Biomarkers of Ecotoxicological Effects in Social Insects

Oksana Skaldina and Jouni Sorvari

Abstract Complementing ecotoxicity testing, a biomarker approach is widely used in ecological risk assessment programs. Biomarkers provide information about early warning biological responses to one or several chemical pollutants and can be revealed in an organism or its products. Biochemical, morphological or behavioral parameters of living organisms can be set to biomarkers of exposure, effect or susceptibility or biomarkers of defense and damage. This concept is more developed within aquatic than terrestrial ecotoxicology and social hymenopterans insect (ants, bees, bumblebees, wasps and termites), which are already actively used as bioindicator species, can be furtherly studied for revealing novel sets of biomarkers. They can provide sufficient information about ecosystem health because social insects usually occupy high trophic levels and are important predators, pollinators, scavengers and ecological engineers. Social insect colonies stay in a certain place (except army ants), which makes them excellent model group for biomarker studies on ecotoxicological effects in nature. Despite their high ecological significance, wide spread distribution and sampling convenience, social insects are not intensively studied within biomonitoring programs and still not widely used as sentinel species. Revealing direct biological responses of social insects to toxic substances at the different levels of biological organization, systematization of scientific data and creating of simplified recommendations for practical biomonitoring purposes may facilitate progress in current terrestrial ecotoxicology.

Keywords Bioindicators · Biological responses · Terrestrial ecotoxicology · Sentinel species · Social hymenopterans

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1 Introduction

Over the last past century numerous classes of contaminants have been elaborated and released into environment. The most harmful of them are persistent organic pollutants (POPs) and inorganic compounds such as heavy metals and nonmetallic toxic substances, pharmaceuticals and personal care products. Aerial pollutants, such as aerosols and dust particles, are widely spread from tropics to the Arctic, and emissions still remain at high level (Gong and Barrie 2005; Chen et al. 2008) as well as POPs and polycyclic aromatic hydrocarbons (PAHs) are widely distributed, producing global adverse effects (Bartrons et al. 2016). However, at the same time advanced techniques and new approaches for monitoring environmental changes and preserving nature have been discovered. One of them is a biomarker approach, which was intensively developed over last 20 years and now significantly complements ecotoxicity testing (Romeo and Giamberini 2013). Biological responses toward toxic substances can be revealed at different levels of biological organization. However, at the lowest: molecular, cellular and tissular, which are the most sensitive, early warning signals can be discovered long before visible effects at the population level (Amiard-Triquet et al. 2013). Because of high sensitivity these early warning responses are especially useful for ecological risk assessment and preventing the harmful effects of pollution for the whole ecosystem. As soon as a xenobiotic is released into environment, it starts to affect it gradually from the level of an organism to the level of the whole ecosystem. That is why bioindicator organisms, or sentinel species, are useful for revealing the presence and toxicological effects of one or several contaminants (Berhet 2013) and biomarkers of these species may facilitate progress of ecotoxicological studies.

To be a sentinel species an organism should meet several requirements and the most desirable characteristics are: (1) sedentarity in the studied site; (2) ease of collection and identification; (3) sufficient population size, so the impact of specimens collection can be minimized; (4) wide and well known distribution range; (5) existence of a resistance to pollutants, when a species is able to tolerate stress within several years; (6) well established dose-effect and cause-effect relationships; (7) well-known biology and ecology allowing clear differentiation between the signal and the background noise (Berhet 2013). Social insects, which dominate in their environments, and frequently are at the top of food chains, can be a proper group to indicate ecotoxicological effects in general and biomagnification effects in particular. However, they remain poorly studied in this case. Modern advances in studies of social insect ecology, biology, physiology, genetics, conservation and management practice, allow review directions for revealing potential biomarkers in this unique group of living organisms.

2 Biomarker Approach in Revealing Ecotoxicological Effects

The initial idea of using early warning signals for the description of the health state have been derived from human medicine and was closely related to biology of vertebrates (Amiard-Triquet et al. 2013). In the early 90s European Science Foundation (ESF) considered four review papers, published in the same year and concerning possible usage of early warning responses in invertebrates, vertebrates, terrestrial plants and communities of invertebrates. After that, ESF had come to conclusions to what extent biomarkers are useful to evaluate environmental change and recommended to use them in biomonitoring programs (Romeo and Giamberini 2013).

The biomarkers approach is quite new, thus several different definitions and terminological concepts are simultaneously in use. According to one of the most early raised and widely used definitions given by Depledge (1994), a biomarker is a biochemical, cellular, physiological or behavioral change which can be measured in body tissues or fluids or at the level of the whole organism that reveals the exposure at or the effects of one or more chemical pollutants". Some authors, for example Hansen (2003), restrict biomarkers only to biochemical parameters, but current understanding of a term «biomarker» is much wider. Biomarkers can be any biological responses towards chemical contaminant, which are measured in an organism or its products and not occurring in non-exposed organisms (Romeo and Giamberini 2013). Consequently, there are biochemical, physiological, morphological or behavioral biomarkers (Amiard-Triquet et al. 2013). As it can be seen in a review concerning history of biomarkers by Romeo and Giamberini (2013), some authors refer biomarkers only to sub-organismal level of biological organization, while changes occurring in a whole organism they propose to consider as bioindicators. Bartell (2006) proposes more functional approach and states that biomarkers indicate measures of exposure or dose, while bioindicators—measures of effects.

There are several classifications of biomarkers, the most widely used are one, proposed by Manahan (2003) in which there are biomarkers of exposure, effect and susceptibility, and the other one created by De Lafontaine (2000) with biomarkers of defense, signaling about adaptation capacities of the species to survive in polluted environments and biomarkers of damage, revealing biological impairments which may indicate detrimental effects on reproduction and survival. Biomarkers varies from those of high specificity, like enzyme aminolevulinic acid dehydratase (ALAD) inhibited only by lead to those, which can be caused by numerous chemical agents (Amiard-Triquet et al. 2013). Biomarkers are capable to reveal presence of environment contamination, however there is a significant lack of specificity and only a few of biomarkers are really specific (Amiard-Triquet et al. 2013).

3 Social Insects as Bioindicator Species

Among invertebrate insects comprise the largest class with the estimated number of extant species between six and 10 million (Chapman 2009). Insects from the orders Hymenoptera and Isoptera (ants, wasps, bees, bumblebees and termites) exhibit advanced level of the community organization—eusociality. Eusociality is characterized by three main features, which are: (a) division of labor within reproductive and non-reproductive individuals; (b) cooperative brood care; (c) simultaneous co-existence of several generations within one nest (Wilson 1971). Besides being unique representatives of the top-level social structures among animals, social insects play crucial and complex ecological role in many terrestrial ecosystems. They are generalist and specialist predators, pollinators, parasites, and they are significant as a prey in food webs. Social insects differ from solitary insects in that case, that they possess additional organizational level—colonial. Their colonies can be either annual (bumblebees, social wasps) or multiannual (ants, honeybees, termites). The colony stays in a certain place (except in army ants) and occupies one (monodomy) or several nests (polydomy). Nests of social insects provide excellent, environmentally buffered, habitat opportunities for numbers of other organisms (parasites, commensals, mutualists) and so function as biodiversity hotspots for particular ecosystem (Hughes et al. 2008; Härkönen and Sorvari 2014). All points, mentioned above, make them proper bioindicator species of the habitat and environmental conditions they live, and excellent model organisms to study the general effects of pollution on animals.

Ants are widespread insects. Occupying high trophic levels and in many cases specialized niches, they possess numerous opportunities to be used as environmental, ecological and biodiversity indicators (Ellison 2012). Ants are predators, omnivores and fungivores as well as significant ecological engineers (Frouz and Jilková 2008; Sanders and van Veen 2011; Frouz et al. 2016). They are highly sensitive and quickly reactive to various environmental changes. For terrestrial ecosystems ants can provide information about ecotoxicological effects as a significant part of the ground-layer indicator sets, also they can be indicators of foliage-inhabiting communities and open habitats (Gerlach et al. 2013). Ants were used as bioindicators of mining site rehabilitation (Majer 1983; Andersen and Majer 2004).

Bees and bumblebees are important ecosystem providers and they can be widely used in agricultural ecosystems to monitor biological responses towards pesticides and other agricultural chemicals spreading in non-target species (Gerlach et al. 2013). Honeybees (*Apis mellifera*) have been shown to respond to biopesticide spinosad biochemically within first 24 h of exposure (Rabea et al. 2010). It has been shown that such economically important pollinators as bumblebees are suffering from neonicotinoid insecticides, widely used for herbivore pests. Thus, neonicotinoid exposure results in reduced colony growth rate and decreased queen production (Whitehorn et al. 2012).

Due to specific biological features, social wasps are promising bioindicators. Many species build their nests in urban or suburban areas close to human dwellings. Social wasps collect and use many types of food from nectar, pollen and honey dew, to many herbivorous pests, caterpillars and other insects. Thus, social wasps are widely involved into food chains. Although colonies in most cases possess annul life cycles new queens frequently stay in the location of their maternal colonies and this phenomenon have been known as philopatry. Therefore, effects on a population can be monitored over several years. Yet, there are not so many studies about wasps as environmental indicators, but interestingly, larval fecal mass of *Polistes dominulus* paper wasp have been shown to lead concentrations (Urbini et al. 2006).

4 Biochemical and Physiological Biomarkers in Social Insects

Being invertebrate arthropods, social insects possess several specific morphological, physiological and biochemical features. First, adults (imagos) through the process of metamorphosis always acquire a three-part-body, consisting of the head, thorax and abdomen. In some cases, semi-independent physiological processes can arise in these body parts, allowing search for biomarkers in a direct place. Second, insects have resilient external chitin skeleton, supporting and protecting their organs, and numerous physiological and biochemical processes are involved in its formation and functioning. Consequently, the reflection of these biological processes can be revealed in the surface of an insect body. Finally, due to the interior fluid hemolymph (analogous to blood in vertebrates), which is in direct contact with tissues, insects are able for immediate physiological and biochemical responses towards various external stresses, at least at the cellular level. Details of current knowledge about insects' physiology and biochemistry are systematically organized by Nation (2015), and should be considered before search for biomarkers in social insects.

Biochemical biomarkers in social insects are in their developmental stage. There are several studies concerning biological responses in these organisms to various toxic substances, however the majority of them report the need of validation that is more precise and the necessity for future studies.

Perfluorinated organic chemical perfluorooctane sulfonic acid (PFOS) is considered to be the most widespread of the perfluorinated organic compounds. It is released into environment mainly from plastic waste. After that as a particulate matter it can enter terrestrial ecosystems, affecting soil properties and even transporting from soil to crops and penetrating into nectar and pollen (Stahl et al. 2009). Being semi-volatile, phthalates are easily adsorbing to atmospheric particles, and then trapped by insect cuticle, so these toxic substances can affect social insects both via terrestrial and atmospheric pathways. Mitochondrial electron transport activity and lipid amounts of the bumblebee *Bombus terrestris* showed significant decrease in respond to (PFOS) chronic exposure via drinking or treated sugar (Mommaerts et al. 2011). Decreased mitochondrial membrane potential can lead to quick cell death and be a significant reason for an organism mortality.

Cuticular hydrocarbons (CHCs) act as water insulator and protection against pathogens (Martin and Drijfhout 2009; Ortiz-Urquiza and Keyhani 2013). In addition, CHCs are significantly responsible for chemical communication, e.g., nestmate recognition in social insects (Thomas et al. 1999; Sorvari et al. 2008). The cuticular wax layer and body fat reserves of ants have shown to easily absorb phthalates that are commonly used in plastic products (Lenoir et al. 2012, 2014, 2016). Black garden ant (*Lasius niger*) showed activation of immune genes *defensin*, *vitellogenin*, *histone-2A*, and *superoxide dismutase 1* in workers and degreased egg-laying in queens (Cuviller-Hot et al. 2014). Many of the phthalates are harmful for health, also for humans (Tickner et al. 2001; Matsumoto et al. 2008). In social wasp *Polistes dominulus* CHCs were shown to correlate with fertility of foundresses, conveying valuable information about their fecundity (Izzo et al. 2010). However, the effects of environmental pollutants on CHC-layer of social wasps remain unstudied. In addition to phthalates, the binding of other compounds on CHC layer of ants and social wasps might be worth of further research.

Although modern insecticides are developed far away from the harmful DDT, they still tend to affect non-target species and cause sub-lethal and lethal effects to economically important insects. Biochemical studies on honeybees (*Apis mellifera*), concerning early warning responses towards chemical exposure, showed significant inhibitory effects of insect growth regulators chlorfluazuron and oxymatrine and biopesticide spinosad on acetylcholine esterase (AChE) and adenosine triphosphatase (ATPase) (Rabea et al. 2010). Consequently, enzymes acetylcholinesterase (AChE), which is the nerve cell enzyme responsible for the acetylholine destruction, and adenosine triphosphatases (ATPases), which represent enzymes, catalyzing decomposition of ATP to ADP and a phosphate ion, can be promising biomarker to insecticide exposure.

Heavy metal poisoning also is an important ecotoxicological problems, and responses of social insects towards various environmental stresses can be quickly visible at the cellular level as well. Thus, encapsulation rate, which is an indicator of immune defense, of the red wood ant *Formica aquilonia* was elevated at the moderate levels of heavy metals, but suppressed in high levels of contamination (Sorvari et al. 2007). Encapsulation rate can be measured with a very simple method, by inserting a rubbed nylon mono-filament implant (Fig. 1) into the haemocoelon of insect and allow the circulating cells, including melanocyte cells, to encapsulate the object for certain time period. After the removal the implant can be photographed and its darkness refers the amount of cells participating the encapsulation reaction (Fig. 1). Encapsulation reaction is one of the major component in insect immunity, targeted especially against parasites and bacterial and fungal pathogens.

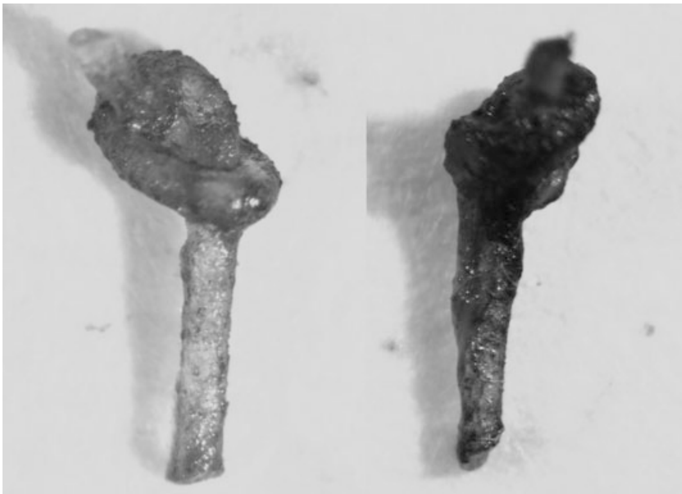

Fig. 1 Nylon monofilament implants used for encapsulation rate assay in insects. The darker the implant the stronger is the encapsulation reaction

Another cellular marker, hepato-nephrocitic system (HNS) has recently been found to be a promising biomarker. It has been shown that the HNS of the bumble bee *Bombus morio* responds to cadmium pollution and possible possessing broader practical application in the other bumblebees and bees (Abdalla and Domingues 2015).

5 Morphological Biomarkers in Social Insects

While search for biomarkers of ecotoxicological effects, insect morphology can be assumed in a broad sense, so many aspects of the outward appearance like size, shape, structure, pattern or colour can be considered. Social insects show visible size variation, which is typically measured as lengths or widths of different body parts as well as dry body weight. As it was mentioned before, social insects possess segmented body structure, thus some body parts or appendages may be more sensitive to various environmental stresses and influences of the toxic substances than the others may.

Generally, social insects are able to tolerate heavy-metal pollution and maintain their individual body resources. Thus, in workers of red wood ants (*Formica* s. str.) morphological characters, such as body mass and head width were not associated with the level of heavy metals: Al, Cu, Cd, Ni, Zn, As, Pb and Hg (Eeva et al. 2004). However, an individual body size can respond in long-term effects, as it was shown by Fedoseeva (2011) or can react in a biased frequency. Black garden ant (*Lasius niger*), which colonies were located along a metal pollution gradient, demonstrated the bias towards the higher frequencies of small workers in more heavily polluted areas (Grześ et al. 2015a). This was suggested to be a consequence of increased proportion of young colonies with small first generation workers in polluted environment, but physiological stress could also play a role in growth.

Although fluctuating asymmetry is considered to be a reliable measure of developmental instability for many vertebrate and invertebrate taxa (De Anna et al. 2013), it has not yet detected in ants (Rabitsch 1997; Grześ et al. 2015b). In addition to asymmetry, allometry, i.e., the size/shape relationship, could be used as a biomarker. Allometries are outcomes of growth of different body parts in a different rate. Typically allometric relationships are similar between colonies in ants (e.g., Tschinkel et al. 2003), but sometimes such relationships, can differ between colonies as Perl and Niven (2016) found differences in size of compound eyes between red wood ant *Formica rufa* colonies. The different scaling can reflect differences in environmental conditions, however, associations between scaling differences and environmental pollutants has not been studied in social insects.

Melanin-based color polyphenism is a promising direction of future search for morphological biomarkers in social insects. The key enzyme, responsible for the melanin production, is phenoloxidase, and it is present in insect cuticle, midgut as well as in the hemolymph (Sugumaran 2002). Therefore, for insects melanin play an important role in numerous physiological processes, such as physical protection, thermoregulation, regulation of water balance and immunity. Consequently, coloration can reflect biological responses of an organism and is a promising candidate to be an indicator for heavy-metal pollution, because melanin could bind metals and store them in pigmented cells (McGraw 2003). Among social insects, social wasps and many ant species show variable color patterns (Fig. 2) that could be promising early warning biomarkers of environmental pollution.

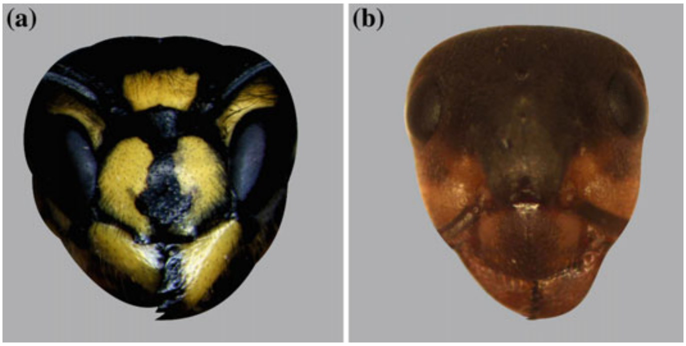

Fig. 2 Facial colour patterns of **a** a social wasp *Vespula vulgaris* and **b** a *red* wood ant *Formica lugubris*

6 Behavioral Biomarkers in Social Insects

Behavior is an integral part of any animal organisms and behavioral changes may indicate possible biochemical or physiological impairments such as dysfunction of a sensory system, endocrine disruption, metabolic disorders, neurotoxicity or some other adverse processes. Review of recent studies concerning behavioral biomarkers have shown that they are particularly responsive to nanoparticles contamination, as well as to antibiotics, brominated flame retardants and nonylphenols (Amiard-Triquet and Amiard 2013). That is why exactly behavior can be a key connecting link between responses at the infra-individual and supraorganismal levels (Amiard-Triquet and Amiard 2013).

Concerning social insects, behavioral biomarkers of ecotoxicological effects not systematically organized, yet there is a strong background allowing the rapid development of this direction. Thus, well-elaborated ethograms exist, like for example developed for the *Formica* ants (Wallis 1962). Social hymenopterans exhibit various behavior types concerning nest building and brood care, cooperative task performance and foraging activity, social organization and nestmate recognition, colony defense and grooming, etc. Impairments in these and the other types of behavior may indicate the presence of environmental stress. Behavioral tests were recommended to be used in risk assessment programs for toxic pesticides (e.g. neonicotinoids); as it was proved that chemical concentrations that might be considered safe, would cause adverse effects on foraging behaviour in bumblebees (Mommaerts et al. 2010) and pollen collecting efficiency in bees (Gill et al. 2012).

Such behavioral characteristics as passivity, aggressiveness and repeatability of various behavioral patterns worth considering when search for behavioral biomarkers in social insects. Heavy metal pollution has shown to be associated with passive behavior in territorial ant *Formica aquilonia* (Sorvari and Eeva 2010). Interestingly, repeatability as an independent parameter can be studied in different types of behavior: mating displays, male-female preferences, parental care, foraging, learning for different types of food, nest-building behavior, nest mate recognition and grooming behavior (Boake 1989).

7 Conclusions

Biomarkers of ecotoxicological effects in social insects are still in the stage of their development and systematization. To create efficient and informative biomarkers one should consider specificity of biological responses to toxicant in a particular species and determine the level of a biomarker specificity. Simultaneous assessment of biological responses at the different levels of biological organization may help to create biomarkers with higher specificity.

Practical application of biomarkers in social insects in terrestrial ecotoxicology requires simple and efficient protocols, which are easy in use and meet the needs of environmental experts and land managers. Concerning terrestrial invertebrates as bioindicators, the existing gap between scientific data and their real functionality is a general problem, so the development of relevant but simplified techniques are needed (Andersen et al. 2002).

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