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Investigation on the Blood Differential Formula in *Rana ridibunda* (Anura, Amphibia) from the Area of the Maritsa – Iztok 1 Steam Power Plant

Zhivko M. Zhelev[1]

[1] University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Human Anatomy and Physiology, 24 Tsar Assen St., 4000 Plovdiv, Bulgaria, e-mail: zhivko_m@pu.acad.bg

Abstract: This is a comparative study of the leucogram of two populations of *Rana ridibunda*: one in polluted industrial area, in proximity to the Maritsa – Iztok - 1 Steam Power Plant, and another in an ecologically clean area, located to the south of the town of Harmanli. The indicators of the "white blood" were traced in spring and in autumn. The plasmatic cells of the animals from the polluted area significantly increased, most likely as a specific immune response to the antigens derived from the toxic environment. The monocytes in the animals from the polluted area were also above the normal levels, while the basophile cells were reduced. These changes could be explained as increased phagocytosis of products of tissue decomposition, consequence of the presence of environmental toxins, and could be regarded as a protective adaptive response to the hazardous environment.

Key words: Leucogram, *Rana ridibunda*, industrial pollution, adaptation, South Bulgaria

Introduction

Due to its transportation function, blood guarantees the connection between every cell in the multi-cellular organism and the environment. All changes spurred by the metabolism and the adaptive reactions of the organism to the dynamically changing environment have an impact on the blood (Shvarts 1986). The increased industrial production nowadays and the intensified anthropogenic impact on the biocenoses, being a major cause for their transformation, spurs interest in the natural reaction of organism communities to the changes of the surroundings (Shvarts 1976). The application of ecological expertise and ecological monitoring in industrial and agricultural production is required in order to maintain the overall balance of the biosphere at a level securing optimal development of the human society (Vershinin 1990). In relation to that, studies that provide not only assessment of the level of anthropogenic impact, but also allow for predictions for the state of environment, are extremely interesting (Toktamyssova, Kaydulova 1996).

Many authors suggest various morphological and physiological indicators in the amphibians, which can be used as "test-criteria" for bio-indication (Shvarts, Pyastolova 1970, Kossareva, Vasyukov 1976, Kubantsev, Zhukova 1982, Pyastolova *et al*. 1982, Pyastolova, Danilova 1986, Pyastolova, Trubetskaya 1989, Vershinin 1990, 2004). There is data that characterizes the inter-species changeability of hematological indicators in amphibians (Hutchison, Szarski 1965, Zhukova, Kubantsev 1978, Atatür *et al.* 1998, 1999). However, the studies explaining changes in the qualitive and quantitive indices of blood from amphibians living in areas with industrial pollution are limited (Sherstneva 1978, Tarasenko 1981, Zhukova, Kubantsev 1982, 1984, Zhukova 1987, Kalashnikov 1984, Toktamyssova, Kaydaulova 1996). Likewise, the hematological indicators of amphibians in Bulgaria are poorly studied (Tachev *et al.* 1975, Boyadzhieva *et al.* 2001, Zhelev *et al.* 2001, 2002a,b). There is only one study concerning the differential blood formula of some amphibian species (Nikolov, Darakchiev 1988) and there are only few works considering possibilities for the application of the amphibian hematological parameters in bio-indication and bio-monitoring (Zhelev *et al*. 2005, Zhelev *et al*. 2006). Moreover, there are no extensions considering any possibilities for their application in bio-indication and bio-monitoring.

The current paper is a part of a study on the hematological parameters in *Rana ridibunda* Pallas, 1771 (number of erythrocytes, metric parameters of erythrocytes, hemoglobin content, haematocrit value erythrocyte sedimentation speed, etc.). The research was carried out in the area of the Maritsa – Iztok - 1 Steam Power Plant (Zhelev *et al*. 2001, 2002). The aim is to analyze the blood differential formula in Rana ridibunda caught in the industrial area of the Maritsa – Iztok - 1 SPP, in the vicinity of the town of Galabovo, and to compare it to that of animals from the area of the town of Harmanli, which is considered clean and non-polluted (Bulletin for the condition of atmospheric air of the town of Harmanli, 2001). The spring and autumn time of the year were chosen in order a wide temporal gap to be set between the research periods, and thus to be considered the seasonal changes in the traced parameters. The Maritsa Iztok – 1 SPP together with the briquette factory not far away from it cover an area of 3-4 km to the southeast of the town of Galabovo, 100 m above sea level. The region is affected by significant industrial pollution, caused mainly by the two plants (Bulletin for the condition of atmospheric air of the town of Galabovo, 2001). According to 1992 data from the spatial arrangement plan of the Eastern Maritsa Coal Complex, the main sources of annual pollution in the area are sulfur oxides (96%), nitrogen oxides (12.5%) as well as carbon oxide (1.5%). Over 6000 tons of ashes are released into the atmosphere annually.

Materials and Methods

The object of examination were 120 *Rana ridibunda* specimens from both sexes with a body size ranging from 7 to 13 cm. Sixty of them were caught and tested in May 2001 and the remaining sixty in October 2001. Thirty specimens were caught and tested during both study seasons from the Rozov kladents Reservoir, located near the Maritsa Iztok – 1 SPP: 19 ♀ and 11♂ in May and additional 22♀ and 8♂ in October. Table 1 presents data from the physical chemistry analysis of the water. The tests were carried out in the SPP laboratory as a part of the research project. The values of the reservoir pollutants exceeding the allowed levels pointed in the Maximum Acceptable Concentration (MAC) are given below. The remaining 60 specimens, 21♀ and 9♂ in May and 23♀ and 7♂ in October, were caught in a micro-reservoir located 25 km to the south of the town of Harmanli in an area with similar climatic and geographical characteristics and with no records of industrial pollution. The wetland is detached from settlements, industrial objects and agricultural land. It hosts a great variety of animal species and therefore physical chemistry analysis of water pollutants was not carried out. These specimens were used as a control group.

The tests were carried out immediately after the animals were captured and heart blood was used for the preparation of blood smears, stained by Romanovsky-Gimza (Pavlov 1977). Blood differential formula was determined using 100 leucocytes from each sample, and the Shiling's method, microscoping by Erdval microscope and magnification of 10 x 100 (immersion lens) (Pavlov 1989). Digital data obtained in the course of these tests was statistically processed and variations

Table 1. Contents of pollutants above limit admissible concentration in Rozov kladenez Dam Lake

Pollutants	MAC	MAX	Times above LAC
May 2001			
Total hardness, mg/ekv/l	40.00	48.00	1.20
Nitrates, mg/l	0.8	2.20	2.60
Nitrites, mg/l	20.00	65.00	3.00
Sulphates, mg/l	300.00	780.00	2.60
October 2001			
Total hardness, mg/ekv/l	40.00	51.00	1.30
Nitrates, mg/l	0.8	2.40	3.00
Nitrites, mg/l	20.00	62.00	3.1
Sulphates, mg/l	300.00	820.00	2.70

were calculated. The authenticity of the differences was verified through the Student-Fisher t-criteria according to which variations are considered significant if t ≥3 (Sepetliev 1972).

Results and Discussion

The results from the research are based on 12 000 processed leucocytes. No sex variations were found among the animals from the Maritsa Iztok – 1 SPP area, as well as among those from the town of Harmanli, for both of the study seasons. No particular differences were detected between the two groups either. Therefore, in the table and graph illustrating the results, both sexes are pooled as a sum of the two groups. The blood differential formula in the animals from the Maritsa Iztok – 1 SPP has proven lymphoid characteristics during the spring season. The dominant cell group is lymphocytes (54, 27%), the relatively uniformly distributed basophiles – Ba (13, 07%), the segmented neutrophiles – Sg (12, 7%) and the plasmatic cells – Pl (10, 19%) (Table 2). The control group of animals from the region of Harmanli showed similar type of leucocyte blood formula in spring. Leucocytes (41,95 %), basophiles (21, 08%) and segmented neutrophiles were most abundant (Table 2).

Other authors have also mentioned the lymphoid character of the blood differential formula in Rana ridibunda (Kudryatsev *et al.* 1969, Grebenikova 1980, Zhukova 1984, 1988, Nikolov, Darakchiev 1988, Peskova, Zhukova 2003, Vershinin 2004). According to Peskova (2001), two main types of changes in the amphibian white blood are likely to occur as a result of the presence of toxic agents (pesticides), with the type of reaction independent from the nature of the chemical, but influenced by the duration and concentration of the exposure. The first type of changes on the leucogram is characterized by leucocytosis with a marked neutrophilia, displaced leftwards (growing of the stab cells), while monocytosis is observed when the concentration of the pollutant does not exceed any critical level, typical of the current toxicant, that is why this type is not considered as an adaptive reaction of the amphibians to a life in a polluted environment. The second type of reaction determined for the amphibian white blood (neutropenia combined with monocytosis and eosinophilia or lymphocytosis) is observed as a result of short exposure to highly concentrated toxic agents and is also considered a pathological type of reaction with a lethal outcome.

A comparative analysis of the data on the leucocyte blood formula in the animals from the Maritsa Iztok – 1 SPP and those from the area of Harmanli determined the following differences (Table 2.). The neutrophile granulocytes in the “white blood” of the animals from the industrial area (17, 24) are fewer than those within the control group (24, 29). The differences in the segmented neutrophile

– Sg cells are statistically significant, whereas the significance of the band neutrophile – St cells is ambiguous (t=2.95). The variations calculated on the basis of the number of eosinophiles (Eo), basophiles (Ba) and monocytes (Mo) are similar: their quantity in the blood of the Maritsa Iztok – 1 SPP animals is significantly less than that in the blood stream of the Harmanli area animals (t=9.07- Eo; 13, 81- Ba; 4, 86- Mo). The variations calculated on the basis of the quality of lymphocytes (Ly) and plasmocytes (Pl) represent a contrasting picture. Their number in the blood stream of the animals from the industrial area significantly exceeds that of the animals from the control group (54, 27 to 41, 95 Ly; 10, 19 to 1,

Table 2. Leucocyte blood formula of *Rana ridibunda* from the studied areas. Spring 2001.

Indicator		Harmanli n=30	Maritsa Iztok -1 SPP	t
Stab cells **St**	Limits of fluctuations	3 – 12	2 – 11	2.95
	$\overline{X} \pm m$	7.00 ± 0.47	5.17 ± 0.41	
	S	2.58	2.27	
	Cv%	36.86	43.90	
Cells with segmented nuclei **Sg**	Limits of fluctuations	14 – 22	4 – 24	7.68
	$\overline{X} \pm m$	17.29 ± 0.47	12.07 ± 0.49	
	S	2.57	2.71	
	Cv%	14.97	22.45	
Eozinophile cells **Eo**	Limits of fluctuations	3 – 10	1 – 8	9.07
	$\overline{X} \pm m$	6.50 ± 0.31	2.60 ± 0.30	
	S	1.69	1.64	
	Cv%	26.00	63.08	
Basophile cells **Ba**	Limits of fluctuations	17 – 25	3 – 24	13.81
	$\overline{X} \pm m$	21.08 ± 0.40	13.07 ± 0.42	
	S	2.18	2.29	
	Cv%	10.43	17.52	
Lymphocytes **Ly**	Limits of fluctuations	32 – 54	44 – 68	9.40
	$\overline{X} \pm m$	41.95 ± 0.81	54.27 ± 1.03	
	S	4.41	5.65	
	Cv%	10.51	10.41	
Monocytes **Mo**	Limits of fluctuations	2 – 8	1 – 7	4.86
	$\overline{X} \pm m$	4.38 ± 0.26	2.63 ± 0.25	
	S	1.42	1.38	
	Cv%	32.42	52.47	
Plasmatic cells **Pl**	Limits of fluctuations	1 – 4	7 – 15	21.51
	$\overline{X} \pm m$	1.8 ± 0.17	10.19 ± 0.34	
	S	0.95	1.87	
	Cv%	52.78	17.31	

8 Pl). In both cases the differences are statistically significant (t=9,40 and 21,51 respectively).

The inter-population variations observed in the spring could be caused by the presence of industrial pollutants in the area of the Maritsa Iztok – 1 SPP, despite the fact that no reaction of white blood, similar to the one described by Peskova (2001) was observed. Mainly chemicals, the polluting agents most likely stimulate the lymphoid branch in hemopoesis instead of the myeloid one. This is the most plausible explanation of the increased quantities of lymphocytes and plasmocytes in the blood stream of that group of animals. Such a reaction of the "white blood" elements to small pesticide doses in the organism of *Rana ridibunda* was reported by Kalashnikov (1984). He recorded sudden lymphocytosis together with a decrease in the number of neutrophile granulocytes and monocytes. His observations correspond to our results obtained from the spring data.

According to Kudryavtsev, Kudryavtseva (1984), toxic leucocytosis is usually accompanied by an increase in the number of leucocytes from the neutrophile range. In support of such conclusions are the findings of Peskova, Zhukova (2003), who reported marked neutrophilia in the leucogram of a *Rana ridibunda* taken from a rice field treated with pesticides. Tarasenko (1981) reports a sudden increase in the percent content of monocytes along with the increase of the St- and Sg- cells in the leucocyte blood formula in *Rana ridibunda* collected in a wetland, polluted by industrial waste waters. Such changes in the blood stream of the animals from the area of Maritsa Iztok – 1 SPP studied in the spring were not encountered. A plausible explanation for the lack of such an effect could be the nature of the toxicants present in the environment – mostly sulfur and nitrogen compounds, which stimulate the increase of the cells, participating in the humoral immune response.

The great number of plasmocytes found in the leucocyte blood formula of the animals from the Maritsa Iztok – 1 SPP in the spring is very intriguing, because there is almost no data in the literature for the presence of plasmocytes in the differential blood formula of *Rana ridibunda*. Their increased number in the white blood of the animals from the industrial area could be ascribed to the specificity of the humoral immune response, which correlates with the character of the "toxicant anti-genes" in the environment. Given that plasmocytes are the final result of the anti-gene stimulation and are the last stage of the differentiation of the B-lymphocytes, which are specialized in the synthesis of highly-specific immune globulines, it is undoubtedly interesting to note the immune reactions related to the participation of plasmocytes and the antibodies. Such findings lay the basis for a more detailed biochemical and immunological analysis in any future studies.

As far as the basophile cells (Ba) are concerned, our results suggest reduction in their numbers in the blood differential formula in the animals from the industrial area (13.07) compared to the control group (21.08). Such findings are supported by the data published by Zhukova (1987) for *Rana ridibunda* from a wetland polluted with pesticides. It is also noteworthy that the values for frogs captured in Russia are several times lower (5.00) than our estimates for the animals from our studied sites. A likely explanation is the geographical distance, according to Nikolov, Darakchiev (1980), as basophilia is reported in the blood stream of *Rana ridibunda* in Bulgaria compared to other groups of vertebrate animals and men. Their estimation of the value of the indicator for *Rana ridibunda* collected in Plovdiv is 19,22.

The seasonal transition from spring to autumn caused significant changes in the blood differential formula in the animals from the area of the Maritsa Iztok – 1 SPP, in comparison to the control group (Table 3).

The dynamics of the intra-population seasonal changes in the traced parameters of the white blood of the two groups should be noted (Table 4). Compared to the frogs collected around Harmanli, an increase was observed in the cells from the myeloid branch of the hemopoesis in the leucocyte blood formula of the animals from the Maritsa Iztok – 1 SPP in the autumn. The number of cells of the neutrophile and the mononuclear phagocyte systems in the blood of the animals from the industrial area (Sg St-29,44; Mo-6.90) exceed those in the blood of the animals from the control group (Sg St-25,44; Mo-3,27). The increase in the number of neutrophile granulocytes is at the expense of the statistically significant increase of the Sg-cells, while the number of St-cells in the autumn is still lower in the animals from the industrial area (Table 4).

The autumn changes in the neutrophile granulocyte system show similar trends within the investigated populations from both regions in comparison to those found out in spring:

– A reduction in the St-cells: Maritsa Iztok – 1

Table 3. Leucocyte blood formula of *Rana ridibunda* from the studied areas. Autumn 2001.

Indicator		Harmanli *n*=30	Maritsa Iztok -1 SPP	*t*
Stab cells **St**	Limits of fluctuations	2 – 9	1 – 5	4.66
	$\overline{X} \pm m$	4.57 ± 0.33	2.80 ± 0.19	
	S	1.79	1.04	
	Cv%	39.17	37.14	
Cells with segmented nuclei **Sg**	Limits of fluctuations	18 – 30	18 – 38	6.21
	$\overline{X} \pm m$	20.87 ± 0.52	27.02 ± 0.84	
	S	2.85	4.59	
	Cv%	13.66	16.53	
Eozinophile cells **Eo**	Limits of fluctuations	8 – 21	1 – 6	14.14
	$\overline{X} \pm m$	13.26 ± 0.70	2.80 ± 0.23	
	S	3.82	1.28	
	Cv%	28.79	45.71	
Basophile cells **Ba**	Limits of fluctuations	16 – 24	9 – 21	9.95
	$\overline{X} \pm m$	19.30 ± 0.30	13.83 ± 0.68	
	S	1.65	3.70	
	Cv%	8.55	26.75	
Lymphocytes **Ly**	Limits of fluctuations	32 – 43	34 – 64	5.22
	$\overline{X} \pm m$	37.33 ± 0.64	43.44 ± 0.98	
	S	3.50	5.39	
	Cv%	9.38	12.36	
Monocytes **Mo**	Limits of fluctuations	1 – 6	4 – 10	9.81
	$\overline{X} \pm m$	3.27 ± 0.24	6.90 ± 0.28	
	S	1.34	1.56	
	Cv%	40.98	22.61	
Plasmatic cells **Pl**	Limits of fluctuations	1 – 3	2 – 7	7.54
	$\overline{X} \pm m$	1.40 ± 0.11	3.21 ± 0.24	
	S	0.61	1.30	
	Cv%	43.57	38.58	

SPP (2.80), Harmanli (4.57). The differences with the spring values of the indicator, which are 5.17 and 7.00 are statistically significant (t=5, 27; t=4, 26 respectively).

– An increase in the Sg-cells, more strongly expressed in the animals from the industrial area (20.87), (17.29) for the autumn and spring respectively (Table 4) in comparison to those from the control group (20.87, respectively 17.29; t=5.11).

The statistically significant (t=9.81) increase in the monocytes in the blood differential formula of the animals from the area of Maritsa Iztok – 1 SPP in comparison to the control group is due to a greater increase within the group (6.90 in autumn in parallel to 2.63 in spring) given a reduction of the indicator in the population from the area of the town of Harmanli in the autumn (3.27, towards 4.38; t=3.08).

Similar reaction of monocytosis is reported by Isaeva, Vyazov (1997) in *Rana ridibunda* exposed to chemical pollution and by Vershinin (2004) in juveniles from the same species, living in a water basin, located in an area of increased radiation. According to these authors, the increased numbers of monocytes could be explained by the need to enhance the phagocytosis of products of tissue decomposition, caused by chemical agents and radiation.

It is very likely that increased waste products, caused by tissue necrosis due to hypoxia as a result of long-term presence of the pollutants in the environment, have resulted in such an adaptive mechanism that triggers the processes of an active increase in the level of cellular phagocytes in the blood of the animals from the area of the Maritsa Iztok – 1 SPP. Our hypothesis is supported by data describing changes in the condition of other quantitative and qualitative morpho-physiological parameters in Rana ridibunda collected in the same area at the same time; the changes were caused by the decrease of oxygen in the tissues (Zhelev *et all.* 2002a,b, Zhelev, Mollov 2004).

The autumn changes in the lymphoid branch, if the haemopoesis have the lymphocytes variations reported in the spring: the animals from the industrial area have a statistically significant higher value (t=5.22) of the indicator (43.44) compared to the control group (37.33).

Within both of the target animal groups, the lymphocyte reduction occurs in the autumn with the differences between autumn and the spring values being statistically significant (t=7.63 for the Maritsa Iztok – 1 SPP animal group and t=4.49 for that from the area of the town of Harmanli).

Table 4. Comparative seasonal analysis of leucocyte blood formula of *Rana ridibunda* from the industrial area of the Maritsa Iztok – 1 SPP and Harmanli (2001).

Indicator	Maritza Iztok – 1 SPP, 2001 Spring / Autumn, t-criterium	Harmanli, 2001 Spring / Autumn, t-criterium
Stab cells **St**	5.27	4.26
Cells with segmented nuclei **Sg**	15.41	5.11
Eozinophile cells Eo	0.54	8.78
Basophile cells **Ba**	0.95	3.56
Lymphocytes **Ly**	7.63	4.49
Monocytes **Mo**	11.54	3.08
Plasmatic cells **Pl**	16.62	2.00

The plasmocytes in the blood differential formula in the animals from the industrial area (3,21) significantly decrease in comparison to the spring values of the indicator (10.19), but are also (t=7.54) much greater than those in the blood of the animals from the control group (1.40) in the autumn.

The autumn reduction of the indicator value reported for the animal group from the area of the Maritsa Iztok – 1 SPP is statistically significant (t=16.62), while the minimal decrease from 1.8 in the spring to 1,4 in the autumn, observed in the sample taken from the area of Harmanli is not (t=2.00).

The autumn neutrophilia, combined with monocytosis in the specimens from the area of the Maritsa Iztok – 1 SPP towards the control group, points out that the anti-gene impact, prolonged in time, involves the more active participation of the cells from the neutrophile and mononuclear phagocyte systems in the immune processes of the organism. At the same time, the dominant role of the cells needed for the humoral immune response is preserved, despite the considerable decrease in the highly-specialized plasmocytes.

In the autumn the eospinophile and the basophile cells in the leucocyte blood formula of the animals from the area of the Maritsa Iztok – 1 SPP showed lower values (Eo-2.8; Ba-13.83) than those in the blood of the control group animals (Eo-13.26; Ba-19.30). The differences are statistically significant (t=14.14 - Eo; 9.95 - Ba) and outline a constant retaining of the eosinopeny and basopeny in the blood of the animals from the industrial area compared to those from the area of Harmanli. This trend becomes more evident within the group from the Maritsa Iztok – 1 SPP, whose autumn results (Eo-2.80; Ba-13.83) show an almost unchanged value of the indicator from the one reported in the spring (Eo-2.60; Ba-13.07).

Considering the fact that the Eo- and Ba- cells are derived from a common predecessor in the hemopoesis (an uni-polar progenitor cell) and that they are involved mainly with "alosteric" type of reactions in the organism's immune protection, it can be concluded that the possible lack of an "allergenic stimulus" causes their decreased numbers in the blood stream of these animals.

The increase in the levels of eosinophiles in the blood stream of amphibians from the genus of *Rana* inhabiting pure biotopes, in comparison to specimens living in urbanized areas, is described by Lebedinski, Rizhkova (1994) And Vershinin (2004). A possible explanation for that could be a parasite invasion, which affects more often amphibians in "natural" populations. Therefore, there is a likely correlation between the toxicants present in the habitat of the amphibians from the area of the "Maritsa Iztok" – 1 SPP and the extent of the parasite invasion. The overall increase in the protective functions of the organism of the animals from the industrial area is most probably a barrier against parasites, which could be related to the decreased number of Eo-cells in their blood as compared to the animals from the control group. To support such a hypothesis, additional data is needed on the extent of parasite invasion on the populations in both areas. Such an analysis was not among the goals of the current study.

Given similar changes in the blood of the animals from the industrial area, the autumn blood changes in the control group compared to the spring season can be considered normal: there was an increase in the Eo-cells (13.26, towards 6.50; t=8.78) as well as a slight decrease in the Ba-cells (18.30, towards 21.08; t=3.56). The autumn changes in the white blood of amphibians from the industrial areas of the Maritsa Iztok – 1 SPP (neutrophilia with dominance of Sg-cells and monocytosis) are a typical "first type" response, as described by Peskova (2001). These illustrate the development of a protective type of mechanism in the response the amphibian organisms in time, allowing the group to adapt to polluted environments. It is likely that the autumn occurrence of these changes in leucocyte blood formula in amphibians from the industrial area is a result of the close interaction of the specific local climatic factors (air and water temperature, direction of wind, rains, etc.) and the volley release of sulfur oxides that causes frequent acid rains in the June – September period. It is evident that, given the intransient presence in the environment of pollutants in a moderate amount, amphibians respond to the physiological stress by conserving the homeostasis of the organism and adapting to environment with less favourable conditions for life.

This research will contribute to the acceptance of "white blood" characteristics and their changes as test-criteria in bio-indication and to their use in ecological expertise and bio-monitoring of the environment. Another important aspect of the application is the possibility of using similar developments to describe trends for clarifying the mechanisms and the specifications of intra-species adaptogenesis in amphibians.

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Проучване на диференциалната формула на кръвта у *Rana ridibunda* (Anura, Amphibia) от района на ТЕЦ „Марица-Изток“ – 1

Ж. Желев

(Резюме)

В настоящата работа е извършено изследване на диференциалната формула на кръвта при две популации на *Rana ridibunda*: едната от промишления район на ТЕЦ „Марица Изток“ – 1, до гр. Гълъбово, а другата от незамърсен водоем, намиращ се южно от гр. Харманли. Изследваните показатели на „бялата кръв“ са проследени през два годишни сезона: пролет и есен. Броят на плазматичните клетки на животните произхождащи от замърсения район беше значително увеличен, най-вероятно като специфичен имунен отговор към антигените в замърсения водоем. Моноцитите при животните от замърсения район също бяха над нормалното ниво, докато броя на базофилните клетки беше понижен. Тези промени биха могли да бъдат обяснени с увеличената фагоцитоза на продуктите от тъканното разлагане, следствие от присъствието на токсини във водоема, и може да се счита като защитна адаптивна реакция към опасните условия на средата.