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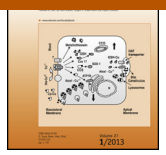

Multi-elemental analysis of human lung samples using inductively coupled plasma mass spectrometry

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ABSTRACT

The aim of this study was to establish concentrations of a wide range of elements in human lung samples to allow better identification of potential exposures in subsequent cases. This study reports concentrations of 48 elements (Al, As, Au, B, Ba, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Gd, Ge, Hf, Hg, In, Li, Mn, Mo, Nb, Ni, Os, Pb, Pd, Pt, Rb, Re, Ru, Sb, Se, Sm, Sn, Sr, Ta, Te, Ti, Tl, Tm, V, W, Y, Zn and Zr) in fresh lung tissue samples from 54 hospital patients, of which 93% exhibited various forms of neoplasia. The lung samples were taken from unaffected, background tissue. The samples were stored as fresh tissue in alcohol, dried and microwave digested before analysis by inductively coupled mass spectrometry (ICP–MS). It was possible to establish 95th percentiles for all elements except for rhenium and for 40 elements mixed effects modelling was undertaken.

Overall, the levels reported are commensurate with ranges for those elements that had been reported previously. The data were examined for gender, smoking and occupational exposures to metals. The results show that males have higher lung concentrations of Ni, Cr, Gd, Au and Be than females, but significantly lower lung concentrations of Co, Sn, W and In. Cadmium lung concentrations were significantly higher in smokers. Platinum lung concentrations were higher in those who had undergone chemotherapy and gadolinium concentrations were predictably high in those who had undergone imaging scans. More essential elements such as Cu, Br, Fe and also Ge varied the least within lung samples from individuals whilst Be, Hf and Pt had the greatest variances. Between individuals V and Li lung concentrations varied the most, whilst Cu varied least. Analysis of the data for those who reported as having previously worked with metals showed 24 of the 48 elements determined were higher than those from those who had not reported working with metals.

1. Introduction

Workplace exposure to metals can occur by inhalation, ingestion or dermal uptake; or a combination thereof. Inhalation of small metal particles is common in workplaces where airborne particulates are produced from activities such as welding, grinding and smelting. Such exposures can lead to the development of respiratory disease and general ill health. It is also thought that small metal particles can remain in lung tissue following chronic inhalation of relatively insoluble metal particles. Particles of metals such as nickel and chromium may remain undissolved in lung samples for many decades [1]. From the disease perspective, the International Agency for Research on Cancer (IARC) has classified hexavalent chromium and nickel compounds and arsenic (and arsenic compounds), beryllium, cadmium (and cadmium compounds) as Group 1 carcinogens. Such carcinogens are defined as carcinogenic to humans. Inorganic lead compounds are classified as Group 2A (i.e. probably carcinogenic to humans) and many other metals are classified as Group 2 B (i.e. possibly carcinogenic to humans) [2–6].

The use of lung samples to assess differences in exposures mostly occurs in post mortem samples and is a fairly rare occurrence in the UK. The collection and analysis whilst technically is a simple concept given modern analysis methods, involves strict ethical protocol and consent be adhered when collecting the samples. To date there is no standardised method for the sample storage, pre-treatment or analysis; thus making data comparison between different studies a considerable challenge. It has been suggested that older methodology had potentially resulted in both analytical and sample preparation anomalies [7]. It is believed that a number

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of previous studies have reported results with poor specificity and questionable accuracy, as well as artificially high results reported through contamination with elements found in fixative and storing reagents and reported losses of volatile elements through freeze drying [7].

More recent studies have attempted to address these anomalies [8,9]. Catalani et al. undertook a *meta*-analysis of relevant publications and usefully summarised common approaches and results [8]. Indeed, the main suggestions from Catalani *et al*'s review were to avoid steel cutting devices, to use plastic storage, to be consistent in the anatomical location of the sample and to desiccate the lung sample to minimise the variability of water content.

Differences in lung composition, since lung is not homogenous and may have background pathology, are likely to affect the concentrations of the elements. This can further add to the variability observed between studies. Differences are also likely between geographical habitats of donors; as it is known that persons from different locations can be exposed through environmental and dietary routes to varying levels of elements and this can make interpretation of results between different geographical regions difficult [10,11].

Recent research has shown that elevated environmental exposures to certain metals in urban areas can be associated with different sources of pollution. For example, iron, chromium and manganese were found to be associated with exposures in underground rail systems whilst calcium, titanium, tin, antimony and lead were associated with roadways from tyre, brake and oil sources [12]. This finding suggests that the overall body burden of elements could potentially vary considerably even within relatively small geographical areas. These possible variables perhaps may make the idea of a 'normal range' in lung tissue for a wide range of elements ambitious.

Specifically, the aim of this study was to collect lung samples from living donors and analyse for current 'normal' values for a wide range of elements. Collecting a lung sample from a living donor is only possible as part of a more significant surgical procedure. In this study the lung samples were collected from patients undergoing elective thoracic surgeries. The samples were removed from lung tissue deemed to be from unaffected areas of the biopsy/tumour removal. The samples were taken from lung that was macroscopically unaffected, being distal to any focal pathology. Anything that was a diffuse process meant that the sample was not taken. The main objective was to define concentrations of a wide of range of elements in freshly collected lung samples, which could then be used to compare against future clinical, environmental, nutritional, toxicological and forensic investigations.

A secondary objective of the project was to produce a standard method to collect and analyse fresh lung tissue from 'unaffected' tissue (in our project, from patients undergoing lung surgery – serving as our surrogate for normal lung). This methodology was designed to be easily applicable to other laboratories internationally. The intention was to replicate sample sizes of fresh tissue normally received for case/incident analysis and to use equipment containing no stainless steel/metal and reagents with high purity to prevent possible contamination.

2. Materials and methods

2.1. Cohort

Ethical approval was obtained from a NHS Research Ethics Committee (11/YH/0267 and Sheffield Teaching Hospitals STH Number 16163).

Using a structured questionnaire; details were collected regarding gender, age, smoking status and occupational history were collated. Details of chemotherapy treatments were also noted where relevant. All subjects gave their written informed consent to participate in the study.

Lung sections were removed during routine operations in 54 patients at Sheffield Teaching Hospital NHS Trust. The cohort was comprised 31 male and 23 female donors aged between 18 and 83 years, with a mean age of 67.8 years. Fourteen of the 54 donors stated that they currently smoked or had previously smoked. Fifteen donors stated they had previously worked with metals (mostly steels). The local area of the city of Sheffield, located in central England, is an area known historically for its steel making industry.

Cube-shaped fragments of lung tissue samples were collected from patients undergoing lung surgery, fresh from the operating theatre. The tissue used was removed from background tissue, as defined by the pathologist and had to be free macroscopically from any disease process for which surgery was undertaken.

Among the donors, the lung pathologies prompting surgery were 22 (41%) adenocarcinomas, 19 (35%) squamocellular carcinomas, three (6%) neuroendocrine carcinomas and three (6%) carcinoids. In addition to these primary lung carcinomas there were three (6%) cases of metastasectomy and four (7%) non-cancerous lung diseases.

Table 1
Microwave programme for acid digestion of dried lung samples.

Stage	Power	Ramp	Temperature control	Hold
1	300 W 65%	1:00	50 °C	2:00
2	300 W 5%	1:00	1 °C	2:00
3	300 W 100%	2:00	120 °C	5:00
4	300 W 5%	1:00	1 °C	2:00
5	600 W 100%	5:00	140 °C	5:00

2.2. Sample collection and storage

The samples for metals analysis were excised from the removed tissue sample by the pathologist approximately 10 min post-surgery in the laboratory. Upon removal the samples were placed in absolute alcohol in sterilin tubes. The samples were anonymised and delivered to the Health and Safety Laboratory where they were refrigerated.

2.3. Sample analysis

The sterilin tubes containing the lung sample (approximately 2 cm^3) were opened and using nylon tweezers (Fisher Scientific, Loughborough, UK) the lung sample was placed on a sterile petri dish. Using a ceramic blade (Safety Knife Services, Hertfordshire, UK) the lung samples were cut into smaller sections from different areas of the sample and placed into a fresh sterilin tube. This tube was then placed in a laboratory drying oven at a temperature of 70 °C (LEEC, Nottingham, UK). The samples were dried in the oven for up to three days until they reached constant weight.

The dried lung samples were then microwave digested and for each initial lung sample, three sub samples were digested. Each small dried section was weighed into a cleaned microwave vessel; the average sample weight digested and the standard deviation of this was 10 ± 0.6 mg. The samples were microwave digested by adding 2 ml concentrated nitric acid (Romil, Cambridge, UK) and 1 ml hydrogen peroxide (Aristar, BDH Laboratory Supplies, Poole, UK) into each vessel, which were then sealed and heated using the programme stated in Table 1 (Mars Xpress, CEM, Buckingham, UK). The digest was then diluted with water to 10 ml in 50 ml conical capped plastic tubes (Sarsedt, Nümbrecht, Germany). There were 161 digests of the 54 donated lung samples; all but one tissue sample had three sections digested.

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The samples were analysed using inductively coupled plasma mass spectrometry (X Series 2 ICP–MS and ICAP Q ICP–MS, Thermo Fisher Scientific, Bremen, Germany). The sample digests were then further diluted (1 in 10) and analysed for a total of 48 elements using four different ICP–MS methods; in standard mode using Thermo X Series 2 with 1% v/v nitric acid solution (method 1), in collision cell mode using kinetic energy discrimination with a collision cell gas of 7% hydrogen in helium at a flow of 3.5 ml/min (KED/CCT) mode using Thermo X Series 2 with 1% v/v nitric acid solution (method 2), in standard mode using ICAP Q with 1% v/v nitric acid solution (method 3) and in standard mode using ICAP Q with 1% v/v HCl solution (method 4). Six point calibration graphs were used for each analysis, concentration ranges were 0.01–10 μg/L (method 1), 0.01–100 μg/L (100 μg/L for Br and Fe only) (method 2), 0.002–0.2 μg/L (method 3) and 0.01–2.5 μg/L (method 4). The ICP–MS instruments were tuned daily to ensure that oxides (using CeO/Ce) and doubly charged ions (using Ba++) were less than 2%.

The concentration of each element (μg/g) in the lung sample was reported as a ratio of the dry weight of the lung sample.

2.4. Quality control

External certified powdered reference materials (CRMs) were analysed in each round of digestions as well as an ovine (sheep) liver sample obtained from a supermarket. It was not possible to obtain lung samples from a commercial source. There are a limited number of quantified elements in externally purchased certified reference materials so it was necessary to spike a liver sample and the blank digestion matrix to optimise the methodology to assess the elemental recoveries of all the elements being determined in this study. This was achieved by analysing the digested blank and spiked ovine liver sample by all the ICP–MS methods. The blank digestion matrix was also spiked to help with optimising the methodology. The quality control samples were CRMs of pig kidney ERM-BB186 and lyophilised bovine liver BCR-185R (LGC standards, Teddington, UK). There were no lung CRMs available for use in this study.

The ovine liver sample was sectioned and dried in the same way as the study lung samples, and prior to digestion a number of the liver sub-samples were spiked with the elements being analysed at concentrations of 0.1–10 μg/L. The blank liver and spiked liver digests were analysed in each method. A blank digestion matrix and a spiked digestion matrix were determined at the start and end of each analysis. A urine CRM was also analysed, ClinChek levels 1 and 2 (lot 122 Recipe, Germany) to ensure the accuracy of the analysis.

The full details of the CRMs, spiked liver samples and spiked blank digest matrices recoveries and limits of detection are listed in Supplementary Table 1 (see Supplementary Table 1 in the online version at DOI: 10.1016/j.jtemb.2016.11.008). The ovine liver sample, spiked with all of the 48 elements, showed a range of recoveries between 84.5 ± 1.2% for Hg and 118 ± 1.7% for Tm; the Se recoveries for the spiked liver samples were 92.0 ± 5.1%. The recovery of Se in the CRMs is lower at 60–70%, possibly as a result of an under recovery because of the presence of an organo-selenium species in the samples and this could have benefitted from the addition of an organic solvent to the reagents. It may also be related to the higher concentrations of Se present in the CRMs.

2.5. Limit of detection

The limit of detection (LOD) for each analyte was calculated as the mean + three times the standard deviation of the blank matrix run throughout all analyses. Conversions to μg/g could be made by dividing by 0.01 g (mean weight of sample) and multiplying by the dilution factor (×10) of the analysis. In Table 2 approximate LOD corrected for weight (μg/g) in for the lung samples have been calculated using the mean weight of sample 0.01 g.

2.6. Statistical analysis

Statistical analysis was carried out by fitting mixed effects models to the data in WinBUGs (Version 1.4.3) [13], using Markov Chain Monte Carlo methods within a Bayesian framework. The models were specified on the log scale, and included age, gender, smoking and metal exposure as fixed effects that were considered in combination with each other. This allowed us to determine whether any, or all, of the variables were significant determinants of elemental concentrations.

As up to three replicates were taken per sample, a mixed effects model was used in the analysis to account for correlation and to model inter-sample variability via random effects. The measurements were assumed to be log-normally distributed. Values below the LOD were treated as left censored and substituted by values imputed using Bayesian methods. For elements where a large proportion of measurements fall below the LOD, the mixed effects modelling may result in biased estimates of the fixed effects and variability. Although there is no standard cut-off point, the decision was thus made to limit the mixed effects analysis to only those elements where no more than one third of measurements were below the LOD to minimise the bias arising from censored data. The mixed effects model was of the form

$$\ln\left(Y_{ij}\right) = \mu + \beta_g I_{g,ij} + \beta_s I_{s,ij} + w_i + \varepsilon_{ij}$$

$$w_i \sim N\left(0, \sigma_1^2\right)$$

$$\varepsilon_{ij} \sim N\left(0, \sigma_2^2\right)$$

where elemental concentrations are denoted by Y_{ij}, (the subscripts denote the j^{th} replicate on the i^{th} sample). These elemental concentrations were log-transformed prior to analysis and are expressed in the statistical model as a sum of fixed effects, random effects and residual error; μ represents the mean concentration of a female non-smoker, $I_{g,ij}$ and $I_{s,ij}$ are indicator variables which take the value 1 if the individual is a male and a smoker respectively, and 0 otherwise. β_g and β_s are corrections to the overall mean and measure consistent adjustments for males and smokers respectively, i.e. they represent differences between males and females; and differences between smokers and non-smokers. The random effects w_i are assumed to be normally distributed with a mean of zero and standard deviation σ_1^2, with σ_1^2 quantifying the inter-individual variability. The term ε_{ij} represents the residual errors which are assumed to be normally distributed (on the log-scale) with mean zero and standard deviation σ_2^2, with σ_2^2 quantifying intra-individual variability.

3. Results

3.1. Human lung sample data

The mean, median and range of all 48 elements determined in the lung samples analysed in this study are reported in Table 2. For 41 elements, over half of the measurements were greater than the LOD. For 29 elements, over 95% of measurements were greater than the LOD. For Re it was not possible to establish 95th percentiles as too high a proportion of the data was below the limit of detection (>96%). For Hg, Tl, As, Ru, Te, Th, Re and Os over a third of samples were below the LOD. Thus, mixed effects modelling was not carried out on these elements due to the bias that may arise from the large proportion of censored measurements. For the other 40 elements,

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Table 2
Results of the 48 elements analysed in the lung samples from 54 donors including medians, 95th percentiles, ranges and percentage of results below the limit of detection.

Element	% < LOD	LOD[a] in μg/g	Median in μg/g	95th prctile in μg/g	Range in μg/g
Al	1	0.388	14.27	116.64	<LOD–638.125
As	52	0.016	*	0.168	<LOD–0.804
Au	9	0.001	0.01	0.03	<LOD–0.0068
B	14	0.47	2.04	8.18	<LOD–12.541
Ba	3	0.025	0.22	2.58	<LOD–14.970
Be	28	0.0001	0.00	0.01	<LOD–0.0005
Bi	14	0.002	0.01	0.04	<LOD–0.667
Br	0	2.168	55.14	98.79	10.075–115.786
Cd	1	0.002	0.27	3.43	<LOD–5.700
Ce	0	0.0008	0.29	2.32	0.008–7.168
Co	2	0.001	0.11	0.42	<LOD–1.096
Cr	0	0.024	0.48	5.18	0.092–51.143
Cs	4	0.0009	0.02	0.06	<LOD–0.213
Cu	0	0.049	6.02	10.51	2.186–40.561
Fe	0	0.089	745.56	1692.49	201.484–2979.310
Ga	0	0.0007	0.017	0.088	0.004–0.454
Gd	5	0.001	0.009	0.272	<LOD–3.119
Ge	0	0.001	0.052	0.122	0.014–0.179
Hf	0	0.0002	0.011	0.065	0.001–0.153
Hg	64	0.037	*	0.28	<LOD–0.701
In	2	0.0002	0.004	0.024	<LOD–0.071
Li	3	0.004	1.637	5.929	<LOD–6.814
Mn	0	0.018	0.621	2.015	0.142–7.776
Mo	16	0.01	0.080	0.522	<LOD–2.523
Nb	0	0.0005	0.006	0.084	<LOD–1.866
Ni	1	0.016	0.221	1.127	<LOD–3.364
Os	83	0.013	*	0.030	<LOD–0.208
Pb	3	0.002	0.08	0.23	<LOD–0.621
Pd	16	0.002	0.004	0.012	<LOD–0.029
Pt	25	0.0005	0.003	5.140	<LOD–11.646
Rb	2	0.021	5.50	9.20	<LOD–14.152
Re	96	0.0002	*	**	<LOD–0.001
Ru	88	0.017	*	0.036	<LOD–0.062
Sb	0	0.002	0.03	0.12	0.001–0.593
Se	8	0.120	0.89	2.37	<LOD–3.963
Sm	30	0.002	*	0.02	<LOD–0.090
Sn	1	0.004	1.01	6.66	<LOD–14.903
Sr	1	0.036	0.32	0.83	<LOD–8.778
Ta	22	0.002	0.0035	0.140	<LOD–7.836
Te	71	0.006	*	0.023	<LOD–0.035
Ti	1	0.048	1.59	15.71	<LOD–43.647
Tl	45	0.0005	0.0005	0.003	<LOD–0.006
Tm	81	0.0006	*	0.003	<LOD–0.005
V	1	0.014	4.86	22.13	<LOD–45.043
W	0	0.074	1.75	5.14	0.096–42.012
Y	0	0.0006	0.03	0.14	0.003–0.267
Zn	0	0.580	49.44	82.95	1.131–338.103
Zr	2	0.008	0.51	1.76	<LOD–4.854

*More than a third of values below the LOD not possible to determine a median value.
*More than 96% of values below the LOD not possible to determine a 95th percentile value.
[a] The LOD expressed as μg/g was calculated from the ICP-MS analysis (mean + 3SD of the blank) and then assumed a mass of 10 mg and a dilution to 10 ml.

the mixed effects modelling takes into account the replicates taken from each sample.

Only Cd was significantly different in smokers with a four-fold increase in median values, from 0.23 μg/g to 1.608 μg/g for non-smokers compared to smokers. Males were found to have significantly higher concentrations of Ni (52% higher), Cr (109% higher), Gd (129% higher), Au (58% higher) and Be (85%) than females, and significantly lower concentrations of Co (56% lower), Sn (55% lower), W (60% lower) and In (65% lower).

The intra-individual and inter-individual geometric coefficients of variation (GCV_{intra} and GCV_{inter}) are indications of the extent of variability within and between individuals in relation to the mean, for log-normally distributed data. After adjusting for variation due to gender and smoking, the elements that displayed the greatest GCV_{intra} were Be (199%), Pt (128%) and Hf (111%). Those that displayed the lowest were Cu (21%), Fe (24%), Br (25%) and Ge (27%).

In terms of variability between individuals, GCV_{inter} was greatest for Pt (7100%), Li (472%), V (303%) and Gd (300%), and lowest for Cu (30%). The difference between male and females and their relative variations are shown in the bubble plot in Fig. 1. Relatively large variances were seen for Au, Be, Cd, Gd, Li, Pt, Ta and V, of >1.0 (on the log scale) whilst the more essential trace elements selenium, zinc, iron and copper showed smaller variances of <0.5. The variances in Pt and Gd concentrations are most likely as a direct result of treatments and diagnostic tests received i.e. chemotherapy and magnetic resonance imaging (MRI), respectively.

Significant effects (at the 95% level of significance) of increased concentration with increased age (shown in Fig. 2), were observed for Al, Be, Br, Ce, Co, Cr, Ga, In, Nb, Se, Sm, Sn and Ti, with the greatest effect being for Be where a 5.0% (95% CI [2.3%, 7.9%]) increase in concentration per year was seen, after adjusting for gender. One element, Pt, showed a borderline significant negative trend with age, with a −5.6% (95% CI [−11.3%, 0.14%]) decrease in concentration per year being found; again this is believed to be as a result of the

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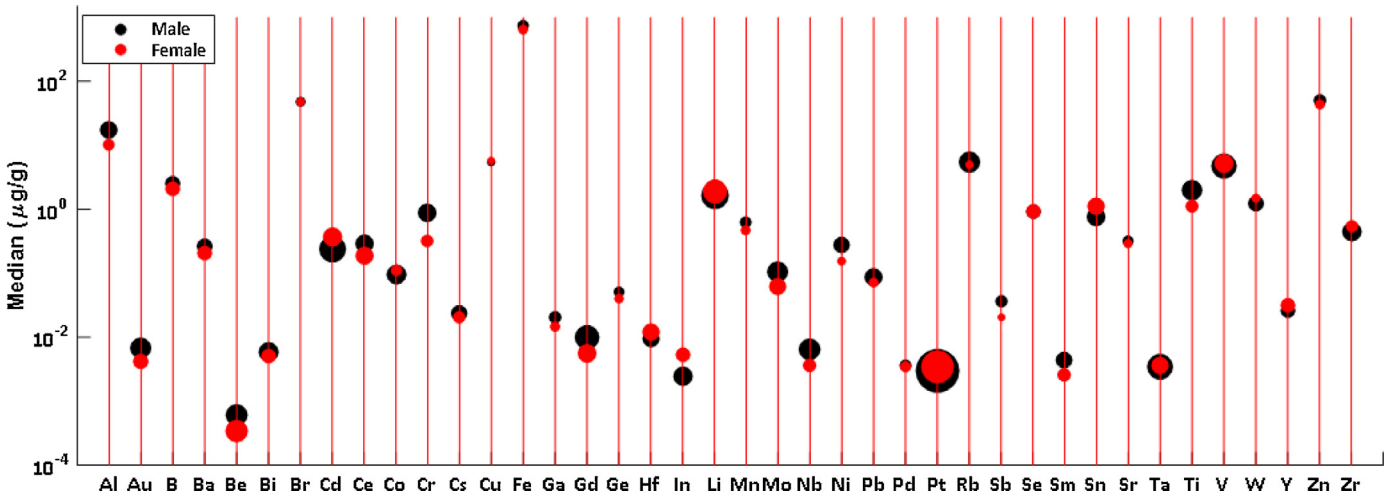

Fig. 1. Median of elemental concentrations measured in lung samples from 54 donors. The size of the bubble represents the relative variation on the log-transformed data (the larger the bubble, the greater the variation).

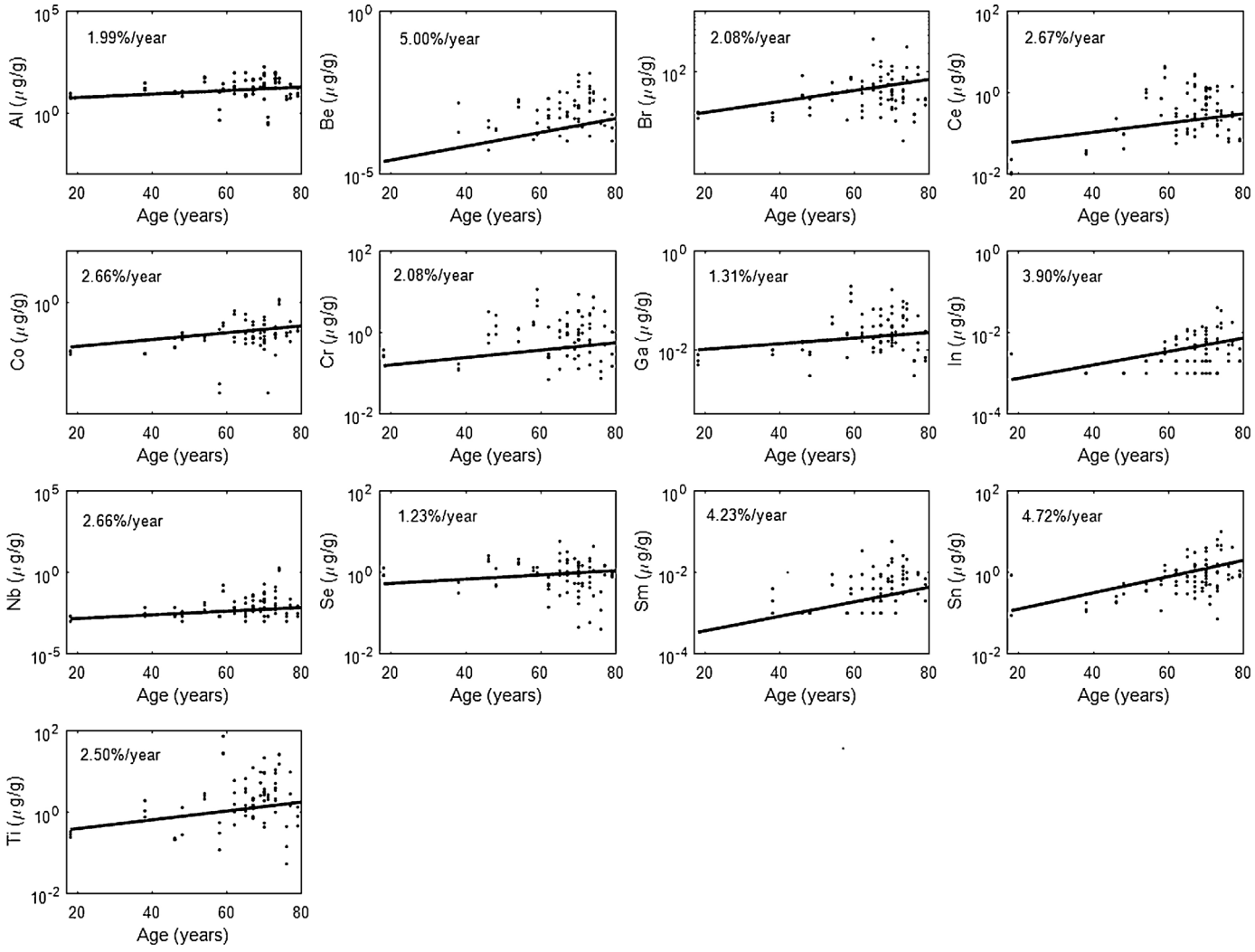

Fig. 2. Plots to show the trends in elements in lungs when modelled for age where increased concentrations were observed with increased age (at the 95% level of significance).

influence of Pt containing treatment on the lung tissue in those who underwent chemotherapy. The summary of the differences observed with age is listed in Table 3.

The patients who had undergone chemotherapy showed significantly higher concentrations of Pt, Bi and Mo than those who had not. In particular, concentrations of Pt were 2300% higher (95% CI

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Table 3
Effects of smoking, gender, age and metal-work on elemental concentrations using mixed effects modelling. 95% credible intervals are provided in brackets. The asterisk (*) indicates the effects that are not statistically significant at the typically used 95% level of significance, but are considered to be borderline significant.

Element	Percentage increase for smokers	Percentage increase for males	Percentage increase per year of age	Percentage increase for metal work
Al			1.99 (1.79, 3.83)	
Au		58.0* (−10, 168)		
B				
Ba				
Be		85.4* (−8.4, 200)	5.00 (2.30, 7.88)	
Bi				97.5* (−10.8, 340)
Br			2.08 (1.40, 2.78)	16.5* (−9.1, 50.0)
Cd	300 (−3.6, 1660)*			163* (−7.6, 650)
Ce			2.67 (0.30, 5.09)	
Co		−55.6 (−76.4, −17.1)	2.66 (0.08, 4.60)	175 (37.5, 443)
Cr		109 (25.5, 253)	2.08 (0.24, 4.01)	
Cs				
Cu				15.6* (−8.6, 47.3)
Fe				39.3* (−1.2, 98.9)
Ga			1.31 (0.03, 2.65)	89.1 (17.5, 210)
Gd		129* (−3.75, 464)		
Ge				37.2* (−2.30, 94.0)
Hf				
In		−65.3 (−79.3, −41.6)	3.90 (2.33, 5.55)	269 (108, 537)
Li				
Mn				79.2 (20.4, 169)
Mo				
Nb			2.66 (0.54, 4.93)	200 (37.0, 566)
Ni		51.6* (−2.10, 141)		
Pb				86.4 (4.69, 223)

[280%, 14700%]). For Bi and Mo, concentrations were 104% (95% CI [2%, 304%]) and 106% (95% CI [2%, 310%]) higher respectively.

Fifteen of the 54 donors stated that they had worked with metals; potentially exposed job descriptions included steel workers, builders, joiners, grinders/fettlers and plumbers. When stratification for working with metals was undertaken 14 elements were seen to significantly differ from those who had not worked with metals; for another 10 elements, the difference was borderline significant. Investigating common metals found in 'steel works' found Fe, W, Mn and Ti concentrations were elevated in metal-exposed workers (see Fig. 3). In particular, median Fe concentrations in metal workers were 1087 μg/g compared with 728 μg/g in non-metal workers. No significant difference in concentrations of V, Ni and Cr was found between metal-exposed and non-metal exposed. The summary of the differences observed between those who had and had not worked with metals are listed in Table 3.

The largest effect of metal work was seen in Ta, In and W. For Ta, concentrations were 386% higher in metal-exposed workers (95% CI [59%, 1390%]); for In, concentrations were 269% higher in metal-exposed workers (95% CI [108%, 537%]); for W, concentrations were 255% higher in metals exposed (95% CI [88%, 574%]). All but one of those 24 elements showed higher concentrations in metal-exposed workers than non-exposed workers; the element whose concentrations were found to be significantly lower in metal-exposed workers was Se, where concentrations were 27% lower (95% CI [1%, 47%]) after adjusting for age and gender.

Pearson's correlation between pairs of elements was calculated (on the log-transformed values). Where the concentration fell below the LOD, the value of half of the LOD was used. The strongest association was between Ge and Fe, with a correlation coefficient of 0.95 on the log-transformed values. Large positive associations were also observed for the following pairs of elements; Rb and Zn ($r = 0.87$), Cs and Rb ($p = 0.86$), Rb and Pb ($r = 0.81$), In and Sn (0.81), and Hf and Y (0.80). For these elements, no more than 4% of the concentrations fell below the LOD and therefore their substitution with half of the LOD would have had relatively little influence on the correlation.

4. Discussion

Analysis of the lung samples showed that 52% of As and 64% of Hg results determined were below their limits of detection (0.0165 μg/L for As and 0.0366 μg/L for Hg). It would have been possible to establish concentrations by digesting a larger lung sample or using a smaller post digest dilution. Often the amount of tissue sample available from potential exposure 'cases' is limited, and so low sample weights typical of those obtained in real cases were digested and analysed in this study. De Palma et al. also report a significant amount of As results below the limit of detection [9] whilst Mari et al. reported all of their Hg results below the limit of detection [14].

In recent years the standard approach for sample analysis is commonly microwave digestion with nitric acid and subsequent analysis by ICP–MS. However, there are differences still in the sample treatment and whether the weight used in the calculation is dry, or wet, weight. Studies have reported concentrations in wet weight [15,16] and some in dry weight [9].

In order to reduce the variation and allow the results to be directly compared to samples from different sources we would recommend the analysis of dry weight samples only. It has been reported that typically dry weight has concentrations 5 times higher than wet weights [17]. With a similar conversion rate Barregard et al. established transformed dry weight concentrations of kidney samples to wet weight concentrations by multiplying by 0.18 [18]. It is also important to consider changes that can occur in samples post-mortem (such as cell lysing, variation in tissue volume and contamination from locally leaking body compartment fluids). Results published [19] from liver tissue sampled at various times post-mortem showed that elements could alter by up to 40% depending on the element, storage temperature and number of days since autopsy. There is a need to standardise the sample storage post collection – Iyengar suggested a freeze drying approach. In this study no samples were taken from post mortems, all samples were from living donors.

In Supplementary Table 2 (see Supplementary Table 2 in the online version at DOI: 10.1016/j.jtemb.2016.11.008), data from this

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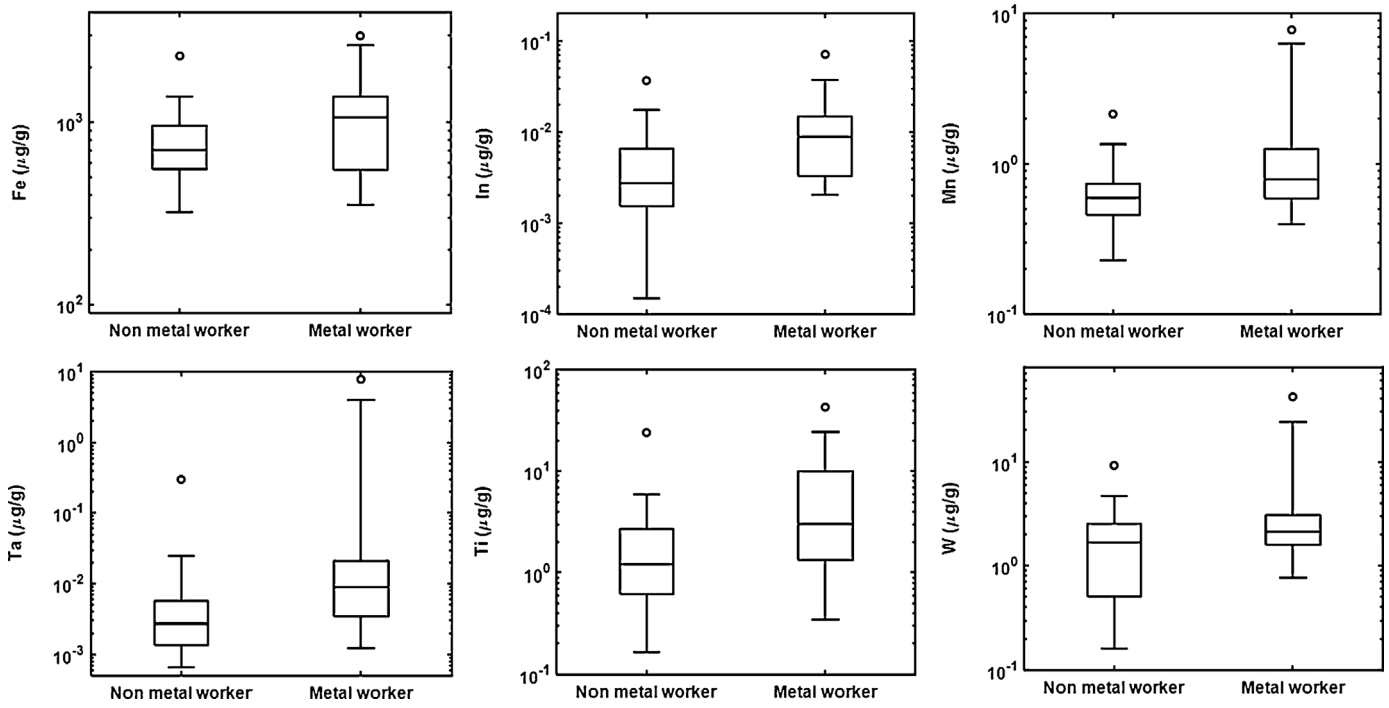

Fig. 3. Boxplots of iron, indium, manganese, tantalum, titanium and tungsten concentrations in lung samples from metal exposed workers and non-metal exposed. The boxes represent the 25th, 50th and 75th percentiles whilst the bears extend to the 5th and 95th percentiles. The single point represents the highest concentration.

study and four other studies have been compared. Three of the four studies used dry weight lung samples to calculate the results whereas Brune et al. used wet weight [20]. Whilst Brune et al. reported results from 1980 using wet weight calculations and a variety of analytical methods, their study was extensive, measuring 23 elements and valid comparing controls and workers in a Swedish population [20]. The data showed that median results from this study compared well for concentrations of Al, Ba, Be, Cd, Co, Cr, Cs, Pb, Rb, Sb, Sr and Ti in the lung samples with those published by Zhu et al. [21]. Elements such as As, Au, Hg and Sn where values are only available from one other study are difficult to compare.

Across all the studies there are wide ranges of varying concentrations reported for those elements more commonly measured in lung tissue samples, such as Cu, Fe, Mn, Ni, Se, V, W and Zn. It was not possible to compare concentrations for following elements that have not been reported elsewhere- Bi, Br, Ce, Ga, Gd, Ge, Hf, In, Li, Nb, Os, Pd, Pt, Re, Ru, Sm, Ta, Te, Tl, Tm, Y and Zr.

Investigating the range of external exposures and variables, the more 'essential' elements, such as Cu, varied the least between different donors in this study. The median Cu concentration in this study was 6.02 μg/g which compares well to the Cu concentration of 4.75 μg/g found in benign lung samples in a Polish study where the samples had been frozen and analysed by X-Ray Fluorescence [22]. Majewska et al. also reported similar Se concentrations, 0.803 μg/g, compared with our reported median of 0.89 μg/g whereas the Fe and Zn concentrations were lower. Brune et al. found a median Cu concentration in wet tissue of 1.1 μg/g, which if the factor of five were applied, would compare well with our study [20]. The Cu concentration in our study was much higher than those of 0.44 ug/g found by Farah et al; their sample treatment was very different, for example samples were stored in formalin fixed blocks [23]. Formalin has been reported to alter the concentrations of Al and Mn over time by increasing and decreasing elemental concentrations respectively [7]. In fact Farah et al. determined 15 elements in normal lung samples from a US cohort and only the Cr concentration compares to this study [23]. A study by DePalma et al. using similar pre-treatment and analysis methodologies showed similar results to those found in this study for As, Be, Cd, Co,and Pb but higher results for Cr and Ni [9].

Data from the Zhang et al. study compared control and cancer lung samples from two locations in China [24]. Results showed similar concentrations for Fe, Co, Cu, Zn and Se, higher concentrations of Ti, Ni, As, Rb, Cd, Sb, Cs, Ba and Pb but lower V than the concentrations seen in our study. Comparing all the studies, variations in the Cu and Fe concentrations between the studies are evident which could well be from environmental/dietary differences. Other concentrations that differ between the studies are Ni, Cd, Pb, Zn, Ba, Ti, Sr and Rb.

In our study with samples analysed from three separate pieces of the dried lung tissue it was possible to assess the within individual sample variation which showed that essential elements Cu, Br, Fe and Ge varied the least whilst Be, Hf and Pt had the greatest variances. Between individuals V, Li, Gd, Pt and Cd varied the most whilst Cu varied the least (see Fig. 1). The elevated Gd and Pt results are thought to be as result of chemotherapy and scanning procedures; in this study 24% of the donors had been exposed to Pt containing chemotherapy drugs resulting in a wide range of platinum concentrations (<LOD–11646 μg/g).

In the review of metallic elements in lung samples undertaken by Catalani et al. considerable intra- and inter-individual variability between samples and elements [8] were observed. Raithel et al. undertook a detailed study analysing different anatomical sections of lung samples for Ni and Cr [1]. They found that the highest concentrations of Ni and Cr were found in the upper regions of the lungs with the highest levels in the hilum. Ni median dry weight concentrations were 159 ng/g compared with a median of 221 ng/g in our study and Cr median concentrations dry weight 967.4 ng/g compared with a median of 480 ng/g in our study [1].

Overall, the relationship with elemental concentrations and gender were found to be on the whole inconclusive in the review by Catalani et al. [8]. In our study males were found to have significantly higher concentrations of Ni, Cr, Au, Be and Gd than females, and significantly lower concentrations of Co, Sn, W and In after adjusting for age, smoking and metal work. Kollmeier et al. found

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Cd, Cr and Ni concentrations to be twice as high in males than females in a German study comparing exposures from different locations [25].

As in other studies Cd was found to be higher in lung samples from smokers than lung samples from non-smokers in this study [15]. The difference of a four-fold increase was observed in the Cd measured in the lung tissue of smokers compared to those of non-smokers, with a median value of 0.23 μg/g in non-smokers compared to 1.608 μg/g in smokers.

Kollmeier et al. also found a four-fold increase of Cd in the lung samples from smokers [25] whilst in an Australian study average levels of Cd in non-smokers was 0.008 μg/g and in smokers 0.41 μg/g as wet weight [26]. Other common elements to be associated with smoking are Ni, Cr and Pb but these showed no significant difference in this study.

The largest percentage increases with age per year observed in this study were for Sn, Be, Nb and In. Other studies have investigated the relationship between elemental concentrations and age. Kollmeier et al. found that Cr increased by 2.45% per year and Ni increased by 3% per year [25]; whilst our study found a 1.89% increase per year of Cr the same trend is not seen for Ni. Raithel et al. also reported an increase with age for Cr, in tissues taken from the upper lobes of the lungs and in the hilum region; but no effect with age was observed for Ni [1]. As with results from this study, Kollmeier et al. did not find an age related effect for Cd [25].

The disease type and therefore viability of lung tissue from donors may play a part in the elemental profile found in lung samples. Generally, Catalani et al. found that tissue concentrations were similar in unaffected tissues from both controls and lung cancer patients but that lower elemental concentrations were found in actual lung tumour samples [8]. Zhang et al. determined 15 elements in normal and malignant tumour tissue in lung samples from Chinese adults, the results showed that the concentrations of V, Fe, Zn, Cd, Ni, Cu, Se and Pb in lung cancer tissues were significantly different from those in benign tissues. Specifically, significantly lower concentrations of V, Fe, Zn and Cd were found whilst significantly higher concentrations of Ni, Cu, Se and Pb in the tumour tissue compared to the control tissue [24].

De Palma et al. undertook a study that demonstrated that unaffected lung tissue is more representative of the elements found in the lung rather than those found in cancerous tissue [9]. Their study found higher concentrations of Cd, Ni and Pb in unaffected tissue samples than in controls; Pb concentrations in lung cancer tumours were seen to be higher in squamous carcinoma, compared with adenocarcinomas. Overall, the concentrations for the control samples (not lung cancer tissue) reported by DePalma et al. compare well with the results in this study. Median Cr and Ni concentrations being slightly higher and Cd being slightly lower in the DePalma et al. study than those reported here [9]. Raithel et al. found that there was no difference in the concentrations of Cr and Ni in lung tissue from lung cancer patients and patients with healthy lungs [17].

Determining occupational exposure in lung samples has been undertaken in a number of studies from Pb, Cd, Cu and Zn in smelter workers [16,28] to lanthanides such La, Ce, Nd and Pr in the lung of a man who worked as a printer [28].

Generally, the studies have determined specific elements for known exposures. Our study questionnaire asked if the donors had ever worked with metals. The respondents indicated that 24% had worked with metals. In this cohort, higher concentrations of 24 elements were observed in samples from those who had worked with metals. In particular, the Fe results showed a 100% increase at the 95th percentile level, from 1380 μg/g in non-metal workers compared to 2672 μg/g in those who stated they worked with metals. No occupational exposure effect was observed for Cr or Ni. It was not possible to examine As and Hg for exposure effects due to the high proportion of results below the limit of detection. It is also interesting that Ta and In had significantly higher concentrations in the lung samples from those who worked with metals. Ta and In are not elements traditionally measured as a result to exposure to 'steel metal work', but it is possible that both elements were present in some other industrial processes. This study saw only a decrease in the Se concentrations in lung samples from metal-exposed workers. Lower Se levels in lung tissue from metal workers have not been previously reported, however, there have been reports of elevated concentrations of Se in lung tumour samples compared to normal lung samples [23,24].

A Swedish study analysed tissue samples from both controls and smelter workers and found higher concentrations of Cd and Cu but not for Zn in the smelter workers with a 4.2 higher Cd concentration and a 1.3 higher copper concentration [27]. In a previous study Gerhardsson et al. had reported that Pb from occupational sources can accumulate in tissues including lung, liver and kidney of smelter workers [16]. Though Gerhardsson et al. reported wet values the median lead in lung value of the referent range for Pb in lungs was 0.06 μg/g whilst in this study we are reporting a median value of 0.08 μg/g. The smelter workers were seen to have up a five-fold increase of Pb in their lungs, whereas our study (with no specific lead workers) shows that metal-exposed workers had a 1.86 increase in lead levels compared to those who had not worked with metals. Significant increases in lung samples from stainless steel welders have been reported, of up to 500 times for Ni and 60 times for Cr. Elevated Ni levels were also reported in both foundry and nickel refinery workers [1].

In this study Be showed the greatest year on year increase in the lung samples of 5% per year. Larivière et al. reported Be concentrations in the lung samples taken from 19 nuclear workers occupationally exposed to beryllium [29]. The average Be wet tissue concentration was 0.81 μg/kg which is higher than both the value obtained by Zhu et al. of 0.86 ng/kg in 'unexposed' Chinese persons [21] and those in this study with a median value of 0.22 μg/g. De Palma et al. reported no beryllium was detectable in control lung samples of control lung samples in Italy with a slight increase observed in cancerous tissue [9].

Exposure from environmental sources can also be identified from tissue samples. The concentrations reported in this paper compare well with lung samples (using wet weight samples) collected from autopsied subjects who resided near a waste incinerator in Spain. Comparing the values from our study with those from their 2013 results all elemental concentrations were similar with the exception that our V, Ni and Mn concentrations are higher. Interestingly, the Spanish study looked at samples analysed from different donors over the time period 1998–2013 and saw that decreases were observed for Pb, Cd and Sn levels and that Mn and Cr levels were increasing in human lung tissue samples [14].

5. Conclusion

Whilst this study reports concentrations of 48 elements in lung samples, some elements being reported for the first time, the limitation of the demographics of the study must be borne in mind. The lung samples analysed in this study, whilst deemed unaffected tissue, were nevertheless taken from patient where 93% of the samples were removed from patients with neoplasia. With these limitations borne in mind the study has defined a background range from a group of patients across a wide range of elements that is generally consistent with previous studies. The simple protocol used in this study has helped to produce valid data for assessing future cases.

The main findings from this study show that males have higher concentrations of Cr, Ni, Au and Be and lower concentrations of Co,

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Sn, W and In than females. Only Cd was seen to be higher in smokers and there was less variation in essential elements like Cu, Fe and Br.

Thirteen elements showed a year on year increase in the elemental composition found in the lung samples, with Be having the largest increase. There were higher concentrations of Pt, Bi and Mo in those samples where the patients had undergone chemotherapy.

Those who stated they had been occupationally exposed showed significantly higher concentrations of Fe, Mn, Ti, Ta and In; whilst there was no significant different in the concentrations of Cr, Ni and V. Whilst not exhaustive it is hoped that the information and the elemental concentrations reported in study will provide useful comparative data for future lung sample analysis.

We would recommend that any future studies use a similar analytical protocol for producing reproducible and reliable data.

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