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Novel approach to ecotoxicological risk assessment of sediments cores around the shipwreck by the use of self-organizing maps

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ABSTRACT

Marine and coastal pollution plays an increasingly important role due to recent severe accidents which drew attention to the consequences of oil spills causing widespread devastation of marine ecosystems. All these problems cannot be solved without conducting environmental studies in the area of possible oil spill and performing chemometric evaluation of the data obtained looking for similar patterns among pollutants and optimize environmental monitoring during eventual spills and possible remediation actions – what is the aim of the work presented. Following the chemical and ecotoxicological studies self-organising maps technique has been applied as a competitive learning algorithm based on unsupervised learning process.

Summarizing it can be stated that biotests enable assessing the impact of complex chemical mixtures on the organisms inhabiting particular ecosystems. Short and simple application of biotests cannot easily explain the observable toxicity without more complex chemometric evaluation of datasets obtained describing dependence between xenobiotics and toxicological results.

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1. Introduction

One of the basic features of pollutants reaching the marine and ocean water bodies is their spreading and transport in the omni ocean. For this reason for decades it was believed that degradation of marine environment was very slow process. Not sooner than after several severe accidents the society started to realize the consequences of oil spills causing widespread devastation of marine ecosystems. It has drawn attention of politicians, scientists and ecologists to water transport as plausible source of marine ecosystems pollution. It was reflected in introducing a set of legal acts of both regional and global character. Such regular strategies of actions however do not take under consideration threat posed by shipwrecks already lying at the sea beds. Such wrecks still contain fuel, ammunition and chemical weapons.

Presence of civilian and military ships wrecks is resulting mostly from I and II WW actions with the greatest intensity of their locations in the waters of northern Atlantic and southern Pacific (Michel et al., 2005). In this involuntary way the sea bottoms are in many cases endangered with these "ecological bombs". The risk of leakage is dependent on numerous factors: condition in the moment of accident, amount and type of fuel, time elapsed since sinking, water depth, water currents, salinity, action of waves and winds, amount and type of ammunition (HelCom Report on Chemical Munitions Dumped in the Baltic Sea, 1994). Most of it has been subsequently sank in the sea (it is estimated that that over 13,000 tons of chemical agents were sank in the Baltic Sea after WW2) (Sanderson et al., 2009).

Oil spills also have serious economic and social consequences, just to mention the following:

- loss of nature value and loosing attractiveness of the seaside belt (reduction of touristic income),
- temporary cessation of fishering due to contamination, and
- acute costs of cleaning the oil spill region.

It results in slowing down the economic growth of the region of interest and increment of protests of local societies. Assessing such cultural costs are very complex and very often impossible to determine (Garza-Gil et al., 2006; Hassellov, 2007), the best example of which is latest tragedy of the Deepwater Horizon (http://www.deepwaterhorizoneconomicsettlement.com/docs/statistics.pdf, 2013).

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All these problems cannot be solved without conducting environmental studies in the area of possible oil spill and what is even more important to conduct chemometric evaluation of the data obtained in order to look for similarities patterns among pollutants and optimize not only environmental monitoring during eventual shipwrecks spills but also possible remediation actions – what is the aim of the work presented.

It is of utmost importance to apply multivariate statistical methods in studies like this in order to assess the results of chemical and toxicological monitoring. They turn to be specific source of information about hidden relationships between locations studied, chemical or biological features etc. (Kudłak et al., 2014). The classification, the data compression and projection lead to better data interpretation and modeling.

2. Materials and methods

Wreck of s/s Stuttgart is located in the western part of the Gdańsk Bay (southern Baltic Sea) ca. two nautical miles from the Gdynia harbor. This area is particularly prone to anthropogenic pollution due to high level of urbanization and industrialization. Large agglomerations of Gdańsk and Gdynia as well as harbors of Gdańsk and Gdynia and the Vistula River greatly contribute to the pollution of the area of interest. The wreck is sunk at 21–23 m depth as presented elsewhere (Rogowska et al., 2012).

2.1. Sampling and samples handling

For sampling and studies nine core localizations (presented in Fig. 1) have been selected. Being sampled all of them were subdivided into 6 subsamples with the following depth schemes: 0–20, 20–40, 40–60, 60–80, 80–120, 120–160 cm below the sea bed level. All cores were collected with vibro corer (VKG-4 type) in the vicinity of the wreck of the s/s Stuttgart, frozen and lyophilized, the sampling methodology and hydrogeological situation is presented elsewhere (Rogowska et al., 2012) (Fig. S1).

2.2. Analytical procedures

2.2.1. Ecotoxicological studies

To assess the toxicity of the samples collected, three toxicity tests were applied: Microtox^{R} (Strategic Diagnostics Inc., USA), Phytotoxkit^{FM} (MicroBioTests Inc., Belgium) and Ostracodtoxkit^{FM} (MicroBioTests Inc., Belgium).

2.2.1.1. Acute toxicity test – MT. The acute toxicity of aqueous extracts of sediments was assessed on the basis of the Microtox^{R} test utilizing the marine bacteria *Vibrio fischeri* according to the ISO 11348:2 procedure (ISO 11248:2, Water Quality—Determination of the Inhibitory Effect of Water Samples on the Light Emission of *V. fischeri* (Luminescent Bacteria Test)-Part 2: Method Using Liquid-Dried Bacteria, 2002.). The aqueous extracts were prepared by diluting one volume unit of the sediment tested (weighed prior to dilution) with four volume units of aerated distilled water. Samples prepared in this way were shaken for 24 h. The Microtox^{R} Model 500 analyzer was used to determine the bioluminescence level according to the '81.9 percent Basic Test' procedure available with the MicrotoxOmni™ analyzer software.

2.2.1.2. Chronic toxicity test – SA1 and SA2. Briefly speaking, the Phytotoxkit^{FM} is a plant test based on measurement of number of seeds sprouting (SA1) and growth inhibition of roots (SA2) after three days of exposure to toxic substances or polluted soil. The *Sinapis alba* was chosen from the available three plants in the Phytotoxkit^{FM}. The endpoints are determined by analysis of the roots' pictures by software (Image Tools). The results are given as the percentage of seeds germination and root growth inhibition compared to those for control soil samples.

2.2.1.3. Sub-chronic direct contact test – HI. The chronic toxicity of the sediments was assessed with respect to *Heterocypris incongruens*. The Ostracodtoxkit ^{FM} test is a six day direct-contact test that determines the chronic toxicity of sediments from an evaluation of the mortality and growth inhibition of organisms after contact with the test sample. The test was run according to the standard operational procedure supplied by the manufacturer. The test is considered to have been performed reliably and reproducibly if the reference sediment mortality is < 20 percent and the mean growth increment in the reference sample exceeds 400 mm. The toxicity test with *H. incongruens* is applicable mainly to fresh water sediments and marine sediments with a salinity < 0.7‰, which is the case in the Gdańsk Bay of the Baltic Sea (what is more due to proper sample preparation method after extraction and/or other steps the samples can be considered as brakish/freshwater sediments).

2.2.2. Chemical studies

2.2.2.1. Persistent organic pollutants. Determination of sixteen PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(123-cd)pyrene, dibenzo(ah)anthracene, benzo(ghi) perylene) and 7 PCB ((IUPAC nos.: 28, 52, 118,101, 138, 152, and 180) has been performed according to procedure described elsewhere (Rogowska et al., 2012) with small modification: instead of PCB209 the mixture of six PCBs isotopically labeled with $^{12}C_{13}$ (IUPAC nos.: 28, 52, 101, 138, 152, and 180) dissolved in the nonane has been used at concentration level of 5 μg/ml for each substance (Resteck Corporation, USA). Qualitative and quantitative determinations have been conducted with gas chromatograph equipped with a mass spectrometer (GC–MS model 7890A with MS model 5975C, Agilent Technologies, USA) working in the SIM mode.

2.2.2.2. Metals. Lyophilized sediments samples were homogenized and sieved to obtain fraction < 0.063 mm. 0.5 g of this fraction (0.25 g in case of Hg determination) was digested with 65 percent HNO_3 and mineralized (Microwave Digestion System: Multiwave 3000, Anton Paar, Austria). The concentrations of Cu, Mo, Mg, Zn in the liquid extract were determined by an Atomic Absorption Spectrometer with the graphite cuvette for Zn, Cu, Mg (SavantAA Z, GBC, Polska) and flame atomizer (SenasAA Dual, GBC, Poland) for Mo, while Cd, Co, Cr, Cu, Mn, Ni and Pb content was determined with inductively coupled plasma-mass spectrometry

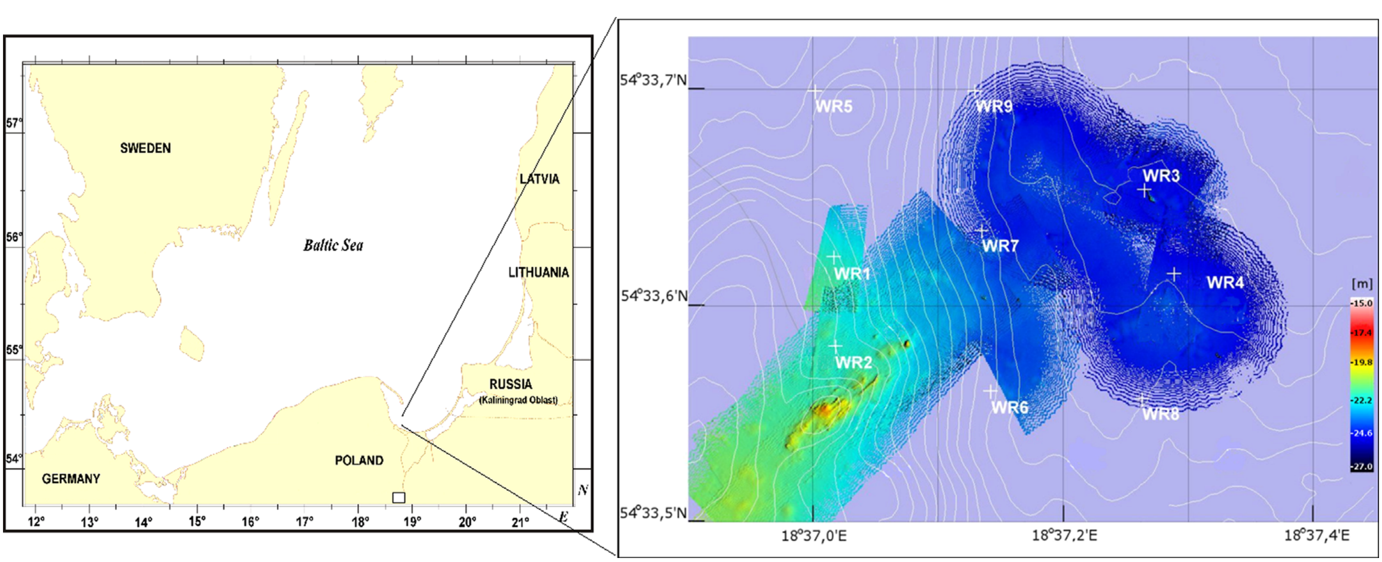

Fig. 1. Location of s/s Stuttgart wreck near the Polish coast and position of cores' sampling in its vicinity.

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