Multinuclear NMR and toxic heavy elements

***S. Aravamudhan**
Department Of Chemistry, North Eastern Hill University
Shillong 793022, Meghalaya,
**Corresponding author (inboxnehu_sa@yahoo.com)*

Studies related to biological toxicity due to chemicals, in particular inorganic cations (of transition metal elements, actinides and rare earths elements) require the identification of the elements responsible for the toxic effects by recognizing the safety levels beyond which the elements become toxic. Detoxification studies would require (i) trying to find methods by which the levels of occurrences of such chemicals can be controlled kept below the limits of toxicity (ii) trying to find the actual ways by which the presence of these chemicals interfere in the normal metabolic processes and circumventing these interferences by appropriately providing substitutes. This could mean also trying to design drugs which can undo the abnormalities caused by the excessive chemicals. For example if an enzyme action is activated by a metal ion complexing, the presence of the metal ion would be indicated by the biological functions which require the activation of enzymes. However when the enzyme activation is excessive over the required level, then unwanted or even harmful consequences would result. The symptoms of abnormality would become evident as illness and diseases.

In the enumeration that follows some of the heavy elements and the toxic effects due to them have been listed out:

Long term exposure to **cadmium** is associated with renal dysfunction. Cadmium is bio-persistent and once absorbed remains resident for many years. High exposure can lead to obstructive lung diseases and has been linked to lung cancer. Cadmium may also cause bone defects in humans and animals. The average daily intake for humans is estimated as 0.15μg from air and 1μg from water.

Low exposure to **chromium** can irritate the skin and cause ulceration. Long term exposure can cause kidney and liver damage. It can also cause damage to circulatory and nerve tissues.

High doses of **copper** can cause anemia, liver and kidney damage, and stomach and intestinal irritation. People with Wilson's disease are at greater risk for health effects from overexposure to copper;

Exposure to **lead** can lead to a wide range of biological defects in human depending on duration and level of exposure. The developing fetus and infants are far more sensitive than adults. High exposure can cause problems in the synthesis of hemoglobin's, damage to the kidneys, gastrointestinal tract, joints, reproductive system and the nervous system. Studies have suggested that exposure to lead can cause up to a loss of 2 IQ points;

Inorganic **mercury** poisoning is associated with tremors, gingivitis and/or minor psychological changes together with spontaneous abortion and congenital malformation. Mono methyl mercury causes damage to the brain and the central nervous system while fetal and post-natal exposure have given rise to abortion, congenital malformation and development changes in young children.

Excessive amounts of **nickel** can be mildly toxic. Long term exposure can cause

decreased body weight, heart and liver damage and skin irritation; Exposure to high levels of **arsenic** can cause death. All types of arsenic exposure can cause kidney and liver damage and in the most severe exposure there is erythrocyte hemolytic.

Manganese is known to block calcium channels and with chronic exposure results in CNS dopamine depletion. This duplicates almost the entire symptomology of Parkinson's disease.

Aluminium toxicity is associated with the development of bone disorders including fractures, osteopenia and osteomalacia.

There are several techniques by which the presence of such heavy elements can be tested. For example the above enumeration is part of the literature cited with the ***Heavy Metals Test Kit*** as can be displayed from the webpage **http://www.heavymetalstest.com**. One of the aspect to be reckoned with is the fact that all the consequences of these transition metals is because of the electrons present in the elemental atom or ion because of which these metals can exhibit chemically binding characteristics. It is known that the atoms and ions of such elements have their characteristic nucleus around which the electrons of the system revolve in orbits. For the chemical consequences there is not any significant role assigned to the nuclear characteristics unless it is a radioactive element and the nuclear radiations can make it possible to be tracked by radioactive tracer techniques. The radioactivity itself can be hazardous besides the toxic effects of such elements by chemical reactions. It is at this juncture it is worth trying to inquire the possibilities of using the **Nuclear Magnetic Resonance [NMR]** spectroscopy to follow the characteristics due to the presence of such nuclei invariably with the electron system to be identified as an element. An elementary description of Nuclear Magnetic Resonance [NMR] phenomena is given in the following paragraph.

This NMR phenomenon is due to the fact that nuclei, placed in a strong external magnetic field, can resonate with externally applied electromagnetic radiations in the radio frequency range of the electromagnetic spectrum. Such of those nuclei which have nuclear magnetic moments are the candidates which can be detected by this resonance phenomenon. The frequency of the electromagnetic radiation at which the resonance can occur is governed by a specific equation which relates the frequency to the strength of the external magnetic field. Since protons are naturally abundant in occurrence, and since being the lightest element, it also has the highest frequency of resonance at a given magnetic field as compared to any other nucleus; proton-NMR has the maximum sensitivity for detection by NMR techniques. The nuclei detected by NMR, depending on the electronic structure around the nucleus, also display variations in the resonance characteristics in part per million measures. Since the atomic nuclei are present in molecular systems, and since the molecular systems have the characteristic electronic structures, detecting the presence of a nuclear species by NMR also can reveal the electronic environment in which that species is placed in the molecules. Since the biological structural consequences and functional variations are intricately related to the electronic structure variations, the NMR spectroscopic technique can be a useful technique for toxicity related studies, in particular due to the metal ions. As is prevailing in scientific studies, in certain contexts NMR might prove to be superior method even though in certain other contexts any of the other techniques might be preferable.

The advantage of the NMR technique is the rate at which advances in the instrumentation and the development in the theoretical approaches make possible elegant methods to study several aspects of chemistry and biology.

It is conventional to specify a NMR spectrometer system by the resonant frequency for the Proton Magnetic Resonance. Thus magnetic fields of the order of 1Tesla, 2Tesla, 7Tesla, 9Tesla, 14 Tesla, 21 Tesla (1T=10KG) are used for NMR studies and the corresponding frequencies for proton resonance can typically cover the range 60 MHz to 900 Mhz. A Spectrometer system with a Magnet of **7.0483 T** would be operating with Proton Resonance Frequency of 300MHz.

Table 1 lists the NMR frequency data for several nuclei at this field of 7.0483 T. In this table all the heavy elements listed earlier with their toxic effects find the place with their characteristic NMR characteristic specified.

Usually the NMR measurements had been made with spectrometer system tuned to detect Proton NMR Spectra. This is a case of single nuclear NMR study. Subsequently, the NMR instrumentation improved and a single spectrometer system could be tuned to Proton and Carbon (13) nuclei. Further improvements made it possible for single spectrometer system to be tuned for ^{1}H, ^{13}C, ^{15}N, and ^{31}P nuclei. Till about 20 years before the NMR spectrometer system did not have **Multi Nuclear** capabilities. By **multi nuclear system** it is conveyed that the NMR system and the NMR Probe can be tuned most of the NMR frequencies as it can be available from NMR Periodic table. This NMR table is available on the internet currently at the URL:
http://rmn.iqfr.csic.es/guide/eNMR/chem/NMRnuclei.html

Mostly only High Resolution Liquid State NMR spectra are analyzed. But, with the current technological trends, several NMR techniques for samples in Solid State have been devised and most of the present day NMR spectrometers routinely perform several tasks for high through put data acquisition in a variety of samples.

The High Magnetic Field values as high as 21 Tesla, has made it possible to extract NMR information from High Molecular weight bio molecules. Thus the potential of NMR spectroscopy in several contexts, not only for toxicity studies, has been growing and more and more NMR users have been enlisted. From the manufacturers site at http://www.bruker.de it is possible to find technical notes which typically illustrate the most recent state of the art in the trends in NMR spectroscopy and Instrumentation. Current day analysis by NMR includes multi dimensional NMR information and multi nuclear NMR information on a particular system for a detailed study.

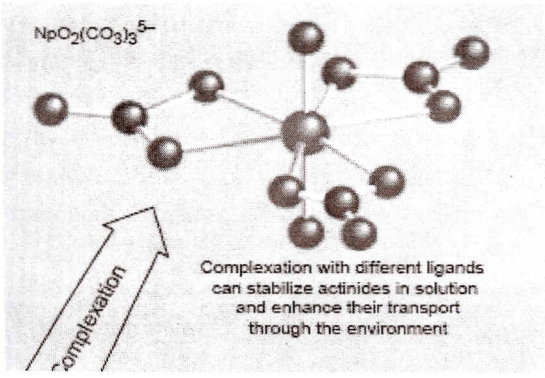
Some of the NMR results related to Bio molecular systems are listed under the topic of Metabonomics. The main problems in using NMR spectroscopy for study of biological systems arise from the low sensitivity in detection, crowded spectra where each peak is to be assigned to individual resonating nuclei, and that water, the milieu of biological systems, gives huge ^{1}H signal against which the weak signals from the molecules of interest have to be detected. Fourier Transform (FT) NMR techniques have made it possible to overcome most of the above difficulties. Metabonomics is defined as *"quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification"*(G.Govil, Invited Talk,IT24 at the NMRS2004, Symposium on NMR, Drug Design and Bioinformatics, held at Saha Institute of Nuclear Physics,Kolkat; organized by Bose Institute and S..N.Bose National Centre for Basic Sciences). This powerful and emerging technology characterizes complex time-dependent metabolic profiles in biofluids, cells, tissues and whole bodies (in particular whole body Magnetic Resonance imaging) and this helps in following metabolic changes. In the remaining part of the article

some of the NMR results from the published literature would be reviewed to highlight the capabilities of NMR in the field of toxic studies.

Some of the metal ions are paramagnetic and have unpaired electrons in their'd' or 'f ' set of atomic orbital. These unpaired electrons have much larger magnetic moments than the nuclear magnetic moments. Thus in a Nuclear Magnetic Resonance spectrum, typically of the lighter element, Proton, the presence of these heavy paramagnetic element at a farther location in a binding site of a macro molecule would be indicated conspicuously by distant dependent *"pseudo contact shifts"* and *"line-widths"* which are typically NMR spectral parameters. In the literature such instances are abundant where in these information has been used with success.

The results on toxicity effects have enabled systematically classified information to be made available on the heavy elements to provide a guide line for toxicity related issues Biogeochemical cycles of elements: (Bio-inorganic) by By Astrid Sigel, Helmut Sigel, Roland K.O. Sigel E-book available in the internet downloadable from: **http://books.google.co.in/books?id=C3tt3uMosS4C&pg=PT26&lpg=PT26&dq=NMR+Studies+of+TOXIC+HEAVY+ELEMENTS&source=web&ots=NR9zvnPLHc&sig=EpB2caN75EQ-JuofJqlxeIY9B-M&hl=en&sa=X&oi=book_result&resnum=2&ct=result#PPT26,M1** Volume 21 and Volume 22 are discussions on Magnetic Resonance Experiments with Heavy Elements. Volume 21 particularly deals with Applications of Nuclear Magnetic Resonance to Paramagnetic Species.

These available data on heavy elements should enable the adaption of those methods and procedures for similar studies for the location-dependent parameters of toxic effects. For example the following category of documentation Actinides is retrievable from the internet resources: (*from* "The Chemical Interactions of Actinides in the Environment" by *Wolfgang Runde: downloadable article "00818040.pdf")*



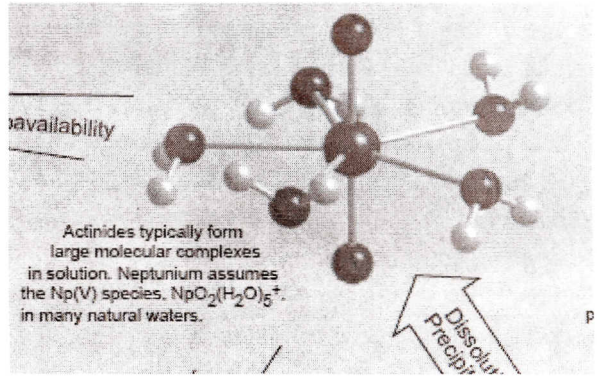


Table I. Oxidation States of Light Actinides[a]

Th	Pa	U	Np	Pu	Am	Cm
III	III	III	III	III	III	III
IV	IV	**IV**	IV	**IV**	IV	IV
	V	V	**V**	**V**	V	
		VI	VI	**VI**	VI	
			VII	VII		

[a]The environmentally most important oxidation states are bolded.

With these documentations it is only necessary to consult the NMR data and find out the possibilities of designing experiments for locally relevant toxicity parameters. It is this kind of adapting available results by the awareness of available resources, which makes the scientific activity indigenous, useful and not a redundant activity. In this context the awareness about NMR Spectroscopy and its potential lags far behind the satisfactory utilization criteria.

For Comprehensive NMR data on which of such actinides are good NMR candidates; visit pages at
http://rmn.iqfr.csic.es/guide/eNMR/chem/NMRnuclei.html
Where the page title itself is obviously mentioned as "NMR periodic table" with a notation "eNMR".

From such data it would be possible to know and specify what kind of NMR experiments can be advantageous for a particular context.

For those who are in search of availing NMR services the documentations at http://www.intertek-cb.com/nmr/index.shtml could be providing a lead information on the kind of NMR services available globally from which it would be possible to get to know how to seek and avail NMR services without really knowing much about NMR spectrometers. A variety of NMR spectrometers are located at the national NMR facilities like at TIFR, Mumbai or IISc., Bangalore http://sif.iisc.ernet.in/ which regularly provide NMR services conveniently within the country besides whatever the needy can find from the Analytical facilities located in SHILLONG.

The research results to be reviewed in the following pages would fall under two major categories.

(1). Infer the toxic effects of heavy elements (listed out in Table-1), by the Conventional ^{1}H, ^{13}C, ^{15}N, ^{31}P NMR spectroscopy without the necessity to directly detect the Heavy Element Nuclei by Multi nuclear NMR spectrometers.

(2). Multi Nuclear NMR category of experiments.

The literature references would be cited so that the readers can access the original articles from the literature documentations.

Item-1: This is based on the research publication in *"Clays and Clay Minerals, Vol.48, No.5, 495-502, 2000"Title: A Nuclear Magnetic Resonance (NMR) and Fourier TransformInfrared (FTIR) Study of Glycine Speciationon a Cd-rich Montmorillonite"*

This article reports that in the Montmorillonite, only in the Cd-rich form interlayer complexation of Glycine occurs and in Ca-rich Montmorillonite such complexation is not observed. This result has the significance to reveal that because of the structure of clay materials, which consists of expandable tetrahedral and octahedral sheets bearing negative charges, of necessity require charge compensation by cations. In this context the innocuous elements like Ca and Na present in clay can get exchanged by heavy metals such as Cd and Pb. This can affect the interaction of organic pollutants with clay materials. These organic pollutants and their intermolecular interactions with Clay-mineral surfaces are crucial for the chemical and biological transformations. This article reports a study under laboratory conditions, the intermolecular interaction of organic molecule Glycine with Montmorillonite surfaces. The article relies on Data from ^{13}C *CP-MAS and* ^{113}Cd *MAS Solid State NMR* studies to draw conclusions on the retention of organic molecule and the highly toxic heavy metal cadmium. The results are that in Ca- rich Montmorillonite the adsorbed Glycine undergoes only protonation whereas in the Cd-rich specimen the adsorbed ligand complexes with interlayer cation. More details about the nature of these reactions are available from the original paper and the significance and consequences of such transformations prove the important role that the NMR technique plays in such determinations. For the sake of illustration selected NMR spectra (Fig.7 as in original) from these publications are reproduced where it is possible to find that the relevant changes have been marked on the spectrum which indicates the above transformations.

The points to note are how the interlayer Cd^{+2} peaks and interlayer $CdCl^{+}$ peaks undergo changes with the concentration of the Organic compound. Such trends of shift in resonant lines reflect the changes in electronic structure of the corresponding molecules and hence evidence the chemical nature of interactions and the consequences could be biologically significant.

Thus the soil structure and transformations in such media because of organic contaminants and heavy element pollutions can be studied with such detail and at any location the constituents causing pollutions and the extent of contamination would indicate local environmental factors. This publication is only an indicator as to how NMR can be utilized for such purposes.

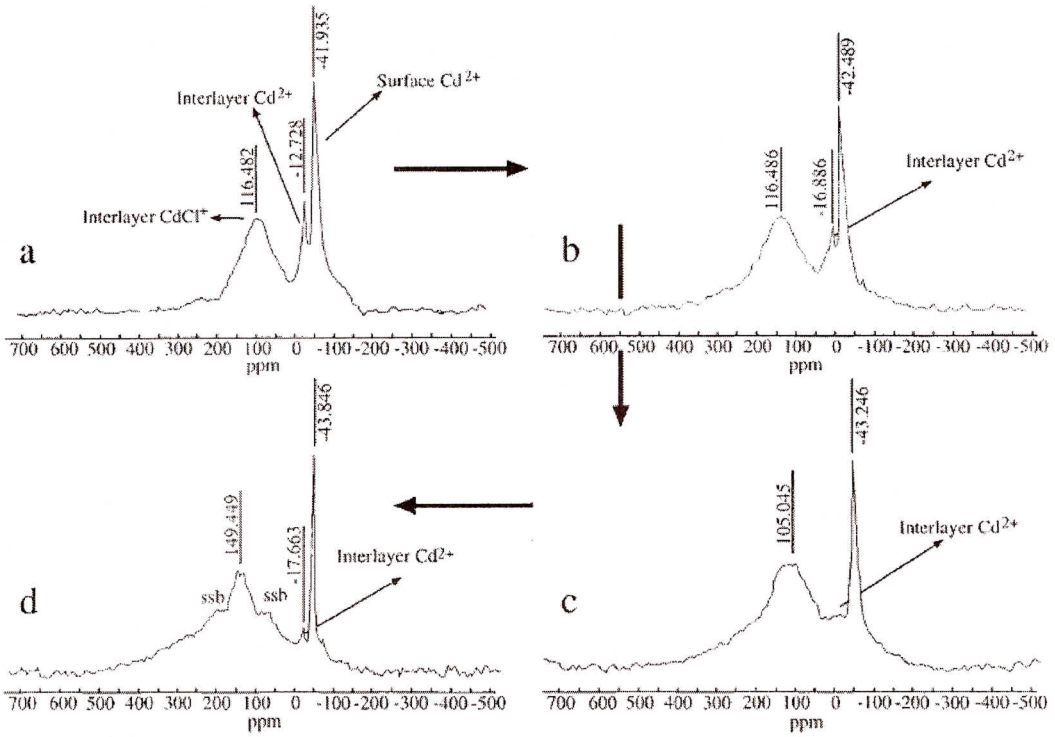


Figure 7. Scheme indicating changes in the ^{113}Cd MAS NMR spectra of Cd-rich montmorillonite treated with b) 0.01, c) 0.1, and d) 1 M Gly solutions. The spectrum relative to Cd-rich montmorillonite not treated with glycine (a) is from Di Leo and O'Brien (1999). All spectra have comparable signal areas.

Item-2 Based on the research publication in *"Chinese Journal of Chemistry, **2004, 22, 849-853"** Title: "NMR and Pattern Recognition Studies on the acute Biochemical Effects of Lu $(NO_3)_3$"*

This investigation can come under the category of Metabonomics. Pattern Recognition is a recent trend being applied to Data Processing and interpretation of NMR peaks in biologically significant macro molecules. An indication of how assignments in NMR spectra metabolites are made evident with the application to the effects of the Lu complex. The spectrometer used is BrukerAv 600. This specifies that the spectrometer has a Magnet system at which the Proton NMR frequency is 600MHz. This comes under the category of a High Field NMR system. (Frequencies upto 100MHz can be equipped with *Electromagnet* systems, upto about 2.3T and such NMR Spectrometers are classified as the Low field systems. High filed NMR systems typically require *Superconducting Magnets*. This article also lists out spectrometer conditions used and those who want to acquaint with these terms can refer to the full paper as cited above. The conditions under which the ^1H NMR spectra of *rat urine* were recorded are well enumerated. Below a NMR spectra of rat urine under a specified condition are reproduced from which it should be evident as to how pattern recognition and principal component analysis can be applied to such complex spectra of a biological sample, thus resulting in such evident assignments of the metabolites. One may well be enlightened by comparing such complex spectra with the proton NMR spectra of small molecules even in which case it becomes an ordeal to make unambiguous assignments. The changes in spectral features with time and nature of dosage of the chemical contamination.

Table 1 Assignments of urinary metabolites

Metabolite	Chemical shift δ^a
Valine (Val)	0.99 (d)
Ethanol (Eth)	1.17 (t)
Lactate (Lac)	1.35 (d)
Succinate (Suc)	2.43 (s)
α-Ketoglutarate (α-Kg)	2.46 (t)
Citrate (Cit)	2.61 (AB)
Dimethylamine (DMA)	2.72 (s)
Dimethylglycine (DMG)	2.81 (s)
Creatinine (Cn)	3.06 (s)
Taurine (Tau)	3.25 (t)
Trimethylamine-*N*-oxide (TMAO)	3.27 (s)
Glycine (Gly)	3.57 (s)
Sarcosine (Sar)	3.61 (s)
Glutamine (Glu)	3.77 (t)
Allantoin	5.43 (s)
Phenylalanine (Phe)	7.36 (m)
Hippurate (Hip)	7.87 (m)

[a] s, singlet; d, doublet; t, triplet; m, complex multiplet; AB, second order.

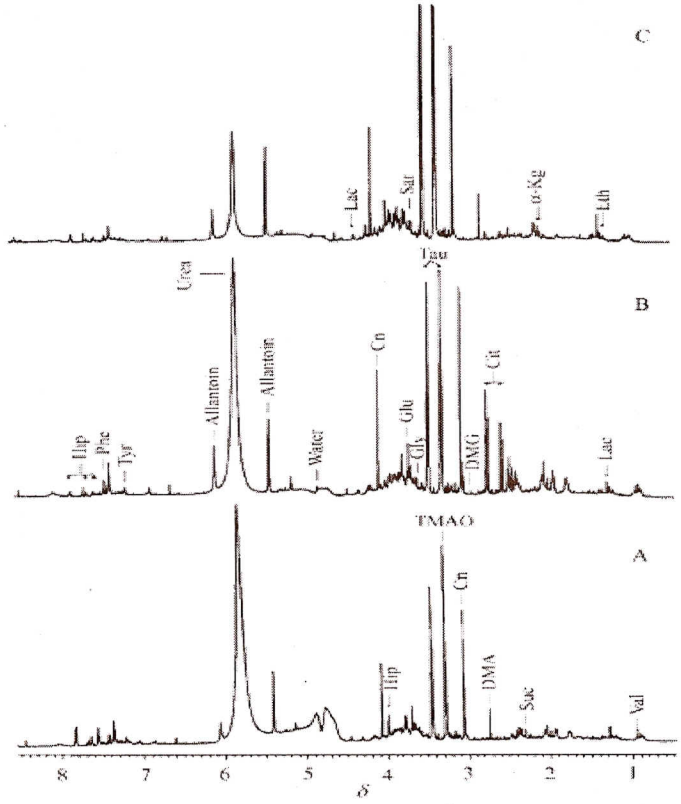


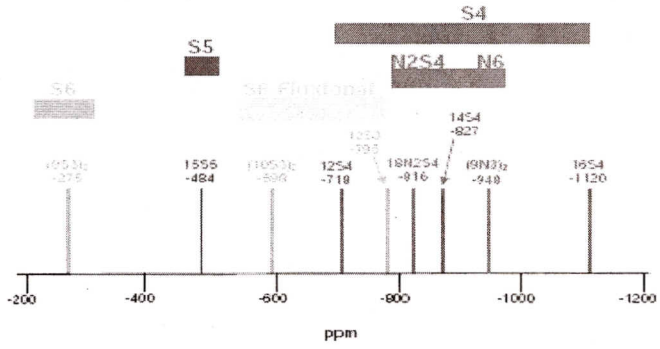
Figure 1 A comparison of the 600 MHz ^{1}H NMR spectra of the urine of 4—8 h time point from Wistar rats (A) control (0.9 % saline), (B) $HgCl_2$ (2 mg/kg body weight) and (C) CCl_4 (1.5 mL/kg body weight).

Some what a similar study of a Malabsorption syndrome was presented at the **NMRS 2004 symposium** on NMR: *"Drug design and Bioinformatics"* This study was carried out at the Center of Biomedical Magnetic Resonance, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow.

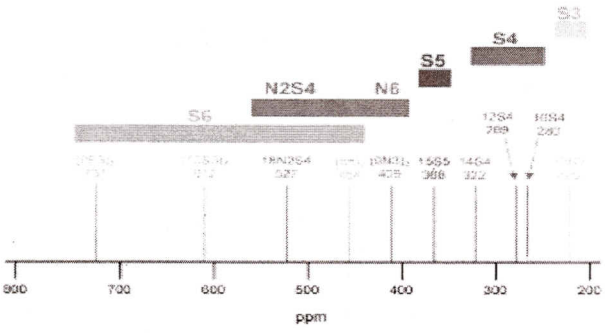
This was on the D-Xylose consumption and this study could establish that the ^{1}H NMR technique compared much better to the conventional calorimetric method. Thus D-xylose test for malabsorprtion syndrome in Humans could be well set with the NMR of urine specimen from 35 patients.. This is an instance of similar metabolite study as above for the human symptoms, even though this is not specifically due to any toxic heavy element.

Item-3: Based on the *Tennessee Water Research Center: Annual Technical Report FY2004*

The specific goal of the research project was to examine correlations between x-ray crustal structures and NMR Chemical Shifts for4 complexes containing the divalent heavy metal ions Hg(II) and Cd(II). ^{113}Cd chemical shifts in the range of 730-225 ppm and ^{199}Hg chemical shifts in the range -275 to -1120 ppm for a series of macrocyclic complexes are reported. Their results graphically are copied below:



$^{113}Cd\{^{1}H\}$ NMR Shifts of Various Thioether Complexes



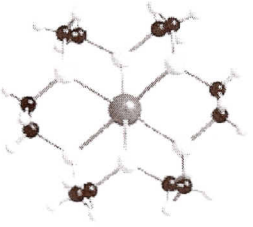
To summarize these data -- Upfield chemical shifts in the NMR spectra of either nucleus are seen whenever:

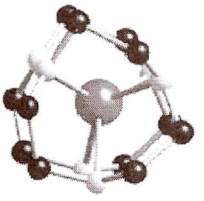
1. the number of thioether sulfur donors in the complex is decreased.
2. a thioether sulfur donor is replaced by a secondary nitrogen donor.
3. the size of the macrocycle ring increases without a change in the nature or number of the donor atoms.

Further the report includes the following:

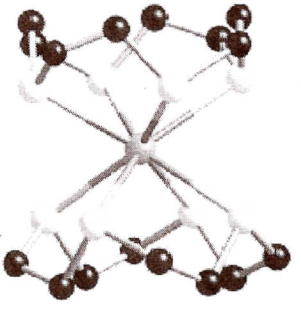
Thus, we have developed a novel means of clearly identifying the surrounding ligand environment for mercury or cadmium in solution.

We report the crystal structures of the mercury(II) complexes with the crown thioether 18S6 as well as the bis complex with azacrown 9N3. The two structures are shown below. An interesting structural result that we have obtained is complexes with six sulfur donors bonded to mercury(II) have an octahedral structure while complexes with six nitrogen donors have a trigonal prismatic structure as shown for the 9N3 complex (2nd structure) below.





We also report the crystal structure for the bis cadmium(II) complex with the tetrathiacrown 12S4. An unusual octakis(thioether) coordination of Cd(II) with a square anti-prismatic geometry is exhibited by [Cd(12S4)2](ClO4)2is shown.



Summary

We have established clear trends in how changes in the ligand environment around a mercury or cadmium center affect its NMR chemical shift. This information will be invaluable for the design of ligand systems which can selectively bind these heavy metals and in chemosensors that are used to detect them.

Item-4 Based on the publication in *"Plant Physiology, 1998 July, 117(3), 753-759" Title: High Aluminum Resistance in Buckwheat II. Oxalic Acid Detoxifies Aluminum Internally.*

This is a research report on the Detoxification phenomenon occurred due to the presence of organic compounds and pertains to Al toxicity. Buck wheat is an important crop in Asia.

This reports the detoxification in terms of the changes observed in ^{13}C NMR and ^{27}Al NMR. The figure captions explain the conditions and the changes are evident in the spectra. And, the detoxification in presence of oxalic acid could be established conclusively. Below are the spectra copied from the original source:

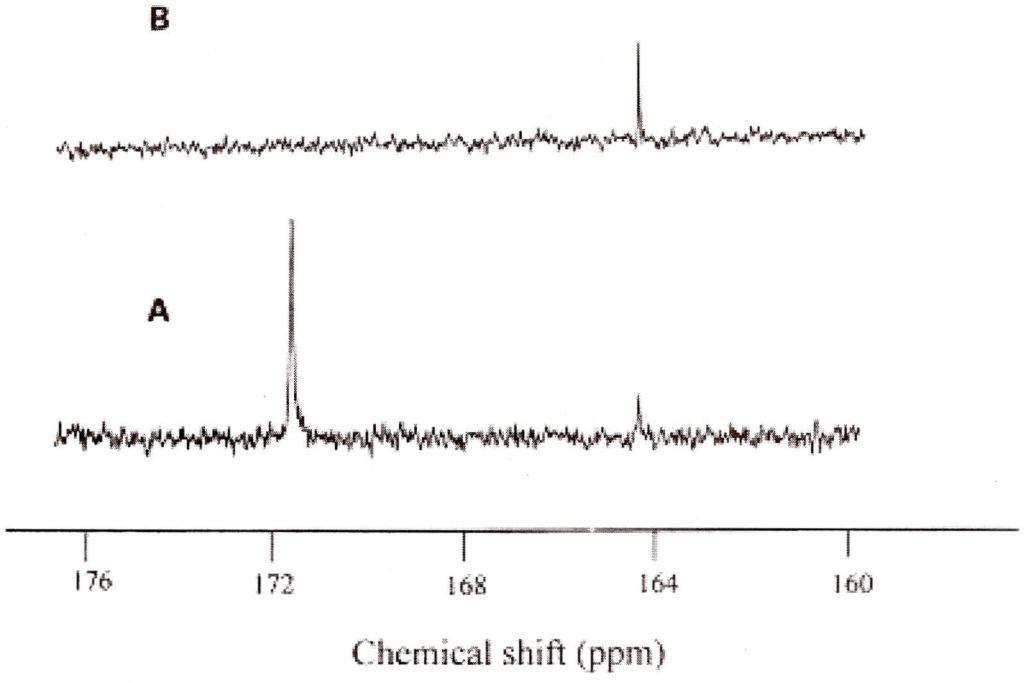


Figure 3

13C-NMR spectra of Al-oxalate (1:25) complex (A) and the purified cell sap of buckwheat leaves (B). Buckwheat was intermittently exposed to 50 μm Al in 0.5 mm CaCl2, pH 4.5, for 10 d. The crude cell sap was purified four times by Sephadex G-10 column chromatography. Spectra were measured at 150.8 MHz. See **Methods** for the purification process and measurement conditions.

Below are spectra of ^{27}Al NMR, and on the right side (Fig.5) the spectra of Al complexed with higher molar ratio of oxalic acid resembles the un-complexed Al spectra on the left side (Fig.4).

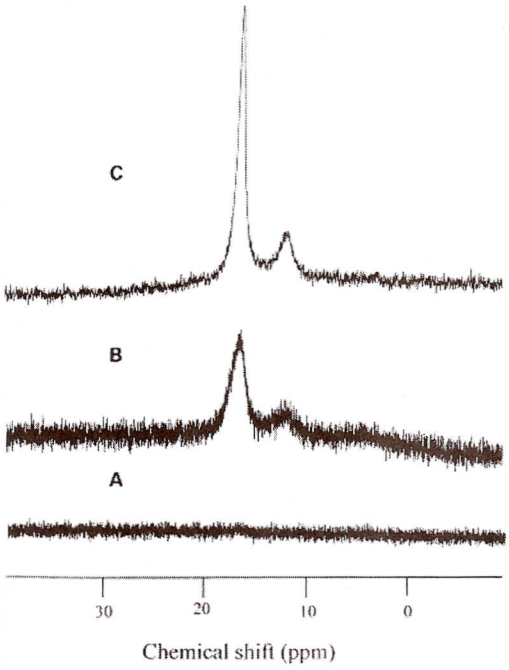


Figure 4

27Al-NMR spectra of intact roots exposed to 0 (A) or 50 µm (B) Al for 20 h or the cell sap (C) extracted from the roots exposed to 50 µm Al for 20 h. Spectra were measured at 156.3 MHz. See **Methods** for measurement

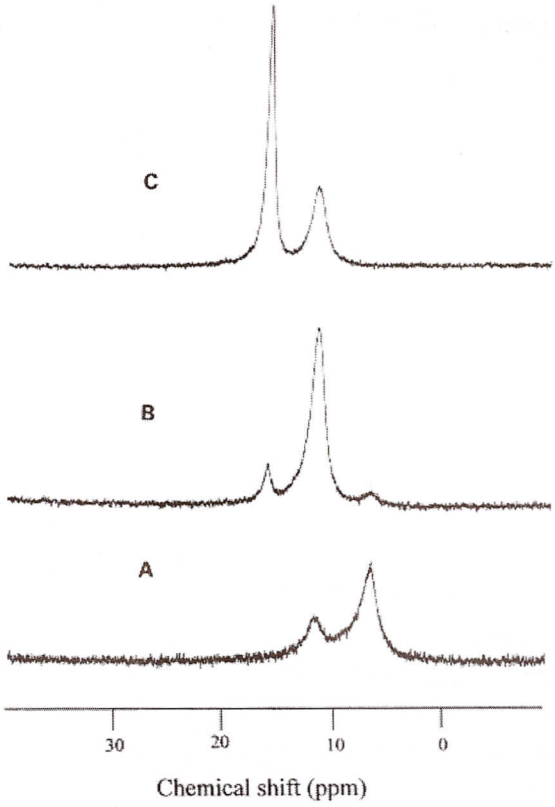


Figure 5

27Al-NMR spectra of Al-oxalate complexes with a 1:1 (A), 1:2 (B), or 1:3 (C) molar ratio of Al to oxalic acid. The pH of the solution was 4.6. Spectra were measured at 156.3

From the above illustrations of 4 items, the advantages, and versatility of the Multi Nuclear NMR technique for study of Heavy element Toxicity stand vindicated.