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PARADOXAL EFFECTS OF MORPHO-PHYSIOLOGICAL PARAMETERS OF DANDELION (*TARAXACUM OFFICINALE WIGG.S.L.*) URBAN POPULATIONS

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Abstract:

The influence of atmospheric pollution, caused by road transport, on the physiological and biochemical,ecological and morphological parameters of common dandelion plants (TaraxacumofficinaleWiggs.l.) is considered in the article. Multiphase dependences were obtained with a change in the direction of response,under the conditions of increasing traffic, in the germination energy and the weight of seeds, ripened in inflorescences, cut off on the fourth day after flowering. In the gradient of atmospheric pollution by motor vehicles, a complex three-phase dependence of the mass of seeds in the calathide, and the energy of their germination, is observed. The extreme values of these parameters are 13-20% higher, than the control values,and they are manifested when the traffic intensity is 2100 vehicles per hour, that allows to referthe dependence data to paradoxical effects. The critical mass of viable seeds for their germination was 0.23 ± 0.007 mg, which was 10% higher, than the previously defined critical mass of Taraxacumofficinale seeds. According to the Cheddock scale, the extent of interrelation between the weight of seeds and their germination is high (R^2 = 0.80).

Excessive activation of protective systems is one of the signs of paradoxical effects. With anthropogenic load of 2100 vehicles per hour, a sixfold increase in the activity of guaiacol peroxidases was recorded. At the same time, the level of lipid peroxidation in plant leaves from the considered cenopopulation did not differ from control.

Keywords: *bioindication, plant adaptation, anthropogenic load, energy of germination.*

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INTRODUCTION:

It is considered, that plants are reliable indicators of environmental pollution by various toxic substances. They are forced to adapt to stressors with the help of physiological-biochemical and anatomic-morphological reconstructions of the organism, since they are not able to move. Fixation and evaluation of these changes provide a reliable picture of the conditions of the place of growth of plants and reflect the state of the urban environment [1]. This thesis is valid only in case of direct or monotonic dose-response dependence.

Up to the present, when choosing bioindication factors, most of researchers have not considered the possibility of developing of nonmonotonic dose-response relationships in various physiological, biochemical, and ecological-morphological parameters of plants. The problem of hormesis and paradoxical effects under the influence of various environmental factors, including anthropogenic ones, is paid very little attention, therefore researchers, who are faced with paradoxical effects in plants, do not use these terms [2]. However, without taking into account these phenomena, the probability of inadequate estimation of the anthropogenic load level during bioindication is sharply increased.

Paradoxical effects relate to nonmonotonic dose-effect relationships (multiphase), under which the direction of response of the biosystem changes with increasing the intensity of the operating factor [3; 4; 5]. The current definition of paradoxical effects includes any nonmonotonic dose-effect relationships, except for two-phase hormeticcurve. At present, the mechanisms and consistent patterns, underlying the nonmonotonic responses of biosystems, especially in plants [6], remain unknown [7].Therefore there are a sufficiently large number of different hypotheses, which, however, consider the mechanism of development of nonmonotonic responses of biosystems only at the molecular and cellular levels. At the same time, it is generally accepted, that paradoxical effects are adaptive [8; 9; 3]. The stimulating phase of paradoxical effects is suggested to consider as a phenomenon of overcompensation, that is, excessive activation of the body's defense systems. The extremum of the stimulating effect can not be lower than 110%, and above than 130-160% of the control value [9].

Common dandelion (*Taraxacumofficinale*Wigg.S.l.) is widely used as a test object for environmental studies [10;11; 12; 13; 14]. Herewith, nonmonotonic dose-response relationships, as a rule morphological, are discussed only in recent years.

The purpose of this study was to determine the dependence of germination energy and the weight of dandelion seeds (*Taraxacumofficinale*Wigg. S.), matured in the inflorescences, cut off on the fourth day after flowering, under the conditions of periodic mowing from the intensity of automobile traffic,and to correlate the extreme indicators of these parameters with the activity of antioxidant system.

MATERIALS AND METHODS:

The object of the study

Common dandelion *Taraxacumofficinale*Wigg.s.l. was chosen as the object of the research.It is the plant, belonging to the family AsteraceaeDumort (CompositaeGiseke), the genus *Taraxacumofficinale*Wigg [15].

The places of plant material collection

Collection of plant material was carried out at the end of May 2015, at sites, located at a distance 1-4 m from roads, with different levels of pollution, in Kazan, Russia. Collecting grounds were chosen in such a way, that the traffic intensity varied over a wide range. The reference area was located in 7 km from the city, in the territory of the gardening company “Tatarstan” (Table 1).

Table 1. The intensity of traffic in the studied grounds of Kazan (average ± SD, n = 10).

№	Studyplots	Traffic intensity, vehicles per hour
1	Control	50±6
2	Ippodromnaya Street	380±45
3	PavlyukhinaStreet	720±111
4	TatarstanStreet	965±98
5	OstrovskyStreet	1180±107
6	HalturinStreet	1460±88
7	AkademikaParinaStreet	1632±74
8	MavlutovaStreet	2100±103
9	GorkovskoeShosseStreet	2596±112
10	OrenburgtractStreet	2992±108

Assessment of pollution by vehicles

Pollution, caused by automobile traffic, was estimated by traffic intensity (vehicles per hour). The cars were counted in the morning (from 8 to 10) and in the evening (from 17 to 19) [16]. The intensity of traffic correlates with the content of the main pollutants (oxides of sulfur, nitrogen, carbon, gasoline, kerosene, benz[a]pyrene and formaldehyde) in air along the motor roads [11, 12].

Assessment of parameters of reproduction and germination of seeds

To analyze the impact of pollution, the plants of young generative (q_1) ontogenetic state were used, the inflorescence shoot of which was cut off on the fourth day after flowering [17]. Ten inflorescences with a pedicel (10 cm) were cut randomly within areas 10×40 m. Each inflorescence was placed in a separate paper bag. The ripe seeds were stored in the refrigerator, at a temperature -18^{0}C.

The seeds were germinated in Petri dishes, for 50 seeds in settled tap water. Counting of germinating seeds was carried out on the seventh day of germination.

Evaluation of theantioxidant system effectiveness

The plant material for the analysis of peroxidation and peroxidase activity was collected from all grounds simultaneously. Placed in liquid nitrogen, the leaves were transported from the collection ground to the laboratory. Samples were stored at a temperature -80^{0}C.

The level of lipid peroxidation in leaves (0.2 g) was determined by reaction with thiobarbituric acid, in accordance with Heath and Packer [18]. Concentration of malonicdialdehyde (MDA) was defined, using an extinction coefficient - 155 mMсм$^{-1}$. The measurements were performed using thespectrophotometer Unico-2800 UV/VIS (USA), at a wavelength of 532 nm, as well as at 600 nm for the adjustment of nonspecific absorption [19].

The activity of guaiacol peroxidase was determined by the method of Kumar and Knowles (1993), with modifications on the same spectrophotometer [20].

Statistical analysis

Statistical analysis was carried out using the program OriginPro 9. The box plots, average values and their standard deviations were used to display the statistically processed data graphically. The significance of differences in the results was evaluated according to the U test of Mann Whitney at p = 0.05.

RESULTS AND DISCUSSION:

One of the indicators of seeds quality is their mass. Figure 1 shows the dependencies of seed weights on the intensity of motor traffic. The mass of seeds in the inflorescences, cut off on the fourth day after the end of flowering, significantly depended on the degree of contamination of the atmosphere. The decrease in mass was observed up to an intensity of 720 vehicles per hour, inclusive. Then, with the growth of automobile traffic, the mass of seeds has increased, while the weight of seeds from contaminated cenopopulation (2100vehicles per hour) was 17% higher, than control. The further increase in automobile traffic led to a sharp decrease in the total mass of seeds.

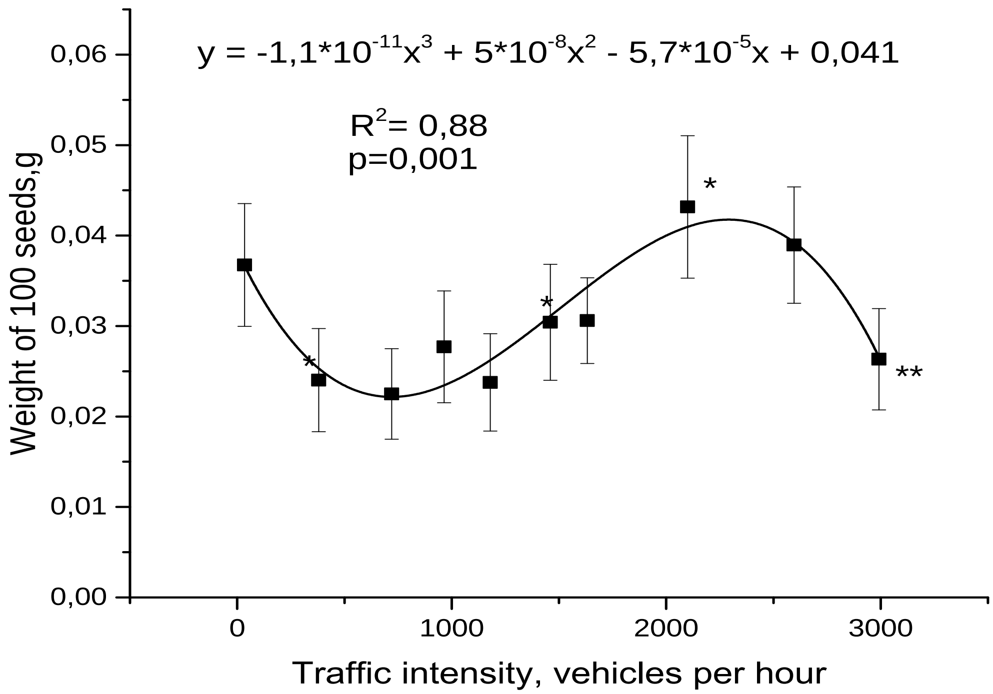

Figure 1. Dependence of the mass of seeds on traffic intensity (* indicates significant differences in values from previous ones, * p<0.05, ** p<0.001).

At present, the reasons for the nonmonotonic response in living organisms are insufficiently studied. At the same time, it has been shown, that at various stages of nonmonotonic dose-response, the contaminant can have an influence on various types of receptors, or different signaling cellular pathways, which cause a non-monotonic nature of dependency [3]. It is logical to assume, that thenonmonotonicityof the observed relationships is due to the work of the enzymes of the plant's antioxidant system, thatdoes not contradict the hypothesis of the gradual involvement of various adaptive mechanisms in the process of phenotypic adaptation to the factor.

It is known, that the widespread unspecific reaction of plants to unfavorable environmental factors is the production of reactive oxygen species (ROS) [21] and, as a consequence, the activation of processes of lipid peroxidation (LPO) [22].

The main role in the control of the level of ROS is played by peroxidases, reducing the level of hydrogen peroxide by restoring it to water [23; 24; 25]. The antioxidant system enzymes are more important in the elimination of ROS in plants,in comparison with non-enzyme antioxidants, since their operation can be highly controlled by the cell [24]. The activity of guaiacol peroxidases is often considered as a common peroxidase activity. These peroxidases (guaiacol-dependent peroxidases) are able to use aromatic compounds as hydrogen donors [26]. They participate in the processes of plant protection from biotic and abiotic stress factors of the environment [26]. Sulfur dioxide (sulfurous gas) [27; 28; 29; 30], motor vehicle emissions [31] contribute to the increase in the activity of guaiacol peroxidases. Based on these facts, peroxidase activity is used in bioindication [32; 33; 31; 34].

Figure 2 presents the results of the research of the influence of air pollution, caused by motor transport, on the activity of guaiacol-dependent peroxidases in dandelion leaves. The patternof the change in the activity of these peroxidases in the gradient of contamination was not revealed by us.However, with the intensification of traffic flow to 2100 vehicles per hour, a multiple increase in peroxidase activity was recorded (Figure 2). This coincides with an increase in the mass of seeds in the inflorescences, cut off on the fourth day after the end of flowering (Figure 1).

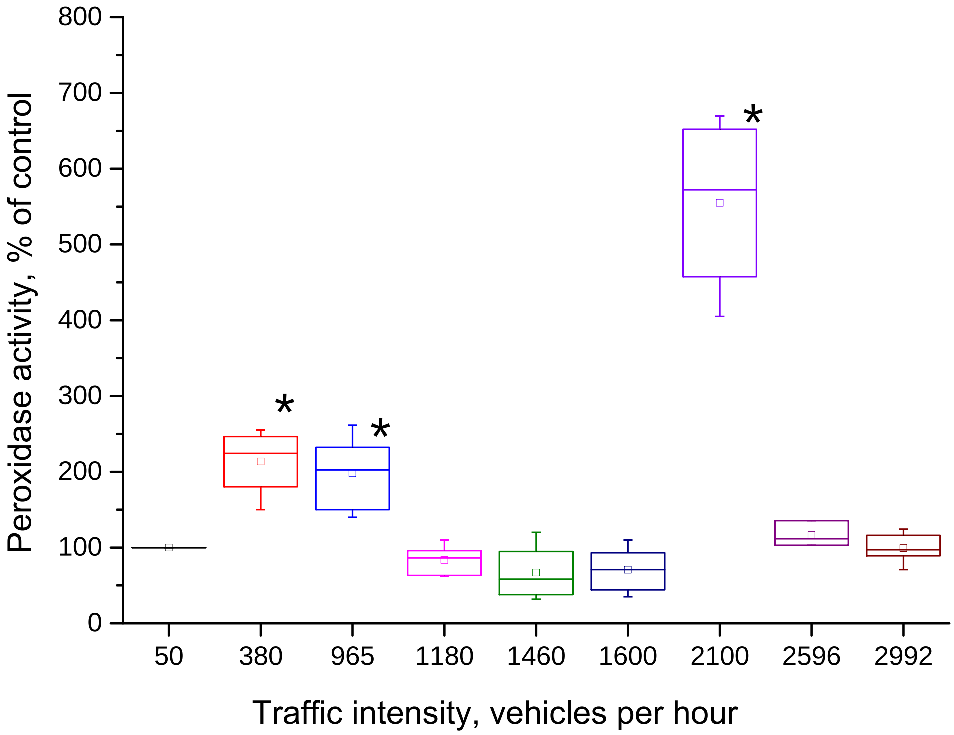

Figure 2. Change in guaiacol-dependent peroxidase activity with increasing traffic intensity (* indicates a significant difference in this parameter between plants, growing in contaminated and control populations, * p<0.05).

Restoring hydrogen peroxide, peroxidases reduce the possibility of forming a hydroxyl radical •OH, which is the main initiator of lipid peroxidation (LPO). The inverse correlation was expected between the activity of peroxidases and the concentration of malonicdialdehyde (MDA). The results of the study do not fully conform to this assumption (Figure 3). So, with the intensity of motor traffic 380, 965 vehicles per hour, the activity of peroxidases increased (Figure 2), and the concentration of MDA did not change (Figure 3). Under these conditions, the weight of seeds decreased (Figure 1). Completely different dependence was observed in the cenopopulation with anthropogenic load of 2100 vehicles per hour. The increased content in the atmosphere of pyrene and sulfur dioxide, contained in the exhaust gases, acting as pro-oxidants [35; 36],shouldcause an increase in LPO. However, the concentration of MDA in leaves of plants from this coenopopulationwas not higher, than in plants from the control cenopopulation (Figure 3), and the activity of peroxidases increased by 600%. In addition, the mass of seeds in the inflorescences, cut off on the fourth day after the end of flowering, has been increased (Figure 1).

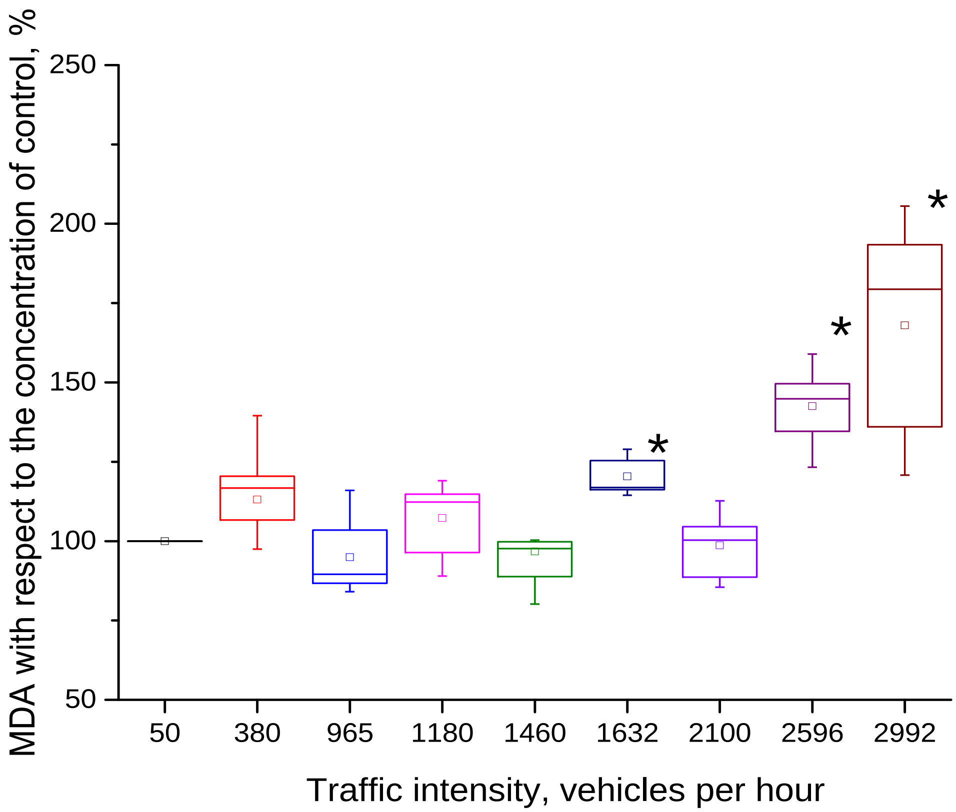

Figure 3. Change in the concentration of malonicdialdehyde (MDA) with an increase in the intensity of motor traffic (* indicates a significant difference in this parameter between plants, growing in contaminated and control populations, * p<0.05).

The implementation of the adaptive potential of dandelion at the considered level of anthropogenic load was manifested in the activity of peroxidases, which minimized the processes of LPO. It is known, that MDA is a regulator of gene expression [37; 38; 39]. It is likely, that significant increase in the peroxidase activity is associated with the synthesis of de novo peroxidases. It can be assumed, that stimulation of the expression of peroxidase genes is possible, when a certain threshold level of MDA is reached. In this case, with a lower anthropogenic load, an elevated MDA level should be revealed. Indeed, in the previous cenopopulation (1632 vehicles per hour), the MDA level was 20% higher, than the control values (Figure 3). With the growth of motor traffic (2596 vehicles per hour and more), the MDA content in dandelion leaves increases (Figure 3), and the peroxidase activity remains at the level of control plants (Figure 2). At the same time, all indicators of seed productivity decrease.

It was assumed, that these conditions would affect not only the weight, but also the quality of ripened seeds after mowing.

Adaptation to the pollution of atmosphere, caused by road transport, is associated with the metabolic cost of the body [11]. It is obviously, that in the inflorescences, cut off on the fourth day of ripening, the process of seed formation shows less tolerance to the anthropogenic stressor, due to the limited energy resources. Under these conditions, the accumulation of nutrients in seeds should be inversely proportional to the energy costs of adapting to anthropogenic stressor.

Figure 4 shows the dependence of the seeds number, germinated on the seventh day of spouting, on the intensity of air pollution, caused by motor transport. Germinative capacity of the ripened seeds in a cut inflorescence is 42 ± 8% (Figure 4).

The dose-response relationship in the seed germination energy is three-phase. At the first stage, the inhibition of germination was observed. The seeds of cut inflorescences did not germinate with the intensity of traffic 720 vehicles per hour.

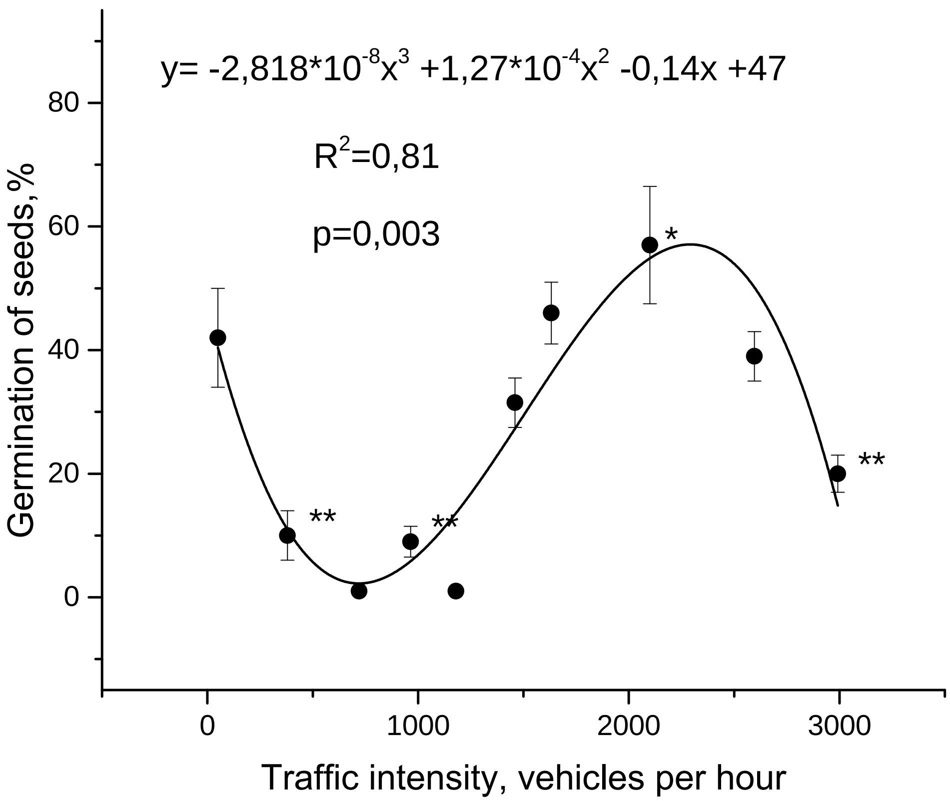

Figure 4.The percentage of seed germination, depending on the air pollution, caused by the road transport (* indicates significant differences in values, compared to control plants, * p<0.05, ** p<0.001).

At the second stage, the further increase in air pollution, due to the increment of traffic intensity, led to an increase in the germination of seeds. The maximum germination index was 15% higher, than the control (p = 0.03), with an automobile traffic intensity of 2100 vehicles per hour.

The third phase of dependence was characterized by a sharp decrease in the germination energy. The level of decline from control was 53%, and the weight of seeds in the most polluted cenopopulation decreased by 28% from the control (Figure 1).

It is known, that the critical mass of *Taraxacumofficinale* seeds for their viability in ecologically safe conditions is 0.212 ± 0.006 mg [17]. In the investigated cenopopulations, it was 0.23 ± 0.007 mg. At the same time, the mass of seeds correlated with their germination. According to the Cheddock scale, the extent of interrelation between the above processes is high R^2 = 0.80

SUMMARY:

In a wide range of the level of atmospheric pollution by motor vehicles, the dose-response relationship manifests a nonmonotonicity of the responses, which is satisfactorily described by a polynomial function of the third degree. The extremum valueof seed productivity is achieved when the plant's antioxidant protection system is activated, while the coefficient of determination between the weight and the energy of seeds germination remains high.

CONCLUSIONS:

According to the results of the study, the following conclusions can be drawn:

1. In the gradient of atmospheric pollution, caused by motor vehicles, a complex three-phase dependence of the mass and energy of seed germination, ripened in cut inflorescences, is observed. The extreme values of these parameters are 13-20% higher, than the control values, and they are manifested when the traffic intensity is 2100 vehicles per hour, that allows to refer the dependence data to paradoxical effects.

2. Excessive activation of protective systems is one of the signs of paradoxical effects. With an anthropogenic load of 2100 vehicles per hour, a sixfold increase in the activity of guaiacol peroxidases was recorded.
3. The critical weight of viable seeds for their germination was 0.23 ± 0.007 mg, thatis 10% higher, than the previously defined critical weight of *Taraxacumofficinale* seeds. At the same time, the extent of interrelation between the mass of seeds and their germination is high (R^2 = 0.80).

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