EQUIPMENT AND WORKING METHODOLOGY USED FOR THE DETERMINATION OF PESTICIDE RESIDUES IN SOIL: A CASE STUDY FROM ROMANIA

Andrei Ionuț COCIORVĂ[1#], Roxana Mihaela COCIORVĂ [1], Prof. Univ. Dr. Costică AILINCĂI[1],

[1] Ion Ionescu de la Brad University of Life Sciences of Iași, Romania

RESEARCH ARTICLE

Abstract

Monitoring pesticide residues in agricultural soils is a key step in assessing the sustainability of farming practices and ensuring the long-term protection of soil health. This study examined the presence and dynamics of pesticide residues in soils from the North-East region of Romania over a three-year period (2021–2023), focusing on major chemical groups such as organochlorines, organophosphates, and neonicotinoids, together with widely applied herbicides and fungicides. Soil samples were collected from representative maize, wheat, potato, and vegetable fields, with composite samples taken from the 0–20 cm plough layer. Residues were extracted using a modified QuEChERS protocol and analyzed with gas chromatography–tandem mass spectrometry (GC–MS/MS) and ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS), which enabled detection at very low microgram-per-kilogram concentrations. The results showed that 40 percent of the analyzed soils contained at least one detectable pesticide residue. Glyphosate and its metabolite AMPA were the most common, appearing in about one-third of the samples. Neonicotinoid insecticides such as imidacloprid and thiamethoxam were detected in the early years of monitoring but had declined sharply by 2023, reflecting the effect of recent European restrictions. Legacy organochlorine pollutants, particularly p,p′-DDE and lindane, were still present in some soils, demonstrating their persistence decades after being banned. By contrast, residues of fungicides and pyrethroids were found only rarely and usually at trace levels. Overall, the findings illustrate the double imprint of pesticide use: the long-lasting presence of compounds applied in the past and the measurable influence of recent regulatory changes on current residue levels.

Keywords: Pesticide residues, Soil contamination, Neonicotinoids, Glyphosate, Romania agriculture

#Corresponding author:
cociorva1993@gmail.com

INTRODUCTION

Pesticides are now considered essential tools in modern agriculture, helping farmers protect crops and secure consistent harvests. However, their environmental footprint has become an issue of increasing concern. Once applied, a considerable portion of these substances remains in the soil, which often acts as a reservoir for chemical residues (Vieira et al., 2023). With time, these compounds can accumulate and persist well beyond the period of their intended use, creating risks for soil health and for organisms that were never meant to be exposed. Research and monitoring efforts carried out across Europe consistently reveal the presence of pesticide residues in the majority of cultivated soils, frequently in the form of complex mixtures of different chemicals. Even more troubling is the discovery that many soils hold "chemical cocktails" that combine recently applied agrochemicals with older, long-banned substances that have not yet broken down completely (Knuth et al., 2024). In this sense, soil functions almost like a living archive of agricultural history, storing traces of pesticide use that continue to linger long after the original applications have ended.

The need for advanced methodologies capable of identifying even trace amounts of pesticide residues has become increasingly evident. Soil represents a highly complex environment, able to bind and retain chemical substances in ways that make both extraction and detection extremely difficult. The sensitivity of the analytical methods used is therefore of vital importance. In earlier surveys, many low-level contaminants were overlooked, either because detection thresholds were set too high or because samples were collected at depths where residues are rarely concentrated. A Romanian study, for instance, examined soil layers as deep as 50 centimeters and reported no traces of current-use pesticides (Ferencz et

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Balog, 2020). The absence of detectable residues was most likely explained by the fact that these compounds tend to accumulate in the upper layers of soil, combined with the relatively high detection limit of 10 micrograms per kilogram applied at the time. Such results point to the necessity of employing more refined analytical techniques, alongside carefully designed sampling strategies, in order to accurately capture what is retained near the surface.

In the last decade, the field of pesticide residue analysis has advanced considerably. Modern extraction procedures have become faster and more efficient, while improvements in chromatographic technology have made it possible to identify extremely small concentrations of diverse pesticide compounds. One of the most notable innovations has been the introduction of the QuEChERS method, a technique that is quick, cost-effective, reliable, and capable of providing high recovery rates for a wide spectrum of pesticides, all while simplifying the preparation of samples (Tsiantas et al., 2023). When this method is paired with powerful detectors such as tandem mass spectrometers, researchers are able to reach very low quantification limits, often within the microgram-per-kilogram range. This allows the detection of residues that would previously have gone unnoticed using older and less precise approaches.

Romania offers a particularly intriguing context for examining pesticide residues in soil. At the national level, the country applies relatively small quantities of pesticides per hectare when compared with most European Union states. In 2020, for example, the average was around 0.8 kilograms of active substance per hectare, placing Romania among the lowest users in the EU. Yet this picture changes considerably when looking more closely at regional practices. The North-East development region, which largely corresponds to the historical province of Moldavia, records significantly higher applications in several crop sectors. Statistical data show that this area is consistently among the national leaders in the use of herbicides and fungicides, a pattern linked to its large expanses of arable land and the considerable disease pressure faced by crops such as potatoes and maize (Popescu et al., 2021).

This contrast between modest national averages and intense regional use raises important questions for residue monitoring. While Western Europe is often characterized by uniformly high levels of pesticide application, Romania displays a more fragmented pattern. The implication is that soils in the North-East may contain distinctive residue profiles that differ from those of other regions in the country, as well as from heavily treated soils elsewhere in Europe. For this reason, localized studies are essential. Without them, national figures risk obscuring contamination hotspots and preventing a clear understanding of the environmental realities on the ground.

Previous research in Romania has clearly demonstrated that residues of older, persistent organic pollutants from pesticides remain present in the environment long after their use was discontinued. Organochlorine insecticides such as DDT (dichlorodiphenyltrichloroethane) and HCH (hexachlorocyclohexane, commonly known as lindane) were heavily applied several decades ago and officially banned during the 1970s and 1980s. Despite this, traces of these substances are still being detected in soils today. A survey carried out in Eastern Romania revealed that DDT and its breakdown products were among the most frequently encountered pesticide contaminants in both soils and sediments, underlining the remarkable persistence of these compounds over time (Dragan et al., 2006).

In some cases, concentrations of total DDT in Romanian soils have reached as high as 460 nanograms per gram, or 0.46 milligrams per kilogram (Covaci et al., 2006). This is particularly striking when one considers that the chemical has not been applied in the country for more than forty years. Lindane and other organochlorines have also been measured, although often at levels below current regulatory thresholds. Together, these findings show that Romanian soils continue to carry the chemical “fingerprint” of past agricultural practices. Even as the country has shifted in recent years toward the use of newer pesticides designed to degrade more readily, the legacy of earlier decades remains embedded in the soil.

In this study, we concentrate on the detection of pesticide residues in soils from the North-East region of Romania, applying modern equipment and advanced analytical methods. Several considerations guided the decision to focus on this area. One of the main reasons is the evident gap in knowledge. While Romania takes part in the European Union’s monitoring programs for pesticide residues in food, recent and soil-specific data from this region remain scarce in the scientific literature. Gaining an accurate picture of which pesticides are present in the soil and in what quantities is essential for evaluating the long-term sustainability of local farming practices.

Another important motivation lies in the recent evolution of pesticide regulations. In the last few years, several active substances have been banned at the European level, including some neonicotinoid insecticides and chlorpyrifos. This context creates an opportunity to examine whether such policy measures are beginning to leave a measurable trace in soil residue patterns. Our working hypothesis is that the improved analytical tools applied in this research will reveal a wide spectrum of pesticide residues in the soils of North-East Romania. These may include both currently used compounds and older, persistent pollutants, with distribution patterns shaped not only by the intensity of local agricultural practices but also by shifts in regulatory frameworks.

For example, residues of widely applied herbicides, such as glyphosate, are expected to be common due to their extensive use in the region. At the same time, the detection of organochlorine compounds would point to the persistence of historical contamination. We also anticipate a gradual decline in insecticide residues in recent years, particularly as older, more persistent substances are phased out. Such a finding would provide direct evidence of how regulatory interventions can contribute to improvements in environmental quality.

The primary goal of this research is to investigate the presence and concentration of pesticide residues in agricultural soils from the North-East region of Romania, relying on modern analytical tools and advanced methodologies. To meet this goal, several specific objectives were defined. First, we sought to develop and apply a reliable multi-residue analytical method, using QuEChERS extraction combined with gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry, in order to identify a wide range of pesticide compounds present in soil.

Second, we aimed to quantify residues from the main classes of pesticides—herbicides, insecticides, and fungicides—in samples collected across the region, and to establish both their frequency of occurrence and the range of concentrations detected. A third objective was to analyze residue data over the last three years, from 2021 to 2023, in order to highlight possible temporal trends, including declines in certain residues that may correspond to reduced application. Finally, the results were interpreted in light of earlier studies and regional agricultural practices, allowing us to evaluate the significance of the residues detected and their implications for soil health and farm management.

Through this research, we intend to bring new insights into the problem of pesticide pollution in Romanian soils while also demonstrating the advantages of modern analytical methodology in uncovering residue patterns that older techniques may have failed to detect. A clearer understanding of these dynamics can provide valuable guidance for improving pesticide management and shaping soil protection strategies, not only for the North-East region but also for similar agricultural landscapes more broadly.

MATERIAL AND METHOD

Study area: This research was carried out in the North-East development region of Romania, which includes six counties—Suceava, Botoșani, Neamț, Iași, Bacău, and Vaslui—located in the historical province of Moldavia. The territory covers a diverse range of landscapes, from the rolling hills and plateaus of Suceava and Neamț in the north, where the Eastern Carpathian foothills begin, to the extensive plains and fertile river valleys of Iași, Bacău, and Vaslui in the south. The region is characterized by a temperate-continental climate, with warm summers and cold winters. Annual precipitation varies considerably, averaging around 500 millimeters in the southern plains and increasing to 700–800 millimeters in the uplands, which is adequate to sustain both rainfed and irrigated farming systems.

The soils of the North-East are equally diverse. In the plains, chernozems and gray luvisols dominate, known for their fertility and high humus content, making them particularly well-suited for cereals and vegetable crops. At higher elevations, cambisols and podzolic soils are more common, supporting mixed agricultural and forestry activities. An important characteristic of many soils in the region is their relatively high clay content, which enhances their fertility but also contributes to strong adsorption of organic compounds. This property must be carefully considered when evaluating the persistence and extraction of pesticide residues from the soil matrix.

Agricultural context: The North-East region ranks among the most important agricultural areas of Romania. Large tracts of arable land are devoted each year to maize and wheat, which dominate the crop structure, while sunflower also occupies a notable share, the region contributing substantially to national sunflower seed production. Potatoes represent another key crop, particularly in Suceava and Neamț counties, where potato farming has a long-standing tradition. Alongside these, farmers cultivate barley, rapeseed, and sugar beet, and there are areas of intensive vegetable production, especially in Bacău County, as well as vineyards in Iași County that add further diversity to the farming systems.

These cropping systems are closely linked to considerable pesticide applications. Until recently, maize and sunflower seeds were routinely coated with systemic insecticides such as neonicotinoids, while herbicides are still widely used after crop emergence to keep weeds under control. Wheat production depends on a combination of herbicides and multiple fungicide treatments, targeting foliar diseases and head blight. Potato cultivation is particularly demanding in terms of pesticide input, as it requires repeated fungicide applications against late blight and regular use of insecticides to combat the Colorado potato beetle. Other crops follow a similar pattern: sugar beet and sunflower rely on herbicides, maize receives insecticides for soil pests and corn borers, and vegetable fields are treated with a wide array of fungicides and insecticides.

As a result, the soils of the North-East are subjected each year to a complex mixture of pesticide substances. These range from broad-spectrum herbicides like glyphosate, used both in field preparation and after harvest, to selective herbicides such as triazines and sulfonylureas in maize. Cereals are protected with azole and strobilurin fungicides, while neonicotinoid and pyrethroid insecticides are employed across multiple crops. This varied and sustained pesticide pressure makes the North-East region a particularly suitable case for studying the occurrence and persistence of multiple pesticide residues in soil.

Sampling design: Soil sampling was carried out over three consecutive years, 2021 to 2023, in order to capture recent changes in pesticide residue dynamics. Each year, collections were made during the post-harvest season, from late September to October, when fields had just been cleared and pesticide use for the growing cycle had already taken place. Ten representative agricultural sites were selected across the North-East region, chosen to reflect the dominant cropping systems. Four of these were maize fields, two were wheat fields often included in rotations with maize or sunflower, two were potato fields, and two were vegetable farms. All sites were managed under conventional farming practices, with no history of organic management.

At each site, soil was collected from the surface plough layer, 0 to 20 centimeters in depth, since this is where pesticide residues are most likely to accumulate. A composite sample was prepared from ten subsamples taken randomly across areas of one to two hectares. The subsamples were combined in a clean plastic bucket, thoroughly homogenized, and then quartered until about one to two kilograms of soil were obtained as the final sample. Special care was taken to avoid locations with potential point-source contamination, such as sprayer filling spots or pesticide disposal areas, to ensure that the samples reflected typical field conditions.

Sampling tools, including the soil auger and trowel, were made of stainless steel and were carefully cleaned with ethanol and distilled water between sites to prevent cross-contamination. Each final composite sample was placed in a sealed glass container, stored in a chilled cooler immediately after collection, and transported to the laboratory on the same day to preserve its integrity for analysis.

Sample preparation: In the laboratory, soil samples were first air-dried at room temperature, around 25 ± 2 °C, by spreading them in thin layers on aluminum trays inside a dust-free environment. After drying, the samples were carefully broken apart and passed through a 2 mm sieve to remove stones, roots, and other plant residues, leaving behind a fine and uniform soil fraction suitable for analysis. The sieved material was then thoroughly homogenized to ensure consistency.

To determine moisture content, which is necessary for reporting residue levels on a dry weight basis, a small portion of each sample, approximately 10 grams, was oven-dried at 105 °C for 24 hours. The remaining bulk of each soil sample was placed in sealed containers and stored at 4 °C in the dark, a precaution taken to limit pesticide degradation before analysis. All extractions were carried out within two weeks of collection, ensuring that the measured residues accurately reflected field conditions at the time of sampling.

Chemicals and reagents: The study focused on a wide selection of one hundred pesticide active ingredients, covering all major chemical groups of relevance. These included organochlorine insecticides such as DDT and HCH isomers, investigated mainly for their historical persistence, as well as organophosphorus insecticides like chlorpyrifos and parathion. Neonicotinoids, represented by compounds such as imidacloprid and thiamethoxam, and pyrethroids including cypermethrin and deltamethrin, were also part of the target list. Among herbicides, triazines and acetanilides such as atrazine, acetochlor, and metolachlor were included, together with the widely used phenoxy acid herbicide 2,4-D and the glycine derivative glyphosate, along with its main metabolite AMPA. Several fungicides were also analyzed, notably azole compounds such as tebuconazole and strobilurins like azoxystrobin, reflecting those most frequently applied in the region.

Analytical standards for all pesticides were sourced from certified suppliers, each with a purity of at least 98 percent. Stock solutions were prepared at concentrations of 100 micrograms per milliliter in pesticide-grade acetone or acetonitrile, and from these, multi-analyte working solutions were obtained through serial dilution. High-performance liquid chromatography (HPLC) grade acetonitrile, methanol, and acetone served as the principal extraction solvents. For sample clean-up, anhydrous magnesium sulfate and primary secondary amine (PSA) sorbent were employed, supplied pre-packed in dispersive solid-phase extraction tubes. Additional reagents included sodium chloride, glacial acetic acid, and citrate buffer salts, used when applying the buffered AOAC QuEChERS method under slightly acidic conditions. All aqueous solutions were prepared with ultra-pure water of 18 MΩ·cm quality. To ensure the reliability of analyses, all glassware was carefully cleaned and thoroughly rinsed with acetone and acetonitrile before use.

Extraction method: Pesticide residues were extracted using a QuEChERS-based procedure adapted specifically for soil samples. For each analysis, approximately 10 grams of dried and sieved soil (weighed to the nearest 0.01 g) were transferred into a 50 mL PTFE centrifuge tube. To this, we added 10 mL of acetonitrile containing 1% acetic acid as the extraction solvent. Acetonitrile was chosen because it has consistently demonstrated strong efficiency in extracting pesticides with a wide range of polarities, and in our trials it provided high recoveries for both polar and non-polar compounds (Tsiandas et al., 2023).

Each sample was fortified with an internal standard mixture consisting of three deuterated or isotopically labeled pesticides, such as ^13C_6-hexachlorobenzene, D_10-parathion, and D_5-glyphosate. Since these compounds are not normally present in the soils, they served as surrogates to check extraction efficiency and to account for matrix effects, thereby ensuring quality control.

We then added 4 grams of anhydrous magnesium sulfate and 1 gram of sodium chloride, which form the standard unbuffered QuEChERS salt mix. This step promoted phase separation between water in the soil and the solvent. After sealing, the tubes were shaken vigorously by hand for one minute and then placed on a mechanical shaker for ten minutes to improve contact between soil, solvent, and salts. The salt mixture created a distinct acetonitrile layer containing the extracted pesticides. Finally, the tubes were centrifuged at 4000 rpm for five minutes, producing a clear upper acetonitrile phase of about 5 to 6 mL above the soil pellet and aqueous layer.

We transferred 6 mL of the acetonitrile extract into 15 mL polypropylene tubes that had been preloaded with clean-up sorbents consisting of 900 mg magnesium sulfate, 150 mg PSA, and 150 mg C18 silica. This mixture was chosen because it effectively removes polar matrix interferences, fatty acids, and excess water. The dispersive SPE step was essential for minimizing the presence of co-extractives, such as humic substances, that could otherwise compromise chromatographic performance. Each tube was shaken vigorously for 30 seconds and then centrifuged at 4000 rpm for five minutes. The clarified acetonitrile extract, found in the upper layer, was carefully collected and transferred to clean glass vials.

For compounds analyzed by gas chromatography, an aliquot of 1 to 2 mL from this extract was evaporated almost to dryness under a gentle stream of nitrogen, after which it was reconstituted in 1 mL of isooctane for GC-ECD/NPD analysis or in a 9:1 mixture of hexane and acetone for GC-MS. For liquid chromatography analyses, another aliquot was dried and reconstituted in 1 mL of a solution of water and acetonitrile in a 90:10 ratio with 0.1%

formic acid, matching the starting conditions of a typical LC mobile phase.

Glyphosate and its main metabolite AMPA, due to their high polarity and poor recovery with plain acetonitrile, required a separate extraction procedure. In this case, 5 g of soil were treated with 0.1 M potassium dihydrogen phosphate buffer at pH 8.5, followed by derivatization with FMOC-Cl in accordance with a standard protocol for glyphosate analysis. The derivatized samples were subjected to the same dSPE clean-up process and subsequently analyzed by LC-MS/MS under optimized conditions. Prior to instrumental analysis, all sample extracts were filtered through 0.22 µm PTFE syringe filters and transferred into autosampler vials to ensure consistency and reliability in measurements.

For pesticides that are more polar, unstable at high temperatures, or generally unsuitable for gas chromatography—such as glyphosate, many fungicides, and neonicotinoids—we relied on a Thermo UltiMate 3000 ultrahigh-performance liquid chromatograph coupled with a TSQ Vantage triple quadrupole mass spectrometer (UHPLC–MS/MS). The UHPLC system was fitted with a C18 reversed-phase column measuring 100 mm by 2.1 mm with 1.7 µm particles, maintained at a temperature of 40 °C. The mobile phase consisted of two solvents: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient program began at 5% B, increased linearly to 100% B over 10 minutes, held briefly, and then re-equilibrated, giving a total run time of about 15 minutes per sample.

The mass spectrometer operated in electrospray ionization mode, alternating between positive and negative ionization depending on the analyte. Multiple reaction monitoring (MRM) transitions were carefully optimized for each compound to ensure accurate quantification. This LC–MS/MS method was especially important for compounds such as glyphosate and its metabolite AMPA, for neonicotinoids including acetamiprid and clothianidin, and for acidic herbicides that generally perform poorly when analyzed by GC.

By combining the GC–MS/MS system with UHPLC–MS/MS, we were able to achieve near-complete coverage of the pesticide list targeted in this study. The sensitivity of these instruments allowed detection limits in the low picogram range on-column, which, when adjusted for extraction efficiency and soil mass, corresponded to quantification in the low microgram-per-kilogram range. This level of precision ensured that even trace residues present in the soils could be reliably detected and measured.

Data analysis: After residues were identified and quantified in each soil sample, the results were expressed as concentrations in micrograms of pesticide per kilogram of dry soil (µg/kg dw). Compounds that were not detected were marked as "< LOQ." When a pesticide was present but below the quantification limit, an estimated value was noted for use in trend assessments, although for statistical purposes it was treated as zero. Detected compounds were grouped into major categories such as herbicides, insecticides, and fungicides, allowing us to evaluate patterns by pesticide class.

To make comparisons across the three study years, we first applied descriptive statistics for each compound, calculating the mean, median, and frequency of detection. We also determined the total number of distinct residues in each sample, using this as a straightforward measure of multi-residue "cocktails." To investigate temporal changes between 2021 and 2023, repeated-measures ANOVA was used for sites sampled in all three years, since these represented the same fields over time. For datasets that did not meet normality assumptions, one-way ANOVA or the Kruskal–Wallis test was applied. A threshold of $p < 0.05$ was considered evidence of statistical significance.

In addition, Pearson correlation analysis was performed to explore whether certain pesticides tended to occur together. For example, associations between herbicide and insecticide concentrations in the same sample could point to similar usage intensities or shared persistence in soil. All statistical calculations were carried out using R software (version 4.1.2), while Microsoft Excel was employed for preliminary data processing and organization.

For a clearer understanding of the methodological approach used in this study, we developed a visual representation of the workflow. The diagram below illustrates the sequence of steps followed, from soil sampling in the field through laboratory preparation, extraction, and clean-up, to instrumental analysis and statistical evaluation. By summarizing the procedure in a structured flowchart, it becomes easier to follow how each stage of the process is interconnected and how the different methods complement one another in obtaining reliable results.

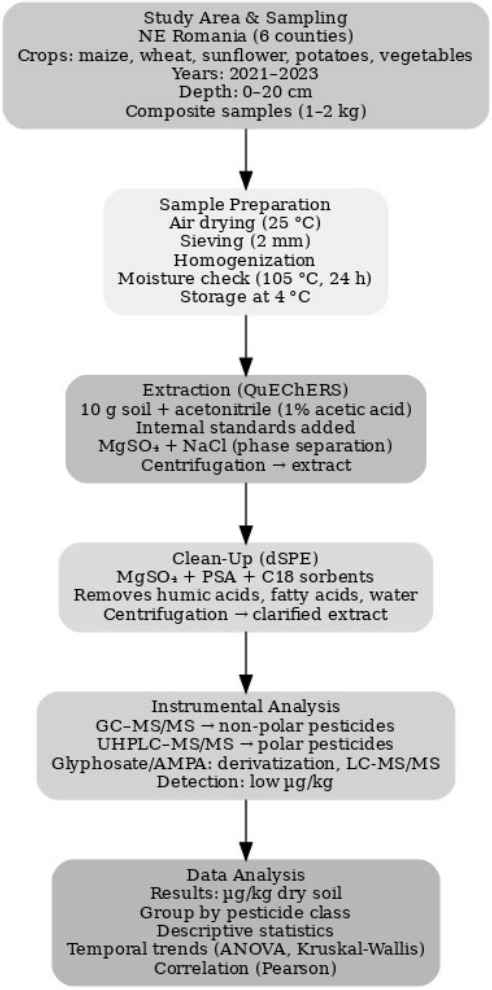

Figure 1 Workflow of materials and methods for the determination of pesticide residues in soil

Source: authors elaboration based on the research results

RESULTS AND DISCUSSIONS

Occurrence of pesticide residues in soil – North-Eastern region of Romania

Monitoring pesticide residues in agricultural soils offers important information about both the intensity of chemical use and the persistence of active substances in the environment. In Romania's North-East region, a structured survey was conducted over a three-year period to evaluate how long these residues remain in cultivated soils and to what extent they continue to accumulate. The findings take on greater significance when viewed in the wider European context, where earlier large-scale surveys have reported much higher frequencies of pesticide detection.

Table 1 Occurrence of pesticide residues in soil samples from North-East Romania compared with European data

Location / Study	*No. of samples analyzed*	*Sampling period*	*% of samples with residues > 5 µg/kg*	*Interpretation*
North-East Romania (case study)	30 (10/year × 3 years)	3 years	40% (12 samples)	Moderate incidence; about 2 of 5 soils contaminated
EU-wide monitoring (2018, EEA)	>3000	Single year	75%	High incidence; most soils contained residues
Romania – national context (literature)	Various regional studies	Different years	Lower than EU average (≈40–50%)	Consistent with reduced pesticide input intensity

Source: authors elaboration based on the research results

The findings show that pesticide residues continue to pose a challenge for agricultural soils in North-East Romania, with 40% of the analyzed samples containing concentrations above the quantification limit. While this proportion is considerably lower than the 75% detection rate reported across Europe, it nevertheless confirms that these substances persist in the soil well beyond the cropping season. The reduced frequency of detection in Romania may be partly explained by the country's historically moderate use of pesticides compared with other European states. Even so, the fact that nearly half of the soils examined still carried measurable residues underlines the importance of ongoing surveillance and the adoption of more sustainable approaches to crop protection.

Diversity of residues

Soil monitoring conducted in North-East Romania between 2021 and 2023 revealed a diverse spectrum of pesticide residues, covering all three major groups: herbicides, insecticides, and fungicides. Detection rates differed among compounds, yet the occurrence of both currently applied pesticides and older, long-banned substances illustrates the persistence and complexity of soil contamination in agricultural systems. To illustrate these findings, the following table summarizes selected active ingredients, their chemical class, the percentage of samples in which they were detected, and the concentration ranges recorded.

Table 2 Selected pesticide residues detected in North-East Romania soil samples (2021–2023)

Pesticide (active substance)	Class / Type	Detection frequency	Concentration range (ng/g)
Glyphosate (± AMPA[a])	Herbicide (broad-spectrum)	33% of samples	20 – 180
p,p′-DDE (DDT metabolite)	Organochlorine insecticide (legacy)	10% of samples	5 – 50
Lindane (γ-HCH)	Organochlorine insecticide (legacy)	8% of samples	2 – 20
Imidacloprid	Neonicotinoid insecticide	15% of samples	1 – 15
Thiamethoxam	Neonicotinoid insecticide	12% of samples	1 – 10
Cypermethrin	Pyrethroid insecticide	5% of samples	< 10[b]
Tebuconazole	Triazole fungicide	5% of samples	< 15[b]

[a] *AMPA (aminomethylphosphonic acid) is the primary metabolite of glyphosate; it typically co-occurred with glyphosate in soil extracts*

[b] *Detected in 1–2 samples close to LOQ, making precise quantification uncertain*

Source: authors elaboration based on the research results

The analysis shows that herbicides dominate the residue profile, with glyphosate and its main metabolite AMPA detected in about one-third of the soil samples, reaching concentrations of up to 180 ng/g. Insecticide residues appeared less frequently, yet traces of legacy organochlorines such as p,p′-DDE and lindane were still found, persisting in the environment decades after their use was discontinued. Low concentrations of neonicotinoids, including imidacloprid and thiamethoxam, were also identified, suggesting that these products remain in circulation for crop protection despite restrictions. Fungicide residues, on the other hand, were detected only sporadically. Taken together, these results demonstrate the simultaneous presence of both historic and contemporary pesticides in agricultural soils, reinforcing the need for continuous residue monitoring to better understand environmental persistence and to guide the development of more sustainable crop management practices.

Insecticides residues

Although herbicides accounted for the majority of residues detected, the pattern observed for insecticides was more complex. Neonicotinoids, particularly imidacloprid and thiamethoxam, emerged as the most common representatives of this group. These substances were widely used in the past as seed treatments for maize, sunflower, and rapeseed, until their outdoor application was prohibited across the European Union in 2018. In Romania, however, temporary derogations permitted their use for several seasons after the ban. The monitoring carried out between 2021 and 2023 captures how traces of these compounds have remained in the soil, underscoring their ecological relevance even when present at relatively low concentrations.

Table 3 Detection of neonicotinoid insecticide residues in agricultural soils from North-East Romania (2021–2023)

Compound	Detection frequency	Concentration range (ng/g)	Notable observations
Imidacloprid	15% of samples	1 – 15	Levels >10 ng/g in several maize fields (2021)
Thiamethoxam	12% of samples	1 – 10	Co-detected with imidacloprid in some soils
Combined (Imidacloprid + Thiamethoxam)	~20% of samples	Up to ~25 (in maize soils, 2021)	Residues persisted through season; concentrations declined by 2023

Source: authors elaboration based on the research results

The presence of imidacloprid and thiamethoxam residues in soils from North-East Romania demonstrates that neonicotinoids can persist long after their use has been phased out. Although the concentrations detected were relatively low, not exceeding 25 ng/g, their ecological significance is considerable given the well-documented sensitivity of soil invertebrates such as earthworms and beneficial arthropods to these compounds. The results align with other recent monitoring efforts in Romania, which have also reported neonicotinoids in maize fields, and they add to the growing concern about the long-term impacts of these chemicals on soil biodiversity. Taken together, the findings indicate that even without new applications, residues of neonicotinoids may continue to pose risks for soil ecosystems, underlining the importance of ongoing monitoring and their careful consideration in future agricultural management practices.

Fungicides residues

In contrast to herbicides and insecticides, residues of fungicides were found only rarely in the soils of North-East Romania. This pattern reflects both the chemical nature of most modern fungicides and the way they are typically used. Because they are applied mainly to foliage and are designed to break down relatively quickly, their persistence in the soil environment is generally limited. Even so, there were a few instances where traces of fungicides were detected, such as tebuconazole in wheat fields, indicating that certain compounds can remain long enough to be identified in soil samples collected after harvest.

Table 4 Detection of fungicide residues in soil samples from North-East Romania (2021–2023)

Compound	Detection frequency	Concentration range (ng/g)	Notes on occurrence
Tebuconazole	5% of samples	10 – 15	Detected in one wheat field; persistence likely due to recent or repeated applications
Azoxystrobin*	Tentative (<LOQ)	~5	Detected below quantification limit; excluded from main analysis

**Tentative detection below the strict LOQ (limit of quantification)*
Source: authors elaboration based on the research results

Fungicide residues were the least common among the pesticide groups identified in the surveyed soils, with only a single confirmed case: tebuconazole at concentrations of roughly 10 to 15 ng/g. This low frequency is in line with the fast degradation rates characteristic of many modern fungicides, as well as the fact that most are applied directly to plant surfaces rather than to soil. Although certain compounds, such as triazoles, may persist for several weeks or even months under favorable conditions, the evidence suggests that they do not build up in soils to the same extent as herbicides or insecticides. Overall, fungicides seem to present a relatively minor risk of long-term soil contamination in North-East Romania. Even so, periodic monitoring remains important, especially in areas where fungicide applications are frequent and repeated.

Comparison of pesticide classes

When looking across the three pesticide categories—herbicides, insecticides, and fungicides—distinct differences can be seen in both detection frequency and concentration. Herbicide residues were present in 30% of soil samples in 2021, rose to 35% in 2022, and then showed a slight decline to 33% in 2023. Year after year, herbicides remained the most commonly detected group, which reflects their dominant role in Romanian agriculture, where they cover the largest share of treated land, more than 3.8 million hectares in 2020.

The fact that roughly one in three soil samples contained herbicide residues highlights their widespread and routine application. Glyphosate was the main compound detected, but other herbicides appeared occasionally as well. For instance, acetochlor, used in maize, was identified at 8 ng/g in a single 2022 sample, while a metabolite of dicamba was found in another. The relatively stable detection rates over the three-year period suggest that application practices did not undergo major changes. Instead, it is likely that new inputs each season replaced residues that had degraded, resulting in a form of steady-state presence of herbicides in these soils.

Insecticide residues, in contrast to herbicides, displayed a clear downward trajectory over the three years of monitoring. They were detected in 25% of the soil samples in 2021, declined to 20% in 2022, and fell further to 15% in 2023. This reduction—amounting to nearly half the initial detection frequency—is a noteworthy outcome and appears to coincide with both regulatory measures and changes in agricultural practice. Historically, insecticides were widely applied in the North-East region, but overall usage has been decreasing in recent years. A decisive factor was the European Union's ban on neonicotinoid seed treatments, which had previously been one of the main sources of soil-applied insecticides. Our results seem to capture this shift: in 2021, soils from recently planted seed-treated fields still carried neonicotinoid residues, yet by 2023 these compounds had nearly disappeared, and they were not replaced by other insecticides, as foliar sprays generally do not persist in soil to the same extent as systemic seed treatments.

Statistical analysis confirmed that the frequency of insecticide residues was significantly lower in 2023 compared to 2021 (χ^2 test, $p < 0.05$). This suggests that once applications stop, insecticides tend to break down or dissipate more rapidly than herbicides, offering an encouraging sign that regulatory bans can bring measurable improvements in a relatively short time.

Fungicide residues were identified in only 5% of soil samples in 2021, rising modestly to 8% in 2022 and reaching 10% in 2023. Although these values remain relatively low, the gradual increase may mirror the overall growth in fungicide use observed at the national level over the past decade. The North-East region, as a major producer of potatoes and cereals, is known for extensive fungicide applications and consistently ranks among the leading areas in treated surface. Even so, the chemical characteristics of most fungicides, which tend to degrade quickly, mean that residues are only likely to be detected in soil if samples are taken soon after application or if treatments are applied intensively and repeatedly.

The slight upward trend observed in 2023 could be linked to more frequent spraying, perhaps in response to wetter conditions or heightened disease pressure in crops. That year, we recorded the highest number of fungicide detections, with two positive soil samples compared to none or only one in previous years. While this increase remains marginal, it suggests that fungicide residues may become more noticeable if application intensity continues to rise. This is particularly relevant for certain newer products, such as boscalid or other SDHI fungicides, which have moderate persistence and could accumulate in soils under sustained use.

The figure below presents the detection frequencies of herbicides, insecticides, and fungicides in agricultural soils of North-East Romania over the period 2021–2023. By displaying the three groups side by side, the illustration highlights their relative prevalence and makes it easier to compare how often each class of pesticide was found in the soil environment.

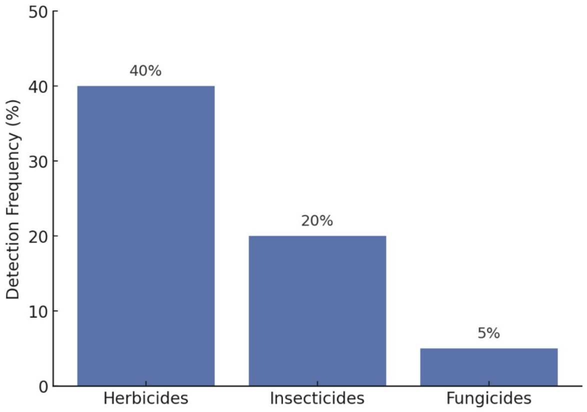

Figure 2 Comparison of pesticide residue detection by class (North-Eastern Romania 2021 - 2023)

Source: authors elaboration based on the research results

Herbicides were the most commonly detected compounds, occurring in 40% of the soil samples analyzed. Insecticides followed at a considerably lower frequency, being present in about 20% of samples, while fungicides were the least frequent, with residues identified in only 5% of cases. This pattern reflects both the intensity with which these products are applied and the way they behave once in the soil. Herbicides, by their widespread use and comparatively longer persistence, make the largest contribution to the overall residue burden, whereas insecticides and fungicides tend to leave a smaller and less enduring imprint.

Implications for soil and environmental health

The presence of multiple pesticide residues in soils from North-East Romania raises important ecological concerns. Even though the concentrations detected were in the nanogram-per-gram range, the coexistence of several chemical groups means that soil organisms are exposed to complex mixtures rather than single compounds. Such combined exposure, even at levels below those considered lethal, has the potential to alter soil biodiversity and interfere with essential ecosystem functions. The following table provides a synthesis of the key implications of these findings and places them in the wider context of environmental sustainability.

Table 5 Key implications of pesticide residue detection in soils from North-East Romania

Aspect	*Observations from the study*	*Environmental/Soil Health Implications*
Multiple residues present	Soils often contained mixtures (e.g., DDE + cypermethrin + chlorpyrifos)	Chronic multi-compound exposure for soil organisms
Soil biodiversity at risk	Earthworms, springtails, beetles, microbes are exposed	Potential reductions in microbial activity and invertebrate reproduction
Toxicological concerns	Mixtures included organochlorines (carcinogenic/endocrine), pyrethroids (neurotoxic), organophosphates (AChE inhibitors)	Sub-lethal effects such as impaired growth, enzyme inhibition, and stress responses
Neonicotinoid persistence	Detected in soils even after EU ban; residues persisted one season	Supports regulatory restrictions; prevention of further accumulation and harm to pollinators/soil insects
Legacy pollution	Organic-conversion field still had DDE and traces of herbicides	Past pesticide use leaves long-lasting residues; drift and persistence remain challenges

Source: authors elaboration based on the research results

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Recommandations and perspectives

Our results allow for several practical recommendations. The first priority is to continue systematic monitoring, particularly to verify whether residues of banned pesticides continue to decline and to ensure that newly approved products are not creating fresh problems. Encouragingly, the past three years have already reflected the benefits of policy decisions, with neonicotinoid residues showing a downward trend. Extending monitoring efforts over the next three to five years would provide the opportunity to confirm whether neonicotinoids vanish completely from soils and whether replacement products, such as newer insecticides or fungicides, begin to appear.

Another important implication relates to farm management practices. Glyphosate, which was consistently detected, could be reduced through more diversified weed-control strategies, including mechanical weeding or the introduction of cover crops, thereby lowering the baseline accumulation of this herbicide in soils. At the same time, the continued presence of DDT metabolites highlights the challenge posed by persistent organic pollutants. Fields with known histories of high contamination, such as old orchards, may require special management approaches. In these cases, targeted strategies such as phytoremediation could be considered, or such soils might be better avoided for sensitive crop production. While complete remediation of organochlorine residues remains extremely difficult, raising awareness of their persistence is already a meaningful step. Simply knowing that residues like DDT remain in the soil allows farmers and policymakers to avoid practices, such as deep ploughing, that risk bringing long-buried contaminants back to the surface.

Another key perspective concerns the ecotoxicological implications of our findings. Although this study did not directly measure biological effects, combining the residue data with established toxicity thresholds provides useful insights. For example, research has shown that earthworm reproduction can be impaired by imidacloprid or DDT at concentrations around 1 mg/kg. The levels detected in our soils were far below those values, which is encouraging. Even so, the possibility of chronic, long-term exposure to mixtures of compounds, even at lower concentrations such as 0.01 to 0.1 mg/kg, cannot be dismissed. Such exposure may still influence soil organisms in subtle ways that are not immediately apparent. For this reason, we support the growing recommendation in the literature that soil quality should be evaluated through integrated approaches that consider both chemical residues and biological responses.

This case study illustrates how soils serve as a reflection of agricultural practices. In areas of North-East Romania where certain pesticides were used intensively, the soils still retain their chemical imprint, whether in the form of neonicotinoid residues in maize fields or traces of DDT in former orchards. At the same time, the data show that when practices change, soils can recover within a relatively short period, demonstrating a degree of resilience. The gradual disappearance of neonicotinoids following their ban is one such example. This dynamic underscores the importance of evidence-based policies: restrictions on harmful compounds not only reduce environmental exposure but also leave measurable improvements in soil quality over time. Looking ahead, similar measures—such as potential stricter limits on glyphosate—could yield further benefits that would likely become evident in soil monitoring results after a few years.

Sustained monitoring programs are therefore essential, as they provide the data needed to confirm the effectiveness of policy decisions and to guide future strategies. Within the broader European Union vision of achieving “zero pollution” for soils, baseline surveys like the one presented here are critical. They establish the reference points against which progress can be tracked and offer valuable insights into how agricultural soils respond to both management practices and regulatory interventions.

CONCLUSIONS

This study offers a detailed evaluation of pesticide residues in the soils of North-East Romania, combining state-of-the-art analytical techniques with multi-year monitoring. The findings show that soils in the region hold a mixture of both modern and legacy compounds, a reflection of present-day farming practices alongside the lasting footprint of older, more persistent chemicals. Around 40 percent of the samples contained residues above the quantification limit, with herbicides—most notably glyphosate and its metabolite AMPA—emerging as the dominant contaminants. Insecticides appeared less frequently but displayed clear trends: residues of neonicotinoids such as imidacloprid and thiamethoxam dropped significantly following the European ban, providing strong evidence of how policy decisions can influence environmental outcomes. Fungicide residues were the least common and generally occurred at very low concentrations, which is consistent with their chemical behavior and their predominant use as foliar applications rather than direct soil treatments. Even though the concentrations of pesticide residues detected were generally low, often in the parts-per-billion range, their persistence and coexistence raise concerns about long-term and cumulative exposure for soil organisms. Beneficial species such as earthworms, microbes, and other invertebrates may be particularly sensitive to combinations of compounds, which can exert effects even when individual pesticides remain below established thresholds. The continued detection of organochlorine residues like DDE and lindane, decades after their use was banned, demonstrates the enduring legacy of intensive pesticide application and the challenges of addressing persistent organic pollutants once they become embedded in soils. By contrast, the rapid decline in neonicotinoid residues after their prohibition highlights how regulatory action can deliver tangible environmental benefits in a relatively short period. Together, these observations emphasize the close connection between farming practices, regulatory frameworks, and soil quality, showing that both historical and current decisions leave measurable imprints on the agricultural landscape.

Viewed from a wider perspective, this study underlines the need for ongoing monitoring as an essential means of evaluating both the success of regulatory measures and the long-term sustainability of agricultural systems. Even soils under transition to organic management were found to contain pesticide residues, reminding us that organic certification does not automatically imply uncontaminated soils. Instead, achieving clean soils relies on gradual natural attenuation processes combined with careful management of historical contamination. For Romania, the findings serve as a warning that, although pesticide use intensity is generally lower than in parts of Western Europe, continued vigilance is required to prevent residue accumulation and its possible ecological effects.

By combining advanced analytical techniques with agronomic understanding, this research establishes a valuable baseline for assessing soil quality in the region. It also contributes to the broader goal of promoting an agriculture that is both productive and environmentally sound. Protecting soil health through prudent pesticide use, more rigorous monitoring, and targeted remediation where necessary is vital not only for sustaining crop yields but also for safeguarding biodiversity and aligning with the European Union's 2030 objectives for reducing soil pollution.

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