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STRUCTURE OF BENTHIC DIATOMS TAXOCENES IN MODERN CONDITIONS (Crimea, The Black Sea)

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ABSTRACT

The total updated list of benthic diatoms from the Crimean coast, including 409 species and intra-species taxa has been prepared. More than a half (55%) of general floristic richness of the Black Sea benthic diatoms is formed by species of the Crimean coast. 48 new and 21 rare species have been revealed for Crimean coast, five of them were recognized as newly-found for the whole Black Sea and 4 species were new for science.

The comparative structural analysis of benthic diatoms taxocenes from two water areas of Crimean coast have been carried out and based on methods of multivariate statistics. Those areas (Laspi and Sevastopol bays) have substantially differed by content of heavy metals and other pollutants in bottom sediments.

The features of spatial organization of benthic diatoms habitats have been investigated for both bays. Statistically significant taxocenotic complexes and subcomplex groupings of diatoms were revealed in each of the bay. Development of diatom taxocene in Laspi bay is caused by worsening of optimal environmental conditions from the central part of the bay towards the both more shallow and deep-water zones. The peak of species richness values coincides with 16-20 m depth, and characterizes the middle sublittoral zone that is the most optimal one for diatom algae inhabitation.

In Sevastopol bay the level of toxicants' content in bottom sediments and water depth are the leading abiotic factors influencing on peculiarities of diatom taxocene structure. The differences in the structural pattern can be caused by presence an eurybiontic species and species having the highest parameters of development within the certain biotope at all stations of the investigated water area.

Lists of principal species contributing the most input into similarity within taxocenotic complexes of each bay were compounded. Inter-complex differences in taxocenes structure are mostly pronounced and probably caused by different response of discriminating species to a high level of

toxicants. Structural differences at sub-complex level are less pronounced and can be conditioned by similar reaction of discriminating species on joint influence of key environmental factors within a certain bay. The most significant discriminating species can also be considered as indicators of the diatom taxocenes condition under comparative assessment of biotopes subjected to miscellaneous anthropogenic load. It is proposed to consider *Tabularia tabulata, Amphora proteus* and *Nitzschia reversa* as indicators of conventionally healthy biotopes, whereas *Tryblionella punctata, Diploneis smithii* and *Nitzschia sigma* can be considered as indicators of biotopes subjected to persistent technogenic impact.

Keywords: benthic diatoms, *BACILLARIOPHYTA*, multivariate statistics, pollutant, Crimea, the Black Sea.

INTRODUCTION

Benthic diatom algae *(BACILLARIOPHYTA)* are leading among all other groups of microphytobenthos by abundance of population and species richness. They are dwelling in all biotopes of sublittoral from a surf zone up to depth of 50-70 m. They have an important role in matter and energy transformation, self-purification processes and in an oxygen balance of coastal water areas. Benthic diatoms are closely associated with certain biotope and directly subjected by environmental factors. It allows consider them as the appropriate indicator of anthropogenic impact during the complex monitoring of sublittoral ecosystems.

Benthic diatom taxocenes in the Western and North-western sectors of the Black Sea are most examined, whereas the shores of Crimea and Caucasus are relatively poorly investigated. The information about diatom’s flora is almost lacking for the Southern and South-eastern parts of the Black Sea.

MATERIAL AND METHODS

The results of studies based on the review of literary data (Nevrova, 2003) and our own materials on benthic sampling survey performed in August 1994 nearby mouth of Sevastopol bay (Nevrova, 1999) and in July 1996 in Laspi bay (Nevrova, Revkov, 2003) (Figure 1).

Samples were taken by the Petersen grab on various types of substrate within range of depths 0.5-52 m (Nevrova et al., 2003). The quantitative counting of mass species, i.e. having abundance more than $7{,}86\times10^4$ cells per cm^2, was performed and recalculated per 1 sm^{-2} of substrate. Density of those species which have not been included in to the quantitative calculation, but have found in samples, was considered to be equal to 10 cells/cm^2 in the further counting. Complete taxonomic analysis of diatoms on slides prepared by standard technique of cold burning in acids was carried out

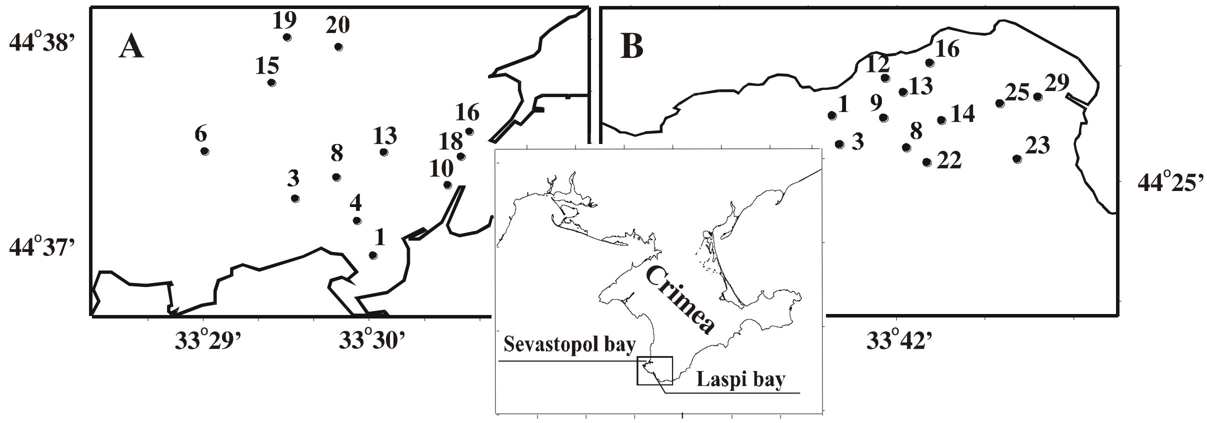


Figure 1. The schematic map of the sampling areas nearby Sevastopol bay mouth (A) and in Laspi bay (B)

The comparative analysis in diatoms taxocene structure features have been fulfilled by application of multivariate statistical algorithms and software package PRIMER (Clarke, Warwick, 2001). Clustering, PCA and nMDS ordination techniques were used for distinguish the group of stations in relation to different environmental conditions (Carr, 1997). Significance of differences between separated group of stations was tested by using permutation/randomization methods (ANOSIM test). The Spearman rank correlation coefficient (ρ) have been evaluated for detection the combination of environmental factors which attains a best match of the high similarities (low rank) in the biotic (abundance data) and abiotic matrices, i.e. to recognize a set of abiotic variables "best-explaining" the spatial alterations in benthic diatoms community patterns across the surveyed bottom area.

Based on the results of PCA analysis, two principal environmental components (PCs) have been revealed: PC1 (making 58 % of total variation explained) is associated with gradient of several heavy metals (Pb, Cu, Mn and Cr) concentration across study area, and PC2 (23 %) can be associated with changes in COC (DDT and PCBs) content in upper 2-4 cm layer of sediments. The contamination gradient has formed by 7 heavy metals: Hg, Cu, Pb, Zn, Ni, Cr, Mn and chlorine-organic compounds was investigated in surveyed water area and possible effect of toxicants on structural characteristics of benthic diatom taxocene was assessed.

SIMPER data analysis was performed for to provide additional information concerning which species are principally responsible for similarity within distinguished benthic assemblages (indicator species) and for differences between such taxocenotic complexes (discriminating species).

Species Diversity of the Benthic Diatoms Taxocene of the Crimean Coast

The list of benthic diatom algae of Crimean coast has been prepared on the basis of literary and own data (Nevrova et al., 2003) and in accordance with the system of higher taxa proposed by Round F.E. (Round et al., 1990). The list includes 409 species and intra-species taxa of benthic diatoms. Meanwhile, in the North-Western region of the Black Sea were found 341 species and intra-species taxa, at the Romanian coast – 353, at the Bulgarian coast – 272 and at the Caucasian shelf – 266.

The highest species richness of diatoms is registered near Crimea that makes about 55 % of total number of the Black Sea benthic diatom species (table 1). Regarding other investigated coastal areas, this relative index was much lower: 36.5 % (Bulgarian coast); 47.3 % (Romanian coast); 35.6 % (Caucasian coast); 45.6 % (North-Western shelf, but without consideration of species from brackish-water estuaries and lagoons). By reviewing of all species dwelling in hypersaline and brackish-water lagoons (Guslyakov, 2003), total updated list of diatoms from NW region includes 604 species and intra-species taxa (i.e. about 80 % of total number of species registered for the Black Sea).

Under comparing diatom species composition of Crimea with other Black Sea regions, the highest extent of species similarity was revealed for North-western region, where Chekanowsky similarity index was 71.3% (for presence/absence transformed species data matrix). This index had a little lower value– 67.4 % in comparison between Crimean and Caucasian coasts. The similarity index of diatom flora between Crimean and Bulgarian coasts was 52.7 %. Among all investigated regions, the lowest degree in species composition similarity index was marked between NW region of the Black Sea and Bulgarian coast (46.1 %).

The list includes 409 species and intra-species taxa, belonging to 81 genera, 45 families, 24 orders, 6 subclasses and 3 classes of division *BACILLARIOPHYTA*. Representatives of class Bacillariophyceae bring 77.5 % of the total number of taxons found belonging to 9 orders, 23 families, 42 genera, 271 species (317 intra-species taxa) of benthic diatoms. Class Coscinodiscophyceae (10.8 %) is represented by 7 orders, 13 families, 19 genera, 39 species (44 intra-species taxa), class Fragilariophyceae (11.8 %) - by 8 orders, 9 families, 20 genera and 42 species (48 intra-species taxa).

The following families are the most representative ones the near Crimean coast: *Bacillariaceae* (4 genera, 58 species), *Catenulaceae* (2 genera, 41 species) and *Naviculaceae* (3 genera, 35 species). The highest richness at genera level have marked for family *Fragilariaceae* (10 genera, 17 species).

The most mass species of benthic diatoms at the Crimean coast, determining the quantitative development of microphytobenthos assemblages are *Striatella delicatula* (Kutzing) Grunow, *Rhabdonema adriaticum* Kutzing, *Grammatophora marina* (Lyngbye) Kutzing, *Tabularia tabulata* (Agardh) Snoeijs, *Licmophora ehrenbergii* (Kutzing) Grunow, *Achnanthes brevipes* Agardh, *Achnanthes longipes* Agardh, *Cocconeis scutellum* Ehrenberg, *Navicula pennata* A. Schmidt var. *pontica* Mereschkowsky, *Navicula* *ramosissima* Agardh*, Berkeleya rutilans* (Trentepohl) Grunow*, Diploneis smithii* (Brebisson) Cleve, *Caloneis liber* (W. Smith) Cleve*, Trachyneis aspera* (Ehrenberg) Cleve*, Pleurosigma angulatum* (Queckett) W. Smith*, Amphora proteus* Gregory*, Amphora coffeaeformis* (Agardh) Kutzing*, Bacillaria paxillifera* (O. Muller) Hendey*, Nitzschia closterium* (Ehrenberg) Reimer et Lewis*, Nitzschia hybrida* Grunow*, Campylodiscus thuretii* Brebisson.

Table 1. Representativeness of benthic diatoms in different regions of the Black Sea

Compared areas	Total number of species and intra-species taxa	References
Laspi bay	208	(Nevrova, Revkov, 2003)
Sevastopol area	247	(10 issues reviewed by Nevrova et al., 2003)
Karadag area	146	(8 issues reviewed by Nevrova et al., 2003)
Total for Crimean coast	**409**	(21 issues reviewed by Nevrova et al., 2003)
NW region of the Black Sea	341	(Guslyakov et al., 1992; Black Sea …Ukraine, 1998)
Romanian coast	353	(Bodeanu, 1979)
Bulgarian coast	273	(Black Sea … Bulgaria, 1998)
Caucasian coast	266	(Proshkina-Lavrenko, 1963; Nevrova, unpubl. data)
Totally for the Black Sea	**747**	

By results of studies through the last 10-15 years, 4 following species were discovered as a new for science: *Amphora karajeae* Guslyakow, *Amphora macarovae* Guslyakow, *Gomphonemopsis domniciae* (Guslyakow) Guslyakow and *Cymbella odessana* Guslyakow. Five new species for the whole Black Sea were found: *Achnanthes pseudogroenlandica* Hendey, *Cocconeis britannica* Naegeli, *Navicula finmarchica* Cleve et Grunow, *Nitzschia sigmoidea* (Ehrenberg) W.Smith, *Undatella quadrata* (Brebisson) Paddock et Sims. Besides, 21 rare species and 48 new ones for the Crimean coastal water areas were also marked (Nevrova et al., 2003).

In the coast water area of western Crimea benthic diatom taxocenes on the different types of natural and artificial substrates in the near-shore zones of Sevastopol are widely studied since the end of XIX- XX centuries.

The flora of diatoms in the investigated part of Sevastopol's shore accounts 247 species and intra-species taxa, belonging to 3 classes, 23 orders, 40 families, 65 genera of *BACILLARIOPHYTA*. Class Conscinodiscophyceae is represented by 24 species (i.e. 10 % of the total number of species), belonging to 6 orders, 11 families, and 13 genera. Fragilariophyceae is represented by 36 species (14.5 %), relating to 8 orders, 8 families, and 14 genera. Genera *Licmophora* and *Diatoma* are represented by 10 and 5 species respectively, the rest of genera - by 1-3 species. Class *BACILLARIOPHYTA* dominates (75.5 % of total species number), 187 species and intra-species taxa, relating to 9 orders, 21 families, 37 genera are included (Nevrova et al., 2003).

Order Naviculales is the most diversed in number of taxons found : 4 orders, 8 families, 12 genera, 54 species and intra-species taxa. Representativeness of other orders is lower: Baccillariales – 1 family, 4 genera, 34 species and intra-species taxa, Achnanthales – 2 families, 3 genera, 27 species and intra-species taxa, Thalassiophysales – 1 family, 2 genera, 26 species and intra-species taxa. Considerable contribution into the species structure of taxocene makes the genera of *Nitzchia, Amphora, Navicula, Cocconeis* and *Diploneis* (25, 25, 16, 16, 11 species and intra-species taxa, respectively).

As a result of studies in the Sevastopol zone 13 rare species for the Black Sea and 26 new ones for Crimean coast were marked, and one new species for Black Sea was registered - *Achnanthes pseudogroenlandica* (Guslyakov et al., 1992).

The diatom flora of the Southern coast of Crimea was represented by results of studies in Laspi bay (June 1996). The flora of benthic diatoms in the bay is represented by 193 species (208 intra-species taxa), relating to 63 genera, 40 families, 22 orders, 5 subclasses, and 3 classes of *BACILLARIOPHYTA*. The class Coscinodiscophyceae was represented by small number of taxons: 15 species (7.2 % of total number), relating to 3 subclasses, 5 orders, 8 families, 11 genera. In class Fragilariophyceae have been 27 species (13 %), relating to 1 subclass, 8 orders, 9 families, 16 genera. Genus *Licmophora* is represented by 6 species, the rest of genera -by 1-3 species. The class Bacillariophyceae is dominant (79.8% of total number of species) and represented by 166 species and intra-species taxa, 9 orders, 23 families, 36 genera (Nevrova,Revkov, 2003).

Order Naviculales is the most representative in the number of found taxons - 4 suborders, 10 families, 14 genera, 55 species (57 intra-species taxa). Representation of other orders is lower: Bacillariales - 1 family, 4 genera, 26 species and (29 intra-species taxa), Achnanthales - 2 families, 4 genera, 22 species (27 intra-species taxa), Thalassiophysales - 1 family, 1 genera, 22 species (24 intra-species taxa). The significant contribution to the species structure of taxocene is brought by genera of *Nitzschia*, *Amphora*, *Navicula*, *Cocconeis* and *Diploneis* (respectively 24, 21, 1, 17, 14, 10 species and intra-species taxa, respectively).

In the water area of Laspi bay 11 rare species for the Black Sea and 25 new ones for Crimean coast were marked . Among them two species were discovered earlier only in fossils (*Raphoneis amphiceros* Ehrenberg and *Diploneis vetula* (A.S.) Cleve), we have found them as alive. Two new species for Black Sea basin have been discovered for the first time: *Cocconeis britannica* Naegeli and *Navicula finmarchica* Cleve et Grunow (Nevrova, Revkov, 2003).

In the area of eastern Crimea benthic diatoms are mostly investigated in Karadag Natural Reserve' water area. By the present time the list of benthic diatoms of Karadag coast includes 146 species and intra-species taxa, belonging to 48 genera, 34 families, 21 orders, 3 classes of *BACILLARIOPHYTA*. In class Coscinodiscophyceae 5 orders, 7 families, 7 genera, 17 species and intra-species taxa are marked, in Fragilariophyceae - 8 orders, 8 families, 11 genera, 25 species and intra-species taxa, in Bacillariophyceae - 9 orders, 19 families, 30 genera, 104 species and intra-species taxa. Genera *Nitzschia* (16 species and intra-species taxa), *Amphora* (14), *Licmophora* (10), *Cocconeis* (8) and *Navicula* (7) are most widely represented (Nevrova et al., 2003).

Two new species for Black Sea basin were discovered there: *Undatella quadrata* (Brebisson) Paddock et Sims and *Nitzschia sigmoidea* (Roschin et al., 1992).

The Structure of Diatom Taxocene in ecologically healthy biotope (on example of Laspi bay)

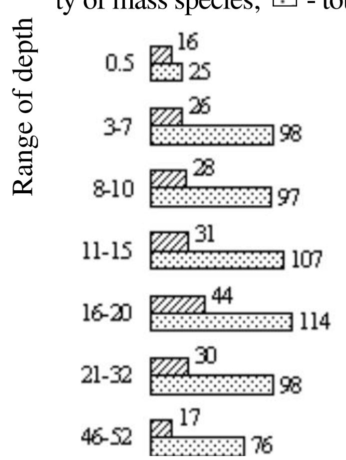
By present, the distinquish of taxocenotic complexes in algology based on predominance of main species and density indexes assemblages. At the present work the analysis of the structural organization of diatoms' assemblages is executed by application of multivariate statistical technique along the routine methods of estimation of species' distribution and alteration of quantitative characteristics of diatoms by depth (Revkov, Nevrova, 2004).

Quantitative estimation of diatoms' development and distribution on depth in Laspi bay. The density of diatoms assemblages ranged from 15.72×10^4 up to 2307.7×10^4 cells•cm^{-2}, averaging 398.9×10^4 cells•cm^{-2} of the bottom area, that is comparable to the similar data for other water areas. For example, in the mouth of Sevastopol bay (the western coast of Crimea) these values changing from 94.32 up to 901.43×10^4 cells•cm^{-2}, averaging 340.64×10^4 cells•cm^{-2}, along the open coast of South-western Crimea is 174.4×10^4 cells•cm^{-2}, within the urban zone where water areas are impacted by moderate level of municipal sewage is 288.0×10^4 cells•cm^{-2}; in the inner, most polluted part of the bay is 18.7×10^4 cells•cm^{-2} (Nevrova et al., 2003). Along the Romanian coast nearby Danube river delta the average density of benthic diatoms was 26.7×10^4 cells•cm^{-2}; while a maximum value 184×10^4 cells•cm^{-2} was registered at 20 m depth (Bodeanu, 1978).

The most mass species determining the general pattern of quantitative development of bottom diatoms in Laspi bay, are colonial *Tabularia tabulata* and *Licmophora gracilis* (average densities are 105.1×10^4 и 58.4×10^4 cells•cm^{-2} , respectively). Other species bring altogether about 61 % of average density of taxocene. The ranged list of the first 20 species is following (in brackets, the percentage of average species density from the total average density of diatom's taxocene is marked): *Tabularia* *tabulata* (25 %), *Licmophora gracilis* (14 %), *Navicula ramosissima* (9 %), *Licmophora abbreviata* (7 %), *Navicula pennata* var. *pontica* (6 %), *Grammatophora marina* (5 %), *Cocconeis scutellum* var. *parva* (5 %), *Cocconeis scutellum* var. *scutellum* (3 %), *Amphora proteus* (2 %), *Licmophora hastata* (2 %), *Nitzschia closterium* (2 %), *Navicula palpebralis* var. *semiplena* (2 %), *Amphora coffeaeformis* (2 %), *Cocconeis euglypta* (1 %), *Bacillaria paxillifera* (1 %), *Diploneis smithii* var. *smithii* (1 %), *Pleurosigma angulatum* (1 %), *Caloneis liber* (1 %), *Thalassionema nitzschioides* (1 %), *Fallacia forcipata* (1 %).

At an estimation of diatoms' distribution on different depth, the total number of species and an abundance of mass forms were taken into account. Quantitative distribution of diatom species in Laspi bay (investigated depth range from 0.5 to 52 м) has bell-shaped trend (Figure 2). A maximal species richness (114 species and intra-species taxa) is registered on the depth of 16-20 m, minimal (25 species and intra-species taxa) is revealed at most shallow zone (depth 0.5 m). The similar tendency is also marked in representativeness (44 species) of mass forms of diatoms. The share of the mass diatom species is rather constant; 3 m deeper it changes within 22-39 % and reaches 57 % at depth of 0.5 m.

Figure 2. Distribution of diatom species number within depth range 0.5 to 52 m:
- quantity of mass species; - total quantity of species



The received data allowed to reveal more exactly the tendency in changes of diatom species diversity by depth. So, following to previously postulated opinion (Proshkina-Lavrenko, 1963; Bodeanu, 1979; Nevrova, 1999) species richness increases up to 20 m depth. Our results have shown that the maximum number of species was found out within depth range 16-20 m and gradually decrease towards more deep waters (up to 50 m).

By the results of clustering and nMDS ordination analysis based on Bray-Curtis similarity index all stations were subdivided into two complexes on 30 % similarity level. Complex I include 5 stations sited at depth 0.5 m on rocky substrate and macrophytes; complex II combines 20 stations at depths 3-52 м on soft substrate and macrophytes. Stations of complex II cover almost whole bottom area of Laspi bay (Figure 3) (Revkov, Nevrova, 2004). At 54% Bray-Curtis similarity level the complex II is subdivided to the core zone and group of the marginal stations. The core zone includes two subareas (II_a and II_b) located in the central part of the bay within depth range 8-46 m. The group of marginal stations (subarea II_c) is located both in nearshore (depths 3-5 and 16 м), and in deeper offshore zone (32-52 м) of Laspi bay. The subarea II_c is the least homogeneous and distanced from complex I more far, than the subareas II_a and II_b. This determines the least floral similarity between the complex I and the marginal zone of the complex II (subarea II_c).

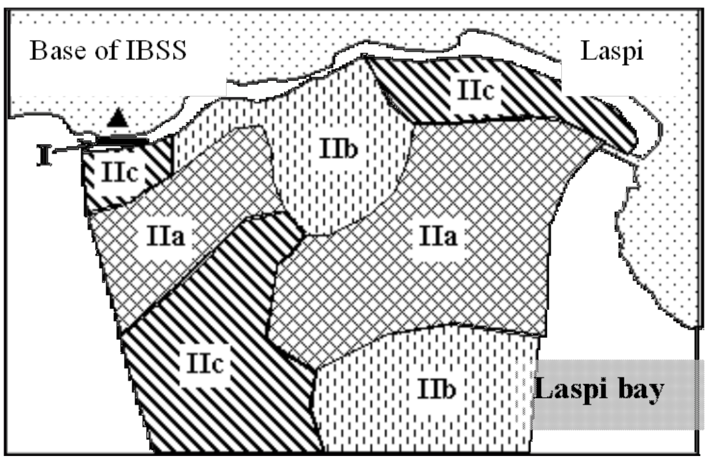


Figure 3. Schematic map of benthic diatom taxocenotic complexes distribution pattern in Laspi bay (by results of the clustering and nMDS ordination analysis) (from Revkov, Nevrova, 2004)

According to the results of clustering and MDS-analysis, the floristic similarity has been revealed for stations located in the neighbouring sites at similar depths but on different type of substrates (soft bottom or macrophytes).

Average similarity within the first complex in comparison with the second complex is appeared to be higher (62.2 against 40.3 %). In the complex I the first top ranged five species are mainly characterized the features of its internal organization, determining 64 % of similarity: *Navicula ramosissima*, *Licmophora gracilis, Grammatophora marina, Tabularia tabulata* and *Navicula pennata* var. *pontica*. In the complex II the similar cumulative percent is achieved at the level of 18 species and the most significant among them is *T. tabulata*.

We determine complex I as *Navicula ramosissima* + *Licmophora gracilis* + *Grammatophora marina* and complex II as *Tabularia tabulata* by dominating species and estimating the species significance by their contribution to intercomplex Bray-Curtis similarity.

In the both complexes among the first top ranged eight species five common ones are marked: *Grammatophora marina, Tabularia tabulata, Navicula pennata* var. *pontica, Cocconeis scutellum* and *Amphora coffeaeformis*. It specifies relative similarity of the complexes, but average distinction between the complexes according to Bray-Curtis similarity is rather high - 75.1 %. There are no pronounced leaders among the species determining this dissimilarity: the contribution of each of the first ten species is changing within 1.79 - 2.99 %. It makes only 29.1 % of the cumulative contribution of all species. The first five of such species are: *Licmophora gracilis* (the contribution to the average dissimilarity between complexes is 2.99 and makes 3.98 % of the cumulative contribution of all species), *Navicula ramosissima* (2.52 and 3.35 %), *Amphora proteus* (2.41 and 3.20 %), *Licmophora abbreviata* (2.37 and 3.16 %) and *Navicula palpebralis* var. *semiplena* (2.21 and 2.94 %). Among the mentioned above species the highest values of dissimilarity has *Licmophora gracilis*, that is the additional basis for its consideration as discriminating species of the examined complexes. Average values of *L. gracilis* population density in the II and the I complexes are different (8087 and 80680 cells•cm^{-2}, respectively). Relative heterogeneity of complex II also characterized by a high level of dissimilarity (64.36%) between its core and marginal zone. The most essential contribution made by such species, as *A. proteus* (1.93 and 3.0 %), *T. tabulata* (1.83 and 2.84 %) and *C. scutellum* var. *parva* (1.71 and 2.65 %).

Earlier marked floristic difference between of complex I and marginal zone of complex II (subarea II_c) proves to be true also at a level of the quantitative data. Stations of complex I are less similar to the stations of marginal zone (subarea II_c), than with the core stations of complex II (sub areas II_a and II_b): corresponding values of dissimilarity I–II_a, I–II_b and I–II_c by Bray-Curtis index are 77.85, 66.70 and 79.54 %, respectively.

Ranked distribution of species is one of the methods for estimation of species diversity. The curve of the rank species' distribution for complex II lies above on the dominance diversity plot and is more flat (i.e. represented by the higher number of species) comparatively with the corresponding curve for the complex I (Figure 4 A). After fractional consideration of complex II (Figure 4 B) distinction in position of curves corresponding to central (II_a, II_b) and marginal (II_c) subareas have been marked. Affinity between II_c curve and curve of complex I have also been shown.

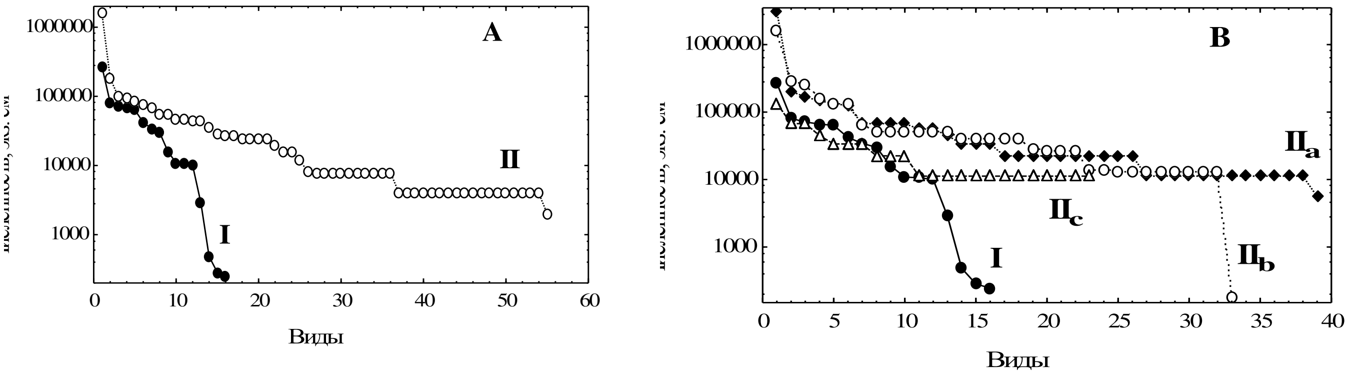


Figure 4. Species rank distribution curves at benthic diatom taxocene in Laspi bay: A – consideration of taxocene at a level of complexes I and II, B – position of species rank distribution curves, corresponding to complex I, to central subareas (II_a, II_b), and to marginal zone (II_c) of a complex II are shown.

As it was mentioned above, the peak on the curve of species richness and representativeness of mass diatom species in Laspi bay have corresponded to depth range 16-20 m. In this case the biotopes corresponding to specified depth can be considered as the optimal zones for development of benthic diatoms in comparison with upper sublitoral. The core of taxocenotic complex II is allocated there (average depth of stations 17±6 м).

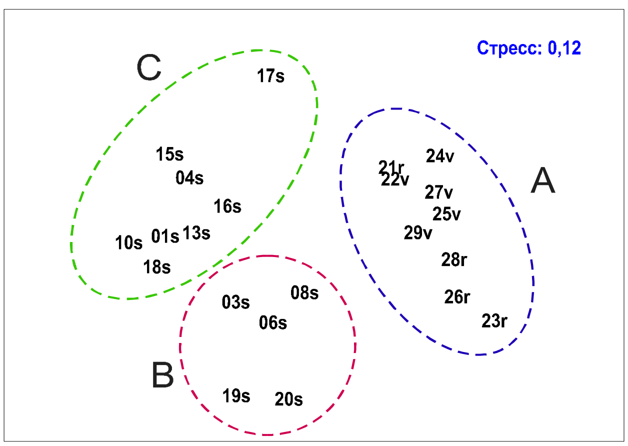
The decrease of diatom species richness from central part towards both more deep water and coastal zones of a bay that can be caused by deviation of ecological conditions from optimum and it can be proved by the position and shaped of dominance-diversity curves corresponding to complexes II_c and I.

The affinity of floristic structure between subareas II_a and II_b is revealed by position of dominance-diversity curves (12 species are common within the list of the first 16 top ranged species). There are: *Tabularia tabulata*, *Amhora proteus*, *Navicula pennata* var. *pontica*, *Navicula palpebralis* var. *semiplena*, *Pleurosigma angulatum*, *Navicula ramosissima*, *Bacillaria paxillifera*, *Amphora coffeaeformis*, *Diploneis smithii* var. *smithii*, *Striatella unipunctata*, *Caloneis liber* and *Nitzschia reversa*. Under the comparison of complex I with subarea II_c, 6 common species were found from the top ranged 16 ones: *Navicula pennata* var. *pontica, Grammatophora marina*, *Cocconeis scutellum* var. *scutellum*, *Tabularia tabulata*, *Auricula insecta* and *Amphora coffeaeformis*. Comparison between complex I and subarea II_c testifyed the similarity of responses of different benthic diatoms' complexes in stressful conditions of habitat. Such adverse factors can be: influence of surf activity, wide range of temperature changes and high level of insulation for shallow water biotopes as well as the unsufficient level of solar radiation for deep-water zone.

The structure of diatom taxocene in polluted biotopes (on the example of Sevastopol bay)

Identification the groups of stations in accordance with level of pollution. Three groups of stations (clusters), corresponding to sites with different pollution levels were distinguished within the Sevastopol bay studied area. Respectively, three certain taxocenotic complexes of diatoms develop within every group of stations (Figure 5). The 1st group (A) corresponded to most shallow zone (average depth 0.5 m, substrate: shell debris and small pebble, dominant species are *Navicula ramossissima* and *Navicula pennata* var. *pontica)*, where concentrations of all toxicants were 10-100 times lower then for two other groups. 2nd group (B) of stations (average depth 22,6 ± 3,0 m) characterized by fine sand substrate and highest concentrations of COC and lead. *Nitzschia sigma* and *Cocconeis scultellum* var *scultellum* are the most predominant species. For stations separated into 3rd group (C) (17,5 ± 2,4 m, silty sediments, *N. pennata* var. *pontica*) the highest level of heavy metals content in sediments was found.

Figure 5. The results of ordination (MDS) analysis: grouping of stations into complexes from Bray-Curtis similarity of diatom algae abundance. Literal notation: samples are taken from



sandy/silty substrate (s); from rocks (r) and mussel valves (v).

The average values of concentration of most toxicants (excepting zinc and PCB) were higher of 10-15 % for stations of group B co Stress 0.12 th group C. Average values of taxocene diversity parameters are represented in Table 2.

Table 2. Average values of diatom algae abundance and species richness indices for three groups of stations.

Group (number of stations)	Average depth (м)	Average Abundance (ind/m2)	Total number of species	Number of mass species	Number of rare species
A (9)	0.5 ± 0.1	290800 ± 59190	58	27	31
B (5)	22.6 ± 3.0	1129430 ± 118970	78	13	65
C (8)	17.5 ± 2.4	4265040 ± 1123840	124	43	81

Rather not high values of stress function (0.11-0.12) have been receiving from MDS analysis, has evidenced about reliable allocation of sample projection on 2-D plot. Besides, there is well pronounced separation of stations into 3 main groups. Differences between groups were statistically significant: global R-statistics = 0.88 at a significance level of 0,1 %; pairwise testing gives R_p values from 0.70 to 0.98, (0.1 %). These results testify statistically reliable differentiation of three complexes of stations within the investigated water area. Such pattern can be explained by influence of pronounced environmental gradient upon structure and quantitative parameters of diatom complexes in surveyed part of the bay.

The results of comparative evaluation of Spearman rank correlation coefficient (ρ) have shown that combination of variables "**Depth+Pb+Mn+Cu+DDT**" have mostly influenced upon structural alteration of diatom taxocene (ρ =0,73-0,75).

The analysis of changes in structure of taxocenotic complexes under toxicants' impact gradient. The lists of principal species contributing the most input into similarity within each pollution-related taxocenotic complexes as well as into dissimilarity between complexes were prepared. The average similarity of stations within every allocated complex, evaluated by Bray-Curtis similarity index, appeared to be rather high: for complex A - 54.5 %, B - 56.3 % and C - 52.2 %.

In complex A four top ranged species bring more than 54 % of the total input into determination of diatom assemblage structure similarity. The relative contribution of two most dominating species *Navicula ramosissima* and *N. pennata var. pontica* (19.09 % and 18.28 %, respectively) 2-5 times exceeds the value of contributions of other indicator species from the leading group the determine the structural features in benthic diatom taxocene. Such indicator forms have highest values of the similarity function, that evidenced about most constant parameters of these species development under adverse influence of environmental conditions comparatively with other species.

Under favorable living conditions these two indicator species are able to form colonies, achieving the maximum in density and biomass.

Navicula ramosissima, N. pennata var. *pontica* as well as other indicator species *Amphora coffeaeformis, Cocconeis scutellum* var. *parva* are euritherm, euribiotic and photophylic forms, living mainly in the upper sublittoral zone (0-10 m) and adapted to its stressful conditions (surf activity, high insulation of seabed, wide amplitude of temperature fluctuations, etc).

In complex B, containing stations with highest level of COC and rather not wide range of heavy metals concentration, the cumulative contribution at a level of 50% form 7 species, among them *Navicula pennata* var. *pontica* also dominates. The relative input of this species into similarity within group makes 19.1%, that 2-4 times exceeds contributions of other species. Besides *N. pennata* var. *pontica, Diploneis smithii var. smithii, Tryblionella punctata var. punctata,* and *Ardissonea crystalline* are the most significant species of this complex. These species are adapted to the low level of bottom illumination, they are inhabitants mainly of the middle (10-20 m) and deeper (20-30 м) zones of sublitoral.

In complex C, uniting stations with the widest range of heavy metals concentration, but the lower level of COC, the two most significant species are *Nitzschia sigma* var. *sigma* and *Cocconeis scutellum* var. *scutellum.* They have identical values of the relative input into intra-complex similarity (7.8 %). The cumulative contribution at the 50 % level is achieved due to 10 top ranged species. Five of them - *Diploneis smithii* var. *smithii, Tryblionella punctata* var. *punctata*, *Navicula pennata var. pontica, Grammatophora marina, Tabularia tabulata* - are common with the list of the most significant species from complex B, that specifies quite close eco-floristic similarity of these complexes. *Tryblionella punctata* var. *coarctata* and *Pleurosigma angulatum* species are shade requiring ones, and being adapted to the low level of insulation can vegetate mainly within the middle and deep water zones of sublitoral.

Thus, using a principle of allocation of biocenotic complexes by dominating species and taking into account the maximal values of similarity function, it is possible to designate complex A as *Navicula ramosissima* + *N. pennata* var. *pontica*, complex B - as *N. pennata* var. *pontica*, and complex C - as *Nitzschia sigma var. sigma* + *Cocconeis scutellum* var. *scutellum.*

Besides the marked top ranged species, the following species can also be considered as indicators of certain spatial groupings of benthic diatoms: in complex A - *Amphora coffeaeformis* and *Caloneis liber,* in complex B - *Tryblionella punctata var. punctata, Diploneis smithii* var. *smithii* and *Ardissonea crystallina,* in complex C - *Diploneis smithii* var. *smithii, Tryblionella punctata var. punctata, Navicula pennata var. pontica* and *Grammatophora marina.* These species are characterized by the most substantial input into similarity within corresponding complexes as well as the most constant parameters of development in diatom's taxocene in polluted water areas of Sevastopol bay.

The contribution of the certain species to the dissimilarity between each pair of distinguished taxocenotic complexes is evaluated by values of dissimilarity function ***D.*** The greatest dissimilarity has been revealed under comparison of complex A with complexes C and B, that can be explained due to the differences in the leading abiotic factors (depth, substrate), and also in the average level of toxicants accumulation in the biotope. The content of heavy metals in bottom sediments for stations of complex A was 5-240 times lower (level of COC was 1.2-62 times lower) in comparison with levels of similar variables for complexes C and B. Additionally, "variability increasing" effect of diatom taxocene structure under conditions of high content of toxicants has been shown.

At the analysis of possible combinations of paired comparison between three examined complexes 6 discriminating species have been revealed: *D. smithii* var. *smithii, N. sigma* var. *sigma, T. punctata* var. *punctata, N. ramosissima, C. scutellum* var. *scutellum* and *A. coffeaeformis*. All of them can also be considered as principal indicator species of allocated taxocenotic complexes of benthic diatoms.

Comparative Assessment of Changes in Structure of Benthic Diatoms under different Levels of Technogenic Pollution Impact

The purpose of this study was to assess comparatively the effect of anthropogenic impact mostly by heavy metals and chlorine-organic compounds on the structure and diversity characteristics of benthic diatom taxocenes from two above-described near-shore water areas of southwest Crimea: Laspi bay and Sevastopol bay. Laspi bay is located near boundaries of marine reserve and is unaffected by technogenic pollution, while Sevastopol bay water area is situated within industrial zone of Sevastopol port where average level of toxicant's content in silty bottom sediments was higher of 5-13-fold (heavy metals) and 22-270-fold (other toxicants) comparatively with Laspi bay.

The further analysis has been performed to test whether such differences in environmental conditions can be influencing upon peculiarities in structure of benthic diatoms assemblages in compared bays.

Allocation of inter-regional taxocenotic complexes and intra-complex groupings of diatoms. Results of multivariate statistical analysis have shown that at similarity level about 25 % all sampling stations are subdivided into 2 separate groups (clusters). Each of group consisted of stations located either in Laspi bay or in Sevastopol bay only. At a similarity level about 37 % each of two clusters is subdivided, in one's turn, into 2 subclusters. In Laspi bay (cluster I) subclusters are A and B, each of them contains 6 stations. Cluster II (Sevastopol bay) is also splitted into two subclusters C and D (5 and 7 stations, respectively).

Results of MDS ordination have also revealed the presence of two not overlapped areas on 2-D ordination plot in which the stations are taken in Laspi bay (I) and in Sevastopol bay (II) have been included (Figure 6).

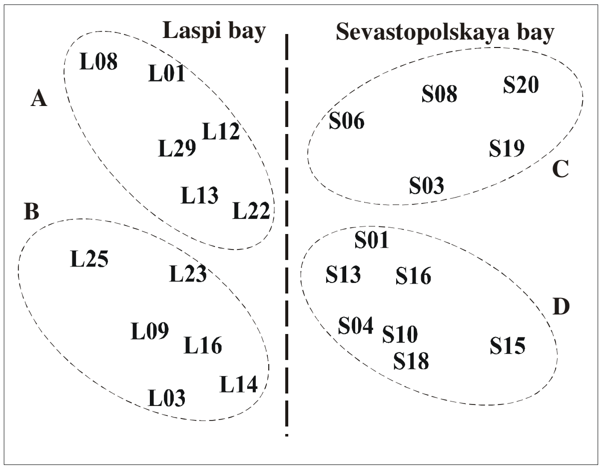


Figure 6. Results of ordination (MDS) analysis: grouping of stations in Sevastopol bay and in Laspi bays into complexes based on Bray-Curtis similarity of diatom algae abundance. The dotted line shows separation of stations between areas and sub-regional groupings (A-D).

Results of ANOSIM test statistically confirmed a differentiation between taxocenotic diatom complexes corresponding to each of two compared locations. Value of global R-statistics was rather high (0.691; at significance level of 0.1%), values of R-statistics for pairwise test have altered within range from 0.79 to 0.98, at significance level of 0.1%. These results also have verified that in each of the compared bays the taxocenotic complexes can be subdivided into two statistically different groupings which characterize by the certain features of diatom structure.

By comparison of 2 surveyed areas (as a whole) there have been was revealed that differences in average abundance of diatoms are insignificant, though average values of total species richness, number of mass and rare species in the healthy bay appear to be higher than in the polluted bay. At the same time, the quantitative characteristics of diatom assemblages in comparison between the allocated interregional groupings are also greatly different (Table 3).

Table 3. Average abundance and other species diversity parameters for 2 main complexes and allocated subcomplex groupings (A-D) of benthic diatoms

Region, grouping	Average abundance (10^6 cells x cm^{-2})	Total number of species	Number of mass species	Number of rare species
Laspi bay (as whole)	3.020±0.562	176	53	123
A	1.079±0.330	145	24	121
B	4.960±2.288	140	47	93
Sevastopol bay (as whole)	2.572±0.413	128	38	90
C	1.132±1.190	78	13	65
D	3.772±0.891	119	36	83

Comparison of structural features of taxocenotic complexes. In the taxocenotic complex of Laspi bay the 11 most significant species (indicator species), determining structural features of taxocene, bring about 48% of total input into average similarity within this complex. *Tabularia tabulata* and *Amphora proteus* are the most top ranged species of this list. The relative contribution of other nine indicator species is less sizeable and decrease from 5.83 % for *Navicula pennata* var. *pontica* up to 2.41 % for *Bacillaria paxillifera.*

In the complex of Sevastopol bay the similar part of total contribution (47.6 %) to average similarity within complex is determined by group of 8 top ranged indicator species (of the total list 128). *N. pennata var. pontica, Diploneis smithii var. smithii* and *Tryblionella punctata* var. *punctata* are leading forms displaying the highest values of their relative contribution (11.23, 9.51 and 5.98 %, respectively). These parameters define the indicator role of the marked species in the given taxocenotic complex which is formed under strong technogenic impact of the biotope. The relative input into average Bray-curtis similarity within this complex of other five significant species is gradually reduced from 4.75 % (*Cocconeis scutellum* var. *scutellum)* up to 3.70 % (*Ardissonea crystallina).*

While comparing the lists of indicator species of two complexes, from 16 species and intra-species taxa only 4 ones appeared to be common. Such low affinity level (1/4) evidences about pronounced eco-floristic difference between the comparing complexes, probably caused by different tolerance of the most indicator species to the severe pollution extent.

For example, *T. punctata* var. *punctata, N. sigma var. sigma* and *A. crystallina* (marked as leading indicator forms only for Sevastopol bay), usually are met in great density in heavily impacted biotopes. Meantime, significant species, common for both bays (*N. pennata* var. *pontica, C. scutellum, D. smithii* var. *smithii* and *F. forcipata),* are eurytherm and eurybiotic forms, widely developing in different zones of sublitoral. A high dissimilarity level was revealed at comparison of taxocenotic complexes in surveyed bays (average dissimilarity is 68.3 %). It testifies to significant differences between the compared water areas in species structure of taxocenes and quantitative development of key species (Table 4).

Table 4. Contribution from the most significant species (discriminating species) into average dissimilarity between ecological-taxocenotic complexes of diatoms at the Laspi bay and Sevastopol bay

Species	*N*, cells•см$^{-2}$ *		D_i.	D	D_i.(%)
Complexes of Laspi bay and Sevastopol bay – average dissimilarity 68.3 %	**Laspi bay**	**Sevastopol-skaya bay**			
Tabularia tabulata (Agardh) Snoeijs	1139775	69825	2.65	1.55	3.88
Amphora proteus Gregory	150667	69817	1.86	1.51	2.76
Navicula pennata var. *pontica* Mereschkowsky	216392	349108	1.82	1.35	2.69
Tryblionella punctata W. Smith var. *punctata*	33	104625	1.79	1.29	2.62
Diploneis smithii (Brebisson) Cleve var. *smithii*	45867	209275	1.80	1.24	2.63
Bacillaria paxillifera (O. Muller) Hendey	52392	104750	1.49	1.19	2.23
Nitzschia sigma (Kutz.) W. Smith var. *sigma*	6592	104542	1.47	1.16	2.15
Caloneis liber (W. Smith) Cleve var. *liber*	45908	226975	1.48	1.02	2.18
Cocconeis scutellum Ehrenberg var. *scutellum*	72117	122050	1.43	1.06	2.10
Fallacia forcipata (Greville) Stick et Mann	32800	157133	1.45	0.95	2.13
Cocconeis scutellum Ehrenberg var. *parva* Grunow	45908	34875	1.24	1.05	1.81
Ardissonea crystallina (Agardh) Grunow	50	104625	1.41	0.92	2.07
Tryblionella punctata W. Smith var. *coarctata* Grunow	50	52383	0.94	0.92	1.38
Rhabdonema adriaticum Kutzing	0	69917	0.93	0.93	1.36
Cocconeis euglipta Ehrenberg	86825	17458	1.16	0.86	1.70
Amphora coffeaeformis (Ag.) Kutzing var. *coffeaeformis*	58975	17483	1.03	0.85	1.50
Nitzschia reversa W. Smith	39292	17425	0.98	0.87	1.44
Pinnularia quadratarea (A. Schmidt) Cleve	8	104800	1.02	0.77	1.49
Lyrella abrupta (Donkin) Guslyakov et Karaeva	6625	69817	0.98	0.72	1.43
Nitzschia lanceolata W. Smith var. *minor* Van Heurck	6550	52258	0.95	0.68	1.44

Note: * ***N*,** cells•см$^{-2}$- average abundance of i-th species in comparing complexes, D_i– absolute and D_i*(%)* – the relative contribution of i-th species in average Bray-Curtis dissimilarity between the benthic eco-taxocenotic complexes, D – dissimilarity function

The most significant indicator species evaluated by their relative contribution into average similarity within complex can also be considered as discriminating species, determining the most contribution to species structure dissimilarity between taxocenotic complexes in compared biotopes. There are *T. tabulata* + *A. proteus* in Laspi bay and *N. pennata var. pontica* + *D. smithii* + *T. punctata var. punctata* in Sevastopol bay.

By consideration of structural-taxonomic differences at the intra-complex level, i.e. between all pair of groupings, the highest average dissimilarity values were recorded for pairs “B-C” and “A-C” (73% and 69%, respectively). For these both pairs *T. tabulata* and *A.proteus* are the leading discriminating forms, bringing the most valuable input into dissimilarity between comparing groupings. These two species are sharply dominated by density in Laspi bay (2-4 times higher than in Sevastopol bay). The similar differences in species structure are also revealed under comparison of other pair of innercomplex taxocenotic groupings (Figure 7). At general, structural differences at subcomplex level are less pronounced and can be conditioned by similar reaction of the discriminating forms, defining differences between groupings, upon joint influence of leading environmental factors within a certain bay.

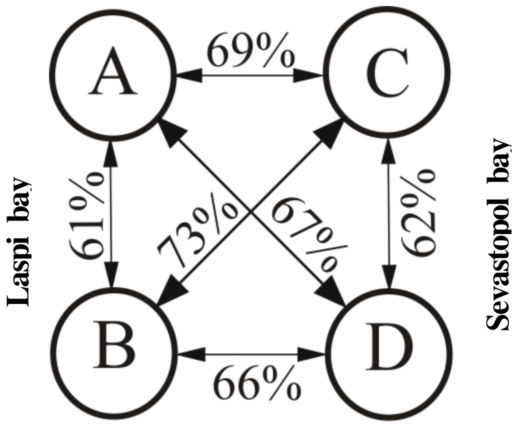


Figure 7. Average dissimilarity (%) between all pairs of intra-complex taxocenotic groupings in compared biotopes

Thus, based on the highest values of dissimilarity function reflecting the high stability of species development in certain ecological conditions, and also taking into account the individual contribution of species (by density) to inter complex differences, several discriminating species have been extracted from the total list of species (see Table 4). Those species can be considered as indicators of the diatom taxocene' condition at a comparative assessment of coastal biotopes subject to persistent technogenic pollution. It is proposed to consider *Tabularia tabulata, Amphora proteus* and *Nitzschia reversa* as indicators of conventionally healthy biotopes (Laspi bay), whereas, *Tryblionella punctata* var. *punctata, Diploneis smithii* var. *smithii, Nitzschia sigma* var. *sigma, Fallacia forcipata, Ardissonea crystallina* and *Pinnularia quadratarea* can be marked as indicators of the polluted habitats.

CONCLUSION

Thus, the implemented inventory of the data has showed contemporary state of species richness of benthic diatoms along Crimean coastal zone of the Black Sea. More than a half (55%) of total floristic richness of the Black Sea benthic diatoms is formed by species richness of the Crimean coast diatoms. 48 new and 21 rare species have been found for Crimean coast. Five of them were newly-found for the whole Black Sea and 4 species were new for science. The increase of diatom species richness has recorded through the last decades can be caused by intensification of studies as well as by more active introduction of new species into the Black Sea. The total updated list, including 409 species and intra-species taxa, can be used for the further research on quantitative development and diversity aspects of the Black Sea benthic diatom algae.

By application of algorithms of multivariate statistics the comparative analysis in taxocene structure features of benthic diatoms from two near shore water areas of southwest Crimea is fulfilled. Those areas (Laspi and Sevastopol bays) have substantially differed by levels of heavy metals and other pollutants content in bottom sediments.

The features of spatial organization of benthic diatoms habitats have been investigated for both bays. In each of the bay statistically significant taxocenotic complexes and sub-complex groupings of diatoms were revealed. Development of diatom taxocenes in Laspi bay is caused by worsening of optimal conditions from the central part of the bay towards more shallow and deep-water zones. The peak of species richness values coincides with the middle sublittoral zone (16-20 m depth), which can be considered as the most optimal one for diatom algae inhabitation.

In Sevastopol bay well-pronounced distinctions in the structural organization of benthic taxocenes corresponding to 3 locations with different level of pollution, have been revealed. Such differences can be caused by both presence at all stations of the investigated water area certain eurybiontic species and indicator species having the highest parameters of development within the certain complex (biotope). The basic abiotic factors influencing on peculiarities of diatom taxocene structure are level of toxicants' content in bottom sediments and water depth.

Lists of main species contributing the most input into similarity within taxocenotic complexes of the each bay were compounded. There were *Tabularia tabulata, Amphora proteus, Fallacia forcipata* and others for conventionally healthy Laspi bay; while *Navicula pennata* var. *pontica, Diploneis smithii* var. *smithii* and *Triblionella punctata* var. *punctata* - for polluted Sevastopol bay.

Inter-complex differences in structure of taxocenes are mostly pronounced and caused by different response of discriminating species, i.e. determining the most contribution to dissimilarity between complexes, to a high level of toxicants. Structural differences at sub-complex level are less pronounced and can be conditioned by similar reaction of respective discriminating species on joint influence of key environmental factors within a certain bay.

The most significant discriminating species can also be considered as indicators of the diatom taxocene' condition at a comparative assessment of biotopes subjected to various anthropogenic load. It is proposed to consider *Tabularia tabulata, Amphora proteus* and *Nitzschia reversa* as indicators of conditionally healthy biotopes, whereas *Tryblionella punctata* var. *punctata, Diploneis smithii* var. *smithii* and *Nitzschia sigma* var. *sigma* can be considered as indicators of biotopes under persistent technogenic impact.

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