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# Snails as Temporal Biomonitors of the Occurrence and Distribution of Pesticides in an Apple Orchard

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**Abstract:** The intensive use of pesticides in agricultural areas and the resulting effects have created a need to develop monitoring programs for their active assessment at low cost. This research entails a biomonitoring study of the pesticides in an apple orchard, using juvenile *Cornu aspersum* (O. F. Müller, 1774) snails exposed in field microcosms. The snails were deployed at three different locations in the orchard area and were used to assess the temporal biomonitoring of 100 different semi-volatile and non-volatile pesticides. The study was performed over an 18-week period and targeted the center, the border, and the outside of the orchard. Results showed that greater levels of pesticides were detected at the center of the orchard as compared to the other sites. The type and level of the applied pesticide influenced its environmental dissipation, as significantly greater levels of semi-volatile pesticides were accumulated by the caged snails in comparison to non-volatile pesticides. The presence of semi-volatile pesticides in the snails outside the orchard revealed the usefulness of these species in the biomonitoring of off-site pesticide emissions. The findings of this study showed that *C. aspersum* can serve as a reliable and effective model organism for the active biomonitoring of pesticide emissions in agricultural sites.

**Keywords:** snails; biomonitoring; pesticides; apple orchard



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## 1. Introduction

The increase in global population growth and decrease in available farmland areas have led to an increase in reliance on pesticide-based solutions for pest control and food security [1]. About 5 million tons of pesticides are applied in agricultural practices around the world each year [2]. Pesticides are simple-to-use and fast-acting chemical compounds that have been intensively employed in order to protect crop production from pests, weeds, and diseases, regulate plant growth, and improve the overall quality and productivity of crops [3–5]. This is particularly the case for apple orchards, which are known for their wide growing areas and their high economic value [6,7]. Apple trees are classified among the most-treated fruit crops and require a large number of pesticides yearly (average of 30 pesticides) to safeguard against pest-induced losses [8,9]. The recurrent intensive use of pesticides during the apple growing season, which extends about 8 months per year, allows a significant prevention of losses, while preserving quality and yield [10,11].

However, the uncontrolled massive application of these chemicals can lead to severe adverse effects [12,13]. Studies have shown that, during their application, a very small fraction of the sprayed chemical reaches the targeted crop, while the remaining fraction is emitted to the different environmental compartments (air, soil, water), which ultimately

affects non-targeted organisms [14–16]. The emission of pesticides into the environment occurs via several processes such as volatilization, drifts, chemical degradation, microbial uptake, and soil erosion [17].

Accordingly, pesticides can pose a direct threat to the environment, where they can persist and bioaccumulate [18]. For example, pesticides that are characterized by a high octanol-water partition coefficient ($K_{OW}$), low solubility (<1 mg $L^{-1}$), and a soil half-life greater than 30 days tend to accumulate in the biomass [19]. In addition, pesticides can have devastating effects on human health, depending on the level and type of the exposure [17,20]. Pesticides have been reported to induce different types of cancers, endocrinological disorders, neurological diseases, and respiratory diseases, while approximately 355,000 deaths from pesticide poisoning occur yearly [21–24]. Given the hazardous effects associated with pesticide emissions and exposure (particularly in agricultural areas), a regular monitoring of their presence in the environment is urgently needed in order to devise and implement procedures that ensure their proper use and control.

While the environmental persistence and fate of pesticides in the soil of these agricultural areas is largely reported through well-known sampling and analytical techniques, their analysis in the surrounding environment is still lagging [25]. Atmospheric pesticide measurements could be conducted via continuous monitoring programs, which allow real-time continuous measurement of the pesticides' concentration in the atmosphere over a period of time [26], or by integrated monitoring programs where pesticides in the air are accumulated on a collecting media for later analyses [25]. While continuous monitoring programs allow fast and direct measurement of pesticides in the atmosphere, their use is still limited and atmospheric pesticide measurements are mainly conducted using integrated measurement approaches [27]. These integrative approaches rely either on an active or a passive sampling technique. Active sampling techniques make use of pumps that operate at a defined flow rate to collect pesticides through a filter and an adsorbent at a high frequency over short periods of time. Conversely, passive sampling techniques rely on the free flow of analytes, which is mainly driven by chemical potential (i.e., flow of the analyte from the sampled medium with a high concentration to the collection medium with a lower concentration) [28–30]. Despite providing reliable data on pesticide concentrations in the air and their distribution among the particulate and gaseous phases, active sampling techniques suffer from several limitations, in particular: their expensive maintenance costs, transportation difficulties, and reliance on a stable source of electricity, thus limiting their use in rural areas [29,31]. Therefore, integrative passive sampling techniques for pesticide monitoring were introduced to overcome these challenges. These techniques include samplers to collect airborne drift samples or sedimented (deposited) drift samples. Examples of airborne sample collectors include badges, diffusion tubes, packed diffusion tubes and cartridges, and polyvinyl chloride (PVC) lines, whereas examples of collectors for deposited samples include filter paper collectors, polyethylene collectors, steel plates, and plastic Petri dishes. These techniques are low-cost, easy to handle, and lack any dependence on power sources [31–36].

More recently, several living organisms have been reported as reliable passive samplers for the released pesticides [37]. These organisms can provide an efficient assessment of the environmental conditions for areas surrounding the application zones [38–40]. Snails are known for their wide distribution, pollutant bioaccumulation capacity, and easy sampling, which makes them valuable in environmental biomonitoring studies [41,42]. These invertebrates live at the soil–plant–air interface, where they can be exposed, through various dermal, oral, and respiratory routes, to different classes of pollutants and chemicals such as heavy metals, industrial chemicals, pesticides, and hydrocarbons [43–47]. Snails are able to bioaccumulate pollutants in their soft tissues and can, therefore, transfer them to higher trophic levels, as they are important prey for large invertebrates, birds, and mammals (including humans) [44]. As a result, the analysis of snails as potential biomonitors for pesticide pollution, especially in rural agricultural areas, is of interest, as it could reflect the true impact of these chemicals on the total ecosystem. In fact, snails could be exposed to

pesticides directly at the time of the spray application, through cutaneous and respiratory routes, and indirectly at later times through their digestive, cutaneous, and respiratory routes [48]. It is worth noting that pesticides have to reach close to the soil level before their uptake can be through the respiratory route of the snails. All the aforementioned exposure pathways could serve as an alternative to the passive collectors, which are used for assessing pesticides' airborne emission and deposition [48,49].

Even though snails are well-suited for the biomonitoring of environmental pollution, studies in this field are limited and are mostly focused on the toxicity of organic pollutants [50–53]. Moreover, passive monitoring of environmental pollution using wild snails (not reared) is hampered by their unknown exposure history, age, and prior movements. Therefore, active biomonitoring approaches using reared snails were introduced to circumvent these limitations [48]. Such active approaches rely on the transplantation of snails, reared under defined conditions, in cages or microcosms that allow for realistic field biomonitoring studies [50]. The microcosm approach has been successfully used for the monitoring of metal pollution, but limited data are available on environmental pollution assessment [42,45]. For this, the use of snails as active biomonitors for environmental pollution to assess pesticide emissions could serve to address the limitations of the commonly applied integrative sampling techniques. Such use could also be advantageous, as it could reflect not only the emission and deposition of the pesticides but also the bioavailability of the chemicals through the respiratory, cutaneous, and digestive system pathways of the snails.

To the authors' knowledge, no studies to date have reported on the temporal active biomonitoring of pesticides in an apple orchard using the garden snail *C. aspersum.* Therefore, the aim of the present work is to assess the potential use of this snail species, exposed in field microcosms, to accumulate different pesticide residues in an apple orchard over an 18-week period. The selected apple orchard, which is located in Aabdine, North Lebanon, was treated with different types of pesticides that are typically used on other crops, thus applying similar agricultural practices that can serve to assess the impact of pesticides' release in comparable agricultural areas.

## 2. Materials and Methods

### *2.1. C. Aspersum Müller Snails and Field Microcosms*

Juvenile land snails *C. aspersum* Müller, aged two months and weighing between 2.5 and 2.7 g, were used in this study. The snails were reared on the Helix Lebanon farm, situated in Aamiq, Lebanon (33°42′46.3″ N 35°47′14.2″ E), under a temperature of 20 ± 3 °C, with a photoperiod of 10 h/day and a hygrometry between 70% and 80%. They were fed organic chard, which was cultivated under the same conditions on the farm.

Prior to their field transfer, the snails were analyzed for their potential contamination by the assessed pesticides and were considered as the starting point for field pesticides' accumulation (Sampling-S0). The snails were then transferred to microcosms in groups of 50. The microcosms consisted of stainless-steel cages (0.6 m (L) × 0.3 m (W) × 0.4 m (H)) fully fitted with fiberglass wire mesh (mesh size 6) allowing free airflow through the cages, while avoiding the escape of the snails. The floor of each microcosm was packed with 20 cm of soil collected from each sampling site. The soil and organic chard (harvested from Helix Lebanon farm) made up the only diet sources for the caged snails during their entire field exposure. In addition, the snails were regularly sprayed with mineral water to avoid dehydration.

### *2.2. Study Site*

The apple orchard assessed in this study is located in Aabdine, North Lebanon (34°16′14.0″ N 35°53′21.3″ E). It is at 1000 m altitude, covers 0.2 ha, and is surrounded by several treated orchards and agricultural lands. Several pesticides were regularly applied by the farmer prior to the sampling campaign including Alpha- Syper (Cypermethrin), Evex (buprofezin), Emperor (Pymetrozine), Flint (Trifloxystrobin), Germino (Chlorpropham),

Malathion, and Methalin (Pendimethalin). In addition, Insecta (INSECTA LB, Beirut-Lebanon) at 0.3 mg/L and Myclo 24 (SIAD, Adonis- Lebanon) at 0.75 mL/L were used during the monitoring campaign. The pesticides' application was conducted using a backpack at a volume rate of 100 L/ha. The treatment of both pesticides, applied together, occurred at the same sampling date for the first four intervals. The active ingredient of these two pesticides were Lambda Cyhalotrin (10%) and Myclobutanil (24%), respectively.

The experiment targeted the center of the apple orchard (Site-A), where three different microcosms, distanced one meter from each other, were placed. A fourth microcosm was placed at the border of the orchard (Site-B). This microcosm was located approximately 10 m from the last apple tree cultivated in the orchard. In addition, a fifth microcosm was placed outside of the orchard (35 m from Site-A), which served as a local control (Site-C). Helix Lebanon farm, which is located approximately 140 km away from the apple orchard, was used as a reference site for the study (Site-R). The study area is illustrated in Figure 1.

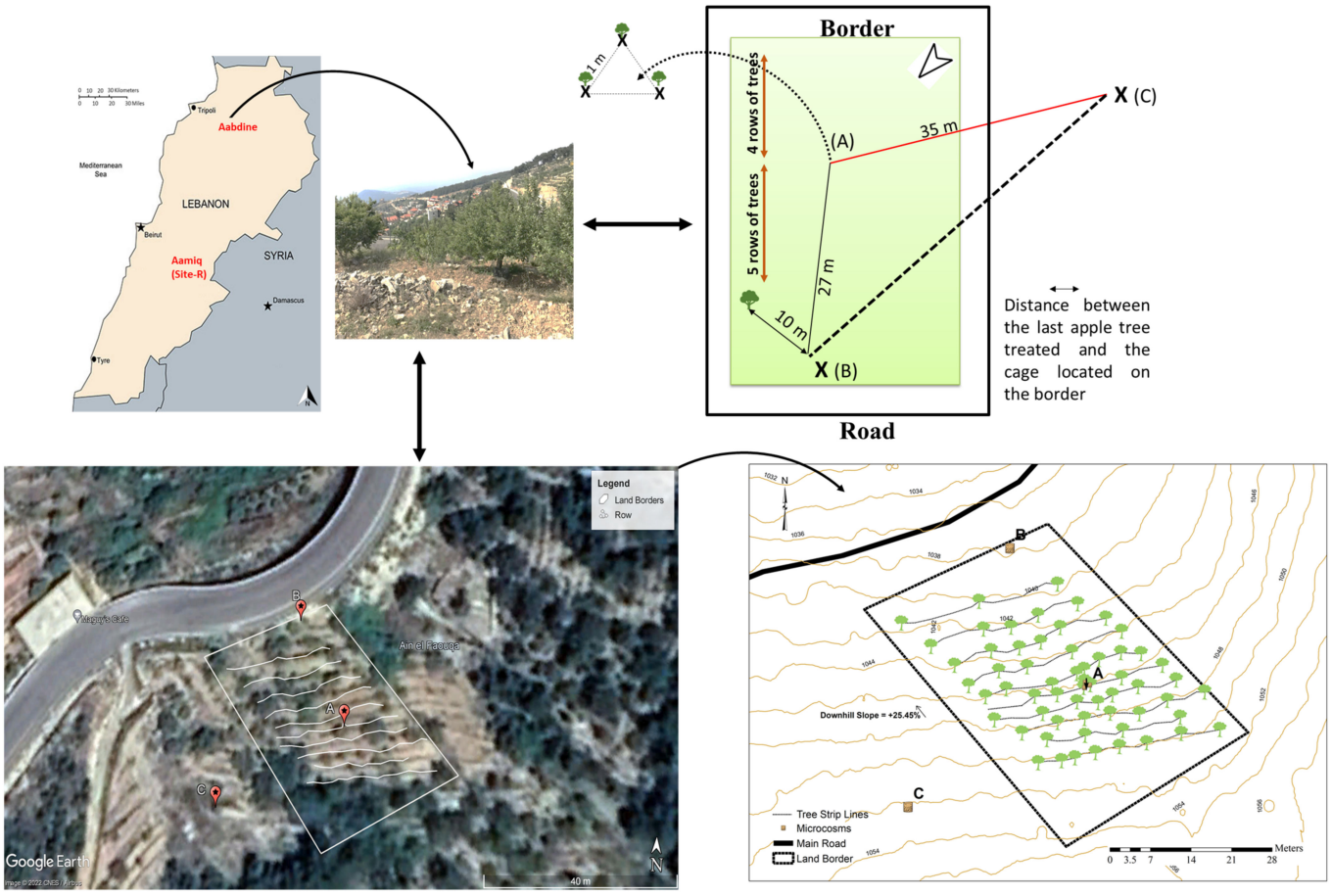


**Figure 1.** Study site showing the location of the three locations, A, B, and C, of the orchard. X indicates the location of the microcosms; the three microcosms installed at Site-A were 1 m distant from each other and were installed directly under the trees, as illustrated.

Soil samples were collected at the beginning and the end of the campaign from each microcosm at the three sites at the orchard. The soil samples at sites A, B, and C have pH values of 7.37 ± 0.07, 7.45 ± 0.04, and 7.58 ± 0.06, respectively. The corresponding organic matter (OM) content, in percentage (%), conducted per ASTM D 2974 [54], were 9.27% ± 0.67, 9.60% ± 0.25, and 27.71% ± 0.67, for sites A, B, and C, respectively. Site-R was characterized by a pH of 7.65 ± 0.02 and an OM content of 12.89 ± 1.07.

### 2.3. Sampling Campaign

Sampling was conducted once every three weeks from 7 June to 9 October 2020, making a total of 6 samplings. During the treatments, the cages were relocated away from the impact area to avoid direct exposure to pesticides and were then returned to their initial position within a few minutes.

At each sampling event, five snails were randomly removed from each microcosm and were then transferred to clean sterile containers for a 48-h fasting period, after which they were sacrificed by freezing and kept at −20 °C until analysis.

Meteorological parameters including temperature, wind speed, and precipitation, provided by Time and Date AS (timeanddate.com), were recorded over the campaign as illustrated in Figure 2. It is worth noting that the predominant wind direction in the orchard area during the study was from south to north and that no precipitation occurred over the duration of the study.

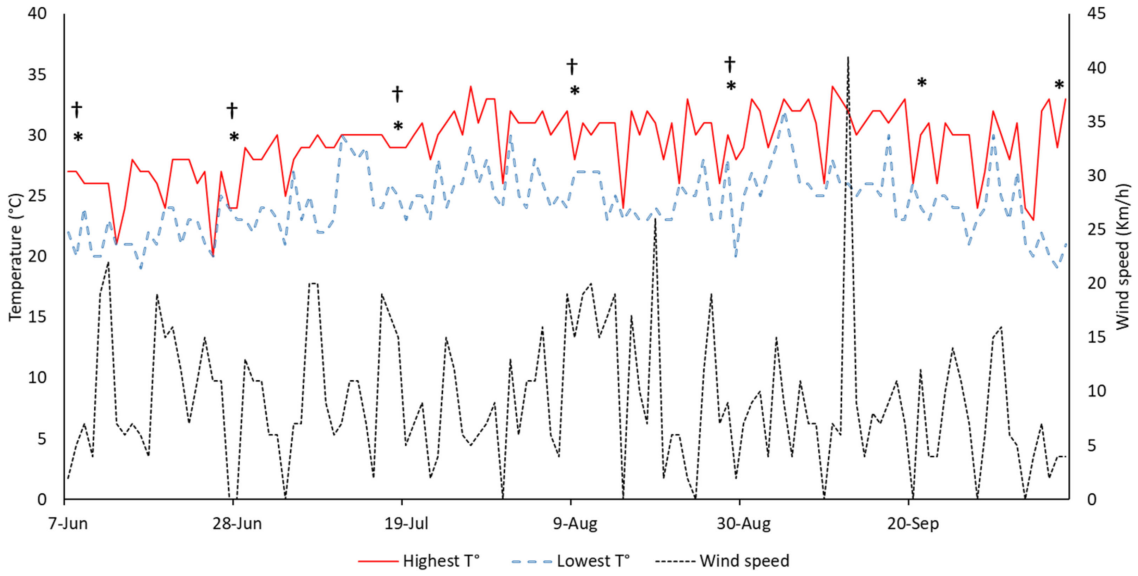


**Figure 2.** Meteorological parameters during the four-month sampling campaign. * denotes the sampling dates and † denotes pesticides' treatment.

### 2.4. Analytical Procedure of Soil and Snails Samples

Samples were analyzed for their potential contamination by 100 pesticides, including 30 non-volatile pesticides analyzed by LC-MS/MS and 70 semi-volatile pesticides analyzed by GC-MS/MS. These pesticides are listed in Supplementary Information Section S1.

The analytical procedure used for the extraction and analysis of pesticides from sampled snails and soils were based on the work of Al-Alam et al., for the multiresidue analysis of organic pollutants in snails [55]. All analyses were performed in triplicate (three different extractions per sample with separate analysis per extract).

#### 2.4.1. Samples Treatment

For snails samples: each set of five snails sampled from each microcosm at each sampling event were maintained in clean sterile containers for a 48-h fasting period, after which they were sacrificed by freezing at −20 °C and stored until analysis. Afterwards, snails were thawed, and the soft body, used for pesticides analysis, was separated from the shell. Each set of 5 snails was mixed together, and the resulting mixture was extracted and analyzed in triplicate (three different extractions per sample with separate analysis per extract).

The snails' water content was determined by drying the snail samples at 60 °C to a constant weight (about 3 days).

For soils samples: soils were collected at the beginning and at the end of the study from each microcosm. The soil samples collected from each microcosm were mixed well in order to ensure homogeneity, and the resulting mixture was extracted and analyzed in triplicate.

#### 2.4.2. Extraction Procedure

Briefly, the extraction was based on the QuEChERS extraction procedure, followed by a concentration of semi-volatile pesticides by solid phase microextraction (SPME) using a polyacrylate (PA) fiber. For this, five grams of homogenized samples were extracted with acetonitrile and QuEChERS citrate buffered extraction kits (EN 1566 method). The obtained extract was cleaned by dispersive solid phase extraction (d-SPE) using sample clean-up kits (AOAC 2007 method). The resultant extract was then concentrated to 1 mL by evaporation. About 0.1 mL of this extract was analyzed by LC-MS/MS, following the addition of the appropriated internal standard (IS) solution, while the remaining 0.9 mL was diluted with salted water (1.5% NaCl), treated with the IS solution, and concentrated with SPME on a PA fiber (65 µm) prior to their analysis by GC-MS/MS. SPME was carried out by direct immersion for 40 min at 60 °C under agitation.

#### 2.4.3. Chromatographic Analysis

Non-volatile pesticides were analyzed by an LC system (Thermo Scientific, Waltham, MA, USA, Surveyor pump and autosampler) coupled with a tandem MS/MS system (TSQ Quantum Access Max equipped with a Hyper Quads Driven) operating in electrospray ionization (ESI) mode. Chromatographic separation was done using a Macherey-Nägel Nucleodur $C_{18}$ Pyramid column (150 mm × 3 mm; 3 µm) thermostated at room temperature with a mobile phase of acetonitrile/water (0.1% formic acid) at 300 µL $min^{-1}$. The gradient used started with 30:70 (*v/v*) for 5 min, followed by 50:50 (*v/v*) for 6 min, 80:20 (*v/v*) for 7 min, to finally achieve 95:5 (*v/v*) for 10 min. Afterwards, 30:70 (*v/v*) for 8 min was used for column stabilization. The injection volume was 20 µL.

Semi-volatile pesticides were analyzed by a Thermo Scientific Trace GC coupled with an MS/MS system (ITQ 700, temperature source: 210 °C, transfer-line temperature: 300 °C) operating in electron impact (EI) mode. The temperature source was 210 °C, and the transfer line temperature was 300 °C. Chromatographic separation was conducted on an XLB (50% phenyl/50% methylsiloxane) capillary column (30 m × 0.25 mm; 0.25 µm). Injections were conducted by thermal desorption of the PA fiber in splitless mode at 250 °C for 15 min, with helium used as a carrier gas at 1 mL $min^{-1}$. The oven temperature was initially set at 50 °C for 3 min, then was raised to 160 °C at 36.6 °C $min^{-1}$, and then was programmed to 300 °C at 5.8 °C $min^{-1}$, where it was held for 10 min.

#### 2.4.4. Quality Assurance (QA)/Quality Control (QC)

Pesticides' quantification was conducted using matrix-matched calibration curves. Peaks were positively identified if the retention time corresponded with that of the standard compound, and if Multiple Reaction Monitoring (MRM) ratios and ion fragmentations agreed with those of the standards for non-volatile and semi-volatile pesticides, respectively. Blank snails used were verified for their potential contamination by the analyzed pesticides and were considered as sampling S0 in the study. All validation parameters including details on the quality control (QA/QC) procedure, the limits of detection and quantification and uncertainties are shown in Table S1.

### *2.5. Data Treatment and Analysis*

The total concentration of pesticides accumulated by the snails caged at each site was calculated by adding the single concentration of both non- and semi-volatile pesticides quantified at each sampling date.

Statistical analysis was conducted using Minitab version 19.0 (Minitab for Windows, LLC, Pennsylvania, PA, USA). The means of replicates were analyzed by One-Way Analysis

of Variance (ANOVA) and Fisher pairwise comparisons at a 95% confidence level for post hoc analysis. A $p$-value < 0.05 was considered significant.

## 3. Results and Discussion

### *3.1. Pesticides' Analysis in Soils*

The soils collected from the four tested sites all showed detectable levels of pesticides. The average concentration of semi-volatile pesticides was higher than that of non-volatile pesticides at all four sites, with sites A and R being the most- and the least-contaminated sites, respectively. The concentrations of pesticides detected in the soils at the four sites A, B, C, and R are shown in Table 1.

**Table 1.** Concentration of pesticides (ng/g) in soils collected from the three sites of the orchard at the beginning (S0) and the end (SF) of the sampling campaign (Site-A: center of the orchard, Site-B: border of the orchard, Site-C: control site outside the orchard, Site-R: reference site). The data are presented as mean ± RSD (n = 3 for each site).

| | | Site-A | | Site-B | | Site-C | | Site-R | |
|---|---|---|---|---|---|---|---|---|---|
| | **Pesticide** | **S0** | **SF** | **S0** | **SF** | **S0** | **SF** | **S0** | **SF** |
| non-volatile pesticides | Carbendazim | 14.3 ± 0.1 * | 8.84 ± 1.9 * | 81.11 ± 1.01 * | 17.91 ± 1.84 * | 7.3 ± 0.2 * | 4.5 ± 0.1 * | 7.4 ± 0.1 | 7.63 ± 0.98 |
| | Diflubenzuron | 3.4 ± 0.2 * | 0.84 ± 0.51 * | 8.2 ± 0.2 * | 0.6 ± 0.2 * | 7.37 ± 0.06 * | 3.87 ± 0.25 * | 0.7 ± 0.1 * | N.D. |
| | Diflufenican | <DL | <DL | <DL | <DL | <DL | <DL | <DL | N.D. |
| | Epoxiconazole | 18.2 ± 0.2 * | 14.87 ± 1.27 * | N.D. | N.D. | N.D. | N.D. | 2.3 ± 0.2 * | N.D. |
| | Flufenoxuron | 2.7 ± 0.2 * | 1.29 ± 0.48 * | 7.3 ± 0.2 * | <DL | <DL | <DL | <DL | N.D. |
| | Foramsulfuron | 2.7 ± 0.1 * | <DL | 36.2 ± 2.43 * | <DL | 4.27 ± 0.05 * | <DL | <DL | <DL |
| | Isoxadifen | 5 ± 0.14 * | 3.17 ± 0.25 * | 12.67 ± 2.02 * | N.D. | 2.5 ± 0.1 * | N.D. | <DL | N.D. |
| | Nicosulfuron | 18.14 ± 1 * | <DL | 63.01 ± 2.5 * | 30.59 ± 1.44 * | 6.47 ± 0.06 * | <DL | N.D. | N.D. |
| | Penconazole | 1.87 ± 0.25 * | <DL | 47.27 ± 2.2 * | 11.23 ± 1.1 * | 2.44 ± 0.05 * | <DL | <DL | <DL |
| | Pymetrozine | 3.4 ± 0.1 * | <DL | 74 ± 2 * | 62.99 ± 3.21 * | 7.3 ± 0.1 * | <DL | <DL | N.D. |
| | Pyraclostrobine | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL |
| | Tebuconazole | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL |
| | Thiacloprid | <DL | <DL | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Triflusulfuron Methyl | <DL | N.D. | 13.74 ± 2.14 * | <DL | 3.2 ± 0.1 * | <DL | <DL | <DL |
| ***Σ non-volatile pesticides*** | | ***69.71 ± 0.2*** | ***29.01 ± 0.5*** | ***343.5 ± 1.09*** | ***123.32 ± 0.65*** | ***40.85 ± 0.1*** | ***8.37± 0.2*** | ***10.4 ± 0.07*** | ***7.63 ± 0.2*** |
| Semi-volatile pesticides | Acetochlor | 22.41 ± 1.1 * | 14.71 ± 4.3 * | <DL | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Alachlor | 814.32 ± 5.5 * | 712.79 ± 7.23 * | 743.29 ± 8.55 * | 624.81 ± 10.3 * | N.D. | N.D. | N.D. | N.D. |
| | Benoxacor | 55.81 ± 1.86 * | 32.16 ± 10.22 * | 12.49 ± 1.13 * | 9.29 ± 1.05 * | 90.97 ± 3.48 * | 15.39 ± 1.1 * | 34.72 ± 1.88 * | 12.51 ± 0.69 * |
| | Bifenthrin | 929.42 ± 10.87 * | 854 ± 17.98 * | 829.16 ±7.83 | 735.48 ± 6.28 | N.D. | N.D. | N.D. | N.D. |
| | Bromoxynil octanoate | <DL | N.D. | N.D. | N.D. | < DL | N.D. | N.D. | N.D. |
| | Buprofezin | 331.71 ± 5.14 * | 114.73 ± 13.4 * | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Chlorpropham | 7.51 ± 1.04 * | <DL | N.D. | N.D. | <DL | N.D. | N.D. | N.D. |
| | Cypermethrin | 1184.94 ± 12.64 * | 994.92 ± 27.32 * | 1084.36 ± 7.53 * | 958.33 ± 13.95 * | N.D. | N.D. | N.D. | N.D. |
| | Dimethachlore | 41.89 ± 2.48 * | 27.43 ± 0.05 * | 6.8 ± 0.56 * | N.D. | N.D. | N.D. | 39.57 ± 1.67 * | 20.85 ± 0.63 * |
| | Dimethenamid-P | <DL | N.D. | <DL | N.D. | 79.63 ± 1.36 * | 61.89 ± 3.09 * | 45.4 ± 2.8 * | 19.55 ± 2.16 * |
| | Dimoxystrobin | 52.91 ± 2.95 * | 38.54 ± 2.9 * | 4.14 ± 0.1 * | 0.43 ± 0.2 * | <DL | N.D. | <DL | <DL |
| | Diphenylamine | <DL | <DL | <DL | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Ethofumesate | 339.48 ± 14.5 * | 257.37 ± 11.73 * | <DL | < DL | N.D. | N.D. | N.D. | N.D. |
| | Fenoxycarb | 277.11 ± 3.64 * | 71.32 ± 9.40 * | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Fenpropidin | 642.67 ± 4.4 * | 593.16 ± 6.47 * | 647.47 ± 5.95 * | 605.23 ± 6.23 * | 656.26 ± 11.4 | 645.83 ± 5.7 | N.D. | N.D. |
| | Flurochloridon | <DL | <DL | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Lambda cyhalothrin | 790.72 ± 1.6 * | 731.6 ± 6.69 * | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Malathion | 816.54 ± 3.89 * | 737.42 ± 10.7 * | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Metolachlor | 11.64 ± 1.20 * | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Myclobutanil | 2241.08 ± 24.17 * | 1737.52 ± 34.14 * | 1397.90 ± 6.57 * | 709.82 ± 21.1 * | N.D. | N.D. | N.D. | N.D. |
| | Pyrimethanil | <DL | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Spiroxamine | 11.7 ± 0.94 * | 5.32 ± 4.64 * | 23.17 ± 0.97 * | 13.74 ± 1.48 * | 86.46 ± 2.05 * | 50.53 ± 1.59 * | N.D. | N.D. |
| | Tebufenpyrad | 821.57 ± 4.73 * | 737.45 ± 13.57 * | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | Tebutam | 66.93 ± 1.81 * | 46.95 ± 2.9 * | 12.57 ± 0.94 * | 7.76 ± 0.38 * | 30.13 ± 1.48 | 22.92 ± 3.85 | 8.9 ± 0.42 * | 4.73 ± 0.89 * |
| | Trifloxystrobine | 544.23 ± 5.21 * | 466.55 ± 16.89 * | 684.69 ± 1.68 * | 579.24 ± 2.85 * | 482.13 ± 1.95 | 473.6 ± 5.26 | N.D. | N.D. |
| ***Σ semi-volatile pesticides*** | | ***10,004.59 ± 4.97*** | ***8173.94 ± 14.44*** | ***5546.04 ± 1.38*** | ***4244.13 ± 3.1*** | ***1425.58 ± 1.16*** | ***1270.16 ± 1.15*** | ***128.59 ± 0.3*** | ***57.64 ± 0.2*** |
| **Σ pesticides (ng/g)** | | 10,074.3 ± 2.6 | 8202.95 ± 7.5 | 5789.54 ± 1.3 | 4367.45 ± 1.83 | 1466.43 ± 0.7 | 1278.53 ± 0.7 | 138.99 ± 0.2 | 65.27 ± 0.2 |

N.D. = not detected, <DL: below detection limit, asterisks denote means that are significantly different at * $p < 0.05$.

The results showed that the orchard was mainly contaminated by alachlor, bifenthrin, buprofezin, cypermethrin, ethofumesate, fenpropidin, lambda cyhalothrin, malathion, myclobutanil, tebufenpyrad, and trifloxystrobine. These pesticides, with the exception of alachlor, bifenthrin, ethofumesate, fenpropidin, and tebufenpyrad, were reported to be frequently used by the farmer as well as in the vicinity of the orchard.

At the start of the sampling campaign, myclobutanil and cypermethrin were the most abundant pesticides detected at Site-A, with a concentration of 2241.08 ± 24.17 and 1184.94 ± 12.64 ng/g, respectively. These pesticides belong to the fungicide and insecticide classes, respectively, which are known to be particularly used for orchard treatments and were previously reported as residues in apple fruits harvested in Lebanon [56].

The analysis of the soil samples collected at the start and the end of the sampling campaign showed a significant decrease in the concentration of all pesticides in the soils sampled from sites A, B, and R after the 18-week sampling period. Conversely, a non-significant decrease in the concentration of fenpropidin, tebutam, and trifloxystrobine was

observed for the soil sampled from Site-C. The decrease observed in the pesticide concentrations at the four sites is mainly governed by their biotic (throughout microorganisms) and abiotic (throughout photolysis and hydrolysis) degradation as well as their volatilization from the soils throughout the sampling period [57,58]. However, the difference in the significant decrease in the concentration of pesticides among the different soils could be related to soil properties such as OM content [59]. In fact, it was shown that the adsorption of pesticides in soils was highly correlated to the OM content, and, therefore, the high OM content calculated for Site-C (27.71 ± 0.67) could be the main reason for the observed non-significant decrease. In addition, the uptake of the pesticides by the organic fraction of the soil is reported to be highly correlated with the partitioning coefficient $K_{OW}$ of the pesticide [60]. Indeed, fenpropidin, tebutam, and trifloxystrobine are characterized by a relatively high log $K_{OW}$ (>3) and were found to be mostly adsorbed onto the organic content of the soil at this site. This contrasted with benoxacore, Dimethenamid-P, and spiroxamine, characterized by a log $K_{OW}$ of 2.7, 1.89, and 2.89, respectively, which were found to be significantly decreased at the end of the sampling period.

The presence of various residues of pesticides in the orchard was reflective of the intensive use of different types of these phytopharmaceutical products for crop protection, especially considering that drift losses can occur during application and could lead to severe environmental contaminations [25]. International regulations and thresholds for the majority of these currently used pesticides do not exist and are mainly reported for highly persistent and no-longer-approved pesticides [61,62]. Ecotoxicological studies are usually used to establish threshold for these chemicals.

The results obtained by the multiresidues analysis of the pesticides in the tested soils were similar to the results in previous studies reporting on the levels of currently used pesticides in agricultural soils. For example, bifenthrin reached up to 884 $\mu g \cdot kg^{-1}$ in the soils collected from Pakistani farmlands [63], and alachlor and cypermethrin were detected in all the soil samples collected from farm fallows in Kenya, each having a different pesticide-application history, in the range of 580–4120 $\mu g \cdot kg^{-1}$ and 290–3130 $\mu g \cdot kg^{-1}$, respectively [64]. In addition, it was shown that carbendazim was the main fungicide detected in more than half of the soils in both arable and vegetable farming systems, and it could still be detected for long durations after its application [65].

### *3.2. Pesticides' Analysis in Snails*

All pesticides' concentrations were initially calculated in ng/g fresh weight (fw) and were then reported in ng/g dry weight (dw) by considering the water content in the snails, which was measured and averaged at 68 ± 4.5%.

#### 3.2.1. Non-Volatile Pesticides' Analysis

The concentration of non-volatile pesticides in ng/g dw in the snails sampled from the four sampling sites over the sampling period (sampling-S0–sampling-S6) are shown in Figure 3.

Among the 30 non-volatile pesticides analyzed, 3 were found above the detection limits at sites A and B, whereas only 2 were found above the detection limits at Site-C at the end of the sampling period (sampling-S6). The results reported in Figure 3 showed that pymetrozine and carbendazim were detected at the three sites in the orchard, while epoxiconazole and foramsulfuron were detected only at sites A and B, respectively.

The results of the total non-volatile pesticides accumulated by the snails at the three different locations of the orchard revealed non-significant differences between sites A and B ($p > 0.05$), whereas significant differences were observed between Site-C and the other two sites ($p < 0.05$). The obtained results show that Site-A accumulated the highest concentration of these pesticides, with a total concentration of approximately 170 ± 2.54 ng/g dw, with carbendazim and epoxiconazole recording the most-accumulated pesticide. As for sites B and C, the total concentration of accumulated pesticides was approximately 150 ± 4.12 and 94.5 ± 2.6 ng/g dw, respectively, with pymetrozine being the major non-volatile

pesticide found at these sites. As for Site-R, the sole accumulation of carbendazim by the caged snails could be due to its presence in the soil used beneath the snails at this site. The physico-chemical properties of the aforementioned pesticides, including their high solubility in water and their low log $K_{OW}$ (Table S2), did not favor their accumulation in the snails' tissues. Accordingly, the occurrence of these non-volatile pesticides in the tested snails during the study could be due to the numerous exposure routes, particularly the cutaneous and digestive ones [48]. As for the major presence of pymetrozine at the three sites of the orchard, this could be mainly due to its prior use, as reported by the farmer, for protection against homopteran insects that may feed on the apple foliage and lead to the development of sooty fungus [66]. It is noteworthy that the final concentration of the non-volatile pesticides quantified in the snails at the end of the sampling period at the three sites did not exceed the European Union (EU) Maximum Residues Limits (MRL) threshold [67].

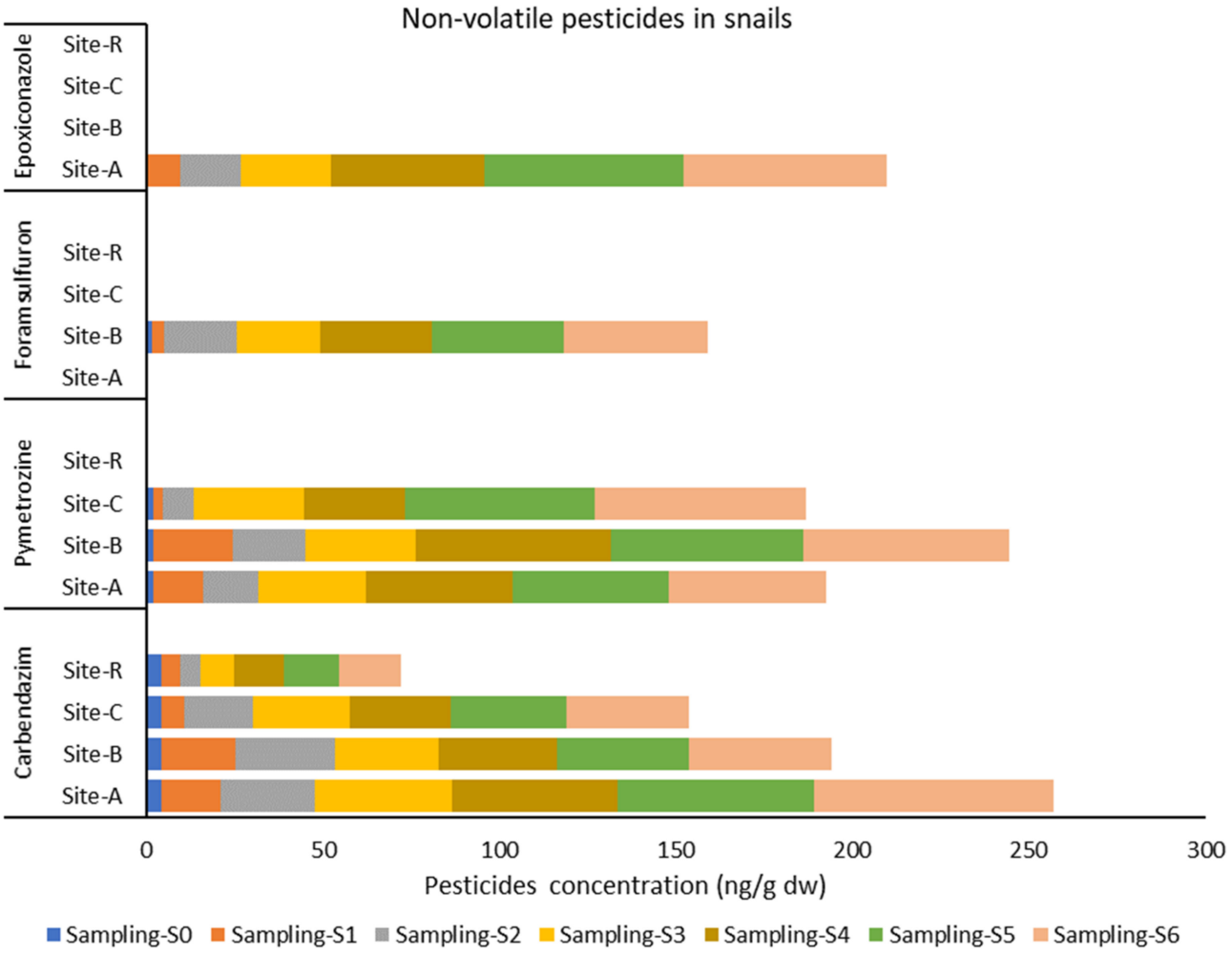


**Figure 3.** Concentration of non-volatile pesticides (ng/g dw) in the snails sampled from the four sampling sites.

### 3.2.2. Semi-Volatile Pesticides Analysis

The concentration of the semi-volatile pesticides in ng/g dw in the snails that were sampled at the four sampling sites over the sampling period (sampling-S0–sampling S6) are shown in Figure 4.

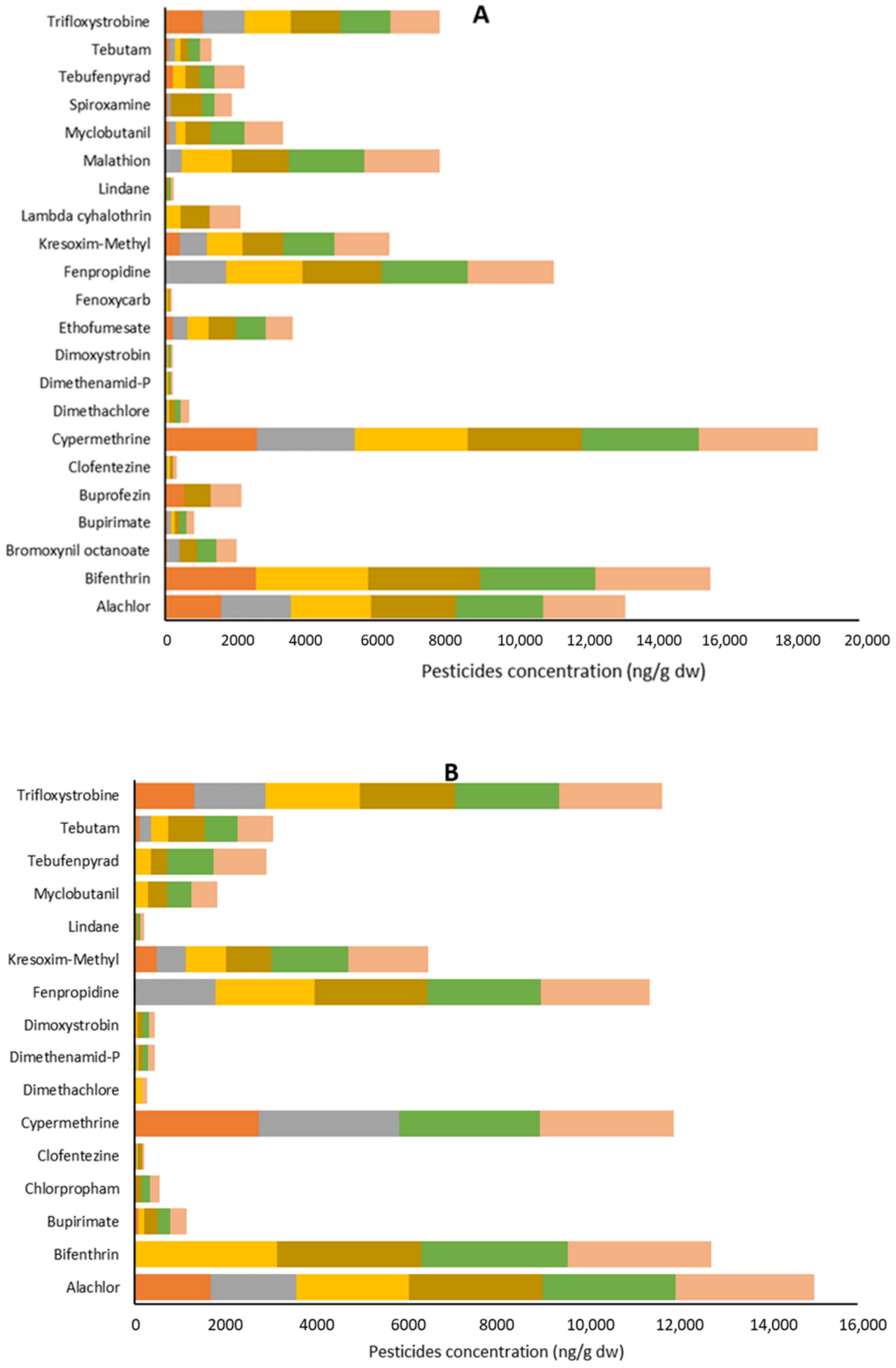


**Figure 4.** *Cont.*

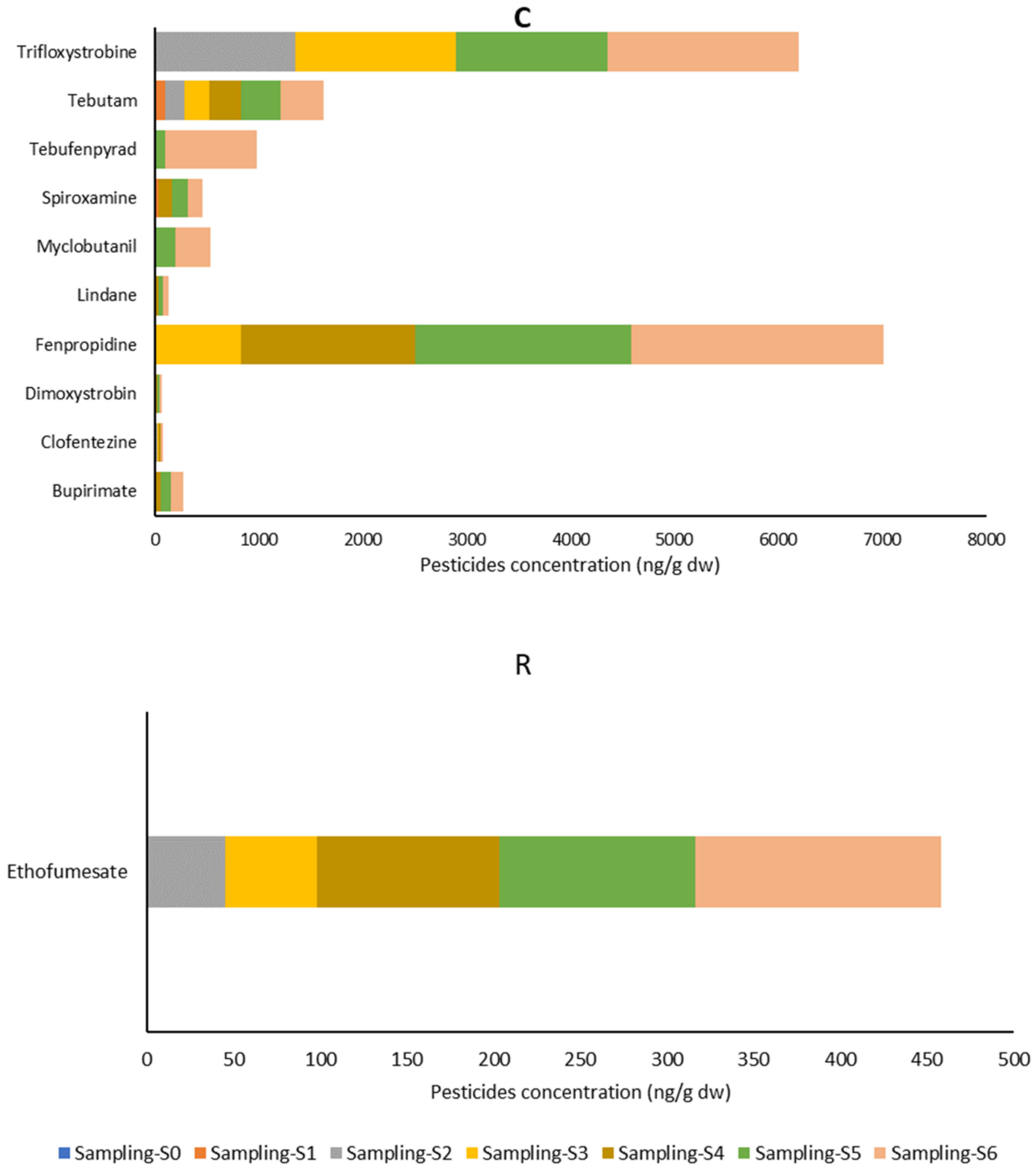


**Figure 4.** Concentration of semi volatile pesticides (ng/g dw) in the snails sampled from the four sampling sites, A: site-A, B: Site-B, C: Site C and R: Site-R.

Among the 70 targeted semi-volatile pesticides, 22 were quantified at the end of the study in the snails caged at Site-A, which was the most contaminated site of the orchard, as expected (Figure 4, see site-A). The overall classification of these pesticides, presented in Section S2, included nine insecticides, six herbicides, and seven fungicides. The concentrations of these 22 pesticides ranged from 57.32 ± 0.45 to 3427.14 ± 13.26 ng/g dw. Cypermethrin and bifenthrin accounted for around 14.5% of the 22 semi-volatile pesticides, respectively, and were the main pesticides accumulated by the tested snails. In addition,

Fenpropidin, alachlor, and malathion were also found at relatively high concentrations in the tested snails, where they each accounted for about 9–10% of the 22 semi-volatile pesticides quantified at sampling-S6. Out of these five pesticides, cypermethrin and malathion were previously used for the treatment of the orchard and the neighborhood as well. In addition, all these pesticides were found at high concentration in the soil sampled at Site-A (Table 1). Regarding lambda-cyhalothrin and myclobutanil, which were applied during the campaign, they each accounted for approximately 4–5% of the 22 semi-volatile pesticides, with a final concentration of about 907.04 ± 10.7 and 1119.44 ± 17.44 ng/g dw, respectively.

For Site-B, which was located at the border of the orchard and 27 m from Site-A, 16 out of the 70 semi-volatile pesticides assessed were quantified in the snails at sampling-S6 (Figure 4, see site-B). These pesticides encompassed five insecticides, five herbicides, and six fungicides. The concentrations of the 16 pesticides at this site ranged between 50.32 ± 0.26 and 3166.1 ± 12.58 ng/g dw, with bifenthrin, alachlor, and cypermethrin being the major compounds, where each contributed about 16% of the 16 quantified pesticides. In addition, trifloxystrobin and fenpropidin were also found at a high concentration at this site, where each accounted for about 12% of the 16 pesticides quantified at the end of the study. However, among the two pesticides applied during the sampling period, myclobutanil was the only one found at the border of the orchard, with a concentration of 570.2 ± 5.68 ng/g dw (sampling-S6).

As for Site-C, which was located outside the orchard, 10 out of the 70 semi-volatile assessed pesticides were quantified at sampling-S6. Overall, three insecticides, one herbicide, and six fungicides were found at this site. The concentration of these 10 pesticides ranged between 11.2 ± 1.32 and 2423.4 ± 3.25 ng/g dw (Figure 4, see site-C). Trifloxystrobin and fenpropidin were the major pesticides found at Site-C, accounting for about 68% of the 10 identified pesticides. Lambda-cyhalothrin was not detected at Site-C, whereas the concentration of myclobutanil reached 342.25 ± 3.89 ng/g dw at Site-C at the end of the study.

Ethofumesate was the only chemical detected at Site-R starting at sampling-S2, even though it was not detected in the soil sample. This could be due to off-site emission that may have occurred in the surrounding area or due to the presence of this pesticide in the soil at a concentration below the detection limit, which progressively accumulated in the snails over the sampling periods.

The analysis of the accumulated total semi-volatile fraction revealed similar trends as those for the non-volatile ones, where non-significant differences were found between sites A and B ($p > 0.05$). Conversely, significant differences were found between Site-C and sites A and B ($p < 0.05$). The microcosm placed at Site-B was located 27 m downwind of the center of the orchard and 10 m away from the last treated apple tree and, thus, could be influenced by pesticides' emissions and drifts (the dominant wind direction in the area during the study was from south to north). Moreover, the soils that were placed at the floor of the cages underneath the snails revealed significant background levels of these pesticides (Table 1). These soils could, in addition to the digestive and cutaneous exposure tracks, contribute to respiratory exposure of the invertebrate through the volatilization of these semi-volatile pesticides and their transfer to the ambient air above the field [68,69]. The differences in the behavior of myclobutanil and lambda-cyhalothrin for the different sites could be attributed to the vapor pressure pertaining to these two compounds and could potentially explain the absence of lambda-cyhalothrin at sites B and C [59]. In fact, at ambient temperature, myclobutanil (vapor pressure: $1.6 \times 10^{-6}$ mmHg) could exist at both the vapor and particulate phases of the atmosphere, while lambda-cyhalothrin (vapor pressure: $3.35 \times 10^{-9}$ mmHg) is found mostly in the particulate phase [70].

In addition, it is noteworthy that the final concentration of the reported semi-volatile pesticides quantified in the caged snails at the end of the study exceeded the MRL for pesticides in terrestrial invertebrates promulgated by the EU. This could potentially threaten the entire ecosystem in the treated areas [67,71].

The comparison between both targeted classes of pesticides (semi-volatile and non-volatile pesticides) showed a significantly higher level of semi-volatile pesticides ($p < 0.05$). These results are in accordance with previous studies, showing that semi-volatile pesticides are frequently detected in both gas and particulate phases of the atmosphere, while non-volatile pesticides are typically found to be associated with the particulate phase [25,72].

#### 3.2.3. Total Pesticides' Accumulation in Snails

The results (Figure 5) showed that *C. aspersum* is an efficient biomonitor for environmental pollution, as they immediately accumulated significant amounts of pesticides (S0 to S1) that are bioavailable in their surroundings. At the end of the sampling campaign, the total concentration of pesticides in the snails reached 23,717.1 ± 30.2 and 19,396.9 ± 29.1 ng/g dw at sites A and B, respectively, whereas the corresponding concentration at sites C and R reached 6368.8 ± 9.5 and 159.5 ± 3.5 ng/g dw, respectively. The relatively high amount of pesticides found at sites A and B could be mainly due to the broad spectrum of these compounds that were conventionally used about 10–15 times/year for the treatment of apple orchards [73]. Conversely, no pesticides were applied at sites C and R. The difference in the levels of pesticides quantified in the caged snails among the different sites showed that snails could be used as active biomonitors for environmental pollution and as a reliable alternative for commonly used integrative sampling techniques, despite the limited amount of the literature on this topic [40]. These results are in accordance with previously reported ones by Baroudi et al., 2021, where *H. aspersa* accumulated significant levels of pesticides when they were exposed to agricultural sites [42]. Itziou and Dimitriadis also reported that land snails could serve as early detectors of pollutants, due to the significant changes that might occur in their haemolymph and their digestive glands while exposed to these pollutants [74].

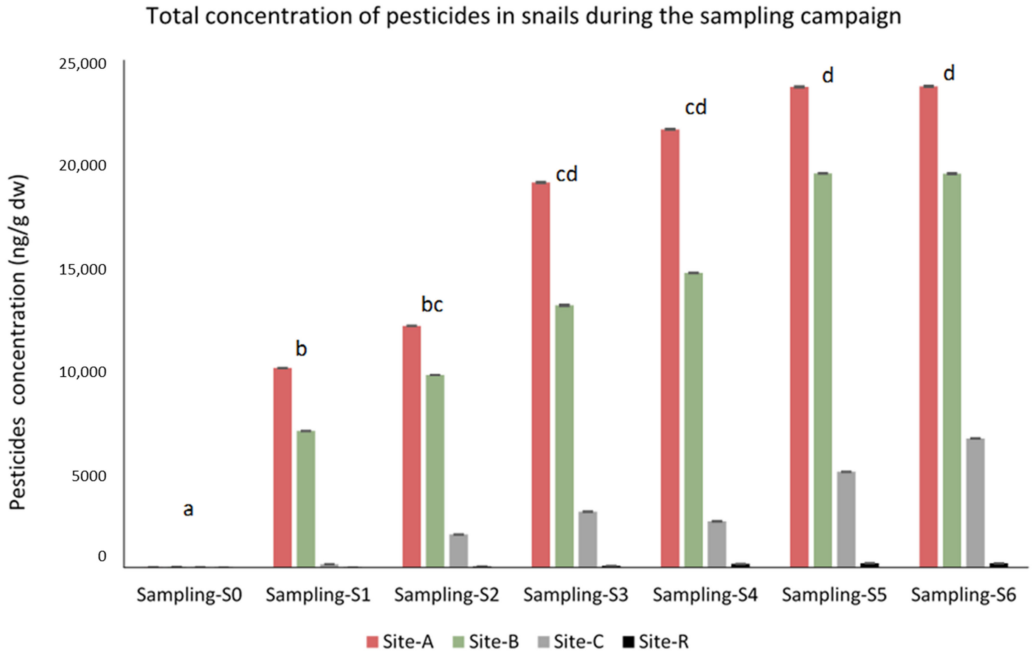


**Figure 5.** Concentration of total pesticides (ng/g dw) in the snails sampled from the four sampling sites, Site-A: middle of apple orchard, Site-B: border of apple orchard, Site C: control spot of apple orchard, Site-R: reference site (Aamiq). The data are presented as mean ± RSD (n = 3 for each site). Differing lowercase letters shown represent significant difference between the total concentration of pesticides (semi-volatile and non-volatile) accumulated by the snails and quantified on each sampling date based on Fisher pairwise comparisons (95% confidence level).

The presence of significant amounts of semi-volatile pesticides at Site-C (6274.4 ± 7.8 ng/g dw), even though it was located outside the orchard, indicated that

these pesticides originated from sites A and B and from the other orchards in the surrounding area. Accordingly, off-site pesticide emissions originating from the direct impact zone could contaminate surrounding areas. Similar observations have been reported by other studies, showing that drifts and deposition of pesticides could occur in off-target areas beyond 60 m from the target pesticide-application area [75]. These airborne drift losses could reach an average of 35% of the applied pesticides in apple orchards [76]. It is worth noting that the absence of agricultural production and pesticide emissions at Site-R allowed its use as a reference site, where non-significant pesticides emissions and uptakes were observed.

Despite showing an increasing trend in pesticide concentration in the snails with increased field exposure (irrespective of the sampling site), the observed increase appeared to be less significant over two consecutive sampling dates ($p > 0.05$), whereas extended exposure could result in a more significant accumulation. In addition, the results presented in Figure 5 showed that at the end of the study a non-significant increase in the concentration of total pesticides (comparable to a plateau) was observed ($p > 0.05$). This phase represented a steady-state condition, where an equilibrium between the uptake and elimination processes could occur during a prolonged exposure to chemicals [77]. This steady state represents where the bioaccumulation factors of the pollutants could be determined [78]. However, the observed condition appeared to be slightly increasing, showing that a perfect equilibrium was not achieved. These results are similar to previous observations, showing that steady state is never effectively achieved in real field measurements [79–81]. It was shown that the changes in the bioavailability of the pollutants, as well as the presence of unaccounted routes for the uptake of the pollutant, could alter and prevent the establishment of steady-state conditions [82]. These two factors appear to match the conditions in this study, where the concentrations of pesticides in the environment surrounding the caged snails are undetermined and where several uptake routes (air-soil) could have been used by the snails.

## 4. Conclusions

In this study, the land snail *C. aspersum* Müller was used as an efficient active biomonitor for the environmental contamination of an apple orchard by pesticides. The snails, caged in microcosms and installed at different locations, were tested for their ability to biomonitor 100 different pesticides during an 18-week sampling campaign. The results revealed increased levels of pesticides in the snails with increased exposure. Greater levels of pesticides were accumulated by snails sampled from the center of the orchard, which was intensively exposed to pesticides. In addition, the results obtained for the snails located outside the orchard (Site-C) demonstrated the suitability of *C. aspersum* as a potential biomonitor for pesticide emissions, off-site drifts, and deposition. However, the almost stable meteorological conditions for the duration of this study did not favor further investigation of the implications of these meteorological parameters on the transfer of pesticides off site. Additional long-term monitoring studies under more controlled conditions could be, therefore, considered to investigate the fate of pesticides in the environment and the efficiency of these species to provide, under more fluctuating meteorological conditions, realistic monitoring of environmental pollution by pesticides.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos13081185/s1, Section S1: List of assessed pesticides; Table S1: Method validation parameters; Table S2: Partitioning coefficient log $K_{OW}$ of assessed pesticides; Section S2: Non-volatile pesticides classification.

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