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Comparison between Microwave Digestion and Conventional Open Digestion Method for Heavy Metals determination

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ABSTRACT

In the present study a comparison between microwave assisted digestion and conventional wet acid open digestion system for dissolution of bivalve tissue samples was carried out. Sample matrix decomposition was done by using a mixture of concentrated nitric acid and hydrogen peroxide. The determination of trace elements (Zn, Co, Cu, Cd and Pb) in commercially popular bivalve species from two important fish landing centers in Mumbai was used for this purpose. The recovery of heavy metals after analysis was done using certified reference material TORT 2- Lobster hepatopancreas for both the digestion methods. The results of the present study indicate that microwave digestion system for heavy metal analysis is the preferred method for rapid and good quality results. It provides better recovery and precision for heavy metal analysis. The sample preparation step is much easier by microwave digestion system compared to conventional open digestion methods for heavy metal analysis. It is particularly ideal for trace metals like lead and cadmium as they are generally present in very small concentration in biological samples. In the current study microwave digestion proved to be better method than conventional open digestion method with rapid sample digestion process and least sample contamination problem.

Key words: *Metal pollution, Microwave digestion, Metal analysis, Open digestion, Sample preparation.*

Introduction

To detect heavy metals in environmental samples there is a need of an appropriate and sensitive analytical technique. As a result, there has been a significant technological improvement. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are examples of such advances in analytical instruments for metal analysis in different environmental samples. They are sensitive analytical tools with low detection limits that provide rapid multielement analysis of samples (Samanta *et al.*, 2021).

However, most analytical techniques require a prerequisite step of converting solid samples into liquid form by thermal digestion (Wilschefski and Baxter, 2019). For this, acid digestion procedure is used which help in decomposing the solid sample matrix to solubilize the analytes of interest. Until recently conventional methods of sample digestion like wet acid open digestion using hot plate or dry ashing using muffle furnace have been used on a large scale. The advantage of these methods is that it is cost effective. However, there are several drawbacks like high occurrences of sample contamination, labor intensive process, loss of volatile elements, a greater quantity of acids required for sample digestion. Specially designed fume hoods are required to remove the toxic fumes. It also requires extreme care, skill and expertise on part of the analyst in order to get accurate results (Sánchez López *et al.*, 2003).

Thus, in order to overcome these problems, there has been advances in sample digestion techniques. At present many researchers use the microwave digestion system for sample pretreatment (Qin *et al.*, 2021; Usman, Al-Ghouti, and Abu- Dieyeh, 2019). This technique has several advantages over conventional methods: rapid digestion time, less use of acid reagents, lower sample amount, lower chances of sample contamination. Being a closed procedure, it allows the use of higher pressure as well as temperature to obtain an efficient decomposition of sample and dissolve the trace metals. There is no loss of volatile elements, no requirement of fume hood chamber as well as better safety of the analyst while preparing the sample(Perelonia *et al.*, 2021).

Heavy metals like manganese (Mn), iron (Fe), magnesium (Mg), copper (Cu), cobalt (Co), Zinc (Zn) are important for living organisms thus called as essential heavy metals. However, they are only required in trace amounts for proper functioning and can have toxic effects at higher concentrations in the body (Weerasinghe & Kaumal, 2018). Certain non-essential heavy metals namely lead (Pb), cadmium (Cd) and mercury (Hg) becomes toxic to humans even when present in trace amounts having no role in function of living organisms (Singh and Gupta, 2021).

Fish and shellfish are consumed on a global scale for their exceptional nutrition significance. They are an important source of protein, good fats like omega-3 fatty acids, vitamins and minerals (Kommuri, Naresh, and Kondamudi, 2019; Maurya *et al.*, 2019). Due to increasing awareness about several nutritional benefits of seafood products, its consumption has increased on a large scale. However, fish and shellfish tend to accumulate the contaminants like heavy metals along with their food present in the water. In recent years several researchers have studied the toxic effects of heavy metal pollution in different varieties of seafood (Vinothkannan *et al.*, 2022). Molluscs particularly bivalves have also been used as one of the most important environmental bio monitors used widely for trace metals analysis (Dar *et al.*, 2018).

Thus, in order to analyze the presence of toxic heavy metals in fish and shellfish it is very important to use the most efficient method of sample preparation. The aim of the present study was to compare the two different methods of sample digestion – conventional wet acid open digestion using hot plate and microwave digestion system for the heavy metals analysis in bivalve tissues using Inductively coupled plasma (ICP) Spectroscopy.

Materials and Methods

Instrumentation

For the analysis of trace metals Zn, Co and Cu inductively coupled optical emission spectrometer (ICP OES- Perkin Elmer Optima 7300 DV) while for Cd and Pb, ICP Mass Spectrometer (ICP MS- iCAP RQ) was used. For sample pre-treatment by close digestion method, microwave digestion system CEM MARS 5 (CEM, Matthews, NC, USA) was used. Wet acid open digestion was done using hot plate method. For sample digestion suprapure grade chemicals were used. All the beakers and glassware were cleaned thoroughly by soaking them in 10 % (v/v) nitric acid solution overnight. It was then rinsed with ultrapure water before using them for sample pretreatment. Nitric acid (65%) and Hydrogen peroxide (30%) (Merck, Germany) were used for sample pretreatment. For instrument calibration multi-element standard stock solution of 1000 mg/L of elements (Merck, Germany) was used by proper dilution.

Sample collection and digestion

Bivalve samples were collected from two fish landing centers in Mumbai (New Ferry Wharf and Arnala). They were put in ice box and brought to the laboratory for analysis. Using a plastic knife the shells were separated from the tissue. The tissue samples were cleaned with distilled water and put in oven for drying till constant weight. It was powered using mortar and pestle to make a homogenized sample which was further used for wet ashing and microwave digestion procedures in replicates. For wet acid open digestion, 0.5 g of dried powdered sample was predigested overnight with 5 ml of nitric acid at room temperature. Following the pre- digestion step the sample was treated with mixture of Nitric acid (65%) and Hydrogen Peroxide (30%) (3:1) at 120°C for 10 hours on hot plate. The completely solubilized sample was cooled and made up to 25 ml volume using deionized water.

Along with the tissue samples, certified reference material (TORT 2 Lobster hepatopancreas) obtained from National Research Council Canada (NRCC) was also digested in the same way. Blanks were also prepared with each batch but without the tissue sample. For Microwave digestion, 0.5 g of certified reference material and powdered bivalve tissue samples were digested with 2 ml HNO_3 (65%) and 1 ml H_2O_2 (30%) in polytetrafluoroethylene (PTFE) vessels using Microwave digestion system. Digestion conditions for the microwave system is shown in Table 1. Once cooled the resulting sample solutions were diluted to 25 ml with deionized water.

Statistical analysis

The data from both digestion methods was subjected to statistical analysis. Significance of values was tested using Student's t-test ($p < 0.05$).

Results and Discussions

The results for the concentration of trace metals Pb, Cd, Cu, Co, Zn in certified reference material (TORT 2 – Lobster hepatopancreas) and bivalve tissue samples using microwave digestion and conventional wet acid open digestion using hot plate are given in Table 2 and 3 respectively. Concentrations of heavy metals are presented on a dry weight basis. The average certified reference materials concentration was used to obtain the recovery percentage. Overall, there was a good agreement between the certified and observed values of CRM by both sample digestion methods. On comparison between the two methods for CRM, the recoveries of heavy metals were found to be higher by microwave digestion system for all elements except Cu at one fish landing center. Similar results were observed by (Alsehli, 2021; Mico *et al.,* 2007). The recoveries of heavy metals using microwave digestion method ranged from 94.05 % to 101.96 %, whereas for open digestion method it ranged from 88.57 % to 102.83%. The relative standard deviation (RSD) values for microwave digestion were within 6%. However, for open digestion system it was within 20 % except for Cu which was 23%. Thus, based on CRM digestion, microwave digestion system was shown to be a more precise method with lower RSD values and better recovery as compared to wet acid open digestion method. This result was similar to the results observed by (Adebiyi and Tedela (2020). The higher variability in open digestion system may be due to the splashing of sample solution between the open

Table 1. Digestion program of microwave digestion system.

Stage	Power (W)	Power (%)	Temperature (˚C)	Ramp time (min)	Hold time (min)
1	1200	100	220	15	10
2	1200	100	180	10	10
3	1000	100	150	5	8

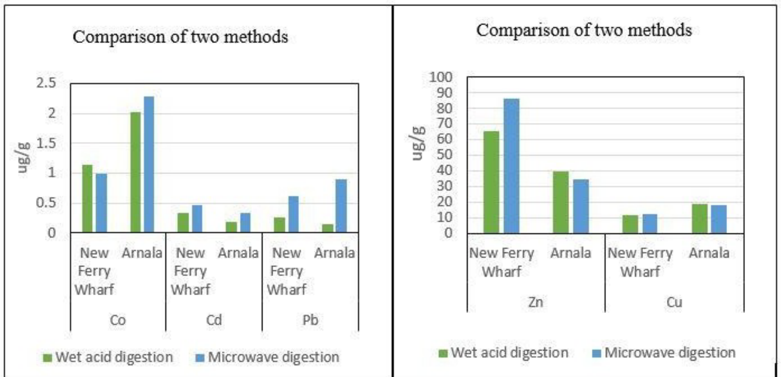
Table 2. The results of CRM 2 (TORT- Lobster hepatopancreas) analysis with microwave and open digestion system (mean ± S.D., µg/g, dry weight), N = 5.

Heavy metals	Certified values	Observed values (MD)	Recovery (%)	Observed value (OD)	Recovery (%)	RSD (%)	
						MD	OD
Pb	0.35 ± 0.13	0.35 ± 0.02	100	0.31 ± 0.07	88.57	6	23
Cd	26.7 ± 0.6	25.92 ± 0.51	97.07	23.69 ± 1.8	88.72	2	8
Zn	180 ± 6.0	174 ± 3.74	96.66	173 ± 5.9	96.11	2	3
Co	0.51 ± 0.09	0.52 ± 0.03	101.96	0.47 ± 0.09	92.15	6	19
Cu	106 ± 10.0	99.7 ± 4.95	94.05	109.25 ± 6.9	102.83	5	6

MD – Microwave Digestion, OD – Open Digestion, RSD- Relative Standard Deviation

Table 3. Mean concentration of metals (µg/g, n = 5) in bivalve tissue samples.

Location	Method	Pb	Cd	Zn	Cu	Co
New Ferry Wharf	Wet acid open digestion	0.26 ±0.13	0.34 ± 0.15	65.75 ± 2.71	11.83 ± 1.19	1.15 ± 0.22
	Microwave digestion	0.61 ±0.10	0.46 ± 0.11	86.62 ± 2.28	12.11 ± 1.05	0.99 ± 0.01
Arnala	Wet acid open digestion	0.15 ±0.24	0.19 ± 0.63	39.23 ± 2.13	18.40 ± 2.37	2.03 ± 0.63
	Microwave digestion	0.90 ±0.16	0.33 ± 0.29	34.34 ± 1.8	17.75 ± 2.0	2.58 ± 0.59



(a) Concentration of Co, Cu and Pb (b) Concentration of Zn and Cu

Fig. 1 Comparison of heavy metal contents after digestion using the two methods.

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beakers which is prevented in closed microwave digestion system. Alsehli (2021) concluded that heavy metals determination after microwave digestion is a more precise method than conventional wet acid open digestion method.

The concentration of essential heavy metals was found to be higher in the bivalve samples than the non-essential ones. Similar results were found by (Nekhoroshkov *et al.*, 2021; (Kapranov *et al.*, 2021). Statistical analysis revealed significant difference in the concentration of metals Pb, Co, Zn between the two digestion methods. Similar observations were concluded by (Ishak *et al.*, 2015 and Kilic and Soylak 2020). Wet acid digestion using open digestion system took 10–12 hours for sample digestion step only while for microwave digestion system it took less than 70 mins for the entire process. Fig. 1 shows the comparison of heavy metal digestion using the two methods.

Conclusion

The present study was conducted to compare the two sample digestion methods of conventional open digestion system and closed microwave digestion system for the determination of heavy metals (Zn, Co, Cu, Cd, Pb) in bivalve tissue samples. The recoveries of the certified reference material TORT 2 for the heavy metals (Zn, Cu, Co, Pb and Cd) using microwave digestion and open digestion were in the range of 88.57 % to 102.83%. Satisfactory recoveries were observed in both systems. However, microwave digestion system gave better recoveries of trace metals when compared to open wet acid digestion system. This inference was further established when bivalve tissue samples were digested by the two methods for heavy metals. Along with digestion efficiency, microwave digestion system provides better recovery, precision and is less complicated than wet acid open digestion system. Although the wet acid open digestion method helps in digestion of larger number of samples at a time but it is more time consuming and has higher chances of contamination**.** It also uses more amount of acids which might lead to introduction of interferences while sample analysis by ICP. Thus, with microwave assisted digestion, sample decomposition becomes faster, easier, and safer process with no loss of volatile elements. As a result of the present study, it can be concluded that microwave digestion system is the preferred method for sample digestion prior to heavy metal analysis.

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