Neutron Activation Analysis of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn Contents and Their Content to Zn Content Ratios in Prostate Adenocarcinoma

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Introduction

Prostate cancer (PCa) is the most common non-cutaneous male cancer in most populations.[1] Although the etiology of PCa is unknown, several risk factors including age and diet (Ca, Zn and some other nutrients) have been well identified.[2,3] It is also reported that the risk of having PCa drastically increase with age, being three orders of magnitude higher for the age group 40–79 years than for those younger than 40 years.[3,4]

Trace elements have essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively.[5] Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration or death.[5,6,7] High intraprostatic Zn concentrations are probably one of the main factors acting in both initiation and promotion stages of prostate carcinogenesis.[8-11] A significant tendency of age-related increase in Ba, Bi, Cd, Co, Fe, Hg, Pb, Sc, Sn, Th, U, and Zn, mass fraction in the prostate was recently demonstrated by us.[12-22] Thus, it seems fair to suppose that besides Zn, many other trace elements also play a role in the pathophysiology of the prostate.

The trace element contents in tissue of the normal and cancerous prostate have been studied, producing contradictory results.[23-38] The majority of these data are based on measurements of processed tissue and in many studies tissue samples are ashed before analysis. In other cases, prostate samples are treated with solvents (distilled water, ethanol etc) and then are dried at a high temperature for many hours. There is evidence that certain quantities of trace elements are lost as a result of such treatment.[39-41] Moreover, only a few of these studies employed quality control using certified reference materials for determination of the trace element mass fractions. Thus, the questions about the differences between trace element contents in normal and cancerous prostate tissue remained open.

This work had three aims. The first was to assess the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, Zn contents in prostate adenocarcinoma and the nonhyperplastic prostate of healthy men aged over 40 years using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The second aim was to calculate the ratios of trace element contents to Zn content in normal and cancerous prostate, and the third was to compare the levels of trace elements and their ratios to Zn level in the malignant prostate with those in normal gland. All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Experimental

All patients suffered from adenocarcinoma of prostate (n=37, mean age M±SD was 63±11 years, range 40-79) were hospitalized in the Urological Department of the Medical Radiological Research Centre. Transrectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis adenocarcinoma has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Normal prostates for the control group samples were removed at necropsy from 37 men (mean age 55±11 years, range 41-87), who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer.

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The sample weighing about 10 mg (for biopsy materials) and 50 mg (for resected materials) was used for chemical element measurement by instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with double rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

To determine the contents of the trace elements by comparison with known data for standard, aliquots of commercial, chemically pure compounds, synthetic and natural reference materials were used.[42] Ten sub-samples of international certified reference material (CRM) IAEA H-4 (animal muscle) and ten sub-samples of CRM IAEA HH-1 (human hair) weighing about 50 mg were treated and analyzed under exactly the same conditions as the prostate samples, to allow estimation of the precision and accuracy of results.

The vertical channel of a nuclear reactor was used to determine the samples' content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule containing prostate samples, standards, and certified reference materials was soldered, positioned in an aluminum transport container and exposed to a 24-hour neutron irradiation in the vertical channel with a neutron flux $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. Ten days after the irradiation samples were reweighed and repacked.

Details of the relevant facility for INAA-LLR, nuclear reactions, radionuclides, gamma energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the trace elements of nonhyperplastic human prostate tissue.[13]

A dedicated computer program for INAA mode optimization was used.[43] All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. Using the Microsoft Office Excel programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for trace element contents in normal and cancerous prostate tissue. The reliability of difference in the results between the two groups of prostate tissue samples was evaluated by Student's *t*-test. For the estimation of the Pearson correlation coefficient between different pairs of the trace element mass fractions in the normal and cancerous prostate tissue the Microsoft Office Excel program was also used.

Results and discussion

Table 1 depicts certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions as well as Ag/Zn, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Rb/Zn, Sb/Zn, Sc/Zn, and Se/Zn mass fraction ratios in normal prostate tissue and adenocarcinoma of prostate.

As was shown by us[13] the use of CRM IAEA H-4 and CRM IAEA HH-1 as a certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents analyzed by INAA-LLR with the certified data of CRMs IAEA indicates an acceptable accuracy of the results obtained in the study of trace elements of the adenocarcinoma of prostate gland presented in Table 1. The mean values and all selected statistical parameters were calculated for 10 chemical elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) (Table 1). The mass fraction of these elements were measured in all, or a major portion of normal and cancerous prostate samples.

The ratios of means and the reliability of difference between mean values of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions as well as Ag/Zn, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Rb/Zn, Sb/Zn, Sc/Zn, and Se/Zn mass fraction ratios in normal and cancerous prostate tissue are presented in Table 2.

From Tables 1 and 2, it is observed that in adenocarcinoma the mass fractions of Ag (p<0.00012), Cr (p<0.00059), Fe (p<0.012), Hg (p<0.040), and Sb (p<0.0000002) are significantly higher while the mass fractions of Co (p<0.015), Rb (p<0.0076), Sc (p<0.0064), and Zn (p<0.0000000016) are significantly lower than in normal tissues of the prostate. Except for Se, the mass fractions of all trace elements investigated in the study show significant variations in cancerous tissues when compared with normal tissues of the prostate. For example, in adenocarcinoma the Sb mass fractions were 10 times, and the Ag, Cr, Fe, and Hg mass fractions were approximately 1.6–5 times, greater than in normal prostate tissue (Table 2).

In contrary, the Zn mass fractions were almost 8 times, and the Co, Rb, and Sc mass fractions were approximately 30-60%, lower in adenocarcinoma than in normal prostate tissue (Table 2). It was found that Zn and some other trace elements are involved in functional features of prostate tissue.[44]

Because malignant transformation is accompanied by a loss of tissue-specific functional features, this process leads to a significant reduction in the contents of trace elements such as Zn and, probably, Co, Rb, Sc, and Se associated with functional characteristics of the human prostate tissue.

All ratios of trace element mass fraction to Zn mass fraction in cancerous tissues were significantly higher than in normal tissues. Published data referring to inter-element correlations of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal and cancerous tissues of the human prostate gland were not found.

The comparison of our results with published data for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal and cancerous prostate tissue is shown in Table 3. The results for all trace element contents in the prostates of the control group (mean age 55±11 years, range 41-87) are in accordance with our earlier findings in prostates of apparently healthy men aged 41-60.[13]

Table 1. Some statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions (mg/kg, dry mass basis) and trace element mass fraction/ Zn mass fraction ratio in normal and cancerous prostate

Tissue	Parameter	Mean	SD	SEM	Min	Max	Med	Per. 0.025	Per. 0.975
Norm	Ag	0.048	0.046	0.009	0.010	0.223	0.0344	0.0100	0.156
n=37	Co	0.045	0.024	0.004	0.017	0.106	0.0400	0.0169	0.099
	Cr	0.532	0.422	0.081	0.030	1.81	0.392	0.0300	1.54
	Fe	111	51	9	35.0	267	100	35.8	221
	Hg	0.056	0.057	0.011	0.008	0.242	0.0320	0.0114	0.216
	Rb	12..7	5.0	0.9	4.70	25.3	12.0	5.69	24.6
	Sb	0.045	0.037	0.007	0.005	0.158	0.0380	0.0087	0.154
	Sc	0.029	0.024	0.005	0.005	0.077	0.0200	0.0066	0.077
	Se	0.696	0.240	0.044	0.318	1.43	0.691	0.346	1.15
	Zn	1002	664	109	231	3513	915	252	2185
	$(Ag/Zn)10^3$	0.072	0.076	0.015	0.009	0.329	0.0424	0.0115	0.249
	$(Co/Zn)10^3$	0.065	0.055	0.010	0.012	0.236	0.0471	0.0145	0.209
	$(Cr/Zn)10^3$	0.676	0.851	0.164	0.019	4.04	0.380	0.0355	3.11
	Fe/Zn	0.157	0.131	0.023	0.033	0.593	0.115	0.0335	0.491
	$(Hg/Zn)10^3$	0.071	0.081	0.016	0.007	0.394	0.0360	0.0119	0.266
	$(Rb/Zn)10^3$	17.9	12.9	2.2	3.50	54.3	13.0	3.74	52.0
	$(Sb/Zn)10^3$	0.061	0.066	0.012	0.005	0.341	0.0397	0.0048	0.213
	$(Sc/Zn)10^4$	0.308	0.186	0.042	0.045	0.701	0.269	0.0627	0.634
	$(Se/Zn)10^3$	0.927	0.592	0.108	0.274	2.37	0.749	0.291	2.26
Cancer	Ag	0.242	0.165	0.039	0.024	0.527	0.197	0.0293	0.520
n=37	Co	0.032	0.015	0.0032	0.005	0.067	0.0291	0.0104	0.062
	Cr	2.31	1.98	0.43	0.165	6.48	1.42	0.255	6.28
	Fe	172	113	21	15.0	427	128	28.5	401
	Hg	0.091	0.055	0.012	0.016	0.203	0.0797	0.0202	0.201
	Rb	9.31	5.07	0.88	1.00	18.5	8.20	2.12	17.9
	Sb	0.445	0.289	0.057	0.015	1.03	0.453	0.0355	0.957
	Sc	0.013	0.009	0.002	0.001	0.044	0.0107	0.0013	0.032
	Se	0.629	0.431	0.092	0.063	1.54	0.507	0.0719	1.43
	Zn	127	74	12	20.0	311	120	20.0	307
	$(Ag/Zn)10^3$	3.14	4.20	0.99	0.089	15.5	2.02	0.114	14.4
	$(Co/Zn)10^3$	0.348	0.236	0.050	0.065	0.937	0.314	0.0791	0.896
	$(Cr/Zn)10^3$	25.8	24.3	5.3	0.988	88.0	15.6	1.88	82.5
	Fe/Zn	2.48	3.61	0.68	0.337	18.4	1.28	0.346	10.8
	$(Hg/Zn)10^3$	0.915	0.632	0.138	0.100	2.94	0.789	0.214	2.48
	$(Rb/Zn)10^3$	128	173	30	12.0	770	68.7	18.5	730
	$(Sb/Zn)10^3$	5.88	6.81	1.34	0.107	26.9	2.92	0.258	25.1
	$(Sc/Zn)10^4$	1.23	1.05	0.22	0.037	4.50	0.965	0.0938	3.93
	$(Se/Zn)10^3$	5.27	2.88	0.61	1.42	14.4	4.44	1.81	12.2

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Med* median *Per.0.025* percentile with 0.025 level, *Per.0.975* percentile with 0.975 level.

Table 2. Comparison of mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions (mg/kg, dry mass basis) and trace element mass fraction/ Zn mass fraction ratio in normal and cancerous prostate

Parameter	Prostatic tissue			Ratio Adenocarcinoma to Normal
	Normal 41-87 year n=37	Adenocarcinoma 40-79 year n=37	Student's *(*t-test) $p\leq$	
Ag	0.0478±0.0089	0.242±0.039	0.00012	5.06
Co	0.0452±0.0043	0.0317±0.0032	0.015	0.70
Cr	0.532±0.081	2.31±0.43	0.00059	4.34
Fe	111±9	172±21	0.012	1.55
Hg	0.056±0.011	0.091±0.012	0.040	1.63
Rb	12.7±0.9	9.31±0.88	0.0076	0.73
Sb	0.0445±0.0067	0.445±0.057	0.0000002	10.0
Sc	0.0294±0.0053	0.0126±0.0019	0.0064	0.43
Se	0.696±0.044	0.629±0.092	0.516 (NS)	0.90
Zn	1002±109	127±12	0.0000000016	0.13
$(Ag/Zn)10^3$	0.072±0.015	3.14±0.99	0.0066	43.6
$(Co/Zn)10^3$	0.065±0.010	0.348±0.050	0.000013	5.35
$(Cr/Zn)10^3$	0.68±0.16	25.8±5.3	0.00013	37.9
Fe/Zn	0.16±0.02	2.48±0.68	0.0021	15.5
$(Hg/Zn)10^3$	0.071±0.016	0.915±0.138	0.0000055	12.9
$(Rb/Zn)10^3$	18±2	128±30	0.00092	7.11
$(Sb/Zn)10^3$	0.061±0.012	5.88±1.34	0.00020	96.4
$(Sc/Zn)10^4$	0.31±0.04	1.23±0.22	0.00053	3.97
$(Se/Zn)10^3$	0.93±0.11	5.27±0.61	0.00000050	5.67

M arithmetic mean, *SEM* standard error of mean, *NS* not significant difference.

In data from the literature a number of values for trace element mass fractions in prostate tissue were not expressed on a dry mass basis. Therefore, we calculated these values using published data for water - 83% [45] and ash - 1% on wet mass basis [46] contents in the prostate of adult men and also for water – 80% in prostate cancer tissue.[47] This reported data also includes samples obtained from patients who died from different non-urological diseases. Values obtained for Ag, Cr, Fe, Rb, Se, and Zn contents agree well with median of mean values cited by other researches for the human prostate (Table 3). However, calculated means for Co, Hg, and Sb in this work are an order of magnitude lower than the median of previously reported data. No published data referring to Sc content in the human prostate was found. In the adenocarcinoma of prostate our results were comparable with published data for Cr, Fe and Zn contents and one or two orders of magnitude lower for Co and Se (Table 3). No published data referring to Ag, Hg, Rb, Sb, and Sc contents of cancerous prostate tissue were found.

Compared to other soft tissues, the prostate of young adults has higher levels of Zn and many other trace elements.[15-18] Moreover, the level of Zn and some other trace elements continue to increase with age.[19-22] In our earlier publications[8,10,11] it was discussed in detail that the age-related excessive Zn level in prostatic tissue is probably one of the main factors influencing the initiation and progression of PCa. In addition to the elevated Zn level, an age-related increase and excess in Cr, Fe, Hg, and Sb mass fractions in prostatic tissue may

contribute to harmful effects on the gland. There are good reasons for such speculations since many reviews and numerous papers raise the concern about toxicity and carcinogenicity of these metals.[48-57] Each of these metals is distinct in its primary mode of action. Moreover, there are several forms of synergistic action of these metals as a part of intracellular metabolism, during which several reactive intermediates and byproducts are created.[48,49,56] These reactive species are capable of potent and surprisingly selective activation of stress-signaling pathways, inhibition of DNA metabolism, repair, and formation of DNA crosslinks, which are known to contribute to the development of human cancers.[49,50,54] In addition to genetic damage via both oxidative and nonoxidative (DNA adducts) mechanisms, metals can also cause significant changes in DNA methylation and histone modifications, leading to alterations in gene expression.[50,51,53,] In vitro and animal carcinogenic studies provided strong support for the idea that metals can also act as co-carcinogens in combination with nonmetal carcinogens.[50].

Table 3. Median, minimum and maximum value of means of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions (mg/kg, dry mass basis) in normal and cancerous prostate according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Prostate tissue	Element	Published data [References]			This work
		Median of means (n)[a]	Minimum of means M or M±SD, (n)[b]	Maximum of means M or M±SD, (n)[b]	M±SD
Norm	Ag	≤0.1 (2)	<0.05 (48) [25]	0.2 (7) [23]	0.048±0.046
	Co	0.55 (3)	<0.09 (50 [25]	12 (9) [24]	0.045±0.024
	Cr	0.56 (3)	0.042 (50) [25]	1.4 (8) [23]	0.53±0.42
	Fe	150 (14)	5.7±0.1 (5) [26]	1040±65 (10) [30]	111±51
	Hg	0.65 (1)	0.65±0.58 (5) [27]	0.65±0.58 (5) [27]	0.056±0.057
	Rb	34.5(3)	4.7 (9) [24]	58±33 (4) [28]	12.7±5.0
	Sb	0.42 (1)	0.42±0.56 (10) [27]	0.42±0.56 (10) [27]	0.045±0.037
	Sc	-	-	-	0.029±0.024
	Se	0.625 (7)	0.27 (129) [32]	1.5 (15) [31]	0.70±0.24
	Zn	482 (48)	111 (-) [29]	3218±41 (10) [30]	1002±664
Cancer	Ag	-	-	-	0.24±0.17
	Co	5.1 (1)	4.7±0.4 (1) [33]	5.4±0.6 (1) [33]	0.032±0.015
	Cr	7.0 (4)	0.33±0.06 (1) [33]	217±8 (27) [35]	2.3±2.0
	Fe	195 (9)	12.5±5.0 (23) [36]	3530±45 (27) [35]	172±113
	Hg	-	-	-	0.091±0.055
	Rb	-	-	-	9.3±5.1
	Sb	-	-	-	0.45±0.29
	Sc	-	-	-	0.013±0.009
	Se	5.5 (7)	0.90±0.55 (29) [37]	11.5±3.5 (3) [34]	0.63±0.43
	Zn	200 (34)	16.7±3.5 (3) [34]	840±85 (13) [38]	127±74

M - arithmetic mean, SD – standard deviation, (n)[a] – number of all references, (n)[b] - number of samples.

Our findings show that mass fraction of Ag, Cr, Sb and Zn are significantly different in most adenocarcinomas as compared to normal prostate tissues (Tables 2). Thus, it is plausible

to assume that levels of these trace elements in prostate tissue can be used as tumor markers. However, this subjects needs in additional studies.

Conclusions

In this work, trace elemental analysis was carried out in the tissue samples of normal prostate and adenocarcinoma of prostate using INAA-LLR. It was shown that INAA-LLR is an adequate analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn content in the tissue samples of human prostate, including needle-biopsy cores. It was observed that in cancerous tissues contents of Co, Rb, Sc, and Zn were significantly lower and those of Ag, Cr, Fe, Hg, and Sb were significantly higher than in normal tissues. All ratios of trace element mass fraction to Zn mass fraction in cancerous tissues were significantly higher than in normal tissues. In our opinion, the abnormal decrease in levels of Co, Rb, Sc, and Zn in cancerous tissue could be a consequence of malignant transformation. It was supposed that elevated levels of Ag, Cr, Fe, Hg, and Sb as well as an inadequate level of Se are involved in an initiation and a promotion of prostatic adenocarcinoma.

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References

1. Rebbeck T.R., Haas G.P. *Can. J. Urol.* **2014**, *21*(5), 7496-7506.
2. Yamada K., Araki S.**,** Tamura M. et al. *Int. J. Cancer* **2000**, *89*, 259-264.
3. Rebbeck T.R. *Cancer Epidemiol Biomarkers Prev*. **2006,** *15*, 1569-1571.
4. Jemal A., Murray T., Samuels A., et al. *CA: A Cancer J. for Clinicians* **2003**, *53*, 5-26.
5. Zaichick V. *J. Radioanal. Nucl. Chem.* **2006**, *269*, 303-309.
6. Ektessabi A., Shikine S., Kitamura N. et al. *X-Ray Spectrom*. **2001**, *30*, 44-48.
7. Yoshida S., Ektessabi A., Fujisawa S. *J. Synchrotron Radiat*. **2001**, *8*, 998-1000.
8. Zaichick,V., Zaichick S. In: *Mengen und Spurenelemente, 19* Arbeitstagung (M Anke et al., Eds.). Friedrich-Schiller-Universitat, Jena, **1999**, pp.104-115.
9. Leitzmann M.F., Stampfer M.J., Wu K. et al. *JNCI* **2003**, *95*, 1004-1007.
10. Zaichick V. *J. Radioanal. Nucl. Chem*. **2004**, *262*, 229-234.
11. Zaichick V., Zaichick S. *Age* **2014**, *36*(1), 167-181.
12. Zaichick S., Zaichick V. *J. Radioanal. Nucl. Chem*. **2011**, *288*(1), 197-202.
13. Zaichick S., Zaichick V. *Appl Radiat Isot*. **2011**, *69*(6), 827-833.
14. Zaichick V., Nosenko S., Moskvina I. *Biol. Trace Elem. Res*. **2012**, *147*(1-3), 49-58.
15. Zaichick V., Zaichick S. *Appl. Radiat. Isot*. **2013**, *82*, 145-151.
16. Zaichick V., Zaichick S. *J. Radioanal. Nucl. Chem*. **2013**, *298*(3), 1559-1566.
17. Zaichick V., Zaichick S. *Biol. Trace Elem. Res*. **2013**, *156*(1), 357-366.
18. Zaichick V., Zaichick S. *American Journal of Analytical Chemistry* **2013**, *4*, 696-706.
19. Zaichick V., Zaichick S. *Appl. Radiat. Isot*. **2014**, *90*, 62-73.
20. Zaichick V., Zaichick S. *Open Journal of Biochemistry* **2014**, *1*(2), 16-33.
21. Zaichick V., Zaichick S. *J. Radioanal. Nucl. Chem*. **2014**, *301*(2), 383-397.
22. Zaichick V. *Biol. Trace Elem. Res*. **2015**, *168*(1), 44-60.

23. Tipton J.., Steiner R.L., Foland W.D. et al. USAEC-ORNL-Report-CF-54-12-66, **1954**.
24. Stitch S.R. *Biochemistry Journal* **1957**, *67*, 97-103.
25. Tipton J.H., Cook M.J. *Health Physics*, **1963**, *9*, 103-145.
26. Sangen H. *Jap. J. Urol.* **1967**, *58*, 1146–1159.
27. Liebscher K., Smith H. *Arch. Environ. Health* **1968**, *17*, 882-891.
28. Soman S.D., Joseph K.T., Raut S.J. et al. *Health Physics* **1970**, *19*, 641-656.
29. Anspaugh L.R., Robinson W.L., Martin W.H., Lowe O.A. Compilation of Published Information on Elemental Concentrations in human Organs in Both Normal and Diseased States, No. UCRL-51013Pt. 1971-1973, **1973**, pp. 1-4.
30. Jafa A., Mahendra N.M., ChowdhuryA.R. et al. *Indian J. of Cancer*, **1980**, *17*, 34-37.
31. Sarafanov A.G., Todorov T.I., Kajdacsy-Balla A. et al. *Journal of Trace Elements in Medicine and Biology*, **2008**, *22*, 305-314.
32. Schöpfer J., Drasch G., Schrauzer G.N. *Biol. Trace Elem. Res*. **2010**, *134*, 180-187.
33. Kwiatek W.M. et al. *J. of Alloys and Compounds* **2005**, *401*, 173-177.
34. Kwiatek W.M. et al. *J. of Alloys and Compounds* **2004**, *362*, 83-87.
35. Guntupalli J.N.R. et al. Eur. J. Cancer Prev. 2007, *16*, 108–115.
36. Kiziler A.R., Aydemir B., Guzel S. et al. *Trace Elements & Electrolytes* **2010**; *27*, 65–72.
37. Sapota A., Daragó A., Taczalski J. et al. *Biometals* **2009**, 22, 1041–1049.
38. Dhar N.K., Goel T.C., Dube P.C. et al. *Exp. Mol. Pathol.* **1973**, 19, 139-142.
39. Zaichick,V. In: *Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques*. International Atomic Energy Agency, Vienna, **1997**, pp 123-133.
40. Zaichick V., Zaichick S. *J. Radioanal. Nucl. Chem*. **1997**, *218*(2), 249-253.
41. Zaichick,V. *Trace Elements in Medicine* **2004**, *5*(3), 17–22.
42. Zaichick V. *Fresenius J. Anal. Chem.* **1995**, *352*, 219-223.
43. Korelo A.M., Zaichick V. In: *Activation Analysis in Environment Protection*. Joint Institute for Nuclear Research, Dubna (Russia), **1993**, pp 326–332.
44. Zaichick V., Zaichick S. *Andrology & Gynecology: Current Research* **2014**, *2*(2) doi:10.4172/2327-4360.1000121 http://dx.doi.org/10.4172/2327-4360.1000121
45. Woodard H.Q., White D.R. *Br. J. Radiol.* **1986**, *59*, 1209–1218.
46. Saltzman B.E., Gross S.B., Yeager D.W. et al. *Environ. Res.* **1990**, *52*, 126–145.
47. Györkey F., Min K.-W., Huff J.A. et al. *Cancer Res.* **1967**; *27*(8 Part 1), 1349–1353.
48. Sunderman F.W. *Biol. Trace Elem. Res*. **1979**, *1*, 63–86.
49. Snow E.T. *Pharmacology Ther.* **1992**, *53*, 31–65.
50. Salnikow K., Zhitkovich A. *Chem. Res. Toxicol.* **2008**, *21*, 28–44.
51. Toyokuni S. *Cancer Sci.* **2009**, *100*, 9–16.
52. Martinez-Zamudio R., Ha H.C. *Epigenetics* **2011**, *6*, 820–827.
53. Tokar E.J., Benbrahim-Tallaa L., Waalkes M.P. *Met. Ions Life Sci.* **2011**, *8*, 375–401.
54. Chervona Y., Arita A., Costa M. *Metallomics* **2012**, *4*, 619–627.
55. Tchounwou P.B., Yedjou C.G., Patlolla A.K. et al. *Molecular, Clinical and Environmental Toxicology* **2012**, *101*, 133–164.
56. Koedrith P., Kim H., Weon J.I. et al. *Int. J. Hyg. Environ. Health* **2013**, *216*, 587–598.
57. Tabrez S., Priyadarshini M., Priyamvada S. et al. *Mutation Research* **2014**, *760*, 1–9.