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Article

Novel Non-Invasive Biomonitoring Using Avian Faecal Sacs Reveals Dependence of Pesticide Exposure on Field Distance

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Abstract

Pesticides remain among the most significant threats to biodiversity and natural ecosystems. Non-invasive methods, such as the analysis of bird faeces, have shown great potential for detecting pesticide exposure. In this study with a new approach, we analysed faecal sacs from nestlings of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) to gain deeper insights into pesticide contamination during the breeding period. Samples were collected from three distinct sites near Münster, Germany. In total, we detected 65 substances from 57 different pesticides, as well as caffeine, with pesticides present in 16.07% of the 168 samples. Concentrations varied between species and sites and were higher for fungicides and insecticides in nests located closer to agricultural fields. While no direct effects on reproductive success were found, our results underscore the potential of faecal sac analysis as a valuable tool for spatially resolved pesticide monitoring. Importantly, we show that pesticide exposure also occurs in nestlings and birds breeding outside of intensive farmland. To better understand the ecological consequences, future studies should incorporate environmental variables and conduct a separate analysis of urate and faeces of faecal sacs to precisely determine concentrations.

Keywords: breeding birds; nest; blue tit; great tit; pesticides; caffeine

1. Introduction

Human activities have an increasingly negative impact on biodiversity [1]. Besides land-use change and microplastic, one major anthropogenic driver on terrestrial ecosystems are pesticides [2]. Their main field of application is modern agriculture, but substances are also increasingly emitted in urban environments [3,4]. Pesticides spread everywhere via air and water, so they can have unintended negative effects far from where they are used [5–7].

Overall, it is suspected that a large proportion of wildlife is exposed to pesticides [8]. In Central Europe, residues such as the herbicide terbuthylazine, the fungicide azoxystrobin, and the insecticide thiacloprid have been found in insects in nature reserves [9], which could lead to trophic cascade effects on birds [10–12]. Birds play a crucial role in ecosystems, providing a range of essential ecosystem services including predation, pollination, scavenging, seed dispersal and ecosystem engineering [13]. Furthermore, birds are highly effective bioindicators as very mobile consumers, offering valuable insights into ecosystem health and environmental change [14]. The potential risks of pesticides to birds must be evaluated separately from other animals, given their metabolism with unique working enzymes [15]. Several pesticides such as insecticides [16,17], fungicides and herbicides [18,19] have been found mainly in farmland birds.

The potential of non-invasively collected faeces as an indicator for multiple pesticide exposure have been shown [18]. However, during the different seasons, many captured individual birds lack a clear area reference, as they often change both their feeding and roosting sites. The breeding period is one of the few stages where it can be safely assumed that birds stay close to the same places – the nest area – for an extended period. Nests are particularly useful for pesticide testing, as they allow for the analysis of both adult and young birds, the latter of which tend to be easier to catch, due to their not fully developed wings. The ringing of fledglings in the nest is a commonly used method in scientific bird ringing, which establishes a straightforward sampling possibility [20].

The adult birds have a strong influence on the brood, building the nest and raising the young until they fledge [21]. In songbirds, the young are fed mostly with insects as they are high in protein and energy and therefore a particularly important source of nutrition [22]. Fledglings excrete droppings, which the adults can easily carry out of the nest thanks to a protective covering, the so-called faecal sacs. This makes the faeces less susceptible to external contamination than the droppings of adult birds [23]. In the past, faecal sacs have been successfully used for the analysis of metal exposure [24]. Since all the young of a bird pair are raised side by side in a nest, influences often affect all the young at a very early stage, which can affect their future life. The nidobiome is a fragile yet indispensable ecosystem for the reproduction and therefore the survival of bird species [21]. This highlights the importance of studying this life period.

The most severe consequence of pesticide contamination is the death of a bird, and if it affects an adult, it has a devastating effect on the brood [25]. Furthermore, a plethora of other mostly negative effects are possible [26,27], which are complicated by natural factors influencing broods such as weather [28] and timing [29]. To assess the consequences of pesticides on breeding birds, it is necessary to determine the breeding success and other performance parameters precisely during different stages of the brood in a real-life environment.

This study selected two co-occurring species of passerine birds, the Blue tit (*Cyanistes caeruleus*) and Great tit (*Parus major*). The selection of these species was based on their abundance and wide distribution across a range of habitats [30]. Both species are cavity-nesting songbirds and reach their highest population densities in settlements and in closed forests [31]. Pesticide residues have so far only been studied in connection with direct application of substances in the nest environment of Blue tits. The fungicides propiconazole and tridemorph had no effect on several fitness parameters such as survival of nestlings. However, the insecticide cypermethrin had significant negative influence on nestling weight and survival [32,33]. The insecticide malathion was applied in Blue tits nesting areas with no effect on breeding parameters, such as nest success, hatching success and nestling mortality [34]. Most of the studies on pesticides in birds concentrate on the direct farmland context, as a major known source of emissions [8,35]. However, large parts of the load profile and the consequences of pesticides on birds in the whole range of their natural habitat remain poorly understood [36].

Understanding the emission pathways and, as a next step, the effects of the substances from the individual bird to the population level in the real environment is crucial to assess the risks to wildlife and implement targeted measures. For a better understanding of the impact of pesticides on a population scale, faecal sacs were collected from Blue and Great tit fledglings in nest boxes in three different areas around Münster, Germany. Faecal sacs provide a direct insight into the nidobiome ecosystem during one of the most critical phases in a bird's life. This is the first time the influence of pesticides on nestlings has been analysed in real environmental conditions. We studied in detail which pesticide substances can be found in the nestlings of the two species, and which factors should be considered in future analyses. Answering these questions is essential to contribute to a holistic understanding of the impact of pesticides on our ecosystems.

2. Materials and Methods

Sampling

The study areas are located in and around the city of Münster in North Rhine-Westphalia, Germany. Three distinct areas with nest boxes were selected to sample Blue and Great tits nests in different land use environments (Figure 1 & Table S1): a) JKI: A research institute with park-like grounds and cultivated fields in an urban area adjacent to agricultural land (6.58 ha), b) WL: a mixed forest, used as a cemetery (85.60 ha), and c) ZDM: a recultivated landfill site with mostly permanent herbaceous areas, managed by late mowing once a year (47.87 ha).

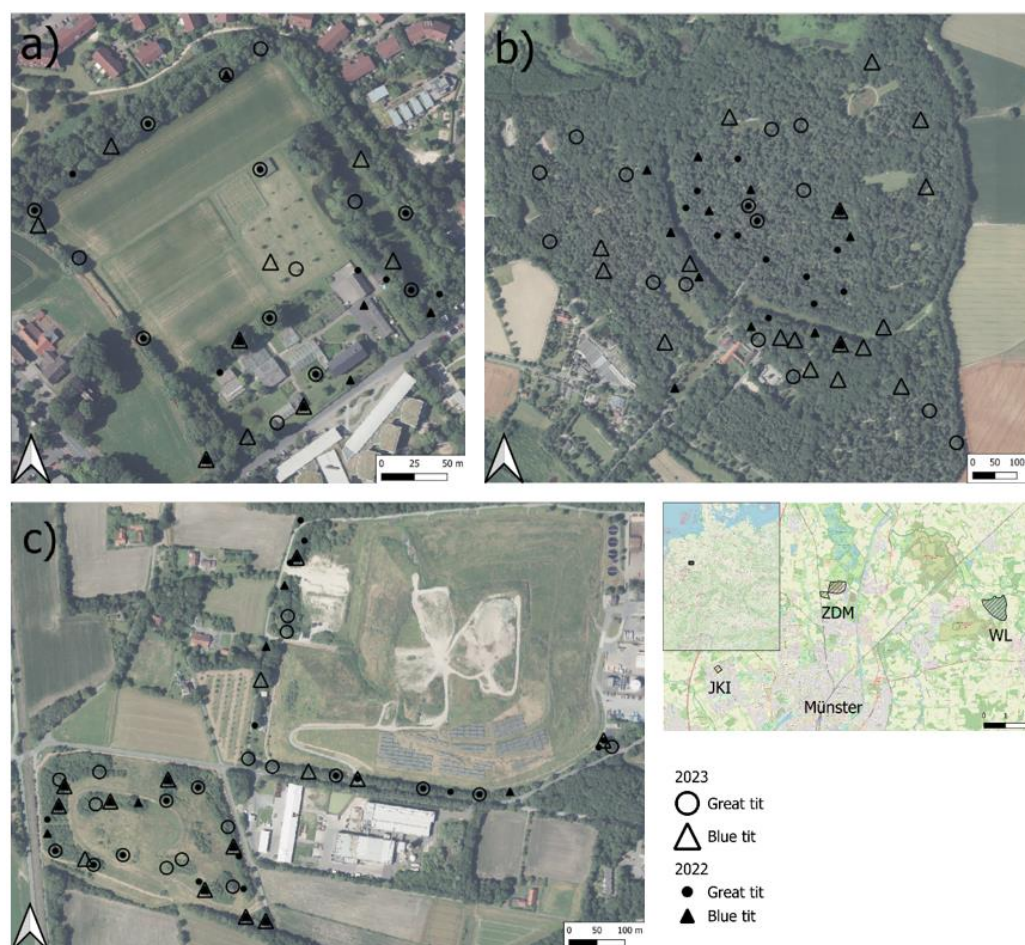


Figure 1. Map showing sampling points across the three study areas: a) JKI, b) WL and c) ZDM categorised by year and species [37].

The sampling took place during the scientific bird ringing of nestlings, which is part of a programme by the Helgoland bird ringing centre. Field work was conducted during 2022 and 2023. Nest boxes were checked weekly starting from the end of April. The young birds were ringed between ages of 8 to 15 days. The breeding success for each nest box resulted from the number of young birds ringed. In addition, unfertilised eggs and dead juveniles in the nest box were counted. The two species compete for nesting facilities in areas where both species co-occur. Great tits tend to prevail in the competition for nesting sites due to their larger size. Conversely, Blue tits have been observed to have an advantage when competing for food during the breeding season [38]. Great tits typically lay 6 to 12 eggs in mid-April, with hatching commencing 13 to 15 days later. The young are fed for a further 18 to 21 days in the nest until they fledge. Blue tits lay slightly more eggs (7 to 13) with similar timing [39]. The hatching success rate varies between 82% and 98% for both species. Breeding success fluctuates from year to year due to several environmental factors but declines

during the course of a breeding season [40]. Second broods are a common occurrence in both species, with third broods also occasionally observed [41]. During the breeding season, caterpillars play a significant role in providing nutrition for the young. Additionally, spiders and other larvae and imagines of insects are fed [41].

The samples were collected between 27 April and 30 May, with a notable peak in the second May decade. Samples were collected during the process of ringing by placing the young on chlorine-free bleached paper bags. The sampling and ringing took under 2 minutes per nest depending on the number of fledglings, after which they were all immediately returned to their nest. All faecal samples from a single brood were collected in one sample tube without physical contact and were immediately cooled. Although pesticide residues persist for at least 30 days in weathered bird droppings, the samples were placed in a cool bag to protect them from heat and direct sunlight [42]. The samples were frozen as soon as possible at -20 degrees Celsius until laboratory analysis.

Several factors potentially influencing the results were recorded: weight of the sample, time of sampling (day of the year and year) and location of the nest box. The distance to the next fields was measured for each nest box, while the land use within a defined radius of 50 metres was also determined using the Corine Land Cover dataset (resolution of 10 metres per grid cell). Every cell is assigned to one of eleven land cover classes, seven of which were present in the study area (sealed, woody needle-leaved trees, woody broadleaved deciduous trees, low-growing woody, permanent herbaceous, periodically herbaceous, non- and sparsely vegetated) [43]. QGIS was used to determine the percentage of each land cover class within the radius (50 m) of each nest box. [44]. The radius of 50 m represents the most frequented foraging habitat [45]. It is probable that Blue and Great tits will forage beyond this radius, although this is dependent on food supply and therefore habitat structure. The likelihood of a bird foraging during the breeding season decreases with distance from the nest box [45]. Therefore, areas further away should not be treated in the same manner as the immediate vicinity of the box. In addition, the closest distance from the nest box to the nearest field was measured using QGIS.

Substance analysis

The Julius Kühn Institute for Ecological Chemistry, Plant Analysis and Stock Protection in Berlin prepared and analysed the samples. The faecal samples were freeze-dried and weighed upon transfer to extraction stubs. Sample preparation followed the modified QuEChERS method based on DIN EN 15,662 [46] (Table S2). An internal standard was added to the samples, which were then mixed with cooled solvents and left to cool overnight. The next day, the QuEChERS salt was added and vigorously shaken. The sample was then centrifuged, and a portion of the supernatant was purified using dispersive solid phase extraction (dSPE). Subsequently, an analysis was conducted utilising LC QTRAP-MS/MS [47]. Quality assurance and control samples were also analysed. A total of 108 substances were analysed. These consist of all substances that are particularly toxic and environmentally harmful, such as the herbicides diflufenican, nicosulfuron, and terbuthylazine, as well as other substances included in the national action plan for sustainable use [48]. These included several substances classified as fungicides, herbicides or insecticides, as well as caffeine. Concentrations are expressed in ng/g of the sample. Substances' limit of detection (LOD) range between 0.001 and 0.081 ng/g. Limits of quantification (LOQ) range between 0.0001 and 4.115 ng/g.

Statistical analysis

The statistical analysis was performed with R [49]. Firstly differences between the two species were analysed using the Bray-Curtis dissimilarity index of the package vegan [50]. Although the differences were significant, the observed R^2 values were minimal. To establish a more robust foundation for subsequent analysis, the two species were therefore considered collectively.

The substances were assigned to the pesticide categories (fungicides/herbicides/insecticides) according to their field of application. As caffeine is not a pesticide, but a contaminant linked to anthropogenic influence, it was analysed separately.

We analysed the differences between the areas by boxplots dividing the data per area and the pesticide categories. Kruskal-Wallis tests were performed for the different pesticide categories, post-hoc tests with the Holm method provided further insights.

To further investigate the causal relationships and effects of pesticides on Blue and Great tits, we performed a series of generalised linear mixed-effects models (GLMMs) using the glmmTMB package [44]. All models were considered with the concentration of pesticides per nestbox by pesticide category and the sum of all pesticides (herbicides + fungicides + insecticides) as well as presence/absence of pesticides. Given that the samples were taken in different areas and years, they were incorporated as a random effect. The concentrations were determined by setting the zero values to LOD/2 and the values between LOD and LOQ to (LOD + LOQ)/2. This made the analysis more accurate and robust. In addition to the day of the year, the analysed independent variables included sample weight, distance to the nearest field, and the area of all land cover classes found within 50 m of the nest boxes. As the distance to the next field showed an influence around the threshold ($p=0.05$), we used boxplots to gain a clearer picture. This was tested again using a Kruskal–Wallis test and a post-hoc test with the Holm method.

Moreover, an analysis was conducted to ascertain the effect of pesticide contamination (concentrations and presence/absence) on the total number of young birds, the number of unfertilised eggs, and the number of dead juveniles. In all models the best model fit was chosen using the Akaike Information Criterion ($AIC \leq 2$). All graphical statistic elements were generated using ggplot2 with the help of ggeffects [52,53]. The full details of all GLMMs are provided in the appendix.

3. Results

3.1. Residues in Blue and Great tits

In total, 168 samples (Blue tit = 73, Great tit = 95) from the three areas (JKI = 43, WL = 57, ZDM = 68) contained 65 substances (39 herbicides, 17 fungicides, 8 insecticides and caffeine) of 57 pesticides and 8 corresponding metabolites. Significant differences in residues were found between the two species ($F = 6.09$, $R^2 = 0.035$, $p = 0.007$, Bray-Curtis test). There were differences in occurrence of pesticide substances between the three areas (Table S3). In JKI, 45 substances were identified in 14 samples ($44 > LOQ$). In WL, 16 substances were detected in nine samples, while in ZDM, 29 substances were identified in four samples. Overall, pesticide substances were identified in 16.07% of the samples. Most of the loads could be quantified, except for two values of the fungicide fenpropimorph, and one for the herbicides chlorotoluron and diflufenican respectively, as the concentrations were below the limit of quantification (LOQ) (Table S3). The concentrations reached a maximum in dimefuron with 208.92 ng/g for herbicides, in thiacloprid-amid with 206.46 ng/g for insecticides and in dimethomorph with 194.92 ng/g for fungicides (Table 1, Table S4).

Caffeine, which was identified in 4.76% of the samples, reached a maximum of 1287.70 ng/g. This would represent 1.29 mg/kg body weight, however most of the pesticide values found do not exceed 0.2 mg/kg body weight of a bird (Table S4).

Table 1. Concentrations of detected pesticides and their metabolites, in more than 2% of the faecal sac samples from Great and Blue tits (see Table S4 for findings less than 2%), represented by detection frequency per species (%), as well as median and maximum (all in ng/g) of the concentrations found.

substance	Blue tit (n=73)			Great tit (n=95)		
	det. Freq. (%)	median (ng/g)	max (ng/g)	det. Freq (%)	median (ng/g)	max (ng/g)
Caffeine	6.85	65.71	152.45	3.16	19.41	1287.70
Carbendazim, fungicide	5.48	5.48	9.63	8.42	6.07	15.02
Chlorantraniliprole, insecticide	4.11	19.60	21.69	7.37	11.11	44.72
Chloridazon, herbicide	1.37	1.89	1.89	6.32	12.03	31.93
Diflufenican, herbicide	4.11	8.76	14.18	5.26	2.46	89.85

Dimefuron, herbicide	5.48	12.46	25.61	10.53	5.80	208.92
Diuron, herbicide	4.11	8.04	17.14	4.21	4.49	207.23
Fenuron, herbicide	2.74	13.43	20.92	5.26	4.20	11.33
Fluopicolide, fungicide	5.48	11.17	36.38	6.32	2.65	135.70
Flupyrsulfuron-methyl, herbicide	4.11	35.34	74.18	7.37	30.86	92.36
Foramsulfuron, herbicide	4.11	1.96	31.67	1.05	5.49	5.49
Imidacloprid, insecticide	1.37	3.04	3.04	3.16	1.42	18.11
Methiocarb, insecticide	4.11	55.19	111.53	7.37	27.20	111.19
Prosulfuron, herbicide	1.37	1.94	1.94	6.32	4.53	171.08
Terbutylazine-2-hydroxy-desethyl, herbicide	2.74	5.66	6.22	3.16	12.45	140.46
Terbutylazine-desethyl, herbicide	4.11	7.60	31.04	5.26	9.11	134.92
Thiacloprid, insecticide	2.74	18.27	26.53	6.32	10.55	36.22
Thiacloprid-Amid, insecticide	4.11	16.43	40.90	8.42	5.02	206.46
Zoxamide, fungicide	4.11	6.84	7.88	6.32	6.90	14.08

3.2. Influencing factors

The concentrations of fungicides in contaminated samples (median: 13.88 (JKI) vs. 34.17 (WL) vs. 380.81 (ZDM) ng/g), herbicides (79.21 vs. 54.26 vs. 1310.84 ng/g) and insecticides (66.69 vs. 40.9 vs. 162.14 ng/g) exhibited no significant differences (Kruskal-Wallis test) between the sites (Figure 2). Insecticides were almost all detected in JKI, where caffeine (0 vs. 45.95 vs. 85.93 ng/g) was not detected at all. There were no differences in sample weight, but there were differences in sampling day between the areas ($H = 8.53$, $df = 2$, $p < 0.05$, Kruskal-Wallis test), which were significantly earlier in JKI (mean: 13 May) than in ZDM (17 May, $Z = -2.88$, $p < 0.05$, post-hoc test), though not WL (14 May).

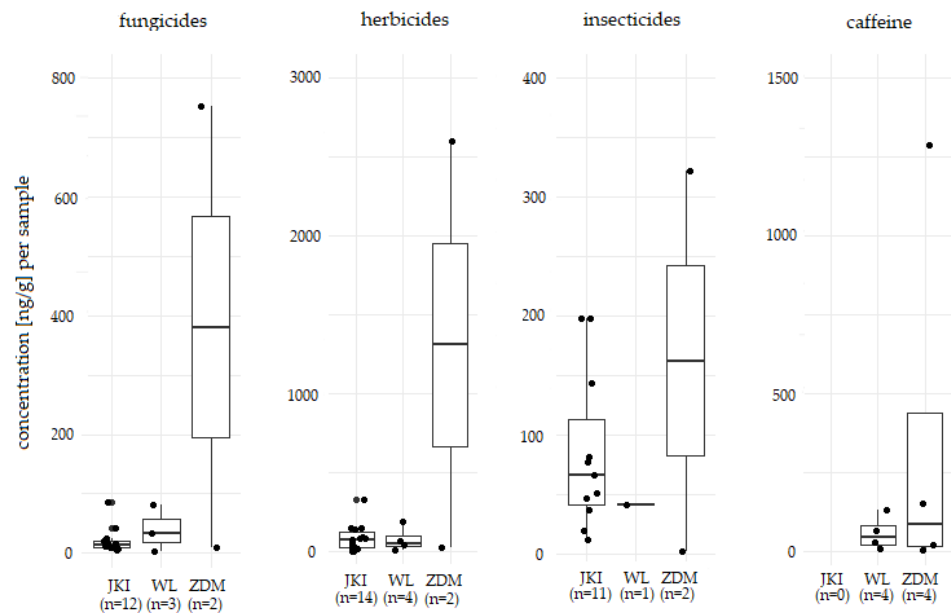


Figure 2. Boxplots showing the concentrations of different substances (fungicides, herbicides, insecticides, and caffeine) in contaminated samples across the three study areas.

The total concentration of fungicides, herbicides, and insecticides was found to be significantly negatively affected by the day of the year ($p < 0.05$) and sample weight ($p < 0.001$) (Figure 3).

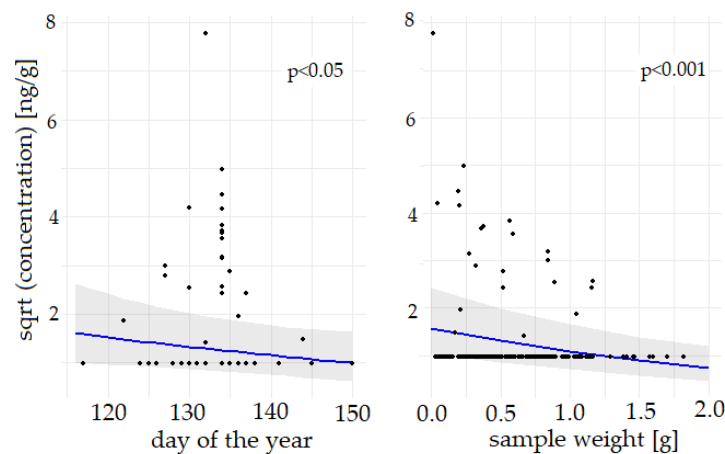


Figure 3. Effect plots of the generalised linear mixed model (GLMMs) showing the relationships between the day of the year and the sample weight with the total concentration of fungicides (f), herbicides (h), and insecticides (i) for each sample. The regression line is presented with 95% confidence intervals, which account for the random effects of year and area (Table S6).

The fledglings contaminated with fungicides were raised in nest boxes significantly closer to the next field than the uncontaminated ones (median: 71.3 vs. 97.7, $W = 1864$, $p < 0.05$, Wilcoxon test). The same could be observed for insecticides (60.4 vs. 97.6, $W = 1465$, $p < 0.05$). However, caffeine (136.0 vs. 90.0, $W = 477$, $p = 0.23$) and herbicide contamination (73.5 vs. 98.4, $W = 1847$, $p = 0.07$) showed no distinct relation with the distance to the nearest field (Figure 4).

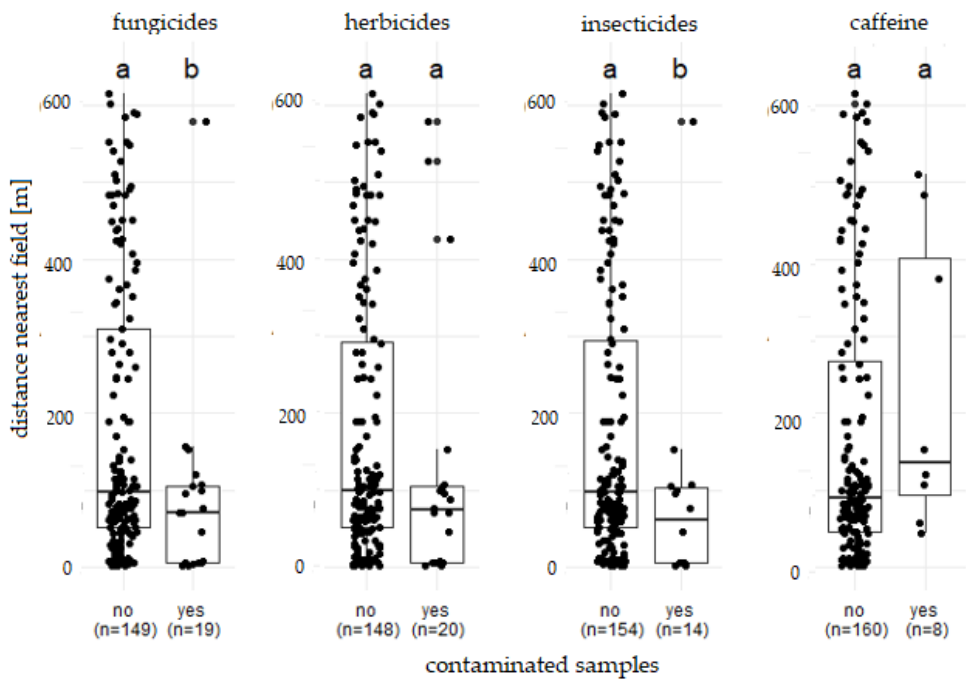


Figure 4. Boxplots showing the distance to the nearest field for all samples, divided into contaminated (yes) and uncontaminated (no) categories for different substances: fungicides, herbicides, insecticides, and caffeine.

The exposure of fledglings to pesticides did not result in a significant change in the number of fledglings, the number of unfertilised eggs or the number of dead juveniles in the nests (Table S6).

4. Discussion

In total, 65 substances from 57 pesticides were detected, with significant differences between the two species. Caffeine exhibited an appearance pattern that was distinct from the other substances with residues found in eight broods up to 1287.7 ng/g. There was no significant influence of landscape composition on pesticide contamination. The most influential factors on pesticide contamination were sample size and the distance to the nearest field. We found no effects of the pesticide residues on the broods' performance.

4.1. Residues in Blue and Great tits

Several substances were identified which present a significant toxicological risk to birds. These include the banned herbicide prosulfocarb [2], persistent neonicotinoids such as thiamethoxam, thiacloprid [3] and imidacloprid, which showed adverse effects in Northern Bobwhites (*Colinus virginianus*) such as eggshell thinning and bodyweight reduction [54]. Neonicotinoids are also known to reduce feeding and accumulation of body mass and fat stores [55]. Other persistent substances, including the herbicides dimefuron and flupyr-sulfuron-methyl, were found in elevated concentrations that have been prohibited for some time.

The concentrations identified in this study do not exceed the established limits for the substances, including the acute oral LD50 (Lewis et al., 2016). However, the specified limits are provided for non-songbirds. Consequently their interpretation is not-target-oriented, due to the variability in toxicity among bird species, depending on factors such as body size, feeding habits, and the activity of metabolic enzymes [15]. Due to the mixed samples, it is possible that some of the individual contaminations of juveniles may be considerably higher. The concentrations obtained are merely a proxy for the intake as substances can be absorbed and distributed to tissues [57]. Furthermore, it should be noted that the faecal samples collected may not provide a comprehensive representation of the entire development period from birth to maturity, as they are only collected at specific points in time. Among other factors, the nutrition of the fledglings is dependent on the habitat structure, brood timing and nestling age, resulting in a variable individual nutrition profile for each brood [58–60]. Consequently, it can be concluded that the concentrations of pesticides obtained are merely a snapshot of the total load to which the young tits are exposed.

Caffeine serves as an urban tracer and increases in areas with a higher human density, which consequently can lead to a higher consumption rate by birds [61]. Its adverse effects include oxidative stress, metabolic disruption, and reproductive impacts [62]. Although the focus of caffeine research is primarily on wastewater and marine coastal systems, the impact on terrestrial animals should not be overlooked [62]. Approximately 35% of reported environmental concentrations exceed predicted no-effect levels [61]. Small concentrations (<50 mg/kg) of caffeine seem to increase growth in chicken (*Gallus domesticus*) [63], but also cause pulmonary hypertension syndrome [64]. At 366 mg/kg [63], the LD50 for feral pigeons (*Columba livia domestica*) is relatively low compared to pesticides that are still in use [65].

4.2. Influencing factors

The areas analysed do not differ sufficiently to draw definitive conclusions about the origin and entry routes of the different pesticides. It is noteworthy that the JKI, which has the most substantial evidence of herbicides, fungicides and insecticides, is the only area with a proportion of periodically herbaceous areas in the vicinity of the nest boxes. However, in view of the wider suburban environment of the area, other routes of entry for pesticides are also possible [4]. The resolution of the employed landscape classification is low (10 m grid) when analysing a 50 metre radius around the nest boxes. Higher resolution products may offer enhanced capacity to account for actual fine-scale landscape composition, thereby more effectively highlighting differences. The landscape classes utilised in this study were predetermined by the Corine Land Cover classification system. The creation of classes based on the landscape present in the study area has the potential to enhance the

precision through more sensible classification. The employment of drones in conjunction with machine learning landscape classification is a promising methodology for conducting fine-scale landscape analysis [66,67].

Lower concentrations were found in heavier samples. The deposition of pesticides may be analogous to metals, which exhibit concentrations that vary between the urate and faeces parts of faecal sacs [24]. Naturally, every faecal sac is different in size, but also in urate and faeces proportion with their differing loads. This would mean that the proportions of urate and faeces, as well as their individual contamination profiles, would have to be quantified in each faecal sample by laboratory analysis. Only then would it be possible to compare different samples.

Concentrations were found to be higher the earlier the samples were taken. The young birds must have ingested the pesticides. Most likely, this occurred through the insects they were fed. Temporal variability can possibly be explained by differences in insect exposure or in the locations where the insects were caught [9]. Great tits tend to collect more insects from the ground during the early stages of the breeding season. At this time, they may come into more frequent contact with pesticide-contaminated insects than in later stages, when they mostly forage amongst vegetation [68].

The agricultural land surrounding Münster is primarily used for the cultivation of cereal crops, maize, and potatoes [69]. Most of the substances found can be traced back to agricultural activities, especially to those frequently cultivated crops. The significant correlation between the substances with the distance to the next field suggests that a substantial proportion of the pesticides identified originate from agricultural activities. Numerous other studies have already linked contamination of birds with agricultural pesticides [8,17,18]. However, those species analysed are frequently representative of the typical avifauna of agricultural landscapes [35]. In contrast, Blue and Great tits only sporadically visit these structures for foraging purposes during the breeding season [70]. Naturally, they tend to follow food sources, demonstrating a preference for organic over conventional agricultural practices [70]. Nevertheless, it can be reasonably assumed that breeding that takes place near arable land (as in JKI) will result in the birds foraging in or near the field with greater frequency. Consequently, these birds will be more susceptible to contamination by pesticides. Broods that are situated at a greater distance from the field may, for instance, become contaminated by pesticide-laden insects that carry the substances from the field into the surrounding area [9].

However, other entry routes might be possible, for example the materials utilised in the construction of the nest have the potential to introduce pesticides into the nidobiome. Insecticides were found in all Blue and Great tit nests in a study from the UK [71]. For instance, imidacloprid, thiacloprid and thiamethoxam are brought into the nest with fur from companion animals used for the nest construction [71]. Consequently, juvenile birds in the nest are directly exposed to these substances.

5. Conclusions

The sampling of faecal sacs from juvenile birds represents a straightforward and non-invasive approach to pesticide monitoring, offering opportunities to analyse the influence of the surrounding environment. Our findings highlight the widespread presence of pesticides in the food web. We found pesticides in all areas with a diverse range of land uses, highlighting the widespread exposure of birds to these substances.

Future studies should consider the number of faeces sacs and differentiate between urate and faeces to eliminate sample weight dependency. The selection of monotypic study areas with distinct habitat types is crucial to better identify entry pathways of contaminants. Additionally, close brood monitoring together with the consideration of a broad set of ecological variables is essential to assess potential impacts on nestling development and survival. Furthermore, future research should investigate possible entry routes of caffeine into the environment, as its presence in faecal samples raises questions about its sources and ecological implications.

Supplementary Materials: The following data and supporting information can be downloaded at the website of this paper posted on Preprints.org., Table S1: mean land use; Table S2: analytic procedure; Table S3: substances detected; Table S4: substances per species; Table S5: application, load etc.; Table S6: models.

Author Contributions: Conceptualisation, M.M. and A.E.; methodology, M.M. and A.E.; formal analysis, M.M.; investigation, M.M., J.A. and S.F.; resources, K.F.; data curation, M.M.; writing—original draft preparation, M.M.; writing—review and editing, A.E., S.B., K.F. and J.A.; supervision, A.E. and S.B. All authors have read and agreed to the published version of the manuscript.

Data availability statement: Data are available as Supplementary Materials and from corresponding authors.

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Conflicts of interest: The authors declare no conflicts of interest.

References

1. Díaz, S.; Settele, J.; Brondízio, E.S.; Ngo, H.T.; Agard, J.; Arneth, A.; Balvanera, P.; Brauman, K.A.; Butchart, S.H.M.; Chan, K.M.A.; et al. Pervasive Human-Driven Decline of Life on Earth Points to the Need for Transformative Change. *Science* **2019**, *366*, eaax3100, doi:10.1126/science.aax3100.
2. Rillig, M.C.; Ryo, M.; Lehmann, A. Classifying Human Influences on Terrestrial Ecosystems. *Glob. Change Biol.* **2021**, *27*, 2273–2278, doi:10.1111/gcb.15577.
3. Alavanja, M.C.R. Introduction: Pesticides Use and Exposure, Extensive Worldwide. *Rev. Environ. Health* **2009**, *24*, doi:10.1515/REVEH.2009.24.4.303.
4. Md Meftaul, I.; Venkateswarlu, K.; Dharmarajan, R.; Annamalai, P.; Megharaj, M. Pesticides in the Urban Environment: A Potential Threat That Knocks at the Door. *Sci. Total Environ.* **2020**, *711*, 134612, doi:10.1016/j.scitotenv.2019.134612.
5. Kruse-Platz, M.; Hofmann, F.; Wosniok, W.; Schlechtriemen, U.; Kohlschütter, N. Pesticides and Pesticide-Related Products in Ambient Air in Germany. *Environ. Sci. Eur.* **2021**, *33*, 114, doi:10.1186/s12302-021-00553-4.
6. Mojiri, A.; Zhou, J.L.; Robinson, B.; Ohashi, A.; Ozaki, N.; Kindaichi, T.; Farraji, H.; Vakili, M. Pesticides in Aquatic Environments and Their Removal by Adsorption Methods. *Chemosphere* **2020**, *253*, 126646, doi:10.1016/j.chemosphere.2020.126646.
7. Silva, V.; Gai, L.; Harkes, P.; Tan, G.; Ritsema, C.J.; Alcon, F.; Contreras, J.; Abrantes, N.; Campos, I.; Baldi, I.; et al. Pesticide Residues with Hazard Classifications Relevant to Non-Target Species Including Humans Are Omnipresent in the Environment and Farmer Residences. *Environ. Int.* **2023**, *181*, 108280, doi:10.1016/j.envint.2023.108280.
8. Tassin De Montaigu, C.; Goulson, D. Identifying Agricultural Pesticides That May Pose a Risk for Birds. *PeerJ* **2020**, *8*, e9526, doi:10.7717/peerj.9526.
9. Brühl, C.A.; Bakanov, N.; Köthe, S.; Eichler, L.; Sorg, M.; Hörren, T.; Mühlethaler, R.; Meinel, G.; Lehmann, G.U.C. Direct Pesticide Exposure of Insects in Nature Conservation Areas in Germany. *Sci. Rep.* **2021**, *11*, 24144, doi:10.1038/s41598-021-03366-w.
10. Hallmann, C.A.; Foppen, R.P.B.; Van Turnhout, C.A.M.; De Kroon, H.; Jongejans, E. Declines in Insectivorous Birds Are Associated with High Neonicotinoid Concentrations. *Nature* **2014**, *511*, 341–343, doi:10.1038/nature13531.
11. Rigal, S.; Dakos, V.; Alonso, H.; Auniņš, A.; Benkő, Z.; Brotons, L.; Chodkiewicz, T.; Chylarecki, P.; De Carli, E.; Del Moral, J.C.; et al. Farmland Practices Are Driving Bird Population Decline across Europe. *Proc. Natl. Acad. Sci.* **2023**, *120*, e2216573120, doi:10.1073/pnas.2216573120.

12. Møller, A.P. Parallel Declines in Abundance of Insects and Insectivorous Birds in Denmark over 22 Years. *Ecol. Evol.* **2019**, *9*, 6581–6587, doi:10.1002/ece3.5236.
13. Whelan, C.J.; Wenny, D.G.; Marquis, R.J. Ecosystem Services Provided by Birds. *Ann. N. Y. Acad. Sci.* **2008**, *1134*, 25–60, doi:10.1196/annals.1439.003.
14. Smits, J.E.G.; Fernie, K.J. Avian Wildlife as Sentinels of Ecosystem Health. *Comp. Immunol. Microbiol. Infect. Dis.* **2013**, *36*, 333–342, doi:10.1016/j.cimid.2012.11.007.
15. Katagi, T.; Fujisawa, T. Acute Toxicity and Metabolism of Pesticides in Birds. *J. Pestic. Sci.* **2021**, *46*, 305–321, doi:10.1584/jpestics.D21-028.
16. Badry, A.; Schenke, D.; Treu, G.; Krone, O. Linking Landscape Composition and Biological Factors with Exposure Levels of Rodenticides and Agrochemicals in Avian Apex Predators from Germany. *Environ. Res.* **2021**, *193*, 110602, doi:10.1016/j.envres.2020.110602.
17. Fuentes, E.; Gaffard, A.; Rodrigues, A.; Millet, M.; Bretagnolle, V.; Moreau, J.; Monceau, K. Neonicotinoids: Still Present in Farmland Birds despite Their Ban. *Chemosphere* **2023**, *321*, 138091, doi:10.1016/j.chemosphere.2023.138091.
18. Angelier, F.; Prouteau, L.; Brischoux, F.; Chastel, O.; Devier, M.-H.; Le Menach, K.; Martin, S.; Mohring, B.; Pardon, P.; Budzinski, H. High Contamination of a Sentinel Vertebrate Species by Azoles in Vineyards: A Study of Common Blackbirds (*Turdus Merula*) in Multiple Habitats in Western France. *Environ. Pollut.* **2023**, *316*, 120655, doi:10.1016/j.envpol.2022.120655.
19. Esther, A.; Schenke, D.; Heim, W. Noninvasively Collected Fecal Samples as Indicators of Multiple Pesticide Exposure in Wild Birds. *Environ. Toxicol. Chem.* **2022**, *41*, 201–207, doi:10.1002/etc.5260.
20. Crick, H.Q.P.; Baillie, S.R.; Leech, D.I. The UK Nest Record Scheme: Its Value for Science and Conservation. *Bird Study* **2003**, *50*, 254–270, doi:10.1080/00063650309461318.
21. Campos-Cerda, F.; Bohannan, B.J.M. The Nidobiome: A Framework for Understanding Microbiome Assembly in Neonates. *Trends Ecol. Evol.* **2020**, *35*, 573–582, doi:10.1016/j.tree.2020.03.007.
22. Wilman, H.; Belmaker, J.; Simpson, J.; De La Rosa, C.; Rivadeneira, M.M.; Jetz, W. EltonTraits 1.0: Species-level Foraging Attributes of the World's Birds and Mammals: Ecological Archives E095-178. *Ecology* **2014**, *95*, 2027–2027, doi:10.1890/13-1917.1.
23. Ibáñez-Álamo, J.D.; Rubio, E.; Soler, J.J. Evolution of Nestling Faeces Removal in Avian Phylogeny. *Anim. Behav.* **2017**, *124*, 1–5, doi:10.1016/j.anbehav.2016.11.033.
24. Eeva, T.; Raivikko, N.; Espín, S.; Sánchez-Virosta, P.; Ruuskanen, S.; Sorvari, J.; Rainio, M. Bird Feces as Indicators of Metal Pollution: Pitfalls and Solutions. *Toxics* **2020**, *8*, 124, doi:10.3390/toxics8040124.
25. Santema, P.; Kempnaers, B. Complete Brood Failure in an Altricial Bird Is Almost Always Associated with the Sudden and Permanent Disappearance of a Parent. *J. Anim. Ecol.* **2018**, *87*, 1239–1250, doi:10.1111/1365-2656.12848.
26. Richard, F.-J.; Southern, I.; Gigauri, M.; Bellini, G.; Rojas, O.; Runde, A. Warning on Nine Pollutants and Their Effects on Avian Communities. *Glob. Ecol. Conserv.* **2021**, *32*, e01898, doi:10.1016/j.gecco.2021.e01898.
27. Jiménez-Peñuela, J.; Santamaría-Cervantes, C.; Fernández-Vizcaíno, E.; Mateo, R.; Ortiz-Santaliestra, M.E. Integrating Adverse Effects of Triazole Fungicides on Reproduction and Physiology of Farmland Birds. *J. Avian Biol.* **2024**, e03313, doi:10.1111/jav.03313.
28. Marques-Santos, F.; Dingemanse, N.J. Weather Effects on Nestling Survival of Great Tits Vary According to the Developmental Stage. *J. Avian Biol.* **2020**, *51*, jav.02421, doi:10.1111/jav.02421.
29. Rodríguez, S.; Van Noordwijk, A.J.; Álvarez, E.; Barba, E. A Recipe for Postfledging Survival in Great Tits *Parus Major*: Be Large and Be Early (but Not Too Much). *Ecol. Evol.* **2016**, *6*, 4458–4467, doi:10.1002/ece3.2192.
30. *European Breeding Bird Atlas*; European bird census council, Ed.; Lynx Edicions: Barcelona, 2020; ISBN 978-84-16728-38-1.
31. Grüneberg, C.; Nordrhein-Westfälische Ornithologengesellschaft *Die Brutvögel Nordrhein-Westfalens*; 1. unveränd. Nachdruck.; LWL-Museum für Naturkunde: Münster, 2014; ISBN 978-3-940726-24-7.
32. Odderskær, P.; Sell, H. Survival of Great Tit (*Parus Major*) Nestlings in Hedgerows Exposed to a Fungicide and an Insecticide: A Field Experiment. *Agric. Ecosyst. Environ.* **1993**, *45*, 181–193, doi:10.1016/0167-8809(93)90069-2.

33. Pascual, J.A.; Peris, S.J. Effects of Forest Spraying with Two Application Rates of Cypermethrin on Food Supply and on Breeding Success of the Blue Tit (*Parus Caeruleus*). *Environ. Toxicol. Chem.* **1992**, *11*, 1271–1280, doi:10.1002/etc.5620110907.
34. Pascual, J.A. No Effects of a Forest Spraying of Malathion on Breeding Blue Tits (*Parus Caeruleus*). *Environ. Toxicol. Chem.* **1994**, *13*, 1127–1131, doi:10.1002/etc.5620130713.
35. Bonneris, E.; Gao, Z.; Prosser, A.; Barfknecht, R. Selecting Appropriate Focal Species for Assessing the Risk to Birds from Newly Drilled Pesticide-treated Winter Cereal Fields in France. *Integr. Environ. Assess. Manag.* **2019**, *15*, 422–436, doi:10.1002/ieam.4112.
36. Lamonica, D.; Charvy, L.; Kuo, D.; Fritsch, C.; Coeurdassier, M.; Berny, P.; Charles, S. A Brief Review on Models for Birds Exposed to Chemicals. *Environ. Sci. Pollut. Res.* **2024**, doi:10.1007/s11356-024-34628-5.
37. Open Street Map contributors Map Data for Muenster 2025.
38. Minot, E.O.; Perrins, C.M. Interspecific Interference Competition--Nest Sites for Blue and Great Tits. *J. Anim. Ecol.* **1986**, *55*, 331, doi:10.2307/4712.
39. *Methodenstandards zur Erfassung der Brutvögel Deutschlands*; Südbeck, P., Ed.; DDA Verlag: Steckby, 2005; ISBN 978-3-00-015261-0.
40. Handbuch der Vögel Mitteleuropas. Teil 4,[1]: Passeriformes Muscicapidae—Paridae. In; Haffer, J., Glutz von Blotzheim, U.N., Niethammer, G., Eds.; Aula-Verl: Wiesbaden, 1993 ISBN 978-3-89104-022-5.
41. Südbeck, P.; Andretzke, H.; Fischer, S.; Gedeon, K.; Pertl, C.; Linke, T.J.; Georg, M.; König, C.; Schikore, T.; Schröder, K.; et al. *Methodenstandards zur Erfassung der Brutvögel Deutschlands*; 1. überarbeitete Auflage.; Eigenverlag DDA: Münster, 2025; ISBN 978-3-9819703-3-3.
42. Vyas, N.B.; Henry, P.F.P.; Binkowski, L.J.; Hladik, M.L.; Gross, M.S.; Schroeder, M.A.; Davis, D.M. Persistence of Pesticide Residues in Weathered Avian Droppings. *Environ. Res.* **2024**, *259*, 119475, doi:10.1016/j.envres.2024.119475.
43. European Environment Agency Corine Land Cover Change CLC+ Backbone 2018 (Raster 10 m), Europe, 3-Yearly, Feb. 2023 2023.
44. QGIS Development Team QGIS Geographic Information System, Version 3.34.6 2024.
45. Naef-Daenzer, B. Patch Time Allocation and Patch Sampling by Foraging Great and Blue Tits. *Anim. Behav.* **2000**, *59*, 989–999, doi:10.1006/anbe.1999.1380.
46. Comité Européen de Normalisation DIN EN 15662:2018-07, *Pflanzliche Lebensmittel_ Multiverfahren Zur Bestimmung von Pestizidrückständen Mit GC Und LC Nach Acetonitril-Extraktion/Verteilung Und Reinigung Mit Dispersiver SPE_ Modulares QuEChERS-Verfahren; Deutsche Fassung EN_15662:2018*; **2018**. DOI: 10.31030/2748453.
47. Trau, F.N.; Fisch, K.; Lorenz, S. Habitat Type Strongly Influences the Structural Benthic Invertebrate Community Composition in a Landscape Characterized by Ubiquitous, Long-Term Agricultural Stress. *Inland Waters* **2023**, *13*, 473–485, doi:10.1080/20442041.2023.2242084.
48. Umweltbundesamt *Umsetzung Des Nationalen Aktionsplans Zur Nachhaltigen Anwendung von Pestiziden Teil 2: Konzeption Eines Repräsentativen Monitorings Zur Belastung von Kleingewässern in Der Agrarlandschaft*; Dessau-Roßlau, 2019;
49. R Core Team R: A Language and Environment for Statistical Computing 2024.
50. Oksanen, J.; Simpson, G.L.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'Hara, R.B.; Solymos, P.; Stevens, M.H.H.; Szoecs, E.; et al. *Vegan: Community Ecology Package* 2001, 2.6-10.
51. Brooks, M., E.; Kristensen, K.; Benthem, K., J. ,van; Magnusson, A.; Berg, C., W.; Nielsen, A.; Skaug, H., J.; Mächler, M.; Bolker, B., M. glmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling. *R J.* **2017**, *9*, 378, doi:10.32614/RJ-2017-066.
52. Lüdtke, D. Ggeffects: Create Tidy Data Frames of Marginal Effects for “ggplot” from Model Outputs 2017, 2.0.0.
53. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Use R!; Second edition.; Springer international publishing: Cham, 2016; ISBN 978-3-319-24277-4.
54. Sabin, L.B.; Mora, M.A. Ecological Risk Assessment of the Effects of Neonicotinoid Insecticides on Northern Bobwhites (*Colinus Virginianus*) in the South Texas Plains Ecoregion. *Integr. Environ. Assess. Manag.* **2021**, *18*, 488–499, doi:10.1002/ieam.4479.

55. Eng, M.L.; Stutchbury, B.J.M.; Morrissey, C.A. A Neonicotinoid Insecticide Reduces Fueling and Delays Migration in Songbirds. *Science* **2019**, *365*, 1177–1180, doi:10.1126/science.aaw9419.
56. Lewis, K.A.; Tzilivakis, J.; Warner, D.J.; Green, A. An International Database for Pesticide Risk Assessments and Management. *Hum. Ecol. Risk Assess. Int. J.* **2016**, *22*, 1050–1064, doi:10.1080/10807039.2015.1133242.
57. Gross, M.S.; Bean, T.G.; Hladik, M.L.; Rattner, B.A.; Kuivila, K.M. Uptake, Metabolism, and Elimination of Fungicides from Coated Wheat Seeds in Japanese Quail (*Coturnix Japonica*). *J. Agric. Food Chem.* **2020**, *68*, 1514–1524, doi:10.1021/acs.jafc.9b05668.
58. Arnold, K.E.; Ramsay, S.L.; Henderson, L.; Larcombe, S.D. Seasonal Variation in Diet Quality: Antioxidants, Invertebrates and Blue Tits *Cyanistes Caeruleus*: ANTIOXIDANTS IN INVERTEBRATES AND CHICKS. *Biol. J. Linn. Soc.* **2010**, *99*, 708–717, doi:10.1111/j.1095-8312.2010.01377.x.
59. García-Navas, V.; Ferrer, E.S.; Sanz, J.J. Prey Selectivity and Parental Feeding Rates of Blue Tits *Cyanistes Caeruleus* in Relation to Nestling Age. *Bird Study* **2012**, *59*, 236–242, doi:10.1080/00063657.2012.662939.
60. Serrano-Davies, E.; Sanz, J.J. Habitat Structure Modulates Nestling Diet Composition and Fitness of Blue Tits *Cyanistes Caeruleus* in the Mediterranean Region. *Bird Study* **2017**, *64*, 295–305, doi:10.1080/00063657.2017.1357678.
61. Quadra, G.R.; Paranaíba, J.R.; Vilas-Boas, J.; Roland, F.; Amado, A.M.; Barros, N.; Dias, R.J.P.; Cardoso, S.J. A Global Trend of Caffeine Consumption over Time and Related-Environmental Impacts. *Environ. Pollut.* **2020**, *256*, 113343, doi:10.1016/j.envpol.2019.113343.
62. Li, S.; He, B.; Wang, J.; Liu, J.; Hu, X. Risks of Caffeine Residues in the Environment: Necessity for a Targeted Ecopharmacovigilance Program. *Chemosphere* **2020**, *243*, 125343, doi:10.1016/j.chemosphere.2019.125343.
63. Kamely, M.; Karimi Torshizi, M.A.; West, J.; Niewold, T. Impacts of Caffeine on Resistant Chicken's Performance and Cardiovascular Gene Expression. *J. Anim. Physiol. Anim. Nutr.* **2022**, *106*, 566–574, doi:10.1111/jpn.13608.
64. Kamely, M.; Torshizi, M.A.K.; Rahimi, S.; Wideman, R.F. Caffeine Causes Pulmonary Hypertension Syndrome (Ascites) in Broilers. *J. Anim. Sci.* **2016**, *94*, 1493–1500, doi:10.2527/jas.2015-0152.
65. Sedghi, S.; Mosleh, N.; Shomali, T.; Dabiri, S.A.H. A Study on Acute Oral Caffeine Intoxication and Its Treatment Strategies in Domestic Pigeons *Columba Livia Domestica*). *Vet. Res. Forum* **2022**, *13*, doi:10.30466/vrf.2020.122469.2881.
66. Cruzan, M.B.; Weinstein, B.G.; Grasty, M.R.; Kohn, B.F.; Hendrickson, E.C.; Arredondo, T.M.; Thompson, P.G. Small Unmanned Aerial Vehicles (micro-UAVs, Drones) in Plant Ecology. *Appl. Plant Sci.* **2016**, *4*, 1600041, doi:10.3732/apps.1600041.
67. Gislason, P.O.; Benediktsson, J.A.; Sveinsson, J.R. Random Forests for Land Cover Classification. *Pattern Recognit. Lett.* **2006**, *27*, 294–300, doi:10.1016/j.patrec.2005.08.011.
68. Betts, M.M. The Food of Titmice in Oak Woodland. *J. Anim. Ecol.* **1955**, *24*, 282, doi:10.2307/1715.
69. Landesbetrieb Information und Technik Nordrhein Westfalen *Ackerland Der Landwirtschaftlichen Betriebe in NRW Nach Kulturarten 2022 Und 2023 (Ergebnisse Für Regierungsbezirke)*; Düsseldorf, 2023;
70. Bouvier, J.-C.; Delattre, T.; Boivin, T.; Musseau, R.; Thomas, C.; Lavigne, C. Great Tits Nesting in Apple Orchards Preferentially Forage in Organic but Not Conventional Orchards and in Hedgerows. *Agric. Ecosyst. Environ.* **2022**, *337*, 108074, doi:10.1016/j.agee.2022.108074.
71. Tassin De Montaigu, C.; Glauser, G.; Guinchard, S.; Goulson, D. High Prevalence of Veterinary Drugs in Bird's Nests. *Sci. Total Environ.* **2025**, *964*, 178439, doi:10.1016/j.scitotenv.2025.178439.

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