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*Original Research*

# Pharyngeal Tonsil as New Biomarker of Pollution on Example of Barium

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**Abstract**

The mainly purpose of our study was to determine Ba in pharyngeal tonsils of children living in the influence of dust emission from power stations in the following regions in southern Poland: Jura Krakowsko-Częstochowska, Upper Silesia, and Kraków. The concept of investigation was based on the fact of high levels of Ba in suspended dust emitted by power stations (about 800 µg/g). Moreover, these children are exposed to secondary emissions of Ba from soil dust (500-1,000 µg/g) during outdoor recreation. Ba concentrations in pharyngeal tonsils may be involved in indicating the pollution of some plants.

Ba concentrations in pharyngeal tonsils were determined using the ACP-AES method. The characteristic feature of pharyngeal tonsils is their ability and high specificity of Ba accumulation from inhaled air.

The average level of Ba concentration in tonsils was similar in all groups of children (0.03-0.09 µg/g) with the exception of boys from Jura Krakowsko-Częstochowska – 0.22 µg/g. The differences of Ba content in pharyngeal tonsils measured as the maximum concentration range depends on sex and place of residence. Moreover, the interaction between Ba with other pollution elements was described.

**Keywords:** pharyngeal tonsils, barium concentration, power station dust pollution, children, ICP-AES method

## Introduction

The appearance of barium as an alkaline metal in the earth's crust is relatively high in the natural state. Barium enters the environment through the weathering of rocks and minerals. Anthropogenic-related releases are primarily associated with industrial processes. Barium is present in suspended dust in the atmosphere, as a result of secondary emissions in urban pollution, surface water, soils, and many foods [1, 2].

In industry, Barium forms useful alloys with aluminum and magnesium, which are used in electronic tubes to remove residual gases [3]. Barium is also used as a deoxidizer for steel and other metals [4].

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The content of barium in soils of Poland diverges, ranging between 20 and 1,000 μg/g [5]. The amount of barium found in soil of the USA ranges from 15 to 3.500 μg/g [6].

Most Polish soils contain no more than 50 μg/g of barium [5], but in submontane regions its content may increase up to 100 μg/g. For comparison, the barium measured in dust samples taken from Ottawa, Canada, had mean concentrations of 405.56 μg/g dust [7, 8]. The mean barium concentrations in background air, collected between April and October 2002 in the campus of the University of Birmingham, United Kingdom, were 0.32 and 1.4 ng/$m^3$ in the <0.5 and 3.0-7.2 μm particular matter fractions, respectively [9]. In the nearby industrial plants, the amount of barium in soils may reach 800 μg/g [5, 10].

The content of barium in inland waters is relatively low and only in contaminated waters reaches higher levels, e.g. in the Przemsza River – 136 μg/l [5]. In drinking waters of the Polish natural environment, the content of barium ranges from 3 to 60 μg/l [5]. To compare, the content of barium in the waterworks of the cities, with the average value of 7.3 mg/l, gives 14.6 mg Ba/24 h in a 2-liter daily water intake. In the areas of lower barium content, the amount of this element in water was below 0.2 (on average 0.1) mg Ba/l. It was observed that in populations exposed to high concentrations of barium in drinking water, the frequency of death due to heart diseases increased significantly [11].

In the air the amount of barium occurrence may range to approximately 100 ng/$m^3$, as the result of electric power plant dust emissions [12-14]. The concentration of barium in ambient air is estimated to be <0.05 μg/$m^3$ (IPCS 1991).

The daily intake of barium in a normal diet has little toxic effect, and Ba is slightly secreted with urine [5]. The reports on barium accumulation in tissues are not clear, although according to Sapota [11], Ba gets built into bones 5 times quicker than Ca. Moreover, barium reacts with K, Mg, and Ca [1, 2]. The U.S. EPA has determined that drinking water should not contain more than 2.0 mg/$dm^3$ [15].

The daily intake of barium amounts to 500 μg, while the lethal dose is 200 mg [5]. To compare others samples of tissues, the average barium deposition in the kidneys is 100 μg/kg, in the liver 30 μg/kg, in muscles 50 μg/kg [16]. According to Sowden [17, 18], total value of barium in a human organism amounts to approximately 23.8 mg, which is comparable to the reports of Tipton – 16 mg [16]. In some other publications the following results of Ba occurrence in some tissues were found: bone – 2 μg/g, the level of barium in blood, urine, and feces will vary with daily intake of barium. The following levels have been reported: feces – 690-1,215 μg/day, urine 17-50 μg/day, urine 1.5 μg/L [1].

According to the National Research Council (1980) [19], barium does not have any physiological functions in human organisms. There is little information on the toxicity of barium to children.

This study is the result of previous investigations on properties of accumulating of biomarkers by pharyngeal tonsils in relation to different behavioral and environmental parameters [12, 13, 20-23].

## Material and Methods

The pharyngeal tonsils of children living in Jura Krakowsko-Czestochowska, Upper Silesia, and Kraków were the material for this study. Pharyngeal tonsils were removed by adenotomy in accordance with and due to medical indications.

The tonsil samples were divided with respect to the residence (Jura Krakowsko-Czestochowska n=13, Upper Silesia n=27, Region of Kraków n=9), and to sex (girls n=21, boys n=28).

The removed tonsils were dried in the Wamed KBC 65 G drier after their humidity was determined. The next stage consisted of pressure mineralization with Merck 65% $HNO_3$ (V) in the closed system (PDS 6). Barium was marked by emission spectrometry with inductively coupled plasma (ICP-AES), with the use of a PerkinElmer Optima 5300DV™ Spectrometer [24].

The accuracy of the markings related to the expected levels in the tonsils was checked using the Merck Company method. Detectability of barium was 0.003 μg/g; the precision of the markings was 2.0%.

The results were analyzed statistically by means of Statistica ver. 7.1.

## Results and Discussion

Anatomical features and the location in the ventilation way of air make pharyngeal tonsils (due to the higher accumulation of elements for example barium) the biological material for the studies on the scope of the harmful influence of dust containing aluminium [25]. The anatomical structure, and location of pharyngeal tonsils in the upper part of respiratory tract causes a higher accumulation of different elements (for example barium) in this tissue. The pharyngeal tonsil was used as a material for studies of the harmful influence of dust containing aluminium [25].

There are no established biomarkers of barium exposure and its effects. There are no data correlating levels of barium in human tissues and fluids with exposure, and there are no quantitative studies correlating toxicity effects (hypokalemia, gastrointestinal disfunctions, hyper – or hypotension, ventricular tachycardia, numbness, and tingling around mouth and neck) that are recognized as not specific to barium toxicity [1, 2].

The main exposure of children to barium is expected to occur from diet or by dermal contact with barium-containing dust, with minor exposure of barium through inhaled air [1]. Average daily intake of barium in children has been determined in Canadian studies that show the average barium intake in young male and female children to increase with age of children 1-4 years (25.251 μg/kg body weight/day) and then down to 11,759 μg/kg body weight/day for males (5-11 years) and 9.280 μg/kg body weight/day for females age 12-19 [1].

Sources of barium for human organisms are power plants dust emissions [12, 13] and drinking water from heavily salted surface water intakes [26].

Table 1. Statistical characteristic of Ba occurrence in pharyngeal tonsils in children [μg/g].

| Examined group | Arithmetic mean ± standard deviation | Statistically most probable variety range | Geometric mean | Observed variety range | Contents in percentiles | | | Distribution coeficient | | Coeficient of variation |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 10 | 50 | 95 | skewness | kurtosis | |
| The whole examined population (n=49) | 0.19 ± 0.40 | 0.08-0.31 | 0.07 | 0.01-2.00 | 0.01 | 0.06 | 1.34 | 3.58 | 12.68 | 207 |
| Region – Jura Krakowsko-Częstochowska | | | | | | | | | | |
| Girls (n=7) | 0.05±0.05 | 0.00-0.09 | 0.03 | 0.01-0.12 | 0.01 | 0.03 | 0.12 | 0.81 | -1.26 | 98 |
| Boys (n=5) | 0.56±0.82 | 0.46-1.58 | 0.22 | 0.04-2.00 | 0.04 | 0.29 | 2.00 | 2.03 | 4.24 | 147 |
| Region – Upper Silesia | | | | | | | | | | |
| Girls (n=9) | 0.20±0.43 | 0.14-0.53 | 0.05 | 0.01-1.34 | 0.01 | 0.04 | 1.34 | 2.93 | 8.66 | 220 |
| Boys (n=18) | 0.20±0.38 | 0.01-0.39 | 0.09 | 0.02-1.66 | 0.02 | 0.11 | 1.66 | 3.75 | 14.86 | 190 |
| Region – Kraków | | | | | | | | | | |
| Girls (n=4) | 0.13±0.17 | 1.39-1.64 | 0.05 | 0.01-0.25 | 0.01 | 0.13 | 0.25 | - | - | 130 |
| Boys (n=5) | 0.09±0.11 | 0.05-0.22 | 0.04 | 0.01-0.24 | 0.01 | 0.02 | 0.24 | 0.90 | -1.63 | 122 |

The frequency of barium presence in children pharyngeal tonsils is in a non-parametrical distribution. After finding the logarithm of the concentrations values, the range became a normal distribution (Fig. 1).

When comparred with Kolgomorow-Smirnow's test, the geometric means of barium concentration in pharyngeal tonsils of children from different regions did not differ significantly (0.04-0.05), with the exception of boys from Jura Krakowsko-Czestochwoska (0.22 μg/g).

Average barium content in pharyngeal tonsils in children living in Jura Krakowsko-Czestochowska was 0.03 μg/g for girls and 7 times higher for boys – 0.22 μg/g. The general barium concentration in tonsils of children living in Kraków maintained at the similar level (0.04-0.25 μg/g) in girls, as well as in boys. On the contrary, barium content in Silesian children was twice higher for boys (0.09 μg/g) than for girls (0.05 μg/g) – Table 1. Those values are comparable with concentrations observed in muscles – on average 0.1-0.3 μg/g [5, 16]. Kabata-Pendias reports that barium concentration in animal tissues falls between 0.1-700 μg/g [5]. Hard tissue contains the greatest barium concentration – 700 μg/g in the bones. The research of Sowden [17], demonstrated different average barium content in specific organs of dead children aged 4 months to 12 years. The results quoted in μg/g of ash were as follows: liver – 3.3, brain – 2.3, spleen – 3.5, heart – 20.3, kidneys – 5.0, lungs – 11.3, pancreas – 8.0, urinary bladder – 5.1, gallbladder – 5.1, muscles – 6.3, tongue – 6.3, skin – 10.3, and thyroid – 1.2 [18].

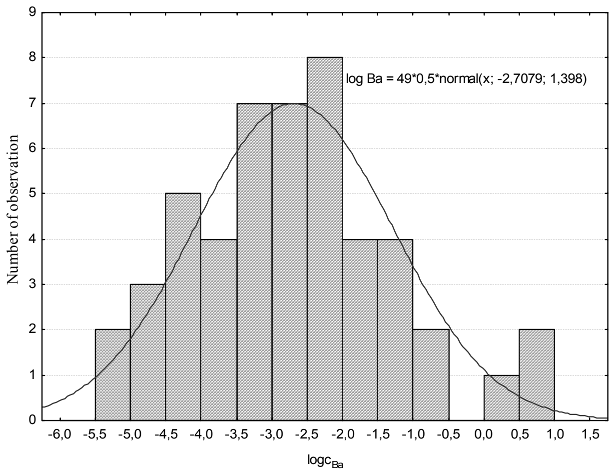

Fig. 1. Frequency of Ba occurrence in pharyngeal tonsils in children (total population).

Environmental barium contents corresponding to the tenth percentile, which is interpreted as the smallest levels of the metal, are the result of environmental emission influence from dusts suspended in air, drinking water, and soil maintained at a similar level of 0.01 μg/g. Pharyngeal tonsils of boys living in Jura Krakowsko-Częstochowska are the exception (0.04 μg/g) – Table 1.

Maximum as well as extreme values (corresponding to 95 percentiles resulting from environmental influence) were observed in boys from rural areas (2.00 μg/g), probably as a result of the secondary emission of barium fertilized soils. Pharyngeal tonsils in girls from Jura Krakowsko-Czestochowska contained less barium, which is explained as a result of a smaller liveliness in comparison to the boys. In children from Kraków, barium content maintained the same level (0.24-0.25 μg/g). In boys from Silesia, barium content in pharyngeal tonsils (1.66 μg/g) was greater than in girls (1.34 μg/g).

The range of barium occurrence differences in pharyngeal tonsils in children are of great value for evaluating the fluctuation of environmental barium occurrence. The range

Table 2. Ba co-existence with other elements in pharyngeal tonsils of children living in selected regions.

| Group | Al | Ca | Cr | Cu | Fe | Mg | Mn | Zn | K | Na | Cd |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Region – Jura Krakowsko-Częstochowska | | | | | | | | | | | |
| Girls | - | -0.26 | -0.82 | 0.96 | -0.79 | -0.63 | 0.78 | -0.52 | -0.70 | -0.81 | 0.86 |
| Boys | - | -0.72 | 0.91 | -0.21 | -0.35 | 0.23 | 0.52 | 0.85 | 0.90 | -0.45 | -0.31 |
| Region – Upper Silesia | | | | | | | | | | | |
| Girls | - | 0.41 | -0.22 | 0.24 | 0.52 | -0.19 | 0.43 | -0.12 | -0.39 | 0.48 | 0.10 |
| Boys | - | -0.06 | -0.03 | -0.07 | 0.06 | -0.24 | -0.16 | -0.29 | -0.26 | -0.30 | -0.42 |
| Region – Kraków | | | | | | | | | | | |
| Girls | - | - | - | - | - | - | - | - | - | - | - |
| Boys | -0.66 | -0.41 | 0.13 | 0.55 | -0.66 | -0.14 | -0.76 | -0.20 | 0.13 | -0.63 | 0.21 |

of different barium occurrences were between 0.01 μg/g to 2.00 μg/g – in Jura Krakowsko-Czestochowska regions, from 0.006 μg/g to 1.66 μg/g in the Upper Silesia region, and in Kraków – 0.01-0.24 μg/g – Table 1.

The greatest diversity of barium occurrence in pharyngeal tonsils was observed in girls living in Upper Silesia (220%), while in girls from Jura Krakowsko-Czestochowska the diversity was the smallest (98%).

Data suggest that barium may interact with other cations, for example with potassium, calcium, and magnesium. Barium exposure may cause a buildup of potassium inside the cell, resulting in extracellular hypokalemia, which is belived to mediate barium-induced paralysis [1]. Barium in low concentrations may stimulate calcium uptake in pancreatic cells [1].

The other problem analyzed in the study regarded the correlation of barium with other metals in pharyngeal tonsils at the $p \leq 0.05$ significance level (Table 2). Pearson's correlation coefficient was used in the study. The correlation coefficient above 0.20 for n=27 was considered significant. The greatest number of significant correlation coefficients was observed in rural areas. Synergic reactions of pharyngeal tonsils in girls concerned barium with: Cu (0.96), Mn (0.78), Cd (0.86), and for boys from rural regions concerned Ba with: Cr (0.91), Mg (0.23), Mn (0.52), Zn (0.85), and K (0.9). Antagonistic relations in pharyngeal tonsils of girls from Jura Krakowsko-Czestochowska concerned Ba with: Ca (-0.26), Cr (-0.82), Fe (-0.79), Mg (-0.63), Zn (0.52), K(-0.70), and Na (-0.81), and with boys Ca (-0.72), Cu (-0.21), Fe (-0.35), Na (-0.45), and Cd (-0.31). Antagonism between the contents of Ba and Ca, well-known from references [5, 11], was confirmed in the case of children living in rural areas. But this relation was antagonistic for girls and synergic for boys. Analyzing the issue of barium co-existence in pharyngeal tonsils of Silesian children, the synergic correlation of the studied element with Ca (0.41), Cu (0.24), Fe (0.52), Mn (0.43), and Na (0.48) was characteristic in girls, while in boys only antagonistic relations of Ba with Mg (-0.24), Zn (-0.29), and Cd (-0.42) were observed. Lack of a correlation between

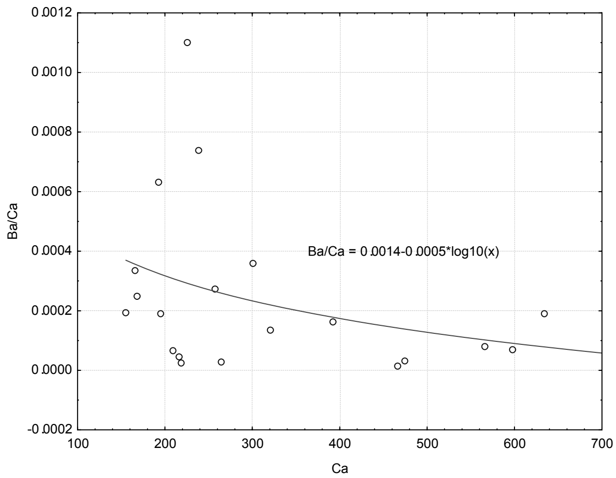


Fig. 2. The quotient of Ba/Ca content in the Ca function changes in the pharyngeal tonsils of girls.

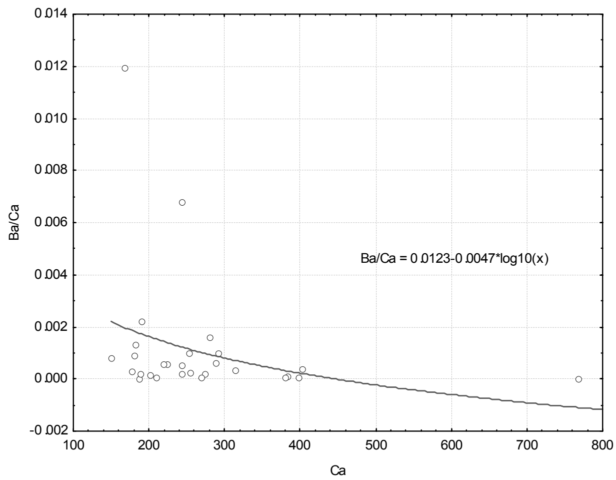


Fig. 3. The quotient of Ba/Ca content in the Ca function changes in the pharyngeal tonsils of boys.

Table 3. The relationships between Ba and other elements in pharyngeal tonsils.

| Synergism | | Antagonism | |
|---|---|---|---|
| Region Jura Krakowsko-Częstochowska – girls | | | |
| Ba = -0.09 + 0.06 * Cu | r = 0.96 | Ba = 0.08 - 0.1E-3 * Ca | r = -0.26 |
| Ba = -0.02 + 0.18 * Mn | r = 0.78 | Ba = 0.12 - 0.0226 * Cr | r = -0.82 |
| Ba = -0.03 + 0.61 * Cd | r = 0.86 | Ba = 0.14 - 0.6E-3 * Fe | r = -0.79 |
| | | **Ba = 0.29 - 0.3E-3 * Mg** | **r = -0.63** |
| | | Ba = 0.29 - 0.0032 * Zn | r = -0.52 |
| | | **Ba = 0.27 - 0.1E-4 * K** | **r = -0.70** |
| | | Ba = 0.16 - 0.4E-4 * Na | r = -0.81 |
| Region Jura Krakowsko-Częstochowska – boys | | | |
| **Ba = -0.65 + 0.32 * Cr** | **r = 0.91** | Ba = 2.33 - 0.01 * Ca | r = -0.72 |
| **Ba = -0.18 + 0.74E-3 * Mg** | **r = 0.23** | **Ba = 0.44 - 0.13 * Cu** | **r = -0.21** |
| Ba = -0.07 + 0.49243 * Mn | r = 0.52 | **Ba = 0.98 - 0.002 * Fe** | **r = -0.35** |
| Ba = -10.06 + 0.14908 * Zn | r = 0.85 | **Ba = 0.96 - 2.55 * Cd** | **r = -0.31** |
| Ba = -3.40 + 0.28E-3 * K | r = 0.90 | | |
| Region Upper Silesia – girls | | | |
| Ba = -0.18 + 0.001 * Ca | r = 0.41 | Ba = 0.11 - 0.02 * Cr | r = -0.22 |
| Ba = -0.32 + 0.18 * Cu | r = 0.24 | **Ba = 1.11 - 0.7E-4 * K** | **r = -0.39** |
| Ba = -0.12 + 094E-3 * Fe | r = 0.52 | | |
| **Ba = -0.43 + 0.95 * Mn** | **r = 0.43** | | |
| **Ba = -0.48 + 0.17E-3 * Na** | **r = 0.48** | | |
| Region Upper Silesia – boys | | | |
| | | Ba = 0.63 - 0.5E-3 * Mg | r = -0.24 |
| | | **Ba = 0.76 - 0.01 * Zn** | **r = -0.29** |
| | | **Ba = 0.66 - 0.3E-4 * K** | **r = -0.26** |
| | | Ba = 0.64 - 0.1E-3 * Na | r = -0.30 |
| | | Ba = 0.57 - 1.87 * Cd | r = -0.42 |
| Region Kraków – boys | | | |
| Ba = -0.34 + 0.15 * Cu | r = 0.55 | **Ba = 0.13 - 0.002 * Al** | **r = -0.66** |
| Ba = -0.02 + 0.56 * Cd | r = 0.21 | Ba = 0.14 - 0.2E-3 * Ca | r = -0.41 |
| | | Ba = 0.23 - 0.9E-3 * Fe | r = -0.66 |
| | | Ba = 0.62 - 0.97 * Mn | r = -0.76 |
| | | Ba = 0.38 - 0.004 * Zn | r = -0.20 |
| | | Ba = 0.25 - 0.4E-4 * Na | r = -0.63 |

barium and other metal content changes in pharyngeal tonsils of girls from Kraków was surprising. However, only two synergic relations of Ba with Cu (0.55) and Cd (0.21) were characteristic for boys from Kraków, as well as inversely proportional correlations of Ba with Al (-0.66), Ca (-0.41), Fe (-0.66), Mn (-0.76), Zn (-0.20), and Na (-0.63). The above-mentioned results confirmed step by step the changes of some elements in dust contents, and in pharyngeal tonsils.

The influence of Ca presence on Ba content in pharyngeal tonsils for boys and girls is shown in Figs. 2 and 3. Despite the increase of Ca content in pharyngeal tonsils, barium concentration increases, and the role of barium is higher in percentage terms by 50-300 μgCa/g.

The change of barium content in pharyngeal tonsils as a function of chosen elements content changes are presented in Table 3.

The interpretation of achieved regression equations let us assess the change of barium content, e.g. in the function of Ca content changes in the case of boys living in Jura Krakowsko-Czestochowska, which could be described with the equation: Ba=0.01Ca+2.33, which means that the average barium content in pharyngeal tonsils, determined by its physiology, is 2.33 μg/g. The rise of Ca concentration by 100 μg/g in pharyngeal tonsils causes barium concentration rise by 1 μg/g.

## Conclusions

1. Pharyngeal tonsils may be involved in proportionally controlling pollution by some plants.
2. The main feature of pharyngeal tonsils is their high ability and specificity of barium accumulation from the inhaled air.
3. The changes of barium content of pharyngeal tonsils depend on gender and residence of children, which confirms their possible application as a biomarker.

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