters in the Charting coastal area, the green oysters collected from the Erhjin Chi estuary on 26 January 1989 gave the highest copper concentration of 4.401±79 $\mu g\ g^{-1}$ dry wt. (Han *et al.*, 1994; Han and Hung, 1990). The area around the Erhjin Chi estuary was especially affected by large discharges of heavy metals from acid cleaning of metal scrap on the riverbanks. All seafood industry suffered from the green oyster incident because consumers were so afraid of its products. People consuming large amounts of contaminated seafood may have elevated concentration of heavy metals in their tissues compared to the general population (Asplund *et al.*, 1994; Dewailly *et al.*, 1994).

The mean Cd and Zn concentration were 2.53±0.19 and 228.00±16.75 $\mu g\ g^{-1}$, respectively and which were higher than the acceptable limit proposed by EPA (Anonymous, 2005), EU(Anonymous, 2001) and TFC (2002) in *M. galloprovincialis* (Table 4). It should be emphasized that Zn, Cu and Cd are accumulated in human tissues and they are harmful to human health. It is known that most human exposure to Cd is from food. Cd can be accumulated with natural compound as metallothioneins and 3.00-330.00 mg day^{-1} is toxic and 1.5-9 mg day^{-1} is lethal to man (Bowen, 1979).

Most living organisms need small amount of some essential metal such as iron (Fe), manganese (Mn), Cu and Zn for their vital processes (Bryan, 1976). However, these metals become toxic when they exceed certain limits (Rainbow, 1985). The non-essential metals such as Cd, lead (Pb), mercury (Hg) and silver (Au) are toxic even at relatively low concentration (Bryan, 1976). Especially, Cd occurs naturally in ores together with Zn, Pb and Cu. Cd compounds are used as stabilizers in PVC products, colour pigment, several alloys and, now most commonly, in rechargeable nickel-cadmium (Ni-Cd) batteries. Cd is also present as a pollutant in phosphate fertilizers. Natural as well as anthropogenic sources of Cd, including industrial emissions and the application of fertilizer and sewage sludge to farm land, may lead to contamination of soils and to increased Cd uptake by crops and vegetables, grown for human consumption. Cigarette smoking is also a major source of Cd exposure. Food is the most important source of Cd exposure in the general non-smoking population in most countries. Long-term high Cd exposure may cause skeletal damage, first reported from Japan, where the itai-itai (ouch-ouch) disease (a combination of osteomalacia and osteoporosis) was discovered in the 1950s (Järup, 2003).

Bellinger and Benham (1978) and Young *et al.* (1979) showed that human activities or anthropogenic sources of metal input into the marine environment include contamination from ships in docks and harbor activities that involve the use some heavy metals in antifouling

Table 3: Comparison of reported concentration (μg g^{-1}) of zinc (Zn), copper (Cu) and Cadmium (Cd) in *Mytilus galloprovincialis* and *Rapana venosa* from regional studies with the present results and those from other studies done in Black Sea region

Species	Region	Zn	Cu	Cd	Reference
R. venosa	Bosporus	83.00±5.000	82.00±14.00	4.90±1.30	Topçuoglu *et al.* (1994)
R. venosa	Fatsa	49.00±6.000	57.00±8.000	1.00±0.50	Topçuoglu *et al.* (1994)
R. venosa	Sinop coasts	11.00-78.00	1.00-10.000	0.04-0.22	Bat *et al.* (2000)
R. venosa	Marmara sea	20.50-41.50	29.40-39.30	0.02-0.04	Altug and Guler (2002)
R. venosa	Amasra	40.60±0.200	17.01±0.030	2.19±0.02	Topçuoglu *et al.* (2002)
R. venosa	Persembe	44.60±0.100	35.02±0.140	0.37±0.03	Topçuoglu *et al.* (2002)
R. venosa	Rize	68.30±0.300	57.83±0.190	<0.02	Topçuoglu *et al.* (2002)
R. venosa	Sinop coasts	59.85±4.440	44.25±2.810	3.44±1.45	Çulha *et al.* (2007)
M. galloprovincialis	Sinop coasts	--	0.81-0.9300	--	Ünsal *et al.* (1995)
M. galloprovincialis	Trabzon coasts	87.89-326.530	6.80-122.40	--	Boran and Karaçam (1997)
M. galloprovincialis	Sinop coasts	71.00-188.000	1.20-1.9000	0.33-1.05	Ünsal *et al.* (1998)
M. galloprovincialis	Istanbul coasts	28.98-2035.71	1.39-442.43	--	Atayeter and Koksal (1998)
M. galloprovincialis	Sinop coasts	01.58-7.28000	0.10-1.8900	0.03-0.27	Bat *et al.* (1999)
M. galloprovincialis	Sinop coasts	256.40±1.3000	8.01±0.0200	1.79±001	Topçuoglu *et al.* (2002)
M. galloprovincialis	Amasra	512.00±2.6000	7.26±0.0200	6.44±0.01	Topçuoglu *et al.* (2002)
M. galloprovincialis	Rize	78.12±0.15000	11.52±0.020	<0.02	Topçuoglu *et al.* (2002)
M. galloprovincialis	Marmara sea	208.00-319.00	6.70-9.5000	1.26-2.88	Topcuoglu *et al.* (2004)
M. galloprovincialis	Bosporus	48.00-54.0000	16.40-29.30	0.05-0.08	Altug and Guler (2004)
M. galloprovincialis	Sinop coasts	150.32±17.410	1.21±0.2500	6.83±0.30	Çulha *et al.* (2007)
M. galloprovincialis	Sinop coasts	228.00±16.250	6.98±0.3100	2.53±0.19	This study

Table 4: Guidelines on heavy metal for food safety set by different countries

	Cu	Zn	Cd
Anonymous (2005) (EPA)	54.00 mg kg^{-1}	410.00 mg kg^{-1}	1.40mg kg^{-1}
FAO limits fresh weight[a]	10-30 μg g^{-1}	40-100 μg g^{-1}	2.00 μg g^{-1}
FAO limits dry weight[a]	50-150 μg g^{-1}	200-500 μg g^{-1}	10.00 μg g^{-1}
Anonymous (2001) (EU)	--	--	1.00 μg g^{-1}
TFC (2002)	20.00 mg kg^{-1}	50.00 mg kg^{-1}	1.00mg kg^{-1}
This study	6.98 μg g^{-1}	228 μg g^{-1}	2.53 μg g^{-1}

Wagner and Boman (2004)

paints and other metals, including Pb, Cu and Zn, in preservative paints. The local population in Sinop is about 25000, increasing to 60000 in summer. Thus, untreated domestic wastes and human activity along the coastal zone increase in July and August and probably give rise to high metal concentrations (Bat *et al.*, 1999). In the last decade, urbanization, domestic wastes, the dumping of ship wastes, harbour activities and other coastal activities and human activity have been taking place in Sinop inner harbour areas, accompanied by an increase in heavy metal pollution associated with organic pollution in the coastal environment, especially near estuaries (Çulha *et al.*, 2007; Bat and Öztürk, 1997). Although, the Cd and Zn levels were high in *M. galloprovincialis* for human consumption, the study of Çulha *et al.* (2007) showed that the accumulation of trace metals in fish is much lower than mollusc and so fish are in consumable limit in this area.

CONCLUSION

From the public health point of view, Cd and Zn levels in the raft cultivated mussels in this study were generally higher than the permitted levels inner Sinop Harbour, in the Black Sea region. Therefore, offshore mussel aquaculture, the last trend on the worlwide, is advised in this region. So, it is thought that, the offshore cultivated mussels is more healty for human consumption due to its distance to domestic and solid wastes in the coastal waters.

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