

Metal concentrations and metallothionein metal detoxification in blue sharks, *Prionace glauca* L. from the Western North Atlantic Ocean



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OBJECTIVE

In this paper, the lack of information regarding metal concentrations and detoxification in blue sharks was addressed through the sampling of blue sharks in the North Atlantic Ocean. Through the use of ICP-MS, the liver and muscle metal concentrations of the sharks were determined. By using the UV-vis spectrophotometer, the effects of detoxification and oxidative stress endpoints were measured in these sharks. This paper establishes baseline data and measurements on metal concentrations for biomonitoring effects and changes in the population.

The choice of ICP-MS for the determination of metal concentrations was due to the high sensitivity of the instrument on trace metal element detection. By dissolving a sample, one is able to target low concentrations and the instrument can be used to quantify low trace elements, such as metals in animals. UV-vis spectrophotometry was used to determine a difference between composition of samples and reference standards. This allows for the measurement of the effects of detoxification and oxidative stress in the blue sharks.

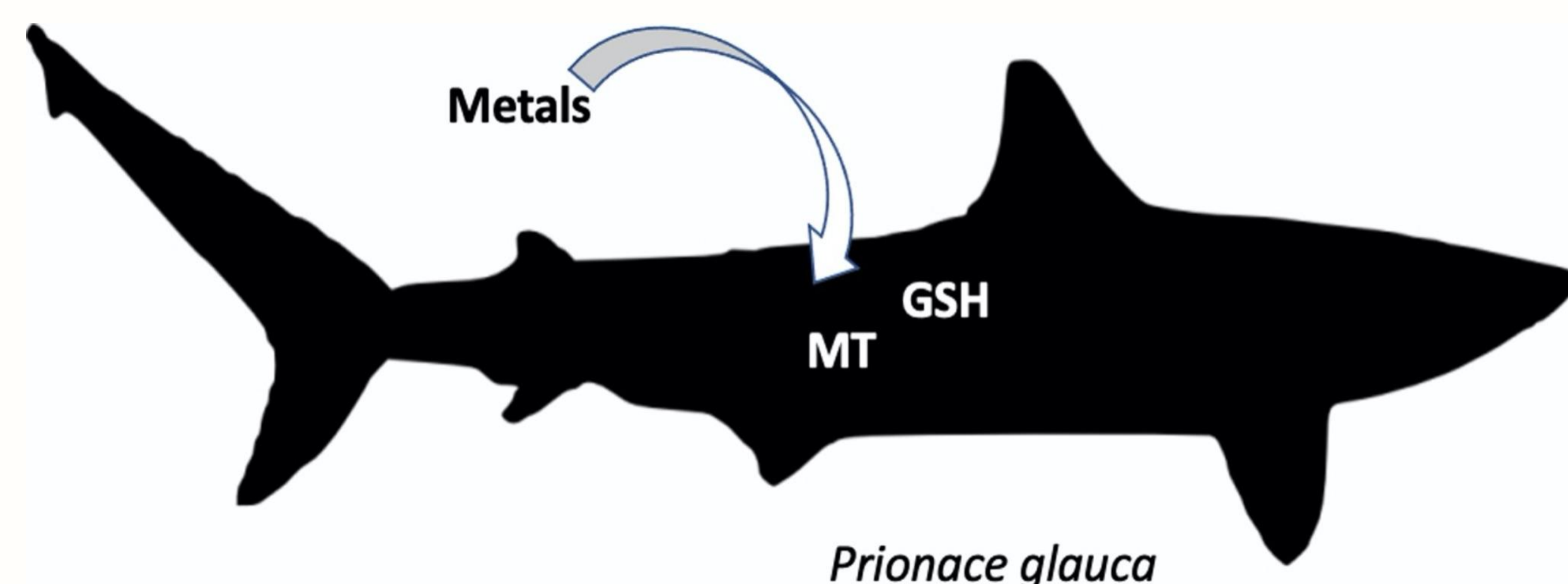


Figure 1. Image depicting the species of blue shark studied, *Prionace glauca* (Hauser-Davis et al.).

BACKGROUND

This research paper goes into detail regarding elasmobranchs and their vulnerability towards environmental metal contamination. Specifically, how blue sharks, *Prionace glauca* L., detoxify the contaminated waters through their liver and muscles. This paper also determines which specific metals are being secreted through muscles and the liver. Although the research is being done on cartilaginous species, it is significantly important for other species. This is relevant to humans due to the common consumption of these metals. For example, if fish being consumed have an excess of mercury in their system due to environmental metal contamination, this contamination could, in turn, impact the consumers. Keeping track of these environmental metal contamination levels aids in the determination of the toxicity of various species. Through an increased awareness of the contaminants and the process of contamination, the risk of potential harm to humans and other animals in the ecosystem is reduced.

FUTURE WORK

The results from this research created baseline data for metal concentrations in this blue shark species. This data can be used for further studies on environmental metal contamination in the ecosystem and as reference for future biomonitoring projects. A baseline data set could be determined for various other shark species to allow for a biomonitoring of the ecosystem.

METHODOLOGY

Study area, shark sampling, and sample processing

Blue sharks were collected from the Northern Atlantic Ocean using standard rod and reel techniques. Sex, fork length and total weight of each shark was recorded. White dorsal muscle (N=5) and liver tissue (N=8) were collected and stored at -20°C. The samples were then dried at 60°C for 48 hours and homogenized with a mortar and pestle.

Reduced glutathione (GSH) extraction and determination

Each muscle and liver sample were homogenised and centrifuged. The filtered supernatants were transferred to sterile polypropylene microtubes and incubated. The absorbances of the GSH extraction values were determined at 412 nm on a UV-vis spectrophotometer.

Metallothionein (MT) extraction and determination

MT was thermally extracted, and the liver and muscle tissue samples were homogenised and centrifuged. The supernatants were transferred to fresh sterile polypropylene microtubes and heated in a heating block, denaturing non-thermal resistant proteins. The MT sample absorbances was quantified using a UV-vis spectrophotometry to determine MT concentrations.

Metal determinations

Metal assays were performed in both dried tissue and thermally purified supernatants containing MT, to confirm total metal body load and subcellular MT-bound metals, as well as detoxification and accumulation pathways. The dried tissue and MT-containing purified supernatants were prepared for subsequent ICP-MS analysis using an Elan DRC II spectrometer. External calibration in the quantitative mode was performed for all assessed elements, using appropriate dilutions of the ICP multi-element standard solution Merck IV.

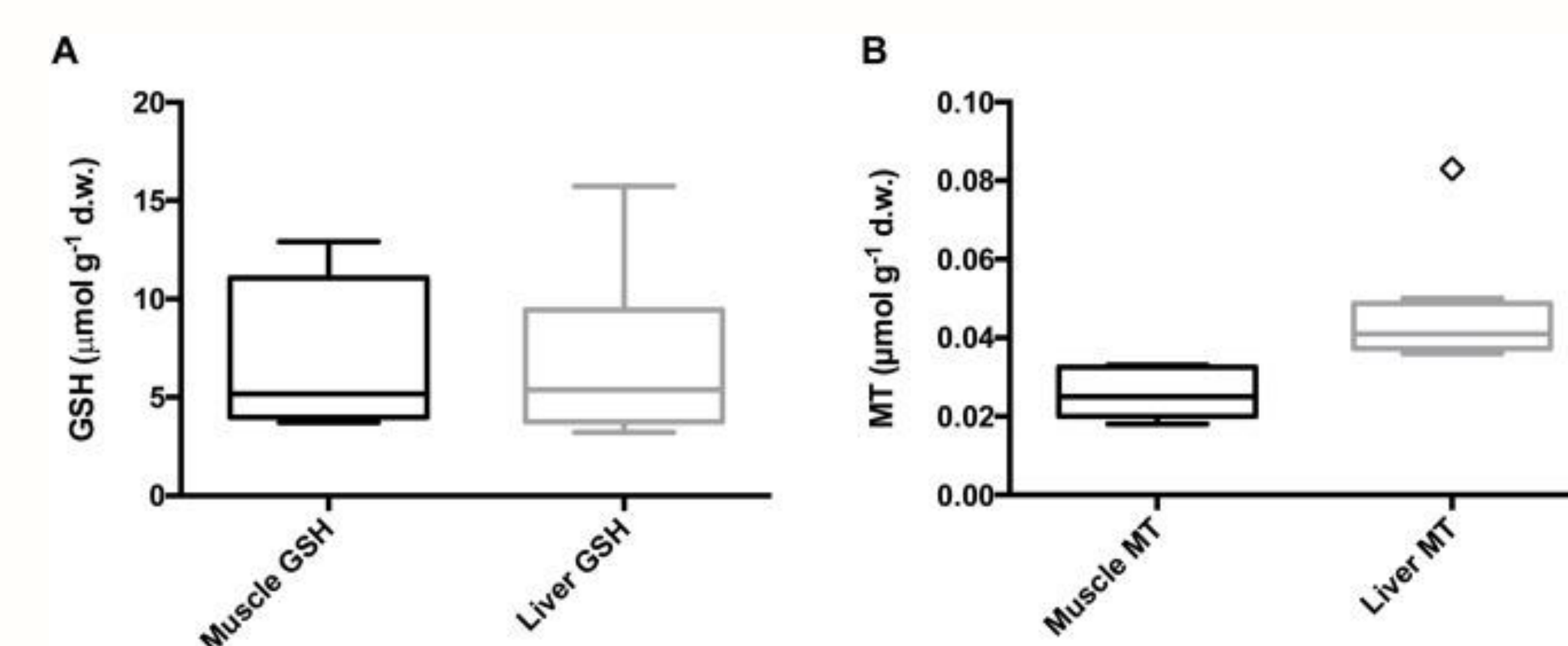


Figure 2. Blue shark liver and muscle tissue (A) glutathione (GSH) and (B) metallothionein (MT) concentrations ($\mu\text{mol g}^{-1}$ d.w.) (Hauser-Davis et al.).

REFERENCES

- Hauser-Davis RA, Rocha RCC, Saint PTD, Adams DH. 2021. Metal concentrations and metallothionein metal detoxification in blue sharks, *Prionace glauca* L. from the Western North Atlantic Ocean. *Journal of Trace Elements in Medicine and Biology*. 68.
- Kashani A, Mostaghimi J. 2010. Aerosol characterization of concentric pneumatic nebulizer used in inductively coupled plasma-mass spectrometry (ICP-MS). *Atomization and Sprays*. 20:415-433.

RESULTS

All analytical curves presented correlation coefficients greater than 0.995. To analyze data, the Shapiro-Wilk test and the Levenes test was applied to data variance. Differences between metal concentrations, MT and GSH levels were assessed by the Kruskal-Wallis ANOVA. MT and GSH data were investigated using Spearman's correlation test. All differences and correlations were significant when $p < 0.05$. No significant difference was found between muscle and liver ($p > 0.05$) GSH levels. MT concentrations were slightly lower in muscle compared to the liver.

Total metal concentrations in blue shark liver and muscle tissue were significantly different ($p < 0.05$) were observed in Al, As, Cd, Co, Cr, Cs, Cu, Fe, Hg, Mn, Sb, Sr, and Ti. Significantly higher ($p < 0.05$) concentrations in muscle were found for Al, As, Cr, Cs, Hg, Ni, Pb, Sb, Sr, Ti, V and Zn. Concerning metal levels, Al, Cr, Cu, Fe, Pb and Zn were lower compared to literature data. Ag, Co, Cs, Sb, Ti and V are all non-essential to sharks and pose risks if present above toxic concentrations.

Metallothionein is not involved in the detoxification of all assessed metals, but does work in both the muscle and liver for most present. Reduced glutathione is especially important to the detoxification of Co and Zn.

Table 1. Thermostable, MT-bound and Metal Content in blue shark liver and muscle tissue. Data is reported as means \pm standard deviation (Hauser-Davis et al.).

Element	mg kg^{-1}		% of total metal content	
	Liver	Muscle	Liver	Muscle
As	9.741 \pm 6.475	15.06 \pm 6.28	41.5	25.06
Cd	0.082 \pm 0.054	0.00106 \pm 0.00026	4.5	0.56
Cs	0.006 \pm 0.002	0.03 \pm 0.01	85.7	38.96
Cu	0.268 \pm 0.320	<LOD	25.5	<LOD
Hg	0.004 \pm 0.003	<LOD	1.5	<LOD
Pb	0.623 \pm 1.106	0.24 \pm 0.04	1780	461.54
Se	0.084 \pm 0.038	0.07 \pm 0.03	6.7	2.27
Ti	1.063 \pm 0.296	<LOD	46.0	<LOD
Zn	1.499 \pm 0.844	0.063 \pm 0.08	22.9	0.80

<LOD – Below the method limit of detection.

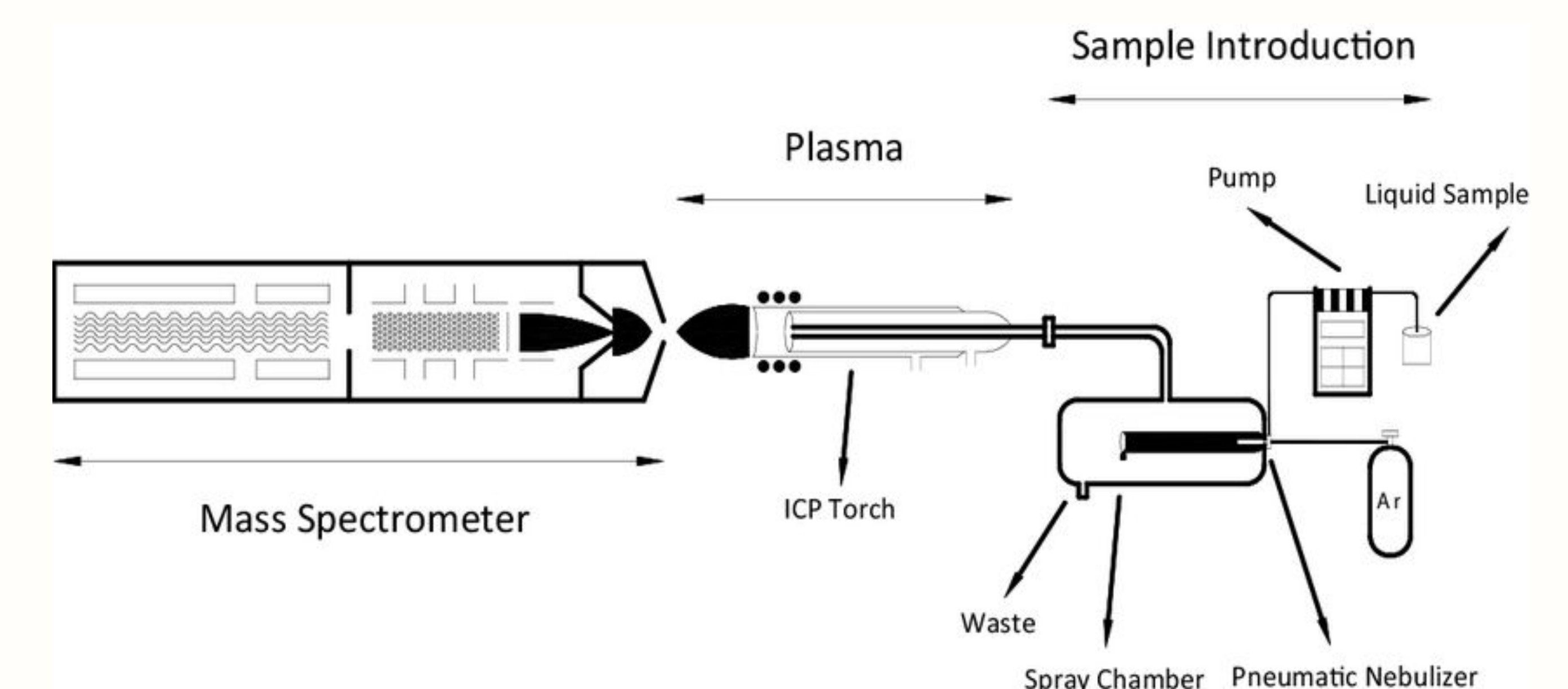


Figure 3. Schematic diagram of inductively coupled plasma mass spectrometry used to analyze trace elements (Kashani et al.).