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Original Research

Bioaccumulation of Heavy Metals (Zn, Pb, Cd) in *Polistes nimphus* (Christ, 1791) (Hymenoptera, Vespidae) Living on Contaminated Sites

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Abstract

The aim of the study was to determine the extent of heavy metal accumulation (Zn, Cd and Pb) in the bodies of females of the predatory species *Polistes nimphus* (Christ, 1791) (Hymenoptera, Vespidae). The insects were captured in areas affected by the proximity of ZGH "Bolesław" – an industrial complex located in Bukowno near Olkusz (southern Poland), whose main activity is mining and processing of lead and zinc ores.

Three sites that differed in terms of distance from the source of contamination and also in the concentrations of Zn, Cd and Pb in the top soil layer were selected. The heavy metal content of the soil was determined for each site. The most contaminated site was located in the immediate vicinity of ZGH "Bolesław" (4326.50 mg/kg Zn, 56.96 mg/kg Cd, 3977.00 mg/kg Pb); the least contaminated was the site furthest away from the source of contamination (48.75 mg/kg Zn, 0.72 mg/kg Cd, 25.43 mg/kg Pb).

On all the sites, during the two-year study (2015-2016), individuals of the genus Polistes were captured and female wasps of the species *P. nimphus* were isolated from among them. Then the extent of accumulation of Zn, Cd and Pb in their bodies was determined. Correlations between the concentrations of Cd, Zn and Pb in the soil and those in insect bodies were calculated.

On all three sites, in both years of the study, the concentrations of heavy metals in insect bodies changed depending on their concentrations in the soil. The highest levels of the accumulated Zn, Cd and Pb were always observed on the site located in the vicinity of ZGH "Bolesław". The lowest values were observed on the site furthest away from the source of contamination. The concentrations of all three metals in the bodies of insects increased with their increasing concentrations in the soil, but the differences were not statistically significant.

Keywords: paper wasps, social insects, heavy metals, trophic levels, bioaccumulation

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Introduction

Everyday human activity is closely related to the ongoing economic progress associated with the development of various industries, including mining and metallurgical industries. Although the development of these sectors generates unquestionable financial benefits, it also causes a number of negative effects, such as environmental pollution with heavy metals [1, 2]. Despite the fact that some metals are necessary for the normal development of an organism (Zn, Cu), they become toxic after exceeding a certain level. Other metals (Cd, Pb, Ni) are not used by organisms [3]. These elements have the ability to migrate between different levels of trophic networks [4]. Consequently, they also accumulate in the bodies of insects of various species.

Despite the fact that insects are very often used as bioindicators of the environment pollution, no permissible limits of heavy metals in their bodies compared to the background of contamination were estimated yet. Determination of the critical content of heavy metals in their bodies is based, among others on the use of indicators defining the toxicity of a given substance, e.g. LC50, EC50. Their values differ depending on the insect species [5-8].

A lot of attention in research conducted so far has been devoted to pollinating insects because of the role they play in maintaining plant biodiversity [9]. However, other groups of insects, through bioaccumulation of heavy metals, can be a valuable source of information on environmental contamination with these elements. Social and predatory insects, which include wasps of the genus Polistes, seem to be particularly useful for this purpose.

The aim of the study was to determine the extent of Zn, Cd and Pb accumulation by female *Polistes nimphus* (Christ, 1791) as dependent on the distance from the source of pollution and the concentrations of these heavy metals in the soil.

Materials and Methods

Research Sites

The research was conducted in the vicinity of ZGH "Bolesław" – a mining and processing complex located in Bukowno near Olkusz, Poland (50°30'28''N, 19°28'17''E). The company is engaged in mining and processing of lead and zinc ores, thus being a source of pollution with heavy metals, i.e. cadmium, zinc and lead. These metals occur in this region in significant quantities, which facilitates the detection of these elements in soil and living organisms. In addition, they have been studied for bioaccumulation in other living organisms developing in this area (plants, animals) [10-12]. Three sites that differed in terms of heavy metal content in the soil and the distance from the source of contamination were marked out. Site 1 was located in the immediate vicinity of ZGH "Bolesław" (0.44 km). It was a warm, very sunny area of turf surrounded by tree stands dominated by Scots pine (*Pinus sylvestris* L.). Site 2 was located 1.5 km away from the source of contamination, in a north-westerly direction. It was also a sunny area of grassland surrounded by pine forests with an admixture of various deciduous and coniferous species (birch, beech, larch, etc.). The last site, Site 3, located to the north of the industrial complex (smelter) and the furthest away from it (19.62 km), also represented a warm area of turf; it was surrounded by trees with a species composition similar to that of the first two sites. All three sites were located on sandy soils.

Insects that were the subject of the study were caught alive on plants of the family Apiaceae, including lesser burnet (*Pimpinella saxifraga* L.) and carrot (*Daucus carota* L.), present on all the selected sites. The strongly sunlit, warm and dry grassland on each site provided an environment suitable for the development of the genus Polistes. The experimental sites were selected on the basis of the data on the concentrations of Zn, Cd and Pb in the soil in the vicinity of ZGH "Bolesław", contained in the works published by Grześ [13] and Szentgyörgyi et al. [14].

Insects Studied

Wasps of the species *P. nimphus* were selected as the subject of the study. In justifying this choice, it should be emphasized that:

– This species is a predatory species, which makes it particularly vulnerable to heavy metal bioaccumulation. Both the larvae and imagines feed on animal food from a wide range of phytophagous and other species. In addition, Polistes are also known to ingest plant food in the form of pollen and nectar.
– Wintering females establish colonies in which numerous individuals develop, thus facilitating collection of an adequate number of wasps [15].
– Individuals of the species *P. nimphus* are social insects, bound to the area strictly defined by the location of the nest. They are not very good flyers. It is assumed that their flights in search of food are limited to an area of about 72-150 m^2, depending on the species [16, 17].
– *P. nimphus* is a species poorly studied in terms of bioaccumulation of heavy metals.

Insect Sampling

In late July and early August of 2015 and 2016, individuals belonging to the genus Polistes were captured on each site. During this period, Polistes occur in the highest numbers, but still remain close to their nests. The catching was performed on sunny days, at approximately weekly intervals. On each site, the catching took place on three times in each year of the study. The adults were collected with the seeping net using the method of hand collecting at a specific time catching individuals during flight or foraging on plants. The collected individuals were placed in separate Eppendorf vials with air access and thus transported to the laboratory, placed individually in PVC boxes with perforated lids labelled with the date and place of collection, provided with cotton wads soaked in distilled water. The insects were kept at 25°C for 48 hours without food to make them empty the contents of their intestines. Then the insects were killed by freezing. Individuals belonging to the genus Polistes were identified to the level of species using the entomological key compiled by Dvorak & Roberts [18].

Soil Sampling

At the end of 2015, samples of topsoil were collected on each of the three sites to estimate its contamination with heavy metals. The samples were taken randomly from an area of 1 km^2 from the closest nesting area and feeding places of *P. nimphus*. The topsoil was taken using a spatula, discarding the part that was in contact with the surface of the sampling tool. Each sample was placed in a separate bag, labelled with the date and place of collection, and transferred to the laboratory. In total, 40 soil samples were collected at each of the sites, from which 3 mixed samples corresponding to individual sites were then created. The samples were dried to an air dry condition, then ground and sieved through a 0.2 mm sieve.

Insect Analysis

During the collection of material, insects belonging to the genus Polistes were caught, but only females belonging to the species *P. nimphus* , being the most abundant species, were selected for analysis. During the sampling, male specimens were also collected, but they were not very numerous in the total sample and more importantly they were not caught in all tested sites. Considering that for the determination of heavy metals in insects bodies, an appropriate size of sample was needed, the authors decided to focus only on female individuals. The insects were separated by sex, washed thoroughly in distilled water, to remove from their bodies plant and soil pollutions that could affect the result of analysis, and dried in the laboratory at 20ºC. Next, the concentrations of heavy metals (Zn, Cd, Pb) were determined in the bodies of the insects: samples were digested by wet mineralization in a semi-open system with heating plates in quartz glass. After mineralization, all sub-samples, blanks and reference materials were poured over with 2 ml of acid water (0.2% nitric acid) according to the AAS determination procedure. The solutions prepared in this way were then used to determine in them the concentrations of heavy metals (Cd, Pb and Zn) using atomic absorption spectrophotometers: PerkinELmer PinAAcle 900Z (Pb, Cd) in a graphite cuvette and a PerkinElmer AAnalyst 200 (Zn) in an acetylene-air flame. The following wavelengths were used for the individual elements: Cd – 228.8 nm, Pb – 283.3 nm, Zn – 213.9 nm.

Soil Analysis

The soil samples collected from each site were mixed together to obtain three bulk samples for further analysis. Granulometric composition was determined by the Casagrande method modified by Prószyński, and soil pH was measured by the potentiometric method in a water : soil (1 : 2) solution. For the determination of heavy metals (Zn, Cd, Pb) soil samples were dried and next homogenized. Approximately 0.5g of sample were weighted and transferred to digestion vessel and a 10 ml aquaregia was added. Samples were mineralized. The obtained solutions were filtered, collected in 50 ml flasks and diluted with distillated water and next determined by ICP-OES (inductively coupled plasma optical emission spectrometry- spectrometer recommended for metals in soils determination).

Statistical Analysis

To determine the significance of differences in the concentrations of individual heavy metals in the soil on each site, univariate ANOVA significance tests were conducted. The relationships between the Zn, Cd and Pb concentrations in the soil and those in the bodies of female *P. nimphus* were determined using Pearson's linear correlation. The statistical analyses were performed on $\log_{10}$-transformed data using Statistica 13.1 software.

Results

Soil

All of the selected sites were located on sandy soils, with an acidic or slightly acidic pH of 5.59 on Site 3 to 6.8 on Site 1. The concentrations of heavy metals in the soil changed with the concentration gradient and the distance between the site and the source of pollution. The most contaminated soils were found on Site 1 in the immediate vicinity of ZGH "Bolesław". The heavy metal content in that area was 4326.50 mg/kg Zn, 56.96 mg/kg Cd and 3977.0 mg/kg Pb.

The lowest concentrations of the analyzed elements were recorded on Site 3, the one furthest away from the source of contamination, where they amounted to: 48.75 mg/kg Zn, 0.72 mg/kg Cd and 25.43 mg/kg Pb (Table 1).

Insects

In the two years of the study, individuals of the genus Polistes were collected, and 294 females of the

Table 1. Soil pH and the concentrations of Zn, Cd and Pb in the topsoil.

Site	Location	Distance from smelter (km)	pH	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
1	50°16'N 19°28'E	0.44	6.87	4326.50	56.96	3977.00
2	50°17'N 19°27'E	1.5	6.02	1856.30	35.21	915.88
3	50°26'N 19°35'E	19.63	5.59	48.75	0.72	25.43

dominant species, *P. nimphus*, were selected from among them. Companion species included *P. dominulus* and *P. biglumis*.

On all three sites, in both years of the study, the concentrations of heavy metals in insect bodies changed depending on the concentrations of those elements in the soil. The highest levels of Zn, Cd and Pb were always observed on Site 1, where they were, respectively, in 2015: 368.68 mg/kg, 17.94 mg/kg, 5.19 mg/kg, and in 2016: 317.27 mg/kg, 5.10 mg/kg and 4.88 mg/kg.

The lowest Zn content in the bodies of *P. nimphus* females was recorded in 2016 on Site 3, where it was 186.04 mg/kg. In the case of Cd, the lowest level of accumulation was also observed on Site 3 in the second year of testing (1.62 mg/kg). Lead showed the lowest accumulation level in 2015 on Site 3, where its value was 0.63 mg/kg.

The percentage share of metals accumulated by insects in relation to the content of these elements in soil was the lowest in the case of lead (from 0.12% to 5.03%) and the highest in the case of Zn and Cd in both years of research in the third site (418.09% and 381.62% Zn and 420.83% and 225% Cd).

In most cases, the concentrations of heavy metals in insect bodies decreased with increasing distance from the source of contamination, and thus also with a decrease in their amounts in the topsoil. Only in 2015, a lower cadmium content was observed on Site 2 than on Site 3.

Table 2. Concentrations of Zn, Cd and Pb in the bodies of female wasps of the species *P. nimphus* (Christ, 1791).

Site	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
2015			
1	368.68	17.94	5.19
2	234.84	2.03	2.41
3	203.82	3.03	0.63
2016			
1	317.27	5.10	4.88
2	270.20	2.47	1.76
3	186.04	1.62	1.28

Due to the fact that the insects analyzed in the study were thoroughly washed in distilled water to remove any pollution from the surface of their bodies before performing chemical analyzes, authors assume that the content of heavy metals obtained in the analysis of their bodies results only from the process of bioaccumulation of these elements from food and plant material.

The concentrations of all three heavy metals in the bodies of *P. nimphus* females caught on the individual sites differed significantly (Cd p = 0.008660, Pb p = 0.035609, Zn p = 0.017202) (Table 2., Fig. 1).

The Pearson correlations showed a statistically insignificant relationship between the levels of heavy metals in the topsoil and in the bodies of *P. nimphus* females (Zn_{2015} p = 0.4061, Zn_{2016} p = 0.0758; Cd_{2015} p = 0.7132, Cd_{2016} p = 0.3658; Pb_{2015} p = 0.0517, Pb_{2016} p = 0.03381) (Fig. 2).

Discussion

The study showed that females of the species *P. nimphus* (Christ, 1791) accumulated zinc, cadmium and lead in their bodies, and that the level of this accumulation depended on the concentration of these elements in their habitat. A similar relationship had been found earlier by other authors conducting research in the area affected by the activities of ZGH "Bolesław", although they had focused on other taxa of insects. Those studies mainly concerned the order Coleoptera (beetles) represented by the predatory species *Pterostichus oblongopunctatus* [19-21] and Hymenoptera - those species that feed on pollen and nectar of plants (*Osmia rufa* [22, 23]), bumblebees [14], and those that feed on varied food (various species of ants [24]) – all of which are insects representing different trophic groups, which is extremely important because heavy metals released into the environment have the ability to move across different trophic levels [25]. They migrate from the soil to the vegetation growing on the contaminated land. A unique place in science has been assigned to plants called metallophytes, i.e. plants accumulating heavy metals in their tissues [26]. They become a source of food for phytophagous insects, especially of the species that specialize in this group of plants, although it was detected that the part of plant species use heavy metals as protection against herbivores [27]. In addition, heavy

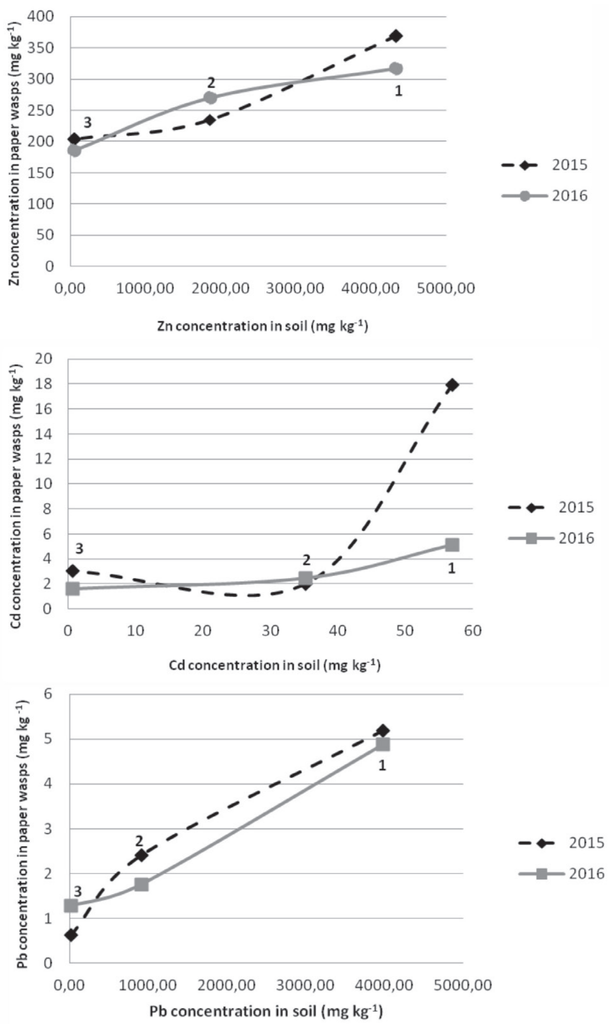

Fig. 1. Relation between the concentrations of heavy metals (Zn, Cd, Pb) in insect bodies and soil in two years of the study; 1 – Site 1, 2 – Site 2, 3 – Site 3.

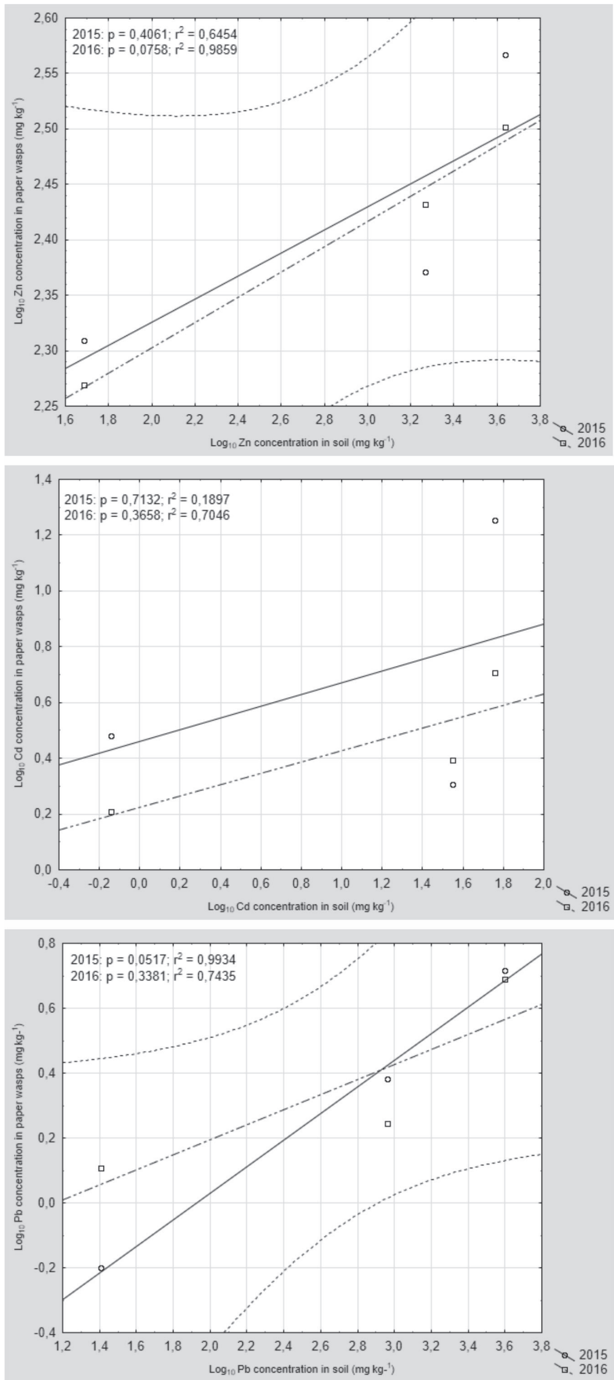

Fig. 2. Correlation between the levels of Zn, Cd and Pb in topsoil and their concentrations in the bodies of female P. nimphus (Christ, 1791) in 2015 and 2016.

metals (Zn, Cd and Pb) make their way into pollen which is a source of food for pollinating species [22]. All this leads to the accumulation of harmful elements at higher trophic levels, and thus (in this case) in the bodies of predatory and omnivorous insects (such as *P. nimphus*), whose food is highly varied and their chance of exposing the body to the harmful effects of metals is greater.

Bioaccumulation of heavy metals has been found in many insect taxa, including the beetles (Coleoptera) *Blaps polychresta* and *Trachyderma hispida* [28]. The authors had found higher concentrations of Cd, Zn and Pb in the tissues of the middle intestine of beetles captured in industrial areas in relation to those from a reference area [28]. Accumulation of the same heavy metals has also been observed in Hymenoptera. The concentrations of these elements in the soil and in the bodies of ants of the species *Crematogaster scutellaris* have always significantly correlated with each other. Interestingly, in the case of Zn and Cd, their concentrations in insect bodies were found to be higher than in the soil [29]. A similar relationship was also observed in our study. Both Zn and Cd found in *P. nimphus* caught in the least polluted site tended to increase the accumulation of these elements in their bodies in comparison to more contaminated areas. Interestingly, no similar trend was found for lead.

Also, representatives of another species of ants, *Formica lugubris*, are known to accumulate Zn, Cd and Pb in their bodies. The metals have also been found in the building material of their nests [30]. According to

Zhelazkova [31], the honey bee (*Aphis mellifera* L.), too, accumulates Zn, Cd and Pb, with the lead content in the droppings of individuals of this species exceeding the concentration of this element in their bodies. In the honey bee, Ruschioni et al. [32] detected accumulation of Cd and Pb that depended on the place, year and position of sampling. Among the termites (Isoptera), the ability to accumulate heavy metals has been detected in the species *Macrotermes bellicosus*, although only the caste of soldiers showed vulnerability to contamination with lead ions, and the species was characterized by a general low tendency to accumulate heavy metals from the soil [33]. Bioaccumulation of heavy metals has also been found in other orders of insects, including: Hemiptera – *Myzus persicae* (Cd and Zn) [34]; Diptera – *Hermetia illucens* (Pb and Cd) [35], (Cd, Zn and Pb) [36]; Odonata – *Crocothemis servilia*; Orthoptera – *Oxya hyla*; Lepidoptera – *Danaus chrysippus* [37]; and others.

To check to what extent *P. nimphus* accumulates heavy metals in comparison with other insect taxa, the concentrations of Zn, Cd and Pb found in the soil and insect bodies by other authors conducting research in the area around ZGH "Bolesław" were compared with the values obtained in this study.

Stone et. al. [19] had found concentrations of heavy metals in the top soil layer similar to those obtained in our study (51.1 mg/kg Cd, 0.84 mg/kg Cd, 870 mg/kg Pb, 1522 mg/kg Zn, and in our study 56.92 mg/kg Cd, 0.72 mg/kg Cd, 915.88 mg/kg Pb, 1856.30 mg/kg Zn). Corresponding to these values, the concentrations of heavy metals in the bodies of female beetles of the species *Pterostichus oblongopunctatus* analyzed by the above authors were lower than the concentrations recorded by us in *P. nimphus* females, and were respectively: 3.8 mg/kg Cd (with 17.94 and 5.19 mg/kg Cd in *P. nimphus*), 1.3 mg/kg Cd (3.03 and 1.62 mg/kg Cd), and 131 mg/kg Zn (234.84 and 270.20 mg/kg Zn). Only for lead, the value obtained by us in 2016 was lower and amounted to 1.76 mg/kg Pb compared to 1.9 mg/kg Pb in *P. oblongopunctatus*. In the case of male *P. oblongopunctatus* analyzed by the cited authors, the concentrations of individual metals were even lower than for the females of this species.

Beetles of the species *P. oblongopunctatus* had also been studied by Bednarska and Stachowicz [20]. The concentrations of heavy metals in the soil were at a level of 1763 mg/kg Zn and 39.1 mg/kg Cd (in our study: 1856.30 mg/kg and 35.21 mg/kg, respectively). They corresponded to the following concentrations in the bodies of male beetles: 107 mg/kg Zn and 1.6 mg/kg Cd (234.84 and 270.20 mg/kg Zn, and 2.03 and 2.47 mg/kg Cd).

In the case of two species of ants, *Formica cunicularia* and *Lasius flavus* [24], a greater accumulation of zinc had been observed in comparison with the results for *P. nimphus* (Christ, 1791) in the present study. The concentration of 4644.5 mg/kg Zn in the soil as reported by the cited author was higher than the value presented in this paper (4326.50 mg/kg). It corresponded to 907.66 mg/kg Zn accumulated in *Formica cunicularia* and 882.31 mg/kg in *Lasius flavus*. For comparison, *P. nimphus* (Christ, 1791) accumulated 368.68 and 317.27 mg/kg Zn.

Based on the examples cited above, one could make a far-reaching conjecture that the species *Pterostichus oblongopunctatus* accumulate less heavy metals (Zn, Cd, Pb) in their tissues than *P. nimphus*. The opposite situation is observed in *F. cunicularia* and *L. flavus*. This could be indicative of different ways of heavy metal interception in these species, which is reflected in some scientific studies.

For example, Aydogan et al. [38] compared the extent of heavy metal accumulation between species of Hydrophilidae (Coleoptera). Their results indicate a different degree of zinc and lead accumulation between the species. *Paracymus chalceolus* accumulated these elements to a greater extent than *Berosus spinosus*.

Grześ [24], too, confirms the variation in heavy metal interception and regulation by different species of ants. In her study, she found differences in Zn capture and maintenance of a stable Zn level in the bodies of *Formica cunicularia*, *Lasius flavus* and *Myrmica rubra*. It was *M. rubra* that proved to exhibit the highest effectiveness in Zn regulation.

The above-cited authors see the reasons for the observed differences primarily in the type of accumulated metal and insect species.

The study presented here is the first to focus on the accumulation of Zn, Cd and Pb in the bodies of insects of the species *P. nimphus* found in the area impacted by the activities of ZGH "Bolesław". Studies on the bioaccumulation of heavy metals in the bodies of wasps of the genus Polistes have so far been based on the species *Polistes dominula*/*dominulus*, but they were conducted in another region of Europe and concerned the accumulation of only one metal – lead. Both Polidori et al. [39] and Urbini et al. [40] confirmed the ability of individuals of the species *Polistes dominulus* to accumulate Pb, comparing its concentrations in wasps captured in contaminated and reference areas.

The results obtained by Urbini et al. [40] are particularly interesting. They had recorded the accumulation of lead in *P. dominulus* imagines at an average level of 0.197 μg/g Pb for control areas and at 0.67 μg/g Pb for industrial areas. What is also interesting in their study is that they observed a significantly higher lead content in the excreta of larvae in comparison with its concentration in the bodies of the individual developmental stages (larvae, pupae, imagines), which may indicate effective regulation of this metal by *Polistes dominulus* at the lower stages of development. The slope of the regression curves in our study is a reason to conclude that in the case of imagines of female *P. nimphus* such effective regulation does not occur. For both the year 2015 and 2016, this species shows strong correlations between the concentration of lead in the soil and in the bodies of the insects.

Considering the results obtained by us and the data on another species of the genus Polistes, it can be concluded that this taxon can be successfully used in biomonitoring of heavy metal pollution of the natural environment. As representatives of social insects, they are characterized by considerable usefulness as bioindicators due to their habit of building nests (thanks to which they live in a strictly defined place) and the ease with which they can be sampled for testing. In addition, this taxon occupies high trophic levels [41]. It is advisable, of course, to continue studies, especially on the species *P. nimphus*, which until now has not received too much attention in research on bioaccumulation of heavy metals, and which is a common insect species in Poland. These studies could concern, for example, determination of the effectiveness of heavy metal detoxification by this species, investigation of the impact of these elements on its representatives, or differences between the sexes in the accumulation of Zn, Cd and Pb.

Conclusions

Females of the species *P. nimphus* accumulate zinc, cadmium and lead in their bodies, and that the level of this accumulation depends on the concentration of these elements in their habitat.

Polistes can be successfully used in biomonitoring of heavy metal pollution of the natural environment.

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Conflicts of Interest

Authors declare no conflicts of interest.

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