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My Hair Guides My Bioelement Dietary Requirements

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Abstract: Background. The bio element nutritional status may be defined as deficient, adequate, or excessive/toxic. Non-invasive method of hair collection makes it a tissue of choice for multi bio element analysis in precision medicine and nutritional epidemiology. Methods. Hair multi bio element profile of healthy 1073 subjects (339 Men. 734Women) was analyzed with the ICP MS. The frequency distribution of the hair log concentration data was used to assess the bio element nutritional status with the median derivatives bioassay. The assay allows for the reliable assessment of the bio element deficiency, adequacy, and excess. Results. Nutritional status of P, Zn, and the bio element pairs of I and Se, Ca and Mg, and Na and K are fully exposed and illustrated. The nutritional status of all twenty two hair bio elements are presented in Table 1 in their decreasing order of abundance ($\mu g \cdot g^{-1}$): Calcium, Zinc, Phosphorus, Sodium, Potassium, Magnesium, Silicon, Copper, Iron, Aluminum, Boron, Strontium, Chromium, Iodine, Selenium, and in ($ng \cdot g^{-1}$) for Nickel, Manganese, Molybdenum, Silver, Lithium, Cobalt and Gold. Conclusion. Hair bio element median derivatives bioassay proved to be a rapid, reliable, accurate, and fully reproducible method for assessing the bio element nutritional status, the nutritional health surveillance, and maintenance in precision medicine.

Keywords: Hair, Essentialbio Elements, Entanglement, Interactions, Median Derivatives Bioassay, Reference Standards, Reliability, Nutritional Status

1. Introduction

In the last three quarters of a century the definition of malnutrition has evolved from a simple "Faulty and imperfect nourishment" [1], to the current elaborate WHO definition.

"Malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients. The term malnutrition covers two broad groups of conditions. One is "under nutrition". which includes stunting (low height for age), wasting (low weight for height), underweight (low weight for age) and lack of micronutrient vitamins and minerals, their deficiencies or insufficiencies. The other diet related malnutrition subgroups are the no communicable diseases, such as heart disease, stroke, diabetes, and cancer" [2]. This broad WHO definition of malnutrition tell us that too much of the nutrients is as bad as is too little of them. Moreover, a complex situation may arise, like having one nutrient in excess and the other being deficient. Today, the time has come that everybody may look for his personal bio element precise nutrition or so called Rx (receipt) nutrition. And beyond the surrent Recommended Dietary Allowances (RDA's).

The aim of this article is to demonstrate how to assess the bio element nutritional status and to allow us for acceptable level of differentiation between the deficient, adequate and excessive bio element nutritional status. Here the term bio element is used as a common denominator for the major elements (electrolytes), trace elements and ultra-trace elements. Some of the bio elements are essential, some are conditionally essential, some were not shown to actively participants in the metabolism, and some are considered to be toxic at the extremely low concentrations. The situation is even the more complex since the essential bio elements may be also toxic depending upon their concentration [3]. Indeed, the rate at which the same dose of the bio element is

administered may result in their different metabolic response [4]. Thus, the purpose of this article is to define how the level of the nutritional bio element exposure, i.e. it's nutritional status, can be accurately assessed over their entire spectrum of ranges, from an bio element deficient, adequate, and excessive dietary intake, respectively.
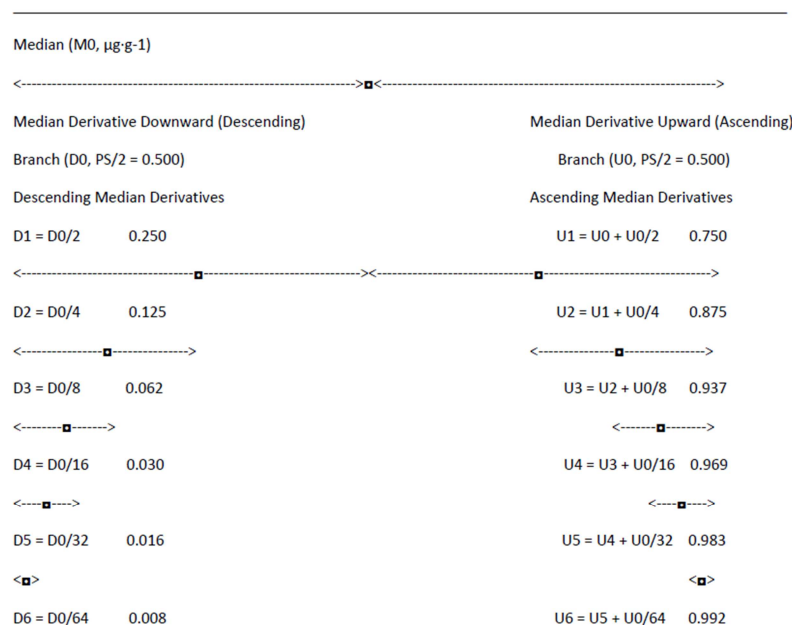
Median (M0, µg·g-1)

Descending	Ascending
Median Derivative Downward (Descending) Branch (D0, PS/2 = 0.500)	Median Derivative Upward (Ascending) Branch (U0, PS/2 = 0.500)
Descending Median Derivatives	Ascending Median Derivatives
D1 = D0/2 0.250	U1 = U0 + U0/2 0.750
D2 = D0/4 0.125	U2 = U1 + U0/4 0.875
D3 = D0/8 0.062	U3 = U2 + U0/8 0.937
D4 = D0/16 0.030	U4 = U3 + U0/16 0.969
D5 = D0/32 0.016	U5 = U4 + U0/32 0.983
D6 = D0/64 0.008	U6 = U5 + U0/64 0.992

Figure 1. The median derivatives bioassay for assessing the bioelements nutritional status.

Our biological indicator tissue of choice for assessing the bio element nutritional status is human hair [5]. Hair is a biological indicator tissue that can be non- invasively (painlessly) sampled with a simple scissors, and the collected hair samples are easily stored and transported to be later analyzed at some adequately equipped analytical laboratory [6, 7]. Hair follicle is a natural collector of the bio elements in our body (Figure 2), some kind of our, always running, personal metabolic ward facility. The numbers shown are the logarithms since the hair bio element concentrations ranges over the eight orders of magnitude. The hair bio element analysis has an advance in comparison with the alternative bio indicators. The superiority of hair iodine against the iodine in the whole blood and urine biological indicator tissues is demonstrated in Figure 3 and Figure 4. The highest concentrations of iodine were observed in the hair, then in urine, and they were the lowest in the whole blood. Today, urinary iodine is considered to be the bio indicator tissue of choice, the so called "golden standard", for assessing the iodine bio element body status, but the difficulties in collecting, transporting, and analyzing urinary samples are notorious [8]. Of all the here tested twenty two hair bio elements, only the blood iron concentrations were higher in the whole blood than they were in the hair. However, great hopes for broader use of the hair bio element analysis in the bio-medicine wane soon when the actual analytical data of supposedly replicated samples, were shown to be unacceptably dispersed. This article shows that the observed unacceptable dispersion of the analytical data between the replicates, is not due to the chemical analysis inadequacy, but due to the Inappropriate way of analyzing their frequency distribution. Indeed, the hair samples from even the same head are not homogenous entities, as it was taken for granted, but a sex dependent mixture of hair treads in different stages of hair cyclic follicular growth [9].

Vignette 1. Solving the riddle of analytical variability of calcium in hair replicate samples

Laboratory	Mean	1	2	3	4	5
Calcium (µg·g^{-1})	802 ± 37	844	1555	431	686	1024

Miekeley et al. (1988) (1) published an article which discussed how reliable are human hair reference intervals for trace elements. They gave (supposedly) the same hair samples to five different analytical laboratories.

Numerous studies showed similar inconsistency in analyzing duplicate hair samples, and what is used as a principal objection for wider use of hair bio elements in clinical medical practice.

However, Dinh and Wang (2022) (2) revealed that the replicate samples are not a homogenous set of hair threads, but a complex mixture generated by hair follicles in different phases of their sex dependent hair cell cycle. Indeed, the here presented study (Table 1) showed that hair calcium has a distinct sex dependent statistical data distribution, which resulted in a wide range of adequate nutritional status concentrations.

Calcium (µg·g⁻¹)	Men				Women.			
	Min	Median	Max	Optimum	Min	Median	Max	Optimum
	281,0	492.0	816.4	599 - 761	487.7	1721.4	4 426	971 – 4 195

This study proved the sex dependent difference in calcium intermediary metabolism. Apparently, women have a higher internal turnover of calcium than men. Also, our results revealed substantial difference between duplicate hair samples in Mekeley et al study (1). Our study indicates that Labs 1 and 4 analyzed mostly the mixed men and women hair, Lab. 2 and 5 analyzed mostly women hair, and Lab 3 analyzed predominantly men hair. Thus, the reported inadequacy in the analysis of calcium in the hair of replicates or duplicates, reflects the inhomogeneity of the analyzed hair threads mix.

1) Miekeley B, Dias Carneiro MTW, Porto de Silveira CL. How reliable are human hair reference intervals for trace elements? Sci Total Environment 1998; 218: 9-17.
2) Dinh K, Wang Q. A probabilistic Boolean model on hair follicle cell fate regulation by TGF-β, Biophysical J 2022; 121: 1-15.
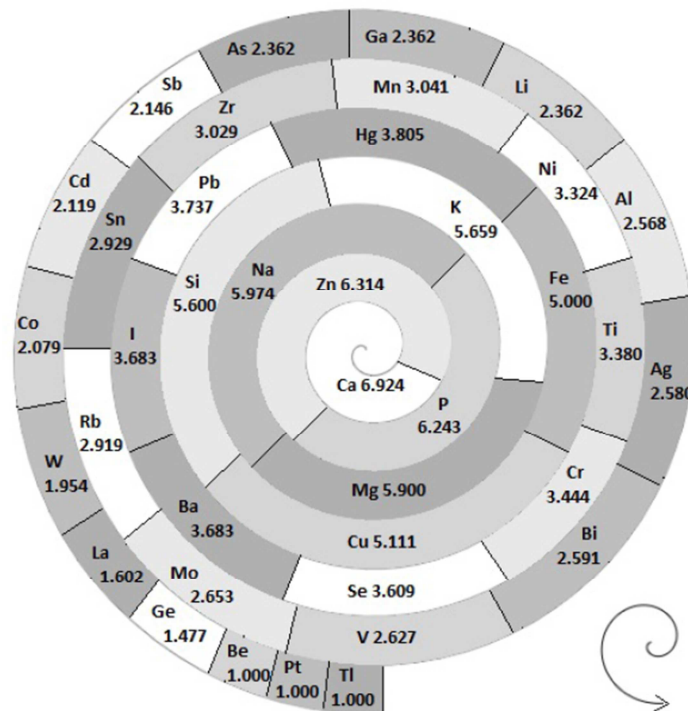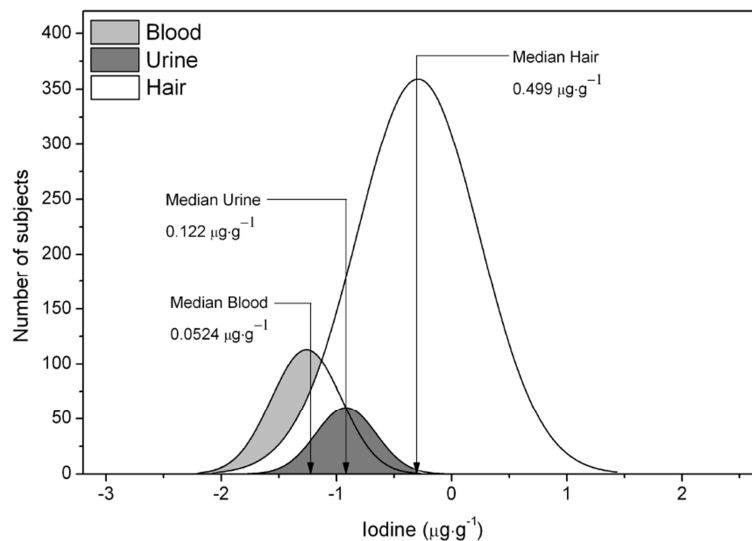
Figure 2. Hair Bio elements abundance (log µg·g⁻¹).

Figure 3. Iodine in the hair, whole Blood and ur. Gausian (µg·g⁻¹).

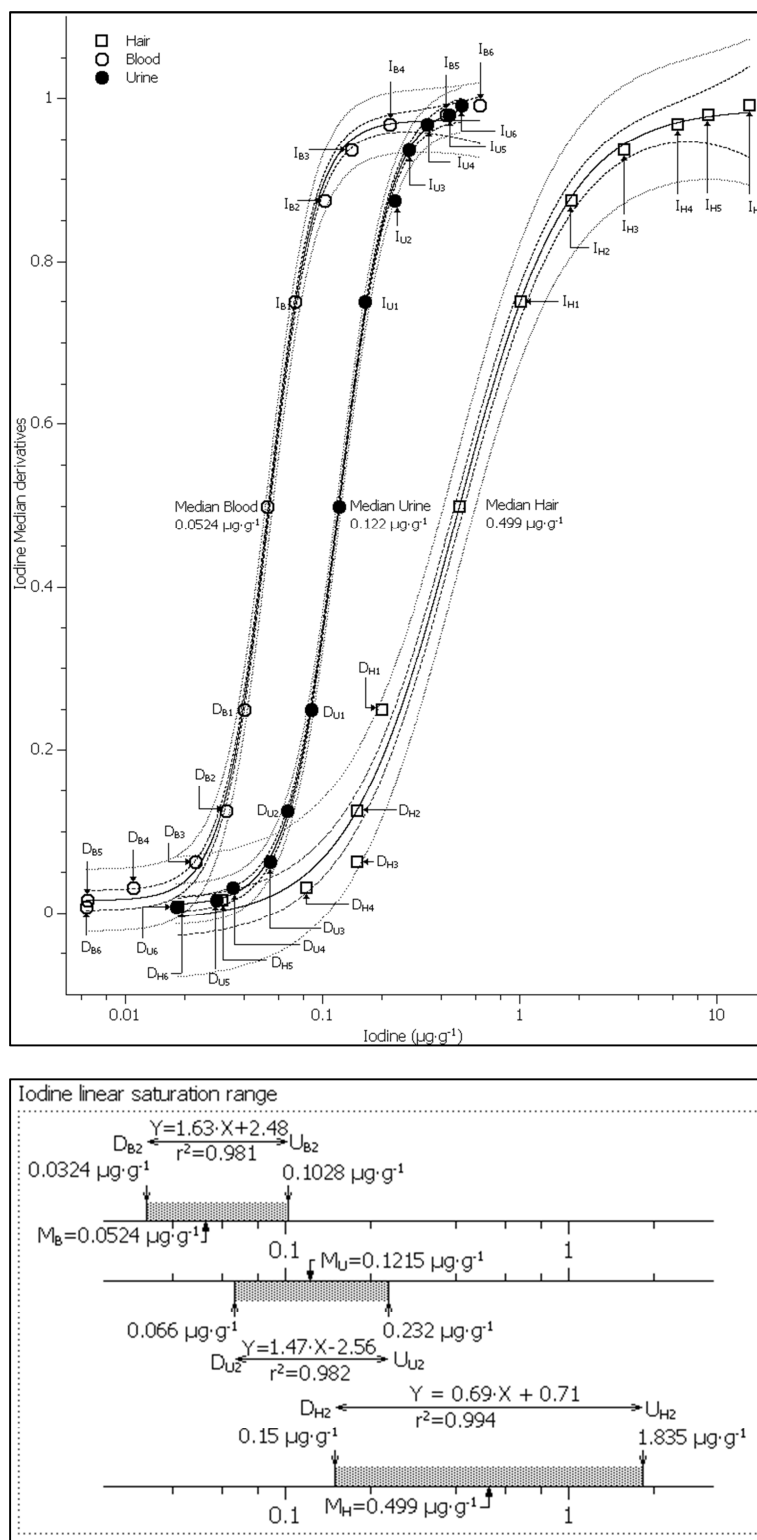**Figure 4.** *Iodine in the hair, whole blood and urine. Golden Range Yardstick (log $\mu g \cdot g^{-1}$).*

We studied the frequency distribution of hair bioelement concentrations, their medians and median its derivatives to assess the particular bioelement nutritional status. First we assess the median (M0) hair bioelement concentration of the subject population. By definition, one half of the studied population was above the median (upward median branch, U0), and the other half was below the median (downward median branch, D0). Hence, the population size (PS) for M0 is the sum of the respective upward and downward median branches around the central inflection "hinge" M0, i.e., PS = U0 + D0 = 0.5 + 0.5 = 1.0. Both the respective upward and downward median branches can be further divided in the same "median of median" way into a series of sequential median derivatives (U 0,1,2,3…n-1, n and D0,1,2,3,…n-1, n).

For every median derivative of the population, the actual hair bioelement concentration can be identified. Thus, instead of mechanically throwing the preconceived percentile grid upon the observed data, we inferred the median derivative grid out from the data set itself.

2. Subjects and Methods

In this article we studied multi bio element profile in the hair of 1073 apparently healthy men (n=330) and women (n=734) in Croatia (Figure 1) in order to assess their bio element nutritional status for the precision medicine (!0). All the hair bio elements in this study were analyzed at the Center for biotic Medicine (CBM), Moscow, Russia. CBM is an internationally accredited commercial bio element analytical laboratory. Data on hair preparation for analysis are shown separately in Appendix 1.

Mediaan Derivatives Biossay

The skewed cumulative exponential distribution curve of the initial data set was log transformed, what generated a standard Gaussian bell shaped data distribution. Such "normalized" frequency data distribution of the hair bio elements was analyzed with the median derivative bioassay. This log transformed data were fitted to form the sigmoid logistic regression function (power function) [5, 10]:

$$A2 + (A1 - A2)/[1+(x/x0)p].$$

Where A1 is the initial value (lower horizontal asymptote), A2 is the final value (upper horizontal asymptote), x0 is the center (point of inflection) of the detected median (M0), p is the power (the parameter that affects the slope of the area about the inflection point). The OriginPro 8.0 data analysis and graphing software was used for this analysis (OriginLab Corp., OriginPro Version 8.0., Northampton, MA). As a rule, one standard deviation of the total tested population formed the emerged linear segment of the sigmoid saturation curve.

Visual examination of the bioassay sigmoid dose-response saturation curve allows for the determination of the natural cut off points of the central linear portion and their upper (Maximum, Max) and lower (Minimum, Min) tails. The central linear segment indicates the adequate range of a given bio element hair incorporation. That linear segment is considered to reflect the phase of a transient equilibrium between the input of the available circulating bio elements to the hair follicle, and the bio element actual uptake by the hair follicle. Thus, the level of a bio element below that cut off point of linearity, is considered t to be a border separating the deficient and adequate nutritional status. Similarly, the bio element concentrations above the linear portion of the sigmoid saturation curve, represents an excessive nutrient (bio element) intake which is no more regulated in a linear fashion. Indeed, the central linear portion of the sigmoid saturation curve is considered to represent the adequate bio element nutritional status. It should be noted that this natural bio element response pattern, suggests that the adequate nutritional status reference values, need to be presented in the range format. [12].

Also, by identifying this linear segment of the sigmoid saturation curve allows us to further subdivide the adequate nutritional status, into the different sub-segments, i.e., low adequate, ADEQUATE GOLDEN RANGE (conditionally optimal range), and supra adequate regions. The low adequate segment would be comparable with the concept of a subclinical deficiency, whereas the supra adequate region, or the sub pharmacological region, would be more important to the environmental exposure studies. In this study the adequate nutritional status range is divided into (a) low adequate, (b) ADEQUATE GOLDEN RANGE, and (c) supra adequate regions with a 30:60:10 percent ratio. All the defined cut off points for every tested bio element are shown in Table 1, where they are assigned as Minimum (Min) and Maximum (Max). All the concentrations below the Minimum are considered to be overtly deficient, whereas all the concentrations above the Maximum are considered to be excessive.

Table 1. Adequate bio lement Nutritional status of men (M) and women (W) as assessed with a hair Median Derivatives Bioassay.

Element		ADEQUATE (LINEAR PORTION of a SIGMOID CURVE) RANGE (µg·g⁻¹)							
		Minimum (Min) GOLDEN RANGE YARDSTICK (Lowewr Limit) Conditionally optimal range (30 – 90% of Adequate range)						Maximum (Max) (Upper Tolerance Limit)	
		Men	Women			Men	Women		
		Man	Women	MEDIAN M	W				
Calcium	Ca	291.0	487.7	492.0	1721.4	816.4	4 426.	599 - 761	971 -4195
Zinc	Zn	126	131	179	183	247	234	103 – 198	181 - 224
Phosphorus	P	121.9	124.5	150.8	154	184.1	199.2	131 – 188	147 -177
Sodium	Na	84.0	55.6	471.4	254.0	1450.0	1397.0	411 – 1313	272 -1117
Potassium	K	25.8	8.9	31.5	14.25	1079.0	467.0	315 -974	137 -330
Magnesium	Mg	20.5	29.7	47.1	137.2	90.2	279.6	23.9 – 32.2	75.0 - 254.6
Silicium	Si	1 1.01	11.58	12.99	30.58	59.86	85.76	14.67 – 54.97	23.21 – 78.34
Copper	Cu	8.50	9.11	11.1	11.8	19.0	18.0	15.85 – 17.95	15.33 – 17.11
Iron	Fe	6.09	6.11	12.72	32.19	28.23	12.42	8.4 –26.02	6.74 – 11.79
Aluminum	Al	2.70	1.86	6.54	4.74	17.49	14.29	5.14 -16.01	5.59 – 13.06
Boron	B	0.771	0.472	2.21	1.04	6.51	3.89	2.49 -5.94	1.48 -2.55

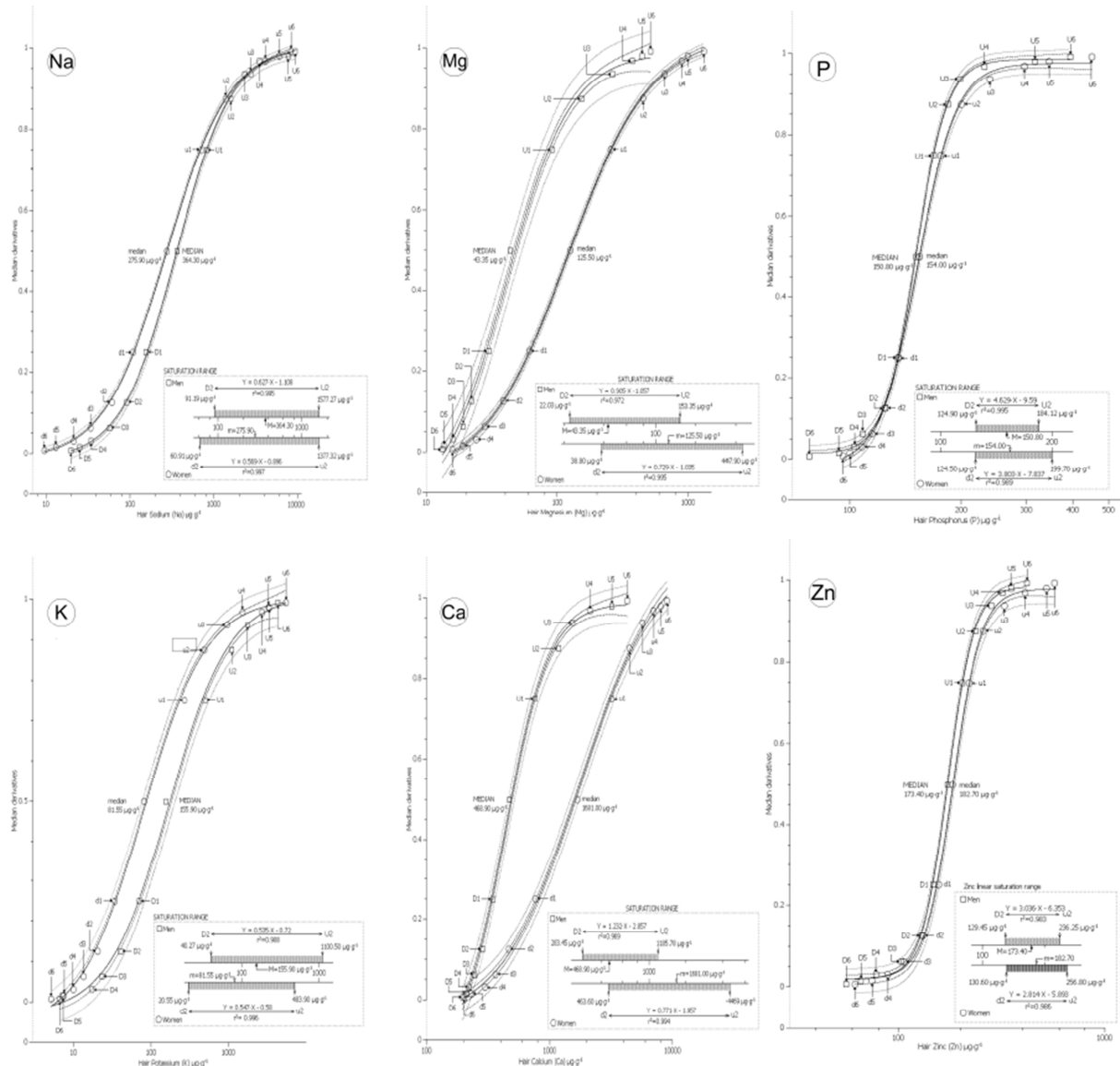| Element		ADEQUATE (LINEAR PORTION of a SIGMOID CURVE) RANGE ($\mu g \cdot g^{-1}$)						
Strontium	Sr	0.420	0.850	0.867	1.28	3.120	7.26	1.23 -2.85	2.77 -6.62
Chromium	Cr	0,212	0.389	0.397	0.317	0.867	0.626	0.197 – 0.588	0.231 – 0.395
Iodine	I	0.150	0.150	0.482	0.508	1.765	1.960	0.436 – 1.603	0.257 – 0.531
Selenium	Se	0.134	0.078	0.346	0.269	0.701	0.623	0.304 -0.644	0.233 - 0.569
Nickel	Ni	0.098	0.150	0.215	0.351	0.432	0.926	0.100 - 0.300	0.233 - 0.600
Manganese	Mn	0,090	0.091	0.100	0.118	0.749	0.628	0.288 - 0.863	0.253 – 0.574
							nano	$g \cdot g^{-1}$	
Molybdenum	Mo	34	27	49	39	71	57	45 - 67	44 – 76
Silver	Ag	15	15	50	76	22	69.5	75 -195	22-63
Lithium	Li	15	14	28	25	100	86	42 - 92	36 - 79
Cobalt	Co	6	9	14	28	22	74	49 – 74	41 - 79
Gold	Au	7	7	30	34	61	90	16 - 55	27 -87

Figure 5. *Hair (log $\mu g \cdot g^{-1}$) Sodium and Potassium) (Left column), Magnesium and Calcium (Middle column), Magnesium and calcium, Phosphorus and Zinc (Right column).*

3. Results

The results of assessing the bio element nutritional status with the aid of the median derivatives bioassay are shown for phosphorus and zinc *(Figure 5 Left column))*, for the hair phosphorus invariance across the Earth globe (Figure 6), and for the physiologically entangled bio element pairs of iodine (Figure 5 Left) and selenium (Figure 5 Right), for sodium and potassium *(Figure 5 Middle column)*, and calcium and

magnesium *(Figure 5. Right column)*. The combined comparative box-plot of Ca, K, Mg, Na, P, and Zn is shown in Figure 9. The assessment of the nutritional status of the other tested bio elements is presented in the tabular form without the graphic presentation. The other tested bio elements have identical pattern of sigmoid saturation curves, i.e., in decreasing order of concentration: Silicon, Copper, Iron, Aluminum, Boron, Strontium, Chromium, Nickel, Manganese, Molybdenum, Silver, Lithium, Cobalt and Gold. All the hair bio element concentration data are expressed in $\mu g \cdot g^{-1}$ and in $ng \cdot g^{-1}$ for Mo, Ag, Li, Co and Au. (Table 1). The so far studied bio element interactions are shown in Figure 11. The hair median derivatives bioassay is presented in Figure 1.
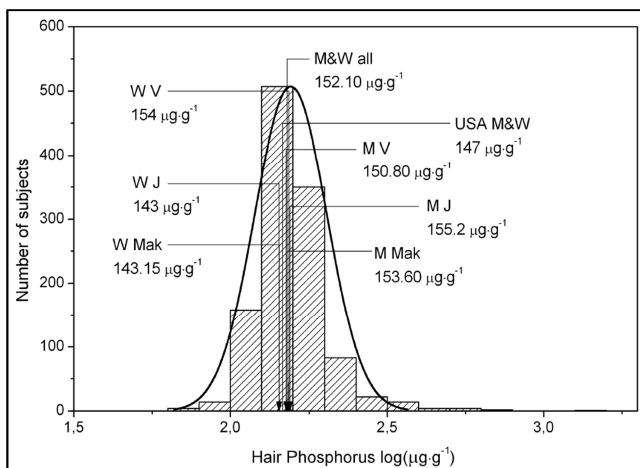

Figure 6. *Invariance of hair phosphorus ($\mu g \cdot g^{-1}$). W Mak · women Northern Macedonia, W J · women from Japan, W V · Croatian women, ALL M&W · all Croatian men and women, M V · Croatian men, U M&W · Texas men and women,, M J · Japan men, M Mak · men Northern Macedonia.*

PHOSPHORUS and ZINC (Figure 5 Left column). Major bio element phosphorus and the trace element zinc are two essential bio elements [13]. The low limit threshold of adequate phosphorus nutritional status is ($\mu g \cdot g^{-1}$) 121.9 in men and 124.5 for women, respectively (Table 1). That is shown in a Minimum (Min) column and it is considered to represent a breaking point between the deficient and adequate phosphorus nutritional status, respectively. From that Min point or lower limit, the hair phosphorus concentration rises straight up to its Maximum (Max) or upper tolerance limit of 194.1 and 199.2 for men and women, respectively (Table 1). The entire adequate (linear) range may be further subdivided by an 30:60:10 ratio, The first 30%s of that subdivision of the phosphorus linear segment of the sigmoid saturation curve, represents the sub- adequate or sub-clinical bio element nutritional status. The next subdivision of 60% represents the true ADEQUATE GOLDEN RANGE (Conditionally Optimal Range) of the bio element phosphorus nutritional status, and the final upper 10% of the subdivision is a supra adequate phosphorus nutritional status. Let us remind that all of these three subdivisions actually belong to the adequate phosphorus nutritional status range. Indeed, hair phosphorus concentrations were almost identical for both men (M) and women (W) in two different population groups in Croatia

(initials J and V), North Macedonia (M), Japan, and the US (Texas) where mostly Mexican were involved (Figure 6) [14]. Apparently, sex and race does not measurably affect the human races phosphors nutritional status. It is pertinent to note here that both phosphorus and zinc showed almost identical hair zinc concentration invariance. Indeed, zinc is like phosphorus, an essential bio element, but zinc was never directly associated with the metabolic cycling of ADP and ATP, respectively.

Since zinc is very much involved in body growth. i.e., an energy high demanding process, it is not surprising that zinc and phosphorus are so tightly related). Indeed, zinc essentiality was recognized after stunted growth of Iranian children was proved back in 60' to be due to zinc nutritional deficiency. The border between the deficient and adequate zinc nutritional status, as assessed with the median derivatives bioassay is Min 126 and 131 for men and women, respectively, whereas, the upper Max level off of the linear part of the sigmoid saturation curve, is 184.1 and 247 for men and women, respectively. The optimal hair zinc nutritional status, lies between 173-198 and 191-224 for men and women, respectively. The previous idiorrhythmic experiments demonstrated that dietary zinc reference values should be considered in their range format and beyond their current daily defined dietary allowances [12].

Thus, the hair phosphorus concentrations below 121.9 or above 199.3 should be considered as ominous warning sign of somebody metabolic health. Apparently, the invariant stability of hair phosphorus may serve as an internal standard against which other bio elements may be compared, like namely Na and, K, and Mg and Ca. The summary comparative box-plot enlist six pairs of bio elements, i.e., P·Zn, Ca·Mg, and Na·K are visually displayed; an intriguing network pattern comparing several bio elements simultaneously (Figure 10).

IODINE and SELENIUM (Figures 5A and 5B). Historically, iodine is the first identified trace element (oligo element in French, spurn element in German), or bio element in this article annotation [15]. Although the iodine deficiency was known since the end of the18th century, it was rampant in the Great Lakes of the USA all the way in the 20th century and even now is a problem in the lowlands of Holland and Belgium, but also in the river Poe valley in Italy, Himalayan countries, and wide areas of China and India. All over the world, iodine deficiency is frequently observed but preventable human health deficiebct. The iodine status is so far, mostly assessed by analyzing its urine concentrations; a cumbersome and low sensitivity biological indicator tissue, often missing to detect a low urine iodine concentrations, even when analyzed with a highly sensitive analytical instruments like the ICP MS. When our Croatian hair iodine analytical data were subjected to a median derivatives bioassay analysis, the characteristic sigmoid saturation curve was obtained (Figure 7). Our data showed that adequate linear region of the sigmoid saturation curve covers the range starting at Min of 0.150 for both men and women, whereas the Max appears to be at 0.765 and 0.509 for men and women, respectively.
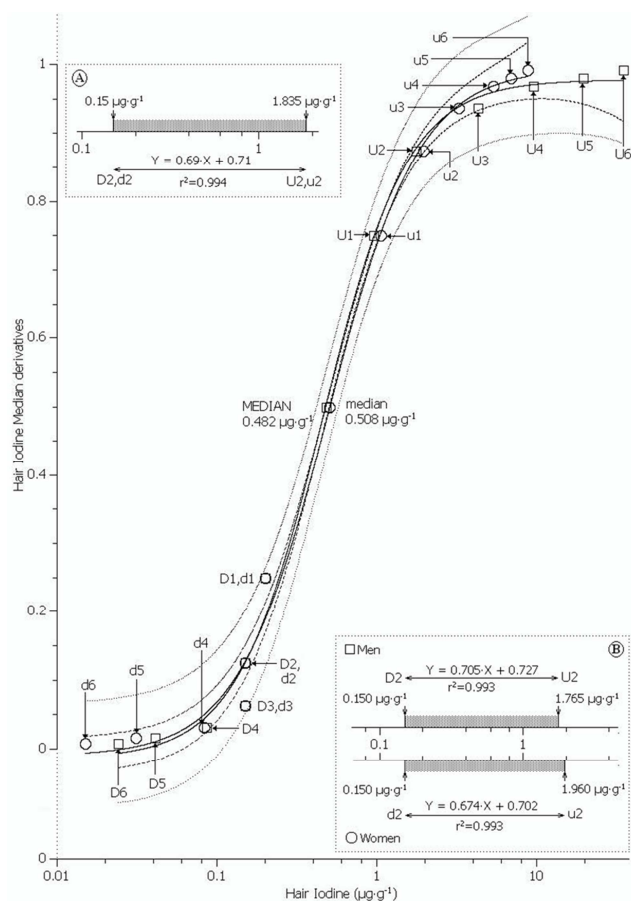
Figure 7. Hair Iodine Sigma.

The linear part of the iodine sigmoid dose-response saturation curve may be further divided into its subclinical, optimal (GDOLDEN RANGE), and supra saturated part with a 30:60:10 partition ratio, as already explained. Indeed, the follicular hair is growing linearly, but the rate of hair follicular iodine incorporation depends upon the equilibration of blood iodine with iodine hair follicular uptake. Indeed, the iodine hair incorporation changes depending upon the already reached level of the hair iodine saturation. Here we are facing a currently ill-defined area of diagnosing the subclinical bio element deficit, and its antipode of the bio element over abundance (the upper end of the linear part of the sigmoid dose-response saturation curve). Therefore, we propose that the area of the initial 30% of the linear range of the iodine dose-response sigmoid saturation curve should be considered as a subclinical iodine (and other bio elements) nutritional status. Similarly, the liner region above the 90% of the upper part of the linear range should be considered as an over saturated region. Indeed, the position of amy given bio element median, may slide up or down along the tilted linear segment of the sigmoid saturation curve. Essentially, the tilting angle of the median derivatives slope is constant for an bio element, so that the bio element medians from the various parts of the world would be sliding up or down along it. However, the linear bio element tilt may vaey with sex, as
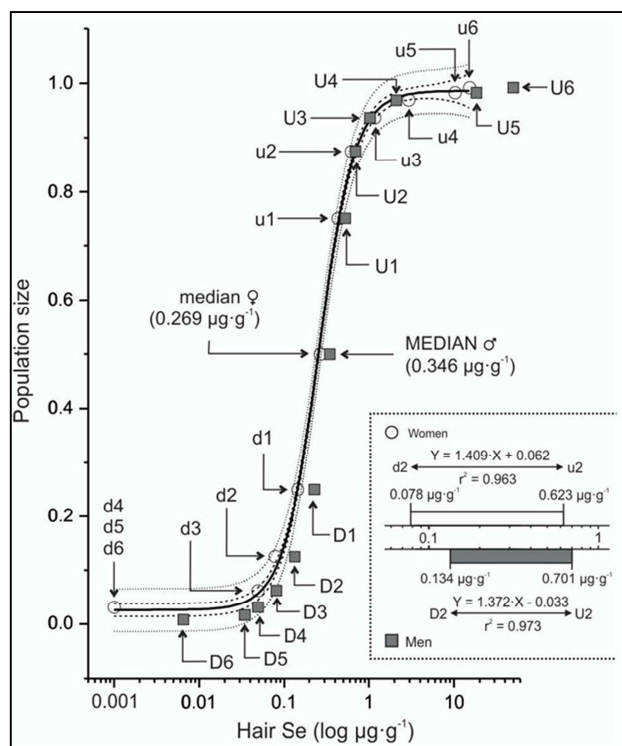
is demonstrated in this study.

Figure 8. Hair Selenium Sigma.

Selenium, is another what we may call a "proper" essential trace element, or what we here name the bio element. Selenium is shown here paired with iodine, since both of these bio elements are critical partners involved in the normal thyroid hormone biosynthesis [16, 17]. Hair selenium sigmoid saturation curve is shown in Figure 8. The adequate linear part of the selenium sigmoid dose-rate saturation curve Min to Max range lies between 0.134 and 0.078 (Min), and 0.701 and 0.623 (Max) for men and women, respectively. The suggested optimal GOLDEN RANGE for selenium nutritional status, lies between 0.304-0.644 and 0.233-0.569 for men and women, respectively. The subjects who belong to the upper 10% part of the supra adequate linear part of the sigmoid saturation curve, have the increased risk of developing diabetes, as observed in China. So the supra adequate level of a bio element in the human body may be a "mixed blessing". In the case of Se, when our bio element nutritional status is supra adequate, we may have to pay for this good result by having the increased chance of getting ill with the diabetes mellitus [17]. One more fact is interesting. When data on position of the hair median Se along the adequate linear range of the sigmoid response curve were plotted for various countries, Croatia and Tajikistan appeared to have similarly positioned their selenium medians, whereas it was different in comparison with Italy [18, 19]. Thus, the hair bio element median derivatives allows for direct visual comparison of the bio element nutritional status across the different countries (Vignette 2).

Vignette 2. International Selenium nutritional status (see text on Se and Table 1)
GOLDEN RANGE CROATIA (LM Low limit, UM upper limit)

Nutr Status	Sub adequate	Adequate		Supra adequate	
Grading (%)	0-30%	Min	30-90%	Max	90=100%
Men	LM 0.134	0.304 –	[Median 0.346]	0.664 -	UL 0.701
Women	LM 0.078	0.233	[Median 0.269]	-0.569	UL 0.623

Selenium Nutritional status from Europe to Central Asia (GOLDEN RANGE)

0.100	0.269,	0,346	0.34.0 0.369 0.394	0.37, 0.78	0.39	[6]0.54[e]	0.54	0.60[g]
	Women	Men		4a	4b			
(1) Belgium,	(2) Croatia	[3] Tajikistan	(4) Italy	(5) Italy	(6) France.	(7) Italy (8) Italy		

Hair selenium nutritional status appeared satisfactory for tested countries sxcept Belgium where it was at the bottom of the subclinical adequate selenium nutritional status zone. We recommend selenium supplementation for all subjects whose hair selenium is in the subclinical selenium nutritional status range. For hair selenium levels above the subclinical adequate selenium nutritional status but below the median derivative concentration, selenium supplementation is optional. We consider hair selenium supra adequate concentrations shold not attempt selenium supplementation.

(1) Carneiro M. F. H., Grotto D., Batista M. L., Rhoden C. R., Barbosa F. (Jr.). Biol Trace Element Res 2011; DOI 10.1007/s12011-011-9102—1, (2) Prejac J, Morović D, Drmić S, Morović Pišl Z, Momčilović B., Trace Eledments in Medicine, 2022; 23: 6-15, (3) Skalny AV., Burtseva T, Salnikova EV, Ajsuvakova OP, Skalnaya MG, Kirichuk AA., Tinkova AA. Environ. Res. 2019; 171: 293−301. (4a) Dongarra G., Lombardo M., Tamburo E., Varrica D., Cibella F., Cuttitta G. X12 Palermo. Environ. Toxicol. Pharmacol. 2011; 32: 27−34. (4b) Dongarra G., Varrica D., Tamburo E., Ándrea D. Environ Toxicol Pharmacol 2012; 34: 160-169, (5) Tamburo E., Varrica D., Dongarra G. Environ Tooxicol Pharmacol 2015; 19: 70-76. (6) Goullé J. P., Mahieu L., Castermant J., Neveu N., Bonneau L., Lainé G., Bouige D., Lacroix C. Forensic Science Intl. 2005; 153: 39−44. (7) Tamburo E., Vaeeica D., Dongarra G. Sci. Tota Environ. 2016; 63: 996−1002. (8) Astolfi M. L., Protano C., Marconi E., Massimi L., Brunori M., Piamonti D., Migliara G., Vitali M., Canepari S. A, Royal Soc. Chem. 2020; 12: 1906−1918.
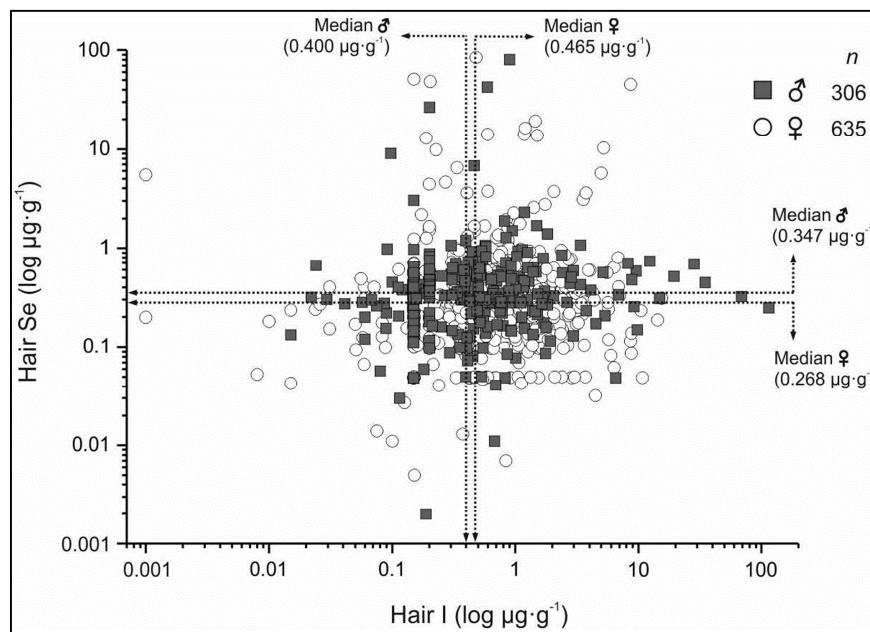

Figure 9. *Hair Se and I.*

We assume that the lower 30% of the adequate selenium linear part of the sigmoid curve should be considered as subclinical deficiency, whereas the upper 10% of the sigmoid linerpart should be considered as an over saturated. Thus, the suggested optimal selenium nutrition status is 0.304-0.644 for men, and 0.233-0.569 for women (Table 1).

It should be noted that hair follicles does not actively discriminates between iodine and selenium (Figure 9). This is an important point how it is essential to control the iodine nutritional status if somebody supplements himself with selenium, and vice verse.

SODIUM and POTASSIUM (Figure 5 Middle column).

These two major electrolytes of the human body are essential bio elements that we found in the human hair [20, 21]. The Min sodium hairconcentrations are 94.0 and 56.6 for men and women, respectively, whereas their Max hair concentrations are 1450 and 1357 for men and women, respectively. It is amazing how our metabolism operates under such a concentration gap range (over the two orders of (magnitude). However, the suggested optimal sodium concentrations linear segment of the dose-response sigmoid curve is 411-1313 and 272-1117 for men and women respectively. These two essential bio elements, sodium and potassium, are the key participants in maintaining the cell membrane osmotic gradient. Indeed, sodium and potassium intracellular metabolic balance is a dynamic state of constant cellular membrane out flux of sodium with exchange for potassium cellular influx. Indeed, It was quite unexpected to see how this two bio elements of fundamental importance were increased in the hair of depressed people [21]. Evidently, the metabolic cause of that, the most prevalent human mental health impairment, needs further elucidation. Moreover, and contrary to the currently prevailing understanding, dietary sodium apparently is not the cause of the idiopathic essential hypertension, but the consequence of the failure of the cell membrane Na K ATPase regulation of in and out cellular
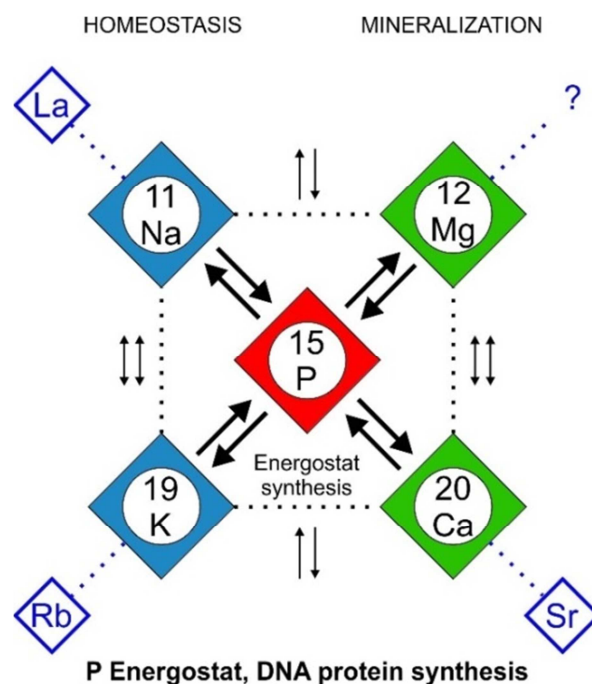
Vignette 3. Phosphorus metabolic node (hub)

The field of bio element metabolic interactions is waste and complex (Figure 11). Moreover, these interactions were studied by following the paradigm that we may vary only one parameter at a time. However, the life is evidently a much more complex phenomenon than the simple mechanical cause and effect relationships. Indeed, our bodies are simultaneously exposed to numerous metabolic stimuli of varied intensity. And all these metabolic stimuli are dispersed across the other numerous metabolic receptors. Evidently, the life is not a simple linear flow process, but a network of numerous different streams which may changeably mingle to keep the organ homeostasis steady.

After studying hair multi bio element profiles with principal component analysis, we identified their grouping in definitive clusters (1). Two clusters were prominent: (a) sodium and potassium cluster (Na·K). and (b) magnesium and calcium cluster (Mg·Ca). However, we also observed that beside the Na·K and Mg·Ca pairing, the members of this affinity pairs have around themselves the other elements of similar chemical characteristics, some kind of their "shadow". Indeed, we need a new way shown in this Vignette to present bio element interactions and beyond the current model of linear diagonal display. Apparently, a web-like cross-balanced models like this where Na·K and Mg·Ca pairs are cross=balanced over the centrally positioned phosphorus as shown in this Vignette. Here, we have a model involving five elements at a time. A new statistical methods need to be developed to handle such web-like structures.

Na and K transport [23]. Evidently, this observation opposes the current hysterical international crusade to decrease the sodium dietary intake, and increase that of potassium in our daily diet. Indeed, studying the role of Na K ATPase as a fundamental core set conformer factor in the idiopathic hypertension human disease, requires a rigorous biomedical study [24]. At this moment, it should be noted, that decreased hair sodium, but adequate hair potassium, was observed in cystic fibrosis, an chlorine ionic channel disease [25]. This findings should great ly improve the diagnosis of that inherited genetic disease. Indeed, cystic fibrosis is currently definitively confirmed only by finding high levels of chlorine in the human sweat, a technically very demanding diagnostic procedure that may be carried on in only a few laboratories in the entire world. It is pertinent to note here, that according to our congress reported data, the bio element lanthanum somehow mimics sodium metabolism, whereas the bio element rubidium acts like a potassium internal metabolic "shadow" (Vignette 3). Here presented data suggests that the optimal GOLDEN RANGEsodium concentrations along the linear segment of the dose-response sigmoid saturation curve are 411-1313 and 272-1117 for men and women, respectively. Similarly, the optimal hair potassium concentrations are 315-974 and 137-330 for men and women, respectively (Table 1).

It is reasonable to assume that energy production has evolved around phosphorus, and that this energy drives the homeostatic energy necessary for Na·K ionic exchange, and signal transduction and ossification mediated by Mg·Ca.

1. MOMČILOVIĆ B, PREJAC J, SKALNY AV, MIMICA N. IN SEARCH OF DECODING THE SYNTAX OF THE BIOELEMENTS IN GYMAN HAIR. A CRITICAL OVERVIEW, J TRACE ELEKEJTS BIOL MED 2018; 50: 543-553.

P Energostat, DNA protein synthesis

CALCIUM and MAGNESIUM (Figure 5 Middle column). Calcium and magnesium are another two essential electrolytes in our family of bio elements [26, 27]. The Min adequate hair calcium concentrations are 291.0 and 487.7 for men and women, respectively. Calcium is evidently the most abundant mineral bio element in the human hair (Figure 2), and also it is a bio element that has a distinctly high sex dependent accumulation in the women hair. Indeed, hair Max for calcium concentrations are 816.4 and 4426.0 for men and women, respectively. It isreally amazing how our metabolism operates under the such a concentration diversity (over the two orders of magnitude). What are the energy costs of maintenance of such a homeostasis range and its adaption capacity remains to be explored. However, the suggested GOLDEN RANGEoptimal calcium concentrations range along the linear segment of the dose-response sigmoid saturation curve is 591-761 and 971-4195 for men and women respectively. At the same time, the corresponding GOLDEN RANGE hair magnesium levels were Min 20.5 and 29.7 for men and women, respectively, whereas the hair magnesium Max were 90.2 and 270.6 for men and women, respectively. The suggested optimal GOLDEN RRANGE hair magnesium concentrations were 21.9-32.2 and 75.0-254.6 for men and women, respectively. Our results showed a marked sex difference in the hair concentration of calcium and magnesium;, both bio elements were higher in women than men.

Of course, there is a wide overlapping area between the two bio elements for both sexes, but the linear segments of the sigmoid saturation curves for these two bio elements were distinctly different in their slope tilts (Figure 3). Indeed, some human diseases, especially the stone forming pathology, are more prevalent in women than men [27]. As a matter of fact, the mineral metabolism differences of calcium in men and women, were sporadically observed and reported in the literature, but generally neglected and avoided in discussion.

We also observed an illustrative case of a women subject (No. 161) who had very high Ca hair concentrations (above 4200) in two separate hair sample analysis. Further medical diagnostic procedures revealed that she had a hyperparathyroidism. It should be noted that diagnosing hyperparathyroidism is a considerable diagnostic endeavor [29]. Later on it was found, that our hair multi bio element profile analysis confirmed other author findings of high hair calcium in hyperparathyroidism [30]. Also, the osteotrophic bio elements (bone seekers), like Sr showed identical sex dependent behavior as did Ca and Mg; they are all higher in women. The sex dependent differences in bone metabolism need to be acknowledged since osteoporosis is more prevalent in women. Moreover, Hans Selye, the father of the human "stress" health paradigm, demonstrated how the systematic tissue calcification, i.e. calciphylaxis (internal calcification of tissues). may be induced with the combined administration od high doses of calcium, parathyroid hormone, and vitamin D. Interestingly enough, such a calciphylaxis may be avoided

or alleviated by concurrent administration of magnesium salts [31]. We may consider calciphylaxis to be a special case of malnutrition where the complex high and imbalanced doses of the participant nutrients may induce disastrous health effects,
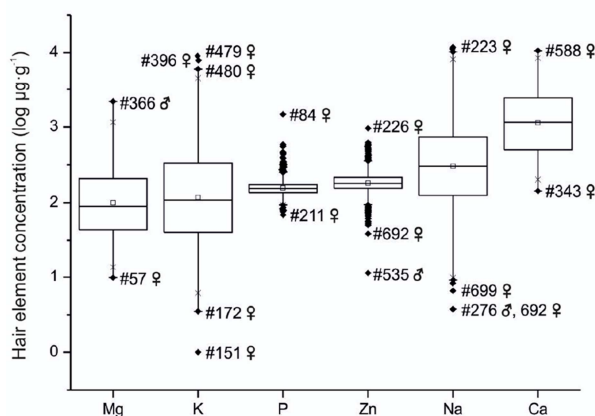

Figure 10. Box –plot comparison of six bio element pairs: Mg·Ca, K·Na and P·Zn.

The box-plot Figure 10 is shown here to allow a cross comparison of the entangled bio element pairs P·Zn, Ca·Mg, and Na·K. Essentially, the Figure 10 demonstrated the potential role of the hair as an integrative multi bio elements sensor organ in the human body.

Silicon, Copper, Iron, Aluminum, Boron, Strontium, Chromium, Nickel, Manganese, Molybdenum, Silver, Lithium, Chromium, Nickel, Cobalt. mand Gold.

In the preceding text, the eight exemplary bio element pairs, i.e., P·Zn, I·Se, Na·K, and Ca·Mg, in the human hair were analyzed in a great detail and accompanied with figures. For the rest of the fourteen bio elements, i.e. Silicon, Copper, Iron, Aluminum, Boron, Strontium, Chromium, Nickel, Manganese, Molybdenum, Silver, Lithium, Cobalt, and Gold, the respective reader will find their defined nutritional status determinants in Table 1. There, the data are shown as reference values to differentiate between the bio element deficiency and excess, and (more important), what is their boundaries of the adequate and optimal nutritional status for men and women, respectively. The more detailed information about the metabolic role off these bio elements in the human body, health and the diet may be found in a WHO informative book on vitamins and minerals [32] and on their toxvicity [3].

Indeed, bio elements are involved or help us image numerous health impairments like the relationship of zinc deficiency and alopecia [33], the great allergy inducing potential of Co, Cr, and Ni [34]; the role of copper in urticaria [35], the place of boron in the rheumatic diseases [36], the role of skin absorption of bio elements [37], the metabolic role of metals in human depression [38], and the effect of sub-pharmacological doses of Li in depression

[39]. The sensitivity of infabt brain on lead exposure [40], and olfactory route of vectored bio element particle matter in the brain [41], to name a few related research subjects. Special attention needs to be paid to the study of the physiology of bio element entanglement phenomenon alike that observed for Na and K, and Ca and Mg, in this paper.

This story on bio element metabolic peculiarities may go on, from bio element to the bio element, and it will only show how lacking is our knowledge about their mutual interactions in a complex body metabolic "internet" network (Figure 11). It should be noted that the here shown metabolic bio element interactions were induced with the high doses of the bio elements that were unusually high in comparison to those that we may find in the normal human diet. Moreover, the question of the various bio element composed mixtures on their nutritional status also needs a novel approach for its elucidation.
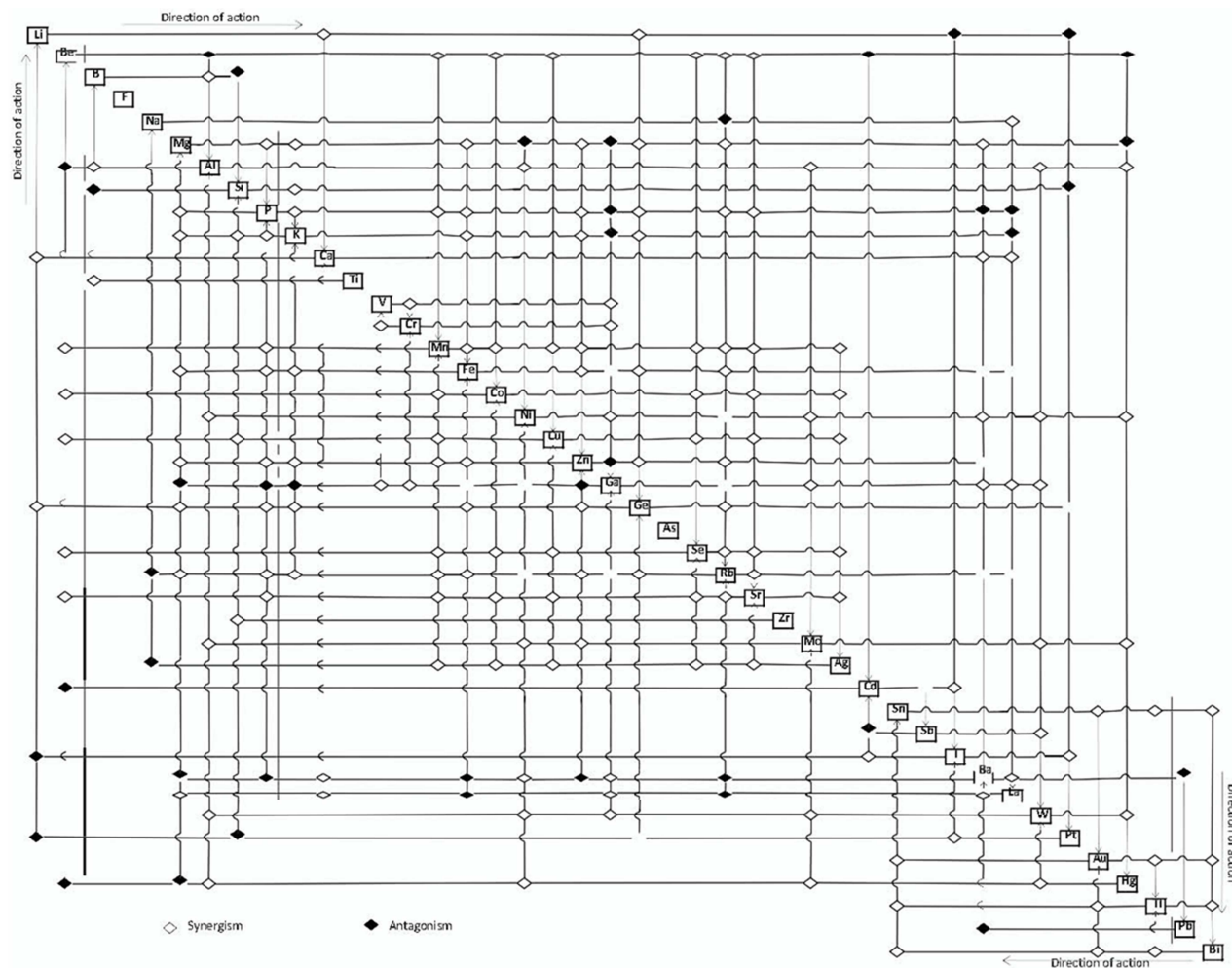

Figure 11. *Bio element interactions.*

4. Conclusion

At the end of this article on assessing the bio elements nutritional status we may conclude how hair is a superb biological indicator tissue of a great potential in precision medicine. Once we accurately did the chemical analytical work, there remains for us to focus our mind and reflect on the biological meaning of our analytical chemical results. The hair median derivatives bioassay provide us with an insight of how much of a dietary bio element became actually incorporated in our body.. Hair is itself a complex biological multi-sensor of the bio element metabolic behavior, and, in a way our personal metabolic study laboratory. Npw, we get the possibility to fully control an individual dietary bio element intake and tailor made an Rx and, moreover, to control bio element nutritional supplementation. Indeed, surveying the multi bio element profiles proves to be a useful simple screening diagnostic test for numerous metabolic and other health disturbances. Let us conclude with a statement, that adequate, quantitative and qualitative, bio element precision medicine nutrition, is the essential prerequisite for human health.

Acknowledgements

Appendix

Hair Bioelement Analysis

A strand of hair 5-7 cm long and weighting less than one gram would be cut with titanium coated scissors over the anatomically well-defined bone prominence at the back of the skull (lat. protuberantia occipitalis externa). The individual hair samples were further minced into strands less than 1 cm long prior to chemical analysis, stirred 10 min in an ethylether/acetone (3:1, w/w), rinsed three times with the deionized H_2O (18 MΩ·cm), dried at 85°C for one hour to constant weight, immersed one hour in 5% EDTA, rinsed again in the deionized H_2O, dried at 85°C for twelve hours, wet digested in HNO_3/H_2O_2 in a plastic tube, sonicated, and microwaved. The digested solutions were quantitatively transferred into 15 ml polypropylene test tubes. They were rinsed three times with the deionized water, and the rinses were transferred into the individual test tubes. These test tubes were filled up to 15 ml with deionized water and thoroughly shaken to mix. The samples were run in NexION 300 + NWR 2013 spectrometer (Perkin Elmer, USA). Graduation of the instrument was carried out with a monelement Perkin Elmer reference solution. We used certified GBW09101 Human Hair Reference Material (Shanghai Institute for Nuclear Research, Academia Sinica, Shanghai 201849, China to validate the quality of the analytical work.

References

[1] The Merriam Webster Pocket Dictionary. Pocket Books Inc. New York 1947 p. 205.

[2] Shetty P. Malnutrition and under nutrition. Medicine. 2003; 31: 18–22.

[3] Hayes AW Principles and methods of toxicology, 4th Ed., Taylor and Francis, Philadelphia, PA, 2001.

[4] Momčilović B, Reeves PG, Blake MJ. Idiorrhythmic dose-rate variability in dietary zinc intake generates different response pattern of zinc metabolism than conventional dose- response feeding. Brit. J. Nutr. 1997; 78: 173-191.

[5] Momčilović B, Prejac J, Mimica N. In search of decoding the syntax of the bioelements in human hair. A critical overview, JTEMB 2017; 49: 1-11.

[6] Harpley FW, Stewart GA, Young PA. Principles of biological assay. In International Encyclopedia of Pharmaceutical sciences and Therapeutics (Delanouis AL Ed.), Vpl. 1, Pergamum Press, Oxford 1973.

[7] Chittleborough G. A chemist's view of the analysis of human hair for trace elements. Sci. Total Environ. 1980; 14: 63-75.

[8] Momčilović B. Lykken GI, Tao L, Wielopoloski L. Comparative analysis of 65Zn and 40K in human urine by library least square and window methods using a personal computer. J Radioanalyt Nucl Chem 1995; 195: 315-319.

[9] Dinh K, Wang Q. A probabilistic Boolean model on hair follicle cell fate regulation by TGF-β. Biophysical Journal 2022; 121: 1-15.

[10] Alpers DA, Bier DM, Carpenter KJ, McCormick DB, Miller AB, Jacques PF. History and impact of nutritional epidemiology. Adv Nutr 2014, doi: 10.3945/an.113.006353.

[11] Finey AJ. Probit analysis. A statistical treatment of the sigmoid response curve, 2nd Ed. Cambridge Univ Press, Cambridge, UK 1952.

[12] Momčilović B.: "Dietary zinc dose-rate idiorrhythm is a powerful tool for detection of subtle mineral interactions. A case for the expression of Recommended Dietary Allowances (RDA's) and Safety Limits (RfD) in the range format", Trace Elements in Man and Animals 9 (Fischer PW, LÁbbe MR, Cockell KA, Gibson RA, eds.), NRC Research Press, Ottawa, Canada, 1997; 9: 403-405.

[13] Linder MC. Nutritional biochemistry and metabolism. Elsevier, Amsterdam, Netherland 1985.

[14] DiPietro ES, Phillips DL. Paschal DC, Beese JW. Determination of trace elements in human hair, Biological Trace FElement Research 1989; 22: 83-100.

[15] Braverman LE, Utiger RD (Eds.). Werner &Ingbar's thyroid. A fundamental and clinical text, 9th. Ed, Lippincott Williams & Wilkins, Philadelphia, PA 2005.

[16] Preedy VR, Burrow GN, Watson RR (Eds.). The comprehensive handbook of iodine. Nutritional biochemical, pathological and therapeutic aspects, Academic Press (imprint of Elsevier), Amsterdam, Netherlands 2009.

[17] Prejac J, Morović S,, Drmić S, Morović J, Pisl Z, Momčilović B. New way to assess the bioelement selenium nutritional status non-invasively in vivo, Trace Elements in Medicine, 2022; 23 (2): 6-15.

[18] Skalny AV, Burtseva T, Salnikova EV, Ajsuvakova OP, Skalnaya MG, Kirichuk AA, Tinkova Geographic variation of environmental, food, and human hair selenium content in an industrial region of Russia. Environ Res 2019; 171: 293-301.

[19] amburo E, Varrica D, Dongarra G. Coverage intervals for trace elements in human scalp hair are [geographic] site specific. Environ Toxicol Pharmacol 2015; 19: 70-76.

[20] Strazzullo P, Leclercq C. Sodium, Adv. Nutr 2014; 5: 188-190.

[21] Traber MG, Manor D. Potassium, Adv Nutr 2012; 3: 820-821.

[22] Momčilović B, Prejac J, Višnjević V, Drmić S, Mimica N, Bukovec-Megle Ž, Brundić S, Skalny AV. The muscle immobility of depression-The weightlessness within, Psychology 2012; 3: 825- 833.

[23] Momčilović B, Dietary salt in the whirl of nutritional science, public health and food processing industry, Trace elements in Medicine 2021; 22 (4): 3-13.

[24] Langappa VR, Farttarey K. Physiological Medicine, A clinical approach to basic medical physiology, McGrew Hill, New York, NY 2000.

[25] Sperelakis N. Regulation of ionic channels by phosphorylation, in Cell physiology. Source book, Ch. 32, Academic Press, San Diego, CA, `1998: 499=509.

[26] Momčilović B. Case HR 0101 144 Increased hair K/Na ratio in cystic fibrosis, Trace elements in medicine, 2021; 22 (3): 58-59.

[27] Weawer CM, Heaney RR. Calcium, in Modern Nutrition in health and disease (Shills ME, Shike M, Ross AC, Caballero B, Cousins RJ, Eds) m 10th Ed., Lippincott Williams & Wilkins, Philadelphia, PA 2006: 194-210.

[28] Rude RK, Shils ME. Magnesium. in Modern Nutrition in health and disease (Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, Eds), in 0th Ed., Lippincott Williams & Wilkins, Philadelphia, PA 2006: 223-247.

[29] Smith R Disorders of the skeleton, in Conscience Oxford Textbook of Medicine (Ledingham JGG, Warnell, Eds.), Section 9, Oxford Univ Press, Oxford, UK 2000: 941-965.

[30] Kanis JA. Disorders of calcium metabolism,. Disorders of the skeleton, in Conscience Oxford Textbook of Medicine (Ledingham JGG, Warnell, Eds.), Ch. 7.5, Oxford Univ Press, Oxford, UK 2000: 839-850.

[31] Porto de Silveira CL, Mikeley L, de Ca\rrvalho Portes L<. de Paula FA. A; la; ime earth element and phosphorus anomalies in human hair samples asd an indicator for osteoporosis, Fifyh COMTOX Sym; posium in Toxicology and Clinical Chemistry of Metals, Vancouver, Canada, Abstract 311, 1995.

[32] Selye H. Calciphylaxis, Chicago Univ Press, Chichago, IL 1962.

[33] WHO and FAO (UN)/ Vitamin and mineral requirements to human nutrition, 2nd Ed., World Health Organization and Food and Agriculture Organization of the United Nation Geneva, Switzerland 2004.

[34] Momčilović B, Handl S, Zja i V. A case of alopecia in man associated with zinc deficiency, Trace Element Metabolism in Man and Animals 4 (J. McC. Howell, J. M. Gawthorne, C. L. White, eds.). Austral Acad Sci, Canberra 1981; 4: 491-494.

[35] Momčilović B (Plenary Lecture). The epistemology of trace element balance and interaction, Plenary Lecture. Trace Elements in Man and Animals 6 (L. S. Hurley, C. L. Keen, B. Lonnerdal, R. B. Rucker, eds.). Plenum Publ Co, NY 1988; 6: 173-177.

[36] Mom ilovi B. Low serum copper in urticaria and post virus B hepatitis, 7th International Symposium on Trace Elements in Man and Animals (TEMA 7). Dubrovnik 1990; 7: 23.

[37] Prejac J, Skalny AV, Grabeklis AR, Uzum S, Mimica N, Momčilović B. Assessing the boron nutritional status by analyzing its cumulative frequency distribution in the hair and whole blood, J Trace Element Med Biol, 3018; 45: 50-56.

[38] Guy RH, Hostynek JJ, Hunz RS, Lorence CR. Metals and the skin. Topical effects and systematic absorption. Marcel Dekker Inc., New York 1999.

[39] Momčilović B.: "A case report of acute human molybdenum toxicity from a dietary molybdenum supplement - A new member of the "*lucor metallicum*" family, Arh.hig.rada toksikol 1999; 50: 289-297.

[40] Momčilović B, Prejac J, Ivičić N. Trace element fingerprint of human depression, Trace Elements and Electrolytes, 2007; 25: 225 (Abstract).

[41] Mimica N, Prejac J, Skalny AV, Grabeklis AR, Momčilović B. Assessing the lithium nutritional status by analyzing its cumulative frequency distribution in the hair and whole blood, J Trace Element Med Biol, 3017; 44: 46.

[42] Momčilović B, Kostial K. Kinetics of lead retention and distribution in suckling and aduly rays, Environ Res 1974; 8: 214-220.

[43] Lykken GI, Ward T, Momčilopvić B, Traudt J. Analysis of human tissues, including brain, containing environmental toxic metal-tagged cp, bustion particulate matter PM 2,5, Academia Letters 2021, Article 4212. https://doi.org/10.20936/ALA212.