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Heavy Metals Contents in *Caulerpa racemosa* var.*cylindracea* from Turkish Coastline

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Abstract

Caulerpa racemosa var.*cylindracea* is a well-known invasive marine seaweed in the Mediterranean sea since 1991. Inasmuch as there is no a valid eradication method for *Caulerpa racemosa* var.*cylindracea* so far, this species still continues to invade the Mediterranean sublittoral ecosystem. Seven heavy metal (Cu, Cd, Zn, Mn, Fe, Cr and Pb) contents from four season in the *Caulerpa racemosa* var.*cylindracea* were investigated in the present study. While some metal contents varied seasonally, Cr and Pb were remained under detection limits in *Caulerpa racemosa* var.*cylindracea*. The possible frond abnormalities are tried to be associated with metal levels in the present study. Further researches on the uptaking mechanisms of the heavy metals in *Caulerpa racemosa* var.*cylindracea* are strongly recommended in the present study.

INTRODUCTION

Several studies have been reported on the relationship between heavy metals and marine algae [1-3]. Since some of marine algae such as *Ulva* and *Enteromorpha* are readily able to accumulate the heavy metals, they are used as biomonitors for heavy metal contamination in marine systems [3,4-8]. *Caulerpa* family possesses over 100 species in all around the world. Among them, *Caulerpa racemosa* had been accepted as a lessepsien migrant in Mediterranean Sea, when it had firstly been observed in Mediterranean. However, last comprehensive studies showed that invasive strain of *C.racemosa* in Mediterranean Sea is quite similar to *C.cylindracea* in Australian coastlines [9]. After a comprehensive genetic and morphological analysis, invasive strain of *Caulerpa racemosa* in Mediterranean Sea was identified as *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman, et Boudouresque *(Caulerpales, Chlorophyta)*. In the last decade, the interest by scientists on *Caulerpa racemosa* var. *cylindracea* increased because of its effective invasion property [10]. *Caulerpa racemosa* var. *cylindracea* has exhibited invasive properties and affected the sublittoral ecosystem of 12 Mediterranean countries

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(Albania, Algeria, Croatia, Cyprus, France, Greece, Italy, Libya, Malta, Spain, Tunusia and Turkey) and some large islands (Balearic Islands, Corsica, Crete, Cyprus, Sardinia and Sicily) [9-11]. Like all members of *Caulerpa* family, *Caulerpa racemosa* var. *cylindracea* has also important secondary metabolite called caulerpenyne (CPN), negative effects of this secretion on some marine organisms have been reported [12]. Inasmuch as *Caulerpa racemosa* var. *cylindracea* invades *Posidonia oceanica* habitat especially in winter seasons, a ecological problems with respect to decreasing *Posidonia oceanica* areas might be faced in near future. Although some physicochemical preventive methods have been applied, there is -still- no valid eradication method for *Caulerpa racemosa* var. *cylindracea* in the scientific literature. There are very limited studies in the literature on the concentrations of heavy metals in *Caulerpa* species. Therefore, the following research questions were aimed to be answered in the present study:

* What are the levels of Cu, Cd, Zn, Mn, Fe, Cr and Pb in *Caulerpa racemosa* var. *cylindracea*?
* Do these metal levels vary dependent on season?
* Could morphological changes in *Caulerpa racemosa* var. *cylindracea* be associated with the seasonal metal variations?

As far as we know, this research is the first attempt for the determination of heavy metals in invasive *Caulerpa racemosa* var. *cylindracea*.

MATERIALS AND METHODS

Caulerpa racemosa var. *cylindracea* samples were hand-picked by SCUBA divings from Seferihisar (Turkey) coastline in May-2003, September-2003, December-2003 and March-2004. The deep of collection area was 2 meters. The geographical coordinates are 38° 02’ 39.79’’ N, 27° 00’ 51.93’’ E. There is no record with respect to pollution of collection area by any industrial pollutants because of lacking industrial factories. After collection, the samples were firstly washed with seawater to remove epiphytes-impurities and then deionised water to remove salty water and contamination with respect to metals.

Analysis of Metals

After rinsing of algae samples with ultra deionised water, they were left on the towel paper for 1 hour to remove water. Then, about 0.3 g algae material was weighed by using special cups of Millestone Ethos plus 900 microwave sample preparation oven. 5 mL HNO_3 was added and final volume was adjusted to 25 mL in terms of appropriate oven software.

Perkin-Elmer AA800 Atomic absorption spectrophotometer was used for determination of trace elements. For Cu, Cd, Pb and Cr analysis, graphite oven mode was applied. Pd + $Mg(NO_3)_2$ solutions for Cu; $NH_4H_2PO_4$ + $Mg(NO_3)_2$ for Cd and Pb; $Mg(NO_3)_2$ solution for Cr were used as matrix modifiers. Analysis of Fe, Zn and Mn was carried out by using flame mode. Cathode lamb was used as light source. The wavelenghts were 324.8 nm, 228.8 nm, 283.3 nm, 357.9 nm, 248.3 nm, 213.9 nm, and 279.5 nm for Cu, Cd, Pb, Cr, Fe, Zn, and Mn, respectively.

Statistical Analysis

Data are subjected to student t-test. The values are the means of three separate experiments of three individuals. The statistical difference set up at $p<0.05$. The Pearson correlation test was used to check possible correlation among the variations.

RESULTS

The seven heavy metals were determined in invasive *Caulerpa racemosa* var. *cylindracea* dependent on season. The results were shown in the Table 1.

Table 1. Seasonal Variations in the levels of heavy metals in invasive *Caulerpa racemosa* var. *cylindracea* (µg / g wet weight).

Samples	Cu	Cd	Zn	Mn	Fe	Cr	Pb
C.racemosa May 2003	0.10 ± 0.03[a]	< 0.01	2.55 ± 0.09[a]	13.61 ± 0.35[a]	66.67 ± 0.20[a]	< 0.01	< 0.04
C.racemosa September 2003	0.75 ± 0.03[b]	< 0.01	< 0.1	28.65 ± 0.39[b]	276.63 ± 1.25[b]	< 0.01	< 0.04
C.racemosa December 2003	0.85 ± 0.02[b]	0.01± 0.001[a]	2.10 ± 0.25[a]	38.25 ± 0.13[c]	60.40 ± 0.25[c]	< 0.01	< 0.04
C.racemosa March 2004	0.41 ± 0.01[c]	0.02 ± 0.013[a]	2.75 ± 0.20[a]	15.66 ± 0.18[a]	130.26 ±0.20[d]	< 0.01	< 0.04

The different superscript lowercase (a,b,c and d) letters in the table showed statistical significance at 0.05.

As can be seen from Table 1, Cu concentration in December-2003 was significantly ($p<0.05$) higher than those of other months. Minimum Cu concentration was observed in May-2003. Cd concentration in May-2003 and September-2003 were under our determination limits. However, Cd levels in March-2004 and December-2003 were detectable but they were very low levels. The highest and lowest Zn levels were observed in the samples which were collected in March-2004 and September-2003, respectively. A gradual increase was observed in Mn levels from May-2003 to December-2004 then, a sharp decrease was determined. The maximum Fe concentration in *Caulerpa racemosa* var. *cylindracea* tissue was observed in September- 2003 as 276.63 ± 1.25 mg / g wet weight. Fe concentrations showed a fluctuation between May 2003 and March 2004. Both Cr and Pb in *Caulerpa racemosa* var. *cylindracea* were remained determination limits. No significant correlation ($p>0.05$) was found among metals investigated.

DISCUSSION

Heavy metal bio-accumulation in marine algae is of great importance in the evaluation of heavy metal contamination in the aquatic ecosystems. In the present study, seven heavy metal contents such as Cu, Cd, Zn, Mn, Fe, Cr and Pb in invasive *Caulerpa racemosa* var. *cylindracea* were investigated dependent on the season. Lacking of significant correlation among heavy metals may reveal that no relationship in bio accumulation of these heavy metals exists in *Caulerpa racemosa* var. *cylindracea*. Fe is an important metal for photosyntetic organisms. It plays important roles in algae physiology. However, this metal shows a fluctuation during an annual analysis. An increase from May-2003 to September-2003 might be associated with increased metabolic rate. Seawater temperature is increased about 2^{o}C during this period (May-2003 22^{o}C-September 24^{o}C). Increased temperature might have been caused increased metabolic rate. Therefore, it may have been bio-accumulated by *Caulerpa racemosa* var. *cylindracea* to use it for metabolic events.

Similarly, Mn levels in September-2003 and December-2003 were also significantly higher than those of March-2004 and May-2003 samples. Observation of undetectable Cr and Pb levels in the samples might indicate that the levels of these heavy metals in the collection site exist under detection limits. Observation of fluctuations in Zn level of samples can be an evidence of different regulation mechanism for this metal compared to other metals. On the other hand, as Villares et al (2002) mentioned in their report that different reasons can be underlied in the observation of different concentrations of heavy metals in different seasons. These factors can be environmental factors, variations in the metal concentrations of the sea, salinity, pH or metabolic factors such as growth. *Caulerpa racemosa* var. *cylindracea* might be affected by the salinity and nutrient levels of seawater [13]. In the excess conditions, fronds and stolons of *Caulerpa racemosa* var. *cylindracea* might be broken down and this dead biomass can be accumulated different metals. Therefore, *Caulerpa racemosa* var. *cylindracea* might be a good biomarker for metal contamination in seawater. On the other hand, inasmuch as *Caulerpa racemosa* var. *cylindracea* is an invasive marine alga, different levels of metals might have been affecting its regulatory mechanisms. In our previous studies [14-15], we seasonally analyzed some antioxidant enzymes such as superoxide dismutase and catalase which contain some metals such as Fe, Cu and Zn for their enzymatic activities, however, no significant correlation was found among metals and antioxidant enzymes (comparison was done with total enzyme activities). This results may show that enzyme regulatory mechanisms in *Caulerpa racemosa* var. *cylindracea* might not have been effected by metal levels in water body. According to our diving observations, this invasive species exhibits quite different morphological properties (Figure 1-4).

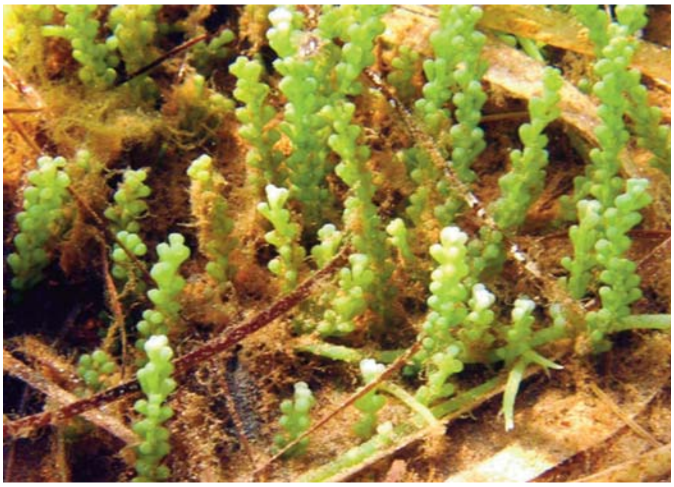

Figure 1. Well-known morphological view of *Caulerpa racemosa* var. *cylindracea.*

Figure 4. *Caulerpa racemosa* var. *cylindracea* normal and abnormal fronds.

Figure 2. *Caulerpa racemosa* var. *cylindracea* with different morphological view. The sea slug is *Oxynoe olivacea* which is a consumer of *Caulerpa racemosa* var. *cylindracea*. *Caulerpa racemosa* var. *cylindracea* was subjected to low light and low micronutrients in an aquarium.

Figure 3. *Caulerpa racemosa* var. *cylindracea* with different morphological fronds structures. The fronds exhibits lots of ramifications. The samples were collected in February-2006 from Seferihisar-Turkey. This is the characteristic winter frond view of *Caulerpa racemosa* var. *cylindracea* in Seferihisar-Turkey.

Especially frond structures of *Caulerpa racemosa* var. *cylindracea* amazingly change during our one-year observation. Morphological parameters are used for identification of marine algae. However, as Stam et al (2006) mentioned [16], identification of *Caulerpales* by using morphological characters may sometimes be resulted with wrong identification compared to molecular methods. As can be seen in Figure 1-4, we witnessed quite different morphological differences in *Caulerpa racemosa* var. *cylindracea*. These morphological views could be associated with metal variations. Among the metals tested, Fe, Mn and Cu might have been played in the formation of these abnormal frond structures. On the other hand, nutrients biomarkers in water body such as C:N, C:P ratios, PO_4^{3-} and NO_3^{2-} should be taken into consideration for these abnormal frond structures. The first attempt to measure trace element levels in *Caulerpa racemosa* was done by Harriss (1965) [17]. High amount of Fe and Mn compared to Ni, Co, Sr, and Rb was reported in the study of Harriss (1965) [17]. Another metal survey on the metal contents of *Caulerpa racemosa* was investigated by Khristoforova and Bogdanova (1980) [18]. These researchers determined Fe, Mn, Zn, Pb and Cu levels in *Caulerpa racemosa, Caulerpa urvilliana* and *Caulerpa peltata*. They reported no Pb levels in *Caulerpa racemosa* and *Caulerpa urvilliana* collected from Suvorov Atoll and Pukapuka Atoll however they reported 3.69 mg/g dry matter in *Caulerpa peltata* collected from Bio island. On the other hand, they also reported 2.8-fold higher Fe, 2.41-fold higher Zn and 1.81-fold higher Cu in the *Caulerpa urvilliana* collected from Pukapuka Atoll compared to *Caulerpa urvilliana* collected from Suvorov Atoll. A comprehensive trace metal analysis of marine macroalgae from Aegean sea was done by Sawidis et al (2000) [1]. They determined trace metal levels such as Cu, Zn, Cd, Pb, Mn and Ni in commonly occuring seaweeds in their regions from three classes of *Chlorophycea*, *Rhodophycea* and *Phaeophycea*. The authors of the paper reported that the investigated seaweeds had different affinities for different metals. And they also underlined that metal levels in seaweeds may be associated with their morphology. Sanchiz et al (2001) investigated the heavy metal bio accumulation in the Mediterranean soft-bottom macrophytes [19]. They found that Pb in *Caulerpa prolifera* is correlated with the Pb content of sediment. They also found Hg level in the fronds of *Caulerpa prolifera* since the sediment contained Hg levels. In an another study of Sanchiz et al (1999) they compared Hg, Cd, Pb and Zn levels in between *Caulerpa prolifera* and four marine phanerogams. Equal distribution of metal in rhizoids, stolons and fronds was reported in *Caulerpa prolifera* [2]. *Caulerpa taxifolia*, known as “killer algae”, accidentaly introduced western-Mediterranean via an aquarium in 1984 and exhibited a sigmoidal growth around French riviera and other six Mediterranean countries [20-21]. The heavy metal contents in this killer algae was comprehensively studied by Gnassia-Barelli et al (1995) [22]. They sampled *C.taxifolia* from four different seasons and separated fronds and stolons. Gnassia-Barelli et al (1995) also sampled their samples from different depths. They found very low Cd levels in both fronds and stolons. Effects of season and depth on the all metal concentration were shown in fronds of *C.taxifolia* in the study of Gnassia-Barelli et al (1995). They also concluded that although the heavy metal concentrations are changed dependent on season and depth in *Caulerpa taxifolia*, concentration of heavy metals are low in *Caulerpa taxifolia* when the levels are compared with other the Mediterranean algae.

In conclusion, the present study shows the first data on the metal content variations in invasive *Caulerpa racemosa* var. *cylindracea* dependent on season and also provides a hypothesis on the observation of abnormal frond morphology observed especially winter season. Because of importance of the field-observation on these invasive species, any chemical attempts which are associated with biological observation will provide a big contribution to complete the puzzle of *Caulerpa racemosa* var. *cylindracea* phenomena in the Mediterranean Sea. Therefore, further researches on the metal levels and morphological view of *Caulerpa racemosa* var. *cylindracea* are strongly recommended to scientists who are interested in *Caulerpa racemosa* var. *cylindracea* invasion.

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