DOI: 10.24850/j-tyca-2021-03-04

Articles

Lead, chromium, nickel, copper and zinc levels in *Sargassum* species reached the coasts of Dominican Republic during 2019: A preliminary evaluation for the use of algal biomass as fertilizer and animal feeding

Niveles de plomo, cromo, níquel, cobre y zinc en especies de *Sargassum* llegadas a las costas de República Dominicana durante 2019: una evaluación preliminar para el uso de la biomasa algal como fertilizante y en alimentación animal

Pamela Tejada-Tejada[1], ORCID: https://orcid.org/0000-0002-8707-5755

Yaset Rodríguez-Rodríguez[2], ORCID: https://orcid.org/0000-0003-3937-2031

Luis Enrique Rodríguez de Francisco[3], ORCID: https://orcid.org/0000-0001-9146-8634

Omar Paíno-Perdomo[4], ORCID: http://orcid.org/0000-0002-9937-0153

Carlos José Boluda[5], ORCID: https://orcid.org/0000-0001-8778-700X

[1]Instituto Tecnológico de Santo Domingo (INTEC), Basic and Environmental Sciences Area, Santo Domingo, Dominican Republic, pamelatejada11@gmail.com

[2]Instituto Tecnológico de Santo Domingo (INTEC), Basic and Environmental Sciences Area, Dominican Republic, yaset.rodriguez@intec.edu.do

[3]Instituto Tecnológico de Santo Domingo (INTEC), Basic and Environmental Sciences Area, Santo Domingo, Dominican Republic, luis.defrancisco@intec.edu.do

[4]Instituto Tecnológico de Santo Domingo (INTEC), Basic and Environmental Sciences Area, Santo Domingo, Dominican Republic, omar.perdomo@intec.edu.do

[5]Instituto Tecnológico de Santo Domingo (INTEC), Basic and Environmental Sciences Area, Santo Domingo, Dominican Republic, carlos.boluda@intec.edu.do

Corresponding author: Carlos José Boluda, carlos.boluda@intec.edu.do

Abstract

The repetitive invasive episodes of *Sargassum natans* and *Sargassum fluitans* on the Caribbean coasts during the last years have become a first-order problem threatening tourism, fishing, and local fauna. New massive seaweed arrivals are nowadays expected and could be considered in the near future a normal event, with its associated problems. Appropriate

solutions, taking advantage of algal biomass, are necessary to overcome this problem. Due to the well-known ability of these algae to accumulate heavy metals, applications related to animal feeding and agriculture must necessarily be preceded by chemical analysis that guarantees the harmlessness of the algal material. In this research, the contents of lead (Pb), chromium (Cr), nickel (Ni), copper (Cu), cadmium (Cd) and the less toxic zinc (Zn) in *S. natans* and *S. fluitans* arrived in the dominican coast during 2019 were analyzed, by using flame atomic absorption spectroscopy (FAAS). The results showed significant levels of copper, although the concentration of all detected metals were within the normal values, reflecting the safety of the algal material as far as these metals are concerned, for use as fertilizers and animal feed. No significant differences in the contents of these elements were found between both species.

Keywords: FAAS, heavy metals, quantification, *Sargassum*, toxicity.

Resumen

Las invasiones repetidas de *Sargassum natans* y *Sargassum fluitans* en las costas del Caribe durante los últimos años se han convertido en un problema de primer orden que amenaza al turismo, pesca y fauna local. Se esperan nuevas llegadas masivas de algas que podrían considerarse, en un futuro cercano, un evento normal, con su problemática asociada. Para resolver este problema resulta necesario encontrar soluciones apropiadas que aprovechen la biomasa algal. No obstante, debido a la conocida capacidad de estas algas para acumular metales pesados, las

aplicaciones relacionadas con la alimentación animal y la agricultura deben ir precedidas necesariamente de un análisis químico que garantice la inocuidad de la biomasa. En esta investigación se determinaron las concentraciones de plomo (Pb), cromo (Cr), níquel (Ni), cobre (Cu), cadmio (Cd) y el zinc menos tóxico (Zn) en la biomasa de *S. natans* y *S. fluitans* que llegaron a las costas de República Dominicana durante 2019. Para ello se utilizó la técnica de espectroscopía de absorción atómica de llama (FAAS). Los resultados mostraron niveles significativos de cobre, aunque la concentración de todos los metales detectados estuvo dentro de los valores considerados normales, lo que refleja la seguridad del material algal, en lo que a estos metales se refiere, para su utilización como fertilizantes y alimentación animal. No se encontraron diferencias significativas en el contenido de estos elementos entre ambas especies.

Palabras clave: FAAS, metales pesados, cuantificación, *Sargassum*, toxicidad.

Received: 20/05/2020

Accepted: 10/08/2020

Introduction

The genus *Sargassum* comprises about 359 taxa (species and infraspecific taxa) of brown algae (Aouissi *et al*., 2018) (Fucales, Phaeophyceae, Heterokontophyta) that owe their colour to the carotenoid fucoxanthin, that masks the chlorophylls (Peng, Yuan, Wu, & Wang, 2011; Gutiérrez, González, Valdés, Hernández, & Acosta, 2016). *Sargassum natans* (Linnaeus) Gaillon and *Sargassum fluitans* Børgesen (Børgesen) are highly branched pelagic brown seaweeds provided with long phyllodes and serrated edge that reproduce by vegetative fragmentation of the thallus (Széchy, Guedes, Baeta-Neves, & Oliveira, 2012). The free-floating ability of these species is due to the presence of spherical air bladders, provided by a spicule in the case of *S. natans*, known as aerocyst. The clear morphological differences in the phyllodes and air bladders make possible to easily differentiate both species (Moreira & Alfonso, 2013; Oyesiku & Egunyomi, 2014). These algae form thick rugs and house a high marine biodiversity with multiple endemic and non-endemic species including cnidarians, platyhelminthes, crustaceans, molluscs, and fish, thus constituting an important ecosystem (CIT, 2015). Its pelagic existence combined with sea currents makes possible these algae massively reach the coasts of the Caribbean Region. The sargasso arrival happened in 2011 and 2015, affecting deeply the Greater and Lesser Antilles (Direction de l´Environnement, de l´Aménagement et du Logement, 2015), show the importance of these events for the economy and ecology of these islands. Both algae have a wide geographical distribution, especially *Sargassum fluitans* (Széchy *et al*., 2012), and its origin was typically

attributed to the oligotrophic waters of the Sargassum Sea, located in western North Atlantic Ocean. Nevertheless, the origin of the 2011 sargasso invasion, which largely affected the Caribbean, originated much further south, in a region, with an approximate latitude range of 5-10 °N and a latitude range of 60-10 °W and related with Amazon and Congo Rivers discharge (Gower, Young, & King, 2013; Johnson, Ko, Franks, Moreno, & Sánchez-Rubio, 2013). Since then, satellital images seem to confirm the periodic blooms of sargasso in an equatorial region between the African coast and Brazil forming a great Atlantic *Sargassum* belt (GASB). This may mean a new pattern of recurrent outbreaks and invasions of the Caribbean coast by this *Sargassum* species (Wang *et al*., 2019).

The accumulations of these species exert a negative impact on tourism (León, 2015; Mohammed *et al*., 2019) and fishing (Mohammed *et al*., 2019) and suppose relevant risks to human health by inhalation of hydrogen sulfide, produced by the decomposition of algal biomass (Oyesiku & Egunyomi, 2014; Resiere *et al*., 2018; Resiere, Mehdaoui, Névière, & Mégarbane, 2019). Additionally, the massive accumulation and decomposition of algae material on the shore have a negative effect on local fauna, with a high incidence in fish and crustaceans. This is due to the combined action of a lack of oxygen and a high concentration of hydrogen sulfide and ammonium ion (Rodríguez-Martínez *et al*., 2019).

However, algal biomass can be valuable since it can be used as fertilizer after desalination. In addition, species of this genus contain different compounds of interest such as alginates, polysaccharides of wide

applications in cosmetics, pharmaceutical, and food industries (Moreira & Alfonso, 2013).

The extracts of *Sargassum natans* and other *Sargassum* species have shown significant therapeutic potential, suggesting these seaweeds could provide novel functional ingredients for pharmaceuticals as an application in the treatment and prevention of several disorders (Milledge & Harvey, 2016).

In any case, the use of algal biomass as fertilizer and human and/or animal feeding requires an analysis that guarantees its chemical safety. The ability of marine algae to effectively retain heavy metals is well documented (Sheng, Ting, Chen, & Hong, 2004; Khan *et al*., 2015). *Sargassum* can absorb heavy metals as oxides, hydroxides, and oxyhydroxides from seawater, hence, its consumption is of concern (Oliveira, Hammer, Guibal, Taulemesse, & García, 2014; Barquilha, Cossich, Taveres, & Silva, 2019; Fernández-Martínez *et al*., 2015). Algaes accumulate free metal ions, the concentrations of which depend on the nature of suspended particulate matter, which, in turn, is formed by both organic and inorganic complexes (Tamayo, Guas, Leyte-Vidal, & Maccini, 2014). It is known, the cell walls of algae are composed of polysaccharides, proteins, and lipids, which contain functional groups with high affinity to heavy metal ions (Zhao, Wang, Zhang, Gu, & Gao, 2018; Trica *et al*., 2019). The biosorption capacity of these algae is mainly attributed to alginates, acidic polysaccharides biosynthesized by polymerization of guluronic and manuroic acids, with a strong affinity with heavy metal ions (Zhao *et al*., 2018). These anionic polysaccharides are

absent in terrestrial plants and allow these algae to accumulate metals by ion exchange (Raize, Argaman, & Yannai, 2004).

The concentrations of heavy metals in seaweed biomass could reach values higher than their corresponding concentrations in seawater (Milledge & Harvey, 2016). Knowing the levels of heavy metals in *S. natans* and *S. fluitans*, through chemical analysis, is necessary to guarantee the safety of the algal material in certain applications that could endanger human and animal health. In this sense, some sensitive techniques can be used for this purpose (Fernández-Martínez *et al*., 2015), such as electrothermal atomic absorption spectroscopy (ETAAS), inductively-coupled plasma optical emission (ICP-OES) and atomic absorption spectroscopy (AAS) (Tamayo *et al*., 2014). This last is a technique specific and sensitive (Gallegos, Vega, & Noriega, 2012), and have been used for quantifying more than sixty elements of the periodic table. Additionally, several reasons such as its robustness, precision, accuracy and low cost, justify its use for quantifying metals. Furthermore, this technique has proven useful for evaluating the heavy metal's biosorption capacity in *Sargassum* species (Ibrahim, 2016; Ali, Idris, Ebrahim, & Eltayeb, 2017). AAS has also been used to determine the affinity order of the sargassum alginate for different metallic ions (Moino, Costa, Da-Silva, & Vieira, 2017). The main objective of this research is to evaluate the levels of heavy metals in *S. natans* and *S. fluitans* that arrived at the Dominican beaches during 2019, using flame atomic absorption spectroscopy (FAAS).

Methodology

Sampling and pre-processing

Sargassum samples were collected at the following dominican beaches: Caleta, Juan Dolio, San Andrés, Monte Río and La Saladilla (Figure 1), from May to June 2019. Two samples by each beach were taken with a temporal difference of 15 to 21 days. The distance between the sampling point and the seashore were (5-15) meters and both species of *Sargassum* were found. Afterward, the samples were placed in a plastic portable container for transferring to the laboratory.

Tecnología y ciencias del agua, ISSN 2007-2422, 12(3), 124-163. DOI: 10.24850/j-tyca-2021-03-04

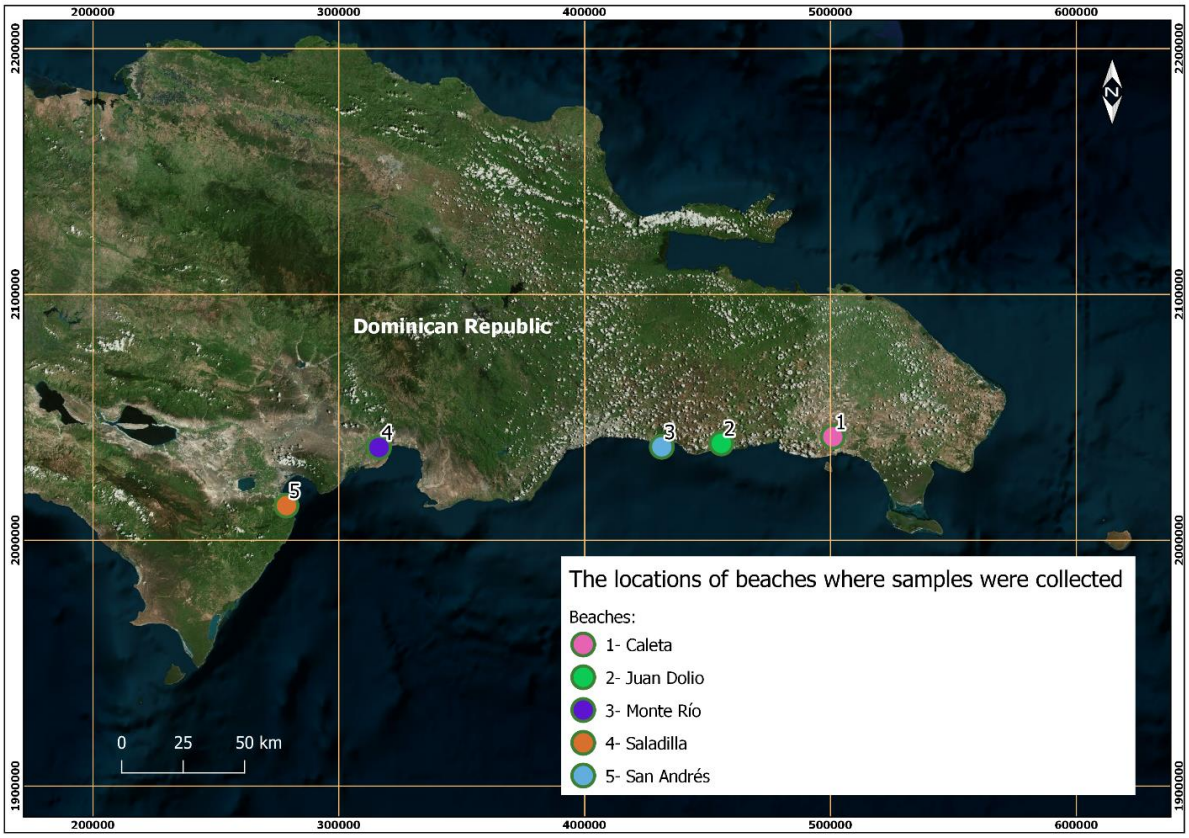

Figure 1. The location of beaches where samples were collected.

The samples were washed with distilled water to eliminate the sand, attached organisms and other undesired materials. The algal material was dried in the shade for one day and then finished drying in an oven model (Thelco) at 60 °C for 24 hours. After that, a manual porcelain mill was used for pulverizing them, following the procedure described (Zhao *et al*., 2018), and then sifted in the sieve shaker machine (Fischer Scientific Company) for 25 minutes. The sieve used was 600 µm to achieve a small and controlled particle size.

From this biomass, 2.5 g of each sample was accurately weighed using an analytical balance model CP225D (Sartorius, Germany) and transferred to a beaker for the digestion procedure. All digestions were conducted in triplicate with 25 ml concentrated nitric acid on the hot plate for 3 hours at 300 °C and were cooled, filtered and deionized water was added to complete 100 ml of solution. The solutions were analyzed by Atomic Absorption Spectroscopy (AAS) technique for flame model PinAAcle500 (PerkinElmer). The digestion procedure was based on the 968.08 method of the Association of Official Analytical Chemists (Association of Official Analytical Chemists, 1990).

Preparation of calibration curve

The linearity test is necessary to proof for quantitative determination studies in the chemical sciences. Through this, the proportionality between the analyte concentration and its response signal can be verified in an instrumental chemical analysis method, in the concentration range of sample solutions. The linear curve must be repeatable in-between day measurements (Gumustas & Ozkan, 2011).

The calibration curve points were obtained from a stock standard 3 (Multi-element calibration) supplied by PerkinElmer. It has a 10 µg/ml of

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metallic element concentration (Cd, Cu, Cr, Ni, Pb, Zn) dissolved in 5 % HNO_3. Corresponding aliquots were taken from this standard, to obtain a new standard solution of the appropriate concentration of each metallic element, for building the corresponding calibration curves. A nitric acid solution at 5 % was used for completing to 25 ml solution. Absorbance readings were obtained at the corresponding wavelength for each element according to the procedure described by the equipment manufacturer, in a flame atomic absorption spectrophotometer model PinAAcle500. Three replicates were measured for each standard solution on different days.

Repeatability and intermediate precision

Repeatability and intermediate precision were studied following the proceeding described in Perez-Rodriguez *et al*. (2019). For this, different solutions of each metal, containing (0.02, 0.12 and 0.2) mg/L were prepared and analyzed for FAAS in repeatability condition (the same day with six replicates for each metal). On the other hand, the reproducibility studies were carried on three different days at the same concentrations for each metal, with six replicates for a day. Then, the coefficient of variation between days ($CV_{\text{between/day}}$) and the theoretical variation coefficient according to Horwitz (Horwitz *CV*%) were calculated (González & Herrador, 2007).

Veracity

The method veracity was determined through the recovery test, according to the proceeding described in Perez-Rodriguez *et al*. (2019). Nine solutions with a concentration of 0.1 mg/l of each metal were prepared (this point is an intermediate point on the calibration curve). After that, these solutions are analyzed by the FAAS technique for each metal. Subsequently, the values obtained are compared with the expected value for each case, and it is calculated the corresponding recovery percentage (R%).

In order to verify the linearity of each curve, several linearity tests were carried out to each metallic element studied (Table 1). The results indicated that the linearity parameters are within the accepted ranges according to different analytical validation guidelines (Tettey, 2010; Anexo, 2014). Linear regression by the least-squares method is widely used to estimate the regression coefficients (R^2) of an analytic curve (Gumustas & Ozkan, 2011). In this sense, the values obtained for this coefficient ($R^2 > 0.98$) and the correlation coefficient ($r > 0.99$) (Kazusaki, Ueda, Takeuchi, & Ohgami, 2012) indicate a strong linear relationship between absorbance and concentration, within the studied range for each element.

Tecnología y ciencias del agua, ISSN 2007-2422, 12(3), 124-163. DOI: 10.24850/j-tyca-2021-03-04

Table 1. Values of linearity parameters evaluated: correlation coefficient (*r*), determination coefficient (R^2), coefficient of variation (CV_f), a relative standard deviation of the slope (Sb_{rel}), and quality coefficient (*QC*) of each calibration curve compared with the acceptance requirements (Tettey, 2010; Anexo, 2014).

Linear range (0.02-0.2) mg/l of heavy metals calibration curves studied							
Parameters	**Cd**	**Cu**	**Ni**	**Pb**	**Cr**	**Zn**	**Acceptance requirements**
Correlation coefficient (*r*)	0.9982	0.9988	0.9976	0.9951	0.9962	0.9987	**> 0.99**
Determination coefficient (R^2)	0.9963	0.9977	0.9953	0.9902	0.9923	0.9974	**> 0.98**
Coefficient of variation (CV_f)	4.92	2.96	4.5	2.55	2.23	3.32	**≤ 5 %**
Relative standard deviation of the slope (*b*)	0.65	0.54	1.05	1.70	1.22	0.29	**≤ 2 %**
Quality coefficient (*QC*)	3.04	2.78	3.30	3.56	1.76	2.16	**≤ 5 %**

The deviation of the slope of the regression line (Sb_{rel}) (Kazusaki *et al*., 2012; Souza-de, & Junqueira, 2005), and coefficient of variation of

response factors (CV_f) (Gumustas & Ozkan, 2011), also were determined for estimating the linearity of the calibration curves for each heavy metal. These other tests are used for evaluating the quality of calibration curves (Rodríguez, Valentín, Prieto, De-La-Torre, & Acosta, 2014; Rodríguez *et al*., 2018). In these cases, both parameters were less than the acceptance criteria established for validation guidance selected (Tettey, 2010; Anexo, 2014).

The quality coefficient (*QC*) is another chemometric tool that was recently applied to calibration curves to evaluate their linearity (Van-Loco, Elskens, Croux, & Beernaert, 2002). The corresponding contrast indicated that there is a high correlation between the variables over the concentration range.

Table 2 shows the corresponding results of the repeatability and intermediate precision tests. So, the variation coefficients of repeatability (CV_r%) in all concentrations were less than 3 % for each metal, this value is considered such as the acceptable requirement in spectroscopy methods. Also, results show the variation coefficients of reproducibility (CV_R%) in all concentrations were satisfying, due they are less than the corresponding theoretical Horwist's coefficients obtained between days (CV_{Horwit}%) (González & Herrador, 2007). All these results indicate that the method used for quantifying the metals studied in sargassum samples has high precision.

Table 2. Values of repeatability and intermediate precision tests, at different concentration levels of each metal.

Metals	**Cd**			**Cu**			**Pb**		
c(M) mg/l	CV_r **%**	CV_R **%**	CV_{Horwit} **%**	CV_r **%**	CV_R **%**	CV_{Horwit} **%**	CV_r **%**	CV_R **%**	CV_{Horwit} **%**
0.02	2.39	0.71	16.68	2.43	0.44	14.80	2.29	0.72	16.01
0.12	0.85	0.23	12.63	2.24	0.59	12.67	2.55	2.92	12.43
0.2	0.48	0.52	11.70	1.09	0.43	11.69	2.36	1.36	11.59
Metals	**Cr**			**Ni**			**Zn**		
c(M) mg/l	CV_r **%**	CV_R **%**	CV_{Horwit} **%**	CV_r **%**	CV_R **%**	CV_{Horwit} **%**	CV_r **%**	CV_R **%**	CV_{Horwit} **%**
0.02	2.85	1.28	16.18	2.48	0.58	16.28	2.49	0.49	16.36
0.12	1.98	0.72	12.63	1.01	0.46	12.70	0.88	0.52	12.63
0.2	0.78	1.22	9.83	0.75	0.63	11.88	0.58	0.44	11.70

Table 3 shows the results of the veracity test, through recovery percentages. All results were in the range to 97-103 %, this interval is the acceptance criteria for this parameter (Perez-Rodriguez *et al*., 2019). The experimental statistic *t*-Student values were lower than the *t*-tabulated (t_{tab}) for a 95 % confidence level (1.860) in all cases. These results guarantee the veracity of the method used for determining the heavy metals in sargassum samples.

Table 3. Values of veracity tests at the concentration 0.1 mol/l of each metal.

Metals	Recovery percentage (*R*%)	Experimental $t_{student}$
Cd	99.73 ± 0.62	1.011
Cu	99.30 ± 1.26	1.277
Pb	99.56 ± 0.81	1.259
Cr	99.55 ± 2.57	0.402
Ni	99.38 ± 1.20	1.185
Zn	99.20 ± 1.05	1.751

Results

Table 4 shows the results of heavy metals concentration in the *Sargassum* samples. The results show the highest levels for metals analyzed correspond *Sargassum natans* with 45.8808 ± 0.0117 mg/kg for Cu in

Caleta (sample LCN1). The capacity to absorb this toxic metal has been previously described for *Sargassum angustifolium* (Niad, Rasoolzadeh, & Zarei, 2014) and *Sargassum fusiforme* (Huang & Lin, 2015).

Table 4. Heavy metals concentration in *Sargassum* species.

Samples	Cd (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn(mg/kg)
LSN1	1.4890 ± 0.1091	7.9476 ± 0.0830	6.3997 ± 0.3314	11.3048 ± 0.1273	5.4091 ± 0.7091	19.0306 ± 0.0666
LSF1	1.5311 ± 0.0796	8.1482 ± 0.2435	6.9668 ± 0.1325	28.2665 ± 0.2832	6.5979 ± 0.4889	18.9011 ± 0.0392
LSN2	1.0793 ± 0.0454	4.7837 ± 0.0456	7.3972 ± 0.2537	12.2746 ± 0.2316	6.6418 ± 0.6167	16.3366 ± 0.0182
LSF2	0.7424 ± 0.2000	6.4727 ± 0.0765	9.4472 ± 0.5911	29.6237 ± 0.5299	6.8488 ± 0.4307	17.3856 ± 0.0208
MRN1	1.3888 ± 0.0641	9.6191 ± 0.2986	17.6599 ± 0.5623	14.4117 ± 0.4477	9.2254 ± 0.2444	20.0058 ± 0.0628
MRF1	1.2757 ± 0.4554	12.1823 ± 0.3921	20.9607 ± 0.5882	13.5043 ± 0.3579	9.8687 ± 0.1907	19.8335 ± 0.0780

MRN 2	1.3649 ± 0.0423	8.2045 ± 0.2054	14.6157 ± 0.0903	10.8996 ± 0.0482	5.3689 ± 0.0605	18.821 1 ± 0.0617
MRF 2	1.4189 ± 0.0873	17.4560 ± 0.3191	26.1843 ± 0.6977	15.6371 ± 0.3793	11.8455 ± 0.4882	20.924 6 ± 0.0382
JDN 1	1.2910 ± 0.0665	8.0225 ± 0.1046	10.3038 ± 0.1519	13.1802 ± 0.3296	8.1241 ± 0.2811	20.248 3 ± 0.0313
JDF1	1.5456 ± 0.0427	9.8623 ± 0.2085	19.1185 ± 0.3881	39.7820 ± 0.8125	9.9110 ± 0.4664	23.175 1 ± 0.0498
JDN 2	1.0788 ± 0.0301	13.0828 ± 0.1259	7.3807 ± 0.4269	16.3434 ± 0.2187	4.4933 ± 0.6698	18.267 0 ± 0.0192
JDF2	1.0495 ± 0.0762	8.8529 ± 0.2494	6.7996 ± 0.1834	9.5024 ± 0.2304	5.9609 ± 0.5447	18.274 4 ± 0.0127
SAN 1	1.2766 ± 0.0742	15.4873 ± 0.3411	9.2189 ± 0.2879	38.3927 ± 0.7228	8.6155 ± 0.1083	27.010 6 ± 0.0623
SAF1	1.6172 ± 0.0346	26.6124 ± 0.1620	11.9849 ± 0.8400	13.8533 ± 0.6523	8.6761 ± 0.3311	26.918 2 ± 0.0528
SAN 2	1.6439 ± 0.0555	23.1942 ± 0.4238	11.1565 ± 0.3131	10.1297 ± 0.1687	8.3094 ± 0.2232	27.045 8 ± 0.1126

SAF2	1.5741 ± 0.0666	28.7445 ± 0.2803	11.8834 ± 0.6138	11.4414 ± 0.2917	7.8262 ± 0.3621	25.7614 ± 0.0623
LCN1	1.5209 ± 0.0456	45.8808 ± 0.4670	10.9738 ± 0.6287	13.1639 ± 0.3302	8.1397 ± 0.2805	22.2868 ± 0.1194
LCF1	1.2359 ± 0.0463	23.2059 ± 0.2073	11.3273 ± 0.2251	12.3647 ± 0.2202	10.1885 ± 0.2916	22.1613 ± 0.0351
LCN2	1.5611 ± 0.0448	10.0340 ± 0.1675	8.1023 ± 0.2135	11.7880 ± 0.2439	8.0068 ± 0.2784	23.3132 ± 0.0312
LCF2	1.0052 ± 0.1410	8.8656 ± 0.3334	10.8526 ± 0.4921	11.7001 ± 0.3976	8.2787 ± 0.2491	24.3525 ± 0.1264

Note: LSN1: *S. natans*, La Saladilla beach; **LSF1:** *S. fluitans*, La Saladilla beach; **LSN2:** *S. natans*, La Saladilla beach; **LSF2:** *S. fluitans*, La Saladilla beach; **MRN1:** *S. natans*, Monte Río beach; **MRF1:** *S. fluitans*, Monte Río beach; **MRN2:** *S. natans*, Monte Río beach; **MRF2:** *S. fluitans*, Monte Río beach; **JDN1:** *S. natans*, Juan Dolio beach; **JDF1:** *S. fluitans*, Juan Dolio beach; **JDN2:** *S. natans*, Juan Dolio beach; **JDF2:** S. fluitans, Juan Dolio beach; **SAN1:** *S. natans*, San Andrés beach; **SAF1:** *S. fluitans*, San Andrés beach; **SAN2:** *S. natans*, San Andrés beach; **SAF2:** *S. fluitans*, San Andrés beach; **LCN1:** *S. natans*, Caleta beach; **LCF1:** *S. fluitans*, Caleta beach; **LCN2:** *S. natans*, Caleta beach; **LCF2:** *S. fluitans*, Caleta beach.

On the one hand, these results suggest a possible application of *Sargassum* biomass as biosorbent, in the treatment of wastewater polluted by cooper. On the other hand, and considering the concentrations

observed for copper, the ability of *Sargassum* to capture and accumulate Cu must be taken into account to ensure the safety of algal materials in certain applications. However, as shown in Table 4, samples from Caleta Beach contained a wide range of differences between copper concentrations. It should be noted that the first sample collection (samples LCN1 and LCF1) obtained a higher concentration compared to the second one (samples LCN2 and LCF2).

For *S. fluitans* the highest concentration was observed for Ni, with 39.7820 ± 0.0204 mg/kg in the locality of Juan Dolio (sample JDF1), however for the second sample collection (sample JDF2), this concentration was decreased by an order of magnitude. A similar value of 38.3927 ± 0.7228 mg/kg was presented on the beach of San Andrés, for the species *S. natans* (sample SAN1). The nickel biosorption capacity has been registered for several *Sargassum* species such as *S. angustifolium* (Ahmady-Asbchin, Tabaraki, Jafari, Allahverdi, & Azhdehakoshpour, 2013) and *S. muticum* (González, Rodríguez, Gutiérrez, & Guibal, 2011). It shows that algal biomass could also be used to remove nickel from contaminated water.

Discussion

All samples showed quantifiable concentrations of the metals studied (Pb, Cr, Ni, Cu and Zn). The concentration values considered normal and the limit of acceptance for the analyzed heavy metals in the foliar tissue of plants, were selected from the literature (Kabata-Pendias, 2001) to compare with the obtained results.

The Figure 2 shows that except for Zn, the metals are found in *Sargassum* in a higher concentration than in the foliar tissues of plants. This result is consistent with the well-known tendency of these algae for metals bioaccumulating, due to the presence of polysaccharides and polyphenols in their cellular structures. These cations can pass through the membranes of these brown algae, by using ionic carriers such as Ca channels or through a transfer amino acid such as cysteine. Furthermore, carboxyl, sulfate, hydroxyl, amino groups, and polysaccharides play an important role in the cell wall as acceptor sites for metals (Sinaei, Loghmani, & Bolouki, 2018).

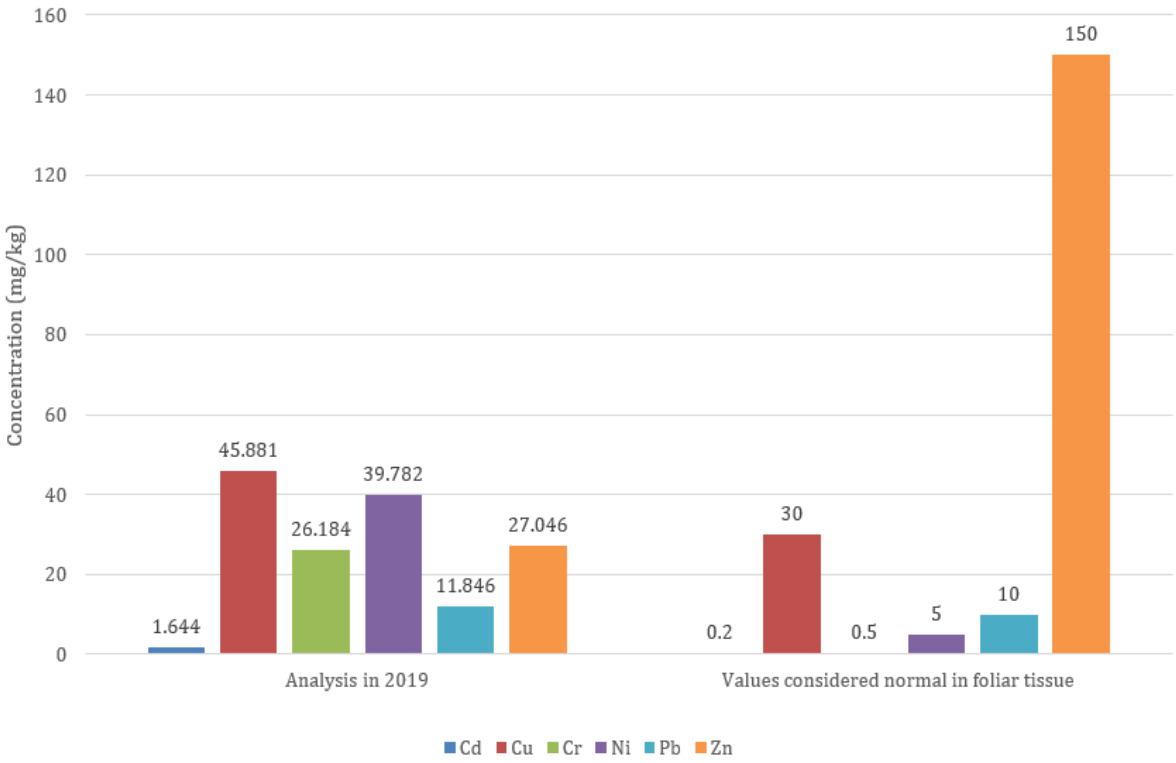

Figure 2. Comparison of the maximum concentration ranges of heavy metals obtained in this analysis and de the values considered normal in foliar tissue (mg/kg).

However, as shown in Table 5, the levels of these elements found in both *Sargassum* species and all the studied beaches 2019 are below the acceptability limits (Kabata-Pendias, 2001; Addico, & De-Graft-Johnson, 2016).

Table 5. Values considered normal and the limit of acceptance in foliar tissue for the heavy metals of interest. Data extracted from Kabata-Pendias (2001), and Galán and Romero (2008).

Elements	Normal geochemical (mg/kg)	Values considered normal in foliar tissue (mg/kg)	Limit of acceptance in foliar tissue (mg/kg)
Cd	<1-2	0.05-0.2	5-30
Cu	60	5-30	20-100
Cr	N.R.	0.1-0.5	5-30
Ni	2-100	0.1-5	10-100
Pb	10-150	5-10	30-300
Zn	25-200	27-150	100-400

Note: Unreported N.R.

Except for metalliferous soils, the normal ranges of concentration of heavy metals are not a threat to living beings (Gardea-Torresdey, Peralta-Videa, De-la-Rosa, & Parsons, 2005). None of the levels of the metals analyzed were higher than normal geochemical concentrations (Galán &

Romero, 2008). This can be evidenced in the metals Ni, Pb, and Zn, for which the maximum value of the geochemical range is greater by 1 order of magnitude. However, in all samples Cd, is within the normal geochemical range (1-2 mg/kg). Cr, with maximum values of 26.1843 mg/kg, is also below the normal and acceptable levels in foliar tissue (Table 5), and far from the range considered critical in soil (75-100 mg/kg) (Gardea-Torresdey *et al*., 2005).

In previous prospective research, Fernández *et al*. (2017) analyzed, by using inductively coupled plasma mass spectrometry (ICP-MS), the content of heavy metals and lanthanides of *S. natans* and *S. fluitans* arrived on the coast of the Dominican Republic during 2015. The results obtained in the present research show, in comparison, similar results for Ni and Zn, but relevant differences for Cu, Cr, Pb and Cd, especially significant in the last two metals (Figure 3).

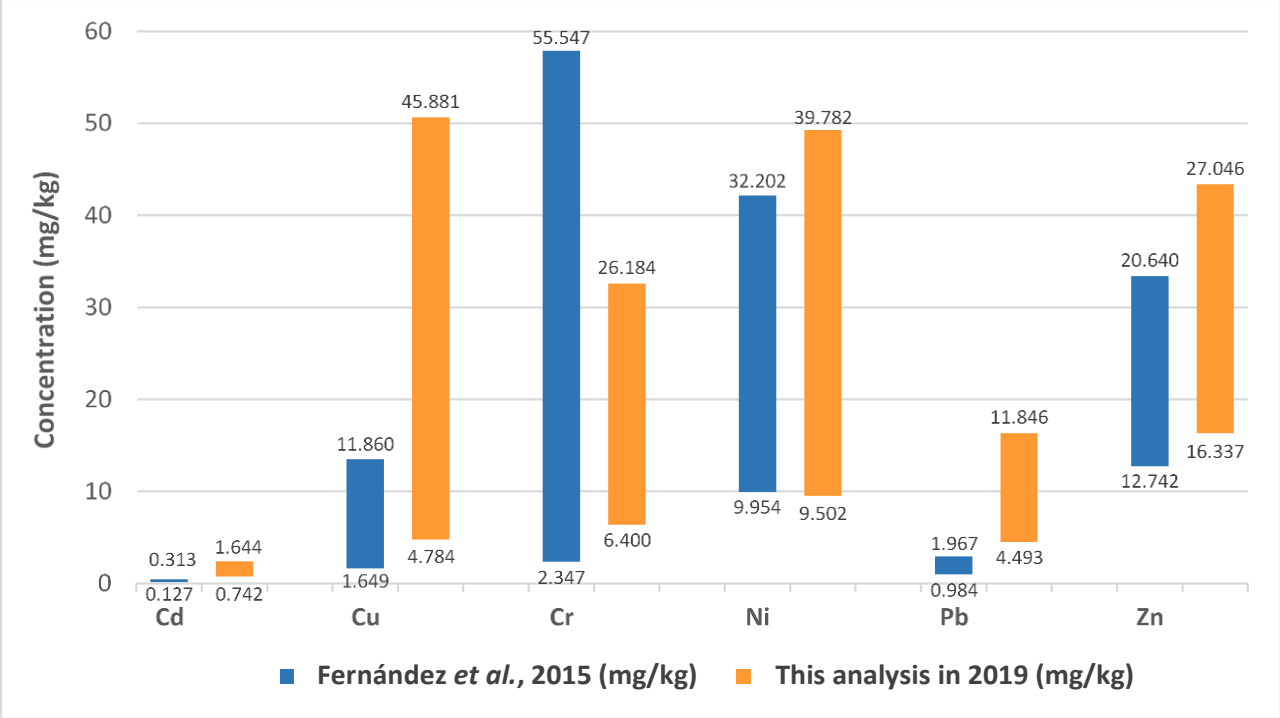

Figure 3. Comparison of the concentration ranges of heavy metals, from the prospective analysis in 2015 (Fernández *et al*., 2017) and this analysis.

As shown in Table 6 the levels of Ni and Zn were similar in both investigations. The concentrations obtained in 2019 for Ni and Zn remained close to those values shown by the study of 2015, although, occasionally, their levels were slightly higher for 2019 shore seaweed arrival. The maximum value obtained for Ni was 39.7820 mg/kg versus 32.2015 mg/kg in the analysis of 2015, while for Zn it was 27.0458 mg/kg compared to 20.6399 mg/kg obtained in the prospective study carried out by Fernández *et al*. (2017).

Table 6. Comparison of the concentration ranges of heavy metals, from the prospective analysis in 2015 (Fernández *et al*., 2017) and this analysis.

Element	Concentration ranges from the analysis of Fernández *et al*. (2015) (mg/kg)	Concentration ranges from this analysis in 2019 (mg/kg)
Cd	0.1271 – 0.3130	0.7424 – 1.6439
Cu	1.6493 – 11.8601	4.7837 – 45.8808
Cr	2.3465 – 55.5471	6.3997 – 26.1843
Ni	9.9543 – 32.2015	9.5024 – 39.7820
Pb	0.9837 – 1.9674	4.4933 – 11.8455
Zn	12.7417 – 20.6399	16.3366 – 27.0458

Nevertheless, the Cu content was much higher in the present research, with 45.8808 mg/kg (Figure 3), in contrast with the maximum concentration reported in 2015 (11.8601 mg/kg). An opposite example was observed in the case of Cr, with maximum concentrations values of 55.5471 mg/kg in the prospective study of 2015, in contrast with the levels detected in the research of 2019 (26.1843 mg/kg).

As shown in Table 6, the concentration for Pb and Cd presented differences in an order of magnitude over the values of the prospective analysis of 2015. The latter showed 1.9674 mg/kg for Pb, and 0.3130 mg/kg for Cd, which contrasts with the value obtained in the present research: 11.8455 mg/kg for Pb, and 1.6439 mg/kg for Cd, the highest value for this metal.

Comparative statistical analysis of the biosorption capacity for each metal between the species

The *t*-Student was used as a test that allows two groups of data to be compared to determine if they have any statistical significance (Gutiérrez, & De-La-Vara, 2008). To evaluate the existence of any significant difference between the samples analyzed, it was compared to the *p*-value of the test and the level of significance (α = 0.05) (Table 7).

Table 7. The *p*-value for the *t*-Student test in the comparison of the biosorption between *S. natans* and *S. fluitans* with significant value (α) = 0.05.

Elements	The *p*-value for the *t*-Student test

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Cd	0.537
Cu	0.930
Cr	0.184
Ni	0.430
Pb	0.099
Zn	0.734

Therefore, based on the fact that the *p*-value for all the samples was higher than the level of significance, with 95 % confidence it is established that there is no significant difference between the *S. natans* and *S. fluitans*, found on the five beaches, for the metals analyzed: Cd, Cu, Cr, Ni, Pb, and Zn.

Conclusions

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The concentrations of the analyzed metals in the studied species are below the acceptable limits. This indicates that the species *S. natans* and *S. fluitans* that reached the Dominican coast in 2019 could be used as fertilizer and in animal feeding without risks of poisoning for the analyzed elements (Cd, Cu, Cr, Ni, Pb, and Zn). Anyhow, additional analysis, including other toxic elements such as Hg and As, are required to assure the safety of the algal material for these purposes.

In the case of Cu and Cr, there are important differences with the 2015 prospective study (Fernández *et al*., 2017) that become of an order of magnitude for Cd and Pb. Further studies should clarify whether these differences are correlated with normal fluctuations in heavy metal levels between different arrivals at the coast of the algal material.

Regarding the comparison of the biosorption capacity of both species, the statistical analysis of the data allows concluding that there is no significant difference in the concentrations of the metals studied between *S. natans* and *S. fluitans*. This could indicate that the biochemical mechanisms and factors involved in biosorption capacity are similar between both species.

Acknowledgments

The authors thank the ISO:9001:2015 certified Laboratorio de Servicios Analíticos y Ambientales del INTEC for technical support. We are very grateful to laboratory manager A. Rojas for her helpful contribution to AAS acquisition.

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