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Original Research

Ecotoxicological Assessment of Freshwater Sediments

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Abstract

Human activity causes a serious increase in the variability and concentrations of chemical compounds present in bottom sediments and sorptive properties of sediments, and intensifies the deposition of hard-to-decompose and toxic substances, including heavy metals, radionuclides, and hydrophobic organic compounds. Those substances may cause elimination of ecologically important groups of benthic organisms, and disrupt the function of the water ecosystem.

A significant development of methods of ecotoxicological studies in aqueous environments has been seen during the last two decades. Monitoring concentrations of selected metals and organic compounds has been supplemented by bioindicative methods. Routine use of batteries of ecotoxicological tests allows for complex hazard and risk assessment of sediment pollution. According to the law, it is necessary to evaluate the toxic effects of newly developed and existing compounds on bottom biocenoses. The presented study describes problems of ecotoxicological sediment quality assessment and toxic substances, and is mainly focused on selecting proper test methods and a clear and compact classification of sediments.

Keywords: sediment, ecotoxicological assays, pore water, sediment classification methods

Introduction

Bottom sediments constitute a habitat for vegetable and animal life. They are rich in nutritional compounds. Therefore, they constitute an important component in the circulation of matter and energy in water basins. They are composed of mineral and organic particles and water contained between those particles.

Pursuant to the definition provided in the 2005 Annex to the Regulation of the Polish Ministry of Health on methods of realisation of tests of physical and chemical properties, toxicity, and ecotoxicity of chemical substances and preparations [1], sediment is a "mixture of mineral and organic chemical compounds, the latter being composed of high molecular-weight and compounds of high carbon and nitrogen content. It is formed in natural waters and has an interface with water."

Water basin sediments are formed as a result of sedimentation of allochthonic and autochthonic substances. Allochthonic material is in the main part composed of sand, slime, and gravel formed as a result of the destruction of river and lake beds, and mineral and organic suspensions reaching ground waters with surface flow, with side streams, and with industrial and communal wastewater. Autochthonic materials are inorganic and organic substances precipitating from water, including calcium carbonate, iron and manganese hydroxides, phosphorus compounds, and dead plants and animals sinking to the bottom. Sediments in rivers and in the onshore zone of lakes are

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composed mainly of allochthonic substances, including humus, and abyssal sediments in lakes are predominantly composed of material of autochthonic origin [2].

In aqueous environments, sediments have several functions important for the ecosystem. One of them is supporting primary production. Hard bottom sediments, characteristic of fast flowing streams, contain significant amounts of gravel, stones and cobbles – forming a stable base for colonization and development of periphyton. Soft sediments characteristic of ponds, lakes, river mouths, and slowly flowing sections of rivers and streams, are composed mostly of sands, slime, and clay, and constitute a base for developing aqueous macrophytes. Animals feeding on organic matter, such as nematodes, mayflies, caddisflies, crustaceans, annelidas, sponges, coelenterate, clams, and snails, also appear in the sediment.

Human activity causes a serious increase in variability and concentration of chemical compounds present in bottom sediments, and sorptive properties of sediments intensify deposition of hard-to-decompose and toxic substances, including heavy metal compounds, radionuclides, and hydrophobic organic compounds. Those substances may cause elimination of ecologically important groups of bentos organisms, and disrupt function of the ecosystem.

A significant development of methods of ecotoxicological studies in aqueous environments has been seen during the last two decades, [3, 4] accompanied by methods for assessment of risk caused by the presence of pollution in basins collecting wastewater and flows from land areas [5]. Ecotoxicological criteria have been introduced to legal acts in many countries, concerning the testing of wastewater and dangerous waste. At present a tendency is observed to estimate exposure of biocenoses in bottom sediments to the presence of substances cumulating there, and which may also become released to the aqueous phase [6]. In Poland there are no valid legal regulations regarding evaluation of harmful effects of chemical substances in bottom sediments, and there is no classification of sediments based on ecotoxicological assessments.

The aim of this paper is to present the current status of the ecotoxicological assessment of the bottom sediments in the world, in the context of Polish conditions, and to demonstrate that ecotoxicological tests of the bottom sediments should pose a base in aquatic ecosystems' hazard assessment for hydrophobic pollutants. A brief historical review, problems connected with sediment evaluation (e.g. test battery or reference sediment selection), and methodological aspects of sediment testing were addressed. Common approaches to ecotoxicological evaluation of sediments as a whole sediment, pore water, and solvent extracts are described. A large part of this review is dedicated to methods used for classification of sediments based on ecotoxicological tests.

Ecotoxicological Studies of Bottom Sediments

Studies on toxicity of bottom sediments started in the early 1970s. The studies involved observation of amphipods (*Amphipoda*), crustaceans belonging to an order in a sub-class of *Malacostraca*, in polluted bottom sediments and in humid land environments. The American Society for Testing and Materials (ASTM), the United States Environmental Protection Agency (USEPA), and Environment Canada (EC) standards appeared in the 1990s, to determine toxicity and bioaccumulation of pollution in bottom sediments, with use of other organisms [7-18].

At first they were associated only with acute (<10 days), mostly lethal tests. But it was soon obvious that they are not a good measure of toxicity of compounds accumulated in sediments. Therefore, much longer times of tests (up to 28 days) and control of other end points of test reactions (e.g. growth, weight, or reproduction) have been implemented. Toxicological tests on organisms inhabiting sediments are carried out in many countries [19-27]. Lethal, effective [28], and threshold [29] concentrations of pollution are determined based on them, as well as admissible concentrations of chemicals defined in valid standards of bottom sediment quality and new factors introduced to legal regulations [30].

Three main methods are used all over the world for toxicological assessment of sediments: whole-sediment tests [19, 20, 22-26, 28, 31-38], organic solvent washed out fraction tests (using acetone and DMSO, and other solvents) [19, 22, 24], and tests using pore water contained in sediments [19, 21, 22, 25, 27, 29, 36].

Use of both pore water tests and whole sediment tests for complex assessment of bottom sediment toxicity testing is important due to the various ways of ecosystem organism exposure, such as:

- Direct contact with flowing ground waters
- Direct contact with polluted sediments
- Direct contact with sediment particles present in supernatant water
- Digestion of polluted sediments
- Consumption by higher organisms (birds, mammals) [39]

Whole-Sediment Tests

Review of publications regarding toxicity of sediments indicates how important and often harmful to the whole ecosystem's role the compounds associated with solid fraction are. Burton et al. [25] studied bottom sediments from various depths of Lake Orta (Italy) using 6 toxicological tests using both pore water and sediments without previous "processing." The authors demonstrated a strong correlation between results of individual test methods and metal concentrations (Pb, Zn, Cu, Ni, Cr) in sediments. Dutka et al. [20] used a modified method of the SOS-Chromotest test to detect the presence of bio-available geno- and cyto-toxic compounds in samples of sediment, without a procedure of extraction of those compounds to a solution. They achieved a very low (9.8 ng/cm^3) threshold of detectability of mutagenic effect of 4-nitroquinoline 1-oxide (4NQO) for *E. coli*. Suedel and Rodgers [28] used *Daphnia magna*, *Hyalella azteca*, *Chironomus tentans*, and *Stylaria lacustris* as bioindicators in toxicity testing of a multi-cyclic aromatic hydrocarbon – fluoranthene in toxicity test of both aqueous and sediment fraction. They showed that the obtained values of LC(EC)50-48h[1], NOEC-48h[2], LC(EC)50-10d, and NOEC-10d were in the majority of cases lower in cases of tests with sediment compared to those with aqueous fraction. Based on the obtained results, the authors question valid US EPA criteria for fluoranthene as too liberal and not realistic. Guzzella [22] tested toxicity of ten sediment samples from the Po River (Italy) using bioluminescence inhibition test on *Vibrio fischeri* bacteria. Her tests regarded both solid phase (Microtox test) and pore water and organic extracts (Microtox and Lumistox tests). The author demonstrated higher sensitivity of the Lumistox compared to the Microtox. Moreover, she found a significantly higher toxicity of the solid phase, and therefore low usability of the liquid phase for estimation of toxicity of sediments. Vigano [24] also observed that the Po is characterized by variability in physical and chemical composition of sediments between summer and winter, and that the variability has a significant effect on toxicity of sediments for *Ceriodaphnia dubia*.

The most polluted areas in river flow have been determined based on tests of toxicity of sediments of the Po. A similar analysis was performed by Bettinetti et al. [26] for one of the tributaries of the Po River – Lambro River. They used highly sensitive, sub-lethal end points in chronic toxicological tests on *Chironomus riparius* and *Tubifex tubifex* – body weight increase, number of cocoons produced, and count of young organisms born. The authors demonstrated a strong correlation between concentrations of organic micropollutants (PCB, DDT, DDE, DDD, HCH, and HCB) and toxic effects observed in bioindicators. Bottom sediments from the Australian river Colo were used by Hyne and Everett [36] for development of sediment toxicity test both for fresh-water areas and estuaries, using a representative of amphipods – *Corophium* sp. The authors demonstrated usability of the organism for assessment of toxicity of metals in bottom sediments on the example of copper, for a broad spectrum of sample salinity. Naylor and Rodrigues [33] typed an artificial bottom sediment as a reference medium for 10-day toxicity tests using *Chironomus riparius* larvae. The sediment was composed of sand, kaolin clay, peat, and calcium carbonate. Tests indicated the necessity for use of an additional source of food in quantity of 1mg/larva/day to achieve optimal parameters of survival and growth of bioindicators. It was also found that food given to larvae has to be located on the surface of the tested sediment.

Naylor and Howcroft [35] used an analogous artificial sediment in order to determine the value of LOEC-10d[3] for 3,4-dichloroaniline (DCA). Tests were performed to determine the effects of larval stage of *Chironomus riparius* and density of bioindicators on obtained values of LOEC-10d. The authors demonstrated that larvae in the second and third stage of development are less susceptible to the effects of DCA compared to larvae in the first stage. The study demonstrated that organism body weight gain is a much more sensitive end point of the test compared to body length increase. The effect of reduction of volume of food introduced to test vessels on limitation of bioindicator growth, and therefore on sensitivity of the test, was also determined.

Day et al. [31] removed organisms inhabiting sediments collected from natural water basins by sieving out, gamma irradiation, freezing out, and autoclaving, and assessed the effects of these physical processing methods on survival rate, growth, and reproduction of invertebrates *Hyalella azteca*, *Chironomus riparius*, and *Tubifex tubifex*. The authors proved that *Hyalella azteca* is characterized by a higher susceptibility to those "manipulations." It was found that the applied processing methods had no effect on the survival rate of *Chironomus riparius*, and the species was susceptible only to the presence of pollution.

Belgis et al. [38] in their paper suggested a rapid and simple micro-test of a Toxkit type using *Heterocypris incongruens*. In their study they made a comparison between susceptibility of *Heterocypris incongruens* and *Hyalella azteca,* and *Chironomus riparius*, using 33 samples of sediments obtained from the Grand Lake region in Canada. The majority of sediments were classified as non-toxic (mortality <20%) for all three bioindicators. A high and statistically significant correlation was found for test results for individual species. In the case of 20% of samples, higher susceptibility of *Heterocypris* was demonstrated, compared to *Hyalella* and *Chironomus*. Studies on toxicity of sediments are carried out not only in laboratories, but also in field or semi-field conditions (micro- and mesocosms). Castro et al. [40] presented capsules for field tests using *Chironomus riparius*. Studies completed by the authors demonstrated usability of applied test vessels for toxicological tests in natural basins at variable environmental conditions. The authors found a significant concordance between laboratory and field results, allowing application of extrapolation techniques between those two types of results. Chappie and Burton [41] in their review present several structures allowing for the realization of tests in field conditions, providing results of both toxicity and bioaccumulation of compounds in organisms. The authors indicated a possible use of field tests for realization of the TIE procedure (toxicity identification evaluation) aimed at determination of groups of compounds responsible for the highest toxicity, depending on abiotic and biotic conditions (e.g. surrounding water, bottom sediment, light availability, suspensions, flow intensity, predators). A significance of

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[1] LC(EC)50-t – Lethal (Effective) Concentration – a concentration causing an increase in mortality (or another tested effect) by 50% compared to the control sample that had not been exposed to a toxic compound during the test time - t.

[2] NOEC-t – No Observable Effect Concentration – the highest concentration tested using a toxicological test having no statistically observed effects in bioindicators used, compared to a control sample during the test time - t.

[3] LOEC-t – Lowest Observable Effect Concentation – the lowest toxicology-tested concentration causing statistically observable effects in selected bioindicators, compared to a control sample during test time t.

Table 1. Phenanthrene LC50-10d for *Eohaustorius estuaries* and *Leptocheirus plumulosus* according to different organic carbon content [43].

Organism				TOC [%]
Eohaustorius estuaries		*Leptocheirus plumulosus*		
Sediment LC50-10 d [μg/g dw]	Pore water LC50-10 d [μg/dm^3]	Sediment LC50-10 d [μg/g dw]	Pore water LC50-10 d [μg/dm^3]	
39.2	138	92.4	387	0.82
97.2	139	162	306	2.47
122	146	255	360	2.97

physical parameters of bottom sediments in reactions of bioindicators was demonstrated, among others, by Sardo et al. [42] in relation to reproduction of an oligochaete *Lumbriculus variegatus* commonly used in toxicological studies in fractions of sediments of various granulation (<1 mm, 1 mm< x <2mm, >2 mm and mixed). It was found that in the finest sediment the organism count was almost 1.5-times higher ($p<0.05$) than in other ones during a five-month-long culture. The authors also stressed that long-term, non-renewed inbreed (and "cloning" of test organisms by cutting them in halves) may negatively affect locomotion and growth of bioindicators. Irwin [43] tested toxicity of phenanthrene for two freshwater species of amphibians: *Eohaustorius estuarius* and *Leptocheirus plumulosus* with various organic carbon content (Table 1).

Presented results indicate that both in the cases of *Eohaustorius estuarius* and *Leptocheirus plumulosus*, phenanthrene toxicity in sediment is significantly reduced with reduced organic carbon content. This correlation is not equally clear for pore water. Toxicology tests with bottom sediments are usually completed in static conditions, using supernatant to sediment ratio of 4:1, pursuant to U.S. EPA guidelines [44]. Some studies indicate, however, that in the case of some specific bottom sediments that course of action is improper. Borgman and Norwood [23] stated that in tests with *Hyalella azteca* (duration 28 days) and *Hexagenia* sp. (duration 21 days), pH of the supernatant may drop to 4 (probably as a result of oxidation of sulphides contained in sediments), causing 100% mortality of bioindicators exposed to action of relatively pure bottom sediments. They suggested a change of test vessels from beakers to Imhoff cones and change of supernatant to sediment ratio from 4:1 to 67:1. The authors observed a significant increase in survival rate of selected test species, up to 97% in the case of *Hyalella azteca*, and up to 100% in the case of *Hexagenia* sp. Control tests completed in those conditions with insect larvae *Chironomus* sp. and annelids *Tubifex tubifex* also showed high survival rates of those organisms, 90% and 100%, respectively. A necessary increase of water to sediment ratio is also postulated by Novelli et al. [45]. The authors stated that in the case of tests on sea urchin *Paracentrotus lividus* embryos and sperm cells and on oyster *Crassostrea gigas* embryos, sediments from 6 posts in the Lagoon of Venice were more toxic for the sediment to water ratio 1:20 and 1:50 compared to 1:4. They recommend use of several different sediment to water ratios within the range 1:4 to 1:200 for routine toxicological assessment of bottom sediments, especially if chemical analyzes parallel to bioindication indicate the presence of water-soluble pollution.

Various sediment-to-water proportions are also used practically by other researchers, including: 1:2 [46, 47], 1:3 [48], 1:5 [49, 50], 1:8 [51], 1:10 [50, 52], 1:20 [49, 52], 1:25 [49], 1:50 [50, 53-55], 1:200 [50, 56], 2:1 [57], and 2:5 [58].

Studies with Pore Waters and Solvent Extracts

Toxicity assessment of bottom sediment samples based on pore waters or solvent-based extracts does not pose any serious problems, except for selection of extraction or separation of aqueous phase from solid phase methods. Those assessments, however, do not consider the whole content of pollution accumulated in sediment. Pollution is often closely associated with organic fraction of sediment, and the a.m. methods detect only the part of pollution that may be washed out with solvents (water or organic solvents). That method of toxicological assessment of bottom sediments is not different from methods using aquatic organisms, and is justified by bioavailability of pollution for living organisms, which usually may absorb them only from the dissolved phase. Two main assumptions are made adopting that attitude to sediment toxicity assessment, and those assumptions are not always observed. First, it is assumed that biologically available fraction of pollution, bioaccumulation and toxicity are closely associated with concentrations in pore water; and second, that susceptibility of aquatic and sediment-based organisms to toxic compounds is comparable. However, variable physical and chemical conditions of water over sediment may cause the release of previously unavailable portions of pollution, especially that organic compounds accumulated in bottom sediments may become biodegraded and biotransformed there to highly toxic metabolites. Pore water sampling methods from sediments may be divided into two categories: *in situ* methods consisting of collection of pore water using samplers directly introduced to sediments, and left there until they are naturally or suction-filled, and *ex situ* methods using pneumatic pressure or centrifugation, although extraction with negative pressure may also be used [59]. Pore water extraction from sediments is often labour-consuming. This poses a problem in obtaining volumes sufficient for toxicological tests and chemical assays [60, 61].

Changes in chemical and physical composition may occur during sampling and extraction. However, the effect of those factors is not fully understood. Pore water testing in assessment of quality of bottom sediments has several advantages, including:

- Short exposure time compared to tests of whole sediments;
- Low costs of test completion;
- Pore waters constitute the main exposure route to pollution for the majority of organisms.

Table 2. Artificial sediments used in ecotoxicological assays.

Inorganic fraction [%]								Organic fraction [%]					Food supply [%]	Reference
Sand	Kaolin clay	$CaCO_3$	Benthonite	Chitin	FeO(OH) – Goethite	MnO_2	Dolomite	α-cellulose	Peat	Compost	Humic acid	Urtica powder	Tetramin	
60	33.25	0.1							6.5				0.15	[62]
65	30	0.1						4.85					0.15	[63]
58			30	5	5	2								[64]
80	20													[65]
70	20								10					[66]
80	5									15				
65	5									30				
76.87	19.78	0.49						2.35					0.15	[67]
85	14								1		0.01			[68]
76	22	0.05							2					[69]
69	20	1							10					[33]
82	18								5					[70]
100														[71]
50	50													
69	20	1							10					[33]
76.1	19.02	0.1						4.78						[30]
75	20								5					[72]
70	20								10					[73]
74.3	19.8	0.5							5			0.4		[74]
68.5	19.8	1.4							10.3					[75]
14.17	80.34						0.49	5.00			0.01			[76]

In their studies on toxicity of pore water components from nine bottom sediments collected in the area of Amsterdam, Heida and Oost [21] used four toxicological tests (Microtox, Rotoxkit F, Thamnotoxkit F, SOS-Chromotest). Based on results of toxicological analyses and values of standards valid in the Netherlands, three tested sediments were classified as toxic, and one as highly toxic. The results did not correlate with chemical tests, based on which only one of those sediments could be assessed as dangerous for biocenosis. It was demonstrated that Microtox and Thamnotoxkit F tests were the most sensitive of the applied battery of toxicological tests. Thomas et al. [27] assessed toxicity of pore water from six estuaries in Great Britain using a copepod *Tisbe battagliali* as bioindicators. The purpose of the study was to type toxic sediments for TIE procedure. Based on those studies it was stated that organic compounds, metal kations, and ammonia have the highest share in general toxicity of sediments.

Reference Sediment

Studies of bottom sediments, both in context of assessment of toxicity of chemical substances and monitoring of the environment, require use of a reference sediment to compare effects observed in bioindicators. Natural sediments collected from unpolluted water basin, or artificial, standardized mixtures of chemical components imitating natural sediments are used for that purpose. Kwan and Dutka [34] demonstrated that neutral bottom sediment collected from unpolluted areas, sieved through a 0.25 mm mesh, washed with tap water and frozen out, may be used as reference medium for toxicological studies. Examples of artificial reference bottom sediments are presented in Table 2.

Use of sediments from natural water basins in toxicology studies is associated not only with higher costs (selection of a water basin, sampling, transport), but also with much

Table 3. Bioindicators used in ecotoxicological assessment of sediments.

Producers	Consumers	Decomposers
Cucumis sativus	*Brachionus calyciflorus*	*Escherichia coli*
Lemna minor	*Ceriodaphnia dubia*	*Pseudomonas fluorescens*
Lepidium sativum	*Chironomus riparius*	*Vibrio fischeri*
Selenastrum capricornutum	*Chironomus tentans*	
Sinapis alba	*Daphnia magna*	
Sorghum saccharatum	*Diporeia* sp	
	Hexagenia sp.	
	Heterocypris incongruens	
	Hyalella azteca	
	Lumbriculus variegatus	
	Tubifex tubifex	
	Pimephales promelas	
	Stylaria lacustris	
	Stylodrilus heringianus	
	Thamnocephalus platyurus	
	Tisbe battagliai	

higher uncertainty associated with heterogeneity of their composition. Use of a standard bottom sediment for toxicological tests is therefore postulated, due to a better control of test organism exposure conditions and much higher repeatability of results of laboratory tests.

Test Organisms Used for Toxicological Assessment of Bottom Sediments

Another important factor associated with toxicological studies is selection of an appropriate battery of test organisms – bioindicators. Just like in the case of toxicology tests with aquatic organisms, bioindicators should represent all links in the food chain – producers, consumers, and decomposers. Test organisms have to possess the following properties: representation of ecologically significant groups of organisms, susceptibility to a broad spectrum of pollution, clear and repeatable test reactions, and easy culture in laboratory conditions. Several species of organisms have been used all over the world for assessment of toxicity of sediments. Those are both benthic and aquatic organisms. Examples of the most commonly used bioindicators are presented in Table 3.

The presented list is not exhaustive and does not contain all organisms used for sediment toxicity testing. Part of the above-mentioned species inhabit some limited areas of various climatic zones, therefore their application in Polish conditions is unjustified. Blaise and Ferad [77] demonstrated in their analysis of 75 reference reports regarding bottom sediment toxicity assessment (total 109 samples) that the most commonly used bioindicators are invertebrates (61.3% of all organisms used) and bacteria (23.9%). Analysis of fractions used for tests, whole sediments, and pore waters are most popular (37.6% and 25.7%, respectively).

Table 4. Toxicity classification by Persoone et al. [78].

Toxicity class	Threshold criteria	Toxicity evaluation
1	TU<1	Non toxic
2	1<TU<10	Toxic
3	10<TU<100	Acute toxic
4	TU>100	Very toxic

TU is acute toxic unit (TU=100/LC(EC)50-t)

Table 5. Oleszczuk's toxicity classification in Ostracodtoxkit test [81].

Toxicity class	Threshold criteria	Toxicity evaluation
1	TS<0.4	No chronic toxicity
2	0.4<TS<1	Slight chronic toxicity
3	1<TS<10	Chronic toxicity
4	10<TS<100	High chronic toxicity
5	TS=100	Very high chronic toxicity

TS, test score;
No chronic toxicity – none of the parameters (mortality, growth inhibition) shows a toxic effect;
Slight chronic toxicity – a statistically significant ($p < 0.05$) percentage effect is reached in at least one parameter but the effect level is below 50%;
Chronic toxicity – the percentage effect (50%) is reached or exceeded in at least one parameter, but the effect level is below 100%;
High chronic toxicity – the percentage effect (100%) is reached in at least one parameter;
Very high chronic toxicity – the percentage effect (100%) is reached in all the parameters.

Classification of Bottom Sediments

At present there is no simple classification of bottom sediments from the point of view of ecotoxicology. One applied method is an evaluation system proposed by Persoone et al. [78] (Table 4) for natural waters and waste waters, and used also for assessment of toxicity of composts and sludge, because of the lack of appropriate criteria [79, 80].

A modification of the a.m. classification for the Ostracodtoxkit test and sludges, and resulting compost, was proposed by Oleszczuk [81] (Table 5).

Table 6. Sediment toxicity classification for *Amphipoda* and *Polychaeta* [82].

Threshold criteria	Toxicity evaluation
Average organism survival differed significantly from laboratory controls ($p<0.05$) and was less than 80% for amphipods or 64% for polychaetes of the survival in laboratory controls	Highly toxic
Average organism survival significantly lower than laboratory sediment controls ($p<0.05$) but exceeded 80% (*amphipod*) or 64% (*polychaete*) of the survival in the laboratory controls	Marginally toxic
Average organism survival that was not significantly different ($p>0.05$) from the laboratory controls	Non toxic

Table 7. Toxicological classification of sediments based on pT index [83, 84].

Highest dilution level without effect	pT value	Toxicity class	Toxicity designation
Original sample	0	0	Toxicity not detected
1:2	1	I	Very slightly toxic
1:4	2	II	Slightly toxic
1:8	3	III	Moderately toxic
1:16	4	IV	Distinctly toxic
1:32	5	V	Highly toxic
≤1:64	≥6	VI	Extremely toxic

Table 8. Ecotoxicological classification of sediments based on PE Indicator [85].

Toxicity class	Numerical Value of the PE Indicator
I	PE≤40% for all analyzed organisms
II	40%<PE<50% for one organism
III	40%<PE<50% for two organisms
IV	PE>50% for one organism
V	PE>50% for two organisms

PE – the analyzed effect for a determined organism [%]

Iannuzzi et al. [82] developed classification criteria for estuary bottom sediments based on results of standardized toxicity tests with amphipods (*Ampelisca abdita*) and polychaetes (*Neanthes arenaceodentata*) (Table 6).

Another example of bottom sediment classification developed for monitoring purposes is an approach based on multiplicity of pore water or solvent-based extract dilution developed in the German Institute of Hydrology [83, 84]. For each test end point in a selected battery of tests, a threshold value of effect was determined. If the value was exceeded, it is assigned a so-called toxicity exponent (pT – *Potentia Toxicologiae*) depending on multiplicity of sample dilution. Classification of sediment toxicity to one of seven classes is based on criteria presented in Table 7.

Wolska et al. [85] proposed a simplified classification system used to evaluate the quality of sediments (Table 8). The system is based on numerical value of the so-called, PE Indicator – that is defined as the analyzed effect for a determined organism and is expressed as a percentage.

The HOCNF (harmonized offshore chemical notification format) [86] scheme, suggested for classification and assessment of the effect of chemical substances on the environment by the Common Norwegian-French Commission OSPARCOM (Oslo and Paris Commission for Environmental Regulations in the European Community) for management of the off-coast natural gas and oil processing and production industries, assumes classification of chemical substances based on a battery of three bottom sediment ecotoxicity tests into one of five classes (Table 9).

Assessment is based on results of tests using algae *Skeltonema costatum* (72-hour growth test), crustaceans *Acartia tonsa* (48-hour survival test), and young forms of amphipods *Corophium volutator* (10-day survival test). Chemical substances belonging to category A are characterized by the highest harmful potential, and substances belonging to category E pose the lowest risk.

Kalinowski and Załęska-Radziwiłł [87] suggested a modification of Dutch [88] classification of bottom sediments (Table 10), along with evaluation of sediment qual-

Table 9. Ecotoxicological classification of pollutants in sediments based on test battery [86].

Toxicity class	Threshold criteria [ppm]
A	LC(EC)50-t<10
B	10>LC(EC)50-t<100
C	100>LC(EC)50-t <1,000
D	>1,000>LC(EC)50-t<10,000
E	LC(EC)50-t>10,000

Table 10. Dutch sediment classification criteria according to Henning-de Jong [88].

Toxicity class	Threshold criteria	Sediment quality description
I	Environmental concentration $< HC_5/A$	Very good
II	$HC_5/A <$ Environmental concentration $< HC_5$	Good
III	$HC_5 <$ Environmental concentration $< \sqrt{HC_5 \cdot HC_{50}}$	Average
IV	$\sqrt{HC_5 \cdot HC_{50}} <$ Environmental concentration $< HC_{50}$	Bad
V	$HC_{50} <$ Environmental concentration	Very bad

HC_5 – hazardous concentration for 5% of species, A – assessment factor depended on HC5 calculation method, HC_{50} – hazardous concentration for 50% of species

Table 11. Assessment factors selections criteria suggested by Kalinowski and Załęska-Radziwiłł [87].

Assessment factor A	Hazardous concentration derive method
100	Statistical models based on LC50-t
50	Statistical models based on NOEC-t
10	Data from multispecies laboratory tests
2	Data from multispecies field tests

ity, based on species sensitivity distribution (SSD) obtained from a battery of ecotoxicological tests using whole sediment, and calculated values of hazardous concentrations HC5 and HC50, by use of assessment factors (Table 11).

The proposed scheme of assessment factors applied to the methodology of obtaining threshold criteria allow to take into account uncertainty of results. This uncertainty is connected with different methods used in hazardous concentrations calculations. Therefore assessment factors consider different quality of such data. The classification of bottom sediments based on species sensitivity distributions requires use of much higher amounts of ecotoxicological data compared to other methods, but allows a more realistic assessment of purity of bottom sediments. It is clear that the availability of sufficient (for SSD approach) numbers of sediment toxicity test results is strongly dependent on costs carried out by manufacturers and scientists and it is allowed to use this kind of approach only in limited cases.

Conclusions

The problem of ecotoxicological assessment of bottom sediments is highly complex. This complexity is caused both by physical and chemical properties and by biological properties of lower parts of water basins. Continuous processes on the water-sediment interface, exchange of matter associated with supernatant water movement and activity of benthic organisms, as well as inertness and buffer properties of sediments themselves pose a serious challenge to researchers of that environment.

Future actions in the field of ecotoxicological assessment of chemical substances in sediments should be led in two directions. The first should be developing proper test methodologies and data requirements (e.g. species choice, clear classification criteria, proper calculations procedures) for newly occurring substances of concern like medicines or nanoparticles, especially for whole sediment toxicity tests. The second should be legal force on the manufacturers to provide laboratory test results for existing and newly developed compounds that will make environmental hazard and risk assessment procedures much more real and effective.

Toxicity assessments of bottom sediments have been successfully realized since the 1970s. Poland is clearly backward in this area, both compared to other European countries and world tendencies in ecotoxicology. It should also be implemented into Polish law using a small bioindicator-based test battery as a parameter in monitoring lake and river sediments that will greatly complement chemical analysis.

The introduction of bioindicator tests with bottom sediments for routine monitoring of the environment, and development of a clear and compact classification of sediments, would allow for an unambiguous assessment of quality, and would facilitate comparison of various sediment-based environments, as well as making decisions, for example, on processes of reclamation of water basins.

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