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Analysis and Determination of Polyphenol and Iron Concentrations in Some Selected Seaweed Species using UV-Vis

J. I. Bungudu[1*] and Lorrie M. Murphy[2]

[1]Department of Pure and Industrial Chemistry, Faculty of Science, Federal University Birnin Kebbi, Kebbi State, Nigeria.
[2]Chemistry Unit, School of Natural sciences, Bangor University, Wales, United Kingdom.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Seaweeds readily absorb heavy metals that are present in their environment, because of this; they are an effective way of monitoring heavy metal pollution in an area. The metal that is absorbed can be damaging to the seaweed cell walls. Seaweeds have evolved over time to have excellent antioxidant systems to combat this damage. One antioxidant they produce is polyphenol, a chemical molecule containing many phenol rings that bind to metals and keep them from the damaging the cell walls. They have been shown to be linked to the health benefits of red wine, fruit and vegetables. Samples of different species of seaweed from the Bangor area were collected, processed and analysed for their iron and polyphenol concentrations. Samples were collected from multiple points along the Bangor coastline, they were dried and processed into a fine, dry powder. The iron concentrations were analysed by Atomic Absorption Spectroscopy after digestion by HNO3. Different species were analysed for the iron and polyphenol content. The whole of the algae was analysed, as well as the nodules and the rest (stipe and blades). The polyphenol concentrations were analysed by a colourimetric assay using the Ragan and Glombitza method and quantified by UV/Vis spectroscopy. Different species were tested in order to see if the

**Corresponding author: E-mail: jibungudu@gmail.com;*

concentrations of iron and polyphenol change between species. Different parts of the seaweed were also tested to see where the higher concentrations of the metals were located. Iron concentration statistically significantly changed between all species with the concentrations ranging from 58.0 ± 3.5 mg/Kg to 796.0 ± 10.6 mg/Kg. The Polyphenol concentration changed statistically significantly between some species, but statistically not significant between others. Polyphenol concentration ranged from 63.7 ± 0.3 mg (g dw)-1 to 202.1 ± 6.7 mg (g dw)-1.

Keywords: Atomic absorption spectroscopy; UV Vis; seaweeds; iron and polyphenol.

1. INTRODUCTION

Many seaweeds are large, multicellular systems and with complex structures e. g kelp plants, often referred to as brown seaweeds, while others are reported to be unicellular with uniform and identical cells such as a green seaweed *Ulva lactuca*.[1] Seaweeds vary in size from very small number of millimetres to metres[1]. It was reported that about 11,000 seaweed species are widely distributed across the earth surface with 644 species believed to be found on the coastal areas of United Kingdom [2]. There are 14 species of seaweeds that are commonly found across the beaches of north Wales [2]. Seaweed species have been reported to constitute significant quantity of polysaccharides, minerals, proteins, fibres, vitamins and lipids, polyphenol and some trace elements [3]. Polyphenols and polysaccharides from seaweeds have been found to possess antiviral, antibacterial and antifungal properties as well as their potentiality in preventing some serious diseases including diabetes, cardiovascular diseases (CVD), obesity and cancer [4]. Seaweed growth and photosynthesis efficiency can be affected by a broad range of temperature and some environmental stressors such as UV radiation [5]. The continuous and constant exposure to these stressors caused the formation of radicals and oxidizing agents. However, seaweeds possess an effective antioxidant system that reduce the impact and damage being caused to DNA, proteins, lipids and amino acids by the oxidizing agents [5].

Higher concentrations of toxic metals such as Pb and Cd concentration on seaweeds will re-duce the growth rate, chloroplast content and it leads to death of cells [5]. Polyphenol compounds are heterogeneous group of compounds containing many phenol rings with antioxidant activity, the ring can vary from small to large compounds depending on the number of rings present in the compound [6]. Fig. 1 show examples of a small and large polyphenol compounds.

There are many different polyphenol compounds that are present in the seaweed such as hydroxycinnamic acid and phlorotannin including phloroglucinol, eckol and eckstolonol [6].

Fig. 2 provide an overview of polyphenol compounds found in the seaweed. Health benefit of polyphenols compounds have been reported to increase significantly over the years and they are used against the aggressive pathogens and Ultraviolet radiation [7].

Research conducted shows potential health benefit of polyphenols consumption in the human diet and can prevent serious diseases such as diabetes, cancer development, neurodegenerative disease and osteoporosis among others [7]. Polyphenols can be used in the diet to contribute to the flavour, bitterness, oxidative stability, odour and astringency [7].

Seaweed rich- antioxidants can be used to prevent chronic diseases and also provide health benefit of some food properties and enhanced shelf life [8].

Polyphenols and polysaccharides cells wall that are mostly found in brown seaweeds could provide binding sites and therefore can be easily bonded to metals through chelation [5].

Natural environments are in continuous harm causing an environmental pollution as a result of mining and other anthropogenic activities [8]. Some metals including molybdenum, nickel, manganese, copper, lead, and zinc are considered to be essentials for normal seaweed growth and development.9 However, some of these metals can be harmful and dangerous to the growth and development of seaweed when the required amount of concentration is exceeded [9]. Since metals are considered as non-biodegradable substances and at higher concentrations, these metals can accumulate into human body via the diet and through the food chain [9]. Heavy metals such as arsenic

(As), cadmium (Cd), lead (Pb) and mercury (Hg) can be toxic to human even at trace levels [8]. However, the level of toxicity can vary depending on factors such as exposure route, dose, age, and even the health status of affected individual [8].

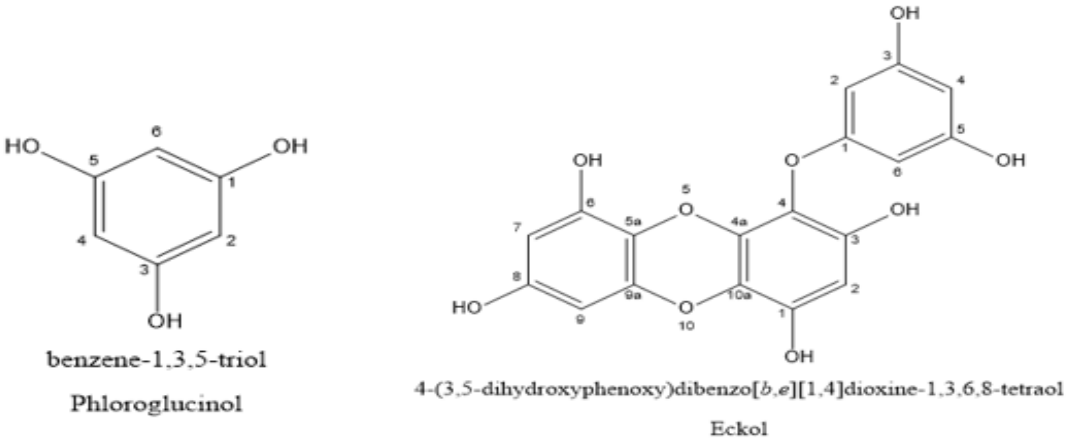

Fig. 1. Example of small and Large polyphenol compounds

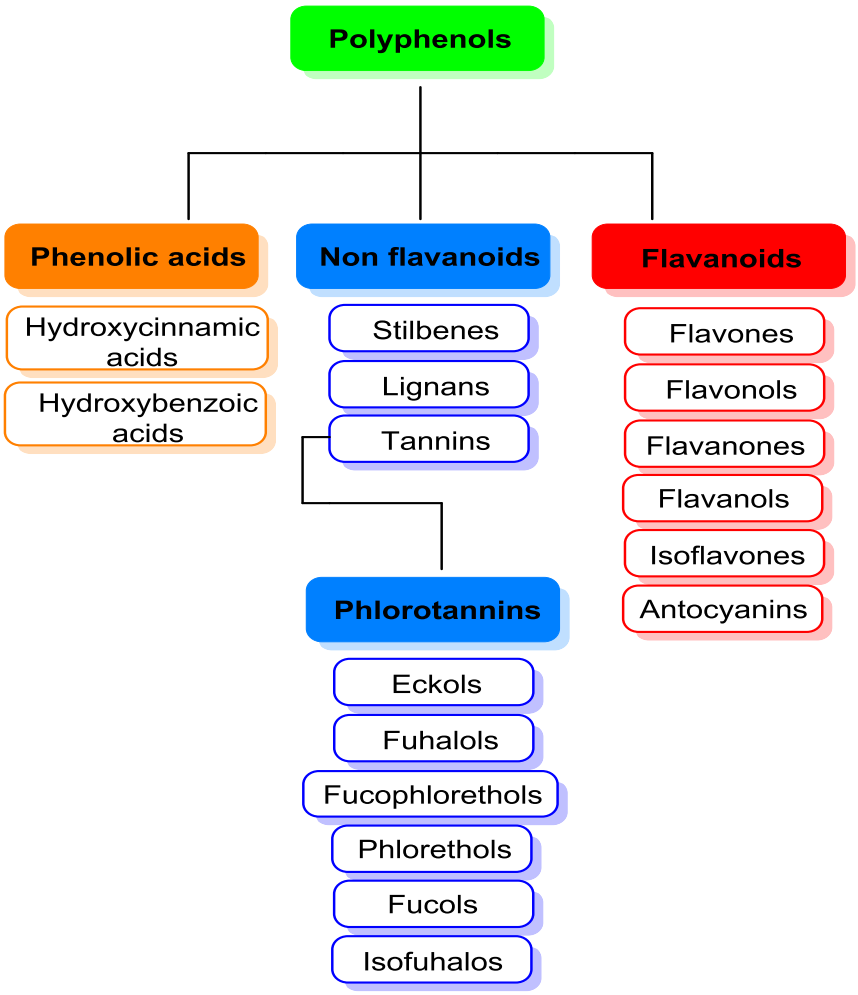

Fig. 2. Polyphenol compounds found in seaweed (Redrawn from.6)

Seaweeds are considered as a good biomonitoring agent because of their ability to accumulate many compounds. Seaweed can be used directly to investigate the levels of metals contamination over an area for a specified time.8 In order to analyse and determine the concentrations of polyphenols in the seaweed species, a spectroscopic technique known as UV/Vis is used. UV/Vis is simply the interaction of light and matter. In UV/Vis, light is being separated into it different components through the process of diffraction where a beam of single light of selected wavelength is passed via a standard and sample simultaneously [10].

The whole idea of using UV/Vis spectroscopy is that different compounds might absorb photons of different wavelengths based on their electronic structures. We might be able to look at the UV spectrum of a compound and tell its identity or structure [10]. Both the standard or reference and sample on one side is measured by a detector and transmittance produced as photon [10]. From this transmittance, an absorbance can be calculated. UV/Vis has been used to identify different groups of compounds in simple and complex samples [10]. Atomic absorption spectroscopy (AAS) is one of the most powerful and sensitive analytical methods to absorb ultraviolet or visible radiation by atoms in gas phase, the sample is converted into atoms by aspirating the aqueous sample into the flame, those atoms formed absorbs electromagnetic radiation emitted from a source (a hollow cathode lamp). The choice AAS is justified because it provides a high degree of accuracy. Normally results fall within a range of 0.5 per cent to 5 per cent accuracy, but this may improve further depending on the standards set for testing and analysis. It is a highly sensitive method of analysis. In a given material, it can measure parts per billion of a gram

2. MATERIALS AND METHODS

2.1 Sample Preparation

The polyphenolic content of the seaweed extracts was determined colorimetrically using the Folin-Ciocalteu reagent according to the Regan and Glombitza (1986).5 The process of seaweed extraction involves using 250 ± 0.5 mg of well-grounded seaweed powder and put in 10 mL of 80/20 (v/v) acetone/water. The mixture was then incubated for an hour in the dark at room temperature for cells realignment. The supernatant was recovered and the pellet reextracted for the second time under same condition as above. Supernatants from first and second extraction were pooled and filtered using o.45 µm filter paper. 200 µL of filtrate were mixed with 1300 µL deionised water plus 100 µL Folin-Ciocalteu reagents followed by addition of 400 µL of 29 % Na2CO3 to achieve alkaline condition. After samples incubation at 45°C for another 30 minutes in the dark, the absorbance was measured at 760nm uisng UV/Vis with phloroglucinol as a standard reference for brown seaweed phenolic determination. The use of phloroglucinol as standard reference is important because it has been reported to constitute secondary metabolite of phlorotannins in brown seaweeds.5 Gallic acid has been identified to constitute secondary metabolite of phlorotannins in red seaweeds.5 Approximately 50 mg of phloroglucinol was dissolved in 10 mL deionised water to make 5000 mg/L phloroglucinol and galic stock solutions. Standard of 0.0 to 100 mg/L standards were made from the stock solution. The polyphenol concentrations of the red species were analysed against the standard of gallic acid.

Furthermore, for iron determination, powdered seaweed sample in triplicate (500 ± 0.5 mg) was digested using borosilicate glass digestion tube plus 5 mL conc. HNO3 that stand for 4 hours. Blank samples were prepared with only 5 mL conc. HNO3 and placed into an empty digestion tube. The samples were heated for an hour at 80°C, the temperature was increased to 100°C for another 1 hour and finally the temperature was raised to 120°C for additional 2 hours in digestion heating blocks. Open completion of digestion, sample were allowed to cool before dilution to 50 mL with denoised water and the samples were then filtered using 0.22 µm filter to ensure no solid seaweed remained. Iron standards of 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L were prepared from commercially purchased pure iron standard solution for AAS (Fisher Scientific, UK) by diluting the stock solution further with deionised water. AAS spectrometer was used to analyse the solutions.

3. RESULTS AND DISCUSSION

Polyphenolic concentrations of the five different seaweeds species were calculated using the standard calibration curve. An example of calibration curve used in present study has been shown in Fig. 3. Both polyphenol and iron concentrations were determined, and results presented.

3.1 Analysis of Polyphenol Concentration between the Species

The mean concentrations of polyphenol for all the five species have been presented in Table 1.

Statistical analysis of the results using T-test shows that the concentrations of polyphenol varied statistically significant between some species ($P < 0.05$) and no significant difference between others. The polyphenol concentrations ranged from 64.0 ± 0.3 mg (gdw)-1 to 202.0 ± 5.9 mg (gdw)-1 for the *Ascophyllum nodosum* from B and *Fucus serratus* collected, respectively.

The two species of *Ascophyllum nodosum* samples obtained from location B (53°19'04.5"N 3°50'40.4"W) and L (53˚19´17.9" N 3˚51´01.3" W) were statistically significant as shown in Table 2 ($P < 0.05$). The other three species for genus *Fucus* (*Fucus vesiculosus*, *Fucus serratus* and *Fucus spiralis*) were not statistically significant ($P > 0.05$) for polyphenol concentrations between the species as shown in Table 2.

The concentrations of polyphenol in the three species were close to each other as shown in Table 1 with highest concentration found in *F. serratus* (202.0 ± 5.9 mg (gdw)-1) and *F. spiralis* (201.0 ± 5.8 mg (gdw)-1) fell in between the other two species. In all the seaweed species investigated the lowest mean concentration of polyphenol was found in *Ascophyllum nodosum* collected from L and highest polyphenol average concentration was found with *Fucus serratus* as shown in Table 1. The concentrations of polyphenol of six sample species were *Ascophyllum nodosum* (L) < *Ulva lactuca* < *Ascophyllum nodosum* (B) < *Fucus vesiculosus* < *Fucus spiralis* < *Fucus serratus*. Literature scan shows limited studies conducted to determine the content of polyphenol and trace metals on same species across selected locations.[5,11,12,13] However, in comparison with the literature values for other brown species (*Alaria esculenta* and *Saccharina latissimi)*, the polyphenol concentration of present study was much higher. The values published for the species in that study are in the range of 5-61 mg (g dw)-1.5 Even though studies regarding determination of total concentration of polyphenol in seaweeds is limited, there are reviews conducted for the identification, extraction and quantification phenolic content of a brown seaweed species.[11] Three brown seaweeds basically *Ascophyllum nodosum, Fucus vesiculosus* and *Bifucaria bifurcate* from Galicia in New Spain has their phenolic content determined.[12] In that study, it was mentioned that in all the three species phlorotannins were identified as the major phenolic constituent of the extract.[12] Another study conducted by Morrison *et al* to investigate the variation of heavy metals concentrations in *Ascophyllum nodosum* along the Irish coastal reported mean concentrations of Pb, Cd, Co and Cr.[13] The authors reported the mean concentrations ranged from 0.054 to 2.144 µg (g dw)-1 over six season and the metals was found to be in the order of Pb > Cr > Co > Cd.[13].

3.2 Analysis of iron concentration between the species

Concentrations of iron were determined using AA spectrometer according to the method described in the experimental section above. The mean concentrations of iron in five species and standard error were presented in Table 3.

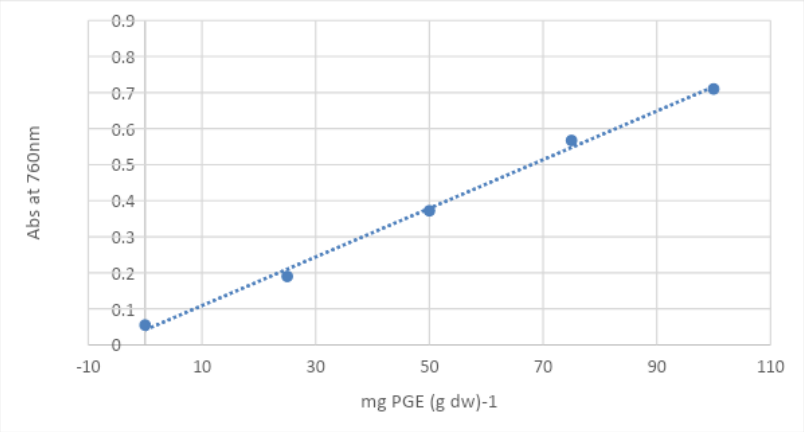

Fig. 3. An example of calibration curve for phloroglucinol standards

Table 1. Calculated mean for polyphenol concentrations for all investigated species and standard error

Seaweed	mg (g dw)-1	Standard Error
Ascophyllum nodosum (B)	145.0	± 5.0
Ascophyllum nodosum (L)	64.0	± 0.2
Ulva lactuca	125.0	± 15.8
Fucus vesiculosus	188.0	± 19.2
Fucus serratus	202.0	± 5.9
Fucus spiralis	201.0	± 5.8

B = Bangor (53°19'04.5"N 3°50'40.4"W) and L = Llandudno (53˚19´17.9" N 3˚51´01.3" W)

Table 2. Statistically significant differences in polyphenol concentration, *P* values for each species vs other species, the mean difference highlighted in bold is significant at 0.05 level

Species	other species	p.value
Ascophyllum nodosum (B)	*Ascophyllum nodosum (L)*	0.0030
	Ulva lactuca	0.8570
	Fucus vesiculosus	0.1590
	Fucus serratus	0.0390
	Fucus spiralis	0.0420
Ascophyllum nodosum (L)	*Ascophyllum nodosum (B)*	0.0030
	Ulva lactuca	0.0220
	Fucus vesiculosus	0.0000
	Fucus serratus	0.0000
	Fucus spiralis	0.0000
Ulva lactuca	*Ascophyllum nodosum (B)*	0.8570
	Ascophyllum nodosum (L)	0.0220
	Fucus vesiculosus	0.0250
	Fucus serratus	0.0060
	Fucus spiralis	0.0060
Fucus vesiculosus	*Ascophyllum nodosum (B)*	0.1590
	Ascophyllum nodosum (L)	0.0000
	Ulva lactuca	0.0250
	Fucus serratus	0.9470
	Fucus spiralis	0.9580
Fucus serratus	*Ascophyllum nodosum (B)*	0.0390
	Ascophyllum nodosum (L)	0.0000
	Ulva lactuca	0.0060
	Fucus vesiculosus	0.9470
	Fucus spiralis	1.0000
Fucus spiralis	*Ascophyllum nodosum (B)*	0.0420
	Ascophyllum nodosum (L)	0.0000
	Ulva lactuca	0.0060
	Fucus vesiculosus	0.9580
	Fucus serratus	1.0000

Table 3. Average concentrations of iron investigated between the species

Species	Fe (mg/Kg)	Standard Error
Ascophyllum nodosum (B)	66.4	± 4.7
Ascophyllum nodosum (L)	57.9	± 3.4
Ulva lactuca	794	± 10.5
Fucus vesiculosus	235.2	± 3.0
Fucus serratus	184	± 3.7
Fucus spiralis	257.9	± 5.1

Table 4. Statistically significant differences in iron concentration, *P* values for each species vs other species, the mean difference highlighted in bold is significant at 0.05 levels

Species	other species	p. values
Ascophyllum nodosum (B)	*Ascophyllum nodosum (L)*	0.755
	Ulva lactuca	0.000
	Fucus vesiculosus	0.000
	Fucus serratus	0.000
	Fucus spiralis	0.000
Ascophyllum nodosum (L)	*Ascophyllum nodosum (B)*	0.755
	Ulva lactuca	0.000
	Fucus vesiculosus	0.000
	Fucus serratus	0.000
	Fucus spiralis	0.000
Ulva lactuca	*Ascophyllum nodosum (B)*	0.000
	Ascophyllum nodosum (L)	0.000
	Fucus vesiculosus	0.000
	Fucus serratus	0.000
	Fucus spiralis	0.009
Fucus vesiculosus	*Ascophyllum nodosum (B)*	0.000
	Ascophyllum nodosum (L)	0.000
	Ulva lactuca	0.000
	Fucus serratus	0.000
	Fucus spiralis	0.000
Fucus serratus	*Ascophyllum nodosum (B)*	0.000
	Ascophyllum nodosum (L)	0.000
	Ulva lactuca	0.000
	Fucus vesiculosus	0.000
	Fucus spiralis	0.000
Fucus spiralis	*Ascophyllum nodosum (B)*	0.000
	Ascophyllum nodosum (L)	0.000
	Ulva lactuca	0.009
	Fucus vesiculosus	0.000
	Fucus serratus	0.000

The concentration of iron among all the investigated species were statistically significant as shown in Table 4 ($P < 0.05$). The limit of detection (LoD) was 0.38 mg/L.

The statistical analysis of the results using T-test shows that the concentrations of iron between the two species of *Ascophyllum nodosum* collected from locations B and L were not statistically significant ($P > 0.05$), but these species were statistically significant to the remaining four species as presented in Table 4. The iron concentrations of three genus species were relatively close to each other as shown in Table 4 with highest concentration found in *F. spiralis* (257.9.0 ± 5. mg /kg, lowest in *F. serratus* (184 ± 3.7) and *Fucus vesiculosus* fell in between the other two species. The lowest and highest iron mean concentrations was recorded with *Ascophyllum nodosum* (L) 57.9 ± 3.4 mg/Kg and *Ulva lactuca* 794.0 ± 10.5 mg/Kg. The mean iron concentration of all the five species were found to be in the increasing order of *Ascophyllum nodosum* (L) < *Ascophyllum nodosum* (B) < *Fucus serratus* < *Fucus vesiculosus* < *Fucus spiralis* < *Ulva lactuca.* In general, iron concentrations between all investigated species were statistically significant ($P < 0.05$). Limited published literature values were available for Wales for comparison. [14] The mean concentrations of iron in *Ascophyllum nodosum* found in present study were within published values of 101-176 mg/Kg reported from Norway.[15] The red species of *Ulva lactuca* values reported from Sri Lanka were lower (73-158 mg/Kg)[16] compared to the values obtained in this study (794 ± 10.5 mg/kg). North and West Wales studies reported values for *Fucus vesiculosus* to be 9-350 mg/Kg and these are higher than values of 33-145 mg/Kg cited from the conducted in Norway.15,14 73-197 mg/Kg was reported from North Wales for *Fucus serratus* and our values fall within [14,16].

4. CONCLUSION

In conclusion, five different species of seaweed were analysed for their iron and polyphenols concentrations. Iron concentrations were statistically significant ($P < 0.05$) between all the five species. A concentrations range were measured as shown in Tables 1 and 3. Concentrations of polyphenol measured ranged from 64.0 ± 0.2 mg (g dw)-1 for *Ascophyllum nodosum* collected from L to 202.0 ± 5.9 mg (g dw)-1 for *Fucus serratus*. The concentrations of polyphenol in increasing order among the species was *Ascophyllum nodosum* (L) *Ulva lactuca* < *Ascophyllum nodosum* (B) < *Fucus vesiculosus* < *Fucus spiralis* < *Fucus serratus*.

A project like this one is recommended to be conducted over different locations across the Wales and possibly wider UK. Seaweed is readily available as good biomonitoring agent for metals remediation and can also be used in determination of many bioactive compounds for nutrition and medicine

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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