Heavy Metals Under-Reporting in Water Environment Importance of Method Selection

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Owing to low-cost labour availability, the manufacturing sectors are moving from developed to less developed countries. Often, the less developed nations are less equipped (as well as aware) for reliable monitoring frameworks. Generally, the standard methods by US EPA are followed for monitoring heavy metal pollution in water environment. Since, the heavy metal determination is method dependent, accuracy and applicability of heavy metal detection were evaluated, which can improve the effectiveness of water management strategies. US EPA methods 3015A and 3005A were evaluated for their ability to detect heavy metals from aqueous phase. The detection was carried out using Flame Atomic Absorption Spectroscopy (FAAS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The heavy metal recovery using different methods was significantly ($P < 0.05$) different. Recovery of certain heavy metals (Pb, Mn, Cr, and Zn) was significantly low with some standard method. In view of the development of technological solutions for heavy metal pollution mitigation (for water environment), the results of this study offer valuable insights for designing the monitoring studies that can ensure correct determination of heavy metals. The consequences of under-reporting of heavy metals (such as Pb, Mn, Cr and Zn) are discussed in view of their toxicity potential and ecological and other risks.

Key words: *Heavy metals, pollution, recovery, toxicity, risk*

Introduction

Due to its toxic properties, presence of heavy metals in water (above certain limits) is a serious global health concern for humans[1] as well as aquatic animals.[2-4] The bioavailability and subsequent toxicity of heavy metals are dependent upon the geochemical partitioning of the metals to sediment components[5] (referred to as metal speciation) [6,7]. Therefore, adequate and accurate knowledge of heavy metal concentration in water environment (occurring naturally and those added through anthropogenic activities) has enormous importance for delineating effective heavy metal pollution mitigation policies.[8-10] In view of the currently available technologies for heavy metal detection, often, the validity and reliability of the results is questionable (especially for environmental samples such as water, soil and sediment). The importance of valid and reliable estimates of heavy metals is important, especially in the context of demotechnic growth of humans, which is responsible for immense contribution of heavy metal to different environmental compartments.[11,12] Although different types of industrial effluents (cause of pollution) contain heavy metals, its (heavy metal's) qualitative and quantitative distribution is often distinctly different (Table 1).[13] However, the methods used for heavy metal determination are often same and do not take into account the environmental and other conditions prevailing at the sampling sites.

Current heavy metal detection techniques treat heavy metals as a group and are often individual laboratory based (with respect to use of chemical and equipments). It is obvious that the conditions prevailing at various areas are primarily responsible for qualitative and quantitative variation of heavy metals in the concerned environment. Furthermore, the most common hurdles in accurate detection of heavy metals are partial (inadequate) digestion and substandard reagent quality. Also, the detection and quantification (especially low levels) of heavy metals require highly sensitive instrumental techniques.[14] Conventionally, heavy metal concentration in aqueous samples has been evaluated by numerous techniques; notable amongst them are Atomic Absorption Spectroscopy (AAS), ICP-AES,

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Table 1: Presence of heavy metals in particular industrial effluents

Industries	Ag	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Ti	Zn
General Industry and Mining				√	√	√		√		√			√
Plating			√	√	√				√	√			√
Paint Products				√						√		√	
Fertilizers		√	√	√	√	√	√	√	√	√			√
Insecticides / Pesticides		√			√		√						
Tanning		√		√									
Paper Products				√	√		√		√	√		√	√
Photographic	√			√									
Fibers					√								√
Printing / Dyeing				√						√			
Electronics	√										√		
Cooling Water				√									
Pipe Corrosion					√					√			

Source: Ramachandra, T.V., Ahalya, N. and Kanamadi, R.D., Biosorption : Techniques and Mechanisms, Technical Report : 110, CES Technical Report I 110, Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore – 560 012 (2005)

Microwave Plasma Torch - AES and ICP-MS. Other analytical techniques, such as electrochemical analysis,[15-18] fluorescence measurement,[19-21] absorption measurement,[22] X-ray fluorescence technique[23-24] and electrolyte cathode atmospheric glow discharge[25] are also used. The selection of instrument and sample preparation method (acid digestion and thermal decomposition) used for detection of trace heavy metals is responsible for analyte losses, incomplete recoveries, and/or sample contamination,[26-29] which affects correct heavy metal reporting.

Thus, it is apparent that the currently available analytical techniques have certain limitations, which affect the validity of reported heavy metal concentration in environmental samples.[30] As a result, total recoverable methods have become common methods for sample preparation, such as those proposed by the USEPA.[31] For aqueous samples, two digestion methods are commonly used: USEPA method 3005A (Hot-Plate, HNO_3, total recoverable)[32] and 3015A (Microwave, HNO_3, total recoverable).[33] USEPA method 3015A, that is microwave-assisted acid digestion for aqueous sample, is designated as a regulatory alternative to

method 3005A, which is a hot plate digestion technique. Therefore "similar or equivalent" results for the analysis of metals in the aqueous samples are expected in case of use of methods 3005A and 3015A. In both the methods, multi-element acid digestion of sample matrices is carried out prior to the instrumental analysis by FAAS-AAS or ICP-AES.[34] However, there are certain variations in sample preparation method 3005A (temperature, digestion time and acid additions), which may affect elemental recoveries.[35-38] Since 1975, many studies on microwave digestion (for heavy metal analysis) have been reported,[39,40] however, most of the studies[31,41-44] are related to heavy metal leaching from soil or sediment samples. On the other hand, studies related to performance assessment of analytical methods of heavy metal determination (used for water samples) are very scarce. For this reason, there is a need for evaluating analytical methods recommended by various scientific bodies for improving the reliability of heavy metal estimates.

Since, the water samples from different environment show considerable variation, the studies performed using only natural water samples may

Table 2: Initial concentration of heavy metals in spiked samples

Heavy metal	HNO_3 digestion (mg/L)	HNO_3+HCl digestion (mg/L)
Zn	0.5	1.0
Pb	1.0	1.0
Cd	0.5	1.0
Co	1.0	1.0
Ni	1.0	1.0
Mn	1.0	1.0
Fe	2.0	2.0
Cr	2.0	2.0
Cu	1.0	1.0

interfere with the statistical inference, and may also negatively affect the reliability of the results. Hence, in the present study, heavy metal recovery from aqueous samples was assessed as a function of the leaching procedure and analytical techniques (3005A and 3015A) using synthetic samples to avoid the interferences in recovery of metal caused by sample matrix.[45,46] Performance of two USEPA methods (3005A and 3015) was assessed for their suitability to determine concentration of 9 trace metals (Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr and Cu) from synthetic water using FAAS and ICP-AES equipments and the digestion procedure involved use of HNO_3 and HCl.

Materials and methods

Chemicals, reagents and standards

Concentrated HNO_3 (70%) and HCl (35.5-36.5%) were obtained from Qualigens (Mumbai, India). All standard metal solutions (ICP-multi element standard solution and AAS standard with concentration

of 1000 mg/L) were obtained from E. Merck, Germany. To avoid interferences in synthetic samples, high purity (ultra pure) water having high resistivity (18 mega ohm/cm at 25°C) was used (Millipore QUF plus along with RO-10). The initial concentrations of different heavy metals in spiked samples are given in **Table 2**.

FAAS

An FAAS (model Perkin Elmer-USA, AAnalyst 800) with Deuterium and Zeeman Background Correctors, Hollow cathode lamps and air-acetylene (carrier gas) was used for heavy metal detection.

ICP-AES

Heavy metals were also determined using plasma emission spectrometry; ICPAES (model Jobin Yvon JY-24), with main features, like multi-elemental analysis in sequential mode, wavelength range 165-800 nm with wide linear dynamic range, concentric glass nebulizer with peristaltic pump and carrier gas argon.

Microwave digester

Microwave digester (Milestone, Italy, Model ETHOS 1600), power range of 1600 W in 10 Watt increments with single magnetron equipped with static microwave diffuser (for homogeneous microwave distribution) and a capacity of 10 vessels made up to TFM Teflon fitted on a rotary table was used. The digestion vessels fitted with a pressure relief device were capped and then heated.

Hot plate

The hot plate (Tempo make, Mumbai, India) with maximum temperature of 200°C was used for sample digestion.

Table 3: Sample preparation

Criterion	FAAS		ICP-AES	
Method Used	3005A	3015	3005A	3015
Nature of sample	SW sample	SW sample	SW sample	SW sample
Digestion equipment	Hot plate	MCW digester	Hot plate	MCW digester
Digestion medium	S,M	S,M	S,M	S,M
Time/Temperature	7-8 hrs/95°C	20 min 2cycles160-170°C	7-8 hrs/95°C	20 min/ 2cycles160-170°C

SW: Synthetic water; **MCW**: Microwave digester; **S**: Single acid; **M**: Acid mixture

Glassware and filter papers

All glassware and plastic containers used throughout this experiment were immersed in dilute HNO_3 solution overnight and rinsed with ultra pure water, and finally dried in a clean bench prior to use.. Sample filtration was carried out using Whatman No.42 filter papers.

Sample preparation

The standard solutions were prepared from a 1000 mg/L standard for ICP-AES and AAS (Merck, Germany) by dilution with 1M HNO_3. Single metal synthetic samples were prepared from standard solution (1000 mg/L) with subsequent dilutions (working standards). Synthetic samples and working standards were prepared in 5% HNO_3 solution to keep similar composition as of the standards procured. Sample blank for the experiment was prepared in 5% HNO_3 so that metal interferences or addition due to HNO_3 can be nullified in the final result. Spiked samples were prepared using multi element standards. The heavy metal concentration in standards varied between 0.5 and 2.0 mg/L, however, sample volume was kept constant (Table 4).

Heavy metal determination from synthetic water samples

EPA Method 3005A: EPA Method 3005A was used for determination of heavy metals from the synthetic water samples. For this method, digestion procedure involved use of hot plate and the acids i.e. HNO_3 (singly) and acid mixture of HNO_3 and HCl (2:5 ratio).

EPA Method 3015A: Method 3015A was also used for determination of heavy metals from the synthetic water samples. The digestion was carried out using microwave digester and the acids i.e. HNO_3 (singly) and acid mixture of HNO_3 and HCl (2:5 ratio) (Table 4).

Heavy metal detection

The ICP-AES and FAAS were used for heavy metal detection from samples processed by following methods; 3005A and 3015A.

Statistical analysis of data

The data generated during this study was analyzed using various statistical tests with the aid of PASW 18.0 statistical software. The data characteristics (descriptive statistics) were determined and the comparative assessment was carried out using one way Analysis of Variance (ANOVA) test. The significance level was chosen to be 0.05 (or equivalently, 5%) by keeping in view the consequences of such an error and to make the significance level as small as possible in order to protect the null hypothesis and to prevent, as far as possible, from inadvertently arriving at false conclusions.

Results

The following section presents information on the results and findings. The results are expressed in the form of concentration as well as percentage recovery of different heavy metals.

Sample digestion using acid mixture

The heavy metal concentration data showed that maximum mean for Zn was $0.499 \pm 0.0024 mgL^{-1}$ (FAAS; method 3015A), Pb $0.972 \pm 0.0083 mgL^{-1}$ (ICP-AES; method 3005A), Cd $0.498 \pm 0.0023 mgL^{-1}$ (FAAS; method 3005A), Co $0.958 \pm 0.334 mgL^{-1}$ (ICP-AES; method 3015A), Ni $0.992 \pm 0.0178 mgL^{-1}$ (ICP-AES; method 3005A), Mn $0.992 \pm 0.0044 mgL^{-1}$ (FAAS; method 3005A), Fe $1.980 \pm 0.1643 mgL^{-1}$ (FAAS; method 3005A), Cr $1.966 \pm 0.0134 mgL^{-1}$ (ICP-AES; method 3005A), Cu $0.980 \pm 0.0212 mgL^{-1}$ (FAAS; method 3005A). The comparative assessment of heavy metal concentrations observed as a function of various

Table 4: Protocol

	3005A		3015A	
	Single acid	Acid Mixture	Single acid	Acid Mixture
Volume of sample	100 mL	100 mL	45 mL	45 mL
Volume of HNO_3	5 mL	2 mL	5 mL	2 mL
Volume of HCl	–	5 mL	–	5 mL
Total volume made after ligestion	100 mL	100 mL	100 mL	100 mL

ngle acid (HNO_3); Acid mixture (HNO_3 + HCl)

Table 5: Heavy metal concentration obtained by digestion using acid mixture

		Mean	SD	SE	Min	Max	F	P
Zn	ICP-AES-3005A	0.497	0.0010	0.0004	0.496	0.498	2.680	.082
	ICP-AES-3015A	0.496	0.0013	0.0006	0.495	0.498		
	FAAS-3005A	0.497	0.0024	0.0011	0.495	0.500		
	FAAS-3015A	0.499	0.0024	0.0010	0.497	0.500		
	Total	0.497	0.0021	0.0004	0.495	0.500		
Pb	ICP-AES-3005A	0.972	0.0083	0.0037	0.960	0.980	1301.3	.000
	ICP-AES-3015A	0.692	0.0130	0.0058	0.680	0.710		
	FAAS-3005A	0.970	0.0070	0.0031	0.960	0.980		
	FAAS-3015A	0.686	0.0108	0.0048	0.670	0.700		
	Total	0.830	0.1449	0.0324	0.670	0.980		
Cd	ICP-AES-3005A	0.497	0.0008	0.0004	0.496	0.498	.856	.484
	ICP-AES-3015A	0.496	0.0013	0.0005	0.495	0.498		
	FAAS-3005A	0.497	0.0028	0.0012	0.492	0.499		
	FAAS-3015A	0.498	0.0023	0.0010	0.495	0.500		
	Total	0.497	0.0019	0.0004	0.492	0.500		
Co	ICP-AES-3005A	0.955	0.0313	0.0119	0.910	0.970	8.236	.002
	ICP-AES-3015A	0.958	0.0334	0.0149	0.900	0.980		
	FAAS-3005A	0.921	0.0167	0.0074	0.910	0.950		
	FAAS-3015A	0.890	0.0100	0.0044	0.880	0.900		
	Total	0.931	0.0374	0.0083	0.880	0.980		
Ni	ICP-AES-3005A	0.992	0.0178	0.0080	0.970	1.000	5.561	.008
	ICP-AES-3015A	0.976	0.0114	0.0051	0.960	0.990		
	FAAS-3005A	0.970	0.0093	0.0041	0.960	0.985		
	FAAS-3015A	0.961	0.0074	0.0033	0.950	0.970		
	Total	0.974	0.0159	0.0035	0.950	1.000		
Mn	ICP-AES-3005A	0.946	0.0433	0.0193	0.900	0.990	5.146	.011
	ICP-AES-3015A	0.968	0.0130	0.0058	0.950	0.980		
	FAAS-3005A	0.992	0.0044	0.0020	0.990	1.000		
	FAAS-3015A	0.940	0.0100	0.0044	0.930	0.950		
	Total	0.961	0.0299	0.0067	0.900	1.000		
Fe	ICP-AES-3005A	1.970	0.0158	0.0070	1.950	1.990	11.048	.000
	ICP-AES-3015A	1.972	0.0164	0.0073	1.950	1.990		
	FAAS-3005A	1.980	0.1643	0.0734	1.800	2.000		
	FAAS-3015A	1.640	0.1516	0.0678	1.400	1.800		
	Total	1.890	0.1807	0.0404	1.400	2.000		
Cr	ICP-AES-3005A	1.966	0.0134	0.0060	1.950	1.980	25.223	.000
	ICP-AES-3015A	1.966	0.0207	0.0092	1.940	1.990		
	FAAS-3005A	1.720	0.0836	0.0374	1.600	1.800		
	FAAS-3015A	1.660	0.1140	0.0509	1.500	1.800		
	Total	1.828	0.1576	0.0352	1.500	1.990		
Cu	ICP-AES-3005A	0.952	0.0311	0.0139	0.900	0.980	1.097	.379
	ICP-AES-3015A	0.968	0.0130	0.0058	0.950	0.980		
	FAAS-3005A	0.980	0.0212	0.0094	0.950	1.000		
	FAAS-3015A	0.962	0.0301	0.0134	0.920	0.985		
	Total	0.965	0.0251	0.0056	0.900	1.000		

SD: Standard deviation; SE: Standard Error; Min: Minimum; Max: Maximum; F: F ratio; P: Probability

digestion and detection techniques indicated a significant (P<0.05) difference in six out of nine heavy metals (**Table 5**). The data of heavy metal concentration and percent recovery from synthetic samples (digestion with acid mixture) is presented in **Table 5** and **Fig. 1** respectively.

Heavy metal recovery

The heavy metal recovery data indicated a total variation between 68.6 and 99.8 percent. The lowest metal recovery of 68.6% was observed for heavy metal Pb, when the samples were processed using method 3015A and detection equipment being FAAS. However, highest recovery of 99.8% was observed for heavy metal Zn with method 3015A and detection equipment being FAAS.

The results of heavy metal concentrations from synthetic samples digested using acid mixture revealed that the recovery of Pb was significantly (P<0.05) lower than other heavy metals for methods CP-AES-3015A and FAAS-3015A. However, the comparative assessment of the heavy metal recovery following 3005A and 3015A indicated that the percent recovery of Pb was significantly (P<0.05) high with 3005A than that recorded with 3015A method (confirmed with Post Hoc test). Besides Pb, recovery of Fe and Cr by 3015A using FAAS was observed to be relatively less (<85.0%), while with other methods

it was moderately high. Furthermore, the results indicated that the recovery of different heavy metals is dependent on the type of method (**Fig. 1**).

Sample digestion using only HNO₃

The heavy metal recovery data indicated a total variation between 52.2 and 99.8 percent. The lowest metal recovery of 52.2% was observed for heavy metal Pb, when the samples were processed using method 3015A and detection equipment being FAAS. However, highest recovery of 99.8 % was observed for heavy metals Cd and Mn with method 3015A and 3005A with detection equipment being FAAS respectively. The heavy metal concentration data showed that maximum mean for Zn is 0.974 ± 0.015 mgL^{-1} (FAAS; method 3005A), Pb 0.980 ± 0.007 mgL^{-1} (ICP-AES; method 3005A), Cd 0.998 ± 0.018 mgL^{-1} (ICP-AES; method 3015A), Co 0.974 ± 0.018 mgL^{-1} (ICP-AES; method 3015A), Ni 0.990 ± 0.007 mgL^{-1} (ICP-AES; method 3015A), Mn 0.998 ± 0.008 mgL^{-1} (FAAS; method 3005A), Fe 1.978 ± 0.008 mgL^{-1} (ICP-AES; method 3015A), Cr 1.992 ± 0.019 mgL^{-1} (ICP-AES; method 3015A) and Cu 0.994 ± 0.009 mgL^{-1} (ICP-AES; method 3015A). The data of heavy metal concentration and percent recovery from synthetic samples (digestion with HNO₃) is presented in **Table 6** and **Fig. 2** respectively.
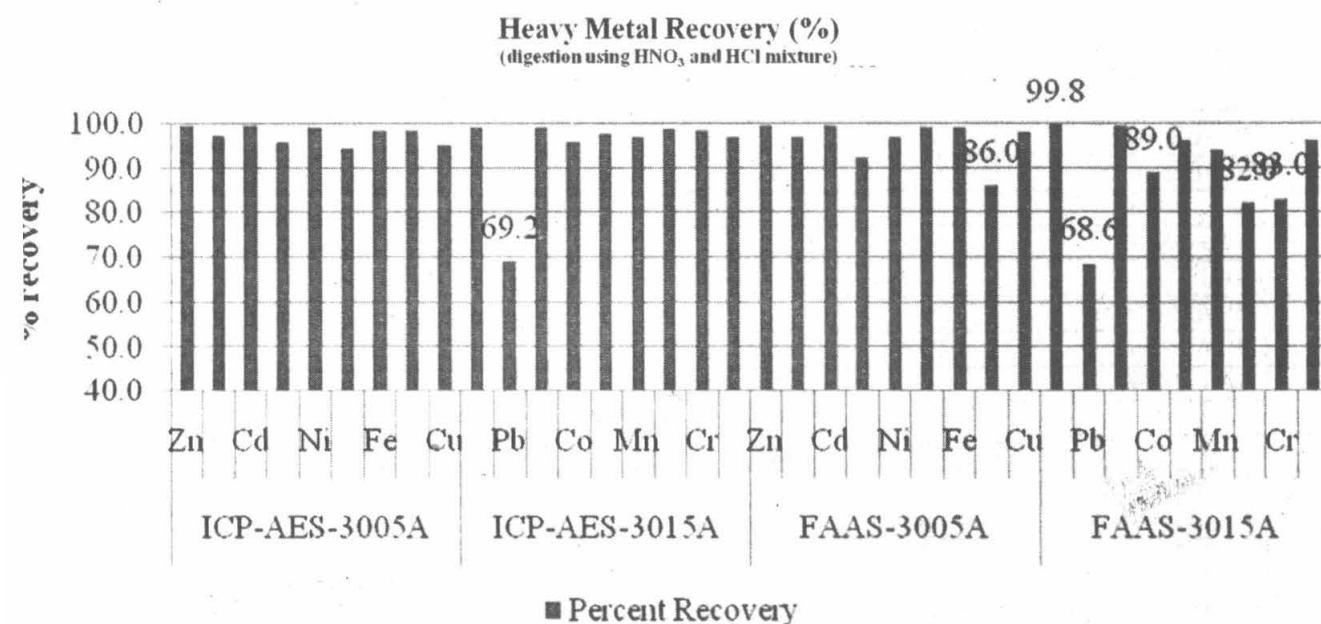

Fig. 1: Heavy metal recovery using acid mixture (HNO₃ : HCl) for digestion

Table 6: Heavy metal recovery using HNO_3 digestion

		Mean	SD	SE	Min	Max	F	P
Zn	ICP-AES-3005A	0.952	0.008	0.004	0.940	0.960	28.473	.000
	ICP-AES-3015A	0.972	0.013	0.006	0.960	0.990		
	FAAS-3005A	0.974	0.015	0.007	0.950	0.990		
	FAAS-3015A	0.910	0.012	0.005	0.900	0.930		
	Total	0.952	0.029	0.006	0.900	0.990		
Pb	ICP-AES-3005A	0.980	0.007	0.003	0.970	0.990	2160.9	.000
	ICP-AES-3015A	0.883	0.010	0.004	0.870	0.890		
	FAAS-3005A	0.950	0.007	0.003	0.940	0.960		
	FAAS-3015A	0.522	0.015	0.007	0.500	0.540		
	Total	0.834	0.188	0.042	0.500	0.990		
Cd	ICP-AES-3005A	0.978	0.013	0.006	0.960	0.990	3.594	.037
	ICP-AES-3015A	0.980	0.007	0.003	0.970	0.990		
	FAAS-3005A	0.988	0.008	0.004	0.980	1.000		
	FAAS-3015A	0.998	0.013	0.006	0.980	1.010		
	Total	0.986	0.013	0.003	0.960	1.010		
Co	ICP-AES-3005A	0.962	0.013	0.006	0.950	0.980	318.0	.000
	ICP-AES-3015A	0.974	0.018	0.008	0.950	0.990		
	FAAS-3005A	0.910	0.012	0.005	0.900	0.930		
	FAAS-3015A	0.742	0.008	0.004	0.730	0.750		
	Total	0.897	0.096	0.021	0.730	0.990		
Ni	ICP-AES-3005A	0.976	0.011	0.005	0.960	0.990	1.388	.283
	ICP-AES-3015A	0.990	0.007	0.003	0.980	1.000		
	FAAS-3005A	0.980	0.010	0.004	0.970	0.990		
	FAAS-3015A	0.990	0.021	0.009	0.960	1.010		
	Total	0.984	0.014	0.003	0.960	1.010		
Mn	ICP-AES-3005A	0.976	0.011	0.005	0.960	0.990	669.7	.000
	ICP-AES-3015A	0.980	0.010	0.004	0.970	0.990		
	FAAS-3005A	0.998	0.008	0.004	0.990	1.010		
	FAAS-3015A	0.703	0.017	0.008	0.680	0.720		
	Total	0.914	0.126	0.028	0.680	1.010		
Fe	ICP-AES-3005A	1.966	0.011	0.005	1.950	1.980	106.0	.000
	ICP-AES-3015A	1.978	0.008	0.004	1.970	1.990		
	FAAS-3005A	1.974	0.013	0.006	1.960	1.990		
	FAAS-3015A	1.440	0.114	0.051	1.300	1.600		
	Total	1.840	0.243	0.054	1.300	1.990		
Cr	ICP-AES-3005A	1.976	0.011	0.005	1.960	1.990	48.71	.000
	ICP-AES-3015A	1.992	0.019	0.009	1.970	2.020		
	FAAS-3005A	1.680	0.084	0.037	1.600	1.800		
	FAAS-3015A	1.540	0.114	0.051	1.400	1.700		
	Total	1.797	0.209	0.047	1.400	2.020		
Cu	ICP-AES-3005A	0.978	0.008	0.004	0.970	0.990	3.513	.040
	ICP-AES-3015A	0.994	0.009	0.004	0.990	1.010		
	FAAS-3005A	0.974	0.009	0.004	0.960	0.980		
	FAAS-3015A	0.970	0.020	0.009	0.940	0.990		
	Total	0.979	0.015	0.003	0.940	1.010		

SD: Standard deviation; SE: Standard Error; Min: Minimum; Max: Maximum; F: F ratio; P: Probability
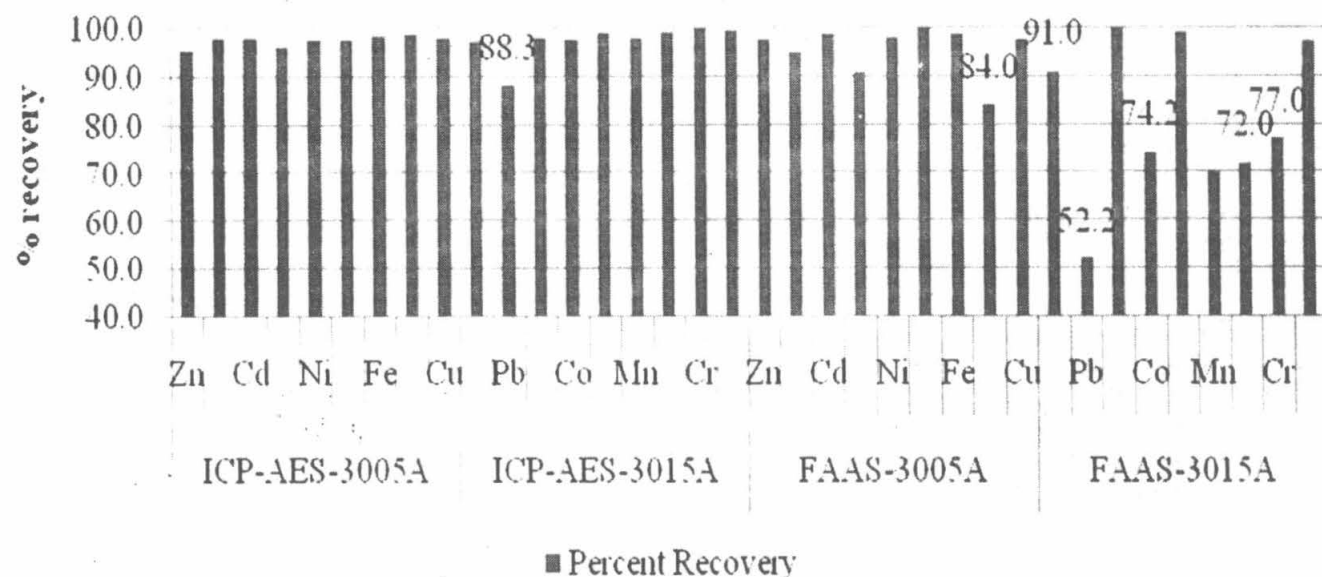
Table 7: Heavy metal recovery

Instrument	Digestion medium	Method	Heavy metal recovery (%)
ICP-AES	HNO₃ + HCl	3005A	Zn>Cd>Ni>Fe>Cr>Pb>Co>Cu>Mn
		3015A	Zn>Cd>Cr>Fe>Ni>Cu>Mn>Co>Pb
HNO₃		3005A	Cr>Fe>Pb>Cu>Cd>Mn>Ni>Co>Zn
		3015A	Cr>Cu>Ni>Fe>Mn>Cd>Co>Zn>Pb
FAAS	HNO₃ + HCl	3005A	Zn>Cd>Mn>Fe>Cu>Pb>Ni>Co>Cr
		3015A	Zn>Cd>Cu>Ni>Mn>Co>Cr>Fe>Pb
HNO₃		3005A	Mn>Cd>Fe>Ni>Zn>Cu>Pb>Co>Cr
		3015A	Cd>Ni>Cu>Zn>Cr>Co>Fe>Mn>Pb

The results of heavy mental concentrations from synthetic samples digested using only HNO₃ revealed that the recovery of Pb was significantly (P<0.05) lower than other heavy metals for methods ICP-AES-3015A and FAAS-3015A. This observation was similar to that for the other method i.e. digestion using the acid mixture. In addition to this, the comparative assessment of the heavy metal recovery following 3005A and 3015A indicated that the percent recovery of Pb was significantly (P<0.05) low for method 3015A (FAAS) than that recorded with other methods (confirmed with Post Hoc test). Besides Pb, recovery of Mn, Fe, Cr and Co by 3015A using FAAS was observed to be remarkably less (<85.0%), while with other methods (ICP-AES 3005A, ICP-

AES 3015A and FAAS 3005A), it was significantly (P<0.05) high. Hence, from the data it is evident that the heavy metal recovery from aqueous samples is dependent on the type of method used (**Fig. 2**).

Discussion

The data obtained in this study clearly indicated that the heavy metal recovery from aqueous samples is a function of method selected. The heavy metal recovery trends (high to less) observed, are presented in **Table 7**. Natural as well as anthropogenic sources are responsible for presence of heavy metals in surface waters.[47-49] Presently, at many places, anthropogenic inputs of metals exceed natural inputs resulting in excess metal levels in surface water, which

Fig. 2: Heavy metal recovery using only HNO₃ for digestion

poses a severe health risk to biotic components in general and humans in particular and also to the complete ecosystem.[50] Though, living organisms require trace amounts of some heavy metals (including Co, Cu, Fe, Mn, Mo, V and Zn), their excessive levels in water can be detrimental. Non-essential heavy metals of particular concern to surface water systems are Cd, Cr, Hg, Pb, Ar, and Sb.[51] Hence, the variation (in the heavy metal recovery) observed in present study needs to be given due importance. Since, not all the environments contain similar trends of heavy metal presence, it is clear that the existing conditions play an important role in prevailing heavy metal concentrations in the respective areas.

Presence and behavior of heavy metals in natural waters

All heavy metals exist in surface waters in colloidal, particulate, and dissolved phases, however, the dissolved concentrations are generally low[51]. There are many factors, which govern the behavior of heavy metals in natural waters. The soluble forms of heavy metals are generally ions or unionized organometallic chelates or complexes. The colloidal and particulate metal may be found in: (a) hydroxides, oxides, silicates, or sulfides, and (b) adsorbed to clay, silica, or organic matter. All these factors govern the leaching of metals and their subsequent availability for detection by using different techniques. The solubility of trace metals in surface waters is predominately controlled by the water pH, the type and concentration of ligands on which the metal could adsorb, and the oxidation state of the mineral components and the redox environment of the system.[52] Furthermore, the presence of heavy metals in natural waters is a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry. Metals also have a high affinity for humic acids, organo-clays, and oxides coated with organic matter.[52]

The water chemistry of the system controls the rate of adsorption and desorbtion of metals to and from sediment.[52] Metals may be desorbed from the sediment if the water experiences increase in salinity (creates increased competition between cations and metals for binding sites, and often, metals will be driven off into the overlying water), decrease in redox potential (decreased redox potential will change the composition of metal complexes and release the metal ions into the overlying water), or decrease in pH (low pH increases the competition between metal and hydrogen ions for binding sites). A decrease in pH may also dissolve metal-carbonate complexes, releasing free metal ions into the water column. Hence, the results obtained in this study point towards the need for novel approaches (rather than having very specific test method) for quantitative determination of heavy metals.

Health effects to humans

High concentration of heavy metals in water has serious health consequences. Ingestion of metals such as Pb (nephrotoxicity, neurotoxicity, and hypertension), Cd, Hg (pharyngitis, gasteroenteritis, vomiting, nephritis, hepatitus, liver damage, neural damage, and teratogenesis)[53] and Cr (adverse health effects are respiratory and dermatologic),[54] poses great risks to human health. Trace metals, such as Pb and Cd are known to interfere with essential nutrients of similar appearance, such as Ca^{2+} and Zn^{2+}.

Effects of heavy metals on aquatic organisms

Elevated metal levels in natural waters may cause numerous sublethal effects in aquatic organisms, notable amongst them are: (1) histological or morphological change in tissues; (2) changes in physiology [suppression of growth and development, poor swimming performance, changes in circulation]; (3) change in biochemistry [enzyme activity and blood chemistry]; (4) change in behavior; and (5) changes in reproduction.[47] The adverse effects of harmful chemical in aquatic ecosystems can be understood from the lethal concentration and lethal dose concepts. The knowledge of lethal concentration (LC_{50}) helps in determining the risk of exposure to various organisms. LC_{50} is widely used to assess the potential hazards/ threats posed by various chemicals (heavy metals) to the different biotic components of the aquatic ecosystem. On the basis of the published literature, it is evident that the LC_{50} values for various organisms for single heavy metal vary enormously[55-61] (Table 8). Thus, it will be prudent to select and use system specific analytical method for detection of desired heavy metals (which if not done results in under-reporting of the heavy metal concentration) on the basis of the dominant species in the area under investigation.

Effects on irrigation water

Irrigation water may transport dissolved heavy metals to agricultural fields. Although most heavy metals do not pose a threat to humans through crop consumption, cadmium may be incorporated into plant

Table 8: LC_{50} values for various aquatic species

Heavy metal	Test organism	Exposure time (hrs)	EC_{50}(ppm)	LC_{50}(ppm)	Reference
Cd	*Mysis.sp*	24	–	0.8	[55]
	T. fuscatus	24	–	424.33	[56]
	C. africanus	24	–	72.75	[56]
	S. huzardi	24	–	352.24	[56]
	Tubifex tubifex	24	62.68		[57]
Cu	*T. fuscatus*	24	–	84.51	[56]
	C. africanus	24	–	33.996	[56]
	S. huzardi	24	–	463.41	[56]
	Barytelphusa cunicularis	24	–	282	[58]
	Tubifex tubifex	24	0.579		[57]
	M.Cephalus	24	–	6.3	[55]
	T.Zillii	24	–	9.2	[55]
	Mysis sp.	24	–	4.86	[55]
Pb	*T. fuscatus*	24	–	1144.51	[56]
	C. africanus	24	–	380.75	[56]
	S. huzardi	24	–	2518.89	[56]
	Tubifex tubifex	24	166.48		[57]
	Dapnia sp.	24		2.51	[59]
	Cyclop sp.	24		3.11	[59]
Zn	*T. fuscatus*	24	–	459.75	[56]
	C. africanus	24	–	118.324	[56]
	S. huzardi	24	–	794.31	[56]
	Tubifex tubifex	24	36.11		[57]
	M.Cephalus	24	–	37.0	[56]
	T.Zillii	24	–	78.5	[56]
	Culex quinquefasciatus	24		0.41	[60]
Cr	*Tubifex tubifex*	24	29.77		[57]
Co	*Tubifex tubifex*	24	694.8		[57]
Fe	*Tubifex tubifex*	24	29.30	–	[57]
	Cyclops viridis			33.2	[61]
	Branchiura soerbyi			560	[61]
Mn	*Tubifex tubifex*	24	124.0	–	[57]
Ni	*Tubifex tubifex*	24	<560	–	[57]
Hg	*T. fuscatus*	24	–	11.764	[56]
	C. africanus	24	–	13.466	[56]
	S. huzardi	24	–	1.707	[56]
	Tubifex tubifex	24	0.075		[57]

tissue[62] (accumulation usually occurs in plant roots, but may also occur throughout the plant).

Summary

Environmental compartments are dynamic entities and present a challenging condition with respect to its assessment. From the results of this study, it is evident that the standard methods used for determination of heavy metals do not help in accurate determination of heavy metals. This was evident in the present study, as there was a significant (P<0.05) difference in the heavy metal recovery of same sample when processed by different methods (methods 3005A and 3015A). The sample digestion technique, i.e. choice of acid (singly or acid mixture), heating method (hot plate or microwave digester) along with detection equipments were also found to affect the heavy metal recovery and detection. The current analytical knowhow (available methods) thus, appears to be one of the

causes for under-reporting of the heavy metals present in the environmental samples. Thus, on the basis of study results, it may be concluded that the heavy metal determination from aqueous samples needs to be planned by keeping in view the conditions prevailing at the sampling sites and the expected dominance of a particular heavy metal. This practice will help the researchers and higher authorities to understand the accurate quantity of heavy metals in different aquatic ecosystems, which will aid them in better management of the respective systems.

Acknowledgements

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