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Metals Content in Green Algae *Ulva lactuca* from Dakar coast (Senegal)

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Abstract

Green algae *Ulva lactuca* from Dakar coast (Senegal) were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Microwave acid digestion was also employed for metals (As, Mn, Ni, Co) determination. Dakar coast usually receives numerous domestic and industrial discharges without prior treatment. The contents of nickel were, in all cases, higher than other metals. However, green algae Ulva lactuca present themselves as effective bio-monitors when assessing marine aquatic pollution by contaminants in Dakar coast. Pearson's correlation shows that most of the four metals studied have significant correlation coefficients at the 0.01 level. ANOVA analysis allows concluding that significant differences were found between algae from different sampling points during different season. In all cases, the April samples have a higher content than those collected in January, August and December.

Keywords:* *Ulva lactuca; Metals content; Dakar coast; Pollution

Introduction

The increase in pollutants in estuaries and oceans, in particular that of heavy metals, has prompted numerous studies in different parts of the world. These studies investigated the accumulation of trace metals in algae [1]. Algae play an important role in the functioning and balance of all aquatic ecosystems. They also participate significantly in the economic activities of certain countries [2,3].

Dakar is an important urban center situated in the Atlantic coast of Senegal (Africa) and, besides the port, account with diverse industrial units largely involved in the manufacture, storage and use of chemicals, petrochemicals, agrochemicals, etc. [4]. Important discharges both treated and untreated coming from these industrial activities together with other anthropogenic inputs (coastal tourism, fishing activities, etc.) dumping to the Dakar coast can threaten the aquatic ecosystem [5,6]. For these reasons, some scientific studies have been developed in the zone to become aware of the situation, but any of them have been done about heavy metals contamination in marine algae.

It seemed important to us to carry out research on the levels of metals in seaweed to avoid possible contamination of the population. The main objective of this work is to evaluate the content of metals (As, Mn, Ni, Co) in green algae samples of Ulva lactuca from Dakar coast. These results provide essential information to assess the state of pollution of the study area and the impact of various human activities on the marine environment.

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Materials and Methods

Instrumentation and reagents

Heavy metal determination was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A domestic microwave oven (Moulinex, 900 W power) was used for microwave heating. A 45 mL capacity Parr reactor (model 4782) was used for acid digestion of all analysed samples. A ball-mill (Retsh, model S100) equipped with a 250 mL capacity agate cup was used for grinding the algae samples. A nylon sieve (70 µm) was used to homogenize the particle size of the samples.

All reagents employed were of analytical reagent grade and all of them were supplied by Merck. High purity water (Millipore Milli-Q System) was used throughout. Pearson correlation coefficient was employed for the better understanding of relationship between the concentrations of various metals by using statistical package of IBM-SPSS (version 19). The ANOVA analysis was used to understand the variation in the concentration of heavy metals studied compared to algae samples taken from the study area.

Study area

Analysis Dakar region is located on the Cape Verde peninsula and covers an area of 550 km², or 0.28% of the national territory. It is between 17° 10' and 17° 32' West longitude and 14° 53' and 14° 35' North latitude. It is limited to the east by the region of Thies and by the ocean Atlantic in its northern parts, West and South. The study area is characterized by high human pressure estimated at 3.529.300 inhabitants, intensive fishing activity and several bathing areas; however, also receives domestic and industrial waste from the city of Dakar and surrounding industrial zones. This pollution is materialized by an ocean considered as a wastewater discharge, bays receiving water from the wastewater evacuation channels [7,8].

Sampling sites

In **FIG. 1** are shown the sampling points in the corresponding localities. Green algae were sampled at four stations selected on the coast of Dakar. The choice of sites is based, in addition to the presence of algae, on the proximity of the effluent discharges, in order to assess their pollutant flow and their impact on the receiving marine environment [9]. The description and nature of the wastewater outlets are summarized as follows:

- Locality of Mbao (M1) is located next to the African Society of Petroleum Refining (SAR) and the power station (Cap des Biches)
- Municipality of Hann (M2) houses a textile factory, as well as factories processing marine products and the east channel draining wastewater to the sea
- Soumbedioune beach (M3) is a place of landing of fishery products and receives numerous urban water discharges through the western channel or channel IV
- Port of Dakar (M4) is currently active with frequent transshipment of oil. From there depart the pipelines that supply various oil companies of the capital

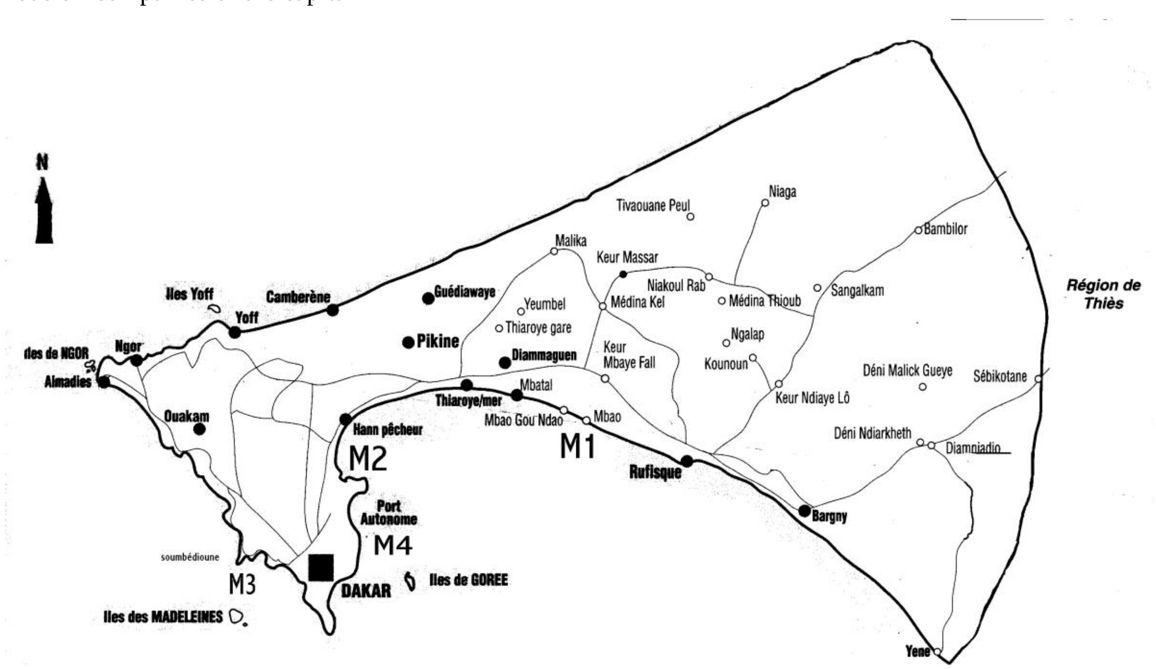

FIG. 1. **Location of sampling sites algae along the coast of Dakar (Senegal).**

Sample collection and preparation

All marine algae were collected in each sampling position shown in **FIG. 1** between January, April, August and December 2020. The collected algae were stripped of their epiphytes and debris adhering to their thalli and then placed in plastic bags. In the laboratory, the algae are dried at 40°C for 72 h. The dried seaweed is then crushed to obtain a fine powder which will be used for mineralization [10].

Chemical analysis

The mineralization of algae samples for heavy metals determination was performed by acid digestion using a microwave procedure. A portion of dry algae samples (about 0.15 g) was weighted and placed in the PFA vessel of the Parr reactor and 4 mL of nitric acid (65%) were added. The vessel was closed and heated in the microwave oven during 2 minutes at 450 W of power. After cooling, the reactor was opened and 1 mL of hydrogen peroxide (30%) was added to complete the sample decomposition by heating again during 1 minute at the same power. Finally, the resultant solution was quantitatively transferred into a 25 mL volumetric flask and diluted to volume with ultrapure water. The final solution was stored, at 4°C, in stoppered glass bottles until analysis by ICP-MS. The corresponding blank solution was prepared in a similar way but without any sample added [11].

Results and Discussion

Characteristics of seawater on the coast of the city of Dakar during the January-December 2020 campaign are reported in **TABLE 1**. The average values of the physico-chemical parameters (pH, temperature, salinity and conductivity) are given for a series of four measurements and their relative standard deviation.

TABLE 1. **Parameters (pH, temperature, salinity and conductivity) found in samples studied**

Parameters	M1	M2	M3	M4
pH	7.75 ± 0.12	7.11 ± 0.23	7.34 ± 0.3	7.60 ± 0.2
Temperature (ºC)	28.4 ± 1.2	27.6 ± 1.6	24.8 ± 0.9	29.5 ± 1.4
Salinity (%)	29.4 ± 1.2	26.8 ± 0.9	27.4 ± 1.1	30.1 ± 2.3
Conductivity (µS/cm)	1922 ± 10.5	1634 ± 20.2	1545 ± 11.1	1696 ± 14.3

The study of the physico-chemical parameters of seawater during the annual cycle provides information on the state of the quality of marine water on the Dakar coast. During sampling, pH, temperature, salinity and conductivity were measured for the different samples studied. The temperature measured in the waters coming from the coasts of Dakar varies between 24.8°C and 29.5°C, which complies with European standards for liquid. We notice that the average pH values obtained (7.11-7.75) in the four studied sites M1, M2, M3 and M4 are less than 8, indicating a slight acidification of the sampling media. The salinity of the surface waters of the Dakar coast varies between 26.8% and 30.1% during the annual cycle, while the relatively high values of conductivities (1545-1922) indicate the highly mineralized character of the coastal water. of the city of Dakar. These preliminary results can therefore introduce the presence of mineral pollutants because this increase in conductivity is not associated with significant acidity.

The physico-chemical parameters measured above were followed by qualitative assays of the metal ions likely to be present in the samples. According to our means of analysis, we focused on four metallic elements (As, Mn, Ni, Co). The results of the assay revealed the presence of these metals in the algae studied. These elements were then chosen for further study.

In the present study, four trace elements were analyzed in green algae Ulva lactuca: arsenic (As), manganese (Mn), nickel (Ni), cobalt (Co). The average levels of trace metals studied in green algae at four sites are shown in **TABLE 2**. All the values are expressed in µg/g (dry weight) and they are given as mean of four separated determinations and their standard deviation.

TABLE 2. **Mean concentration of heavy metals (µg/g) in algae sampled in four stations (M1 to M4) of Dakar coast during different season from Dakar coast.**

Metal	Sampling points	Season			
		January	April	August	December
As	M1	10.7 ± 0.9	13.2 ± 0.4	9.3 ± 0.5	7.9 ± 0.4
	M2	14.3 ± 0.8	16.4 ± 0.9	12.7 ± 0.5	11.2 ± 0.6
	M3	8.7 ± 0.6	9.5 ± 0.8	6.9 ± 0.8	8.3 ± 0.4
	M4	6.5 ± 0.5	5.2 ± 0.2	8.4 ± 0.9	5.6 ± 0.4
Mn	M1	12.5 ± 0.6	14.7 ± 0.9	11.6 ± 0.8	9.4 ± 0.5
	M2	20.2 ± 1.1	24.3 ± 0.9	21.2 ± 0.6	18.9 ± 1.3

	M3	15.3 ± 0.9	17.5 ± 0.7	18.1 ± 0.6	16.2 ± 0.8
	M4	10.2 ± 0.8	14.5 ± 1.0	12.7 ± 0.6	9.8 ± 0.4
Ni	M1	42.2 ± 1.8	39.7 ± 0.9	43.6 ± 1.2	41.3 ± 0.8
	M2	17.2 ± 0.4	20.3 ± 0.7	15.6 ± 0.9	14.8 ± 0.8
	M3	30.4 ± 0.7	28.6 ± 1.5	33.3 ± 0.9	31.5 ± 0.6
	M4	10.2 ± 0.8	14.5 ± 0.9	11.8 ± 0.7	10.8 ± 1.1
Co	M1	3.6 ± 0.8	6.7 ± 0.7	4.3 ± 0.8	5.4 ± 0.9
	M2	8.8 ± 0.4	10.2 ± 0.9	9.1 ± 0.3	7.5 ± 0.6
	M3	3.4 ± 0.9	2.6 ± 0.7	4.5 ± 0.5	3.7 ± 0.4
	M4	2.9 ± 0.6	4.4 ± 0.2	3.2 ± 0.4	2.3 ± 0.5

The levels of metals, found in samples of algae from the Dakar coasts, vary between 2.3 and 43.6 µg/g dry weight. The locality of Mbao has the lowest values, while the port of Dakar has the highest values. Samples were taken in four campaigns carried out within one year (January, April, August, December). According to the results obtained, the nickel had the highest contents and the lowest contents were noted at the level of cobalt (**FIG. 2**). The considerable levels of nickel obtained in green algae collected from the studied areas of the city of Dakar may be linked to the abundance of this metal in seawater [12]. This abundance is also linked to the sea currents responsible for the upwelling of deep waters, especially rich in trace metals [13]. The low cobalt values can be attributed to the dilution effect caused by the tide.

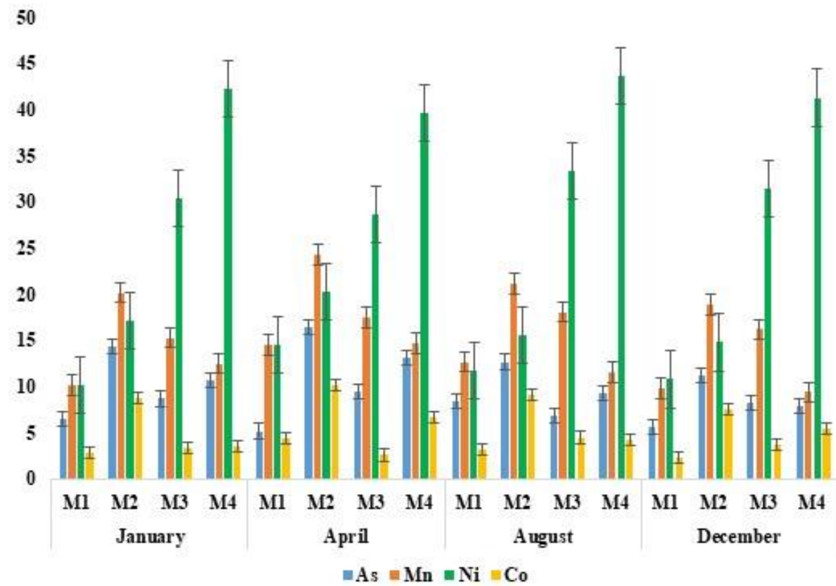

FIG. 2. **Metals content (As, Mn, Ni, Co) in algae samples taken in January 2020 until December 2020, as determined using ICP-MS analysis.**

This variability in results can be attributed to the cell wall of macroalgae, which is considered an important site for the complexation of metal cations [14]. For this reason, algae can be considered as good bioindicators of metal contamination [15-17]. To this end, several studies, using different species of algae (Ulva and Enteromorpha), have been developed [18,19].

Some macroalgae are able to produce exudates (low molecular weight proteins, glutathione, phytochelatins and phyto-metallothionins) which will compete with algal sites for metals, thus reducing the incorporation of metals into cells [20,21].

The levels of metals in algae sampled in different countries have been summarized by various authors, but it is difficult to compare the results of these studies with the present research due to differences in sample handling and analytical procedures.

The overall results presented in **TABLE 2**, are summarized in the box diagram of **FIG. 3**, which shows that there are significant differences between the samples of algae collected during the months of January, April, August, and December. In fact, the percentiles (between 5% and 75%) do not show a coincidence in any way. It seems that the samples of green algae collected during the month of April have in all cases the highest metal contents than those collected in other months (January, August, December).

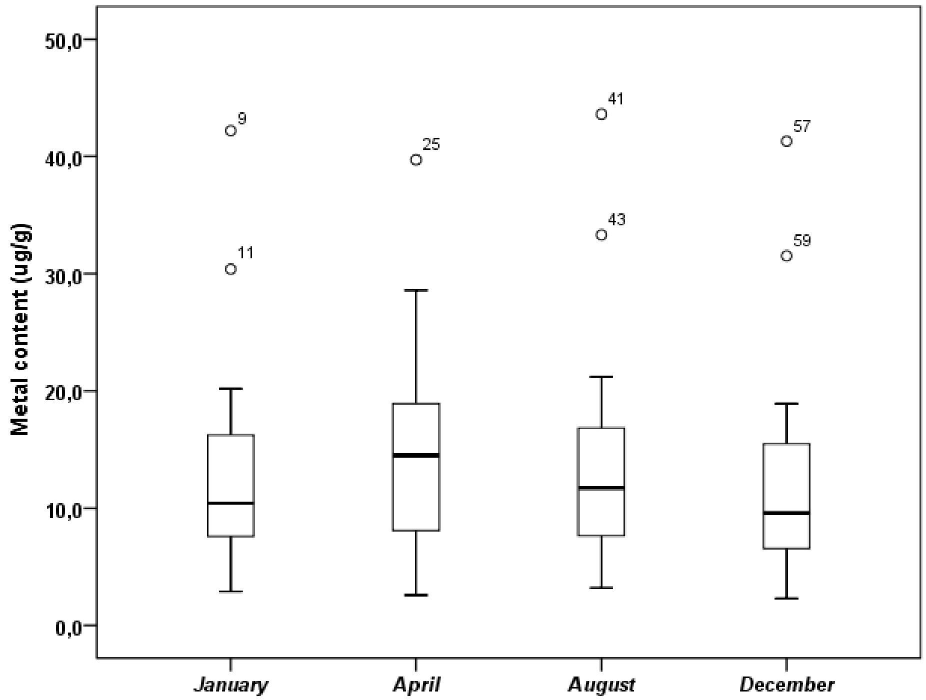

FIG. 3. **Box diagrams to see the difference in metals between the four samplings.**

The metallic contents found in samples of green algae Uva lactuca, from the Dakar coast, do not seem high enough to lead to a certain risk of contamination [22]. Nickel, an essential metal, appears to be the most abundant. The comparison of our data with those provided by the literature, for the same species, collected in polluted areas or not, shows that the levels of the metals As, Mn, Ni, Co are lower than the limit values of the literature [23].

In order to obtain a better interpretation of the results obtained between the metals studied in the green algae of the Dakar coast, a principal component analysis was applied. In the principal component analysis, metals are divided into 2 groups, with a cumulative variance of 89.77% of the system. The factor is associated with a cumulative variance of 63.60% and a variance of 26.17%. In a group, only Ni would go, because it is fundamentally associated with component or factor 2 (0.983) and barely associated with factor 1 (-0.137). Other metals (As, Co and Mn) can be included in the other group, because they are fundamentally associated with component or factor 1 (between 0.898 and 0.941) and very little associated with factor 2 (between -0.125 and 0.254). The factor loadings obtained by principal component analysis are listed in **TABLE 3**.

TABLE 3. **Factor loadings obtained by Principal Component Analysis (PCA)**

Metal	**Component Matrix**	
	PC1	**PC2**
Co	0.941	0.015
As	0.913	0.254
Mn	0.898	-0.125
Ni	-0.137	0.983

The graphic representation of the two main components extracted is shown in **FIG. 4**. It is observed that the metals (As, Co and Mn) exhibit a similar behavior in all the algae studied from the Dakar coast and therefore, are positioned together. This indicates a positive relationship between the contents of arsenic (As), cobalt (Co) and manganese (Mn) in all the algae analyzed. This fact can be attributed to similar sources of metal contamination, probably linked to anthropogenic activities developed in the study areas (chemical and agrochemical industries, seafood factories, petroleum refining, etc.). Wan et al. (2017) stated that the concentrations of the metals found in Ulva lactuca algae were site dependent [24]. Overall, the comparison of heavy metal contents in the studied samples showed that Ulva lactuca is a suitable plant for bio-monitoring studies.

Finally, it is interesting to make a correlation analysis between heavy metal contents in all analyzed samples in order to make predictions in algae samples. In the case of algae samples from the coast Dakar, the highest concentrations of nickel Ni (43.6 µg/g) were measured around the port of Dakar. Pearson's correlation shows that most of the four metals studied have significant correlation coefficients at the 0.01 level (**TABLE 4**).

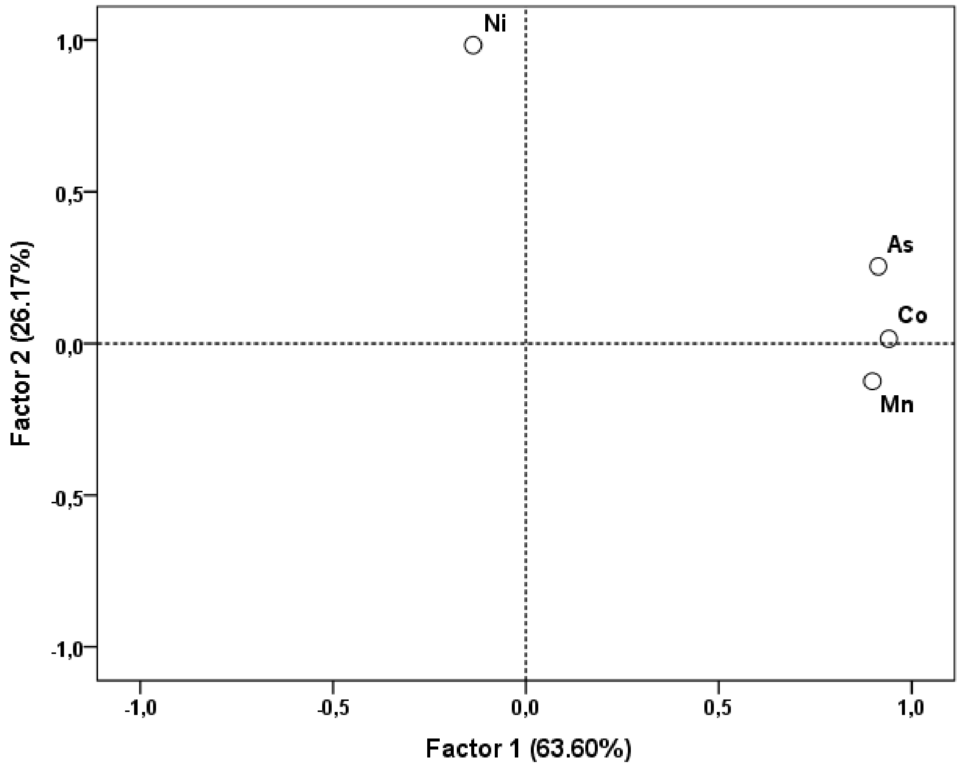

FIG. 4. **Box diagrams to see the difference in metals between the four samplings**.

TABLE 4. **Pearson correlations between the metals analysed**

Correlations					
		As	**Mn**	**Ni**	**Co**
As	Correlation de Pearson	1	0.713**	0.089	0.832**
	Sig. (bilatérale)		0.002	0.742	0
	N	16	16	16	16
Mn	Correlation de Pearson	0.713**	1	-0.2	0.753**
	Sig. (bilateral)	0.002		0.453	0.001
	N	16	16	16	16
Ni	Correlation de Pearson	0.089	-0.202	1	-0.119
	Sig. (bilateral)	0.742	0.453		0.661
	N	16	16	16	16
Co	Correlation de Pearson	0.832**	0.753**	-0.12	1
	Sig. (bilateral)	0	0.001	0.661	
	N	16	16	16	16
**. The correlation is significant at the 0.01 level (bilateral)					

Conclusion

Metal content (Mn, Ni, Co, As) was measured in green algae Uva lactuca from Dakar coast during four campaigns carried out in an interval of one year (January, April, August and December). The results obtained indicate that algae accumulate much more nickel than other metals. However, the highest levels of nickel have been observed around the port of Dakar, near the port's multipurpose ore loading facilities, where large amounts of these metallic ores are loaded. This provided reasonable indications of marine pollution caused by spills during loading. Our current study confirms that marine algae are good indicators for evaluating marine aquatic pollution, especially when looking for trace elements. The algae monitoring program in the Dakar region will continue and the results of our study will serve as guidelines for further sampling, as well as a basis for estimating future trends. Pearson's correlation shows that most of the four metals studied have significant correlation coefficients at the 0.01 level. The ANOVA analysis allows to conclude that significant differences were found between the samples of algae taken between the different periods (January, April, August, December). In all cases, samples taken in April have higher levels than those collected in other months.

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