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Contents

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Determination of Effects of Seasonal and Sampling Area on Ulva Rigida's Elemental Composition

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ABSTRACT

Macroalgae is used as a bioindicator for their accumulation capacity of potentially hazardous elements. Ulva spp. was used extensively in previous studies as in the recent study. Along the Mersin coast, sampling was made in four different locations (Çamlıbel Marina, Pozcu Marina, Deniz Feneri, Karaduvar) during the spring and summer seasons between 2015-2016. For elemental analyses, ICP-MS was used, and Mg, Al, K, Ti, Cr, Mn, As, Se, Sr, Mo levels were analyzed in Ulva rigida samples. The highest level (134960.0 µg g-1) was found in the elemental analysis of Mg, and the lowest levels (20.83 µg g-1) were found in the elemental analysis of Se. Potentially hazardous trace elements (Al, Ti, Cr, Sr, As, Mn) were found in high levels in all seasons and sampling points in comparison to other studies. The abundance in other elements (Mg, K, Mo, and Se) was also notable in the same sense. The seasonal difference seems to have little effect on the accumulation of trace elements for our study. Pozcu Marina and Karaduvar stations are good sampling points in regard to monitor potentially toxic elements in macroalgae tissue.

1. INTRODUCTION

Macroalgae is used as a term for seaweeds and other benthic marine algae that can be seen by the naked eye. Although they are not really "weeds", larger macroalgae are also referred to as seaweeds (Diaz-Pulido and McCook, 2008). Seaweeds are abundant in the aquatic environment, they are sedentary and can be easily collected and identified (Campanella et al., 2001). They are mostly distributed in the rocky intertidal zone in most of the marine environment, therefore seaweeds are very important ecologically (Murphy, 2007). Based on their chemical composition, macroalgae are classified into three divisions: green (Chlorophyta), red (Rhodophyta), and brown (Phaeophyceae) algae (Gupta and Abu-Ghannam, 2011). Ulva genus is one of the green macroalgae and they grow in shallows waters and are specifically found in the marine environment (Loughnane et al., 2008).

Ulva sp. contain protein, essential fatty acids, minerals, polysaccharides, carotenoids, etc. in their structure (Fleurence, 1999). Environmental (salinity, temperature, pH, season, etc.) and biological (age, thallus morphology, etc.) factors can affect the accumulation levels of these elements (Lobban and Harrison, 1997). In addition, it is known that several macroalgae can accumulate high levels of metals and they can grow in the coastal waters which contain high levels of metal levels (Pawlik-Skowronska et al., 2007). Macroalgae can indicate pollution in the marine environment thanks to their characteristics such as (i) most of them are sessile; (ii) they are widely distributed and available all year round; (iii) they can tolerate wide ranges of salinity, turbidity, and high levels of pollutants; (iv) they are easy to collect and process; (v) they can be kept in laboratory conditions (Haug et al. 1974; Phillips 1990).

Macroalge's usage of bioindicators has started in the early 1950s, and mainly it was in UK and Canada at the beginning (Black and Mitchell 1952; Fuge and James 1973; Wort 1955; Bohn 1975). Because of its easy implantation today, it is used worldwide. It can be either native (passive biomonitoring) or transplanted species (active biomonitoring) (Garcia-Seoane et al. 2018).

Some metals such as Cu, Fe, Zn, Cr, Mn, etc. are essential for the human body to continue its functions. However, most heavy metals such as Cd, Pb, Hg, As, Cr,

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etc. are dangerous substances for they are harmful to human metabolism. Because they are toxic, non-biodegradable, have a very long half-life in soil (Singh et al., 2011). Heavy metals can accumulate in living systems through the active food chain.

In Mersin coastal zone, Alp et al. (2012) have investigated some of the heavy metal levels (Al, Cr, Mn, Fe, Ni, Cu, Zn, Pb, Cd) in Ulva sp. and Enteromorpha sp. and in sediment. Furthermore, Altun (2017) has also investigated some of the heavy metals (Fe, Cu, Cd, Zn, Pb) in the Mersin coastal zone.

Although there were several studies in the past years about heavy metal levels that accumulated in macroalgae in Mersin coastal area, the elemental composition of macroalgae may change constantly over the years. Furthermore, previous studies have concluded that the studies about the heavy metal levels in macroalgae should continue to cover reliable information to the researchers and authorities and thus, there are several macroalgae species to be investigated in this regard. The aim of this study was the determination of the elemental composition of Ulva rigida regarding the seasonal changes and sampling area.

2. MATERIALS AND METHODS

2.1. Collection of samples

The study was carried out in Mersin which has the largest port in Turkey. The territorial border of Mersin is 608 km with an area of 15.953 km2 and the sea border is 321 km. The study was carried out by sampling monthly, between April 2015 and May 2016 at the coastal stations, which were selected in different parts of Mersin province's coastline. The stations were as follows; Çamlıbel Marina (36°47' 27.6"N 34° 37' 36.2"E), Pozcu Marina (36°46' 06.1"N 34°34' 00.5"E), Deniz Feneri (36°47'05.0"N 34°37'15.8"E), and Karaduvar (36°48'27.1"N 34°41'31.4"E). The sample stations were shown in Figure 1. During the study, the samples are taken from the littoral zone.

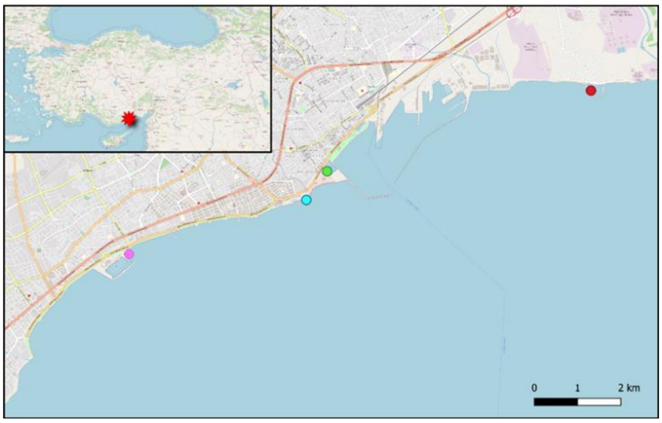

Figure 1. Sampling Stations in Mersin Bay (Pozcu marina purple, Deniz feneri as cyan, Çamlıbel marina as green, Karaduvar as red)

2.2. Storage and preparation of samples for analysis

The samples were put into polyethylene bags and labeled to indicate the date and stations. They were washed to remove the sand and other materials then dried at room temperature.

To make the samples dried, the samples were put in an incubator at 70 °C for 2 hours. After the drying process was done, the samples were shredded into little pieces with the shredder and again put back to the polyethylene bags. They were kept at +4 °C in a refrigerator until the time that they will be analyzed.

2.3. Elemental analysis

In this study, ten elements that accumulated in U. rigida's tissue were analyzed; those were Mg, Al, K, Ti, Cr, Mn, As, Se, Sr, and Mo. For that purpose, the samples were made soluble. From each U. rigida sample, 0.1 g was taken. 4 ml HNO3, 2 ml HClO4, 2 ml H2O2, and 2 ml H2SO4 were added to each sample's tube, and then they were heated on the hot-plate until they become homogenized. Then dilutions and pH adjustments of the samples were made. The samples were diluted in a ratio of 1:100 and the analysis was made via inductively coupled plasma mass spectrometry (ICP-MS, 7500-Ce) at the Mersin University Advanced Technology Education, Research and Application Center (MEITAM).

2.4. Statistics

Prior to the analyses, all data were checked for outliers, and homogeneity of variance was also tested. Statistical analysis of data was carried out with the IBM SPSS STATISTICS 22 statistical program. ANOVA (Analysis of Variance) was used to evaluate the effect of seasons and stations on the elemental profiles.

3. RESULTS

Maximum, minimum, and mean values of elemental levels and statistical differences between seasons and stations were presented in Table 1. Mg (182000.0 µg g-1) is the most abundant element, and it was at Pozcu Marina station in summer, Se (20.26 µg g-1) was the least found element in the structure of U. rigida and it was found at Deniz Feneri station in summer.

The highest level of Mg (182000 µg g-1) was found at Pozcu Marina in the summer season, while the lowest level of Mg (37002.72 µg g-1) was found at Karaduvar station in spring. There is a statistically significant difference between Çamlıbel Marina, Deniz Feneri, Karaduvar, and Pozcu Marina stations for the samples both in the spring and summer seasons. The highest and lowest levels of Al (2882.47 µg g-1 - 230.69 µg g-1, respectively) were found at Çamlıbel Marina in spring. There is no statistically significant difference between stations, both in the spring and summer seasons.

Table 1. The effects of season and sampling area on element levels of *U. rigida* (μg g^{-1})

	Çamlıbel Marina	**Pozcu Marina**	**Deniz Feneri**	**Karaduvar**	**Season**
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	
Mg	67544.0±2792.24^{x} (54501.99-78240.47)	105420.0±25011.1^{y} (62104.23-149000.00)		37119.0±66.95^{x} (37002.72-37234.66)	**Spring**
		134960.0±27448.5^{y} (87413.33-182000.00)	59745.0±716.60^{x} (58503.40-60985.78)		**Summer**
Al	1297.5±340.05^{x} (230.69-2882.47)	1087.5±362.18^{x} (460.18-1714.83)		1570.1±71.67^{x} (1445.96-1694.26)	**Spring**
		1651.6±265.88^{x} (1191.11-2112.15)	663.47±29.57^{x} (612.24-714.69)		**Summer**
K	32181.0±1707.32^{y} (23440.86-39840.64)	44746.0±9915.66^{z} (27571.29-61920.15)		36383.0±176.98^{yz} (36076.29-36689.39)	**Spring**
		11691.0±2295.37^{x} (7715.56-15666.95)	8026.0±28.76^{x} (7679.19-8075.83)		**Summer**
Ti	1183.1±34.28^{x} (1053.20-1342.86)	1931.5±423.70^{y} (1197.64-2265.40)		1201.3±3.49^{x} (1195.28-1207.40)	**Spring**
		1923.6±397.26^{y} (1235.56-2611.72)	1159.6±52.87^{x} (1068.03-1251.18)		**Summer**
Cr	97.18±5.72^{x} (77.20-123.84)	176.41±43.36^{y} (101.30-251.52)		95.96±4.93^{x} (87.41-104.52)	**Spring**
		195.65±41.06^{y} (124.52-266.78)	100.17±5.14^{x} (91.26-109.08)		**Summer**
Mn	95.32±18.12^{x} (32.65-179.39)	105.9±25.81^{x} (61.20-150.61)		88.61±2.63^{x} (84.05-93.18)	**Spring**
		224.08±47.21^{y} (142.29-305.86)	129.44±7.39^{x} (116.63-142.26)		**Summer**
As	59.51±14.09^{x} (20.44-157.51)	34.21±7.36^{x} (21.46-46.98)		127.24±34.66^{y} (67.21-187.28)	**Spring**
		60.63±7.26^{x} (48.05-73.22)	23.66±0.53^{x} (22.74-24.59)		**Summer**
Se	65.40±21.29^{x} (24.89-220.62)	22.24±0.34^{x} (21.65-22.83)		181.43±62.42^{y} (73.30-289.55)	**Spring**
		35.16±2.22^{x} (31.32-39.02)	20.83±0.33^{x} (20.26-21.42)		**Summer**
Sr	177.79±19.05^{x} (86.31-289.14)	238.83±60.78^{x} (133.55-344.11)		123.36±4.59^{x} (115.40-131.31)	**Spring**
		453.08±120.14^{y} (244.98-661.17)	255.89±24.31^{x} (213.78-298.01)		**Summer**
Mo	188.65±47.48^{xy} (53.00-525.43)	82.32±0.16^{x} (82.05-82.60)		330.10±95.41^{y} (164.83-495.37)	**Spring**
		84.58±4.24^{x} (77.23-91.95)	130.08±8.40^{x} (117.00-154.77)		**Summer**

Different letters (x,y,z) in the same rows and columns for each metal significant differences ($p<0.05$). : mean±standard error; () shows min-max levels

The highest level of Cr (266.78 μg g-1) was found in Pozcu Marina in summer, while the lowest level (77.20 μg g-1) were found at Çamlıbel Marina in spring. Mean levels from Çamlıbel Marina and Karaduvar in spring, and from Deniz Feneri in summer are in the same group and there is a statistically significant difference with the mean levels from Pozcu Marina both in spring and summer seasons. The highest level of Mn was found (305.86 μg g-1) in Pozcu Marina in spring, while the lowest level was found in (32.65 μg g-1) at Çamlıbel Marina in spring. Mean levels from Çamlıbel Marina, Pozcu Marina, Karaduvar in spring, and from Deniz Feneri in summer are in the same group. There is a statistically significant difference in the mean level from Pozcu Marina in summer.

The highest level of As (187.28 μg g-1) was found in Karaduvar in spring. The lowest values (20.44 μg g-1) were found at Çamlıbel Marina in spring. Mean values from Çamlıbel Marina, Pozcu Marina, Deniz Feneri in both spring and summer seasons are in the same group and there is a statistically significant difference with mean values from Karaduvar station in the spring season. The highest level of Se (289.55 μg g-1) Karaduvar in spring, while lowest values (20.26 μg g-1) were found at Deniz Feneri in summer. Mean values from Çamlıbel Marina, Pozcu Marina, Deniz Feneri in both spring and summer seasons are in the same group and there is a statistically significant difference with mean values from Karaduvar station in spring.

The highest level of Sr was found (661.17 μg g-1) in Pozcu Marina in summer, while the lowest level (86.31 μg g-1) were found at Çamlıbel Marina in spring. Mean levels from Çamlıbel Marina and Karaduvar in spring, and from Deniz Feneri in summer are in the same group and there is a statistically significant difference with mean levels from Pozcu Marina in spring and summer seasons. The highest and lowest levels (525.43 - 53.00 μg g-1) were found at Çamlıbel Marina in spring. There is no statistically significant difference in mean level from Çamlıbel Marina in spring. Mean levels from Pozcu Marina and Deniz Feneri in both spring and summer seasons are in the same group and there is a statistically significant difference with mean levels from Karaduvar in spring.

4. DISCUSSION

As a consequence of anthropogenic activities such as mining, agricultural, domestic water disposal, heavy metals contaminate terrestrial and aquatic systems. Also, intense industrial activities produce exhaust gases that include heavy metals (As, Ti, Mo, Mn, Cr, Fe, Zn, Pb, etc.). They are given into the atmosphere, in the form of fine particles heavy metals travel and settle to soil and water. From terrestrial to aquatic environments, these contaminants enter to the food chain and are accumulated reaching levels a thousand times more than the seawater (Castillo 2016). Heavy metals can also enter into environment through natural sources such as; forest fires, volcanic activities, erosion, etc. (Jaishankar et al. 2014). Where there is human population and industry, it can be expected that some level of heavy metal contamination may occur. Therefore biomonitoring of these elements is of great importance for a sustainable environment.

The use of marine organisms as biomonitoring tools is strongly recommended by the Water Framework Directive (2000/60/EC) and the Marine Strategy Framework Directive (2008/56/EC). The green algae from the genus Ulva are the most used macroalgae for the biomonitoring of the levels of trace elements in the marine environment (Chakraborty et al. 2014). While collecting the samples for our study, Ulva genus is abundant and distributed along the coastal line of Mersin. Elemental analyses in our study show that U. rigida accumulates a very high level of heavy metals. These features of U. rigida can present it as an important and valuable indicator in regards to biomonitoring of toxic metals in the coastal area of Mersin (Bonanno and Orlando-Bonaca 2018).

The importance of our analyses and the usage of Ulva genus as a bioindicator in Mersin bay can be better understood when similar studies were reviewed. Malea et al. (2015) also have investigated trace element levels in seawater, sediment, and several macroalgae species including U. rigida. They have made analyses for various heavy metal levels in seawater, sediment, and in four macroalgae species. Despite both studies have the same genus, the results for As levels were notably concentrated in our study. Malea et al. (2015) present mean value of As in U. rigida was 1.449 µg g-1, while in our study U. rigida's lowest value was 23.66 µg g-1. Though it was the lowest value, it was almost 15 times higher than the mean values found in Malea et al. (2015). The mean value of As in seawater was 2.030 µg L-1 in their study. Most notably among our As results, the highest mean value of As was obtained from the Karaduvar station in spring (127.24 µg g-1). The same study also investigated Cr, Mn, Mo, Se, and Sr levels along with several other trace elements, and found mean values respectively Cr: 9.383 µg g-1, Mn: 37.33 µg g-1, Mo: 37.92 µg g-1, Se: 0.281 µg g-1, Sr: 2.707 µg g-1. In recent study, however, lowest mean levels (given with the standard errors for each element) were found respectively; Cr: 95.96 ± 4.93 µg g-1, Mn: 88.61 ± 2.63 µg g-1, Mo: 82.32 ± 0.16µg g-1, Se: 20.83 ± 0.33µg g-1, Sr: 123.36 ± 4.59 µg g-1.

Another study about macroalgae trace element accumulation capacity by Malea and Kevrekidis (2014), also presents trace elements levels on previous studies that investigated Ulva spp., includes six trace elements (As, Cr, Mn, Mo, Se, Sr) in common with the present study. Table 2. shows the range of those trace elements and the results of our study in dry weight.

Table 2. Trace element levels of Ulva spp. from various geographical areas and present study (µg g-1)

Trace element	Levels found in previous studies	The present study (Lowest and highest levels)
As	0.87 - 86	23.66 - 187.28
Cr	0.06 - 84.4	77.20 - 266.78
Mn	0.012 - 1600	32.65 - 305.86
Mo	0 - 58	53.00 - 525.43
Se	< 0.2 - 1.4	20.26 - 289.55
Sr	81 - 700	86.31 - 661.17

There is a clear trace element abundance in all U. rigida samples in Mersin Bay comparing with Malea et al. (2015), also Table 2. clearly shows As, Cr, Mo, Se levels are much higher than they were found in previous studies.

The molybdenum is also an essential element but, in its abundance reduces the intake of copper (Castillo 2016). It should be considered that higher levels of Mo may be already present in the seawater of Mersin Bay. While low selenium status for humans may result in mortality, poor immunity, and cognitive decline, its supplementation, even when daily uptake is adequate, may cause serious human health problems (Rayman 2012). High magnesium content doesn't relate to any kind of hazardous effect on humans, but its deficiency is a serious dietary problem for humans in regards to their physiological functions (Vormann 2003). Potassium also plays a very important physiological role. WHO recommends at least 3510 mg/d K intake for adults (Whelton and He 2014). For their carcinogenic effects, levels of Cr, As, Ti, Al, Mn, and Sr found in the present study should be noted as a potential hazard to human health.

Arsenic is an essential element for life, up to a level (10 µg L-1). However, when it is more than 10 µg L-1, there can be toxicological effects to humans (Fawzy 2008). Arsenic exists in the environment naturally and it can be organic or inorganic form. The toxic effects can occur when the arsenic is in inorganic form (Castillo 2016). Inorganic arsenic is usually an outcome of the use of pesticides which is widely used in agriculture in Mersin. The used irrigation water might be discharged uncontrollably and without any treatment in some areas of Mersin bay. This is very likely to happen for Mersin has a very long coastal area which makes it harder for the authorities to control the irrigation waters. It is also known that arsenic is widely used in industrial activities (processing of glass, textiles, paper, and metal adhesive, etc.) (Garelick et al. 2009). Most of these industrial activities are present in Mersin industrial zones. International Agency for Research on Cancer (IARC) has classified arsenic and arsenic compounds as carcinogenic to humans (Roy and Saha 2002; Castillo 2016)

Strontium-90 is a radioactive contaminant, due to its ~29 year half-time in soil, it is an environmental concern. It occurs in the natural environment solely as the Sr^{2+} ion, and its geochemical behavior is similar to Ca^{2+} (Thorpe et al. 2012). With the Chernobyl and Fukushima accidents, and with disposal of wastewater and sludge related to nuclear activities, large quantities of 90Sr enter the aquatic environments where it can easily be soluble (Martignier et al. 2018). Macroalgae have great strontium accumulation potential, thus they represent great potential for bioremediation as well. In a recent study, Ulva's Sr accumulation capacity in the present study shows similar results with the previous studies. It also shows that Mersin bay may be subject to serious Sr levels. So, its root causes shall be investigated with further research that focuses to monitor the level of this contaminant over the years.

Chromium can be observed in compounds in the environment naturally, but not in elemental form. Contamination of chromium occurs both by electroplating processes and the disposal of Cr-containing waters. Chromium may be transported by water in its soluble form, also precipitated form. Despite Cr is another essential element, Chromium (VI) is carcinogenic to humans (Pathnia 2016). Cr^{3+} is used in the production of leather, steel, and textile while Cr^{6+} is used in electro painting and chemical manufacturing. However, water contamination may be limited because of soil adherence of Cr. When such water contamination occurs, it should be observed whether there is improper disposal of industrial manufacturing equipment (Castillo 2016). EPA set the limit for chromium to be 100 µg L^{-1} in drinking water (EPA, 1999). Glassmaking, cement, and textile manufacturing are some of the sources that can be listed to present in Mersin. The levels found in this study may be linked to industrial activities in Mersin.

High levels of iron and manganese in drinking water may be toxic for organisms. While in lower levels, they play an important role in hemoglobin synthesis. As in the drinking water, their extensive levels accumulated in macroalgae may pose threat through the food chain, and affect neural and muscle systems in humans (Sanjay 2014).

Titanium is known to be inert metal and is used for various implications in the medical industry. Titanium can be found on the earth's crust, also in animals, plants, and natural waters. TiO_2 is used as a food additive and in the cosmetic industry etc. Though in some forms, it can be harmful to human health (Tibau et al. 2019).

Aluminum, when at high levels, is toxic to organisms in aquatic environments. For mammals and birds, aluminum might also be dangerous interfering with their metabolic processes. Thus, its control and monitoring are very important for marine environments (Rosseland et al. 1990).

Considering the hazardous effects of these trace elements in Mersin Bay, our study reveals some seasonality and spatial differences. From our results; Mg levels showed no seasonal difference, but it can be seen that there is a spatial difference between Pozcu Marina and with other three stations. There was no spatial nor seasonal difference regarding Al levels throughout our sampling stations. The most significant spatial difference was found in the accumulation of K levels, which also shows lower levels with a seasonal difference. It can be said that algae might be accumulating less potassium in the summer season. Titanium levels don't show a seasonal difference but due to spatial differences, Pozcu Marina shows the highest levels. Cr and Sr, both showed spatial difference with the accumulated levels in Ulva, particularly high levels were found at Pozcu Marina where it is very close to the city center and a heavily populated area. Mn levels showed no spatial difference, but an increase was found with seasonality. For As, Se, and Mo levels, there was no seasonal difference. Levels of those three elements showed an increase with a spatial difference, in particular at Karaduvar station. It should be also noted due to the possible decay of algae, there was no sampling in the summer season at Karaduvar Station.

5. CONCLUSION

Potentially hazardous trace elements (Al, Ti, Cr, Sr, As, Mn) were found in high levels in all seasons and sampling points in comparison to other studies. The abundance in other elements (Mg, K, Mo, and Se) was also notable in the same sense. The seasonal difference seems to have little effect on the accumulation of trace elements for our study. It should be noted that Mersin bay is subject to high water temperature and salinity. Those factors may play an important role in the decay of algae in the summer season, due to these limitations, sampling in every season was not possible. Though, it can be seen Pozcu Marina and Karaduvar stations are good sampling points in regards to monitor potentially toxic elements in macroalgae tissue. The present study was also conducted comparatively in a short period, longer periods of sampling may result in a better understanding about the accumulation of trace elements. Also in up-to-date studies, levels might be expected to rise due to the recent events (floods and forest fires in Turkey). Those negative chain of events may drive researchers to increase monitoring studies' scope and the time period in the future.

Author contributions

Nahit Soner BÖREKÇİ: Conceptualization, Investigation, Writing- Original draft preparation, Writing Reviewing and Editing.
Mısra BAKAN: Investigation
Büşra PEKSEZER: Investigation
Mehmet Tahir ALP: Writing Reviewing and Editing.
Deniz Ayas: Investigation, Writing- Original draft preparation, Writing-Reviewing and Editing.

Conflicts of interest

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

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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Gill Ectoparasites of Goldfish (Carassius auratus) Imported from Syria

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ABSTRACT

This study was carried out to determine species of ectoparasites from goldfish (Carassius auratus) imported from Syria. One hundred of live imported goldfish from Syria, May and June between month in 2019, were examined in the laboratory of Aquaculture Faculty, Mersin University. Of the seven species of ectoparasites isolated in this study, four were monogeneans (Dactylogyrus sp. Dactylogyrus vastator, Gyrodactylus sp. and Gyrodactylus chinensis) and two were protistan (Trichodina sp., Ichthyophthirius multifiliis) and one were crustacean (Argulus japonicus). The fish examined were imported from Syria. Although goldfishes were carried by trucks many of which came from the single exporting region of Syrian Arab Rebuplic, the all trucks examined were found to have high prevalence and intensity of parasites. It is suggested that proper quarantine procedure was not performed before retail selling of these imported fish. According to consequences of this study, It is recommended that before transporting internationally, fish should be examined for parasitic risk and other pathogens to prevent the spread of parasitic diseases.

1. INTRODUCTION

The ornamental fish industry in Turkey has grown in recent years (Hekimoğlu, 2006. 237-241). Gold fish (Carassius auratus) is considered among the most important ornamental fish in Turkey. Most of these fish are imported from Asian countries. Lately, these fish are the largest of all aquarium fish imported from Syria into Turkey.

In many countries, the tropical ornamental fish trade operates without appropriate quarantine practices. These fish may cause problems in the importing country, since they can die of infections soon after their arrival, or during transportation, resulting in economic losses. Recently, mortalities have occurred in Gold fish (Carassius auratus) imported from Syria into Turkey and a number of parasites have been observed in these fishes.

The presence of parasites on ornamental fishes and their transport to other countries has been reported worldwide; in China (Kuo,vd.1994. 227-238), Germany (Moravec,vd.1999: 296-310), Australia (Dove & Ernst, 1998 : 1755-1764, Evans & Lester, 2001: 51-55), South Africa (Mouton, vd., 2001: 327-333), France (Michell,vd.,2002:253-263), Korea (Kım,vd.2002: 231-235), Norway (Levsen,vd. 2003:639-649) and Sri Lanka (Thilakaratne vd.2003; 154-162). The most important ectoparasites of freshwater ornamental fishes are ciliate protozoans such as; Ichthyophthirius multifiliis, Trichodinids, Chilodonella . Monogeneans are typically parasites of the gills and skin of the fishes and are generally host specific Gyrodactylids are skin and fins while Dactylogyrids live on gill. Argulus foliaceus and Lernaea cyprincea has been reported parasitizing several freshwater fishes (Woo ,1995).

In this study, we aimed to diagnose the parasites in freshwater Gold fish (Carassius auratus) and determine the prevalence of some of these parasites imported into Turkey from Syria.

2. METHOD

One hundred live imported goldfish from Syria, May and June between month in 2012, were collected from a quarantine facility (with their original water) and transfered by trucks into Syria to examine in the laboratory of diagnosis of Department of Aquaculture, Faculty of Fisheries Mersin University. Fish samples were weighed and measured. Routine laboratory examinations, like examination of wet mount of skin and

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direct of gills examination were performed. Wet mounts from both left and right gill arches were examined from each fish. Then for more precise observation, stereo and light microscopies were used in examination of gill arches. Photo of any infected fish was taken with the aid of phase contrast microscopy (Nikon). Monogenean parasites were fixed in 70 % ethanol, and preserved on slides using Malmberg's method (ammonium picrate glycerine) and protozoans were airdried onto slides and stained with Gieamsa solution. Identification of monogenean species was made by using morphological (hook, marginal hook, their bars and shape/number of copulatory organs) and morphometric(hook, marginal hook and bars parameters) characteristics. The parasitic arthropod were fixed in 70% ethanol and identified using by gross morphology (size, appendage morphology, urosome,and respiratory areas) (Rushton-Mellor, 1994: 51-63; Lom .& Dykova 1992:253).

3. RESULTS

The parasites identified from gill of one hundered samples were found comprising: four species belonging to two genera of, monogeneans trematodes, Dactylogyrus sp, Dactylogyrus vastator, Gyrodactylus sp., and Gyrodactylus chinensis, two protistan species Trichodina sp., Ichthyophthirius multifiliis, and one arthropoda species Argulus japonicus. D. vastator was the parasite showing the highest prevalence rate in one parasitized with almost 50% followed by Gyrodactylus sp. with 16% indicating high and low prevalences among monogeneans. Trichodina sp. and Ichthyophthirius multifiliis show prevelances of 31 and 25% among the protozoan respectively. Argulus japonicus show 80% prevalence among the arthropod.

Table 1. Prevalence of parasites isolated from goldfish (*C. auratus*).

Parasite	Prevalence (%)	Parasite taxonomy
Metazoans		
Dactylogyrus sp.	28	Monogenea
Dactylogyrus vastator	50	Monogenea
Gyrodactylus sp.	16	Monogenea
Gyrodactylus chinensis	29	Monogenea
Protozoans		
Trichodina sp	31	Ciliata
Ichthyophthirius multifiliis	25	Ciliata
Arthropoda		
Argulus japonicus	80	Branchiura

4. DISCUSSION

This study is the first report of Gold fish imported from Syria into Turkey . We found a total of seven species of parasites among Gold fish in this study (Tablo1).

Ichthyophthirius multifiliis is a widely distributed ectoparasite, and probably occurs worldwide (Koyuncu, 2009: 25-27). In this study all trucks originating from the same region were found to have I. multifiliis and Trichodina sp.intensity of ectoparasites.

It was difficult to determine the cause of death in this case, however, because we also found Trichodina sp. in the same stock. Trichodinids are essentially commensals and never occur in large numbers on healthy fish. However, in stressed conditions caused by some other factors such as poor water quality, overcrowding, they can proliferate massively and behave like serious ectoparasite (Koyuncu, 2009: 25-27). We suspect that both poor conditions and I. multifiliis infection facilitated the proliferation of Trichodina sp. and caused the death of the host in this outbreak.

The monogeneans Dactylogyrus sp. and D. vastator were described from Gold fish (Carassius auratus). Another monogenean, G. chinensis, was first reported as Gold fish (Carassius auratus) in Turkey. This monogeneans are likely to have harmful effects not only on Gold fish (Carassius auratus), but also on other Gold fish, because it can lead to other hosts in confined environments or in stressful conditions.

The copepod A. japonicus has a broad host range including Gold fish (Carassius auratus), and can cause serious damage (Rushton-Mellor, 1994: 51-63). This copepod has a worldwide distribution, partly because of the international trade of tropical fishes (Rushton-Mellor,1994: 51-63; Lom .& Dykova 1992: 253). Its pathogenicity is well-known because it can cause serious mortality due to hemorrhages and secondary bacterial infections, especially in cases of heavy infestations (Şahin , 2004). In this study all trucks originating from the same region were found to have intensity of these ectoparasites.

The Gold fish trade constitutes a significant portion of worldwide trade in aquatic animals and a large number of imported Gold fish originate from Southeast Asian countries. (Evans & Lester , 2001: 51-55). Turkey also imports various kinds of Gold fishes from Syria; the scale and number of imported Gold fish are increasing. However, most fishes are imported without proper quarantine measures, and consequently, the fishes infected with undetected pathogens can be distributed to retailers and sold to consumers. Thus, many undescribed parasites could be entering importing countries with imported fish.

Mousavi studied parasites of ornamental fish imported in Iran and reported ten parasite species (Mousavi vd.2009:175-180). Other investigator and authors have described many of isolated parasites in this study, which can infect ornamental fish of many species (Evans & Lester , 2001: 51-55; Thilakaratne vd.2003:

154-162; Şahin, 2004: 63: 17; Mousavi vd.2003: 297-300; Mousavi vd.2009: 175-180.).

In the present study, it has been confirmed that parasites are prevalent in Gold fish (Carassius auratus), imported into Turkey from Syria.

Author contributions

All contributions belong to the author in this paper.

Conflicts of interest

The author declares no conflicts of interest.

Statement of Research and Publication Ethics

The author declare that this study complies with Research and Publication Ethics

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Three-Dimensional Modeling of an Object Using Underwater Photogrammetry

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3d model
Camera Calibration

ABSTRACT

Since the use of cameras in the underwater environment, underwater photogrammetry (UP), a sub-science of photogrammetry, has emerged. Methods and software that can produce three-dimensional (3D) data from moving recordings of objects known as Structure from Motion-SfM, which are used in the same photogrammetry, are also used in underwater photogrammetry. It can be used to create 3D models of shipwrecks, marine ecosystems and archaeological remains with UP. In this article, an object was placed in a 1.5-meter deep pool and it was aimed to create a (3D) model of the object by underwater photogrammetry using the obtained data set. The 3D visual of the model was created by combining the images taken under water in the software.

1. INTRODUCTION

With the developing technology, the present and future generations are provided with opportunities to collect and evaluate environmental information in various ways. One of these fields is photogrammetric studies. Photogrammetry is a branch of technology, science and art in which reliable information is obtained because of recording, measuring and interpreting the images shaped by physical objects and the rays reflected from the environment they form and the electromagnetic energies they emit (Yakar et al., 2009; Ulvi et al., 2020). The photogrammetry process, which aims to collect three-dimensional information about the geometry, color and texture of an object, is a technique that allows the creation of a 3D virtual model by obtaining digital images (Yakar et al., 2016). In this way, 3D models are frequently used in many areas such as documentation studies, smart city applications and the production of city models, modeling of organs for medical purposes, modeling of architectural designs, and archaeological studies, since they contain many details of the building (Yiğit et al., 2020).

With the production of cameras that can photograph underwater, underwater has also been added to these areas. In this way, 3D modeling has begun to be used in different areas such as modeling the underwater topography, marine ecosystem, archaeological remains and shipwrecks (Alyılmaz et al., 2010; Van Damme, 2015). Experiments in water alto photogrammetry can be performed both in laboratory conditions and in pools a few meters deep or in natural waters.

The usage areas of underwater photogrammetry are very wide. Of these, it is now widely used in marine biology and archeology to study sunken objects (such as shipwrecks and prehistoric settlements). Additionally, hydrographic measurements and bathymetric surveying include the maintenance of underwater cables and pipelines (Yılmaz et al., 200; Kanki et al., 2021; Hamal and Ulvi, 2020).

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Underwater photogrammetry is the creation of a 3D model with the help of 2D photographs taken underwater without contact with the object (Drap, 2012; Drap et al., 2015). Digital cameras are used in underwater photogrammetry. However, most digital cameras require a special underwater housing with a lens port suitable for the underwater environment to be suitable for underwater inspections. (Capra et al., 2015; Skarlatos et al., 2021). For this reason, it is obtaining popularity by supplying comparatively high resolution in relation to low-cost devices such as the GoPro camera and its maintenance costs. Requires professional divers during underwater expeditions to use either expensive digital cameras or inexpensive GoPro cameras. Due to the dangerous features of the measurements (divers being under water for a long time, difficult measurement conditions, etc.), unmanned underwater vehicles such as unmanned aerial vehicles or underwater drones are increasingly needed. They can be controlled entirely remotely or with little diver intervention. (Raoult et al., 2016; Kujawa, 2021).

There are many factors that affect the quality and accuracy of measurements taken in underwater photogrammetry (Korumaz et al., 2011). The first of these; The blowing from the wind causes the sun's rays on the water surface to vibrate, which creates shiny textures called caustics on the water floor or on the objects on the bottom. When light comes to still water, the water acts as a reflective mirror, while the light coming into wavy water is more absorbed by the water. Light rays are refracted by forming caustics on wavy surfaces, which is undesirable for photogrammetric applications. This is because it affects the extraction of two-dimensional content data from the photo as well as creating poor quality object texture. The intensity of the light caustics depends on the slope of the sun angle, the water turbidity and the depth, but after a few meters the effect gradually decreases. In underwater environments, especially in deep places, it is necessary to use artificial light sources such as electronic flashes. Because it is argued that both the colors can be reflected as they are and the clarity caused by the limited viewing angle can be compensated (Menna et al., 2016; Menna et al., 2016). Various particles such as phytoplankton, organic substances, and pollution suspended in the water cause the water to become cloudy, and light is scattered in the water for these reasons. Scattering, or diffuse reflection, occurs due to the random deflection of light from its direction. Scattering; it limits the view quality, lowers the contrast and causes blurry images.

The second factor affecting underwater photogrammetry is the clarity of the water (Köseoğlu and Kocaman, 2018). It is advised to measure in the early morning or late afternoon to avoid reflection of sunlight from the water surface and the research object (in areas with shallower depths) (Agrafiotis et al., 2018). Shooting distance also varies depending on viewing conditions. Insufficient water transparency decrease the contrast of the image. This need the camera to be closer to the subject when taking pictures (Drap et al., 2007; Gambin et al., 2021).

The third factor affecting underwater photogrammetry is the refraction of light. In most underwater imaging systems, a beam of light travels through water, housing (glass or plastic), and air. Light rays are refracted twice at the air and water interfaces (Taşdemir et al., 2009; Treibitz et al., 2012; Song et al., 2019). The principle of refraction is given in the following equation (1) according to Snell's law:

$$\frac{sin\theta_i}{sin\theta_t} = \frac{n_2}{n_1} \quad (1)$$

Snell's law states that for a light ray, the ratio of the sine of the angle of incidence to the sine of the angle of refraction is constant (Bhattacharjee, 2021). Here n_1 is the scale of refraction of the medium from which the light comes, and n_2 is the scale of refraction of the medium in which the light is refracted. In addition, θ_i and θ_t are the angles of the incident and refracted ray with the normal, respectively.

The light beam is refracted as it passes through various mediums (water, glass, air). This causes a refractive error that must be taken into account in the calibration process. Two solutions have been proposed for this problem. The first solution is to use a modified linearity model with geometric interpretation of light in multimedia (camera housing - water). The second solution requires the use of standard system calibration software, including a camera and a waterproof housing device. (Telem and Filin, 2010; Elnashef and Filin, 2019; Kujawa, 2021).

The process of geo-referencing photographs in underwater photogrammetry is a problematic issue because of the more complex logistics underwater than in the air (Yilmaz et al., 2004). Several techniques are used in the literature in the geo-referencing process. In the measurement of small objects, grids made of scaled iron rods are made before the measurement to make the measurement easier and faster. For large areas, depending on the topography of the measured place, grid-shaped strips are drawn in the area to be measured underwater and placed with iron bars at the control points. In order to measure these points accurately and precisely (fixed), these buoys were measured with the help of GPS before placing buoys with varying weights on the surface and starting the measurement. The lengths of the iron rods are subtracted for the depth calculation of the measured points.

2. METHOD

2.1. Data acquisition

In the study, overlay photographs covering the entire study area were taken to create a 3D model with the underwater photogrammetry technique. The photos are in similar weather conditions, the sky is cloudy, the temperature is around 23 ^{o}C and the height of the pool is 1.5 meters. For this purpose, the go pro black hero 9 camera was used to collect the photographic data of the underwater location (Figure 1). The technical information of the camera used in the study is shown in table 1.

Figure 1. Go Pro Hero Black 9 camera and housing

Table 1. Technical specifications of the Go Pro Hero Black 9 camera

Technicial Specifications	Value
Sensor	CMOS Sensor
Sensor Resolution	23,6 MP
Media Recording	1 x microSD / HC / XC (256 GB Maksimum)
Still Image Support	JPEG - 20 MP
Audio format	WAV
Display type	LCD
Dimension	5.7 cm / 2.27 inç
Secondary Display	Front: Live View Monitor
Shutter speed	1/25 - 1/2000 second (photografy)
Photo ISO Range	100 - 6400
Video ISO Range	100 - 6400
Burst Photo	30 Photos / 3 Seconds
Image stabilization	Digital
Waterproof Depth	33.0 '/ 10.0 m (camera)
White Balance Modes	Oto
Built-in Microphone	Yes
Built-in speaker	Yes
Wireless Internet	Yes
Battery	Rechargeable Battery Pack, 1720 mAh
Charging Method	USB
Weight	5,6 oz / 158 g

Care has been taken to take the photographs with an overlay so that they can see the object from different angles.

During the photo shoot, attention was paid to ensure that each control point marked around the object in the appropriate number and angle for the production of the 3D model of the work with high resolution and accuracy is visible and selectable in three photographs. The number of photos varies according to the size of the model to be made 3D model. In the study, 80 overlay photographs were taken.

2.2. Data processing

Context Capture software was preferred for photogrammetric evaluation and creating the 3D model of the object.

The intersection points of the ceramics around the placed object were used for the detail points in the photographs taken (Figure 2).

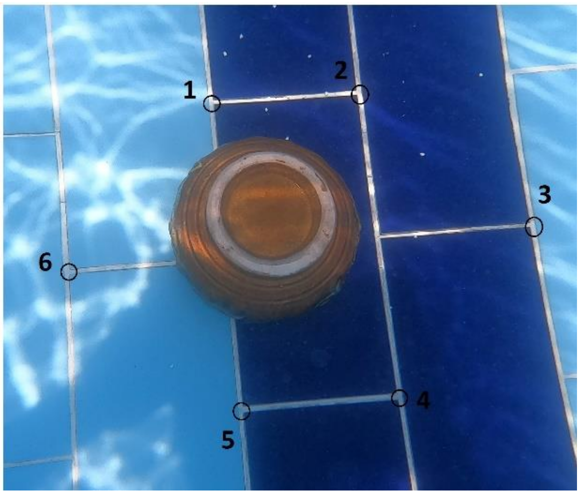

Figure 2. Tie point

3. RESULTS

First, the photographs were loaded into the software and the alignment process was performed. Then, the detail points are marked in the photos to create an accurate and precise model. Finally, dense point cloud and mesh were produced (Figure 3 and Figure 4).

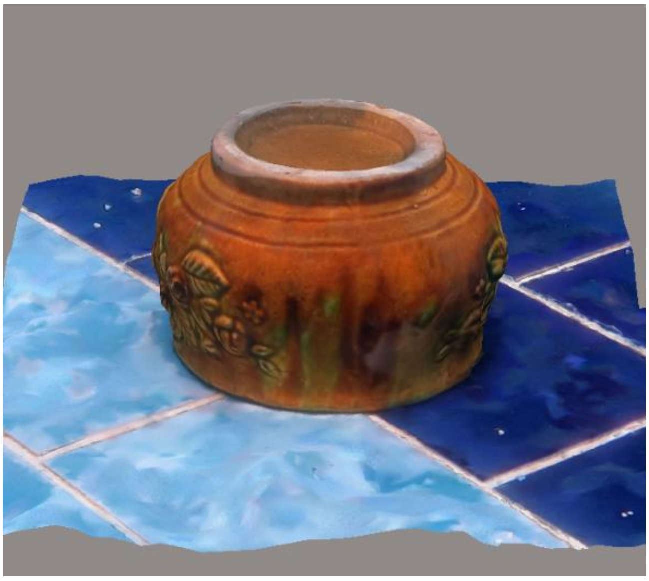

Figure 3. The resulting dense point clouds.

Figure 4. The resulting mesh.

The mean square error of the created model is ±1 cm.

However, when the positions of the point clouds are examined, even though the shape and details of the model provide a meaningful unification, there are fluctuations in the ceramics around the model. As can be seen in Figure 5, while the junction values of the model were 0.00097 cm, its circumference increased up to 0.14157 cm values. There are two reasons for this situation. The positions of the ceramics may be deviated due to the object-oriented shooting during the first photo shoot. Secondly, due to taking underwater photos, it was concluded that the waves were effective even if they were mild.

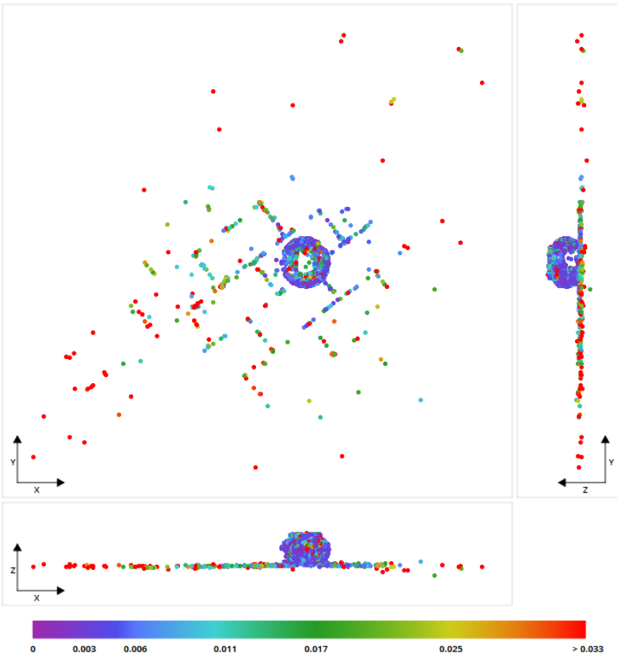

Figure 5. Top view (XY plane), side view (ZY plane) and front view (XZ plane) displays of all tie points

4. DISCUSSION and CONCLUSION

In this article, the processing stages of the photographs obtained in the underwater environment are presented. Photograph taken at shallow depth and under good environmental conditions (no water turbidity and good lighting conditions). However, in 10 of the photographs taken, caustics occurred in the images due to fluctuations. These photos were not used in merging.

The mean square error of the research model was ±1 cm, and it was seen that it provided convenience in terms of obtaining the data. The software used was quite effective in giving a detailed and realistic image. When the 3D point cloud and mesh model of the research model are examined, the details of the patterns on the model are given clearly.

This shows that underwater photogrammetry can be used in cultural heritage documentation. The presented research model validates the use of underwater cameras in photogrammetry. It can be seen as a new beginning for more professional research in the context of underwater photogrammetry.

Author contributions

The contributions of the authors to this article are equal.

Conflicts of interest

The authors declare no conflicts of interest.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics

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The First Record of Argulus japonicus Thiele, 1900 Infestations on Telescope fish (Carassius auratus) of Mersin in Turkey

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Keywords
Argulus japonicas
Ectoparasite
telescope fish
Carassius auratus
Mersin

ABSTRACT

Argulus japonicus (Crustacea: Branchiura), or the fish louse, is an ectoparasite of the skin or gill of the fresh water fish species. Clinical signs in infected fish include scratching or flashing on pond aguarium or other objects. It causes pathological changes due to direct tissue damage and secondary infections. In the present study, telescope fish (Carassius auratus), taken from a goldfish aquarium with symptoms such as abnormal swimming, poor growth and death, were examined for ectoparasites. The parasites collected from the skin and fins of fish were identified as A.japonicus. Treatment was carried out by trichlorfon. After administration, no parasite was observed on the fish. This study is the first report of infection with A. japonicus of telescope fish (Carassius auratus)of Mersin in Turkey.

1. INTRODUCTION

Ornamental fish culture has rapidly developed in different countries. Crustaceaparasitic infestation is the most important disease affecting ornamental fish and it causes economical losses for this growing industry in intensive culture systems. (Roberts, 2010)

Argulids have been recognized as pest of cultured trout in Europe and carp in China since the 17 th centrury(Kabata 1985).The three best known species are Argulus foliaceus, A. japonicus and A. coreconi.They cause mortalities of fish in aguarium, in lakes and estuaries, and occasionally cause problems in sea-caged salmonids (Menezes et al.1990; Rushton-Mellor, 1992). Secondary infestions by fungi and bacteria reduce the commercial value of parasitized carp and goldfish (Shimura, 1983a).

Argulus japonicus has a worldwide distribution. It has been introduced with aguarium fish from orient and now is common wherever Goldfish but has found (Cressey, 1978). In North America it infests primary goldfish but has been found on Cyprinus and Ictalurus(Amin, 1981). In Europa it infests Carassius, Cyprinus and other genera inculuding Exos,Perca,Tinca and Sardinus (Freyer, 1982, Woo, 1995). It feeds by piercing the skin of their host, injecting a toxin and drawing off blood (Kabata, 1970). Heavy infestations can cause serious damage to the skin and subsequent mortality (Kabata, 1970; 1985).

This study is the first report of infection with A. japonicus of Telescope fish (Carassius auratus) of Mersin in Turkey. Also, in this study was determination of causes of death in telescope fish and treatment of infested fish as well.

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2. MATERIALS AND METHOD

In May month 2019a goldfish producer referred to, Department of Aquaculture of Faculty of FisheriesMersin University,Mersin in Turkey. He complained of abnormal swimming, poor growth and death in telescope fish aquarium. Examination of fish showed around the head, typically behind the fins and small red spots on the skin .Fish samples were weighed, measured and thereafter the operculum, fins and body surface were examined for ectoparasites.The research material consisted of 100 telescope fish (Carassius auratus). Parasites were easily observed with the naked eyes and were removed using a small probe or forceps and fixed in 70% ethanol. A hand lens was used to distinguish the external parts and general structure. Under the light microscope, these parasites were identified at Argulus foliaceus according to the rounded lobes of abdomen and the posterior emargination not reaching the mid-line and posterior lobes cephalothoracic carapace not extended beyond the beginning of abdomen.Specimens were examined and photographed using a phase contrast microscopy (Nikon H550L). Parasites identification was made using the following specific key: Wilson 1903, Bykhovskaya-Pavlovskaya et al.1962;Fryer, 1982; Kabata 1985;Gresty et al.,1993;Rushton-Mellor,1994; Kabata1996 ;Wadeh et al.2008on the basis of main morphological features (size, appendage morphology, urosome, respiratory areas).

3. RESULTS

The fish weighed 4.2-5.5 g and were 5.2-5.7 cm in size.Fish infested with A. japonicushad brown-grey points on the skin and fins due to parasitic irritation and tissue damage. Parasites were collected from around the operculum and body surface).The mean number of parasites per fish was 1-5. From body surface of 33 (33%) out of 100 subjects examined, only argulid crustaceans were detected. The prevalence of A.japonicus was 28 % on telescope fish. All the argulids found during this survey were identified as adult females and males of A. japonicus.

The dimensions were male (n=10)a length range of 3,7(2.9–4.4) mm and a width range of 2.6(2.2-3) mm, abdomen a length range of 1.2 (1.1–1.3) mm and abdomen a width range of 1.0 (0.9–1.1) mm. The dimensions were female (n=27) a length range of 5,45(4,9-6,0) mm and a width range of 4,5(3,8-5,1)mm. abdomen a length range of 1.5 (1.3–1.7) mm and abdomen a width range of 1.15(0.8–1.5) mm.The parasite has rounded lobes of abdomen and the posterior emargination reaching the mid-line and posterior lobes cephalothoracic carapace extended beyond level of the middle of abdomen. The head show features are typical of A.japonicus in dorsal view.Legs of the males of species of Argulus japonicus to show the differences in the clasping apparatus .The parasite identified as A. japonicus by the looking morphologic key characteristics under phase contrast microscopy .

The owner was recommended to disinfect the aquariums and equipment to remove completely eggs, and treatment of fish with trichlorfon (0.25 mg/l at temperatures below 27 ºC, or with 0.50 mg/l above 27 ºC) . Yıldız and Kumantaş 2002;Tokşen, 2006). During treatment, neither adverse effects nor mortality was observed throughout the trichlorfon bath. All fish were checked in terms of parasite following the treatment. No parasite was observed on the fish.

4. DISCUSSION

Goldfish is one of the major ornamental fish for which more than 100 varieties have been produced with selective hatching and many people are interested in their breeding and rearing. Besides their breeding in different fish farms in Turkey, different species of goldfish are imported annually from Southeast Asian countries.Studies carried out on goldfish and koi confirms the Argulus as the most prevalent parasite (Noga 2010).Argulus are good swimmers. Adults and larvae can easily migrate among many hosts. So, Argulus can induce morbidity and mortality in cultured fish populations (Kabata 1985).Also, this ectoparasitic species is widely adaptable and can live in freshwater habitats.

Argulus sp. was reported from different fish species worldwide (Woo 1995, Buchmann&Bresciani 1997).A.foliaceus has been reported parasitizing several freshwater fishes from the different regions of Turkey (Geldiay & Balık 1974; Sarieyyüpoğlu &Sağlam 1991; Özerand Erdem,1999; Yıldızet al2002,Koyuncu,2002;Öztürk&Aydoğdu2003;Kahveci, 2004; Karatoy, 2004;Kır et.al. 2004;Tabakoğlu, 2004;Tekinet.al. 2005;Öztürk, 2005,Uzunay & Soylu 2006; Karatoy & Soylu 2006; Öktemeret.al 2006,Tokşen2006,Alaş et al. 2010, Öktener et al. 2010, Öztürk, 2010; Pekmezci, et al, 2011). In the present study, A. japonicuswas first reported fromorandagoldfish(Carassıus auratus)of Mersin in Turkey.

In this study, it was determined that the general morphology and dimensions of both sexes of A. japonicus are similar to those described by other authors (Fryer, 1982; Kabata, 1985; Gresty et al.1993; Rushton-Mellor, 1994; Kabata 1996, Wadeh et. al.2008).

A.japonicus infestations cause the skin irritation manifested by flicking of the fins (Richards, 1977; Bauer 1991). This is often accompanied by increased mucus production over the skin surface and the appearance of small haemorrhages (Richards, 1977). In this study, abnormal swimming, rubbing themselves against the wall of aquarium and lack of appetite were observed in diseased fish. The skin and fins have numerous brownish grey points and hemorrhagic areas.

It is known that Argulus infections lead to secondary parasitic infestation of the skin (Bauer 1991). Some authors reported that Costia necatrix accompanied by A.foliaceus in infected fish, and also Trichodina sp., Trichodinella sp.,Apiosoma sp.andDactylogyrus sp. were observed in skin and gills preparation (Burgu &Oğuz, 1984; Bauer, 1991; Yıldız &Kumantas 2002). In this study, no other parasites were observed on the body surface.

There are several reports of hundreds of Argulus species occurring on a single fish, and Fryer (1982) stated that a tench in Europe was found with thousands

Argulus sp. In this study, 1 to 5 parasites were found on the fish examined. This might be related to the early stage of infection. Pathogenesis was severe because these fish were small a lot of parasites being found on the fish. Also aquarium fish were affected heavily by ectoparasites due to the very fine structure of the skin.

The treatments of Argulus infestations include the use of common chemicals such as salt (NaCl) . potassium permanganate (2-5 mg/l bath), formaldehyde and formalin . The most effective treatment against argulusosis is organophosphates. Organophosphates, usually 2-3 doses at one-week intervals, are needed to treat the emerging larvae and juveniles. Treatments such as trichlorfon (0.25 ppm for several hours) and emamectin benzoate have been used to eradicate Argulus japonicus. (Öge, 2002).The owner was recommended to treatment of fish with trichlorfon (0.25 mg/l at temperatures below 27 ºC, or with 0.50 mg/l above). All fish were checked in terms of parasite following the treatment. No parasite was observed on the fish.

5. CONCLUSION

As a result, importation of ornamental fish is carried out in many countries without any special management and strict quarantine. So, in the case of any infestations, diseases come into the country through these infested fish, especially parasitic infestations, that threatens native fish and aquaculture industry of that country. One of the important issues related to parasitic infestations of ornamental fish is the infestation transmission from imported fish to native fish and their habitation as natives in new region. Therefore, imported fish should be examined for their health and for parasitic infestations in order to prevent the burst of new parasitic fauna to different countries and stop direct economic losses caused by mortality derived from infestations appeared in relocation.

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Author contributions

All contributions belong to the author in this paper.

Conflicts of interest

The author declares no conflicts of interest.

Statement of Research and Publication Ethics

The author declare that this study complies with Research and Publication Ethics

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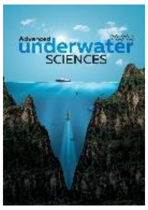

The Economic Importance of Macroalgae

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Keywords
Algae
Macroalgae
Taxa
Species

ABSTRACT

In this study, we discussed algae with economic importance. Information was given about the use of macroalgae in the food industry, medicine and pharmacy, additives, organic agriculture, wastewater treatment, and bioplastic production, which are the areas of use in the economy. The taxa and species used in these areas were investigated. Macroalgae in the seas of our country were examined, and information was given about how they can contribute to the economy.

1. INTRODUCTION

Algae are primary producers in the aquatic environment and form an essential part of the food chain (Aktar and Cebe, 2010). Industrial uses of algae differ in terms of micro and macroalgae. Marine macroalgae are mostly preferred as raw materials in the industry due to the risk of contamination in the production of microalgae (as in bacterial sources) and the difficulties experienced in their harvest (Oğur, 2016).

Algae are used in almost every field of industry. Algae, which are used as food in the Far East and South Asian countries, also have wide use in medicine, pharmacy and cosmetics industries, in agriculture, and in fertilizer production. In addition to collecting algae from nature, they are also cultured (Oğur, 2016). Macroalgae are used as food in Japan, China, Korea, the Philippines, and similar places, and as raw materials in many areas of industry in Europe and America. For this reason, macroalgae stand before us as organisms worth examining and focusing on in all aspects (Yazıcı and Kaynak, 2012).

1.1. Ecology of Algae

Algae, which perform the first production by photosynthesis, have a very important role in the marine ecosystem as they form the first link of the food chain (Aktar and Cebe, 2010). Algae show a geographical distribution depending on the physical and chemical changes of the environment. Human activities, domestic, industrial, and agricultural wastes have had a direct impact on eutrophication in recent years. In addition, nitrogen mixing with the water from the atmosphere, nutrients carried by rainwater, and substances carried to the environment through drainage are natural developments that accelerate the pollution process. One of the most obvious consequences of eutrophication is the occurrence of excessive algal blooms. The increase in phytoplankton populations causes the color, smell, and ecological balance of the water to deteriorate. In addition, the excessive growth of algae causes toxic effects for many living things in the aquatic environment and causes death (Aktar and Cebe, 2010; McHugh, 2003).

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1.2. Ecological Factors Affecting the Distribution of Algae

The factors affecting the distribution of algae are grouped under three groups (Aktar and Cebe, 2010):

1.2.1. Physical Factors

Substrate: The physical structure of the substrate is important for the adhesion and development of algae. Algae living in the substrate are called “endolytic algae” and algae living on the substrate are called “epiphytic algae”.

Temperature: In seas, the seasonal temperature difference decreases as the depth increases. For this reason, some algae are not resistant to temperature changes in the depths. On the other hand, since the temperature changes are very high in the water bodies in the mediolittoral zone, only algae resistant to temperature changes such as Ulva sp. and Enteromorpha sp. are encountered. In the Mediterranean, cold sea species such as Bangia sp., Nemalion sp., Ulothrix sp. are found in winter, and warm sea species such as Acetabularia sp., Anadyomene sp., Halimeda sp., and Digenea sp. are seen in summer.

Light: The wavelength and amount of light is a limiting factor in the deep distribution of algae. Non-living particles and planktonic organisms in seawater cause a decrease in the light transmittance of seawater. Blue and violet light are the rays that can reach the deepest the most. According to their tolerance to light, marine algae are divided into two groups: "light-loving algae" and "dark-loving algae".

Turbidity: Suspended solid matter causes the water to lose its clarity. This is called “turbidity of waters”. Turbidity limits the intensity and spread of light. As a result, photophilous organisms move away from the environment as primary production efficiency decreases.

1.2.2. Chemical Factors

Salinity: Salinity is one of the most important factors in the distribution of marine organisms. NaCl, which is dominant in seawater, regulates the osmotic pressure in living things. Oligo-elements such as N, P, S, K, Ca, Mg, Si is also important in the physiological activities of living things. Elements such as I and Br, which accumulate in large amounts in various algae species such as Laminaria sp., increase the economic value of these plants (Aktar and Cebe, 2010; Cirik and Cirik, 2011).

pH: Seawater is generally basic and its pH varies between 8.1 and 8.3. Eurichalin, euritherm, and eurionic green algae species, Ulva sp. and Enteremorpha sp., are seen to grow abundantly in environments in the supralittoral and mediolittoral zones with little contact with the sea (Casal et al., 2009).

As a result of the photosynthesis activity of these algae and the excessive use of CO_2, the bicarbonate in the environment decomposes and turns into neutral carbonate. This event causes the alkalinity in the environment to increase and the pH to reach 10. Therefore, stenoionic algae such as red algae cannot grow in such environments (Cirik and Cirik, 2011).

Dissolved Gases in Sea Water: O_2 has a limiting effect on the distribution of algae as well as in all sea creatures. The main factors that increase the amount of O_2 are photosynthesis, the contact of oxygen-poor surface waters with the atmosphere, the effect of currents and winds. Among the factors that reduce the amount of O_2, it can be said that the respiration of plants and animals, various chemical and biological events including oxidation, O_2 loss from surface waters that are in contact with the atmosphere and richer in oxygen. Algae are absent in oxygen-free zones (Aktar and Cebe, 2010; Cirik and Cirik, 2011).

Nutrient salts, oligo-elements, and vitamins: Lack of nutritive salts such as nitrate and phosphate limits the growth of phytoplankton, and excess causes the proliferation of nitrophilous algae such as Ulva sp. and Enteromorpha sp.. Some oligo-elements support the growth of algae. As a result of bacterial activities in seawater, various vitamins, especially B_{12}, occur. While some algae do not require vitamins for their growth, others require vitamins such as B_{12}, biotin, and thiamine. Some algae such as Corallina sp., Halimeda sp., Lagora sp., and Diatomae sp. store $CaCO_3$ and silica Cirik and Cirik, 2011; Casal Garcia et al.,2009; Farasat et al., 2014; Hassan and Ghareib, 2009).

1.2.3. Dynamic Factors

Sea Turbulence (Agitation): Although the algae in environments with high water movements such as Cytoseira fimbriata are unbranched, it has been observed that the algae in calm waters are more branched. The turbulence of the water is important for the ecological factors to become homogeneous. In the observations made on algae, it was observed that the intensity of photosynthesis and respiration change depending on the water movements (Aktar and Cebe, 2010; Cirik and Cirik, 2011).

Sea Level Change and Staying Out of Water (Emersion): Periodic changes in sea level are seen depending on the gravitational force of the moon, the effect of winds, air pressure, and the location of the coast. Although being out of the water is a requirement for some algae such as Fucus sp., Pelvetia sp., Ascophyllum sp., most of them cannot stay out of the water for a long time (Aktar and Cebe, 2010; Cirik and Cirik, 2011; Hassan and Ghareib, 2009).Water Movements (Currents, Waves): In the observations made on benthic algae, it was determined that thallus size is related to water movement. Water movements can affect the growth and development of algae in two ways. The first of these is the transport of reproductive cells to various places with water movements and the facilitation of their fertilization, and the other is the negative or positive effects of young individuals at the time of settling. It is accepted that the biogeographical distributions of planktonic algae are related to currents (Aktar and Cebe, 2010; Cirik and Cirik, 2011).

Pressure: In a study on the effect of hydrostatic pressure on algae distribution; Although the superficial forms of Codium bursa are a few cm in diameter, it has been observed that the forms living at 40-50 m depths are approximately 1 m in diameter (Aktar and Cebe, 2010; Cirik and Cirik, 2011).

1.3. Usage Areas of Algae

One of the most important living resources of the seas is algae. Algae are used in the fields of food, agriculture, medicine, pharmacy, cosmetics, and industry. In addition, algae are of great importance to other sea creatures.

1.3.1. Use of Algae in Medicine and Pharmacy

It is known that algae are used in injuries, heavy metal poisoning, balancing the immune system, reducing "high fever, regulating blood circulation, skin regeneration, removing vascular occlusions, and lowering cholesterol (Aktar and Cebe, 2010; Meenakshi ,et al.,2012). In some societies, Ulva species are used as a low-calorie diet for weight loss because of their rich fiber, mineral, protein, low fat and digestible carbohydrate content. There is information that algae are widely used in the treatment of goiter, in the treatment of various kidney diseases, and as an anthelmintic, especially in countries with a coastline. For example, in South America, Ulva lactuca is used to increase resistance to goiter because it is rich in vitamin A (Aktar and Cebe, 2010; Gümüş, 2006). Antimicrobial agents could be obtained from Fucus serratus species. In addition, it is rich in iodine and is used against goiter disease and obesity (Aktar and Cebe, 2010; Taşkın, et al., 2010).

S. vulgare, one of the Sargassum species found in our country, contains an antilipidemic substance in its structure. Other Sargassum species are also algae with anticoagulant and analgesic properties. Cyctoseria barbata is also one of the species that have an antilipidemic substance in its structure and spreads in our seas. Macrocystis pyrifera is used in anemia because it is rich in vitamin B12. Acanthopeltis japonica, one of the red algae, is used as a food and is also known to reduce the cholesterol ratio. Some red algae, such as Porphyra atropurpurea, are used as "poultice" (Aktar and Cebe, 2010; Devi,et al. 2008). Chondrus crispus species has been used as medicine and food in Europe for a long time (Taşkın, et al., 2010; El Baky,et al., 2008; Taşkın, et al., 2007).

The important uses of algae in pharmacy are mainly due to their phycocolloids (Brownlee, et al., 2005). It is known that phycocolloids affect drug absorption in the body due to their colloid-forming properties. Phyllophora nervosa found in the Black Sea is used as a source of phycocolloids in medicine and pharmacy (Aktar and Cebe, 2010).

Alginates obtained from algae are used as raw materials or auxiliary materials in the pharmaceutical industry. Alginates are used in injectable and oral drug forms of some active substances (such as insulin, antibiotics, hormones, vitamins); in aqueous solutions of oils and waxes; providing homogenization and stability of oily creams; It is used in making lotions, ointments, soaps, shampoos, tampons, toothpaste and coating drug forms that dissolve in the intestine. Alginates form the main material of plasters, dressings, and bandages (Aktar and Cebe, 2010; Gümüş, 2006).

Compounds responsible for antibiotic activity are common in macroalgae. The most important of them are; halogenated compounds were alcohols, aldehydes, terpenoids, hydroquinones, and ketones. These macroalgae used in antibiotic activity: Cystophora sp, Bryopsis sp., Ascophyllum nodosum, Jania rubens, Dictyopteris membranacea, Cystoseira barbata, Ulva rigida, Corallina officinalis.

Macroalgae used in anticancer and antitumor activities are Scytosiphon lomentaria, Lessonia nigricans, Laminaria japonica, Bryopsis sp., Ulva sp., Chondria atropurpurea, Caulerpa taxifolia, Cystoseira mediterranea, Cystophora usneoides species.

In recent studies, it has been found that algae can be effective in the treatment of diabetes, especially in TYPE2 diabetes. TYPE2 diabetes is the most common form of diabetes, a metabolic disorder in insulin resistance that accounts for more than 90% of all cases. In pharmacological studies, it has been shown that substances obtained from macroalgae as natural products affect the disorders in antidiabetic metabolism (Yan, et al.,2019).

1.3.2. Use of Macro Algae in Cosmetics

Algae products prepared in the form of various dough, flour, or powder are also preferred in Thalassotherapy centers due to minerals such as calcium, magnesium, sodium, potassium, trace elements such as iron, copper, zinc, and manganese, and vitamins (Turan, 2007). Agar gels are also used in various products such as perfumed underarm creams, sunscreens, and dermatological creams containing zinc oxide or penicillin. It has also been stated in the studies that the alginates used in the creams give a feeling of freshness and relaxation to the skin due to the rapid evaporation effect on the skin. It also provides economic advantages in the preparation of low-fat creams. In soaps and shaving foams, sodium alginate is used as a lubricant, to add an oily feature to non-foaming shaving creams, and to maintain the foam in foamy ones. In hair lotions, alginate spreads over the entire hair, giving the hair a shiny feature and making it easy to shape. Sodium alginate is also used in the production of band-aids due to its adhesive properties (Ak, 2015).

It holds most of the water in toothpaste and is one of the basic ingredients of suntan oils. They are used as fixatives in hair balms and as absorption enhancers in products such as face masks, preserving the foam in shampoos, shaving foams, and soaps. Underarm deodorants with carrageenan inhibit the growth of bacteria that cause undesirable sweat odors (Fitton, et al., 2007).

Macroalgae genera that can be used for these purposes in the field of cosmetics, Porphyra sp., Gelidium sp., Hypnea sp., Halopteris sp., Dictyopteris sp, Stilophora sp, Sargassum sp., Cystoseira sp., Ulva sp., Enteromorpha sp., Codium sp., Gigartina sp., Gracilaria sp., and their species are found in the seas of our country (Cirik and Cirik, 2011).

1.3.3. Use of Algae in Organic Agriculture

The oldest known usage area of algae is fertilizer and it was used mostly in the Far East. Although it is known that algae have been used as fertilizer since ancient times, it has been understood that the application of algae extracts by spraying the leaves for 40-50 years also increases the yield and product quality. Algae extracts are used more in organic agriculture, especially in developed countries, in areas such as increasing the amount of product, reducing fruit storage losses, increasing the uptake of inorganic nutrients from the soil, increasing seed germination, and increasing resistance to stress conditions.

As a result of the use of algae extracts in world agriculture; To increase germination, to provide better root development, to increase the storage life of fruits and vegetables, to provide darker and larger flowers and leaves, to provide resistance against diseases and pests, to increase resistance to stress conditions such as frost, drought and adverse soil conditions, and to increase the nutrient elements in the soil such as effects have been determined (Yazıcı and Kaynak, 2012).

1.3.4. Use of Algae in Food

About 160 species of algae are consumed by humans as food. Algae, which are widely used in Far East countries, especially in China, Korea, and Japan, are on the list of alternative foods against the danger of hunger predicted in the future (Ünver et al. 2017). While 800 thousand tons of 28 million tons of seaweed produced in 43 countries around the world are collected from nature, 94% is obtained through aquaculture. Although there are academic studies on macroalgae in our country, commercial cultivation has not yet started (Ak, 2015). Among marine macroalgae, red and green algae contain higher levels of protein than most brown algae. However, the reduction of nutrient salts in coastal waters during summer reduces the macroalgal protein levels and changes the relative ratios of amino acids. There is a need for better replication of proteins and amino acid profiles and for all nutritional components in macroalgae studies, as well as better commercial identification of the analyzed samples (Wells, et al., 2017).

Algae are the source of industrial polysaccharides such as carrageenan (Chondrus sp., Eucheuma sp., and Kappaphycus species), agar-agar (Gelidium sp., Gracilaria species), and alginate (Laminaria sp., Ascophyllum sp., and Macrocystis species) with a market size of approximately 1 billion USD (Ak, 2015).

Carrageenan: Among the red algae, Chondrus crispus contains 71% and Kappaphycus species up to 88% carrageenan (Ak, 2015). Carrageenan is used as a food additive in canned foods, salad dressing, filling material in pastry products, ice cream, and canned cat-dog foods (Kraan, 2012). The Gigartina species from which this substance is obtained are found in our seas and it is thought that it can be commercially cultivated.

Agar-agar: It is obtained from the red algae Gracilaria sp. and Gelidium species. 90% of the produced agar is used in food applications and 10% is used in microbiological and biotechnological applications (Kraan, 2012). Agar, which is mostly used as a coating gel in canned meat and fish in foods, is widely used in the field of pastry, such as cake fillings and creams, due to its high-temperature resistance (Cirik and Cirik, 2011). In addition, it is used in bakery products to control water activity and delay staling, in processed cheeses and fruit juices due to its stabilizing properties (Ak, 2015; Kraan, 2012).

Alginate: Alginate is obtained from brown algae such as Laminaria digitata. Only 20% of the produced alginate is used as a food additive and 80% is used in many industries (Kraan, 2012). Alginates, which are used at a rate of 0.3 - 0.5% in fruit cake coatings due to their film-forming and water-holding properties, prevent the formation of ice crystals as well as providing a smooth structure and volume increase during ice cream production. In addition, due to their rapid gelation and structural diversity, these stabilizers are widely used in cream cheese, whipped cream, cheese, and sour cream (Yılsay,et al., 2001). Alginates are used as film-forming agents to prevent oxidative rancidity of meat and fish products and to minimize evaporative losses during storage (Ak, 2015). It is possible to obtain alginate from algae called Cystoseira sp. and Sargassum sp. in our seas (Cirik and Cirik, 2011).

1.3.5. Use of Algae in Wastewater Treatment

It is known that algae have an important role in the process of self-purification of natural waters as a result of the continuous supply of oxygen to the environment by photosynthesis. However, it has been reported by many researchers that algae remove pollutants that are dangerous for the aquatic environment such as nutrients, heavy metals, pesticides, organic and inorganic toxins, pathogenic organisms, especially nitrogen and phosphorus in water and wastewater, by using or accumulating pollutants in their cells and cells (Şen et al., 2003).

1.3.6. Use of Algae in Bioplastic Production

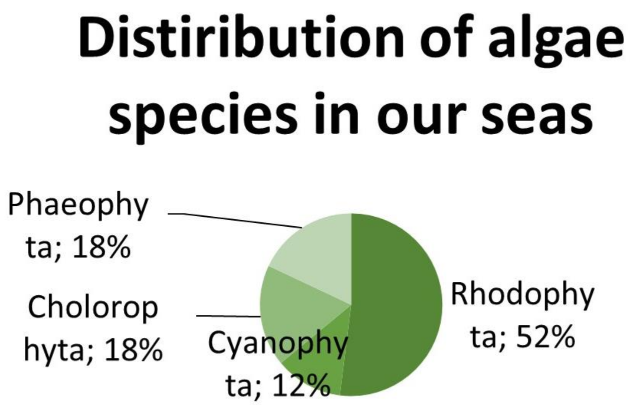
The main element used in the production of bioplastics is polysaccharides (Rajendran et al., 2012). Therefore, in production, polysaccharides must be determined and extracted. Polysaccharides are polymers of monosaccharides linked by glycosidic bonds with stabilizing, thickening (gelling), and emulsifying properties (Özdemir and Erkmen, 2013). Marine algae contain high amounts of structural polysaccharides (cellulose, hemicellulose, neutral polysaccharides) as well as mucopolysaccharides and storage polysaccharides (laminarin (β-1,3-glucan) (Rajendran,et al., 2012; Özdemir and Erkmen, 2013). Since the amount and types of polysaccharides are species-specific, different extraction methods have been developed (Özdemir and Erkmen, 2013).

1.4. Macroalgae Diversity in Turkey

It has been reported that 92 Cyanophyta, 413 Rhodophyta, 144 Phaeophyta, and 139 Chlorophyta species are distributed in the seas of our country (FAO, 2015). The taxa numbers of macrobenthic algae distributed in our Black Sea, Marmara, Mediterranean, and Aegean seas are given in Table 1.

Table 1. Algae distribution in our seas (Durucan and Turna, 2010)

Taxon	Marmara	Karadeniz	Ege	Akdeniz
Cyanophyta	43	12	71	45
Chlorophyta	90	55	92	82
Phaeophyta	103	53	99	85
Rhodophyta	264	139	253	228
Total	500	259	515	440



2. CONCLUSION

Algae, which have been used in many areas throughout human history, continue to be used in many countries today. According to 2014 FAO data, macroalgae constitute 28% of the total marine products obtained through aquaculture, and the economic size of production reaches 5 billion dollars (FAO, 2015).

Studies on algae have demonstrated the presence of antimicrobial, cytotoxic, antimitogenic, anticancer, and antitumoral activities. The presence of phenolic compounds such as phenol and flavonoid tannin in algae indicates antioxidant activity and free radical scavenging effect (Meenakshi, et al., 2012).

In studies on species that are distributed in our seas and have economic importance in terms of their composition; alginic acid, agar, carrageenan, vitamin B12, some organic acids, and cellulose were obtained from algae (Aktar and Cebe, 2010).

In today's world, where the nutritional problem is getting bigger, the efforts to benefit from algae are increasing, and besides benefiting from naturally reproducing algae, they are also benefiting from the cultures of these algae (Cirik and Cirik, 2011).

Author contributions

Büşra PEKSEZER: Conceptualization, Investigation, Writing- Original draft preparation, Writing Reviewing and Editing.
Mehmet Tahir ALP: Writing Reviewing and Editing.
Deniz Ayas: Investigation, Writing- Original draft preparation, Writing-Reviewing and Editing.

Conflicts of interest

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

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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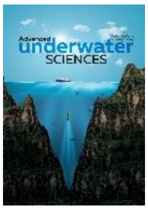

Toxic Effects of Copper Nanoparticles in Aquatic Organisms

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ABSTRACT

Although copper is an essential element for animal organisms, its potentially toxic effects have been known for centuries. In recent years, nanoparticles, which are widely used in many fields, have been added to the sources that allow copper to participate in aquatic ecosystems. The difference between nanoparticles containing Cu, Zn, Fe, Ag, Ti, and Al ions from known metal toxicity is not clear enough yet, and the toxic effects of metal oxide nanoparticles continue to be investigated. In this study, it was aimed to compile the toxic effects of copper nanoparticles in aquatic organisms living in different trophic zones of the food chain and with different organizational levels.

1. INTRODUCTION

1.1. Source of Environmental Pollution

Although environmental pollution caused by metals in the world is based on human history, technological development has also increased the sources of participation. Nanotechnology, which has an important place in human life and causes developments in health, engineering, and basic sciences, is among the new sources of pollutants. Metal oxide nanoparticles have entered human life in recent years with the increase in nanotechnology. These materials range in size from 1-100 nm and mostly contain metal ions such as Ag, Ti, Cu, Zn, Fe, and Al. Nanoparticles are used in many fields with their high surface-volume ratio, electronic properties, reactivity, surface structures, and crystal properties (Handy and Shaw, 2007). It took many years for the concept of nanotechnology, which was first used by Norio Taniguchi in 1974, to become widespread. The development of nanotechnological products has been divided into four generations. The second-generation products containing active nanostructures have started to take place in medical applications, and fourth-generation products containing molecular size tools and atomic design have been added to the applications in the last few years, and it is believed that it will make a greater contribution to the development in the health sector (Turgut et al., 2011).

The use of nanomaterials in many areas, from the use of contrast agents in medical imaging to gene transfer to specific cells, enables analyzes and treatments that cannot be carried out with a different application in medicine. On the other hand, developments in the energy, electronics, automotive, textile, food, and paint industries have also been applications that facilitate human life (Song et al., 2015). In addition to these positive effects, increased metal pollution with nanotechnology causes great concerns (Turgut et al., 2011).

Metals used as raw materials in the industry are discharged to aquatic ecosystems. In addition to agricultural practices, domestic wastes, increase in fossil fuel use are factors that increase metal incorporation into aquatic ecosystems. The long half-lives of these pollutants and the continuity of discharges pose a great threat to aquatic ecosystems.

1.2. Copper Toxicity in Animals

Although copper is an essential element for animal organisms, it is a potentially toxic substance. For this reason, a cellularly evolved homeostatic control mechanism has developed over time. Under the influence of a high concentration of copper through water and food, protein functions and homeostatic mechanisms are impaired due to cellular copper increase. Cellular copper increase leads to physiological disorders and toxicity. Increasing copper concentration in the environment is

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not a simple parameter that affects water quality but affects the bioavailability, physiology, and sensitivity of metal by organisms.

The redox potential of copper makes it essential for all aerobic organisms. Copper is involved in the structure of many enzymes such as cytochrome c oxidase. However, the redox potential causes the formation of reactive oxygen species (ROS) under the influence of high copper concentration.

1.3. Copper Nanoparticle Toxicity in Animals

Copper nanoparticles are a form of insoluble copper with a wide range of applications such as air and liquid filtration, wood protection, bioactive coatings, integrated circuits, coatings on batteries, thermal and electrical conductivity, textiles (Shaw and Handy, 2011; Gomes et al., 2011). Although insoluble in water, copper nanoparticles release copper ions into the surrounding water due to the small particle size. Nanoparticles and copper alone are toxic to aquatic organisms. Studies are trying to determine the difference between the toxic effects of CuO-NPs and the known metal toxicity.

In order to adequately understand the toxicity mechanism of copper nanoparticles, researches were carried out on aquatic organisms living in different trophic zones of the food chain and with different organizational levels, and cytotoxic effects were determined in bacteria (Vibrio fischeri, Escherichia coli, Staphylococcus aureus, Bacillus subtilis), protozoa (Tetrahymena thermophila), crustacean (Daphnia magna, Thamnocephalus platyurus, Daphnia pulex, Ceriodaphnia dubia), algae (Pseudokirchneriella subcapitata) and zebrafish (Danio rerio) (Gomes et al., 2011). It has been determined that copper nanoparticles cause the formation of reactive oxygen species and oxidative stress. It has been reported that lipid peroxidation, oxidative stress, antioxidant enzyme activity, and metallothionein synthesis increase in Mytilus galloprovincialis under the influence of CuO-NPs (Gomes et al., 2012).

1.4. Factors affecting tissue metal toxicity

Metabolically active tissues are responsible for the uptake, detoxification, and excretion of metals. Gills are target organs that function in ion regulation and interact directly with the environment (Evans et al., 2005). The uptake and accumulation of metals from the gills vary depending on the physical and chemical properties of the water, as well as the competition between metals for the attachment points in the gill epithelium. It is known that metals accumulate in the gills at high concentrations at the beginning of the effective period in aquatic organisms, causing hyperplasia, hypertrophy, and proliferation in gill epithelial cells, and inhibition of Na+/K+ ATPase activity (Evans, 1987; Xiong, et al., 2011; Zhao et al., 2011). Inhibition of enzyme activity, ammonia excretion from the gills, and a decrease in sodium uptake, resulting in impaired osmoregulation and mortality (Grosell et al., 2007).

In freshwater fish, the gills filter more water than in marine fish to provide osmoregulation. For this reason, they are more affected by the toxic substances in the environment. It has been reported that CuNP and copper nitrate cause deformations in the gill filaments and gill pavement cells in Oncorhynchus mykiss, Pimephales promelas, and Danio rerio, and the toxic effect varies depending on the sensitivity of the examined species (Song et al., 2015).

The initial effect of metals that accumulated in the gills is transmitted to the liver via the circulatory system by binding to carrier proteins when the carrying capacity of the gills is exceeded. Liver in vertebrates or hepatopancreas in invertebrates is a metabolically active tissue that functions in the conversion of nutrients absorbed from the intestine, the synthesis of bile salts that function in the digestion of fatty acids, and the storage of glycogen, which is the main source of blood glucose, and the regulation of heavy metals. In addition, It is one of the main synthesis sites of metallothionein and glutathione which functions in the elimination of toxic effects by binding heavy metals. Metallothionein is a cytoplasmic protein with low molecular weight, rich in cysteine, devoid of aromatic amino acids, soluble in acids, resistant to precipitation by alcohol, and trichloroacetic acid. Glutathione is a tripeptide containing large amounts of cysteine with a sulfhydryl group (Heath, 1995).

Increasing in exposure time, accumulation in liver tissue increases, and it is known that there is an increase in vacuolization, number of lysosomal vesicles, and gluconeogenic enzyme (Aspartate aminotransferase, Alanine aminotransferase) activity in liver cells in fish. It was determined that Cu+2 accumulated at higher concentrations in the hepatopancreatic tissue at the beginning of the exposure time in M. galloprovincialis compared to mussels under the influence of CuO-NP, but the concentration decreased due to the increase in the exposure time. In mussels exposed to CuO-NP, the accumulation showed a linear increase. The decrease in the concentration of Cu+2 in the hepatopancreas at the end of the experiment can be explained by detoxification (Gomes et al., 2012).

Cu-NPs tend to both release metal ions and form aggregates in water. Many studies have suggested that the toxicity of metallic NP suspensions may be due to the release of metal ions (Griffitt et al., 2009; Hoheisel et al., 2012). In M. galloprovincialis, CuO-NPs were found to accumulate twice as much in the hepatopancreas than in the muscle tissue. This was explained by the release of CuO from NPs and its transport to the hepatopancreas for detoxification (Gomes et al., 2012). It is also supported by previous research that copper ions can be released from the nanoparticle surface, thereby exposing fish to dissolved Cu as well as nano-form (Gomes et al., 2011; Shaw and Handy, 2011).

It was determined that CuO-NPs accumulated in tissues of Cyprinus carpio, respectively, in intestine>gill>muscle>skin & scale>liver>brain tissue. Intestines and gills may have accumulated in higher concentrations than other tissues since they constitute the main uptake route from food and the environment. Higher concentration in muscle tissue than liver may indicate a chronic effect of CuO-NP. Increased accumulation in the intestines due to an increase in exposure time may indicate that CuO-NPs are removed from the body through feces. Meanwhile, in fish exposed to CuO-NP, it is thought that soluble Cu+2 ions accumulate in the brain and inhibit cholinesterase (ChE) activity and cause neurotoxicity (Zhao et al., 2011).

It has been reported that CuNPs have more negative effects on intestinal tissue and copper sulfate has more negative effects than gill and liver tissues of Epinephelus coioides (Hoseini et al., 2016). These findings show that the chemical form of the toxic substance, as well as the metabolic function of the tissues, is effective on the accumulation. In mammals, the liver, kidney, and spleen are target tissues for CuNPs and cause tissue damage, but micro Cu particles do not cause damage. This indicates that toxicity varies depending on particle size (Chen et al., 2006).

1.5. The toxicity effects of Cu-NPs and CuSO4 in animals

It was determined that the effect of Cu-NPs and CuSO4 in juvenile Oncorhynchus mykiss caused similar tissue damage in fish. Hyperplasia, aneurisms, and necrosis in the secondary lamellae, in gill; swelling of goblet cells, necrosis in the mucosa layer and vacuole formation, in the gut; hepatitis-like injury and cells with pyknotic nuclei, in the liver; damage to the epithelium of some renal tubules and increased Bowman's space, in the kidney; some mild changes were observed in the nerve cell bodies in the telencephalon, alteration in the thickness of the mesencephalon layers, and enlargement of a blood vessel on the ventral surface of the cerebellum, in the brain; changes in the proportional area of muscle fibers in skeletal muscle were observed (Al-Bairuty et al., 2013).

In the gill, CuSO4 and Cu-NPs were found to cause similar pathological changes in juvenile rainbow trout, but the damage was more due to the CuSO4 effect. Intestines are responsible for functional absorption, and it has been determined that damages are more severe under the influence of Cu-NPs. Effects in the brain are explained by indirect toxicity and/or hypoxia on neuroendocrine functions. It has been reported that Cu, Ag, and Al-NPs cause damage to the blood-brain barrier and edema formation in the brain in rodents. Enlarged blood vessels on the surface of the brain are consistent with an attempt to increase blood flow to offset the effects of hypoxia. It was emphasized that the thickening caused by Cu-NPs and CuSO4 in the midbrain layers could not be caused by different tissue growth, since the trial period (10 days) was not long, and the thickening was definitely associated with edema. Although more research is needed to adequately understand the etiology of Cu and Cu-NP pathology in the brain, the findings show that various physiological dysfunctions (osmotic, metal homeostasis, oxidative stress, vascular) may contribute to the pathology.

2. CONCLUSION

Consequently, copper toxicity in gills and viscera in water-borne exposures has a significant effect on cellular metabolism and respiration. Copper can promote mitochondrial dysfunction and exert detrimental effects on fish metabolic energy production (Linder and Hazegh-Azam, 1996; Braz-Mota et al., 2018). Identifying changes caused by copper toxicity in species living in different trophic zones of the water column and with different levels of the organization will be valuable in understanding the effects of copper nanoparticles and ions in more detail. The continuity of the discharge of metals and nanomaterials into the aquatic ecosystem causes an increase in the risk of toxicity in aquatic organisms. Investigating the toxic effect mechanisms of these substances is important for the environment and human health.

Author contributions

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Deniz Ayas: Investigation, Writing- Original draft preparation, Writing-Reviewing and Editing.

Conflicts of interest

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

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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