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# Application of biomarkers in brown algae (*Cystoseria indica*) to assess heavy metals (Cd, Cu, Zn, Pb, Hg, Ni, Cr) pollution in the northern coasts of the Gulf of Oman

Mahmood Sinaei[a,*], Mehran Loghmani[b], Mehdi Bolouki[c]

[a] Department of Fisheries, Chabahar Branch, Islamic Azad University, Chabahar, Iran
[b] Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, Iran
[c] Department of Environmental, P.O. Box 14155-7383, Tehran, Iran

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ABSTRACT

In this study, selected biomarkers-Phytochelatin (PC) and Metallothionein (MT)- were measured in the brown algae (i.e., *Cystoseria indica*) obtained from the Gulf of Oman. Chemical analyses were performed to measure the concentration of heavy metals (Cd, Cu, Zn, Pb, Hg, Ni, and Cr) in the brown algae. Zn had the highest concentration followed by Cr > Ni > Cu > Pb > Cd > Hg. Performing a spatial analysis revealed that heavy metals had a significant difference ($p < 0.05$) among sampling sites. Mean PC and MT ranged between 5 and 13 amol/cell and 105–134 μg/g ww, respectively. Significant correlations were found between heavy metal (Cd, Cu, Zn, Pb, Ni, and Cr) concentrations and MT. However, statistically significant correlations were only found between heavy metals (Cd, Zn) and PC ($p < 0.01$). The results showed that PC reacts to heavy metals less than MT in brown algae (*C. indica*) which limits the use of PC in heavy metals biomonitoring.

## 1. Introduction

Advancement of technology and development of various industries and agricultural development have led to the introduction of a huge amount of industrial and municipal wastewater with various chemical compounds, especially heavy metals, into aquatic ecosystems. Heavy metals are one of the most important environmental pollutants that enter the sea through coastal areas and rivers and may become bioavailable to fish and other marine organisms through the food chain (Sinaie et al., 2010; Benkdad et al., 2011). Heavy metals are also naturally found in the crust of the earth. However, despite their low concentrations and low solubility, they generally are separated from the crust by weathering and erosion processes and introduced to aquatic ecosystems (Chakraborty et al., 2014). Due to bioaccumulation of these elements and the interference in the physiological function of various aquatic organisms, there have been many studies on the rate of adsorption and accumulation of heavy metals in aquatic animals. In recent years, the use of algae as a biochemical indicator has been of great interest in monitoring and controlling contaminants (Anusha et al., 2017). Algae are used as biological indicators because of their role at the beginning of the food chain, wide distribution, presence in contaminated areas, high absorption of metals, and ease of measurement of metals in them (Anusha et al., 2017). Accordingly, they have been often used as an appropriate bioindicator of the chemical contaminants. In this regard, biomarkers respond almost more rapidly to chemical stress and have high toxicological relevance (Sinaei and Rahmanpour, 2012; Tuvikene, 1995). Pandey et al. (2003) stated that exposure to the toxic effects of environmental chemicals can be evaluated by quantitative measures of changes in the biological system.

Macroalgae help to reduce the toxicity of non-essential trace metals (e.g., Hg and Cd) and lead to metal homeostasis in the cytoplasm by employing a variety of biochemical strategies (Hall, 2002; Cobbet and Goldsbrough, 2002; Szivak et al., 2009). Some of these strategies include using heavy metal-binding ligands such as the metallothioneins (MTs) and phytochelatins (PCs). PCs are produced enzymatically by PC synthase from the glutathione, a tripeptide of glutamate, cysteine, and glycine. PCs are indeed small polypeptides with molecular weight ranges from 2 to 10 kDa and with the amino acid structure y-(Giu-Cys) n-Gly, where *n* ranges from 2 to 11 (Cobbett, 2000; Szivak et al., 2009). Induction of PC synthesis is a metal-specific response rather than

*Abbreviations:* MTs, Metallothioneins; PCs, Phytochelatins; Cd, Cadmium; Hg, Mercury; Cu, Copper; Pb, Lead; Ni, Nickel; Cr, Chromium; SPSS, Statistical Package for Social Sciences

* Corresponding author.
*E-mail address:* oceanography.sina@gmail.com (M. Sinaei).

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inducing by other chemicals. The role of PC in detoxification of metals is unclear because, for most metals found to induce PC synthesis, the formation of metal-phytochelatin complexes (Me-PC) was only examined in vitro but not in vivo in algae (Ahner and Morel, 1995; Scarano and Morelli, 2002; LeFaucheur et al., 2006; Andra et al., 2009; Rauser, 1999; Schmoger et al., 2000; Alberich et al., 2007; Chekmeneva et al., 2007). Metallothionein with the low molecular weight of 6–8 (kDa) among proteins class and in its structure confers a unique metal-binding property (Pandey et al., 2003; Verkleij et al., 2003; Sinaie et al., 2010). MTs class II (MTII) does not show a strict arrangement of Cys, contrary to what is seen in fungi and photosynthetic organisms. PC and MT production has been suggested as a biomarker due to its specificity as a detoxification against metal stress in the aquatic system (Sinaie et al., 2010; Chen et al., 2007).

The Gulf of Oman with its rich plant and animal resources is considered as one of the most important ecological resources with the highest biological diversity. The northern coast of the Gulf of Oman is known as the richest sources of macroalgae. Brown algae (*Cystoseria indica*) are important groups of bioactive compounds in the northern coast of the Gulf of Oman. These algae (*C. indica*) are importance due to their high density and abundance in the region, growth in large sizes, and their valuable and valuable compounds such as alginic acid, iodine, vitamins, and minerals (Hashim and Chu, 2004). The Gulf of Oman is a developing area; traditionally, this area is used for fisheries, aquaculture activities, import and export, economy, urbanization, and industries. Heavy metals discharging into the marine ecosystem of the Gulf of Oman have the potential to negatively affect the marine organism.

Although the area is still growing and its especial weather seems to greatly affect distribution and redistribution of metals in coastal ecosystems, there is a lack of information concerning trace metals contamination. Overall, there is a clear need to improve knowledge of heavy metal pollution and biomarker response in Iranian territorial waters of the Gulf of Oman. Many studies have also reported uptake of heavy metals by different macro algae. The use of PCs and MTs production in marine macroalgae in vivo studies is very limited. Although it has been shown that these macroalgae are good bioindicators of heavy metal pollution. To the best of our knowledge, this is the first study to investigate PC and MT production in the Brown algae (*C. indica*) as a bioindicator of heavy metal pollution in the northern coast of the Sea of Oman. Therefore, this study was carried out:

1) to determine heavy metal (Cd, Cu, Zn, Pb, Hg, Ni, Cr) concentration in the Brown algae (*C. indica*), to examine their grade of pollution;
2) to study the potentiality of MT and PC activity to detoxify heavy metal in the Brown algae (*C. indica*);
3) to evaluate the potential MT and PC as a biomarker of heavy metal pollution; and
4) to assess the relationships between the selected biomarkers and heavy metal pollution in Brown algae (*C. indica*)

## 2. Materials and methods

### 2.1. Field sampling strategy

This study was carried out on the northern coast of the Gulf of Oman in November 2017. Ten different stations were chosen along the northern coasts of Gulf of Oman (Fig. 1). Sites selection was based on the earlier information available in reports about the local contaminant levels (Ravanbakhsh et al., 2009).

A total of 50 g algae were randomly collected at several places from each of the 10 sampling sites. Immediately after transportation to the laboratory, the algae were washed twice with NaCI (3.5%, w/v) and blotted to remove excess water. Microscopical examination of the algae represented no noticeable presence of saprophytic organisms. The algae were divided into two parts. A section of algae was frozen in liquid nitrogen and stored at −80 °C until the MT and PC assessments could be performed. The remaining parts were stored at −20 °C for the following heavy metals pending analyses.

### 2.2. Chemical analysis

Heavy metal contents in the algae were determined from 10 sampling sites in the Gulf of Oman. To extract and analyze heavy metals, the method proposed by Rodenas et al. (2009) was adopted. About 3 cm of front tips of algae samples were freeze-dried (72 h at −50 °C and 133 9 $10^{-3}$ mbar). About 0.5 g of powdered samples were digested in hot plate equipment (model: HPA2235M) with 10 ml quartz-distilled concentrated nitric acid. Finally, 50 ml deionized water was added to digest the material and then the mix was stored in polyethylene containers. All samples were analyzed in triplicate for Cd, Pb, Cu, Zn, Ni, and Cr by Atomic Absorption Spectrophotometer (model: Lovibond 712005, Vermont, and United States). To determine the amount of mercury, cold vapor analysis was performed. About 0.3 g of wet algae subsamples–used to avoid metal losses due to volatilization – were put in closed quartz vessels and then were added by 2 ml of concentrated redistilled $HNO_3$, concentrated $H_2SO_4$, and finally oxidized with 10 ml of a saturated solution of $KMnO_4$. In a hydride generator apparatus (model: Lovibond 712005, Vermont, the United States), excess oxidizing agents and mercury ions were reduced using 10 ml of a reducing solution (3% NaBHa in 1% NaOH). After preparing, mercury was vaporized and measured in an atomic absorption spectrophotometer (model: Lovibond 712005, Vermont, United States).

### 2.3. MT analysis

The UV-spectrophotometric method devised by Viarengo et al. (1997) with modifications by Aly et al. (2014) was employed to extract and purify MT. The procedure was run in triplicate for each sample.

About 3 cm of the front tips of seaweed were dissected. Samples were prepared each one individually by homogenization buffer (15 mM cold Tris–HCl pH = 7.0) in which 10 mM M2-mercaptoethanol and phenylmethanesulfonyl fluoride (PMSF), respectively, were added in volumes of 1:3.0 (w/v) using a Teflon homogenizer (Sigma-Aldrich, Z659428) at 1000 rpm. The obtained homogenates solutions were then centrifuged (Lovibond, Model Z323K) at 12,000*g* for 40 min at 4 °C. The supernatants were heated at 80 °C for 10 min to denature the thermolabile proteins and then centrifuged again at 12,000*g* for 40 min at 4 °C.

### 2.4. PC analysis

To extract and purify PC, the same method proposed earlier by Esmaeili (2015) was followed. However, it was done by applying some slight modifications based on the context of the present research. To avoid the degradation of polypeptides by enzymes, the extraction of thiols was made at 4 °C. For the extraction process, HCl was added to promote the denaturation of enzymes and diethylene triamine Pentetic acid (DTPA) was added as a metal-chelating agent. After centrifugation, the supernatant was removed and 1.2 ml of 0.1 M HCl containing 5 mM of DTPA was added. Then, glass beads were added to the mixture, which was swirled through a vortex (Digital vortex mixer, 3000 rpm, Fisher Scientific) for 2 min. The extract was disrupted by ultrasonication (0 °C, 5 min) two times each lasting 2.5 min and centrifuged at a high speed (12,500*g* for 20 min at 4 °C). The supernatant was used for reduction and derivatization reactions. An aliquot of 250 µl of the supernatant was removed and buffered to a pH of 8–9 by adding 625 µl of 200 mM HEPES and 5 mM DTPA. Then, 25 µl of 20 mM TCEP (tris (2-carboxyethyl) phosphine) was added to break disulfide bonds before derivatization by mBrB (monobromobimane) fluorescent tag. The derivatization of PCs with rnBrB (in acetonitrile, final concentration 1 mM) was done under dim light conditions at room temperature. After 15 min in the dark, 10 µl of mBrB of 100 mM was added and the mixture