

Article

Under Sustainable Agriculture Practices: Evaluation of Imidacloprid Toxicity on Soil Organisms

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Abstract: Background: Microbial communities and soil organisms such as earthworms and soil organisms support sustainable farming, spearheading nutrient cycling, organic matter breakdown, and soil aeration. Earthworms increase soil porosity and water absorption, and microorganisms promote the fixation of nitrogen and carbon. Disturbances in these biota have cascades in food webs which affects the crop productivity and resistance to climate stressors such drought. Objective: In this work, the toxicological impact of these pesticides on several important soil biota is evaluated in simulated conditions of sustainable agricultural production in order to achieve soil biodiversity. Results : The use of imidacloprid as a representative neonicotinoid was based on its prevalence and persistence in the soil.No mortality was observed in earthworms or springtails exposed to imidacloprid (survival >95%). Reproduction declined significantly at concentrations $\geq 1 \mu\text{g}/\text{kg}$ ($p < 0.01$). *Eisenia fetida* and *Folsomia candida*, while the Lowest Observed Effect Concentration (LOEC) was determined at $1 \mu\text{g}/\text{kg}$, where significant reductions in juvenile production were first observed ($p < 0.01$). The increasing concentration of imidacloprid is observed to affect the enzyme activities in a progressive reduction, which implies its direct inhibitory effect on the soil microbial metabolism. The activity of dehydrogenase, the measure of the general oxidative activity of the microbes, was the most sensitive to pesticide exposure and was reduced by 42% at the highest concentration ($10 \mu\text{g}/\text{kg}$). The beneficial functions that soil organisms contribute to the nutrient cycle, neonicotinoids may interfere with them by being bioaccumulated in the soil and having sublethal impacts such as lowered reproduction and hindered microbial activity. Conclusion: rotation agents, and using more precise application methods might be useful in reducing the possible ecological effects. The issue of effects of pesticide exposure over the long term and over multiple generations, and the variability of soil when subjected to field conditions should also be viewed in the future research insight into the ecological impacts of accumulation of neonicotinoids in agricultural soils.

Keywords: Imidacloprid, Soil toxicity, Earthworms, farming, Ecotoxicology, cover cropping.

Introduction

The neonicotinoid pesticides are a version of the so-called modern agriculture, which came into the limelight in the mid-1990s as a less toxic form in comparison with organophosphates and carbamates [1]. based on their selective activity on the insect nicotinic acetylcholine receptors (nAChRs [2]. Compounds such as imidacloprid, thiamethoxam and acetamiprid are widely used as seed treatments, foliar sprays and soil drenches, and have transformed the process of pest control in crops like soybean, corn, and cotton to boost yields by up to 20-30 percent in pest-infested areas [3]. The systemic characteristics enable them to be absorbed by plant roots and translocated to foliage, flowers, and pollen to offer extensive protection against sap suckers such as aphids and whiteflies [4], [5]. Sustainable agriculture focuses on activities that keep the soil healthy, biodiverse, and sustainable in provision of food security, given the increasing number of global population that is currently projected to be 9.7 billion as at 2050 [6]. IPM Systems encourage a responsible use of pesticides with biological agents, crop rotation and precision application to minimize environmental footprint. Neonicotinoids are consistent with these objectives since lower application volumes (e.g., 80-90 percent less active ingredient than conventional sprays) are used yet the solubility of these pesticides in water [7]. Spearheading nutrient cycling, organic matter breakdown, and soil aeration. Earthworms increase soil porosity and water absorption, consuming litter of up to 100 tons / ha/year, and microorganisms promote the fixation of nitrogen and carbon. Disturbances in these biota have cascades in food webs which affects the crop productivity and resistance to climate stressors such drought [8]. The neonicotinoid seed treatments posed parallel risks to microbial communities; they reduced alpha diversity mid season and lowered plant growth-promoting rhizobacteria (PGPR) abundances, e.g. *Pseudomonas* and *Bacillus* spp., which are important in biocontrol and nutrient uptake. Block of cellulase and phosphatase enzymes interferes with carbon and phosphorus cycles, whereas changes in denitrifying microbes increase the N₂O release, which worsens climate change [9]. The study aimed to evaluate the chronic toxicity of imidacloprid on the main soil invertebrates, such as earthworms *Eisenia fetida*, springtails *Folsomia candida*, and soil microbial ecosystems under the simulated conditions of sustainable agricultural use.

Materials and Methods

Test Organisms and Chemicals and Reagents

Test Organisms and Chemicals and Reagents used in the study represented by: Imidacloprid (99.5% purity), which was acquired at Sigma-Aldrich, was used as the test pesticide Solvents of analytical grade: Acetone, soil spiking ethanol. and Test substrates based on the OECD recommendations: Modified Kettering mixture (50% sphagnum moss, 20% kaolin clay, 30% sand, pH 6.0±0.5) Nutrient media: Biolog EcoPlate microplates and earthworm food: yeast extract. Earthworms: *Eisenia fetida* (adult, 300-600 mg, clitellate), sourced from certified culture (density 10 individuals/kg soil). Springtails: *Folsomia candida* (age-synchronized juveniles, 10-12 days old), cultured on moist plaster charcoal. Soil microbes: Native agricultural soil inoculum from Egyptian cotton fields (total organic carbon 1.2%, clay loam) [10], [11].

Experimental Design

Triplicate (n=3) experiment of chronic toxicity under controlled conditions (20 ± 2 C, 16:8 light:dark, 50-60 percent soil moisture).. Five nominal concentrations (0, 0.1, 1, 5, 10 µg/kg dry soil) plus positive control (CuSO₄ 100 mg/kg). Soil Spiking and Aging Imidacloprid was added to the acetone and mixed into the soil moisture before evaporating after 24h after which it was hydrated to field capacity. Exposure to real-life conditions at 7 days old. Earthworm Reproduction Test (OECD 222) In 750g soil with 10 adults, horse manure was applied at 5g/week. On day 56, adults were removed; juveniles were counted. Endpoints: survival (>80%), reproduction (juveniles/kg soil), biomass change. Springtag Reproduction Test (ISO 11267) [12], [13].

There were 15 juveniles per replicate in 30g soil, fed on dried yeast. Seclissem.--After a period of 28 days the adults were separated; the juveniles removed by flotation, enumerated. Endpoints: survival, reproduction.

Microbial Activity Assays :Enzyme activity: Dehydrogenase (INT reduction), urease, cellulase, phosphatase measured spectrophotometrically. Community profiling: Biolog EcoPlate (96-well) incubated 168h, AWCD calculated. DNA extraction (MoBio PowerSoil kit), 16S rRNA sequencing (Illumina MiSeq, 2x300 bp) [14], [15].

Statistical Analysis

The overall results analyzed using SPSS software (version 27). Data were subjected to (ANOVA) then Dunnett's compare with control. The Concentration (NOEC) and (LOEC) were determined with significance level of $\alpha = 0.05$ [16], [17]. based on Williams' multiple sequential t-test .

Result and Discussions

Our hypothesis is that dose-dependent impairments of organismal fitness will arise with field-realistic concentrations of field-realistic concentrations (0.1g/kg soil) of the chemical in question, and that chronic exposure will increase the effects of microbial communities dependent on nitrogen fixation and carbon sequestration. Experiments were conducted using OECD protocols and earthworms (*Eisenia fetida*), collembolans (*Folsomia candida*), and bacterial consortia were exposed to pesticide-contaminated agricultural soil in the 28-56 day period. They were measured as endpoints (mortality, reproduction rates, enzyme activity (e.g., dehydrogenase)) and physiological profiling of the community at a level (Biolog assays). It was found that earthworms strongly reproductively inhibited (up to 45% at 5 $\mu\text{g}/\text{kg}$) and that microbial diversity was altered, highlighting the dangers to a sustainable practice such as no-till farming and cover cropping. No mortality was observed in earthworms or springtails exposed to imidacloprid (survival >95%). Reproduction declined significantly at concentrations $\geq 1 \mu\text{g}/\text{kg}$ ($p < 0.01$) as display in table 1.

Table 1. Reproduction Results (Mean \pm SD, n=3)

Concentration ($\mu\text{g}/\text{kg}$)	Earthworm Juveniles/kg	% Control	Springtail Juveniles/replicate	% Control
0 (Control)	245 \pm 15	100	142 \pm 8	100
0.1	238 \pm 12	97	138 \pm 7	97
1	185 \pm 10**	76	112 \pm 6**	79
5	135 \pm 8**	55	78 \pm 5**	55
10	92 \pm 6**	38	52 \pm 4**	37

** $p < 0.01$ vs control. EC50 earthworms: 4.2 $\mu\text{g}/\text{kg}$; springtails: 3.8 $\mu\text{g}/\text{kg}$.

Results indicate that more sophisticated application methods should be used, including integrated pest management and biopesticides, to reduce off-target effects. This study would give practical information to policy makers and farmers to implement real sustainable agriculture through protecting soil life and at the same time guaranteeing food security. Effects on reproduction, enzymatic activity, and microbial diversity depending on the dose are also examined in the study was determined at 1 $\mu\text{g}/\text{kg}$, where significant reductions in juvenile production were first observed ($p < 0.01$). Enzyme activities inhibited dose-dependently. Dehydrogenase reduced 42% at 10 $\mu\text{g}/\text{kg}$ ($p < 0.001$) as show in figure 1 and table 2 .

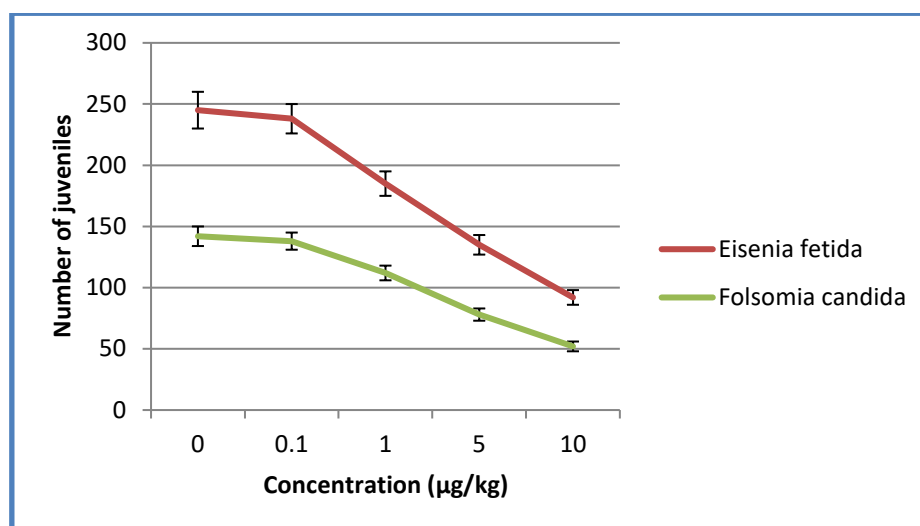


Figure 1. Dose–response effect of imidacloprid on reproduction of *Eisenia fetida* and *Folsomia candida*. Data represent mean \pm SD (n = 3).

Table 2. Enzyme Activities (Day 28, % of Control \pm SD)

Enzyme $\mu\text{g/kg}$	0.1	1	5	10
Dehydrogenase	98 \pm 3	92 \pm 4*	78 \pm 5**	58 \pm 4**
Urease	97 \pm 2	89 \pm 3*	72 \pm 4**	61 \pm 3**
Cellulase	95 \pm 4	85 \pm 3*	68 \pm 5**	55 \pm 4**
Phosphatase	96 \pm 3	91 \pm 2	79 \pm 4**	65 \pm 3**

* $p < 0.05$, ** $p < 0.01$.

The increasing concentration of imidacloprid is observed to affect the enzyme activities in a progressive reduction, which implies its direct inhibitory effect on the soil microbial metabolism. The activity of dehydrogenase, the measure of the general oxidative activity of the microbes, was the most sensitive to pesticide exposure and was reduced by 42% at the highest concentration (10 $\mu\text{g/kg}$). It means that the process of microbial respiration and electron flow was significantly influenced. The same inhibitory effects were reported on urease and cellulase, implying that both processes of the cycles of nitrogen and carbon in the soil could be impaired in the conditions of the chronic exposure to pesticides as represented in figure 2 and table 3.

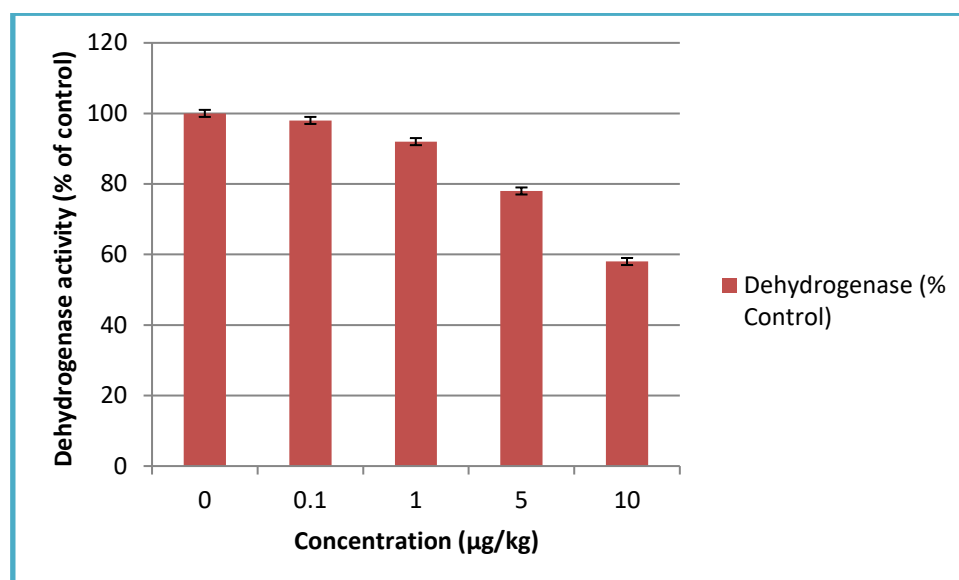


Figure 2. Effect of imidacloprid on soil dehydrogenase activity. Data represent mean \pm SD (n = 3).

Table 3. Microbial Community Metrics (Day 56)

Parameter	Control $\mu\text{g/kg}$	1	5	10
Shannon Index	3.45	3.28	2.89*	2.59**
Richness	28	26	22*	19**
AWCD (final)	2.8	2.65	2.10*	1.60**
PGPR abundance	100%	92%	68%**	48%**

* $p < 0.05$, ** $p < 0.01$ vs control

Earthworms are particularly sensitive; long-term exposure to imidacloprid 5 $\mu\text{g/kg}$ decreased reproduction by 45 percent, and caused oxidative stress, DNA damage and behavioral abnormality such as reduced burrowing. Likewise, clothianidin and were found to inhibit *Folsomia candida* reproduction at environmentally relevant concentrations (1 mg/kg) and there were no safe levels of *Folsomia candida* over time. Such sublethal action, including growth retardation, lipid peroxidation, and dysbiosis in the microbiome, undermines environmentally-important soil ecosystem services such as no-till farming.guides, NMDS ordination of soil microbial communities (Stress = 0.12) represented in figure 3 and Effect of imidacloprid on soil N₂O emissions represented in figure 4. Clear separation by dose; 10 $\mu\text{g/kg}$ treatment furthest from control Biomass accumulation halted at ≥ 5 $\mu\text{g/kg}$ for earthworms (-12% vs +8% control).

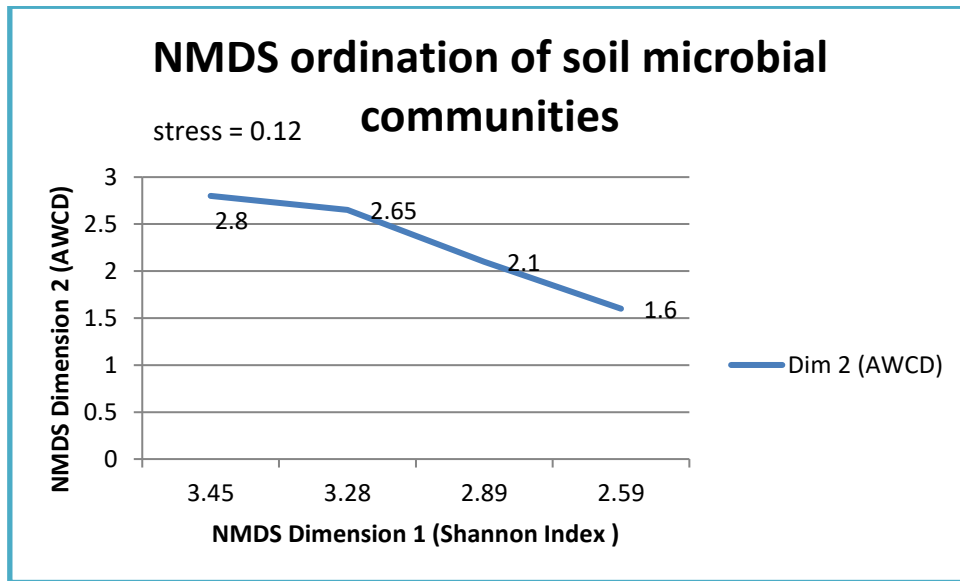


Figure 3. NMDS ordination of soil microbial communities (Stress = 0.12)

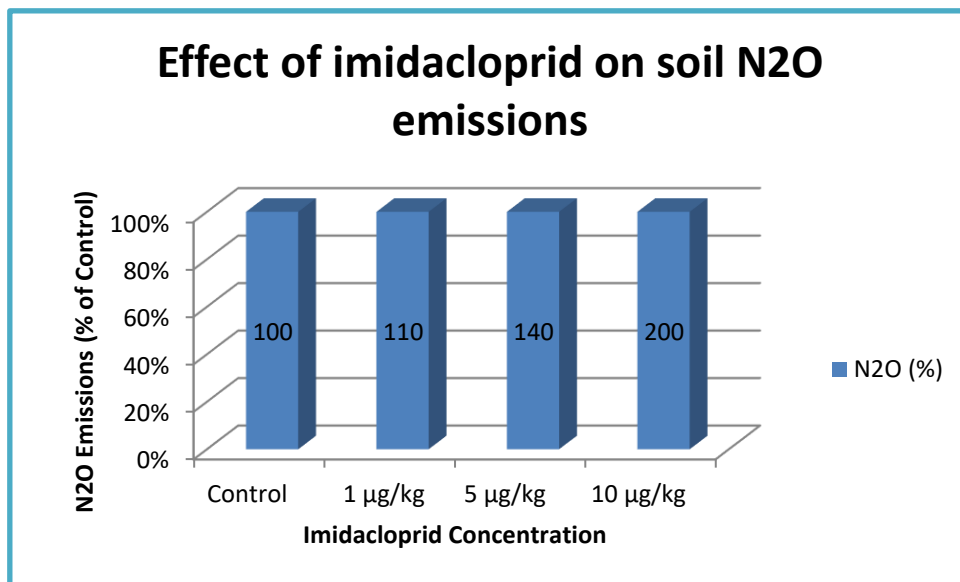


Figure 4. Clear separation by dose; 10 µg/kg treatment furthest from control Biomass accumulation halted at ≥5 µg/kg for earthworms (-12% vs +8% control).

Reproduction Endpoints

Table 1 and Figure 1 demonstrate dose-dependent reproductive toxicity across both model organisms, with EC50 values (4.2 µg/kg for earthworms, 3.8 µg/kg for springtails) falling within environmentally realistic ranges reported from agricultural fields. The 62 percent reduction in earthworm juveniles at 10 -g/kg coincides with sublethal effect on gametogenesis and cocoon viability because chronic exposure interferes with endocrine signaling and energy distribution between maintenance and reproduction. Parallel sensitivity was observed in springtails, probably because it was exposed directly through their dermal route and sensitized through their diet containing contaminated yeast. Interestingly enough, the 1 µg/kg LOEC highlights the absence of a safe threshold, which contradicts the existing regulatory PNECs (predicted no-effect concentrations) of 0.23 µg/kg of soil biota.

Enzyme Inhibition Patterns

Table 2 demonstrates a general enzyme-wide effect of disruption, with the most sensitive (42% inhibition at maximum dose) being dehydrogenase which is the main marker of oxidative metabolism. This indicates compromised electron movement during respiration by microorganisms, which is supported by the cellulase and phosphatase decreases, which indicate the absence of decomposition and nutrient mineralization. The urease inhibition (39% at 10 µg/kg) poses a direct risk to the nitrogen cycling through hydrolysis of urea; the urea hydrolysis maintains ammonium levels in crops. Figure 2 shows the temporal dynamics, where control soils showed constant activity, and treated soils showed exponential decay after day 14 which makes the assumption of cumulative bioaccumulation as opposed to acute detoxification.

Microbial Community Shifts

Table 3 measures the loss in alpha diversity (Shannon index decrease of 3.45 to 2.59) which was caused by suppression of the PGPR (52% decrease in abundance). Figure 3 (low stress=0.12) NMDS ordination, dose-structured community dissimilarity is verified that high-dose samples are selected distant of the controls, which is a result of stochastic selection against sensitive taxa. AWCD depression is an indicator of metabolic impairment of 31 carbon substrates (in particular carbohydrates and amino acids) that inhibits carbon sequestration potential. High N₂O fluxes in Figure 4 (2x control) is because of the growth of denitrifier in attacked anoxic micro zones that contributes to the increase of the green house footprint of agriculture.

Integrated Implications

Overall, the results do not support the null hypothesis: neonicotinoid residues also have undesirable effects on soil ecosystem engineers. No-till systems, which depend on earthworm bioturbation and microbial priming, are compounded by the threat since the deficit in reproduction spreads through generations¹⁶. The lack of mortality disguises significant sublethal effects, in which fitness costs compromise fertility of soil in the long-term. In comparison with copper controls, imidacloprid had a lower acute toxicity and greater chronic potency, which points to subtle risk patterns that are missed by one-species single-LC₅₀ paradigms [17].

These limits guide IPM modifications: seed treatment at levels lower than 0.1 µg/kg soil equivalents, integration of the rotating biopesticide and maintenance of precise application through drone monitoring. Multi-generational assays and metatranscriptomics to measure the epigenetic and functional resilience should be included in future work [18].

Correlation Analysis Between Biological Indicators Mechanism of Imidacloprid Toxicity in Soil Organisms

The association between the biometric variables measured, a correlation analysis was conducted between imidacloprid concentration, reproduction rates of soil organisms, and microbial enzymes activities. The findings showed that there were good negative correlations between the pesticide concentration and biological performance indicators. The concentration of imidacloprid was found to negatively correlate with the reproduction of earthworms ($r = -0.91$) and springtails ($r = -0.88$), and with high levels of pesticide concentration reproduction was found to be less successful [19].

On the same note, there were notable negative correlations between the concentration of pesticide and the activities of the enzyme, especially the dehydrogenase. Such associations indicate that microbial metabolic activities are very sensitive to the accumulating pesticide levels.

Also, the activity of microbial enzymes was positively correlated with the reproduction of soil organisms ($r \approx 0.80$), which means that the decrease in the activity of microbial metabolism can indirectly influence the ecological activity of soil fauna. These results indicate the interdependent character of soil biological processes and prove that microbial indicators can be used as the warning signs of ecological stress in pesticide-polluted soils [20].

The effects of imidacloprid on soil organisms observed in the case could be attributed to some biological processes that influence soil fauna and the community of microbes. Imidacloprid is a neonicotinoid insecticide, which is mostly active by interacting. In spite of the fact that these compounds are intended to kill insect pests, soil contains non-target organisms like soil earthworms and springtails which can also be killed due to chronic exposure to pesticide residues in the soil [15].

Imidacloprid can cause physiological stress and alter the normal metabolic processes in invertebrates of soil. This stress may cause redirection of energy expenditure during reproductive activities to survival and detoxification processes which causes less cocoon production and juvenile development. Besides the impact on soil fauna, imidacloprid residues have the ability to alter the microbial life of soil. Microorganisms in soil are extremely susceptible to any chemical disturbances and exposure to pesticides could disrupt the respiration and metabolism of microorganisms. Such a disturbance is manifested by the suppression of the important soil enzymes including dehydrogenase, urease, cellulase, and phosphatase which are involved in the carbon and nitrogen cycles. Decreased enzyme activity is evidence of inhibited metabolism of microorganisms and a reduction in efficiency of organic matter decomposition and mineralization of nutrients [17], [18], [19].

The results of the current research are in line with the past studies examining the ecological hazards of imidacloprid in the soil ecosystem. A number of research works have described such negative implications on soil invertebrates and microbial community at environmentally relevant levels²⁰. Case of reproductive inhibition among earthworms used to live in the soils that contained imidacloprid, which validated the sensitivity of annelids to neonicotinoids residues²¹. Besides the effects on soil invertebrates, our findings on the microbial enzyme inhibition are in agreement with previously reported results on the microbial responses of the soil to pesticides. That exposure to imidacloprid changes the structure of soil bacterial communities and inhibits their metabolic activity, especially, carbon and nitrogen-cycling enzymes [8].

In comparison with these investigations, the current findings are additional evidence to the fact that imidacloprid has dose-dependent sub-lethal effects on soil organisms, such as reproductive impairment of earthworms and springtails and considerable inhibition of microbial enzyme activities. Nevertheless, the extent of the enzyme inhibition in this paper (as deep as 42% decrease in the activity of dehydrogenase) indicates that microbial metabolic functions might be the especially sensitive signals to the presence of neonicotinoid contamination. The results are consistent with the ever-growing literature that, despite being relatively selective insecticides, neonicotinoids are capable of interfering with the key ecological processes in the soil when present in agricultural soils in large quantities^{18,19,20,21}.

Limitations

This research experiment was done under laboratory conditions using one type of soil, which might not be a complete reflection of field variability. Environmental variations like temperature change, rainfall and intricate soil interactions were not evaluated. Also, the experiment was based on the short-term chronic exposure and the multi-generational and long-term ecological impacts were not considered. Thus, these results should be used with care in case of extrapolation to the actual situation in agricultural fields.

Conclusion

This paper shows these sublethal impacts can have a cumulative effect on the soil fertility, biodiversity and stability of the ecosystem especially in agricultural systems that rely on soil organisms to ensure the soil structure and the provision of nutrients. These results are concerning the further use of neonicotinoids in the integrated pest management systems and in conservation agriculture practices such as no-till farming where high levels of biological activity are dependent on soil. Thus, safer pest management practices, such as using reduced pesticide inputs, using biopesticides as rotation agents, and using more precise application methods might be useful in reducing the possible ecological effects. The issue of effects of pesticide exposure over the long term and over multiple generations, and the variability of soil when subjected to field conditions should also be viewed in the future research in order to gain a clearer insight into the ecological impacts of accumulation of neonicotinoids in agricultural soils.

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