From hair to body liquids – TXRF analysis of biological and medical samples

Bruker Nano Analytics, Berlin
Webinar, December 18th, 2019

Welcome

Speakers

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Applications Scientist TXRF
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Itinerary

Part I: Analysis of human hair

- Motivation
- TXRF spectroscopy

Part II:

- Bone as matrix
- Se-Supplementation Trial in Humans
- Diagnostic Trial of serum Se and Cu in TSCI
- Analytics of recombinant or purified Protein
- Nested case-control Study => predictive Biomarker

Part III: Summary/Q & A

Part I: Analysis of human hair

Analysis of human hair
Motivation

Significance of micronutrients in human hair

The distribution of micronutrients like Mg, K, Ca, Mn, Cu, Zn, Se, Mo and I gives information on diseases, metabolic disorders, environmental exposures, and nutritional status.

Examples:

- Breast cancer patients show low hair Ca, Mg and Zn, but high As, Na and K concentrations compared with the normal controls. The Fe level is shown to be significantly low and associated with Ca and Mn levels [1]
- A cross-sectional analysis on 343 subjects showed that those with metabolic syndrome had significantly lower contents of Ca, Mg and Cu, whereas the amounts of Na, K and Hg are higher [2]
- Hair Zn, Se and Cu deficiencies are noted in chronic gastrointestinal diseases [3]

Analysis of human hair
Motivation

Control of toxic metals in human hair

- Hair analysis has been considered as one of the most important biomarkers according to the Environmental Protection Agency (EPA) [4].
- As an excretory system, human hair can accumulate and incorporate toxic metals into its structure during its growth process. Therefore, concentrations of heavy metals in hair can reflect the mean level in the human body, recording the population’s exposure to heavy metals [5]

[1] Joo NS, Kim SM, Jung YS, Kim KM. Hair iron and other minerals’ level in breast cancer patients. Biol Trace Elem Res. 2009;129:28–35.
[2] Park SB, Choi SW, Nam AY. Hair tissue mineral analysis and metabolic syndrome. Biol Trace Elem Res. 2009;130:218–28.
[3] Bhat YJ, Manzoor S, Khan AR, Qayoom S. Trace element levels in alopecia areata. Indian J Dermatol Venereol Leprol. 2009;75:29–31
[4] Rashed M.N., Hossam F. Heavy metals in fingernails and scalp hair of children, adults and workers from environmentally exposed areas at Aswan. Egypt. Environ. Bioindic. 2007;2:131–145
[5] Sera K., Futatsugawa S., Murao S. Quantitative analysis of untreated hair samples for monitoring human exposure to heavy metals. Nucl. Instrum. Methods B. 2002;189:174–179.

Analysis of human hair
Motivation

Benefits of human hair analysis

- Hair is easier to collect, transport and store than other medical samples like blood or serum
- As elements are fixed in the hair, element control over longer time period is possible

Analyzed samples

- Certified reference sample NIES 13 (National Institute for Environmental Studies)
- 5 human hair samples (cleaned with acetone and ultrapure water)

Analysis of human hair
Samples and sample preparation

Cutting (~ 5 mm segments)

Grinding in ball mill
(Retsch MM400, Zr-vessel/ball)

Weighing in Teflon vials
(100 ± 5 mg)

Suspension

+ 5 ml Triton X100 solution (1 vol.-%)

+ 10 µl Ga (0,1 g/l)
+ 5 µl Pd (1 g/l)

Homogenization

Pipetting 10 µl on siliconized quartz carrier, while samples are shaken at 1000 r/min

Digestion

+ 1 ml HNO_3/H_2O_2 mixture (3:1), filled up to 5 ml

+ 10 µl Ga (0,1 g/l)
+ 5 µl Pd (1 g/l)
+ 100 µl PVA (0,3 g/l)

Heating 120 °C, 30 min

Pipetting 10 µl on siliconized quartz carrier

Drying in vacuum

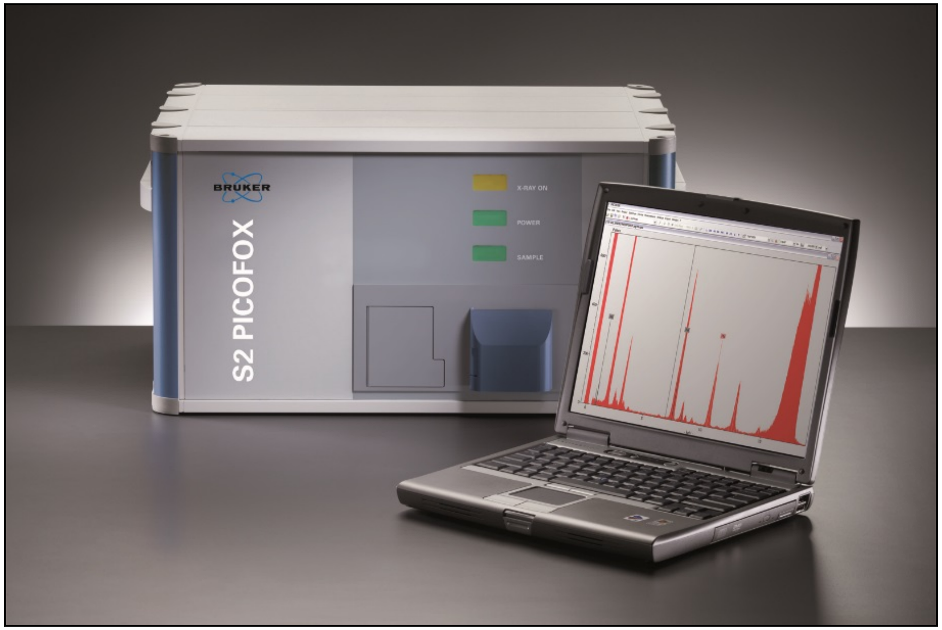
Analysis of human hair
Measurements

S2 PICOFOX

- Mo tube, 50 kV/1000 µA
- 60 mm^2 XFlash SDD

Measurement conditions

- Mo-K excitation, 1000 s



Measurements

Measurement program

Analysed using K-lines

Analysed using L-lines

1 H Hydrogen																	2 He Helium
3 Li Lithium	4 Be Beryllium											5 B Boron	6 C Carbon	7 N Nitrogen	8 O Oxygen	9 F Fluorine	10 Ne Neon
11 Na Sodium	12 Mg Magnesium											13 Al Aluminium	14 Si Silicon	15 P Phosphorus	16 S Sulphur	17 Cl Chlorine	18 Ar Argon
19 K Potassium	20 Ca Calcium	21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	29 Cu Copper	30 Zn Zinc	31 Ga Gallium	32 Ge Germanium	33 As Arsenic	34 Se Selenium	35 Br Bromine	36 Kr Krypton
37 Rb Rubidium	38 Sr Strontium	39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	47 Ag Silver	48 Cd Cadmium	49 In Indium	50 Sb Antimony	51 Sn Tin	52 Te Tellurium	53 I Iodine	54 Xe Xenon
55 Cs Cesium	56 Ba Barium	57 La Lanthanum	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	79 Au Gold	80 Hg Mercury	81 Tl Thallium	82 Pb Lead	83 Bi Bismuth	84 Po Polonium	85 At Astanine	86 Rn Radon
87 Fr Francium	88 Ra Radium	89 Ac Actinium															
			L Lanthanides	58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolynium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium	69 Th Thulium	70 Yb Ytterbium	71 Lu Luthetium
			Ac Actinides	90 Th Thorium	91 Pa Proactinium	92 U Uranium	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium	101 Md Mendelevium	102 No Nobelium	103 Lr Lawrencium

Analysis of human hair
Measurements

S4 T·STAR

- Mo tube, 50 kV/1000 µA
- W-tube, 50 kV/1000 µA
- 60 mm^2 XFlash SDD

Measurement conditions

- Mo-K excitation, 1000 s
- W-Brems excitation, 1000 s

Analysis of human hair
Measurements

Excitation modes used for quantification

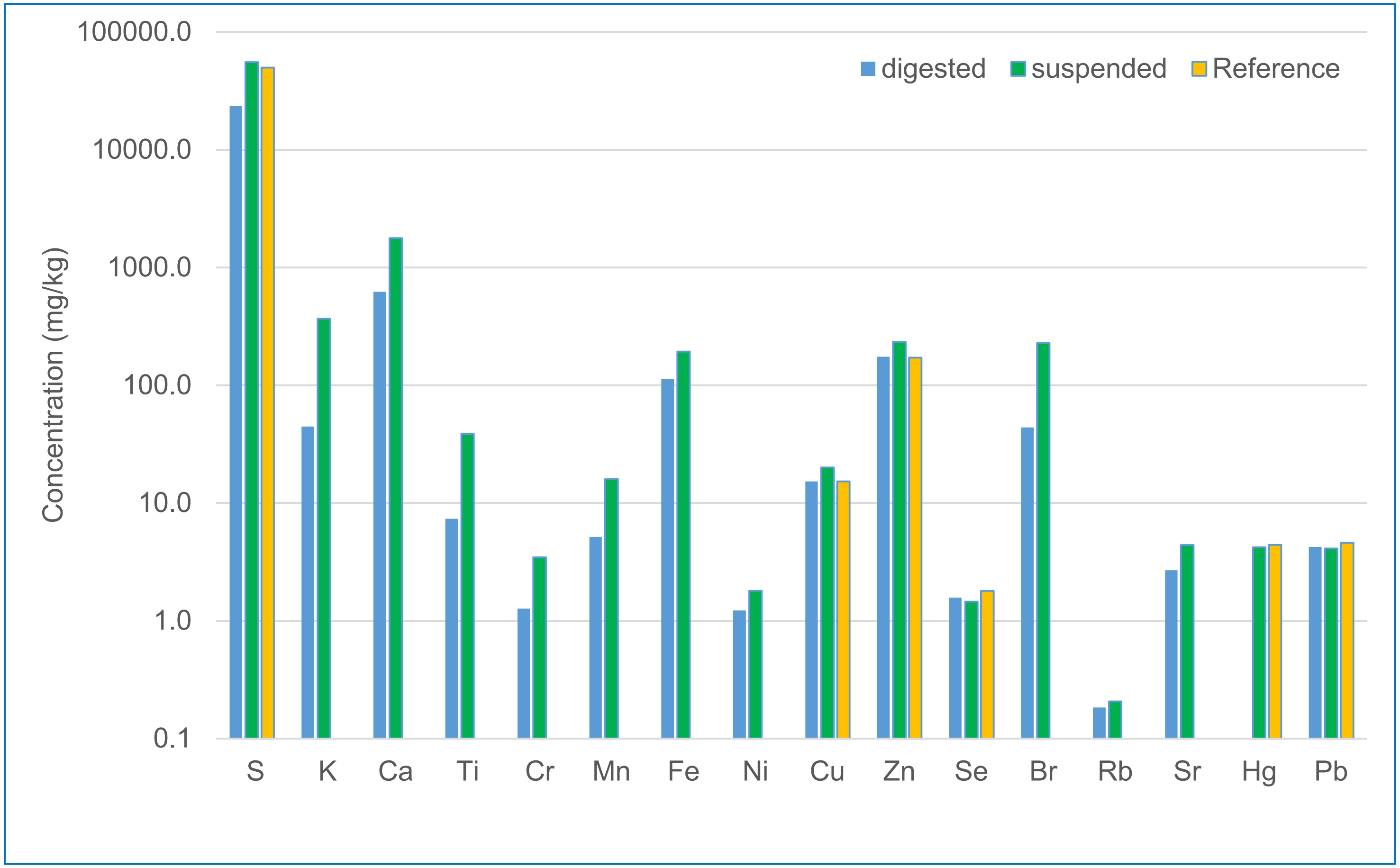
Mo-K excitation

W-Brems Excitation

1 H Hydrogen																	2 He Helium
3 Li Lithium	4 Be Beryllium											5 B Boron	6 C Carbon	7 N Nitrogen	8 O Oxygen	9 F Fluorine	10 Ne Neon
11 Na Sodium	12 Mg Magnesium											13 Al Aluminium	14 Si Silicon	15 P Phosphorus	16 S Sulphur	17 Cl Chlorine	18 Ar Argon
19 K Potassium	20 Ca Calcium	21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	29 Cu Copper	30 Zn Zinc	31 Ga Gallium	32 Ge Germanium	33 As Arsenic	34 Se Selenium	35 Br Bromine	36 Kr Krypton
37 Rb Rubidium	38 Sr Strontium	39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	47 Ag Silver	48 Cd Cadmium	49 In Indium	50 Sb Antimony	51 Sn Tin	52 Te Tellurium	53 I Iodine	54 Xe Xenon
55 Cs Cesium	56 Ba Barium	57 La Lanthanum	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	79 Au Gold	80 Hg Mercury	81 Tl Thallium	82 Pb Lead	83 Bi Bismuth	84 Po Polonium	85 At Astanine	86 Rn Radon
87 Fr Francium	88 Ra Radium	89 Ac Actinium															
			L Lanthanides	58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolynium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium	69 Tm Thulium	70 Yb Ytterbium	71 Lu Luthetium
			Ac Actinides	90 Th Thorium	91 Pa Proactinium	92 U Uranium	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium	101 Md Mendelevium	102 No Nobelium	103 Lr Lawrencium

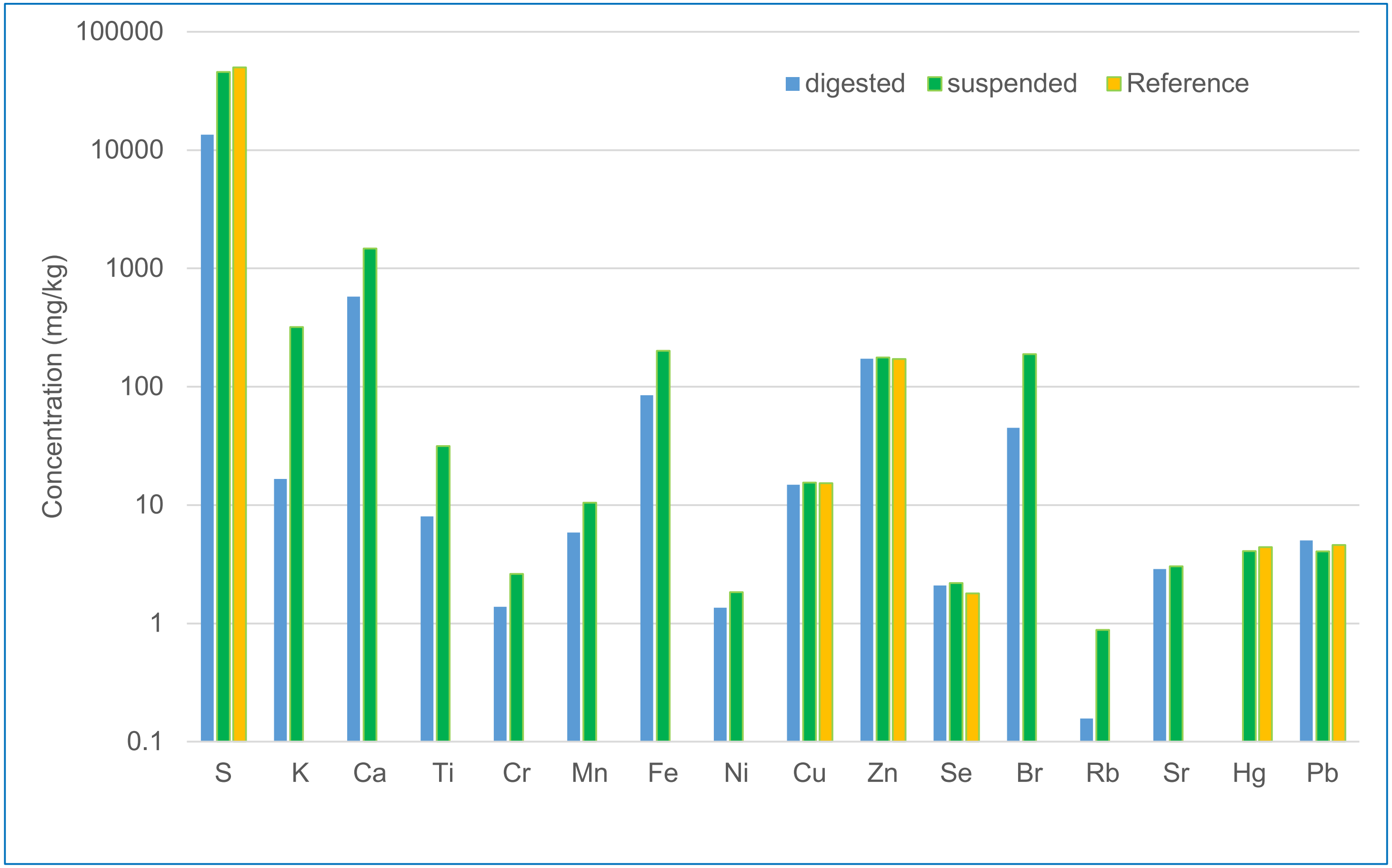
Analysis of human hair
Results

Reference standard NIES 13 – S2 PICOFOX



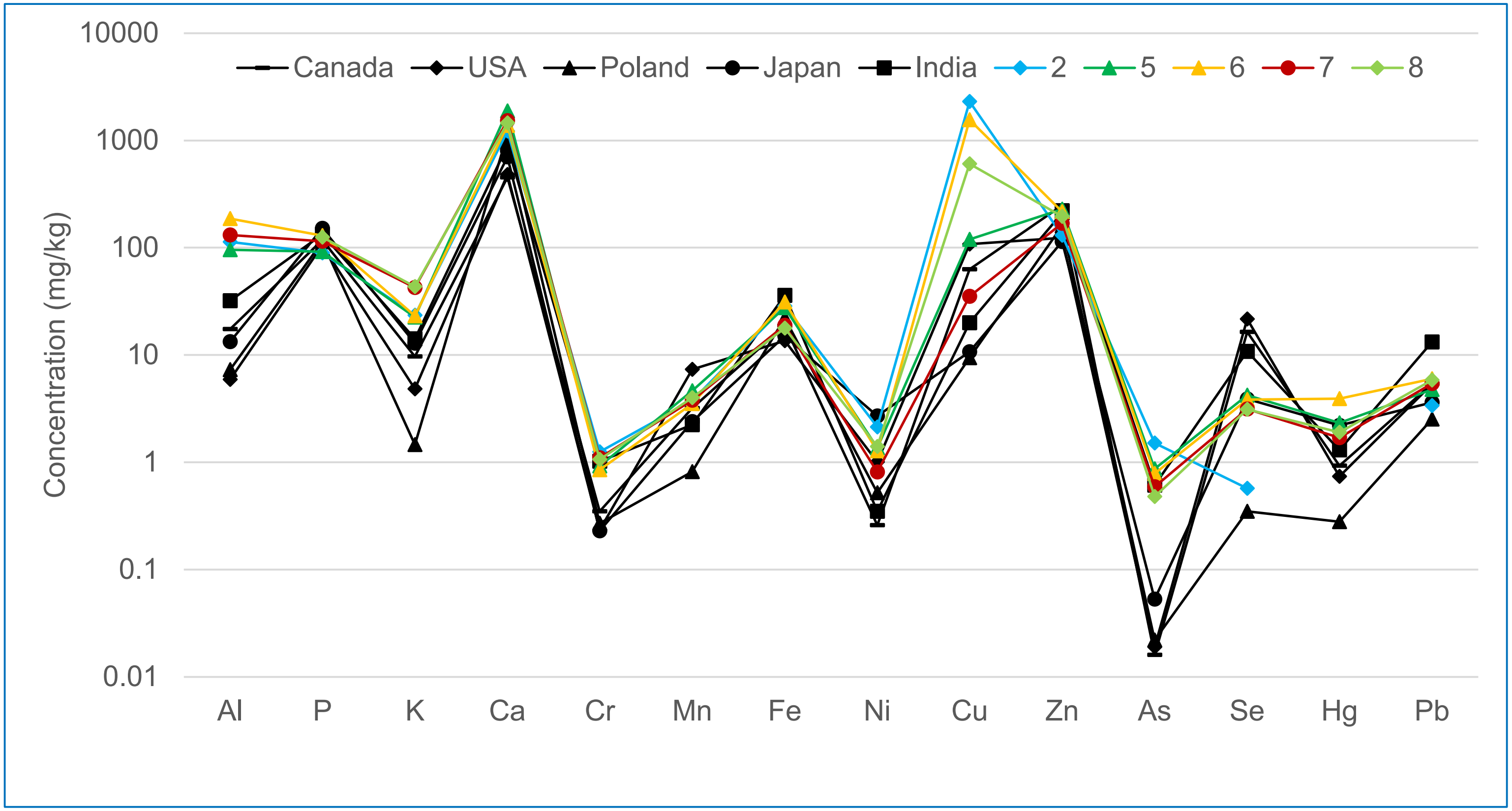
Analysis of human hair
Results

Reference standard NIES 13 – S4 T·STAR



Analysis of human hair
Results

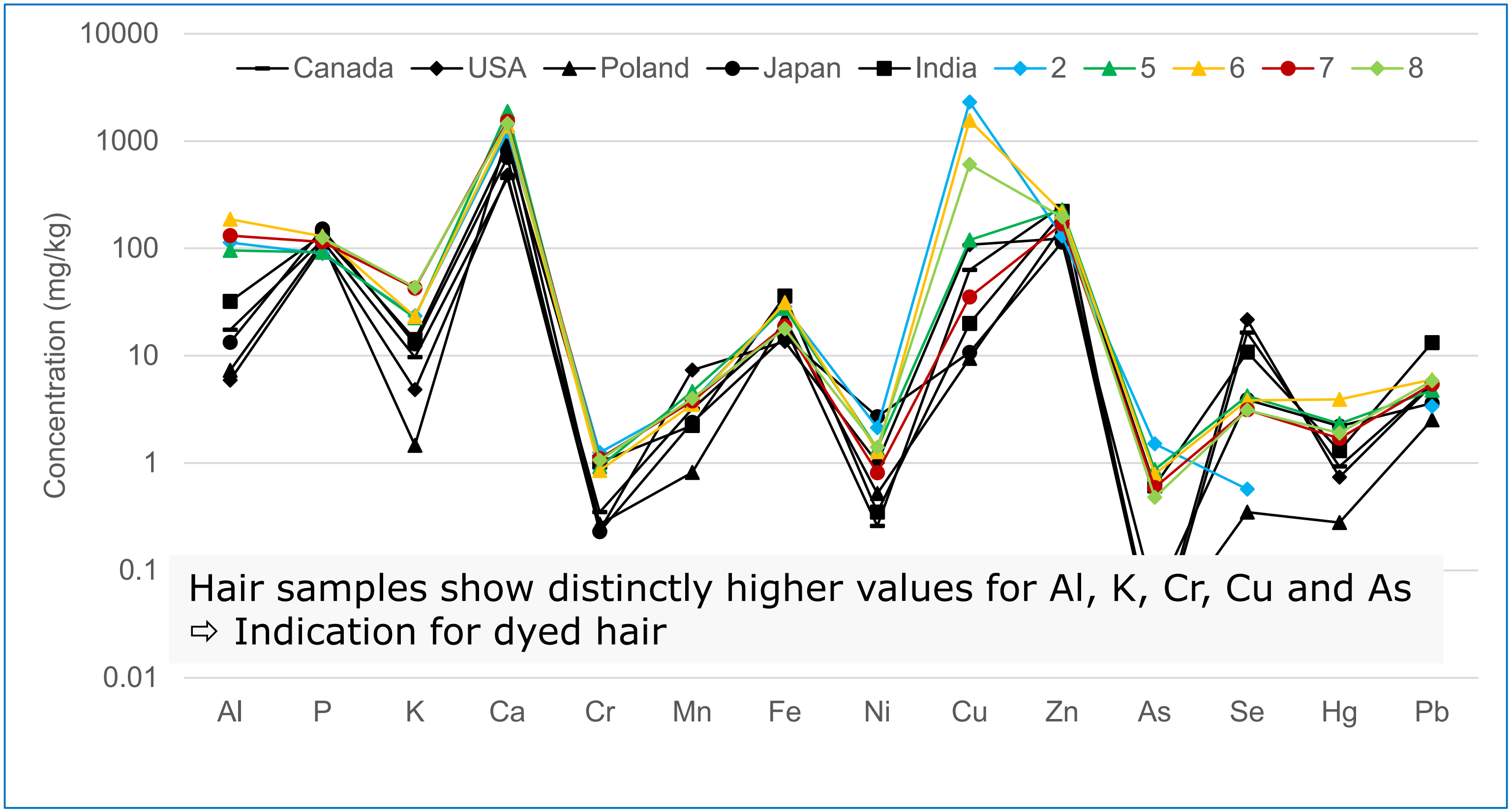
Hair samples results compared to literature values – S2 PICOFOX



Literature values: Tagagi et al (1986)

Analysis of human hair
Results

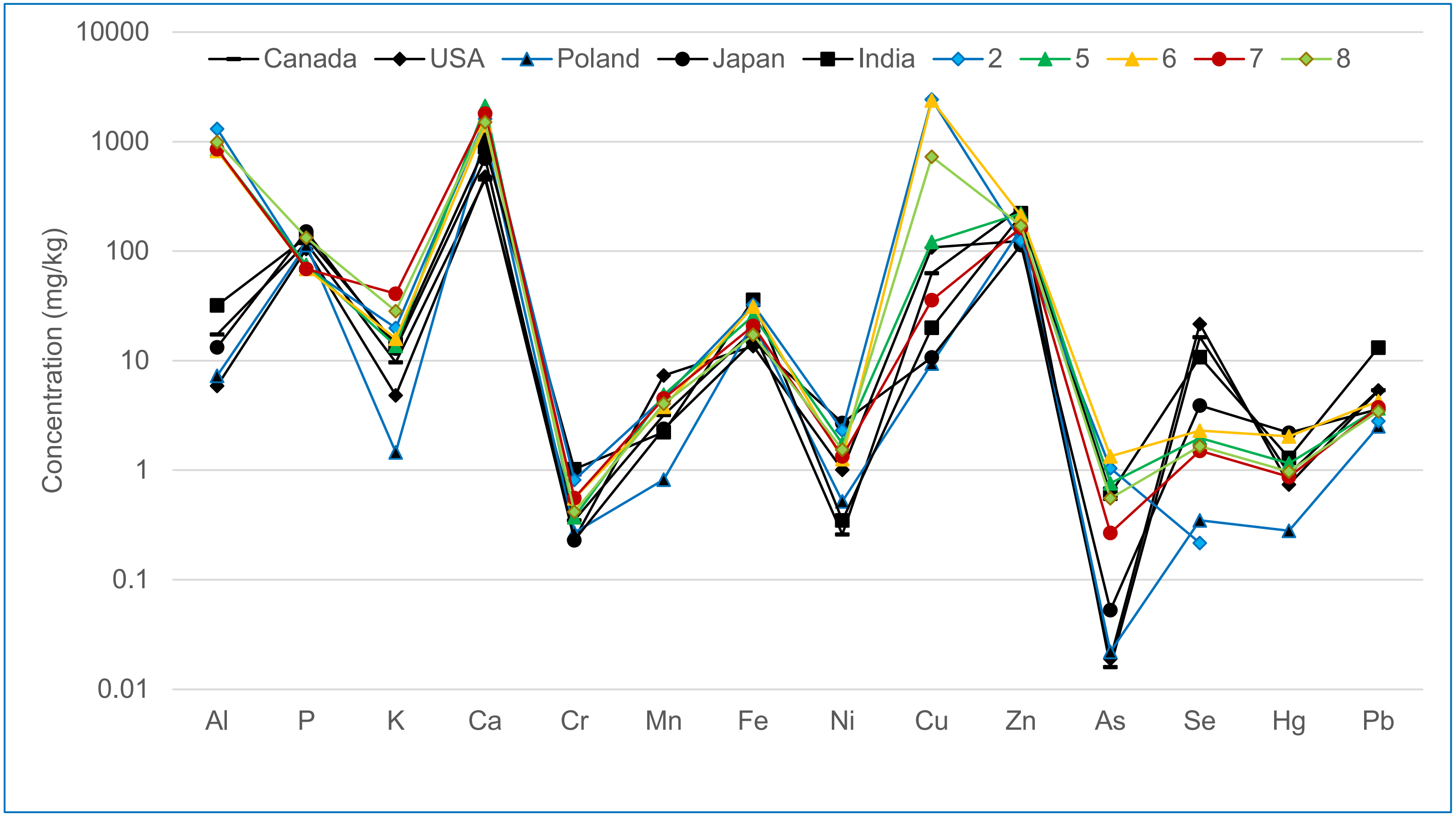
Hair samples results compared to literature values– S2 PICOFOX



(6) Literature values: Tagagi et al (1986)

Analysis of human hair
Results

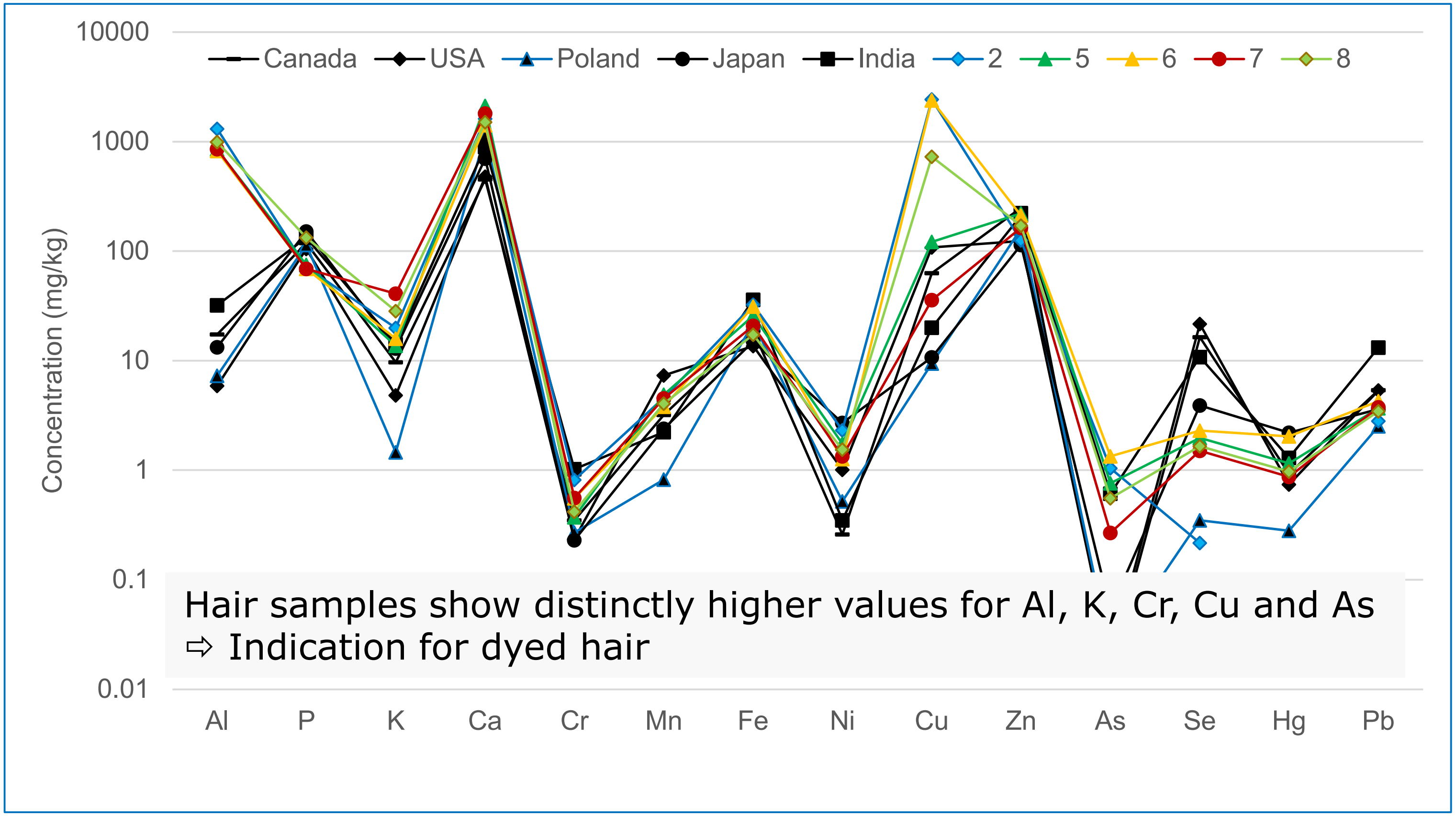
Hair samples results compared to literature values– S4 T·STAR



Literature values: Tagagi et al (1986)

Analysis of human hair
Results

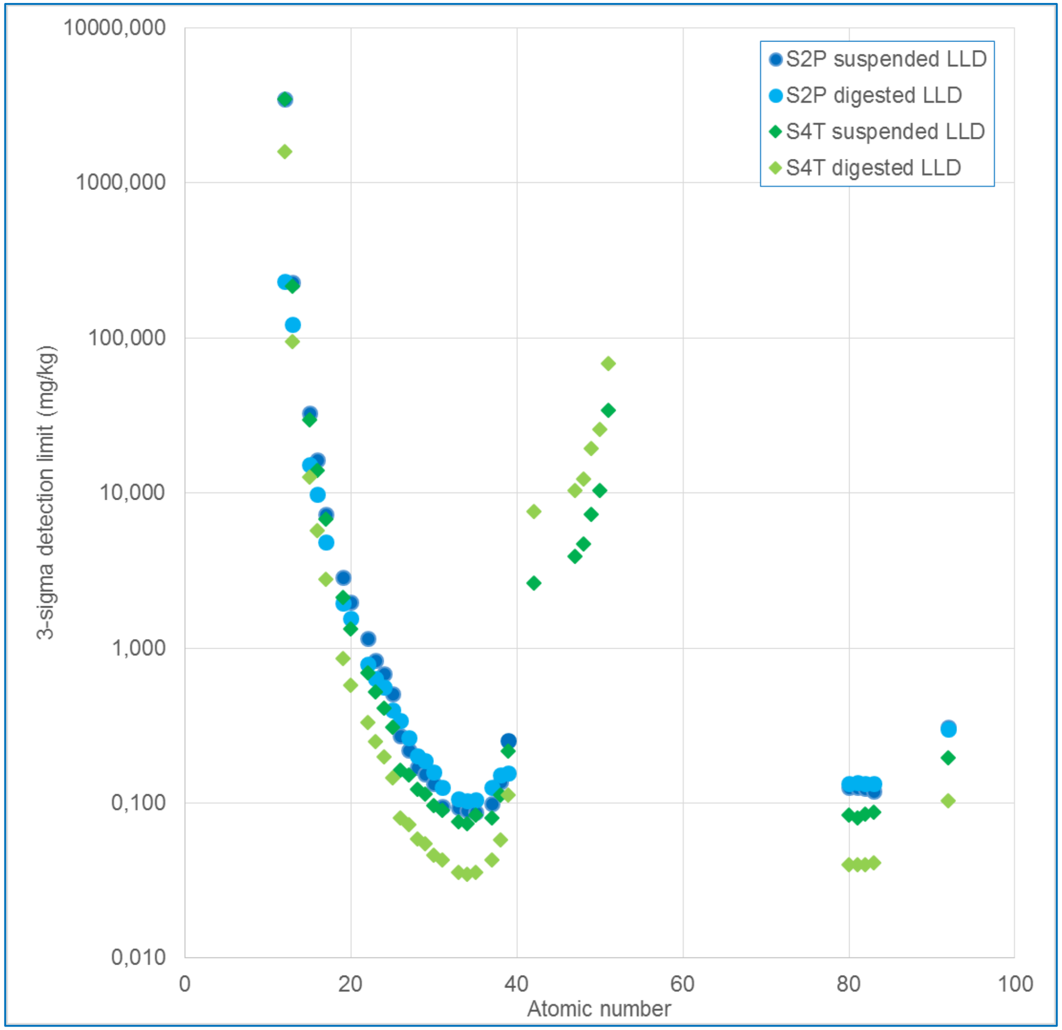
Hair samples results compared to literature values– S4 T·STAR



(6) Literature values: Tagagi et al (1986)

Analysis of human hair
Results

3-sigma LLDs



Analysis of human hair
Results & Discussion

- All elements of interest can easily be analyzed with the S2 PICOFOX and the S4 T·STAR, peapred as suspensions or after acid digestion
- Acid digestion gives better sensitivities but elements like Hg get lost during preparation
- The S4 T·STAR gives distinctly better sensitivities compared to the S2 PICOFOX
- A possible application strategy could be to use the S2 PICOFOX for a fast mobile analysis and the S4 T·STAR for a detailed lab-based analysis
- The TXRF method generally offers a high flexibility for enhanced analytical tasks (additional nutrients, toxic metals etc.)

Part II: Application of TXRF in medical research

Selenoprotein P is the essential selenium transporter for bones† *Metallomics*, 2014, 6, 1043

Nicole Pietschmann,[ab] Eddy Rijntjes,[a] Antonia Hoeg,[ab] Mette Stoedter,[a] Ulrich Schweizer,[c] Petra Seemann[d] and Lutz Schomburg*[a]

Metallomics

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Bone as matrix

Selenoprotein P is the essential selenium transporter for bones†

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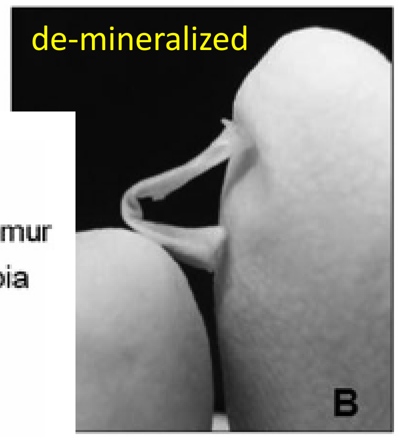

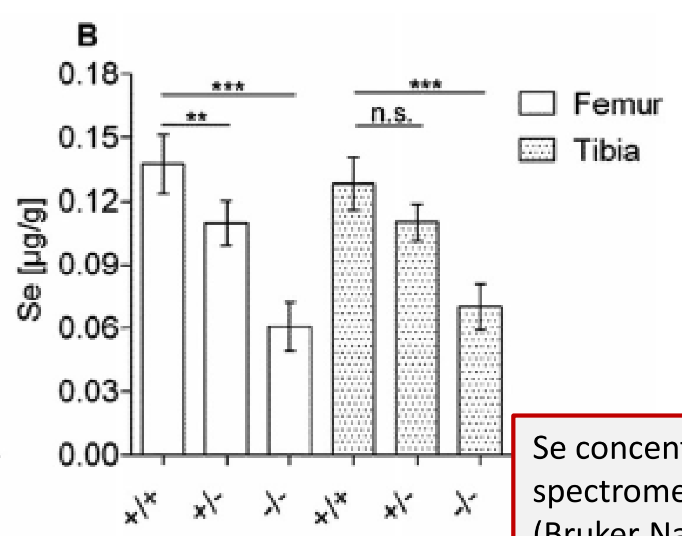

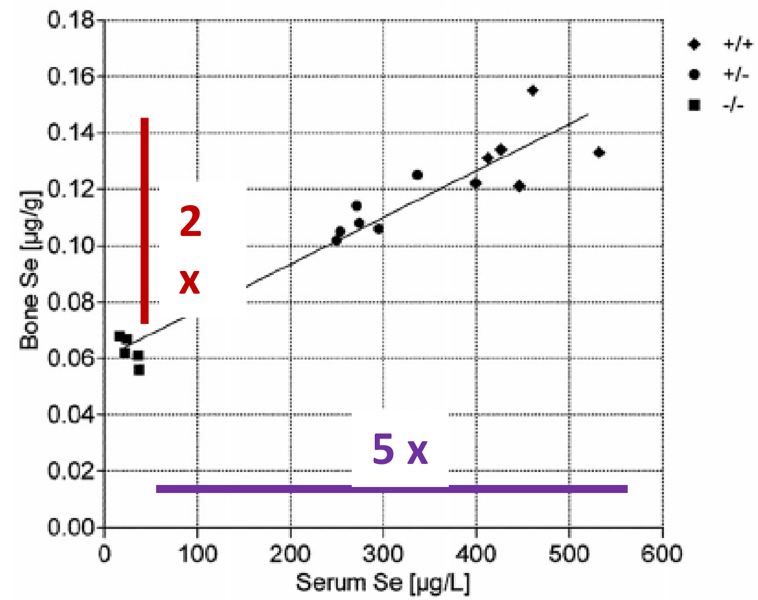

Se concentrations were quantified by **TXRF spectroscopy** using a **PICOFOX S2** spectrometer
(Bruker Nano GmbH, Berlin, Germany).
Briefly, bones were **decalcified** in 2.5% nitric acid and supplemented with **Gallium** (Ga) …
Alternatively, bones were treated with **proteinase K** and analysed as above.

DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED TRIAL OF SELENIUM IN GRAVES' HYPERTHYROIDISM

George J Kahaly, Michaela Riedl, Jochem König, Tanja Diana, Lutz Schomburg

ENDOCRINE SOCIETY

Se-Supplementation Trial in Humans

DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED TRIAL OF SELENIUM IN GRAVES' HYPERTHYROIDISM

George J Kahaly, Michaela Riedl, Jochem König, Tanja Diana, Lutz Schomburg

	Selenium + MMI	Placebo + MMI	P[a]
N	35	35	
Age, y, mean (SD)	44.5 (13.8)	44.5 (13.4)	1.0
Height, m, mean (SD)	1.69 (0.091)	1.69 (0.066)	0.988
Weight, kg, mean (SD)	68.8 (11.2)	71.8 (11.7)	0.270
Systolic blood pressure, mm Hg, mean (SD)	118.0 (11.5)	120.4 (14.2)	0.435

Table 2. Serological Results

	Selenium + MMI					Placebo + MMI				
	Week 0	Week 4	Week 12	Week 24	Week 36	Week 0	Week 4	Week 12	Week 24	Week 36
Selenium, µg/L	109 (25)	189 (209)	209 (261)	160 (51)	117 (43)	115 (29)	107 (34)	107 (27)	111 (27)	110 (26)
Vs baseline		$P = 0.030$	$P = 0.035$	$P < 0.001$	$P = 0.395$		$P = 0.036$	$P = 0.024$	$P = 0.574$	$P = 0.373$
Selenium vs placebo	$P = 0.384$	$P = 0.026$	$P < 0.001$	$P < 0.001$	$P = 0.406$					
SELENOP, mg/L	3.8 (0.8)	5.2 (1.1)	5.1 (1.1)	5.1 (1.3)	3.3 (0.9)	3.7 (1.1)	3.6 (1.0)	3.6 (1.0)	3.6 (1.0)	2.9 (0.8)
Vs baseline		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.008$		$P = 0.248$	$P = 0.413$	$P = 0.509$	$P < 0.001$
Selenium vs placebo	$P = 0.828$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.082$					

10 µl of serum was spiked with an internal Gallium and applied to polished quartz glass carriers.
Inter-assay and intra-assay CV were below 15% as determined using a commercial human reference serum sample (Seronorm; SERO AS, Billingstad, Norway).

Selenium and copper status - potential signposts for neurological remission after traumatic spinal cord injury

Julian Seelig[a], Raban Arved Heller[a,b], Julian Hackler[a], Patrick Haubruck[b,c], Arash Moghaddam[d], Bahram Biglari[e], Lutz Schomburg[a,*]

ELSEVIER

Diagnostic Trial of serum Se and Cu in TSCI

Selenium and copper status - potential signposts for neurological remission after traumatic spinal cord injury

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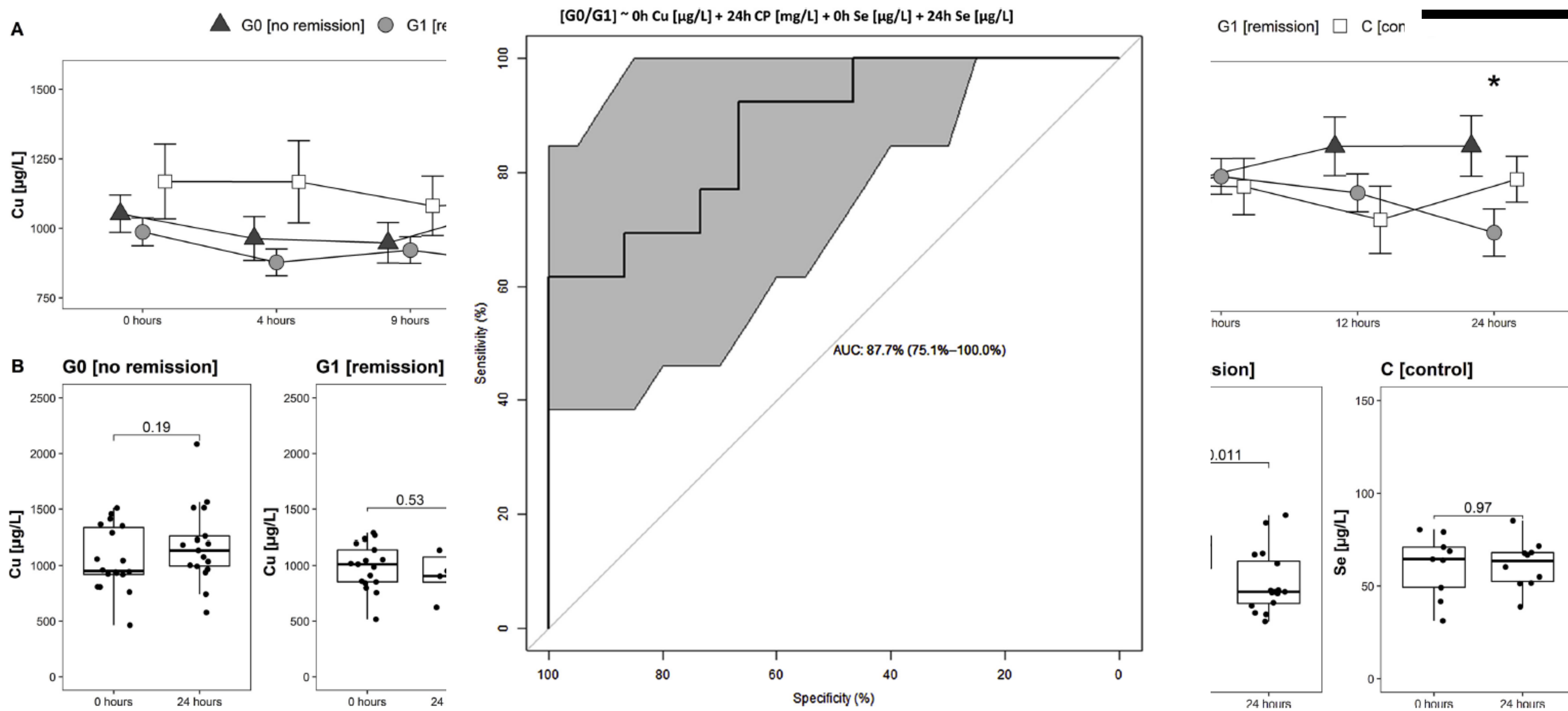

Aminoglycoside-driven biosynthesis of selenium-deficient Selenoprotein P

Kostja Renko[1], Janine Martitz[1], Sandra Hybsier[1], Bjoern Heynisch[1], Linn Voss[1], Robert A. Everley[2], Steven P. Gygi[2], Mette Stoedter[1], Monika Wisniewska[1], Josef Köhrle[1], Vadim N. Gladyshev[3] & Lutz Schomburg[1]

SCIENTIFIC REPORTS

Analytics of recombinant or purified Protein

Aminoglycoside-driven biosynthesis of selenium-deficient Selenoprotein P

SCIENTIFIC REPORTS

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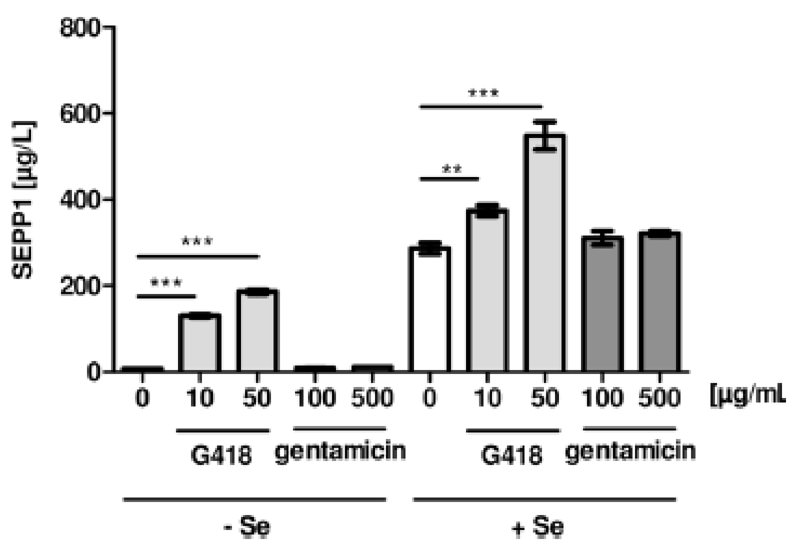

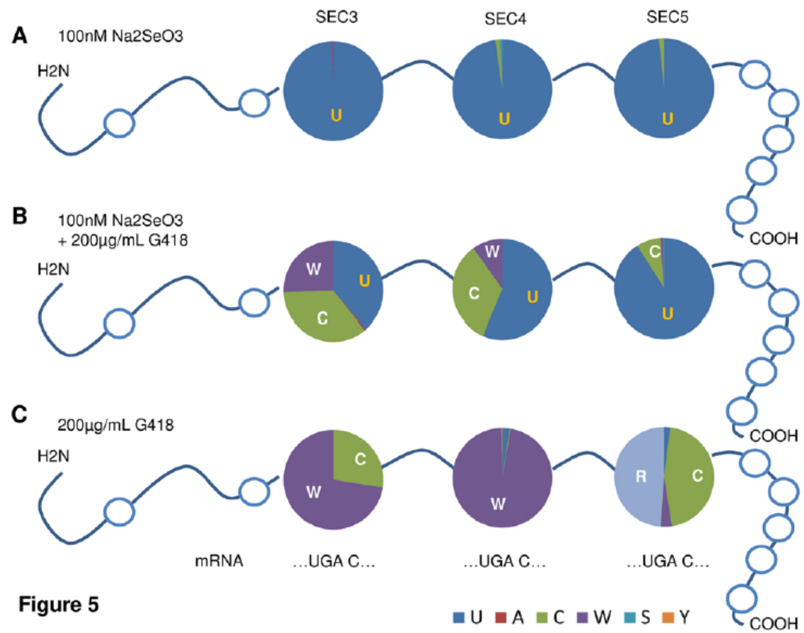

Figure 5

SELENOP was purified by immuno-affinity and subjected to LC-MS/MS analysis. (A) Sec in SELENOP was detected almost exclusively at the positions SEC3, SEC4 and SEC5 when cells were supplemented with selenite. (**B**) The pattern of amino acids inserted at the three Sec codons varied strongly when cells were grown in the presence of 100 nM selenite and 200 µg/mL G418.

Pre-diagnostic copper and zinc biomarkers and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

Carcinogenesis, 2017, Vol. 38, No. 7, 699–707

doi:10.1093/carcin/bgx051
Advance Access publication June 1, 2017
Original Article

Nested case-control Study => predictive Biomarker

Pre-diagnostic copper and zinc biomarkers and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

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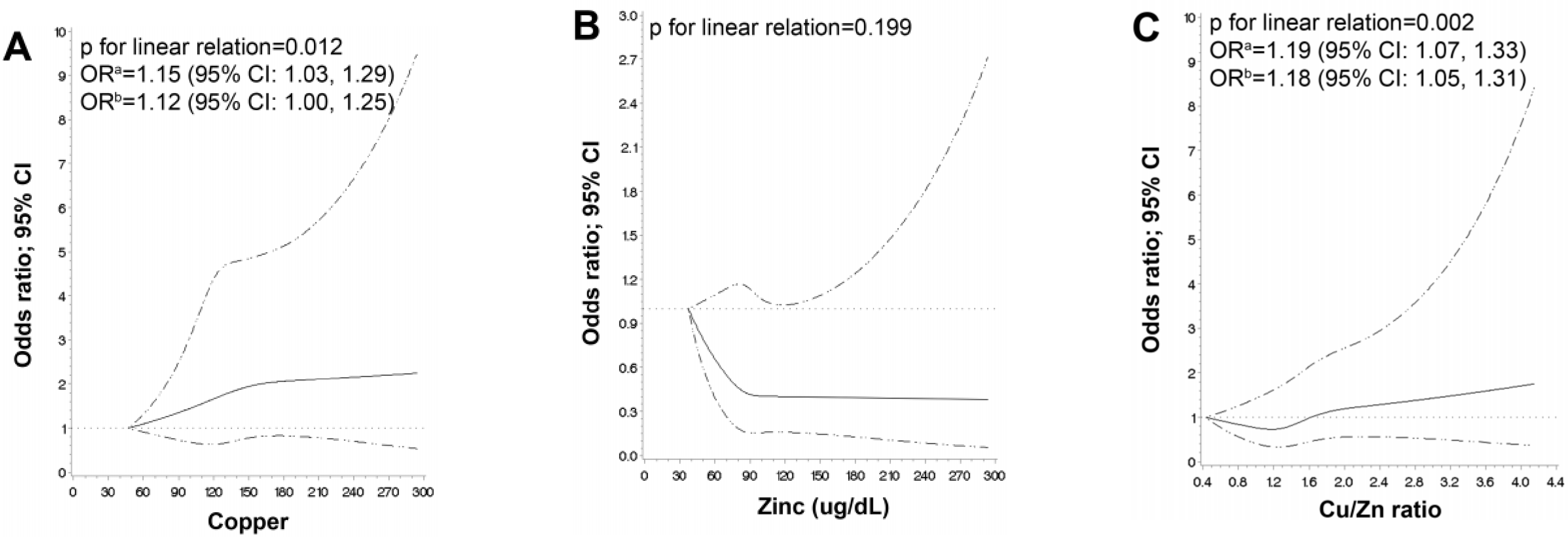


Bench-top total reflection X-ray fluorescence (TXRF) spectrometer (PicofoxTM S2, Bruker Nano GmbH, Berlin, Germany) was used to analyse the serum sample.

Part III: Summary & Q/A

Summary

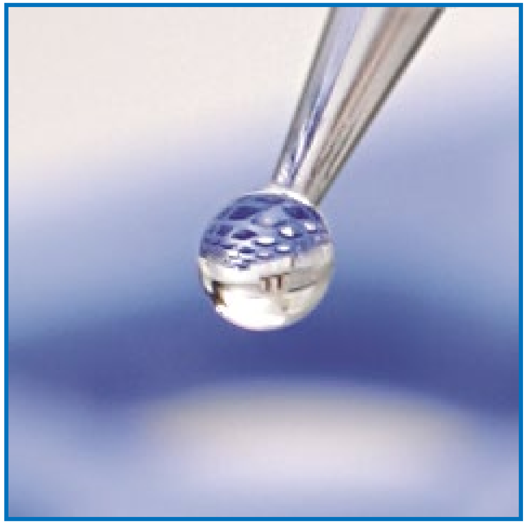
TXRF offers an ideal analytical solution for elemental analysis in medicical and biological research

- Analysis of small sample amounts in the low µl-range
- Simultaneous analysis of main- and trace elements
- Simultaneous analysis of other important samples types like buffer solutions
- Instruments can be operated in normal laboratory environments (small footprint, no external gases or cooling water necessary)
- Moderate analytical demands on laboratory staff
- Low analytical and lifetime costs

Q & A

Any Questions?

Please **type in** the questions you may have for our speakers in the **Questions Box** and click **Submit**



Innovation with Integrity

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