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PERSONAL MONITORING AND BIOINDICATORS OF EXPOSITION TO SOME CHEMICAL POLLUTANTS IN INDUSTRY AND RESIDENTIAL AREAS: ECOTOXICOLOGY OF PROTECTIVE ZONES

Simple method of NO_2 analyses was applied to estimation of spatial differentiation of the air pollution in some regions of Poland. Samplers could be applied to measurement of both personal exposition to nitrogen oxide and nitrogen dioxide as well as for detection of risk of deterioration of culture and nature heritage. SEM and X-ray microanalyses of elements in the single blood cells are very useful for individual monitoring of patients and healthy people exposed to environmental pollutants. There were observed relations between industrial pollution of residential areas in regions of steel-works and levels of Pb, Cd, Zn in the whole blood of their inhabitors under similar professional exposition.

1. INTRODUCTION

Estimation of risk factors responsible for characteristics of human environment requires the improvement of methods for measurement of personal exposition to various pollutants in working places, residential districts, recreation areas, etc. It is also necessary to detect indirect intoxication by the food chain. This ecotoxicological aspect was considered with respect to the level of oligo and trace elements (including heavy metals) in soil, drinking water, food of animals, cattle blood and cow milk, as well as in human blood in cancer and leukemic cluster [5].

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There were also conducted interdisciplinary "case studies" on the pollution of natural environment with some metals and on the intoxication of people and animals living in the biggest in Poland region of lead and zinc mining and metallurgy center "Bolesław" [7], as well as in the protective zone of former the Lenin Steel Plant [8].

Such bioindicators as blood, placenta and hair samples are especially useful for the evaluation of biocummulation of many environmental pollutants. Coupling of ecotoxicological and epidemiological studies with the measurement of concentrations of selected pollutants in individual persons seems to be necessary, both for the estimation of professional hazard in industry as well as pollution of the natural environment and food chain.

Some methods may be recommended for a wider application, e.g. for measurement of individual exposition (bot in- and outdoors) to some toxic gases like nitrogen oxides and also for analyses of the amounts of selected elements in blood and even in single blood cells of human beings and animals. In order to estimate the background level of the elements subject to analysis, it is necessary to know the amounts of these elements in healthy individuals and in those suffering from various diseases. There are also some biophysical methods useful in comparative studies (e.g. stimulated photon emission measurement, chemiluminescence, bioluminescence).

2. METHOD OF NO_2 AND SO_2 MEASUREMENT

There is a simple and economical method of measuring individual exposure to NO_2 and SO_2. A plastic sampler, ca. 2 cm in diameter, covered with a lid, has been constructed. When the sampler is opened, a hydrophobic membrane protects the adsorbent against the influence of rainfall and wind. The original Japanese filter paper, Toyo Roshi No. 50, present in the sampler is impregnated with 0.06 cm^3 of aqueous solution of 20% v/v triethanolamine. It is possible to select any time for exposure, e.g. during working hours in a specific chemical plant or at any time in any particular place (e.g. indoor environment or in areas for recreation, etc). After the exposure, colorimetric analysis is carried out in the standard way (by colorimeter or specol).

This method is very useful, both to study the correlation between personal exposure to NO_2 and SO_2 and the occurrence of bronchitis and asthma and to screen and monitor these gases. This method allows also an epidemiological survey, regarding for instance plant protective zones and living districts. The samplers can be used additionally in the investigation of spatial and temporal differences in the mean levels of NO_2 and SO_2 and in studies on the deterioration of historic buildings, sculptures, trees and other natural monuments. This simple method, enabling simultaneous analyses made in a great number of selected points, may be helpful in determining proper locations of new habitat, living houses, green areas, recreation centers, parking facilities and for the protection of cultural heritage (including the old city of Cracow, recognised by UNESCO as the World Culture Heritage). Amaya's method, described here, was applied in Japan and later on larger scale in Poland to determine, e.g. the impact of traffic on air pollution (see table 1). For preparation of samples take a piece of filter paper impregnated with 20% v/v aqueous solution of triethanolamine and next put it in a sampler (plastic vessel of about 2.5 cm diameter, made of polyethylene) or in a frame for slides, put on a cover of hydrophobic porous film (e.g. made of polypropylene) and for measurement of NO_2 concentration (usually for 24 h) take off the lid and expose the sampler at a desired place. After finishing the exposure, put on the lid and store the sampler at a tight vessel. For analysis, add 3 cm^3 of Salzman solution and leave the sampler for 15 min at room temperature, thereupon measure the intensity of reddish-purple colour (indicated NO_2 in the air) by colorimeter in green light.

Table 1

Results of application of simple colorimetric method (Amaya's) to screening and monitoring mean NO_2 concentration in the air during 24 h in different regions of Poland

Place	Mean concentration of NO_2 (ppm)	
	Working days	Holidays
Chrząszczewo		
Wrzosowo (rural areas near center of crude oil production	0.009–0.150	0.004
Dziwnów (area of recreation in the coastal region)	0.002	0.022
Biecz (small town)	0.049	0.015
Jaworki (village near the Pieniny Mts. National Park and recreation areas)	0.005	0.010

Maximum NO_2 concentrations (mean value for 24 h) in:	
Kamień Pomorski (town on the Baltic coastal region)	0.023
Dziwnów (as above)	0.022
Świnoujście (near harbour and hospital, on the beach)	0.040
	0.007
Szczawnica (center recreation in Pieniny Mts., near the bus stop)	0.200
Homole valley (reserve of nature)	0.009
Szczawnica (at the main street)	0.015
Szczawnica (in the park for recreation)	0.005

There is significant correlation between 24 h mean concentration of NO_2 in the air and traffic intensity, e.g. in Wrzosowo on the Baltic coast, during working days this concentration was 0.150 ppm and in holidays, 0.004 ppm. Such a correlation has been also stated for localisation of samplers, e.g. in the park in Szczawnica in the border of the oldest in Europe park, 0.005 ppm and at the bus stop, 0.200 ppm.

3. METHODS OF ESTIMATION OF PERSONAL HEALTH HAZARD

In the selected working places of one industrial center (the biggest steel plant in Poland), studies of air containing lead-bearing steel dusts were conducted by standard methods of aspiration and AAS analyses, as well as by the use of Rtg-diffractograms. As a result of these studies, some technological recommendations were elaborated for promoting clean air and health conditions. AAS analyses were carried out in blood samples taken from workers of similar professional exposure but coming from different residential areas and from other inhabitants forming control groups. An important influence of the natural environment pollution has been stated in residential regions, where people are exposed to lead and other metals poisoning. Mean level of lead in blood samples of the people living in one protective zone of a great metallurgical center was 6.23 ppm, and that of cadmium was 1.10 ppm. For people inhabiting another district, the concentrations of these elements in blood were 1.78 and 0.22 ppm of lead and cadmium, respectively. Distinct differences in concentrations of some trace elements have been also found in samples of human placentas collected within the protective zone of this steel work. These concentrations ranged from 0.01 to 0.038 ppm of lead and from 0.015 to 0.055 ppm of cadmium. Such a bioindicator as human placenta is especially recommended because of a high sensitivity of foetus to various environmental pollutants and the risk factors of congenital malformations (HARADA, NIWELIŃSKI et al., personal information). Therefore, AAS analyses of placenta and maternal blood and hair samples are being conducted in relation to personal exposition. Results of the analyses of lead, cadmium and zinc in the samples of blood of workers living in various regions are shown in table 2.

Table 2

Mean contents of lead, cadmium and zinc in the blood of the workers in big metallurgical center, depending on living places (determined by AAS)

Place	Element (μg/g)		
	Lead	Cadmium	Zinc
Zawiercie	$\bar{x}$ 1.20	$\bar{x}$ 0.06	$\bar{x}$ 6.19
	SD 2.48	SD 5.87	SD 0.97
Ząbkowice	$\bar{x}$ 1.13	$\bar{x}$ 0.07	$\bar{x}$ 6.21
	SD 2.05	SD 6.98	SD 1.34
Dąbrowa Górnicza	$\bar{x}$ 0.54	$\bar{x}$ 0.04	$\bar{x}$ 6.56
	SD 0.96	SD 0.08	SD 1.87
Strzemieszyce	$\bar{x}$ 0.59	$\bar{x}$ 0.05	$\bar{x}$ 8.66
	SD 0.58	SD 0.07	SD 2.14
Gołonóg	$\bar{x}$ 0.65	$\bar{x}$ 0.05	$\bar{x}$ 6.41
	SD 0.58	SD 0.12	SD 2.02
Sosnowiec	$\bar{x}$ 0.53	$\bar{x}$ 0.03	$\bar{x}$ 7.99
	SD 0.43	SD 0.02	SD 3.98
Będzin	$\bar{x}$ 0.45	$\bar{x}$ 0.02	$\bar{x}$ 6.41
	SD 0.48	SD 0.02	SD 2.31

$\bar{x}$ – mean, SD – standard deviation.

Some metals have been determined in zooindicators at different distances from the selected industrial centers. Mean amounts of lead and cadmium in the samples of cattle hair (in the area of the protective zone) ranged from 1.80 to 5.41 ppm and from 0.10 to 0.27 ppm, respectively [8]. High levels of both metals were detected in similar areas. Such coincidence may increase the risk factor of chronic toxicity. In some zones, the amounts of lead and cadmium in the hair samples of dogs were different depending upon the distance from the mining and metal-working plants. The excess or deficiency of certain elements in soil, water, cultivated plants and animals influences the contents of these elements in both samples of whole blood and individual blood cells of man and cattle [5]. This method of investigation permits us to associate certain morphological and cytochemical differences between normal and leukemic blood cells with the differences in the contents of some oligo- and trace-elements. It has been found that the improved powder electrodes of Tomassi and Janczarski's construction and the measurements of oxidation–reduction potential and bioluminescence are really useful in comparative studies of blood of healthy people and patients suffering from selected blood diseases (see *New horizons of health aspects of elements*, S. B. VOHORA., J. W. DOBROWOLSKI (eds.), Hamdard University, New Delhi, in press). Qualitative and quantitative differences were repeatedly observed between photon-emission curves for normal and neoplastic (leukemic and cancer) cells [6], especially for blood cells of patients suffering from acute leukemia, breast cancer and some other solid tumors. Chemiluminescence studies included spontaneous and induced photon emission. It was found that exposure to UV and addition of Fe^{2+} to the suspension of lymphocytes or blood serum multiply the differences in photon-emission curves between leukemic and control groups. Chemiluminescence method allows us also to determine more precisely certain air pollutants, mutagens included. Other methods, such as EPR, or NMR, are also of importance in determining biophysical changes during the development of leukemia or cancer. The studies performed at the submolecular level permit an evaluation of the hazards associated with the synergistic effects of various chemical, physical and biological carcinogens. A combination of various research methods gives the possibility of evaluating the sensitivity of human organism to many environmental factors, depending, e.g. on the content of scavengers in the body.

Apart from original studies conducted by our interdisciplinary team and other Polish scientists, the use of such equipment as "Beprom" introduced by Beckenkamp, Müller, Rosenberg and Wilke should be also discussed, since it makes it possible to evaluate accurately the exposure to NO_2 and SO_2 in any workplace and to study their influence on the activity of selected enzymes in the phytoindicator, *Hypogymia physodes*. Beckenkamp also combined the results of ecological monitoring with the localisation of dwellings of people suffering from lung cancer and leukemia living in the Sarland industrial area in Germany.

Amaya developed a calorimetric method and constructed samplers permitting screening monitoring of mean NO_2 and SO_2 concentrations in the air and the evaluation of personal exposure to these gases and some other pollutants (O_3, acid rains, etc.). He also used successfully a high sensitivity calorimeter, type MPC-III made by Hagiwara, in order to detect the presence of certain radioactive isotopes and to determine quickly both the number of bacteria in a sample and its dependence on low concentration of chemical pollutants in water. Such a calorimeter, in addition to the identification of certain risk factors, makes it possible to study the changes in the cellular metabolism caused by environmental pollution. The application of new and different research and prognostic methods, including computer simulation, gives a higher probability of forecasting health hazards and undertaking prophylactic measures in time.

We would like to recommend the application of the above-mentioned methods as connection with the measurement of the contents of different elements in single blood cells of healthy people (living in both polluted and unpolluted areas) as well as sick persons.

4. CELL MONITORING OF ELEMENTS

In determination of the content of a given element in intracellular space, various techniques are used, namely: absorption atomic spectrophotometry (AAS), flame spectrophotometry, ion selective electrodes, Quin-2 method, nuclear magnetic resonance (NMR) and neutron activation [9], [12], [15], [18], [19], [20]. Erythrocytes, leukocytes and platelets are used as biological material for cell monitoring of elements [11], [12], [13], [16]. In our study we have chosen electron X-ray microanalyse because of its pecularity. It allowed us to analyse chemically the content of single cells under scanning electron microscope.

The elctron X-ray microanalysis techniques [10], [14] are fluorescent spectral analysis in the microarea. The electron beam accelerated to 20 keV bombards the sample inducing in the irradiation site a number of physical effects, the most siginificant of which is the emission of X-radiation characteristic of a given element. The intensity of X-radiation is proportional to the element mass in the volume of the sample irradiated by the electron beam, that is to the total element concentration. Comparing the intensity of X-radiation emitted by the sample of the unknown content of the element with X-radiation emitted from the known model, one may determine total concentration of any element ranging from boron to uranium [1], [2].

The contents of some elements were determined in peripheral blood lymphocytes isolated by the modified BOYUM's method [4]. Serum separated from the blood was put in layers of lymphoprep and the samples were centrifuged. Next, the lymphocyte layer was collected and suspended in 2 cm^3 of 0.9% NaCl with 2 cm^3 of 5% glutaraldehyde and the sample was left overnight in a refrigerator (+4°C). Next day, the degreased surface of the aluminium holder (99.99% Al) was covered with 0.1% water solution of polylysine (Serva) in 0.1% Triton X-100 (Windsor Laboratories Ltd.).

The solution was left on the holder surface for 10–15 min and then its excess was removed and dried. The holder surface prepared in this way was covered with lymphocytes centrifuged for 5 min at 800 rev/min. Then the preparation was washed with 50%, 70%, 80%, 90%, and 100% ethanol.

Using the X-ray microanalysis method we stated statistically significant differences in concentration of the selected elements in blood cells of leukemic patients in comparison with the control [3]. We observed an excess of magnesium and deficiency of zinc in lymphoblasts of people suffering from chronic lymphatic leukemia (CLL), as well as high concentration of calcium in patients suffering from acute leukemia. The link between chemical composition of the natural environment, food chain and level of some elements in human and animal bodies was also reported [5].

In patients with primary arterial hypertension, total potassium content in peripheral blood lymphocytes was $\bar{x} = 0.129 \pm 0.041\%$, being significantly lower than in healthy persons where $\bar{x} = 0.156 \pm 0.042\%$, $p < 0.002$. Total intracellular magnesium content in patients with primary arterial hypertension and in healthy persons did not differ significantly, being $\bar{x} = 0.053 \pm 0.011\%$ and $\bar{x} = 0.052 \pm 0.020\%$, respectively. There was a low, significant correlation between intracellular magnesium and potassium concentrations both in hypertensives ($r = 0.337$, $p < 0.05$) and in healthy persons ($r = 0.292$, $p < 0.05$) [17]. Results of X-ray microanalyses of control lymphocytes are shown in figs. 1 and 2.

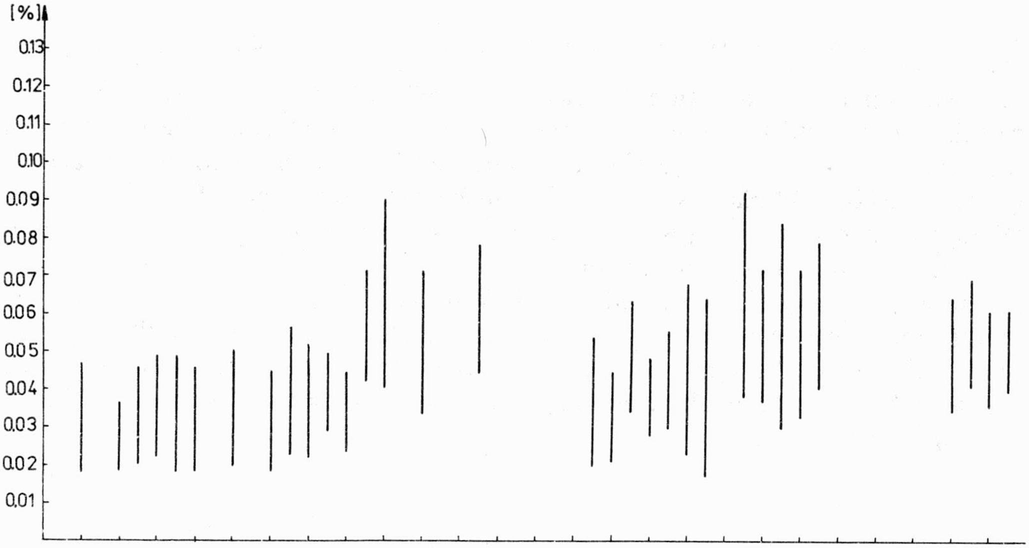

Fig. 1. Concentration of magnesium in human control lymphocytes

X-ray microanalysis was also used to determine the influence of environmental pollutants on the level of selected elements in blood cells. It was found a correlation

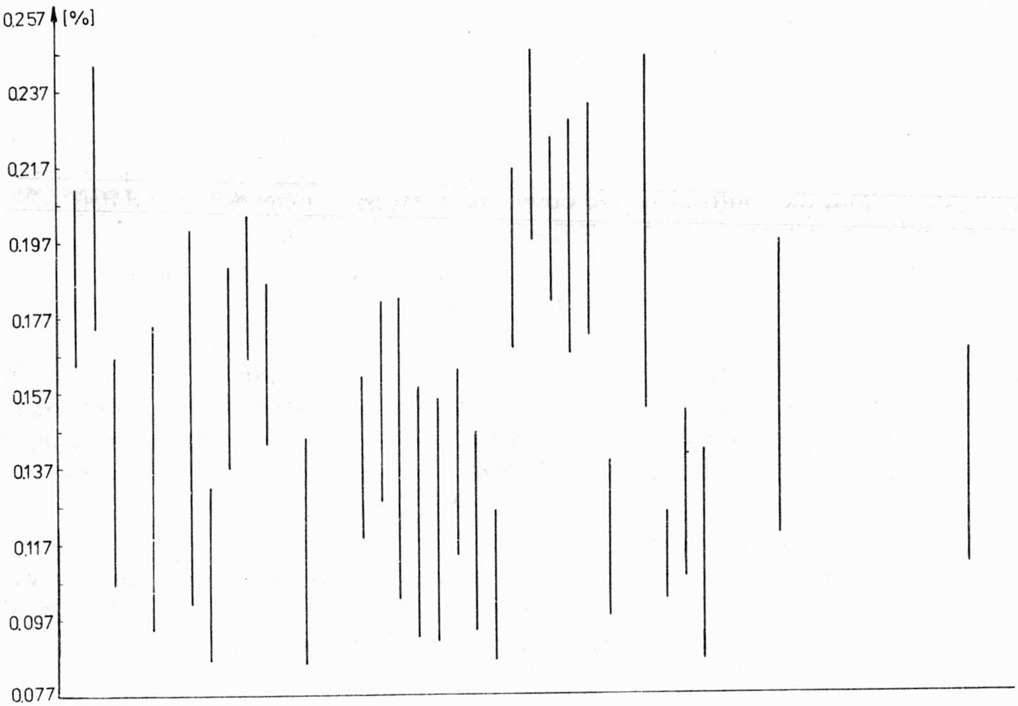

Fig. 2. Concentration of potassium in human control lymphocytes

between individual exposition to metals as well as to sulphur coumpounds in working places and the content of these elements in blood cells. The pollution of residential areas was also examined. It appeared that the mean content of zinc in lymphocytes of steel plant workers living in the protective zones of the Lenin Steel Work was 1.5 percentage by weight, whereas in the workers living in distant places, it was only 0.3 percentage by weight.

"Classical" analytical methods, applied to ecotoxicological studies and cell monitoring of elements constitute a new useful tool for studying health hazard in industrial regions. Personal monitoring of environmental pollutants seems to be necessary because of great biocumulation diversity of the same pollutants (e.g. heavy metals) in individuals.

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INDYWIDUALNY MONITORING I BIOINDYKATORY EKSPOZYCJI NA NIEKTÓRE SKAŻENIA CHEMICZNE W PRZEMYŚLE I NA TERENACH MIESZKANIOWYCH EKOTOKSYKOLOGIA STREF OCHRONNYCH

Omówiono nowe metody analiz umożliwiające ocenę narażenia na wybrane zanieczyszczenia środowiskowe (np. dwutlenek azotu oraz metale) w zależności od odległości od głównego emitora.

Przedstawiono przykłady zastosowania tych metod w monitoringu komórkowym pierwiastków u ludzi zdrowych i chorych. Uwzględniono wpływ skażenia zarówno środowiska pracy, jak też zamieszkania w rejonach dużych zakładów przemysłowych na zawartość niektórych metali w ludzkich i zwierzęcych bioindykatorach (krew, łożysko, włosy). Zasygnalizowano też możliwość zastosowania metod biofizycznych do wykrywania submolekularnych zmian w organizmie, związanych ze skażeniem środowiska przyrodniczego i żywności.

ИНДИВИДУАЛЬНЫЙ МОНИТОРИНГ И БИОИНДИКАТОРЫ ЭКСПОЗИЦИИ НА НЕКОТОРЫЕ ХИМИЧЕСКИЕ ЗАРАЖЕНИЯ В ПРОМЫШЛЕННОСТИ И НА ЖИЛИЩНЫХ ТЕРРИТОРИЯХ ЭКОТОКСИКОЛОГИЯ ЗАЩИТНЫХ ЗОН

Обсуждены новые методы анализов, дающие возможность оценки подвержения избранным заражениям в среде (напр. двуокись азота и металлы) в зависимости от расстояния от главного источника заражения. Представлены примеры применения этих методов в области клетчатого мониторинга химических элементов у здоровых и больных людей. Учтено влияние заражения как в среде работы, так и жилищной в окружении больших промышленных предприятий на содержание некоторых металлов в биоиндикаторах человека и животных (кровь, плацента, волосы). Засигнализирована также возможность применения биофизических методов для обнаружения субчастичных изменений в организме, связанных с заражением естественной среды и пищи.