



# Investigation of the nutritional environment of the differences in toxicity levels of some heavy metals and pesticides examined in gilthead bream fishes

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## Abstract

In this study, we investigated the differences between the gilthead bream fishes that live in cages and feed by fish feed (CF), had escaped from the cages and started to live in their natural medium (EF), and live in their natural medium and do not feed by fish feed (NF). We would like to demonstrate the micro pollutants and heavy metal residues in CF, EF and NF and reveal out the diversity in living in three different ambient conditions. We analyzed Mn, Cr, Zn, Sn, Ag, Hg, Cd, Cu, Ni and Pb residues and p-p'-DDE,  $\alpha$ -BHC, endosulfan-sulfate, endrin, heptachlor, heptachlor epoxide, methoxychlor, p-p'-DDD, p-p'-DDT,  $\beta$ -BHC, aldrin, cypermethrin, dieldrin ve endosulfan pesticides in three types of gilthead bream (CF, EF and NF) and also researched the heavy metal residues in the marine sample that provided from Guvercinlik Cove in three different time intervals by gas chromatography-mass spectroscopy (GC-MS) and Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Physical and chemical parameters of sea water samples were studied to reveal out the effect of fish feed between the regions. Heavy metal and organochlorine pesticides residues of gilthead bream muscle tissues from CF are remarkable higher than other regions when we evaluate the all results. The results show an indication of the significant health risks associated with the consumption of these contaminated fish in the cages. The morphology of gilthead bream fish liver tissue samples were studied by using scanning electron microscopy (SEM).

**Keywords:** heavy metal; organochlorine pesticides; toxicity; gilthead bream fish; seawater.

**Practical Application:** Determination of toxicity in fishes that living in three different ambient conditions.

## 1 Introduction

Today, the effects of heavy metals have a great importance for human health and the environment, especially in aquatic food products. Heavy metals can be classified in three groups which are potentially toxic (arsenic, cadmium, lead, mercury, silver, etc.), probably necessary (nickel, cobalt) and required (copper, zinc, iron, manganese) (Dural et al., 2007, 2006). Heavy metals can leave a long lasting effect on the body even if they are taken to the body in low concentrations (Suomi et al., 2018). Organochlorine pesticides (OCPs), which have been used extensively since the 1940s, are a major risk factor for human health. This type of pesticide is used for industrial and agricultural purposes and causes environmental pollution (Parrino et al., 2021; Witzczak et al., 2018). OCPs are used in Turkey since 1945; the use of them has been restricted since 1983. Unfortunately, the data that obtained by other researchers demonstrated the widespread use of these compounds in Turkey.

In our country, gilthead bream farming began in the 1980s. Gilthead bream is the most cultivated fish species in our country. Bodrum, Guvercinlik is one of the most important coves where gilthead bream cultivating is done. Gilthead bream cultivating is carried out either by water taken from the sea in the pools, or by the pools (cages) that placed in the sea. Fresh and frozen fish

with low economic value or cheap price are passed through the meat grinder to make a fish feed and fishes are fed by these fish feed. It is mixed powdered gilt head bream fry (1:1 w/w) to this fish feed and it is obtained a new feed which can be consumed by juvenile fishes. They fed with this feed 4-6 times a day and 10-15% of their weight. It is generally added antibiotics and pesticides (especially fungicides) into these feeds to protect the fishes from diseases and this is an important issue that be considered and taken precautions at this point. The overuse of antibiotics and pesticides threatens a danger for the fishes that lives in the cage.

In this study, we investigated the differences between the gilthead bream fishes that live in cages and feed by fish feed (CF), the gilthead bream fishes that had escaped from the cages and started to live in their natural medium (EF) and the gilthead bream that live in their natural medium and do not feed by fish feed (NF). We would like to demonstrate the micro pollutants and heavy metal residues in CF, EF and NF and reveal out the diversity in living in three different ambient conditions. We analyzed the Mn, Cr, Zn, Sn, Ag, Hg, Cd, Cu, Ni and Pb residues and p-p'-DDD, p-p'-DDT, p-p'-DDE,  $\alpha$ -BHC,  $\beta$ -BHC, heptachlor epoxide, heptachlor, endosulfan-sulfate, dieldrin,

Received 29 Apr., 2021

Accepted 13 May, 2021

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endrin, aldrin, methoxychlor, cypermethrin, and endosulfan pesticides in three types of gilthead bream (CF, EF and NF) and also researched the heavy metal residues in the sea water sample that provided from Guvercinlik cove. Additionally, the analyzing of temperature, dissolved oxygen, pH, conductivity, hardness, turbidity, salinity, total organic carbon (TOC), UV,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and  $\text{PO}_4^{3-}$  anions of the sea waters that obtained from the sample collection areas were carried out and physical and chemical parameters of the Guvercinlik cove's sea waters were determined. It was tried to be investigated the effect of sea water on gilthead bream fishes (CF, EF and NF) that lives in three different mediums with the analyzing of sea water.

## 2 Material and methods

### 2.1 Sample preparation and collection

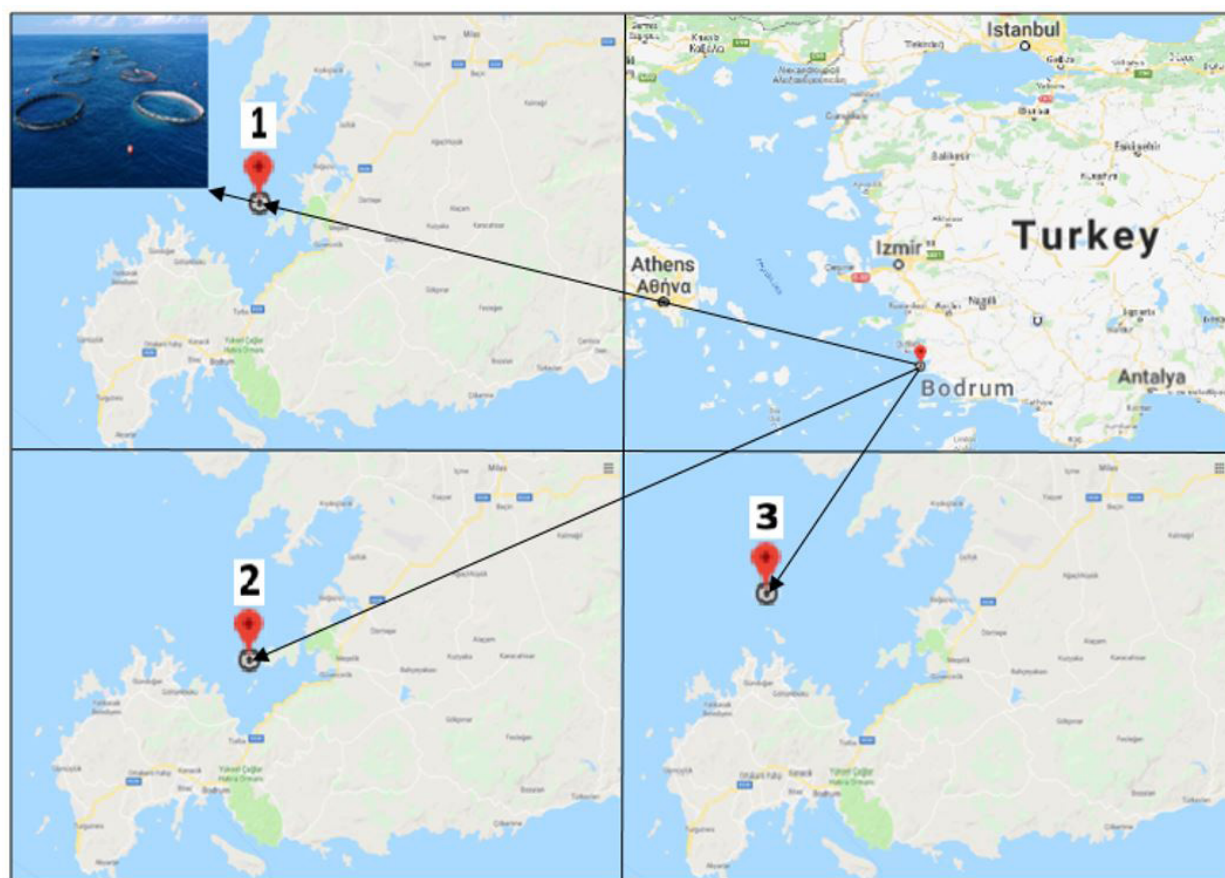
Bodrum is one of the 13 districts of Mugla where is located in the south of Turkey. Bodrum is one of the most important tourism centers of Turkey and the World. Additionally, Bodrum is a locomotive of Turkey and Europe in fish farming as well as being an important tourism center. The fish farms are located in the vicinity of Guvercinlik Cove and Salih island of Bodrum and they are 20 km far away from the center of Bodrum. CF fishes were grown in these fish farms and provided from a local market. Figure 1 presents the sample collection areas of CF, EF and NF. Figure 1(1-3) show the coordinates of sampling area

of CF (37° 16' N, 27° 48' E), EF (37° 14' N, 27° 48' E) and NF (37° 21' N, 27° 35' E), respectively. The samples of EF and NF were collected 3 pieces for each of them in February 2019 by the support of fishermen. Sea water samples were provided in three different time intervals in August 2018, November 2018 and February 2019 according to the coordinates that presented in Figure 1(1-3) and 10-30 cm deep from the surface. The samples were transferred to our laboratory in polyethylene boxes at +4 °C. Each of fish samples were dried with filter paper and washed with purified water and kept at -20 °C until they were analyzed. The sea water samples also were kept at +4 °C until they were analyzed too (Karaca et al., 2014). The fish samples were teared to pieces by mixer before starting to analyze and got ready to extraction and microwave solubilization process for the determination of pesticides and heavy metals.

The samples were extracted according to AOAC QuEChERS, method (Lehotay et al., 2007). And the following procedure is same as our previous study (Topal & Onac, 2020).

### 2.2 Instruments and chemicals

The determination of the target elements and pesticides was achieved by GC-MS (Thermo TRACE™ 1300 ISQ LT Single Quadrupole Mass Spectrometer (Thermo Fisher, Inc., Waltham, MA, USA) and ICP-MS (NexION 2000 B Perkin Elmer). We used microwave digestion system (CEM MARS-6,



**Figure 1.** The coordinates of sampling area of CF (37° 16' N, 27° 48' E) (1), EF (37° 14' N, 27° 48' E) (2) and NF (37° 21' N, 27° 35' E) (3).

closed vessel system, Matthews, NC in USA) preliminary of the fish samples for the analysis of ICP-MS. All organic solvents and chemical were provided from Sigma Aldrich as analytical grades. (ACN (purity  $\geq 99.7\%$ ), Acetone (purity  $\geq 99.9\%$ ), Methanol (MeOH) (purity  $\geq 99.9\%$ ), Acetic acid (purity  $\geq 99.7\%$ ), Toluene (purity  $\geq 99.8\%$ ),  $\text{MgSO}_4$ , NaOAc). The concentration of calibration solutions were prepared with ICP-MS standard solutions that provided from Perkin Elmer. All pestanal<sup>®</sup> analytical standards were purchased from Dr. Ehrenstorfer Reference Materials. All laboratory equipment were washed  $\text{HNO}_3$  solution before use (1/6, v/v) for inorganic determinations.

### 2.3 GC-MS analyses

The separation and determination of target pesticides was achieved by GC-MS according to the same procedure in our previous study (Topal & Onac, 2020). All the selective pesticides were presented in Table 1 with their retention times and selected ions (m/z volume). We obtained the calibration curve of the target pesticides were obtained the mass detection of each compounds with three fragment ions and equations for each compounds.

### 2.4 ICP-MS analyses

The muscle tissues each fish samples digested by microwave system, before analyzing by ICP-MS. 0.5 g (wet weight) of muscle tissue was weighted and added 10 mL  $\text{HNO}_3$  in One Touch Food method. Iprep vessels were preferred to use during digestion. Ramp time of the method is 30 min at 210 °C and the samples hold this temperature for 15 min. Table 2 presents ICP-MS spectrometer parameters for the determination of fish samples. The concentration of calibration solutions were diluted from 1000 mg/kg to 1  $\mu\text{g}/\text{kg}$ , 5  $\mu\text{g}/\text{kg}$ , 10  $\mu\text{g}/\text{kg}$ , 30  $\mu\text{g}/\text{kg}$ , and 500  $\mu\text{g}/\text{kg}$  respectively. The determination of Hg was achieved by spiking Au standard to the blank and standard solutions. Accuracy of the results was applied using different statistical methods at 95% confidence interval, significance,  $p < 0.05$ .

### 2.5 Other analyses

Anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ ) analyses of sea water samples that provided in three different time intervals in August 2018, November 2018 and February 2019, were achieved by Ion Chromatography System (DIONEX ICS-1000, United States of America with Ion-pac AG9-SC column. WTW InoLab pH7110 and Cond 7110 apparatus were used for pH, conductivity, and salinity determination of the sea water samples by using SM 2510 B, SM 4500-H<sup>+</sup> B and SM 2520 B methods (American Public Health Association, 1985, 2018b, 2018c; Eaton et al., 1996). Turbidity was determinate by Velp Scientifica TB1 (Italy) Turbidimeter apparatus, SM 2130B method (American Public Health Association, 2018a). Hardness of the sea water samples carried out by titrimetric determination based on SM2340 C method (American Public Health Association, 2018d). The determination of dissolved oxygen was obtained WTW inolab Oxi 730 by using SM 4500-O G method (American Public Health Association, 2018c). Total organic carbon analyses were achieved with Sievers M5310 instrument (United States

of America). Shimadzu 1800 spectrometer was used for  $\text{UV}_{254}$  and  $\text{UV}_{272}$  analyses at 254 and 272 nm wavelengths.

### 2.6 Surface characterization of fish liver

The morphology of gilthead bream fish liver tissue samples were studied by field emission scanning electron microscope (FESEM, Zeiss Supra 40VP). The magnification of FESEM image was 1000 $\times$  with 7 kV (Figure 2). EDS analysis of the gilthead bream liver tissue was compatible with heavy metal analysis by ICP-MS. We observed more heavy metal residues by ICP-MS due to limit of detection of mass spectrometry when we compared to EDS analysis of liver tissue by FESEM.

### 2.7 Ethics statement

The authors confirm that no ethical approval was needed in this study.

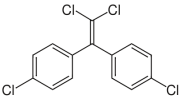
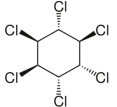
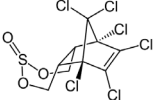
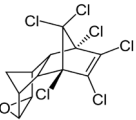
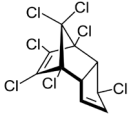
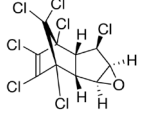
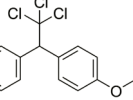
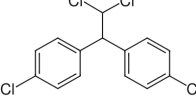
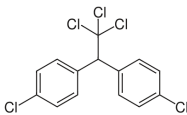
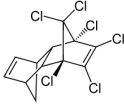
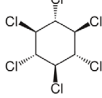
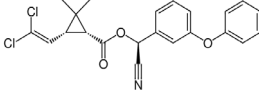
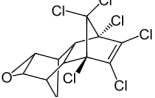
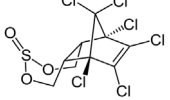
## 3 Results and discussion

### 3.1 Physical and chemical parameters of sea water samples in specified months

Seawater is a mixture of 72 elements, most of them in very small quantities, and therefore it is described as an almost "infrequent solution of all things". In other words, almost all of the elements naturally exist in the sea water in the world. The most abundant elements in seawater are chloride (Cl), sodium (Na), sulfur (S), magnesium (Mg), calcium (Ca) and potassium (K), respectively. Apart from these six elements, which constitute 90 percent of all minerals, there are various elements starting from bromine (Br) and carbon © to gold (Au), iridium (Ir) and even platinum (Pt). Regardless of the density of the salt, whether in polar seas or tropical seas, the proportions of the inorganic (carbon-free) compounds it contains are almost identical.

In this study, we would like to reveal out a detailed investigation regarding with Guvercinlik Cove's sea waters and the difference between the gilthead bream fishes that lives in CF, EF and NF. For this purpose, we investigated the physical and chemical parameters of Guvercinlik Cove's sea water samples that collected in three different time intervals, firstly (Table 3). pH of sea water samples did not change seriously between three different time intervals from each sea sampling area, CF, EF and NF. The pH values in each area are closed together; pH is changing between 8.24 and 7.68 in CF, EF and NF regions. According to the general quality a criterion of seawater pH parameter varies are between 6.0 and 9.0 and the values obtained are within these limits (Turkey, 2004). The temperature of sea water samples is nearby 25 °C in August 2018 and 17 °C in February 2019 for each region. This phenomenon presents the effect of seasonal change in sea water. The turbidity parameter increased to the highest value in CF in February as 4.34 NTU, while the lowest value was determinate as 3.13 NTU in November in EF. We suggest that the high turbidity during the winter period depends on the circulation of organic and inorganic substances in the seas with surface and dip flow depending on the heavy rainy weather conditions in this period. Furthermore, it is thought that turbidity is higher in CF than EF and NF regions due to the

**Table 1.** Retention times and selected ions of target pesticides.

Pesticide Molecular Structure	MW	Retention time, min	<i>m/z</i>
	318.03 g/mol	20.41	246, 318, 248
	290.83 g/mol	15.41	181, 183, 219
	422.9 g/mol	21.95	272, 229, 387
	380.907 g/mol	20.91	81, 263, 241
	373.32 g/mol	17.75	100, 272, 237
	389.3 g/mol	19.28	353, 263, 183
	345.65 g/mol	23.12	227, 212, 196
	354.49 g/mol	21.27	235, 165
	320.04 g/mol	21.19	235, 165
	364.90 g/mol	18.46	66, 263, 193
	290.83 g/mol	16.13	181, 219, 109
	416.3 g/mol	25.26	163, 181, 165
	380.91 g/mol	20.48	79, 108, 263
	406.93 g/mol	19.98	195, 241, 277



dissolution of feeds in seawater and this increases the colloidal and suspended solids content.

Conductivity of sea water increased to its highest value (48.9 mS/cm) in August in CF and the lowest 46.9 mS/cm in NF in February. The high conductivity in the CF is interpreted as the maximum conductivity depending on the amount of dissolved substance in the water. It was determined that the conductivity parameter of the water supplied from the regions was higher in August periods due to the fact that evaporation was higher in summer than in winter.

The highest salinity parameter was 39.9% in the CF in August period and 37.9% in the EF in February. The high salinity in the cage (CF) is thought to be due to anion or cation salts by dissolving the synthetic feeds that used for fish (Kalkan & Altuğ, 2015).

Sulfate concentrations of EF and NF samples were observed as 3196-3310 mg/L, while 3780-3900 mg/L was observed in CF. It is thought that there are anionic and cationic sulfate salts in feeds and of course in sea water. The highest nitrite concentration was 0.0423 mg/L in February in CF and the lowest nitrite

concentration was determinate as 0.0176 mg/L in November in EF. The high values in the CF are in agreement with the results that obtained in other parameters. It is thought that gilthead bream's feces that grown in cages and nitrogen compounds which produced by fertilizers increase the concentration of nitrogen

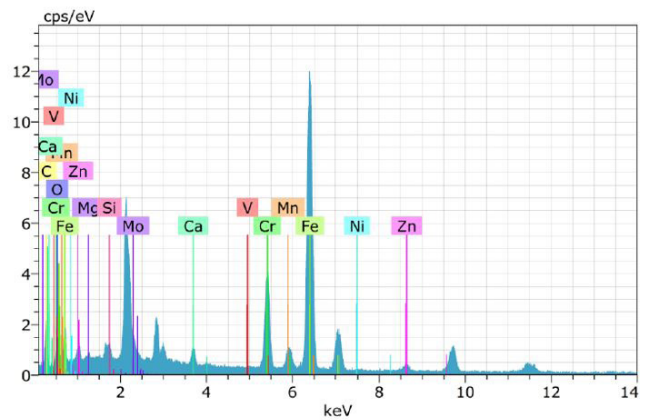
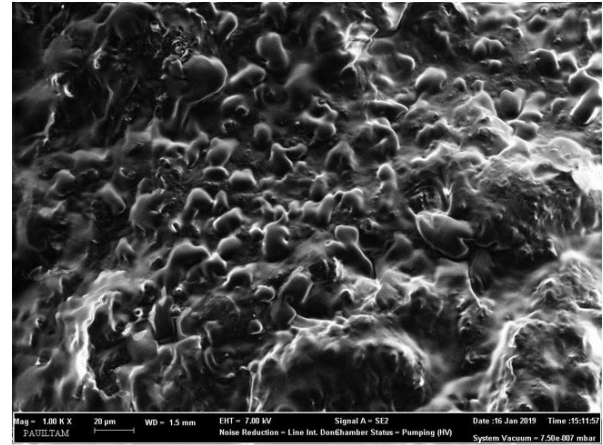


Figure 2. SEM image and EDS analysis of gilthead bream liver tissues.

Table 2. ICP-MS parameters for determination of target heavy metals in fish samples.

Rf Power (W)	1300
Gas flow rate (mL/min)	1.5
Coolant gas (mL/min)	15
Auxiliary gas (mL/min)	0.2
Nebuliser gas (mL/min)	0.65
Sample flow rate (mL/min)	1.5
Flush time (sec)	20
Read time (s)	3

Limit of detections of target heavy metals were Pb: 0.04 ngL<sup>-1</sup>, Hg: 1 ngL<sup>-1</sup>, Cu: 0.2 ngL<sup>-1</sup>, Zn: 0.7 ngL<sup>-1</sup>, Ag: 0.09 ngL<sup>-1</sup>, Cd: 0.07 ngL<sup>-1</sup>, Mn: 0.1 ngL<sup>-1</sup>, Cr: 0.3 ngL<sup>-1</sup>, Sn: 0.2 ngL<sup>-1</sup> and Ni: 0.2 ngL<sup>-1</sup>; The concentration of calibration solutions were 1 µg/kg, 5 µg/kg, 10 µg/kg, 30 µg/kg, and 500 µg/kg respectively; Certified levels of standard Bi, In, Sc and Ge were used as internal standards for ICP-MS analysis.

Table 3. Physical and chemical parameters of sea water samples in specified months.

Parameter		CF			EF			NF		
		2018 August	2018 November	2019 February	2018 August	2018 November	2019 February	2018 August	2018 November	2019 February
pH	-	8.04	7.94	8.0	8.24	8.06	7.90	7.68	7.70	7.72
Temperature	°C	25.4	24.4	17.6	26.1	23.6	16.5	27.6	26.8	16.6
Turbidity	NTU	3.72	3.92	4.34	3.36	3.13	4.25	3.45	3.15	3.80
Conductivity	mS/cm	48.9	48.8	48.2	47.9	47.6	47.4	47.4	47.5	46.9
Salinity	%	39.9	39.1	39.6	38.2	38.3	37.9	38.1	38.0	38.9
Sulfate	(mg/l)	3900	3896	3780	3254	3296	3214	3196	3299	3310
Nitrite	(mg/l)	0.0415	0.0391	0.0423	0.0198	0.0176	0.0254	0.0219	0.0294	0.0314
Nitrate	(mg/l)	15.7569	16.8934	17.3578	6.2332	5.1449	4.6634	3.5885	4.6970	3.9889
Phosphorus	(mg/l)	4.0563	3.9863	4.0330	0.6932	0.4408	0.2825	0.3289	0.6891	0.3941
Chlorine	(mg/l)	25550	26000	25650	24140	24002	24390	24260	24583	24790
Dissolved O <sub>2</sub>	(mg/l)	5.99	5.86	5.74	6.08	6.19	6.34	7.05	7.00	6.94
UV <sub>254</sub>	cm <sup>-1</sup>	0.203	0.246	0.198	0.105	0.163	0.145	0.096	0.084	0.101
UV <sub>272</sub>	cm <sup>-1</sup>	0.141	0.136	0.154	0.74	0.98	0.64	0.66	0.85	0.91
Hardness	(French degrees)	724	702	690	688	695	687	713	696	705
TOC	mg/l	3.24	3.16	2.98	2.65	2.85	2.59	2.66	2.71	2.80

concentration in sea water sample from CF. The highest and lowest nitrate concentration were determinate as 17.3578 mg/L in February (in CF) and 3.5885 mg/L in August (in NF), respectively. Nitrite and nitrate concentrations of the sea water samples from each region were below the limit values when we consider the Seawater general quality criteria (Turkey, 2004). Total phosphorus concentrations in fish farming; depending on excessive stocking and feeding rates, amount of suspended solids, intensive phosphate fertilizer usage. It is observed that there is 10 times difference between CF and other regions when compared the results. The use of dense phosphate fertilizers, feed rate, suspended solids content and fish feces could be cause a high phosphate concentration. The highest chloride concentration was determinate as 26000 mg/L in CF (in November), the lowest was determinate as 24002 mg/L in EF (in November). There is not a big difference in chloride concentrations between the regions. The highest and lowest dissolved oxygen concentration was 7.05 mg/L in August, in NF and 5.74 mg/L in February in CF due to high amount of fish in the cages.

The highest  $UV_{254}$  value was measured in CF with a value of  $0.246\text{ cm}^{-1}$  in November, while the lowest  $UV_{254}$  absorbance level was measured in NF water with  $0.084\text{ cm}^{-1}$ . The  $UV_{272}$  absorbance parameter was also measured with the highest value of  $0.154\text{ cm}^{-1}$  in February in NF. The lowest  $UV_{272}$  value was determined as  $0.64\text{ cm}^{-1}$  in February in EF. As the amount of organic matter in the water increased, the amount of organic matter with aromatic content increased as well. These compounds absorb UV light at 254 and 272 nm due to double bonds in their structure. For this reason,  $UV_{254}$  and  $UV_{272}$  parameters are used as an indicator parameter in order to determine the content of aromatic substances in water. The high  $UV_{254}$  and  $UV_{272}$  values indicate a high humic content in the CF. The highest value for the hardness parameter was measured 724 (French degrees) in August in the CF and the lowest value was measured 687 (French degrees) in February in the EF (1 mg  $\text{CaCO}_3/\text{L}=0.1$  French degrees).

Physical and chemical parameters values of the CF were higher than other regions' sea water samples when we evaluate the all results. Parallel to these results, TOC values also are expected to be high in CF. The highest TOC value was analyzed as 3.24 mg/L in August in the CF and the lowest one was analyzed 2.59 mg/L in the EF in February too. It is thought that the high TOC value in the CF is due to the high amount of organic matter.

### 3.2 Heavy metal analysis of sea water samples and gilthead bream by ICP-MS

Table 4 presents the threshold limit values of heavy metals in marine water according to different standards. It was studied Cr, Zn, Sn, Hg, Cd, Cu, Ni, Pb, Fe and Mn residues of Guvercinlik Cove's marine water samples from three different regions (CF, EF, NF) in three different time intervals. Some of the heavy metals that we studied in this paper are within the scope of national and international general quality criteria but some heavy metals do not have limit values. With this study, we would like to draw attention to these heavy metals which have high toxicity for the environment and do not have limit values in national and international general quality criteria.

EC No 1881/2006 presents ML of Sn, Cd, Pb and Hg in a foodstuff that depicts the ML of chemical pollutants in the muscle meat of fish (European Union, 2006b). Table 5 presents the permissible upper limits of heavy metal in various standards in fishes.

The absorption of chromium by food depends on the load of chromium but is less than 10%. It is thought that most of the hexavalent chromium entering our body and it is transformed into trivalent chromium, which has low bioavailability (Türkmen et al., 2009). However, this conversion occurs when exposed to a low dose of chromium. This transformation is important because the hexavalent chromium cannot pass through the cell membrane, while the trivalent chromium can pass through the cell membrane and has a much higher toxic effect. Unfortunately, there is no upper limit of chromium that can be found in foods, while the limit for drinking water is set at 50 ppb by 98/83/EC (European Union, 1998). The toxic effect of hexavalent chromium on humans has been fully demonstrated by cancer cases from textile and mining workers. Table 6 and Table 7 present heavy metal residues of CF, EF and NF regions of sea water samples and gilthead bream fishes that live in CF, EF and NF. The concentrations of Cr in muscle tissues of gilthead bream 0.96  $\mu\text{g/g}$  (in CF), 0.51  $\mu\text{g/g}$  (in EF) and 0.23  $\mu\text{g/g}$  (in NF) (Figure 3). Cr concentration of sea water samples are 0.71 mg/L, 0.65 mg/L, 0.72 mg/L in CF, 0.09 mg/L, 0.08 mg/L, 0.12 mg/L in EF and 0.06 mg/L, 0.08 mg/L, 0.10 mg/L for three different time intervals. The high chromium concentration is not surprising when chromium concentration amounts are examined in the sea water samples. Besides all these toxic effects of chromium there is not determine any maximum level (ML) for Cr in any

**Table 4.** The threshold limit values of heavy metals in marine water.

Concentration of heavy metal ( $\mu\text{g/L}$ ) ppb	Cr	Zn	Sn	As	Hg	Ag	Cd	Cu	Ni	Pb	Mn
Nature concentration of marine water (Environmental Protection Agency, 2002) <sup>1</sup>	0.05	10	NA	3	0.03	NA	0.11	3	5.4	0.3	NA
International Marine EQS <sup>2</sup>	20	100	NA	69	4	7	2	10	50	20	NA
National Recommended Aquatic Life Criteria table (EPA) <sup>3</sup>	1.1	90	NA	69	1.8	1.9	33	4.8	74	140	NA
General Quality Criteria of Marine Water (in Turkey) <sup>4</sup>	100	100	NA	100	4	NA	10	10	100	100	NA

<sup>1</sup>EPA (United States Environmental Protection Agency, 2002) (United States Environmental Protection Agency) National Recommended Water Quality Criteria EPA-822-R-02-047, Office of Science and Technology; <sup>2</sup>Environmental Standards for Marine Quality, report by Dr. Michal Green and Dr. Marcelo Huaniko, The Technion, April 2000; <sup>3</sup>National Recommended Water Quality Criteria - Aquatic Life Criteria Table (United States Environmental Protection Agency) EPA (United States Environmental Protection Agency, 2002); <sup>4</sup>Ministry of Environment and Forestry, Regulation on Water Pollution Control, General Quality Criteria of Marine Water, Table 4, Abolished:RG-13/2/2008-26786, 2004 (Turkey, 2004). NA= non applicable.

standard. We especially draw attention to the samples taken from cages (CF). There is a big difference in Cr residues of the gilthead bream and sea water samples between CF and NF. The high chromium concentration is serious issue for gilthead bream fishes that cultivating in these cages (CF).

Copper concentration is determined as 7.56 µg/g (CF), 1.56 µg/g (EF) and 2.00 µg/g (NF) in muscle meat of gilthead bream fishes (Table 7, Figure 3). But there is no any ML for Cu in any various standards that presented in Table 5. Copper plays a major role in the formation of red blood cells and the synthesis of

hemoglobin. It removes free radicals away from the body. However, the risk of cancer increases due to excessive accumulation in the body (Dalman et al., 2006). So the concentration of copper should be take consideration in muscle meat of fish products. Copper concentration of sea water samples are 0.13 mg/L, 0.15 mg/L, 0.12 mg/L in CF, 0.05 mg/L, 0.04 mg/L, 0.03 mg/L in EF and 0.03 mg/L, 0.02 mg/L, 0.02 mg/L for three different time intervals (Table 6). There is an extremely big difference when we compare the concentration of copper with various standards in Table 4. The ML of copper is listed 3µg/L at EPA 2002 (United States Environmental Protection Agency, 2002) but in this study, especially in CF, copper concentration is almost five decades higher than ML of EPA standards. This is a really serious issue that should be control. The differences between the regions are threatening. Excessive use of fish feed could be the reason of high copper concentration in CF region.

Zinc-containing wastes have a direct toxic effect on aquatic organisms. Fishes are affected by zinc, zinc-copper or other metal compounds of zinc. Since these metals are elements, they do not suffer loss or degradation in the environment, they are transported only from one place to another. Once Zn enters the body, it can bind to specific proteins found in the blood and become insoluble grants, stored or discharged. In the presence of high levels of Zn, the detoxification mechanism will be insufficient to eliminate the excess in the body and in this case Zn will exhibit a direct toxic effect (Korkmaz Görür et al., 2012). So it is necessary to investigate the toxicity of Zn in fish products. Zinc

**Table 5.** Heavy metal limits in various standards.

Standards in fish (µg/g) ppm	Pb	Cd	Hg	Sn
Commission Regulation (EC No78/2005) <sup>1</sup>	0.2	0.05	0.5	-
Commission Regulation (EC No1881/2006) <sup>2</sup>	0.3	0.05	1.0	-
Codex Standards 193-1995 (Amendment in 2015) <sup>3</sup>	0.3	-	-	-
Turkish Food Codex (TFC) <sup>4</sup>	0.3	0.05	0.5	-
Food Standard Australia (FSANZ) <sup>5</sup>	0.5	-	0.5	-

<sup>1</sup>Commission Regulation (EC) No 78/2005 amending Regulation (EC) No 466/2001 as regards heavy metals; <sup>2</sup>Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (European Union, 2006b); <sup>3</sup>General Standard For Contaminants And Toxins In Food And Feed (CODEX STAN 193-1995) Adopted in 1995 Revised in 1997, 2006, 2008, 2009 Amended in 2010, 2012, 2013, 2014, 2015; <sup>4</sup>(TFC) Turkish Food Codex Regulation, Food Ingredients Communique on Maximum Limits; <sup>5</sup>Food Standards Australia New Zealand (2015, Federal Register of Legislation, ) (FSANZ).

**Table 6.** Heavy metal residues of CF, EF and NF regions of sea water samples (mg/L).

	Ni	Mn	Cr	Cu	Zn	Sn	Cd	Pb	Hg	Fe
<b>CF August</b>	1.24±0.11	1.26±0.03	0.71±0.05	0.13±0.009	2.45±0.047	0.26±0.017	0.05±0.002	1.86±0.092	0.0034±0.0001	24.30±0.68
<i>RSD</i>	8.6	2.0	7.1	7.2	1.9	6.6	5.0	4.9	5.0	2.8
<b>November</b>	1.38±0.09	1.35±0.08	0.65±0.02	0.15±0.003	2.36±0.061	0.24±0.018	0.04±0.002	1.98±0.143	0.0035±0.0001	39.14±1.36
<i>RSD</i>	6.2	6.0	2.8	2.0	2.6	7.5	3.6	7.2	3.8	3.5
<b>February</b>	1.58±0.09	1.45±0.08	0.72±0.02	0.12±0.003	1.96±0.059	0.15±0.004	0.04±0.001	2.14±0.075	0.0037±0.0001	39.06±1.83
<i>RSD</i>	6.0	5.7	2.1	2.6	3.0	2.4	2.5	3.5	3.6	4.7
<b>EF August</b>	0.56±0.02	0.42±0.01	0.09±0.006	0.05±0.001	0.27±0.011	0.08±0.005	0.008±0.0004	0.18±0.005	< LOD	9.56±0.17
<i>RSD</i>	1.4	1.1	5.9	2.5	4.2	5.7	5.7	2.8		1.8
<b>November</b>	0.60±0.02	0.36±0.02	0.08±0.002	0.05±0.001	0.28±0.013	0.07±0.002	0.009±0.0002	0.24±0.007	< LOD	9.44±0.10
<i>RSD</i>	2.7	6.0	2.8	1.9	4.5	3.2	2.6	3.1		1.1
<b>February</b>	0.58±0.02	0.40±0.01	0.12±0.004	0.04±0.003	0.21±0.007	0.06±0.002	0.007±0.0002	0.12±0.008	< LOD	8.74±0.17
<i>RSD</i>	4.2	1.9	3.1	8.6	2.9	3.5	3.6	7.1		2.0
<b>NF August</b>	0.26±0.01	< LOD	0.06±0.002	0.03±0.001	0.12±0.006	< LOD	0.006±0.0002	0.11±0.006	< LOD	8.16±0.12
<i>RSD</i>	2.7		2.8	1.5	5.0		2.7	5.2		1.5
<b>November</b>	0.22±0.01	< LOD	0.08±0.007	0.02±0.001	0.19±0.008	< LOD	0.003±0.00007	0.16±0.002	< LOD	8.45±0.25
<i>RSD</i>	2.7		7.6	2.2	4.0		2.5	1.1		3.0
<b>February</b>	0.25±0.01	< LOD	0.10±0.006	0.02±0.001	0.11±0.005	< LOD	0.005±0.00001	0.21±0.004	< LOD	9.86±0.26
<i>RSD</i>	2.8		6.4	2.9	4.2		1.9	2.0		2.7

Time intervals: August 2018, November 2018, August 2019.

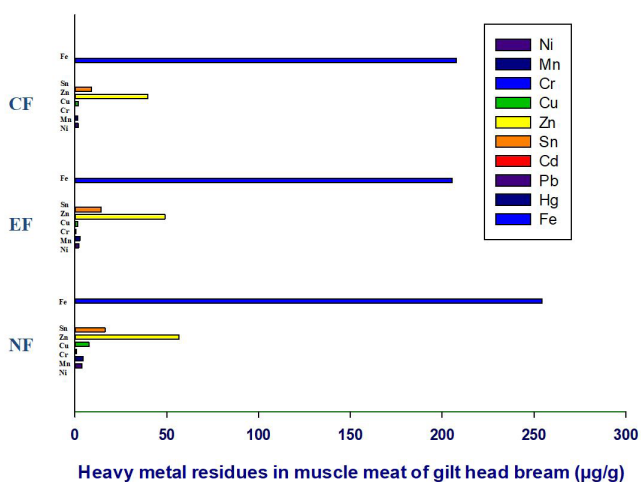
**Table 7.** Heavy metal residues (µg/g) of gilt head bream fishes in CF, EF and NF (at February 2019).

	Ni	Mn	Cr	Cu	Zn	Sn	Cd	Pb	Hg	Fe
<b>CF</b>	3.80±0.22	4.56±0.25	0.96±0.041	7.56±0.019	56.78±1.70	16.37±0.039	0.012±0.0003	0.009±0.0003	0.042±0.001	254.5±2.79
<i>RSD</i>	6.0	5.7	4.3	2.6	3.0	2.4	2.5	3.5	2.5	1.1
<b>EF</b>	2.09±0.008	2.68±0.05	0.51±0.019	1.56±0.134	48.96±1.41	14.38±0.50	< LOD	< LOD	< LOD	205.4±3.69
<i>RSD</i>	4.2	1.9	3.9	8.6	2.9	3.5				1.8
<b>NF</b>	1.72±0.048	1.56±0.029	0.23±0.006	2.00±0.058	39.77±1.67	9.11±0.25	< LOD	< LOD	< LOD	207.6±4.56
<i>RSD</i>	2.8	1.9	2.8	2.9	4.2	2.8				2.2



concentration of sea water samples were determinate 2.45 mg/L, 2.36 mg/L, 1.96 mg/L in CF, 0.27 mg/L, 0.28 mg/L, 0.21 mg/L in EF and 0.12 mg/L, 0.19 mg/L, 0.11 mg/L in NF regions for three different intervals (Table 6). ML of Zn concentrations are listed 10 µg/L (United States Environmental Protection Agency, 2002), 100 µg/L (EQS), 90 µg/L (EPA) and 100 µg/L (GQC in Turkey) in the standards. Zn concentrations of the sea water samples are over ML of various standards in all three regions (CF, EF and NF). Especially in CF, zinc concentrations are two times higher than other regions. When we analyzed zinc concentrations of gilthead bream in muscle tissues the results are not surprising (Table 7). Zn concentrations of gilthead bream are 56.78 µg/g in CF, 48.96 µg/g in EF and 39.77 µg/g in NF (Figure 3). Unfortunately there is no maximum limit value for muscle meat of fish while the maximum limit value for zinc metal is present in sea waters.

Lead is a toxic element that is environmentally common and naturally present in soil but accumulates in the body and has serious toxic effects. Normally lead is not desired in the body and blood. In adults, 94% of the body's lead is accumulated in the bones. The half-life of this lead in the bone is 27 years on average, and even if the exposure ends, the lead remains in the body for many years. In this study, the lead concentrations of sea water samples were determinate as 1.86 mg/L, 1.98 mg/L, 2.14 mg/L in CF, 0.18 mg/L, 0.24 mg/L, 0.12 mg/L in EF, 0.11 mg/L, 0.16 mg/L, 0.21 mg/L in NF for three different time intervals (Table 6). The ML of lead is listed very different levels in the various standards (Table 4). The maximum lead concentration of each region is over the permitted level according to the standards in Table 4. There is a serious issue with the lead concentration of CF region. The high lead concentration of CF region is supported and compatible with the lead concentration of muscle tissues of gilthead bream in CF. It is determine 0.009 µg/g in CF. In the literature, the amount of lead determinate as 0.22-0.85 µg/g in muscle meat of fish in the middle Black Sea region (Tüzen, 2003), while it is observed 0.33-0.93 µg/g (Uluozlu et al., 2007), 1.41-3.92 µg/g (Dural et al., 2007), 0.38-5.20 µg/g (Tepe et al., 2008) and 0.83-3.71 µg/g (Türkmen et al., 2008) in Aegean and Black Sea regions.



**Figure 3.** Heavy metal residues in muscle meat of gilt head bream fish (µg/g).

Mercury is a naturally occurring heavy metal. It can accumulate in fish bodies in the form of methylmercury, which is highly toxic. Eating fish and shellfish is an important source of mercury exposure in humans and animals. Exposure to mercury can cause serious health problems. Higher levels of mercury can harm brain function and heart health. In fact, larger, predatory fish may contain 10 times higher mercury concentrations than the fish they consume. We had determined 0.042 µg/g Hg which is under the ML in gilthead bream muscle tissues (CF region). The concentration of mercury of sea water samples had a serious issue in CF region. Hg concentration was determined as 0.0034 mg/L in August, 0.0035 mg/L in November, 0.0037 mg/L in February. We did not determine any Hg residues in the other regions.

Cadmium is one of the most dangerous heavy metal pollutants in the ecosystem and living organisms. The compounds are also highly toxic. The ML of Cd is 0.11 µg/L in EPA (United States Environmental Protection Agency, 2002) and 10 µg/L in GQC in Turkey). In Table 6, a high concentration of Cd (0.05 mg/L in August, 0.04 mg/L in November and February) in sea water samples from CF are especially remarkable different from other regions (EF and NF). This phenomenon is attributing the fish farms and using excessive fish feed in the cages. The results are appropriate with gilthead bream samples results in CF. Cd concentration was determined as 0.012 µg/g while it was not determine any in other regions. The other researchers reported the concentration of Cd in fish samples as 0.02-0.24 µg/g at Black Sea (Topcuoğlu et al., 2002), 0.02-0.35 µg/g at Marmara Sea (Dural et al., 2007), 0.13-0.47 µg/g at Marmara (Istanbul), Aegean and Mediterranean Sea (Türkmen et al., 2008, 2009).

The highest Ni concentration (1.24 mg/L in August, 1.38 mg/L in November, 1.58 mg/L in February) was observed in sea water samples which is 15 times more than other regions from CF region (Table 6). The ML of Ni is listed 0.0054 mg/L in EPA 2002(United States Environmental Protection Agency, 2002), 0.05 mg/L in EQS and 0.1 mg/L in GQC in Turkey. The amount of Ni in all sea water samples from each region are over the ML of each standard. Ni concentration of gilthead bream samples were 3.80 µg/g in CF, 2.09 µg/g in EF, 1.72 µg/g in NF (Table 7, Figure 3). It is difficult to compare the amounts of Ni in our study gilthead bream muscle samples with EC or TFC because there is not reported any ML for Ni at EC No.1881/2006 commission nor in the TFC (Regulation, 2008). There must be stated a permissible upper limit in EC and TFC when we consider of the toxicity of nickel.

Despite the toxic effects of Mn, there is no report of any maximum limit at the standards listed in Table 4. The highest Mn concentrations were observed in sea water samples (1.26 mg/L in August, 1.35 mg/L in November, 1.45 mg/L in February) from CF and muscle meat of gilthead bream (4.56 µg/g) from CF too (Table 6 and 7). In the other studies, Mn concentration was reported as 2.76-9.10 µg/g from Black Sea (Tuzen, 2009), 0.18-2.78 µg/g from Aegean Sea (Türkmen et al., 2009) and 0.10-0.99 µg/g from Mediterranean Sea (Türkmen et al., 2008).

Tin is used in various organic materials. The most dangerous forms of tin are organic tin bonds for humans. Tin compounds are used in agricultural field and in many industries such as pesticides, plastic industry, and paint industry. The number of areas where organic tin materials are used is increasing every



day. High concentrations of organotin in marine units, especially in mussels and oysters, which are hunted from waters with high concentrations of organotin, are very important because of the toxic effects of these compounds on humans. The highest tin concentration was determined 16.37 µg/g in gilthead bream muscle from CF region. The same applies to the sea water samples. Sn concentration of sea water samples are 0.26 mg/L in August, 0.24 mg/L in November, 0.15 mg/L in February at CF, 0.08 mg/L in August, 0.07 mg/L in November, 0.06 mg/L in February at EF. We did not determine any Sn amount from NF. Unfortunately, there is not listed any ML for Sn at General Quality Criteria of Marine Water (in Turkey) nor EPA (Table 4). According to the results, Sn concentration of sea water and gilthead bream increases in CF region which is related with fish farming.

Iron is an essential element for all cells. The most important task is to carry oxygen through hemoglobin. High doses of iron may cause toxic effects on the body. The most affected organ is the liver in iron poisoning. Toxic effects caused by iron poisoning are more than 20 mg/kg of elemental iron (Pestaner et al., 1999). These effects can be expressed according to different classifications; <30 mg/kg mild, 30-60 mg/kg moderate, 60-150 mg/kg heavy, > 150 mg/kg can be defined as fatal intoxications (Ioannides & Panisello, 2000). The highest sea water Fe concentration was observed in CF (24.30 µg/g in August, 39.14 µg/g in November, 39.06 µg/g in February) (Table 6). There is a remarkable and quite high Fe concentration in CF when we compared to other regions. The results in sea water samples, are quite consistent with the results in gilthead bream muscle tissues. The highest Fe concentration was observed (254.2 µg/g) in CF too (Table 7).

### 3.3 Analytical performance and real sample application by GC/MS

The analysis method for in-house validation was validated based on the internationally accepted guidance documents in the Fitness for Purpose of Analytical Methods (Magnusson &

Örnemark, 2014) before real sample analysis. In this context, linearity, precision, limits of detection (LOD) and quantification (LOQ), and recovery were studied and calculated. Linearity was performed by an external calibration curve, which adopted between 10-500 µg kg<sup>-1</sup>. All calibration solutions were diluted step by step from stock solutions of selected pesticides (1000 µg kg<sup>-1</sup>). The prepared calibration standards were injected into GC-MS with randomly. The procedure Linearity was found from 10 to 500 µg kg<sup>-1</sup> except for p-p'-DDT, endosulfan, and cypermethrin with a good correlation coefficient. The linear range for these pesticides was calculated 10 to 400 µg kg<sup>-1</sup> by linear regression of the peak area as a function of the standard concentration. LOD and LOQ were defined as a signal-to-noise (S/N) ratio equal to 3.3σ and 10σ with the slope of the calibration curve (σ is the standard deviation of the blank sample) under MS-SIM mode. The repeatability and intermediate precision of the method was calculated at three replicated analysis of a spiked blank sample at a concentration level of 100 µg kg<sup>-1</sup> for five different days with one-way analysis of variance (ANOVA). The recoveries of the method spiked in a blank sample at 100 µg kg<sup>-1</sup> and were found above 87.4%, and repeatability relative standard deviations (RSD<sub>r</sub>) and intermediate precision relative standard deviations (RSD<sub>R</sub>) were at the range of 3.01–6.56%. The results of analytical performance merits were given in Table 8.

OCPs are obtained by chlorination of organic substances and are very stable compounds. They do not dissolve in water, do not evaporate easily, and their persistence in soil is long-lasting. Organochlorine pesticides dissolve very quickly in oil and do not decompose easily. Because of these properties, they can accumulate in adipose tissue and damage living organisms. Organochlorine pesticides are compounds with a wide range of action. Heptachlor, p-p'-DDT, aldrin, α-BHC, endosulfan, p-p'-DDD, β-BHC and endrin, are the most known organochlorine pesticide species. Due to their environmental pollution, non-target species and their effects on humans, their widespread presence

**Table 8.** In-house validation results of each target pesticides.

Pesticide	Linearity, µg/kg	Regression equation	R <sup>2</sup>	<sup>a</sup> LOD	<sup>a</sup> LOQ	Recovery %	<sup>b</sup> Precision (%) (ANOVA)	
							With- in day (RSD <sub>r</sub> ) (N=3)	Intermediate Precision (RSD <sub>R</sub> )
p-p'-DDE	10-500	4.49e <sup>2</sup> x+2.265e <sup>3</sup>	0.9914	3.011	9.033	94.8	2.9	2.8
α- BHC	10-500	5.21e <sup>2</sup> x+7.124e <sup>3</sup>	0.9902	2.205	6.615	95.1	2.2	3.2
endosulfan- sulfate	10-500	1.684e <sup>2</sup> x+4.185e <sup>3</sup>	0.9960	3.315	9.945	96.0	1.9	2.1
endrin	10-500	2.83e <sup>2</sup> x+1.785e <sup>3</sup>	0.9992	2.989	8.967	101.0	3.7	3.4
heptachlor	10-500	3.528e <sup>3</sup> x+3.954e <sup>5</sup>	0.9947	2.727	8.181	89.5	6.4	6.1
heptachlor epoxide	10-500	2.59e <sup>2</sup> x+2.73e <sup>3</sup>	0.9987	1.732	5.196	100.6	3.6	3.7
methoxychlor	10-500	1.786e <sup>3</sup> x+1.994e <sup>4</sup>	0.9982	1.863	5.589	92.8	1.5	2.2
p-p'-DDD	10-500	2.394e <sup>3</sup> x+1.209e <sup>5</sup>	0.9927	2.123	6.369	90.6	2.8	3.2
p-p'-DDT	10-400	8.52e <sup>2</sup> x+8.378e <sup>4</sup>	0.9911	1.658	4.974	87.4	3.7	3.8
Aldrin	10-500	5.15e <sup>2</sup> x+6.393e <sup>4</sup>	0.9989	2.489	7.467	103.5	3.8	3.5
β-BHC	10-500	2.64e <sup>2</sup> x+2.952e <sup>3</sup>	0.9957	1.560	4.680	98.2	3.9	4.1
cypermethrin	10-400	5.603e <sup>2</sup> x+5.902e <sup>3</sup>	0.9972	1.991	5.973	101.4	3.0	3.4
dieldrin	10-500	7.249e <sup>2</sup> x+1.28e <sup>5</sup>	0.9983	1.923	5.769	96.8	2.1	2.2
endosulfan	10-400	2.093e <sup>2</sup> x+1.825e <sup>5</sup>	0.9897	3.483	10.449	89.8	3.6	4.0

<sup>a</sup>Blank samples are used for reference to content sample; <sup>b</sup>One-way ANOVA is used to calculate precision.

in the environment still requires continuous monitoring of the residues of these compounds although they are abandoned. For this purpose, we studied the residues of these pesticides and also  $\alpha$ -BHC,  $\beta$ -BHC, p-p'-DDE and methoxychlor that appears predominantly in the fish liver and muscle tissues of fishes.

Endosulfan, cypermethrin, heptachlor and endrin were stated in the list of priority substances in the field of water policy in directive 2013/39/EU (European Union, 2013). Cypermethrin, endrin, dieldrin, heptachlor, p-p'-DDT, p-p'-DDD, and endosulfan were listed in Annex I environmental quality standards for priority substances and certain other pollutants in directive 2013/39/EU. Pesticides resulted by biological accumulation in the fish species. Table 9 presents the residues of some of pesticides in the muscle meat of gilthead bream from CF, EF and NF regions.

We observed remarkable  $\beta$ -BHC residues in gilthead bream samples from each region (Table 9) which are 16.18  $\mu\text{g}/\text{kg}$ , 16.11  $\mu\text{g}/\text{kg}$ , 16.20  $\mu\text{g}/\text{kg}$ , respectively.  $\beta$ -BHC residues values are very close to each other in each region. Unfortunately, there is no report any max allowable concentration (MAC) in 2013/39/EU, EQS bioata neither for  $\beta$ -BHC nor for the others (Directive 2006/77/EC, TFC) (European Union, 2006a). When we evaluated the residues of  $\beta$ -BHC regionally, it should be noted that  $\beta$ -BHC residue is quite high, although no limit value is specified. We observed  $\alpha$ -BHC residues only in the EF region (16.54  $\mu\text{g}/\text{kg}$ ).

p-p'-DDE was determined in every fish sample of each region. The highest concentration of p-p'-DDE was observed in the grey mullet samples (21.70  $\mu\text{g}/\text{kg}$ ) from EF region. Directive 2006/77/EC listed the maximum level of p-p'-DDE as 50  $\mu\text{g}/\text{kg}$  for all feeding stuffs (European Union, 2006a). When we evaluated the residues of p-p'-DDE for each region, p-p'-DDE residues are under the MAC but the values is quite high. This is a serious issue that should be taken control. It is known that the form of p-p'-DDT in oxygen-free environments is caused by p-p'-DDD and p-p'-DDE is caused by oxygen metabolisms (Calamari et al., 1994), therefore, the conversion of p-p'-DDT to p-p'-DDE can occur in the living body. In this study we did not observe the presence of p-p'-DDT residues in fish samples, not observing any residue is attributing this situation. Moreover, the high

amount of DDE compared to DDT presents that the pollution in the ecosystem is very new (Yatagan, 2008) (Table 9).

Endrin residues were determinate in each region. When we compared the endrin residues of the region they are very close to each other (20.56  $\mu\text{g}/\text{kg}$  in CF, 20.47  $\mu\text{g}/\text{kg}$  in EF, 20.45  $\mu\text{g}/\text{kg}$  in NF). Endrin residues of grey mullet samples are concerning in this study. Endrin is very toxic to many wildlife species, especially aquatic organisms. Endrin has high accumulation potential in aquatic organisms. It is strongly bound to soil particles and may remain in some soils for years. In some cases, groundwater seepage may occur, but this is rare due to the tendency to strongly bind to most soil types and its relatively low solubility in water. Due to its ability to evaporate in the atmosphere and its stability in the environment, endrin can be transported away from its release point. Endrin is classified as a "permanent organic pollutant" (POP) (Topal & Onac, 2020). Unfortunately, there is no stated any MAC for EQS biota in 2013/39/EU although the usage of it is prohibited in all European countries. Only directive 2006/77/EC is mentioned the maximum level for endrin as 10  $\mu\text{g}/\text{kg}$  for all feedingstuffs (European Union, 2006a). In this study, endrin residues observed two times more than 2006/77/EC. Caliskan and Yerli (Çalişkan & Yerli, 2000) were determine  $\beta$ -HCH and  $\gamma$ -HCH in all of the fish samples examined and they have identified,  $\gamma$ -HCH > endrin >  $\beta$ -HCH >  $\alpha$ -HCH > Aldrin, five different organochlorinated pesticide residues in their study.

Dieldrin is also classified as a "permanent organic pollutant" (POP). Like other organochlorine pesticides, it is very toxic to aquatic organisms and many other wildlife species and accumulates in the environment, especially in animal fats. Dieldrin has been banned internationally under the UNECE POP protocol and has been proposed for its elimination under the UNEP POP Convention. Its use is now banned in the EU. European Directives regulate the levels of dieldrin. It is also listed as a candidate substance protecting the marine environment of the north-eastern Atlantic Ocean under the convention for the protection of the marine environment of the north-east Atlantic (OSPAR). Unfortunately, there is no maximum limit for the dieldrin residue in the respective protocols. Directive 2006/77/EC is listed ML of dieldrin as 10  $\mu\text{g}/\text{kg}$  for all feedingstuffs. In this study, we determined quite high dieldrin residues in grey mullet

**Table 9.** The residues ( $\mu\text{g}/\text{kg}$ ) of some of pesticides in the muscle meat of in gilt head bream from CF, EF and NF regions.

Pesticide Species	CF (mean $\pm$ SD)	RSD	EF (mean $\pm$ SD)	RSD	NF (mean $\pm$ SD)	RSD
p-p'-DDE	21.69 $\pm$ 0.80	3.7	21.70 $\pm$ 0.60	2.8	21.69 $\pm$ 0.88	4.1
$\beta$ - BHC	16.18 $\pm$ 1.06	6.6	16.11 $\pm$ 0.30	1.9	16.20 $\pm$ 0.84	5.4
p-p'DDD	<LOD		<LOD		<LOD	
p-p'DDT	<LOD		<LOD		<LOD	
Endrin	20.56 $\pm$ 0.28	1.4	20.47 $\pm$ 1.98	9.7	20.45 $\pm$ 0.57	2.8
Heptachlor	18.75 $\pm$ 0.30	1.6	18.77 $\pm$ 1.68	9.0	18.77 $\pm$ 0.56	3.0
Heptachlorepoide	<LOD		<LOD		<LOD	
Methoxychlor	24.34 $\pm$ 2.09	8.6	<LOD		24.56 $\pm$ 1.74	7.1
Aldrin	18.32 $\pm$ 0.32	1.8	<LOD		<LOD	
$\alpha$ - BHC	<LOD		16.54 $\pm$ 0.79	4.8	<LOD	
Dieldrin	20.44 $\pm$ 0.59	2.9	20.47 $\pm$ 0.51	2.5	20.48 $\pm$ 0.38	1.9
Cypermethrin	<LOD		<LOD		<LOD	
Endosulfan	<LOD		<LOD		<LOD	
Endosulfan sulfat	<LOD		<LOD		<LOD	

samples from each region, 20.44 µg/kg in CF, 20.47 µg/kg in EF, 20.48 µg/kg in NF and the values are close to each other. The high concentration of dieldrin is alarming and must be under control.

Heptachlor is an insecticide that is effective through gastric and contact. It is highly volatile, so it can be found in the atmosphere. It easily binds to water sediments and becomes bioconcentrated in the oils of living organisms. Heptachlor is metabolized to heptachlor epoxide in animals and this substance can also be stored in animal fats. The maximum allowable concentration EQS biota of heptachlor is defined as  $6.7 \times 10^{-3}$  µg/kg in 2013/39/EU. 18.75 µg/kg is the lowest, 18.77 µg/kg is the highest heptachlor residues were observed from CF and from NF region respectively (Table 9). The highest heptachlor concentration is nearly three thousand more than MAC EQS biota of heptachlor that listed in in 2013/39/EU. This is a serious issue that should be taken under control urgently.

Methoxychlor is a pesticide that causes health problems by altering the functioning of the endocrine system in a healthy organism or its future generation. Oxidative stress occurs in kidney and liver tissue during exposure to methoxychlor. Fish concentrate the organochlorine pesticides in their bodies and the insecticide density in fish can reach 1000-10000 times that in water. This level reaches higher levels in creatures that feed on fish. Methoxychlor residues were observed in two regions (Table 9). The amount of methoxychlor residue is really close in CF and NF regions (24.34 and 24.56 µg/kg). Methoxychlor is a pesticide that does not accumulate in living organisms and has recently been used in place of DDT. Since it is insoluble in water, this spray-applied pesticide is generally removed from the body in a short time, even if it is transmitted through the body's breathing, contact or digestion. Detection in fish samples can be considered as a result of the introduction of new methoxychlor in the ecosystem in the near future, ie it is still in use in this environment. There is no maximum allowable limit for methoxychlor in 2006/77/EC, 2013/39/EU (European Union, 2013) nor TFC (Regulation, 2008).

Although aldrin is prohibited in the EU, its release to the environment may consist of products or materials processed elsewhere. In countries where it is still used as a pesticide, it directly pollutes the soil. There is no natural aldrin source to the environment. Because it is highly toxic, it is not considered a suitable pesticide in the fight against parasites in domestic animals. In both plant and animal and insect organisms, aldrin is converted to dieldrin, a toxic substance by epoxidation. This suggests that metabolic deviation of aldrin is inevitable. A single dose of deposition occurs as well as repeated small doses. The use of aldrin is prohibited in the EU. The European Directives governing residues of Aldrin include those related to the marketing and use of certain hazardous chemicals (79/117/EEC) (European Union, 1979) and pollution of the water environment (76/464/EEC) (European Union, 1976). Directive 2006/77/EC is regulated the maximum content of Aldrin as 10 µg/kg for all feedingstuffs. In this study, Aldrin was determined only in CF region as 18.32 µg/kg which is nearly two times more than the ML listed in 2006/77/EC. It was not determine any Aldrin residues in EF and NF regions. This phenomenon is attributed to converting Aldrin to dieldrin by epoxidation. EF and NF

represent fishes escape from cages and live in their natural medium, not feed by fish feed.

Endosulfan-sulfate, p-p'-DDT, heptachlor epoxide, p-p'-DDD, endosulfan and cypermethrin were studied in gilthead bream muscle tissues from each regions and we did not determine any residues in the samples.

#### 4 Conclusion

In this study, it was tried to be investigated the effect physical and chemical parameters of the Guvercinlik cove's sea waters on gilthead bream fishes (CF, EF and NF) that lives in three different mediums with the analyzing of sea water. We would like to demonstrate the micro pollutants and heavy metal residues in CF, EF and NF and reveal out the diversity in living in three different ambient conditions. We analyzed the Mn, Cr, Zn, Sn, Ag, Hg, Cd, Cu, Ni and Pb residues and cypermethrin, p-p'-DDE, α-BHC, endosulfan-sulfate, endrin, dieldrin heptachlor epoxide, heptachlor, methoxychlor, p-p'-DDD, endosulfan, p-p'-DDT, aldrin, and β-BHC pesticides in three types of gilthead bream (CF, EF and NF) and also researched the heavy metal residues in the sea water sample that provided from Guvercinlik cove.

The high conductivity in the CF is interpreted as the maximum conductivity depending on the amount of dissolved substance in the water. The high salinity in the cage (CF) is thought to be due to anion or cation salts by dissolving the synthetic feeds that used for fish. The highest nitrite concentration was 0.0423 mg/L in February in CF which is in agreement with the results that obtained in other parameters. Total phosphorus concentration is 10 times difference between CF and other regions when compared the results. The use of dense phosphate fertilizers, feed rate, suspended solids content and fish feces could be cause a high phosphate concentration. Physical and chemical parameters values of the CF were higher than other regions' sea water samples when we evaluate the all results.

Some of the heavy metals that we studied in this paper are within the scope of national and international general quality criteria but some heavy metals do not have limit values. With this study, we would like to draw attention to these heavy metals which have high toxicity for the environment and do not have limit values in national and international general quality criteria.

We especially draw attention to the samples taken from cages (CF). Cr, Cu, Zn, Pb, Hg, Cd, Ni, Mn, Sn, and Fe residues in CF are especially remarkable different from other regions (EF and NF). This phenomenon is attributing the fish farms and using excessive fish feed in the cages. Copper concentration (in CF) is almost five decades higher than ML of EPA standards. The maximum lead concentration of each region is over the permitted levels according to the standards in Table 4. EDS analysis and SEM image of gilthead bream liver tissues also demonstrate the heavy metal residues.

We observed remarkable β-BHC residues in gilthead bream samples from each region although no limit value is specified in any directive. p-p'-DDE was determined in every fish sample of each region. Endrin and dieldrin residues of grey mullet samples are concerning in this study which are two times more than



2006/77/EC. The highest heptachlor concentration is nearly three thousand more than MAC EQS biota of heptachlor that listed in in 2013/39/EU. Detection of metoxychlor residues in fish samples can be considered as a result of the introduction of new metoxychlor in the ecosystem in the near future, ie it is still in use in this environment. There is no maximum allowable limit for metoxychlor in 2006/77/EC, 2013/39/EU nor TFC. Aldrin was determined only in CF region which is nearly two times more than the ML listed in 2006/77/EC. It was not determine any Aldrin residues in EF and NF regions. This phenomenon is attributed to converting Aldrin to dieldrin by epoxidation.

The consumption of nutrient needs increases with increasing in the human population. In order to meet the increasing demand for consumption, it is used different types of methods to have more products in fish farming. In our study, the difference between the fishes grown in cages and grown in their natural feeding environments is clearly seen. Most of the heavy metals and organochlorinate pesticides that are determined in this study do not have maximum limit values that are of concern for human, marine organisms and environmental health. We wanted to create awareness for micro pollutants and heavy metals that have no limit values in any directive. In the light of the data obtained in our study, we believe that more healthy seafood can be obtained by increasing the controls and controls of the feed that used in the fish cultivating.

### Conflict of interest

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

### Acknowledgements

Thanks to Selim Sakrak and Duygu Takanoglu Bulut.

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