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REVITALIZATION OF PESTICIDE-POLLUTED AGRICULTURAL SOIL FUNCTIONS BY MICROBIOME TRANSPLANTATION

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Abstract

The continuous application and often the overuse of pesticides in agricultural soils influence in time soil microbiota abundance and metabolic activities, with final potential negative effects on soil functioning. Recently, bioaugmentation has emerged as an advantageous method for revitalizing agricultural soils affected by chemicals use. In this study, the hypothesis that transplantation of soil cores with optimal microbiota community structure could serve in affected soil microbiota restoration and pesticides degradation enhancement was evaluated. To assess soil core transplantation efficiency, in this study were investigated implication of soil microbiota structure and abundance in pyrethroids degradation in soil, as well pyrethroids impact on soil microbiota metabolic activity. The presence of pyrethroid pesticides as cypermethrin, deltamethrin and fenvalerate in agricultural soils from Turda was evidenced within range of 119 – 845 $\mu g \cdot kg^{-1}$. They negatively impacted both soil bacterial and fungal community abundance, decreasing them with approximately 50%. The impact of transplanted soil cores on both soil microbiota abundance as well on cypermethrin, deltamethrin and fenvalerate concentration was also evidenced. A revitalization of microbiota abundance in contaminated soil was observed after transplant (increase with 1.5-fold generally). These data positively sustain that affected soil microbiota due to use of pyrethroid pesticide could be revitalized through transplant of soil cores with no contamination.

Keywords: biodegradation, catabolic activity, microbiota, pyrethroid pesticide, soil transplant

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1. Introduction

Use of pesticides has become a requirement in current agriculture practices that must meet the demands of a continuously growing population. The continuous application, and often the overuse of pesticides in agricultural soils influence in time soil microbiota abundance and metabolic activities, with final potential negative effects on soil functioning. According to literature, pesticide use could impact soil microbiota community abundance and structure in different ways. Once, it could decrease their abundance because of direct toxic effects on them (Bhatt et al., 2021; Diaz-Lopez et al., 2019). Secondly, it could increase their abundance once that applied pesticides could supply a specific nutrient source (Bhatt et al., 2019; Cui et al., 2018; Wakelin et al., 2010) or because of the negative impact on microbial predators (Atwood et al., 2018; Rasmussen et al., 2013). Also, reports show no evidence of direct or indirect impacts of pesticides on soil microbiota community abundance and structure (Zabaloy et al., 2016; Zhang et al., 2009).

In addition, a mixture of pesticides at different doses under different environmental conditions (climatic, pedologic etc.) can deviate from the

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behavior of an individual pesticide due to the occurrence of synergistic (Zhang et al., 2020), antagonistic (Janssens and Stoks, 2013) or additive interactions (Madakka et al., 2017) between and among them (Singh et al., 2018). Microbiota is recognized as an important component of soil which is involved in several key functions (organic matter decomposition, nutrients cycling, chemicals degradation, etc.) that assure optimal soil properties and provision with ecosystem services (Birolli et al., 2019; Jouquet et al., 2011; Wu et al., 2020). However, studies have revealed that use of pesticides could alter the soil microbiota community structure (Mastro et al., 2020; Milan et al., 2018) and metabolic activities (Bhatt et al., 2021; Delorenzo et al., 1999; Madakka et al., 2017), with end results as the alteration of soil properties (El-Mageed et al., 2020, Saez et al., 2018), and loss of soil functions (Berner et al., 2011). These in turn could lead in loss or diminish of several provided ecosystem services (Jouquet et al., 2011; Wu et al., 2020).

Bioaugmentation, defined as an improvement of potential contaminated soil properties and functioning by introducing specific microorganisms or communities, has emerged as an advantageous method for revitalizing agricultural soils affected by chemicals use (either organic or inorganic pesticides) (Bhatt et al., 2021; Cycon et al., 2017; Saez et al., 2018). Considering that previous research studies evidenced in agricultural soils with pesticides use management a loss of autochthonous microorganisms that enable their degradation (Chen et al., 2014; Huang et al., 2020; Karpouzas et al., 2014; Verhagen et al., 2014) or loss of their catabolic pathways required to metabolize pesticides (Delorenzo et al., 1999; Widenfalk et al., 2008), use of bioaugmentation could rely on the enhancement of the catabolic potential of microbial communities' structure for the degradation of pesticides (Cycon and Piotrowska-Seget, 2016; Sessitsch et al., 2019). Although the application of soil inoculation with selected single strains of bacteria/fungi or with genetically engineered microorganisms, which exhibit an enhanced ability to degrade a wide range of toxic pollutants could have positive effects on soil functions restoration, it could also be improper for application in common affected agricultural soils due to the involved costs and required infrastructure (Shahid et al., 2018).

This work aimed to find optimal solutions, based on bioaugmentation and inoculum theories, to improve properties of pesticides use affected agricultural soils. We hypothesized that transplantation of soil cores with optimal microbiota community structure could serve in affected soil microbiota restoration and pesticides degradation enhancement. Moreover, use of this technique may also be applicable even to more limited budgets. To assess soil core transplantation efficiency, in this study were investigated implication of soil microbiota structure and abundance in pyrethroids degradation in soil, as well pyrethroids impact on soil microbiota metabolic activity.

2. Material and methods

2.1. Case study and sampling

Contaminated soils with pyrethroid pesticides were taken from the surface horizon (0 – 50 cm) from an agricultural land from Turda (46°34'15"N, 23°46'45"E; elevation: 362 m; slope 11°), Romania. Samples were a vertic Phaeozem (USST) soil type with a silty clay loam texture (sand 13.2 %, silt 56.3% clay 30.5%). Soil main physicochemical parameters vary as follows between 0 – 50 cm depth: pH 5.3 – 6.1 (1:1 H_2O); humus content: 1.34 – 1.94% (Walkley-Black method); total nitrogen: 0.118 – 0.157% (Kjeldahl method), mobile phosphorous 0.3 – 3.2 ppm (spectrophotometric method developed by Murphy and Riley, 1962); and 89 – 135 ppm potassium (flame photometer method). During study period (2018), in Turda 8.2 °C was the mean annual temperature and 522 mm was the mean annual precipitation.

Free of pyrethroid pesticides residues soils as cores for transplant were collected from the surface horizon (0 – 50 cm) from a natural grassland from Jucu (46°51'05"N, 23°27'24"; elevation 325 m, slope 9°). Selection was made considering similarities as climatic characteristics of region, soil texture, and soil physicochemical properties. Transplant cores were entic hapludoll (USST) soil samples with silty clay loam texture (sand 11%, silt 57.2% clay 31.8%). Physicochemical properties between 0-50 cm varied as follows: pH: 6.3-6.9 (1:1 H_2O); humus content: 2.21-2.94% (Walkley-Black method); total nitrogen: 0.151-0.217% (Kjeldahl method); mobile phosphorous: 7-22 ppm (colorimetric method with ammonium molybdate); and potassium: 117-196 ppm (flame photometer method). Mean annual temperature and mean annual precipitation in Jucu for the study period (2018) were 9.2 °C and 549 mm. Contaminated soils samples were collected in March 2018 and transplant soil cores were collected in April (5 cores) and September (4 cores) 2018, respectively.

2.2. Soil core transplantation experiment

Contaminated soil was placed in a 50 cm (height) x 50 cm (width) x 70 cm (length) dark colour plastic box without disturbing soil distribution on layers. This box with contaminated soil was placed in a controlled temperature (20-30°C) and humidity chamber. The water holding capacity (WHC) of soil was maintained at 57% during the whole period of the experiment, March-November 2018. The following day-night cycle was applied: 14 h of light/10 h of night in period of March-May; 16 h of light/8 h night for period of June – August; and 14 h of light/10 h of night in the period of September-November. In April, the collected 5 soil cores (C1-C5, each with 10 cm diameter) were transplanted in contaminated soil box according to Fig. 1, representing the first transplant). In similar conditions, other 4 cores (C6-C9) were transplanted in September (these were the second transplant).

Soil box samples were collected for analysis according to Fig. 2. Approximately 25 g of soils were collected from each sampling point at established sampling date during the experiment. During each sampling time 4 samples were collected from transplanted soil cores (0 – top, 15 cm, 30 cm and 50 cm depth) and six samples from approximately 7 – 10 cm near to core (three samples from 0 position – the top, and 3 samples in soil depth profile as previously detailed). Samples were divided as follows: 15 g of soil for analysis of microbiota structure/abundance, microbiota catabolic activity, basal respiration analysis, esterase, and dehydrogenase soil extracellular enzyme analysis; and 10 g for pyrethroid pesticides analysis.

2.3. Pesticide GC-ECD analysis

Pyrethroid pesticides as cypermethrin, fenvalerate, and deltamethrin were targeted through this study. Their selection was based on a previous screening of the site from where the contaminated soil was collected. Obtained results have shown an average value (n = 15) for cypermethrin, fenvalerate, and deltamethrin of 608.2, 210, and 388.6 $\mu g \cdot kg^{-1}$, respectively (Table 1).

An amount of 5 g of soil samples were used for targeted pyrethroid pesticides extraction. Soil sample with 10 mL of acetone and 3 g of NaCl was placed in a 50 mL Falcone tube. This mixture was centrifuged at 5000 rpm for 5 min. Other 10 mL of mixture of ethyl acetate and cyclohexane was added (1:1, v/v) to the centrifuged mixture, and allowed again for centrifugation for 5 min at 10 000 rpm. The solvent layer was filtered through a glass fiber filter with 5 g of anhydrous sodium sulphate. The collected solvent effluent was evaporated under vacuum to 1 mL. For solid phase extraction, this extract aliquot was diluted with 5 mL of n-hexane. Florisil cartridges were used for the separation of pyrethroid pesticides extraction. Elution was performed using an ethyl acetate and n-hexane mixture (5 mL, 5 + 95%, v/v).

Eluted pyrethroids were concentrated under vacuum to 1 mL, of that 1 µL was injected into a gas chromatograph with electron capture detector (7890A GC-µECD, Agilent Technologies, CA, USA) with split/splitless injector. DB-5MS (5% phenyldimethylsiloxane) fused-silica capillary column with 30m, 320 µm i.d. x 0.25 µm film thickness was used for the separation of target compounds. He and N_2 were used as carrier and make up gas at flow rates of 1 and 5 $mL \cdot min^{-1}$, respectively. The injector and detector temperature were set at 250°C and 300 °C. The applied temperature program for oven was as follows: 80 °C for 5 min, followed by an increase with $10°C \cdot min^{-1}$ until 270°C. This final temperature was maintained isotherm for 10 min. For quantitative analysis, an external standard quantification mode was applied. Main parameters of method are given in Table 2.

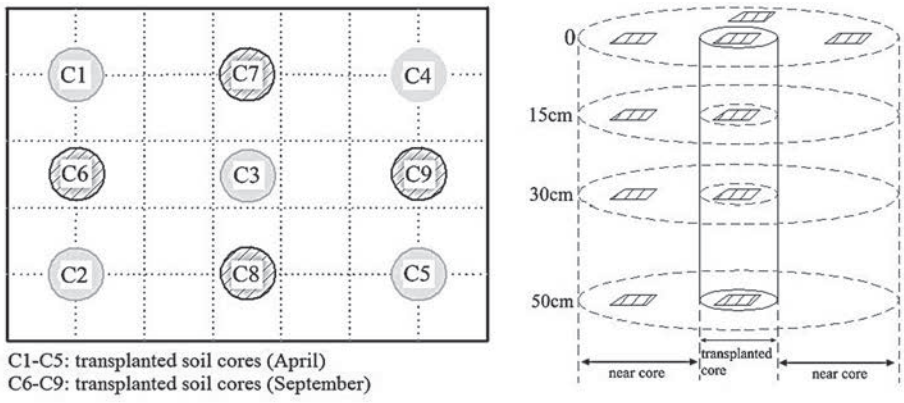


Fig. 1. Schematic presentation of soil core transplants and sampling points in soil experiment box

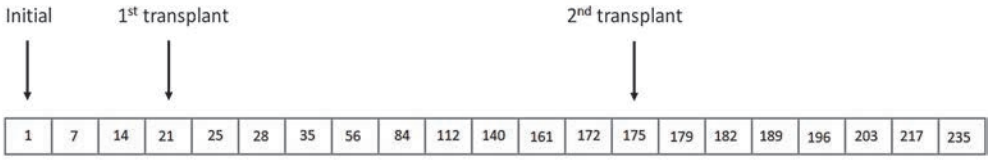


Fig. 2. Schematic presentation of soil samples collections from experimental box (numbers represent the days of experiment)

Table 1. Cypermethrin, fenvalerate and deltamethrin concentrations (n = 15) of agricultural land from Turda

Pyrethroid pesticide	***Average*** ***($\mu g \cdot kg^{-1}$)***	***Min*** ***($\mu g \cdot kg^{-1}$)***	***Max*** ***($\mu g \cdot kg^{-1}$)***	***RSD*** ***(%)***
Cypermethrin	608.2	328.9	895.2	25.3
Deltamethrin	388.6	219.6	558.9	26
Fenvalerate	210	119.1	304.9	24.3

Table 2. Cypermethrin, fenvalerate and deltamethrin gas chromatographic analysis method performance

Pyrethroid pesticides	*Linearity (µg·kg^{-1})*	*r^2*	*LOD (µg·kg^{-1})*	*LOQ (µg·kg^{-1})*	*Recovery (%)*	*Precision (%)*
Cypermethrin	10 - 1000	0.9967	0.162	0.54	82.3	7.2
Fenvalerate	10 - 1000	0.9992	0.219	0.73	89.2	5.8
Deltamethrin	10 - 1000	0.9991	0.098	0.33	105.2	3.7

Targeted pyrethroid pesticides analytical method for soil was evaluated in terms of linearity, precision, accuracy, and detection limits. The method was validated according to procedures described before, specifically based on the extraction of spiked and non-spiked vegetables samples. Spiked soil samples were prepared with accurately weighted 5 g of soil free of interest pesticides. For each pyrethroid pesticide in part, the soil sample mixed with 2 mL of n-hexane containing an appropriate amount of pesticide to give the final concentration in the soil samples of 50 µg·kg^{-1}, were placed into 50 mL Falcon tube. The solvent blank was run with each set of samples extraction to check for contamination from the preparative steps and laboratory background levels. Six spiked soil samples for each pyrethroid pesticide with interest were used in the recovery assay. Repeatability and reproducibility were determined at 25 and 250 µg·kg^{-1} theoretical spiked amount in soil. For repeatability experiment, six extraction and gas chromatographic analysis of spiked soils was performed in the same day. Reproducibility experiment was performed two times per day for a 5 five consecutive days period. Both repeatability as well reproducibility experiment was realized for each mentioned spiking levels. The limits of detection (LOD) were calculated as three times the signal of the background noise obtained in the lowest spiked sample (10 µg·kg^{-1}) at the retention times of the corresponding analytes, and the limits of quantification (LOQ) were determined considering a value of ten times the background noise.

These results are summarized in Table 1. Because obtained LOQs were lower than 0.5 µg·kg^{-1}, therefore, the applied method was considered with good sensitivity and selectivity for the determination of target pyrethroid pesticides from soil matrices. Hence, the method was considered suitable for proposed experimental applications.

The calibration curve of targeted pharmaceuticals active ingredients was realized analyzing seven concentrations from 10-1000 µg·kg^{-1}. Calibration curves correlation coefficient (r^2), and recoveries (%) are listed also in Table 1. The precision of the method, expressed by the relative standard deviation of mean recovery values, when spiked samples were analyzed (within and between days) ranged from 1 to 20% for all matrices (Table 1).

2.4. Soil microbiota PLFA analysis

Soil microbiota structure and abundance were assessed based on phospholipids derived fatty acids (PLFA) analysis by gas chromatography with flame ionization detector (7890A GC-FID, Agilent Technologies, CA, USA). 1.5 g of freeze-dried soil samples were used for PLFA extraction according to the method described by Frostegard et al. (2011) and Frostegard et al. (1993). Microbial lipids were extracted with Blight and Dyer mixture. Lipids were fractionated into phospholipids, glycolipids, and neutral lipids with a silicic acid column (500 mg). Mild alkaline methanolysis was used to recover fatty acid methyl esters. 150 µL of final extract was used for gas chromatographic analysis. Fatty acid methyl esters separation was done on a polyethylene glycol stationary phase DB - WAX column (Agilent J&W) with 30 m x 0.25 mm I.D., 0.25 µm characteristics.

The applied temperature program for the oven was: 80 °C (hold for 3 min), increased at 150°C with 7 °C·min^{-1}, hold at this temperature for 5 min and then followed by an increase with 15 °C·min^{-1} at 250 °C. This final temperature was maintained isotherm for 8 min. The splitless mode was used for sample injection at 250 °C. Helium flow was set at 1 mL·min^{-1}. In Table 3 are presented the PLFA markers for soil microbiota structure and abundance identification and quantification, respectively.

2.5. Assessment of soil microbiota metabolic activity

Potential changes in soil microbiota functional diversity were identified by microbiota catabolic fingerprint assessment estimated from carbon (C), nitrogen (N) and phosphate (P) consumption from sole nutrient sources. The following sources were used:

(i) The sole C – carbohydrates (d-cellobiose, α-d-lactose, β-methyl-d-glucoside, d-xylose, i-erythriol, d-manitol, d-galactonic acid γ-lactone), phenolic compounds (2-hydroxy benzoic acid, 4-hydroxy benzoic acid), polymers (tween 40, tween 80, α-cyclodextrin, glycogen);

(ii) C x N – carbohydrates (N-acetyl-D-glucosamine), carboxylic acid (d-glucosaminic acid), amino acids (l-arginine, l-asparagine, l-phenylalanine, l-serine, l-threonine, glycyl-l-glutamic acid), amines (phenylethylamine, putrescine);

(iii) C x P – carbohydrates (glucose-1-phosphate, d,l-α-glycerol phosphate).

A sample of 1 g of soil was used for bacterial cell isolation with low enriched phosphate buffer (0.1 M). Inoculation of substrates containing redox dye tetrazolium salt was accomplished with 150 µL of cell extract. Incubation was performed at 30 °C for 168 h. Absorbances at 590 nm were taken at every hour during first 24 h followed by absorbance measurements at every 6 h for the rest of period.

2.6. Soil basal respiration analysis

Basal respiration of soil heterotrophic microbiota was performed using a 40 mL glass chamber closed with Teflon faced rubber liner caps. A sample of 5 g soil (adjusted at 60% water holding capacity) was placed with 0.2% KOH solution in the 40 mL glass chamber and allowed for incubation for 10 days period at 21 °C (LabCompanion, CA, USA). 1 mL of gas samples from the headspace of closed chambers were sampled with a gas-tight syringe through chamber septum and analyzed with a thermal conductivity detector of a 7890 Agilent gas chromatograph (7890A GC-FID/TCD/TCD, Agilent Technologies, CA, USA). Gas chromatographic analysis conditions were as follows: inlet temperature 200°C, splitless injection mode, detector 250°C, He flow 2 mL·min^{-1}. The oven temperature program started with 60°C (hold for 2 min), increase with 15°C·min^{-1} until 190 °C (hold for 3 min). Released CO_2 was quantified with external standard quantification mode using gas mixtures certified reference materials (Linde, Germany). Calibration curve correlation coefficient was 0.9998 and limit of quantitation was 0.12% mmol CO_2. Blank measurement was performed using closed chambers without soil samples on each batch analysis. Basal respiration variation (V_{BR}, %) of soil heterotrophic microbial biomass was determined by Eq. (1):

$$V_{BR}(\%) = \left(\frac{CO_{2,soil} - CO_{2,blank}}{CO_{2,blank}}\right) \cdot 100 \quad (1)$$

2.7. Soil esterase and dehydrogenase assay

Soil esterase (*E.C. 3.1.1.1.*) analysis was performed according with method described by Sakai et al. (2002) and Tsuboi et al. (2018) with modifications as follows: 0.5 g of moist soil samples were placed in a 5 mL plastic tube with 1 mL of 2 mmol·L^{-1} p-Nitrophenol valerate in tris (hidroxymethyl aminomethane (Tris)-maleic buffer (0.5 mol·L^{-1}, pH 6.0). Tubes were incubated at 30 °C for 30 min and shacked continuously during this time at 15 rpm (Cole-Parmer, IL, USA). Incubated soil samples were centrifuged at 15 000 x g for 7 min. From each tube, 100 µL of supernatant was collected and mixed and vortexed for few seconds with 250 µL ethanol (100%) and 75 µL of 2 mol·L^{-1} Tris. The absorbance of solutions mixture was measured at 405 nm by microplate reader (SpectraMax iD3, Molecular Devices, USA). Blank was the absorbance of pNP-valerate in the buffer without soil while background was the absorbance of pNP-valerate of each soil extract mixture without the substrate. Esterase amount was expressed in nmol · g^{-1}min^{-1}.

Soil dehydrogenase (*E.C. 1.1.1.*) analysis was determined according to the method described by Garcia et al. (1997). 1 g of soil was placed in 5 mL test tube with 200 µL of 0.4% 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) solution. This was allowed for incubation at 22 °C for 20 h. 10 mL of methanol was used to extract formed iodo-nitrotetrazolium formazan (INTF). Prior measurement at 490 nm (SpectraMax iD3, Molecular Devices, USA), the methanolic extract was vortexed for few seconds, followed by a filtration step through a filter paper (Whatman No. 5). Results were expressed as µg INTF·g^{-1}.

2.8. Statistical interpretation of data

Effect of microbiota phenotypic structures and abundance on pyrethroids degradation in soil was considered under first-order kinetic model adapted after Cycon et al. (2009). Eq. (2) was used to describe the rate of reduction of each studied pyrethroid pesticide.

$$C_t = C_0 \cdot e^{-kt} \quad (2)$$

Table 3. Used PLFA biomarkers for soil microbiota phenotypic structure and abundance assessment

Microorganisms	***PLFA biomarkers***	***References***
Gram positive bacteria	i14:0; a14:0; i15:0; a15:0; i16:0; a16:0; i17:0; a17:0; (Branched PLFA)	Frostegard et al. (1993); Kourtev et al. (2002); Zheng et al. (2013)
Gram negative bacteria	i15:0-3OH; i15:1; 2OH-16:1; i16:1; 16:1ω5c; 16:1ω7c; 16:1ω9c; i17:1; 17:1ω8c; 18:1ω5c; 18:1ω7c; 18:1ω9c; cy17:0; 18:0; cy19:0;	Frostegard et al. (1993); McKinley et al. (2005); Zheng et al. (2013)
Aerobe	18:1ω10; 18:1ω9; 18:1ω7; 16:1ω7c;	Vestal and White (1989)
Anaerobes	cy17:0, cy19:0;	Molineux et al. (2015)
Methanotrophs	16:1ω8c; 16:1ω5c;	Piotrowska-Seget and Mrozik (2003)
Nitrate reducing bacteria	18:2ω6c; 18:3ω3	Veuger et al. (2013)
Sulfate reducing bacteria	17:1 ω7c; 10Me16:0; 17:1ω6; 15:1; i17:1ω7c; cy18:0ω7,8; i15:1ω7c; i19:1ω7c;	Frostegard and Baath (1996); Molineux et al. (2015)
Actinomycetes	10Me16:0; 10Me18:0; 10Me17:0;	Yu et al. (2009)
Fungi	18:2ω9; 18:1ω9; 18:3ω6; 18:3ω3	Frostegard and Baath (1996)
Arbuscular mycorrhizal fungi	16:1ω5	Kaiser et al. (2010); Olsson (1999)
Saprotrophic fungi	18:2ω6c	Frostegard et al. (2011)
Ectomycorrhizal fungi	18:2ω9c	Joergensen and Wichern (2008)

In Eq. (2), C_0 was the initial concentration of considered pyrethroid pesticide (cypermethrin, deltamethrin, fenvalerate) at time zero, C_t is the concentration of pyrethroids at time t, k is the degradation rate constant (day^{-1}), t is the degradation period in days. The half-life ($t_{1/2}$) for pyrethroids was determined with Eq. (3):

$$t_{1/2} = \frac{\ln 2}{k} \quad (3)$$

Microbiota corresponding phospholipid derived fatty acids biomarkers were used to determine numerically ecological parameters as species richness (Simpson diversity (Eq. 4), Shannon-Wiener diversity (Eq. 5) and Alatalo evenness (Eq. 6)).

$$D = 1 - \sum P_i^2 \quad (4)$$

$$H = -\sum P_i \cdot ln(P_i) \quad (5)$$

$$J = \frac{\left[\frac{1}{\sum(P_i^2) - 1}\right]}{[exp(H) - 1]} \quad (6)$$

where: D is Simpson diversity index, H is Shannon-Wiener diversity index, J is the Alatalo evenness index. P_i is the ratio between N_i and N, where N_i is the i^{th} kind of PLFA, while N is the total amount of determined PLFA in a soil sample (Luo et al., 2019).

The correlation between pyrethroids concentration and microbial indicators in soil was established with Spearman coefficient analysis. Measurement of microbiota community structure and abundance change after soil cores transplant was done with principal component analysis. These analyses were performed using Statistica 10 software (Stat. Soft. Inc., USA).

3. Results

3.1. Contaminated soil abiotic and biotic property

In soil samples being studied, collected from an agricultural land from Turda, the major quantified pyrethroid pesticides were cypermethrin, Fenvalerate and deltamethrin. Their quantity varied within a range of 119.1-895.2 µg·kg^{-1} with the following pattern: Cypermethrin > Deltamethrin > Fenvalerate (Table 1). Before the start of the experiment (t = 0), laboratory soil box was tested for all targeted pyrethroid pesticides content at 4 depth levels (0 – top, 0-15 cm, 15-30 cm and 30 – 50 cm) according to Fig. 1. and sampling diagram presented in Fig. 3. Quantitative amount of cypermethrin, deltamethrin and fenvalerate in experimental box soil varied between 335.9 -584.5, 198.2-381.2, 68.5-157.2 µg·kg^{-1}, respectively. Differences in quantitative amount distribution with depth (0-50 cm) were observed for all three pyrethroids (Fig. 4).

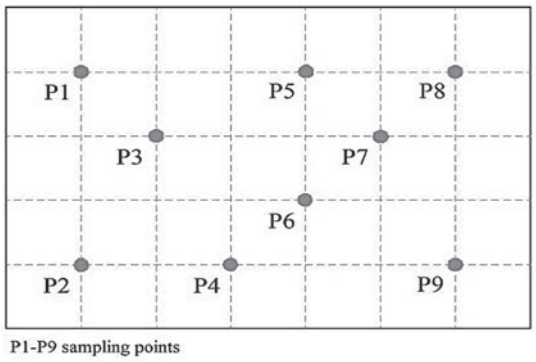


Fig. 3. Schematic presentation of sampling points from soil box before transplant of soil cores

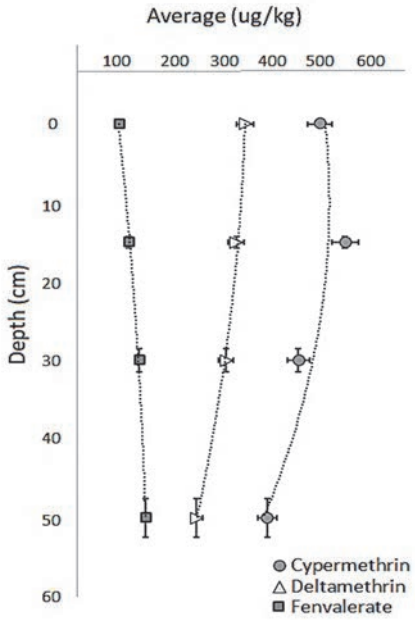


Fig. 4. Pyrethroids distribution with soil depth in soil box

Homogeneity test was performed on measured values for each horizon and each targeted pyrethroids with aim to evidence if there are significant differences between pyrethroids concentrations. p-values (> 0.05) obtained after chi-square test has shown that there are no significant differences in this sense (Table 4).

Table 4. Homogeneity test parameters

Pyrethroid pesticides	χ^2	***p-value***	***RSD$^{(a)}$ (%)***
Cypermethrin	18.11	0.8	3.9 – 8.2
Deltamethrin	30.59	0.17	5.3 – 13.2
Fenvalerate	7.39	0.95	11.3 – 14.6

RSD$^{(a)}$ – relative standard deviation range of each pesticide in part measured for the same horizon

Fenvalerate concentration was lower in top horizon compared with lowest ones (Fig. 4.). This could be attributed to potential photodegradation processes due to light cycle periods applied through experiment. This is in accordance with data presented by Tariq et al. (2017) and Liu et al. (2010), where fenvalerate was found more photolabile than

deltamethrin with approximately 1.5-fold. Zhu et al. (2020) in their review found also deltamethrin as the most photostable pyrethroid pesticide, followed by cypermethrin. Also, the silty clay loam soil had a retention effect on pyrethroid pesticides in the site.

Soil physicochemical parameters as total N, mobile P, and K presented a decreasing tendency through soil layers. The highest values for these parameters were reported in the 0-15 cm horizon, after that started to decrease. Only K concentration started to decrease from the third horizon (30 cm).

The average value of soil microbiota abundance in contaminated soil was 432.2 nmol·g^{-1} soil (dry weight). The dominance of bacterial community was observed in case of each samples from all horizons analyzed in the contaminated soil box (Fig. 5.), with an average value of bacterial community of 327.5 nmol·g^{-1} (dry weight) and an average F:B ratio of 0.315. Microbiota community composition and relative abundance varied within soil horizons (depths). In first two horizons (top – H 0, and H 0 – 15 cm) aerobic bacteria dominance could be observed, the average value of dominance indices for them being 0.47 and 0.51, respectively. Starting with depth 15 cm (H 15 – 30 cm and H 30 – 50 cm) this dominancy was changed, showing an anaerobe bacterial dominance (dominance indices: 1.38 and 5.25).

3.2. Transplanted soil core biotic and abiotic property

Soil cores used for transplant were collected from a natural grassland where no contamination with pyrethroid pesticides was evidenced before. Two sessions of sampling were performed, one in spring (April) and the second in the fall (September). The average value of microbiota abundance in all transplanted cores, including all horizons, was 740.1 nmol·g^{-1} soil (dry weight). The selection of these two periods was motivated by the fact that there are changes in soil microbiota structure and abundance due to seasonality.

Literature reported in many cases a higher abundance in the fall season compared with spring (Luo et al., 2019; Silas and Margesin, 2017). Microbiota abundance in collected soil cores for transplant differed, the average value of total microbiota abundance in fall (683.1 nmol·g^{-1} soil dry weight) being higher than in spring (629.6 nmol·g^{-1} soil dry weight).

According to that hypothesis, abundance of bacterial and fungal communities differed between seasons, in soil cores collected in fall the average abundance of total bacteria was higher with 7.2% than in spring while and average abundance of total fungi was higher with 11.2% in soil cores sampled in fall than in spring period. These data concurred with the results of previous works studying seasonality in microbiota abundance (Luo et al., 2019; Siles and Margesin, 2017).

As in contaminated box soil, bacterial dominance was evidenced in all transplanted cores. The average F:B varied within 0.16-0.21. Differences in microbiota abundance between the two seasons could relate to the vegetation stage and abundance at each site. Plant composition and development stage influence belowground conditions by driving changes in soil environmental properties such as soil pH, litter quantity and quality, soil nutrient contents and soil moisture. Soil cores pH and humus content were a little bit higher in the fall season (average pH: 6.5, and humus: 2.51%) than in spring (average pH: 6.3; and humus: 2.25%).

Bacterial community, including actinomycetes also, are primary decomposers in soil. They decompose labile tissues and compounds. Fungal community, secondary decomposers in soil, grow slowly and are responsible for more recalcitrant carbon tissues and compounds decomposition (Dhakal and Islam, 2018). The higher soil fungal biomass (104.7 nmol·g^{-1} dry weight) and F:B ratio (0.315) in contaminated soil than in transplanted soil cores could be due to the presence of more recalcitrant substrates, which favors fungal growth and increase F:B ratio.

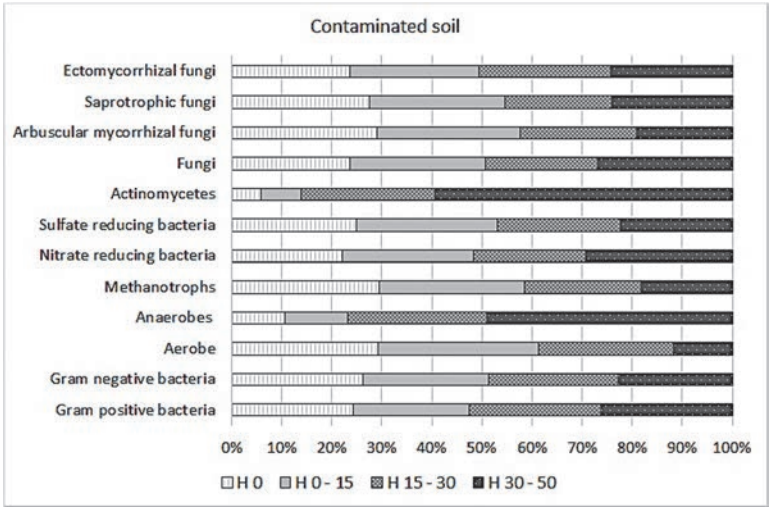


Fig. 5. Microbial community composition and relative abundance in the contaminated box soil from different depts (horizons H 0 – top, H 0 – 15 cm, H 15 – 30 cm and H 30 – 50 cm)

The F:B ratio in soil is increased inversely with organic and inorganic compounds cycling and results in more recalcitrant carbon in soil (Marschner et al., 2003).

4. Discussions

4.1. Pyrethroids changes over experiment

Pyrethroids can be degraded in the environment through multiple processes, including hydrolysis (Heidari et al., 2005), photodegradation (Liu et al., 2010; Tariq et al., 2017) and biodegradation (Sakai et al., 2002; Verhagen et al., 2014). While previous research has been conducted on photodegradation (Diaz-Lopez et al., 2019; Zhu et al., 2020) and hydrolysis (Diaz-Lopez et al., 2019) mechanisms of pyrethroids in the environment, experiments are minor considering natural biodegradation processes in soil ecosystems (Karpouzas et al., 2014; Zhu et al., 2020). To study the influence of soil microbiota structure and abundance (initial – contaminated soil, first transplant of soil cores and second transplant of soil cores) on the degradation of cypermethrin, deltamethrin and fenvalerate in contaminated soil samples incubated for 235 days under controlled conditions were monitored. The results are shown in Fig. 6 and Fig. 7. The initial high pyrethroid concentration resulted in faster degradation in the early stages (Fig. 6.) and short half-lives (Table 5) after soil cores transplant. These data were in accordance with previous data presented by Pose-Juan et al., (2017) for Triasulfuron, Gregory et al., (2015) for organochlorine pesticides, and by Chen et al., (2012) for bifenthrin.

Degradation rates of the monitored pyrethroids varied as follows: cypermethrin > deltamethrin > Fenvalerate. Faster degradation was observed after the second transplant of the soil cores (Table 5, Fig. 6). This could be attributed to the higher microbiome abundance in the second transplanted soil cores (683.1 nmol·g^{-1} soil dry weight) than in the first transplanted soil cores (average 629.6 nmol·g^{-1} soil dry weight), consequence of seasonality. Considering pyrethroids degradation rate with soil depths, differences were observed. In horizons H 0 – 15 and H 15 – 30 the degradation of pesticides presented a higher rate. This could be attributed to the higher abundance and diversity of soil microbiota in that layer (0.534-682 nmol·g^{-1} soil dry weight, $p < 0.05$) in case of all monitored pyrethroids.

In Fig. 7, the Napierian logarithm of the concentration of undegraded compounds vs. exposure time at soil microbiota was plotted and found to be linear. Squared regression coefficients (R^2) for cypermethrin, deltamethrin and fenvalerate were 0.884, 0.873, and 0.877, respectively (Fig. 7a). The rates of biodegradation increased in proportion once with soil cores transplant, higher biodegradation rates being achieved for all pyrethroids after the second soil core transplant. After soil cores transplant the squared regression coefficients varied within 0.808-0.966 (Fig. 7.b. and c). These results suggest that the degradation process was of first order.

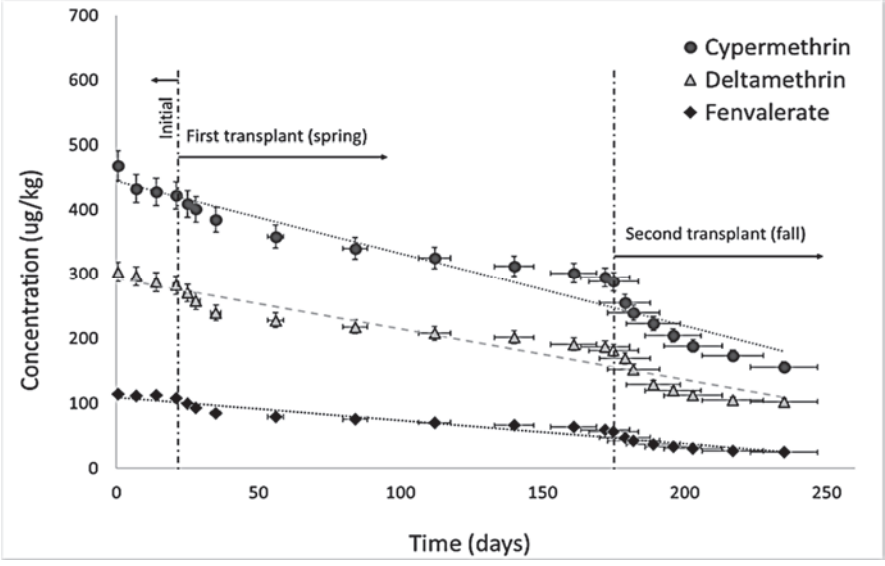


Fig. 6. Variation of pyrethroids concentration in time also considering the moments of the two soil cores transplant

Table 5. Half-life of pyrethroid pesticides before and after soil cores transplant

Pyrethroid pesticide	***$t_{1/2}$ – initial (days)***	***$t_{1/2}$ – first transplant (days)***	***$t_{1/2}$ – second transplant (days)***
Cypermethrin	140	59.2	19.4
Deltamethrin	141.2	57.2	18.3
Fenvalerate	69.3	56.9	16.8

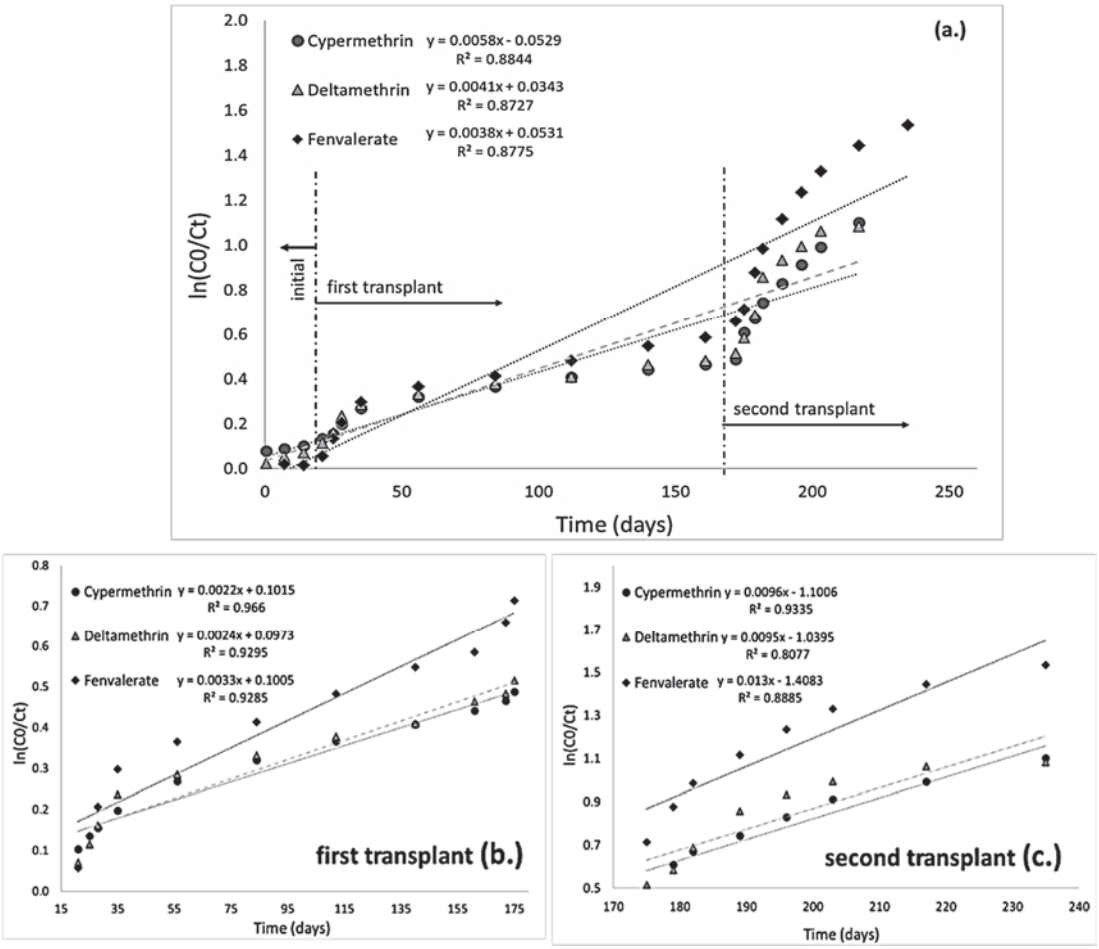


Fig. 7. Pyrethroids degradation in soil environment. (a.) whole period of study; (b.) after first transplant; (c.) after second transplant

4.2. Bacterial and fungal abundance and structure changes before and after transplant of soil cores

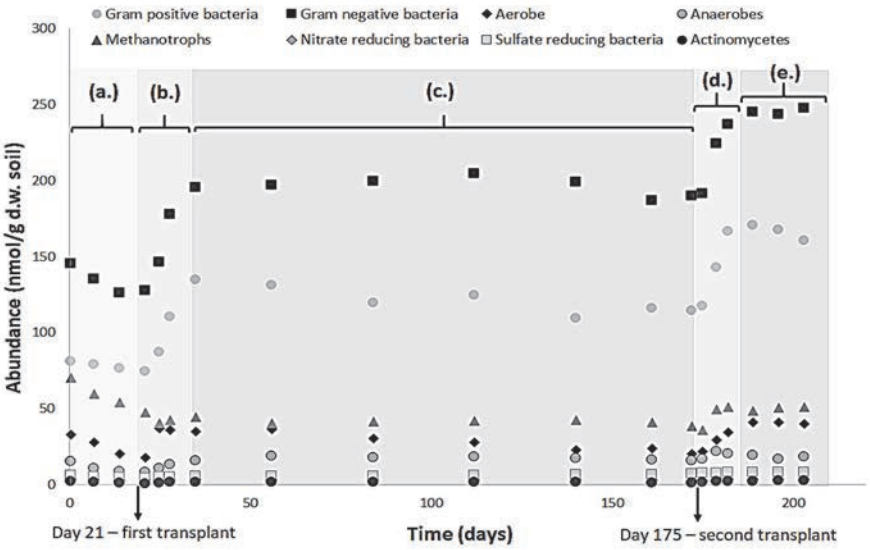
Microbial degradation is one of the major routes through for pesticides elimination from ecosystems (Huang et al., 2020; Karpouzas et al., 2014; Sakai et al., 2002; Verhagen et al., 2014; Zhu et al., 2020). Despite the ecological relevance of soil microbiota, there is minor information about the potential toxicological effects of pyrethroid pesticides in general toward these organisms (Bhatt et al., 2021; Bhatt et al., 2020). This study presents a well-defined tiered microcosm-to-field approach (contaminated soil box experiment) for assessing the impact of field level pyrethroids on microbiota abundance, structure, and metabolic activities. Our data evidenced that soil microbiota community structure abundance is changed in soils contaminated with cypermethrin, deltamethrin and fenvalerate. Both bacterial and fungal communities show a decrease tendency in their abundance (Fig. 8.a and b). Most affected bacterial structure was actinomycetes, and their abundance decreasing with 73%. Anaerobes and aerobes bacterial abundance was reduced at almost half, 46.6 and 46.4%, respectively (Fig 8.a. – zone (a).). Methanotroph and sulphate reducing bacteria decreased also with approximately 30%. Gram positive and negative bacterial abundance was reduced by approximately 10% in contaminated soil, showing a higher resistance at these pyrethroid pesticides (Fig. 8.a. – zone a.). It was observed that fungal communities are more affected when are exposed at pyrethroid pesticides as cypermethrin, deltamethrin and fenvalerate (Fig. 8.b. – zone a.). Their abundance was reduced within 49.2 – 64%.

Transplanted cores influenced the microbial community structure abundance in the contaminated soil – transplanted soil cores ecotones (Fig. 8.a. and b. – zone b., c., e). Among bacterial community higher revitalization efficiency due to transplanted cores (first transplant) was observed in case of actinomycetes, anaerobe and gram-negative bacteria. Their abundance increased with 1.8, 1.2 and 1.6-fold, respectively. Slight increases in abundance were registered also in case aerobe and sulphate reducing bacteria, while a decrease with approximately 25% was observed in case of methanotrophs and nitrate reducing bacteria (Fig. 8.a. – zone b.). The first transplanted cores showed a more pronounced impact on fungal community. In their case, increases with 1.7-2.3-fold was registered for all monitored phenotypic structures (Fig. 8.b. – zone b.). The second soil core transplant also influenced soil microbiota community abundance but in more lessened way. Bacteria as gram positive, gram negative, aerobes, and nitrate reducing increased approximately with 30%. No significant

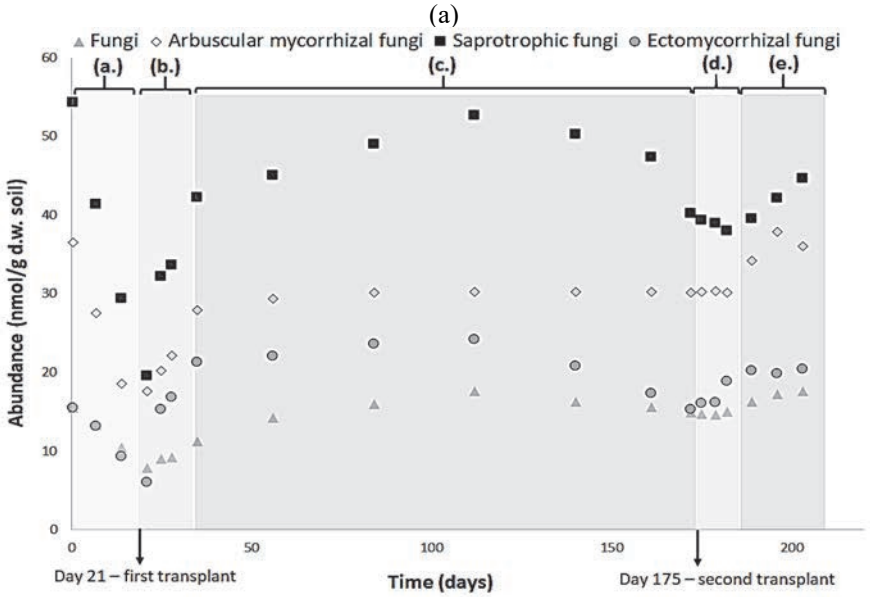
increases were observed in case of methanotroph bacteria ($p < 0.05$), while actinomycetes showed an increase only with 3%, which is significantly lower compared with the impact of first transplanted soil cores where a 1.8-fold increases was observed after transplant (Fig. 8.a. – zone e.). Arbuscular mycorrhizal, saprotrophic, and ectomycorrhizal fungi abundance increased with 16%, after the second transplant of soil cores (Fig. 8.b. – zone e.).

Although after the first transplant most phenotypic groups of monitored microbiota presented a stabilization in their abundance, minor decreases tendency was observed in case of gram-positive bacteria and ectomycorrhizal and saprotrophic fungi (Fig. 8.a. and b. – zone c. and d.). The correlation coefficient between their abundance and pyrethroid pesticides amount (0.652, 0.811 and 0.724) in soil suggests that they can be directly involved in pesticides decomposition in soil media.

These were also sustained by principal component analysis (Fig. 9), where first axis explained with 57.3% and the second axis with 28.5% contribution of transplanted soil core microbiota involvement in pyrethroid pesticides degradation in contaminated soil. The microbial diversity analysis of the contaminated soil, transplanted soil cores and corresponding near ecotones showed that the richness (number of microbial species) was in the range of 13 to 42, while the Simpson index, Shannon-Wiener index and Alatalo index ranged between 0.62 – 0.91, 2.48-3.65 and 0.48-0.74, respectively. The microbial diversity of the monitored soils varied. At the top (H0) and the first layer horizons of soil depth (H0-15 cm), the richness and Simpson, Shannon-Wiener and Alatalo indexes peaked in the transplanted soil cores, especially in the transplanted soil cores from fall season, with values of 38.2, 0.87, 3.47 and 0.67, respectively.



(a)



(b)

Fig. 8. Microbiota abundances change in contaminated – transplanted soil cores ecotones: (a) bacterial community; (b) fungal community

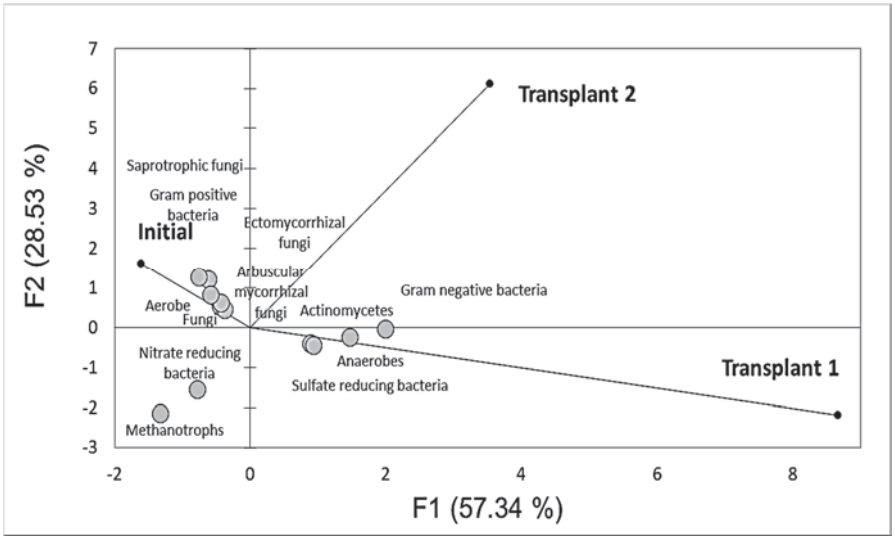


Fig. 9. Principal component analysis of transplanted soil cores microbiota community impact on pyrethroids degradation in soil

The Simpson and Shannon-Wiener indexes in the transplanted soil cores from fall season and spring season were significantly different from those in the contaminated soil ($P < 0.05$). A significant difference in richness found between the transplanted soil cores of the two seasons but with no significant differences in the Alatalo index considering seasons of the transplanted soil cores ($P > 0.05$). In the second soil layer (H 15 – 30 cm), the largest values of richness and Simpson and Shannon-Wiener indexes were still found in the transplanted soil cores from fall season, with values of 31.5, 0.85 and 3.37, respectively. Overall, the microbial diversity of the transplanted soil cores was greater than that in the contaminated soil.

4.3. Pyrethroids and transplanted soil core impact on bacterial and fungal biomass metabolic activity

The nontarget effects of cypermethrin, deltamethrin and fenvalerate on microbiota in soil ecosystems are of great interest because of their essential role in soil functions. Also, microbial degradation is one of the major elimination routes of pesticides, as pyrethroids, from ecosystems (Bhatt et al., 2019; Birolli et al., 2019; Karpouzas et al., 2014; Zhu et al., 2020). Despite the ecological relevance of soil microbiota, there is minor information about the effects of pyrethroid pesticides in general toward these organisms.

Zhang et al., (2020) has evidenced nontoxic effects of permethrin on in situ populations of microbiota in both mineral and organic soils. Bacterial and fungi number in soil did not change significantly when were exposed at 100 $\mu g \cdot kg^{-1}$ for long-term period. Other studies (Zabaloy et al., 2016; Zhang et al., 2009; Zhan et al., 2020; Zhan et al., 2018) revealed that in cased of short-term exposure the effect of permethrin on soil microbiota depending upon the soil type. Their observed effects included both inhibition as well stimulation on microbiota abundance expressed as numbers of cells.

Similarly, the study by Farenhorst et al., 2010 on pure cultures of soil fungi revealed a decrease of their life period. Pose-Juan et al., (2017) and Cycon et al (2009) showed that the parent compound and its degradation products can interact to produce synergistic and antagonistic responses with these organisms.

Because of the shortage of data on the toxicity of pyrethroid insecticides toward microorganisms and microbial processes in the biosphere necessitates more research into this aspect of ecotoxicology. In this study impact of cypermethrin, deltamethrin and fenvalerate on soil microbiota catabolic activity was studied by considering C, C x N, and C x P consumption rate from carbohydrates, carboxylic acids, amino acids, amines, phenolic compounds, and polymers used as substrates.

The effect of cypermethrin, deltamethrin and Fenvalerate on microbial community catabolic activity was evaluated considering the average well color development ($AWCD_{590}$) during 168 h of incubation period. There were statistically significant differences found in the AWCD index in transplanted soil cores and contaminated soils (Table 6).

The highest AWCD (AWCD – 1.829) value was measured in soil cores from the second transplant. However, the lowest AWCD value was recorded in the contaminated soil (AWCD – 0.359). The effect of pyrethroid pesticides on microbial community catabolic activity as evaluated by substrate use incubated for 0 – 168 h was measured. The highest absorbance values were measured after 72 – 120 h (Table 6).

Among substrate carbohydrates under study, (2.428) and carboxylic acids (2.115) were most easily consumed by microorganisms. The lowest absorbance values were observed in case of polymers (0.359). Statistically significant differences were observed in the use of particular groups of C, C x N, and C x P compounds by microorganisms in soils depending on soil contamination with pyrethroid pesticides.

Table 6. AWCD index of contaminated soil, transplanted soil cores, and contaminated soil cores – transplanted soil cores ecotones (near ecotones)

Time (h)	*AWCD*			
	Contaminated soil	*1st transplanted soil*	*2nd transplanted soil*	*Near ecotones*
6	0.359 ± 0.085	0.401 ± 0.067	0.511 ± 0.084	0.367 ± 0.038
12	0.372 ± 0.077	0.469 ± 0.064	0.548 ± 0.094	0.388 ± 0.067
24	0.511 ± 0.092	0.805 ± 0.112	0.789 ± 0.105	0.553 ± 0.062
48	0.714 ± 0.088	0.985 ± 0.091	1.052 ± 0.088	0.806 ± 0.154
72	1.002 ± 0.135	1.284 ± 0.124	1.418 ± 0.176	1.104 ± 0.123
96	1.295 ± 0.104	1.488 ± 0.215	1.566 ± 0.194	1.205 ± 0.207
120	1.322 ± 0.096	1.428 ± 0.186	1.829 ± 0.302	1.315 ± 0.149
144	1.289 ± 0.127	1.512 ± 0.173	1.821 ± 0.216	1.568 ± 0.167
168	1.305 ± 0.207	1.517 ± 0.189	1.826 ± 0.274	1.557 ± 0.231

The values are means ± standard error (n = 3)

Enzymes catalyze metabolic reactions through hydrolysis, oxidation, the addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, the addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, the replacement of a sulphur with an oxygen, the metabolism of side chains, the ring cleavage etc. (Garcia et al., 1997). Pyrethroid pesticides biodegradation processes are directly dependent on the metabolic potential of microorganisms to detoxify or transform these molecules. Through this study it was observed that soil enzyme activity as dehydrogenase and esterase was significantly affected by pyrethroids. Dehydrogenase occurs in living soil microorganism cells, and its linked with microbial respiratory processes (Riah et al., 2014). Spearman correlation coefficient between dehydrogenase activity and soil respiration in contaminated soil, first transplanted soil cores, second transplanted soil cores and near ecotones were 0.812, 0.888, 0.924, and 0.814, respectively. Dehydrogenase in soil was inhibited by pyrethroid pesticides for several days due to their repressive effects on soil microbiota. In contaminated soil and near transplanted soil cores, dehydrogenase activity was lower with an average of 42% than that from transplanted soil cores. These were in accordance with soil microbiota abundance (Spearman correlation coefficient 0.792, $p = 0.017$).

Esterase is an enzyme which is involved in the metabolism of xenobiotics. Esterase protects contaminated soils by catalyzing the hydrolysis of pyrethroids (Garcia et al., 1997). Esterase activity varies within 41 – 110 nmol · $g^{-1}min^{-1}$, with higher values registered in near ecotones of contaminated soils – transplanted soil cores (27-36% higher than in contaminated or transplanted soil cores).

These data sustain our hypothesis that soil microbiota contributes at pyrethroid pesticides degradation as well that transplant of soil cores could revitalize affected soil microbiota. However, understanding of the mechanisms of how microorganisms biodegrade pollutants and how they interact with the environment need more attention thus to successfully implement soil cores transplant methodology for in situ remediation. Also, attention to resulted pyrethroids degradation products effects on soil microbiota should be a further interest.

4. Conclusions

The presence of cypermethrin, deltamethrin and Fenvalerate in agricultural soils from Turda negatively impacted soil microbiota.

Considering soil microbiota phenotypic structure, actinomycetes community was more affected, their abundance decreasing by 73%. Anaerobes and aerobes bacterial abundance was also reduced by approximately 50%. Compared with bacterial community, Soil fungal community abundance was also affected when were exposed at pyrethroid pesticides as cypermethrin, deltamethrin and fenvalerate. Their abundance was reduced within 49.2-64%.

In this study a positive impact of transplanted soil cores on both soil microbiota abundance as well on cypermethrin, deltamethrin and fenvalerate concentration was observed. Thus, a revitalization on microbiota abundance in contaminated soil was observed after transplant. Among bacterial community higher revitalization efficiency due to transplanted cores was observed in case of actinomycetes, anaerobe and gram-negative bacteria. Their abundance increased with 1.8, 1.2 and 1.6-fold, respectively. Also, fungal community abundance increased generally with 16%, after transplant of soil cores.

These data positively sustain that affected soil microbiota due to use of pyrethroid pesticide could be revitalized through transplant of soil cores with no contamination.

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