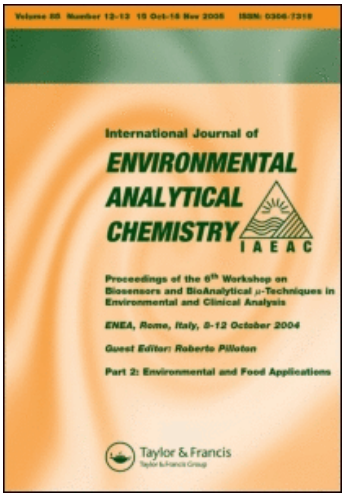

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Toxicity of Molybdenum and its Trace Analysis in Animal Tissues and Plants

Shahid Abbas Abbasi[a]
[a] Water Quality and Environment Division, Centre for Water Resources Development and Management, Kunnamangalam, Calicut, India

To cite this Article Abbasi, Shahid Abbas(1981) 'Toxicity of Molybdenum and its Trace Analysis in Animal Tissues and Plants', International Journal of Environmental Analytical Chemistry, 10: 3, 305 — 308
To link to this Article: DOI: 10.1080/03067318108071554
URL: http://dx.doi.org/10.1080/03067318108071554

Intern. J. Environ. Anal. Chem., 1981, Vol. 10, pp. 305–308
0306-7319/81/1004-0305 $06.50/0

Printed in Great Britain

Toxicity of Molybdenum and its Trace Analysis in Animal Tissues and Plants

SHAHID ABBAS ABBASI

Water Quality and Environment Division, Centre for Water Resources Development and Management, Kunnamangalam, Calicut-673571, India

(*Received February 16, 1981; in final form May 15, 1981*)

A sensitive, selective, rapid and reproducible method is presented for the analysis of sub-microgram levels of molybdenum in animal tissues (Liver) and plants. The method is based on solvent extraction of Molybdenum (VI) using isoamyl alcohol solution of *N*-*o*-tolyl-*o*-methoxy-benzohydroxamic acid at pH 1.5–2.5, and subsequent spectrophotometric determination of the yellow extract at 350 nm.

KEY WORDS: Toxicity; Molybdenum; Trace Analysis; Animal Plants; Tissues.

INTRODUCTION

Molybdenum is one of the essential trace metals for animals and plants but the need for this element varies from species to species.[1,2] Similarly the levels of molybdenum which may cause toxicity vary widely amongst different species of plants and animals.[2,3] Molybdenum concentrations which may be optimal in a plant may cause toxicity to the livestock grazing upon those plants. Molybdenum accumulates in bones and soft tissues and finds its way in the human system through consumption of plants, flesh of livestock, and cattle milk.[1]

In ruminant animals the toxicity, metabolism, and excretion of molybdenum are greatly affected by the simultaneous intake of copper and sulfur.[1,4,5] In sheep, for example, the intake of molybdenum has direct bearing on the chronic copper poisoning. The zinc status is also of interest in this connection due to copper/zinc antagonism. The accurate determination of molybdenum in biological materials in presence of other metals is thus essential for an understanding of the role of molybdenum in biological phenomena as also for detecting the instances of molybdenum pollution, if any.

During an ongoing program of studies on the uptake of molybdenum and other metals by living systems the need was felt to develop a suitable method for the determination of molybdenum in such systems. The prevailing methods[6-8] are either tedius or they fall short in sensitivity and selectivity. Recently we have reported a method[9] for the extractive separation and direct spectrophotometric determination of molybdenum in steels. The method was sensitive and rapid and tolerated the presence of high levels of copper and zinc. The same method was modified and applied in biological analysis and is reported here.

MATERIALS AND METHODS

All chemicals were reagent grade unless otherwise stated. Goat liver was used for the present studies because of its easy availability. The weighed liver samples (100 gm) were digested in a mixture containing concentrated sulfuric acid and nitric acid in the volume ratio 1:2. If charring occured, more nitric acid was added. Finally 1 ml of perchloric acid was added and the digestion continued until a clear solution was obtained. The bulk of the remaining nitric acid were removed by distilling off 5–10 ml of water from the solution. Plant samples were oven dried, ashed, and brought into solution as per standard method.[10] The solutions were made up to 200 ml.

The reliability of the method was tested by adding varying but known quantities of molybdenum (VI) in the form of measured volumes of an standard solution of ammonium molybdate. Copper and Zinc were similarly added to some samples.

The reagent N-o-tolyl-o-methoxy benzohydroxamic acid (TMHA) was freshly prepared.[9] The reagent was recrystallised repeatedly from ethanol to a constant, sharp, melting point. It was characterised by gas-liquid chromatography and IR and UV spectroscopy. A stock solution (0.01 M) of the reagent was prepared in isoamyl alcohol.

The pH measurements were carried out with a radiometer pH meter model PHM-29 (Hungary). The absorption spectra were recorded on a Perkin–Elmer 492–5000 spectrophotometer.

EXTRACTION PROCEDURE

To a 25 ml aliqot solution of sample containing 0.5–10.5 μg of Mo/ml, 5 ml of 0.1 M potassium hydrogen phthalate solution was added and with the help of 2 M NaOH and 2 M HCl its pH was adjusted between 1.5–2.5. The mixture was transfered to a 100 ml separatory funnel. To it about 10 ml of reagent solution was added and, after stoppering the funnel, the contents were shaken vigorously for 5 minutes. The aqueous

and non-aqueous phases were allowed to separate and the isoamyl alcohol extract was removed into a beaker containing anhydrous sodium sulphate. The aqueous layer was retained in the separatory funnel and extracted again with a fresh 10 ml portion of TMHA solution for 5 minutes. The extract, after phase separation, was removed and mixed with the first extract, transferred to 25 ml volumetric flask and made up to the mark with TMHA solution. The absorbance of the yellow extract was measured

TABLE I

Analysis of molybdenum in liver and plant samples

Matrix	Molybdenum added ppm	Copper added ppm	Zinc added ppm	Molybdenum found[a] ppm
Goat Liver	0.10	0	0	0.1 ± 0.003[b]
	0.10	2	4	0.1 ± 0.002[b]
	1.00	0	0	1.0 ± 0.02
	1.00	10	10	1.0 ± 0.02
	5.00	0	0	5.0 ± 0.06
	5.00	10	10	5.0 ± 0.09
	5.00	50	50	5.0 ± 0.09
Fodder Plant	0.10	0.2	0.4	0.1 ± 0.003[b]
(*Melilotus indica*)	1.00	0	0	1.0 ± 0.02
	1.00	10	10	1.0 ± 0.02
	5.00	0	0	5.0 ± 0.07
	5.00	50	50	5.0 ± 0.10
Grass	2.00	0	0	2.0 ± 0.04
(*Chloria barbata*)	2.00	20	40	2.0 ± 0.09
	10.00	50	100	10.0 ± 0.14

[a]Average of six determinations

[b]Quartz cells with 10 cm path length were used in these cases.

at 350 nm. The reagent blank does not have significant absorbance at this wavelength.[9] A calibration curve was set by extraction-determination of several known amounts of molybdenum and plotting the optical density values against concentration of Mo (VI).

To test the reliability of molybdenum determination in the liver and plant samples, the dissolved samples were extracted with excess of TMHA at pH 1.2–2.5 to remove any molybdenum already present. The samples were then treated with known concentrations of molybdenum and the recovery was carried out from the spiked samples. The results are summarised in Table I.

RESULTS AND DISCUSSION

The absorption spectra of Mo-TMHA system in isoamyl alcohol shows a peak at 350 nm and all measurements were done at this wavelength. At this wavelength Beer's law is obeyed in the range 0.5–10.5 μg/ml. The molar absorptivity is 9.1×10^3 litre/mole^{-1} cm^{-1}. The sensitivity of the method, calculated according to the definition of Sandell[6] is 0.010 μg/of Mo/ml. Studies on the optimisation of pH, reagent concentration, and diverse ions have been described before.[9]

The results (Table I) indicate that the present method has a high degree of precision and accuracy in absence as well as presence of high levels of Copper (II) and Zinc (II) in the liver and plant matrices.

Acknowledgement

The author is thankful to the authorities of IIT Bombay and Bhopal University for providing facilities.

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