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801

Bioakumulacja metali ciężkich pochodzenia antropogenicznego w wybranych gatunkach owadów

Rozprawa doktorska

Rozprawa doktorska wykonana pod kierunkiem

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Podziękowania

Składam serdeczne podziękowania dla dr hab. inż. Elżbiety Wojciechowicz-Żytko za okazaną pomoc w trakcie pisania pracy, cierpliwość oraz nieustanne wsparcie.

Spis treści

1. Wykaz publikacji stanowiących rozprawę doktorską

Przedstawiona rozprawa doktorska ma formę spójnego tematycznie cyklu trzech prac naukowych:

Mielczarek A., Mielczarek Ł., Wojciechowicz-Żytko E. 2021. The influence of heavy metals on the shape and asymmetry of wings of female *Polistes nimpha* (Hymenoptera, Vespidae) living on contaminated sites. Ecotoxicology 30, 1854-1861.

Punktacja MNiSW (2021): 70
IF (2020) 2.823

Mielczarek A., Mielczarek Ł., Wojciechowicz-Żytko E. 2021. Hoverflies (Syrphidae: Diptera) in areas contaminated with heavy metals (Cd, Zn, Pb). Folia Horticulturae 33(2), 1-18.

Punktacja MNiSW (2021): 20
IF (2020) 1.873

Mielczarek A., Wojciechowicz-Żytko E. 2020. Bioaccumulation of heavy metals (Zn, Pb, Cd) in *Polistes nimphus* (Christ, 1791) (Hymenoptera, Vespidae) living on contaminated sites. Polish Journal of Environmental Studies 29(6), 4249-4256.

Punktacja MNiSW (2019): 40
IF (2020) 1.699

2. Streszczenie

Celem rozprawy doktorskiej było określenie stopnia akumulacji Zn, Cd, Pb w ciałach samic drapieżnego gatunku *Polistes nimpha* (Christ, 1791) (Hymenoptera, Vespidae) żyjących w środowisku skażonym metalami ciężkimi oraz określenie poziomu asymetrii fluktuacyjnej pierwszej pary ich skrzydeł. Dodatkowo badano różnorodność gatunkową bzygowatych (Diptera: Syrphidae) żyjących na obszarze znajdującym się pod wpływem emisji metali ciężkich.

Zawartość metali ciężkich w ciałach samic *P. nimpha* wzrastała wraz ze wzrostem ich zwartości w glebie. W wyniku przeprowadzonych analiz stwierdzono istotne różnice w wartościach asymetrii pierwszej pary skrzydeł w zależności od poziomu Zn, Cd i Pb zakumulowanych przez osy.

Podczas badań odłowiono 1180 bzygowatych należących do 165 gatunków, co stanowi 39,76% gatunków Syrphidae występujących w Polsce. Dominowały zoofagi.

Badania potwierdziły podatność samic *P. nimpha* do akumulacji Zn, Cd i Pb w swoich ciałach. Akumulacja ta może powodować zmiany w asymetrii fluktuacyjnej pierwszej pary ich skrzydeł. Badania nie potwierdziły jednak negatywnego oddziaływania skażenia środowiska na bogactwo gatunkowe Syrphidae.

3. Summary

The aim of the study was to determine the extent of Zn, Cd, Pb in the bodies of females of the predatory species *Polistes nimpha* (Christ, 1791) living in an environment contaminated with heavy metals and to determine the fluctuating asymmetry of the first pair of each wings. We also investigated the diversity of syrphids living in areas under the influence of heavy metal emissions.

The content of heavy metals in the bodies of *P. nimpha* females increased with the increase of their concentration in the soil. As a result of the analyses, significant differences were found in the asymmetry values of the first pair of wings depending on the level of Zn, Cd, Pb accumulated by the wasps.

During the studies a total of 1,180 syrphids belonging to 165 species were caught (39.76% of the Syrphidae species of Poland). Dominated zoophagous species.

The research confirmed the susceptibility of female *P. nimpha* to the accumulation of Zn, Cd, Pb in their bodies. This accumulation may cause changes in the fluctuation asymmetry of the first pair of their wings this institution. The research did not confirm the negative impact of environmental contamination on the species richness of Syrphidae.

4. Wstęp

4.1. Źródła metali ciężkich

Obecność metali ciężkich w środowisku jest zjawiskiem naturalnym - występują w skałach metalonośnych, z których są uwalniane w procesie wietrzenia; do środowiska trafiają również na skutek erupcji wulkanów (Fergusson 1990, He i in. 2005, Nriagu 1989). Jednak od początku rewolucji przemysłowej ich emisja zwiększyła się, doprowadzając do zanieczyszczenia środowiska (Siwek 2008). Są to źródła antropogeniczne, ściśle powiązane z działalnością człowieka (Alloway 2013). Do źródeł zanieczyszczeń metalami ciężkimi wynikającymi z działalności człowieka należą, m.in. górnictwo, hutnictwo, transport, działalność przemysłowa i rolnicza.

Znaczące wzbogacenie gleb w metale ciężkie zostało odnotowane na obszarach o wyższym stopniu nawożenia (głównie nawozami fosforowymi) w porównaniu do gleb pierwotnych (Domagała-Świątkiewicz i Sady 2001). Na obszarach miejskich, charakteryzujących się zwiększonym ruchem motoryzacyjnym, gleby mogą być zanieczyszczone metalami ciężkimi, głównie ołowiem (Czarnowska i Bednarz 2000, Sternbeck i in. 2002, Zereini i in. 2007, Wiseman i in. 2013). Także ścieki komunalne i przemysłowe oraz emisje związane z ogrzewaniem domostw w sektorze komunalno-bytowym (emisje powierzchniowe) przyczyniają się do antropogenicznego wzbogacenia środowiska w metale ciężkie (WIOŚ Szczecin 2015, Mahmood i Malik 2014, Jan i in. 2010, Khan i in. 2008).

Metale ciężkie uwalniane do atmosfery podczas wydobycia rud, hutnictwa i innych procesów przemysłowych powracają następnie do środowiska poprzez suchą i mokrą ekspozycję. Zanieczyszczenie środowiska jest bardzo dobrze widoczne na obszarach źródeł punktowych, takich jak górnictwo, odlewnie i huty oraz inne zakłady przemysłowe oparte na przetwórstwie metali (Fergusson 1990, Bradl 2002).

Pomimo niewątpliwych korzyści ekonomicznych oraz udogodnień w życiu codziennym jakie niosą ze sobą antropogeniczne źródła metali ciężkich, nie można zapominać o szeregu szkodliwych dla środowiska i organizmów żywych konsekwencji skażenia metalami ciężkimi. Substancje pochodzące z tych źródeł zanieczyszczają glebę, wodę, powietrze i kumulują się w organizmach żywych na kolejnych poziomach troficznych (Gorlach i Gambuś 2000).

4.2. Szkodliwość metali ciężkich

Niektóre metale ciężkie, w odpowiednich ilościach, są ważne dla prawidłowego funkcjonowania organizmów żywych. Przykładowo, Mn, Fe, Cu, Zn i Mo to mikroelementy lub pierwiastki śladowe wykorzystywane przez rośliny. Są niezbędne do prawidłowego przebiegu procesów metabolicznych. Niedobór lub nadmiar któregoś z tych metali prowadzi do zakłócenia homeostazy i chorób. Trzeba jednak pamiętać, iż metale ciężkie niezbędne do prawidłowego rozwoju są zróżnicowane dla poszczególnych grup organizmów, a ich wyższe stężenia przedostające się do organizmów żywych mogą również powodować niekorzystne dla nich skutki (Wang i in. 2003, Pueyo i in. 2004, Wójcik i in. 2004).

Metale ciężkie takie jak Cd, Pb i Hg nie odgrywają żadnej znanej roli biologicznej w organizmach żywych; są toksyczne i już w niewielkich stężeniach wywołują zmiany w funkcjonowaniu organizmów (Boyd i Rajakaruna 2013).

Skażenie metalami ciężkimi jest poważnym i powszechnym zagrożeniem dla środowiska. Jego negatywne oddziaływanie opiera się w szczególności na wysokiej toksyczności metali ciężkich, ich trwałości w środowisku, braku zdolności do biodegradacji, zdolności do akumulacji w organizmach żywych oraz przenoszeniu z jednego poziomu troficznego na kolejne (Yang-Guang i in. 2016, Wojciechowska-Mazurek i in. 2008, Zhang i in. 2021).

Metale ciężkie zawarte w glebie oddziałują na żyjące w niej mikroorganizmy, powodując m.in. spadek biomasy bytujących w glebie mikroorganizmów (Wang i in. 2007). Zmiany w ilości i zróżnicowaniu tych mikroorganizmów mogą w konsekwencji prowadzić do zakłócenia procesu rozkładu i przemiany substancji organicznej, co z kolei może powodować zwiększenie udziału biodostępnych form metali ciężkich dla roślin (Bååth i in. 1997, Becker i in. 2006, Hander i in. 2001).

Wysokie stężenie metali ciężkich w roślinach prowadzi, m.in. do hamowania działania enzymów cytoplazmatycznych i uszkadzania struktur komórkowych na skutek stresu oksydacyjnego (Assche i Clijsters 1990, Jadia i Fulekar 2009). Prowadzi również do skarłowacenia roślin (Kibra 2008), opóźnienia wzrostu roślin i ich starzenia (Choi i in. 1996, Sudhakar i in. 1992), ograniczenia kiełkowania (Manivasagaperumal i in. 2011), zakłócenia pobierania i transportu wody oraz pierwiastków przez rośliny (Das i in. 1997), zmiany w funkcjonowaniu błon komórkowych (Fodor i in. 1995) i inne.

Część roślin wykształciła zdolność do obrony przed szkodliwym oddziaływaniem metali ciężkich, np. poprzez strategie unikania stresu, pobierania i neutralizacji szkodliwych substancji (Siwek 2008). Istnieją wśród nich gatunki akumulujące wysokie stężenia metali w tkankach, używające ich w celu ochrony przed fitofagami (Poschenrieder i in. 2006a, Poschenrieder i in. 2006b) oraz taksony wyspecjalizowane do życia w wyjątkowo skażonym środowisku, gdzie znalazły własną niszę (hiperakumulatory) (Siwek 2008). Większość z nich stanowi jednak źródło pożywienia dla zwierząt, przez co metale ciężkie przedostają się do wyższych ogniw łańcucha pokarmowego.

Szkodliwe oddziaływanie metali ciężkich dotyka każdej części ekosystemu. Z oczywistych względów szeroko zbadany został wpływ ekspozycji organizmu ludzkiego na metale ciężkie. Istnieje coraz więcej dowodów na to, że zanieczyszczenie metalami ciężkimi na terenach górniczych spowodowało szkody dla zdrowia miejscowej ludności (Nawab i in. 2016, Nuapia i in. 2018, Roba i in. 2016, Wang i in. 2017). Należy także pamiętać o innych zwierzętach, dla których kontakt z metalami ciężkimi również stanowi zagrożenie i prowadzi do wielu niekorzystnych zmian.

4.3. Wpływ metali ciężkich na owady

Swoje miejsce w badaniach nad szkodliwym oddziaływaniem metali ciężkich na organizmy żywe znalazły również owady. Szczególną uwagę skupiono – ze względu na pełnioną przez nie funkcję ekosystemową - na owadach zapylających, ale także na owadach społecznych, rozwijających się w glebie oraz na drapieżcach zajmujących wyższe poziomy troficzne (Heikens i in. 2001, Moroń i in. 2013, Nummelin i in. 2007).

Część owadów wykazuje silną reakcję na skażenie środowiska metalami ciężkimi, podczas gdy pozostałe zdają się wykazywać odporność na ich szkodliwe działanie poprzez wypracowanie szeregu mechanizmów obronnych (Grześ 2010, Bednarska i in. 2013).

Wśród owadów wykazujących negatywną reakcję na podniesione zawartości metali ciężkich w środowisku znaleźli się przedstawiciele owadów zapylających. Moroń i in. (2013) wykazali negatywny wpływ metali ciężkich na liczbę budowanych przez *Osmia rufa* (Linnaeus, 1758) komórek czerwiowych oraz wzrost śmiertelności osobników tego gatunku. Moroń i in. (2012) odnotowali spadek zróżnicowania gatunkowego dzikich pszczół oraz wzrost śmiertelności osobników gatunku *Megachile ligniseca* (Kirby, 1802) wraz ze wzrostem poziomu skażenia środowiska.

Według Meindla i Ashmana (2013) metale ciężkie, głównie Ni, zaburzają pobieranie pokarmu przez trzmiele żyjące na obszarach skażonych.

Badania nad szkodliwością metali ciężkich prowadzono również na gatunkach drapieżnych owadów.

Zygmunt i in. (2006) wykazali, iż masa ciała chrząszczy z gatunku *Pterostichus oblongopunctatus* (Fabricius, 1787) wzrastała wraz ze wzrostem zawartości metali ciężkich w glebie. Polidori i in. (2018) stwierdzili podwyższone stężenia Pb w nabłonku jelita środkowego os z rodzaju *Polistes*.

Skaldina i in. (2018) odkryli, że *Formica lugubris* reaguje zmianami melanotycznymi w obrębie głowy oraz zmniejszeniem masy ciała na skutek ekspozycji na metale ciężkie. Grześ i in. (2015b) odnotowali zmiany w wielkości osobników kasty robotnic *Lasius niger* z obszarów skażonych w odniesieniu do osobników bytujących na obszarach nieskażonych - na terenach skażonych dominowały mniejsze osobniki. Sorvari i in. (2007) wykazali istnienie zaburzeń w odpowiedzi immunologicznej, a tym samym większe narażenie na infekcje mrówek z obszarów skażonych. U *Formica aquilonia* żyjących na obszarach skażonych odnotowano zmiany zachowania robotnic - z agresywnego na łagodne, co mogło wpływać na ich wydajność podczas polowania i w konsekwencji przekładać się na rozwój całej kolonii (Sorvari i Eeva 2010).

W przypadku asymetrii fluktuacyjnej nie wszystkie gatunki mrówek wykazują zależność między FA a stopniem skażenia środowiska. Do gatunków odpornych należą m.in. *Formica pratensis* i *Lasius flavus*, a ich odporność tłumaczy się posiadaniem przez nie mechanizmów regulacji metali ciężkich (Grześ i in. 2015a, Rabitsch 1997).

Analizom poddawano również taksony owadów fitofagicznych, uznawanych za ważne szkodniki roślin.

Zhan i in. (2017) wykazali wpływ kadmu na przedłużenie rozwoju larw *Helicoverpa armigera* (Hübner, 1808). Wraz ze wzrostem stężenia tego metalu w pożywieniu zmniejszał się również wskaźnik przeżywalności gatunku, płodność samic, zmianie ulegała aktywność enzymatyczna. Görür (2006) stwierdził zależność pomiędzy poziomem metali ciężkich w roślinie żywicielskiej a zaburzeniami morfologicznymi u mszyc.

Według Noret i in. (2007) gąsienice *Issoria lathonia* (Linnaeus, 1758) odżywiające się pokarmem skażonym metalami ciężkimi charakteryzowały się spowolnionym wzrostem w porównaniu do kontroli. Gąsienice *Parnassius apollo* (Linnaeus, 1758) odżywiające się roślinami skażonymi cierpiały na biegunki i ginęły w trakcie doświadczenia (Nieminen i in. 2001).

5. Hipotezy badawcze i cele prowadzonych badań

W pracach stanowiących rozprawę doktorską weryfikowano hipotezę, iż Cd, Zn i Pb ulegają bioakumulacji w ciałach samic *P. nimpha*. Poziom tej akumulacji jest zależny od stopnia skażenia środowiska metalami ciężkimi oraz wpływa na poziom asymetrii fluktuacyjnej pierwszej pary ich skrzydeł. W drugim badaniu założono, iż bogactwo gatunkowe muchówek z rodziny Syrphidae jest zależne od poziomu skażenia środowiska.

Celem pracy było określenie wpływu metali ciężkich na stopień asymetrii fluktuacyjnej pierwszej pary skrzydeł samic *Polistes nimpha* oraz skład gatunkowy muchówek z rodziny Syrphidae występujących na terenach skażonych metalami ciężkimi, a tym samym zbadanie czy owady te mogą być wykorzystywane w badaniu wpływu oddziaływania metali ciężkich pochodzenia antropogenicznego na owady.

6. Materiały i metody

6.1. Przedmiot badań

Jako przedmiot badań wytypowano samice drapieżnego gatunku os *Polistes nimpha* (Christ, 1791) oraz muchówki z rodziny Syrphidae.

P. nimpha (Christ, 1791) to drapieżny gatunek os społecznych. Budowanie przez nie gniazd oznacza, że są owadami bytującymi w danym środowisku, co w połączeniu ze stosunkowo słabą zdolnością lotu oraz dużą liczebnością kolonii czyni je dobrymi bioindykatorami stanu środowiska (Hunt 2007; Prezoto i Gobbi 2005; Suzuki 1978). Larwy *Polistes* są karmione głównie gąsienicami (Sumner i Cini 2021) żerującymi na różnych gatunkach roślin. W związku z tym są narażone na ekspozycję na zanieczyszczenia i akumulację znacznych ilości metali ciężkich.

Syrphidae są cennymi zapylaczami roślin wyższych (Wojciechowicz-Żytko i Jankowska 2017, Wojciechowicz-Żytko 2019) i mają duże znaczenie w utrzymaniu bioróżnorodności, szczególnie w dobie kryzysu pszczół (Zych i in. 2018). Ich larwy rozwijają się w różnorodnych siedliskach (próchnie, dziuplach wypełnionych wodą, płytkich zbiornikach wodnych, w tkankach roślinnych), odżywiając się różnorodnym typem pożywienia: martwą materią organiczną, odchodami, tkankami roślin, mszycami itp. (Wnuk 1978).

Zarówno badany gatunek *P. nimpha*, jak i muchówki z rodziny *Syrphidae* (w aspekcie bioróżnorodności) nie były wcześniej badane pod kątem oddziaływania na nie metali ciężkich.

6.2. Miejsce badań

Badania przeprowadzono w sąsiedztwie Zakładów Górniczo-Hutniczych (ZGH) „Bolesław" – kompleksu wydobywczo-przetwórczego zlokalizowanego w Bukownie koło Olkusza, zajmującego się wydobyciem oraz przetwórstwem rud cynku i ołowiu. Ich działalność przyczynia się do zanieczyszczenia środowiska metalami ciężkimi, takimi jak Zn, Cd i Pd. W związku z działalnością ZGH „Bolesław", jak również z naturalnym występowaniem na tym terenie dolomitów kruszconośnych, gleby rejonu charakteryzują się stężeniami metali ciężkich odbiegającymi w sposób znaczący od naturalnych zawartości metali notowanych dla gleb Polski (Cabała 2009, Kapusta i in. 2011, Kicińska 2009, Trafas i in. 2006).

6.3. Metody badań

W badaniu wykorzystano samice drapieżnego gatunku osy *P. nimpha*, pozyskane ze stanowisk zróżnicowanych pod względem odległości od źródła skażenia (ZGH Bolesław) i stopnia skażenia gleby Zn, Cd i Pb. Określono stopień akumulacji metali ciężkich w ich ciałach oraz korelację pomiędzy skażeniem gleby na poszczególnych stanowiskach a zawartością zakumulowanych w ciałach os metali ciężkich (Zn, Cd i Pb). Następnie określono czy istnieje związek pomiędzy akumulacją metali ciężkich w ciałach os a poziomem asymetrii fluktuacyjnej pierwszej pary ich skrzydeł. W tym celu dorosłe samice gatunku odłowiono na trzech stanowiskach wytypowanych w obrębie obszaru oddziaływania ZGH „Bolesław".

Dorosłe osobniki *Polistes* odławiano w latach 2015–2017, na przełomie lipca i sierpnia, przy pomocy siatki entomologicznej, metodą „na upatrzonego". Schwytane osy przetrzymywano pojedynczo w pudełkach z perforowaną pokrywą w temperaturze 25˚C przez okres 48 h. W celu opróżnienia zawartości jelit, owady zaopatrywano wyłącznie w sterylne waciki nasączone wodą destylowaną. Osy uśmiercono przez zamrożenie, rozdzielono według płci i rozpoznano do gatunku na podstawie cech morfologicznych zawartych w kluczu Dvořáka i Robertsa (2006).

Samice *P. nimpha* zostały pozbawione skrzydeł. Pierwsza para skrzydeł tworzy główną powierzchnię lotu, dzięki czemu jest dobrym punktem odniesienia do porównań cech biometrycznych (rozmiar i kształt). Z analiz wykluczono punkty zlokalizowane w wierzchołkowej części skrzydła, ponieważ u wielu osobników powierzchnia ta uległa zniszczeniu. Analizom morfometrycznym nie poddano również osobników, których skrzydła zostały poważnie uszkodzone. Skrzydła umieszczono na slajdach fotograficznych i zeskanowano (w rozdzielczości 2400 dpi) skanerem Nikon Coolscan 5000 ED. Następnie skrzydła łączono z odpowiednią próbką, którą płukano w wodzie destylowanej (w celu usunięcia pozostałości zanieczyszczeń mogących zawyżać wyniki analiz) i suszono w warunkach laboratoryjnych w temperaturze 20°C.

Ciała owadów analizowano na obecność metali ciężkich (Zn, Cd, Pb). Próbki mineralizowano metodą mineralizacji na mokro w układzie półotwartym. Po trawieniu wszystkie podpróbki, ślepe próby i materiały odniesienia zadano 2 ml kwaśnej wody. Wytworzone w ten sposób roztwory posłużyły w dalszej kolejności do oznaczenia w nich stężenia Cd, Pb i Zn za pomocą spektrofotometrów absorpcji atomowej: PerkinELmer PinAAcle 900Z (Pb, Cd) w kuwecie grafitowej oraz PerkinElmer AAnalyst 200 (Zn) w płomieniu acetylenowo-powietrznym.

Fotografie skrzydeł poszczególnych osobników przyporządkowano do odpowiedniej strony ciała. Za pomocą programu IdentiFly (Przybyłowicz i in. 2016) na uzyskanym obrazie pierwszej pary skrzydeł zaznaczono 17 punktów przecięcia żyłek. Uzyskane w ten sposób współrzędne punktów orientacyjnych zostały nałożone przy użyciu pełnego dopasowania Procrustes w programie MorphoJ (Klingenberg 2011). Do obliczenia poziomu asymetrii za pomocą programu MorphoJ obliczono również Procrustes Anova (Klingenberg 2015). Uzyskane dane poddano testom statystycznym MANOVA. Rozmiar skrzydła analizowano zgodnie z wielkością Centroid (Zelditch i in. 2004). Kształt został opisany przez współrzędne Prokrustesa, które zostały przeskalowane do tego samego rozmiaru. Asymetrię wielkości mierzono jako bezwzględną różnicę między wielkościami centroidów prawego i lewego przedniego skrzydła podzieloną przez średnią wielkość centroidów. Asymetrię kształtu mierzono jako odległość Prokrustesa między kształtami prawego i lewego skrzydła. Rozmiary centroidów i wyniki Procrustes FA obliczono za pomocą oprogramowania MorphoJ (Klingenberg 2011).

W badaniach nad wpływem metali ciężkich na skład gatunkowy Syrphidae, dorosłe osobniki odławiano na stanowiskach zlokalizowanych w obrębie Olkuskiego Regionu Rudnego (ORR) oraz pobliskich terenów w latach 2015–2021. W pierwszym roku badań pobrano próbki ilościowe bzygowatych. W kolejnych latach materiał zbierano jakościowo. Owady odławiano w okresie od wczesnej wiosny do późnego lata przy użyciu siatki entomologicznej. Schwytane owady zostały zabite, spreparowane i zidentyfikowane do gatunku. Uzyskany skład gatunkowy został porównany z listą gatunków odłowionych w Ojcowskim Parku Narodowym, jako stanowisku najbliższym rejonowi badań, najlepiej zbadanym pod kątem Syrphidae, nie znajdującym się pod bezpośrednim wpływem oddziaływania antropogenicznych źródeł skażenia. Porównano strukturę troficzną bzygowatych pomiędzy terenem badanym a terenem referencyjnym. Przy zastosowaniu testu-G określono istotność różnic pomiędzy poszczególnymi grupami troficznymi bzygowatych odłowionych na obu obszarach badań.

7. Najważniejsze wyniki przeprowadzonych badań

Uzyskane dane pozwoliły na przygotowanie trzech publikacji naukowych (Mielczarek i Wojciechowicz-Żytko 2020, Mielczarek i in. 2021a, Mielczarek i in. 2021b). Wszystkie dotyczyły określenia związku pomiędzy metalami ciężkimi (Zn, Cd i Pb) a owadami. W pracach skupiono się na określeniu stopnia akumulacji metali ciężkich w ciałach samic drapieżnego gatunku *P. nimpha*, powiązaniu poziomu zakumulowanych w ich ciałach metali ze stopniem asymetrii fluktuacyjnej pierwszej pary skrzydeł os oraz na oszacowaniu bogactwa gatunkowego bzygowatych na terenach skażonych metalami ciężkimi w porównaniu z obszarem kontrolnym.

W trakcie prowadzenia badań odłowiono 416 samic *P. nimpha*. Średnie stężenie metali ciężkich w ciałach owadów zmieniało się w zależności od odległości od źródła zanieczyszczenia, osiągając najwyższe wartości na stanowisku zlokalizowanym najbliżej źródła zanieczyszczenia, charakteryzującym się najwyższą zawartością metali w wierzchniej warstwie gleby (stanowisko 1: 385,45 µmg/kg Zn, 9,62 mg/kg Cd i 6,61 mg/kg Pb), a najniższy w najbardziej oddalonym, najmniej zanieczyszczonym miejscu (stanowisko 3: 194,65 mg/kg Zn, 2,27 mg/kg Cd i 0,84 mg/kg Pb).

W analizach morfometrycznych wykorzystano łącznie 268 par skrzydeł samic *Polistes*. W wyniku przeprowadzonych analiz stwierdzono istotne różnice w wartościach asymetrii pierwszej pary skrzydeł w zależności od poziomu Zn, Cd i Pb zakumulowanych w ciałach os. W przypadku asymetrii kształtu różnice stwierdzono dla wszystkich badanych efektów (rok i miejsce schwytania) ($p = 0,001$), z istotną różnicą między stanowiskiem 1. i 3. ($p = 0,01378$).

Znaczące różnice stwierdzono również w wielkości skrzydeł pomiędzy osobnikami schwytanymi na stanowisku 1. i 2., a schwytanymi na stanowisku 3. ($p = 0,0004$). Skrzydła osobników odłowionych na stanowisku 3. okazały się istotnie większe niż u osobników zebranych na pozostałych stanowiskach (Mann Whitney parami $p = 0.0001455$; $p = 0,01527$). Nie stwierdzono różnic w liczebności osobników pomiędzy poszczególnymi latami prowadzonych odłowów ($p = 0,2684$).

Na obszarze badań wykazano występowanie 165 gatunków muchówek z rodziny Syrphidae. Stanowi to 39,76% gatunków bzygowatych wykazanych z naszego kraju. Wśród gatunków tych dominowały gatunki drapieżne (48,48%), następnie najliczniej występujące były saprofagi wodne, lądowe oraz fitofagi. Nie stwierdzono różnic statystycznych pomiędzy udziałem procentowym poszczególnych grup troficznych Syrphidae odłowionych podczas badań a bzygowatymi wykazanymi z terenu Ojcowskiego Parku Narodowego.

8. Podsumowanie i wnioski

Na podstawie przeprowadzonych badań stwierdzono możliwość wykorzystania samic *P. nimpha* w badaniach nad oddziaływaniem metali ciężkich na owady. Badania potwierdziły podatność samic *P. nimpha* do akumulacji Zn, Cd i Pb w swoich ciałach. Poziom tej akumulacji mógł powodować istotny wzrost poziomu asymetrii fluktuacyjnej pierwszej pary skrzydeł. Jest to bardzo istotne ze względu na fakt, iż zdolność lotu u *Polistes* jest kluczowa dla prawidłowego rozwoju tworzonych przez nie kolonii.

Kontynuacja badań nad wpływem Cd, Zn i Pb na poziom asymetrii fluktuacyjnej skrzydeł samic *P. nimpha* mogłaby potwierdzić przydatność analiz morfometrycznych w określaniu skażenia środowiska metalami ciężkimi.

Z kolei biorąc pod uwagę skład gatunkowy odłowionych bzygowatych nie stwierdzono znacznego zmniejszenia liczby gatunków na badanym terenie w porównaniu z obszarem, gdzie zanieczyszczenie metalami ciężkimi nie było tak duże (OPN). Co więcej, pobierane próby zwykle cechowały się stosunkowo dużą różnorodnością, choć niektóre gatunki jak np. *Syritta pipiens* czy *Eristalinus aeneus* dominowały, szczególnie na terenach otwartych. Uzyskane przez nas wyniki badań mogą sugerować, iż działalność antropogeniczna w Olkuskim Regionie Rudnym nie powoduje całkowitego zniszczenia różnorodności gatunkowej bzygowatych.

Niniejsze badania stanowią przyczynek wzbogacający wiedzę na temat oddziaływania metali ciężkich na organizmy żywe i ich transferu w łańcuchu troficznym.

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10. Dorobek naukowy

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The influence of heavy metals on the shape and asymmetry of wings of female *Polistes nimpha* (Hymenoptera, Vespidae) living on contaminated sites

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Abstract

The aim of the present study was to determine the fluctuating asymmetry of the first pair of wings in females *Polistes nimpha* (Christ, 1791) living in an environment contaminated with heavy metals. The average concentration of Zn, Cd and Pb in the bodies of the insects varied depending on the distance from the source of contamination, reaching the highest values on the site closest to the source of contamination and the lowest at the most distant site. As a result of the morphometric analyses, significant differences were found in the asymmetry values of the first pair of wings depending on the level of Zn, Cd, Pb accumulated by the wasps. In the case of shape asymmetry, differences were found for all the effects studied (year of capture and site). Significant differences were also found in the size of wings between individuals captured on Sites 1 and 2 and those caught on Site 3. Specimens caught on site characterized by the lowest concentration of heavy metals in the topsoil, proved to be significantly larger than the insects collected on the other sites. There were no differences in the size of individuals between the different years of capture. Based on the results obtained by us, it can be assumed that the wings of *P. nimpha* females may become a useful object in studying the impact of environmental stress of Zn, Cd and Pb pollution on the symmetry of their wings.

Keywords Heavy metals · Fluctuating asymmetry · *Polistes nimpha* · Wings · Pollution

Introduction

Heavy metals are widely distributed in the environment through natural sources (e.g. bedrock weathering), but more and more attention is now being paid to this group of metals as they are inextricably linked with everyday human activities (mining, processing and automotive industries, agriculture, etc.) (Alloway 2013).

Despite the fact that some metals are used by living organisms and are necessary for their proper development, they become toxic to them after exceeding critical levels. The remaining metals are toxic regardless of the amount absorbed by living organisms (Boyd and Rajakaruna 2013). Too high a concentration of both these groups of metals in the habitat of living organisms may constitute for them a strong stress factor determining their development. For these reasons, heavy metals have been widely studied in terms of their negative effects on the human body (Liu et al. 2013; Qing et al. 2015), but research is also conducted on other living organisms, such as insects. Evaluation of the impact of heavy metals on insects is based on determining, *inter alia*, the influence of these elements on the reproductive capacity and proliferation in contaminated areas (Moroń et al. 2013), the extent of parasitization, species diversity (Szentgyörgyi et al. 2011), etc.

One of the tools also used to estimate the influence of heavy metals on living organisms is analysis of fluctuating asymmetry in individual specimens. In theory, any organism with a bilateral structure is characterized by the presence of perfect bilateral symmetry (a normally distributed pattern of symmetry in a population) (Freeman et al. 1993). In reality, however, small, randomly occurring deviations

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from the symmetry of bilateral features are widespread in nature, resulting from, for example, low developmental stability of the organism. They are called fluctuating asymmetry. An organism that develops under unfavourable exo- or endogenous conditions is often characterized by a greater degree of asymmetry (Daloso 2014; Palmer and Strobeck 1986; Van Valen 1962).

Research on fluctuating asymmetry in response to a variety of stresses has been conducted with a variety of organisms: plants (Alves-Silva and Del-Claro 2013; Ivanov et al. 2015), birds (Herring et al. 2016; Minias et al. 2013), fish (Özsoy et al. 2007; Tocts et al. 2016), mammals (Cánovas et al. 2015; Sánchez-Chardi et al. 2013). They have also concerned various types of stress factors, e.g. temperature (Bjorksten et al. 2001; Chang et al. 2007), population density (Gibbs and Breuker 2006; Mpho et al. 2000), the extent of parasitization (Ward et al. 1998), exposure to contamination with pesticides (Abaga et al. 2011; Hardersen et al. 1999) and heavy metals (Graham et al. 1993; Polak et al. 2004).

The aim of the present study was to determine the fluctuating asymmetry (FA) of the first pair of wings of females of the predatory species Polistes *nimpha* (Christ, 1791) living in an environment contaminated with heavy metals (Zn, Cd and Pb).

The results presented in this article are an extension of part of the research by Mielczarek and Wojciechowicz-Żytko (2020).

Materials and methods

Study sites

The study was carried out in the vicinity of Zakłady Górniczo-Hutnicze (ZGH) "Bolesław"—a mining and processing complex located in Bukowno near Olkusz (southern Poland) (50°30′28″N, 19°28′17″E). These plants, operating since 1967, are engaged in mining and processing of zinc and lead ores. Their operations contribute to environmental contamination with heavy metals such as Zn, Cd and Pd.

Based on the concentrations of heavy metals in the topsoil, which had been reported in the works by Grześ (2009) and Szentgyörgyi et al. (2011), and on personal observations, three sites were designated for the study, differing in terms of heavy metal concentrations in the soil and the distance from the source of contamination (0.44 km, 1.5 km, and 19.62 km, respectively). All three sites were warm, sunlit grasslands surrounded by Scots pine (*Pinus sylvestris* L.) trees with an admixture of other pioneering types of trees and shrubs (e.g. *Betula*, *Larix*, *Prunus*). Among the plants flourishing on the grasslands in great numbers were plants of the family Apiaceae (including *Pimpinella saxifraga* and *Daucus carota* L.), which were used by *P. nimpha* for hunting the prey.

In 2015, samples of topsoil at depths up to 20 cm were collected from all three sites and analyzed for Zn, Cd and Pb content. The samples were taken at random from an area of 1 km^2, giving special consideration to the nesting sites of *P. nimpha* and their feeding grounds. The top layer of soil was sampled with a metal spatula, discarding the part of soil that came in contact with it (to eliminate the risk of sample contamination). Each sample was placed in a separate bag and transported to the laboratory. The samples were dried in the open air, milled and sieved through 0.2 mm sieves.

Forty topsoil samples were collected from each of the three sites; they were mixed to obtain 3 bulk (pooled) samples to represent each site in further analyses. The bulk soil samples were used to determine their granulometric composition by the Casagrande method, as modified by Prószyński, and soil pH using the potentiometric method in a 1:2 water:soil solution.

To determine the concentrations of heavy metals (Zn, Cd, Pb), the soil samples were dried, milled and homogenized. Weighed amounts of 0.5 g were each transferred to a vessel into which 10 ml of aqua regia was added, and mineralized. The resulting solutions were filtered, transferred to 50 ml flasks and rinsed with deionized water. Metal content was determined using the ICP-OES method (optical emission spectrometry with inductively coupled plasma – as recommended for the determination of metals in soil).

Insects studied

The study was concerned with females of the predatory species *P. nimpha* (Christ, 1791). *P. nimpha* like other species of the genus Polistes is a social vespid wasp known to build relatively small paper nests which also reflect the English name of these insects – paper wasps. The species is abundant throughout Poland, living in warm, sunny places, where they build their nests. Numerous individuals of wasps develop in these nests. Their building of nests means that they are insects that persist in a given environment, which, combined with relatively poor flying ability, makes them ideal as bioindicators of the state of the environment (Hunt 2007; Prezoto and Gobbi 2005; Suzuki 1978).

Although flying close to the nest, females of the Polistes as predators penetrate its territory very actively in order to feed its larvae. The Polistes are known to feed its larvae mainly on caterpillars (Sumner and Cini 2021) which eat various plants so that it is expected that they have plenty of opportunity to be exposed to pollutants and accumulate a substantial amount during its relatively long life.

Imagines of the genus Polistes were caught in 2015–2017, in late July and early August, when they reach

Table 1 Soil pH and Zn, Cd and Pb content in the topsoil of individual sites (Mielczarek and Wojciechowicz-Żytko 2020)

Site	Location	Elevation [m a.s.l.]	pH	Distance to the source of contamination [km]	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
1	50°16′N 19°28′E	326	6.87	0.44	4326.50	56.96	3977.00
2	50°17′N 19°27′E	346	6.02	1.5	1856.30	35.21	915.88
3	50°26′N 19°35′E	412	5.59	19.63	48.75	0.72	25.43

the highest numbers and still remain close to their nests. The "on sight" catching of live individuals was carried out with an entomological net, on warm days conducive to active foraging of adults. The insects were caught during active flight or while feeding on plants. The specimens were kept in separate Eppendorf vials with air access. The caught individuals were transported to the laboratory, where they were kept individually in boxes with a perforated lid at 25 °C for 48 h. The wasps were not fed to make them empty their intestinal contents; they were provided only with sterile swabs soaked in distilled water. The insects were then sacrificed by freezing, segregated by sex and recognized to the species on the basis of the morphological features contained in the Dvořák and Roberts (2006) entomological key, which allowed them to be easily and quickly distinguished from other Polistes species (antennae distinctly darkened above, last sternum black, clypeus with transverse black strip).

Females of the species *P. nimpha*, as the most abundant species, were selected for further analyses. The selected individuals were de-winged. The wings were placed in photo slides and scanned (at a resolution of 2400 dpi) with a Nikon Coolscan 5000 ED scanner, saving the image obtained in this way. Later, the wings were put together with the corresponding specimen, which was rinsed in distilled water (to remove remnants of impurities that could overestimate the results of analyses), and dried in laboratory conditions at a temperature of 20 °C.

The bodies of the insects were analyzed for heavy metals (Zn, Cd, Pb); the samples were mineralized by wet digestion in a semi-open system with heating plates (quartz glass). After digestion, all sub-samples, blanks and reference materials were flooded with 2 ml of acidic water (0.2% nitric acid). The solutions produced in this way were then used to determine the concentration of heavy metals (Cd, Pb and Zn) in them using atomic absorption spectrophotometers: PerkinELmer PinAAcle 900Z (Pb, Cd) in a graphite cuvette and 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.

Photographs of the wings of the tested individuals were assigned to the appropriate side of the body. The image obtained by scanning the wings was prepared for further analyses using the IdentiFly software (Przybyłowicz et al. 2016); http://drawwing.org/identifly. Using the IdentiFly programme, 17 points of intersection of the veins were marked on the obtained image of the first pair of wings which are much larger than the hindwings and form the main flight surface, so that they are a good focus for comparisons of biometric characteristics like size and shape. Moreover it is known that wing size is strongly correlated with body size and has been used as an measure of body size in insects living in natural (Bullock 1999) and polluted areas (Moroń et al. 2013, Szentgyörgyi et al. 2017). Szentgyörgyi et al. (2017) report the correlation of body mass and wing size of mason bee in areas polluted by heavy metals.We expected that similar correlation of wing size, body size and body mass should occur also in individuals of Polistes.

No points were measured in the apical part of the wing because it had been damaged in many specimens over the course of their lifetime, and this would have reduced the size of the available test sample. Specimens whose wings were badly damaged were not subjected to morphometric analyses. Estimation of the shape based on geometric morphometrics requires relatively large sample sizes (Cardini and Elton 2007). It is recommended that, in multivariate analysis, the sample size of each group should markedly exceed the number of variables (Arnold 1983).

The coordinates of the landmarks were superimposed using full Procrustes fit in MorphoJ software (Klingenberg 2011). To calculate asymmetry Procrustes Anova (Klingenberg 2015) was calculated also using MorphoJ. The obtained data were subjected to MANOVA statistical tests. Wing size was analyzed as represented by the Centroid Size (Zelditch et al. 2004). Shape was described by Procrustes coordinates, which were scaled to the same size. Size asymmetry was measured as the absolute difference between the centroid sizes of the right and the left forewing divided by the mean centroid size. Shape asymmetry was measured as the Procrustes distance between the shapes of the right and the left wing, and it is further called Procrustes FA score. Centroid sizes and Procrustes FA scores were calculated with MorphoJ software (Klingenberg 2011).

Table 2 Average concentration of Zn, Cd and Pb in the bodies of female *P. nimpha* wasps caught on different sites in years 2015–2017

Site	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
1	385.45	9.62	6.61
2	258.24	2.43	2.04
3	194.65	2.27	0.84

Results

Soil

Each site was characterized by the presence of sandy soils, which were acidic or slightly acidic (pH 6.8, 6.02, and 5.59, respectively). The highest concentrations of heavy metals in the topsoil were recorded on Site 1, located in the immediate vicinity of ZGH "Bolesław", where they were 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 the site furthest away from the source of contamination (Site 3: 48.75 mg/kg Zn, 0.72 mg/kg Cd, and 25.43 mg/kg Pb) (Table 1.).

Insects

Individuals of the genus Polistes were collected in all three years of the study. They were classified into three species: *P. nimpha*, *P. dominula* and *P. biglumis* which are the only representatives of the genus in the studied area. The dominant species was *P. nimpha*, and that is why female specimens of this species were selected for further analyses—a total of 416 females of this species were caught.

The average concentration of heavy metals in the bodies of the insects varied depending on the distance from the source of contamination (Table 2), reaching the highest values on the site closest to the source of contamination (Site 1: 385.45 mg/kg Zn, 9.62 mg/kg Cd, and 6.61 mg/kg Pb), and the lowest at the most distant site (Site 3: 194.65 mg/kg Zn, 2.27 mg/kg Cd and 0.84 mg/kg Pb).

A total of 268 pairs of wings were used in the morphometric analyses (Site 1 – 112, Site 2 – 74, Site 3 – 82). As a result of the analyses, significant differences were found in the asymmetry values of the first pair of wings depending on the level of heavy metals (Zn, Cd and Pb) accumulated by the wasps.

In the case of shape asymmetry, differences were found for all the effects studied (year of capture and site) ($p = 0.001$), with a significant difference between Site 1 and Site 3 ($p = 0.01378$) (Fig. 1).

Significant differences were also found in the size of wings between individuals captured on Sites 1 and 2 and those caught on Site 3 ($p = 0.0004$). Specimens caught on Site 3, the farthest away from the source of contamination and characterized by the lowest concentration of heavy metals in the topsoil, proved to be significantly larger than the insects collected on the other sites (Mann Whitney pairwise $p = 0.0001455$; $p = 0.01527$). There were no differences in the size of individuals between the different years of capture ($p = 0.2684$) (Table 3).

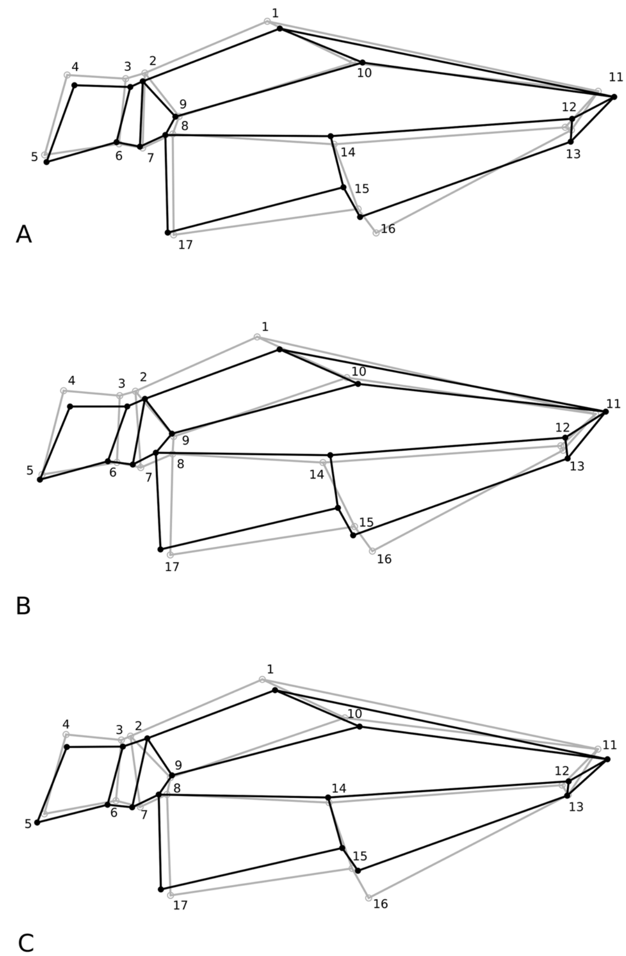

Fig. 1 Diagram showing the differences in the shape of the veins between the right and left wings of *P. nimpha* females captured on Site 1 (**A**), 2 (**B**), and 3 (**C**) (Procrustes ANOVA). The differences are magnified 20× to make them more apparent. The black lines indicate the right wing, and the grey lines indicate the left wing

Discussion

Analysis of fluctuating asymmetry is recognized as one of the methods of assessing the impact of environmental stress on living organisms. Although not all the studies conducted so far confirm the occurrence of changes in the symmetry of individuals with respect to various stressors, other results clearly indicate the usefulness of FA analysis as a valid tool

Table 3 Results of ANOVA: sums of squares (SS), mean squares (MS), degrees of freedom (df), *F* statistics and parametric *P*-values for each of the effects

Centroid size:

Effect	SS	MS	*df*	*F*	*P* (param.)
Locality	307365.206020	153682.603010	2	8.04	0.0004
Year	50557.512899	25278.756449	2	1.32	0.2684
Individual	5029782.792646	19124.649402	263	317.94	<0.0001
Side	1026.884533	1026.884533	1	17.07	<0.0001
Ind*Side	16060.527544	60.151789	267		

Shape, Procrustes ANOVA:

Effect	SS	MS	*df*	*F* (Goodall's)	*P* (param.)	Pillai trace	*P* (param.)
Locality	0.00335033	0.0000558388	60	2.20	<0.0001	0.46	<0.0001
Year	0.00251390	0.0000418984	60	1.65	0.0013	0.42	<0.0001
Individual	0.20067318	0.0000254339	7890	9.55	<0.0001	24.47	<0.0001
Side	0.00172228	0.0000574092	30	21.56	<0.0001	0.59	<0.0001
Ind*Side	0.02132472	0.0000026623	8010				

for studying environmental stress (Beasley et al. 2013). Hoffmann et al. (2005) state that any stress affecting insects during their life may change the shape of their wings in a specific way.

The aim of our study was to determine whether females of the predatory species *P. nimpha* would be characterized by increased levels of FA in response to elevated concentrations of heavy metals (Zn, Cd, and Pb) in their habitat. To verify our hypotheses, we had chosen to model their wings as a practically two-dimensional organ, characterized by the presence of characteristic points (intersections of the veins) to facilitate the analyses performed.

Insect wings have already been successfully used by other authors in this type of research (Benìtez et al. 2013 – *Diabrotica virgifera*, Costa et al. 2015 – *Drosophila antionetae*, Galbo and Tabugo 2014 – *Culex quinquefasciatus*, Nunes 2015, Quirog and Tabugo 2015 – *Aedes albopictus* Szentgyörgyi et al. 2016 – *Apis mellifera*).

As a result of our analyses, we found significant differences in the asymmetry values of the first pair of wings depending on the level of heavy metals (Zn, Cd and Pb) accumulated by wasps which are living in its natural but contaminated habitat.

A higher level of fluctuating asymmetry of various organs of the body in response to stress related to exposure to heavy metals has already been observed in aphids *Brevicoryne brassicae* (Görür 2006, 2009), *Chironomus* spp. (Al-Shami et al. 2010), dragonfly *Calopteryx maculata* (Kelliher 2004), and honeybee *Apis mellifera* (Abaga et al. 2011). On the other hand, there are reports in the literature that negate the occurrence of symmetry disorders caused by exposure to heavy metals, e.g. *Lasius flavus* (Grześ et al. 2015), *Formica pratensis* (Rabitsch 1997), *Chironomidae* spp. (Arambourou et al. 2012). When interpreting the cited studies, it should be remembered, however, that they were concerned with different taxa, and the analyses were performed on various external organs (wings, eyes, etc.).

In our study, we analyzed the size and shape of the wings of *P. nimpha* females captured along a concentration gradient. The results showed significant differences in the centroid size of the wings of individuals caught in the most contaminated areas (Site 1 and 2), compared to individuals collected on the site with the lowest degree of contamination (Site 3). In our study there were also no significant differences between years of capture. Similarly, Szentgyörgyi et al. (2017) had demonstrated significant differences in the size and shape of the wings of *Osmia bicornis* (Hymenoptera) captured on sites with different levels of contamination with heavy metals, but they did not show significant differences in the asymmetry of their wings depending on the year of capture.

Nijhout, Callier (2015); Nijhout, Grunert (2010) report that under stable environmental conditions the body size of a given species is relatively constant. However, it changes when a stress factor appears in the environment, such as temperature fluctuations or food availability. The wings forming during metamorphosis develop proportionally to the size of the entire insect. Therefore, based on the results obtained by us, we made an assumption that heavy metals (Zn, Cd and Pb) can be a stress factor affecting wing size of the adults of this species in its natural environment. This thesis is supported by the fact that there were no significant differences in the size of the wings depending on the year of capture, which theoretically excludes the impact of, for example, differences in atmospheric conditions between individual years that could have determined the development of larvae in Polistes colonies. However, this hypothesis would require laboratory tests to exclude the influence of other stress factors that could play a significant role in the individual development of wasps (e.g. food deficits, different calorific value of food consumed by the larvae). As this was a field experiment, we do not have data on the quantity and quality of food collected in each year of the study by individuals developing on the different sites.

A laboratory experiment on the effect of food availability on the size of insect wings had been conducted by Szentgyörgyi et al. (2016) and concerned representatives of the *Apis mellifera* species. In that study, only for drones were there significant differences in wing size between the families denied pollen and the control group. In the case of the worker caste, no similar relationship was found. However, the limited availability of food did not significantly change the size symmetry of the wings, regardless of sex.

Turillazzi (1980) reports that in the species *Polistes gallicus* there is a seasonal variation in body size; he recorded a gradual increase in the body size of females (queens and workers) in the summer. In the autumn, he did not observe statistically significant differences in size between the representatives of the two groups. Haggard and Gamboa (2012) also indicated that the body size of the species *Polistes metricus* varies during the season.

Taking into account the research results of the above-cited authors, the *P. nimpha* specimens captured by us were collected in the same period on all three sites to exclude the possibility of a similar phenomenon of different size of *P. nimpha* specimens, which could reduce the reliability of the wing size analyzes of individuals.

In our study, only females of the species *P. nimpha* were analyzed because of the insufficient material representing males of this species. Abbasi (2009) reports on the dimorphism of the front pairs of wings between the sexes in the genus *Polistes*. Also Szentgyörgyi et al. (2017) report on differences in the size and shape of wings in both sexes of *Osmia bicornis* (Hymenoptera) developing in an environment contaminated with heavy metals. In this case, the asymmetry of shape and size was smaller in females than in males; therefore, future research should also focus on male *P. nimpha*.

The research carried out so far indicates the existence of a relationship between the symmetry of features of bilateral insects and the basic aspects of their life f.e. sexual selection, mating success (McLachlan and Cant 1995, Pavković-Lučić and Kekić 2011), susceptibility to disease or predatory attack (Møller 1996).

We can presume that features of the wings, including bilateral symmetry, are crucially important in flight performance, which in turn is of main importance in the life of the colony.

Based on the results obtained by us, it can be assumed that the wings of *P. nimpha* females may become a useful object in studying the impact of environmental stress of heavy metal pollution on the symmetry of their wings. It should be remembered that these are preliminary studies, and the results obtained by us require confirmation of this effect by subsequent observations in relation to the above-mentioned species.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Consent to participate All authors consent to participate.

Consent for publication All authors consent for publication.

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Hoverflies (Syrphidae: Diptera) in areas contaminated with heavy metals (Cd, Zn, Pb)

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ABSTRACT

The aim of the research was to study the diversity of hoverflies (Syrphidae: Diptera) living in areas under the influence of heavy metal emissions (Cd, Zn and Pb). Although the area of the Olkusz Ore-bearing Region (OOR), where the research was partially conducted, belongs to the contaminated regions, where a relatively high species richness of Syrphids has been noted. During the research period, a total of 1,180 syrphids, belonging to 165 species were caught, which constitutes 39.76% of all the Polish Syrphidae fauna. These species represented all the trophic groups with the dominance of zoophagous species (48.48%); however, the most numerous (quantitatively abundant) were saprophagous. Among the collected syrphids, common and numerous species, such as: *E. arbustorum* (LINNAEUS, 1758), *E. tenax* (LINNAEUS, 1758), *Episyrphus balteatus* (DE GEER, 1776) and *S. pipiens* (LINNAEUS, 1758), as well as rare species such as *Callicera aenea* (FABRICIUS, 1777) (NT), *Chalcosyrphus piger* (FABRICIUS, 1794) (DD), *Epistrophe ochrostoma* (ZETTERSTEDT, 1849) (VU), *Orthonevra geniculata* (MEIGEN, 1830) (DD), *Rhingia rostrata* (LINNAEUS, 1758) (DD), *Sphegina sibirica* STACKELBERG, 1953 (LC) and *Spilomyia diophthalma* (LINNAEUS, 1758) (NT), were noted. Eleven of the collected species have been included in the Polish Red List of Endangered Species. *Melangyna ericarum* (COLLIN, 1946) is recorded as a new one among the Polish fauna. Numerous observations did not indicate that the area covered by the study was characterised by a significantly lower species diversity compared to other sites. In fact, the samples taken were usually characterised by a relatively high biodiversity. The obtained results may suggest that anthropogenic activities, including those leading to environmental pollution with heavy metals and to a strong transformation of natural habitats, do not completely destroy biodiversity, and in some cases leave space for nature to create habitats where even rare species of organisms such as Syrphidae can develop.

KEYWORDS: biodiversity, calamine grasslands, heavy metals, Syrphidae

INTRODUCTION

Syrphidae is one of the most diverse families of flies, with 415 species reported from Poland (Żóralski, 2015; Żóralski and Kowalczyk, 2015). The diversity of this group is distinguished not only by the number of species, but also by the variety of trophic groups or ecological niches occupied, especially by larval forms. Predatory forms, actively feeding most often on aphids, phytophages that dig or mine plants, aquatic and terrestrial saprophages feeding on microorganisms or decaying wood and less common mycophages feeding

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on mushrooms are all included. Syrphidae are very active insects, whose adult forms obtain the energy needed to remain active from nectar and honeydew, while pollen enables the females to produce their eggs (Wnuk, 1978; Wojciechowicz-Żytko and Jankowska, 2017; Wojciechowicz-Żytko, 2019). These features make them, apart from bees, some of the most important pollinating insects. The numerous occurrences of Syrphidae in almost every terrestrial environment make them a good object for research and assessment of biodiversity. Areas strongly influenced by human pressure due to activities, including industrial areas, are places where the occurrence of hoverflies has been poorly known so far, compared to, for example, protected areas. Contaminated areas are characterised by the disappearance of natural systems due to, for example, changes in the landscape, movements of earth masses, impoverishment of the surface water network, environmental pollution, changes in land use, etc. This leads to the creation of new types of habitats that may become a site of the occurrence of a large number of species of plants, animals and other living organisms, and even a refugium of rare taxa, which in more natural habitats are not as successful (Godzik, 2015).

In the Olkusz Ore-bearing Region (OOR), exploitation and processing of zinc-lead ores have been carried out for many centuries. This activity has caused far-reaching changes to the landscape, soil, water and land use. New, often heavily polluted, habitats have arisen and are still being created there.

One of the examples of this type of habitat are calamine grasslands, which consist of species that tolerate or are associated with high levels of heavy metals in the soil (Kapusta et al., 2010; Kowolik et al., 2010) and other transformed, unused areas. The emergence of this type of habitat has resulted in the enrichment of the species composition of the flora in the vicinity of Olkusz. Evaluation of the vegetation has shown that the area is inhabited by plant taxa that are rare in Poland and Europe, and the vegetation in this area should be considered as diverse (Nowak et al., 2011). In the research area, there are plant species under protection and rare in the country, for example, *Biscutella laevigata*. Species diversity and the presence of many rare species may lead to a richness of insect species, including Syrphidae, and consequently the presence of syrphid species – pollinators – in this area would be a valuable factor in maintaining the diversity of rare plant taxa.

The aim of the research was to study the diversity of hoverflies (Syrphidae: Diptera) living in contaminated areas under the influence of heavy metal emissions (Cd, Zn and Pb).

Additionally, the study determined the extent to which the species composition of the hoverflies caught in the OOR was similar to the species composition of the hoverflies recorded in the Ojcowski National Park (ONP) – a region not contaminated with heavy metals.

MATERIALS AND METHODS

Site

Habitats located in the area of the current and historical exploitation of zinc-lead (Zn-Pb) ores in the vicinity of Zakłady Górniczo-Hutnicze 'Bolesław' (southern Poland) were selected as the research area. This region is part of the so-called Olkusz OOR located within the macroregion of the Silesian Upland (Kondracki, 2011). It is characterised by the presence of high concentrations of trace elements in the soil, due to their presence in the bedrock, and contamination of the area with anthropogenic sources (mining and processing of Zn-Pb ores). Both natural and anthropogenic sources of metals make the Zn, Cd and Pb content in the soils of the region at the level of 150–10,500 mg · kg^{-1}, 0.8–100 mg · kg^{-1} and 140–2,600 mg · kg^{-1}, respectively, which many times exceed the standards allowed for Polish soils (Stone et al., 2002). The average annual temperature in this area is 8 °C (Szczypek, 1997). Annual rainfall (for the Olkusz region) is around 700–800 mm, with a peak in the period from June to September (Dziechciarz, 2002).

The OOR area is characterised by a very poor hydrological network. The main drainage watercourses are Biała Przemsza and Sztoła (Godzik, 2015), and the mining activities cause additional limitation of surface waters. Metalliferous soils are accompanied by specific plant complexes, forming the so-called calamine grasslands (Kapusta et al., 2010). They include plant species adapted to growth and development in an environment enriched with heavy metals (including Cd, Zn and Pb), for example, *Biscutella laevigata* subsp. Woycickii, *Armeria maritima* subsp. *halleri*. The unique soil conditions were most likely the reason to the survival of the local population of *B. laevigata* even in the maximum extent of the last glaciation (Wierzbicka et al., 2020). To protect these unique habitats, the areas NATURA 2000 'Armeria' PLH120091 and 'Pleszczotka' PLH120092 have been created here. Also, the NATURA 2000 'Błędowska Desert' as well as other large areas of sand exploitation is strongly associated with mining activities in this area. The calamine grasslands of the Olkusz area are accompanied by plantings of Scots pine (*Pinus sylvestris*), Banks pine (*Pinus banksiana*), black pine (*Pinus nigra*), Eurasian larch *(Larix* spp.), warty birch *(Betula pendula*) and American bird cherry (*Prunus serotina*). Over half of the OOR area is covered by non-forest communities namely meadows, xerothermic grasslands, psammophilous grasslands, segetal and ruderal communities (Godzik, 2015). The areas elevated on limestone, which are often occupied by beech forests or rock-plant communities, are of a completely different character.

The research was conducted in the following localities: Bukowno, Hutki, Klucze, Pazurek, Rasztyn, Ryczów, Rodaki and Ujków Stary (US) (Figure 1).

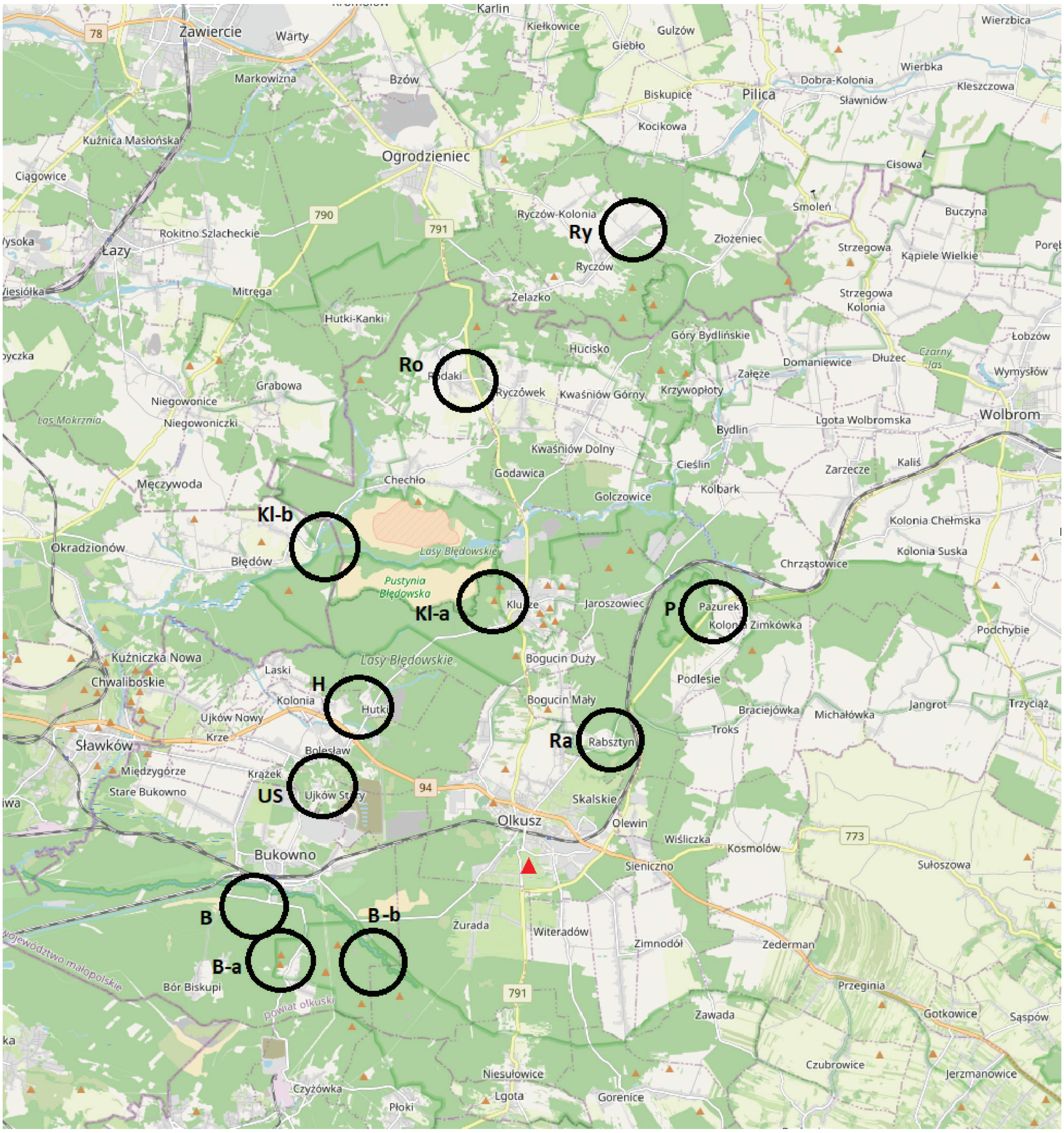

Figure 1. Locality of the research area (©OpenStreetMap authors). B, Bukowno (sand mine); B-a, Bukowno (Diabla Góra); B-b, Bukowno (Sztoła); H, Hutki; Kl-a, Czubatka; Kl-b, Kuźnica Błedowska; P, Pazurek; Ra, Rabsztyn, Ro, Rodaki; Ry, Ryczów; two specimens collected from Olkusz.

Methods

Syrphidae were collected in the years 2015–2021. In the first year of research, quantitative samples of syrphids were collected. In the following years, the material was collected qualitatively. The insects were caught in the period from early spring to late summer using an entomological net in the morning hours. Most of the insects were caught during active flight, while feeding on nectar or pollen, or in food traps – a sugar solution spread over the leaf surface. Captured insects were killed, pinned and identified to species by using the entomological keys: Stubbs and Falk (2002), Bartsch (2009a, 2009b), Van Veen (2004), Speight (2020).

Other collections available to the authors were also analysed for specimens from the area under study (collections of the Museum and Institute of Zoology of the Polish Academy of Sciences in Warsaw and the collection of Bogusław Soszyński [BS]. Only two specimens of species were not confirmed in the current research and were from these two collections *Melangyna ericarum* (COLLIN, 1946) and *Brachypalpus chrysites* EGGER, 1859.

To determine the trophic structure of the caught adults and to facilitate its comparison with the results of research conducted by Klasa and Soszyński (2011) in the ONP, the following division was adopted, taking into account the diet of the larvae: predators and parasites, aquatic saprophages (SW), terrestrial saprophages, nest saprophages, phytophages, mycophages, coprophages. The significance difference among trophic groups was checked using G-test (Sokal and Rohlf, 1981).

RESULTS

During the years 2015–2021 a total of 1,180 syrphids belonging to 165 species were caught, which constitutes 39.76% of the Syrphidae species of Poland (Table 1).

These species represented all the trophic groups described in the family, dominated by the prescence of zoophagous species (48.48% of all the species). The remaining trophic groups accounted for: 16.37% – species belonging to SW, 15.76% – phytophagous species, 13.94% – terrestrial saprophages, 3.03% – mycophagic species and 1.21% each of coprophagic species and saprophages living in nests.

The percentages for thropic groups in ONP were as follows: zoophagous species (46.59% of all the species). The remaining trophic groups accounted for: 13.07% – species belonging to SWs, 18.75% – phytophagous species, 18.18% – terrestrial saprophages, 0.57% – mycophagic species, 1.70% – coprophagic species and 1.14% – saprophages living in nests.

There were no significant differences between the trophic groups in OOR and ONP on using G-test: G 0.122, P: 0.2731, G 0.7409, P: 0.6106; G 0.5334, P: 0.5348; G 1.1372 P: 0.7138; G 3.2201, P: 0.9273; G 0.143, P: 0.2947; G 0.0036, P 0.0478, respectively.

Although the zoophagous species dominated in the list of species, the most numerous specimens were saprophages quantitatively (e.g. *Syritta pipiens* (LINNAEUS, 1758) – 122 individuals, *Eristalis tenax* (LINNAEUS, 1758) – 113, *E. arbustorum* (LINNAEUS, 1758) – 71, *Eristalinus aeneus* (SCOPOLI, 1763) – 58).

Dominant among the collected flies were the Palearctic species (50%), while the Holarctic (16.46%) and Western Palearctic (15.85%) species also had a large share. The smallest share belongs to the species with a partial Oriental and Afrotropical range, and cosmopolitan species (0.61–2.44%).

Particularly noteworthy are the Syrphidae species that are rarely observed in Poland or occurring only locally in large numbers.

Blera fallax (LINNAEUS, 1758)

Material: 01.05.2020, Bukowno (Diabla Góra), 1M (male), on hawthorn flowers.

Occupies habitats located in forests with pine or spruce. Larvae develop in hollows in pine trees (Vujić et al., 2020).

Brachypalpus chrysites (EGGER, 1859)

Material: 'IX' 1968 (Pustynia Błędowska), 1M, leg. R. Januszek, det. Bańkowska R. (coll, MiIZ PAN). Record is based on a museum collection. According to label data, the specimen was probably collected in the Błędów Desert in 1968. The label was handwritten and there is confusion if 'IX' refers to September which is very unlikely for *B. chrysites* or it is just miswriting of 'IV' April. It is a rare mountain and spring species associated with rich Beech-*Abies* forests. Larvae develop in wet humus on tree cavities (Speight, 2011). Suitable habitats still occur in the east part of Błędów Desert where old beech forest is still present. Numbers of field surveys in spring time during this study in order to rediscover this species in the area were unsuccessful.

Callicera aenea (FABRICIUS, 1777)

Material: 19.05.2015, Ujków Stary (road by the park), 1M, leg. Mielczarek Ł.; 19.05.2015, Ujków Stary, 1M, leg. Mielczarek A.

This is a rare species that prefers deciduous forests with old trees. Larvae are still unknown, but most probably they develop in hollows of deciduous trees. Flight period is from late April to August (Speight, 2011). Threat category – NT on the Red List of Endangered Animals in Poland.

Chalcosyrphus piger (FABRICIUS, 1794)

Material: 18.05.2015, Klucze (Czubatka), 3M, leg. A. Mielczarek.

Although this species prefers pine and spruce stands, which in Poland occupy huge areas, adults are rarely caught. It can be abundant locally under appropriate conditions. Imagines can be found on pine wood yards and on flowers of *Ranunculus*, *Seseli* and *Potentilla erecta*. Flight period is from May to August. Larvae are known to develop under pine bark in wet tree humus formed from the frass of *Ips* and *Acanthocinus* (Speight, 2011). It is a rarely caught species. Threat category is DD on the Red List of Endangered Animals in Poland.

Cheilosia aerea (DUFOUR, 1848)

Material: 19.05.2015, Ujków Stary, 1M, leg. Mielczarek Ł.; 01.05.2020, Pustynia Błędowska (Chechło), 4F (females), leg. Mielczarek Ł.; 06.06.2021, Ujków Stary (ecological site), 1F, leg. Mielczarek Ł.; 06.06.2021, Ujków Stary (ecological site), 9M, leg. Mielczarek Ł.; 10.06.2021, Ujków Stary (Armeria), 2M, leg. Mielczarek Ł.; 10.06.2021, Ujków Stary (Armeria), 1F, leg. Mielczarek Ł.; 23.05.2021, Ujków Stary (ecological site), 1M, leg. Mielczarek Ł.; 23.05.2021, Ujków Stary (ecological site), 4F, leg. Mielczarek Ł.

This species occupies thermophilic deciduous forests and dry grasslands; found in March–June and August–September. They are undescribed larvae, but most likely associated with *Verbascum* (Vujić et al., 2020).

Cheilosia barbata (LOEW, 1857)

Material: 02.07.2015, Ujków Stary, 1M, leg. Mielczarek Ł.; 16.08.2015, Ujków Stary, 3F, leg. Mielczarek Ł.; 28.05.2020, Olkusz (Krucza Góra), 1M, leg. Mielczarek Ł.

It occurs locally in moist deciduous forests and at edges of meadows, clearings and undeveloped pastures and other open areas. Phytophagous larvae; imagines feed on pollen, nectar, honeydew. Flight period is from May to June. Larvae not described, but most likely associated with the roots of *Primula* plants (Vujić et al., 2020).

Table 1. Hoverfly species recorded from the study area.

No.	Species	EZ	GT	DET.	Site									
					US	H	B	Kl	Ry	Ro	Ra	P	Σ	OPN
1	*Anasimyia contracta* CLAUSSEN & TORP, 1980	Pal	SW	ŁM			+						1	
2	*Blera fallax* (LINNAEUS, 1758)	Pal	S	ŁM			+						1	+
3	*Brachypalpus chrysites* EGGER, 1859	E-K	S	RJ				+					1	
4	*Brachyopa insensilis* COLLIN, 1939	W-Pal	S	ŁM	+								4	
5	*Brachypalpus laphriformis* (FALLEN, 1816)	E	S	ŁM			+						1	+
6	*Callicera aenea* (FABRICIUS, 1777)	Pal	S	AM, ŁM	+								2	
7	*Chalcosyrphus nemorum* (FABRICIUS, 1805)	Hol	S	ŁM				+					1	
8	*Chalcosyrphus piger* (FABRICIUS, 1794)	Hol	S	AM				+					3	
9	*Chalcosyrphus valgus* (GMELIN, 1790)	Pal	S	ŁM	+								1	+
10	*Cheilosia aerea* DUFOUR, 1848	W-Pal	F	ŁM	+			+					23	
11	*Cheilosia albitarsis* (MEIGEN, 1822)	Hol	F	ŁM	+	+							5	+
12	*Cheilosia barbata* LOEW, 1857	E	F	ŁM	+								4	+
13	*Cheilosia bergenstammi* (BECKER, 1894)	W-Pal	F	ŁM	+		+						3	+
14	*Cheilosia caerulescens* (MEIGEN, 1822)	E	F	ŁM				+	+				7	+
15	*Cheilosia carbonaria* EGGER, 1860	Pal	F	AM, ŁM	+	+		+					7	+
16	*Cheilosia chrysocoma* (MEIGEN, 1822)	Pal	F	ŁM				+					1	+
17	*Cheilosia fasciata* SCHINER & EGGER, 1853	E	F	ŁM								+	5	+
18	*Cheilosia flavipes* (PANZER, 1798)	W-Pal	F	AM, ŁM		+							2	
19	*Cheilosia gigantea* (ZETTERSTEDT, 1838)	Pal	F	ŁM			+	+					3	+
20	*Cheilosia himantopus* (PANZER, 1798)	E	F	AM				+					1	+
21	*Cheilosia illustrata* (HARRIS,1776)	Pal	F	AM, ŁM	+		+	+					8	+
22	*Cheilosia impressa* LOEW, 1840	Pal	F	ŁM				+					3	+
23	*Cheilosia latifrons* (ZETTERSTEDT, 1843)	Pal	F	ŁM					+				1	+
24	*Cheilosia longula* (ZETTERSTEDT, 1838)	Pal	M	ŁM	+								17	
25	*Cheilosia morio* (ZETTERSTEDT, 1838)	Pal	F	ŁM				+					1	+
26	*Cheilosia nigripes* (MEIGEN, 1822)	Pal	F	ŁM	+								6	+
27	*Cheilosia pagana* (MEIGEN, 1822)	Hol	F	AM, ŁM	+	+							6	+
28	*Cheilosia proxima* (ZETTERSTEDT, 1843)	Pal	F	AM, ŁM	+								3	+

(*Continued*)

Table 1. Continued.

No.	Species	EZ	GT	DET.	Site									
					US	H	B	Kl	Ry	Ro	Ra	P	Σ	OPN
29	*Cheilosia scutellata* (FALLÉN, 1817)	Pal	M	AM, ŁM	+			+					3	+
30	*Cheilosia ruffipes* (PREYSSLER, 1793) = *soror* (ZET.)	Pal	M	ŁM	+						+		3	
31	*Cheilosia semifasciata* BECKER, 1894*	E	F	ŁM									1	+
32	*Cheilosia urbana* (MEIGEN, 1822)	W-Pal	F	ŁM				+					2	+
33	*Cheilosia variabilis* (PANZER, 1798)	Pal	F	ŁM	+			+					2	+
34	*Cheilosia vernalis* (FALLÉN, 1817)	Pal	F	ŁM	+								7	+
35	*Cheilosia vicina* (ZETTERSTEDT, 1849)	W-Pal	F	AM, ŁM			+	+					7	+
36	*Chrysotoxum bicinctum* (LINNAEUS, 1758)	Pal	Z	AM	+					+			2	+
37	*Chrysotoxum cautum* (HARRIS,1776)	W-Pal	Z	AM, ŁM	+			+					14	+
38	*Chrysotoxum festivum* (LINNAEUS, 1758)	Pal	Z	AM, ŁM	+			+		+			13	+
39	*Chrysotoxum vernale* LOEW, 1841	Pal	Z	AM, ŁM	+		+	+					17	+
40	*Chrysotoxum verralli* COLLIN, 1940	W-Pal	Z	ŁM					+				1	
41	*Criorhina asilica* (FALLEN, 1816)	E	S	ŁM			+						1	+
42	*Criorhina berberina* (FABRICIUS, 1805)	W-Pal	S	ŁM	+								1	+
43	*Criorhina ranunculi* (PANZER, 1804)	Pal	S	ŁM			+						1	+
44	*Dasysyrphus albostriatus (*FALLÉN, 1817)	Pal	Z	AM, ŁM	+								9	+
45	*Dasysyrphus hilaris* (ZETTERSTEDT, 1843)	Pal	Z	AM, ŁM	+			+					7	+
46	*Dasysyrphus neovenustus* SOSZYŃSKI & MIELCZAREK, 2013	Pal	Z	AM, ŁM	+			+					9	
47	*Dasysyrphus pauxillus* (WILLISTON, 1887)	Hol	Z	ŁM			+						1	
48	*Dasysyrphus tricinctus* (FALLÉN, 1817)	Pal	Z	AM, ŁM	+			+					13	+
49	*Dasysyrphus venustus* (MEIGEN, 1822)	Hol	Z	AM, ŁM	+			+					4	+
50	*Didea alneti* (FALLÉN, 1817)	Hol	Z	AM, ŁM	+		+	+					8	+
51	*Didea intermedia* LOEW, 1854	Pal	Z	AM, ŁM	+								3	
52	*Epistrophe diaphana* (ZETTERSTEDT, 1843)	Pal	Z	ŁM	+								2	
53	*Epistrophe eligans* (HARRIS, 1780)	W-Pal	Z	AM, ŁM	+		+	+					5	+
54	*Epistrophe flava* DOCZKAL AND SCHMID, 1994	Pal	Z	AM, ŁM	+		+						3	
55	*Epistrophe melanostoma* (ZETTERSTEDT, 1843)	W-Pal	Z	ŁM				+					2	
56	*Epistrophe nitidicollis* (MEIGEN, 1822)	Hol	Z	AM, ŁM	+		+						5	+
57	*Epistrophe ochrostoma* (ZETTERSTEDT, 1849)	Pal	Z	AM	+								1	

(Continued)

Table 1. Continued.

No.	Species	EZ	GT	DET.	Site									
					US	H	B	Kl	Ry	Ro	Ra	P	Σ	OPN
58	*Epistrophella euchroma* (KOWARZ, 1885)	W-Pal	Z	AM, ŁM	+		+						4	+
59	*Episyrphus balteatus* (DE GEER, 1776)	Pal+Orient	Z	AM, ŁM	+			+					20	+
60	*Eristalinus aeneus* (SCOPOLI, 1763)	C	SW	AM, ŁM	+								58	+
61	*Eristalinus sepulchlaris* (LINNAEUS, 1758)	Pal+Orient	SW	AM	+								1	+
62	*Eristalis arbustorum* (LINNAEUS, 1758)	Hol	SW	AM, ŁM	+			+					71	+
63	*Eristalis nemorum* (LINNAEUS, 1758)	Hol	SW	AM, ŁM	+			+					15	+
64	*Eristalis pertinax* (SKOPOLI, 1763)	E-K	SW	AM, ŁM	+			+					19	+
65	*Eristalis picea* (FALLÉN, 1817)	Pal	SW	AM				+					2	
66	*Eristalis pseudorupium* KANERVO, 1938	Pal	SW	ŁM				+					1	
67	*Eristalis similis* (FALLÉN, 1817)	Pal	SW	AM	+								6	
68	*Eristalis tenax* (LINNAEUS, 1758)	C	SW	AM	+			+					113	+
69	*Eumerus ovatus* (LOEW, 1848)	E-K	F	ŁM	+								15	
70	*Eupeodes bucculatus* (RONDANI, 1857)	E-K	Z	AM	+								1	
71	*Eupeodes corollae* (FABRICIUS, 1794)	Pal+Afr	Z	AM, ŁM	+								5	+
72	*Eupeodes luniger* (MEIGEN, 1822)	Pal	Z	AM, ŁM	+			+					2	+
73	*Eurinomyia lineata* (FABRICIUS, 1787)	Pal	SW	ŁM	+								8	
74	*Fagisyrphus cinctus* (FALLÉN, 1817)	E-K	Z	AM, ŁM	+								1	+
75	*Ferdinandea cuprea* (SCOPOLI, 1763)	Pal	S	ŁM	+		+	+					5	+
76	*Hammerschmidtia ferruginea* (FALLÉN, 1817)	Hol	S	ŁM				+					1	
77	*Helophilus hybridus* LOEW, 1846	Hol	SW	ŁM	+								1	+
78	*Helophilus pendulus* (LINNAEUS, 1758)	Pal	SW	AM, ŁM	+			+					9	+
79	*Helophilus trivittatus* (FABRICIUS, 1805)	Pal	SW	AM, ŁM	+			+					6	+
80	*Lapposyrphus lapponicus* (ZETTERSTEDT, 1838)	Hol	Z	AM, ŁM	+			+				+	6	+
81	*Leucozona inopinata* DOCZKAL, 2000	Pal	Z	ŁM				+					1	
82	*Leucozona lucorum* (LINNAEUS, 1758)	Hol	Z	ŁM			+						1	+
83	*Megasyrphus erraticus* (LINNAEUS, 1758)	Pal	Z	AM				+					2	+
84	*Melangyna ericarum* (COLLIN, 1946)	E	Z	H- BS				+					2	
85	*Melangyna lasiophthalma* (ZETTERSTEDT, 1843)	Hol	Z	ŁM				+				+	10	+
86	*Melangyna lucifera* NIELSEN, 1980)	Pal	Z	ŁM				+					4	+

(*Continued*)

Table 1. Continued.

No.	Species	EZ	GT	DET.	Site									
					US	H	B	Kl	Ry	Ro	Ra	P	Σ	OPN
87	*Melangyna pavlovskyi* (VIOLOVITSH, 1956)	Pal	Z	ŁM				+				+	9	+
88	*Melangyna quadrimaculata* VERRALL, 1873	Pal	Z	AM, ŁM				+					1	+
89	*Melangyna umbellatarum* (FABRICIUS, 1794)	Hol	Z	AM, ŁM	+								3	+
90	*Melanogaster hirtella* (LOEW, 1843)	E	SW	ŁM			+						1	
91	*Melanogaster nuda* (MACQUART, 1829)	W-Pal	SW	AM	+								1	+
92	*Melanogaster parumplicata* (LOEW, 1840)	E	SW	AM, ŁM	+			+					2	
93	*Melanostoma mellinum* (LINNAEUS, 1758)	Hol	Z	ŁM	+								18	+
94	*Meligramma triangulifera* (ZETTERSTEDT, 1843)	Pal	Z	AM		+							1	+
95	*Merodon equestris* (FABRICIUS, 1794)	C	F	ŁM		+							2	+
96	*Merodon moenium* (WIEDEMANN, 1822)	E-K	F	ŁM				+					2	
97	*Microdon analis* (MACQUART, 1842)	Pal	Z	ŁM			+						1	
98	*Microdon mutabilis* (LINNAEUS, 1758)	Pal	Z	ŁM	+			+					10	
99	*Myathropa florea* (LINNAEUS, 1758)	Pal	S	AM	+			+					5	+
100	*Neoascia meticulosa* (SCOPOLI, 1763)	W-Pal	SW	ŁM		+	+						2	+
101	*Neoascia podagrica* (FABRICIUS, 1775)	Pal	SW	ŁM	+								1	+
102	*Neoascia tenur* (HARRIS, 1780)	W-Pal	SW	ŁM	+								2	+
103	*Neocnemodon larusi* (VUJIĆ, 1999)	E	Z	AM				+					2	+
104	*Neocnemodon vitripennis* (MEIGEN, 1822)	Pal	Z	AM	+								2	+
105	*Orthonevra brevicornis* (LOEW, 1843)	W-Pal	SW	ŁM			+						1	+
106	*Orthonevra geniculata* (MEIGEN, 1830)	Pal	SW	ŁM	+		+						7	
107	*Orthonevra nobilis* (FALLEN, 1817) *	Pal	SW	ŁM									1	+
108	*Paragus albifrons* (FALLEN, 1817)	Pal	Z	AM						+			1	
109	*Paragus constrictus* SIMIC, 1986	Pal	Z	ŁM	+		+						4	
110	*Paragus haemorrhous* MEIGEN, 1822	Hol+Afr	Z	ŁM	+		+	+					9	+
111	*Paragus pecchiolii* RONDANI, 1857	W-Pal	Z	ŁM			+	+	+				3	
112	*Parasyrphus annulatus* (ZETTERSTEDT, 1838)	Pal	Z	AM	+		+	+					16	+
113	*Parasyrphus punctulatus* (VERRALL, 1873)	Pal	Z	AM, ŁM	+		+						3	+
114	*Parasyrphus vittiger* (ZETTERSTEDT, 1843)	Pal	Z	AM, ŁM	+								3	+
115	*Parhelophilus frutetorum* (FABRICIUS, 1775)	W-Pal	SW	AM, ŁM			+	+					5	

(*Continued*)

Table 1. Continued.

No.	Species	EZ	GT	DET.	Site									
					US	H	B	Kl	Ry	Ro	Ra	P	Σ	OPN
116	*Parhelophilus versicolor* (FABRICIUS, 1794)	Pal	SW	AM, ŁM			+	+					3	+
117	*Pelecocera scaevoides* (FALLEN, 1817)	W-Pal	M	AM, ŁM	+		+						10	
118	*Pelecocera tricincta* MEIGEN, 1822	W-Pal	M	AM, ŁM	+		+						4	
119	*Pipiza austriaca* MEIGEN, 1822	Pal	Z	AM, ŁM				+					3	+
120	*Pipiza festiva* MEIGEN, 1822	Pal	Z	AM	+								3	
121	*Pipiza lugubris* (FABRICIUS, 1775)	E-K	Z	AM, ŁM	+								2	+
122	*Pipiza noctiluca* (LINNAEUS, 1758)	W-Pal	Z	ŁM			+						1	+
123	*Pipiza quadrimaculata* (PANZER, 1804)	Pal	Z	AM				+					1	+
124	*Pipizella annulata* (MACQUART, 1829)	E	Z	AM, ŁM	+								10	
125	*Pipizella viduata* (LINNAEUS, 1758)	W-Pal	Z	AM, ŁM	+		+	+					29	+
126	*Pipizella virens* (FABRICIUS, 1805)	Pal	Z	ŁM				+			+		2	+
127	*Platycheirus albimanus* (FABRICIUS, 1781)	Hol	Z	AM, ŁM	+	+	+	+					17	+
128	*Platycheirus ambiguus* (FALLEN, 1817)	Pal	Z	AM	+								1	
129	*Platycheirus angustatus* (ZETTERSTEDT, 1843)	Pal	Z	ŁM	+								1	+
130	*Platycheirus clypeatus* (MEIGEN, 1822)	Hol	Z	ŁM				+					1	+
131	*Platycheirus discimanus* (LOEW, 1871)	Hol	Z	ŁM			+						3	+
132	*Platycheirus europaeus* GOELDLIN, MAIBACH & SPEIGHT, 1990	Pal	Z	AM, ŁM	+	+	+						8	+
133	*Platycheirus fulviventris* (MACQUART, 1829)	Pal	Z	ŁM	+								1	
134	*Platycheirus immarginatus* (ZETTERSTEDT, 1849)	Hol	Z	ŁM			+						1	
135	*Platycheirus peltatus* (MEIGEN, 1822)	Pal	Z	ŁM	+								3	+
136	*Platycheirus scutatus* (MEIGEN, 1822)	Hol	Z	AM, ŁM	+			+					11	
137	*Psilota atra* (FALLÉN, 1817)	W-Pal	S	AM, ŁM				+					2	
138	*Rhingia rostrata* (LINNAEUS, 1758)	W-Pal	K	AM, ŁM	+								3	+
139	*Scaeva pyrastri* (LINNAEUS, 1758)	Hol	Z	AM, ŁM	+								2	+
140	*Scaeva selenitica* (MEIGEN, 1822)	Pal	Z	AM, ŁM	+			+					9	+
141	*Sericomyia lappona* (LINNAEUS, 1758)	Pal	SW	ŁM			+						1	
142	*Sphaerophoria batava* GOELDLIN, 1974	Pal	Z	ŁM	+		+	+					8	+
143	*Sphaerophoria rueppellii* (WIEDEMANN, 1830)	Pal+Afr	Z	AM	+								1	

(*Continued*)

Table 1. Continued.

No.	Species	EZ	GT	DET.	Site									
					US	H	B	Kl	Ry	Ro	Ra	P	Σ	OPN
144	*Sphaerophoria scripta* (LINNAEUS, 1758)	Pal	Z	AM, ŁM	+	+	+	+					25	+
145	*Sphaerophoria taeniata* (MEIGEN, 1822)	Pal	Z	ŁM	+	+	+	+					6	+
146	*Sphegina sibirica* STACKELBERG, 1953	Pal	S	ŁM			+						1	
147	*Sphiximorpha subsesilis* (ILLIGER IN ROSSI, 1807)	W-Pal	S	ŁM	+								2	+
148	*Spilomyia diophtalma* (LINNAEUS, 1758)	Pal	S	ŁM	+			+					2	+
149	*Syritta pipiens* (LINNAEUS, 1758)	C	K	AM, ŁM	+			+					122	+
150	*Syrphus ribesii* (LINNAEUS, 1758)	Hol	Z	AM, ŁM	+								8	+
151	*Syrphus torvus* OSTEN-SACKEN, 1875	Hol	Z	AM, ŁM	+	+	+	+					30	+
152	*Syrphus vitripennis* MEIGEN, 1822	Hol	Z	AM	+								9	+
153	*Temnostoma meridionale* KRIVOSHEINA et MAMAEV, 1962	E-K	S	ŁM				+					2	+
154	*Temnostoma vespiforme* (LINNAEUS, 1758)	Pal	S	ŁM	+			+					2	+
155	*Triglyphus primus* LOEW, 1840	Pal	Z	ŁM	+								1	
156	*Tropidia scita* (HARRIS, 1780)	Pal	SW	ŁM			+						3	
157	*Volucella inanis* (LINNAEUS, 1758)	Pal	Z	AM, ŁM	+								4	+
158	*Volucella pellucens* (LINNAEUS, 1758)	Pal	SG	AM, ŁM	+								4	+
159	*Volucella zonaria* (PODA, 1761)	Pal	SG	ŁM									1	
160	*Xanthandrus comtus* (HARRIS, 1776)	Pal	Z	AM, ŁM	+								5	+
161	*Xanthogramma citrofasciatum* (DE GEER, 1776)	W-Pal	Z	ŁM				+					1	+
162	*Xanthogramma pedissequum* (HARRIS, 1780)	E	Z	AM, ŁM	+		+			+			17	+
163	*Xylota florum* (FABRICIUS, 1805)	Pal	S	ŁM			+						1	
164	*Xylota segnis* (LINNAEUS, 1758)	Hol	S	AM, ŁM	+		+	+					17	+
165	*Xylota sylvarum* (LINNAEUS, 1758)	Pal	S	ŁM				+					1	+

*Specimen caught in Olkusz.

Σ, number of specimens collected; AM, Anna Mielczarek; Afr, Afrotropical; BS, Bogusław Soszyński; B, Bukowno; C, cosmopolitan; DET., detected; E, European; E-K, Euro-Caucasian; EZ, zoogeographical element; F, phytophagous; GT, tropic group; H, Hutki; H, historical statement; Hol, Holarctic; ŁM, Łukasz Mielczarek; K, coprophage; Kl, Klucze; M, mycophagus; ONP, Ojcowski National Park; Orient, Oriental; P, Pazurek; Pal, Palearctic; Ra, Rabsztyn; RJ, R. Januszek; Ro, Rodaki; Ry, Ryczów; S, terrestrial saprophage; SW, aquatic saprophage; SG, nest-dwelling saprophage; US, Ujków Stary; W-Pal, West-Palearctic; Z, zoophagus.

Cheilosia caerulescens (MEIGEN, 1822)

Material: 05.08.2017, Ryczów (limestone inselbergs), 1F, leg. Mielczarek Ł.; 06.2020, Klucze (limestone inselbergs), 3M, leg. Mielczarek Ł.; 08.2020, Klucze (limestone inselbergs), 3F, leg. Mielczarek Ł.

A species found in calcareous grasslands, gardens with *Sempervivum*. Larvae undescribed. The peak of the outbreak is in May and August (Speight, 2011).

Cheilosia fasciata (SCHINER ET EGGER, 1853)

Data published in Żóralski and Mielczarek (2021).

The species occurs in mountainous areas in moist, shady deciduous forests with *Allium ursinum* L. and *A. victorialis* (Schmid and Grossmann, 1998). Larvae (one per leaf) mine the leaves of *Allium* (Renema, 1999).

Cheilosia himantopus (PANZER, 1798)

Material: 31.05.2015, Klucze (Pustynia Błędowska), 1F, leg. Mielczarek A.

Found in moist deciduous forests with a host plant of the *Petasites* genus. Imagines found in May on the flowers of the Apiaceae and Asteraceae families (Vujić et al., 2020).

Cheilosia latifrons (ZETTERSTEDT, 1843)

Material: 05.08.2017, Ryczów, 1M, leg. Mielczarek Ł.

It occurs in deciduous mesophilic forests and within mountain grasslands. Flight period runs from May to August. Imagines visit flowers from the Apiaceae, Asteraceae families. Biology of the larvae remains unknown (Vujić et al., 2020).

Cheilosia longula (ZETTERSTEDT, 1838)

Material: 02.07.2015, Ujków Stary, 1F, leg. Mielczarek Ł.; 16.08.2015, Ujków Stary, 11F, 5M, leg. Mielczarek Ł.

It is found in deciduous and coniferous forests. Flight period is in August, when imagines can be found on flowers of Apiaceae, Asteraceae, *Achillea, Euphorbia* and *Galium*. Larvae develop on fungi of the family Boletaceae (Vujić et al., 2020).

Cheilosia morio (ZETTERSTEDT, 1838)

Material: 30.03.2019, Kuźnica Błędowska, 1M, leg. Mielczarek Ł.

The larvae were described on the basis of the material collected on spruce, in resinous leakages caused by *Dendroctonus micans* (Bańkowska, 1961). Flight period is from March till beginning of May.

Cheilosia nigripes (MEIGEN, 1822)

Material: 19.05.2015, Ujków Stary, 1M, leg. Mielczarek Ł.; 23.05.2021, Ujków Stary (ecological site), 1F, leg. Mielczarek Ł.; 23.05.2021, Bolesław (staw na ‘‘Pasterniku’’), 1M, leg. Mielczarek Ł.; 23.05.2021, Bolesław (staw na “Pasterniku”), 1F, leg. Mielczarek Ł.; 23.05.2021, Ujków Stary (Armeria), 1F, leg. Mielczarek Ł.; 23.05.2021, Ujków Stary (Armeria), 1M, leg. Mielczarek Ł.

Adult forms can be found in grasslands or in open deciduous forests from April to June. Imagines visit flowers from Apiaceae, *Euphorbia*, *Prunus* and *Ranunculus*. Larvae are unknown (Vujić et al., 2020).

Cheilosia scutellata (FALLÉN, 1817)

Material: 31.05.2015, Pustynia Błędowska, 1M, leg. Mielczarek A.; 26.07.2015, Klucze (Czubatka), 1M, leg. Mielczarek Ł.; 16.08.2015, Ujków Stary, 1M, leg. Mielczarek Ł.

It is a forest species. Flight is from May to October. Imagines visit plants from Apiaceae, *Crataegus*, *Galium*, *Hedera*, *Hieracium*, *Ranunculus* and *Sorbus*. Larvae develop in fungi of the Boletaceae family (Vujić et al., 2020).

Cheilosia semifasciata (BECKER, 1894)

Observation: 05.2015, Olkusz, 1F, obs. Mielczarek Ł. on *Hylotelephium spectabile*,

Data presented in Żóralski and Mielczarek (2021).

The species occurs in dry deciduous forests, cliffs, strongly sunlight. It is associated with the sites of *Hylotelephium telephium* and *Hylotelephium maximum* (Rotheray, 1988).

Cheilosia ruffipes (PREYSSLER, 1793) = *soror* (ZETTERSTEDT, 1843)

Material: 21.07.2018, Rabsztyn (castle hill), 2M, leg. Mielczarek Ł.; 23.07.2017, Ujków Stary, 1M, leg. Mielczarek Ł.

This species prefers deciduous forests. Imagines found from April to October when they visit flowers from Apiaceae, Asteraceae, *Foeniculum vulgare*, *Taraxacum* and *Cirsium*. Larvae are in the fruiting bodies of *Tuber* truffle*s* (Speight, 2011).

Epistrophe diaphana (ZETTERSTEDT, 1843)

Material: 16.08.2015, Ujków Stary, 1F, leg. Mielczarek Ł.; 06.06.2021, Ujków Stary (ecological site), 1M, leg. Mielczarek Ł.

It cccurrs in wetlands/forests and rivers and streams in deciduous woodland, including carr; also in unimproved, montane grassland. Aduls visit white umbellifers: *Foeniculum*, *Senecio*. Flight period is in May/August (with peak in July) and on into September in southern Europe (Speight, 2011).

Epistrophe flava (DOCZKAL AND SCHMID, 1994)

Material: 23.06.2014, Ujków Stary, 1ex., leg. Mielczarek A.; 06.06.2021, Ujków Stary (ecological site), 1F, leg. Mielczarek Ł.; 06.06.2021, Bukowno (Diabla Góra), 1M, leg. Mielczarek Ł.

It Preferred deciduous forest. Found on meadows, roadsides, forest edges. Flower visited: *Berberis vulgaris*, *Crataegus*, *Tilia cordata*, *Ranunculus* etc. Found to be active in June and July. Larvae described from aphid galls on *Malus* (Speight, 2011).

Epistrophe ochrostoma (ZETTERSTEDT, 1849)

Material: 02.05.2014, Ujków Stary, 1F, leg. Mielczarek A.

Species occur in the conifer and deciduous forest. They visit flowers of *Salix repens*, *Prunus padus*, *Ranunculus ficaria*. Adults can be found during the period from May to June. Larvae undescribed (Speight, 2011). Threat category – VU on the Red List of Endangered Animals in Poland.

Eumerus ovatus (LOEW, 1848)

Material: 19.05.2015, Ujków Stary, 1M, leg. Mielczarek Ł.; 10.06.2021, Ujków Stary (Armeria), 10M, 4F, leg. Mielczarek Ł.

It occupies open, thermophilic forests and dry, open, calcareous grasslands rich in vegetation. Flight period is from May to July and it is a rare species. Larvae, probably phytophagous, develop in bulbs or rhizomes of host plants. Imagines visit flowers (Vujić et al., 2020).

Eupeodes bucculatus (RONDANI, 1857)

Material: 26.07.2015, Ujków Stary, 1M, leg. Mielczarek A.

Found in deciduous riverside forests with the participation of poplars, willows and birches. Flight period runs in April/May and July/August. Imagines visit flowers from the Asteraceae, *Euphorbia*, *Nartecium*, *Salix*, *Sorbus* and *Stellaria*. Aphidophagous larvae are found on aphids on *Cirsium arvense* (Vujić et al., 2020).

Megasyrphus erraticus (LINNAEUS, 1758)

Material: 18.05.2015, Klucze (Czubatka), 2F, leg. Mielczarek A.

It is found in coniferous forests with *Abies*, *Picea* and *Pinus*. It visit flowers of various plant eg. *Calluna vulgaris*, *Cirsium vulgare*, *Crataegus*, *Prunus spinosa*, *Stellaria* sp. in the period from May to September (Speight, 2011). Although sparse, locally occurring populations tend to be numerous.

Melangyna aff. ericarum (COLLIN, 1946)

Material: 02.05.1994, Pustynia Błędowska, 1F, leg. Soszyński B. det. Soszyński B. det. Mielczarek Ł.

This is new for Poland, but known in Great Britain (Scottish highlands), Denmark, Netherlands, Germany, Czech Republic, France, Switzerland, and Italy (Speight, 2011). Despite probably being associated with Scots Pine forest, it is a rarely collected species. Stubbs and Falk (2002) stated that species occur in native woods of Scots Pine but also in a fen far away from stands with pines. In Sweden occur in alpine heaths close to *Pinus mugo* (Bartsch, 2009b). Features of our specimen fit well with existing identification keys to *Melangyna* species although in general appearance (e.g. broader abdomen) it resembles more *M. lucifera* and *M. lasiophthalma* than the summer *M. umbellatarum*. Stubbs and Falk (2002) stated that the posterior part of the fore and middle femora should have black hairs. In our specimen, the second femur has completely white hairs and the first one only a few. *Melangyna* are presumably univoltine in Europe and occurrence of *M. ericarum* in spring in Poland is quite confusing despite records from the Western Europe show summer months. In our opinion, it is worth to review scarce *M. ericarum* material to check if it is the only one probable bivoltine species or it is a complex of two or more species differing in flight period.

Melangyna pavlovskyi (VIOLOVITSH, 1956)

Material: 10.04.2021, Pazurek, 2M, leg. Mielczarek Ł.; 10.04.2021, Pazurek, 1F, leg. Mielczarek Ł.; 11.04.2021, Kuźnica Błędowska, 1F, leg. Mielczarek Ł.; 01.03.2020, Pazurek, 3M, leg. Mielczarek Ł.; 01.03.2020, Pazurek, 2F, leg. Mielczarek Ł.

It is a common species, inhabiting forest clearings, forest edges, parks and thickets. It occurs in March and April. Larvae are aphidophagous; adults feed on pollen and nectar. The species behaves like an invasive and recently recorded as new for Europe (Bygebjerg, 2011; Mielczarek, 2011) and rapidly extending its range in other countries (van de Meutter et al., 2015). Recorded from Belgium, Denmark, Netherlands, Slovakia; France (Langlois and Speight, 2020), Asiatic Russia (Sakhalin) and Japan.

Melanogaster hirtella (LOEW, 1843)

Material: 01.05.2020, Bukowno (Diabla Góra), 1F, leg. Mielczarek Ł.

It occurs in wetlands, fens, marshes, poorly-drained pastures, along woodland streams or field drains, beside lakes, ponds and rivers. Adults visit white umbellifers, *Caltha*, *Euphorbia*, *Iris pseudacorus*, *Menyanthes*, *Mimulus guttatus*, *Potentilla erecta*, *Pyrus communis Ranunculus*, *Sorbus aucuparia*, *Taraxacum* and *Viburnum opulus*. Flight period: end April/July. Larvae aquatic, associated with various aquatic plants, including *Glyceria* and *Typha* (Speight, 2011). The species was recorded only at Białowieża Primeval forest in Poland by Soszyński (1999). This record was based on one specimen reported by Theo Zeegers and Sander Turrnhout (Dijkstra and Kalkman, 1996). Record of this Atlantic species from Białowieża PF is unlikely and needs further study. The specimen from Diabla Góra was collected on flowering *Crataegus monogyna* at the top of the hill. Areas with marshes – potential habitat of *Melanogaster* larvae are present very close to Diabla Góra, in old sand mine Szczakowa where other aquatic hoverflies were present in numbers.

Melanogaster parumplicata (LOEW, 1840)

Material: 01.05.2020, Bukowno (Diabla Góra), 1F, leg. Mielczarek Ł.

Environment: open ground/forest/freshwater; close to water-bodies in river-flood plain grassland and alluvial forest. Adults visit flowers of umbellifers, *Caltha*, *Crataegus*, *Prunus spinosa*, *Ranunculus*, *Rhamnus cathartica* and *Taraxacum*. Flight period: Mid May/ Mid August, with peak in June. Larvae: undescribed (Speight, 2011).

Microdon mutabilis (LINNAEUS, 1758)

Material: 25.05.2018, Klucze (Czubatka), 1M, leg. Mielczarek Ł.; 10.06.2021, Ujków Stary (Armeria), 6M, leg. Mielczarek Ł.; 10.06.2021, Ujków Stary (Armeria), 3F, leg. Mielczarek Ł.

It is a rare species. It develops in open ground; sparsely-vegetated, dry, rocky ground appropriate for ant nests; unimproved pasture and grassy clearings in forest. It occurs in May to July. Larvae develop in colonies of *Formica* ants, feeding on their eggs and larvae (Speight, 2011).

Neocnemodon larusi (VUJIĆ, 1999)

Material: 18.05.2015, Klucze (Czubatka), 2F, leg. Mielczarek A.

The species found in clearings in deciduous and mixed forests (Bartsch, 2009a). It is an undescribed larvae, collected by Ł. Mielczarek from spruce galls.

Neocnemodon vitripennis (MEIGEN, 1822)

Material: 13.07.2014, Ujków Stary, 1F, leg. Mielczarek A.; 13.07.2014, Ujków Stary, 1M, leg. Mielczarek A.

The larvae of the species are predators found in the *Dreyfusia piceae* colonies on fir trees and on coccidia on the black poplar, Italian form. A typical habitat is forest paths and forest edges. It flies from May to October with peak occurrence in June and July (Stubbs and Falk 2002).

Orthonevra geniculata (MEIGEN, 1830)

Material: 03.05.2021, Bukowno (sand mine), 2F, leg. Mielczarek Ł.; 01.05.2020, Bukowno (Sztoła), 4M, leg. Mielczarek Ł.

It is a Spring species, often found on the edge of forests with *Salix* or *Alnus* spp. (Bartsch, 2009a). It prefers mid-forest lakes, springs and periodically flooded area (Żóralski and Trzciński, 2015).

Pelecocera scaevoides (FALLÉN, 1817)

Material: 13.09.2015, Ujków Stary, 1F, leg. Mielczarek A.; 05.2019, Bukowno (Diabla Góra), 9F, leg. Mielczarek Ł.

They prefer coniferous forests, especially Pinus. Adults flight in open woodland, clearings etc. They visited flowers of *Myosotis*, *Cerastium* and *Potentilla* in June/September. Larvae are apparently phytophagous (Speight, 2011).

Pelecocera tricincta (MEIGEN, 1822)

Material: 13.09.2015, Ujków Stary, 1F, leg. Mielczarek A.; 07.2018, Bukowno (Spacerowa), 1M, leg. Mielczarek Ł.; 10.06.2021, Bolesław (Armeria), 1M, leg. Mielczarek Ł.; 23.05.2021, Bolesław (Staw na "Pasterniku"), 1M, leg. Mielczarek Ł.

Species found in conifer forests, tracksides, clearings etc. They visited flowers: *Calluna vulgaris*, *Cirsium palustre*, *Hieracium*, *Ranunculus* and *Sedum acre*. Fly period is from June to September (Speight, 2011).

Pipizella annulata (MACQUART, 1829)

Material: 13.07.2014, Ujków Stary, 1M leg. Mielczarek A.; 13.07.2015, Ujków Stary, 1M, leg. Mielczarek A.; 26.07.2015, Ujków Stary, 1F, leg. Mielczarek A.; 02.07.2015, Ujków Stary, 2M, leg. Mielczarek Ł.; 02.07.2015, Ujków Stary, 1F, leg. Mielczarek Ł.; 16.08.2015, Ujków Stary, 1F, leg. Mielczarek Ł.; 26.07.2015, Ujków Stary, 2M, leg. Mielczarek Ł.; 26.07.2015, Ujków Stary, 1F, leg. Mielczarek Ł.

Rare thermophilic species, and can therefore be found on xerothermic grasslands (Stennis and Stennis, 1997).

Platycheirus ambiguus (FALLÉN, 1817)

Material: 19.05.2015, Ujków Stary, 1F, leg. Mielczarek A.

Occurs in deciduous forests; scrub-invaded clearings in woodland and forests, scrub-edged tracks in woodland. Adults visit flowers: *Acer pseudoplatanus*, *Crataegus*, *Prunus mahaleb*, *P. spinosa*, *Pyrus communis*, male flowers of *Salix* spp. (including *S. repens*), *Sorbus aucuparia, Viburnum*. Flight period: beginning April/end May. Larva is aphidophagous (Speight, 2011).

Psilota atra (FALLÉN, 1817)

Material: 07.05.2019, Klucze (Czubatka), 1F, leg. Mielczarek Ł.; 01.05.2014, Klucze (Czubatka), 1M, leg. Mielczarek A. (data published in Żóralski, 2018).

Species is connected with coniferous forests of *Pinus* spp. Adults often drink at damp mud in hot weather. They visited flowers of *Crataegus*, *Salix* etc. in the period May to June (Speight, 2011).

Rhingia rostrata (LINNAEUS, 1758)

Material: 16.08.2015, Ujków Stary, 2F, leg. Mielczarek Ł.; 02.05.2014, Ujków Stary, 1F, leg. Mielczarek A.

Species connected with deciduous forest and scrubs with rich grand flora. Flies visiting flowers in small glades: *Centaurea*, *Cirsium*, *Geranium*, *Hypericum* and *Veronica*. Flight period is from May to August (Speight, 2011).

Sphegina sibirica (STACKELBERG, 1953)

Material: 14.06.2021, Bukowno, 1F, leg. Mielczarek Ł.

The species occurs in moist, deciduous forests with birch and coniferous trees. Adults visit pignut flowers and possibly other umbellifers. It occurs in June and July (Stubbs and Falk, 2002). Larva is undescribed (Bartsch, 2009a).

Spilomyia diophtalma (LINNAEUS, 1758)

Material: 26.07.2015, Klucze, 1F, leg. Mielczarek Ł.; 23.07.2017, Ujków Stary, 1F, leg. Mielczarek Ł.

Species is similar to *S. manicata*. Imagines visit flowers such as *Angelica sylvestris*. It occurs in mixed and deciduous forests with old aspen in the period from July to August. The larvae develop in rotten hollows of deciduous trees (Bartsch, 2009a).

DISCUSSION

Syrphid adults belonging to the Diptera order are valuable pollinators (Dunn et al., 2020). As many as 415 species of hoverflies have already been recorded that are living in Poland (Żóralski and Kowalczyk, 2015). They occupy various types of habitats including meadows, forest clearings and gardens (Oosterbroek, 2006; Soszyński, 2007). In Polish literature, there are no reports on hoverflies in industrial and post-industrial areas characterised by a high degree of environmental contamination with heavy metals (Zn, Cd and Pb).

Heavy metals have a negative effect on living organisms, including insects. Their toxicity is manifested, for example, in the increased mortality of insects (Moroń et al., 2012), changes in the sex structure and a decrease in the number of brood cells in the species *Osmia rufa* (Moroń et al., 2013), shortening of the lifespan (Stone et al., 2001), a change in body weight of individuals caught in contaminated areas compared to those in uncontaminated ones (Moroń et al., 2013; Stone et al., 2001; Zygmunt et al., 2006), changes in morphological structures (Polidori et al., 2018) or wing asymmetry in *Polistes nimpha* (Mielczarek et al., 2021). Moroń et al. (2012) observed a decrease in the number of species of the genus *Osmia* with an increase in the concentration of heavy metals in their living environment. However, a similar relationship was not found by Szentgyörgyi et al. (2011) in bumblebee taxa. Grześ (2009), in her study of ants from areas contaminated with heavy metals, found an increase in the species with richness of ants along with an increase in the Zn content in the soil. Eeva et al. (2004) found no differences in the number of *Formica* ant species caught in a contaminated area in relation to the control area. Also, Migliorini et al. (2004) included ants in a study comparing the responses of various arthropod communities to contamination with heavy metals, mainly Pb and Sb, but found no clear relationship. Similarly, Nahmani and Lavelle (2002) analysed the diversity of species with the richness of arthropods (Coleoptera, Diptera, Lepidoptera, Hymenoptera, and Hemiptera) developing in soils contaminated with heavy metals. The authors did not consider species richness in relation to individual taxa, but only determined its total size for individual sites. The authors found the lowest species richness on the site characterised by the highest degree of contamination.

As a result of our research work, we collected 165 syrphid species out of 415 Syrphidae species found in Poland (39.76%). As a comparative site, we selected the area of the Ojców National Park, 20 km away from Olkusz, as the closest and the best studied area in terms of the Syrphidae fauna. In the ONP, 176 (excluding questionable species such as *Pipiza bimaculata*, noted in ONP) species from this family had been found (Klasa and Soszyński, 2011; Mielczarek and Klasa, 2017) – 11 species more than those reported in our research area (OOR). When comparing the results of the two studies, it should be emphasised that the research conducted in the ONP lasted almost 30 years (1986–2008, 2008–2015), while the present study is conducted for only 7 years.

The variety of habitat types within which the sites were selected should also be emphasised – in the ONP, the research area was more varied (it covered various types of habitats, such as: rock grasslands, hay meadows, herbaceous plants, mixed forests, oak-hornbeam forest, stream valley and Carpathian beech forest). Our research was conducted mainly in ruderal areas or ones heavily transformed by industrial activities.

As a result of our observations in the research area, we recorded 112 species with syrphid taxa that were the same as those identified in the ONP (68.29%). Like in the ONP, zoophagous species also dominated in our study, which is consistent with the tendency of the hoverfly trophic dominance. The percentages of individual trophic groups in the research area and the ONP were similar i.e.: predators accounted for 48.48% in the research area and 46.59% in the ONP, water saprophages – 16.37% and 13.07%, terrestrial saprophages – 13.94% and 18.18%, nest saprophage – 1.21 and 1.14, phytophages – 15.76 and 18.75, mycophages – 3.03% and 0.57% and coprophages – 1.21% and 1.7%, respectively. There were no significant differences among groups on using the G test. Heavy metals tend to accumulate at successive trophic levels (Zhang et al., 2021). Predatory insects seem to be more exposed to the negative effects of exposure to these substances than, for example, phytophages or saprophages. The effects of heavy metal bioaccumulation in the bodies of insects are well-known and may influence on their mortality, physiology etc. All this factors should lead to decrease the abundance of predatory species in the contaminated areas compared to the control areas. Gospodarek and Jaworska (2001) showed that on *Vicia fabae* plants growing on soils enriched with heavy metals, more numerous colonies of *Aphis fabae* Scop. occurred. Authors also observed lower number of syrphid larvae feeding in aphid colonies compared to the control. Stolpe et al. (2017) report that in the case of hyperaccumulators, heavy metals contained in plant tissues reduce the feeding of phytophages. In our research we did not find significant differences among trophic groups in OOR and ONP. The obtained results do not indicate that heavy metals have an effect on trophic structure of Syrphidae. Nevertheless, it seems justified to extend the research on syrphid larvae which are less mobile than adults and thus constitute a better model to study the impact of heavy metals on this taxon.

Among the collected syrphids, common and numerous species, such as: *Eristalis arbustorum* (LINNAEUS, 1758), *Eristalis tenax* (LINNAEUS, 1758), *Episyrphus balteatus* (DE GEER, 1776), *Syritta pipiens* (LINNAEUS, 1758) and *Myathropa florea* (LINNAEUS, 1758), as well as rare species were noted. Eleven of the collected species have been included in the 'Red List of Endangered Animals in Poland': *Callicera aenea* (FABRICIUS, 1777) (NT category), *Chalcosyrphus piger* (FABRICIUS, 1794) (DD), *Brachypalpus chrysites* EGGER, 1859 (LC), *Epistrophe ochrostoma* (ZETTERSTEDT, 1849) (VU), *Hammerschmidtia ferruginea* (FALLÉN, 1817) (DD), *Orthonevra geniculata* (MEIGEN, 1830) (DD), *Rhingia rostrata* (LINNAEUS, 1758) (DD), *Sericomyia* *lappona* (LINNAEUS, 1758) (NT), *Sphegina sibirica* STACKELBERG, 1953 (LC), *Spilomyia diophtalma* (LINNAEUS, 1758) (NT), and *Temnostoma vespiforme* (LINNAEUS, 1758) (DD) (Banaszak et al., 2002).

A very rare species *Melangyna ericarum* (COLLIN, 1946) was caught in this region (the Błędowska Desert) by Soszyński 27 years ago and it is still the only record of this rare species in Poland.

Interestingly, on our research site, *Volucella bombylans* (LINNAEUS, 1758) – a common species whose larvae are associated with nests of bumblebees, was not found. Bumblebees are present in the investigated research area, which was confirmed by the research of Szengyorgyi et al. (2011). It would be valuable to investigate the sensitivity of this species to heavy metals in future research.

Klasa and Soszyński (2011) state that the number of hoverflies indicated by them in the ONP may indirectly result from the large diversity of the fauna of vascular plants occurring in this area (approximately 950 species). Taking into account of the existing food webs, the individual elements such a plant on which aphids – source of food for zoophagous larvae – develop, are crucial for the occurrence of this group of hoverflies. Therefore, the species diversity of the flora in the study area seems to be significant. On the other hand, white or yellow flowers characterised by a shallow calyx structure are a source of pollen necessary for laying eggs by female Syrphidae.

Our research was conducted in a theoretically hostile environment due to strong anthropopressure, but despite this fact, this environment was also rich in diverse vegetation (736 species). It should be emphasised that 47% of the plants available there were rare or very rare species, and the lowest percentage of all the taxa were common species (Nowak et al., 2015). Therefore, in the future, it would be valuable to conduct field observations determining the role of hoverflies in the pollination of rare and protected plants occurring in the area of this study.

The richness of Syrphidae species has also been studied in other protected areas. Żóralski et al. (2017), in their research, which was conducted since 1978, reported 163 Syrphidae species from the Świętokrzyski National Park. Among them were 17 species from the Red List. Also, Żóralski and Kowalczyk (2019) reported 168 species caught in the Trójmiejski Landscape Park. Trzciński and Sienkiewicz (2006), during 1-year observations, recorded the occurrence of 42 species of hoverflies in the area of the Wielkopolski National Park. The richness of the syrphid species has also been studied in the area of the Wigry National Park – 171 species (Żóralski et al., 2017), the Western Beskids – 106 species (Żóralski and Dubiel, 2020) and in the massif of Babia Góra – 101 species (Palaczyk and Klasa, 2003).

Comparing our results with the species richness in the protected areas mentioned above, it can be concluded that the area studied by us is rich and diverse in terms of Syrphidae fauna, not inferior in its richness even to areas recognised as unique in terms of nature.

CONCLUSIONS

On the basis of the obtained results, it can be concluded that the studied area was not characterised by a lower species diversity in comparison with regions where contamination with heavy metals is not so significant. Moreover, the collected samples were usually characterised by a relatively large variety, although indeed some species, such as *Syritta pipiens* (LINNAEUS, 1758) or *Eristalinus aeneus* (SCOPOLI, 1763), dominate, especially in open areas.

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AUTHOR CONTRIBUTIONS

A.M. – collection, identification of research material, writing the paper. Ł.M. – collection, identification of research material, writing an article. E.W.-Ż. – designed the experiment, writing and correcting the manuscript.

CONFLICT OF INTEREST

We inform that the manuscript has not been submitted for publication elsewhere. All co-authors have contributed to this article and all have agreed to submit it into this journal. There is no conflict of interests.

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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

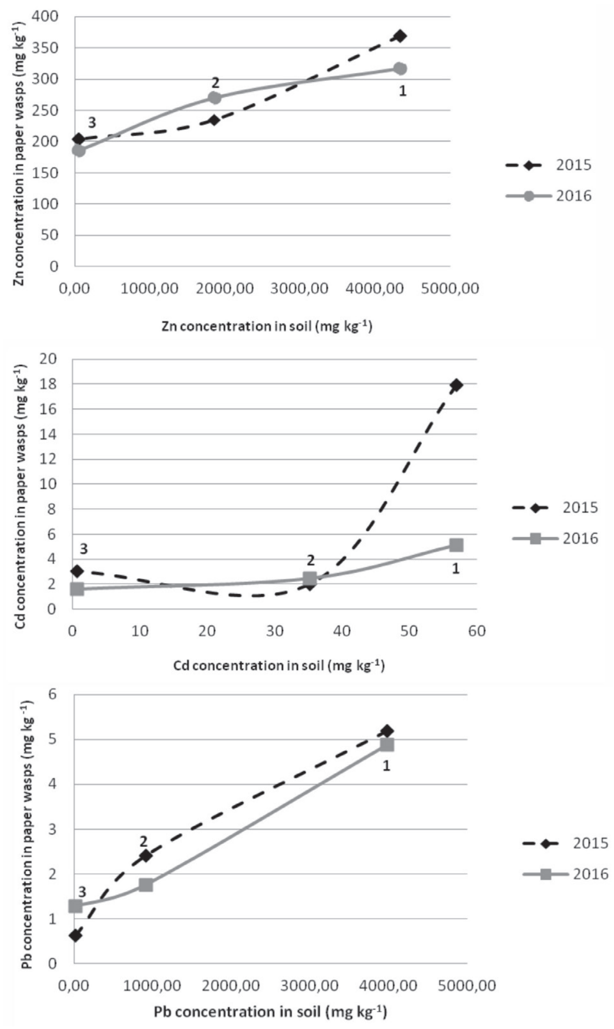

Fig. 1. Relation between the concentrations of heavy metals (Zn, Cd, Pb) in insect bodies and soil in two years of the study; 1 – Site 1, 2 – Site 2, 3 – Site 3.

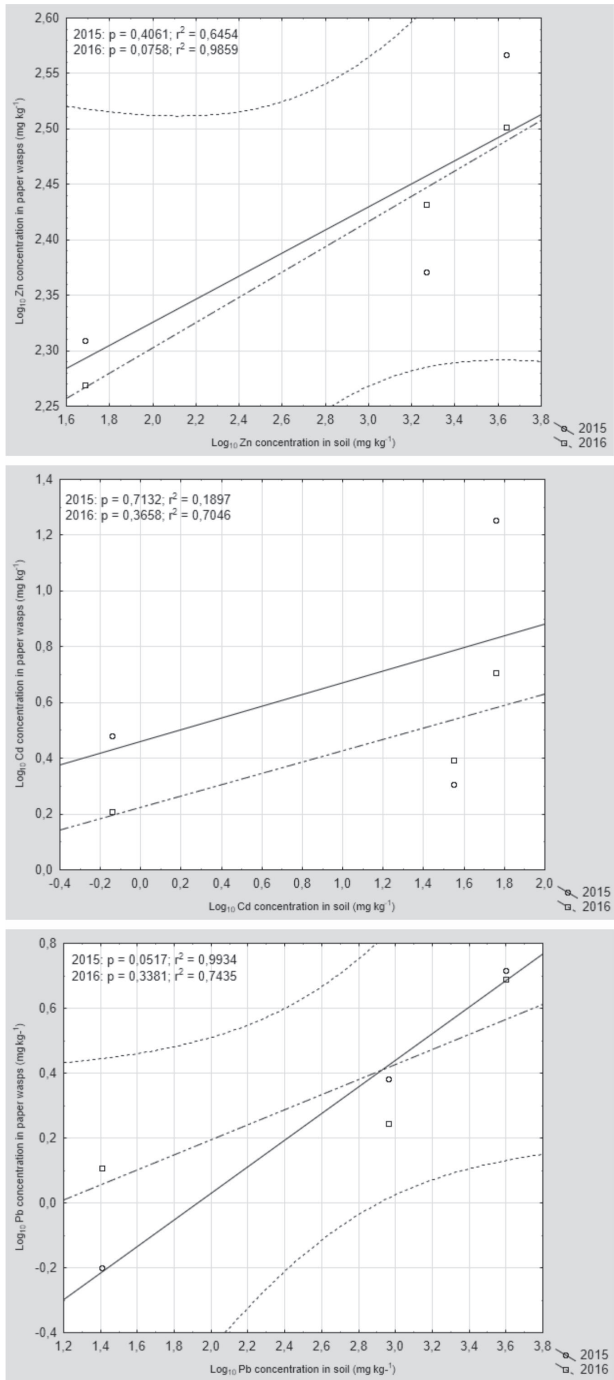

Fig. 2. Correlation between the levels of Zn, Cd and Pb in topsoil and their concentrations in the bodies of female P. nimphus (Christ, 1791) in 2015 and 2016.

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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