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**Relationship between air pollution and metal levels
in cancerous and non-cancerous lung tissues**

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ABSTRACT

We aimed to check the relationships between levels of metals (Ca, Cd, Cu, Fe, Hg and Zn) in cancerous and non-cancerous lung tissues and their link to air pollution, expressed as particulate matter (PM) concentrations. The study also examines the influence on metal concentration in the lung tissue of patients' sex and the distance of their homes from the nearest emitter. We found that the general pattern of ascending concentrations in tumor tissue was as follows: $Hg < Cd < Cu < Ca < Zn < Fe$. In non-affected lung tissue the order of concentrations of Ca and Fe was reversed. With the exception of Cd and Cu, levels of metals were found in higher accumulations in non-cancerous tissue (e.g. Fe 326.423 and Ca 302.730 $\mu\text{g/g d.w}$) than in tumorous tissue (Fe 150.735 and Ca 15.025 $\mu\text{g/g d.w}$). Neither the PM₁₀ (PM of a diameter of 10 μm) concentration nor sex revealed any connection with metal concentrations. The shorter the distance from the emitter, the higher the metal concentrations that tended to be observed for almost all metals, but a statistically significant (but weak) relationship was noted only for Cu in tumor tissue ($r_s -0.4869$).

Keywords: metal; copper; air pollution; cancer; biomonitoring

INTRODUCTION

Since the industrial revolution, a considerable increase in anthropogenic air pollution has been noted. Among the polluting substances, dangerous to human health are particulate matter (PM) which includes metals, as among others Cd and Hg ^[1, 2] along

with other substances and compounds. A characteristic feature of PM, suspended dust with an electrostatic dry, dust ventilation, is the range of variation in its chemical composition and scale of diverse size fractions. ^[3] Frequent emission of PM10 (particulate matter of a diameter of 10 µm) is associated mainly with combustion, road transport and the urban sprawl. Many studies show that suspended dust in urban air has a significant impact on human health and may increase the risk of stroke, cardiovascular diseases and respiratory diseases. It may also be a significant risk factor for lung cancer and cancerogenesis. ^[4-7]

Lung cancer is the major cause of cancer mortality around the world. This is also the most common cancerogenic disease since 1985 and since then the number of cases has increased by 51%. The disease is more predominant among smokers, but also other factors, as air pollution, occupational exposure and diet may increase the risk of the disease. ^[8-9] Silesia is one of the most polluted areas in Poland and a high incidence of cancer has been noted among its inhabitants. The cause of the cancer rate in the area is still not fully confirmed, but the link to environmental pollution is suspected. ^[10-11] Anthropogenic emissions, including energy and industrial production are the main source of air pollution in Silesia where the highest number of point sources are to be found in Poland. The PM10 level in Silesia is high and shows variability depending on the meteorological conditions and degree of urbanization. ^[12] The varying fraction size of dust released from emitters also causes a variation in the distance the emissions may travel. ^[3]

Epidemiological evidence of the relationship between lung cancer and exposure to metallic compounds may be found in the literature. ^[13-15] Metal contents in lung tumors may, moreover, be different from those in non-cancerous tissue. Assessing the impact of PM10 exposure on metal concentrations in both cancerous and non-

cancerous tissue types therefore would appear to be of utmost importance. There is, however, no comprehensive evaluation of the possible association between these factors for cancerous and non-cancerous lung tissue. Since we also know that the PM10 concentrations may vary geographically, the other potential link to sources of air pollution (e.g. distance from the emitter) to lung tissue investigations should be taken into account. Our studies address this lack of research and verify the connection mentioned.

The major aim of the research was to estimate the concentrations of common metals, such as calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg) and zinc (Zn), in cancerous and non-cancerous lung tissue in patients from Silesia. We evaluated the differences in metal concentrations according to tissue type, the sex of the patient and PM10 pollution in the area the patient lived. We also verified the relationship between the levels of metals examined and the distance from the nearest emitter, as well as the relationships in concentrations between metals and tissues.

MATERIALS AND METHOD

The patients (n=56) whose data were used in the study were patients of the Pulmonology and Thoracic Surgery Center in Bystra, Poland. They represented two study groups: one suffering from lung cancer (n=42; non-small cell lung cancer) and the other (the control) suffering from non-cancerous pulmonary disease (i.e. chronic obstructive pulmonary disease, tuberculosis, idiopathic pulmonary fibrosis; n=14). Some patients were smokers, but they were spread evenly among studied groups. None of the patient was exposed to PM or metals occupationally. All the patients live

in the Silesia region which is heavily urbanized and highly populated area. The main source of PM₁₀ air pollution here is the energy industry, emission from the residential sector, agriculture and emissions from road transport.

Samples and analytical procedure

All the samples were collected between 2013 and 2014 in the hospital while the patients were being treated. Patients with non-cancerous disease gave lung tissue samples during biopsy. Patients suffering from cancer gave samples of tumors and of adjacent non-affected tissue during surgery. After the samples were collected, some parts were sent for pathological analyses (unconnected with this study) and the rest were used in metal assessment protocol. Those samples were stored at a temperature of -20°C.

The concentrations of the metals, Ca, Cd, Cu, Fe and Zn, were measured in ca. 2 g of wet weight, firstly oven-dried (60°C, SUP-100W dryer, WAMED), then mineralized with hot nitric acid (65%, Baker Analyzed, JT Baker) in the open mineralization system (Velp Scientifica DK20). Mineralized solutions were diluted with ultrapure water (18.2 MΩ·cm at 25°C, Direct-Q 3, Merck-Millipore) up to 10 mL and analyzed with a flame atomic absorption spectrometer (AAAnalyst 200, PerkinElmer). The final results, after comparison with the limits of quantification and recalculations, were presented as μg of metal per 1 g of the dry sample (μg/g d.w.). Hg measurements were taken in the automated Hg analyzer (NIC MA-2) without the external mineralization in ca. 100 mg of each sample (with two repetitions). The initial Hg results were obtained in μg/g wet weight, but they were then recalculated and also expressed as

$\mu\text{g/g d.w.}$ ^[16] The whole procedure was checked against the certified reference material analysis (Table 1).

External data

We investigated the connection between the distance from a patient's place of residence to the nearest big emitter, such as a mine or smelter, and metal concentrations in the patient's tissues. The distance was calculated as the straight line between the two points on a map.

Data regarding the concentrations of PM10 in the air where patients lived were taken from the system of air quality monitoring carried out by the Regional Inspectorate for Environmental Protection in Katowice. ^[17] In accordance with that we have distinguished five classes of PM10 (metric tons per year: 2-100; 101-155; 156-220; 221-410 and 411-3690) in the area studied and in further statistical analysis we evaluated the influence of the PM10 factor on the concentrations found in the tissue studied.

Statistical analysis

The distribution of the data and variance homogeneity among the study groups were checked with the Shapiro-Wilk and Levene tests. ^[18] Since deviations from the assumptions were observed, factorial ANOVA on ranks were carried out. ^[19] The relationships between metal concentrations and other parameters were tested with the Spearman correlation analysis (r_s). In all the statistical tests, the significance level was

set at 0.05. All the calculations and analyses were performed with StatSoft Statistica 10 EN and Microsoft Excel 2016 EN for Mac.

RESULTS

All the metals were found in both tissue types examined. Values lower than the limit of quantifications were observed in the frequencies: 12% for Ca, 8% for Cd, 4% for Cu, 0% for Fe, 6% for Hg and 3% for Zn. PM10 and sex factors revealed no connection with metal concentrations (Table 2). On that basis, the data were pooled and the results were presented without the division. Additionally, since there were no differences in metal concentrations in non-affected lung tissue between patients from the group suffering non-cancerous diseases and the group suffering from cancer (the Mann Whitney test, the lowest p was noted for Fe 0.2070), all the non-tumor data (non-affected tissue) were used in the comparison with the tumor tissue.

Metal concentrations

The general scheme of ascending concentrations in tumor tissue was as follow: Hg < Cd < Cu < Ca < Zn < Fe. A similar trend was noted in the case of non-affected lung tissue with the exception of Ca whose median concentration was higher than the Zn level (Table 2). Concentrations of Ca, Cu, Fe and Hg differed significantly between tumor tissue and non-affected tissue (

Table 3). Higher levels were generally noted in non-affected lung tissue, with the exception of Cu whose concentrations were higher in tumorous samples. Of all the metals studied Fe achieved the highest median levels in both tissue-types tested (150.740 $\mu\text{g/g}$ d.w. for tumor and 326.42 $\mu\text{g/g}$ d.w. for non-affected lung). Unlike Fe, the median concentrations were the lowest for Hg (0.0203 $\mu\text{g/g}$ d.w. and 0.0340 $\mu\text{g/g}$ d.w. for tumorous and non-affected tissues, respectively).

Correlations between metals tested and distances from emitters

For all the metals studied (with the exception of Ca and Fe in tumor) negative trends regarding the distance to the nearest emitter were observed, but almost all of them were statistically insignificant (

Table 3). The only significant, but still weak relationship occurred for Cu concentrations in tumor tissues (r_s -0.4869, $p < 0.05$, Figure 1, Table 3).

Statistically significant correlations of metal concentrations between tissues were observed (Table 4). The strongest relationships were detected in Cu and Zn concentrations in tumors (r_s 0.5449). We also noted a significant, but weak correlation of Hg concentrations between cancerous and non-cancerous tissue (r_s 0.3811).

DISCUSSION

We found that neither sex nor PM10 were differentiating factors in the metal concentrations in tumor tissue and non-affected lung tissue. Despite this, we observed

the negative correlation between environmental pollution (expressed as the distance of the nearest emitter to the patient's city of residence) and Cu concentrations in tumors. In other cases, similar trends, albeit statistically insignificant, were noted. We also observed several relationships between the concentrations of metals in tissues studied.

Air pollution and metals

Since we know that mining and manufacturing have been major industrial activities in Silesia for several decades, the contamination of this region with various pollutants is of special importance.^[20-21] We also know that air pollution may be harmful to people's health. Generally, women are more susceptible than men to the harmful influence of air quality on respiratory health. It is not yet well explained whether observed distinctions are attributable to biological differences (e.g. hormonal composition), differences in exposure (e.g. occupational) or to a combination of the two.^[22] Our results did not support these hypotheses, since we reported no differences between gender groups of patients studied.

An imbalance in the homeostasis of metals may generate reactive oxygen species (ROS) production leading to oxidative stress, and consequently cause pathological conditions.^[23-24] Since we know that Fe may be involved in tumor initiation and that Zn supported tumor growth, we suppose that the levels of these metals could be higher in the tissue before a tumor is diagnosed.^[25-26] Some investigations have confirmed that high Fe levels in breast tissue positively correlate with an impending risk of cancer.^[27] Declining Zn concentrations were also seen in malignant lung tissue when compared to non-affected tissue.^[28] This may explain the observation of higher Fe and Zn levels in lung tissue. We also suppose that higher Ca levels in the non-

affected tissue studied may be linked with subsequent cancer development because Ca^{2+} overload induces mitochondrial dysfunction, as well as increasing ROS production and cellular damage.^[29] Transition metals such as Hg under pathological conditions additionally accumulate in target organs, lead to ROS generation and lipid peroxidation, promoting oncogenesis.^[30] This may explain the higher Hg concentrations detected in non-cancerous lung tissue, which is also consistent with other studies.^[31]

Generally, we observed higher concentrations of metal (with the exception of Cd and Cu) in non-affected lung tissue. Higher Cu levels in tumor tissue may be explained by the fact that Cu-thionein may promote angiogenesis throughout the supplementation of Cu ions for enzymes involved in the formation of new blood vessels^[32] as one stage of the process of carcinogenesis. The mechanism of the accumulation of Cd in tumor tissue, already known in the literature, is unclear, but it may be linked with the increasing total accumulation according to age and the effect of cumulative exposure from various sources, such as environmental pollution, occupational exposure and lifestyle.^[33-34]

Correlations

Negative weak trends between the levels of metals in non-affected and in cancerous lung tissue and distance from emitters were found. These results pointed to the tendency that the further from the source of pollution, the lower the metal levels detected. The same tendency has already been observed in plants and crabs.^[34-35] The strongest, but still weak correlation we observed linked the distance with Cu concentrations in tumor tissue (Figure 1). Cu is one of the main products of smelting

and its concentrations in the tissues decrease as the distance from the source increases.

^[36] This along with our observation may be very useful in biomonitoring, but one should bear in mind that emitters such as mines and smelters are not the only cause of air pollution. Today a great many point sources of air pollution exist, such as on-road mobile sources (e.g. cars and buses), non-road mobile sources (e.g. trains, aircraft,) and immobile sources (e.g. household coal boilers). All these carriers add to the general air pollution and affect organisms (including the stimulation of cancerogenesis). ^[37] Factors such as occupational exposure or daily movement may influence the inference and the method may thus be useful for large areas, such as provinces or areas with a very few, but centralized emitters.

Zn plays a role in regulating Cu levels in the body which may explain the correlation we noted between them. ^[38] Essential elements protect against intoxication by other metals, and this may explain the significant relationship between Cd and Cu. ^[39] We also saw the positive correlations Zn-Fe and Zn-Ca. Correlations between metals in the same organs may represent evidence of similar distribution of those metals throughout the system. ^[39] Interestingly, Cobanoglu ^[40] found a negative relationship between Zn and Fe levels in cancerous lung tissue, which contradicts Yoo's ^[39] and our results.

We observed a significant, positive correlation between Hg levels in tumors and in non-cancerous tissue. Tumors grow inside the lung tissue and may even be considered to be changed tissue, so this could explain the relationship observed. This hypothesis, however, needs further examination.

CONCLUSIONS

The study revealed that the PM10 factor seems to have no direct relationship with the levels of metal in the tissues studied. The tendency that the shorter the distance from the emitter, the higher the metal concentrations in target organs, however, was observed for copper in tumor tissue ($r_s -0.4870$). Non-cancerous tissue seems to reach higher levels of metals in comparison with tumorous tissue. This may suggest that the metals studied, if found at elevated concentrations, may promote cancerogenesis and air contamination may be a co-factor behind cancerogenesis. The results also indicate the importance of the distance of the emitter as a tool in evaluating exposure, however a stronger relationship is to be observed for areas with very few, but centralized emitters, or large areas, such as provinces.

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Table 1. Characteristics of the analytical method used: limits of quantification (LoQ) in the mineralized solutions [mg/L], recoveries for certified reference material (CRM) analyses (n=11) with relative standard deviations (RSD) between replicates

Metal	Wave λ [nm]	LoQ	CRM**	Recovery [%]	RSD [%]
Ca	422.70	0.514	SRM1577b	100.3	2.1
Cd	228.80	0.010	SRM1577b	106.8	2.7
Cu	324.80	0.035	SRM1577b	92.2	1.3
Fe	248.30	0.415	SRM1577b	92.3	6.2
Hg	253.70	0.208*	BCR-463	99.2	1.7
Zn	213.90	0.024	SRM1577b	108.1	2.4

*LoQ value for Hg expressed as ng per sample.

** SRM1577b - bovine liver, National Institute of Standards & Technology, USA; BCR-463 – tuna fish, Joint Research Centre, Institute for Reference Materials and Measurement, Belgium).

Table 2. Concentrations of metals ($\mu\text{g/g}$ d.w.) and the significance of sex and PM10 factors (factorial ANOVA on ranks) in tumor and non-affected lung tissue of patients studied

	n	Median	Minimum	Maximum	Q ₁	Q ₃	Sex factor p	PM10 factor p
Ca tumor	36	15.025	0.000	377.767	0.000	76.167	0.2334	0.1563
Cd tumor	36	1.239	0.000	8.108	0.728	3.096	0.9023	0.4669
Cu tumor	30	9.151	3.700	18.633	7.819	11.239	0.4935	0.7413
Fe tumor	36	150.735	38.265	1021.244	97.497	220.476	0.9821	0.7579
Hg tumor	40	0.020	0.000	0.299	0.010	0.034	0.3374	0.4137
Zn tumor	36	77.382	0.000	233.449	58.602	93.511	0.1808	0.2107
Ca lung	38	302.730	53.557	6420.290	142.765	660.225	0.6720	0.3036
Cd lung	38	1.084	0.000	11.111	0.160	3.182	0.2696	0.2559
Cu lung	38	5.474	0.000	49.684	4.680	6.960	0.4030	0.9122
Fe lung	38	326.423	105.378	3251.029	217.729	571.977	0.3129	0.2925
Hg lung	53	0.034	0.000	0.606	0.027	0.046	0.5863	0.5310
Zn lung	38	79.990	29.576	204.545	60.927	99.849	0.3873	0.3107

Q₁ – lower quartile, Q₂ – higher quartile.

Table 3. The differences in metal concentrations between tumor tissue and non-affected lung tissue (Mann Whitney test; p) in the population studied and the correlations between metal concentrations and distance from the emitter (Spearman correlation r_s)

	Tumor vs lung concentrations p	Distance with tumor concentrations r_s	Distance with lung concentrations r_s
Ca	<u><0.0001</u>	0.1452	-0.1032
Cd	0.6302	-0.0596	-0.2018
Cu	<u><0.0001</u>	<u>-0.4870</u>	-0.1353
Fe	<u><0.0001</u>	0.0389	-0.1529
Hg	<u><0.0001</u>	-0.0047	-0.0907
Zn	0.7610	-0.2966	-0.0146

Underlining indicates statistically significant differences and correlations.

Table 4. Significant Spearman correlations (r_s) between metal concentrations in tissues studied

Correlation	r_s
Ca lung vs Zn lung	0.4264
Ca tumor vs Cd tumor	0.4411
Cu tumor vs Zn tumor	0.5449
Fe lung vs Zn tumor	0.3977
Hg lung vs Hg tumor	0.3811

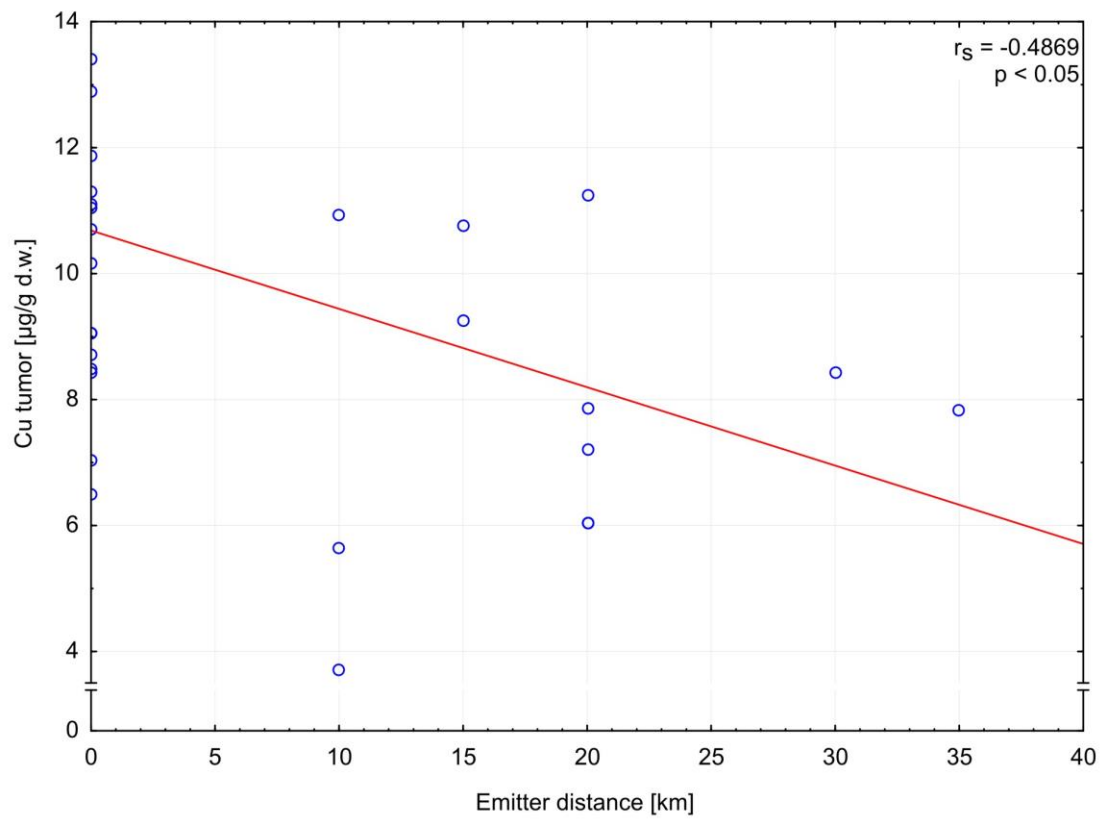


Figure 1.

FIGURE CAPTION

Figure 1. Statistically significant correlation between levels of copper in tumor tissue and distance from the nearest emitter.