Environ Sci Pollut Res (2016) 23:21536–21553
DOI 10.1007/s11356-016-7298-5

RESEARCH ARTICLE

Styela plicata: a new promising bioindicator of heavy metal pollution for eastern Aegean Sea coastal waters

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Received: 7 March 2016 / Accepted: 20 July 2016 / Published online: 11 August 2016
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Abstract As part of a research project, the concentrations of Cd, Cu, Pb, V, and Zn in the tissues of *Styela plicata* were investigated for the first time to determine if *S. plicata* is a suitable biological indicator for biomonitoring of heavy metals in eastern Aegean Sea coastal waters. To examine the relationships, heavy metal levels in suspended particulate matters (SPMs) and sediments were also determined. According to the results, the mean metal levels in SPM, sediments, and *S. plicata* samples could be arranged in the following order of abundance: Zn > Cu > Pb > V > Cd. As for heavy metal levels, significant positive correlations were noted between Cd-Pb, Cd-V, Cd-Zn, Cu-V, and Pb-V in SPM; Cd-Zn, Cu-Zn, Pb-Cd, Pb-Cu, and Pb-Zn in sediment; and Cu-Pb, Cu-Zn, and Pb-Zn in *S. plicata* samples. Positive relationships between these metals showed that they were originated from same sources and that they were associated with each other. Based on the findings, Zn, Cu, and Pb concentrations in suspended particulate matters, sediments, and *S. plicata* samples were generally represented with higher levels at stations that were used for boating, shipping, and related activities. As *S. plicata* is a strongest accumulator of V, the relatively low V levels observed in this study may indicate the lack of anthropogenic sources of this metal in the sampling stations. In conclusion, suspended particulate matter and sediment can be useful tool to detect the pollution status of the marine environment. Furthermore, the findings of this study highlighted that *S. plicata* is a promising alternative for the monitoring of heavy metal pollution for eastern Aegean Sea coasts.

Keywords Heavy metals · *Styela plicata* · Suspended particulate matter · Sediment · Bioindicator · Aegean Sea

Responsible editor: Philippe Garrigues

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Introduction

From an environmental point of view, heavy metal pollution is a global phenomenon because of their deleterious effects on biota and also on human health. Heavy metals enter aquatic food webs in several ways (benthic or planktonic trophic levels), and they potentially bioaccumulate and biomagnify through all levels of the aquatic food chains (Wang 2002). Because of its impact on living organisms, pollution is essentially a biological problem (Wright et al. 1994), so analysis of total heavy metal content in water and sediment is not enough to predict the toxicity of contaminants to biota (Rainbow 2006; Wang et al. 2010). In this sense, Arienzo et al. (2014) reported that sediment and especially biota are the most suitable variables because they integrate the pollution of a specific water body in time and partly in space.

Environmental factors such as pH, dissolved oxygen, electrical conductivity, type of metal species, organic ligands, the oxidation state of mineral components, the redox environment of the aquatic system, and the available surface area for adsorption caused by the variation in grain size distribution may affect the accumulation of heavy metals from the overlying water to the sediment (Lalah et al. 2008; Koffi et al. 2014).

In addition, metals cannot always be fixed by sediments permanently. Acidification, redox potential conditions, the organic ligand levels, and impose adverse effects on living organisms may remobilize some of the sediment-bound metals

and then heavy metals be released back to the waters (Liu et al. 2009).

Studies on bioaccumulation of heavy metals in different marine organisms are essential. Because using a suite of different biomonitors can give information about a more reliable breakdown of relative metal bioavailabilities in different sources (e.g., solution, suspended material, sediment; Rainbow 2006).

Ascidians are benthic filter feeders that are particularly abundant in marine environments; occur in a variety of habitats, microhabitats; and include both solitary and colonial species (Monniot et al. 1985; Lambert and Lambert 1998). They are most abundant in polluted environments such as ports, harbors, and industrial areas, where the excess of particulate organic matter is high (Monniot et al. 1991). As filter feeders, because of their suspension (Au et al. 2001) or filter-feeding habitat (Moriarty 1983), they process large volumes of water selectively and accumulate certain toxicants such as heavy metals or hydrocarbons in their tissue (Monniot et al. 1993, 1994).

Ascidians feed on suspended particulate matter including phytoplankton and large bacteria, and their diet depends in part, on the mucus net structure (Petersen 2007). All ascidians accumulate heavy metals from water in dissolved and particulate forms (de Moreno et al. 1997), though to varying levels, but substantial levels for some elements (Fe, Co, Zn, Se, and V) have been reported in some species of the suborder *Stolidobranchia*. For this reason, they are considered as good indicators of water quality (Papadopoulou and Kanias 1977; Monniot et al. 1994; Cheney et al. 1997; Gallo and Tosti 2015).

Our study organism, *Splicata plicata* (Lesueur 1823), is a solitary hermaphroditic species commonly found in ports and marinas around the world (Maltagliati et al. 2015). It is an Atlanto-Mediterranean species and also a solitary ascidian found in shallow, protected environments in tropical and warm-temperate oceans, brackish and polluted waters, frequently being found in estuarine environments (Kott 1952, 1972; Kott and Goodbody 1980). Although *S. plicata* has been historically classified as a cosmopolitan species, in the past few decades, it has been considered as an introduced or invasive species in some regions of the world (de Barros et al. 2009; David et al. 2010).

The distribution of *S. plicata* in ports all over the world is favored by its adaptive potential in terms of its tolerance to wide variations in temperature and salinity (Sims 1984; Thiyagarajan and Qian 2003) and ability to cope with high levels of pollution (Naranjo et al. 1996; Pineda et al. 2012). The ascidian *S. plicata* is ubiquitous, sessile benthic habit, and easy to collect, so it fulfill the criteria as a good bioindicator (Rainbow 2006). It can be considered as an indicator species in areas that have experienced intense stress (substrate transformation, water stagnation, and excessive sedimentation) for extended periods of time (Naranjo et al. 1996; de Barros et al. 2009).

It is well known that ascidians are useful species because many substances such as antitumorigen and against Alzheimer's disease and also cytotoxic compounds of therapeutic interest such as pyridoacridine alkaloids (*Cystodytes dellechiajei*), aplidin (*Aplidium* sp.) extract from ascidians (Saad et al. 2011), and bioactive compounds possessing anticancer activity extract from *Ciona intestinalis* (Russo et al. 2008). Physiological variables of ascidians have allowed them to be a peculiar model utilized all over the world (cell cycle, huge amount of gametes produced, synchrony of maturation, peculiar metamorphosis, the availability of the genome for some of them, etc.). So, they have been largely used as models for developmental biology (Satoh 1994). Furthermore, tunicates, commonly known as sea squirts, have been investigated for use in biomonitoring studies and have been shown to accumulate especially Cd and V (Jiang et al. 2009; Treberg et al. 2012) and also a range of metals such as Cu and Mn (Michibata et al. 1986), Ni (Rayner-Canham et al. 1985), Pb and Zn (Philp et al. 2003; Denton et al. 2006). Moreover, these tunicates have been used to compare availability of metals such as Cu and Pb in contaminated and natural harbors (de Caralt et al. 2002).

Ascidians are filter feeder organisms and they have the ability to accumulate and concentrate harmful toxicants present in low concentrations in the surrounding water column, and they trapped these substances on the pharyngeal mucus which is produced by the organ homologous to the mammalian thyroid (Villa et al. 2003; Gallo and Tosti 2015).

Ascidians accumulates V in their tissues to a higher degree than other aquatic invertebrates (Chasteen 1983), in some species of tunicates at concentrations in excess of a million times that of seawater (Dingley et al. 1981). It accumulates in the blood cells of ascidians in association with vanadocytes, where it is an essential element (Chasteen 1983). Presence of vanadocytes and the absence of kidneys cause them to accumulate metals (Abdul Jaffar Ali et al. 2015).

Hazardous accumulation of heavy metals in the different tissues of the marine fouling ascidian has profound effects. Heavy metal accumulation in *C. intestinalis* alters the molecular characterization proteins and exerts harmful effects (Saad et al. 2011). The heavy metals can have effects on the hemocytes of tunicates (Saad et al. 2011). Sublethal doses of Cu decrease cell proliferation and viability in *S. plicata* and reduce the cytotoxic activity of hemocytes (Raftos and Hutchinson 1997; Radford et al. 2000). Cu, another biocidal metal often used as an antifoulant, has also been shown to suppress tunicate pharyngeal cell proliferation and to reduce the cytotoxic activity of tunicate hemocytes (blood cell equivalents; Raftos and Hutchinson 1997).

Preliminary works on accumulation of heavy metals in ascidians have been studied by Carlisle (1968), Krishnan

(1992), Michibata et al. (1986), de Moreno et al. (1997), Kim et al. (2001), Denton et al. (2006), Odate and Pawlik (2007), Webb (2009), Lebar et al. (2011), Noël et al. (2011), Saad et al. (2011), Choi et al. (2014), Radhalakshmi et al. (2014), Abdul Jaffar Ali et al. (2015), etc. In contrast, little work that has focused on ascidian species in Turkey such as existence, structure, and localization of membraneous and myelinated bodies which appear within the cerebral ganglion and neural gland, which are the components of the neural complex of *C. intestinalis* (Tunicata, Ascidiacea) (Öber 1999), dorsal tubercule structures in some Ascidiacea (Tunicata) species live in Izmir Bay (Dinçaslan and Öber 2004), morphological investigation of the pharynx structure of *Ascidiella aspersa* (Müller 1776; Dinçaslan et al. 2007), neural complex variations in ascidians (Dinçaslan and Öber 2010), ascidian species in Turkey Seas (Kocak et al. 1999; Kocak and Kucuksezgin 2000; Dinçaslan and Öber 2005; Aslan 2006; Çınar et al. 2006a, b; Okuş et al. 2007; Aslan-Cihangir et al. 2011; Çınar et al. 2011; Çınar 2014), and virtually nothing, is known about the utility of *S. plicata* as a biomonitor for eastern Aegean Sea coastal waters, despite its abundance.

The primary goal of this study was to determine the concentrations of Cd, Cu, Pb, V, and Zn in suspended particulate matters, sediments, and in the tissues of *S. plicata* collected from stations situated in eastern Aegean Sea coasts. Heavy metal concentrations of suspended particulate matters and sediments were compared with tissue concentrations to evaluate the usefulness of *S. plicata* as biomonitors of eastern Aegean Sea coastal waters. A further objective of this study was to examine the relationships

- between heavy metal values in suspended particulate matters and in sediment,
- between heavy metal levels in suspended particulate matters and in biota,
- and also between heavy metal concentrations in sediments and biota samples.

Material and methods

Study area

Samples of suspended particulate materials, sediments, and specimens of the ascidian *S. plicata* were collected twice during August 2015 to November 2015 at seven stations situated along the coastal area of eastern Aegean Sea, Turkey. The sampling stations were selected on the basis of possible sources of heavy metal pollution along the eastern Aegean Sea. Moreover, they were chosen from marinas and from places generally using by recreational or commercial fishing boats. And stations were labeled as S1, S2, S3, S4, S5, S6, and S7 in Fig. 1.

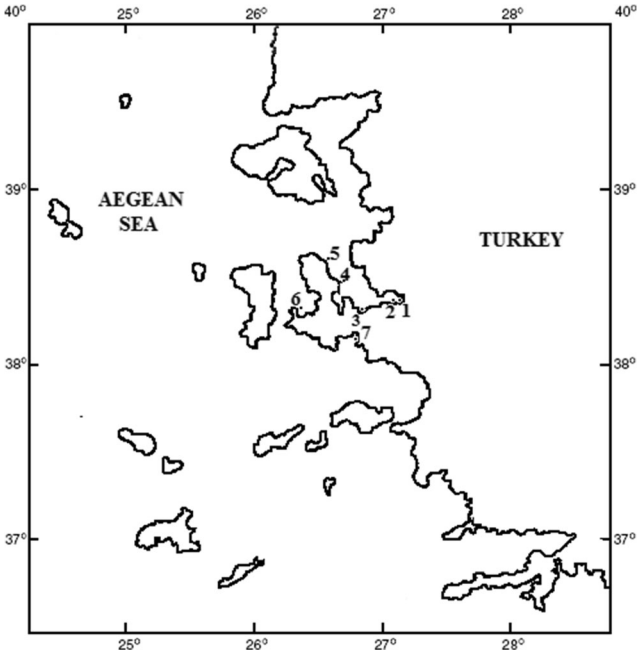


Fig. 1 Map of the study area showing the locations of sampling points. 1 Pasaport, 2 Levent Marina, 3 Urla, 4 Mordoğan, 5 Karaburun, 6 Çeşme, and 7 Sığacık (Zyadah and Chouikhi 1999)

Sampling took place at seven stations located throughout the study area, two of which placed in marinas. Two stations (stations S1 and S2) are situated in Pasaport and Levent Marina located in the inner İzmir Bay. İzmir Bay serves as receptor for domestic and industrial wastes such as food processing, leather tanning, paint, chemicals, textile manufacture, and petroleum refining (Fig. 1). It is therefore imperative to continuously monitor the levels of heavy metal pollution in the Bay. As for station S2, this marina is the site of intense tourism and yachting activities especially during hot seasons. Station 3 is located in Urla. This station receives urban wastewater, and in this station, boating activities increase during tourism period. Station 4 is placed in outer İzmir Bay. The contamination sources in this station are thus mainly derived from the boats, ships, shipping activities, and the sewage disposal points. Station 5 is located in Karaburun. This station can be considered a clean reference site; it is generally used for recreational and small fishing boat launch site and activities. Station 6 is situated in Çeşme (Dalyan). This selected station is located in a marina exposed to pressure from increasing boats, ship-repairing operation tourism activities, and urbanization especially in summer period. Station 7 is located in Sığacık Bay. This station is generally used for recreational and fishing boat activities (Fig. 1).

Sample collection

Surface water samples were collected with sampling bottles (precleaned and rinsed twice with seawater). Whatman GF/C

filters were used to filter seawater (2–3 lt*)* samples for heavy metal analysis in suspended particulate materials. And then, the filters were labeled and frozen at −20 °C until analysis.

Superficial sediments were collected by using Van Veen grab, and to minimize metal contamination from the sampling devices, top sediments were scraped with a Teflon-coated spatula. Then, the samples were placed in polyethylene bags; after that, they were transported to the laboratory and stored at −20 °C until treatment.

Ten to fifteen specimens of the *S. plicata* (Chordata, Ascidiacea), belonging to the subphylum Urochordata or Tunicata, were collected by hand from mooring ropes, wheels, walls, etc., from five (stations S2, S3, S4, S6, and S7) of the seven stations during the study.

Analytical procedure

In the laboratory, sampling and lab treatments were done using non-metal tools. All glassware and plastic bottles used for sample treatment were cleaned prior to use by presoaking in 10 % *v*/*v* HNO_3 for 24 h and rinsed with distilled water. The filters were dried at 40 °C to determine the amount of suspended particulate matter and weighed again. As for heavy metal analysis, suspended particulate matter filters were digested in acid mixture (HCl-$HClO_4$-HNO_3; UNEP 1985a, b, c).

Sediment samples were dried (40 °C) to constant weight and homogenized and reduced to a fine powder by using a sieve (63 μm). For determining total heavy metal levels in sediment, 0.1–0.2 g of finely powdered and dried sample were digested in microwave digestion system with a HCl-$HClO_4$-HNO_3 acid mixture solutions (UNEP 1985a, b, c). Grain size analysis was performed using standard sieving and settling procedures according to Häkanson and Jansson (1983). Sulfochromic oxidation method was used to determine the amount of organic matter (%) in dried sediment spectrophotometrically. The accuracy of this method is ±0.017 % organic matter (Hach 1988). All metal concentrations were expressed on a dry tissue weight basis. Residual water content of samples was determined by oven drying (105 °C for 24 h).

Specimens were examined and only whole, undamaged *S. plicata* individuals were chosen in the laboratory. Furthermore, *S. plicata* specimens were identified by using stereomicroscopes and the most recent literature for the taxon (Van Name 1921, 1945; Vasseur 1967; Millar 1970). Taxonomic identification was carried out by Dr. Noa Shenkar, in Tel Aviv University, Department of Zoology, George S. Wise Faculty of Life Science, Israel.

The biomass was recorded and the whole body of the ascidians was dissected by using a stainless steel scalpel and scissors, and then, they were weighted again. The samples were then oven dried at 35 °C, and finely powdered tissues (0.5–1 g) were accurately weighed and digested with HCl-HNO_3, 5:1 by using Milestone microwave system, and then, the obtained solution was taken to a final volume of 25 ml with distilled water (UNEP 1982, 1984).

All chemicals used in sample treatments were of ultrapure grade (HCl, $HClO_4$, and HNO_3 Merck Suprapur). All solutions were prepared by using ultrapure water (Milli-Q System, Millipore). The stock standard solutions of ultrapure grade supplied by Merck were used to prepare the standard solutions of heavy metals.

Determination of the heavy metals (Cd, Cu, Pb, V, and Zn) in all studied samples was carried out by using inductively coupled plasma–mass spectrometer (ICP-MS; Agilent Technologies 7500ce Series, USA). The instrumental conditions are presented in Table 1.

The accuracy of analytical procedure was checked by analyzing the standard reference materials (suspended material and sediment IAEA 433, International Atomic Agency Analytical Quality; biota 2976 NIST, National Institute of Standards and Technology).

Statistical analysis

The data analysis was performed using the STATISTICA v.7.1 (STATSOFT) software package, and statistical significance was defined at the $p < 0.05$ level. Normality and homogeneity of variance tests were carried out by using Cochran-C and Levene tests, and transformation of data was conducted when necessary. If data were found to be non-normally distributed, non-parametric tests were preferred for the comparisons of changes in metal concentrations. One-way analysis of variance (ANOVA; parametric data) and the Kruskal-Wallis test (non-parametric data) were applied to find any significant differences in metal concentrations among different stations and seasons. The Tukey (parametric data) and the Mann-Whitney *U* tests (non-parametric data) were used to discriminate significant differences.

To verify existing relationships between suspended particulate matter and sediment metal levels, between suspended particulate matter metal contents and *S. plicata* metal values, sediment metal levels and biota metal contents, and between heavy metal contents in *S. plicata* tissues, Spearman's rank order correlation test was applied.

Results

In the present study, the concentrations of heavy metals (Cd, Cu, Pb, V, and Zn) in suspended particulate matters, in sediments, and in *S. plicata* collected from seven stations positioned along the eastern coast of Aegean Sea during between August 2015 and November 2015 are given in Tables 1, 2, and 3, respectively.

Table 1 The instrumental conditions of ICP–MS

Instrument	Agilent 7500ce
Nebulizer	Micromist
Spray chamber	Quartz
Plasma	
RF generator	Frequency 10 MHz, power output 1500 W
Air flow rate (l/min)	Plasma 15, auxiliary 0.9, nebulizer 1–1.1
Solution uptake rate	1.8 ml/min
Interface	
Sampler cone	Nickel, i.d. 1.1 mm
Skimmer	Nickel, i.d. 0.9 mm
Vacuum	İnterface 4 Torr, quadrupole 2×10^{-5} Torr
Data acquisition	Peak hopping, replicate time 200 ms, dwell time 200 ms, sweeps/reading 3, readings/replicate 3, number of replicates 3

Heavy metals in suspended particulate matter

The mean heavy metal concentrations in suspended particulate matter decreased in the following order: Zn > Cu > Pb > V > Cd. In seawater, the lowest Cd concentration in suspended particulate matter (0.017 μg/l) was measured at stations S3 and S7 in summer, while the highest value was noted at station S3 (0.118 μg/l) in autumn (Table 2). The ANOVA test showed that there were statistically significant variations between seasons for Cd values in suspended particulate matter ($r = 0.0000$, $p < 0.001$); however, no statistically significant differences were found between stations.

In terms of Cu in suspended particulate matter, the minimum value (0.69 μg/l) was noticed at station S5 in summer and the maximum level was (14.03 μg/l) determined at station S4 in autumn period (Table 2). On the other hand, no remarkable spatiotemporal changes were exhibited for Cu values in suspended particulate matter ($p > 0.05$).

Table 2 Heavy metal concentrations in suspended particulate matter samples (μg/l) collected from eastern Aegean Sea coasts

Stations	Season	Cd (μg/l)	Cu (μg/l)	Pb (μg/l)	V (μg/l)	Zn (μg/l)
S1	Summer	0.033	1.22	0.27	0.183	2522
S2	Summer	0.033	1.12	0.13	0.150	2565
S3	Summer	0.017	1.36	1.81	0.175	1258
S4	Summer	0.020	2.30	0.60	0.390	1391
S5	Summer	0.020	0.69	0.53	0.090	1609
S6	Summer	0.050	3.40	0.17	0.325	4025
S7	Summer	0.017	2.44	0.26	0.167	1318
S1	Autumn	0.100	1.62	1.88	0.433	2655
S2	Autumn	0.075	0.99	1.04	0.288	2005
S3	Autumn	0.118	3.90	2.22	0.397	2747
S4	Autumn	0.088	14.03	1.73	0.338	2343
S5	Autumn	0.088	1.14	1.13	0.238	2278
S6	Autumn	0.088	3.33	4.99	0.338	2308
S7	Autumn	0.088	1.90	1.41	0.325	2304

The peak value of Pb in suspended particulate matter (4.99 μg/l) was recorded in autumn at station S6 corresponding to the area selected from marina. And, the minimum content (0.13 μg/l) was observed at station S2 in summer (Table 2). There were no clear spatial patterns that were detected for Pb level in suspended particulate matter; furthermore, a significant temporal variation was observed between sampling seasons ($r = 0.0188$, $p < 0.05$).

Recorded V levels in suspended particulate matter changed between 0.090 and 0.433 μg/l (Table 2). And, the lowest level was noted at station S5 in summer, whereas the highest content was measured at station S1 in autumn. It can be noticed that V levels in suspended particulate matter exhibited meaningful differences for sampling periods ($r = 0.0208$, $p < 0.05$). On the other hand, determined values did not show significant changes between stations.

Concerning Zn levels in suspended particulate matter, the highest level (4025 μg/l) was detected at station S6 in summer, and it is approximately four times greater than the lowest concentration (1258 μg/l) measured at station S3 in the same period (Table 2). Concerning sampling stations and seasons, samples did not exhibit, in general, statistically significant variations in the measured Zn levels.

With regard to suspended particulate matter (SPM), significant positive moderate relations were noted between Cd and Pb ($r = 0.6155$, $p < 0.05$), Cd and V ($r = 0.7118$, $p < 0.05$), Cd and Zn ($r = 0.6601$, $p < 0.05$), Cu and V ($r = 0.6344$, $p < 0.05$), and Pb and V contents ($r = 0.6344$, $p < 0.05$).

Heavy metals in sediment

The mean heavy metal levels in sediment samples could be arranged in the following sequence: Zn > Cu > Pb > V > Cd.

Table 3 Heavy metal values in sediment samples [μg/g dry weight (d.w.)] collected from eastern Aegean Sea coasts

Stations	Seasons	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	V (μg/g)	Zn (μg/g)	Gravel (%)	Sand (%)	Silt + clay (%)	Silt (%)	Clay (%)	Organic matter (%)
S1	Summer	1.14	290.1	358.2	73.8	1134.8	0.0	28.4	71.6			8.25
S2	Summer	0.47	399.4	88.0	88.0	745.5	5.6	56.5	38.0	31.2	6.8	6.81
S3	Summer	0.48	253.7	69.2	42.4	339.6	3.3	77.3	19.5	16.7	2.8	5.70
S4	Summer	0.25	697.6	96.1	112.0	623.9	87.7	1.2	11.1			5.86
S5	Summer	0.21	98.6	49.4	78.2	182.0	48.5	42.7	8.8	7.2	1.6	2.61
S6	Summer	0.31	164.0	35.0	31.3	136.0	70.3	27.5	2.3			4.41
S7	Summer	0.49	1872.2	149.7	44.6	1768.6	1.4	29.3	69.3	55.4	13.8	3.14
S1	Autumn	1.29	222.5	285.2	47.1	890.9	0.0	58.7	41.3			7.98
S2	Autumn	0.36	195.7	60.2	42.6	320.7	10.4	72.5	17.1			5.24
S3	Autumn	0.37	113.4	42.2	18.6	163.4	1.0	82.0	17.0			3.59
S4	Autumn	0.22	90.7	27.2	26.6	109.1	73.8	15.6	10.6			3.35
S5	Autumn	0.36	136.5	103.5	41.5	161.7	44.2	49.9	5.8			3.93
S6	Autumn	0.34	1081.7	62.3	24.6	889.3	28.6	66.7	4.8			4.41
S7	Autumn	1.32	214.9	50.8	64.5	399.4	4.6	31.3	64.1	44.3	19.0	5.86

As for Cd contents in sediment samples, detected value (0.21 μg/g) at station S5 in summer was slightly lower than the recorded concentration (1.32 μg/g) at station S7 in autumn (Table 3).

Stations located at southern part of the study area represented with higher Cu concentrations in sediment compared to other sampling stations. The peak levels 1872.2 and 1081.7 μg/g were recorded at station S7 in summer and S6 in autumn, respectively. Moreover, the minimum concentration (90.7 μg/g) was found at station S4 in autumn sampling (Table 3).

Comparison between sampling seasons showed that the maximum Pb value in sediment (358.2 μg/g) measured at station S1 in summer period was extremely higher than the minimum level (27.2 μg/g) observed at station S4 in autumn (Table 3).

The concentrations of V in sediment showed fluctuations with the lowest to the highest measured values (18.6–112.0 μg/g) at station S3 in autumn and station S4 and summer periods, respectively (Table 3).

Zn concentrations in sediment samples showed a wide range of variations. The maximum values were recorded at station S7 (1768.6 μg/g) and station S1 (1134.8 μg/g) in summer sampling, while the minimum concentration (109.1 μg/g) was determined at station S4 in autumn (Table 3). The higher Zn levels in sediment were generally recorded at summer period, except noted Zn level at station S6. The statistical analysis highlighted that no significant spatial and temporal differences were detected for all studied heavy metal levels in sediment samples collected from seven stations.

According to the results, Cd in sediment directly related with Zn in sediment ($r = 0.6132$, $p < 0.05$). Cu in sediment showed strong correlation with Zn in sediment ($r = 0.8593$, $p < 0.01$). In terms of Pb in sediment, there were positive relations with Cd in sediment ($r = 0.5429$, $p < 0.05$), with Cu in sediment ($r = 0.6571$, $p < 0.05$), and also with Zn in sediment ($r = 0.7802$, $p < 0.01$).

The textural distribution of sediments showed that stations S1 and S7 were characterized by higher percentage of silt + clay (41.3–71.6 and 64.1–69.3 %) and sand (28.4–58.7 and 29.3–31.3 %), respectively. Moreover, stations S2 and S3 were primarily composed of sand (56.5–72.5 and 77.3–82.0 %) and silt + clay (17.1–38.0 and 17.0–19.5 %), respectively. In addition, station S4 was covered by gravel (73.8–87.7 %) with moderate percentages of silty clay (fine fraction; 10.6–11.1 %). Station S5 was characterized by gravel (44.2–48.5 %) and sand (42.7–49.9 %), and also, station S6 was predominantly composed of gravel (28.6–70.3 %) and sand (27.5–66.7 %; Table 3).

With regard to organic matter (OM; %) values in sediment, measured levels at seven sampling stations are quite similar. Furthermore, the minimum OM (%) level was recorded (2.61 %) at station S5 in summer and the maximum value was found at station S1 (8.25 %) in the same period (Table 3). The results showed that the pattern of organic matter (%) levels in sediment samples could be arranged in the following sequence: S1 > S2 > S3 > S4 > S7 > S6 > S5. In addition, the stations located in the inner parts of Izmir Bay presented with relatively higher percentages of OM (%) levels compared to other stations. However, no statistically significant spatio-temporal variations were existed in sedimentary organic matter (%) contents.

Heavy metals in *S. plicata*

According to the findings, *S. plicata* can accumulate the studied metals in the following order of abundance: Zn > Cu > Pb > V > Cd. Comparison of measured Cd levels in *S. plicata* specimens showed that observed values in autumn are a bit higher than the levels recorded in summer period. Cd in *S. plicata* reached its highest level (0.158 μg/g) at station S7 in autumn (Table 4). In contrast, Cd in *S. plicata* was not detected at stations S2 and S6 in summer.

Obtained Cu levels in *S. plicata* showed an obvious increase in the selected stations from northern to southern part of the study area. Higher Cu values were detected both in summer period (338.7 and 276.6 μg/g) at stations S6 and S7, respectively. Furthermore, the lowest Cu content in biota samples (26.6 μg/g) was observed at station S2 in autumn (Table 4).

Pb level in *S. plicata* (21.2 μg/g) was peaked in autumn at station S3, while its minimum value (1.6 μg/g) was obtained at station S2 in the same sampling period (Table 4). Elevated level of V (19.0 μg/g) in *S. plicata* was detected at station S6 in summer, and the lowest V content (1.6 μg/g) in this sample was found at station S2 in autumn (Table 4).

Zn concentration in *S. plicata* showed fluctuations from a minimum level of 35.5 μg/g at station S4 noticed in summer and the maximum content of 361.5 μg/g at station S6 detected in the same period (Table 4). Regardless of all studied metals, only Cu content in the tissues of *S. plicata* exhibited statistically significant variations ($r = 0.3908$, $p < 0.05$) between seasons.

The highest coefficients were found between Cu in *S. plicata* and Pb in *S. plicata* ($r = 0.6849$, $p < 0.01$) and Cu in *S. plicata* and Zn in *S. plicata* ($r = 0.7576$, $p < 0.01$). Moreover, significant positive correlation was established between Zn in *S. plicata* and Pb in *S. plicata* ($r = 0.7939$, $p < 0.01$).

Discussion

In coastal ecosystem, use of biomonitors for heavy metal analysis to understand the environmental health of an area can lead to an early warning system. Moreover, SPM and sediments are the main sources of heavy metals in the marine ecosystem and play an important role in the transport and storage of potentially hazardous metals (Cuong et al. 2008).

Concentrations of heavy metals in SPM at the seven stations are expressed in terms of water volume (μg/l), and recorded levels ranged between 0.017 and 0.118 μg/l for Cd, 0.69 and 14.03 μg/l for Cu, 0.13 and 4.99 μg/l for Pb, 0.090 and 0.433 μg/l for V, and 1258 and 4025 μg/l for Zn (Table 2).

In aquatic systems, partitioning of heavy metals within SPM is an important factor influencing heavy metal adsorption, biogeochemistry, bioavailability, fate, toxicity, and transport (Lu and Allen 2001; Saeedi et al. 2004).

According to the results of ANOVA, performance with Cd data showed that recorded levels in SPM exhibited temporal variations ($p < 0.001$). However, no significant spatial differences were found between stations. Regarding seven sampling stations, recorded Zn, Cu, and Pb values in suspended particulate matter were elevated among the studied heavy metals and station S6 represented with higher heavy metal levels in SPM. These activities explained the source of Cu and Zn in SPM (Chouba and Mzoughi 2013). Moreover, measured heavy metal concentrations in autumn were generally higher compared to other sampling periods, and Pb contents in SPM showed seasonal changes ($p < 0.05$). These can be due to an increase in boating and related activities and also antifouling paints. According to Turner (2010), the fine particles generated by sanding or blasting of boat hulls or that is produced gradually by antifouling paints can be probably the source of high SPM values. With respect to V level in SPM, no overall significant stational variations were observed; however, statistically significant changes between seasons were detected ($p < 0.05$). Regarding SPM, variations in concentration and characteristics of particles along with possible physicochemical

Table 4 Heavy metal levels in *Styela plicata* samples [μg/g dry weight (d.w.)] collected from eastern Aegean Sea coasts

Stations	Seasons	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	V (μg/g)	Zn (μg/g)
S2	Summer	0.000	67.9	4.0	4.3	103.8
S3	Summer	0.088	32.8	4.2	3.6	279.1
S4	Summer	0.098	35.2	9.1	12.0	35.5
S6	Summer	0.000	338.7	18.1	19.0	361.5
S7	Summer	0.090	276.6	8.0	10.5	135.9
S2	Autumn	0.053	26.6	1.6	1.6	79.9
S3	Autumn	0.146	130.7	21.2	9.2	165.3
S4	Autumn	0.150	48.6	6.6	8.3	142.9
S6	Autumn	0.105	197.2	13.1	11.7	146.1
S7	Autumn	0.158	239.5	17.3	12.4	165.1

modifications play an important role in estuarine biogeochemical cycles (Zwolsman and Van Eck 1999; Turner and Millward 2002).

The general increase in mean concentrations of heavy metals in the water during autumn could be attributed to more bioaccumulation due to the metal concentration arising from increasing rains during this season. Based on the annual rainfall data for the study area, it can be seen that the mean monthly precipitation was peaked in autumn, while the lowest mean monthly precipitation was measured in summer period (https://weather-and-climate.com/average-monthly-Rainfall-Temperature Sunshine; Izmir, Turkey).

As for heavy metal levels in SPM, significant positive correlations were noted between Cd and Pb ($r = 0.6155$, $p < 0.05$), Cd and V ($r = 0.7118$, $p < 0.05$), Cd and Zn ($r = 0.6601$, $p < 0.05$), Cu and V ($r = 0.6344$, $p < 0.05$), and Pb and V contents ($r = 0.6344$, $p < 0.05$). The findings indicated that heavy metal correlations in SPM suggest that these metals originate from the same sources.

Little work has been done on the accumulation of heavy metal levels in SPM in Turkey coasts. Türkmen and Türkmen (2004) studied the heavy metal levels in SPM. They collected SPM samples monthly from the Arsuz (ARZ), Iskenderun Harbor Area (IHA), İsdemir (İSD), Dörtyol Botaş (DBT), and Petrotrans (PTS) stations. They reported that the distribution of the heavy metal levels in SPM ranged as follows: Cd 5.88–66.7, Cu 110–725, Pb 65.0–783, and Zn 466–1547 mg/kg dry weight, respectively. Comparing our results showed that our recorded heavy metal values in SPM were lower than the measured levels in SPM by Türkmen and Türkmen (2004) (Table 5).

Demirak et al. (2012) investigated the Cd, Cu, Fe, Pb, and Zn values in SPM, and sediments taken from nine stations were selected in the inner Gökova Bay, Kadın Creek, and Akçapınar Creek. The results showed that recorded levels in this study were lower than the measured values in SPM by Demirak et al. (2012) (Table 5).

Kontas (2012) investigated the concentrations of heavy metals (Cu, Fe, Mn, Ni, and Zn) in SPM and biota samples selected from stations in Izmir Bay (eastern Aegean Sea) in order to evaluate the environmental impact of the anthropogenic metals before building of wastewater treatment plant. Comparing the results showed that our findings were higher than the reported Cu (0.36–2.19 μg dm^{-3}) and Zn levels in SPM (7.33–269 μg dm^{-3}) by Kontas (2012) (Table 5).

As for SPM levels, Cu (0.27 μg dm^{-3}) and Zn levels (0.18 μg dm^{-3}) recorded at stations situated in Gulf of Gera by Doukakis et al. (1993) and also Cu (0.20 μg dm^{-3}) and Zn concentrations (1.18 μg dm^{-3}) detected in Gulf of Riga by Poikane et al. (2005) were lower than the given values in this study (Table 5).

A comparison of our findings showed that our measured Cd levels in SPM were lower than the recorded values for Cd (0.25–0.60 μg dm^{-3}) and higher than the noted levels for Zn (1.70–2.44 μg dm^{-3}) at stations selected from Saranikos Gulf by Scoullos et al. (1994) (Table 5).

Chouba and Mzoughi (2013) investigated the heavy metal levels in SPM collected from La Goulette, Rades, and Sidi Bou Said harbors in the Gulf of Tunis. The results exhibited that measured Cu (0.43–11.72 μg/g), Pb (3.09–27.49 μg/g), and Zn (101–149 μg/g) values were lower than that reported levels in the present study (Table 5).

Sediments often act as both carriers and potential sources for heavy metals in the aquatic environment (Eggleton and Thomas 2004). The fate (speciation) of the metal released from sediments is affected by the overlying water condition, in particular, the pH, salinity, dissolved oxygen concentration, and amount of suspended solids (Simpson and Batley 2003).

In this study, heavy metal concentrations in sediment samples were varied from 0.21 to 1.32 μg/g for Cd, 90.7 to 1872.2 μg/g for Cu, 27.2 to 358.2 μg/g for Pb, 18.6 to 112.0 μg/g for V, and 109.1 to 1768.6 μg/g for Zn (Table 3).

With regard to ANOVA findings, no spatial and temporal differences were noted for all studied heavy metal levels in sediment samples. The difference in the distribution of heavy

Table 5 Literature comparisons of metal concentrations (μg/g d.w.) in suspended particulate materials from different geographical areas in the world

Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	V (μg/g)	Zn (μg/g)	Sampling Areas	References
5.88–66.7	110–725	65.0–783		466–1547	İskenderun, Turkey	Türkmen and Türkmen 2004
0.048–6.466	0.867–5.800	0.017–40.786		30–749	Akçapınar Creek, Turkey	Demirak et al. 2012
0.038–7.813	1.061–7.850	0.363–88.50		184–987	Kadın Creek, Turkey	Demirak et al. 2012
0.078–0.579	0.427–2.876	0.276–36.784		78–408	Gökova Inner Bay, Turkey	Demirak et al. 2012
	0.36–2.19			7.33–269	İzmir Bay, Turkey	Kontas, 2012 (μg dm^{-3})
	0.27			0.18	Gulf of Gera, Greece	Doukakis et al. 1993 (μg dm^{-3})
	0.20			1.18	Gulf of Riga, Baltic Sea	Poikane et al. 2005 (μg dm^{-3})
0.25–0.60				1.70–2.44	Saranikos Gulf, Greece	Scoullos et al. 1994 (μg dm^{-3})
	0.43–11.72	3.09–27.49		101–149	Gulf of Tunis, Tunisia	Chouba and Mzoughi 2013

metals among the seven locations can be attributed to the differences in inflow of effluents from anthropogenic wastes, increasing tourism, fishing and boat activities, and increase in the use of antifouling paints in summer which is the probable source of heavy metals especially Cd and Zn.

Generally, the highest concentrations of all studied metals in sediment samples were analyzed in summer period. Higher Cd values in sediment samples were observed at stations S1 and S2 in summer and at station S7 in autumn. The higher concentrations of Cd levels in these stations were mainly due to the relative increase in boat activities and their maintenance which includes painting and cleaning.

Cd in sediment samples was high at stations (stations S1 and S7) represented with higher silt + clay (%) levels (Table 3). This can be due to the tendency of Cd to increase with decrease in size and increase in density in terms of partition of sediment samples by size and density (Fergusson 1990).

Especially recorded levels at stations S7, S1, S2, and S4 in summer and stations S6 and S1 in autumn period were represented with higher Cu and Zn levels in sediment. Yacht (pleasure) boating activities and boat maintenance can be source of metallic particle contamination. Cu- and Zn-based antifouling paints have been previously identified as important sources of these metals in these stations. Cu levels in heavily polluted sediments have been reported to exceed 200 μg/g (Denton et al. 1997). Observed Cu values in sediment were generally exceeded the given value by Denton et al. (1997). Moreover, they reported the 15–50-μg/g range for Pb levels in sediment in coastal and estuarine sediments around the world. Comparing our results with this range showed that our noted values at studied stations especially at stations S1 were generally higher than the given concentrations.

Grain size in particular fine fractions plays an important role in controlling the heavy metal levels in sediments because it has a large surface area to ratio and contains large amounts of interstitial water (Horowitz 1991). Sediments composed of fine particles bind heavy metals and pollutants due to their high specific surface areas (Keil et al. 1994). According to the results of this study, gravel (%) levels exhibited a negative relation with silt + clay (%) values ($r = -0.7335$, $p < 0.05$).

In this study, it is observed that investigated sediments differ in their grain size characteristic. Stations S1 and S7 have showed higher Cd, Cu, Pb, and Zn concentrations in sediment samples as well as high silt + clay (%) values (Table 3); in contrast, no significant relationship was detected between Cd, Cu, Pb, and Zn levels in sediment samples and silt + clay (%) contents.

Generally, organic matter content of sediments increase as the sediment texture becomes finer (Denton et al. 2001). The recorded organic matter (%) results of this study were in agreement with the findings given by Denton et al. (2001). Decomposition of organic material produces organic ligands that may extract heavy metals from the sediments. Furthermore, organic materials can affect metal species solubilization by complexing the metal ions; they can also take metal ions out from the solution and contribute to the sediments (Fergusson 1990).

According to the results, it can be seen that higher heavy metal values were generally detected at stations that have higher organic matter (%) contents (Table 3). However, no significant relationships were exhibited between heavy metal levels in sediment samples and organic matter (%) values.

Recorded V contents in sediment were generally higher in summer periods. Furthermore, high Pb levels were examined at station S1 in both periods. This variation could be related to the changes in the pollution load of the studied sites. Stations S4 and S2 showed the highest values of vanadium, which were 112 and 88 μg/g, respectively (Table 3). These stations suffered from pollution through different sources such as many industrial effluents, shipping activities, and discharge of domestic wastewater. The peak value of V in sediment was in the range of the sediment quality guideline value of 20–150 mg/kg (Moore 1991). In general, sediments collected near the sewage outlet, cities, harbors, marinas, and shipping activities seemed to have high concentrations of V (Abdel Ghani et al. 2013).

Significant differences were observed among seasons for both heavy metals in suspended particulate matter and sediments. This reflected the contribution of various heavy metals from different watersheds by runoff during rainy and hot seasons.

Cd in sediment showed a moderate positive relation with Zn in sediment ($r = 0.6132$, $p < 0.05$). Cu in sediment showed a high degree correlation with Zn in sediment ($r = 0.8593$, $p < 0.01$). In terms of Pb in sediment, there were positive relationships with Cd in sediment ($r = 0.5429$, $p < 0.05$), with Cu in sediment ($r = 0.6571$, $p < 0.05$), and also with Zn in sediment ($r = 0.7802$, $p < 0.01$). All the metal pairs in the surface sediments that exhibit positive relations may suggest a common pollution source or a similar geochemical behavior for these metals.

This study shows that our recorded heavy metal levels in sediment samples were higher than those reported levels in sediment samples collected from stations paced in Urla (İzmir), except recorded Cd levels (1.68–1.98 μg/g) noted by Sunlu et al. (1998), the sediments in the eastern Aegean shelf by Batki et al. (1999), and also the sediment samples taken during September 1995 from 100 stations in Izmir Bay by Atgın et al. (2000) (Table 6).

Bergin et al. (2006) analyzed the heavy metal concentrations in sediment samples collected from various stations in the Gulf of Izmir. Results of the investigation showed that Cd levels (0.05–0.138 μg/g), Cu contents (2.6–50 μg/g), Pb values (14–76 μg/g), and Zn levels (20–249 μg/g) were lower than those observed in the present study (Table 6).

Table 6 Literature comparisons of metal concentrations (μg/g d.w.) in sediments from different geographical areas in the world

Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	V (μg/g)	Zn (μg/g)	Sampling areas	References
1.68–1.98	11.75–14.90	28.79–32.21		29.94–33.48	Urla (İzmir,Turkey)	Sunlu et al. 1998
0.20–0.42	14–40	30–50		27–106	The eastern Aegean shelf, Turkey	Batki et al. 1999
0.22 ± 0.13–0.42 ± 0.22	32 ± 12–70 ± 38	36 ± 5–62 ± 29		99 ± 37–260 ± 100	Izmir Bay, Turkey	Atgın et al. 2000
0.05–0.138	2.6–50	14–76		20–249	Gulf of Izmir, Turkey	Bergin et al. 2006
0.005–0.82		14–113		7.33–269	İzmir Bay, Turkey	Kucuksezgin et al. 2006
	66–993	82–203		217–1031	İzmir Bay, Turkey	Güven and Akinci 2008
0.028–0.096	1.18–23.81	1.97–23.74		5.98–102.28	İzmir Bay, Turkey	Erdoğan 2009
0.005–0.042	3.2–18.7	8.1–51.1		17.6–83.6	The eastern Aegean Sea coast, Turkey	Akçali and Kucuksezgin 2011
1.2–2.3	239–343	252–570		557–681	Pasaport, İzmir Bay, Turkey	Aydin Onen et al. 2011
0.19–0.44	219–316	68–100		289–387	Levent Marina, İzmir Bay, Turkey	Aydin Onen et al. 2011
0.009–0.82	2.2–109	3.1–119		14–412	İzmir Bay, Turkey	Kucuksezgin et al. 2011
0.02–0.49	31.6–81.6	81.2–172.8		105.4–265.3	İzmir Bay, Turkey	Özkan 2012
0.03–1.04	2.87–407.93	2.51–79.78	10.82–1215.81		Black Sea	Sur et al. 2012
		bdl–265.92		5.25–928.76	İzmir Bay, Turkey	Kükrer 2013
0.1–2.18	0.49–181	0.6–324		1–552	Guam Harbors,	Denton et al. 2006
			3.76–168		Mediterranean Sea, Egypt	El-Moselhy 2006
			8.5–214.4		Red Sea, Egypt	El-Moselhy 2006
			11.42–69.98		Western Mediterranean Sea, Egypt	Abdel Ghani et al. 2013
			25.04–574.75		Eastern Mediterranean Sea, Egypt	Abdel Ghani et al. 2013
21.3–107.7	54.4–356.6			98.7–664.8	Fusaro Lagoon, Naples Harbor, Italy	Arienzo et al. 2014

Kucuksezgin et al. (2006) studied the heavy metal levels in samples collected from the stations located in the outer, middle, and inner İzmir Bay. Comparison of Cd and Pb levels in the present study revealed that our detected concentrations in this study were higher than the reported Cd (0.005–0.82 μg/g) and Pb (14–113 μg/g) values in sediments by Kucuksezgin et al. (2006) (Table 6).

In this study, the Cu, Pb, and Zn levels in the observed sediment samples, however, were higher than the reported Cu (66–993 μg/g), Pb (82–203 μg/g), and Zn (217–1031 μg/g) values in sediments collected from seven stations located in the inner Bay (Güven and Akinci 2008; Table 6).

Erdoğan (2009) determined the concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn in surface sediments which act as contamination indicators. They collected the samples bimonthly between July 2008 and June 2009 from 13 locations (Karaburun Plaj, Karaburun Iskele, Mordogan Plaj, Mordogan, Karapinar, Gulbahce, Urla Plaj, Sahilevleri, Melez Deltasi, Bostanli, Kus Cenneti, Foca, Yeni Foca) along İzmir Bay. Results of the study showed that our detected values in this study were higher than the measured Cd, Cu, Pb, and Zn values in sediment samples by Erdoğan (2009) (Table 6).

Akçali and Kucuksezgin (2011) analyzed heavy metal concentrations in seawater, sediment, and macroalgal species at eight coastal stations along the eastern Aegean coast. The findings showed that the detected Cd (0.005–0.042 μg/g), Cu (3.2–18.7 μg/g), Pb (8.1–51.1 μg/g), and Zn levels (17.6–83.6 μg/g) in sediment samples collected from Foça, Bostanlı, Narlıdere, and Urla stations were lower than that reported concentrations in our study (Table 6).

Aydin Onen et al. (2011) investigated the heavy metal concentrations in seawater, sediment, and barnacles (*Amphibalanus amphitrite*) taken from different stations situated in eastern Aegean Sea coasts. Comparison to our findings exhibited that recorded Cd (1.2–2.3 μg/g) and Pb values in sediment (252–570 μg/g) were lower, while Cu (239–343 μg/g) and Zn levels in sediment samples (557–681 μg/g) collected from Pasaport station were higher than that reported values at station S1. Moreover, Cu values (219–316 μg/g) were lower; however, Cd (0.19–0.44 μg/g), Pb (68–100 μg/g), and Zn (289–387 μg/g) contents in sediment at Levent Marina station were higher than the reported values at station S2 (Table 6).

Kucuksezgin et al. (2011) collected sediment from the outer, middle, and inner Izmir Bay. And, they reported 0.009–0.82 μg/g for Cd, 2.2–109 μg/g for Cu, 3.1–119 μg/g for Pb, and 14–412 μg/g for Zn in the samples. A comparison of the findings showed that our recorded heavy metal values were higher than that found by Kucuksezgin et al. (2011) (Table 6).

Özkan (2012) collected the sediment samples from the 21 stations, of which 14 samples were collected from the inner Bay and also 7 sediment samples were collected from creeks (Melez, Manda, Bayrakli, Bostanlı, Bornova, and Balcova). A comparison of the findings showed that our recorded Cd, Cu, Pb, and Zn contents were higher than that reported concentrations by Özkan (2012) (Table 6).

Sur et al. (2012) studied the Al, Cd, Cu, Hg, V, and Pb levels in surface sediments collected from 26 stations from the Turkish coasts of the Black Sea. By comparing the present findings with that of Sur et al. (2012), it can be seen that our results were higher than the given results (Cd 0.03–1.04 μg/g, Cu 2.87–407.93 μg/g, Pb 2.51–79.78 μg/g, V 10.82–1215.81 μg/g). And, the measured values are also higher than the detected levels for Pb (bdl–265.92 mg/kg) and Zn (5.25–928.76 mg/kg) in the inner part of Izmir Bay (eastern Aegean Sea) by Kükrer (2013) (Table 6).

Denton et al. (2006) investigated the heavy metal levels in sediment and in flora and fauna samples collected from stations selected from Guam Harbors. They reported Cd, Cu, Pb, and Zn levels in sediment (0.1–2.18 μg/g), (0.49–181 μg/g), (0.6–324 μg/g), and (1–552 μg/g), respectively. Compared to the our results, it can be said that our noted Cd values in sediment were lower, while Cu, Pb, and Zn contents in sediment were higher than the measured concentrations (Table 6).

El-Moselhy (2006) aimed to measure the concentration of vanadium in 73 sediment samples collected along the coastal area of the Egyptian seas. The highest V levels in sediment samples were reported in this study, however, lower than those noted V contents in sediments collected from Mediterranean Sea, Egypt (3.76–168 mg/kg), and Red Sea, Egypt (8.5–214.4 mg/kg), by El-Moselhy (2006) (Table 6).

Moreover, our detected V levels in sediment samples were higher than the given V values for Western Mediterranean Sea, Egypt (11.42–69.98 mg/kg), and considerably lower than the V content (25.04–574.75 mg/kg) found at Eastern Mediterranean Sea, Egypt (Abdel Ghani et al. 2013; Table 6).

Arienzo et al. (2014) investigated the heavy metal levels in sediment and ascidian samples in the Fusaro Lagoon, Naples Harbor, Italy. Findings clearly showed that our study results were lower than the reported Cd level (21.3–107.7 μg/g) and consistently higher than the given results for Cu (54.4–356.6 μg/g) and Zn (98.7–664.8 μg/g; Table 6).

In terms of heavy metals in *S. plicata*, recorded levels ranged between 0.000 and 0.158 μg/g for Cd, 26.6 and 338.7 μg/g for Cu, 1.6 and 21.2 μg/g for Pb, 1.6 and 19.0 μg/g for V, 35.5 and 361.5 μg/g for Zn (Table 4).

The little work that has focused on cadmium in tunicates, including the results of the present study, indicates that levels normally encountered in this group range between 0.1 and 3.0 μg/g (Denton et al. 1999). It is noteworthy that recorded Cd levels in *S. plicata* in this study were higher lower end of this range (0.1 μg/g), except recorded values at stations S2 and S6 in summer period (Table 4).

The marina population collected from station S6 and also specimens collected from station S4, where numbers of boats and commercial ships were high, accumulated significantly more Cu and Zn. On the other hand, *S. plicata* accumulated a similar amount of V, a metal involved in ascidian metabolism (de Caralt et al. 2002). The relatively low levels of V determined in this study pointed the lack of anthropogenic sources of this metal in the studied stations.

Marinas and harbors make a large contribution to the introduction and invasion of ascidians in particular (Lambert and Lambert 1998). In addition, marinas are frequently polluted by heavy metals, hydrocarbons, and other organic pollutants (Commendatore et al. 2000).

Pb and V were present in ascidian tissues in smaller amounts and did not show clear cycles, although minimum values were generally found in summer. This behavior may correspond to the dynamics of the metals in the sampling stations, but more likely, it is the result of the dynamics of the ascidian.

A study carried by Carlisle (1968) at 11 coastal stations distributed around Algeciras Bay indicated that some species such as *C. intestinalis*, *Diplosoma spongiforme*, *Phallusia mammillata*, *Microcosmus squamiger*, *Styela plicata*, and *Synoicum argus* could be considered as indicators of areas, which have been subject to intense stress.

Michibata et al.(1986) studied the vanadium, iron, and manganese contents of test, mantle, branchial basket, stomach, liver, gonads, corpuscles, and plasma of 15 species of solitary ascidians belonging to the suborders Phlebobranchia (*Polycarpa cryptocarpa* var. *kuruboja*, *Styela plicata*, *Pyura saccjformis*, *Halocynthia roretzi*, *Halocynthia aurantium*, *Halocynthia papillosa*, *Microcosmus sulcatus*, and *Molgula manhattensis*) and Stolidobranchia (*C. intestinalis*, *Ciona savignyi*, *Ascidia malaca*, *Ascidia ahodori*, *Ascidia sydneiensis samea*, *Phallusia mammillata*, and *Chelyosoma siboja*) by thermal neutron activation analysis. A comparison of the findings showed that our recorded result was higher than the measured V level (0.3 ± 0.03 μg/g) in mantle of *S. plicata* collected from Mutsu Bay, Japan (Table 7).

According to the results, only Cu in *S. plicata* exhibited seasonal variation ($p < 0.05$). Physiological parameters such as water pumping and metabolic rates that fluctuate temporally may contribute to seasonal differences in metal accumulation (Stacey and Driedzic 2010).

Seasonal changes for copper levels in *S. plicata* can be due to the changes in reproductive cycle. The seasonal distribution

Table 7 Comparisons of recorded heavy metals levels in sea water (μg/l), sediment, *C. barbata* (μg/g dry weight), *P. caerulea*, and *M. auratus* (μg/g wet weight), during the present study with literature reported from different areas

	Site	Cd	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Sea water									
	Gulf of Gaeta, central Italy	0.214-0.301	0.00212-0.00305				0.002-0.003	0.0075-0.0104	Conti and Cecchetti (2003)
	Vigo estuary area (Galicia, NW Spain)	0.01-0.05	0.12-0.88				0.17-2.05	38.47-52.85	Pérez-López et al. (2003)
	Gulf of Suez	0.04-0.27	1.15-4.78	6.33-32.78	1.28-4.56	1.36-3.65	0.56-3.17	6.79-25.19	Hamed and Emara (2006)
	Lebanese coasts (Eastern Mediterranean Sea)	0.062					0.221		Nakhle et al. (2006)
	Mersin Bay (Turkey)	0.06-0.73					0.21-15.79		Ayas et al. (2009)
	Linosa Island, Sicily (southern Tyrrhenian Sea, Italy)	0.33±0.03	2.34±0.26				0.97±0.15	13.0±1.0	Conti et al. (2010)
Sediment	Marmara Sea (Turkey)	0.02-0.05	12.7±0.4-30.6±0.1	5956±36-14896±45	273.6±0.5-384.2±1.9	20.5±0.47-53.9±0.70	21.6±3.6-31.9±3.7	34.1±0.1-50.9±0.1	Topcuoglu et al. (2004)
C. barbata	Sinop (Turkey)	1.30±0.02	4.15±0.03	446±88			5.3±2.4	12.1±5.1	Guven et al. (1998)
	Şile (Turkey)			231±89				18.6±7.7	Guven et al. (1998)
	Riva (Turkey)	1.08±0.02	12.33±0.18	604±101			8.3±1.2	7.0±2.8	Guven et al. (1998)
	İğneada (Turkey)	<100						<7	Guven et al. (1998)
	N. Fokaea (Turkey)	0.47	6.1		70.5	8.7	2.1	22.3	Sawidis et al. (2001)
	Kalandra (Turkey)	0.83	8.8		40.5	14.2	2.5	16.4	Sawidis et al. (2001)
	A Trias (Turkey)	0.06	1.7		22.2	9.7	0.02	29.8	Sawidis et al. (2001)
	Alykes (Turkey)	0.4	2.1		30.9	4.3	0.02	18.7	Sawidis et al. (2001)
	Lehonia (Turkey)	2.6	3.2		181.3	28.5	0.02	58.1	Sawidis et al. (2001)
	Monolithos (Turkey)	0.4	2.7		47.0	14.6	0.02	17.2	Sawidis et al. (2001)
	Perivolos (Turkey)	1.4	0.7		7.9	6.8	0.02	8.8	Sawidis et al. (2001)
	Heraklion (Turkey)	1.1	3.8		5.6	4.3	0.02	14.2	Sawidis et al. (2001)
	Chania (Turkey)	2.7	1.7		34.0	16.0	0.02	24.5	Sawidis et al. (2001)
	Venice lagoon (Italy)	<0.1-0.2±0.0	2±0-21±0	199±7-609±100		1.4±0.1-2.4±0.6	1.4±0.0-5.6±0.7	15±4-88±11	Caliceti et al. (2002)
	Pazar (Turkey)	0.64±0.06	4.12±2.18		5.4±2.1		6.7±1.87	12.0±2.6	Topcuoglu et al. (2003a)
	Rize (Turkey)	0.66±0.12	6.30±0.04		22.2±1.3		14.1±1.21	61.2±1.1	Topcuoglu et al. (2003a)
	Şile (Turkey)	<0.02-0.78±0.1	2.2±0.08- 5.7±0.12	130±1-427±3	6.7±0.1-32.1±0.2	<0.1-9.1±0.1	<0.1-1.4±0.2	3.9±0.3-35.1±0.3	Topcuoglu et al. (2003b)

of heavy metal accumulation explicitly reveals that the organisms are differentially selective. Reproductive mechanisms of marine organisms appear to be very sensitive to pollutants and can be used as biomarkers to detect ecological risk at the level of populations and communities (Gallo et al. 2011).

S. plicata is characterized by two spawns (two ovulations), i.e., two ovarian cycles. So, the reproductive cycle of this species can be investigated as follows: it begins with the beginning of spring, and the first ovulation occurs during early autumn while the second one occurs at the end of the following winter. March can be considered the end of the cycle or the interbreeding period. The pleurogoneate ascidians *S. plicata* has ripe gonads during the period of autumn and winter, while spring and summer are considered interbreeding periods (El Bawab et al. 2012).

The correlation analysis applied to assess the relationships between heavy metal concentrations in biota samples shown that Cu in *S. plicata* revealed positive relationships with Pb in *S. plicata* ($r = 0.6849$, $p < 0.01$) and with Zn in *S. plicata* ($r = 0.7576$, $p < 0.01$). Moreover, significant positive correlation was established between Zn in *S. plicata* and Pb in *S. plicata* ($r = 0.7939$, $p < 0.01$). Positive relations between metals indicate that these metals are derived from same sources and that these metals associated with each other.

de Moreno et al. (1997) analyzed Cd, Cu, Hg, and Zn levels in 34 species of organisms that included algae, filter feeders, omnivorous invertebrates, and vertebrates from different areas close to the Antarctic Peninsula. They reported lower concentrations for measured Cd (0.01–1.01 μg/g w.w.) and higher values for Cu (0.29–0.30 μg/g) and Zn (44.74–58.56 μg/g) in ascidian *Distaplia cylindrica* collected from Artigas Base (Antarctic Ocean). And also, there were lower contents for Cd (0.26–0.30 μg/g) and higher concentrations for Cu (0.14–0.52 μg/g) and Zn (40.00–44.20 μg/g) in *D. cylindrica* taken from Pratt Base (Antarctic Ocean) than that recorded in the present study (Table 7).

Kim et al. (2001) measured Cd, Cu, and Pb levels in gill, viscera, gonad, muscle, and mantle of *Halocynthia roretzi* collected from Wolsung, Korea. They reported the accumulation order in *H. roretzi* as Cu > Pb > Cd. Comparison of Cd, Cu, and Pb levels in the present study revealed that our detected values in this study were lower than the reported Cd (0.33 μg/g) levels and higher than the given Cu (40.96 μg/g) and Pb (1.42 μg/g) contents by Kim et al. (2001) (Table 7).

Denton et al. (2006) collected *Ascidia* sp. and *Rhopalaea* sp. from stations situated in Apra Harbor, Guam. Findings clearly showed that recorded results for *S. plicata* were lower than Cd level in *Ascidia* sp. (0.08–0.36 μg/g), while our findings were higher than Cu (3.10–5.58 μg/g), Pb (0.54–1.47 μg/g), and Zn values (15.2–95.8 μg/g) in *Ascidia* sp. Moreover, our findings were lower than given Cd levels (0.13–0.44 μg/g) in *Rhopalaea* sp. and also considerably higher than Cu (6.46–9.87 μg/g), Pb (1.06–2.91 μg/g), and Zn contents (20.7–34.1 μg/g) in *Rhopalaea* sp. (Table 7).

Odate and Pawlik (2007) investigated the V levels in blood, tunic, and internal tissues *Phallusia nigra* collected from the seawalls and pilings in the Port Largo Canal, Key Largo, FL, USA. Studied heavy metal levels in *P. nigra* decreased in the following order of abundance: internal tissue > blood > whole tunic. It can be seen that our recorded highest V level in *S. plicata* was markedly lower than the given V (2280 ± 822 μg/g) content for internal tissues of *P. nigra* (Table 7).

To evaluate the usefulness of *M. edulis* and *C. intestinalis* as biomonitors, Webb (2009) collected seawater and biota samples from Nova Scotia, Halifax Harbor, and compared heavy metal concentrations of water samples and diffusive gradients in thin films (DGTs) with tissue concentrations. Comparing the results with Webb (2009) showed that our noted Cu levels (13–23 μg/g) in *S. plicata* were higher and V concentration (28–120 μg/g) in *S. plicata* was lower than the given data by Webb (2009) (Table 7).

Lebar et al. (2011) investigated the concentrations of the heavy metals such as manganese, Ni, and V in the body tissues of tunicates from the western Antarctic Peninsula. Our findings were slightly lower than those reported V levels in *Aplydium* sp. (1; 40.0 ± 3.1 μg/g), *Aplydium* sp. (2; 254.4 ± 3.0 μg/g), *Ascidia* sp. (567.2 ± 16.3 μg/g), *Distaplia colligans* (24.3 ± 1.7 μg/g), *Distaplia cylindrica* (1455.2 ± 13.8 μg/g), and *Synoicum adareanum* (26.1 ± 1.2 μg/g; Table 7).

Noël et al. (2011) measured Pb, Cd, and Hg concentrations in marine gastropods *Littorina littorea*, *Buccinum undatum Bolinus brandaris* (*Murex brandaris*), echinoderms (*Paracentrotus lividus* and *Holothuria leucospilota*), and tunicates (*Microcosmus sabatieri*) investigated by the French National Reference Laboratory (NRL) for trace elements in foodstuffs of animal origin. Cd content in the present study revealed that our detected values in this study were lower than the reported Cd level (0.020–0.033 μg/g) in *M. sabatieri* (Table 7).

Saad et al. (2011) collected *C. intestinalis* specimens from stations located in Alexandria, Mediterranean Sea. And, they measured copper, iron, magnesium, cadmium, zinc, and tributyl tin (TBT) accumulations in both seawater and in the tissues of *C. intestinalis* using the atomic absorption spectrophotometer. They have also done RAPD-PCR analysis, and determined levels of lipid peroxidation, superoxide dismutase, catalase, glutathione transferase, glutathione reductase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and proteins were recorded as well. Comparasion of our findings with that of Saad et al. (2011) showed that our recorded values were slightly

lower than Cd levels (5.2–10.6 μg/g) and higher than in Cu (2.1–3.1 μg/g) and Zn (8.2–13.3 μg/g) values in *C. intestinalis* (Table 7).

Prabhu et al. (2012) studied the heavy metal in water; sediment; and *Polyclinum madrasensis*, *Polyclinum indicum*, *Herdmania pallida*, *Microcosmus squamiger*, and *Microcosmus exasperates* tissues collected from Palk Bay, India. They found the accumulation order in studied ascidians as Fe > Zn > Pb > Cd (Table 7).

Choi et al. (2014) studied heavy metal values in commonly consumed food species of Echinodermata (*Anthocidaris crassispina* and *Stichopus japonicus*) and Chordata (*Halocynthia roretzi* and *Styela plicata*) collected from all over South Korea. According to the findings, our measured Cd values in biota samples were similar than the reported Cd levels in *H. roretzi* (0.01–0.16 μg/g) and Cd contents in *S. plicata* (0.01–0.08 μg/g). On the other hand, our obtained values were higher than noted Pb concentrations in *H. roretzi* (0.01–0.65 μg/g) and also Pb values in *S. plicata* (0.06–0.51 μg/g; Table 7).

Arienzo et al. (2014) studied the heavy metal values in water, sediments, and specimens of corpus and tunic of the ascidian *C. intestinalis* collected from the Fusaro Lagoon, Naples Harbor, Italy. The findings of present study for Cu levels in corpus of *C. intestinalis* (0–125 μg/g) and tunic of *C. intestinalis* (0–124 μg/g) were higher and also V contents in corpus of *C. intestinalis* (2.5–666 μg/g) and tunic of *C. intestinalis* (2.5–375 μg/g) were lower than that reported values by Arienzo et al. (2014) (Table 7).

Radhalakshmi et al. (2014) is aimed to analyze the concentration of heavy metals (Cd, Cu, Pb, and Zn) in selected five species of ascidians (*Microcosmus squamiger*, *Microcosmus exasperatus*, *Herdmania pallida*, *Phallusia arabica*, and *Styela canopus*) and their environment in Thoothukudi coast, India. All studied metals in acidians, Cu, Pb, and Zn values were low compared with the data obtained in the present study (Table 7).

Abdul Jaffar Ali et al. (2015) studied the heavy metal levels in tunic and body of *P. nigra* from Thoothukudi coast and Vizhinjam Bay, India. They reported higher levels of Cd (0.063–9.13 μg/g) and V (79.75–116.82 μg/g) in tunic of *P. nigra* and in mantle body of *P. nigra* [Cd (0.35–29.96 μg/g), Pb (4.59–62.4 μg/g), and V (229.06–423.12 μg/g)]. Furthermore, they found lower level of Cu in tunic of *P. nigra* (0.9–1.8 μg/g) and Cu in mantle body of *P. nigra* (3.2–5.3 μg/g) than that recorded in the present study (Table 7).

Conclusion

Measuring heavy metal levels in the tissues of organisms, sediments, and suspended particulate matters offers an indicative view of the ecosystem's health, assessing the bioavailability of the pollutants.

According to the results, the mean heavy metal levels in suspended particulate matters, sediments, and *S. plicata* samples could be arranged in the following order of abundance: Zn > Cu > Pb > V > Cd.

As for heavy metal levels, significant positive correlations were noted between Cd-Pb, Cd-V, Cd-Zn, Cu-V, and Pb-V in SPM; Cd-Zn, Cu-Zn, Pb-Cd, Pb-Cu, and Pb-Zn in sediment; and Cu-Pb, Cu-Zn, and Pb-Zn in *S. plicata* samples. Positive relationships between heavy metals showed that these metals originated from same sources and that these metals associated with each other.

Zn, Cu, and Pb concentrations in suspended particulate matters, sediments, and *S. plicata* samples were generally represented with higher levels at stations that served as for boating, shipping, and related activities. This can be due to the Cu- and Zn-based antifouling paints used in these stations. *S. plicata* is a strongest accumulator of V; the relatively low V levels observed in this study may indicate the lack of anthropogenic sources of this metal in the sampling stations.

In this study, detected heavy metal levels in suspended particulate matters were generally higher compared with the previous studies conducted in different areas in the world. Moreover, findings of this study exhibited that measured Cd and V levels in sediment and *S. plicata* samples were generally lower, while Cu, Pb, and Zn levels were higher than that reported values from other parts of the world.

To our knowledge, no previous study was conducted on the heavy metal accumulation in the tissues of *S. plicata*. Further researches that are necessary with regard to this issue should focus on actual accumulation patterns of these organisms. This investigation provides a pioneer picture of a new bioindicator to detect heavy metal contaminations of eastern Aegean Sea coasts.

Overall, the results of this study indicate that *S. plicata* is one of the most promising alternative for the monitoring of environmental pollution caused by heavy metals for eastern Aegean Sea coasts.

Acknowledgments The author is grateful to the Scientific and Technological Research Council of Turkey (TUBITAK) for providing the financial support (project ID 115Y417) to conduct this research. I would like to express my deep gratitude to Dr. Noa Shenkar for her hospitality, valuable helps, and kind assistance in identification of *S. plicata* specimens. Thanks are due to the several anonymous reviewers for their informative comments and suggestions that helped to improve the original version of this manuscript. The author is indebted to Engineer (M.sc) Zülfikar Ata Karakoç, ARGEFAR Laboratory (Ege University, İzmir) team, and Dokuz Eylül University Institute of Marine Science and Technologies Marine Chemistry Laboratory and Geology Laboratories for their technical support in heavy metal and grain size analysis. I wish to express my sincere gratitude to Prof. Dr. Filiz Küçüksezgin, Prof. Dr. Ferah Koçak, and Gamze Kordacı Uzkuç for their

helps during the study. Finally, I thank all the people who contributed in some aspect to this work.

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Table 7 Literature comparisons of metal concentrations (μg/g d.w.) in ascidians from different geographical areas in the world

	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	V (μg/g)	Zn (μg/g)	Sampling Areas	References
Boltenia villosa				520-750		Bamfield, British Columbia	Danskin, 1977
Styela plicata (mantle)				0.3±0.03		Mutsu Bay, Japan	Michibata et al., 1986
Distaplia cylindrica (μg/g w.w.)	0.01-1.01	0.29-0.30			44.74-58.56	Artigas Base (Antarctic Ocean)	Moreno et al., 1997
Distaplia cylindrica (μg/g w.w.)	0.26-0.30	0.14-0.52			40.00-44.20	Pratt Base (Antarctic Ocean)	Moreno et al., 1997
Halocynthia roretzi	0.33	40.96	1.42			Wolsung, Korea	Kim et al., 2001
Ascidia sp.	0.08-0.36	3.10-5.58	0.54-1.47		15.2-95.8	Apra Harbour, Guam, USA	Denton et al., 2006
Rhopalaea sp	0.13-0.44	6.46-9.87	1.06-2.91		20.7-34.1	Apra Harbour, Guam, USA	Denton et al., 2006
Phallusia nigra (Internal tissues)				2,280±822		Port Largo Canal, USA	Odate and Pawlik, 2007
Ciona intestinalis		13-23		28-120		Nova Scotia, Halifax Harbour	Webb et al., 2009
Aplydium sp. 1 (pink small, <10 cm)				40.0±3.1		The Western Antarctic Peninsula	Lebar et al., 2011
Aplydium sp. 2 (pink massive, >25 cm)				54.4±3.0		The Western Antarctic Peninsula	Lebar et al., 2011
Ascidia sp.				567.2±16.3		The Western Antarctic Peninsula	Lebar et al., 2011
Distaplia colligans				24.3±1.7		The Western Antarctic Peninsula	Lebar et al., 2011
Distaplia cylindrica				1,455.2±13.8		The Western Antarctic Peninsula	Lebar et al., 2011
Synoicum adareanum (2007)				26.1±1.2		The Western Antarctic Peninsula	Lebar et al., 2011
Microcosmus sabatieri	0.020-0.033		0.042-0.505			France	Noël et al., 2011
Ciona intestinalis (mg/g)	5.2-10.6	2.1-3.1			8.2-13.3	Alexandria, Meditteranean Sea	Saad et al., 2011
Ciona intestinalis		0-124		2.5-666		The Fusaro Lagoon, Naples Harbour, Italy	Arienzo et al., 2014
Halocynthia roretzi	0.01-0.16		0.01-0.65			South Korea	Choi et al., 2014
Styela plicata	0.01-0.08		0.06-0.51			South Korea	Choi et al., 2014
Microcosmus exasperatus	0.0109	1.11	0.38	0.44		Thoothukudi coast, India	Radhalakshmi et al., 2014
Microcosmus squamiger	0.0064	2.07	0.53	0.56		Thoothukudi coast, India	Radhalakshmi et al., 2014
Herdmania pallida	0.0035	8.45	0.33	0.55		Thoothukudi coast, India	Radhalakshmi et al., 2014
Phallusia arabica	0.0199	4.57	1.54	22.23		Thoothukudi coast, India	Radhalakshmi et al., 2014
Styela canopus	0.0322	3.38	1.74	3.55		Thoothukudi coast, India	Radhalakshmi et al., 2014
Phallusia nigra (tunic)	0.063-9.13	0.9-1.8		79.75-116.82		Thoothukudi coast and Vizhinjam Bay, India	Abdul Jaffar Ali et al., 2015
Phallusia nigra (mantle body)	0.35-29.96	3.2-5.3	4.59-62.4	229.06-423.12		Thoothukudi coast and Vizhinjam Bay, India	Abdul Jaffar Ali et al., 2015