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

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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 el turismo, la pesca y la 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.
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.
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 *et al.*, 2011; Gutiérrez *et al.*, 2016). *Sargassum natans* (Linnaeus) Gaillon and *Sargassum fluitans* Børjesen (Børjesen) are highly branched pelagic brown seaweeds provided with long phyllodes and serrated edge that reproduce by vegetative fragmentation of the thallus (Széchy *et al.*, 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, Convención Interamericana para la protección y Conservación de las Tortugas Marinas, 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 sargassso 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 et al., 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 et al., 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 et al., 2015). Sargassum can absorb heavy metals as oxides, hydroxides, and oxyhydroxides from seawater, hence, its consumption is of concern (Oliveira et al., 2014; Barquilha et al., 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 et al., 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 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 et al., 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 et al., 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 et al., 2017). AAS has also been used to determine the affinity order of the sargassum alginate for different metallic ions (Moino et al., 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.
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 metallic element concentration (Cd, Cu, Cr, Ni, Pb, Zn) dissolved in 5% HNO₃. 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_{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 et al., 2012), indicate a strong linear relationship between absorbance and concentration, within the studied range for each element.

The deviation of the slope of the regression line (Sbrel) (Kazusaki et al., 2012; Souza de, & Junqueira, 2005), and coefficient of variation of response factors (CVf) (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 et al., 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 et al., 2018). The corresponding contrast indicated that there is a high correlation between the variables over the concentration range.
Table 1. Values of linearity parameters evaluated: correlation coefficient (r), determination coefficient (R²), coefficient of variation (CVᵋ), a relative standard deviation of the slope (Sᵋrel), and quality coefficient (QC) of each calibration curve compared with the acceptance requirements (Tettey, 2010; Anexo, 2014).

| 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 (R²)                  | 0.9963 | 0.9977 | 0.9953 | 0.9902 | 0.9923 | 0.9974 | > 0.98                 |
| Coefficient of variation (CVᵋ)                  | 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%                   |

Table 2 shows the corresponding results of the repeatability and intermediate precision tests. So, the variation coefficients of repeatability (CVᵋ,%) 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 <sub>R</sub>%) in all concentrations were satisfying, due they are less than the corresponding theoretical Horwist's coefficients obtained between days (CV <sub>Horwist</sub>%) (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 <sub>r</sub> % | CV <sub>R</sub> % | CV <sub>Horwist</sub> % | CV <sub>r</sub> % | CV <sub>R</sub> % | CV <sub>Horwist</sub> % | CV <sub>r</sub> % | CV <sub>R</sub> % | CV <sub>Horwist</sub> % |
|        | 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 <sub>r</sub> % | CV <sub>R</sub> % | CV <sub>Horwist</sub> % | CV <sub>r</sub> % | CV <sub>R</sub> % | CV <sub>Horwist</sub> % | CV <sub>r</sub> % | CV <sub>R</sub> % | CV <sub>Horwist</sub> % |
|        | 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 |
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 *et al.*, 2014) and *Sargassum fusiforme* (Huang & Lin, 2015). 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 *et al.*, 2013) and *S. muticum* (González *et al.*, 2011). It shows that algal biomass could also be used to remove nickel from contaminated water.
### 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|
| MRN2    | 1.3649 ± 0.0423| 8.2045 ± 0.2054| 14.6157 ± 0.0903| 10.8996 ± 0.0482| 5.3689 ± 0.0605| 18.8211 ± 0.0617|
| MRF2    | 1.4189 ± 0.0873| 17.4560 ± 0.3191| 26.1843 ± 0.6977| 15.6371 ± 0.3793| 11.8455 ± 0.4882| 20.9246 ± 0.0382|
| JDN1    | 1.2910 ± 0.0665| 8.0225 ± 0.1046| 10.3038 ± 0.1519| 13.1802 ± 0.3296| 8.1241 ± 0.2811| 20.2483 ± 0.0313|
| JDF1    | 1.5456 ± 0.0427| 9.8623 ± 0.2085| 19.1185 ± 0.3881| 39.7820 ± 0.8125| 9.9110 ± 0.4664| 23.1751 ± 0.0498|
| JDN2    | 1.0788 ± 0.0301| 13.0828 ± 0.1259| 7.3807 ± 0.4269| 16.3434 ± 0.2187| 4.4933 ± 0.6698| 18.2670 ± 0.0192|
| JDF2    | 1.0495 ± 0.0762| 8.8529 ± 0.2494| 6.7996 ± 0.1834| 9.5024 ± 0.2304| 5.9609 ± 0.5447| 18.2744 ± 0.0127|
| SAN1    | 1.2766 ± 0.0742| 15.4873 ± 0.3411| 9.2189 ± 0.2879| 38.3927 ± 0.7228| 8.6155 ± 0.1083| 27.0106 ± 0.0623|
| SAF1    | 1.6172 ± 0.0346| 26.6124 ± 0.1620| 11.9849 ± 0.8400| 13.8533 ± 0.6523| 8.6761 ± 0.3311| 26.9182 ± 0.0528|
| SAN2    | 1.6439 ± 0.0555| 23.1942 ± 0.4238| 11.1565 ± 0.3131| 10.1297 ± 0.1687| 8.3094 ± 0.2232| 27.0458 ± 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|
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 et al., 2018).
Figure 2. Comparison of the maximum concentration ranges of heavy metals obtained in this analysis and 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).

Except for metalliferous soils, the normal ranges of concentration of heavy metals are not a threat to living beings (Gardea-Torresdey et al., 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).

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; 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.
In previous prospective research, Fernández et al., 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 (Fernández et al., 2017). 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](image-url)  
**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.

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.

**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 and collaborators in 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                           |
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 ($\alpha = 0.05$) (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 ($\alpha$)=0.05.

| Elements | The p-value for the t-Student test |
|----------|-----------------------------------|
| Cd       | 0.537                             |
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**

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.

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