

Grzegorz ŁAGÓD<sup>\*1</sup>, Mariola CHOMCZYŃSKA<sup>\*</sup>, Agnieszka MONTUSIEWICZ<sup>\*</sup>  
Jacek MALICKI<sup>\*</sup> and Andrzej BIEGANOWSKI<sup>\*\*</sup>

## PROPOSAL OF MEASUREMENT AND VISUALIZATION METHODS FOR DOMINANCE STRUCTURES IN THE SAPROBE COMMUNITIES

### PROPOZYCJA POMIARU PODOBIENSTWA STRUKTURY DOMINACJI ZBIOROWISK SAPROBÓW I WIZUALNEJ PREZENTACJI ZMIAN TEJ CHARAKTERYSTYKI

**Abstract:** A large taxonomic diversification of saprobes causes difficulties in practical use of the saprobic system for biomonitoring purpose. In such a case taxonomic levels higher than species level became more popular. Methods based on biocenotic structure can be also used in bioindication. It is known that application of the Shannon biodiversity index based not only on numbers and abundances of species but also on numbers and abundances of easily identified morphological-functional groups gives the same information as saprobe measurements. Moreover, the other structural indices together with the Shannon index can be used to obtain more complete characteristics of saprobe communities. It enables more precise interpretation of biomonitoring results based on dominance structure of organism groups settled at the examined object. The obtained results of the quantitative nature can be compared with a chosen accuracy, however they are difficult to be perceived. The aim of the present work is calculating a similarity of dominance structures characterizing saprobe communities as well as presenting modified methods for visualisation of these structure changes.

**Keywords:** saprobes, activated sludge, biofilm, bioindication, similarity coefficient, dominance structure, dentrite, physical-chemical sewage parameters

Urban sewer systems are settled by saprobe communities which form biofilm on the walls of the sewers and backbones alike to an activated sludge. These organisms cause decrease in pollutant load in sewage before they reach a wastewater treatment plant [1-4]. Species structure of mentioned communities is similar to activated sludge or biofilm in the bioreactor of sewage treatment plant. It is also similar to the structure of organism communities of saprobic zones specified for water bodies. The sewage parameters can be

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<sup>\*</sup> Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, Poland, tel. 081 538 43 22

<sup>\*\*</sup> Institute of Agrophysics, Polish Academy of Sciences, ul. Doświadczalna 4, 20-290 Lublin, Poland

<sup>1</sup> Corresponding Author: G.Lagod@wis.pol.lublin.pl

established based on the presence of microorganisms from the saprobic system living in the sewerage and using sewage as a source of nourishment [5-7].

A large taxonomic diversification of saprobes causes difficulties in the practical use of saprobic system. Thus, taxonomic levels higher than species level became more popular for biomonitoring purposes. Methods based on biocenotic structure (organism distribution among species) can be also used in bioindication [8]. It has been shown that application of the Shannon biodiversity index gives the same information as saprobe measurements. It is known that this information can be obtained using index calculation based not only on numbers and abundances of species but also on numbers and abundances of easily identified morphological-functional groups [5, 9].

The Shannon index  $H$  is calculated according to the equation [10]:

$$H = -\sum_{i=1}^S \Pi_i \log_2 \Pi_i$$

where:  $S$  - species (or morphological-functional group) richness, number of species (or number of morphological-functional groups) and  $\Pi_i$  - relative abundance of the "i-th" species (or the "i-th" morphological-functional group).

Relative abundances, necessary for the calculation of the Shannon index and derived indices, are determined on the basis of the following equation [10]:

$$\Pi_i = \frac{n_i}{n_T}$$

where:  $n_i$  - number of individuals in the "i-th" species or in "i-th" morphological-functional group; and  $n_T$  - total number of individuals in a sample.

Relative abundances take values in the range 0-1; after multiplication by 100 they are expressed as percentages.

Besides the Shannon index other structural indices can be used for bioindication purposes. Among them species richness, maximal value of the Shannon index, MacArthur's index and proportionality index are used most frequently.

Species richness  $\Delta_{Sp}$ , or taxon richness  $S$  is determined by simply summing all taxa belonging to a community [10]. Maximum value of the Shannon index  $H_{max}$  [11, 12] is calculated using the following formula:

$$H_{max} = \log_2 S$$

where  $S$  - number of species (or morphological-functional groups).

Evenness index  $V$  [11, 12] is calculated as:

$$V = \frac{H}{H_{max}}$$

where:  $H$  - observed value of the Shannon index for the studied saprobe community and  $H_{max}$  - value of the Shannon index when all taxa are equally abundant in the community.

MacArthur's index  $E$  [13] is calculated on the basis of the following equation:

$$E = 2^H$$

where 2 - the base of the logarithm.

The value of MacArthur's index,  $E$  is the taxon richness of a community for which the observed value of  $H$  equals  $H_{max}$ . Proportionality index  $P$  [14, 15] is calculated using the formula:

$$P = E/S \cdot 100$$

where:  $E$  - value of MacArthur's index and  $S$  - species richness or morphological-functional group richness for studied community.

The index  $P$  can express "shortage in the taxa number" in the investigated community. The studies may also be based on the other biodiversity indices eg Simpson index [16] determining the probability that two individuals "allotted" in the single trial belong to the same species. The estimator of this index is as follows:

$$D = 1 - \sum_{s=1}^S \frac{n_i(n_i - 1)}{n_T(n_T - 1)}$$

The mentioned indices give more complete characteristics of saprobe communities, in so doing they permit for more precise interpretation of biomonitoring results based on dominance structure of organism groups settled at an examined object. The obtained results of quantity nature can be compared with a chosen accuracy, however they are difficult to be perceived. The graphical methods for comparison of the dominance structure for saprobe communities have been presented in our previous publications as suitable visualisation tools [7, 9]. However, these methods have some inconveniences which implicate the necessity of their modifications. Thus, the aim of this paper is to calculate a similarity of dominance structures characterizing saprobe communities as well as present modified method for visualisation of these structure changes.

## Material and methods

The material used for our previous and present study came from Klimowicz's elaboration [17]. The author presented species composition and individual abundances for communities of activated sludge in specified wastewater classes (characterized by biological oxygen demand ranges: 0÷10, 11÷20, 21÷30 and > 30 g O<sub>2</sub> m<sup>-3</sup>). On the basis of the Klimowicz's data set relative abundances of distinguished morphological-functional groups were calculated ( $\Pi_i$ ). The relative abundances mentioned above were multiplied by 100 to obtain percent fractions. The percent fractions were used in calculations of Renkonen's similarity coefficients for the compared communities of activated sludge [18]. The obtained values of Renkonen's coefficients were sorted using Czekanowski's diagram [19]. Both Czekanowski's and Renkonen's methods are used in phytosociology to sort results of the floral inventory and to specify plant associations.

There are also possible, different than Renkonen measures of studied communities similarity. For example - the factor of similarity [16, 18-21]:

Jaccard and Steinhaus:

$$P = \frac{w}{a+b+w} \cdot 100$$

Marczewski and Steinhaus:

$$P = \frac{w}{a+b-w} \cdot 100$$

Kulczyński:

$$P = \frac{100}{2} \cdot \left( \frac{w}{a} + \frac{w}{b} \right)$$

Sorensen:

$$P = \frac{2w}{a+b} \cdot 100$$

William and Mantford:

$$P = \frac{2w}{2ab - (a+b)w} \cdot 100$$

where:  $P$  - obtained species similarity [%] of two compared communities,  $a$  - number of species in the first community,  $b$  - number of species in the second community,  $w$  - number of common species appearing in both studied communities.

The calculations may be also conducted in the more precise way - after calculation of  $\Pi_i = n_i/n_T$ , for every species in every community, where  $a$  and  $b$  are equal to 1 and  $a$  is a sum of lower values of  $\Pi_i$  I i  $\Pi_i$  II (eg species 1 in agglomeration I  $\Pi_1 = 0.2$ , species 1 in community II  $\Pi_1 = 0.1$ , so the value of 0.1 is selected to the calculations).

The percent fractions of morphological-functional groups were graphically visualized using “radar” plots also called “AMOEBAs” since the publication of the Ten Brink’s paper [22]. During preparation of “AMOEBAs” plots original fractions and their natural logarithms were marked.

## Results

The study results are presented in Figures 1 and 2. It can be seen that changes in pollution level influence dominance structure of the described communities (Fig. 1). The changes in dominance structure are clearly visualized by radar plots with original fractions (grey colour). In the community I (BOD<sub>5</sub> range: 0÷10 g O<sub>2</sub> m<sup>-3</sup>) attached ciliates are dominants and rotifers are subdominants (Fig. 1a). The community II (BOD<sub>5</sub> range: 11÷20 g O<sub>2</sub> m<sup>-3</sup>) is characterized by attached ciliates as dominants and swimming ciliates as subdominants (Fig. 1b). In the community III (BOD<sub>5</sub> range: 21÷30 g O<sub>2</sub> m<sup>-3</sup>) attached ciliates also play role of dominants and new subdominants as flagellates appear (Fig. 1c). Finally, in the community IV (BOD<sub>5</sub> range: >30 g O<sub>2</sub> m<sup>-3</sup>) the dominance of flagellates is observed and amoebas become subdominants (Fig. 1d). The described changes of dominance structure are not presented so clearly using logarithms of fractions.

However, their application enables the extremely low fractions of morphological-functional groups to be observed (Fig. 1a and 1b - black colour).

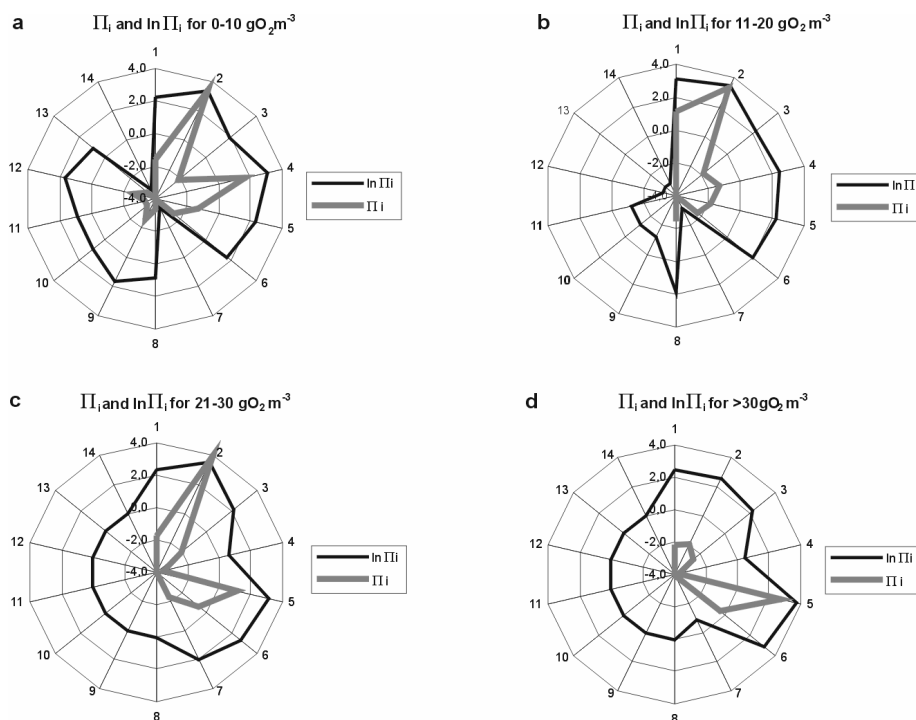


Fig. 1. Relative abundances of morphological-functional groups in specified classes of purified sewage. Explanation: 1 - swimming ciliates, 2 - attached ciliates, 3 - crawling ciliates, 4 - rotifers, 5 - flagellates, 6 - amoebae, 7 - nematodes, 8 - oligochaetes, 9 - gastrotriches, 10 - arachnids, 11 - tardigrades, 12 - copepods, 13 - cladocers, 14 - turbellarians

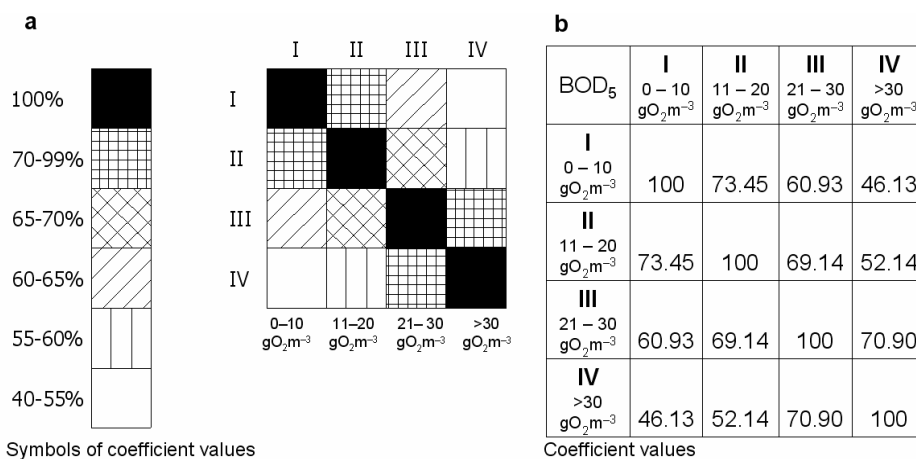


Fig. 2. Coefficients of taxa similarity for specified classes of purified sewage

In studied material two different groups of an activated sludge communities can be distinguished (Fig. 2a and 2b). They are present in wastewater classes with BOD<sub>5</sub> range: 0÷20 g O<sub>2</sub> m<sup>-3</sup> and > 21 g O<sub>2</sub> m<sup>-3</sup>, respectively. Parallely, the community present in class with BOD<sub>5</sub> range: 21÷30 g O<sub>2</sub> m<sup>-3</sup> is similar to those from classes characterized by BOD<sub>5</sub> range: 11÷20 and >30 g O<sub>2</sub> m<sup>-3</sup> to the same degree (about 70%).

Beside Czekanowski's diagram, the other manners for visualization of studied communities' similarity are also possible [16, 21]. Mentioned manners (dendrite and dendrogram) are presented in this paper with use of results obtained with Renkonnen method. A reason for this is that calculation of Renkonnen's coefficient is the most convenient as compared with other methods for determination of taxon similarity coefficient (Jaccard and Steinhaus'es coefficient, Marczewski and Steinhaus'es coefficient, Kulczyński coefficient, Sorensen's coefficient, William and Mantford's coefficient [16, 19-21]).

A dendrite of mutual similarities is obtained by selection of the highest similarity coefficients from Table b (Fig. 2). The community I has one high coefficient and is similar to the community II in 73.45%. The community II has two high values of similarity coefficients and is also in 69.14% similar to community III, which is characterised by the 70.90% similarity to the community IV. The distances inside the dendrite among the communities are: 26.55% between community I and II (because  $100 - 73.45 = 26.55$ , for the 100% similarity the distance would be equal to 0), 30.86% between community II and III and 29.10% between community III and IV - Figure 3.

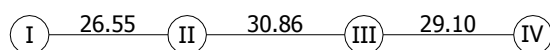


Fig. 3. Distances between communities I, II, III and IV presented in a form of dendrite

Obviously, each of compared communities may have more than 2 high similarity coefficients. In this case, a dendrite becomes branched. It seems, that a dendrite does not give a lot of information but in the case of higher number of the studied communities, a dendrite construction makes easier on arrangement of Czekanowski's diagram.

The highest available level of information may be obtained from a dendrogram. It is created by the gradual connection of compared communities, but joining subsequent communities requires the calculation of mean coefficient on all its values already existing in a dendrogram. The dendrogram presented in Figure 4 may be constructed basing on data presented in Table b (Fig. 2). The sum of species similarity coefficient's equals to:  $73.45 + 70.90 + 57.085 = 201.435$  (Fig. 4). At assumption of different dimensions (Fig. 5) the sums are equal to:  $73.45 + 65.035 + 56.39 = 194.875$  and  $70.90 + 60.64 + 60.17 = 191.71$ . In such cases the sums appear to be lower, thus array of communities according to taxon similarity is worse.

The dendrogram helps to observe the dependence similar to the one visible in Figure 1 where communities a and b are more similar one to another than to communities c and d. The latter show the mutual similarity especially when the values of  $\ln \Pi_i$  are presented.

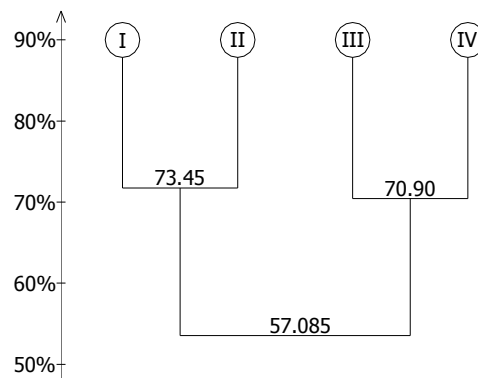


Fig. 4. Distances between communities presented in a form of dendrogram

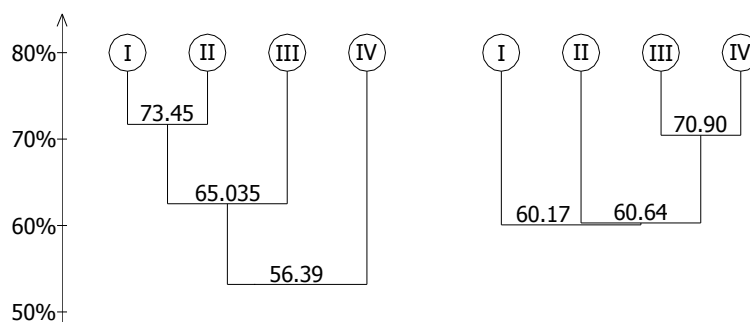


Fig. 5. Distances between communities presented in a form of dendrograms with different construction

## Conclusions

1. Changes in wastewater pollution level cause differences in dominance structure of saprobe communities.
2. Percent fractions of taxa calculated for biomonitoring purposes can be also used for determination of similarity coefficients between compared communities.
3. Saprobe fractions below 1% are clearly visualized as logarithm values.
4. Changes in dominance structure are the best observed using radar plots called "AMOEBAs".
5. Czekanowski's diagrams can be used for sorting of communities considering their taxa similarity.
6. The satisfactory diversification may be obtained when dendrogram is used to the similarity visualization.

## Acknowledgements

This work was supported by the Ministry of Science and Higher Education of Poland, No. 4949/B/T02/2008/34

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## **PROPOZYCJA POMIARU PODOBIENSTWA STRUKTURY DOMINACJI ZBIOROWISK SAPROBÓW I WIZUALNEJ PREZENTACJI ZMIAN TEJ CHARAKTERYSTYKI**

Wydział Inżynierii Środowiska, Politechnika Lubelska  
Instytut Agrofizyki PAN

**Abstrakt:** Zróżnicowanie taksonomiczne systemu saprobów wiąże się z trudnościami w jego zastosowaniu do celów biomonitoringu. Dlatego też wprowadzenie do bioindykacji jednostek taksonomicznych wyższych rangą od gatunku oraz metod opartych na strukturze biocenotycznej staje się powszechne. Zastosowanie indeksu bioróżnorodności Shannona, bazującego nie tylko na liczbie i ilościowości gatunków, lecz również na liczbie i liczebności łatwo identyfikowalnych grup morfologiczno-funkcjonalnych, jest tak samo przydatnym źródłem informacji jak pomiary saprobowości. W celu otrzymania pełniejszej charakterystyki badanego obiektu obok indeksu Shannona stosowane są także inne indeksy struktury biocenotycznej. Użycie tych indeksów umożliwia bardziej precyzyjną interpretację wyników biomonitoringu uwzględniającego strukturę dominacji. Ze względu na ilościowy charakter danych wyniki mogą być porównywane z dowolną dokładnością, jednakże są mało czytelne w odbiorze. Celem prezentowanej pracy jest wyznaczenie podobieństwa struktury dominacji zbiorowisk saprobów i przedstawienie metod wizualizacji zmian badanych struktur.

**Słowa kluczowe:** system saprobów, osad czynny, błona biologiczna, bioindykacja, współczynniki podobieństwa, struktury dominacji, dendryty, parametry ścieków