Biomarkers and kits for early and rapid testing – current knowledge and gaps

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Abstract The use and development of biomarkers is related to the establishment of hidden toxic effect on molecular, sub-cellular, cellular and tissue level. The development of new biomarkers is very important for successful early diagnosis of toxic effects on the macro-biological systems. The effects on these levels often come from complex impact and are hard to spot until the onset of extinction of the population or degradation of the community. Developing of a novel biomarker is time consuming and in most cases very expensive, but very perspective issue. The other contemporary tool for rapid assessment of toxicity is the biomarker assay kits. They are very often used due to their ease of operation and representativeness of obtained results. The discussed information about early and rapid ecotoxicological testing was obtained by Review Publication screener software using published developments for recent five years. The frequency and characteristics of biomarkers and kits used in the recent studies are examined and statistically processed. The main advantages, disadvantages and trends in developments are outlined.

Keywords: ecotoxicology, biomarker, kit, advantages, gaps

1. Introduction

Over the past two decades, knowledge of biomarkers is widely spread (Peakall 1992) and can be assessed by using different responses covering molecular, biochemical, physiological, histopathological, organismic, population or community levels (Khoshnood 2016). We are witnesses of explosion of scientific papers, which are published in printed magazines and much more uploaded on web-based editions. It is almost impossible to follow all published scientific information and a lot of important scientific information can be unobserved and missed. There are a large number of articles concerning the application of biomarkers in ecotoxicology during the past few years (Furuhagen 2015, Khoshnood 2016, Kaviraj *et al*. 2014, Mussali-Galante *et al*. 2013, Hook *et al*. 2014). Despite that fact, fully established and working platform for semantic review and store for further aggregation of information related with basic ecotoxicology categories like the kind of ecotoxicological test, test object, test environment, biomarkers, etc. could not be found in the web. In order to achieve this goal, we outlined the current trends in biomarkers for assessing the impact of environmental pollution by using a developed web application allowing us in a semi automatically way to review and store of all categories described in screened publications.

2. Materials and method

A large amount of scientific papers (8826) from the period 2012 -2016 were observed. From all of them, those containing the word "biomarker" were selected. The main sources used for collecting information were the web pages of some journals for ecotoxicology (Chemosphere https://www.journals.elsevier.com/chemosphere/; Ecotoxicology and Environmental Safety https://www.journals.elsevier.com/ecotoxicology-and-environmental-safety; Ecotoxicology https://link.springer.com/journal/10646 and Toxicology https://www.journals.elsevier.com/toxicology). Results were processed using a portal based on the Model-View-Controller (MVC) technology. That technology is developed by the combination of programming language PHP and MySQL database. The developed web application is based on Apache 2.2.15, PHP 5.3.2, MySQL 5.1.73 and runs on Linux and Windows platforms. The forms, for which the user has access rights, are visible. After selecting the review type, visualized data already entered in tabular form. The visualized table can be further sorted if needed. For larger volumes of information and longer identifiers appears pop-up text with complete description, when hovering over them.

3. Results and discussion

The results show that from all observed articles, 2.87% (253) contained the word "biomarker". It was found that during the recent years the number of scientific articles decreased with approximately 50% (Fig. 1).

This tendency did not coincidenced with the findings of Jemec *et al*. (2009), who found a significant increase in the number of scientific publications containing the keyword 'biomarker' since the infancy of the discipline at the beginning of the 1980s. After the research boom, a decline in the scientific community's interest in publishing new biomarkers has been observed. The focus has been shifted to gene modifications for solving certain problems associated with negative toxic effects.

We investigated also the publication frequency of five groups of selected biomarkers, namely: oxidative stress biomarkers, biomarkers for Genotoxicology, histopathological biomarkers, behavioral biomarkers and physiological biomarkers – Fig. 2. On average, in 46.2% of the cases the oxidative stress biomarkers were used. The next are geno-toxicological and physiological biomarkers – 20% and 18.5%, respectively, histopathological (12.5%) biomarkers and finally behavioral biomarkers with 2.8% relative participation.

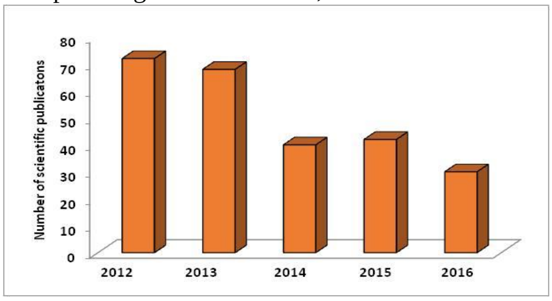

Fig. 1. Number of scientific papers published annually containing keyword “biomaker”.

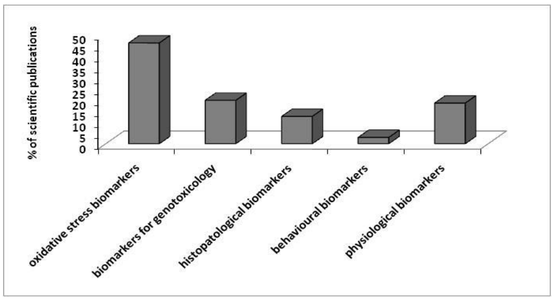

Fig. 2. Average publication frequency of some basic biomarkers.

The most frequently used are ezymatic oxidative stress biomarkers like catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and acetycholinesterase (AchE). They are mentioned in 144 of the observed scientific papers, which is about 46% from their total number. Based on results obtained for the activity of these enzymes, the tested species can be used for environmental risk assessment as early warning indicators like fish[1] (***), mussels (**), microalgae (**); for biomonitoring like mussels (**, ***), crustaceans (***), plants (**, ***), insects (**) or for assessment of environmental pollution like earthworms (**), reptiles (***), mussels (**). Although these biomarkers are widely used, there are also new findings like the pi-class GST homolog from *Mytilus coruscus* that can potentially be used as indicators and biomarkers for detection of marine environmental pollution (Liu *et al*. 2015).

Scientific articles using biomarkers for genotoxicology and physiological biomarkers comprise the second and third group with 20 and 18.5%, respectively. Biomarkers for genotoxicology are mainly related with gene expression (*,****, **) and DNA damage (**) as in approximately 50% fish were used as test objects.

Behavioural biomarkers are the least concerned type in the scientific publications – 2.8% (9 articles). As biomarkers, feeding (***) and burrowing (***) of different groups of species like molluscs (***), fish (***), bees (**) are most often used.

A very high ammount of articles (25%) published results from the application of a combination of biomarkers at different level of organisation – from molecular to organism (**). Also the multi-biomarker approach is often used for environmental risk assessment (***, **).

Even authors (Palais *et al*. 2012) recommend the use of multi-marker approach in order to allow data interpretation. An integrated biomarker response was also very often used for evaluating the ecological risk of different polluted areas (**). It is also applied as an evaluation system of the toxicity effects from certain pollutants like ZnO NPs, WWTP, Benzophenone-type UV filters, Cu and benzo[a]pyrene (**). Moreover, Kim *et al*. (2014) pionted out that multi-level IBR index was more correlated with dose than conventional IBR index. Although the neutral red method have been originally developed by Borenfreund and Puerner (1985) few decades ago Neutral Red Uptake (NRU) still remains simple, accurate and yields reproducible results (https://www.sigmaaldrich.com). We found two articles (*, **), where authors apply the Neutral Red Retention Time (NRRT) assay. Also Irizar *et al*. (2015) developed in vitro assays with primary cultures of coelomocytes based on the NRU as promising tools for toxicity assessment of chemical in a reproducible and cost-effective manner.

Modelling also finds application in analyzing the results obtained as for example Karami *et al*. (2012) examined the potential of artificial neural network (ANN) modeling to infer timing, route and dose of contaminant exposure from biomarkers in a freshwater fish. Bradley (2012) stated that finding biomarkers is getting easier, but there still must be confirmation by multivariable statistics and with field studies.

Based on the results obtained from biomarkers, new indicator species were found. For example the endemic wild cyprinid *Petroleuciscus esfahani* was used as a bioindicator of estrogenic exposure in the Zayandeh Roud River, Iran (Gilannejad *et al*. 2016). Rodrigo *et al*. (2013) found a novel potential bioindicator (*Sepia officinalis*) for risk assessment in impacted estuaries and due its properties it could be a candidate to meet the European Union’s requirements for efficient biomonitoring programmes according to its better reflection to anthropogenic stressors impact and a wider area than sedentary organisms.

[1] Note: Due to the large number of scientific articles, only the web source will be cited as follows:

* https://www.journals.elsevier.com/chemosphere/

** https://www.journals.elsevier.com/ecotoxicology-and-environmental-safety

*** https://link.springer.com/journal/10646

**** https://www.journals.elsevier.com/toxicology

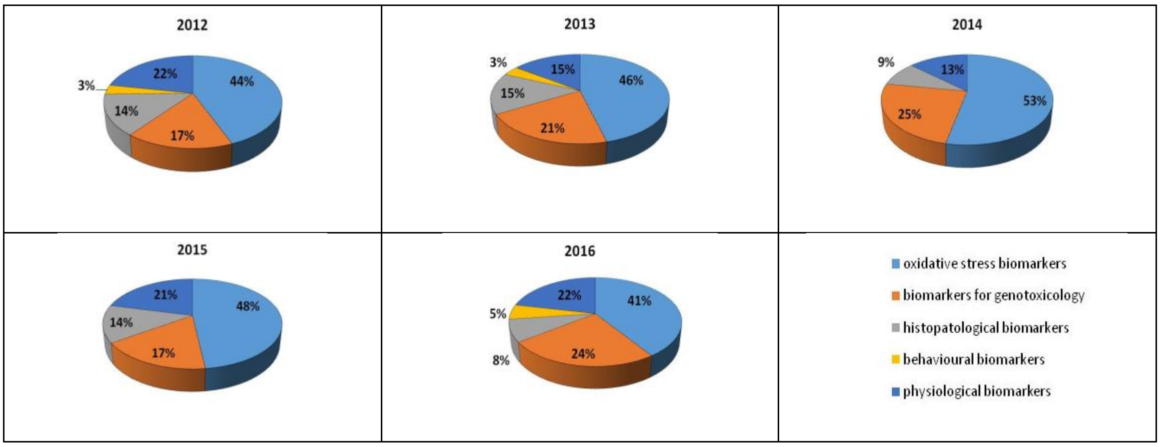

Fig. 3. Publication frequency of some basic biomarkers by years.

The analysis of trends in the usage of different types of biomarkers for a 5-year period showed an increase by 2014 in the frequency of usage of oxidative stress and geno-toxicological biomarkers, respectively to 53% and 25% - Figure 3. For the last two years, the frequency of usage of oxidative stress biomarkers steadily decreased, while the usage of geno-toxicological biomarkers decreased in 2015 and rose sharply over the past year. There is no pronounced trend in the frequency of physiological and histopathological biomarkers usage. The rate of behavioral biomarkers usage increased at the end of the period.

Kits for ecotoxicological analyses are another tool that is widely used. They are rapid, sensitive, easy for operation and in most cases cost effective, which makes them siutable for environmental assessment. Depending on the type of envoronment and the purpose of application, a variety of kiths have been developed as most of them are trade marks of certain companies. A brief summary of the kits used in ecotoxicology with some of their characteristics is given in Table 1.

Table 1. Kits for ecotoxicological analyses

Type of kit	**Components of the test or model organism used**	**Application**
ECOTOX	*Euglena gracilis*	For quality monitoring of water and waste water (Tahedl and Hёder 1999)
SOS-*LUX*-TEST	Genetically modified *Salmonella typhimurium* TA1535 bacteria	For detection and quantification of genotoxic substances (Rettberg *et al*. 2001)
LAC-*FLUORO*-TEST	Based on the constitutive expression of green fluorescent protein (GFP) mediated by the bacterial protein expression vector pGFPuv (Clontech, Palo Alto, USA)	For detection of cellular responses to cytotoxins (Rabbow *et al*. 2002)
Microtox assay	*Photobacterium phosphoreum* or *Vibrio fischeri*	Gives a screening assessment of the likelihood of contamination being present as can be applied in all types of water. (De Zwart and Sloof 1983); (Lebsack *et al*. 1981)
PolyTox® Rapid Toxicity Test	Contains specialized microbial cultures	For measuring the toxicity of wastewater to biological wastewater treatment systems (http://www.polyseed.com/)
Thamnotox	Crustacean *Thamnocephalus platyurus*	Used for routine screening of chemicals; specifically sensitive to biotoxins produced by blue-green algae in environmental samples (Lai 2013)
The Salmonella/E. coli Mutagenicity Test or Ames Test	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538	For testing of chemicals for mutagenicity (http://ntp.niehs.nih.gov/testing/types/genetic/invitro/sa/index.html)
Umu-genotoxicity-test	Genetically engineered bacterium *Salmonella typhimurium* TA1535pSK1002	For determination of the genotoxic potential of water samples
DAPHTOXKITS	Daphnid species *Daphnia magna*, *Daphnia pulex* and *Ceriodaphnia dubia*	Suited for routine toxicity testing of chemicals and wastes released in aquatic as well as in terrestrial environments http://www.microbiotests.be/
ALGALTOXKIT	Microalgae which are immobilized in a	Suited for toxicity of all chemicals and wastes released in aquatic as

	special matrix	well as terrestrial environments http://www.microbiotests.be/
ARTOXKIT M	Marine crustacean *Artemia franciscana*	For regulatory marine toxicity testing; for routine screening of chemicals and environmental samples http://www.microbiotests.be/
CERIODAPHTOXKIT F ACUTE and DAPHTOXKIT F MAGNA	*Daphnia magna, Daphnia pulex* and *Ceriodaphnia dubia*	For routine toxicity testing of chemicals and wastes released in aquatic as well as in terrestrial environments http://www.microbiotests.be/
DAPHTOX KIT F TM PULEX	*Daphnia pulex*	Crustacean toxicity test for freshwater http://www.biohidrica.cl/
MARINE ALGALTOXKIT	Microalgae inoculum	Suited for toxicity testing of all chemicals and wastes released in aquatic as well as terrestrial environments http://www.microbiotests.be/
OSTRACODTOXKIT F	Neonates of benthic ostracod crustacean *Heterocypris incongruens*	To detect and quantify the toxicity of freshwater sediments and by extension also soils contaminated by inorganic or organic pollutants http://www.microbiotests.be/
Phytotoxkit	Monocotyl *Sorghum saccharatum* (Sorgho) and the dicotyls *Lepidium sativum* (garden cress) and *Sinapis alba* (mustard)	Measures the decrease (or the absence) of seed germination and of the growth of the young roots of selected higher plants to toxicants or to contaminated soils; suited for sludges, sediments, composts and effluents for irrigation, as well as for toxicity determinations and toxicity ranking of pure chemicals and biocides http://www.microbiotests.be/
PHYTOTESTKIT	the monocotyl *Sorghum saccharatum* (Sorgho) and the dicotyls *Lepidium sativum* (garden cress) and *Sinapis alba* (mustard)	A variant of the Phytotoxkit and allows to determine the "direct" (= intrinsic) effects of "growth inhibiting" (toxic) chemicals and "growth promoting" chemicals on the germination and early growth of plants, without prior incorporation of the chemicals into a (reference) soil http://www.microbiotests.be/
PROTOXKIT F	Ciliate protozoan *Tetrahymena thermophila*	Growth Inhibition Microbiotest for Toxicity Screening of Pure Compounds - Effluents - Sediments - Surface and Ground Waters - Wastewaters http://www.microbiotests.be/
RAPIDTOXKIT	Larvae of the freshwater crustacean *Thamnocephalus platyurus*	Microbiotest For Rapid Detection of Water Contamination http://www.microbiotests.be/
ROTOXKIT M	Rotifer *Brachionus plicatilis*	Toxicity Test for Marine and Estuarine waters http://www.microbiotests.be/
ROTOXKIT F CHRONIC	Freshwater rotifer *Brachionus calyciflorus*	A Chronic Rotifer Toxicity Test for Freshwater http://www.microbiotests.be/
MOLTOX® UMU Genotoxicity Test Kit	Measures the ability of chemical treatmentsto induce *umu* gene expression in *S. typhimurium*	Application of the method for water and waste water samples is described in ISO 13829. http://moltox.com/
ABRAXIS Microcystin Test Strip	The test device consists of a vial with specific antibodies for mycrocystins and nodularins labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached	A rapid immunochromatographic test, designed solely for the use in the qualitative screening of Mycrocystins and Nodularins in finished drinking water; provides only preliminary qualitative test results http://www.dlwid.org/
ENVIROLOGIX QualiTube Kit for Microcystins	Microcystin toxin in the sample competes with enzyme (horseradish peroxidase)-labeled Microcystin for a limited number of antibody binding sites on the inside surface of the test tubes	Designed for semi-quantitative field screening of Microcystin toxin in surface water samples http://www.envirologix.com/

4. Conclusions

Summarizing and analyzing available information on the usage of modern tools in ecotoxicological research is crucial to outline current issues and trends in this area. The need for early diagnosis of negative anthropogenic impacts at all levels requires rapid and accurate assessments. An interesting trend associated with reducing the frequency of usage of oxidative stress biomarkers and increasing the frequency of usage of geno-toxicological and behavioral biomarkers has been established. The geno-toxicological biomarkers occupy second place in frequency of use, displacing physiological biomarkers. This trend is related to the general increase in genome interests in addressing environmental and human health problems. The used unique portal streamlines such assessments. It has a very simple and intuitive end user interface for author's research on the web. Given the fact that this is the first attempt in using such portal for analyzing scientific information, it should be mentioned that the latter has the opportunity to be improved in the course of work and by processing as much scientific articles as possible and by expanding the features added according to the relevant aim of the user. Also, it can be used not only for ecotoxicological papers but also for scientific articles with different topics.

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