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Phytochemical Content and Heavy Metals Level in *Cystoseira Spicata* and *Cystoseira Compressa* from Annaggaza Seacoast

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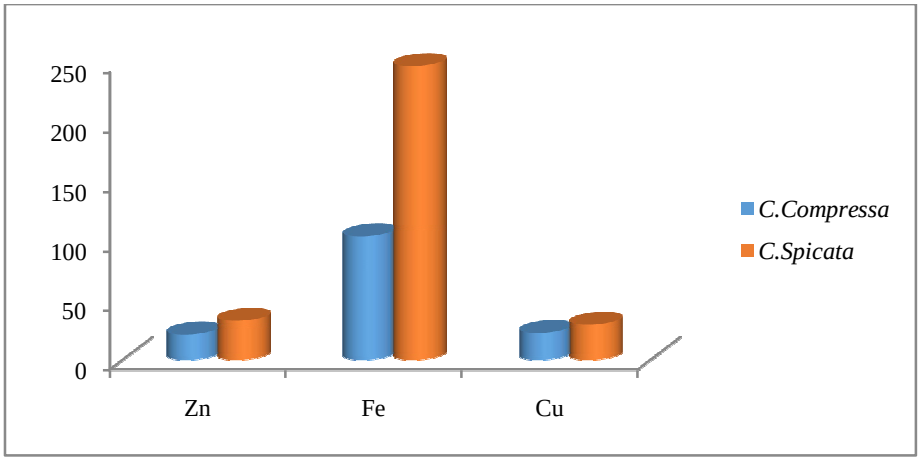
ABSTRACT

In the present study, phytochemical screening and heavy metals levels analysis of two brown algae; Cystoseira spicata and Cystoseira compressa from Annaggaza sea coast, were presented. The phytochemical screening indicated the presence of quinines, proteins, diterpenes, phytosterols, alkaloids, carbohydrates, flavonoids, tannins, glycosides, phenols and saponins. Major and minor elements levels in both algae were also investigated. As denoted in previous studies, iron was detected as a major element in both algae. The elements levels in C. spicata were in the following order: Fe> Zn> Cu> Ni> Cd> Pb, while in C. compressa were Fe> Cu> Zn> Ni> Pb> Cd. In both algae, the levels of toxic metals had the similar order. According to our results, the accumulation of heavy metals in C. spicata was higher than C. compressa.

Graphical Abstract



Basic nutrients Zn, Fe, Cu concentrations (ppm) in studied algae.

Keywords: Phytochemical Screening, *Cystoseira spicata*, *Cystoseira compressa*, Heavy metals.

INTRODUCTION

Approximately 30,000 kinds of algae were found in the sea where sufficient light and moisture present. More than 16,000 marine natural compounds have been isolated from aquatic organisms [**1**]. The four types of algae (brown, blue, red and green algae) are rich in molecules with considerable biological activities such as antiviral, antioxidant, antifungal and antimicrobial [**2**]. In addition to their biological importance as a potential source of new drugs, algae are a source of food for fishes, cattle and man. Seaweeds are traditionally consumed in the orient as part of the daily diet. Currently, human consumption of green algae (5%), brown algae (66.5%), and red algae (33%) is elevated in Asia; mainly in Japan, China and Korea [**3**]. Algae are also used as fertilizers and in cosmetic industry. There are few algae that excrete toxic substances pollute marine water [**1**]. Extensive series of bioactive metabolites were found in seaweeds including brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides, proteins, halogenated ketone, alkanes and cyclic polysulphides [**4**].

Plants and living organisms need heavy metals in different concentrations, some of them are essential in very low concentrations because of their essential nutritious value but they may have harmful effects [**5**]. In aquatic environment, seaweeds absorb heavy metals from the water, and can accumulate high amounts of them, subsequently, they reflect the toxicity of the water environment, and may serve as a tool for the bioindicator of polluted waters [**6**].

Brown algae contain high concentrations of alginic acid and sulfated poly saccharides, which are absent in terrestrial plants, these compounds allow the agae to soak up some metallic ions in aquatic medium *via* ion exchange mechanism [**7**].

In our previous studies, the phytochemical screening and heavy metal contents in Zizyphus Lotus, Codium Tomentosum, Sargassum Hornschuchi, and Nicotiana glauca were conducted [**5, 8, 9**]. The aim of the current study was to investigate the organic and inorganic components of *Cystoseira spicata* and *Cystoseira compressa* (Figure 1) by performing phytochemical screening and monitoring the heavy metal levels in order to evaluate their nutritional and environmental file.

Figure 1. *Cystoseira spicata* and *Cystoseira compressa* .

MATERIALS AND METHODS

Algal Material: Algal material was collected from rocky seaside on nearly 1.5 m depth of Alnaggaza coast near Alkoms city (32°43'00.7"N 14°05'55.0"E) during October 2015. In order to remove epiphytes, debris and other marine organisms, the collected samples were washed thoroughly with tap water then with distilled water. The washed samples were kept in fridge. The taxonomic identity of the samples was confirmed by Marine Biology Research Centre, in Tajuora, Libya. The collected samples were dried in darkness and grinded into fine powder using electrical blender (Hommer 350 Watt) then kept in desiccator.

Preparation of algal extracts: 15.0 grams of *C. Spicata* powder was deposited in soxhlet apparatus thimble and 150 mL of solvent was transferred into 250 mL distillation flask of the soxhlet. Thermal continues extraction process was performed using gradual polarity solvents starting from petroleum ether, chloroform, ethyl acetate and finally methanol. For each solvent, extraction process was held for 6 h till solvent in the flask become colorless. For *C. compressa*, 10.0 g were adapted. Aqueous extract in both cases was prepared separately by heating 10.0 g of algal powder in 150 mL of distilled water at 60ºC for 20 min, the mixture was cooled to room temperature, filtrated, then kept in the fridge to prevent any possible biological reactions.

Wet Digestion Procedure for Heavy Metals Analysis: Algal sample was digested using wet digestion method [**10**]. To 2.0 g of algal powder in 250 mL covered beaker, 3.0 mL of 65 % nitric acid was added then heated gradually to near dryness. After cooling to room temperature, 3.0 ml portion of 65 % nitric acid was added until the sample solution became clear with bright brown color. Clear solution was evaporated and 5.0 mL of conc. HCl and distilled water (1:1) was added then warmed to ensure complete digestion. After cooling, resultant solution was diluted to 250 ml with deionized water. Three samples were prepared using same digestion protocol. The samples were analyzed using atomic absorption spectrometer Shimadzu AA-7000 (Figure 2), the instrument was supplied with Deuterium lamp (D2-lamp) background correction and hollow cathode lamps, air/acetylene flame mixture was used for determination of elements.

Figure 2. Atomic Absorption Spectrophotometer AA-7000 (Flame Model).

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening applied on *C. spicata* extracts has revealed the presence of some secondary metabolites, such as flavonoids, alkaloids, glycosides while other secondary metabolites were absent as illustrated in table 1.

Alkaloids were observed in all extracts when Wagner test was used forming clear reddish brown precipitate, also Hager test was used for all extracts. Dragendroff test was positive with chloroform extract only while Mayer test was positive only with aqueous extract forming yellow precipitate. Alkaloids are the final products of nitrogen compounds metabolism, and stored in the plants for using as a first defence strategy against insects and animals, this important property attributed to the bitter test of these compounds [**11**].

The presence of carbohydrates was noticed in all extracts using Molisch and Fehling tests showing clear results of violet circle and red precipitate respectively, meanwhile, the third carbohydrates test; Benedict test, gave negative results for all extracts except methanolic extract

giving red precipitate. Carbohydrates are very important for all biological processes and are considered the basic source of energy in the body. Algal extracts were poor in glycosides, Borntrager test was negative for all types of extracts, Legal test was negative for all extracts except for ethyl acetate and aqueous extracts whereas pink colour was observed, the presence of glycosides was noticed in methanolic extract only using Keller-Kelani test forming brown circle. Glycosides play an important role in cardiac muscles activation and strengthen blood vessels, in addition to treatment of arthritis [12].

Table 1. Phytochemical Screening of *C.Spicata*

Extract	Test	Pet. ether	$CHCl_3$	EtOAc	MeOH	Aqueous
Alkaloids	Mayer	-	-	-	-	+
	wagner	+	+	+	+	+
	Dragendroff	-	+	-	-	-
	Hager	+	+	+	+	+
Carbhydrates	Molisch	+	+	+	+	+
	Benedict	-	-	-	+	-
	Fehling	+	+	+	+	+
Glycosides	Borntrager	-	-	-	-	-
	Legal	-	-	+	-	+
	Keller-kelani	-	-	-	+	-
Saponines		-	-	-	-	+
phenols		-	+	-	-	-
Tannines		-	+	+	-	-
Phlobatannines		-	-	-	-	-
Phytosterols	Salkowski	-	-	+	+	-
	Libermann-Borchard	+	+	+	+	+
Flavonoids	Basic test	-	+	-	-	+
	Lead acetate	-	+	-	+	+
	Shenoda	-	-	-	-	-
Proteins and	Melon	-	-	-	+	+
amino acids	Buiret	-	-	-	-	-
	Xanthoprotein	-	-	-	+	+
	Ninhydrine	-	-	-	+	+
Diterpenoids		+	+	+	+	+
Quinones		-	-	-	-	-
Anthraquinones		-	-	-	-	-

Saponines are water soluble compounds with thick foam forming property. The performing foam test showed formation of 1.0 cm foam persisting 15 min but only with aqueous extract. Saponines are used mostly in teeth paste and shampoo manufacturing [13]. Libermann-Borchard test for phytosterols gave positive results for all extracts, a clear brown circle was formed. Salkowski test gave positive results only with ethyl acetate and methanolic extracts forming golden yellow color. Phytosterols are present only in plant kingdom and work as natural anti-cholesterol, so if our food contains vegetable products rich in phytosterols, it will work to resist and block the absorption of intestinal cholesterol [14].

Phenolics were appeared only in chloroform extract forming black color. Phenolic class; tannins, was observed only in chloroform and ethyl acetate extracts where white precipitate was formed. Phlobatannins were absent in all extracts. Tannins are well known in leather tanning industry, also Tannins used in the treatment of gastrointestinal disorders due to their effect on the intestines and because of its disinfectant property which cause constriction of blood vessels and also used to stop bleeding and treatment of wounds and sores [15].

Basic test for flavonoids showed positive result only with methanol and chloroform extracts, while lead acetate test gave similar results in addition to aqueous extract forming strong yellow color in both tests. Flavonoids are a class of chemical compounds responsible of some fruits and seeds and vegetables colors, Flavonoids show important biological activity including Anti-inflammatory, anti-

histamine, anti- viral viruses and anti- oxidation. Flavonoids have strong effects on the protection of low-density lipoproteins from oxidation and lowering the level of cholesterol, which represent additional protection against heart disease and various cancers [16].

Proteins and amino acids tests revealed presence of them in methanolic and aqueous extracts forming white precipitate with Melon test and yellow precipitate with Xanthoprotein test. Buiret and Ninhydrine tests were negative with all extracts. Diterpenes were found in all extracts as green color was formed with cupper acetate test. This type of terpenes exhibits important biological activity against bacteria and cancer cells and have significant cardiovascular effects, also work as growth hormones in some plants, in addition to using as insecticide [17]. Quinones and anthraquenones tests were negative with all extracts.

Phytochemical screening of *C. compressa* was shown in table 2, alkaloids were found in all extracts when Wagner and Hager tests were applied while Dragendroff and Mayer tests were negative for all extracts. Carbohydrates were also exist in this kind of algae, Molisch and Fehling tests were positive with all extracts while red precipitate was formed with methanolic and aqueous extracts only using Benedict test.

Table 2. Phytochemical screening of *C. Compressa*

Extract	Test	Pet. ether	$CHCl_3$	EtOAc	MeOH	Aqueous
Alkaloids	Mayer	-	-	-	-	-
	wagner	+	+	+	+	+
	Dragendroff	-	-	-	-	-
	Hager	+	+	+	+	+
Carbhydrates	Molisch	+	+	+	+	+
	Benedict	-	-	-	+	+
	Fehling	+	+	+	+	+
Glycosides	Borntrager	-	-	-	-	-
	Legal	-	-	-	-	+
	Keller-kelani	-	-	-	-	-
Saponines		-	-	-	-	-
phenols		-	+	-	-	+
Tannines		-	+	+	-	-
Phlobatannines		-	-	-	-	-
Phytosterols	Salkowski	+	-	-	+	+
	Libermann-Borchard	-	+	+	+	+
Flavonoids	Basic test	-	+	+	-	+
	Lead acetate	-	+	-	+	+
	Shenoda	-	-	-	-	-
Proteins and amino acids	Melon	-	-	-	-	+
	Buiret	-	-	-	-	-
	Xanthoprotein	-	-	-	+	+
	Ninhydrine	-	-	-	+	+
Terpenes		-	-	-	-	-
Diterpenoids		+	+	+	+	+
Quinones		-	-	-	-	+
Anthraquinones		-	-	-	-	-

All glycosides tests (Borntrager, Legal, Keller-Kelani) were negative with all extracts except for aqueous extract with Legal test as pink color was observed.

The Froth formation, the usual saponines presence indication test, was negative for all extracts. Phytosterols were clearly present in this species, Salkofwky test was positive with all extracts except chloroform and ethyl acetate extracts. Liberman-Borchard test was positive with all extracts as brown circle was formed, except ether extract.

The polar compounds; phenolics were found only in chloroform and aqueous extracts forming bluish black color. The polyphenols; tannins, present in chloroform and ethyl acetate extracts as white precipitate was formed while phlobatannines were absent in all extracts. Other polar class, flavonoids were found in all extracts except ether extract using basic and lead acetate tests. Shenoda test was negative for all extracts.

Proteins and amino acids tests were negative mostly, positive results observed with aqueous extract using Melon test. Xanthoprotein and ninhydrine tests gave positive results with both methanolic and aqueous.

General terpenes test was negative except diterpenes which were present in all extracts as green color was observed using cupper acetate test. Anthraquinones were absent in all extracts but quinones were observed only aqueous extract.

Heavy Metals levels: Algae are considered as significant indicator for heavy metals poisonings in aquatic environments due to their ability of accumulating the heavy metals. Algae binds with metals only in free ionic state and the absorption process may effected with evolution season, sample collection location, water salinity and water temperature [18].

Heavy metals levels assessment in algae is better than in sea waters or sediment due to small and changeable concentrations of metals in sea water. This change in the concentration attributed to many factors such as; acidity and salinity of water, temperature, light, oxygen and basic nutrients. Same issue considered in measurement of heavy metals in sediment due to effect of sediment particles size, pH and organic matter content. The accumulation process occurs in two stages; firstly during physical then chemical adsorption on algal material surface, the final step is slow metabolism in algae cells leading to accumulating the metals continuously with time [19]. Six heavy metals concentrations (in ppm) were assessed, Fe, Cu, Zn, Ni, Cd and Pb as shown in table 3.

Table 3. Heavy metal concentrations in *C. Compressa* and *C. Spicata*

The Metal	*C. Compressa*		*C. Spicata*	
	Conc. (ppm)	SD	Conc. (ppm)	SD
Pb	0.37	± 0.014	0.19	± 0.03
Cd	0.17	± 0.001	0.23	± 0.02
Zn	21.30	± 1.42	32.75	± 1.28
Fe	103.82	± 0.65	246.99	± 1.47
Cu	22.54	± 0.17	29.46	± 0.17
Ni	2.08	± 0.04	3.84	± 0.02

From the results illustrated in table 3, the concentrations of basic elements Cu, Fe and Zn in *C. spicata* (29.46, 246.99, 32.75 ppm) are higher than their level in *C. compressa* (22.54, 103.82, 21.30 ppm respectively), a graphical comparison shown in figure 3.

Clearly, the concentration of iron in both algae was the highest in both algae which is similar to Al-Khafji-Saudi Coast results in Saudi Arabia [20]. For *C. myrica*, comparable levels of iron, 533 ppm to our results. In the study conducted in Egypt, high level of iron, 467 ppm, was found in *C. crinite* which collected from Marsa Matrouh seacoast [21]. Zinc and copper levels in the investigated algae were comparable to previous studies. High Zn levels were recorded in Dammam coast in Saudi Arabia study (75.25 ppm) and lower level of Cu in Marsa Matrouh coast study (3.26 ppm), heavy metals levels comparison with other studies was shown in table 4.

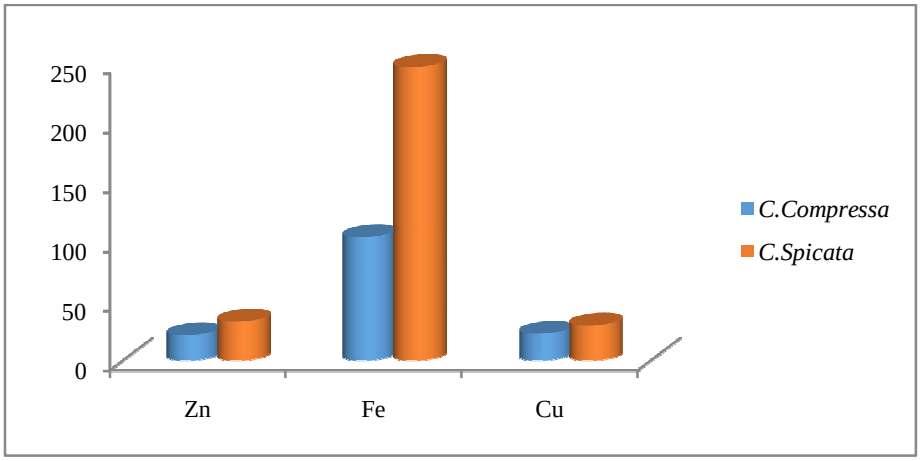


Figure 3. Basic nutrients Zn, Fe, Cu concentrations (ppm) in studied algae.

Table 4. A comparison between the examined metal levels in different brown alga

Algae	Cd	Cu	Fe	Ni	Pb	Zn	Location
C. myrica	1.33	10.80	533	-	8.84	28.22	Al-Khafji-Saudi Coast of the Arabian Gulf.
	0.62	6.65	1.002	-	14.00	75.25	Al-Dammam -Saudi Coast of the Arabian Gulf
C .crinite	0.34	3.46	73.48	5.41	19.75	27.00	Romila- Marsa Matrouh- Egypt.
	0.44	19.25	467.58	8.79	18.86	25.74	Alam El-Roum- Marsa-Matrouh- Egypt.
	0.83	3.26	72.38	7.32	16.32	30.98	Mina Hashish- Marsa-Matrouh- Egypt
C.compressa	0.1704	22.54	103.82	2.08	0.3715	21.3	Al nnagazza coast –Libya
C. spicata	0.2304	29.46	246.99	3.84	0.1935	32.75	Al nnagazza coast –Libya

Zinc and Copper levels were similar in both algae, but by comparing with other algae in the table 4, Cu levels in studied samples were considerably high (except Alam Elrom sample, 19.25 ppm) while Zn levels were in the same range. According to table 3, Cadmium concentrations in both algae were comparable, but Lead levels in *C.compressa* were nearly two fold as in *C. spicata*. Nickel concentration was notably higher than the other two toxic elements and was in *C. spicata* as high as double in *C.compressa*. A graphical comparison was shown in figure 4.

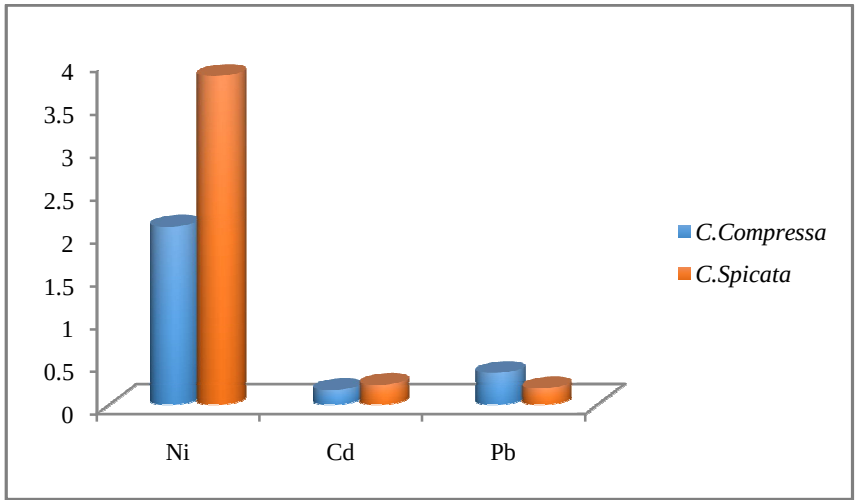


Figure 4. Toxic metals levels (ppm) Pb, Cd and Ni in studied algae.

By comparing with the above mentioned studies; as shown in table 4, the study area in this research clearly is less contaminated by toxic metals. The toxic metals ions are not only potential human health hazards but also to other life forms. Toxic metal ions cause physical discomfort and sometimes life-threatening problems by intervening some vital biological reactions in our bodies. Generally, the most

dangerous metals in this study are lead and cadmium followed by the less toxicity metals Cu, Zn and Ni then Fe [22].

The dangerous aspect of metals is their highly reactive free radical state, they attacking cellular structures causing disruption of the fundamental biological processes in cellular molecules, such as protein, enzyme and DNA. Also, replacement of certain essential metals with similar metals could cause serious damage to the cell. For example cadmium can substitute for the essential metal zinc in certain protein that requires zinc for their structure and function. The modification in protein chains also can lead to toxic consequences. In the same way, lead could replace calcium in bones and in other sites where calcium is required [23]. Lead is extremely toxic and can damage the nervous system, kidneys, and reproductive system, particularly in children while chronic exposure to high levels of cadmium is known to cause renal dysfunction, bone degeneration, liver damage, and blood cells damage. Cadmium has a half-life of 10-30 years, and its accumulation in human body affects kidney, bone and also causes cancer [22].

APPLICATION

The metals concentrations data could be used as a bio alert for toxic metals accumulation and very important indicator for aquatic environment pollution.

CONCLUSION

Phytochemical analysis of *Cystoseira spicata* and *Cystoseira compressa* extracts reveled the richness of marine life in this area with useful chemicals which may use in pharmacological industry. Additionally, important heavy metals (Fe, Cu, Zn, Ni, Pb, Cd) levels measurements showed low content of the toxic metals Ni, Pb and Cd.

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