4. Elemental analysis

4.1 Determination of trace element concentration by means of INAA and GFAAS

In studies carried out on rats, element analytical methods were combined with biochemical separation procedures. Subcellular separation of the tissues into nuclear, mitochondrial, microsomal and cytosolic fractions was achieved by differential ultracentrifugation. Instrumental neutron activation analysis (INAA) was used to determine the concentrations of several trace elements in the lung, trachea, kidney, liver, heart and blood and in subcellular fraction of the lung and trachea. Graphite furnace atomic absorption spectrometry (GFAAS) was employed for the determination of copper and nickel in the mitochondrial and cytosolic fractions of the lung and trachea.

4.1.1 **Determination of trace element concentrations in the tissues of selenium - sufficient and selenium - deficient rats**

In these experiments INAA has been used to determine the concentrations of selenium, arsenic, zinc, iron, manganese and rubidium in the lung, trachea, liver, kidney and blood of the rat and to investigate the effects of changes in the selenium status on their distribution. The tissues were obtained from rats which had been fed either a low selenium diet (about 10 µg Se/kg) for several generations or a selenium-sufficient diet (which was produced from the basal diet by adding 300 µg Se/kg as sodium selenite). The elemental concentrations in the whole tissues and subcellular fractions were related to the dry weight of the samples. The dry weight of the Tris buffer used in the preparation of the subcellular fraction was subtracted before calculation.

The concentrations found in 5 rats fed the low selenium diet and in 5 animals supplied with adequate amounts of selenium were calculated as mean ± standard deviation. Figure 4-1 shows the data for the six elements.

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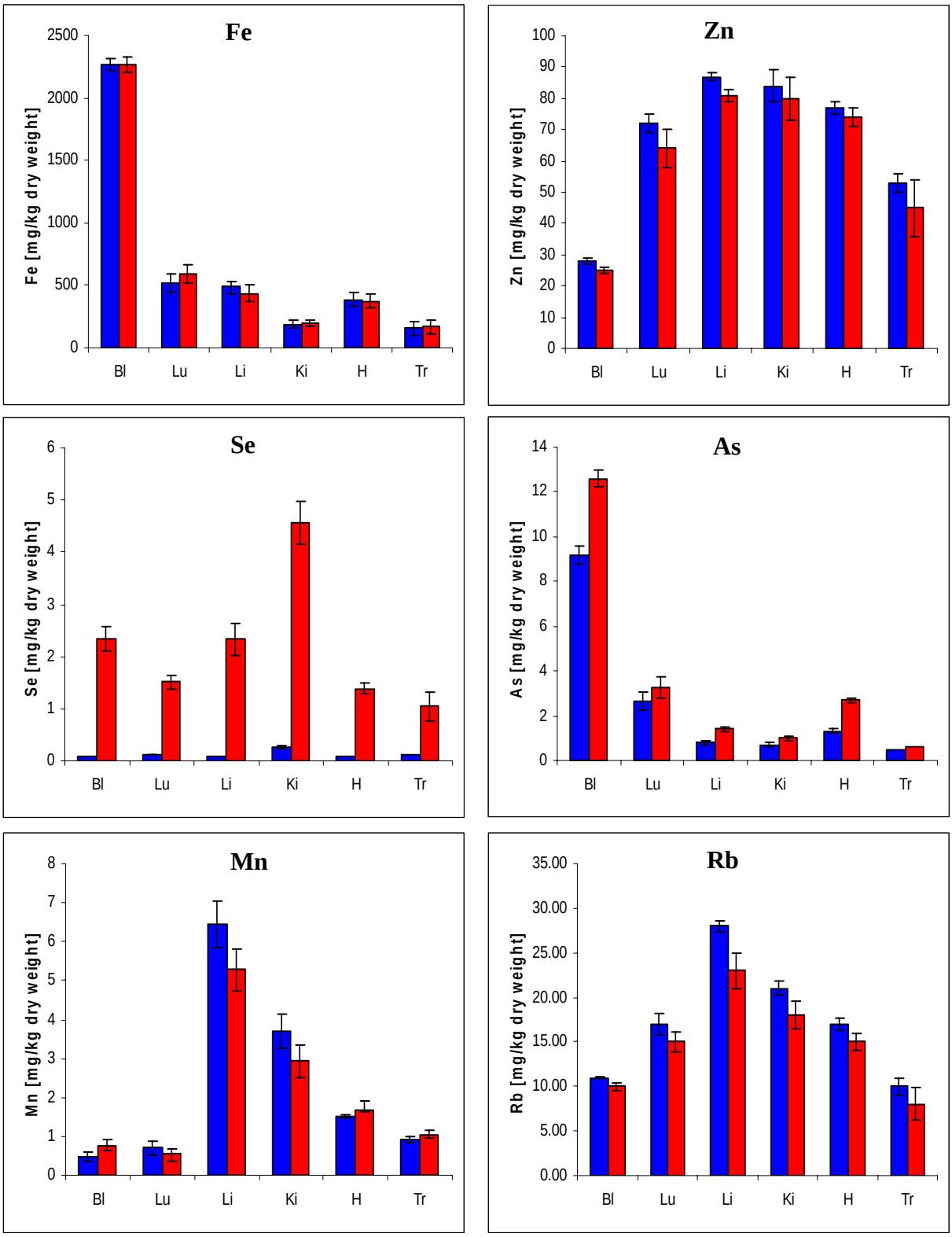

Figure 4-1 Concentrations of iron, zinc, selenium, arsenic, manganese and rubidium (mean ± SD, n = 5) in blood, lung, liver, kidney, heart and trachea of rats fed either a low selenium diet or a selenium-adequate diet.

The elements were found to be distributed inhomogeneously among the body compartments. As was to be expected, the selenium concentrations in the tissues of the rats fed the low selenium diet were considerably lower than those in the adequate group. Selenium, zinc, manganese and rubidium were mainly present in the liver and kidney in both groups. The highest concentrations of arsenic and iron were found in the blood. With the exception of the manganese and arsenic concentrations in the blood and the arsenic concentration in the heart, which were significantly higher in the selenium-adequate animals ($P < 0.01$), no significant differences between the two groups were observed. It is therefore very likely that pathological changes found in selenium deficiency are not related to secondary effects caused by changes in the tissue concentrations of these elements.

4.1.2 Distribution of the trace elements in the lung and trachea and in their subcellular fractions

Further experiments were carried out to determine the concentrations of selenium, arsenic, iron, manganese, zinc, cobalt, rubidium, chromium, potassium and sodium in the lung and trachea and in their subcellular fractions of rats fed either a selenium-deficient or a selenium-sufficient diet. The concentrations found in the experimental animals were calculated as mean ± standard deviation. The results are shown in the Tables 4-1, 4-2, and 4-3 for the lung and Tables 4-4, 4-5, and 4-6 for the trachea.

Table 4-1 Concentrations of arsenic, cobalt, chromium, iron, manganese, rubidium, selenium, zinc, potassium and sodium (mean ± SD, n = 5) in the lung of rats fed either a selenium-deficient or a selenium-adequate diet

Elements	Element concentration [mg kg^{-1} dry mass]	
	Selenium-deficient group	Selenium-sufficient group
As	2.66 ± 0.40	3.26 ± 0.49
Co	0.043 ± 0.014	0.048 ± 0.018
Cr	< 0.8	< 0.8
Fe	520 ± 75	592 ± 76
Mn	0.71 ± 0.18	0.55 ±0.12
Rb	17.0 ± 1.2	15.0 ± 1.1
Se	0.06 ± 0.01	1.52 ± 0.13
Zn	72 ± 3	64 ± 6
K	12760 ± 700	11580 ± 735
Na	6440 ± 516	6880 ± 397

Table 4-2 Concentrations of arsenic, cobalt, chromium, iron, rubidium, selenium, zinc, potassium and sodium (mean ± SD, n=5) in the supernatant, nuclei, mitochondria, microsomes and cytosol of the lung of rats fed a selenium-deficient diet

Elements	Element concentration [mg kg^{-1} dry mass] in selenium-deficient group				
	Supernatant 200g	Nuclei	Mitochondria	Microsomes	Cytosol
As	0.99 ± 0.24	0.58 ± 0.28	0.74 ± 0.22	0.70 ± 0.21	1.27 ± 0.20
Co	0.192 ± 0.009	0.432 ± 0.078	0.256 ± 0.050	0.248 ± 0.080	0.155 ± 0.072
Cr	1.14 ± 0.21	5.92 ± 1.38	< 2.4	< 2.4	< 1.1
Fe	1607 ± 226	987 ± 164	1730 ± 116	4593 ± 124	1651 ± 117
Rb	53.0 ± 5.8	12.3 ± 1.9	49.8 ± 5.6	40.7 ± 4.1	73.6 ± 6.8
Se	0.44 ± 0.10	0.84 ± 0.17	0.68 ± 0.17	0.89 ± 0.10	0.35 ± 0,.07
Zn	304 ± 14	466 ± 33	427 ± 6	567 ± 40	216 ± 7
K	3507 ± 150	514 ± 83	2840 ± 265	2205 ± 301	4210 ± 285
Na	1713 ± 60	273 ± 56	1622 ± 97	1473 ± 78	2430 ± 163

Table 4-3 Concentrations of arsenic, cobalt, chromium, iron, rubidium, selenium, zinc, potassium and sodium (mean ± SD, n = 5) in the supernatant, nuclei, mitochondria, microsomes and cytosol of the lung of rats fed a selenium-adequate diet

Elements	Element concentration [mg kg^{-1} dry mass] in selenium-sufficient group				
	Supernatant 200g	Nuclei	Mitochondria	Microsomes	Cytosol
As	0.96 ± 0.08	0.45 ± 0.10	0.80 ± 0.15	0.71 ± 0.10	1.28 ± 0.11
Co	0.092 ± 0.007	0.354 ± 0.033	0.196 ± 0.025	0.272 ± 0.058	0.096 ± 0.018
Cr	1.00 ± 0.32	2.6 ± 0.38	< 1.8	< 1	< 0.8
Fe	1573 ± 146	805 ± 128	1707 ± 172	7866 ± 1026	1585 ± 42
Rb	41.2 ± 5.3	10.4 ± 4.7	38.2 ± 2.2	31.2 ± 2.9	48.4 ± 4.4
Se	4.56 ± 0.25	6.38 ± 0.35	5.96 ± 0.60	7.94 ± 0.32	3.90 ± 0.41
Zn	263 ± 18	482 ± 10	389 ± 9	552 ± 30	175 ± 15
K	3210 ±320	765 ± 137	2820 ± 356	2131 ± 168	3721 ± 363
Na	1756 ± 184	434 ±74	1560 ± 166	1458 ± 115	2059 ± 216

As was to be expected, the selenium concentration in the lungs of the deficient animals was lower than that in the rats fed the selenium-sufficient diet. With the other elements no significant differences between the two groups were observed. All elements investigated were distributed inhomogeneously among the subcellular fractions. All elements except chromium were mainly present in the mitochondria and microsomes. Interestingly, the chromium concentration was found to be higher in the nuclei. The cytosol contained the largest concentration of arsenic and rubidium, while the highest contents of zinc, iron and selenium were in the microsomal fraction. In all subcellular fractions of the selenium-deficient animals more zinc, iron, and cobalt but fewer selenium and arsenic was found than in the whole organ. The samples were washed and centrifuged at low speed to remove red blood cells, membranes and connective tissue not homogenized. In this way the contamination of selenium and arsenic could be avoided, as those two elements are mostly present in the blood. Of interest is the very high concentration of iron in the subcellular fractions, as it is also one of the main elements present in the blood. A possible explanation for this could be an enrichment of iron-containing substances. The microsomal fraction contains catalase, one of the iron-binding enzymes.

Table 4-4 Concentrations of arsenic, cobalt, chromium, iron, rubidium, selenium, zinc, potassium and sodium (mean ± SD, n = 5) in the trachea of rats fed either a selenium-deficient or a selenium-sufficient diet

Elements	Element concentration [mg kg^{-1} dry weight]	
	Selenium-deficient group	Selenium-sufficient group
As	< 1	< 1
Co	0.075 ± 0.026	0.097 ± 0.036
Cr	< 0.6	< 0.6
Fe	159 ± 30	168 ± 35
Mn	0.93 ± 0.08	1.02 ± 0.13
Rb	10.0 ± 1.0	8.0 ± 1.8
Se	0.13 ± 0.03	1.04 ± 0.28
Zn	53 ± 3	45 ± 9
K	7120 ± 306	7966 ± 464
Na	7680 ± 387	6675 ± 742

Table 4-5 Concentrations of arsenic, cobalt, chromium, iron, rubidium, selenium, zinc, potassium and sodium (mean ± SD, n = 5) in the supernatant, nuclear, mitochondria, microsomes and cytosol of the trachea of rats fed a selenium-deficient diet

Elements	Element concentration [mg kg^{-1} dry mass] in selenium-deficient group				
	Supernatant 200 g	Nuclei	Mitochondria	Microsomes	Cytosol
As	< 2	< 1	< 9	< 1	< 7
Co	0.192 ± 0.009	0.432 ± 0.078	0.256 ± 0.050	0.248 ± 0.080	0.155 ± 0.072
Cr	< 2	4.9 ± 1.4	< 1	< 2	< 2
Fe	225 ± 35	45 ± 4	141 ± 32	966.7 ± 73.9	199 ± 11
Rb	10.3 ± 1.6	< 5	< 5	< 5	17.7 ± 2.1
Se	0.155 ± 0.015	< 0.1	< 0.1	< 0.5	0.16 ± 0.04
Zn	66 ± 5	140 ± 22	39.5 ± 2.6	76 ± 7	40.5 ± 3.3
K	890 ± 80	53 ± 15	162.5 ± 15.3	217.5 ± 64.0	1506 ± 175
Na	1100 ± 260	133 ± 6	177.5 ± 25.2	245 ± 58	1704 ± 149

Table 4-6 Concentrations of arsenic, cobalt, chromium, iron, rubidium, selenium, zinc, potassium and sodium (mean ± SD, n = 5) in the supernatant, nuclear, mitochondria, microsomes and cytosol of the trachea of rats fed a selenium-sufficient diet

Elements	Element concentration [mg kg^{-1} dry mass] in selenium-sufficient diet				
	Supernatant 200 g	Nuclei	Mitochondria	Microsomes	Cytosol
As	< 0.7	< 0.1	< 0.9	< 0.2	< 1.2
Co	0.192 ± 0.007	0.354 ± 0.033	0.196 ± 0.025	0.272 ± 0.058	0.096 ± 0.018
Cr	< 2	2.4 ± 0.98	< 1	< 2	< 2
Fe	285.6 ± 76.8	41 ± 4	217 ± 36	1140 ± 106	183 ± 14
Rb	9.8 ± 1.7	< 7	< 5	< 7	16.6 ± 2.8
Se	1.04 ± 0.16	0.38 ± 0.05	0.82 ± 0.11	1.09 ± 0.16	1.45 ± 0.27
Zn	60.6 ± 15.6	104 ± 28	45.6 ± 5	74 ± 5	43 ± 4
K	870 ± 78	94 ± 37	280 ± 52	220 ± 31	1484 ± 3237
Na	948 ± 99	172 ± 23	308 ± 166	248 ± 47	1765 ± 173

In the trachea too, only the selenium concentrations of the deficient animals were lower than those in the rats fed the selenium-adequate diet. With the other elements no significant differences between the two groups were observed. All elements investigated were distributed inhomogeneously among the subcellular fractions. With most of these elements the highest concentrations were found in the nuclei and microsomes. Chromium, cobalt and zinc were enriched in the nuclei, and selenium and iron were mainly present in the microsomes.

4.1.1.3 Distribution of copper and nickel in cytosolic and mitochondrial fractions of the lung, and trachea

The copper and nickel concentrations found in the cytosolic and mitochondrial fraction of lung and trachea of selenium-deficient and selenium-sufficient rats by means of GFAAS (as described in the section 2.2.1.3) are shown in the Tables 4-7 and 4-8.

Table 4-7 Concentration of copper (mean ± SD, n = 4) in the mitochondria and cytosol of the lung and trachea of rats fed either a selenium-sufficient or a selenium-deficient diet.

Tissue	Cu [µg g^{-1}dry mass]			
	Selenium-sufficient diet		Selenium-deficient diet	
	Mitochondria	Cytosol	Mitochondria	Cytosol
Lung	216 ± 38	159 ± 25	232 ± 32	173 ± 26
Trachea	37.5 ± 8.5	48.4 ± 7.5	38.6 ± 8.6	74 ± 12

Table 4-8 Concentration of nickel in the mitochondria and cytosol of the lung and trachea of rats fed either a selenium-sufficient or a selenium-deficient diet (n = 1).

Tissue	Ni [µg g^{-1}dry mass]			
	Selenium-sufficient diet		Selenium-deficient diet	
	Mitochondria	Cytosol	Mitochondria	Cytosol
Lung	12.0	13.1	7.8	11.0
Trachea	38.9	34.6	6.0	3.9

Interestingly, the copper concentration was found to be higher in the lung. This element was mainly present in the mitochondria of the lung, but not in the of the trachea, where it was enriched in the cytosol. There were no distinct differences between the two groups. Nickel was distributed homogeneously between these subcellular fractions. Remarkable differences in nickel concentration between selenium-deficient and selenium-sufficient fractions of the trachea were observed. Much less nickel in the selenium-deficient animals was found.

4.2 Summary

Trace elements play an important biochemical and physiological function in the organism which elated also to their concentrations and chemical speciation in the organs. The first important step in the metallomics is therefore the determination of the concentration of the trace elements present in the tissues and their distribution among cellular compartments.

This series of experiments was carried out on the tissues of the respiratory tract, their subcellular fractions and some other important organs (liver, kidney, heart) of the rat to

analyze the trace elements presence and to obtain information on their possible sites of action within the different cell compartments.

The use of the different microtechniques of element analysis allowed the quantitative determination of several trace elements such as arsenic, chromium, cobalt, copper, iron, manganese, nickel, rubidium, selenium and zinc.

INAA, as a non-destructive method, allowed the quantitative determination of a larger number of trace elements in small amounts of biological material by irradiating the samples repeatedly at different irradiation conditions. This method was used to determine the concentrations of arsenic, chromium, cobalt, iron, manganese, rubidium, selenium and zinc in the rat lung and trachea and their distribution among the subcellular compartments of these tissues. With manganese the limit of detection was not low enough to detect it in the subcellular fractions. It is not possible to use this method for the determination of copper and nickel. Therefore GFAAS was applied.

In the same way the distribution of the arsenic iron, manganese, rubidium, selenium and zinc in the selenium-deficient and selenium-sufficient organs were studied. These elements were distributed inhomogenously among the body compartments. Except for the manganese and arsenic concentrations in blood, no significant differences between these two groups were observed. It is therefore very likely that pathological changes found in selenium deficiency are not related to secondary effects caused by changes in the tissue concentrations of these essential elements.

Of special interest was the high concentration of arsenic which in the lung exceeded that in the liver by a factor of 3 and the fact of the novel finding that both arsenic and nickel were present in relatively high amounts in the subcellular compartments of the lung and trachea.

4.3 Speciation analysis of the lung and trachea cytosols

Monitoring elemental species requires analytical technology with sufficient selectivity and sensitivity to resolve and quantitate the individual species at ultratrace levels. One of the most promising technologies for meeting those requirements is high performance liquid chromatography (HPLC) linked to inductively coupled plasma mass spectrometry (ICP MS). This technique is very useful in carrying out automated speciation analysis to obtain information on the trace elements bound to biomolecules in the cytosolic fraction [125, 126].

4.3.1 Trace element-containing proteins in the lung and trachea cytosols

After fractionation of the cytosolic proteins by size exclusion chromatography (SEC), inductively coupled plasma mass spectrometry (ICP-MS) was employed to determine the trace element-containing proteins on-line in the eluate. The separation method permits the investigation of the native metal composition of the trace element-containing proteins. The distribution profiles of copper, manganese, molybdenum, cobalt, arsenic, selenium, zinc, iodine, nickel, cadmium, iron and silver after chromatographic separation of rat lung and trachea cytosols are shown in Figures: 4-2, 4-3, 4-4, 4-5 and 4-6.

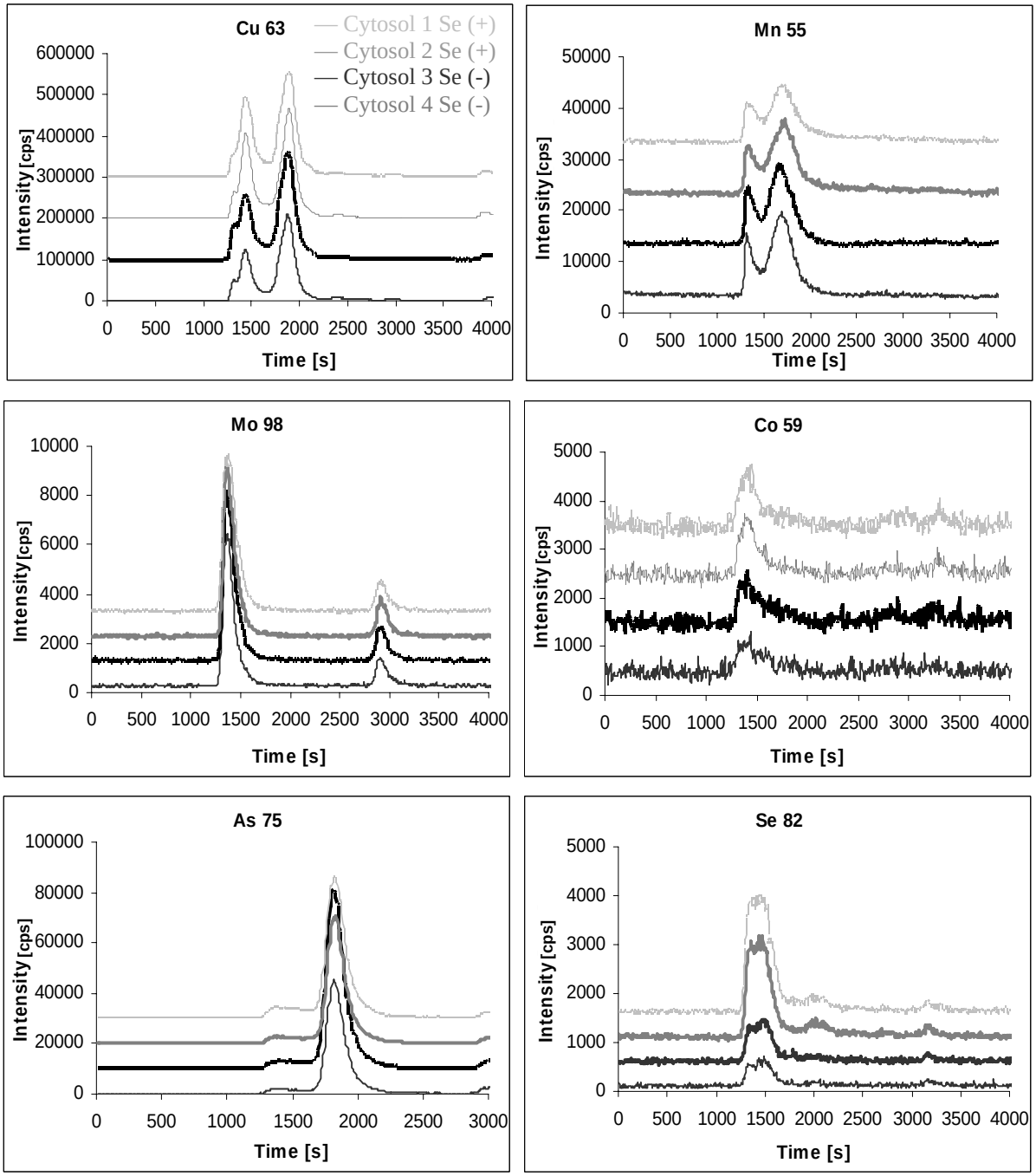

Figure 4-2 Distribution profiles of copper, manganese, molybdenum, cobalt, arsenic and selenium after chromatographic separation of trachea cytosols of two rats fed a selenium-deficient Se (-) diet or a selenium-sufficient Se (+) diet

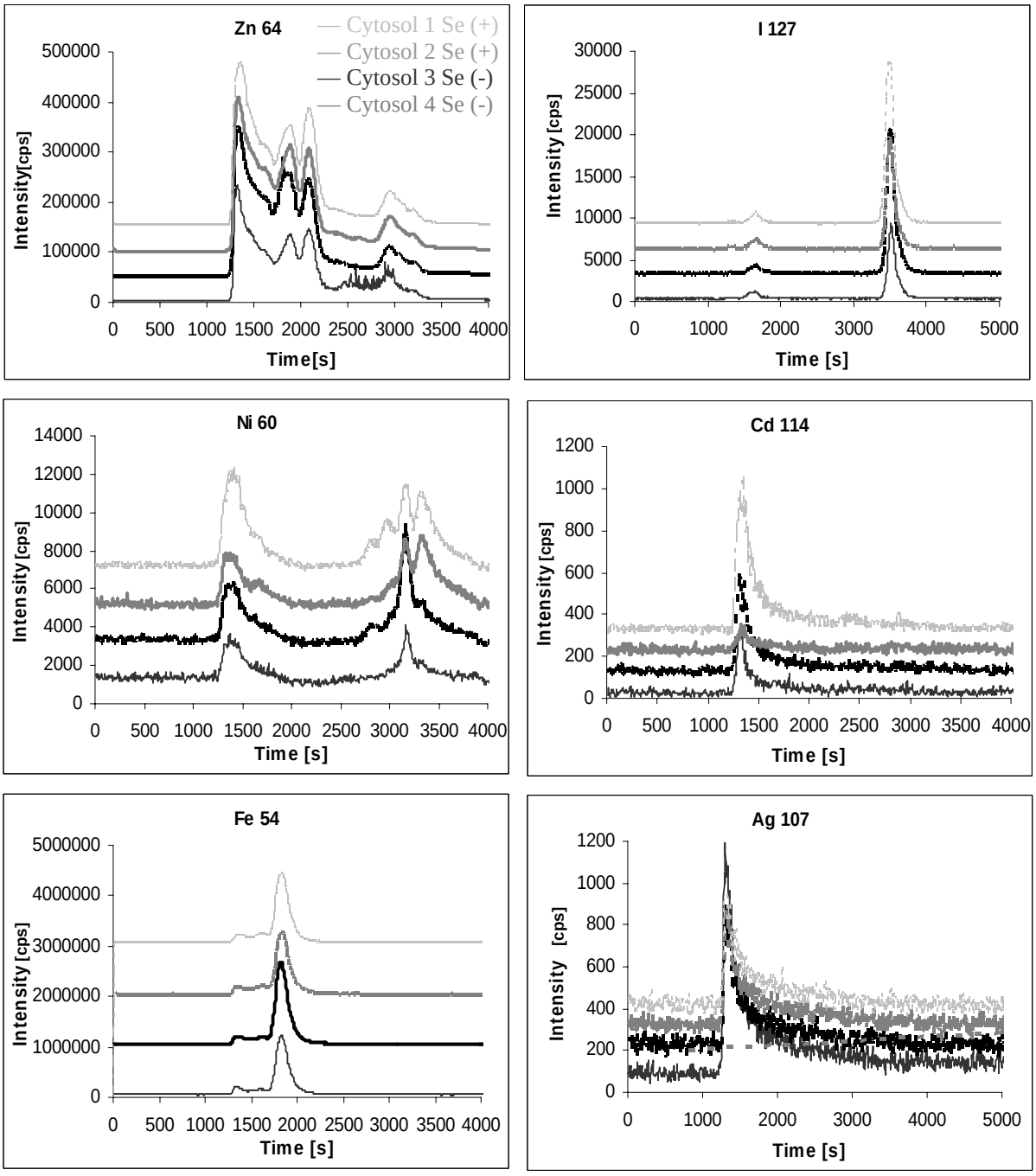

Figure 4-3 Distribution profiles of zinc, iodine, nickel, cadmium, iron and silver after chromatographic separation of lung cytosols of two rats fed a selenium-deficient Se (-) diet or a selenium-sufficient Se (+) diet

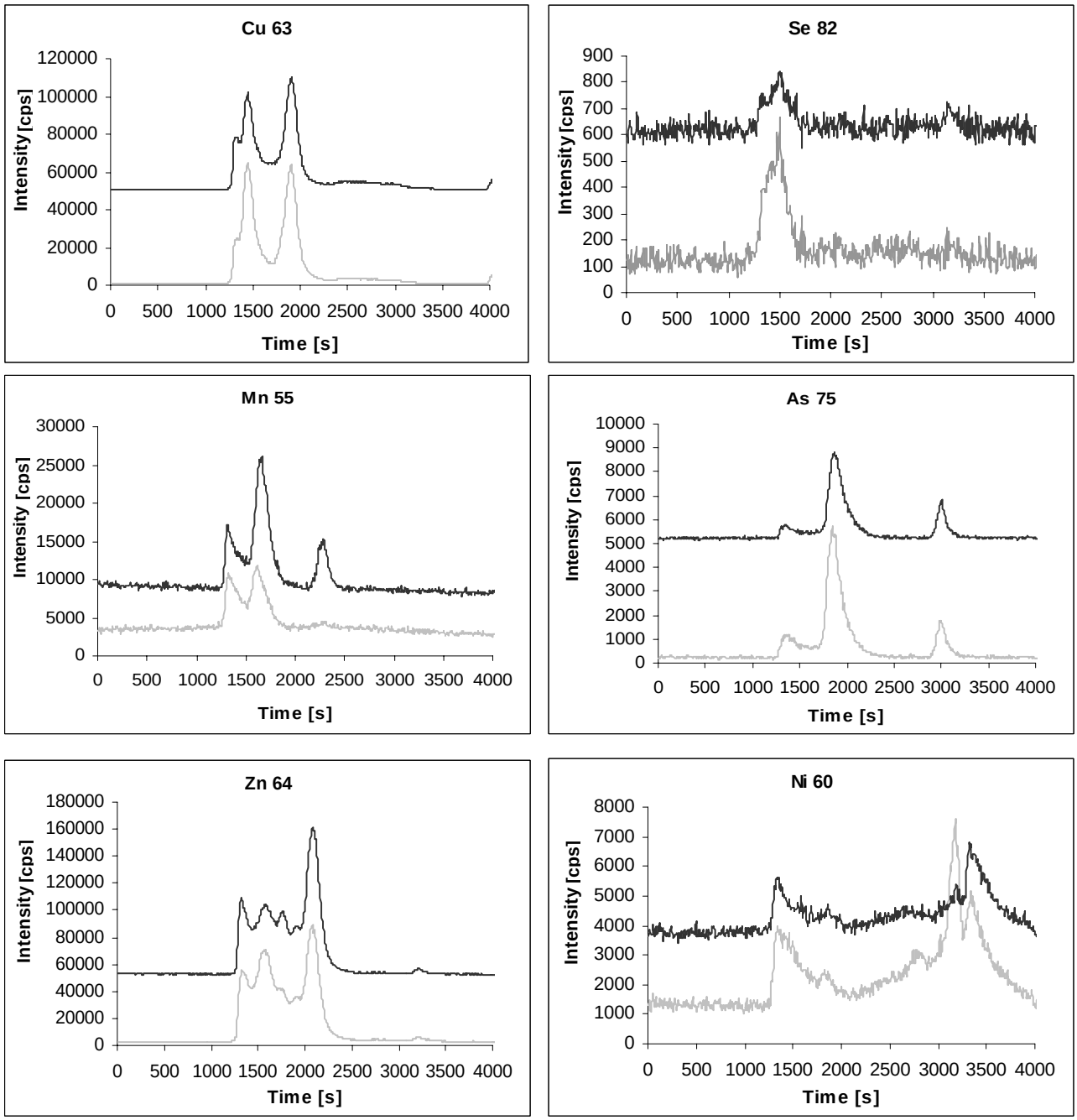

Figure 4-4 Distribution profiles of copper, selenium, manganese, arsenic, zinc and nickel after chromatographic separation of trachea cytosols of two rats fed a selenium-deficient Se (-) diet or a selenium-sufficient Se (+) diet

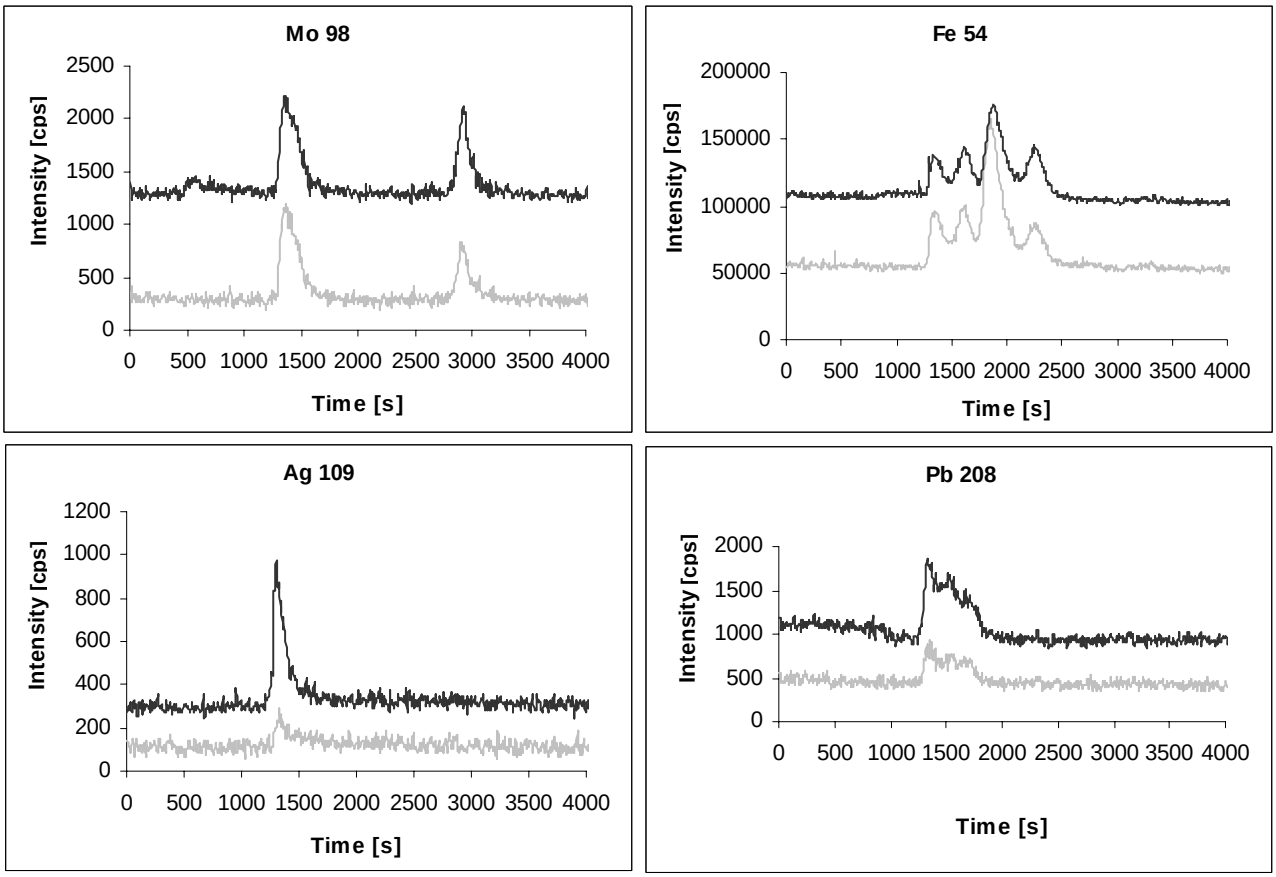

Figure 4-5 Distribution profiles of molybdenum, iron, silver and lead after chromatographic separation of trachea cytosols of two rats fed a selenium-deficient Se (-) diet or a selenium-sufficient Se (+) diet

The chromatographic fractionation of the proteins present in the liquid cytosolic phase of the lung and trachea cells and the determination of the element distribution in the separated protein fractions by means of ICP MS indicated that all the elements investigated were bound to proteins. These profiles and also those of iron, copper, manganese, cadmium, silver, molybdenum and cobalt showed that they were associated with various cytosolic proteins.

The distribution profiles of zinc, copper and manganese after chromatographic separations of lung and trachea cytosols were comparison with cytosols obtained from human lung epithelium and human trachea fibroblasts (see Figure 4-6). In all samples the same element distribution profile in the separated proteins was observed. The separated fractions differ only in the peak intensity.

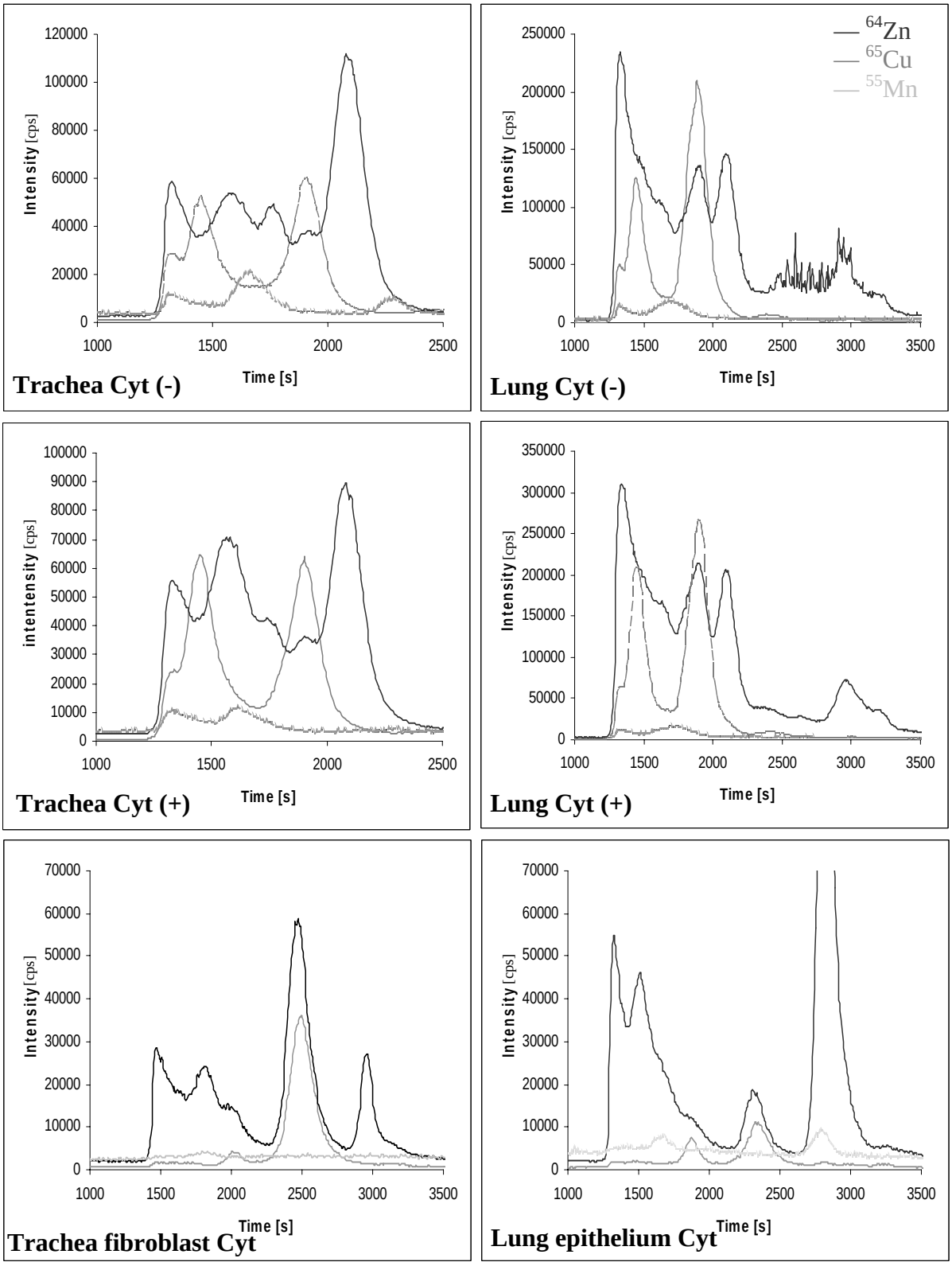

Figure 4-6 Distribution profiles of zinc, copper, and manganese after chromatographic separation of trachea, lung, human tracheal fibroblast, human lung epithelium cytosols from two rats fed a selenium-deficient or a selenium-sufficient diet.

4.3.2 Summary

First information on the presence of protein-bound forms of arsenic, cadmium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium and zinc in the rat lung and trachea cytosol was obtained by chromatographic separation of the cytosolic proteins and determination of the distribution of these elements among the separated proteins. It could be assumed that with the exception of rubidium all the elements investigated are attached to proteins. Copper, iron, manganese, selenium and zinc are known to be essential constituents of redox-active enzymes, but there is the possibility that in the lung these elements may also be contained in further proteins not yet identified. Interestingly, arsenic and nickel were also found to be protein-bound. So far nothing is known about interactions between these elements and lung proteins. Further studies were therefore carried out to investigate more closely the biological functions of these elements and especially the role of arsenic in the lung and trachea.

4.4 Studies on arsenic metabolites in the cytosols by SEC-ICP MS

In the following experiments the human cell lines: A549 – human lung epithelium, CRL - human tracheal fibroblasts and HepG2 - human hepatocytes were exposed to an arsenic solution in a concentration that did not lead to toxic effects during the exposure. The cytosolic fraction of those cells was obtained, and together with culture media, analyzed by HPLC-ICP-MS. The combination of these two methods allowed the detection of the trace element-containing proteins. In this way the metabolic pathway of arsenic in those cells and also the effect of arsenic on the expression of other trace element-binding proteins (e.g. Cu- or Zn-proteins) could be examined.

4.4.1 Choosing the appropriate arsenic concentration

The toxicity of the arsenicals was examined in the human lung epithelial cells A549 and human tracheal fibroblasts CRL7422. Cells were exposed to trivalent and pentavalent inorganic arsenic (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 25 or 50 µM) for 24 hours. The cell viability was determined by the MTT assay during the exposure, as described in the section 2.2.5.6. The MTT- test results are shown in Figure 4-6.

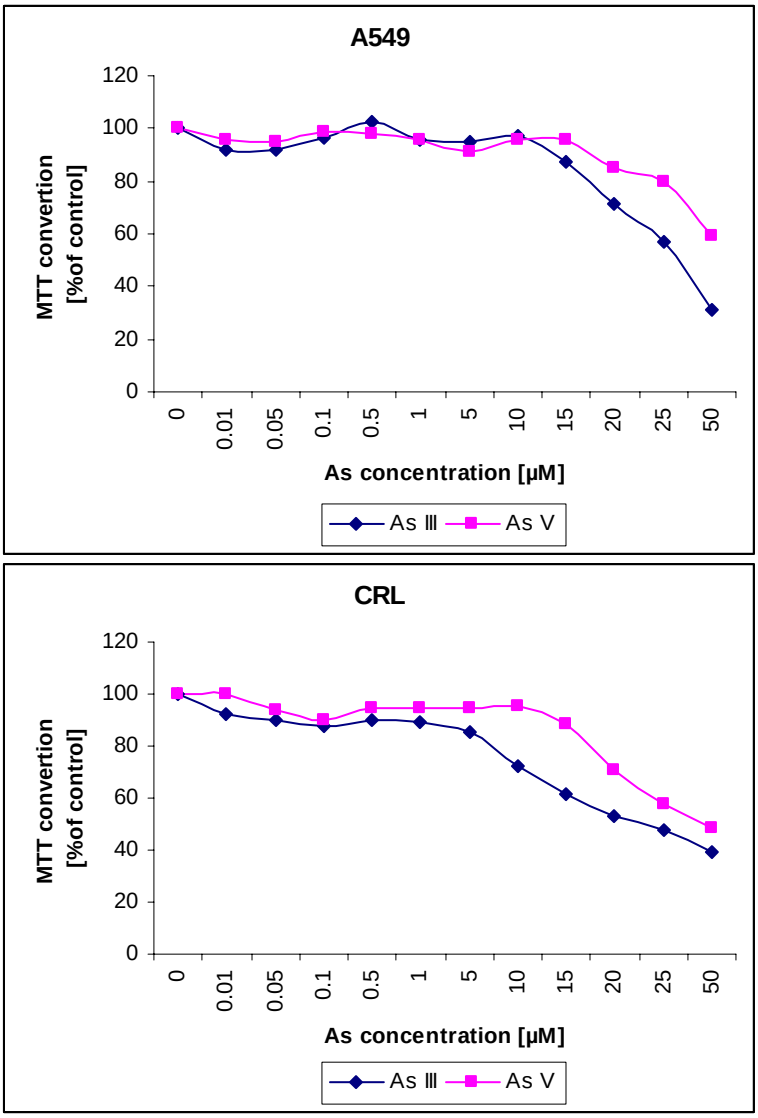

Figure 4-6 Changes in the cell viability of the lung epithelia (A549) and human tracheal fibroblasts (CRL7422) exposed to different concentrations of trivalent and pentavalent arsenicals. The viability of the cells was determined by the MTT assay.

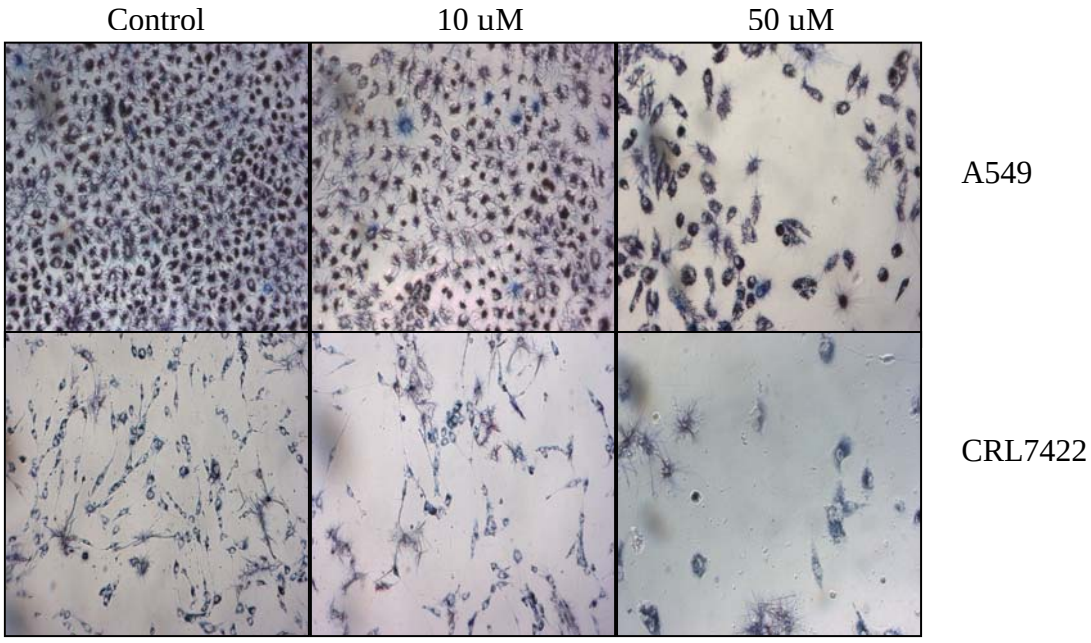

Figure 4-7 Photos of the human lung epithelia (A549) and human tracheal fibroblasts (CRL7422) exposed to pentavalent arsenical 3 hours after MTT addition; control cells without arsenical - left, cells treated with 10 µM arsenical- middle, and cells treated with 50 µM arsenical- right.

The exposure of the cells to solutions in concentrations from 0.01 to 20 µM of inorganic arsenicals had similar effects. It decreased the rate of MTT conversion by 20% to 25% without apparent changes in the cell morphology. The microscopic observation of the cells after treatment with pentavalent arsenical is shown in Figure 4-7. In contrast, exposure to an arsenical concentration of 20 µM resulted in morphological changes and a decrease in the rate of MTT conversion by 40% to 60%. The 10 µM solution of pentavalent arsenical was chosen for further experiments.

4.4.2 Separation of the cell cytosols and culture media by HPLC MS

After platting (1.5×10^4 – 4×10^4cells / cm^2) the human lung epithelial cells A549, rat hepatocytes HepG2, and human tracheal fibroblasts CRL7422 cells were cultured for 24 hours, after addition of the arsenic solution (final concentration 10µM). The cells were incubated for another 24 hours. Simultaneously the experiment without arsenic application was carried out. The culture medium was then removed and the cell monolayer was rinsed with sterile phosphate-buffer saline (PBS) and harvested by scraping. The cells were homogenized in 20 mM Tris-HNO_3 pH 7.4 by sonification. The homogenates were parted into

pellet and cytosolic fraction by centrifugation for one hour at 100000×g. The cytosolic proteins and the proteins present in the culture medium were separated by HPLC according to their native molecular masses using Superdex 75PG column (see section 2.2.5.7). The elements attached to them were then analyzed by ICP-MS. The element profiles are shown in the Figures 4-8 to 4-13.

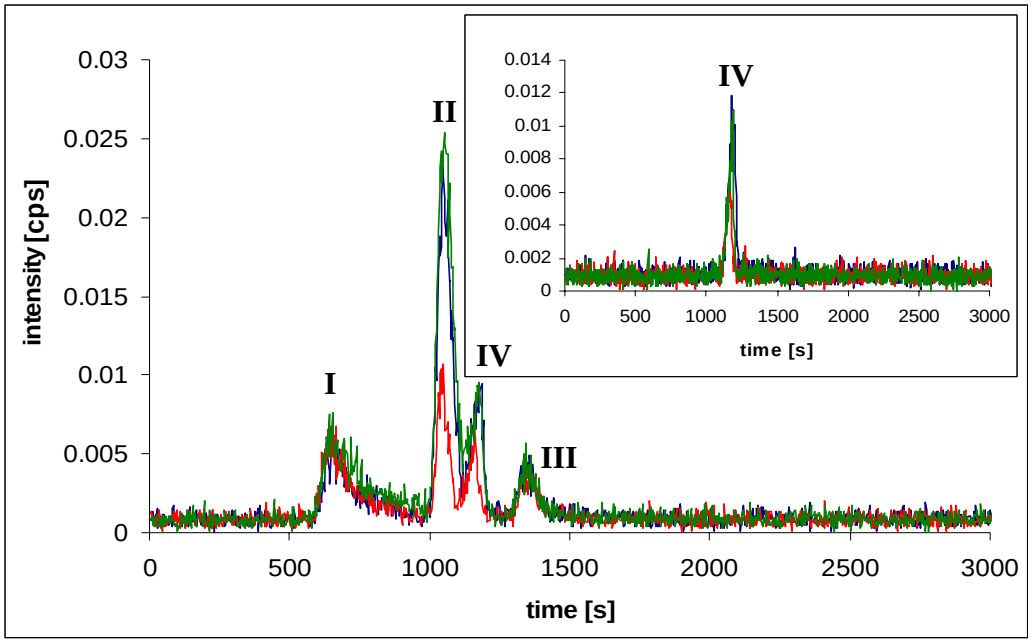

Figure 4-8 Chromatographic arsenic profile of cells cytosols treated with arsenical (large figure) and of untreated cytosols (small figure); — A549, — CRL, — HepG2

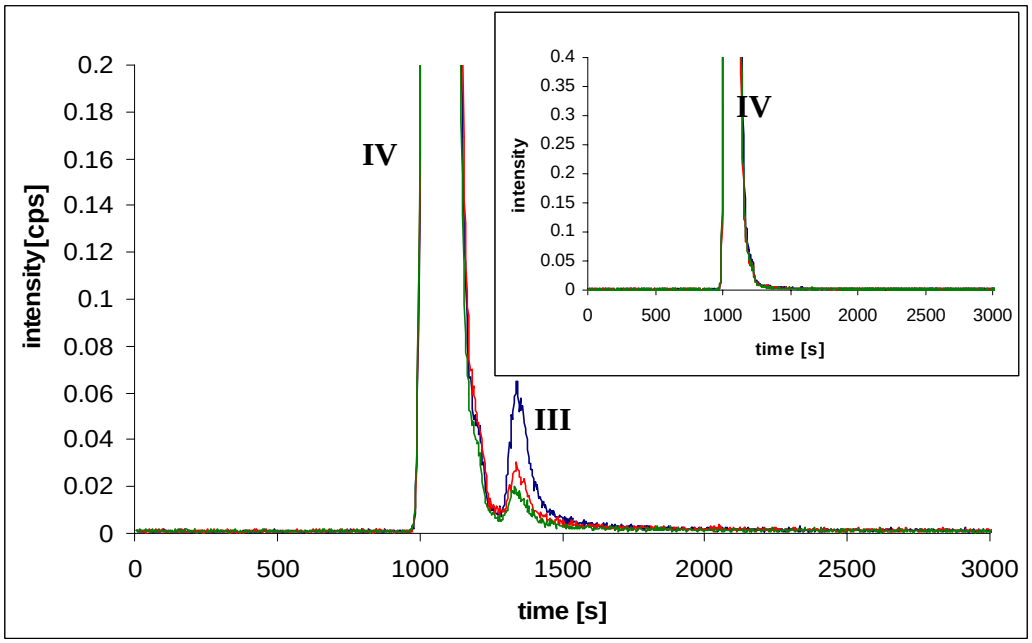

Figure 4-9 Chromatographic arsenic profile of the cell media treated with arsenical (large figure) and of the pure media (small figure); — A549, — CRL, — HepG2

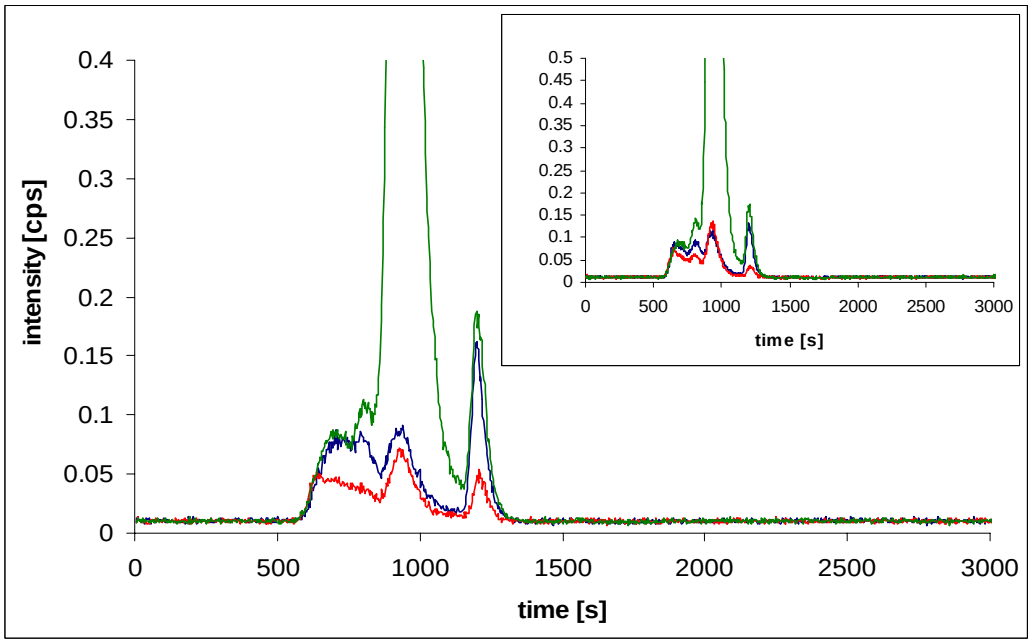

Figure 4-10 Chromatographic copper profile of the cell cytosols treated with arsenical (large figure) and of the untreated cytosols (small figure); —A549, —CRL, —HepG2

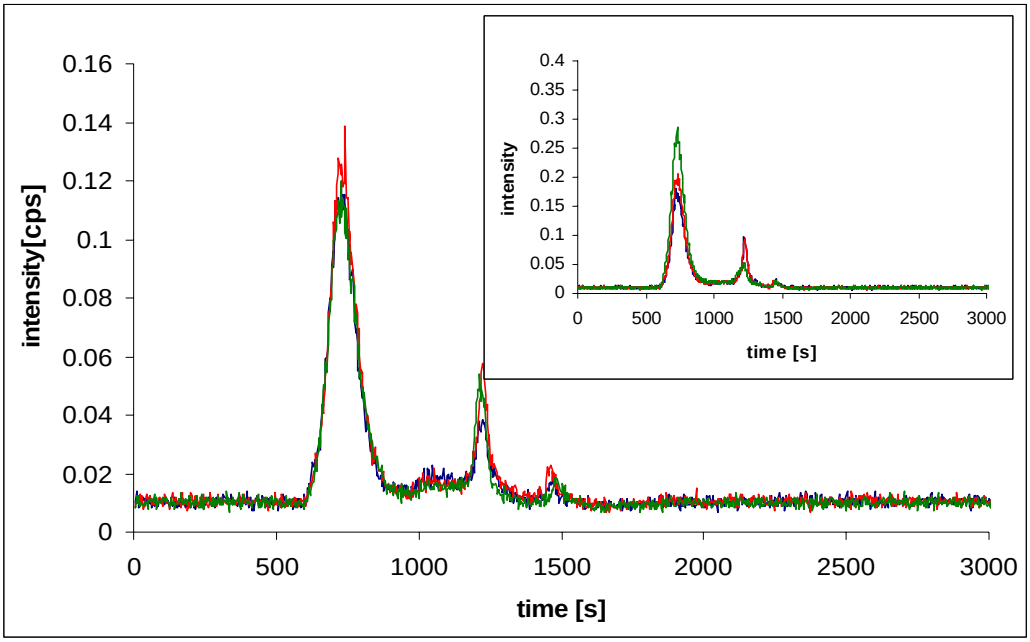

Figure 4-11 Chromatographic copper profile of the cell media treated with arsenical (large figure) and of the pure media (small figure); — A549, — CRL, — HepG2

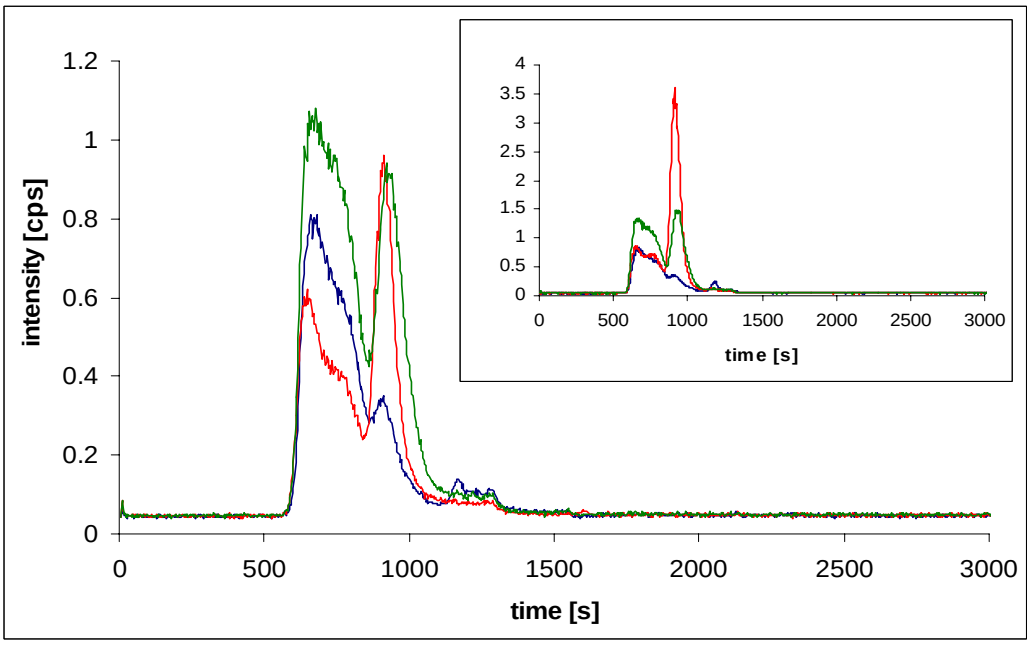

Figure 4-12 Chromatographic zinc profile of the cell cytosols treated with arsenical (large figure) and of the untreated cytosols (small figure); —A549, —CRL, —HepG2

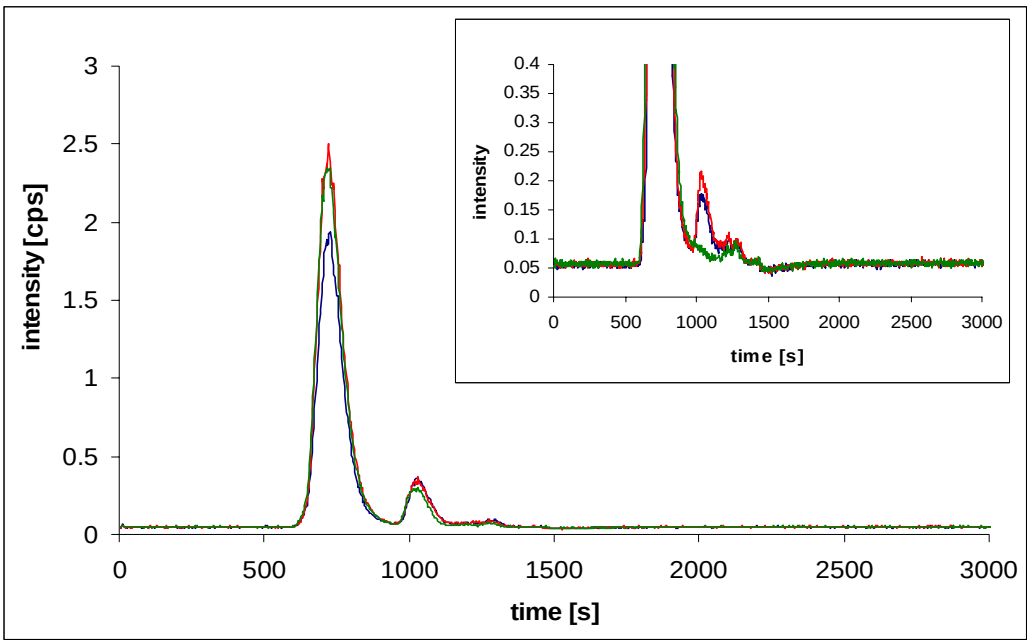

Figure 4-13 Chromatographic zinc profile of the cell media treated with arsenical (large figure) and of the pure media (small figure); — A549, — CRL, — HepG2

In the chromatographic arsenic profiles of cell cytosols after treatment with arsenic four peaks were detected (I, II, III, IV), while in the profiles of untreated cells only one peak (IV) was observed. The peak IV stemmed from the $^{40}Ar^{35}Cl$ interference. The other peaks present in the three fractions had molecular masses of: I > 80 kDa, II ~ 8 kDa, III < 3 kDa. In the cytosols of

untreated cells no arsenic - containing species were detected. For more information on the metabolism of the treated und untreated cells the culture media were also analyzed. As a control a pure medium were taken. The peak IV was detected in the pure medium and also in the medium after cell growth and arsenic treatment, while the peak III was present only in the medium from the treated cells. This suggested that this fraction could contain arsenic metabolites, which were excreted by the cells. The molecular mass of fraction III is < 3 kDa suggesting it might contain the methylated arsenic metabolites. The changes in metabolism of the other trace metals after treatment with arsenic were also investigated. In this case the chromatographic element profiles from the cell cytosols and culture media treated and untreated with arsenic were monitored. In all cultured media from cells treated with arsenic a decreased intensity of copper, zinc, iron and manganese was observed, that connoted of concentration reducing of those elements after cell growth.

4.5 Comparison of the element binding pattern in cytosols of human cells treated with selenium

After platting ($1.5 \times 10^4 - 4 \times 10^4$ cells / cm^2) the human lung epithelial cells A549, rat hepatocytes HepG2, and human tracheal fibroblasts CRL7422 cells were cultured for 24 hours after adding of selenium solution (final concentration 10µM). The cells were incubated for another 24 hours. Simultaneously the experiment without selenium application was carried out. The treatment of the samples, the protein separation and the element analysis was performed as described in the section 4.4.2.

The element profiles of selenium, copper, nickel and zinc are shown in the Figures 4-14 to 4-25.

The chromatographic selenium profiles obtained from cytosols of selenium-treated cells differ from those of untreated cells. In the formed profile two peaks were observed, one in the area of high molecular masses (retention time ~ 730 s, the corresponding molecular mass ~ 60 kDa) and the other in the small mass range (retention time ~ 2300 s, the corresponding molecular mass <3 kDa). In the chromatograms from the untreated cells the first peak appeared after 730 s, but the second was detected after 1200 s. The peak with the high molecular masses had the highest intensity in the cytosols of human lung epithelium and the peak found in the low molecular range - in the cytosols of human hepatocytes. In the cultured media after cell growth the lowest selenium intensity was found in the chromatographic profile obtained from the human lung epithelium. It may indicate that these cells metabolize selenium quite fast. The application of selenium influenced the expression of selenium -

containing proteins in the cells. Of special interest is the selenium-containing species which appeared in the low molecular range. Its intensity was found to be high in the hepatocytes, which synthesize many selenoproteins including selenoprotein P. This small species might be of importance in the selenium transport. Until now the studies on the selenium transport have mainly focused on selenoprotein P.

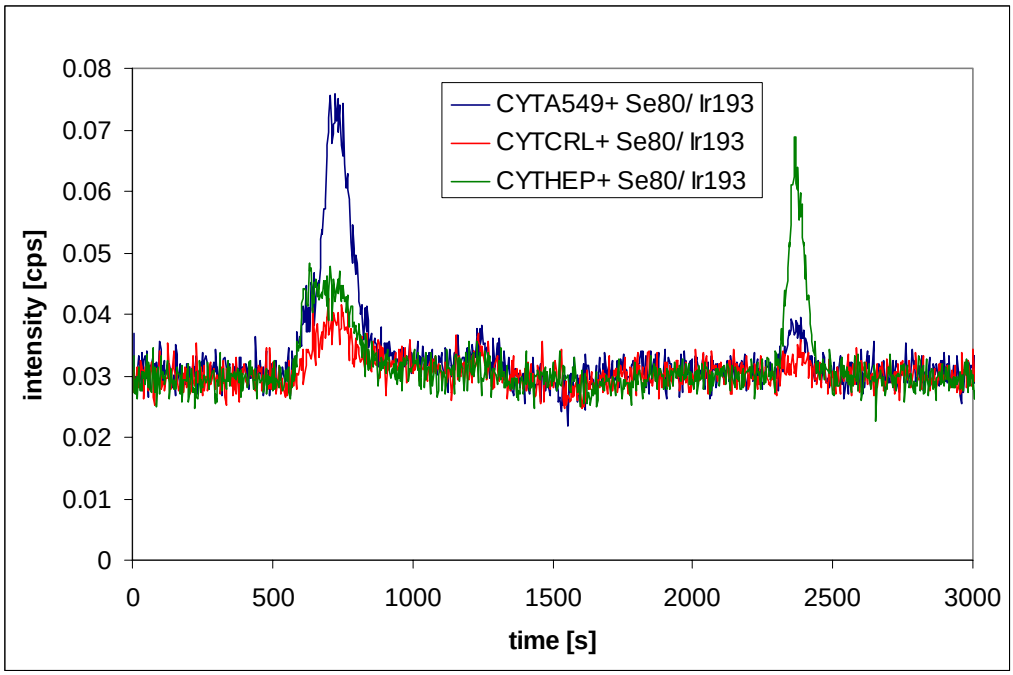

Figure 4-14 Chromatographic selenium profile of the cell cytosols treated with selenium

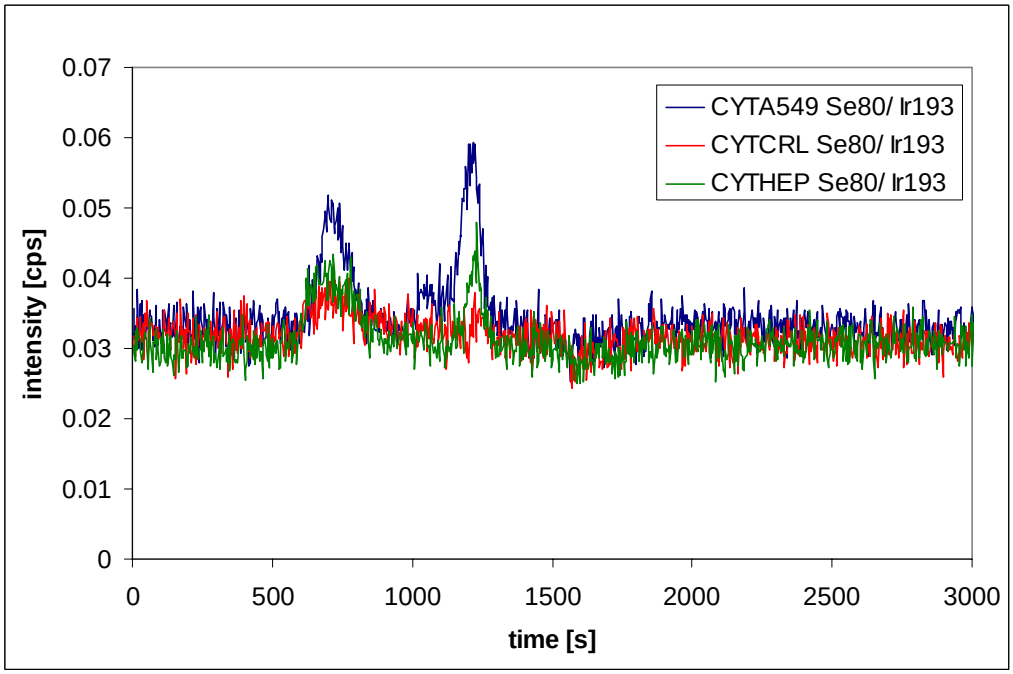

Figure 4-15 Chromatographic selenium profile of the untreated cell cytosols

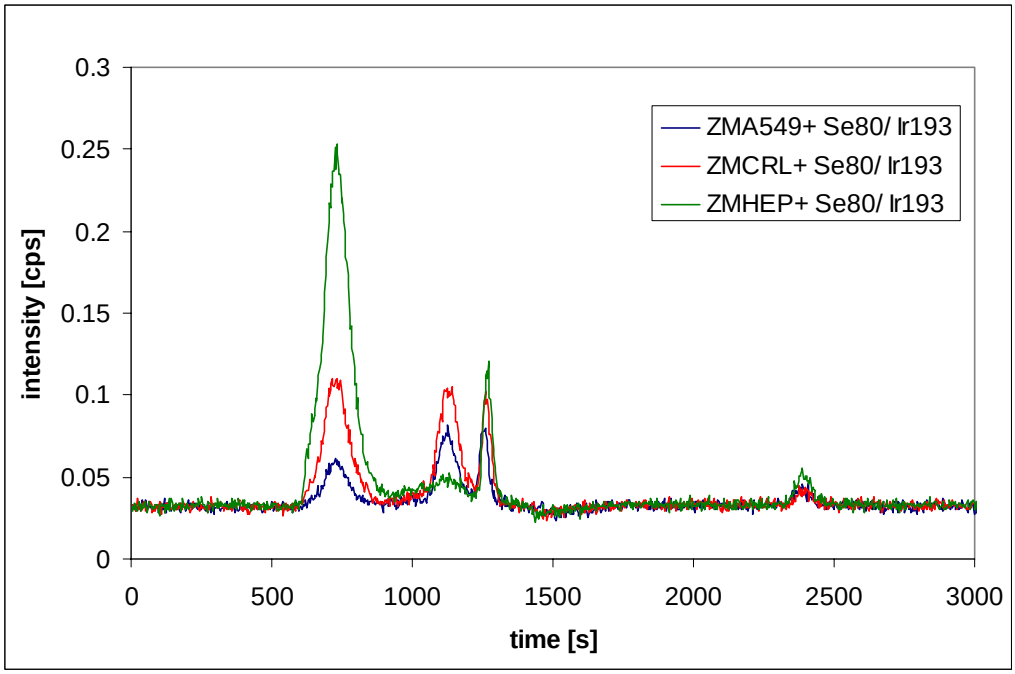

Figure 4-16 Chromatographic selenium profile of the cell media treated with selenium

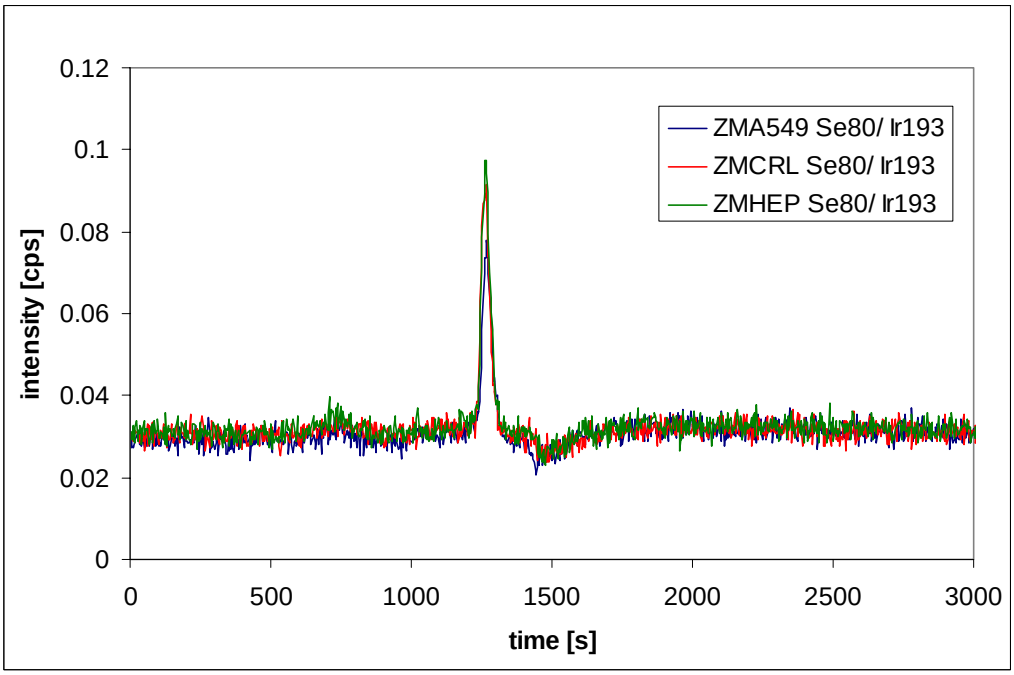

Figure 4-17 Chromatographic selenium profile of the untreated cell media

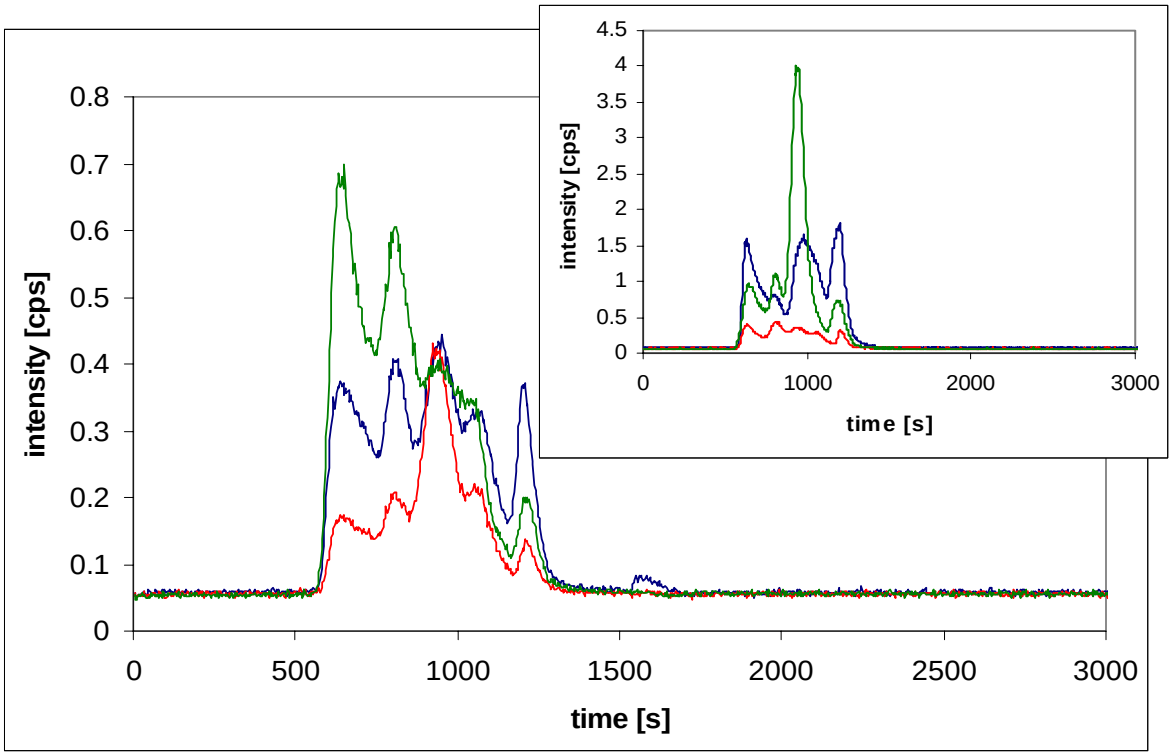

Figure 4-18 Chromatographic copper profile of the cell cytosols treated with selenium (large figure) and the untreated cytosols (small figure); — A549, — CRL, — HepG2

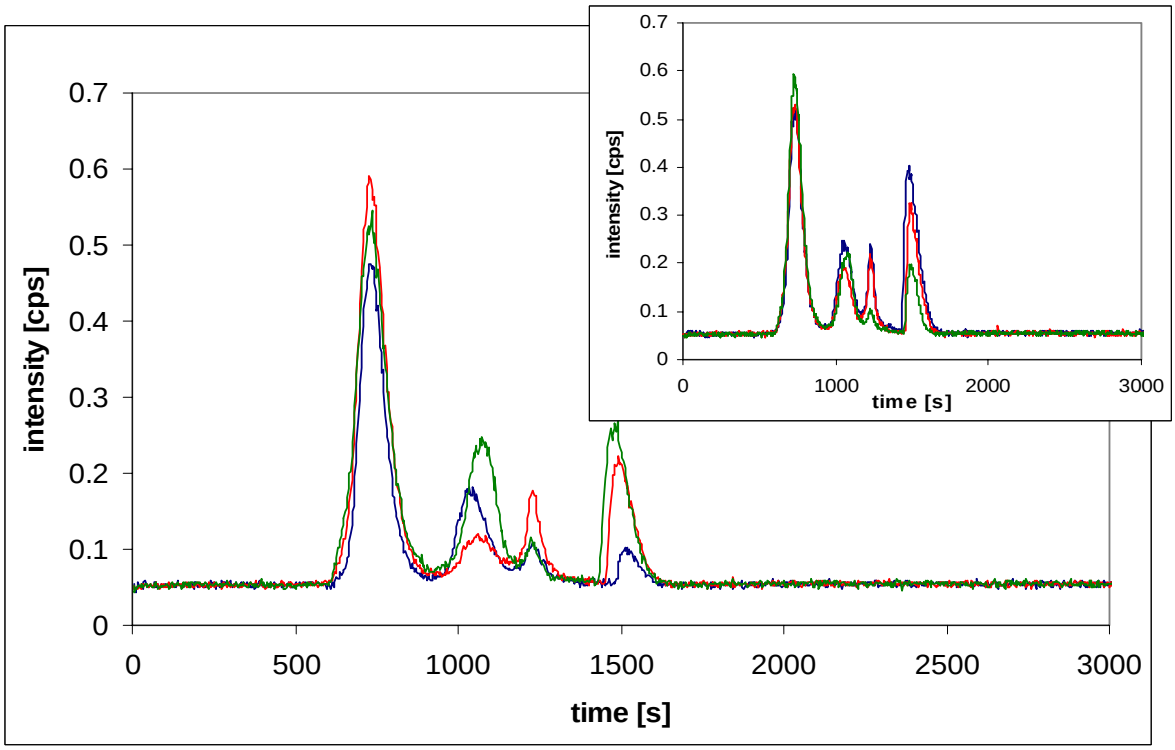

Figure 4-19 Chromatographic copper profile of the cell media treated with selenium (large figure) and of the untreated media (small figure); —A549, —CRL, —HepG2

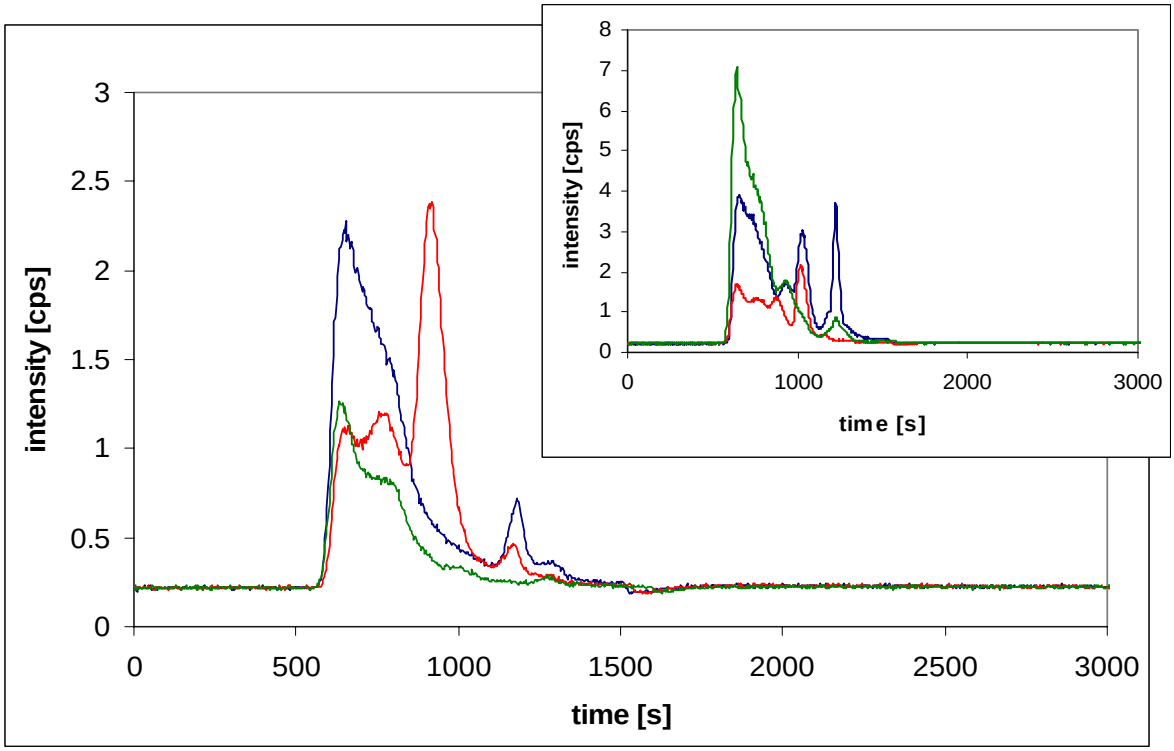

Figure 4-20 Chromatographic zinc profile of the cell cytosols treated with selenium (large figure) and of the untreated cytosols (small figure); —A549, —CRL, —HepG2

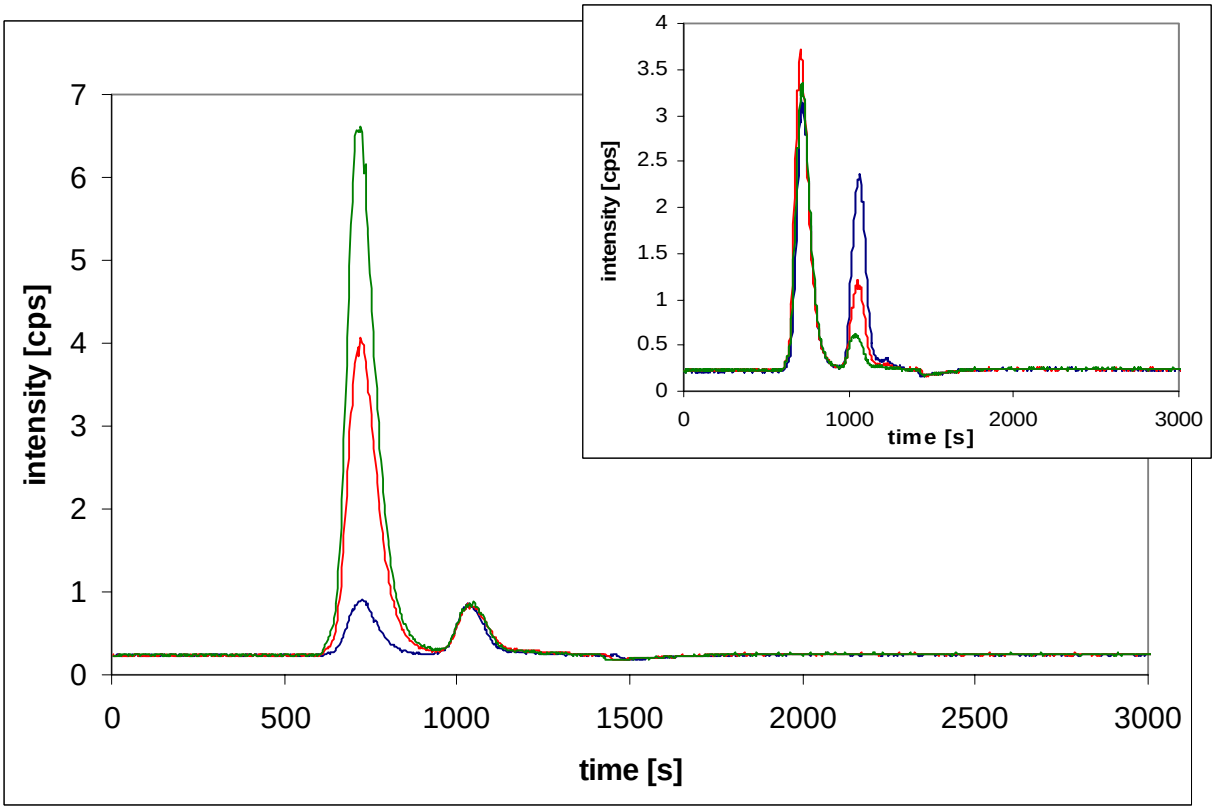

Figure 4-21 Chromatographic zinc profile of cell the media treated with selenium (large figure) and of the untreated media (small figure); —A549, —CRL, —HepG2

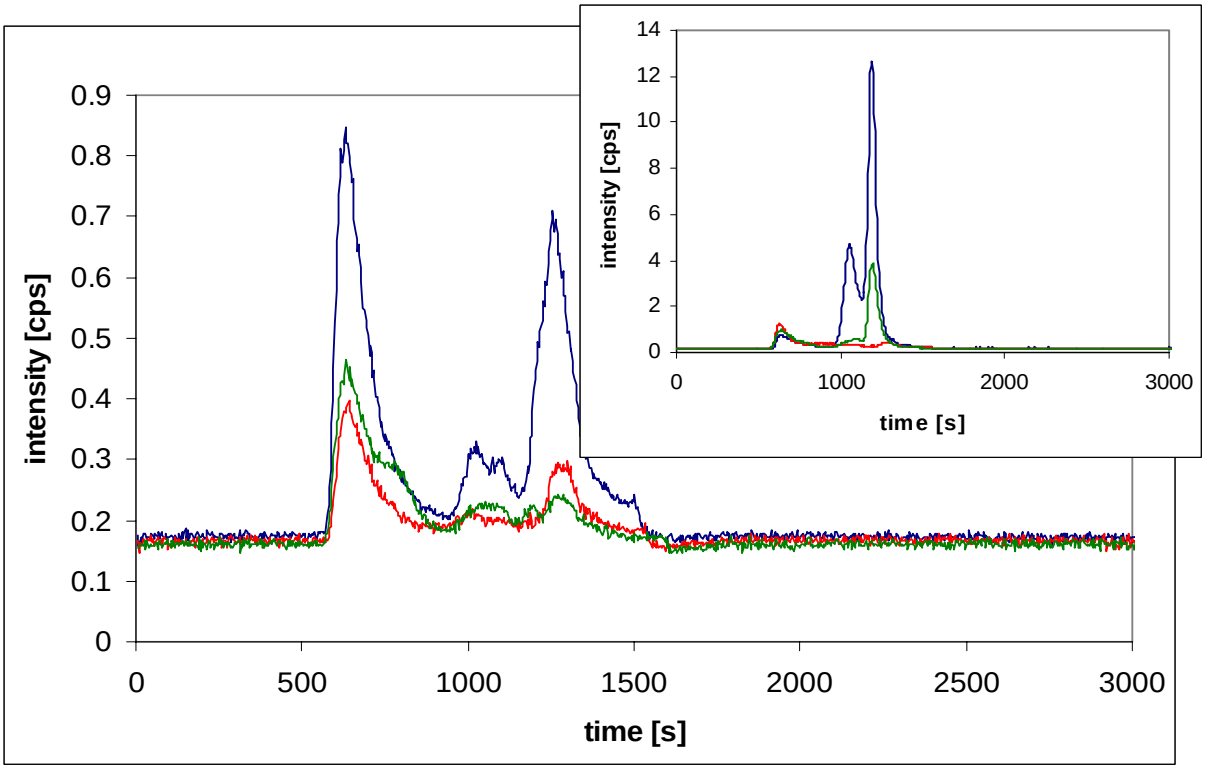

Figure 4-22 Chromatographic nickel profile of the cell cytosols treated with selenium (large figure) and of the untreated cytosols (small figure); —A549, —CRL, —HepG2

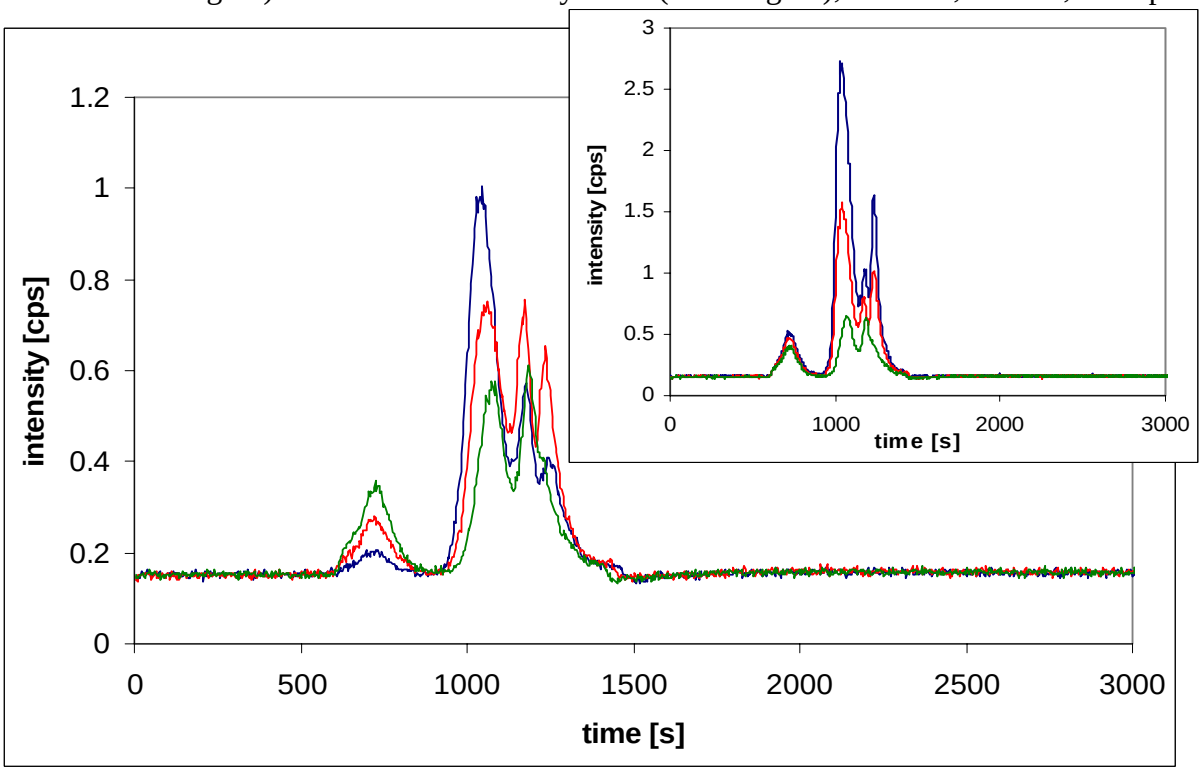

Figure 4-23 Chromatographic nickel profile of the cell media treated with selenium (large figure) and of the untreated media (small figure); —A549, —CRL, —HepG2

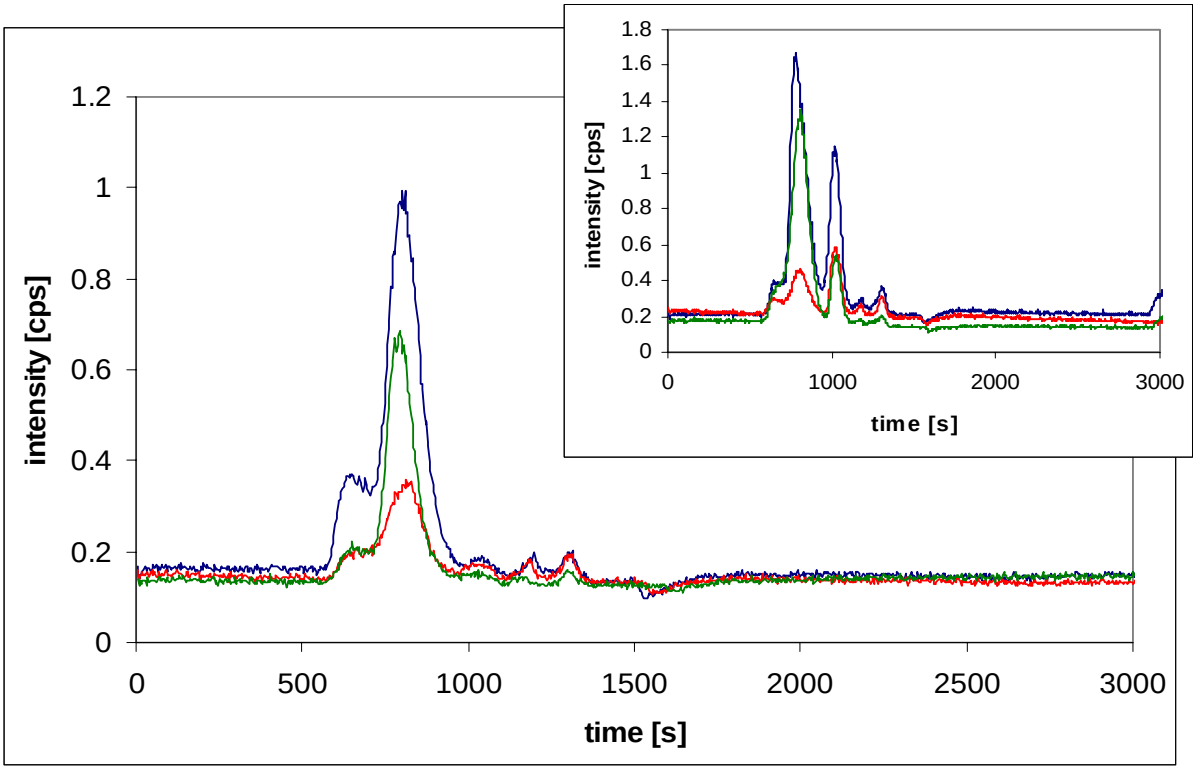

Figure 4-24 Chromatographic manganese profile of the cell cytosols treated with selenium (large figure) and of the untreated cytosols(small figure); —A549, —CRL, — HepG2

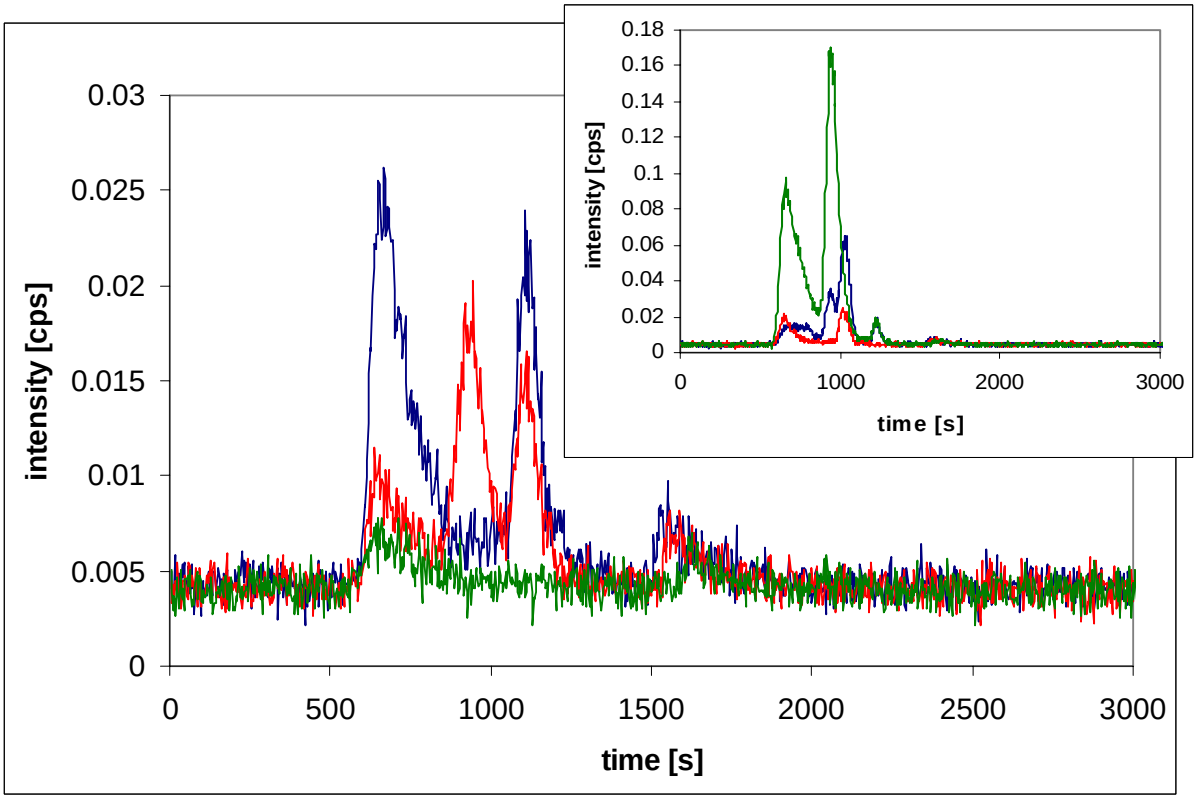

Figure 4-25 Chromatographic lead profile of the cell cytosols treated with selenium (large figure) and of the untreated cytosols (small figure); — A549, — CRL, — HepG2

Selenium addition affected the expression of other trace element-containing proteins. The comparison of the chromatographic profiles of copper, zinc, nickel and manganese of treated and untreated cell cytosols showed remarkable differences. A decrease in the profile intensity of the treated cell cytosols was observed. The retention of those elements in the control cells was clearly higher. Similarly, the element profile of the media of the treated cells a higher intensity of these elements was detected.

There were also changes in the profile characteristics. Some peaks were clearly lower after selenium treatment than the corresponding peaks found in the control sample. A good example is the lead profile (Figure 4-25). In the control cell cytosols of human hepatocytes two peaks were observed, while in the cytosols of the cells exposed to selenium only one peak with very low intensity was detected. In contrast, in the cytosol of human lung epithelium three peaks were found and after treatment with selenium the lead profile yielded only two peaks with a distinctly lower intensity.

Significant changes in the characteristics of the nickel profile were also observed. In the case of the cytosol obtained from human lung cells treated with selenium, the peak eluted at 1200 s was distinctly lower. Changes of the intensity were also observed in the other two cells types.

Selenium interactions with those elements influenced their retention in the cytosolic cell fraction. Selenium might influence the expression of proteins containing these trace elements and their metabolism.