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Freshwater animal models of the toxicity of novel substances. Effect of the history of population

Modele toksyczności nowych substancji u zwierząt słodkowodnych. Wpływ historii populacji

INTRODUCTION

Fish and molluscs have long provided valuable models for the study of basic biological processes in higher vertebrate systems [1, 7, 9]. Particularly, similarities in the types of oxidative damage and defenses between mammalian and piscine systems suggest that fish can provide model systems for further understanding of oxidative damage. Bivalve molluscs concentrate many contaminants and in this manner they serve as potential early bioindicators of human exposure risk [6]. The main novel kinds of water pollution can not often be appreciated in the water due to their rapid and spontaneous degradation, or they act even at low ppt–ppb concentrations [9]. Besides that, the complex pollution by municipal and agricultural wastes in the studied area [3, 4, 8] represent danger to human health.

The key goal of this study was to develop a sensitive approach to assess the relative risks associated with novel man-made substances by biotesting methods on widely explored freshwater teleost *Cyprinidae* and bivalve mollusk *Unionidae* with respect to the previous history of the population. Appreciation of biological responses included a set of biomarkers of general stress (oxidative stress, DNA damage) and of specific effects (neurotoxicity, metal-related toxicity, endocrine disruption).

MATERIAL AND METHODS

The effects of carbamate fungicide TATTU (0.005; 0.050 and 0.091 mg L^{-1}) was studied in fish *Carassius auratus gibelio* and bivalve mollusc *Anodonta cygnea* from clean (reference, R) and chronically polluted by municipal and agricultural wastes (P) sites after ten days of exposure. Superoxide dismutase (Cu,Zn- and Mn-SOD, EC 1.15.1.1) and cholinesterase (ChE, EC 3.1.1.7) activity, Redox index of glutathione (RI GSH), lipid peroxidation (LPO) determined by the production of TBA-reactive substances (TBARS), concentration of protein carbonyl (PC) and metallothionein (MT) in the gills, vitellogenin-like proteins (Vtg-LP) in plasma/hemolymph were determined as

described thoroughly in [1, 4, 5]. The micronucleated erytrocytes/hemocytes (MN) were calculated by the presence of miniature nuclei in the cytoplasm of postmitotic cells [2]. All measurements were expressed as mean ± standard deviation (SD) from 8 individuals. All statistical calculations were performed with SPSS 15.0 software, Statistica v 7.0 and Excel for Windows-2000.

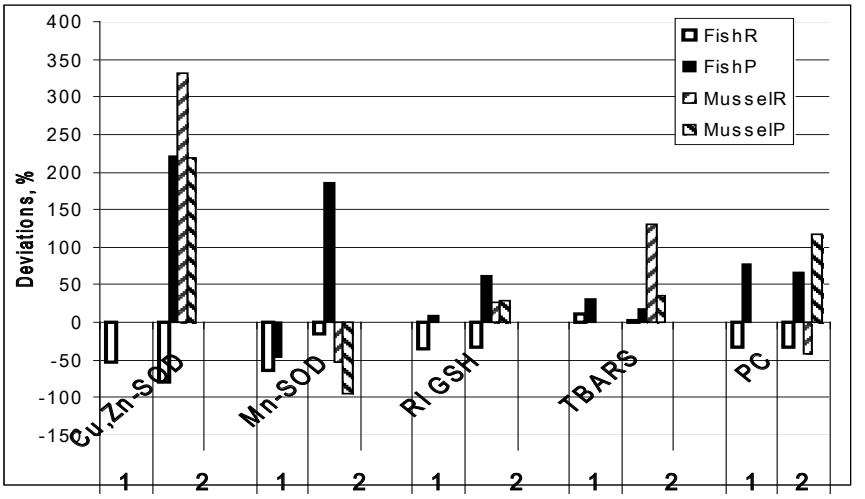
RESULTS

The untreated fish from site P demonstrated signs of oxidative destruction (low levels of SOD, RI GSH and high LPO) as compared to fish from site R (Table 1). The differences between two groups of molluscs were opposite. A higher concentration of MT at site P was shown for both species. The effect of TATTU provoked in fish from site R the inhibition of antioxidant defence and a decrease of MT, but elevation of Vtg-LP concentration, whilst in the fish from site P, the prominent activation of both antioxidative defence and LPO, a decreased level of Vtg-LP and higher activity of ChE were indicated (Fig.1). The difference in the effect of TATTU on molluscs from the two sites were less revealed. The concentration of PC was decreased by different concentrations of TATTU in both species from site R and increased in site P. Centroid grouping and discriminant analyses distributed all animals into separate sets confirming the dependence of their response of the history of population ($F=22.8$, $p<0.0001$ for fish and $F=16.7$, $p<0.0001$ for mollusc).

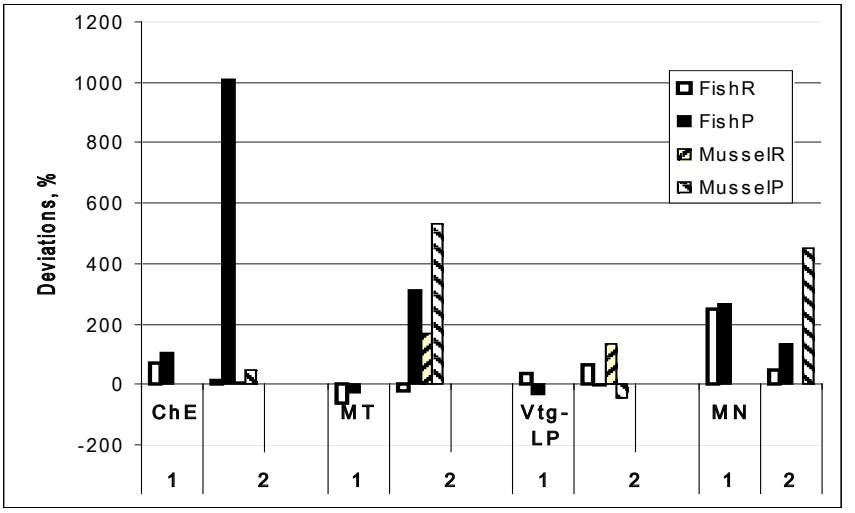
Table 1. Biomarkers of general stress and exposure in the fish and mollusk from two sites, mean ± SD (n=8)

Parameter	*Carassius auratus gibelio*		*Anodonta cygnea*	
	reference site	polluted site	reference site	polluted site
	gills			
Cu,Zn-SOD, U·mg^{-1} proteins	0.42±0.04	0.20±0.02*	0.13±0.02	0.34±0.12*
Mn-SOD, U·mg^{-1} proteins	0.25±0.03	0.22±0.03	1.06±0.08	2.20±0.08*
RI GSH	0.94±0.02	0.50±0.02*	0.58±0.08	0.93±0.01*
TBARS, nmol·g^{-1} of tissue	62.1±4.5	109.8±3.7*	20.7±0.3	28.3±4.1*
PC, μmol·g^{-1} of tissue	1.5±0.2	0.9±0.1*	1.2±0.1	1.2±0.1
ChE, nmol·min^{-1}, $^{-1}$ proteins	4.3±0.7	5.4±0.2	0.29±0.06	0.40±0.10
MT, μg mg^{-1} proteins	0.5±0.1	1.0±0.1*	0.03±0.01	0.14±0.02*
	blood plasma		hemolymph	
Vtg-LP, μg P_i mg^{-1} proteins	5.2±1.0	2.6±0.2	13.6±1.9	85.1±23.2
	erythrocytes		hemocytes	
Micronuclei, ‰	2.0±0.5	3.0±0.5	2.0±0.5	4.0±1.0

* Between sites statistically significant difference, $P < 0.05$



A



B

Fig. 1. Markers of the oxidative stress (A) and exposure (B) in the tissues of fish and mussel from the sites R and P under the effect of TATTU; 1, 0.005 mg L^{-1}; 2, 0.05–0.09 mg L^{-1} of TATTU

DISCUSSION

In the field conditions, the casual character of some kinds of pollution, wide variations in the time of induction, adaptation and recovery complicated the appreciation of relationship between the biomarker response and reasons for it [9]. The additional loading by inappropriate factor in low, environmentally realistic concentrations allows us to determine the comparative tolerance of the specimens due to the previous history of the population and also to the species-specific adaptive mechanisms. MN test confirmed the worst living conditions for both species at site P by high level of genotoxicity. Nevertheless, hardy fish *Carassius* demonstrated higher tolerance in the polluted area, due to the morphological and biochemical peculiarities mainly in the gills, whilst in the clean area the activity of stress response was not so effective. The difference between the two populations of mussels was less prominent. The similarity of the response of PC probably reflected the activation of the apoptosis as the general feature of the effect of TATTU at site R, whilst at site P the oxidative modifications of proteins were not removed. The activation of MT, ChE and Cu,Zn-SOD as a stress protein in the gills was the most prominent response to the inappropriate effect in the polluted area.

CONCLUSIONS

Low concentrations of contaminants can provoke stress response in aquatic animals that is more dependent on the history of population and animal species than on the nature of the contaminant. This approach may be applied for the appreciation of local environmental impacts on health status.

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SUMMARY

The effects of carbamate fungicide TATTU was studied in fish *Carassius auratus gibelio* and bivalve mollusc *Anodonta cygnea* from reference (R) and chronically polluted (P) sites after ten days of exposure by measuring a set of biomarkers of general stress and specific effects. Stress response was more dependent on the history of the population and animal species than on the nature of the contaminant.

Key words: biosecurity, adaptation, oxidative stress, metallothionein, endocrine disruption, micronuclei, aquatic animal models.

STRESZCZENIE

Zbadano wpływ karbaminianowego środka grzybobójczego TATTU na ryby z gatunku *Carassius auratus gibelio* oraz mięczaki *Anodonta cygnea* w lokalizacji referencyjnej (R) i długotrwale skażone (P) po 10 dniach ekspozycji poprzez pomiar biomarkerów stresu ogólnego i efektów specyficznych. Odpowiedź na stres była bardziej uzależniona od historii populacji i gatunku zwierząt niż od rodzaju zanieczyszczenia.

Słowa kluczowe: biobezpieczeństwo, adaptacja, stres oksydacyjny, metalotioneiny, zaburzenia endokrynne, mikrojądra, modele zwierząt wodnych