



# Article Assessment of Extraction Methods of Trace Metallic Elements in Plants: Approval of a Common Method

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**Abstract:** The question of trace metal elements (TME) is still relevant and causes several environmental problems. Moreover, the digestion methods of TME have a significant impact on ecosystems. Sample preparation is an important step of any analytical procedure. In fact, defining the levels of TME in vegetal tissues requires various steps: drying samples, crushing, extraction, and dosage. The use of chemical extraction solutions can be put into question. Other than their lack of specificity, they are susceptible to provoking the redistribution and/or re-fixation of a part of the metallic ions between the various vegetal components. Our study aims to test the procedures of extraction that are specific for the metals Cu, Zn, Fe, Ca, Cr and Ba. Our results show an outstanding difference in the levels of TME derived from the same vegetal sample, according to the various techniques of extraction. In fact, cold extraction by nitric acid diluted at 10% is the most efficient way to put the ions of Cu, Zn, Fe and Ca into a solution. Meanwhile, the use of heated extraction using different acid digestion approaches is the most efficient for Cr and Ba solubilization from *Atriplex portulacoides*, *Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279.

Keywords: trace metal elements; vegetal tissues; acid digestion; % extractability

## 1. Introduction

Trace metal elements (TME) are found naturally on the Earth's crust, but their polluting impacts on the environment are increasingly becoming a problem, which has become of great concern due to the adverse effects they are causing around the world [1]. In fact, industry, agriculture, extensive mining, and military operations have led to the accelerated release of metals into the ecosystems, causing serious environmental problems and harmful effects on human and ecosystem health [2].

Trace metal elements are known to be naturally occurring compounds, but anthropogenic activities introduce them in large quantities into different environmental compartments [3,4]. They are characterized by a persistence that lasts for a long period in aquatic and land ecosystems [3,5].

According to the literature, several methods have been used to extract TME from plant tissues. The solubilization of the internalized elements is of outmost importance, since the analytical processes are normally based on liquid solutions only. Wet mineralization methods, in which organic matter is mineralized in an aqueous phase by adding oxidizing agents (for example, single acid and hydrogen peroxide addition), do not allow the complete decomposition of this organic fraction, which will then remain partially present in the final solution, and could, in some techniques, interfere with the assay [6]. However, studies by Idera et al. [7] have reported that the use of mixtures of acids is generally fast



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and more efficient, and the most commonly used are nitric ( $HNO_3$ ), sulfuric ( $H_2SO_4$ ), perchloric (HClO<sub>4</sub>) and hydrochloric (HCl) acids, as well as hydrogen peroxide. For the extraction of metals from plant tissues, a variety of acid mixtures have been used, such as a nitric–sulfuric–perchloric acid mixture (10:1:0.5, v/v/v) [8,9]; hydrochloric–nitric acids known as aqua regia mixture (3:1, v/v) [10]; nitric–perchloric acid mixture (3:1, v/v) [11]; nitric–sulfuric acid mixture (2:1, v/v) [12]; nitric–hydrochloric–perchloric acid mixtures (5:1:0.5, v/v/v) [13] and nitric-hydrochloric-sulfuric acid mixtures (5:1:1, v/v/v) [14]. The choice of an individual acid or a combination of acids depends on the nature of the matrix to be decomposed [14]. Hydrochloric acid is useful for salts of carbonates, phosphates, some oxides and some sulfides. Nitric acid, HNO<sub>3</sub>, oxidizes many samples that cannot be dissolved by HCl. Hydrogen peroxide,  $H_2O_2$ , is a strong oxidizer, which is used in combination with  $HNO_3$  to dissolve matter that is not fully decomposed by  $HNO_3$  [15,16]. According to Shaibur et al. [17], HClO<sub>4</sub> acid prevents excessive frothing, which occurs frequently when  $HNO_3$  or  $H_2SO_4$  acid is used alone. Hydrofluoric acid is very effective for use in the decomposition of silicate matrices, especially in roots. In order to improve the decomposition, the hydrofluoric acid is routinely mixed with other acids [18].

Considering this multitude of procedures, this study aims to investigate the levels of heavy metal contents in different plant species by testing eight different extraction methods, aiming to determine the best method for the quantification of each heavy metal with the highest extraction efficiency and analytical accuracy.

#### 2. Experimental

#### 2.1. Plant Material

Our work has focused on different plants: *Atriplex portulacoides, Arthrocnemum indicum, Ulva lactuca* (field samples) and two certified reference materials from the Community Bureau of Reference—BCR: *Olea europaea* BCR-62 and *Ulva lactuca* BCR-279. The sampling of these plants was carried out in areas polluted with TMEs.

*Atriplex portulacoides* was taken from plants at the edge of the Bizerte lagoon situated in North Tunisia (37.214162 N, 9.939034 E). This species has been identified in the African Plant Database (Record number 131513).

*Arthrocnemum indicum* was taken from plants at the edge of Soliman sebkha situated in North Tunisia (36.708654 N, 10.436224 E). This species has been identified by the African Plant Database (Record number 26680).

*Ulva lactuca* was taken from the coast of the Mediterranean sea in the region of Bizerte situated in North Tunisia (37.284153 N, 9.879823 E). *Ulva lactuca* has been identified by the UBC Herbarium Algae Database (Accession number: A8586).

Regarding the field samples, shoots were collected from twelve individuals per species and per station. The sampling area was limited to 20 m<sup>2</sup> per species. After drying for two weeks at 60 °C until a constant weight, the plant samples (aerial parts) were reduced to a fine powder using an agate ball mill.

#### 2.2. Test Methods

Overall, extraction methods of metals are easier than selective extraction based on the use of reagents [19]. For this reason, we have tested eight methods of chemical extraction: cold extraction with nitric acid diluted at 1%, cold extraction with nitric acid diluted at 10%, and heated extraction using different acid digestion methods, as detailed in point 2.4.

#### 2.3. Nitric Acid Digestion (1% and 10%)

Extraction was preformed according to the method of Larson et al. [20]. In short, a subsample of 25 mg of plant sample fine powder was additionally dried for 24 h at 60 °C, after which 10 mL of nitric acid was poured into the mixture and left at room temperature for 15 days to ensure the good digestion of organic matter and to obtain a better dissolution of metallic cations. All the samples were stirred every 3 days to ensure better contact between the plant sample and the acid solution. Finally, the mineral deposit was filtered

through ashless filter paper (Whatman N°1, 90 mm diameter circles). The extracts were stored at 4 °C until analysis. Two different cold extraction methods were used, differing in the nitric acid concentration: 1% (method A) and 10% (method B).

### 2.4. Acid Mixtures Digestion

The extraction was achieved via a heated attack using six different acid digestion methods: nitric–sulfuric–perchloric acid digestion (method C) in a proportion of 10:1:0.5, v/v/v [8,9]; hydrochloric–nitric acid digestion, known as aqua regia mixture (method D), in a proportion of 3:1, v/v [10]; nitric–perchloric acid digestion (method E) in a proportion of 3:1, v/v [11]; nitric–sulfuric acid digestion (method F) in a proportion of 2:1, v/v [12]; nitric–hydrochloric–perchloric acid digestion (method G) in a proportion of 5:1:0.5, v/v/v [13], and nitric–hydrochloric–sulfuric acid digestion (method H) in a proportion of 5:1:1, v/v/v [14].

About 40 to 50 mg of plant powder, previously dried in an incubator at 60 °C for 24 h, was poured into a Kjeldahl flask with 3 mL of the mixture of acids, as explained above, and was then placed on a heated ramp. To ensure good mineralization, the temperature increase was performed gradually from 100 to 350 °C, by increments of 50 or 100 °C every 15 min. Incineration was subsequently maintained for one hour at 350 °C. After two hours of mineralization, we obtained a white ash that was to be dissolved in 2 mL of nitric acid (0.05%). Hence, the solutions obtained were kept at 4 °C until the analysis.

#### 2.5. Measurements of Trace Metal Elements

Metal quantification was performed by atomic absorption spectroscopy (AAS). This work aimed to measure six metals: copper, zinc, iron, calcium, chromium and barium. The assay was carried out by the method of atomic absorption spectrophotometry with a flame (FAAS, Perkin Elmer PinAAcle 900T, Boston, MA, USA). The TME contents were calculated by relating the concentration obtained in the extraction solution (by atomic absorption spectrometer) to the mass of dry matter used for the extraction.

For each metal element, a standard curve was prepared from a master solution (1000 mg/L) using appropriate dilutions with ultrapure water. The concentration ranges varied with the nature of the element being assayed and its expected abundance in the mineralized sample. The calibration ranges of Cu, Zn, Fe, Ca, Cr and Ba are  $0-5 \text{ mg} \cdot \text{L}^{-1}$ ,  $0-1 \text{ mg} \cdot \text{L}^{-1}$ ,  $0-5 \text{ mg} \cdot \text{L}^{-1}$ ,  $0-5 \text{ mg} \cdot \text{L}^{-1}$  and  $0-20 \text{ mg} \cdot \text{L}^{-1}$ , respectively. We used at least 4 points of range.

#### 2.6. Statistical Analysis

The analysis of variance (ANOVA) and pairwise Tukey HSD tests were used in order to evaluate the existence of significant differences (p < 0.05) between the contents obtained by different extraction procedures.

#### 3. Results

## 3.1. Copper Assay

The copper contents in the different tested samples showed a significant variation between digestion methods (Figure 1). Thus, the maximum levels of Cu were obtained when extraction was performed via method B and method C on *Atriplex portulacoides* and *Arthrocnemum indicum*, with Cu content values of 135.0 and 155.7  $\mu g \cdot g^{-1}$  dry weight (DW), respectively, with method B, and 122.0 and 209.9  $\mu g \cdot g^{-1}$  DW, respectively, with method C.



**Figure 1.** The copper contents ( $\mu g \cdot g^{-1}$  DW) in *Atriplex portulacoides, Arthrocnemum indicum, Olea europea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 using methods A, B, C, D, E, F, G, and H. Bars marked with different letters are significantly different at p < 0.05.

In *Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279, the results show that the maximum levels of Cu were obtained during mineralization with method B, showing values of 196.1, 158.4 and 159.7  $\mu$ g·g<sup>-1</sup> DW, respectively.

## 3.2. Zinc Assay

Our results show that higher Zn levels were obtained using method B and method C from *Atriplex portulacoides* and *Arthrocnemum indicum*. Using extraction method B, the measured Zn values were 236 and 350  $\mu$ g·g<sup>-1</sup> DW in *Atriplex portulacoides* and *Arthrocnemum indicum*, respectively, while with the application of extraction method C, the Zn content values were 299 and 300  $\mu$ g·g<sup>-1</sup> DW, respectively, for each species. On the other hand, extraction method B proved to be the most reliable for Zn mineralization in *Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 (Figure 2), presenting Zn content values of 617, 440 and 483  $\mu$ g·g<sup>-1</sup> DW, respectively.



**Figure 2.** The zinc contents ( $\mu g \cdot g^{-1}$  DW) in *Atriplex portulacoides, Arthrocnemum indicum, Olea europea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 using methods A, B, C, D, E, F, G, and H. Bars marked with different letters are significantly different at *p* < 0.05.

# 3.3. Iron Assay

Figure 3 shows the levels of Fe according to the extraction process in plants studied beforehand. Overall, the maximum Fe levels were obtained upon the application of extraction method B, presenting Fe content values of 1189, 1119, 1082, 2186 and 1788  $\mu$ g·g<sup>-1</sup> DW in *Atriplex portulacoides, Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279, respectively.

# 3.4. Calcium Assay

The results show that the Ca contents were higher using the extraction methods C and F in *Atriplex portulacoides*. On the other hand, methods B, E and F proved to be more efficient when extraction was performed on *Arthrocnemum indicum* samples. Method A, method B and method E were the best for *Olea europaea* BCR-62 (Figure 4), while method A and method B were the most reliable for Ca extraction in *Ulva lactuca* and *Ulva lactuca* BCR-279 (Figure 4).



**Figure 3.** The iron contents ( $\mu g \cdot g^{-1}$  DW) in *Atriplex portulacoides, Arthrocnemum indicum, Olea europea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 using methods A, B, C, D, E, F, G and H. Bars marked with different letters are significantly different at p < 0.05.

For example, the highest Ca contents recorded were 5814, 7016, 10,148, 11,995 and 11,927  $\mu$ g·g<sup>-1</sup> DW in *Atriplex portulacoides, Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279, respectively.

## 3.5. Chromium Assay

Generally, the assay results of Cr show that heated extraction, using different acid combinations, enabled the highest levels of Cr recovery in all studied species (Figure 5). In fact, the highest Cr contents recorded were 641.6, 698.1, 634.5, 699.7 and 642.1  $\mu$ g·g<sup>-1</sup> DW in *Atriplex portulacoides, Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279, respectively.



**Figure 4.** The calcium contents ( $\mu g \cdot g^{-1}$  DW) in *Atriplex portulacoides, Arthrocnemum indicum, Olea europea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 using methods A, B, C, D, E, F, G, and H. Bars marked with different letters are significantly different at *p* < 0.05.

## 3.6. Barium Assay

Similarly to chromium, the determination of barium using different acid digestion methods showed that the heated extraction methods achieved the highest Cr levels in all studied species (Figure 6). Method D (aqua regia, HCl-HNO<sub>3</sub>) and method G (HNO<sub>3</sub>-HCl-HClO<sub>4</sub>) achieved the maximum levels of Ba extraction, achieving values of 21935, 22489, 23440, 23576 and 23864  $\mu$ g·g<sup>-1</sup> DW in *Atriplex portulacoides, Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279, respectively, using the extraction method G (Figure 6). Via the application of extraction method D, the Ba content values were 19828, 22152, 23577, 22471 and 21661  $\mu$ g·g<sup>-1</sup> DW, respectively, for each species.



**Figure 5.** The chromium contents ( $\mu g \cdot g^{-1}$  DW) in *Atriplex portulacoides, Arthrocnemum indicum, Olea europea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 using methods A, B, C, D, E, F, G and H. Bars marked with different letters are significantly different at p < 0.05.



**Figure 6.** The barium contents ( $\mu g \cdot g^{-1}$  DW) in *Atriplex portulacoides, Arthrocnemum indicum, Olea europea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 using methods A, B, C, D, E, F, G and H. Bars marked with different letters are significantly different at p < 0.05.

# 4. Discussion

## 4.1. Drying and Crushing

Two dehydration methods were used to remove the water from plant tissues: freezedrying and oven-drying. In our work, samples were dried in an oven at 60 °C for 15 days. Similarly, Bankaji and Sleimi [21] used oven-drying at 60 °C for drying the fresh plant material. However, Lefèvre et al. [22], for example, used a temperature of 70 °C for drying plant material, while Redondo-Gómez et al. [23] used 80 °C to achieve similar dehydration levels.

Homogenization is a necessary step to obtain a homogeneous sample for analysis, and this can be achieved by grinding. For our samples (field samples), we used a ball mill, which is used for fibrous plants [24]. However, several other types of mills can be used, such as the oscillating ring mill [25].

#### 4.2. Extraction

Sample mineralization into an acidic solution is essential for quantification procedures, since most analytical technologies use solutions for metal quantification. The main problem of this step lies in the disintegration of organic matter, which represents the major part of

plants' dry weight. Cellulose and lignin are not attacked by classic acids used to mineralize conventional soil [26]. For example, aqua regia has a low oxidizing power towards organic matter, and its application in highly organic matrixes generates a carbon-based precipitate in the reaction vessels, so preliminary calcinations or the addition of oxidized agents are made beforehand to alleviate this problem [27].

Wet dissolution methods, for which organic matter is mineralized in an aqueous phase by the addition of oxidizing agents (for example, mixture of acid and hydrogen peroxide), can sometimes lead to the incomplete decomposition of the organic matrix, which will remain partially present in the final solution and thus can interfere with the quantification procedure [6]. However, studies by Idera et al. [7] reported that the use of an acid mixture or a mixture of acids and oxidants is generally fast and efficient.

To test for the best solubilization of metal ions, we opted for eight extraction methods using nitric acid at 1%, nitric acid at 10%, as well as a mixture of different acids, such as nitric acid, sulfuric acid, perchloric acid and hydrochloric acid, as explained above. Indeed, the attack of nitric acid alone (diluted or not) is used in several methods employing solutions of heavy metals [26]. Similarly, extraction protocols using acid mixtures have been described as fast and efficient [7]. For example, extraction by a mixture containing sulfuric acid and other oxidizing agents makes it possible to degrade the most resistant organic compounds. The main disadvantages associated with the use of sulfuric acid are associated with its tendency to form insoluble compounds and, paradoxically, its high boiling point [28].

The dissolution of trace metals by heated nitric acid alone or in combination with other acid and oxidizing agents is widely used to decompose organic samples, which is consistent with our extraction protocol. Indeed, Mokoena et al. [29] used the wet process for the extraction of minerals by mixing hydrogen peroxide/nitric acid ( $H_2O_2/HNO_3$ ). These methods are never preceded by calcinations because the hydrogen peroxide will let the organic matter volatilize in a closed environment.

Other processes of extraction are also mentioned in the literature, such as the dry ashing procedure [30,31]. This method consists of calcinations followed by recovery from the ashes with an appropriate acid. The calcinations carried out at a conveniently chosen temperature ensure the decomposition of organic matter. The organic matter-bound minerals are then transformed into carbonates or oxides. When selecting the temperature of calcinations, the complete removal of the organic matter must be assured, without causing the partial or total volatilization of elements to be dosed [32]. The temperature of calcinations varies from 450 °C [28] to 550 °C [25]. The transition metals that form complexes with entities are called electron-rich ligands. The stability of these complexes depends on several parameters, such as pH, temperature, and the nature of the ligands and metal.

The results obtained in the present work indicate that method B, using 10% HNO<sub>3</sub>, and method C, which represented the mixture of HNO<sub>3</sub>–HClO<sub>4</sub>–H<sub>2</sub>SO<sub>4</sub>, gave the highest contents of Cu (135.0 and 209.9  $\mu$ g·g<sup>-1</sup> DW) and Zn (299.7 and 347.7  $\mu$ g·g<sup>-1</sup> DW) in the two halophytes *Atriplex portulacoides* and *Arthrocnemum indicum*. Likewise, method B was better (p < 0.05) for Cu and Zn extraction in *Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279. However, Sleimi et al. [33] found lower Cu contents—14.5, 15.1, 24.2 and 14.9  $\mu$ g·g<sup>-1</sup> DW—in *Atriplex portulacoides*, *Arthrocnemum indicum*, *Salicornia arabica* and *Sueada fruticosa*, respectively, harvested at the edge of the lagoon of Bizerte.

We even noticed that method B achieved the highest Cu and Zn levels compared to the certified reference values of 46.6  $\mu g \cdot g^{-1}$  DW and 16  $\mu g \cdot g^{-1}$  DW, respectively, in *Olea europaea* BCR-62, and 13.1 and 51.3  $\mu g \cdot g^{-1}$  DW, respectively, in *Ulva lactuca* BCR-279 (Table 1). This difference between the results obtained and the certified reference values could be explained essentially by the use of different extraction methods and assay techniques.

**Table 1.** Comparison of results obtained by tested digestion methods with certified reference values in *Olea europaea* BCR-62 and *Ulva lactuca* BCR-279. The "certified reference values" are published by the Community Bureau of Reference—BCR of the Commission of the European Communities, Brussels 1986 and 1987, respectively, for BCR-62 and BCR-279.

Olea europaea BCR-62	Cu ( $\mu g \cdot g^{-1}$ DW)	Zn ( $\mu g \cdot g^{-1}$ DW)
Method A	97.07	209.40
Method B	196.11	616.67
Method C	38.78	127.21
Method D	55.47	51.53
Method E	78.99	52.11
Method F	48.05	46.78
Method G	46.87	54.09
Method H	79.87	270.99
Certified reference value	46.60	16.00
Ulva lactuca BCR-279	Cu ( $\mu g \cdot g^{-1}$ DW)	Zn ( $\mu g \cdot g^{-1}$ DW)
Method A	79.79	290.93
Method B	159.66	482.75
Method C	39.80	159.80
Method D	54.51	70.43
Method E	68.25	73.54
Method F	30.68	66.36
Method G	42.52	66.57
Method H	30.51	54.63
Certified reference value	13.10	51.30

Compared to all other methods, the highest contents of Fe were recorded using method B in all studied species. In general, the same results were observed for Ca extraction, which shows that method B was the most remarkable for the mineralization of this element.

On the other hand, the results show that heated extraction leads to a greater level of Cr and Ba in *Atriplex portulacoides*, *Arthrocnemum indicum*, *Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279.

## 5. Conclusions

The shoots of *Atriplex portulacoides, Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279 were collected, rinsed and dried by oven, and after that they were crushed and homogenized. Eight procedures of extraction have been tested to show the best way create solutions of copper, zinc, iron, calcium, chromium and barium.

Our results show that cold extraction by nitric acid diluted at 10% (method B) is the most efficient way to bring the copper, zinc, iron and calcium ions into the solution in *Atriplex portulacoides, Arthrocnemum indicum, Olea europaea* BCR-62, *Ulva lactuca* and *Ulva lactuca* BCR-279. Besides this, compared to the certified reference values in *Olea europaea* BCR-62 and *Ulva lactuca* BCR-279, method B achieved the highest Cu and Zn levels. Meanwhile, the use of the heated extraction procedure is best for the solubilization of chromium and barium ions into a solution. Method B uses nitric acid diluted at 10%, so it has a lower impact on the ecosystem than methods using acids or a mixture of concentrated acids. Finally, TMEs were dosed by atomic absorption spectroscopy, and these are the best when dosing low levels of metallic ions. **Author Contributions:** Conceptualization, N.S. and I.C.; methodology, N.S., I.B. and R.K.; formal analysis, I.B. and N.D.; writing-original draft preparation, N.S. and I.B.; writing-review and editing, N.S., B.D. and I.C.; visualization, B.D. and I.C.; supervision, N.S. and I.C. All authors have read and agreed to the published version of the manuscript.

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## References

- 1. Briffa, J.; Sinagra, E.; Blundell, R. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon* **2020**, *6*, e04691. [CrossRef] [PubMed]
- Hou, D.; O'Connor, D.; Igalavithana, A.D.; Alessi, D.S.; Luo, J.; Tsang, D.C.W.; Sparks, D.L.; Yamauchi, Y.; Rinklebe, J.; Sik Ok, Y. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. *Nat. Rev. Earth Environ.* 2020, 1, 366–381. [CrossRef]
- Masindi, V.; Muedi, K.L. Environmental Contamination by Heavy Metals. In *Heavy Metals*; Saleh, H., Aglan, R., Eds.; IntechOpen: London, UK, 2018; pp. 115–133. [CrossRef]
- Diarra, I.; Prasad, S. The current state of heavy metal pollution in Pacific Island Countries: A review. *Appl. Spectrosc. Rev.* 2021, 56, 27–51. [CrossRef]
- 5. Al Naggar, Y.; Khalil, M.S.; Ghorab, M.A. Environmental Pollution by Heavy Metals in the Aquatic Ecosystems of Egypt. *Open Acc. J. Toxicol.* **2018**, *3*, 555603. [CrossRef]
- 6. Vassileva, E.; Docekalova, H.; Baeten, H.; Vanhentenriijk, S.; Hoenig, M. Revisitation of mineralization modes for arsenic and selenium determinations in environmental samples. *Talanta* **2001**, *54*, 187–196. [CrossRef]
- Idera, F.; Omotola, O.; Adedayo, A.; John Paul, U. Comparison of acid mixtures using conventional wet digestion methods for determination of heavy metals in fish tissues. J. Sci. Res. Rep. 2015, 8, 1–9. [CrossRef]
- 8. Sghaier, D.B.; Bankaji, I.; Pedro, S.; Caçador, I.; Sleimi, N. Photosynthetic behaviour and mineral nutrition of *Tamarix gallica* cultivated under aluminum and NaCl combined stress. *Phyton-Int. J. Exp. Bot.* **2019**, *88*, 239–252. [CrossRef]
- 9. Labidi, O.; Vives-Peris, V.; Gómez-Cadenas, A.; Pérez-Clemente, R.M.; Sleimi, N. Assessing of growth, antioxidant enzymes and phytohormone regulation in *Cucurbita pepo* under cadmium stress. *Food Sci. Nutr.* **2021**, *9*, 2021–2031. [CrossRef]
- 10. Abbruzzini, T.F.; Silva, C.A.; de Andrade, D.A.; de Oliveira Carneiro, W.J. Influence of digestion methods on the recovery of iron, zinc, nickel, chromium, cadmium and lead contents in 11 organic residues. *Rev. Bras. Ciência Solo* **2014**, *38*, 166–176. [CrossRef]
- 11. Caçador, I.; Caetano, M.; Duarte, B.; Vale, C. Stock and losses of trace metals from salt marsh plants. *Mar. Environ. Res.* 2009, 67, 75–82. [CrossRef]
- 12. Momen, A.A.; Zachariadis, G.A.; Anthemidis, A.N.; Stratis, J.A. Investigation of four digestion procedures for multi-element determination of toxic and nutrient elements in legumes by inductively coupled plasma-optical emission spectrometry. *Anal. Chim. Acta* 2006, 565, 81–88. [CrossRef]
- 13. Micó, C.; Recatalá, L.; Peris, M.; Sanchez, J. A comparison of two digestion methods for the analysis of heavy metals by flame atomic absorption spectroscopy. *Spectrosc. Eur.* **2007**, *19*, 23–26.
- 14. Asher, A.S.; Samuel, K.E.; Mary, D.S. Analytical method for comparison of suitable wet digestion methods for heavy metal analysis in soil around a cement industry. *Int. J. Res. Sci. Innov.* **2020**, *7*, 2321–2705.
- 15. Ghanthimathi, S.; Aminah, A.; Salmijah, S.; Ujang, T.; Nurul Izzah, A. Comparison of microwave assisted acid digestion methods for ICP-MS determination of total arsenic in fish tissue. *Sains Malays.* **2012**, *41*, 1557–1564.
- 16. Ranasinghe, P.; Weerasinghe, S.; Kaumal, M.N. Determination of heavy metals in tilapia using various digestion methods. *Int. J. Sci. Res. Innov. Technol.* **2016**, *3*, 2313–3759.
- Shaibur, M.R.; Md Shamim, A.H.; Imamul Huq, S.M.; Kawai, S. Comparison of digesting capacity of nitric acid and nitric acid-perchloric acid mixture and the effect of lanthanum chloride on potassium measurement. *Nat. Sci.* 2010, *8*, 157–162.
- Müller, E.I.; Mesko, M.F.; Moraes, D.P.; Korn, M.d.G.A.; Flores, É.M.M. Wet digestion using microwave heating. In Microwave-Assisted Sample Preparation for Trace Element Analysis; Flores, É.M.M., Ed.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 99–142. [CrossRef]
- 19. Baize, D. Teneurs totales en "métaux lourds" dans les sols français. Le Courrier De L'environnement De L'inra 2000, 39, 39–54.
- 20. Larson, K.D.; Dejong, T.K.; Johnson, R.S. Physiological and growth responses of mature peach trees to postharvest water stress. *J. Am. Soc. Hortic. Sci.* **1988**, 133, 296–300.

- 21. Bankaji, I.; Sleimi, N. Polymorphisme biochimique chez quelques halophytes autochtones du nord Tunisien. *Revue Ecologie* **2012**, 67, 29–39.
- 22. Lefèvre, I.; Marchala, G.; Meerts, P.; Corréal, E.; Lutts, S. Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex, halimus* L. *Environ. Exp. Bot.* **2009**, *65*, 142–152. [CrossRef]
- Redondo-Gómez, S.; Mateos-Naranjo, E.; Andrades-Moreno, L. Accumulation and tolerance characteristics of cadmium in a halophytic Cd-hyperaccumulator, *Arthrocnemum macrostachyum. J. Hazard. Mater.* 2010, 184, 299–307. [CrossRef] [PubMed]
- 24. Barbaste, M. Analyse des teneurs en métaux dans les végétaux, analyse foliaires de diverses espèces végétales Al, As, B, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Zn. In *Comparaison de Méthodes D'analyse des Éléments Traces Métalliques (ETM) et des Hydrocarbures Aromatiques Polycycliques (HAP) sur les Sols et les Végétaux;* Hébrard-Labit, C., Meffray, L., Eds.; [Rapport de Recherche]; Centre d'études sur les réseaux, les Transports, l'urbanisme et les Constructions Publiques (CERTU): Bordeaux, France, 2004; pp. 63–75.
- 25. Des Ligneris, L.; Douay, F.; Sterckeman, T. Teneurs en métaux (Pb, Cd et Zn) de végétaux cultivés dans les exploitations agricoles et les jardins familiaux. In *Programme de Recherches Concertées, Environnement et Activités Humaines, Etude d'un Secteur Pollué par les Métaux, Lère Partie: Métaux Polluants des Sols (Cd, Pb, Zn) et Organismes Vivants. Qualité des Productions Végétales;* Ministère de l'Enseignement Supérieur et de la Recherche, Région Nord-Pas de Calais: Nord-Pas-de-Calais, France, 1999; Volume 3, p. 131.
- 26. C.E.R.T.U.: Centre d'Etude sur les Réseaux, les Transports, l'Urbanisme et les Constructions Publiques. Comparaison de Méthodes D'analyse des Éléments Traces Métalliques et des Hydrocarbures Aromatiques Polycycliques (HAP) sur les Sols et les Végétaux 2004; Centre d'Etude sur les Réseaux, les Transports, l'Urbanisme et les Constructions Publiques (CERTU): Lyon, France, 2004.
- Uddin, A.H.; Khalid, R.S.; Alaama, M.; Abdualkader, A.M.; Kasmuri, A.; Abbas, S.A. Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. *J. Anal. Sci. Technol.* 2016, 7, 6. [CrossRef]
- 28. Hoenig, M. Preparation steps in environmental trace element analysis—Facts and traps. Talanta 2001, 54, 1021–1038. [CrossRef]
- Mokoena, D.P.; Mngadi, S.V.; Nomngongo, P.N. Microwave-Assisted extraction of trace metals from sediments using dilute hydrogen peroxide and dilute nitric acid prior to their determination by Inductively Couple Plasma-Optical Emission Spectrometry. *Curr. Anal. Chem.* 2020, 16, 970. [CrossRef]
- 30. Yang, L.; Li, Y.; Xj, G.; Ma, X.; Yan, Q. Comparison of dry ashing, wet ashing and microwave digestion for determination of trace elements in *periostracum serpentis* and *periostracum cicadae* by ICP-AES. *J. Chil. Chem. Soc.* **2013**, *58*, 3. [CrossRef]
- Anugrahwati, M.; Falahudin, A.; Khoirul Anas, A. Comparative analysis of dry ashing and wet digestion methods for determination of Pb metal in tobacco leaves. *AIP Conf. Proc.* 2020, 2229, 030040. [CrossRef]
- 32. Bui, X.T. Use of Matrices Agro-Foodstuff as Indicators of Environmental Pollution: Example of Milk. Ph.D. Thesis, Sciences of the Universe, Paris, France, 2007.
- Sleimi, N.; Bankaji, I.; Dallai, M.; Kefi, O. Accumulation des éléments traces et tolérance au stress métallique chez les halophytes colonisant les bordures de la lagune de Bizerte. *Revue Ecologie* 2014, 69, 49–99.