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Stefan TSAKOVSKI[1], Pavlina SIMEONOVA[2] and Vasil SIMEONOV[1*]

SEDIMENT POLLUTION ASSESSMENT BY CHEMOMETRIC METHODS

OCENA STANU ZANIECZYSZCZENIA OSADÓW Z POMOCĄ METOD CHEMOMETRYCZNYCH

Abstract: The application of chemometric methods and strategies for sediment pollution is presented. Since the sediments are an important environmental phase collecting the history of polluting events, the correct data analysis, modeling and interpretation of monitoring results from lake, marine and river sediments is performed. Variety of chemometric techniques like cluster analysis, principal components analysis, fuzzy principal components analysis, self-organizing maps of Kohonen, end-point mixing models are used in order to detect sources of pollution in a certain environment, linkage between sampling sites and chemical parameters, relationships between ecotoxicity values with chemical pollutants etc.

Keywords: chemometric methods, sediment samples, pollution, risk assessment

Introduction

Careful environmental monitoring requires data collection from lake, river, and marine bottom sediments, as they reveal important characteristics of aquatic ecosystems.

Sediments can serve both as reservoirs and as potential sources of contaminants to the water column, and can adversely affect sediment-dwelling organisms, aquatic-dependent wildlife and human health [1]. Effective pollution assessment requires finding a relationship between sediment chemistry, sediment location and toxicity endpoints. In the present state-of-art one common approach, for instance, is the direct comparison between chemical concentrations to toxicity data [2]. However, this is a traditional univariate strategy which produces traditional *Sediment Quality Guidelines* (SQG). The problems of the SQG estimation procedure are connected with the bioavailability of sediment contaminants, sediment locations in various environments and the effects of covarying chemicals and

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chemical mixtures. The “mixture paradox” is somehow resolved by “grouping” contaminants, using empirically derived SQG’s [3]. It is our conviction that the above-mentioned problems can be solved, to a large extent, simply with the application of multivariate statistical (chemometrical or environmetric) methods. Moreover, these methods could be applied even to smaller data sets (usually collected during short-term monitoring).

Assessment of the impact of pollution on biological diversity in water bodies requires not only good quality bottom sediment datasets, but also a complete multivariate statistical data analysis. Recently, many studies have been performed using chemometric approaches for monitoring datasets as the best way for classification, modeling and interpretation of various environmental compartments, just to cite a limited number of own studies [4-14].

The big variety of sediment samples (estuarine, river, lake, marine, oceanic) requires careful interpretation of the monitoring data by the use of multivariate strategies. That is why the application of different environmetric strategies for *intelligent data analysis* (IDA) seems to be an appropriate tool in risk and quality assessment theory and practice. Chemometrics (Environmetrcs) as part of IDA deals with application of multivariate statistical methods in environmental data mining. The models offered by the environmetric approaches lack the theoretical limitations of the dynamic process modeling and offer a useful way to understand many features of the system in consideration, among them the following:

- similarities and dissimilarities between sampling sites;
- detection of site outliers in the monitoring net and their explanation;
- identification of data structure features;
- detection of latent factors which could be interpreted as anthropogenic or natural sources responsible for the chemical content of the environmental samples;
- trends in the behavior of pollutants;
- seasonal effects of the pollutants distribution;
- apportioning of the contribution of each identified source to the total species mass;
- finding of the necessary chemical mass balance and relationships in the environmental systems in consideration.

All these opportunities offered by the environmetric approaches turn to be a very important tool for decision making and economic, health and political solutions. We shall try to present some real-time examples about the application of environmetrics to everyday problems on different environmental scales. In all of the case studies a problem arises which needs a quick assessment and even quicker response. Keeping a certain local equilibrium in a local environment should be considered as a global action in the modern world where the relations and the interactions, the atmospheric transfer processes and the mutual dependence on “hot spot” pollution is unavoidable. Therefore, risk or quality assessment at a given location is much more than a local episode. Environmetrics tries to be useful as specific metrics of the sustainable development.

The main goal of the present communication is to give a short outlook of the major chemometric methods as applied to sediment pollution assessment and the results of several environmental studies on sediment pollution modeling for various sediment samples (estuarine, lake and marine). Special attention will be paid to the relationship between chemical pollution and ecotoxicity mode of the lake sediments.

Experimental

Chemometric methods used

In the data treatment approaches of the chemonmetrics both unsupervised and supervised techniques are used. In the first case the data mining is performed spontaneously, in a hierarchical way, from the data set. In the latter case a preliminary step of learning (training) is necessary to derive a treatment (classification) rule based on grouping of objects with known origin or behavior. This rule allows interpreting new objects with unknown origin or behavior in the classes offered by the classification rule.

Cluster analysis is a well-known and widely used classification approach for environmetrical purposes with its hierarchical and non-hierarchical algorithms [15, 16].

In order to cluster objects characterized by a set of variables (eg sampling sites by chemical concentrations or pollutants), one has to determine their similarity. To avoid influence of the data size, a preliminary step of data scaling is necessary (eg autoscaling or z-transform, range scaling, logarithmic transformation) where normalized dimensionless numbers replaces the real data values. Thus, even serious differences in absolute (concentration) values are reduced to close numbers. Then, the similarity (or more strictly, the distance) between the objects in the variable space can be determined. Very often the Euclidean distance (ordinary, weighted, standardized) is used for clustering purposes. Another way of measuring similarity is calculation of the correlation coefficient between two row-vectors $\mathbf{x_1}$ and $\mathbf{x_2}$ characterizing objects 1 and 2. Thus, from the input matrix (raw data) a similarity matrix is calculated. There is a wide variability of hierarchical algorithms but the typical ones include the single linkage, the complete linkage and the average linkage methods. The representation of the results of the cluster analysis is performed either by a tree-like scheme called dendrogram comprising a hierarchical structure (large groups are divided into small ones) or by tables containing different possible clusterings. The hierarchical methods of clustering mentioned above are called agglomerative. Good results are obtained also by the use of hierarchical divisive methods, ie methods that first divide the set of all objects in two so that two groups (clusters) are formed. Then each group (cluster) is again divided in two etc, until all objects are separated.

The aim of classification by non-hierarchical clustering is to classify the objects in consideration into certain number of preliminary intended groups, eg K clusters. For instance, in order to obtain 2 clusters, one selects 2 seed points among the objects and classifies each of the objects with the nearest seed point. Thus, an initial cluster is obtained. For each of these clusters, one determines the centroid (the point of mean values of the variables $\mathbf{x_i}$ for each cluster). The whole procedure is repeated; new centroids are calculated for the new clusters. The new centroids have new coordinates and it leads to reclassification of the objects. Widely applied are: the Forgy's method, MacQueen's K-means method etc.

All clustering methods mentioned up to now have a general feature in the classification: they consider each object to be part of only one single cluster. A different strategy is typical for the so-called fuzzy clustering, which permits objects to be part of more than one cluster. In the classification fuzzy procedure each object i is given a value f_{ik} for a membership function in cluster k.

New original clustering algorithms named *density-based spatial clustering of application with noise* (DBSCAN) and *ordering points to identify the clustering structure* (OPTICS) was recently offered [17]. In the first case an important advantage is the possibility to detect outliers. A cluster with DBSCAN is defined as a region of the data space where the objects' neighborhood of certain radius ε, contains at least k objects. Only one input parameter is required, namely, the minimal number of objects in the neighborhood, k. Three categories of objects are distinguished - core, border and outlier object, with respect to the neighborhood density. Core and border objects are grouped into clusters.

With OPTICS approach a unique order of objects in the n-dimensional data set is established, which reveals the data set structure. It allows finding zones where many objects are close together (high density zones) using a so-called reachability plot. The OPTICS algorithm calculates the similarity between the objects by reachability distances. In a very first approximation they can be considered as Euclidean distances. Outliers could be easily detected since their reachability distances are higher than those of the other objects. The OPTICS plot can be visualized as a color map. The information of this map can be used to study the inter-variable and inter-object relations and to estimate the contribution of each variable to the data set structure.

Principal components analysis (PCA) is a typical display method, which allows to estimate the internal relations in the data set and to model the ecosystem in consideration. There are different variants of PCA but basically, their common feature is that they produce linear combination of the original columns in the data matrix (data set) responsible for the description of the variables characterizing the objects of observation. These linear combinations represent a type of abstract measurements (factors, principal components) being better descriptors of the data structure (data pattern) than the original (chemical or physical) measurements. Usually, the new abstract variables are called latent factors and they differ from the original ones named manifest variables. It is a common finding that just a few of the latent variables account for a large part of the data set variation. Thus, the data structure in a reduced space can be observed and studied [18].

Generally, when analysing a data set consisting of *n* objects for which m variables have been measured, PCA can extract m principal components PCs (factors or latent variables) where m < n. The first PC represents the direction in the data, containing the largest variation. PC 2 is orthogonal to PC 1 and represents the direction of the largest residual variation around PC 1. PC 3 is orthogonal to the first two and represents the direction of the highest, residual variation. Around the plane formed by PC 1 and PC 2. The projections of the data on the plane of PC 1 and PC 2 can be computed and shown as a plot (score plot). In such a plot it is possible to distinguish similarity groups. According to the theory of PCA the scores on the PCs (the new coordinates of the data space) are a weighted sum of the original variables (eg chemical concentrations):

$$\text{Score (value of object I along a PC p)} = \gamma_{1p}\, Y_1 + \gamma_{2p}\, Y_2 + \ldots + \gamma_{kp}\, Y_k$$

Where Y is indication of the variable value (eg concentration) and γ are the weights (called loadings). The information hidden in the loadings can also be displayed in loadings plots. It is important to note that PCA requires very often scaling of the input raw data to eliminate dependence on the scale of the original values.

When time parameters are taken into account quite suitable approaches for three-way data analysis are Tucker3 model and the PARAFAC model [19]. The basic principle of the first method is the decomposition of the three-way data set into a three-way core matrix and three two-way loading matrices (one of each mode, eg objects, variables, time parameters).

To each mode a certain number of factors are assigned chosen in such a way as to be less than the dimensions of the original three-way data set in order to achieve a considerable amount of data reduction. Again, plots for each kind of factors could be constructed showing the relationships of objects, variables or time parameters (seasonality). In PARAFAC again a decomposition of the data set is aimed but the three loading matrices obtained are not necessary orthogonal.

Kohonen network Kohonen [20] has the general idea of mapping data from a high dimensional space onto a low dimensional space. The latter consists of a layer of i nodes arranged in a 2D plane as neighboring hexagons or rectangles. The mapping preserves the data topology, ie objects, which are similar in the original data space are mapped on the same node or onto neighboring nodes. A weight vector represents each node. The training of the net is done in an iterative way by updating the weights of the nodes. At each iteration a randomly selected object is presented to each node and the node whose weight is closest to this object is determined. This is the winner node. The weights of the winner are updated together with the weights of the nodes in its neighborhood, which size is defined by neighborhood function. Once the network is trained, for each object the winning node is found and the object is assigned to this node.

Neural gas network is suggested by Martinez et al [21]. In contrast to Kohonen network, the neural net has no defined topology. Each neuron is associated with vector of weights, which correspond to its coordinates in variable space. At each iteration the input vector (object) is presented to the network and the weight vectors are ranked according to their distances to the input vector. Then the nodes are moved according to the rank. After a predetermined number of iterations, the nodes are distributed over the space. During the last step of the procedure a clustering is performed, ie for each object from the data set its closest node is found. Similar objects are grouped in the same node but there is no additional information about similarities between the nodes.

Multiple regression on principal components (apportioning models) is a very important environmetric approach [22]. It makes it possible to apportion the contribution of each identified by PCA latent factor (emission source) to the total mass (concentration) of a certain chemical variable. The first step is performance of PCA, identification of latent factors, then determination of the *absolute principal components scores* (APCS) and multiple regression of the total mass (dependent variable) on the APCSs (independent variables).

Fuzzy PCA - optimizing the first component (FPCA-1). For the data collected on p variables for n cases, PCA performs analyses in the *n*-dimensional space defined by p variables and *p*-dimensional space defined by n cases. In PCA straight lines are sought which best fit the clouds of points in the vector spaces (of variables and cases), according to the least squares criterion. This, in turn, yields the principal components (factors) that result in the maximum sums of squares for the orthogonal projections. Consequently, a lower dimensional vector subspace is recovered that best represents the original vector space. Although the first factor is extracted so as to capture the variance to the maximum extent, it can seldom capture the variance in its entirety. What remains should, therefore, be recovered by another (second) factor, a third, etc. However, the number of factors thus extracted will never exceed the number of original variables.

Fuzzy clustering is an important tool for identifying the structure in data [23]. According to the choice of prototypes and the definition of the distance measure, different fuzzy clustering algorithms are obtained. If the prototype of a cluster is a point - the cluster center - it will produce spherical clusters; if the prototype is a line, it will produce tubular clusters, and so on. Also, elements with a high degree of membership in the *i*-th cluster (ie, close to the cluster's center) will contribute significantly to this weighted average, while elements with a low degree of membership (far from the center) will contribute almost nothing. In what follows we briefly review the Fuzzy (first component) PCA algorithm proposed in reference [24]. It is important to determine the particular membership degrees A(x) such that the first principal component is best fitted along the points of the data set X. The algorithm is a natural extension of the Fuzzy Regression Algorithm [25, 26]. The fuzzy set in this case may be characterized by a linear prototype, denoted L(u,v), where v is the center of the class and u, with $\|u\| = 1$, is the main direction. This line is named the first principal component for the set, and its direction is given by the unit eigenvector u associated with the largest eigenvalue λ_{max} of, for example, the covariance matrix given in relation below, which is a slight generalization for fuzzy sets of the classical covariance matrix:

$$C_{kl} = \frac{\sum_{j=1}^{n} \left[A_i(x^j)\right]^2 (x_{jk} - \bar{x}_k)(x_{jl} - \bar{x}_l)}{\sum_{j=1}^{n} \left[A_i(x^j)\right]^2}$$

The algorithm defined in this way permits the determination of the $A(x^j)$ values that best describe the fuzzy set *A* and the relation with its linear prototype (the *first principal component*).

Fuzzy PCA - orthogonal (FPCA-O). The fuzzy approach could be extended one step more. A Fuzzy PCA algorithm is written that would extend the fuzzy clustering scheme with computing each particular principal component, not just the first one. Let us denote λ_1, …, λ_p, and e^1, ..., e^p the eigenvalues and the eigenvectors, respectively, that will finally be produced by our suggested algorithm. The first fuzzy principal component is computed as with the FPCA-1 algorithm, ie, by finding the optimal fuzzy membership degrees and the optimal linear prototype for the data set. Let us denote λ'_1, …, λ'_p and e'^1, ..., e'^p the eigenvalues and eigenvectors, respectively, produced in this way. Therefore,

$$\lambda_1 = \lambda'_1$$

and

$$e^1 = e'^1$$

The major novelty of this algorithm is in the way the other fuzzy principal components are computed. The original data set is projected onto the hyperplane orthogonal to the first fuzzy principal component, i.e., determined by all the other principal components, as determined by the Fuzzy First Component PCA algorithm. Practically, this may be done by computing the scores and removing the first item from the data vectors. Therefore, the scores are computed:

$$x'^{j^T} = x^{j^T} \cdot (e'^1, \ldots e'^p)$$

and then the first component of x'^j is removed, thus producing a subset X' of R^{p-1}:

$$X' = \left\{ (x'_2, \ldots x'_p \mid \exists j : x'^j = (x'^1, x'^2, \ldots, x'^p) \right\}$$

This produces a data set in a Euclidean space of dimension p-1, where p is the size of the original data set. Denote $\lambda''_1, \ldots, \lambda''_{p-1}$ and $e''^1, \ldots, e''^{p-1}$ the eigenvalues and eigenvectors, respectively, produced in this way. The first fuzzy principal component of this projected data set, after being rewritten in terms of the original space, is orthogonal on the first fuzzy principal component, as computed originally. In order to account for the fuzziness in the fuzzy data sets, when rewriting the components in terms of the original space, the eigenvalues computed in the p-1 sized space will be multiplied by the fuzzy set fuzziness index f_A, given by:

$$f_A = \frac{1}{n} \sum_{i=1}^{n} A(x^j)^m$$

Thus

$$\lambda_2 = \lambda''_1 \cdot f_A$$

and

$$e^{2T} = (0, e''^1)^T \cdot (e'^1, \ldots, e'^p)^T$$

where $(0; e''^1)$ denotes a vector having 0 for the first component, and the components of the vector e''^1 for the other components. In order to determine the third fuzzy principal component one will reason in the same way, but here it has to be started with the projected data set and project it onto the hyperplane orthogonal to the first two fuzzy principal components. This twice-projected data set will be in a Euclidean space of dimension p-2. Let us suppose that, after proper transformations have been made at the superior level, these newly produced eigenvectors and eigenvalues (now in the R^{p-1} space) and still denoted by $\lambda''_1, \ldots, \lambda''_{p-1}$ and by $e''^1, \ldots, e''^{p-1}$. These notations will replace the already computed values. Now we need only a final transformation: to revert these eigenvectors and eigenvalues to the original space.

It has to be mentioned that ***empirical models*** are also used for assessment of sediment pollution as in the case of a three end-member mixing model used to quantify terrestrial, marine and salt-marsh contributions to the bottom sediments of Winyah Bay, South Carolina. If n sources (end-members) contribute to the sediments, then a system of n mixing equations, constrained by (n-1) tracers of organic carbon, is needed. This approach requires that the composition of the end-members is well known. For example, if stable isotopes of carbon, nitrogen and sulfur are used to trace the flow of organic matter in estuaries, then the contributions of a maximum of four end-members can be calculated using the following system of four mixing equations keeping in mind that the sum of all fractional contributions F_i is equal to 1 (100%):

$$\Sigma\delta^{13}C_iF_i = \delta^{13}C_s$$

$$\Sigma\delta^{15}N_iF_i = \delta^{13}N_s$$

$$\Sigma\delta^{34}S_iF_i = \delta^{34}S_s$$

$$\Sigma F_i = 1$$

Sampling region, sampling and sample analysis - lake sediments

The Turawa Lake sampling region is located in southwestern Poland (Opole province) and the main pollution sources are industry, agricultural activity and urban and domestic sewage. The bottom sediment dataset was obtained during a sampling campaign in 2004. From the initial collection of a total 34 sediment cores (0.00÷8.00 m in length), out of 260 samples, only 59 were chosen for the present multivariate statistical analysis, as they comprise all the necessary data without any missing results for both chemical and ecotoxicity parameters (44 variables in total, including exotoxicity parameters, pesticides, congeners, PAHs, heavy metals as well as two physical markers of the sediment samples, namely ignition loss and fraction size). The samples were representative for the surface layer of the sediment and involved 59 sampling sites from the bottom sediment of Turawa Lake. In Figure 1, the sampling grid is presented.

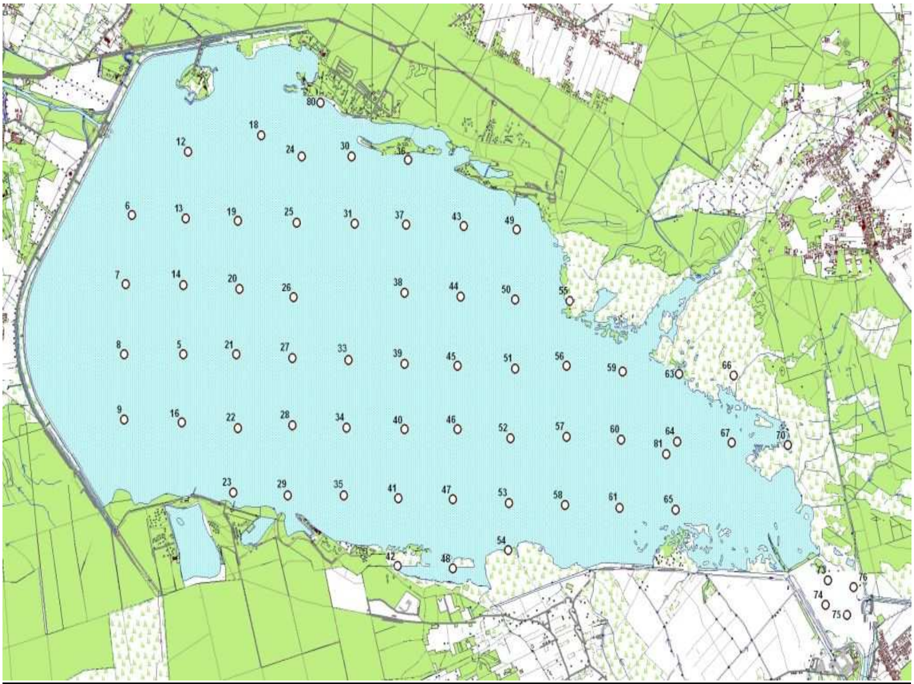

Fig. 1. Turawa Lake sampling grid

Chemical analysis of the samples included various instrumental methods complied with different chemical and ecotoxicity variables. Gas chromatography coupled with mass spectrometry was used for polychlorinated biphenyl congeners (PCB), organochlorine pesticides and polycyclic aromatic hydrocarbons (PAHs) determination; inductively coupled plasma-atomic emission spectrometry - for Cr, Zn, Cu, Ni, V, Fe, Mn, Al and Li; electrothermal atomic absorption spectrometry - for Cd and Pb; hydride generation atomic absorption spectrometry - for As, and cold vapour atomic absorption spectrometry - for Hg. All the methods applied are described in more details elsewhere [14].

Table 1

Chemical species and their abbreviations used in the Turawa Lake study

Acronym	**Name**	**Gross average concentration**
PCB28	2,4,4'-trichlorobiphenyl	1.219
PCB52	2,2',5,5'-tetrachloro-1,1'-biphenyl	0.174
PCB101	2,2',4,5,5'-pentachlorobiphenyl	0.115
PCB118	2,3',4,4',5-pentachlorobiphenyl	0.130
PCB138	2,2',3,4,4',5'-hexachlorobiphenyl	0.561
PCB153	2,2',4,4',5,5'-hexachloro-1,1'-biphenyl	3.076
PCB180	2,2',3,4,4',5,5'-heptachlorobiphenyl	0.357
a-HCH	alpha-1,2,3,4,5,6-hexachlorocyclohexane	1.384
b-HCH	beta-1,2,3,4,5,6-hexachlorocyclohexane	0.686
g-HCH	gamma-1,2,3,4,5,6-hexachlorocyclohexane	0.863
hepta-Cl	heptachlor	2.169
aldrine	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene	0.876
hepta-Cl-B	heptachlor epoxide isomer B	0.070
p,p-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene	4.314
o,p-DDD	*o,p*-dichlorodiphenyl dichloroethane	1.334
dieldrine	(1aα,2ß,2aα,3ß,6ß,6aα,7ß,7aα)-3,4,5,6-9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene	2.175
endrine	3,4,5,6,9,9,-hexachloro-1a,2,2a,3,6,6a,7,7a--octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene	5.823
p,p-DDD	*p,p*-dichlorodiphenyl dichloroethane	6.451
o,p-DDT	*o,p'*-dichloro-1,1-diphenyl-2,2,2-trichloroethane	41.06
p,p-DDT	*p,p'*-dichloro-1,1-diphenyl-2,2,2-trichloroethane	1.591
HCB	hexachlorobenzene	0.556
BaA	benzo[a]anthracene	202.6
BbF	benzo[b]fluoranthene	319.5
BkF	benzo[k]fluoranthene	178.7
BaP	benzo[a]pyrene	319.1
IndP	indeno[1,2,3-c,d]pirene	320.6
DahA	dibenzo[a,h]antracene	37.59
BPer	benzo[g,h,i]perylene	232.4
FR	Fraction size <63	17.66
IGN	Ignition losses	8.735
As	Arsenic	11.06
Hg	Mercury	0.156
Cd	Cadmium	47.75
Pb	Lead	79.66
Cr	Chromium	20.06
Zn	Zinc	1142
Cu	Copper	34.22
Ni	Nickel	11.94
V	Vanadium	15.86
Fe	Iron	7901
Mn	Manganese	95.91
Al	Aluminium	8426
Li	Lithium	5.516

Note: The gross average concentration for each species is presented for information (all units are in mg kg^{-1} dry weight, with exception for FR and IGN where units are % and % dry weight, respectively)

The acute and chronic toxicity of all the samples was determined using ToxAlert 100 and Microtox model 500 instruments and the bioluminescent bacteria *Vibrio fisheri* as acute toxicity bioindicating species. Chronic toxicity was tested in the presence of *Heterocypris incongruens* crustacean. Bioluminescence inhibition, growth inhibition, mortality, EC_{20} and EC_{50} were the numerical output of the toxicity tests. In Table 1, the coded names of the chemical and ecotoxicity parameters are given.

Black Sea coastal sediments

Sediment samples were taken from four different sampling sites: Lake Beloslavsko (10 sites, sample number 1-10, close to a location of glass production factory), Lake Varnensko (11 sites, sample number 11-21, close to a location of steel-work), Varna Gulf, close to Lake Varnensko (7 sites, sample number 22-28), Varna Gulf near to coast (7 sites, sample number 29-35; close to a location of cement and chemical plant Solvey Soda) and Bourgas Gulf, near to the waste inlets caused by the local oil-refinery (4 sites, sample number 36-39). It is worth noting that in the configuration of the coastal line, the two lakes (Beloslavsko and Varnensko) serve as a natural buffer zone between the industrial zone and the gulf of Varna. For the gulf of Bourgas no such zone exists, and there is a direct inlet of contaminated waters into the sea.

The sampling was performed with a standard bottom grab of Smith-McIntyre and the elements measured throughout this study were Cu, Pb, Mn, Zn, Co, Cd, Cr, Fe, Ni and As. Digestion in concentrated hydrofluoric acid and subsequent analysis by atomic absorption were used for quantification. ETAAS (graphite furnace AAS, Perkin Elmer Z/3030) was the analytical method to determine Cu, Pb, Co, Cd, Cr, Ni, As and flame AAS (Perkin Elmer 603) was used for Mn, Fe and Zn. Certified reference materials (MESS-1, BCSS-1 and NBS 1646) were run with each series of samples. Precision for Mn, Fe and Zn was $\leq 5\%$ (as *relative standard deviation*); for the other analyses the RSD was $\leq 10\%$. The experimental procedure is fully described by Simeonov et al. The aim of the study is to perform a multivariate statistical analysis of metal concentrations in coastal sediments collected at different "hot spots" sites of the Bulgarian Black Sea coast in order to gain information on the marine water quality.

Winyah Bay estuary sediments

Bottom sediments from Winyah Bay were collected during five cruises in 1996: winter (February, high freshwater discharge), early spring (March, high freshwater discharge), late spring (April, falling freshwater discharge), summer (September) and fall (November, low freshwater discharge). Freshwater discharge in the summer of 1996 was higher than the historical average of 500 m^3/s due to intense hurricane and tropical storm season on the East coast of the United States.

Sediment grab samples were collected along a longitudinal transect outside of the 8 m deep navigational channel in Winyah Bay. The transect extended from near the mouth of Winyah Bay to stations in the tidal parts of the Sampit and Pee Dee Rivers. No samples were collected from the Waccamaw River (Fig. 2). The sediment oxic layer was carefully removed from each sample and was stored frozen until analysis. In the lab, the sediments were wet-sieved into coarse (>63 μ, sand) and fine (<63 μ, silt and clay) fraction. Prior to isotopic and elemental analyses, inorganic carbon was removed with 1N H_3PO_4 and the samples were then rinsed thrice with deionized water. The processed sediments were stored in acid-washed glass vials at 60°C.

Several new leaves and leaf litter from riparian woody and non-woody species, typical of the area, were collected approximately during the same part of the day and from similar tree canopy positions. The plant samples were thoroughly washed with deionized water, dried at 60°C and homogenized with a mortar and pestle. Then the samples were stored in acid-washed glass vials at 60°C [12].

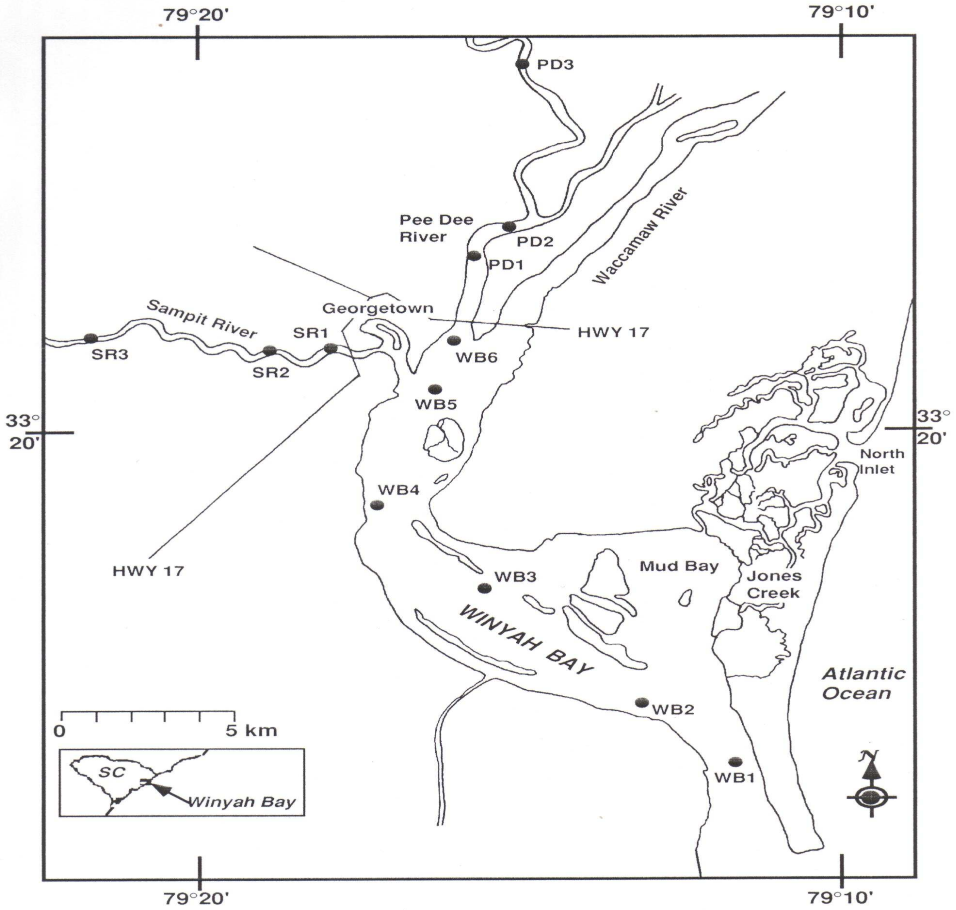

Fig. 2. Winyah Bay sampling area

Carbon and nitrogen isotopic composition of plant and sediment samples was measured using an OPTIMA stable isotope ratio mass-spectrometer. The average analytical precision of carbon isotopic data, reported as ±1S.D., is: ±0.1‰ (fine sediment), ±0.4‰ (coarse sediment) and ±0.2‰ (plant samples). The average analytical precision of nitrogen isotopic data, reported as 1S.D., is: ±0.3‰ (fine sediment), ±0.4‰ (coarse sediment) and ±0.2‰

(plant samples). Isotopic values, referenced to the *Pee Dee belemnite* (PDB) or atmospheric nitrogen standards, are expressed using the conventional δ-notation:

$$\delta X = (Rs/R_{std} - 1)\cdot 1000‰$$

where X is ^{13}C or ^{15}N, R_s is the mass ratio of the heavier to the lighter isotope in the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), R_{std} is the isotopic ratio of the corresponding standard.

Sampling and sample analysis - oceanic sediments

The sampling was performed according to the requirements of the program NS&T, which consists of two major projects: The National Benthic Surveillance and the Mussel Watch. The aim of the program is to estimate the environmental pollution along the coastal line of the USA and to localize the pollution sources by analysis of coastal and estuary systems including marine sediments.

The data used in this study are sediment data collected along the coastal line of Gulf of Mexico. Altogether 126 sampling sites are used and from each site three samples are taken. Each of these samples is a mean from three samplings. In this way for sediment analysis three samples are available to determine organic compounds, three - for inorganic components and three for grain size estimation.

The sediment sampling is performed at depths between 0.1 and 3 m at 1 to 400 m distance from shore. For lesser depths the sample collection is carried out manually by the use of a Teflon spoon but for bigger depths with a special device described in [27].

Table 2

Basic statistics for oceanic sediment data

Component	Mean	SD	SD/mean	Min.	Max
TOC	19729	23477	1.19	900	118773
Al	42239	25400	0.60	790	108703
As	6.82	5.17	0.76	0.35	23.54
Cd	0.19	0.16	0.84	0.01	0.91
Cr	47.70	26.76	0.56	3.56	170.38
Cu	12.97	12.59	0.97	0.92	87.67
Fe	19708	14500	0.73	823	66049
Pb	20.50	17.65	0.86	1.26	115.13
Mn	343	324	0.95	4.0	1560
Hg	0.06	0.07	1.17	0.001	0.24
Ni	14.27	9.25	0.65	0.84	36.23
Se	0.36	0.27	0.75	0.007	1.21
Ag	0.12	0.17	1.42	0.008	1.42
Sn	1.69	1.18	0.70	0.20	6.65
Zn	66.37	49.26	0.74	3.16	372.53
Grain size	0.58	0.24	0.42	0.10	0.99

Note: The dimension of the chemical components is in ppm; grain size is in mm. SD means standard deviation. The total number of samples is 378

Sample of 0.10 to 0.45 g dry sediment is located in a Teflon vessel and treated by mixtures like HNO_3–HF, HNO_3–KCl–HF or HNO_3–$HClO_4$–HF at heating with conventional or microwave heaters. Solution of H_3BO_3 is added to dissolve the insoluble fluorides in the sample. The solution obtained is analyzed by ETAAS, ICP-AES, AAS (cold vapor method) and XRF for Ag, Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn. Altogether 16 parameters (chemical variables) were determined in each sample. A full description of sampling, sample preparation and analysis (including TOC and grain size) could be found elsewhere [27]. A summarized statistics of the analytical results is presented in Table 2. All calculations were performed by applying Statistica 7.0 and Matlab 6.5 software packages using respective Toolboxes.

Results and discussion

Chronic toxicity assessment of lake sediments

The initial step in this sediment data classification was the construction of SOMs (Self-Organizing Maps) for each of the 44 sediment quality parameters. The intention was to project on a plane the complex dataset having 59x44 elements. The projection procedure makes it possible to compare the distribution of each parameter concentration in each one of the 59 sampling sites. In Figure 3, the SOMs for a selection of 30 parameters out of all 44 are presented. Some of the parameters are not presented on the diagram due to their close similarity to each other (eg some PCBs, most of the PAHs, several heavy metals).

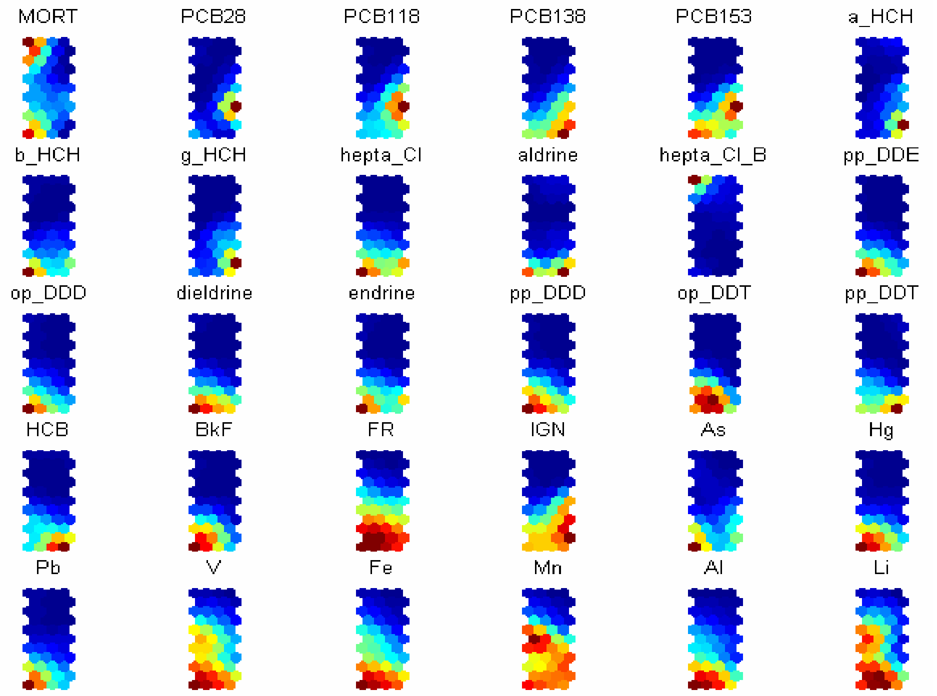

Fig. 3. SOM for all Turawa Lake sites and 30 parameters in chronic toxicity mode

It is readily seen that the values included in the mortality SOM form a specific pattern where a limited number of sites reveal a high mortality. Altogether, 4 groups of sites with relatively high levels of mortality could be observed and their spatial vicinity is presented in

Figure 4. Additionally, in Table 3 the membership of each sampling site (marked by its site number) to each group is indicated.

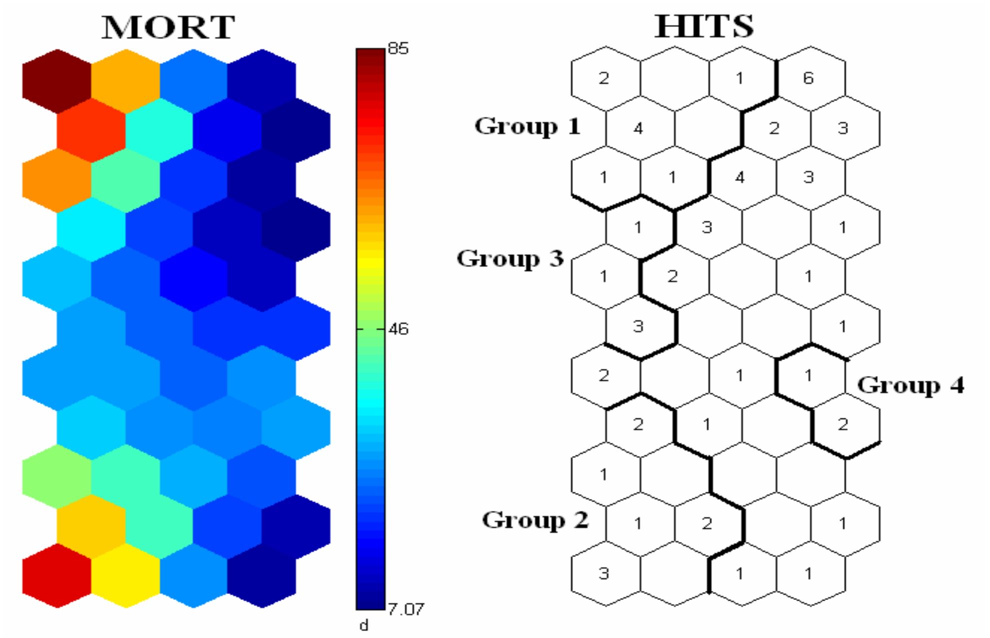

Fig. 4. Mortality SOM and hits diagram for the identified 4 chronic toxicity groups

Table 3

Membership of Turawa Lake sites to the groups of chronic toxicity patterns

Group	Discriminating pollutants	Sampling sites
1	heptaCl_B	18, 34, 55, 64, 70, 73, 75, 76, 81
2	pesticides - *p,p*-DDE, *o,p*-DDD, dieldrine, endrine, *p,p*-DDD, *o,p*-DDT; PAHs - BaA, BbF, BkF, BaP, IndP, DahA, BPer; Heavy metals - Hg, Cd, Pb, Zn, Cu	6, 7, 8, 9, 13, 14, 19, 20, 25
3	FR Heavy metals - Cr, V, Fe, Mn, Al, Li	27, 40, 53, 58, 65
4	PCB - PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180	16, 51, 57

As known, mortality values are an indication of the level of chronic toxicity of the samples. It was interesting to detect a relationship between this ecotoxicity parameter and the chemicals' concentrations of the samples using the visual classification patterns of the SOMs. The four groups, having enhanced mortality, were compared with respect to their "concentration indices", introduced by the authors. Each index is formed as a ratio of the average concentration of the group, divided by the gross average of all data for a certain parameter. The fraction was multiplied by 100 to get the index in percentage. With this data normalisation, it is relatively easy to visualize the connection between mortality and a given chemical parameter. It could be assumed that a high mortality index is achieved if other quality parameters have enhanced indexes. Thus, the overall chronic toxicity could be attributed to certain chemicals for each group of sites indicated on the SOM pattern of mortality. It has to be emphasized that only enhanced mortality values were of interest for this classification mode. So, 26 out of all 59 sites were involved in the assessment procedure. Those which are not taken in consideration possess mortality lower than 30%.

The four individual high-mortality groups formed on the SOM could be interpreted as follows:

Group 1: Nine sites are included in this pattern, having the numbers 18, 34, 55, 64, 70, 73, 75, 76, 81. The mortality index for the group is 268%, having the highest value compared with all other values. As seen in Figure 5, the only chemical parameter with a very high index (nearly 500%) is heptachlor B. It may be assumed that for this particular group (and location) of sites, chronic toxicity is due mainly to the toxic effect of specific accumulation of this pesticide. Conditionally, this pattern of chronic toxicity could be named "***heptachlor B"*** produced toxicity. Most of the sites with very high "heptachlor B" chronic toxicity are located at the southeastern side of Turawa Lake, where Mala Panew River flows into the lake carrying pollutants from agricultural activity.

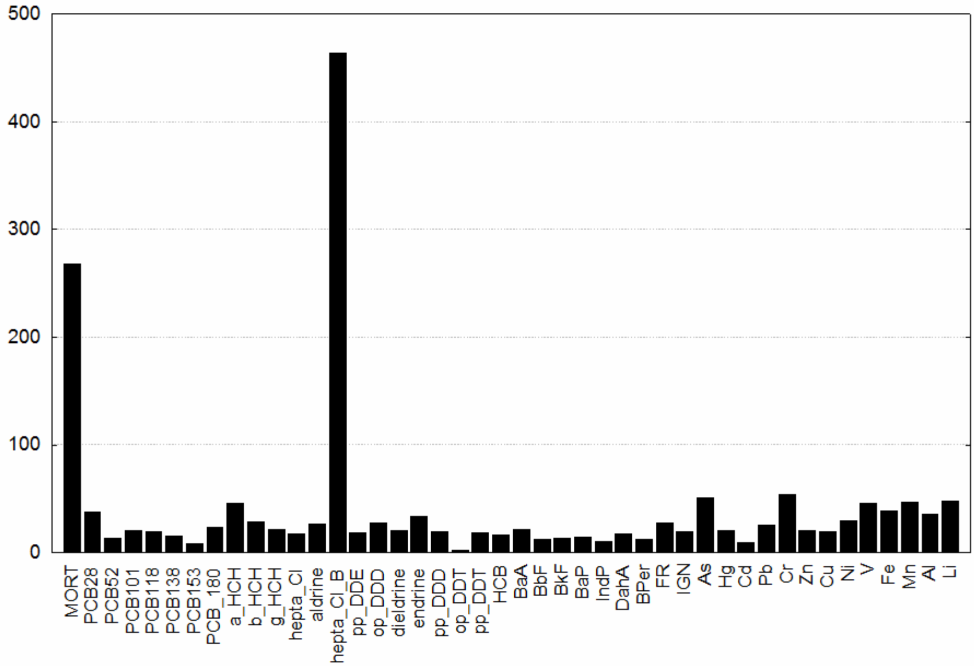

Fig. 5. Indices distribution for all parameters with respect to mortality for group 1

Group 2: Another 9 sites are included in the second classification group (sites 6, 7, 8, 9, 13, 14, 19, 20, 25). They are located in the western side of the lake and characterized by a relatively lower chronic toxicity index (nearly 200% or two and a half times lower than that of group 1). In this case, the contribution of heptachlor B or HCH to the mortality is obviously negligible (with indices lower that 100%). All other chemicals, however, show high indices: between 100 and 200% are many PCB chemicals (PCB28, PCB52, PCB101, PCB118), hexachlorocyclohexanes HCH, V, Mn, Li; all other quality parameters are over 200%, as extremely high indices are found for *p,p*-DDE, *o,p*-DDD, dieldrine, endrine, *p,p*-DDT, *o,p*-DDT, all PAHs, Hg, Cd, Pb, Zn, Cu. Thus, this cluster of sites could be conditionally related to a ***"pesticide and PAH"*** caused chronic toxicity, but without any specific effect of a certain chemical as in the previous case. Probably, the sedimentation of different chemicals (agricultural and industrial anthropopressure) is facilitated exactly in this part of the lake due to stream dynamics reasons since the lake outlet starts in this part of the reservoir.

In Figure 6, the values of the indices for the quality parameters significantly influencing the sediment quality (and chronic toxicity) are shown.

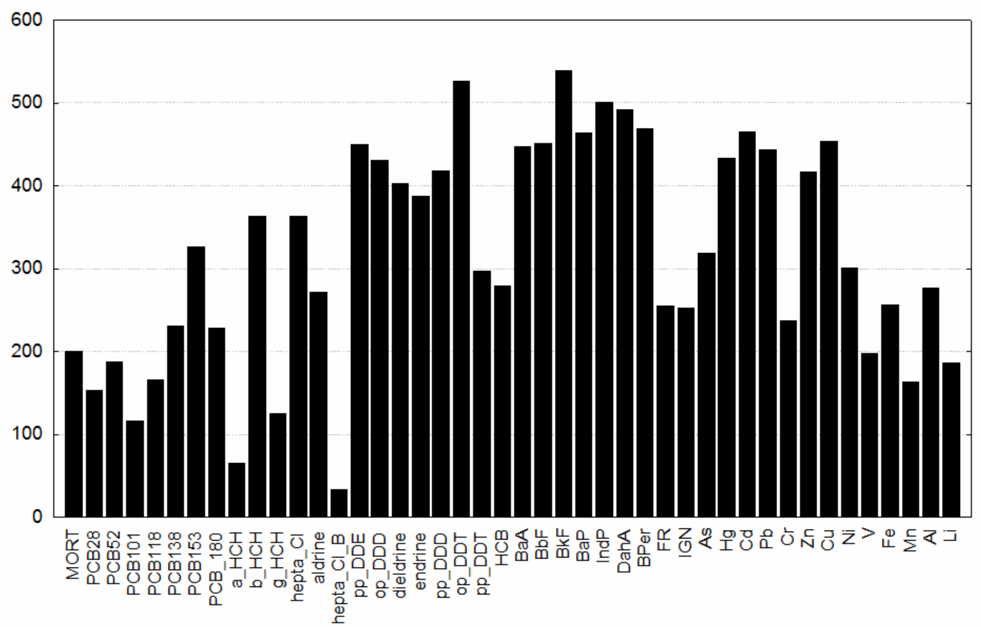

Fig. 6. Indices distribution for all parameters with respect to mortality for group 2

Group 3: Five sites are included in the next level of the chronic toxicity pattern. The mortality index is again lower than before (nearly 140%) and it is related to relatively high indices of several metals - V, Fe, Mn, Al and Li. Also, the fractional size parameter possesses a high index. It may be assumed that chronic mortality is linked directly in this situation with the sediment structure (all metals are mainly major components of the sediments, the fraction size is also a structural characteristics). Therefore, this pattern of chronic toxicity is ***"structure"*** caused. The sites' identification numbers are 27, 40, 53, 58, 65 forming a relatively compact group of sites near the southern bench of the Lake, close to the sites of the first group. It could be suggested that the "structural" group accumulates suspended material with a lower concentration of pollutants, which are either absorbed by group 1 or quickly transported to group 2. Thus, only specific structural features determine the additional absorption. In Figure 7, the indices of the parameters responsible for the formation of group 3 are shown.

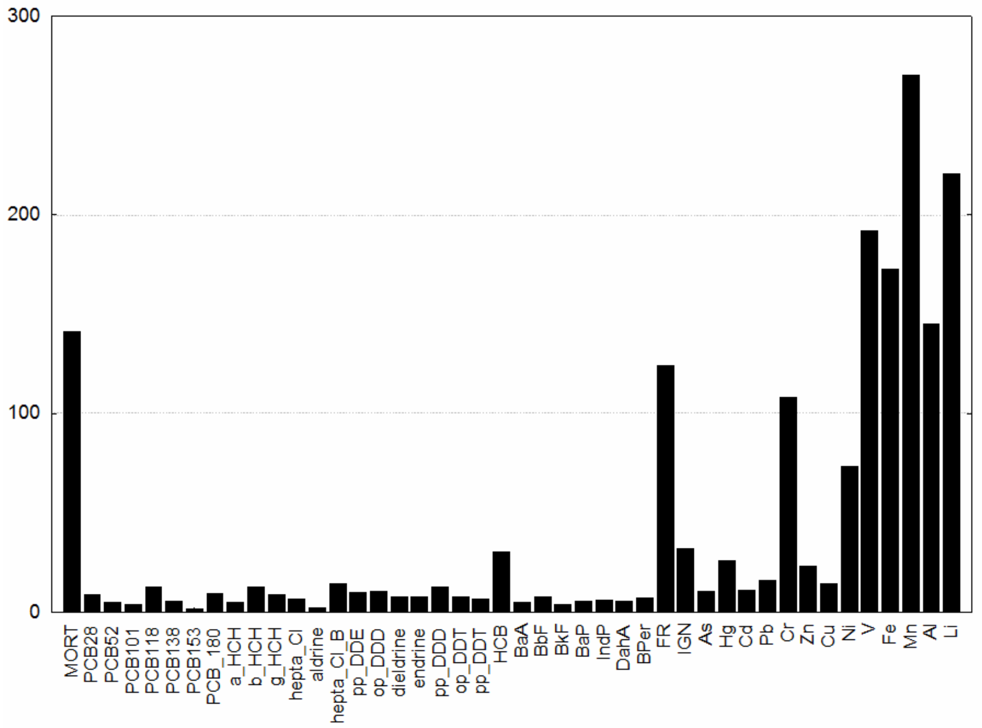

Fig. 7. Indices distribution for all parameters with respect to mortality for group 3

Group 4: The last obvious pattern of sites grouped in the mortality SOM includes only 3 sites (16, 51, 57). The group has an index of 130% and the calculations indicate that this chronic mortality is probably related to the very high concentrations (indices) of all PCBs (between 600 and 1100%). The conditional name of this group could be ***"PCB congeners"*** caused mortality. The location of the three sites in this case is quite occasional. The indices are presented in Figure 8.

The rest of the sites (33 more) show very low levels of mortality and they form a big uniform region on the SOM.

This classification, aiming to find a relationship between mortality parameters of the sediment samples and their chemical composition, proves unambiguously that this relationship is quite complex and multivariate. The chronic toxicity is affected by different chemical pollutants, but individually dependent on spatial, structural and hydrodynamic parameters.

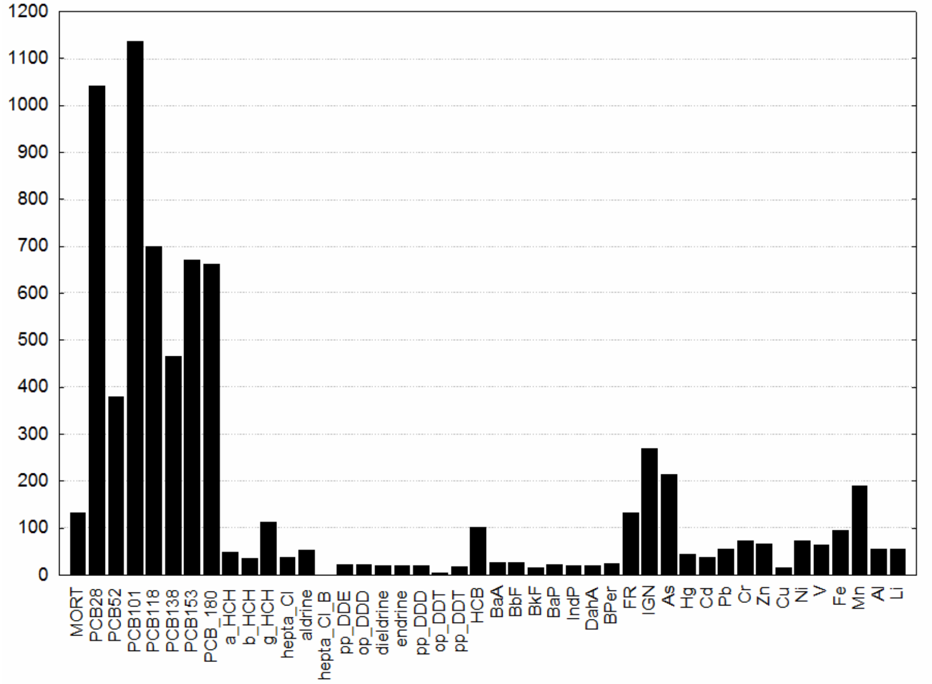

Fig. 8. Indices distribution for all parameters with respect to mortality for group 4

Black Sea sediment data

The cluster analysis results (hierarchical clustering, Ward's method) of the sampling sites as objects are shown in Figure 9.

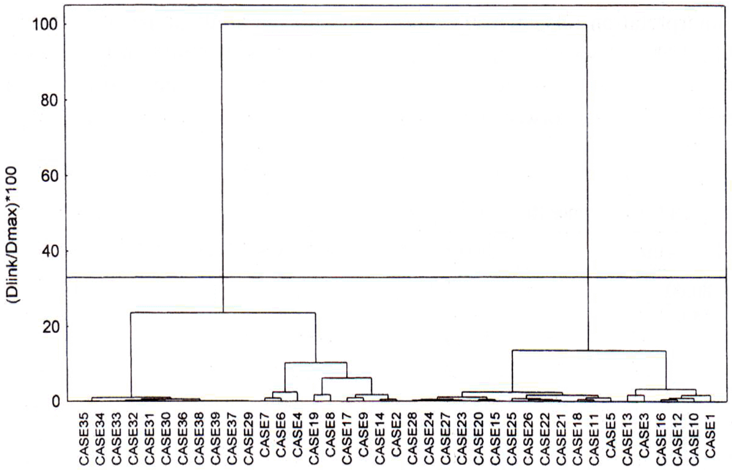

Fig. 9. Hierarchical dendrogram for Black Sea sediment data clustering of sampling sites

Altogether four clusters could be interpreted divided into two bigger subgroups: the first contains heavily polluted sites from Varna and Bourgas Gulf (near to the coastal line and waste inlets, sites 29-39 located near a big chemical and cement plant - Varna and an oil refinery - Bourgas) and several sites from both coastal lakes located near to industrial sources (sites 2, 4, 6, 7, 8, 9 from the Lake Beloslavsko located near to a glass production factory; sites 14, 17, 19 from the Lake Varnensko located near to a steel-work); the second one indicates a moderately polluted buffer zone consisting of lake and near to the lake Varna Gulf sites. In both big clusters two subgroups could be found. In the first one they represent the most severely polluted gulf areas (sites from Varna Gulf 29-35 and from Bourgas Gulf 36-39) and the less contaminated lake industrial inlets (sites 2, 4, 6-9 from Lake Beloslavsko and sites 14, 17, 19 from Lake Varnensko). In the second one they reflect the separation between one (Varnensko lake and non-affected Varna Gulf parts, sites 11-13, 15, 16, 18, 20, 21 and sites 22-28, respectively) or another part (Lake Beloslavsko, sites 1, 3, 5, 10) of the buffer zone moderately affected by pollutants.

The next step in the multivariate statistical analysis was application of PCA in order to group the chemical components by the loadings plots and the sites by the score plots. It is interesting to note that the site score plot (Fig. 10) reveals a more detailed description of the polluted coastal region.

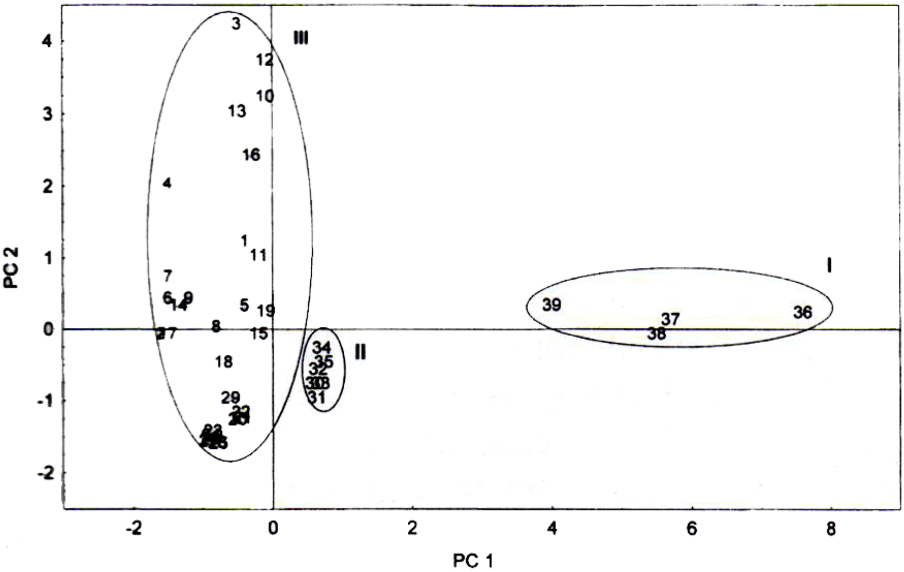

Fig. 10. PC score plot of sampling site for Black Sea sediments

The sites in the Bourgas Gulf (36-39) represent an independent group (I) of heavily polluted area (oil refinery). They are definitely separated from all other sites and this is due to the enhanced determinant concentrations. The next well-formed group (III) comprises sites from the moderately contaminated lake buffer zones (sites 1-28), which indicates sites from the two lakes and Varna Gulf sites located near to Lake Varnensko. The third group

(II) indicates the intermediate level of pollution (higher than the buffer zone contamination but less than the Bourgas gulf area) of the sites originating mainly from the Varna gulf area (sites 29-35).

The factor loading matrix is listed in Table 4.

Table 4

Factor loadings (Varimax normalized; marked loadings are higher than 0.7) for four principal components in Black Sea sediment data

Element	**PC1**	**PC2**	**PC3**	**PC4**
Cu	0.95	0.08	–0.09	0.04
Pb	0.04	–0.04	–0.88	–0.04
Mn	0.96	0.04	0.16	0.17
Zn	0.27	0.90	0.04	0.11
Co	0.31	–0.04	0.17	0.89
Cd	–0.15	0.88	–0.26	–0.05
Cr	–0.13	0.92	0.05	0.27
Fe	0.95	–0.14	0.07	0.11
Ni	–0.01	0.48	–0.04	0.81
As	–0.14	0.16	–0.86	–0.06
[%] Expl. var	29.5	27.1	16.5	15.8

Four factors describe almost 90% of the total variance of the system. The first one contains dominantly copper, manganese and iron and could be conditionally named “natural” since these elements are typical major constituents of Black Sea coastal sediments. The second factor includes zinc, cadmium and chromium, the third - lead and arsenic and the fourth - nickel and cobalt. The last three factors reflect typical anthropogenic influences of heavy metals from various sources such as chemical and glass production plants, oil refineries, steel-works and smelting plants. The detected pollution pattern indicates in a semi-quantitative way the emission sources.

Oceanic sediment pollution interpretation

In the next figures (Figs 11-16) the scatter plot diagrams for all sampling sites and all chemical variables treated by various PCA approaches (classical and fuzzy) are presented.

Each one of the plots requires a careful interpretation. When one applies classical PCA to the data plot four principal components are found (scree plot check) to describe over 85% of the total variance of the system (126 sediment samples x 18 variables; 15 of them are chemical components, one is a physical parameter - grain size and the last two are the geographical coordinates of the sites - longitude and latitude). The first latent factors could be conditionally named “natural” since it indicates high factor loadings for Al, As, Cr, Fe, Mn, Ni, and grain size. These are major sediment components, which play a substantial role in the sedimentation process - iron, manganese, chromium, nickel, arsenic as typical constituents in iron - containing natural materials. The belonging of grain size to this latent factor is an indication for the significant contribution of the major components to the sediment topology.

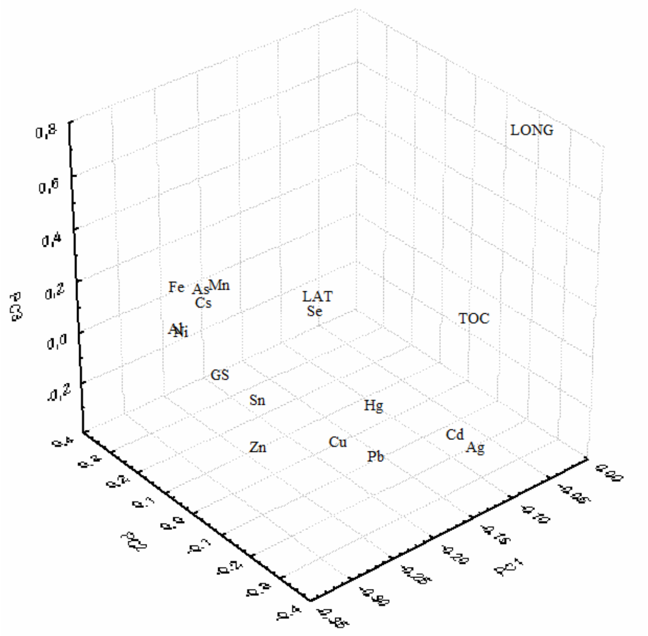

Fig. 11. Scatter plot of loadings corresponding to the first three principal components (classical PCA) in oceanic sediment data interpretation

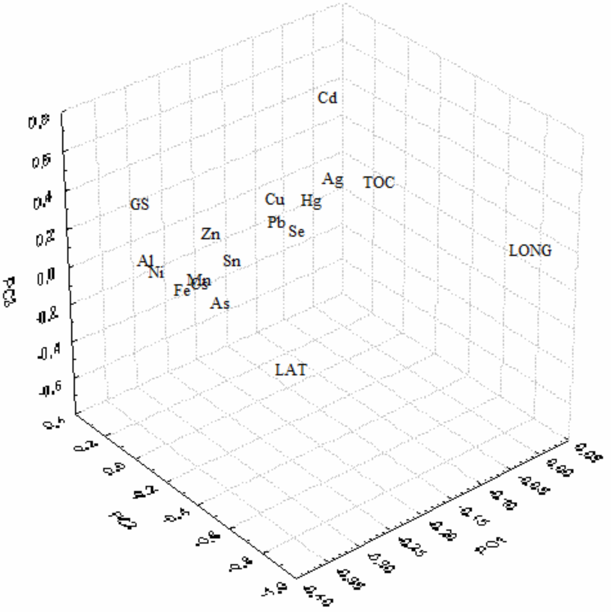

Fig. 12. Scatter plot of loadings corresponding to the first three principal components (Fuzzy PCA, first direction) in oceanic sediment data interpretation

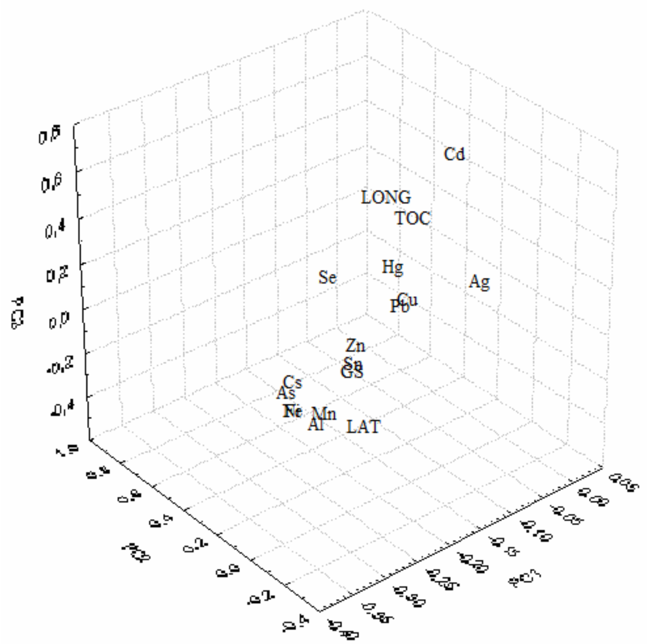

Fig. 13. Scatter plot of loadings corresponding to the first three principal components (Orthogonal Fuzzy PCA) in oceanic sediment data interpretation

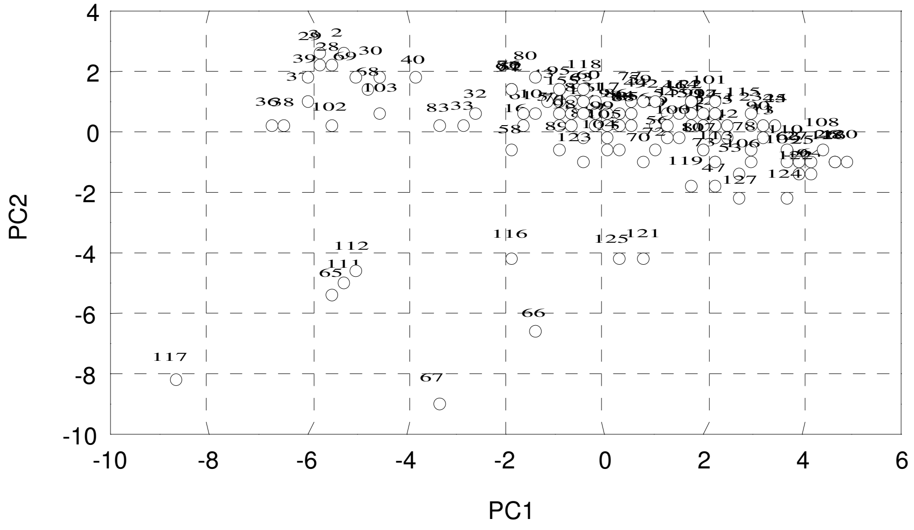

Fig. 14. Scatter plot of scores corresponding to the first two principal components (classical PCA) in oceanic sediment data interpretation

The second latent factor contains as significant contributors polluting heavy metals (zinc, mercury, copper, lead, cadmium and tin). It could be conditionally called "anthropogenic". The third latent factor represents the participation of the organic matter in the sediment formation (high factor loadings for TOC) and could be conditionally named "bioorganic". The bioorganic contribution to the sediment formation is obviously split into two parts: the first one explained by PC3 informs on natural organic matter impact to the sediment mass and the second (explained by PC 4) on the role of the organometallic polluting species to the same impact. Higher factor loadings in PC 4 possess silver and lead, but also TOC and cadmium. It allows calling the fourth latent factor conditionally "organic anthropogenic".

The geographical parameters are included in the plots just to indicate their independence on the chemical variables and are not commented in this study.

It was of substantial interest to show if the application of the fuzzy PCA (first direction and orthogonal) changes the way of variables projection. The 3D plots for both cases are presented in Figures 12 and 13.

It is readily seen that in case of fuzzy PCA (first direction approach, explained total variance for 4 PCs approximately 80%) the identified sources for the sediment formation are almost the same as in the case with the classical PCA. One could separate the conditional "natural" factor (with high loadings of Al, Ni, Fe, Mn, Cr, As, and grain size parameter (GS)); further the "anthropogenic" one (significant loading values for Cu, Hg, Zn, Sn, and Cd). In the next latent factor one detect high contributions of *total organic carbon* (TOC) and Se, which holds for the conditional "bioorganic" tracer in the sediment formation as well as relation between Pb and Ag to make the latent factor named "organic anthropogenic" due to the relatively high loading for TOC in PC 4. Therefore, no difference between classical PCA and fuzzy PCA (first direction) is found in identification of the sources of sediment formation in the region of interest.

In the third option of PCA (orthogonal fuzzy PCA, explained total variance approximately 78%) the results obtained for the latent factors identification do not differ substantially from the other two options. The first two principal components are almost the same and resemble the "natural" and the "anthropogenic" impact in sediment formation. The only difference is that in PC 2 a clear relation between Pb, Cu, Zn, Hg, and Sn is established as Cd changes its belonging with Pb to PC 4, respectively. Thus, the total organic carbon amount is related to Se as in the previous cases but the "organic anthropogenic" factor now is, in deed, another anthropogenic impact (high correlation between Ag and Cd with weaker correlation to TOC). In such a way, the orthogonal fuzzy PCA gives better information on the role of the bioorganic impact to the sediment formation. Probably, the identified PC4 is resulting in specific anthropogenic activity, rather than in formation of organometallic compounds.

In the next three plots the projection of the sampling stations as objects of interest is given. In Figure 14 the scatter plot of the 126 sites obtained by the application of classical PCA is represented. It may be seen that several sites appear to be typical outliers (sites with code numbers 117, 65, 111, 112, 116, 66, 67, 125, 121) since the rest form a large group of obviously close related in properties sites. The interpretation of the outliers from the scatter plot (Fig. 14) does not seem very simple. For instance, site 117 is located near to a small settlement (less than 1000 inhabitants) and could be accepted as background site with very low level of pollution. On the other hand, site 67 is a typical urban site (above 10000 inhabitants and chemical industry). Considered in such a way, the outliers indicated by the classical approach of PCA indicated either background site with clean environment or urban sites with higher level of pollution impact. The sediments sampled in the neighborhood of similar sites reflect, therefore, the general environmental situation and, maybe, lack information on some important details like type and nature of separate pollution sources.

A further attempt was made to improve the information on the characteristics of the sampling areas by using fuzzy PCA (first direction). The scatter plot for the factor scores is presented in Figure 15. It is seen that a slight change of the grouping of the sampling sites is at hand. For instance, the former outlier site 117 is in one group of similar objects like 36, 37, 39, 68, and 69. It could mean that the little village with code number 117 resembles sites with different urban profile (eg 68, 69 which are bigger towns). The common feature of these sites is that they belong to one and the same geographical region (Mississippi Delta). Similar considerations could be made for other groups of sites to find general features like sites from Texas, from the western part of the Mexican Gulf etc.

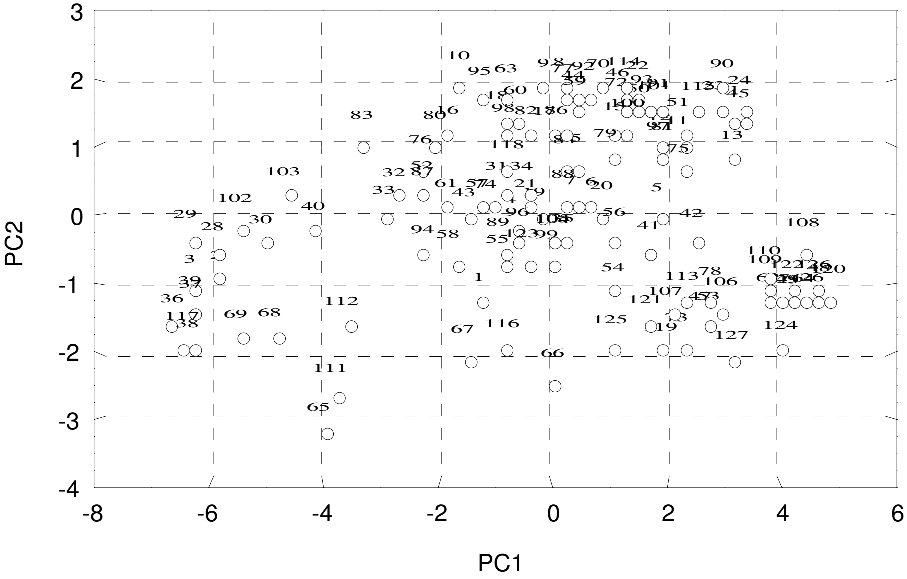

Fig. 15. Scatter plot of scores corresponding to the first two principal components (Fuzzy PCA, first direction) in oceanic sediment data interpretation

The application of the third mode of PCA used in this study, namely the orthogonal fuzzy PCA adds some more interesting aspects of the objects interpretation. The data treatment in this case indicates that no typical outliers are already available. Since the classical PCA is very sensitive to outliers and this fact can spoil any classification attempt, the results obtained by the orthogonal fuzzy PCA make it possible to find the following factors, which regulate the sediment formation in the region of interest (additionally to the identified possible pollution sources):

- the geographical location of the site (with respect to big areas like Mississippi Delta sites, Texas sites, Louisiana sites, Florida sites, sites from the southern or western part of the Mexican Gulf);
- the urban situation within the geographical regions: typical urban sites, small settlements, background sites, industrial sites;
- the population factor.

River sediments modeling

The sampling region is indicated in Figure 2.

Next, we examined the isotopic composition of all individual C-3 plants whose isotopic composition was used to calculate the carbon and nitrogen isotopic values of the terrestrial end-member (Table 5).

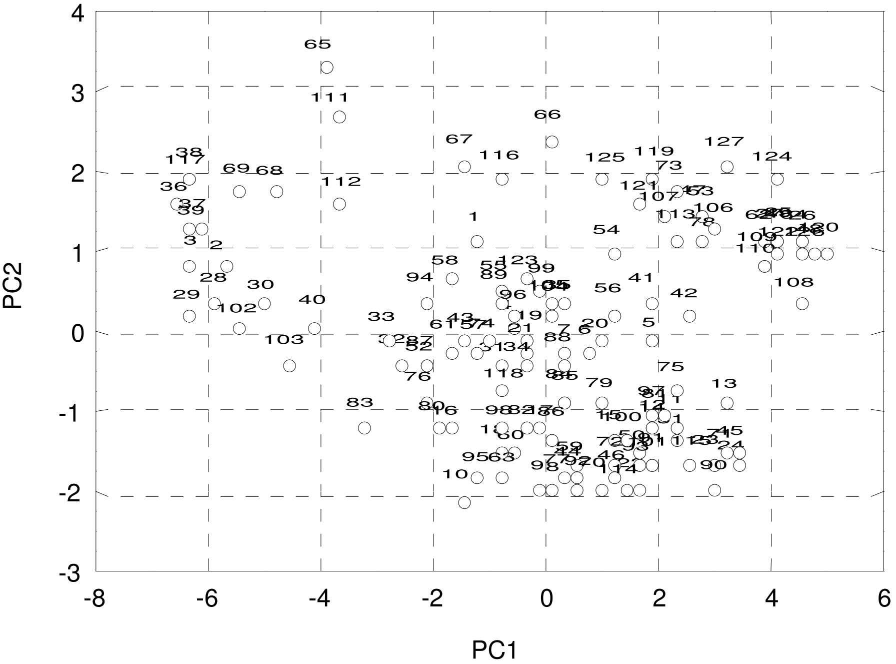

Fig. 16. Scatter plot of scores corresponding to the first two principal components (Orthogonal Fuzzy PCA) in oceanic sediment data interpretation

The 11.3‰ range of $\delta^{15}N$, compared with that of 4.3‰ for $\delta^{13}C$, suggested that the composite terrestrial end-member used in our calculations may consist of two new end-members which reflect the two main types of biotic communities in the Winyah Bay watershed. These new end-members could be distinguished by $\delta^{15}N$ but not by $\delta^{13}C$. The average $\delta^{13}C$ and $\delta^{15}N$ of the first one, terrestrial end-member I, were - 28.4±1.0‰ and 3.4±1.9‰ respectively. This end-member represented plants typical of freshwater marshes/cypress swamps in Winyah Bay: bald cypress, tupelo gum, common cattail. Black needlerush was also added to this end-member because of its similar isotopic composition. The average δ^{13}C and δ^{15}N of terrestrial end-member 11 were –29.1±1.4‰ and –1.8±1.9‰ respectively. Terrestrial end-member II included mesophytic species, such as red maple, sweet gum and black gum, as well as plants typical of Winyah Bay pine-oak forests, such as loblolly pine, longleaf pine, live oak, blackjack oak and turkey oak. Because the three end-member model was constrained by the δ^{13}C and δ^{15}N of its end-members, the model yielded negative results for any data points that plotted outside of the mixing triangle.

Table 5

Stable isotopic composition of carbon and nitrogen, reported as X±S.D.(the number of parallel analytical measurements is indicated in parentheses), in new leaves and leaf litter from riparian C-4 and C-3 plants in the Winyah Bay, South Carolina, area

Plant	**δ^{13}C [‰]**		**δ^{15}N [‰]**	
Latin name (common name)	**New leaf**	**Leaf litter**	**New leaf**	**Leaf litter**
C-4 plants				
Spartina alterniflora (Saltmarsh cordglass)	–13.4±0.1(2)	–13.3±0.1(3)	4.3±0.4(3)	1.31±0.5(5)
Spartina cynosuroides (Big cordgrass)	–12.91±0.1 (4)	–13.01±0.1 (3)	6.71±0.4(3)	3.91±0.1 (3)
C-3 plants				
Pinus taeda (Loblolly pine)	–30.51±0.1(3)	–29.11±0.2(3)	–5.41±0.5(3)	–3.41±0.9(3)
Pinus palustris (Longleaf pine)	–26.21±0.9(3)	–26.91±0.3(3)	–1.91±0.3(3)	–0.71±0.1(5)
Quercus virginiana (Live oak)	–28.41±0.1(3)	–30.31±0.2(3)	–2.6±0.2(2)	–3.9±0.2(3)
Quercus laevis (Turkey oak)	–28.3±0.1 (3)	–31.0±0.2(3)	–2.21±0.1 (6)	–0.41±0.1 (6)
Quercus marilandica (Blackjack oak)	–29.31±0.1(3)	–30.61±0.1(3)	–2.01±0.5(3)	–1.01±0.4(4)
Nyssa sylvatica (Black gum)	–29.5±0.1(3)	–27.61±0.3(3)	–0.81±0.3(3)	–2.9(1)
Acer rubrum (Red maple)	–29.81±0.1(6)	–28.31±0.8(3)	0.01±0.4(3)	–0.51±0.2(3)
Liquidambar styraciflua (Sweet gum)	–30.4±0.1(3)	–30.8±0.3(3)	0.81±0.1(6)	0.6±0.2(6)
Taxodium distichum (Baldcypress)	–27.01±0.2(3)	–28.11±0.3(3)	1.7±0.2(3)	0.91±0.1(3)
Nyssa aquatica (Tupelo gum)	–29.21±0.1(3)	–29.71±0.2(3)	2.11±0.1(6)	2.0±0.1(6)
Juncus roemerianus (Needle rush)	–29.11±0.1(3)	–28.5±0.5(3)	3.81±0.1(3)	0.31±0.3(3)
Typha latifolia (Common cattail)	–28.21±0.1(3)	–27.1±0.1(3)	5.91±0.1(6)	3.21±0.1(6)

If sedimentary organic carbon in Winyah Bay was viewed as a mixture of five end-members (terrestrial I, terrestrial II, anthropogenic, salt-marsh and marine), rather than the initially hypothesized three ones, then the corresponding five end-member model would be constrained by a five-point polygon, which would include 93% of the sediment data (Fig. 17). Thus, if end-member contributions were recalculated using a system of five equations, only 7% of end-member contributions would be expected to yield negative results, ie unexplained by a five end-member mixing model. At present we are unable to recalculate percent contributions using a system of five equations because we need two additional tracers of organic carbon. Thus the results presented should be viewed as upper limits to terrestrial, salt-marsh and marine contributions to Winyah Bay bottom sediments.

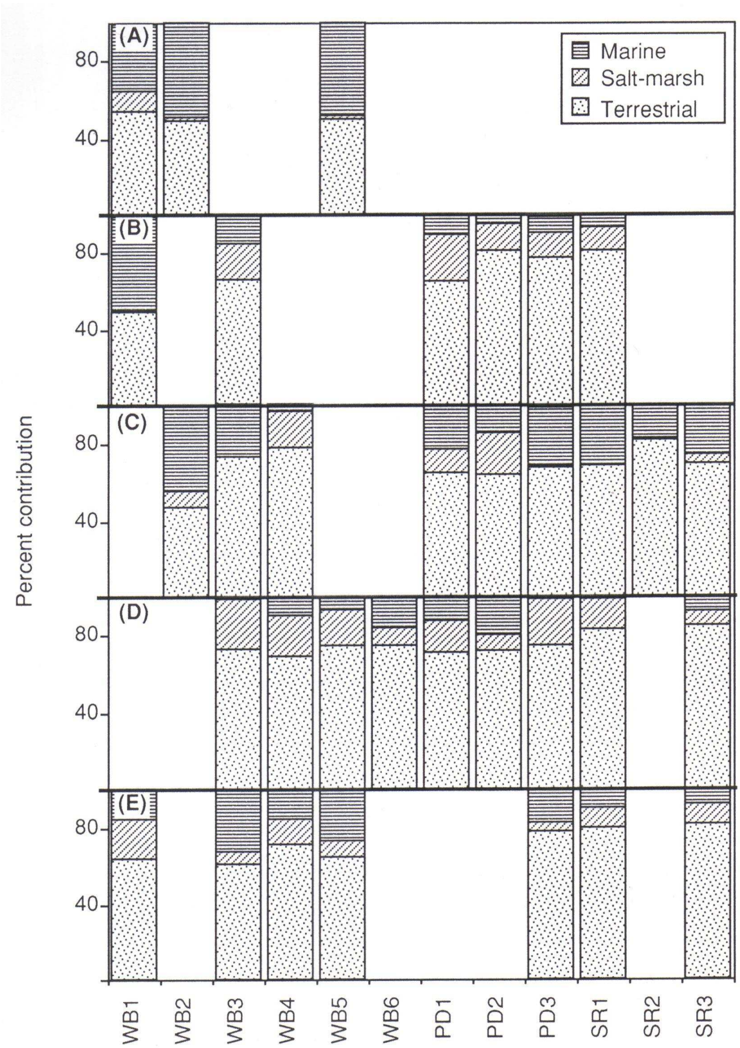

Fig. 17. Calculated seasonal contributions of terrestrial, marine and salt-marsh organic carbon to the fine fraction (≤63 μ) of bottom sediments from Winyah Bay, South Carolina: (A) winter, (B) early spring, (C) late spring, (D) summer and (E) fall

Examination of mixing model assumptions

The assumptions of the mixing model are that the isotopic composition of each plant end-member is constant over the plant lifetime and does not change after the plant dies and decomposes. There are two sources of uncertainty to the first assumption: intra- and interspecies isotopic variability. Intraspecies isotopic fluctuations are a function of the canopy density and height, chemical conditions for growth, light availability and stage of the plant growing season. For example, $\delta^{13}C$ can vary up to 1.8‰ from early leaf out to just prior to leaf abscission for some riparian species along blackwater streams of South Carolina [28]. In order to minimize the effects of intraspecies variability on our calculation, we collected more than one leaf from several plants (where possible), approximately from the same canopy position, during the same time of day. Unlike intraspecies variability, interspecies isotopic fluctuations could not be minimized.

To test the validity of the second assumption, we collected leaf litter in addition to new leaves. The carbon isotopic composition of new leaves was more ^{13}C-enriched than that of leaf litter by an average of 0.2‰ and 0.1‰ for C-3 and C-4 plants, respectively. The nitrogen isotopic composition of new leaves was more ^{15}N-enriched than that of leaf litter by an average of 0.4‰ and 3.2‰ for C-3 and C-4 plants, respectively. This assumption was constrained better by the carbon than by the nitrogen isotopic composition.

Limitations of the mixing model

The mixing model does not account for non-linear mixing processes such as enrichment or depletion of organic carbon in the sediment. These processes would introduce variability in the isotopic data, which cannot be explained as a linear function of isotopic composition with distance upstream. For example, organic carbon is enriched by sorption on finer-grained sediments (eg silt and clay) relative to coarser ones (eg sand). The sorption of carbon to the fine sediment matrix may facilitate the preferential preservation of labile organic compounds. As organic carbon in bottom sediments is a complex mixture of individual compounds, each with its own isotopic composition, bulk isotopic analysis would yield only a weighted isotopic average. The weighted average can be shifted towards lighter or heavier isotopic values depending on the isotopic composition of the preferentially preserved organic compounds. Furthermore, these processes may act parallel to hydrodynamic grain size sorting and linear end-member mixing to establish spatial gradients of sedimentary isotopic composition.

Other potential processes, which may contribute to the spatial variability of the data, include: bottom sediment resuspension, transport and redistribution in the estuary, respiration of organic carbon, bioturbation, mixing of carbon from potential sources which are not accounted for in the mixing model, such as: bacteria, benthic algae, macroalgae, anthropogenic non-point pollution.

Conclusions

Some specific conclusions could be made when chemometrics is applied to sediment pollution monitoring data. The application of multivariate statistical approaches for data projection and classification seems to be quite a useful tool to reveal specific relationships between very different groups of assessment parameters such as chemical concentrations and toxicity. In case of classification of large data sets from monitoring results SOM indicates some additional advantages, whose demonstration is one of the goals of the present study. SOM makes it possible not only to cluster the objects of interest (or the variables characterizing the objects) but to demonstrate their spatial proximity. Further, SOM data interpretation allows better understanding of the discriminating tracers for each one of the identified groups of similarity between the sampling areas.

The traditional latent factor identification, which is normally achieved by estimating of the factor loadings in the classical PCA, could be improved if fuzzy PCA is applied. Very often fuzzy PCA gives better models with higher level of explained total variation. But more important improvement is achieved in the interpretation of the projection scatter plots of the objects (sampling sites). The fuzzy approach eliminates some outliers and makes it possible to determine in a more reliable way the role of “location” factors in sediment formation - the geographical coordinates of the sampling site, the level of population, the total environment of the site.

The modern world exists in a very dynamic mode. The environmental problems, despite their already long history, are not completely solved but the strategy of the sustainable development is a fact, which helps in solving problems. It is our deep conviction that a serious contribution to the overall solution is the application of chemometrics to the environmental monitoring observations, which makes the solution finding and the problem solving a more intelligent and reliable process.

Acknowledgement

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OCENA STANU ZANIECZYSZCZENIA OSADÓW Z POMOCĄ METOD CHEMOMETRYCZNYCH

Abstrakt: Przedstawiono zastosowanie strategii i metod chemometrycznych do oceny zanieczyszczenia osadów dennych. Osady te są ważnym elementem środowiska, gromadzącym informacje o przeszłych wydarzeniach polegających na wprowadzaniu zanieczyszczeń do środowiska. Wykonano odpowiednią analizę danych, modelowano oraz zinterpretowano wyniki badań monitoringowych osadów jeziornych, rzecznych i morskich. W celu wykrycia źródeł zanieczyszczeń w określonym środowisku, powiązania miejsc pobierania próbek oraz parametrów chemicznych, zależności ekotoksyczności od rodzaju zanieczyszczenia i in. zastosowano różnorodne techniki chemometryczne, takie jak: analiza skupień, analiza głównych składowych, analiza rozmytych głównych składowych, samoorganizujące się mapy Kohonena oraz modele mieszania punktu końcowego.

Słowa kluczowe: metody chemometryczne, próbki osadów, zanieczyszczenia środowiska, oceny ryzyka

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APPLICATION OF ENRICHMENT FACTOR (EF) TO THE INTERPRETATION OF RESULTS FROM THE BIOMONITORING STUDIES

ZASTOSOWANIE WSPÓŁCZYNNIKA WZBOGACENIA (EF) DO INTERPRETACJI BADAŃ BIOMONITORINGOWYCH

Abstract: This paper discusses the relations between the concentrations of micro- and macroelements accumulated in the lichen *Hypogymnia physodes*, the moss *Pleurozium schreberi* and soil humus. The concentrations of the elements were measured using the *Neutron Activation Analysis* (NAA) and the *Atomic Absorption Spectrometry* (AAS). In addition, the activity of ^{137}Cs in the samples was measured using the gamma spectrometry. For many metals - Mg, Al, Ti, V, Cr, Fe, Co, As, Se, Sr, Mo, Sb, I, Ba, La, Ce, Sm, Tb, Tm, Yb, Ta, Th and U - the linear relations were observed between the metal concentration and the concentration of scandium in each matrix (lichens, mosses and soil). These linear relations and the analysis of the ^{137}Cs activity in the samples indicated that the assessing of the origins of pollutants based on the enrichment factors (EF) is highly uncertain when the factors are smaller than 10.

Keywords: lichens, mosses, soil, biomonitors, Enrichment Factors (EF)

Introduction

Interactions between various components of a single ecosystem have been examined for a long time. The efforts had been aimed at the identification of mutual relations between the chemical composition of the lithosphere, hydrosphere and atmosphere, and the fauna and flora inhabiting the particular ecosystem. [1-3]. The results were applied in the biomonitoring of the air, water and soil pollution, and in the assessment of the translocation of pollutants across the successive phases of the trophic chains [4, 5]. Many plant and animal species were identified that displayed physiological, anatomic and morphological changes resulting from the accumulation of pollutants.

The biomonitoring of pollution by the atmospheric aerosol most commonly utilizes lichens and mosses, which have no root parts and collect nutrients and pollutants through the entire body surface. Analysis of trace elements accumulated in lichens and mosses provides much information on the emission of pollutants into the environment, enables the

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assessment of a changing quality of the environment, and allows the determination of directions in which the pollutants propagate. One of the aims in the biomonitoring of the atmospheric aerosol is the assessment of the time-resolved variation of the deposition of pollutants over a given area. This is realized either by comparing how the concentrations of various analytes in lichens and mosses inhabiting the examined area change in time (*passive biomonitoring*) or by analyzing the changes of the concentration of analytes in lichens exposed to pollutants in the contaminated areas (*active biomonitoring*).

In order to assess the origins of the analytes accumulated in mosses or lichens, the results are often analyzed using the Enrichment Factor (EF) [6-9], which compares the relative concentration of an analyte accumulated in lichens or mosses to that in soil:

$$EF = \frac{(c_x/c_{Sc})_{lichen/moss}}{(c_x/c_{Sc})_{soil}} \tag{1}$$

where: c_x - concentration of an examined analyte, c_{Sc} - concentration of the *reference element* (scandium or aluminium) in lichens or mosses and in soil.

The reference elements - scandium and aluminium - rarely enter the atmospheric aerosols from anthropogenic sources. The sum of the concentrations of the rare-earth elements was also suggested as a suitable reference quantity [10, 11].

By definition, the enrichment factor close to unity (EF = 1) indicates, that the element considered did originate from the soil [12]. However, some authors argue that the values of EF < 10 are merely the measurement background, hence they cannot show, whether the source of the analytes accumulated in lichens and mosses was the local soil, or rather some remote source of emission [12-14].

The present study discusses the factors affecting the uncertainty of assessing the origins of analytes accumulated in mosses and lichens when EF < 10.

Materials and methods

Samples of the lichen *Hypogymnia physodes*, the moss *Pleurozium schreberi* and the soil ectohumus were collected in the course of the biomonitoring of the area of Bory Stobrawskie located in southern Poland, north-east of the city of Opole. The samples were collected at 5 measurement sites located in the areas with similar geological structure of the soil and similar vegetation (Fig. 1).

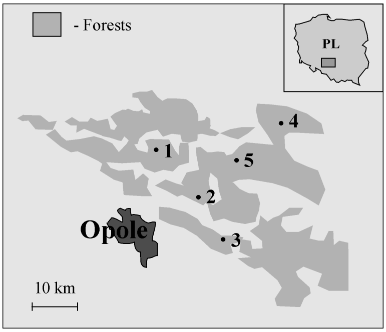

Fig. 1. Location of the measurement sites and neighbouring localities: 1 - Zagwizdzie; 2 - Rzedow; 3 - Staniszcze Wielkie; 4 - Olesno; 5 - Szumirad

Bory Stobrawskie partly overlap with the area of the Opole Anomaly, in which the activity of caesium 137 isotope in soil tends to exceed the average for Poland, as confirmed by measurements taken since 1994 [15]. Forests and wastelands contain large amounts of ^{137}Cs released during meltdown at Chernobyl. Translocation of radioceasium was discussed in numerous research papers [eg 16-18].

Methods of collecting and preparing samples

At each measurement site, samples of the lichen *Hypogymnia physodes*, the moss *Pleurozium schreberi*, and the soil were collected from six different sampling points and mixed together to produce the composition-averaged samples weighing about 50 g (lichen), 100 g (moss) and 500 g (soil). The lichen and moss samples were cleaned from foreign material and dried at temperatures not exceeding 303 K. The soil samples were sieved (mesh diameter - 2 mm) and dried at 373 K. From the averaged lichen, moss and soil samples, the respective portions of 30, 40 and 150 g were used for the ^{137}Cs radioactivity measurements. The remaining parts of the samples were homogenized in agate mortars and analyzed using the INAA and AAS methods.

Analysis

The content of elements in the lichen, moss and soil samples was determined by a combined analytical method including the *Instrumental Neutron Activation Analysis* (INAA), the *Atomic Absorption Spectrometry* (AAS) and the *Gamma Spectrometry* (GS).

The lichen, moss and soil samples for the INAA, which weighed about 0.3 g each, were heat-sealed in polyethylene foil bags for short irradiation, and additionally packed in aluminium cups for long irradiation. The INAA was carried out at the IBR-2 reactor in the Frank Laboratory of Neutron Physics in Dubna, Russian Federation. The analytical procedures and the basic characteristics of the pneumatic system employed were described in detail elsewhere [19]. The gamma-spectra of the samples were measured with a Ge-Li detector providing the resolution of 2.5÷3 keV for the ^{60}Co 1332 keV line. An HPGe detector with the resolution of 1.9 keV for the ^{60}Co 1332 keV line was also used. The processing of the data and determination of the content of elements were performed using the certified reference materials and flux comparators, as well as the software developed in FLNP, JINR [20].

The content of some metals, namely Cu, Cd, Pb and ^{137}Cs, was determined at the Opole University in Poland, using a SOLAAR 969 Atomic Absorption Spectrometer from UNICAM. Samples of lichen, moss and soil were mineralised with *aqua regia* in a MARS-X microwave mineraliser from CEM.

The ^{137}Cs activity in the samples was measured with a gamma-spectrometer equipped with a high-resolution germanium detector HPGe (Canberra) providing 1.29 keV FWHM at 662 keV and 1.70 keV FWHM at 1332 keV, as well as the relative efficiency of 21.7%. The measurements and the analysis of spectra were controlled with a computer running the GENIE 2000 software.

Quality control

The quality control of NAA was performed by carrying out the concurrent analyses of three reference materials: Lichen 336 IAEA (RM) from the International Atomic Energy

Agency; a standard reference material SRM-1575 (Pine Needles) from the US National Institute of Standards and Technology; and NORD DK-1, the moss reference samples prepared for the calibration purposes in the laboratories participating in the 1990 Nordic Survey. In long irradiations, the three reference materials were analyzed along with 10-12 regular samples. Table 1 compares the NAA results obtained for the reference materials with the corresponding certified values. Basing on the best agreement of the data, the RM reference material was chosen for further use in the analysis. There were no certified reference values available for Mg, I, Hf, and Ta, so the corresponding results were calculated using the Nordic moss reference sample DK-1 [21]. The content of Mo and W was determined by the absolute method based on the nuclear constants [20].

Table 1

NAA data for the reference materials [µg/g] [22]

Element	**Lichen 336**				**SRM 1575**			
	NAA		**Certified**	**D* [%]**	**NAA**		**Certified**	**D* [%]**
	Mean	**±SD**			**Mean**	**±SD**		
Na	346	14	320	8.2	60	7		
Mg	636	209			897	202		
Al	643	76	680	–5.5	469	50	645	–27.2
Cl	1731	218	1900	–8.9	234	46		
K	2021	346	1840	9.8	3951	324	3700	6.8
Ca	2340	606			3176	866	4100	–22.5
Sc	0.19	0.17	12.9	0.044	0.044	0.008	0.030	46.7
V	1.19	0.15	1.47	–18.9	0.27	0.06		
Cr	1.17	0.25	1.06	10.1	2.52	0.48	2.60	–3.1
Mn	71	7	63	13.2	596	63	675	–11.7
Fe	438	59	430	1.9	218	20	200	9.1
Co	0.34	0.13	0.29	15.7	0.15	0.06	0.10	50.0
Ni	1.15	0.25			2.97	0.36	3.5	–15.3
Zn	30.5	3.7	30.4	0.2	66.0	5.5		
As	0.66	0.09	0.63	4.7	0.24	0.03	0.21	14.5
Br	14.8	3.4	12.9	14.8	7.9	2.2	9	–12.4
Rb	1.76	0.33	1.76	0.1	13.5	1.6	11.7	15.2
Sr	9.58	2.85	9.30	3.0	5.90	1.83	4.80	22.9
Mo	0.075	0.008			0.11	0.01		
Sb	0.083	0.014	0.073	13.4	0.19	0.02	0.20	–4.0
I	2.08	0.48						
Cs	0.12	0.02	0.11	6.9	0.14	0.01		
Ba	6.8	1.6	6.4	6.9	8.1	1.2		
La	0.76	0.05	0.66	14.9	0.20	0.02	0.2	1.7
Ce	1.64	0.47	1.28	27.8	0.49	0.11	0.4	22.9
Sm	0.137	0.019	0.106	29.1	0.034	0.008		
Tb	0.017	0.005	0.014	24.6	0.0046	0.0010		
Yb	0.049	0.009	0.037	32.4				
Hf	0.060	0.011			0.025	0.006		
Ta	0.021	0.004			0.007	0.001		
W	1.01	0.73			1.47	1.36		
Th	0.16	0.03	0.14	14.5	0.043	0.006	0.037	15.6
U	0.043	0.005			0.024	0.002	0.020	18.1

* Deviation: the difference between the NAA and the certified values, divided by the certified value, and expressed in %

The AA spectrometer was calibrated against a standard source from Czech company ANALYTIKA Ltd.

Table 2 shows the concentrations of metal elements in the reference material BCR-482 *lichen* obtained using AAS spectrometry.

Table 2

AAS data for reference materials [µg/g]

Element	AAS		BCR-482 *lichen*		D*
	Mean	**±SD**	**Certified value**	**±Uncertainty**	
	[mg/kg]				**[%]**
Cu	6,54	0.18	7.03	0.19	–7.0
Cd	0.50	0.04	0.56	0.02	–6.3
Pb	37.6	0.9	40.9	1.4	–7.6

* Deviation: the difference between the AAS and the certified values, divided by the certified value, and expressed in [%]

The energy and efficiency calibration of the gamma spectrometer was performed using a standard solution type MBSS 2 from the Czech Metrological Institute in Prague, which covers the energy range from 59.54 keV (^{241}Am) to 1836.06 keV (^{88}Y). Geometry of the calibration source and samples was a 450 cm^3 Marinelli container.

Results and discussion

Tables 3-9 show the measured concentrations (activities) of the analytes in samples of the lichen, moss and soil. The results were grouped after the method of analysis, namely INAA, AAS and GS. Most of the results were also presented elsewhere [22]. By permission of the authors [22], Table 3 contains also the unpublished data obtained in the Frank Laboratory of Neutron Physics in Dubna, Russian Federation.

Table 3

Concentration of analytes [µg/g] determined in 5 samples of lichen using the INAA (% denotes the measurement uncertainty)

Sample	Na	Mg	Al	Cl	K	Ca	Sc	Ti	V	Cr
1	196	1187	1281	1874	4050	4935	0.19	185	2.0	3.80
2	169	964	517	1582	4481	6883	0.14	75	0.9	1.57
3	231	940	478	1497	4470	8451	0.10	94	0.8	1.77
4	134	1113	595	1507	4290	8076	0.14	147	1.1	1.23
5	125	1517	673	1723	5837	19460	0.13	81	1.2	2.32
[%]	10	30	5	30	20	10	12	20	20	10
Sample	**Mn**	**Fe**	**Co**	**Zn**	**As**	**Se**	**Br**	**Rb**	**Sr**	**Mo**
1	180	676	0.33	114	0.90	0.30	7.2	29	20.4	0.34
2	144	458	0.35	134	0.81	0.27	5.4	32	14.9	0.28
3	190	443	0.34	192	0.71	0.35	5.2	37	27.1	0.34
4	347	391	0.39	107	0.81	0.19	8.3	15	21.0	0.14
5	332	438	0.40	91	1.13	0.30	8.9	28	17.3	0.22
[%]	5	13	20	6	10	20	25	10	5	30
Sample	**Sb**	**I**	**Cs**	**Ba**	**La**	**Ce**	**Sm**	**Eu**	**Tb**	**Dy**
1	0.22	1.65	0.71	34	1.07	2.13	0.14	0.40	0.014	0.23
2	0.17	1.21	0.81	23	1.02	2.75	0.08	0.38	0.009	0.23
3	0.20	1.17	1.21	37	0.44	0.92	0.08	0.37	0.018	0.26

4	0.26	1.33	0.19	32	0.47	1.54	0.09	0.15	0.012	0.49
5	0.19	1.70	0.67	28	0.48	1.21	0.11	0.10	0.008	0.25
[%]	10	30	13	6	8	15	6	30	15	30
Sample	**Tm**	**Yb**	**Hf**	**Ta**	**W**	**Th**	**U**			
1	0.010	0.043	0.056	0.016	0.55	0.20	0.08			
2	0.020	0.053	0.053	0.010	1.04	0.10	0.06			
3	0.010	0.027	0.040	0.010	0.97	0.08	0.04			
4	0.013	0.024	0.050	0.012	0.66	0.08	0.05			
5	0.059	0.030	0.067	0.005	0.92	0.09	0.04			
[%]	30	20	15	37	30	15	10			

Table 4

Concentration of analytes [µg/g] determined in 5 samples of moss using the INAA (% denotes the measurement uncertainty)

Sample	**Na**	**Mg**	**Al**	**Cl**	**K**	**Ca**	**Sc**	**Ti**	**V**	**Cr**
1	474	2390	3896	415	8252	3592	0.32	99	4.4	2.4
2	278	1444	1320	408	9454	3518	0.15	97	1.9	4.2
3	694	1478	2214	849	6702	4369	0.33	130	3.5	4.6
4	270	1600	1017	443	9133	2113	0.14	56	1.5	0.8
5	322	1531	1172	480	7292	1907	0.15	207	1.8	2.8
[%]	10	30	5	30	20	10	12	20	20	10
Sample	**Mn**	**Fe**	**Co**	**Zn**	**As**	**Se**	**Br**	**Rb**	**Sr**	**Mo**
1	216	646	0.43	61	0.59	0.67	2.1	37	17	0.24
2	183	495	0.41	45	0.67	0.35	2.3	32	20	0.31
3	171	1117	0.50	68	0.94	0.41	3.4	32	22	0.58
4	290	450	0.40	45	0.58	0.33	2.1	28	19	0.24
5	423	97	0.30	41	0.54	0.18	2.3	16	19	0.22
[%]	5	13	20	6	10	20	25	10	5	30
Sample	**Sb**	**I**	**Cs**	**Ba**	**La**	**Ce**	**Sm**	**Eu**	**Tb**	**Dy**
1	0.28	1.0	1.06	32	1.19	2.95	0.27	0.12	0.030	0.22
2	0.89	1.0	0.83	16	0.58	1.61	0.12	0.25	0.011	0.27
3	0.80	1.8	1.29	51	1.08	2.15	0.22	0.44	0.021	0.19
4	0.34	1.1	0.40	23	0.54	1.70	0.10	0.10	0.009	0.14
5	0.49	1.3	0.31	15	0.64	1.14	0.11	0.24	0.020	0.37
[%]	10	30	13	6	8	15	6	30	15	30
Sample	**Tm**	**Yb**	**Hf**	**Ta**	**W**	**Th**	**U**			
1	0.07	0.07	0.16	0.044	0.91	0.38	0.18			
2	0.01	0.02	0.10	0.019	1.75	0.15	0.12			
3	0.07	0.06	0.21	0.035	1.13	0.28	0.14			
4	0.05	0.03	0.16	0.012	1.29	0.13	0.07			
5	0.04	0.02	0.10	0.020	0.87	0.13	0.07			
[%]	30	15	15	37	30	15	10			

Table 5

Concentration of analytes [µg/g] determined in 5 samples of soil using the INAA (% denotes the measurement uncertainty)

Sample	**Na**	**Mg**	**Al**	**Cl**	**K**	**Ca**	**Sc**	**Ti**	**V**	**Cr**
1	641	3992	8820	402	2951	4906	1.9	808	14.7	35
2	871	5685	13850	327	4012	2705	3.1	1067	23.9	51
3	957	4516	11400	350	4344	5242	2.4	1059	16.2	39
4	952	4979	12840	312	3842	3738	2.9	1197	22.4	33
5	725	5082	10050	227	2378	2927	1.9	791	14.4	37
[%]	10	30	5	30	20	10	12	20	20	10

Sample	**Mn**	**Fe**	**Co**	**Zn**	**As**	**Se**	**Br**	**Rb**	**Sr**	**Mo**
1	122	6886	2.9	94	7.0	1.4	17.8	14	36	0.8
2	98	11930	3.6	112	13.7	2.3	22.4	20	53	1.4
3	238	8386	2.5	199	9.2	1.1	18.5	21	59	1.4
4	248	10120	3.8	107	9.1	1.7	19.2	16	48	1.1
5	324	6985	2.5	80	7.2	1.2	19.1	13	24	0.9
[%]	5	13	20	6	10	20	25	10	5	30
Sample	**Sb**	**I**	**Cs**	**Ba**	**La**	**Ce**	**Sm**	**Eu**	**Tb**	**Dy**
1	2.0	10.6	1.7	114	5.0	12.2	0.96	1.1	0.20	0.7
2	3.5	13.3	2.3	179	8.7	17.8	1.53	1.0	0.32	1.1
3	2.3	10.0	2.6	202	6.8	12.6	1.31	0.2	0.30	1.1
4	2.9	10.7	1.6	179	9.5	18.8	1.52	0.8	0.36	1.1
5	2.3	9.9	1.3	112	6.1	13.7	1.12	1.1	0.23	1.3
[%]	10	30	13	6	8	15	6	30	15	30
Sample	**Tm**	**Yb**	**Hf**	**Ta**	**W**	**Th**	**U**			
1	0.22	0.33	1.4	0.26	3.6	1.53	0.85			
2	0.36	0.47	2.3	0.30	13.5	2.70	1.45			
3	0.29	0.57	3.1	0.34	4.2	2.14	1.04			
4	0.33	0.51	4.6	0.38	5.0	2.38	1.43			
5	0.20	0.37	2.2	0.25	4.2	1.87	1.03			
[%]	30	15	15	37	30	15	10			

Table 6

Concentrations of heavy metals [µg/g] determined in 5 samples of lichen using the AAS
(mean ± SD and (minimal - maximal) values)

Sample	**1**	**2**	**3**	**4**	**5**
Cu	6.61 ±0.19 (6.32÷6.81)	8.17 ±0.12 (8.01÷8.33)	7.17 ±0.15 (6.99÷7.38)	7.52 ±0.23 (7.12÷7.68)	5.73 ±0.17 (5.51÷5.93)
Cd	1.19 ±0.18 (0.88÷1.31)	1.14 ±0.13 (0.96÷1.32)	1.60 ±0.25 (1.42÷1.76)	1.52 ±0.13 (1.32÷1.64)	1.80 ±0.18 (1.64÷2.04)
Pb	20.60 ±0.44 (20.06÷21.12)	15.79 ±0.39 (15.31÷16.24)	21.70 ±0.44 (21.27÷22.31)	21.33 ±0.44 (20.8`÷21.84)	23.27 ±0.35 (22.75÷23.72)

Table 7

Concentrations of heavy metals [µg/g] determined in 5 samples of moss using the AAS
(mean ±SD and (minimal - maximal) values)

Sample	**1**	**2**	**3**	**4**	**5**
Cu	3.63 ±0.43 (3.12÷4.22)	4.87 ±0.42 (4.36÷5.43)	10.0 ±0.25 (9.66÷10.28)	8.95 ±0.15 (8.88÷9.21)	8.37 ±0.16 (8.11÷8.52)
Cd	0.321 ±0.031 (0.281÷0.349)	0.81 ±0.14 (0.69÷1.04)	0.85 ±0.12 (0.68÷0.98)	0.668 ±0.084 (0.547÷0.781)	0.740 ±0.060 (0.652÷0.812)
Pb	12.5 ±1.1 (10.8÷13.6)	16.4 ±1.2 (14.2÷17.1)	20.8 ±1.4 (19.2÷22.6)	19.68 ±0.85 (18.66÷20.92)	21.0 ±1.2 (19.8÷22.8)

Figure 2 shows the linear relation between the concentrations of Sc and Al in samples of lichens, mosses and soil. The logarithmic scale was used to provide better resolution at small values of the concentration. Error marks in the charts correspond to the uncertainty of the INAA measurements (Tables 3-5).

Table 8

Concentrations of heavy metals [µg/g] determined in 5 samples of soil using the AAS (mean ±*SD* and (minimal - maximal) values)

Sample	1	2	3	4	5
Cu	13.34 ±0.24 (12.89÷13.51)	22.20 ±0.37 (21.71÷22.49)	10.61 ±0.37 (10.21÷11.09)	22.66 ±0.31 (22.47÷23.21)	15.59 ±0.24 (15.31÷15.89)
Cd	0.68 ±0.12 (0.55÷0.81)	0.84 ±0.12 (0.71÷0.98)	1.90 ±0.10 (1.79÷2.03)	1.20 ±0.13 (1.06÷1.33)	1.07 ±0.10 (0.98÷1.13)
Pb	91.3 ±2.1 (89.6÷94.1)	173.6 ±2.0 (171.5÷176.2)	113.9 ±1.4 (112.6÷116.2)	144.1 ±1.4 (142.0÷144.7)	105.1 ±2.0 (102.8÷107.2)

Table 9

Mean activity of ^{137}Cs [Bq/kg] in samples of the lichen, moss and soil

Sample	Lichen		Moss		Soil	
	Mean	±SD	Mean	±SD	Mean	±SD
1	221	11	404	19	627	21
2	1010	41	1075	47	1010	20
3	13.2	1.1	133.3	3.0	155.2	4.6
4	696	16	731	11	1327	31
5	769	34	748	22	1765	40

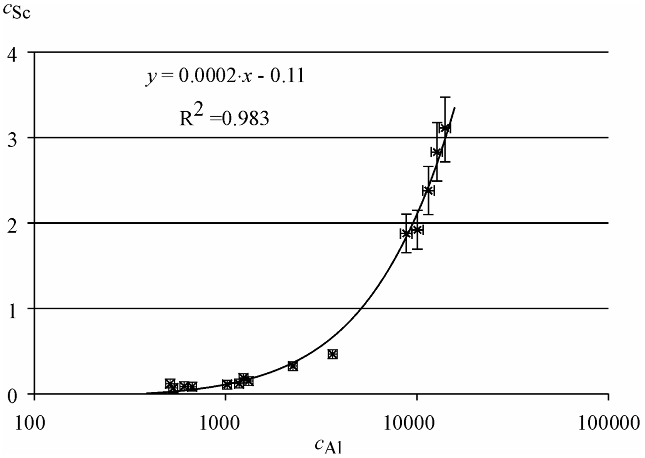

Fig. 2. Linear relation between the concentrations of Sc and Al accumulated in soil, lichens and mosses [µg/g]

Table 10 shows the parameters of linear relations between the concentration of scandium and the concentrations of several other analytes accumulated in lichens, mosses and soil, at the level of statistical correlation $R^2 > 0.9$.

The linear relations between the concentrations of analytes and the concentration of Sc occurred mostly for the lithophilic elements of the Goldschmidt's classification [23], ie the elements contained in the rock-forming minerals present in the Earth crust mostly in the form of oxygen compounds such as silicates, aluminosilicates, oxides and other oxy-acid salts. In order to assess the similarities in accumulation of the analytes in lichens, mosses and soil, the EF values were determined for all analytes, with scandium as a reference element (equation 1). The results are presented in Figures 3 and 4. The enrichment factors for elements, whose concentration in samples was linear against that of scandium were shown in Table 10. The value of EF determined for ^{137}Cs was distinguished with dark gray colour.

Table 10

Parameters of linear relations y = bx + a between the concentration of scandium ($y = c_{Sc}$) and the concentrations of other elements ($x = c_x$) accumulated in lichens, mosses and soil

Element (x)	**Goldschmidt classification**	**Group of the periodic system**	**Function: y = bx + a**	**R^2**	**±SD$_b$**	**±SD$_a$**
Mg	Lith	2 (IIA)	$y = 64\cdot10^{-5}x - 0.71$	0.943	$4\cdot10^{-5}$	0.13
Ba	Lith		$y = 0.0167x - 0.27$	0.942	0.0011	0.11
La	Lith	3 (IIIB)	$y = 0.341x + 0.069$	0.983	0.012	0.053
Ce	Lith		$y = 0.1702x - 0.130$	0.977	0.0072	0.065
Sm	Lith		$y = 1.970x - 0.089$	0.994	0.042	0.032
Tb	Lith		$y = 8.42x + 0.050$	0.985	0.28	0.047
Tm	Lith		$y = 8.90x + 0.129$	0.948	0.58	0.099
Yb	Lith		$y = 4.40x + 0.04$	0.921	0.36	0.11
Th	Lith		$y = 1.156x - 0.015$	0.994	0.025	0.032
U	Lith		$y = 2.100x - 0.004$	0.992	0.053	0.036
Ti	Lith	4 (IVB)	$y = 0.00260x - 0.129$	0.974	0.00012	0.069
V	Lith	5 (VB)	$y = 0.1361x - 0.078$	0.992	0.0033	0.036
Ta	Lith		$y = 7.86x + 0.033$	0.961	0.44	0.079
Cr	Lith	6 (VIB)	$y = 0.0611x + 0.028$	0.945	0.0041	0.093
Mo	Sider		$y = 2{,}54x - 0.50$	0.903	0.23	0.16
Fe	Sider	8 (VIIIB)	$y = 270\cdot10^{-6}x - 0.034$	0.996	$5\cdot10^{-6}$	0.025
Co	Sider	9 (VIIIB)	$y = 0.832x - 0.143$	0.973	0.038	0.070
Al	Lith	13 (IIIA)	$y = 223\cdot10^{-6}x - 0.115$	0.983	$8\cdot10^{-6}$	0.055
Pb	Chalc	14 (IVA)	$y = 0.02072x - 0.206$	0.981	0.00080	0.061
As	Chalc	15 (VA)	$y = 0.258x - 0.000$	0.970	0.013	0.070
Sb	Chalc		$y = 0.978x - 0.174$	0.961	0.054	0.086
Se	Chalc	16 (VIA)	$y = 1{,}67x - 0.31$	0.918	0.14	0.14
I	Lith	17 (VIIA)	$y = 0.238x - 0.147$	0.965	0.013	0.080

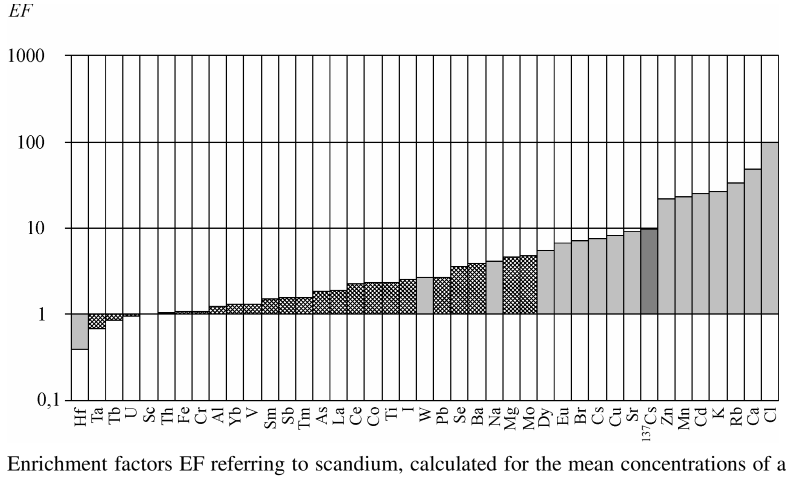

Fig. 3. Enrichment factors EF referring to scandium, calculated for the mean concentrations of analytes accumulated in the lichen and soil samples collected at sampling sites 1-5

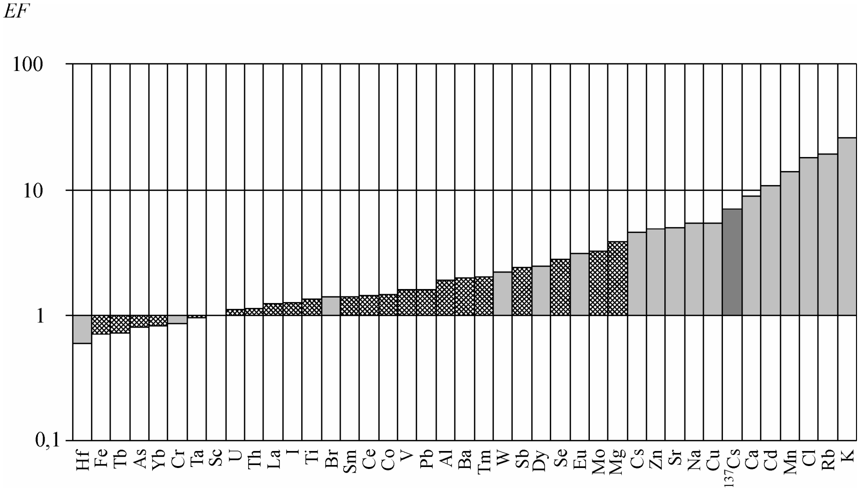

Fig. 4. Enrichment factors EF referring to scandium, calculated for the mean concentrations of analytes accumulated in the moss and soil samples collected at sampling sites 1-5

Figures 3 and 4 show that for several analytes the $EF > 1$ indicated the remote sources, while the linear correlations most probably indicated these analytes originated only from the local soil. Moreover, no remote sources are known for ^{137}Cs, which had the EF close to 10. In Poland, ^{137}Cs has not been found in the global fallout for a number of years [24]. In 2006, its radioactivity in air was 0.1÷10.7 (mean 1.4) μBq/m^3 in the 2nd quarter of the year, and 0.1÷6.7 (mean 1.0) μBq/m^3 in the 3rd quarter of the year [25, 26]. Therefore, the ^{137}Cs pollution is now negligible. The observed enrichment can be explained only by the transfer of ^{137}Cs from the particulate matter in the local soil to the lichen. This conclusion has been confirmed by other studies [16].

The disagreement between the real sources of the analytes and the remote sources indicated by high values of the EF can result from several factors: the uncertainty of the measurement methods, which for some analytes, eg Cl and Mo, reached 30% (Tables 3-9); the inhomogeneity of the chemical composition of the samples; and the very definition of the EF. The enrichment factors are calculated from the concentrations of analytes in the soil, lichens and mosses, but mosses and lichens accumulate only the bioaccessible forms of pollutants. Moreover, bioaccumulation is affected by the chemical characteristics of compounds which contain the examined analytes, mainly by the ability of these compounds to convert into ionic forms, as well as by the affinity of the ionic forms of compounds to the sorption structures of mosses and lichens [27]. One example of poorly bioaccessible elements with $EF < 1$ is hafnium ($EF_{\text{lichen}} = 0.39$, $EF_{\text{moss}} = 0.60$).

In the examined area, the following values of EF_x larger than the value for caesium, EF_{Cs}, were determined for lichens: Zn (EF = 21), Mn (23), Cd (25), K (26), Rb (33), Ca (49) and Cl (100); and for mosses: Ca (9), Cd (11), Mn (14), Cl (18), Rb (19) and K (26). All these elements, with the exception of Ca, accumulated better in lichens. On the basis of the EF values, it is possible to conclude that they have the remote character.

Conclusions

Biomonitoring methods are gaining popularity in the assessment of environmental pollution. Their advantages include simple and inexpensive collection of samples, as well as direct delivery of data on bioaccessible pollutants, ie those present in active chemical forms.

One of the subjects in the biomonitoring studies is the assessment of the origin of pollutants accumulated in lichens or mosses. Atmospheric aerosol is often enriched by pollutants originating from remote sources, but it can also be enriched locally by the material lifted up from the soil. In order to assess the effect of these sources of pollution on the composition of micro- and macroelements accumulated in lichens and mosses, the measurements are commonly interpreted using the enrichment factor (EF). By definition, values of EF greater than 1 should indicate the permanent remote nature of pollutant origin.

The presented studies conducted for the area of Bory Stobrawskie woods, show, in agreement with other works [12-14], that the biomonitoring results are uncertain when EF < 10. A good example is ^{137}Cs, which deposited temporarily after the Chernobyl disaster in 1986, and now is not linked to any remote sources. The values of EF determined for this radionuclide in lichens and mosses was 9.8 and 7.0, respectively. Similarly, for many analytes whose concentration in soil, lichens and mosses was linear against the concentration of Sc (Table 10), the EF factors were greater than 1, eg $EF_{\text{Mo, lichen}} = 4.8$, $EF_{\text{Mg, moss}} = 3.9$ (Figs 3 and 4).

The uncertainty in assessing the origins of analytes from EF < 10 can result form the uncertainty of the analytical methods used, from inhomogeneity of the chemical composition of samples collected for the analysis, and from the very definition of EF, which includes the total concentration of the analytes in the soil and the concentration of only the bioaccessible forms of these analytes in lichens or mosses.

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ZASTOSOWANIE WSPÓŁCZYNNIKA WZBOGACENIA (EF) DO INTERPRETACJI BADAŃ BIOMONITORINGOWYCH

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Abstrakt: Zbadano relacje między stężeniami mikro- i makropierwiastków zakumulowanych w porostach (*Hypogymnia physodes*), mchach (*Pleurozium schreberi*) i w glebie (próchnicy nadkładowej). Próbki pobrano w 5 miejscach, na obszarze Borów Stobrawskich, położonych na północny wschód od miasta Opola (Polska Południowa). Pierwiastki oznaczano metodą instrumentalnej neutronowej analizy aktywacyjnej (37 pierwiastków) oraz absorpcyjnej spektrometrii atomowej (6 pierwiastków). W próbkach, metodą spektrometrii gamma, zbadano także aktywność ^{137}Cs. Wskazano na prawdopodobne, liniowe zależności między stężeniami: Mg, Al, Ti, V, Cr, Fe, Co, As, Se, Sr, Mo, Sb, I, Ba, La, Ce, Sm, Tb, Tm, Yb, Ta, Th i U a stężeniem skandu w porostach, mchach i w glebie. Na tej podstawie oraz na podstawie analizy wyników aktywności ^{137}Cs wskazano na dużą niepewność oceny pochodzenia zanieczyszczeń poprzez wyznaczenie współczynników wzbogacenia (EF), kiedy ich wartość jest mniejsza od 10.

Słowa kluczowe: porosty, mchy, gleba, biomonitoring, współczynnik wzbogacenia (EF)

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NANOSILVER - MAKING DIFFICULT DECISIONS

NANOSREBRO - PODEJMOWANIE TRUDNYCH DECYZJI

Abstract: Nanotechnology, a fast-growing discipline of science, is widespread in various areas of life around the world. One of the examples of nanocompounds is nanosilver, which is used in medicine, electronics, the construction industry and chemical technology as an exceptionally efficient antibacterial and antifungal agent. For centuries silver has been known for its biocidal properties, much desired when creating aseptic conditions. On the other hand, the toxic effects of nanosilver have also been observed. Silver may accumulate in the food chain, which creates the risk of a direct effect on living organisms. What is more, silver nanoparticles may cause necrosis in human tissues, and can also distort the activity of elementary components in human cells. This paper presents the benefits and risks from the use of nanosilver.

Keywords: antimicrobial activity, disinfection, nanosilver, nanotoxicology

Introduction

Nanotechnology is a dynamically growing discipline of science which has been particularly intensively researched from the beginning of the 1990s. The prefix "*nano*" originates from Greek and means "*dwarf*", which refers to the microscopic size that nanotechnology deals with. The invention of the *scanning tunnel microscope* (STM), enabling the manipulation of atomic structures, is considered an exceptionally valuable achievement contributing to the development of nanotechnology [1].

The major focus of nanotechnology is on the design, development and application of materials whose dimensions range from 1÷100 nm. With the possible manipulation of nanostructure size, particularly the reduction of the particles forming them, the designed materials can have predictable physical, chemical or biological properties. If a structure is characterised by the dimensions of 1÷100 nm, ie the dimensions relevant to single atoms (10^{-9} m) and crystals (10^{-7} m), the material can have properties which are considerably different from those typical for individual atoms or crystals [2]. In structures whose dimensions range from 1 to 100 nm, the ratio of surface area to weight, and the ratio of the total number of atoms to the surface of the structure attain sufficiently high values.

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Therefore, surface properties, playing an important role, may have considerably modified chemical activity, thermal and electrical conductivity, and tensile strength. Modifications of material properties resulting from changes in the structure's size make nanomaterials very interesting from a commercial point of view. It is expected that in the future the scope of nanotechnology research will enable the development of structures demonstrating innovative properties. Today nanotechnology is a science broadly applied in various disciplines, such as biotechnology, biomedicine, molecular medicine, pharmacology, ecotoxicology, electronics and many others. Agriculture, veterinary science and the food industry also benefit from nanotechnology research to a comparable degree [3]. It has been estimated that because of its multiple applications the broadly defined nanotechnology industry will achieve a considerable profitability of ca 3 trillion USD before 2015.

Nanosilver (NAg), whose application has recently attained the profitability margin, is considered one of the most useful commercial products from the group of nanomaterials [4]. Colloidal nanosilver of a concentration of 300÷500 ppm fetches a catalogue price of ca 2000 EUR per litre, which in calculation for pure silver is ca. 60 million EUR per kilogram. However, the price for metallic silver is ca 600 EUR per kilogram.

Silver is a metal which has been used from early times, since before the Neolithic revolution. In ancient Greece, silver vessels were used for the storage of water. A pioneering method for the application of silver for medicinal purposes was described as early as in the 8th century. Silver was known only in its basic form until very recently. However, with the advent of the nanotechnology era the concept of manufacturing silver on a nano-scale was born. In this way nanosilver has proven to be a fundamental nanotechnology product. Silver nanoparticles stimulate the deep interest of scientists because they offer essential advantages, such as thermal conductivity, chemical stability, and catalytic and antibacterial activity [5]. Nevertheless, there are many concerns regarding unlimited confidence in nanosilver, which are attributed to increasingly frequent reports on the toxic properties of this structure. The specific nature of a nanomolecule should be thoroughly analysed and understood in order to make the right decision about the utility of nanosilver [6]. This paper presents the benefits of the application of nanosilver in various fields of science and highlights the various risks emerging during its use.

Obtaining nanomaterials

Work to develop manufacturing methods and identify nanostructures formed by noble metals has been carried out in many laboratories from the very beginning of the nanotechnology era. Cubes, prisms, discs, plates, triangles, rods and nanotubes are the major shapes of noble metals analysed on the nanoscale and being the focus of attention of scientists, who continue to search for more innovative areas of science where these forms can be applied. However, they have already found very many applications in photography, catalysis, biological marking, optoelectronics, sensors, antibacterial products and others. The reaction method is one of parameters affecting the quality of obtained nanomolecules (eg reduction can be a chemical, photochemical or electrochemical process). The quantitative and qualitative composition of used reagents, the presence of stabilizers, pH value, temperature, order of mixing components, the use of electrophilic or nucleophilic compounds, as well as the proportion of the used reducer also have considerable influence.

These parameters are crucial for the size, morphology, shape, stability and colour, as well as physical, chemical and magnetic properties of obtained nanocompounds [7, 8].

Obtaining nanosilver

To expand and improve knowledge of the methods for nanosilver synthesis, scientists usually refer to nanotechnology, a discipline of modern chemical synthesis. Each of the methods for the manufacture of nanosilver has its particular advantages, but also its limitations. Parameters which largely depend on the chosen reaction method concern molecule shape, its stability and the presence of agents protecting its core, as well as yield and impurities in the final product. Although the literature describes a broad range of superb and innovative methods for obtaining nanosilver, only a few of them are concerned with the mainstream production. The most popular and best known method to obtain nanosilver is the reduction of silver nitrate with a reducing agent (eg sodium borohydride). Photoreduction with UV light is also one of the leading methods. In addition, regardless of the vast number of publications on “green” technologies with which to obtain nanosilver, research into developing a fully environmentally-friendly production method for NAg is still in progress [9, 10]. The characteristics of the most popular methods for obtaining nanosilver are presented below.

Chemical reduction is the most common of all methods to obtain silver nanoparticles. The most important advantages it offers include high yield of non-aggregated nanoparticles, low price and ease of performance. This method is based on the reduction of silver nitrate ($AgNO_3$) with a reducing agent in the presence of a suitable amount of stabilizer, which controls the growth of silver nanoparticles undisturbed by aggregation. Example reducing agents include sodium borohydride ($NaBH_4$) and hydrazine (N_2H_4). Panigrahi et al used glucose, a weak reducing agent, but the obtained product was not uniform [11]. Polyoxyethylene sorbitan monolaurate, a stabilizer commercially known as TWEEN 20, was used by Nia in research to obtain colloidal silver [12]. The most important parameters of this method are: starting concentration of $AgNO_3$, ratio of molal concentrations of reducing agent to silver nitrate, and the concentration of the stabilizer [13, 14].

Another method of obtaining nanosilver is based on photochemical reduction. This method is valuable as it allows the blending of all the reacting substrates before the reaction is initiated. The core of the method is the fast reduction of silver cations, and small-size nanoparticles are seized by kinetic methods [3]. Kempa and collaborators developed a photoreduction method [15] in which they used *N*-methylnifedipine as a reducing agent. For that purpose, the mixture of silver chlorate(VII) ($AgClO_4$) and *N*-methylnifedipine was irradiated with a UV lamp, and silver nanoparticles of 1÷7 nm diameter were obtained [15].

Laser ablation, a new method to obtain nanosilver which has been frequently analysed in the last decade, involves the superficial reaction between liquid and a solid body suspended in it [16]. In this process a pulsating laser beam directed at the surface of a solid body causes a “discharge” of material from the surface of the solid body, which then migrates to the surrounding liquid (reducing liquid medium) in a bubble form. Ethylene or diethylene glycol are used as the liquid medium. The quality and appearance of molecules depend on the laser parameters and the concentration of the liquid medium. The advantage offered by this method is the ability of the migration of a considerable amount of material from the solid body to the liquid. Moreover, nanomolecules obtained using this method are characterised by high purity, stability, spherical shape and resistance to aggregation [3, 17].

Vacuum ion sputtering is another method used to synthesize nanosilver. For that purpose, a 30 μA/cm^{-2} current is directed at a target located at a 10 cm distance under variable energy levels. A beam diameter of approx. 50 mm is one of the key parameters. Nanosilver particles are formed on a silica gel matrix after irradiation with ions. A positive aspect of this method is the high purity of the obtained nanoparticles [3].

Reetz and Helbig were the first to develop, in 1994, an innovative method for obtaining nanoparticles by an electrochemical method [18]. In principle, this method is based on the dissolution of metal on an anode, and then the reduction of the obtained transitional metal salt on the cathode, which leads to the formation of metal particles stabilized with tetraalkyl ammonium salts. In a similar procedure nanosilver was obtained through the potentiostatic or galvanostatic polarization of silver in an ethanol solution. The major advantage of the above method is the high purity of particles and the control of their size through the compensation of the current density, with no need to add surface active agents. In addition, it does not require expensive equipment and instrumentation [18].

Microwave irradiation is another method used to synthesize nanoparticles, and it was successfully used to obtain nanosilver and nanogold. When a microwave beam goes through the dielectric coating of the material, radiation energy transforms into thermal energy and the material's temperature increases [19].

Green synthesis is an interesting method used to obtain nanosilver and is based on the synthesis of silver nanoparticles using *Bacillus subtilis* bacteria. The enzymatic reaction is enabled by the mediation of nitrate reductase in the bacterial culture. The reaction mechanism involves the reduction of metal ions stimulated by bacteria. However, this process has not been researched in detail. It is likely that the reduction of Ag^+ ions and further growth of silver nanoparticles are stimulated by both reductase and electrons originating from the compound, as well as peptides or proteins [20]. Enzyme synthesis of nanosilver offers a great number of advantages for large-scale production, and the process is also uncomplicated to perform [21].

Properties of nanosilver

Silver, together with gold and other rare and precious metals, has been used by people for thousands of years. It has found various applications in medicine, dentistry, the jewellery industry, photography, the production of explosives and others. Vessels coated with silver were used in ancient times for the storage of water and wine, and Hippocrates, the "father of medicine", wrote that silver had beneficial healing and anti-disease properties [22]. Silver compounds were used as the main cure for infections during World War I, until the advent of antibiotics. In 1884 C.S.F. Crede, a German obstetrician, administered silver nitrate solution to treat *Gonococcal Ophthalmia*, and this was probably the first scientifically documented use of silver. Later on, silver sulfadiazine cream was used for the antibacterial treatment of burns. With the advent of the antibiotic age, the popularity of silver as an antibacterial agent gradually decreased. However, in later periods advances in modern science strengthened the role of silver as a highly important material in many areas [23].

Despite the very popular positive perception of the attractive properties of nanosilver, the toxicology and health hazards resulting from the use of silver nanoparticles should also be understood and emphasized. The toxicity of silver has been known for centuries [24]. Scientists have claimed that the use of nanosilver may cause side effects, and the evaluation of silver's toxicity requires a profound knowledge of its metabolism and decomposition in living organisms [6]. Unfortunately, toxicology aspects are frequently overlooked or ignored [25]. Recent *in vivo* and *in vitro* studies demonstrated that nanosilver absorbed via the skin or inhaled may cause negative health effects, and the use of medicinal products containing silver nanoparticles may increase the risk of chronic diseases [26, 27]. For that reason world supervisory authorities and scientific public information centres began to focus their attention on the potential harmful effects of nanocompounds. The *US Environmental Protection Agency* (USEPA) has taken action in order to explain the reasons for adding nanosilver to various consumer products available in supermarkets. Concerns regarding the uncontrolled effects of nanotechnology have lead to the establishment of environmental and political organizations whose operation is focused on reducing the use of silver nanoparticles, the introduction of stricter regulations and, in extreme cases, a total ban on the use of nanosilver [6]. A more detailed understanding of the nature of the emerging hazards caused by nanotechnology will enable everybody concerned to make reasonable decisions concerning the use of nanosilver and other nanomaterials.

Benefits of nanosilver applications

The human body is frequently exposed to microorganisms such as bacteria, fungi, yeast and viruses. A considerable number of materials containing organic and nonorganic substances with potential antibacterial properties have been analysed. Silver nanoparticles, characterised by specific surface and a large proportion of superficial atoms, have been thoroughly investigated owing to their unique physical and chemical properties. Research has demonstrated that silver ions have a remarkably strong bacteriostatic effect. The results of recent studies demonstrated that metal binds with microbial proteins in the reaction with a thiol group (-SH), which leads to protein inactivation. This process is considered the most important among all others resulting in protein inactivation.

In their paper Cho et al presented [28] the effect of nanosilver against *Staphylococus aureus* and *Escherichia coli*. In an experiment they used two solutions of colloidal 10 nm nanosilver stabilised with *poly-*(*N-vinyl-2-pyrrolidone*) (PVP) and *sodium dodecyl sulphate* (SDS). *S. aureus* and *E. coli* were used as Gram-positive and Gram-negative bacteria, respectively. It was found that PVP-stabilised nanosilver demonstrates especially strong antibacterial activity against both bacteria. Moreover, the nanosilver solution considerably inhibited the growth of both *S. aureus* and *E. coli*. The study demonstrated the lowest effective concentration of nanosilver solution to be 50 ppm for *Staphylococus aureus* and 100 ppm for *Escherichia coli.*

Yan et al have developed a method for the production of nanosilver-coated granules to be used as an antibacterial and antifungal agent. The product inactivates a broad spectrum of bacteria, including *Staphylococus aureus*, *Chlamydia trachomatis*, *Providencia stuarti*, *Vibrio vulnificus*, *Bacillus subtitilis*, and *Streptococus paratyphi* [29].

Building materials are a characteristic application of nanosilver as a biocidal agent. In their patent Horner et al reported that silver nanoparticles used as a component of building materials for roofing, insulation, cladding, wall lining, etc. considerably contributed to the inactivation of bacteria, fungi and harmful algae in comparison with silver-free materials

[30]. Kwon et al developed a paint formula containing 30 ppm silver nanoparticles, which is used for coating cast surfaces [31].

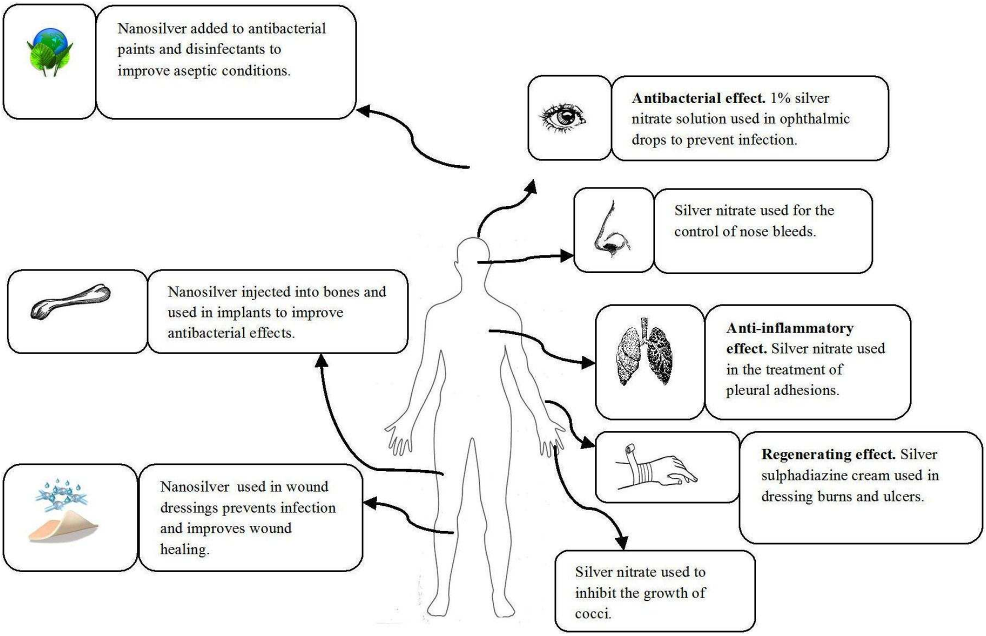
The application of nanosilver in medicine is a particularly important area. Roe et al reported the development of nanosilver-coated polymers which are used for plastic catheters. A coating of silver nanoparticles has an antibacterial and disinfecting effect. The *in vitro* tests confirmed growth inhibition in the bacterial layer which was sustained for 72 hours [32]. It is expected that after relevant studies on humans this method of using nanosilver will be introduced on a broad scale [9].

Nanosilver is also applied as an antibacterial additive for *poly*(*methyl methacrylate*) (PMMA), used in bone implants [33]. This concerns, for example, prosthetics of the knee or hip joint. Scientists have suggested that the use of elements made of nanosilver and PMMA will considerably reduce bacterial resistance to antibiotics administered during treatment [9].

Studies have also been conducted to explain the anti-inflammatory mechanisms of action for nanosilver. Anti-inflammatory properties of silver nanoparticles were evaluated in dermal contact in pigs. The study demonstrated that nanosilver significantly reduced inflammatory states, which was reflected in reduced levels of neutrophil infiltration found during biopsy [34].

There is also a dressing with nanosilver used on wounds and scalds. The hydrophilic coating formed by nanocomposite fibre with nanosilver incorporated in its structure comes into a direct contact with a wound or skin surface and its major purpose is disinfection and blood absorption [35]. Pictorial applications of nanosilver and silver nitrate in medicine are presented in Figure 1.

Another interesting application of silver nanoparticles is in the treatment of air from air-conditioning systems in meat processing plants. One of the problems faced by the meat industry is the contamination of products with microorganisms which migrate during slaughter, cold room storage or during meat processing. Microbial growth creates high risk and the air becomes a significant source of contamination, because proliferating microorganisms, mostly pathogenic, migrate mainly into the ambient air. There are various sources of contamination: natural, eg water, soil, putrefying vegetable or animal parts, and anthropogenic sources, eg storage of municipal waste and wastewater treatment. Air is the natural living environment for microorganisms, but because of various chemical and physical factors and the deposition of nutrients it becomes nothing more than the medium for the migration of microbes, not an ecological niche. Therefore, the air supplied to production halls, as one of the microbial risk factors, must be filtered, which also involves another risk of contamination, because filters operating under conditions favourable to microorganisms (moisture and high temperature) may be a site for their growth. This results in low quality air and unpleasant odours, leading to the proliferation of bacteria, fungi and mould on meat products. To counteract this problem a system was designed for the coating of filters with chemical substances characterised by a strong antibacterial activity. Nanosilver was used for this purpose, and studies demonstrated that filters with incorporated silver nanoparticles allow for the almost complete elimination of microorganisms from the air [36]. Also in this case, the antimicrobial efficiency of nanosilver was confirmed, and an additional advantage was the use of nanosilver in low concentrations, ie expressed in ppm, which results from its very large specific surface and high biological activity [36].



Nanosilver added to antibacterial paints and disinfectants to improve aseptic conditions.

Antibacterial effect. 1% silver nitrate solution used in ophthalmic drops to prevent infection.

Silver nitrate used for the control of nose bleeds.

Nanosilver injected into bones and used in implants to improve antibacterial effects.

Anti-inflammatory effect. Silver nitrate used in the treatment of pleural adhesions.

Regenerating effect. Silver sulphadiazine cream used in dressing burns and ulcers.

Nanosilver used in wound dressings prevents infection and improves wound healing.

Silver nitrate used to inhibit the growth of cocci.

Fig. 1. Applications of nanosilver and silver nitrate in medicine [9]

Another current problem is the optimisation of conditions under which farm animals are kept. More specifically this concerns gas emissions, particularly those generating odours, like ammonia. Gas emission sources include animal faeces and fodder, as well as process equipment. Unpleasant gases released from the ground remain in the air and in the vicinity of farms, which is problematic for the environment and for local inhabitants. This problem has lead to the development of a preparation containing nanosilver as the major component to eliminate both the ammonia emission and microbial growth [37].

For a long time nanosilver has been used in animal breeding as a component of disinfecting agents for transport chambers or facilities for keeping animals. Owing to the lack of information concerning the direct effect of nanosilver on animals, a laboratory study was performed to evaluate the level of ammonia emissions from ovine faeces after the use of a preparation comprising silver nanoparticles and a mineral sorbent. The study demonstrated that the use of the preparation reduced ammonia emissions from the ground. In addition, the study revealed that increasing the preparation dose did not increase its effectivity, which indicates the pointlessness of using higher doses. It is believed that the manufacture of preparations containing nanosilver and environmentally-friendly natural sorbent offers a promising perspective for counteracting ammonia emissions from animal faeces. In this case the biocidal properties of nanomolecular silver are an additional advantage, as they also prevent ammonia formation in the process of bacterial synthesis [37].

Nanosilver is used in agriculture to a wide extent because of its specific properties. Nia described a number of studies monitoring the reaction of plants after contact with colloidal nanosilver obtained by chemical reduction [38]. The study concerned testing a broad range of plant products, eg citrus and other fruits, cereals and olive trees. The experiment demonstrated that in all cases spraying nanosilver on plants resulted in their faster growth and the formation of longer roots. In addition, the size of leaves increased and they regenerated.

Progress in the germination of wheat grains after treatment with silver nanoparticles was the subject of a detailed study. For that purpose nanosilver solutions of 0, 20, 40, 60 and 80 ppm concentrations were used. After one week wheat grains grew by 70, 78, 90, 96 and 90% respectively. The study demonstrated that nanosilver significantly accelerates the germination of wheat grain. Similar experiments were also carried out on rape, cotton and maize grains, and the results were comparable. Another study investigated the reaction of olive trees after the immersion of juvenile plants in nanosilver solution and planting in a glasshouse. The study demonstrated that the use of nanomolecular silver solution reduced the incidence of root diseases. Nia also reported that strawberry plants sprayed with nanosilver solution were resistant to diseases and their fruits and leaves were larger [38].

These examples demonstrate that the use of a colloidal nanosilver solution may considerably improve the growth and health of various plants.

Hazards associated with the use of nanosilver

Potential toxicity is the major feature of nanosilver raising concerns. Four aspects should be considered when analysing the emerging risk: hazard identification, toxicity assessment levels, exposure assessment levels, and hazard characteristics. Additional external factors may influence the toxicity of nanosilver, such as size, particle shape or deviations in the intensity of its biological activity [6].

The migration of nanostructures into the natural environment is one of the currently encountered problems. Depending on the type, nanoparticles may be absorbed by air, water or soil [39]. In each of these media, silver particles may remain for a long time without significant activity, or they can be absorbed by living organisms. In other words silver nanoparticles may create an ecotoxic hazard due to biodegradation, or as a compound accumulated in living organisms in the food chain [40]. The US Environmental Protection Agency reported that nanosilver migrates to the trophic chain from body care products containing silver nanoparticles, such as sunbathing creams, which easily migrate to water bodies; in this process nanosilver may be absorbed by living organisms [41].

Blaster and collaborators carried out an analysis and its results contributed to the assessment of the effects of nanosilver released from plastic materials on the ecosystem of the Rhine river [43]. The scientists demonstrated that silver nanoparticles were absorbed by sludge and wastewater, bringing a future risk of spreading on farming lands, which can consequently lead to bioaccumulation and toxicology hazard [1, 42]. Bilberg and collaborators studied the effects of nanosilver on respiration levels in Eurasian perch [43]. Fish gills are in direct contact with the ambient water, making them potentially exposed and vulnerable to suspended silver nanoparticles. Scientists investigated the effect of silver nanoparticles on oxygen consumption. Perch were exposed to concentrations of 63, 129 and 300 μg/l nanosilver, plus controls for comparison, which were fish not exposed to nanosilver. Oxygen consumption was measured by automated intermittent closed respirometry. Final findings demonstrated that exposure to nanosilver results in impairment of tolerance to hypoxia (oxygen shortage) [43]. *In vitro* studies demonstrated that silver nanoparticles also cause other toxic damage to cells originating from various living organisms (Table 1).

Table 1

Toxic effects of nanosilver on mammalian cells [4]

NAg size [nm]	Type of mammalian cells	Dose	Exposure time [h]	Observations
15	mouse spermatogonial stem cells	5÷10 μg/cm^3	24	reduced mitochondrial function, increased leakage of LDH (*lactic dehydrogenase* [44])
15	rat neuroendocrine cells	50 μg/cm^3	24	reduced mitochondrial function and dopamine level
7-20	human skin neoplastic cells	6.25÷50 μg/cm^3	24	reduced cellular function, oxidative stress, DNA fragmentation
100	human mesenchymal stem cells	2.5÷5 μg/cm^3	24	reduced chemotaxis of cells
5-10	human liver cells	0.5÷10 μg/cm^3	28	oxidative stress
25	human full thickness abdominal skin	0.46÷2.32 ng/cm^2	24	NAg particles are able to migrate via damaged skin to inside the cells
18	hamster liver cells	11 μg/cm^3	2.9	apoptosis

The negative effects of nanosilver on human health are a crucial problem. The use of nanosilver in body care products and fabrics essentially increases the exposure of human skin to it [4]. Kulthong et al demonstrated in their study on artificial human skin that silver may be transferred from deodorants to human sweat. The dynamics of nanosilver release are determined by its concentration in a product, product structure, pH value and sweat formulation [45]. Paddle-Ledinek et al investigated the interaction of keratinocytes with a wound dressing containing nanosilver. Study results demonstrated that the wound dressing containing silver nanoparticles had the strongest cytotoxic effect. In addition, Lam and collaborators reported that dressings containing nanocrystalline silver are cytotoxic to keratinocytes and fibroblasts and should not be used for local skin treatment. Moreover, it was found that fibroblasts are more sensitive to nanosilver than keratinocytes [4]. Arora et al concluded that silver nanoparticles caused cell necrosis and oxidative stress in human neoplastic cells. In another study the same team of scientists found that nanosilver migrated to cells and caused damage to DNA and apoptosis in liver cells [46]. Studies identified the liver and lungs to be the major organs prone to the effects of silver nanoparticles. Hussain et al, in their studies on rat liver cells, demonstrated that nanosilver caused considerable deterioration in cell structure [47]. The results exhibited, eg reduced mitochondrial membrane potential. These findings provide convincing evidence that nanosilver cytotoxicity potentially contributes to the development of oxidative stress in liver cells. A study was carried out using a non-cytotoxic dose of nanosilver <0.5 μg/cm^3. Results demonstrated that even this concentration caused the expression of genes responsible for the apoptosis of human liver cells. Therefore it can be concluded that nanosilver is a harmful substance even if the exposure dose is not cytotoxic [4].

Experiments investigating the effect of inhaled nanomaterials demonstrated that lungs are an easy target for nanosilver, which may additionally migrate via the nasal pathway to the brain. However, there is a limited amount of evidence concerning toxic effects on lung cells [48].

Nanosilver may also have a negative effect on the human reproductive system. This is possible due to the use of various commercially available products, such as intimate body care preparations. Braydich-Stolle et al demonstrated that nanosilver has a toxic effect on germinal stem line cells, causing reduced mitochondrial function and permeability of the cellular membrane [4].

The advantages of nanosilver used in building materials [30] were analysed from the different angle of negative effects of silver nanoparticles in the construction industry. In their study Kaegi et al evaluated the levels of released metallic silver nanoparticles from paints coating the facade of a building which was specially designed for the experimental purpose [49]. The facade was exposed to natural weather conditions for one year. The core of the experiment was to measure the amount of nanosilver contained in run-off water surrounding the building after rainfall. A strong leaching of silver nanoparticles was observed at the initial stage of the experiment. The maximum measured concentration of nanosilver was 145 $\mu g/dm^3$. It was found that after 12 months approx. 30% of nanosilver particles size <15 nm had migrated into the environment from the coating.

Conclusions

The properties of nanosilver are very important in industry, medicine and other disciplines where biocidal activity plays a significant role. Unfortunately, studies demonstrated that nanosilver also creates a hazard to human health. In addition, silver nanoparticles also create a toxicology hazard to the natural environment and living organisms.

The objective assessment of silver's benefits and potential hazards resulting from its use can certainly help in making reasonable choices and key decisions in various areas of nanotechnology. This should lead to the full exploitation of the bacteriocidal and fungicidal properties of silver in concentrations which are non-toxic to humans and animals.

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NANOSREBRO - PODEJMOWANIE TRUDNYCH DECYZJI

Instytut Chemii i Technologii Nieorganicznej, Wydział Inżynierii i Technologii Chemicznej
Politechnika Krakowska

Abstrakt: Nanotechnologia jako szybko rozwijający się obszar nauki jest rozpowszechniona w różnych dziedzinach życia na całym świecie. Jednym z przykładów nanosubstancji jest nanosrebro, które wykorzystuje się w medycynie, elektronice, budownictwie, technologii chemicznej oraz jako wyjątkowo skuteczny środek przeciwbakteryjny i antygrzybiczy. Od wieków znane są jego właściwości biobójcze, które są bardzo pożądane przy tworzeniu warunków aseptycznych. Jednak zaobserwowano wręcz toksyczne w skutkach działanie nanosrebra. Może ono kumulować się w łańcuchu pokarmowym, co stwarza ryzyko bezpośredniego oddziaływania na organizmy żywe. Co więcej, nanosrebro może spowodować obumieranie tkanki ludzkiej, a także zaburzyć działanie elementarnych składników komórek człowieka. Poniższe opracowanie ma na celu przedstawienie korzyści i szkód płynących ze stosowania nanosrebra.

Słowa kluczowe: nanosrebro, dezynfekcja, aktywność antymikrobiologiczna, nanotoksykologia

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ANTIBACTERIAL ACTIVITY AND SCANNING ELECTRON MICROSCOPY (SEM) EXAMINATION OF ALGINATE-BASED FILMS AND WOUND DRESSINGS

WŁAŚCIWOŚCI ANTYBAKTERYJNE I SKANINGOWA MIKROSKOPIA ELEKTRONOWA (SEM) W BADANIACH FILMÓW ALGINIANOWYCH I OPATRUNKÓW AKTYWNYCH

Abstract: Natural polymers widely used to produce drug carriers and active dressings include alginates, gelatine, chitosan and hyaluronic acid. In this study, alginate films were obtained bypassing the process of lyophilization. Produced from the common bladder wrack (*Fucus vesiculosus* L.), they can be used in food chemistry, wound treatment (tissue infections, burns) and in skin care; they are also good drug carriers. The films were examined for their bacteriostatic effects. Alginate gels exhibit bacteriostatic properties against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Tests conducted for active dressings revealed that Aquacel Ag and gauze soaked with 1% $AgNO_3$ exhibit bacteriostatic properties against *E. coli* and no resistance against *S. aureus*. Examination using scanning electron microscopy (SEM) confirmed the bacteriostatic properties of the Aquacel Ag dressing and gauze with 1% $AgNO_3$. Dead cultures of *Staphylococcus aureus* were observed on the fibre surface of both dressings.

Keywords: antibacterial, scanning electron microscopy, alginate films, wound dressings

Alginate, a polysaccharide extracted from marine brown algae (*Phaeophyceae*), is a common type of gelling agent employed in the food industry [1, 2]. It is of interest as a potential film or coating component because of its unique colloidal properties. These include thickening, stabilizing, suspending, film forming, gel producing and emulsion stabilizing properties [3, 4].

Microbial growth on food surfaces is a major cause of food spoilage. In particular, bacterial contamination of ready-to-eat products is of serious concern to human health. Antimicrobial agents used in food application include organic acids, bacteriocins, enzymes, alcohols and fatty acids [5]. The beneficial effects obtained by using edible film and coating in terms of physical, mechanical and biochemical benefits have been reported [6]. Edible

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films can improve shelf life and food quality by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation and losses of volatile aromas and flavours [7, 8]. Their use is gaining importance in food protection and preservation as they provide advantages compared with films made from synthetic materials [9]. Addition of hydrocolloids such as alginate may improve the barrier and tensile properties of fruit-based films [1].

Biopolymers from marine sources have also been studied and utilized in pharmaceutical and biotechnological products. Carrageenans are watersoluble galactose polymers extracted from red seaweed, which are extensively used in food and pharmaceutical industries as gelling and stabilizing agents. Carrageenan has one negative charge per disaccharide with a tendency to form excellent gel and film forming properties, and exhibits the highest tensile strength. Alginic acid is a copolysaccharide extracted from brown algae consisting of D-mannuronic and L-guluronic acid monomers. The ability of alginates to react with di- and trivalent cations is being utilized in alginate film formation. Na-alginate is a water soluble salt of alginic acid, a naturally occurring non-toxic polysaccharide. With regard to the film forming properties of Na-alginate and k-carrageenan, numerous controlled or sustained-delivery systems have been described in literature [10-13] whereas the activity of these biopolymers as antimicrobial agents in such films has not been thoroughly researched.

Many modern wound dressings have a variety of properties that are designed to create an environment to encourage conditions that support wound healing. These include the ability to absorb exudate, provide optimum moisture balance at the wound surface and prevent maceration of surrounding tissue. Since bacteria are often present in high numbers in wound fluid, it is also important that dressings with high fluid retention levels be able to absorb and retain bacteria. Once the skin barrier is broken, there is a much greater risk for infection as the majority of wounds provide a favourable environment for both aerobic and anaerobic bacteria [14]. It is self-evident that if a wound is bacteria-free then infection cannot occur. The prevention of wound infection and a reduction in cross-infection of wound pathogens are primary concerns in infection control.

Lawrence [15] demonstrated that the dispersal of bacteria was reduced by 20% when a moisture retentive hydrocolloid was removed from a simulated wound, compared with a gauze dressing. If a wound remains unhealed for more than six weeks, it becomes chronic and in these cases complications become more frequent because of bacterial colonization [14]. Some of these dressings are medicated, containing active substances to manage the microbial load in the area of the wound. Typical actives include antibiotics (such as neomycin, bacitracin or polymyxin combinations) and broad-spectrum germicidal agents (silver, iodine, chlorhexidine, etc.).

Silver in its numerous forms, including metallic silver, silver nitrate, silver sulfadiazine and silver calcium phosphate, has been used for over 200 years in the treatment of burn injury [16]. It is effective against a broad range of aerobic, anaerobic, Gram-negative and Gram-positive bacteria, especially *Staphylococcus aureus*, *S. epidermidis* and *Klebsiella pneumoniae*, as well as yeast, filamentous fungi and viruses [17]. Silver nitrate has been used historically as an antiseptic agent. In concentrated form, it is highly toxic to tissues. However, an aqueous solution of 0.5% silver nitrate offers significant antimicrobial activity without tissue toxicity.

It is very important to investigate the possibility of producing antimicrobial alginate film by incorporation of $AgNO_3$ and H_4SiO_4. The objective of this study was to assess the antibacterial activity of alginate films and wound dressings against the pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus.*

Experimental

Materials

Film preparation

Films were extracted from the common bladder wrack (*Fucus vesiculosus* L.), which belongs to marine brown algae. Weeds from two producers were used for examination, packed and distributed by the Zakład Konfekcjonowania Ziół Flos, Morsko, Poland and by the Witherba S.C. Zioła, Piotrkow Trybunalski, Poland.

Dried *Fucus vesiculosus* L. was dipped in an aqueous formaldehyde solution (3.7%, 100 cm^3) and kept in a closed flask at 30°C overnight to remove lipophyllic compounds. The suspension was stirred and extracted at 80°C for 4 hours with 0.1 M HCl (100 cm^3), 1 M HCl (100 cm^3), 1% HCl (100 cm^3) or 0.085 M Na_2CO_3. Then it was filtered through the Büchner funnel. The filtrate was vacuum-condensed to about ¼ volume. During and just before the end of condensation, the solution was rinsed with a small amount of 96% ethanol. The gelling precipitate was poured onto Petri plates. Some films were poured over with 10% $CaCl_2$, taken out and dried. All films were dried overnight on the plates at 25°C or 50°C.

Also examined were 100% sodium carboxymethylcellulose commercial active dressings: Aquacel (ConvaTec) and Aquacel Ag (ConvaTec), as well as cotton or viscose gauze soaked with 1% $AgNO_3$ or 1% H_4SiO_4.

Antimicrobial treatment

Two kinds of alginate gels, produced from the common bladder wrack by Witherba and Flos, were examined. They were exposed to bacteria that can cause nosocomial infections, that is the Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli.*

Physiological salt (2 cm^3) was poured into two sterile test tubes. Using a sterile (red hot) inoculation loop, *E. coli* sample was taken from its culture on enriched agar (used for growing particularly demanding bacteria strains), inserted into one of the test tubes and diluted in the salt. Using a pipette, 3 drops of the suspension were transferred onto enriched agar; then, using a cooled sterile bacteria spreader, they were spread all over the agar surface. After that, the spreader was sterilized again and 3 drops of alginate gel were placed in the centre of the Petri plate, using a dropper. The same procedure was repeated for *Staphylococcus aureus*, which was placed on mannitol salt agar (containing 7.5% NaCl for inhibiting growth of other bacteria). The Petri plates were subsequently placed in a tube and then kept in a laboratory heater at 37ºC for 24 h and 48 h.

Dressing materials were exposed to the same bacteria as the alginate gels: *Escherichia coli* on MacConkey agar (containing salts of bile acids and crystal violet inhibiting growth of Gram-positive bacteria) and *Staphylococcus aureus* on mannitol salt agar (with high concentration of NaCl inhibiting growth of other bacteria). The samples were kept in a laboratory heater at 37°C for 24 h, and then photos were taken.

Scanning electron microscopy (SEM)

Fibre surface was also examined using a JSM 5500LV scanning electron microscope supplied by JEOL. Secondary electrons (SE) and back-scattered electrons (BSE) observations were conducted, with the accelerating voltage of 5 kV. Microphotographs were taken at magnifications ranging from 500× to 10,000×.

Results and discussion

The objective of this study was to produce from the common bladder wrack (*Fucus vesiculosus* L.), bypassing the process of lyophilization, a series of alginate films and to select a gel exhibiting good antibacterial effects. Figure 1 shows some examples of such films.

Fig. 1. Examples of alginate films: 1.5% alginic acid gel (a); non-defatted alginate film (b); defatted alginate film (c and d)

Antimicrobial activity of alginate films

Corrected zone of inhibition tests were used to determine the antimicrobial activity of the alginate films. They proved that both Flos and Witherba gels inhibit growth of Gram-negative *Escherichia coli*. There were no inhibition zones in samples of

Staphylococcus aureus. Results of these observations are shown in Table 1 and, as an example, Figures 2 and 3.

Table 1

Antibacterial activity of alginate films

Samples	Bacteria	Inhibitory zone
Flos	*Escherichia coli*	+
Flos	*Staphylococcus aureus*	-
Witherba	*Escherichia coli*	+
Witherba	*Staphylococcus aureus*	-

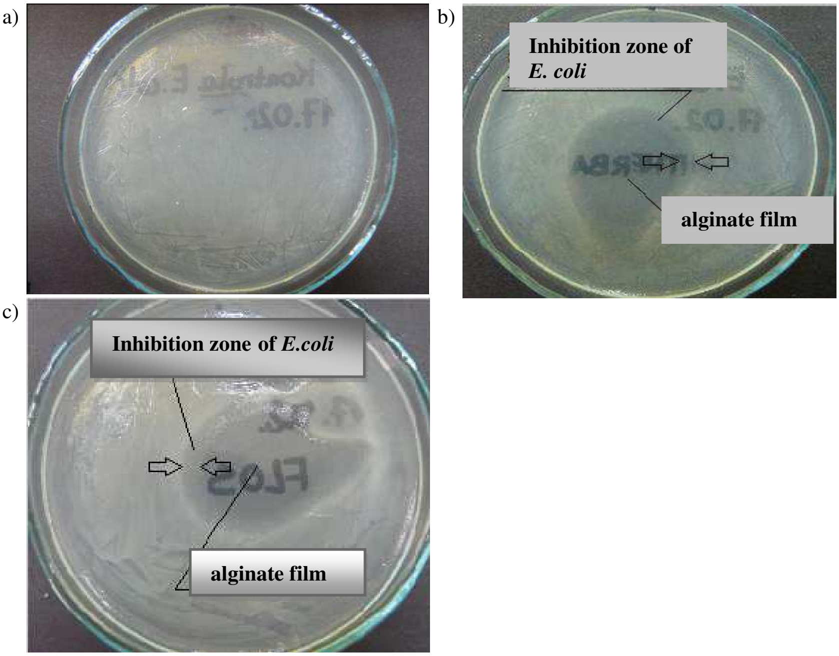

Fig. 2. Inhibition zone of Witherba (b) and Flos (c) alginate films compared with reference (a) against *Escherichia coli* (24 h incubation)

Table 2

Antibacterial activity of wound dressings

Samples	Bacteria	Inhibitory zone
Aquacel (1)	*Escherichia coli*	–
Aquacel (1)	*Staphylococcus aureus*	–
Aquacel Ag (2)	*Escherichia coli*	+
Aquacel Ag (2)	*Staphylococcus aureus*	+
Gauze + 1% H_4SiO_4 (3)	*Escherichia coli*	–
Gauze + 1% H_4SiO_4 (3)	*Staphylococcus aureus*	–
Gauze + 1% $AgNO_3$ (4)	*Escherichia coli*	+
Gauze + 1% $AgNO_3$ (4)	*Staphylococcus aureus*	+

A parallel examination of commercial active dressings was also conducted in order to compare the results with those of examining the antibacterial activity of the alginate films. Both results are shown in Table 2 and Figure 4.

Fig. 3. Inhibition zone of Flos (b) and Witherba (c) alginate films compared with reference (a) against *Escherichia coli* (48h incubation)

The antibacterial tests proved that Aquacel Ag and gauze soaked with 1% $AgNO_3$ inhibit Gram-negative *Escherichia coli*. Inhibition zones appeared along the edges of the dressings. For neither Aquacel Ag nor gauze soaked with 1% $AgNO_3$, there were any such zones against *Staphylococcus aureus.*

Silver has found particular application in medicated wound dressings as it shows broad antimicrobial (against both Gram-negative and Gram-positive organisms) and anti-fungal activity [18]. It has been suggested that alginate wound dressings may immobilize bacteria within their fibrous matrix. Bowler et al [19] have demonstrated that phenomena whereby a hydrated carboxymethylcellulose (AQUACELs Hydrofibers) wound dressing immobilized exudate containing bacterial populations within its cohesive gel structure. The composition, the arrangement of fibres, the fibre density and the relative proportion of individual groups (for example, guluronic and mannuronic acid groups which form fibres in alginate wound dressings) are important considerations and can influence the ability of a dressing to handle large volumes of exudate by absorbing and retaining microorganisms.

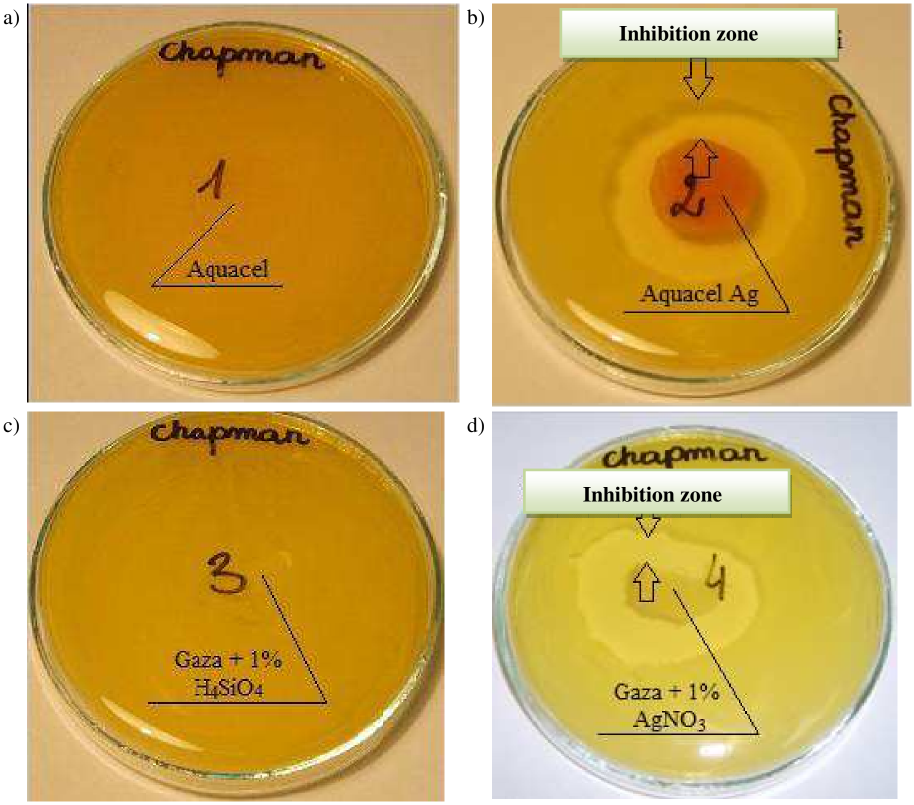

Fig. 4. Inhibition zone of Aquacel Ag (2) and gauze + 1% $AgNO_3$ (4) compared with Aquacel reference (1) and gauze + 1% H_4SiO_4 (3) against *Escherichia coli*

Several proposals have been developed to explain the inhibitory effects of Ag^+ ions on bacteria. In aqueous environments, silver ions are released and antimicrobial activity depends on the intracellular accumulation of their low concentrations. The ions avidly bind to negatively charged components in proteins and nucleic acids, thereby effecting structural changes in bacterial cell walls, membranes and nucleic acids that affect viability. In particular, silver ions are thought to interact with thiol groups, carboxylates, phosphates, hydroxyls, imidazoles, indoles and amines, either singly or in combination, so that multiple deleterious events rather than specific lesions simultaneously interfere with microbial processes [20]. Microbiological and chemical experiments imply that the interaction of Ag^+ ions with thiol groups plays an essential role in bacterial inactivation [21].

Perhaps the most unique form of silver developed for wound dressings is nanocrystalline silver, which differs in both physical and chemical properties from micro- or macrocrystalline silver and from silver salts. A unique property of nanocrystalline silver is that, according to Fan and Bard [22], it dissolves to release Ag^0 clusters and Ag^+, whereas other silver sources release only Ag^+. This difference in the dissolution properties of nanocrystalline silver dressings appears to alter the biological properties of the solution, including both antimicrobial and anti-inflammatory activity. Nanocrystalline silver dressings have been demonstrated in vitro as effective antifungal agents, antibacterial agents [23] and antibacterial agents for antibiotic-resistant bacteria [24]. In vivo studies have shown that nanocrystalline silver is very effective at preventing infections and healing wounds [25].

Cotton and viscose gauze, although still much in use for acute wounds, has largely been replaced for the treatment of chronic wounds by modern wound dressings produced from alginate and, more recently, fibres made from carboxymethylated cellulose (NaCMC fibre, Hydrofibers, Aquacel ConvaTec Ltd). *Sodium carboxymethylcellulose* (NaCMC) fulfils these criteria, forming a soft gel or viscous solution with wound fluid. This gel-forming property observed for NaCMC has led to its use for wound care, in gel formulations and in hydrocolloid dressings that contain NaCMC in an insoluble matrix. Fibrous dressings made from alginate combine the properties of fluid absorbency and gel formation and some also retain their integrity so that they can be readily removed. The presence of the methyl carboxyl group (-CH_2COO-) in carboxymethyl gauze permits further chemical modification by partial cation exchange of sodium by silver to develop a post-treated antimicrobial product [26]. Silver cations are microcidal at low concentrations and are used to treat burns wounds and ulcers. These new dressings contain various forms of silver, ranging from pure metallic silver (Silverlon) to compounds such as silver carboxymethylcellulose (Aquacel Ag), silver phosphate (Arglaes) and silver chloride (Silvasorb) [27].

Scanning electron microscopy (SEM)

Scanning electron microscopy has demonstrated the immobilization of bacteria within Hydrofibers [28]. Therefore, the aim of this study was to develop a suitable SEM technique that would allow for the visualization of bacteria within fibrous wound dressings.

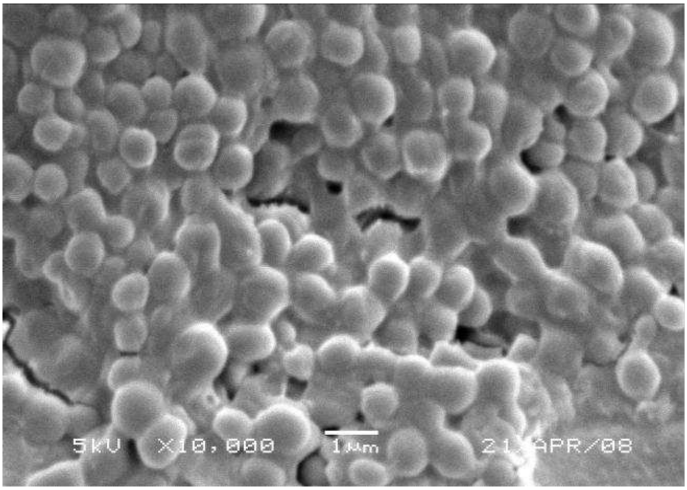

Fig. 5. *Staphylococcus aureus* adhering to the surface of Aquacel fibres

A key challenge for maximizing the healing response in wounds has been the advent of modern fibrous dressings to absorb large volumes of exudate, while still providing moisture balance in the wound environment [29]. Equally important, however, is the ability of these dressings to immobilize wound exudate, which may contain pathogenic bacteria (such as *P. aeruginosa* or *Staphylococcus aureus*). Bowler et al [19] have suggested that as Hydrofibers dressings absorb wound exudate, this in turn reduces the interstitial spaces between individual fibres within the dressing as they coalesce, resulting in bacterial immobilization. Subsequent studies using SEM have confirmed these findings [28].

In this study, since there were no inhibition zones against *S. aureus* in any of the samples examined, fibre surface was investigated using SEM. The results are shown in Figures 5-7. In Figure 6, *S. aureus* cultures fill the interstitial spaces between individual fibres of the Aquacel dressing and grow on their surface.

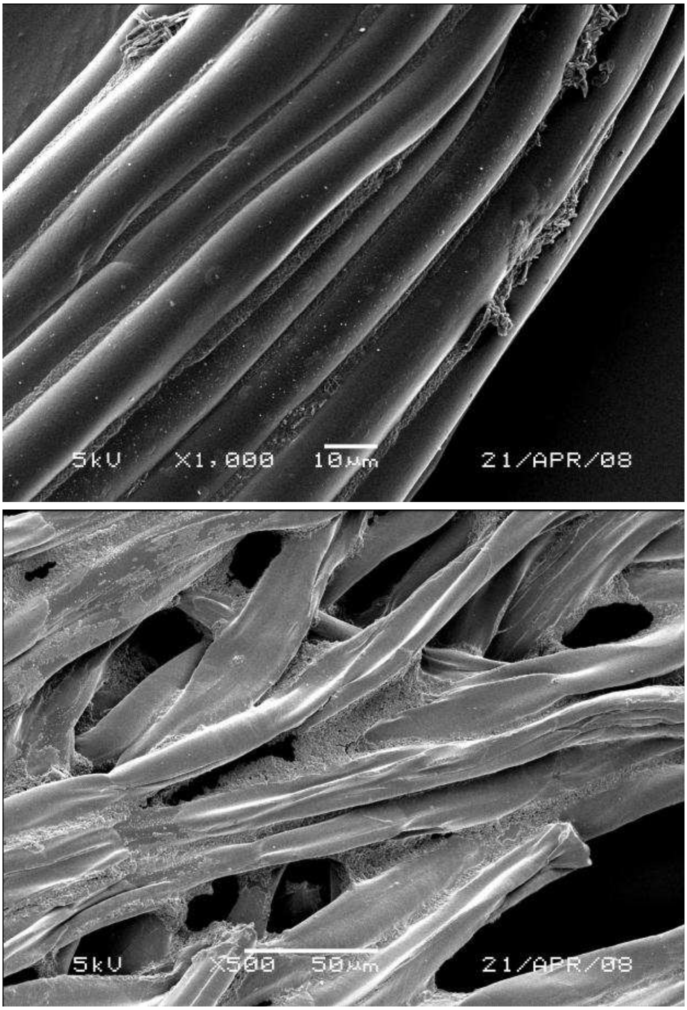

Fig. 6. Bacteria adhering to the surface of Aquacel fibres

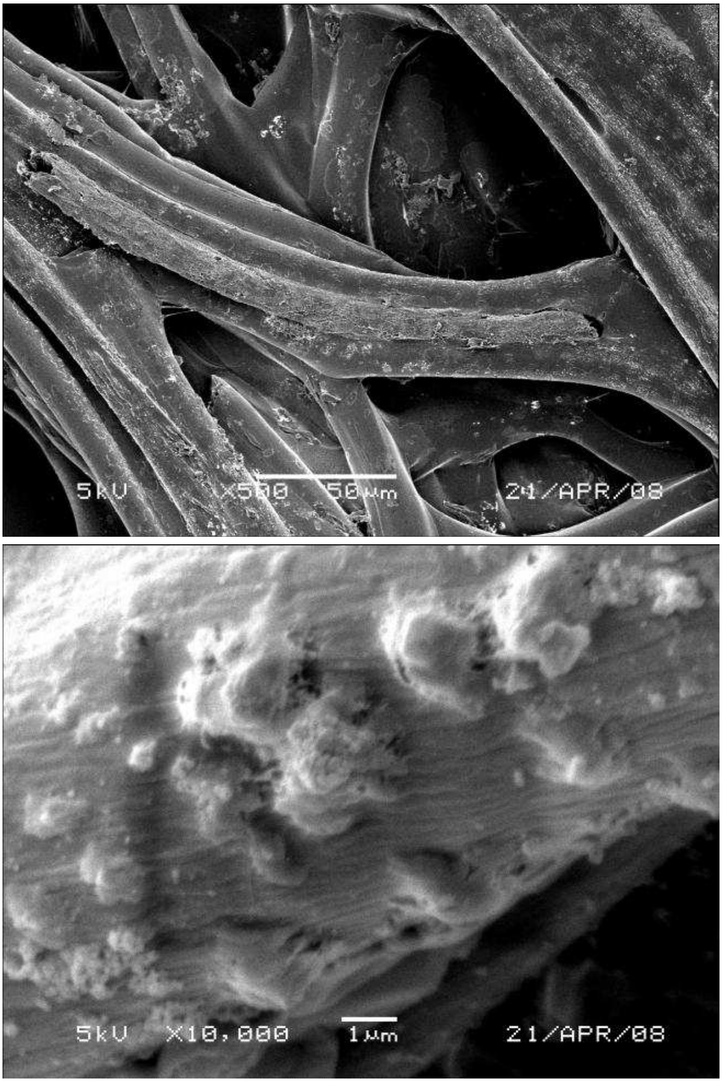

Fig. 7. Bacteria adhering to the surface of and filling the spaces between Aquacel fibres

Figure 7 proves that the Aquacel Ag dressing is effective against Gram-positive bacteria (as it releases bioactive Ag^+ cations). The amount of the bacteria growing on the fibre surface is substantially lower and degraded cultures of *Staphylococcus aureus* can also be seen. One possible explanation for this could be related to the presence of a thinner cell wall in Gram-negative bacteria compared with the much thicker and more complex cell wall present in Gram-positive bacteria. Feng and Kim [30] have suggested that the presence of the thicker cell walls "is of immense practical importance in protecting the cell from penetration of silver ions into the cytoplasm". Studies are under way to examine the effects of ionic silver on the bacterial cell wall. Generally, the bacteria closest to the Aquacel Ag hydrating fibres interact with available silver ions first and the bactericidal effect continues as bacterial suspensions move along the fibres throughout the extended experimental periods (Fig. 7).

Earlier it has been demonstrated that both the alginate films produced for this study and the commercial active dressings of the Aquacel Ag type, as well as gauze soaked with a solution of silver nitrate, exhibit bacteriostatic properties. The results of these examinations are shown in Figures 2-4. Bacteriostatic effects of gauze soaked with 1% $AgNO_3$ and H_4SiO_4 were also observed. The results are shown in Figures 8-10.

Gauze soaked with 1% H_4SiO_4 (Fig. 8) reveals the presence of *Staphylococcus aureus* growing on its surface while gauze soaked with 1% $AgNO_3$ (Fig. 9) is more inhibitory to this growth. Gauze soaked with 1% $AgNO_3$ (Fig. 10) exhibits, in the same way as the Aquacel Ag dressing, bacteriostatic properties, as confirmed by the dead bacteria cultures.

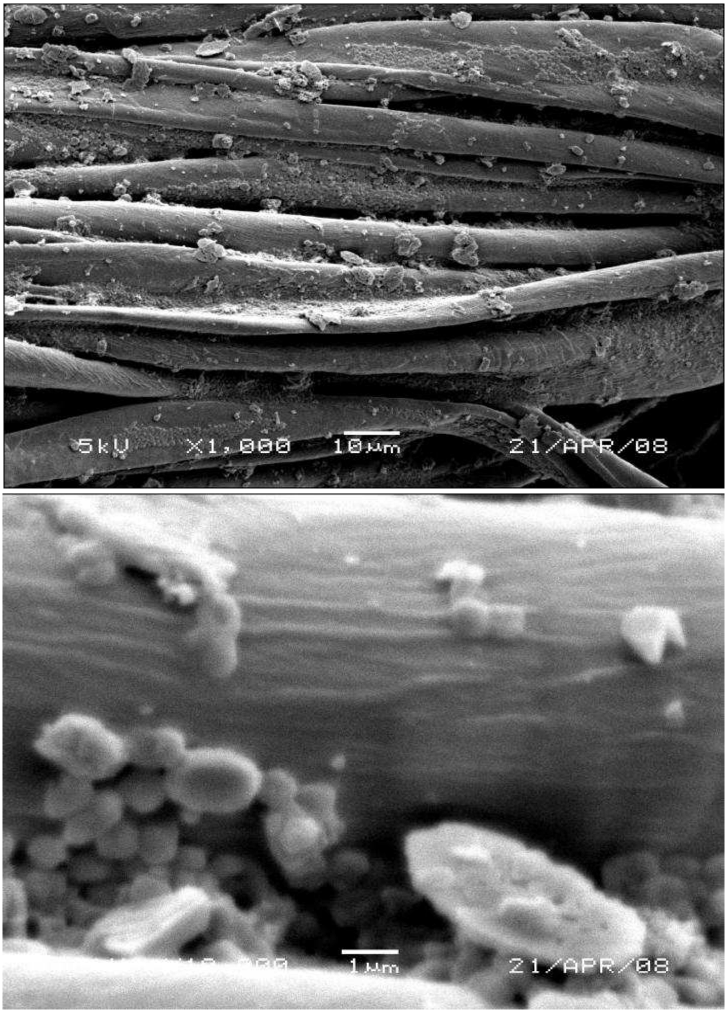

Fig. 8. Bacteria adhering to the surface of cotton gauze fibres

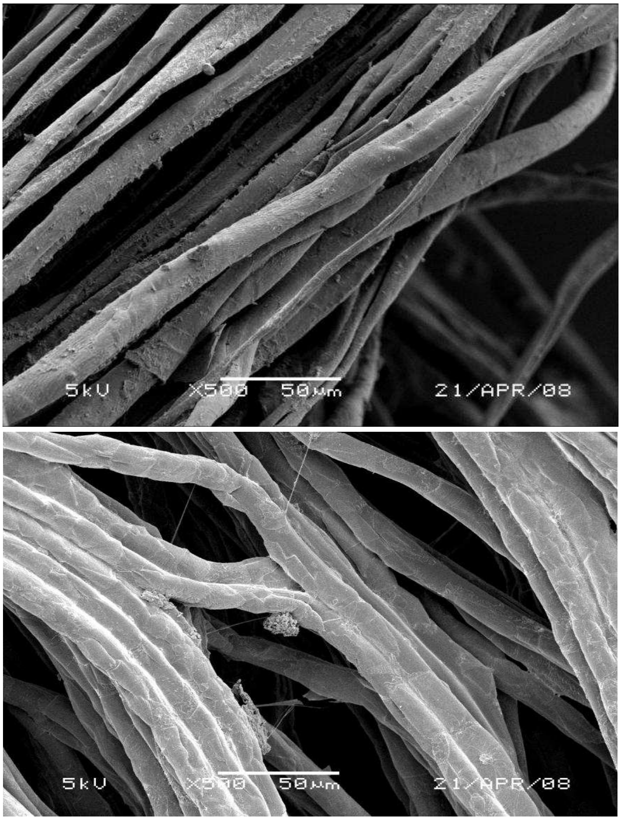

Fig. 9. Bacteria adhering to the surface of cotton gauze fibres

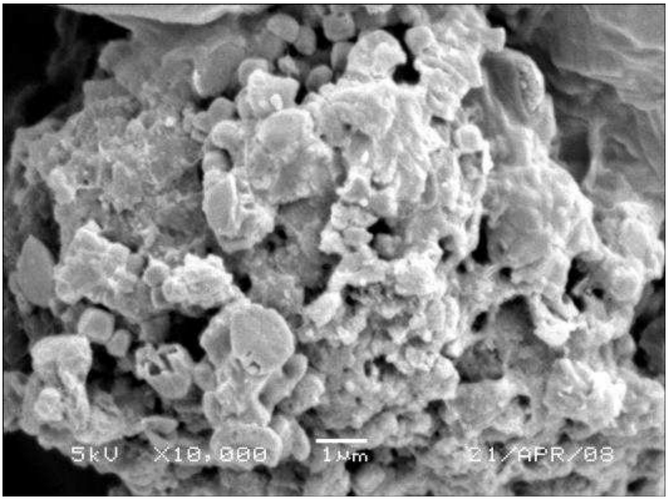

Fig. 10. Bacteria adhering to the surface of cotton gauze fibres

To summarize these findings, it should be said that the alginate films exhibit bacteriostatic properties against Gram-negative bacteria *Escherichia coli* and no resistance against Gram-positive *Staphylococcus aureus*. Tests with inhibitory spaces conducted for active dressings show that Aquacel Ag and gauze soaked with 1% $AgNO_3$ have bacteriostatic effects against *E. coli* and no resistance against *Staphylococcus aureus*. Compared with alginate gels, Aquacel Ag and gauze soaked with 1% $AgNO_3$ offer more effective antimicrobial activity, as demonstrated by larger inhibition zones around the dressings. SEM examination has confirmed the antibacterial properties of the Aquacel Ag dressing and gauze soaked with 1% $AgNO_3$. Dead cultures of *Staphylococcus aureus* have been observed on the surface of both dressings. Aquacel and gauze soaked with 1% H_4SiO_4 have proved to offer no resistance against *E. coli* and *S. aureus*.

Conclusions

The alginate films/gels produced from the common bladder wrack (*Fucus vesiculosus* L.), bypassing the costly process of lyophilization, exhibit good antibacterial effects. These gels can be used in food chemistry, pharmacy, medicine and cosmetology along with the widely produced active dressings.

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WŁAŚCIWOŚCI ANTYBAKTERYJNE I SKANINGOWA MIKROSKOPIA ELEKTRONOWA (SEM) W BADANIACH FILMÓW ALGINIANOWYCH I OPATRUNKÓW AKTYWNYCH

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Abstrakt: Naturalne polimery są szeroko używane do opatrunków aktywnych na bazie: alginianów, żelatyny, chitozanu i kwasu hialuronowego. W przedstawionych badaniach błonę alginianową otrzymuje się, omijając liofilizację. Żel otrzymano z morszczynu pęcherzykowatego (*Fucus vesiculosus* L.) wykorzystywanego powszechnie w chemii żywności, leczeniu ran (zakażeniach tkanek, oparzeniach), pielęgnacji skóry oraz jako nośnik leków. Badane żele alginianowe wykazywały właściwości bakteriostatyczne w stosunku do bakterii Gram-dodatnich *Staphylococcus ureus* oraz bakterii Gram-ujemnych *Escherichia coli*. Testy przeprowadzone dla aktywnych opatrunków wykazały, że Aquacel Ag i gaza nasączona 1% $AgNO_3$ wykazują właściwości bakteriostatyczne w stosunku do *S. ureus* i *E. coli*. Badanie za pomocą skaningowego mikroskopu elektronowego (SEM) potwierdziło właściwości bakteriostatyczne Aquacel Ag i opatrunku z gazy nasączonym 1% $AgNO_3$. Na powierzchni włókien obu opatrunków zidentyfikowano obumarłe kultury *S. aureus* i *E. coli*.

Słowa kluczowe: właściwości antybakteryjne, skaningowy mikroskop elektronowy, błona alginianowa, opatrunki

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MICROBIOLOGICAL HAZARD TO THE ENVIRONMENT POSED BY THE GROUNDWATER IN THE VICINITY OF MUNICIPAL WASTE LANDFILL SITE

ZAGROŻENIA MIKROBIOLOGICZNE DLA ŚRODOWISKA POWODOWANE PRZEZ WODY PODZIEMNE W STREFIE ODDZIAŁYWANIA SKŁADOWISKA ODPADÓW KOMUNALNYCH

Abstract: In the area and environs of the municipal waste dump Barycz in Krakow, all analyzed groundwater samples, taken from piezometers C-1, P-3, P-6, P-8 and G revealed occurrence of bacteria - indicators of bad sanitary conditions. Even periodical pollution of groundwater results in its bad quality. Presence of fecal Coliform bacteria indicates on anthropogenic origin of the groundwater contamination. The number of cfu of fecal Coliform bacteria in water samples taken from piezometers was decreasing with growing distance from the waste dump borders. However, even in a 1230 m distance north from borders of the waste dump in the piezometer G, in spite of the absence of fecal Coliform bacteria, bad sanitary condition was ascertained, according to other microbiological indicators.

Keywords: microbial contamination, groundwater, landfill leachate

Landfilling is still the most often used method for solid waste disposal in Poland. However, this method is associated with several problems. Among these are pollution of air, soil and groundwater, high cost associated with landfill operation and than with their closing and remediation. An important issue is leachate which is both chemically and microbiologically contaminated [1, 2]. Landfill leachate consists of the liquid generated by the breakdown of waste, and the infiltrating precipitation [3]. A broad range of xenobiotic compounds occurring in leachate can be linked to household hazardous waste deposited in the landfill [4]. The environmental impacts caused by municipal solid waste degradation are mainly associated with liquid and gaseous emissions of heavy metals and other toxic chemicals [5]. The landfill age has a significant effect on leachate composition especially on organics and ammonia concentrations [6]. According to Christensen et al [7] landfill

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leachates may contain 1000÷5000 higher concentration of dissolved organic matter and inorganic macrocomponents than groundwater. The quality of leachate is determined primarily by the composition and solubility of the waste constituents [8].

Microbiological pollutants may largely affect surface water quality eg eutrophication processes and may pose threat to life or health through proliferation of pathogenic microorganisms. The main threat to animal and human health poses the direct contact of leachates and surface waters [9]. Landfills may contain pathogenic and opportunistic bacteria, due to the presence of domestic human origin waste [10, 11]. The fecal bacteria survival in municipal solid waste may be favored by constant moisture as well as high levels of organic matter that induce bacterial growth [12]. Because pathogenic bacteria may contaminate drinking water their presence must be controlled to avoid potential health hazards [12].

The effect of municipal landfill sites on ground and surface water is often various: among point polluters are leachates, surface runoffs, draining by underflow and aquifers, and waste leaching during rainfall flood. These factors can occur individually or in complex [13, 14]. Microbiological contamination may persist in the environment dozen years [1]. According to Marchwinska and Budka [15] the prolonged danger is caused by inevitable degradation of plastic membrane used to seal landfill base which causes pollutants infiltration into the ground. Floods, intensive downpours and snowfalls in recent years increases the threat of leachetes to environment. The most dangerous are landfills with damaged basal sealing, where pollutants may infiltrate deep into the ground and at long distance [16, 17].

The disturbance of homeostasis caused by pollutants leads to activation of biological regulation mechanism. The qualitative and quantitative dominance of microorganisms is changing during the process. The homeostasis is retain when the concentration of pollutants are kept below the threshold of biocenosis regulatory potential. Destruction of the biotic elements of the system leads to total inhibition of autoregulatory processes [18]. However, landfill sites can be good habitats if plants and animals can tolerate leachate contamination [19].

Influence of a waste storage process on atmospheric air pollution was evaluated in many analyses [20]. Fewer analyses concerned soil, water and soaking water. These analyses mainly considered chemical pollution, ignoring microbiological aspect. The reason for such state is the lack of international legal directives and commonly obeyed and unitary methods of evaluation of waste dumps effect on the environment and people’s health [21, 22]. However public concerns and awareness regarding environmental issue have grown in most countries [23]. Moreover, it is necessary to perform proper evaluation methods of risk combined with municipal waste dumps. An integrated risk assessment methodology for landfill gas, leachate or degraded waste was no developed yet [23].

Microbiological contamination of groundwater by the waste dump is the constant threat towards its quality and depends on the waste composition and the load of waste, technological infrastructure for the waste intakes, isolation properties of the ground (depending on the natural ground structure and applied technical protection) and also on environment’s capability to purify itself [9, 24]. The important element of the waste dump’s proper protection is the correct sewage treatment process. Groundwater is usually treated by mechanical filtration, biological breakdown and assimilation, sorption, ion exchange and dilution. When it comes to physicochemical properties of the leaching water, the method of reverse osmosis is of particular meaning. Treatment technology for leachate should be considered after fully understanding the composition and concentration of leachate [6]. Water need at least several years before they return to their natural properties [1, 25]. Sustainable, environmentally passive and inexpensive methods of landfill leachate treatment are in growing demand. Energy crops such as willows may offer a solution, fulfilling these criteria [3].

It is crucial to develop the proper methods of the risk evaluation related to the municipal waste dumps' operation [3]. According to the latter, performing extensive microbiological research focused on municipal waste dumps and surroundings is required to know the important factors influencing human environment. Appreciating this, the main scientific aim of complex microbiological analyses was to evaluate existing microbiological hazard for the water environment by a big municipal waste dump - the Barycz near Krakow.

Material and methods

Microbiological analyses of groundwater and leaching water were carried out in the region of the Barycz waste dump near Krakow, encompassing a total area of 37 ha. The area is divided into three parts: the first one was exploited from the end of 1974 to 1992, second to 2005, nowadays the third section is exploited - 11 ha. The tertiary miocene aquifier (Bogucice sands) is predominant in the vicinity of the waste dump. The anthropogenic threat centers origin from the possibility of contaminations and pollutions infiltration from the surface to the groundwater. The physicochemical analyses published by the MPO in Krakow in 2004 show that the quality of groundwater is permanently low in the piezometers located within the water flow direction, due to the over-normative values of: electrolytic conductivity, chlorides, nitrogen and organic compounds' concentration characterized by the general organic carbon index.

In order to perform microbial analyses, samples of groundwater were taken once every month over a year period (from June 2004 to May 2005) from 5 piezometers and one leachate sample was taken from the drainage ditch. Before the sample was taken, the trial pumping was performed in order to exchange the water from the piezometer for the fresh water from the aquifier layer. The representative samples were taken with the bail sampler (PB1 model, Biosphera, Poland). Each time the water level in piezometers was measured. Immediately after the sample was taken, the temperature was measured with the digital thermometer (PT-101 type, Elmetron, Poland). Microbiological analyses were performed in the laboratory of the Department of Microbiology, University of Agriculture in Krakow. The measurement points' location depended on the piezometers' location, which was consistent with the groundwater flow direction and reflected the spatial representativeness. The chosen standard sample points were defined as following:

C-1 - piezometer, placed nearby the II section, 450 m (North-East) from the entry gates of the waste dumps,
P-3 - piezometer placed near the road - 770 m from the entry gates of the North-East Section, about 70 m from an unused leachate collector,
P-6 - piezometer located nearby the buildings, on a small height on a dry area, 570 m to the North from the borders of the II section of the waste dump,
P-8 - a piezometer hidden in dense reed beds, in the well of the damp area, near the Malinowka stream, 660 m to the North from the II section of the waste dump,

G - the furthest located piezometer, on the Malinowka stream bank, by a gravel road, 1230 m to the North from the II section.

It needs to be stressed, that the piezometers signed as: P-6, P-8 and G were located beyond the 500 m wide zone, spreading the waste dump borders.

The carried out bacteriological and mycological analyses included the following evaluations:

- total number of mesophilic bacteria - grown on the nutrient agar, in the temperature of 37°C for 24 hours,
- total number of relatively psychrophilic bacteria - grown on the nutrient agar, in the temperature of 20°C for 72 hours,
- total number of microscopic fungi - grown on *Malt Extract Agar* (MEA), in the temperature of 28°C for 5 days.

The evaluation of the sanitary state was based on the detection and quantitative analyses of the general coliforms and fecal coliforms (thermotolerant). The membrane filtration method was applied for the analysis. According to the method, the amount of water (100 cm^3) from the sampling points was each time filtered through the membrane filter with the pores diameter of 0.45 μm with the filtration kit. Then the filters with bacteria were placed on the selective medium - Endo agar. General coliforms and fecal coliforms (thermo tolerant) were cultured in 37 and 44°C for 24÷48 hours.

After the incubation period, the cfu was determined - colony forming units - for the tested microorganisms in cm^3 or 100 cm^3 of water ($cfu \cdot cm^{-3}$ or $cfu \cdot 100\ cm^{-3}$).

Isolated strains of microorganisms were identified using morphological and macroscopic analysis (colonies' morphology) and microscopic analysis of cells stained by the Gram's method. Investigated strains of bacteria were differentiated on the basis of metabolic characteristics using Bergey's Manual of Determinative Bacteriology [26] and biochemical test system API. Fungi were identified on the basis of the macroscopic and microscopic traits of colonies compared with the data from the available taxonomic manuals [27-30].

Results

The present paper aims at describing the influence of the municipal waste dump Barycz in Krakow on the microbiological quality of groundwater in its closest vicinity. Mesophilic bacteria abundance in the processed groundwater samples varied a lot depending on the sample location and date of sampling and varied from 10 to 1 360 cfu in 1 cm^3 of water and the abundance of psychrophilic bacteria varied from 25 to 2 240 cfu in 1 cm^3 of water (Table 1). Maximum amount of both groups of bacteria was found in the P-3 piezometer (near the old leaching water collector and the approach road)) in June 2004 and in August 2004, at water temperature 15.4 and 16.4°C respectively and the water table at 1,5 and 2 m. The lowest values on the other hand were found in January 2005 in the P-6 piezometer, located on a small height by the dwelled houses at Barycka Street, at the water temperature 5.3°C and water table at 0.6 m. On other dates of sampling, water in this piezometer revealed also a low count of microorganisms, mainly during the winter period (November 2004 - February 2005). The average number of mesophilic bacteria in tested water samples (Table 1) occurred to be the highest in the P-3 piezometer (located by the old leaching water collector), whereas in the C-1 piezometer (located next to the II section of the waste dump) occurred to be about 25.5% lower, in piezometer P-8 (660 m to the North from the 2nd section border) 46.3% lower, in piezometer P-6 (located next to the buildings) 62.8% lower and in the G piezometer (1230 m to the North from the 2nd section) as much as 71.1% lower. This indicates an important impact of the waste dumps on the microbiological quality of groundwater, as well as still high microbiological threat near the unused leaching water collector.

Table 1

Number of microorganisms in groundwater nearby the municipal waste dump Barycz in Krakow

Measuring points	**Average number of cfu in 1 cm^3 of water**											
	June 2004	**July 2004**	**Aug. 2004**	**Sept. 2004**	**Oct. 2004**	**Nov. 2004**	**Dec. 2004**	**Jan. 2005**	**Feb. 2005**	**March 2005**	**April 2005**	**May 2005**
Mesophilic bacteria												
C-1	850	630	1 100	980	540	250	180	160	110	320	410	380
P-3	1 360	870	1 200	850	690	310	95	130	210	430	680	1 120
P-6	740	320	450	300	180	90	40	10	35	110	400	230
P-8	1 010	500	930	380	120	110	90	75	110	230	120	510
G	680	320	260	150	160	80	80	20	25	60	240	220
Psychophilic bacteria												
C-1	910	730	980	820	600	110	230	210	360	470	450	390
P-3	1 800	1 380	2 240	980	780	640	240	470	690	880	1 340	1 180
P-6	380	410	570	600	310	110	95	25	100	215	480	150
P-8	890	610	780	970	250	310	110	115	90	310	230	620
G	890	420	380	310	130	200	90	45	90	120	220	560

It needs to be stressed, that in the analyzed groundwater samples, usually psychrophilic microbes outnumbered the mesophilic microorganisms, which is characteristic of the water microflora developed in lower temperatures. The measured water temperature in the piezometers never exceeded 20°C, which favors psychrophilic bacteria. Only in the C1 piezometer, located by the 2nd section, the number of psychrophilic bacteria was almost the same as mesophilic bacteria. It has to be stressed, that the number of microorganisms analyzed in groundwater was very variable. The smallest changes occurred during the winter season. This was related to the temperature of groundwater. The lowest temperatures (4.8÷8.1°C) were logged between December 2004 and February 2005. Maximum temperature of 18.5°C was recorded in August 2004, in the P-6 measuring point.

Diversity of microorganisms in the tested groundwater was pretty slight. The following bacteria genera were found: *Pseudomonas*, *Flavobacterium*, *Desulfovibrio*, *Bacillus*, *Clostridium*, *Enterobacter*, *Enterococcus, Salmonella* and *Escherichia*. Among them occurred microorganisms specified by the pathogens' list defined by the Ministry of Environment, issued on 29th November 2002. Species: *Enterobacter cloacae*, *Escherichia coli, Salmonella* were included in the 2nd category, *Clostridium perfringens* in the 3rd category.

Basing on mesophilic and relatively psychrophilic bacteria occurrence, as well as on the other microbiological indicators, it may be stated that all groundwater samples taken from piezometers C-1, P-3, P-6, P-8 and G were bacteriologically contaminated.

During the yearly analyses of the area and vicinity of the municipal waste dump Barycz in Krakow, periodical occurrence of general coliforms was observed reaching from 0 to

110 cfu · 100 cm^{-3} as well as fecal coliforms (thermotolerant) from 0 to 95 cfu · 100 cm^{-3} (Table 2). Strong tendency of decreasing of fecal coliforms number (thermotolerant) in water taken from piezometers more distant from waste dump was observed. Maximum number - 95 cfu of these bacteria in 100 cm^3 of water was found in August 2004 in the C-1, piezometer, located by the II section. This sampling point had the highest average number of general coliforms (46 cfu · 100 cm^{-3}) and thermotolerant coliforms (19 cfu · 100 cm^{-3}), which indicated the highest level of microbiological contamination in relation to the other sampling points. In the P-8 sampling point, located 660 m from the waste dump, only four of 12 measurements revealed the occurrence of fecal coliforms (average number: 9 cfu /100 cm^3). However in the G piezometer, located the furthest from the waste dump (1230 m) no fecal coliforms were found during the whole analysis period. In the samples taken from the C-1 piezometer, located by the II section of the waste dump fecal coliforms were not observed when the area was shut out of exploitation.

Table 2

Number of coliforms in groundwater nearby the municipal waste dump Barycz in Krakow

Measuring points	**Average number of cfu in 100 cm^3 of water**											
	June 2004	**July 2004**	**Aug. 2004**	**Sept. 2004**	**Oct. 2004**	**Nov. 2004**	**Dec. 2004**	**Jan. 2005**	**Feb. 2005**	**March 2005**	**April 2005**	**May 2005**
General coliforms												
C-1	45	98	110	85	50	80	0	10	0	20	25	31
P-3	40	55	90	0	0	20	0	15	10	45	10	69
P-6	60	105	100	0	0	15	0	10	0	25	55	103
P-8	0	0	85	10	15	10	0	10	23	25	60	110
G	0	0	0	0	0	10	0	15	0	16	10	10
Fecal coliforms												
C-1	31	85	95	10	0	10	0	2	0	0	0	0
P-3	15	35	52	0	0	13	0	6	5	15	4	3
P-6	10	18	10	0	0	0	0	0	0	0	5	48
P-8	0	0	25	0	0	0	0	0	13	0	30	45
G	0	0	0	0	0	0	0	0	0	0	0	0

Basing on the microbiological research it was ascertained, that the tested samples of groundwater revealed low number of yeast. The maximum count - 20 cfu · cm^{-3} was observed in August 2004 in the C-1 piezometer and in November 2004 in the P-6 piezometer. Yeast were most rarely isolated from groundwater in the P-3 and G sampling points. Yeast most often appeared in groundwater from the C-1 piezometer, placed by the II section (Table 3). Other sampling points revealed only 3 cfu in 1 cm^3 in groundwater. Mould fungi on the other hand were most often found in the P-3 piezometer in June 2004 (70 cfu · cm^{-3}), C-1 (35 cfu · cm^{-3}) and P3 (also 35 cfu · cm^{-3}) in July 2004. Taking into account the average cfu numbers of these microorganisms, water from the P-3 piezometer (by the Krzemieniecka Street and next to the old effluent collector tank) and from the C-1 (next to the 2nd section of the waste dump) are the most fungally contaminated. Only in the furthest G sampling point no mould fungi were found for the whole analysis period (Table 4).

The diagnostic analyses of the *Micromycetes* fungi shows, that the following microscopic fungi genera were isolated from the processed groundwater samples:

Aspergillus, *Candida*, *Penicillium*, *Toprulopsis* and *Trichoderma*. The research confirmed the results gained by the researchers from other countries that the most common fungi species in the area of the waste dumps are *Alternaria, Aspergillus* and *Penicillium*. It was found that the sampling points located in the vicinity of the waste dump (piezometers P-3 and C-1) presented the highest differentiation of the microbiocenotic composition of fungi.

Table 3
Number of mould fungi and yeasts in groundwater nearby the municipal waste dump Barycz in Krakow

Measuring points	**Average number of cfu in 1 cm^3 of water**											
	June 2004	**July 2004**	**Aug. 2004**	**Sept. 2004**	**Oct. 2004**	**Nov. 2004**	**Dec. 2004**	**Jan. 2005**	**Feb. 2005**	**March 2005**	**April 2005**	**May 2005**
Moulds												
C-1	35	20	25	19	12	5	2	0	0	12	3	10
P-3	70	35	30	17	10	5	20	8	5	28	3	5
P-6	2	4	25	15	0	0	0	0	5	5	0	15
P-8	8	12	30	0	4	6	0	0	0	8	2	20
G	0	0	0	0	0	0	0	0	0	0	0	0
Yeast												
C-1	5	10	20	0	0	5	3	0	0	0	3	18
P-3	8	14	7	0	0	0	0	0	3	0	3	0
P-6	3	3	4	0	0	20	0	0	0	2	3	0
P-8	13	13	3	0	0	0	0	0	0	0	4	5
G	0	3	0	7	0	0	0	0	0	3	8	11

Table 4
Average number of microorganisms in the tested groundwater samples in the period of June 2004 - May 2005

Measuring point	**Average number of cfu in 1 cm^3 of water**					**Average number of cfu in 100 cm^3 of water**	
	mesophiles	**psychrophiles**	**proteolytic bacteria**	**yeasts**	**moulds**	**coliforms**	**thermotolerant coliforms**
C-1	493	522	234	5	12	46	19
P-3	662	1052	336	3	20	30	12
P-6	246	287	66	3	6	39	8
P-8	349	440	163	3	8	29	9
G	191	288	80	3	0	5	0

Due to evaluation of impact of the temperature and groundwater table level on microorganisms' amount, Pearson's correlation coefficient (r) and its statistical significance ($p \leq 0.05$) were calculated using the Statistica software for Windows PL, v. 5.1 G. The statistical analysis revealed that the groundwater temperature influence on the number of mesophilic and psychrophilic bacteria in each sampling point was statistically important and the influence of water table level was statistically important only in C-1, P-6, and P-8 piezometers. Nevertheless, the tested environmental factors' influence on the general coilforms or fecal coliforms abundance in groundwater was not statistically important.

Discussion

It was often stated in many papers, that the groundwater contamination caused by the waste dumps depends on: waste physicochemical properties amount and composition, waste dump location, applied technological solution of ground protection and leachate intake.

Nevertheless, spreading the contamination depends on: groundwater flow speed, hydraulic gradient of water, filtration coefficient, permeable formations, properties and quantity of leaching water, and sorption ability of the ground. Water pollution by the waste dump may last for several years, depending, among others, on its size and exploitation time [31-35].

According to Olanczuk-Neyman [18] relative slow flow rates, longer detention times and very slow pollution elimination, eg related to the physicochemical and biological properties, the most crucial features of groundwater, differentiating them from the surface water.

According to the PIOŚ (*Inspectorate of Environmental Protection*) [36] the infiltrating water, occurring on the ground surface, mostly origins from atmospheric precipitation. Rainfall water, leaching from waste dumps may microbiologically contaminate groundwater. However it was noticed that microorganisms migration is limited by their relatively short life. According to the presented results, the number of bacteria 100 times exceeded the amount of these microorganisms in non-contaminated water in some cases. For example, the amount of mesophilic bacteria in groundwater samples from measuring points placed nearby the waste dumps in Sobniow and Smoczka reached 1÷860, though coliform index and fecal coliform index were often higher than 50.

The carried out research revealed that within the area and in the surroundings of the municipal waste dump Barycz in Krakow all analyzed groundwater samples contained the coliform bacteria. Though bacteria were found in different numbers and frequency, nevertheless their periodical presence resulted in the tested water being qualified into "bad quality" category. There was a strong tendency of decreasing number of fecal coliforms in the water samples taken from the piezometers located further from the waste dump. Among all tested groundwater samples, only the sample taken from G piezometer did not reveal the presence of coliforms. Coliforms are usually composed of *Escherichia coli* strains but also of *Enterobacter*, *Citrobacter* and *Klebsiella*. Fecal coliforms (thermotolerant) revealed mainly strains of *Escherichia coli* and just few strains of the above-mentioned genera. Presence of these bacteria suggests relatively recent fecal, sewage, soil or rotten plants contamination [37, 12].

The highest number and the highest frequency of indicators' as well as other microorganisms' occurrence was found nearby the active section and in the vicinity of the leaching water collector and the approach road. Mor et al [24] found fecal coliforms in leaching water from the municipal waste dump as well. Christensen et al [7] show that the amount of bacteria in water reservoirs nearby the municipal waste dumps is higher than in the neutral, non-contaminated reservoirs.

It has to be stressed that the examined groundwater samples, apart from containing contaminant microorganisms and bioindicators of fecal contamination, were characterized by high amount of mesophilic, psychrophilic and proteolitic bacteria, yeasts and fungi of low diversity. Often occurrence of these microorganisms was positively correlated with low water levels from 0.1 to 2.9 m in piezometers: C-1, P-3, P-6, P-8 and slightly higher in the G piezometer - between 3 and 5 m, and with high concentration of organic compounds. Water temperature from 4.8 to 18.5°C did not impact psychrophilic bacteria, because their growth optimum is 15°C or lower, and maximal growth temperature is less than 20°C. Moreover, the microorganisms' diversity decreases with the water environment's contamination increase.

The same relation was observed by Olanczuk-Neyman [18], who found, that the effectiveness of allochtonous microorganisms elimination may by decreased by strong and long-lasting contamination, which causes damage in antagonistic autochtonous microbial microflora. Microorganisms' migration to groundwater may be caused by active or passive drainage from the surface or migration from the supply areas.

Groundwater and surface water as well as soil contamination is mostly influenced by the leaching water. The amount of leachate depends on eg type of atmospheric precipitation, ways of collection, period of exploitation of the waste dump and the size of precipitation. The leaching water composition depends on the stage of waste decay and the waste substance [34]. According to data presented by PIOS [36] the leaching water is much more loaded than the urban sewage and it presents large variability of bacteria count. The municipal waste dumps' leachate may be also harmful to the environment. The improvement of leaching water quality was achieved usually after protection of the waste heap by eg reinforcing the slopes, impregnation of the ground and enclosing it by the screen as deep as the impermeable level [38, 39]. The microbiological research was performed between June 2004 and May 2005, which is the period when the II section, and then the III section were operating. Comparing the groundwater samples collected from examined area, it may be concluded that the leaching water influenced the contamination of the water environment in the area and nearby this waste dump.

Conclusions

1. On the basis of the carried out research it was stated that all tested groundwater samples taken from C-1, P-3, P-6, P-8 and G piezometers were bacteriologically contaminated. The strong tendency was observed that the number of bacteria decreased in water taken from piezometers more distant from waste dump.
2. In all tested groundwater samples the bacteria - low sanitary state indicators were observed.
3. The factor which mainly influenced the microbiological quality of groundwater was the distance of the sampling point (piezometer) from the waste dump.
4. The necessity of performing microbiological analyses, not only physicochemical ones, should be legally recommended.

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ZAGROŻENIA MIKROBIOLOGICZNE DLA ŚRODOWISKA POWODOWANE PRZEZ WODY PODZIEMNE W STREFIE ODDZIAŁYWANIA SKŁADOWISKA ODPADÓW KOMUNALNYCH

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Abstrakt: Na terenie i w okolicy składowiska odpadów komunalnych Barycz w Krakowie we wszystkich badanych wodach podziemnych, pobieranych z piezometrów C-1, P-3, P-6, P-8 i G, stwierdzono występowanie bakterii - wskaźników złego stanu sanitarnego. Nawet okresowe zanieczyszczenie wód podziemnych kwalifikuje je do kategorii złej jakości. Obecność bakterii coli typu kałowego wskazuje na antropogenne pochodzenie zanieczyszczeń wód podziemnych. Liczba baterii coli typu fekalnego w wodach pobieranych z piezometrów zmniejszała się wraz ze wzrostem odległości od granic składowiska. Jednak nawet w odległości 1230 m na północ od obrzeży sektorów składowiska w piezometrze G, pomimo braku w nim bakterii coli typu fekalnego, stwierdzono zły stan sanitarny wody podziemnej na podstawie innych wskaźników mikrobiologicznych.

Słowa kluczowe: zanieczyszczenia mikrobiologiczne, wody podziemne, odciek

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TESTING OF TOXIC ELEMENTS MIGRATION FROM THE MATERIALS USED AS TOY COATINGS

BADANIE MIGRACJI TOKSYCZNYCH PIERWIASTKÓW Z MATERIAŁÓW STOSOWANYCH JAKO POWŁOKI W ZABAWKACH

Abstract: Materials from which toxic elements, including heavy metals, can penetrate children organisms, in doses dangerous for their health should not be used for production of toys. That especially concerns the materials used for production of components that are exposed to children. The results of testing migration of selected elements like: barium, chromium, cadmium, mercury, antimony and selenium from the materials used to cover wooden and metal toys are discussed in the paper. Tests of elements migration were carried out in years 2009÷2010 in the accredited Laboratory of Material Engineering and Environment at KOMAG. Requirements of the PN-EN 71-3:1998 Standard on toys safety was the basis to conduct testing. The results were assessed in the light of limits of elements migration, identifying the types of material in which exceeding of permissible values of migration were found. The elements, which migration is characteristic for a given type of coating material, were specified.

Keywords: testing toys safety, migration of elements from toy materials, coatings, paints, varnishes, impregnates, colorants

Toys as the products intended for children up to 14 years of age should not, according to the requirements of 88/378/EEC Directive, cause any threat to health and life of their users [1]. They should be designed and manufactured in such a way that during their use as intended, they do not cause any health danger to people, including a danger of exposure to chemical substances and mixtures being in toys [2]. Such a risk can appear in the case of using materials containing toxic elements such as: antimony (Sb), arsenic (As), barium (Ba), chromium (Cr), lead (Pb), cadmium (Cd), selenium (Se), mercury (Hg) and other heavy metals. They can penetrate children organism in the result of sucking, licking and swallowing a toy or its component as well as in the result of long-lasting contact with the skin [3].

Migrating elements affects children health causing acute and chronic disease depending on type and dose of absorbed element and its form (as organic or inorganic compound), its

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oxidation number as well as on child metabolism. The following elements are the most dangerous ones: cadmium, lead, antimony and mercury, that apart of their toxic properties can accumulate in the child organism [4-7]. Such a danger is especially important in the case of babies and small children, which have a greater ability for absorption of dangerous elements and their retention in organism [8]. In the consequence that can lead to permanent deterioration of organism condition already at the early stage of the child growth [9, 10].

Studies on migration of elements from toy materials are carried out in many research projects. In majority the studies concern assessment of the quality of tests including measuring methods and interpretation of results. Such an assessment is made on the basis of comparable tests realized in the laboratories accredited for toys testing. Migration of elements is identified for the reference samples of the materials of known composition [11-13].

In the published project results, proper determination of limiting values of elements migration as regards their accepted daily absorption by the child organism, is also analyzed [14, 15]. The available literature data do not indicate the scale of the risks associated with migration of toxic elements from different types of material used for toys. Published reports from toys audits conducted by market surveillance authorities as well as data from dangerous products files are not sufficient [15-17]. The present state-of-the-art does not enable to take any measures to eliminate from toys production the dangerous materials, which can release toxic elements in doses affecting children health. Special attention has to be drawn to those materials that have a direct contact with child body.

Laboratory of Material Engineering and Environment at KOMAG Institute of Mining Technology realized the project, in the light of current requirements [18], aiming at specification and assessment of amount of toxic elements migration from materials used as toys coatings to complete information about that problem.

The project was realized within the research task financed by KOMAG entitled: “Analysis of results of tests for determination of dangerous substances in products intended for children, carried out in the Laboratory of Material Engineering and Environment in the years 2009/2010” [18]. Results from migration of elements from toy coatings materials, determined upon order of KOMAG’s Laboratory clients, were the essential basis of the project [19]. Results of this research project are presented in the paper.

Materials and methods

Identification and determination of migration of the elements such as: Sb, As, Ba, Cr, Pb, Cd, Se and Hg from materials used as the coatings for toys were the main objective of tests. Testing of migration was carried out according to the requirements of PN-EN 71-3:1998 standard on toys safety, which is harmonized with 88/378/EEC Directive [20]. Simulation of conditions that are in the stomach after swallowing of the toy material was the method for testing migration of the elements [1].

Layer of the material formed or embedded on the basic toy material like: paints, varnishes, polymers or other substances of similar properties that can contain metallic particles, irrespectively to the method of covering and possibility of their removal, were according to a definition of the above-mentioned standard, the tested coatings.

The materials used in coatings of wooden and metal toys intended for children up to 6 years were tested.

Coating materials for testing purposes were divided into the following types: paints, including powder paints (polyester-epoxy, epoxy, polyester) as well as paints in a liquid form, varnishes (acrylic, nitrocellulose, polyurethane, alkyl urethane), enamel paints (water-based, acrylic, nitrocellulose), impregnates and colorants.

Totally 163 coating materials were tested, majority of which were paints. Percentage share of types of tested materials is given in Figure 1.

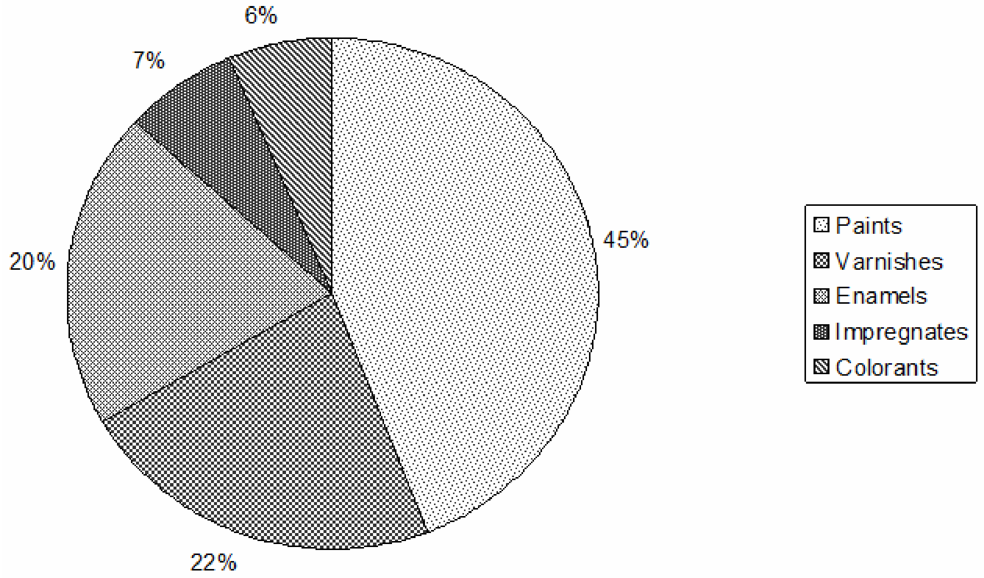

Fig. 1. Percentage share of types of tested materials [own source]

Samples of coating were taken by scratching from the available parts of toys. The resulting coatings were subjected to a process of disintegration and sieving through the screen with holes 0.5 mm. In the case of oily components found in the sample they were removed by *n*-heptan. In the case of liquid or powder coatings they were solidified before taking samples for testing. The samples were then extracted in conditions simulating action of stomach acid in the case of swallowing part of a toy by the child. The extraction was carried out using hydrochloric acid of concentration 0.07 mol/dm^3 in a heated bath with stirring, without light access, in a temperature 37°C, for 1 hour, when the extracted solution reaches pH from 1.0 to 1.5.

After extraction the solution was separated from the solid particles by filtering through a filter paper of pores 0.45 μm. Obtained solutions were quantitatively analyzed using the methods in which lower limit of detection of elements is not over 0.1 of limit migration value. *Inductively coupled plasma atomic emission spectrometry* (ICP-AES) was used for detection of antimony, arsenic, barium, chromium, lead, cadmium and selenium. *Cold vapor atomic absorption spectrometry* (CV-AAS) was used for detection of mercury. General algorithm for testing migration of elements from coatings is given in Figure 2.

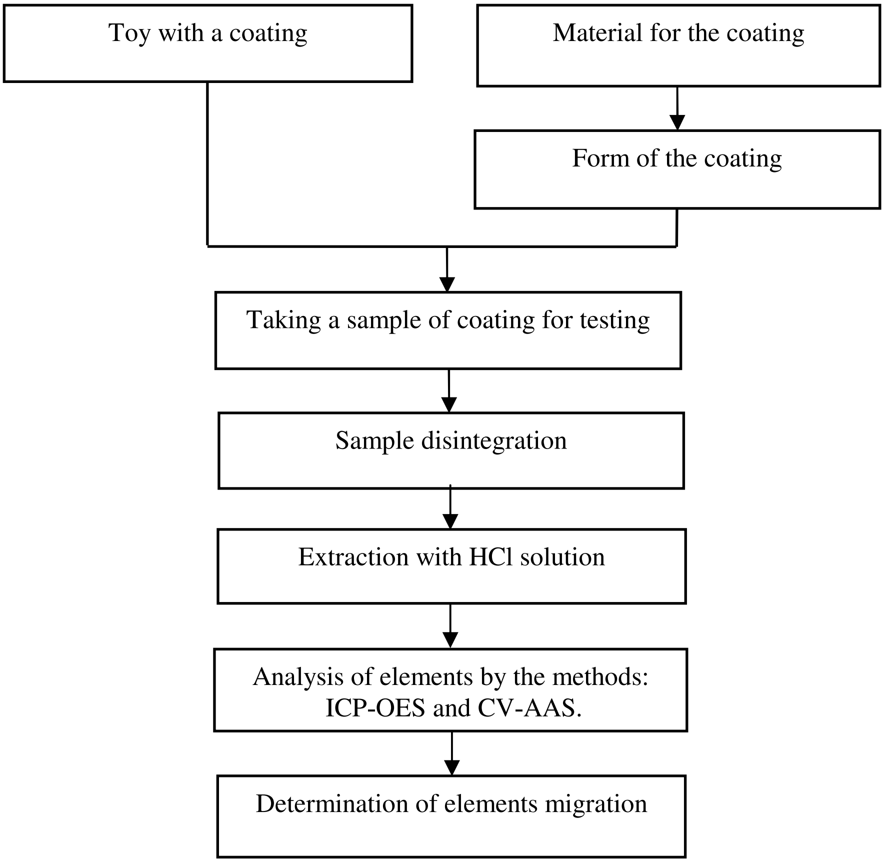

Fig. 2. Algorithm for testing migration of elements from the materials used as toy coatings [own source]

Results and discussion

Analysis of test results shown that each type of tested coating material is characterized by migration of certain group of elements. That indicates for individual profile of migration for the given material. On that basis we can conclude that in the case of covering the toy with the following type of coating we can expect migration of those elements:

- polyester-epoxy and epoxy powder paints: Sb, Ba, Cr, Pb and Se;
- polyester powder paints: Sb, Ba, Cd, Cr, Pb and Se;
- paints in a liquid form: Ba, Cr, Pb and Hg;
- varnishes: Sb, Ba, Cr, Pb, Hg and Se;
- enamel paints: Sb, Ba, Cr, Pb, Hg and Se;
- impregnates: Sb, As, Ba, Cd, Cr, Pb and Se;
- colorants: Sb, As, Ba and Se.

Barium is the element, migration of which was found in all coating materials. Migration of chromium, lead and antimony was also found to be frequent. Migration of these elements was reported in the majority of coatings except colorants.

Detailed results from testing of migration of elements are given in Table 1.

Table 1

List of results from testing of elements migration [18]

Type of tested material		No. of samples	Statistical parameters	Elements							
				Sb	**As**	**Ba**	**Cd**	**Cr**	**Pb**	**Hg**	**Se**
Paints	polyester-epoxy powder paints	25	Size	2		25		19	16		14
			x_{max} [mg/kg]	158.0		642.0		726.7	3541.0		31.1
			$x_{min.}$ [mg/kg]	1.4		26.5		0.9	0.4		1.2
			$x_{avg.}$ [mg/kg]	79.7		428.6		40.0	223.5		9.2
	epoxy powder paints	7	Size	2		7		4	4		2
			x_{max} [mg/kg]	34.7		543.0		218.9	985.0		29.6
			$x_{min.}$ [mg/kg]	1.3		28.3		1.4	1.5		5.5
			$x_{avg.}$ [mg/kg]	18.0		391.6		56.2	474.1		17.5
	polyester powder paints	17	Size	1		17	1	14	9		11
			x_{max} [mg/kg]	1.7		731.7	0.9	20.7	144.3		27.8
			$x_{min.}$ [mg/kg]	1.7		304.0	0.9	1.1	0.7		2.7
			$x_{avg.}$ [mg/kg]	1.7		413.1	0.9	4.0	17.4		11.1
	in a liquid form	17	Size			9		4	5	1	
			x_{max} [mg/kg]			12.7		3.9	7.0	0.7	
			$x_{min.}$ [mg/kg]			0.8		0.8	1.6	0.7	
			$x_{avg.}$ [mg/kg]			5.2		2.4	3.0	0.7	
Varnishes		39	Size	3		22		4	4	2	3
			x_{max} [mg/kg]	2.8		132.5		3.2	6.3	0.4	12.2
			$x_{min.}$ [mg/kg]	0.8		0.5		1.2	1.0	0.2	0.9
			$x_{avg.}$ [mg/kg]	1.9		19.1		1.8	2.9	0.3	4.7
Enamels		35	Size	2		29		24	26	1	4
			x_{max} [mg/kg]	1.5		480.6		26.0	19.2	2.3	324.9
			$x_{min.}$ [mg/kg]	0.9		2.3		0.8	0.8	2.3	1.4
			$x_{avg.}$ [mg/kg]	1.2		180.9		4.1	5.3	2.3	84.2
Impregnates		12	Size	3	1	3	1	3	2		1
			x_{max} [mg/kg]	706.9	244.4	1.4	52.3	2510.0	19.6		1.0
			$x_{min.}$ [mg/kg]	1.0	244.4	0.9	53.2	2.7	2.1		1.0
			$x_{avg.}$ [mg/kg]	236.5	244.4	1.2	53.2	874.2	10.9		1.0
Colorants		11	Size	1	1	4					1
			x_{max} [mg/kg]	196.3	19.7	66.0					3.0
			$x_{min.}$ [mg/kg]	196.3	19.7	0.9					3.0
			$x_{avg.}$ [mg/kg]	196.3	19.7	18.0					3.0

The obtained tests results were compared with limits of elements migration given in PN-EN 71-3:1998 Standard (Table 2).

Table 2

Limits of selected, dangerous elements migration from toys according to PN-EN 71-3:1998 Standard [3]

Element	**Sb**	**As**	**Ba**	**Cd**	**Cr**	**Pb**	**Hg**	**Se**
Maximal value of element migration from toy material [mg/kg]	60	25	1000	75	60	90	60	500

Comparison of test results have proved that accepted values of chromium, lead, antimony and arsenic migration are exceeded in some cases. Exceeding was reported in the following samples: powder paints (polyester-epoxy, epoxy and polyester), impregnate and colorant (Fig. 3).

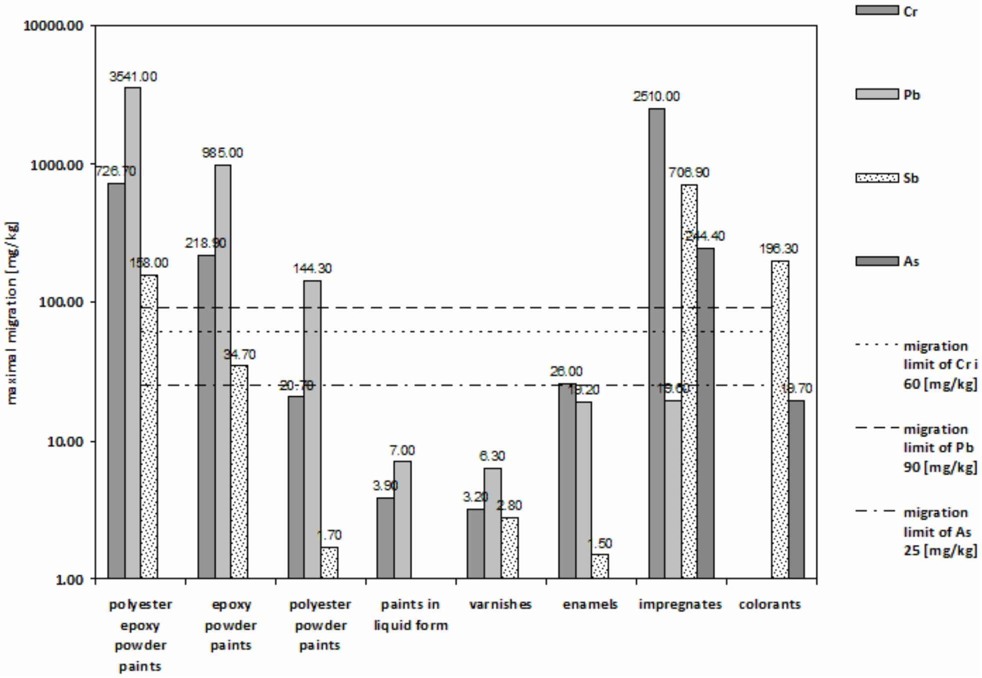

Fig. 3. Exceeding of migration limits of Cr, Pb, Sb and As [own source]

The values of migration, which exceeded the standard limits, were corrected for interpretation of tests results, according to the procedure given in PN-EN 71-3:1998 standard by including analytical corrections, relevant to each element (Table 3).

Obtained reduced results were compared again with the accepted migration values. The results were still much above the permissible values. For example, the result of chromium analysis x_{max} = 726.7 mg/kg, after analytical correction AC = 30%, got the value 508.69 mg/kg, which is much higher than the limit 60 mg/kg.

Table 3

Analytical corrections according to PN-EN 71-3:1998 Standard [3]

Element	Sb	As	Ba	Cd	Cr	Pb	Hg	Se
Analytical correction AC [%]	60	60	30	30	30	30	50	60

It should be noted that the total number of identified exceedances of migration occurred only in about 7% of tested coating samples. The percentage of the number of exceedances of the migration of each element in all the test results was: Cr - 1.8%, Pb - 2.4%, Sb - 1.8%, As - 0.6%.

During analyses of tests results, high differentiation of migration of each element has been found, what is illustrated on the example of Cr and Pb migration, in Figures 4 and 5.

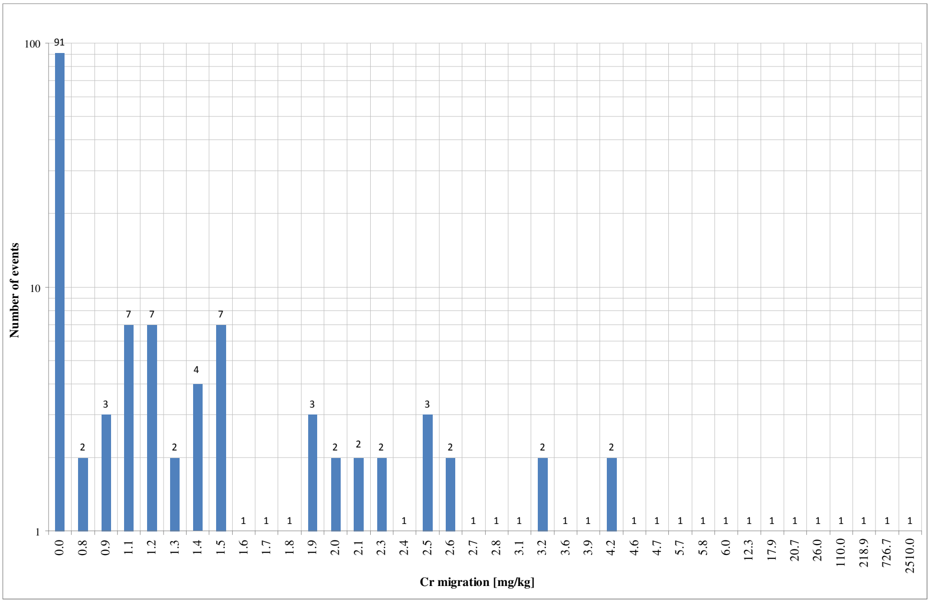

Fig. 4. Frequency of Cr migration [own source]

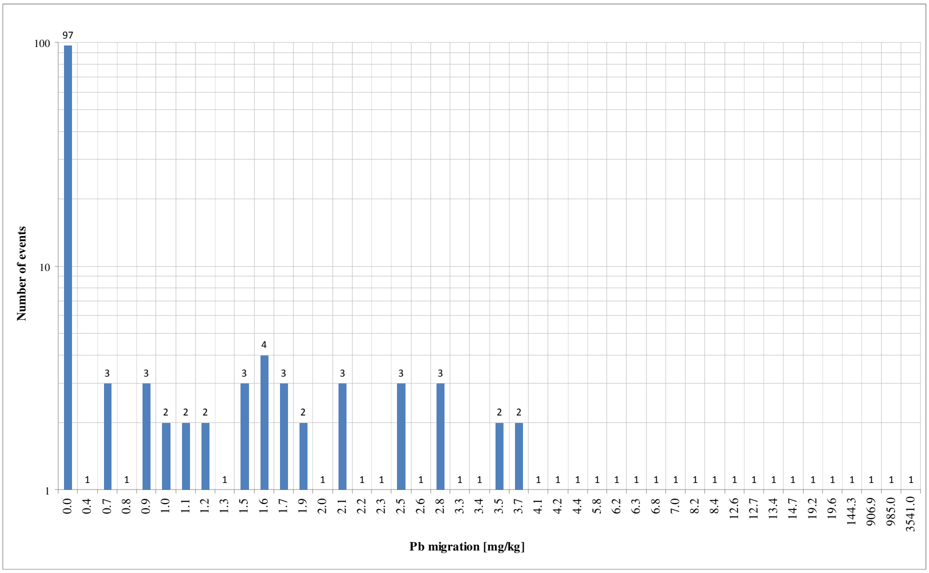

Fig. 5. Frequency of Pb migration [own source]

The analyses show frequent migrations of Cr and Pb with values significantly lower than limits as well as the cases with no migration of given element from tested samples. On the basis of Figures 4 and 5 we can see that in 91 samples of coatings migration of Cr was not reported and in the case of Pb migration was not found in 97 samples.

Conclusions

Materials used as toys coatings contain such toxic elements as: Sb, As, Ba, Cr, Pb, Cd, Hg and Se, which can penetrate child organism in amounts exceeding permissible values what can pose a threat to its health and life. Because of that tests of toys safety are carried our according to the PN-EN 71-3:1998 Standard, which specifies conditions of testing of migration of the above- mentioned elements as well as their migration limits.

The tests carried out by the author have shown that each type of material, used as the toy coating, due to the substances included, has its own migration profile, characterized by the selected elements. Analysis of test results identified few exceedances of migration limits of the following elements: chromium, lead, antimony and arsenic. They have been reported in the following samples: powder paints, impregnate and colorant, used in manufacture of wooden toys. That fact indicates for a necessity of carrying out further tests on migration of elements for this group of materials as regards meeting its safety limits included in PN-EN 71-3:1998 Standard.

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BADANIE MIGRACJI TOKSYCZNYCH PIERWIASTKÓW Z MATERIAŁÓW STOSOWANYCH JAKO POWŁOKI W ZABAWKACH

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Abstract: Do produkcji zabawek nie powinny być stosowane materiały, z których mogą przedostawać się do organizmu dziecka, w dawkach szkodliwych dla jego zdrowia i życia, toksyczne pierwiastki, w tym metale ciężkie. Dotyczy to szczególnie materiałów znajdujących się na dostępnych dla dziecka elementach zabawek. W artykule przedstawiono wyniki badań migracji wybranych pierwiastków, takich jak: bar, chrom, ołów, kadm, rtęć, arsen, antymon i selen, z materiałów, którymi powlekane są powierzchnie zabawek drewnianych i metalowych. Badania migracji przeprowadzono w latach 2009-2010 w akredytowanym Laboratorium Inżynierii Materiałowej i Środowiska ITG KOMAG. Podstawą wykonywania badań były wymagania normy PN-EN 71-3:1998, dotyczącej bezpieczeństwa zabawek. Wyniki badań oceniano w świetle granicznych wartości migracji pierwiastków, identyfikując rodzaje materiałów, w których występują przekroczenia wartości dopuszczalnych migracji. Wyspecyfikowano pierwiastki, których migracja jest charakterystyczna dla danego rodzaju materiału powłoki.

Słowa kluczowe: badania bezpieczeństwa zabawek, migracja pierwiastków z materiałów zabawek, powłoki, farby, lakiery, impregnaty, koloranty

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BIOCONCENTRATION OF MERCURY AND HEAVY METALS BY THE BARK OF MAPLE-LEAF PLANE TREE

BIOKONCENTRACJA RTĘCI I METALI CIĘŻKICH PRZEZ KORĘ PLATANA KLONOLISTNEGO

Abstract: Continuous civilization progress, urbanization, and growing level of industrialization cause the fact that the contents of mercury and heavy metals in the natural environment is increasing, posing a threat to proper ontogenesis of all living organisms. Thus it is justified to monitor and control the accumulation of the above-mentioned elements in the environment. The main goal of our research was to determine whether maple-leaf plane tree (*Platanus x acerifolia*) may be used as a bio-indicator of the air pollutants. This tree may be found all across Central Europe, it is quite resistant to different environment pollutions, and, what is very important, it sheds the bark every autumn. The research covered a determination of the analytical procedure capable of quantitive analysis of the contents of some given heavy metals: copper, zinc, iron, mercury, and also sulfur. The results and correctness of the procedure of the preparation of the bark samples for the analysis were confirmed by the research of a certified material (BCR-062 Olive leaves). Particular attention was put to the influence of the separated stages of the sample preparation (washing, drying, grinding down), especially for the mercury analysis due to the fact that this element is characterized by high volatility. To broaden the analysis, the samples were taken from different parts of the trees: limbs, trunks, and roots, as well as from different places, such as high-traffic streets, parks, and from different cities of Poland and Europe. Total mercury contents were measured by means of automatic Mercury Analyzer MA-2. The quantification determination of the transient-metal ions was performed on a emission spectrometer with inductively coupled plasma VARIAN VISTA-MPX.

Keywords: mercury, heavy metals, ICP-OES

Mercury, among other chemical elements, stands out from very high level of chemical and biological activity [1]. This element is very toxic, moreover, it is not subjected to biodegradation [2]. The toxicity of mercury is strongly correlated with its presence form, while the exposure depends on the way this element gets to the organisms. The emission of mercury to the atmosphere may be a result of some natural geochemical processes, biological methylation, as well human activities. Basic source of environmental-pollution mercury, being a result of human activities, is related with burning of the crude oil and coal, some smelting processes, battery and fluorescent-lamp production, and burning of waste materials [3-5]. Agriculture also participates in the pollution of nature environment by

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means of mercury - its derivatives are used for the production of seed grounds and some plant-protection preparations [1, 6]. For the non-industrial areas, elemental mercury dominates (98%) in comparison with mercury(II) in the aerosol and gaseous forms [7]. Small amount of mercury in the atmosphere is of organic nature, such as dimethylmercury and monomethyl mercury. Once emitted to the atmosphere as either bonded with some compounds, or as a vapour, mercury may be transported with the wind at a high distance. Mercury vapour is usually bonded by atmospheric dust, which in turn may be settled at the surface of the soil, water, and plants, polluting them [8]. Mercury is quite easily absorbed by the plants (mainly the roots - 95%), also from the air.

Recently, in European Union the emission of mercury considerably lowered; however, the concentration of this element in the environment still remains high. The report for the European Commission and Parliament in Brussels in the year 2005 pointed out an important issue for Poland as a leader in the liberation of mercury as a result of coal burning. Thus, it is appropriate to monitor and to control the accumulation of mercury in the natural environment in Poland and across Europe.

This work is devoted to the determination of total concentration of mercury and some heavy metals in the bark of the maple-leaf plane tree. For many years the bark of trees is a target for many researches on the elimination of pollution aspects related with heavy metals and organic compounds to be present in the air, in water solutions and sewage. As reported in the literature, so far the research was concentrated on such trees as: eucalyptus - *Eucalyptus camaldulensis* [9], guava - *Psydium guajawa* [10], pine tree [11], oak, fir tree, and spruce European tree [12, 13]. The main goal of the work was to determine whether maple-leaf plane tree (Platanus x acerifolia) may be used as an effective bioindicator of the air pollution. This tree is to be found across almost all Middle Europe, is quite resistant to the pollution, and it throws down the bark every autumn and winter. Our research was concentrated on the determination of an analytical procedure capable of quantitive analysis of contents of such heavy metals, as copper, zinc, iron, manganese, mercury and, in addition, sulfur.

The results obtained as well as the accuracy of the proposed procedure of the preparation of the bark samples for the analysis was verified by the research on a certified material (BCR-062 Olive leaves). Particular attention was put on the influence of the stages of sample preparation (washing, drying, grinding, etc.), especially for such volatile element as mercury. In addition, the samples were (1) taken from different parts of a tree: branches, the trunk, and roots, (2) collected at different places (heavy-traffic streets, parks), and (3) in different cities and countries (Poland, Europe). Total concentration of mercury was determined by means of automatic mercury analyser Merkury Analyzer MA-2. The other above-mentioned heavy metals we determined by means of emission spectrometer with inductively-coupled plasma VISTA - MPX (VARIAN).

Reagents and methods

Sample preparation

Sample collection

The samples of the bark of maple-leaf plane tree were collected from May to October 2008. The choice of the collection places was dictated by a differentiation of the places, from cities (Poland: Poznan, Kornik, Miedzyzdroje, Sopot, Europe: Vienna, Berlin, Padova, Rome, Slovakia - Tatrzanske Lomnice), to green areas such as parks. The samples were taken at the approximate height of 1 meter above the ground level, as well as directly from the ground, where the bark was shed.

Preparation of the samples for the analysis

Samples of the bark were purified from the lichen by means of a ceramic knife. Next, the samples were cleaned by a tap water and further deionized water. Next, the bark was subjected to drying at the temperature 50°C, and grinded in an agate mortar. The grinded sample was drying till solid state at the temperature 110°C (approximately during 3 hours).

Determination of total concentration of mercury

To determine total concentration of mercury, the method of cold vapours of atomic absorption spectroscopy CV AAS was applied, by means of the automatic mercury analyzer MA-2. The determination procedure was composed of the following steps:

✓ Determination of concentration of metals (Cu, Fe, Mn, S, Zn)

Once the bark was prepared according to the above-described procedure, the samples were mineralized by means of the microwave mineralizer MDS - 2000. The mineralization was performed inside Teflon bombs, in a mixture of 65% $HNO_3 + H_2O_2$ (5 : 2, 10 cm^3), at PSI 60 and POWER 40. After the mineralization the samples were put quantively to the 25 cm^3 flasks, supplemented by distilled water, and used for the determination of the above-mentioned heavy metals. The analysis was performed by means of the emission spectrometer with inductively couples plasma ICP-MS of VARIAN company.

✓ Testing the quality of the determination method

To inspect the quality of the measurement, reference material BCR® - 062 Olive leaves (*Olea europaea*) was applied. The determination of mercury concentration in the reference material was performed at the beginning and at the end of each experiment series. The certified concentration of mercury in the reference material was equal to 0.28 ± 0.02 $mg \cdot kg^{-1}$. The measured (according to the above-described method) concentration was exactly 0.28 ± 0.01 $mg \cdot kg^{-1}$. The certified concentration of copper in the reference material was equal to 46.6 ± 1.8 $mg \cdot kg^{-1}$, manganese - 57.0 ± 2.4 $mg \cdot kg^{-1}$, and zinc - 16.0 ± 0.7 $mg \cdot kg^{-1}$, while the measured values for these metals 46.2 ± 0.5 $mg \cdot kg^{-1}$, 56.8 ± 0.4 $mg \cdot kg^{-1}$, and 15.8 ± 0.5 $mg \cdot kg^{-1}$, respectively.

Results and discussion

The characteristics issue of the maple-leaf plane tree is related with shedding of the bark every year. Such a bark was inspected in our research, to determine the concentration of mercury and other elements, such as copper, iron, manganese, zinc and sulfur.

To determine a structure of the bark, some pictures were taken of its surface by means of the electron microscope Zeiss EV040. The preparation of the bark to take a picture aimed in a deposit of a sample on a double-glued table (carbon tape), and further a atomic-gold spray by means of ionization sprayer Balzers 050. Microscope tension was set to 17.93 kV.

Figure 1 presents a sample photo with a magnification factor equals to 250 times (Fig. 1a), 500 times (Fig. 1b and 1c), and 830 times (Fig. 1d). Figures 1a and 1b present photos of the external surface of the bark, while Figures 1c and 1d - its sideline. At it may be seen the structure of the bark is porous, with large specific surface.

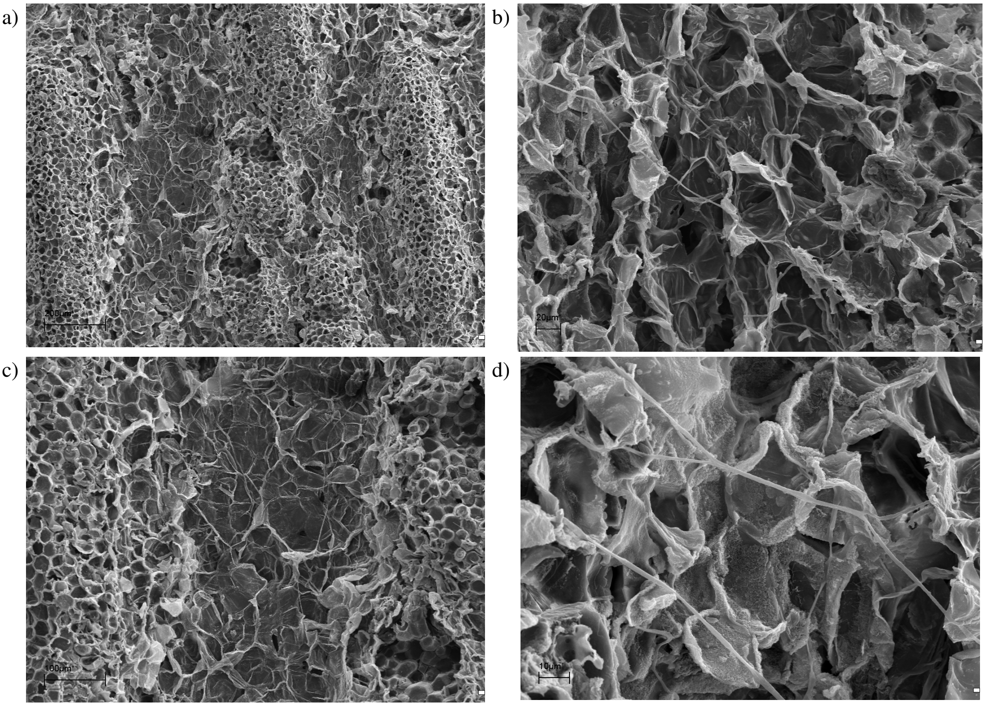

Fig. 1. Sample photos of the bark taken by electron microscope: a) surface magnified 250 times, b) surface magnified 500 times, c) borderline magnified 500 times, d) borderline magnified 830 times

In all the environmental research the key to get right results aims in sample preparation phase. This phase is particularly important in the case of mercury, due to a high volatility of this metal. Thus, in this research the mercury samples were collected in different ways (with cleaning or without) and at different places (directly from the tree and from the ground below the tree) and geographical locations. To provide a comparison of results, the research was also taken for the bark of alder tree and hornbeam tree.

In Table 1 some results are presented of a determination of mercury and some other metals, such as iron, manganese, sulfur, and zinc, in the bark of plane tree, for several geographical localizations (Bydgoszcz and Poznan in Poland, and Vienna in Austria). Graphical presentation of the results is given in Figure 2. Similar results are presented in Table 2, except the samples were collected from the ground under the tree. As it may be seen while analyzing the results given in Tables 1 and 2, a way of sample preparation influences the determined content of mercury and other metals. In the clean samples the metal concentrations were lowered in comparison with the non-cleaned samples. It was caused by the fact that in the case of non-cleaned samples the concentration was determined not only for the bark itself, but also for the dust covering the bark surface.

Table 1

Results of determination of metals in the bark collected directly from the plane tree, depending on the way of obtaining the sample

Bark collected from the tree	**Hg [mg·kg^{-1}]**		**Cu [mg·kg^{-1}]**		**Fe [mg·kg^{-1}]**	
	Cleaned	**Not cleaned**	**Cleaned**	**Not cleaned**	**Cleaned**	**Not cleaned**
Bydgoszcz, Poland (sample I)	0.35	0.43	3.1	9.7	61.0	166.8
Bydgoszcz, Poland (sample II)	0.31	0.40	2.1	3.1	58.2	103.5
Poznan, Poland	0.31	0.39	5.1	11.6	151.8	267.9
Vienna, Austria (sample I)	0.50	0.66	5.9	6.5	78.5	223.6
Vienna, Austria (sample II)	0.40	0.47	10.3	10.7	143.0	175.5
Vienna, Austria (sample III)	0.22	0.26	16.1	18.6	254.8	379.3

Bark collected from the tree	**Mn [mg·kg^{-1}]**		**S [mg·kg^{-1}]**		**Zn [mg·kg^{-1}]**	
	Cleaned	**Not cleaned**	**Cleaned**	**Not cleaned**	**Cleaned**	**Not cleaned**
Bydgoszcz, Poland (sample I)	12.2	15.4	441.6	642.9	6.9	19.4
Bydgoszcz, Poland (sample II)	10.5	11.9	434.5	882.9	154.3	18.2
Poznan, Poland	16.8	26.4	1160.6	1067.5	10.2	21.1
Vienna, Austria (sample I)	14.5	21.8	1406.8	908.0	18.3	21.8
Vienna, Austria (sample II)	23.2	27.2	1119.2	1242.1	34.8	37.9
Vienna, Austria (sample III)	22.5	27.1	706.6	757.8	25.7	32.2

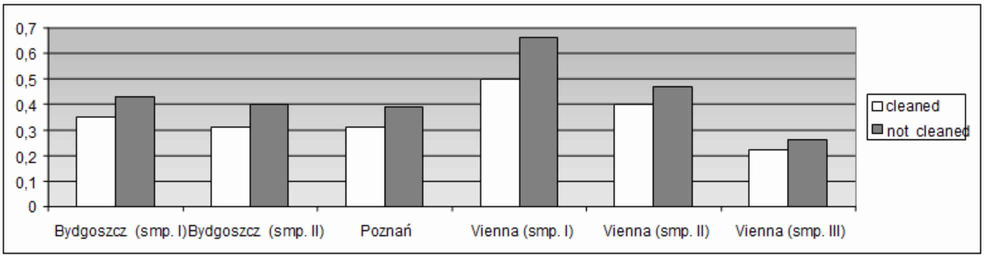

Fig. 2. Mercury content [mg·kg^{-1}] in the bark, samples collected directly from the tree at different places

Graphical presentation of mercury contents in the bark collected both directly from the tree and from the ground below the tree is presented in Figure 3 (cleaned samples). It is evident that the concentration of mercury is higher in the bark on the tree in comparison with the shed bark collected from the ground. Thus, to determine a level of environmental pollution it is very important to collect the samples from the right place - the biological material is continuously evolving over time, and the bark leaved on the ground may decay the analytes.

Table 2

Results of determination of metals in the bark collected from the ground under the plane tree, depending on the way of obtaining the sample

Bark collected from the ground	**Hg [mg·kg^{-1}]**		**Cu [mg·kg^{-1}]**		**Fe [mg·kg^{-1}]**	
	Cleaned	**Not cleaned**	**Cleaned**	**Not cleaned**	**Cleaned**	**Not cleaned**
Bydgoszcz, Poland (sample I)	0.15	0.24	6.7	16.4	155.7	450.5
Bydgoszcz, Poland (sample II)	0.35	0.41	3.4	11.2	122.5	717.3
Poznan, Poland	0.28	0.30	5.7	6.8	148.1	287.0
Vienna, Austria (sample I)	0.35	0.54	2.9	4.6	47.9	81.9
Vienna, Austria (sample II)	0.35	0.40	7.9	10.3	267.6	415.7
Vienna, Austria (sample III)	0.15	0.24	6.7	16.4	155.7	450.5

Bark collected from the ground	**Mn [mg·kg^{-1}]**		**S [mg·kg^{-1}]**		**Zn [mg·kg^{-1}]**	
	Cleaned	**Not cleaned**	**Cleaned**	**Not cleaned**	**Cleaned**	**Not cleaned**
Bydgoszcz, Poland (sample I)	18.6	23.2	387.0	587.6	21.5	36.1
Bydgoszcz, Poland (sample II)	14.3	29.8	508.5	1182.1	149.8	39.1
Poznan, Poland	19.3	21.2	700.3	673.2	7.9	13.5
Vienna, Austria (sample I)	14.5	15.9	897.0	877.1	6.5	7.9
Vienna, Austria (sample II)	38.7	39.6	774.7	908.9	40.6	42.8
Vienna, Austria (sample III)	18.6	23.2	387.0	587.6	21.5	36.1

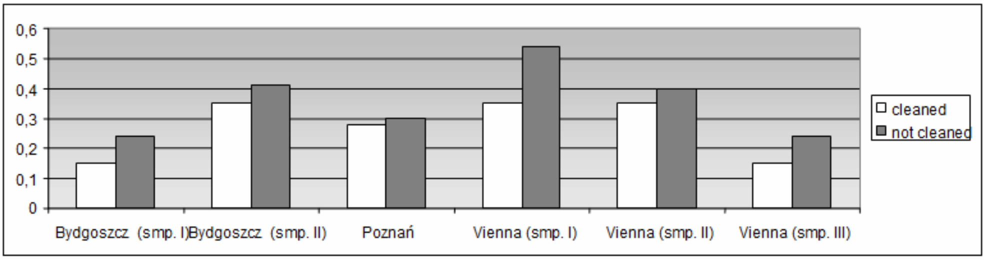

Fig. 3. Mercury content [mg·kg^{-1}] in the bark, samples collected from the ground under the tree at different places

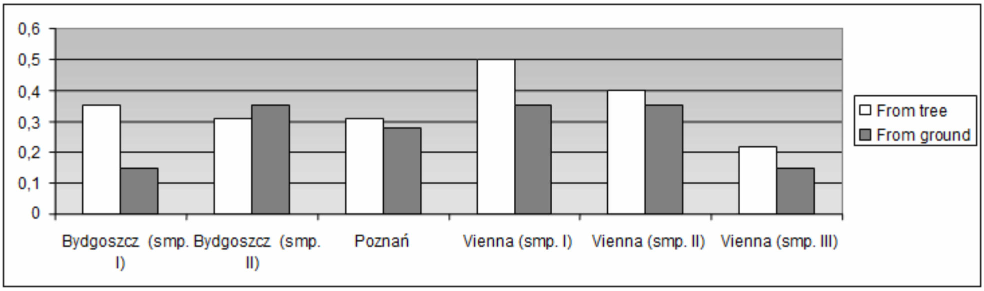

Fig. 4. Mercury content [mg·kg^{-1}] in the bark, cleaned samples collected from the ground under the tree at different places

The samples were taken from different parts of the tree: branches, trunk, and roots, as well as at different places across Poland and Europe. In Table 3 some results of determination of mercury and other heavy metals are given, for the samples collected in Poland, while in Table 4 - for the samples collected across Europe.

Table 3

Concentration of the metals under study [ppm] in the bark of maple-leaf plane tree, at some localization in Poland

Place	**Concentration [ppm]**					
	Hg	**Cu**	**Fe**	**Mn**	**Zn**	**S**
Miedzyzdroje 1	0.084 ± 0.010	4.1	84.5	12.1	9.0	700.7
Miedzyzdroje 2	0.062 ± 0.008	2.5	21.0	6.1	3.2	765.0
Sopot	0.065 ± 0.004	5.9	64.9	15.8	11.9	670.8
Kornik	0.14 ± 0.03	3.3	31.9	23.8	4.4	730.4
Poznan:						
PUE park 1	0.063 ± 0.004	2.9	49.6	4.4	5.7	843.3
PUE park 2	0.049 ± 0.005	2.1	31.4	4.4	2.2	592.2
Niepodległosci Str. 1	0.13 ± 0.02	2.7	39.6	12.7	21.1	523.1
Niepodległosci Str. 2	0.12 ± 0.02	1.7	12.2	4.5	7.5	494.1
Niepodległosci Str. 3	0.14 ± 0.02	2.5	25.1	5.4	3.6	621.4
Niepodległosci Str. 4	0.09 ± 0.04	8.6	156.9	19.9	17.6	584.8
Sniadeckich Str.	0.16 ± 0.03	10.0	222.9	19.1	24.4	1067.2

Table 4

Concentration of the metals under study [ppm] in the bark of maple-leaf plane tree, at some localization in Europe

Place	**Concentration [ppm]**					
	Hg	**Cu**	**Fe**	**Mn**	**Zn**	**S**
Berlin	0.063 ± 0.003	4.6	39.8	25.1	16.5	1016.6
Padova	0.086 ± 0.001	16.5	128.8	17.2	14.7	1075.5
Rome	0.067 ± 0.005	12.6	171.3	14.6	9.5	1345.3
Vienna	0.067 ± 0.004	9.0	80.8	24.7	21.8	920.6
Slovakia - Tatrzanske Lomnice	0.078 ± 0.006	4.5	46.0	201.7	52.0	485.9

As it may be deduced from an analysis of the results presented in Table 3, the concentration of the metals is different for different samples. This situation may be caused by the fact that the samples were collected from the places of different nature, such as the streets or the green areas (parks). The highest concentration of all the elements under study was detected at Sniadeckich Str. in Poznan. This place is located very close to the city center, moreover, Faculty of Chemistry and a big hospital are nearby. The lowest concentration was detected for a park near Poznan University of Economics (PUE) - this place is quite separated from the city traffic. Sample No. 4 was taken from a young tree at Niepodleglosci Str., Poznan. In this case large concentration was detected of such elements as copper, zinc, and iron. These metals are responsible for proper ontogenesis, growth, and metabolism of the tree, as well as for photosynthesis process. High concentration levels are probably caused by intensive growth of this young tree. At the seaside, concentration levels of mercury are very similar (0.06÷0.08 ppm). Such low concentration is probably related with low emission to the atmosphere, and strong winds to and from the sea, forcing the replacement of the pollutants at a higher distance.

As it may be deduced from an analysis of the results presented in Table 3, the concentration of the metals is from 0.06 to 0.08 ppm. The highest concentration was detected in Padova (Italy) - this fact is probably related with a presence of large mercury deposits nearby. The concentration of manganese for the sample from Slovakia (Tatrzanske Lomnice) is pretty high - 201.7 ppm. It may be justified by the presence of large deposits of the ore of this element in this region, as well as related industry. Unfortunately, this area is poor in pyrite deposits, thus low concentration levels determined for sulfur and iron, in comparison with other European cities. On the contrary, Rome (Italy) area is reach in pyrite deposits, thus the concentration levels of sulfur and iron are high. In the bark collected in Berlin (Germany), lowest concentration levels were detected for such elements as manganese, iron, zinc, and sulfur. Probably, the soil there was drain of its nutrients, these elements included. It proofs the fact that the concentration levels of the metals in the bark are related with the corresponding levels for the soil. Heavy metals are cumulated by the vascular plants, however, in smaller amounts than the moss.

In Table 5 some results are given of the analysis of concentration of heavy metals in different parts of the maple-leaf plane tree (branches, trunk and roots). The samples were taken at Sniadeckich Str., Poznan, Poland.

Table 5

Concentration of the metals under study [ppm] in the parts of the plane tree

Maple-leaf plane tree	**Concentration [ppm]**					
	Hg	**Cu**	**Fe**	**Mn**	**Zn**	**S**
branches	0.26 ± 0.03	6.5	106.3	17.4	9.9	870.1
trunk	0.16 ± 0.03	10.0	222.9	19.1	24.4	1067.2
roots	0.21 ± 0.06	2.0	264.3	14.0	14.5	727.2

As it may be deduced, the highest concentration of the mercury was detected in the branches, while the lowest - in the trunk. It is probably caused by the fact that the adsorption of the pollutants by the bark is conditioned by wet and dry deposition, which in turn is conditioned by the size of the tree crown. Thus, the pollutants carried by the wind are mainly deposited at the branches and leaves. The high concentration of mercury in the roots may be explained by the fact that the pollutants are present in the soil, as a result of the contact with the rain water, and the city-polluted atmosphere.

Conclusions

While analyzing the bark of the maple-leaf plane tree, one may uncover important information about a level of degradation of the natural environment, from the moment of the development of the new bark, to its shedding. What is particularly important is the procedure of sample preparation, as improper treatment of the samples may significantly rise the detected concentration levels. By means of the proposed method, while undertaking the research on plant samples we are able to determine the contents of the pollutants not only at the plant surface, but also inside the plants.

To determine the pollution level one has to collect the samples from different, well-matched places, taking into account not only the localization of the place, but also tree part. One must note that some parts of the tree are subjected to decomposition, sometimes fast.

According to the samples we collected for this research, the highest concentration of the pollutants is usually determined for the crowded city streets, while the lowest - for the parks, even inside urban area.

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BIOKONCENTRACJA RTĘCI I METALI CIĘŻKICH PRZEZ KORĘ PLATANA KLONOLISTNEGO

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Abstrakt: Ciągły rozwój cywilizacyjny, urbanizacja, postępujące uprzemysłowienie sprawiają, iż zawartość rtęci i metali ciężkich w środowisku naturalnym wzrasta i stwarza zagrożenie dla prawidłowego rozwoju wszystkich organizmów. Wydaje się więc celowe, aby monitorować i kontrolować akumulowanie wspomnianych pierwiastków w naszym środowisku. Celem naszych badań było określenie, czy platan klonolistny (*Platanus x acerifolia*) może służyć jako bioindykator zanieczyszczeń powietrza. Drzewo to występuje prawie w całej Europie Środkowej, jest stosunkowo odporne na zanieczyszczenia środowiska, a jego cechą charakterystyczną jest zrzucanie kory. Przeprowadzone badania obejmowały opracowanie procedury analitycznej pozwalającej na analizę ilościową zawartości wytypowanych metali ciężkich: miedzi, cynku, żelaza, manganu, rtęci oraz dodatkowo siarki. Wyniki i poprawność stosowanej procedury przygotowania próbek kory do analizy potwierdzono badaniami materiału certyfikowanego (BCR-062 Olive leaves). Zwrócono szczególną uwagę na wpływ poszczególnych etapów przygotowania próbek (mycie, suszenie, rozdrabnianie itd.), co zwłaszcza w przypadku rtęci ma istotne znaczenie z uwagi na jej dużą lotność. Ponadto przebadano próbki pochodzące z różnych części drzewa: konary, pień i korzenie. Kolejną zmienną były miejsca pobierania próbek, kora drzew rosnących przy ruchliwych ulicach, w parkach, pobrane w różnych miastach Polski i Europy. Całkowitą zawartość rtęci oznaczono, wykorzystując automatyczny analizator rtęci Merkury Analyzer MA-2. Pozostałe metale ciężkie oznaczono na spektrometrze emisyjnym z indukcyjnie sprzężoną plazmą VISTA - MPX firmy VARIAN.

Słowa kluczowe: rtęć i metale ciężkie, ICP-OES

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WASTE MANAGEMENT IN LATVIA

GOSPODARKA ODPADAMI NA ŁOTWIE

Abstract: Waste composition study provides information on the waste composition which can be further used for successive economic, political and organizational decision-making. In waste composition studies important criteria is the determination of proper stratification factors: type of dwelling, source of waste, socio-economic status. Upon waste composition study within the limits of stratification factors which are most important under Latvian conditions, household and related waste are sorted by weight into 14 categories of different material types. From the waste composition research data and the experience of old EU member states, Latvia in theory could reduce the amount of landfilled waste by 275000 Mg, burying only 48% out of the generated waste amount, which could decrease also generated gas (SEG) emissions by more than 8% yearly.

Keywords: waste management, waste composition, waste composition methodology, greenhouse gases

Proper waste management is of essential importance in the effective utilization of resources and development of sustainable environmental planning. The most important element of the waste management system is waste sorting. The most of recycled materials from the total waste flow are found in household and related waste. In Latvia the sorting of household waste is in the initial stage with most of unsorted waste still arriving at waste deposit areas and dumpsites. Such an approach to the household waste management cannot be maintained in the long term since it has a negative effect upon the environment and human health as well as is indicative of the lack of experience and desire by respective institutions towards more effective utilization of waste.

Methodology

One of the most important elements ensuring the analysis of the efficiency of the waste management system planning is the inventory of the existing waste management system which is based on waste composition study. Waste composition study provides information on the waste composition which can be further used for successive economic, political and organizational decision-making. Waste composition studies executed on a regular basis

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provide information about waste flow changes and help to evaluate the projects started in the field of waste management. In order to ensure the assessment of the efficiency of introduced waste management projects it is important to ensure that a unified waste composition study methodology is applied. The development of such methodology is a pending matter also in EU member-states which historically apply different methodologies to waste composition studies.

In the EU several methodologies have been developed and applied, such as ARGUS methodology in research in Austria; Brandenburg guideline, Saxony guideline, North Rhine-Westphalia guideline - in Germany; Nordtest Method - in Denmark; and MODECUM TM (MODE de Caracterisation des Ordures Menageres), developed in 1992 in France, used during research in France and Austria, as well as in several European Commission (EC) research, REMECOM-Project [1].

On the basis of research executed by this methodology EC has performed their analysis, attempting to define the most important waste composition criteria. One of the most important criteria is the determination of proper stratification factors (which of used factors are statistically significant) for the formation of research selection.

Under recommendations provided by EC on the significance of stratification factors used in the waste composition research, on the basis of the outcome resulting from the research by four pilot projects, waste composition research methodology which was in conformity with Latvian conditions was developed. Upon analysis of research outcome, the results as to the significance of stratification factors vary in EU and Latvia (Table 1).

Table 1

Comparison of the most significant stratification factors in EU and Latvia

Stratification factor	Types of factor	EU	Latvia
Type of dwelling	Multi-storey houses	Significant	Significant
	Semidetached houses	Significant	Insignificant
	Private houses	Significant	Significant
	Country estates	Significant	Significant
Size of container	Containers with less than 240 dm^3 capacity	Significant	Insignificant
	Containers with more than 240 dm^3 capacity	Significant	Insignificant
Source of waste	Industry	Significant	Significant
	Private sector	Significant	Significant
Waste collection system		Insignificant	Insignificant
Socio-economic status		Insignificant	Significant

In common with EU provided recommendations also in Latvia the division of the population into groups (or stratification) according to the type of dwelling is significant - division of households into groups of private houses and blocks of flats enhances the outcome of the research. Unlike in EU where the size of a container (less or more than 240 dm^3 capacity) is indicative of the contained waste composition differences, no such a trend has been observed in Latvia. In EU provided recommendations the population socio-economic status has no impact on waste composition, but in Latvia there are statistically significant differences.

Upon waste composition study within the limits of stratification factors which are most important under Latvian conditions, household and related waste are sorted by weight into

14 categories of different material types, with recycled raw materials (paper/cardboard, metal, glass, etc) distinguished apart from other types. The classifier of waste composition is a significant tool for waste composition study since it determines the division of waste, and hence the accessible outcome. The waste classifier used in the research complies with the waste classification prescribed in EU and Latvian laws and regulations. This information is supplemented with classification criteria prescribed in European List of Wastes (EURLW) and Regulation of Waste Statistics. Apart from other plastic waste, for instance, plastic used packing is distinguished, with a more detailed division according to manufacturing technology and chemical composition PET, HDPE, LDPE (Table 2) etc.

Table 2

Classification of plastic waste

Category	Subcategory	Division	Designation	Subdivision
Plastic	Packaging	PET	01 PET	Transparent
				Brown
				Blue
				Green
		Polyethylene HDPE	03 HDPE	
		Polyethylene LDPE	04 LDPE	
		Polypropylene	05 PP	
		Polystyrene	06 PS	
		Foam polystyrene	N/A	
		Other	(07-20)	
	Other (than packaging)	*Other*		*Other*

For selection of the sample to be sorted a random selection is used in the amount of $2\% < x < 1\%$ out of the amount referable to the time period (such a selection amount is sufficient, on the ground of the pilot project research outcome). Out of the total waste flow, within the limits of stratification factors which are most important under Latvian conditions, a random selection of a waste load takes place since selecting a waste load for sorting makes it easier to meet selection formation requirements, decreases the expenditure of time and enables to make a better assessment of the total situation within a wider area, as well as the variation of results does not exceed the rate limits, providing population representing results. The amount to be sorted is selected by *coining and quartering* method:

a) waste of the selected population group is collected in the basket of a lorry;
b) the load is dumped in the area prepared in advance;
c) the dumped load is divided into two equal parts (*coining*);
d) by random selection technique one of the waste heaps is selected;
e) the selected heap is mixed;
f) the heap is divided into four equal parts (*quartering*);
g) by random selection technique one of four distinguished heaps is selected;
h) in accordance with the developed waste classifier, a certain amount of the selected heap is sorted (240 dm^3).

By applying the developed methodology 111 measurements of waste composition were executed in various waste handling and management regions. Measurements were executed in 4 waste handling and management regions out of 10, with the aim to cover, as far as possible, more diverse parts of the territory of Latvia.

The results of the waste composition research executed on the ground of the developed methodology have been used in the preparation of Latvia's statistical reports with regard to types of packaging generated in 2005, 2006 and 2007 and, the resource recovery capacity in the Republic of Latvia, in compliance with Commission Regulation 2005/270/EC [2]. The results can also be used to assess the operation of the segregated waste collection system, to analyze the efficiency of the recovery of recycled resources contained in household and related waste, as well as to assess the running of waste decomposition caused greenhouse gas emissions decrease programmes, etc.

Results

Upon accession to the EU, Latvia undertook to comply with a chain of requirements regarding the decrease of landfilled municipal waste; by the middle of 2010 the amount of biologically treated landfilled waste shall be reduced to 75% out of the amount of biodegradable waste landfilled in 1995; in 2010 regeneration of the used packaging shall be ensured in the amount of 54% out of the amount placed in the market in 2010 [3].

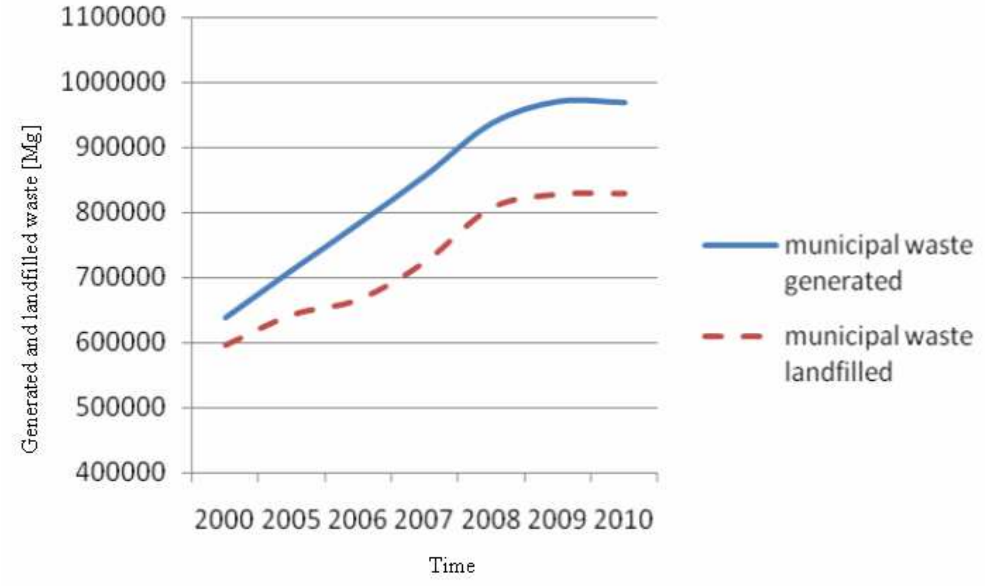

Fig. 1. Projection of the increase in the amount of generated and landfilled waste

Following projections of the difference between the waste amount generated and the waste amount landfilled which have been performed by using the correlation of the waste amount increase with changes of the National Gross Domestic Product (Fig. 1), in order to achieve the prescribed quantitative objectives in respect of the decrease of only biodegradable waste and used packaging landfilling, Latvia shall by the middle of 2010 reduce the amount of landfilled waste by 150000 Mg out of the 2007 level. Taking into account that Latvia already in 2007 failed to meet the requirements for regeneration of used packaging by 25000 Mg (the above amount of used packaging was not collected and thus arrived in waste deposit areas), as well as the fact that at present the landfilling of biological waste in Latvia is underdeveloped, meeting the deadline for these objectives will be very complicated [2]. To ensure their accomplishment the existing waste handling and management system shall be changed, furthering the recovery of recycled raw materials.

856000 Mg of household and related waste were generated in Latvia in 2007 [4]. Out of this amount 724000 Mg or 85% were buried in waste deposit areas or waste dumps. While applying the developed methodology to waste composition studies it was concluded that out of the total buried waste amount, in theory 258000 Mg or 36% could be used as recycled raw materials. Most of recycled raw materials having arrived in waste deposit areas or waste dumps by weight comprise glass, plastic and paper/cardboard. The proportion of biodegradable waste in landfilled waste constitutes 203000 Mg or 28% (Fig. 2).

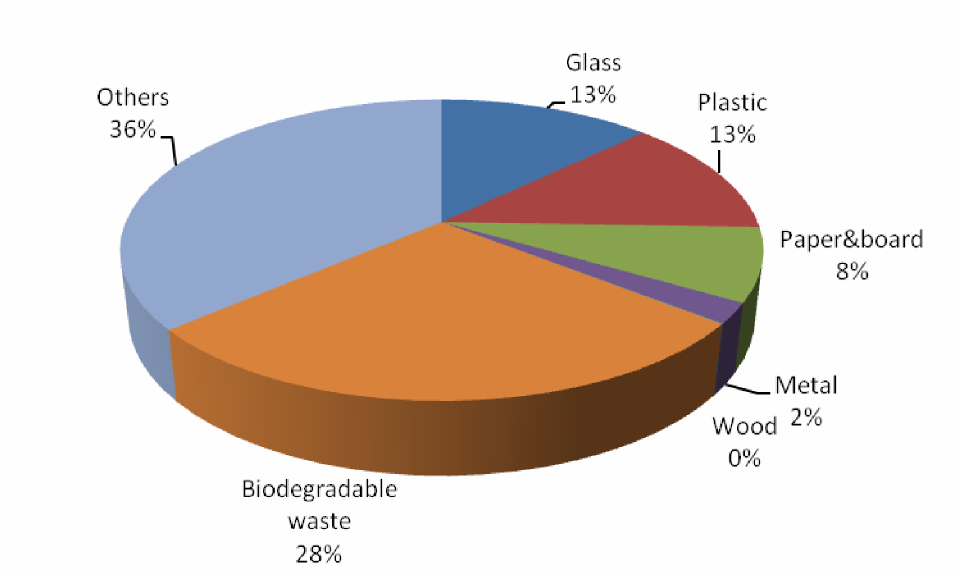

Fig. 2. Composition of landfilled waste in Latvia in 2007

Discussion and conclusion

Being guided by the experience of old EU member states where recycled raw materials are recovered in the amount of 75% [4] and where biodegradable waste is channelled to composting equipment in the amount of nearly 60% [5], as well as following the data obtained from the waste composition research and the experience of old EU member states, Latvia in theory could reduce the amount of landfilled waste by 275000 Mg, burying only 48% out of the generated waste amount. To ensure the above objective a segregated waste collection system shall be developed by prescribing the number of necessary segregated waste collection containers and planning their placement according to the population distribution structure and the number of population, as well as by raising the community motivation to participate in the sorting of waste (to change the tariff policy).

On the ground of the results obtained from the waste composition study and waste density showings, determined by assessing the weight of specific type of waste material in the container with 240 dm^3 capacity (5 measurements have been executed for each type of material), for accepted container capacities in the three container segregated waste collection system and, their removal frequency, it can be calculated on a provisional basis that in order to ensure the maximum reduction in the amount of landfilled waste according to showings of 2007, nearly 30000 extra segregated collection containers shall be placed (Table 3).

Table 3

Number of extra containers necessary for segregated waste collection

Type of material	**Total weight of landfilled waste [Mg]**	**Density [Mg/m^3]**	**Total capacity of landfilled waste [m^3]**	**Capacity of containers to be placed [m^3]**	**Frequency of container removal [times per year]**[**]	**Necessary number of segregated waste collection containers**
Glass	20124	0,4074	49396	1.1	12	3742
Plastic	20123		698685	1.1	52	13002
PET	*4024*	*0.0207*	*194256*			
LDPE	*1867*	*0.0327*	*332324*			
Other	*5232*	*0.0304*	*172105*			
Paper&board	12384	0.0562	220355	1.1	24	8346
Metal[*]	3096	0.0687	45065			
Biodegradable waste	119770	0.2294	522101	1.1	78	6085

[*] In three containers segregated waste collection system, metal along with plastic is collected in one container

[**] Accepted showing

Taking into account the present economic situation in the state, so considerable increase of the number of segregated waste collection containers is not possible without heavy expenses. In order to purchase plastic containers with 1.1 m^3 capacity, more than 5,000,000 EUR [6] shall be invested in the segregated waste collection system. However, if these expenses are compared with total resources shifted by the state into waste handling and management, with projects often implemented without any detailed analysis of their further running, EUR 5,000,000 is not a large amount at all.

Investment of financial resources in the increase of the number of segregated waste collection containers would not only contribute to the economy of primary resources for generation of various new products, but also would enable a diminished environmental mechanical pollution from waste, lessen the utilization of land for the construction of waste deposit areas, as well as decrease greenhouse gas emissions.

Irrespective of the fact that recently the waste disposal system in Latvia was put in order, by closing waste landfills which did not meet environmental protection requirements and creating modern waste disposal areas, not all of them ensure effective collection and usage of CH_4, which is a lot stronger SEG gas than CO_2. Waste composition has the main role as to the amount of generated gas in the waste disposal area. Paper&board and biodegradable waste are types of materials which during their decomposition generate considerable amount of CH_4. Irrespective of the fact that plastic, glass and metal waste do not generate or generate very little amount of CH_4, the presence of these types of materials also has a negative effect upon the collection of CH_4. They substantially make the construction of the gas extractive system difficult [7].

Maximum decrease of Paper&board and biodegradable waste disposal, following their CH_4 generation potential (Table 4) would ensure the decrease of methane generation by 32,000 Mg in 2007, equivalent to 672,000 Mg of CO_2. It is prognosticated that in 2010 this showing could already reach 36,500 Mg or 766,500 Mg of CO_2.

Table 4

Potential of methane for various types of waste materials [7]

Type of material	Possible extraction of CH_4 [m^3/kg]
Mixed municipal waste	0.204
Paper&board	0.369
Biodegradable waste	0.343

By ensuring maximum effective operation of the segregated waste collection system, Latvia could decrease generated SEG emissions by more than 8% yearly. In such a way the total amount of Latvia's SEG emissions could be reduced from 9,275,000 Mg CO_2 [8] equivalent to 8,600,000 Mg.

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GOSPODARKA ODPADAMI NA ŁOTWIE

Abstrakt: Badanie odpadów pozwala na uzyskanie informacji na temat ich składu, które mogą być wykorzystywane przy podejmowaniu decyzji ekonomicznych, politycznych i organizacyjnych. W badaniach składu odpadów ważnym kryterium jest określenie właściwego czynnika stratyfikacyjnego, tj.: rodzaju mieszkania, źródła odpadów, statusu społeczno-gospodarczego. Po badaniu składu odpadów w zależności od czynnika stratyfikacji, który jest najważniejszy w warunkach łotewskich, odpady z gospodarstw domowych są sortowane według masy w 14 kategoriach różnych rodzajów materiałów. Wykorzystując badania dotyczące składu odpadów i doświadczenia starych krajów członkowskich UE, na Łotwie w teorii można zmniejszyć ilość składowanych odpadów o 275 000 Mg, składując tylko 48% ich ilości, co mogłoby doprowadzić do zmniejszenia emisji gazowych o ponad 8% rocznie.

Słowa kluczowe: gospodarka odpadami, skład odpadów, metodologia składu odpadów, gazy cieplarniane

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Natalia KUTSEVOL[1*] and Tetiana BEZUGLA[1]

INFLUENCE OF STRUCTURE PECULIARITIES OF DEXTRAN SULPHATE-*g*-POLYACRYLAMIDE ON FLOCCULATION PHENOMENA

WPŁYW WŁAŚCIWOŚCI STRUKTURY SIARCZANU DEKSTRANU-*g*-POLIAKRYLAMIDOWEGO NA ZJAWISKO FLOKULACJI

Abstract: Brush-like copolymers of Polyacrylamide grafted onto Dextran Sulphate backbone were tested as flocculation aids in polydisperse kaolin suspension. It was shown that flocculation efficiency of these copolymers is significantly dependent upon their internal structure. Both the kinetics of flock sedimentation and supernatant clarification of suspensions are the function of macromolecule conformation of copolymers in water solution. The most efficient flocculants are copolymers with high molecular mass but low value of R_z^2/M_w.

Keywords: graft copolymers, polyacrylamide, dextran sulphate, flocculation, kaolin suspension

Flocculant-assisted processes are commonly used in mineral industry, ceramic manufacturing, wastewater treatment, etc. Water-soluble polymers such as high-molecular-weight non-ionic and ionic *Polyacrylamides* (PAA) are known to be effective flocculation aids. The flocculation phenomena are affected by a bundle of factors such as flocculant characteristics, dosage, dilution and slurry properties (pH, ionic strength of solution, temperature, solid content, particle concentration, their size, etc.) [1]. But the most important factors are the conformation and adsorption of polymeric flocculants. These two characteristics of polymers and polymer-surface interaction substantially determine a mechanism of flocculation: a) depletion flocculation [2] is caused by the exclusion of the free polymer from the antiparticles region; b) bridging flocculation [3] is caused by a polymer adsorbed on the surface of a particle in an extended conformation. PAA chains adsorb on kaolin particle surface, so being in expanded conformation in water solution PAA-grafted copolymers may act as bridging agent.

Copolymers obtained by grafting Polyacrylamide chains onto polysaccharide backbone are thought to improve the flocculation efficiency of linear PAA due to their branched

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architecture. Moreover, these copolymers seem to be perspective as task-oriented materials because they can be undergone controlled transformations through the variation of numerous parameters such as initial polymer architecture, average degree of polymerization, nature and flexibility of backbone, distance between grafts, their lengths, etc [4].

To obtain polymer systems corresponding to the theoretical model of cylindrical polymer brushes [5] we have synthesized water-soluble branched polymers by grafting of flexible synthetic Polyacrylamide chains onto Dextran Sulphate backbone. Dextran Sulphate molecule due to strong repulsion of charged functional groups of polyion chain (2,3 sulphate groups per one polysaccharide ring) has extremely extended conformation in aqueous solution [6]. This study is aimed to investigate the effect of copolymer internal structure of branched polymers Dextran Sulphate-*graft*-Polyacrylamide, which corresponds to the theoretical model of cylindrical brushes on their flocculation efficiency in kaolin polydisperse suspension.

Materials and methods

The samples of graft copolymers of Polyacrylamide grafted to Dextrane Sulphate backbone (M_w = 500 000), designated as DS-*g*-PAA, were synthesised by radical polymerization using Ce(IV)/HNO_3 redox system. Dextran Sulphate (Leuconostoc, M_w = 500 000) was purchased from Fluka, Cerium(IV) ammonium nitrate from Aldrich (CAN), Acrylamide from Aldrich.

The average number of grafting sites per backbone molecule depends on the ratio of concentration of ceric ions to Polysaccharide [7]. The ratio of mol Ce(IV) to mol Dextrane Sulphate was equal to 25 and 50. The length of grafted chains was regulated by amount of monomer AA added to the reactive mixture (Table 1). To obtain the copolymers with “short” and “long” PAA-grafting chains the amount of monomer Acrylamide was double increased (Table 1). All PAA-*g*-DS copolymers were synthesized and purified according to the procedures described in detail in [4].

Table 1

Molar proportion of initiator and polysaccharide ([Ce^{4+}]/[DS]), amount of acrylamide monomer (AA) and Dextrane Sulphate (DS) in graft copolymer syntheses

Sample	**[Ce^{4+}]/[DS]**	**AA [mol/dm^3]**	**DS [mmol/dm^3]**
DS-*g*-PAA, 25 grafts (short)	25	0.1	0.2
DS-*g*-PAA, 25 grafts (long)	25	0.2	0.2
DS-*g*-PAA, 50 grafts (short)	50	0.2	0.2
DS-*g*-PAA, 50 grafts (long)	50	0.4	0.2

The molecular parameters of DS-*g*-PAA in water solution were determined by the static *light scattering* (LS) experiments carried out by using SEMATECH apparatus with laser source He-Ne of wavelength λ = 632.8 nm and the scattering angle range between 30 and 150°. Light scattering results were analyzed in terms of the excess Rayleigh ratio R(q) by using a classical Zimm-plot which yielded the weight-average molar mass M_w and the z-average radius of gyration R_z [8].

All samples were tested as flocculants in kaolin polydisperse suspensions (3 g/dl). Flocculation process parameters were determined for a wide range of flocculant concentrations. All procedures were strongly standardized and were carried out as described in [9]. For the characteristic of supernatant clarification the optical density (D) of supernatant liquid was determined by spectrometer (Varian, Cary 50) at $\lambda = 540$ nm.

Results and discussion

In accordance with the synthesis conditions the SD-*g*-PAA copolymers have theoretically 25 or 50 PAA-grafts per Polysaccharide backbone (Table 1). The increase of AA monomer amount results in the more high macromolecular weight of copolymers. Some differences in expected M_w values, according to the amount of monomer AA added to the reaction mixture (Table 1), can be accounted for the fact that ceric initiator can form the radicals on the polysaccharide backbone and is an interrupter of polymerization simultaneously.

The molecular parameters obtained by light scattering for the copolymers synthesized are reported in Table 2. These molecular parameters have characterized the properties of individual macromolecules in water solution because the concentrations used for LS ($C \leq 8\times10^{-4}$ g/dl) are less in three orders of magnitude than the critical "overlap" concentration $C^* = 1/[\eta]$ ($[\eta]$ - intrinsic viscosity) [6]. Thus the intermolecular interaction can be neglected.

Table 2

Molecular parameters of graft copolymers determined by LS

Sample	**$M_w \cdot 10^{-6}$ [g/mol]**	**R_z [nm]**	**$R_z^2/M_w \cdot 10^3$ [nm^2·mol/g]**
DS-*g*-PAA, 25 grafts (short)	1.31	145	16.05
DS-*g*-PAA, 25 grafts (long)	2.85	142	7.08
DS-*g*-PAA, 50 grafts (short)	1.89	108	6.17
DS-*g*-PAA, 50 grafts (long)	3.83	110	3.16

According to the experimental results all copolymers are characterized by high values of the radii of gyration R_z (Table 2), that indicates the extended conformation of copolymer macromolecules in solution. The samples with 25 grafts have close R_z values in spite of the fact that their molecular weights M_w are practically differ twice. The same tendency is observed for samples with 50 grafts. Comparing the samples with different number of grafts one can see that R_z values for samples with 25 grafts are essentially higher than R_z values of samples with 50 grafts (Table 2). It is obvious that the conformation of the samples with 25 grafts is rather more extended than the conformation of copolymers with 50 grafts. So, these data show that the increase of the PAA-grafts number causes the decrease of macromolecule size in solution.

According to the theoretical model of cylindrical polymer brushes [5] and the experimental results for polymer brushes with non-ionic main chain [10], the increase of grafts number causes the increase of the main chain rigidity. Also, it is shown [10] that the size of cylindrical brush-like macromolecules is a superposition of the sizes of the main and grafted chains. Thus, with increasing of the length of grafts the size of macromolecules should increase, but for series of DS-*g*-PAA the opposite effect is observed. Obviously with increase of the content of PAA-component in DS-*g*-PAA copolymers the electrostatic repulsion between charged groups of polysaccharide component becomes lesser, so the main chain loses rigidity and becomes more flexible and more compact. So, it seems that the highest value of R_z of DS-*g*-PAA samples with 25 short grafts is caused by the more extended conformation of polysaccharide main chain. It is known, that branched polymers have more compact internal structure in comparison with linear ones. The compactness can be estimated by ratio of R_z^2/M_w (Table 2). The less R_z^2/M_w value corresponds to the more compact internal macromolecule structure. So, the compactness of the copolymers increases with the increase of content of PAA-component in graft copolymers.

The flocculation activity of copolymers depends on the macromolecule conformation in water solution, so the internal structure of DS-*g*-PAA copolymers that defines the macromolecule conformation is very important for the flocculation performance.

To find the relationship between the internal structure and the flocculation efficiency of DS-*g*-PAA copolymers we tested them in kaolin flocculation process. The polymer solutions used were very dilute, thus the application of the molecular parameters of copolymers obtained by LS (Table 2) for analysis the flocculation process was correct.

The flocculation activity of DS-*g*-PAA copolymers was revealed to be very high (Tables 3 and 4). The kinetic characteristic of flocculation process is the sedimentation rate (V) of flocks formed after dispersion treatment with flocculation aids. Even low concentrations of copolymers accelerate the aggregation and sedimentation of suspension particles. As usual [11] the sedimentation rate (V) directly depends on the dose of flocculant (Table 3). In accordance with bridging mechanism of flocculation the polymer chains adsorb on the particles and form the flocks [12]. Their dimensions affect their setting. The increase of flocculant concentration causes the formation of the fair-sized flocks those settle faster. Such correlation remains for all copolymers examined.

Table 3

Floc sedimentation rate in kaolin suspensions (C = 3 g/dl*)

Samples	**DS-*g*-PAA 25 grafts (short)**	**DS-*g*-PAA 25 grafts (long)**	**DS-*g*-PAA 50 grafts (short)**	**DS-*g*-PAA 50 grafts (long)**
C [g/dl*]	**V [mm/s]**			
0	0.80	0.80	0.80	0.80
0.00001	1.00	0.80	0.94	0.98
0.00005	1.42	1.60	1.45	1.57
0.0001	1.83	2.11	2.94	3.12
0.0005	2.21	3.25	3.05	4.94
0.001	3.12	4.42	3.17	6.70

* 1 dl = 0.1 dm^3

Table 4

Optical density of supernatant liquid

Samples	**DS-*g*-PAA 25 grafts (short)**	**DS-*g*-PAA 25 grafts (long)**	**DS-*g*-PAA 50 grafts (short)**	**DS-*g*-PAA 50 grafts (long)**
C [g/dl*]	**A_{540}**			
0	1.222	1.222	1.222	1.222
0.00001	1.266	1.115	0.328	1.055
0.00005	1.650	1.240	0.470	0.820
0.0001	1.491	0.980	0.759	0.507
0.0005	1.006	0.329	0.417	0.101
0.001	0.447	0.184	0.186	0.121

* 1 dl = 0.1 dm^3

It is known that flocculation efficiency of Polyacrylamide rises with increasing of its molecular weight. In contrast to linear Polyacrylamide the non-linear copolymers have not direct relations between their molecular weight and sedimentation rate of kaolin flocks. The increase of their M_w not always causes the improvement of their flocculation efficacy (Tables 2, 3). The linear Polyacrylamide macromolecules are of random coil conformation in water solution, but the DS-*g*-PAA polymer brushes have the initial complicated architecture that determines the macromolecule conformation in water solution and shows some peculiarities of flocculation behaviour.

The degree of water clarification was estimated by the optical density of supernatant over the kaolin flock sediment in 20 minutes after treatment with flocculant aids. As it resulted from experimental data more fine clarification occurred when we used the DS-*g*-PAA copolymers having 50 grafts per macromolecule (Table 4). When compare all samples the degree of clarification is in direct dependence on the compactness of DS-*g*-PAA macromolecules in solution (Fig. 1). The fine purification requires the removal of very small particles so the flocculation process is expected to settle down the most of impurities. As it resulted from experimental data the best clarification (the lowest value of supernatant optical density) was achieved when using the copolymer samples with low R_z^2/M values - copolymers DS-*g*-PAA, 50 grafts in a proper concentration.

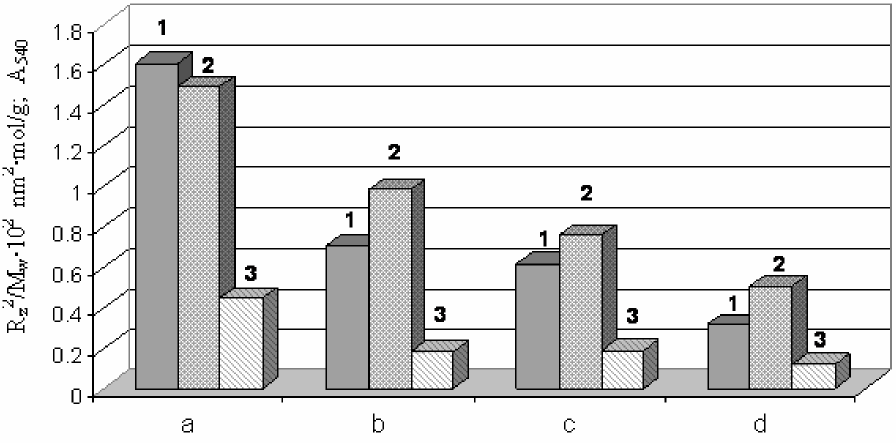

Fig. 1. Molecular parameter R_z^2/M_w (1) and supernatant optical density (A_{540}) after treatment the kaolin dispersion with 0.0001 g/dl (2) and 0.001 g/dl (3) of copolymer flocculants: a - DS-*g*-PAA, 25 grafts (short); b - DS-*g*-PAA, 25 grafts (long); c - DS-*g*-PAA, 50 grafts (short); d - DS-*g*-PAA, 50 grafts (long)

The brush-like polymers with high compactness of macromolecule structure in water solution has an ability to interact with a great number of suspension particles including very small ones. The best results in flocculation process may be achieved when the optimal dose of flocculant aid ensures both the rate of sedimentation and supernatant clarification of required level. Thus, the better flocculant must have high content and suitable density of functional groups that trap suspension fine particles.

Conclusions

The initial architecture and the internal structure of branched DS-*g*-PAA macromolecules determine the flocculation efficiency against the kaolin polydisperse suspension. The high compactness of DS-*g*-PAA, 50 grafts macromolecules ensures high degree of clarification due to the high concentration of functional groups trapping suspension fine particles. Adding in optimal dose these copolymers ensure the high rate of flock sedimentation and high level of supernatant clarification of kaolin polydisperse suspensions.

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WPŁYW WŁAŚCIWOŚCI STRUKTURY SIARCZANU DEKSTRANU-*g*-POLIAKRYLAMIDOWEGO NA ZJAWISKO FLOKULACJI

Abstrakt: Badano wpływ kopolimeru złożonego z poliakrylamidu szczepionego w strukturę siarczanu dekstranu (o strukturze przypominającej szczotkę) na flokulację polidyspersyjnej zawiesiny kaolinu. Wykazano, że skuteczność flokulacji tych kopolimerów zależy od ich struktury wewnętrznej. Zarówno kinetyka sedymentacji osadu, jaki i klarowania się roztworu nad osadem są funkcjami konformacji makromolekuł kopolimerów w roztworze wodnym. Najbardziej efektywnymi flokulantami są kopolimery o dużej masie molekularnej i małej wartości R_z^2/M_w.

Słowa kluczowe: kopolimery szczepione, poliakryloamid, siarczan dekstranu, flokulacja, zawiesina kaolinu

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Polecamy symbolikę zalecaną przez PTChem (Symbole i terminologia wielkości i jednostek stosowanych w chemii fizycznej, Ossolineum, Wrocław 1989; Pure Appl. Chem., 1979, **51**, 1-41).

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Przypisy i tabele, podobnie jak rysunki, zapisujemy jako osobne pliki.

Literaturę prosimy zamieszczać wg poniższych przykładów:

[1] Kowalski J. i Malinowski A.: Polish J. Chem., 1990, **40**(3), 2080-2085.
[2] Nowak S.: Chemia nieorganiczna. WNT, Warszawa 1990.

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