Estimating of the bioaccumulation of some heavy metals in *Posidonia oceanica* at the coast of Al Khums City

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تقدير التراكم الحيوي لبعض العناصر الثقيلة في أعشاب البوسيدونيا (Posidonia oceanica) النامية بشاطئ مدينة الخمس

الملخص:

تهدف هذه الدراسة إلى تقدير التراكم الحيوي لبعض المعادن الثقيلة مثل الرصاص (Pb)، الكادميوم (Cd) والزنك (Zn) في أعشاب البوسيدونيا (Posidonia oceanica) وفي عينات مياه البحر. وقد تم جمع هذه العينات من ثلاثة مواقع على ساحل مدينة الخمس (شاطئ الخمس وشاطئ باركو والشاطئ المقابل لمحطة الكهرباء وتحلية المياه)، خلال الفترة من خريف 2017 إلى صيف 2018. في هذه الدراسة تم استخدام جهاز التحليل الطيفي للامتصاص الذري (CONTRAA700 analytikjena). وقد أظهرت النتائج أن تركيز (Pb) كان أعلى من الحد المسموح به من قبل منظمة الصحة العالمية ومنظمة الأغذية والزراعة WHO/FAO و كان أعلى تركيز للرصاص (12.1) ميكروجرام/لتر خلال فصل الصيف في موقع باركو بماء البحر و(18.5) ميكروغرام/غرام من الوزن الجاف في أعشاب البوسيدونيا خلال فصل الربيع على الشاطئ المقابل لمحطة توليد الكهرباء وتحلية المياه. كان أعلى تركيز للكادميوم (0.08) ميكروجرام/لتر خلال فصل الصيف على شاطئ الخمس وفي فصل الربيع على الشاطئ المقابل لمحطة توليد الكهرباء وتحلية المياه. وبالمثل زاد تركيز الكادميوم (Cd) حيث بلغ أعلى تركيز له هو (12.3) ميكروجرام/غرام كوزن جاف في فصل الصيف على الشاطئ المقابل لمحطة توليد الكهرباء وتحلية المياه. بينما كان أعلى تركيز للزنك (13.3) ميكروجرام/لتر بماء البحر بينما في أعشاب البوسيدونيا كان أعلى تركيز له (167.9) ميكروجرام/جرام كوزن جاف بفصل الصيف على شاطئ باركو. بالإضافة إلى ذلك تم حساب عامل التراكم الحيوي (BCF) و كانت أعلى قيمة للتراكم الحيوي للرصاص (225) ملجم/لتر مرة و للكادميوم (205) ملجم/لتر مرة و للزنك كانت (330) ملجم/لتر مرة وفقاً لتركيزه في الماء .

الكلمات المفتاحية: *المعادن الثقيلة، مياه البحر، التراكم الحيوي، Posidonia oceanica.*

Abstract:

The aim of the study is to estimate the bioaccumulation of some heavy metals Pb,Cd and Zn in *Posidonia oceanica* and samples of sea water. These samples have been collected from three sites at the coast of Al-Khums city (Beach of Al-Khums Park, Barco Beach and the beach opposite to the Power and Desalination plant). These samples have been collected during the period from Autumn 2017 to Summer 2018. In this study used the Atomic Absorption Spectroscopy (CONTRAA700analytikjena) to determine the concentrations of heavy metals at the Sadeem Laboratory. Results shown that, the concentration of Pb in most samples of sea water in all sites was above the permissible limit by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). The highest concentration of Pb (12.1) µg/L in Summer at the Barco Beach in sea water and (18.5) µg/g as dry weight in *Posidonia oceanica* in Spring at beach opposite to the Power and Desalination plant. The highest concentration of Cd was (0.08) µg/L in Summer at Al Khums Park Beach in sea water and it was (12.3) µg/g in Summer at the beach opposite to the Power and Desalination plant in Spring. While the highest value of Zn was (13.3) µg/L Concerning the studied seagrass *Posidonia oceanica* and its highest value (167.9) µg/g as a dry weight was in Summer at the Barco beach. Bioaccumulation factor (BCF) values were high in seagrass. The highest BCF values of lead, cadmium and zinc were 225, 205 and 330 mg/L times in accordance to its concentration in water respectively.

Keywords: *Heavy Metals, seawater, Posidonia oceanica, Bioaccumulation.*

Introduction:

Environmental pollution is a growing problem at the global level that is directly caused by anthropogenic factors. Among the many types of pollution, marine pollution stands out, which is especially recognized issue within EU Marine Strategy Framework Directive (MSFD 2008/56/EC). The Mediterranean Sea is surrounded by three continents and is subject to pronounced anthropogenic influences due to limited water exchange (Durrieu de Madron *et al*., 2011). The city beach Al Khums marine ecosystem is threatened by various negative impacts which contribute to the pollution of the area, especially heavy metal contamination. Negative impacts that effect the sea pollution are increasing intensive urbanization of the coastal area, large influx of sewage and industrial waste waters and agricultural activities (Zoller and Hushan ،2000؛ Usero *et al*. ،2005; Alqadami *et al*., 2018). Due to their toxicity, persistence, low biodegradability, and propensity to accumulate in aquatic organisms, trace metals are regarded as contaminants of the marine environment (Schüürmann and Markert, 1998; Conti et al., 2010). Increased levels of hazardous elements entering the food chain and decreased species diversity are two severe consequences of higher heavy metal concentration values in marine environments (Liu et al., 2008). Trace metals' bioaccumulation and hazardous qualities are heavily influenced by both their intrinsic characteristics and the environment, which regulates how bioavailable these metals are (Moiseenko and Gashkina, 2020). In order to precisely assess the level of heavy metal pollution of marine ecosystems, it is needed to conduct the analysis of the heavy metal content not only in living organisms, but also of the abiotic components (Conti *et al*., 2002; Gray, 2002; Majer *et al*., 2014). The use of biological species in the monitoring of marine environment quality permits the evaluation of the biologically available levels of pollutants in the ecosystem on the effects on pollutants on living organisms. The analysis of environmental matrices such as water or sediment provides a picture of the total pollution load rather than of that fraction of direct ecotoxicological relevance (Akcali & Kucuksezgin, 2011). While biomonitoring cannot completely replace chemical monitoring, it does combine the two and make a special contribution to the assessment of pollutants and their toxicity. As a result, there were more research looking at the

quantities of saltwater, sediment, and one or more sea grasses (Serrano et al., 2011; Cozza et al., 2013; Jovic and Stankovic, 2014; Saliha et al., 2017) . P. oceanica, an indigenous seaweed species to the Mediterranean, is significant to the region's ecology and is typically found in protected, shallow coastal waters with sand or mud bottoms (Zoller and Hushan, 2000; Usero et al., 2005). Meadows are used by a variety of plant and animal species as a spawning ground, habitat, or hunting ground. It has a high potential to acquire trace metals and concentrate contaminants found in the environment. It may absorb trace elements directly from the water column and/or from interstitial water in sediments (Calmet et al., 1988; Malea and Horitonidis, 1989; Di Leo et al., 2013). Seagrass Posidonica oceanica (L.) has thus been used for many years in the Mediterranean as a gauge of the level of pollution in the marine ecosystem (Ferrat et al. 2003; Lafabrie et al., 2007; Di Leo et al., 2013; El Zrelli et al., 2017; Bertini et al., 2019).
The main aim of this study was to determine the concentration of Pb, Cd and Zn in seawater and sea grasses *P. oceanica* sampled from three locations at Al Khums coast and assess the pollution considering sea straw is an essential component of the aquatic food chain. The obtained results were compared with the findings of the similar researches done Mediterranean region.

Materials and methods:

Study Area:

This study was performed in the beach city of Al-Khums located Northern part of Libya. About 120 km from Tripoli city, the beach city of Al-Khums is Located at the Mediterranean sea on the North coast, extending from Al Khums Park Beach (19°14'E and 39°32'N) in the West, to Beach opposite the desalination station (20°14'E and 37°32'N) in the East , with a distance of 11 km in between. The samples were collected from three sites (Al Khums Park Beach, Barco Beach, Beach opposite the desalination station)) Fig. 1(These samples have been collected during the period from Autumn 2017 to Summer. 2018.

Fig. 1: Map of the the beach city of Al-Khums and sampling sites First site (Al Khum Park Beach), Second site(Barco Beach), Third site (Beach opposite the desalination station).

The plants description:

Posidonia oceanica is a vascular plant, so contrary to the seaweed, it has leaves, stems and roots, and it produces flowers and fruit, but it is submerged. These plants make meadows, more or less big clusters which has leaves that grow up to the sediments. Leaves are in the shape of flat stripes that are 1 cm wide and from 10 to 150 cm long, depending on the season. Posidonia oceanica's clusters make large underwater meadows, which are stable and long-lasting, but which can sometimes be affected by a delicate environmental balance.

Table (1): Scientific classification of the plant (Guiry, 2019).

Kingdom:	**Plantae**
Order:	**Alismatales**
Family:	**Posidoniaceae**
Genus:	***Posidonia***
Species:	***P. oceanica***

Fig. 2: *Posidonia oceanica*

Samples collection: At each sites 5 leaves beams of *Posidonia oceanica*, which it was randomly collected from diving by hand per sites and per season at a depth ranging between 1 and 7 m depending on the sites, collected were three repetitions (n= 36). At the same time and place, about the *P. oceanic* samples and two liters of seawater from the bottom were collected at the all studied locations. Samples were brought to the laboratory to be analyzed.

Samples preparation: *Posidonia oceanica* samples were dissected in the laboratory to separate their roots (ra), rhizomes (rh) and leaves, these last have been separated using the Giraud method (29) in juvenile leaves (Jl), intermediate (Il) and mature ones (Ml). Only mature leaves have been cleaned of their epiphytes by a plastic ruler and rinsed with distilled water. All tissues of plant were then dried at 70°C until a constant weight for 24 h, then mashed and wet-mineralized. A known amount of each sample 0.5g dry wt. of seagrass was digested by 65% the nitric acid and%60 Prochloric ($HNO3$ and $HCLO4$ in an amount of 5/3 mL) to the mash and heating them at 100°C until a clear solution was obtained (Lytle and Smik, 1995). The latter was then filtered through a filter paper (Whatman No. 45) and the filtrate obtained was transferred to the volumetric flasks and made up to 25 mL with 2% HNO3. The resulting product was stored in polyethylene bottles tightly sealed until analysis. Accurate extraction of 100 ml Standard Seawater in 250 ml beaker, adding 7 ml of the nitric acid to digest, then Heat the beaker on a hot plate, After that adding few drops of hydrochloric acid HCL . Finally adding ultrapure water to make the solution volume is 50 ml (APHA, 1995). The resulting product was stored in polyethylene bottles tightly sealed until analysis.

Chemical analysis: The Pb, Cd and Zn concentrations in seawater and sea grasses *P. oceanica* were run with quality assurance procedures at the Laboratory of Sadeem (Tripoli, Libya), were analyzed by using Flame Atomic Absorption Device (CONTRAA700analytikjena) obtained results of the investigated elements in seawater are expressed in µg/l and sea grasses *P. oceanica* mg/kg. The efficiency of metal bioaccumulation of *P. oceanica* was evaluated by calculating the bioconcentration factor (BCF) and biota-sediment factor (BSAF) which are defined as ratio between metal concentration in the organism and in seawater (Geyer *et al*., 2000; Lafabrie *et al*.,2007). The bioconcentration factors BCF) of the heavy metals in sea grasses *P. oceanica* samples were obtained using equation (Vassiliki and Konstantina, 1984).

BCF = Corg / Csed.

Where BCF = bioconcentration factor.

Corg= concentration of metal in the organism.

Csed = Concentration of the same metal in the ambient environment, seawater in this case. The coefficient of variation was calculated to determine whether or not the BCF obtained for the various heavy metals in the *P. oceanica* were different from one another.

Statistical analysis: The data of the present study analyzed statistically by using the software package SPSS (23.0) (Sanchiz *et al*. 2001). Significant differences between seasons and between sites were determined by a two-way analysis of variance (ANOVA). Duncan's test (Homogeneity) was run to estimate the homogeneity and difference in the studied groups between the different seasons and stations.

Results:

The metal concentrations of Pb, Cd and Zn found in seawater from the different locations and seasons are given in Table 2. In three sites, the mean seasonal concentrations for Pb, Cd and Zn follow the order summer > spring > fall > winter. The highest Pb concentrations are recorded at Third site in the summer, whereas the highest Cd concentrations are recorded at first site during the summer, while the highest Zn concentrations are recorded at the second site in summer. The concentration of zinc was highest in sea grass leaves and seawater of the second site followed by Pb and Cd. The "ANOVA" in Duncan test indicated highly significant differences in the content of the seawater metals according season and metal dose.

Even in mature leaves of *Posidonia oceanica*, recorded heavy metal content also varies with the seasons and sites of study. Highest metal concentrations Pb in leaves are recorded at Third site in the spring whereas the highest Cd concentrations are recorded at Third site during the summer, while the highest Zn concentrations are recorded at second site during the summer. As with the seawater, the metal concentrations recorded in summer were the most outstanding and, in all sites, the high concentrations were observed at "Zn" metal (essential micronutrients to organisms).

Table 2: Heavy metals concentration in seawater (Mean± SD) (µg/l dry weight) samples collected from three sites during seasons and permissible limit (WHO,1985; Obasohan, 2007).

Elements	Seasons / Locations	Autumn	Winter	Spring	Summer
Lead Pb **Allowed limit WHO/1985 o.o5 µg/l**	First site	0.05±0.02^{b}	0.04±0.03^{a}	0.5±0.03^{c}	7.13±1.0^{d}
	Second site	0.04± 0.01^{b}	0.02±1.0^{a}	3.8±0.02^{c}	11.6±0.2^{d}
	Third site	0.07±0.03^{b}	0.05±0.04^{a}	10.3±0.03^{c}	12.1±0.04^{d}
Cadmium Cd Allowed limit WHO/1985 0.05 µg/l	First site	0.05±0.03^{a}	0.06±0.02^{b}	0.06±0.01^{b}	0.08±0.03^{c}
	Second site	0.06±0.01^{b}	0.05±0.01^{a}	0.064±0.04^{b}	0.06±0.02^{b}
	Third site	0.06±0.02^{b}	0.05±0.01^{a}	0.08±0.02^{d}	0.07±0.04^{c}
Zinc Zn **Allowed limit WHO/1985** 5.0 µg/l	First site	2.3±0.3^{c}	0.6±0.03^{a}	1.2±0.2^{b}	2.1±0.2^{c}
	Second site	1.35±0.2^{a}	2.3±0.02^{b}	11.2±0.5^{c}	13.3±1.0^{d}
	Third site	0.11±0.3^{a}	0.4±0.1^{c}	0.39±0.03^{b}	1.1±0.03^{d}

Values are given as mean ± SD. The same letters in the row are not significantly difference. First site (El Khums Park Beach), second site (Barco Beach), third site (Beach opposite the desalination station).

The "ANOVA" in Duncan test indicated highly significant $p<0.05$ differences in the content of the leaves metals according season and sites (Table). The bioconcentration factors (BCF) of heavy metals for sea grass in (Tab. 3). For most metals, a BCF value of less than 1.00 is usually expected; otherwise, bio-accumulation of the metals by organisms will occur (Vassiliki and Konstantina, 1984). From the results obtained, it was observed that Pb, Cd and Zn have BCF values ranging from 1 – 330. All these values were considered too high when compared with the highest value of 1.00 expected for any metal The high BCF values obtained for Pb, Cd and Zn, therefore indicated that the metals were highly bioaccumulated and bio-magnified in *P. oceanica*. The metal which presents the highest BCF for *P. oceanica* are Zn and Cd from Third site in the Autumn and the Summer, respectively. while Pb from Second site in the Autumn. High values of BCF indexes obtained for Pb, Cd and Zn *in P. oceanica* indicate at their ability to accumulate heavy metals which classifies them as good bioindicators of marine ecosystems pollution. Comparing the values of the accumulation capacity. This is in accordance with the researches done by Bonnano *et al*. (2017) and Bonnano & Borg (2018). This indicates that *P. oceanica* can be used as bioindicators of Pb, Cd and Zn metals in seawater, especially in conditions when these metals are present in higher concentration.

Table 3: Heavy metals concentation in *P. oceanic* (Mean±S.D.)(mg/g dry weight) samples that collected from three sites during seasons n=36 and permissible limit (FAO/ WHO, 1983) (Kumar,*et al*., 2013).

Elements	Seasons / Locations	Autumn	Winter	Spring	Summer
Lead Pb FAO/WHO1983 Allowed limit 6-0.5 (mg/g)	First site	9.5 ± 1.4^{b}	3.7 ± 0.01^{a}	13.09 ± 0.5^{c}	10.3 ± 0.8^{b}
	Second site	8.2 ± 2.2	4.5 ± 0.8	17.05 ± 0.8	8.8 ± 0.12
	Third site	4.1 ± 0.3^{b}	1.37 ± 0.02^{a}	18.5 ± 0.01^{d}	15.01 ± 2.1^{c}
Cadmium Cd FAO/ WHO 1983 Allowed limit 5.5-0.05 (mg/g)	First site	7.3 ± 0.002^{d}	0.06 ± 0.001^{a}	1.8 ± 0.001^{b}	3.7 ± 0.001^{c}
	Second site	1.32 ± 0.01^{c}	0.67 ± 0.08^{b}	0.53 ± 0.03^{a}	2.03 ± 0.08^{d}
	Third site	4.2 ± 0.05^{c}	0.084 ± 0.003^{a}	0.561 ± 0.006^{b}	12.3 ± 0.003^{d}
Zinc Zn FAO/WHO1983 Allowed limit 30-100 (mg/g)	First site	42.2 ± 0.6^{c}	3.6 ± 0.3^{a}	7.2 ± 0.5^{b}	66.4 ± 0.008^{d}
	Second site	21.9 ± 4.2^{a}	34.4 ± 2.7^{b}	116.9 ± 2.5^{c}	167.9 ± 0.002^{d}
	Third site	36.4 ± 2.3^{c}	5.03 ± 0.3^{a}	9.00 ± 0.3^{b}	106 ± 0.02^{d}

Values are given as mean ± SD. The same letters in the row are not significantly difference. First site (El Khums Park Beach), second site (Barco Beach), third site (Beach opposite the desalination station).

Discusion:

The results of the current study showed that the concentrations of heavy metals differ from one season to another, perhaps the reason is due to the difference in temperature and speed (El-Serafy *et al*,. 2003). Overall, the concentrations of heavy metals in coastal water were the following sequence: Pb > Zn > Cd. The maximum value of Pb, Cd were in the Third site, First site and Third site. Zn in Second site. The high content of heavy metals in the seawater samples can be explained by the increased impact of various anthropogenic activities that take place along the El Khums coast during the summer months, when sampling was performed, Causes high temperatures (Kargin, 1995), Which causes an increase in the solubility of gases and elements in water(Smith, 2004 ; Kennish, 2002) and also discharge of untreated municipal wastewater from households and touristic *facilities*(Georgopoulos *et al*., 2001) into the sea and vessel activities in the coastal region (Pergent, 1990; Tranchina *et al*., 2005; Davenport & Davenport, 2006; Joksimović *et al*., 2011), As well as any waste from the power generation and water desalination plant and the waste of ships that supply the station with fuel (Pourang *et al*., 2018), Which spreads for wide distances due to sea currents, which leads to a high concentration in the water (Peltier, *et al*., 2008). The study indicates that concentrations of Zn and Pb the seawater samples are significantly higher compared to the data obtained for the same metals in the previous researches (Mihajlović *et al*.2002) and are lower than the concentrations of these metals in the seawater in (Mihajlović *et al*. ‹2002 ; Joksimović *et al*., 2011; Komar *et al*., 2017). With regard to Cd, values significantly lower than (Tan, *et al*., 2016). In this study, different amounts of trace metals (Pb, Cd and Zn) in the leaf *P. oceanic* were obtained. Whose uptakes occurs through a passive process that depends on the leaf surface exposed to a subsequent translocation in the roots and rhizomes (Villares and Carballeira, 2001). the fact that Pb Cd and Zn concentrations were found in the seagrass may mean that primarily uptake these three elements from the water column (Lafabrie *et al*., 2007; Stanković *et al*., 2015). In fact, marine macrophytes absorb metals in two ways: By direct absorption from water through the leaf surface or from the sediment and interstitial water through the roots (Brinkhuis *et al.*, 1980). but the effectiveness of metal uptake from these sources may differ in relation to the ecological needs, the contamination gradients of water and sediment, as well as other factors such as salinity, temperature and interacting agents (Madkour *et al*., 2011). As *P. oceanica* may reflect both contaminations in the water column and in sediment (Joksimović and Stanković, 2012). This therefore leads to the hypothesis of a preferential uptake of Pb, Cd, Co, Hg, Ni and Zn from the water column to the photosynthetic tissue (Pergent-Martini *et al*., 2006), confirming that the metabolic condition and the lifecycle stage of the organ may influence the metal uptake and accumulation. As general role, the data highlighted that the apical tissues of leaves showed the highest levels of trace metals (Lin *et al*, 2005; Kim and Triplett, 2008). The distribution of Pb is normally controlled by atmospheric deposition and its concentration is directly linked to human activities (Besada *et al*., 2002). Because of cumulative land and sea traffic and the end of the tourist season fall is the period of year with most anthropogenic, activity in the sea, which can be a major source of Pb pollution of coastal seawater. Hence, it comes as no surprise that the highest Pb concentrations were in the leaf *P. oceanica* metal concentrations in plant population are the highest in the warm seasons, Zn is element found in higher concentrations in *P. oceanica.* The increased presence of Zn in this area could result from galvanizing operations of vessels, because within the sea there is a small marina and a small area for mooring vessels. The concentration range of Zn rated by other authors (Stanković *et al.*, 2012) appears to be greater this indicates a lower contamination of Zn in our study area. The presence of Zn concentrations higher in some areas is linked to the use of this element as antifouling agent in boat paints, as our study area has a heavy vessel traffic. Zn uptake by plants can be inhibited due to complex formation between nutrients and the metal ions in environments with high nutrient levels (Joksimović and Stanković, 2012). This can explain the lowest concentration of Zn in *P. oceanica* in the winter period of the year. The highest variations of concentrations by location were obtained for Pb, Cd and Zn in the case of *P. oceanica.* For these metals in every season the maximum concentration was obtained for other locations. Variations in the metal concentrations during the year by location suggest that they are influenced by number of variable environmental factors such as salinity, temperature, pH, oxygen content, nutrient level, precipitation, inflow of fresh water, currents upwelling, etc., rather than by a constant source of pollution. However, the levels of Pb, Cd and Zn observed in this study are higher compared to previous studies on *P. oceanica* (Lafabrie *et al*. 2008; Serrano *et al*. 2011; Copat *et al.* 2012; Cozza *et al*. 2013, Jović and Slavka, 2014; Saliha and Mourad, 2016). Concerning Zn, our values are below than those found (Warnau *et al*. 1995). In addition, the levels of Pb, Cd and Zn observed in this study are higher compared to previous studies on *P. oceanica*, suggesting that our sites are exposed to these metals. This study even if at a local scale, confirms that the *P. oceanica* is useful tool to provide evidence of past metal contamination in marine environment. Finally, we suggest that *P. oceanica* would be a suitable biomonitor to assess the metal contamination.

Conclusions:

This study provided information on the concentrations, distributions, levels of pollution, and ecological dangers of heavy metals (Pb, Cd, and Zn) in seawater as well as P. oceanica along the coast of Al Khums. Lead, Cd, and Zn were the predominant pollutants in the ocean. The main causes of heavy metal pollution in the marine habitats along the Al Khums coast are wastewater discharges and coastal discharges from abandoned local industry.

Seagrass P. oceanica has a high capacity for heavy metal bioaccumulation and is a prospective bioindicator of heavy metals in saltwater (higher values of BCF).

The researches of accumulation of heavy metals in seagrasses will be significant in the upcoming period, especially given the need to establish continuous monitoring of the Al Khums marine ecosystem using appropriate indicator organisms, as well as the application of legislation governing this area.

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