E-ISSN 2618-6365 Vol. 2 Issue 3 2019

AQUATIC RESEARCH

ScientificWebJournals (SWJ)

AQUATIC RESEARCH

Publisher

Adress: Abdi Bey Sok. No:24B D. 435 Kadıköy/İstanbul, Turkey
E-mail: swj@scientificwebjournals.com
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http://aquatres.scientificwebjournals.com

AQUATIC RESEARCH

Aims and Scope

AQUATIC RESEARCH

Abbreviation: **Aquat Res**

e-ISSN: 2602-2834

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AQUATIC RESEARCH

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Aquatic Research 2(3), 134-142 (2019) • https://doi.org/10.3153/AR19011 *Research Article*

EVALUATING THE ESSENTIAL AND NON-ESSENTIAL METAL REMEDIATION EFFICIENCY OF *Chlorella vulgaris*, AND PHOTOSYNTHETIC GENE EXPRESSION LEVEL CHANGES DURING THE PROCESS

Tuğba Şentürk[1], Muhammet Burak Batır[1], Çisil Çamlı[2], Şükran Yıldız[1]

Cite this article as:

Şentürk, T., Batır, M.B., Çamlı, C., Yıldız, Ş. (2019). Evaluating the essential and non-essential metal remediation efficiency of *Chlorella vulgaris*, and photosynthetic gene expression level changes during the process. *Aquatic Research*, 2(3), 134-142. https://doi.org/10.3153/AR19011

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Submitted: 15.04.2019
Revision requested: 09.05.2019
Last revision received: 18.05.2019
Accepted: 20.05.2019
Published online: 26.05.2019

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Available online at
http://aquatres.scientificwebjournals.com

ABSTRACT

Algal populations hold great potential for encountering water pollution problem due to their remediation abilities. Thus, using them for removing the pollutants stands as a powerful approach. However, it is crucial to investigate the negative effects of these pollutants on algal populations as well as understanding the removal capacity of these populations in order to benefit their abilities. In the present study, *Chlorella vulgaris* was used as a candidate for metal removal. Besides the remediation capacity for certain essential (Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mo^{6+}) and non-essential (Cd^{2+}, Pb^{2+}, Sn^{4+}, Ba^{2+}, As^{5+}) metals, chlorophyll and carbohydrate contents, and photosynthetic gene (psaB, Photosystem I reaction center protein subunit B) expression levels were also evaluated. Results indicated that remediation efficiency of *C. vulgaris* for essential metals was Cu>Co>Zn>Mo>Mn and for non-essential metals was Sn>Pb>Ba>Cd>As, respectively. It was also observed that psaB expression was increased after the essential and non-essential metal treatment. It can be concluded that *C. vulgaris* can be used as a bioindicator for Mn and As while it is also suitable to be used for metal removal.

Keywords: Bioremediation, Phytoremediation, Metal pollution, Metal removal, Algal removal

Introduction

In recent years, most of the available water resources are facing metal pollution. Metals are one of the significant pollutants of the water ecosystems. The main reason for this problem is the increased industrial discharge due to the increased human population. The negative effects of metal pollution are specifically crucial for algal populations. Metal pollution caused by the human activities accelerate the toxicity of water organisms. Since they are the primary producers of water ecosystems, changes in their vitally would cause changes in other living organisms vital rates as well (Franklin et al., 2000). Thus, it is critical to balance the metal discharge levels in non-toxic levels.

Metals can be a group as essential and non-essential based on the levels of metabolic usage. Non-essential metals for algae, like Cd, Pb, As, and Hg, cause toxicity even in low doses, while essential metals like Cu, Zn, and Mn are necessary for metabolic activities, yet they also show toxicity in high doses (Provasoli, 1958).

Although the negative effects of metal pollution on algal populations have been studied widely, the use of algae as bio-indicators, their effect of self-purification as a result of oxygen production, as well as their waste removal effect have been also shown in several studies (Islam et al., 2007; Khan et al., 2008; Wang & Chen, 2009; Hong et al., 2011; Kumar et al., 2015; König-Peter et al., 2015). Based on these properties, extensive and detailed studies in these fields, specifically on the negative effects of pollution and the removal capacity of algae would help to benefit algae more efficiently for pollutant removal in the future (Mehta & Gaur, 2005). *Chlorella vulgaris*, as a cosmopolitan species, carry a significant potential for this purpose.

The aim of this study is to evaluate and compare the essential (Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mo^{6+}) and non-essential (Cd^{2+}, Pb^{2+}, Sn^{4+}, Ba^{2+}, As^{5+}) removal and adsorption capacity of *C. vulgaris*, observe the changes in chlorophyll-a (chl-a), chlorophyll-b (chl-b) and carbohydrate content, as well as the photosynthetic gene (psaB, Photosystem I reaction center protein subunit B) expression levels.

Material and Methods

Algal Culture Growth and Preparation

C. vulgaris was obtained from the Culture Collection of Microalgae at the University of Ege, Izmir, Turkey. A standard initial inoculum of the algae was inoculated to culture flasks (200 mL each) that contained BG-11 Medium (Stanier et al., 1979), and incubated at 28 ±1ºC under 12 h light (20 E m^{-2} s^{-1} ± 20%), with magnetic stirring (100 rpm). The pH value was adjusted to 6–6.5 using 1 M NaOH and 1 M HCI. The metal removing capacities of the *C. vulgaris* was determined using 50 ml aliquots of ten-day-old bacterial cultures, when they were in the linear phase of growth (De Philippis et al., 2007). 5 mL algae were filtered from 0.45 µm Whatman GF/C filters, oven dried for 3-4 h at 105ºC and weighted in order to calculate dry weight was as mg/mL.

Metal Solution Preparation

Metal solutions was prepared for the essential metals (Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mo^{6+}) and non-essential (Cd^{2+}, Pb^{2+}, Sn^{4+}, Ba^{2+}, As^{5+}) for 5 different concentrations as 0.5; 1; 2.5; 5 and 10 mg/L (De Philippis et al., 2007). Another metal mix solution containing both essential and non-essential metals was also prepared with same concentrations. By using these metal solutions algal culture was treated for 10 days.

Chlorophyll and Carbohydrate Content

Total carbohydrate contents were determined by using the phenol-sulfuric acid assay and using glucose as a standard. 1 mL culture aliquots were used for spectrophotometrically quantification of the total carbohydrate content by the phenol-sulfuric acid assay (Skoog et al., 2000).

In order to measure the chlorophyll content, 10 mL samples of each culture were collected. Acetone and magnesium carbonate were used to extract the chlorophyll from the samples. According to the method of Parsons and Strickland (1963), chl-a and chl-b contents were measured spectrophotometrically.

ICP Analysis

Supernatants collected from the samples after metal treatment were analyzed in ICP-MS (Agilent 7700 Series, US) with 3 replicates. Metal removal amount (q, given as mg/g) and metal removal percentage was calculated as follows (König-Peter et al., 2015);

$q\ (mg/mL) = (c_i - c_t) * V/m$

$\text{Metal removal \%} = 100 * (c_i - c_t) / c_i$

V: solution volume (mL)

m: dry weight of the adsorbent (g)

c_i and c_t: initial and final metal concentrations

Gene Expression Analysis

Total RNA Miniprep Kit (GMbiolab Co. Ltd., Taiwan) was used for RNA isolation. RNA quality and integrity were observed by bleach agarose with SAFE-T stain (Aranda et al., 2012). cDNA synthesis was performed via cDNA Reverse

Transcription Kit (AppliedBiosystems). With the aim of keeping the expression levels equal for each sample, RNA amount was fixed to 9 ng/µL.

In order to determine the expression levels of 18S rRNA (housekeeping gene) and psaB (Photosystem I reaction center protein subunit B) with RT-PCR, 18S rRNA and psaB sequences were determined from the National Center for Biotechnology Information (NCBI) database. Based on this, specific primers were designed with Primer3Plus software (Table 1).

Table 1. 18S rRNA and psaB primer sequences

Table 1. 18S rRNA and psaB primer sequences

18S rRNA	Forward 5'- ATTGGAGGGCAAGTCTGGTG -3'
18SrRNAR	Reverse 5'- GTCCCACCCGAAATCCAACT -3'
psaBF	Forward 5`-TGCCACTGGGTTTATGTTCC-3`
psaBR	Reverse 5`-GCCATCGTACGAGATTTGCT-3`

RT-PCR is performed by using 2XSYBR Green Kit (GMbiolab Co. Ltd., Taiwan) in 20 µl reaction volume. Cycle Threshold (Ct) value was calculated based on Pfaffl (2004).

Statistical Analysis

All experiments were performed in 3 replicates. The data are presented as the mean±standard deviation of the mean (SDM). Spectrophotometrically obtained results and ICP-MS results were supported by Freundlich (Freundlich, 1907) and Langmuir adsorption isotherms (Langmuir, 1916).

Cycle Threshold gene expression values obtained by RT-PCR were calculated by SPSS 16.0 One-Way ANOVA test.

Results and Discussion

Chlorophyll and Carbohydrate Contents

Chl-a and b levels for the control group were measured 0.6812 and 0.2441 µg/L respectively. Since 10 mg/L is the highest concentration, chlorophyll level changes were observed most clearly for this concentration. Essential and non-essential metal treatments caused an increase in the chl-a levels. While chl-b levels were decreased with essential metal treatment except for Mo^{6+} and generally increased with non-essential metal treatment (Figure 1).

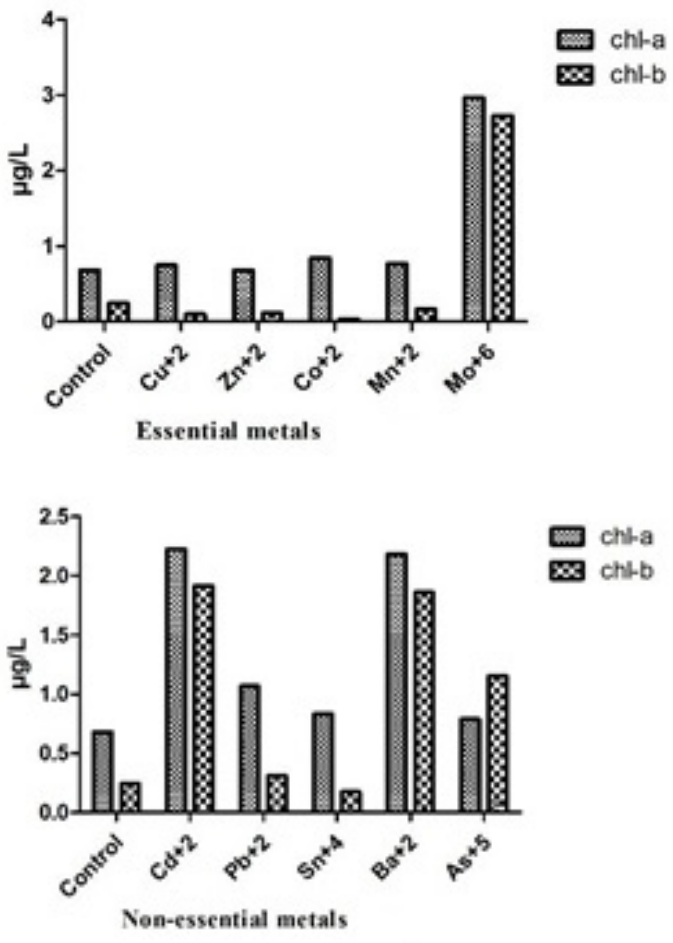
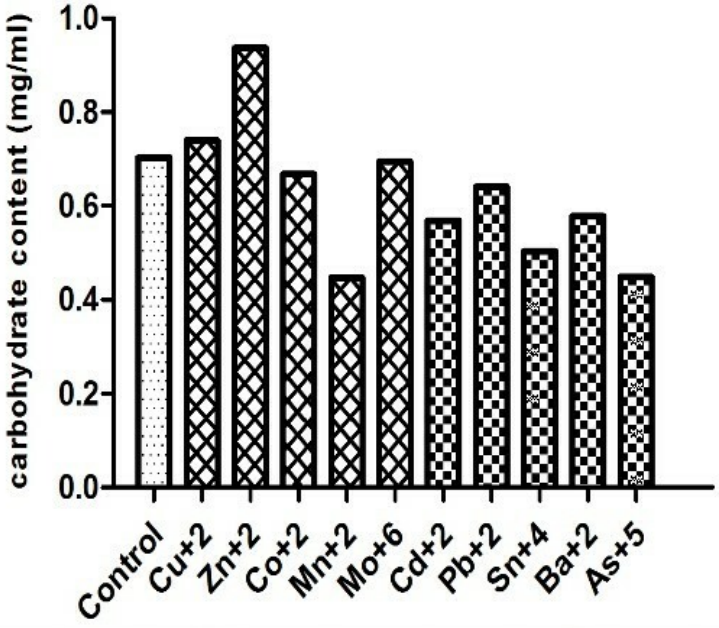


Figure 1. Chl-a and b changes in µg/L after the essential and non-essential metal treatment

Carbohydrate content of the *C. vulgaris* samples were measured at 0.7035 mg/mL for the control group. Measurements for the carbohydrate content, metal concentration showed that Zn and Cu treatment causing an increase in carbohydrate levels. On the other hand carbohydrate content for Mn and As treatment measured as 0.4475 and 0.4492 mg/mL, showing that these metals cause 63% of a decrease on the carbohydrate levels (Figure 2).



Control	Cu	Zn	Co	Mn	Mo	Cd	Pb	Sn	Ba	As
0,7035	0,7402	0,937	0,6677	0,4475	0,694	0,5686	0,6406	0,5037	0,5785	0,4492

Figure 2. Carbohydrate content changes in mg/mL after the essential and non-essential metal treatment

Metal Removal

Metal removal results obtained from essential-metal treatment showed that average removal order was Cu> Co> Zn> Mo> Mn (0.2483; 0.2482; 0.2442; 0.2374 and 0.0820 mg/g) respectively. For the non-essential metals average results obtained as Sn> Pb> Ba> Cd> As (0.2549; 0.2548; 0.2474; 0.2463 and 0.2412 mg/g) respectively (Table 2).

Metal removal capacity of *C. vulgaris* was also evaluated in mg/g for all the metals separately for 5 different concentrations (0.5; 1; 2.5; 5 and 10 mg/L). Lowest removal capacity was observed in Mn treatment (Figure 3).

Gene Expression Analysis

RT-PCR results indicated that essential metal treatment (1.0, 2.5, 5, 10 mg/L) increased the psaB expression as compared to the control group. Similarly, non-essential metal treatment (0.5, 1, 2.5, 5, 10 mg/L) also caused an in an increase in psaB expression in a dose-dependent manner (Figure 4).

Statistical Results

Metal removal capacity of the biomass was determined by using Freundlich and Langmuir isotherms (Freundlich, 1907; Langmuir, 1916) (Table 3).

Correlation coefficient constants (K_L, K_f) of essential metal removal was determined as ranging between 0.029-1.767 and 0.121-2.761 respectively. Lowest and highest q_m values (maximum adsorption capacity of the adsorbent) was observed as 1.767 mg/g for Co and 0.001 mg/g for Mn. Calculated results showed that *C. vulgaris* is an effective adsorbent for essential metals (Table 4).

Correlation coefficient constants (K_L, K_f) of non-essential metal removal was determined as ranging between 0.868-43.088 and 0.3591-6.124 respectively. Lowest and highest q_m values (maximum adsorption capacity of the adsorbent) was observed as 8.296 mg/g for Pb and 0.075 mg/g for Cd. Calculated results showed that *C. vulgaris* is relatively less effective adsorbent for non-essential metals as compared to essential metals (Table 5).

Table 2. Metal removal results for the essential and non-essential metal mix treatment (mg/g)

	***C. vulgaris* essential metal mix removal (mg/g)**				
mg/L	Cu	Zn	Co	Mn	Mo
0.5	0.032	0.031	0.033	0.031	0.030
1	0.065	0.063	0.066	0.058	0.061
2.5	0.164	0.161	0.163	0.035	0.156
5	0.327	0.323	0.328	0.037	0.313
10	0.653	0.644	0.651	0.249	0.627
average	**0.2483**	**0.2442**	**0.2482**	**0.082**	**0.2375**
	***C. vulgaris* non-essential metal mix removal (mg/g)**				
mg/L	Cd	Pb	Sn	Ba	As
0.5	0.032	0.033	0.033	0.033	0.032
1	0.065	0.067	0.067	0.066	0.064
2.5	0.162	0.168	0.168	0.163	0.161
5	0.323	0.335	0.335	0.326	0.318
10	0.649	0.671	0.671	0.649	0.631
average	**0.2463**	**0.25479**	**0.25485**	**0.2474**	**0.2412**

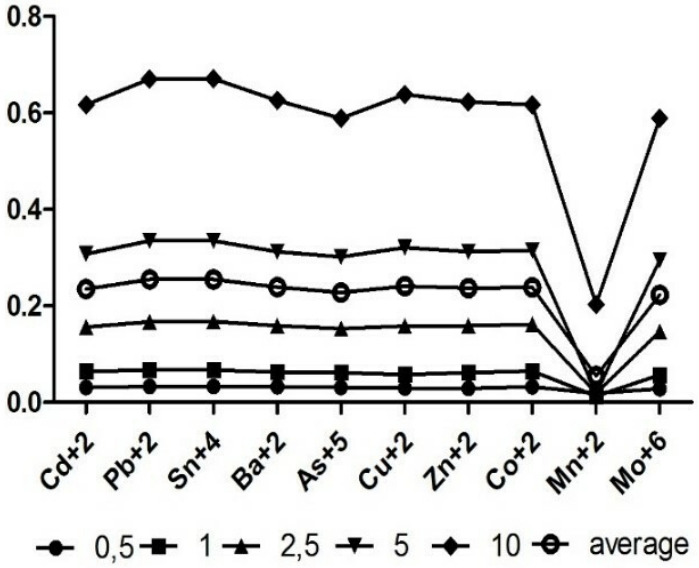


Figure 3. Metal removal levels measured for 0.5; 1; 2.5; 5 and 10 mg/L and average values

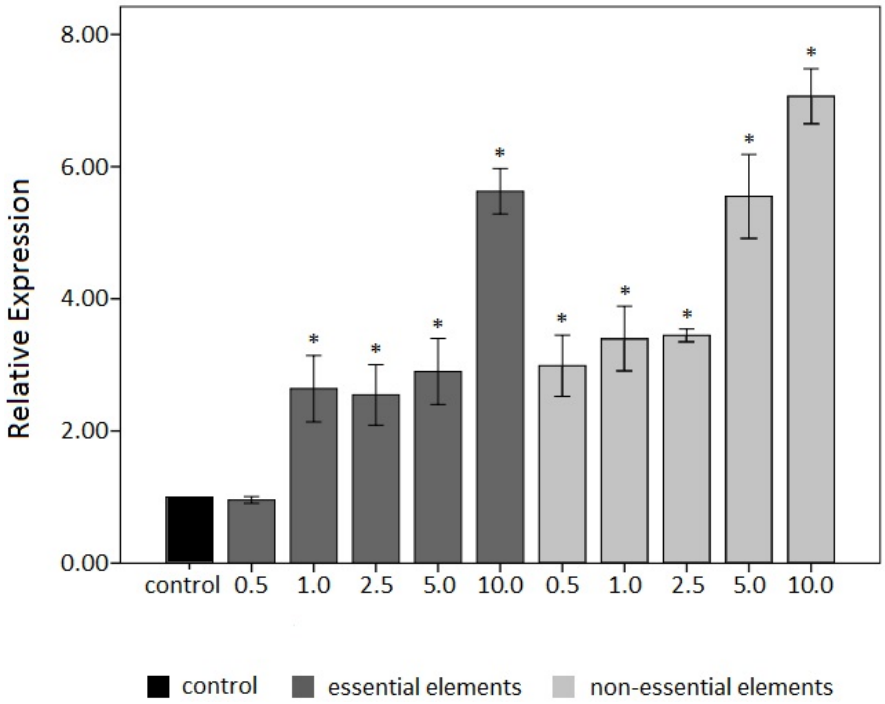


Figure 4. Relative expression changes of psaB mRNA values (fold change) of essential and non-essential heavy metal (0.5-10 mg/L) treated *C. vulgaris* cells according to the control cells. Error bars indicate the standard errors. Asterisk indicates significant differences (*p<0.01-0.001)

Table 3. Langmuir and Freundlich isotherm models

Langmuir: $q_e=(K_L.C_e)/(1+K_L.C_e)$
Freundlich: $Inq_e= InK_F+ 1/n * InC_e$

Table 4. Adsorption isotherm constant for essential metals

	Cu	Zn	Co	Mn	Mo
Langmuir					
q_m (mg/g)	1.3549	0.2382	1.6610	0.0011	0.2035
K_L (L/mg)	0.6443	0.7986	1.7671	0.0292	0.3924
R^2	0.5635	0.0548	0.6664	0.0618	0.5340
Freundlich					
n (g/L)	0.6317	0.8381	1.2315	0.4381	0.7859
K_F (L/mg)	0.5908	0.1214	0.1862	2.7618	0.3234
R^2	0.9102	0.9405	0.9676	0.4919	0.9921

Table 5. Adsorption isotherm constant for non-essential metals

	Cd	Pb	Sn	Ba	As
Langmuir					
q_m (mg/g)	0.0758	8.296	7.820	0.8453	0.7154
K_L (L/mg)	1.1558	31.492	96.384	1.359	0.8687
R^2	0.2967	0.1126	0.001	0.4978	0.8904
Freundlich					
n (g/L)	1.1500	0.9619	0.2700	1.1712	1.1075
K_F (L/mg)	0.3591	3.5704	6.1240	0.2086	0.5051
R^2	0.9698	0.9715	0.7330	0.9920	0.9944

Heavy metals are the crucial pollutants of the water environment. Algal populations hold great potential for monitoring and reducing the metal pollution due to their metal holding capacity (Çetinkaya et al., 1999). In order to understand and use algal populations for metal pollution, it is crucial to understand metal uptake mechanisms and its effects on algae.

In this study, *C. vulgaris* was used for understanding the metal removal capacity of the organism as well as the effects of removal. Remediation capacity, changes in chl-a, chl-b and carbohydrate contents and the photosynthetic gene expression levels were observed in *C. vulgaris* in the presence of essential and non-essential metals.

It has been showed that photosynthesis is relatively more vulnerable to metal toxicity as compared to other processes in algae (Lu et al., 2000). Metals can affect the photosynthesis by directly affecting the photosynthetic pathways, ion distribution, and enzyme activity disruption or via affecting the membrane permeability (Rai at al., 1981). Chlorophyll content results showed that metal treatment caused an increase in chl-a levels, while chl-b content decreased in essential metal treatment except for Mo, and increased in non-essential metal treatment except Sn. These results indicated that while non-essential metals could be relatively tolerated, non-essential metals affects the chl-b content negatively. Heavy metals are known to affect chlorophyll pigment biosynthesis and enzymes adversely (Shioi et al., 1978). Observed changes in chl-a and b contents also shows that photosynthetic pigment processes work mutually. The total carbohydrate content of *C. vulgaris* was measured lowest for Mn and As treatment (63.61% and 63.58% respectively). These results can be explained by the fact that metal pollution causing the algal growth inhibition though affecting the necessary element uptake (Shioi et al., 1978; Gaur, & Kumar, 1981; Lu et al., 2000; Bajguz, 2011).

Remediation efficiency of *C. vulgaris* was observed as Cu>Co>Zn>Mo>Mn for essential metals and Sn>Pb>Ba>Cd>As for non-essential metals. Low levels of biorption for Mn and As can be explained by the toxic effect of these metals on *C. vulgaris*, and shows consistency with total carbohydrate results. Highest uptake level was observed for Cu. Related conducted studies was also showed that *C. vulgaris* has a high capacity of Cu uptake and store Cu through specific metal binding proteins (Rachlin & Grosso, 1993; Knauer et al., 1997; Lopez et al., 2000; Soldo et al., 2005).

Gene expression levels of psaB for 0.5 mg/L essential metal treatment did not show a significant increase of the psaB relative expression level (0.95) ($p>0.05$) compared to the control group. On the other hand, other concentrations of essential (1.0, 2.5, 5.0, 10.0 mg/L) and non-essential (0.5, 1.0, 2.5, 5.0, 10.0 mg/L) metals caused increasing in the relative expression level of the psaB gene. 1.0, 2.5, 5.0, 10.0 mg/L essential metal treatment increased the expression level of the psaB gene as a 2.6, 2.5, 2.9, 5.6, respectively, compared to the control group ($p<0.001$). Also, 0.5, 1.0, 2.5, 5.0, 10.0 mg/L non-essential metal treatment increased the expression level of the psaB gene as a 3.0, 3.4, 3.4, 5.4, 7.0, respectively, compared to the control group ($p<0.001$). This result could be explained by the fact that, most of these metals act as cofactors for metalloproteins and plays role in photosyn-

thetic electron transport, respiration, and cell wall metabolism in small doses (Fayed et al., 1983). Metals like Cu and Zn takes part in oxidoreduction reactions as catalyzers for ROS. Thus, in high doses even the essential metals leads a toxic effect and decreases psbA gene expression level (responsible from the expression of D1 protein of the photosystem II (PSII)) while increases the psaB expression level (responsible from the expression of P700 chlorophyll A2 apoprotein of photosystem I (PSI)) (Raven et al., 1999; Mediouni et al., 2006). On the other hand, since non-essential metals do not take part in cell functions, they show a toxic effect even at the low doses (Qian et al., 2009). Results of the study showed that psaB expression levels increased with the essential and non-essential metal treatment. Considering the fact that psaB expression is related to P700 chlorophyll A2 apoprotein of photosystem I (PSI), obtained results related to the gene expression level showed consistency with the chlorophyll-a analysis results.

Conclusion

To conclude, this report describes the metal removal efficiency of *C. vulgaris* while illustrating the effect of essential and non-essential metals on carbohydrate and chlorophyll content as well as its relation between photosynthetic gene expression levels. Results indicate that *C. vulgaris* can be used as a bioindicator for Mn and As and it is also suitable to be used for metal removal from the polluted water environments.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Financial disclosure: This research was funded by the Manisa Celal Bayar University Scientific Investigation Project, Grant Nr. FEF 2015–154.

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AQUATIC RESEARCH

E-ISSN 2618-6365

Aquatic Research 2(3), 143-153 (2019) • https://doi.org/10.3153/AR19012 *Research Article*

THE LIVER LIPID FATTY ACID COMPOSITION OF TWO CARTILAGINOUS FISH, THE THORNBACK RAY (*Raja clavata*) AND THE COMMON SMOOTH-HOUND (*Mustelus mustelus*)

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Cite this article as:

Yığın, C.Ç., Çakır, F., Cabbar, K., Kızılkaya, B., Ormancı, H.B., Öztekin, A., Özüdoğru, Y. (2019). The liver lipid fatty acid composition of two cartilaginous fish, the thornback ray (*Raja clavata*) and the common smooth-hound (*Mustelus mustelus*). *Aquatic Research*, 2(3), 143-153. https://doi.org/10.3153/AR19012

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Submitted: 16.05.2019

Revision requested: 14.06.2019

Last revision received: 28.06.2019

Accepted: 28.06.2019

Published online: 02.07.2019

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ABSTRACT

We have evaluated the fatty acid composition of the livers from two cartilaginous fish species *Raja clavata* (thornback ray) and *Mustelus mustelus* (common smooth-hound) caught off the Northern Aegean Sea. While there was generally little variation between species, *Mustelus mustelus* indicated low saturated (SFA) in summer (29.61%), in spring (32.57%), in autumn (30.07%) and in winter (31.81%) and high polyunsaturated fatty acid (PUFA) in summer (40.35%), in spring (36.50%), in autumn (30.21%) and in winter (27.26%) levels. The dominant fatty acids were palmitic acid (C16:0), oleic acid (C18:1 (n-9)), eicosapentaenoic acid (EPA; C20:5 (n-3)), and docosahexaenoic acid (DHA; C22:6 (n-3)) in both cartilaginous fish species in all seasons. The ratio of DHA/EPA with respect to the total of fatty acids in livers oils was ranged from 2.66% to 4.44% for *Mustelus mustelus* and 2.89% to 4.46% for *Raja clavata*. The n:3/n:6 ratio of thornback ray was higher compared to smooth-hound shark in all seasons. The liver oil of *R. clavata* and *M. mustelus* represent a valuable source of omega-3 PUFA that can be used for human and animal nutrition.

Keywords: Cartilaginous fish, Fatty acids, Liver, *Raja clavata*, *Mustelus mustelus*

Introduction

The fish oils, constitute an important source of omega-3 polyunsaturated fatty acids (PUFA), mainly the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA). The omega-3 PUFA provides several benefits to the human health; they have supporting effect on brain and retina development, especially premature children (Navarro-García et al., 2004b; Hoffman and Uauy, 1992). Studies have shown that consumption of fish and fish oil rich in long chain polyunsaturated fatty acids not only reduces the risk of cardiovascular and coronary heart failure (Bigger, 2001; Lee and Lip, 2003), but also cancers, immune system regulators (Side et al., 1998) and supports the development of the brain (Haag, 2003). When the fish is thought to be healthier, both lipid content and PUFA composition should be considered (Aidos, 2002; Nuñez, 2007). However, liver oils from elasmobranchs have the effect of strengthening the immune system in humans, but it can also be used for the prevention of colds, infections, allergies, sinusitis, asthma, low blood pressure, blood sugar reduction and pain relief (Solomon et al., 1997). The liver oil contains high levels of vitamin A and vitamin D, which is very important in preventing diseases such as blindness and rashchitis (Hall, 1992). Cartilaginious fishes are traditionally caught around the world. However, only a few parts are eaten, the caudal fins, and most of the rest is considered as waste (body, viscera, skin) (Le Néchet et al., 2007). Few authors have studied the lipid composition of those by-products, notably liver and gonads, but they contain a high proportion of lipids (Navarro-García et al., 2004a; Navarro-García et al., 2004b; Ould El Kebir, 2003; Pal et al., 1998; Tufan et al., 2013). Also, the studies on lipid and fatty acids in liver oils of cartilaginous fishes in the world have been mostly investigated in deep species (Bordier et al., 1996; Bakes and Nichols, 1995; Deprez et al., 1990).

Information has been reported for thornback ray and smoothhound, liver oils. In the present investigation, the lipids and fatty acids of the liver of the commercial ray and shark species were studied for the first time in the Northern Aegean Sea. In Turkish fishery data, landings of elasmobranchs appear under generic names as “sharks” and “rays” and do not reflect the diversity of Elasmobranchs in Turkish waters at species level. Elasmobranch (sharks and rays) landings reduced from 4040 tonnes in 2000 to 104 tonnes in 2018 that corresponds to a 97.4% decrease (TUIK, 2018). In Turkey, shark and ray meat consumption is rather limited and elasmobranch catch is mainly processed for export. Meat of *S. acanthias* and *M. mustelus* are smoked or salted or marketed fresh as whole carcasses for export. Similarly, the wings of rays and skates are processed and marketed skinned and frozen (Kabasakal, 1998). *Raja clavata*, which is a member of the Rajidae family, is found in the coastal and neritic areas of the Mediterranean; It is a demersal species (Fischer, 1973; Tortonese, 1975). Its distribution areas; Iceland, Norway, North Sea, Mediterranean and Black Sea in the East Atlantic (Compagno et al., 1989). It is distributed in the Marmara Sea, the Aegean Sea, the Mediterranean Sea and the Black Sea in Turkish waters (Mater et al., 2005). Although the coasts prefer sandy-muddy areas with especially soft grounds, It can also be found in various substrates. It ranges from shallow water to a depth of 600 m (Stehmann, 1990; Mytilineou et al., 2005; Mater et al., 2005). *Raja clavata* individuals are among the species with high economic importance. Especially in Europe and Far East countries, these fish can be marketed as whole or as a whole along the vertebral system, including the tail and the head, which can be marketed as skinned or skinless with their fins. As canned, it is preferred in hot and cold fuels. It can be prepared by special methods from head and bones in "Meikotsu", a special dish of Chinese and Japanese. Up to 60% of the liver can be obtained in fat and high in vitamin A, as well as in pipes of some species, vessels in combs, shells, etc. such as ornamental items . In some countries, only internal organs are used in fish flour and fertilizer industry (Akşıray, 1987). Other important cartilaginous fish, *Mustelus mustelus*, is a benthic species. It is distributed on the sandy-muddy grounds of coastal waters, between 5-150 m. depths (Branstetter, 1984). Its distribution areas; ranges from Azore, Madeira, Angola to South Africa, including the East Atlantic, England to the Mediterranean, Morocco and Canary islands, and the Indian Ocean and is distributed in the Marmara Sea, the Aegean Sea and the Mediterranean Sea in Turkish waters (Mater et al., 2005). It is tasty meats with high economic value are prepared by various methods such as fresh, frozen, salted and brine. Also soups and varieties made of fins are preferred in the world. Because of the fat and vitamins found in the liver, it is used in the pharmaceutical industry (Akşıray, 1987).

Sharks and rays liver oil are important raw material which they are rich in EPA and DHA polyunsatured fatty acids (Navarro-García et al., 2004b; Navarro-García et al., 2010). The former authors showed that livers in ray species indicated around 5-11% of the total fish weight, with an oil content of approximately 50% of its weight. EPA and DHA represented 16-18% of the fatty acids present in the oil (Navarro-García et al., 2010). Unfortunately, available literature on liver oil studies from ray and shark species are limited in Turkish Seas (Tufan et al., 2013; Özyılmaz and Öksüz, 2015; Cabbar and Yığın, 2015; Özyılmaz, 2016). The aims of the present study

explore the composition of liver oils derived from the thornback rays and smooth-hound.

Material and Methods

The thornback rays and smooth-hound were obtained seasonal from commercial trawl vessels in the Northern Aegean Sea, off the Babakale and Yeniköy, Turkey. While the mean length and weight of thornback rays were 65.69 cm and 1868.15 g, the mean length and weight of smooth-hound were 97.95 cm and 3297.85 g, respectively. The livers of the fish were removed, weighted and stored at -20ºC for further analysis. The hepatosomatic index was calculated as the the ratio of liver weight to total body weight. Lipid extraction was carried out according to Bligh and Dyer (1959) method. Homogenized tissue sample is mixed with 1 volume of chloroform and 2 volumes of methanol. After thoroughly vortexing, 1 volume of chloroform is added to the homogenate followed by another mixing step. Afterwards, 1 volume of distilled water is added and subsequently the suspension is stirred for an additional incubation period. The resulting suspension consists of non-extractable residues in a chloroform/methanol/water mixture with volumetric ratios 2:2:1.8 (v/v/v). This suspension is subsequently filtered through a medium flow filter paper. After a short incubation period, the filtrate is completely phase separated and the upper aqueous layer can be removed. Approximately 10 g of minced liver samples were used for oil extraction. Chloroform was evaporated using a vacuum rotary evaporator at 40ºC. The remaining fish liver oil were dried at 60ºC for 30 min. Fatty acid methyl ester (FAME) preparation, chromatographic conditions and fatty acid determination were performed as described (IUPAC, 1979; Özyılmaz and Öksüz, 2015).

Shimadzu GC (Gas Chromatography) was used to determine fatty acids. The system consists of a FID detector (Flame Ionization Detector), a gas chromatograph (Shimadzu, GC 2014, Japan) and an autoinjector (AOC-20i, Shimadzu, Japan). The gas chromatography is controlled by GC solution software (Version 2.41.00 su_1). It was used FAME-WAX (polyethylene glycol, 30 m*0.25 mm I.D*0.2 µm, GC Columns Restek) as chromotographic column. The chromatography operating conditions were identied as follows; 5 minutes at 70°C, reach 5°C/min increase up to 250°C, waiting time of 20 minutes at 250°C. Helium was used as the carrier gas with a flow and split rates of 1.0 ml/min and 1:10, respectively. All analyses were conducted in triplicate. Supelco 37 Component FAMEs Mix was used for determination of peaks as standard of fatty acids.

The results were statistically evaluated using SPSS 19.0 package program. Significance of difference ($P<0.05$) between seasons was determined by one-way ANOVA. Differences between means were determined by Tukey's test.

Results and Discussion

The descriptive data on the common smoothhound and thornback ray (Table 1) reveal that their average lengths are 97.95 ±10.58 cm and 67.1 ±8.47 cm and average weights 3297.85 ±1127.11 g and 1868.15 ±766.41 g, respectively. Golani et al. (2006) report that common smooth-hound species having been detected at 50-100 m weight up to 120 kg. Ismen et al. (2009) report that the lengths of male *M. mustelus* species in the Northern Aegean Sea range between 46.8 cm and 148.3 cm, while females may measure 49 cm to 152.2 cm. They also express that the male and female members may weight 390-10270 g and 382-14431 g, respectively. Özyılmaz and Öksüz (2015) indicate the length of the common smoothhound they captured in the Northwestern Mediterranean Sea as 107.67 cm. Yıgın and Ismen (2009) note that the minimum and maximum lengths of the thornback rays they caught in the Northern Aegean Sea are 10-88 cm (disc width: 6-60 cm) for the females and 11-76 cm (disc width: 7-50 cm) for the males. As reported by Yıgın and Ismen (2009), their total weights range from 5 g and 4622 g. Özyılmaz (2016) the total lengths and weights provides that the female *R. clavata* individuals from the Mediterranean Sea measure 53.9 cm (disc width: 37.1 cm) and weight 980.0 g and the males 49.5 cm (disc width: 33.8 cm) and 778.5 g, respectively.

Table 1. The total length, disc width, total weight, liver weight and hepatosomatic index of the *R. clavata* and *M. mustelus*.

Measurements	*R. clavata* (n=51)			*M. mustelus*(n=12)		
	Min.	Max.	Mean	Min.	Max.	Mean
Total length (cm)	50.2	81.9	65.69 ±8.47	78	113.8	97.95±11.30
Disc width (cm)	33.4	64.2	45.60 ±6.88	-	-	-
Total weight (g)	705.71	3717.27	1868.15 ±766.41	1442.26	5559.85	3297.85±1127.11
Liver weight (g)	12.89	241.4	78.55 ±51.99	51.82	384.01	211.12±104.23
HSI (%)	1.83	7.53	3.94 ±1.27	3.31	9.88	6.03±1.78

n: Sample size, Min.:Minimum, Max: Maximum

Hepatosomatic index (HSI) is a measure of the energy reserves in fish and fish generally have smaller livers in poor environmental conditions (Avşar, 2005) Liver is key in reproduction particularly to vitellogenesis in females (Kousteni and Megalogonou, 2011). In cartilaginous fish, females have larger livers than males do to supply the energy they need for the formation of oocytes during vitellogenesis and pregnancy period. Females store more lipid in their livers during the reproductive cycle (Capapé and Reynaud, 2011). In the present study while the HSI values of the *R. clavata* individuals ranged between 1.83% and 7.53%, those of *M. mustelus* between 3.31% and 9.88%. The analyzed values revealed that the liver weights of *M. mustelus* were higher. Özyılmaz and Öksüz (2015) calculated the HSI values of *M. mustelus* species captured in Northwestern Mediterranean Sea to be 6.34%. The HSI values were calculated in the other examined ray species as well and found to be 4.24% in common guitarfish (*Rhinobatos rhinobatos*), 8.25% in common stingray (*Dasyatis pastinaca*), 5.36% in common eagle ray (*Myliobatis aquila*), and 5.15% in lusitanian cownose ray (*Rhinoptera marginata*).

The fatty acid compositions of *M. mustelus* and *R. clavata* are provided in Table 2 and Table 3. The unsaturated fatty acids (ΣMUFA and ΣPUFA) in the liver lipids of both cartilaginous fish species were observed to be more than the saturated fatty acids (ΣSFA). The total saturated fatty acids (ΣSFA) in *M. mustelus* were realized to be more than those in *R. clavata* in summer, spring, autumn, and winter. The percentages concerning the saturated fatty acids in each species in this study showed that the dominant ones were myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), respectively. The results of previous studies on the liver fatty acid profiles of cartilaginous fish supports this finding (Ould El Kebir, 2003; Navarro-García et al., 2004b; Navarro-García et al., 2009; Sellami et al., 2014; Özyılmaz and Öksüz, 2015, Özyılmaz, 2016).

While a statistically significant difference in the myristic acid (C14:0) content of the liver lipids of both species was observed in spring, autumn, and winter ($P<0.05$), the levels were found to be similar in summer ($P>0.05$). Whereas the difference between both species in palmitic acid (C16:0) content was not statistically significant in summer, spring, and winter ($P>0.05$), a significant difference was detected in autumn ($P<0.05$). The difference between *M. mustelus* and *R. clavata* in terms of stearic acid (C18:0) content was found statistically significant ($P<0.05$). Oleic acid (C18:1n9) was discovered to be the most dominant monounsaturated fatty acid in all the seasons. The oleic acid values in *M. mustelus* were 14.21%, 19.28%, 24.74%, and 23.67% in spring, summer, autumn, and winter, respectively. On the other hand, those in *R. clavata* were 17.66%, 20.11%, 22.65%, and 22.67%, respectively. The analysis of the seasonal differences revealed a statistically significant difference between summer and spring in the oleic acid contents of both species ($P<0.05$), while no statistical differences was observed between autumn and winter ($P>0.05$). Nichol et al. (1998), Ould El Kebir et al. (2007), Le Néchet et al. (2007), Turan et al. (2007), Navarro-García et al. (2009), Tufan et al. (2013), Sellami et al. (2014), Özyılmaz and Öksüz (2015), and Özyılmaz (2016) report that the oleic acid (C18:1n9) has the highest value among all the monounsaturated fatty acids in *R. clavata* and other stingrays. Besides, in their study on the liver fatty acids in *Dasyatis brevis* and *Gymnura marmorata*, Navarro-García et al. (2004b) found higher palmitoleic acid (C16:1) and eicosenoic acid (C20:1) values than oleic acid. The comparison between *M. mustelus* and *R. clavata* in terms of oleic acid content indicated a statistically significant difference between the values in summer and autumn ($P<0.05$), but none was observed in spring and winter ($P>0.05$).

Table 2. Fatty acid (%) profiles of the *R.clavata* livers

Fatty acids	Summer	Spring	Autumn	Winter
$C_{14:0}$	2.31±0.12^{ab}	2.04±0.05^{c}	2.21±0.16^{bc}	2.47±0.15^{a}
$C_{15:0}$	0.83±0.05^{c}	0.86±0.21^{c}	1.14±0.14^{b}	1.54±0.04^{a}
$C_{16:0}$	18.23±0.32^{c}	21.80±0.26^{a}	19.81±1.37^{b}	22.59±0.69^{a}
$C_{17:0}$	0.95±0.03^{c}	1.08±0.46^{c}	3.21±0.14^{a}	2.17±0.01^{b}
$C_{18:0}$	9.23±0.27^{a}	9.17±0.81^{a}	4.11±0.05^{b}	4.10±0.15^{b}
$C_{23:0}$	1.16±0.03^{a}	1.09±0.08^{a}	0.82±0.38^{a}	0.27±0.02^{c}
$C_{14:1}$	0.08±0.01^{c}	0.05±0.01^{c}	0.30±0.03^{b}	0.52±0.01^{a}
$C_{16:1}$	5.64±0.14^{c}	5.64±0.71^{c}	6.84±0.24^{b}	8.06±0.05^{a}
$C_{17:1}$	0.67±0.05^{a}	0.00±0.00^{d}	0.41±0.03^{c}	0.59±0.01^{b}
$C_{18:1}$ n9+n7	14.21±0.61^{c}	19.28±0.81^{b}	24.74±0.83^{a}	23.67±0.57^{a}
$C_{20:1}$ n9	3.72±0.02^{b}	4.90±0.31^{a}	3.76±0.75^{b}	3.33±0.33^{b}
$C_{22:1}$ n9	1.68±0.25^{c}	2.63±0.20^{a}	2.08±0.21^{b}	0.55±0.15^{d}
$C_{24:1}$ n9	0.38±0.07^{b}	0.49±0.00^{ab}	0.61±0.17^{a}	0.33±0.13^{b}
$C_{18:2}$ n6c	1.57±0.05^{b}	1.53±0.18^{b}	3.64±0.26^{a}	3.77±0.40^{a}
$C_{18:3}$ n6	0.59±0.03^{ab}	0.46±0.08^{b}	0.71±0.18^{a}	0.53±0.02^{ab}
$C_{18:3}$ n3	1.43±0.04^{a}	1.26±0.07^{ab}	0.87±0.25^{c}	1.09±0.11^{bc}
$C_{20:2}$	0.72±0.00^{a}	0.52±0.11^{b}	0.31±0.02^{c}	0.50±0.06^{b}
$C_{20:3}$ n6	0.06±0.00^{c}	0.06±0.01^{c}	0.93±0.04^{a}	0.28±0.01^{b}
$C_{20:3}$ n3	3.38±0.08^{a}	1.45±0.59^{b}	1.51±0.42^{b}	1.12±0.41^{c}
$C_{20:4}$ n6	0.14±0.03^{b}	0.20±0.03^{a}	0.20±0.03^{a}	0.00±0.00^{c}
$C_{20:5}$ n3	8.40±0.05^{a}	4.63±0.20^{c}	5.15±0.24^{b}	5.32±0.21^{b}
$C_{22:2}$	0.36±0.08^{a}	0.24±0.02^{b}	0.37±0.15^{a}	0.36±0.02^{b}
$C_{22:6}$ n3	24.26±0.37^{a}	20.62±1.41^{b}	16.29±1.74^{c}	16.83±0.91^{c}
ΣSFA	32.9	36.03	31.28	33.15
ΣMUFA	26.56	32.99	38.74	37.04
ΣPUFA	40.55	30.98	29.97	29.81
ΣUFA	67.10	63.97	68.72	66.85
Σ ω-3	37.08	27.96	23.82	24.36
Σ ω-6	2.38	2.25	5.48	4.59
Σ ω-9	20.12	27.3	31.20	27.88
Σ ω-3/ Σ ω-6	15.58	12.43	4.35	5.31
DHA/EPA	2.89	4.46	3.16	3.17

a, b, c indicated statistical differences between seasons (P<0.05). Mean values ±S.D. of determination for triplicate samples. SFA- saturated fatty acids; PUFA- polyunsaturated fatty acids; UFA- unsaturated fatty acids; DHA-docosahexaenoic acid; EPA-eicosapentaenoic acid.

Table 3. Fatty acid (%) profiles of the *M. mustelus* livers

Fatty acids	Summer	Spring	Autumn	Winter
$C_{14:0}$	2.65±0.20^{b}	3.19±0.07^{a}	2.75±0.04^{b}	3.14±0.29^{a}
$C_{15:0}$	0.11±0.02^{d}	0.84±0.04^{a}	0.25±0.04^{b}	0.19±0.03^{c}
$C_{16:0}$	18.64±0.61^{b}	22.12±1.37^{a}	22.94±1.04^{a}	23.75±0.98^{a}
$C_{17:0}$	1.17±0.11^{c}	1.38±0.10^{b}	1.17±0.02^{c}	1.71±0.14^{a}
$C_{18:0}$	6.84±1.08^{a}	4.92±1.17^{b}	2.84±0.04^{c}	3.02±0.33^{c}
$C_{20:0}$	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
$C_{21:0}$	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
$C_{22:0}$	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
$C_{23:0}$	0.21±0.13^{a}	0.12±0.03^{ab}	0.12±0.03^{ab}	0.00±0.00^{b}
$C_{14:1}$	0.78±0.02^{a}	0.06±0.01^{d}	0.53±0.04^{b}	0.27±0.13^{c}
$C_{15:1}$	0.00±0.00^{c}	0.01±0.02^{c}	0.79±0.06^{b}	1.19±0.04^{a}
$C_{16:1}$	4.47±0.24^{c}	6.39±0.43^{b}	8.16±0.38^{a}	8.85±1.06^{a}
$C_{17:1}$	0.41±0.06^{c}	0.01±0.02^{d}	0.86±0.08^{b}	1.49±0.06^{a}
$C_{18:1}$ n9+n7	17.66±0.28^{c}	20.11±0.16^{b}	22.65±0.49^{a}	22.67±1.25^{a}
$C_{20:1}$ n9	3.64±0.18^{a}	2.37±0.20^{b}	2.11±0.08^{b}	3.63±0.28^{a}
$C_{22:1}$ n9	2.96±0.17^{b}	1.92±0.21^{c}	3.59±0.39^{a}	2.83±0.11^{b}
$C_{24:1}$ n9	0.12±0.21^{b}	0.04±0.01^{b}	1.03±0.02^{a}	0.00±0.00^{b}
$C_{18:2}$ n6c	0.98±0.04^{b}	1.06±0.08^{b}	3.83±0.09^{a}	3.08±0.99^{a}
$C_{18:3}$ n6	0.38±0.03^{c}	0.36±0.01^{c}	0.54±0.10^{b}	0.73±0.09^{a}
$C_{18:3}$ n3	0.77±0.06^{a}	1.06±0.54^{a}	0.81±0.02^{a}	1.00±0.24^{a}
$C_{20:2}$	0.81±0.11^{a}	0.36±0.05^{b}	0.91±0.08^{a}	0.36±0.26^{b}
$C_{20:3}$ n6	0.08±0.01^{c}	0.27±0.01^{c}	1.80±0.46^{a}	0.92±0.03^{b}
$C_{20:3}$ n3	0.14±0.01^{bc}	0.10±0.02^{c}	0.20±0.06^{b}	0.38±0.05^{a}
$C_{20:4}$ n6	3.61±0.19^{a}	2.37±0.10^{b}	0.93±0.25^{c}	0.70±0.07^{c}
$C_{20:5}$ n3	6.13±0.45^{b}	7.45±0.55^{a}	5.37±0.26^{bc}	4.81±0.36^{c}
$C_{22:2}$	0.26±0.04^{c}	0.30±0.04^{c}	1.57±0.46^{a}	0.81±0.08^{b}
$C_{22:6}$ n3	27.20±1.03^{a}	23.18±0.46^{b}	14.27±0.24^{c}	14.46±0.25^{c}
ΣSFA	29.61	32.57	30.07	31.81
ΣMUFA	30.04	30.93	39.72	40.93
ΣPUFA	40.35	36.50	30.21	27.26
ΣUFA	70.39	67.43	69.93	68.19
Σ ω-3	34.24	31.79	20.65	20.65
Σ ω-6	5.05	4.06	7.1	5.43
Σ ω-9	24.38	24.44	29.38	29.13
Σ ω-3/ Σ ω-6	6.78	7.83	2.91	3.80
DHA/EPA	4.44	3.11	2.66	3.00

a, b, c indicated statistical differences between seasons ($P<0.05$). Mean values ±S.D. of determination for triplicate samples. SFA- saturated fatty acids; PUFA- polyunsaturated fatty acids; UFA- unsaturated fatty acids; DHA-docosahexaenoic acid; EPA-eicosapentaenoic acid.

DHA and EPA of the polyunsaturated fatty acids (ΣPUFA) were the most dominant fatty acids in a common smoothhound (*M. mustelus*) and a thornback ray (*R. clavata*). The EPA values in *M. mustelus* were revealed to be significantly different in each season ($P<0.05$) and the values accounted for 6.13%, 7.45%, 5.37%, and 4.81%, respectively. The EPA values in *R. clavata* were realized to be similar in autumn (5.15%) and winter (5.32%) ($P>0.05$), while a statistically significant difference was observable in summer (8.40%) and spring (4.63%) ($P<0.05$). The DHA values in *M. mustelus* were discovered to be 27.20%, 23.18%, 14.27%, and 14.46% in summer, spring, autumn, and winter, respectively. It was realized that the seasonal difference in summer and spring was statistically significant ($P<0.05$), while the difference in the shark's liver lipid was not in autumn and winter ($P>0.05$). Similar to the findings related to *M. mustelus*, the seasonal difference in *R. clavata* individuals were statistically significant in summer and spring ($P<0.05$) but not in autumn and winter ($P>0.05$). The DHA values of the thornback ray was calculated to be 24.26%, 20.62%, 16.29%, and 16.83% in summer, spring, autumn, and winter. The comparison between *R. clavata* and *M. mustelus* in terms of EPA values indicated a statistically significant difference between these two species in summer and spring ($P<0.05$) and none in autumn and winter ($P>0.05$). Similarly, the differences in DHA values between both species were observed to be significant in summer, spring, and winter ($P<0.05$). But no statistically significant difference in DHA values was observed between *R. clavata* and *M. mustelus* in autumn ($P>0.05$). Özyılmaz and Öksüz (2015) reported the EPA and DHA values in *M. mustelus* captured at the Iskenderun Gulf in the Northwestern Mediterranean Sea as 2.31% and 11.81%, respectively. The EPA and DHA values in *M. mustelus* specimens retrieved in the Northern Aegean Sea for the purpose of this research study were observed to be twice as high as these values. In the study on *R. clavata* individuals captured in the Black Sea and Mediterranean Sea, Özyılmaz (2016) reports the EPA values in the females and males captured in the Black Sea as 7.7% and 8.9% and the DHA values as 23.4 and 19.9%, respectively. Özyılmaz (2016) notes that the EPA values in the females and males of the same species account for 4.5% and 5.8% and the DHA values for 26.1% and 22.4%, respectively. These differences in the EPA and DHA values of the sharks might have resulted from several critical factors such as nutrient composition and water temperature. Planktonic crustaceans are among the primary nutrient resources of sharks; therefore, the EPA and DHA concentrations are influenced by temperature swings in the species habitats. Any increase in water temperature reduces EPA and DHA levels in sharks' livers (Malins et al. 1965; Nuñez 2007). Differences in EPA and DHA levels are also dependent on many other factors such as species, region, age, sex, nutrients, water temperature, feeding environment, and seasons. Moreover, Özyılmaz (2016) revealed statistically significant differences between males and females in terms of the DHA values in *R. clavata* captured in the Black Sea and Mediterranean Sea and noted that these differences resulted from sex and region. Omega-3 and Omega-6 polyunsaturated fatty acids have antagonistic effects on human body. Allen and Harris (2001) remark that the higher amount of the polyunsaturated fatty acid ω-3 than that of ω-6 is good for human health. ω-3/ω-6 ratio is a determinative and crucial factor in the formation of nutritional values of fats. In the present study, ω-3/ω-6 ratios in *M. mustelus* were 6.78, 7.83, 2.91, and 3.80 in summer, spring, autumn, and winter while those in *R. clavata* were 15.58, 12.43, 4.35, and 5.31 respectively. Navarro-García et al. (2014) report this ratio to range between 2.32 and 4.03 in the stingray species *U. chilensis*, *U. halleri*, *R. glaucostigma*, *R. steindachneri*, and *D. dipeteura* and the highest value in *R. glaucostigma*. The distribution of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in sharks and stingrays in various studies and this research study are presented in Table 4.

Conclusion

In this study, the ΣPUFA values in *R. clavata* and *M. mustelus* were found to range from 32.83% to 33.58% and ΣMUFA values from 33.83% to 35.41%. The results suggest that the fatty acid compositions in the livers of cartilaginous fish vary according to species, regions, seasons, sex, age, habitats, nutrient compositions, and water temperature. In conclusion, two cartilaginous fishes has favourable amount of polyunsaturated fatty acid. The major fatty acids identified in *R. clavata* and *M. mustelus* were 16:0 (palmitic), 18:1 n9 (oleic), and 22:6 n3 (DHA) in all seasons. Indeed, such oil, which could be obtained in relatively high amounts, is an excellent source of omega-3, MUFAs and PUFAs, particularly DHA. Therefore, thornback ray and smoothhound liver oil can be considered to be an alternative to fish oil as a source of EPA and DHA.

Table 4. Fatty acid composition of liver from various cartilaginous fish species (%) (M: Male; F: Female)

Species	SFA	MUFA	PUFA	Region	Source
Somniosus pacificus	12.50	72.00	13.30		
Centroscymnus plunketi	11.50	83.60	2.60		
Etmopterus granulosus	15.00	80.00	2.50	Southern Avustralian waters	Bakes and Nichols (1995)
Deania calcea	26.10	64.80	7.20		
Centroscymnus crepidater	15.70	80.20	1.41		
Centrophorus scalpratus	25.60	62.20	0.60		
Carcharhinus falciformis	35.68	19.46	37.63	Gulf of California,	Navarro-García et al. (2000)
Galeocerdo cuvier	30.13	33.74	14.38	Caribbean waters	
Gymnura spp.	3.63	3.88	18.06	Malaysian waters	Osman et al. (2001)
Centroscymnus coelolepis	18.54	53.41	28.04		
Centroscyllium fabricii	26.81	47.82	25.38	North Atlantic	Remme et al. (2005)
Centrophorus squamosus	25.59	44.70	29.47		
Dasyatis marmorata	27.47 M	32.3 M	39.79 M		
	39.08 F	26.83 F	33.1 F		
Rhinoptera marginata	51.89 M	20.74 M	27.36 M	East Atlantic Ocean	Ould El Kebir et al. (2007)
	43.31 F	24.11 F	31.93 F		
Rhinobatos cemiculus	41.55 M	20.48M	37.20 M		
	42.34 F	22.98F	34.17 F		
Rhinoptera bonasus	34.40	17.90	28.60		
Aetobatus narinari	38.90	19.60	20.70	Gulf of Mexico	Navarro-García et al. (2009)
Dasyatis americana	34.50	16.10	30.30		
Raja clavata	27.1-32.1	14.9-19.0	34.3-39.5	Black Sea	Tufan et al. (2013)
Urotrygon chilensis	29.30	18.15	23.61		
Urobatis halleri	27.29	26.47	22.62		
Rhinobatos glaucostigma	24.72	15.67	21.73	Sinaloa, México	Navarro-García et al. (2014)
Rhinoptera steindachneri	9.01	7.84	10.16		
Dasyatis dipeteura	35.62	36.25	17.28		
Mustelus mustelus	34.79	43.30	20.04		
Rhinobatos rhinobatos	36.84	35.70	27.34		
Dasyatis pastinaca	34.97	41.22	22.07	Northeastern	Özyılmaz and Öksüz (2015)
Myliobatis aquila	35.10	28.61	30.43	Mediterranean	
Rhinoptera marginata	34.95	24.19	34.19		
Carcharhinus altimus	24.28	55.98	8.87		
Raja clavata	22.50 F	30.70 F	45.70 F	Black Sea	
	23.00 M	32.20 M	43.30 M		Özyılmaz (2016)
Raja clavata	28.40 F	23.90 F	46.30 F	Mediterranean	
	29.40 M	25.87 M	43.90 M		
Raja clavata	33.34	33.83	32.83	Aegean Sea	This study
Mustelus mustelus	31.02	35.41	33.58		

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Financial disclosure: This research was financially supported by the Canakkale Onsekiz Mart University Scientific Research Projects Coordination Unit, Turkey (FBA-2015-533).

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AQUATIC RESEARCH
E-ISSN 2618-6365
Aquatic Research 2(3), 154-160 (2019) • https://doi.org/10.3153/AR19013 Short Communication

TRACE TOXIC MINERAL LEVELS OF SEA LETTUCE (*Ulva* spp.) FROM COAST OF ISTANBUL

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Cite this article as:

Özden, Ö., Orhan, Y., Kaplan, M., Parıldar, S., Erkan, N. (2019). Trace toxic mineral levels of sea lettuce (*Ulva* spp.) from coast of Istanbul. *Aquatic Research*, 2(3), 154-160. https://doi.org/10.3153/AR19013

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Submitted: 19.03.2019
Revision requested: 23.04.2019
Last revision received: 06.05.2019
Accepted: 06.05.2019
Published online: 08.07.2019

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ABSTRACT

Concentrations of Nickel (Ni), Copper (Cu), Zinc (Zn), Iron (Fe), Arsenic (As), Mercury (Hg), Lead (Pb) and Cadmium (Cd) were determined in the macro algae sea lettuce (*Ulva* spp.), sampled from the coastline of Istanbul old city (Cankurtaran) in summer 2016. The abundance of trace toxic mineral concentrations in sea lettuce were in the following order: Fe > Zn > As > Cu> Ni >Pb> Cd. However, mercury was not detected in any sample. The present study provides a new information to the consumer on the distribution of trace toxic minerals in sea lettuce.

Keywords: Marmara Sea, Istanbul coast, Trace toxic minerals, *Ulva* spp., Sea lettuce

Introduction

Trace toxic minerals are natural substances that have a high atomic weight and a density at least 5 times greater than that of water and these minerals are termed as heavy metals. Some trace minerals taken together with the food have a very important role in human life and are known to be necessary up to a certain concentration for human metabolism, as in the example iron, zinc and copper. Whereas, the majorities of heavy metals are toxic even at low concentrations such as arsenic, cadmium, chromium, lead and mercury. The multiple factors such as industrial, agricultural, medical and technological applications of trace toxic minerals have led their wide distribution in the environment. The trace toxic minerals, in particular, mercury, lead, cadmium and arsenic are frequently detected in aquatic organisms and raised concerns regarding the potential human health impacts. The toxic effects of these trace minerals may depend upon a variety of factors such as dose, exposure route and chemical structure, as well as age, gender, genetics and nutritional status of the exposed individuals (Belitz et al., 2009; Özcan, 2004; Soylak et al., 2005; Tchounwou et al., 2012).

The degree of pollution by trace toxic minerals in marine environments can be estimated by analysis of water, sediment and tissues of organisms (Morillo et al., 2005). Marine algae species are generally used to determine coastal waters trace toxic mineral grades in worldwide. In this process, it takes advantage of the key role of algae in the food chain and its temporal relationship with the pollutants (Topcuoğlu et al., 2010). The analysis of sediments in marine environments always faces limitations and the concentration of a trace toxic mineral in the sediment varies, among other factors, depending on the rate of deposition and the nature of the particles. This does not reflect the bioavailability. Macro algae appear to be the most appropriate indicators of both active and passive minerals (Villares et al., 2010). Sea lettuce (*Ulva lactuca*) is a macro algae which has the potential importance in terms of bio indicators with the tendency to absorb trace toxic minerals and spread in cytoplasmic cells in marine contamination with toxic pollutants (Davis et al., 2003).

Trace toxic mineral-related pollution in coastal regions of Marmara Sea has become a significant problem because of intensive industrial activity, ship wastes, dense population and construction origin pollution and municipality wastewater discharges. Sea lettuce is a type of macro algae and continues its vital activities by fixing itself to rocks and taking nourishment from marine environment. Figure 1 shows the general distributions of this species, which has a large habitat from tropical to polar areas. This species is frequently seen in the rocky coastal lanes of the Black Sea, Marmara and Aegean Sea in Turkey.

Therefore, the aim of the present study is to evaluate the pollution in the Marmara Sea through determination of trace toxic metals in the sea lettuce obtained from old city region Cankurtaran Istanbul coast of the Marmara Sea.

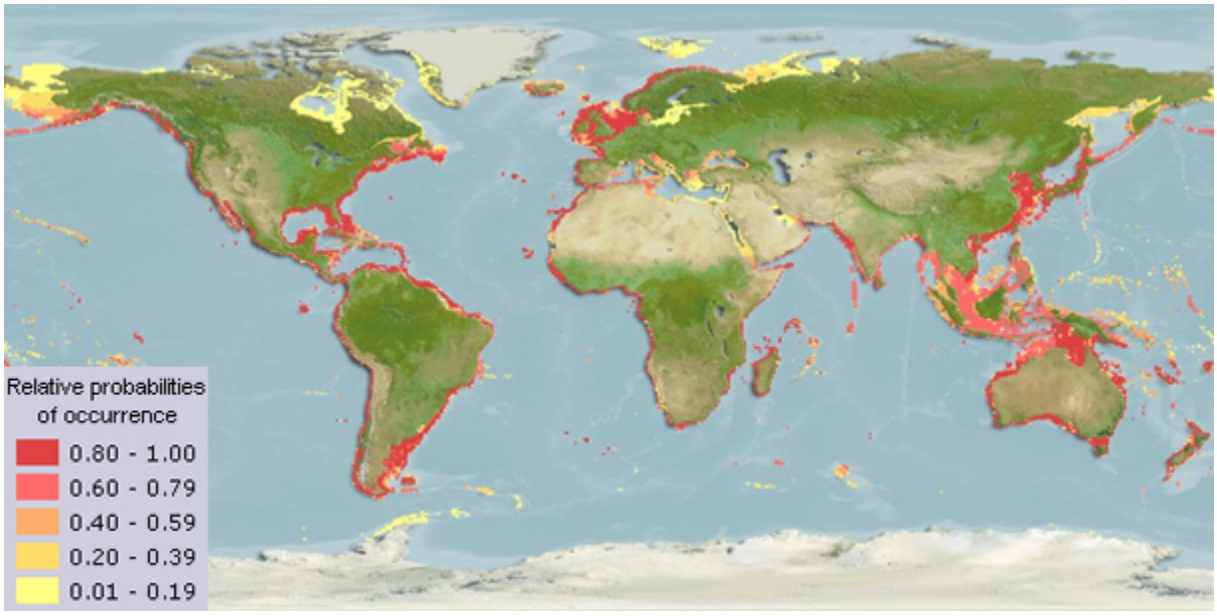

Figure 1. The habitats and distribution (native range) of *Ulva lactuca* in world (AquaMaps, 2016a)

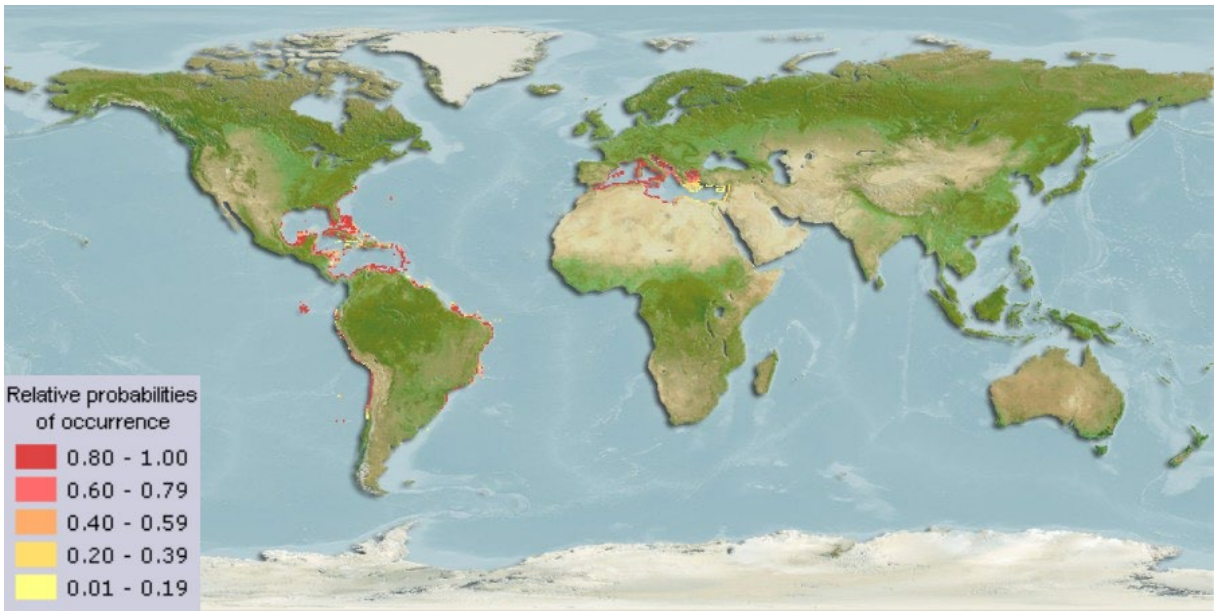

Figure 1. The habitats and distribution (native range) of *Ulva rigida* in world (AquaMaps, 2016b)

Material and Methods

Sea lettuce (*Ulva* spp.) were obtained (5 kg wet sample) from old city region Cankurtaran, Istanbul coast of the Marmara Sea (Figure 1) in year 2016 (end of summer) and dried under shadow (end dry product ~1 kg).

Determination of trace toxic minerals in dry sea lettuce (Ni, Cu, Zn, Fe, As, Hg, Pb, Cd) was carried out using an Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) (Analytic Jena PlasmaQuant® MS, ICP-MS). An aliquot of 250 mg dried sea lettuce sample was weight into a pre-cleaned Teflon tube and 8 mL of concentrated nitric acid (65%) added. The samples digested by microwave assisted digestion system. (Table 1.). After digestion, samples diluted to 50 mL with ultrapure water. The analysis performed with "NMKL No: 186 - Trace Elements - As, Cd, Hg, Pb and Other minerals. Determination by ICP-MS After Pressure Digestion, 2007" method (NMKL "Nordic Committee On Food Analysis", 2007) (Table 2., 3.). The accuracy and precision of the analytical method checked using the certified reference material, powdered muscle tissue (Catalogue No. ERM-BB422). It was found that RSD % did not exceed 5.0%. All trace toxic mineral concentrations were determined on a mg/kg dry weight basis.

Table 1. Microwave Digestion program for sea lettuce sample preparation

Berghof Microwave Unit (Berghof - Speedwave SW4)				
Temperature (°C)	**Pressure (bar)**	**Ramp (min)**	**Time (min)**	**Power (%)**
155	50	8	8	90
205	50	8	30	90
50	0	1	15	0

Figure 1: Istanbul old city coast (Cankurtaran) of Marmara Sea.

Table 2. ICP-MS operating parameters for the quantification of trace toxic minerals in sea lettuce

ICP-MS (Analytic JENA Plasma Quant MS Elite) application parameters	Parameters Used
Nebulizer Gas Flow	1.06 L/ min.
Auxiliary Gas Flow	1.3 L/ min.
Plasma Gas Flow	9.0 L/min.
ICP RF Power	1400 watts
Pump Rate	10 rpm
Stabilization Delay	40 second

Table 2. Trace toxic mineral standards used to establish ICP-MS calibration curve

Specifications of Standards used in ICP-MS					
Element Name	**Trademark**	**Catalogue Number**	**Main Stock Concentration**	**Measured in ICP-MS mass value**	**LOQ (ng/mL)**
Ni	VHG	PNIN-100	999 µg/mL	60	125.36
Cu	VHG	ACUN-100	1001 µg/mL	65	76.77
Zn	VHG	PZNN-100	1000 µg/mL	66	428.05
Fe	VHG	PFEN-100	1000 µg/mL	56	601.42
As	VHG	PASN-100	1008 µg/mL	75	105.04
Hg	VHG	PHGN-100	1002 µg/mL	200	44.57
Pb	VHG	PPBN-100	1005 µg/mL	206	31.50
Cd	VHG	PCDN-100	1000 µg/mL	111	18.53
Y	Inorganic Ventures	GGY1-1	1001 µg/mL	89	-
Y (Ittrium) element is used as internal standard in the device					

The internal standard concentration used was 2.0 ng/mL

Table 3. Concentration levels of the standards for ICP-MS Calibration curve

Element Name	Calibration Points of the Elements						
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	
Ni	0.5	2.5	5	10	20	50	
Cu	0.3	2.5	5	10	50	100	
Zn	2	10	50	100	250	500	
Fe	2.5	10	20	50	100	250	**ng/mL**
As	0.5	1	2,5	5	10	50	
Hg	0.2	0.5	1	2.5	5	10	
Pb	0.15	0.5	1	2.5	5	10	
Cd	0.08	0.3	1	2.5	5	10	

Results and Discussion

The present study provides new information on the distribution of trace toxic minerals in sea lettuce (*Ulva* spp.*)* obtained from Cankurtaran, the old city coast of Istanbul (European seaside). The trace toxic mineral concentrations determined in the algae samples studied are given in Table 1.

The present study reports the trace toxic minerals pollution in the samples collected from the Istanbul old city (Cankurtaran) coastline in the summer of 2016. Similarly, in 2014, Ozyigit et al. studied trace toxic mineral contents of *sea lettuce* samples collected from different parts of Istanbul including Istanbul old city coast (Cankurtaran) station, Büyükada, Fenerbahçe, Maltepe, Bakırköy and Beylikdüzü stations (Ozyigit et. al., 2017). Table 5, shows the trace toxic mineral contents of sea lettuce samples collected from different coastal cities of Marmara Sea. The results of their study is presented in Table 5. As can be seen from the Table, the sea lettuce samples collected from Cankurtaran coastline were found to contain much lower levels of Cu, Zn, Fe, Pb and Cd than other coastal cities of Istanbul including Büyükada, Fenerbahçe, Maltepe, Bakırköy and Beylikdüzü. Consequently, it can be concluded that the Istanbul old city (Cankurtaran) coast is less affected from trace toxic mineral pollution than its other cities with coasts to Marmara Sea.

Trace toxic mineral contents of sea lettuce collected from other cities with coasts to Marmara Sea were also reported in the literature (Table 5 and Table 6).

Culha et al. studied the trace toxic mineral contents of sea lettuce samples collected from city of Yalova located on the coast of Marmara Sea (Culha et al., 2013). They reported lower Cd, Zn, Ni and Pb contents compared to our results for the sea lettuce samples obtained from Cankurtaran, coast of Istanbul old city (Table 6). However, Cu and Fe levels of sea lettuce collected from Cankurtaran were much lower than the samples collected from Yalova. In the same study, no information is reported for As and Hg in the samples of Yalova region.

In another study, Ergul et al. determined the trace toxic mineral contents of sea lettuce collected from **Dilovası** coast shore of Izmit Bay in 2009 (Ergul et al., 2010). The amounts of trace toxic minerals contained in the sea lettuce samples collected during the summer and autumn of 2009 were much higher (Zn:125.95-373.1 mg/kg, Fe: 1518.9-5249.8 mg/kg and Pb: 1.05-1.95 mg/kg) than the samples collected from old city coast of Istanbul (Cankurtaran) during the Summer of 2016 (Table 6).

Table 4. Toxic elements determined in *Ulva* spp. samples

Toxic Lements (mg/kg)	Ni	Cu	Zn	Fe	As	Hg	Pb	Cd
Sea lettuce	1.32 ±0.04	4.92 ±0.10	6.92 ±0.25	88.74 ±3.57	3.65 ±0.11	n.d.	0.26 ±0.01	0.05 ±0.00

n.d. Not Detected <0.02 ng/kg

Table 5. Trace toxic mineral contents (mg/kg) of the sea lettuce samples obtained from present study and those reported by Özyiğit et al. (2017) and Culha et al. (2013)

Sea lettuce	Büyükada	Fenerbahçe	Maltepe	Bakırköy	Beylikdüzü	Istanbul old city coast (Cankurtaran)[a]
Cd	0.45 ±0.01	1.01 ±0.01	1.49 ±0.03	2.22 ±0.04	3.22 ±0.05	**0.05** ±0.00
Cu	6.67 ±0.10	10.92 ±0.13	13.01 ±0.15	15.28 ±0.17	18.31 ±0.20	**4.92** ±0.10
Fe	553.3 ±11.00	686.2 ±13.10	721.23 ±16.12	775.3 ±19.20	989.3 ±29.20	**88.74** ±3.57
Pb	4.93 ±0.07	7.63 ±0.09	8.92 ±0.10	12.35 ±0.20	19.32 ±0.25	**0.26** ±0.01
Zn	15.16 ±0.22	22.23 ±0.52	26.87 ±0.73	31.88 ±0.81	41.23 ±1.02	**6.92** ±0.25
Ni	No Data	No Data	No Data	No Data	No Data	**1.32** ±0.04
As	No Data	No Data	No Data	No Data	No Data	**3.65** ±0.11
Hg	No Data	No Data	No Data	No Data	No Data	**n.d**

n.d. Not Detected <0.02 ng/kg

Table 6. Comparison of the results (mg/kg) obtained from the present study and the data reported in the literature.

Sea lettuce	Istanbul old city coast (Cankurtaran)[a]	Yalova (Culha et al., 2009)	Dilovası/Kocaeli (Ergul et al., 2010) Summer	/ Autumn
Cd	**0.05** ±0.00	<0.01	No Data	No Data
Cu	**4.92** ±0.10	12.44	No Data	No Data
Fe	**88.74** ±3.57	358.36	1518.9	5249.8
Pb	**0.26** ±0.01	<0,01	1.05	1.95
Zn	**6.92** ±0.25	5.99	125.95	373.1
Ni	**1.32** ±0.04	1.25	No Data	No Data
As	**3.65** ±0.11	No Data	No Data	No Data
Hg	**n.d**	No Data	No Data	No Data

[a] the results obtained from present study n.d. Not Detected <0.02 ng/kg

Conclusion

This study presents new information about the distribution of trace toxic minerals in sea lettuce from the old city coastal area of Istanbul (Cankurtaran). According to the results of the current study, there is no risk for health in the consumption and evaluation of this species. However, if the consumption and evaluation of this product is considered in the future, it is recommended that the water environment of the Black Sea, Marmara and Aegean Sea coastal production / harvest potentials must be monitored periodically.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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AQUATIC RESEARCH

E-ISSN 2618-6365

Aquatic Research 2(3), 161-169 (2019) • https://doi.org/10.3153/AR19014 *Review Article*

MACHINE LEARNING APPLICATIONS IN OCEANOGRAPHY

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Cite this article as:

Ahmad, H. (2019). Machine learning applications in oceanography. *Aquatic Research*, 2(3), 161-169. https://doi.org/10.3153/AR19014

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Submitted: 16.06.2019
Revision requested: 17.06.2019
Last revision received: 24.06.2019
Accepted: 28.06.2019
Published online: 08.07.2019

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ABSTRACT

Machine learning (ML) is a subset of artificial intelligence that enables to take decision based on data. Artificial intelligence makes possible to integrate ML capabilities into data driven modelling systems in order to bridge the gaps and lessen demands on human experts in oceanographic research .ML algorithms have proven to be a powerful tool for analysing oceanographic and climate data with high accuracy in efficient way. ML has a wide spectrum of real time applications in oceanography and Earth sciences. This study has explained in simple way the realistic uses and applications of major ML algorithms. The main application of machine learning in oceanography is prediction of ocean weather and climate, habitat modelling and distribution, species identification, coastal water monitoring, marine resources management, detection of oil spill and pollution and wave modelling.

Keywords: Machine learning, Application, Oceanography, Data driven

Introduction

Machine Learning (ML) is a discipline of the computer science that develops dynamic algorithms capable to produce data-driven decisions (Thessen, 2016). ML has proven itself to be an answer to many real world problems with it capabilities. ML has advantage over the traditional methods because it is able to a build model, which is highly dimensional and nonlinear data with complex relations and missing values. ML has proven useful for a very large number of applications in many parts of the Earth system (land, ocean, and atmosphere) and beyond, from retrieval algorithms, crop disease detection, new product creation, bias correction and code acceleration (Yi and Prybutok, 1996).

Large amount of data which is collected by scientific instruments then separated into train set and test set. Therefore ML algorithms are trained by this data .then build model with high accuracy and its parameters are optimized based on sample data during the learning step. During prediction, the model parameters are used to infer results on the previously unseen data.

ML has multiple algorithms, techniques and methodologies that can be used to build models to solve real world problems using oceanographic data. A supervised Learning (SL) is a type of ML algorithm that uses labelled data. After that, the machine is provided with new set of data so that SL algorithms analyses the training data and produces a correct outcome from the labelled data. SL mainly trials to model the relationship between the inputs and their corresponding outputs from the training data so that we would be able to predict the output based on the knowledge it gained earlier with regard to relationships. SL are classified into two major categories. A. classification and B. regression.

Unsupervised learning (USL) is the training of the machine using data that is neither classified nor labelled. The task of the machine is to group unsorted data based on the similarities, patterns and differences without any guidance. USL can be classified into following the categories a. clustering, b. dimensionality reduction c. anomaly detection.

The reinforcement learning (RL) methods are slightly different from SL or USL. RL is a type of ML where an agent learns how to behave in the environment by performing actions and thereby drawing intuitions and seeing the results.

Deep learning (DL) is the subset of ML concerned with algorithms inspired by the structure and function of the human brain called artificial neural network. Neural networks (NNs) come in several forms such as recurrent neural networks, convolutional neural networks, and artificial neural networks and feed forward neural networks. An ANN is an interconnected group of nodes. Here, each circular node represents an artificial neuron and an arrow represents a connection from the output of one artificial neuron to the input of another. Model comprises synaptic links which allow the inputs (x_1, x_2,……x_n) to be measured by applying the weights (w_1, w_2, …. w_n).

Methodology

This study was based on the syntheses of secondary information. To collect data, an intensive literature review related to the machine learning applications and scope of machine learning in oceanography was done. The context were conducted through an online and offline mode .In addition, relevant documents and reports were also collected from the websites and published research articles personal contacts. Open source software python and R as well as commercial software adobe illustrator were used for data analysis and visualization (Figure 1).

Necessity of the Machine Learning Approach for Oceanographic Research

The ocean is vast, dynamic and complex. Data structure of the ocean becomes increasingly complex and large. Generally, coastal zone is vulnerable to natural diesters like sea level rise (SLR), coastal flooding, erosion etc. For the coastal zone management and flood erosion control, a reliable and accurate tool for prediction and forecasting of coastline evolution and inundation by water is needed in order to minimize coast protection and conservation. For this reason, traditional data analysing methods are time consuming and costly, even in some cases, analysis is not possible in conventional way. ML techniques are robust, fast and highly accurate.

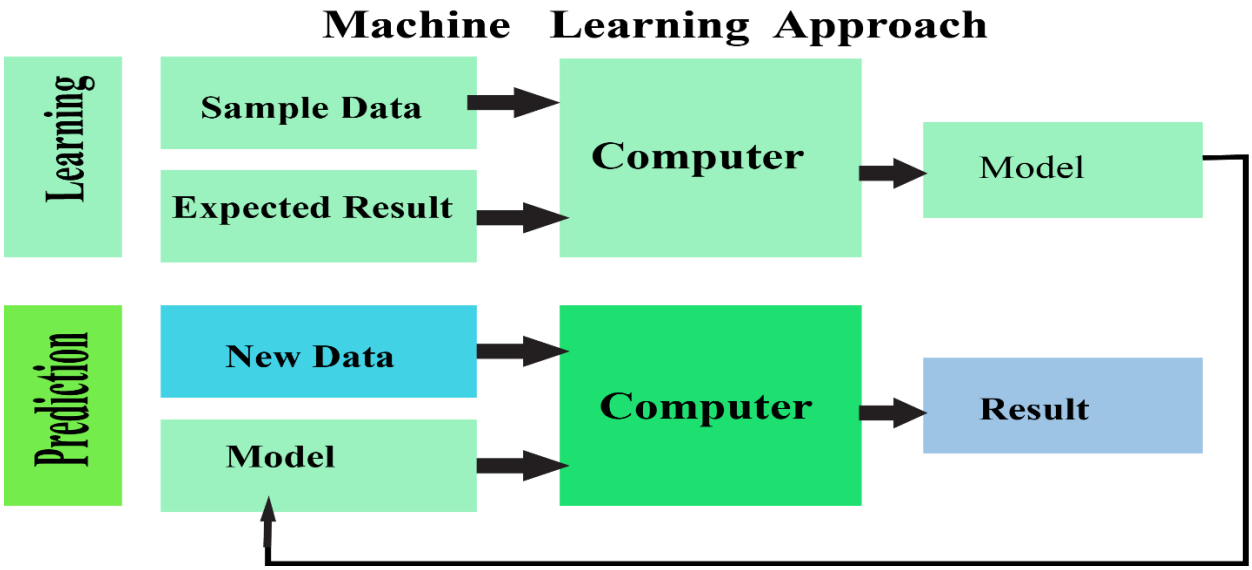

Figure 1. Simple Machine learning working approach (created by adobe illustrator CS6)

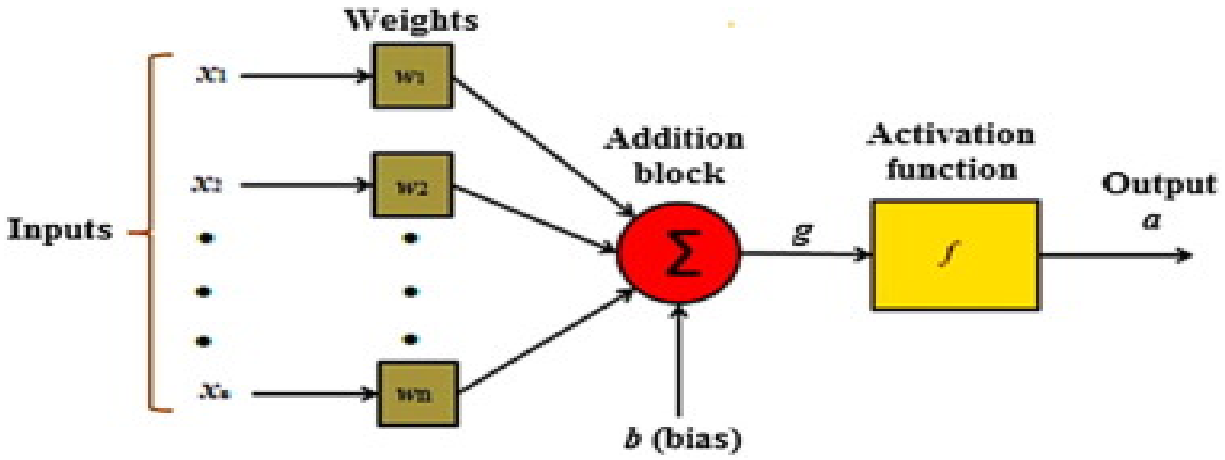

Figure 2. simple artificial neural network (Burkitt, 2006; Oja, 1982; Turkson et al., 2016)

Common Machine Learning Applications in Oceanography

Oceanic climate prediction and forecasting

Advancements in ML, in combination with optimization methods are promising to balance the performance of forecast and the earliness of those forecasts (Mori et al., 2017). The most common ML methods used in meteorological forecasting are genetic algorithms, which have been used to model rainy vs. non-rainy days (Haupt, 2009). Machine learning methods have been applied to forecast coastal sea level fluctuations (Hsieh, 2009). ML is used to study important processes such as El Niño, sea surface temperature anomalies, and monsoon models (Cavasos et al., 2002; Hsieh, 2009; Krasnopolsky, 2009; Thessen, 2016). The oceanography community makes extensive use of neural networks for forecasting sea level, waves, and sea surface temperature (Hsieh, 2008; Forget et al., 2015).Wu et al. (2006) developed an MLP NN model to forecast the sea surface temperature (SST) of the entire tropical pacific ocean where sea level pressure and SST were used as predictor to predict.

Species identification

Identification of small and large size marine taxa require specialized knowledge, which is one of the bottlenecks in oceanographic studies. This limitation can be solved by ML approach with high accuracy (automatic identification techniques). Recent advances in the ML are promising with regard to improving accuracy of automated detection and classification of marine organisms from high volume data such as images and video (Olson and Sosik 2007). Generally, ML algorithms are trained on images, videos, sounds and other types of data labelled with taxon names. Trained algorithms can then automatically annotate new data and this methods are used to identify plankton, shellfish larvae from images, bacteria from gene sequences, cetacean from audio, fish and algae from acoustic and optical characteristics (Simmonds and Armstrong, 1996; Boddy, 1999; Jennings et al., 2008; Goodwin and North 2014).

Detection of ocean pollution

ML can be used in detection of ocean pollution with the help of satellite and radar images such as oil spills, plastics pollution, algal bloom etc. Oil spill detection currently requires a highly trained human operator to assess each region in each image (Kubat et al., 1998). Del Frate et al. (2000) used MLP NN models to detect oil spill on the ocean surface from synthetic aperture radar (SAR) images.

Marine and coastal water monitoring

A multilayer preceptor neural networks model was developed to derive the concentrations of phytoplankton pigment, suspended sediments and gelbstoff, and aerosol over turbid coastal waters from satellite data (Tanaka et al., 2004). ML methods are also used in coastal water monitoring (Kim et al., 2014). Machine learning applications to electronic monitoring of fishery-dependent data are of increasing interest to management bodies in the United States and Europe. It has the potential to reduce the cost associated with observers and streamline the processing of video data (Lewis et al., 2001).

Sedimentation modelling

Sedimentation is an important phenomenon in the coastal oceanography among ML methods, ANN has widely used in various water related research such as rain runoff modelling, modelling stage discharge relationship (Bhattacharya and Solomatine 2005). ML models that predict sedimentation in the harbor basin of the port of Rotterdam (Bhattacharya and Solomatine, 2006). Random forest ML approach has been applied to the mapping marine substrates (Hasan et al., 2012; Diesing et al., 2014).

Coastal morphological and morphodynamic modeling

A variety of coastal morphology and morphodynamic models have been built by using the ML (Goldstein et al., 2018). ML models are widely used in the applications of sediment transport, morphology and detection of coastal changes through videos, images. Nonlinear ML forecasting techniques were used to predict suspended sediment concentration based on instantaneous water velocity (Goldstein et al. 2018). ANN was also used to predict the depth integrated alongshore sediment transport using water depth, wave height, wave period and alongshore current velocity (van Maanen et al., 2010). ANN was used to determine the correlation between sandbar morphology and a given wave climate, culminating in examining the nonlinear dependencies of bar position on past wave conditions (Múnera et al., 2014).

Habitat modelling and species distribution

Understanding the habitat and distribution of marine species are important tasks for management and conservation of oceanography. An algorithm can be trained using a large data set matching environmental variables to taxon abundance or presence/absence data. If the algorithm tests well, it can be given a suite of environmental variables from a different location to make predictions on what taxa are present. This technique has been used to identify current suitable habitat for specific taxa, model future species distributions including predicting invasive and rare species presence, and predict biodiversity of an area (Thessen, 2016).

Wind and wave modelling

Ocean wave modelling and prediction are important for a maritime country because there are numerous reasons behind this. For example shipping routes can be optimized by avoiding rough sea thereby reducing time spent during transportation (James et al., 2018). Accurate forecasts of ocean wave heights and directions are a valuable resource for many marine- based industries (O'Donncha, 2017). We may apply machine learning techniques is to predict wave conditions in order to replace a computationally intensive physics-based model by straightforward multiplication of an input vector by mapping matrices resulting from the trained machine learning models (James et al., 2018). Horstmann et al. (2003) used multilayer perceptron (MLP) NN models to retrieve wind

speed s globally at about 30 m resolution from SAR data (Horstmann et al., 2003).

Ocean current prediction

Generally, ROMS is widely used for ocean dynamic process analysis. It is possible to improve the prediction of ocean currents using (historical data) data-driven machine learning methods (Hollinger et al., 2012). For example, neural networks have been used to build Reynolds average turbulent models (Bolton and Zanna, 2019).

Marine and coastal resources management

ML models have ability to capture complex, nonlinear relationships in the input data which are the crucial building blocks for the implementation of ecosystem based fisheries management (Lewis et al., 2001). Taking right inferences about marine conservation and management can be very difficult as there is not sufficient data for certainty and the consequences of their existence can be disastrous. ML methods can provide a tool for increasing certainty and improving results especially techniques that incorporate Bayesian probabilities (Thessen, 2016). ML and more specifically Bayesian networks are being used for marine spatial planning in cooperation with GIS (Lewis et al., 2001).

The goal of this review paper is to give a clear idea about ML applications in oceanographically different areas. Traditional Data driven research is time consuming, even not integrated and dynamic nature. Furthermore, the extent of our training, testing, and field evaluation data ensures that the approach is robust and reliable across a range of conditions (i.e., changes in taxonomic composition and variations in image quality related to lighting and focus (Olson and Sosik, 2007). ML methods has great potentials for applications in oceanography but effective adoption is limited by several factors that need to be eliminated. This concerns not only the methods themselves, which can often seem opaque or are not well understood, but also the necessary data sources, as well as deployment and how methods are integrated into the existing advisory and scientific process (Headquarters, 2018).

Common ML methods for resources management are genetic algorithms (Haupt, 2009), neural networks (Brey and Jarre-Teichmann, 1996), support vector machines (Guo and Kelly, 2005), fuzzy inferences systems (Tscherko and Kandeler, 2007), decision tree (Jones and Fielding, 2006) and random forest (Quintero et al., 2014).

Table 1. Machine learning algorithms and scope of applications in oceanography

No.	Types	Major Machine learning algorithms	Scope and potentials of application
1	Supervised	Linear regression	
2		Support vector machine Support vector regression	1.Oil spill mapping and detection 2.Satellite image processing for land use 3.Retrieve ocean surface chorophyll concentration 4.Habitat modeling
3		Decision tree	1.Resources management 2.Sediment properties
		Random forest	Mapping of marine substrates
		Naïve Bayes	
4	Unsupervised	k-means	Clustering ocean biomes
5		PCA	
6	Reinforcement	Markov decision process	1.Quickly detect hazardous weathers 2.Detection of whale acoustics
7	Deep learning		1.Wave modelling 2.Coastal water monitoring 3. prediction of coastal morphologic properties

Recommendations: Some steps can be taken to improve ML models in oceanography.

1. Constant Engagement of oceanographic expertise in ML.
2. Preservation and sharing acquired knowledge of ML among community.
3. Collected data of Ocean should be available for ML model experiments such as "www.kaggle.com".
4. Communication between oceanographers and machine learning scientist is needed for awareness and potentials of applications.
5. Machine learning scientists could cooperate ocean scientists for data collection and equipment designing.
6. Motivation and encourage for long term ML research in oceanographic applications.
7. Some events in schools, college and university, competition of ML in oceanography can be effective.

Conclusion

This work investigates various machine learning techniques for the oceanographic data analysis and future opportunities. ML offers a diverse number of methods that are accessible to researchers and fitted in oceanographic applications which is heavily based on data. This approach offers significant advantages in real life operational applications. They have great potential to improve the quality of oceanographic research approaches by creating more accurate models. ML might be used in large oceanographic datasets to discover hidden patterns and trends. The success of the ML approach strongly depends on the adequacy of the data set used for the training. The data availability, precision, quality, representativeness, and amount are the crucial elements for success in this type of ML application. ML also requires interdisciplinary collaboration, communication, technical knowledge on programming and financial support.

Compliance with Ethical Standard

Conflict of interests: The author declare that for this article they have no actual, potential or perceived conflict of interests.

Acknowledgement: I would like to express my sincere thanks to all those who provided me documents and published papers to complete this review. And I am highly motivated by the popularity of "https://www.kaggle.com". This website provided me with the taste of machine learning. And there has been no financial support for this work.

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E-ISSN 2618-6365

Aquatic Research 2(3), 170-173 (2019) • https://doi.org/10.3153/AR19015 *Short Communication*

FIRST RECORD OF *Jujubinus errinae* OUTSIDE OF THE TYPE LOCALITY

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Cite this article as:

Giacobbe, S., Renda, W. (2019). First record of *Jujubinus errinae* outside of the type locality. *Aquatic Research*, 2(3), 170-173. https://doi.org/10.3153/AR19015

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Submitted: 20.07.2019
Revision requested: 27.07.2019
Last revision received: 30.07.2019
Accepted: 31.07.2019
Published online: 31.07.2019

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ABSTRACT

Revision of formerly studied samples showed that the top-shell, *Jujubinus errinae* Smriglio et al., 2016, known so far only from the Strait of Messina, also occurs in the Ustica seafloors, south-western Tyrrhenian Sea. Both areas are characterized by *Laminaria rodriguezii* kelp beds, which may represent an Atlantic-like habitat hosting *J. errinae* together with other benthic species having in the Strait of Messina their type locality.

Keywords: Mollusk, Mediterranean, Laminariales, Biogenic seafloor, Biogeography

Introduction

The recently described top-shell, *Jujubinus errinae* Smriglio et al., 2016, is known so far only from the type locality, the Strait of Messina, an area that is recognized as a hotspot of biodiversity hosting numerous presumed endemism. In the eventuality that *J. errinae* might occur in other Mediterranean districts, samples of *Jujubinus* collected elsewhere in related habitats were reexamined and related published and unpublished data carefully revised.

Aim of the paper is to report the second finding of this poorly known species, suggesting more extensive investigation on the Mediterranean bioclastic environments.

Material and Methods

Kelp bed communities and related death assemblages have been described by Di Geronimo et al. (1988) from the "Apollo Bank", south-western Tyrrhenian Sea. The bank, belonging to the Ustica volcanic system, has a 40 m shallower depth, reaching -150 m towards the 3 km distant Ustica Isle (Figure 1). A dense *Laminaria rodriguezii* Bornet 1888 kelp forest characterizes the rocky floors, locally covered by biogenic gravelly sediments, which testify of a strong current regime. A 7 dm^3 sample of sediment, dredged from 70 m to 50 m depth, has been sieved on 1 mm mesh and entirely examined.

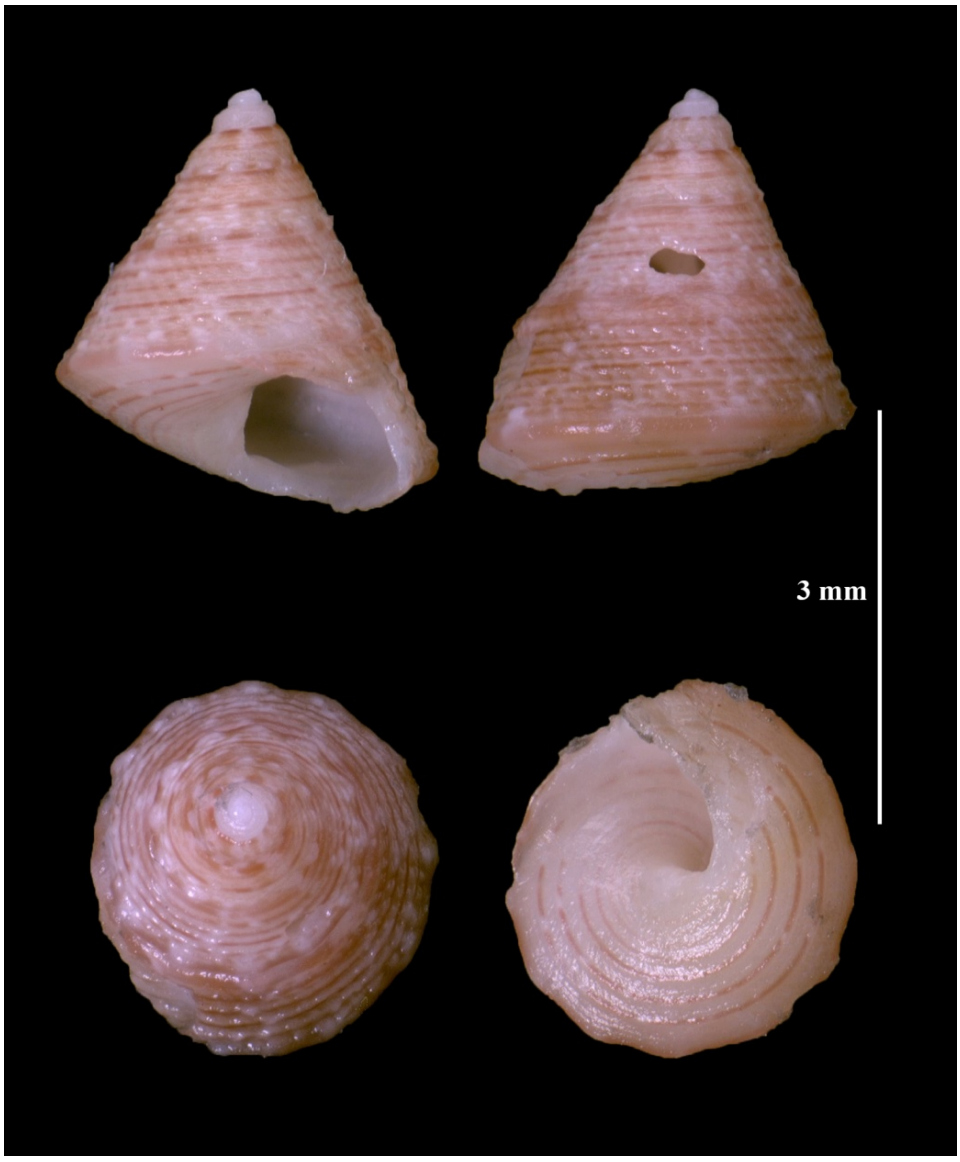

Figure 1. Four views of *Jujubinus errinae* entire specimen from Ustica. The cephalopod predation hole is recognizable.

Results and Discussion

The mollusc death assemblages, deposited at the Messina University Benthic Ecology Laboratory, provided a specimen (BEL137BA1988Je1) (Figure 2) and two incomplete shells of this species (BEL137BA1988Je2/3), formerly classified as *Jujubinus elenchoides* (Monterosato) (sic). The complete specimen, 3 mm height, showed a small elliptical hole indicating cephalopod predation, while the fragmented shells indicated durophagous fish predation.

The Strait of Messina, type locality of *J. errinae*, is a complex and diversified area of tidal-induced upwelling, whose nutrient enrichment and temperature lowering support peculiar "Pliocene Atlantic remnants", as wide kelp beds and dense colonies of the Hydrozoan *Errina aspera* (Linnaeus, 1767) (Smriglio et al., 2016).

In agreement with Di Geronimo et al. (1988), contamination by shallower levels in the Apollo Bank may be excluded, suggesting that the species is linked to the habitat in which it was collected. The initial hypothesis that *J. errinae* might represent an accessory species in the *Errina aspera* assemblages (Smriglio et al., 2016), should be thus implemented, including this species in those kelp bed communities known to give a marked "Atlantic" connotation to the Strait of Messina (Assis et al., 2016), and recognizable in other localized Mediterranean sites, as recently suggested for the polyplacophoran *Callistochiton (Allerychiton) pachylasmae* (Monterosato, 1869), also having the Strait of Messina as type locality (Dell'Angelo et al., 2018).

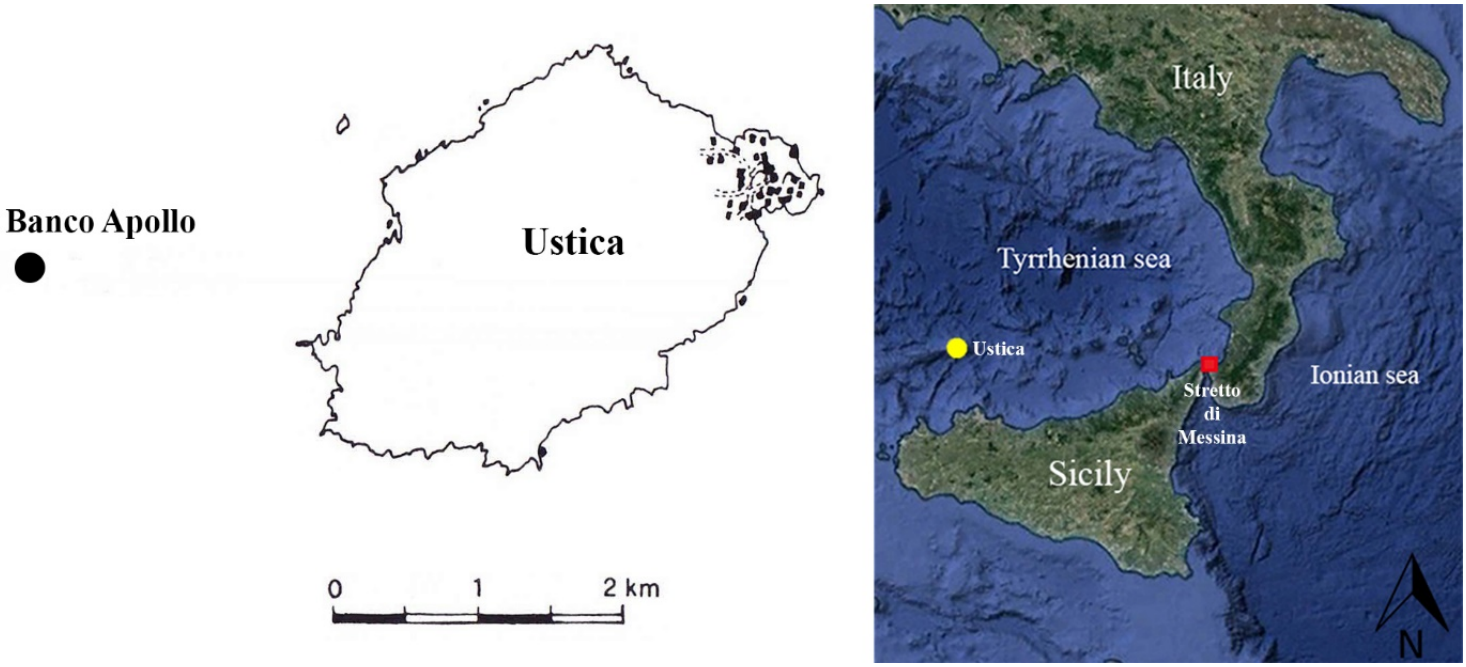

Figure 2. Sampling stations of *Jujubinus errinae* (modified from Di Geronimo et al., 1988).

Conclusion

The report of *Jujubinus errinae* from Ustica Island is a further indication that Mediterranean biogeography is more articulated and complex than currently described; in this respect, more extensive investigations on habitats that are still considered marginal, and an accurate revision of deposited samples, will might provide important contributions.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labelled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

AQUATIC RESEARCH

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

Reference System is APA 6th Edition

In-text Citation with APA

The APA style calls for three kinds of information to be included in in-text citations. The **author's last name** and the work's **date of publication** must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Crockatt, 1995).

Direct quote from the text

"The potentially contradictory nature of Moscow's priorities surfaced first in its policies towards East Germany and Yugoslavia," (Crockatt, 1995, p. 1).

Major Citations for a Reference List in Table 2.

Table 2.

Material Type	Reference List/Bibliography
A book in print	Baxter, C. (1997). *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
A book chapter, print version	Haybron, D.M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
An eBook	Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.2015)
An article in a print journal	Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy,* 14(1), 38-48.
Preview article in a journal with DOI	Gaudio, J.L. & Snowdon, C.T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (*Saguinus oedipus*) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
Websites - professional or personal sites	*The World Famous Hot Dog Site.* (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015).
Websites - online government publications	U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.15).
Photograph (from book, magazine or webpage)	Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015).
Artwork - from library database	Clark, L. (c.a. 1960's). *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor.
Artwork - from website	Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015).

Note: All second and third lines in the APA Bibliography should be indented.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.