



# Relationship between concentrations of elements and geographic location in Poland

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## Abstract

**Introduction.** Interactions occur between concentrations of metals in the human body and the environment in various geographic locations, which can be of importance for both the proper development and the course of pregnancy.

**Objective.** The aim of the study was to assess the concentrations of Zn, Cu and Cd and Zn:Cu and Zn:Cd molar ratios in the placenta, umbilical cord and in the foetal membrane, and to examine the relationship between concentrations of these elements and the place of residence.

**Materials and method.** The research material was obtained from 99 healthy women from north-western and central Poland. Data for the study were collected from the medical history and documents of admission to the ward and documents confirming the birth of the newborn. Concentrations of zinc (Zn), copper (Cu) and cadmium (Cd) were determined using the ICP–AES method (spectrophotometry of atomic absorption) in inductively coupled argon plasma.

**Results.** The average concentration of Zn, Cu and Cd in afterbirths was ~ 0.01 mg/kg of dry weight (dw). In central Poland, the results revealed a significant increase in zinc in the membrane (OR=1.098, p=0.002), cadmium in the placenta (OR=1.324, p=0.006), Zn:Cu in the membrane (OR=1.012, p<0.001). In north-western Poland, an increase in copper in the membrane (OR=1.239, p=0.025) was revealed.

**Conclusions.** The use biological materials, such as the placenta, foetal membrane and umbilical cord to assess exposure to heavy metals and necessary elements is justified.

## Key words

placenta, umbilical cord, foetal membrane, newborn, zinc, copper, cadmium

## INTRODUCTION

Zinc (Zn) is one of the elements found in people in the largest amounts [1]. It is of particular importance during pregnancy, lactation, and in periods of increased metabolism and convalescence [2]. It is also necessary for proper growth [3, 4]. A deficiency of zinc may have a negative effect on embryonic development, birth weight, brain development or the wrong breaking time of the amniotic sac [2, 5–7]. Full-term infants have zinc reserves in their bodies [8] as opposed to preterm infants. In developing countries, over half-a-million deaths of infants and small children are caused by a deficiency of zinc [9]. Special attention is paid to the influence of alcohol during pregnancy, which changes the metabolism of zinc and causes serious consequences for the foetus [10].

Copper (Cu) is a necessary cell component in almost all organisms. The demand for Cu increases particularly during pregnancy and lactation [11, 12]. An insufficient amount of

Cu may lead to anaemia due to inferior absorption (Fe) and in a reduction in the number of erythrocytes [13]. Moreover, Cu deficiency in pregnant and breast-feeding women may cause various developmental aberrations or the death of foetuses or infants at an early stage [11].

The main component of chemical pollution of the environment is cadmium which is considered redundant in the human body [14]. Its greatest source which poses a significant threat to the body is nicotine [15]. More Cd may accumulate in women's bodies, in particular during pregnancy, which affects the kidneys, blood pressure and causes many pathologies in mothers and the foetuses, such as preterm birth or lower birth weight [16–21]. Despite numerous studies, the toxicity mechanisms are still not fully known [22; however, it has been shown that the mother's placenta is an effective barrier that protects the foetus against cadmium compounds [23].

Consumption of excessive amounts of zinc (>20 mg/kg/day) may have a negative effect on the absorption of elements such as Cu and vitamin A [24]. Zn may induce a Cu deficiency, and Cu supplementation also accelerates Zn coupling and reduces the Zn ratio, which in turn stimulates antagonistic actions in the transport of these two elements [25]. In addition, Zn

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has an antagonistic action for Cd in a detoxifying manner [26, 27, 28].

A low amount of zinc and copper compounds, calcium, iron and protein, in one's diet have a considerable influence on Cd accumulation in the body and its absorption from the gastrointestinal tract [29]. It is also suggested that an appropriate amount of Zn and Fe in the body can alleviate the effects caused by Cd [30]. Therefore, zinc (Zn) supplementation has a considerable influence when the exposure to Cd is moderate or relatively high as it reduces the burden of this toxic metal on the body and prevents some effects of its toxic action [31].

Due to the way in which Cd, Zn and Cu interact with each other, it seems very important that they should be considered in research. The transportation of metals in the human body is not yet scientifically known, although the processes of transporting elements, also in the placenta, between Zn and Cd is known. Cadmium interacts with Fe and Zn membrane transporter, which, as a result, may cause Cd to reduce the efficiency of Zn transport to the foetus by stimulating the production of metallothionein, which binds and inhibits the transport of Zn and Cd to the foetus [32, 33], which may slow down its growth and development [34]. Thus, cadmium has a significant influence on the metabolism of elements such as Zn and Cu in the body, which change the morphology and function of some organs. Copper, on the other hand, is a Zn antagonist [33]. As a result, its excess leads to zinc deficiencies and also a copper deficit causes interactions with zinc [33, 35].

Interactions between metal concentrations in the human body and environmental conditions exist in various geographic locations. These interactions can be of importance for the proper development of pregnancy and its course. In the current study, special attention was paid to two regions – central and north-western Poland. Both areas differ in terms of road traffic intensity, the number of industrial plants, as well as the number of power plants and CHP plants which emit large amounts of harmful gases. The Łódź Province has a typically industrial character and ranks in the last five in terms of environmental conditions. Protected areas constitute only a small part of its surface area. The accumulation of types of economic activity which constitute an ecological burden results in a relatively high environmental burden and exceed the average values for the whole country. The Łódź Province is one of the provinces with the highest production of gas pollution in the country. The level of development and quality of life is classified on a level of 'Class 3' out of four available classes. A positive aspect is the quality of public utilities in which the Łódź Province ranks among provinces with the best quality, followed by the West Pomeranian Province. However, the West Pomeranian Province has an industrial and agricultural character and is on a moderate level in terms of environmental conditions. In recent years, investments are being made to improve environmental protection, which is an advantage over the Łódź Province. The West Pomeranian Province produces less waste than the Łódź Province and this amount has been decreasing in recent year; dust pollution is also at a lower level than in the Łódź Province. The average level of development and quality of life is classified as 'Class 2' [36]. There were over 2,700 industrial economic entities in the Łódź Province during the research, compared to less than half of this number, i.e. approx. 1,250 in the West Pomeranian Province [37].

The quality of atmospheric air in central Poland is mostly affected by emissions of pollutants from local sources and by long-distance pollution from adjacent areas. The following local sources of pollution have the greatest influence on the deterioration of air quality: pollution emissions from business entities, road transport, illegal waste incineration (e.g. in household furnaces and others) as well as low emissions from coal-fuelled furnaces in single-family buildings, industrial plants, etc. The main threats to the environment resulting from agricultural activity are the pollution of soil and water environments in these areas [38].

Dust and gas pollution emissions into the atmosphere are predominant in north-western Poland. This is related to the fact that the main sources of emissions are situated nearby, i.e. a power plant complex and CHP plants, as well as chemical plants [39].

The aim of the study was to assess the concentrations of and relationship between Zn, Cu and Cd in the placenta, the foetal membrane and umbilical cord, and the geographic location in Poland.

Consent to the research was obtained from the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/76/14).

## MATERIALS AND METHOD

The study group consisted of 99 people aged 18 – 44 ( $29 \pm 5.5$  years) from central ( $n=52$ ) and north-western Poland ( $n=47$ ). Women who gave a single birth to a live born infant qualified for the study. Data for the research was collected from the medical history and hospital admittance documents and documents confirming the birth of a child. This included information about the place of residence, mother's age, date of the child's birth, the child's gender, number of pregnancies and births, as well as duration of the pregnancy. The study also included the type of birth (natural or C-section), size of the placental (expressed in centimetres) and placental weight (expressed in grams). Before collection of the material, each patient was informed about the type of research and expressed voluntary written informed consent to participate in the study. Placentae, foetal membranes and umbilical cords were collected from patients at the Gynaecology and Obstetrics Department, Dr A. Troczewski Local Government Hospital in Kutno (Central Poland) and John Paul II District Hospital in Gryfino (Northwest Poland). Both areas have similar populations, but a higher degree of pollution is recorded in Kutno, which is affected by road transport, waste incineration, pollution from paint shops, the food industry, and municipal and construction plants [40]. The level of heavy-metal pollution from particularly burdensome plants in the Łódź and West Pomeranian Provinces was 842 kg/year and 173kg/year for Zn, respectively, and 17 kg/year for Cd in the Łódź Province. No measurements of Cd were recorded in the West Pomeranian Province, nor data available for Cu [41].

The research material was stored at  $-27^{\circ}\text{C}$  until analysis. The tested material was described, weighed and dried at  $105^{\circ}\text{C}$  to obtain a solid mass for determination of these elements. After fragmenting in a mortar, the dried material was weighed using an analytical scale Sartorius YPD03-OCE (Sartorius AG, Germany) with an accuracy up to 0.0001 g. Next, the material was placed in tubes for further analysis.

Determination of zinc, copper and cadmium. 0.5g – 1.0g weighed-out samples of biological material were prepared. The material was mineralised under wet conditions in the glass tubes of a Velp Scientifica mineraliser (Italy) in 10 ml or 20 ml (depending on sample size) of a mixture of concentrated nitric acid HNO<sub>3</sub> (65%) and perchloric acid HClO<sub>4</sub> (70%) Suprapur Merck®, in a 4:1 ratio [42, 43]. After 12 hours of storing at room temperature, the samples were placed in a heating block and digested at 50–60 °C. The mineralisation was performed by slowly increasing the temperature by 200 °C. After the mineralisation stage, the samples were transferred quantitatively to 10 ml or 20ml certified class A volumetric flasks (flasks with a DURAN® DIN EN ISO 1042 compliance certificate) which were then filled up to the mark with distilled water (Pure Lab Option ELGA, certif. Genuine ELGA®, Global Operation Center).

The concentrations of zinc (Zn), copper (Cu) and cadmium (Cd) were determined using the ICP–AES method (spectrophotometry of atomic absorption) in inductively coupled argon plasma with the use of a Perkin-Elmer Optima 2000 DV device at the laboratory of the Department of Farm and Ornamental Bird Breeding, Faculty of Biotechnology and Animal Breeding of the West Pomeranian University of Technology in Szczecin. The limit of quantification for the device for Zn, Cu, Cd is 0.2, 0.4 and 0.1 µg/l, respectively.

Correctness of the analytical procedure was controlled by determining the tested elements in reference materials with known concentrations of these elements: NIST 8414 Bovine Muscle, BCR-185R, and DOLT-4 Dogfish Liver. These materials are manufactured by the National Institute of Standards and Technology (NIST), the Commission of the European Communities (BCR 185R) and the Canadian Irradiation Centre (DOLT-4). The characteristics of the study group from central and north-western Poland are presented in Table 1.

Statistical analysis. The arithmetical mean (AM), standard deviation (SD) and median (Me) were calculated for zinc, copper and cadmium in three types of biological material. The consistency of the concentration distributions for these elements with the expected normal distribution was tested using the Shapiro–Wilk test. The comparisons of average concentrations of elements between the place of origin of the sample were performed using the Mann–Whitney U test. Analysis of the interdependencies between individual elements was performed using Spearman's rank correlation coefficient. Univariate logistic regression was performed. The unit was changed for the purpose of calculating the odds ratio for cadmium.

The molar ratios were calculated as per Kalisinska et al. (2017) [44]. Dry and wet weight data was calculated into nmol/g by the formula: molar concentration (nmol/g) = concentration (nmol/g) × 1000/atomic weight (g/mol). The atomic weights of Zn, Cu and Cd were 65.38, 63.55 and 112.41 g/mol, respectively. The calculated Zn, Cu and Cd concentrations were used to calculate the Cu:Zn and Cu:Cd molar ratios. Statistical significance was adopted for a value of p<0.05. Statistica 13 Stat Soft software was used for statistical analysis.

Table 2 presents comparisons of average values for zinc, copper and cadmium concentrations and the molar ratio of Zn:Cu and Zn:Cd between women living in central and north-western Poland.

**Table 1.** Group characteristics

	Central Poland (n=52)	North West Poland (n=47)	p	
No. of pregnancies (n,%)	First	21 (40.4%)	13 (27.7%)	0.562
	Second	22 (42.3%)	22 (46.8%)	
	Third	5 (9.6%)	7 (14.9%)	
	Fourth	4 (7.7%)	4 (8.5%)	
	Seventh	0 (0.0%)	1 (2.1%)	
No. of births (n,%)	First	26 (50.0%)	15 (31.9%)	0.241
	Second	23 (44.2%)	28 (59.6%)	
	Third	3 (5.8%)	3 (6.4%)	
	Seventh	0 (0.0%)	1 (2.1%)	
Type of birth (n,%)	Natural childbirth	13 (43.3%)	32 (68.1%)	0.031
	Cesarean section	17 (56.7%)	15 (31.9%)	
Gender (n,%)	Male	24 (57.1%)	28 (59.6%)	0.816
	Female	18 (42.9%)	19 (40.4%)	
Gestational age [weeks], (AM±SD; Me)	38.0±3.2; 38.5	38.8±1.4; 39.0	0.566	
Placenta parameters (AM±SD; Me)	Weight [g]	657.8±140.3; 685.0	467.9±143.3; 450.0	<0.001
	Length [cm]	18.9±3.4; 18.0	16.8±3.5; 16.0	0.005
	Breadth [cm]	18.0±3.5; 18.0	18.0±3.8; 18.0	0.933
Newborn parameters (AM±SD; Me)	Weight [g]	3424.0±645.5; 3500.0	3529.1±514.0; 3530.0	0.765
	Height [cm]	53.6±4.3; 54.0	54.5±2.9; 55.0	0.362
	Head circuit [cm]	34.7±2.3; 35.0	34.9±1.8; 35.0	0.746
	Chest circumference [cm]	33.3±3.1; 33.0	34.0±1.9; 34.0	0.483

AM - arithmetic mean; SD - standard deviation; Me - median; n - number of participants; p - statistical significance value

## RESULTS

Statistically significant differences were observed between children born in central Poland and north-western Poland for zinc concentrations in the foetal membrane (p<0.001), copper in the foetal membrane (p=0.039), copper in the umbilical cord (p=0.042), cadmium in the placenta (p=0.003), cadmium in the umbilical cord (p=0.006), and the molar ratio Zn:Cu in the foetal membrane (p<0.001), Zn:Cu in the umbilical cord (p=0.006), Zn:Cd in the placenta (p=0.017), Zn:Cd in the umbilical cord (p=0.010).

Assessment of the relationship between zinc, copper and cadmium and the Zn:Cu, Zn:Cd ratios in the placenta, umbilical cord and foetal membrane and the mothers' place of residence is presented in Table 3. Among mothers living in central Poland, the results revealed a significant increase in zinc in the membrane (OR=1.098, p=0.002), cadmium in the placenta (OR=1.324; p=0.006), Zn:Cu in the membrane (OR=1.012; p<0.001). In women living in north-western Poland, in turn, an increase in copper in the membrane (OR=1.239; p=0.025) was revealed.

**Table 2.** Assessment of zinc, copper and cadmium concentrations and of Zn:Cu, Zn:Cd ratios in the placenta, umbilical cord and foetal membrane according to the mothers' place of residence

		Total			Central Poland (n=52)			North-West Poland (n=47)			p*
		AM	SD	Me	AM	SD	Me	AM	SD	Me	
Zn [mg/kg]	placenta	66.260	11.250	66.020	67.722	12.234	66.892	64.297	9.596	65.442	0.174
	foetal membrane	63.095	11.822	63.115	66.554	8.973	66.864	56.176	13.875	57.284	<0.001
	umbilical cord	54.653	11.633	52.389	55.480	15.161	51.046	54.410	10.949	53.197	0.938
Cu [mg/kg]	placenta	5.992	1.642	5.638	6.286	1.841	5.699	5.597	1.246	5.511	0.099
	foetal membrane	8.933	2.970	8.250	8.321	2.486	7.619	10.158	3.507	9.119	0.039
	umbilical cord	4.320	2.127	3.838	5.326	1.743	5.067	4.025	2.184	3.589	0.042
Cd [mg/kg]	placenta	0.010	0.003	0.009	0.011	0.004	0.010	0.009	0.002	0.008	0.003
	foetal membrane	0.009	0.003	0.009	0.010	0.003	0.009	0.009	0.003	0.008	0.194
	umbilical cord	0.008	0.005	0.008	0.013	0.007	0.011	0.007	0.002	0.007	0.006
Zn:Cu	placenta	733.7	165.8	730.5	718.8	178.6	721.6	753.8	147.0	743.1	0.431
	foetal membrane	488.0	157.2	459.2	546.9	151.7	528.8	370.2	87.2	358.6	<0.001
	umbilical cord	901.3	256.7	954.0	676.7	90.0	644.1	967.3	253.1	1001.3	0.006
Zn:Cd	placenta	819277.0	221346.5	811925.8	774349.3	239124.1	763539.6	879608.4	181170.6	889446.9	0.017
	foetal membrane	814386.1	229554.5	805228.8	827115.1	210080.0	813723.9	788928.2	267800.6	673608.4	0.229
	umbilical cord	851698.2	309880.8	894442.7	536548.3	263983.8	489135.4	944389.3	261622.7	991029.0	0.010

\* statistical significance value refers to the comparison between central Poland and north-western Poland

AM - arithmetic mean; SD - standard deviation; Me - median; n - number of participants; Zn - zinc;

Cu - copper; Cd - cadmium; p - statistical significance value

**Table 3.** Univariate logistic regression depending on the mothers' place of residence

		Central Poland				North-West Poland			
		p	OR	OR -95%	OR +95%	p	OR	OR -95%	OR +95%
Zn [mg/kg]	placenta	0.178	1.029	0.987	1.073	0.178	0.972	0.932	1.013
	foetal membrane	0.002	1.098	1.035	1.165	0.002	0.911	0.858	0.966
	umbilical cord	0.853	1.008	0.925	1.099	0.853	0.992	0.910	1.082
Cu [mg/kg]	placenta	0.068	1.407	0.975	2.030	0.068	0.711	0.493	1.025
	foetal membrane	0.025	0.807	0.669	0.974	0.025	1.239	1.027	1.494
	umbilical cord	0.265	1.289	0.825	2.015	0.265	0.776	0.496	1.212
Cd [g/kg]	placenta	0.006	1.324	1.084	1.617	0.006	0.755	0.619	0.922
	foetal membrane	0.162	1.157	0.943	1.420	0.162	0.864	0.704	1.061
	umbilical cord	0.129	3.540	0.693	18.098	0.129	0.282	0.055	1.444
Zn:Cu	placenta	0.345	0.999	0.996	1.001	0.345	1.001	0.999	1.004
	foetal membrane	<0.001	1.012	1.006	1.019	0.000	0.988	0.981	0.994
	umbilical cord	0.064	0.994	0.989	1.000	0.064	1.006	1.000	1.012
Zn:Cd	placenta	0.039	1.000	1.000	1.000	0.039	1.000	1.000	1.000
	foetal membrane	0.522	1.000	1.000	1.000	0.522	1.000	1.000	1.000
	umbilical cord	0.039	1.000	1.000	1.000	0.039	1.000	1.000	1.000

Zn - zinc; Cu - copper; Cd - cadmium; p - statistical significance value; OR - odds ratio

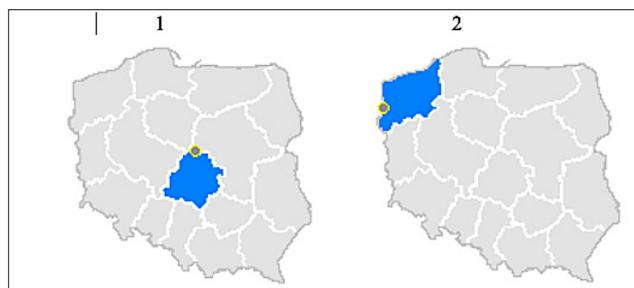
## DISCUSSION

In the developing world, the degree of the environmental exposure of the human body - especially during the prenatal period - is a very important aspect. One of the factors which influence the concentrations of elements in the human body is the place of residence. The majority of researchers focus on determining elements in the blood and in the mother's serum. Few of them specify the levels of elements and their molar ratios in afterbirths; therefore, the current study aimed to assess the profile of prenatal exposure to a toxic element

(Cd), and essential elements such as Zn and Cu in terms of environmental exposure. Moreover, the Zn:Cu and Zn:Cd molar ratios were specified which may help in assessing the risk of exposure in two Polish regions.

The average consumption of Zn for adults aged 20 - 50 is approx. 8.8-14.4 mg/day [45] and the average Cu consumption is approx. 1 mg/day [46]. The limit of Cd in the diet is estimated at 0.8 µg/kg of body weight, or 25 µg/kg of body weight per month due to a long half-life period [47].

In Poland, Kubala-Kukuś et al. (2003) conducted similar research to the presented study by comparing urban and



**Figure 1.** Location of the Łódź Province in Central Poland (Fig. 1) and the West Pomeranian Province in North-West Poland (Fig. 2) from which the study data was obtained

rural areas [48]. They demonstrated differences in Cu concentrations in the foetal membranes which amounted to 2.9 and 4.0  $\mu\text{g/g}$  for samples from the Bieszczady region (rural) and Legnica region (urban), respectively. Statistically significant differences were found for Cu concentrations in foetal membranes. They also demonstrated higher Zn concentrations in the placentae than in the membranes in rural and urban areas, which is consistent with the results for Zn obtained in the current study. In a study by Cerrillos et al. (2019), the average value of elements in the placenta was 17.24, 0.97, 8.43  $\mu\text{g/g}$  for Cd, Cu and Zn, respectively, and was 1.5 times and twice as low for Cu and Zn, respectively, compared to the research by Kubala-Kukus et al. (2003) [48, 49]. Amaya et al. (2013), on the other hand, examined placentae from women from urban (3.70 ng/g mm) and rural (3.85 ng/g mm) areas in Spain and did not demonstrate a significance between the place of residence and Cd concentrations. On the other hand, they revealed higher Cd concentrations in the placenta of women who smoked [50].

An aspect that deserves attention is research conducted with the participation of non-smoking women from rural areas in Bangladesh. The average Cd concentration in the placentae was 0.13 mg/kg dw., which was exceptionally high [34]. Cd concentrations in women from Bangladesh were comparable to concentrations observed in smokers or women living in areas with industrial pollution [51]. The source of exposure to cadmium pollution in this region is most likely food, namely, rice consumed in larger amounts [52] in which high cadmium concentrations are accumulated from soil [53].

The use of umbilical cord blood and the mother's blood, which is easily available, is appropriate for the assessment of foetal exposure to Cu, Zn and Cd. Cottrell et al. (2018) researched inorganic elements in umbilical cord blood in rural and urban areas in the USA. They found significant differences between rural and urban samples in which the Cu content was much higher in urban samples than in rural samples. However, the authors did not find any significant differences between the groups for Cd and Zn [54].

Tissues are an equally valuable material due to the ability of long-term accumulation of individual elements. Therefore, a valuable aspect of the research is demonstration of the content of elements in the placenta, which is a barrier in other tissues such as the foetal membrane and the umbilical cord [55].

Numerous research studies have shown the ability of the placenta to protect the foetus against hazardous elements. However, it was observed that the placenta not only protects, but can also limit the transfer of necessary elements. In individual studies it was demonstrated that the placental

barrier is present, to a certain extent, for elements such as Zn or Cu [56, 56, 58]. However, a study on the basis of which it can be concluded that the placenta is not a barrier for zinc exists [59]. For a harmful element such as cadmium, it is shown that it can partly pass through the placenta [60, 61], and also that the placenta can be a barrier for Cd to protect the foetus [62]. However, more extensive research is necessary to confirm this thesis.

Authors from Beijing in China conducted research using samples of the mother's blood and umbilical cord blood. It was shown that Cd in the mother's blood was at a much higher level than in the cord blood, which could imply that it was difficult for Cd to cross the placental barrier [63]. Using umbilical cords as the biological material, Sakamoto et al. (2013), Kozikowska et al. (2013) and Ni et al. (2019) examined Zn, Cu and Cd concentrations [64–66]. Sakamoto et al. (2013) demonstrated higher Zn, Cu and Cd concentrations in the placentae than in umbilical cords, which is consistent with the current study for all the presented elements, with the exception of Cd concentrations. The aforementioned differences in concentration values can be caused by the method of collecting the material and the various regions which differ in levels of industrialisation. Moreover, another reason could be exposure to harmful elements in the environment – in food products and in water. The similarity, on the other hand, may depend on individual conditions and needs of the body, which may regulate the optimal values of concentrations for necessary elements.

In the current study, two different regions, situated in north-western and central Poland, were considered. However, these are still large regions inhabited by up to 100,000 people. Statistical significance was demonstrated for these regions in Cd ( $p=0.003$ ) concentrations in placentae, in Zn ( $p<0.001$ ) and Cu ( $p=0.039$ ) concentrations in foetal membranes, as well as Cu ( $p=0.042$ ), Cd ( $p=0.006$ ) in umbilical cords.

Despite the different geographical locations, the degree of industrial development, culture and other factors, in the presented study the values of concentrations for Zn and Cu did not differ significantly from one another: Zn and Cu in placentae in Kutno (67.7 mg/kg d.m.; 6.29 mg/kg d.m.) and Gryfino (64.3 mg/kg d.m.; 5.6 mg/kg d.m.), compared to the study by Kippler et al. (2010) in Bangladesh (67 mg/kg d.m.; 5.3 mg/kg d.m.) [34].

Zn, Cu and Cd interact with each other [29]. The appropriate amount of Zn in the body may tone the toxicity caused by Cd [30]. Cd and Zn interact with each other in the human body and their co-existence causes a reduction in their levels. Therefore, zinc (Zn) supplementation has a considerable influence when the exposure to Cd is moderate or relatively high, as it reduces the burden of this toxic metal on the body and protects it against some effects of its toxic action [31]. The interaction between metals depends on numerous factors and the type of transport of some elements. A vicious circle also exists which is shown by Wisniewska et al. (2017) [67] who stated that Cu or Zn deficiencies may cause immune deficiency. Acute infections can also cause an increase in Cu concentrations in the serum and a decrease in Zn concentrations in the serum. This is particularly important in preterm infants without a fully developed immune system [67].

In the presented study, the molar ratio was tested as the most adequate in relation to descriptions of the tested elements. Statistical significance of the molar ratio in

biological material from mothers from central Poland and north-western Poland was revealed for the Zn:Cu ratio ( $p=0.010$ ), Zn:Cu ratio ( $p=0.006$ ) in the umbilical cord, the Zn:Cu ratio ( $p=0.017$ ) in the placenta and the Zn:Cu ratio ( $p<0.001$ ) in the foetal membrane. However, no similar study has been found in the available literature which shows similar relationships.

There are few studies in the available literature which assess element concentrations in afterbirths by comparing them between distinct regions. Comparisons between rural and urban areas would be valuable. The current study has focused on two large urban areas situated at a distance of approx. 430 km from each other, and with different degrees of industrial development. The results of this research, and other research described in the available literature, could have been influenced by a large number of factors, such as physical activity, stress, access to healthcare and education, nutritional behaviour, the use of stimulants, among others. In addition, it could also have been influenced by marital status, access to prenatal care, place of residence, industrialisation and many other factors.

In the contemporary dynamically developing environment it is necessary to control the concentrations of bio-elements in the human body, because the excess or deficiency of individual elements may pose a serious hazard to human health or life. There is also a considerable threat of exposing mothers and newborn infants to heavy metals which affect the children's neurological development when the exposure occurs in the prenatal period, and can also have a negative influence on intrauterine growth [11, 68]. However, due to the hazard resulting from the presence of toxic metals in biological materials, further research should be conducted to examine the influence of elements on the health of pregnant women and newborn infants [69, 70], as environmental exposure can have long-term consequence for the development of the foetus and newborn infant [71].

Limitations of the study. The results of the study may have been influenced by the small number of people in the study group, too high a blood content in tissue, and even the fact that newborn infants were born in different seasons and the availability and quality of consumed products can be limited depending on the season. Environmental conditions in the area where the study was conducted must also be taken into consideration. This area is situated in one country with similar environmental conditions, which can prevent the demonstration of higher significant differences. Moreover, one would need to take into account data concerning the environmental exposure considering the occupation and information on nutritional habits, including the consumption of fish and seafood, as well as amalgam dental fillings, which can influence the concentrations of toxic metals. However, there is still insufficient scientific research on the concentrations of elements in specific biological items; therefore, there is a justified need to continue the research.

## CONCLUSIONS

The study justifies the use of biological materials such as the placenta, foetal membrane and umbilical cord to assess the exposure to heavy metals and other necessary elements. There are significant differences in the accumulation of cadmium, zinc and copper, depending on the place of residence of the

study participants. Zinc and cadmium concentrations are higher in central Poland than in north-western Poland, which could be caused by a nearly twice as high number of industrial plants. Further research is justified regarding the influence of factors on prenatal exposure to necessary elements and heavy metals which are of considerable importance for a child's development. An analysis of trace elements in populations living in rural and urban areas would be appropriate.

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