The influence of heavy metals in the green zone oak coppice on the fertility of *Neuroterus numismalis* Fourc and *Diplolepis quercus folii* L.

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**Abstract.** The problem of pollution effect on insects requires careful research. It is necessary to work out ways to reduce this impact on insects, if the pollution does not aim to destroy it. The purpose of the study was to investigate the effect of heavy metals on the species composition and population of *Cynips* (*Neuroterus numismalis* and *Diplolepis quercus folii*) in the overgrown oak forests of green zones. The main test - the object was a *Neuroterus numismalis* and *Diplolepis quercus folii*. The main research was carried out in coppice oak forests of the green zone of Voronezh (Russian Federation) in the Pravoberezhnoye forestry in quarters 33 and 35 along the M-4 “Don” highway. The ability of a *Neuroterus numismalis* to selectively populate oak leaves with an increased concentration of heavy metals puts it in the category of the most effective bio-indicators among the *Cynips*. The gall of the *Diplolepis quercus folii* is most susceptible to chemical contamination. With a low level of chemical contamination, the fecundity of the *Diplolepis quercus folii* and *Neuroterus numismalis* reaches a maximum level and decreases (in the *Neuroterus numismalis* one more sharply) with an average level of pollution.

1. **Introduction**

It is known that herbivorous insects occupy a central position in food chains and ecological pyramids. Under certain conditions, insects can influence the state of the habitat stage and the ecosystem as a whole [1, 2]. It was established that the process of the effect of chemical pollution on *Hymenoptera* (*Neuroterus numismalis* and *Diplolepis quercus folii*) hasn’t been studied properly [1, 3]. One of the possible effects of urbanization on insects is due to the influence of solid and gaseous emissions on *Hymenoptera* (*Neuroterus numismalis* and *Diplolepis quercus folii*). Air pollution by soot and solid oxides directly affects the insect population as a result of changes in their trophic regime [3, 4]. The timely detection and identification of these changes can provide the necessary information about a possible danger and allow it to be prevented in time, and, therefore, oak nutcrackers, to a greater extent than any other organisms, depend on the environment. A characteristic feature of the studied group of insects is the formation of galls on the leaves and other organs of trees of various species of oak. Galls are diverse in form and they are fairly reliable diagnostic signs of the species affiliation of their pathogens. Good visual detectability of galls makes them promising to use as bioindicators of the environment [5, 6]. The main experimental object was an *Neuroterus numismalis* and *Diplolepis quercus*.
The average lifespan of imago was determined at different distances from the pollution source, according to the adopted scheme. Adults were obtained from mature galls according to a well-established technique [7, 8]. As a result of contamination of plants with heavy metals, there is a danger not only of reducing their fertility, but also the appearance of genetically caused diseases [9, 10].

Researchers [11, 12] are of the opinion that walnuts can only weaken plants, but are not able to lead to their death even in the case of mass reproduction. This is primarily due to the fact that in the temperate zone the formation of galls of most nut growers occurs at the end of the growing season, when plants complete the active life cycle. For these reasons, this ecological group of arthropod phytophages as pests developing in forest ecosystems is not given due attention. On the other hand, the fact that outbreaks of the number of these insects cannot but affect the state of forest ecosystems is obvious. However, due to the fact that any significant damage by these insects is observed very often, the question of the effect of nut growers on oak groves remains open at present.

Revealing the influence of heavy metals on population dynamics *Cynips* in coppice oak forests in green zones of large cities (for example, the green zone of Voronezh, Russian Federation).

2. Materials and methods

The main research was carried out in coppice oak forests of the green zone of Voronezh in the Pravoberezhnoye forestry in quarters 33 and 35 along the M-4 “Don” highway (coordinates 51°45′N 39°11′E). The negative impact of the dense network of roads is especially noticeable, causing chemical pollution of plantings. Additional data were obtained in the course of sporadic surveys in the Shipovy forest, in the oak forests of Usmansky pine and Tsinsky forestry.

*Cynips* is constantly accompanied by *Quercus robur*. Their characteristic feature is the formation of galls on the leaves and other organs of the *Quercus robur*. Galls are large enough, specific for each species, and they represent a convenient and reliable diagnostic sign of the species belonging of their pathogens.

To study the effect of chemical pollution of coppice oak forests on the species diversity and population parameters of oak walnut trees along the road, a network of registration points was created. The distances between the counting points along the roads were taken equal within 100-300 m (depending on the composition of oak plantations), on average - 200 m, in the transverse direction 50-150 m (depending on the completeness of oak plantations), on average 100 m Thus, the survey of 1 km² of the roadside required the selection of 50 samples for chemical analysis. This approach ensured the principle of randomness, and therefore objectivity, of the research. A total of 360 observation points were established. The configuration of the counting tapes naturally changed depending on the location of the plantings.

Large clearings were excluded from the inventory. At each observation point in the autumn-spring period, samples of oak leaves from growing trees were taken for analysis for the content of heavy metals (lead, zinc, cadmium, copper and nickel), and samples of the forest litter were taken to determine the species composition and abundance of *Cynips* (by galls).

Studies were carried out among the *Cynips*: *Diplolepis quercus folii; Neuroterus numismalis; Cynips quercustolii; Biorriza pallida; Andricus inflator; Andricus foecundatrix; Andricus callidoma; Diplolepis longiventris; Andricus ostreus; Diplolepis longiventris; Neuroterus albipes; Frigonaspis megaptera; Neuroterus quercus-baccarum.*

The affection of *Quercus robur* trees by *Cynips* was assessed on a 5-point scale: 0 – no damage, 1 – low incidence (< 10%, on average 5%), 2 – medium susceptibility (10-50%, on average 30%), 3 – high susceptibility (> 50%, on average 75%), 4 – complete affection (100%) [13].

Analyzes for the contents of heavy metals in the selected samples were performed on an atomic absorption analyzer with 115M in duplicate at the laboratory of the Voronezh State Agricultural University. Determination of heavy metals is based on the absorption of light of the corresponding wavelength by the atoms of the element under study in a low-temperature plasma. Atomization of a substance in a graphite furnace is achieved by heating to a temperature of 2600–2700 °C with a current
of 400 A in an inert gas (argon) atmosphere. The average abundance was determined on the basis of 12-16 counting samples, providing a 95% level of significance of observations. To assess the reliability between the values, the Student's t-criterion (t(st)) was applied.

Table 1. Conventional levels of contamination of coppice oak forests with heavy metals in the green zone of Voronezh.

| Pollution level | Contents of heavy metals, mg/kg |
|-----------------|---------------------------------|
|                 | Zn     | Pb     | Cd     | Cu     | Ni     | Σ       |
| 1. Low          | < 27.0 | < 2.0  | < 0.20 | < 6.0  | < 2.0  | < 37.20 |
| 2. Middle       | 27.1-29.0 | 2.1-3.0 | 0.21-0.30 | 6.1-7.0 | 2.1-3.0 | 37.61-42.3 |
| 3. High         | > 29.0 | > 3.0  | > 0.30 | > 7.0  | > 3.0  | > 42.30 |

3. Results and discussion
When conducting research, special attention was paid to the Neuroterus numismalis and Diplolepis quercus folii. Table 2 shows the contents of heavy metals in oak leaves.

Table 2. The contents of chemical elements in the leaves of the oak colonized and uninhabited.

| Sample options                     | Contents of heavy metals, mg/kg |
|------------------------------------|---------------------------------|
|                                    | Zn     | Pb     | Cd     | Cu     | Ni     | Σ       |
| Unpopulated leaves                 | 27.1   | 2.1    | 0.48   | 6.05   | 3.74   | 39.47   |
| Populated leaves                   | 39.2   | 5.26   | 0.95   | 8.68   | 3.63   | 57.72   |
| Conditionally leaf values (table 1)| 26.4   | 3.1    | 0.18   | 6.0    | 1.5    | 37.18   |

As follows from table 1, the significance of differences between the total contents of heavy metals in unaffected and affected Neuroterus numismalis leaves of oak is proved at a fairly high level of significance t(st)= 3.8 (at confidence levels t(st)= 2.18 for the probability level 0.95). For certain metals, lead is released according to the significance of the differences. In terms of nickel contents, the differences are not statistically significant (t(st)= 1.8 ≤ 2.18). For comparison, we used samples affected by an oak Cynips, since the highest contents of heavy metals was found in them. Cynips chose oak leaves which were more saturated with heavy metals, their own influence was localized by chemical reactions.

Galls of oak nut growers are able to deposit heavy metals, but in smaller quantities compared to oak leaves. As follows from table 3, the Diplolepis quercus folii is most susceptible to the accumulation of heavy metals. The significance of differences in the accumulation of heavy metals in the galls of the Neuroterus numismalis was proved only with respect to lead.

The highest level of abundance in the presented complex of species of Cynips is characteristic of the average level of pollution (Σ39.47 mg / kg) (table 2). At low (<37.2 mg / kg) and high (>42.3 mg / kg) pollution levels, abundance levels decrease (according to the methodology - table 1).

Table 3. The contents of chemical elements in the galls of the Diplolepis quercus folii and Neuroterus numismalis.

| Kinds Cynips                | Contents of heavy metals, mg/kg |
|-----------------------------|---------------------------------|
|                             | Pb near the motorway | in the forest | Ni near the motorway | in the forest |
| Diplolepis quercus folii    | 2.81                     | 1.41          | 1.72            | 0.58          |
| Neuroterus numismalis       | 1.86                     | 1.23          | 0.32            | 0.21          |

From the data of table 3 it follows that Neuroterus numismalis tree sensitively reacts to chemical pollution. With increasing chemical pollution (within the studied levels), it increases its presence in the plantation. The galls of the Diplolepis quercus folii are largely susceptible to the accumulation of heavy
metals and stably maintain a high level of abundance at high levels of chemical pollution when other species of the Cynips cease to occur [7].

The effect of chemical pollution on the fecundity of oak Cynips from a biological standpoint is of particular interest [6]. This most important biological parameter was investigated by taking into account chemical pollution zones. Figure 1 shows the fertility diagram of the studied species of Cynips at various levels of chemical pollution. With further increase in pollution, the fertility of the Diplolepis quercus folii decreased slightly, while the Neuroterus numismalis increase. Thus, the nature of the influence of heavy metal pollution on the fertility of the studied species of Cynips is characterized by a rather complex pattern. The increase in the density of populations of Diplolepis quercus folii and Neuroterus numismalis under conditions of high levels of chemical pollution can most reliably be explained by a decrease in competition from other species of Cynips. So our hypothesis becomes most acceptable which also is confirmed in the works of foreign authors [14, 15].

As follows from the diagram (figure 1), the total number of species of oak Cynips and their average abundance depend on the level of chemical pollution of oak groves: with increasing pollution the number of species (from 11 to 4 types) and their abundance decrease (from 15 to 13 pieces). At the same time, the average level of abundance of one species found increases markedly (Diplolepis quercus folii) due to the disappearance of some species (Andricus foecundatrix) under conditions of a high level of chemical pollution. Therefore, in order to monitor chemical pollution, it is advisable to use not all species of oak Cynips found in the study region, but only those found at all pollution levels. These include the following five species: Andricus foecundatrix, Andricus ostreus, Diplolepis quercus folii, Neuroterus albipes, Neuroterus numismalis. The level of their abundance depending on the level of chemical pollution is characterized by the following diagram (figure 2).
Figure 2. Dependence of the abundance level of constantly occurring oak *Cynips* (*Neuroterus numismalis, Neuroterus albipes, Diplolepis quercus folii, Andricus ostreus, Andricus foecundatrix*) on the level of chemical pollution of the stands.

As shown in figure 2 that the average score for the abundance of oak nut growers has significantly decreased, but the abundance indices of certain species of *Cynips* (*Neuroterus numismalis, Diplolepis quercus folii, Andricus testaceipes*) wherein have increased. Other species (*Andricus curvator, Andricus ostreus*) became less common [9]. The above comparison is not strictly justified, since the studies were carried out using slightly different methods, but wherein it gives an idea of the trend in the dynamics of the abundance of oak *Cynips* - a decrease in the total number of species and an increase in the "weight" of individual species.

It is practically convenient in many cases to assess the level of profusion of *Cynips* in the form of the following scale (which allows one to obtain objective and fairly accurate information about the state of the environment quickly and without special costs) (table 4).

**Table 4.** Scale of abundance of galls of *Cynips* [13].

| Abundance score kind of | Accounting results in the crowns of trees (average / 10 m of the route) | Accounting results in forest litter (average / m² of litter) |
|------------------------|-------------------------------------------------|-------------------------------------------------|
| 0 – absent             | No galls detected                               | No galls detected                               |
| 1 – single             | 0.1 – 0.2                                       | 0.1 – 0.3                                       |
| 2 – rare               | 0.3 – 0.4                                       | 0.4 – 0.6                                       |
| 3 – usual              | 0.5 – 1.0                                       | 0.7 – 1.0                                       |
| 4 – numerous           | > 1                                             | > 1                                             |

Distribution features of some types of *Cynips*: *Diplolepis quercus folii* is distributed throughout the green zone of the city of Voronezh. *Neuroterus numismalis* occurs in the studied objects, alternating with *Andricus foecundatrix* and *Cynips quercustolii*. *Biorriza pallida* is generally found in large quantities in oak cultures, in glades. *Biorriza pallida*. *Andricus inflator* is distributed not all over the green zone, but only in some of its areas. *Andricus foecundatrix* is found everywhere, along with *Neuroterus numismalis* and *Cynips quercustolii*. *Andricus callidoma* occurs in the green zone singly. *Diplolepis longiventriss* occurs sporadically. *Andricus ostreus* is not found. *Neuroterus albipes* is not found everywhere and in small quantities. *Frigonaspis megaptera* is not found on experimental objects.
Thus, the fauna of oak *Cynips* in the green zone of Voronezh currently includes 12 species, three of which are numerous, five are common, two are rare and two are single; the numerous are *Diplolepis quercus folii*, *Neuroterus numismalis* and *Biorriza pallida*.

Five types of *Cynips* — *Andricus curvator*, *Andricus callidoma*, *Andricus testaceipis*, *Frigonapsis megaptera*, *Neuroterus guercusbaccarum* — are not found in the zone of high chemical pollution. The liberated ecological niche is occupied by *Diplolepis quercus folii* and *Neuroterus numismalis*. Their reproductive potential, although reduced, is sufficient to maintain an increased population density under conditions of high pollution background [16, 17]. Certain differences in biological and environmental features do not exclude completely competitive relations in oak *Cynips* complexes.

![Figure 3. The dependence of the fertility of the *Diplolepis quercus folii* on the levels of chemical pollution.](image)

Monitoring of chemical pollution of oak forests should be carried out in the second half of summer (by galls in the crowns of trees and shrubs) or in spring (by galls in the litter). The preference for the imago of the *Neuroterus numismalis* leaves of pedunculate oak with a high contents of heavy metals (figure 3) is probably that such leaves are physiologically weakened and are not able to resist insect infestation sufficiently. The positive effect of this phenomenon is that the affected leaves fall prematurely and free the tree from excessive pollution with heavy metals. The most susceptible to the accumulation of heavy metals are the galls of the *Diplolepis quercus folii*. The significance of differences in the accumulation of heavy metals by the galls of the *Neuroterus numismalis* was proved only with respect to lead. In general, galls of *Neuroterus numismalis* are more protected from heavy metals.

Under conditions of a low level of chemical pollution of the environment, the fertility of the *Diplolepis quercus folii* and *Neuroterus numismalis* is maximum and decreases (in the *Diplolepis quercus folii* tree more sharply with an average level of pollution. With further increase in pollution, the fertility of the *Diplolepis quercus folii* grows slightly, but *Neuroterus numismalis* even increases. Thus, a clear conclusion about the effect of pollution by heavy metals on the fecundity of the studied species of *Cynips* is not possible.

With all levels of heavy metals identified in the overgrown oak forests, two types of oak *Cynips* are found and increase in abundance with increased pollution: *Diplolepis quercus folii*, *Neuroterus numismalis*. In the natural range, the damage from *Cynips* (*Diplolepis quercus folii* and *Neuroterus numismalis*) is great, this is confirmed by the results of other researchers, while *Cynips* react to chemical pollution, increasing the number of individuals in the stands [18-20]. In the studies of many authors, it
was proposed to carry out biomonitoring of chemical pollution using oak *Cynips* as a more effective method [14, 2].

Recommended monitoring of chemical pollution of *Cynips* oak groves is not an alternative to other types of monitoring and is proposed as practically convenient. Thus, an increase in the density of populations of *Diplolepis quercus folii* and *Neuroterus numismalis* under conditions of high levels of chemical pollution can most reliably be explained by a decrease in competition from other species of *Cynips*.

4. Conclusion

The highest average abundance score (2.8) was noted for *Diplolepis quercus folii* and *Neuroterus numismalis* oak *Cynips*, the minimum (0.8) for *Andricus foecundatrix*. In conditions of an average level of chemical pollution, all studied *Cynips* species are also present, but the minimum average abundance score is already decreasing to 0.3 in relation to *Andricus callidoma* and *Frigonapsis magaptera*. The significance of differences in the accumulation of heavy metals by the galls of the *Neuroterus numismalis* was proved only with respect to lead. With a low level of chemical contamination, the fecundity of the *Diplolepis quercus folii* and *Neuroterus numismalis* reaches a maximum level and decreases with an average level of pollution. The highest level of *Cynips* is characteristic of the average level of pollution (Σ39.47 mg/kg). The total number of species of oak *Cynips* and their average abundance depend on the level of chemical pollution of oak groves: with increasing pollution (to high), the number of species (from 11 to 4 types) decreases and their abundance (from 15 to 13 pieces) also decreases. At the same time, the average level of abundance of one species found increases markedly (*Diplolepis quercus folii*) due to the disappearance of some species (*Andricus foecundatrix*) under conditions of a high level of chemical pollution.

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